Evidence base of diagnosis of Corona Virus...

The scandal of the millennium

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&

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India Book of Records

Extraordinary feats... Extraordinary people

...and the treatment of COVID-19

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Evidence Base of Diagnosis of Corona Virus & the treatment of COVID-19

Evidence Base of Diagnosis of Corona Virus
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the treatment of COVID-19

Ques-1: Why the panic of corona virus? Projection Vs reality

U.S Centre for disease Control and Prevention predicted in the month of **February**[1] from 200000 to 1.7 million deaths due to corona virus this season in US alone.

In contrast the total flu (Covid-19 is also a Flu) death this season is about **20000 to 50000**[2] which is less than the number of flu death in the previous four seasons (2018, 2017, 2016, 2015).

Flu Hospitalization in U.S.A[1]	
Year	**Number**
2016-2017	500000
2017-2018	800000
2018-2019	500000
2019-2020	525000

If we extrapolate the US model to India, the total corona deaths should have been between one lakh to 70 lakhs. In contrast the total Covid-19 death so far is 72 (till 3rd April) and 80% of them were having comorbid conditions and average age more than 60 years.[3] Here we must keep in mind the average life expectancy of India is 68 Yrs ***(Source World Bank)***.

Similarly ahead in this article you will see even in Italy there is no excess death this season. Infact every year in winter the ICU is 85% -90% full [4].

Ques-2: What is Covid-19 / Corona Virus?

A Corona Virus is like any influenza virus and the disease it causes is called COVID-19.

COVID-19 can be put in ILI (Influenza like Illness) group as it shares many features of Influenza including:

- Mortality rate about 0.1%
- The virus attacked the respiratory tract.
- Common symptoms include fever, cough weakness and shortness of breath.
- It's a single strand RNA segment
- It's airborne / waterborne like any other flu virus.

Ques-3: Now the question arises how do we get to know whether a person is a patient of corona virus or covid?

There is only one way-rtPCR Test-Reverse transcription Polymerase Chain Reaction Test- a test kit by which you can diagnose whether a patient has contracted coronavirus or not. Whenever a machine or gadget is bought, whether car, camera or laptop, a manufacture's manual is provided. Similarly, when a rtPCR kit is bought, a manufacturer's manual will be provided. If you look at the manual it clearly says under Regulatory Status – '**For Research Use Only, Not For Use In Diagnostic Purpose**.[5]

It is very clearly mentioned that it is only for research and not to be used for diagnostic purpose. This is the manufacturer's mandate. Not only the manufacturer's mandate, it has also been said by the inventor of this kit***, Kary Mullis, who is a Noble Prize winner***[6]

Why this is not for diagnostic purpose ? The answer to this question is that- specificity of this test kit is at the most 99%. Specificity means that you can subject any random 100 healthy persons to undertake this test, and then it will declare any one person as false-positive. This was proved on 18th March 2020 in Iceland. **On the 18th this test**[7] was carried out on 1800 healthy people and 19 people were identified as coronavirus patients. This is when the test kit was functional with full efficiency. If we listen to the advice of White House then the coordinator of corona virus in the White House, Dr. Birx, is of the opinion that the kit has ***50% chance of proving false***[8]. That means every second test could be proved wrong. For this very reason in Finland on 20th March, the health ministry ***in Finland, rejected this test kit***[9].

To understand the confusion, we have to turn some pages of the medical journal. According to the ***American Journal of Medical Association of 27th Feb 2020***[10], 4 patients of Wuhan were tested with this kit. They were declared corona virus negative by this test kit just before being released from the hospital. They were discharged from hospital and allowed to go home. After about 13 days, they were tested with the same kit and were discovered to be corona-positive. What does this mean? Either they were never negative in the first place and they had coronavirus, secondly, they were cured but again contracted infection on reaching home, thirdly, their body is coronavirus free and the test is wrong, hereby meaning that there is no conclusive answer with anyone.

Now let us move on to 4th March ***-The Lancet***[11]- a very important journal. There is a case study of a patient in Singapore in this journal. This patient was taken to the hospital in high fever, where he was tested for Dengue and declared dengue-positive, for which treatment began. The doctor decided to test him for corona virus and was found to be coronavirus -positive as well. The question is whether to consider him a dengue patient or a coronavirus patient? Was it false positive in both the cases? Simply put, there can be no conclusive answer.

Let us move onto 5th March- ***New England Journal of Medicine***[12]- the first patient to get Coronavirus In the U.S. A sample was taken from his nose and it was tested corona positive. A

sample from his mouth was tested corona- negative. It is up to you to draw your conclusion. I can only say that the reliability of this test is zero.

Even the founder of Cochrane Collaboration Peter Gøtzsche had written in a report in the ***British Medical Journal***[13] of the 6th March wherein he stated that the only way to come out of the present environment is by removing the testing-kit. This testing kit is the root cause of all problems..

Actually, I have also invented a test kit by which you can by sitting at home determine whether you are a corona patient or not. Are you interested? This test only requires 15 seconds to find out whether you are a corona patient or not. Your 15 seconds start now. Akkad bakkad bambe bo , Assi nabbe poore sau, Sau mein nikla dhaaga Chor nikal ke bhaaga. The finger is pointing towards you at the end of this counting, that means you are coronavirus patient. If there are 10 people and you want to find out the patient, then it is very simple. Just memorize this counting style and wherever the finger points, that person is the patient.

You will say this is a big joke. This is a fluke. What will you believe in – science or fluke? What is the meaning of science – a manufacturer's manual, or inventor? All medical journals? Science is saying that this test kit is illogical, illegal and crime. Who is recommending this test kit? Only one organization is doing it and that is WHO (World Health Organisation) which was declared a thief by ***PACE-Parliamentary Assembly of Council of Europe***[14]-10 years ago. Remember about 10 or 11 years ago there was a similar kind of situation and the villain then was H1N1 pandemic. After a few months the Director of WHO let out some secrets to unearth a big medical scam. So to adhere to any advice from such an organization is a sin.

Ques-4: The question is if this is a scam then why are so many people dying in the world?

11500 (as on 31st march 2020) people have died in Italy in 2 months. Will you follow the media or the health ministry of Italy? of Italy? Yesterday I got in touch with people of Italy to know the **real time status of Italy**[15]. Besides this, the summary report of the ***National Health Institute of Italy*** [16]dated 20th March 2020, states that 48.6% people had 3 other ailments besides corona, 26.6% people had 2 other ailments besides corona, 23.5% patients had 1 other ailment other than corona. Only 1.2 % had corona. If we have a look at the death certificates of the patients then ***only 12% person***[17] had died due to corona virus, as per the report of NHI. In reality only 1380 have succumbed due to corona virus.

There is a difference- dying with corona virus or dying from corona. They mean that 12% people died from corona virus and the rest died due to some other disease.

If you are not yet convinced then please visit website-***Euromomo.eu***[18]where you will find data of the mortality rates of the last 5 years within Europe. You can see mortality rate in Italy or any other country during the last 5 years is almost the same . The only difference is that this time each death figure was reported in real time through social media and panic was created.

The question is what to do in this situation. What is the media saying, what is the public saying? You have a choice.

Actually ***UK govt. in January***[19] released a statement that 22 Lakhs death in USA and 5 lakhs deaths in UK will occur due to corona. A special status was given to coronavirus-HCID (High Consequence Infectitious Disease). Now 2 months later, after compiling results from all over the world UK govt has realized that coronavirus is only a simple flu virus and can be treated in any hospital or clinic.

Quietly on 19th March they removed coronoavirus from ***HCID***[20] and put into normal virus. But they hid this fact from public and social media. So I made a video on the 28th and reached out to you all with this report where UK Govt admitted that they were wrong about Corona Virus Pandemic. And today with your help this report has reached every household. On 26th March the ***New England Journal of Medicine***[21] and 27th March Research Paper in ***The Lancet***[22], reported that this Corona virus is not so deadly as was expected and the death rate is 0.1% which is equivalent in case of any normal flu

Ques-5: Status of CDC 2019 – nCov- Real Time rtPCR diagnostic panel as on 30th March 2020 [23]

- Detection of viral RNA may not indicate the presence of infectious virus or that 2019-nCov is the causative agent for clinical symptoms.
- False positive is more likely when prevalence is moderate or low.
- The performance of this test has not been established for monitoring treatment of 2019-n Cov infection.

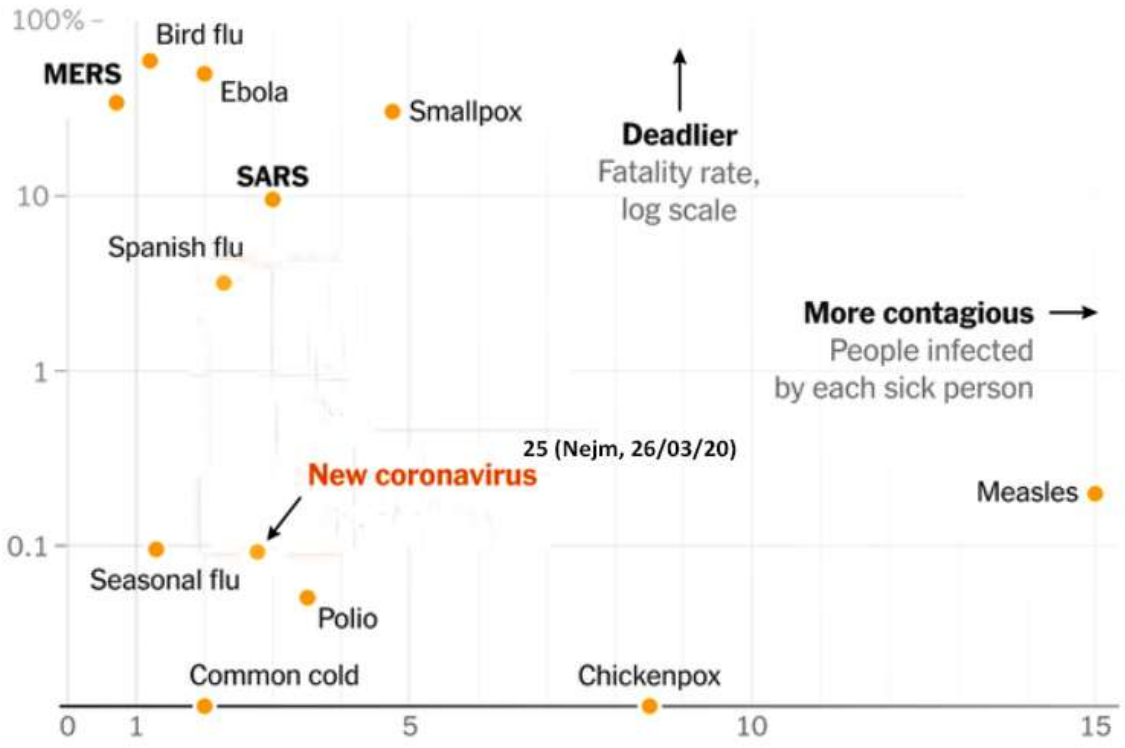
Ques-6: Corona Virus Vs Flu

The new corona virus causing COVID 19 has led to more than 454,000 ilnesses and more than 20550 deaths worldwide. For comparison in the US alone the Flu (also called influenza) has caused an estimated 38 million illnesses, 390,000 hospitalizations and more than 23,000 deaths this season according to *CDC*[24] (as of 25th march 2020)

The death rate of COVID-19 and Flu is 0.1%[21]. The R_o of corona virus is 2.2[21] whereas R_o of Flu Virus is 1.3[24].
Here we must remember the R_o is not an intrinsic feature of the virus. It can be lowered through containment, mitigation and ultimately "herd immunity", as the people who have recovered become less susceptible to infections or serious illnesses. For the epidemic to begin to end the reproduction rate has to drop below 1[25]

Based on the above facts and figures, here is how it compares in terms of the death rate and transmission rate (R_O) to other viruses.

Reference [26]



Symptoms : Corona V/s Flu[24]

The new corona virus and the seasonal flu are similar in many ways. Both are respiratory diseases that spread through droplets of fluid from mouth and nose of someone who is infected. Both are contagious and produce similar symptoms such as fever, cough, muscle ache, weakness and are particularly hard on elderly.

The Difference

1. They come from different family of virus.
2. People have more protection from flu virus because they are exposed to flu virus repeatedly every year.

Ques-7: Yearly Flu death figures in London & Wales

Looking at the year-to-date, the number of deaths is currently lower than the five-year average. The current number of deaths is 150,047, which is 3,350 fewer than the five-year average. Of the deaths registered by 27 March 2020, 647 mentioned the coronavirus (COVID-19) on the death certificate; this is 0.4% of all deaths. [27]

Ques-8: WHO recommendation of RT-PCR test

WHO has recommended to use RT-PCR test to diagnose Corona Virus.[28]

3 Most Important Questions

Ques-9: Not a single extra death due to Covid 19 across the World?

Looking at the above data directly from respective Government Website we can see there is no excess mortality in this season in US or Italy and in fact it is lower the five year average in case of UK. Similar trend can be seen in all European countries through EUROMOMO.EU
In India every year respiratory infarction (Influenza like Illness) kill nearly 3,50000 people[29] i.e regularly 1000 deaths every day due to influenza like illness (ili). Now if you compare the total death of COVID-19 patients till now (not every death is due to corona itself, it could be due to the side effects of medications as explained below), it is coming to be a mere fraction of the total death due to ILI.

Ques-10: It is impossible to stop corona virus with lockdown because…

1) 80%[30] of the corona virus carrier are asymptomatic and so cannot be detected through present thermal screening method, employed initially airport and subsequently in colonies etc.
2) Among the symptomatic COVID patients 57% do not have fever[31]
3) The present thermal scanner used is the industrial thermal scanner[32] with an acceptable error of -4^0 F.
   If we combine the above three points its conclusive that 95% of the cornona carrier will never be detected with the present mass screening strategy employed since last week of January 2020.
   So inspite of lockdown there can be active transmission of virus through essential services like vegetables/fruits/milk etc. So according to the Oxford model[33], inspite of the lockdown 50% of the UK population is already infected, leading to developing "herd immunity" which will finally lead to protection from Corona Virus deaths. The same scenario can be assumed for India as well.

Ques-11: If not corona what is the true cause of death of COVID-19 patients.

To get the answer we have to go back 100 years. In the year 1920 H1N1 Spanish Flu the world's worst flu outbreak in which 10 crore people died, which was approx.5 % of the total world population then. Among them were 1 crore 80 lakh Indians. It was believed that the virus was very strong, deadly and dangerous to have killed crores of people. But now medical science is very clear that the actual cause of deaths was not virus but some other reason. Which means that between 1918-1920, whenever anyone came down with flu, that person was dumped in the hospital-into a cramped room without fresh air, sunlight and adequate nutrition, and that was the cause of the death. Just then, there were very few hospitals in the world which were given the name of ***'Open Air Hospitals[34]'***. This meant that the patient was kept in fresh air and also provided with sunlight and these patients walked out of such hospitals alive. So if we take that example and compare it with the present context, then if anyone is detected with corona virus, then that person is quarantined completely in a way that the person is cut off from fresh air and sunlight. Also the food is processed and packed or cooked in a way that the person can fill his stomach

but nutrition is almost negligible. In such a situation the patient takes a longer time to recover. Also, if the patient has been suffering from other ailments like kidney failure, diabetes, heart disease or high blood pressure and administered with ***anti-malarial drug***[35]along with antibiotic then it is seen the combination of these 2 medicines in the last 40 years has resulted in ***QT prolongation of heart***[36]meaning the heart beat dangerously rises and causes sudden cardiac death. This has been seen in the research paper of ***27th March of Journal of American Medical Association***[37]**,** according to which the cause of death is myocardial injury, meaning injury in the heart. This happens in 1/3 of the patients. This means it is clear that the cause is either coronavirus or the treatment for coronavirus. Also we have cut ourselves from fresh air and sunlight in our quest to recover. Remember, fresh air and sunlight are 2 precious gifts to us from GOD and are antiviral. Not only that, these 2 plays an important role in boosting our immunity.

Wherever there is lockdown, people suffer from blood sugar, blood pressure, weight issues and depression. The rate of depression has risen. Meaning that the people are falling sick because of the lock down protocol . Locking up in the house has its own hazards. At least 1/3 of the people are worrying about losing their jobs. The Economic Times quotes that 30 % of the Indian population is on the verge of losing their jobs, in such a situation, depression has already set in over and above the fear of losing their job. This is a dangerous situation. So the need of the hour is to come out of this situation by empowering yourself with the right kind of knowledge

Ques-12: Lockdown V/s No Lockdown (as adapted from the Video of the same name)

There are only 2 ways to tackle an enemy in this world. 1. Defence and 2. Offence. Defence means to protect oneself, to live in shield or hide till the enemy runs away or dies. 2. Offence meaning the attack is from your end. Today our enemy is coronavirus. Here also there are 2 strategies-defence and offence. In this world 90% of the world is on the first strategy that is defence-lockdown. What are we achieving by a LOCKDOWN? We are shielding ourselves from the coronavirus, as in defence, so that it can't attack us and we will continue to do so till it either vanishes or dies.

Today there are 21 countries or 10 % of the world in which corona came at the same time and rightly thought of following the second strategy-offence, a strategy to attack, known in medical language as HERD IMMUNITY.

There are 2 ways to fight with coronavirus. One is Lockdown and second is No lockdown. You have all the data to inform you about what we have achieved with a lockdown whether in Spain, Italy, US, UK, India or China. In all these countries they thought of their own action plan to win through defence meaning that we lock ourselves in while coronavirus is waiting, gets tired and runs away. How many people suffered or died with this? I am not providing the statistics because all of it is coming on the TV in real time. You would have memorized by now.

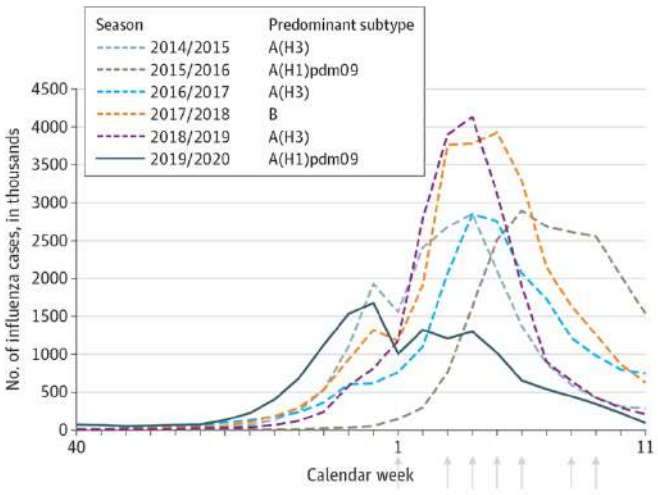
But there are 21 countries in the world where there has been no Lockdown. You will be surprised to know

NO LOCKDOWN			
Country	Corona Death	Country	Corona Death
Bhutan	0	Sweden	990
Maldives	0	Equatorial Guinea	0
Brunei	1	Zambia	2
Iceland	8	Cambodia	0
Latvia	5	Taiwan	6
Jamaica	4	Belarus	33
Guyana	6	Japan	143
Uruguay	8	Hong Kong	4
St Vincent Grenadines	0	Singapore	9
Belize	2	Macao	0
Cameroon	12		

that after following strategy 2- offence, they have not reached even double digit figures in death, in some cases there have been no deaths. If you study the list carefully you will find 2 countries- Japan and Sweden where corona death has reached 3 digit figures. To find out why there have been such few deaths in these 2 countries where they followed herd immunity, I investigated at my end.

You have to understand that when coronavirus enters the body, then we are inflicted with COVID-19.

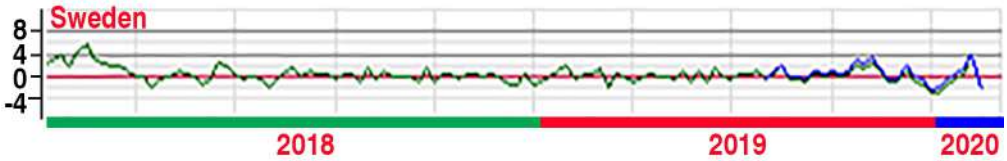
Covid-19 is like influenza falling in the category – Influenza like illness in which category also falls H1N1. Every year thousands of people all over the world die due to this in Japan, US and India. I have studied the past 6 years in ***Japan***[38] to find out how many deaths have occurred due to influenza.



JAMA Published online April 10, 2020

In 2014-15 wherein the influenza season occurs from November-April, 2015-16, 2016-17, 2017-18, 2018-19, 2019-20 till April 10, I have tried to analyse how many deaths have occurred due to influenza like illness. All the data is on the screen in between the dotted lines. In the last 5 years the death this year has been very less as compared to previous years. This is the story of Japan.

Now let us talk about ***Sweden***[18]. I have studied the total mortality of Sweden and you can find it on the screen.



If you carefully look at the graph on the screen you will find that death due to corona is more but if you compare figures with previous year and year before that you will see that the deaths due to influenza is more than corona deaths. In Sweden and Japan the figures have crossed 3 digits

but if we compare previous years then the situation today is much better. Meaning to say that the situation is much better in the 21 countries where there was NO LOCKDOWN rather than in those countries where LOCKDOWN was imposed to win over the fight with coronavirus.

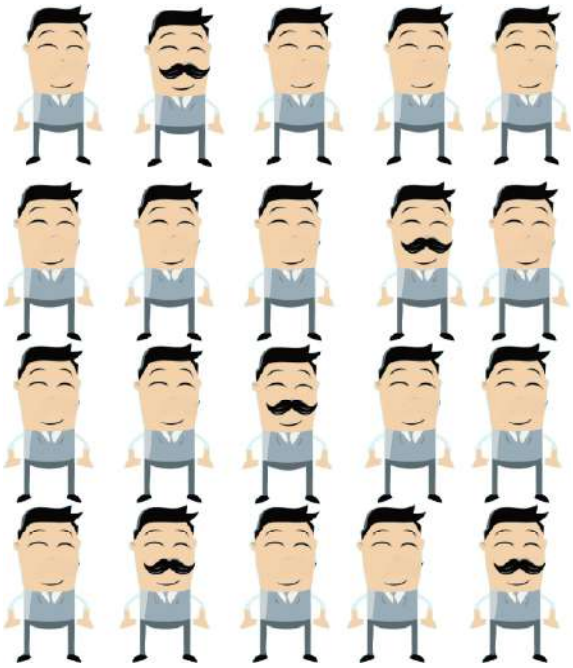
Now what I want to ask is why is there no story circulating in the media about these 21 countries? I tried to seek its answer. Here is a story. Imagine that like every year, this year will also see the mango season. People climb mango trees and pluck mangoes to eat. Some people however fall from the trees while plucking mangoes. Then there is rumour in the administration that this year there has been an unusual crop of mangoes such that if those mangoes are eaten it will probably result in the death of people. This means that everyone has been warned not to step outside till the mango season is over. You are craving for mangoes so the administration said that we will provide you with mangoes. They started filling the bottles in a way like this, so that you can have as many mangoes as possible. This mango juice contains many deadly chemicals. On one side is God given gift the mangoes. And on the other hand here is the manmade mango juice. It may be 100 % natural yet can never equal God's mango.

Let us talk in terms of vaccine in today's context. Those 21 countries never needed any medicines nor vaccines to increase their herd immunity against corona virus.

What is vaccine? Let me explain with an example. Let us assume this is coronavirus and when this enters the body, remember everyone will not fall sick. Statistics show that 80% people will not fall ill due to coronavirus. They will not even know that they are the carriers of coronavirus. Only 20% people fall sick. Even among them 10% have fever and the other 10% develop cough and these recover fast. The other 10% who had fever out of these only 1 will die and the other 999 will recover. What I want to say is that when coronavirus enters your body through nose, mouth or contact then 80 % of the people will not know. Only a few will know and they will hope to recover soon. The benefit you will gain is that your body develops immunity or antivirus to fight the coronavirus so that the next time it enters your body, your body will be in a better position to fight. This is known as natural immunization. This is what the pharma company wants to achieve through vaccine. They want you to stay locked in as there is a virus outside that will kill you. But then they will prepare vaccine for you by putting the virus in a bottle and then put this virus into your body and body will fight the virus and develop immunity.

This vaccine is not going to be bought by 21 countries because herd immunity is already developed. If death due to corona occurred then pandemonium would be there and lakhs of people would have died. Fortunately this did not happen.

Let's Talk about India! In India Lockdown has been implemented since March 25-2020. Lets rewind and go back to January 2020, and consider this imaginary situation. Imagine you have been given the responsibility to provide security to the entire nation and you are alert not to let any enemy enter into your country, loot or attack it. And you have spies all over the world and you are security in charge of the entire nation. Your spies inform you that smugglers are going to enter from different parts of the world and will eat away the whole country. You have to save the country so you ask how I will recognize them. Spies tell you that the smugglers look just like normal citizens and cannot be spotted easily. So you ask if I cannot identify them how will I catch them? They tell you that some of the smugglers have moustaches.



You get an idea atleast we can catch the smugglers with moustaches. So you ask how many of the smugglers have moustaches. Spy tells you that 10% of them have moustaches and 90% donot have. . So you make up your mind to catch at least 10% of these smugglers. So you put a high alert at the airport and other ports and announce that any passenger with moustaches should be detained and scanned and after due scrutiny should be allowed to go. May be they are smugglers! Also you give your security guards special glasses through which any passenger with moustache can be easily seen and spotted. But unfortunately the glasses were in such a bad condition that passengers with moustaches could not be spotted at all. So the smugglers entered into the country even after you were on high alert crossed the airport and entered into the country and ransacked the whole country. Now this example can be related in today's context. The smugglers here are the carriers of the corona virus and not patients. I told you that 80% of the people are carriers of the virus without even knowing about it. And the Glasses here are the 'THERMAL SCANNERS' means only 10% of the people whose temperature is high can be detected through this thermal scanner. January last week onwards these thermal scanners were used at the airports to scan the passengers coming from abroad and only after scanning people were allowed to go.

Now this thermal scanner has some real problems and to know about these problems I have here with me Thermal Scanner expert Mr. Ashutosh Mittal who is the owner of the Company Gibson that manufactures ***Thermal Scanners***. Lets hear what he has to say about Thermal Scanners used at the airports that time from January onwards.

Ashutosh ji I have this thermometer manufactured in your company. In January /February when I was coming back from Malaysia, at the airport this kind of thermometer was used for scanning. I want to know what kind of thermometer is this. And this one manufactured by your company

used for which purpose. This I can understand is meant for measuring human body temperature and this one is for industrial purpose. But can this industrial thermal scanner be used for measuring human? No , Dr Saab this is Industrial Thermometer. Measuring range of this thermometer is 50 degree Celsius to 550 degree Celsius. If we talk in Fahrenheit its range is minus 58 degree to 1022 degree and the variation is 2% plus minus + 2 degree Fahrenheit which means 4 degree Fahrenheit Plus minus is its tolerance. And because this one is industrial and this one is medical thermometer. And medical thermometer performs a calculation after taking temperature from human body surface. But this industrial thermometer doesn't use this calculation and can show variation as high as 10 degrees. Which means it can show the temperature of 99 degrees as 89 degrees or 109 degrees. This is industrial thermometer and using these thermometers for measuring temperature is a completely futile exercise. But I saw that at airports and also in our colonies these industrial thermometers were being used. So that means it was useless! Actually in January / February when corona started, these infra red or Medical thermometer were exported out of India and when guidelines were received in India to measure temperature these were not available and only industrial thermometers were available and people did not have much knowledge about it and used these industrial thermometer unknowingly. It cannot be used to diagnose high temperature. That means in the month of January, February and March the entire exercise of scanning done at the airports with this thermometer was useless or completely futile. Those scanned with industrial one is futile. Those that used Medical thermometer could be correct but in this Medical thermometer too there was this issue that measuring distance of this medical thermometer is 125cm. Different manufacturers have different distances and this one says 125 cms which means the distance should be this close but as we saw it in television or videos measurement was done from this far. From this distance this will not give the right measurement as the distance should be 125cm. You mean when the temperature was measured and if you remember it was done from this far this will not give the right picture and will be less which is again Futile. That means when this one (Industrial Thermometer) was used it was absolutely futile and using medical thermometer a distance was quite far which again resulted in less measurement and a distances of 125 cm was not maintained and was measured from far which would have resulted in temperature difference.

You just heard that the strategy to scan through thermal scanner to stop the corona patients failed completely. Which means before lockdown since January February and till march end these corona virus carriers kept coming into the country from all over the world and spread throughout the country. Though we were quite alert but they still spread into the country. Today at this point of lockdown its obvious that these corona virus carriers and not corona patients who themselves are unaware about it are spread throughout this country. Now take this imaginary situation that I am a corona virus carrier and despite lockdown I am allowed to go to buy vegetables and fruits during 3-4 hrs breaks. I take this mango and hold it and I do not like it so keep it back. Then I buy some mangoes while I keep the mango back that I did not like.I am carrier of Corona virus and not its victim or patient. So is this mango infected with corona virus. Yes it is! Now another healthy person is also there to shop for mango and buys this infected mango unknowingly which means corona virus reached his home. What I want to say that in spite of lockdown corona virus is spreading and in a country like India it is not possible to stop its spread. India has a population of 140 crores and has a police force of approximately 15 lakhs and a good sizeable number of these policemen are engaged in VIP security and other work. With limited number of policemen

it is nearly impossible to keep an Indian population of 140 crores locked inside homes. And whatever tits and bits you are watching on the TV and around you cannot be expanded and generalized for the whole country. So that means according to me corona virus has spread all over the country. And if its has spread then it's a good news because that would means India has achieved "herd immunity". You can also see that corona deaths in India are 360. In a country of 140 crores 360 deaths is a miniscule number. And these 360 deaths are because or corona or not is also questionable. As I explained in my previous video that RT PCR test for corona virus diagnosis is questionable and not a reliable test. Secondly I also explained that the treatment of corona after keeping the patient in quarantine, the medicines used for treatment is the very cause of deaths. The evidences say so… So even if the patient died of corona virus the number is just 360 but the truth is we cannot be sure if they died of corona virus or due to treatment or due to other medical complications . But overall the numbers are very less. So India has achieved "Herd Immunity" so we do not have to worry much about falling sick with corona virus as corona has transmission rate of 2 and mortality rate of 0.1% which is just like any other flu. Now I have a question for you. Imagine this is corona virus with mortality rate of 0.1% which means out of 1000 people affected with corona virus only 1 will die with this virus and 999 people will recover and its transmission rate is 2.2, which means 1 person will infect 2 people further. Imagine there is another situation in India or anywhere in the world where another virus or bacteria many times stronger and deadlier than corona which means infectious agent whose transmission rate is 10 i.e will spread to 10 people from a single infected person 5 times stronger than corona virus and whose mortality rate is 20 times more than Corona. A virus which can kill 5 lakh people in a year or 1 person every minute! If this kind of bacteria or virus arrives in our country what to do in such a situation? You will suggest that when we were quarantined for corona it's obvious that in the other case of virus or bacteria too we should lock ourselves in our homes and there should be a lockdown till it is contained or dies or goes away. I would like to tell you here that this is a bacteria here in this case and the disease is Tuberculosis. Very sadly I have to say that every year 4-5 lakh people die due to tuberculosis. Almost 1 person dies every minute. Which means 10 people have lost their life due to tuberculosis while you are done watching this video. So my question to you whose answer you have find as I could not find any answer to this. The way every death due to corona virus is reported every minute and highlighted on the TV . Same way Tuberculosis death toll which is 1000 per day is not reported and Highlighted on TV as 1000 people died of tuberculosis, now 1001, 1002… reaching 5 lakhs, reached a figure of 5 lakhs! Why such a high figure of Tuberculosis deaths are not reported and highlighted on TV. The answer to this question will not be given by me! You will give the answer and reason through comment section of this video…

Now I ask you the second question. Whenever a patient is infected with corona virus then that patient is quarantined and given allopathic treatment. Let me tell you that HIV medicine given in allopathic, remdesivir, an experimental drug, which has never been approved for any ailment or anti -malarial drug which has no link with coronavirus whatsoever. In fact it has been seen in the last 40 years that anti malarial drug abnormally increases heart beat resulting in sudden cardiac death. It is clear that this medicine may result in heart attack or cardiac arrest. There is no evidence that a coronavirus patient can recover.

1. On one hand these medicines are being administered t patients and on the other hand Ayurveda and homeopath is being kept separate. These 2 branches are also part of Indian culture and also legal. But such doctors are not allowed to treat corona patients, patients are kept at a distance. They are using Ayurveda for prevention. If a person is inflicted with coronavirus does he have the option of choosing allopathy, ayurveda, unani, naturopathy or homeopathy. He has no choice simply because he is a guinea pig. He is administered allopathic medicines which have no relevance to coronavirus. Whenever an Ayurvedic practitioner approaches the health ministry for patients to be handed over to them for treatment then in reply he is questioned if he has ever treated a corona patient. What would happen if I had to ask an allopathic doctor the same question. Do you have any evidence of having treated a corona patient. He also does not have evidence and nor do you. You have not treated a corona patient before and nor have they.But there is evidence that the medicine being used for treatment has resulted in many people affected with heart attack or cardiac arrest. On 29th March [39] , Dr Utpal Barman, a senior anaesthetist from Guwahati complained of chest pain after taking anti malaria drug (as a preventive measure from COVID-19) leading to death due to cardiac arrest.

2. On the basis of which evidence are people being administered anti malarial drugs? Why isn't Ayurveda being given importance. In Tehran-Iran[40] 200 people in a hospital were treated with the strategy of Ayurveda and within 1 week 190 people recovered and the remaining 10 were observed to be recovering fast. On one hand in some part of the world Ayurveda is being used to treat patients and on the other hand in our country patients are kept away from Ayurveda and homoeopathic doctors. Why so.

Ques-13: Shehanshah The Body-Guard

(Adapted from the video with the same Name)

You have an 'Emperor' who single- handedly catches culprits, fights for justice and passes the *judgement.* I am talking about a very important cell of our body, DENDRITIC CELL. DENDRITIC CELL means 95% immunity of your body. Its function is to catch hold of enemies for you. Enemies could be virus, bacteria, pathogen, chemical or poison-meaning fighting your own case and giving the verdict too. Maybe that is why you and I are alive today. These dendritic cells are spread out in our entire body, like a body guard, especially just below the skin. But a few of us unknowingly kill these dendritic cells or make them inactive by feeding them with liquor. I am talking here about SANITIZERS. SANITIZER means about 72% alcohol. When you are applying sanitizer on your hand, you are actually rubbing alcohol on your hands. This alcohol is not limited to the skin but penetrates your skin to reach the dendritic cells to make them inactive or completely destroy them. It does not stop here. This alcohol mixes with the blood and spreads throughout the body. If you don't believe what I'm saying then please carry out an experiment after watching this video.

Take sanitizer and rub it well on your hands, and immediately go out to drive your car. While driving pray that you meet a policeman with a breath analyzer. If the policeman stops you and puts a breath analyzer in front of your face and say that you are challaned or fined and your

licence will be taken away as alcohol content has been detected in your breath. But wait, you will not be challaned or fined nor lose your license.

If you want to verify what I'm saying then please go to the link on the screen and read the article given **in the journal**[41].

You will be surprised to know that each time you rub your hands with alcohol, this alcohol content penetrates the skin to reach the dendritic cell and actually kills the dendritic cell. If the dendritic cell dies or weakens then that is the time you will turn weak and be attacked by the virus and bacteria and fall ill.

If you want to remain strong then you have to keep this EMPEROR of your body, meaning the dendritic cell, strong. If you want to keep the dendritic cell of your body strong, then you just have to do 2 things.

1. To avoid those things which weaken or kill the dendritic cell. I**n this first is alcohol, either by drinking**[42] **or applying**[41]. It affects the dendritic cell directly and it can also die. Secondly, animal protein. Any food that has animal protein such as eggs, meat fish or dairy products like milk, cheese, butter milk, butter, paneer or curd. **When these milk products are consumed then it directly affects the dendritic cell meaning one's own immunity**[43]. This weakens the body's immunity. These are things that must be avoided.
2. Now you have to adopt this, that is daily 0.2% Vitamin C, not in the form of tablet, capsule or tonic, but in the form of vegetables and fruits. If you eat one large guava, 0.2 gm Vitamin C will enter your body. 2 medium size orange or even 3 mangoes, 0.2 gm vit. C will go inside your body. Or 4 tomatoes will provide 0.2 gm vitamin C. Do one thing – take about 3or 4 varieties of fruit to make it 300-400 grams. If you do this daily then 0.2 gm or more Vitamin C will enter your body daily. If you are able to do only this much daily then your immunity will be strong, the EMPEROR or body guard of your body will be strong and you will be able to tackle boldly any virus attack.

Ques-14: WHO Exposed

Imagine a scene where there are 4 neighbours-neighbour 1, 2, 3 and 4. Suddenly a baba -an old wise man- arrives and predictsthat they will be dead in the next 4 months. Neighbour no. 1 worries and says that he will do what the baba says. Baba demands Rs 10, 000 for saving his life. The first neighbour immediately hands the cash to him. Neighbour 2 negotiates with the baba and hands him Rs. 5000. Neighbor no. 3 also negotiates further and gives the baba only Rs. 2500 and thinks of ways to save his own life. Neighbor no. 4 doesn't trust the baba, will not open his door nor listen to him. Now 4 months have passed. The 4 neighbors come out of their houses to see that nothing has changed. Everything is the same as before. It means baba's prediction failed.They felt that the baba cheated them. Suddenly they remembered that he had visited them 10 years ago.Then also he had said something similar but nothing had changed.What is the moral of thestory?

The moral of this story is not to believe this babain future. In today's scenario the baba's role is being played by WHO-World Health Organization. WHO had predicted in March this year that 4 crore deaths will occur in the world due to coronavirus. If all precautions are taken like lockdown, social distancing, masking and everything shut down then 2 crore people will die. In such a situation many countries behaved like neighbor no.1. Let us take for example what was predicted for India. WHO had predicted that if no prevention was taken by India then 60 lakh people would die.With lockdown, social distancing and masking 30 lakh people would die. The reality is before you- not even 2000 people died.

Death Prediction			
	WHO Prediction		Reality
	Min	Max	
India	23,75803	60,96,359	1,986

Some countries were behaving like neighbour no. 2 and one of these countries is Japan. It did not agree to all the terms of WHO and did not opt for a lockdown, only observed social distancing and masking. The prediction for Japan was 14 lakh deaths. If all conditions were observed then 2 lakh 33 thousand deaths would occur. The fact is only 590 people died.

Death Prediction			
	WHO Prediction		Reality
	Min	Max	
India	23,75803	60,96,359	1,986
Japan	2,33,148	14,74,438	590

Now we talk about neighbour no. 3. The behaviour of Sweden is like neighbour no. 3. It didn't agree for a lockdown, social distancing, closing schools, maybe only agreed to protect their elderly citizens. WHO predicted for Sweden that if it didn't follow any intervention then 90,000 people will die. 16000 people will die after observing all interventions like lockdown, social distancing and masking. The actual deaths that occurred in Sweden are approx. 3175.

Death Prediction			
	WHO Prediction		Reality
	Min	Max	
India	23,75803	60,96,359	1,986
Japan	2,33,148	14,74,438	590
Sweden	16,192	90,157	3,175

Taiwan is not amember of WHO and WHO did not make predictions for Taiwan.Its behaviour is similar to neighbor no.4. Only 6 deaths occurred. Taiwan didn't implement any intervention because Taiwan doesn't know what WHO is saying.

Death Prediction			
	WHO Prediction		Reality
	Min	Max	
India	23,75803	60,96,359	1,986
Japan	2,33,148	14,74,438	590
Sweden	16,192	90,157	3,175
Taiwan	-	-	6

Know more about the prediction by WHO for all the countries [44]

Ques-15: How to Cure Covid-19

If you are with science then you need not worry or need to be scared just as incase of common cold or flu. Now if you want to know how to cure Corona Virus ,you will need to follow the s protocol of common cold or flu . If you want to cure in 3 days all you need to do is follow ***3 step diet protocol.***

3 Step Diet Protocol

(Based on 161 reference papers from 1920-2020)[31]

Day 1: Liquid Day.

If your body weight is 70 kg. The divide it by 10 to get 7.

This means in the whole day drink 7 glasses of fruit juice + 7 glasses of coconut water

Day 2 : Fluid day.

Body wt. divided by 20. 60 divided by 20= 3 glasses of citrus fruit juice+ 3 glasses of coconut water + tomato and cucumber by weight (body weight multiplied by 5).

Day 3: Solid Day.

This means 60 divided by 30=2, which means 2 glasses of citrus fresh fruit juice without straining + 2 glasses of coconut water till 12 noon

After that for lunch tomato + cucumber as you had yesterday 350 grams, for a 70 kg person , that is 350 grams of vegetable .

By dinner you will be able to eat normal home cooked vegetarian food.

SECTION - 2

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Evidence Base of Diagnosis of Corona Virus
&
The treatment of COVID-19

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2019-2020 U.S. Flu Season: Preliminary Burden Estimates

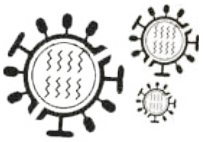
CDC estimates* that, from **October 1, 2019**, through **March 28, 2020**, there have been:

39,000,000 – 55,000,000
flu **illnesses**

18,000,000 – 26,000,000
flu **medical visits**

400,000 – 730,000
flu **hospitalizations**

24,000 – 63,000
flu **deaths**



*Because influenza surveillance does not capture all cases of flu that occur in the U.S., CDC provides these estimated ranges to better reflect the larger burden of influenza. These estimates are calculated based on CDC's weekly influenza surveillance data and are preliminary.

**Influenza testing across the United States may be higher than normal at this time of year because of the COVID-19 pandemic. These estimates may partly reflect increases in testing in recent weeks and may be adjusted downward once the season is complete and final data for the 2019/20 season are available.

This web page provides weekly, preliminary estimates of the cumulative in-season numbers of flu illnesses, medical visits, hospitalizations, and deaths in the United States. CDC does not know the exact number of people who have been sick and affected by influenza because influenza is not a reportable disease in most areas of the U.S. However, CDC has estimated the burden of flu since 2010 using a mathematical model that is based on data collected through the U.S. Influenza Surveillance System, a network that covers approximately 8.5% of the U.S. population (~27 million people).

Limitations

The estimates of the cumulative burden of seasonal influenza are subject to several limitations.

First, the cumulative rate of laboratory-confirmed influenza-associated hospitalizations reported during the season may be an under-estimate of the rate at the end of the season because of identification and reporting delays.

Second, rates of laboratory-confirmed influenza-associated hospitalizations were adjusted for the frequency of influenza testing and the sensitivity of influenza diagnostic assays. However, data on testing practices during the 2019-2020 season are not available in real-time. CDC used data on testing practices from the past influenza seasons as a proxy. Burden estimates will be updated at a later date when data on contemporary testing practices become available.

Third, estimates of influenza-associated illness and medical visits are based on data from prior seasons, which may not be accurate if the seriousness of illness or patterns of care-seeking have changed.

Frequently Asked Questions

What does the cumulative burden of influenza for the 2019-2020 season mean?

The cumulative burden of influenza is an estimate of the number of people who have been sick, seen a healthcare provider, been hospitalized, or died as a result of influenza since October 01, 2018. CDC does not know the exact number of people who have been sick and affected by influenza because influenza is not a reportable disease in most areas of the United States. However, these numbers are estimated using a mathematical model, based on observed rates of laboratory-confirmed influenza-associated hospitalizations.

How does CDC estimate the cumulative burden of seasonal influenza?

Preliminary estimates of the cumulative burden of seasonal influenza during the 2019-2020 season in the United States are based on crude rates of laboratory-confirmed influenza-associated hospitalizations, reported through the Influenza Hospitalization Surveillance Network (FluSurv-NET), which were adjusted for the frequency of influenza testing during recent prior seasons and the sensitivity of influenza diagnostic assays. Rates of hospitalization were then multiplied by previously estimated ratio of hospitalizations to symptomatic illnesses, and frequency of seeking medical care to calculate symptomatic illnesses, medical visits, and deaths associated with seasonal influenza, respectively.

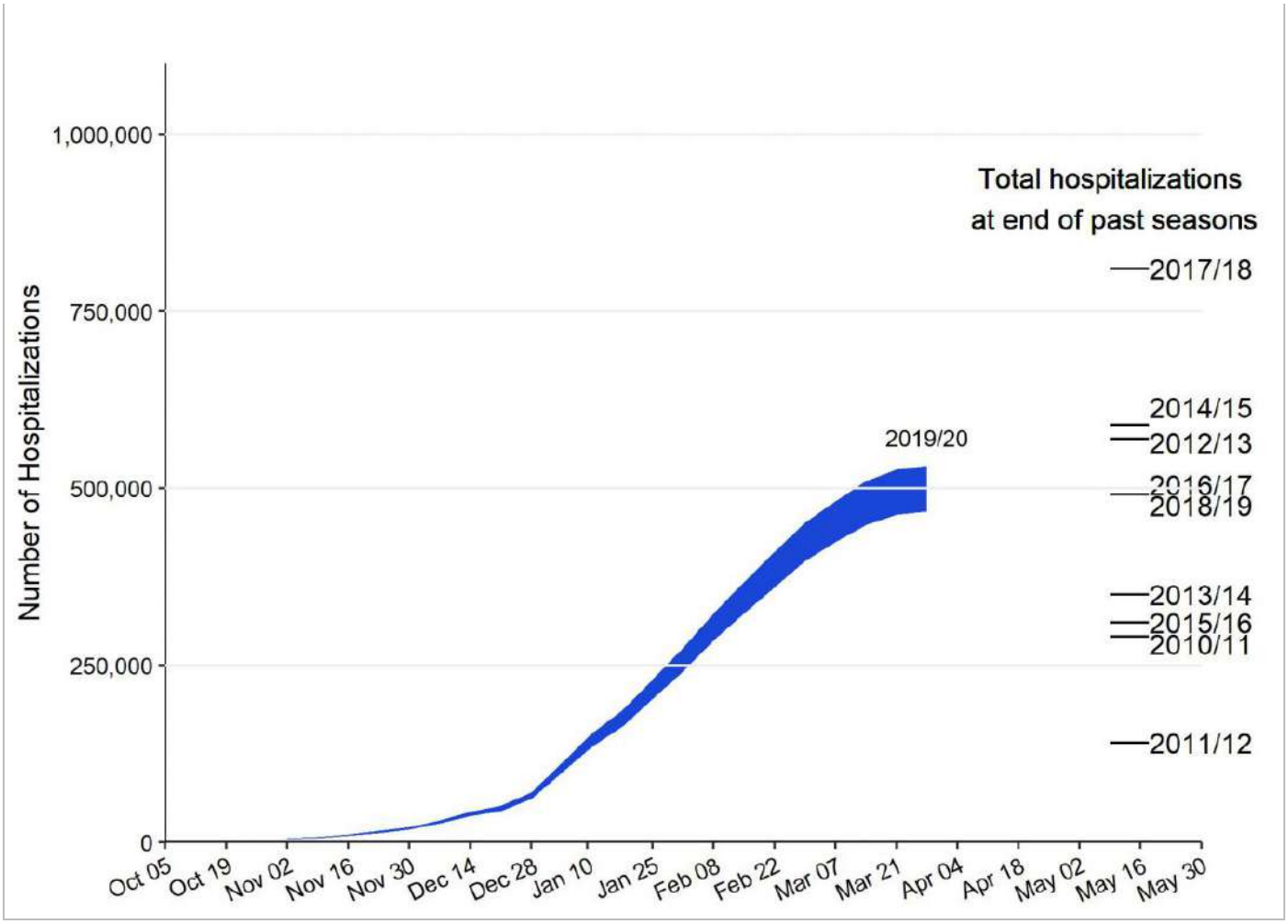
Why does the estimate of cumulative burden change each week?

The estimates of cumulative burden of seasonal influenza are considered preliminary and may change each week as new laboratory-confirmed influenza-associated hospitalizations are reported to CDC. New reports include both new admissions that have occurred during the reporting week and also patients admitted in previous weeks that have been newly reported to CDC.

How does the number of flu hospitalizations estimated so far this season compare with previous end-of-season hospitalization estimates?

The number of hospitalizations estimated so far this season is lower than end-of-season total hospitalization estimates for any season since CDC began making these estimates. This table also summarizes all estimated influenza disease burden, by season, in U.S. from 2010-11 through 2017-18.

Preliminary Cumulative Estimates of Hospitalizations in the U.S. 2019-2020 Flu Season



*These estimates are preliminary and based on data from CDC's weekly influenza surveillance reports summarizing key influenza activity indicators.

Estimated number of influenza-associated hospitalizations

The y-axis extends from 0 to 1 million.

The x-axis is a timeline starting October 5, 2019 and extending to May 30, 2020.

There is a single blue-shaded curve labeled with "2019/20".

There are several other lines on the right side of the graph under Total hospitalizations at end of past seasons. The lines are labeled, from top to bottom, as 2018/19, 2017/18, 2014/15, 2016/17, 2012/13, 2013/14, 2015/16, 2010/11, and 2011/12 and represent the estimated burden for these seasons. This allows for the comparison of the current season to past seasons.

Page last reviewed: April 3, 2020
Content source: Centers for Disease Control and Prevention, National Center for Immunization and Respiratory Diseases (NCIRD)

The New York Times | https://nyti.ms/2w1vRKE

Worst-Case Estimates for U.S. Coronavirus Deaths

Projections based on C.D.C. scenarios show a potentially vast toll. But those numbers don't account for interventions now underway.

By Sheri Fink

Published March 13, 2020 Updated March 18, 2020

Officials at the U.S. Centers for Disease Control and Prevention and epidemic experts from universities around the world conferred last month about what might happen if the new coronavirus gained a foothold in the United States. How many people might die? How many would be infected and need hospitalization?

One of the agency's top disease modelers, Matthew Biggerstaff, presented the group on the phone call with four possible scenarios — A, B, C and D — based on characteristics of the virus, including estimates of how transmissible it is and the severity of the illness it can cause. The assumptions, reviewed by The New York Times, were shared with about 50 expert teams to model how the virus could tear through the population — and what might stop it.

The C.D.C.'s scenarios were depicted in terms of percentages of the population. Translated into absolute numbers by independent experts using simple models of how viruses spread, the worst-case figures would be staggering if no actions were taken to slow transmission.

Between 160 million and 214 million people in the United States could be infected over the course of the epidemic, according to a projection that encompasses the range of the four scenarios. That could last months or even over a year, with infections concentrated in shorter periods, staggered across time in different communities, experts said. As many as 200,000 to 1.7 million people could die.

And, the calculations based on the C.D.C.'s scenarios suggested, 2.4 million to 21 million people in the United States could require hospitalization, potentially crushing the nation's medical system, which has only about 925,000 staffed hospital beds. Fewer than a tenth of those are for people who are critically ill.

The assumptions fueling those scenarios are mitigated by the fact that cities, states, businesses and individuals are beginning to take steps to slow transmission, even if some are acting less aggressively than others. The C.D.C.-led effort is developing more sophisticated models showing how interventions might decrease the worst-case numbers, though their projections have not been made public.

"When people change their behavior," said Lauren Gardner, an associate professor at the Johns Hopkins Whiting School of Engineering who models epidemics, "those model parameters are no longer applicable," so short-term forecasts are likely to be more accurate. "There is a lot of room for improvement if we act appropriately."

Those actions include testing for the virus, tracing contacts, and reducing human interactions by stopping mass gatherings, working from home and curbing travel. In just the last two days, multiple schools and colleges closed, sports events were halted or delayed, Broadway theaters went dark, companies barred employees from going to the office and more people said they were following hygiene recommendations.

The Times obtained screenshots of the C.D.C. presentation, which has not been released publicly, from someone not involved in the meetings. The Times then verified the data with several scientists who did participate. The scenarios were marked valid until Feb. 28, but remain "roughly the same," according to Ira Longini, co-director of the Center for Statistics and Quantitative Infectious Diseases at the University of Florida. He has joined in meetings of the group.

The C.D.C. declined interview requests about the modeling effort and referred a request for comment to the White House Coronavirus Task Force. Devin O'Malley, a spokesman for the task force, said that senior health officials had not presented the findings to the group, led by Vice President Mike Pence, and that nobody in Mr. Pence's office "has seen or been briefed on these models."

Latest Updates: Coronavirus Outbreak

- Debate roils White House over an untested drug the president insists on promoting.

- As many as half of those with the coronavirus could be asymptomatic, Fauci says.
- As cases rise in Japan, the prime minister considers declaring an emergency.

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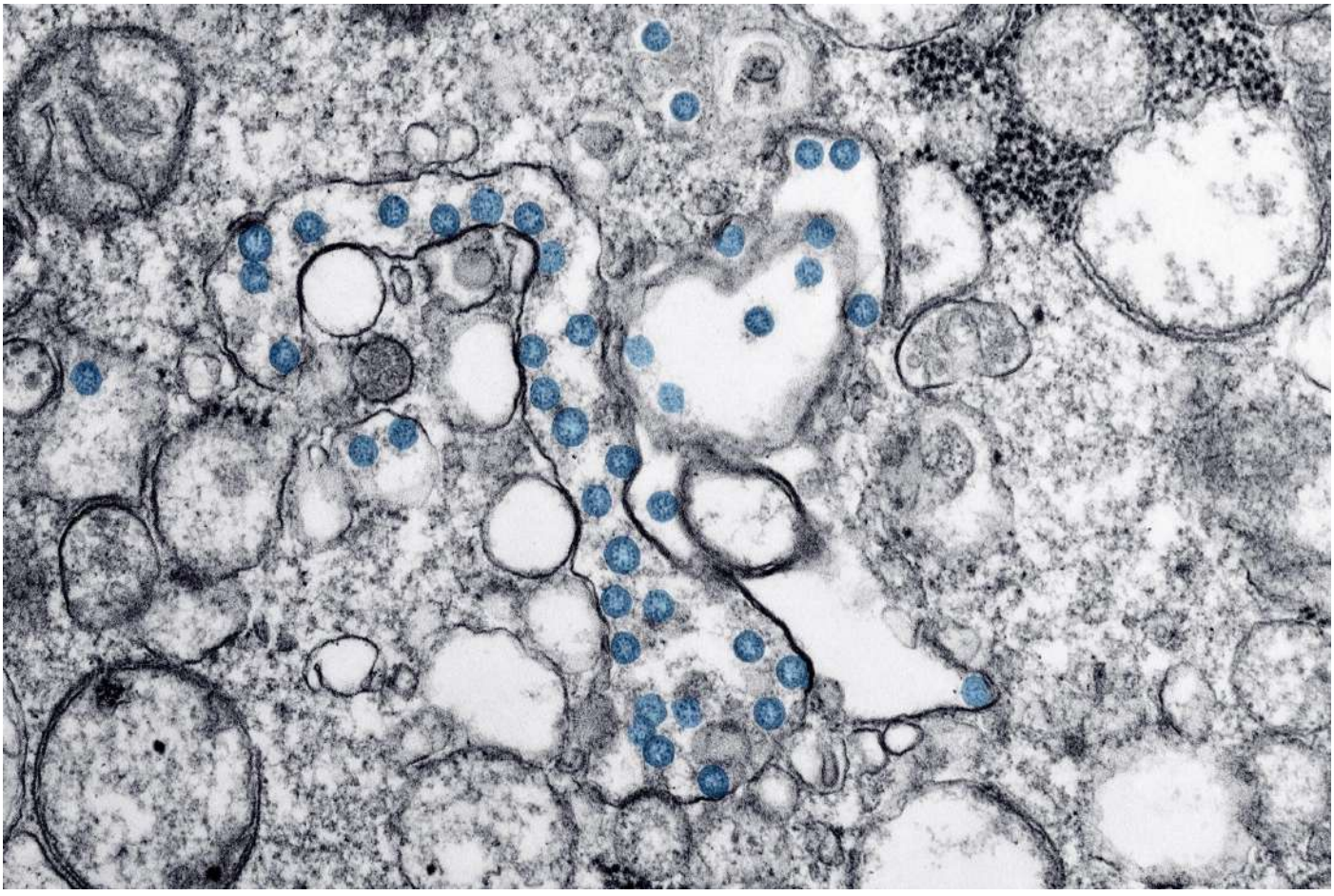
The assumptions in the C.D.C.'s four scenarios, and the new numerical projections, fall in the range of others developed by independent experts.

Dr. Longini said the scenarios he helped the C.D.C. refine had not been publicly disclosed because there remained uncertainty about certain key aspects, including how much transmission could occur from people who showed no symptoms or had only mild ones.

"We're being very, very careful to make sure we have scientifically valid modeling that's drawing properly on the epidemic and what's known about the virus," he said, warning that simple calculations could be misleading or even dangerous. "You can't win. If you overdo it, you panic everybody. If you underdo it, they get complacent. You have to be careful."

But without an understanding of how the nation's top experts believe the virus could ravage the country, and what measures could slow it, it remains unclear how far Americans will go in adopting — or accepting — socially disruptive steps that could also avert deaths. And how quickly they will act.

Studies of previous epidemics have shown that the longer officials waited to encourage people to distance and protect themselves, the less useful those measures were in saving lives and preventing infections.



An isolate from the first U.S. case of Covid-19, the illness caused by coronavirus. Centers for Disease Control via Reuters

"A fire on your stove you could put out with a fire extinguisher, but if your kitchen is ablaze, that fire extinguisher probably won't work," said Dr. Carter Mecher, a senior medical adviser for public health at the Department of Veterans Affairs and a former director of medical preparedness policy at the White House during the Obama and Bush administrations. "Communities that pull the fire extinguisher early are much more effective."

From Flu to Coronavirus

Dr. Biggerstaff presented his scenarios in a meeting held weekly to model the pandemic's effects in the United States, Dr. Longini said. Its participants had been at work for several months before the emergence of the virus, modeling a potential influenza pandemic. "We just kind of retooled, re-shifted," said Dr. Longini. "The priority's now coronavirus."

The four scenarios have different parameters, which is why the projections range so widely. They variously assume that each person with the coronavirus would infect either two or three people; that the hospitalization rate would be either 3 percent or 12; and that either 1 percent or a quarter of a percent of people experiencing symptoms would die. Those assumptions are based on what is known so far about how the virus has behaved in other contexts, including in China.

Other weekly C.D.C. modeling meetings center on how the virus is spreading internationally, the impact of community actions such as closing schools, and estimating the supply of respirators, oxygen and other resources that could be needed by the nation's health system, participants said.

In the absence of public projections from the C.D.C., outside experts have stepped in to fill the void, especially in health care. Hospital leaders have called for more guidance from the federal government as to what might lie in store in the coming weeks.

Even severe flu seasons stress the nation's hospitals to the point of setting up tents in parking lots and keeping people for days in emergency rooms. Coronavirus is likely to cause five to 10 times that burden of disease, said Dr. James Lawler, an infectious diseases specialist and public health expert at the University of Nebraska Medical Center. Hospitals "need to start working now," he said, "to get prepared to take care of a heck of a lot of people."

Dr. Lawler recently presented his own "best guess" projections to American hospital and health system executives at a private webinar convened by the American Hospital Association. He estimated that some 96 million people in the United States would be infected. Five out of every hundred would need hospitalization, which would mean close to five million hospital admissions, nearly two million of those patients requiring intensive care and about half of those needing the support of ventilators.

Dr. Lawler's calculations suggested 480,000 deaths, which he said was conservative. By contrast, about 20,000 to 50,000 people have died from flu-related illnesses this season, according to the C.D.C. Unlike with seasonal influenza, the entire population is thought to be susceptible to the new coronavirus.

Dr. Anthony Fauci, director of the National Institute of Allergy and Infectious Diseases, speaking at a congressional hearing on Thursday, said predictions based on models should be treated with caution. "All models are as good as the assumptions that you put into the model," he said, responding to a question from Representative Rashida Tlaib about an estimate from the attending physician of Congress that the United States could have 70 million to 150 million coronavirus cases.

What will determine the ultimate number, he said, "will be how you respond to it with containment and mitigation."

Clues From 1918

Independent experts said these projections were critically important to act on, and act on quickly. If new infections can be spread out over time rather than peaking all at once, there will be less burden on hospitals and a lower ultimate death count. Slowing the spread will paradoxically make the outbreak last longer, but will cause it to be much milder, the modelers said.

A preliminary study released on Wednesday by the Institute for Disease Modeling projected that in the Seattle area, enhancing social distancing — limiting contact with groups of people — by 75 percent could reduce deaths caused by infections acquired in the next month from 400 to 30 in the region.

A recent paper, cited by Dr. Fauci at a news briefing on Tuesday, concludes that the rapid and aggressive quarantine and social distancing measures applied by China in cities outside of the outbreak's epicenter achieved success. "Most countries only attempt social distancing and hygiene interventions when widespread transmission is apparent. This gives the virus many weeks to spread," the paper said, with the average number of people each new patient infects higher than if the measures were in place much earlier, even before the virus is detected in the community.

"By the time you have a death in the community, you have a lot of cases already," said Dr. Mecher. "It's giving you insight into where the epidemic was, not where it is, when you have something fast moving." He added: "Think starlight. That light isn't from now, it's from however long it took to get here."

He said a single targeted step — a school closing, or a limit on mass gatherings — cannot stop an outbreak on its own. But as with Swiss cheese, layering them together can be effective.

This conclusion is backed up by history.

The most lethal pandemic to hit the United States was the 1918 Spanish flu, which was responsible for about 675,000 American deaths, according to estimates cited by the C.D.C.

The Institute for Disease Modeling calculated that the new coronavirus is roughly equally transmissible as the 1918 flu, and just slightly less clinically severe, and it is higher in both transmissibility and severity compared with all other flu viruses in the past century.

Dr. Mecher and other researchers studied deaths during that pandemic a century ago, comparing the experiences of various cities, including what were then America's third- and fourth-largest, Philadelphia and St Louis. In October of that year Dr. Rupert Blue, America's surgeon general, urged local authorities to "close all public gathering places if their community is threatened with the epidemic," such as schools, churches, and theaters. "There is no way to put a nationwide closing order into effect," he wrote, "as this is a matter which is up to the individual communities."

The mayor of St. Louis quickly took that advice, closing for several weeks "theaters, moving picture shows, schools, pool and billiard halls, Sunday schools, cabarets, lodges, societies, public funerals, open air meetings, dance halls and conventions until further notice." The death rate rose, but stayed relatively flat over that autumn.

By contrast, Philadelphia took none of those measures; the epidemic there had started before Dr. Blue's warning. Its death rate skyrocketed.

The speed and deadliness of the pandemic humbled doctors then much as the coronavirus pandemic is doing now. Some commented on the difficulty of getting healthy people to take personal precautions to help protect others at greater risk.

Modern societies have tools that did not exist then: advanced hospitals, the possibility of producing a vaccine in roughly a year, the production of diagnostics. But other signs are more worrying.

The world population is about triple the size it was the year before the 1918 flu, with 10 times as many people over 65 and 30 times as many over 85. These groups have proven especially likely to become critically ill and die in the current coronavirus pandemic. In Italy, hospitals are so overwhelmed that ventilators are being rationed.

"It's so important that we protect them," said Dr. Gabriel Leung, a professor in population health at Hong Kong University. In work accepted for publication in the journal Nature Medicine, he estimated that 1.5 percent of symptomatic people with the virus died. He and others who have devoted their careers to modeling said that looking at the experiences of other countries already battling the coronavirus was all it took to know what needed to be done in the United States.

"All U.S. cities and states have the natural experiment of the cities that have preceded us, namely the superb response of Singapore and Hong Kong," said Dr. Michael Callahan, an infectious disease specialist at Harvard. Those countries implemented school closures, eliminated mass gatherings, required work from home, and rigorously decontaminated their public transportation and infrastructure. They also conducted widespread testing.

They were able to "reduce an explosive epidemic to a steady state one," Dr. Callahan said.

As in the case of an approaching hurricane, Dr. Mecher said, "You've got to take potentially very disruptive actions when the sun is shining and the breeze is mild."

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Questioning the HIV-AIDS Hypothesis: 30 Years of Dissent

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Since 1984, when the hypothesis that HIV-causes-AIDS was announced, many scholars have questioned the premise and offered alternative explanations. Thirty years later, competing propositions as well as questioning of the mainstream hypothesis persist, often supported by prominent scientists. This article synthesizes the most salient questions raised, alongside theories proposing non-viral causes for AIDS. The synthesis is organized according to four categories of data believed to support the HIV-AIDS hypothesis: retroviral molecular markers; transmission electron microscopy (EM) images of retroviral particles; efficacy of anti-retroviral drugs; and epidemiological data. Despite three decades of concerted investments in the mainstream hypothesis, the lingering questions and challenges synthesized herein offer public health professionals an opportunity to reflect on their assumptions and practices regarding HIV/AIDS.

"The HIV/AIDS hypothesis is one hell of a mistake", wrote Kary Mullis in 1996 [(1), p. 14]. Mullis – Nobel Laureate in Chemistry, 1993 – and other distinguished scientists have claimed the HIV-causes-AIDS hypothesis is false, unproductive, and unethical. They have done so since 1984, when the hypothesis was proposed. Thirty years after countless studies, resources, and attempts to cure have been poured into the HIV-AIDS hypothesis, it may be fruitful to ask: What happened to those views

and voices that once disagreed? Have the past three decades, with their scientific, technological, and public health developments, been sufficient to convince critics of the hypothesis' value? Have these advances been able to silence the questioning?

Here, I synthesize the main criticisms aimed at the HIV-AIDS hypothesis, alongside select unorthodox[1] theories proposing non-viral cause(s) for AIDS, to argue: far from being condemned to extinction, competing explanations for, and thorough questioning of the mainstream premise persist. Perhaps better known by the lay public than by health professionals, many explanations are, in fact, attracting a growing number of sympathizers. To support the argument, I employ historical research and data synthesis methods. I utilize, as data, trade and professional publications in tandem with authoritative scientific sources.

It is important to note that my purpose is not to review the state of the science regarding HIV/AIDS, nor to persuade readers to reject the mainstream hypothesis. Instead, I aim to expose readers to the persisting controversies, and to motivate them to raise questions of their own. Ultimately, then, this article invites the public health workforce to reflect on prevailing assumptions and practices regarding HIV-AIDS. Reflecting on assumptions and practices represents a central task for public health professionals; a vital step to ensure their (our) practice continually grounds itself in the most rigorous ethical standards (3).

HIV-Causes-AIDS: How Valid are the DATA?

In 1984, Margaret Heckler (then Secretary of the Department of Health and Human Services) announced a retrovirus was the "probable cause" of the alarming immune system collapse emerging in the US since 1981 (4). When scientists identified antibodies to a retrovirus known as LAV, or HTLV-III, in 48 persons (from a sample of 119, with and without immune deficiency symptoms), the retrovirus became the culprit of what would be perceived as "the most urgent health problem facing the country" in recent history [(5, 6), p. 1].

The announcement intended to assure the public: the mystery surrounding this apparently contagious and decidedly fatal illness – later labeled AIDS for acquired immune deficiency syndrome – was solved. The newly identified virus – soon renamed HIV, for human immunodeficiency virus – was, almost certainly, responsible for debilitating people's immune system and making them vulnerable to infections which, before AIDS, were either rare or not particularly dangerous. Now, however, infections such as Kaposi's Sarcoma and *Pneumocistis carinii* Pneumonia had morphed into vicious killers (4, 6). By identifying the perpetrator, scientists' attention and government resources could then focus on treatment, cure, and vaccine development.

Yet almost immediately, scientists who knew a great deal about retroviruses and immunology began to voice misgivings regarding the HIV-causes-AIDS hypothesis, and to question it. They highlighted the difficulties, flaws, and contradictions they saw in the hypothesis, and offered alternative explanations. Many of the original misgivings have survived, and others have been raised, in the past three decades.

In this paper, therefore, I summarize some of these difficulties, and present what critics propose as alternative causes of AIDS. I organize the challenges put forth by unorthodox scholars into four categories of data that support the HIV-AIDS hypothesis[2] : (1) retroviral molecular markers; (2) transmission electron microscopy (EM) images of retroviral particles; (3) efficacy of anti-retroviral (ARV) drugs; and (4) epidemiological data (7, 8). Because these data are proffered as solid evidence for HIV's role in causing AIDS, it is useful to examine how critics question the evidence in each category, specifically.

Retroviral molecular markers

Mainstream scientists and physicians claim the molecular evidence for HIV-as-the-cause-of-AIDS is irrefutable (8, 9) and comprises: (a) HIV antibodies and (b) viral load. As incontrovertible as these molecular markers appear to be, unorthodox scientists have meticulously examined each one and detected significant problems in both (7).

HIV antibodies

The first available tests to screen blood banks for HIV detected HIV antibodies (10). Physicians still use these tests when screening blood for infection and, since 2004, direct-to-consumer home tests have become available for identifying antibodies to HIV using only a saliva sample (e.g., OraQuick) (11). Yet, from the time the first tests appeared, scientists in both orthodox and unorthodox camps reiterated that, according to established immunology principles, antibodies to a virus indicate the immune system has acted to control the invading virus. Antibodies point to previously occurring infection and do not signal active infection. In 1984, CDC scientists (mainstream) wrote:

> *A positive test for most individuals in populations at greater risk of acquiring AIDS will probably mean that the individual has been infected at some time with HTLV-III/LAV [the names originally used for HIV]. Whether the person is currently infected or immune is not known, based on the serologic test alone [(12), p. 378].*

It is not only this simple argument – antibodies suggest the immune system has controlled the invading agents – that unorthodox scientists have debated. The tests themselves remain the target of critic's intense scrutiny. For instance, in 1996 Johnson reported 60-plus factors capable of causing a false-positive result on tests for HIV antibodies [either an ELISA or a western blot (WB) test] (13). Because they react to these factors, the tests may not be detecting HIV at all. Worthy of notice, among the list, are elements ubiquitous among all populations such as the flu, flu vaccinations, pregnancy in women who have had more than one child, tetanus vaccination, and malaria (an important element to consider in the case of the AIDS epidemic in Africa). Supporting each factor, Johnson provides scientifically valid evidence – published in reputable peer-reviewed journals such as *AIDS, the Proceedings of the National Academy of Sciences of the United States of America, The Lancet, the Canadian Medical Association Journal*, and the *Journal of the American Medical Association (JAMA)* (13).

Celia Farber's book, Serious Adverse Events: An Uncensored History of AIDS (14) – an exposé of the epidemic's ethically questionable history – contains an interesting appendix authored by Rodney Richards. Richards – who helped to develop the first ELISA test for HIV – outlines the "evolution" of CDC's stances regarding the role of antibodies, infection, and HIV tests. First, the CDC aligned itself with the traditional view of antibodies signaling past/prior infection (as evidenced in the quote above, from 1984). In 1986, the CDC moved toward a qualified claim, stating:

> *... patients with repeatedly reactive screening tests for HTLV-III/LAV antibody ... in whom antibody is also identified by the use of supplemental tests (e.g., WB, immunofluorescence assay) should be considered both infected and infective [(15), p. 334].*

Finally, in 1987, CDC adopted a non-qualified claim that antibodies signify active infection and/or illness: "The presence of antibody indicates current infection, though many infected persons may have minimal or no clinical evidence of disease for years" [(16, 17), p. 509].

A more specific measure than the ELISA test, the WB detects antibodies by identifying proteins believed to be associated with HIV, and only with HIV. A person undergoes a confirmatory WB after a prior ELISA screening test reacts positively (but it is important to remember: over 60 conditions can yield a false-positive ELISA) (13, 18).

Critics of the orthodox view decry the lack of standardized criteria for a positive result in a WB, across countries, world-wide (19). Bauer (Table 1), in a 2010 article titled “HIV tests are not HIV tests” claims, “no fewer than five different criteria have been used by different groups in the United States” [(18), p.7]. Moreover – adds Bauer – included in the contemporary criteria for a positive WB are p41 and p24, protein–antigens “found in blood platelets of healthy individuals.” This means some of the biological markers being used to “flag” the presence of HIV are not “specific to HIV or AIDS patients [and] p24 and p41 are not even specific to illness.” In other words, healthy persons may test positive on a WB but not carry HIV at all [(18), p. 6].

Table 1

Credentials and professional experience of select critics of the HIV-AIDS hypothesis.

Name (alphabetical order by last name)	Credentials
Henry Bauer, Ph.D.	Professor Emeritus of Chemistry and Science Studies
	Dean Emeritus of Arts and Sciences
	Virginia Polytechnic Institute and State University (Virginia Tech)
James Chin, MD, MPH[a]	Chief of Infectious Disease Section, California State Department of Health Services, Berkeley, CA, USA (1970s–1987)
	Former Chief of Surveillance, Forecasting and Impact Assessment (SFI), Unit of the Global Program on AIDS (GPA) of the World Health Organization Editor: APHA's "Control of Communicable Diseases Manual"
Ettiene de Harven, MD	Emeritus Professor of Pathology: University of Toronto, ON, USA
	Specialized in electron microscopy at the "Institute du Cancer" in Paris
	Published first images of budding virus through EM (1960)
	Member: Sloan Kettering Institute, New York, NY, USA in 1968
	Former President: The Electron Microscopy Society of America (in 1976)
	Former President: Rethinking AIDS
Peter Duesberg, Ph.D.	Professor of Molecular and Cell Biology: The University of California, Berkeley, CA, USA
	Isolated the first cancer gene and mapped the genetic structure of retroviruses (1970)
	Member: National Academy of Sciences (since 1986)
	Outstanding Investigator Award – National Institutes of Health 1986
Heinrich Kremer, MD	Founder and Senior Consultant: Cell Symbiosis Therapy Academy® (based on his work on NO and its association with chronic inflammatory and degenerative disease)
	Collaborating Member: Study Group for Nutrition and Immunity (Bern, Germany)
	Extensive clinical work with youth drug addiction
Kary Mullis, Ph.D.	Nobel Laureate – Chemistry – 1993
	Developed: polymerase chain reaction
	Founder and Chief Scientific Advisor: Altermune
David Rasnick, Ph.D.	Biochemist with >25 years of work with proteases and protease inhibitors
	Former President: Rethinking AIDS: the group for the scientific reappraisal of the HIV hypothesis

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[a] *Chin agrees with the mainstream hypothesis that HIV is the cause of AIDS. His critique centers on the collection and interpretation of the epidemiological data for HIV/AIDS, in the US and world-wide*.

An example may clarify: if tested in Africa, a WB showing reactivity to any two of the proteins p160, p120, or p41, would be considered positive for HIV. In Britain, the test would be positive only if it showed reactivity to one of these three proteins, together with reactions to two other proteins, p32 and p24 (see mention of p24, above, as occurring in healthy individuals). Therefore, someone whose test reacts to p160 and p120 would be considered HIV-positive in Africa, but not in Britain. A test reaction to p41, p32, and p24 would be considered positive in Britain, but negative in Africa, leading author Celia Farber to comment: "… a person could revert to being HIV-negative simply by buying a plane ticket from Uganda to Australia [or in our example, from Uganda to London" (14), p. 163].

According to critics, a definitive answer regarding which protein–antigens are specific to HIV and HIV alone can only come from successful virus isolation and purification. Isolating and purifying "would be required to verify that all of these proteins actually originate from HIV particles" [(7), p. 70]. Attempts at purifying have been made (20, 21), but have been criticized for their ambiguous findings (22), or for their use of cultured samples (see discussion below on EM images). To date, the issue of HIV isolation in purified samples has not been addressed to critics' satisfaction (23).

Viral load The expression "viral load" refers to the quantity of virus found in HIV-infected blood. According to the mainstream perspective, information on viral load helps monitor the infection's progress, "decide when to start treatment, and determine whether or not … HIV medications are working" (24).

The technique for measuring viral load is known as RNA PCR – ribonucleic acid polymerase chain reaction (25). Mainstream scientists regard this test as the most specific documentation of HIV's presence in a person's body. It is often used when the ELISA and WB tests are negative, because PCR can detect the virus' genetic material (or its RNA/DNA fragments), before the human body has had a chance to recognize the virus, produce antibodies in defense, and react positively in an antibodies-only test (26).

Despite its enhanced specificity, many mainstream scientists and practitioners recommend caution when using PCR for screening or diagnosing infection (27). For instance, authors of a study published in JAMA in 2006, in which PCR was used with a sample of almost 3,000 people, concluded: "The PCR assay is not sufficiently accurate to be used for the diagnosis of HIV infection without confirmation" [(28), p. 803].

PCR technology evolved quickly since it was introduced in 1983 (25). Although being employed, mostly, for assessing viral load (less for screening and diagnosis), it should give us pause to learn, however, that Dr. Kary Mullis – the scientist who won the 1993 Nobel Prize for inventing the PCR test and whose quote introduced this article (Table 1) – has strongly opposed using the technique for determining the amount of virus circulating in plasma. Lauritsen explains:

> *Kary Mullis … is thoroughly convinced that HIV is not the cause of AIDS. With regard to the viral-load tests, which attempt to use PCR for counting viruses, Mullis has stated: "Quantitative PCR is an oxymoron." PCR is intended to identify substances qualitatively, but by its very nature is unsuited for estimating numbers. Although there is a common misimpression that the viral-load tests actually count the number of viruses in the blood, these tests cannot detect free, infectious viruses at all; they can only detect proteins that are believed, in some cases wrongly, to be unique to HIV. The tests can detect genetic sequences of viruses, but not viruses themselves [(29), p. 3].*

If to this picture we add human endogenous retroviruses (or HERVs) (30) as potential confounders, the genetic sequences detected in a PCR test may not be those from an exogenous virus, at all, and may explain the test's substantial false-positive rates (18, 27). HERVs consist of retrovirus-like particles produced by host cells that are stressed or dying. In other words, when various infections assail the body, and certain cells experience stress or die in large numbers, they can manufacture by-products similar to retroviruses. These by-products can be reactive when testing for HIV antibodies, protein antigens, and viral loads (31). Culshaw summarizes it well:

> *A retrovirus is nothing more than RNA with an outer protein shell. The shell enables it to bind to cells of the type it infects, and once it gains entry, the outer coating disappears and the RNA is transcribed to DNA and incorporated as provirus into the host cell's own genome. It is for this reason that retroviruses are called enveloped viruses, and it is also the reason that it is very difficult to distinguish between exogenous retroviruses (those that originate outside the body from a foreign invader) and endogenous retroviruses (those that are manufactured from our own retroviral-like genetic sequences under conditions of cellular stress, including diseases) … Much of the genetic material attributed to HIV is in fact DNA or RNA from [these] decaying cells (…) Human beings are filled with such endogenous retroviruses [(32), pp. 53, 55–56].*

Transmission electron microscopy images of retroviral particles

Although it seems intuitive that photographing HIV would provide undeniable evidence of its presence in the host's plasma, the reality is much more complex. Adequately interpreting images obtained through EM is, even for the most skilled scientists, challenging. EM generates highly amplified images of cells and viral particles. An electron-microscope uses "beams of electrons focused by magnetic lenses instead of rays of light" to produce images magnified up to 10,000,000× (a light microscope has difficulty exceeding 2000× magnification) (33).

The first images of what researchers believed to be HIV particles budding out of human cells were published in the journal Science, in 1983, by the French team that co-discovered HIV (headed by Luc A. Montagnier) (34). These images, and the computer graphics based on them, were printed in textbooks and articles discussing AIDS, extensively. Despite their popularity, the images were obtained from a "pre-AIDS" patient (not a patient with AIDS), and the sample furnishing the images had not been purified according to standard procedures (35).

It would be 14 years later, in 1997, when EM images from purified samples were produced (20). Yet another study (22), published simultaneously with these images (in fact, printed as an adjoining article), reported: even purified HIV samples harbor protein particles (called microvesicles), considered to be contaminants. These microvesicles do not disappear during the purifying process. In other words, even when technicians purify HIV samples, certain "cellular proteins bound to non-viral particles (i.e., microvesicles) can copurify with [the] virus," and appear in the EM images. The question, then, remains: are the EM images seen in these purified samples, pictures of HIV itself, or of other elements/particles? (36).

In 2010, Ettiene de Harven – the scientist who "produced the first electron micrograph of a retrovirus (the Friend leukemia virus)" [(32), p.13] through EM research in 1960 (Table 1) (37) – added to the debate:

> *All the images of particles supposedly representing HIV and published in scientific as well as in lay publications derive from EM studies of cell cultures. They never show HIV particles coming directly from an AIDS patient [(7), p. 70 – emphasis added].*

Why is it important to obtain EM images of HIV from AIDS patients, as opposed to images of HIV cultured in a laboratory? According to de Harven, non-viral micoorganisms frequently contaminate cell cultures and show up very easily in EM. It is quite difficult to obtain absolutely pure cell cultures, especially because the culturing process itself – the growth factors added to the culture, such as "T cell lymphocyte growth factor (TCGF), interleukin 2, or corticosteroid hormones" [(23), p. 4] – can introduce potential contaminants. HERVs, for example, are often generated by cells that have been stressed or hyperstimulated to grow in cultures. HIV cultures obtained from patients with AIDS may not require as much stimulation or addition of growth factors, thus resulting in less contaminated, purer cultures.

Montagnier also acknowledges the problems with relying on EM to identify a retrovirus, given the difficulties with purifying viral samples. In an interview given in 1997, he reflects on those first HIV images from cultured samples, produced in his laboratory at the Pasteur Institute:

> *DT (Djamel Tahi): Why do the EM photographs published by you, come from the culture and not from the purification?*
>
> *LM (Luc Montagnier): There was so little production of virus it was impossible to see what might be in a concentrate of virus from a gradient. There was not enough virus to do that …*
>
> *(…)*
>
> *DT: How is it possible without EM pictures from the purification, to know whether these particles are viral and appertain to a retrovirus, moreover a specific retrovirus?*
>
> *LM: Well, there were the pictures of the budding. We published images of budding which are characteristic of retroviruses. Having said that, on the morphology alone one could not say it was truly a retrovirus … (38).*

It appears, therefore, there is little consensus regarding what the existing EM images reflect: are the visualized particles HIV or something else? According to Papadopulos-Eleopulos and colleagues, "some of the best known retrovirologists including Peter Duesberg, Robert Gallo, and Howard Temin have been telling us that particles may have the morphological characteristics of retroviruses but are not viruses" [(39), p. 2]. It is feasible, therefore, that EM images are, in fact, depictions of (a) microvesicles (or protein particles), not viral or infectious in nature, but not eliminated even when using purified samples (22); or (b) human endogenous retroviruses – defective, non-infectious retroviruses associated with the host's own genome (see discussion above on HERVS).

Efficacy of anti-retroviral drugs

From the epidemic's onset, researchers worked relentlessly to find a vaccine to keep the virus from spreading and to develop drugs for managing the symptoms from opportunistic infections (40). The challenges inherent in developing both vaccine and treatment were daunting: post-infection, HIV appears to mutate and recombine continually, thus making it difficult to design an effective vaccine (41, 42). Furthermore, designing treatments for a retrovirus is a tricky feat, given it shares many of the same characteristics of the host's immune cells – thus, an attack on the virus can become a simultaneous attack on the healthy host cells (14, 32, 35).

After the public announcement regarding the probable cause of AIDS, various pharmaceutical companies tried to develop drugs to thwart the action of the virus' reverse transcriptase enzyme (an enzyme essential for the replication of retroviruses). AZT became the first medication of this kind, approved specifically for treating AIDS patients in 1987 (43). Azidothymidine (AZT) – also known as

Retrovir, a drug originally designed, but proven unsuccessful, for treating leukemia – made history not only because it was the first available treatment specifically for AIDS, but also due to how quickly it was approved: AZT received "investigational new drug (IND) status (initial approval for testing) within 5 days of application" [(44), p. 134]. Given the desperate need for specific treatment, the drug's placebo-controlled trials also moved fast, lasting "only 6 months before approval was given for general sale" [(44), p. 134]. Phase II trials were interrupted, mid-way, due to findings that fewer patients taking AZT were dying of AIDS when compared to the control group not taking the drug (44, 45).

Approving AZT, however, did not prevent scientists from trying to develop other drugs, during the following decade; but most attempts would make little headway into the treatment of AIDS. Adding to these difficulties, AZT was proving to be extremely toxic and not as effective as initially anticipated. Researchers did learn, meanwhile, that prescribing AZT in lower dosages and in combination with other, well-known drugs such as heparin, acyclovir, and bactrim, was beginning to curb mortality rates (44).

Thus, in the mid-90s "combination therapy" became available. Also referred to as the "drug cocktail," combination therapy comprised a joint attack on HIV using three main classes of drugs, simultaneously: (a) those inhibiting reverse transcriptase's ability to duplicate the virus' genetic material using host DNA sub-divided into two classes – nucleoside and non-nucleoside inhibitors; (b) protease inhibitors (designed to limit certain proteins needed for HIV assembly); and (c) myristoylation or entry/fusion inhibitors (blocking the virus from entering the host cells). These three classes of drugs – known collectively as HAART (highly active ARV therapy) or antiretrovirals (ARVs) – have been praised for their ability to restore the health of patients with AIDS who become extremely ill [(24, 44, 46), p. 240].

Antiretrovirals also are praised for their ability to reduce patients' viral loads and, therefore, their level of infection and ability to transmit the virus (or infectivity). This reduction in viral load has been deemed so significant that, in 2012, the FDA approved using one of the combination drugs (Truvada) for pre-exposure prophylaxis or PrEP (47).

PrEP or "HIV treatment-as-prevention" (48) involves administering to non-infected persons one pill of the antiretroviral, daily, to stave off infection: an initiative crowned *Breakthrough of the Year* by the journal Science, in 2011 (47). Trials conducted world-wide have consistently demonstrated low rates of HIV infection among people taking PrEP (41, 48). The 2011 breakthrough, therefore, was the conclusion: "The early initiation of ARV therapy reduced rates of sexual transmission of HIV-1 and clinical events, indicating both personal and public health benefits from such therapy" [(41), p. 493].

Yet, as with most treatment drugs, ARVs also produce important side-effects. Even mainstream scientists who praise the drugs by saying, "Combination theory [*sic*] was a miracle, comparable with antibiotics, anesthesia, and the polio vaccine in the annals of the history of medicine … a 'quantum leap"' – candidly admit: "The miracle was not without complications." [(44), pp. 246, 247]. Because these drugs also attack non-infected cells, they can destroy the immune systems' healthy T-cells, and even cause a collapse identical to AIDS. Authors of a study reporting on the first decade of ARV use concluded,

> *The results of this collaborative study, which involved 12 prospective cohorts and over 20,000 patients with HIV-1 from Europe and North America, show that the virological response after starting HAART has improved steadily since 1996. However, there was no corresponding decrease in the rates of AIDS, or death, up to 1 year of follow-up. Conversely, there was some evidence for an increase in the rate of AIDS in the most recent period [2002–2003] [(49), p. 454 – emphasis mine].*

Critics' concerns center on the potential association between use of HAART and a depressed immune system. This association carries significant implications for the prophylactic use of ARVs. For instance, studies have documented patients' compromised immune systems as *preceding* their seroconversion (50, 51). Therefore, having non-infected persons take HAART as prophylaxis may, over time, impact their immune systems negatively, and predispose them to becoming infected with various agents, including HIV itself. Moreover, there is evidence that ARVs can accelerate aging of cells in ways that promote progressive multi-organ disease (52). Critics also point to data on patients taking ARVs who develop *Pneumocystis Carinii*, and *Candida albicans* (opportunistic infections typical of patients with AIDS) while on the drugs, despite the fact the protease inhibitors have "marked anticandidal and antipneumocystis effects" [(7), p. 71]. Equally vexing, are the deaths among ARV-treated patients, resulting from acute liver failure. These deaths point to the ARVs' detrimental effects, given that HIV, itself, does not cause liver toxicity (7, 53, 54).

Critics also highlight studies documenting the reduction of plasma HIV RNA among patients treated with ARVs, but the non-reduction in HIV DNA, suggesting there is "continued expression of viral agents" even after 1 year of treatment [(55), p. 320]. Compounding these difficulties are the often debilitating side effects (45), the drugs' extremely high costs (AZT alone cost around $6,000 a year and the cocktails can easily tally $12,000 – 13,000 a year per patient) [(44), pp. 245–246] and the oftentimes daunting regimen some prescriptions require, leading to patients' less-than-optimal compliance during treatment.

Despite this host of problems, orthodox scientists and practitioners still claim HAART has changed the face of the AIDS epidemic: once considered a lethal syndrome, testing positive for HIV does not equate to a death sentence any longer; merely to a lifetime of managing a chronic infection (56, 57). Critics, on the other hand, assert: because the drugs are anti-viral and anti-bacterial in nature, they give a false impression of being effective for treating HIV infection. What appears a miraculous recovery in many patients is, in fact, the drugs' effects upon the opportunistic infectious agents the person may harbor at the time, other than HIV. Contrary to the reigning enthusiasm for ARVs' effectiveness for prevention and treatment, critics will argue the risks associated with ARVs appear to outweigh the benefits, especially if these drugs are consumed over long periods of time. In short, unorthodox scholars believe the appearance of effectiveness of ARVs does not represent strong evidence for the role of HIV in AIDS and, in a paradoxical manner; ARVs may actually be the cause of AIDS-defining illnesses and non-AIDS-defining ones.

Epidemiological data

It is easy to obtain current statistics describing the HIV-AIDS distribution, world-wide. One has only to access the website of the Joint United Nations Program on HIV to learn: "In 2012, there were 35.3 million [32.2–38.8 million] people living with HIV" and that, in the same year, "1.6 million [1.4–1.9 million] people died from AIDS-related causes worldwide compared to 2.3 million [2.1–2.6 million] in 2005" (58).

Scholars on both sides of the debate agree: "epidemiologic studies and data can show only that a risk factor is statistically associated (correlated) with a higher disease incidence in the population exposed to that risk factor" [(59), p. 42]. Epidemiological data do not provide evidence for causation. All the data can do is reveal risk factors and illness co-occurring in a given group. Despite this well-known caveat, mainstream scientists argue that because HIV has spread among high-risk groups as expected, the AIDS epidemic has, indeed, a viral, infectious agent: its "epidemic curves resemble … such infectious agents as hepatitis B and genital herpes viruses" [(59), p. 53]. These scientists also will

explain the differences observed in the frequency of certain illness in specific geographic regions (e.g., higher numbers of HIV-related Tuberculosis in sub-Saharan Africa) as caused by the "background flora of infectious disease agents" present in these regions [(59), p. 54].

Curiously, however, even among mainstream scholars who believe epidemiological data constitute valuable evidence of a viral cause for AIDS, there are those who have turned a critical eye toward the data the US and the WHO have compiled. James Chin – one such critic (Table 1) writes in his book, *The AIDS Pandemic: The Collision of Epidemiology with Political Correctness*:

> *Estimation and projection of HIV infections and AIDS cases and deaths (HIV/AIDS) can be considered more of an art than a science because of the marked limitations of both available data and methods for estimation and projection. These limitations make it possible for UNAIDS and other AIDS program advocates and activists to issue misleading and inflated estimates and projections [(59), p. 137].*

The questions regarding the validity and reliability of epidemiological data emerging from within the mainstream/orthodox views have been echoed and amplified by unorthodox scholars. Both camps' concerns center on four problems plaguing the estimates of incidence (new cases), prevalence (remaining cases), and projection (future cases) of HIV infections, AIDS diagnoses, and AIDS-related deaths: (a) the varying clinical definitions of AIDS (the official definition has changed four times since 1982) (60); (b) variability in the criteria for seropositivity in HIV tests; (c) the absence of testing in many regions of the world (many developing countries do not have the laboratories needed to test every single AIDS case); and (d) the mistakes in estimation, data management and reporting (e.g., the revision of projections for year 2006 by UNAIDS) (59–62).

This article's space limitations do not allow an expanded treatment of each problem-area, but readers can find further details within the works cited. For instance, in Rebecca Culshaw's book – *Science Sold Out: Does HIV Really Cause AIDS* (32) – readers will find 13 "failed predictions" regarding the spread of HIV and AIDS, including the prediction that HIV infection would spread randomly among populations (i.e., outside specific risk groups). Culshaw also tells her personal story of having written a master's thesis, received a Ph.D. based on her work with "mathematical models of the immunological aspects of HIV infection," and eventually concluding "there is good evidence that the entire basis for this theory is wrong" [(32), p.7].

Unorthodox Theories: If not HIV, Then What?

If the criticisms outlined above pinpoint significant problems with each type of data used to support the HIV-AIDS hypothesis, they only contribute to deconstructing the hypothesis, not to providing explanations for what might cause AIDS if not a retrovirus. However, alternative hypotheses abound. Anchoring themselves in well-established causes of immune system malfunction, these hypotheses point to pharmacological (drug) factors, immune dis-balance factors, latent infection overload, and malnutrition as culprits.

Although several scientists investigated the role drugs might play in causing immune suppression *before* HIV was identified [see a list of these studies in Duesberg et al. (46)], the main proponent of the drug-AIDS hypothesis in the epidemic's early years was Peter Duesberg, a professor of Molecular and Cell Biology at UC Berkeley. According to Seth Kalichman, who wrote *Denying AIDS* (a harsh critique of unorthodox views and of Duesberg in particular), "In every respect, HIV/AIDS denialism starts and ends with Peter Duesberg" [(63), p. 175]. Duesberg's arguments gained notoriety among unorthodox

theories not only due to his expertise and prominence (see Table 1), but also to his challenge of the medical and scientific establishments early in the history of the epidemic, employing clear empirical logic.

Duesberg began challenging the viral hypothesis for AIDS soon after the publication (in 1984) of the four seminal articles pointing to HIV as the "probable" cause (64–67). In two key publications in 1987 and 1989 – in *Cancer Research* and in the *Proceedings of the National Academy of Sciences* (68, 69) – Duesberg cogently argued: retroviruses are not known for killing cells. In other words, retroviruses are not "cytocidal." If anything, retroviruses were once thought to be associated with cancer because they cause precisely the opposite of cell death; they contribute to cells' growth or proliferation. In Duesberg's words, "… retroviruses are … considered to be plausible natural carcinogens because they are not cytocidal and hence compatible with neoplastic growth and other slow diseases." [(68), p. 1200]. In his view, HIV's inability to kill cells could not explain the suppression of the T-cells in the immune system, as proposed by the teams who discovered HIV[3]. According to Farber,

> *In other fields, such as gene therapy, it is axiomatic that retroviruses are the ideal carriers for genetic materials, because they 'don't kill cells'. Incredibly, this is where the so-called HIV debate first forked in 1987, and where the camps remain bitterly divided to this day [(14), p. 50].*

For Duesberg and scientists agreeing with him, then, other agents would have to be responsible for the disastrous immune function collapse seen in AIDS patients. These scientists saw as prominent among such causes, the use of drugs, both recreational and routinely prescribed ones. As author Gary Null points out, even before AIDS, researchers were documenting the immune-suppressing effects of amyl nitrites or "poppers" (the form of amyl nitrites popular among gay men in the early and mid-80s) and determining both their toxicity and carcinogenic properties in humans and animals (45). However, two studies CDC published in 1983, one in which they were unable to detect any toxicity from amyl nitrites, the other, unable to document a significant association between inhaled nitrates and Kaposi's sarcoma or *Pneumocystis carinii* pneumonia, led the search to a halt (70, 71). Investigators later tried to determine if certain *batches* might have been contaminated with toxic agents but, when they found no contamination, the focus on poppers/amyl nitrites themselves ceased (1). Nonetheless, in 1998 Duesberg and Rasnick (Table 1) (72) reviewed evidence published since 1909, "which prove[s] that regular consumption of illicit recreational drugs causes all AIDS-defining and additional drug-specific diseases at time and dose-dependent rates" [(46), p. 393].

Other drugs such as those given to transplant patients to prevent organ rejection, as well as routinely prescribed antibiotics, also have been implicated as potential causes of immune dysfunction. Studies have shown that transplant patients who develop Kaposi's sarcoma will go into remission, once taken off the drugs required to avoid organ rejection. Immune-suppressing drugs (as well as amyl nitrites) have, for instance, been directly correlated with Kaposi's sarcoma, the rare skin cancer found frequently among AIDS patients during the epidemics' early days [see reviews by Null (45) and Kremer (35)].

Anti-retroviral drugs used to treat HIV infection/disease, also, are indicted by Duesberg and those who agree with him as potentially causing AIDS (43, 62). Because the drug cocktails include "DNA chain-terminators and protease inhibitors" that affect healthy cells as well as the virus, and because "many studies find that people receiving ARV medications experience AIDS-defining diseases to a greater extent than controls not receiving those medications" [(73), p. 122], antiretrovirals are viewed as potential immune suppressors.

In a review of the chemical bases for AIDS, published in 2003, Duesberg and his colleagues (46) outlined the epidemiological and bio-chemical evidence supporting different causes for the AIDS epidemics in the US/Europe and in Africa, none of which are viral or contagious. The authors concluded:

> *The chemical-AIDS hypothesis proposes that the AIDS epidemics of the US and Europe are caused by recreational drugs, alias lifestyle, and anti-HIV drugs … and by other non-contagious risk factors such as immunosuppressive proteins associated with transfusions of blood clotting factors … pediatric AIDS is due to prenatal consumption of recreational and anti-HIV drugs by unborn babies together with their pregnant mothers … The chemical basis of African AIDS is proposed to be malnutrition and lack of drinkable water … exactly as proposed originally by the now leading HIV-AIDS researchers Fauci and Seligman: "The commonest cause of T-cell immunodeficiency worldwide is protein-calorie malnutrition" … and others … [(46), p. 392].*

Alongside a drug hypothesis, another proposed cause for AIDS is the iNOS hypothesis, or immune dis-balance hypothesis. In his book, *The Silent Revolution in Cancer and AIDS Medicine*, Kremer (35) (Table 1) explains that much of what scientists now know about the immune system and its functions was not well understood at the time they identified HIV. In particular, the research on NO, or nitric oxide, was still in its infancy: NO is "an important intracellular and intercellular signaling molecule" acting as "…an important host defense effector in the immune system" [(74), p. 639]. Even though NO (and its derivative iNOs) is "involved in the regulation of diverse physiological and pathophysiological mechanisms in cardiovascular, nervous, and immunological systems," researchers have shown it can also become a harmful, "cytotoxic agent in pathological processes, particularly in inflammatory disorders" [(74), pp. 639–640]. Put simply, at adequate levels NO helps regulate blood pressure as well as "wound repair and host defense [*sic*] mechanisms" [(75), p. 277]. Excessive amounts, however, lead to T-cell depletion, "inflammation, infection, neoplastic diseases [cancer] liver cirrhosis, [and diabetes" [(75), p. 277]. This change from adequate-to-excessive amounts of NO in the human body results from multiple factors, including "nitrite inhalation [e.g., using 'poppers'], microbial antigen, and toxin stimulation [e.g., suffering repeated infections with different viruses/bacteria], immunotoxic medications [e.g., taking ARVs and antibiotics], [and] many other stress factors" [(35), p. 49].

A closely related perspective, placing the blame for AIDS on bio-chemical processes gone awry within human cells is the oxidative stress (or redox) hypothesis. Oxidative stress is a cellular-level electro-chemical phenomenon that diminishes a cell's ability to absorb oxygen. This diminished capacity to process oxygen at optimal levels leads to the cell's disruption and death. Scientists have either hypothesized or empirically connected oxidative stress to many diseases, including type 2 diabetes and cancer (35, 45, 76). According to this hypothesis' main proponents,

> *At first sight it appears that there is no common factor, apart from HIV infection, linking the various AIDS risk groups. However, homosexuals are exposed to relatively high levels of nitrites and anally deposited sperm, drug abusers to opiates and nitrites, hemophiliacs to factor VIII. All these are known potent oxidizing agents … [(77), p. 147 – emphasis mine].*

For these proponents of the redox hypothesis even Luc Montagnier (the head of the French team that discovered HIV) agrees "that anti-oxidants should be used for treatment of HIV/AIDS patients" [(78, 79), p. 6].

Viewing a person's immune system as a complex dynamic balancing act among various elements, which sometimes behave as defenders, other times, as offenders, is also consistent with the "latent infection overload hypothesis" proposed by Kary Mullis (Table 1). According to Mullis, as people

become infected with multiple viruses and experience many latent infections, the immune system embarks on a chain-reaction-response to each virus. Latent infections are those without visible symptoms, and according to Mullis, "at a given time most viral infections in an individual are latent" [(80), p. 196]. Eventually, the system overloads itself and becomes dysfunctional. AIDS, he says, "may be the result of such a chain reaction." This hypothesis assumes:

> *... there is not a single organism that is the cause of AIDS, and there should exist AIDS patients who do not test positive for HIV*[4] *. It is an overwhelming number of distinct organisms, which causes the immune dysfunction. These may individually be harmless [(80), p. 197].*

Perhaps the most intriguing alternative hypothesis, however – if not from its bio-chemical perspective, at least from the perspective of who supports it – is the one proposing HIV may not be the primary villain, but merely an accomplice in causing AIDS (83). Joseph Sonnabend – a prominent physician/researcher responsible for encouraging his gay patients to lead a healthy lifestyle to avoid developing AIDS, and one who "did not accept HIV = ADS theory for many years" – recently changed his views and "has come to think that HIV, together with other factors, may play a subsidiary causative role" [(73, 84), p. 120]. Even Montagnier and Gallo (leaders of the French and American teams, respectively, that discovered HIV), at various times since the epidemic began, have suggested HIV might be a co-factor in AIDS, not its exclusive causative agent (85).

Other hypotheses have been proposed over the years, but none have garnered as much attention as those outlined above. Some of these other hypotheses claim AIDS is caused by (a) multiple factors; some factors explaining some cases, other factors accounting for other cases; (b) undiagnosed or untreated syphilis infection; (c) autoimmunity; (d) selenium deficiency, and (e) psychological factors, including stress and trauma [see Bauer (73), pp. 124, 136–139 for details on these hypotheses].

The positive or reassuring aspect of these alternative hypotheses is the tangible hope for prevention, treatment, and cure they embody. Nevertheless, it is difficult not to agree with Bauer when he concludes, "…it is hardly reassuring that this array of suggestions has been in circulation for something like (three) decades without having been adequately explored" [(73), p. 139].

Discussion

At this point, readers might be wondering: given the problems with the mainstream hypothesis, how did we get here? How did we come so far, tethered to such a problematic perspective? The complexity of the answers to these questions aside, it may help to bear in mind the notion that HIV-causes-AIDS emerged and developed within a very specific scientific-cultural-historic context. Although the scope of this article precludes dealing with this complex context, for our purposes it is important to recall at least one element: Funding for President Nixon's War on Cancer campaign ended in 1981 with very little achieved in the quest for an infectious cancer agent (15, 85–87). The only exception was the discovery connecting select retroviruses to a few, rare cancers. Other than this, scientists had a handful of "orphaned" viruses which, they suspected, might play a role in causing illnesses, but no known diseases to which these viruses could be connected. Proposing a connection between an emerging syndrome and one of these viruses (even if only a circumstantial connection) proved enticing enough to pursue. And pursue they did, as soon as AIDS began to appear in larger-than-expected numbers among otherwise healthy adults.

If viewed from this perspective, then, why scientists so quickly and assuredly "jumped on the HIV bandwagon" may not be very difficult to understand. That the scientific establishment world-wide insistently refuses to re-examine the HIV-AIDS hypothesis, however, is more difficult to accept, especially when one examines the credentials of those proposing such a revision. Their expertise

carries as much weight as the teams who defend the orthodox hypothesis (Table 1). Seth Kalichman, a critic of AIDS "denialists," recommends adamantly: anyone who entertains alternative views should "consider the source: credibility of where the article is reported as well as the researchers themselves must be weighed" [(63), p. 159]. I could not agree more: taking into account the credibility of the scholars who question the HIV-AIDS hypothesis is, perhaps, the strongest argument *in favor* of seriously considering their critiques, not against it.

Furthermore, credibility as an argument works both ways: if to question the trustworthiness of unorthodox scholars is vital, it is equally crucial to question the reliability of those supporting the HIV-AIDS hypothesis. Readers who care to learn about HIV-AIDS' history will encounter ethically questionable actions carried out by some of the most notable orthodox researchers, as well as ethical misconduct charges made against them [for an extensive treatment of these ethical and legal issues, backed by extensive official documentation, see Crewdson (88)].

If it is difficult to dismiss the unorthodox views due to the credibility of their sources, then, why are not orthodox scientists and practitioners more willing to rethink the hypothesis or, at the very least, test the unorthodox arguments in a scientific, open debate? Although there have been, in fact, several attempts to engage the orthodox community in dialog, nearly all have been unsuccessful [for examples, see Ref. (14, 85, 88)]. Most likely, reasons for denying the calls to re-examine the orthodox stance lie in the complex, synergistic dynamics within the scientific, medical, economic, and political systems or ideologies worldwide. Even brief speculation about these reasons would exceed the scope of this article, therefore I refer the reader, once again, to the sources referenced [in particular, see Epstein (89) and Bauer (73)].

Here I would argue, nonetheless, that the debate between orthodox and unorthodox scientists comprises much more than an intellectual pursuit or a scientific skirmish: it is a matter of life-and-death. It is a matter of justice. Millions of lives, worldwide, have been and will be significantly affected by an HIV or AIDS diagnosis. If we – the public health workforce – lose sight of the social justice implications and the magnitude of the effect, we lose "the very purpose of our mission" [(3, 90), p. 690].

In particular, a pressing concern for public health is the move or push toward (a) HIV screening for "patients in all health-care settings" (with opt-out screening) (91) and (b) placing persons-at-risk (even if not yet infected with HIV), on retroviral medication as a form of prophylaxis (see discussion about PrEP, above) (92). If in 1986 the CDC recommended voluntary testing for people in high-risk groups, in 2013 the U.S. Preventive Services Task Force "gave routine HIV screening of all adolescents and adults, ages 15–65, an 'A' rating" [(93), p. 1]. The recently approved Affordable Care ACT "requires or incentivizes new private health plans, Medicare, and Medicaid to provide preventive services rated 'A' or 'B' at no cost to patients" [(93), p. 1]. Thus, routine screening of every adolescent and adult in all populations is, now, the goal (91, 94).

If, to this goal we juxtapose the problems with the HIV tests, with the definition(s) of AIDS, and with the toxicity of the ARVs currently prescribed, we begin to understand the potential for harm inherent in them. Put blatantly: these recommendations can be harmful or iatrogenic (95).

Public health workforce: Our role

What can the public health workforce do, given such potential for harm? As stated in the introduction, this paper represents a call to reflect upon our public health practice vis-à-vis HIV-AIDS. Reflecting upon and questioning the *status quo* constitute important dimensions of public health professionals' competencies and practice. If the only hope the HIV-AIDS hypothesis can offer, 30 years later, is to provide highly toxic drugs to treat HIV infection and to prevent high-risk but healthy persons from becoming infected, health promoters have a professional duty to reflect on the available data and

question the usefulness of the hypothesis. Only in doing so can public health professionals maintain their professional integrity, tend to public health's roots in social justice, and contribute to developing knowledge using ethical methods.

James Jones, in his book *Bad Blood: The Tuskegee Syphilis Experiment* (96), reminds us poignantly that not asking whether health professionals "should be doing" something, but continuing to do it uncritically, because "it can be done" was, ultimately, the mind-set sustaining the Tuskegee syphilis study for 40 years – unquestionably one of the worst cases of scientific misconduct in American history. The AIDS epidemic – if managed without questioning or without the dialogical process of action-reflection – may, with time, overshadow Tuskegee in the magnitude of its negative impact.

Specifically, I propose the public health workforce can undertake such an action-reflection process by engaging in the following tasks:

(1) Learning about the history of the HIV/AIDS epidemic, of the problems surrounding the discovery of HIV, and about the development of drug therapies and PrEP. Publications recording this history abound in the professional and trade literatures, representing both mainstream and unorthodox view-points. To understand the forces shaping the HIV/AIDS epidemic, we currently experience represents a crucial responsibility of a competent and ethics-driven workforce.

(2) Conducting its own research to test alternative theories for the cause(s) of AIDS and/or to portray the inconsistencies and contradictions in the orthodox hypothesis. Qualitative inquiry, for instance, exploring unorthodox views and the practices of providers, patients, and scientists, might be a fruitful option for challenging prevailing assumptions.

(3) Fostering and mediating a debate among HIV-infected persons, scientists, and health-care providers, to critically assess current beliefs and practices. Public health professionals – who are well-informed about the orthodox and unorthodox perspectives' strengths and weaknesses – could play an important role as facilitators in this much-needed dialog.

Although carrying out the tasks outlined above may represent a novelty for many public health professionals, for the scientists, practitioners, and investigators who believe a viral hypothesis for AIDS is unproductive, none of this is new. They have combed historical documents (or played a role in the history, themselves); they have amassed substantial amounts of data, and they have made numerous calls for debate. They have held to their beliefs, steadfastly, for the past 30 years. Twenty four years after the first article challenging HIV, Duesberg and colleagues, for instance, still claimed HIV is only a "passenger virus" (one "not sufficient and not necessary to cause a disease") [(62), p. 81]. While not all unorthodox scholars agree with Duesberg, most still actively defend their critiques of the HIV-AIDS hypothesis and persist in their questioning. As we face the next decade with AIDS still rampant, then, it becomes vital that public health professionals attend to the debate and embark in a questioning of their own.

Conflict of Interest Statement

The author declares that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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Footnotes

[1]In this article, I will use the terms unorthodox, non-orthodox, non-mainstream, and alternative, to refer collectively to those who disagree with the prevalent view, and to their propositions (despite their variability). I will favor the term "unorthodox" for it carries the notion of intention or willful deviation from the norm and connotes a power differential in which one set of theories (the orthodox or mainstream) dominates another – what Delborne calls "the epistemological tyranny of the intellectual majority" [(2), p. 510].

[2]I am indebted to E. de Harven (7) for suggesting these categories.

[3]In fact, evidence supporting the notion "HIV kills T-cells" has been so conspicuously absent that, currently, scientists don't believe HIV "kills T-cells in any way. Rather, they believe HIV primes T-cells to commit suicide at some later time" [(32), p. 73]

[4]Some would argue this is the strongest evidence against the HIV-AIDS hypothesis: cases of AIDS with no documentable presence of HIV. However, say the critics, the difficulty with this argument lies in the definition of AIDS: because AIDS is defined as "the final stage of HIV infection" (81), AIDS presupposes infection with HIV, making the definition a circular one (i.e., AIDS = final stage of HIV infection = opportunistic infections + high viral load + low CD_4 counts). Due to the circularity in the logic, if there is no HIV, there can be no AIDS. Nonetheless, cases of patients with AIDS-defining opportunistic infections and low CD_4 counts without HIV do exist (see, for example, the review by Green and colleagues (82).

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Viewpoint

March 13, 2020

Critical Care Utilization for the COVID-19 Outbreak in Lombardy, Italy

Early Experience and Forecast During an Emergency Response

Giacomo Grasselli, MD[1,2]; Antonio Pesenti, MD[1,2]; Maurizio Cecconi, MD[3]

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JAMA. Published online March 13, 2020. doi:10.1001/jama.2020.4031

On February 20, 2020, a patient in his 30s admitted to the intensive care unit (ICU) in Codogno Hospital (Lodi, Lombardy, Italy) tested positive for a new coronavirus, severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2), the virus that causes coronavirus disease 2019 (COVID-19). He had a history of atypical pneumonia that was not responding to treatment, but he was not considered at risk for COVID-19 infection.[1] The positive result was immediately reported to the Lombardy health care system and governmental offices. During the next 24 hours, the number of reported positive cases increased to 36. This situation was considered a serious development for several reasons: the patient ("patient 1") was healthy and young; in less than 24 hours, 36 additional cases were identified, without links to patient 1 or previously identified positive cases already in the country; it was not possible to identify with certainty the

source of transmission to patient 1 at the time; and, because patient 1 was in the ICU and there were already 36 cases by day 2, chances were that a cluster of unknown magnitude was present and additional spread was likely.

On February 21, an emergency task force was formed by the Government of Lombardy and local health authorities to lead the response to the outbreak. This Viewpoint provides a summary of the response of the COVID-19 Lombardy ICU network and a forecast of estimated ICU demand over the coming weeks (projected to March 20, 2020).

Setting the Priorities and the Initial Response

In Lombardy, the precrisis total ICU capacity was approximately 720 beds (2.9% of total hospital beds at a total of 74 hospitals); these ICUs usually have 85% to 90% occupancy during the winter months.

The mission of the COVID-19 Lombardy ICU Network was to coordinate the critical care response to the outbreak. Two top priorities were identified: increasing surge ICU capacity and implementing measures for containment.

Increasing ICU Surge Capacity

The recognition that this outbreak likely occurred via community spread suggested that a large number of COVID-19–positive patients were already present in the region. This prediction proved correct in the following days. Based on the assumption that secondary transmission was already occurring, and even with containment measures that health authorities were establishing, it was assumed that many new cases of COVID-19 would occur, possibly in the hundreds or thousands of individuals. Thus, assuming a 5% ICU admission rate,[2] it would not have been feasible to allocate all critically ill patients to a single COVID-19 ICU. The decision was to cohort patients in 15 first-responder hub hospitals, chosen because they either had expertise in infectious disease or were part of the Venous-Venous ECMO Respiratory Failure Network (RESPIRA).[3]

The identified hospitals were requested to do the following.

1. Create cohort ICUs for COVID-19 patients (areas separated from the rest of the ICU beds to minimize risk of in-hospital transmission).

2. Organize a triage area where patients could receive mechanical ventilation if necessary in every hospital to support critically ill patients with suspected COVID-19 infection, pending the final result of diagnostic tests.

3. Establish local protocols for triage of patients with respiratory symptoms, to test them rapidly, and, depending on the diagnosis, to allocate them to the appropriate cohort.

4. Ensure that adequate personal protective equipment (PPE) for health personnel is available, with the organization of adequate supply and distribution along with adequate training of all personnel at risk of contagion.

5. Report every positive or suspected critically ill COVID-19 patient to the regional coordinating center.

In addition, to quickly make available ICU beds and available personnel, nonurgent procedures were canceled and another 200 ICU beds were made available and staffed in the following 10 days. In total, over the first 18 days, the network created 482 ICU beds ready for patients.

Containment Measures

Local health authorities established strong containment measures in the initial cluster by quarantine of several towns in an attempt to slow virus transmission. In the second week, other clusters emerged. During this time, the ICU network advised the government to put in place every measure, such as reinforcing public health measures of quarantine and self-isolation, to contain the virus.

ICU Admissions Over the First 2 Weeks

There was an immediate sharp increase in ICU admissions from day 1 to day 14. The increase was steady and consistent. Publicly available data indicate that ICU admissions (n = 556) represented

16% of all patients (n = 3420) who tested positive for COVID-19. As of March 7, the current total number of patients with COVID-19 occupying an ICU bed (n = 359) represents 16% of currently hospitalized patients with COVID-19 (n = 2217). All patients who appeared to have severe illness were admitted for hypoxic respiratory failure to the COVID-19 dedicated ICUs.

Surge ICU Capacity

Within 48 hours, ICU cohorts were formed in 15 hub hospitals totaling 130 COVID-19 ICU beds. By March 7, the total number of dedicated cohorted COVID-19 ICU beds was 482 (about 60% of the total preoutbreak ICU bed capacity), distributed among 55 hospitals. As of March 8, critically ill patients (initially COVID-19–negative patients) have been transferred to receptive ICUs outside the region via a national coordinating emergency office.

Forecasting ICU Demand Over the Next 2 Weeks

During the first 3 days of the outbreak, starting from February 22, the ICU admissions were 11, 15, and 20 in the COVID-19 Lombardy ICU Network. ICU admissions have increased continuously and exponentially over the first 2 weeks. Based on data to March 7, when 556 COVID-19–positive ICU patients had been admitted to hospitals over the previous 15 days, linear and exponential models were created to estimate further ICU demand (eFigure in the **Supplement**).

The linear model forecasts that approximately 869 ICU admissions could occur by March 20, 2020, whereas the exponential model growth projects that approximately 14 542 ICU admissions could occur by then. Even though these projections are hypothetical and involve various assumptions, any substantial increase in the number of critically ill patients would rapidly exceed total ICU capacity, without even considering other critical admissions, such as for trauma, stroke, and other emergencies.

In practice, the health care system cannot sustain an uncontrolled outbreak, and stronger containment measures are now the only realistic option to avoid the total collapse of the ICU system. For this reason, over the last 2 weeks, clinicians have continuously advised authorities to augment the containment measures.

To our knowledge, this is the first report of the consequences of the COVID-19 outbreak on critical care capacity outside China. Despite prompt response of the local and regional ICU network, health authorities, and the government to try to contain the initial cluster, the surge in patients requiring ICU admission has been overwhelming. The proportion of ICU admissions represents 12% of the total positive cases, and 16% of all hospitalized patients. This rate is higher than what was reported from China, where only 5% of patients who tested positive for COVID-19 required ICU admission.[2,4] There could be different explanations. It is possible that criteria for ICU admission were different between the countries, but this seems unlikely. Another explanation is that the Italian population is different from the Chinese population, with predisposing factors such as race, age, and comorbidities.[5]

On March 8 and 9, planning for the next response, which includes defining a new hub and spoke system for time-dependent pathology, increasing ICU capacity further, and reinforcing stronger containment measurement in the community, has begun, as well as discussions of what could have been done differently.

First, laboratory capacity to test for SARS-CoV-2 should have been increased immediately. Laboratory capacity reached saturation very early. This can add extra stress to a system and affect the ability to make accurate diagnoses and allocate patients appropriately.

Second, in parallel to the surge ICU capacity response, a large, dedicated COVID-19 facility could have been converted more quickly. On day 1 of the crisis, it was not possible to predict the speed and extent of the contagion. Importantly, the forecasts show that increasing ICU capacity is simply not enough. More resources should be invested to contain the epidemic.

As of March 8, Lombardy was quarantined and strict self-isolation measures were instituted. This may be the only possible way to contain the spread of infection and allow resources to be developed for the time-dependent disease.

As of March 10, Italy has been quarantined and the government has instituted stronger containment measures, including strict self-isolation measures. These containment measures and individual citizen responsibility could slow down virus transmission.

While regional resources are currently at capacity, the central Italian government is providing additional resources, such as transfers of critically ill patients to other regions, emergency funding, personnel, and ICU equipment. The goal is to ensure that an ICU bed is available for every patient who requires one. Other healthcare systems should prepare for a massive increase in ICU

demand during an uncontained outbreak of COVID-19. This experience would suggest that only an ICU network can provide the initial immediate surge response to allow every patient in need for an ICU bed to receive one. Health care systems not organized in collaborative emergency networks should work toward one now.

Article Information

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March 14, 2020

Emergency Response of a Western Country to the COVID-19 "Tsunami"

Giuliano Ramadori, Professor of Medicine | University Clinic,Internal Medicine,Göttingen,Germany

This is an impressive report about the challenge the Lombardy Health care system had to face after the outbreak of COVID-19 became clear in an area of Italy with a large Chinese minority. In fact it was supposed that the virus originated from China but the first patient with COVID-19 pneumonia is a young marathon runner of 38 year of age and not a person belonging to the Chinese minority. It is still unclear how he, his wife and his parents became infected.

The number of ICU-patients is impressive. Even more impressive is the velocity of the increase of ...

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March 14, 2020

Mild COVID-19 Cases: Who Might Be Hospitalized And Who Can Be Quarantined?

Arturo Tozzi, Pediatrician | University of North Texas

The escalating number of Italian patients with positive COVID-19 test results causes an unmanageable increase of hospital admissions, including of mild/moderate cases. Indeed, about three fifths of the patients with confirmed SARS-CoV-2 are currently hospitalized in Italy, while the rest are home quarantined. Therefore, it would be useful to grasp who of the patients affected by mild to moderate symptoms require hospital admission instead of household follow-up.

White blood cell counts in SARS-CoV-2-positive but not critically ill patients might be a way to determine who requires hospitalization. Indeed, lower lymphocyte counts have ...

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March 15, 2020

Behavioral factors; clinical COVID-19 exacerbation; prevention and recommendations

Stefano Olgiati, PhD (Epidemiology) | University of Bergamo, Bergamo, Italy

Dear Fellow Researchers,

a. In the article, Grasselli et al (2020) report: "with predisposing factors such as race, age, and comorbidities"

b. In the Comments, Ramadori (2020) observes that: "... the first patient with COVID-19 pneumonia is a young marathon runner of 38 year of age."

c. Fragmented health data report that the marathon runner (and other critically or severely ill patients) practiced high performance sports and / or occupational activities during the asymptomatic and /or mild symptomatic period;

d. Zhoukun et al (2020) report that: " ... clinical symptoms and radiological abnormalities are not ...

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March 19, 2020

What about Non Invasive Ventilation in ICU/Sub-Intensive Units

Paolo Bonazza, MD (Internal Medicine) | Karolinska University Hospital Huddinge

First of all I send you great thanks for taking the time to share your experiences just a few days after you began to manage the COVID outbreak.

As an internist working in a COVID high-dependency unit (HDU) is important to try to help our critical care colleagues and try to know, since the beginning of the outbreak, indications for, and other experiences with, use of non invasive ventilation.

What do you have to say about non invasive ventilation (NIV)? Both in ICU as well HDU/Sub-in-tensive units. I read already that the majority of patients with advanced disease ...

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March 20, 2020

What was the required number of ICU beds per 100.000 inhabitants?

Ignacio Garcia Doval, MD, MSc Epid, PhD | Complexo Hospitalario Universitario de Vigo. Spain

Thank you very much for this description of an impressive, and frightening, effort.

The results would be more valuable elsewhere, and useful to plan for the emergency, if they were related to the population in the area. What is the source population of these hospitals? What was the required number of ICU beds per 100.000 inhabitants? Could the authors answer?

CONFLICT OF INTEREST: None Reported

March 23, 2020

ACE2 and COVID-19

ISKANDAR MONEM ISKANDAR BASAL, medstudent | Università di Roma La Sapienza

Today is the 22rd of March and it is the second day in which the report of the "Protezione Civile" here in Italy registers a small reduction either in the number of infected persons or the number

of deaths. We all hope and intensely pray this trend to continue in the following days.

What is happening in Italy has been actually very unusual and the heroic efforts of the Italian health system to face this tsunami of epidemic is already evident to everybody.

However, many are asking a question. Even the JAMA Editor in his video meeting with ...

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SARS-CoV-2 Coronavirus Multiplex RT-qPCR Kit

(CD019RT)

Regulatory status: For research use only, not for use in diagnostic procedures.

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Summary Documentation

Specificity

non-specific interference of Influenza A Virus (H1N1), Influenza B Virus (Yamagata), Respiratory Syncytial Virus (type B),
Respiratory Adenovirus (type 3, type 7), Parainfluenza Virus (type 2), Mycoplasma Pneumoniae, Chlamydia Pneumoniae, etc.

Species Reactivity
Human

Application
Qualitative

Size
100T

Storage
All reagents should be stored at -30°C~-15°C with protection from light.
The reagents are stable for 12 months when stored at the recommended condition.
The expiration date will not change if the kit is opened and stored at the recommended condition.
The expiration date will not change if the kit is transported with ice-packs for 4 days and/or treated with 10 freeze-thaw cycles.

Intended Use
This product is intended for the detection of 2019-Novel Coronavirus (2019-nCoV).
The detection result of this product is only for clinical reference, and it should not be used as the only evidence for clinical diagnosis and treatment.

Principles of Testing
This product is a dual-color multiplex fluorescent probe-based Taqman® RT-qPCR assay system. The Taqman fluorescent probe is a specific oligonucleotide based on a reporter-quencher mechanism. For each probe, the 5'-end is labeled with a fluorophore, while the 3'-end was labeled with a quencher. When the probe is intact, the fluorescence emitted by the fluorophore is absorbed by the quencher, and no fluorescent signal is detected. However, during amplification of the template, the probe will be degraded due to the 5'-3' exonuclease activity of Taq DNA polymerase, and the fluorescent reporter and the quencher are cleaved and separated, then a fluorescent signal can be detected. The generation of each molecular amplicon is accompanied by the generation of a fluorescent signal. Real-time monitoring of the entire PCR process can be assessed by monitoring the accumulation of fluorescent signals.

This product provides dual-detections of two independent genes of 2019-nCoV in a

single tube. Specific primers and probes were designed for the detection of conserved region of 2019-nCoV's ORF1ab gene and N gene, respectively, avoiding non-specific interference of SARS2003 and BatSARS-like virus strains.

Detection Limit

500 copies /mL.

Reagents And Materials Provided

1. Detection Buffer (900 μL × 2 tubes), including Buffer, dNTPs, Primers, Probes.
2. Enzyme Mix (200 μL × 1 tube), including RNase Inhibitor, UDG, Reverse Transcriptase, Taq DNA polymerase.
3. Positive Control (200 μL × 1 tube), plasmid containing target fragment.
4. Negative Control (500 μL × 1 tube), DEPC-Treated Water.

Note: Do not mix the components from different batches for detection.

Materials Required But Not Supplied

Real-time PCR instrument with both FAM and TEXAS RED channels,
such as ABI7500, ABI Q3, ABI Q6, Roche LightCycler480, Bio-Rad CFX96.

Specimen Collection And Preparation

1. Suitable specimen type: upper respiratory specimen (including nasal swabs, nasopharyngeal swabs / aspirates / washes, and sputum) and lower respiratory specimen (including respiratory aspirates, bronchial washes, bronchoalveolar lavage fluids, and lung biopsy specimens).
2. For detailed methods of specimen collection, please refer to the protocol in the "Microbiology Specimen Collection Manual".
3. The collected specimen should be used for detection within the same day.

Otherwise, please store the specimen as follows:
Store at 2°C - 8°C for no more than 24 hours;
Store at < -20°C for no more than 10 days;
Store at < -70°C for long-term, avoiding repeated freeze-thaw cycles.

4. The specimen should be transported using sealed foam box with dry ice.

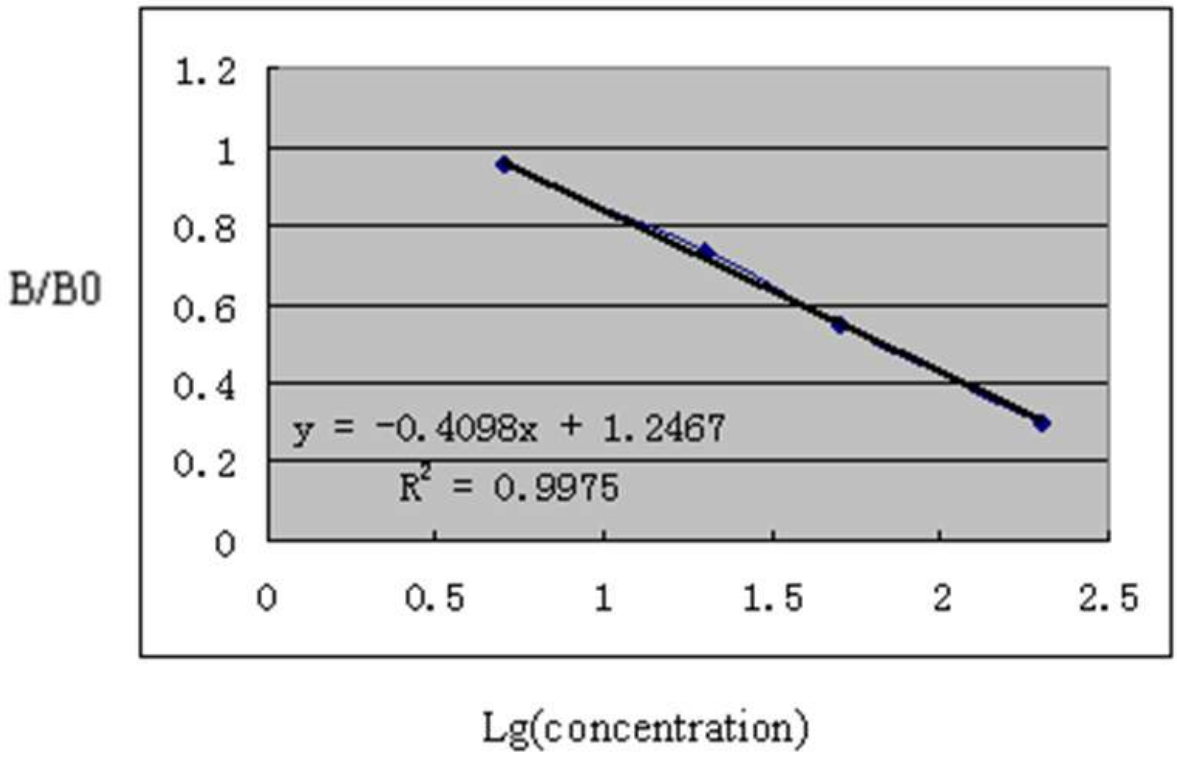
Specimen Preparation

The samples should be extracted according to the corresponding requirements and procedures of viral RNA extraction kits. The extracted RNA can be directly used for

detection. If the extracted RNA is not used for detection immediately, please store the RNA at below -70°C, avoiding repeated freeze-thaw.

Reagent Preparation

Thaw the required reagents, mix by shaking, and centrifuge briefly before use. Prepare the mixture in a RNase-free centrifuge tube as follows:



Note: It is recommended to set both negative and positive controls for each test. Mix the above mixture thoroughly, and make aliquots of 20 μL into different PCR reaction tubes. Then, move to the Specimen Preparation Area.

Assay Procedure

1. Template Addition (Specimen Preparation Area)

Add 5 μL of Negative Control (no extraction required), 5 μL of Positive Control (no extraction required), and 5 μL of extracted RNA from specimen to different PCR reaction tubes which contained 20 μL of PCR mix.

2. RT-PCR Amplification (Detection Area)

Put the reaction tubes on a PCR instrument, setup and run the following cycling protocol:

Settings of detection fluorescence: ORF1ab gene (FAM), N gene (TEXAS RED / ROX). Please set the internal reference parameter of fluorescence of the instrument to "None". For example: for ABI series instruments, please set "Passive Reference" to "None".

3. Data Analysis (refer to Instrument User Manual)

Take ABI7500 as an example: after the qPCR reaction, the results were saved automatically. According to the analyzed image, please adjust the Start value, End value, and Threshold value of the Baseline (Start value: 3 ~ 15; End value: 5 ~ 20; Threshold value could be set in the Log window, and the threshold line should be in the exponential phase of the amplification curve; the amplification curve of the negative control should be straight or below the threshold line). Click "Analysis" to obtain the analysis result automatically, and read the detection result in the "Report" window.

Quality Control

The result is valid if ALL the above criteria is met. Otherwise, the result is invalid.

Interpretation Of Results

If the criteria of QUALITY CONTROL is met, analysis the data of sample as follows:

Precision

Using two cases of high and low positive quality products to test for 10 consecutive times, the CV of their Ct values is ≦5%.

Precautions

1. Please read this manual carefully before beginning the experiment, and strictly follow the instructions.
2. This product should be only used by trained labor personnel in safety protected laboratories and wear appropriate protective equipments.
3. This product should be protected from light. Please use sterile, DNasefree, and RNase-free tubes and tips during the detection.
4. The tested specimen of this product is regarded as infectious material. The operation and treatment should meet the requirements of the local regulations and laws.

Limitations

1. The detection result of this product is only for clinical reference, and it should not be used as the only evidence for clinical diagnosis and treatment. The clinical management of patients should be considered in combination with their symptoms/signs, history, other laboratory tests and treatment responses. The detection results should not be directly used as the evidence for clinical diagnosis, and are only for the reference of clinicians.
2. The detection result can be affected by operations, including specimen collection, storage and transportation. False negative result may occur if there is any mistakes in the operation. Cross contamination during specimen treatment may lead to false positive result.
3. The detected target sequences of this products are the conservative region of 2019-nCoV's ORF1ab gene and N gene. However, target sequence variations may lead to false negative result.

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This article has been retracted.

Retraction in: Front Public Health. 2019 October 29; 7: 334 See also: PMC Retraction Policy

Questioning the HIV-AIDS Hypothesis: 30 Years of Dissent

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Since 1984, when the hypothesis that HIV-causes-AIDS was announced, many scholars have questioned the premise and offered alternative explanations. Thirty years later, competing propositions as well as questioning of the mainstream hypothesis persist, often supported by prominent scientists. This article synthesizes the most salient questions raised, alongside theories proposing non-viral causes for AIDS. The synthesis is organized according to four categories of data believed to support the HIV-AIDS hypothesis: retroviral molecular markers; transmission electron microscopy (EM) images of retroviral particles; efficacy of anti-retroviral drugs; and epidemiological data. Despite three decades of concerted investments in the mainstream hypothesis, the lingering questions and challenges synthesized herein offer public health professionals an opportunity to reflect on their assumptions and practices regarding HIV/AIDS.

"The HIV/AIDS hypothesis is one hell of a mistake", wrote Kary Mullis in 1996 [(1), p. 14]. Mullis – Nobel Laureate in Chemistry, 1993 – and other distinguished scientists have claimed the HIV-causes-AIDS hypothesis is false, unproductive, and unethical. They have done so since 1984, when the hypothesis was proposed. Thirty years after countless studies, resources, and attempts to cure have been poured into the HIV-AIDS hypothesis, it may be fruitful to ask: What happened to those views

and voices that once disagreed? Have the past three decades, with their scientific, technological, and public health developments, been sufficient to convince critics of the hypothesis' value? Have these advances been able to silence the questioning?

Here, I synthesize the main criticisms aimed at the HIV-AIDS hypothesis, alongside select unorthodox[1] theories proposing non-viral cause(s) for AIDS, to argue: far from being condemned to extinction, competing explanations for, and thorough questioning of the mainstream premise persist. Perhaps better known by the lay public than by health professionals, many explanations are, in fact, attracting a growing number of sympathizers. To support the argument, I employ historical research and data synthesis methods. I utilize, as data, trade and professional publications in tandem with authoritative scientific sources.

It is important to note that my purpose is not to review the state of the science regarding HIV/AIDS, nor to persuade readers to reject the mainstream hypothesis. Instead, I aim to expose readers to the persisting controversies, and to motivate them to raise questions of their own. Ultimately, then, this article invites the public health workforce to reflect on prevailing assumptions and practices regarding HIV-AIDS. Reflecting on assumptions and practices represents a central task for public health professionals; a vital step to ensure their (our) practice continually grounds itself in the most rigorous ethical standards (3).

HIV-Causes-AIDS: How Valid are the DATA?

In 1984, Margaret Heckler (then Secretary of the Department of Health and Human Services) announced a retrovirus was the "probable cause" of the alarming immune system collapse emerging in the US since 1981 (4). When scientists identified antibodies to a retrovirus known as LAV, or HTLV-III, in 48 persons (from a sample of 119, with and without immune deficiency symptoms), the retrovirus became the culprit of what would be perceived as "the most urgent health problem facing the country" in recent history [(5, 6), p. 1].

The announcement intended to assure the public: the mystery surrounding this apparently contagious and decidedly fatal illness – later labeled AIDS for acquired immune deficiency syndrome – was solved. The newly identified virus – soon renamed HIV, for human immunodeficiency virus – was, almost certainly, responsible for debilitating people's immune system and making them vulnerable to infections which, before AIDS, were either rare or not particularly dangerous. Now, however, infections such as Kaposi's Sarcoma and *Pneumocistis carinii* Pneumonia had morphed into vicious killers (4, 6). By identifying the perpetrator, scientists' attention and government resources could then focus on treatment, cure, and vaccine development.

Yet almost immediately, scientists who knew a great deal about retroviruses and immunology began to voice misgivings regarding the HIV-causes-AIDS hypothesis, and to question it. They highlighted the difficulties, flaws, and contradictions they saw in the hypothesis, and offered alternative explanations. Many of the original misgivings have survived, and others have been raised, in the past three decades.

In this paper, therefore, I summarize some of these difficulties, and present what critics propose as alternative causes of AIDS. I organize the challenges put forth by unorthodox scholars into four categories of data that support the HIV-AIDS hypothesis[2] : (1) retroviral molecular markers; (2) transmission electron microscopy (EM) images of retroviral particles; (3) efficacy of anti-retroviral (ARV) drugs; and (4) epidemiological data (7, 8). Because these data are proffered as solid evidence for HIV's role in causing AIDS, it is useful to examine how critics question the evidence in each category, specifically.

Retroviral molecular markers

Mainstream scientists and physicians claim the molecular evidence for HIV-as-the-cause-of-AIDS is irrefutable (8, 9) and comprises: (a) HIV antibodies and (b) viral load. As incontrovertible as these molecular markers appear to be, unorthodox scientists have meticulously examined each one and detected significant problems in both (7).

HIV antibodies

The first available tests to screen blood banks for HIV detected HIV antibodies (10). Physicians still use these tests when screening blood for infection and, since 2004, direct-to-consumer home tests have become available for identifying antibodies to HIV using only a saliva sample (e.g., OraQuick) (11). Yet, from the time the first tests appeared, scientists in both orthodox and unorthodox camps reiterated that, according to established immunology principles, antibodies to a virus indicate the immune system has acted to control the invading virus. Antibodies point to previously occurring infection and do not signal active infection. In 1984, CDC scientists (mainstream) wrote:

> *A positive test for most individuals in populations at greater risk of acquiring AIDS will probably mean that the individual has been infected at some time with HTLV-III/LAV [the names originally used for HIV]. Whether the person is currently infected or immune is not known, based on the serologic test alone [(12), p. 378].*

It is not only this simple argument – antibodies suggest the immune system has controlled the invading agents – that unorthodox scientists have debated. The tests themselves remain the target of critic's intense scrutiny. For instance, in 1996 Johnson reported 60-plus factors capable of causing a false-positive result on tests for HIV antibodies [either an ELISA or a western blot (WB) test] (13). Because they react to these factors, the tests may not be detecting HIV at all. Worthy of notice, among the list, are elements ubiquitous among all populations such as the flu, flu vaccinations, pregnancy in women who have had more than one child, tetanus vaccination, and malaria (an important element to consider in the case of the AIDS epidemic in Africa). Supporting each factor, Johnson provides scientifically valid evidence – published in reputable peer-reviewed journals such as *AIDS, the Proceedings of the National Academy of Sciences of the United States of America, The Lancet, the Canadian Medical Association Journal*, and the *Journal of the American Medical Association (JAMA)* (13).

Celia Farber's book, Serious Adverse Events: An Uncensored History of AIDS (14) – an exposé of the epidemic's ethically questionable history – contains an interesting appendix authored by Rodney Richards. Richards – who helped to develop the first ELISA test for HIV – outlines the "evolution" of CDC's stances regarding the role of antibodies, infection, and HIV tests. First, the CDC aligned itself with the traditional view of antibodies signaling past/prior infection (as evidenced in the quote above, from 1984). In 1986, the CDC moved toward a qualified claim, stating:

> *... patients with repeatedly reactive screening tests for HTLV-III/LAV antibody ... in whom antibody is also identified by the use of supplemental tests (e.g., WB, immunofluorescence assay) should be considered both infected and infective [(15), p. 334].*

Finally, in 1987, CDC adopted a non-qualified claim that antibodies signify active infection and/or illness: "The presence of antibody indicates current infection, though many infected persons may have minimal or no clinical evidence of disease for years" [(16, 17), p. 509].

A more specific measure than the ELISA test, the WB detects antibodies by identifying proteins believed to be associated with HIV, and only with HIV. A person undergoes a confirmatory WB after a prior ELISA screening test reacts positively (but it is important to remember: over 60 conditions can yield a false-positive ELISA) (13, 18).

Critics of the orthodox view decry the lack of standardized criteria for a positive result in a WB, across countries, world-wide (19). Bauer (Table 1), in a 2010 article titled “HIV tests are not HIV tests” claims, “no fewer than five different criteria have been used by different groups in the United States” [(18), p.7]. Moreover – adds Bauer – included in the contemporary criteria for a positive WB are p41 and p24, protein–antigens “found in blood platelets of healthy individuals.” This means some of the biological markers being used to “flag” the presence of HIV are not “specific to HIV or AIDS patients [and] p24 and p41 are not even specific to illness.” In other words, healthy persons may test positive on a WB but not carry HIV at all [(18), p. 6].

Table 1

Credentials and professional experience of select critics of the HIV-AIDS hypothesis.

Name (alphabetical order by last name)	Credentials
Henry Bauer, Ph.D.	Professor Emeritus of Chemistry and Science Studies
	Dean Emeritus of Arts and Sciences
	Virginia Polytechnic Institute and State University (Virginia Tech)
James Chin, MD, MPH[a]	Chief of Infectious Disease Section, California State Department of Health Services, Berkeley, CA, USA (1970s–1987)
	Former Chief of Surveillance, Forecasting and Impact Assessment (SFI), Unit of the Global Program on AIDS (GPA) of the World Health Organization Editor: APHA's "Control of Communicable Diseases Manual"
Ettiene de Harven, MD	Emeritus Professor of Pathology: University of Toronto, ON, USA
	Specialized in electron microscopy at the "Institute du Cancer" in Paris
	Published first images of budding virus through EM (1960)
	Member: Sloan Kettering Institute, New York, NY, USA in 1968
	Former President: The Electron Microscopy Society of America (in 1976)
	Former President: Rethinking AIDS
Peter Duesberg, Ph.D.	Professor of Molecular and Cell Biology: The University of California, Berkeley, CA, USA
	Isolated the first cancer gene and mapped the genetic structure of retroviruses (1970)
	Member: National Academy of Sciences (since 1986)
	Outstanding Investigator Award – National Institutes of Health 1986
Heinrich Kremer, MD	Founder and Senior Consultant: Cell Symbiosis Therapy Academy® (based on his work on NO and its association with chronic inflammatory and degenerative disease)
	Collaborating Member: Study Group for Nutrition and Immunity (Bern, Germany)
	Extensive clinical work with youth drug addiction
Kary Mullis, Ph.D.	Nobel Laureate – Chemistry – 1993
	Developed: polymerase chain reaction
	Founder and Chief Scientific Advisor: Altermune
David Rasnick, Ph.D.	Biochemist with >25 years of work with proteases and protease inhibitors
	Former President: Rethinking AIDS: the group for the scientific reappraisal of the HIV hypothesis

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[a] *Chin agrees with the mainstream hypothesis that HIV is the cause of AIDS. His critique centers on the collection and interpretation of the epidemiological data for HIV/AIDS, in the US and world-wide*.

An example may clarify: if tested in Africa, a WB showing reactivity to any two of the proteins p160, p120, or p41, would be considered positive for HIV. In Britain, the test would be positive only if it showed reactivity to one of these three proteins, together with reactions to two other proteins, p32 and p24 (see mention of p24, above, as occurring in healthy individuals). Therefore, someone whose test reacts to p160 and p120 would be considered HIV-positive in Africa, but not in Britain. A test reaction to p41, p32, and p24 would be considered positive in Britain, but negative in Africa, leading author Celia Farber to comment: "… a person could revert to being HIV-negative simply by buying a plane ticket from Uganda to Australia [or in our example, from Uganda to London" (14), p. 163].

According to critics, a definitive answer regarding which protein–antigens are specific to HIV and HIV alone can only come from successful virus isolation and purification. Isolating and purifying "would be required to verify that all of these proteins actually originate from HIV particles" [(7), p. 70]. Attempts at purifying have been made (20, 21), but have been criticized for their ambiguous findings (22), or for their use of cultured samples (see discussion below on EM images). To date, the issue of HIV isolation in purified samples has not been addressed to critics' satisfaction (23).

Viral load The expression "viral load" refers to the quantity of virus found in HIV-infected blood. According to the mainstream perspective, information on viral load helps monitor the infection's progress, "decide when to start treatment, and determine whether or not … HIV medications are working" (24).

The technique for measuring viral load is known as RNA PCR – ribonucleic acid polymerase chain reaction (25). Mainstream scientists regard this test as the most specific documentation of HIV's presence in a person's body. It is often used when the ELISA and WB tests are negative, because PCR can detect the virus' genetic material (or its RNA/DNA fragments), before the human body has had a chance to recognize the virus, produce antibodies in defense, and react positively in an antibodies-only test (26).

Despite its enhanced specificity, many mainstream scientists and practitioners recommend caution when using PCR for screening or diagnosing infection (27). For instance, authors of a study published in JAMA in 2006, in which PCR was used with a sample of almost 3,000 people, concluded: "The PCR assay is not sufficiently accurate to be used for the diagnosis of HIV infection without confirmation" [(28), p. 803].

PCR technology evolved quickly since it was introduced in 1983 (25). Although being employed, mostly, for assessing viral load (less for screening and diagnosis), it should give us pause to learn, however, that Dr. Kary Mullis – the scientist who won the 1993 Nobel Prize for inventing the PCR test and whose quote introduced this article (Table 1) – has strongly opposed using the technique for determining the amount of virus circulating in plasma. Lauritsen explains:

> *Kary Mullis … is thoroughly convinced that HIV is not the cause of AIDS. With regard to the viral-load tests, which attempt to use PCR for counting viruses, Mullis has stated: "Quantitative PCR is an oxymoron." PCR is intended to identify substances qualitatively, but by its very nature is unsuited for estimating numbers. Although there is a common misimpression that the viral-load tests actually count the number of viruses in the blood, these tests cannot detect free, infectious viruses at all; they can only detect proteins that are believed, in some cases wrongly, to be unique to HIV. The tests can detect genetic sequences of viruses, but not viruses themselves [(29), p. 3].*

If to this picture we add human endogenous retroviruses (or HERVs) (30) as potential confounders, the genetic sequences detected in a PCR test may not be those from an exogenous virus, at all, and may explain the test's substantial false-positive rates (18, 27). HERVs consist of retrovirus-like particles produced by host cells that are stressed or dying. In other words, when various infections assail the body, and certain cells experience stress or die in large numbers, they can manufacture by-products similar to retroviruses. These by-products can be reactive when testing for HIV antibodies, protein antigens, and viral loads (31). Culshaw summarizes it well:

> *A retrovirus is nothing more than RNA with an outer protein shell. The shell enables it to bind to cells of the type it infects, and once it gains entry, the outer coating disappears and the RNA is transcribed to DNA and incorporated as provirus into the host cell's own genome. It is for this reason that retroviruses are called enveloped viruses, and it is also the reason that it is very difficult to distinguish between exogenous retroviruses (those that originate outside the body from a foreign invader) and endogenous retroviruses (those that are manufactured from our own retroviral-like genetic sequences under conditions of cellular stress, including diseases) … Much of the genetic material attributed to HIV is in fact DNA or RNA from [these] decaying cells (…) Human beings are filled with such endogenous retroviruses [(32), pp. 53, 55–56].*

Transmission electron microscopy images of retroviral particles

Although it seems intuitive that photographing HIV would provide undeniable evidence of its presence in the host's plasma, the reality is much more complex. Adequately interpreting images obtained through EM is, even for the most skilled scientists, challenging. EM generates highly amplified images of cells and viral particles. An electron-microscope uses "beams of electrons focused by magnetic lenses instead of rays of light" to produce images magnified up to 10,000,000× (a light microscope has difficulty exceeding 2000× magnification) (33).

The first images of what researchers believed to be HIV particles budding out of human cells were published in the journal Science, in 1983, by the French team that co-discovered HIV (headed by Luc A. Montagnier) (34). These images, and the computer graphics based on them, were printed in textbooks and articles discussing AIDS, extensively. Despite their popularity, the images were obtained from a "pre-AIDS" patient (not a patient with AIDS), and the sample furnishing the images had not been purified according to standard procedures (35).

It would be 14 years later, in 1997, when EM images from purified samples were produced (20). Yet another study (22), published simultaneously with these images (in fact, printed as an adjoining article), reported: even purified HIV samples harbor protein particles (called microvesicles), considered to be contaminants. These microvesicles do not disappear during the purifying process. In other words, even when technicians purify HIV samples, certain "cellular proteins bound to non-viral particles (i.e., microvesicles) can copurify with [the] virus," and appear in the EM images. The question, then, remains: are the EM images seen in these purified samples, pictures of HIV itself, or of other elements/particles? (36).

In 2010, Ettiene de Harven – the scientist who "produced the first electron micrograph of a retrovirus (the Friend leukemia virus)" [(32), p.13] through EM research in 1960 (Table 1) (37) – added to the debate:

> *All the images of particles supposedly representing HIV and published in scientific as well as in lay publications derive from EM studies of cell cultures. They never show HIV particles coming directly from an AIDS patient [(7), p. 70 – emphasis added].*

Why is it important to obtain EM images of HIV from AIDS patients, as opposed to images of HIV cultured in a laboratory? According to de Harven, non-viral micoorganisms frequently contaminate cell cultures and show up very easily in EM. It is quite difficult to obtain absolutely pure cell cultures, especially because the culturing process itself – the growth factors added to the culture, such as "T cell lymphocyte growth factor (TCGF), interleukin 2, or corticosteroid hormones" [(23), p. 4] – can introduce potential contaminants. HERVs, for example, are often generated by cells that have been stressed or hyperstimulated to grow in cultures. HIV cultures obtained from patients with AIDS may not require as much stimulation or addition of growth factors, thus resulting in less contaminated, purer cultures.

Montagnier also acknowledges the problems with relying on EM to identify a retrovirus, given the difficulties with purifying viral samples. In an interview given in 1997, he reflects on those first HIV images from cultured samples, produced in his laboratory at the Pasteur Institute:

> *DT (Djamel Tahi): Why do the EM photographs published by you, come from the culture and not from the purification?*
>
> *LM (Luc Montagnier): There was so little production of virus it was impossible to see what might be in a concentrate of virus from a gradient. There was not enough virus to do that …*
>
> *(…)*
>
> *DT: How is it possible without EM pictures from the purification, to know whether these particles are viral and appertain to a retrovirus, moreover a specific retrovirus?*
>
> *LM: Well, there were the pictures of the budding. We published images of budding which are characteristic of retroviruses. Having said that, on the morphology alone one could not say it was truly a retrovirus … (38).*

It appears, therefore, there is little consensus regarding what the existing EM images reflect: are the visualized particles HIV or something else? According to Papadopulos-Eleopulos and colleagues, "some of the best known retrovirologists including Peter Duesberg, Robert Gallo, and Howard Temin have been telling us that particles may have the morphological characteristics of retroviruses but are not viruses" [(39), p. 2]. It is feasible, therefore, that EM images are, in fact, depictions of (a) microvesicles (or protein particles), not viral or infectious in nature, but not eliminated even when using purified samples (22); or (b) human endogenous retroviruses – defective, non-infectious retroviruses associated with the host's own genome (see discussion above on HERVS).

Efficacy of anti-retroviral drugs

From the epidemic's onset, researchers worked relentlessly to find a vaccine to keep the virus from spreading and to develop drugs for managing the symptoms from opportunistic infections (40). The challenges inherent in developing both vaccine and treatment were daunting: post-infection, HIV appears to mutate and recombine continually, thus making it difficult to design an effective vaccine (41, 42). Furthermore, designing treatments for a retrovirus is a tricky feat, given it shares many of the same characteristics of the host's immune cells – thus, an attack on the virus can become a simultaneous attack on the healthy host cells (14, 32, 35).

After the public announcement regarding the probable cause of AIDS, various pharmaceutical companies tried to develop drugs to thwart the action of the virus' reverse transcriptase enzyme (an enzyme essential for the replication of retroviruses). AZT became the first medication of this kind, approved specifically for treating AIDS patients in 1987 (43). Azidothymidine (AZT) – also known as

Retrovir, a drug originally designed, but proven unsuccessful, for treating leukemia – made history not only because it was the first available treatment specifically for AIDS, but also due to how quickly it was approved: AZT received "investigational new drug (IND) status (initial approval for testing) within 5 days of application" [(44), p. 134]. Given the desperate need for specific treatment, the drug's placebo-controlled trials also moved fast, lasting "only 6 months before approval was given for general sale" [(44), p. 134]. Phase II trials were interrupted, mid-way, due to findings that fewer patients taking AZT were dying of AIDS when compared to the control group not taking the drug (44, 45).

Approving AZT, however, did not prevent scientists from trying to develop other drugs, during the following decade; but most attempts would make little headway into the treatment of AIDS. Adding to these difficulties, AZT was proving to be extremely toxic and not as effective as initially anticipated. Researchers did learn, meanwhile, that prescribing AZT in lower dosages and in combination with other, well-known drugs such as heparin, acyclovir, and bactrim, was beginning to curb mortality rates (44).

Thus, in the mid-90s "combination therapy" became available. Also referred to as the "drug cocktail," combination therapy comprised a joint attack on HIV using three main classes of drugs, simultaneously: (a) those inhibiting reverse transcriptase's ability to duplicate the virus' genetic material using host DNA sub-divided into two classes – nucleoside and non-nucleoside inhibitors; (b) protease inhibitors (designed to limit certain proteins needed for HIV assembly); and (c) myristoylation or entry/fusion inhibitors (blocking the virus from entering the host cells). These three classes of drugs – known collectively as HAART (highly active ARV therapy) or antiretrovirals (ARVs) – have been praised for their ability to restore the health of patients with AIDS who become extremely ill [(24, 44, 46), p. 240].

Antiretrovirals also are praised for their ability to reduce patients' viral loads and, therefore, their level of infection and ability to transmit the virus (or infectivity). This reduction in viral load has been deemed so significant that, in 2012, the FDA approved using one of the combination drugs (Truvada) for pre-exposure prophylaxis or PrEP (47).

PrEP or "HIV treatment-as-prevention" (48) involves administering to non-infected persons one pill of the antiretroviral, daily, to stave off infection: an initiative crowned *Breakthrough of the Year* by the journal Science, in 2011 (47). Trials conducted world-wide have consistently demonstrated low rates of HIV infection among people taking PrEP (41, 48). The 2011 breakthrough, therefore, was the conclusion: "The early initiation of ARV therapy reduced rates of sexual transmission of HIV-1 and clinical events, indicating both personal and public health benefits from such therapy" [(41), p. 493].

Yet, as with most treatment drugs, ARVs also produce important side-effects. Even mainstream scientists who praise the drugs by saying, "Combination theory [*sic*] was a miracle, comparable with antibiotics, anesthesia, and the polio vaccine in the annals of the history of medicine … a 'quantum leap"' – candidly admit: "The miracle was not without complications." [(44), pp. 246, 247]. Because these drugs also attack non-infected cells, they can destroy the immune systems' healthy T-cells, and even cause a collapse identical to AIDS. Authors of a study reporting on the first decade of ARV use concluded,

> *The results of this collaborative study, which involved 12 prospective cohorts and over 20,000 patients with HIV-1 from Europe and North America, show that the virological response after starting HAART has improved steadily since 1996. However, there was no corresponding decrease in the rates of AIDS, or death, up to 1 year of follow-up. Conversely, there was some evidence for an increase in the rate of AIDS in the most recent period [2002–2003] [(49), p. 454 – emphasis mine].*

Critics' concerns center on the potential association between use of HAART and a depressed immune system. This association carries significant implications for the prophylactic use of ARVs. For instance, studies have documented patients' compromised immune systems as *preceding* their seroconversion (50, 51). Therefore, having non-infected persons take HAART as prophylaxis may, over time, impact their immune systems negatively, and predispose them to becoming infected with various agents, including HIV itself. Moreover, there is evidence that ARVs can accelerate aging of cells in ways that promote progressive multi-organ disease (52). Critics also point to data on patients taking ARVs who develop *Pneumocystis Carinii*, and *Candida albicans* (opportunistic infections typical of patients with AIDS) while on the drugs, despite the fact the protease inhibitors have "marked anticandidal and antipneumocystis effects" [(7), p. 71]. Equally vexing, are the deaths among ARV-treated patients, resulting from acute liver failure. These deaths point to the ARVs' detrimental effects, given that HIV, itself, does not cause liver toxicity (7, 53, 54).

Critics also highlight studies documenting the reduction of plasma HIV RNA among patients treated with ARVs, but the non-reduction in HIV DNA, suggesting there is "continued expression of viral agents" even after 1 year of treatment [(55), p. 320]. Compounding these difficulties are the often debilitating side effects (45), the drugs' extremely high costs (AZT alone cost around $6,000 a year and the cocktails can easily tally $12,000 – 13,000 a year per patient) [(44), pp. 245–246] and the oftentimes daunting regimen some prescriptions require, leading to patients' less-than-optimal compliance during treatment.

Despite this host of problems, orthodox scientists and practitioners still claim HAART has changed the face of the AIDS epidemic: once considered a lethal syndrome, testing positive for HIV does not equate to a death sentence any longer; merely to a lifetime of managing a chronic infection (56, 57). Critics, on the other hand, assert: because the drugs are anti-viral and anti-bacterial in nature, they give a false impression of being effective for treating HIV infection. What appears a miraculous recovery in many patients is, in fact, the drugs' effects upon the opportunistic infectious agents the person may harbor at the time, other than HIV. Contrary to the reigning enthusiasm for ARVs' effectiveness for prevention and treatment, critics will argue the risks associated with ARVs appear to outweigh the benefits, especially if these drugs are consumed over long periods of time. In short, unorthodox scholars believe the appearance of effectiveness of ARVs does not represent strong evidence for the role of HIV in AIDS and, in a paradoxical manner; ARVs may actually be the cause of AIDS-defining illnesses and non-AIDS-defining ones.

Epidemiological data

It is easy to obtain current statistics describing the HIV-AIDS distribution, world-wide. One has only to access the website of the Joint United Nations Program on HIV to learn: "In 2012, there were 35.3 million [32.2–38.8 million] people living with HIV" and that, in the same year, "1.6 million [1.4–1.9 million] people died from AIDS-related causes worldwide compared to 2.3 million [2.1–2.6 million] in 2005" (58).

Scholars on both sides of the debate agree: "epidemiologic studies and data can show only that a risk factor is statistically associated (correlated) with a higher disease incidence in the population exposed to that risk factor" [(59), p. 42]. Epidemiological data do not provide evidence for causation. All the data can do is reveal risk factors and illness co-occurring in a given group. Despite this well-known caveat, mainstream scientists argue that because HIV has spread among high-risk groups as expected, the AIDS epidemic has, indeed, a viral, infectious agent: its "epidemic curves resemble … such infectious agents as hepatitis B and genital herpes viruses" [(59), p. 53]. These scientists also will

explain the differences observed in the frequency of certain illness in specific geographic regions (e.g., higher numbers of HIV-related Tuberculosis in sub-Saharan Africa) as caused by the "background flora of infectious disease agents" present in these regions [(59), p. 54].

Curiously, however, even among mainstream scholars who believe epidemiological data constitute valuable evidence of a viral cause for AIDS, there are those who have turned a critical eye toward the data the US and the WHO have compiled. James Chin – one such critic (Table 1) writes in his book, *The AIDS Pandemic: The Collision of Epidemiology with Political Correctness*:

> *Estimation and projection of HIV infections and AIDS cases and deaths (HIV/AIDS) can be considered more of an art than a science because of the marked limitations of both available data and methods for estimation and projection. These limitations make it possible for UNAIDS and other AIDS program advocates and activists to issue misleading and inflated estimates and projections [(59), p. 137].*

The questions regarding the validity and reliability of epidemiological data emerging from within the mainstream/orthodox views have been echoed and amplified by unorthodox scholars. Both camps' concerns center on four problems plaguing the estimates of incidence (new cases), prevalence (remaining cases), and projection (future cases) of HIV infections, AIDS diagnoses, and AIDS-related deaths: (a) the varying clinical definitions of AIDS (the official definition has changed four times since 1982) (60); (b) variability in the criteria for seropositivity in HIV tests; (c) the absence of testing in many regions of the world (many developing countries do not have the laboratories needed to test every single AIDS case); and (d) the mistakes in estimation, data management and reporting (e.g., the revision of projections for year 2006 by UNAIDS) (59–62).

This article's space limitations do not allow an expanded treatment of each problem-area, but readers can find further details within the works cited. For instance, in Rebecca Culshaw's book – *Science Sold Out: Does HIV Really Cause AIDS* (32) – readers will find 13 "failed predictions" regarding the spread of HIV and AIDS, including the prediction that HIV infection would spread randomly among populations (i.e., outside specific risk groups). Culshaw also tells her personal story of having written a master's thesis, received a Ph.D. based on her work with "mathematical models of the immunological aspects of HIV infection," and eventually concluding "there is good evidence that the entire basis for this theory is wrong" [(32), p.7].

Unorthodox Theories: If not HIV, Then What?

If the criticisms outlined above pinpoint significant problems with each type of data used to support the HIV-AIDS hypothesis, they only contribute to deconstructing the hypothesis, not to providing explanations for what might cause AIDS if not a retrovirus. However, alternative hypotheses abound. Anchoring themselves in well-established causes of immune system malfunction, these hypotheses point to pharmacological (drug) factors, immune dis-balance factors, latent infection overload, and malnutrition as culprits.

Although several scientists investigated the role drugs might play in causing immune suppression *before* HIV was identified [see a list of these studies in Duesberg et al. (46)], the main proponent of the drug-AIDS hypothesis in the epidemic's early years was Peter Duesberg, a professor of Molecular and Cell Biology at UC Berkeley. According to Seth Kalichman, who wrote *Denying AIDS* (a harsh critique of unorthodox views and of Duesberg in particular), "In every respect, HIV/AIDS denialism starts and ends with Peter Duesberg" [(63), p. 175]. Duesberg's arguments gained notoriety among unorthodox

theories not only due to his expertise and prominence (see Table 1), but also to his challenge of the medical and scientific establishments early in the history of the epidemic, employing clear empirical logic.

Duesberg began challenging the viral hypothesis for AIDS soon after the publication (in 1984) of the four seminal articles pointing to HIV as the "probable" cause (64–67). In two key publications in 1987 and 1989 – in *Cancer Research* and in the *Proceedings of the National Academy of Sciences* (68, 69) – Duesberg cogently argued: retroviruses are not known for killing cells. In other words, retroviruses are not "cytocidal." If anything, retroviruses were once thought to be associated with cancer because they cause precisely the opposite of cell death; they contribute to cells' growth or proliferation. In Duesberg's words, "… retroviruses are … considered to be plausible natural carcinogens because they are not cytocidal and hence compatible with neoplastic growth and other slow diseases." [(68), p. 1200]. In his view, HIV's inability to kill cells could not explain the suppression of the T-cells in the immune system, as proposed by the teams who discovered HIV[3]. According to Farber,

> *In other fields, such as gene therapy, it is axiomatic that retroviruses are the ideal carriers for genetic materials, because they 'don't kill cells'. Incredibly, this is where the so-called HIV debate first forked in 1987, and where the camps remain bitterly divided to this day [(14), p. 50].*

For Duesberg and scientists agreeing with him, then, other agents would have to be responsible for the disastrous immune function collapse seen in AIDS patients. These scientists saw as prominent among such causes, the use of drugs, both recreational and routinely prescribed ones. As author Gary Null points out, even before AIDS, researchers were documenting the immune-suppressing effects of amyl nitrites or "poppers" (the form of amyl nitrites popular among gay men in the early and mid-80s) and determining both their toxicity and carcinogenic properties in humans and animals (45). However, two studies CDC published in 1983, one in which they were unable to detect any toxicity from amyl nitrites, the other, unable to document a significant association between inhaled nitrates and Kaposi's sarcoma or *Pneumocystis carinii* pneumonia, led the search to a halt (70, 71). Investigators later tried to determine if certain *batches* might have been contaminated with toxic agents but, when they found no contamination, the focus on poppers/amyl nitrites themselves ceased (1). Nonetheless, in 1998 Duesberg and Rasnick (Table 1) (72) reviewed evidence published since 1909, "which prove[s] that regular consumption of illicit recreational drugs causes all AIDS-defining and additional drug-specific diseases at time and dose-dependent rates" [(46), p. 393].

Other drugs such as those given to transplant patients to prevent organ rejection, as well as routinely prescribed antibiotics, also have been implicated as potential causes of immune dysfunction. Studies have shown that transplant patients who develop Kaposi's sarcoma will go into remission, once taken off the drugs required to avoid organ rejection. Immune-suppressing drugs (as well as amyl nitrites) have, for instance, been directly correlated with Kaposi's sarcoma, the rare skin cancer found frequently among AIDS patients during the epidemics' early days [see reviews by Null (45) and Kremer (35)].

Anti-retroviral drugs used to treat HIV infection/disease, also, are indicted by Duesberg and those who agree with him as potentially causing AIDS (43, 62). Because the drug cocktails include "DNA chain-terminators and protease inhibitors" that affect healthy cells as well as the virus, and because "many studies find that people receiving ARV medications experience AIDS-defining diseases to a greater extent than controls not receiving those medications" [(73), p. 122], antiretrovirals are viewed as potential immune suppressors.

In a review of the chemical bases for AIDS, published in 2003, Duesberg and his colleagues (46) outlined the epidemiological and bio-chemical evidence supporting different causes for the AIDS epidemics in the US/Europe and in Africa, none of which are viral or contagious. The authors concluded:

> *The chemical-AIDS hypothesis proposes that the AIDS epidemics of the US and Europe are caused by recreational drugs, alias lifestyle, and anti-HIV drugs … and by other non-contagious risk factors such as immunosuppressive proteins associated with transfusions of blood clotting factors … pediatric AIDS is due to prenatal consumption of recreational and anti-HIV drugs by unborn babies together with their pregnant mothers … The chemical basis of African AIDS is proposed to be malnutrition and lack of drinkable water … exactly as proposed originally by the now leading HIV-AIDS researchers Fauci and Seligman: "The commonest cause of T-cell immunodeficiency worldwide is protein-calorie malnutrition" … and others … [(46), p. 392].*

Alongside a drug hypothesis, another proposed cause for AIDS is the iNOS hypothesis, or immune dis-balance hypothesis. In his book, *The Silent Revolution in Cancer and AIDS Medicine*, Kremer (35) (Table 1) explains that much of what scientists now know about the immune system and its functions was not well understood at the time they identified HIV. In particular, the research on NO, or nitric oxide, was still in its infancy: NO is "an important intracellular and intercellular signaling molecule" acting as "…an important host defense effector in the immune system" [(74), p. 639]. Even though NO (and its derivative iNOs) is "involved in the regulation of diverse physiological and pathophysiological mechanisms in cardiovascular, nervous, and immunological systems," researchers have shown it can also become a harmful, "cytotoxic agent in pathological processes, particularly in inflammatory disorders" [(74), pp. 639–640]. Put simply, at adequate levels NO helps regulate blood pressure as well as "wound repair and host defense [*sic*] mechanisms" [(75), p. 277]. Excessive amounts, however, lead to T-cell depletion, "inflammation, infection, neoplastic diseases [cancer] liver cirrhosis, [and diabetes" [(75), p. 277]. This change from adequate-to-excessive amounts of NO in the human body results from multiple factors, including "nitrite inhalation [e.g., using 'poppers'], microbial antigen, and toxin stimulation [e.g., suffering repeated infections with different viruses/bacteria], immunotoxic medications [e.g., taking ARVs and antibiotics], [and] many other stress factors" [(35), p. 49].

A closely related perspective, placing the blame for AIDS on bio-chemical processes gone awry within human cells is the oxidative stress (or redox) hypothesis. Oxidative stress is a cellular-level electro-chemical phenomenon that diminishes a cell's ability to absorb oxygen. This diminished capacity to process oxygen at optimal levels leads to the cell's disruption and death. Scientists have either hypothesized or empirically connected oxidative stress to many diseases, including type 2 diabetes and cancer (35, 45, 76). According to this hypothesis' main proponents,

> *At first sight it appears that there is no common factor, apart from HIV infection, linking the various AIDS risk groups. However, homosexuals are exposed to relatively high levels of nitrites and anally deposited sperm, drug abusers to opiates and nitrites, hemophiliacs to factor VIII. All these are known potent oxidizing agents … [(77), p. 147 – emphasis mine].*

For these proponents of the redox hypothesis even Luc Montagnier (the head of the French team that discovered HIV) agrees "that anti-oxidants should be used for treatment of HIV/AIDS patients" [(78, 79), p. 6].

Viewing a person's immune system as a complex dynamic balancing act among various elements, which sometimes behave as defenders, other times, as offenders, is also consistent with the "latent infection overload hypothesis" proposed by Kary Mullis (Table 1). According to Mullis, as people

become infected with multiple viruses and experience many latent infections, the immune system embarks on a chain-reaction-response to each virus. Latent infections are those without visible symptoms, and according to Mullis, "at a given time most viral infections in an individual are latent" [(80), p. 196]. Eventually, the system overloads itself and becomes dysfunctional. AIDS, he says, "may be the result of such a chain reaction." This hypothesis assumes:

> *... there is not a single organism that is the cause of AIDS, and there should exist AIDS patients who do not test positive for HIV*[4] *. It is an overwhelming number of distinct organisms, which causes the immune dysfunction. These may individually be harmless [(80), p. 197].*

Perhaps the most intriguing alternative hypothesis, however – if not from its bio-chemical perspective, at least from the perspective of who supports it – is the one proposing HIV may not be the primary villain, but merely an accomplice in causing AIDS (83). Joseph Sonnabend – a prominent physician/researcher responsible for encouraging his gay patients to lead a healthy lifestyle to avoid developing AIDS, and one who "did not accept HIV = ADS theory for many years" – recently changed his views and "has come to think that HIV, together with other factors, may play a subsidiary causative role" [(73, 84), p. 120]. Even Montagnier and Gallo (leaders of the French and American teams, respectively, that discovered HIV), at various times since the epidemic began, have suggested HIV might be a co-factor in AIDS, not its exclusive causative agent (85).

Other hypotheses have been proposed over the years, but none have garnered as much attention as those outlined above. Some of these other hypotheses claim AIDS is caused by (a) multiple factors; some factors explaining some cases, other factors accounting for other cases; (b) undiagnosed or untreated syphilis infection; (c) autoimmunity; (d) selenium deficiency, and (e) psychological factors, including stress and trauma [see Bauer (73), pp. 124, 136–139 for details on these hypotheses].

The positive or reassuring aspect of these alternative hypotheses is the tangible hope for prevention, treatment, and cure they embody. Nevertheless, it is difficult not to agree with Bauer when he concludes, "…it is hardly reassuring that this array of suggestions has been in circulation for something like (three) decades without having been adequately explored" [(73), p. 139].

Discussion

At this point, readers might be wondering: given the problems with the mainstream hypothesis, how did we get here? How did we come so far, tethered to such a problematic perspective? The complexity of the answers to these questions aside, it may help to bear in mind the notion that HIV-causes-AIDS emerged and developed within a very specific scientific-cultural-historic context. Although the scope of this article precludes dealing with this complex context, for our purposes it is important to recall at least one element: Funding for President Nixon's War on Cancer campaign ended in 1981 with very little achieved in the quest for an infectious cancer agent (15, 85–87). The only exception was the discovery connecting select retroviruses to a few, rare cancers. Other than this, scientists had a handful of "orphaned" viruses which, they suspected, might play a role in causing illnesses, but no known diseases to which these viruses could be connected. Proposing a connection between an emerging syndrome and one of these viruses (even if only a circumstantial connection) proved enticing enough to pursue. And pursue they did, as soon as AIDS began to appear in larger-than-expected numbers among otherwise healthy adults.

If viewed from this perspective, then, why scientists so quickly and assuredly "jumped on the HIV bandwagon" may not be very difficult to understand. That the scientific establishment world-wide insistently refuses to re-examine the HIV-AIDS hypothesis, however, is more difficult to accept, especially when one examines the credentials of those proposing such a revision. Their expertise

carries as much weight as the teams who defend the orthodox hypothesis (Table 1). Seth Kalichman, a critic of AIDS "denialists," recommends adamantly: anyone who entertains alternative views should "consider the source: credibility of where the article is reported as well as the researchers themselves must be weighed" [(63), p. 159]. I could not agree more: taking into account the credibility of the scholars who question the HIV-AIDS hypothesis is, perhaps, the strongest argument *in favor* of seriously considering their critiques, not against it.

Furthermore, credibility as an argument works both ways: if to question the trustworthiness of unorthodox scholars is vital, it is equally crucial to question the reliability of those supporting the HIV-AIDS hypothesis. Readers who care to learn about HIV-AIDS' history will encounter ethically questionable actions carried out by some of the most notable orthodox researchers, as well as ethical misconduct charges made against them [for an extensive treatment of these ethical and legal issues, backed by extensive official documentation, see Crewdson (88)].

If it is difficult to dismiss the unorthodox views due to the credibility of their sources, then, why are not orthodox scientists and practitioners more willing to rethink the hypothesis or, at the very least, test the unorthodox arguments in a scientific, open debate? Although there have been, in fact, several attempts to engage the orthodox community in dialog, nearly all have been unsuccessful [for examples, see Ref. (14, 85, 88)]. Most likely, reasons for denying the calls to re-examine the orthodox stance lie in the complex, synergistic dynamics within the scientific, medical, economic, and political systems or ideologies worldwide. Even brief speculation about these reasons would exceed the scope of this article, therefore I refer the reader, once again, to the sources referenced [in particular, see Epstein (89) and Bauer (73)].

Here I would argue, nonetheless, that the debate between orthodox and unorthodox scientists comprises much more than an intellectual pursuit or a scientific skirmish: it is a matter of life-and-death. It is a matter of justice. Millions of lives, worldwide, have been and will be significantly affected by an HIV or AIDS diagnosis. If we – the public health workforce – lose sight of the social justice implications and the magnitude of the effect, we lose "the very purpose of our mission" [(3, 90), p. 690].

In particular, a pressing concern for public health is the move or push toward (a) HIV screening for "patients in all health-care settings" (with opt-out screening) (91) and (b) placing persons-at-risk (even if not yet infected with HIV), on retroviral medication as a form of prophylaxis (see discussion about PrEP, above) (92). If in 1986 the CDC recommended voluntary testing for people in high-risk groups, in 2013 the U.S. Preventive Services Task Force "gave routine HIV screening of all adolescents and adults, ages 15–65, an 'A' rating" [(93), p. 1]. The recently approved Affordable Care ACT "requires or incentivizes new private health plans, Medicare, and Medicaid to provide preventive services rated 'A' or 'B' at no cost to patients" [(93), p. 1]. Thus, routine screening of every adolescent and adult in all populations is, now, the goal (91, 94).

If, to this goal we juxtapose the problems with the HIV tests, with the definition(s) of AIDS, and with the toxicity of the ARVs currently prescribed, we begin to understand the potential for harm inherent in them. Put blatantly: these recommendations can be harmful or iatrogenic (95).

Public health workforce: Our role

What can the public health workforce do, given such potential for harm? As stated in the introduction, this paper represents a call to reflect upon our public health practice vis-à-vis HIV-AIDS. Reflecting upon and questioning the *status quo* constitute important dimensions of public health professionals' competencies and practice. If the only hope the HIV-AIDS hypothesis can offer, 30 years later, is to provide highly toxic drugs to treat HIV infection and to prevent high-risk but healthy persons from becoming infected, health promoters have a professional duty to reflect on the available data and

question the usefulness of the hypothesis. Only in doing so can public health professionals maintain their professional integrity, tend to public health's roots in social justice, and contribute to developing knowledge using ethical methods.

James Jones, in his book *Bad Blood: The Tuskegee Syphilis Experiment* (96), reminds us poignantly that not asking whether health professionals "should be doing" something, but continuing to do it uncritically, because "it can be done" was, ultimately, the mind-set sustaining the Tuskegee syphilis study for 40 years – unquestionably one of the worst cases of scientific misconduct in American history. The AIDS epidemic – if managed without questioning or without the dialogical process of action-reflection – may, with time, overshadow Tuskegee in the magnitude of its negative impact.

Specifically, I propose the public health workforce can undertake such an action-reflection process by engaging in the following tasks:

(1) Learning about the history of the HIV/AIDS epidemic, of the problems surrounding the discovery of HIV, and about the development of drug therapies and PrEP. Publications recording this history abound in the professional and trade literatures, representing both mainstream and unorthodox view-points. To understand the forces shaping the HIV/AIDS epidemic, we currently experience represents a crucial responsibility of a competent and ethics-driven workforce.

(2) Conducting its own research to test alternative theories for the cause(s) of AIDS and/or to portray the inconsistencies and contradictions in the orthodox hypothesis. Qualitative inquiry, for instance, exploring unorthodox views and the practices of providers, patients, and scientists, might be a fruitful option for challenging prevailing assumptions.

(3) Fostering and mediating a debate among HIV-infected persons, scientists, and health-care providers, to critically assess current beliefs and practices. Public health professionals – who are well-informed about the orthodox and unorthodox perspectives' strengths and weaknesses – could play an important role as facilitators in this much-needed dialog.

Although carrying out the tasks outlined above may represent a novelty for many public health professionals, for the scientists, practitioners, and investigators who believe a viral hypothesis for AIDS is unproductive, none of this is new. They have combed historical documents (or played a role in the history, themselves); they have amassed substantial amounts of data, and they have made numerous calls for debate. They have held to their beliefs, steadfastly, for the past 30 years. Twenty four years after the first article challenging HIV, Duesberg and colleagues, for instance, still claimed HIV is only a "passenger virus" (one "not sufficient and not necessary to cause a disease") [(62), p. 81]. While not all unorthodox scholars agree with Duesberg, most still actively defend their critiques of the HIV-AIDS hypothesis and persist in their questioning. As we face the next decade with AIDS still rampant, then, it becomes vital that public health professionals attend to the debate and embark in a questioning of their own.

Conflict of Interest Statement

The author declares that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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Footnotes

[1]In this article, I will use the terms unorthodox, non-orthodox, non-mainstream, and alternative, to refer collectively to those who disagree with the prevalent view, and to their propositions (despite their variability). I will favor the term "unorthodox" for it carries the notion of intention or willful deviation from the norm and connotes a power differential in which one set of theories (the orthodox or mainstream) dominates another – what Delborne calls "the epistemological tyranny of the intellectual majority" [(2), p. 510].

[2]I am indebted to E. de Harven (7) for suggesting these categories.

[3]In fact, evidence supporting the notion "HIV kills T-cells" has been so conspicuously absent that, currently, scientists don't believe HIV "kills T-cells in any way. Rather, they believe HIV primes T-cells to commit suicide at some later time" [(32), p. 73]

[4]Some would argue this is the strongest evidence against the HIV-AIDS hypothesis: cases of AIDS with no documentable presence of HIV. However, say the critics, the difficulty with this argument lies in the definition of AIDS: because AIDS is defined as "the final stage of HIV infection" (81), AIDS presupposes infection with HIV, making the definition a circular one (i.e., AIDS = final stage of HIV infection = opportunistic infections + high viral load + low CD_4 counts). Due to the circularity in the logic, if there is no HIV, there can be no AIDS. Nonetheless, cases of patients with AIDS-defining opportunistic infections and low CD_4 counts without HIV do exist (see, for example, the review by Green and colleagues (82).

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Everyone In Iceland Can Get Tested For The Coronavirus. Here's How The Results Could Help All Of Us.

The small island nation's large-scale testing strategy includes people who don't have any symptoms.

Posted on March 18, 2020, at 4:53 p.m.


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As countries around the world scramble to fight back the spread of the coronavirus, Iceland is doing things a little differently from the rest — and the approach could have a much larger impact on our understanding of the virus.

The small island nation of 364,000 is carrying out large-scale testing among its general population, making it the latest country to put aggressive testing at the heart of its fight against the pandemic.

But — crucially — the testing also includes people who show no symptoms of the disease.

Iceland's government said it has so far tested a higher proportion of its citizens than anywhere else in the world.

The number of individuals tested by the country's health authorities and the biotechnology firm deCode Genetics — 3,787 — roughly translates to 10,405 per million, which compares to about 5,203 in South Korea, 2478 in Italy, and 764 in the UK.

"Iceland's population puts it in the unique position of having very high testing capabilities with help from the Icelandic medical research company deCode Genetics, who are offering to perform large scale testing," Thorolfur Guðnason, Iceland's chief epidemiologist, told BuzzFeed News.

"This effort is intended to gather insight into the actual prevalence of the virus in the community, as most countries are most exclusively testing symptomatic individuals at this time."

Of 3,787 individuals tested in the country, a total of 218 positive cases have been identified so far. "At least half of those infected contracted the virus while travelling abroad, mostly in high-risk areas in the European Alps (at least 90)," the government said on Monday.

Those numbers include the first results of the voluntary tests on people with no symptoms, which started last Friday. The first batch of 1,800 tests produced 19 positive cases, or about 1% of the sample.

"Early results from deCode Genetics indicate that a low proportion of the general population has contracted the virus and that about half of those who tested positive are non-symptomatic," said Guðnason. "The other half displays very moderate cold-like symptoms."

"This data can also become a valuable resource for scientific studies of the virus in the future," he added.

Marco Sabadin / Getty Images

Italian soldiers patrol by a checkpoint at the entrance of the small town of Vo Euganeo, situated in the red zone of the coronavirus outbreak in northern Italy.

Mass testing on the scale adopted in Iceland is unlikely to be feasible across larger countries. However, it has proved crucial in some of the other areas hardest hit by the novel coronavirus so far. The testing has provided evidence revealing that a significant portion of those who catch the disease do so with no or mild symptoms — and confirmed multiple pieces of research that have shown that asymptomatic individuals contribute to the transmission of the disease in great numbers.

In the small northern Italian town of Vo, one of the communities where the outbreak first emerged, the entire population of 3,300 people was tested — 3% of residents tested positive, and of these, the majority had no symptoms, researchers said.

The population was tested again after a two-week lockdown and isolation. Researchers found that transmission was reduced by 90% and all those still positive were without symptoms and could remain quarantined.

Luca Zaia, the governor of the Veneto region told Italian media this week: "We tested everyone, even if the 'experts' told us this was a mistake: 3,000 tests. We found 66 positives, who we isolated for 14 days, and after that 6 of them were still positive. And that is how we ended it."

Zaia wants to now extend mass testing, which started as a contingency measure in Vo, to the whole region. The Veneto governor told newspaper Corriere della Sera that the region has the ability to carry out 20-25,000 swabs a day.

The initial data from Iceland and Veneto appears to be in line with authoritative studies that have attempted to model the novel coronavirus.

Heikki Saukkomaa / Getty Images

A person wearing protective clothes takes samples from people arriving in their cars at a testing drive-in station in Espoo, Finland.

A study published on Monday in the magazine Science found that for every confirmed case of the virus there are likely another five to 10 people with undetected infections in the community. The scientists, which based their model on data from China, reported that these often milder and less infectious cases are behind nearly 80% of new cases.

Another report published this week by the Imperial College COVID-19 Response Team — a group of experts who have been advising the British and other European governments on how the disease could spread — makes a similar case.

It states: “Analyses of data from China as well as data from those returning on repatriation flights suggest that 40-50% of infections were not identified as cases. This may include asymptomatic infections, mild disease and a level of under-ascertainment.” The model also assumes that infectiousness occurs more quickly in symptomatic individuals and that they are more infectious than asymptomatic ones.

The finite testing capacity available to governments is mostly focussed on testing those symptoms and tracing their contacts, while other measures to slow the virus and not overwhelm health services cover the population at large.

But the volume of testing has become a critical issue as the virus has spread to countries around the world and new cases are growing exponentially across much of western Europe.

The World Health Organization has urged countries to test more suspected cases. “You cannot fight the fire blindfolded, and we cannot stop this pandemic if we don’t know who is infected," director-general Dr Tedros Adhanom Ghebreyesus said this week. "We have a simple message for all countries: Test, test, test. Test every suspected case.”

And the governments fighting back against the coronavirus say that extensive testing has led to substantial results — and saved lives.

Ed Jones / Getty Images

A woman watches from a waiting area as a nurse administers a COVID-19 novel coronavirus test at a testing booth outside Yangji hospital in Seoul, March 17. A South Korean hospital has introduced phone booth–style coronavirus testing facilities that avoid medical staff having to touch patients directly and cut down disinfection times.

South Korea, one of the countries first and worst hit after China, quickly put in place the most aggressive testing regime in the world after a cluster of a few dozen cases in early February exponentially ballooned to almost 5,000 cases by the end of that month. The country now has the ability to test about 20,000 people a day. A diagnosis takes about five to six hours and patients usually get results within a day. 268,000 South Koreans have been tested for the virus — about one in every 200 citizens, according to South Korean foreign minister Kang Kyung-wha.

After surpassing 8,000 cases, the number of new cases is now smaller than the number of those fully cured. The South Korean foreign minister told the BBC that testing was key. "Testing is central because that leads to early detection, it minimises further spread and it quickly treats those found with the virus," she said. "That is the key behind our very low fatality rate as well."

The data from South Korea is in stark contrast to countries like the UK, where there is currently no community testing of people with symptoms self-isolating at home. The government is under mounting pressure to do more.

Although Britain has carried out more tests when compared to many others around the world it is still lagging well behind the likes of South Korea and Italy, which as of March 17 had carried out 148,657 tests. Yesterday that figure stood at just under 138,000 and five days ago it was 86,000.

As of 9am on Tuesday, a total of 50,442 tests had been carried out in the UK, with 1,950 positive results and 48,492 negative. Health secretary Matt Hancock tweeted that a record 7,500 tests had been done in the past 24 hours.

Matt Hancock
@MattHancock

A record 7,500 tests done over the past 24 hours. More to come
https://t.co/EoQVsSEakk

03:45 PM - 17 Mar 2020

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The actual number of cases in the UK was estimated on Monday to be between 35,000 and 50,000, with this number expected to grow rapidly in the coming weeks.

This week the UK government shifted to a strategy to "suppress" the outbreak and scaling up testing could prove challenging. There has been growing criticism of its approach on testing; as the number of cases soars, people with mild symptoms are now being advised to stay at home without being tested. It means that many coronavirus sufferers will never know for sure whether or not they had it.

At the moment, testing is largely restricted to all those in intensive care units, and those with pneumonia or significant respiratory infections in hospital. This is because there is limited testing capability, according to the government scientists, which must be directed to the most serious patients where doctors need to make decisions based on clinical need.

On Tuesday, the government's chief scientific adviser Patrick Vallance told the Commons health select committee that he had been "pushing for" a "big increase in testing" in the UK.

He said there was a lot of work going on within Public Health England, the NHS and the Department of Health and Social Care to select a test that could be used more widely in the community.

The government should work closely with the private sector, he added, so “we can get things out there faster on the community side”.

There are also deep concerns among NHS workers that they are not getting the tests they need, amid fears that they are unwittingly spreading the virus to vulnerable patients.

Doctors and nurses, as well as frontline health and emergency personnel, in China and Italy are among the many that have died from the disease.

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Did Federal Officials Really Question W.H.O. Tests for Coronavirus?

Dr. Deborah Birx said she did not mean to suggest the widely used diagnostic tests generated frequent false-positive results.

By Donald G. McNeil Jr.

March 17, 2020

At a time when the Trump administration is facing intense criticism for its failure to make coronavirus tests available to millions of nervous Americans, remarks by a federal health official on Tuesday appeared to suggest that the World Health Organization's diagnostic tests were wildly inaccurate.

In a somewhat rambling answer to a question related to W.H.O. tests, Dr. Deborah Birx, the White House coronavirus response coordinator, said: "It doesn't help to put out a test where 50 percent or 47 percent were false positives. Imagine what that would mean to the American people. Imagine what that would mean to tell someone they were positive when they weren't."

It was not clear where Dr. Birx got those figures, but obviously such an inaccurate test would be worthless. Late on Tuesday night, Dr. Birx confirmed that although she was responding to a question about the W.H.O. test, she was referring to a study of an early diagnostic test used in China.

The paper found that, in a specific subset of those tested in China — asymptomatic contacts of known cases — the tests wrongly found them to be positive 47 percent of the time.

But there have been no suggestions that the W.H.O. test, distributed worldwide, has such significant accuracy problems. On Tuesday night, Dr. Birx said she has not looked into the W.H.O. test, "but I assume it is functional."

Dr. Birx was asked several questions by reporters about the lack of tests during the news conference, and came and went to the microphone several times.

Early on, she was asked a question that the administration has struggled to deal with: If federal officials have shipped millions of tests, as White House officials have said several times, why have only 60,000 Americans been tested?

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Dr. Birx answered that tests in the United States were now being made by many producers, which is correct.

Differing diagnostic tests are now made by state laboratories, medical school laboratories and private companies like Thermo Fisher, which she mentioned as an example.

Dr. Birx said she was strongly urging commercial providers to get their tests out, but of course, they first had to prove to the Food and Drug Administration that they were of high quality.

Later, she was asked about a criticism made by former Vice President Joseph R. Biden Jr. in Monday's night's debate. He said the W.H.O. had "offered tests to the United States but we didn't buy them."

In her answer, she did not refer to the W.H.O. tests at all, but said, "We don't buy tests that haven't been quality-controlled and they show us the data," then adding that a test with high rates of inaccuracy would be a disaster.

A coronavirus test kit.
Kamran Jebreili/Associated Press

A spokeswoman for the W.H.O. said she did not know what Dr. Birx was referring to, but the agency had been supplying kits to member nations since January.

The accuracy of the test was validated by three laboratories before it was rolled out, the spokeswoman said, and it had consistently showed "good performance in laboratory and clinical use, and neither a significant number of false positive nor false negative results have been reported."

In any case, Mr. Biden's assertion that the Trump administration refused tests offered by the W.H.O. appears to be wrong. The W.H.O. does not sell tests to wealthy countries, which usually prefer to make their own.

Dr. Anne Schuchat, deputy principal director of the Centers for Disease Control and Prevention, confirmed that the W.H.O. gave test kits "primarily to underresourced countries." Another administration official, speaking on the condition of anonymity, confirmed that the W.H.O. had never offered to sell or give tests to the United States.

China, Hong Kong, France, Germany, Thailand and the United States have all designed their own tests, according to the W.H.O. website. Each one looks for the presence of two or three short stretches of viral genes.

For example, the C.D.C's test looks at three targets on the N gene, while the tests ordered by the W.H.O. look at bits of the N gene, the RdRP gene and the E gene. Each gene performs a different function in helping the virus break into cells, hijack their DNA machinery and reproduce million of copies of itself.

For countries that are unable to make the tests or buy them from other countries, the W.H.O. asks academic

or government laboratories to make tests.

It then delivers them to poor and middle-income countries at low or no cost, paying for them out of emergency funds or loans from institutions like the World Bank.

The test ordered by the W.H.O. was designed in a lab run by Dr. Christian Drosten at the medical school of Berlin's Charity Hospital, which is considered one of the world's top genomic laboratories.

According to a detailed description of the test posted on the W.H.O. website, in its initial rollout, it was accurate 100 percent of the time.

In a Feb. 21 email, another W.H.O. spokesman said the test's accuracy had been verified by three other laboratories before it was sent to a German diagnostics company for manufacturing. There had been no problems with the first shipment of 250,000 doses, he said.

Dr. Michael Mina, an assistant professor of epidemiology at the Harvard School of Public Health, said both the W.H.O. test and the initial C.D.C. tests were "exceptional" in their accuracy.

The problems with the C.D.C. test have been attributed to flaws in the manufacturing of reagents for kits, not in the C.D.C.'s design.

No test is accurate 100 percent of the time, but the errors are usually introduced by medical personnel who fail to take samples correctly or lab personnel who run the test incorrectly or accidentally contaminate it with stray DNA.

For example, in February an American passenger released from the cruise ship Westerdam, which went from port to port for many days before Cambodia allowed it to dock, tested positive for the virus as she passed through Malaysia, setting off a crisis.

The C.D.C. later said she did not have the virus and judged the Malaysian test to be a likely false positive.

Since Malaysia did not have its own test, it presumably used the W.H.O.'s. But Malaysia does not have a top-quality lab, and many labs make initial errors when they are rolling out a new test.

Sheri Fink and Ellen Gabler contributed reporting from New York. Abby Goodnough contributed reporting from Washington.

Finland scoffs at WHO's coronavirus testing protocol, suggests organization doesn't understand how pandemics work

20 Mar, 2020 10:20 / Updated 16 days ago

A medical worker in Finland administers a coronavirus test to a driver © Lehtikuva/Heikki Saukkomaa via REUTERS

A senior Finnish health official has dismissed a World Health Organization (WHO) advisory to test as many people as possible for coronavirus,

arguing that such a measure would be completely illogical when combating a pandemic.

Finland's head of health security, Mika Salminen, took aim at the notion that stopping the spread of Covid-19 requires testing on a mass-scale.

"We don't understand the WHO's instructions for testing. We can't fully remove the disease from the world anymore," she said, adding: *"If someone claims that, they don't understand pandemics."*

Citing limited supplies, Finland has narrowed coronavirus testing to high-risk individuals and medical workers. Salminen told local media that screening for the virus should be done where it will be *"effective,"* not simply *"where there is concern"* about the respiratory disease.

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"Those who may be sick at home do not benefit from testing," she said.

The Finnish health official noted that administering the test drains valuable medical resources and personnel from those who need it most.

Finland has 400 confirmed cases of coronavirus but no reported deaths, according to a tally compiled by Johns Hopkins University.

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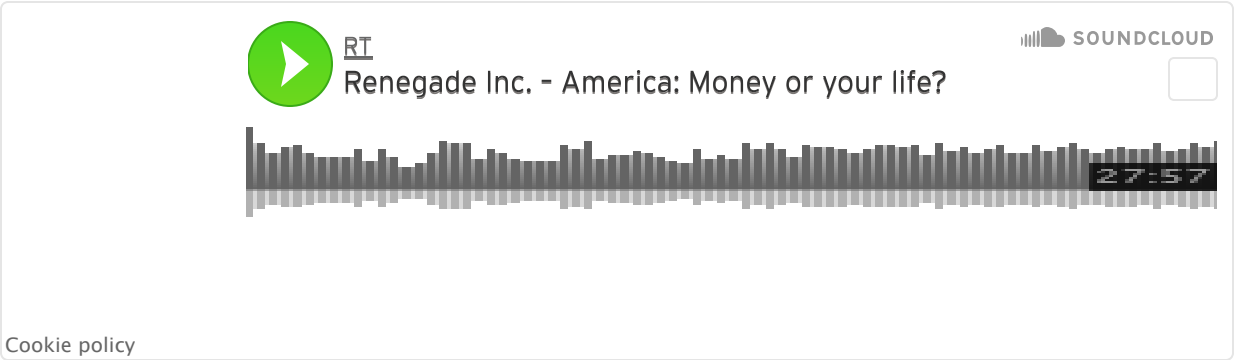

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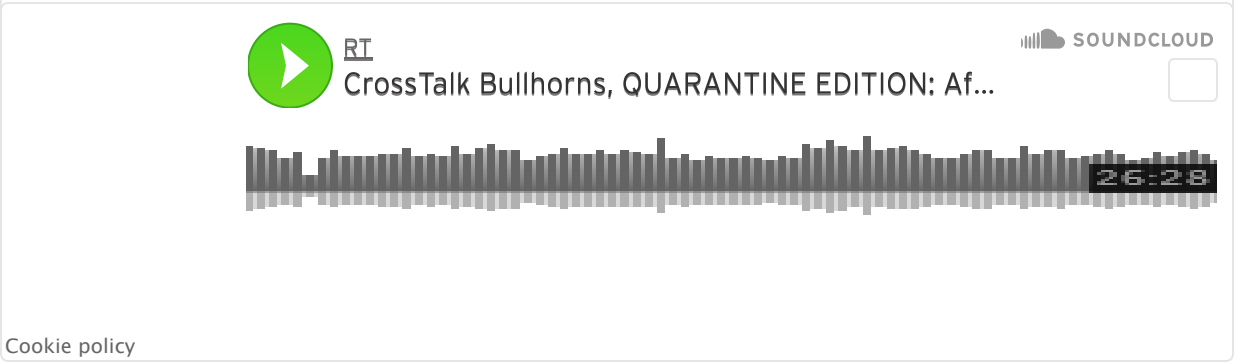

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RESEARCH LETTER

Positive RT-PCR Test Results in Patients Recovered From COVID-19

Previous studies on coronavirus disease 2019 (COVID-19) mainly focused on epidemiological, clinical, and radiological features of patients with confirmed infection.[1-4] Little attention has been paid to the follow-up of recovered patients.

Methods | One hospitalized patient and 3 patients (all medical personnel) quarantined at home with COVID-19 were treated at Zhongnan Hospital of Wuhan University, Wuhan, China, from January 1, 2020, to February 15, 2020, and evaluated with real-time reverse transcriptase–polymerase chain reaction (RT-PCR) tests for COVID-19 nucleic acid to determine if they could return to work. All the following criteria[5] had to be met for hospital discharge or discontinuation of quarantine: (1) normal temperature lasting longer than 3 days, (2) resolved respiratory symptoms, (3) substantially improved acute exudative lesions on chest computed tomography (CT) images, and (4) 2 consecutively negative RT-PCR test results separated by at least 1 day.

The RT-PCR tests were performed on throat swabs following a previously described method.[1] The RT-PCR test kits (BioGerm) were recommended by the Chinese Center for Disease Control and Prevention. The same technician and brand of test kit was used for all RT-PCR testing reported; both internal controls and negative controls were routinely performed with each batch of tests.

Demographic information, laboratory findings, and radiological features were collected from electronic medical records. After recovery, patients and their families were contacted directly, and patients were asked to visit the hospital to collect throat swabs for the RT-PCR tests.

This study was approved by the Zhongnan Hospital of Wuhan University institutional review board and the need for informed consent was waived.

Results | All 4 patients were exposed to the novel 2019 coronavirus through work as medical professionals. Two were male and the age range was 30 to 36 years. Among 3 of the patients, fever, cough, or both occurred at onset. One patient was initially asymptomatic and underwent thin-section CT due to exposure to infected patients. All patients had positive RT-PCR test results and CT imaging showed ground-glass opacification or mixed ground-glass opacification and consolidation. The severity of disease was mild to moderate.

Antiviral treatment (75 mg of oseltamivir taken orally every 12 hours) was provided for the 4 patients. For 3 of the patients, all clinical symptoms and CT imaging abnormalities had resolved. The CT imaging for the fourth patient showed delicate patches of ground-glass opacity. All 4 patients had 2 consecutive negative RT-PCR test results. The time from symptom onset to recovery ranged from 12 to 32 days.

After hospital discharge or discontinuation of quarantine, the patients were asked to continue the quarantine protocol at home for 5 days. The RT-PCR tests were repeated 5 to 13 days later and all were positive. All patients had 3 repeat RT-PCR tests performed over the next 4 to 5 days and all were positive. An additional RT-PCR test was performed using a kit from a different manufacturer and the results were also positive for all patients. The patients continued to be asymptomatic by clinician examination and chest CT findings showed no change from previous images. They did not report contact with any person with respiratory symptoms. No family member was infected.

Discussion | Four patients with COVID-19 who met criteria for hospital discharge or discontinuation of quarantine in China (absence of clinical symptoms and radiological abnormalities and 2 negative RT-PCR test results) had positive RT-PCR test results 5 to 13 days later. These findings suggest that at least a proportion of recovered patients still may be virus carriers. Although no family members were infected, all reported patients were medical professionals and took special care during home quarantine. Current criteria for hospital discharge or discontinuation of quarantine and continued patient management may need to be reevaluated. Although false-negative RT-PCR test results could have occurred as suggested by a previous study,[6] 2 consecutively negative RT-PCR test results plus evidence from clinical characteristics and chest CT findings suggested that the 4 patients qualified for hospital discharge or discontinuation of quarantine.

The study was limited to a small number of patients with mild or moderate infection. Further studies should follow up patients who are not health care professionals and who have more severe infection after hospital discharge or discontinuation of quarantine. Longitudinal studies on a larger cohort would help to understand the prognosis of the disease.

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Acquisition, analysis, or interpretation of data: Lan, Ye, Wang, Li.
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Covert COVID-19 and false-positive dengue serology in Singapore

Dengue and coronavirus disease 2019 (COVID-19) are difficult to distinguish because they have shared clinical and laboratory features.[1,2] We describe two patients in Singapore with false-positive results from rapid serological testing for dengue, who were later confirmed to have severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) infection, the causative virus of COVID-19.

The first case is a 57-year-old man with no relevant past medical, travel, or contact history, who presented to a regional hospital on Feb 9, 2020, with 3 days of fever and cough. He had thrombocytopenia (platelet count 140×10^9/mL) and a normal chest radiograph. He was discharged after a negative rapid test for dengue NS1, IgM, and IgG (SD Bioline Dengue Duo Kit; Abbott, South Korea). He returned to a public primary healthcare clinic with persistent fever, worsening thrombocytopenia (89×10^9/mL), and new onset lymphopenia ($0{\cdot}43 \times 10^9$/mL). A repeat dengue rapid test was positive for dengue IgM and IgG (Dengue Combo; Wells Bio, South Korea). He was referred to hospital for dengue with worsening cough and dyspnoea. A chest radiograph led to testing for SARS-CoV-2 by RT-PCR (in-house laboratory-developed test detecting the *N* and *ORF1ab* genes) from a nasopharyngeal swab, which returned positive. The original seropositive sample and additional urine and blood samples tested negative for dengue, chikungunya, and Zika viruses by RT-PCR,[3–5] and a repeat dengue rapid test (SD Bioline) was also negative. Thus, the initial dengue seroconversion result was deemed a false positive.

The second case is a 57-year-old woman with no relevant past medical, travel, or contact history, who presented to a regional hospital on Feb 13, 2020, with fever, myalgia, a mild cough of 4 days, and 2 days of diarrhoea. She had thrombocytopenia (92×10^9/mL) and tested positive for dengue IgM (SD Bioline). She was discharged with outpatient follow up for dengue fever. She returned 2 days later with a persistent fever, worsening thrombocytopenia (65×10^9/mL), and new onset lymphopenia ($0{\cdot}94 \times 10^9$/mL). Liver function tests were abnormal (aspartate aminotransferase 69 U/L [reference range 10–30 U/L], alanine aminotransferase 67 U/L [reference range <55 U/L], total bilirubin 35·8 µmol/L [reference range 4·7–23·2 µmol/L]). Chest radiography was normal and she was admitted for dengue fever. She remained febrile despite normalisation of her blood counts and developed dyspnoea 3 days after admission. She was found to be positive for SARS-CoV-2 by RT-PCR from a nasopharyngeal swab. A repeat dengue test (SD Bioline) was negative and an earlier blood sample also tested negative for dengue by RT-PCR.[6] The initial dengue IgM result was deemed to be a false positive.

Failing to consider COVID-19 because of a positive dengue rapid test result has serious implications not only for the patient but also for public health. Our cases highlight the importance of recognising false-positive dengue serology results (with different commercially available assays) in patients with COVID-19. We emphasise the urgent need for rapid, sensitive, and accessible diagnostic tests for SARS-CoV-2, which need to be highly accurate to protect public health.

We declare no competing interests.

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BRIEF REPORT

First Case of 2019 Novel Coronavirus in the United States

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SUMMARY

An outbreak of novel coronavirus (2019-nCoV) that began in Wuhan, China, has spread rapidly, with cases now confirmed in multiple countries. We report the first case of 2019-nCoV infection confirmed in the United States and describe the identification, diagnosis, clinical course, and management of the case, including the patient's initial mild symptoms at presentation with progression to pneumonia on day 9 of illness. This case highlights the importance of close coordination between clinicians and public health authorities at the local, state, and federal levels, as well as the need for rapid dissemination of clinical information related to the care of patients with this emerging infection.

On December 31, 2019, China reported a cluster of cases of pneumonia in people associated with the Huanan Seafood Wholesale Market in Wuhan, Hubei Province.[1] On January 7, 2020, Chinese health authorities confirmed that this cluster was associated with a novel coronavirus, 2019-nCoV.[2] Although cases were originally reported to be associated with exposure to the seafood market in Wuhan, current epidemiologic data indicate that person-to-person transmission of 2019-nCoV is occurring.[3-6] As of January 30, 2020, a total of 9976 cases had been reported in at least 21 countries,[7] including the first confirmed case of 2019-nCoV infection in the United States, reported on January 20, 2020. Investigations are under way worldwide to better understand transmission dynamics and the spectrum of clinical illness. This report describes the epidemiologic and clinical features of the first case of 2019-nCoV infection confirmed in the United States.

CASE REPORT

On January 19, 2020, a 35-year-old man presented to an urgent care clinic in Snohomish County, Washington, with a 4-day history of cough and subjective fever. On checking into the clinic, the patient put on a mask in the waiting room. After waiting approximately 20 minutes, he was taken into an examination room and underwent evaluation by a provider. He disclosed that he had returned to Washington State on January 15 after traveling to visit family in Wuhan, China. The patient stated

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*A full list of the members of the Washington State 2019-nCoV Case Investigation Team is provided in the Supplementary Appendix, available at NEJM.org.

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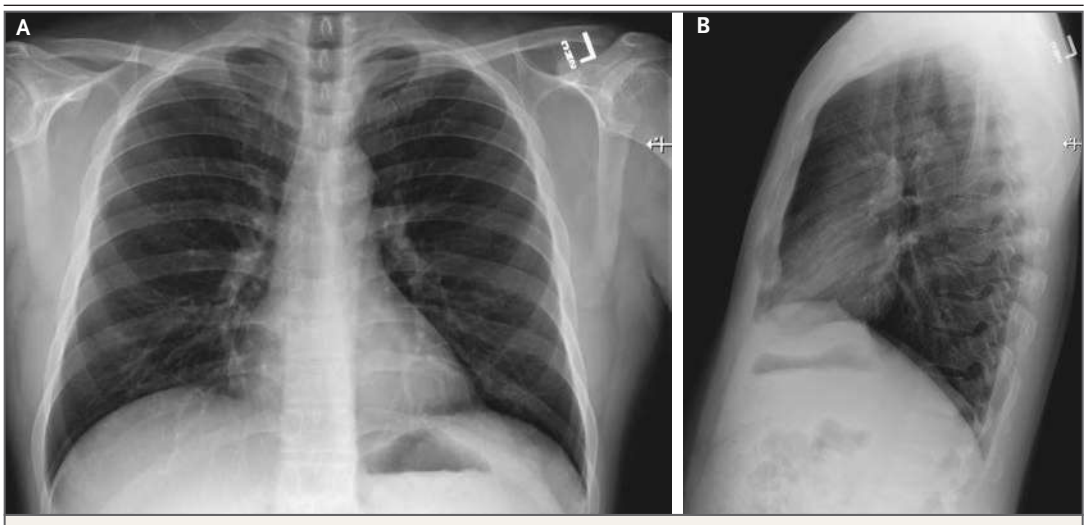

Figure 1. Posteroanterior and Lateral Chest Radiographs, January 19, 2020 (Illness Day 4).
No thoracic abnormalities were noted.

that he had seen a health alert from the U.S. Centers for Disease Control and Prevention (CDC) about the novel coronavirus outbreak in China and, because of his symptoms and recent travel, decided to see a health care provider.

Apart from a history of hypertriglyceridemia, the patient was an otherwise healthy nonsmoker. The physical examination revealed a body temperature of 37.2°C, blood pressure of 134/87 mm Hg, pulse of 110 beats per minute, respiratory rate of 16 breaths per minute, and oxygen saturation of 96% while the patient was breathing ambient air. Lung auscultation revealed rhonchi, and chest radiography was performed, which was reported as showing no abnormalities (Fig. 1). A rapid nucleic acid amplification test (NAAT) for influenza A and B was negative. A nasopharyngeal swab specimen was obtained and sent for detection of viral respiratory pathogens by NAAT; this was reported back within 48 hours as negative for all pathogens tested, including influenza A and B, parainfluenza, respiratory syncytial virus, rhinovirus, adenovirus, and four common coronavirus strains known to cause illness in humans (HKU1, NL63, 229E, and OC43).

Given the patient's travel history, the local and state health departments were immediately notified. Together with the urgent care clinician, the Washington Department of Health notified the CDC Emergency Operations Center. Although the patient reported that he had not spent time at the Huanan seafood market and reported no known contact with ill persons during his travel to China, CDC staff concurred with the need to test the patient for 2019-nCoV on the basis of current CDC "persons under investigation" case definitions.[8] Specimens were collected in accordance with CDC guidance and included serum and nasopharyngeal and oropharyngeal swab specimens. After specimen collection, the patient was discharged to home isolation with active monitoring by the local health department.

On January 20, 2020, the CDC confirmed that the patient's nasopharyngeal and oropharyngeal swabs tested positive for 2019-nCoV by real-time reverse-transcriptase–polymerase-chain-reaction (rRT-PCR) assay. In coordination with CDC subject-matter experts, state and local health officials, emergency medical services, and hospital leadership and staff, the patient was admitted to an airborne-isolation unit at Providence Regional Medical Center for clinical observation, with health care workers following CDC recommendations for contact, droplet, and airborne precautions with eye protection.[9]

On admission, the patient reported persistent dry cough and a 2-day history of nausea and vomiting; he reported that he had no shortness of breath or chest pain. Vital signs were within normal ranges. On physical examination, the patient was found to have dry mucous membranes. The remainder of the examination was generally unremarkable. After admission, the patient received supportive care, including 2 liters of normal saline and ondansetron for nausea.

On days 2 through 5 of hospitalization (days

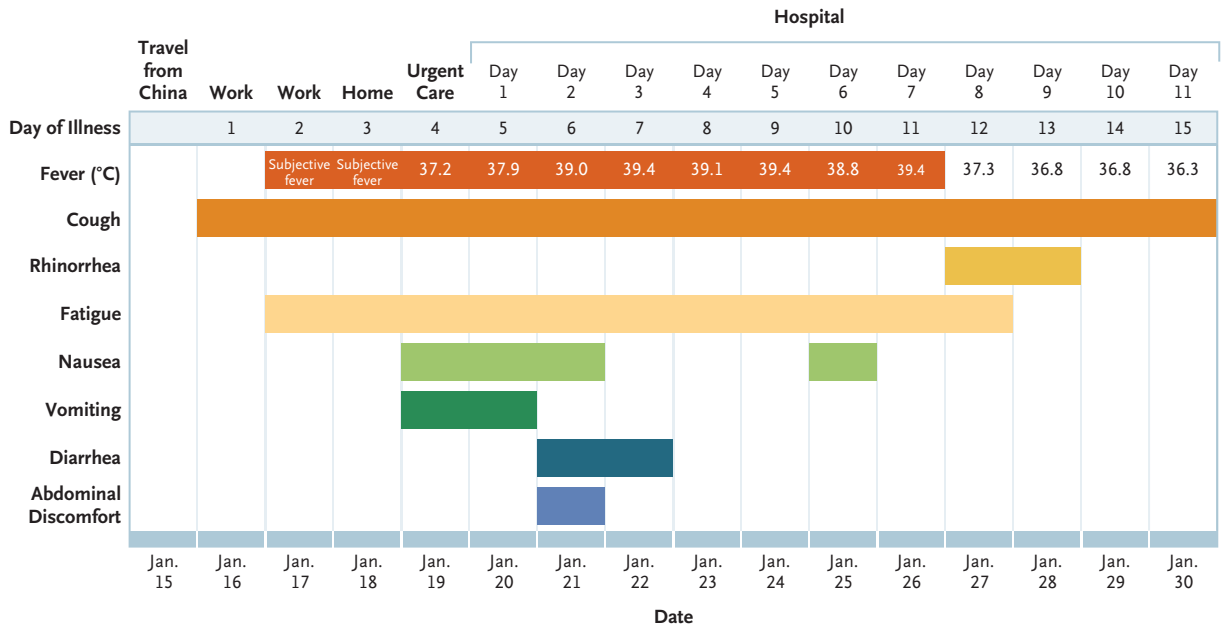

Figure 2. Symptoms and Maximum Body Temperatures According to Day of Illness and Day of Hospitalization, January 16 to January 30, 2020.

6 through 9 of illness), the patient's vital signs remained largely stable, apart from the development of intermittent fevers accompanied by periods of tachycardia (Fig. 2). The patient continued to report a nonproductive cough and appeared fatigued. On the afternoon of hospital day 2, the patient passed a loose bowel movement and reported abdominal discomfort. A second episode of loose stool was reported overnight; a sample of this stool was collected for rRT-PCR testing, along with additional respiratory specimens (nasopharyngeal and oropharyngeal) and serum. The stool and both respiratory specimens later tested positive by rRT-PCR for 2019-nCoV, whereas the serum remained negative.

Treatment during this time was largely supportive. For symptom management, the patient received, as needed, antipyretic therapy consisting of 650 mg of acetaminophen every 4 hours and 600 mg of ibuprofen every 6 hours. He also received 600 mg of guaifenesin for his continued cough and approximately 6 liters of normal saline over the first 6 days of hospitalization.

The nature of the patient isolation unit permitted only point-of-care laboratory testing initially; complete blood counts and serum chemical studies were available starting on hospital day 3. Laboratory results on hospital days 3 and 5 (illness days 7 and 9) reflected leukopenia, mild thrombocytopenia, and elevated levels of creatine kinase (Table 1). In addition, there were alterations in hepatic function measures: levels of alkaline phosphatase (68 U per liter), alanine aminotransferase (105 U per liter), aspartate aminotransferase (77 U per liter), and lactate dehydrogenase (465 U per liter) were all elevated on day 5 of hospitalization. Given the patient's recurrent fevers, blood cultures were obtained on day 4; these have shown no growth to date.

A chest radiograph taken on hospital day 3 (illness day 7) was reported as showing no evidence of infiltrates or abnormalities (Fig. 3). However, a second chest radiograph from the night of hospital day 5 (illness day 9) showed evidence of pneumonia in the lower lobe of the left lung (Fig. 4). These radiographic findings coincided with a change in respiratory status starting on the evening of hospital day 5, when the patient's oxygen saturation values as measured by pulse oximetry dropped to as low as 90% while he was breathing ambient air. On day 6, the patient was started on supplemental oxygen, delivered by nasal cannula at 2 liters per minute. Given the changing clinical presentation and concern about hospital-acquired pneumonia, treatment with vancomycin (a 1750-mg loading dose followed

Table 1. Clinical Laboratory Results.*

Measure	Reference Range	Illness Day 6, Hospital Day 2†	Illness Day 7, Hospital Day 3	Illness Day 9, Hospital Day 5	Illness Day 11, Hospital Day 7	Illness Day 13, Hospital Day 9	Illness Day 14, Hospital Day 10
White-cell count (per μl)	3800–11,000	"Slight decrease"	3120‡	3300‡	5400	5600	6500
Red-cell count (per μl)	4,200,000–5,700,000	—	4,870,000	5,150,000	5,010,000	4,650,000	5,010,000
Absolute neutrophil count (per μl)	1900–7400	—	1750‡	1700‡	3700	3800	3200
Absolute lymphocyte count (per μl)	1000–3900	—	1070	1400	1400	1400	2100
Platelet count (per μl)	150,000–400,000	"Adequate"	122,000‡	132,000‡	151,000	150,000	239,000
Hemoglobin (g/dl)	13.2–17.0	12.2‡	14.2	14.8	14.8	13.5	14.2
Hematocrit (%)	39.0–50.0	36.0‡	42.0	43.0	43.0	39.3	42.0
Sodium (mmol/liter)	136–145	134‡	136	138	138	135‡	138
Potassium (mmol/liter)	3.5–5.1	3.3‡	3.6	3.4‡	3.6	4.1	3.9
Chloride (mmol/liter)	98–107	99	101	105	106	100	103
Calcium (mg/dl)	8.7–10.4	—	8.5‡	9.3	9.0	8.6‡	9.3
Carbon dioxide (mmol/liter)	20–31	—	26	24	25	23	36§
Anion gap (mmol/liter)	5–16	—	9	9	7	12	9
Glucose (mmol/liter)	65–140	104	103	120	96	148§	104
Blood urea nitrogen (mg/dl)	9–23	15	10	13	13	22§	18
Creatinine (mg/dl)	0.7–1.3	1.0	1.06	1.06	0.88	1.08	0.84
Total protein (g/dl)	5.7–8.2	—	6.9	7.1	6.8	6.9	6.8
Albumin (g/dl)	3.2–4.8	—	4.2	4.7	4.5	2.9‡	4.4
Total bilirubin (mg/dl)	0.3–1.2	—	1.0	1.1	1.5§	0.8	1.0
Procalcitonin (ng/ml)	<0.05	—	—	<0.05	<0.05	—	—
Alanine aminotransferase (U/liter)	10–49	—	68§	105§	119§	219§	203§
Aspartate aminotransferase (U/liter)	≤33	—	37§	77§	85§	129§	89§
Alkaline phosphatase (U/liter)	46–116	—	50	68§	88§	137§	163§
Fibrinogen (mg/dl)	150–450	—	477§	—	—	—	—
Lactate dehydrogenase (U/liter)	120–246	—	250§	465§	—	—	388§
Prothrombin time (sec)	12.2–14.6	—	11.9‡	11.9‡	—	—	12.7
International normalized ratio	0.9–1.1	—	0.9	0.9	—	—	1.0
Creatine kinase (U/liter)	62–325	—	353§	332§	—	—	—
Venous lactate (mmol/liter)	0.4–2.0	—	1.3	1.7	—	—	—

* To convert the values for calcium to millimoles per liter, multiply by 0.250. To convert the values for blood urea nitrogen to millimoles per liter of urea, multiply by 0.357. To convert the values for creatinine to micromoles per liter, multiply by 88.4. To convert the values for total bilirubin to micromoles per liter, multiply by 17.1.
† Results are from point-of-care blood analyzer (iStat) testing.
‡ The value in the patient was below normal.
§ The value in the patient was above normal.

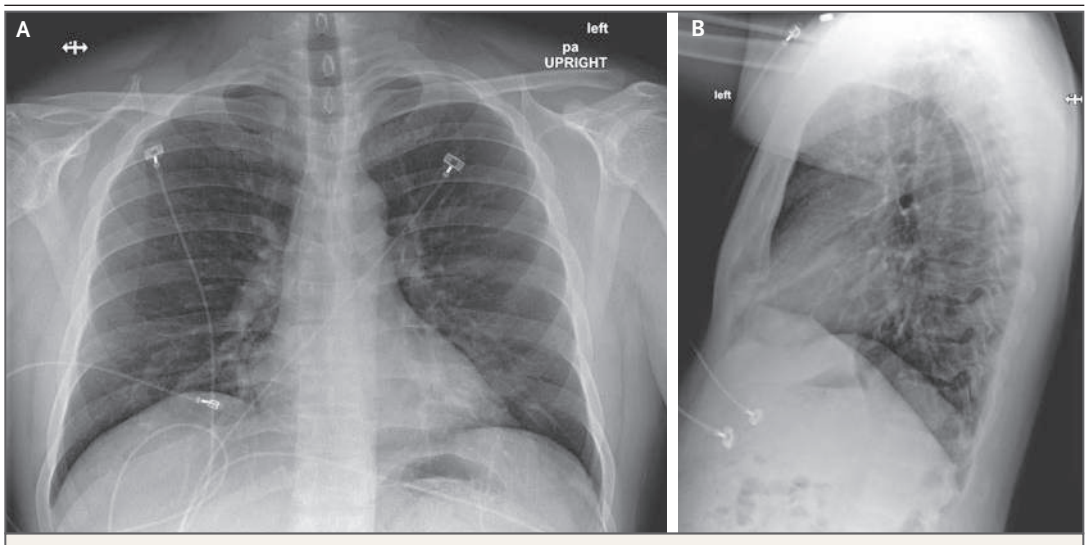

Figure 3. Posteroanterior and Lateral Chest Radiographs, January 22, 2020 (Illness Day 7, Hospital Day 3).
No acute intrathoracic plain-film abnormality was noted.

by 1 g administered intravenously every 8 hours) and cefepime (administered intravenously every 8 hours) was initiated.

On hospital day 6 (illness day 10), a fourth chest radiograph showed basilar streaky opacities in both lungs, a finding consistent with atypical pneumonia (Fig. 5), and rales were noted in both lungs on auscultation. Given the radiographic findings, the decision to administer oxygen supplementation, the patient's ongoing fevers, the persistent positive 2019-nCoV RNA at multiple sites, and published reports of the development of severe pneumonia[3,4] at a period consistent with the development of radiographic pneumonia in this patient, clinicians pursued compassionate use of an investigational antiviral therapy. Treatment with intravenous remdesivir (a novel nucleotide analogue prodrug in development[10,11]) was initiated on the evening of day 7, and no adverse events were observed in association with the infusion. Vancomycin was discontinued on the evening of day 7, and cefepime was discontinued on the following day, after serial negative procalcitonin levels and negative nasal PCR testing for methicillin-resistant *Staphylococcus aureus*.

On hospital day 8 (illness day 12), the patient's clinical condition improved. Supplemental oxygen was discontinued, and his oxygen saturation values improved to 94 to 96% while he was breathing ambient air. The previous bilateral lower-lobe rales were no longer present. His appetite improved, and he was asymptomatic aside from intermittent dry cough and rhinorrhea. As of January 30, 2020, the patient remains hospitalized. He is afebrile, and all symptoms have resolved with the exception of his cough, which is decreasing in severity.

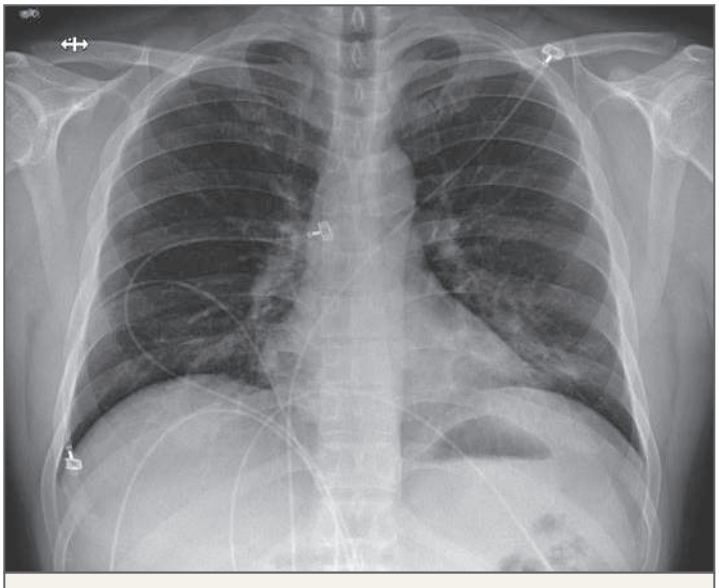

Figure 4. Posteroanterior Chest Radiograph, January 24, 2020 (Illness Day 9, Hospital Day 5).
Increasing left basilar opacity was visible, arousing concern about pneumonia.

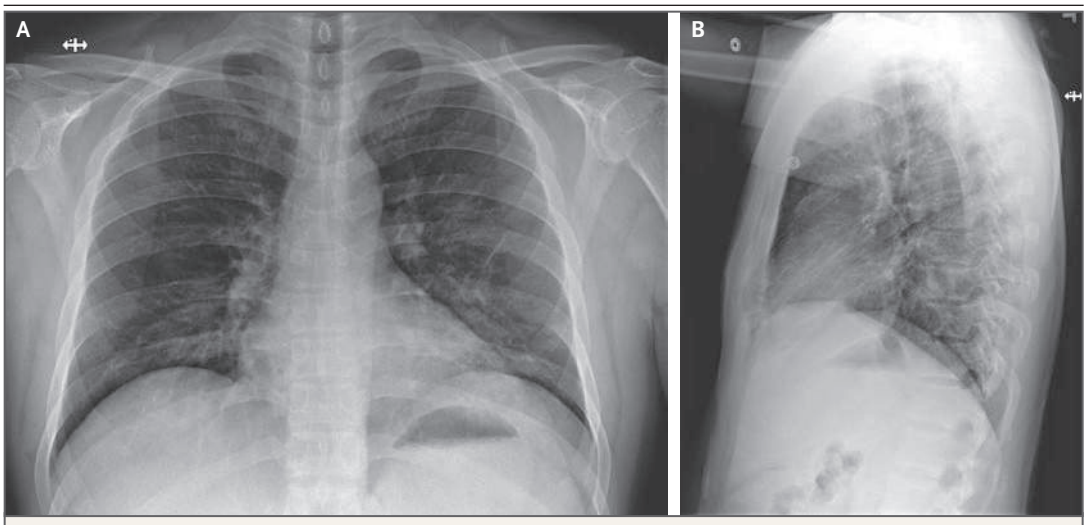

Figure 5. Anteroposterior and Lateral Chest Radiographs, January 26, 2020 (Illness Day 10, Hospital Day 6).
Stable streaky opacities in the lung bases were visible, indicating likely atypical pneumonia; the opacities have steadily increased in density over time.

METHODS

SPECIMEN COLLECTION

Clinical specimens for 2019-nCoV diagnostic testing were obtained in accordance with CDC guidelines.[12] Nasopharyngeal and oropharyngeal swab specimens were collected with synthetic fiber swabs; each swab was inserted into a separate sterile tube containing 2 to 3 ml of viral transport medium. Serum was collected in a serum separator tube and then centrifuged in accordance with CDC guidelines. The urine and stool specimens were each collected in sterile specimen containers. Specimens were stored between 2°C and 8°C until ready for shipment to the CDC. Specimens for repeat 2019-nCoV testing were collected on illness days 7, 11, and 12 and included nasopharyngeal and oropharyngeal swabs, serum, and urine and stool samples.

DIAGNOSTIC TESTING FOR 2019-NCOV

Clinical specimens were tested with an rRT-PCR assay that was developed from the publicly released virus sequence. Similar to previous diagnostic assays for severe acute respiratory syndrome coronavirus (SARS-CoV) and Middle East respiratory syndrome coronavirus (MERS-CoV), it has three nucleocapsid gene targets and a positive control target. A description of this assay[13] and sequence information for the rRT-PCR panel primers and probes[14] are available on the CDC Laboratory Information website for 2019-nCoV.[15]

GENETIC SEQUENCING

On January 7, 2020, Chinese researchers shared the full genetic sequence of 2019-nCoV through the National Institutes of Health GenBank database[16] and the Global Initiative on Sharing All Influenza Data (GISAID)[17] database; a report about the isolation of 2019-nCoV was later published.[18] Nucleic acid was extracted from rRT-PCR–positive specimens (oropharyngeal and nasopharyngeal) and used for whole-genome sequencing on both Sanger and next-generation sequencing platforms (Illumina and MinIon). Sequence assembly was completed with the use of Sequencher software, version 5.4.6 (Sanger); minimap software, version 2.17 (MinIon); and freebayes software, version 1.3.1 (MiSeq). Complete genomes were compared with the available 2019-nCoV reference sequence (GenBank accession number NC_045512.2).

RESULTS

SPECIMEN TESTING FOR 2019-NCOV

The initial respiratory specimens (nasopharyngeal and oropharyngeal swabs) obtained from this patient on day 4 of his illness were positive for 2019-nCoV (Table 2). The low cycle threshold (Ct) values (18 to 20 in nasopharyngeal specimens and 21 to 22 in oropharyngeal specimens) on illness day 4 suggest high levels of virus in these specimens, despite the patient's initial mild symptom presentation. Both upper respiratory

Table 2. Results of Real-Time Reverse-Transcriptase–Polymerase-Chain-Reaction Testing for the 2019 Novel Coronavirus (2019-nCoV).*

Specimen	Illness Day 4	Illness Day 7	Illness Day 11	Illness Day 12
Nasopharyngeal swab	Positive (Ct, 18–20)	Positive (Ct, 23–24)	Positive (Ct, 33–34)	Positive (Ct, 37–40)
Oropharyngeal swab	Positive (Ct, 21–22)	Positive (Ct, 32–33)	Positive (Ct, 36–40)	Negative
Serum	Negative	Negative	Pending	Pending
Urine	NT	Negative	NT	NT
Stool	NT	Positive (Ct, 36–38)	NT	NT

* Lower cycle threshold (Ct) values indicate higher viral loads. NT denotes not tested.

specimens obtained on illness day 7 remained positive for 2019-nCoV, including persistent high levels in a nasopharyngeal swab specimen (Ct values, 23 to 24). Stool obtained on illness day 7 was also positive for 2019-nCoV (Ct values, 36 to 38). Serum specimens for both collection dates were negative for 2019-nCoV. Nasopharyngeal and oropharyngeal specimens obtained on illness days 11 and 12 showed a trend toward decreasing levels of virus. The oropharyngeal specimen tested negative for 2019-nCoV on illness day 12. The rRT-PCR results for serum obtained on these dates are still pending.

GENETIC SEQUENCING

The full genome sequences from oropharyngeal and nasopharyngeal specimens were identical to one another and were nearly identical to other available 2019-nCoV sequences. There were only 3 nucleotides and 1 amino acid that differed at open reading frame 8 between this patient's virus and the 2019-nCoV reference sequence (NC_045512.2). The sequence is available through GenBank (accession number MN985325).[16]

DISCUSSION

Our report of the first confirmed case of 2019-nCoV in the United States illustrates several aspects of this emerging outbreak that are not yet fully understood, including transmission dynamics and the full spectrum of clinical illness. Our case patient had traveled to Wuhan, China, but reported that he had not visited the wholesale seafood market or health care facilities or had any sick contacts during his stay in Wuhan. Although the source of his 2019-nCoV infection is unknown, evidence of person-to-person transmission has been published. Through January 30, 2020, no secondary cases of 2019-nCoV related to this case have been identified, but monitoring of close contacts continues.[19]

Detection of 2019-nCoV RNA in specimens from the upper respiratory tract with low Ct values on day 4 and day 7 of illness is suggestive of high viral loads and potential for transmissibility. It is notable that we also detected 2019-nCoV RNA in a stool specimen collected on day 7 of the patient's illness. Although serum specimens from our case patient were repeatedly negative for 2019-nCoV, viral RNA has been detected in blood in severely ill patients in China.[4] However, extrapulmonary detection of viral RNA does not necessarily mean that infectious virus is present, and the clinical significance of the detection of viral RNA outside the respiratory tract is unknown at this time.

Currently, our understanding of the clinical spectrum of 2019-nCoV infection is very limited. Complications such as severe pneumonia, respiratory failure, acute respiratory distress syndrome (ARDS), and cardiac injury, including fatal outcomes, have been reported in China.[4,18,20] However, it is important to note that these cases were identified on the basis of their pneumonia diagnosis and thus may bias reporting toward more severe outcomes.

Our case patient initially presented with mild cough and low-grade intermittent fevers, without evidence of pneumonia on chest radiography on day 4 of his illness, before having progression to pneumonia by illness day 9. These nonspecific signs and symptoms of mild illness early in the clinical course of 2019-nCoV infection may be indistinguishable clinically from many other common infectious diseases, particularly during the winter respiratory virus season.

In addition, the timing of our case patient's progression to pneumonia on day 9 of illness is consistent with later onset of dyspnea (at a median of 8 days from onset) reported in a recent publication.[4] Although a decision to administer remdesivir for compassionate use was based on the case patient's worsening clinical status, randomized controlled trials are needed to determine the safety and efficacy of remdesivir and any other investigational agents for treatment of patients with 2019-nCoV infection.

We report the clinical features of the first reported patient with 2019-nCoV infection in the United States. Key aspects of this case included the decision made by the patient to seek medical attention after reading public health warnings about the outbreak; recognition of the patient's recent travel history to Wuhan by local providers, with subsequent coordination among local, state, and federal public health officials; and identification of possible 2019-nCoV infection, which allowed for prompt isolation of the patient and subsequent laboratory confirmation of 2019-nCoV, as well as for admission of the patient for further evaluation and management. This case report highlights the importance of clinicians eliciting a recent history of travel or exposure to sick contacts in any patient presenting for medical care with acute illness symptoms, in order to ensure appropriate identification and prompt isolation of patients who may be at risk for 2019-nCoV infection and to help reduce further transmission. Finally, this report highlights the need to determine the full spectrum and natural history of clinical disease, pathogenesis, and duration of viral shedding associated with 2019-nCoV infection to inform clinical management and public health decision making.

The findings and conclusions in this report are those of the authors and do not necessarily represent the official position of the Centers for Disease Control and Prevention.

Disclosure forms provided by the authors are available with the full text of this article at NEJM.org.

We thank the patient; the nurses and clinical staff who are providing care for the patient; staff at the local and state health departments; staff at the Washington State Department of Health Public Health Laboratories and at the Centers for Disease Control and Prevention (CDC) Division of Viral Disease Laboratory; CDC staff at the Emergency Operations Center; and members of the 2019-nCoV response teams at the local, state, and national levels.

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Intended for healthcare professionals

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Practice Practice Pointer

Coronavirus disease 2019 (covid-19): a guide for UK GPs

BMJ 2020; 368 doi: https://doi.org/10.1136/bmj.m800 (Published 06 March 2020) Cite this as: BMJ 2020;368:m800

Read our latest coverage of the Coronavirus outbreak

On 12 March 2020 Public Health England published new guidance to the public for people with confirmed or possible covid-19 infection. The article and infographic will be updated once new guidance for primary care is published.

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Rapid Response:

Covid-19: Are we the victims of mass panic?

Case-fatality rates for respiratory virus infections are highly uncertain. Many mild infections pass unnoticed, and if an elderly frail patient with serious heart disease is pushed over the edge by an infection, was it then a virus death or a cardiac death?

I have suspected for a long time that we are the victims of mass panic. Two days ago, I read in a newspaper that the average age of those who died after coronavirus infection was 81 and that they also often had comorbidity.

What if the Chinese had not tested their patients for coronavirus or there had not been any test? Would we have carried on with our lives, without restrictions, not worrying about some deaths here and there among old people, which we see every winter? I think so.

The estimate for the case-fatality rate for coronavirus infections is around 2% (1). For the mild influenza pandemic in 2009, and the following years, the median case-fatality rate in the studies was around 1% for laboratory confirmed influenza (2, figure 3).

WHO estimates that seasonal influenza may result in 290,000 to 650,000 deaths each year due to respiratory diseases alone (3). About 4,000 have died so far from coronavirus.

Why all the panic? Is it evidence-based healthcare to close schools and universities, cancel flights and meetings, forbid travel, and to isolate people wherever they happen to fall ill? In Denmark, the government recommends cancellation of events with over 1000 participants. When some organisers crept just below 1000, they were attacked by professors in virology and microbiology. But if it is wrong to invite 990 people, it should also be wrong to invite 980, and so forth. Where does this stop? And should big shopping centres be closed, too? (4)

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Competing interests: No competing interests

08 March 2020
Peter C Gøtzsche
Director
Institute for Scientific Freedom, Copenhagen

Feb 5, 2010, 04:35pm EST

Why The WHO Faked A Pandemic

Michael Fumento Subscriber

This article is more than 10 years old.

The World Health Organization has suddenly gone from crying "The sky is falling!" like a cackling Chicken Little to squealing like a stuck pig. The reason: charges that the agency deliberately fomented swine flu hysteria. "The world is going through a real pandemic. The description of it as a fake is wrong and irresponsible," the agency claims on its Web site. A WHO spokesman declined to specify who or what gave this "description," but the primary accuser is hard to ignore.

The Parliamentary Assembly of the Council of Europe (PACE), a human rights watchdog, is publicly investigating the WHO's motives in declaring a pandemic. Indeed, the chairman of its influential health committee, epidemiologist Wolfgang Wodarg, has declared that the "false pandemic" is "one of the greatest medicine scandals of the century."

Even within the agency, the director of the WHO Collaborating Center for Epidemiology in Munster, Germany, Dr. Ulrich Kiel, has essentially labeled the pandemic a hoax. "We are witnessing a gigantic misallocation of resources [$18 billion so far] in terms of public health," he said.

They're right. This wasn't merely overcautiousness or simple misjudgment. The pandemic declaration and all the Klaxon-ringing since reflect sheer dishonesty motivated not by medical concerns but political ones.

Unquestionably, swine flu has proved to be vastly milder than ordinary seasonal flu. It kills at a third to a tenth the rate, according to U.S. Centers for Disease Control and Prevention estimates. Data from other countries like France and Japan indicate it's far tamer than that.

Indeed, judging by what we've seen in New Zealand and Australia (where the epidemics have ended), and by what we're seeing elsewhere in the world, we'll have considerably fewer flu deaths this season than normal. That's because swine flu muscles aside seasonal flu, acting as a sort of inoculation against the far deadlier strain.

Did the WHO have any indicators of this mildness when it declared the pandemic in June?

Absolutely, as I wrote at the time. We were then fully 11 weeks into the outbreak and swine flu had only killed 144 people worldwide--the same number who die of seasonal flu worldwide every few *hours*. (An estimated 250,000 to 500,000 per year by the WHO's own numbers.) The *mildest* pandemics of the 20th century killed at least a million people.

But how could the organization declare a pandemic when its own official definition required "simultaneous epidemics worldwide with enormous numbers of deaths and illness." Severity--that is, the number of deaths--is crucial, because every year flu causes "a global spread of disease."

Easy. In May, in what it admitted was a direct response to the outbreak of swine flu the month before, WHO promulgated a new definition matched to swine flu that simply eliminated severity as a factor. You could now have a pandemic with zero deaths.

Under fire, the organization is boldly lying about the change, to which anybody with an Internet connection can attest. In a mid-January virtual conference WHO swine flu chief Keiji Fukuda stated: "Did WHO change its definition of a pandemic? The answer is no: WHO did not change its definition." Two weeks later at a PACE conference he insisted: "Having severe deaths has never been part of the WHO definition."

They did it; but why?

In part, it was CYA for the WHO. The agency was losing credibility over the refusal of avian flu H5N1 to go pandemic and kill as many as 150 million people worldwide, as its

Around the world nations heeded the warnings and spent vast sums developing vaccines and making other preparations. So when swine flu conveniently trotted in, the WHO essentially crossed out "avian," inserted "swine," and WHO Director-General Margaret Chan arrogantly boasted, "The world can now reap the benefits of investments over the last five years in pandemic preparedness."

But there's more than bureaucratic self-interest at work here. Bizarrely enough, the WHO has also exploited its phony pandemic to push a hard left political agenda.

In a September speech WHO Director-General Chan said "ministers of health" should take advantage of the "devastating impact" swine flu will have on poorer nations to get out the message that "changes in the functioning of the global economy" are needed to "distribute wealth on the basis of" values "like community, solidarity, equity and social justice." She further declared it should be used as a weapon against "international policies and systems that govern financial markets, economies, commerce, trade and foreign affairs."

Chan's dream now lies in tatters. All the WHO has done, says PACE's Wodart, is to destroy "much of the credibility that they should have, which is invaluable to us if there's a future scare that might turn out to be a killer on a large scale."

Michael Fumento is director of the nonprofit Independent Journalism Project, where he specializes in health and science issues. He may be reached at fumento@pobox.com.

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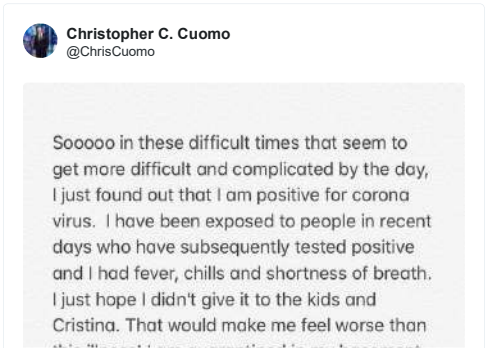
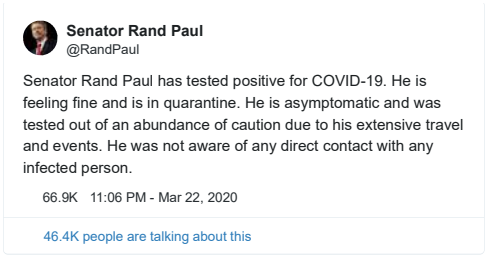
(Updated 11:57am ET, March 31, 2020)

Topline: Novel coronavirus is everywhere—with over 438,000 positive Covid-19 cases around the world; this is a list of celebrities who have announced they've tested positive for it.

- **Chris Cuomo:** The CNN news anchor and brother of New York Governor Andrew Cuomo announced via Twitter he is positive for COVID-19. "In his job, he's combative and argumentative...but that's his job, that's not who he is. He's a really sweet, beautiful guy, and he's my best friend," said Governor Cuomo in Tuesday's press conference.

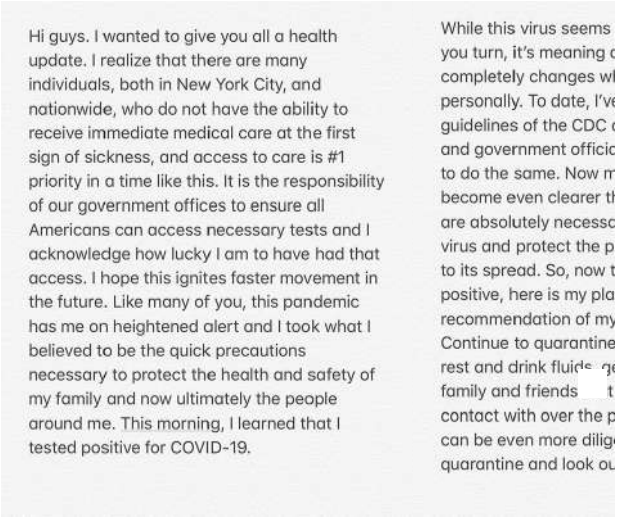


- **Jeff Shell**: The CEO of NBCUniversal—who took over the executive role of the nearly $34 billion entertainment giant on January 1—announced he had tested positive for COVID-19 in an email to his staff on March 26.
- **Prince Charles:** The 71-year-old heir to the British throne tested positive for novel coronavirus on March 25 and is self-isolating in Scottish royal estate, according to a Clarence House statement. His last public engagement was March 12.
- **Harvey Weinstein:** The recently convicted Hollywood mogul, who is serving a 23-year prison sentence for rape and sexual assault near Buffalo, New York, was announced positive for Coronavirus on Sunday and is doing time in isolation.
- **Rand Paul:** The Kentucky Senator became the first senate member to announce positive results for COcVID-19. He's asymptomatic and self-quarantined,

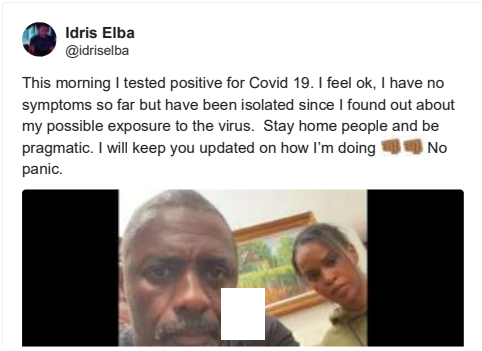


- **Andy Cohen:** Bravo's "Watch What Happens Live" host Andy Cohen announced to his 3.7 million Instagram followers that he had tested positive for Covid-19 on March 21 with a selfie and message thanking medical professionals.

- **Prince Albert II of Monaco**: tested positive yesterday, according to a statement from the Palace parlayed to CNN.
- **Kevin Durant:** The Basketball-turned-investor mogul announced he had Covid-19 on March 17.
- **Arielle Charnas:** The social media influencer announced to her 1.3 million followers on Wednesday that she was experiencing symptoms of coronavirus. She tested positive on Wednesday, and documented the experience on Instagram.



- **Idris Elba**: The actor best known for his roles in TV shows "The Wire", "Luther" and for playing Nelson Mandela in *Nelson Mandela: Long Walk to Freedom*, announced on Twitter that he tested positive for Covid-19. Though he bore no symptoms, he came into contact with someone who tested positive on Tuesday, and thus the actor sought the test, according to CNN.



- **Kristofer Hivju:** Perhaps best known as Tormund Giantsbane, the Game of Thrones star tested positive for Covid-19 on March 17 with an Instagram post:

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422,778 likes

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- **Olga Kurylenko:** Bond girl Kurylenko tested positive for the virus five days ago
- **Donovan Mitchell:** A Utah Jazz teammate of early Covid-19 Rudy Gobert, announced that he had tested positive for the virus, despite experiencing no symptoms.
- **Sophie Gregoire Trudeau:** The Canadian Prime Minister's wife tested positive for novel coronavirus six days ago, and Justin Trudeau announced he would go into 14 days of self-isolation to prevent the spread.
- **Francis Suarez:** The mayor of Miami announced that he tested positive for novel coronavirus last Thursday and posted this opinion piece on the New York Times, discussing his isolation from his wife and children and decision to shut restaurants, nightclubs etc. amid the coronavirus crisis, saying, "While this may seem inconvenient in the short term, it can make all the difference in the long run. We must practice social isolation now to flatten the curve."
- **Rudy Gobert:** The Utah Jazz player tested positive for COVID-19 on March 11, a key factor in the NBA's hiatus.
- **Tom Hanks, Rita Wilson:** Both tested positive on March 11, becoming the first Hollywood a-listers to confirm they had the virus

View More on Instagram

3,199,034 likes

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Background: Celebrities and wealthy people have a significantly easier time accessing coronavirus testing, according to The New York Times. The shortage and failures of Covid-19 testing has been well-documented by national media and individuals on social media.

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Alexandra Sternlicht

I'm the Under 30 Editorial Community Lead at Forbes. Previously, I directed marketing at a mobile app startup. I've also worked at The New York Times and New York... **Read More**

Characteristics of COVID-19 patients dying in Italy
Report based on available data on March 20th, 2020

1. Sample

The present report describes characteristics of 3200 COVID-19 patients dying in Italy.* Geographic distribution across the 19 regions and 2 autonomous provinces of Trento and Bozen is presented in the table below. Data are update to March 20th, 2020.

REGIONS	N	%
Abruzzo	7	0.2
Bolzano	14	0.4
Calabria	1	0.0
Campania	17	0.5
Emilia-Romagna	524	16.4
Friuli-Venezia Giulia	35	1.1
Lazio	31	1.0
Liguria	90	2.8
Lombardia	2175	68.0
Marche	36	1.1
Molise	3	0.1
Piemonte	69	2.2
Puglia	27	0.8
Sardegna	2	0.1
Sicilia	3	0.1
Toscana	14	0.4
Trento	12	0.4
Umbria	4	0.1
Veneto	136	4.3
Total	**3200**	**100.0**

* COVID-19 related deaths presented in this report are those occurring in patients who test positive for SARSCoV-2 RT by PCR, independently from pre-existing diseases.

2. *Demographics*

Mean age of patients dying for COVID-2019 infection was 78.5 (median 80, range 31-103, IQR 73 -85). Women were 942 (29.4%). *Figure 1* shows that median age of patients dying for COVID-2019 infection was more than 15 years higher as compared with the national sample diagnosed with COVID-2019 infection (median age 63 years). *Figure 2* shows the absolute number of deaths by age group. Women dying for COVID-2019 infection had an older age than men (median age women 82 - median age men 79).

Figure 1. *Median age of patients with COVID-2019 infection and COVID-19 positive deceased patients*

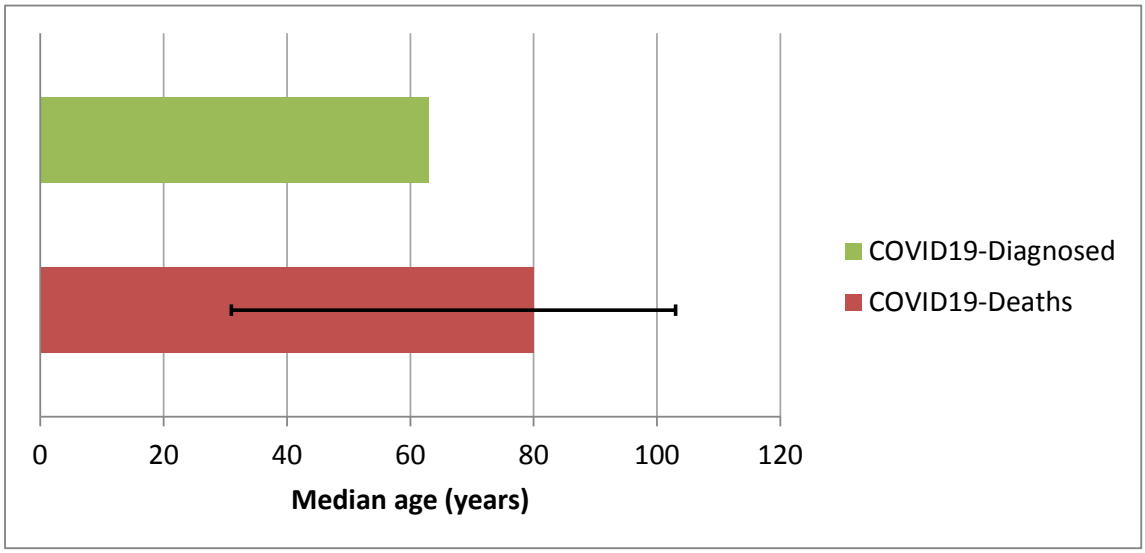
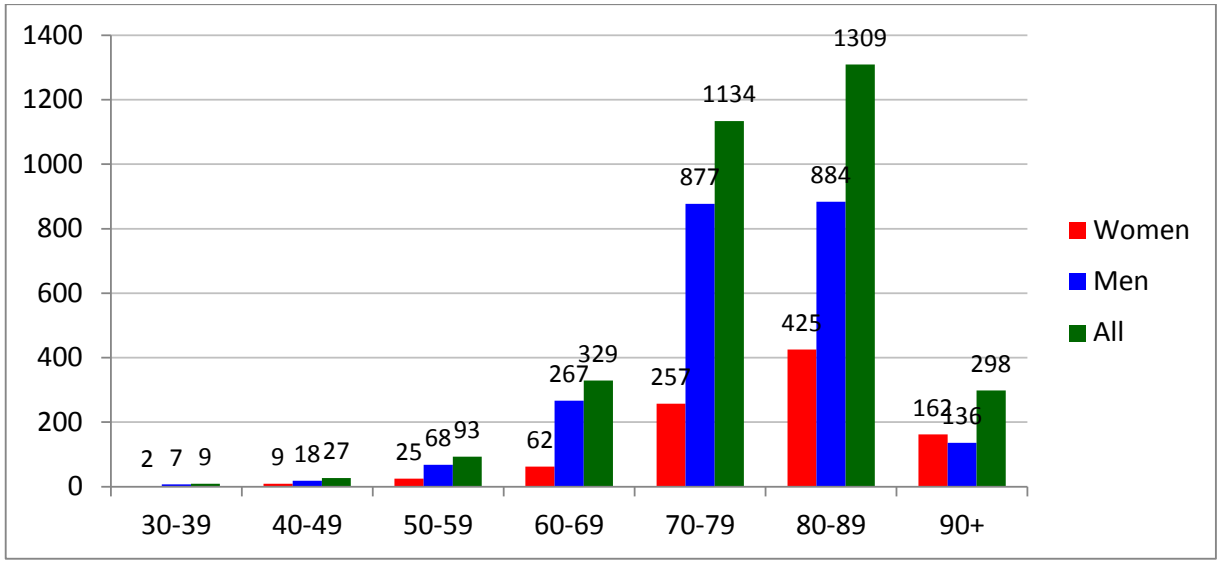


Figure 2. *Absolute number of deaths by age group*



3. Pre-existing conditions

Table 1 presents most common comorbidities diagnosed before COVID-2019 infection. Data on diseases were based on chart review and was available on 481/3200 patients dying in-hospital (15.0% of the sample). Mean number of diseases was 2.7 (median 2, SD 1.6). Overall, 1.2% of the sample presented with a no comorbidities, 23.5% with a single comorbidity, 26.6% with 2, and 48.6% with 3 or more.

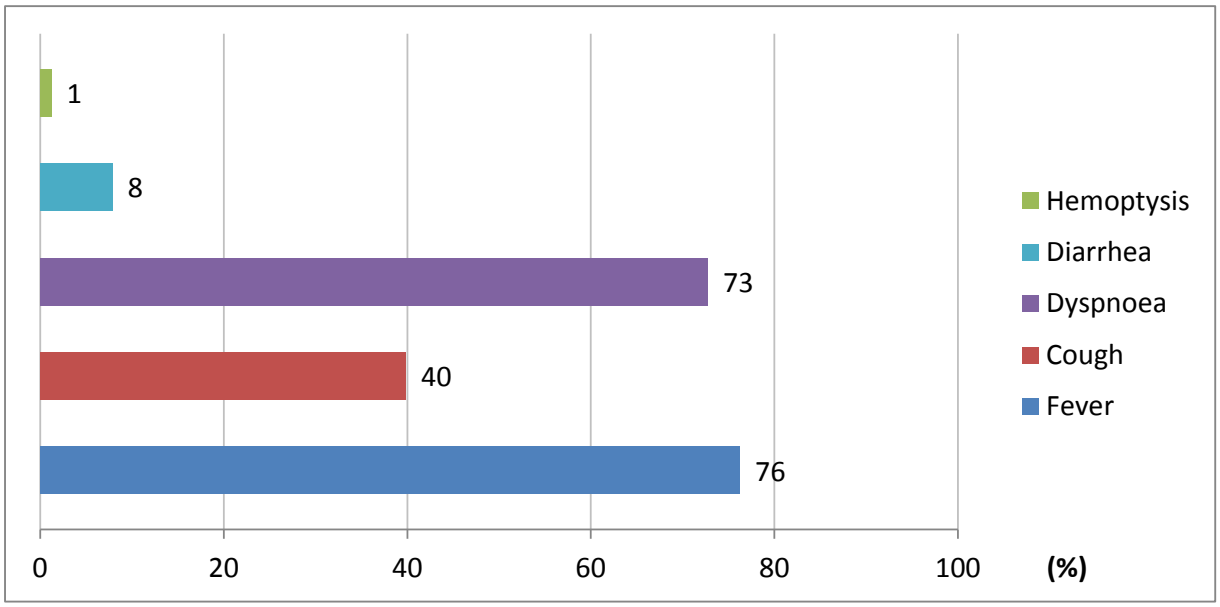
Table 1. *Most common comorbidities observed in COVID-19 positive deceased patients*

Diseases	***N***	***%***
schemic heart disease	*145*	*30.1*
Atrial Fibrillation	*106*	*22.0*
Stroke	*54*	*11.2*
Hypertension	*355*	*73.8*
Diabetes	*163*	*33.9*
Dementia	*57*	*11.9*
COPD	*66*	*13.7*
Active cancer in the past 5 years	*94*	*19.5*
Chronic liver disease	*18*	*3.7*
Chronic renal failure	*97*	*20.2*
Number of comorbidities		
0 comorbidities	*6*	*1.2*
1 comorbidity	*113*	*23.5*
2 comorbidities	*128*	*26.6*
3 comorbidities and over	*234*	*48.6*

4. *Symptoms*

Figure 3 shows symptoms most commonly observed at hospital admission. Fever and dyspnoea were the most commonly observed symptoms, while cough, diarrhoea and haemoptysis were less commonly observed. Overall, 5.7% of patients did not present any symptoms at hospital admission.

Figure 3. *Most common symptoms observed in COVID-19 positive deceased patients*



5. *Acute conditions*

Acute Respiratory Distress syndrome was observed in the majority of patients (**96.5% of cases**), followed by acute renal failure (29.2%). Acute cardiac injury was observed in 10.4% of cases and superinfection in 8.5%.

6. *Treatments*

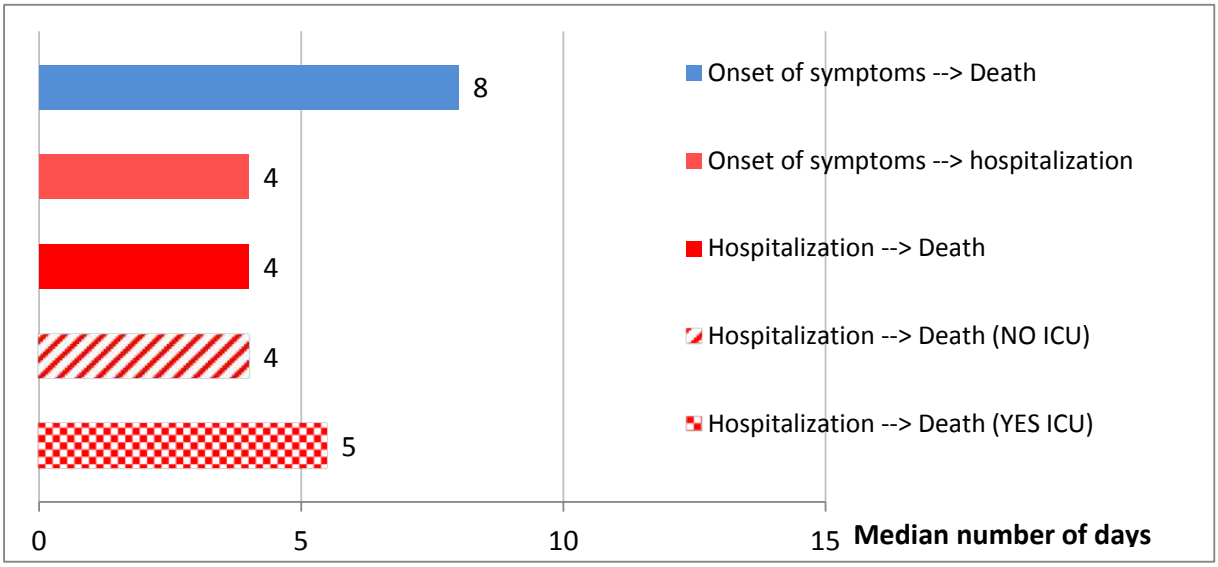
Antibiotics were used by 84% of patients during hospital stay, while less used were antivirals (54%) and corticosteroids (31%). Concomitant use of these 3 treatments was observed in 18.6% of cases.

Before hospitalization, 36% of COVID-19 positive deceased patients followed ACE-inhibitor therapy and 16% angiotensin receptor blockers-ARBs therapy. This information can be underestimated because data on drug treatment before admission were not always described in the chart.

7. Time-line

Figure 4 shows, for COVID-19 positive deceased patients, the median times, in days, from the onset of symptoms to death (8 days), from the onset of symptoms to hospitalization (4 days) and from hospitalization to death (4 days). The time from hospitalization to death was 1 day longer in those who were transferred to intensive care than those who were not transferred (5 days vs. 4 days).

Figure 5. *Median hospitalization times (in days) in COVID-19 positive deceased patients*



8. Deaths under the age of 50 years

To date (March the 20th), 36 of 3200 (1.1%) COVID-19 positive patients under the age of 50 have died. In particular, 9 of these were younger than 40 years, 8 men and 1 woman (age range between 31 and 39 years). For 2 patients under the age of 40 years, no clinical information is available; the remaining 7 had serious pre-existing pathologies (cardiovascular, renal, psychiatric pathologies, diabetes, obesity).

This report was produced by COVID-19 Surveillance Group

Members of the COVID-19 Surveillance Group

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euroMOMO

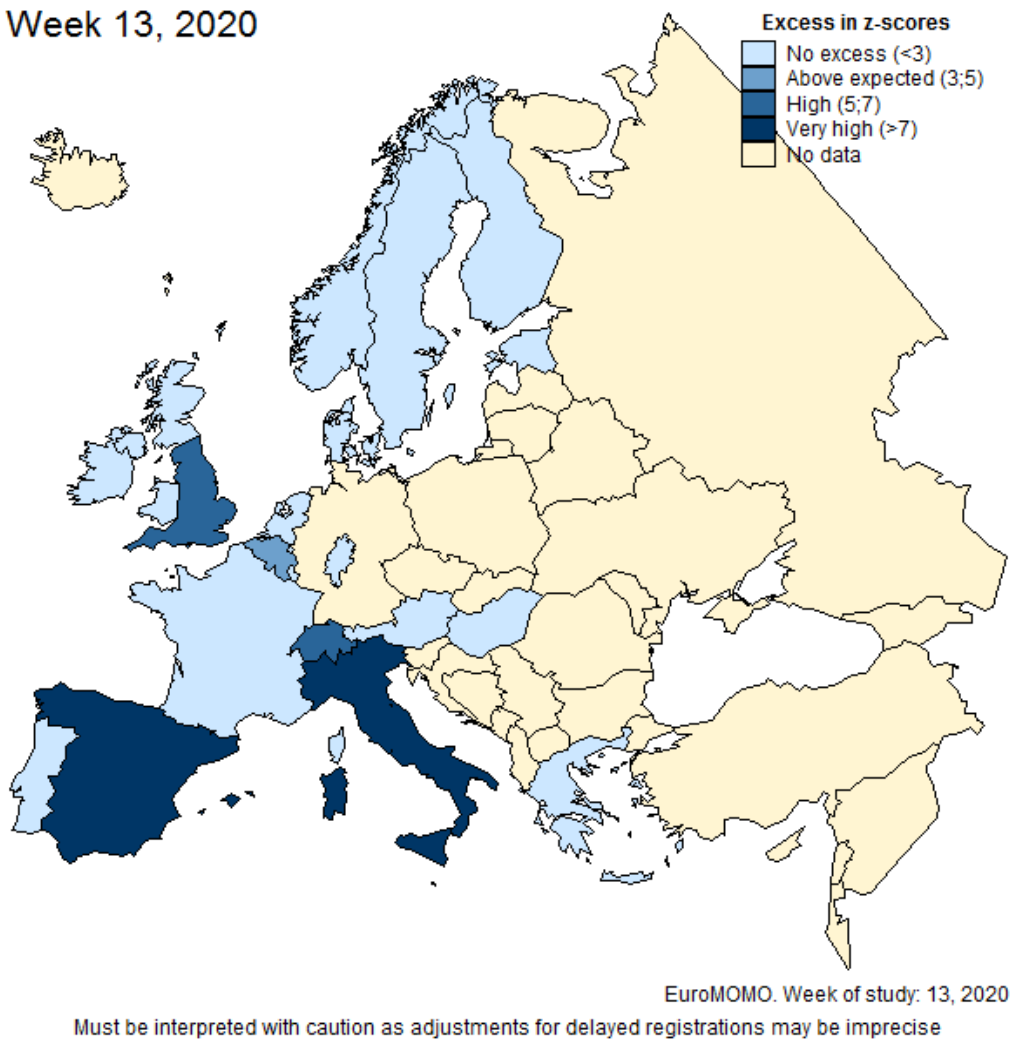
European monitoring of excess mortality for public health action

European Mortality Bulletin, week 13, 2020:

Pooled estimates from the EuroMOMO network show excess all-cause mortality, overall, for the participating countries; however, this pooled excess mortality is driven by a particularly high excess mortality in some countries, primarily seen in the age group of 65 years and above.

Data from 24 participating countries or regions were included in this week's pooled analysis of all-cause mortality in Europe.

The number of deaths in the recent weeks should be interpreted with caution as adjustments for delayed registrations may be imprecise. Furthermore, results of pooled analyses may vary depending on countries included in the weekly analyses. Pooled analyses are adjusted for variation between the included countries and for differences in the local delay in reporting. Further details are available on http://www.euromomo.eu.



euroMOMO

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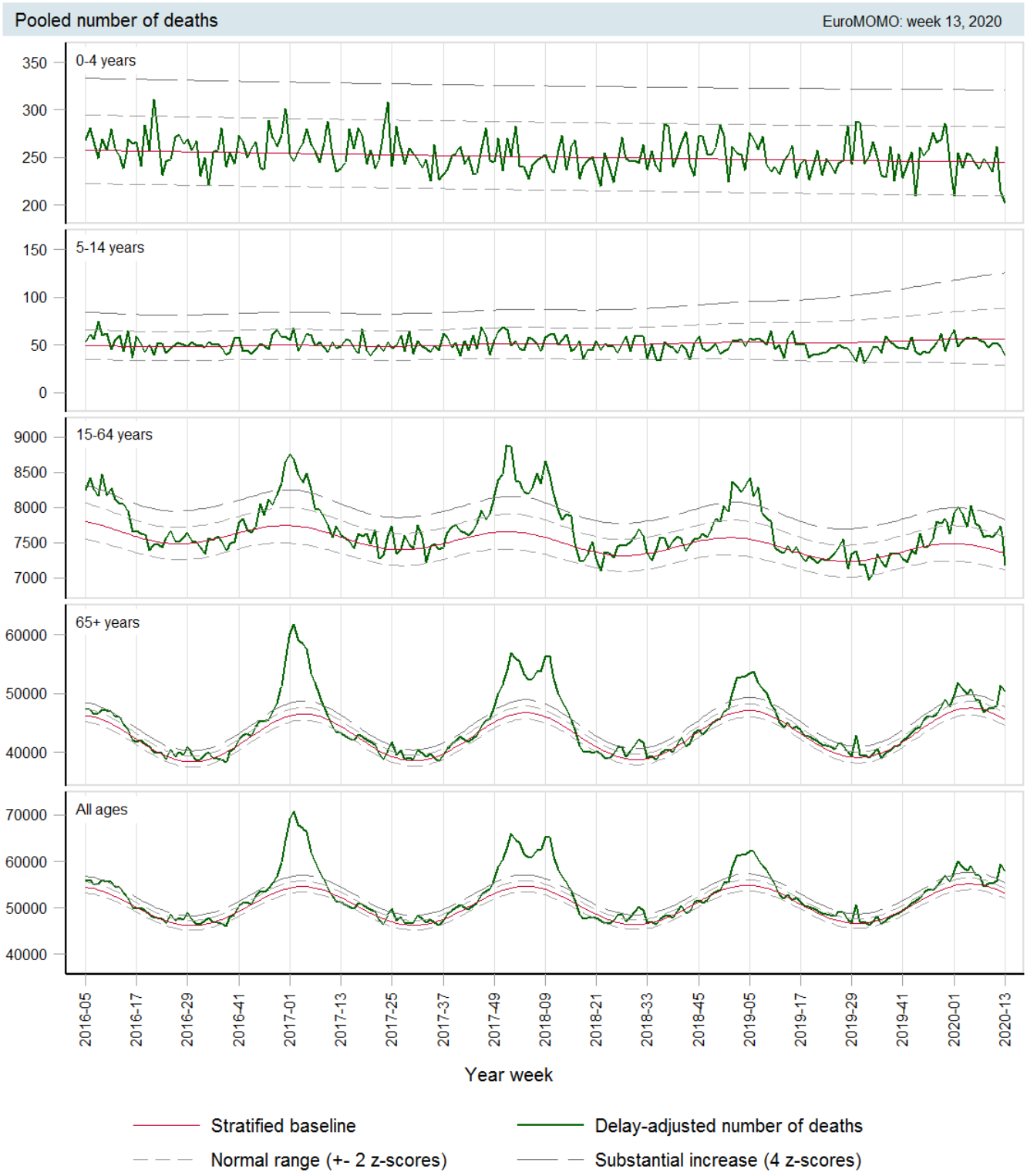

Participating countries:
Austria, Belgium, Denmark, Estonia, Finland, France, Germany (Berlin), Germany (Hesse), Greece, Hungary, Ireland, Italy, Luxembourg
Malta, Netherlands, Norway, Portugal, Spain, Sweden, Switzerland, UK (England), UK (Northern Ireland), UK (Scotland), UK (Wales)

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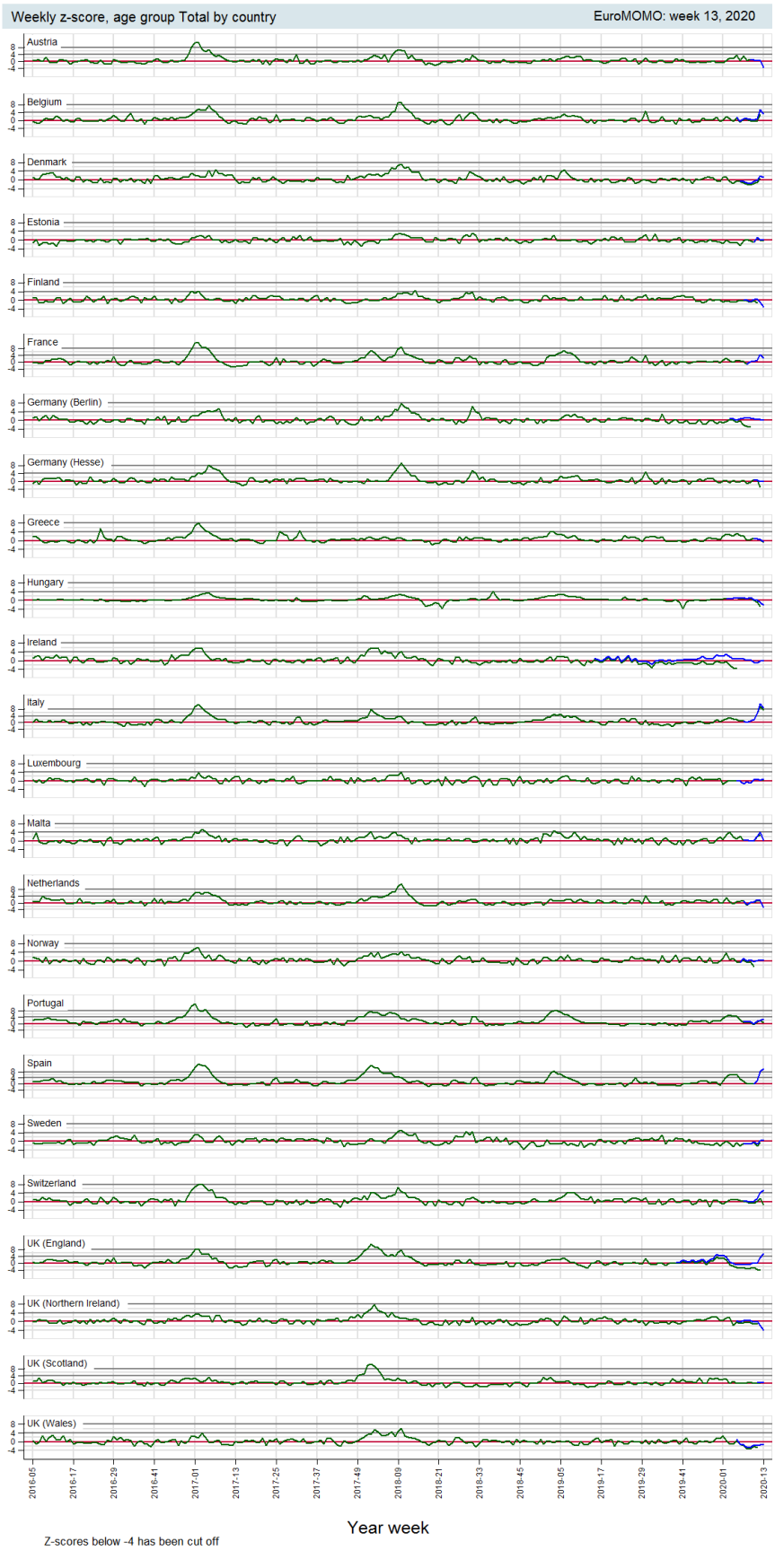
European monitoring of excess mortality for public health action



GOV.UK

Guidance

High consequence infectious diseases (HCID)

Guidance and information about high consequence infectious diseases and their management in England.

Published 22 October 2018
Last updated 21 March 2020 — see all updates

From:
Public Health England (https://www.gov.uk/government/organisations/public-health-england)

Contents

Status of COVID-19

As of 19 March 2020, COVID-19 is no longer considered to be a high consequence infectious diseases (HCID) in the UK.

The 4 nations public health HCID group made an interim recommendation in January 2020 to classify COVID-19 as an HCID. This was based on consideration of the UK HCID criteria about the virus and the disease with information available during the early stages of the outbreak. Now that more is known about COVID-19, the public health bodies in the UK have reviewed the most up to date information about COVID-19 against the UK HCID criteria. They have determined that several features have now changed; in particular, more information is available about mortality rates (low overall), and there is now greater clinical awareness and a specific and sensitive laboratory test, the availability of which continues to increase.

The Advisory Committee on Dangerous Pathogens (ACDP) is also of the opinion that COVID-19 should no longer be classified as an HCID.

The need to have a national, coordinated response remains, but this is being met by the government's COVID-19 response (https://www.gov.uk/coronavirus).

Cases of COVID-19 are no longer managed by HCID treatment centres only. All healthcare workers managing possible and confirmed cases should follow the updated national infection and prevention (IPC) guidance for COVID-19 (https://www.gov.uk/government/publications/wuhan-novel-coronavirus-infection-prevention-and-control), which supersedes all previous IPC guidance for COVID-19. This guidance includes instructions about different personal protective equipment (PPE) ensembles that are appropriate for different clinical scenarios.

Definition of HCID

In the UK, a high consequence infectious disease (HCID) is defined according to the following criteria:

- acute infectious disease
- typically has a high case-fatality rate
- may not have effective prophylaxis or treatment
- often difficult to recognise and detect rapidly
- ability to spread in the community and within healthcare settings
- requires an enhanced individual, population and system response to ensure it is managed effectively, efficiently and safely

Classification of HCIDs

HCIDs are further divided into contact and airborne groups:

- contact HCIDs are usually spread by direct contact with an infected patient or infected fluids, tissues and other materials, or by indirect contact with contaminated materials and fomites
- airborne HCIDs are spread by respiratory droplets or aerosol transmission, in addition to contact routes of transmission

List of high consequence infectious diseases

A list of HCIDs has been agreed by a joint Public Health England (PHE) and NHS England HCID Programme:

Contact HCID	Airborne HCID
Argentine haemorrhagic fever (Junin virus)	Andes virus infection (hantavirus)
Bolivian haemorrhagic fever (Machupo virus)	Avian influenza A H7N9 and H5N1
Crimean Congo haemorrhagic fever (CCHF)	Avian influenza A H5N6 and H7N7
Ebola virus disease (EVD)	Middle East respiratory syndrome (MERS)
Lassa fever	Monkeypox
Lujo virus disease	Nipah virus infection
Marburg virus disease (MVD)	Pneumonic plague (Yersinia pestis)
Severe fever with thrombocytopaenia syndrome (SFTS)	Severe acute respiratory syndrome (SARS)*

*No cases reported since 2004, but SARS remains a notifiable disease under the International Health

Regulations (2005), hence its inclusion here

**Human to human transmission has not been described to date for avian influenza A(H5N6). Human to human transmission has been described for avian influenza A(H5N1), although this was not apparent until more than 30 human cases had been reported. Both A(H5N6) and A(H5N1) often cause severe illness and fatalities. Therefore, A(H5N6) has been included in the airborne HCID list despite not meeting all of the HCID criteria.

The list of HCIDs will be kept under review and updated by PHE if new HCIDs emerge that are of relevance to the UK.

HCIDs in the UK

HCIDs, including viral haemorrhagic fevers (VHFs), are rare in the UK. When cases do occur, they tend to be sporadic and are typically associated with recent travel to an area where the infection is known to be endemic or where an outbreak is occurring. None of the HCIDs listed above are endemic in the UK, and the known animal reservoirs are not found in the UK.

As of February 2020, 2019, the UK has experience of managing confirmed cases of Lassa fever, EVD, CCHF, MERS and monkeypox. The vast majority of these patients acquired their infections overseas, but rare incidents of secondary transmission of MERS and monkeypox have occurred in the UK.

HCID risks by country

For health professionals wishing to determine the HCID risk in any particular country, an A to Z list of countries and their respective HCID risk is available.

See HCID country risks (https://www.gov.uk/guidance/high-consequence-infectious-disease-country-specific-risk)

Monthly summaries of global HCID events

PHE's epidemic intelligence activities monitor global HCID events. These are published in a monthly summary (https://www.gov.uk/government/publications/high-consequence-infectious-diseases-monthly-summaries).

Infection prevention and control in healthcare settings

Specific infection prevention and control (IPC) measures are required for suspected and confirmed HCID cases, in all healthcare settings (specialist and non-specialist).

IPC guidance appropriate for suspected and confirmed cases of Lassa fever, EVD, CCHF, MVD, Lujo virus disease, Argentinian haemorrhagic fever, Bolivian haemorrhagic fever and SFTS, is available in the ACDP guidance (https://www.gov.uk/government/publications/viral-haemorrhagic-fever-algorithm-and-guidance-on-management-of-patients).

IPC guidance for MERS, avian influenza, Nipah virus infection, monkeypox and pneumonic plague, can be found in the relevant PHE guidance listed below.

Links to relevant PHE guidance for healthcare professionals

- avian influenza (https://www.gov.uk/government/collections/avian-influenza-guidance-data-and-analysis)

- MERS (https://www.gov.uk/government/collections/middle-east-respiratory-syndrome-coronavirus-mers-cov-clinical-management-and-guidance)
- monkeypox (https://www.gov.uk/guidance/monkeypox)
- Nipah virus infection (https://www.gov.uk/guidance/nipah-virus-epidemiology-outbreaks-and-guidance)
- plague (https://www.gov.uk/guidance/plague-epidemiology-outbreaks-and-guidance)
- VHF, including Ebola (https://www.gov.uk/government/collections/viral-haemorrhagic-fevers-epidemiology-characteristics-diagnosis-and-management)

Specialist advice for healthcare professionals

The Imported Fever Service (IFS) (https://www.gov.uk/guidance/imported-fever-service-ifs) provides 24-hour, 7-days a week telephone access to expert clinical and microbiological advice. Hospital doctors across the UK can contact the IFS after discussion with the local microbiology, virology or infectious disease consultant.

Hospital management of confirmed HCID cases

Once an HCID has been confirmed by appropriate laboratory testing, cases in England should be transferred rapidly to a designated HCID Treatment Centre. Occasionally, highly probable cases may be moved to an HCID Treatment Centre before laboratory results are available.

Contact HCIDs

There are 2 principal Contact HCID Treatment Centres in England:

- the Royal Free London High Level Isolation Unit (HLIU)
- the Newcastle Royal Victoria Infirmary HLIU.

Further support for managing confirmed contact HCID cases is provided by the Royal Liverpool Hospital and the Royal Hallamshire Hospital, Sheffield.

Airborne HCIDs

There are 4 interim Airborne HCID Treatment Centres in England. Adult and paediatric services are provided by 6 NHS Trusts:

- Guy's and St Thomas' NHS Foundation Trust (adult and paediatric services)
- Royal Free London NHS Foundation Trust, with a paediatric service provided by Imperial College Healthcare NHS Foundation Trust
- Royal Liverpool and Broadgreen University Hospitals NHS Trust, with a paediatric service provided by Alder Hey Children's NHS Foundation Trust
- Newcastle upon Tyne Hospitals NHS Foundation Trust (adult and paediatric services)

Case transfer arrangements

Hospital clinicians seeking to transfer confirmed HCID cases, or discuss the transfer of highly probable HCID cases, should contact the NHS England EPRR Duty Officer. It is expected that each case will have been discussed with the Imported Fever Service (https://www.gov.uk/guidance/imported-fever-service-ifs) before discussing transfer.

Travel health advice for HCIDs

The National Travel Health Network and Centre (NaTHNaC) provides travel health information about a number of HCIDs, for healthcare professionals and travellers. Advice can be accessed via the Travel Health Pro website (https://travelhealthpro.org.uk/).

Published 22 October 2018
Last updated 21 March 2020 + show all updates

1. 21 March 2020
   Added explanation of the removal of COVID-19 from the list of HCIDs in the UK.
2. 16 January 2020
   Added Wuhan novel coronavirus
3. 13 May 2019
   Amended the definitions for HCID.
4. 17 April 2019
   Added explanation for inclusion of avian influenza H5N6 as an HCID.
5. 30 January 2019
   Added link to information on HCID risks by country.
6. 22 October 2018
   First published.

Related content

- High consequence infectious disease: country specific risk (https://www.gov.uk/guidance/high-consequence-infectious-disease-country-specific-risk)
- COVID-19: investigation and initial clinical management of possible cases (https://www.gov.uk/government/publications/wuhan-novel-coronavirus-initial-investigation-of-possible-cases)
- COVID-19: guidance for sampling and for diagnostic laboratories (https://www.gov.uk/government/publications/wuhan-novel-coronavirus-guidance-for-clinical-diagnostic-laboratories)
- Viral haemorrhagic fevers: epidemiology, characteristics, diagnosis and management (https://www.gov.uk/government/collections/viral-haemorrhagic-fevers-epidemiology-characteristics-diagnosis-and-management)
- Avian influenza: guidance, data and analysis (https://www.gov.uk/government/collections/avian-influenza-guidance-data-and-analysis)
- MERS-CoV: clinical management and guidance (https://www.gov.uk/government/collections/middle-east-respiratory-syndrome-coronavirus-mers-cov-clinical-management-and-guidance)

Detailed guidance

- Crimean-Congo haemorrhagic fever: origins, reservoirs, transmission and guidelines (https://www.gov.uk/guidance/crimean-congo-haemorrhagic-fever-origins-reservoirs-transmission-and-guidelines)
- Ebola and Marburg haemorrhagic fevers: outbreaks and case locations (https://www.gov.uk/guidance/ebola-and-marburg-haemorrhagic-fevers-outbreaks-and-case-locations)
- Lassa fever: origins, reservoirs, transmission and guidelines (https://www.gov.uk/guidance/lassa-fever-origins-reservoirs-transmission-and-guidelines)
- Marburg virus disease: origins, reservoirs, transmission and guidelines (https://www.gov.uk/guidance/marburg-virus-disease-origins-reservoirs-transmission-and-guidelines)

- Monkeypox (https://www.gov.uk/guidance/monkeypox)
- + 3 more
- Viral haemorrhagic fevers: origins, reservoirs, transmission and guidelines (https://www.gov.uk/guidance/viral-haemorrhagic-fevers-origins-reservoirs-transmission-and-guidelines), Nipah virus: epidemiology, outbreaks and guidance (https://www.gov.uk/guidance/nipah-virus-epidemiology-outbreaks-and-guidance), and Plague: epidemiology, outbreaks and guidance (https://www.gov.uk/guidance/plague-epidemiology-outbreaks-and-guidance)

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- Infectious diseases (https://www.gov.uk/topic/health-protection/infectious-diseases)

EDITORIAL

Covid-19 — Navigating the Uncharted

Anthony S. Fauci, M.D., H. Clifford Lane, M.D., and Robert R. Redfield, M.D.

The latest threat to global health is the ongoing outbreak of the respiratory disease that was recently given the name Coronavirus Disease 2019 (Covid-19). Covid-19 was recognized in December 2019.[1] It was rapidly shown to be caused by a novel coronavirus that is structurally related to the virus that causes severe acute respiratory syndrome (SARS). As in two preceding instances of emergence of coronavirus disease in the past 18 years[2] — SARS (2002 and 2003) and Middle East respiratory syndrome (MERS) (2012 to the present) — the Covid-19 outbreak has posed critical challenges for the public health, research, and medical communities.

In their *Journal* article, Li and colleagues[3] provide a detailed clinical and epidemiologic description of the first 425 cases reported in the epicenter of the outbreak: the city of Wuhan in Hubei province, China. Although this information is critical in informing the appropriate response to this outbreak, as the authors point out, the study faces the limitation associated with reporting in real time the evolution of an emerging pathogen in its earliest stages. Nonetheless, a degree of clarity is emerging from this report. The median age of the patients was 59 years, with higher morbidity and mortality among the elderly and among those with coexisting conditions (similar to the situation with influenza); 56% of the patients were male. Of note, there were no cases in children younger than 15 years of age. Either children are less likely to become infected, which would have important epidemiologic implications, or their symptoms were so mild that their infection escaped detection, which has implications for the size of the denominator of total community infections.

On the basis of a case definition requiring a diagnosis of pneumonia, the currently reported case fatality rate is approximately 2%.[4] In another article in the *Journal*, Guan et al.[5] report mortality of 1.4% among 1099 patients with laboratory-confirmed Covid-19; these patients had a wide spectrum of disease severity. If one assumes that the number of asymptomatic or minimally symptomatic cases is several times as high as the number of reported cases, the case fatality rate may be considerably less than 1%. This suggests that the overall clinical consequences of Covid-19 may ultimately be more akin to those of a severe seasonal influenza (which has a case fatality rate of approximately 0.1%) or a pandemic influenza (similar to those in 1957 and 1968) rather than a disease similar to SARS or MERS, which have had case fatality rates of 9 to 10% and 36%, respectively.[2]

The efficiency of transmission for any respiratory virus has important implications for containment and mitigation strategies. The current study indicates an estimated basic reproduction number (R_0) of 2.2, which means that, on average, each infected person spreads the infection to an additional two persons. As the authors note, until this number falls below 1.0, it is likely that the outbreak will continue to spread. Recent reports of high titers of virus in the oropharynx early in the course of disease arouse concern about increased infectivity during the period of minimal symptoms.[6,7]

China, the United States, and several other countries have instituted temporary restrictions on travel with an eye toward slowing the spread of this new disease within China and throughout the rest of the world. The United States has seen a dramatic reduction in the number of travelers from China, especially from Hubei province.

At least on a temporary basis, such restrictions may have helped slow the spread of the virus: whereas 78,191 laboratory-confirmed cases had been identified in China as of February 26, 2020, a total of 2918 cases had been confirmed in 37 other countries or territories.[4] As of February 26, 2020, there had been 14 cases detected in the United States involving travel to China or close contacts with travelers, 3 cases among U.S. citizens repatriated from China, and 42 cases among U.S. passengers repatriated from a cruise ship where the infection had spread.[8] However, given the efficiency of transmission as indicated in the current report, we should be prepared for Covid-19 to gain a foothold throughout the world, including in the United States. Community spread in the United States could require a shift from containment to mitigation strategies such as social distancing in order to reduce transmission. Such strategies could include isolating ill persons (including voluntary isolation at home), school closures, and telecommuting where possible.[9]

A robust research effort is currently under way to develop a vaccine against Covid-19.[10] We anticipate that the first candidates will enter phase 1 trials by early spring. Therapy currently consists of supportive care while a variety of investigational approaches are being explored.[11] Among these are the antiviral medication lopinavir–ritonavir, interferon-1β, the RNA polymerase inhibitor remdesivir, chloroquine, and a variety of traditional Chinese medicine products.[11] Once available, intravenous hyperimmune globulin from recovered persons and monoclonal antibodies may be attractive candidates to study in early intervention. Critical to moving the field forward, even in the context of an outbreak, is ensuring that investigational products are evaluated in scientifically and ethically sound studies.[12]

Every outbreak provides an opportunity to gain important information, some of which is associated with a limited window of opportunity. For example, Li et al. report a mean interval of 9.1 to 12.5 days between the onset of illness and hospitalization. This finding of a delay in the progression to serious disease may be telling us something important about the pathogenesis of this new virus and may provide a unique window of opportunity for intervention. Achieving a better understanding of the pathogenesis of this disease will be invaluable in navigating our responses in this uncharted arena. Furthermore, genomic studies could delineate host factors that predispose persons to acquisition of infection and disease progression.

The Covid-19 outbreak is a stark reminder of the ongoing challenge of emerging and reemerging infectious pathogens and the need for constant surveillance, prompt diagnosis, and robust research to understand the basic biology of new organisms and our susceptibilities to them, as well as to develop effective countermeasures.

Disclosure forms provided by the authors are available with the full text of this editorial at NEJM.org.

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The many estimates of the COVID-19 case fatality rate

Since the outbreak of coronavirus disease 2019 (COVID-19) began in December, a question at the forefront of many people's minds has been its mortality rate. Is the mortality rate of COVID-19 higher than that of influenza, but lower than that of severe acute respiratory syndrome (SARS)?

The trend in mortality reporting for COVID-19 has been typical for emerging infectious diseases. The case fatality rate (CFR) was reported to be 15% (six of 41 patients) in the initial period,[1] but this estimate was calculated from a small cohort of hospitalised patients. Subsequently, with more data emerging, the CFR decreased to between 4·3% and 11·0%,[2,3] and later to 3·4%.[4] The rate reported outside China in February was even lower (0·4%; two of 464).[5]

This pattern of decreasing CFRs is not surprising during the initial phase of an outbreak. Hard outcomes such as the CFR have a crucial part in forming strategies at national and international levels from a public health perspective. It is imperative that health-care leaders and policy makers are guided by estimates of mortality and case fatality.

However, several factors can restrict obtaining an accurate estimate of the CFR. The virus and its clinical course are new, and we still have little information about them. Health care capacity and capability factors, including the availability of health-care workers, resources, facilities, and preparedness, also affect outcomes. For example, some countries are able to invest resources into contact tracing and containing the spread through quarantine and isolation of infected or suspected cases. In Singapore, where these measures have been implemented, the CFR of 631 cases (as of March 25, 2020) is 0·3%. In other places, testing might not be widely available, and proactive contact tracing and containment might not be employed, resulting in a smaller denominator and skewing to a higher CFR. The CFR can increase in some places if there is a surge of infected patients, which adds to the strain on the health-care system and can overwhelm its medical resources.

A major challenge with accurate calculation of the CFR is the denominator: the number of people who are infected with the virus. Asymptomatic cases of COVID-19, patients with mild symptoms, or individuals who are misdiagnosed could be left out of the denominator, leading to its underestimation and overestimation of the CFR.

A unique situation has arisen for quite an accurate estimate of the CFR of COVID-19. Among individuals onboard the Diamond Princess cruise ship, data on the denominator are fairly robust. The outbreak of COVID-19 led passengers to be quarantined between Jan 20, and Feb 29, 2020. This scenario provided a population living in a defined territory without most other confounders, such as imported cases, defaulters of screening, or lack of testing capability. 3711 passengers and crew were onboard, of whom 705 became sick and tested positive for COVID-19 and seven died,[6] giving a CFR of 0·99%. If the passengers onboard were generally of an older age, the CFR in a healthy, younger population could be lower.[7]

Although highly transmissible, the CFR of COVID-19 appears to be lower than that of SARS (9·5%) and Middle East respiratory syndrome (34·4%),[8] but higher than that of influenza (0·1%).[9,10]

We declare no competing interests.

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CDC 2019-Novel Coronavirus (2019-nCoV) Real-Time RT-PCR Diagnostic Panel

For Emergency Use Only

Instructions for Use

Catalog # 2019-nCoVEUA-01
1000 reactions

For *In-vitro* Diagnostic (IVD) Use

Rx Only

Centers for Disease Control and Prevention
Division of Viral Diseases
1600 Clifton Rd NE
Atlanta GA 30329

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Intended Use

The CDC 2019-Novel Coronavirus (2019-nCoV) Real-Time RT-PCR Diagnostic Panel is a real-time RT-PCR test intended for the qualitative detection of nucleic acid from the 2019-nCoV in upper and lower respiratory specimens (such as nasopharyngeal or oropharyngeal swabs, sputum, lower respiratory tract aspirates, bronchoalveolar lavage, and nasopharyngeal wash/aspirate or nasal aspirate) collected from individuals who meet 2019-nCoV clinical and/or epidemiological criteria (for example, clinical signs and symptoms associated with 2019-nCoV infection, contact with a probable or confirmed 2019-nCoV case, history of travel to geographic locations where 2019-nCoV cases were detected, or other epidemiologic links for which 2019-nCoV testing may be indicated as part of a public health investigation). Testing in the United States is limited to laboratories certified under the Clinical Laboratory Improvement Amendments of 1988 (CLIA), 42 U.S.C. § 263a, to perform high complexity tests.

Results are for the identification of 2019-nCoV RNA. The 2019-nCoV RNA is generally detectable in upper and lower respiratory specimens during infection. Positive results are indicative of active infection with 2019-nCoV but do not rule out bacterial infection or co-infection with other viruses. The agent detected may not be the definite cause of disease. Laboratories within the United States and its territories are required to report all positive results to the appropriate public health authorities.

Negative results do not preclude 2019-nCoV infection and should not be used as the sole basis for treatment or other patient management decisions. Negative results must be combined with clinical observations, patient history, and epidemiological information.

Testing with the CDC 2019-nCoV Real-Time RT-PCR Diagnostic Panel is intended for use by trained laboratory personnel who are proficient in performing real-time RT-PCR assays. The CDC 2019-Novel Coronavirus (2019-nCoV) Real-Time RT-PCR Diagnostic Panel is only for use under a Food and Drug Administration's Emergency Use Authorization.

Summary and Explanation

An outbreak of pneumonia of unknown etiology in Wuhan City, Hubei Province, China was initially reported to WHO on December 31, 2019. Chinese authorities identified a novel coronavirus (2019-nCoV), which has resulted in thousands of confirmed human infections in multiple provinces throughout China and many countries including the United States. Cases of asymptomatic infection, mild illness, severe illness, and some deaths have been reported.

The CDC 2019-nCoV Real-Time RT-PCR Diagnostic Panel is a molecular *in vitro* diagnostic test that aids in the detection and diagnosis 2019-nCoV and is based on widely used nucleic acid amplification technology. The product contains oligonucleotide primers and dual-labeled hydrolysis probes (TaqMan®) and control material used in rRT-PCR for the *in vitro* qualitative detection of 2019-nCoV RNA in respiratory specimens.

The term "qualified laboratories" refers to laboratories in which all users, analysts, and any person reporting results from use of this device should be trained to perform and interpret the results from this procedure by a competent instructor prior to use.

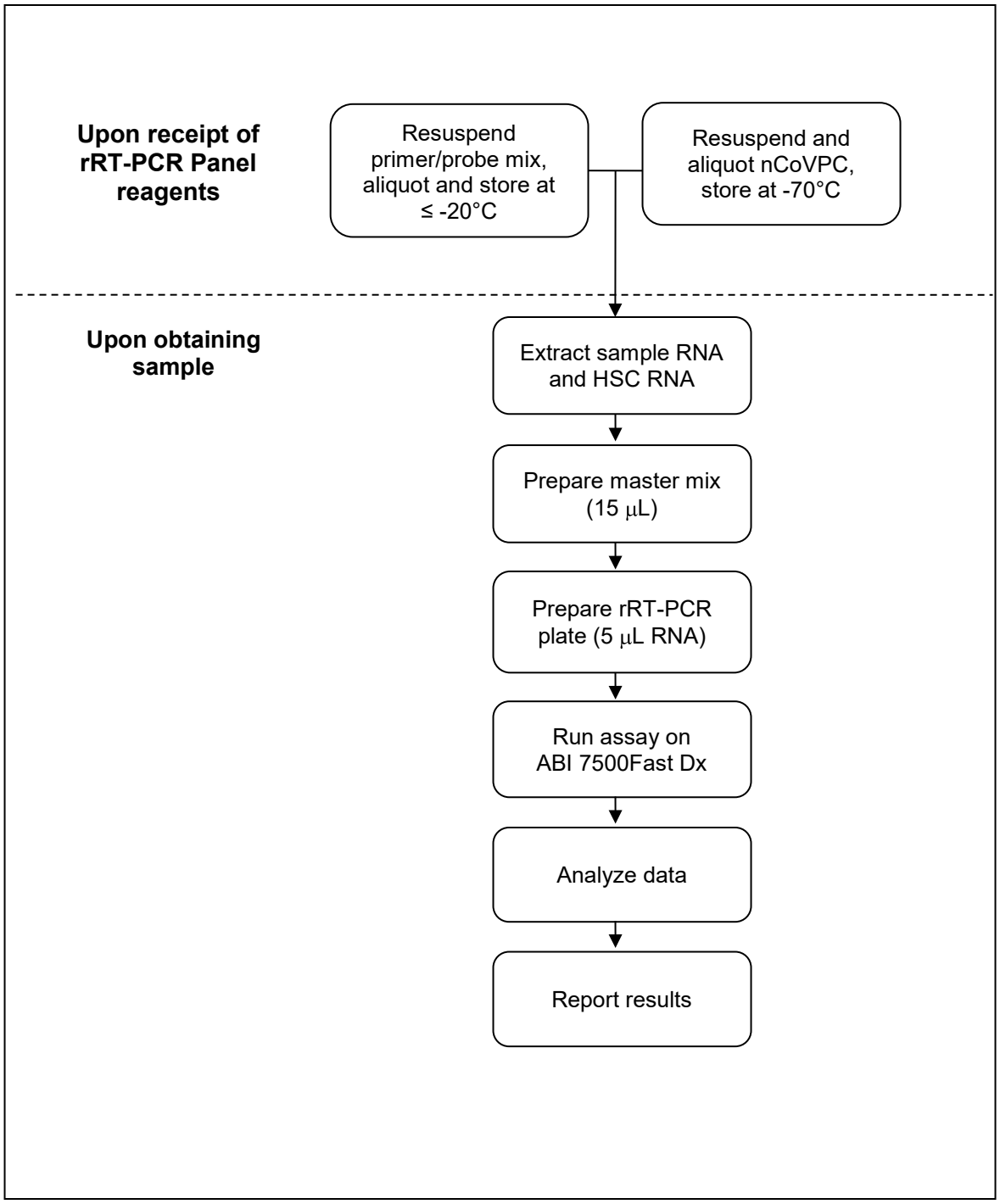
Principles of the Procedure

The oligonucleotide primers and probes for detection of 2019-nCoV were selected from regions of the virus nucleocapsid (N) gene. The panel is designed for specific detection of the 2019-nCoV (two primer/probe sets). An additional primer/probe set to detect the human RNase P gene (RP) in control samples and clinical specimens is also included in the panel.

RNA isolated and purified from upper and lower respiratory specimens is reverse transcribed to cDNA and subsequently amplified in the Applied Biosystems 7500 Fast Dx Real-Time PCR Instrument with SDS version 1.4 software. In the process, the probe anneals to a specific target sequence located between the forward and reverse primers. During the extension phase of the PCR cycle, the 5' nuclease activity of Taq polymerase degrades the probe, causing the reporter dye to separate from the quencher dye, generating a fluorescent signal. With each cycle, additional reporter dye molecules are cleaved from their respective probes, increasing the fluorescence intensity. Fluorescence intensity is monitored at each PCR cycle by Applied Biosystems 7500 Fast Dx Real-Time PCR System with SDS version 1.4 software.

Detection of viral RNA not only aids in the diagnosis of illness but also provides epidemiological and surveillance information.

Summary of Preparation and Testing Process



Materials Required (Provided)

Note: CDC will maintain on its website a list of commercially available lots of primer and probe sets and/or positive control materials that are acceptable alternatives to the CDC primer and probe set and/or positive control included in the Diagnostic Panel. Only material distributed through the CDC International Reagent Resource and specific lots of material posted to the CDC website are acceptable for use with this assay under CDC's Emergency Use Authorization.

This list of acceptable alternative lots of primer and probe materials and/or positive control materials will be available at:
https://www.cdc.gov/coronavirus/2019-nCoV/lab/index.html

Primers and Probes:

Catalog #2019-nCoVEUA-01 Diagnostic Panel Box #1:

Reagent Label	*Part #*	*Description*	*Quantity / Tube*	*Reactions / Tube*
2019-nCoV_N1	RV202001 RV202015	2019-nCoV_N1 Combined Primer/Probe Mix	22.5 nmol	1000
2019-nCoV_N2	RV202002 RV202016	2019-nCoV_N2 Combined Primer/Probe Mix	22.5 nmol	1000
RP	RV202004 RV202018	Human RNase P Forward Primer/Probe Mix	22.5 nmol	1000

Positive Control (either of the following products are acceptable)

Catalog #2019-nCoVEUA-01 Diagnostic Panel Box #2:

Reagent Label	*Part #*	*Description*	*Quantity*	*Notes*
nCoVPC	RV202005	2019-nCoV Positive Control (nCoVPC) For use as a positive control with the CDC 2019-nCoV Real-Time RT-PCR Diagnostic Panel procedure. The nCoVPC contains noninfectious positive control material supplied in a dried state and must be resuspended before use. nCoVPC consists of *in vitro* transcribed RNA. nCoVPC will yield a positive result with each assay in the 2019-nCoV Real-Time RT-PCR Diagnostic Panel including RP.	4 tubes	Provides (800) 5 µL test reactions

Catalog #VTC-04 CDC 2019-nCoV Positive Control (nCoVPC)

Reagent Label	*Part #*	*Description*	*Quantity*	*Notes*
nCoVPC	RV202005	2019-nCoV Positive Control (nCoVPC) For use as a positive control with the CDC 2019-nCoV Real-Time RT-PCR Diagnostic Panel procedure. The nCoVPC contains noninfectious positive control material supplied in a dried state and must be resuspended before use. nCoVPC consists of *in vitro* transcribed RNA. nCoVPC will yield a positive result with each assay in the 2019-nCoV Real-Time RT-PCR Diagnostic Panel including RP.	4 tubes	Provides (800) 5 µL test reactions

Materials Required (But Not Provided)

Human Specimen Control (HSC)

Description	Quantity	CDC Catalog No.
Manufactured by CDC. For use as an RNA extraction procedural control to demonstrate successful recovery of RNA as well as extraction reagent integrity. The HSC consists of noninfectious (beta-Propiolactone treated) cultured human cell material supplied as a liquid suspended in 0.01 M PBS at pH 7.2-7.4.	10 vials x 500uL	KT0189

Acceptable alternatives to HSC:

- Negative human specimen material: Laboratories may prepare a volume of human specimen material (e.g., human sera or pooled leftover negative respiratory specimens) to extract and run alongside clinical samples as an extraction control. This material should be prepared in sufficient volume to be used across multiple runs. Material should be tested prior to use as the extraction control to ensure it generates the expected results for the HSC listed in these instructions for use.
- Contrived human specimen material: Laboratories may prepare contrived human specimen materials by suspending any human cell line (e.g., A549, Hela or 293) in PBS. This material should be prepared in sufficient volume to be used across multiple runs. Material should be tested prior to use as the extraction control to ensure it generates the expected results for the HSC listed in these instructions for use.

CDC will maintain on its website a list of commercially alternative extraction controls, if applicable, that are acceptable for use with this assay under CDC's Emergency Use Authorization, at: https://www.cdc.gov/coronavirus/2019-nCoV/lab/index.html

rRT-PCR Enzyme Mastermix Options

Reagent	Quantity	Catalog No.
Quantabio qScript XLT One-Step RT-qPCR ToughMix	100 x 20 µL rxns (1 x 1 mL)	95132-100
	2000 x 20 µL rxns (1 x 20 mL)	95132-02K
	500 x 20 µL rxns (5 x 1 mL)	95132-500
Quantabio UltraPlex 1-Step ToughMix (4X)	100 x 20 µL rxns (500 µL)	95166-100
	500 x 20 µL rxns (5 x 500 µL)	95166-500
	1000 x 20 µL rxns (1 x 5 mL)	95166-01K
Promega GoTaq® Probe 1- Step RT-qPCR System	200 x 20 µL rxns (2 mL)	A6120
	1250 x 20 µL rxns 12.5 mL	A6121
Thermofisher TaqPath™ 1-Step RT-qPCR Master Mix, CG	1000 reactions	A15299
	2000 reactions	A15300

RNA Extraction Options

For each of the kits listed below, CDC has confirmed that the external lysis buffer is effective for inactivation of SARS-CoV-2.

Instrument/Manufacturer	Extraction Kit	Catalog No.
QIAGEN	[2]QIAmp DSP Viral RNA Mini Kit	50 extractions (61904)
	[2]QIAamp Viral RNA Mini Kit	50 extractions (52904) 250 extractions (52906)
QIAGEN EZ1 Advanced XL	[2]EZ1 DSP Virus Kit	48 extractions (62724) Buffer AVL (19073) EZ1 Advanced XL DSP Virus Card (9018703)
	[2]EZ1 Virus Mini Kit v2.0	48 extractions (955134) Buffer AVL (19073) EZ1 Advanced XL Virus Card v2.0 (9018708)
[1]Roche MagNA Pure LC	[2]Total Nucleic Acid Kit	192 extractions (03 038 505 001)
[1]Roche MagNA Pure Compact	[2]Nucleic Acid Isolation Kit I	32 extractions (03 730 964 001)
[1]Roche MagNA Pure 96	[2]DNA and Viral NA Small Volume Kit	576 extractions (06 543 588 001) External Lysis Buffer (06 374 913 001)
[1]QIAGEN QIAcube	[2]QIAmp DSP Viral RNA Mini Kit	50 extractions (61904)
	[2]QIAamp Viral RNA Mini Kit	50 extractions (52904) 250 extractions (52906)
[1, 3]bioMérieux NucliSENS® easyMAG® and [1, 3]bioMérieux EMAG® (Automated magnetic extraction reagents sold separately. Both instruments use the same reagents and disposables, with the exception of tips.)		EasyMAG® Magnetic Silica (280133) EasyMAG® Lysis Buffer (280134) EasyMAG® Lysis Buffer, 2 mL (200292) EasyMAG® Wash Buffers 1,2, and 3 (280130, 280131, 280132) EasyMAG® Disposables (280135) Biohit Pipette Tips (easyMAG® only) (280146) EMAG®1000µL Tips (418922)

[1]Equivalence and performance of these extraction platforms for extraction of viral RNA were demonstrated with the CDC Human Influenza Virus Real-Time RT-PCR Diagnostic Panel (K190302). Performance characteristics of these extraction platforms with 2019-nCoV (SARS CoV-2) have not been demonstrated.

[2] CDC has confirmed that the external lysis buffer used with this extraction method is effective for inactivation of SARS-CoV-2.

[3] CDC has compared the concentration of inactivating agent in the lysis buffer used with this extraction method and has determined the concentration to be within the range of concentrations found effective in inactivation of SARS-CoV-2.

Equipment and Consumables Required (But Not Provided)

- Vortex mixer
- Microcentrifuge
- Micropipettes (2 or 10 µL, 200 µL and 1000 µL)
- Multichannel micropipettes (5-50 µl)
- Racks for 1.5 mL microcentrifuge tubes
- 2 x 96-well -20°C cold blocks
- 7500 Fast Dx Real-Time PCR Systems with SDS 1.4 software (Applied Biosystems; catalog #4406985 or #4406984)
- Extraction systems (instruments): QIAGEN EZ1 Advanced XL
- Molecular grade water, nuclease-free
- 10% bleach (1:10 dilution of commercial 5.25-6.0% hypochlorite bleach)
- DNA*Zap*™ (Ambion, cat. #AM9890) or equivalent
- RNAse AwayTM (Fisher Scientific; cat. #21-236-21) or equivalent
- Disposable powder-free gloves and surgical gowns
- Aerosol barrier pipette tips
- 1.5 mL microcentrifuge tubes (DNase/RNase free)
- 0.2 mL PCR reaction plates (Applied Biosystems; catalog #4346906 or #4366932)
- MicroAmp Optical 8-cap Strips (Applied Biosystems; catalog #4323032)

Warnings and Precautions

- For *in vitro* diagnostic use (IVD).
- For emergency use only.
- Follow standard precautions. All patient specimens and positive controls should be considered potentially infectious and handled accordingly.
- Do not eat, drink, smoke, apply cosmetics or handle contact lenses in areas where reagents and human specimens are handled.
- Handle all specimens as if infectious using safe laboratory procedures. Refer to Interim Laboratory Biosafety Guidelines for Handling and Processing Specimens Associated with 2019-nCoV https://www.cdc.gov/coronavirus/2019-nCoV/lab-biosafety-guidelines.html.
- Specimen processing should be performed in accordance with national biological safety regulations.
- If infection with 2019-nCoV is suspected based on current clinical and epidemiological screening criteria recommended by public health authorities, specimens should be collected with appropriate infection control precautions.
- Performance characteristics have been determined with human upper respiratory specimens and lower respiratory tract specimens from human patients with signs and symptoms of respiratory infection.
- Perform all manipulations of live virus samples within a Class II (or higher) biological safety cabinet (BSC).
- Use personal protective equipment such as (but not limited to) gloves, eye protection, and lab coats when handling kit reagents while performing this assay and handling materials including samples, reagents, pipettes, and other equipment and reagents.

- Amplification technologies such as PCR are sensitive to accidental introduction of PCR product from previous amplifications reactions. Incorrect results could occur if either the clinical specimen or the real-time reagents used in the amplification step become contaminated by accidental introduction of amplification product (amplicon). Workflow in the laboratory should proceed in a unidirectional manner.
    - Maintain separate areas for assay setup and handling of nucleic acids.
    - Always check the expiration date prior to use. Do not use expired reagent. Do not substitute or mix reagent from different kit lots or from other manufacturers.
    - Change aerosol barrier pipette tips between all manual liquid transfers.
    - During preparation of samples, compliance with good laboratory techniques is essential to minimize the risk of cross-contamination between samples, and the inadvertent introduction of nucleases into samples during and after the extraction procedure. Proper aseptic technique should always be used when working with nucleic acids.
    - Maintain separate, dedicated equipment (e.g., pipettes, microcentrifuges) and supplies (e.g., microcentrifuge tubes, pipette tips) for assay setup and handling of extracted nucleic acids.
    - Wear a clean lab coat and powder-free disposable gloves (not previously worn) when setting up assays.
    - Change gloves between samples and whenever contamination is suspected.
    - Keep reagent and reaction tubes capped or covered as much as possible.
    - Primers, probes (including aliquots), and enzyme master mix must be thawed and maintained on cold block at all times during preparation and use.
    - Work surfaces, pipettes, and centrifuges should be cleaned and decontaminated with cleaning products such as 10% bleach, "DNA*Zap*™" or "RNase AWAY®" to minimize risk of nucleic acid contamination. Residual bleach should be removed using 70% ethanol.
- RNA should be maintained on cold block or on ice during preparation and use to ensure stability.
- Dispose of unused kit reagents and human specimens according to local, state, and federal regulations.

Reagent Storage, Handling, and Stability

- Store all dried primers and probes and the positive control, nCoVPC, at 2-8°C until re-hydrated for use. Store liquid HSC control materials at ≤ -20°C.
  Note: Storage information is for CDC primer and probe materials obtained through the International Reagent Resource. If using commercial primers and probes, please refer to the manufacturer's instructions for storage and handling.
- Always check the expiration date prior to use. Do not use expired reagents.
- Protect fluorogenic probes from light.
- Primers, probes (including aliquots), and enzyme master mix must be thawed and kept on a cold block at all times during preparation and use.
- Do not refreeze probes.
  Controls and aliquots of controls must be thawed and kept on ice at all times during preparation and use.

Specimen Collection, Handling, and Storage

Inadequate or inappropriate specimen collection, storage, and transport are likely to yield false test results. Training in specimen collection is highly recommended due to the importance of specimen quality. CLSI MM13-A may be referenced as an appropriate resource.

- Collecting the Specimen
  - Refer to Interim Guidelines for Collecting, Handling, and Testing Clinical Specimens from Patients Under Investigation (PUIs) for 2019 Novel Coronavirus (2019-nCoV) https://www.cdc.gov/coronavirus/2019-nCoV/guidelines-clinical-specimens.html
  - Follow specimen collection device manufacturer instructions for proper collection methods.
  - Swab specimens should be collected using only swabs with a synthetic tip, such as nylon or Dacron®, and an aluminum or plastic shaft. Calcium alginate swabs are unacceptable and cotton swabs with wooden shafts are not recommended. Place swabs immediately into sterile tubes containing 1-3 ml of viral transport media.
- Transporting Specimens
  - Specimens must be packaged, shipped, and transported according to the current edition of the International Air Transport Association (IATA) Dangerous Goods Regulation. Follow shipping regulations for UN 3373 Biological Substance, Category B when sending potential 2019-nCoV specimens. Store specimens at 2-8°C and ship overnight to CDC on ice pack. If a specimen is frozen at -70°C or lower, ship overnight to CDC on dry ice.
- Storing Specimens
  - Specimens can be stored at 2-8°C for up to 72 hours after collection.
  - If a delay in extraction is expected, store specimens at -70°C or lower.
  - Extracted nucleic acid should be stored at -70°C or lower.

Specimen Referral to CDC

For state and local public health laboratories:

- Ship all specimens overnight to CDC.
- Ship frozen specimens on dry ice and non-frozen specimens on cold packs.
- Refer to the International Air Transport Association (IATA - www.iata.org) for requirements for shipment of human or potentially infectious biological specimens. Follow shipping regulations for UN 3373 Biological Substance, Category B when sending potential 2019-nCoV specimens.
- Prior to shipping, notify CDC Division of Viral Diseases (see contact information below) that you are sending specimens.
- Send all samples to the following recipient:

Centers for Disease Control and Prevention
c/o STATT
Attention: Dr. Stephen Lindstrom (Unit 84)
1600 Clifton Rd., Atlanta, GA 30329-4027
Phone: (404) 639-3931

The emergency contact number for CDC Emergency Operations Center (EOC) is 770-488-7100.

All other laboratories that are CLIA certified and meet requirements to perform high complexity testing:

- Please notify your state and/or local public health laboratory for specimen referral and confirmatory testing guidance.

Reagent and Controls Preparation

NOTE: Storage information is for materials obtained through the CDC International Regent Resource. If using commercial products for testing, please refer to the manufacturer's instructions for storage, handling and preparation instructions.

Primer and Probe Preparation:

1) Upon receipt, store dried primers and probes at 2-8°C.
2) Precautions: These reagents should only be handled in a clean area and stored at appropriate temperatures (see below) in the dark. Freeze-thaw cycles should be avoided. Maintain cold when thawed.
3) Using aseptic technique, suspend dried reagents in 1.5 mL of nuclease-free water (50X working concentration) and allow to rehydrate for 15 min at room temperature in the dark.
4) Mix gently and aliquot primers/probe in 300 µL volumes into 5 pre-labeled tubes. Store a single aliquot of primers/probe at 2-8°C in the dark. Do not refreeze (stable for up to 4 months). Store remaining aliquots at ≤ -20°C in a non-frost-free freezer.

2019-nCoV Positive Control (nCoVPC) Preparation:

1) Precautions: This reagent should be handled with caution in a dedicated nucleic acid handling area to prevent possible contamination. Freeze-thaw cycles should be avoided. Maintain on ice when thawed.
2) Resuspend dried reagent in each tube in 1 mL of nuclease-free water to achieve the proper concentration. Make single use aliquots (approximately 30 µL) and store at ≤ -70°C.
3) Thaw a single aliquot of diluted positive control for each experiment and hold on ice until adding to plate. Discard any unused portion of the aliquot.

Human Specimen Control (HSC) (not provided)

1) Human Specimen Control (HSC) or one of the listed acceptable alternative extraction controls must be extracted and processed with each specimen extraction run.
2) Refer to the Human Specimen Control (HSC) package insert for instructions for use.

No Template Control (NTC) (not provided)

1) Sterile, nuclease-free water
2) Aliquot in small volumes
3) Used to check for contamination during specimen extraction and/or plate set-up

General Preparation

Equipment Preparation

Clean and decontaminate all work surfaces, pipettes, centrifuges, and other equipment prior to use. Decontamination agents should be used including 10% bleach, 70% ethanol, and *DNAzap*™ or *RNase AWAY*® to minimize the risk of nucleic acid contamination.

Nucleic Acid Extraction

Performance of the CDC 2019-nCoV Real-Time RT-PCR Diagnostic Panel is dependent upon the amount and quality of template RNA purified from human specimens. The following commercially available RNA extraction kits and procedures have been qualified and validated for recovery and purity of RNA for use with the panel:

Qiagen QIAamp® DSP Viral RNA Mini Kit or QIAamp® Viral RNA Mini Kit

Recommendation(s): Utilize 100 µL of sample and elute with 100 µL of buffer or utilize 140 µL of sample and elute with 140 µL of buffer.

Qiagen EZ1 Advanced XL

Kit: Qiagen EZ1 DSP Virus Kit and Buffer AVL (supplied separately) for offboard lysis
Card: EZ1 Advanced XL DSP Virus Card
Recommendation(s): Add 120 µL of sample to 280 µL of pre-aliquoted Buffer AVL (total input sample volume is 400 µL). Proceed with the extraction on the EZ1 Advanced XL. Elution volume is 120 µL.

Kit: Qiagen EZ1 Virus Mini Kit v2.0 and Buffer AVL (supplied separately) for offboard lysis
Card: EZ1 Advanced XL Virus Card v2.0
Recommendation(s): Add 120 µL of sample to 280 µL of pre-aliquoted Buffer AVL (total input sample volume is 400 µL). Proceed with the extraction on the EZ1 Advanced XL. Elution volume is 120 µL.

Equivalence and performance of the following extraction platforms were demonstrated with the CDC Human Influenza Virus Real-Time RT-PCR Diagnostic Panel (K190302) and based on those data are acceptable for use with the CDC 2019-nCoV Real-Time RT-PCR Diagnostic Panel.

QIAGEN QIAcube
Kit: QIAGEN QIAamp® DSP Viral RNA Mini Kit or QIAamp® Viral RNA Mini Kit
Recommendations: Utilize 140 µL of sample and elute with 100 µL of buffer.

Roche MagNA Pure LC
Kit: Roche MagNA Pure Total Nucleic Acid Kit
Protocol: Total NA External_lysis
Recommendation(s): Add 100 µL of sample to 300 µL of pre-aliquoted TNA isolation kit lysis buffer (total input sample volume is 400 µL). Elution volume is 100 µL.

Roche MagNA Pure Compact
Kit: Roche MagNA Pure Nucleic Acid Isolation Kit I
Protocol: Total_NA_Plasma100_400
Recommendation(s): Add 100 µL of sample to 300 µL of pre-aliquoted TNA isolation kit lysis buffer (total input sample volume is 400 µL). Elution volume is 100 µL.

Roche MagNA Pure 96
Kit: Roche MagNA Pure 96 DNA and Viral NA Small Volume Kit
Protocol: Viral NA Plasma Ext Lys SV Protocol
Recommendation(s): Add 100 µL of sample to 350 µL of pre-aliquoted External Lysis Buffer (supplied separately) (total input sample volume is 450 µL). Proceed with the extraction on the MagNA Pure 96. (**Note: Internal Control = None)**. Elution volume is 100 µL.

bioMérieux NucliSENS® easyMAG® Instrument
Protocol: General protocol (not for blood) using "Off-board Lysis" reagent settings.
Recommendation(s): Add 100 µL of sample to 1000 µL of pre-aliquoted easyMAG lysis buffer (total input sample volume is 1100 µL). Incubate for 10 minutes at room temperature. Elution volume is 100 µL.

bioMérieux EMAG® Instrument
Protocol: Custom protocol: **CDC Flu V1** using "Off-board Lysis" reagent settings.
Recommendation(s): Add 100 µL of samples to 2000 µL of pre-aliquoted easyMAG lysis buffer (total input sample volume is 2100 µL). Incubate for 10 minutes at room temperature. Elution volume is 100 µL. The custom protocol, **CDC Flu V1**, is programmed on the bioMérieux EMAG® instrument with the assistance of a bioMérieux service representative. Installation verification is documented at the time of installation. Laboratories are recommended to retain a record of the step-by-step verification of the bioMérieux custom protocol installation procedure.

Manufacturer's recommended procedures (except as noted in recommendations above) are to be followed for sample extraction. HSC must be included in each extraction batch.

Disclaimer: Names of vendors or manufacturers are provided as examples of suitable product sources. Inclusion does not imply endorsement by the Centers for Disease Control and Prevention.

Assay Set Up

Reaction Master Mix and Plate Set Up

Note: Plate set-up configuration can vary with the number of specimens and workday organization. NTCs and nCoVPCs must be included in each run.

1) In the reagent set-up room clean hood, place rRT-PCR buffer, enzyme, and primer/probes on ice or cold-block. Keep cold during preparation and use.
2) Mix buffer, enzyme, and primer/probes by inversion 5 times.
3) Centrifuge reagents and primers/probes for 5 seconds to collect contents at the bottom of the tube, and then place the tube in a cold rack.
4) Label one 1.5 mL microcentrifuge tube for each primer/probe set.
5) Determine the number of reactions (N) to set up per assay. It is necessary to make excess reaction mix for the NTC, nCoVPC, HSC (if included in the RT-PCR run), and RP reactions and for pipetting error. Use the following guide to determine N:
   - If number of samples (n) including controls equals 1 through 14, then N = n + 1
   - If number of samples (n) including controls is 15 or greater, then N = n + 2
7) For each primer/probe set, calculate the amount of each reagent to be added for each reaction mixture (N = # of reactions).

Thermofisher TaqPath™ 1-Step RT-qPCR Master Mix

Step #	Reagent	Vol. of Reagent Added per Reaction
1	Nuclease-free Water	N x 8.5 µL
2	Combined Primer/Probe Mix	N x 1.5 µL
3	TaqPath™ 1-Step RT-qPCR Master Mix (4x)	N x 5.0 µL
	Total Volume	**N x 15.0 µL**

Promega GoTaq® Probe 1- Step RT-qPCR System

Step #	Reagent	Vol. of Reagent Added per Reaction
1	Nuclease-free Water	N x 3.1 µL
2	Combined Primer/Probe Mix	N x 1.5 µL
3	GoTaq Probe qPCR Master Mix with dUTP	N x 10.0 µL
4	Go Script RT Mix for 1-Step RT-qPCR	N x 0.4 µL
	Total Volume	**N x 15.0 µL**

Quantabio qScript XLT One-Step RT-qPCR ToughMix

Step #	Reagent	Vol. of Reagent Added per Reaction
1	Nuclease-free Water	N x 3.5 µL
2	Combined Primer/Probe Mix	N x 1.5 µL
3	qScript XLT One-Step RT-qPCR ToughMix (2X)	N x 10.0 µL
	Total Volume	**N x 15.0 µL**

Quantabio UltraPlex 1-Step ToughMix (4X)

Step #	Reagent	Vol. of Reagent Added per Reaction
1	Nuclease-free Water	N x 8.5 µL
2	Combined Primer/Probe Mix	N x 1.5 µL
3	UltraPlex 1-Step ToughMix (4X)	N x 5.0 µL
	Total Volume	**N x 15.0 µL**

8) Dispense reagents into each respective labeled 1.5 mL microcentrifuge tube. After addition of the reagents, mix reaction mixtures by pipetting up and down. ***Do not vortex***.
9) Centrifuge for 5 seconds to collect contents at the bottom of the tube, and then place the tube in a cold rack.
10) Set up reaction strip tubes or plates in a 96-well cooler rack.
11) Dispense 15 µL of each master mix into the appropriate wells going across the row as shown below (**Figure 1**):

Figure 1: Example of Reaction Master Mix Plate Set-Up

	1	2	3	4	5	6	7	8	9	10	11	12
A	N1	N1	N1	N1	N1	N1	N1	N1	N1	N1	N1	N1
B	N2	N2	N2	N2	N2	N2	N2	N2	N2	N2	N2	N2
C	RP	RP	RP	RP	RP	RP	RP	RP	RP	RP	RP	RP
D												
E												
F												
G												
H												

12) Prior to moving to the nucleic acid handling area, prepare the No Template Control (NTC) reactions for column #1 in the assay preparation area.
13) Pipette 5 µL of nuclease-free water into the NTC sample wells (**Figure 2**, column 1). Securely cap NTC wells before proceeding.
14) Cover the entire reaction plate and move the reaction plate to the specimen nucleic acid handling area.

Nucleic Acid Template Addition

1) Gently vortex nucleic acid sample tubes for approximately 5 seconds.
2) Centrifuge for 5 seconds to collect contents at the bottom of the tube.
3) After centrifugation, place extracted nucleic acid sample tubes in the cold rack.
4) Samples should be added to columns 2-11 (column 1 and 12 are for controls) to the specific assay that is being tested as illustrated in **Figure 2**. Carefully pipette 5.0 µL of the first sample into all the wells labeled for that sample (i.e. Sample "S1" down column #2). *Keep other sample wells covered during addition. Change tips after each addition.*
5) Securely cap the column to which the sample has been added to prevent cross contamination and to ensure sample tracking.
6) Change gloves often and when necessary to avoid contamination.
7) Repeat steps #4 and #5 for the remaining samples.

8) If necessary, add 5 µL of Human Specimen Control (HSC) extracted sample to the HSC wells (**Figure 2**, column 11). Securely cap wells after addition. NOTE: Per CLIA regulations, HSC must be tested at least once per day.
9) Cover the entire reaction plate and move the reaction plate to the positive template control handling area.

Assay Control Addition

1) Pipette 5 µL of nCoVPC RNA to the sample wells of column 12 (**Figure 2**)**.** Securely cap wells after addition of the control RNA.

NOTE: *If using 8-tube strips, label the TAB of each strip to indicate sample position.* ***DO NOT LABEL THE TOPS OF THE REACTION TUBES!***

2) Briefly centrifuge reaction tube strips for 10-15 seconds. After centrifugation return to cold rack.

NOTE*: If using 96-well plates, centrifuge plates for 30 seconds at 500 x g, 4°C.*

Figure 2. 2019-nCoV rRT-PCR Diagnostic Panel: Example of Sample and Control Set-up

	1	2	3	4	5	6	7	8	9	10	11[a]	12
A	NTC	S1	S2	S3	S4	S5	S6	S7	S8	S9	S10	nCoV PC
B	NTC	S1	S2	S3	S4	S5	S6	S7	S8	S9	S10	nCoV PC
C	NTC	S1	S2	S3	S4	S5	S6	S7	S8	S9	S10	nCoV PC
D												
E												
F												
G												
H												

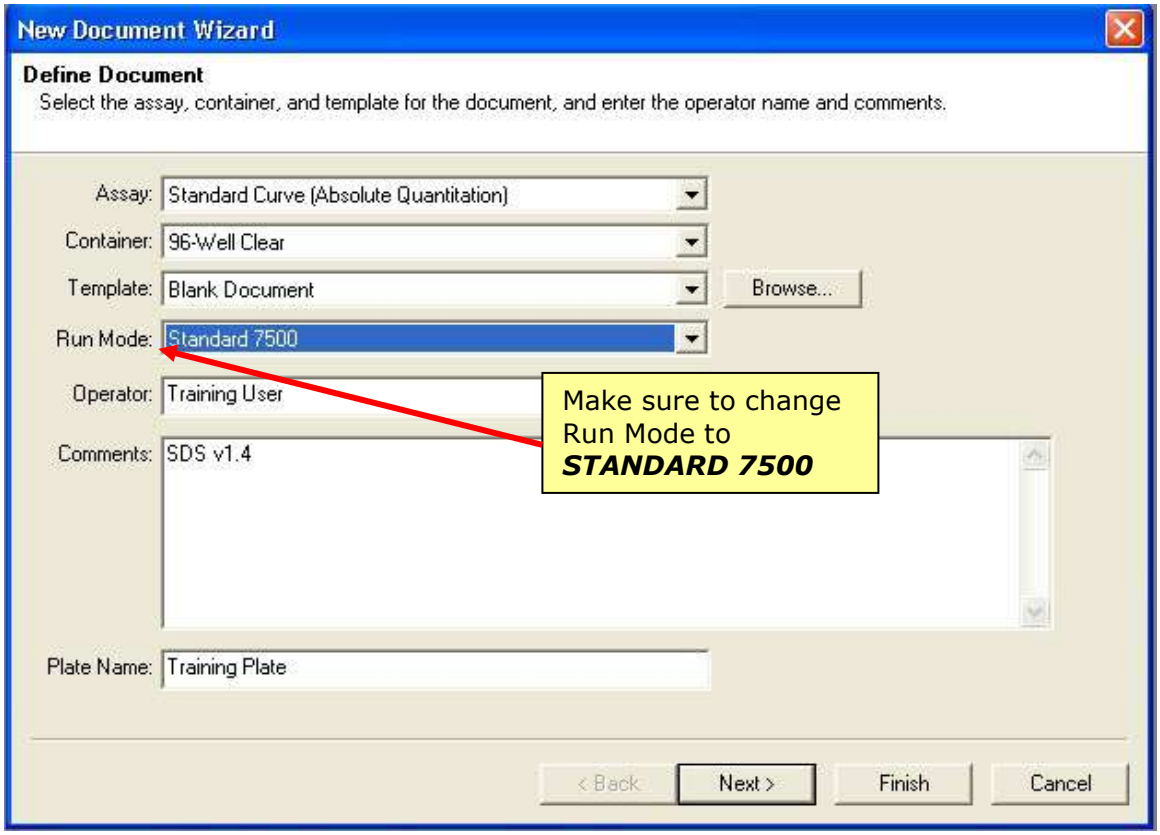
[a]Replace the sample in this column with extracted HSC if necessary

Create a Run Template on the Applied Biosystems 7500 Fast Dx Real-time PCR Instrument (Required if no template exists)

If the template already exists on your instrument, please proceed to the **RUNNING A TEST** section.

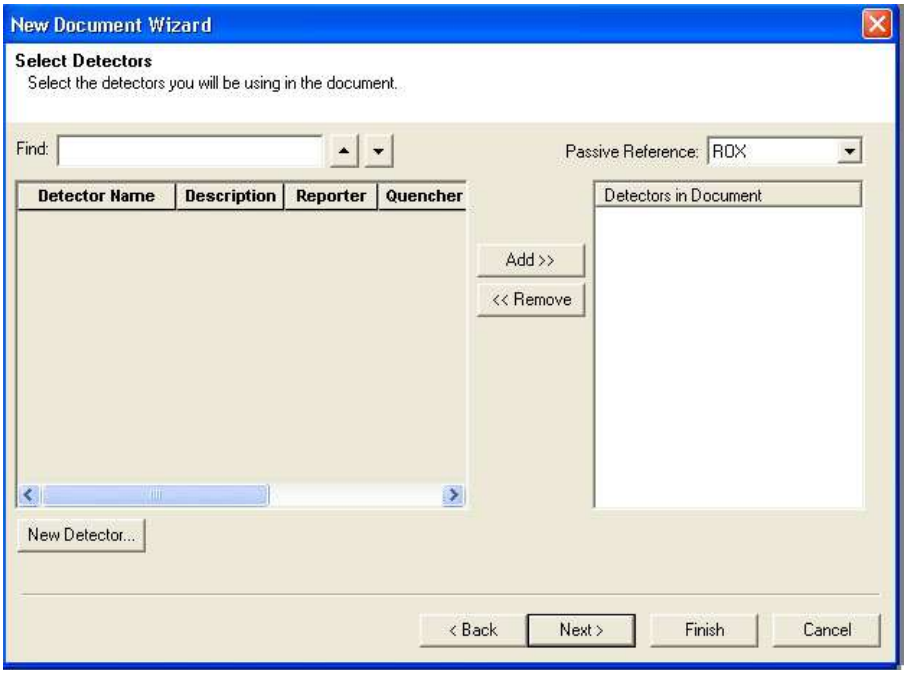
1) Launch the Applied Biosystems 7500 Fast Dx Real-time PCR Instrument by double clicking on the Applied Biosystems 7500 Fast Dx System icon on the desktop.
2) A new window should appear, select **Create New Document** from the menu.

Figure 3. New Document Wizard Window



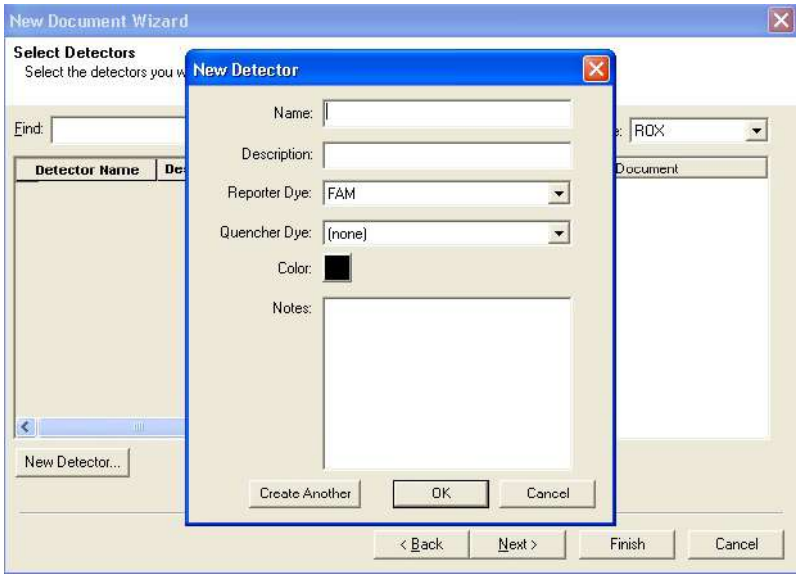
3) The **New Document Wizard** screen in **Figure 3** will appear. Select:
    a. Assay: **Standard Curve (Absolute Quantitation)**
    b. Container: **96-Well Clear**
    c. Template: **Blank Document**
    d. Run Mode: **Standard 7500**
    e. Operator: ***Your Name***
    f. Comments: **SDS v1.4**
    g. Plate Name: ***Your Choice***
4) After making selections click **Next** at the bottom of the window.

Figure 4. Creating New Detectors



5) After selecting next, the ***Select Detectors*** screen (**Figure 4**) will appear.
6) Click the **New Detector** button (see **Figure 4**).
7) The **New Detector** window will appear (**Figure 5**). A new detector will need to be defined for each primer and probe set. Creating these detectors will enable you to analyze each primer and probe set individually at the end of the reaction.

Figure 5. New Detector Window

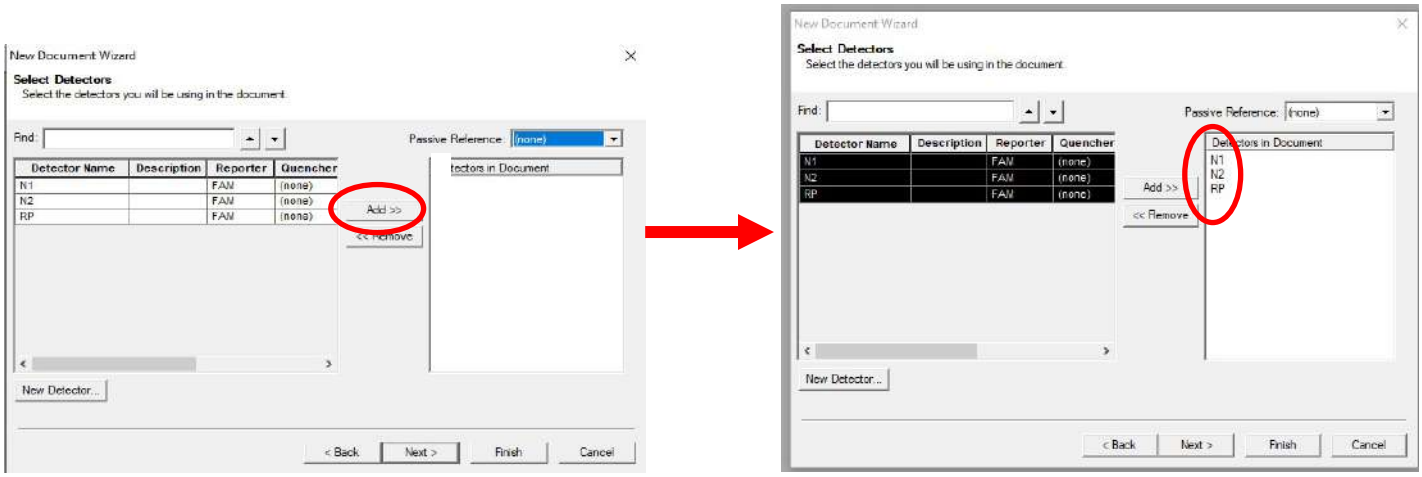


8) Start by creating the N1 Detector. Include the following:
    a. Name: **N1**
    b. Description: *leave blank*
    c. Reporter Dye: **FAM**
    d. Quencher Dye: **(none)**
    e. Color: *to change the color of the detector indicator do the following:*
        ⇒ Click on the color square to reveal the color chart
        ⇒ Select a color by clicking on one of the squares
        ⇒ After selecting a color click **OK** to return to the New Detector screen
    f. Click the **OK** button of the New Detector screen to return to the screen shown in **Figure 4**.
9) Repeat step 6-8 for each target in the panel.

Name	Reporter Dye	Quencher Dye
N1	FAM	(none)
N2	FAM	(none)
RP	FAM	(none)

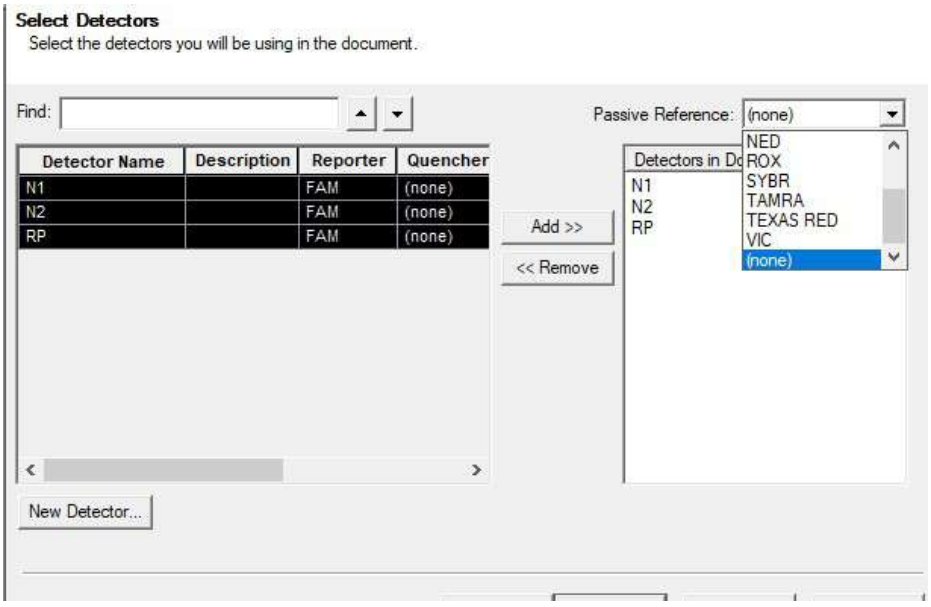
10) After each Detector is added, the **Detector Name**, **Description**, **Reporter** and **Quencher** fields will become populated in the **Select Detectors** screen (**Figure 6**).
11) Before proceeding, the newly created detectors must be added to the document. To add the new detectors to the document, click **ADD** (see **Figure 6**). Detector names will appear on the right-hand side of the **Select Detectors** window (**Figure 6**).

Figure 6. Adding New Detectors to Document



12) Once all detectors have been added, select **(none)** for **Passive Reference** at the top right-hand drop-down menu (**Figure 7**).

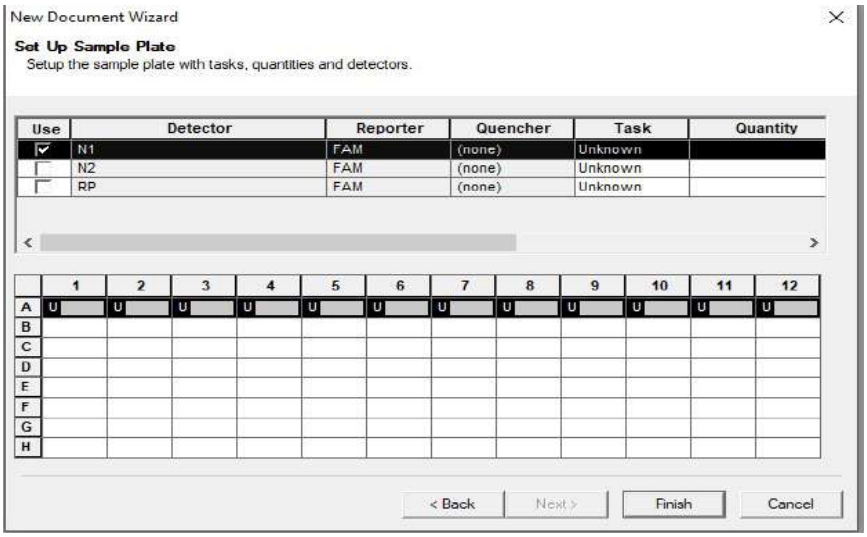
Figure 7. Select Passive Reference



Passive reference should be set to "(none)" as described above.

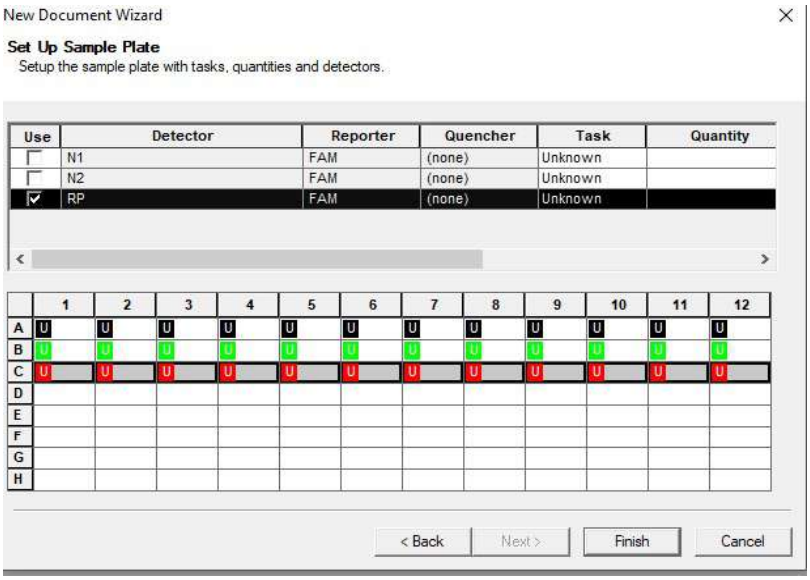
13) Click **Next** at the bottom of the **Select Detectors** window to proceed to the **Set Up Sample Plate** window (**Figure 8**).
14) In the **Set Up Sample Plate** window (**Figure 8**), use your mouse to select row A from the lower portion of the window, in the spreadsheet (see **Figure 8**).
15) In the top portion of the window, select detector **N1**. A check will appear next to the detector you have selected (**Figure 8**). You will also notice the row in the spreadsheet will be populated with a colored "U" icon to indicate which detector you've selected.
16) Repeat step 14-15 for each detector that will be used in the assay.

Figure 8. Sample Plate Set-up



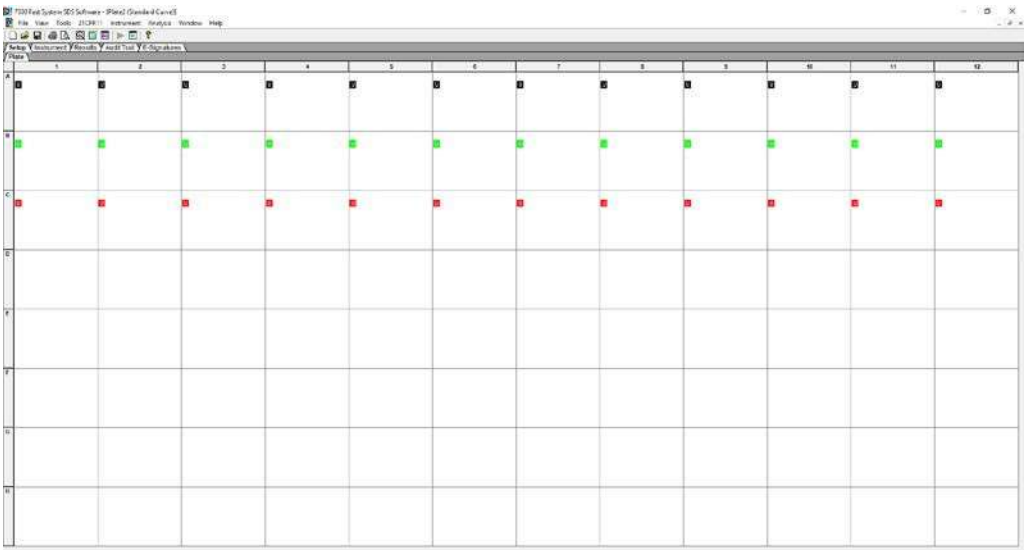
17) Select **Finish** after detectors have been assigned to their respective rows. (**Figure 9**).

Figure 9. Finished Plate Set-up



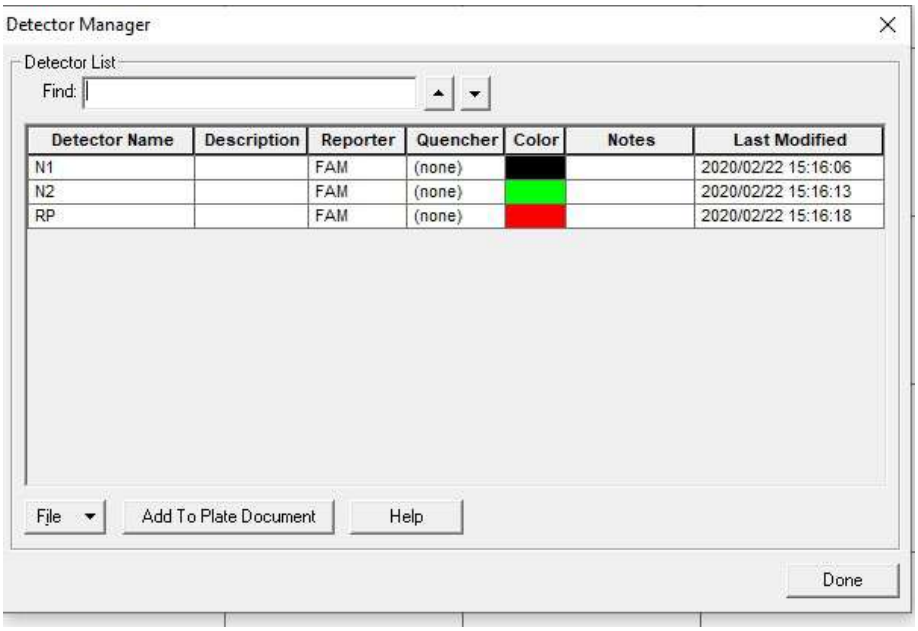
18) After clicking “Finish”, there will be a brief pause allowing the Applied Biosystems 7500 Fast Dx to initialize. This initialization is followed by a clicking noise. ***Note: The machine must be turned on for initialization.***
19) After initialization, the **Plate** tab of the Setup (**Figure 10)** will appear.
20) Each well of the plate should contain colored U icons that correspond with the detector labels that were previously chosen. To confirm detector assignments, select **Tools** from the file menu, then select **Detector Manager.**

Figure 10. Plate Set-up Window



21) The Detector Manager window will appear (**Figure 11**).

Figure 11. Detector Manager Window



22) Confirm all detectors are included and that each target has a **Reporter** set to **FAM** and the **Quencher** is set to **(none)**.
23) If all detectors are present, select **Done**. The detector information has been created and assigned to wells on the plate.

Defining the Instrument Settings

1) After detectors have been created and assigned, proceed to instrument set up.
2) Select the **Instrument** tab to define thermal cycling conditions.
3) Modify the thermal cycling conditions as follows (**Figure 12**):

Thermofisher TaqPath™ 1-Step RT-qPCR Master Mix, CG

a. In Stage 1, Set to 2 min at **25°C**; **1 Rep**.
b. In Stage 2, Set to 15 min at **50°C**; **1 Rep**.
c. In Stage 3, Set to 2 min at **95°C, 1 Rep.**
d. In Stage 4, Step 1 set to **3 sec** at **95°C**.
e. In Stage 4, Step 2 set to **30 sec** at **55.0°C.**
f. In Stage 4, Reps should be set to **45.**
g. Under **Settings** (**Figure 12**), bottom left-hand box, change volume to 20 µL.
h. Under **Settings**, **Run Mode** selection should be **Standard 7500**.
i. Step 2 of Stage 4 should be highlighted in yellow to indicate data collection (see **Figure 12**).

OR

Quantabio qScript™ XLT One-Step RT-qPCR ToughMix or UltraPlex 1-Step ToughMix

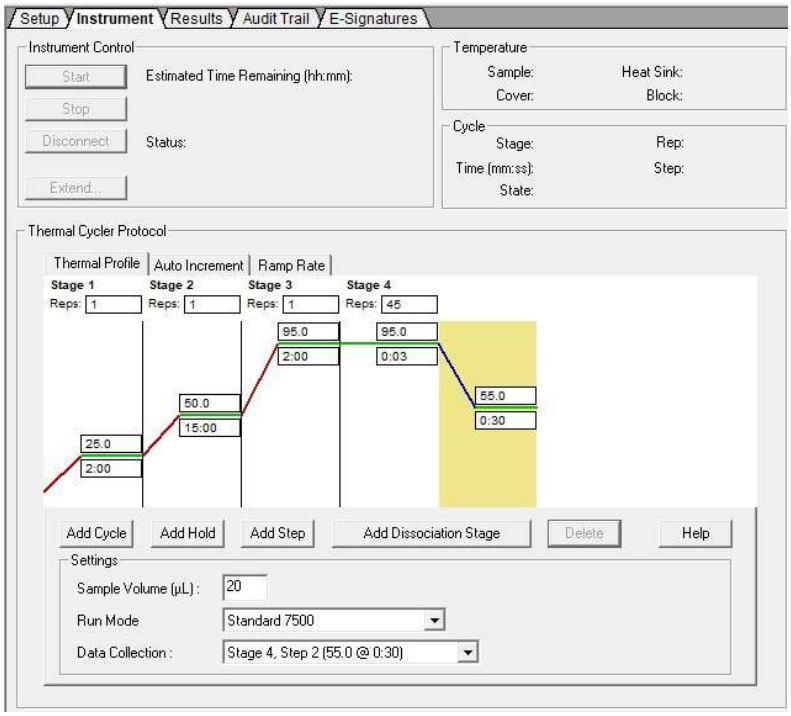
a. In Stage 1, Set to 10 min at **50°C**; **1 Rep**.
b. In Stage 2, Set to 3 min at **95°C, 1 Rep.**
c. In Stage 3, Step 1 set to **3 sec** at **95°C**.
d. In Stage 3, Step 2 set to **30 sec** at **55.0°C.**
e. In Stage 3, Reps should be set to **45.**
f. Under **Settings** (**Figure 12**), bottom left-hand box, change volume to 20 µL.
g. Under **Settings**, **Run Mode** selection should be **Standard 7500**.
h. Step 2 of Stage 4 should be highlighted in yellow to indicate data collection (see **Figure 12**).

OR

Promega GoTaq® Probe 1-Step RT-qPCR System

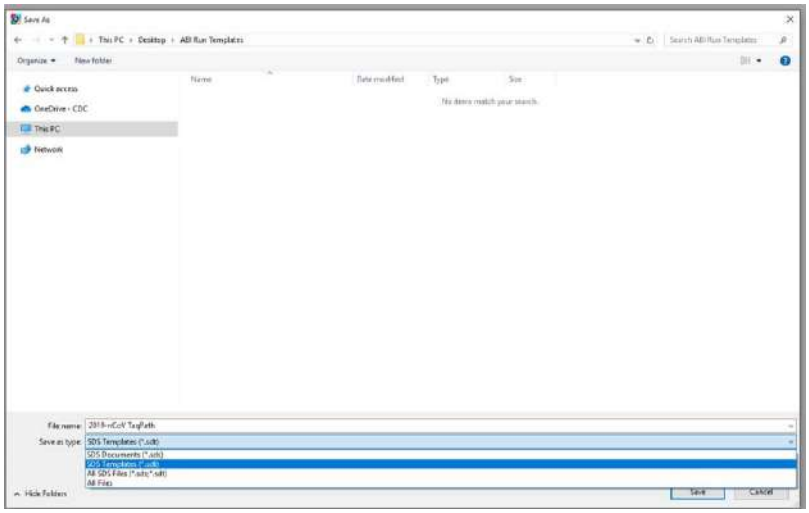
a. In Stage 1, Set to 15 min at **45°C**; **1 Rep**.
b. In Stage 2, Set to 2 min at **95°C, 1 Rep.**
c. In Stage 3, Step 1 set to **3 sec** at **95°C**.
d. In Stage 3, Step 2 set to **30 sec** at **55.0°C.**
e. In Stage 3, Reps should be set to **45.**
f. Under **Settings** (**Figure 12**), bottom left-hand box, change volume to 20 µL.
g. Under **Settings**, **Run Mode** selection should be **Standard 7500**.
h. Step 2 of Stage 4 should be highlighted in yellow to indicate data collection (see **Figure 12**).

Figure 12. Instrument Window



4) After making changes to the **Instrument** tab, the template file is ready to be saved. To save the template, select **File** from the top menu, then select **Save As**. Since the enzyme options have different instrument settings, it is recommended that the template be saved with a name indicating the enzyme option.
5) Save the template as **2019-nCoV Dx Panel TaqPath** or **2019-nCoV Dx Panel Quanta** or **2019-nCoV Dx Panel Promega** as appropriate in the desktop folder labeled "***ABI Run Templates***" (*you must create this folder*). Save as type should be SDS Templates (*.sdt) (**Figure 13**).

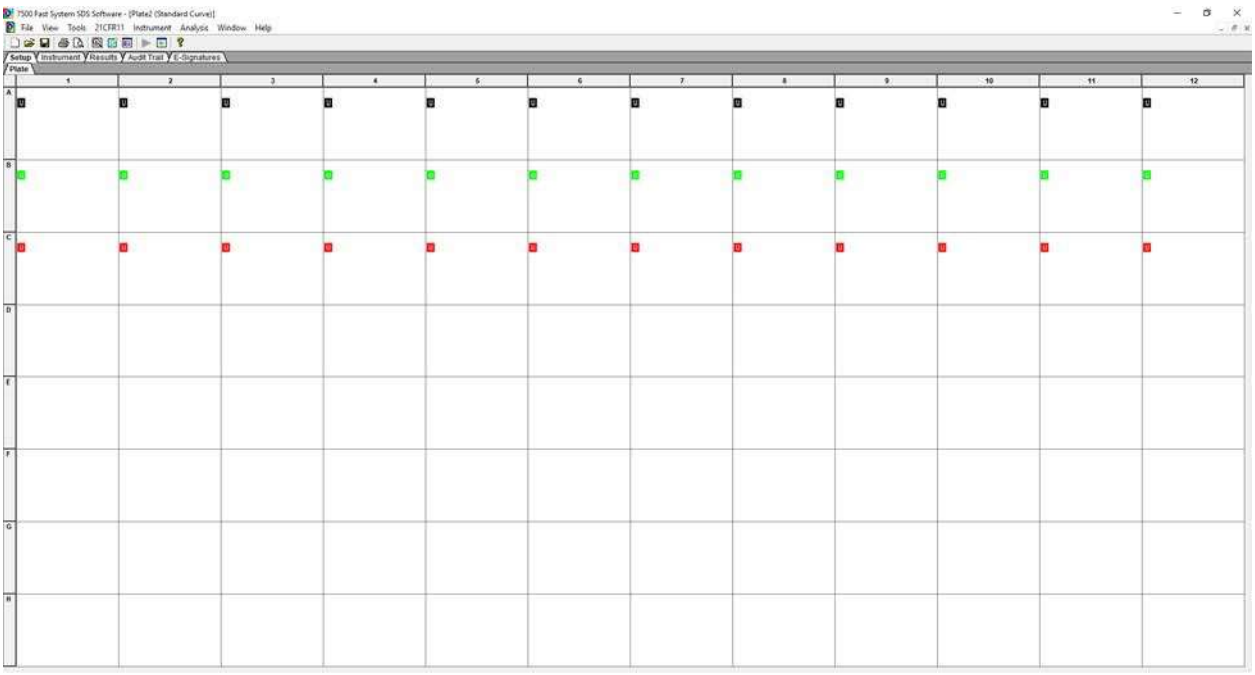
Figure 13. Saving Template



Running a Test

1) Turn on the ABI 7500 Fast Dx Real-Time PCR Instrument.
2) Launch the Applied Biosystems 7500 Fast Dx Real-time PCR System by double clicking on the 7500 Fast Dx System icon on the desktop.
3) A new window should appear, select **Open Existing Document** from the menu.
4) Navigate to select your ABI Run Template folder from the desktop.
5) Double click on the appropriate template file (**2019-nCoV Dx Panel TaqPath** or **2019-nCoV Dx Panel Quanta** or **2019-nCoV Dx Panel Promega)**
6) There will be a brief pause allowing the Applied Biosystems 7500 Fast Dx Real-Time PCR Instrument to initialize. This initialization is followed by a clicking noise. ***Note: The machine must be turned on for initialization.***

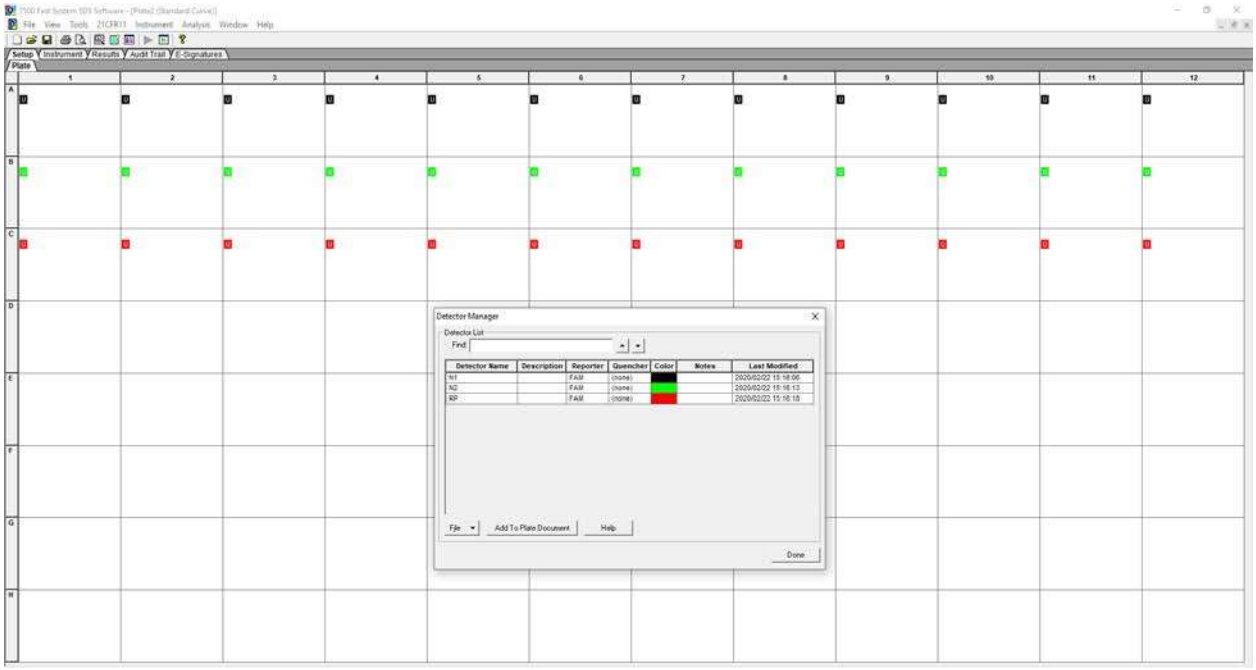
Figure 14. Plate Set-up Window



7) After the instrument initializes, a plate map will appear (**Figure 14**). The detectors and controls should already be labeled as they were assigned in the original template.

8) Click the **Well Inspector** icon  from the top menu.
9) Highlight specimen wells of interest on the plate map.
10) Type sample identifiers to **Sample Name** box in the **Well Inspector** window (**Figure 15**).

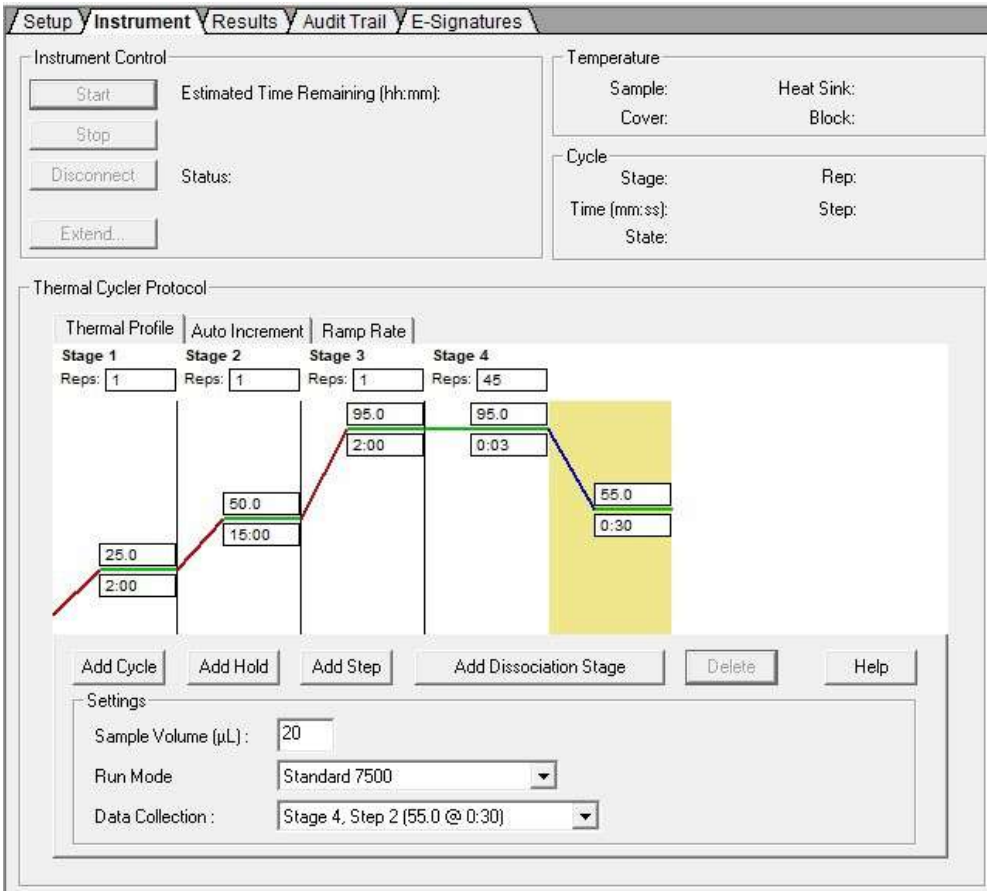
Figure 15. Labeling Wells



11) Repeat steps 9-10 until all sample identifiers are added to the plate setup.

12) Once all specimen and control identifiers are added click the **Close** button on the **Well Inspector** window to return to the **Plate** set up tab.
13) Click the **Instrument** tab at the upper left corner.
14) The reaction conditions, volumes, and type of 7500 reaction should already be loaded (**Figure 16**).

Figure 16. Instrument Settings

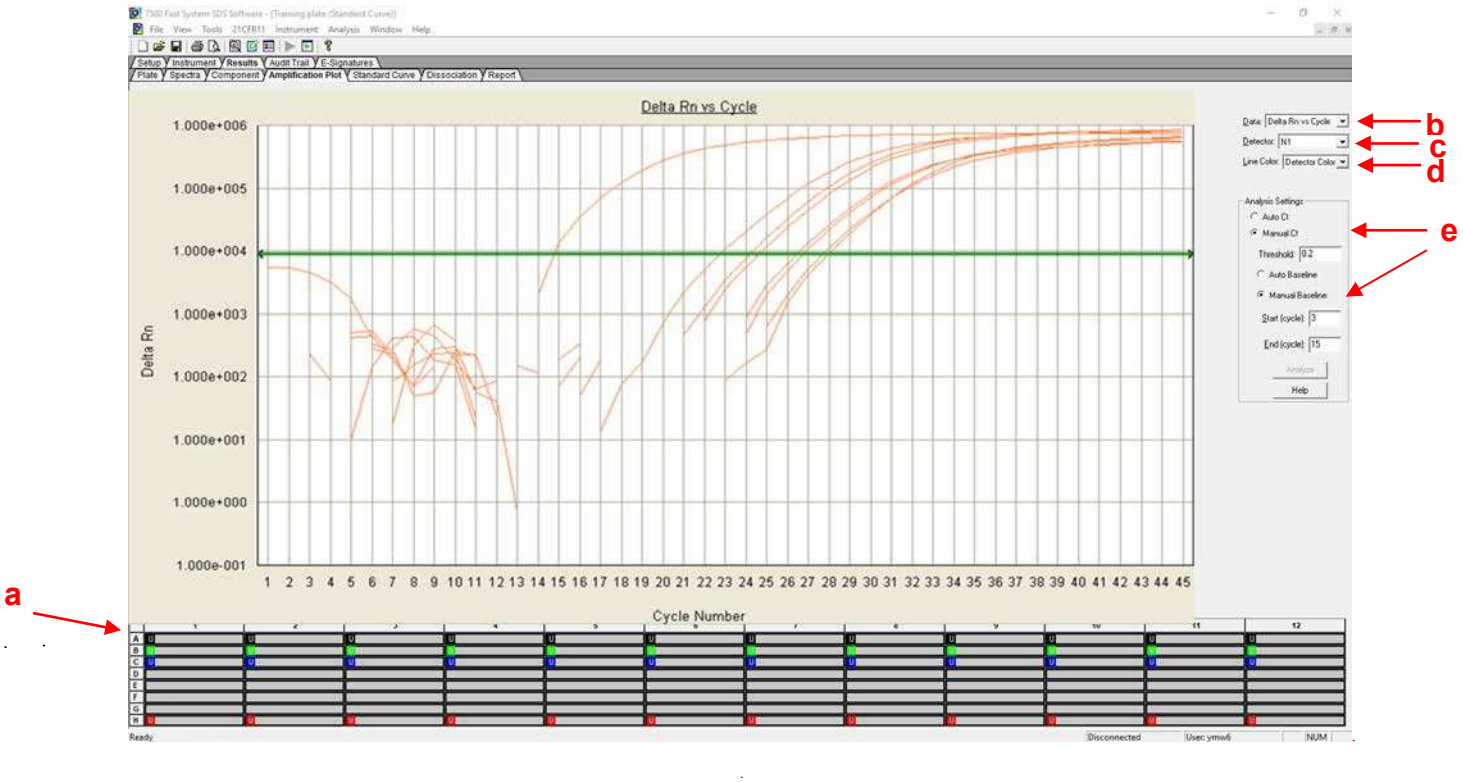


15) Ensure settings are correct (refer to the *Defining Instrument Settings*).
16) Before proceeding, the run file must be saved; from the main menu, select **File,** then **Save As**. Save in appropriate run folder designation.
17) Load the plate into the plate holder in the instrument. Ensure that the plate is properly aligned in the holder.
18) Once the run file is saved, click the **Start** button. *Note: The run should take approximately 1hr and 20 minutes to complete.*

Data Analysis

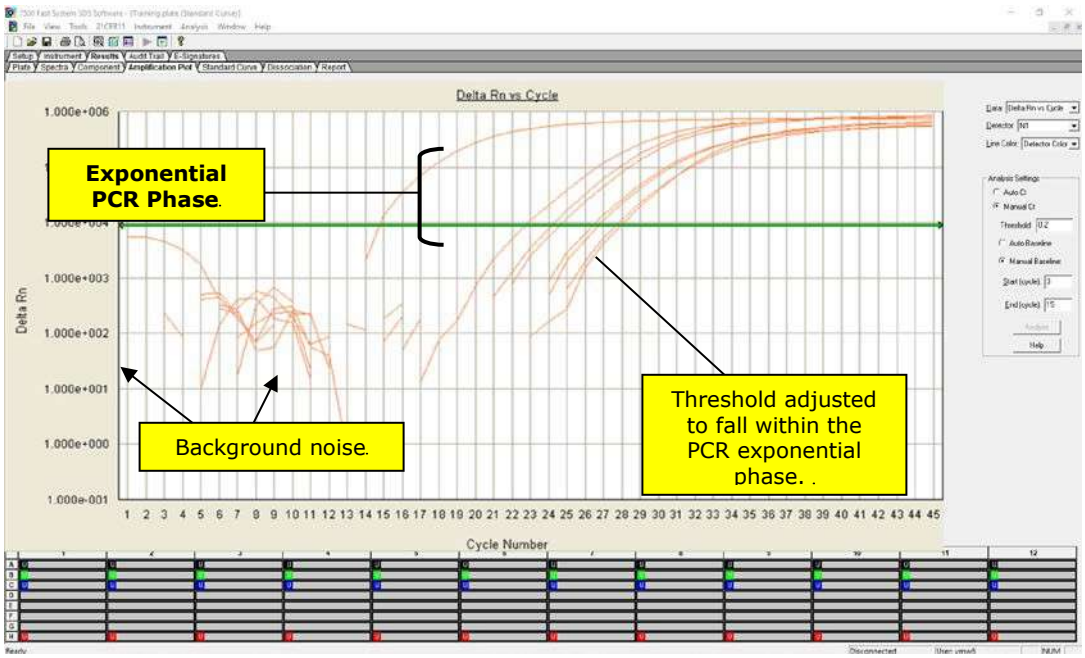
1) After the run has completed, select the **Results** tab at the upper left corner of the software.
2) Select the **Amplification Plot** tab to view the raw data (**Figure 17**).

Figure 17. Amplification Plot Window



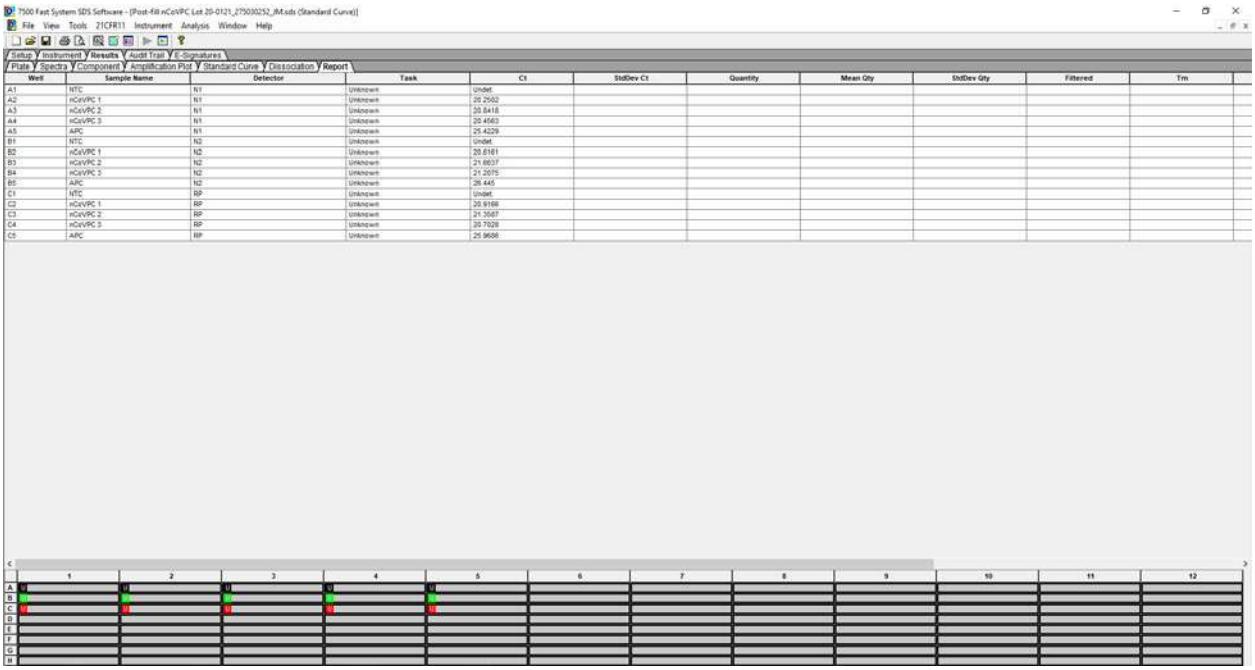
3) Start by highlighting all the samples from the run; to do this, click on the upper left-hand box **(a)** of the sample wells (**Figure 17**). All the growth curves should appear on the graph.
4) On the right-hand side of the window **(b)**, the **Data** drop down selection should be set to **Delta Rn vs. Cycle**.
5) Select **N1** from **(c)**, the **Detector** drop down menu, using the downward arrow.
    a. Please note that each detector is analyzed individually to reflect different performance profiles of each primer and probe set.
6) In the **Line Color** drop down **(d)**, **Detector Color** should be selected.
7) Under **Analysis Settings** select **Manual Ct (e)**.
    b. Do not change the **Manual Baseline** default numbers.
8) Using the mouse, click and drag the red threshold line until it lies within the exponential phase of the fluorescence curves and above any background signal (**Figure 18)**.

Figure 18. Amplification Plot



9) Click the **Analyze** button in the lower right corner of the window. The red threshold line will turn to green, indicating the data has been analyzed.
10) Repeat steps 5-9 to analyze results generated for each set of markers (N1, N2, RP).
11) Save analysis file by selecting **File** then **Save As** from the main menu.
12) After completing analysis for each of the markers, select the **Report** tab above the graph to display the Ct values (**Figure 19**). To filter report by sample name in ascending or descending order, simply click on **Sample Name** in the table.

Figure 19. Report



Interpretation of Results and Reporting

Extraction and Positive Control Results and Interpretation

No Template Control (NTC)

The NTC consists of using nuclease-free water in the rRT-PCR reactions instead of RNA. The NTC reactions for all primer and probe sets should not exhibit fluorescence growth curves that cross the threshold line. If any of the NTC reactions exhibit a growth curve that crosses the cycle threshold, sample contamination may have occurred. Invalidate the run and repeat the assay with strict adherence to the guidelines.

2019-nCoV Positive Control (nCoVPC)

The nCoVPC consists of in vitro transcribed RNA. The nCoVPC will yield a positive result with the following primer and probe sets: N1, N2 and RP.

Human Specimen Control (HSC) (Extraction Control)

When HSC is run with the CDC 2019-nCoV rRT-PCR Diagnostic Panel (see previous section on Assay Set Up), the HSC is used as an RNA extraction procedural control to demonstrate successful recovery of RNA as well as extraction reagent integrity. The HSC control consists of noninfectious cultured human cell (A549) material. Purified nucleic acid from the HSC should yield a positive result with the RP primer and probe set and negative results with all 2019-nCoV markers.

Expected Performance of Controls Included in the CDC 2019-nCoV Real-Time RT-PCR Diagnostic Panel

Control Type	External Control Name	Used to Monitor	2019 nCoV_N1	2019 nCoV_N2	RP	Expected Ct Values
Positive	nCoVPC	Substantial reagent failure including primer and probe integrity	+	+	+	< 40.00 Ct
Negative	NTC	Reagent and/or environmental contamination	-	-	-	None detected
Extraction	HSC	Failure in lysis and extraction procedure, potential contamination during extraction	-	-	+	< 40.00 Ct

If any of the above controls do not exhibit the expected performance as described, the assay may have been set up and/or executed improperly, or reagent or equipment malfunction could have occurred. Invalidate the run and re-test.

RNase P (Extraction Control)

- All clinical samples should exhibit fluorescence growth curves in the RNase P reaction that cross the threshold line within 40.00 cycles (< 40.00 Ct), thus indicating the presence of the human RNase P gene. Failure to detect RNase P in any clinical specimens may indicate:
  - Improper extraction of nucleic acid from clinical materials resulting in loss of RNA and/or RNA degradation.
  - Absence of sufficient human cellular material due to poor collection or loss of specimen integrity.
  - Improper assay set up and execution.
  - Reagent or equipment malfunction.
- If the RP assay does not produce a positive result for human clinical specimens, interpret as follows:
  - If the 2019-nCoV N1 and N2are positive even in the absence of a positive RP, the result should be considered valid. It is possible, that some samples may fail to exhibit RNase P growth curves due to low cell numbers in the original clinical sample. A negative RP signal does not preclude the presence of 2019-nCoV virus RNA in a clinical specimen.
  - If all 2019-nCoV markers AND RNase P are negative for the specimen, the result should be considered invalid for the specimen. If residual specimen is available, repeat the extraction procedure and repeat the test. If all markers remain negative after re-test, report the results as invalid and a new specimen should be collected if possible.

2019-nCoV Markers (N1 and N2)

- When all controls exhibit the expected performance, a specimen is considered negative if all 2019-nCoV marker (N1, N2) cycle threshold growth curves DO NOT cross the threshold line within 40.00 cycles (< 40.00 Ct) AND the RNase P growth curve DOES cross the threshold line within 40.00 cycles (< 40.00 Ct).
- When all controls exhibit the expected performance, a specimen is considered positive for 2019-nCoV if all 2019-nCoV marker (N1, N2) cycle threshold growth curves cross the threshold line within 40.00 cycles (< 40.00 Ct). The RNase P may or may not be positive as described above, but the 2019-nCoV result is still valid.
- When all controls exhibit the expected performance and the growth curves for the 2019-nCoV markers (N1, N2) AND the RNase P marker DO NOT cross the cycle threshold growth curve within 40.00 cycles (< 40.00 Ct), the result is invalid. The extracted RNA from the specimen should be re-tested. If residual RNA is not available, re-extract RNA from residual specimen and re-test. If the re-tested sample is negative for all markers and RNase P, the result is invalid and collection of a new specimen from the patient should be considered.
- When all controls exhibit the expected performance and the cycle threshold growth curve for any one marker (N1 or N2 but not both markers) crosses the threshold line within 40.00 cycles (< 40.00 Ct) the result is inconclusive. The extracted RNA should be retested. If residual RNA is not available, re-extract RNA from residual specimen and re-test. If the same result is obtained, report the inconclusive result. Consult with your state public health laboratory or CDC, as appropriate, to request guidance and/or to coordinate transfer of the specimen for additional analysis.
- If HSC is positive for N1 or N2, then contamination may have occurred during extraction or sample processing. Invalidate all results for specimens extracted alongside the HSC. Re-extract specimens and HSC and re-test.

2019-nCoV rRT-PCR Diagnostic Panel Results Interpretation Guide

The table below lists the expected results for the 2019-nCoV rRT-PCR Diagnostic Panel. If a laboratory obtains unexpected results for assay controls or if inconclusive or invalid results are obtained and cannot be resolved through the recommended re-testing, please contact CDC for consultation and possible specimen referral. See pages 10 and 40 for referral and contact information.

2019 nCoV_N1	2019 nCoV_N2	RP	Result Interpretation[a]	Report	Actions
+	+	±	2019-nCoV detected	Positive 2019-nCoV	Report results to CDC and sender.
If only one of the two targets is positive		±	Inconclusive Result	Inconclusive	Repeat testing of nucleic acid and/or re-extract and repeat rRT-PCR. If the repeated result remains inconclusive, contact your State Public Health Laboratory or CDC for instructions for transfer of the specimen or further guidance.
-	-	+	2019-nCoV not detected	Not Detected	Report results to sender. Consider testing for other respiratory viruses.[b]
-	-	-	Invalid Result	Invalid	Repeat extraction and rRT-PCR. If the repeated result remains invalid, consider collecting a new specimen from the patient.

[a]Laboratories should report their diagnostic result as appropriate and in compliance with their specific reporting system.

[b]Optimum specimen types and timing for peak viral levels during infections caused by 2019-nCoV have not been determined. Collection of multiple specimens from the same patient may be necessary to detect the virus. The possibility of a false negative result should especially be considered if the patient's recent exposures or clinical presentation suggest that 2019-nCoV infection is possible, and diagnostic tests for other causes of illness (e.g., other respiratory illness) are negative. If 2019-nCoV infection is still suspected, re-testing should be considered in consultation with public health authorities.

Quality Control

- Quality control requirements must be performed in conformance with local, state, and federal regulations or accreditation requirements and the user's laboratory's standard quality control procedures. For further guidance on appropriate quality control practices, refer to 42 CFR 493.1256.
- Quality control procedures are intended to monitor reagent and assay performance.
- Test all positive controls prior to running diagnostic samples with each new kit lot to ensure all reagents and kit components are working properly.
- Good laboratory practice (cGLP) recommends including a positive extraction control in each nucleic acid isolation batch.
- Although HSC is not included with the 2019-nCov rRT-PCR Diagnostic Panel, the HSC extraction control must proceed through nucleic acid isolation per batch of specimens to be tested.
- Always include a negative control (NTC), and the appropriate positive control (nCoVPC) in each amplification and detection run. All clinical samples should be tested for human RNAse P gene to control for specimen quality and extraction.

Limitations

- All users, analysts, and any person reporting diagnostic results should be trained to perform this procedure by a competent instructor. They should demonstrate their ability to perform the test and interpret the results prior to performing the assay independently.
- Performance of the CDC 2019-nCoV Real-Time RT-PCR Diagnostic Panel has only been established in upper and lower respiratory specimens (such as nasopharyngeal or oropharyngeal swabs, sputum, lower respiratory tract aspirates, bronchoalveolar lavage, and nasopharyngeal wash/aspirate or nasal aspirate).
- Negative results do not preclude 2019-nCoV infection and should not be used as the sole basis for treatment or other patient management decisions. Optimum specimen types and timing for peak viral levels during infections caused by 2019-nCoV have not been determined. Collection of multiple specimens (types and time points) from the same patient may be necessary to detect the virus.
- A false negative result may occur if a specimen is improperly collected, transported or handled. False negative results may also occur if amplification inhibitors are present in the specimen or if inadequate numbers of organisms are present in the specimen.
- Positive and negative predictive values are highly dependent on prevalence. False negative test results are more likely when prevalence of disease is high. False positive test results are more likely when prevalence is moderate to low.
- Do not use any reagent past the expiration date.
- If the virus mutates in the rRT-PCR target region, 2019-nCoV may not be detected or may be detected less predictably. Inhibitors or other types of interference may produce a false negative result. An interference study evaluating the effect of common cold medications was not performed.
- Test performance can be affected because the epidemiology and clinical spectrum of infection caused by 2019-nCoV is not fully known. For example, clinicians and laboratories may not know the optimum types of specimens to collect, and, during the course of infection, when these specimens are most likely to contain levels of viral RNA that can be readily detected.
- Detection of viral RNA may not indicate the presence of infectious virus or that 2019-nCoV is the causative agent for clinical symptoms.

- The performance of this test has not been established for monitoring treatment of 2019-nCoV infection.
- The performance of this test has not been established for screening of blood or blood products for the presence of 2019-nCoV.
- This test cannot rule out diseases caused by other bacterial or viral pathogens.

Conditions of Authorization for the Laboratory

The CDC 2019-nCoV Real-Time RT-PCR Diagnostic Panel Letter of Authorization, along with the authorized Fact Sheet for Healthcare Providers, the authorized Fact Sheet for Patients and authorized labeling are available on the FDA website:
https://www.fda.gov/MedicalDevices/Safety/EmergencySituations/ucm161496.htm
Use of the CDC 2019-nCoV Real-Time RT-PCR Diagnostic Panel must follow the procedures outlined in these manufacturer's Instructions for Use and the conditions of authorization outlined in the Letter of Authorization. Deviations from the procedures outlined are not permitted under the Emergency Use Authorization (EUA). To assist clinical laboratories running the CDC 2019-nCoV Real-Time RT-PCR Diagnostic Panel, the relevant Conditions of Authorization are listed verbatim below, and are required to be met by laboratories performing the EUA test.

- Authorized laboratories[1] will include with reports of the results of the CDC 2019-nCoV Real-Time RT-PCR Diagnostic Panel, all authorized Fact Sheets. Under exigent circumstances, other appropriate methods for disseminating these Fact Sheets may be used, which may include mass media.

- Authorized laboratories will perform the CDC 2019-nCoV Real-Time RT-PCR Diagnostic Panel as outlined in the CDC 2019-Novel Coronavirus (2019-nCoV) Real-Time RT-PCR Diagnostic Panel Instructions for Use. Deviations from the authorized procedures, including the authorized RT-PCR instruments, authorized extraction methods, authorized clinical specimen types, authorized control materials, authorized other ancillary reagents and authorized materials required to perform the CDC 2019-nCoV Real-Time RT-PCR Diagnostic Panel are not permitted. [2]

- Authorized laboratories that receive the CDC 2019-nCoV Real-Time RT-PCR Diagnostic Panel must notify the relevant public health authorities of their intent to run the test prior to initiating testing.

- Authorized laboratories will have a process in place for reporting test results to healthcare providers and relevant public health authorities, as appropriate.

- Authorized laboratories will collect information on the performance of the test and report to DMD/OHT7-OIR/OPEQ/CDRH (via email: CDRH-EUA-Reporting@fda.hhs.gov) and CDC

[1]Authorized Laboratories: For ease of reference, the Letter of Authorization refers to "laboratories certified under the Clinical Laboratory Improvement Amendments of 1988 (CLIA), 42 U.S.C. § 263a, to perform high complexity tests" as "authorized laboratories."
[2]If an authorized laboratory is interested in implementing changes to the CDC 2019-nCoV Real-Time RT-PCR Diagnostic Panel that are not in the scope (Section II) of this letter of authorization FDA recommends you discuss with FDA after considering the policy outlined in *Immediately in Effect Guidance for Clinical Laboratories and Food and Drug Administration Staff: Policy for Diagnostics Testing in Laboratories Certified to Perform High Complexity Testing under CLIA prior to Emergency Use Authorization for Coronavirus Disease-2019 during the Public Health Emergency* (https://www.fda.gov/media/135659/download).

(respvirus@cdc.gov) any suspected occurrence of false positive or false negative results and significant deviations from the established performance characteristics of the test of which they become aware.

- Authorized laboratories will report adverse events, including problems with test performance or results, to MedWatch by submitting the online FDA Form 3500 (https://www.accessdata.fda.gov/scripts/medwatch/index.cfm?action=reporting.home) or by calling 1-800-FDA-1088

- All laboratory personnel using the test must be appropriately trained in RT-PCR techniques and use appropriate laboratory and personal protective equipment when handling this kit and use the test in accordance with the authorized labeling.

- CDC, IRR, manufacturers and distributors of commercial materials identified as acceptable on the CDC website, and authorized laboratories will ensure that any records associated with this EUA are maintained until otherwise notified by FDA. Such records will be made available to FDA for inspection upon request.

Performance Characteristics

Analytical Performance:

Limit of Detection (LoD):

LoD studies determine the lowest detectable concentration of 2019-nCoV at which approximately 95% of all (true positive) replicates test positive. The LoD was determined by limiting dilution studies using characterized samples.

The analytical sensitivity of the rRT-PCR assays contained in the CDC 2019 Novel Coronavirus (2019-nCoV) Real-Time RT-PCR Diagnostic Panel were determined in Limit of Detection studies. Since no quantified virus isolates of the 2019-nCoV are currently available, assays designed for detection of the 2019-nCoV RNA were tested with characterized stocks of in vitro transcribed full length RNA (N gene; GenBank accession: MN908947.2) of known titer (RNA copies/µL) spiked into a diluent consisting of a suspension of human A549 cells and viral transport medium (VTM) to mimic clinical specimen. Samples were extracted using the QIAGEN EZ1 Advanced XL instrument and EZ1 DSP Virus Kit (Cat# 62724) and manually with the QIAGEN DSP Viral RNA Mini Kit (Cat# 61904). Real-Time RT-PCR assays were performed using the ThemoFisher Scientific TaqPath™ 1-Step RT-qPCR Master Mix, CG (Cat# A15299) on the Applied Biosystems™ 7500 Fast Dx Real-Time PCR Instrument according to the CDC 2019-nCoV Real-Time RT-PCR Diagnostic Panel instructions for use.

A preliminary LoD for each assay was determined testing triplicate samples of RNA purified using each extraction method. The approximate LoD was identified by extracting and testing 10-fold serial dilutions of characterized stocks of in vitro transcribed full-length RNA. A confirmation of the LoD was determined using 3-fold serial dilution RNA samples with 20 extracted replicates. The LoD was determined as the lowest concentration where ≥ 95% (19/20) of the replicates were positive.

Table 4. Limit of Detection Confirmation of the CDC 2019-nCoV Real-Time RT-PCR Diagnostic Panel with QIAGEN EZ1 DSP

Targets	2019-nCoV_N1			2019-nCoV_N2		
RNA Concentration[1]	$10^{0.5}$	$10^{0.0}$	$10^{-0.5}$	$10^{0.5}$	$10^{0.0}$	$10^{-0.5}$
Positives/Total	20/20	19/20	13/20	20/20	17/20	9/20
Mean Ct[2]	32.5	35.4	NA	35.8	NA	NA
Standard Deviation (Ct)	0.5	0.8	NA	1.3	NA	NA

[1] Concentration is presented in RNA copies/µL

[2] Mean Ct reported for dilutions that are ≥ 95% positive. Calculations only include positive results.

NA not applicable

Table 5. Limit of Detection Confirmation CDC 2019-nCoV Real-Time RT-PCR Diagnostic Panel with QIAGEN QIAmp DSP Viral RNA Mini Kit

Targets	2019-nCoV_N1			2019-nCoV_N2			
RNA Concentration[1]	$10^{0.5}$	$10^{0.0}$	$10^{-0.5}$	$10^{0.5}$	$10^{0.0}$	$10^{-0.5}$	$10^{-1.0}$
Positives/Total	20/20	20/20	6/20	20/20	20/20	20/20	8/20
Mean Ct[2]	32.0	32.8	NA	33.0	35.4	36.2	NA
Standard Deviation (Ct)	0.7	0.8	NA	1.4	0.9	1.9	NA

[1] Concentration is presented in RNA copies/µL

[2] Mean Ct reported for dilutions that are ≥ 95% positive. Calculations only include positive results.

NA not applicable

Table 6. Limit of Detection of the CDC 2019-nCoV Real-Time RT-PCR Diagnostic Panel

Virus	Material	Limit of Detection (RNA copies/µL)	
		QIAGEN EZ1 Advanced XL	QIAGEN DSP Viral RNA Mini Kit
2019 Novel Coronavirus	N Gene RNA Transcript	$10^{0.5}$	10^{0}

FDA Sensitivity Evaluation: The analytical sensitivity of the test will be further assessed by evaluating an FDA-recommended reference material using an FDA developed protocol if applicable and/or when available.

In Silico Analysis of Primer and Probe Sequences:

An alignment was performed with the oligonucleotide primer and probe sequences of the CDC 2019 nCoV Real-Time RT-PCR Diagnostic Panel with all publicly available nucleic acid sequences for 2019-nCoV in GenBank as of February 1, 2020 to demonstrate the predicted inclusivity of the CDC 2019 nCoV Real-Time RT-PCR Diagnostic panel. All the alignments show 100% identity of the CDC panel to the available 2019-nCoV sequences with the exception of one nucleotide mismatch with the N1 forward primer in one deposited sequence. The risk of a single mismatch resulting in a significant loss in reactivity, and false negative result, is

low due to the design of the primers and probes with melting temperatures > 60°C and run conditions of the assay with annealing temperature at 55°C to tolerate one to two mismatches.

Specificity/Exclusivity Testing: In Silico Analysis

BLASTn analysis queries of the 2019-nCoV rRT-PCR assays primers and probes were performed against public domain nucleotide sequences. The database search parameters were as follows: 1) The nucleotide collection consists of GenBank+EMBL+DDBJ+PDB+RefSeq sequences, but excludes EST, STS, GSS, WGS, TSA, patent sequences as well as phase 0, 1, and 2 HTGS sequences and sequences longer than 100Mb; 2) The database is non-redundant. Identical sequences have been merged into one entry, while preserving the accession, GI, title and taxonomy information for each entry; 3) Database was updated on 10/03/2019; 4) The search parameters automatically adjust for short input sequences and the expect threshold is 1000; 5) The match and mismatch scores are 1 and -3, respectively; 6) The penalty to create and extend a gap in an alignment is 5 and 2 respectively.

2019-nCoV_N1 Assay:
Probe sequence of 2019-nCoV rRT-PCR assay N1 showed high sequence homology with SARS coronavirus and Bat SARS-like coronavirus genome. However, forward and reverse primers showed no sequence homology with SARS coronavirus and Bat SARS-like coronavirus genome. Combining primers and probe, there is no significant homologies with human genome, other coronaviruses or human microflora that would predict potential false positive rRT-PCR results.

2019-nCoV_N2 Assay:
The forward primer sequence of 2019-nCoV rRT-PCR assay N2 showed high sequence homology to Bat SARS-like coronaviruses. The reverse primer and probe sequences showed no significant homology with human genome, other coronaviruses or human microflora. Combining primers and probe, there is no prediction of potential false positive rRT-PCR results.

In summary, the 2019-nCoV rRT-PCR assay N1 and N2, designed for the specific detection of 2019-nCoV, showed no significant combined homologies with human genome, other coronaviruses, or human microflora that would predict potential false positive rRT-PCR results.

In addition to the *in silico* analysis, several organisms were extracted and tested with the CDC 2019-nCoV Real-Time RT-PCR Diagnostic Panel to demonstrate analytical specificity and exclusivity. Studies were performed with nucleic acids extracted using the QIAGEN EZ1 Advanced XL instrument and EZ1 DSP Virus Kit. Nucleic acids were extracted from high titer preparations (typically ≥ 10^5 PFU/mL or ≥ 10^6 CFU/mL). Testing was performed using the ThemoFisher Scientific TaqPath™ 1-Step RT-qPCR Master Mix, CG on the Applied Biosystems™ 7500 Fast Dx Real-Time PCR instrument. The data demonstrate that the expected results are obtained for each organism when tested with the CDC 2019-nCoV Real-Time RT-PCR Diagnostic Panel.

Table 7. Specificity/Exclusivity of the CDC 2019-nCoV Real-Time RT-PCR Diagnostic Panel

Virus	Strain	Source	2019-nCoV_N1	2019-nCoV_N2	Final Result
Human coronavirus	229E	isolate	0/3	0/3	Neg.
Human coronavirus	OC43	isolate	0/3	0/3	Neg.
Human coronavirus	NL63	clinical specimen	0/3	0/3	Neg.
Human coronavirus	HKU1	clinical specimen	0/3	0/3	Neg.
MERS-coronavirus		isolate	0/3	0/3	Neg.
SARS-coronavirus		isolate	0/3	0/3	Neg.
bocavirus	-	clinical specimen	0/3	0/3	Neg.
Mycoplasma pneumoniae		isolate	0/3	0/3	Neg.
Streptococcus		isolate	0/3	0/3	Neg.
Influenza A(H1N1)		isolate	0/3	0/3	Neg.
Influenza A(H3N2)		isolate	0/3	0/3	Neg.
Influenza B		isolate	0/3	0/3	Neg.
Human adenovirus, type 1	Ad71	isolate	0/3	0/3	Neg.
Human metapneumovirus	-	isolate	0/3	0/3	Neg.
respiratory syncytial virus	Long A	isolate	0/3	0/3	Neg.
rhinovirus		isolate	0/3	0/3	Neg.
parainfluenza 1	C35	isolate	0/3	0/3	Neg.
parainfluenza 2	Greer	isolate	0/3	0/3	Neg.
parainfluenza 3	C-43	isolate	0/3	0/3	Neg.
parainfluenza 4	M-25	isolate	0/3	0/3	Neg.

Endogenous Interference Substances Studies:

The CDC 2019-nCoV Real-Time RT-PCR Diagnostic Panel uses conventional well-established nucleic acid extraction methods and based on our experience with CDC's other EUA assays, including the CDC Novel Coronavirus 2012 Real-time RT-PCR Assay for the presumptive detection of Middle East Respiratory Syndrome Coronavirus (MERS-CoV) and the CDC Human Influenza Virus Real-Time RT-PCR Diagnostic Panel-Influenza A/H7 (Eurasian Lineage) Assay for the presumptive detection of novel influenza A (H7N9) virus that are both intended for use with a number of respiratory specimens, we do not anticipate interference from common endogenous substances.

Specimen Stability and Fresh-frozen Testing:

To increase the likelihood of detecting infection, CDC recommends collection of lower respiratory and upper respiratory specimens for testing. If possible, additional specimen types (e.g., stool, urine) should be collected and should be stored initially until decision is made by CDC whether additional specimen sources should be tested. Specimens should be collected as soon as possible once a PUI is identified regardless of symptom onset. Maintain proper infection control when collecting specimens. Store specimens at 2-8°C and ship overnight to CDC on ice pack. Label each specimen container with the patient's ID number (e.g., medical record number), unique specimen ID (e.g., laboratory requisition number), specimen type (e.g., nasal swabs) and the date the sample was collected. Complete a CDC Form 50.34 for each specimen submitted.

Clinical Performance:

As of February 22, 2020, CDC has tested 2071 respiratory specimens from persons under investigation (PUI) in the U.S. using the CDC 2019-nCoV Real-Time RT-PCR Diagnostic Panel. Specimen types include bronchial fluid/wash, buccal swab, nasal wash/aspirate, nasopharyngeal swab, nasopharyngeal/throat swab, oral swab, sputum, oropharyngeal (throat) swab, swab (unspecified), and throat swab.

Table 8: Summary of CDC 2019-Novel Coronavirus (2019-nCoV) Real-Time RT-PCR Diagnostic Panel Data Generated by Testing Human Respiratory Specimens Collected from PUI Subjects in the U.S.

Specimen Type	2019 nCoV Negative	2019 nCoV Positive	Inconclusive	Invalid	Total
Bronchial fluid/wash	2	0	0	0	2
Buccal swab	5	1	0	0	6
Nasal wash/aspirate	6	0	0	0	6
Nasopharyngeal swab	927	23	0	0	950
Nasopharyngeal swab/throat swab	4	0	0	0	4
Oral swab	476	9	0	0	485
Pharyngeal (throat) swab	363	10	0	1	374
Sputum	165	5	0	0	170
Swab (unspecified)[1]	71	1	0	0	72
Tissue (lung)	2	0	0	0	2
Total	2021	49	0	1	2071

[1]Actual swab type information was missing from these upper respiratory tract specimens.

Two thousand twenty-one (2021) respiratory specimens of the 2071 respiratory specimens tested negative by the CDC 2019-nCoV Real-Time RT-PCR Diagnostic Panel. Forty-nine (49) of the 2071 respiratory specimens tested positive by the CDC 2019-nCoV Real-Time RT-PCR Diagnostic Panel. Only one specimen (oropharyngeal (throat) swab) was invalid. Of the 49 respiratory specimens that tested positive by the CDC 2019-nCoV Real-Time RT-PCR Diagnostic Panel, seventeen (17) were confirmed by genetic sequencing and/or virus culture (positive percent agreement = 17/17, 95% CI: 81.6%-100%)

During the early phase of the testing, a total of 117 respiratory specimens collected from 46 PUI subjects were also tested with two analytically validated real-time RT-PCR assays that target separate and independent regions of the nucleocapsid protein gene of the 2019-nCoV, N4 and N5 assays. The nucleocapsid protein gene targets for the N4 and N5 assays are different and independent from the nucleocapsid protein gene targets for the two RT-PCR assays included in the CDC 2019-nCoV Real-Time RT-

PCR Diagnostic Panel, N1 and N2. Any positive result from the N4 and/or the N5 assay was further investigated by genetic sequencing.

Performance of the CDC 2019-nCoV Real-Time RT-PCR Diagnostic Panel testing these 117 respiratory specimens was estimated against a composite comparator. A specimen was considered comparator negative if both the N4 and the N5 assays were negative. A specimen was considered comparator positive when the N4 and/or the N5 assay generated a positive result, and the comparator positive result(s) were further investigated and confirmed to be 2019-nCoV RNA positive by genetic sequencing.

Table 9: Percent Agreement of the CDC 2019-nCoV Real-Time RT-PCR Diagnostic Panel with the Composite Comparator

CDC 2019-nCoV Panel Result	**Composite Comparator Result**	
	Positive	Negative
Positive	13[1]	0
Inconclusive	0	0
Negative	0	104

[1]Composite comparator results were available for 13 of 49 CDC 2019-nCoV Panel positive specimens only.

Positive percent agreement = 13/13 = 100% (95% CI: 77.2% - 100%)
Negative percent agreement = 104/104 = 100% (95% CI: 96.4% - 100%)

Enzyme Master Mix Evaluation:

The limit of detection equivalence between the ThermoFisher TaqPath™ 1-Step RT-qPCR Master Mix and the following enzyme master mixes was evaluated: Quantabio qScript XLT One-Step RT-qPCR ToughMix, Quantabio UltraPlex 1-Step ToughMix (4X), and Promega GoTaq® Probe 1- Step RT-qPCR System. Serial dilutions of 2019 novel coronavirus (SARS CoV-2) transcript were tested in triplicate with the CDC 2019-nCoV Real-time RT-PCR Diagnostic Panel using all four enzyme master mixes. Both manufactured versions of oligonucleotide probe, BHQ and ZEN, were used in the comparison. The lowest detectable concentration of transcript at which all replicates tested positive using the Quantabio qScript XLT One-Step RT-qPCR ToughMix and Quantabio UltraPlex 1-Step ToughMix (4X) was similar to that observed for the ThemoFisher TaqPath™ 1-Step RT-qPCR Master Mix. The lowest detectable concentration of transcript when using the Promega GoTaq® Probe 1- Step RT-qPCR System was one dilution above that observed for the other candidates when evaluated with the BHQ version of the CDC assays. The candidate master mixes all performed equivalently or at one dilution below the ThemoFisher TaqPath™ 1-Step RT-qPCR Master Mix when evaluated with the ZEN version of the CDC assays.

Table 10: Limit of Detection Comparison for Enzyme Master Mixes – BHQ Probe Summary Results

Copy Number	ThemoFisher TaqPath™ 1-Step RT-qPCR Master Mix		Quantabio qScript XLT One-Step RT-qPCR ToughMix		Quantabio UltraPlex 1-Step ToughMix (4X)		Promega GoTaq® Probe 1- Step RT-qPCR System	
	2019-nCoV_N1	2019-nCoV_N2	2019-nCoV_N1	2019-nCoV_N2	2019-nCoV_N1	2019-nCoV_N2	2019-nCoV_N1	2019-nCoV_N2
10^2 copies/µL	3/3	3/3	3/3	3/3	3/3	3/3	3/3	3/3
10^1 copies/µL	3/3	3/3	3/3	3/3	3/3	3/3	3/3	3/3
10^0 copies/µL	3/3	3/3	3/3	3/3	3/3	3/3	3/3	2/3
10^{-1} copies µL	2/3	0/3	1/3	1/3	1/3	1/3	0/3	0/3

Table 11: Limit of Detection Comparison for Enzyme Master Mixes – ZEN Probe Summary Results

Copy Number	ThemoFisher TaqPath™ 1-Step RT-qPCR Master Mix		Quantabio qScript XLT One-Step RT-qPCR ToughMix		Quantabio UltraPlex 1-Step ToughMix (4X)		Promega GoTaq® Probe 1- Step RT-qPCR System	
	2019-nCoV_N1	2019-nCoV_N2	2019-nCoV_N1	2019-nCoV_N2	2019-nCoV_N1	2019-nCoV_N2	2019-nCoV_N1	2019-nCoV_N2
10^2 copies/µL	3/3	3/3	3/3	3/3	3/3	3/3	3/3	3/3
10^1 copies/µL	3/3	3/3	3/3	3/3	3/3	3/3	3/3	3/3
10^0 copies/µL	3/3	2/3	3/3	3/3	3/3	2/3	3/3	3/3
10^{-1} copies µL	1/3	1/3	0/3	0/3	0/3	1/3	1/3	1/3

Retrospective positive (18) and negative (17) clinical respiratory specimens were extracted using the QIAGEN EZ1 Advanced XL instrument and EZ1 DSP Virus Kit and were tested with the CDC 2019-nCoV Real-time RT-PCR Diagnostic Panel using the Quantabio qScript XLT One-Step RT-qPCR ToughMix, Quantabio UltraPlex 1-Step ToughMix (4X), and Promega GoTaq® Probe 1- Step RT-qPCR System master mixes. All three enzyme master mixes performed equivalently, demonstrating 100% positive and 100% negative agreement with expected results and a 95% confidence interval of 82.4%-100% and 81.6%-100%, respectively.

Table 12: Clinical Comparison – Retrospective Study Summary Results

CDC 2019-nCoV Real-time RT-PCR Diagnostic Panel Result	Quantabio qScript XLT One-Step RT-qPCR ToughMix		Quantabio UltraPlex 1-Step ToughMix (4X)		Promega GoTaq® Probe 1-Step RT-qPCR System	
	Positive	Negative	Positive	Negative	Positive	Negative
Positive	18	0	18	0	18	0
Negative	0	17	0	17	0	17

Disposal

Dispose of hazardous or biologically contaminated materials according to the practices of your institution.

References

1. Ballew, H. C., *et al*. “Basic Laboratory Methods in Virology,” DHHS, Public Health Service 1975 (Revised 1981), Centers for Disease Control and Prevention, Atlanta, Georgia 30333.

2. Clinical Laboratory Standards Institute (CLSI), “Collection, Transport, Preparation and Storage of Specimens for Molecular Methods: Proposed Guideline,” MM13-A

3. Lieber, M., *et al*. "A Continuous Tumor Cell Line from a Human Lung Carcinoma with Properties of Type II Alveolar Epithelial Cells." *International Journal of Cancer* 1976, 17(1), 62-70.

Revision History

Revision #	Effective Date	Summary of Revisions
1	February 4, 2020	Original Instructions for Use
2	March 15, 2020	• Intended use update • Removal of N3 primer and probe set from Diagnostic Panel • Performance data update • Addition of alternative nucleic acid extraction platforms • Addition of acceptable alternatives to HSC and addition of QIAGEN RUO extraction reagents • Positive results no longer presumptive. No confirmation of positive results required
3	March 30, 2020	• Addition of alternative enzyme master mix options

Contact Information, Ordering, and Product Support

For technical and product support, contact the CDC Division of Viral Diseases directly.

Send email to: respvirus@cdc.gov

Note: If your laboratory is using reagents sourced from someone other than the CDC International Reagent Resource, please refer to the manufacturer's instructions provided with the commercial materials.

DO NOT DISCARD: Important product-specific information

CDC 2019-nCoV Real-Time RT-PCR Diagnostic Panel

For use under EMERGENCY USE AUTHORIZATION (EUA) only.

Rx only

CATALOG: 2019-nCoV EUA-01

KIT LOT:

EXPIRATION DATE: YYYY-MM-DD (3 Years from DOM)

INTENDED USE

The CDC 2019-Novel Coronavirus (2019-nCoV) Real-Time RT-PCR Diagnostic Panel is a real-time RT-PCR test intended for the qualitative detection of nucleic acid from the 2019-nCoV in upper and lower respiratory specimens (such as nasopharyngeal or oropharyngeal swabs, sputum, lower respiratory tract aspirates, bronchoalveolar lavage, and nasopharyngeal wash/aspirate or nasal aspirate) collected from individuals who meet 2019-nCoV clinical and/or epidemiological criteria (for example, clinical signs and symptoms associated with 2019-nCoV infection, contact with a probable or confirmed 2019-nCoV case, history of travel to a geographic locations where 2019-nCoV cases were detected, or other epidemiologic links for which 2019-nCoV testing may be indicated as part of a public health investigation). Testing in the United States is limited to laboratories certified under the Clinical Laboratory Improvement Amendments of 1988 (CLIA), 42 U.S.C. § 263a, to perform high complexity tests.

Results are for the identification of 2019-nCoV RNA. The 2019-nCoV RNA is generally detectable in upper and lower respiratory specimens during infection. Positive results are indicative of active infection with 2019-nCoV but do not rule out bacterial infection or co-infection with other viruses. The agent detected may not be the definite cause of disease. Laboratories within the United States and its territories are required to report all positive results to the appropriate public health authorities.

Negative results do not preclude 2019-nCoV infection and should not be used as the sole basis for treatment or other patient management decisions. Negative results must be combined with clinical observations, patient history, and epidemiological information.

Testing with the CDC 2019-nCoV Real-Time RT-PCR Diagnostic Panel is intended for use by trained laboratory personnel who are proficient in performing real-time RT-PCR assays. The CDC 2019-Novel Coronavirus (2019-nCoV) Real-Time RT-PCR Diagnostic Panel is only for use under a Food and Drug Administration's Emergency Use Authorization .

PACKAGE CONTENTS

PACKAGING	COMPONENT	PART NUMBER	COMPONENT LOT NUMBER	VIALS PER KIT	QUANTITY /VIAL	STATE
Oligonucleotide Box	2019-nCoV_N1 Combined Primer/Probe Mix	RV202001		1	22.5 nmol	Dried
	2019-nCoV_N2 Combined Primer/Probe Mix	RV202002		1	22.5 nmol	Dried
	RP Combined Primer/Probe Mix	RV202004		1	22.5 nmol	Dried
Control Box	nCoVPC 2019-nCoV Positive Control (non-infectious)	RV202005		4	1×10^4 copies/µL	Dried

STORAGE INSTRUCTIONS

Upon receipt, store at 2-8°C. Refer to the CDC 2019-Novel Coronavirus (2019-nCoV) Real-Time RT-PCR Diagnostic Panel Instructions for Use before opening and preparing reagents for use.

PROCEDURE/INTERPRETATION/LIMITATIONS

Users should refer to the **CDC 2019-Novel Coronavirus (2019-nCoV) Real-Time RT-PCR Diagnostic Panel Instructions for Use** posted on the FDA website for all IVD products used under Emergency Use Authorization, http://www.fda.gov/MedicalDevices/Safety/EmergencySituations/ucm161496.htm.

IVD

PRECAUTIONS

This reagent should be handled in an approved BSL-2 handling area to avoid contamination of laboratory equipment and reagents that could cause false positive results. This product is non-infectious. However, this product should be handled in accordance with Good Laboratory Practices.

REAGENT COMPLAINTS/QUESTIONS

If you have a question/comment about this product, please contact the CDC Division of Viral Diseases/Respiratory Viruses Branch by email at respvirus@cdc.gov.

DISTRIBUTED BY

Manufactured by the Centers for Disease Control and Prevention, 1600 Clifton Road, Atlanta, Georgia, 30329, USA

IVD

****** DO NOT DISCARD: Important product-specific information ******

CDC 2019-nCoV Real-Time RT-PCR Diagnostic Panel – Verification Requirements

Please consult the following guidance from CMS regarding Emergency Use Authorized diagnostic tests: https://www.cms.gov/Medicare/Provider-Enrollment-and-Certification/SurveyCertificationGenInfo/Policy-and-Memos-to-States-and-Regions-Items/QSO18-19-CLIA

INTENDED USE

The CDC 2019-Novel Coronavirus (2019-nCoV) Real-Time RT-PCR Diagnostic Panel is a real-time RT-PCR test intended for the qualitative detection of nucleic acid from the 2019-nCoV in upper and lower respiratory specimens (such as nasopharyngeal or oropharyngeal swabs, sputum, lower respiratory tract aspirates, bronchoalveolar lavage, and nasopharyngeal wash/aspirate or nasal aspirate) collected from individuals who meet 2019-nCoV clinical and/or epidemiological criteria (for example, clinical signs and symptoms associated with 2019-nCoV infection, contact with a probable or confirmed 2019-nCoV case, history of travel to a geographic locations where 2019-nCoV cases were detected, or other epidemiologic links for which 2019-nCoV testing may be indicated as part of a public health investigation). Testing in the United States is limited to laboratories certified under the Clinical Laboratory Improvement Amendments of 1988 (CLIA), 42 U.S.C. § 263a, to perform high complexity tests.

Results are for the identification of 2019-nCoV RNA. The 2019-nCoV RNA is generally detectable in upper and lower respiratory specimens during infection. Positive results are indicative of active infection with 2019-nCoV but do not rule out bacterial infection or co-infection with other viruses. The agent detected may not be the definite cause of disease. Laboratories within the United States and its territories are required to report all positive results to the appropriate public health authorities.

Negative results do not preclude 2019-nCoV infection and should not be used as the sole basis for treatment or other patient management decisions. Negative results must be combined with clinical observations, patient history, and epidemiological information.

Testing with the CDC 2019-nCoV Real-Time RT-PCR Diagnostic Panel is intended for use by trained laboratory personnel who are proficient in performing real-time RT-PCR assays. The CDC 2019-Novel Coronavirus (2019-nCoV) Real-Time RT-PCR Diagnostic Panel is only for use under a Food and Drug Administration's Emergency Use Authorization.

REQUIRED MATERIALS

The 2019 novel coronavirus positive control (nCoVPC) is provided with the CDC 2019-nCoV Real-Time RT-PCR Diagnostic Panel and should be prepared according to the instructions for use. The nCoVPC consists of an RNA transcript of the 2019-nCoV N gene as well as human RNase P gene segment. nCoVPC will yield a positive result with the following primer and probe sets: 2019-nCoV_N1, 2019-nCoV_N2, and RP.

Approximately 2 mL of an upper respiratory specimen (e.g. nasopharyngeal swabs (NPS) in transport media) will be needed for testing. Specimens may be pooled if less than 2mL of one specimen is available.

Refer to CDC 2019-nCoV Real-Time RT-PCR Diagnostic Panel package insert (manufacturer instructions) for additional reagents, materials, and instructions.

PRECAUTIONS

This reagent should be handled in an approved BSL-2 handling area to avoid contamination of laboratory equipment and reagents that could cause false positive results. This product is an

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RNA transcript and is non-infectious. However, the nCoVPC should be handled in accordance with Good Laboratory Practices.

Store reagent at appropriate temperatures (see instructions for use) and hold on ice when thawed.

Please use standard precautions when handling respiratory specimens.

INSTRUCTIONS FOR PREPARING SAMPLES BEFORE EXTRACTION WITH THE QIAamp DSP VIRAL RNA MINI KIT OR THE QIAamp VIRAL RNA MINI KIT

- Refer to the 2019-nCoV Real-Time RT-PCR Diagnostic Panel instructions for use for reconstitution of the materials for use. RNA should be kept cold during preparation and use.
- Make a 1/10 dilution of nCoVPC by adding 5 µL of nCoVPC into 45 µL of nuclease-free water or 10 mM Tris
- Aliquot 560 µL of lysis buffer into each of nine tubes labeled 1-9.
- Add 140 µL of upper respiratory specimen (e.g. NPS in viral transport media) into each of the nine labeled tubes with lysis buffer
- To prepare samples at a moderate concentration, spike 14 µL of undiluted nCoVPC (rehydrated as described in the CDC 2019-nCoV Real-Time RT-PCR Diagnostic Panel instructions for use) into each tube labeled 1-3 containing lysis buffer and specimen
- To prepare samples at a low concentration, spike 14 µL of 1/10 dilution of nCoVPC into each tube labeled 4-6 containing lysis buffer and specimen
- To prepare negative samples, spike 14 µL of nuclease-free water into each tube labeled 7-9 containing lysis buffer and specimen
- Perform extractions of all nine samples according to the CDC 2019-nCoV Real-Time RT-PCR Diagnostic Panel instructions for use

INSTRUCTIONS FOR PREPARING SAMPLES BEFORE EXTRACTION WITH THE QIAGEN EZ1 ADVANCED XL

- Refer to the 2019-nCoV Real-Time RT-PCR Diagnostic Panel instructions for use for reconstitution of the materials for use. RNA should be kept cold during preparation and use.
- Make a 1/10 dilution of nCoVPC by adding 5 µL of nCoVPC into 45 µL of nuclease-free water or 10 mM Tris
- Aliquot 280 µL of lysis buffer into each of nine tubes labeled 1-9.
- Add 120 µL of upper respiratory specimen (e.g. NPS in viral transport media) into each of the nine labeled tubes with lysis buffer
- To prepare samples at a moderate concentration, spike 12 µL of undiluted nCoVPC (rehydrated as described in the CDC 2019-nCoV Real-Time RT-PCR Diagnostic Panel instructions for use) into each tube labeled 1-3 containing lysis buffer and specimen
- To prepare samples at a low concentration, spike 12 µL of 1/10 dilution of nCoVPC into each tube labeled 4-6 containing lysis buffer and specimen
- To prepare negative samples, spike 12 µL of nuclease-free water into each tube labeled 7-9 containing lysis buffer and specimen
- Perform extractions of all nine samples according to the CDC 2019-nCoV Real-Time RT-PCR Diagnostic Panel instructions for use

INSTRUCTIONS FOR PREPARING SAMPLES BEFORE EXTRACTION WITH THE ROCHE MagNA PURE TOTAL NUCLEIC ACID KIT OR THE ROCHE MagNA PURE NUCLEIC ACID ISOLATION KIT I

- Refer to the 2019-nCoV Real-Time RT-PCR Diagnostic Panel instructions for use for reconstitution of the materials for use. RNA should be kept cold during preparation and use.
- Make a 1/10 dilution of nCoVPC by adding 5 µL of nCoVPC into 45 µL of nuclease-free water or 10 mM Tris
- Aliquot 300 µL of lysis buffer into each of nine tubes labeled 1-9.
- Add 100 µL of upper respiratory specimen (e.g. NPS in viral transport media) into each of the nine labeled tubes with lysis buffer

**** DO NOT DISCARD: Important product-specific information ****

- To prepare samples at a moderate concentration, spike 12 µL of undiluted nCoVPC (rehydrated as described in the CDC 2019-nCoV Real-Time RT-PCR Diagnostic Panel instructions for use) into each tube labeled 1-3 containing lysis buffer and specimen
- To prepare samples at a low concentration, spike 12 µL of 1/10 dilution of nCoVPC into each tube labeled 4-6 containing lysis buffer and specimen
- To prepare negative samples, spike 12 µL of nuclease-free water into each tube labeled 7-9 containing lysis buffer and specimen
- Perform extractions of all nine samples according to the CDC 2019-nCoV Real-Time RT-PCR Diagnostic Panel instructions for use

INSTRUCTIONS FOR PREPARING SAMPLES BEFORE EXTRACTION WITH THE ROCHE MagNA PURE 96 DNA AND VIRAL NA SMALL VOLUME KIT

- Refer to the 2019-nCoV Real-Time RT-PCR Diagnostic Panel instructions for use for reconstitution of the materials for use. RNA should be kept cold during preparation and use.
- Make a 1/10 dilution of nCoVPC by adding 5 µL of nCoVPC into 45 µL of nuclease-free water or 10 mM Tris
- Aliquot 350 µL of lysis buffer into each of nine tubes labeled 1-9.
- Add 100 µL of upper respiratory specimen (e.g. NPS in viral transport media) into each of the nine labeled tubes with lysis buffer
- To prepare samples at a moderate concentration, spike 12 µL of undiluted nCoVPC (rehydrated as described in the CDC 2019-nCoV Real-Time RT-PCR Diagnostic Panel instructions for use) into each tube labeled 1-3 containing lysis buffer and specimen
- To prepare samples at a low concentration, spike 12 µL of 1/10 dilution of nCoVPC into each tube labeled 4-6 containing lysis buffer and specimen
- To prepare negative samples, spike 12 µL of nuclease-free water into each tube labeled 7-9 containing lysis buffer and specimen
- Perform extractions of all nine samples according to the CDC 2019-nCoV Real-Time RT-PCR Diagnostic Panel instructions for use

INSTRUCTIONS FOR PREPARING SAMPLES BEFORE EXTRACTION WITH THE BIOMÉRIEUX NucliSENS easyMAG OR THE BIOMÉRIEUX EMAG

- Refer to the 2019-nCoV Real-Time RT-PCR Diagnostic Panel instructions for use for reconstitution of the materials for use. RNA should be kept cold during preparation and use.
- Make a 1/10 dilution of nCoVPC by adding 5 µL of nCoVPC into 45 µL of nuclease-free water or 10 mM Tris
- Aliquot 1000 µL or 2000 µL of pre-aliquoted easyMAG lysis buffer into each of nine tubes labeled 1-9 for the easyMAG or eMAG, respectively.
- Add 100 µL of upper respiratory specimen (e.g. NPS in viral transport media) into each of the nine labeled tubes with lysis buffer
- To prepare samples at a moderate concentration, spike 12 µL of undiluted nCoVPC (rehydrated as described in the CDC 2019-nCoV Real-Time RT-PCR Diagnostic Panel instructions for use) into each tube labeled 1-3 containing lysis buffer and specimen
- To prepare samples at a low concentration, spike 12 µL of 1/10 dilution of nCoVPC into each tube labeled 4-6 containing lysis buffer and specimen
- To prepare negative samples, spike 12 µL of nuclease-free water into each tube labeled 7-9 containing lysis buffer and specimen
- Perform extractions of all nine samples according to the CDC 2019-nCoV Real-Time RT-PCR Diagnostic Panel instructions for use

PROCEDURE

Follow the CDC 2019-nCoV Real-Time RT-PCR Diagnostic Panel instructions for use for testing the 9 extracted samples at least once.

EXPECTED RESULTS

Moderate nCoVPC samples should be positive for 2019-nCoV.
Low nCoVPC samples should be positive for 2019-nCoV.
Negative upper respiratory samples should be negative for 2019-nCoV.

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≥ 90% of test results should be in agreement with the expected results. If test results are less than 90% in agreement with expected results, contact CDC at respvirus@cdc.gov.

QUESTIONS

Please send questions or comments by email to respvirus@cdc.gov.

DISTRIBUTION:

Distributed to qualified laboratories by Centers for Disease Control and Prevention, 1600 Clifton Road, Atlanta, GA, 30329 USA

The New York Times | https://nyti.ms/2vTRFbf

How Does the Coronavirus Compare With the Flu?

As new cases appear in the U.S., some — including the president — have compared it to the seasonal flu. Here's a close look at the differences.

By Denise Grady

March 27, 2020

As coronavirus infections began appearing across the United States, in cities from Seattle to New York, Americans wondered how to measure this new threat against a more familiar foe: influenza.

President Trump, a self-described germophobe, has said he was amazed to learn that tens of thousands of Americans died from the flu each year. On several occasions, Mr. Trump has accused the news media and Democrats of exaggerating the dangers of the coronavirus.

"The flu kills people," Mick Mulvaney, the acting White House chief of staff, said in February. "This is not Ebola. It's not SARS, it's not MERS. It's not a death sentence."

To many public health officials, that argument misses the point.

Yes, the flu is terrible — that's exactly why scientists don't want another contagious respiratory disease to take root. If they could stop the seasonal flu, they would. But there may yet be a chance to stop the coronavirus, or at least slow its spread.

In many ways, the flu is the best argument for throwing everything at the coronavirus. Here's a closer look at the similarities and differences.

Which virus is deadlier?

The coronavirus seems to be more deadly than the flu — so far.

On average, seasonal flu strains kill about 0.1 percent of people who become infected. The 1918 flu had an unusually high fatality rate, around 2 percent. Because it was so contagious, that flu killed tens of millions of people.

Early estimates of the coronavirus death rate from China were about 2 percent. But a later report on 1,099 cases from many parts of China, published in The New England Journal of Medicine, found a lower rate: 1.4 percent.

In a recent speech, Dr. Tedros Adhanom Ghebreyesus, director-general of the World Health Organization, asserted that the global case fatality rate for people infected with coronavirus was 3.4 percent, a startling figure.

W.H.O. officials later clarified that Dr. Tedros's figure was a crude "snapshot" based on incomplete data and heavily skewed by the intensity of the initial outbreak in Wuhan, China.

The true death rate could turn out to be similar to that of a severe seasonal flu, below 1 percent, according to an editorial published in the journal by Dr. Anthony S. Fauci and Dr. H. Clifford Lane, of the National Institute

of Allergy and Infectious Diseases, and Dr. Robert R. Redfield, director of the Centers for Disease Control and Prevention. But more recently, Dr. Fauci has cited the 1 percent estimate, emphasizing that it is 10 times the death rate from seasonal flu.

Even a disease with a relatively low death rate can take a huge toll if enormous numbers of people catch it. As of Friday, there were more than 135,000 coronavirus cases and nearly 5,000 deaths. In the United States, there have been more than 1,200 coronavirus cases and about 36 deaths.

But because of the lack of testing capacity in the United States, the true case count and number of deaths are not known for sure.

Which virus is more contagious?

So far, the new coronavirus seems to be more contagious than most strains of the flu, and roughly as contagious as strains that appear in pandemic flu seasons.

Each person with the coronavirus appears to infect 2.2 other people, on average. But the figure is skewed by the fact that the epidemic was not managed well in the beginning, and infections soared in Wuhan and the surrounding province. As an epidemic comes under control, the reproduction number, as it's called, will fall.

By comparison, the figure for the seasonal flu is roughly 1.3. The reproduction number for the flu of 1918 was about the same as that of the new coronavirus, perhaps higher, but that was before modern treatments and vaccines were available.

In both flu and the illness caused by the coronavirus, people may be contagious before symptoms develop, making it difficult or even impossible to control the spread of the virus. Nobody knows yet how many people infected with the coronavirus have only very mild symptoms or none at all.

Who is most at risk from infection?

People who are older than 60, or have a weakened immune system or chronic illnesses like lung disease, heart disease or diabetes, have the highest risk of becoming severely ill if they contract the coronavirus or the flu. Each underlying illness adds to the risk.

Many people in the United States have an increased risk of becoming seriously ill if they are infected: about 60 percent of adults have at least one underlying health condition, and 40 percent have two or more underlying conditions. Approximately 25 million have diabetes, which can lower immunity.

Death rates among men infected with the coronavirus in China, particularly those in their late 40s and older, have exceeded those among women, a pattern not seen in the seasonal flu. The reason for the discrepancy is not known, although Chinese men do smoke more, often resulting in compromised lung function.

There seems to be another important difference: The flu appears far more dangerous to children, particularly very young ones, who can become severely ill. Children infected with the new coronavirus tend to have mild or no symptoms.

The flu is also especially dangerous for pregnant women, who can become severely ill from it. Whether the new coronavirus poses as serious a threat to pregnant women is not known.

Not everyone who becomes seriously ill fits the high-risk profile. In every infectious disease outbreak, there are unexplained cases that defy the statistics, such as severe illness striking a young, healthy person who would have been expected to become just mildly sick. The physician in China who was penalized for alerting colleagues to the outbreak there, Dr. Li Wenliang, died from the disease at age 34.

Which virus makes you sicker?

In the current season, there have been at least 34 million cases of flu in the United States, 350,000 hospitalizations and 20,000 flu deaths, according to the C.D.C. Hospitalization rates among children and young adults this year have been unusually high.

There would be even more illnesses and deaths if there were no flu vaccine. Most people recover in less than two weeks, and sometimes in just days.

By contrast, at least 90,000 people in the United States have been infected with the new coronavirus by late March, and there have been at least 1,400 deaths. There are no treatments or vaccines for the coronavirus, only supportive care for infected people.

Most cases of coronavirus infection are not severe, but some people do become quite sick. Data from the largest study of patients to date, conducted in China, suggests that of coronavirus patients receiving medical attention, 80 percent had mild infections, about 15 percent had severe illnesses, and 5 percent were critical. (But many of the mild infections included patients with pneumonia, experts later learned.)

The first symptoms, fever and cough, are similar to that of the flu, so the diseases can be hard to tell apart without a test to identify the virus. Pneumonia is common among coronavirus patients, even among those whose cases are not severe.

Experts think there may also be many people with no symptoms at all, or such mild ones that they never bother to seek medical attention. Because those cases have not been counted, it's not possible now to know the real proportion of mild versus severe cases.

Antibody tests, which can determine whether someone has ever been infected, may eventually help to establish how many people had mild or asymptomatic coronavirus infections.

Can people become immune to the coronavirus?

After viral infections, people generally develop antibodies in their blood that will fight off the virus and protect them from contracting it again. It's reasonable to assume that people who have had the new coronavirus will become immune to it.

But it is not known how long that immunity will last. With other coronaviruses, which cause the common cold, immunity can wane.

There are vaccines for the seasonal flu, of course, and these induce at least some immunity to influenza.

What treatments are available?

There is no approved antiviral drug for the coronavirus, though several are being tested. Doctors can recommend only the usual remedies for any viral illness: rest, medicine to reduce pain and fever, and fluids to avoid dehydration.

Coronavirus patients with pneumonia may also need oxygen, and a ventilator if breathing trouble worsens.

For the flu, however, there are four prescription medicines. All work best if they are taken within a day or two of when symptoms start.

They're not miracle cures: They can lessen the severity of the illness and shorten its course by a day or so, and they may lower the risk of serious complications.

The drugs are also recommended for people who have been exposed to a flu patient, to try to prevent the illness.

The flu, like the coronavirus illness, can also cause pneumonia and breathing trouble. Anyone who becomes short of breath needs medical attention quickly.

Can I get vaccinated?

An experimental vaccine for the coronavirus may be ready for safety testing in humans soon, but will take much longer, at least a year or two, to become available for widespread use — if it works.

Flu vaccines, on the other hand, are widely available and generally 40 percent to 60 percent effective, which means they will reduce cases by that amount in a population that has been vaccinated, compared with one that has not.

The vaccine for the current season falls into that range, according to the C.D.C., which said in February that people who have not been vaccinated should still get the shot, because the flu season is ongoing.

Experts have been urging people to get the flu shot for all the usual reasons. But now there's another: As the coronavirus spreads in the United States, hospitals will need all the beds, equipment and staff they can muster.

It will be important not to have those resources taken up by patients with flu that could have been prevented.

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Will the coronavirus go away when the weather warms?

Mr. Trump has said repeatedly that the coronavirus will retreat as weather warms, just as influenza does. In fact, because this is a new virus, there is no information about how the weather might affect it.

Even if the virus were to diminish in the spring, it might rebound later in the fall, as the weather cools. This is a pattern often seen in severe flu seasons.

Containment is becoming less likely, because of the contagiousness of the virus, the possibility that people can spread it before they have symptoms and the increasing number of outbreaks around the world.

Cases in California, New York, Oregon and Washington State without known links to overseas travel indicate the new coronavirus has already begun to circulate.

Reporting was contributed by Gina Kolata and Knvul Sheikh.

The Coronavirus Outbreak

Frequently Asked Questions and Advice

Updated April 4, 2020

- **Should I wear a mask?**
  The C.D.C. has recommended that all Americans wear cloth masks if they go out in public. This is a shift in federal guidance reflecting new concerns that the coronavirus is being spread by infected people who have no symptoms. Until now, the C.D.C., like the W.H.O., has advised that ordinary people don't need to wear masks unless they are sick and coughing. Part of the reason was to preserve medical-grade masks for health care workers who desperately need them at a time when they are in continuously short supply. Masks don't replace hand washing and social

READ MORE

The Washington Post

Democracy Dies in Darkness

Three months into the pandemic, here's how likely the coronavirus is to infect people

By **Joel Achenbach**

March 28, 2020 at 8:16 p.m. GMT+5:30

PLEASE NOTE

The Washington Post is providing this story for free so that all readers have access to this important information about the coronavirus. For more free stories, sign up for our daily Coronavirus Updates newsletter.

Three months into this pandemic, scientists are coming to understand the novel coronavirus. They know, for example, that as horrible as this virus is, it is not the worst, most apocalyptic virus imaginable. Covid-19, the disease caused by the virus, is not as contagious as measles, and although it is very dangerous, it is not as likely to kill an infected person as, say, Ebola.

But there is one critically important, calamitous feature of SARS-CoV-2: the novelty. When it jumped from an animal host into the human population sometime late last year, no one had immunity to it. That is one reason the new coronavirus is not comparable to a harsh strain of the flu going around.

The first cluster of mysterious, pneumonia-like respiratory illnesses was reported in Wuhan, China, at the end of December, and in the days that followed, it spread explosively. With astonishing speed, this submicroscopic pathogen has contaminated the planet, infecting more than 600,000 as of Saturday and killing at least 28,000, grinding global commerce to a near standstill and rattling the nerves of everyone brave enough to be following the news.

“This is a new virus that has landed in the human community. We are a brand-new, naive population. We’re kind of sitting ducks, right?” said Ilhem Messaoudi, a virologist at the University of California at Irvine.

Most viral contagions in circulation face obstacles in the form of people with at least partial immunity. But this coronavirus is a bulldozer. It can flatten everyone in its path.

When the virus infects people, they don't get sick right away. Researchers believe the incubation period before symptoms is roughly five days on average. In studying the pattern of illness, epidemiologists have made the dismaying discovery that people start shedding the virus — potentially making others sick — in advance of symptoms. Thus, the virus has a gift for stealth transmission. It seeds itself in communities far and wide, where vulnerable human beings represent endless fertile terrain.

At the genetic level, the new virus is not terribly different from the SARS virus that emerged in China in 2002 — which is why the new one has the derivative name SARS-CoV-2. SARS killed nearly 1 in 10 patients. But people with SARS infections did not shed the virus until they were already quite sick, and victims were typically hospitalized. SARS was snuffed out after causing about 8,000 infections and 774 deaths worldwide.

That successful fight may have led to some complacency; researchers say funding for SARS research dried up in recent years.

“We thought we cured it. We thought the virus disappeared. Well, the virus didn’t disappear, did it?” said Michael Buchmeier, a UC Irvine virologist who has studied coronaviruses for three decades.

Because this is such a contagious virus, a large percentage of the world’s population, potentially billions of people, could become infected within the next couple of years. Frantic efforts to develop a safe and effective vaccine are likely to take a year or more.

President Trump and others have repeatedly downplayed the threat of covid-19 by comparing its lethality to seasonal influenza, which claims tens of thousands of lives in the United States every year. But covid-19 may be many times as lethal for an infected person as seasonal flu.

Messaoudi noted that the health system is set up to deal with the seasonal flu, but not with a new, pandemic disease.

“We have a vaccine for the flu. And antivirals. It’s seasonal, we prepare for it, we try to get vaccination coverage; this is already what our system is dealing with,” she said. “This is the wrong time to deal with another surge of a respiratory disease that causes a lot of morbidity and potentially mortality.”

The bulldozer nature of coronavirus means widespread severe illnesses and deaths from covid-19 can happen with terrifying speed. This happened in northern Italy, where hospitals become overwhelmed and many patients couldn’t get standard lifesaving treatment.

The pandemic appears to be largely driven by direct, human-to-human transmission. That is why public health officials have told people to engage in social distancing, a simple but effective way to drive down virus’s reproductive number — known as R0, pronounced “R naught.” That is the average number of new infections generated by each infected person.

The R0 is not an intrinsic feature of the virus. It can be lowered through containment, mitigation and ultimately “herd immunity,” as people who have recovered become less susceptible to infections or serious illnesses. For the epidemic to begin to end, the reproduction rate has to drop below 1.

In the early days in China, before the government imposed extreme travel restrictions in Wuhan and nearby areas, and before everyone realized exactly how bad the epidemic might be, the R0 was 2.38, according to a study published in the journal Science. That is a highly contagious disease.

But on Jan. 23, China imposed extreme travel restrictions and soon put hundreds of millions of people into some form of lockdown as authorities aggressively limited social contact. The R0 plummeted below 1, and the epidemic has been throttled in China, at least for now.

The virus does have an innate infectivity, based on how it binds to receptors in cells in the respiratory tract and then takes over the machinery of those cells to make copies of itself. But its ability to spread depends also on the vulnerability of the human population, including the density of the community.

“If you have a seriously infectious virus and you’re sitting by yourself in a room, the R naught is zero. You can’t give it to anybody,” says Jeffery Taubenberger, a virologist with the National Institute of Allergy and Infectious Diseases.

Without a vaccine or a drug to stop infections, the best hope is to break the chain of transmission one infection at a time. There is no way to combat the virus through aerial spraying, dousing the public drinking water with a potion or simply hoping that it will magically go away.

“Social distancing is building speed bumps so that we can slow the spread of the virus. We have to respect the speed bumps,” Messaoudi said.

Melissa Nolan, an epidemiologist at the University of South Carolina, said the efficacy of social distancing “is the million-dollar question right now.”

She compared the current public measures to what happened during the 1918 influenza pandemic that killed an estimated 675,000 people in the United States, and in which some cities were more careful than others about enforcing social distancing.

“The USA is currently in a natural experiment of sorts, which each state implementing their own version of social distancing,” she said. “We will be able to compare the efficacy of these various public health policies, but not until more time has passed.”

The social distancing effort requires individual participation on behalf of a collective need. But it is self-interested first and foremost: No one wants to catch this virus. It can be deadly, and even if not, many victims are miserable for days or even weeks on end.

Not only must people limit their direct contact, they need to limit the amount that their paths overlap, because the virus can linger on surfaces.

The virus degrades outside a host because of exposure to moisture and sunlight, or from drying out. But a study published in the New England Journal of Medicine showed that in pristine laboratory conditions, some SARS-CoV-2 particles can remain potentially viable on metal or plastic for up to three days.

It is unclear to what degree contact with contaminated surfaces is playing a role in the contagion. This is obviously something everyone would like to know when they handle the pump at a gas station or go to a grocery store. Absent hard data, limiting contact with shared surfaces, such as door handles or checkout machines, and frequent hand-washing is highly advisable.

Even though we do not have a vaccine, and no one had immunity to this novel pathogen, people have some innate, mechanical defenses against viruses just like they do against pollen and dust, Taubenberger noted. Cells in the respiratory tract have tiny hairlike projections, called cilia, that move mucus toward the throat in a manner that helps clear invasive particles. This is not our body's first viral rodeo.

Coronavirus: What you need to read

The Washington Post is providing some coronavirus coverage free, including:

Updated April 5, 2020

Live updates: The latest in the U.S. and abroad | The latest from the D.C. region

More news today: Across the U.S., the coronavirus is killing more men than women | Rate of infection among Navajos is a major concern

Mapping the spread: Cases and deaths in the U.S. | Map of cases worldwide

What you need to know: How to make your own fabric mask | What to do if you get laid off or furloughed | Calculate how much money you might receive from the stimulus bill | Follow all of our coronavirus coverage and sign up for our daily newsletter (all stories in the newsletter are free).

How to help: Your community | Seniors | Restaurants | Keep at-risk people in mind

Share your story: Has someone close to you died from covid-19?

How does coronavirus compare to flu, Sars, and other diseases?

Over 100 countries have now confirmed cases of coronavirus, with more than 19,000 deaths. How does it compare to other diseases?

By Dominic Gilbert, DATA JOURNALIST

25 March 2020 • 4:55pm

More than 8,000 cases of coronavirus are now confirmed in the UK, and early estimates are being made of how quickly the disease is likely to spread.

Experts have been rushing to assess the spread of the Covid-19 virus, which has so far killed more than 19,000 people - mostly in Italy.

Scientists believe Covid-19 has mutated into two strains: the older 'S-type' appears to be milder and less infectious, while the 'L-type' which emerged later, spreads quickly and currently accounts for around 70 per cent of cases. It may also be possible to be infected with both types.

In January, the World Health Organisation (WHO) estimated the current trend of the spread, analysing how many people would be infected per case.

According to early WHO estimates, the average reproductive rate (r0) of coronavirus ranged between 1.4 and 2.5. That meant, on average, each confirmed case of coronavirus would infect between 1.4 and 2.5 other people.

Any disease with an r0 of more than one will spread and need effective control measures. WHO said control measures would need to block at least 60 per cent of transmissions to be effective in keeping the coronavirus in check.

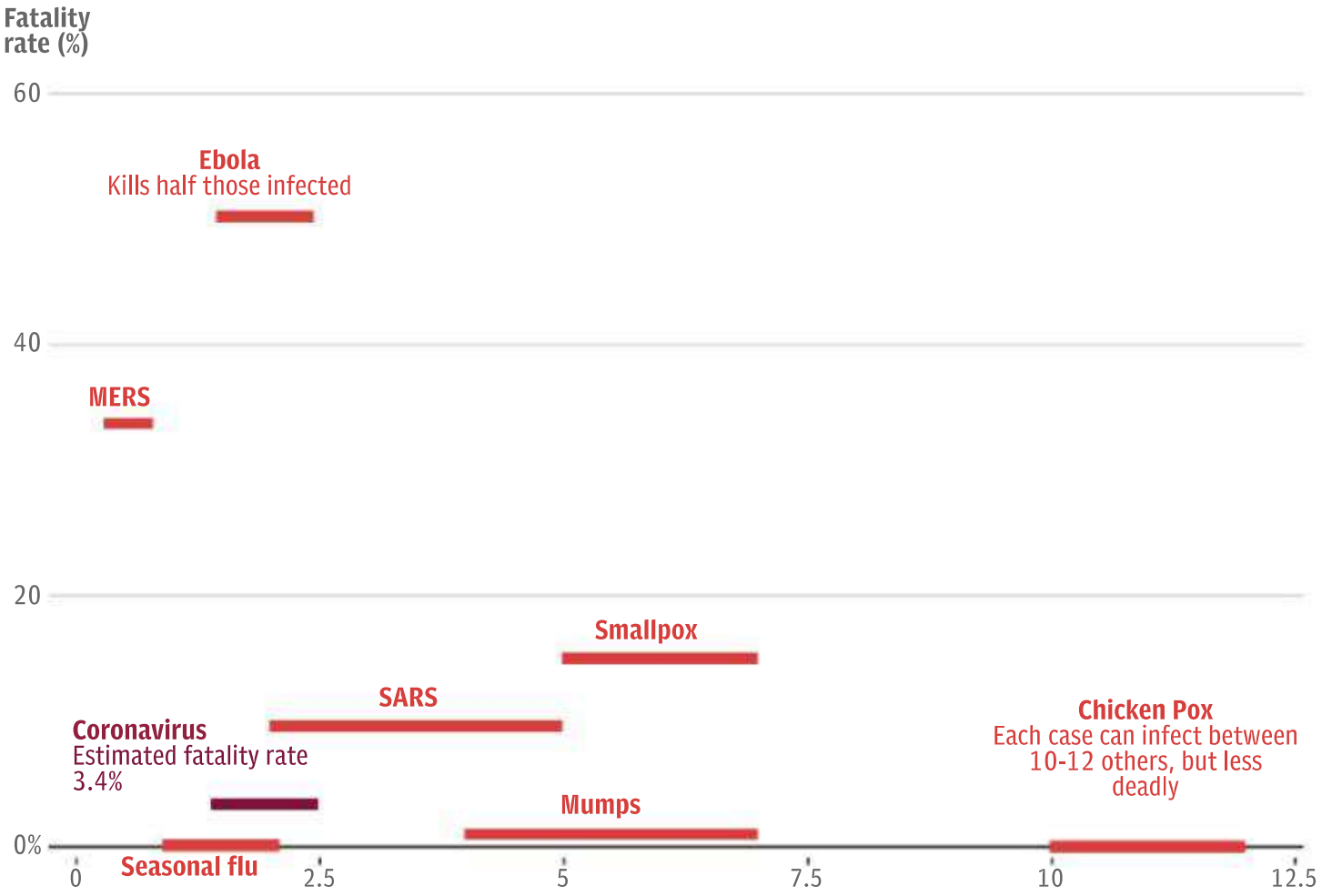
The r0 measure is an average - meaning 'super spreaders' could infect many more, and others could infect no other people. Early estimates are also dynamic and could vary

significantly as the disease develops.

Risk factors including age and location are also significant variables.

But measured against other viral outbreaks and common diseases, coronavirus appears at the first estimate to be less contagious or deadly than many others, giving hope for containment.

How does it compare to other diseases?
Average reproductive rate (r0) of infectious diseases and their fatality rate



With a mortality rate currently estimated at around 3.4 per cent according to the latest WHO estimates, it is less deadly to those who become affected than Ebola, Sars or Mers.

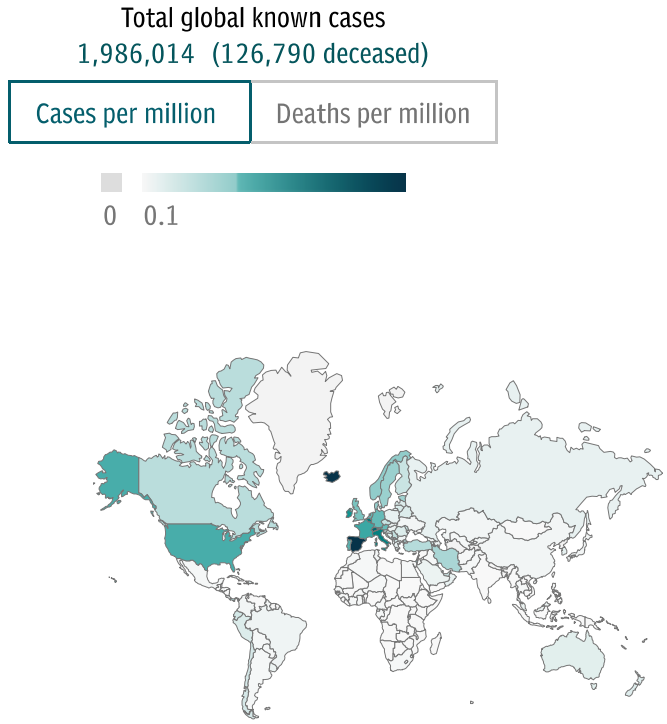
It is more contagious than some of the most deadly airborne viruses, however. Mers has an r0 of between 0.3 and 0.8, and a fatality rate of around 35 per cent.

At the other end of the scale, chicken pox is very contagious, with each case on average infecting between 10 and 12 others, but with an extremely low fatality rate.

Initial estimates are already being contested. A study from the MRC centre at Imperial College London estimated that, up until January 18, the r0 for coronavirus was between 1.5 and 3.5, higher than the WHO estimate.

That would match it more closely with Sars, which infects on average two to five people per confirmed case.

Coronavirus cases tracker



Source: WHO, CDC, ECDC, NHC, DXY.

- Coronavirus Live Tracker: latest figures for your local area, the UK and worldwide

Related Topics

Bird Flu, Data story, Flu, Global Health Security, Coronavirus

Office for National Statistics

Statistical bulletin

Deaths registered weekly in England and Wales, provisional: week ending 27 March 2020

Provisional counts of the number of deaths registered in England and Wales, including deaths involving the coronavirus (COVID-19), by age, sex and region, in the latest weeks for which data are available.

Contact:
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+44 (0)1633 456 490

Release date:
7 April 2020

Next release:
14 April 2020

Table of contents

1 . Main points

- The provisional number of deaths registered in England and Wales in the week ending 27 March 2020 (Week 13) was 11,141; this represents an increase of 496 deaths registered compared with the previous week (Week 12) and 1,011 more than the five-year average.
- A total of 150,047 deaths were registered in England and Wales between 28 December 2019 and 27 March 2020 (year to date), and of these, 647 involved the coronavirus (COVID-19) (0.4%); including deaths that occurred up to 27 March but were registered up to 1 April, the number involving COVID-19 was 1,639.
- For deaths that occurred up to 27 March, there were 1,568 deaths in England registered by 1 April involving COVID-19 compared with 1,649 deaths reported by NHS England for the same period in a newly published dataset.
- Of the deaths registered in Week 13, 539 mentioned "novel coronavirus (COVID-19)", which is 4.8% of all deaths; this compared with 103 (1.0% of all deaths) in Week 12.
- This is slightly lower than the figures reported by the Department of Health and Social Care (DHSC) for Week 13 (739) as it takes time for deaths to be reported and included in Office for National Statistics (ONS) figures.
- Of deaths involving COVID-19 in Week 13, 92.9% (501 deaths) occurred in hospital with the remainder occurring in hospices, care homes and private homes.
- Please note, where Easter falls in previous years will have an impact on the five-year average used for comparison.

2 . Comparisons of COVID-19 death counts

The Department of Health and Social Care (DHSC) release daily updates on the GOV.UK website counting the total number of deaths reported to them that have occurred in hospitals among patients who have tested positive for the coronavirus (COVID-19) up until 5pm the day before.

Since 2 April, NHS England have been releasing daily updates of deaths in hospitals among patients who have tested positive for COVID-19 in England, which includes updates on previous days numbers.

The Office for National Statistics (ONS) provides figures based on all deaths registered involving COVID-19 according to death certification, whether in or out of hospital settings. More information can be found in the Measuring the Data section.

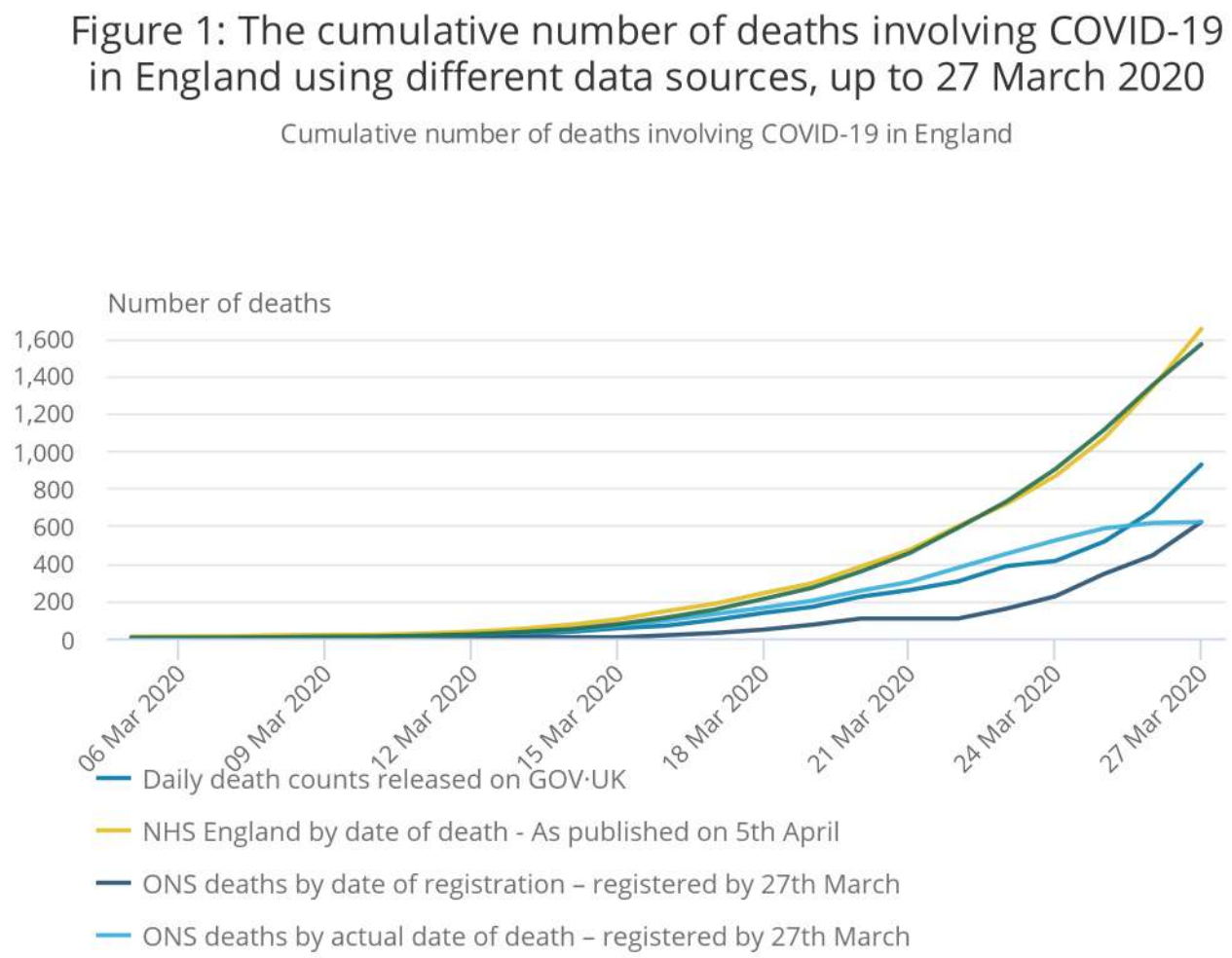
Using these three sources for England only, Figure 1 shows for each day:

- the numbers of deaths involving COVID-19 that were announced each day by DHSC
- the numbers of deaths that occurred each day, as released by NHS England (the same data as DHSC announce, but counted by date of death)
- the numbers of deaths that occurred each day for those that were registered by and informed to the ONS by 1 April

At the time of publication, further work is in progress across government to reconcile all sources of COVID-19 deaths data. We will be reviewing the comparisons section in light of these developments in the coming weeks.

Figure 1: The cumulative number of deaths involving COVID-19 in England using different data sources, up to 27 March 2020

Cumulative number of deaths involving COVID-19 in England



Source: Department of Health and Social Care, NHS England, Office for National Statistics

Notes:

1. DHSC figures.
2. NHS England figures.
3. Figures include deaths of non-residents.
4. Estimates are provisional.
5. The ICD-10 definitions for COVID-19 are U07.1 and U07.2.

Figure 1 shows that on 27 March, the DHSC reported 926 total deaths had taken place in hospitals in England (deaths by 5pm on the 27 March as announced on the 28 March). NHS England's reconciled figures now report 1,649 deaths in hospitals by the same date (published on 5 April). The number of deaths registered by 1 April involving COVID-19, by the same date of death, was 1,568 occurring both within and outside of hospitals. This is more than double that published by the DHSC but slightly lower than NHS England's latest reconciled figures. This is because of the time taken for deaths to be registered.

We have undertaken some preliminary analysis to understand how many deaths registered in England and Wales so far have taken place outside of hospital settings. The analysis shows that of deaths involving COVID-19 in Week 13, 92.9% (501 deaths) occurred in hospital with the remainder occurring in hospices, care homes and private homes.

Table 1: The majority of COVID-19 deaths occurred within hospitals
England and Wales

	Number of deaths	Number of COVID-19 deaths
Home	2,785	15
Hospitals (acute or community not psychiatric)	5,105	501
Hospice	504	2
Care Home	2,489	20
Other communal establishments	33	0
Elsewhere	225	1
Total	11,141	539

Source: Office for National Statistics – Deaths registered weekly in England and Wales, provisional: week ending 27 March 2020

Notes

1. For all deaths registered from 20 to 27 March 2020. Back to table
2. Figures include deaths of non-residents. Back to table
3. Estimates are provisional. Back to table
4. The International Classification of Diseases and Related Health Problems (ICD-10) definitions for COVID-19 are U07.1 and U07.2. Back to table

The figures published on GOV.UK are valuable because they are available very quickly and give an indication of what is happening day by day. Their definition is also clear, so the limitations of the data can be understood. But they will not necessarily include all deaths involving COVID-19, such as those in England that are not in a hospital or where no test result was available. Although the main GOV.UK figure reported is for the whole UK, breakdowns by area are available.

NHS England's reconciled numbers are valuable as they give a good indication of the lags in the daily deaths in hospital reporting process. They allow analysis by date of death to be carried out, which is a better indicator of the growth in the number of deaths.

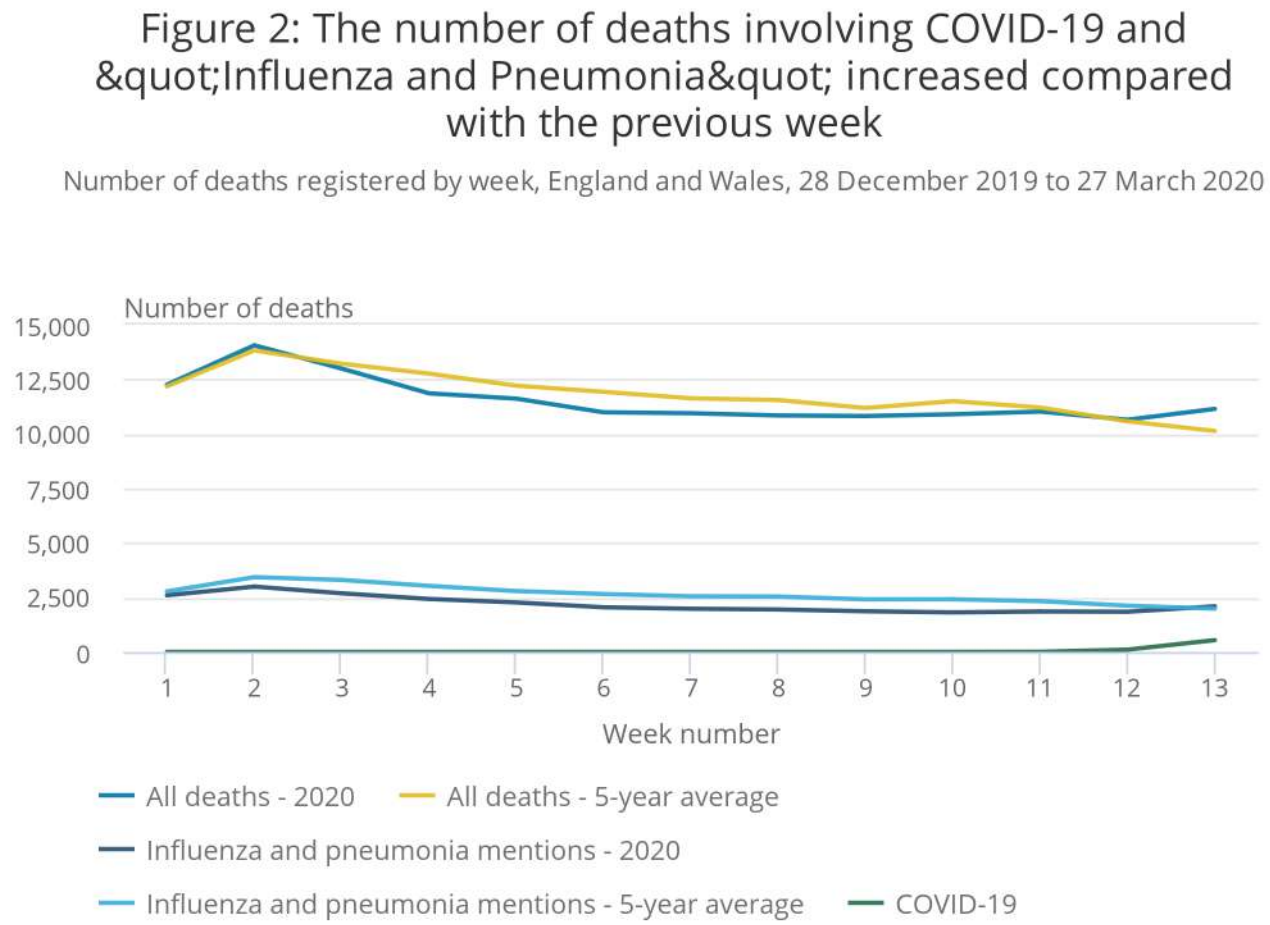
Numbers produced by the ONS take longer to prepare because they have to be certified by a doctor, registered and processed. But once ready, they are the most accurate and complete information. The ONS provides figures based on deaths registered in England and Wales with COVID-19 (more information can be found in the Measuring the data section).

Comparisons of data sources at the England and Wales level are available in the accompanying datasets.

3 . Deaths registered by week

Figure 2: The number of deaths involving COVID-19 and "Influenza and Pneumonia" increased compared with the previous week

Number of deaths registered by week, England and Wales, 28 December 2019 to 27 March 2020



Source: Office for National Statistics – Death registrations

Notes:

1. Figures include deaths of non-residents.
2. Based on date a death was registered rather than occurred.
3. Estimates for 2020 are provisional.
4. The ICD-10 definitions are as follows: COVID-19 (U07.1 and U07.2), Influenza and Pneumonia (J09-J18).
5. A death can be registered with both COVID-19 and Influenza and Pneumonia mentioned on the death certificate, therefore a death may be counted in both categories.

The provisional number of deaths registered in England and Wales in Week 13 (week ending 27 March 2020) increased from 10,645 in Week 12 (week ending 20 March 2020) to 11,141. This is 1,011 more deaths than the five-year average of 10,130.

The number of death registrations involving coronavirus (COVID-19) increased from 103 in Week 12 to 539 in Week 13. Including deaths that occurred in Week 13 but were registered up to 1 April, the number involving COVID-19 was 1,268 (this is not shown in Figure 2).

The number of deaths mentioning “Influenza or pneumonia” on the death certificate increased from 1,841 in Week 12 to 2,090 in Week 13.

In Week 13, 18.8% of all deaths mentioned “Influenza or Pneumonia”, COVID-19, or both. In comparison, for the five-year average, 19.6% of deaths mentioned “Influenza and Pneumonia”. “Influenza and Pneumonia” has been included for comparison, as a well-understood cause of death involving respiratory infection that is likely to have somewhat similar risk factors to COVID-19.

4 . Deaths registered by age group

Figure 3: Deaths involving COVID-19 were registered in all age groups apart from those aged under 15 years

Deaths by age group, England and Wales, week ending 27 March 2020

Download the data

In Week 13 (week ending 27 March 2020), there were no deaths registered involving the coronavirus (COVID-19) in the two youngest age groups (that is, those aged 1 year or under and those aged 1 to 14 years). There were 99 deaths among those aged 65 to 74 years, which was 5.5% of deaths of that age group, the highest proportion. The highest number of deaths in a specific age group occurred in those aged 85 years and over, with 188 deaths (4.2% of deaths in this age group).

5 . Deaths by region

Figure 4: The highest number of deaths involving COVID-19 was recorded in London, while the lowest number was in the East and Yorkshire and The Humber

Deaths by regions in England and Wales, week ending 27 March 2020

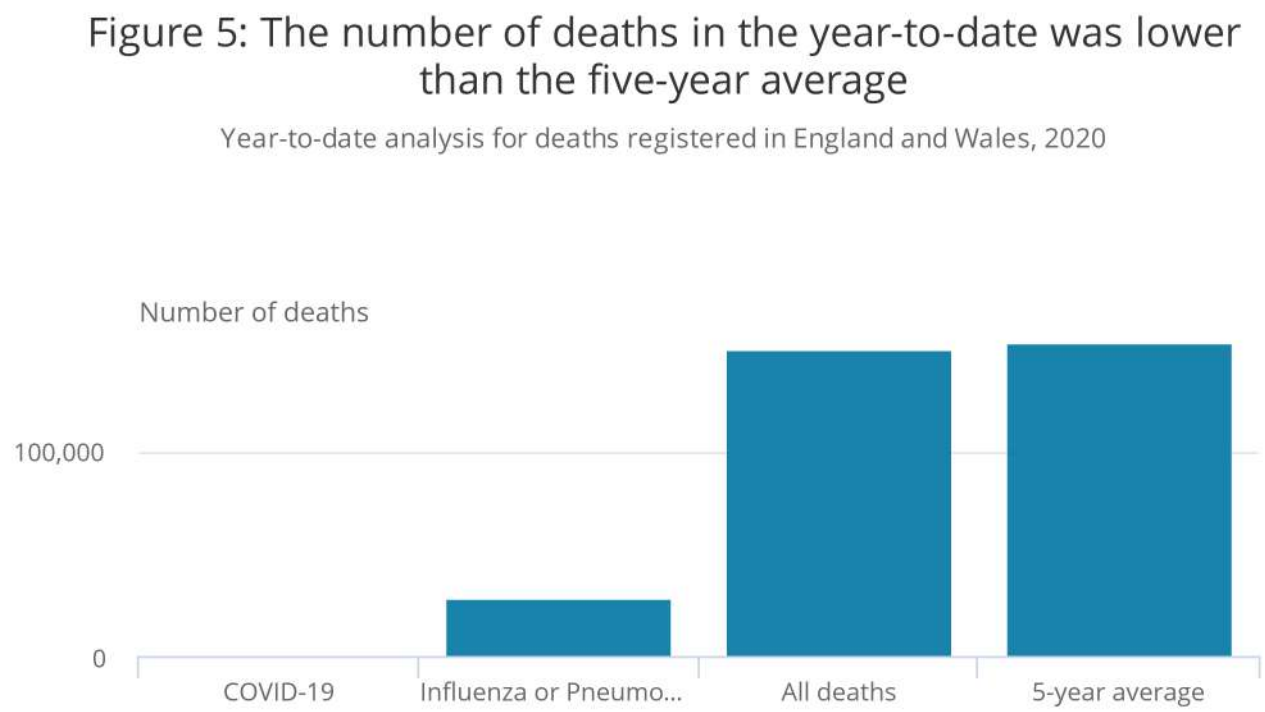
Download the data

In Week 13 (week ending 27 March 2020), there were 12 deaths involving coronavirus (COVID-19) registered in both the East of England region and Yorkshire and The Humber region. The region with the largest number and proportion of deaths involving COVID-19 was London with 237 deaths; 18.3% of all London deaths.

6 . Deaths registered in the year-to-date, Week 1 to 13

Figure 5: The number of deaths in the year-to-date was lower than the five-year average

Year-to-date analysis for deaths registered in England and Wales, 2020



Source: Office for National Statistics – Death registrations

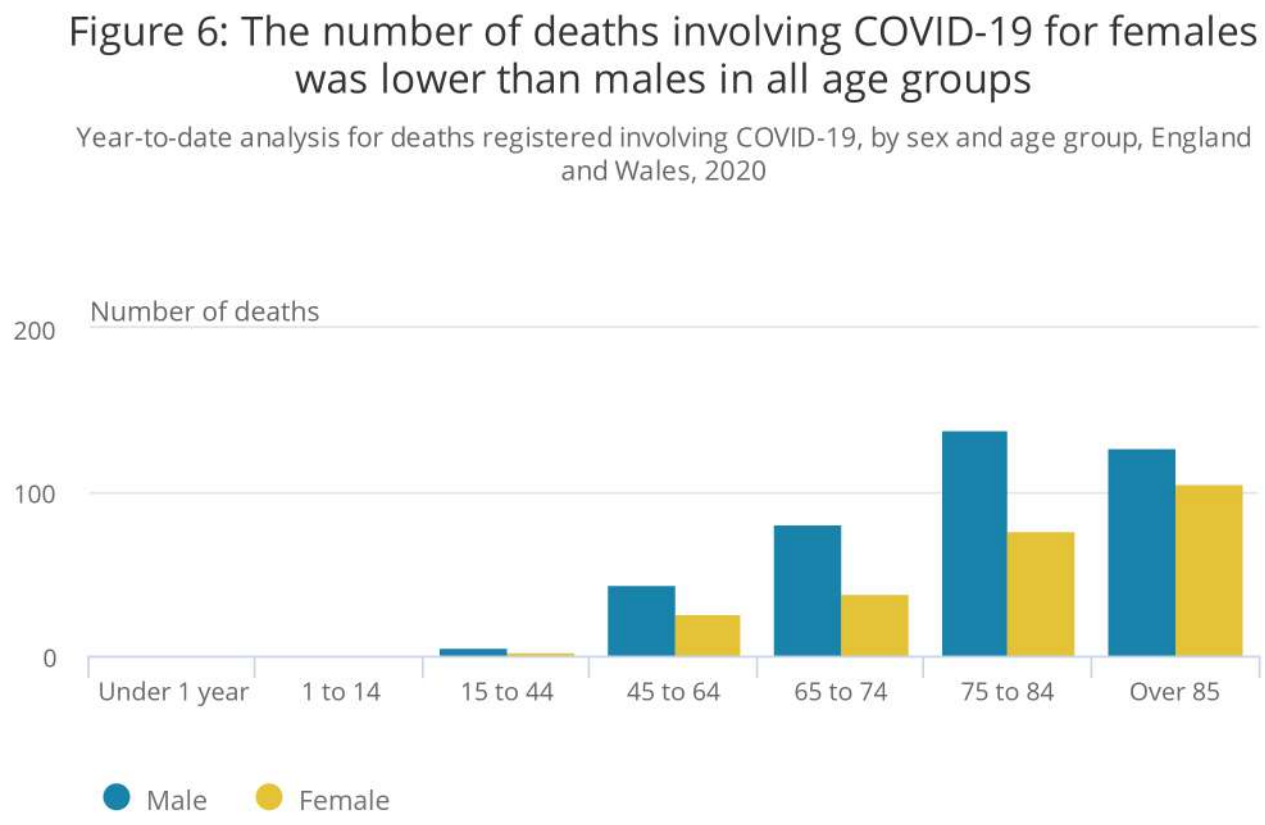
Notes:

1. Figures include deaths of non-residents.
2. Based on date a death was registered rather than occurred.
3. Estimates for 2020 are provisional.
4. The ICD-10 definitions for COVID-19 are U07.1 and U07.2.
5. Individual weeks may not sum to the year-to-date analysis as previous weeks have been recalculated in order to have the most up-to-date estimates.

Looking at the year-to-date (using refreshed data to get the most accurate estimates), the number of deaths is currently lower than the five-year average. The current number of deaths is 150,047, which is 3,350 fewer than the five-year average. Of the deaths registered by 27 March 2020, 647 mentioned the coronavirus (COVID-19) on the death certificate; this is 0.4% of all deaths.

Figure 6: The number of deaths involving COVID-19 for females was lower than males in all age groups

Year-to-date analysis for deaths registered involving COVID-19, by sex and age group, England and Wales, 2020



Source: Office for National Statistics – Death registrations

Notes:

1. Figures include deaths of non-residents.
2. Based on date a death was registered rather than occurred.
3. Estimates for 2020 are provisional.
4. The ICD-10 definitions for COVID-19 are U07.1 and U07.2.
5. Individual weeks may not sum to the year-to-date analysis as previous weeks have been recalculated in order to have the most up-to-date estimates.

In each age group there have been more deaths involving COVID-19 in males than in females. The largest difference was in age group 75 to 84 years where there were 138 deaths involving COVID-19 in males and 77 in females.

7 . Deaths data

Deaths registered weekly in England and Wales, provisional
Dataset | Released 7 April 2020
Provisional counts of the number of deaths registered in England and Wales, by age, sex and region, in the latest weeks for which data are available. Includes data on the coronavirus (COVID-19) deaths.

8 . Glossary

Coronavirus (COVID-19) deaths

Coronavirus (COVID-19) deaths are those deaths registered in England and Wales in the stated week where COVID-19 was mentioned on the death certificate as “deaths involving COVID-19”. A doctor can certify the involvement of COVID-19 based on symptoms and clinical findings – a positive test result is not required.

9 . Measuring the data

More quality and methodology information on strengths, limitations, appropriate uses, and how the data were created is available in the Mortality statistics in England and Wales QMI.

To meet user needs, we publish very timely but provisional counts of death registrations in England and Wales in our Deaths registered weekly in England and Wales, provisional dataset. These are presented by sex, age group and regions (within England) as well as for Wales as a whole. To allow time for registration and processing, these figures are published 11 days after the week ends. Because of the rapidly changing situation, in this bulletin we have also given provisional updated totals based on the latest available death registrations, up to 1 April 2020.

Because of the coronavirus (COVID-19) pandemic, our regular weekly deaths release now provides a separate breakdown of the numbers of deaths involving COVID-19: that is, where COVID-19 or suspected COVID-19 was mentioned anywhere on the death certificate, including in combination with other health conditions. If a death certificate mentions COVID-19 it will not always be the main cause of death, but may be a contributory factor. This new bulletin summarises the latest weekly information and will be updated each week during the pandemic.

These figures are different from the daily surveillance figures on COVID-19 deaths published by the Department of Health and Social Care (DHSC) on the GOV.UK website, for the UK as a whole and constituent countries. Figures in this report are derived from the formal process of death registration and may include cases where the doctor completing the death certificate diagnosed possible cases of COVID-19, for example, where this was based on relevant symptoms but no test for the virus was conducted. Our figures also include any deaths that occur outside hospital.

In contrast to the GOV.UK figures, we include only deaths registered in England and Wales, which is the legal remit of the Office for National Statistics (ONS). Table 1 provides an overview of the differences in definitions between sources.

Table 2: Definitions of COVID-19 deaths between different sources

	DHSC COVID-19 (as published on Gov.uk)	ONS COVID-19 deaths registered	ONS COVID-19 death occurrence (actual date of death)
Coverage	UK (however we only include England and Wales breakdowns for comparable coverage to ONS data)	Registrations in England & Wales	Registrations in England & Wales
		In discussions with devolved nations to create UK estimates in the near future	In discussions with devolved nations to create UK estimates in the near future
Inclusion	Deaths in hospitals	Any place of death, including Nursing homes	Any place of death, including Nursing homes
	Deaths where patient has been tested for COVID-19	Deaths where COVID-19 has been mentioned on the death certificate	Deaths where COVID-19 has been mentioned on the death certificate
Timeliness	Provided daily but not officially registered. Data is provided to NHS-E directly by hospitals.	Weekly registrations are 11 days behind due to the time taken to register, process and publish.	Weekly registrations are 11 days behind due to the time taken to register, process and publish.
	Data only published once confirmed family have been notified of death	Registered in the week ending the 20th March (week 12)	Deaths which occurred in week 12 but were registered up to 26 March

Source: Office for National Statistics

We will publish accompanying articles periodically, giving enhanced information such as age-standardised and age-specific mortality rates for recent time periods and breakdowns of deaths involving COVID-19 by associated pre-existing health conditions.

There is usually a delay of at least five days between occurrence and registration. More information on this issue can be found in our impact of registration delays release.

Our User guide to mortality statistics provides further information on data quality, legislation and procedures relating to mortality and includes a glossary of terms.

10 . Strengths and limitations

Figures are based on the date the death was registered, not when it occurred. There is usually a delay of at least five days between occurrence and registration. More information on this issue can be found in our impact of registration delays release.

11 . Related links

Deaths registered in England and Wales: 2018
Bulletin | Released 6 August 2019
Registered deaths by age, sex, selected underlying causes of death and the leading causes of death. Contains death rates and death registrations by area of residence and single year of age.

Coronavirus (COVID-19) product page
Product page | Updated when new data are available
Brings together the latest data and analysis on the coronavirus (COVID-19) pandemic in the UK and its effect on the economy and society.

World Health Organization

WHO lists two COVID-19 tests for emergency use

7 April 2020 | Departmental news

WHO has listed the first two diagnostic tests for emergency use during the Covid-19 pandemic. The move should help increase access to quality-assured, accurate tests for the disease. It also means that the tests can now be supplied by the United Nations and other procurement agencies supporting the COVID-19 response.

Both *in vitro* diagnostics, the tests are *genesig Real-Time PCR Coronavirus (COVID-19*) and *cobas SARS-CoV-2 Qualitative assay for use on the cobas® 6800/8800 Systems*.

“The emergency use listing of these products will enable countries to increase testing with quality assured diagnostics,” says Dr Mariângela Simão, WHO Assistant-Director General for Medicines and Health Products. “Facilitating access to accurate tests is essential for countries to address the pandemic with the best tools possible.”

The Emergency Use Listing procedure (EUL) was established to expedite the availability of diagnostics needed in public health emergency situations. It is intended to help procurement agencies and countries navigate the large presence of different devices on the market and, by assessing them, provides assurance of the products’ quality and performance.

The ***genesig Real-Time PCR Coronavirus (COVID-19)*** (Primerdesign, United Kingdom) is an open system more suitable for laboratories with moderate sample testing capacity, while the ***cobas® SARS-CoV-2 for use on the cobas® 6800/8800 Systems*** (Roche, United States of America) is a closed system assay for larger laboratories.

EUL listed products:

https://www.who.int/diagnostics_laboratory/200407_eul_sars_cov2_product_list.pdf?ua=1

Roche test:

https://www.who.int/diagnostics_laboratory/eul_0504-046-00_cobas_sars_cov2_qualitative_assay_ifu.pdf?ua=1

Primerdesign test:

https://www.who.int/diagnostics_laboratory/eul_0489_185_00_path_covid19_ce_ivd_ifu_issue_2.0.pdf?ua=1

National Burden Estimates of healthy life lost in India, 2017: an analysis using direct mortality data and indirect disability data

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Summary

Background Many countries, including India, seek locally constructed disease burden estimates comprising mortality and loss of health to aid priority setting for the prevention and treatment of diseases. We created the National Burden Estimates (NBE) to provide transparent and understandable disease burdens at the national and subnational levels, and to identify gaps in knowledge.

Methods To calculate the NBE for India, we combined 2017 UN death totals with national and subnational mortality rates for 2010–17 and causes of death from 211 166 verbal autopsy interviews in the Indian Million Death Study for 2010–14. We calculated years of life lost (YLLs) and years lived with disability (YLDs) for 2017 using published YLD–YLL ratios from WHO Global Health Estimates. We grouped causes of death into 45 groups, including ill-defined deaths, and summed YLLs and YLDs to calculate disability-adjusted life-years (DALYs) for these causes in eight age groups covering rural and urban areas and 21 major states of India.

Findings In 2017, there were about 9·7 million deaths and 486 million DALYs in India. About three quarters of deaths and DALYs occurred in rural areas. More than a third of national DALYs arose from communicable, maternal, perinatal, and nutritional disorders. DALY rates in rural areas were at least twice those of urban areas for perinatal and nutritional conditions, chronic respiratory diseases, diarrhoea, and fever of unknown origin. DALY rates for ischaemic heart disease were greater in urban areas. Injuries caused 11·4% of DALYs nationally. The top 15 conditions that accounted for the most DALYs were mostly those causing mortality (ischaemic heart disease, perinatal conditions, chronic respiratory diseases, diarrhoea, respiratory infections, cancer, stroke, road traffic accidents, tuberculosis, and liver and alcohol-related conditions), with disability mostly due to a few conditions (nutritional deficiencies, neuropsychiatric conditions, vision and other sensory loss, musculoskeletal disorders, and genitourinary diseases). Every condition that was common in one part of India was uncommon elsewhere, suggesting state-specific priorities for disease control.

Interpretation The NBE method quantifies disease burden using transparent, intuitive, and reproducible methods. It provides a simple, locally operable tool to aid policy makers in priority setting in India and other low-income and middle-income countries. The NBE underlines the need for many more countries to collect nationally representative cause of death data, paired with focused surveys of disability.

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Introduction

In 1993, the World Bank proposed using burden of disease estimation paired with cost-effectiveness and economic analyses as quantitative tools to set priorities for disease control.[1] The Bank's measure of the global burden of disease drew upon three inputs: earlier work at WHO on consistent estimates of death by cause worldwide,[2] methodologies developed in the 1970s to combine fatal and non-fatal health events[3]—now known as disability-adjusted life-years (DALYs)—and an illustration of national burden in Ghana that combined non-fatal outcomes with cause of death estimates.[4,5] Many governments, especially of low-income and middle-income countries (LMICs), now conduct local cost-effectiveness studies.[5] By contrast, most LMICs lack nationally representative mortality data, and hence most burden of disease estimates are done by the Global Burden of Diseases, Injuries, and Risk Factors Study (GBD) secretariat in Seattle, USA.[5,6]

GBD is an important advance by ensuring consistent estimates of the global numbers of death by cause, and attempting to combine death and disability into a single metric.[1,6] At the national level, GBD estimates for LMICs of death by cause rely primarily on econometric models. Where no consistent and reliable national cause of death data are available, GBD or similar might be the only choice.[5,7,8] Where such data are available, however, they can be used for independent and locally relevant estimates, based on actual deaths. Here, we report a simple method

Research in context

Evidence before this study

We searched MEDLINE, Popline, CABI Global Health, and websites of WHO and the Global Burden of Diseases, Injuries, and Risk Factors Study (GBD) using the terms "burden of disease", "DALY", "India", and "causes of death" for national studies in people of all ages in India, from Jan 1, 2010, to March 1, 2019, with no language restrictions. From 795 articles screened, we found that GBD and WHO published modelled annual national estimates of disability-adjusted life-years (DALYs) for more than five diseases in 2013, 2015, 2016, and 2017. Ischaemic heart disease was consistently the leading cause of DALYs in GBD estimates, but the rank of other causes varied by year. It was difficult to separate changes in model specifications from changes in actual disease burdens. We were unable to reproduce the GBD method for burdens in India.

Added value of this study

We have developed and implemented an indigenous, simple, and intuitive method to calculate deaths and disability at national and state levels in India. The National Burden Estimates (NBE) establishes the plausible distribution of the major causes of death and disability across the major states of India. In 2017, there were about 9·7 million deaths and 486 million DALYs in India. Non-communicable diseases comprised 46·6% of national DALYs, but a notably higher 55·0% in urban areas. Injuries comprised 11·4% of DALYs. The conditions that accounted for the top 15 DALYs were led mainly by deaths in childhood and early adulthood. Together, these conditions accounted more than 70% of total DALYs—a proportion consistent with WHO and GBD results. The remarkable variation in years of life lost across India suggests that diseases common in one part of the country are relatively uncommon elsewhere, for reasons that are not well understood. Five conditions comprise much of the uncertainty in years lived with disability, and should be the focus of future research to derive better disability estimates. The NBE and GBD results for years of live lost and overall DALYs were moderately comparable, and the gaps identified in disability should help to improve future modelling and inform direct surveys of the major conditions causing disability.

Implications of all the available evidence

Much of Indian disease burden is avoidable. The NBE method is simple, locally operable, and widely replicable within India and in many other low-income and middle-income countries to track progress in human health.

to create a measure called National Burden Estimates (NBE), which combines nationally representative cause of death data from the Million Death Study (MDS) with UN demographic totals and WHO estimates of deaths and disability.[9,10] We provide details on the methodology to encourage replication in other LMICs.

About a fifth of all deaths worldwide occur in India.[10,11] The NBE was created in response to a request from India's Ministry of Health and Family Welfare to the Indian Council of Medical Research (ICMR) to provide transparent and understandable disease burdens at the national and subnational levels, and to identify gaps in knowledge, particularly from disability.[12]

Methods

Data sources

To calculate our estimates, we used national-level population and mortality data for 2017 from the UN Population Division[11] and state-level population and mortality data for 2010–17 from the Registrar General of India's Sample Registration System,[13,14] a continuous demographic surveillance system that reports state-level vital rates every year. For cause of death data, we used 2010–14 data from the MDS,[14] to which we applied the classifications of specific disease groups used in the WHO Global Health Estimates (GHE) for 2016.[10] We drew on the average of 2010–14 deaths, which are the latest available, for stability across age groups and cause of death categories.

See Online for appendix

Full details, including data limitations, of the UN demographic data, the Sample Registration System vital rates, and the WHO GHE have been published elsewhere.[9–11,13] The methods, strengths, and limitations of the MDS and key results for various diseases have also been extensively reviewed and published.[14–17] Briefly, in collaboration with the Registrar General of India, the MDS monitored approximately 14 million people in 2·4 million nationally representative households in India from 1998 to 2014.[18] About 900 non-medical surveyors recorded the details of each death that occurred in these households during the preceding 6 months using a well validated verbal autopsy instrument, which is based on the 2012 WHO instrument and includes a half-page local language narrative. Each record is converted to an electronic form and randomly assigned to two of 400 trained physicians, who assign a cause according to the International Classification of Diseases, 10th revision (ICD-10). Disagreements in assignment undergo anonymous reconciliation, and persisting differences undergo adjudication by a third physician.

Subnational analyses focused on the 21 major states of India, comprising the 20 most populous states as defined by the Registrar General of India plus seven northeastern states which we grouped as one state.[14] We included the recently created state of Telagana within Andhra Pradesh. These 21 states were home to more than 99% of India's total population in 2017.

Causes of death

We grouped ICD-10 codes into 44 overarching categories (appendix pp 5–7), informed by public health goals, in consultation with ICMR's Burden of Disease Technical

Advisory Group.[12] These 44 categories were further grouped into three main disease categories: communicable, maternal, perinatal, and nutritional diseases (13 causes); non-communicable diseases (NCDs; 24 causes); and injuries (seven causes). We retained ill-defined deaths as an additional category. By contrast, the GBD reassigns ill-defined deaths using unpublished algorithms whereas the GHE redistributes them to a published list of other specific causes.[6,9,10] Ill-defined deaths are a check on the quality of a cause of death system, with generally low levels before old age in the MDS.[15]

The NBE method

Calculation of the NBE involves seven steps (figure 1). First, we obtained UN age-specific and sex-specific country population and death counts for 2017 and deaths and population by state and for rural and urban strata for 2010–17. Second, we summed the subnational deaths and adjusted these (usually upwards by small amounts) to match the UN national total for each age and sex stratum.

In the third step, we applied the cause of death proportions from the MDS for 2010–14,[14] weighted by the sampling probability for rural and urban strata for each state, to these adjusted death totals to obtain age-specific and sex-specific numbers of deaths for each cause. We aggregated the death and population totals into eight age groups: 0–4 years, 5–14 years, 15–29 years, 30–49 years, 50–59 years, 60–69 years, 70–79 years, and 80 years or older. Fourth, we mapped the MDS classification of ICD-10 codes to the WHO GHE classification for India (appendix pp 5–7).[10] For each condition in the GHE, we derived the years lived with disability (YLDs) and years of life lost (YLLs) and calculated the YLD–YLL ratio for the specified age groups (appendix p 8). The GHE assigns no deaths to major depression; hence, to calculate YLDs for depression, we applied the GHE proportion of YLDs due to depression to the estimated overall YLDs from neuropsychiatric conditions.

Fifth, we calculated the median age at death for each cause from the MDS, subtracted this from the WHO standard life expectancy of 92 years, and multiplied this by the number of deaths from step 3 to obtain YLLs. Thus, the YLLs for cause i for age group j are given by

$$YLLs_{i,j} = (92 - \text{median age at death}_{i,j}) \times \text{adjusted UN deaths}_{i,j}$$

Sixth, we multiplied the YLLs by the GHE YLD–YLL ratios from step 4 to obtain YLDs. The final step summed YLLs and YLDs to obtain DALYs for each cause by age and sex. A worked example of the calculations for respiratory infection deaths at ages 5–14 years is shown in the appendix (p 4).

For subnational (rural or urban and state-specific) estimates, we used the same method, applying the national median age of deaths and 684 age-specific and sex-specific YLD–YLL ratios. We summed state-level vital rates to national totals in step 2, and applied the state-specific proportion of deaths in step 3. We compared state variation in DALY, YLL, and YLD rates after standardising for age using the World Standard Population 2000–25.[19]

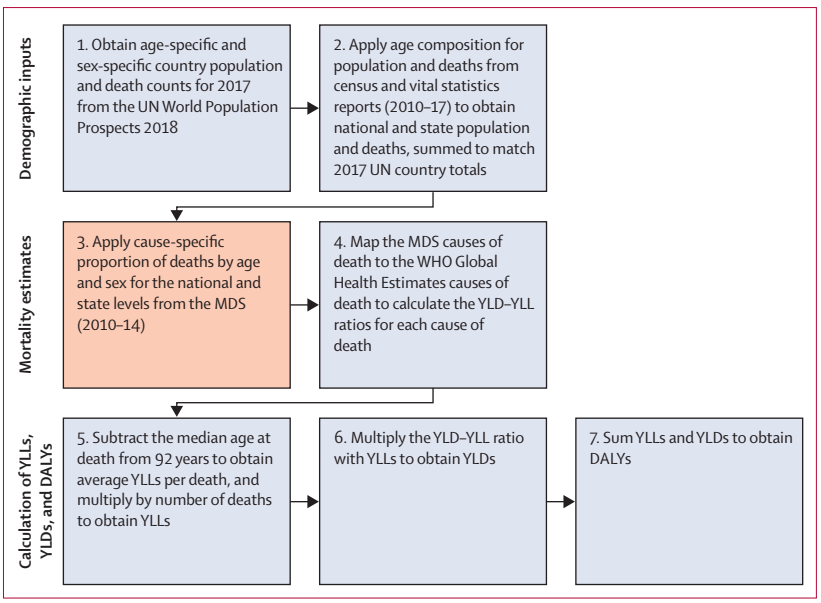

Figure 1: **Summary of the steps in the National Burden Estimates of combined death and disability**
The orange tinted box (ie, step 3) indicates the required input dataset on country-specific causes of death. All other steps use publicly accessible datasets from the UN Population Division[11] or the WHO Global Health Estimates.[10] MDS=Million Death Study. YLD=year lived with disability. YLL=year of life lost. DALY=disability-adjusted life-year.

Statistical analysis

We applied chance-corrected mortality fraction accuracy to calculate the population-level concordance between the NBE and GBD, taking into account chance agreement.[20] 100% concordance would mean identical cause of death distribution in the two comparisons. The major source of uncertainty in the NBE does not arise from random errors: the sample size for the MDS is very large and completeness of the sources of vital rates is high, as evaluated independently by the UN.[13,14,21] Rather, uncertainty arises mostly from the misclassification of causes of death. The appendix (p 114) presents the uncertainty bounds based on dual or single physician agreement on the underlying cause of death. We used Stata version 15.1 for statistical analyses. The ICMR has developed a user-friendly estimation and visualisation tool. The Stata code and tools are available on written request to the first author.

Role of the funding source

The sponsors of the study had no role in the study design, data collection, or data interpretation. The corresponding authors had full access to the study data and had final responsibility for the decision to submit for publication.

	Sex			Location	
	Both	Male	Female	Urban	Rural
Population, millions	1339	694	645	418	921
Deaths, thousands	9652	5298	4354	2397	7255
DALYs at all ages, millions	486	264	222	114	372
DALYs at age <70 years, millions	427	234	193	99	328
MDS deaths, 2010–14	211166	120912	90254	47695	163471
DALYs per 100 000 population*					
By age, years					
All ages	36300	38100	34400	27400	40400
0–4	84400	83800	85000	58100	93700
5–14	13300	14400	12100	9300	14800
15–29	17400	16800	18100	16100	18100
30–49	27900	31000	24600	20400	31900
50–59	52200	59200	44900	36800	60600
60–69	85000	94000	76000	66800	92500
70–79	127600	137900	118400	109700	135100
≥80	112900	120400	106800	99600	118600
By major cause groups					
Communicable, maternal, perinatal, and nutritional	13000	12900	13000	7600	15400
Non-communicable	16900	18000	15800	15100	17800
Injuries	4100	5100	3100	3100	4600
Ill-defined at age <70 years	1100	1000	1200	800	1200
By top 15 causes of DALYs					
Ischaemic heart disease	3500	4300	2500	4000	3200
Perinatal conditions	3100	3200	3000	1800	3700
Nutritional deficiencies	2200	2200	2200	1200	2600
Chronic respiratory diseases	2100	2300	1800	1200	2500
Neuropsychiatric conditions	2000	1800	2300	1500	2300
Diarrhoea	1700	1600	1800	900	2100
Vision and other sensory loss	1600	1500	1900	1300	1800
Respiratory infections	1600	1600	1600	1000	1900
Cancers	1400	1400	1500	1300	1500
Stroke	1300	1400	1200	1100	1400
Road traffic accidents	1200	1900	400	1100	1200
Tuberculosis	1100	1500	800	700	1300
Liver and alcohol-related conditions	1100	1500	600	1000	1100
Musculoskeletal disorders	1000	800	1200	1000	1000
Fever of unknown origin	900	800	1000	500	1100

DALYs=disability-adjusted life-years. MDS=Million Death Study. *Rounded to nearest 100. Totals might not sum due to rounding.

Table: **Burden of disease in India due to major causes in different age groups, by sex and location, 2017**

Results

We analysed 211166 deaths from 2010 to 2014 in the MDS covering the whole of India (table). The full results for deaths, DALYs, YLLs, and YLDs by sex and age for each major state, and for rural and urban areas nationally, are provided in the appendix (pp 9–112). For ease of understanding, we present these results in formats identical to WHO GHE tables, the only difference being the number of causes (45 major causes in NBE *vs* 136 major or subcauses in the GHE).

In 2017, India had about 9·7 million deaths and 486 million DALYs, so the ratio of DALYs to deaths was about 50 to one (table). More than three quarters of deaths and DALYs occurred in rural areas, and males accounted for 54·3% of all DALYs. At all ages, the DALY rate per 100 000 population was 36 300, but rates were higher among rural residents and among males (table). DALY rates in rural areas were at least twice those of urban areas for perinatal and nutritional conditions, chronic respiratory diseases, diarrhoea, and fever of unknown origin. By contrast, DALY rates for ischaemic heart disease were considerably greater in urban areas (table). DALY rates showed a U-shaped relationship with age, starting high at ages 0–4 years, dropping to their lowest among children aged 5–14 years, and rising again to highest levels at 70–79 years. 35·7% of total national DALYs arose from communicable, maternal, perinatal, and nutritional causes, and this proportion was greater among females and rural residents (appendix pp 89–90). NCDs comprised 46·6% of DALYs overall, which increased to 55·0% in urban areas. Injuries comprised 11·4% of DALYs. Ill-defined causes comprised 3·3% of all DALYs before age 70 years but a higher proportion (27·9%) above age 70 years (appendix pp 89, 113). NCD and injury DALY rates were higher in males than females (table).

The top 15 conditions that accounted for the most DALYs at all ages arose mostly from YLLs—namely, ischaemic heart disease (9·6% of all DALYs), perinatal conditions (8·5%), chronic respiratory diseases (5·7%), diarrhoea (4·7%), respiratory infections (4·5%), cancer (4·0%), stroke (3·6%), road traffic injuries (3·3%), tuberculosis (3·1%), and liver and alcohol-related conditions (3·0%). DALYs for five conditions arose mostly from YLDs as opposed to YLLs: neuropsychiatric conditions including epilepsy (6·2% of all DALYs), nutritional deficiencies (6·0%), vision and other sensory loss (4·5%), musculoskeletal disorders (2·7%), and genitourinary diseases excluding renal failure (0·8%).

More than 70% of DALYs at all ages resulted from YLLs (346 million of 486 million years; figure 2), with YLLs dominating DALYs among the communicable, perinatal, maternal, and nutritional disorders and among injuries. By contrast, YLDs constituted 86·8% of DALYs for nutritional deficiencies. YLLs also dominated most of the NCDs, including all cancers and vascular and respiratory diseases. Among the NCDs, YLDs contributed more than the YLLs for four conditions: genitourinary diseases (excluding renal failure), neuropsychiatric conditions (mostly major depression, but also including other psychiatric conditions and epilepsy), musculoskeletal disorders, and vision and other sensory loss. Collectively, these four NCDs plus nutritional deficiencies accounted for 62·8% of all YLDs and fewer than 18·1% of all DALYs (table; appendix p 65, 89).

YLLs continued to dominate DALYS when we restricted analyses to below age 70 years, and for ages 30–69 years

(corresponding to the ages for the UN Sustainable Development Goals for NCDs; appendix p 117), and ages 15–59 years (corresponding to the ages in the current World Bank Human Capital Index;[22] appendix p 118).

We observed a clear geographical distribution across states of YLLs and YLDs (appendix pp 11–14). We present differences in the age-standardised YLL rates per 100 000 population across the major states for selected causes that showed marked variation across states (figures 3, 4); we included smaller states and Union Territories in separate analyses of all remaining states (appendix pp 89–112). We defined the levels of each of the chosen diseases separately to highlight differences. Each is shown in descending order of YLL rates. Nearly every condition that is common in one state was far less common in another state, and hence must be mostly avoidable.

Among the infectious diseases, tuberculosis YLL rates were much higher in the north, particularly in Uttar Pradesh and Rajasthan, than in southern India (figure 3). Respiratory infection YLL rates were high in the northern and northeastern states. By contrast, diarrhoea YLL rates showed an east–west gradient, being much higher in Odisha, Jharkhand, Bihar, and Uttar Pradesh, and comparatively lower in western India. The high-burden states accounted for 52% of the absolute national total YLLs for tuberculosis, 41% for respiratory infections, and 15% for diarrhoea (figure 3).

Among NCDs, cancer YLLs were particularly high in northeastern states, Uttar Pradesh, Rajasthan, West Bengal, Haryana, Assam, Gujarat and Madhya Pradesh, and in the southern states of Kerala and Karnataka (figure 4), but the YLLs from specific causes of cancer varied even within those states with high cancer burden;[12] these high-burden states accounted for 44% of national YLLs from cancer. Chronic respiratory YLL rates were high in Rajasthan and Uttar Pradesh, accounting together for 7% of national YLL totals. Liver and alcohol-related YLL rates were high in the northeastern states, Assam, Bihar, Karnataka, and Maharashtra, accounting for 18% of national YLLs. Suicide YLL rates were highest in the southern states, accounting for 15% of national totals.[23] Road traffic injuries were high in the northern states of Uttar Pradesh, Punjab, Uttarakhand, Haryana and Himachel Pradesh, accounting for 33% of national totals. Drowning YLL rates were highest in the central states of Madhya Pradesh and Chhattisgarh and in Assam in the northeast, accounting for 11% of national totals.

GBD estimates, which we derived from GBD data,[6] and NBE DALY results correlated moderately (figure 5). Compared with the NBE, GBD underestimated absolute totals of nutritional conditions for males, overestimated most NCDs for both sexes, and, surprisingly, underestimated road traffic injury deaths among males. There were differences in both directions for specific conditions, with some overestimates and some underestimates when comparing NBE and GBD estimates. The contribution of

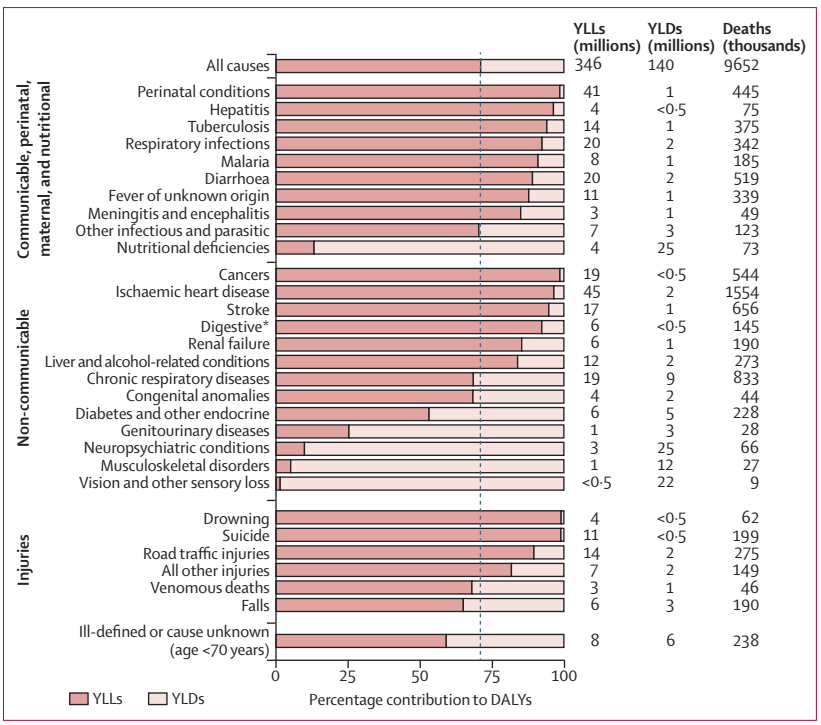

Figure 2: **Contribution of YLLs and YLDs for selected major causes of death in India at all ages, 2017**
Sexually transmitted infections, selected vaccine-preventable diseases, maternal conditions, epilepsy, rheumatic heart diseases, gastro-oesophageal diseases, and interpersonal violence resulted in a total of 181 000 deaths, with total DALYs comprised of 81% YLLs and 19% YLDs. YLLs=years of life lost. YLDs=years lived with disability. DALY=disability-adjusted life-year. *Digestive excludes gastro-oesophageal diseases and liver and alcohol-related conditions.

YLDs to overall DALYs in the NBE is similar to that in the GHE and GBD, at around 30% (appendix p 116). The most notable discrepancies between NBE, GHE, and GBD were for YLDs for just a few conditions (appendix pp 115–116).

There is no reference standard for disability, only the modelled estimates from the GBD, which WHO also uses.[24] We examined our NBE estimates of major depression, which causes much disability but little mortality. At ages 30–59 years, major depression caused 4·1 million YLDs, approximately 40% of all YLDs attributable to neuropsychiatric conditions. Based on GBD median disability weights,[24] this would constitute about 10 million people in India with prevalent depression. This prevalence is close to the estimate of 13 million adults of these ages reporting major depression in a recent multistate survey of mental health.[25]

If we take NBE to be the comparison standard, the GBD yields similar YLD rates for vision loss, underestimates YLD rates for nutritional and other genitourinary diseases, and overestimates YLD rates for neuropsychiatric conditions and musculoskeletal disorders. Had we substituted our NBE rates with the GBD rates, then the total from these conditions would have been 96 million YLDs versus 87 million YLDs in the NBE. This change would add less than 2% to total DALYs.

Discussion

We have developed and implemented an indigenous, transparent, and reproducible method to calculate deaths and disability at national and state levels in India, using a combination of the UN mortality totals for India,[11] disability–mortality ratios published by WHO for many years,[10] and, most importantly, nationally representative cause of death data from the MDS.[14–18] The NBE establishes the plausible distribution of the major causes of death and disability across the major states of India, showing that the largest burdens of disease occur in rural areas, especially from communicable, maternal, perinatal, and nutritional causes, and a large burden of NCDs exists in urban areas. Importantly, premature deaths, expressed as YLLs, account for more than 70% of the total DALYs.

The MDS mortality data have been incorporated recently into GBD analyses, but GBD data and the modelling techniques are not in the public domain and hence have not been reproduced in other studies. Unsurprisingly, this has led to discrepant results between GBD and country-led estimates, even for high-income countries with complete mortality data.[26–28] In India, for example, the availability of MDS data from 2001 onwards should have decreased GBD's reliance on modelled inputs. However, it is not possible to determine how these data were used because changes in model specifications and variable data inputs are not public,[7,9,29] leading to an inability to understand trends or to compare them with estimates using other methods, such as NBE. For example, in the GBD estimates for India, premature birth ranked as the second leading cause of death at all ages in 2015 but seventh in 2016 and fifth in 2017.[6]

The NBE method avoids so-called black boxes of complex econometric models that have uncertain validity,[7] even for countries with high-quality mortality data.[27,28] The NBE will allow the Indian Government to reliably monitor progress in the major states, including the impact on mortality of the new Ayushman Bharat national health insurance programme intended to cover about 500 million Indians.[30]

We observed remarkable variation in YLLs across India, showing that each disease that is common in one part of the country is relatively uncommon elsewhere. This disease variation contributes particularly to marked differences in adult mortality, where differences in life expectancy between districts can exceed a full decade.[31] This variation in disease rates across India indicates the existence of differences in underlying social, behavioural, or biological risk factors, suggesting important avoidable causes that await discovery. Much more remains to be understood about the novel genomic, proteomic, and other biochemical

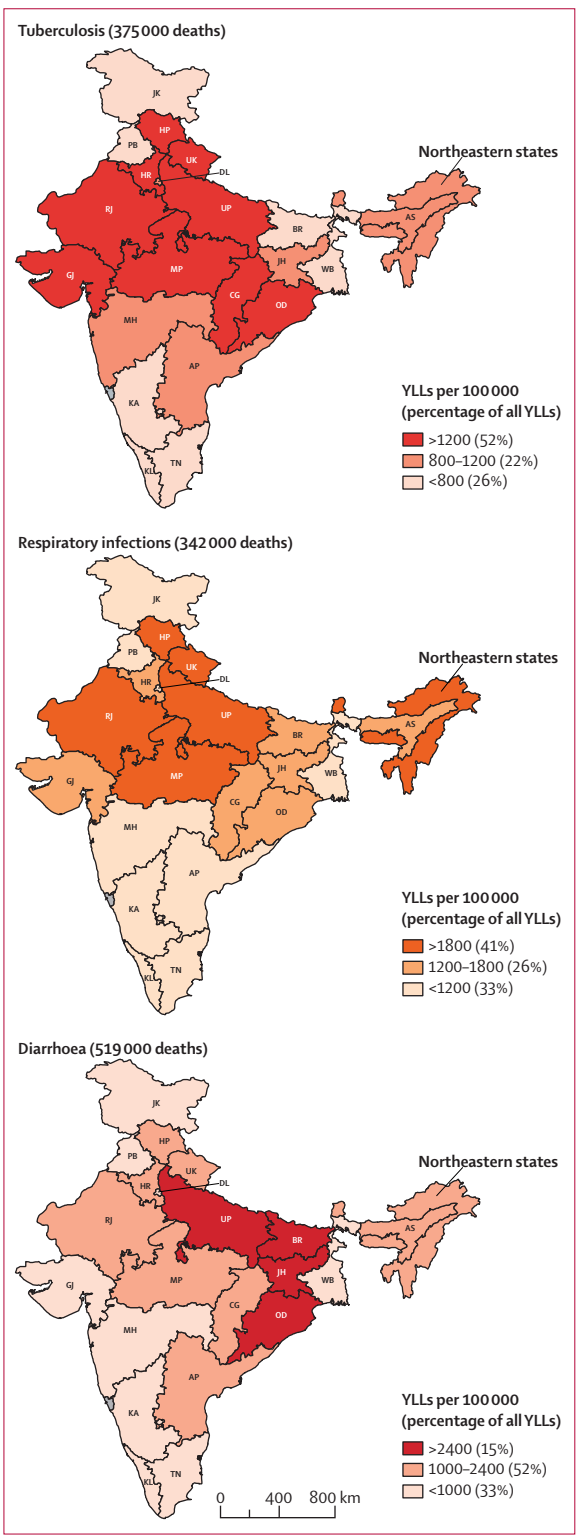

Figure 3: **Variation in YLLs using age-standardised rates for selected communicable causes of death across the major states of India, 2017**
Northeastern states include Tripura, Meghalaya, Manipur, Nagaland, Arunachal Pradesh, Mizoram, and Sikkim. YLLs=years of life lost. AP=Andhra Pradesh. AS=Assam. BR=Bihar. CG=Chhattisgarh. DL=Delhi. GJ=Gujarat. HP=Himachal Pradesh. HR=Haryana. JH=Jharkhand. JK=Jammu and Kashmir. KA=Karnataka. KL=Kerala. MH=Maharashtra. MP=Madhya Pradesh. OD=Odisha. PB=Punjab. RJ=Rajasthan. TN=Tamil Nadu. UK=Uttarakhand. UP=Uttar Pradesh. WB=West Bengal.

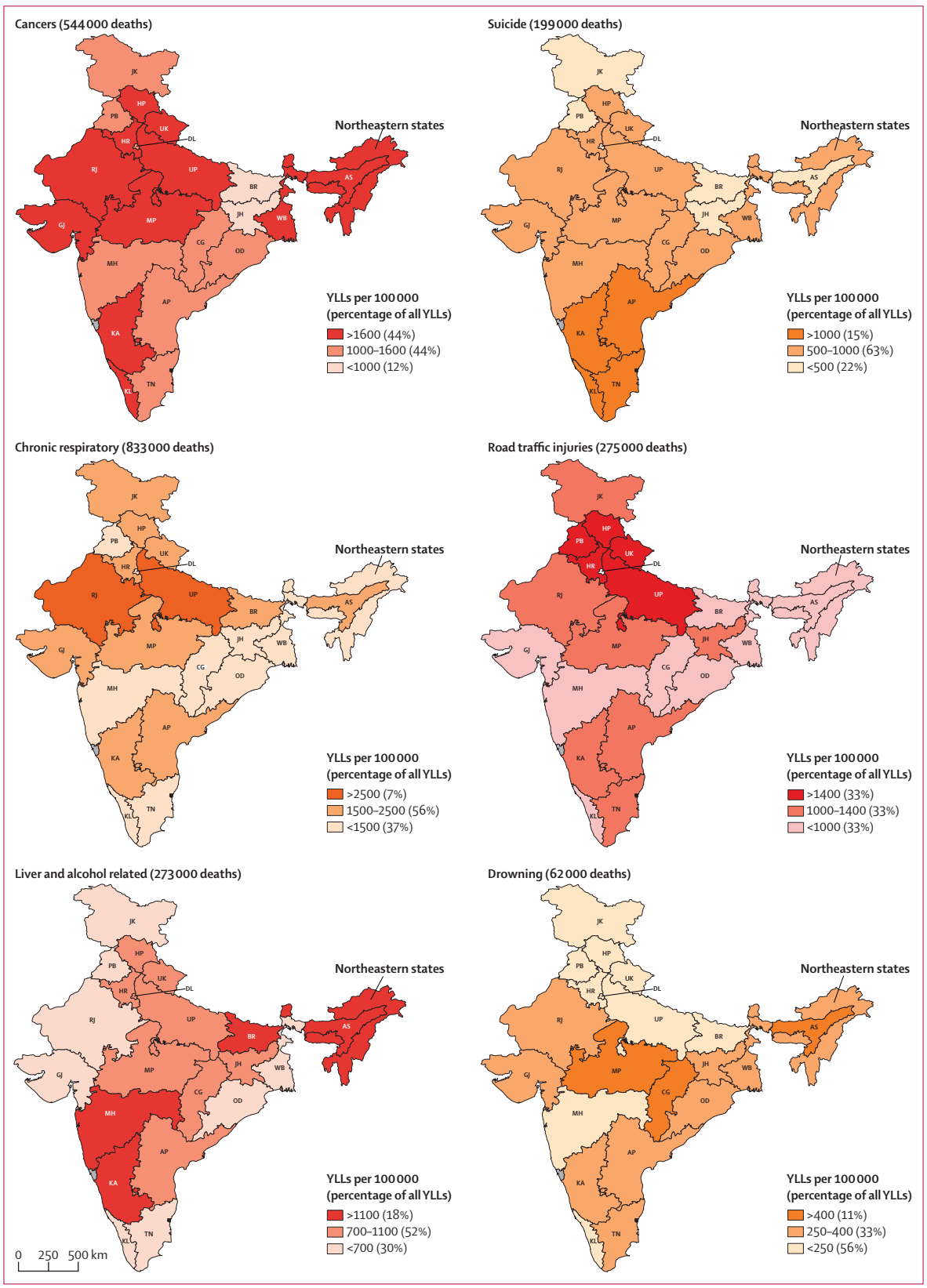

Figure 4: **Variation in YLLs using age-standardised rates for selected non-communicable diseases and injuries across the major states of India, 2017**
Northeastern states include Tripura, Meghalaya, Manipur, Nagaland, Arunachal Pradesh, Mizoram, and Sikkim. YLLs=years of life lost. AP=Andhra Pradesh. AS=Assam. BR=Bihar. CG=Chhattisgarh. DL=Delhi. GJ=Gujarat. HP=Himachal Pradesh. HR=Haryana. JH=Jharkhand. JK=Jammu and Kashmir. KA=Karnataka. KL=Kerala. MH=Maharashtra. MP=Madhya Pradesh. OD=Odisha. PB=Punjab. RJ=Rajasthan. TN=Tamil Nadu. UK=Uttarakhand. UP=Uttar Pradesh. WB=West Bengal.

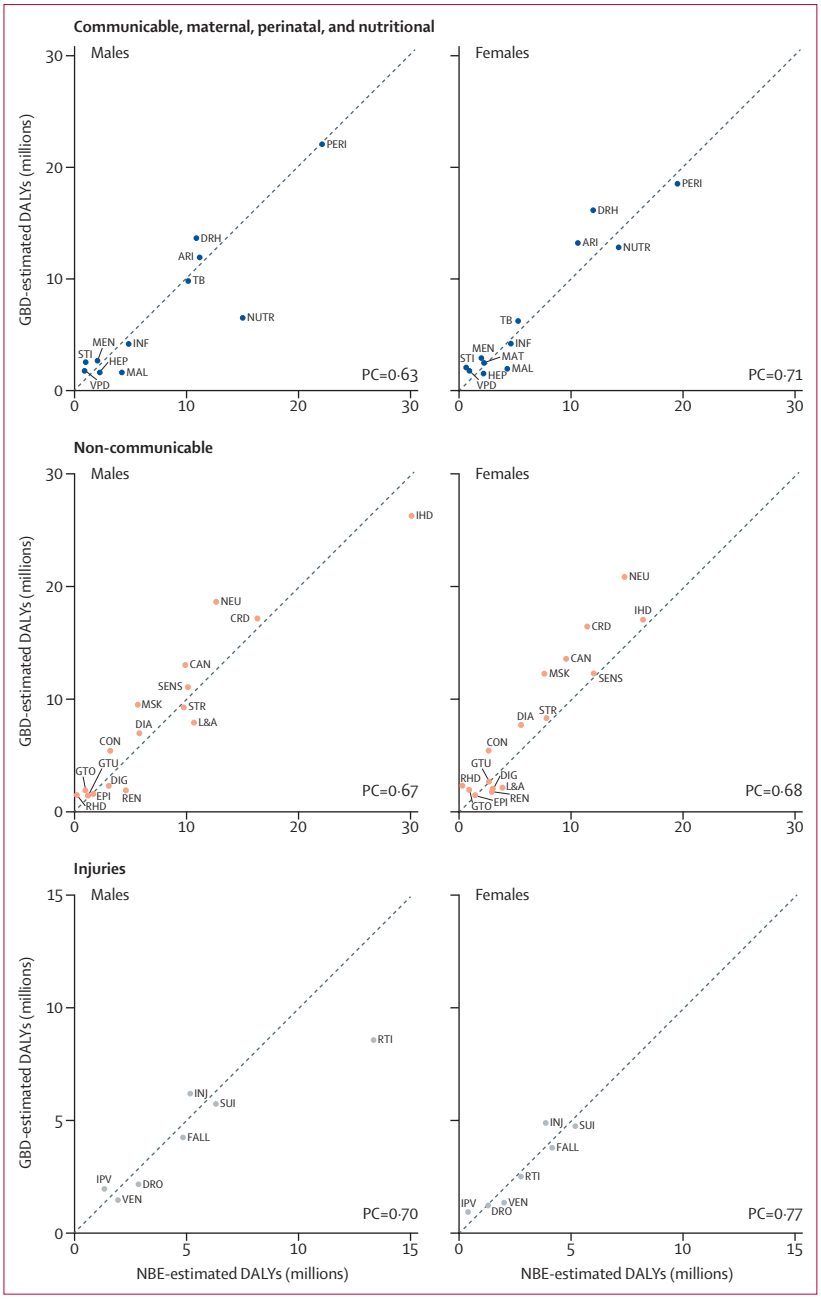

Figure 5: **Comparison of the absolute total of DALYs in India in the GBD model-based estimates to the NBE by condition, 2017**

To calculate concordance in cause of death distribution between NBE and GBD, we excluded the causes fever of unknown origin and ill-defined or cause unknown due to the lack of comparable categories between the NBE and GBD. DALYs=disability-adjusted life-years. GBD=Global Burden of Diseases, Injuries, and Risk Factors Study. NBE=National Burden Estimates. PC=population-level concordance. ARI=respiratory infections. DRH=diarrhoea. HEP=hepatitis. INF=other infectious and parasitic. MAL=malaria. MAT=maternal. MEN=meningitis and encephalitis. NUTR=nutritional deficiencies. PERI=perinatal conditions. STI=sexually transmitted infections. TB=tuberculosis. VPD=selected vaccine preventable. CAN=cancers. CON=congenital anomalies. CRD=chronic respiratory diseases. DIA=diabetes and other endocrine. DIG=digestive. EPI=epilepsy. GTO=gastro-oesophageal diseases. GTU=genitourinary diseases. IHD=ischaemic heart disease. L&A=liver and alcohol-related conditions. MSK=musculoskeletal disorders. NEU=neuropsychiatric conditions. REN=renal failure. RHD=rheumatic heart diseases. SENS=vision and other sensory loss. STR=stroke. DRO=drowning. FALL=falls. INJ=all other injuries. IPV=interpersonal violence. RTI=road traffic injuries. SUI=suicide. VEN=venomous deaths.

correlates of respiratory, intestinal, or other infections in general, and of the avoidable causes of chronic diseases such as cancer, heart attack, stroke, and respiratory disease that currently account for most of the adult mortality in India.[31,32] Even for infections such as tuberculosis, there might be biological causes that make particular infections, or progression from infection to disease, more probable in some people. Variation in secondary treatment and in smoking has already been identified as one explanation for the rising rates over the last 15 years in ischaemic heart disease mortality in rural areas.[33]

YLLs alone can be a robust measure to monitor disease burden, particularly trends over time.[34] Indeed, the inconsistent results between NBE and GBD for disability point to measurement error in disability. This error often exceeds any change in health outcomes that governments might want to monitor. For example, in seeking a 10% annual improvement in health outcomes in children, it is not possible to assess accurately the outcome of a child health programme if the measurement error exceeds 10%. As death is a discernible, objective outcome, focusing analyses of trends on mortality should reduce measurement error and allow reliable monitoring of the impact of disease control programmes.[7] An argument can be made that rather than a composite metric such as DALYs, priority setting could focus on the major causes of mortality for children and adolescents (eg, age ≤19 years) and for adults in middle and older age, and separately consider the major causes of disability at all ages. This would have the specific benefit of tying better survey methods to each of these three outcomes.

Nonetheless, governments commonly demand some reasonable measurement of disability. Most of the GBD and GHE disability data use disability weights that relate a preference of disability relative to mortality, and then apply these to estimated incidence and duration for various diseases.[24] These disability weights come from a multicountry (including India) but non-representative household survey that asked 18–65 year olds to self-report their health states.[35] Aside from the obvious biases in self-reporting, there are other limitations to such weights.[36] The YLDs in our analyses correlated poorly with those in the GBD. However, the uncertainties in disability probably had only a minor effect on overall DALY totals, rates, or the relative ranking of diseases. Verbal autopsies cannot capture all conditions, especially conditions leading mostly to disability.[7,8] We identify five conditions that contributed the most to YLDs but to a relatively small proportion of DALYs: nutritional deficiencies, genitourinary diseases, neuropsychiatric conditions, musculoskeletal disorders, and vision and other sensory loss. Improved estimates of YLDs from major depression can use a recent multistate survey.[25] Similar studies of the most common disabilities are lacking in India and most other countries.[24] Ideally, nationally representative disability surveys should accompany expanded cause of death studies.

Our results are subject to uncertainties in the key demographic inputs, such as the age-specific totals of deaths. The Indian census and Sample Registration System data provide a reasonably robust time series of death rates by age, sex, and location, and we grouped results for 5 years to reduce temporal fluctuations. We used 2010–14 cause of death rates, the latest available, applied to 2017 UN death totals, probably resulting in modest overestimates of the rapidly declining burden of some childhood and infectious conditions.[17] Earlier evaluations of the MDS have shown high comparability with relevant hospital or clinical data, strong reproducibility of the dual physician-coded verbal autopsies, and generally low rates of misclassification in children and young and middle-age adults.[15,16,20] Moreover, the uncertainty in diagnosis on verbal autopsy is not likely to affect the relative ranking of diseases.

The NBE method is replicable in other LMICs, as well as in the districts of India. A benefit of the method is that it draws mostly on well established and respected WHO and UN demographic inputs, which are available widely.[21] Although GBD estimates for India have drawn on MDS data in recent years, this is not the case for many other countries as they do not have nationally representative cause of death data.[7,29] Earlier assessments in Africa have found GBD results to be more plausible when local cause of death data were available.[8] As an interim solution, LMICs without nationally representative cause of death data could use results from similar settings (such as Mozambique's 2007 post-census mortality survey[37] in Africa, or from the MDS in Asia). Another option is to use pooled regional cause of death data from the INDEPTH network, despite these not being nationally representative.[8] However, the main priority for countries is to implement nationwide representative mortality studies.[7,16,29] Well validated cause of death data will decrease reliance on modelled data and improve burden estimates.[38]

Decentralised and improved burden estimates would complement the expanding use of local cost-effectiveness and poverty analyses.[5] The NBE could help countries to address data and reporting needs relevant to the WHO and UN goals for universal health coverage. Countries require open-source, locally operable, transparent, and believable data paired with simple, transparent and reproducible tools to track progress towards the 2030 UN Sustainable Development Goals.[1,29,39]

Contributors

GRM and PJ conceived the idea for the study and developed the study design. GRM, SAF, PSh PY, LKW, and WS contributed to the data analysis. SAF and GRM did the literature review. GRM and PJ wrote the initial draft, and all authors were involved in commenting on subsequent revisions.

Declaration of interests

We declare no competing interests.

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NEWS

Covid-19: four fifths of cases are asymptomatic, China figures indicate

Michael Day

London

New evidence has emerged from China indicating that the large majority of coronavirus infections do not result in symptoms.

Chinese authorities began publishing daily figures on 1 April on the number of new coronavirus cases that are asymptomatic, with the first day's figures suggesting that around four in five coronavirus infections caused no illness. Many experts believe that unnoticed, asymptomatic cases of coronavirus infection could be an important source of contagion.

A total of 130 of 166 new infections (78%) identified in the 24 hours to the afternoon of Wednesday 1 April were asymptomatic, said China's National Health Commission. And most of the 36 cases in which patients showed symptoms involved arrivals from overseas, down from 48 the previous day, the commission said.

China is rigorously testing arrivals from overseas for fear of importing a fresh outbreak of covid-19.

Tom Jefferson, an epidemiologist and honorary research fellow at the Centre for Evidence-Based Medicine at the University of Oxford, said the findings were "very, very important." He told *The BMJ*, "The sample is small, and more data will become available. Also, it's not clear exactly how these cases were identified. But let's just say they are generalisable. And even if they are 10% out, then this suggests the virus is everywhere. If—and I stress, if—the results are representative, then we have to ask, 'What the hell are we locking down for?'"

Jefferson said that it was quite likely that the virus had been circulating for longer than generally believed and that large swathes of the population had already been exposed.

Users of Chinese social media have expressed fears that carriers with no symptoms could be spreading the virus unknowingly, especially now that infections have subsided and authorities have eased curbs on travel for people in previous hotspots in the epidemic.

Zhong Nanshan, a senior medical adviser to the Chinese government, said that asymptomatic infections would not be able to cause another major outbreak of covid-19 if such people were kept in isolation. Officials have said this is usually for 14 days.

Nanshan said that once asymptomatic infected people were identified, they and their contacts would be isolated and kept under observation.

Citing classified data, the *South China Morning Post* said that China had already found more than 43 000 cases of asymptomatic infection through contact tracing.

The latest findings seem to contradict a World Health Organization report in February that was based on covid-19 in China. This suggested that "the proportion of truly asymptomatic infections is unclear but appears to be relatively rare and does not appear to be a major driver of transmission."[1]

But since that WHO report other researchers, including Sergio Romagnani, a professor of clinical immunology at the University of Florence, have said they have evidence that most people infected by the virus do not show symptoms. Romagnani led the research that showed that blanket testing in a completely isolated village of roughly 3000 people in northern Italy saw the number of people with covid-19 symptoms fall by over 90% within 10 days by isolating people who were symptomatic and those who were asymptomatic.[2]

In an article on the website of the Centre for Evidence-Based Medicine, Jefferson and Carl Heneghan, director of the centre and editor of *BMJ EBM*, write, "There can be little doubt that covid-19 may be far more widely distributed than some may believe. Lockdown is going to bankrupt all of us and our descendants and is unlikely at this point to slow or halt viral circulation as the genie is out of the bottle.

"What the current situation boils down to is this: is economic meltdown a price worth paying to halt or delay what is already amongst us?"[3]

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ORIGINAL ARTICLE

Clinical Characteristics of Coronavirus Disease 2019 in China

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ABSTRACT

BACKGROUND

Since December 2019, when coronavirus disease 2019 (Covid-19) emerged in Wuhan city and rapidly spread throughout China, data have been needed on the clinical characteristics of the affected patients.

METHODS

We extracted data regarding 1099 patients with laboratory-confirmed Covid-19 from 552 hospitals in 30 provinces, autonomous regions, and municipalities in mainland China through January 29, 2020. The primary composite end point was admission to an intensive care unit (ICU), the use of mechanical ventilation, or death.

RESULTS

The median age of the patients was 47 years; 41.9% of the patients were female. The primary composite end point occurred in 67 patients (6.1%), including 5.0% who were admitted to the ICU, 2.3% who underwent invasive mechanical ventilation, and 1.4% who died. Only 1.9% of the patients had a history of direct contact with wildlife. Among nonresidents of Wuhan, 72.3% had contact with residents of Wuhan, including 31.3% who had visited the city. The most common symptoms were fever (43.8% on admission and 88.7% during hospitalization) and cough (67.8%). Diarrhea was uncommon (3.8%). The median incubation period was 4 days (interquartile range, 2 to 7). On admission, ground-glass opacity was the most common radiologic finding on chest computed tomography (CT) (56.4%). No radiographic or CT abnormality was found in 157 of 877 patients (17.9%) with nonsevere disease and in 5 of 173 patients (2.9%) with severe disease. Lymphocytopenia was present in 83.2% of the patients on admission.

CONCLUSIONS

During the first 2 months of the current outbreak, Covid-19 spread rapidly throughout China and caused varying degrees of illness. Patients often presented without fever, and many did not have abnormal radiologic findings. (Funded by the National Health Commission of China and others.)

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*A list of investigators in the China Medical Treatment Expert Group for Covid-19 study is provided in the Supplementary Appendix, available at NEJM.org.

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In early December 2019, the first pneumonia cases of unknown origin were identified in Wuhan, the capital city of Hubei province.[1] The pathogen has been identified as a novel enveloped RNA betacoronavirus[2] that has currently been named severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2), which has a phylogenetic similarity to SARS-CoV.[3] Patients with the infection have been documented both in hospitals and in family settings.[4-8]

The World Health Organization (WHO) has recently declared coronavirus disease 2019 (Covid-19) a public health emergency of international concern.[9] As of February 25, 2020, a total of 81,109 laboratory-confirmed cases had been documented globally.[5,6,9-11] In recent studies, the severity of some cases of Covid-19 mimicked that of SARS-CoV.[1,12,13] Given the rapid spread of Covid-19, we determined that an updated analysis of cases throughout mainland China might help identify the defining clinical characteristics and severity of the disease. Here, we describe the results of our analysis of the clinical characteristics of Covid-19 in a selected cohort of patients throughout China.

Methods

Study Oversight

The study was supported by National Health Commission of China and designed by the investigators. The study was approved by the institutional review board of the National Health Commission. Written informed consent was waived in light of the urgent need to collect data. Data were analyzed and interpreted by the authors. All the authors reviewed the manuscript and vouch for the accuracy and completeness of the data and for the adherence of the study to the protocol, available with the full text of this article at NEJM.org.

Data Sources

We obtained the medical records and compiled data for hospitalized patients and outpatients with laboratory-confirmed Covid-19, as reported to the National Health Commission between December 11, 2019, and January 29, 2020; the data cutoff for the study was January 31, 2020. Covid-19 was diagnosed on the basis of the WHO interim guidance.[14] A confirmed case of Covid-19 was defined as a positive result on high-throughput sequencing or real-time reverse-transcriptase–polymerase-chain-reaction (RT-PCR) assay of nasal and pharyngeal swab specimens.[1] Only laboratory-confirmed cases were included in the analysis.

We obtained data regarding cases outside Hubei province from the National Health Commission. Because of the high workload of clinicians, three outside experts from Guangzhou performed raw data extraction at Wuhan Jinyintan Hospital, where many of the patients with Covid-19 in Wuhan were being treated.

We extracted the recent exposure history, clinical symptoms or signs, and laboratory findings on admission from electronic medical records. Radiologic assessments included chest radiography or computed tomography (CT), and all laboratory testing was performed according to the clinical care needs of the patient. We determined the presence of a radiologic abnormality on the basis of the documentation or description in medical charts; if imaging scans were available, they were reviewed by attending physicians in respiratory medicine who extracted the data. Major disagreement between two reviewers was resolved by consultation with a third reviewer. Laboratory assessments consisted of a complete blood count, blood chemical analysis, coagulation testing, assessment of liver and renal function, and measures of electrolytes, C-reactive protein, procalcitonin, lactate dehydrogenase, and creatine kinase. We defined the degree of severity of Covid-19 (severe vs. nonsevere) at the time of admission using the American Thoracic Society guidelines for community-acquired pneumonia.[15]

All medical records were copied and sent to the data-processing center in Guangzhou, under the coordination of the National Health Commission. A team of experienced respiratory clinicians reviewed and abstracted the data. Data were entered into a computerized database and cross-checked. If the core data were missing, requests for clarification were sent to the coordinators, who subsequently contacted the attending clinicians.

Study Outcomes

The primary composite end point was admission to an intensive care unit (ICU), the use of mechanical ventilation, or death. These outcomes

were used in a previous study to assess the severity of other serious infectious diseases, such as H7N9 infection.[16] Secondary end points were the rate of death and the time from symptom onset until the composite end point and until each component of the composite end point.

STUDY DEFINITIONS

The incubation period was defined as the interval between the potential earliest date of contact of the transmission source (wildlife or person with suspected or confirmed case) and the potential earliest date of symptom onset (i.e., cough, fever, fatigue, or myalgia). We excluded incubation periods of less than 1 day because some patients had continuous exposure to contamination sources; in these cases, the latest date of exposure was recorded. The summary statistics of incubation periods were calculated on the basis of 291 patients who had clear information regarding the specific date of exposure.

Fever was defined as an axillary temperature of 37.5°C or higher. Lymphocytopenia was defined as a lymphocyte count of less than 1500 cells per cubic millimeter. Thrombocytopenia was defined as a platelet count of less than 150,000 per cubic millimeter. Additional definitions — including exposure to wildlife, acute respiratory distress syndrome (ARDS), pneumonia, acute kidney failure, acute heart failure, and rhabdomyolysis — are provided in the Supplementary Appendix, available at NEJM.org.

LABORATORY CONFIRMATION

Laboratory confirmation of SARS-CoV-2 was performed at the Chinese Center for Disease Prevention and Control before January 23, 2020, and subsequently in certified tertiary care hospitals. RT-PCR assays were performed in accordance with the protocol established by the WHO.[17] Details regarding laboratory confirmation processes are provided in the Supplementary Appendix.

STATISTICAL ANALYSIS

Continuous variables were expressed as medians and interquartile ranges or simple ranges, as appropriate. Categorical variables were summarized as counts and percentages. No imputation was made for missing data. Because the cohort of patients in our study was not derived from random selection, all statistics are deemed to be descriptive only. We used ArcGIS, version 10.2.2, to plot the numbers of patients with reportedly confirmed cases on a map. All the analyses were performed with the use of R software, version 3.6.2 (R Foundation for Statistical Computing).

RESULTS

DEMOGRAPHIC AND CLINICAL CHARACTERISTICS

Of the 7736 patients with Covid-19 who had been hospitalized at 552 sites as of January 29, 2020, we obtained data regarding clinical symptoms and outcomes for 1099 patients (14.2%). The largest number of patients (132) had been admitted to Wuhan Jinyintan Hospital. The hospitals that were included in this study accounted for 29.7% of the 1856 designated hospitals where patients with Covid-19 could be admitted in 30 provinces, autonomous regions, or municipalities across China (Fig. 1).

The demographic and clinical characteristics of the patients are shown in Table 1. A total of 3.5% were health care workers, and a history of contact with wildlife was documented in 1.9%; 483 patients (43.9%) were residents of Wuhan. Among the patients who lived outside Wuhan, 72.3% had contact with residents of Wuhan, including 31.3% who had visited the city; 25.9% of nonresidents had neither visited the city nor had contact with Wuhan residents.

The median incubation period was 4 days (interquartile range, 2 to 7). The median age of the patients was 47 years (interquartile range, 35 to 58); 0.9% of the patients were younger than 15 years of age. A total of 41.9% were female. Fever was present in 43.8% of the patients on admission but developed in 88.7% during hospitalization. The second most common symptom was cough (67.8%); nausea or vomiting (5.0%) and diarrhea (3.8%) were uncommon. Among the overall population, 23.7% had at least one coexisting illness (e.g., hypertension and chronic obstructive pulmonary disease).

On admission, the degree of severity of Covid-19 was categorized as nonsevere in 926 patients and severe in 173 patients. Patients with severe disease were older than those with nonsevere disease by a median of 7 years. Moreover, the presence of any coexisting illness was more common among patients with severe disease than among those with nonsevere disease (38.7% vs.

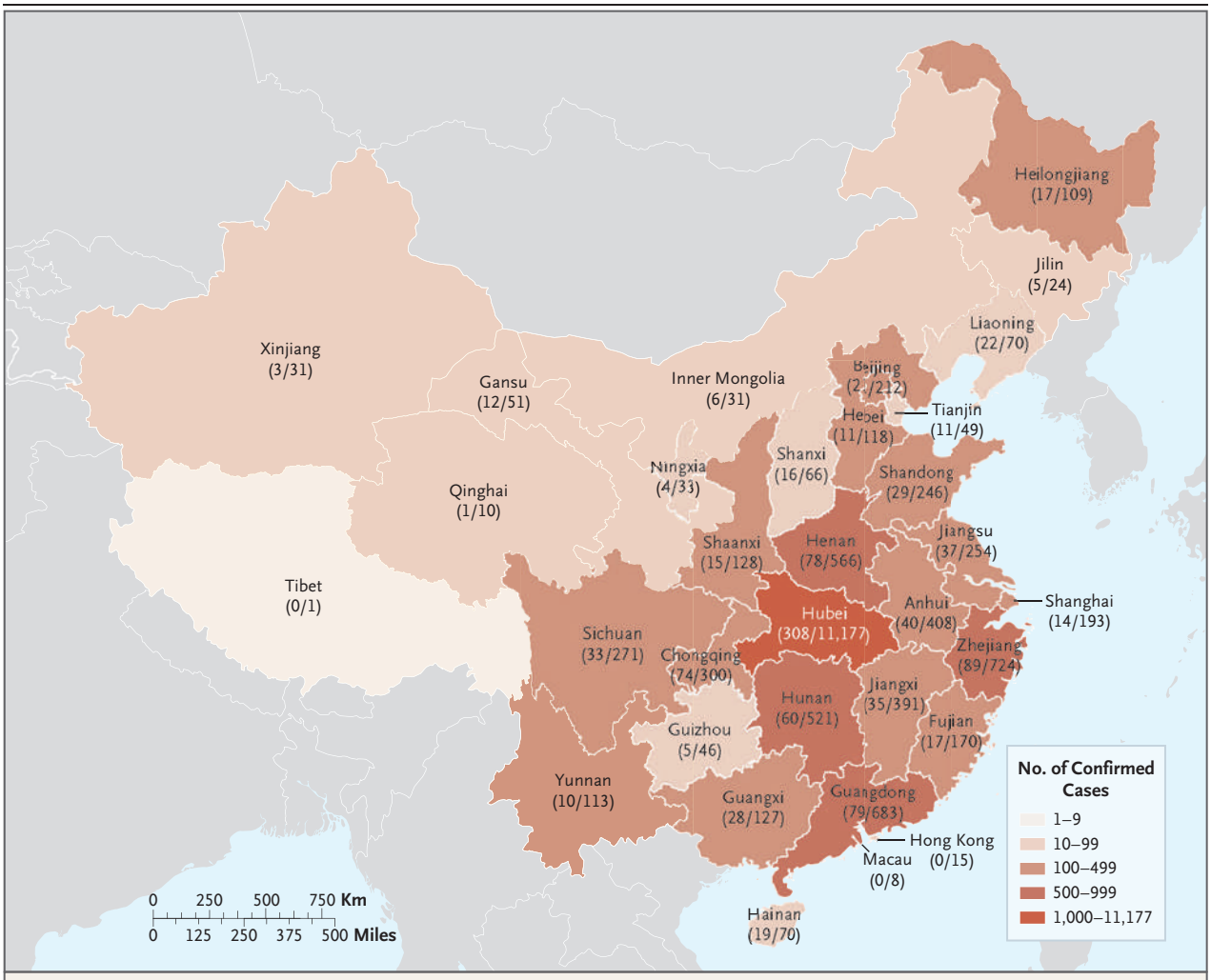

Figure 1. Distribution of Patients with Covid-19 across Mainland China.

Shown are the official statistics of all documented, laboratory-confirmed cases of coronavirus disease 2019 (Covid-19) throughout China, according to the National Health Commission as of February 4, 2020. The numerator denotes the number of patients who were included in the study cohort and the denominator denotes the number of laboratory-confirmed cases for each province, autonomous region, or provincial municipality, as reported by the National Health Commission.

21.0%). However, the exposure history between the two groups of disease severity was similar.

RADIOLOGIC AND LABORATORY FINDINGS

Table 2 shows the radiologic and laboratory findings on admission. Of 975 CT scans that were performed at the time of admission, 86.2% revealed abnormal results. The most common patterns on chest CT were ground-glass opacity (56.4%) and bilateral patchy shadowing (51.8%). Representative radiologic findings in two patients with nonsevere Covid-19 and in another two patients with severe Covid-19 are provided in Figure S1 in the Supplementary Appendix. No radiographic or CT abnormality was found in 157 of 877 patients (17.9%) with nonsevere disease and in 5 of 173 patients (2.9%) with severe disease.

On admission, lymphocytopenia was present in 83.2% of the patients, thrombocytopenia in 36.2%, and leukopenia in 33.7%. Most of the patients had elevated levels of C-reactive protein; less common were elevated levels of alanine aminotransferase, aspartate aminotransferase,

creatine kinase, and D-dimer. Patients with severe disease had more prominent laboratory abnormalities (including lymphocytopenia and leukopenia) than those with nonsevere disease.

CLINICAL OUTCOMES

None of the 1099 patients were lost to follow-up during the study. A primary composite end-point event occurred in 67 patients (6.1%), including 5.0% who were admitted to the ICU, 2.3% who underwent invasive mechanical ventilation, and 1.4% who died (Table 3). Among the 173 patients with severe disease, a primary composite end-point event occurred in 43 patients (24.9%). Among all the patients, the cumulative risk of the composite end point was 3.6%; among those with severe disease, the cumulative risk was 20.6%.

TREATMENT AND COMPLICATIONS

A majority of the patients (58.0%) received intravenous antibiotic therapy, and 35.8% received oseltamivir therapy; oxygen therapy was administered in 41.3% and mechanical ventilation in 6.1%; higher percentages of patients with severe disease received these therapies (Table 3). Mechanical ventilation was initiated in more patients with severe disease than in those with nonsevere disease (noninvasive ventilation, 32.4% vs. 0%; invasive ventilation, 14.5% vs. 0%). Systemic glucocorticoids were given to 204 patients (18.6%), with a higher percentage among those with severe disease than nonsevere disease (44.5% vs. 13.7%). Of these 204 patients, 33 (16.2%) were admitted to the ICU, 17 (8.3%) underwent invasive ventilation, and 5 (2.5%) died. Extracorporeal membrane oxygenation was performed in 5 patients (0.5%) with severe disease.

The median duration of hospitalization was 12.0 days (mean, 12.8). During hospital admission, most of the patients received a diagnosis of pneumonia from a physician (91.1%), followed by ARDS (3.4%) and shock (1.1%). Patients with severe disease had a higher incidence of physician-diagnosed pneumonia than those with nonsevere disease (99.4% vs. 89.5%).

DISCUSSION

During the initial phase of the Covid-19 outbreak, the diagnosis of the disease was complicated by the diversity in symptoms and imaging findings and in the severity of disease at the time of presentation. Fever was identified in 43.8% of the patients on presentation but developed in 88.7% after hospitalization. Severe illness occurred in 15.7% of the patients after admission to a hospital. No radiologic abnormalities were noted on initial presentation in 2.9% of the patients with severe disease and in 17.9% of those with nonsevere disease. Despite the number of deaths associated with Covid-19, SARS-CoV-2 appears to have a lower case fatality rate than either SARS-CoV or Middle East respiratory syndrome–related coronavirus (MERS-CoV). Compromised respiratory status on admission (the primary driver of disease severity) was associated with worse outcomes.

Approximately 2% of the patients had a history of direct contact with wildlife, whereas more than three quarters were either residents of Wuhan, had visited the city, or had contact with city residents. These findings echo the latest reports, including the outbreak of a family cluster,[4] transmission from an asymptomatic patient,[6] and the three-phase outbreak patterns.[8] Our study cannot preclude the presence of patients who have been termed "super-spreaders."

Conventional routes of transmission of SARS-CoV, MERS-CoV, and highly pathogenic influenza consist of respiratory droplets and direct contact,[18-20] mechanisms that probably occur with SARS-CoV-2 as well. Because SARS-CoV-2 can be detected in the gastrointestinal tract, saliva, and urine, these routes of potential transmission need to be investigated[21] (Tables S1 and S2).

The term Covid-19 has been applied to patients who have laboratory-confirmed symptomatic cases without apparent radiologic manifestations. A better understanding of the spectrum of the disease is needed, since in 8.9% of the patients, SARS-CoV-2 infection was detected before the development of viral pneumonia or viral pneumonia did not develop.

In concert with recent studies,[1,8,12] we found that the clinical characteristics of Covid-19 mimic those of SARS-CoV. Fever and cough were the dominant symptoms and gastrointestinal symptoms were uncommon, which suggests a difference in viral tropism as compared with SARS-CoV, MERS-CoV, and seasonal influenza.[22,23] The absence of fever in Covid-19 is more frequent than in SARS-CoV (1%) and MERS-CoV infection

Table 1. Clinical Characteristics of the Study Patients, According to Disease Severity and the Presence or Absence of the Primary Composite End Point.*

Characteristic	All Patients (N=1099)	Disease Severity		Presence of Primary Composite End Point†	
		Nonsevere (N=926)	Severe (N=173)	Yes (N=67)	No (N=1032)
Age					
Median (IQR) — yr	47.0 (35.0–58.0)	45.0 (34.0–57.0)	52.0 (40.0–65.0)	63.0 (53.0–71.0)	46.0 (35.0–57.0)
Distribution — no./total no. (%)					
0–14 yr	9/1011 (0.9)	8/848 (0.9)	1/163 (0.6)	0	9/946 (1.0)
15–49 yr	557/1011 (55.1)	490/848 (57.8)	67/163 (41.1)	12/65 (18.5)	545/946 (57.6)
50–64 yr	292/1011 (28.9)	241/848 (28.4)	51/163 (31.3)	21/65 (32.3)	271/946 (28.6)
≥65 yr	153/1011 (15.1)	109/848 (12.9)	44/163 (27.0)	32/65 (49.2)	121/946 (12.8)
Female sex — no./total no. (%)	459/1096 (41.9)	386/923 (41.8)	73/173 (42.2)	22/67 (32.8)	437/1029 (42.5)
Smoking history — no./total no. (%)					
Never smoked	927/1085 (85.4)	793/913 (86.9)	134/172 (77.9)	44/66 (66.7)	883/1019 (86.7)
Former smoker	21/1085 (1.9)	12/913 (1.3)	9/172 (5.2)	5/66 (7.6)	16/1019 (1.6)
Current smoker	137/1085 (12.6)	108/913 (11.8)	29/172 (16.9)	17/66 (25.8)	120/1019 (11.8)
Exposure to source of transmission within past 14 days — no./total no.					
Living in Wuhan	483/1099 (43.9)	400/926 (43.2)	83/173 (48.0)	39/67 (58.2)	444/1032 (43.0)
Contact with wildlife	13/687 (1.9)	10/559 (1.8)	3/128 (2.3)	1/41 (2.4)	12/646 (1.9)
Recently visited Wuhan‡	193/616 (31.3)	166/526 (31.6)	27/90 (30.0)	10/28 (35.7)	183/588 (31.1)
Had contact with Wuhan residents‡	442/611 (72.3)	376/522 (72.0)	66/89 (74.2)	19/28 (67.9)	423/583 (72.6)
Median incubation period (IQR) — days§	4.0 (2.0–7.0)	4.0 (2.8–7.0)	4.0 (2.0–7.0)	4.0 (1.0–7.5)	4.0 (2.0–7.0)
Fever on admission					
Patients — no./total no. (%)	473/1081 (43.8)	391/910 (43.0)	82/171 (48.0)	24/66 (36.4)	449/1015 (44.2)
Median temperature (IQR) — °C	37.3 (36.7–38.0)	37.3 (36.7–38.0)	37.4 (36.7–38.1)	36.8 (36.3–37.8)	37.3 (36.7–38.0)
Distribution of temperature — no./total no. (%)					
<37.5°C	608/1081 (56.2)	519/910 (57.0)	89/171 (52.0)	42/66 (63.6)	566/1015 (55.8)
37.5–38.0°C	238/1081 (22.0)	201/910 (22.1)	37/171 (21.6)	10/66 (15.2)	228/1015 (22.5)
38.1–39.0°C	197/1081 (18.2)	160/910 (17.6)	37/171 (21.6)	11/66 (16.7)	186/1015 (18.3)
>39.0°C	38/1081 (3.5)	30/910 (3.3)	8/171 (4.7)	3/66 (4.5)	35/1015 (3.4)
Fever during hospitalization					
Patients — no./total no. (%)	975/1099 (88.7)	816/926 (88.1)	159/173 (91.9)	59/67 (88.1)	916/1032 (88.8)
Median highest temperature (IQR) — °C	38.3 (37.8–38.9)	38.3 (37.8–38.9)	38.5 (38.0–39.0)	38.5 (38.0–39.0)	38.3 (37.8–38.9)
<37.5°C	92/926 (9.9)	79/774 (10.2)	13/152 (8.6)	3/54 (5.6)	89/872 (10.2)
37.5–38.0°C	286/926 (30.9)	251/774 (32.4)	35/152 (23.0)	20/54 (37.0)	266/872 (30.5)
38.1–39.0°C	434/926 (46.9)	356/774 (46.0)	78/152 (51.3)	21/54 (38.9)	413/872 (47.4)
>39.0°C	114/926 (12.3)	88/774 (11.4)	26/152 (17.1)	10/54 (18.5)	104/872 (11.9)

Symptoms — no. (%)					
Conjunctival congestion	9 (0.8)	5 (0.5)	4 (2.3)	0	9 (0.9)
Nasal congestion	53 (4.8)	47 (5.1)	6 (3.5)	2 (3.0)	51 (4.9)
Headache	150 (13.6)	124 (13.4)	26 (15.0)	8 (11.9)	142 (13.8)
Cough	745 (67.8)	623 (67.3)	122 (70.5)	46 (68.7)	699 (67.7)
Sore throat	153 (13.9)	130 (14.0)	23 (13.3)	6 (9.0)	147 (14.2)
Sputum production	370 (33.7)	309 (33.4)	61 (35.3)	20 (29.9)	350 (33.9)
Fatigue	419 (38.1)	350 (37.8)	69 (39.9)	22 (32.8)	397 (38.5)
Hemoptysis	10 (0.9)	6 (0.6)	4 (2.3)	2 (3.0)	8 (0.8)
Shortness of breath	205 (18.7)	140 (15.1)	65 (37.6)	36 (53.7)	169 (16.4)
Nausea or vomiting	55 (5.0)	43 (4.6)	12 (6.9)	3 (4.5)	52 (5.0)
Diarrhea	42 (3.8)	32 (3.5)	10 (5.8)	4 (6.0)	38 (3.7)
Myalgia or arthralgia	164 (14.9)	134 (14.5)	30 (17.3)	6 (9.0)	158 (15.3)
Chills	126 (11.5)	100 (10.8)	26 (15.0)	8 (11.9)	118 (11.4)
Signs of infection — no. (%)					
Throat congestion	19 (1.7)	17 (1.8)	2 (1.2)	0	19 (1.8)
Tonsil swelling	23 (2.1)	17 (1.8)	6 (3.5)	1 (1.5)	22 (2.1)
Enlargement of lymph nodes	2 (0.2)	1 (0.1)	1 (0.6)	1 (1.5)	1 (0.1)
Rash	2 (0.2)	0	2 (1.2)	0	2 (0.2)
Coexisting disorder — no. (%)					
Any	261 (23.7)	194 (21.0)	67 (38.7)	39 (58.2)	222 (21.5)
Chronic obstructive pulmonary disease	12 (1.1)	6 (0.6)	6 (3.5)	7 (10.4)	5 (0.5)
Diabetes	81 (7.4)	53 (5.7)	28 (16.2)	18 (26.9)	63 (6.1)
Hypertension	165 (15.0)	124 (13.4)	41 (23.7)	24 (35.8)	141 (13.7)
Coronary heart disease	27 (2.5)	17 (1.8)	10 (5.8)	6 (9.0)	21 (2.0)
Cerebrovascular disease	15 (1.4)	11 (1.2)	4 (2.3)	4 (6.0)	11 (1.1)
Hepatitis B infection¶	23 (2.1)	22 (2.4)	1 (0.6)	1 (1.5)	22 (2.1)
Cancer‖	10 (0.9)	7 (0.8)	3 (1.7)	1 (1.5)	9 (0.9)
Chronic renal disease	8 (0.7)	5 (0.5)	3 (1.7)	2 (3.0)	6 (0.6)
Immunodeficiency	2 (0.2)	2 (0.2)	0	0	2 (0.2)

* The denominators of patients who were included in the analysis are provided if they differed from the overall numbers in the group. Percentages may not total 100 because of rounding. Covid-19 denotes coronavirus disease 2019, and IQR interquartile range.
† The primary composite end point was admission to an intensive care unit, the use of mechanical ventilation, or death.
‡ These patients were not residents of Wuhan.
§ Data regarding the incubation period were missing for 808 patients (73.5%).
¶ The presence of hepatitis B infection was defined as a positive result on testing for hepatitis B surface antigen with or without elevated levels of alanine or aspartate aminotransferase.
‖ Included in this category is any type of cancer.

Table 2. Radiographic and Laboratory Findings.*

Variable	All Patients (N=1099)	Disease Severity		Presence of Composite Primary End Point	
		Nonsevere (N=926)	Severe (N=173)	Yes (N=67)	No (N=1032)
Radiologic findings					
Abnormalities on chest radiograph — no./total no. (%)	162/274 (59.1)	116/214 (54.2)	46/60 (76.7)	30/39 (76.9)	132/235 (56.2)
Ground-glass opacity	55/274 (20.1)	37/214 (17.3)	18/60 (30.0)	9/39 (23.1)	46/235 (19.6)
Local patchy shadowing	77/274 (28.1)	56/214 (26.2)	21/60 (35.0)	13/39 (33.3)	64/235 (27.2)
Bilateral patchy shadowing	100/274 (36.5)	65/214 (30.4)	35/60 (58.3)	27/39 (69.2)	73/235 (31.1)
Interstitial abnormalities	12/274 (4.4)	7/214 (3.3)	5/60 (8.3)	6/39 (15.4)	6/235 (2.6)
Abnormalities on chest CT — no./total no. (%)	840/975 (86.2)	682/808 (84.4)	158/167 (94.6)	50/57 (87.7)	790/918 (86.1)
Ground-glass opacity	550/975 (56.4)	449/808 (55.6)	101/167 (60.5)	30/57 (52.6)	520/918 (56.6)
Local patchy shadowing	409/975 (41.9)	317/808 (39.2)	92/167 (55.1)	22/57 (38.6)	387/918 (42.2)
Bilateral patchy shadowing	505/975 (51.8)	368/808 (45.5)	137/167 (82.0)	40/57 (70.2)	465/918 (50.7)
Interstitial abnormalities	143/975 (14.7)	99/808 (12.3)	44/167 (26.3)	15/57 (26.3)	128/918 (13.9)
Laboratory findings					
Median Pao_2:Fio_2 ratio (IQR)†	3.9 (2.9–4.7)	3.9 (2.9–4.5)	4.0 (2.8–5.2)	2.9 (2.2–5.4)	4.0 (3.1–4.6)
White-cell count					
Median (IQR) — per mm^3	4700 (3500–6000)	4900 (3800–6000)	3700 (3000–6200)	6100 (4900–11,100)	4700 (3500–5900)
Distribution — no./total no. (%)					
>10,000 per mm^3	58/978 (5.9)	39/811 (4.8)	19/167 (11.4)	15/58 (25.9)	43/920 (4.7)
<4000 per mm^3	330/978 (33.7)	228/811 (28.1)	102/167 (61.1)	8/58 (13.8)	322/920 (35.0)
Lymphocyte count					
Median (IQR) — per mm^3	1000 (700–1300)	1000 (800–1400)	800 (600–1000)	700 (600–900)	1000 (700–1300)
Distribution — no./total no. (%)					
<1500 per mm^3	731/879 (83.2)	584/726 (80.4)	147/153 (96.1)	50/54 (92.6)	681/825 (82.5)

Platelet count					
Median (IQR) — per mm^3	168,000 (132,000–207,000)	172,000 (139,000–212,000)	137,500 (99,000–179,500)	156,500 (114,200–195,000)	169,000 (133,000–207,000)
Distribution — no./total no. (%)					
<150,000 per mm^3	315/869 (36.2)	225/713 (31.6)	90/156 (57.7)	27/58 (46.6)	288/811 (35.5)
Median hemoglobin (IQR) — g/dl‡	13.4. (11.9–14.8)	13.5 (12.0–14.8)	12.8 (11.2–14.1)	12.5 (10.5–14.0)	13.4 (12.0–14.8)
Distribution of other findings — no./total no. (%)					
C-reactive protein ≥10 mg/liter	481/793 (60.7)	371/658 (56.4)	110/135 (81.5)	41/45 (91.1)	440/748 (58.8)
Procalcitonin ≥0.5 ng/ml	35/633 (5.5)	19/516 (3.7)	16/117 (13.7)	12/50 (24.0)	23/583 (3.9)
Lactate dehydrogenase ≥250 U/liter	277/675 (41.0)	205/551 (37.2)	72/124 (58.1)	31/44 (70.5)	246/631 (39.0)
Aspartate aminotransferase >40 U/liter	168/757 (22.2)	112/615 (18.2)	56/142 (39.4)	26/52 (50.0)	142/705 (20.1)
Alanine aminotransferase >40 U/liter	158/741 (21.3)	120/606 (19.8)	38/135 (28.1)	20/49 (40.8)	138/692 (19.9)
Total bilirubin >17.1 μmol/liter	76/722 (10.5)	59/594 (9.9)	17/128 (13.3)	10/48 (20.8)	66/674 (9.8)
Creatine kinase ≥200 U/liter	90/657 (13.7)	67/536 (12.5)	23/121 (19.0)	12/46 (26.1)	78/611 (12.8)
Creatinine ≥133 μmol/liter	12/752 (1.6)	6/614 (1.0)	6/138 (4.3)	5/52 (9.6)	7/700 (1.0)
D-dimer ≥0.5 mg/liter	260/560 (46.4)	195/451 (43.2)	65/109 (59.6)	34/49 (69.4)	226/511 (44.2)
Minerals§					
Median sodium (IQR) — mmol/liter	138.2 (136.1–140.3)	138.4 (136.6–140.4)	138.0 (136.0–140.0)	138.3 (135.0–141.2)	138.2 (136.1–140.2)
Median potassium (IQR) — mmol/liter	3.8 (3.5–4.2)	3.9 (3.6–4.2)	3.8 (3.5–4.1)	3.9 (3.6–4.1)	3.8 (3.5–4.2)
Median chloride (IQR) — mmol/liter	102.9 (99.7–105.6)	102.7 (99.7–105.3)	103.1 (99.8–106.0)	103.8 (100.8–107.0)	102.8 (99.6–105.3)

* Lymphocytopenia was defined as a lymphocyte count of less than 1500 per cubic millimeter. Thrombocytopenia was defined as a platelet count of less than 150,000 per cubic millimeter. To convert the values for creatinine to milligrams per deciliter, divide by 88.4.
† Data regarding the ratio of the partial pressure of arterial oxygen to the fraction of inspired oxygen (Pa_{O_2}:F_{IO_2}) were missing for 894 patients (81.3%).
‡ Data regarding hemoglobin were missing for 226 patients (20.6%).
§ Data were missing for the measurement of sodium in 363 patients (33.0%), for potassium in 349 patients (31.8%), and for chloride in 392 patients (35.7%).

Table 3. Complications, Treatments, and Clinical Outcomes.

Variable	All Patients (N = 1099)	Disease Severity		Presence of Composite Primary End Point	
		Nonsevere (N = 926)	Severe (N = 173)	Yes (N = 67)	No (N = 1032)
Complications					
Septic shock — no. (%)	12 (1.1)	1 (0.1)	11 (6.4)	9 (13.4)	3 (0.3)
Acute respiratory distress syndrome — no. (%)	37 (3.4)	10 (1.1)	27 (15.6)	27 (40.3)	10 (1.0)
Acute kidney injury — no. (%)	6 (0.5)	1 (0.1)	5 (2.9)	4 (6.0)	2 (0.2)
Disseminated intravascular coagulation — no. (%)	1 (0.1)	0	1 (0.6)	1 (1.5)	0
Rhabdomyolysis — no. (%)	2 (0.2)	2 (0.2)	0	0	2 (0.2)
Physician-diagnosed pneumonia — no./total no. (%)	972/1067 (91.1)	800/894 (89.5)	172/173 (99.4)	63/66 (95.5)	909/1001 (90.8)
Median time until development of pneumonia (IQR) — days*					
After initial Covid-19 diagnosis	0.0 (0.0–1.0)	0.0 (0.0–1.0)	0.0 (0.0–2.0)	0.0 (0.0–3.5)	0.0 (0.0–1.0)
After onset of Covid-19 symptoms	3.0 (1.0–6.0)	3.0 (1.0–6.0)	5.0 (2.0–7.0)	4.0 (0.0–7.0)	3.0 (1.0–6.0)
Treatments					
Intravenous antibiotics — no. (%)	637 (58.0)	498 (53.8)	139 (80.3)	60 (89.6)	577 (55.9)
Oseltamivir — no. (%)	393 (35.8)	313 (33.8)	80 (46.2)	36 (53.7)	357 (34.6)
Antifungal medication — no. (%)	31 (2.8)	18 (1.9)	13 (7.5)	8 (11.9)	23 (2.2)
Systemic glucocorticoids — no. (%)	204 (18.6)	127 (13.7)	77 (44.5)	35 (52.2)	169 (16.4)
Oxygen therapy — no. (%)	454 (41.3)	331 (35.7)	123 (71.1)	59 (88.1)	395 (38.3)
Mechanical ventilation — no. (%)	67 (6.1)	0	67 (38.7)	40 (59.7)	27 (2.6)
Invasive	25 (2.3)	0	25 (14.5)	25 (37.3)	0
Noninvasive	56 (5.1)	0	56 (32.4)	29 (43.3)	27 (2.6)
Use of extracorporeal membrane oxygenation — no. (%)	5 (0.5)	0	5 (2.9)	5 (7.5)	0
Use of continuous renal-replacement therapy — no. (%)	9 (0.8)	0	9 (5.2)	8 (11.9)	1 (0.1)
Use of intravenous immune globulin — no. (%)	144 (13.1)	86 (9.3)	58 (33.5)	27 (40.3)	117 (11.3)
Admission to intensive care unit — no. (%)	55 (5.0)	22 (2.4)	33 (19.1)	55 (82.1)	0
Median length of hospital stay (IQR) — days†	12.0 (10.0–14.0)	11.0 10.0–13.0)	13.0 (11.5–17.0)	14.5 (11.0–19.0)	12.0 (10.0–13.0)

Clinical outcomes at data cutoff — no. (%)					
Discharge from hospital	55 (5.0)	50 (5.4)	5 (2.9)	1 (1.5)	54 (5.2)
Death	15 (1.4)	1 (0.1)	14 (8.1)	15 (22.4)	0
Recovery	9 (0.8)	7 (0.8)	2 (1.2)	0	9 (0.9)
Hospitalization	1029 (93.6)	875 (94.5)	154 (89.0)	51 (76.1)	978 (94.8)

* For the development of pneumonia, data were missing for 347 patients (31.6%) regarding the time since the initial diagnosis and for 161 patients (14.6%) regarding the time since symptom onset.
† Data regarding the median length of hospital stay were missing for 136 patients (12.4%).

(2%),[20] so afebrile patients may be missed if the surveillance case definition focuses on fever detection.[14] Lymphocytopenia was common and, in some cases, severe, a finding that was consistent with the results of two recent reports.[1,12] We found a lower case fatality rate (1.4%) than the rate that was recently reportedly,[1,12] probably because of the difference in sample sizes and case inclusion criteria. Our findings were more similar to the national official statistics, which showed a rate of death of 3.2% among 51,857 cases of Covid-19 as of February 16, 2020.[11,24] Since patients who were mildly ill and who did not seek medical attention were not included in our study, the case fatality rate in a real-world scenario might be even lower. Early isolation, early diagnosis, and early management might have collectively contributed to the reduction in mortality in Guangdong.

Despite the phylogenetic homogeneity between SARS-CoV-2 and SARS-CoV, there are some clinical characteristics that differentiate Covid-19 from SARS-CoV, MERS-CoV, and seasonal influenza infections. (For example, seasonal influenza has been more common in respiratory outpatient clinics and wards.) Some additional characteristics that are unique to Covid-19 are detailed in Table S3.

Our study has some notable limitations. First, some cases had incomplete documentation of the exposure history and laboratory testing, given the variation in the structure of electronic databases among different participating sites and the urgent timeline for data extraction. Some cases were diagnosed in outpatient settings where medical information was briefly documented and incomplete laboratory testing was performed, along with a shortage of infrastructure and training of medical staff in nonspecialty hospitals. Second, we could estimate the incubation period in only 291 of the study patients who had documented information. The uncertainty of the exact dates (recall bias) might have inevitably affected our assessment. Third, because many patients remained in the hospital and the outcomes were unknown at the time of data cutoff, we censored the data regarding their clinical outcomes as of the time of our analysis. Fourth, we no doubt missed patients who were asymptomatic or had mild cases and who were treated at home, so our study cohort may represent the more severe end of Covid-19. Fifth,

many patients did not undergo sputum bacteriologic or fungal assessment on admission because, in some hospitals, medical resources were overwhelmed. Sixth, data generation was clinically driven and not systematic.

Covid-19 has spread rapidly since it was first identified in Wuhan and has been shown to have a wide spectrum of severity. Some patients with Covid-19 do not have fever or radiologic abnormalities on initial presentation, which has complicated the diagnosis.

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APPENDIX

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Infrared Forehead Thermometer

User Manual

Model： HA-650

Thanks for purchasing Infrared Forehead Thermometer,It is mainly designed for measuring human body temperature. Before using the device, please read this manual carefully to ensure proper and safe operation.
Please take good care of the manual for future reference.

Welcome your advice and support.

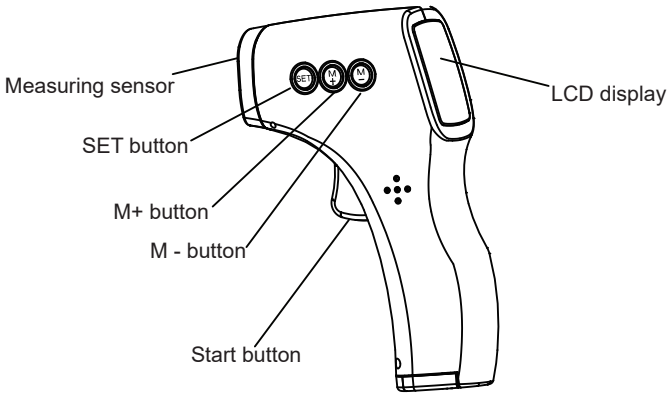
Table of Contents

About the Products

1．The Advantage of this Thermometer

1. Measurement in one second
2. Accurate and reliable
3. 50 memories places
4. Fever alarm
5. Changing between Centigrade and Fahrenheit
6. Beeper function

2. The Constitute of the Product

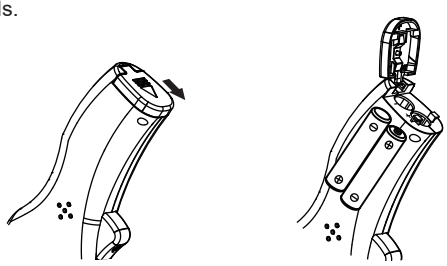


How to Use

1．Batteries Installation

Press the indicator ▼ on the battery cover and slide the cover in the direction of the arrow.

Insert 2 “AAA” size batteries, ensure correct polarity as shown by the symbols.



2. How to setup.

In power off condition, press SET for 2 seconds to enter setup interface with F0 display. press SET to enter into the switch of setup content “ F0->F1-> F2 ”

F0 interface, press M+ to object mode, press M- to body mode .

F1 interface, press M+ to Fahrenheit, press M- to Centigrade.

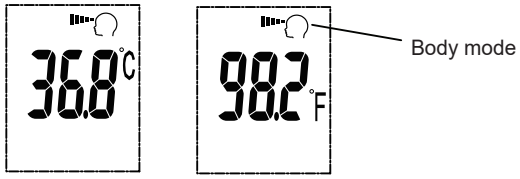
F2 interface, press M+ to beeper off, press M- to beeper on.

Press SET again to save the setup content, the thermometer will be turned off.

3. Direction for Use

Measuring in body mode

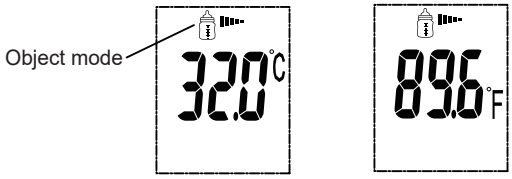
1. Aim the thermometer at center of the forehead with a distance of 1~5 cm. Pls remove the hair and sweat from the forehead before measuring to improve the accuracy of the measurement.
2. Press START button, the measurement result will be displayed within 1 second.



3. The thermometer will be automatically powered off in 10 seconds without any operation.

Measuring in Object mode

1. Aim the thermometer at center of the object with a distance of 1~5 cm.
2. Press START button, the measurement result will be displayed within 1 second.



3. The thermometer will be automaticly powered off in 10 seconds without any operation.

Notes: Object mode can not be used for medical purpose.

Note:

- If thermometer is stored in a location that is cooler or warmer than where it is being used, let it sit in the patient's room for 30 minutes before taking the measurement.
- Do not take the measurement in an extreme condition
- Avoid drinking, exercising, bathing before/ while taking temperature.
- Always taking the temperature in the same position, since temperature readings may vary according to different position.
- Do not move the thermometer during taking temperature.
- It is recommended to take three temperatures, choose average data when three readings are different.

4. How to recall memory

Press M+ button to read last reading.
Press and release M+ button to read more stored memories.

Note:
The thermometer can memorize 50 data. The thermometer will delete the earliest data automatically when the number of data is beyond 50.

5. Safety Instructions

- This device may only be used for the purposes described in these instruction. Do not use the device for any other purpose.
- Do not disassemble or attempt to repair the unit of components.
- Wireless communications equipment such as wireless home network devices, mobile phones, cordless telephones and their base stations, walkie-talkies can affect this equipment and should be kept at least a distance d = 3, 3 m away from the equipment.

WARNING

The measurement results given by this device is not a diagnosis.
It is not replacing the need for the consultation of a physician. Do not rely on the measurement result only, self-diagnosis of measurement results and self-treatment are dangerous.

6. Abnormal Phenomenon

- Display <H> measured temperature too high.
  Measured temperature is higher than 43.0℃/ 109.4℉ in body mode or 50.5℃ / 122.9 ℉ in object mode.
- Display <L> measured temperature too low.
  Measured temperature is lower than 34.0℃/ 93.2℉ in body mode or 10.0℃ / 50 ℉ in object mode
- Display <EH> ambient temperature too high
  Ambient temperature is heigher than 40.0℃/104.0 ℉
- Display <EL> ambient temperature too low
  Ambient temperature is lower than 10.0℃/ 50.0℉
- Display  error function display
  The system has a malfunction, reinstall batteries and start again
- Low battery 
  Please replace the batteries with new batteries

7. Cleaning instruction

Use cotton tissue moistened with alcohol (70%~75%) to clean the thermometer casing, ensure no liquid enters the interior of the device.
Never use abrasive cleaning agents, thinner for cleaning and never immerse the device in water or other cleaning liquids.

8. Technical Specifications

Type:	Infrared Forehead Thermometer
Measurement rage :	Body mode 34.0-43.0℃ (93.2 - 109.4℉) Object mode 10.0-50.5℃ (50 - 122.9℉)
Resolution:	0.1℃/ ℉

Accurancy (Laboratory):	± 0.2℃ (35.0 ~ 42.0℃) / ± 0.4℉ (95.0 ~ 107.6℉) ± 0.3℃ (34.0 ~ 34.9℃) (42.1 ~ 43.0℃) / ± 0.5℉ (93.2 ~ 94.8℉) (107.8 ~ 109.4℉)
Memory:	50 Memories
Backlight:	The display light will be blue when a measurement lower than 37.5℃/99.5℉ The display light will be orange when a measurement between 37.5℃~38.4℃ (99.5℉~101.1℉) The display light will be red when a measurement equal to or higher than 38.5℃ (101.3℉)
Dimensions:	136 x 86 x 39 mm
Operating Condition:	Temperature: 10 - 40 ℃ (50.0- 104.0℉) Humidity: ≤ 85%RH Air Pressure: 700hPa~1060hPa
Storage Condition:	Temperature: -20 - 55 ℃ (-4 - 131.0℉) Humidity: ≤ 93%RH
Automatic Switch off:	Approx 10 seconds after last measurement has been taken
Weight:	About 100g (with batteries)
Battery:	2X 1.5V AAA batteries

Xiamen Ants-Bro Technology Co., Ltd.
4F,5th Building, Technology business establishing center, 289 Wengjiao Road,
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Version 1.0 10/10/2017

The Spectator

How many people have Covid-19 and don't even know it?

4 April 2020, 10:22pm

Just how many of us have Covid-19 and are not even aware of it? It's a question at the heart of this crisis. Epidemiologists are deeply divided, and no-one truly knows. Yesterday came news from China that 130 of the 166 people most recently found to be infected with SARS-CoV-2 there have proved to be asymptomatic. That is to say they had no symptoms whatsoever which would have led them to suspect that they were infected.

This is consistent with research from the village of Vo'Euganeo in Northern Italy where all 3,000 inhabitants were tested for the virus early in the Italian outbreak. There, between 50 and 75 per cent of those infected had no symptoms either.

The proportion of people who are asymptomatic matters hugely because of the implications for the mortality rate and infection rate of the general population. We know that the cases which are being recorded are only the tip of an iceberg – since the UK moved into the 'delay' phase a couple of weeks ago we are no longer even testing people unless they present at hospital with severe symptoms. What we don't know is how large that iceberg is. If it is as big as claimed by modelling by an Oxford team led by Professor Sunetra Gupta – which suggested that up to half the UK population could already be infected – then there may be no point in locking down Britain or any other country: Covid-19 is a chronic disease which has already spread through the population but will be of limited concern because it is not very deadly.

That is the point made by Tom Jefferson and Carl Heneghen of Oxford University's Centre for Evidence-Based Medicine, who did not mince their words when they wrote on the centre's website last Monday: 'Lockdown is going to bankrupt all of us and our descendants and is unlikely at this point to slow or halt viral circulation as the genie is out of the bottle. What the current situation boils down to is this: is economic meltdown a price worth paying to halt or delay what is already amongst us?'

In reaction to the latest data from China, Jefferson goes even further. Noting that the data sample is very small, and thus there is room for some doubt, he tells the BMJ: 'And even if they are 10 per cent out, then this suggests the virus is everywhere. If — and I stress, if — the results are representative, then we have to ask, "What the hell are we locking down for?"'

In short, we are in lockdown because Jefferson's rivals at Imperial College have told the government that it is the only viable way to deal with Covid-19. It was their paper on Monday 16

March, claiming that 250,000 Britons would die if the government stuck to its ‘herd immunity’ strategy, that led to a sharp change in course and inexorably to full lockdown a week later. The Imperial team, led by the ubiquitous Professor Neil Ferguson, has the government’s ear, while the Oxford team is less involved in policy-making.

There is only one way to find out who is closest to the truth – Oxford or Imperial – and that is to test a randomised sample of the UK population for antibodies to see how many of us have had the virus. Matt Hancock said on the *Today* programme on Friday that Porton Down has the facilities to undertake 500 very high quality antibody tests a day – which is enough capacity to undertake a randomised study within a few days. It is a study we desperately need now.

The Open-Air Treatment of *PANDEMIC* INFLUENZA

Richard A. Hobday, PhD and John W. Cason, PhD

Abstract

The H1N1 "Spanish flu" outbreak of 1918–1919 was the most devastating pandemic on record, killing between 50 million and 100 million people. Should the next influenza pandemic prove equally virulent, there could be more than 300 million deaths globally. The conventional view is that little could have been done to prevent the H1N1 virus from spreading or to treat those infected; however, there is evidence to the contrary. Records from an "open-air" hospital in Boston, Massachusetts, suggest that some patients and staff were spared the worst of the outbreak. A combination of fresh air, sunlight, scrupulous standards of hygiene, and reusable face masks appears to have substantially reduced deaths among some patients and infections among medical staff. We argue that temporary hospitals should be a priority in emergency planning. Equally, other measures adopted during the 1918 pandemic merit more attention than they currently receive.

THREE INFLUENZA PANDEMICS occurred during the last century: in 1918, 1957, and 1968. Each was caused by a novel type A influenza virus of avian origin. The H1N1 influenza pandemic of 1918–1919 is notorious because of the infectivity of the virus and the number of lives it claimed. Although the fatality rate was relatively low, the incidence of infection was so great that the number of deaths was high. No other pandemic in history killed so many in such a short time.[1]

Global mortality from the pandemic is not known, because there are large areas of the world for which there is little information. In the 1920s, it was estimated that the disease had killed 21 million people. In 1991, this figure was revised to between 24.7 million and 39.3 million, and more-recent scholarship suggests 50 million to 100 million people may have died.[2] Morbidity was high, at anywhere from 25% to 90%, and the fatality rate was between 1% to 3%.[3] However, some regions reported mortality rates for the entire population as high as 5% to 10%.[2] Most deaths occurred between mid-September and mid-December of 1918.[4] Unusually, many of those who died were young adults, who normally have a low death rate from influenza. Another striking feature was the discoloration of the seriously ill, who often exhibited "heliotrope cyanosis," which is characterized by a blue-gray tinge to the face and other parts of the body.[3,5] Many victims died of pneumonia caused by secondary bacterial infections. Others succumbed to a condition similar to acute respiratory distress syndrome that could kill within days or hours.[5,6] Pleurisy, hemorrhage, edema, inflammation of the middle ear, meningitis, nephritis, and pericarditis were among the many complications reported.[6,7]

There were 3 waves of infection between 1918 and 1919. The first, in the spring of 1918, spread through parts of the United States, Europe, and Asia. This was a fairly mild form of influenza and caused relatively few fatalities. The second wave, which spread around the world in a few months, was disastrous. In less than a year, 220 000 influenza-related deaths occurred in Britain, and between September 1918 and June 1919 it proved fatal to at least half a million US citizens.[1,3] Death rates in Africa were comparable to or higher than those in North America and Europe.[8] Figures suggest that China was spared the worst of the pandemic, although this may simply reflect a lack of accurate records. The mortality in India alone has been estimated at 18 million.[9,10] According to one estimate of the period, 800 of every 1000 people who showed symptoms suffered from uncomplicated influenza. This was more severe than the so-called "three-day fever" of the spring of 1918, but no worse than ordinary influenza. The remaining 200 suffered pulmonary complications; of these, the mortality rate for those developing heliotrope cyanosis was 95%.[7]

With so many infected, and so many dying within a few weeks, the burden on medical staff and the funerary industry were immense, as was the accompanying economic and social disruption.[1,3] There was much debate about the origins of the illness and whether it was indeed influenza. The symptoms were so severe that there was speculation that it was some other disease such as

"trench fever," dengue, anthrax, cholera, or even plague.[1,3,11] Mortality reached alarming levels. The pandemic arrived in Boston, Massachusetts, early in September and by October 19 had claimed 4000 lives out of a total population of less than 800 000.[12] At the peak of the outbreak, more than 25% of patients at an emergency hospital in Philadelphia died each night, many without seeing a nurse or doctor. The bodies of those who succumbed were stored in the cellar of the building, from where they were tossed onto trucks and taken away. Attempts at therapy for those still alive were described as "exercises in futility."[13(p139)]

The demands of wartime meant that many doctors had been called into military service; those not in uniform were caring for the wounded in hospitals at home or inspecting potential recruits at medical boards. The shortage of nurses was even more acute: as they and other medical staff fell ill, patient care rapidly deteriorated.[1,3,14] Hospitals were turning patients away; mortuaries were overflowing, some handling 10 times their normal capacity. Gravediggers, many of whom were ill, could not keep up with the demand for burials.[1,3,15] Early in October 1918, a delegate from a health department in the US Midwest went east to find out how best to combat the infection. Officials there offered the following advice:

> *When you get back home, hunt up your wood-workers and cabinet-makers and set them to making coffins. Then take your street laborers and set them to digging graves. If you do this you will not have your dead accumulating faster than you can dispose of them.*[12(p787)]

This was not meant to cause undue alarm; it was merely a practical solution to a problem that had to be addressed once the pandemic arrived.[12] In an attempt to prevent the infection from spreading, many cities banned public assembly, closed their schools, isolated those infected, and mandated the wearing of surgical face masks.[1,3,6] Recent studies suggest that when such measures were introduced quickly—before the pandemic was fully established—and then sustained, death rates were reduced.[16–19] Yet for those who contracted the disease and went on to develop pneumonia, the prospects were poor. Anyone fortunate enough to gain admission to an "open-air" hospital, however, may have improved their chances of survival.

THE ORIGINS OF THE OPEN-AIR REGIMEN

By the time of the 1918–1919 pandemic, it was common practice to put the sick outside in tents or in specially designed open wards. Among the first advocates of what was later to become known as the "open-air method" was the English physician John Coakley Lettsom (1744–1815), who exposed children suffering from tuberculosis to sea air and sunshine at the Royal Sea Bathing Hospital in Kent, England, in 1791.[20,21] Lettsom's enthusiasm for fresh air attracted little support at the time, and the next doctor to recommend it met with fierce opposition. George Bodington (1799–1882) was the proprietor of the first institution that could be described as a tuberculosis sanatorium, at Sutton Coldfield near Birmingham, England. He treated pulmonary tuberculosis with a combination of fresh air, gentle exercise in the open, a nutritious, varied diet, and the minimum of medicines.

In 1840, Bodington published the results of his work in *An Essay on the Treatment and Cure of Pulmonary Consumption, On Principles Natural, Rational and Successful*.[22] Bodington's essay includes accounts of six cases; one patient died, as he acknowledged, but the others were either cured or greatly improved. This was at a time when, he estimated, one in five people in England were dying of the disease and little was being done to prevent it. Tuberculosis was generally regarded as hereditary, noninfectious, and incurable. Bodington argued otherwise, objecting strongly to the use of blistering, bleeding, and the popular purgative drugs of the day as well as the practice of confining patients in warm, badly ventilated rooms to protect them from the supposedly harmful effects of cold air, "thus forcing them to breathe over and over again the same foul air contaminated with the diseased effluvia of their own persons."[22(p2)]

Bodington had noticed that people who spent their time indoors were susceptible to tuberculosis, whereas those who worked outdoors, such as farmers, shepherds, and plowmen, were usually free of the disease. He reasoned that patients should copy the lifestyles of those who appeared immune to tuberculosis. They should live in well-ventilated houses in the country and spend much

of their time outside breathing fresh air. According to Bodington,

> *The application of cold pure air to the interior surface of the lungs is the most powerful sedative that can be applied, and does more to promote the healing of cavities and ulcers of the lungs than any other means that can be employed.*[22(p17)]

It is not known when Bodington started treating tuberculosis in this way, but there is evidence that he was doing so by 1833. By 1840, he had taken the tenancy of the "White House" at Maney, Sutton Coldfield, to provide suitable accommodation for his tubercular patients. Bodington's tenancy of this seminal building was brief—only three to four years. The *Lancet* published a sarcastic review of his essay and methods, and he abandoned the White House to devote himself to the care of the mentally ill.[23,24]

George Bodington had anticipated the principles of sanatorium treatment that were to become the main line of defense against the disease.[25] By the 1850s, Florence Nightingale (1820–1910) was writing about the importance of sunlight and copious amounts of fresh air in the recovery of hospital patients,[26,27] but her ideas were slow to gain acceptance. And so it was in Germany that the open-air regimen reemerged, most notably at the Nordrach-Kolonie in the Black Forest, a sanatorium established in 1888 by Otto Walter (1853–1919). It was so well known that "Nordrach" became the term for open-air sanatoria. By 1908, there were at least 90 of them in Britain, many of which were enthusiastic imitations of Nordrach.[28] An open-air recovery school for tubercular children, founded in 1904 at Charlottenburg, a suburb of Berlin, was the first of its type and, as with Germany's open-air sanatoria, was widely imitated.[29] In 1884, Edward Livingston Trudeau (1848–1915) opened America's first sanatorium at Saranac Lake in New York State.[30] The first open-air orthopedic hospital was set up in the Shropshire village of Baschurch in England in 1907.[31] In the two decades before World War I, charitable associations, leagues, and societies dedicated to preventing and eliminating tuberculosis among the poor flourished, as did sanatoria.[32]

THE OPEN-AIR TREATMENT OF THE WOUNDED

There is evidence that the open-air regimen may have improved the health of some tuberculosis patients. Records for the Dreadnought Hospital in Greenwich, one of the first British hospitals in which such methods were adopted, appear to show that there were benefits to this approach. From 1900 to 1905, the overall mortality of consumptive patients in open-air wards was less than half that of those who received the orthodox treatment of the day. An improvement in their state of "well-being" was also reported.[33] Later, during World War I, the use of open-air therapy extended to nontubercular conditions, and on a large scale. Temporary open-air hospitals were built to take casualties from the Western Front.

An early example stood on one of Cambridge University's best cricket pitches at the King's and Clare Athletic Ground. The First Eastern General Hospital, which was mobilized in August 1914, was originally designed to provide 520 beds and to be erected in 4 weeks. It proved so popular with the authorities, however, that within 8 weeks its complement of beds more than doubled to 1240. The hospital's wards were completely open to the south except for some low railings and adjustable sun blinds.[34,35]

In June 1915, the eminent scientist and Master of Christ's College, A. E. Shipley (1861–1927), judged the open-air treatment of sick and wounded soldiers at the First Eastern a success, particularly for those with pneumonia. Some 6600 patients had passed through the hospital, with a death rate of 4.6 per 1000. Sixty patients with pneumonia had been treated, and 95% of them recovered. Critics ascribed the low mortality at the hospital to the absence of "bad cases," but according to Shipley, some convoys arrived from the trenches almost entirely made up of them. In his opinion, the open wards produced much better results than closed ones. Instead of patients losing their bodily health and strength during the period of recovery from infections or wounds, they maintained their vigor and even improved it. The only people who felt the cold at the hospital were apparently the nurses, the patients having comfortable beds with plenty of blankets and hot-water bottles.[35] Nearer the front, the British Army put its casualties in tents. As the military surgeon Lieutenant Colonel Sir Berkeley Moynihan observed in 1916,

In the treatment of all gunshot wounds where the septic processes are raging, and the temperature varies through several degrees, an immense advantage will accrue from placing patients out of doors. While in France I developed a great affection for the tented hospitals. There is great movement of air, warmth and comfort; when a sunny day comes the side of the tent may be lifted and the patient enjoys the advantage of open-air treatment."[36(p337)]

INFLUENZA AT THE CAMP BROOKS OPEN-AIR HOSPITAL

When the influenza virus pandemic took hold in the United States in 1918, emergency hospitals were started in schools, halls, and large private houses, and open-air hospitals were being "thrown up" all over the country.[1] In the harbor of East Boston, 1200 out of 5100 merchant sailors onboard training ships had contracted influenza. The seriously ill were too numerous for local hospitals to accommodate. The Massachusetts State Guard responded by building the Camp Brooks Open Air Hospital at Corey Hill in Brookline, near Boston.[37,38] The hospital comprised 13 tents, 12 of which were occupied by one or two patients each and the other by the head nurse. The State Guard took seven hours to erect the tents, make sure the site was properly drained, and provide running water, latrines, and sewerage. Portable buildings were then set up for the medical staff and nurses. From the time the camp opened on September 9, 1918, until its closure a month later on October 12, a total of 351 victims of the pandemic were admitted, one third of whom were diagnosed with pneumonia. In total, 36 of the 351 sailors received at the hospital died.[37]

The treatment at Camp Brooks Hospital took place outdoors, with "a maximum of sunshine and of fresh air day and night."[37(p1747)] The medical officer in charge, Major Thomas F. Harrington, had studied the history of his patients and found that the worst cases of pneumonia came from the parts of ships that were most badly ventilated. In good weather, patients were taken out of their tents and put in the open. They were kept warm in their beds at night with hot-water bottles and extra blankets and were fed every few hours throughout the course of the fever. Anyone in contact with them had to wear an improvised facemask, which comprised five layers of gauze on a wire frame covering the nose and mouth. The frame was made out of an ordinary gravy strainer, shaped to fit the face of the wearer and to prevent the gauze filter from touching the nostrils or mouth. Nurses and orderlies were instructed to keep their hands away from the outside of the masks as much as possible. A superintendent made sure the masks were replaced every two hours, were properly sterilized, and contained fresh gauze.[38]

Other measures to prevent infection included the wearing of gloves and gowns, including a head covering. Doctors, nurses, and orderlies had to wash their hands in disinfectant after contact with patients and before eating. The use of common drinking cups, towels, and other items was strictly forbidden. Patients' dishes and utensils were kept separate and put in boiling water after each use. Pneumonia and meningitis patients used paper plates, drinking cups, and napkins; paper bags with gauze were pinned to pillowcases for sputum. Extensive use was made of mouthwash and gargle, and twice daily, the proprietary silver-based antimicrobial ointment Argyrol was applied to nasal mucous membranes to prevent ear infection.[37]

Of the camp's medical staff—15 doctors, 45 nurses and aids, 20 sanitary corps men, and 74 sailors acting as orderlies—only six nurses and two orderlies developed influenza. In five of these cases, exposure to the virus was reported to have taken place outside the camp. A few medicines were used to relieve the patients' symptoms and aid their recovery, but these were considered less important than were regular meals, warmth, and plenty of fresh air and sunlight.[37]

VENTILATION AND SUNLIGHT

The curative effects of fresh air were investigated at length by the physiologist Sir Leonard Hill (1866–1952) in the years following World War I. He reported favorably on the effects of sun and air when judiciously applied, particularly for tuberculosis.[39,40] In 1919, Hill wrote in the *British Medical Journal* that the best way to combat influenza infection was deep breathing of cool air and sleeping in the open.[41] Whether the patients at Camp Brooks or other temporary hospitals were spared the worst of the influenza

pandemic because they slept in the open is uncertain. The apparent success in reducing the number of infections and deaths reported at this open-air hospital may simply have been caused by patients and staff experiencing levels of natural ventilation far higher than in a conventional hospital ward. Significantly, the minimum amount of ventilation needed to prevent the spread of infectious diseases such as severe acute respiratory syndrome (SARS) and tuberculosis is unknown. Much more fresh air may be needed than is currently specified for hospitals, schools, offices, homes, and isolation rooms.[42–44]

The patients at Camp Brooks recovered in direct sunlight when available. This may have kept infection rates down, because laboratory experiments have shown that ultraviolet radiation inactivates influenza virus and other viral pathogens and that sunlight kills bacteria.[45–50] In addition, exposure to the sun's rays may have aided patients' recovery, because sunlight is known to promote healing in other conditions such as septic war wounds.[35] There is evidence that heart attack victims stand a better chance of recovery if they are in sunlit wards.[51] Depressed psychiatric patients fare better if they get some sun while hospitalized, as do premature babies with jaundice.[52–55] In one study, patients in hospital wards exposed to an increased intensity of sunlight experienced less perceived stress and less pain and took 22% less analgesic medication per hour.[56] One advantage of placing patients outside in the sun is that they can synthesize vitamin D in their skin, which they cannot do indoors behind glass. Rickets, the classic childhood disease of vitamin D deficiency, has long been associated with respiratory infections; it has been hypothesized that low levels of vitamin D may increase susceptibility to influenza.[57,58]

The surgeon general of the Massachusetts State Guard, William A. Brooks, had no doubt that open-air methods were effective at the hospital, despite much opposition to the therapy. Many doctors felt that patients would get the same benefits if the windows of a conventional ward were open or the patients were put in a hospital "sun parlor." Brooks, however, held that patients did not do as well in an ordinary hospital, no matter how well ventilated, as they did outdoors. Patients in indoor sun parlors were not exposed to direct sunlight all day as they were when outdoors. He reported that in one general hospital with 76 cases, 20 patients died within three days and 17 nurses fell ill.[38] By contrast, according to one estimate, the regimen adopted at the camp reduced the fatality of hospital cases from 40% to about 13%.[12] Brooks wrote that "The efficacy of open air treatment has been absolutely proven, and one has only to try it to discover its value."[38(p750)]

Coincidentally, in 1918 a British soldier, Patrick Collins, reached a similar conclusion. When Collins developed the first signs of influenza, he dragged himself and his tent up a hill away from his regiment. There he sweated, shivered, and was delirious for several days, sustained only by his rum ration. He was one of the few survivors of his regiment.[59]

DISCUSSION

The seeming success of the medical team who confronted pandemic influenza on Corey Hill in 1918 was in stark contrast to others' experience of the infection. The high standard of personal and environmental hygiene upheld by staff at the camp may have played a large part in the relatively low rates of infection and mortality there compared with other hospitals. Significantly, the outbreak of SARS in Hong Kong in 2003 showed that basic infection controls, such as those employed at Camp Brooks Hospital, can help to contain the spread of a virulent respiratory infection.[60,61]

Of the measures introduced to combat pandemic influenza at the hospital, the use of improvised facemasks—including their design and the frequency with which they were changed—is noteworthy. Another is the fresh air the patients enjoyed. When Major Harrington, the medical officer at Camp Brooks, discovered that sailors from the most poorly ventilated areas of the ships in East Boston also had the worst cases of pneumonia, he put his patients outdoors. Sailors, such as those on board the ships at East Boston, were particularly vulnerable to influenza infection, because the influenza virus is readily transmitted in confined quarters. In 1977, for example, an influenza outbreak on board a commercial airliner with deficient ventilation resulted in an infection rate of 72%. The aircraft was grounded for over four hours with the passengers on board and the ventilation system turned off.[62]

There is still much uncertainty surrounding the transmission and epidemiology of influenza. As yet, the proportion of influenza infections that occur by the airborne route is not known,[63] nor is there any evidence to support the idea that fresh air helps those infected to recover. Given the threat to public health posed by the avian influenza virus, both merit further study. So too does the part played by sunlight in preventing the spread of the virus. Solar radiation may retard its transmission by directly inactivating virions and by increasing immunity to them. A combination of outdoor air and sunlight could also reduce the likelihood of secondary respiratory infections.

The current H5N1 avian influenza virus has high virulence and lethality but as yet is not readily transmitted from person to person.[64] We do not know how virulent the next type A pandemic will be, but should it prove to be as pathogenic as that of 1918, there could be 180 million to 360 million deaths globally.[65] Vaccines, antiviral drugs, and antibiotics may be effective in controlling avian influenza and dealing with secondary infection; however, for much of the world's population, access to them will be limited. In many countries, the only viable strategy would be to disrupt the transmission of the virus by banning public gatherings, closing schools, isolating infected people, and wearing surgical masks, as was the case during the 1918–1919 pandemic.[66,67]

Epidemiological studies show that the wearing of masks in public places in Hong Kong and Beijing during the SARS outbreak was associated with a lower incidence of infection.[68,69] However, no controlled studies have been undertaken to assess the effectiveness of surgical masks in preventing influenza from passing from one host to the next.[70] In addition, it is uncertain whether transmission of the influenza virus from person to person is chiefly by large droplets or aerosols. If droplets are the main mode of transmission, the isolation of patients in private rooms and the use of ordinary surgical face masks may suffice.[63] If airborne transmission is significant, reusable respirators could be pivotal in preventing infection, because surgical masks do not offer reliable protection from aerosols.[71,72] Also, measures that prevent the influenza virus from spreading through buildings would assume greater importance. Improvements in air-handling equipment, portable filtration units, and the introduction of physical barriers in the form of partitions or doors may offer some protection.[73]

However, more might be gained by introducing high levels of natural ventilation or, indeed, by encouraging the public to spend as much time outdoors as possible. It might also be prudent to stockpile tents and beds, because hospitals in the United Kingdom, the United States, and elsewhere are not prepared for a severe pandemic.[74–80] Temporary accommodation would be required to deal with the most seriously ill, just as it was in 1918. The Camp Brooks Open Air Hospital might serve as a useful model.

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Contributors

R. A. Hobday originated the study and led the writing. J. W. Cason assisted with the study and analyses. Both authors conceptualized ideas, interpreted findings, and reviewed drafts of the article.

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Ventricular Arrhythmia Risk Due to Hydroxychloroquine-Azithromycin Treatment For COVID-19

Mar 29, 2020

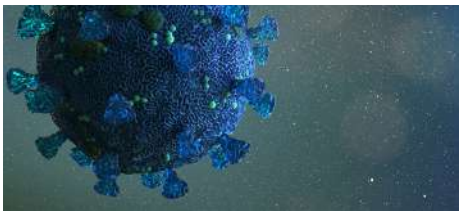
Cardiology Magazine

KEY POINTS

- ✓ Safety considerations for **inpatient** and **outpatient** use of hydroxychloroquine and chloroquine in clinical practice are outlined below.
- ✓ Hydroxychloroquine or chloroquine therapy should occur in the context of a clinical trial or registry, until sufficient evidence is available for use in clinical practice.
- ✓ Hydroxychloroquine or chloroquine use outside of a clinical trial should occur at the direction of an infectious disease or COVID-19 expert, with cardiology input regarding QT monitoring.
- ✓ **Additional sources of expert guidance** with detailed and general arrhythmia monitoring considerations are also available.
- ✓ The intensity of QT and arrhythmia monitoring should be considered in the context of potential drug benefit, drug safety, resource availability and quarantine considerations.
- ✓ IRB-approved protocols should guide use of hydroxychloroquine or chloroquine for pandemic research; suggestions for researchers are outlined **here**.

In vitro and preliminary clinical research have suggested that hydroxychloroquine alone and in

combination with azithromycin could prove to be an effective treatment for COVID-19.



A small study in France enrolling 26 treated patients and 16 non-randomized controls showed that hydroxychloroquine alone or in combination with azithromycin shortened the time to resolution of viral shedding of COVID-19.[1]

Based on this study, clinicians in many countries have begun using these medications in clinical practice, and multiple randomized trials are being initiated. However, chloroquine, hydroxychloroquine and azithromycin all prolong QT interval, raising concerns about the risk of arrhythmic death from individual or concurrent use of these medications.

Both the concerns regarding mortality risk, and the intensity of QT and arrhythmia monitoring should be considered in the context of several important mitigating factors:

1. The duration of use for these medications for COVID-19 infection is short (5 to 10 days for acute illness).
2. While QT-prolonging medication use has been associated with increased risk of death, this risk may be smaller than the potential benefit from treatment of COVID-19 for some patients.
3. There are large potential population-health benefits from hastening viral clearance of COVID-19.

We strongly encourage enrollment of patients in clinical research protocols, whenever available. All clinical use that occurs outside of a research setting should incorporate anticipated benefits balanced against risks.

Currently, there is hope for benefit from hydroxychloroquine, yet there is little evidence. That is likely to rapidly change, given many pending clinical studies.

Arrhythmogenicity of Hydroxychloroquine and Azithromycin

Drug-induced QT prolongation has long served as a surrogate indicator for increased risk of drug-associated torsades de pointes (TdP), a potentially lethal polymorphic ventricular tachycardia. However, the relationship between QT prolongation and risk

of TdP is imperfect and complex. The risk of TdP is not a linear function of QT duration nor the extent of change; some drugs which prolong QTc are not associated with increased arrhythmic death.[2,3]

Although only a small proportion of patients with QTc prolongation suffer TdP, drug-associated QT prolongation is associated with increased arrhythmic and non-arrhythmic mortality and it therefore continues to be an important metric of drug safety.[4,5]

Chloroquine, and its more contemporary derivative hydroxychloroquine, have remained in clinical use for more than a half-century as an effective therapy for treatment of some malarias, lupus, and rheumatoid arthritis. Data show inhibition of iKr and resultant mild QT prolongation associated with both agents.

Despite these suggestive findings, several hundred million courses of chloroquine have been used worldwide making it one of the most widely used drugs in history, without reports of arrhythmic death under World Health Organization surveillance.[4]

Nonetheless, the absence of an active drug safety surveillance system in most countries limits reassurance from these observations.

Azithromycin, a frequently used macrolide antibiotics lacks strong pharmacodynamic evidence of iKr inhibition. Epidemiologic studies have estimated an excess of 47 cardiovascular deaths which are presumed arrhythmic per 1 million completed courses, although recent studies suggest this may be overestimated.[6-7]

There is limited data evaluating the safety of combination therapy, however in vivo studies have shown no synergistic arrhythmic effects of azithromycin with or without chloroquine.[8]

A number of factors are known to contribute to increased risk of drug-induced TdP including female sex, structural heart disease, congenital long-QT syndromes, electrolyte disturbances, hepatic/renal failure and concomitant QT prolonging medications.[6]

The safety of QT prolonging medications may be maximized by close monitoring and optimization of these factors. A risk score has been derived and validated by Tisdale et al., for prediction of drug-associated QT prolongation among cardiac-care-unit-hospitalized patients (Table 1).[9]

Table 1. Risk Score For Drug-Associated QTc Prolongation[9]

Risk Factors	Points
Age ≥68 y	1
Female sex	1
Loop diuretic	1
Serum K+ ≤3.5 mEq/L	2
Admission QTc ≥450 ms	2
Acute MI	2
≥2 QTc-prolonging drugs	3
sepsis	3
Heart failure	3
One QTc-prolonging drug	3
Maximum Risk Score	21
K+ indicates potassium; and MI, myocardial infarction.	

A Tisdale score of ≤ 6 predicts low risk, 7-10 medium risk, and ≥ 11 high risk of drug-associated QT prolongation (Table 2).

Table 2. Risk Levels For Drug-Associated QT Prolongation[9]

Low risk = ≤6 points
Moderate risk = 7-10 points
High-risk = ≥11 points

Suggested Monitoring For Inpatient Clinical Use

Patients admitted with COVID-19 are likely to have longer baseline QTc and have higher potential arrhythmic risks as a result of the metabolic and

Table 3. QTc Formulas; Consider

physiologic sequelae of their illness, and a typically greater burden of comorbid disease.

However, given the severity of illness, hospitalized and critically ill patients may also derive the most benefit from potentially effective therapies.

The goal of QTc screening in this setting is not to identify patients whom are not candidates for therapy, but to identify those who are at increased risk for TdP so aggressive countermeasures may be implemented.

If otherwise ready for discharge, patients who have had QT intervals that are well within normal range and have had no concerning arrhythmias on telemetry should not be held in the hospital exclusively for the purpose of hydroxychloroquine-related arrhythmia monitoring.

Using Fridericia or Framingham Correction, Especially for Heart Rates Over 90 BPM[10]

Fridericia	$QTc = QT / \sqrt[2]{RR}$
Framingham	$QTc = QT + 0.154(1-RR)$
Hodges	$QTc = QT + 1.75(HR-60)$
Bazett	$QTc = QT / \sqrt[3]{RR}$

1. Baseline
    a. Discontinue and avoid all other non-critical QT prolonging agents.
    b. Assess a baseline ECG, renal function, hepatic function, serum potassium and serum magnesium.
    c. When possible, have an experienced cardiologist/electrophysiologist measure QTc, and seek pharmacist input in the setting of acute renal or hepatic failure.
2. Relative contraindications (subject to modification based on potential benefits of therapy)
    a. History of long QT syndrome, or
    b. Baseline QTc >500 msec (or >530-550 msec in patients with QRS greater than >120 msec)
3. Ongoing monitoring, dose adjustment and drug discontinuation
    a. Place on telemetry prior to start of therapy.
    b. Monitor and optimize serum potassium daily.
    c. Acquire an ECG 2-3 hours after the second dose of hydroxychloroquine, and daily thereafter.

d. If QTc increases by >60 msec or absolute QTc >500msec (or >530-550 msec if QRS >120 msec), discontinue azithromycin (if used) and/or reduce dose of hydroxychloroquine and repeat ECG daily.

e. If QTc remains increased >60 msec and/or absolute QTc >500 msec (or >530-550 msec if QRS >120 msec), reevaluate the risk/benefit of ongoing therapy, consider consultation with an electrophysiologist, and consider discontinuation of hydroxychloroquine.

Suggested Monitoring For Outpatient Clinical Use

Patients who are stable for outpatient therapy may be less at risk for complications, but are unlikely to have access to close monitoring.

As for inpatients, QTc screening should be incorporated into an individualized risk-benefit consideration for treatment.

If outpatient ECG assessment is impossible or poses undue risk of infection for others, the necessity of treatment should be balanced against risk when considering alternative monitoring methods or omitting monitoring.

1. Baseline
    a. Discontinue and avoid all other non-critical QT prolonging agents.
    b. Assess a baseline ECG, renal function, hepatic function, serum potassium and serum magnesium.
    c. When possible, have an experienced cardiologist/electrophysiologist measure QTc.
    d. Avoid outpatient initiation in the setting of acute renal or hepatic failure.
2. Relative contraindications (subject to modification based on potential benefits of therapy)
    a. History of long QT syndrome, or
    b. Baseline QTc >480 msec (or >510-530 msec if QRS >120 msec), or
    c. Tisdale risk score ≥11
3. Ongoing monitoring, dose adjustment and drug discontinuation

a. If quarantine or resource constraints are prohibitive, consider no further ECG / telemetry assessment if Tisdale risk score ≤6. Also consider use of alternative mechanisms of QT and arrhythmia assessment outlined below.

b. Otherwise, repeat ECG 2-3 hours after dosing on day 3 of therapy. If QTc increases by >30-60 msec or absolute QTc >500msec (or >530-550 msec if QRS >120 msec), consider discontinuing therapy.

Protocol Modifications in the Setting of Limited Resources or Quarantines

QT-prolonging medication initiation may be considered in the absence of ECG, telemetry or in-office assessment capability for patients with Tisdale risk score ≤6, in the setting of resource scarcity.

Additional considerations may include:

1. **Personal protective equipment (PPE) shortages:** To minimize use of PPE, ECGs may be performed to coincide with "clustered" care between 2 and 4 hours after dosing. To further reduce exposure or save PPE resources, QTc monitoring may be performed using surrogates for 12-lead ECG assessment, including QTc monitoring via inpatient telemetry, direct-to-consumer mobile devices (e.g., KardiaMobile 6-lead, KardiaMobile 1-lead and Apple Watch 1-lead), or prescription mobile cardiac outpatient telemetry devices (e.g., iRhythm, BioTel and Preventice).
2. **Telemetry shortages:** If telemetry resources are limited, their use must be triaged based on clinical importance. Local protocols should be created to weigh the arrhythmia risks across the spectrum of hospitalized patients. Patients already on therapy with QTc values in the clearly acceptable range could be considered for ongoing hydroxychloroquine use without telemetry. Patients initiating therapy with Tisdale risk score ≤6 can similarly be considered for use without monitoring. For higher risk patients who would otherwise not have access to inpatient telemetry, mobile cardiac outpatient telemetry could be considered for use in the hospital. In this telemetry-triage context, any syncope should be considered due to polymorphic VT and should prompt ECG and reinitiation of telemetry.
3. **Minimizing exposure/contact:** It may be reasonable to forego ECG screening to allow patients to remain in quarantine if no high-risk features exist (history of long QT syndrome, concomitant QT-prolonging medications, structural or

ischemic heart disease, history of prolonged QTc on any ECG, history of abnormal renal function and/or electrolytes).

4. **Maximizing telephone assessment:** All patients/ research subjects should have close monitoring of symptoms with attention to indicators of arrhythmia risk (syncope, dehydration, initiation of new medications and worsening of health status).

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This article is authored by **Timothy F. Simpson, MD, PharmD**; **Richard J. Kovacs, MD, FACC**; and **Eric C. Stecker, MD, MPH, FACC**.

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Clinical Topics: **Arrhythmias and Clinical EP, Congenital Heart Disease and Pediatric Cardiology, Implantable Devices, Genetic Arrhythmic Conditions, SCD/Ventricular Arrhythmias, Atrial Fibrillation/Supraventricular Arrhythmias, Congenital Heart Disease, CHD and Pediatrics and Arrhythmias, CHD and Pediatrics and Quality Improvement, Novel Agents, Statins**

Keywords: *Cardiology Magazine, ACC Publications, Coronavirus, Acute Disease, Anti-Bacterial Agents, Arrhythmias, Cardiac, Azithromycin, Chloroquine, Clinical Protocols, Communicable Diseases, COVID-19, Dehydration, Electrocardiography, Ethics Committees, Research, Hydroxychloroquine, Inpatients, Long QT Syndrome, Magnesium, Malaria, Outpatients, Quarantine, Registries, Potassium, Severe Acute Respiratory Syndrome, Sodium Chloride Symporter Inhibitors, Torsades de Pointes, Virus Shedding*

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Spectrum of drugs prolonging QT interval and the incidence of torsades de pointes

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The incidence of drug-induced proarrhythmias in the general population is largely unknown. Knowledge regarding incidence and risk factors is mainly derived from studies during clinical development of drugs and is therefore limited to antiarrhythmic compounds with a relatively high incidence. For non-cardiovascular drugs, proarrhythmias are rarely seen during clinical development but usually appear later, several years after registration. Both spontaneous adverse reaction reports and epidemiological studies have severe limitations when used to estimate the incidence of proarrhythmias with non-cardiovascular compounds. QT prolongation and torsades de pointes have been associated with non-sedating antihistamines, antibiotics, antipsychotics, antidepressants and a gastrointestinal prokinetic agent; drugs within these classes constitute the vast majority of non-cardiovascular compounds associated with this potentially serious side-effect. Epidemiological studies on non-sedating antihistamines and on cisapride have largely failed to demonstrate an increased risk for sudden death or ventricular arrhythmias, which is most likely due to the low specificity of the end-points studied. A careful case ascertainment, which requires access to electrocardiograms and clinical records, and prospectively defined, strict definitions for the classification of proarrhythmias, is of great importance in these studies.

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Key Words: Torsades de pointes, QT prolongation, non-cardiovascular drugs, epidemiology.

Introduction

Torsades de pointes (TdP) may be caused by a large number of different drugs and is a well-known side-effect of all antiarrhythmic drugs that prolong cardiac repolarization. In addition, a large number of non-cardiovascular drugs used for a variety of non-related diseases have been associated with or suspected to cause TdP. Examples include drugs used in the treatment of urinary incontinence (terodiline, now withdrawn), antihistamines (terfenadine, now largely withdrawn, and astemizole), antimicrobials (erythromycin), gastric prokinetic (cisapride), antipsychotics and antidepressants. The aim of this presentation is to discuss different classes of drugs that have been associated with QT prolongation or TdP, the incidence of events and certain characteristics of commonly used databases in studies of drug-induced proarrhythmias.

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Incidence of torsade de pointes

The incidence of drug-induced TdP in the general population is largely unknown. Our knowledge of the incidence and risk factors is mainly based on clinical studies during drug development, on epidemiological data and on post-marketing surveillance through spontaneous adverse drug reactions (ADR) reports. The considerable attention paid to drug-induced TdP during the last few years has resulted in an increased number of spontaneous reports, although the absolute total number is still very low (Table 1). The degree of under-reporting of ADRs varies widely and is particularly high when physicians and pharmacists regard the adverse reaction as 'expected' in relation to the underlying disease of the patient[1]. Examples of under-reporting may include an increased incidence of bleeding in patients on anticoagulation therapy, and sudden death or ventricular arrhythmias in patients with coronary artery disease. An episode of polymorphic ventricular tachycardia in a previously healthy female patient on cisapride and erythromycin therapy is likely to be reported as an adverse drug reaction. Such a report will most probably not be made for a male patient with

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Table 1 Annual number of ADR reports submitted to the WHO Drug Monitoring Centre, 1983 to 1999

Year	Reports of TdP
1983	1
1986	1
1987	5
1988	1
1989	3
1990	7
1991	24
1992	19
1993	28
1994	102
1995	162
1996	166
1997	121
1998	62
1999	59

Table 2 The 20 drugs most commonly reported in association with TdP between 1983 and 1999 (based on ADR reports to WHO)

Drug name	TdP (n)	Fatal (n)	Total (n)	TdP/total (%)
Sotalol	130	1	2758	4·71
Cisapride	97	6	6489	1·49
Amiodarone	47	1	13 725	0·34
Erythromycin	44	2	24 776	0·18
Ibutilide	43	1	173	24·86
Terfenadine	41	1	10 047	0·41
Quinidine	33	2	7353	0·45
Clarithromycin	33	0	17 448	0·19
Haloperidol	21	6	15 431	0·14
Fluoxetine	20	1	70 929	0·03
Digoxin	19	0	18 925	0·10
Procainamide	19	0	5867	0·32
Terodiline	19	0	2248	0·85
Fluconazole	17	0	5613	0·30
Disopyramide	16	1	3378	0·47
Bepridil	15	0	384	3·91
Furosemide	15	0	15 119	0·10
Thioridazine	12	0	6565	0·18
Flecainide	11	2	3747	0·29
Loratidine	11	1	5452	0·20

Abbreviations: TdP (n): total number of ADR reports named TdP for this drug. Fatal (n): number of ADR reports named TdP with a fatal outcome. Total (n): total number of ADR reports for this drug.

severe congestive heart failure and previous episodes of monomorphic ventricular tachycardia.

Between 1983 and December, 1999, 761 cases of TdP, of which 34 were fatal, were reported to the WHO Drug Monitoring Centre. ADR reports are sent to this centre from the member states, but the content and clinical information vary widely between different countries and sources. These reports merely represent a suspicion of association between a drug and an adverse event. The likelihood for a specific ADR to be reported is influenced by various factors, such as the patient's underlying disease, whether the ADR is well known or not previously described and, evidently, how much attention is focused on a specific ADR within the medical community. The 20 most commonly reported compounds are shown in Table 2.

As an initial effort to explore the incidence of drug-associated proarrhythmias, the Swedish Medical Products Agency conducted a one-month pilot study in 1999 (DRAMA pilot study, personal communication from Professor B. Beerman, Swedish Medical Products Agency, and B. Wiholm, MD, PhD, December, 1999). The study involved 32 hospitals with a total reference population of approximately 4·2 million inhabitants. The studied end-points were any episode of ventricular fibrillation or polymorphic tachycardia, whether associated with a prolonged QT interval or not, and episodes of monomorphic ventricular tachycardias lasting more than nine beats. Ventricular tachyarrythmias associated with ischaemic events (acute myocardial infarction or unstable angina pectoris) were excluded. The study period was 28 days and all episodes encountered during admission to hospital, or tracked by Holter recordings at the hospitals during this time period, were reported. All collected episodes were evaluated by three cardiologists and were classified according to prospectively defined and previously described algorithm[2]. A total of 68 episodes of ventricular arrhythmias were collected and of these 14 were regarded as 'medium- or high-confidence TdP' by the expert group. This corresponded to an incidence in this population of 3·3 cases per million for the 28 days, which equalled an annual incidence of 4/100 000. Although it is difficult to base any firm conclusions on 14 patients with TdP, it may be worth pointing out that eight of the 14 (57%) were women and 64% of the patients had either a previous history of ventricular arrhythmias or structural heart disease (Table 3). These observations are consistent with previously reported risk factors for drug-induced TdP[3]. Three of the torsade patients were not on any medication. The most common drugs prescribed to the remaining patients (n=11, Table 4) were sotalol and diuretics, which is noteworthy, since only one patient was hypokalaemic at admission.

The annual incidence of TdP in this study, 4/100 000, is strikingly high and in sharp contrast to the spontaneous ADR reporting. If it was assumed that the arrhythmia is drug-induced in only one-third of these cases, an annual incidence in Sweden of more than 100 cases (among 9 million inhabitants) would result. In contrast, the total number of ADR reports with TdP to the Swedish Medical Products Agency during 1991 to 1999 was 62, i.e. less than eight per year. These numbers thus confirm the opinion that post-marketing surveillance via spontaneous reports under-reports the true incidence of serious adverse reactions by a factor of at least 10[4].

Table 3 Clinical characteristics for patients with ventricular tachyarrhythmias in the Swedish DRAMA pilot study

Characteristic	Torsades de pointes (n=14)		Other ventricular tachycardia (n=54)	
	Number	%	Number	%
Sex				
Female	8	57	15	34
Male	6	43	39	66
Age, years (mean)	68		61	
Previous TdP	1	7	1	7
Medical history				
Other VT	2	14	18	35
IHD	4	28	21	39
CHF	6	43	18	33
IHD ± CHF	8	57	29	54
Any heart disease	9	64	35	65
Laboratory findings				
Creatinine >120 mmol . l^{-1}	5	35	12 (n=44)	27
S-K <3·5 mmol . l^{-1}	2	14	5 (n=44)	11

Drugs that may cause QT prolongation or torsade de pointes

In all, 225 pharmaceutical compounds have been associated with torsade de pointes in spontaneous ADR reports collected by the WHO Drugs Monitoring Centre. Of the 20 most commonly reported drugs, 10 were cardiovascular agents and these appeared in 348 of the reports (46%). This presentation will mainly deal with non-cardiovascular drugs (Table 5). The information on drug-associated TdP is constantly growing in line with the increasing awareness and concern, and the reader is asked to use the Internet for updates. Professor R. Woosley, Department of Pharmacology at Georgetown University Medical School in Washington, DC, USA, provides information from the FDA-approved drug labelling and from the medical literature (available at http://www.dml.georgetown.edu/depts/pharmacology/torsades.html). The Sudden Arrhythmia Death Syndromes Foundation (webside http://www.sads.org/) provides updated information on which drugs should be avoided by patients with congenital long QT syndrome or who have previously experienced TdP.

Table 4 Concomitant medication in 14 patients with TdP in the Swedish DRAMA pilot study

Drug	Number on drug
None	3
Diuretics	9
Thrombocyte inhibitor	7
Sotalol	6
Digitalis	4
ACE inhibitors	3
Antidepressants	2
Calcium antagonists	1
Beta-blockers (other than sotalol)	1
Antibiotics	1
Cisapride	1

Torsades de pointes, n=14.

Antiarrhythmic drugs

Class I antiarrhythmic drugs

All class I antiarrhythmic drugs have the potential to cause life-threatening ventricular proarrhythmias. The use of these agents is decreasing because of safety concerns[5–9]. It has been estimated that 1–8% of patients treated with quinidine will develop TdP[10–12]. The proarrhythmia is frequently 'idiosyncratic', occurring after low doses and at low plasma concentrations[3,13]. Disopyramide and procainamide have also been associated with TdP[13]. Furthermore, the Cardiac Arrhythmia Suppression Trial (CAST) demonstrated an increased mortality from class IC drugs in patients with frequent premature ventricular contractions and previous myocardial infarction, and their use should therefore be restricted to patients with structurally normal hearts and preserved left ventricular function[8,14]. The mechanism underlying the results of the CAST study is not fully elucidated, but a likely explanation seems to be one of fatal proarrhythmia in susceptible individuals[15]. In a meta-analysis of quinidine treatment in patients with atrial fibrillation, a threefold mortality was found compared to placebo or no treatment[6]. In the Stroke Prevention in Atrial Fibrillation study, a 2·5-fold increase in mortality was reported in patients with a history of congestive heart failure and who were treated with antiarrhythmic drugs, mainly quinidine and procainamide[16].

Table 5 Drugs which may cause QT prolongation or have been associated with torsades de pointes

- Cardiovascular compounds
  - Antiarrhythmic drugs
    - Class I
    - Class III
  - Calcium antagonists
    - Bepridil
    - Terodilin (withdrawn)
    - Mibefradil (withdrawn)
- Non-cardiovascular compounds
  - Antihistamines
    - Terfenadine
    - Astemizole
  - Antibiotics
    - Macrolide
      - Erythromycin
      - Clarithromycin
    - Quinolone
      - Sparfloxacin
      - Levofloxacin
      - Grepafloxacin (withdrawn)
      - Moxifloxacin
    - Antimalarials
      - Quinine
      - Halofantrine
      - Pentamidine
    - Imidazole antifungals
      - Ketoconazole
    - Other
      - Trimethoprim-sulfamethoxazole
  - Antipsychotic and antidepressant agents
    - Neuroleptic
      - Thioridazine
      - Chlorpromazine
      - Haloperidol
      - Droperidol
      - Pimozide
    - Antidepressants
      - Amitriptyline
      - Desipramine
      - Imipramine
      - Maprotiline
      - Doxepin
      - Fluoxetin
    - Atypical antipsychotics
      - Sertindole
      - Risperidone
      - Clozapine
      - Zimeldine
      - Citalopram
    - Miscellaneous
      - Cisapride
      - Tamoxifen
      - Tacrolimus
      - Sevoflurane
      - Isoflurane
      - Probucol
  - Antimigraine drugs
    - Sumatriptan
    - Naratriptan
    - Zolmitriptan

This list is not meant to be complete, and the reader is asked to update the information on a continuous basis, by using, e.g., the websites referred to in the text. The association between these drugs and QT interval prolongation or TdP is not always clear, is often based on case reports and the methods for heart rate correction of the QT interval may in some instances be criticized.

Class III antiarrhythmic drugs

Both d,l-sotalol and amiodarone have substantial side-effects in addition to their electrophysiological effects. This, and safety concerns surrounding class I compounds, were the rationale for substantial research efforts during the 1980s to develop drugs with 'pure' class III properties. Most of these newer agents, e.g. dofetilide, d-sotalol and sematilide, are powerful I_{Kr} blockers, but other mechanisms may also contribute to the antiarrhythmic effects (as may be the case with, for example, ibutilide and azimilide). For amiodarone the incidence of TdP is very low[17]. In seven clinical trials, with a total of 882 patients, no proarrhythmia occurred during treatment with intravenous amiodarone for conversion of atrial arrhythmias to sinus rhythm[18–24]. For d,1-sotalol the incidence of torsade is about 2% (three trials, 462 patients)[25–28]. For dofetilide (six trials, 567 patients)[29–34], ibutilide (six trials, 1468 patients)[35–39] and almokalant (two studies, 180 patients)[40,41], the incidence varies between 1% and 8% in the different clinical trials.

Calcium antagonists

One of the first non-cardiovascular drugs associated with TdP was terodiline, used for treatment of urinary incontinence. This drug is a calcium antagonist and was launched as such during the 1960s. Due to its anticholinergic effects, terodiline was eventually used for treatment of urinary incontinence, but was withdrawn because of association with TdP[42,43]. Bepridil is a calcium antagonist which in some countries is labelled for use only in patients who are refractory to other antianginal drugs. The drug prolongs the QT interval and several cases of TdP have been described[44,45]. Although several alternative calcium antagonists without proarrhythmic effects are available, bepridil is allowed, since it may be beneficial in selected patients with severe drug-refractory angina. Mibefradil, a T channel blocker, was withdrawn after only one year on the market, largely due to numerous drug-to-drug interactions, since it inhibited both CYP3A4 and 2D6[46] isozymes. Mibefradil also gave rise to QT prolongation and marked T wave morphological changes that resembled those seen with selective class III antiarrhythmics, and this caused a considerable debate as to whether the drug had proarrhythmic potentials. There were several reports of TdP in patients on mibefradil during its short time on the market, but it is not fully clear whether it was a proarrhythmic propensity of the drug or pharmacokinetic interactions with other drugs that prolonged the QT interval. In either case, it is still noteworthy that the combination of mibefradil and class I and III antiarrhythmics was particularly harmful in a large trial on 2590 patients with congestive heart failure[47].

Table 6 End-points in four epidemiological studies of antihistamines

Pratt *et al.*[53]	Cardiac arrest, sudden death, paroxysmal ventricular tachycardia, ventricular fibrillation and flutter (torsades de pointes not separately coded).
Hanrahan *et al.*[54]	Sudden death, torsades de pointes, other ventricular arrhythmia, syncope, ventricular ectopy (graded according to severity).
Lindquist and Edwards[57]	Arrhythmia, ventricular arrhythmia, cardiac arrest, ventricular fibrillation, QT prolongation, supraventricular tachycardia, ventricular tachycardia, torsades de pointes, sudden deaths, deaths related to rhythm disorders.
Staffa *et al.*[55]	Paroxysmal ventricular tachycardia, ventricular fibrillation, ventricular flutter, sudden death (cardiac arrest).

Antihistamines

Antihistamines have received considerable attention since the early 1990s, when terfenadine and astemizole were associated with proarrhythmias[48–52]. The first 25 reported cases with terfenadine-associated TdP indicated that the parent substance, but not its main metabolite, was the problem[52], and the importance of pharmacokinetic interaction with ketaconazole was identified[51]. Since then, several quite large epidemiological studies have been performed in an effort to assess the cardiac safety profile of antihistamines[53–55]. Pharmaco-epidemiological studies are often performed using large databases that include information on medical diagnoses and prescriptions in a specified population. Even though ADR reports often may be useful for initial drug surveillance, the exposed population is often insufficiently known, and the random nature of the reports make their value limited as a measure of the true incidence. For ADRs with a very low incidence, population-based studies using large databases are often the only feasible approach. In some of these studies of antihistamines, risk factors for proarrhythmias could be identified, but the studies largely failed to establish an increased risk. This 'negative' outcome may be explained by factors associated with the primary end-points, the weakness of the 'signal' in relation to background noise and the lack of source data, such as ECG registrations. The primary end-points in the cited studies are shown in Table 6. Common to all studies was that events with a low specificity for drug-associated TdP (such as ventricular arrhythmia without further specifications, sudden death, cardiac arrest, syncope) were pooled with more specific diagnoses (such as prolonged QT interval and TdP). Despite this, the absolute number of events was low (ranging from 53 to 317). The risk for ventricular arrhythmias was lower or identical for terfenadine compared to other antihistamines or ibuprofen[53,54], and there was no difference in the risk for astemizole compared to sedating antihistamines[55]. A markedly increased risk with concomitant use of terfenadine and ketokonazole was, however, identified[53]. In a recently published cohort study with a nested case-control analysis using the U.K.-based General Practice Research Database, 18 cases of validated 'idiopathic' (no alternative cause in the clinical records) ventricular arrhythmias were identified[56]. Using this approach, current use of any antihistamine (astemizole, terfenadine, loratadine, cetirizin and acrivastine) carried a marginally increased risk for ventricular arrhythmias [odds ratio(OR): 1·9; 95% confidence level(CI): 1·0 to 3·6], whereas recent astemizole use carried a markedly increased risk (OR 19·0; (95% CI) 4·8 to 76·0). The risk with terfenadine use was within the range of other antihistamines. The number of cardiac ADR reports, related to the sales, on the same five antihistamines has also been reported[57]. The frequency of selected cardiac events (Table 6) and deaths were all below 0·1 per million defined daily doses sold. In this survey, loratadine, astemizole and terfenadine carried a similar risk for cardiac events, but it should be emphasized that the specificity of the end-points must be regarded as low for correct identification of drug-induced proarrhythmias.

The limitations of epidemiological studies — the cisapride example

The limitations of epidemiological studies using databases without adequate validation of the studied end-points are well illustrated by cisapride, a drug used for treatment of gastro-oesophagel reflux. In July, 1996, the FDA issued a report on 34 patients who had developed proarrhythmias and 23 patients with prolonged QT intervals during medication with cisapride[4]. Four patients died and another 16 survived resuscitation. Fifty-six per-cent of the patients were on concomitant treatment with other drugs that affected the metabolism of cisapride through inhibition of the hepatic CYP3A4 isozyme, namely macrolide antibiotics (e.g. erythromycin) or antifungals (e.g. ketokonazole). The incidence of proarrhythmias or prolonged QT intervals with cisapride was estimated at 1/120 000 based on spontaneous reports and an estimated, substantial under-reporting. These observations were further expanded in a study in which all suspected cases and ECG strips were reviewed and classified into levels of confidence (high-, medium- and low-confidence TdP)[2]. Recognized cofactors for cisapride-related proarrhythmias, such as CYP3A4 inhibitors, electrolyte disturbances and other drugs with a QT prolonging effect, were substantially more common in the group with high- and medium-confidence TdP compared to the

low confidence group. In subsequent studies, it was demonstrated that cisapride affects cardiac repolarization, presumably through blockade of the rapid component of the delayed rectifier potassium current[58]. On the basis of these reports, the drug received a restricted labelling in several countries, and physicians were asked to avoid concomitant treatment with drugs that interacted either pharmacokinetically (through the metabolic inhibition) or pharmacodynamically (other drugs associated with TdP or with known effect on the QT interval). An epidemiological study based on computerized medical claims data on 36 743 patients prescribed cisapride failed, however, to identify an increased risk for ventricular arrhythmias with recent cisapride use[59]. The studied end-points were sustained ventricular tachycardia, ventricular fibrillation, TdP, sudden death or cardiac arrest. There were a total of 52 events, of which 34 occurred during periods of nonrecent cisapride use and 18 during recent use. Male gender (RR 2·6; 95% CI 1·5 to 4·5) and age above 70 years (RR 1·7; 95% CI 1·0 to 3·1) carried an increased risk for ventricular arrhythmias, but not recent cisapride use (RR 1·6; 95% CI 0·9 to 2·9). The authors therefore concluded that the results were 'consistent with an absence of any cisapride-induced increase in rates of arrhythmic events' and furthermore 'by contrast, advanced age, male gender, diabetes, a history of arrhythmia or ischaemic heart disease and the use of a QT prolonging drug did appear to be associated with an increased risk'. Taken into consideration the expected, very low incidence of TdP among cisapride users (1/120 000), a different conclusion might have been considered: the identified end-points may mainly have been associated with other risk factors, such as ischaemic heart disease, and the study was not sufficiently powered to identify an increased risk with cisapride (which was pointed out by the authors). Furthermore, this assumption is supported by the influence of gender; male gender is a known risk factor for ventricular arrhythmias associated with ischaemic heart disease, whereas female gender is a firmly established risk factor for proarrhythmias with antiarrhythmics and non-cardiovascular drugs[60]. The annual incidence of sudden death in the adult general US population has been estimated to range from 84 to 200 per 100 000[61,62], which, most likely, is several orders of magnitude higher than the incidence of fatal TdP. Since the incidence of ventricular arrhythmias and sudden death associated with structural heart disease is markedly higher than the incidence of TdP, any study must enable the correct discrimination between TdP (polymorphic ventricular tachycardia in the setting of prolonged QT interval) and other forms of ventricular arrhythmias.

Antibiotics

Macrolides, quinolones, imidazole antifungals and antimalarials have been associated with prolonged cardiac repolarization and TdP.

Macrolides

Erythromycin exhibits electrophysiological effects that resemble those of class III antiarrhythmic drugs. In transmural strips, arterially perfused wedges and single myocytes isolated from the canine left ventricle, erythromycin prolonged the action potential and induced early after-depolarizations mainly in the M cells, prolonged the QT interval and increased the transmural dispersion of repolarization[63]. Episodes of TdP have been described after intravenous erythromycin[64–66], and 36 cases of TdP or ventricular tachycardia in the presence of prolonged QT were found in a survey of the FDA's Medwatch Database in 1998[67]. Besides the potential for pharmacodynamic interaction with other drugs that also block I_{Kr}, erythromycin is an inhibitor of the CYP3A4 isozyme and causes significant interactions with, for example, the metabolism of cisapride[68]. Two cases of TdP after oral clarithromycin in critically ill patients with hepatic and/or renal impairment have been reported[69], as well as TdP in patients treated concomitantly with clarithromycin and cisapride. This, again, may be an example of both pharmacodynamic and pharmacokinetic interaction, since clarithromycin also inhibits the metabolism of cisapride[70].

Quinolones

Quinolone-associated TdP has been described on rare occasions, and only with sparfloxacin[71,72], levofloxacin and grepafloxacin[73]. All quinolones, however, seem to prolong cardiac repolarization, when adequately studied[74,75], and this has led to restrictions in the labelling. Moxifloxacin, which prolonged the QT interval by approximately 6 ms in early clinical studies[76], received, when recently registered, a labelling of contraindication for concomitant use with antiarrhythmic drugs and proarrhythmic conditions, and should be cautiously given with other drugs that affect the QT interval. At a time when more than one million patients had been treated with moxifloxacin, there had been only a single case of possibly associated torsades in an elderly female patient with several other risk factors[77].

Imidazole antifungals

Ketaconazole is a very potent CYP3A4 inhibitor and has been associated with numerous cases of TdP in patients using drugs that affect the QT interval and which are metabolized via this route, e.g. terfenadine and cisapride. In addition, ketoconazole also blocks HERG (human ether-à-gogo-related gene) and may therefore have an intrinsic effect on the potassium currents[78], which may further accentuate the propensity for proarrhythmias[51].

Trimethoprim-sulfamethoxazole

Trimethoprim-sulfamethoxazole has been associated with TdP in case reports in which the casual relationship, however, was not fully established[79,80].

Antimalarials

Quinine is the optical isomer to quinidine, but clearly has a much smaller effect on cardiac repolarization. Nevertheless, quinine has been shown to prolong the QT interval and to induce morphological changes of the T wave similar to those observed with quinidine[81], but has only occasionally been associated with TdP[82]. Halofantrine prolonged the QT interval in patients with malaria[83], and this effect was particularly pronounced when the drug was instituted as retreatment after failure with mefloquine, which may implicate a drug interaction. TdP has been reported in two patients with congenital long QT syndrome[84]. Inravenous pentamidine has also been shown to prolong the QT interval and to cause TdP[85–87].

Antipsychotic and antidepressant agents

Neuroleptics

It has long been debated whether the unexplained high incidence of sudden death in psychiatric patients could be explained by drug-induced arrhythmias. Dose-dependent QT prolongation has been observed in patients on neuroleptic medication[88]. Phenothiazines (e.g. thioridazine and chlorpromazine), and butyrophenones (droperidol and haloperidol) have been linked to proarrhythmic events[89–92]. In a recently published study, electrocardiograms obtained from 495 psychiatric patients were compared with 101 healthy reference individuals[93]. QTc prolongation, as defined from the healthy group (QTc $\geq$456 ms), was present in 8% of the psychiatric patients. Age above 65 years (OR: 3·0), use of tricyclic antidepressants (OR: 4·4), thioridazine (OR: 5·4) droperidol (OR: 6·7) and dose of neuroleptic drug (high-dose OR: 5·3; very-high-dose OR: 8·2) predicted QTc prolongation. The risk was substantially higher with thioridazine and droperidol compared to other neuroleptics. In contrast to most other antipsychotics, thioridazine may prolong the QT interval at therapeutic concentrations, and both this drug and droperidol have been shown to prolong the cardiac action potential through blockage of the delayed rectifier potassium current[94,95].

Pimozide is a diphenylpiperidine neuroleptic drug that also may prolong the QT interval, and TdP has been described after ingestion of high doses in suicide attempts[96] or as the result of inhibition of its metabolism via the hepatic CYP3A4 isozyme through pharmacokinetic interaction with clarithromycin[97].

Atypical antipsychotics

Several of the so-called atypical antipsychotics, in particular sertindole, risperidone and clozapine, have also been shown to affect the cardiac action potential[98,99]. Sertindole was withdrawn from the market in 1998 due to cardiovascular safety concerns. There seem, however, to be clear differences in the propensity for different atypical antipsychotics to prolong the QT interval, with effects ranging from zero (e.g. olanzapine) to approximately 20 ms (serindole)[100,101]. Zimeldine[102], as well as citalopram[103,104], has also caused TdP after ingestion of toxic doses.

Antidepressants

After intoxication with tricyclic antidepressants the predominant electrocardiographic effect seems to be a widening of the QRS complex, prolongation of the QT interval and evolvement of polymorphic ventricular arrhythmias[105]. TdP has been observed in this setting, but also after pharmacokinetic interaction[106]. Amitriptyline, desipramine[107], imipramine and maprotiline[108] have all been associated with TdP. In a study using signal-averaged electrocardiograms, doxepin, but not fluoxetin, prolonged the QT interval[109], but both drugs have sporadically been associated with TdP[110–112].

Antimigraine drugs

Naratriptan, sumatriptan and zolmitriptan have all been shown to prolong the QT interval, but no cases of TdP are reported in the literature.

Anticancer

Tamoxifen, an anti-oestrogen drug commonly used to treat breast cancer, prolongs the QT interval at high doses[113] and has been demonstrated to block the I_{Kr} and calcium currents in rabbit myocytes[114], but has not been shown to induce TdP.

Miscellaneous

QT prolongation has been described with probucol, a cholesterol-lowering drug, since the early 1980s[115,116]. In a study that reviewed articles and ADR reports filed with the FDA, 16 cases of tachyarrhythmic events were found, of which 11 were TdP[117]. All 11 cases occurred in women and, in a further analysis of 395 probucol-treated patients, an abnormal QT prolongation was

observed more often in women (22%) than in men (7%). Tacrolimus, a macrolide used for prevention of hepatic allograft rejection, has also been described as the cause of TdP in a case report[118], and animal studies have shown a sustained QT prolongation after intravenous administration[119]. Certain inhalation anaesthetics, such as sevoflurane and isoflurane, prolong the QT interval[120,121]. Also worth mentioning is that a Chinese herbal remedy that contains extract from the same root as is used in liquorice[122], as well as liquorice itself, may cause TdP, presumably through hypokalaemia[123].

Conclusions

A whole range of non-cardiovascular compounds from non-related classes has been shown to effect cardiac repolarization and to induce proarrhythmias in susceptible individuals. Non-sedating antihistamines, antibiotics, antipsychotics and antidepressants and cholinergic antagonists (cisapride) are the classes most commonly associated with this potentially fatal side effect. Epidemiological studies have to a large extent failed to identify an increased risk for proarrhythmias with the use of these non-cardiovascular drugs, possibly due to poor specificity of the studied end-points.

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JAMA Cardiology | Original Investigation

Cardiovascular Implications of Fatal Outcomes of Patients With Coronavirus Disease 2019 (COVID-19)

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IMPORTANCE Increasing numbers of confirmed cases and mortality rates of coronavirus disease 2019 (COVID-19) are occurring in several countries and continents. Information regarding the impact of cardiovascular complication on fatal outcome is scarce.

OBJECTIVE To evaluate the association of underlying cardiovascular disease (CVD) and myocardial injury with fatal outcomes in patients with COVID-19.

DESIGN, SETTING, AND PARTICIPANTS This retrospective single-center case series analyzed patients with COVID-19 at the Seventh Hospital of Wuhan City, China, from January 23, 2020, to February 23, 2020. Analysis began February 25, 2020.

MAIN OUTCOMES AND MEASURES Demographic data, laboratory findings, comorbidities, and treatments were collected and analyzed in patients with and without elevation of troponin T (TnT) levels.

RESULT Among 187 patients with confirmed COVID-19, 144 patients (77%) were discharged and 43 patients (23%) died. The mean (SD) age was 58.50 (14.66) years. Overall, 66 (35.3%) had underlying CVD including hypertension, coronary heart disease, and cardiomyopathy, and 52 (27.8%) exhibited myocardial injury as indicated by elevated TnT levels. The mortality during hospitalization was 7.62% (8 of 105) for patients without underlying CVD and normal TnT levels, 13.33% (4 of 30) for those with underlying CVD and normal TnT levels, 37.50% (6 of 16) for those without underlying CVD but elevated TnT levels, and 69.44% (25 of 36) for those with underlying CVD and elevated TnTs. Patients with underlying CVD were more likely to exhibit elevation of TnT levels compared with the patients without CVD (36 [54.5%] vs 16 [13.2%]). Plasma TnT levels demonstrated a high and significantly positive linear correlation with plasma high-sensitivity C-reactive protein levels (β = 0.530, $P < .001$) and N-terminal pro–brain natriuretic peptide (NT-proBNP) levels (β = 0.613, $P < .001$). Plasma TnT and NT-proBNP levels during hospitalization (median [interquartile range (IQR)], 0.307 [0.094-0.600]; 1902.00 [728.35-8100.00]) and impending death (median [IQR], 0.141 [0.058-0.860]; 5375 [1179.50-25695.25]) increased significantly compared with admission values (median [IQR], 0.0355 [0.015-0.102]; 796.90 [401.93-1742.25]) in patients who died ($P = .001$; $P < .001$), while no significant dynamic changes of TnT (median [IQR], 0.010 [0.007-0.019]; 0.013 [0.007-0.022]; 0.011 [0.007-0.016]) and NT-proBNP (median [IQR], 352.20 [174.70-636.70]; 433.80 [155.80-1272.60]; 145.40 [63.4-526.50]) was observed in survivors ($P = .96$; $P = .16$). During hospitalization, patients with elevated TnT levels had more frequent malignant arrhythmias, and the use of glucocorticoid therapy (37 [71.2%] vs 69 [51.1%]) and mechanical ventilation (41 [59.6%] vs 14 [10.4%]) were higher compared with patients with normal TnT levels. The mortality rates of patients with and without use of angiotensin-converting enzyme inhibitors/angiotensin receptor blockers was 36.8% (7 of 19) and 25.6% (43 of 168).

CONCLUSIONS AND RELEVANCE Myocardial injury is significantly associated with fatal outcome of COVID-19, while the prognosis of patients with underlying CVD but without myocardial injury is relatively favorable. Myocardial injury is associated with cardiac dysfunction and arrhythmias. Inflammation may be a potential mechanism for myocardial injury. Aggressive treatment may be considered for patients at high risk of myocardial injury.

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Viewpoint and Editorial
Related articles

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Coronavirus disease 2019 (COVID-19) is a newly recognized infectious disease that has spread rapidly throughout Wuhan, Hubei, China, to other provinces in China and several countries around the world. The number of fatalities owing to COVID-19 is escalating. Previous studies have described the general clinical characteristics and epidemiological findings of patients with COVID-19, and some of the clinical observations have shown that the condition of some patients with COVID-19 deteriorates rapidly.[1-4]

With the increasing number of confirmed cases and the accumulating clinical data, in addition to the common clinical presentation of respiratory failure caused by COVID-19, the cardiovascular manifestations induced by this viral infection has generated considerable concern. Huang et al[5] reported that 12% of patients with COVID-19 were diagnosed as having acute myocardial injury, manifested mainly by elevated levels of high-sensitive troponin I. From other recent data, among 138 hospitalized patients with COVID-19, 16.7% had arrhythmias and 7.2% had acute myocardial injury.[6] However, at present, specific information characterizing whether patients with COVID-19 with underlying cardiovascular disease (CVD) who develop myocardial injury during hospitalization face greater risk and have worse in-hospital outcomes remains unknown. The present study investigated the association of underlying CVD and myocardial injury with fatal outcomes of patients with COVID-19.

Key Points

Question What is the impact of underlying cardiovascular disease (CVD) and myocardial injury on fatal outcomes in patients with coronavirus disease 2019 (COVID-19)?

Findings In this case series study of 187 patients with COVID-19, 27.8% of patients had myocardial injury, which resulted in cardiac dysfunction and arrhythmias. Myocardial injury has a significant association with fatal outcome of COVID-19, while the prognosis of patients with underlying CVD but without myocardial injury were relatively favorable.

Meaning It is reasonable to triage patients with COVID-19 according to the presence of underlying CVD and evidence of myocardial injury for prioritized treatment and even more aggressive strategies.

Methods

Study Design and Participants

This single-center, retrospective, observational study was performed at the Seventh Hospital of Wuhan City, China, which is a designated hospital to treat patients with COVID-19 and supervised by the Zhongnan Hospital of Wuhan University in Wuhan, China. We retrospectively analyzed patients with COVID-19 who were diagnosed according to the interim guidance of the World Health Organization[7] from January 23, 2020, to February 23, 2020, and who were either treated and discharged or died during hospitalization. Clinical information was collected on admission and during hospitalization by attending physicians.

This study complied with the edicts of the 1975 Declaration of Helsinki[8] and was approved by the institutional ethics board of Zhongnan Hospital of Wuhan University and the Seventh Hospital of Wuhan City (no. 2020026). Consent was obtained from patients or patients' next of kin.

Data Collection

The electronic medical records of the patients were reviewed by a trained team of physicians who worked in Seventh Hospital of Wuhan City during the epidemic period. Patient data including demographics, medical history, laboratory examinations, comorbidities, complication, treatment measures (antiviral, antibiotic, corticosteroid therapies, immune glucocorticoid therapy, and respiratory support), and outcomes were collected and analyzed.

Outcome

The end point was incidence of COVID-19–associated death. Successful treatment toward hospital discharge comprised relieved clinical symptoms, normal body temperature, significant resolution of inflammation as shown by chest radiography, and at least 2 consecutive negative results shown by real-time reverse transcription–polymerase chain reaction assay[6] for COVID-19.

Acute respiratory distress syndrome was defined according to the Berlin Definition.[9] Malignant arrhythmia was defined as rapid ventricular tachycardia lasting more than 30 seconds, inducing hemodynamic instability and/or ventricular fibrillation. Patients were considered to have acute myocardial injury if serum levels of troponin T (TnT) were above the 99th percentile upper reference limit.[5]

Statistical Analysis

Categorical variables are shown as frequency rates and percentages, and continuous variables as mean (SD) and median (interquartile range [IQR]). The means for continuous variables were compared using independent group *t* tests when the data were normally distributed, otherwise, the Mann-Whitney test was used. The Pearson correlation coefficient and Spearman rank correlation coefficient were used for liner correlation analysis. Proportions for categorical variables were compared using the χ^2 test, although the Fisher exact test was used when data were limited. Wilcoxon rank sum matched-pair tests were used to assess differences among the admission, hospitalization, and impending death. All statistical analyses were performed with SPSS, version 19.0 (IBM Corp) for Windows. A 2-sided $P < .05$ was considered statistically significant. Analysis began February 25, 2020.

Results

Clinical Characteristics on Admission

Data were collected in consecutive patients hospitalized with COVID-19, including 211 patients who were successfully treated and discharged and 45 patients who died. We excluded 67 discharged patients and 2 patients who died because of incomplete data, leaving 144 discharged individuals and 43 indi-

Table 1. Demographics and Clinical Characteristics of Patients With COVID-19

Characteristic	No. (%)			
		TnT level		
	Total	Normal	Elevated	*P* value[a]
No. of patients	187	135	52	NA
Male	91 (48.7)	57 (42.2)	34 (65.4)	.005
Age, mean (SD), y	58.50 (14.66)	53.53 (13.22)	71.40 (9.43)	<.001
Smoking	18 (9.6)	11 (8.1)	7 (13.5)	.27
Hospitalization, mean (SD), d	16.63 (8.12)	17.27 (7.68)	14.94 (9.03)	.08
Duration, mean (SD), d[b]	26.30 (8.96)	27.49 (8.55)	23.23 (9.35)	.003
Comorbidities				
Hypertension	61 (32.6)	28 (20.7)	33 (63.5)	<.001
CHD	21 (11.2)	4 (3.0)	17 (32.7)	<.001
Cardiomyopathy	8 (4.3)	0 (0)	8 (15.4)	<.001
Diabetes	28 (15.0)	12 (8.9)	16 (30.8)	<.001
COPD	4 (2.1)	0 (0)	4 (7.7)	.001
Malignant neoplasm	13 (7.0)	7 (5.2)	6 (11.5)	.13
Chronic kidney disease	6 (3.2)	1 (0.7)	5 (9.6)	.002
ACEI/ARB use history	19 (10.1)	8 (5.9)	11 (21.1)	.002
Complication				
ARDS	46 (24.6)	16 (11.9)	30 (57.7)	<.001
VT/VF	11 (5.9)	2 (1.5)	9 (17.3)	<.001
Acute				
Coagulopathy	42 (34.1)	17 (20.0)	25 (65.8)	<.001
Liver injury	19 (15.4)	14 (16.5)	5 (13.2)	.89
Kidney injury	18 (14.6)	4 (4.7)	14 (36.8)	<.001
Therapy				
Antivirus	166 (88.8)	120 (88.9)	46 (88.5)	.93
Antibiotic	183 (97.9)	131 (97.0)	52 (100.0)	.21
Glucocorticoid	106 (56.7)	69 (51.1)	37 (71.2)	.01
Immune globulin	21 (11.2)	14 (10.4)	7 (13.5)	.5
Mechanical ventilation	45 (24.1)	14 (10.4)	31 (59.6)	<.001
Clinical outcome				
Death	43 (23.0)	12 (8.9)	31 (59.6)	<.001

Abbreviations: ACEI, angiotensin-converting enzyme inhibitor; ARB, angiotensin receptor blocker; ARDS, acute respiratory distress syndrome; CHD, coronary heart disease; COPD, chronic obstructive pulmonary disease; COVID-19, coronavirus disease 2019; NA, not applicable; TnT, troponin T; VF, ventricular fibrillation; VT, ventricular tachycardia.

[a] Statistical differences between the normal TnT and elevated TnT groups.

[b] Duration indicates days from onset of symptoms to death or discharge.

viduals who died included for final analysis. Of 187 patients, 66 (35.3%) had underlying CVD including hypertension, coronary heart disease, and cardiomyopathy, and 52 (27.8%) exhibited myocardial injury as indicated by elevated TnT levels.

On admission, none showed evidence of acute myocardial infarction, chronic liver disease, thromboembolic diseases, or rheumatism. In patients with elevated plasma TnT levels who eventually were discharged or died, the median (IQR) duration from illness onset to discharge or death was 28 (22-33) and 23.5 (18.25-34.5) days, respectively. Mortality was markedly higher in patients with elevated plasma TnT levels than in patients with normal TnT levels (31 [59.6%] vs 12 [8.9%]) (**Table 1**).

Compared with patients with normal TnT levels (Table 1), those with elevated TnT levels were older (mean [SD] age, 71.40 [9.43] vs 53.53 [13.22]) and had a higher proportion of men (34 [65.4%] vs 57 [42.2%]). Patients with elevated TnT levels had significantly higher rates of comorbidities including hypertension (33 [63.5%] vs 28 [20.7%]), coronary heart disease (17 [32.7%] vs 4 [3.0%]), cardiomyopathy (8 [15.4%] vs 0), diabetes (16 [30.8%] vs 12 [8.9%]), chronic obstructive pulmonary disease (4 [7.7%] vs 0), and chronic kidney disease (1 [0.7%] vs 5 [9.6%]). Rates of smoking and malignant neoplasms did not differ between those with normal (11 [8.1%] vs 7 [13.5%]) and elevated TnT levels (7 [5.2%] vs 6 [11.5%]).

Laboratory Findings on Admission

Patients with elevated TnT levels presented with significantly higher white blood cell count (median [IQR], 4640 [6170-3740] vs 7390 [4890-11 630] /μL [to convert to $\times 10^9$ per liter, multiply by 0.001]) and neutrophil counts (median [IQR], 3070 [2350-4870] vs 6010 [3540-10 120] /μL [to convert to $\times 10^9$ per liter, multiply by 0.001]) ($P < .001$ for both) and lower lymphocyte counts (median [IQR], 840 [630-1130] vs 690 [340-1010] /μL [to convert to $\times 10^9$ per liter, multiply by 0.001; $P = .01$) than those with normal TnT levels (**Table 2**). Patients with elevated TnT levels also had significantly longer prothrombin time (median [IQR], 12.4 [12.0-13.0] vs 13.3 [12.2-15.3] seconds; $P = .005$), shorter activated partial thromboplastin time (median [IQR], 31.2 [27.5-33.2] vs 32.7 [31.0-35.8] seconds; $P = .003$), and a significant higher level of D-dimer (median

Table 2. Laboratory Results Among Different Groups

Characteristic	Median (IQR)			*P* value[a]
		TnT level		
	Total	Normal	Elevated	
No. of patients	187	135	52	NA
Complete blood cell count, /μL				
White blood cell	4970 (3810-7460)	4640 (6170-3740)	7390 (4890-11 630)	<.001
Neutrophil	3700 (2410-6120)	3070 (2350-4870)	6010 (3540-10 120)	<.001
Lymphocyte	810 (560-1060)	840 (630-1130)	690 (340-1010)	.01
Coagulation profiles				
Prothrombin time, s	12.8 (12.0-14.0)	12.4 (12.0-13.0)	13.3 (12.2-15.3)	.005
APTT, s	32.0 (30.1-35.0)	32.7 (31.0-35.8)	31.2 (27.5-33.2)	.003
D-dimer, μg/mL	0.43 (0.19-2.66)	0.29 (0.17-0.60)	3.85 (0.51-25.58)	<.001
Blood lipids and electrolytes				
Cholesterol, mg/dL				
Total, mean (SD)	137.45 (34.75)	139.38 (35.14)	132.82 (33.20)	.27
Triglyceride	85.84 (62.83-123.01)	82.30 (59.29-115.04)	92.04 (69.91-159.29)	.04
HDL, mean (SD)	43.24 (10.42)	44.02 (10.81)	40.93 (8.88)	.08
LDL, mean (SD)	77.99 (25.48)	79.15 (25.87)	75.29 (23.94)	.42
Serum				
Potassium, mEq/L	3.67 (3.35-3.98)	3.67 (3.34-3.96)	3.62 (3.36-4.23)	.51
Calcium, mg/dL	8.52 (8.16-8.96)	8.60 (8.24-9.00)	8.36 (8.08-8.76)	.01
Inflammatory biomarkers				
hsCRP, mg/dL	4.04 (1.64-8.14)	3.13 (1.24-5.75)	8.55 (4.87-15.165)	<.001
Procalcitonin, ng/mL	0.08 (0.04-0.16)	0.05 (0.04-0.11)	0.21 (0.11-0.45)	<.001
Globulin, g/L	27.7 (25.8-31.0)	27.4 (25.6-29.6)	29.7 (27.0-34.6)	<.001
Other cardiac biomarkers				
Creatine kinase–MB fraction, ng/mL	1.14 (0.66-2.95)	0.81 (0.54-1.38)	3.34 (2.11-5.80)	<.001
Myoglobin, μg/L	38.5 (21.0-78.0)	27.2 (21.0-49.8)	128.7 (65.8-206.9)	<.001
NT-proBNP, pg/mL	268.4 (75.3-689.1)	141.4 (39.3-303.6)	817.4 (336.0-1944.0)	<.001
Blood gas analysis				
Pao_2, mm Hg	83.0 (64.8-118.0)	91.0 (75.0-121.0)	64.0 (51.0-93.0)	<.001
Pao_2/FiO_2, mm Hg	366.7 (202.3-447.8)	390.5 (285.7-461.9)	153.3 (103.3-323.8)	<.001
Lactic acid, mm Hg	1.80 (1.40-2.25)	1.80 (1.30-2.10)	2.10 (1.40-3.10)	.004
HCO_3, mEq/L	25.2 (22.9-27.7)	25.7 (23.8-27.9)	23.3 (20.0-27.1)	.001
Liver and renal function				
Aminotransferase, U/L				
Alanine	23.0 (14.0-35.0)	23.0 (14.0-33.0)	28.5 (16.2-39.8)	.11
Aspartate	21.0 (22.0-31.0)	29.0 (21.0-39.0)	39.5 (27.2-57.8)	<.001
Creatinine, mg/dL	0.69 (0.58-0.84)	0.63 (0.55-0.79)	0.79 (0.71-1.17)	<.001

Abbreviations: APTT, activated partial thromboplastin time; HDL, high-density lipoprotein; hsCRP, high-sensitivity C-reactive protein; IQR, interquartile range; LDL, low-density lipoprotein; NA, not applicable; NT-proBNP, N-terminal pro–brain natriuretic peptide; TnT, troponin T.

SI conversion factor: To convert aminotransferase to microkatal per liter, multiply by 0.0167; blood cell counts to $\times 10^9$ per liter, multiply by 0.001; calcium to millimoles per liter, multiply by 0.25; cholesterol to millimoles per liter, multiply by 0.0259; creatinine to μmol/L, multiply by 88.4; creatine kinase–MB fraction to micrograms per liter, multiply by 1; CRP to milligrams per liter, multiply by 10; D-dimer to nanomoles per liter, multiply by 5.476; HCO_3 to millimoles per liter, multiply by 1; myoglobin to nanomoles per liter, multiply by 0.05814; NT-proBNP to ng/L, multiply by 1; triglyceride to millimoles per liter, multiply by 0.0113; potassium to millimoles per liter, multiply by 1.

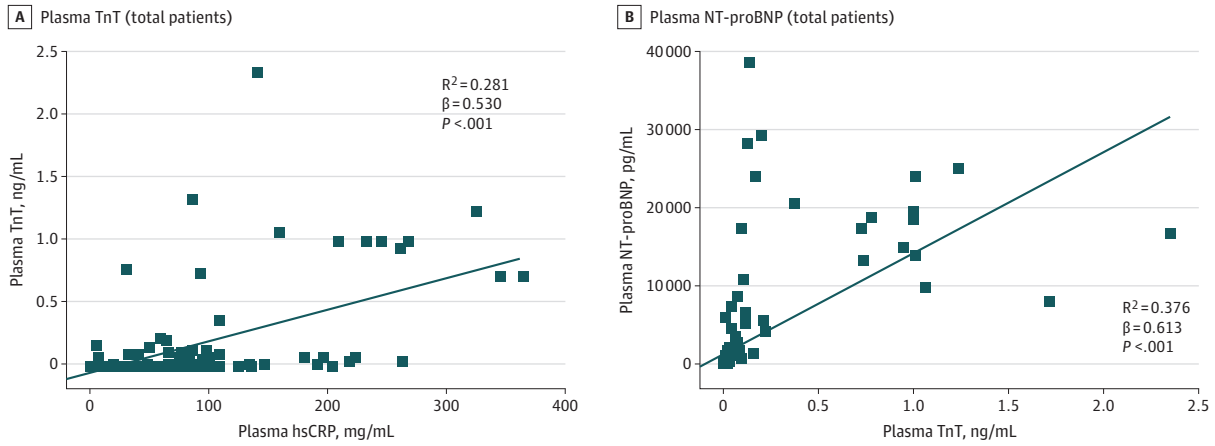
[a] Statistical differences between the normal TnT and elevated TnT groups.

[IQR], 0.29 [0.17-0.60] vs 3.85 [0.51-25.58] μg/mL [to convert to nanomoles per liter, multiply by 5.476]; $P < .001$). Hemoglobin and neutrophil counts of the 2 groups were similar.

Total, high-density lipoprotein, and low-density lipoprotein cholesterol levels did not differ according to TnT levels, but patients with elevated TnT levels had higher triglyceride levels (median [IQR], 92.04 [69.91-159.29] vs 82.30 [59.29-115.04] mg/dL [to convert to millimoles per liter, multiply by 0.0259]; $P=.04$). The inflammatory biomarkers, including high-sensitivity C-reactive protein (median [IQR], 8.55 [4.87-15.165] vs 3.13 [1.24-5.75] mg/dL [to convert to milligrams per liter, multiply by 10]), procalcitonin (median [IQR], 0.21 [0.11-0.45] vs 0.05 [0.04-0.11] ng/mL), and globulin (median [IQR], 29.7 [27.0-34.6] vs 27.4 [25.6-29.6] grams per liter) were significantly higher in patients with elevated TnT levels ($P < .001$ for all).

Notably, patients with normal and elevated TnT levels differed with respect to multiple indexes of organ function including the heart, liver, kidney, and lungs (Table 2). Those with elevated TnT levels had significantly higher levels of other biomarkers of cardiac injury, specifically creatine kinase-myocardial band test (median [IQR], 3.34 [2.11-5.80] vs 0.81 [0.54-1.38], ng/mL [to convert to micrograms per liter, multiply by 1]) and myoglobin (median [IQR], 128.7 [65.8-206.9] vs 27.2 [21.0-49.8] μg/L [to convert to nanomoles per liter, multiply by 0.05814]) ($P < .001$, for all) and also had higher levels

Figure 1. Correlation Between Plasma TnT and NT-proBNP With hsCRP



Plasma troponin T (TnT), high-sensitivity C-reactive protein levels (hsCRP), and N-terminal pro–brain natriuretic peptide (NT-pro BNP) collected on admission.

of N-terminal pro-brain natriuretic peptide (NT-proBNP) (median [IQR], 817.4 (336.0-1944.0] vs 141.4 [39.3-303.6] pg/mL [to convert to nanograms per liter, multiply by 1]). Patients with elevated TnT levels had evidence of more severe respiratory dysfunction, with lower partial pressure of oxygen (PaO_2) (median [IQR], 64.0 [51.0-93.0] vs 91.0 [75.0-121.0] mm Hg), HCO_3 (median [IQR], 23.3 [20.0-27.1] vs 25.7 [23.8-27.9] mEq/L [to convert to to millimoles per liter, multiply by 1]), and PaO_2/fraction of inspired oxygen (FiO_2) (median [IQR], 153.3 [103.3-323.8] vs 390.5 [285.7-461.9] mm Hg), and higher levels of lactic acid (median [IQR], 2.10 [1.40-3.10] vs 1.80 [1.30-2.10] mm Hg) ($P < .001$, $P < .001$, $P = .004$, $P = .001$, respectively). Those with elevated TnT levels also had higher levels of creatinine (median [IQR], 0.79 [0.71-1.17] vs 0.63 [0.55-0.79] mg/dL [to convert to micromoles per liter, multiply by 88.4]) and aspartate aminotransferase (median [IQR], 39.5 [27.2-57.8] vs 29.0 [21.0-39.0] U/L [to convert to microkatal per liter, multiply by 0.0167]) ($P < .001$, both), but alanine aminotransferase did not differ between the 2 groups.

Plasma TnT levels in patients with COVID-19 correlated significantly with both plasma high-sensitivity C-reactive protein levels ($\beta = 0.530$, $P < .001$) (**Figure 1**A) and plasma NT-proBNP levels ($\beta = 0.613$, $P < .001$) (Figure 1B).

Comparison of Complications and Treatment During Hospitalization

Patients with underlying CVD were more likely to exhibit elevation of TnT levels (36 [54.5%]) compared with patients without CVD (16 [13.2%]). During hospitalization, patients with elevated TnT levels developed more frequent complications (Table 1), including acute respiratory distress syndrome (30 [57.7%] vs 16 [11.9%]), malignant arrhythmias (6 [11.5%] vs 7 [5.2%]) including ventricular tachycardia/ventricular fibrillation, acute coagulopathy (25 [65.8%] vs 17 [20.0%]), and acute kidney injury (14 [36.8%] vs 4 [4.7%]), compared with those with normal TnT levels. However, there was no significant differences in incidence of acute liver injury between the 2 groups. Antiviral (oseltamivir, 75 mg twice a day; ribavirin, 0.5 g twice a day; umifenovir, 0.2 g 3 times a day), antibacterial (moxifloxacin, 0.4 g every day), glucocorticoid (methylprednisolone, 40-80 mg every day), and respiratory support were the main treatment approaches for the hospitalized patients (Table 1). During hospitalization, the majority of patients underwent antiviral and antibacterial therapy, with no significant difference in such therapies between patients with normal and elevated TnT levels. However, the rates of glucocorticoid therapy and mechanical ventilation were much higher in patients with elevated TnT levels compared with those with normal TnT levels.

Long-term outpatient medications prior to admission, such as antihypertensive drugs and hypoglycemic drugs, were not discontinued. Notably, the use of angiotensin-converting enzyme inhibitors (ACEIs)/angiotensin receptor blockers (ARBs) was higher in patients with elevated TnT levels (11 [21.1%] vs 8 [5.9%]; Table 1), reflecting the higher rates of CVD. The mortality rates of patients with and without use of ACEIs/ARBs was 36.8% (7 of 19) and 25.6% (43 of 168).

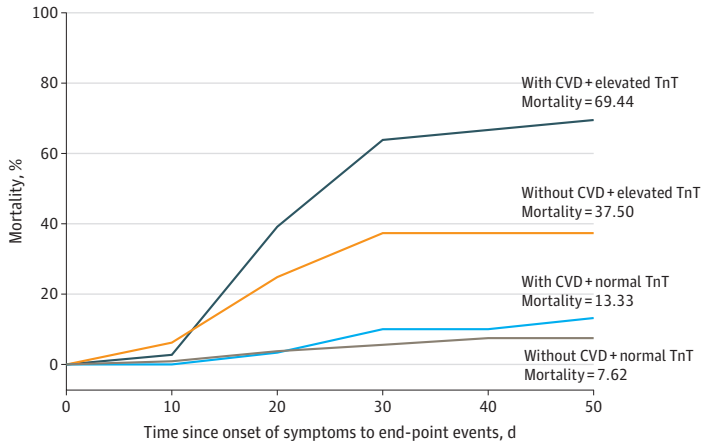
Mortality of Patients With COVID-19 With/Without CVD and With/Without Elevated TnT Levels

Among 187 patients, 7.62% (8 of 105) with normal TnT levels without underlying CVD, 13.33% (4 of 30) with normal TnT levels with underlying CVD, 37.50% (6 of 16) with elevated TnT levels without underlying CVD, and 69.44% (25 of 36) with elevated TnT levels with underlying CVD died during hospitalization (**Figure 2**).

Dynamic Changes of TnT and NT-proBNP Levels During Hospitalization

Figure 3 shows the dynamic escalation of TnT and NT-proBNP levels for patients who died and those who were successfully treated and discharged. Both TnT and NT-proBNP levels increased significantly during the course of hospitalization in those who ultimately died, but no such dynamic changes of TnT or NT-proBNP levels were evident in survivors.

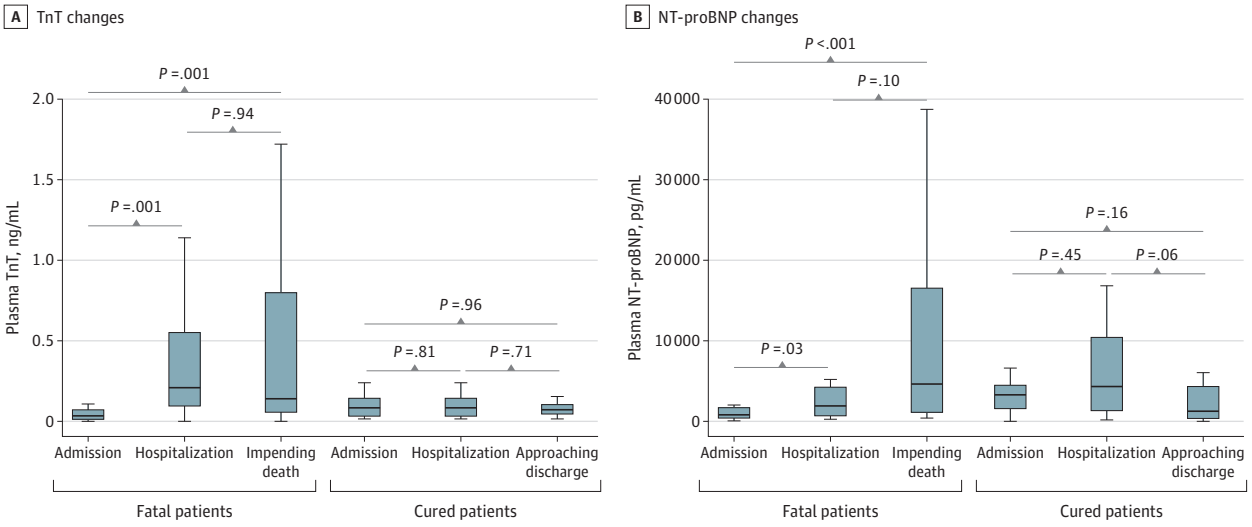
Figure 2. Mortality of Patients With Coronavirus Disease 2019 (COVID-19) With/Without Cardiovascular Disease (CVD) and With/Without Elevated Troponin T (TnT) Levels



No. at risk

Without CVD + normal TnT (n = 105)	102	86	41	10	0
Without CVD + elevated TnT (n = 16)	15	12	7	1	0
With CVD + normal TnT (n = 30)	29	25	10	4	0
With CVD + elevated TnT (n = 36)	34	20	8	2	0

Figure 3. Dynamic Changes of TnT and NT-proBNP During Hospitalization



The horizontal lines represent the median value in each group. NT-proBNP indicates N-terminal pro–brain natriuretic peptide; TnT, troponin T.

Discussion

Association of Myocardial Injury With Prognosis

This report provides detailed cardiovascular information of the association between underlying CVD, myocardial injury, and fatal outcomes of patients with COVID-19. The Chinese Center for Disease Control and Prevention recently published the largest case series to date of COVID-19 in mainland China; the overall case fatality rate was 2.3% (1023 deaths among 44 672 confirmed cases), but the mortality reached 10.5% in patients with underlying CVD.[10]

In the current study, among 187 patients with COVID-19, 52 (27.8%) exhibited myocardial injury as demonstrated by elevation of TnT levels, and the mortality was markedly higher in patients with elevated TnT levels than in patients with normal TnT levels (59.6% vs 8.9%). The median (IQR) duration from illness onset to death was 23.23 (8-41) days in the group with elevated TnT levels. Patients with underlying CVD and escalation of TnT levels had the highest mortality (69.44%) and the shortest survival term. However, patients with underlying CVD but with normal TnT levels during the course of disease experienced a more favorable prognosis, compared with patients with elevated TnT levels but without underlying CVD

(mortality, 13.3% vs 37.5%). The dynamic escalation of NT-proBNP and increased incidence of malignant arrhythmias during the course of disease in patients with elevated TnT levels is evidence that myocardial injury played a greater role in the fatal outcome of COVID-19 than the presence of underlying CVD itself.

NT-proBNP elevation and malignant arrhythmias were significantly more common in patients with elevated TnT levels, and NT-proBNP was significantly correlated with TnT levels (Figure 1). This suggests that those with myocardial injury were more likely to experience impairment in cardiac function.

Potential Mechanism Underlying Myocardial Injury

The current study demonstrates that patients with underlying CVD and other comorbid conditions are more prone to experience myocardial injury during the course of COVID-19. For patients with underlying CVD, including hypertension, coronary heart disease, and cardiomyopathy, viral illness can further damage myocardial cells through several mechanisms including direct damage by the virus, systemic inflammatory responses, destabilized coronary plaque, and aggravated hypoxia. Therefore, patients with CVD are more likely to experience myocardial injury after COVID-19 infection and higher risk of death. However, it is also notable that the 16% of patients with underlying CVD but with normal TnT levels had a relatively favorable outcome in this study. These data suggest that myocardial biomarkers should be evaluated in patients with CVD who develop COVID-19 for risk stratification and possible early and more aggressive intervention.

Although the exact pathophysiological mechanism underlying myocardial injury caused by COVID-19 is not fully understood, a previous report showed that in 35% of the patients with severe acute respiratory syndrome coronavirus (SARS-CoV) infection, the SARS-CoV genome was positively detected in the heart. This raises the possibility of direct damage of cardiomyocytes by the virus.[11] SARS-CoV-2 may share the same mechanism with SARS-COV because the 2 viruses are highly homologous in genome.[12,13] In the current study, plasma TnT levels were significantly positively linear correlated with plasma high-sensitivity C-reactive protein levels (Figure 2), indicating that myocardial injury may be closely associated with inflammatory pathogenesis during the progress of disease. Viral particles spread through respiratory mucosa and simultaneously infect other cells, which could precipitate a cytokine storm and a series of immune responses. Huang et al[5] highlighted that in patients with COVID-19, the imbalance of T helper 1 and T helper 2 responses resulted in a cytokine storm, which may contribute to myocardial injury. The release of inflammatory cytokines after infection may cause reduction in coronary blood flow, decreases in oxygen supply, destabilization of coronary plaque, and microthrombogenesis.

Consideration of Prevention and Treatment for Myocardial Injury

Unfortunately, until now, no specific antiviral drugs or vaccines have been recommended for COVID-19 except for symptomatic supportive treatment and intervention. As patients with underlying CVD are more likely to develop more severe adverse outcomes when myocardial injury occurs after COVID-19 infection and face higher risk of death, it may be reasonable to triage patients with COVID-19 according to the presence of underlying CVD and evidence of myocardial injury for prioritized treatment and even more aggressive treatment strategies. Other cardiac biomarkers such as NT-proBNP and electrocardiograms should be closely monitored for early warning and intervention.

There remains controversy concerning the use of ACEI/ARB for COVID-19. In this study, with a limited number of patients, the mortality of those treated with or without use of ACEI/ARB did not show a significant difference in outcome. Concerns about ACEI/ARB have been raised since angiotensin-converting enzyme 2 (ACE2) is a potential target for COVID-19 infection, and the increased ACE2 expression induced by ACEI or ARB would aggravate lung injury of patients with COVID-19. However, a previous study[14] showed a beneficial effect of ACEI/ARB in patients admitted with viral pneumonia, as it significantly reduced the pulmonary inflammatory response and cytokine release caused by virus infection. The beneficial effect of ACEI/ARB may be related to a compensatory increase in ACE2.[15] However, the evidence regarding the use of ACEI/ARB in patients with COVID-19 infection is still emerging, and larger clinical studies are required. At present, for patients with COVID-19 who previously used ACEI/ARB, the use of these drugs may not need to be discontinued based on current data.

Limitations

Our study has several limitations. First, only 187 patients with confirmed COVID-19 were included, and a larger cohort study is needed to verify our conclusions. Second, as a retrospective study, some other specific information regarding cardiovascular complications and inflammation such as echocardiography and interleukin 6 were not presented in the study because the data were incomplete owing to the limited conditions in the isolation ward and the urgency of containing the COVID-19 epidemic. Third, the data in this study permit a preliminary assessment of the clinical course and outcomes of patients with COVID-19. The causes of death may involve multiple organ dysfunction in most cases, and it is difficult to differentiate the myocardial injury as the main and direct cause in an individual case. Long-term observation and prospective study design on the effectiveness of treatments specific for the myocardial injury are needed.

Conclusions

Myocardial injury has a significant association with fatal outcomes of COVID-19, while the prognosis of patients with underlying CVD but without myocardial injury appears relatively favorable. Myocardial injury is associated with impairment of cardiac function and ventricular tachyarrhythmias. Inflammation may be associated with myocardial injury. Aggressive treatment may be considered for the patients with myocardial injury.

ARTICLE INFORMATION

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Concept and design: Guo, Fan, Zhang, H. Wang, Wan, X. Wang, Lu.
Acquisition, analysis, or interpretation of data: Guo, Fan, Chen, Wu, He, H. Wang, Lu.
Drafting of the manuscript: Guo, Fan, Chen, Zhang, H. Wang, Lu.
Critical revision of the manuscript for important intellectual content: Fan, Wu, He, H. Wang, Wan, X. Wang, Lu.
Statistical analysis: Guo, Fan, Chen, Wu.
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RESEARCH LETTER

Seasonal Influenza Activity During the SARS-CoV-2 Outbreak in Japan

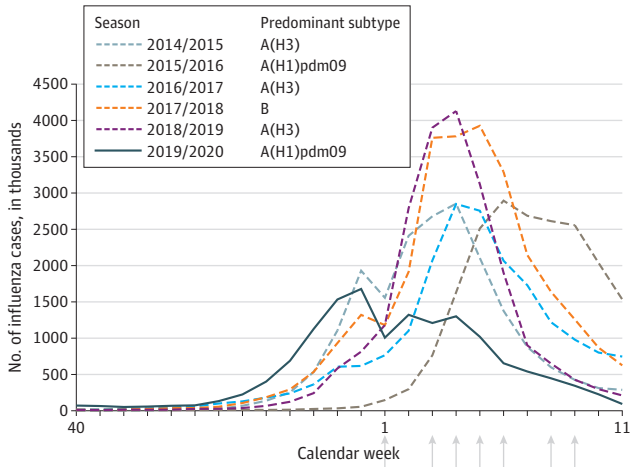
Since the severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) outbreak began, measures for avoiding disease transmission have been widely promoted in Japan, such as use of masks and handwashing, remote work, and cancellation of large events. If effective, these measures may also reduce the spread of other infectious diseases, such as seasonal influenza. We compared the weekly influenza activity in the 2019/2020 season vs 5 previous seasons.

Related article

Audio

Methods | We used data from 2014 to 2020 from the National Institute of Infectious Diseases Japan, which gathers the number of cases of seasonal influenza weekly, diagnosed by physicians based on clinical symptoms or laboratory findings, from approximately 5000 sentinel centers, including hospitals and clinics (60% pediatrics and 40% internal or general medicine clinics).[1,2] We grouped the weekly reports into seasons (week 40 of the year through week 11 of the following year [September 30, 2019, through March 15, 2020, for the 2019/2020 season]; the season was truncated after week 11 because this was the latest available data for 2020). In each season we assessed the weekly influenza activity, presented as a crude standardized estimate of influenza activity nationally, calculated by multiplying the mean number of reported cases per sentinel center with a constant

Figure. Influenza Activity and Predominant Subtype by Influenza Season and Events Related to Measures Taken to Contain or Mitigate the SARS-CoV-2 Outbreak in the 2019/2020 Season



Events during the 2019/2020 season

- WHO informed of a cluster of cases of pneumonia of unknown cause detected in Wuhan, China (Dec 31; week 1).
- The Ministry of Health, Labour and Welfare of Japan asks the public to adhere to "coughing etiquette" and handwashing (Jan 14; week 3).
- First reports of face masks and hand sanitizer being sold out in stores (Jan 24-31; week 3-4).
- Reports of companies implementing remote work (Jan 27; week 5); an increasing number of employers follow suit in the subsequent weeks.
- The Ministry of Health, Labour and Welfare of Japan requests pharmacies and supermarkets to impose restrictions on purchases of face masks and disinfectants to avoid shortages (Feb 7; week 6).
- Cancellations of the Tokyo Marathon for nonprofessional runners and of the emperor's birthday celebrations are announced (Feb 17; week 8).
- The government announces the closure of schools from the following week, requests suspension of large-scale gatherings, and urges individuals with light symptoms to stay at home instead of seeking medical care (Feb 27; week 9).

SARS-COV-2 indicates severe acute respiratory syndrome coronavirus 2; WHO, World Health Organization.

Table. Results From the Difference-in-Difference Model Assessing the Difference in the Estimated Number of Seasonal Influenza Cases in the 2019/2020 Season vs Previous 5 Seasons

Calendar week	Estimated No. of cases[a]		Difference-in-difference value in 2019/2020 vs 2014-2019 seasons (95% CI)[a,b]
	2019/2020 season	2014-2019 seasons	
40	71	10	
41	65	10	
42	52	13	
43	58	17	
44	69	22	
45	74	30	
46	133	44	
47	225	71	
48	402	117	
49	688	185	
50	1127	340	
51	1533	652	
52	1678	947	
1	1006	965	-245 (-1535 to 1046)
2	1322	1703	-667 (-1957 to 624)
3	1209	2634	-1712 (-3002 to -421)
4	1301	3048	-2033 (-3324 to -743)
5	1019	2883	-2150 (-3440 to -859)
6	654	2306	-1937 (-3228 to -647)
7	541	1668	-1413 (-2704 to -123)
8	447	1344	-1182 (-2473 to 108)
9	344	1129	-1071 (-2361 to 220)
10	227	864	-923 (-2214 to 368)
11	93	681	-874 (-2164 to 417)

[a] Numbers are reported in thousands.

[b] Subtraction of differences before week 1 from week 1 to 11 differences. Negative values represent fewer cases in the 2019/2020 season vs the 2014 to 2019 seasons. The difference-in-difference regression model included categorical variables for each week of the season and for the 2019/2020 season (vs previous seasons) and interaction variables between each of weeks 1 to 11 and the 2019/2020 season.

number (n = 72 201) representing the number of outpatient visits to hospitals and clinics in the country in 2019[3] vs the health care institutions in the surveillance system.[1,4] We estimated the change in influenza activity after the SARS-CoV-2 outbreak using a "difference-in-difference" regression model that included a variable for each week, a variable representing the average difference in influenza activity per week for the 2019/2020 season vs the 2014 to 2019 seasons before the outbreak (week 1-11), and interaction variables for each week after the outbreak and the 2019/2020 season. The difference-in-difference value was considered statistically significant if the 95% CI did not overlap 0. Approximately 10% of the sentinel centers provided samples from a subset of influenza cases from week 36 through week 7 in the 2019/2020 season and from week 36 through week 35 in the 2014 to 2019 seasons for analysis using polymerase chain reaction (PCR) testing. Using these data we assessed the predominant subtype of the influenza virus and compared the distribution of cases by age group (aged <15, 15-54, and ≥55 y) in the 2019/2020 season vs the 2014 to 2019 seasons (not including the 2015/2016 season, for which age-specific data were not available) using the χ^2 test. Stata version 16.1 (StataCorp) was used. Institutional board review was not required because no individual-level data were used.

Results | Analyses were based on 8 414 693 cases of influenza (981 373 from the 2019/2020 season). Across all seasons, influenza activity increased toward the end of the year. While influenza activity reached its peak between week 4 and 6 in the 2014 to 2019 seasons, there was a plateau in the beginning of the year and a decrease from week 5 onwards in the 2019/2020 season (**Figure**). In the difference-in-difference analysis, influenza activity was significantly lower from week 3 through week 7 in the 2019/2020 season vs the 2014 to 2019 seasons (**Table**). PCR test results were available on 51 847 samples. The predominant subtypes of influenza virus are shown in the Figure. The number of PCR-confirmed cases in the 2014 to 2019 seasons was 25 930 (63.3%) in individuals younger than 15 years, 10 215 (24.9%) in individuals aged 15 to 54 years, and 4801 (11.7%) in individuals aged at least 55 years; in the 2019/2020 season, the numbers were 2267 (68.9%) in individuals younger than 15 years, 770 (23.4%) in individuals aged 15 to 54 years, and 254 (7.7%) in individuals aged at least 55 years. A lower proportion of cases in the 2019/2020 season vs previous seasons included individuals aged at least 15 years ($P < .001$).

Discussion | Seasonal influenza activity was lower in 2020 than in previous years in Japan. Influenza activity may have been affected by temperature[5] or virulence (although influenza activity in the 2019/2020 season was moderately severe in other parts of the world[6]), but also by measures taken to constrain the SARS-CoV-2 outbreak. While closure of schools and suspension of large events occurred late in the influenza

season, awareness regarding measures to reduce the risk of disease transmission was high among the Japanese public from early in the year. Limitations of this study include lack of availability of age-specific weekly data on influenza activity and information regarding means of diagnosis. Concerns regarding the SARS-CoV-2 outbreak may have changed detection of influenza through changes in symptomatic individuals seeking medical attention or in physicians' inclination to test for influenza.

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Concept and design: All authors.
Acquisition, analysis, or interpretation of data: All authors.
Drafting of the manuscript: All authors.
Critical revision of the manuscript for important intellectual content: All authors.
Statistical analysis: Sakamoto, Ueda.
Administrative, technical, or material support: Sakamoto, Ishikane.
Supervision: Sakamoto, Ishikane.

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Coronavirus in India: Guwahati doctor dies after allegedly taking hydroxychloroquine

Some other doctors had also taken hydroxychloroquine along with the deceased, Dr Utpal Barman.

Hemanta Kumar Nath
Guwahati
March 30, 2020
UPDATED: March 30, 2020 23:11 IST

The colleagues of the deceased doctor said that he died due to a cardiac arrest. (File photo: AP)

A Guwahati-based doctor who allegedly took anti-malaria drug hydroxychloroquine amid the novel coronavirus (Covid-19) outbreak died at a private hospital in the capital city of Assam.

The colleagues of the deceased doctor said that he died due to a cardiac arrest.

According to the reports, 44-year-old Dr Utpal Barman - a senior anaesthetist at Guwahati-based Pratiksha Hospital was admitted at Guwahati Neurological Research Centre (GNRC) on Sunday evening following his heart-related complications.

Pratiksha Hospital Superintendent Dr Nirmal Kumar Hazarika said that on Sunday he and other doctors of Pratiksha Hospital had rushed to the residence of Dr Utpal Barman and admitted him to another hospital in Guwahati.

"He complained of some chest pain and other complications and we immediately admitted him to GNRC hospital. All symptoms have indicated that, it could be marked gum infection," Dr Nirmal Kumar Hazarika said.

Dr Nirmal Hazarika said that earlier Dr Utpal Barman took the anti-malaria drug hydroxychloroquine.

"Many of doctors have taken hydroxychloroquine, they are having this drug. I came to know that one super specialty hospital in Karnataka had directed the employees to have this medicine. They had also directed to their employees to collect the medicine from their store. Many doctors have accepted this. We know that every medicine has adverse effect," Dr Nirmal Kumar Hazarika said.

The senior doctor of Pratiksha Hospital added that some other doctors had also taken hydroxychloroquine along with Dr Utpal Barman.

However, it is not clear if the doctor's death is linked with hydroxychloroquine.

The national taskforce for Covid-19 constituted by Indian Council for Medical Research (ICMR) has recommended the use of hydroxychloroquine for prophylaxis of SARS-CoV-2 infection for high-risk population.

READ | Don't use hydroxychloroquine without prescription: Govt after people panic-buy 'miracle' cure to Covid-19
ALSO READ | Medicines go out of stock as coronavirus lockdown hits Delhi pharmacies
ALSO WATCH | ICMR recommends anti-malarial drug for high-risk coronavirus cases

तेहरान के अस्पताल में अजवायन और अदरक से कोरोना के बीमारों का इलाज, 200 बीमारों में से 190 ठीक होकर डिसचार्ज+वीडियो

Apr ०४, २०२० १४:३७ Asia/Kolkata

ईरान में कोरोना वायरस के संक्रमितों के इलाज के लिए पारम्परिक चिकित्सा शैली का भी प्रयोग किया जा रहा है और इसके लिए राजधानी तेहरान में शोहदाए गुमनाम अस्पताल को विशेष कर दिया गया है और इस अस्पताल से बहुत अच्छी ख़बरें मिल रही हैं।

कोरोना की बीमारी फैली तो पारम्परिक व इस्लामी चिकित्सा विशेषज्ञों ने स्वास्थ्य मंत्रालय से मांग की कि उन्हें भी इस बीमारी के इलाज और इसकी रोकथाम के अभियान में शामिल किया जाए जिसके बाद स्वास्थ्य मंत्रालय ने एक पत्र लिखकर पारम्परिक चिकित्सा शैली के प्रयोग से कोरोना के बीमारों के इलाज की अनुमति दी।

पारम्परिक चिकित्सा विशेषज्ञों ने शोहदाए गुमनाम अस्पताल में कोरोना वायरस से संक्रमित बीमारों को एडमिट करना और उनका इलाज शुरू किया।

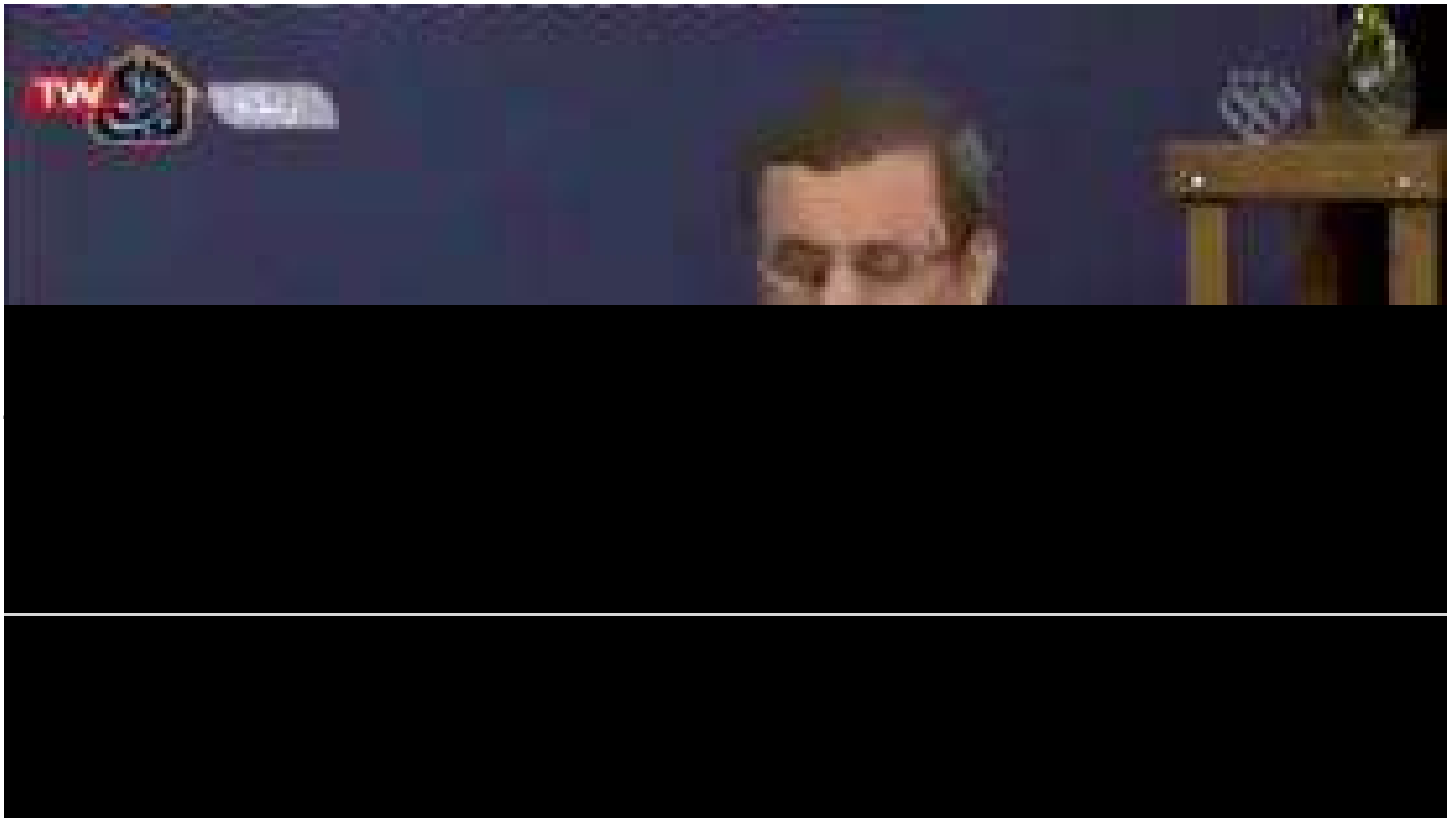
रोचक बात यह है कि पारम्परिक चिकित्सा शैली से किए जाने वाले इलाज का बहुत अच्छा नतीजा मिला है। पारम्परिक व इस्लामी चिकित्सा शैली के विशेषज्ञ डाक्टर रज़ा मुंतज़िर ने बताया कि रोकथाम और इलाज की दो शैलियों के आधार पर हमने काम किया जिसका नतीजा यह निकला कि कोरोना से संक्रमित लोग आठ दिन के बजाए चार दिन में ठीक होकर अस्पताल से डिसचर्ज हो गए।

डाक्टर रज़ा मुंतज़िर ने बताया कि हम माडर्न मेडिकल साइंस के साथ साथ अपनी चिकित्सा शैली को आगे बढ़ा रहे हैं।

संक्रामक रोग विशेषज्ञ और पारम्परिक चिकित्सा शैली के अध्ययनकर्ता डाक्टर फ़सीही दस्तजर्दी ने जो शोहदाए गुमनाम अस्पताल में इस प्रोजेक्ट के डायरेक्टर हैं इस बारे में बताया कि हमने कोविड-19 के लक्षणों की तुलना इनफ़लुएंज़ा के लक्षणों से की तो हमें दोनों में काफ़ी समानता नज़र आई इसलिए हमने कोरोना की रोकथाम और इलाज में अजवायन और अदरक को शामिल किया और बेहतरीन नतीजा मिला है।

डाक्टर दस्तजर्दी ने बताया कि हमने अस्पताल के अधिकारियों से कहा कि कोरोना के बीमारों के खाने में दही का प्रयोग बिल्कुल न किया जाए, इसके साथ ही उन्हें हमने सूप में दारचीनी दी। उन्होंने बताया कि हमारे अस्पताल में दो हफ़्ते के दौरान कोरोना से केवल दो मौतें हुईं।

डक्टर दस्तजर्दी ने बताया कि 200 बीमारों से 190 डिसचार्ज हो चुके हैं।



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Can Alcohol-Based Hand-Rub Solutions Cause You To Lose Your Driver's License? Comparative Cutaneous Absorption of Various Alcohols▽

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We assessed cutaneous ethanol (ETOH) and isopropanol (ISOP) absorption after intensive (30 times per h) use of alcohol-based hand-rub solutions by healthcare workers (HCWs). ETOH was detectable in the breath of 6/20 HCWs (0.001 to 0.0025%) at 1 to 2 min postexposure and in the serum of 2/20 HCWs at 5 to 7 min postexposure. Serum ISOP levels were unrecordable at all time points.

Although hand hygiene culture-change programs using alcohol-based hand-rub solutions (ABHRS) have been associated with a reductions in nosocomial infections, some health care workers (HCWs) remain concerned about potential cutaneous absorption of alcohol from ABHRS (1, 4, 10, 11, 13). In particular, some young HCWs who are required to have a zero serum alcohol level to legally drive automobiles (probationary license) and HCWs of Islamic faith may have reservations about their exposure to alcohol (1, 13). Thus, we aimed to assess the cutaneous absorption of the two most commonly used alcohols (ethanol [ETOH] and isopropanol [ISOP]) among HCWs who used ABHRS intensely (13).

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Consenting HCW volunteers completed a questionnaire recording their age, height, weight, gender, ethnicity, alcohol consumption during the 24 h prior to the study, and prescribed medication usage. Participants' heights and weights were used to calculate their body mass indexes (BMI). HCWs were excluded if they had a evidence of chronic dermatitis (e.g., eczema) or broken/damaged skin or a history of allergy to ABHRS or were currently pregnant.

We assessed two commonly used ABHRS that contained 0.5% chlorhexidine gluconate, a skin emollient and either 70% ETOH (Avagard; 3M Healthcare, Pymble, Australia) or 70% ISOP (DeBug; Orion Laboratories Pty Ltd., Balcatta, Australia) (4, 13). To mimic intensive clinical conditions, HCWs used ABHRS 30 times during a 1-h period on two separate days, with a 1 day "washout" period between (day 1, Avagard use; day 2, washout; day 3, DeBug use). Supervisors coordinated, timed, and advised all participants when to reapply ABHRS and ensured compliance with the correct application (one squirt [1.2 to 1.5 ml] every 2 min) of ABHRS (13). Study room conditions were as follows: room temperature, 24 to 26°C; humidity, 39 to 42%; study room volume, 124 cubic meters.

Breath and serum alcohol levels were assessed as follows. Preexposure (baseline), breath and serum alcohol levels were assessed. Postexposure (time after last application of ABHRS), at 1 to 2 min, breath levels only were tested; at 5 to 7 min, serum levels only were tested; and 10 to 13 min, breath levels only were tested. Breath alcohol levels were assessed by police from the Traffic Alcohol Section, Victoria Police, using a Drager Alcotest 7110 breathalyzer (lower limit of detection, 0.001%), as is used by Victoria Police for all evidential breath alcohol analysis, following preliminary roadside breath testing using a hand-held screening device. Results from this breathalyzer are sufficiently accurate to be legally admissible in court and obviate the need for serum ETOH assessment. The breathalyzer detects ETOH but not ISOP. All breathalyzer analyses were undertaken in a room distant from where ABHRS was in use to avoid potential vapor contamination of breath alcohol tests.

Serum ETOH and ISOP levels were assessed by gas chromatography (lower limit of quantitation, 0.002 g/100 ml [%]; lower limit of detection: 0.0001 g/100 ml [%] for both alcohols) at the Victorian Institute of Forensic Medicine, where all serum/blood alcohol assessments are undertaken for the State Coroner of Victoria. Serum specimens were collected in routine sodium fluoride/EDTA venipuncture tubes and stored at 4°C until analysis. Alcohol-containing skin cleansers were not used to swab the skin before venipuncture. The study protocol was approved by our institution's Human Ethics Committee.

Twenty HCWs (mean age, 40 ± 13 years [median, 36 years; range, 22 to 67 years]; 14 females; ethnic distribution, 18 Caucasian, 2 Asian) participated in the study. Participants' mean BMI was 26 ± 4 (median, 24; range, 21 to 34; acceptable BMI, $n = 11$; overweight BMI, $n = 4$; obese BMI, $n = 5$) (6). One HCW, who regularly used DeBug without any adverse reactions prior to this study, developed a severe cutaneous reaction to Avagard after day 1 such that she could not participate on day 3. Thus, 20 HCWs completed use of Avagard and 19 used DeBug in the study. Both ABHRS groups were sampled at

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TABLE 1. Breath and serum alcohol levels before and after intensive use of alcohol-based hand-rub solution

Time and type of specimen	No. of HCWs with detectable alcohol levels/total no. of HCWs	
	Ethanol (n = 20)	Isopropanol (n = 19)
Preexposure (baseline)		
Breath	0/20	NA[a]
Serum	0/20	0/19
Postexposure		
1–2 min, breath	6/20[b]	NA
5–7 min, serum	2/20[c,d]	0/19[d]
10–13 min, breath	0/20	NA

[a] NA, not assessable by Drager Alcotest 7110 breathalyzer.
[b] Specific levels for these six HCWs were 0.0010%, 0.0012%, 0.0014%, 0.0014%, 0.0018%, and 0.0025%.
[c] Specific levels for these two HCWs were 0.0006% and 0.0015%.
[d] No statistical difference between 2/20 versus 0/19 HCWs (P = 0.49, Fisher's exact test).

similar times postexposure (mean ± standard deviation minutes after last application: ETOH, 2.3 ± 1.2, 6.4 ± 1.6, and 13.4 ± 1.7; ISOP, 1.9 ± 1.2, 7.1 ± 1.6, and 12.0 ± 1.7).

Results are shown in Table 1. ETOH levels were detectable in breath analysis of 6 of the 20 HCWs (range, 0.0010% to 0.0025%) at 1 to 2 min after the final application of Avagard: all would have been recorded as undetectable by Victoria Police performing routine roadside breathalyzer testing. However, two of these six HCWs also had detectable serum ETOH levels at 5 to 7 min postexposure. All breath ETOH levels were zero at 10 to 13 min after Avagard use. Measurable ETOH levels were not associated with HCW age, sex, ethnicity, or BMI, but statistical power was limited due to the low number of participants with detectable levels. All serum ISOP levels were unrecordable at each time point.

This study mimicked clinical settings in which intensive use of ABHRS of up to 30 times per h is required, such as in intensive care units (4, 10). We limited our study to a 1-h duration, since after such periods of intense activity, HCWs frequently wash their hands in soap and water because they have eventually become visibly soiled or because they take a break from clinical activity (2, 4, 10). Unlike one recent case report (5), our study demonstrates that very small amounts of ETOH may be absorbed during intensive use, either via transcutaneous absorption or inhalation of fumes in closed areas. However, none of these levels would be considered positive during either a routine or evidential police breath alcohol test. In comparison, no detectable serum ISOP absorption could be detected during this study.

Our findings appear to differ from those of Turner et al. who detected small levels of ISOP (0.5 to 1.8 mg/liter) in 9 of 10 participants after using ABHRS six times per h for 4 h (11). However, the assay they used had a lower limit of detection of 0.0005% (one dilution more sensitive than our assay) and a number of their participants had very low ISOP levels (0.0005% to 0.001%). Secondly, they applied a larger volume (3 ml) of 52.6% ISOP-containing ABHRS and did not wash their hands with soap and water for >4 h.

Our study has some limitations. First, since 9/20 HCWs were either overweight or obese, we cannot be sure whether lower-body-weight HCWs might not have higher levels. Secondly, we did not assess the routine alcohol consumption of our HCWs and therefore cannot be certain of the impact of increased alcohol metabolism on serum levels. Finally, we cannot be sure that intensive ABHRS use for longer than 1 h without washing may not result in higher absorption or accumulation rates (4, 10, 13).

Although there are many reasons described by HCWs regarding why they exhibit poor hand hygiene compliance (3, 7, 8, 9, 12), fear of alcohol absorption and loss of one's drivers license is no longer valid. Since ISOP appears slightly more predictable in its lack of cutaneous absorption than ETOH, ISOP-containing ABHRS may be preferred by some HCWs and religious groups.

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There are no conflicts of interest. However, DeBug (a trademark for one of the hand hygiene product referred to in this article) was developed by some of the authors (employees of Austin Health) with funding in part from the Department of Human Services, Victoria, Australia. The intellectual property for this development is held by Austin Health, which handles all patent, trademark, and licensing issues. Austin Health, but no individual author, receives a small income stream from the sale of DeBug.

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FOCUS ON: ALCOHOL AND THE IMMUNE SYSTEM

Patricia E. Molina, M.D., Ph.D.; Kyle I. Happel, M.D.; Ping Zhang, M.D., Ph.D.; Jay K. Kolls, M.D.; and Steve Nelson, M.D.

Alcohol abuse suppresses multiple arms of the immune response, leading to an increased risk of infections. The course and resolution of both bacterial and viral infections is severely impaired in alcohol-abusing patients, resulting in greater patient morbidity and mortality. Multiple mechanisms have been identified underlying the immunosuppressive effects of alcohol. These mechanisms involve structural host defense mechanisms in the gastrointestinal and respiratory tract as well as all of the principal components of the innate and adaptive immune systems, which are compromised both through alcohol's direct effects and through alcohol-related dysregulation of other components. Analyses of alcohol's diverse effects on various components of the immune system provide insight into the factors that lead to a greater risk of infection in the alcohol-abusing population. Some of these mechanisms are directly related to the pathology found in people with infections such as HIV/AIDS, tuberculosis, hepatitis, and pneumonia who continue to use and abuse alcohol. *KEY WORDS: Alcohol abuse; alcohol and other drug effects and consequences; immune system; immune response; immunosuppressive effect; infection; bacterial infection; viral infection; communicable disease; host defense mechanisms*

Both acute and chronic alcohol abuse can induce significant defects in the body's defense against microorganisms (i.e., pathogens) by interfering with multiple aspects of the immune response. The resulting increased risk and severity of infections in chronic alcoholics has been recognized as early as 1785, by Benjamin Rush, the first Surgeon General of the United States. The impact of alcohol abuse on risk and severity of infection has been demonstrated particularly well for infections of the respiratory tract, especially bacterial pneumonia and tuberculosis (Zhang et al. 2008). Alcohol consumption also is associated with a higher prevalence of hepatitis C infection (Prakash et al. 2002) and increases the risk of infection with the human immunodeficiency virus (HIV), particularly in binge drinkers (Baliunas et al. 2009). In addition to increasing the risk of infections, alcohol abuse has been reported to contribute to the morbidity and mortality resulting from these infections in alcohol-abusing patients. This is particularly relevant in chronic infections, such as HIV and hepatitis C. After providing a brief overview of the human immune system and its various components, this article summarizes alcohol's diverse effects on these components.

Overview of the Human Immune System

The body constantly is exposed to pathogens that penetrate either our external surface (i.e., the skin), through wounds or burns, or the internal surfaces (i.e., epithelia) lining the respiratory and gastrointestinal (GI) tracts. The body responds to such an infectious challenge with a two-level response. The first line of defense is called the innate immunity;[1] it exists from birth, before the body is even exposed to a pathogen. It is an immediate and rapid response that is activated by any pathogen it encounters (i.e., is nonspecific); in addition, it plays a key role in the activation of the second level of the immune response, termed the adaptive or acquired immunity. This part of the immune response is specific to one particular pathogen and also creates an "immune memory" that allows the body to respond even faster and more effectively if a second infection with the same pathogen occurs. Both innate and adaptive immunity rely on a multitude of different cells and molecules. Thus, both types of immunity are mediated partly by the actions of specific immune cells (i.e., include a cell-mediated response) and partly by the actions of molecules secreted by various immune cells (i.e., include a humoral response).

The Innate Immune Response

The innate immune response comprises five main elements:

- The physical barrier formed by epithelial cells in the skin, gut mucosa, and airways that prevents the entry of pathogens into the body;
- A chemical shield to prevent microbial growth and invasion that is provided by antimicrobial peptides, reactive oxygen species, and the pH and lipid composition of the internal and external surfaces;
- A pathogen recognition system that identifies invading pathogens (e.g., through molecules called Toll-like receptors);
- An inducible response to invading pathogens that includes cell-mediated and humoral components; and
- The coordinated recruitment of other cells that amplify the response.

[1] For a definition of this and other technical terms, see the Glossary, pp. 161–164.

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Virtually all of these components are affected by alcohol; however, the discussion in the following sections will focus on the first and fourth of these elements.

The cell-mediated arm of the innate immunity is orchestrated primarily by granulocytes, monocytes/macrophages, dendritic cells, and natural killer (NK) cells. Granulocytes are white blood cells (i.e., leukocytes) that derive their name from the large granules that are visible when the cells are stained for microscopic analysis. They further are characterized by oddly shaped nuclei with multiple lobes and therefore also are called polymorphonuclear leukocytes (PMNs). They represent approximately 60 percent of all circulating leukocytes. The most abundant type of PMNs is called neutrophils. These cells act as phagocytes—that is, they engulf pathogens and ingest them in a process called phagocytosis. In addition, they can excrete toxic substances from their granules that can kill pathogens. PMNs produce a host of bacteria-killing (i.e., bactericidal) molecules (e.g., myeloperoxidase, defensins, azurophil-derived bactericidal factors, bactericidal permeability-increasing protein, cationic proteins, gelatinase, and lactoferrin). In addition, PMNs participate in the regulation of the local defense response by releasing signaling molecules called cytokines and chemokines (e.g., tumor necrosis factor [TNF]-α; interleukin [IL]-1β, IL-6, and IL-8; and macrophage inflammatory protein [MIP]-2). These molecules help recruit and activate additional PMNs as well as macrophages to the site of an injury or infection.

Monocytes and macrophages are leukocytes with a single-lobed nucleus that also act as phagocytes and which therefore also are called mononuclear phagocytes. Monocytes are an immature form of these cells that circulate in the blood until they are alerted to the presence of a pathogen in a particular tissue. Once they are at the site of infection, they swell in size and develop into the mature defensive cells—the macrophages—that enter the tissues. After eliminating pathogens by phagocytosis, the monocytes exhibit pathogen-derived proteins and other molecules (i.e., antigens) on their surfaces. This is important for activating the cells of the adaptive immune response. Finally, monocytes and macrophages also produce certain cytokines that help regulate immune system activity.

Dendritic cells also are mononuclear phagocytes derived from monocytes. Their main role is to capture, ingest, and process antigens in order to present them on their surface to cells of the adaptive immune response (i.e., to the T-lymphocytes). Thus, dendritic cells play a crucial role in linking innate and adaptive immune responses. Lastly, NK cells are abundant in the liver (Gao et al. 2009) and recognize cells that have low levels of a protein called class I major histocompatibility complex (MHC) on their surface. This reduced class I MHC expression can result from infection with certain types of viruses. NK cells eliminate cells with low class I MHC expression as well as cancer cells.

The most important components of the humoral arm of the innate immune response include the following molecules:

- Cytokines and chemokines. Cytokines are proteins made and released by one cell that affect the behavior of other cells (e.g., activate other cells) and cell–cell interactions. Thus, cytokines released by immune cells control immune processes by regulating the production of new immune cells from precursor cells, activating lymphocytes and phagocytes, coordinating the cell-mediated and humoral immune responses, mediating the process of inflammation, and killing cells directly. Important cytokines are TNF-α and the ILs. Chemokines are similar to cytokines; however, their main function is to attract additional cells (e.g., monocytes and neutrophils) to the site of an infection.

- Interferons (IFNs) are proteins that are involved in the immune response to viral infection. Thus, they participate in inducing a state of resistance to viral replication and upregulate the cell-mediated immune response to viral infection.

- The complement system comprises a large number of distinct plasma proteins that react with one another to cover the surface of a pathogen so that it can be recognized and ingested by phagocytes. This process, which is called opsonization, induces a series of inflammatory responses that help combat the infection. The complement system can be activated through three different biochemical pathways.

- Acute-phase proteins are, as the name implies, produced early during an inflammatory response to infection. They participate in the opsonization of pathogens and of monocytes that have ingested pathogens as well as in the activation of the complement cascade. Important acute-phase proteins are C-reactive protein, mannan-binding lectin, and pulmonary surfactants A and D.

The innate immune response orchestrated by all these components provides the first line of defense against invading pathogens and plays a key role in the activation and orientation of adaptive immunity, as well as in the maintenance of tissue integrity and repair. Only if a pathogen can evade the different components of this response (i.e., structural barriers as well as cell-mediated and humoral responses) does the infection become established and an adaptive immune response ensues.

The Adaptive Immune Response

The innate immune response to a pathogen is followed by an adaptive immune response that is activated only after the body is exposed to the pathogen for the first time and which is specific to that one pathogen. This activation of the adaptive immune response depends on the display of antigens from the invading pathogen (or any other foreign molecule) on the surface of antigen-presenting cells (e.g., monocytes or dendritic cells) in a way that can be recognized by the cells mediating the adaptive immune response—that is, the T-lymphocytes (or T-cells) and the B-lymphocytes (or B-cells).

T-cells are responsible for the cell-mediated arm of the adaptive immune response. After their formation in the bone marrow and maturation in the thymus, they remain in an inactive (naïve) state until they encounter a specific antigen. This encounter activates the T-cells, which then further differentiate into different subtypes. Two important subtypes of T-cells are the following:

- Helper T-cells produce cytokines to stimulate the activity of other immune cells. According to the cytokines they produce, they are categorized into three subsets: (1) Th1 helper cells that produce IFN-γ and mediate immunity against intracellular pathogens; (2) Th2 helper cells that produce IL-4, IL-5, and IL-13 and promote humoral immunity and allergic responses; and (3) Th17 helper cells that produce IL-17, IL-21, and IL-22 and are implicated in host defense and autoimmunity. Helper T-cells (as well as a few other immune cells, such as macrophages) are characterized by the presence of a molecule called CD4 on their surface; this molecule serves as the receptor to which HIV can bind when it infects the cells. Accordingly, CD4-carrying (i.e., CD4+) helper T-cells are the major target of HIV infection. Their depletion leads to the development of the acquired immunodeficiency syndrome (AIDS) and the development of numerous opportunistic infections, including pneumonia caused by infection with the fungus *Pneumocystis* or infections with the yeast *Candida albicans,* the tuberculosis pathogen *Mycobacterium* tuberculosis, and several other pathogens that usually cause no harm in people with a healthy immune system (Phair 1990).

- Cytotoxic T-cells recognize antigens on the surface of virus-infected or transplanted cells and destroy these cells; each cytotoxic T-cell recognizes only one specific antigen. Cytotoxic T-cells are characterized by the presence of a molecule called CD8 on their surface.

B-cells are responsible for the humoral arm of the adaptive immune response. They produce immune molecules called antibodies or immunoglobulins that they can either display on their surface or secrete. The antibodies can recognize and interact with antigens, and each B-cell produces antibodies that recognize only one specific antigen. The antigen–antibody interaction leads to the activation of the B-cell. The activated B-cell then begins to multiply and mature fully in a series of developmental processes that are accompanied by changes in the class of immunoglobulin that the cell produces (i.e., immunoglobulin class switching).[2] In most cases, the resulting daughter cells develop into plasma cells, which secrete many copies of the antibody into the blood or fluid between cells. These antibodies then will bind to any matching antigen molecules they encounter in the blood or on other cells, thereby marking them for destruction. Some B-cells, however, become memory cells that will remain dormant in the body for years and can be activated rapidly if a second infection with the same pathogen occurs. The activities of T-cells and B-cells are intricately intertwined through the actions of various cytokines to orchestrate an effective immune response to any pathogen the organism may encounter.

Both the innate and the adaptive immune response are critical for effective host defense to infectious challenges. Multiple aspects of both arms of the immunity response are significantly affected by alcohol abuse, as described in the following sections.

Alcohol and the Innate Immune Response

Alcohol and Structural Host Defense Mechanisms

The first line of host defense involves both structural (i.e., epithelial) cells and immune cells (i.e., macrophages and dendritic cells) at mucosal surfaces. The epithelial cells function as a physical barrier as well as regulators of the innate and adaptive immunity. Particularly important are the epithelial immune barriers of the reproductive, GI, and respiratory tracts. Several lines of evidence suggest that alcohol abuse significantly disrupts the GI and respiratory tract immune barriers.

Effects on the GI Tract. The GI tract is the organ exposed to the highest concentration of alcohol during acute or chronic ingestion. Therefore, it has been studied extensively with respect to the pathologic effects of alcohol, particularly as they impact the ability of the intestinal barrier to allow passage of certain substances into the blood (i.e., intestinal permeability). Collective evidence from animal and human studies indicates that chronic alcohol abuse results in excessive intestinal permeability, which may underlie several of the health consequences of excessive alcohol consumption (Keshavarzian et al. 1999; Rao et al. 2004). For example, alterations in cell structures called tight junctions in the epithelial cells lining the intestine contribute to the pathophysiology of alcohol-induced intestinal permeability (Rao 2009). These tight junctions are areas where two epithelial cells are closely associated with each other. They serve to hold the cells together and to prevent the direct passage of water and other molecules from the intestine into the blood stream. Thus, if the tight junctions are damaged (e.g., by alcohol's actions), material from the intestine can "leak" into the blood, as has been shown by increased levels of bacterial molecules called lipopolysaccharides (LPSs) in the blood of alcoholic patients (Hanck et al. 1998). Alcohol interferes with tight-junction functioning through several mechanisms. For example, alcohol (or its metabolite acetaldehyde) impairs trafficking of epithelial tight-junction proteins, such as zona occludens (ZO)-1 and occludin (Atkinson and Rao 2001). Moreover, alcohol-induced epigenetic effects may modulate the production of tight-junction protein. Thus, studies

[2] The different immunoglobulin classes are involved in different aspects of the immune response. However, all immunoglobulins produced by one B-cell and its daughter cells specifically recognize the same antigen.

found that alcoholics with liver disease exhibited dramatically increased expression of a small, regulatory molecule called microRNA (miR) 212 in colon tissue samples (Tang et al. 2008). miR-212 can bind to the messenger RNA (mRNA) from which the ZO-1 protein is produced; this binding prevents ZO-1 production, thereby contributing to alcohol-induced increased permeability of the intestinal epithelium and to the "leaky" alcoholic gut.

The consequences of impaired gut structural integrity are significant (see figure 1). Increased intestinal leakage allows bacteria-derived products, such as LPSs, to enter the blood stream supplying the liver (i.e., the portal circulation) and, in the liver, to activate a variety of cells, including endothelial cells, liver macrophages (i.e., Kupffer cells), stellate cells, and the main liver cells (i.e., hepatocytes). This results in a chronic inflammatory environment conducive to liver injury.

In addition to contributing to the pathogenesis of alcoholic liver disease (Rao 2009), other observations suggest that enhanced endothelial permeability is detrimental to HIV disease course in alcohol-abusing patients. In the simian immunodeficiency virus (SIV)/rhesus macaque model of HIV infection, chronic alcohol feeding increased the number of virus particles in the blood (i.e., plasma viral load) and hastened the progression to AIDS (Bagby et al. 2006; Poonia et al. 2006). Both HIV and SIV infection themselves cause extensive intestinal disease and enhanced intestinal permeability during advanced disease stages; moreover, evidence from HIV-infected humans and SIV-infected primates shows a compelling association between

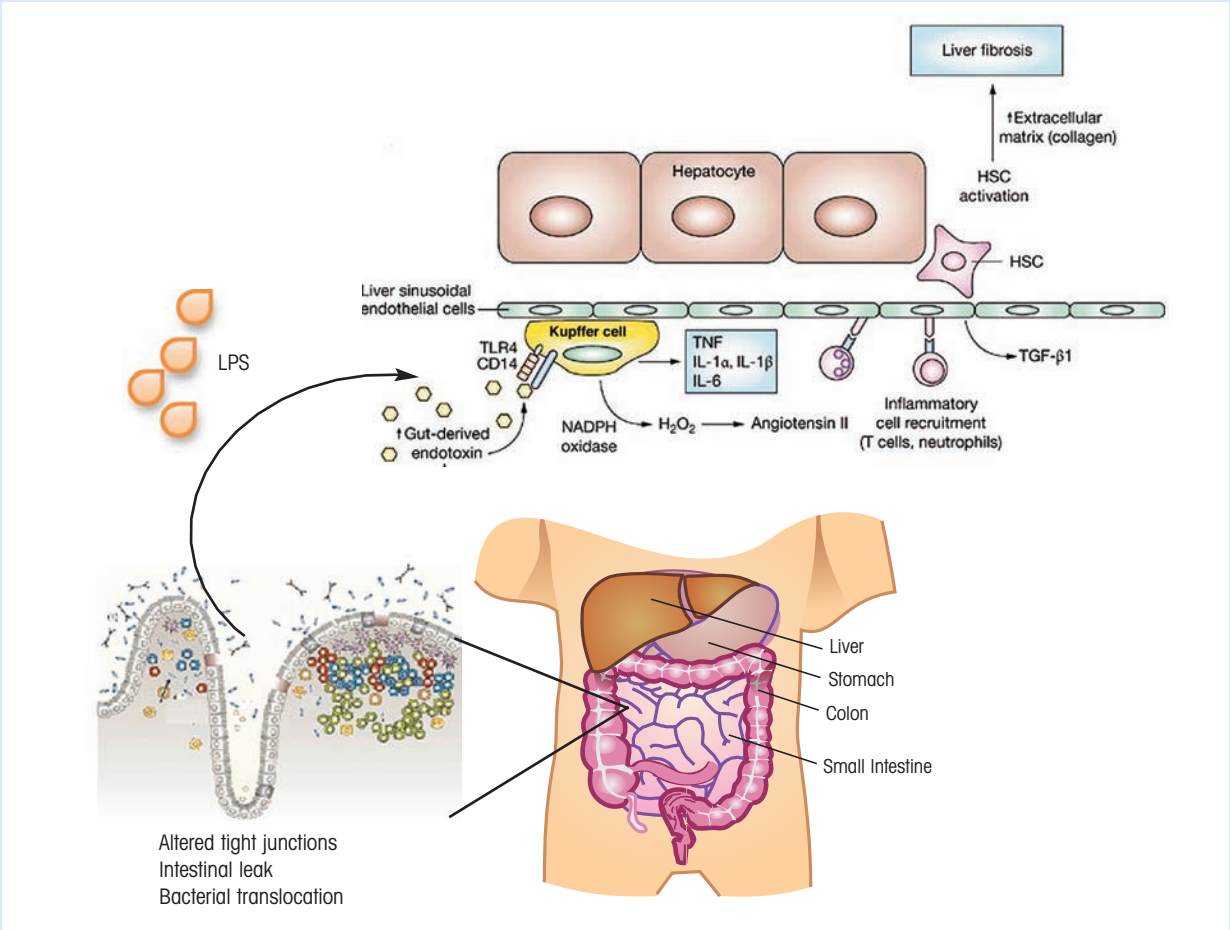

Figure 1 Alcohol's effects on the structural host defense of the gastrointestinal (GI) tract. Alcohol-induced changes in tight junctions cause increased intestinal leaks that lead to translocation of bacteria-derived products such as lipopolysaccharide (LPS). These molecules enter the circulation to the liver where they activate endothelial and stellate cells as well as hepatocytes, resulting in a chronic inflammatory environment aggravating organ injury. This also may contribute to HIV disease pathophysiology.

NOTE: CD14 = cluster of differentiation 14; HSC = hepatic stellate cell; IL = interleukin; NADPH = nicotinamide adenine dinucleotide phosphate; TGF= tissue growth factor; TNF = tumor necrosis factor; TLR4 = toll-like receptor 4.

the entry of microbial antigen into the circulation and the progression of retroviral disease (Brenchley et al. 2006). It is hypothesized that the HIV-related leakage results in chronic activation of the immune system. Because HIV infects primarily immune cells (i.e., CD4+ T-cells and macrophages), this activation leads to the generation of more target cells for the virus; eventually, however, the body's capacity to replenish CD4+ T-cells is exhausted, which results in disease progression to AIDS. The enhanced gut permeability resulting from alcohol abuse is likely to exacerbate the gut leak associated with HIV/SIV infection, thereby further accelerating disease progression.

Effects on the Respiratory System. Mucosal organ "leakiness" resulting from chronic alcohol exposure also contributes, through a variety of mechanisms, to the pathophysiology of acute respiratory distress syndrome (ARDS) or acute lung injury, a serious complication frequently associated with sepsis and trauma in alcohol-abusing patients (Moss et al. 1996) (see figure 2). Chronic alcohol abuse decreases the levels of the antioxidant glutathione in the lung, leading to oxidative injury that predisposes to ARDS (Holguin et al. 1998). Alcohol abuse also affects the tight junctions between the epithelial cells in the small airsacs (i.e., alveoli) where the exchange of oxygen and carbon dioxide occurs in the lung. Moreover, chronic alcohol abuse interferes with the actions of a signaling molecule called granulocyte/macrophage colony–stimulating factor (GM–CSF), which is secreted by various cells (including epithelial cells) and stimulates the production of granulocytes and monocytes. GM–CSF signaling by alveolar epithelial type II (AE2) cells is important for protecting the body against lung infections because it induces macrophage maturation and promotes epithelial barrier maintenance (Joshi and Guidot 2007). Finally, the ciliated epithelium of the airways (i.e., bronchi), which also is a critical structural component of innate lung immunity, has been reported to be impaired by alcohol (Elliott et al. 2007). This increases the risk of airborne bacteria entering the lungs, contributing to the increased risk of infection associated with alcohol abuse.

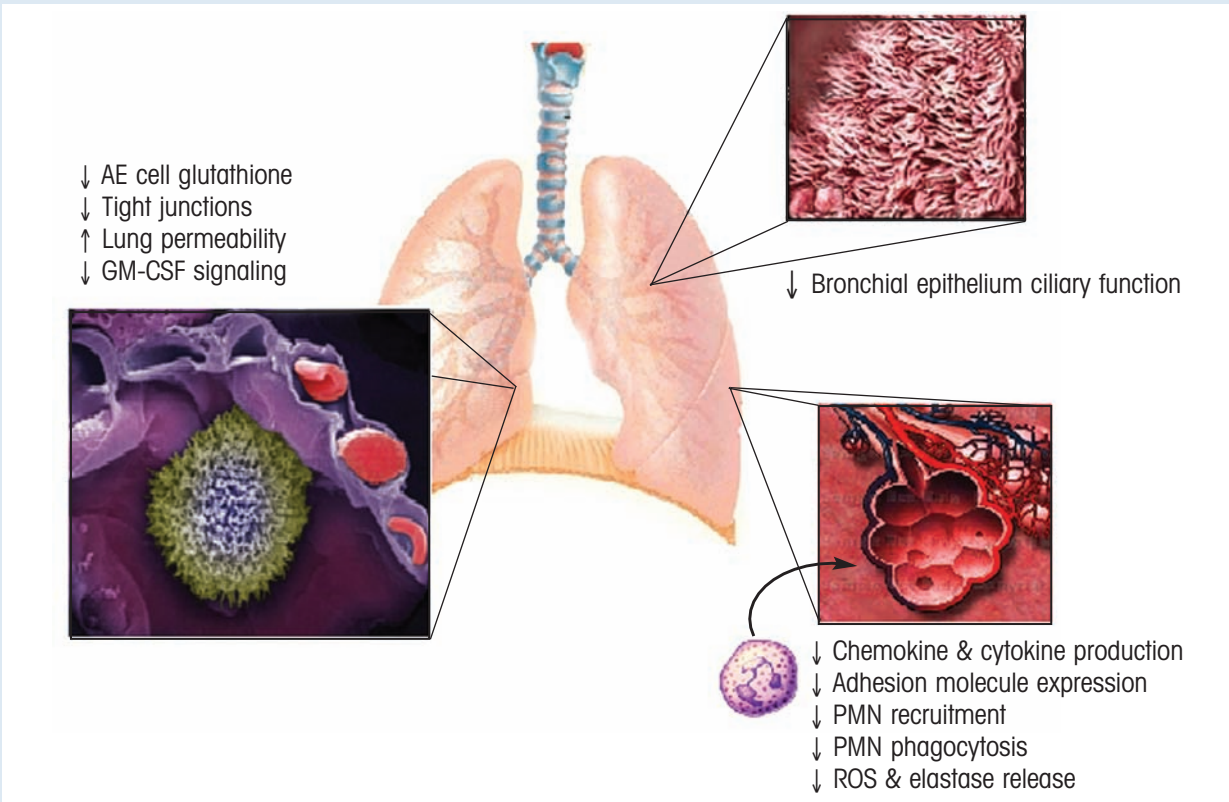

Figure 2 Alcohol abuse decreases host defense against bacterial infections. In the lung, alcohol decreases barrier integrity, antioxidant capacity, chemokine and cytokine production and release in response to infection, and recruitment and activation of polymorphonuclear cells. Together, these defects in host response are associated with increased risk, morbidity, and mortality from infections in the alcohol-abusing host.

NOTE: AE = alveolar epithelial; GM–CSF = granulocyte/macrophage-colony stimulating factor; ROS = reactive oxygen species; PMN = polymorphonuclear.

Alcohol and Cell-Mediated Host Defense Mechanisms

The innate cellular response, which is mediated primarily by monocytes/macrophages and neutrophils, involves the recognition, phagocytosis, and destruction of pathogens—processes essential to subsequent adaptive responses. Acute and chronic alcohol abuse can interfere with the actions of these cells at various levels.

Alcohol's Effects on PMNs. Alcohol abuse results in profound defects in PMN function. For example, alcohol suppresses tissue recruitment of PMNs during infection and inflammation, which can lead to increased susceptibility to bacterial infections (particularly pneumonia), decreased removal of invading bacteria (i.e., bacterial clearance), and increased mortality from pneumonia (Zhang et al. 2002). Thus, alcohol interferes with various processes necessary to deliver neutrophils to the site of an infection, such as expression of a molecule called CD18 on PMNs in response to inflammatory stimuli and PMN "hyperadherence" to endothelial cells following appropriate stimulation (MacGregor et al. 1988). In addition, alcohol significantly inhibits PMN phagocytic activity as well as the production or activity of several molecules (e.g., superoxide or elastase) that are involved in the PMNs' bactericidal activity (Stoltz et al. 1999), so that overall bactericidal activity ultimately is reduced.

Alcohol abuse also profoundly affects the production of new granulocytes (i.e., granulopoiesis), particularly in response to infection (Zhang et al. 2009). In fact, alcohol abusers with severe bacterial infection often present with abnormally low granulocyte levels (i.e., granulocytopenia), which in preclinical and clinical studies was associated with increased mortality (Perlino and Rimland 1985). Moreover, alcohol intoxication can inhibit cell division and the differentiation of precursor cells (i.e., hematopoietic stem cells) into granulocytes, which is a critical step in granulopoiesis triggered by infection (Zhang et al. 2009). These observations suggest that alcohol-mediated effects on PMNs range from the initial stages of primitive hematopoietic precursor commitment to impaired recruitment to and function within infected tissues.

Effects on Mononuclear Phagocytes. Mononuclear phagocytes include monocytes in the blood, macrophages that reside in the tissues, and dendritic cells. Studies found that alcohol abuse impairs the phagocytic function of these cells. This effect is particularly important in the setting of tuberculosis, because in healthy people more than 90 percent of the inhaled tuberculosis pathogens (i.e., mycobacteria) are ingested and destroyed by alveolar macrophages. This initial defense is critical for clearing the infection and preventing the mycobacteria from further proliferating. Chronic alcohol abuse also affects monocytes in the blood: Although the number of these cells increases, their functioning is impaired at various levels. Thus, there are significant reductions in monocyte phagocytosis (Mørland et al. 1988), adherence to other cells (which is essential for their recruitment to the tissues), production of reactive oxygen species, and intracellular microbe killing (Bermudez and Young 1991), as well as alterations in the expression of various proteins (i.e., receptors) on the monocytes' surface.

Alcohol also induces enhanced expression of a molecule called CCR5 on the surface of macrophages, which is particularly important in patients with concurrent HIV infection. This molecule normally serves as a chemokine receptor. In HIV-infected patients, however, it also acts as a coreceptor (together with CD4) for HIV, allowing certain HIV or SIV strains to infect macrophages (Wang et al. 2002). Accordingly, alcohol-induced enhanced expression of CCR5 leads to enhanced infectivity of these HIV strains in the macrophages. Similar effects have been observed in chronic alcohol-fed rhesus macaques that show an increase in the percentage of CCR5-expressing monocytes (Marcondes et al. 2008). This increase correlates with an increase in the SIV viral "set point"[3] in the circulation (Bagby et al. 2003, 2006), which in turn is associated with more rapid SIV disease progression.

Chronic alcohol ingestion also decreases the number of dendritic cells (Laso et al. 2007; Siggins et al. 2009), interferes with their differentiation, and impairs their functions, such as their ability to stimulate other cells (Szabo et al. 2004), absorb and ingest particles from outside the cell, and express co-stimulatory receptors (Lau et al. 2009). This alcohol-mediated dendritic cell dysfunction prevents the organism from generating virus-specific adaptive immune responses involving CD4+ and CD8+ lymphocytes, which may contribute to the acquisition and persistence of hepatitis C infection (Siu et al. 2009).

Effects on NK Cells. NK cells are quantitatively and qualitatively altered by alcohol abuse, particularly in patients with advanced liver cirrhosis (Cook et al. 1997; Zhang et al. 2008). For example, alcohol interferes with the expression of several NK cell proteins (e.g., proteins called perforin and granzymes A and B), and this inhibition leads to a decrease in the NK cells' ability to destroy their target cells. This impairment in NK cell activity may play a role in alcohol-associated tumor development and viral infection (Pan et al. 2006). Moreover, chronic alcohol feeding enhances liver fibrosis in response to treatment with a chemical called carbon tetrachloride (CCl_4). This enhanced fibrotic response is associated with reduced NK cell cytotoxicity and reduced expression of IFN-γ, a cytokine know to inhibit liver fibrosis (Jeong et al. 2008). Finally, alcohol activates a subgroup of NK cells called NKT cells that also express CD3, and the activation of these cells has been associated with enhanced liver injury (Minagawa et al. 2004) and hepatocyte apoptosis (Jaruga et al. 2004).

[3] The HIV (or SIV) set point is the stable viral load that is established in an HIV-infected person after the initial phase of the infection, when the person's immune systems tries to fight the virus. The higher the viral load of the set point, the faster infection will progress to full-blown AIDS.

Alcohol and the Innate Humoral Response to Infections

The induced innate humoral response plays a critical role in clearing or containing infection while an adaptive response develops. It is characterized by the release of mediators of inflammatory reactions, such as cytokines and chemokines, as well as activation of the complement cascade. In addition, viral infections induce the production of various IFNs and acute-phase proteins. Many of these components are affected by acute or chronic alcohol exposure.

Effects on Cytokines and Chemokines. The effects of alcohol on cytokine and chemokine production differ according to duration of alcohol exposure or administration. Acute alcohol exposure generally suppresses cytokine (Pruett et al. 2004) and chemokine responses. Conversely, chronic alcohol exposure frequently is associated with enhanced expression of inflammation-promoting (i.e., proinflammatory) cytokines (Mandrekar et al. 2009), particularly TNF (Nagy 2004). These effects appear to be independent of the type of alcoholic beverage consumed (Romeo et al. 2007). The enhanced expression of pro-inflammatory cytokines induced by chronic alcohol exposure or consumption clearly leads to inflammation-mediated tissue injury. Conversely, the suppression of proinflammatory cytokines and increased expression of anti-inflammatory cytokines resulting from acute alcohol exposure have been associated with impaired host defense against infection.

Acute alcohol reduces the production of proinflammatory cytokines such as TNF-α and IL-1β in macrophages of the spleen and the lungs (Nelson et al. 1989) as well as in human blood monocytes.[4] In addition, both acute and chronic alcohol consumption enhance expression of anti-inflammatory cytokines (Mandrekar et al. 2009). For example, chronic alcoholic patients undergoing cardiac and gastric surgery had higher levels of the anti-inflammatory cytokine IL-10, as well as a lower ratio between the proinflammatory IL-6 and the anti-inflammatory IL-10. These changes were associated with a marked increase in infection rates after the surgery (Sander et al. 2002). Preclinical studies have confirmed that injuries obtained during alcohol intoxication result in increased morbidity and mortality (Greiffenstein and Molina 2008), because the body's ability to elicit an appropriate response to a subsequent inflammatory or infectious challenge (e.g., infection with the bacterium *Klebsiella pneumoniae*) (Zambell et al. 2004) is impaired. Similarly, excess alcohol at the time of burn injury (Choudhry and Chaudry 2006) or prior to a surgical intervention (Spies et al. 2008) is associated with impaired host defense response to infections.

In addition to these changes in cytokine function, investigators also have shown a contribution of barrier dysfunction to the postinjury increase in infections in intoxicated people (Choudhry et al. 2004). Thus, alcohol intoxication can suppress chemokine production and impair the expression of proteins that allow neutrophils to adhere to other cells at the site of infection, which also contributes to increased susceptibility to infection. For example, in a model of lung infection, acute alcohol intoxication suppressed the production of certain chemokines (i.e., CINC and MIP-2) during infection and inflammation, thereby markedly impairing the recruitment of additional neutrophils to the site of infection (Boé et al. 2003). This defective neutrophil recruitment could be partially restored by localized chemokine administration (Quinton et al. 2005).

Effects on IFNs. Various studies in isolated human spleen and blood mononuclear cells (Wagner et al. 1992), alcohol-ingesting rodents (Starkenburg et al. 2001), and nonalcoholic humans (Szabo et al. 2001) have demonstrated that acute alcohol exposure can suppress IFN secretion, which contributes to the risk and severity of infections. For example, the lungs of alcohol-fed rodents infected with *Klebsiella pneumoniae* showed a decreased and delayed production of IFN-γ mRNA and protein, which was associated with reduced bacterial clearance from the lungs and reduced survival of the animals (Zisman et al. 1998).

Effects on Acute-Phase Proteins. Alcohol feeding suppresses the production and secretion of certain acute-phase proteins (i.e., type II cell surfactant). This effect may contribute to lung injury in response to inflammation (Holguin et al. 1998).

Effects on Complement. Few studies have investigated the effects of alcohol abuse on complement activation and its relationship with the incidence and severity of infection; instead, the focus of studies on alcohol-induced alterations in complement has been on liver injury (Pritchard et al. 2008). However, alcoholic patients frequently have abnormally low levels of complement in the blood. In addition, animal studies have indicated that acute alcohol intoxication can decrease complement activation in response to tissue injury resulting from disruptions in blood supply (i.e., ischemic injury). In contrast, chronic alcohol intake can activate the complement response (Roychowdhury et al. 2009), both by inducing the biochemical pathways that lead to activation of the complement cascade and by suppressing processes to terminate or regulate the cascade (Bykov et al. 2007).

Alcohol and the Adaptive Immune Response

Acute and chronic alcohol exposure can interfere with various aspects of the adaptive immune response, including the antigen presentation required to activate T- and B-cells, the activity of CD4+ and CD8+ T-cells, and the activity of B-cells.

[4] Expression of TNF-α and IL-1β requires the actions of a protein called nuclear factor (NF)- B. The activity of this protein is regulated by another molecule, inhibitor of NF- B (I B). Alcohol acts on this molecule (i.e., decreases phosphorylation of I B), thereby allowing I B to attach to NF- B, interfering with its activation of cytokine expression (Mandrekar et al. 1999). In addition, alcohol interferes with TNF expression by inhibiting the normal processing of newly produced TNF that is necessary for normal TNF functioning (Zhao et al. 2003).

Effects on Antigen Presentation

To elicit a response from the cell-mediated arm of the adaptive immunity, antigens need to be presented to the CD4+ and CD8+ T-cells. Studies in rodents found that chronic alcohol feeding can impair presentation of protein antigens in the spleen (Mikszta et al. 1995). Dendritic cells are among the most potent antigen-presenting cells. Acute alcohol intoxication impairs the antigen-presenting ability of these cells (Mandrekar et al. 2004). In addition, alcohol markedly affects the differentiation of dendritic cells in blood and tissues (Ness et al. 2008). The alcohol-induced defects in dendritic cell function include reduced levels of CD80 and CD86 on the cells' surface (which are necessary to induce activation of T-cells) as well as reduced production of IL-12, which is critical for stimulating naïve CD4+ T-cells to become IFN-γ–producing Th1 cells.

Effects on CD4+ (Helper) T-Cells

Numerous studies have demonstrated alcohol-related impairment of T-cell responses to various challenges. For example, in rats that were administered the bacterium *Klebsiella pneumoniae* directly into the lungs, alcohol suppressed the IFN-γ response of Th1 cells; when the animals were genetically modified to express additional IFN-γ, however, their immune response was restored and they were able to clear the pathogen (Kolls et al. 1998). In other studies, chronic alcohol feeding impaired Th1 responses to a hepatitis C virus protein, a defect that was hypothesized to result from impaired secretion of IL-2 and GM–CSF by dendritic and T-cells (Geissler et al. 1997). This alcohol-induced defect in Th1 immunity correlates with suppression of IL-12 secretion by macrophages and dendritic cells (Waltenbaugh et al. 1998). Thus, it appears that alcohol inhibits Th1 immune responses and may predispose the organism to Th2 responses and that this shift is at least partly mediated by suppression of IL-12.

In addition to the Th1 response, alcohol appears to interfere with the Th17 response. For example, following an infectious challenge, acute alcohol can suppress alveolar macrophage expression of IL-23, which helps activate naïve T-cells to differentiate into Th17 cells (Happel et al. 2006). Similarly, as with the Th1 responses, alcohol inhibits the ability of dendritic cells to promote Th17 responses, thereby favoring Th2 responses (Heinz and Waltenbaugh 2007).

Effects on CD8+ (Cytotoxic) T-Cells

Chronic alcohol decreases the numbers of CD4+ and CD8+ T-cells in the thymus and spleen (Saad and Jerrels 1991). In addition, chronic alcoholics with cirrhosis have higher levels of unbound (i.e., soluble) CD8 protein in the blood, which could inhibit CD8+ T-cell activation. It is well documented that chronic alcoholics have more progressive hepatitis C infection as well as a diminished response to treatment, and this may be related to the alcohol-induced suppression of CD8+ T-cell function, which may complicate viral clearance (Jerrells 2002). Evidence supporting this hypothesis includes the observation that chronic alcohol also delays the clearance of another virus (i.e., cytomegalovirus) from the liver in mice, and that this delay is associated with defects in the normal IL-12 and IFN-γ responses. Moreover, chronic alcohol has been associated with increased activation of CD8+ T-cells (Cook et al. 2004), which could reflect homeostatic proliferation of T-cells and increased percentage of peripheral memory cells. However, the CD8+ T-cells that do infiltrate the liver in alcoholics with hepatitis C appear dysfunctional with respect to viral clearance.

Alcohol-mediated effects on CD8+ T-cell function also have been linked to impaired immunity in the lung in response to influenza infection (Meyerholz et al. 2008). Whether the increased viral load measured in SIV-infected chronic alcohol-fed macaques can be attributed to diminished CD8+ T-cell function remains to be established (Bagby et al. 2006; Kumar et al. 2005).

Effects on B-Cells

Several lines of evidence show that the number and function of B-cells are reduced by chronic alcohol. For example, chronic alcoholics exhibit loss of B-cells in the periphery and a reduced capacity to generate protective antibodies (Cook et al. 1996). In addition, chronic alcohol can decrease the number of B-cells that produce an antibody type called IgA[5] in one of the layers of mucous membranes (i.e., the lamina propria), which is indicative of altered mucosal immunity (Lopez et al. 1994). Finally, alcohol inhibits the responsiveness of B-cells at certain developmental stages (i.e., blasts, which are the precursors to the antibody-secreting plasma cells) to various cytokines, particularly to IL-2 and IL-4. However, alcohol may have a dual effect on B-cell function because some studies have reported that B-cells also could be activated in alcohol-consuming people (Drew et al. 1984).

Alcohol's effects on the number and function of B-cells may have several consequences, including the following:

- Because B-cells also can function as antigen-presenting cells, an alcohol-induced reduction in the number of B-cells could inhibit antigen presentation.
- As mentioned earlier, most activated B-cells differentiate into plasma cells; accordingly, alcohol-induced suppression of B-cell differentiation may explain why chronic alcoholics reportedly show a reduced antibody responses to hepatitis B vaccine (Mendenhall et al. 1988).
- Chronic alcoholics have elevated levels of an immunoglobulin type called IgE, which is involved in allergic reactions; this elevation may be related to the previously mentioned

[5] IgA is an antibody that plays a critical role in immune responses in the mucous membranes. These membranes line the body cavities exposed to the external environment (e.g., the GI tract, respiratory tract, nostrils, mouth, or eyelids) and therefore are likely to come in contact with outside pathogens. IgA is the most common type of antibody produced in the body.

shift in T-cell response from a Th1 response to a Th2 response (Dominguez-Santalla et al. 2001).

Despite these observations, which shed some light on alcohol's effects on B-cells and their functions, some questions remain to be answered. For example, the acetaldehyde that is formed during alcohol metabolism can interact with other proteins in the cells, interfering with their function. Therefore, it is possible that acetaldehyde also interacts with antibodies and thereby may alter antibody responses; however, this remains to be established (Thiele et al. 2008). Similarly, more work is needed to determine whether alcohol inhibits specific aspects of B-cell differentiation, such as immunoglobulin class switching and cell survival.

Perspectives, Implications, and Future Research Directions

Alcohol has a broad range of effects on the structural, cellular, and humoral components of the immune system. This alcohol-induced dysregulation of the immune system renders the patient susceptible to a vast array of infectious pathogens, resulting in biomedical consequences such as increased risk of infections after surgery, traumatic injury, or burns; of liver disease, such as hepatitis C infection, fibrosis, and liver cancer; of ARDS and opportunistic infections in the lungs; and of accelerated progression of HIV disease (see figure 3).

Alcohol abuse is particularly prevalent in HIV-infected people, and its ability to interfere with antiviral treatment is now well recognized. In addition, current studies have identified interactions between alcohol and infectious diseases that not only increase risk of and susceptibility to infection but also contribute to comorbidities arising from continued alcohol abuse in infected individuals. The alcohol-induced alterations in the immune environment likely contribute to the pathogenesis and burden of disease in infected people, particularly in the case of HIV and hepatitis C infection (Marcondes et al. 2008). For example, patients who have a compromised immune system resulting from HIV infection and who chronically abuse alcohol are at increased risk for pneumonia. Similarly, chronic alcohol use by HIV-infected patients accelerates the disease course of HIV/AIDS (Shuper et al. 2010). Similar synergistic interactions exist for alcohol and hepatitis C infection. The prevalence of hepatitis C infection is 3- to 30-fold higher in alcoholics compared with the general population (Singal and Anand 2007), and these patients develop more severe fibrosis and have higher rates of cirrhosis and liver cancer compared with nondrinkers. Thus, alcohol abuse is associated not only with increased prevalence of HIV and hepatitis C infection, which can be attributed to behavioral and immune factors but also with more severe pathogenesis and accelerated disease progression that may at least in part result from decreased response rate to antiviral therapy (Siu et al. 2009). Given the substantial additional disease burden that alcohol imparts on people infected with HIV and viral hepatitis, a greater understanding of the precise mechanisms through which acute and chronic abuse alter the multiple facets of the host's immune response to these infections is needed. ■

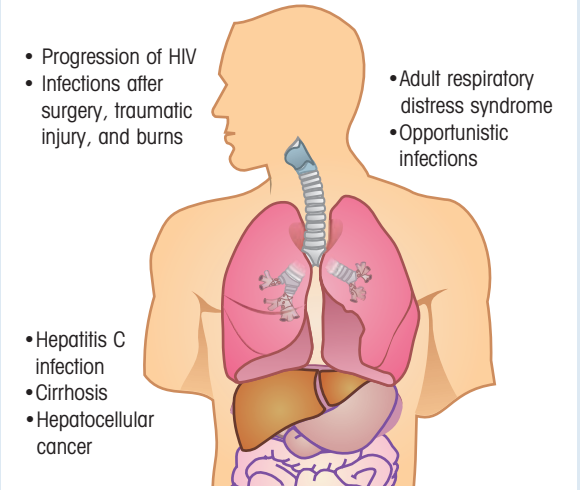

Figure 3 Biomedical consequences of alcohol-induced dysregulation of the immune system. These may include infections after surgery, traumatic injury, or burns; accelerated progression of HIV disease; adult respiratory distress syndrome and other opportunistic lung infections; and infection with hepatitis C virus, cirrhosis, or liver cancer (hepatocellular carcinoma).

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Antigen-specific T cell–mediated apoptosis of dendritic cells is impaired in a mouse model of food allergy

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Background: Dendritic cells (DCs) play a pivotal role in antigen presentation and regulation of immune responses, and strong evidence suggests their involvement in the pathogenesis of allergy. However, hitherto, DC–T-cell cross-talk in relation to IgE-mediated allergic reactions to food has not been investigated.

Objective: Our aim was to investigate T cell–mediated apoptosis of myeloid DCs from spleen and Peyer's patches of mice with cow's milk (CM) allergy after cognate interaction with antigen (CM)–specific T cells.

Methods: Freshly isolated myeloid CD11c$^{+/hi}$/B220^{-} DCs from spleen and Peyer's patches of mice with CM allergy and control mice were cultured with CM-specific T cells in the presence or absence of CM or unrelated antigen as a control. Levels of apoptosis in DCs were evaluated by assessing propidium iodide uptake and annexin V expression by means of flow cytometry.

Results: We observed that both systemic and gastrointestinal-derived DCs showed an increased resistance to T cell–mediated cell death compared with DCs from control but not allergic donors. Further experiments demonstrated that in both allergic and control mice, T cell–mediated DC apoptosis takes place exclusively in the presence of the specific antigen, is MHC II dependent, and is only partially CD95-CD95 ligand dependent.

Conclusion: Here we demonstrate, for the first time, that the reciprocal, finely balanced regulation between these 2 cell types, which plays a central role in controlling immune responses, is altered in allergy. We hypothesize that these events are likely to have a profound influence on the genesis and maintenance of adverse reaction to food. (J Allergy Clin Immunol 2004;113:965-72.)

Key words: *Dendritic cell, food allergy, IgE, T cell*

Allergic reactions to food are very frequent in industrialized countries, and according to recent surveys, there is a rapid increase worldwide.[1,2] Among allergic reactions, those that are IgE mediated are serious and life-threatening conditions. In the past years, it has become evident that allergen-specific T_H2 cells play a central role in the genesis and maintenance of the allergic inflammatory reactions in both human subjects and mice. $CD4^+$ T helper cells from atopic individuals and sensitized laboratory animals belong predominantly to the T_H2 phenotype characterized by production of relatively high levels of IL-4, IL-5, and IL-13 and low amounts of IFN-γ.[3,4] Factors responsible for the polarization of the specific immune response into a predominant T_H2 response in atopic-allergic patients and laboratory animals remain largely undefined. It is well known that T_H1 and T_H2 do not derive from distinct precursors but develop from a common precursor under the influence of both environmental and genetic factors acting at the level of antigen presentation. Dendritic cells (DCs) are the most important and effective professional antigen-presenting cells (APCs), and their role in orchestrating T_H1 and T_H2 responses is now recognized.[5,6] As such, they have the potential to be important players in the pathogenesis of allergic responses.[7] Functional and phenotypic differences in DCs from allergic and nonallergic donors have been reported.[8-10] In addition, allergen-pulsed DCs from atopic donors displayed an increased capability to induce the production of T_H2 cytokines from autologous naive, as well as memory, T cells and IgE antibodies[11,12] compared with DCs from nonatopic donors. DCs can also contribute to the dominant T_H2 response in allergy because of an altered production of IL-12[13] and IL-10.[14] In addition to this, it was recently reported that a DC subset is capable of capturing airborne antigens and remains able to activate T cells a long time after the initial exposure,[15] and in doing so, these cells participate in the chronic T_H2 inflammation typical of the airway hypersensitivity reaction. These data clearly show that DCs are important in allergy, but it is important to highlight the fact that all these data have come

Abbreviations used
APC: Antigen-presenting cell
CM: Cow's milk
CMP: Cow's milk protein
CT: Cholera toxin
DC: Dendritic cell
PP: Peyer's patch

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from studies focused on allergic reactions of the respiratory tract, and nothing is known on the role of DCs in the generation and maintenance of IgE-mediated allergic reactions to food. Furthermore, very little is known about DC–T-cell cross-talk in allergy. It has been reported that DCs might undergo rapid apoptosis after interaction with T cells,[16] and this led to hypothesize that this might be an effective downregulatory mechanism that prevents an otherwise uncontrollable activation of T cells by antigen-loaded DCs. These considerations prompted us to investigate the level of apoptosis in splenic DCs and gastrointestinal Peyer's patch (PP)–derived DCs (PP-DCs) from allergic and nonallergic mice after interaction with antigen-specific T cells in a well-established mouse model of type I hypersensitivity reaction to food components.[3,17,18] Here, we report that both splenic DCs and PP-DCs from mice with cow's milk (CM) allergy showed a significantly reduced degree of apoptosis compared with DCs from control animals without allergy when cultured in the presence of both the antigen and antigen-specific T cells.

METHODS

Mouse model of food allergy

A well-established and well-characterized mouse model of allergic reactions to food, including CM, has been used in our experiments.[3,17,18] This model closely mimics type I hypersensitivity IgE-mediated reactions in human subjects, and it has been recently used to address several issues related to the cellular basis and genetics of food allergy. Female 3-week-old C3H/HeJ mice were purchased from Charles River and maintained in a clean access-restricted room in conventional conditions throughout the experiments, and the number of animals used was kept to a minimum. Mice were immunized with a mixture of homogenized CM and cholera toxin (CT; Calbiochem) that contained 1.0 mg/g body weight of CM together with 0.3 μg/g CT. The CM+CT mixture was administered in PBS (final volume, 0.03 mL/g body weight). Control groups were administered PBS (naive) or the same dose of either CM or CT. Mice were sensitized 5 times at weekly intervals. DCs were isolated from spleen tissue and PPs of sensitized and control mice 24 hours after the delivery of the fifth dose of the sensitizing or control mixture. An additional group of mice was challenged on week 6 with CM to check the percentage of mice that had type I hypersensitivity reactions to CM. These experiments showed that as many as 75% (12/16) of C3H/HeJ mice sensitized with the CM+CT mixture displayed a strong allergic reaction, ranging between 3 and 5 on a scoring system previously described.[17,18]

Preparation of DCs

Isolation and purification of DCs from the spleens and intestinal PPs from allergic and control mice was performed according to a slightly modified procedure that has been previously described.[19] First, PPs were treated with serum free medium containing dithiothreitol, HEPES, and 5 mmol/L EDTA in HBSS for 90 minutes at room temperature (all chemicals were from Sigma Chemical Co) to remove epithelial cells and then extensively washed with HBSS. Spleen and PP tissue were then treated with collagenase D (400 U/mL, Roche) and incubated at 37°C for 10 minutes in the presence of EDTA. A single cell suspension was then prepared, and cells were stained with anti–CD11c-phycoerythrin–labeled (BD Biosciences) and anti–B220 APC–labeled (Ebioscience) antibodies. $CD11c^{+/hi}/B220^{-}$ DCs were isolated with a Coulter Epics Altra (Coulter Becham) flow cytometer. Sorting of DCs was carried out in stringent conditions to exclude $CD11c^{+/lo}$ macrophages,[20] and populations were routinely screened for the presence of $CD19^{+}$ and $CD3^{+}$ cells by using flow cytometry.

Antigen-specific T cells

C3H/HeJ mice were immunized by means of subcutaneous injection of 100 μg of cow's milk protein (CMP) in CFA into the foot pad and boosted twice at biweekly intervals with CMP (100 μg per dose). Seven days after the last injection, spleens were removed and cultured in the presence of irradiated syngeneic splenocytes plus CMP (50-100 μg/mL) and IL-2 (10-30 U/mL) for 10 days. The resulting population was 96% to 98% $CD4^{+}$ and was allowed to stay in culture for an additional 10 days in the presence of antigen and syngeneic splenocytes. T cells harvested showed significant reactivity to autologous CMP-pulsed $CD11c^{+hi}/B220^{-}$ DCs. Antigen-specific T cells were maintained in culture and fed at 3-week intervals with antigen-pulsed splenocytes and IL-2.

Apoptosis assay

The apoptosis assay in splenic DCs and PP-DCs after coincubation with antigen-specific T cells was carried out as described previously.[16] Briefly, splenic DCs or PP-derived DCs (1×10^{5}-1×10^{6}/mL) were cultured alone or with T cells (1×10^{6}/mL) either in the presence or absence of CMP (50-100 μg/mL) or the same dose of the unrelated antigen keyhole limpet hemocyanin for 14 to 20 hours. Twelve to 16 mice per group were used. This relatively large number of mice was due to the low harvest of PP-DCs per mouse. The short-term cocultures were carried out in triplicate in serum free medium to avoid the presence of CM products in FCS. After culture, cells were stained with FITC-conjugated anti-$CD11c^{+}$ antibody. Cells were then stained with propidium iodide or phycoerythrin-conjugated annexin V (BD Biosciences). Propidium iodide uptake and annexin V expression was then evaluated by means of flow cytometry. The influence of MHC class II molecules and CD95-CD95L ligation on T cell–induced DC apoptosis was determined by adding anti-Ia^{b} antibody (Biosciences) or anti-CD95L (K10), respectively, or isotype matching antibody as control to the cocultures.

Statistical analysis

Data are expressed as means ± SDs, and statistical comparison was made by using the Student *t* test. *P* values were considered significant at less than .05.

RESULTS

T cell–mediated killing of systemic (splenic) DCs in allergy

Levels of apoptosis in splenic DCs from all control groups (naive, CM-treated, and CT-treated animals) and the allergic (CM+CT-treated animals) group were determined after cognate interaction with CM-specific T cells. DCs were isolated, and their phenotypes were analyzed by means of flow cytometry. It has been reported that manipulation of DCs by means of isolation from tissue followed by overnight culture can induce maturation and differentiation.[19] Thus DCs isolated in this way might not be representative of the DC function in vivo. To circumvent this problem, we therefore decided to sort $CD11c^{+/hi}/B220^{-}$ DCs by means of flow cytometry (Fig 1) and culture them immediately afterward. First, we determined propidium iodide uptake in $CD11^{c+}$-labeled splenic DCs from each control group (Fig 2, *A-C*) and

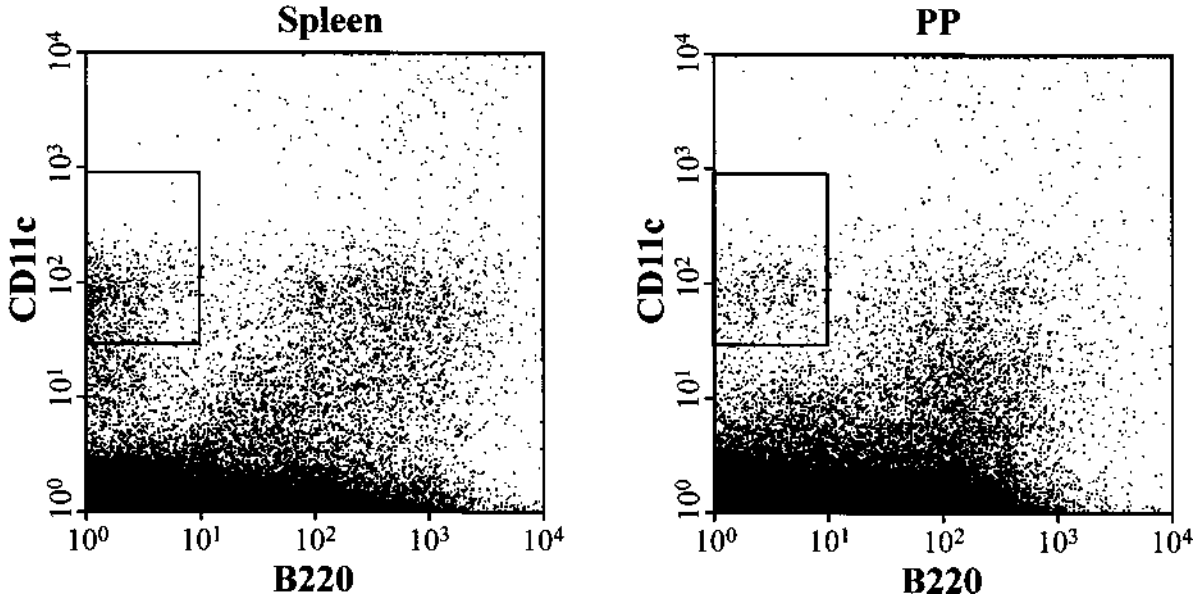

FIG 1. Isolation of myeloid CD11c$^{+/hi}$/B220^{-} DCs from spleen and PP tissue of control mice and mice with CM allergy. No differences were observed in the number of DCs from the control nonallergic groups. DCs were sorted *(boxed area)* by means of flow cytometry and used immediately afterward to avoid nonphysiologic in vitro manipulation.

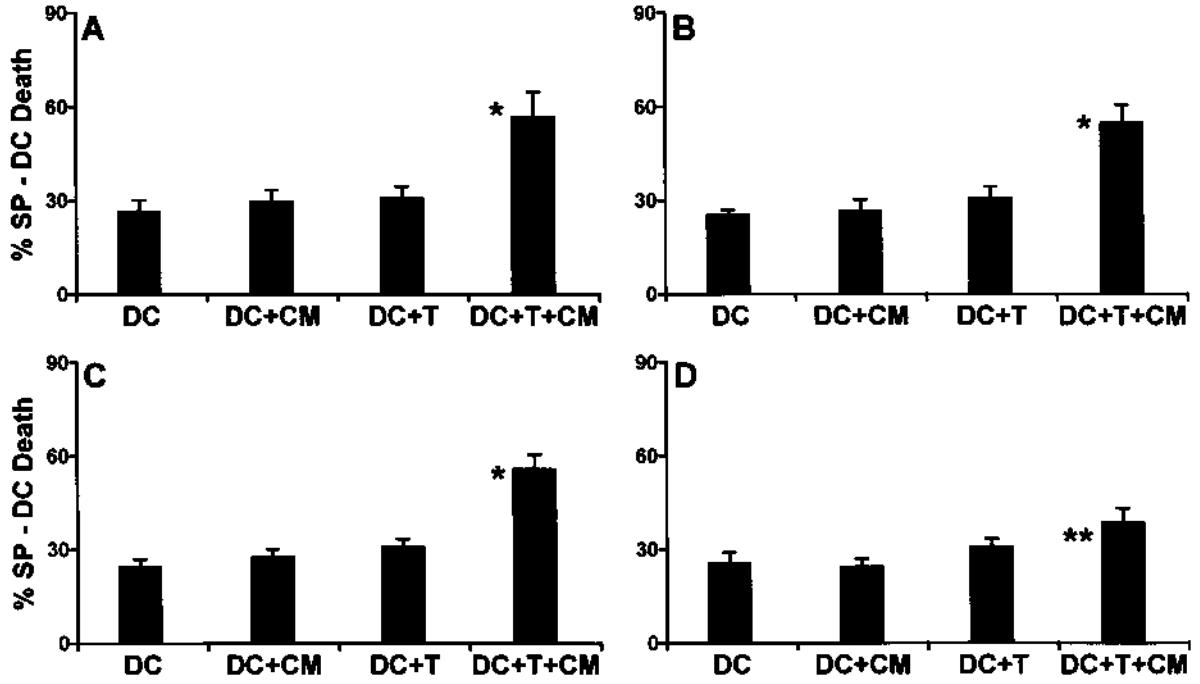

FIG 2. T cell–mediated apoptosis of splenic DCs from nonallergic mice (naive **[A]**, CM treated **[B]**, CT treated **[C]**) and mice with CM allergy (CM+CT treated treated **[D]**). High levels of apoptotic DCs *(*)* were seen only when these were cultured in complete cocultures *(DC+T+CM)*. In contrast, DCs from allergic mice (Fig 2, *D*) evaded T cell–mediated killing *(**)*. Data represent means ± SDs are of 4 independent experiments.

allergic mice (Fig 2, *D*). In accordance with a previous report, we observed that DCs undergo a certain degree of spontaneous apoptosis when cultured alone, and this did not change when DCs were cocultured with the allergen (DC+CM) or with CM-specific T cells alone (DC+T). On the other hand, an increase in the number of apoptotic cells was seen when DCs from all control groups were cultured in the presence of both CM and CM-specific T cells. Values ranged between 50% ± 5% in Fig 2, *A*, and 55% ± 6% in Fig 2, *B* and *C*. Levels of apoptotic CD11c$^{+/hi}$/B220^{-}

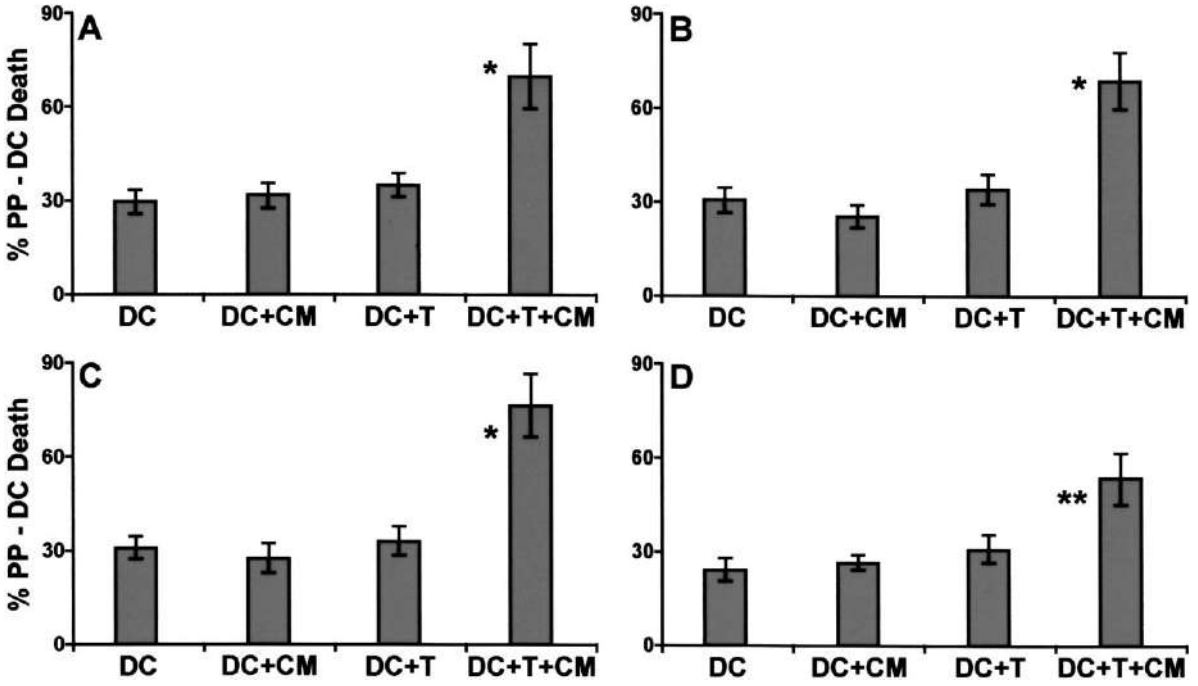

FIG 3. T cell–mediated apoptosis of PP-DCs from control nonallergic and allergic mice. See Fig 2 for details. PP-DCs from control groups showed higher susceptibility *(*)* to T cell–mediated killing compared with their systemic homologues. Also, in this case DCs from allergic mice showed a remarkable capability to survive T cell–mediated apoptosis *(**)*. Data represent means ± SDs are of 4 independent experiments.

DCs from control groups in whole cocultures (DC+T+CM) were found to be significantly higher compared with incomplete (DC+T or DC+CM) cocultures ($P < .01$). We also observed that DC cell death was strictly dependent on the presence of CM in culture because T cell–mediated DC apoptosis was completely abolished by the substitution of CM with unrelated antigen (keyhole limpet hemocyanin, data not shown). The pattern appeared to be different when DCs from allergic mice were used (Fig 2, *D*), and we observed that in whole cocultures such levels were reduced to 40% ± 6%.

T cell–mediated apoptosis of intestinal PP-DCs in allergy

Phenotypic and functional differences between DCs from the systemic and gastrointestinal immune system have been previously observed in mice,[19] and we therefore assessed the levels of T cell–mediated apoptosis in PP-DCs. Results in Fig 3 show that $CD11c^{+/hi}/B220^{-}$ DCs from PPs from all nonallergic control groups were more susceptible to apoptosis than their splenic homologues. Numbers of apoptotic PP-DCs in these groups (Fig 3, *A-C*) did not differ from those of splenic DCs when cultured alone or in the presence of CM alone or T cells alone. Instead, a sharp increase was seen when the numbers of apoptotic DCs were determined in whole cocultures. Nearly 80% of PP-DCs from control groups became apoptotic after coincubation with antigen-specific T cells in the presence of CM. Consistent with what we observed previously in splenic DCs, PP-DCs from allergic mice showed a marked increase in resistance to T cell–mediated apoptosis (Fig 3, *D*), with only 55% ± 7% undergoing cell death. Also in this case, the numbers of apoptotic DCs in whole cocultures differed significantly from those observed in control groups ($P < .01$). These observations were confirmed when the level of expression of another early marker for apoptosis, annexin V,[21] was determined in DCs from both allergic and mice orally immunized with CM (Fig 4). CD95/CD95L cross-linking is not the only pathway involved in the T cell–mediated killing of DCs.

It was reported that CD95-CD95L ligation might be in part accountable for T cell–mediated DC cell death.[19] We then assessed the influence of CD95-CD95L interaction in splenic DCs and PP-DCs from control and allergic mice on T cell–mediated DC cell death. Throughout our experiments, we did not detect any differences in DC phenotype and function between mice treated with either CM or CT alone. With this in mind, we decided, for ethical considerations, to reduce the number of mice used in our experiments, and we selected mice orally immunized with CM as the control group for these experiments. Apoptotic splenic DCs or PP-DCs were enumerated in whole cocultures in the presence of CM and CM-specific T cells in the absence or presence of anti-CD95L antibody. The results are shown in Fig 5, *A* and *B*. Addition of anti-CD95L antibody to cocultures of splenic DCs from immunized nonallergic mice (Fig 5, *A*, black bars) reduced the number of DCs undergoing apoptosis, as measured by means of propidium iodide uptake, from 60% ± 10% to 40% ± 8% ($P < .05$). A similar pattern was observed when

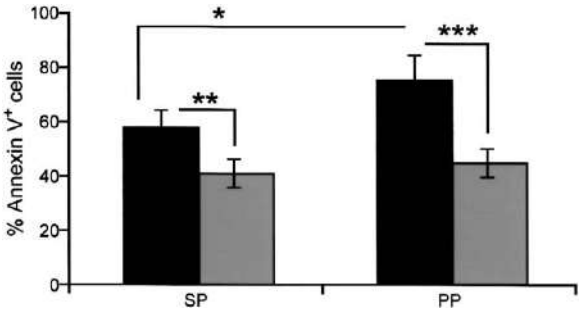

FIG 4. Annexin V in DCs after interaction with T cells. Splenic DCs *(SP, black bar)* and PP-DCs *(PP, black bar)* from nonallergic mice orally immunized with CM significantly differed in their susceptibility to T cell–mediated killing *(*)*. T cell–mediated apoptosis is reduced in both splenic DCs *(gray bar, **)* and PP-DCs *(gray bar, ***)* from allergic mice. Data represent means ± SDs of 3 independent experiments.

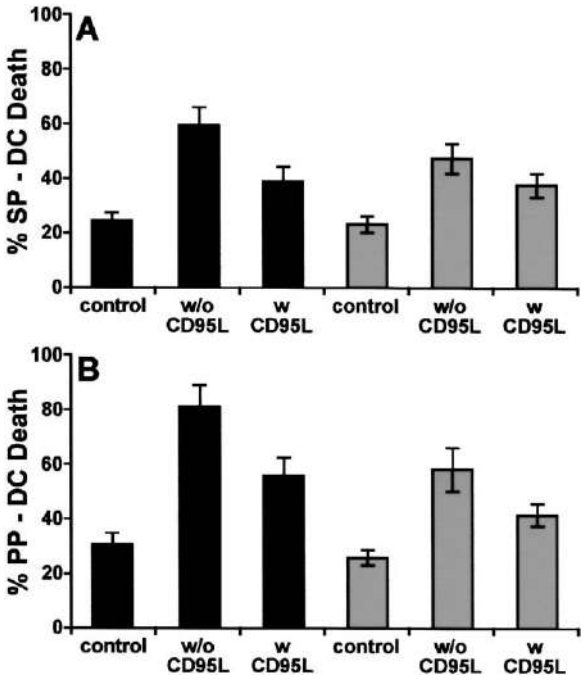

FIG 5. Role of CD95/CD95L cross-linking on T cell–mediated killing. Incubation with anti-CD95L *(wCD95L)* reduced levels of apoptosis of splenic DCs **(A)** and PP-DCs **(B)** of control CM-immunized mice *(black bars)* and allergic mice *(gray bars)* compared with cocultures carried out in the absence of anti-CD95L *(w/oCD95L)*. Spontaneous apoptosis was also evaluated in DCs from all groups *(control)*. Data represent means ± SDs of 4 independent experiments.

anti-CD95L antibody was added to cocultures of splenic DCs from allergic mice (Fig 5, *A*, gray bars). Although in this case the difference was not statistically significant, we observed a consistent trend, and numbers of DCs undergoing apoptosis after interaction with T cells were always higher in the absence of anti-CD95L antibody (48% ± 8% vs 38% ± 6%). PP-DCs show the same profile of response when cultured in the presence of anti-CD95L (Fig 5, *B*). In such a case, the role of CD95-CD95L interaction was more evident as a consequence of the intrinsically higher susceptibility of PP-DCs to T cell–mediated apoptosis. Here the numbers of apoptotic PP-DCs from immunized nonallergic mice and cultured in the absence of anti-CD95L reached 80% (Fig 5, *B*, black bars), whereas it was reduced to 55% ($P < .01$) after addition of anti-CD95L. Thus addition of anti-CD95L to the coculture reduced T cell–mediated DC death by nearly 50%, and this was not modified by increasing the concentration of anti-CD95L antibody.

A similar trend was observed in DCs from allergic mice (Fig 5, *B*, gray bars).

T cell–mediated DC apoptosis is class II dependent

The requirement of antigen-specific T cells to induce apoptosis in DCs prompted us to address the extent of MHC II involvement in these events. Here we report that in both control and allergic mice, T cell–induced DC cell death can be completely prevented by adding anti-Ia antibody to the cocultures (Fig 6), as opposed to isotype (IgG) matching antibody as control.

DISCUSSION

The main finding of this article is that myeloid $CD11^{c+/hi}/B220^{-}$ DCs from both the systemic and gastrointestinal immune system of allergic mice showed an improved resistance to T cell–mediated apoptosis compared with control nonallergic groups. DCs have a unique ability to present antigen to naive T cells. However, activation of T cells by DCs has to be kept under control to avoid an otherwise uncontrollable reaction, and several reports have suggested that some type of regulatory negative feedback loop is in place to serve as a down-regulatory mechanism. One of the most important mechanisms for the maintenance of cellular homeostasis in mammals is apoptosis, and although the mechanism of programmed cell death is relevant to the development of all cell lineages, it is without doubt of paramount importance for the generation of immune cell repertoires and immune responses.[22] It has been shown that T cell–mediated DC cell death exerts a form of control on various subtypes of DCs at least at 2 different time points during their life: first, T_H2 cells downregulated the pre-DC2 subset through IL-4–mediated killing,[23] and second, T cells induced apoptosis on the DC line and freshly isolated murine DCs.[16] In the latter case, a rapid T cell–mediated killing of DCs occurred after cognate interaction with antigen-specific T cells in the presence, but not in the absence, of antigen. Evidence, albeit indirect, also showed that ovalbumin-pulsed DCs labeled with a fluorescent dye, when injected into mice that had been reconstituted with ovalbumin-specific $CD4^{+}$ cells, rapidly migrate to lymph nodes, where they interact with the specific T cells.[24] These DCs rapidly disappeared (48 hours) from the lymph nodes, and although the nature (DC1-DC2) and fate of these cells were not determined, the authors hypothesized that these DCs were eliminated after interaction with

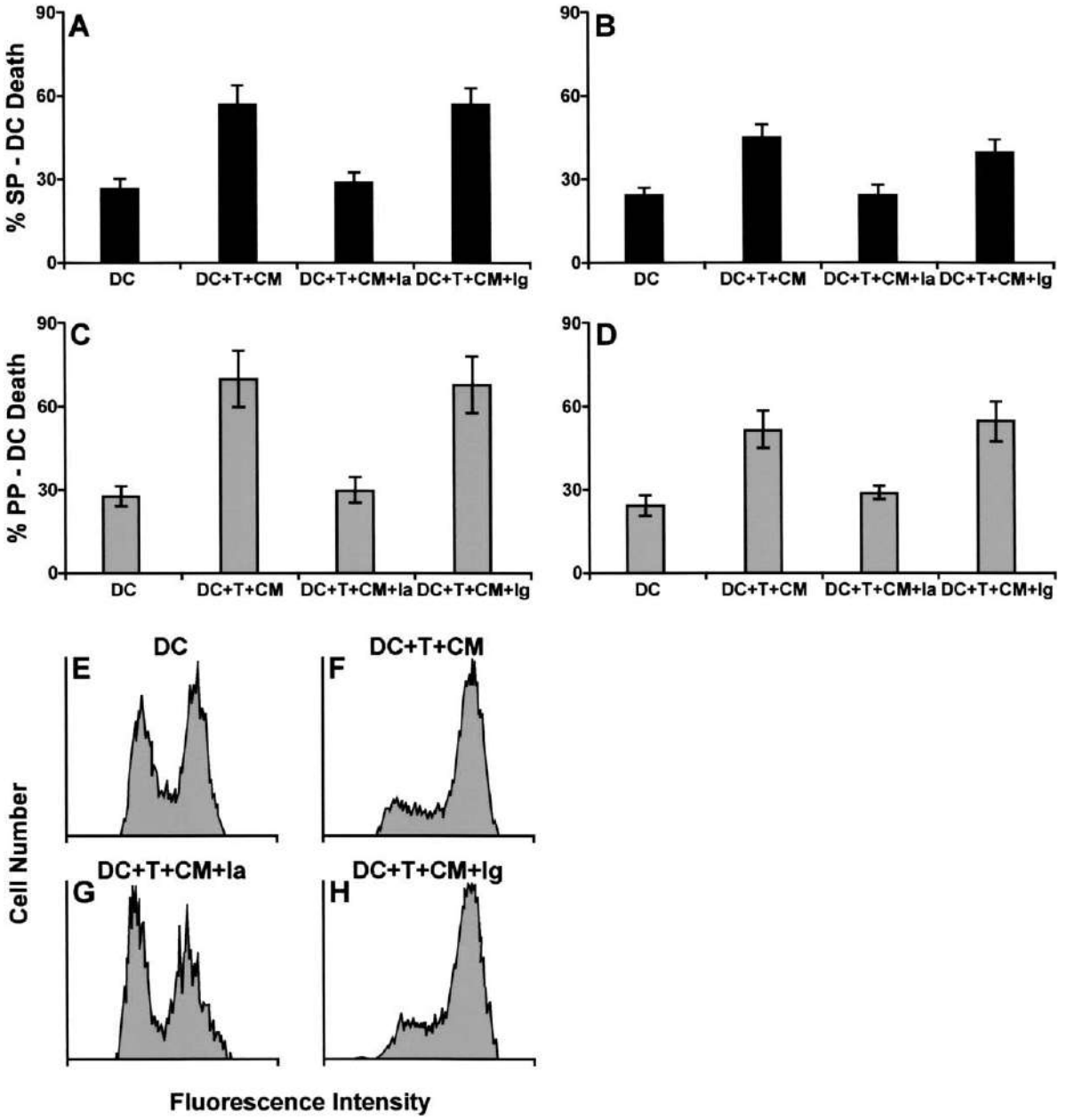

FIG 6. Role of MHC class II in T cell–mediated killing. Addition of anti-Ia^b antibody to whole cocultures *(DC+T+CM+Ia)* of splenic DCs **(A** and **B)** and PP-DCs **(C** and **D)** abolished the capability of T cells to kill DCs from both control (Fig 6, *A-C*) and allergic (Fig 6, *B-D*) mice compared with an isotype control antibody *(DC+T+CM+Ig)*. A representative histogram is shown **(E-H)**. Data represent means ± SDs of 4 independent experiments.

T cells in the presence of antigen. With this in mind, we tested whether DCs from allergic mice could survive the interaction with T cells, thereby escaping the regulatory negative feedback loop. We observed that splenic and PP-derived DCs from allergic mice did not undergo the same level of apoptosis as DCs from control mice, thus suggesting that the mechanism or mechanisms regulating DC–T-cell interactions is altered in food allergy. A differential response to the T_H2 lymphokines has been observed in DCs from atopic individuals.[25] Here the authors reported that IL-4 suppressed IL-13–induced survival of the lymphoid-derived DC2 subset ($CD11c^-$/$CD123^+$) in nonatopic individuals but failed to do so in atopic patients, leading to an increase in the number of cells of the plasmacytoid DC2 subset. Our finding, which is based on the use of myeloid $CD11c^+$ DCs, suggests that both subsets of DCs are affected by the allergic status of the host in regard to T cell–mediated regulation. Although substantial experimental evidence exists to support the notion of myeloid DC1 T_H1 inducer lineages and lymphoid DC2 T_H2-inducer lineages, it has been suggested that the kinetics of DC activation might be

relevant for the DC1-DC2 dichotomy.[26] LPS-challenged myeloid DCs displayed a remarkable ability to induce a T_H1 response. However, when they became exhausted after 48 hours in culture, these DCs predominantly induced T_H2 cells. Therefore it is tempting to speculate that in allergic individuals the survival of allergen-loaded DCs after interaction with antigen-specific T cells might result in an increase of circulating, exhausted myeloid cells of the DC1 subset that have acquired the ability to induce an allergen-specific T_H2 response. In accordance with a previous report,[16] we observed that the mechanism underlying T cell–mediated DC apoptosis is only partially caused by activation of the Fas (CD95) pathway. Although controversial, T cell–mediated apoptosis of DCs through CD95-CD95L ligation has been described by many authors in a variety of experimental models in both human subjects and mice. Conflicting results were mainly obtained by using specific anti-CD95 antibody or soluble CD95L and in vitro generated DCs[27,28]; however, recently, Fas (CD95)–based lysis of DCs, which were apparently resistant to anti-CD95 antibody, has been reported both in immature and mature DC populations.[29] With this in mind, we adopted a more physiologic approach on the basis of the use of antigen-specific T cells in which the combination of other regulatory molecules, such as lymphokines and costimulatory molecules, might play an important role. The relevance of T cell–mediated killing of APCs in the regulation of immune responses is also highlighted by the notion that other APCs, such as B cells and macrophages, are known to be killed by T cells in an antigen-specific and CD95/CD95L-dependent manner.[30,31] Signaling through CD95 is not the only way to induce apoptosis in DCs, and in agreement with our results, evidence does exist for the presence of multiple mechanisms for induction of apoptosis in DCs. Indeed, both CD95-dependent and CD95-independent pathways were found to intervene at different stages of DC maturation and differentiation.[32] Taken together, these data clearly suggest an important role of T cell–mediated DC cell death in the physiologic regulation of immune responses; this mechanism might represent a strategy that allows T cells to control the death or survival of appropriate DC populations in an antigen-specific manner.

We suggest that the malfunctioning of this regulatory pathway in both systemic and gut-derived DCs might have a profound influence on the genesis and maintenance of allergic reactions to food.

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Report 12: The Global Impact of COVID-19 and Strategies for Mitigation and Suppression

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Summary

The world faces a severe and acute public health emergency due to the ongoing COVID-19 global pandemic. How individual countries respond in the coming weeks will be critical in influencing the trajectory of national epidemics. Here we combine data on age-specific contact patterns and COVID-19 severity to project the health impact of the pandemic in 202 countries. We compare predicted mortality impacts in the absence of interventions or spontaneous social distancing with what might be achieved with policies aimed at mitigating or suppressing transmission. Our estimates of mortality and healthcare demand are based on data from China and high-income countries; differences in underlying health conditions and healthcare system capacity will likely result in different patterns in low income settings.

We estimate that in the absence of interventions, COVID-19 would have resulted in 7.0 billion infections and 40 million deaths globally this year. Mitigation strategies focussing on shielding the elderly (60% reduction in social contacts) and slowing but not interrupting transmission (40% reduction in social contacts for wider population) could reduce this burden by half, saving 20 million lives, but we predict that even in this scenario, health systems in all countries will be quickly overwhelmed. This effect is likely to be most severe in lower income settings where capacity is lowest: our mitigated scenarios lead to peak demand for critical care beds in a typical low-income setting outstripping supply by a factor of 25, in contrast to a typical high-income setting where this factor is 7. As a result, we anticipate that the true burden in low income settings pursuing mitigation strategies could be substantially higher than reflected in these estimates.

Our analysis therefore suggests that healthcare demand can only be kept within manageable levels through the rapid adoption of public health measures (including testing and isolation of cases and wider social distancing measures) to suppress transmission, similar to those being adopted in many

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countries at the current time. If a suppression strategy is implemented early (at 0.2 deaths per 100,000 population per week) and sustained, then 38.7 million lives could be saved whilst if it is initiated when death numbers are higher (1.6 deaths per 100,000 population per week) then 30.7 million lives could be saved. Delays in implementing strategies to suppress transmission will lead to worse outcomes and fewer lives saved.

We do not consider the wider social and economic costs of suppression, which will be high and may be disproportionately so in lower income settings. Moreover, suppression strategies will need to be maintained in some manner until vaccines or effective treatments become available to avoid the risk of later epidemics. Our analysis highlights the challenging decisions faced by all governments in the coming weeks and months, but demonstrates the extent to which rapid, decisive and collective action now could save millions of lives.

SUGGESTED CITATION
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1. Introduction

The COVID-19 pandemic is now a major global health threat, with 332,930 cases and 14,510 deaths confirmed worldwide as of the 23rd March 2020[1]. Since the initial identification of the virus in China, global spread has been rapid, with 182 of 202 countries having reported at least one case. The experience in countries to date has emphasised the intense pressure that a COVID-19 epidemic places on national health systems, with demand for intensive care beds and mechanical ventilators rapidly outstripping their availability in even relatively highly resourced settings[2]. This has potentially profound consequences for resource-poor settings, where the quality and availability of healthcare and related resources (such as oxygen) is typically poorer[3].

There remain large uncertainties in the underlying determinants of the severity of COVID-19 infection and how these translate across settings. However, clear risk factors include age, with older people more likely to require hospitalisation and to subsequently die as a result of infection[4], and underlying co-morbidities including hypertension, diabetes and coronary heart disease serving to exacerbate symptoms[5]. Both the age-profile and the distribution of relevant co-morbidities are likely to vary substantially by country, region and economic status, as will age-specific contact patterns and social mixing. Variation in these factors between countries will have material consequences for transmission and the associated burden of disease by modifying the extent to which infection spreads to the older, more vulnerable members of society.

To help inform country strategies in the coming weeks, we provide here summary statistics of the potential impact of mitigation and suppression strategies in all countries across the world. These illustrate the need to act early, and the impact that failure to do so is likely to have on local health systems. It is important to note that these are not predictions of what is likely to happen; this will be determined by the action that governments and countries take in the coming weeks and the behaviour changes that occur as a result of those actions.

2. Demographics and Income Status

Figure 1 summarises two of the demographic and societal factors which are likely to determine the burden of COVID-19 in different countries. First, there is a strong correlation between the gross domestic product (GDP, a measure of the strength of the economy) of a country and its underlying demography (Figure 1A). Higher income countries tend to have the oldest populations; lower income countries in contrast have a much smaller proportion of the population who are above 65 and therefore within the age interval currently observed to be at particularly high risk of mortality from COVID-19[4]. However, we note that these populations also have very different underlying co-morbidities, including a high burden of infectious diseases in low-income (LIC) and low-middle income countries (LMIC) and both infectious and chronic diseases in middle-income countries (MIC). In addition, the burden of many infectious diseases is in young children who may therefore be more at risk than has been observed in China or Europe. The risk profile for COVID-19 could therefore be very different in some low-income settings from that observed to date in China, Europe and North America.

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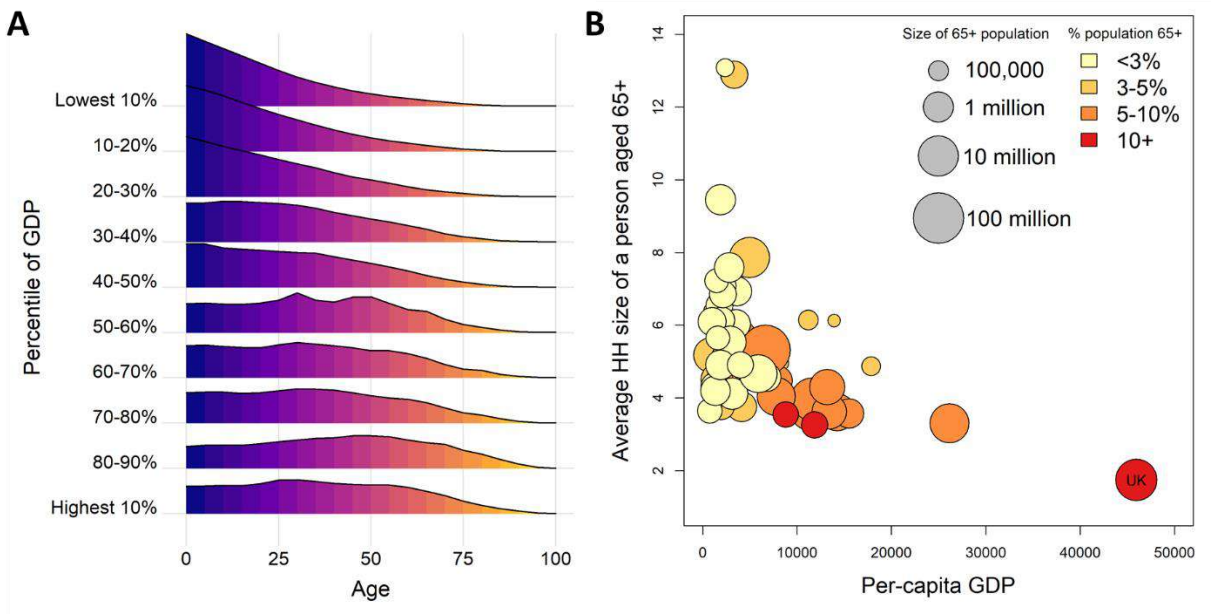

Figure 1: Demographic, societal and mixing patterns relevant to COVID-19 transmission and burden across different countries. A. Aggregated demographic patterns within 2020 World Population Prospects (WPP) projections across countries within each 2018 World Bank (WB) GDP pre-capita decile. B. Average Household size within Demographic Health Surveys (DHS) of individuals aged 65 and over by 2018 WB GDP per-capita. For reference, the average household size of contacts in the UK is also provided.

The household is a key context for COVID-19 transmission[6]. The average size of households that have a resident over the age of 65 years is substantially higher in countries with lower income (Figure 1B) compared with middle- and high-income countries, increasing the potential for spread generally but also specifically to this particularly vulnerable age-group. Contact patterns between age-groups also differ by country; in high-income settings contact patterns tend to decline steeply with age. This effect is more moderate in middle-income settings and disappears in low-income settings (Figure 2), indicating that elderly individuals in these settings (LICs and MICs) maintain higher contact rates with a wide range of age-groups compared to elderly individuals in high-income countries (HICs). These contact patterns influence the predicted COVID-19 infection attack rate across age-groups (Figure 2) with higher attack rates in the elderly predicted in low-income settings compared to high-income settings and middle-income settings showing intermediate patterns.

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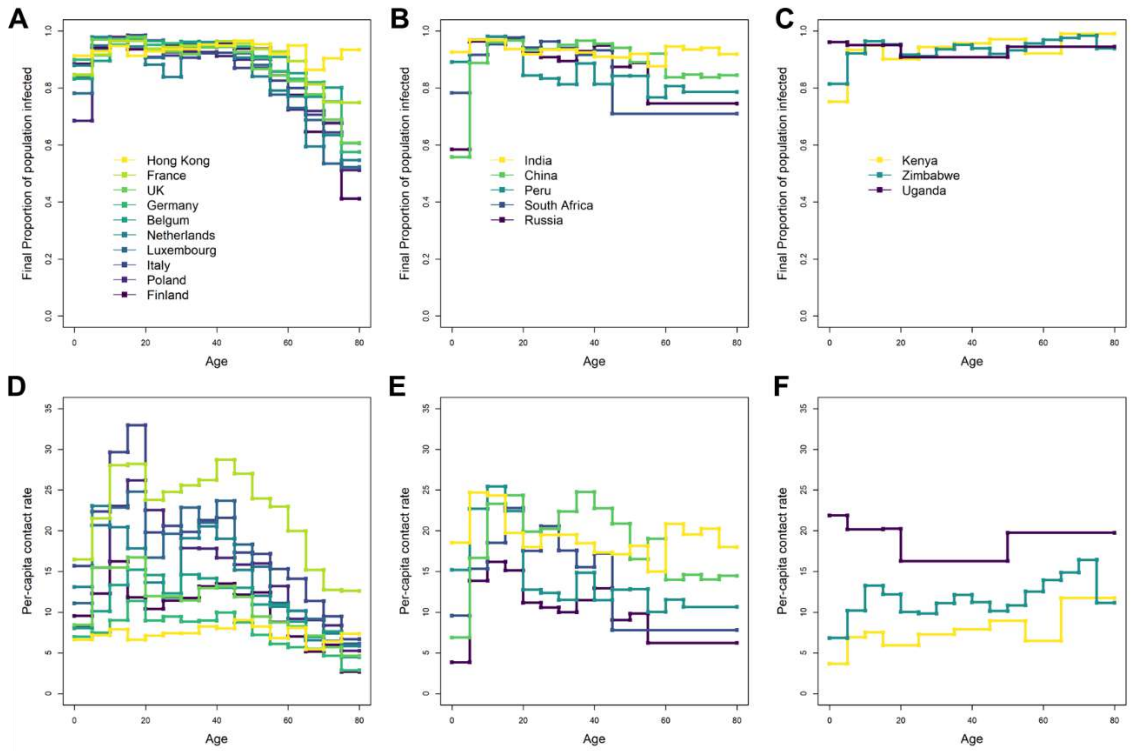

Figure 2: Age-stratified COVID-19 attack rates based upon surveys of age-stratified contact patterns within all-age samples. A-C show estimates of the final attack rate (proportion infected) by age for $R_0 = 2.4$ for contact patterns from surveys in high income, upper middle income and lower middle income/lower income respectively. D-F show the estimated per-capita rates of contact within these surveys adjusted for national-level demography.

3. Healthcare Availability

Figure 3 summarises our estimates of healthcare capacity in different settings. Hospital bed capacity is strongly correlated with the income status of countries (Figure 3B); LICs have the fewest hospital beds per 1000 population (1.24 beds per 1000 population on average) and HICs the highest (4.82 beds per 1000 population on average). Lower and upper middle-income countries (LMIC/UMICs) fall between these two extremes (2.08 and 3.41 beds per 1000 population on average, respectively). We find that the percentage of hospital beds that are in intensive care units (ICU) is lowest in LICs (1.63 on average) and highest in HICs (3.57) with LMICs and UMICs falling in-between (2.38 and 3.32 respectively) (Figure 3C). Note that our estimates of the ICU capacity in HICs are drawn almost exclusively from a recent review of ICU capacity in Asian countries[7] and are not necessarily reflective of ICU capacity in HICs worldwide.

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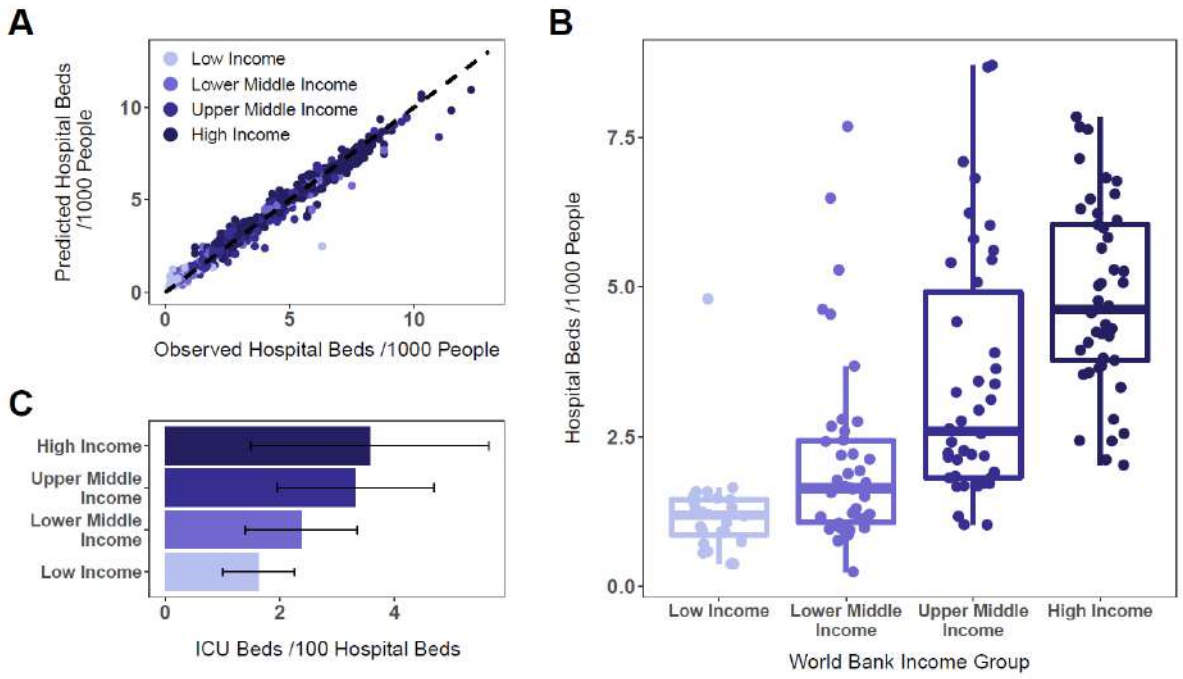

Figure 3: Estimates of Hospital Bed and ICU Capacity, Stratified by World Bank Income Group. Data on hospital beds per 1000 population were modelled using covariates from the World Bank, and data on ICU capacity collated using a systematic review. (A) Comparison of model prediction and empirically observed numbers of hospital beds per 1000 population. Each point represents a country, with the x-axis indicating the observed number of hospital beds per 1000 population for that country, and the y-axis indicating the model predicted number of hospital beds per 1000 population. Colouring of the points indicates which World Bank income strata the country belongs to. (B) Boxplots of the number of hospital beds per 1000 population, stratified by World Bank income group. The points here are the modelled estimates of hospital beds per 1000 population obtained from the boosted regression tree model. (C) Results from a systematic review describing the percentage of all hospital beds that are in ICUs, stratified by World Bank income group. Error bars indicate the 95% confidence interval of the mean.

4. Burden of Disease

We considered the likely scale of four potential scenarios:

A) An unmitigated epidemic – a scenario in which no action is taken.
B) Mitigation including population-level social distancing – we assessed the maximum reduction in the final scale of the epidemic that can be achieved through a uniform reduction in the rate at which individuals contact one another, short of complete suppression.
C) Mitigation including enhanced social distancing of the elderly – as (B) but with individuals aged 70 years old and above reducing their social contact rates by 60%.
D) Suppression –we explore different epidemiological triggers (deaths per 100,000 population) for the implementation of wide-scale intensive social distancing (modelled as a 75% reduction in interpersonal contact rates) with the aim to rapidly suppress transmission and minimise near-term cases and deaths. For these scenarios we do not produce final size estimates but illustrate their impact in representative settings.

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We note that each of these strategies would be, in practice, accompanied by surveillance to test and isolate all identified cases and their household members as rapidly as possible to reduce onward transmission.

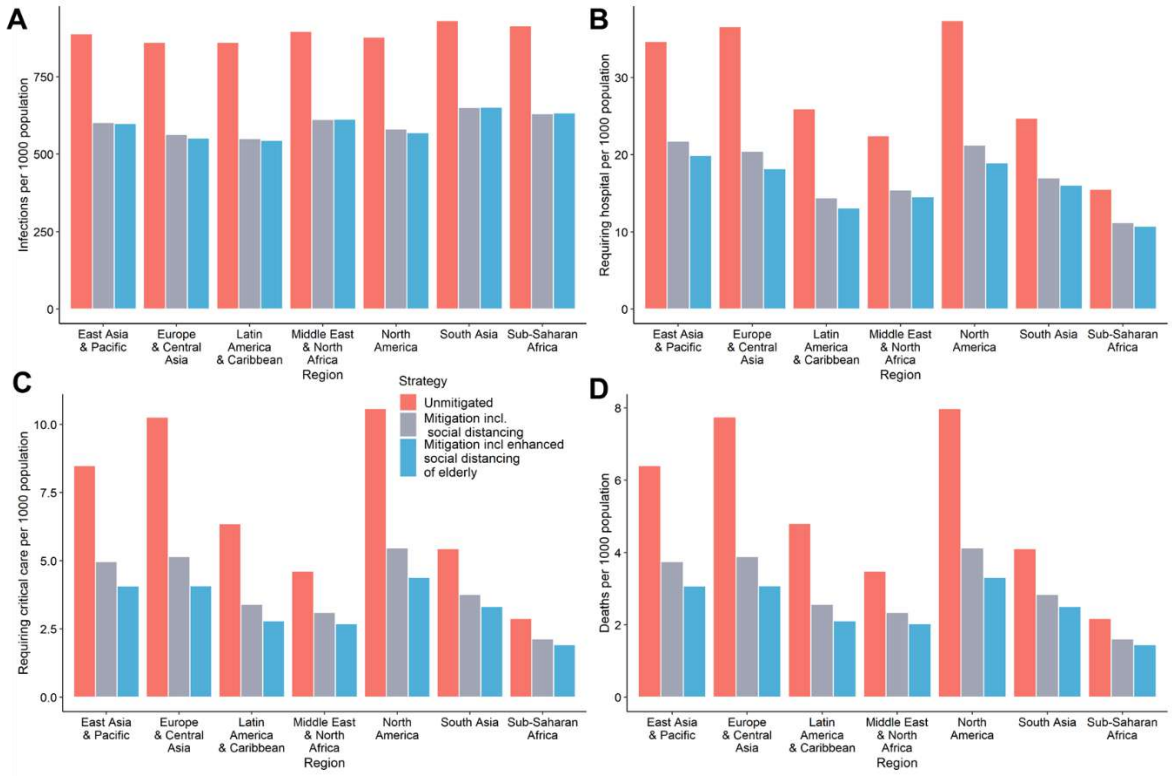

Figure 4. Estimated total number of infections (A), individuals requiring hospitalisation (B) and critical care (c) and deaths (D) in unmitigated and mitigated scenarios by World Bank region.

Figures 4 and 5 summarise these results across World Bank geographic regions and income statuses. The accompanying Excel spreadsheet gives these results for individual countries. Our estimated impact of an unmitigated scenario in the UK and the USA for a reproduction number, R_0 , of 2.4 (490,000 deaths and 2,180,000 deaths respectively) closely matches the equivalent scenarios using more sophisticated microsimulations (510,000 and 2,200,000 deaths respectively)[8]. On the basis of the observed three-day doubling time in the incidence of deaths across Europe, we here use a central estimate of R_0 to 3.0 and investigate scenarios with R_0 between 2.4 and 3.3. Globally, we estimate that a completely unmitigated COVID-19 epidemic would lead to 7.0 (range 6.4-7.2) billion infections for a basic reproduction number, R_0, of 3.0 (range 2.4-3.3). Applying estimates of the age-specific IFR from China[4], this could result in 40 (range 35-42) million deaths.

Despite higher rates of contact across older age-groups, we predict a lower incidence of severe disease, hospitalisation and deaths in lower income settings. This is driven by the younger average age of these populations. It is important to note, however, that these estimates assume no substantive difference in general health/co-morbidity prevalence between Chinese and other populations. Furthermore, the standard of medical care available is likely to vary markedly between settings and be substantially lower within lower-income countries (Figure 3). Neither assumption is likely to hold in practice and as such mortality in unmitigated and mitigated epidemics in LMIC and LIC is likely to be substantially higher.

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If mitigation including enhanced social distancing is pursued, for an R_0 of 3.0, we estimate a maximum reduction in infections in the range 30-38% (median 33%) and a range of reduction in mortality between 19%-55% (median 39%) representing 16 million lives saved for R_0=3 (assuming the mortality patterns observed in China). These optimal reductions in transmission and burden were achieved with a range of reductions in the overall rate of social contact between 40.0%- 44.9% (median 43.9%), with this range increasing to 42.9%-47.9% (median 46.9%) for an R_0 of 3.3 and decreasing to (34.3%-37.3%, median 36.9%) for an R_0 of 2.4.

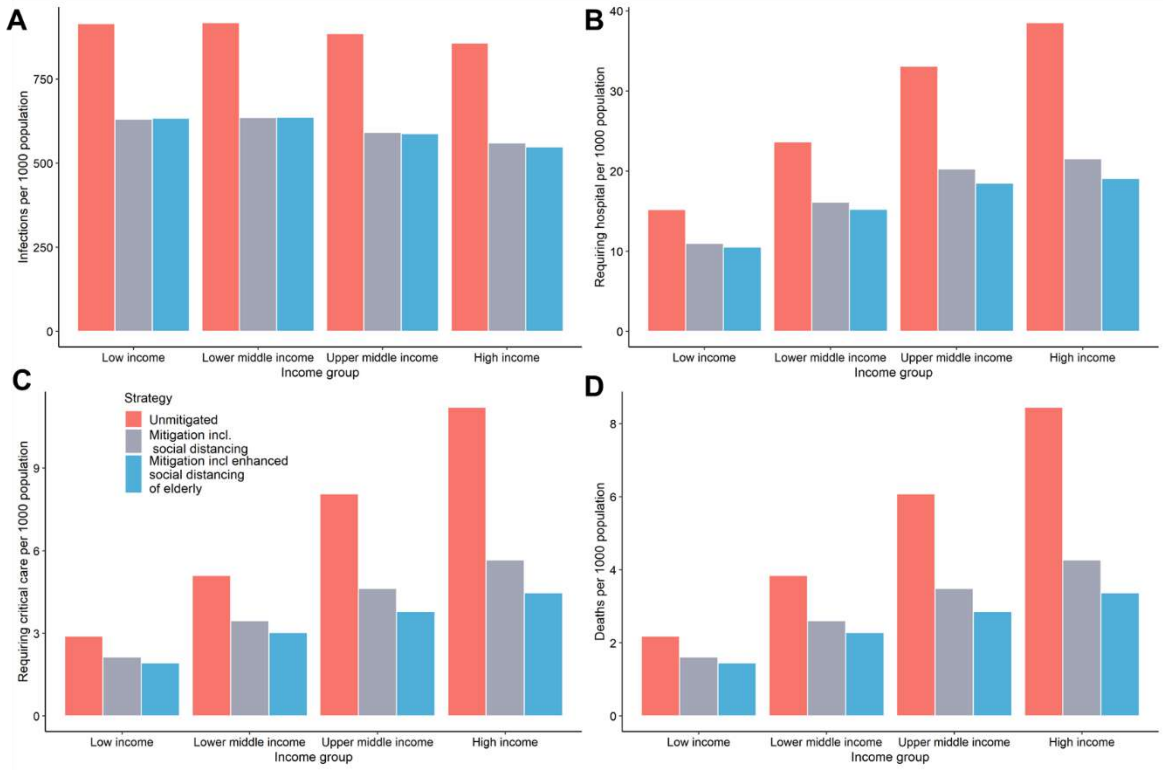

Figure 5 Estimated total number of infections (A), individuals requiring hospitalisation (B) and critical care (c) and deaths (D) in unmitigated and mitigated scenarios by World Bank income group.

Combining mitigation with enhanced social distancing of elderly individuals is predicted to result in higher overall mortality reductions of 23%-67% (median 49%), representing 20 million lives saved for R_0=3. However, these strategies are predicted to have lower proportional impact in lower income settings compared to higher income settings due primarily to lower-income settings possessing a far smaller proportion of elderly individuals. (Figure 1B and Figure 2).

The resulting reduction in burden under optimal mitigation is predicted to substantially reduce the gap between demand for hospital beds and capacity (Figures 6E-H). However, demand for critical care is still predicted to vastly exceed capacity in all countries (here, modelled using demographics and contact patterns for a representative LIC, LMIC, UMIC and HIC) under all mitigation scenarios considered. Although we predict lower demand for critical care in lower income settings due to their younger populations, this is likely to be offset by a much lower level of supply: for our mitigation scenario including population-level social distancing, peak demand for critical care in our simulation for a typical LIC outstrips demand by a factor of 25.4, whereas for the equivalent simulation in a typical HIC this factor was 7.0 (typical LMIC and UMIC produced factors of over-demand of 16.4 and 10.86

respectively). The impact of a lack of adequate care for more severe cases of COVID-19 in these scenarios is difficult to quantify but is likely to significantly increase overall mortality. As a result, we anticipate that those countries pursuing mitigation, lower-income settings are likely to experience a higher degree of excess mortality due to health system failure – this is a factor not currently captured in our projections of total deaths.

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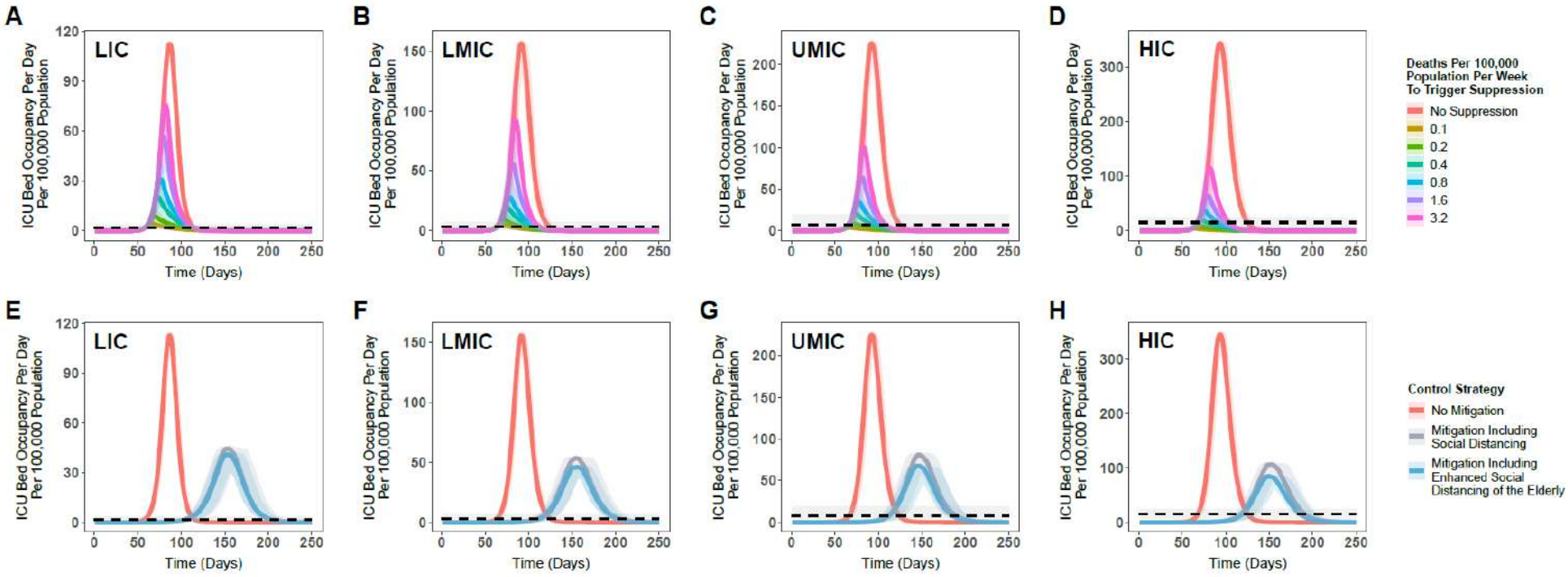

Figure 6: The impact of various control strategies in representative settings. Using an age-structured SEIR model along with demographies and contact patterns representative of LIC, LMIC, UMIC and HIC countries (columns left to right) the impact of different control strategies was. ICU bed occupancy per day per 100,000 population is shown in all figures. The top row shows impact of suppression (triggered at times dependent on when the rate of deaths per week increases beyond certain defined thresholds) and the bottom row shows mitigation (involving either mitigation involving general social distancing across the whole population or mitigation involving whole population social distancing as well as enhanced social distancing of the elderly)

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Table 1: Estimated impact of suppression strategies. The impact on infections and deaths over 250 days for two different suppression strategies triggered according to different thresholds for mortality incidence (0.2 and 1.6 deaths per 100,000 population per week).

	Unmitigated Scenario		Suppression at 0.2 deaths per 100,000 population per week		Suppression at 1.6 deaths per 100,000 population per week	
	Infections	**Deaths**	**Infections**	**Deaths**	**Infections**	**Deaths**
East Asia & Pacific	2,117,131,000	15,303,000	92,544,000	442,000	632,619,000	3,315,000
Europe & Central Asia	801,770,000	7,276,000	61,578,000	279,000	257,706,000	1,397,000
Latin America & Caribbean	566,993,000	3,194,000	45,346,000	158,000	186,595,000	729,000
Middle East & North Africa	419,138,000	1,700,000	30,459,000	113,000	152,262,000	594,000
North America	326,079,000	2,981,000	17,730,000	92,000	90,529,000	520,000
South Asia	1,737,766,000	7,687,000	111,703,000	475,000	629,164,000	2,693,000
Sub-Saharan Africa	1,044,858,000	2,483,000	110,164,000	298,000	454,968,000	1,204,000
Total	7,013,734,000	40,624,000	469,523,000	1,858,000	2,403,843,000	10,452,000

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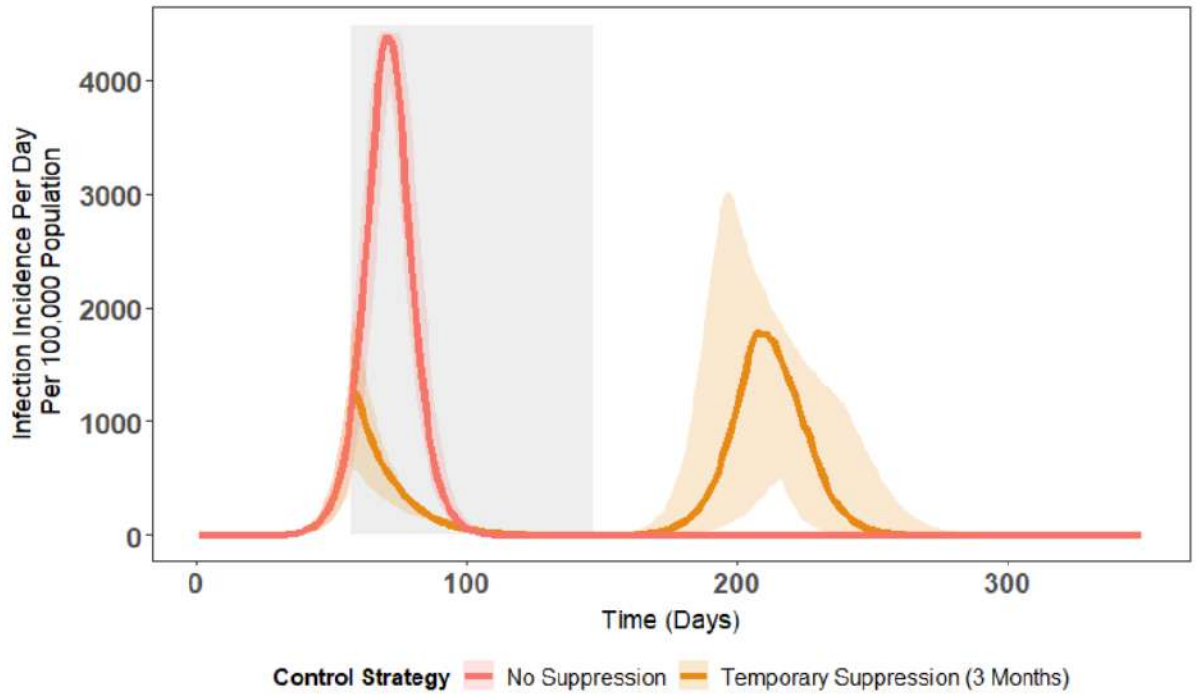

Figure 7: The impact of temporary suppression on infection incidence in a representative lower income setting. In this example, suppression is maintained for 3 months but is then stopped and contact patterns are assumed to return to previous levels.

Even extensive suppression (Figures 6A-D & Table 1), triggered when weekly rate of deaths per 100,000 reaches a given threshold, is predicted to result in critical care demand being exceeded unless suppression is triggered at an early stage of the epidemic in a country. Additionally, the impact of a trigger based upon number of deaths for suppression and its ability to prevent the epidemic exceeding ICU bed capacity differs between settings. Triggering suppression based on deaths or death rates is less sensitive in LICs and LMICs - the younger populations in these settings mean that by the time a certain death rate threshold is reached, they have typically accrued a higher number of cases (and by extension, ICU capacity has already been overwhelmed).

Given these results, the only approaches that can avert health system failure in the coming months are likely to be the intensive social distancing measures currently being implemented in many of the most affected countries, preferably combined with high levels of testing. These approaches are likely to have the largest impact when implemented early (Figure 6, Table 1). It is however important to consider the sustainability of such measures. As illustrated in Figure 7, these interventions will likely need to be maintained at some level in tandem alongside high levels of surveillance and rapid case isolation to avoid the potential for resurgent epidemics.

It is important to note that we do not quantify the wider societal and economic impact of such intensive suppression approaches; these are likely to be substantial. Nor do we quantify the potentially different societal and economic impact of mitigation strategies. Moreover, for countries lacking the infrastructure capable of implementing technology-led suppression maintenance strategies such as those currently being pursued in Asia[6,9], and in the absence of a vaccine or other effective therapy (as well as the possibility of resurgence), careful thought will need to be given to pursuing such strategies in order to avoid a high risk of future health system failure once suppression measures are lifted.

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The results presented here illustrate the potential impact of the COVID-19 pandemic globally. Our analyses give insight into possible trajectories and the impact of measures that can help reduce the spread of the virus based on the experience of countries affected early in the outbreak. However, at the current time, it is not possible to predict with any certainty the exact number of cases for any given country or the precise mortality and disease burden that will result. A full understanding of both will only be available retrospectively.

This analysis highlights the challenging decisions faced by all governments in the coming weeks and months. However, our counterfactual of an unmitigated pandemic clearly demonstrates the extent to which rapid, decisive and collective action can prevent billions of infections and save millions of lives globally.

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5. Methods

Patterns of contact, demography and household size across the World

Population sizes and age distributions by country were taken from the 2020 World Population Prospects, the 27th round of the official United Nations population estimates prepared by the Population Division of the Department of Economic and Social Affairs of the United Nations Secretariat (available here: https://population.un.org/wpp/). Estimates of household size and the age of members of each household were extracted from The Demographic and Health Surveys (DHS) Program using the rDHS package[10]; data from a total of 59 LMIC countries with surveys conducted since 2010 were extracted. In addition, we extracted equivalent household information for the United Kingdom as a representative HIC[11].

Patterns of contact across different populations and countries were drawn from several sources, including previously published estimates of mixing from a number of HICs [12] and a recent systematic review of social contact surveys including MICs and LMICs [13]. Additional data were obtained from surveys included in the socialmixR package (https://github.com/sbfnk/socialmixr), as well as references identified through either the reference lists of included surveys, or through informal searches of Web of Science and PubMed. We identified data from 18 countries. Ten were from HIC settings, with 8 (Belgium, Finland, Germany, Italy, Luxembourg, Netherlands, Poland and the United Kingdom) from the POLYMOD social mixing study[12], and two further surveys from France[14] and Hong Kong[15]. Five surveys were identified in UMIC settings: China[16], India[17], Peru[18], Russia[19] and South Africa[20]. Two surveys were identified in LMIC settings: Kenya[21] and Zimbabwe[22]. One survey was undertaken in a LIC: Uganda[23]. Contact matrices were adjusted to give symmetric age-specific contact rates for each country.

As Figure 2 shows, contact patterns measured within Western Europe suggest attack rates are likely to decline substantially by age. For Hong Kong, the only non-European HIC setting for which contact data were identified, contact rates did not decline sufficiently at older ages to produce a similar decline, which may suggest this is not a consistent trait across all high-income countries. However, we identified additional surveys in the literature from Hong Kong[24] and Japan[25] where contact rates did appear to decline more substantially with age but were not available in readily downloadable format. Our projections for UMIC settings showed declines in projected attack rates by age, though to a lesser extent than HIC settings. Meanwhile the limited data from LMIC did not result in substantial declines in attack rate by age.

Given the sparse availability of contact data, we used representative patterns for countries which do not have survey data. For the USA and Canada we used the UK survey data. For other European and Central Asian countries (with available data from Russia also indicating substantial declines in attack rates in older ages – Figure 2B), as well as countries previously classified as advanced economies by the International Monetary Fund[26], we used the patterns from the European survey producing the median final attack rate within individuals aged 70 and above (the Netherlands POLYMOD survey[12]). Countries from Latin America and the Caribbean were assigned mixing patterns from the Peruvian survey; those from South Asia, mixing patterns from the Indian survey; those from East Asia, mixing patterns from the Chinese survey; those from sub-Saharan Africa, mixing patterns from the Zimbabwean survey (with the exception of South Africa which was assigned patterns from the Chinese survey); whilst those in the Middle-East and North Africa were assigned patterns from the Chinese

survey if they were high or upper-middle income and from the Zimbabwean survey if they were low or lower-middle income. These contact patterns, alongside country-specific demography were then used to provide estimation of number of Infections and deaths, demand for health care in an unmitigated pandemic and the impact of control measures for a given basic reproduction number.

We calculated the final epidemic size generated from an age-structured Susceptible-Infected-Recovered model incorporating both the demographic structure of the population and the rates of contact between different individuals across different age groups[27]. This numerical solution replicates the total number of infected individuals derived from our simulation models for the UK and USA[8]. Final epidemic sizes by age where then generated using a central R_0 value of 3.0, with uncertainty range between 2.4 and 3.3. This value of R_0 was chosen as it results in a 3-day doubling time, consistent with current observations in Europe.

To estimate the demand for health services and overall mortality, we use age-specific estimates of the hospitalisation rate and infection fatality ratio (IFR) obtained from our previous analysis of data from China[4]. Hence, we make the strong assumption that similar levels of medical care to that provided in China are available elsewhere. We also implicitly assume that mortality patterns do not vary given the different co-morbidities. These assumptions may mean that our results may overestimate mortality in some HICs and under-estimate it in some lower income countries.

For each country we estimated the potential maximum benefits from mitigation through a policy of social distancing within the general population. We identified the minimum final size of the epidemic produced by a uniform proportional reduction in social contacts across age categories conditional on this final size achieving a level of herd immunity that would be sufficient to prevent a second wave following the relaxation of the policy and a subsequent return to the levels of social contact prior to the pandemic. In a similar manner, we also assessed the maximum impact of a policy where in addition to overall social distancing, individuals 70 years old and above reduce their social contacts by a substantially larger proportion, here modelled as 60% ("shielding").

To model the impact of these scenarios on the dynamics of likely healthcare demand over time we used an age-structured stochastic Susceptible-Exposed-Infected-Recovered (SEIR) model parameterised to match best estimates of key parameters determining the dynamics of spread of COVID-19. The exposed category was modelled as two separate compartments to produce a gamma-distributed incubation period of mean 4.58 days and standard deviation 3.24 days. A single compartment was used for the infectious compartment, yielding an exponentially distributed infectious period with mean 2.09 days. An R_0 of 3.0 was used for all scenarios explored and presented in this report. Integration with country-specific demographies and patterns of contact between age-groups then enabled setting-specific estimation of the incidence of new infections over time. This incidence of new infections over time is then converted to the incidence of infections requiring hospitalisation and/or critical care. Both the probability that an infected person requires hospitalisation and whether they also require critical care increase with age, matching estimates given in[8]. We assume a delay of 5 days between symptom onset (assumed here to be when individuals progress from the Exposed to the Infectious compartment) and hospitalisation and that hospitalised individuals require a hospital bed for 8 days. If critical care is also required, we assume that individuals remain in hospital and occupy a critical bed for a further 8 days, yielding a total hospital stay of 16 days. Any mortality associated with COVID-19 is assumed to occur 21 days after symptom onset. These

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parameters are based on our current best understanding of the likely progression and severity of COVID-19.

Using this model, we replicated the "unmitigated", "mitigation including social distancing" and "mitigation including enhanced social distancing of the elderly" scenarios from the final size analysis. For the "mitigation including social distancing scenario", contact rates were reduced by a factor determined through our minimum final size calculations described above. For the "mitigation including enhanced social distancing of the elderly" scenario, contact rates were reduced uniformly across age groups less than 70 and then a further, more extreme reduction (60%) applied to the 70-75 and 75+ age groups.

We also explored the impact of more rigorous social distancing approaches aimed at immediate suppression of transmission. We looked at 6 suppression scenarios in which the timing of policy implementation varied according to when the weekly death rate per 100,000 population exceeds a certain threshold (here, either 0.1, 0.2, 0.4, 0.8, 1.6 or 3.2 deaths per week per 100,000 population) – the effects of widespread transmission suppression were modelled as a uniform reduction in contact rates by 75%, applied across all age-groups.

Hospital bed capacity estimation

Data on the number of hospital beds per 1,000 population were available from the World Bank (https://data.worldbank.org/indicator?tab=all) for 201 countries (66 High Income, 58 Upper Middle Income, 47 Lower Middle Income and 30 Low Income). However, many of these records were not recent (earlier than 2015). We therefore use a boosted regression tree-based modelling approach to generate contemporary estimates of hospital beds per 1,000 population using the following covariates: maternal mortality (per 100,000 live births), access to electricity (% of population), population aged 0-14 years (% of population), pupil-teacher ratio in secondary school, rural population (% of population), domestic government health expenditure (% of GDP), infant mortality (per 1,000 live births), the proportion of children enrolled in secondary school, geographical region and income group (with the latter two covariates categorised according to the World Bank's definitions). The model was fitted using the statistical software R and the dismo package, with tree complexity of 12, bag fraction of 0.65, and a learning rate of 0.001. 10-fold cross-validation was implemented to check overfitting, and the model found to predict well both training and held-out (test) datasets.

Review of Intensive Care Unit Capacity

These data were derived from 3 resources. We extracted data from a previously conducted systematic review of ICU capacity in low-income countries[28], as well as a more recently published review of ICU capacity across Asia[7]. In addition to this, we also carried out a systematic review to identify further references containing information on ICU bed capacity in Lower- and Middle-Income Countries. Web of Science was searched on Friday 13th March using the search terms ("critical care" OR "intensive care" OR "ICU" OR "CCU") AND capacity AND (country name) where country name refers to 1 of the 138 countries classified as LMIC by the World Bank. This search yielded 174 results, with 30 texts retained after Abstract screening, and 20 of these retained following screening of the full text. Due to the requirement for contemporary estimates, balanced by the comparative paucity of data for ICU capacity compared to hospital beds, we excluded papers earlier than 2000. These resources provided a total of 57 data points describing the number of ICU beds per 100 hospital beds across countries belonging to the World Bank's 4 income strata (LIC, LMIC, UMIC and HIC).

6. Appendix data sources

Data on global unmitigated, mitigated and suppression scenarios: Imperial-College-COVID19-Global-unmitigated-mitigated-suppression-scenarios.xlsx

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Countries				
Region, subregion, country or area *	**country_code**	**World Bank region**	**World Bank income grou**	**GDP 2018**
Afghanistan	AFG	South Asia	Low income	1955.006208
Albania	ALB	Europe & Central Asia	Upper middle income	13364.1554
Algeria	DZA	Middle East & North Africa	Upper middle income	15481.78762
Angola	AGO	Sub-Saharan Africa	Lower middle income	6452.355165
Antigua and Barbuda	ATG	Latin America & Caribbean	High income	26868.13352
Argentina	ARG	Latin America & Caribbean	Upper middle income	20610.56855
Armenia	ARM	Europe & Central Asia	Upper middle income	10343.17559
Aruba	ABW	Latin America & Caribbean	High income	
Australia	AUS	East Asia & Pacific	High income	51663.36509
Austria	AUT	Europe & Central Asia	High income	55454.68929
Azerbaijan	AZE	Europe & Central Asia	Upper middle income	18044.13678
Bahamas	BHS	Latin America & Caribbean	High income	32087.72915
Bahrain	BHR	Middle East & North Africa	High income	47303.04851
Bangladesh	BGD	South Asia	Lower middle income	4371.754986
Barbados	BRB	Latin America & Caribbean	High income	18554.12928
Belarus	BLR	Europe & Central Asia	Upper middle income	19994.80408
Belgium	BEL	Europe & Central Asia	High income	51407.99834
Belize	BLZ	Latin America & Caribbean	Upper middle income	8648.0888
Benin	BEN	Sub-Saharan Africa	Low income	2424.755845
Bhutan	BTN	South Asia	Lower middle income	10167.93406
Bolivia	BOL	Latin America & Caribbean	Lower middle income	7873.166243
Bosnia and Herzegovina	BIH	Europe & Central Asia	Upper middle income	14623.67405
Botswana	BWA	Sub-Saharan Africa	Upper middle income	18615.79334
Brazil	BRA	Latin America & Caribbean	Upper middle income	16096.40096
Brunei Darussalam	BRN	East Asia & Pacific	High income	80920.04868
Bulgaria	BGR	Europe & Central Asia	Upper middle income	21960.36994
Burkina Faso	BFA	Sub-Saharan Africa	Low income	1984.884306
Burundi	BDI	Sub-Saharan Africa	Low income	744.182072
Cabo Verde	CPV	Sub-Saharan Africa	Lower middle income	7454.063917
Cambodia	KHM	East Asia & Pacific	Lower middle income	4360.854404
Cameroon	CMR	Sub-Saharan Africa	Lower middle income	3785.079978
Canada	CAN	North America	High income	48130.25597

Central African Republic	CAF	Sub-Saharan Africa	Low income	859.9356196
Chad	TCD	Sub-Saharan Africa	Low income	1968.258262
Channel Islands	CHI	Europe & Central Asia	High income	
Chile	CHL	Latin America & Caribbean	High income	25222.52778
China	CHN	East Asia & Pacific	Upper middle income	18236.61298
Hong Kong SAR, China	HKG	East Asia & Pacific	High income	64596.56385
Macao SAR, China	MAC	East Asia & Pacific	High income	123892.1664
China, Taiwan Province of China	NA			
Colombia	COL	Latin America & Caribbean	Upper middle income	15012.93027
Comoros	COM	Sub-Saharan Africa	Lower middle income	2913.387306
Congo, Rep.	COG	Sub-Saharan Africa	Lower middle income	5662.063411
Costa Rica	CRI	Latin America & Caribbean	Upper middle income	17671.09535
Cote d'Ivoire	CIV	Sub-Saharan Africa	Lower middle income	4207.086934
Croatia	HRV	Europe & Central Asia	High income	27579.78083
Cuba	CUB	Latin America & Caribbean	Upper middle income	
Curacao	CUW	Latin America & Caribbean	High income	27743.07005
Cyprus	CYP	Europe & Central Asia	High income	
Czechia	CZE	Europe & Central Asia	High income	39743.59709
Korea, Dem. People’s Rep.	PRK	East Asia & Pacific	Low income	
Congo, Dem. Rep.	COD	Sub-Saharan Africa	Low income	932.1720068
Denmark	DNK	Europe & Central Asia	High income	55671.16527
Djibouti	DJI	Middle East & North Africa	Lower middle income	
Dominican Republic	DOM	Latin America & Caribbean	Upper middle income	17748.18532
Ecuador	ECU	Latin America & Caribbean	Upper middle income	11734.38739
Egypt	EGY	Middle East & North Africa	Lower middle income	12412.3094
El Salvador	SLV	Latin America & Caribbean	Lower middle income	8331.804142
Equatorial Guinea	GNQ	Sub-Saharan Africa	Upper middle income	22743.82443
Eritrea	ERI	Sub-Saharan Africa	Low income	
Estonia	EST	Europe & Central Asia	High income	35973.77646
Eswatini	SWZ	Sub-Saharan Africa	Lower middle income	10637.84173
Ethiopia	ETH	Sub-Saharan Africa	Low income	2022.137961
Fiji	FJI	East Asia & Pacific	Upper middle income	10879.47846
Finland	FIN	Europe & Central Asia	High income	48416.93603
France	FRA	Europe & Central Asia	High income	45342.39574

French Guiana	NA			
French Polynesia	PYF	East Asia & Pacific	High income	
Gabon	GAB	Sub-Saharan Africa	Upper middle income	17875.76115
Gambia	GMB	Sub-Saharan Africa	Low income	2612.000995
Georgia	GEO	Europe & Central Asia	Upper middle income	12004.57383
Germany	DEU	Europe & Central Asia	High income	53074.54012
Ghana	GHA	Sub-Saharan Africa	Lower middle income	4746.684995
Greece	GRC	Europe & Central Asia	High income	29592.15242
Grenada	GRD	Latin America & Caribbean	Upper middle income	15557.54504
Guadeloupe	NA			
Guam	GUM	East Asia & Pacific	High income	
Guatemala	GTM	Latin America & Caribbean	Upper middle income	8462.374008
Guinea	GIN	Sub-Saharan Africa	Low income	2504.869745
Guinea-Bissau	GNB	Sub-Saharan Africa	Low income	1799.067891
Guyana	GUY	Latin America & Caribbean	Upper middle income	8640.73737
Haiti	HTI	Latin America & Caribbean	Low income	1866.617589
Honduras	HND	Latin America & Caribbean	Lower middle income	5138.752457
Hungary	HUN	Europe & Central Asia	High income	31102.50275
Iceland	ISL	Europe & Central Asia	High income	57303.06078
India	IND	South Asia	Lower middle income	7762.88177
Indonesia	IDN	East Asia & Pacific	Lower middle income	13079.6193
Iran (Islamic Republic of)	IRN	Middle East & North Africa	Upper middle income	
Iraq	IRQ	Middle East & North Africa	Upper middle income	17435.85393
Ireland	IRL	Europe & Central Asia	High income	83203.39468
Israel	ISR	Middle East & North Africa	High income	39919.16965
Italy	ITA	Europe & Central Asia	High income	41830.42633
Jamaica	JAM	Latin America & Caribbean	Upper middle income	9326.664059
Japan	JPN	East Asia & Pacific	High income	42797.45852
Jordan	JOR	Middle East & North Africa	Upper middle income	9478.904588
Kazakhstan	KAZ	Europe & Central Asia	Upper middle income	27879.75195
Kenya	KEN	Sub-Saharan Africa	Lower middle income	3467.556478
Kiribati	KIR	East Asia & Pacific	Lower middle income	2293.920333
Kuwait	KWT	Middle East & North Africa	High income	72897.56235
Kyrgyz Republic	KGZ	Europe & Central Asia	Lower middle income	3884.708809

Lao PDR	LAO	East Asia & Pacific	Lower middle income	7439.59936
Latvia	LVA	Europe & Central Asia	High income	30304.8525
Lebanon	LBN	Middle East & North Africa	Upper middle income	13081.10746
Lesotho	LSO	Sub-Saharan Africa	Lower middle income	3219.457157
Liberia	LBR	Sub-Saharan Africa	Low income	1308.629536
Libya	LBY	Middle East & North Africa	Upper middle income	20764.16414
Lithuania	LTU	Europe & Central Asia	High income	35461.3591
Luxembourg	LUX	Europe & Central Asia	High income	113337.4205
Madagascar	MDG	Sub-Saharan Africa	Low income	1891.320668
Malawi	MWI	Sub-Saharan Africa	Low income	1310.995555
Malaysia	MYS	East Asia & Pacific	Upper middle income	31782.15336
Maldives	MDV	South Asia	Upper middle income	15307.72194
Mali	MLI	Sub-Saharan Africa	Low income	2316.6552
Malta	MLT	Middle East & North Africa	High income	42581.09786
Martinique	NA			
Mauritania	MRT	Sub-Saharan Africa	Lower middle income	4150.965246
Mauritius	MUS	Sub-Saharan Africa	Upper middle income	23750.98804
Mayotte	NA			
Mexico	MEX	Latin America & Caribbean	Upper middle income	19844.64567
Micronesia (Fed. States of)	FSM	East Asia & Pacific	Lower middle income	3553.061455
Mongolia	MNG	East Asia & Pacific	Lower middle income	13799.90485
Montenegro	MNE	Europe & Central Asia	Upper middle income	20689.98675
Morocco	MAR	Middle East & North Africa	Lower middle income	8586.6387
Mozambique	MOZ	Sub-Saharan Africa	Low income	1459.698413
Myanmar	MMR	East Asia & Pacific	Lower middle income	6674.028647
Namibia	NAM	Sub-Saharan Africa	Upper middle income	11101.82628
Nepal	NPL	South Asia	Low income	3089.556352
Netherlands	NLD	Europe & Central Asia	High income	56328.94114
New Caledonia	NCL	East Asia & Pacific	High income	
New Zealand	NZL	East Asia & Pacific	High income	41005.42087
Nicaragua	NIC	Latin America & Caribbean	Lower middle income	5533.551064
Niger	NER	Sub-Saharan Africa	Low income	1063.421841
Nigeria	NGA	Sub-Saharan Africa	Lower middle income	5990.850432
North Macedonia	MKD	Europe & Central Asia	Upper middle income	16358.66205

Norway	NOR	Europe & Central Asia	High income	65510.58527
Oman	OMN	Middle East & North Africa	High income	41859.93448
Pakistan	PAK	South Asia	Lower middle income	5567.055608
Panama	PAN	Latin America & Caribbean	High income	25553.69671
Papua New Guinea	PNG	East Asia & Pacific	Lower middle income	4336.244657
Paraguay	PRY	Latin America & Caribbean	Upper middle income	13599.92656
Peru	PER	Latin America & Caribbean	Upper middle income	14418.07067
Philippines	PHL	East Asia & Pacific	Lower middle income	8951.085654
Poland	POL	Europe & Central Asia	High income	31336.6035
Portugal	PRT	Europe & Central Asia	High income	33415.4379
Puerto Rico	PRI	Latin America & Caribbean	High income	39540.58693
Qatar	QAT	Middle East & North Africa	High income	126898.4259
Korea, Rep.	KOR	East Asia & Pacific	High income	40111.77576
Moldova	MDA	Europe & Central Asia	Lower middle income	7271.641919
Réunion	NA	Latin America & Caribbean		
Romania	ROU	Europe & Central Asia	Upper middle income	28206.35705
Russian Federation	RUS	Europe & Central Asia	Upper middle income	27147.33358
Rwanda	RWA	Sub-Saharan Africa	Low income	2251.556388
St. Lucia	LCA	Latin America & Caribbean	Upper middle income	13881.37213
St. Vincent and the Grenadines	VCT	Latin America & Caribbean	Upper middle income	12287.9755
Samoa	WSM	East Asia & Pacific	Upper middle income	6483.534228
Sao Tome and Principe	STP	Sub-Saharan Africa	Lower middle income	3418.58693
Saudi Arabia	SAU	Middle East & North Africa	High income	55335.67959
Senegal	SEN	Sub-Saharan Africa	Lower middle income	3782.538613
Serbia	SRB	Europe & Central Asia	Upper middle income	17434.91613
Seychelles	SYC	Sub-Saharan Africa	High income	30557.07572
Sierra Leone	SLE	Sub-Saharan Africa	Low income	1601.974029
Singapore	SGP	East Asia & Pacific	High income	101531.6302
Slovakia	SVK	Europe & Central Asia	High income	33736.40094
Slovenia	SVN	Europe & Central Asia	High income	38048.78466
Solomon Islands	SLB	East Asia & Pacific	Lower middle income	2422.819533
Somalia	SOM	Sub-Saharan Africa	Low income	
South Africa	ZAF	Sub-Saharan Africa	Upper middle income	13686.88236
South Sudan	SSD	Sub-Saharan Africa	Low income	

Spain	ESP	Europe & Central Asia	High income	39715.43906
Sri Lanka	LKA	South Asia	Upper middle income	13473.66309
State of Palestine	PSE	Middle East & North Africa	Lower middle income	5157.568578
Sudan	SDN	Sub-Saharan Africa	Lower middle income	4759.281882
Suriname	SUR	Latin America & Caribbean	Upper middle income	15510.46464
Sweden	SWE	Europe & Central Asia	High income	53208.88436
Switzerland	CHE	Europe & Central Asia	High income	68060.94105
Syrian Arab Republic	SYR	Middle East & North Africa	Low income	
Tajikistan	TJK	Europe & Central Asia	Low income	3449.779316
Thailand	THA	East Asia & Pacific	Upper middle income	19051.33338
Timor-Leste	TLS	East Asia & Pacific	Lower middle income	7658.434996
Togo	TGO	Sub-Saharan Africa	Low income	1773.896632
Tonga	TON	East Asia & Pacific	Upper middle income	6419.561778
Trinidad and Tobago	TTO	Latin America & Caribbean	High income	32014.78313
Tunisia	TUN	Middle East & North Africa	Lower middle income	12502.82019
Turkey	TUR	Europe & Central Asia	Upper middle income	28068.85941
Turkmenistan	TKM	Europe & Central Asia	Upper middle income	19304.10131
Uganda	UGA	Sub-Saharan Africa	Low income	2038.073892
Ukraine	UKR	Europe & Central Asia	Lower middle income	9233.150469
United Arab Emirates	ARE	Middle East & North Africa	High income	75075.25741
United Kingdom	GBR	Europe & Central Asia	High income	45973.5735
Tanzania	TZA	Sub-Saharan Africa	Low income	3227.034211
United States	USA	North America	High income	62794.58565
United States Virgin Islands	VIR	Latin America & Caribbean	High income	
Uruguay	URY	Latin America & Caribbean	High income	23572.17746
Uzbekistan	UZB	Europe & Central Asia	Lower middle income	8556.051512
Vanuatu	VUT	East Asia & Pacific	Lower middle income	3221.149823
Venezuela (Bolivarian Republic of)	VEN	Latin America & Caribbean	Upper middle income	
Vietnam	VNM	East Asia & Pacific	Lower middle income	7447.814334
Western Sahara	NA	Sub-Saharan Africa		
Yemen	YEM	Middle East & North Africa	Low income	2575.126385
Zambia	ZMB	Sub-Saharan Africa	Lower middle income	4223.906936
Zimbabwe	ZWE	Sub-Saharan Africa	Lower middle income	3029.793005

Mitigation

Country	R0	Strategy	Social_distance	total_pop	total_infected	total_deaths	total_hospital	total_critical
Afghanistan	2.4	Enhanced social distancing of elderly	34%	38928341	22235810	42015	342532	55701
Afghanistan	2.7	Enhanced social distancing of elderly	38%	38928341	24001697	46456	371943	61587
Afghanistan	3	Enhanced social distancing of elderly	44%	38928341	25058690	57529	414970	76266
Afghanistan	3.3	Enhanced social distancing of elderly	47%	38928341	26214010	60640	434649	80429
Afghanistan	2.4	Social distancing whole population	37%	38928341	21576989	55929	378738	74147
Afghanistan	2.7	Social distancing whole population	41%	38928341	23382945	59413	405719	78799
Afghanistan	3	Social distancing whole population	45%	38928341	24841920	62072	426882	82305
Afghanistan	3.3	Social distancing whole population	48%	38928341	26048156	64163	443937	85061
Afghanistan	2.4	Unmitigated	0%	38928341	33234053	74381	535948	98609
Afghanistan	2.7	Unmitigated	0%	38928341	34824650	76059	553653	100832
Afghanistan	3	Unmitigated	0%	38928341	35927979	77072	565164	102168
Afghanistan	3.3	Unmitigated	0%	38928341	36708969	77708	572873	103031
Albania	2.4	Enhanced social distancing of elderly	35%	2877800	1271701	4213	33065	5586
Albania	2.7	Enhanced social distancing of elderly	38%	2877800	1405053	4960	37666	6578
Albania	3	Enhanced social distancing of elderly	42%	2877800	1542120	7325	46576	9712
Albania	3.3	Enhanced social distancing of elderly	45%	2877800	1640318	8253	51000	10940
Albania	2.4	Social distancing whole population	36%	2877800	1323142	7274	42046	9643
Albania	2.7	Social distancing whole population	40%	2877800	1459024	8310	47425	11017
Albania	3	Social distancing whole population	43%	2877800	1571987	9229	52085	12235
Albania	3.3	Social distancing whole population	46%	2877800	1667558	10051	56169	13333
Albania	2.4	Unmitigated	0%	2877800	2178185	15342	80744	20340
Albania	2.7	Unmitigated	0%	2877800	2328478	17299	89134	22939
Albania	3	Unmitigated	0%	2877800	2440567	18935	95875	25111
Albania	3.3	Unmitigated	0%	2877800	2525855	20309	101349	26927
Algeria	2.4	Enhanced social distancing of elderly	34%	43851043	22630780	63329	541065	83956
Algeria	2.7	Enhanced social distancing of elderly	38%	43851043	24684676	72422	598133	96011
Algeria	3	Enhanced social distancing of elderly	43%	43851043	26153264	101950	701426	135151
Algeria	3.3	Enhanced social distancing of elderly	47%	43851043	27485010	111613	747966	148020
Algeria	2.4	Social distancing whole population	37%	43851043	22652248	103677	651591	137447
Algeria	2.7	Social distancing whole population	41%	43851043	24604851	113589	708890	150671
Algeria	3	Social distancing whole population	45%	43851043	26193033	121783	755525	161497
Algeria	3.3	Social distancing whole population	48%	43851043	27512076	128681	794259	170577
Algeria	2.4	Unmitigated	0%	43851043	35364412	171534	1022751	227406
Algeria	2.7	Unmitigated	0%	43851043	37223115	182089	1075192	241430
Algeria	3	Unmitigated	0%	43851043	38561363	189728	1111893	251505
Algeria	3.3	Unmitigated	0%	43851043	39550379	195348	1138076	258972
Angola	2.4	Enhanced social distancing of elderly	35%	32866268	18198225	29066	256110	38534
Angola	2.7	Enhanced social distancing of elderly	39%	32866268	19678221	32307	279022	42842
Angola	3	Enhanced social distancing of elderly	44%	32866268	20656120	40516	312366	53726
Angola	3.3	Enhanced social distancing of elderly	47%	32866268	21632859	42961	328155	56954

Angola	2.4	Social distancing whole population	37%	32866268	17824258	39321	283832	52128
Angola	2.7	Social distancing whole population	41%	32866268	19323398	42035	305221	55726
Angola	3	Social distancing whole population	45%	32866268	20536062	44148	322147	58524
Angola	3.3	Social distancing whole population	48%	32866268	21539422	45838	335886	60776
Angola	2.4	Unmitigated	0%	32866268	27587767	54691	412190	72505
Angola	2.7	Unmitigated	0%	32866268	28954731	56290	427307	74623
Angola	3	Unmitigated	0%	32866268	29918521	57296	437250	75991
Angola	3.3	Unmitigated	0%	32866268	30613383	57944	443936	76828
Antigua and Barbuda	2.4	Enhanced social distancing of elderly	31%	97928	42340	124	947	164
Antigua and Barbuda	2.7	Enhanced social distancing of elderly	35%	97928	47351	148	1101	196
Antigua and Barbuda	3	Enhanced social distancing of elderly	39%	97928	52133	214	1365	284
Antigua and Barbuda	3.3	Enhanced social distancing of elderly	42%	97928	55765	240	1504	319
Antigua and Barbuda	2.4	Social distancing whole population	33%	97928	43615	207	1192	275
Antigua and Barbuda	2.7	Social distancing whole population	37%	97928	48604	238	1362	315
Antigua and Barbuda	3	Social distancing whole population	40%	97928	52792	264	1510	351
Antigua and Barbuda	3.3	Social distancing whole population	43%	97928	56347	288	1640	382
Antigua and Barbuda	2.4	Unmitigated	0%	97928	71817	401	2250	531
Antigua and Barbuda	2.7	Unmitigated	0%	97928	77556	447	2498	593
Antigua and Barbuda	3	Unmitigated	0%	97928	81811	484	2691	642
Antigua and Barbuda	3.3	Unmitigated	0%	97928	85015	514	2843	681
Argentina	2.4	Enhanced social distancing of elderly	34%	45195777	19881939	59872	418845	79373
Argentina	2.7	Enhanced social distancing of elderly	37%	45195777	22132135	71410	485081	94721
Argentina	3	Enhanced social distancing of elderly	42%	45195777	24365226	107249	614649	142225
Argentina	3.3	Enhanced social distancing of elderly	44%	45195777	26005121	120686	677138	159970
Argentina	2.4	Social distancing whole population	36%	45195777	20576774	104787	546644	138918
Argentina	2.7	Social distancing whole population	39%	45195777	22832080	119818	621598	158865
Argentina	3	Social distancing whole population	43%	45195777	24720324	133033	686840	176351
Argentina	3.3	Social distancing whole population	45%	45195777	26321567	144721	743999	191857
Argentina	2.4	Unmitigated	0%	45195777	34471496	212412	1064620	281599
Argentina	2.7	Unmitigated	0%	45195777	36944290	236547	1174102	313586
Argentina	3	Unmitigated	0%	45195777	38770282	255948	1259949	339471
Argentina	3.3	Unmitigated	0%	45195777	40139861	271672	1327912	360208
Armenia	2.4	Enhanced social distancing of elderly	35%	2963234	1397151	4312	34956	5717
Armenia	2.7	Enhanced social distancing of elderly	39%	2963234	1536827	5042	39562	6684
Armenia	3	Enhanced social distancing of elderly	43%	2963234	1674437	7282	47979	9653
Armenia	3.3	Enhanced social distancing of elderly	46%	2963234	1774585	8154	52214	10812
Armenia	2.4	Social distancing whole population	36%	2963234	1442022	7178	43097	9516
Armenia	2.7	Social distancing whole population	40%	2963234	1583109	8148	48309	10801
Armenia	3	Social distancing whole population	43%	2963234	1699312	8997	52769	11932
Armenia	3.3	Social distancing whole population	46%	2963234	1796825	9749	56638	12926
Armenia	2.4	Unmitigated	0%	2963234	2327937	14678	80265	19459
Armenia	2.7	Unmitigated	0%	2963234	2474060	16389	87795	21731
Armenia	3	Unmitigated	0%	2963234	2580964	17793	93722	23588

Armenia	3.3	Unmitigated	0%	2963234	2660711	18951	98440	25123
Aruba	2.4	Enhanced social distancing of elderly	30%	106766	42915	167	1152	221
Aruba	2.7	Enhanced social distancing of elderly	34%	106766	48102	200	1346	266
Aruba	3	Enhanced social distancing of elderly	39%	106766	53507	296	1710	392
Aruba	3.3	Enhanced social distancing of elderly	42%	106766	57383	334	1894	443
Aruba	2.4	Social distancing whole population	33%	106766	45024	289	1516	383
Aruba	2.7	Social distancing whole population	37%	106766	50271	333	1737	441
Aruba	3	Social distancing whole population	40%	106766	54700	371	1932	492
Aruba	3.3	Social distancing whole population	43%	106766	58483	406	2105	538
Aruba	2.4	Unmitigated	0%	106766	75228	576	2939	763
Aruba	2.7	Unmitigated	0%	106766	81576	649	3291	861
Aruba	3	Unmitigated	0%	106766	86380	709	3575	941
Aruba	3.3	Unmitigated	0%	106766	90082	759	3805	1006
Australia	2.4	Enhanced social distancing of elderly	35%	25499881	11505560	38520	296832	51067
Australia	2.7	Enhanced social distancing of elderly	39%	25499881	12679690	45396	337560	60193
Australia	3	Enhanced social distancing of elderly	42%	25499881	13906207	68550	420373	90875
Australia	3.3	Enhanced social distancing of elderly	45%	25499881	14767130	77371	460126	102570
Australia	2.4	Social distancing whole population	36%	25499881	12002262	69102	383450	91610
Australia	2.7	Social distancing whole population	40%	25499881	13202721	78872	431695	104559
Australia	3	Social distancing whole population	43%	25499881	14195271	87514	473316	116067
Australia	3.3	Social distancing whole population	46%	25499881	15030810	95234	509690	126275
Australia	2.4	Unmitigated	0%	25499881	19564996	146707	734250	194492
Australia	2.7	Unmitigated	0%	25499881	20851728	165589	809460	219583
Australia	3	Unmitigated	0%	25499881	21804902	181490	870037	240556
Australia	3.3	Unmitigated	0%	25499881	22525755	194953	919396	258433
Austria	2.4	Enhanced social distancing of elderly	34%	9006400	3795039	14994	111060	19878
Austria	2.7	Enhanced social distancing of elderly	38%	9006400	4206941	17804	127332	23603
Austria	3	Enhanced social distancing of elderly	42%	9006400	4652294	27189	160265	36057
Austria	3.3	Enhanced social distancing of elderly	45%	9006400	4961210	30847	176417	40900
Austria	2.4	Social distancing whole population	35%	9006400	3993988	27404	145412	36330
Austria	2.7	Social distancing whole population	39%	9006400	4418644	31512	164988	41784
Austria	3	Social distancing whole population	43%	9006400	4772589	35182	182055	46639
Austria	3.3	Social distancing whole population	45%	9006400	5072575	38486	197094	51019
Austria	2.4	Unmitigated	0%	9006400	6643425	59573	286532	78977
Austria	2.7	Unmitigated	0%	9006400	7125672	67827	318712	89913
Austria	3	Unmitigated	0%	9006400	7488731	74864	344990	99280
Austria	3.3	Unmitigated	0%	9006400	7767698	80884	366658	107262
Azerbaijan	2.4	Enhanced social distancing of elderly	35%	10139175	5057155	12901	116170	17103
Azerbaijan	2.7	Enhanced social distancing of elderly	39%	10139175	5550308	14916	130575	19780
Azerbaijan	3	Enhanced social distancing of elderly	43%	10139175	5991490	20388	153377	27028
Azerbaijan	3.3	Enhanced social distancing of elderly	46%	10139175	6332566	22527	165471	29863
Azerbaijan	2.4	Social distancing whole population	36%	10139175	5142086	19605	135731	25991
Azerbaijan	2.7	Social distancing whole population	40%	10139175	5632302	22013	151057	29181

Azerbaijan	3	Social distancing whole population	44%	10139175	6033689	24072	163953	31929
Azerbaijan	3.3	Social distancing whole population	46%	10139175	6368602	25857	174966	34293
Azerbaijan	2.4	Unmitigated	0%	10139175	8206246	36990	240260	49038
Azerbaijan	2.7	Unmitigated	0%	10139175	8687967	40415	259126	53602
Azerbaijan	3	Unmitigated	0%	10139175	9033669	43070	273308	57106
Azerbaijan	3.3	Unmitigated	0%	10139175	9287041	45150	284134	59852
Bahamas	2.4	Enhanced social distancing of elderly	33%	393248	174349	475	3747	629
Bahamas	2.7	Enhanced social distancing of elderly	37%	393248	194338	562	4331	745
Bahamas	3	Enhanced social distancing of elderly	41%	393248	212963	799	5304	1059
Bahamas	3.3	Enhanced social distancing of elderly	44%	393248	227323	893	5820	1184
Bahamas	2.4	Social distancing whole population	35%	393248	178492	759	4595	1006
Bahamas	2.7	Social distancing whole population	39%	393248	198300	866	5225	1148
Bahamas	3	Social distancing whole population	42%	393248	214893	959	5772	1272
Bahamas	3.3	Social distancing whole population	45%	393248	228964	1041	6249	1380
Bahamas	2.4	Unmitigated	0%	393248	297852	1480	8780	1963
Bahamas	2.7	Unmitigated	0%	393248	319815	1637	9664	2169
Bahamas	3	Unmitigated	0%	393248	336027	1758	10347	2331
Bahamas	3.3	Unmitigated	0%	393248	348180	1854	10879	2459
Bahrain	2.4	Enhanced social distancing of elderly	34%	1701583	934780	2299	23209	3048
Bahrain	2.7	Enhanced social distancing of elderly	38%	1701583	1011213	2545	25223	3375
Bahrain	3	Enhanced social distancing of elderly	43%	1701583	1054190	3095	27534	4104
Bahrain	3.3	Enhanced social distancing of elderly	47%	1701583	1104824	3277	28908	4344
Bahrain	2.4	Social distancing whole population	37%	1701583	905516	2962	24621	3927
Bahrain	2.7	Social distancing whole population	41%	1701583	983623	3171	26560	4203
Bahrain	3	Social distancing whole population	44%	1701583	1046995	3334	28106	4421
Bahrain	3.3	Social distancing whole population	47%	1701583	1099482	3466	29367	4597
Bahrain	2.4	Unmitigated	0%	1701583	1407811	4155	36341	5509
Bahrain	2.7	Unmitigated	0%	1701583	1480127	4292	37831	5692
Bahrain	3	Unmitigated	0%	1701583	1531341	4381	38833	5809
Bahrain	3.3	Unmitigated	0%	1701583	1568493	4440	39524	5887
Bangladesh	2.4	Enhanced social distancing of elderly	34%	164689383	93855688	264892	2028045	351172
Bangladesh	2.7	Enhanced social distancing of elderly	38%	164689383	101547595	298675	2232628	395935
Bangladesh	3	Enhanced social distancing of elderly	44%	164689383	107207394	395419	2575198	524486
Bangladesh	3.3	Enhanced social distancing of elderly	47%	164689383	112232453	425219	2729019	563912
Bangladesh	2.4	Social distancing whole population	37%	164689383	93052124	388051	2346714	514446
Bangladesh	2.7	Social distancing whole population	41%	164689383	100724508	420078	2543915	557112
Bangladesh	3	Social distancing whole population	45%	164689383	106899433	445820	2703011	591105
Bangladesh	3.3	Social distancing whole population	48%	164689383	111991810	467030	2834506	619114
Bangladesh	2.4	Unmitigated	0%	164689383	142192269	592125	3620453	784990
Bangladesh	2.7	Unmitigated	0%	164689383	148701242	618890	3791939	820416
Bangladesh	3	Unmitigated	0%	164689383	153164342	637191	3910219	844703
Bangladesh	3.3	Unmitigated	0%	164689383	156284908	649967	3993557	862049
Barbados	2.4	Enhanced social distancing of elderly	32%	287371	114233	485	3084	643

Barbados	2.7	Enhanced social distancing of elderly	36%	287371	128097	584	3610	774
Barbados	3	Enhanced social distancing of elderly	40%	287371	142968	892	4674	1183
Barbados	3.3	Enhanced social distancing of elderly	43%	287371	153410	1010	5187	1339
Barbados	2.4	Social distancing whole population	34%	287371	120472	881	4177	1167
Barbados	2.7	Social distancing whole population	38%	287371	134554	1014	4790	1345
Barbados	3	Social distancing whole population	41%	287371	146451	1133	5328	1502
Barbados	3.3	Social distancing whole population	44%	287371	156614	1238	5804	1643
Barbados	2.4	Unmitigated	0%	287371	205098	1808	8307	2397
Barbados	2.7	Unmitigated	0%	287371	221943	2039	9284	2704
Barbados	3	Unmitigated	0%	287371	234697	2229	10073	2955
Barbados	3.3	Unmitigated	0%	287371	244527	2387	10715	3164
Belarus	2.4	Enhanced social distancing of elderly	35%	9449321	4225775	14924	117348	19785
Belarus	2.7	Enhanced social distancing of elderly	38%	9449321	4663827	17563	133619	23280
Belarus	3	Enhanced social distancing of elderly	42%	9449321	5116275	25844	164350	34269
Belarus	3.3	Enhanced social distancing of elderly	45%	9449321	5437274	29103	179798	38597
Belarus	2.4	Social distancing whole population	36%	9449321	4401600	25594	147743	33930
Belarus	2.7	Social distancing whole population	40%	9449321	4848262	29235	166596	38772
Belarus	3	Social distancing whole population	43%	9449321	5218214	32457	182887	43033
Belarus	3.3	Social distancing whole population	46%	9449321	5530050	35333	197136	46838
Belarus	2.4	Unmitigated	0%	9449321	7203722	54090	283709	71708
Belarus	2.7	Unmitigated	0%	9449321	7686944	60982	312939	80841
Belarus	3	Unmitigated	0%	9449321	8045543	66755	336417	88493
Belarus	3.3	Unmitigated	0%	9449321	8317401	71616	355484	95012
Belgium	2.4	Enhanced social distancing of elderly	34%	11589616	5601154	26073	179381	34566
Belgium	2.7	Enhanced social distancing of elderly	38%	11589616	6125285	30374	201615	40281
Belgium	3	Enhanced social distancing of elderly	43%	11589616	6702807	45794	252390	60718
Belgium	3.3	Enhanced social distancing of elderly	46%	11589616	7082309	51136	273942	67788
Belgium	2.4	Social distancing whole population	37%	11589616	5898101	46924	237347	62208
Belgium	2.7	Social distancing whole population	41%	11589616	6426668	52525	262613	69626
Belgium	3	Social distancing whole population	44%	11589616	6858664	57364	283950	76046
Belgium	3.3	Social distancing whole population	47%	11589616	7218935	61600	302260	81697
Belgium	2.4	Unmitigated	0%	11589616	9328730	91586	422196	121417
Belgium	2.7	Unmitigated	0%	11589616	9851326	101122	456797	134165
Belgium	3	Unmitigated	0%	11589616	10229411	108928	483820	144443
Belgium	3.3	Unmitigated	0%	11589616	10509681	115385	505276	152930
Belize	2.4	Enhanced social distancing of elderly	34%	397621	190658	392	3240	519
Belize	2.7	Enhanced social distancing of elderly	38%	397621	210717	458	3691	608
Belize	3	Enhanced social distancing of elderly	42%	397621	228255	641	4429	850
Belize	3.3	Enhanced social distancing of elderly	45%	397621	242236	710	4810	941
Belize	2.4	Social distancing whole population	35%	397621	193011	616	3883	817
Belize	2.7	Social distancing whole population	39%	397621	212775	693	4356	920
Belize	3	Social distancing whole population	43%	397621	229156	759	4759	1007
Belize	3.3	Social distancing whole population	45%	397621	242935	816	5105	1082

Belize	2.4	Unmitigated	0%	397621	313763	1127	6986	1494
Belize	2.7	Unmitigated	0%	397621	334278	1224	7564	1623
Belize	3	Unmitigated	0%	397621	349132	1296	7994	1719
Belize	3.3	Unmitigated	0%	397621	360066	1351	8316	1791
Benin	2.4	Enhanced social distancing of elderly	35%	12123198	6756151	12707	105694	16845
Benin	2.7	Enhanced social distancing of elderly	39%	12123198	7310945	14260	115830	18912
Benin	3	Enhanced social distancing of elderly	44%	12123198	7703590	18630	132622	24701
Benin	3.3	Enhanced social distancing of elderly	47%	12123198	8067945	19940	140046	26433
Benin	2.4	Social distancing whole population	37%	12123198	6669239	18307	121405	24270
Benin	2.7	Social distancing whole population	41%	12123198	7224783	19710	131032	26130
Benin	3	Social distancing whole population	45%	12123198	7673306	20824	138717	27606
Benin	3.3	Social distancing whole population	48%	12123198	8043621	21729	144996	28819
Benin	2.4	Unmitigated	0%	12123198	10270368	26809	181016	35541
Benin	2.7	Unmitigated	0%	12123198	10765529	27818	188410	36884
Benin	3	Unmitigated	0%	12123198	11111328	28481	193344	37773
Benin	3.3	Unmitigated	0%	12123198	11358911	28927	196715	38354
Bhutan	2.4	Enhanced social distancing of elderly	33%	771612	435367	1265	9403	1678
Bhutan	2.7	Enhanced social distancing of elderly	38%	771612	471604	1434	10377	1901
Bhutan	3	Enhanced social distancing of elderly	43%	771612	499940	1948	12154	2582
Bhutan	3.3	Enhanced social distancing of elderly	47%	771612	523795	2104	12914	2791
Bhutan	2.4	Social distancing whole population	37%	771612	434531	1935	11176	2565
Bhutan	2.7	Social distancing whole population	41%	771612	470581	2101	12130	2787
Bhutan	3	Social distancing whole population	45%	771612	499612	2235	12902	2964
Bhutan	3.3	Social distancing whole population	48%	771612	523502	2346	13539	3110
Bhutan	2.4	Unmitigated	0%	771612	663460	3007	17313	3987
Bhutan	2.7	Unmitigated	0%	771612	694374	3157	18159	4186
Bhutan	3	Unmitigated	0%	771612	715580	3262	18744	4324
Bhutan	3.3	Unmitigated	0%	771612	730449	3336	19157	4422
Bolivia	2.4	Enhanced social distancing of elderly	35%	11673029	5541515	12787	96647	16952
Bolivia	2.7	Enhanced social distancing of elderly	38%	11673029	6120190	15055	110424	19964
Bolivia	3	Enhanced social distancing of elderly	42%	11673029	6657592	21994	136154	29163
Bolivia	3.3	Enhanced social distancing of elderly	45%	11673029	7067157	24521	148540	32507
Bolivia	2.4	Social distancing whole population	36%	11673029	5661108	21488	121399	28486
Bolivia	2.7	Social distancing whole population	40%	11673029	6236465	24284	136446	32189
Bolivia	3	Social distancing whole population	43%	11673029	6713530	26704	149333	35400
Bolivia	3.3	Social distancing whole population	46%	11673029	7114835	28816	160472	38212
Bolivia	2.4	Unmitigated	0%	11673029	9250797	41442	224862	54941
Bolivia	2.7	Unmitigated	0%	11673029	9845359	45507	244655	60328
Bolivia	3	Unmitigated	0%	11673029	10276067	48674	259686	64554
Bolivia	3.3	Unmitigated	0%	11673029	10594058	51173	271258	67848
Bosnia and Herzegovina	2.4	Enhanced social distancing of elderly	34%	3280815	1412795	5314	40998	7044
Bosnia and Herzegovina	2.7	Enhanced social distancing of elderly	38%	3280815	1563514	6280	46889	8326
Bosnia and Herzegovina	3	Enhanced social distancing of elderly	42%	3280815	1724462	9339	58332	12380

Bosnia and Herzegovina	3.3	Enhanced social distancing of elderly	45%	3280815	1836821	10554	64063	14000
Bosnia and Herzegovina	2.4	Social distancing whole population	36%	3280815	1481911	9274	52547	12295
Bosnia and Herzegovina	2.7	Social distancing whole population	39%	3280815	1636863	10640	59511	14109
Bosnia and Herzegovina	3	Social distancing whole population	43%	3280815	1765788	11856	65570	15724
Bosnia and Herzegovina	3.3	Social distancing whole population	46%	3280815	1874905	12947	70899	17166
Bosnia and Herzegovina	2.4	Unmitigated	0%	3280815	2449298	19917	102753	26405
Bosnia and Herzegovina	2.7	Unmitigated	0%	3280815	2622463	22576	113990	29931
Bosnia and Herzegovina	3	Unmitigated	0%	3280815	2752281	24818	123112	32899
Bosnia and Herzegovina	3.3	Unmitigated	0%	3280815	2851557	26716	130579	35417
Botswana	2.4	Enhanced social distancing of elderly	34%	2351625	1317160	2940	24250	3898
Botswana	2.7	Enhanced social distancing of elderly	39%	2351625	1427214	3321	26734	4404
Botswana	3	Enhanced social distancing of elderly	44%	2351625	1509966	4412	30968	5851
Botswana	3.3	Enhanced social distancing of elderly	47%	2351625	1582234	4751	32856	6298
Botswana	2.4	Social distancing whole population	37%	2351625	1309423	4340	28280	5753
Botswana	2.7	Social distancing whole population	41%	2351625	1418827	4702	30683	6234
Botswana	3	Social distancing whole population	45%	2351625	1507062	4993	32624	6619
Botswana	3.3	Social distancing whole population	48%	2351625	1579810	5233	34224	6937
Botswana	2.4	Unmitigated	0%	2351625	2010430	6628	43670	8787
Botswana	2.7	Unmitigated	0%	2351625	2105803	6928	45738	9185
Botswana	3	Unmitigated	0%	2351625	2171745	7132	47153	9459
Botswana	3.3	Unmitigated	0%	2351625	2218302	7273	48142	9643
Brazil	2.4	Enhanced social distancing of elderly	33%	212559409	91801981	270693	2023874	358863
Brazil	2.7	Enhanced social distancing of elderly	37%	212559409	102598007	322646	2348908	427709
Brazil	3	Enhanced social distancing of elderly	41%	212559409	112988886	471742	2925842	625338
Brazil	3.3	Enhanced social distancing of elderly	44%	212559409	120836850	529779	3222096	702497
Brazil	2.4	Social distancing whole population	35%	212559409	94554305	452442	2550716	599810
Brazil	2.7	Social distancing whole population	39%	212559409	105309523	518315	2909776	687127
Brazil	3	Social distancing whole population	42%	212559409	114348169	576128	3222624	764105
Brazil	3.3	Social distancing whole population	45%	212559409	122025818	627047	3496359	831381
Brazil	2.4	Unmitigated	0%	212559409	160125948	908009	4974643	1203762
Brazil	2.7	Unmitigated	0%	212559409	172162607	1008804	5490012	1337612
Brazil	3	Unmitigated	0%	212559409	181084337	1088612	5891295	1443116
Brazil	3.3	Unmitigated	0%	212559409	187799806	1152283	6206514	1527536
Brunei Darussalam	2.4	Enhanced social distancing of elderly	33%	437483	239784	705	6249	935
Brunei Darussalam	2.7	Enhanced social distancing of elderly	37%	437483	260576	800	6890	1061
Brunei Darussalam	3	Enhanced social distancing of elderly	43%	437483	273320	1060	7848	1405
Brunei Darussalam	3.3	Enhanced social distancing of elderly	46%	437483	286459	1146	8325	1519
Brunei Darussalam	2.4	Social distancing whole population	37%	437483	236691	1047	7161	1388
Brunei Darussalam	2.7	Social distancing whole population	41%	437483	256544	1138	7772	1509
Brunei Darussalam	3	Social distancing whole population	44%	437483	272595	1212	8266	1607
Brunei Darussalam	3.3	Social distancing whole population	47%	437483	285847	1273	8674	1687
Brunei Darussalam	2.4	Unmitigated	0%	437483	363160	1628	11029	2158
Brunei Darussalam	2.7	Unmitigated	0%	437483	381133	1709	11562	2265

Brunei Darussalam	3	Unmitigated	0%	437483	393789	1764	11929	2340
Brunei Darussalam	3.3	Unmitigated	0%	437483	402932	1804	12186	2392
Bulgaria	2.4	Enhanced social distancing of elderly	34%	6948445	2918669	11559	86088	15324
Bulgaria	2.7	Enhanced social distancing of elderly	38%	6948445	3229297	13716	98608	18186
Bulgaria	3	Enhanced social distancing of elderly	42%	6948445	3575618	21033	124868	27882
Bulgaria	3.3	Enhanced social distancing of elderly	45%	6948445	3810454	23872	137521	31647
Bulgaria	2.4	Social distancing whole population	36%	6948445	3087288	21257	114063	28181
Bulgaria	2.7	Social distancing whole population	39%	6948445	3409768	24427	129307	32383
Bulgaria	3	Social distancing whole population	43%	6948445	3678306	27262	142613	36158
Bulgaria	3.3	Social distancing whole population	46%	6948445	3905831	29817	154360	39535
Bulgaria	2.4	Unmitigated	0%	6948445	5115627	46532	225941	61688
Bulgaria	2.7	Unmitigated	0%	6948445	5481809	53034	251604	70327
Bulgaria	3	Unmitigated	0%	6948445	5758297	58600	272699	77673
Bulgaria	3.3	Unmitigated	0%	6948445	5971359	63377	290197	84014
Burkina Faso	2.4	Enhanced social distancing of elderly	35%	20903278	11640625	19055	168097	25262
Burkina Faso	2.7	Enhanced social distancing of elderly	39%	20903278	12587560	21210	183352	28117
Burkina Faso	3	Enhanced social distancing of elderly	44%	20903278	13223106	26753	206137	35483
Burkina Faso	3.3	Enhanced social distancing of elderly	47%	20903278	13845963	28406	216762	37664
Burkina Faso	2.4	Social distancing whole population	37%	20903278	11423617	26005	187566	34476
Burkina Faso	2.7	Social distancing whole population	41%	20903278	12379017	27830	201826	36902
Burkina Faso	3	Social distancing whole population	45%	20903278	13151569	29254	213131	38782
Burkina Faso	3.3	Social distancing whole population	48%	20903278	13791525	30398	222340	40297
Burkina Faso	2.4	Unmitigated	0%	20903278	17633301	36433	273678	48300
Burkina Faso	2.7	Unmitigated	0%	20903278	18495042	37533	283897	49755
Burkina Faso	3	Unmitigated	0%	20903278	19099112	38226	290622	50682
Burkina Faso	3.3	Unmitigated	0%	20903278	19532860	38674	295155	51293
Burundi	2.4	Enhanced social distancing of elderly	35%	11890781	6575209	10728	93439	14222
Burundi	2.7	Enhanced social distancing of elderly	39%	11890781	7112975	11940	101898	15832
Burundi	3	Enhanced social distancing of elderly	44%	11890781	7473911	15044	114528	19943
Burundi	3.3	Enhanced social distancing of elderly	47%	11890781	7828732	15970	120413	21171
Burundi	2.4	Social distancing whole population	37%	11890781	6450490	14615	104226	19376
Burundi	2.7	Social distancing whole population	41%	11890781	6993605	15640	112140	20734
Burundi	3	Social distancing whole population	45%	11890781	7433296	16441	118413	21804
Burundi	3.3	Social distancing whole population	48%	11890781	7796820	17081	123501	22654
Burundi	2.4	Unmitigated	0%	11890781	9984827	20455	151740	27118
Burundi	2.7	Unmitigated	0%	11890781	10480373	21071	157341	27947
Burundi	3	Unmitigated	0%	11890781	10829296	21459	161016	28452
Burundi	3.3	Unmitigated	0%	11890781	11080859	21708	163478	28777
Cabo Verde	2.4	Enhanced social distancing of elderly	34%	555988	308045	780	6257	1034
Cabo Verde	2.7	Enhanced social distancing of elderly	38%	555988	334224	884	6916	1173
Cabo Verde	3	Enhanced social distancing of elderly	43%	555988	353701	1183	7988	1568
Cabo Verde	3.3	Enhanced social distancing of elderly	46%	555988	370964	1278	8492	1694
Cabo Verde	2.4	Social distancing whole population	37%	555988	305857	1163	7237	1542

Cabo Verde	2.7	Social distancing whole population	41%	555988	331894	1265	7881	1678
Cabo Verde	3	Social distancing whole population	44%	555988	352954	1348	8405	1787
Cabo Verde	3.3	Social distancing whole population	47%	555988	370351	1417	8840	1879
Cabo Verde	2.4	Unmitigated	0%	555988	471210	1823	11415	2417
Cabo Verde	2.7	Unmitigated	0%	555988	494495	1919	12026	2544
Cabo Verde	3	Unmitigated	0%	555988	510675	1987	12456	2634
Cabo Verde	3.3	Unmitigated	0%	555988	522151	2035	12764	2699
Cambodia	2.4	Enhanced social distancing of elderly	34%	16718971	8602848	21107	188432	27981
Cambodia	2.7	Enhanced social distancing of elderly	38%	16718971	9374938	23918	207353	31720
Cambodia	3	Enhanced social distancing of elderly	43%	16718971	9911597	32216	238934	42710
Cambodia	3.3	Enhanced social distancing of elderly	47%	16718971	10416871	34902	253611	46267
Cambodia	2.4	Social distancing whole population	37%	16718971	8547410	32090	219801	42542
Cambodia	2.7	Social distancing whole population	41%	16718971	9293347	34909	238394	46276
Cambodia	3	Social distancing whole population	45%	16718971	9901700	37199	253416	49323
Cambodia	3.3	Social distancing whole population	47%	16718971	10408103	39103	265824	51861
Cambodia	2.4	Unmitigated	0%	16718971	13414629	50146	336478	66480
Cambodia	2.7	Unmitigated	0%	16718971	14137141	52665	352198	69851
Cambodia	3	Unmitigated	0%	16718971	14658802	54410	362969	72142
Cambodia	3.3	Unmitigated	0%	16718971	15045768	55642	370514	73761
Cameroon	2.4	Enhanced social distancing of elderly	35%	26545864	14841373	25550	223250	33872
Cameroon	2.7	Enhanced social distancing of elderly	39%	26545864	16052251	28523	243958	37811
Cameroon	3	Enhanced social distancing of elderly	44%	26545864	16881143	36422	275870	48301
Cameroon	3.3	Enhanced social distancing of elderly	47%	26545864	17676051	38780	290536	51429
Cameroon	2.4	Social distancing whole population	37%	26545864	14597159	35534	251293	47109
Cameroon	2.7	Social distancing whole population	41%	26545864	15814859	38108	270757	50543
Cameroon	3	Social distancing whole population	45%	26545864	16798516	40130	286240	53207
Cameroon	3.3	Social distancing whole population	48%	26545864	17610215	41755	298835	55352
Cameroon	2.4	Unmitigated	0%	26545864	22491386	50535	370107	66996
Cameroon	2.7	Unmitigated	0%	26545864	23577621	52179	384465	69171
Cameroon	3	Unmitigated	0%	26545864	24336360	53226	393966	70560
Cameroon	3.3	Unmitigated	0%	26545864	24878860	53912	400410	71501
Canada	2.4	Enhanced social distancing of elderly	34%	37742157	17361667	74721	519785	99059
Canada	2.7	Enhanced social distancing of elderly	38%	37742157	19107908	87752	590844	116392
Canada	3	Enhanced social distancing of elderly	42%	37742157	21027720	131760	744015	174651
Canada	3.3	Enhanced social distancing of elderly	45%	37742157	22307651	147678	812634	195761
Canada	2.4	Social distancing whole population	36%	37742157	18282575	132687	686167	175905
Canada	2.7	Social distancing whole population	40%	37742157	20061143	149963	767884	198791
Canada	3	Social distancing whole population	44%	37742157	21526603	165036	837820	218788
Canada	3.3	Social distancing whole population	46%	37742157	22755021	178310	898370	236495
Canada	2.4	Unmitigated	0%	37742157	29596636	266741	1277275	353623
Canada	2.7	Unmitigated	0%	37742157	31447344	296840	1396546	393698
Canada	3	Unmitigated	0%	37742157	32802364	321565	1490773	426364
Canada	3.3	Unmitigated	0%	37742157	33814953	342056	1566204	453439

Central African Republic	2.4	Enhanced social distancing of elderly	35%	4829764	2707659	4665	39282	6184
Central African Republic	2.7	Enhanced social distancing of elderly	39%	4829764	2927558	5212	42907	6909
Central African Republic	3	Enhanced social distancing of elderly	44%	4829764	3078928	6699	48742	8882
Central African Republic	3.3	Enhanced social distancing of elderly	47%	4829764	3223221	7139	51330	9468
Central African Republic	2.4	Social distancing whole population	37%	4829764	2664973	6560	44637	8696
Central African Republic	2.7	Social distancing whole population	41%	4829764	2886095	7036	48040	9332
Central African Republic	3	Social distancing whole population	45%	4829764	3064593	7410	50741	9825
Central African Republic	3.3	Social distancing whole population	48%	4829764	3212271	7712	52939	10223
Central African Republic	2.4	Unmitigated	0%	4829764	4097921	9349	65269	12395
Central African Republic	2.7	Unmitigated	0%	4829764	4294243	9658	67719	12803
Central African Republic	3	Unmitigated	0%	4829764	4431311	9855	69331	13065
Central African Republic	3.3	Unmitigated	0%	4829764	4529155	9985	70415	13242
Chad	2.4	Enhanced social distancing of elderly	35%	16425859	9065221	14551	124665	19291
Chad	2.7	Enhanced social distancing of elderly	39%	16425859	9806945	16225	135953	21519
Chad	3	Enhanced social distancing of elderly	44%	16425859	10311144	20755	153830	27518
Chad	3.3	Enhanced social distancing of elderly	47%	16425859	10800257	22074	161783	29262
Chad	2.4	Social distancing whole population	37%	16425859	8905875	20336	140879	26960
Chad	2.7	Social distancing whole population	41%	16425859	9653516	21765	151432	28853
Chad	3	Social distancing whole population	45%	16425859	10258505	22882	159774	30334
Chad	3.3	Social distancing whole population	48%	16425859	10758674	23776	166529	31534
Chad	2.4	Unmitigated	0%	16425859	13779365	28507	203840	37792
Chad	2.7	Unmitigated	0%	16425859	14462189	29369	211130	38950
Chad	3	Unmitigated	0%	16425859	14943752	29910	215879	39665
Chad	3.3	Unmitigated	0%	16425859	15291053	30260	219050	40115
Channel Islands	2.4	Enhanced social distancing of elderly	33%	173859	74935	277	2125	367
Channel Islands	2.7	Enhanced social distancing of elderly	37%	173859	82971	328	2432	435
Channel Islands	3	Enhanced social distancing of elderly	41%	173859	91486	496	3035	657
Channel Islands	3.3	Enhanced social distancing of elderly	44%	173859	97457	562	3335	745
Channel Islands	2.4	Social distancing whole population	34%	173859	78628	504	2759	668
Channel Islands	2.7	Social distancing whole population	38%	173859	86899	579	3127	768
Channel Islands	3	Social distancing whole population	42%	173859	93776	646	3447	856
Channel Islands	3.3	Social distancing whole population	44%	173859	99589	706	3728	936
Channel Islands	2.4	Unmitigated	0%	173859	128194	1063	5287	1409
Channel Islands	2.7	Unmitigated	0%	173859	137550	1210	5880	1604
Channel Islands	3	Unmitigated	0%	173859	144550	1335	6361	1769
Channel Islands	3.3	Unmitigated	0%	173859	149891	1441	6756	1911
Chile	2.4	Enhanced social distancing of elderly	33%	19116209	7948804	27107	189383	35936
Chile	2.7	Enhanced social distancing of elderly	37%	19116209	8903666	32472	220769	43062
Chile	3	Enhanced social distancing of elderly	41%	19116209	9863830	48536	279895	64353
Chile	3.3	Enhanced social distancing of elderly	44%	19116209	10569490	54739	309442	72564
Chile	2.4	Social distancing whole population	35%	19116209	8259484	46981	245940	62283
Chile	2.7	Social distancing whole population	39%	19116209	9217526	54006	281434	71593
Chile	3	Social distancing whole population	42%	19116209	10025920	60214	312541	79824

Chile	3.3	Social distancing whole population	45%	19116209	10715061	65721	339922	87190
Chile	2.4	Unmitigated	0%	19116209	14113562	96205	487728	127540
Chile	2.7	Unmitigated	0%	19116209	15216924	107604	541146	142694
Chile	3	Unmitigated	0%	19116209	16042988	116816	583422	154922
Chile	3.3	Unmitigated	0%	19116209	16670900	124307	617148	164816
China	2.4	Enhanced social distancing of elderly	33%	1439323774	746788386	2826315	22806847	3746892
China	2.7	Enhanced social distancing of elderly	37%	1439323774	821322064	3298809	25700365	4373173
China	3	Enhanced social distancing of elderly	42%	1439323774	874140859	4749585	30753649	6296301
China	3.3	Enhanced social distancing of elderly	46%	1439323774	921282518	5266944	33168816	6985072
China	2.4	Social distancing whole population	37%	1439323774	757128484	4813476	28406024	6381302
China	2.7	Social distancing whole population	41%	1439323774	826543527	5367361	31380232	7119395
China	3	Social distancing whole population	44%	1439323774	878590163	5798045	33657278	7688852
China	3.3	Social distancing whole population	47%	1439323774	923855397	6183672	35670795	8196918
China	2.4	Unmitigated	0%	1439323774	1184943034	8642939	47961274	11458080
China	2.7	Unmitigated	0%	1439323774	1247620433	9304179	51103996	12336119
China	3	Unmitigated	0%	1439323774	1292156050	9794152	53385009	12982831
China	3.3	Unmitigated	0%	1439323774	1324506242	10161559	55065833	13471099
China, Taiwan Province of	2.4	Enhanced social distancing of elderly	33%	23816775	11913712	51670	395266	68500
China, Taiwan Province of	2.7	Enhanced social distancing of elderly	36%	23816775	13183500	61120	449935	81060
China, Taiwan Province of	3	Enhanced social distancing of elderly	42%	23816775	14147563	91275	549321	121049
China, Taiwan Province of	3.3	Enhanced social distancing of elderly	45%	23816775	14968416	102449	597300	135799
China, Taiwan Province of	2.4	Social distancing whole population	37%	23816775	12241249	93712	509489	124235
China, Taiwan Province of	2.7	Social distancing whole population	41%	23816775	13363829	105222	564988	139513
China, Taiwan Province of	3	Social distancing whole population	44%	23816775	14283511	115138	611795	152625
China, Taiwan Province of	3.3	Social distancing whole population	47%	23816775	15051806	123786	651887	164103
China, Taiwan Province of	2.4	Unmitigated	0%	23816775	19368761	179828	896581	238400
China, Taiwan Province of	2.7	Unmitigated	0%	23816775	20456680	196510	964608	260510
China, Taiwan Province of	3	Unmitigated	0%	23816775	21236325	209330	1015373	277624
China, Taiwan Province of	3.3	Unmitigated	0%	23816775	21806631	219270	1053750	290735
Colombia	2.4	Enhanced social distancing of elderly	34%	50882884	22465432	62709	470874	83134
Colombia	2.7	Enhanced social distancing of elderly	37%	50882884	25040425	74533	544667	98804
Colombia	3	Enhanced social distancing of elderly	41%	50882884	27498231	108770	676667	144185
Colombia	3.3	Enhanced social distancing of elderly	44%	50882884	29358446	121940	743669	161696
Colombia	2.4	Social distancing whole population	35%	50882884	23091674	104613	592416	138687
Colombia	2.7	Social distancing whole population	39%	50882884	25654646	119491	673685	158408
Colombia	3	Social distancing whole population	42%	50882884	27802921	132515	744268	175751
Colombia	3.3	Social distancing whole population	45%	50882884	29623911	143964	805892	190877
Colombia	2.4	Unmitigated	0%	50882884	38793185	208179	1143614	275986
Colombia	2.7	Unmitigated	0%	50882884	41611023	230646	1258209	305823
Colombia	3	Unmitigated	0%	50882884	43689722	248369	1347006	329249
Colombia	3.3	Unmitigated	0%	50882884	45247953	262461	1416435	347934
Comoros	2.4	Enhanced social distancing of elderly	34%	869595	487750	929	7968	1231
Comoros	2.7	Enhanced social distancing of elderly	39%	869595	527765	1040	8732	1379

Comoros	3	Enhanced social distancing of elderly	44%	869595	555655	1335	9913	1771
Comoros	3.3	Enhanced social distancing of elderly	47%	869595	581872	1426	10463	1891
Comoros	2.4	Social distancing whole population	37%	869595	480751	1301	9007	1725
Comoros	2.7	Social distancing whole population	41%	869595	520838	1400	9731	1857
Comoros	3	Social distancing whole population	45%	869595	553200	1479	10311	1961
Comoros	3.3	Social distancing whole population	48%	869595	579893	1543	10785	2045
Comoros	2.4	Unmitigated	0%	869595	738089	1894	13493	2511
Comoros	2.7	Unmitigated	0%	869595	773652	1965	14065	2605
Comoros	3	Unmitigated	0%	869595	798396	2011	14450	2667
Comoros	3.3	Unmitigated	0%	869595	816020	2042	14714	2708
Congo, Dem. Rep.	2.4	Enhanced social distancing of elderly	35%	89561404	49508071	88025	733058	116697
Congo, Dem. Rep.	2.7	Enhanced social distancing of elderly	39%	89561404	53574415	98623	802122	130743
Congo, Dem. Rep.	3	Enhanced social distancing of elderly	44%	89561404	56429103	128350	916875	170152
Congo, Dem. Rep.	3.3	Enhanced social distancing of elderly	47%	89561404	59105656	137156	967071	181916
Congo, Dem. Rep.	2.4	Social distancing whole population	37%	89561404	48817470	126170	840947	167266
Congo, Dem. Rep.	2.7	Social distancing whole population	41%	89561404	52894868	135605	906224	179852
Congo, Dem. Rep.	3	Social distancing whole population	45%	89561404	56191670	143064	958176	189698
Congo, Dem. Rep.	3.3	Social distancing whole population	48%	89561404	58924020	149115	1000615	197680
Congo, Dem. Rep.	2.4	Unmitigated	0%	89561404	75372228	182429	1240046	241850
Congo, Dem. Rep.	2.7	Unmitigated	0%	89561404	79061942	188874	1288191	250385
Congo, Dem. Rep.	3	Unmitigated	0%	89561404	81657482	193054	1320022	255916
Congo, Dem. Rep.	3.3	Unmitigated	0%	89561404	83525916	195829	1341545	259652
Congo, Rep.	2.4	Enhanced social distancing of elderly	35%	5518092	3094890	5607	49370	7433
Congo, Rep.	2.7	Enhanced social distancing of elderly	39%	5518092	3347078	6259	54007	8300
Congo, Rep.	3	Enhanced social distancing of elderly	44%	5518092	3519557	7937	60907	10522
Congo, Rep.	3.3	Enhanced social distancing of elderly	47%	5518092	3684892	8452	64191	11205
Congo, Rep.	2.4	Social distancing whole population	37%	5518092	3044312	7697	55202	10204
Congo, Rep.	2.7	Social distancing whole population	41%	5518092	3297700	8265	59572	10957
Congo, Rep.	3	Social distancing whole population	45%	5518092	3502319	8713	63063	11556
Congo, Rep.	3.3	Social distancing whole population	48%	5518092	3671112	9075	65915	12033
Congo, Rep.	2.4	Unmitigated	0%	5518092	4683994	11058	82320	14660
Congo, Rep.	2.7	Unmitigated	0%	5518092	4908754	11439	85718	15170
Congo, Rep.	3	Unmitigated	0%	5518092	5065715	11686	88003	15492
Congo, Rep.	3.3	Unmitigated	0%	5518092	5177507	11849	89571	15708
Costa Rica	2.4	Enhanced social distancing of elderly	33%	5094114	2180650	6718	48922	8906
Costa Rica	2.7	Enhanced social distancing of elderly	37%	5094114	2438196	8019	56846	10631
Costa Rica	3	Enhanced social distancing of elderly	41%	5094114	2688943	11802	71189	15652
Costa Rica	3.3	Enhanced social distancing of elderly	44%	5094114	2876997	13271	78486	17596
Costa Rica	2.4	Social distancing whole population	35%	5094114	2250988	11349	62230	15046
Costa Rica	2.7	Social distancing whole population	39%	5094114	2507999	13016	71046	17258
Costa Rica	3	Social distancing whole population	42%	5094114	2724213	14481	78740	19197
Costa Rica	3.3	Social distancing whole population	45%	5094114	2908086	15776	85488	20914
Costa Rica	2.4	Unmitigated	0%	5094114	3817722	22929	121897	30397

Costa Rica	2.7	Unmitigated	0%	5094114	4107483	25533	134741	33847
Costa Rica	3	Unmitigated	0%	5094114	4322628	27608	144787	36610
Costa Rica	3.3	Unmitigated	0%	5094114	4485157	29276	152724	38826
Cote d'Ivoire	2.4	Enhanced social distancing of elderly	35%	26378275	14722740	26154	225504	34673
Cote d'Ivoire	2.7	Enhanced social distancing of elderly	39%	26378275	15928082	29229	246681	38756
Cote d'Ivoire	3	Enhanced social distancing of elderly	44%	26378275	16761445	37374	279760	49545
Cote d'Ivoire	3.3	Enhanced social distancing of elderly	47%	26378275	17552350	39833	294877	52806
Cote d'Ivoire	2.4	Social distancing whole population	37%	26378275	14497297	36417	254935	48279
Cote d'Ivoire	2.7	Social distancing whole population	41%	26378275	15706291	39103	274868	51837
Cote d'Ivoire	3	Social distancing whole population	45%	26378275	16683810	41222	290772	54667
Cote d'Ivoire	3.3	Social distancing whole population	48%	26378275	17490055	42928	303713	56931
Cote d'Ivoire	2.4	Unmitigated	0%	26378275	22339240	52235	377242	69248
Cote d'Ivoire	2.7	Unmitigated	0%	26378275	23418622	53999	392122	71621
Cote d'Ivoire	3	Unmitigated	0%	26378275	24173326	55132	401989	73097
Cote d'Ivoire	3.3	Unmitigated	0%	26378275	24713260	55876	408684	74070
Croatia	2.4	Enhanced social distancing of elderly	34%	4105268	1713243	6953	50478	9217
Croatia	2.7	Enhanced social distancing of elderly	38%	4105268	1897087	8259	57875	10948
Croatia	3	Enhanced social distancing of elderly	42%	4105268	2101344	12692	73357	16832
Croatia	3.3	Enhanced social distancing of elderly	45%	4105268	2240441	14413	80835	19112
Croatia	2.4	Social distancing whole population	36%	4105268	1811574	12834	66952	17014
Croatia	2.7	Social distancing whole population	39%	4105268	2002239	14757	75955	19570
Croatia	3	Social distancing whole population	43%	4105268	2161215	16478	83822	21841
Croatia	3.3	Social distancing whole population	46%	4105268	2296046	18031	90772	23902
Croatia	2.4	Unmitigated	0%	4105268	3008839	28100	132792	37252
Croatia	2.7	Unmitigated	0%	4105268	3226699	32043	147960	42477
Croatia	3	Unmitigated	0%	4105268	3391471	35424	160440	46961
Croatia	3.3	Unmitigated	0%	4105268	3518531	38325	170788	50830
Cuba	2.4	Enhanced social distancing of elderly	33%	11326616	4434392	18824	124172	24955
Cuba	2.7	Enhanced social distancing of elderly	36%	11326616	4982616	22625	145342	30003
Cuba	3	Enhanced social distancing of elderly	41%	11326616	5565050	34309	186894	45481
Cuba	3.3	Enhanced social distancing of elderly	43%	11326616	5978221	38795	207292	51428
Cuba	2.4	Social distancing whole population	35%	11326616	4665780	33464	165254	44364
Cuba	2.7	Social distancing whole population	39%	11326616	5221247	38555	189626	51110
Cuba	3	Social distancing whole population	42%	11326616	5691513	43067	211064	57112
Cuba	3.3	Social distancing whole population	45%	11326616	6093855	47082	230007	62439
Cuba	2.4	Unmitigated	0%	11326616	8073332	69278	332286	91843
Cuba	2.7	Unmitigated	0%	11326616	8741627	77924	370863	103348
Cuba	3	Unmitigated	0%	11326616	9250029	85035	401974	112743
Cuba	3.3	Unmitigated	0%	11326616	9642828	90923	427258	120528
Curacao	2.4	Enhanced social distancing of elderly	31%	164100	65849	282	1790	374
Curacao	2.7	Enhanced social distancing of elderly	35%	164100	73622	339	2091	449
Curacao	3	Enhanced social distancing of elderly	40%	164100	82101	516	2713	685
Curacao	3.3	Enhanced social distancing of elderly	43%	164100	87956	584	3009	774

Curacao	2.4	Social distancing whole population	34%	164100	69667	512	2446	679
Curacao	2.7	Social distancing whole population	38%	164100	77586	589	2798	781
Curacao	3	Social distancing whole population	41%	164100	84259	657	3107	871
Curacao	3.3	Social distancing whole population	44%	164100	89948	717	3380	950
Curacao	2.4	Unmitigated	0%	164100	116866	1041	4813	1380
Curacao	2.7	Unmitigated	0%	164100	126324	1174	5383	1556
Curacao	3	Unmitigated	0%	164100	133485	1284	5845	1702
Curacao	3.3	Unmitigated	0%	164100	139000	1376	6222	1825
Cyprus	2.4	Enhanced social distancing of elderly	34%	1207361	548936	1750	14129	2319
Cyprus	2.7	Enhanced social distancing of elderly	38%	1207361	605924	2059	16064	2731
Cyprus	3	Enhanced social distancing of elderly	42%	1207361	663819	3059	19785	4055
Cyprus	3.3	Enhanced social distancing of elderly	45%	1207361	705200	3446	21620	4567
Cyprus	2.4	Social distancing whole population	36%	1207361	570336	3064	17905	4062
Cyprus	2.7	Social distancing whole population	39%	1207361	628385	3498	20167	4637
Cyprus	3	Social distancing whole population	43%	1207361	676320	3880	22110	5144
Cyprus	3.3	Social distancing whole population	46%	1207361	716608	4220	23800	5598
Cyprus	2.4	Unmitigated	0%	1207361	929148	6392	33815	8474
Cyprus	2.7	Unmitigated	0%	1207361	990928	7194	37197	9542
Cyprus	3	Unmitigated	0%	1207361	1036463	7863	39891	10425
Cyprus	3.3	Unmitigated	0%	1207361	1070706	8423	42062	11166
Czechia	2.4	Enhanced social distancing of elderly	34%	10708982	4599626	17108	131059	22681
Czechia	2.7	Enhanced social distancing of elderly	38%	10708982	5084014	20258	149782	26853
Czechia	3	Enhanced social distancing of elderly	42%	10708982	5614220	30934	188619	41018
Czechia	3.3	Enhanced social distancing of elderly	45%	10708982	5976795	35057	207332	46491
Czechia	2.4	Social distancing whole population	36%	10708982	4846301	31263	172209	41445
Czechia	2.7	Social distancing whole population	40%	10708982	5347398	35867	194871	47571
Czechia	3	Social distancing whole population	43%	10708982	5763365	39967	214562	52990
Czechia	3.3	Social distancing whole population	46%	10708982	6114855	43653	231880	57865
Czechia	2.4	Unmitigated	0%	10708982	7989785	67843	337512	89940
Czechia	2.7	Unmitigated	0%	10708982	8547125	77116	374694	102227
Czechia	3	Unmitigated	0%	10708982	8965266	85019	405079	112708
Czechia	3.3	Unmitigated	0%	10708982	9285367	91772	430137	121750
Denmark	2.4	Enhanced social distancing of elderly	34%	5792203	2436360	9251	67788	12264
Denmark	2.7	Enhanced social distancing of elderly	38%	5792203	2696341	10982	77654	14563
Denmark	3	Enhanced social distancing of elderly	42%	5792203	2982101	16938	98684	22458
Denmark	3.3	Enhanced social distancing of elderly	45%	5792203	3178440	19231	108721	25492
Denmark	2.4	Social distancing whole population	36%	5792203	2569567	17181	90428	22777
Denmark	2.7	Social distancing whole population	40%	5792203	2838272	19731	102465	26156
Denmark	3	Social distancing whole population	43%	5792203	3062515	22013	112984	29182
Denmark	3.3	Social distancing whole population	46%	5792203	3252934	24073	122287	31937
Denmark	2.4	Unmitigated	0%	5792203	4267420	37521	178910	49743
Denmark	2.7	Unmitigated	0%	5792203	4574654	42735	199151	56671
Denmark	3	Unmitigated	0%	5792203	4806952	47194	215783	62585

Denmark	3.3	Unmitigated	0%	5792203	4986020	51018	229566	67643
Djibouti	2.4	Enhanced social distancing of elderly	34%	988002	547687	1313	10864	1740
Djibouti	2.7	Enhanced social distancing of elderly	38%	988002	594365	1486	12008	1970
Djibouti	3	Enhanced social distancing of elderly	43%	988002	629669	1974	13897	2617
Djibouti	3.3	Enhanced social distancing of elderly	47%	988002	660407	2130	14771	2825
Djibouti	2.4	Social distancing whole population	37%	988002	544819	1935	12610	2566
Djibouti	2.7	Social distancing whole population	41%	988002	591101	2103	13724	2789
Djibouti	3	Social distancing whole population	44%	988002	628514	2239	14629	2969
Djibouti	3.3	Social distancing whole population	47%	988002	659412	2351	15379	3117
Djibouti	2.4	Unmitigated	0%	988002	839799	3004	19807	3983
Djibouti	2.7	Unmitigated	0%	988002	880880	3152	20827	4179
Djibouti	3	Unmitigated	0%	988002	909348	3253	21535	4313
Djibouti	3.3	Unmitigated	0%	988002	929536	3325	22038	4407
Dominican Republic	2.4	Enhanced social distancing of elderly	34%	10847904	5023213	12403	94885	16444
Dominican Republic	2.7	Enhanced social distancing of elderly	38%	10847904	5569166	14648	108969	19424
Dominican Republic	3	Enhanced social distancing of elderly	42%	10847904	6076200	21169	133973	28070
Dominican Republic	3.3	Enhanced social distancing of elderly	45%	10847904	6464759	23627	146532	31322
Dominican Republic	2.4	Social distancing whole population	36%	10847904	5134852	20414	117879	27063
Dominican Republic	2.7	Social distancing whole population	40%	10847904	5676578	23166	133165	30707
Dominican Republic	3	Social distancing whole population	43%	10847904	6127665	25555	146329	33876
Dominican Republic	3.3	Social distancing whole population	46%	10847904	6508675	27646	157768	36661
Dominican Republic	2.4	Unmitigated	0%	10847904	8483595	39710	222156	52644
Dominican Republic	2.7	Unmitigated	0%	10847904	9056063	43690	242722	57920
Dominican Republic	3	Unmitigated	0%	10847904	9473559	46786	258426	62051
Dominican Republic	3.3	Unmitigated	0%	10847904	9782711	49216	270540	65254
Ecuador	2.4	Enhanced social distancing of elderly	34%	17643060	8151054	19921	153071	26409
Ecuador	2.7	Enhanced social distancing of elderly	38%	17643060	9040851	23534	175840	31200
Ecuador	3	Enhanced social distancing of elderly	42%	17643060	9867976	34079	216453	45178
Ecuador	3.3	Enhanced social distancing of elderly	45%	17643060	10501740	38047	236790	50470
Ecuador	2.4	Social distancing whole population	36%	17643060	8333545	32897	190567	43612
Ecuador	2.7	Social distancing whole population	40%	17643060	9216846	37346	215344	49523
Ecuador	3	Social distancing whole population	43%	17643060	9952723	41210	236685	54653
Ecuador	3.3	Social distancing whole population	46%	17643060	10573867	44586	255197	59116
Ecuador	2.4	Unmitigated	0%	17643060	13784683	63996	358957	84841
Ecuador	2.7	Unmitigated	0%	17643060	14719518	70418	392150	93365
Ecuador	3	Unmitigated	0%	17643060	15401072	75410	417461	99966
Ecuador	3.3	Unmitigated	0%	17643060	15906178	79329	436975	105164
Egypt	2.4	Enhanced social distancing of elderly	34%	102334403	56348097	140169	1103114	185825
Egypt	2.7	Enhanced social distancing of elderly	38%	102334403	61155916	159074	1220708	210956
Egypt	3	Enhanced social distancing of elderly	44%	102334403	64907093	214703	1429724	284742
Egypt	3.3	Enhanced social distancing of elderly	47%	102334403	68087459	232185	1521749	307798
Egypt	2.4	Social distancing whole population	37%	102334403	56251333	211731	1307569	280695
Egypt	2.7	Social distancing whole population	41%	102334403	61017008	230323	1422828	305376

Egypt	3	Social distancing whole population	45%	102334403	64869150	245417	1516453	325327
Egypt	3.3	Social distancing whole population	48%	102334403	68050849	257921	1594078	341925
Egypt	2.4	Unmitigated	0%	102334403	86914979	332672	2059077	441029
Egypt	2.7	Unmitigated	0%	102334403	91152645	349534	2164245	463368
Egypt	3	Unmitigated	0%	102334403	94102838	361233	2237309	479098
Egypt	3.3	Unmitigated	0%	102334403	96200339	369515	2289078	489965
El Salvador	2.4	Enhanced social distancing of elderly	34%	6486201	2975788	7602	56188	10079
El Salvador	2.7	Enhanced social distancing of elderly	38%	6486201	3299196	8998	64579	11928
El Salvador	3	Enhanced social distancing of elderly	42%	6486201	3606046	13244	80326	17556
El Salvador	3.3	Enhanced social distancing of elderly	45%	6486201	3837718	14820	87990	19653
El Salvador	2.4	Social distancing whole population	36%	6486201	3052511	12903	71335	17106
El Salvador	2.7	Social distancing whole population	40%	6486201	3374751	14660	80609	19435
El Salvador	3	Social distancing whole population	43%	6486201	3643191	16191	88608	21474
El Salvador	3.3	Social distancing whole population	46%	6486201	3869947	17534	95562	23248
El Salvador	2.4	Unmitigated	0%	6486201	5048386	25395	134981	33667
El Salvador	2.7	Unmitigated	0%	6486201	5391477	28046	147709	37187
El Salvador	3	Unmitigated	0%	6486201	5642343	30137	157501	39950
El Salvador	3.3	Unmitigated	0%	6486201	5828935	31803	165122	42160
Equatorial Guinea	2.4	Enhanced social distancing of elderly	34%	1402985	771715	1278	11904	1695
Equatorial Guinea	2.7	Enhanced social distancing of elderly	39%	1402985	836105	1425	13016	1889
Equatorial Guinea	3	Enhanced social distancing of elderly	44%	1402985	879077	1793	14576	2379
Equatorial Guinea	3.3	Enhanced social distancing of elderly	47%	1402985	921591	1906	15350	2527
Equatorial Guinea	2.4	Social distancing whole population	37%	1402985	756490	1739	13143	2305
Equatorial Guinea	2.7	Social distancing whole population	41%	1402985	821303	1864	14190	2471
Equatorial Guinea	3	Social distancing whole population	44%	1402985	873892	1961	15026	2600
Equatorial Guinea	3.3	Social distancing whole population	47%	1402985	917459	2040	15709	2704
Equatorial Guinea	2.4	Unmitigated	0%	1402985	1175404	2448	19525	3245
Equatorial Guinea	2.7	Unmitigated	0%	1402985	1235310	2525	20335	3348
Equatorial Guinea	3	Unmitigated	0%	1402985	1277483	2574	20880	3414
Equatorial Guinea	3.3	Unmitigated	0%	1402985	1307833	2606	21254	3457
Eritrea	2.4	Enhanced social distancing of elderly	35%	3546427	1989804	4132	32169	5478
Eritrea	2.7	Enhanced social distancing of elderly	39%	3546427	2153324	4671	35381	6193
Eritrea	3	Enhanced social distancing of elderly	44%	3546427	2276712	6347	41490	8414
Eritrea	3.3	Enhanced social distancing of elderly	47%	3546427	2384232	6842	43982	9070
Eritrea	2.4	Social distancing whole population	37%	3546427	1978667	6341	38499	8406
Eritrea	2.7	Social distancing whole population	41%	3546427	2141649	6854	41604	9086
Eritrea	3	Social distancing whole population	45%	3546427	2273014	7265	44093	9636
Eritrea	3.3	Social distancing whole population	48%	3546427	2381508	7604	46138	10082
Eritrea	2.4	Unmitigated	0%	3546427	3030114	9586	58049	12708
Eritrea	2.7	Unmitigated	0%	3546427	3171971	10004	60536	13267
Eritrea	3	Unmitigated	0%	3546427	3270416	10288	62215	13638
Eritrea	3.3	Unmitigated	0%	3546427	3340148	10485	63371	13900
Estonia	2.4	Enhanced social distancing of elderly	34%	1326539	568294	2203	16061	2920

Estonia	2.7	Enhanced social distancing of elderly	38%	1326539	627609	2611	18353	3461
Estonia	3	Enhanced social distancing of elderly	42%	1326539	692685	4022	23200	5334
Estonia	3.3	Enhanced social distancing of elderly	45%	1326539	737134	4562	25509	6049
Estonia	2.4	Social distancing whole population	36%	1326539	598852	4088	21268	5420
Estonia	2.7	Social distancing whole population	40%	1326539	660132	4687	24047	6215
Estonia	3	Social distancing whole population	43%	1326539	711034	5222	26464	6922
Estonia	3.3	Social distancing whole population	46%	1326539	754089	5703	28593	7560
Estonia	2.4	Unmitigated	0%	1326539	984649	8885	41663	11779
Estonia	2.7	Unmitigated	0%	1326539	1053212	10111	46279	13404
Estonia	3	Unmitigated	0%	1326539	1104741	11162	50064	14803
Estonia	3.3	Unmitigated	0%	1326539	1144324	12065	53201	16000
Eswatini	2.4	Enhanced social distancing of elderly	34%	1160164	655231	1292	10501	1712
Eswatini	2.7	Enhanced social distancing of elderly	39%	1160164	708854	1457	11529	1931
Eswatini	3	Enhanced social distancing of elderly	44%	1160164	748195	1960	13380	2599
Eswatini	3.3	Enhanced social distancing of elderly	47%	1160164	783351	2108	14158	2795
Eswatini	2.4	Social distancing whole population	37%	1160164	649519	1955	12355	2592
Eswatini	2.7	Social distancing whole population	41%	1160164	703082	2109	13342	2796
Eswatini	3	Social distancing whole population	45%	1160164	746238	2233	14131	2961
Eswatini	3.3	Social distancing whole population	48%	1160164	781784	2333	14776	3094
Eswatini	2.4	Unmitigated	0%	1160164	993018	2909	18493	3857
Eswatini	2.7	Unmitigated	0%	1160164	1039477	3029	19269	4017
Eswatini	3	Unmitigated	0%	1160164	1071586	3109	19790	4123
Eswatini	3.3	Unmitigated	0%	1160164	1094259	3165	20148	4195
Ethiopia	2.4	Enhanced social distancing of elderly	35%	114963583	64231695	122578	1002806	162505
Ethiopia	2.7	Enhanced social distancing of elderly	39%	114963583	69515414	137853	1099796	182747
Ethiopia	3	Enhanced social distancing of elderly	44%	114963583	73304498	182318	1267008	241801
Ethiopia	3.3	Enhanced social distancing of elderly	47%	114963583	76772885	195501	1339017	259228
Ethiopia	2.4	Social distancing whole population	37%	114963583	63502886	180157	1164449	238838
Ethiopia	2.7	Social distancing whole population	41%	114963583	68783976	194130	1256872	257440
Ethiopia	3	Social distancing whole population	45%	114963583	73048691	205246	1330695	272085
Ethiopia	3.3	Social distancing whole population	48%	114963583	76566099	214285	1390971	284061
Ethiopia	2.4	Unmitigated	0%	114963583	97701712	265429	1736905	351885
Ethiopia	2.7	Unmitigated	0%	114963583	102376081	275678	1807709	365447
Ethiopia	3	Unmitigated	0%	114963583	105633163	282448	1854906	374440
Ethiopia	3.3	Unmitigated	0%	114963583	107956391	287024	1887123	380690
Fiji	2.4	Enhanced social distancing of elderly	34%	896444	471811	1303	11345	1728
Fiji	2.7	Enhanced social distancing of elderly	38%	896444	513950	1484	12535	1968
Fiji	3	Enhanced social distancing of elderly	43%	896444	542033	2002	14489	2654
Fiji	3.3	Enhanced social distancing of elderly	46%	896444	569058	2174	15413	2882
Fiji	2.4	Social distancing whole population	37%	896444	468980	1989	13314	2637
Fiji	2.7	Social distancing whole population	41%	896444	509074	2170	14472	2876
Fiji	3	Social distancing whole population	44%	896444	541624	2318	15412	3073
Fiji	3.3	Social distancing whole population	47%	896444	568636	2441	16191	3237

Fiji	2.4	Unmitigated	0%	896444	728041	3176	20738	4210
Fiji	2.7	Unmitigated	0%	896444	765789	3349	21780	4442
Fiji	3	Unmitigated	0%	896444	792806	3471	22505	4602
Fiji	3.3	Unmitigated	0%	896444	812674	3558	23019	4717
Finland	2.4	Enhanced social distancing of elderly	35%	5540718	2438574	9505	72372	12601
Finland	2.7	Enhanced social distancing of elderly	39%	5540718	2678075	11108	81649	14725
Finland	3	Enhanced social distancing of elderly	43%	5540718	2944141	16711	101896	22166
Finland	3.3	Enhanced social distancing of elderly	46%	5540718	3122128	18823	111234	24964
Finland	2.4	Social distancing whole population	36%	5540718	2573918	17089	94953	22655
Finland	2.7	Social distancing whole population	40%	5540718	2820904	19401	106184	25730
Finland	3	Social distancing whole population	43%	5540718	3024120	21443	115825	28431
Finland	3.3	Social distancing whole population	46%	5540718	3194830	23269	124234	30845
Finland	2.4	Unmitigated	0%	5540718	4157497	36230	178884	48030
Finland	2.7	Unmitigated	0%	5540718	4423852	41029	197145	54384
Finland	3	Unmitigated	0%	5540718	4622437	45167	212138	59877
Finland	3.3	Unmitigated	0%	5540718	4774362	48760	224620	64673
France	2.4	Enhanced social distancing of elderly	33%	65273512	32561112	177332	1104981	235092
France	2.7	Enhanced social distancing of elderly	37%	65273512	35572346	206773	1241042	274241
France	3	Enhanced social distancing of elderly	43%	65273512	38920422	318650	1578916	422341
France	3.3	Enhanced social distancing of elderly	46%	65273512	41082974	355379	1713120	471094
France	2.4	Social distancing whole population	37%	65273512	34438692	332877	1514457	441301
France	2.7	Social distancing whole population	41%	65273512	37445134	370249	1668052	490811
France	3	Social distancing whole population	44%	65273512	39892051	402157	1796685	533139
France	3.3	Social distancing whole population	47%	65273512	41923786	429772	1906150	569982
France	2.4	Unmitigated	0%	65273512	53899241	621256	2618267	823608
France	2.7	Unmitigated	0%	65273512	56748753	677700	2812418	898814
France	3	Unmitigated	0%	65273512	58779677	722189	2960230	957524
France	3.3	Unmitigated	0%	65273512	60260460	757655	3074637	1004367
French Guiana	2.4	Enhanced social distancing of elderly	34%	298682	142781	301	2511	399
French Guiana	2.7	Enhanced social distancing of elderly	37%	298682	157832	352	2867	467
French Guiana	3	Enhanced social distancing of elderly	42%	298682	171198	489	3450	648
French Guiana	3.3	Enhanced social distancing of elderly	45%	298682	181730	541	3753	718
French Guiana	2.4	Social distancing whole population	35%	298682	144907	467	3021	619
French Guiana	2.7	Social distancing whole population	39%	298682	159760	526	3395	697
French Guiana	3	Social distancing whole population	42%	298682	172073	576	3715	764
French Guiana	3.3	Social distancing whole population	45%	298682	182435	620	3991	822
French Guiana	2.4	Unmitigated	0%	298682	235182	860	5491	1140
French Guiana	2.7	Unmitigated	0%	298682	250660	937	5966	1242
French Guiana	3	Unmitigated	0%	298682	261865	994	6322	1318
French Guiana	3.3	Unmitigated	0%	298682	270113	1038	6591	1377
French Polynesia	2.4	Enhanced social distancing of elderly	33%	280904	148456	483	4022	641
French Polynesia	2.7	Enhanced social distancing of elderly	37%	280904	162341	558	4490	741
French Polynesia	3	Enhanced social distancing of elderly	42%	280904	172174	792	5312	1050

French Polynesia	3.3	Enhanced social distancing of elderly	46%	280904	181003	873	5698	1157
French Polynesia	2.4	Social distancing whole population	36%	280904	149504	806	4927	1069
French Polynesia	2.7	Social distancing whole population	40%	280904	162324	890	5393	1180
French Polynesia	3	Social distancing whole population	44%	280904	172723	960	5777	1272
French Polynesia	3.3	Social distancing whole population	47%	280904	181334	1019	6098	1352
French Polynesia	2.4	Unmitigated	0%	280904	230430	1388	8006	1840
French Polynesia	2.7	Unmitigated	0%	280904	242372	1487	8489	1973
French Polynesia	3	Unmitigated	0%	280904	250835	1560	8834	2069
French Polynesia	3.3	Unmitigated	0%	280904	256989	1614	9086	2140
Gabon	2.4	Enhanced social distancing of elderly	34%	2225728	1230490	2472	21187	3277
Gabon	2.7	Enhanced social distancing of elderly	39%	2225728	1333561	2782	23292	3688
Gabon	3	Enhanced social distancing of elderly	44%	2225728	1407358	3647	26654	4835
Gabon	3.3	Enhanced social distancing of elderly	47%	2225728	1475167	3913	28208	5190
Gabon	2.4	Social distancing whole population	37%	2225728	1216055	3578	24222	4743
Gabon	2.7	Social distancing whole population	41%	2225728	1318900	3864	26239	5125
Gabon	3	Social distancing whole population	45%	2225728	1402114	4093	27862	5428
Gabon	3.3	Social distancing whole population	48%	2225728	1470886	4280	29197	5674
Gabon	2.4	Unmitigated	0%	2225728	1879501	5336	36931	7074
Gabon	2.7	Unmitigated	0%	2225728	1972271	5556	38610	7366
Gabon	3	Unmitigated	0%	2225728	2037168	5704	39754	7561
Gabon	3.3	Unmitigated	0%	2225728	2083570	5804	40549	7696
Gambia	2.4	Enhanced social distancing of elderly	35%	2416664	1336862	2208	19340	2927
Gambia	2.7	Enhanced social distancing of elderly	39%	2416664	1446531	2462	21117	3266
Gambia	3	Enhanced social distancing of elderly	44%	2416664	1520888	3136	23847	4158
Gambia	3.3	Enhanced social distancing of elderly	47%	2416664	1593092	3336	25099	4423
Gambia	2.4	Social distancing whole population	37%	2416664	1313148	3061	21739	4058
Gambia	2.7	Social distancing whole population	41%	2416664	1423527	3280	23405	4348
Gambia	3	Social distancing whole population	45%	2416664	1512880	3451	24728	4575
Gambia	3.3	Social distancing whole population	48%	2416664	1586757	3589	25803	4759
Gambia	2.4	Unmitigated	0%	2416664	2030107	4318	31797	5724
Gambia	2.7	Unmitigated	0%	2416664	2130724	4453	33004	5904
Gambia	3	Unmitigated	0%	2416664	2201563	4539	33801	6020
Gambia	3.3	Unmitigated	0%	2416664	2252544	4595	34338	6092
Georgia	2.4	Enhanced social distancing of elderly	35%	3989175	1811750	6050	47179	8020
Georgia	2.7	Enhanced social distancing of elderly	39%	3989175	1995739	7114	53621	9431
Georgia	3	Enhanced social distancing of elderly	43%	3989175	2186015	10565	66261	14006
Georgia	3.3	Enhanced social distancing of elderly	45%	3989175	2320666	11900	72459	15781
Georgia	2.4	Social distancing whole population	36%	3989175	1885923	10543	60035	13977
Georgia	2.7	Social distancing whole population	40%	3989175	2073497	12022	67557	15948
Georgia	3	Social distancing whole population	43%	3989175	2228750	13330	74057	17678
Georgia	3.3	Social distancing whole population	46%	3989175	2359444	14497	79733	19216
Georgia	2.4	Unmitigated	0%	3989175	3069934	22252	114804	29500
Georgia	2.7	Unmitigated	0%	3989175	3270757	25063	126464	33231

Georgia	3	Unmitigated	0%	3989175	3419398	27415	135820	36343
Georgia	3.3	Unmitigated	0%	3989175	3531664	29394	143409	38968
Germany	2.4	Enhanced social distancing of elderly	34%	83783945	39082310	196368	1323896	260329
Germany	2.7	Enhanced social distancing of elderly	38%	83783945	42888917	230241	1496819	305317
Germany	3	Enhanced social distancing of elderly	43%	83783945	47096496	350913	1887223	465308
Germany	3.3	Enhanced social distancing of elderly	46%	83783945	49873153	393716	2057112	521931
Germany	2.4	Social distancing whole population	37%	83783945	41357848	359743	1770509	476917
Germany	2.7	Social distancing whole population	41%	83783945	45175395	404773	1968275	536497
Germany	3	Social distancing whole population	44%	83783945	48310672	444017	2136792	588598
Germany	3.3	Social distancing whole population	47%	83783945	50934909	478622	2282438	634724
Germany	2.4	Unmitigated	0%	83783945	66105817	722405	3234026	957704
Germany	2.7	Unmitigated	0%	83783945	70008049	803387	3521899	1065053
Germany	3	Unmitigated	0%	83783945	72860295	870534	3749889	1154534
Germany	3.3	Unmitigated	0%	83783945	74995460	926777	3933293	1228728
Ghana	2.4	Enhanced social distancing of elderly	35%	31072945	17428811	34397	298503	45602
Ghana	2.7	Enhanced social distancing of elderly	39%	31072945	18862961	38519	327469	51068
Ghana	3	Enhanced social distancing of elderly	44%	31072945	19865493	49124	371082	65121
Ghana	3.3	Enhanced social distancing of elderly	47%	31072945	20805225	52461	391938	69570
Ghana	2.4	Social distancing whole population	37%	31072945	17183300	47538	335592	63022
Ghana	2.7	Social distancing whole population	41%	31072945	18618741	51219	363079	67909
Ghana	3	Social distancing whole population	45%	31072945	19777980	54142	385155	71809
Ghana	3.3	Social distancing whole population	48%	31072945	20736535	56523	403311	74942
Ghana	2.4	Unmitigated	0%	31072945	26449309	69815	508887	92556
Ghana	2.7	Unmitigated	0%	31072945	27716374	72468	531322	96087
Ghana	3	Unmitigated	0%	31072945	28597699	74214	546549	98380
Ghana	3.3	Unmitigated	0%	31072945	29223873	75388	557104	99938
Greece	2.4	Enhanced social distancing of elderly	34%	10423056	4322920	18340	129899	24313
Greece	2.7	Enhanced social distancing of elderly	38%	10423056	4787756	21842	149067	28973
Greece	3	Enhanced social distancing of elderly	42%	10423056	5309649	34127	189989	45258
Greece	3.3	Enhanced social distancing of elderly	45%	10423056	5662180	38839	209552	51479
Greece	2.4	Social distancing whole population	36%	10423056	4582434	34822	174072	46164
Greece	2.7	Social distancing whole population	39%	10423056	5065573	40076	197589	53137
Greece	3	Social distancing whole population	43%	10423056	5468304	44783	218148	59367
Greece	3.3	Social distancing whole population	46%	10423056	5809712	49034	236316	65004
Greece	2.4	Unmitigated	0%	10423056	7616202	76798	346607	101813
Greece	2.7	Unmitigated	0%	10423056	8168967	87776	386634	116363
Greece	3	Unmitigated	0%	10423056	8587116	97231	419649	128953
Greece	3.3	Unmitigated	0%	10423056	8910085	105400	447132	139747
Grenada	2.4	Enhanced social distancing of elderly	31%	112519	49078	144	1054	191
Grenada	2.7	Enhanced social distancing of elderly	35%	112519	54791	172	1223	227
Grenada	3	Enhanced social distancing of elderly	39%	112519	60294	251	1527	333
Grenada	3.3	Enhanced social distancing of elderly	42%	112519	64440	282	1683	375
Grenada	2.4	Social distancing whole population	33%	112519	50613	245	1345	325

Grenada	2.7	Social distancing whole population	37%	112519	56311	281	1534	372
Grenada	3	Social distancing whole population	41%	112519	61091	312	1699	414
Grenada	3.3	Social distancing whole population	43%	112519	65150	340	1844	451
Grenada	2.4	Unmitigated	0%	112519	83336	478	2545	634
Grenada	2.7	Unmitigated	0%	112519	89818	534	2821	708
Grenada	3	Unmitigated	0%	112519	94610	579	3036	767
Grenada	3.3	Unmitigated	0%	112519	98212	614	3205	814
Guadeloupe	2.4	Enhanced social distancing of elderly	32%	400127	159811	739	4431	980
Guadeloupe	2.7	Enhanced social distancing of elderly	36%	400127	178182	886	5166	1175
Guadeloupe	3	Enhanced social distancing of elderly	41%	400127	198850	1367	6767	1813
Guadeloupe	3.3	Enhanced social distancing of elderly	44%	400127	212781	1546	7502	2049
Guadeloupe	2.4	Social distancing whole population	35%	400127	169801	1362	6151	1806
Guadeloupe	2.7	Social distancing whole population	39%	400127	188583	1560	7013	2069
Guadeloupe	3	Social distancing whole population	42%	400127	204391	1736	7771	2301
Guadeloupe	3.3	Social distancing whole population	45%	400127	217870	1892	8441	2509
Guadeloupe	2.4	Unmitigated	0%	400127	286504	2808	12260	3723
Guadeloupe	2.7	Unmitigated	0%	400127	308868	3162	13685	4191
Guadeloupe	3	Unmitigated	0%	400127	325884	3457	14848	4586
Guadeloupe	3.3	Unmitigated	0%	400127	339068	3704	15807	4912
Guam	2.4	Enhanced social distancing of elderly	33%	168783	85303	279	2240	370
Guam	2.7	Enhanced social distancing of elderly	36%	168783	93586	325	2511	431
Guam	3	Enhanced social distancing of elderly	42%	168783	100018	475	3029	630
Guam	3.3	Enhanced social distancing of elderly	45%	168783	105446	528	3268	700
Guam	2.4	Social distancing whole population	36%	168783	86772	492	2840	653
Guam	2.7	Social distancing whole population	40%	168783	94438	547	3122	726
Guam	3	Social distancing whole population	43%	168783	100699	594	3358	788
Guam	3.3	Social distancing whole population	46%	168783	105918	634	3557	841
Guam	2.4	Unmitigated	0%	168783	134728	883	4717	1171
Guam	2.7	Unmitigated	0%	168783	142300	957	5041	1269
Guam	3	Unmitigated	0%	168783	147749	1013	5278	1343
Guam	3.3	Unmitigated	0%	168783	151768	1056	5454	1400
Guatemala	2.4	Enhanced social distancing of elderly	35%	17915567	8794775	16679	135591	22112
Guatemala	2.7	Enhanced social distancing of elderly	39%	17915567	9677214	19424	153473	25750
Guatemala	3	Enhanced social distancing of elderly	43%	17915567	10454162	27493	184710	36461
Guatemala	3.3	Enhanced social distancing of elderly	46%	17915567	11067217	30370	199947	40267
Guatemala	2.4	Social distancing whole population	36%	17915567	8906849	26733	164383	35440
Guatemala	2.7	Social distancing whole population	40%	17915567	9779815	29918	183243	39668
Guatemala	3	Social distancing whole population	43%	17915567	10500066	32627	199168	43252
Guatemala	3.3	Social distancing whole population	46%	17915567	11104053	34956	212773	46340
Guatemala	2.4	Unmitigated	0%	17915567	14396873	48661	291012	64510
Guatemala	2.7	Unmitigated	0%	17915567	15277144	52659	313140	69806
Guatemala	3	Unmitigated	0%	17915567	15911285	55651	329431	73797
Guatemala	3.3	Unmitigated	0%	17915567	16376622	57920	341591	76814

Guinea	2.4	Enhanced social distancing of elderly	35%	13132792	7316955	12784	107755	16948
Guinea	2.7	Enhanced social distancing of elderly	39%	13132792	7915729	14298	117828	18958
Guinea	3	Enhanced social distancing of elderly	44%	13132792	8333020	18430	134249	24432
Guinea	3.3	Enhanced social distancing of elderly	47%	13132792	8726375	19657	141493	26059
Guinea	2.4	Social distancing whole population	37%	13132792	7210381	18048	122974	23926
Guinea	2.7	Social distancing whole population	41%	13132792	7811011	19374	132452	25684
Guinea	3	Social distancing whole population	45%	13132792	8296390	20420	139989	27081
Guinea	3.3	Social distancing whole population	48%	13132792	8698357	21266	146136	28202
Guinea	2.4	Unmitigated	0%	13132792	11110311	25858	180637	34281
Guinea	2.7	Unmitigated	0%	13132792	11647808	26727	187519	35449
Guinea	3	Unmitigated	0%	13132792	12023860	27283	192043	36173
Guinea	3.3	Unmitigated	0%	13132792	12293594	27648	195093	36650
Guinea-Bissau	2.4	Enhanced social distancing of elderly	35%	1967998	1098953	1908	16625	2529
Guinea-Bissau	2.7	Enhanced social distancing of elderly	39%	1967998	1188899	2131	18180	2825
Guinea-Bissau	3	Enhanced social distancing of elderly	44%	1967998	1251294	2727	20622	3616
Guinea-Bissau	3.3	Enhanced social distancing of elderly	47%	1967998	1310345	2905	21731	3852
Guinea-Bissau	2.4	Social distancing whole population	37%	1967998	1082350	2662	18814	3529
Guinea-Bissau	2.7	Social distancing whole population	41%	1967998	1172640	2856	20278	3788
Guinea-Bissau	3	Social distancing whole population	45%	1967998	1245577	3009	21443	3989
Guinea-Bissau	3.3	Social distancing whole population	48%	1967998	1305778	3132	22391	4152
Guinea-Bissau	2.4	Unmitigated	0%	1967998	1665984	3795	27734	5031
Guinea-Bissau	2.7	Unmitigated	0%	1967998	1746620	3920	28817	5196
Guinea-Bissau	3	Unmitigated	0%	1967998	1802967	4000	29533	5303
Guinea-Bissau	3.3	Unmitigated	0%	1967998	1843251	4052	30018	5374
Guyana	2.4	Enhanced social distancing of elderly	34%	786559	367386	887	6894	1176
Guyana	2.7	Enhanced social distancing of elderly	38%	786559	406871	1045	7904	1385
Guyana	3	Enhanced social distancing of elderly	42%	786559	443189	1489	9652	1974
Guyana	3.3	Enhanced social distancing of elderly	45%	786559	471170	1658	10542	2197
Guyana	2.4	Social distancing whole population	36%	786559	374924	1430	8477	1895
Guyana	2.7	Social distancing whole population	39%	786559	414075	1619	9564	2146
Guyana	3	Social distancing whole population	43%	786559	446617	1783	10498	2365
Guyana	3.3	Social distancing whole population	46%	786559	474070	1927	11308	2555
Guyana	2.4	Unmitigated	0%	786559	615600	2744	15838	3637
Guyana	2.7	Unmitigated	0%	786559	656868	3012	17292	3995
Guyana	3	Unmitigated	0%	786559	686908	3220	18399	4269
Guyana	3.3	Unmitigated	0%	786559	709163	3382	19252	4483
Haiti	2.4	Enhanced social distancing of elderly	35%	11402533	5554223	10896	89784	14445
Haiti	2.7	Enhanced social distancing of elderly	39%	11402533	6121119	12707	101934	16845
Haiti	3	Enhanced social distancing of elderly	43%	11402533	6621241	17824	122625	23641
Haiti	3.3	Enhanced social distancing of elderly	46%	11402533	7015916	19695	132960	26119
Haiti	2.4	Social distancing whole population	36%	11402533	5628094	17170	108371	22762
Haiti	2.7	Social distancing whole population	40%	11402533	6188132	19257	121155	25540
Haiti	3	Social distancing whole population	43%	11402533	6650835	21035	131985	27891

Haiti	3.3	Social distancing whole population	46%	11402533	7039651	22569	141280	29918
Haiti	2.4	Unmitigated	0%	11402533	9132705	31462	194362	41710
Haiti	2.7	Unmitigated	0%	11402533	9700804	34086	209723	45186
Haiti	3	Unmitigated	0%	11402533	10110407	36048	221086	47788
Haiti	3.3	Unmitigated	0%	11402533	10411027	37530	229598	49776
Honduras	2.4	Enhanced social distancing of elderly	35%	9904608	4810574	9490	78163	12581
Honduras	2.7	Enhanced social distancing of elderly	38%	9904608	5304735	11073	88762	14686
Honduras	3	Enhanced social distancing of elderly	43%	9904608	5737379	15559	106551	20625
Honduras	3.3	Enhanced social distancing of elderly	45%	9904608	6081048	17194	115511	22793
Honduras	2.4	Social distancing whole population	36%	9904608	4869468	15004	93960	19892
Honduras	2.7	Social distancing whole population	40%	9904608	5357233	16831	105079	22312
Honduras	3	Social distancing whole population	43%	9904608	5760392	18386	114495	24374
Honduras	3.3	Social distancing whole population	46%	9904608	6099073	19723	122562	26157
Honduras	2.4	Unmitigated	0%	9904608	7914408	27412	168266	36341
Honduras	2.7	Unmitigated	0%	9904608	8411056	29682	181516	39366
Honduras	3	Unmitigated	0%	9904608	8769235	31374	191294	41600
Honduras	3.3	Unmitigated	0%	9904608	9032238	32649	198597	43281
Hong Kong SAR, China	2.4	Enhanced social distancing of elderly	27%	7496988	4342046	31060	183003	41177
Hong Kong SAR, China	2.7	Enhanced social distancing of elderly	31%	7496988	4731832	35741	203600	47396
Hong Kong SAR, China	3	Enhanced social distancing of elderly	41%	7496988	4902140	50708	241819	67235
Hong Kong SAR, China	3.3	Enhanced social distancing of elderly	45%	7496988	5131532	55266	258081	73263
Hong Kong SAR, China	2.4	Social distancing whole population	37%	7496988	4284870	52304	229690	69340
Hong Kong SAR, China	2.7	Social distancing whole population	41%	7496988	4632384	56750	248764	75213
Hong Kong SAR, China	3	Social distancing whole population	45%	7496988	4911415	60346	264142	79995
Hong Kong SAR, China	3.3	Social distancing whole population	48%	7496988	5140487	63318	276815	83974
Hong Kong SAR, China	2.4	Unmitigated	0%	7496988	6508912	81551	353691	108113
Hong Kong SAR, China	2.7	Unmitigated	0%	7496988	6798070	85546	370310	113409
Hong Kong SAR, China	3	Unmitigated	0%	7496988	6995095	88319	381774	117133
Hong Kong SAR, China	3.3	Unmitigated	0%	7496988	7132368	90280	389849	119693
Hungary	2.4	Enhanced social distancing of elderly	34%	9660350	4090330	15487	117525	20531
Hungary	2.7	Enhanced social distancing of elderly	38%	9660350	4529552	18367	134558	24346
Hungary	3	Enhanced social distancing of elderly	42%	9660350	5008932	28030	169537	37168
Hungary	3.3	Enhanced social distancing of elderly	45%	9660350	5338225	31786	186519	42154
Hungary	2.4	Social distancing whole population	35%	9660350	4311031	28278	154383	37488
Hungary	2.7	Social distancing whole population	39%	9660350	4765456	32499	175014	43103
Hungary	3	Social distancing whole population	43%	9660350	5143340	36262	192963	48078
Hungary	3.3	Social distancing whole population	45%	9660350	5462949	39644	208751	52552
Hungary	2.4	Unmitigated	0%	9660350	7145369	61425	303392	81432
Hungary	2.7	Unmitigated	0%	9660350	7656682	69900	337256	92660
Hungary	3	Unmitigated	0%	9660350	8041190	77124	364945	102242
Hungary	3.3	Unmitigated	0%	9660350	8336157	83297	387784	110510
Iceland	2.4	Enhanced social distancing of elderly	34%	341250	154410	508	3948	674
Iceland	2.7	Enhanced social distancing of elderly	38%	341250	170113	598	4489	794

Iceland	3	Enhanced social distancing of elderly	42%	341250	186398	895	5570	1187
Iceland	3.3	Enhanced social distancing of elderly	45%	341250	197907	1010	6095	1338
Iceland	2.4	Social distancing whole population	35%	341250	160914	903	5079	1197
Iceland	2.7	Social distancing whole population	39%	341250	176949	1030	5718	1366
Iceland	3	Social distancing whole population	43%	341250	190225	1143	6270	1515
Iceland	3.3	Social distancing whole population	46%	341250	201405	1244	6752	1650
Iceland	2.4	Unmitigated	0%	341250	260373	1892	9638	2508
Iceland	2.7	Unmitigated	0%	341250	277734	2136	10635	2834
Iceland	3	Unmitigated	0%	341250	290590	2341	11437	3104
Iceland	3.3	Unmitigated	0%	341250	300314	2514	12089	3332
India	2.4	Enhanced social distancing of elderly	34%	1380004385	781491691	2375803	17744714	3149647
India	2.7	Enhanced social distancing of elderly	38%	1380004385	846346851	2687837	19600377	3563198
India	3	Enhanced social distancing of elderly	44%	1380004385	898537598	3599172	22937640	4771392
India	3.3	Enhanced social distancing of elderly	47%	1380004385	941237930	3882872	24382304	5149911
India	2.4	Social distancing whole population	37%	1380004385	782715653	3539149	21017000	4691912
India	2.7	Social distancing whole population	41%	1380004385	847115785	3842227	22829100	5095882
India	3	Social distancing whole population	45%	1380004385	898915397	4087442	24297342	5419716
India	3.3	Social distancing whole population	48%	1380004385	941483000	4289966	25511651	5687057
India	2.4	Unmitigated	0%	1380004385	1194241971	5513476	32891009	7309311
India	2.7	Unmitigated	0%	1380004385	1248428445	5781554	34523222	7664071
India	3	Unmitigated	0%	1380004385	1285451521	5966455	35654500	7909145
India	3.3	Unmitigated	0%	1380004385	1311284900	6096359	36453753	8083524
Indonesia	2.4	Enhanced social distancing of elderly	34%	273523621	145903545	419909	3635403	556681
Indonesia	2.7	Enhanced social distancing of elderly	38%	273523621	158895807	478999	4020301	635202
Indonesia	3	Enhanced social distancing of elderly	43%	273523621	167623842	653804	4659004	866911
Indonesia	3.3	Enhanced social distancing of elderly	47%	273523621	175913029	711835	4959702	943646
Indonesia	2.4	Social distancing whole population	37%	273523621	145288435	652399	4283278	864896
Indonesia	2.7	Social distancing whole population	41%	273523621	157599592	712740	4658526	944856
Indonesia	3	Social distancing whole population	45%	273523621	167576039	762192	4963320	1010387
Indonesia	3.3	Social distancing whole population	48%	273523621	175835997	803521	5216086	1065749
Indonesia	2.4	Unmitigated	0%	273523621	225134498	1056765	6722108	1400971
Indonesia	2.7	Unmitigated	0%	273523621	236459937	1115925	7061387	1479645
Indonesia	3	Unmitigated	0%	273523621	244509549	1157729	7297332	1535467
Indonesia	3.3	Unmitigated	0%	273523621	250379720	1187827	7464778	1574920
Iran (Islamic Republic of)	2.4	Enhanced social distancing of elderly	34%	83992953	44805996	131466	1147446	174287
Iran (Islamic Republic of)	2.7	Enhanced social distancing of elderly	38%	83992953	48794433	149899	1267670	198723
Iran (Islamic Republic of)	3	Enhanced social distancing of elderly	43%	83992953	51501318	206108	1469400	273228
Iran (Islamic Republic of)	3.3	Enhanced social distancing of elderly	47%	83992953	54049387	224599	1563806	297860
Iran (Islamic Republic of)	2.4	Social distancing whole population	37%	83992953	44662680	207085	1354122	274536
Iran (Islamic Republic of)	2.7	Social distancing whole population	41%	83992953	48449090	226212	1472093	300044
Iran (Islamic Republic of)	3	Social distancing whole population	45%	83992953	51514982	241884	1567774	320772
Iran (Islamic Republic of)	3.3	Social distancing whole population	48%	83992953	54052119	254991	1647031	338020
Iran (Islamic Republic of)	2.4	Unmitigated	0%	83992953	69167811	335490	2117417	444765

Iran (Islamic Republic of)	2.7	Unmitigated	0%	83992953	72641225	354466	2223242	469982
Iran (Islamic Republic of)	3	Unmitigated	0%	83992953	75105499	367931	2296708	487733
Iran (Islamic Republic of)	3.3	Unmitigated	0%	83992953	76903302	377658	2348790	500658
Iraq	2.4	Enhanced social distancing of elderly	35%	40222503	20203698	41458	384233	54962
Iraq	2.7	Enhanced social distancing of elderly	39%	40222503	21990745	46571	419563	61756
Iraq	3	Enhanced social distancing of elderly	44%	40222503	23232116	61809	477714	81962
Iraq	3.3	Enhanced social distancing of elderly	47%	40222503	24420099	66472	504241	88122
Iraq	2.4	Social distancing whole population	37%	40222503	19982732	61652	440334	81733
Iraq	2.7	Social distancing whole population	41%	40222503	21738463	66545	474760	88217
Iraq	3	Social distancing whole population	45%	40222503	23174681	70463	502250	93408
Iraq	3.3	Social distancing whole population	47%	40222503	24374218	73669	524683	97679
Iraq	2.4	Unmitigated	0%	40222503	31521156	91197	646205	120902
Iraq	2.7	Unmitigated	0%	40222503	33275397	94899	671570	125806
Iraq	3	Unmitigated	0%	40222503	34559017	97371	688433	129140
Iraq	3.3	Unmitigated	0%	40222503	35524762	99054	699885	131338
Ireland	2.4	Enhanced social distancing of elderly	35%	4937796	2316133	7212	58211	9561
Ireland	2.7	Enhanced social distancing of elderly	39%	4937796	2545539	8459	65947	11214
Ireland	3	Enhanced social distancing of elderly	43%	4937796	2780327	12576	81213	16671
Ireland	3.3	Enhanced social distancing of elderly	46%	4937796	2946307	14141	88601	18757
Ireland	2.4	Social distancing whole population	36%	4937796	2405522	12614	73861	16722
Ireland	2.7	Social distancing whole population	40%	4937796	2639086	14342	82868	19018
Ireland	3	Social distancing whole population	43%	4937796	2831259	15862	90596	21035
Ireland	3.3	Social distancing whole population	46%	4937796	2992356	17212	97316	22822
Ireland	2.4	Unmitigated	0%	4937796	3875151	26313	139224	34884
Ireland	2.7	Unmitigated	0%	4937796	4116099	29538	152757	39163
Ireland	3	Unmitigated	0%	4937796	4292377	32224	163535	42716
Ireland	3.3	Unmitigated	0%	4937796	4424147	34476	172226	45704
Israel	2.4	Enhanced social distancing of elderly	35%	8655541	4162243	11183	89491	14826
Israel	2.7	Enhanced social distancing of elderly	39%	8655541	4559199	13055	100747	17314
Israel	3	Enhanced social distancing of elderly	43%	8655541	4954914	19493	123954	25852
Israel	3.3	Enhanced social distancing of elderly	46%	8655541	5239106	21855	134732	28970
Israel	2.4	Social distancing whole population	36%	8655541	4297975	19728	114023	26154
Israel	2.7	Social distancing whole population	40%	8655541	4699956	22288	127081	29552
Israel	3	Social distancing whole population	44%	8655541	5029986	24525	138209	32512
Israel	3.3	Social distancing whole population	47%	8655541	5306312	26507	147841	35141
Israel	2.4	Unmitigated	0%	8655541	6873039	40283	209449	53404
Israel	2.7	Unmitigated	0%	8655541	7282509	44958	228366	59600
Israel	3	Unmitigated	0%	8655541	7581196	48834	243326	64767
Israel	3.3	Unmitigated	0%	8655541	7803732	52069	255320	69038
Italy	2.4	Enhanced social distancing of elderly	32%	60461828	24547997	121648	825320	161271
Italy	2.7	Enhanced social distancing of elderly	36%	60461828	27433613	145308	952491	192612
Italy	3	Enhanced social distancing of elderly	40%	60461828	30641688	223807	1216320	296677
Italy	3.3	Enhanced social distancing of elderly	43%	60461828	32786108	253873	1341062	336666

Italy	2.4	Social distancing whole population	34%	60461828	26174209	227159	1112411	301149
Italy	2.7	Social distancing whole population	38%	60461828	29174591	262230	1268142	347637
Italy	3	Social distancing whole population	41%	60461828	31651631	293021	1401820	388621
Italy	3.3	Social distancing whole population	44%	60461828	33733636	320348	1518082	424732
Italy	2.4	Unmitigated	0%	60461828	43873701	477895	2147426	633553
Italy	2.7	Unmitigated	0%	60461828	47262275	543280	2388895	720335
Italy	3	Unmitigated	0%	60461828	49796409	598529	2584158	793425
Italy	3.3	Unmitigated	0%	60461828	51731221	645541	2743997	855775
Jamaica	2.4	Enhanced social distancing of elderly	34%	2961161	1314820	3681	27334	4880
Jamaica	2.7	Enhanced social distancing of elderly	37%	2961161	1464738	4376	31609	5802
Jamaica	3	Enhanced social distancing of elderly	41%	2961161	1607717	6402	39308	8490
Jamaica	3.3	Enhanced social distancing of elderly	44%	2961161	1715889	7179	43196	9518
Jamaica	2.4	Social distancing whole population	35%	2961161	1351319	6171	34481	8181
Jamaica	2.7	Social distancing whole population	39%	2961161	1500525	7048	39195	9344
Jamaica	3	Social distancing whole population	42%	2961161	1625479	7815	43286	10360
Jamaica	3.3	Social distancing whole population	45%	2961161	1731358	8490	46858	11255
Jamaica	2.4	Unmitigated	0%	2961161	2263767	12284	66432	16285
Jamaica	2.7	Unmitigated	0%	2961161	2427034	13616	73076	18050
Jamaica	3	Unmitigated	0%	2961161	2547241	14668	78222	19459
Jamaica	3.3	Unmitigated	0%	2961161	2637097	15507	82245	20563
Japan	2.4	Enhanced social distancing of elderly	34%	126476458	48835687	233148	1535082	309088
Japan	2.7	Enhanced social distancing of elderly	38%	126476458	54200623	279475	1770624	370567
Japan	3	Enhanced social distancing of elderly	42%	126476458	60683937	452150	2326457	599400
Japan	3.3	Enhanced social distancing of elderly	45%	126476458	64882775	517355	2580921	685854
Japan	2.4	Social distancing whole population	36%	126476458	52627050	469064	2170766	621845
Japan	2.7	Social distancing whole population	39%	126476458	58301095	541514	2472723	717877
Japan	3	Social distancing whole population	43%	126476458	63059135	606892	2738826	804928
Japan	3.3	Social distancing whole population	45%	126476458	67118361	666380	2976014	883535
Japan	2.4	Unmitigated	0%	126476458	88640603	1055426	4424901	1399195
Japan	2.7	Unmitigated	0%	126476458	95474355	1214165	4971635	1609937
Japan	3	Unmitigated	0%	126476458	100742815	1352955	5431341	1793130
Japan	3.3	Unmitigated	0%	126476458	104890164	1474438	5820796	1954588
Jordan	2.4	Enhanced social distancing of elderly	35%	10203140	5359201	12223	111146	16204
Jordan	2.7	Enhanced social distancing of elderly	39%	10203140	5821287	13767	121603	18251
Jordan	3	Enhanced social distancing of elderly	44%	10203140	6135670	18361	138633	24350
Jordan	3.3	Enhanced social distancing of elderly	47%	10203140	6438596	19798	146523	26251
Jordan	2.4	Social distancing whole population	37%	10203140	5301322	18302	127554	24264
Jordan	2.7	Social distancing whole population	41%	10203140	5754071	19804	137735	26261
Jordan	3	Social distancing whole population	45%	10203140	6122129	21015	145891	27859
Jordan	3.3	Social distancing whole population	48%	10203140	6427900	22011	152574	29178
Jordan	2.4	Unmitigated	0%	10203140	8256603	27719	190131	36747
Jordan	2.7	Unmitigated	0%	10203140	8685616	28954	198031	38383
Jordan	3	Unmitigated	0%	10203140	8993478	29796	203331	39508

Jordan	3.3	Unmitigated	0%	10203140	9221060	30381	206974	40291
Kazakhstan	2.4	Enhanced social distancing of elderly	35%	18776707	9397517	23131	202626	30665
Kazakhstan	2.7	Enhanced social distancing of elderly	39%	18776707	10286121	26746	227195	35465
Kazakhstan	3	Enhanced social distancing of elderly	43%	18776707	11105871	37486	269888	49694
Kazakhstan	3.3	Enhanced social distancing of elderly	46%	18776707	11725462	41548	291337	55079
Kazakhstan	2.4	Social distancing whole population	37%	18776707	9590120	36697	242385	48650
Kazakhstan	2.7	Social distancing whole population	41%	18776707	10477763	41176	269115	54585
Kazakhstan	3	Social distancing whole population	44%	18776707	11204622	45033	291660	59732
Kazakhstan	3.3	Social distancing whole population	47%	18776707	11811142	48395	310965	64182
Kazakhstan	2.4	Unmitigated	0%	18776707	15233497	70577	430161	93565
Kazakhstan	2.7	Unmitigated	0%	18776707	16104798	77479	464286	102758
Kazakhstan	3	Unmitigated	0%	18776707	16730927	82946	490246	109977
Kazakhstan	3.3	Unmitigated	0%	18776707	17190820	87317	510292	115749
Kenya	2.4	Enhanced social distancing of elderly	35%	53771300	30435341	52511	472297	69615
Kenya	2.7	Enhanced social distancing of elderly	39%	53771300	32897010	58453	515688	77488
Kenya	3	Enhanced social distancing of elderly	44%	53771300	34543842	73331	577784	97248
Kenya	3.3	Enhanced social distancing of elderly	47%	53771300	36155803	77853	607930	103235
Kenya	2.4	Social distancing whole population	37%	53771300	29871427	70948	522747	94057
Kenya	2.7	Social distancing whole population	41%	53771300	32353985	75985	563430	100771
Kenya	3	Social distancing whole population	45%	53771300	34357519	79926	595831	105960
Kenya	3.3	Social distancing whole population	48%	53771300	36008887	83087	622219	110141
Kenya	2.4	Unmitigated	0%	53771300	45897375	100001	772647	132574
Kenya	2.7	Unmitigated	0%	53771300	48072508	103126	803300	136707
Kenya	3	Unmitigated	0%	53771300	49581887	105112	823741	139346
Kenya	3.3	Unmitigated	0%	53771300	50652190	106409	837695	141130
Kiribati	2.4	Enhanced social distancing of elderly	32%	119446	60975	142	1281	189
Kiribati	2.7	Enhanced social distancing of elderly	37%	119446	66415	161	1406	213
Kiribati	3	Enhanced social distancing of elderly	42%	119446	70078	214	1609	284
Kiribati	3.3	Enhanced social distancing of elderly	45%	119446	73634	231	1705	306
Kiribati	2.4	Social distancing whole population	35%	119446	60340	214	1484	284
Kiribati	2.7	Social distancing whole population	39%	119446	65607	233	1607	308
Kiribati	3	Social distancing whole population	43%	119446	69905	247	1706	328
Kiribati	3.3	Social distancing whole population	46%	119446	73486	260	1787	344
Kiribati	2.4	Unmitigated	0%	119446	93048	324	2208	430
Kiribati	2.7	Unmitigated	0%	119446	98344	340	2311	451
Kiribati	3	Unmitigated	0%	119446	102193	351	2382	466
Kiribati	3.3	Unmitigated	0%	119446	105064	359	2431	476
Korea, Dem. People’s Rep.	2.4	Enhanced social distancing of elderly	34%	25778815	13409930	46807	383507	62053
Korea, Dem. People’s Rep.	2.7	Enhanced social distancing of elderly	37%	25778815	14693307	54269	429571	71971
Korea, Dem. People’s Rep.	3	Enhanced social distancing of elderly	43%	25778815	15577606	76988	507902	102079
Korea, Dem. People’s Rep.	3.3	Enhanced social distancing of elderly	46%	25778815	16392646	85055	545760	112754
Korea, Dem. People’s Rep.	2.4	Social distancing whole population	37%	25778815	13481438	77553	466905	102814
Korea, Dem. People’s Rep.	2.7	Social distancing whole population	41%	25778815	14659232	85920	512606	113897

Korea, Dem. People's Rep.	3	Social distancing whole population	44%	25778815	15617534	92973	550480	123251
Korea, Dem. People's Rep.	3.3	Social distancing whole population	47%	25778815	16413412	99003	582396	131313
Korea, Dem. People's Rep.	2.4	Unmitigated	0%	25778815	21085388	138236	779650	183261
Korea, Dem. People's Rep.	2.7	Unmitigated	0%	25778815	22199630	148791	829508	197372
Korea, Dem. People's Rep.	3	Unmitigated	0%	25778815	22995442	156684	865823	207786
Korea, Dem. People's Rep.	3.3	Unmitigated	0%	25778815	23578292	162674	892754	215635
Korea, Rep.	2.4	Enhanced social distancing of elderly	34%	51269183	22089673	82624	658374	109536
Korea, Rep.	2.7	Enhanced social distancing of elderly	38%	51269183	24517864	97708	754170	129562
Korea, Rep.	3	Enhanced social distancing of elderly	42%	51269183	27034614	143533	928692	190271
Korea, Rep.	3.3	Enhanced social distancing of elderly	45%	51269183	28830870	162073	1019316	214854
Korea, Rep.	2.4	Social distancing whole population	35%	51269183	23067334	141198	826244	187188
Korea, Rep.	2.7	Social distancing whole population	39%	51269183	25550440	162303	937694	215156
Korea, Rep.	3	Social distancing whole population	42%	51269183	27617550	181055	1034460	240175
Korea, Rep.	3.3	Social distancing whole population	45%	51269183	29366663	197844	1119370	262376
Korea, Rep.	2.4	Unmitigated	0%	51269183	38362355	301352	1609540	399507
Korea, Rep.	2.7	Unmitigated	0%	51269183	41134862	341182	1783874	452472
Korea, Rep.	3	Unmitigated	0%	51269183	43205205	374492	1923912	496531
Korea, Rep.	3.3	Unmitigated	0%	51269183	44779577	402466	2037450	533522
Kuwait	2.4	Enhanced social distancing of elderly	32%	4270563	2380826	6819	66547	9040
Kuwait	2.7	Enhanced social distancing of elderly	37%	4270563	2569855	7555	72556	10014
Kuwait	3	Enhanced social distancing of elderly	43%	4270563	2648421	8931	78368	11843
Kuwait	3.3	Enhanced social distancing of elderly	47%	4270563	2773181	9446	82439	12528
Kuwait	2.4	Social distancing whole population	37%	4270563	2267279	8362	69132	11085
Kuwait	2.7	Social distancing whole population	41%	4270563	2463094	8977	74897	11907
Kuwait	3	Social distancing whole population	44%	4270563	2621827	9465	79545	12550
Kuwait	3.3	Social distancing whole population	47%	4270563	2753238	9861	83375	13072
Kuwait	2.4	Unmitigated	0%	4270563	3528147	12045	105518	15969
Kuwait	2.7	Unmitigated	0%	4270563	3709322	12515	110532	16591
Kuwait	3	Unmitigated	0%	4270563	3837656	12835	114010	17014
Kuwait	3.3	Unmitigated	0%	4270563	3930834	13058	116472	17316
Kyrgyz Republic	2.4	Enhanced social distancing of elderly	36%	6524191	3361576	6911	63921	9162
Kyrgyz Republic	2.7	Enhanced social distancing of elderly	39%	6524191	3670633	7907	71118	10488
Kyrgyz Republic	3	Enhanced social distancing of elderly	44%	6524191	3935489	10646	82573	14114
Kyrgyz Republic	3.3	Enhanced social distancing of elderly	47%	6524191	4146279	11656	88431	15451
Kyrgyz Republic	2.4	Social distancing whole population	37%	6524191	3394182	10296	73876	13650
Kyrgyz Republic	2.7	Social distancing whole population	41%	6524191	3700514	11415	81352	15132
Kyrgyz Republic	3	Social distancing whole population	44%	6524191	3950519	12355	87556	16379
Kyrgyz Republic	3.3	Social distancing whole population	47%	6524191	4158612	13158	92793	17451
Kyrgyz Republic	2.4	Unmitigated	0%	6524191	5352834	18179	124203	24100
Kyrgyz Republic	2.7	Unmitigated	0%	6524191	5647665	19553	132365	25929
Kyrgyz Republic	3	Unmitigated	0%	6524191	5858270	20579	138315	27289
Kyrgyz Republic	3.3	Unmitigated	0%	6524191	6012062	21356	142729	28313
Lao PDR	2.4	Enhanced social distancing of elderly	35%	7275556	3779587	8797	79592	11662

Lao PDR	2.7	Enhanced social distancing of elderly	39%	7275556	4109600	9924	87218	13155
Lao PDR	3	Enhanced social distancing of elderly	44%	7275556	4338273	13247	99828	17563
Lao PDR	3.3	Enhanced social distancing of elderly	47%	7275556	4554645	14298	105644	18962
Lao PDR	2.4	Social distancing whole population	37%	7275556	3748150	13185	91918	17480
Lao PDR	2.7	Social distancing whole population	41%	7275556	4069892	14286	99368	18947
Lao PDR	3	Social distancing whole population	45%	7275556	4331731	15175	105350	20121
Lao PDR	3.3	Social distancing whole population	48%	7275556	4549329	15909	110264	21090
Lao PDR	2.4	Unmitigated	0%	7275556	5851431	20135	138043	26693
Lao PDR	2.7	Unmitigated	0%	7275556	6159595	21064	143987	27924
Lao PDR	3	Unmitigated	0%	7275556	6381772	21700	148011	28766
Lao PDR	3.3	Unmitigated	0%	7275556	6546522	22143	150791	29369
Latvia	2.4	Enhanced social distancing of elderly	34%	1886202	790089	3177	23095	4211
Latvia	2.7	Enhanced social distancing of elderly	38%	1886202	874086	3769	26444	4998
Latvia	3	Enhanced social distancing of elderly	42%	1886202	966711	5780	33438	7663
Latvia	3.3	Enhanced social distancing of elderly	45%	1886202	1030105	6558	36813	8694
Latvia	2.4	Social distancing whole population	36%	1886202	833799	5846	30531	7750
Latvia	2.7	Social distancing whole population	39%	1886202	920675	6713	34587	8898
Latvia	3	Social distancing whole population	43%	1886202	993096	7487	38127	9926
Latvia	3.3	Social distancing whole population	46%	1886202	1054532	8186	41254	10857
Latvia	2.4	Unmitigated	0%	1886202	1382227	12762	60316	16918
Latvia	2.7	Unmitigated	0%	1886202	1481694	14543	67143	19295
Latvia	3	Unmitigated	0%	1886202	1556971	16072	72761	21312
Latvia	3.3	Unmitigated	0%	1886202	1615090	17387	77424	23045
Lebanon	2.4	Enhanced social distancing of elderly	34%	6825442	3585019	10631	89181	14094
Lebanon	2.7	Enhanced social distancing of elderly	38%	6825442	3911396	12204	98909	16179
Lebanon	3	Enhanced social distancing of elderly	43%	6825442	4148866	17267	116510	22890
Lebanon	3.3	Enhanced social distancing of elderly	47%	6825442	4359526	18956	124521	25140
Lebanon	2.4	Social distancing whole population	37%	6825442	3601251	17528	108084	23237
Lebanon	2.7	Social distancing whole population	41%	6825442	3909322	19261	117860	25544
Lebanon	3	Social distancing whole population	45%	6825442	4159439	20701	125856	27450
Lebanon	3.3	Social distancing whole population	48%	6825442	4366787	21920	132527	29058
Lebanon	2.4	Unmitigated	0%	6825442	5598538	29649	172773	39306
Lebanon	2.7	Unmitigated	0%	6825442	5885541	31586	182218	41868
Lebanon	3	Unmitigated	0%	6825442	6089973	32998	188898	43743
Lebanon	3.3	Unmitigated	0%	6825442	6239466	34042	193708	45141
Lesotho	2.4	Enhanced social distancing of elderly	34%	2142252	1192780	2802	22208	3715
Lesotho	2.7	Enhanced social distancing of elderly	38%	2142252	1293326	3175	24525	4211
Lesotho	3	Enhanced social distancing of elderly	44%	2142252	1370102	4283	28639	5679
Lesotho	3.3	Enhanced social distancing of elderly	47%	2142252	1436291	4626	30433	6132
Lesotho	2.4	Social distancing whole population	37%	2142252	1187919	4237	26249	5618
Lesotho	2.7	Social distancing whole population	41%	2142252	1287721	4600	28507	6098
Lesotho	3	Social distancing whole population	45%	2142252	1368291	4893	30335	6487
Lesotho	3.3	Social distancing whole population	48%	2142252	1434722	5135	31842	6811

Lesotho	2.4	Unmitigated	0%	2142252	1827033	6562	40750	8699
Lesotho	2.7	Unmitigated	0%	2142252	1914644	6878	42724	9120
Lesotho	3	Unmitigated	0%	2142252	1975316	7095	44080	9410
Lesotho	3.3	Unmitigated	0%	2142252	2018273	7248	45030	9609
Liberia	2.4	Enhanced social distancing of elderly	35%	5057677	2839579	5428	45623	7196
Liberia	2.7	Enhanced social distancing of elderly	39%	5057677	3071776	6088	50006	8071
Liberia	3	Enhanced social distancing of elderly	44%	5057677	3235956	7913	57134	10491
Liberia	3.3	Enhanced social distancing of elderly	47%	5057677	3388099	8466	60333	11228
Liberia	2.4	Social distancing whole population	37%	5057677	2803427	7754	52186	10279
Liberia	2.7	Social distancing whole population	41%	5057677	3035872	8349	56348	11073
Liberia	3	Social distancing whole population	45%	5057677	3223413	8822	59675	11699
Liberia	3.3	Social distancing whole population	48%	5057677	3378017	9206	62394	12204
Liberia	2.4	Unmitigated	0%	5057677	4305227	11366	78085	15068
Liberia	2.7	Unmitigated	0%	5057677	4509818	11796	81326	15640
Liberia	3	Unmitigated	0%	5057677	4652057	12078	83496	16011
Liberia	3.3	Unmitigated	0%	5057677	4753297	12269	84983	16264
Libya	2.4	Enhanced social distancing of elderly	34%	6871287	3714507	9524	86995	12626
Libya	2.7	Enhanced social distancing of elderly	38%	6871287	4030293	10740	95344	14241
Libya	3	Enhanced social distancing of elderly	44%	6871287	4233774	14290	108353	18950
Libya	3.3	Enhanced social distancing of elderly	47%	6871287	4437402	15411	114582	20431
Libya	2.4	Social distancing whole population	37%	6871287	3665629	14215	99266	18846
Libya	2.7	Social distancing whole population	41%	6871287	3973383	15389	107327	20400
Libya	3	Social distancing whole population	45%	6871287	4222482	16332	113793	21651
Libya	3.3	Social distancing whole population	48%	6871287	4428553	17109	119099	22685
Libya	2.4	Unmitigated	0%	6871287	5663452	21621	149588	28664
Libya	2.7	Unmitigated	0%	6871287	5944726	22588	156014	29945
Libya	3	Unmitigated	0%	6871287	6144191	23245	160343	30829
Libya	3.3	Unmitigated	0%	6871287	6289632	23701	163325	31426
Lithuania	2.4	Enhanced social distancing of elderly	34%	2722291	1124367	4657	33368	6174
Lithuania	2.7	Enhanced social distancing of elderly	38%	2722291	1245694	5533	38275	7335
Lithuania	3	Enhanced social distancing of elderly	42%	2722291	1379646	8497	48445	11264
Lithuania	3.3	Enhanced social distancing of elderly	45%	2722291	1471730	9651	53398	12803
Lithuania	2.4	Social distancing whole population	36%	2722291	1187198	8580	44093	11375
Lithuania	2.7	Social distancing whole population	39%	2722291	1312688	9865	50032	13083
Lithuania	3	Social distancing whole population	43%	2722291	1417667	11018	55235	14611
Lithuania	3.3	Social distancing whole population	46%	2722291	1506979	12059	59842	15990
Lithuania	2.4	Unmitigated	0%	2722291	1980449	18810	87690	24937
Lithuania	2.7	Unmitigated	0%	2722291	2126281	21460	97766	28452
Lithuania	3	Unmitigated	0%	2722291	2237002	23734	106061	31462
Lithuania	3.3	Unmitigated	0%	2722291	2322740	25691	112948	34058
Luxembourg	2.4	Enhanced social distancing of elderly	35%	625976	292426	986	8075	1307
Luxembourg	2.7	Enhanced social distancing of elderly	39%	625976	320770	1150	9094	1525
Luxembourg	3	Enhanced social distancing of elderly	43%	625976	350007	1683	11050	2232

Luxembourg	3.3	Enhanced social distancing of elderly	46%	625976	370484	1885	12000	2498
Luxembourg	2.4	Social distancing whole population	36%	625976	303795	1681	10040	2229
Luxembourg	2.7	Social distancing whole population	40%	625976	332664	1903	11206	2524
Luxembourg	3	Social distancing whole population	43%	625976	356393	2098	12202	2781
Luxembourg	3.3	Social distancing whole population	46%	625976	376269	2270	13064	3010
Luxembourg	2.4	Unmitigated	0%	625976	487194	3454	18507	4579
Luxembourg	2.7	Unmitigated	0%	625976	517476	3875	20258	5136
Luxembourg	3	Unmitigated	0%	625976	539742	4227	21657	5606
Luxembourg	3.3	Unmitigated	0%	625976	556496	4525	22791	6000
Macao SAR, China	2.4	Enhanced social distancing of elderly	32%	649342	338606	1381	11067	1830
Macao SAR, China	2.7	Enhanced social distancing of elderly	36%	649342	372942	1612	12479	2137
Macao SAR, China	3	Enhanced social distancing of elderly	42%	649342	395630	2289	14785	3034
Macao SAR, China	3.3	Enhanced social distancing of elderly	45%	649342	416939	2533	15926	3360
Macao SAR, China	2.4	Social distancing whole population	36%	649342	341914	2314	13603	3068
Macao SAR, China	2.7	Social distancing whole population	40%	649342	372368	2569	14974	3406
Macao SAR, China	3	Social distancing whole population	44%	649342	397118	2783	16109	3691
Macao SAR, China	3.3	Social distancing whole population	47%	649342	417644	2965	17066	3932
Macao SAR, China	2.4	Unmitigated	0%	649342	533349	4092	22743	5425
Macao SAR, China	2.7	Unmitigated	0%	649342	561964	4401	24232	5835
Macao SAR, China	3	Unmitigated	0%	649342	582259	4629	25310	6136
Macao SAR, China	3.3	Unmitigated	0%	649342	596987	4799	26105	6362
Madagascar	2.4	Enhanced social distancing of elderly	35%	27691019	15501912	29093	246499	38569
Madagascar	2.7	Enhanced social distancing of elderly	39%	27691019	16771857	32586	269994	43200
Madagascar	3	Enhanced social distancing of elderly	44%	27691019	17657979	42016	307076	55696
Madagascar	3.3	Enhanced social distancing of elderly	47%	27691019	18490551	44876	324026	59501
Madagascar	2.4	Social distancing whole population	37%	27691019	15281700	41022	279723	54384
Madagascar	2.7	Social distancing whole population	41%	27691019	16554279	44128	301956	58500
Madagascar	3	Social distancing whole population	45%	27691019	17581734	46586	319719	61789
Madagascar	3.3	Social distancing whole population	48%	27691019	18429649	48579	334235	64410
Madagascar	2.4	Unmitigated	0%	27691019	23519130	59652	417621	79082
Madagascar	2.7	Unmitigated	0%	27691019	24646766	61817	434778	81969
Madagascar	3	Unmitigated	0%	27691019	25432974	63229	446252	83822
Madagascar	3.3	Unmitigated	0%	27691019	25994120	64170	454102	85067
Malawi	2.4	Enhanced social distancing of elderly	35%	19129955	10709293	17863	154488	23681
Malawi	2.7	Enhanced social distancing of elderly	39%	19129955	11579972	19936	168629	26432
Malawi	3	Enhanced social distancing of elderly	44%	19129955	12173239	25575	190883	33919
Malawi	3.3	Enhanced social distancing of elderly	47%	19129955	12745288	27228	200897	36101
Malawi	2.4	Social distancing whole population	37%	19129955	10527684	25059	174492	33221
Malawi	2.7	Social distancing whole population	41%	19129955	11404606	26848	187754	35598
Malawi	3	Social distancing whole population	45%	19129955	12113027	28251	198275	37450
Malawi	3.3	Social distancing whole population	48%	19129955	12697761	29377	206813	38944
Malawi	2.4	Unmitigated	0%	19129955	16215276	35425	254686	46963
Malawi	2.7	Unmitigated	0%	19129955	16996758	36545	264184	48447

Malawi	3	Unmitigated	0%	19129955	17542970	37258	270437	49413
Malawi	3.3	Unmitigated	0%	19129955	17933338	37722	274650	50025
Malaysia	2.4	Enhanced social distancing of elderly	34%	32365998	17131829	49633	425701	65800
Malaysia	2.7	Enhanced social distancing of elderly	38%	32365998	18669619	56756	470985	75255
Malaysia	3	Enhanced social distancing of elderly	43%	32365998	19785632	79306	551856	105131
Malaysia	3.3	Enhanced social distancing of elderly	47%	32365998	20778280	86765	588680	115023
Malaysia	2.4	Social distancing whole population	37%	32365998	17186696	80183	511467	106300
Malaysia	2.7	Social distancing whole population	41%	32365998	18646900	87855	556772	116466
Malaysia	3	Social distancing whole population	45%	32365998	19830506	94191	593676	124919
Malaysia	3.3	Social distancing whole population	48%	32365998	20810737	99520	624354	131964
Malaysia	2.4	Unmitigated	0%	32365998	26655264	133076	808989	176421
Malaysia	2.7	Unmitigated	0%	32365998	28002253	141272	851498	187345
Malaysia	3	Unmitigated	0%	32365998	28959354	147190	881381	195114
Malaysia	3.3	Unmitigated	0%	32365998	29657013	151538	902812	200881
Maldives	2.4	Enhanced social distancing of elderly	33%	540542	294647	739	6208	980
Maldives	2.7	Enhanced social distancing of elderly	37%	540542	319707	830	6824	1100
Maldives	3	Enhanced social distancing of elderly	43%	540542	335404	1063	7670	1410
Maldives	3.3	Enhanced social distancing of elderly	46%	540542	351839	1136	8108	1507
Maldives	2.4	Social distancing whole population	37%	540542	287085	1031	6858	1366
Maldives	2.7	Social distancing whole population	41%	540542	312151	1112	7444	1475
Maldives	3	Social distancing whole population	44%	540542	332551	1178	7917	1561
Maldives	3.3	Social distancing whole population	47%	540542	349493	1230	8308	1631
Maldives	2.4	Unmitigated	0%	540542	448388	1515	10539	2008
Maldives	2.7	Unmitigated	0%	540542	472283	1575	11059	2088
Maldives	3	Unmitigated	0%	540542	489230	1615	11422	2142
Maldives	3.3	Unmitigated	0%	540542	501497	1642	11681	2178
Mali	2.4	Enhanced social distancing of elderly	35%	20250834	11202474	17885	155665	23710
Mali	2.7	Enhanced social distancing of elderly	39%	20250834	12116430	19927	169747	26423
Mali	3	Enhanced social distancing of elderly	44%	20250834	12737491	25398	191791	33669
Mali	3.3	Enhanced social distancing of elderly	47%	20250834	13339759	26996	201683	35788
Mali	2.4	Social distancing whole population	37%	20250834	11005135	24844	175416	32937
Mali	2.7	Social distancing whole population	41%	20250834	11926824	26583	188577	35240
Mali	3	Social distancing whole population	45%	20250834	12672293	27941	198992	37055
Mali	3.3	Social distancing whole population	48%	20250834	13288502	29027	207426	38495
Mali	2.4	Unmitigated	0%	20250834	17011008	34778	254264	46106
Mali	2.7	Unmitigated	0%	20250834	17849171	35824	263459	47513
Mali	3	Unmitigated	0%	20250834	18439564	36481	269469	48370
Mali	3.3	Unmitigated	0%	20250834	18865183	36905	273496	48922
Malta	2.4	Enhanced social distancing of elderly	34%	441539	184826	722	5320	957
Malta	2.7	Enhanced social distancing of elderly	37%	441539	204669	858	6096	1137
Malta	3	Enhanced social distancing of elderly	41%	441539	226682	1320	7739	1751
Malta	3.3	Enhanced social distancing of elderly	44%	441539	241652	1499	8525	1988
Malta	2.4	Social distancing whole population	35%	441539	195521	1345	7108	1783

Malta	2.7	Social distancing whole population	39%	441539	216146	1547	8062	2052
Malta	3	Social distancing whole population	42%	441539	233312	1728	8894	2290
Malta	3.3	Social distancing whole population	45%	441539	247848	1890	9627	2506
Malta	2.4	Unmitigated	0%	441539	322728	2914	13933	3863
Malta	2.7	Unmitigated	0%	441539	346285	3326	15529	4409
Malta	3	Unmitigated	0%	441539	364067	3678	16841	4876
Malta	3.3	Unmitigated	0%	441539	377764	3981	17930	5280
Martinique	2.4	Enhanced social distancing of elderly	32%	375265	142885	753	4354	998
Martinique	2.7	Enhanced social distancing of elderly	36%	375265	159931	906	5096	1201
Martinique	3	Enhanced social distancing of elderly	40%	375265	179676	1408	6729	1866
Martinique	3.3	Enhanced social distancing of elderly	43%	375265	192773	1594	7477	2113
Martinique	2.4	Social distancing whole population	35%	375265	153147	1406	6123	1864
Martinique	2.7	Social distancing whole population	39%	375265	170675	1615	7003	2140
Martinique	3	Social distancing whole population	42%	375265	185483	1800	7778	2387
Martinique	3.3	Social distancing whole population	45%	375265	198144	1965	8464	2606
Martinique	2.4	Unmitigated	0%	375265	261436	2904	12275	3850
Martinique	2.7	Unmitigated	0%	375265	282960	3279	13746	4349
Martinique	3	Unmitigated	0%	375265	299494	3593	14954	4763
Martinique	3.3	Unmitigated	0%	375265	312422	3857	15955	5113
Mauritania	2.4	Enhanced social distancing of elderly	35%	4649660	2597756	4963	42330	6579
Mauritania	2.7	Enhanced social distancing of elderly	39%	4649660	2811508	5564	46406	7375
Mauritania	3	Enhanced social distancing of elderly	44%	4649660	2961434	7192	52827	9538
Mauritania	3.3	Enhanced social distancing of elderly	47%	4649660	3101627	7689	55782	10197
Mauritania	2.4	Social distancing whole population	37%	4649660	2562175	7024	48068	9312
Mauritania	2.7	Social distancing whole population	41%	4649660	2776128	7563	51937	10031
Mauritania	3	Social distancing whole population	45%	4649660	2948945	7992	55034	10596
Mauritania	3.3	Social distancing whole population	48%	4649660	3091553	8339	57568	11054
Mauritania	2.4	Unmitigated	0%	4649660	3944934	10278	72166	13626
Mauritania	2.7	Unmitigated	0%	4649660	4135147	10663	75214	14136
Mauritania	3	Unmitigated	0%	4649660	4267768	10916	77262	14471
Mauritania	3.3	Unmitigated	0%	4649660	4362362	11086	78667	14702
Mauritius	2.4	Enhanced social distancing of elderly	33%	1271767	641738	2767	18683	3669
Mauritius	2.7	Enhanced social distancing of elderly	37%	1271767	701618	3195	21015	4238
Mauritius	3	Enhanced social distancing of elderly	42%	1271767	759994	4562	25790	6048
Mauritius	3.3	Enhanced social distancing of elderly	46%	1271767	801796	5013	27837	6646
Mauritius	2.4	Social distancing whole population	37%	1271767	660448	4561	23768	6046
Mauritius	2.7	Social distancing whole population	41%	1271767	720034	5037	26203	6676
Mauritius	3	Social distancing whole population	44%	1271767	768658	5436	28235	7206
Mauritius	3.3	Social distancing whole population	47%	1271767	809159	5776	29963	7662
Mauritius	2.4	Unmitigated	0%	1271767	1043823	7934	40748	10518
Mauritius	2.7	Unmitigated	0%	1271767	1101864	8534	43689	11319
Mauritius	3	Unmitigated	0%	1271767	1143285	8987	45887	11917
Mauritius	3.3	Unmitigated	0%	1271767	1173471	9334	47554	12374

Mayotte	2.4	Enhanced social distancing of elderly	34%	272813	153770	326	2585	432
Mayotte	2.7	Enhanced social distancing of elderly	38%	272813	166356	368	2842	488
Mayotte	3	Enhanced social distancing of elderly	43%	272813	175595	495	3296	656
Mayotte	3.3	Enhanced social distancing of elderly	46%	272813	183846	533	3491	707
Mayotte	2.4	Social distancing whole population	37%	272813	152454	494	3038	655
Mayotte	2.7	Social distancing whole population	41%	272813	165018	534	3286	708
Mayotte	3	Social distancing whole population	44%	272813	175142	566	3484	750
Mayotte	3.3	Social distancing whole population	47%	272813	183480	592	3647	785
Mayotte	2.4	Unmitigated	0%	272813	231816	742	4576	984
Mayotte	2.7	Unmitigated	0%	272813	242878	775	4783	1028
Mayotte	3	Unmitigated	0%	272813	250538	798	4923	1058
Mayotte	3.3	Unmitigated	0%	272813	255964	814	5021	1080
Mexico	2.4	Enhanced social distancing of elderly	34%	128932753	59137472	148798	1150908	197265
Mexico	2.7	Enhanced social distancing of elderly	38%	128932753	65655490	175899	1324030	233279
Mexico	3	Enhanced social distancing of elderly	42%	128932753	71714250	253617	1627074	336272
Mexico	3.3	Enhanced social distancing of elderly	45%	128932753	76362508	283182	1781124	375398
Mexico	2.4	Social distancing whole population	36%	128932753	60474818	243724	1425857	323109
Mexico	2.7	Social distancing whole population	40%	128932753	66940433	276968	1613561	367159
Mexico	3	Social distancing whole population	43%	128932753	72332334	305861	1775501	405471
Mexico	3.3	Social distancing whole population	46%	128932753	76887369	331130	1916226	439163
Mexico	2.4	Unmitigated	0%	128932753	100324640	475425	2702534	630280
Mexico	2.7	Unmitigated	0%	128932753	107211257	523460	2956839	694408
Mexico	3	Unmitigated	0%	128932753	112242507	560789	3151367	743660
Mexico	3.3	Unmitigated	0%	128932753	115977950	590083	3301663	782168
Micronesia (Fed. States of	2.4	Enhanced social distancing of elderly	32%	115021	59405	143	1286	189
Micronesia (Fed. States of	2.7	Enhanced social distancing of elderly	36%	115021	64631	161	1413	213
Micronesia (Fed. States of	3	Enhanced social distancing of elderly	42%	115021	68021	208	1606	276
Micronesia (Fed. States of	3.3	Enhanced social distancing of elderly	45%	115021	71450	224	1701	297
Micronesia (Fed. States of	2.4	Social distancing whole population	35%	115021	58591	205	1473	272
Micronesia (Fed. States of	2.7	Social distancing whole population	39%	115021	63688	223	1595	295
Micronesia (Fed. States of	3	Social distancing whole population	43%	115021	67847	237	1695	314
Micronesia (Fed. States of	3.3	Social distancing whole population	46%	115021	71311	248	1776	329
Micronesia (Fed. States of	2.4	Unmitigated	0%	115021	90222	309	2208	410
Micronesia (Fed. States of	2.7	Unmitigated	0%	115021	95340	325	2317	431
Micronesia (Fed. States of	3	Unmitigated	0%	115021	99044	336	2393	445
Micronesia (Fed. States of	3.3	Unmitigated	0%	115021	101802	344	2447	456
Moldova	2.4	Enhanced social distancing of elderly	34%	4033963	1867932	5826	49449	7723
Moldova	2.7	Enhanced social distancing of elderly	38%	4033963	2061207	6821	56120	9042
Moldova	3	Enhanced social distancing of elderly	42%	4033963	2251906	9757	67887	12939
Moldova	3.3	Enhanced social distancing of elderly	45%	4033963	2390597	10924	73949	14486
Moldova	2.4	Social distancing whole population	36%	4033963	1930740	9544	60546	12652
Moldova	2.7	Social distancing whole population	40%	4033963	2126376	10865	68095	14409
Moldova	3	Social distancing whole population	43%	4033963	2287606	12021	74552	15935

Moldova	3.3	Social distancing whole population	46%	4033963	2422846	13043	80146	17289
Moldova	2.4	Unmitigated	0%	4033963	3138766	19480	113136	25825
Moldova	2.7	Unmitigated	0%	4033963	3343097	21766	123951	28854
Moldova	3	Unmitigated	0%	4033963	3492602	23635	132451	31333
Moldova	3.3	Unmitigated	0%	4033963	3604399	25174	139215	33388
Mongolia	2.4	Enhanced social distancing of elderly	34%	3278292	1736372	4581	41252	6073
Mongolia	2.7	Enhanced social distancing of elderly	38%	3278292	1886621	5168	45279	6853
Mongolia	3	Enhanced social distancing of elderly	43%	3278292	1979127	6774	51274	8982
Mongolia	3.3	Enhanced social distancing of elderly	47%	3278292	2075928	7295	54235	9671
Mongolia	2.4	Social distancing whole population	37%	3278292	1707448	6667	46735	8839
Mongolia	2.7	Social distancing whole population	41%	3278292	1853231	7221	50589	9573
Mongolia	3	Social distancing whole population	45%	3278292	1971639	7667	53687	10164
Mongolia	3.3	Social distancing whole population	48%	3278292	2069871	8034	56232	10656
Mongolia	2.4	Unmitigated	0%	3278292	2654760	10124	70672	13421
Mongolia	2.7	Unmitigated	0%	3278292	2792033	10574	73767	14020
Mongolia	3	Unmitigated	0%	3278292	2890518	10879	75858	14428
Mongolia	3.3	Unmitigated	0%	3278292	2963123	11089	77300	14703
Montenegro	2.4	Enhanced social distancing of elderly	34%	628062	282356	945	7436	1253
Montenegro	2.7	Enhanced social distancing of elderly	38%	628062	311452	1112	8463	1475
Montenegro	3	Enhanced social distancing of elderly	42%	628062	341629	1651	10469	2188
Montenegro	3.3	Enhanced social distancing of elderly	45%	628062	362953	1860	11457	2467
Montenegro	2.4	Social distancing whole population	36%	628062	294351	1651	9491	2188
Montenegro	2.7	Social distancing whole population	39%	628062	324078	1885	10697	2501
Montenegro	3	Social distancing whole population	43%	628062	348680	2093	11738	2775
Montenegro	3.3	Social distancing whole population	46%	628062	369400	2278	12649	3019
Montenegro	2.4	Unmitigated	0%	628062	479072	3468	18101	4597
Montenegro	2.7	Unmitigated	0%	628062	511220	3911	19972	5186
Montenegro	3	Unmitigated	0%	628062	535034	4282	21474	5677
Montenegro	3.3	Unmitigated	0%	628062	553058	4595	22694	6091
Morocco	2.4	Enhanced social distancing of elderly	34%	36910558	19221003	56942	478408	75489
Morocco	2.7	Enhanced social distancing of elderly	38%	36910558	20990716	65400	531290	86726
Morocco	3	Enhanced social distancing of elderly	43%	36910558	22270080	92019	626661	122024
Morocco	3.3	Enhanced social distancing of elderly	46%	36910558	23411429	101004	670230	133898
Morocco	2.4	Social distancing whole population	37%	36910558	19310690	93011	580929	123306
Morocco	2.7	Social distancing whole population	41%	36910558	20973820	102267	633944	135566
Morocco	3	Social distancing whole population	45%	36910558	22325887	109964	677382	145770
Morocco	3.3	Social distancing whole population	48%	36910558	23448100	116478	713664	154445
Morocco	2.4	Unmitigated	0%	36910558	30105410	157668	932587	209023
Morocco	2.7	Unmitigated	0%	36910558	31669635	168028	984448	222754
Morocco	3	Unmitigated	0%	36910558	32788459	175580	1021279	232870
Morocco	3.3	Unmitigated	0%	36910558	33609760	181170	1047932	240207
Mozambique	2.4	Enhanced social distancing of elderly	35%	31255435	17394673	29801	252706	39507
Mozambique	2.7	Enhanced social distancing of elderly	39%	31255435	18815979	33331	276162	44188

Mozambique	3	Enhanced social distancing of elderly	44%	31255435	19802361	43159	314498	57214
Mozambique	3.3	Enhanced social distancing of elderly	47%	31255435	20736677	46040	331350	61059
Mozambique	2.4	Social distancing whole population	37%	31255435	17131199	42412	288304	56227
Mozambique	2.7	Social distancing whole population	41%	31255435	18559204	45507	310384	60338
Mozambique	3	Social distancing whole population	45%	31255435	19713093	47942	327918	63585
Mozambique	3.3	Social distancing whole population	48%	31255435	20668307	49910	342194	66174
Mozambique	2.4	Unmitigated	0%	31255435	26409840	60577	422211	80308
Mozambique	2.7	Unmitigated	0%	31255435	27690360	62589	438109	82986
Mozambique	3	Unmitigated	0%	31255435	28588342	63878	448566	84679
Mozambique	3.3	Unmitigated	0%	31255435	29232057	64725	455605	85804
Myanmar	2.4	Enhanced social distancing of elderly	34%	54409794	29047943	81418	708100	107938
Myanmar	2.7	Enhanced social distancing of elderly	38%	54409794	31619898	92761	782414	123012
Myanmar	3	Enhanced social distancing of elderly	44%	54409794	33380436	126306	907327	167511
Myanmar	3.3	Enhanced social distancing of elderly	47%	54409794	35028320	137383	965581	182126
Myanmar	2.4	Social distancing whole population	37%	54409794	28957501	125968	835343	166998
Myanmar	2.7	Social distancing whole population	41%	54409794	31403893	137478	907961	182281
Myanmar	3	Social distancing whole population	45%	54409794	33386173	146902	966921	194733
Myanmar	3.3	Social distancing whole population	48%	54409794	35026707	154767	1015792	205173
Myanmar	2.4	Unmitigated	0%	54409794	44842404	202947	1307584	269050
Myanmar	2.7	Unmitigated	0%	54409794	47089547	214129	1373135	283863
Myanmar	3	Unmitigated	0%	54409794	48685416	222025	1418766	294461
Myanmar	3.3	Unmitigated	0%	54409794	49848836	227698	1451143	301930
Namibia	2.4	Enhanced social distancing of elderly	34%	2540916	1419989	2879	24013	3817
Namibia	2.7	Enhanced social distancing of elderly	39%	2540916	1537651	3239	26380	4294
Namibia	3	Enhanced social distancing of elderly	44%	2540916	1621703	4251	30234	5635
Namibia	3.3	Enhanced social distancing of elderly	47%	2540916	1698907	4560	31982	6046
Namibia	2.4	Social distancing whole population	37%	2540916	1403387	4173	27558	5532
Namibia	2.7	Social distancing whole population	41%	2540916	1520889	4505	29821	5973
Namibia	3	Social distancing whole population	45%	2540916	1615804	4771	31639	6328
Namibia	3.3	Social distancing whole population	48%	2540916	1694109	4987	33131	6613
Namibia	2.4	Unmitigated	0%	2540916	2160157	6219	41781	8244
Namibia	2.7	Unmitigated	0%	2540916	2264232	6474	43627	8585
Namibia	3	Unmitigated	0%	2540916	2336643	6645	44877	8809
Namibia	3.3	Unmitigated	0%	2540916	2388147	6762	45743	8964
Nepal	2.4	Enhanced social distancing of elderly	34%	29136808	16615716	45526	340844	60354
Nepal	2.7	Enhanced social distancing of elderly	38%	29136808	17976976	51374	375340	68115
Nepal	3	Enhanced social distancing of elderly	44%	29136808	19036837	68722	438689	91145
Nepal	3.3	Enhanced social distancing of elderly	47%	29136808	19929941	73976	465308	98083
Nepal	2.4	Social distancing whole population	37%	29136808	16573605	67861	404186	89964
Nepal	2.7	Social distancing whole population	41%	29136808	17929890	73443	437647	97376
Nepal	3	Social distancing whole population	45%	29136808	19019549	77925	464550	103299
Nepal	3.3	Social distancing whole population	48%	29136808	19914885	81605	486673	108182
Nepal	2.4	Unmitigated	0%	29136808	25246764	103435	618530	137125

Nepal	2.7	Unmitigated	0%	29136808	26383379	108048	646608	143235
Nepal	3	Unmitigated	0%	29136808	27158877	111174	665732	147459
Nepal	3.3	Unmitigated	0%	29136808	27699543	113342	679053	150305
Netherlands	2.4	Enhanced social distancing of elderly	34%	17134873	7220136	28058	205761	37197
Netherlands	2.7	Enhanced social distancing of elderly	38%	17134873	7989521	33278	235667	44123
Netherlands	3	Enhanced social distancing of elderly	42%	17134873	8835361	50944	298252	67535
Netherlands	3.3	Enhanced social distancing of elderly	45%	17134873	9416254	57795	328452	76618
Netherlands	2.4	Social distancing whole population	36%	17134873	7613432	51419	272180	68167
Netherlands	2.7	Social distancing whole population	40%	17134873	8408762	59041	308423	78269
Netherlands	3	Social distancing whole population	43%	17134873	9072480	65859	340098	87349
Netherlands	3.3	Social distancing whole population	46%	17134873	9635442	72006	368077	95475
Netherlands	2.4	Unmitigated	0%	17134873	12639509	112170	538723	148706
Netherlands	2.7	Unmitigated	0%	17134873	13546944	127679	599545	169312
Netherlands	3	Unmitigated	0%	17134873	14232679	140922	649456	186784
Netherlands	3.3	Unmitigated	0%	17134873	14760921	152261	690771	201840
New Caledonia	2.4	Enhanced social distancing of elderly	33%	285491	149568	490	4049	650
New Caledonia	2.7	Enhanced social distancing of elderly	37%	285491	163700	568	4525	752
New Caledonia	3	Enhanced social distancing of elderly	42%	285491	174002	813	5388	1078
New Caledonia	3.3	Enhanced social distancing of elderly	45%	285491	183054	898	5788	1191
New Caledonia	2.4	Social distancing whole population	36%	285491	151106	831	5013	1102
New Caledonia	2.7	Social distancing whole population	40%	285491	164165	919	5494	1219
New Caledonia	3	Social distancing whole population	44%	285491	174768	993	5891	1317
New Caledonia	3.3	Social distancing whole population	47%	285491	183562	1056	6225	1400
New Caledonia	2.4	Unmitigated	0%	285491	233557	1451	8207	1923
New Caledonia	2.7	Unmitigated	0%	285491	245824	1559	8717	2066
New Caledonia	3	Unmitigated	0%	285491	254543	1639	9084	2173
New Caledonia	3.3	Unmitigated	0%	285491	260891	1699	9354	2253
New Zealand	2.4	Enhanced social distancing of elderly	35%	4822233	2146106	7270	55542	9638
New Zealand	2.7	Enhanced social distancing of elderly	39%	4822233	2366452	8571	63240	11365
New Zealand	3	Enhanced social distancing of elderly	42%	4822233	2597934	12914	78920	17119
New Zealand	3.3	Enhanced social distancing of elderly	45%	4822233	2760683	14582	86486	19331
New Zealand	2.4	Social distancing whole population	36%	4822233	2240631	12981	71911	17209
New Zealand	2.7	Social distancing whole population	40%	4822233	2465958	14825	81034	19652
New Zealand	3	Social distancing whole population	43%	4822233	2652973	16462	88945	21838
New Zealand	3.3	Social distancing whole population	46%	4822233	2810919	17929	95884	23775
New Zealand	2.4	Unmitigated	0%	4822233	3668162	27680	138773	36695
New Zealand	2.7	Unmitigated	0%	4822233	3914629	31276	153296	41477
New Zealand	3	Unmitigated	0%	4822233	4098244	34309	165041	45489
New Zealand	3.3	Unmitigated	0%	4822233	4237802	36876	174633	48884
Nicaragua	2.4	Enhanced social distancing of elderly	34%	6624554	3148983	6755	54823	8955
Nicaragua	2.7	Enhanced social distancing of elderly	38%	6624554	3483085	7923	62607	10503
Nicaragua	3	Enhanced social distancing of elderly	42%	6624554	3780816	11191	75677	14841
Nicaragua	3.3	Enhanced social distancing of elderly	45%	6624554	4015136	12413	82356	16459

Nicaragua	2.4	Social distancing whole population	36%	6624554	3196939	10735	66357	14232
Nicaragua	2.7	Social distancing whole population	40%	6624554	3526736	12108	74593	16056
Nicaragua	3	Social distancing whole population	43%	6624554	3800392	13286	81623	17611
Nicaragua	3.3	Social distancing whole population	46%	6624554	4030965	14306	87685	18964
Nicaragua	2.4	Unmitigated	0%	6624554	5241043	20112	121652	26663
Nicaragua	2.7	Unmitigated	0%	6624554	5582754	21910	131963	29044
Nicaragua	3	Unmitigated	0%	6624554	5830462	23267	139670	30845
Nicaragua	3.3	Unmitigated	0%	6624554	6013045	24304	145502	32232
Niger	2.4	Enhanced social distancing of elderly	35%	24206636	13215154	21430	181189	28411
Niger	2.7	Enhanced social distancing of elderly	39%	24206636	14303795	23918	197784	31717
Niger	3	Enhanced social distancing of elderly	44%	24206636	15054834	30643	224766	40624
Niger	3.3	Enhanced social distancing of elderly	47%	24206636	15773415	32620	236574	43243
Niger	2.4	Social distancing whole population	37%	24206636	13001399	29995	206210	39764
Niger	2.7	Social distancing whole population	41%	24206636	14095592	32138	221744	42603
Niger	3	Social distancing whole population	45%	24206636	14982011	33817	234036	44836
Niger	3.3	Social distancing whole population	48%	24206636	15717317	35169	244027	46646
Niger	2.4	Unmitigated	0%	24206636	20163748	42351	299128	56145
Niger	2.7	Unmitigated	0%	24206636	21177801	43672	309909	57925
Niger	3	Unmitigated	0%	24206636	21897299	44506	316935	59010
Niger	3.3	Unmitigated	0%	24206636	22420255	45044	321620	59712
Nigeria	2.4	Enhanced social distancing of elderly	35%	206139587	114885822	200111	1749781	265292
Nigeria	2.7	Enhanced social distancing of elderly	39%	206139587	124262817	223244	1912725	295940
Nigeria	3	Enhanced social distancing of elderly	44%	206139587	130717645	282996	2162024	375276
Nigeria	3.3	Enhanced social distancing of elderly	47%	206139587	136877510	301136	2277430	399377
Nigeria	2.4	Social distancing whole population	37%	206139587	113043181	274742	1966856	364231
Nigeria	2.7	Social distancing whole population	41%	206139587	122469733	294734	2120008	390918
Nigeria	3	Social distancing whole population	45%	206139587	130085217	310444	2241970	411607
Nigeria	3.3	Social distancing whole population	48%	206139587	136373807	323103	2341325	428308
Nigeria	2.4	Unmitigated	0%	206139587	174262604	391607	2905604	519163
Nigeria	2.7	Unmitigated	0%	206139587	182701704	404438	3019521	536138
Nigeria	3	Unmitigated	0%	206139587	188619164	412635	3095141	547017
Nigeria	3.3	Unmitigated	0%	206139587	192854728	417990	3146376	554363
North Macedonia	2.4	Enhanced social distancing of elderly	34%	2083380	944537	3078	25306	4080
North Macedonia	2.7	Enhanced social distancing of elderly	38%	2083380	1043099	3618	28801	4799
North Macedonia	3	Enhanced social distancing of elderly	42%	2083380	1143462	5286	35316	7008
North Macedonia	3.3	Enhanced social distancing of elderly	45%	2083380	1215168	5945	38599	7880
North Macedonia	2.4	Social distancing whole population	36%	2083380	981757	5228	31728	6931
North Macedonia	2.7	Social distancing whole population	39%	2083380	1082170	5971	35776	7915
North Macedonia	3	Social distancing whole population	43%	2083380	1165147	6625	39259	8783
North Macedonia	3.3	Social distancing whole population	46%	2083380	1234938	7207	42294	9558
North Macedonia	2.4	Unmitigated	0%	2083380	1602764	10897	60270	14446
North Macedonia	2.7	Unmitigated	0%	2083380	1709817	12249	66343	16246
North Macedonia	3	Unmitigated	0%	2083380	1788721	13369	71172	17727

North Macedonia	3.3	Unmitigated	0%	2083380	1848048	14301	75055	18958
Norway	2.4	Enhanced social distancing of elderly	35%	5421242	2386981	8374	63919	11101
Norway	2.7	Enhanced social distancing of elderly	38%	5421242	2635715	9896	72910	13118
Norway	3	Enhanced social distancing of elderly	42%	5421242	2899872	15027	91318	19926
Norway	3.3	Enhanced social distancing of elderly	45%	5421242	3084046	16998	100193	22542
Norway	2.4	Social distancing whole population	36%	5421242	2499382	15162	83266	20100
Norway	2.7	Social distancing whole population	40%	5421242	2754701	17355	94018	23018
Norway	3	Social distancing whole population	43%	5421242	2966391	19301	103331	25591
Norway	3.3	Social distancing whole population	46%	5421242	3145087	21047	111503	27899
Norway	2.4	Unmitigated	0%	5421242	4102236	32507	161345	43095
Norway	2.7	Unmitigated	0%	5421242	4381931	36813	178518	48801
Norway	3	Unmitigated	0%	5421242	4590474	40458	192438	53633
Norway	3.3	Unmitigated	0%	5421242	4749031	43551	203831	57779
Oman	2.4	Enhanced social distancing of elderly	34%	5106622	2679540	5957	61216	7897
Oman	2.7	Enhanced social distancing of elderly	38%	5106622	2906767	6602	66494	8755
Oman	3	Enhanced social distancing of elderly	44%	5106622	3045284	8231	73065	10914
Oman	3.3	Enhanced social distancing of elderly	47%	5106622	3197195	8741	76726	11588
Oman	2.4	Social distancing whole population	37%	5106622	2609203	8006	65761	10614
Oman	2.7	Social distancing whole population	41%	5106622	2839399	8575	70869	11368
Oman	3	Social distancing whole population	44%	5106622	3027265	9021	74936	11959
Oman	3.3	Social distancing whole population	47%	5106622	3183759	9380	78251	12440
Oman	2.4	Unmitigated	0%	5106622	4110313	11235	96226	14895
Oman	2.7	Unmitigated	0%	5106622	4335529	11601	100042	15387
Oman	3	Unmitigated	0%	5106622	4498014	11838	102598	15699
Oman	3.3	Unmitigated	0%	5106622	4618217	11995	104353	15902
Pakistan	2.4	Enhanced social distancing of elderly	34%	220892331	127182117	311039	2393049	412351
Pakistan	2.7	Enhanced social distancing of elderly	38%	220892331	137388032	348647	2621552	462197
Pakistan	3	Enhanced social distancing of elderly	44%	220892331	144578912	454611	3007547	602675
Pakistan	3.3	Enhanced social distancing of elderly	47%	220892331	151219312	486011	3174760	644596
Pakistan	2.4	Social distancing whole population	37%	220892331	125490674	446288	2757638	591653
Pakistan	2.7	Social distancing whole population	41%	220892331	135724442	480063	2974083	636716
Pakistan	3	Social distancing whole population	45%	220892331	143950576	506780	3146638	671962
Pakistan	3.3	Social distancing whole population	48%	220892331	150711132	528435	3287412	700525
Pakistan	2.4	Unmitigated	0%	220892331	191176051	650513	4104506	862397
Pakistan	2.7	Unmitigated	0%	220892331	199792730	674142	4270345	893663
Pakistan	3	Unmitigated	0%	220892331	205687090	689585	4381286	914123
Pakistan	3.3	Unmitigated	0%	220892331	209805136	699875	4457023	927998
Panama	2.4	Enhanced social distancing of elderly	34%	4314768	1963739	5210	38900	6907
Panama	2.7	Enhanced social distancing of elderly	38%	4314768	2181213	6177	44830	8192
Panama	3	Enhanced social distancing of elderly	42%	4314768	2386777	9037	55570	11982
Panama	3.3	Enhanced social distancing of elderly	45%	4314768	2542643	10118	60939	13412
Panama	2.4	Social distancing whole population	36%	4314768	2014224	8742	48925	11589
Panama	2.7	Social distancing whole population	39%	4314768	2230473	9954	55436	13195

Panama	3	Social distancing whole population	43%	4314768	2410892	11011	61064	14596
Panama	3.3	Social distancing whole population	45%	4314768	2563513	11939	65971	15839
Panama	2.4	Unmitigated	0%	4314768	3345065	17275	93407	22902
Panama	2.7	Unmitigated	0%	4314768	3576681	19096	102433	25323
Panama	3	Unmitigated	0%	4314768	3746140	20528	109386	27224
Panama	3.3	Unmitigated	0%	4314768	3872192	21665	114798	28725
Papua New Guinea	2.4	Enhanced social distancing of elderly	34%	8947027	4607261	10171	93758	13484
Papua New Guinea	2.7	Enhanced social distancing of elderly	39%	8947027	5006154	11410	102498	15127
Papua New Guinea	3	Enhanced social distancing of elderly	44%	8947027	5269467	14773	115985	19584
Papua New Guinea	3.3	Enhanced social distancing of elderly	47%	8947027	5532450	15838	122422	21004
Papua New Guinea	2.4	Social distancing whole population	37%	8947027	4540300	14502	106150	19225
Papua New Guinea	2.7	Social distancing whole population	41%	8947027	4933053	15642	114566	20744
Papua New Guinea	3	Social distancing whole population	45%	8947027	5253173	16554	121305	21953
Papua New Guinea	3.3	Social distancing whole population	48%	8947027	5519749	17300	126821	22935
Papua New Guinea	2.4	Unmitigated	0%	8947027	7112682	21438	157444	28420
Papua New Guinea	2.7	Unmitigated	0%	8947027	7494661	22312	163914	29583
Papua New Guinea	3	Unmitigated	0%	8947027	7771815	22898	168256	30354
Papua New Guinea	3.3	Unmitigated	0%	8947027	7978899	23302	171241	30891
Paraguay	2.4	Enhanced social distancing of elderly	34%	7132530	3352898	7512	58955	9959
Paraguay	2.7	Enhanced social distancing of elderly	38%	7132530	3709919	8836	67427	11717
Paraguay	3	Enhanced social distancing of elderly	42%	7132530	4037160	12695	82519	16833
Paraguay	3.3	Enhanced social distancing of elderly	45%	7132530	4289730	14127	90000	18728
Paraguay	2.4	Social distancing whole population	36%	7132530	3418517	12272	72930	16269
Paraguay	2.7	Social distancing whole population	40%	7132530	3772587	13873	82082	18390
Paraguay	3	Social distancing whole population	43%	7132530	4066776	15257	89922	20225
Paraguay	3.3	Social distancing whole population	46%	7132530	4314704	16460	96695	21826
Paraguay	2.4	Unmitigated	0%	7132530	5616426	23426	134962	31057
Paraguay	2.7	Unmitigated	0%	7132530	5986610	25651	146794	34005
Paraguay	3	Unmitigated	0%	7132530	6255529	27361	155729	36289
Paraguay	3.3	Unmitigated	0%	7132530	6454432	28690	162556	38038
Peru	2.4	Enhanced social distancing of elderly	34%	32971846	14662113	39490	299061	52352
Peru	2.7	Enhanced social distancing of elderly	37%	32971846	16339241	46935	345817	62222
Peru	3	Enhanced social distancing of elderly	42%	32971846	17929656	68568	429283	90898
Peru	3.3	Enhanced social distancing of elderly	44%	32971846	19139167	76863	471638	101959
Peru	2.4	Social distancing whole population	36%	32971846	15052813	65987	375795	87480
Peru	2.7	Social distancing whole population	39%	32971846	16720089	75356	427218	99926
Peru	3	Social distancing whole population	42%	32971846	18117089	83548	471828	110802
Peru	3.3	Social distancing whole population	45%	32971846	19301398	90750	510765	120325
Peru	2.4	Unmitigated	0%	32971846	25256719	131010	723335	173683
Peru	2.7	Unmitigated	0%	32971846	27076802	144991	794843	192237
Peru	3	Unmitigated	0%	32971846	28415434	155974	849987	206763
Peru	3.3	Unmitigated	0%	32971846	29415894	164671	892911	218300
Philippines	2.4	Enhanced social distancing of elderly	35%	109581085	57245042	146877	1279294	194718

Philippines	2.7	Enhanced social distancing of elderly	39%	109581085	62329629	167021	1409432	221495
Philippines	3	Enhanced social distancing of elderly	44%	109581085	65955829	229053	1636685	303771
Philippines	3.3	Enhanced social distancing of elderly	47%	109581085	69268641	249176	1740019	330319
Philippines	2.4	Social distancing whole population	37%	109581085	57108933	229877	1513088	304752
Philippines	2.7	Social distancing whole population	41%	109581085	61998748	250699	1642007	332401
Philippines	3	Social distancing whole population	45%	109581085	65974569	267749	1746466	354930
Philippines	3.3	Social distancing whole population	48%	109581085	69275465	281979	1832865	373815
Philippines	2.4	Unmitigated	0%	109581085	88999387	368084	2337772	487975
Philippines	2.7	Unmitigated	0%	109581085	93619970	388198	2450378	514621
Philippines	3	Unmitigated	0%	109581085	96936593	402413	2528309	533703
Philippines	3.3	Unmitigated	0%	109581085	99377919	412633	2583317	547149
Poland	2.4	Enhanced social distancing of elderly	34%	37846605	16549518	67187	497953	89071
Poland	2.7	Enhanced social distancing of elderly	38%	37846605	18253033	78884	565810	104571
Poland	3	Enhanced social distancing of elderly	42%	37846605	20128007	117154	706058	155297
Poland	3.3	Enhanced social distancing of elderly	45%	37846605	21382623	131363	770820	174189
Poland	2.4	Social distancing whole population	36%	37846605	17429472	116997	645670	155105
Poland	2.7	Social distancing whole population	40%	37846605	19179384	132759	724370	175998
Poland	3	Social distancing whole population	43%	37846605	20622292	146540	791660	194370
Poland	3.3	Social distancing whole population	46%	37846605	21833766	158727	850010	210447
Poland	2.4	Unmitigated	0%	37846605	28508229	239472	1209932	317472
Poland	2.7	Unmitigated	0%	37846605	30415867	268746	1329313	356331
Poland	3	Unmitigated	0%	37846605	31838234	293407	1425408	388964
Poland	3.3	Unmitigated	0%	37846605	32922271	314364	1503846	416742
Portugal	2.4	Enhanced social distancing of elderly	34%	10196707	4163001	17755	126577	23539
Portugal	2.7	Enhanced social distancing of elderly	38%	10196707	4616206	21155	145435	28051
Portugal	3	Enhanced social distancing of elderly	42%	10196707	5128287	32959	185654	43708
Portugal	3.3	Enhanced social distancing of elderly	45%	10196707	5473956	37520	204964	49748
Portugal	2.4	Social distancing whole population	35%	10196707	4420365	33548	169929	44475
Portugal	2.7	Social distancing whole population	39%	10196707	4892453	38650	193141	51244
Portugal	3	Social distancing whole population	43%	10196707	5286825	43229	213474	57303
Portugal	3.3	Social distancing whole population	45%	10196707	5621873	47370	231481	62798
Portugal	2.4	Unmitigated	0%	10196707	7383996	74122	339885	98264
Portugal	2.7	Unmitigated	0%	10196707	7931933	84794	379713	112409
Portugal	3	Unmitigated	0%	10196707	8348397	93995	412652	124718
Portugal	3.3	Unmitigated	0%	10196707	8670980	101941	440103	135176
Puerto Rico	2.4	Enhanced social distancing of elderly	33%	2860840	1100676	5434	32085	7203
Puerto Rico	2.7	Enhanced social distancing of elderly	37%	2860840	1226487	6501	37361	8620
Puerto Rico	3	Enhanced social distancing of elderly	41%	2860840	1372697	10111	49254	13404
Puerto Rico	3.3	Enhanced social distancing of elderly	44%	2860840	1470045	11428	54630	15150
Puerto Rico	2.4	Social distancing whole population	36%	2860840	1175043	10100	44933	13390
Puerto Rico	2.7	Social distancing whole population	40%	2860840	1304161	11536	51134	15294
Puerto Rico	3	Social distancing whole population	43%	2860840	1413549	12814	56611	16996
Puerto Rico	3.3	Social distancing whole population	46%	2860840	1507478	13958	61484	18507

Puerto Rico	2.4	Unmitigated	0%	2860840	2013242	21023	90814	27871
Puerto Rico	2.7	Unmitigated	0%	2860840	2174504	23685	101482	31406
Puerto Rico	3	Unmitigated	0%	2860840	2299114	25927	110284	34363
Puerto Rico	3.3	Unmitigated	0%	2860840	2396873	27826	117593	36887
Qatar	2.4	Enhanced social distancing of elderly	33%	2881060	1555155	3514	38549	4658
Qatar	2.7	Enhanced social distancing of elderly	38%	2881060	1682043	3828	41691	5075
Qatar	3	Enhanced social distancing of elderly	43%	2881060	1750990	4341	44499	5756
Qatar	3.3	Enhanced social distancing of elderly	46%	2881060	1839928	4544	46634	6025
Qatar	2.4	Social distancing whole population	36%	2881060	1487654	4025	39069	5336
Qatar	2.7	Social distancing whole population	40%	2881060	1625404	4292	42266	5692
Qatar	3	Social distancing whole population	44%	2881060	1737425	4500	44816	5966
Qatar	3.3	Social distancing whole population	47%	2881060	1830397	4665	46898	6184
Qatar	2.4	Unmitigated	0%	2881060	2353712	5488	57913	7275
Qatar	2.7	Unmitigated	0%	2881060	2485486	5662	60448	7505
Qatar	3	Unmitigated	0%	2881060	2579111	5775	62163	7656
Qatar	3.3	Unmitigated	0%	2881060	2647107	5851	63351	7760
Réunion	2.4	Enhanced social distancing of elderly	33%	895308	385098	1322	9085	1752
Réunion	2.7	Enhanced social distancing of elderly	37%	895308	428787	1576	10539	2090
Réunion	3	Enhanced social distancing of elderly	41%	895308	473287	2346	13351	3110
Réunion	3.3	Enhanced social distancing of elderly	44%	895308	505408	2640	14727	3499
Réunion	2.4	Social distancing whole population	35%	895308	400640	2282	11843	3025
Réunion	2.7	Social distancing whole population	39%	895308	444585	2611	13486	3461
Réunion	3	Social distancing whole population	42%	895308	481423	2901	14920	3847
Réunion	3.3	Social distancing whole population	45%	895308	512706	3158	16183	4188
Réunion	2.4	Unmitigated	0%	895308	671415	4646	23300	6160
Réunion	2.7	Unmitigated	0%	895308	720791	5191	25816	6885
Réunion	3	Unmitigated	0%	895308	757549	5633	27817	7469
Réunion	3.3	Unmitigated	0%	895308	785378	5994	29422	7946
Romania	2.4	Enhanced social distancing of elderly	34%	19237682	8294956	31019	235281	41122
Romania	2.7	Enhanced social distancing of elderly	38%	19237682	9170086	36727	268919	48687
Romania	3	Enhanced social distancing of elderly	42%	19237682	10117561	55857	337431	74096
Romania	3.3	Enhanced social distancing of elderly	45%	19237682	10770753	63271	370772	83910
Romania	2.4	Social distancing whole population	36%	19237682	8721196	56264	306894	74590
Romania	2.7	Social distancing whole population	40%	19237682	9623958	64542	347280	85595
Romania	3	Social distancing whole population	43%	19237682	10373593	71913	382374	95350
Romania	3.3	Social distancing whole population	46%	19237682	11007199	78539	413251	104114
Romania	2.4	Unmitigated	0%	19237682	14381358	121855	601028	161546
Romania	2.7	Unmitigated	0%	19237682	15384200	138419	667008	183485
Romania	3	Unmitigated	0%	19237682	16135653	152495	720800	202160
Romania	3.3	Unmitigated	0%	19237682	16709965	164499	765070	218173
Russian Federation	2.4	Enhanced social distancing of elderly	35%	145934460	66226950	228291	1797989	302649
Russian Federation	2.7	Enhanced social distancing of elderly	39%	145934460	72991925	268388	2043840	356007
Russian Federation	3	Enhanced social distancing of elderly	42%	145934460	79998761	396230	2515695	525224

Russian Federation	3.3	Enhanced social distancing of elderly	45%	145934460	84941832	446036	2749804	591249
Russian Federation	2.4	Social distancing whole population	36%	145934460	68983206	393846	2270383	522128
Russian Federation	2.7	Social distancing whole population	40%	145934460	75888838	449340	2556307	595661
Russian Federation	3	Social distancing whole population	43%	145934460	81594203	498348	2802799	660656
Russian Federation	3.3	Social distancing whole population	46%	145934460	86393354	542034	3017960	718886
Russian Federation	2.4	Unmitigated	0%	145934460	112346935	830636	4338989	1101187
Russian Federation	2.7	Unmitigated	0%	145934460	119703927	935522	4779253	1240684
Russian Federation	3	Unmitigated	0%	145934460	125138559	1023191	5131993	1356678
Russian Federation	3.3	Unmitigated	0%	145934460	129240369	1096920	5418014	1454158
Rwanda	2.4	Enhanced social distancing of elderly	35%	12952209	7272921	13752	117672	18232
Rwanda	2.7	Enhanced social distancing of elderly	39%	12952209	7868261	15395	128914	20407
Rwanda	3	Enhanced social distancing of elderly	44%	12952209	8284478	19745	146441	26179
Rwanda	3.3	Enhanced social distancing of elderly	47%	12952209	8674437	21079	154534	27958
Rwanda	2.4	Social distancing whole population	37%	12952209	7171310	19218	133186	25477
Rwanda	2.7	Social distancing whole population	41%	12952209	7767640	20675	143821	27422
Rwanda	3	Social distancing whole population	45%	12952209	8249089	21829	152330	28942
Rwanda	3.3	Social distancing whole population	48%	12952209	8647106	22767	159304	30181
Rwanda	2.4	Unmitigated	0%	12952209	11024333	27958	199293	37064
Rwanda	2.7	Unmitigated	0%	12952209	11549493	28971	207540	38408
Rwanda	3	Unmitigated	0%	12952209	11914861	29632	213057	39281
Rwanda	3.3	Unmitigated	0%	12952209	12174758	30071	216822	39880
Samoa	2.4	Enhanced social distancing of elderly	33%	198410	99297	243	2096	322
Samoa	2.7	Enhanced social distancing of elderly	37%	198410	108372	276	2313	367
Samoa	3	Enhanced social distancing of elderly	42%	198410	114787	379	2692	502
Samoa	3.3	Enhanced social distancing of elderly	46%	198410	120728	413	2865	547
Samoa	2.4	Social distancing whole population	36%	198410	98952	382	2495	506
Samoa	2.7	Social distancing whole population	40%	198410	107634	417	2711	553
Samoa	3	Social distancing whole population	44%	198410	114732	446	2886	592
Samoa	3.3	Social distancing whole population	47%	198410	120653	471	3032	624
Samoa	2.4	Unmitigated	0%	198410	154205	608	3830	807
Samoa	2.7	Unmitigated	0%	198410	162974	644	4025	853
Samoa	3	Unmitigated	0%	198410	169378	668	4160	886
Samoa	3.3	Unmitigated	0%	198410	174185	686	4256	910
Sao Tome and Principe	2.4	Enhanced social distancing of elderly	34%	219161	123835	233	1955	308
Sao Tome and Principe	2.7	Enhanced social distancing of elderly	38%	219161	133860	260	2139	345
Sao Tome and Principe	3	Enhanced social distancing of elderly	43%	219161	140801	336	2427	445
Sao Tome and Principe	3.3	Enhanced social distancing of elderly	46%	219161	147364	358	2560	475
Sao Tome and Principe	2.4	Social distancing whole population	36%	219161	121969	329	2216	437
Sao Tome and Principe	2.7	Social distancing whole population	40%	219161	132038	354	2390	470
Sao Tome and Principe	3	Social distancing whole population	44%	219161	140157	374	2529	495
Sao Tome and Principe	3.3	Social distancing whole population	47%	219161	146848	389	2642	516
Sao Tome and Principe	2.4	Unmitigated	0%	219161	185340	475	3270	629
Sao Tome and Principe	2.7	Unmitigated	0%	219161	194306	493	3407	653

Sao Tome and Principe	3	Unmitigated	0%	219161	200527	504	3498	669
Sao Tome and Principe	3.3	Unmitigated	0%	219161	204948	512	3561	679
Saudi Arabia	2.4	Enhanced social distancing of elderly	34%	34813867	19142445	49402	473406	65493
Saudi Arabia	2.7	Enhanced social distancing of elderly	38%	34813867	20714046	55131	516409	73105
Saudi Arabia	3	Enhanced social distancing of elderly	44%	34813867	21597867	69558	572244	92243
Saudi Arabia	3.3	Enhanced social distancing of elderly	47%	34813867	22616329	74148	602292	98300
Saudi Arabia	2.4	Social distancing whole population	37%	34813867	18631136	67619	516635	89644
Saudi Arabia	2.7	Social distancing whole population	41%	34813867	20199925	72643	557503	96303
Saudi Arabia	3	Social distancing whole population	45%	34813867	21469300	76610	590131	101556
Saudi Arabia	3.3	Social distancing whole population	48%	34813867	22518627	79819	616772	105832
Saudi Arabia	2.4	Unmitigated	0%	34813867	28794084	97344	767531	129050
Saudi Arabia	2.7	Unmitigated	0%	34813867	30221250	100778	798710	133598
Saudi Arabia	3	Unmitigated	0%	34813867	31231008	103014	819547	136616
Saudi Arabia	3.3	Unmitigated	0%	34813867	31965343	104503	833784	138570
Senegal	2.4	Enhanced social distancing of elderly	35%	16743930	9346706	16923	142972	22436
Senegal	2.7	Enhanced social distancing of elderly	39%	16743930	10112225	18961	156512	25137
Senegal	3	Enhanced social distancing of elderly	44%	16743930	10649943	24626	178655	32645
Senegal	3.3	Enhanced social distancing of elderly	47%	16743930	11152600	26314	188481	34899
Senegal	2.4	Social distancing whole population	37%	16743930	9218693	24172	163493	32045
Senegal	2.7	Social distancing whole population	41%	16743930	9986081	25987	176305	34456
Senegal	3	Social distancing whole population	45%	16743930	10605746	27421	186512	36369
Senegal	3.3	Social distancing whole population	48%	16743930	11117131	28583	194836	37897
Senegal	2.4	Unmitigated	0%	16743930	14193492	35013	242261	46418
Senegal	2.7	Unmitigated	0%	16743930	14876464	36259	251871	48076
Senegal	3	Unmitigated	0%	16743930	15353470	37069	258250	49140
Senegal	3.3	Unmitigated	0%	16743930	15694281	37607	262581	49854
Serbia	2.4	Enhanced social distancing of elderly	34%	8737370	3775929	13639	104483	18081
Serbia	2.7	Enhanced social distancing of elderly	38%	8737370	4174276	16135	119372	21400
Serbia	3	Enhanced social distancing of elderly	42%	8737370	4604747	24461	149904	32441
Serbia	3.3	Enhanced social distancing of elderly	45%	8737370	4901710	27687	164675	36701
Serbia	2.4	Social distancing whole population	36%	8737370	3968637	24624	136594	32645
Serbia	2.7	Social distancing whole population	40%	8737370	4379559	28234	154515	37435
Serbia	3	Social distancing whole population	43%	8737370	4720640	31444	170072	41683
Serbia	3.3	Social distancing whole population	46%	8737370	5008841	34324	183741	45503
Serbia	2.4	Unmitigated	0%	8737370	6540141	53057	266554	70339
Serbia	2.7	Unmitigated	0%	8737370	6995828	60194	295617	79798
Serbia	3	Unmitigated	0%	8737370	7336836	66243	319271	87859
Serbia	3.3	Unmitigated	0%	8737370	7597409	71386	338715	94650
Seychelles	2.4	Enhanced social distancing of elderly	31%	98340	51673	181	1336	240
Seychelles	2.7	Enhanced social distancing of elderly	35%	98340	56327	207	1493	275
Seychelles	3	Enhanced social distancing of elderly	41%	98340	60295	285	1770	378
Seychelles	3.3	Enhanced social distancing of elderly	44%	98340	63446	311	1899	412
Seychelles	2.4	Social distancing whole population	35%	98340	52184	285	1616	377

Seychelles	2.7	Social distancing whole population	39%	98340	56785	313	1775	415
Seychelles	3	Social distancing whole population	42%	98340	60530	336	1906	446
Seychelles	3.3	Social distancing whole population	45%	98340	63639	356	2017	472
Seychelles	2.4	Unmitigated	0%	98340	80060	467	2635	619
Seychelles	2.7	Unmitigated	0%	98340	84564	500	2817	663
Seychelles	3	Unmitigated	0%	98340	87753	525	2951	696
Seychelles	3.3	Unmitigated	0%	98340	90060	543	3051	720
Sierra Leone	2.4	Enhanced social distancing of elderly	35%	7976985	4479692	8063	69551	10690
Sierra Leone	2.7	Enhanced social distancing of elderly	39%	7976985	4845098	9018	76093	11956
Sierra Leone	3	Enhanced social distancing of elderly	44%	7976985	5097756	11588	86331	15369
Sierra Leone	3.3	Enhanced social distancing of elderly	47%	7976985	5337322	12361	91015	16389
Sierra Leone	2.4	Social distancing whole population	37%	7976985	4411647	11319	78666	15006
Sierra Leone	2.7	Social distancing whole population	41%	7976985	4778405	12159	84838	16121
Sierra Leone	3	Social distancing whole population	45%	7976985	5074518	12820	89758	16995
Sierra Leone	3.3	Social distancing whole population	48%	7976985	5318627	13355	93770	17704
Sierra Leone	2.4	Unmitigated	0%	7976985	6783413	16287	116661	21593
Sierra Leone	2.7	Unmitigated	0%	7976985	7107506	16849	121326	22336
Sierra Leone	3	Unmitigated	0%	7976985	7333102	17212	124431	22827
Sierra Leone	3.3	Unmitigated	0%	7976985	7493801	17452	126546	23144
Singapore	2.4	Enhanced social distancing of elderly	29%	5850343	3444924	20416	135380	27066
Singapore	2.7	Enhanced social distancing of elderly	33%	5850343	3733411	23117	149003	30651
Singapore	3	Enhanced social distancing of elderly	42%	5850343	3826572	30834	170352	40875
Singapore	3.3	Enhanced social distancing of elderly	46%	5850343	3999606	33228	180537	44050
Singapore	2.4	Social distancing whole population	37%	5850343	3324726	31059	158855	41176
Singapore	2.7	Social distancing whole population	41%	5850343	3595474	33485	171543	44391
Singapore	3	Social distancing whole population	45%	5850343	3813061	35423	181712	46979
Singapore	3.3	Social distancing whole population	48%	5850343	3991920	37007	190050	49069
Singapore	2.4	Unmitigated	0%	5850343	5061213	46333	239617	61424
Singapore	2.7	Unmitigated	0%	5850343	5289119	48291	250136	64042
Singapore	3	Unmitigated	0%	5850343	5445127	49628	257342	65783
Singapore	3.3	Unmitigated	0%	5850343	5554108	50565	262391	67028
Slovakia	2.4	Enhanced social distancing of elderly	34%	5459643	2418010	8451	67508	11204
Slovakia	2.7	Enhanced social distancing of elderly	38%	5459643	2672775	9967	76994	13213
Slovakia	3	Enhanced social distancing of elderly	42%	5459643	2939532	14787	95255	19611
Slovakia	3.3	Enhanced social distancing of elderly	45%	5459643	3126860	16682	104352	22119
Slovakia	2.4	Social distancing whole population	36%	5459643	2526936	14719	85915	19513
Slovakia	2.7	Social distancing whole population	39%	5459643	2788087	16854	97079	22348
Slovakia	3	Social distancing whole population	43%	5459643	3004262	18745	106725	24850
Slovakia	3.3	Social distancing whole population	46%	5459643	3186322	20433	115156	27087
Slovakia	2.4	Unmitigated	0%	5459643	4146758	31253	165487	41432
Slovakia	2.7	Unmitigated	0%	5459643	4429913	35313	182848	46811
Slovakia	3	Unmitigated	0%	5459643	4639999	38720	196815	51344
Slovakia	3.3	Unmitigated	0%	5459643	4799183	41587	208165	55154

Slovenia	2.4	Enhanced social distancing of elderly	34%	2078932	877684	3522	26000	4669
Slovenia	2.7	Enhanced social distancing of elderly	38%	2078932	971040	4178	29775	5540
Slovenia	3	Enhanced social distancing of elderly	42%	2078932	1074093	6385	37555	8464
Slovenia	3.3	Enhanced social distancing of elderly	45%	2078932	1144423	7243	41337	9602
Slovenia	2.4	Social distancing whole population	36%	2078932	926602	6444	34200	8543
Slovenia	2.7	Social distancing whole population	39%	2078932	1023272	7403	38763	9813
Slovenia	3	Social distancing whole population	43%	2078932	1103742	8259	42742	10957
Slovenia	3.3	Social distancing whole population	46%	2078932	1171917	9031	46254	11976
Slovenia	2.4	Unmitigated	0%	2078932	1532988	14051	67526	18628
Slovenia	2.7	Unmitigated	0%	2078932	1642457	16006	75147	21226
Slovenia	3	Unmitigated	0%	2078932	1724921	17676	81396	23436
Slovenia	3.3	Unmitigated	0%	2078932	1788413	19109	86569	25332
Solomon Islands	2.4	Enhanced social distancing of elderly	34%	686878	341356	725	6551	961
Solomon Islands	2.7	Enhanced social distancing of elderly	38%	686878	371755	817	7169	1084
Solomon Islands	3	Enhanced social distancing of elderly	43%	686878	392807	1096	8207	1454
Solomon Islands	3.3	Enhanced social distancing of elderly	46%	686878	412959	1183	8679	1569
Solomon Islands	2.4	Social distancing whole population	37%	686878	337922	1097	7580	1455
Solomon Islands	2.7	Social distancing whole population	41%	686878	367611	1188	8185	1576
Solomon Islands	3	Social distancing whole population	44%	686878	391905	1262	8671	1673
Solomon Islands	3.3	Social distancing whole population	47%	686878	412204	1323	9069	1753
Solomon Islands	2.4	Unmitigated	0%	686878	531839	1659	11223	2200
Solomon Islands	2.7	Unmitigated	0%	686878	561888	1735	11691	2300
Solomon Islands	3	Unmitigated	0%	686878	583957	1786	12005	2368
Solomon Islands	3.3	Unmitigated	0%	686878	600656	1822	12220	2417
Somalia	2.4	Enhanced social distancing of elderly	35%	15893219	8765617	15088	125238	20003
Somalia	2.7	Enhanced social distancing of elderly	39%	15893219	9486289	16885	136922	22391
Somalia	3	Enhanced social distancing of elderly	44%	15893219	9989817	21873	156390	28997
Somalia	3.3	Enhanced social distancing of elderly	47%	15893219	10464211	23342	164828	30944
Somalia	2.4	Social distancing whole population	37%	15893219	8638770	21478	143626	28474
Somalia	2.7	Social distancing whole population	41%	15893219	9361531	23057	154622	30565
Somalia	3	Social distancing whole population	45%	15893219	9945981	24300	163348	32220
Somalia	3.3	Social distancing whole population	48%	15893219	10429027	25304	170439	33561
Somalia	2.4	Unmitigated	0%	15893219	13349990	30769	210196	40791
Somalia	2.7	Unmitigated	0%	15893219	14007167	31803	218080	42182
Somalia	3	Unmitigated	0%	15893219	14470843	32465	223260	43044
Somalia	3.3	Unmitigated	0%	15893219	14804896	32900	226740	43613
South Africa	2.4	Enhanced social distancing of elderly	34%	59308690	31291674	80866	718401	107205
South Africa	2.7	Enhanced social distancing of elderly	38%	59308690	34044701	91741	790442	121612
South Africa	3	Enhanced social distancing of elderly	44%	59308690	35976825	125010	914191	165794
South Africa	3.3	Enhanced social distancing of elderly	47%	59308690	37763953	135726	970739	179998
South Africa	2.4	Social distancing whole population	37%	59308690	31172277	125367	844522	166202
South Africa	2.7	Social distancing whole population	41%	59308690	33824271	136474	915581	181000
South Africa	3	Social distancing whole population	45%	59308690	35977239	145536	973006	192965

South Africa	3.3	Social distancing whole population	48%	59308690	37762240	153073	1020390	202915
South Africa	2.4	Unmitigated	0%	59308690	48428245	198365	1296214	262976
South Africa	2.7	Unmitigated	0%	59308690	50901854	208765	1356824	276748
South Africa	3	Unmitigated	0%	59308690	52669882	216064	1398489	286429
South Africa	3.3	Unmitigated	0%	59308690	53966779	221280	1427734	293482
South Sudan	2.4	Enhanced social distancing of elderly	35%	11193729	6247911	11823	97844	15675
South Sudan	2.7	Enhanced social distancing of elderly	39%	11193729	6760918	13278	107254	17610
South Sudan	3	Enhanced social distancing of elderly	44%	11193729	7125228	17410	123028	23081
South Sudan	3.3	Enhanced social distancing of elderly	47%	11193729	7462147	18646	129954	24718
South Sudan	2.4	Social distancing whole population	37%	11193729	6170011	17136	112753	22718
South Sudan	2.7	Social distancing whole population	41%	11193729	6683529	18457	121701	24467
South Sudan	3	Social distancing whole population	45%	11193729	7098145	19505	128848	25858
South Sudan	3.3	Social distancing whole population	48%	11193729	7440299	20357	134683	27001
South Sudan	2.4	Unmitigated	0%	11193729	9496364	25154	168191	33347
South Sudan	2.7	Unmitigated	0%	11193729	9952811	26111	175071	34631
South Sudan	3	Unmitigated	0%	11193729	10270978	26740	179661	35457
South Sudan	3.3	Unmitigated	0%	11193729	10498447	27165	182797	36011
Spain	2.4	Enhanced social distancing of elderly	34%	46754783	19864177	79686	590214	105642
Spain	2.7	Enhanced social distancing of elderly	38%	46754783	21983449	94593	675858	125387
Spain	3	Enhanced social distancing of elderly	42%	46754783	24298814	145272	850622	192643
Spain	3.3	Enhanced social distancing of elderly	45%	46754783	25889670	164891	935964	218664
Spain	2.4	Social distancing whole population	36%	46754783	20931838	146917	773066	194771
Spain	2.7	Social distancing whole population	39%	46754783	23121326	168820	876204	223892
Spain	3	Social distancing whole population	43%	46754783	24944376	188406	966176	249799
Spain	3.3	Social distancing whole population	46%	46754783	26485799	206023	1045369	273104
Spain	2.4	Unmitigated	0%	46754783	34661271	321029	1525437	425593
Spain	2.7	Unmitigated	0%	46754783	37124816	365726	1696355	484817
Spain	3	Unmitigated	0%	46754783	38976531	403936	1836140	535484
Spain	3.3	Unmitigated	0%	46754783	40397739	436748	1951666	579507
Sri Lanka	2.4	Enhanced social distancing of elderly	34%	21413250	11534644	45476	310940	60288
Sri Lanka	2.7	Enhanced social distancing of elderly	38%	21413250	12533868	52046	346726	69030
Sri Lanka	3	Enhanced social distancing of elderly	43%	21413250	13495663	72933	420635	96698
Sri Lanka	3.3	Enhanced social distancing of elderly	47%	21413250	14180070	79657	451374	105594
Sri Lanka	2.4	Social distancing whole population	37%	21413250	11823806	72564	389520	96199
Sri Lanka	2.7	Social distancing whole population	41%	21413250	12820209	79640	426373	105570
Sri Lanka	3	Social distancing whole population	45%	21413250	13625238	85517	456784	113368
Sri Lanka	3.3	Social distancing whole population	48%	21413250	14289447	90483	482345	120012
Sri Lanka	2.4	Unmitigated	0%	21413250	18231448	122991	645725	163052
Sri Lanka	2.7	Unmitigated	0%	21413250	19111269	131325	686202	174197
Sri Lanka	3	Unmitigated	0%	21413250	19722525	137522	715783	182371
Sri Lanka	3.3	Unmitigated	0%	21413250	20156511	142204	737776	188502
St. Lucia	2.4	Enhanced social distancing of elderly	32%	183629	76710	243	1783	322
St. Lucia	2.7	Enhanced social distancing of elderly	35%	183629	86065	291	2079	386

St. Lucia	3	Enhanced social distancing of elderly	40%	183629	95138	428	2601	568
St. Lucia	3.3	Enhanced social distancing of elderly	43%	183629	101989	483	2872	640
St. Lucia	2.4	Social distancing whole population	34%	183629	79242	415	2271	550
St. Lucia	2.7	Social distancing whole population	38%	183629	88580	478	2601	633
St. Lucia	3	Social distancing whole population	41%	183629	96456	533	2890	706
St. Lucia	3.3	Social distancing whole population	44%	183629	103164	581	3143	771
St. Lucia	2.4	Unmitigated	0%	183629	133686	825	4387	1093
St. Lucia	2.7	Unmitigated	0%	183629	144595	923	4873	1224
St. Lucia	3	Unmitigated	0%	183629	152750	1001	5255	1328
St. Lucia	3.3	Unmitigated	0%	183629	158940	1064	5557	1411
St. Vincent and the Grenad	2.4	Enhanced social distancing of elderly	31%	110947	48619	145	1065	193
St. Vincent and the Grenad	2.7	Enhanced social distancing of elderly	35%	110947	54197	173	1234	230
St. Vincent and the Grenad	3	Enhanced social distancing of elderly	40%	110947	59589	254	1538	336
St. Vincent and the Grenad	3.3	Enhanced social distancing of elderly	42%	110947	63633	285	1693	378
St. Vincent and the Grenad	2.4	Social distancing whole population	33%	110947	50158	248	1356	329
St. Vincent and the Grenad	2.7	Social distancing whole population	37%	110947	55729	284	1545	376
St. Vincent and the Grenad	3	Social distancing whole population	41%	110947	60394	315	1709	418
St. Vincent and the Grenad	3.3	Social distancing whole population	43%	110947	64349	343	1853	454
St. Vincent and the Grenad	2.4	Unmitigated	0%	110947	82282	483	2563	640
St. Vincent and the Grenad	2.7	Unmitigated	0%	110947	88608	539	2840	714
St. Vincent and the Grenad	3	Unmitigated	0%	110947	93286	584	3057	774
St. Vincent and the Grenad	3.3	Unmitigated	0%	110947	96807	620	3228	822
State of Palestine	2.4	Enhanced social distancing of elderly	35%	5101416	2541890	5152	47621	6830
State of Palestine	2.7	Enhanced social distancing of elderly	39%	5101416	2767645	5784	51983	7668
State of Palestine	3	Enhanced social distancing of elderly	44%	5101416	2923006	7632	59026	10117
State of Palestine	3.3	Enhanced social distancing of elderly	47%	5101416	3073466	8202	62281	10878
State of Palestine	2.4	Social distancing whole population	37%	5101416	2510291	7586	54301	10056
State of Palestine	2.7	Social distancing whole population	41%	5101416	2732437	8189	58550	10861
State of Palestine	3	Social distancing whole population	44%	5101416	2914387	8672	61941	11500
State of Palestine	3.3	Social distancing whole population	47%	5101416	3066500	9068	64711	12022
State of Palestine	2.4	Unmitigated	0%	5101416	3971129	11219	79600	14873
State of Palestine	2.7	Unmitigated	0%	5101416	4195982	11678	82728	15481
State of Palestine	3	Unmitigated	0%	5101416	4361208	11985	84811	15887
State of Palestine	3.3	Unmitigated	0%	5101416	4486024	12195	86230	16169
Sudan	2.4	Enhanced social distancing of elderly	35%	43849269	24557466	48915	400220	64847
Sudan	2.7	Enhanced social distancing of elderly	39%	43849269	26576890	55022	439360	72972
Sudan	3	Enhanced social distancing of elderly	44%	43849269	28034357	72451	505769	96062
Sudan	3.3	Enhanced social distancing of elderly	47%	43849269	29358151	77708	534849	103012
Sudan	2.4	Social distancing whole population	37%	43849269	24300593	71299	463489	94523
Sudan	2.7	Social distancing whole population	41%	43849269	26316648	76908	500846	101956
Sudan	3	Social distancing whole population	45%	43849269	27942986	81377	530752	107879
Sudan	3.3	Social distancing whole population	48%	43849269	29284078	85022	555242	112765
Sudan	2.4	Unmitigated	0%	43849269	37337141	105847	697445	140324

Sudan	2.7	Unmitigated	0%	43849269	39111081	110065	726967	145938
Sudan	3	Unmitigated	0%	43849269	40344827	112869	746806	149694
Sudan	3.3	Unmitigated	0%	43849269	41222237	114773	760435	152175
Suriname	2.4	Enhanced social distancing of elderly	34%	586634	270682	674	5292	893
Suriname	2.7	Enhanced social distancing of elderly	37%	586634	300349	795	6084	1054
Suriname	3	Enhanced social distancing of elderly	42%	586634	327660	1132	7433	1501
Suriname	3.3	Enhanced social distancing of elderly	45%	586634	348738	1262	8130	1674
Suriname	2.4	Social distancing whole population	35%	586634	276394	1084	6498	1437
Suriname	2.7	Social distancing whole population	39%	586634	305767	1231	7349	1633
Suriname	3	Social distancing whole population	42%	586634	330243	1358	8082	1801
Suriname	3.3	Social distancing whole population	45%	586634	350916	1469	8720	1947
Suriname	2.4	Unmitigated	0%	586634	455685	2090	12223	2771
Suriname	2.7	Unmitigated	0%	586634	487018	2299	13375	3049
Suriname	3	Unmitigated	0%	586634	509878	2461	14255	3262
Suriname	3.3	Unmitigated	0%	586634	526851	2587	14934	3429
Sweden	2.4	Enhanced social distancing of elderly	35%	10099270	4307489	16192	117620	21466
Sweden	2.7	Enhanced social distancing of elderly	38%	10099270	4758792	19210	134492	25475
Sweden	3	Enhanced social distancing of elderly	42%	10099270	5255468	29840	171191	39567
Sweden	3.3	Enhanced social distancing of elderly	45%	10099270	5594824	33878	188432	44911
Sweden	2.4	Social distancing whole population	36%	10099270	4541163	30434	157644	40347
Sweden	2.7	Social distancing whole population	40%	10099270	5007447	34895	178279	46253
Sweden	3	Social distancing whole population	43%	10099270	5395457	38880	196261	51540
Sweden	3.3	Social distancing whole population	46%	10099270	5724141	42473	212131	56324
Sweden	2.4	Unmitigated	0%	10099270	7497281	66393	310257	88019
Sweden	2.7	Unmitigated	0%	10099270	8022344	75560	344765	100170
Sweden	3	Unmitigated	0%	10099270	8417908	83410	373102	110628
Sweden	3.3	Unmitigated	0%	10099270	8722200	90157	396602	119531
Switzerland	2.4	Enhanced social distancing of elderly	34%	8654618	3681355	14271	106501	18920
Switzerland	2.7	Enhanced social distancing of elderly	38%	8654618	4077625	16934	121985	22449
Switzerland	3	Enhanced social distancing of elderly	42%	8654618	4505586	25861	153399	34282
Switzerland	3.3	Enhanced social distancing of elderly	45%	8654618	4802006	29328	168749	38906
Switzerland	2.4	Social distancing whole population	36%	8654618	3872549	26090	139341	34588
Switzerland	2.7	Social distancing whole population	39%	8654618	4280988	29978	157960	39754
Switzerland	3	Social distancing whole population	43%	8654618	4620978	33449	174172	44357
Switzerland	3.3	Social distancing whole population	46%	8654618	4908756	36569	188440	48486
Switzerland	2.4	Unmitigated	0%	8654618	6421334	56606	273707	75043
Switzerland	2.7	Unmitigated	0%	8654618	6881012	64391	304136	85372
Switzerland	3	Unmitigated	0%	8654618	7226229	71022	328951	94146
Switzerland	3.3	Unmitigated	0%	8654618	7490866	76691	349391	101668
Syrian Arab Republic	2.4	Enhanced social distancing of elderly	34%	17500657	9735310	23396	188764	31017
Syrian Arab Republic	2.7	Enhanced social distancing of elderly	39%	17500657	10558309	26500	208542	35144
Syrian Arab Republic	3	Enhanced social distancing of elderly	44%	17500657	11183975	35504	242265	47085
Syrian Arab Republic	3.3	Enhanced social distancing of elderly	47%	17500657	11725947	38325	257459	50806

Syrian Arab Republic	2.4	Social distancing whole population	37%	17500657	9690495	34945	220743	46327
Syrian Arab Republic	2.7	Social distancing whole population	41%	17500657	10507497	37956	239997	50325
Syrian Arab Republic	3	Social distancing whole population	45%	17500657	11167284	40393	255612	53546
Syrian Arab Republic	3.3	Social distancing whole population	48%	17500657	11711660	42407	268536	56218
Syrian Arab Republic	2.4	Unmitigated	0%	17500657	14932320	54349	345683	72052
Syrian Arab Republic	2.7	Unmitigated	0%	17500657	15649697	57006	362980	75572
Syrian Arab Republic	3	Unmitigated	0%	17500657	16146717	58843	374963	78040
Syrian Arab Republic	3.3	Unmitigated	0%	17500657	16498575	60138	383435	79742
Tajikistan	2.4	Enhanced social distancing of elderly	36%	9537642	4949797	8802	84554	11668
Tajikistan	2.7	Enhanced social distancing of elderly	40%	9537642	5395419	9983	93484	13235
Tajikistan	3	Enhanced social distancing of elderly	44%	9537642	5756596	13051	106834	17301
Tajikistan	3.3	Enhanced social distancing of elderly	47%	9537642	6057423	14153	113728	18765
Tajikistan	2.4	Social distancing whole population	37%	9537642	4960133	12535	95541	16618
Tajikistan	2.7	Social distancing whole population	41%	9537642	5401106	13764	104528	18246
Tajikistan	3	Social distancing whole population	44%	9537642	5760903	14778	111895	19600
Tajikistan	3.3	Social distancing whole population	47%	9537642	6060482	15631	118052	20726
Tajikistan	2.4	Unmitigated	0%	9537642	7812224	20747	154177	27504
Tajikistan	2.7	Unmitigated	0%	9537642	8239180	22014	162895	29192
Tajikistan	3	Unmitigated	0%	9537642	8545412	22922	169069	30389
Tajikistan	3.3	Unmitigated	0%	9537642	8770121	23582	173526	31262
Tanzania	2.4	Enhanced social distancing of elderly	35%	59734213	33309296	56897	496047	75430
Tanzania	2.7	Enhanced social distancing of elderly	39%	59734213	36025153	63483	541856	84159
Tanzania	3	Enhanced social distancing of elderly	44%	59734213	37877622	80872	612171	107210
Tanzania	3.3	Enhanced social distancing of elderly	47%	59734213	39661732	86070	644539	114149
Tanzania	2.4	Social distancing whole population	37%	59734213	32747679	78830	557620	104506
Tanzania	2.7	Social distancing whole population	41%	59734213	35480304	84514	600634	112082
Tanzania	3	Social distancing whole population	45%	59734213	37688634	88973	634823	117993
Tanzania	3.3	Social distancing whole population	48%	59734213	39511620	92562	662650	122711
Tanzania	2.4	Unmitigated	0%	59734213	50494476	111893	819796	148339
Tanzania	2.7	Unmitigated	0%	59734213	52943442	115495	851347	153133
Tanzania	3	Unmitigated	0%	59734213	54658902	117792	872227	156149
Tanzania	3.3	Unmitigated	0%	59734213	55888850	119292	886364	158144
Thailand	2.4	Enhanced social distancing of elderly	33%	69799978	35596518	140324	1100680	186030
Thailand	2.7	Enhanced social distancing of elderly	37%	69799978	39233429	164778	1245629	218479
Thailand	3	Enhanced social distancing of elderly	42%	69799978	41902758	241551	1503923	320354
Thailand	3.3	Enhanced social distancing of elderly	45%	69799978	44231173	269518	1628162	357354
Thailand	2.4	Social distancing whole population	37%	69799978	36259189	245967	1389596	326082
Thailand	2.7	Social distancing whole population	41%	69799978	39514303	274808	1535310	364362
Thailand	3	Social distancing whole population	44%	69799978	42172829	299442	1657407	396945
Thailand	3.3	Social distancing whole population	47%	69799978	44388074	320778	1761419	425246
Thailand	2.4	Unmitigated	0%	69799978	57067601	459500	2401245	609166
Thailand	2.7	Unmitigated	0%	69799978	60175218	498933	2571818	661410
Thailand	3	Unmitigated	0%	69799978	62393830	528827	2697601	701545

Thailand	3.3	Unmitigated	0%	69799978	64013943	551759	2791843	731689
Timor-Leste	2.4	Enhanced social distancing of elderly	35%	1318442	649481	1417	12529	1879
Timor-Leste	2.7	Enhanced social distancing of elderly	39%	1318442	708557	1607	13764	2131
Timor-Leste	3	Enhanced social distancing of elderly	43%	1318442	751750	2193	15947	2907
Timor-Leste	3.3	Enhanced social distancing of elderly	47%	1318442	791059	2381	16928	3157
Timor-Leste	2.4	Social distancing whole population	37%	1318442	646976	2201	14764	2918
Timor-Leste	2.7	Social distancing whole population	41%	1318442	704370	2398	16000	3179
Timor-Leste	3	Social distancing whole population	44%	1318442	751385	2559	16998	3394
Timor-Leste	3.3	Social distancing whole population	47%	1318442	790674	2694	17823	3572
Timor-Leste	2.4	Unmitigated	0%	1318442	1023330	3474	22435	4606
Timor-Leste	2.7	Unmitigated	0%	1318442	1081708	3661	23481	4854
Timor-Leste	3	Unmitigated	0%	1318442	1124613	3792	24203	5027
Timor-Leste	3.3	Unmitigated	0%	1318442	1157025	3887	24712	5152
Togo	2.4	Enhanced social distancing of elderly	35%	8278737	4650754	8378	73291	11107
Togo	2.7	Enhanced social distancing of elderly	39%	8278737	5029955	9360	80191	12409
Togo	3	Enhanced social distancing of elderly	44%	8278737	5292220	11935	90787	15830
Togo	3.3	Enhanced social distancing of elderly	47%	8278737	5540638	12718	95704	16863
Togo	2.4	Social distancing whole population	37%	8278737	4580380	11609	82550	15390
Togo	2.7	Social distancing whole population	41%	8278737	4960842	12467	89052	16531
Togo	3	Social distancing whole population	45%	8278737	5267947	13143	94241	17424
Togo	3.3	Social distancing whole population	48%	8278737	5521902	13691	98488	18149
Togo	2.4	Unmitigated	0%	8278737	7040719	16675	122713	22107
Togo	2.7	Unmitigated	0%	8278737	7376583	17243	127669	22858
Togo	3	Unmitigated	0%	8278737	7610354	17609	130973	23347
Togo	3.3	Unmitigated	0%	8278737	7776849	17851	133228	23675
Tonga	2.4	Enhanced social distancing of elderly	32%	105697	53422	131	1121	174
Tonga	2.7	Enhanced social distancing of elderly	37%	105697	58312	150	1239	199
Tonga	3	Enhanced social distancing of elderly	42%	105697	61957	211	1457	280
Tonga	3.3	Enhanced social distancing of elderly	45%	105697	65176	231	1554	307
Tonga	2.4	Social distancing whole population	35%	105697	53565	218	1365	289
Tonga	2.7	Social distancing whole population	39%	105697	58245	239	1485	316
Tonga	3	Social distancing whole population	43%	105697	62065	256	1583	340
Tonga	3.3	Social distancing whole population	46%	105697	65250	271	1665	359
Tonga	2.4	Unmitigated	0%	105697	82414	353	2098	468
Tonga	2.7	Unmitigated	0%	105697	87133	376	2213	499
Tonga	3	Unmitigated	0%	105697	90559	393	2294	521
Tonga	3.3	Unmitigated	0%	105697	93110	406	2352	538
Trinidad and Tobago	2.4	Enhanced social distancing of elderly	33%	1399491	587461	1924	13994	2551
Trinidad and Tobago	2.7	Enhanced social distancing of elderly	37%	1399491	657411	2299	16292	3048
Trinidad and Tobago	3	Enhanced social distancing of elderly	41%	1399491	727097	3381	20493	4485
Trinidad and Tobago	3.3	Enhanced social distancing of elderly	44%	1399491	778609	3806	22630	5047
Trinidad and Tobago	2.4	Social distancing whole population	35%	1399491	609219	3247	17923	4305
Trinidad and Tobago	2.7	Social distancing whole population	39%	1399491	679295	3729	20496	4945

Trinidad and Tobago	3	Social distancing whole population	42%	1399491	738331	4154	22748	5506
Trinidad and Tobago	3.3	Social distancing whole population	45%	1399491	788623	4530	24729	6005
Trinidad and Tobago	2.4	Unmitigated	0%	1399491	1036163	6603	35407	8754
Trinidad and Tobago	2.7	Unmitigated	0%	1399491	1116507	7373	39264	9774
Trinidad and Tobago	3	Unmitigated	0%	1399491	1176562	7991	42309	10594
Trinidad and Tobago	3.3	Unmitigated	0%	1399491	1222155	8491	44733	11262
Tunisia	2.4	Enhanced social distancing of elderly	33%	11818618	6211292	21722	156289	28797
Tunisia	2.7	Enhanced social distancing of elderly	38%	11818618	6772771	24936	174749	33067
Tunisia	3	Enhanced social distancing of elderly	43%	11818618	7266601	34889	209949	46252
Tunisia	3.3	Enhanced social distancing of elderly	46%	11818618	7648406	38133	225414	50550
Tunisia	2.4	Social distancing whole population	37%	11818618	6295653	34649	192139	45934
Tunisia	2.7	Social distancing whole population	41%	11818618	6851559	38080	210913	50479
Tunisia	3	Social distancing whole population	44%	11818618	7303778	40930	226458	54274
Tunisia	3.3	Social distancing whole population	47%	11818618	7678951	43335	239544	57475
Tunisia	2.4	Unmitigated	0%	11818618	9872029	58391	320475	77410
Tunisia	2.7	Unmitigated	0%	11818618	10391562	62297	341108	82625
Tunisia	3	Unmitigated	0%	11818618	10757797	65172	356157	86409
Tunisia	3.3	Unmitigated	0%	11818618	11021077	67323	367307	89245
Turkey	2.4	Enhanced social distancing of elderly	35%	84339067	41868971	105639	923654	140048
Turkey	2.7	Enhanced social distancing of elderly	39%	84339067	45899160	122587	1037988	162504
Turkey	3	Enhanced social distancing of elderly	43%	84339067	49685992	174771	1243144	231803
Turkey	3.3	Enhanced social distancing of elderly	46%	84339067	52506223	194412	1344763	257833
Turkey	2.4	Social distancing whole population	36%	84339067	42866201	172668	1120700	228909
Turkey	2.7	Social distancing whole population	40%	84339067	46908616	194416	1247429	257833
Turkey	3	Social distancing whole population	44%	84339067	50215234	213192	1354386	282672
Turkey	3.3	Social distancing whole population	47%	84339067	52971184	229580	1445939	304340
Turkey	2.4	Unmitigated	0%	84339067	68257784	337078	2004656	446870
Turkey	2.7	Unmitigated	0%	84339067	72224085	371697	2169037	492723
Turkey	3	Unmitigated	0%	84339067	75070796	399369	2294729	529432
Turkey	3.3	Unmitigated	0%	84339067	77162280	421731	2392431	559340
Turkmenistan	2.4	Enhanced social distancing of elderly	35%	6031187	3121639	6467	60448	8574
Turkmenistan	2.7	Enhanced social distancing of elderly	39%	6031187	3409144	7400	67269	9815
Turkmenistan	3	Enhanced social distancing of elderly	43%	6031187	3654644	9960	78016	13203
Turkmenistan	3.3	Enhanced social distancing of elderly	46%	6031187	3850154	10904	83537	14454
Turkmenistan	2.4	Social distancing whole population	37%	6031187	3151105	9633	69712	12771
Turkmenistan	2.7	Social distancing whole population	41%	6031187	3436102	10683	76796	14162
Turkmenistan	3	Social distancing whole population	44%	6031187	3668428	11564	82665	15331
Turkmenistan	3.3	Social distancing whole population	47%	6031187	3861568	12316	87614	16335
Turkmenistan	2.4	Unmitigated	0%	6031187	4962684	16993	117099	22528
Turkmenistan	2.7	Unmitigated	0%	6031187	5234319	18278	124759	24239
Turkmenistan	3	Unmitigated	0%	6031187	5427885	19238	130335	25510
Turkmenistan	3.3	Unmitigated	0%	6031187	5568599	19965	134462	26469
Uganda	2.4	Enhanced social distancing of elderly	35%	45741000	25332930	37656	340227	49922

Uganda	2.7	Enhanced social distancing of elderly	39%	45741000	27389680	41724	369936	55310
Uganda	3	Enhanced social distancing of elderly	44%	45741000	28728218	51836	412124	68726
Uganda	3.3	Enhanced social distancing of elderly	47%	45741000	30088425	54797	432267	72677
Uganda	2.4	Social distancing whole population	37%	45741000	24767992	50272	374481	66646
Uganda	2.7	Social distancing whole population	41%	45741000	26858864	53585	402071	71072
Uganda	3	Social distancing whole population	45%	45741000	28551684	56144	423847	74441
Uganda	3.3	Social distancing whole population	48%	45741000	29951750	58172	441439	77116
Uganda	2.4	Unmitigated	0%	45741000	38381771	68481	537832	90787
Uganda	2.7	Unmitigated	0%	45741000	40294022	70273	556733	93163
Uganda	3	Unmitigated	0%	45741000	41642067	71377	569078	94622
Uganda	3.3	Unmitigated	0%	45741000	42614144	72080	577356	95590
Ukraine	2.4	Enhanced social distancing of elderly	35%	43733759	19457237	69663	545038	92354
Ukraine	2.7	Enhanced social distancing of elderly	38%	43733759	21480926	82161	621199	108963
Ukraine	3	Enhanced social distancing of elderly	42%	43733759	23610944	122546	769413	162484
Ukraine	3.3	Enhanced social distancing of elderly	45%	43733759	25100051	138299	842776	183337
Ukraine	2.4	Social distancing whole population	36%	43733759	20341591	122293	695165	162126
Ukraine	2.7	Social distancing whole population	40%	43733759	22415578	139921	784729	185480
Ukraine	3	Social distancing whole population	43%	43733759	24132231	155542	862177	206199
Ukraine	3.3	Social distancing whole population	46%	43733759	25578449	169512	929966	224811
Ukraine	2.4	Unmitigated	0%	43733759	33300367	260783	1341526	345724
Ukraine	2.7	Unmitigated	0%	43733759	35540535	294787	1482102	391081
Ukraine	3	Unmitigated	0%	43733759	37203041	323404	1595427	428867
Ukraine	3.3	Unmitigated	0%	43733759	38462530	347583	1687722	460705
United Arab Emirates	2.4	Enhanced social distancing of elderly	33%	9890400	5332420	11708	130623	15522
United Arab Emirates	2.7	Enhanced social distancing of elderly	38%	9890400	5762187	12719	141016	16863
United Arab Emirates	3	Enhanced social distancing of elderly	43%	9890400	5981304	14201	149247	18826
United Arab Emirates	3.3	Enhanced social distancing of elderly	46%	9890400	6285635	14821	156233	19653
United Arab Emirates	2.4	Social distancing whole population	36%	9890400	5069148	13056	130289	17309
United Arab Emirates	2.7	Social distancing whole population	40%	9890400	5542229	13901	140950	18433
United Arab Emirates	3	Social distancing whole population	44%	9890400	5928029	14555	149468	19304
United Arab Emirates	3.3	Social distancing whole population	47%	9890400	6248541	15077	156425	19990
United Arab Emirates	2.4	Unmitigated	0%	9890400	8053205	17644	193305	23390
United Arab Emirates	2.7	Unmitigated	0%	9890400	8509756	18189	201871	24117
United Arab Emirates	3	Unmitigated	0%	9890400	8835028	18547	207705	24587
United Arab Emirates	3.3	Unmitigated	0%	9890400	9071765	18790	211769	24911
United Kingdom	2.4	Enhanced social distancing of elderly	34%	67886004	31411840	133654	913618	177188
United Kingdom	2.7	Enhanced social distancing of elderly	38%	67886004	34512550	157036	1037258	208253
United Kingdom	3	Enhanced social distancing of elderly	43%	67886004	37944291	238949	1313637	316815
United Kingdom	3.3	Enhanced social distancing of elderly	46%	67886004	40217603	268084	1434747	355374
United Kingdom	2.4	Social distancing whole population	36%	67886004	33088025	242593	1219928	321610
United Kingdom	2.7	Social distancing whole population	40%	67886004	36249870	273905	1363173	363100
United Kingdom	3	Social distancing whole population	44%	67886004	38851158	301239	1485668	399341
United Kingdom	3.3	Social distancing whole population	47%	67886004	41030656	325345	1591804	431634

United Kingdom	2.4	Unmitigated	0%	67886004	53331345	489828	2268795	649373
United Kingdom	2.7	Unmitigated	0%	67886004	56601671	545684	2480156	723657
United Kingdom	3	Unmitigated	0%	67886004	58995235	591887	2647865	784928
United Kingdom	3.3	Unmitigated	0%	67886004	60787055	630436	2782777	835879
United States	2.4	Enhanced social distancing of elderly	34%	331002647	157164823	623230	4392101	826227
United States	2.7	Enhanced social distancing of elderly	38%	331002647	172412507	729256	4972232	967063
United States	3	Enhanced social distancing of elderly	43%	331002647	188852138	1090267	6232481	1445673
United States	3.3	Enhanced social distancing of elderly	46%	331002647	199911483	1219232	6790097	1616296
United States	2.4	Social distancing whole population	37%	331002647	164661268	1099095	5765915	1457088
United States	2.7	Social distancing whole population	40%	331002647	180136175	1237754	6428279	1640638
United States	3	Social distancing whole population	44%	331002647	192837687	1358164	6992445	1800396
United States	3.3	Social distancing whole population	47%	331002647	203458398	1464020	7479808	1941399
United States	2.4	Unmitigated	0%	331002647	263735706	2186315	10595273	2898432
United States	2.7	Unmitigated	0%	331002647	279390696	2424446	11542084	3214091
United States	3	Unmitigated	0%	331002647	290769042	2619268	12286300	3473985
United States	3.3	Unmitigated	0%	331002647	299216118	2780065	12879412	3685844
United States Virgin Island	2.4	Enhanced social distancing of elderly	31%	104423	41524	186	1136	247
United States Virgin Island	2.7	Enhanced social distancing of elderly	34%	104423	46345	224	1329	296
United States Virgin Island	3	Enhanced social distancing of elderly	39%	104423	51808	345	1752	457
United States Virgin Island	3.3	Enhanced social distancing of elderly	42%	104423	55472	390	1945	518
United States Virgin Island	2.4	Social distancing whole population	33%	104423	44321	348	1610	461
United States Virgin Island	2.7	Social distancing whole population	37%	104423	49270	400	1839	530
United States Virgin Island	3	Social distancing whole population	41%	104423	53434	445	2041	590
United States Virgin Island	3.3	Social distancing whole population	43%	104423	56985	486	2219	644
United States Virgin Island	2.4	Unmitigated	0%	104423	73373	700	3135	928
United States Virgin Island	2.7	Unmitigated	0%	104423	79346	792	3515	1049
United States Virgin Island	3	Unmitigated	0%	104423	83879	868	3825	1150
United States Virgin Island	3.3	Unmitigated	0%	104423	87385	932	4080	1236
Uruguay	2.4	Enhanced social distancing of elderly	33%	3473727	1440954	5457	34704	7235
Uruguay	2.7	Enhanced social distancing of elderly	37%	3473727	1611331	6559	40496	8701
Uruguay	3	Enhanced social distancing of elderly	41%	3473727	1789616	10106	52452	13401
Uruguay	3.3	Enhanced social distancing of elderly	44%	3473727	1916340	11433	58106	15153
Uruguay	2.4	Social distancing whole population	35%	3473727	1509262	9966	46964	13212
Uruguay	2.7	Social distancing whole population	39%	3473727	1681422	11455	53695	15189
Uruguay	3	Social distancing whole population	42%	3473727	1826334	12775	59596	16935
Uruguay	3.3	Social distancing whole population	45%	3473727	1949732	13948	64796	18491
Uruguay	2.4	Unmitigated	0%	3473727	2564862	20633	93491	27353
Uruguay	2.7	Unmitigated	0%	3473727	2762002	23165	103901	30709
Uruguay	3	Unmitigated	0%	3473727	2909521	25241	112220	33477
Uruguay	3.3	Unmitigated	0%	3473727	3021752	26957	118931	35741
Uzbekistan	2.4	Enhanced social distancing of elderly	35%	33469199	17215079	36388	342657	48240
Uzbekistan	2.7	Enhanced social distancing of elderly	39%	33469199	18817956	41656	381762	55226
Uzbekistan	3	Enhanced social distancing of elderly	43%	33469199	20180144	55789	442125	73953

Uzbekistan	3.3	Enhanced social distancing of elderly	46%	33469199	21270530	61072	473622	80975
Uzbekistan	2.4	Social distancing whole population	37%	33469199	17367865	53713	393668	71208
Uzbekistan	2.7	Social distancing whole population	41%	33469199	18956279	59621	434173	79039
Uzbekistan	3	Social distancing whole population	44%	33469199	20252329	64590	467783	85661
Uzbekistan	3.3	Social distancing whole population	47%	33469199	21330461	68826	496125	91259
Uzbekistan	2.4	Unmitigated	0%	33469199	27437261	94968	663895	125900
Uzbekistan	2.7	Unmitigated	0%	33469199	28961720	102220	707992	135547
Uzbekistan	3	Unmitigated	0%	33469199	30050156	107646	740149	142707
Uzbekistan	3.3	Unmitigated	0%	33469199	30843030	111762	764000	148156
Vanuatu	2.4	Enhanced social distancing of elderly	34%	307150	155218	337	3056	447
Vanuatu	2.7	Enhanced social distancing of elderly	38%	307150	168937	380	3344	504
Vanuatu	3	Enhanced social distancing of elderly	43%	307150	178237	503	3809	667
Vanuatu	3.3	Enhanced social distancing of elderly	46%	307150	187293	541	4025	718
Vanuatu	2.4	Social distancing whole population	36%	307150	153355	501	3506	664
Vanuatu	2.7	Social distancing whole population	40%	307150	166774	541	3786	718
Vanuatu	3	Social distancing whole population	44%	307150	177736	574	4010	761
Vanuatu	3.3	Social distancing whole population	47%	307150	186881	601	4194	797
Vanuatu	2.4	Unmitigated	0%	307150	239679	746	5175	989
Vanuatu	2.7	Unmitigated	0%	307150	253112	779	5393	1032
Vanuatu	3	Unmitigated	0%	307150	262915	801	5539	1062
Vanuatu	3.3	Unmitigated	0%	307150	270286	816	5640	1082
Venezuela (Bolivarian Rep	2.4	Enhanced social distancing of elderly	34%	28435943	13213424	33796	259559	44804
Venezuela (Bolivarian Rep	2.7	Enhanced social distancing of elderly	38%	28435943	14630954	39861	298051	52852
Venezuela (Bolivarian Rep	3	Enhanced social distancing of elderly	42%	28435943	15962541	57228	366397	75864
Venezuela (Bolivarian Rep	3.3	Enhanced social distancing of elderly	45%	28435943	16972862	63835	400847	84625
Venezuela (Bolivarian Rep	2.4	Social distancing whole population	36%	28435943	13529910	54955	322158	72855
Venezuela (Bolivarian Rep	2.7	Social distancing whole population	40%	28435943	14939037	62337	363908	82639
Venezuela (Bolivarian Rep	3	Social distancing whole population	43%	28435943	16110940	68752	399927	91184
Venezuela (Bolivarian Rep	3.3	Social distancing whole population	46%	28435943	17099863	74368	431268	98609
Venezuela (Bolivarian Rep	2.4	Unmitigated	0%	28435943	22285075	107419	611782	142408
Venezuela (Bolivarian Rep	2.7	Unmitigated	0%	28435943	23768827	118288	669534	156865
Venezuela (Bolivarian Rep	3	Unmitigated	0%	28435943	24850426	126771	713948	168041
Venezuela (Bolivarian Rep	3.3	Unmitigated	0%	28435943	25652521	133461	748494	176916
Vietnam	2.4	Enhanced social distancing of elderly	34%	97338583	51544002	165555	1375706	219479
Vietnam	2.7	Enhanced social distancing of elderly	38%	97338583	56274816	190434	1528847	252453
Vietnam	3	Enhanced social distancing of elderly	43%	97338583	59521717	268329	1792309	355854
Vietnam	3.3	Enhanced social distancing of elderly	46%	97338583	62528806	294669	1915846	390707
Vietnam	2.4	Social distancing whole population	37%	97338583	51613894	271555	1654584	360005
Vietnam	2.7	Social distancing whole population	41%	97338583	56030494	298664	1806079	396032
Vietnam	3	Social distancing whole population	45%	97338583	59611789	321185	1930004	425793
Vietnam	3.3	Social distancing whole population	48%	97338583	62577131	340220	2033375	451009
Vietnam	2.4	Unmitigated	0%	97338583	80146129	461352	2659283	611622
Vietnam	2.7	Unmitigated	0%	97338583	84225947	491861	2807012	652018

Vietnam	3	Unmitigated	0%	97338583	87124332	514142	2911699	681796
Vietnam	3.3	Unmitigated	0%	97338583	89235141	530665	2987319	703788
Western Sahara	2.4	Enhanced social distancing of elderly	34%	597330	329224	738	6675	979
Western Sahara	2.7	Enhanced social distancing of elderly	38%	597330	357401	829	7361	1099
Western Sahara	3	Enhanced social distancing of elderly	43%	597330	377286	1042	8297	1382
Western Sahara	3.3	Enhanced social distancing of elderly	46%	597330	395808	1115	8792	1478
Western Sahara	2.4	Social distancing whole population	37%	597330	324860	993	7381	1317
Western Sahara	2.7	Social distancing whole population	41%	597330	352885	1076	8038	1426
Western Sahara	3	Social distancing whole population	44%	597330	375603	1143	8572	1515
Western Sahara	3.3	Social distancing whole population	47%	597330	394398	1197	9016	1588
Western Sahara	2.4	Unmitigated	0%	597330	503141	1501	11608	1990
Western Sahara	2.7	Unmitigated	0%	597330	528670	1568	12223	2080
Western Sahara	3	Unmitigated	0%	597330	546494	1614	12654	2140
Western Sahara	3.3	Unmitigated	0%	597330	559210	1646	12963	2181
Yemen	2.4	Enhanced social distancing of elderly	35%	29825968	16727305	29870	258153	39599
Yemen	2.7	Enhanced social distancing of elderly	39%	29825968	18097720	33414	282471	44296
Yemen	3	Enhanced social distancing of elderly	44%	29825968	19045854	43004	320620	57033
Yemen	3.3	Enhanced social distancing of elderly	47%	29825968	19944454	45875	338029	60831
Yemen	2.4	Social distancing whole population	37%	29825968	16473656	42045	292228	55740
Yemen	2.7	Social distancing whole population	41%	29825968	17848124	45162	315172	59891
Yemen	3	Social distancing whole population	45%	29825968	18958476	47617	333453	63126
Yemen	3.3	Social distancing whole population	48%	29825968	19874390	49597	348346	65747
Yemen	2.4	Unmitigated	0%	29825968	25361679	60394	432937	80065
Yemen	2.7	Unmitigated	0%	29825968	26578659	62449	450116	82785
Yemen	3	Unmitigated	0%	29825968	27425691	63769	461520	84538
Yemen	3.3	Unmitigated	0%	29825968	28029353	64638	469270	85731
Zambia	2.4	Enhanced social distancing of elderly	35%	18383956	10249355	15874	142676	21044
Zambia	2.7	Enhanced social distancing of elderly	39%	18383956	11079905	17624	155306	23370
Zambia	3	Enhanced social distancing of elderly	44%	18383956	11624307	22077	173485	29268
Zambia	3.3	Enhanced social distancing of elderly	47%	18383956	12171987	23384	182134	30999
Zambia	2.4	Social distancing whole population	37%	18383956	10031328	21462	157632	28452
Zambia	2.7	Social distancing whole population	41%	18383956	10873833	22912	169403	30372
Zambia	3	Social distancing whole population	45%	18383956	11555399	24037	178713	31870
Zambia	3.3	Social distancing whole population	48%	18383956	12118600	24933	186255	33068
Zambia	2.4	Unmitigated	0%	18383956	15504067	29569	227998	39200
Zambia	2.7	Unmitigated	0%	18383956	16265416	30393	236260	40311
Zambia	3	Unmitigated	0%	18383956	16799549	30908	241686	40981
Zambia	3.3	Unmitigated	0%	18383956	17183007	31238	245339	41411
Zimbabwe	2.4	Enhanced social distancing of elderly	35%	14862927	8370104	14943	126738	19810
Zimbabwe	2.7	Enhanced social distancing of elderly	39%	14862927	9050138	16729	138592	22176
Zimbabwe	3	Enhanced social distancing of elderly	44%	14862927	9522740	21727	157807	28816
Zimbabwe	3.3	Enhanced social distancing of elderly	47%	14862927	9968673	23204	166357	30772
Zimbabwe	2.4	Social distancing whole population	37%	14862927	8246851	21359	144424	28316

Zimbabwe	2.7	Social distancing whole population	41%	14862927	8930321	22944	155628	30430
Zimbabwe	3	Social distancing whole population	45%	14862927	9481786	24195	164543	32079
Zimbabwe	3.3	Social distancing whole population	48%	14862927	9937914	25209	171827	33418
Zimbabwe	2.4	Unmitigated	0%	14862927	12666383	30807	213130	40842
Zimbabwe	2.7	Unmitigated	0%	14862927	13267060	31894	221471	42280
Zimbabwe	3	Unmitigated	0%	14862927	13684271	32600	226999	43217
Zimbabwe	3.3	Unmitigated	0%	14862927	13981038	33073	230755	43864

Suppression											
Country	R0	Strategy	Social_distance	Deaths per week	total_pop	total_infected	total_deaths	total_hospital	peak_hospital_bed_demand	total_critical	peak_critical_bed_demand
Afghanistan	3	Unmitigated	0.75	N/A	38928341	36140196	76973	567916	240272	102693	39621
Albania	3	Unmitigated	0.75	N/A	2877800	2464424	19300	97298	36061	25569	8024
Algeria	3	Unmitigated	0.75	N/A	43851043	38824343	191145	1118956	459923	253553	90068
Angola	3	Unmitigated	0.75	N/A	32866268	30110663	57385	438605	183326	75970	29050
Antigua and Barbuda	3	Unmitigated	0.75	N/A	97928	84247	504	2795	1048	667	215
Argentina	3	Unmitigated	0.75	N/A	45195777	39126816	259966	1276885	489073	342159	110413
Armenia	3	Unmitigated	0.75	N/A	2963234	2603481	18054	95101	35775	24020	7685
Aruba	3	Unmitigated	0.75	N/A	106766	88963	737	3715	1396	981	310
Australia	3	Unmitigated	0.75	N/A	25499881	21991217	185236	881939	330675	244401	76396
Austria	3	Unmitigated	0.75	N/A	9006400	7559177	76313	350262	129800	100950	30976
Azerbaijan	3	Unmitigated	0.75	N/A	10139175	9102969	43606	276438	104967	57585	19143
Bahamas	3	Unmitigated	0.75	N/A	393248	340746	1789	10526	3943	2381	769
Bahrain	3	Unmitigated	0.75	N/A	1701583	1542640	4381	39064	15845	5837	2190
Bangladesh	3	Unmitigated	0.75	N/A	164689383	154022806	640815	3930633	1649241	850522	313157
Barbados	3	Unmitigated	0.75	N/A	287371	238847	2287	10319	3936	3034	951
Belarus	3	Unmitigated	0.75	N/A	9449321	8118548	68045	341478	126904	90009	28279
Belgium	3	Unmitigated	0.75	N/A	11589616	10305691	110545	489543	195265	146624	48557
Belize	3	Unmitigated	0.75	N/A	397621	353490	1316	8122	3134	1748	585
Benin	3	Unmitigated	0.75	N/A	12123198	11180031	28492	194397	81572	38086	14233
Bhutan	3	Unmitigated	0.75	N/A	771612	721036	3279	18912	7959	4366	1588
Bolivia	3	Unmitigated	0.75	N/A	11673029	10362045	49399	263020	102139	65645	21745
Bosnia and Herzegovina	3	Unmitigated	0.75	N/A	3280815	2778822	25239	124898	46051	33536	10404
Botswana	3	Unmitigated	0.75	N/A	2351625	2185830	7172	47521	19740	9495	3476
Brazil	3	Unmitigated	0.75	N/A	212559409	182791505	1102439	5975916	2248613	1465863	469512
Brunei Darussalam	3	Unmitigated	0.75	N/A	437483	397650	1785	12042	4969	2365	865
Bulgaria	3	Unmitigated	0.75	N/A	6948445	5813959	59835	276774	102010	79185	24336
Burkina Faso	3	Unmitigated	0.75	N/A	20903278	19217285	38156	291903	122046	50935	19354
Burundi	3	Unmitigated	0.75	N/A	11890781	10898277	21496	161782	67626	28486	10805
Cabo Verde	3	Unmitigated	0.75	N/A	555988	515151	2008	12581	5193	2647	962
Cambodia	3	Unmitigated	0.75	N/A	16718971	14764027	54949	365432	149919	72454	26304
Cameroon	3	Unmitigated	0.75	N/A	26545864	24484798	53694	395803	165183	70597	26637
Canada	3	Unmitigated	0.75	N/A	37742157	33067353	326322	1509416	588836	433148	140912
Central African Republic	3	Unmitigated	0.75	N/A	4829764	4459268	9953	69496	29236	13092	4958
Chad	3	Unmitigated	0.75	N/A	16425859	15039833	30223	217409	91537	39627	14974
Channel Islands	3	Unmitigated	0.75	N/A	173859	147500	1384	6554	2429	1835	568
Chile	3	Unmitigated	0.75	N/A	19116209	16207664	118765	592342	223725	157185	49782
China	3	Unmitigated	0.75	N/A	1439323774	1300804549	9890457	53821210	21549628	13123394	4531927
Hong Kong SAR, China	3	Unmitigated	0.75	N/A	7496988	7034214	88802	383785	169089	117912	43311
Macao SAR, China	3	Unmitigated	0.75	N/A	649342	587661	4685	25574	10239	6213	2144

China, Taiwan Province of China	3	Unmitigated	0.75	N/A	23816775	21387957	212338	1025106	403488	280895	93378
Colombia	3	Unmitigated	0.75	N/A	50882884	44096507	252064	1364689	515744	333972	108414
Comoros	3	Unmitigated	0.75	N/A	869595	804559	2017	14523	6059	2678	1007
Congo, Rep.	3	Unmitigated	0.75	N/A	5518092	5096722	11779	88504	36711	15512	5842
Costa Rica	3	Unmitigated	0.75	N/A	5094114	4365461	28049	147023	55398	37111	11835
Cote d'Ivoire	3	Unmitigated	0.75	N/A	26378275	24320357	55233	403555	168588	73470	27588
Croatia	3	Unmitigated	0.75	N/A	4105268	3424974	36127	162878	60312	47878	14662
Cuba	3	Unmitigated	0.75	N/A	11326616	9353709	86419	408509	154360	114722	36028
Curacao	3	Unmitigated	0.75	N/A	164100	136542	1328	6030	2311	1759	558
Cyprus	3	Unmitigated	0.75	N/A	1207361	1046888	8029	40519	15089	10657	3347
Czechia	3	Unmitigated	0.75	N/A	10708982	9050655	86700	411771	152187	114839	35369
Korea, Dem. People's Rep.	3	Unmitigated	0.75	N/A	25778815	23152081	157944	872256	350112	209599	72399
Congo, Dem. Rep.	3	Unmitigated	0.75	N/A	89561404	82164295	194156	1322652	557103	255863	97031
Denmark	3	Unmitigated	0.75	N/A	5792203	4854981	48190	219266	81321	63846	19596
Djibouti	3	Unmitigated	0.75	N/A	988002	916137	3278	21705	8949	4335	1585
Dominican Republic	3	Unmitigated	0.75	N/A	10847904	9556193	47536	261450	100437	62769	20674
Ecuador	3	Unmitigated	0.75	N/A	17643060	15536231	76661	422481	162098	101482	33536
Egypt	3	Unmitigated	0.75	N/A	102334403	94671014	362330	2250129	936058	480573	175631
El Salvador	3	Unmitigated	0.75	N/A	6486201	5692694	30611	159333	61416	40371	13239
Equatorial Guinea	3	Unmitigated	0.75	N/A	1402985	1287098	2608	21009	8630	3426	1292
Eritrea	3	Unmitigated	0.75	N/A	3546427	3291069	10340	62547	26480	13680	5099
Estonia	3	Unmitigated	0.75	N/A	1326539	1116452	11444	50954	19039	15137	4693
Eswatini	3	Unmitigated	0.75	N/A	1160164	1079088	3148	19901	8419	4141	1545
Ethiopia	3	Unmitigated	0.75	N/A	114963583	106264340	281678	1863037	784029	375529	138813
Fiji	3	Unmitigated	0.75	N/A	896444	799467	3494	22692	9292	4650	1672
Finland	3	Unmitigated	0.75	N/A	5540718	4662980	46008	215475	80614	61080	19032
France	3	Unmitigated	0.75	N/A	65273512	59173552	731625	2987758	1233274	967239	328557
French Guiana	3	Unmitigated	0.75	N/A	298682	265602	1016	6420	2455	1342	449
French Polynesia	3	Unmitigated	0.75	N/A	280904	253877	1582	8948	3624	2093	734
Gabon	3	Unmitigated	0.75	N/A	2225728	2051004	5726	39934	16536	7602	2805
Gambia	3	Unmitigated	0.75	N/A	2416664	2216662	4520	33958	14166	6071	2287
Georgia	3	Unmitigated	0.75	N/A	3989175	3450187	27941	137794	51619	37006	11652
Germany	3	Unmitigated	0.75	N/A	83783945	73426368	884373	3801734	1502309	1173591	383550
Ghana	3	Unmitigated	0.75	N/A	31072945	28768770	74174	549719	227892	98691	36866
Greece	3	Unmitigated	0.75	N/A	10423056	8671791	99000	426451	159009	131442	40171
Grenada	3	Unmitigated	0.75	N/A	112519	97147	600	3139	1187	795	255
Guadeloupe	3	Unmitigated	0.75	N/A	400127	330901	3553	15188	5912	4687	1500
Guam	3	Unmitigated	0.75	N/A	168783	150254	1037	5385	2150	1374	468
Guatemala	3	Unmitigated	0.75	N/A	17915567	16036279	56167	332414	130238	74486	25443
Guinea	3	Unmitigated	0.75	N/A	13132792	12096855	27353	193090	81113	36354	13698
Guinea-Bissau	3	Unmitigated	0.75	N/A	1967998	1815401	4019	29699	12375	5308	2001
Guyana	3	Unmitigated	0.75	N/A	786559	694407	3268	18666	7151	4338	1434

Haiti	3	Unmitigated	0.75	N/A	11402533	10191506	36299	223219	86669	48189	16339
Honduras	3	Unmitigated	0.75	N/A	9904608	8840695	31599	193064	75205	41866	14151
Hungary	3	Unmitigated	0.75	N/A	9660350	8118426	78816	371119	136791	104324	32001
Iceland	3	Unmitigated	0.75	N/A	341250	294670	2404	11685	4366	3189	1000
India	3	Unmitigated	0.75	N/A	1380004385	1292426271	6022132	35894328	15063300	7972354	2927196
Indonesia	3	Unmitigated	0.75	N/A	273523621	246077180	1168219	7335507	3004233	1543439	557510
Iran (Islamic Republic of)	3	Unmitigated	0.75	N/A	83992953	75590529	370783	2308836	948251	491258	176276
Iraq	3	Unmitigated	0.75	N/A	40222503	34816936	97226	691907	286326	129689	48168
Ireland	3	Unmitigated	0.75	N/A	4937796	4328022	32931	165610	62398	43562	13801
Israel	3	Unmitigated	0.75	N/A	8655541	7641595	49634	246470	94142	65738	21166
Italy	3	Unmitigated	0.75	N/A	60461828	50281111	609676	2625555	972831	809681	246249
Jamaica	3	Unmitigated	0.75	N/A	2961161	2572026	14943	79224	30126	19766	6380
Japan	3	Unmitigated	0.75	N/A	126476458	101788506	1381819	5521836	2066796	1831537	553056
Jordan	3	Unmitigated	0.75	N/A	10203140	9055930	29975	204044	84956	39679	14619
Kazakhstan	3	Unmitigated	0.75	N/A	18776707	16853130	84127	495929	190161	111507	36969
Kenya	3	Unmitigated	0.75	N/A	53771300	49869539	105459	827374	342728	140351	52863
Kiribati	3	Unmitigated	0.75	N/A	119446	104380	358	2420	996	474	173
Kuwait	3	Unmitigated	0.75	N/A	4270563	3863804	12946	114726	45941	17099	6215
Kyrgyz Republic	3	Unmitigated	0.75	N/A	6524191	5900037	20851	139409	54562	27473	9506
Lao PDR	3	Unmitigated	0.75	N/A	7275556	6426976	21991	148444	61392	28777	10601
Latvia	3	Unmitigated	0.75	N/A	1886202	1573512	16431	74056	27507	21747	6706
Lebanon	3	Unmitigated	0.75	N/A	6825442	6131042	33325	190274	77851	44194	15657
Lesotho	3	Unmitigated	0.75	N/A	2142252	1988339	7132	44473	18543	9493	3479
Liberia	3	Unmitigated	0.75	N/A	5057677	4681087	12091	83929	35050	16065	6009
Libya	3	Unmitigated	0.75	N/A	6871287	6184603	23441	160972	66789	30934	11397
Lithuania	3	Unmitigated	0.75	N/A	2722291	2260352	24248	107806	40145	32092	9871
Luxembourg	3	Unmitigated	0.75	N/A	625976	545553	4325	21993	8322	5719	1839
Madagascar	3	Unmitigated	0.75	N/A	27691019	25585584	63480	447454	187086	84096	31356
Malawi	3	Unmitigated	0.75	N/A	19129955	17651581	37367	272012	114232	49573	18738
Malaysia	3	Unmitigated	0.75	N/A	32365998	29147233	148280	885796	362321	196478	70275
Maldives	3	Unmitigated	0.75	N/A	540542	493928	1623	11531	4723	2149	796
Mali	3	Unmitigated	0.75	N/A	20250834	18557804	36576	270505	113207	48639	18536
Malta	3	Unmitigated	0.75	N/A	441539	369237	3772	17199	6349	5008	1535
Martinique	3	Unmitigated	0.75	N/A	375265	304393	3678	15295	5953	4885	1546
Mauritania	3	Unmitigated	0.75	N/A	4649660	4294210	10929	77719	32302	14518	5427
Mauritius	3	Unmitigated	0.75	N/A	1271767	1152776	9130	46371	18983	12031	4193
Mayotte	3	Unmitigated	0.75	N/A	272813	253417	811	4967	2095	1073	395
Mexico	3	Unmitigated	0.75	N/A	128932753	113215128	567846	3186360	1216236	754360	246848
Micronesia (Fed. States of)	3	Unmitigated	0.75	N/A	115021	101212	342	2437	992	452	164
Mongolia	3	Unmitigated	0.75	N/A	3278292	2910923	10970	76238	31598	14496	5390
Montenegro	3	Unmitigated	0.75	N/A	628062	541238	4386	21856	8138	5817	1835
Morocco	3	Unmitigated	0.75	N/A	36910558	33007185	177366	1027568	418234	235102	82813
Mozambique	3	Unmitigated	0.75	N/A	31255435	28765983	64288	450800	189972	84882	32113
Myanmar	3	Unmitigated	0.75	N/A	54409794	48995734	224280	1426350	585376	296811	107239
Namibia	3	Unmitigated	0.75	N/A	2540916	2352352	6679	45192	18841	8876	3289
Nepal	3	Unmitigated	0.75	N/A	29136808	27306425	111877	668977	282618	147773	54748
Netherlands	3	Unmitigated	0.75	N/A	17134873	14365190	144137	659956	245262	191297	58804

New Caledonia	3	Unmitigated	0.75	N/A	285491	257682	1661	9209	3720	2206	769
New Zealand	3	Unmitigated	0.75	N/A	4822233	4135860	34945	167724	62678	46380	14482
Nicaragua	3	Unmitigated	0.75	N/A	6624554	5880597	23524	141121	54323	31231	10448
Niger	3	Unmitigated	0.75	N/A	24206636	22040235	44518	317948	134026	59161	22510
Nigeria	3	Unmitigated	0.75	N/A	206139587	189732638	413526	3104658	1295587	547352	207005
North Macedonia	3	Unmitigated	0.75	N/A	2083380	1805547	13626	72260	26683	18032	5697
Norway	3	Unmitigated	0.75	N/A	5421242	4633217	41268	195366	72851	54643	16989
Oman	3	Unmitigated	0.75	N/A	5106622	4531573	11870	103083	41805	15723	5884
Pakistan	3	Unmitigated	0.75	N/A	220892331	206813615	691824	4403621	1864806	917211	343609
Panama	3	Unmitigated	0.75	N/A	4314768	3780459	20801	110940	42418	27655	9035
Papua New Guinea	3	Unmitigated	0.75	N/A	8947027	7827395	22930	169143	69848	30529	11297
Paraguay	3	Unmitigated	0.75	N/A	7132530	6308582	27638	157099	60631	36816	12199
Peru	3	Unmitigated	0.75	N/A	32971846	28675674	157650	861401	326720	210209	67965
Philippines	3	Unmitigated	0.75	N/A	109581085	97582964	405302	2548034	1050472	537561	194819
Poland	3	Unmitigated	0.75	N/A	37846605	32122997	297828	1445691	551940	395737	126362
Portugal	3	Unmitigated	0.75	N/A	10196707	8431162	95850	419676	155409	127172	38866
Puerto Rico	3	Unmitigated	0.75	N/A	2860840	2325498	26307	112170	43734	35035	11151
Qatar	3	Unmitigated	0.75	N/A	2881060	2598643	5795	62542	24347	7693	2743
Korea, Rep.	3	Unmitigated	0.75	N/A	51269183	43595642	380712	1950636	714282	505263	156081
Moldova	3	Unmitigated	0.75	N/A	4033963	3523427	24013	134447	49890	31935	10184
Réunion	3	Unmitigated	0.75	N/A	895308	766270	5735	28282	10821	7621	2455
Romania	3	Unmitigated	0.75	N/A	19237682	16283637	154904	731837	271767	205971	63579
Russian Federation	3	Unmitigated	0.75	N/A	145934460	126202684	1044154	5210604	1944898	1385319	436235
Rwanda	3	Unmitigated	0.75	N/A	12952209	11987296	29846	214305	89250	39441	14831
St. Lucia	3	Unmitigated	0.75	N/A	183629	155980	1030	5398	2026	1366	436
St. Vincent and the Grenadines	3	Unmitigated	0.75	N/A	110947	95776	605	3164	1200	801	259
Samoa	3	Unmitigated	0.75	N/A	198410	172083	681	4221	1732	899	324
Sao Tome and Principe	3	Unmitigated	0.75	N/A	219161	203133	511	3529	1482	674	252
Saudi Arabia	3	Unmitigated	0.75	N/A	34813867	31434568	103202	822986	340586	137024	51551
Senegal	3	Unmitigated	0.75	N/A	16743930	15447748	37292	259076	108551	49324	18431
Serbia	3	Unmitigated	0.75	N/A	8737370	7405560	67524	323990	119850	89481	27648
Seychelles	3	Unmitigated	0.75	N/A	98340	89831	539	3031	1240	714	252
Sierra Leone	3	Unmitigated	0.75	N/A	7976985	7378611	17280	124770	52121	22779	8564
Singapore	3	Unmitigated	0.75	N/A	5850343	5476249	49923	258746	111912	66099	24426
Slovakia	3	Unmitigated	0.75	N/A	5459643	4682682	39403	199810	73655	52307	16293
Slovenia	3	Unmitigated	0.75	N/A	2078932	1742828	18053	82852	30670	23949	7369
Solomon Islands	3	Unmitigated	0.75	N/A	686878	589862	1799	12101	5035	2390	883
Somalia	3	Unmitigated	0.75	N/A	15893219	14564497	32737	223862	94340	43168	16348
South Africa	3	Unmitigated	0.75	N/A	59308690	53021201	217343	1406692	578915	288338	104612
South Sudan	3	Unmitigated	0.75	N/A	11193729	10332593	26854	180560	75753	35587	13394
Spain	3	Unmitigated	0.75	N/A	46754783	39331479	410968	1865322	693130	545853	167803
Sri Lanka	3	Unmitigated	0.75	N/A	21413250	19841593	138452	721203	299722	183192	64747
State of Palestine	3	Unmitigated	0.75	N/A	5101416	4395175	12013	85259	35359	16026	5942

Sudan	3	Unmitigated	0.75	N/A	43849269	40589416	113695	751642	315495	150596	56112
Suriname	3	Unmitigated	0.75	N/A	586634	515898	2505	14476	5523	3321	1095
Sweden	3	Unmitigated	0.75	N/A	10099270	8496869	84777	379197	141485	112952	34845
Switzerland	3	Unmitigated	0.75	N/A	8654618	7294512	72337	334363	123764	95906	29353
Syrian Arab Republic	3	Unmitigated	0.75	N/A	17500657	16243203	59077	376968	156189	78343	28623
Tajikistan	3	Unmitigated	0.75	N/A	9537642	8607513	23073	170117	67199	30722	10873
Thailand	3	Unmitigated	0.75	N/A	69799978	62828210	536081	2720705	1079038	707576	239456
Timor-Leste	3	Unmitigated	0.75	N/A	1318442	1134428	3842	24366	9988	5065	1823
Togo	3	Unmitigated	0.75	N/A	8278737	7656800	17559	131333	54802	23439	8813
Tonga	3	Unmitigated	0.75	N/A	105697	92665	403	2346	960	535	190
Trinidad and Tobago	3	Unmitigated	0.75	N/A	1399491	1189922	8150	43011	16157	10783	3432
Tunisia	3	Unmitigated	0.75	N/A	11818618	10829721	65681	359179	147277	87060	30688
Turkey	3	Unmitigated	0.75	N/A	84339067	75638628	405452	2321985	886555	538771	177357
Turkmenistan	3	Unmitigated	0.75	N/A	6031187	5466961	19367	131528	51424	25651	8855
Uganda	3	Unmitigated	0.75	N/A	45741000	41902539	71610	572142	239347	95681	36570
Ukraine	3	Unmitigated	0.75	N/A	43733759	37537514	328913	1620489	600963	438024	136218
United Arab Emirates	3	Unmitigated	0.75	N/A	9890400	8900753	18606	209171	80858	24647	8822
United Kingdom	3	Unmitigated	0.75	N/A	67886004	59462912	601823	2683313	1054670	796883	259936
Tanzania	3	Unmitigated	0.75	N/A	59734213	54993789	118017	876453	365633	156898	59504
United States	3	Unmitigated	0.75	N/A	331002647	293011173	2654410	12452700	4896026	3526519	1158327
United States Virgin Islands	3	Unmitigated	0.75	N/A	104423	86386	906	3982	1542	1203	383
Uruguay	3	Unmitigated	0.75	N/A	3473727	2939850	25683	113846	43823	33991	10775
Uzbekistan	3	Unmitigated	0.75	N/A	33469199	30264963	108671	748181	290785	144635	49554
Vanuatu	3	Unmitigated	0.75	N/A	307150	266284	810	5594	2322	1075	400
Venezuela (Bolivarian Republic of)	3	Unmitigated	0.75	N/A	28435943	25059281	128323	721896	276487	170283	56027
Vietnam	3	Unmitigated	0.75	N/A	97338583	87684406	517491	2938204	1200642	688330	242573
Western Sahara	3	Unmitigated	0.75	N/A	597330	551289	1634	12736	5146	2145	788
Yemen	3	Unmitigated	0.75	N/A	29825968	27588973	64276	464007	193408	85061	31918
Zambia	3	Unmitigated	0.75	N/A	18383956	16904019	30969	242707	101116	41261	15592
Zimbabwe	3	Unmitigated	0.75	N/A	14862927	13767424	32726	227962	95738	43543	16371
Afghanistan	3	0.2 deaths per 100,000 per week trigger	0.75	77.856682	38928341	2356700	7208	46011	13428	9773	2635
Albania	3	0.2 deaths per 100,000 per week trigger	0.75	5.7556	2877800	265461	1108	6952	2154	1461	407
Algeria	3	0.2 deaths per 100,000 per week trigger	0.75	87.702086	43851043	2492642	10318	69364	19733	13988	3637
Angola	3	0.2 deaths per 100,000 per week trigger	0.75	65.732536	32866268	3071870	7498	51736	15298	10029	2682

Antigua and Barbuda	3	0.2 deaths per 100,000 per week trigger	0.75	0.195856	97928	5533	21	122	35	27	7
Argentina	3	0.2 deaths per 100,000 per week trigger	0.75	90.391554	45195777	3687144	14481	80469	24605	19730	5435
Armenia	3	0.2 deaths per 100,000 per week trigger	0.75	5.926468	2963234	240499	876	5946	1774	1183	327
Aruba	3	0.2 deaths per 100,000 per week trigger	0.75	0.213532	106766	5844	29	158	47	38	10
Australia	3	0.2 deaths per 100,000 per week trigger	0.75	50.999762	25499881	1653688	6882	42721	12571	9146	2459
Austria	3	0.2 deaths per 100,000 per week trigger	0.75	18.0128	9006400	705814	3493	20782	6386	4844	1323
Azerbaijan	3	0.2 deaths per 100,000 per week trigger	0.75	20.27835	10139175	1047257	3236	23959	7297	4257	1193
Bahamas	3	0.2 deaths per 100,000 per week trigger	0.75	0.786496	393248	26871	90	565	170	121	33
Bahrain	3	0.2 deaths per 100,000 per week trigger	0.75	3.403166	1701583	154004	568	4458	1335	772	217
Bangladesh	3	0.2 deaths per 100,000 per week trigger	0.75	329.378766	164689383	12435481	52141	309764	90867	69062	17358
Barbados	3	0.2 deaths per 100,000 per week trigger	0.75	0.574742	287371	14296	83	398	119	108	28
Belarus	3	0.2 deaths per 100,000 per week trigger	0.75	18.898642	9449321	445504	1864	12121	3406	2574	644
Belgium	3	0.2 deaths per 100,000 per week trigger	0.75	23.179232	11589616	443856	2834	15709	4419	3770	980
Belize	3	0.2 deaths per 100,000 per week trigger	0.75	0.795242	397621	42732	114	736	230	151	42
Benin	3	0.2 deaths per 100,000 per week trigger	0.75	24.246396	12123198	1170030	3340	21847	6589	4458	1232
Bhutan	3	0.2 deaths per 100,000 per week trigger	0.75	1.543224	771612	63003	278	1575	460	364	95

Bolivia	3	0.2 deaths per 100,000 per week trigger	0.75	23.346058	11673029	808289	2401	14497	4265	3279	888
Bosnia and Herzegovina	3	0.2 deaths per 100,000 per week trigger	0.75	6.56163	3280815	151655	685	4267	1208	919	237
Botswana	3	0.2 deaths per 100,000 per week trigger	0.75	4.70325	2351625	183308	604	3833	1117	798	215
Brazil	3	0.2 deaths per 100,000 per week trigger	0.75	425.118818	212559409	11457197	44212	250182	72398	57423	15432
Brunei Darussalam	3	0.2 deaths per 100,000 per week trigger	0.75	0.874966	437483	30777	129	902	258	173	46
Bulgaria	3	0.2 deaths per 100,000 per week trigger	0.75	13.89689	6948445	360375	1764	10607	3112	2414	651
Burkina Faso	3	0.2 deaths per 100,000 per week trigger	0.75	41.806556	20903278	1425866	3570	24747	7083	4818	1243
Burundi	3	0.2 deaths per 100,000 per week trigger	0.75	23.781562	11890781	1184813	3005	20484	6234	3923	1062
Cabo Verde	3	0.2 deaths per 100,000 per week trigger	0.75	1.111976	555988	38566	142	877	254	189	48
Cambodia	3	0.2 deaths per 100,000 per week trigger	0.75	33.437942	16718971	1109183	4116	29104	8344	5480	1510
Cameroon	3	0.2 deaths per 100,000 per week trigger	0.75	53.091728	26545864	2488091	6570	44774	13526	8637	2422
Canada	3	0.2 deaths per 100,000 per week trigger	0.75	75.484314	37742157	1462914	8143	45625	13023	10598	2740
Central African Republic	3	0.2 deaths per 100,000 per week trigger	0.75	9.659528	4829764	462645	1242	8117	2433	1597	433
Chad	3	0.2 deaths per 100,000 per week trigger	0.75	32.851718	16425859	1965297	4915	33291	10422	6606	1928
Channel Islands	3	0.2 deaths per 100,000 per week trigger	0.75	0.347718	173859	14046	67	395	120	87	24
Chile	3	0.2 deaths per 100,000 per week trigger	0.75	38.232418	19116209	1303428	5733	31399	9571	7610	2052

China	3	0.2 deaths per 100,000 per week trigger	0.75	2878.647548	1439323774	43323214	219209	1445585	397037	301466	74053
Hong Kong SAR, China	3	0.2 deaths per 100,000 per week trigger	0.75	14.993976	7496988	206314	2417	10841	3103	3265	813
Macao SAR, China	3	0.2 deaths per 100,000 per week trigger	0.75	1.298684	649342	37169	201	1291	369	269	69
China, Taiwan Province of China	3	0.2 deaths per 100,000 per week trigger	0.75	47.63355	23816775	928287	5485	32690	8987	7126	1739
Colombia	3	0.2 deaths per 100,000 per week trigger	0.75	101.765768	50882884	3111145	11166	64766	18982	14603	3737
Comoros	3	0.2 deaths per 100,000 per week trigger	0.75	1.73919	869595	52657	147	1000	285	193	51
Congo, Rep.	3	0.2 deaths per 100,000 per week trigger	0.75	11.036184	5518092	690619	1864	12887	4018	2483	715
Costa Rica	3	0.2 deaths per 100,000 per week trigger	0.75	10.188228	5094114	267395	1024	6020	1778	1425	378
Cote d'Ivoire	3	0.2 deaths per 100,000 per week trigger	0.75	52.75655	26378275	1992505	5390	36328	10623	7126	1927
Croatia	3	0.2 deaths per 100,000 per week trigger	0.75	8.210536	4105268	152837	760	4416	1302	1038	281
Cuba	3	0.2 deaths per 100,000 per week trigger	0.75	22.653232	11326616	600215	3331	17219	5240	4476	1224
Curacao	3	0.2 deaths per 100,000 per week trigger	0.75	0.3282	164100	7051	40	198	58	53	14
Cyprus	3	0.2 deaths per 100,000 per week trigger	0.75	2.414722	1207361	81404	327	2069	621	432	119
Czechia	3	0.2 deaths per 100,000 per week trigger	0.75	21.417964	10708982	845363	4013	24319	7369	5356	1439
Korea, Dem. People's Rep.	3	0.2 deaths per 100,000 per week trigger	0.75	51.55763	25778815	1525316	7350	47864	13646	9729	2482
Congo, Dem. Rep.	3	0.2 deaths per 100,000 per week trigger	0.75	179.122808	89561404	9608461	26506	171344	52196	35292	10103

Denmark	3	0.2 deaths per 100,000 per week trigger	0.75	11.584406	5792203	279819	1335	7792	2234	1777	462
Djibouti	3	0.2 deaths per 100,000 per week trigger	0.75	1.976004	988002	97837	331	2177	662	442	124
Dominican Republic	3	0.2 deaths per 100,000 per week trigger	0.75	21.695808	10847904	936302	2994	17867	5439	3987	1103
Ecuador	3	0.2 deaths per 100,000 per week trigger	0.75	35.28612	17643060	1418198	4486	26963	8259	5868	1653
Egypt	3	0.2 deaths per 100,000 per week trigger	0.75	204.668806	102334403	7296397	26428	164636	48159	35142	9231
El Salvador	3	0.2 deaths per 100,000 per week trigger	0.75	12.972402	6486201	733049	2483	14392	4655	3349	943
Equatorial Guinea	3	0.2 deaths per 100,000 per week trigger	0.75	2.80597	1402985	129217	334	2365	699	439	120
Eritrea	3	0.2 deaths per 100,000 per week trigger	0.75	7.092854	3546427	172518	554	3377	959	733	191
Estonia	3	0.2 deaths per 100,000 per week trigger	0.75	2.653078	1326539	76776	382	2171	641	511	132
Eswatini	3	0.2 deaths per 100,000 per week trigger	0.75	2.320328	1160164	101459	314	1963	575	415	113
Ethiopia	3	0.2 deaths per 100,000 per week trigger	0.75	229.927166	114963583	8886668	25562	167508	48946	34794	9375
Fiji	3	0.2 deaths per 100,000 per week trigger	0.75	1.792888	896444	50580	203	1397	399	267	70
Finland	3	0.2 deaths per 100,000 per week trigger	0.75	11.081436	5540718	271929	1329	8336	2389	1781	458
France	3	0.2 deaths per 100,000 per week trigger	0.75	130.547024	65273512	2765828	21517	108207	30894	29128	7317
French Guiana	3	0.2 deaths per 100,000 per week trigger	0.75	0.597364	298682	44694	122	807	270	163	49
French Polynesia	3	0.2 deaths per 100,000 per week trigger	0.75	0.561808	280904	19420	92	587	170	119	32

Gabon	3	0.2 deaths per 100,000 per week trigger	0.75	4.451456	2225728	137700	416	2771	783	552	142
Gambia	3	0.2 deaths per 100,000 per week trigger	0.75	4.833328	2416664	231794	602	4055	1214	775	219
Georgia	3	0.2 deaths per 100,000 per week trigger	0.75	7.97835	3989175	327649	1371	8578	2579	1820	509
Germany	3	0.2 deaths per 100,000 per week trigger	0.75	167.56789	83783945	3266137	22571	119882	33878	29014	7587
Ghana	3	0.2 deaths per 100,000 per week trigger	0.75	62.14589	31072945	2554925	7260	50368	14760	9743	2680
Greece	3	0.2 deaths per 100,000 per week trigger	0.75	20.846112	10423056	634950	3451	19408	5776	4664	1269
Grenada	3	0.2 deaths per 100,000 per week trigger	0.75	0.225038	112519	7649	29	165	50	38	11
Guadeloupe	3	0.2 deaths per 100,000 per week trigger	0.75	0.800254	400127	19589	122	565	169	160	43
Guam	3	0.2 deaths per 100,000 per week trigger	0.75	0.337566	168783	6947	33	205	58	42	11
Guatemala	3	0.2 deaths per 100,000 per week trigger	0.75	35.831134	17915567	1751609	4546	28109	8513	5923	1646
Guinea	3	0.2 deaths per 100,000 per week trigger	0.75	26.265584	13132792	1503365	4093	26674	8327	5349	1542
Guinea-Bissau	3	0.2 deaths per 100,000 per week trigger	0.75	3.935996	1967998	179138	482	3257	975	640	175
Guyana	3	0.2 deaths per 100,000 per week trigger	0.75	1.573118	786559	72118	217	1365	417	292	81
Haiti	3	0.2 deaths per 100,000 per week trigger	0.75	22.805066	11402533	1157945	2964	19275	5942	3954	1100
Honduras	3	0.2 deaths per 100,000 per week trigger	0.75	19.809216	9904608	1257000	3334	21041	6770	4395	1325
Hungary	3	0.2 deaths per 100,000 per week trigger	0.75	19.3207	9660350	953786	4702	27799	8812	6220	1785

Iceland	3	0.2 deaths per 100,000 per week trigger	0.75	0.6825	341250	22461	94	570	169	122	33
India	3	0.2 deaths per 100,000 per week trigger	0.75	2760.00877	1380004385	73837342	325819	1907028	555521	430975	115920
Indonesia	3	0.2 deaths per 100,000 per week trigger	0.75	547.047242	273523621	14016991	58302	402381	111762	76764	19065
Iran (Islamic Republic of)	3	0.2 deaths per 100,000 per week trigger	0.75	167.985906	83992953	5079751	22115	150053	42954	29259	7790
Iraq	3	0.2 deaths per 100,000 per week trigger	0.75	80.445006	40222503	3532588	11421	82975	24962	15417	4332
Ireland	3	0.2 deaths per 100,000 per week trigger	0.75	9.875592	4937796	268824	1055	6656	1911	1366	355
Israel	3	0.2 deaths per 100,000 per week trigger	0.75	17.311082	8655541	726102	2546	16312	4883	3437	914
Italy	3	0.2 deaths per 100,000 per week trigger	0.75	120.923656	60461828	2312595	15031	78619	22779	19414	5142
Jamaica	3	0.2 deaths per 100,000 per week trigger	0.75	5.922322	2961161	216973	781	4562	1370	1053	286
Japan	3	0.2 deaths per 100,000 per week trigger	0.75	252.952916	126476458	4858106	30506	154225	44646	40238	10232
Jordan	3	0.2 deaths per 100,000 per week trigger	0.75	20.40628	10203140	827991	2952	20763	6153	3889	1067
Kazakhstan	3	0.2 deaths per 100,000 per week trigger	0.75	37.553414	18776707	2026180	6159	44972	13885	8498	2425
Kenya	3	0.2 deaths per 100,000 per week trigger	0.75	107.5426	53771300	4757910	12421	86480	25633	16699	4511
Kiribati	3	0.2 deaths per 100,000 per week trigger	0.75	0.238892	119446	8630	31	217	63	40	10
Kuwait	3	0.2 deaths per 100,000 per week trigger	0.75	8.541126	4270563	189252	786	5930	1667	1049	273
Kyrgyz Republic	3	0.2 deaths per 100,000 per week trigger	0.75	13.048382	6524191	737551	1944	14494	4474	2630	746

Lao PDR	3	0.2 deaths per 100,000 per week trigger	0.75	14.551112	7275556	614707	2257	15693	4659	2866	790
Latvia	3	0.2 deaths per 100,000 per week trigger	0.75	3.772404	1886202	110624	561	3245	975	770	208
Lebanon	3	0.2 deaths per 100,000 per week trigger	0.75	13.650884	6825442	410756	1731	11626	3267	2319	613
Lesotho	3	0.2 deaths per 100,000 per week trigger	0.75	4.284504	2142252	143559	497	3102	898	657	172
Liberia	3	0.2 deaths per 100,000 per week trigger	0.75	10.115354	5057677	575509	1662	10827	3340	2202	602
Libya	3	0.2 deaths per 100,000 per week trigger	0.75	13.742574	6871287	359546	1400	9903	2825	1867	485
Lithuania	3	0.2 deaths per 100,000 per week trigger	0.75	5.444582	2722291	69988	353	2048	577	491	123
Luxembourg	3	0.2 deaths per 100,000 per week trigger	0.75	1.251952	625976	40120	167	1118	327	223	61
Madagascar	3	0.2 deaths per 100,000 per week trigger	0.75	55.382038	27691019	3037565	8563	56902	17553	11357	3193
Malawi	3	0.2 deaths per 100,000 per week trigger	0.75	38.25991	19129955	1997126	5118	34767	10490	6843	1918
Malaysia	3	0.2 deaths per 100,000 per week trigger	0.75	64.731996	32365998	1815886	7459	51485	14602	10053	2570
Maldives	3	0.2 deaths per 100,000 per week trigger	0.75	1.081084	540542	47105	174	1123	337	237	64
Mali	3	0.2 deaths per 100,000 per week trigger	0.75	40.501668	20250834	2111796	5245	35528	10824	7047	1941
Malta	3	0.2 deaths per 100,000 per week trigger	0.75	0.883078	441539	23586	117	690	203	158	43
Martinique	3	0.2 deaths per 100,000 per week trigger	0.75	0.75053	375265	8969	63	284	81	82	20
Mauritania	3	0.2 deaths per 100,000 per week trigger	0.75	9.29932	4649660	362912	1058	6931	2014	1410	372

Mauritius	3	0.2 deaths per 100,000 per week trigger	0.75	2.543534	1271767	70047	437	2286	655	572	145
Mayotte	3	0.2 deaths per 100,000 per week trigger	0.75	0.545626	272813	15779	53	317	89	69	17
Mexico	3	0.2 deaths per 100,000 per week trigger	0.75	257.865506	128932753	9610873	30280	188313	56653	40569	11230
Micronesia (Fed. States of)	3	0.2 deaths per 100,000 per week trigger	0.75	0.230042	115021	7468	27	192	55	35	9
Mongolia	3	0.2 deaths per 100,000 per week trigger	0.75	6.556584	3278292	230730	933	6360	1844	1187	319
Montenegro	3	0.2 deaths per 100,000 per week trigger	0.75	1.256124	628062	50183	211	1322	398	280	78
Morocco	3	0.2 deaths per 100,000 per week trigger	0.75	73.821116	36910558	1781274	7301	50495	14000	9796	2549
Mozambique	3	0.2 deaths per 100,000 per week trigger	0.75	62.51087	31255435	3426897	9088	60387	18624	12258	3527
Myanmar	3	0.2 deaths per 100,000 per week trigger	0.75	108.819588	54409794	3830050	15654	108069	30899	20793	5444
Namibia	3	0.2 deaths per 100,000 per week trigger	0.75	5.081832	2540916	252679	762	4986	1513	1029	282
Nepal	3	0.2 deaths per 100,000 per week trigger	0.75	58.273616	29136808	2329717	9474	56327	16446	12632	3294
Netherlands	3	0.2 deaths per 100,000 per week trigger	0.75	34.269746	17134873	683884	3293	19461	5565	4400	1135
New Caledonia	3	0.2 deaths per 100,000 per week trigger	0.75	0.570982	285491	22919	106	700	207	144	39
New Zealand	3	0.2 deaths per 100,000 per week trigger	0.75	9.644466	4822233	275888	1179	7229	2113	1568	421
Nicaragua	3	0.2 deaths per 100,000 per week trigger	0.75	13.249108	6624554	729773	2025	12824	4067	2659	757
Niger	3	0.2 deaths per 100,000 per week trigger	0.75	48.413272	24206636	2324664	5788	38892	11875	7769	2177

Nigeria	3	0.2 deaths per 100,000 per week trigger	0.75	412.279174	206139587	21276955	55101	381801	115345	73035	20325
North Macedonia	3	0.2 deaths per 100,000 per week trigger	0.75	4.16676	2083380	162610	649	4294	1310	871	239
Norway	3	0.2 deaths per 100,000 per week trigger	0.75	10.842484	5421242	316775	1435	8422	2473	1829	482
Oman	3	0.2 deaths per 100,000 per week trigger	0.75	10.213244	5106622	296665	1066	8119	2343	1394	369
Pakistan	3	0.2 deaths per 100,000 per week trigger	0.75	441.784662	220892331	19380541	73115	434109	130779	95348	26032
Panama	3	0.2 deaths per 100,000 per week trigger	0.75	8.629536	4314768	308201	1059	6155	1844	1402	379
Papua New Guinea	3	0.2 deaths per 100,000 per week trigger	0.75	17.894054	8947027	881597	2982	21592	6576	3931	1099
Paraguay	3	0.2 deaths per 100,000 per week trigger	0.75	14.26506	7132530	450072	1289	7993	2295	1699	430
Peru	3	0.2 deaths per 100,000 per week trigger	0.75	65.943692	32971846	2833164	9730	58124	18167	12965	3620
Philippines	3	0.2 deaths per 100,000 per week trigger	0.75	219.16217	109581085	4994311	18842	132434	36480	25379	6580
Poland	3	0.2 deaths per 100,000 per week trigger	0.75	75.69321	37846605	1377467	6867	42434	12084	9320	2354
Portugal	3	0.2 deaths per 100,000 per week trigger	0.75	20.393414	10196707	698261	3816	21390	6455	5138	1365
Puerto Rico	3	0.2 deaths per 100,000 per week trigger	0.75	5.72168	2860840	145927	987	4515	1371	1306	347
Qatar	3	0.2 deaths per 100,000 per week trigger	0.75	5.76212	2881060	160624	577	4648	1340	776	216
Korea, Rep.	3	0.2 deaths per 100,000 per week trigger	0.75	102.538366	51269183	3900608	17913	112810	34189	23681	6673
Moldova	3	0.2 deaths per 100,000 per week trigger	0.75	8.067926	4033963	162118	592	4111	1185	784	202

Réunion	3	0.2 deaths per 100,000 per week trigger	0.75	1.790616	895308	41543	181	982	290	242	64
Romania	3	0.2 deaths per 100,000 per week trigger	0.75	38.475364	19237682	669290	3055	19101	5441	4231	1098
Russian Federation	3	0.2 deaths per 100,000 per week trigger	0.75	291.86892	145934460	12135910	50698	326886	98593	68903	19023
Rwanda	3	0.2 deaths per 100,000 per week trigger	0.75	25.904418	12952209	1513443	4145	28570	8837	5631	1588
St. Lucia	3	0.2 deaths per 100,000 per week trigger	0.75	0.367258	183629	13935	58	328	101	77	21
St. Vincent and the Grenadines	3	0.2 deaths per 100,000 per week trigger	0.75	0.221894	110947	8052	31	178	54	41	11
Samoa	3	0.2 deaths per 100,000 per week trigger	0.75	0.39682	198410	12337	45	313	89	60	16
Sao Tome and Principe	3	0.2 deaths per 100,000 per week trigger	0.75	0.438322	219161	25564	73	475	148	97	28
Saudi Arabia	3	0.2 deaths per 100,000 per week trigger	0.75	69.627734	34813867	1668281	6500	47726	13281	8643	2216
Senegal	3	0.2 deaths per 100,000 per week trigger	0.75	33.48786	16743930	1011591	2814	18669	5416	3706	971
Serbia	3	0.2 deaths per 100,000 per week trigger	0.75	17.47474	8737370	539924	2423	14674	4361	3243	875
Seychelles	3	0.2 deaths per 100,000 per week trigger	0.75	0.19668	98340	5777	29	165	48	39	10
Sierra Leone	3	0.2 deaths per 100,000 per week trigger	0.75	15.95397	7976985	478061	1337	8881	2532	1772	457
Singapore	3	0.2 deaths per 100,000 per week trigger	0.75	11.700686	5850343	165717	1641	8120	2232	2137	520
Slovakia	3	0.2 deaths per 100,000 per week trigger	0.75	10.919286	5459643	408089	1763	11182	3323	2323	612
Slovenia	3	0.2 deaths per 100,000 per week trigger	0.75	4.157864	2078932	73220	364	2122	605	473	125

Solomon Islands	3	0.2 deaths per 100,000 per week trigger	0.75	1.373756	686878	49173	167	1168	343	218	59
Somalia	3	0.2 deaths per 100,000 per week trigger	0.75	31.786438	15893219	1706656	4554	29880	9279	5979	1737
South Africa	3	0.2 deaths per 100,000 per week trigger	0.75	118.61738	59308690	3961213	15438	106575	30600	20263	5362
South Sudan	3	0.2 deaths per 100,000 per week trigger	0.75	22.387458	11193729	763103	2197	14264	4073	2891	790
Spain	3	0.2 deaths per 100,000 per week trigger	0.75	93.509566	46754783	3300993	17058	97423	29353	22367	6118
Sri Lanka	3	0.2 deaths per 100,000 per week trigger	0.75	42.8265	21413250	1253461	6961	37859	10936	9201	2452
State of Palestine	3	0.2 deaths per 100,000 per week trigger	0.75	10.202832	5101416	306610	990	7193	2088	1318	357
Sudan	3	0.2 deaths per 100,000 per week trigger	0.75	87.698538	43849269	4131092	12370	80003	23867	16327	4413
Suriname	3	0.2 deaths per 100,000 per week trigger	0.75	1.173268	586634	58455	185	1137	352	243	69
Sweden	3	0.2 deaths per 100,000 per week trigger	0.75	20.19854	10099270	701216	3395	19513	5791	4570	1203
Switzerland	3	0.2 deaths per 100,000 per week trigger	0.75	17.309236	8654618	431744	2053	12306	3535	2772	727
Syrian Arab Republic	3	0.2 deaths per 100,000 per week trigger	0.75	35.001314	17500657	1203371	4190	26658	7701	5687	1543
Tajikistan	3	0.2 deaths per 100,000 per week trigger	0.75	19.075284	9537642	839624	1931	15234	4524	2503	676
Thailand	3	0.2 deaths per 100,000 per week trigger	0.75	139.599956	69799978	3191552	17059	106881	30464	22552	5902
Timor-Leste	3	0.2 deaths per 100,000 per week trigger	0.75	2.636884	1318442	105737	350	2494	747	470	130
Togo	3	0.2 deaths per 100,000 per week trigger	0.75	16.557474	8278737	827218	2251	15316	4608	2961	836

Tonga	3	0.2 deaths per 100,000 per week trigger	0.75	0.211394	105697	6215	23	156	45	31	8
Trinidad and Tobago	3	0.2 deaths per 100,000 per week trigger	0.75	2.798982	1399491	125306	526	3013	945	709	195
Tunisia	3	0.2 deaths per 100,000 per week trigger	0.75	23.637236	11818618	548760	2727	15231	4282	3546	903
Turkey	3	0.2 deaths per 100,000 per week trigger	0.75	168.678134	84339067	10571366	34178	238983	75631	44539	12891
Turkmenistan	3	0.2 deaths per 100,000 per week trigger	0.75	12.062374	6031187	582026	1531	11520	3396	2021	562
Uganda	3	0.2 deaths per 100,000 per week trigger	0.75	91.482	45741000	6292587	14564	102499	33009	19271	5752
Ukraine	3	0.2 deaths per 100,000 per week trigger	0.75	87.467518	43733759	2725250	12202	75679	22250	15891	4292
United Arab Emirates	3	0.2 deaths per 100,000 per week trigger	0.75	19.7808	9890400	786349	2664	22326	6620	3579	1041
United Kingdom	3	0.2 deaths per 100,000 per week trigger	0.75	135.772008	67886004	3805866	21825	117324	34153	28865	7386
Tanzania	3	0.2 deaths per 100,000 per week trigger	0.75	119.468426	59734213	7095696	18589	125484	39275	24781	7153
United States	3	0.2 deaths per 100,000 per week trigger	0.75	662.005294	331002647	16267291	84124	481013	135695	112144	28632
United States Virgin Islands	3	0.2 deaths per 100,000 per week trigger	0.75	0.208846	104423	6426	39	185	56	51	14
Uruguay	3	0.2 deaths per 100,000 per week trigger	0.75	6.947454	3473727	237372	1209	5944	1830	1611	438
Uzbekistan	3	0.2 deaths per 100,000 per week trigger	0.75	66.938398	33469199	2422678	6356	48914	14025	8573	2385
Vanuatu	3	0.2 deaths per 100,000 per week trigger	0.75	0.6143	307150	17754	61	431	121	80	21
Venezuela (Bolivarian Republic of)	3	0.2 deaths per 100,000 per week trigger	0.75	56.871886	28435943	1930068	6081	37577	11208	8053	2124

Vietnam	3	0.2 deaths per 100,000 per week trigger	0.75	194.677166	97338583	5574980	25994	166984	48139	34180	9077
Western Sahara	3	0.2 deaths per 100,000 per week trigger	0.75	1.19466	597330	55077	170	1216	360	223	62
Yemen	3	0.2 deaths per 100,000 per week trigger	0.75	59.651936	29825968	2516141	6630	45759	13635	9107	2504
Zambia	3	0.2 deaths per 100,000 per week trigger	0.75	36.767912	18383956	2181808	5234	36604	11360	7024	2070
Zimbabwe	3	0.2 deaths per 100,000 per week trigger	0.75	29.725854	14862927	1600768	4462	28960	8890	5826	1640
Afghanistan	3	1.6 deaths per 100,000 per week trigger	0.75	622.853456	38928341	12492541	35740	231028	93549	46671	17463
Albania	3	1.6 deaths per 100,000 per week trigger	0.75	46.0448	2877800	791708	3678	22518	9011	4908	1772
Algeria	3	1.6 deaths per 100,000 per week trigger	0.75	701.616688	43851043	11844178	51775	335362	129273	68660	23767
Angola	3	1.6 deaths per 100,000 per week trigger	0.75	525.860288	32866268	14015899	32104	228330	101100	42628	17255
Antigua and Barbuda	3	1.6 deaths per 100,000 per week trigger	0.75	1.566848	97928	16595	67	388	139	87	29
Argentina	3	1.6 deaths per 100,000 per week trigger	0.75	723.132432	45195777	10771885	47284	252448	98319	63057	22150
Armenia	3	1.6 deaths per 100,000 per week trigger	0.75	47.411744	2963234	882093	3757	23823	9476	5010	1809
Aruba	3	1.6 deaths per 100,000 per week trigger	0.75	1.708256	106766	24558	137	728	290	180	65
Australia	3	1.6 deaths per 100,000 per week trigger	0.75	407.998096	25499881	6364134	30453	178754	68468	40158	13846
Austria	3	1.6 deaths per 100,000 per week trigger	0.75	144.1024	9006400	1787891	9925	56037	20875	13144	4403
Azerbaijan	3	1.6 deaths per 100,000 per week trigger	0.75	162.2268	10139175	3751721	12999	92747	38780	17181	6550

Bahamas	3	1.6 deaths per 100,000 per week trigger	0.75	6.291968	393248	129218	505	3078	1301	662	256
Bahrain	3	1.6 deaths per 100,000 per week trigger	0.75	27.225328	1701583	653420	2256	18215	7711	2974	1189
Bangladesh	3	1.6 deaths per 100,000 per week trigger	0.75	2635.030128	164689383	47869670	197281	1198445	461468	264277	94005
Barbados	3	1.6 deaths per 100,000 per week trigger	0.75	4.597936	287371	74457	479	2325	973	642	238
Belarus	3	1.6 deaths per 100,000 per week trigger	0.75	151.189136	9449321	2857561	14226	86703	35254	18898	6871
Belgium	3	1.6 deaths per 100,000 per week trigger	0.75	185.433856	11589616	3062489	21190	113802	43671	28298	9692
Belize	3	1.6 deaths per 100,000 per week trigger	0.75	6.361936	397621	144480	432	2726	1167	566	222
Benin	3	1.6 deaths per 100,000 per week trigger	0.75	193.971168	12123198	3863962	10842	71305	28402	14447	5238
Bhutan	3	1.6 deaths per 100,000 per week trigger	0.75	12.345792	771612	168830	741	4252	1517	975	316
Bolivia	3	1.6 deaths per 100,000 per week trigger	0.75	186.768464	11673029	3074798	10069	59263	23009	13566	4716
Bosnia and Herzegovina	3	1.6 deaths per 100,000 per week trigger	0.75	52.49304	3280815	676479	3414	20763	7701	4539	1536
Botswana	3	1.6 deaths per 100,000 per week trigger	0.75	37.626	2351625	780948	2573	16672	6659	3406	1241
Brazil	3	1.6 deaths per 100,000 per week trigger	0.75	3400.950544	212559409	49599016	206087	1182457	460361	272916	97044
Brunei Darussalam	3	1.6 deaths per 100,000 per week trigger	0.75	6.999728	437483	151838	659	4520	1832	869	327
Bulgaria	3	1.6 deaths per 100,000 per week trigger	0.75	111.17512	6948445	1266255	6892	39682	14341	9113	2961
Burkina Faso	3	1.6 deaths per 100,000 per week trigger	0.75	334.452448	20903278	7192476	17149	121390	49519	22848	8596

Burundi	3	1.6 deaths per 100,000 per week trigger	0.75	190.252496	11890781	5194180	12284	85246	37615	16039	6586
Cabo Verde	3	1.6 deaths per 100,000 per week trigger	0.75	8.895808	555988	198436	754	4596	1884	978	367
Cambodia	3	1.6 deaths per 100,000 per week trigger	0.75	267.503536	16718971	5379295	20144	139277	56525	26309	9888
Cameroon	3	1.6 deaths per 100,000 per week trigger	0.75	424.733824	26545864	10545849	26251	183822	78484	34822	13670
Canada	3	1.6 deaths per 100,000 per week trigger	0.75	603.874512	37742157	7547937	45828	250266	91140	61084	20043
Central African Republic	3	1.6 deaths per 100,000 per week trigger	0.75	77.276224	4829764	1580702	4035	27236	10937	5392	1983
Chad	3	1.6 deaths per 100,000 per week trigger	0.75	262.813744	16425859	7074291	16469	113850	50849	21889	9052
Channel Islands	3	1.6 deaths per 100,000 per week trigger	0.75	2.781744	173859	47063	256	1471	592	340	121
Chile	3	1.6 deaths per 100,000 per week trigger	0.75	305.859344	19116209	4898942	24532	131122	53701	32685	11912
China	3	1.6 deaths per 100,000 per week trigger	0.75	23029.18038	1439323774	380818659	2130919	13249047	4980780	2800348	949378
Hong Kong SAR, China	3	1.6 deaths per 100,000 per week trigger	0.75	119.951808	7496988	840739	9863	44260	14154	13147	3627
Macao SAR, China	3	1.6 deaths per 100,000 per week trigger	0.75	10.389472	649342	153824	913	5637	2061	1196	394
China, Taiwan Province of China	3	1.6 deaths per 100,000 per week trigger	0.75	381.0684	23816775	5556825	35834	208541	76920	47263	15469
Colombia	3	1.6 deaths per 100,000 per week trigger	0.75	814.126144	50882884	9396845	35399	207846	75289	47565	15888
Comoros	3	1.6 deaths per 100,000 per week trigger	0.75	13.91352	869595	307732	846	5788	2365	1121	426
Congo, Rep.	3	1.6 deaths per 100,000 per week trigger	0.75	88.289472	5518092	1838083	4813	33729	13567	6384	2366

Costa Rica	3	1.6 deaths per 100,000 per week trigger	0.75	81.505824	5094114	1230399	5414	30053	11874	7166	2549
Cote d'Ivoire	3	1.6 deaths per 100,000 per week trigger	0.75	422.0524	26378275	10180735	26328	181242	76759	34727	13706
Croatia	3	1.6 deaths per 100,000 per week trigger	0.75	65.684288	4105268	1124214	6799	37028	15101	8977	3253
Cuba	3	1.6 deaths per 100,000 per week trigger	0.75	181.225856	11326616	2672000	16875	84833	34586	22555	8152
Curacao	3	1.6 deaths per 100,000 per week trigger	0.75	2.6256	164100	29401	181	890	333	242	81
Cyprus	3	1.6 deaths per 100,000 per week trigger	0.75	19.317776	1207361	345825	1575	9775	3926	2110	766
Czechia	3	1.6 deaths per 100,000 per week trigger	0.75	171.343712	10708982	2121329	10909	64506	23910	14468	4853
Korea, Dem. People's Rep.	3	1.6 deaths per 100,000 per week trigger	0.75	412.46104	25778815	8429296	44555	276117	110979	58064	21202
Congo, Dem. Rep.	3	1.6 deaths per 100,000 per week trigger	0.75	1432.982464	89561404	28893669	76526	507173	203394	101773	37361
Denmark	3	1.6 deaths per 100,000 per week trigger	0.75	92.675248	5792203	1705374	9853	53966	22362	13079	4799
Djibouti	3	1.6 deaths per 100,000 per week trigger	0.75	15.808032	988002	338221	1189	7682	3108	1580	588
Dominican Republic	3	1.6 deaths per 100,000 per week trigger	0.75	173.566464	10847904	3283642	11722	68431	27936	15264	5641
Ecuador	3	1.6 deaths per 100,000 per week trigger	0.75	282.28896	17643060	4848509	16951	100219	39984	22497	8064
Egypt	3	1.6 deaths per 100,000 per week trigger	0.75	1637.350448	102334403	37489323	139958	858847	358534	184412	70038
El Salvador	3	1.6 deaths per 100,000 per week trigger	0.75	103.779216	6486201	1827341	6837	38772	15624	9006	3267
Equatorial Guinea	3	1.6 deaths per 100,000 per week trigger	0.75	22.44776	1402985	621768	1460	10889	4834	1945	798

Eritrea	3	1.6 deaths per 100,000 per week trigger	0.75	56.742832	3546427	1155789	3699	22669	9046	4934	1802
Estonia	3	1.6 deaths per 100,000 per week trigger	0.75	21.224624	1326539	363654	2083	11480	4646	2789	1000
Eswatini	3	1.6 deaths per 100,000 per week trigger	0.75	18.562624	1160164	368473	1134	7068	2788	1483	537
Ethiopia	3	1.6 deaths per 100,000 per week trigger	0.75	1839.417328	114963583	46677790	135203	866170	374982	176975	69858
Fiji	3	1.6 deaths per 100,000 per week trigger	0.75	14.343104	896444	271813	1126	7624	2996	1487	537
Finland	3	1.6 deaths per 100,000 per week trigger	0.75	88.651488	5540718	1288219	7089	42430	16083	9455	3211
France	3	1.6 deaths per 100,000 per week trigger	0.75	1044.376192	65273512	13733534	114526	555458	201193	153311	49037
French Guiana	3	1.6 deaths per 100,000 per week trigger	0.75	4.778912	298682	145604	456	2980	1387	610	259
French Polynesia	3	1.6 deaths per 100,000 per week trigger	0.75	4.494464	280904	77910	382	2440	931	504	173
Gabon	3	1.6 deaths per 100,000 per week trigger	0.75	35.611648	2225728	684128	2041	13636	5360	2705	978
Gambia	3	1.6 deaths per 100,000 per week trigger	0.75	38.666624	2416664	1014433	2430	17068	7485	3221	1307
Georgia	3	1.6 deaths per 100,000 per week trigger	0.75	63.8268	3989175	924779	4251	25739	9726	5629	1910
Germany	3	1.6 deaths per 100,000 per week trigger	0.75	1340.54312	83783945	19939326	148683	775379	291551	196038	65909
Ghana	3	1.6 deaths per 100,000 per week trigger	0.75	497.16712	31072945	13380089	37539	261535	114101	49481	19926
Greece	3	1.6 deaths per 100,000 per week trigger	0.75	166.768896	10423056	1842494	10957	59315	21621	14582	4703
Grenada	3	1.6 deaths per 100,000 per week trigger	0.75	1.800304	112519	24762	102	577	222	136	47

Guadeloupe	3	1.6 deaths per 100,000 per week trigger	0.75	6.402032	400127	81345	557	2568	999	737	250
Guam	3	1.6 deaths per 100,000 per week trigger	0.75	2.700528	168783	45242	227	1396	533	303	104
Guatemala	3	1.6 deaths per 100,000 per week trigger	0.75	286.649072	17915567	6988370	19902	122558	53516	26038	10274
Guinea	3	1.6 deaths per 100,000 per week trigger	0.75	210.124672	13132792	5031293	13072	88026	37264	17235	6691
Guinea-Bissau	3	1.6 deaths per 100,000 per week trigger	0.75	31.487968	1967998	713806	1838	12684	5267	2429	935
Guyana	3	1.6 deaths per 100,000 per week trigger	0.75	12.584944	786559	257585	887	5376	2241	1188	450
Haiti	3	1.6 deaths per 100,000 per week trigger	0.75	182.440528	11402533	4898851	14297	90639	40742	18858	7735
Honduras	3	1.6 deaths per 100,000 per week trigger	0.75	158.473728	9904608	3025648	8515	53725	21712	11320	4192
Hungary	3	1.6 deaths per 100,000 per week trigger	0.75	154.5656	9660350	3438229	20378	114235	50010	26997	10707
Iceland	3	1.6 deaths per 100,000 per week trigger	0.75	5.46	341250	103217	496	2932	1191	657	242
India	3	1.6 deaths per 100,000 per week trigger	0.75	22080.07016	1380004385	468704827	2085532	12362838	4974364	2783469	1007196
Indonesia	3	1.6 deaths per 100,000 per week trigger	0.75	4376.377936	273523621	78845604	339539	2271395	871351	447211	155293
Iran (Islamic Republic of)	3	1.6 deaths per 100,000 per week trigger	0.75	1343.887248	83992953	33682850	152376	1005480	432018	200130	78533
Iraq	3	1.6 deaths per 100,000 per week trigger	0.75	643.560048	40222503	10036401	32114	232132	88687	42873	15055
Ireland	3	1.6 deaths per 100,000 per week trigger	0.75	79.004736	4937796	1455362	6500	40200	15961	8666	3087
Israel	3	1.6 deaths per 100,000 per week trigger	0.75	138.488656	8655541	2860327	11521	69964	28771	15296	5629

Italy	3	1.6 deaths per 100,000 per week trigger	0.75	967.389248	60461828	17264552	131378	672505	286619	174750	65997
Jamaica	3	1.6 deaths per 100,000 per week trigger	0.75	47.378576	2961161	509114	1930	11144	3980	2581	832
Japan	3	1.6 deaths per 100,000 per week trigger	0.75	2023.623328	126476458	20610477	143380	712619	261598	189285	60886
Jordan	3	1.6 deaths per 100,000 per week trigger	0.75	163.25024	10203140	3097101	10769	76567	30458	14487	5258
Kazakhstan	3	1.6 deaths per 100,000 per week trigger	0.75	300.427312	18776707	6503419	22379	153002	62429	29560	10737
Kenya	3	1.6 deaths per 100,000 per week trigger	0.75	860.3408	53771300	23561108	57613	417515	182519	76014	30682
Kiribati	3	1.6 deaths per 100,000 per week trigger	0.75	1.911136	119446	41719	148	1038	435	196	76
Kuwait	3	1.6 deaths per 100,000 per week trigger	0.75	68.329008	4270563	1315322	5133	40669	16094	6858	2575
Kyrgyz Republic	3	1.6 deaths per 100,000 per week trigger	0.75	104.387056	6524191	1828711	5026	37454	14246	6582	2315
Lao PDR	3	1.6 deaths per 100,000 per week trigger	0.75	116.408896	7275556	2746538	9767	68221	29128	12921	5041
Latvia	3	1.6 deaths per 100,000 per week trigger	0.75	30.179232	1886202	522127	3102	17072	6972	4132	1511
Lebanon	3	1.6 deaths per 100,000 per week trigger	0.75	109.207072	6825442	1850338	8260	53921	20498	11106	3841
Lesotho	3	1.6 deaths per 100,000 per week trigger	0.75	34.276032	2142252	808346	2883	17652	7428	3763	1441
Liberia	3	1.6 deaths per 100,000 per week trigger	0.75	80.922832	5057677	1746870	4963	32778	13278	6585	2442
Libya	3	1.6 deaths per 100,000 per week trigger	0.75	109.940592	6871287	2158800	8438	59047	23392	11109	4072
Lithuania	3	1.6 deaths per 100,000 per week trigger	0.75	43.556656	2722291	708891	4344	23384	9399	5732	2035

Luxembourg	3	1.6 deaths per 100,000 per week trigger	0.75	10.015616	625976	200400	966	6079	2480	1277	471
Madagascar	3	1.6 deaths per 100,000 per week trigger	0.75	443.056304	27691019	8699415	24189	161533	63620	31637	11463
Malawi	3	1.6 deaths per 100,000 per week trigger	0.75	306.07928	19129955	8072850	19646	136616	59517	26389	10661
Malaysia	3	1.6 deaths per 100,000 per week trigger	0.75	517.855968	32365998	9817405	42814	286865	112648	57535	20674
Maldives	3	1.6 deaths per 100,000 per week trigger	0.75	8.648672	540542	182826	671	4374	1786	891	335
Mali	3	1.6 deaths per 100,000 per week trigger	0.75	324.013344	20250834	10462673	23986	168237	78644	31680	13616
Malta	3	1.6 deaths per 100,000 per week trigger	0.75	7.064624	441539	120540	708	3900	1588	929	343
Martinique	3	1.6 deaths per 100,000 per week trigger	0.75	6.00424	375265	84017	674	2980	1213	892	323
Mauritania	3	1.6 deaths per 100,000 per week trigger	0.75	74.39456	4649660	1810801	5092	34416	14634	6713	2636
Mauritius	3	1.6 deaths per 100,000 per week trigger	0.75	20.348272	1271767	325976	2102	11072	4242	2782	962
Mayotte	3	1.6 deaths per 100,000 per week trigger	0.75	4.365008	272813	75183	244	1502	572	324	112
Mexico	3	1.6 deaths per 100,000 per week trigger	0.75	2062.924048	128932753	49765567	187758	1108957	491253	249163	100129
Micronesia (Fed. States of)	3	1.6 deaths per 100,000 per week trigger	0.75	1.840336	115021	32245	114	827	321	151	54
Mongolia	3	1.6 deaths per 100,000 per week trigger	0.75	52.452672	3278292	911590	3555	25239	9742	4762	1683
Montenegro	3	1.6 deaths per 100,000 per week trigger	0.75	10.048992	628062	138564	638	3886	1440	838	277
Morocco	3	1.6 deaths per 100,000 per week trigger	0.75	590.568928	36910558	10337466	46280	301345	115969	61940	21722

Mozambique	3	1.6 deaths per 100,000 per week trigger	0.75	500.08696	31255435	11654408	29794	200397	83860	39646	15407
Myanmar	3	1.6 deaths per 100,000 per week trigger	0.75	870.556704	54409794	13282160	55193	375585	137153	72825	24079
Namibia	3	1.6 deaths per 100,000 per week trigger	0.75	40.654656	2540916	934800	2801	18451	7656	3736	1442
Nepal	3	1.6 deaths per 100,000 per week trigger	0.75	466.188928	29136808	8087491	33206	195807	74733	44005	15224
Netherlands	3	1.6 deaths per 100,000 per week trigger	0.75	274.157968	17134873	4822256	28186	153919	63083	36820	13330
New Caledonia	3	1.6 deaths per 100,000 per week trigger	0.75	4.567856	285491	73813	365	2318	861	480	162
New Zealand	3	1.6 deaths per 100,000 per week trigger	0.75	77.155728	4822233	1504573	7540	43708	18047	9942	3687
Nicaragua	3	1.6 deaths per 100,000 per week trigger	0.75	105.992864	6624554	2541675	7924	50030	21816	10694	4282
Niger	3	1.6 deaths per 100,000 per week trigger	0.75	387.306176	24206636	9736065	23313	158619	68760	31259	12516
Nigeria	3	1.6 deaths per 100,000 per week trigger	0.75	3298.233392	206139587	87600110	218632	1542718	674293	288977	115582
North Macedonia	3	1.6 deaths per 100,000 per week trigger	0.75	33.33408	2083380	633931	2925	18484	7560	3837	1418
Norway	3	1.6 deaths per 100,000 per week trigger	0.75	86.739872	5421242	1510276	7688	44934	17982	10259	3653
Oman	3	1.6 deaths per 100,000 per week trigger	0.75	81.705952	5106622	1412685	4706	37427	14435	6239	2257
Pakistan	3	1.6 deaths per 100,000 per week trigger	0.75	3534.277296	220892331	87105522	314407	1932753	819897	419187	163085
Panama	3	1.6 deaths per 100,000 per week trigger	0.75	69.036288	4314768	1759545	7196	40520	18306	9535	3864
Papua New Guinea	3	1.6 deaths per 100,000 per week trigger	0.75	143.152432	8947027	3072306	10103	73535	30874	13435	5216

Paraguay	3	1.6 deaths per 100,000 per week trigger	0.75	114.12048	7132530	2098906	6665	40521	16375	8885	3245
Peru	3	1.6 deaths per 100,000 per week trigger	0.75	527.549536	32971846	9560177	37133	216767	88846	49768	18502
Philippines	3	1.6 deaths per 100,000 per week trigger	0.75	1753.29736	109581085	44116068	174749	1165647	506571	232263	92070
Poland	3	1.6 deaths per 100,000 per week trigger	0.75	605.54568	37846605	11316780	67077	385822	160137	89055	33131
Portugal	3	1.6 deaths per 100,000 per week trigger	0.75	163.147312	10196707	1846189	11241	60362	22227	14863	4859
Puerto Rico	3	1.6 deaths per 100,000 per week trigger	0.75	45.77344	2860840	716435	5468	24767	10349	7274	2667
Qatar	3	1.6 deaths per 100,000 per week trigger	0.75	46.09696	2881060	952384	2867	26130	10808	3807	1499
Korea, Rep.	3	1.6 deaths per 100,000 per week trigger	0.75	820.306928	51269183	12655406	64648	400012	157307	85863	30487
Moldova	3	1.6 deaths per 100,000 per week trigger	0.75	64.543408	4033963	1333725	5796	38274	15891	7718	2928
Réunion	3	1.6 deaths per 100,000 per week trigger	0.75	14.324928	895308	288127	1481	7820	3366	1967	750
Romania	3	1.6 deaths per 100,000 per week trigger	0.75	307.802912	19237682	5141228	28083	160083	64085	36952	13208
Russian Federation	3	1.6 deaths per 100,000 per week trigger	0.75	2334.95136	145934460	43066149	211955	1273264	515820	277334	100308
Rwanda	3	1.6 deaths per 100,000 per week trigger	0.75	207.235344	12952209	4981005	13651	93194	39061	17936	6917
St. Lucia	3	1.6 deaths per 100,000 per week trigger	0.75	2.938064	183629	63176	307	1702	753	406	160
St. Vincent and the Grenadines	3	1.6 deaths per 100,000 per week trigger	0.75	1.775152	110947	44264	206	1143	519	276	112
Samoa	3	1.6 deaths per 100,000 per week trigger	0.75	3.17456	198410	56163	211	1419	559	281	100

Sao Tome and Principe	3	1.6 deaths per 100,000 per week trigger	0.75	3.506576	219161	70421	196	1301	519	261	96
Saudi Arabia	3	1.6 deaths per 100,000 per week trigger	0.75	557.021872	34813867	10412730	39636	293921	114254	52456	19165
Senegal	3	1.6 deaths per 100,000 per week trigger	0.75	267.90288	16743930	6983707	18625	124689	54139	24771	9865
Serbia	3	1.6 deaths per 100,000 per week trigger	0.75	139.79792	8737370	2722900	14629	84750	35427	19445	7287
Seychelles	3	1.6 deaths per 100,000 per week trigger	0.75	1.57344	98340	20906	107	610	218	142	46
Sierra Leone	3	1.6 deaths per 100,000 per week trigger	0.75	127.63176	7976985	3534860	9281	63787	28233	12224	5011
Singapore	3	1.6 deaths per 100,000 per week trigger	0.75	93.605488	5850343	1027101	9910	49767	16923	13209	4001
Slovakia	3	1.6 deaths per 100,000 per week trigger	0.75	87.354288	5459643	1344414	6509	39972	15358	8559	2945
Slovenia	3	1.6 deaths per 100,000 per week trigger	0.75	33.262912	2078932	570306	3349	18702	7607	4464	1622
Solomon Islands	3	1.6 deaths per 100,000 per week trigger	0.75	10.990048	686878	242602	800	5583	2389	1052	411
Somalia	3	1.6 deaths per 100,000 per week trigger	0.75	254.291504	15893219	5726111	14627	97410	40464	19490	7383
South Africa	3	1.6 deaths per 100,000 per week trigger	0.75	948.93904	59308690	22629957	90469	613931	260261	119747	46365
South Sudan	3	1.6 deaths per 100,000 per week trigger	0.75	179.099664	11193729	4922092	13802	90694	39857	18298	7310
Spain	3	1.6 deaths per 100,000 per week trigger	0.75	748.076528	46754783	12582820	73562	410392	164857	97540	34706
Sri Lanka	3	1.6 deaths per 100,000 per week trigger	0.75	342.612	21413250	4552508	25691	141159	50109	34366	11064
State of Palestine	3	1.6 deaths per 100,000 per week trigger	0.75	81.622656	5101416	1613193	5030	36311	14984	6576	2490

Sudan	3	1.6 deaths per 100,000 per week trigger	0.75	701.588304	43849269	16504567	48953	316627	132346	64581	24863
Suriname	3	1.6 deaths per 100,000 per week trigger	0.75	9.386144	586634	210422	755	4585	1968	999	388
Sweden	3	1.6 deaths per 100,000 per week trigger	0.75	161.58832	10099270	2578740	14518	79040	31355	19166	6792
Switzerland	3	1.6 deaths per 100,000 per week trigger	0.75	138.473888	8654618	1859838	10111	58160	22150	13603	4592
Syrian Arab Republic	3	1.6 deaths per 100,000 per week trigger	0.75	280.010512	17500657	4771873	17075	106850	40457	22562	7859
Tajikistan	3	1.6 deaths per 100,000 per week trigger	0.75	152.602272	9537642	4314552	10643	81607	36226	14183	5823
Thailand	3	1.6 deaths per 100,000 per week trigger	0.75	1116.799648	69799978	15777357	90286	552896	200500	121120	39245
Timor-Leste	3	1.6 deaths per 100,000 per week trigger	0.75	21.095072	1318442	406722	1364	9453	3862	1823	683
Togo	3	1.6 deaths per 100,000 per week trigger	0.75	132.459792	8278737	2976242	7758	54533	22429	10369	3987
Tonga	3	1.6 deaths per 100,000 per week trigger	0.75	1.691152	105697	39901	157	1017	439	210	82
Trinidad and Tobago	3	1.6 deaths per 100,000 per week trigger	0.75	22.391856	1399491	405172	1928	10845	4544	2549	972
Tunisia	3	1.6 deaths per 100,000 per week trigger	0.75	189.097888	11818618	3001658	15612	87058	32726	20619	6892
Turkey	3	1.6 deaths per 100,000 per week trigger	0.75	1349.425072	84339067	24774436	85866	594628	231456	115485	40479
Turkmenistan	3	1.6 deaths per 100,000 per week trigger	0.75	96.498992	6031187	1973188	5635	41229	16402	7327	2718
Uganda	3	1.6 deaths per 100,000 per week trigger	0.75	731.856	45741000	15324598	33823	242183	98691	44597	16711
Ukraine	3	1.6 deaths per 100,000 per week trigger	0.75	699.740144	43733759	11824017	59515	358385	141122	78963	27959

United Arab Emirates	3	1.6 deaths per 100,000 per week trigger	0.75	158.2464	9890400	3469776	9932	92733	38971	12988	5183
United Kingdom	3	1.6 deaths per 100,000 per week trigger	0.75	1086.176064	67886004	19009388	120735	640236	254138	160995	56851
Tanzania	3	1.6 deaths per 100,000 per week trigger	0.75	955.747408	59734213	31069326	75749	532710	248742	100798	43642
United States	3	1.6 deaths per 100,000 per week trigger	0.75	5296.042352	331002647	82980824	474227	2605123	997609	627962	213248
United States Virgin Islands	3	1.6 deaths per 100,000 per week trigger	0.75	1.670768	104423	24863	170	800	323	225	81
Uruguay	3	1.6 deaths per 100,000 per week trigger	0.75	55.579632	3473727	742688	4098	20042	7826	5519	1903
Uzbekistan	3	1.6 deaths per 100,000 per week trigger	0.75	535.507184	33469199	13805024	40299	300361	129338	53229	20813
Vanuatu	3	1.6 deaths per 100,000 per week trigger	0.75	4.9144	307150	104326	346	2445	1029	458	178
Venezuela (Bolivarian Republic of)	3	1.6 deaths per 100,000 per week trigger	0.75	454.975088	28435943	10936165	40968	244006	107440	54533	21667
Vietnam	3	1.6 deaths per 100,000 per week trigger	0.75	1557.417328	97338583	24722462	120476	762331	283435	157620	52796
Western Sahara	3	1.6 deaths per 100,000 per week trigger	0.75	9.55728	597330	202349	625	4533	1818	826	306
Yemen	3	1.6 deaths per 100,000 per week trigger	0.75	477.215488	29825968	10843028	28558	195614	80940	38103	14577
Zambia	3	1.6 deaths per 100,000 per week trigger	0.75	294.143296	18383956	7825201	17377	125150	54947	23019	9356
Zimbabwe	3	1.6 deaths per 100,000 per week trigger	0.75	237.806832	14862927	5670689	14960	100576	42104	19899	7677

Mitigation Sheet	
Variable	**Definition**
Country	Name of country or region
R0	Basic Reproduction number (average number of secondary infection by a typical infection in an unconstrained epidemic and wholly susceptible population)
Strategy	"Unmitigated" = no intervention;
	"Social distancing whole population" = optimal outcome when epidemic is mitigated interventions to limit contacts in the general population including social distancing;
	"Enhanced social distance of elderly"= optimal outcome when epidemic is mitigated through interventions to limit contacts in the general population including social distancing, alongside enhanced social dista
Social_distance	Percentage reduction in contacts in the general population
	For "Unmitigated" this is 0
	For "Social distancing whole population" this reduction applies to the whole population
	For "Enhanced social distancing of elderly" this applies to just individuals under 70. For individuals 70 and abovewe assume a 60% reduction.
total_pop	Total population according to 2020 World Population Prospects (https://population.un.org/wpp/)
total_infected	Final total population infected by the end of the pandemic
total_death	Final total deaths by the end of the pandemic (assuming China-like age-severity profile and health system)
total_hospital	Final total individuals requiring hospitalisation by the end of the pandemic (assuming China-like age-severity profile
total_critical	Final total individuals requiring critical care by the end of the pandemic (assuming China-like age-severity profile)
Suppression Sheet	
Variable	**Definition**
Country	Name of country or region
R0	Basic Reproduction number (average number of secondary infection by a typical infection in an unconstrained epidemic and wholly susceptible population)
Strategy	"Unmitigated" = no intervention
	"0.2 deaths per 100,000 per week trigger" = suppression triggered when weekly death rate reaches this threshold
	"1.6 deaths per 100,000 per week trigger" = suppression triggered when weekly death rate reaches this threshold
Social_distance	Proportional reduction in contact rate in general population modelled
total_pop	Total population according to 2020 World Population Prospects (https://population.un.org/wpp/)
total_infected	Final total population infected by the end of the pandemic.
total_death	Final total deaths by the end of the pandemic (assuming China-like age-severity profile and health system).
total_hospital	Final total individuals requiring hospitalisation by the end of the pandemic (assuming China-like age-severity profile
peak_hospital_bed_demand	Number of hospital beds occupied at the peak of the epidemic (i.e. maximum demand/occupancy during the epidemic)
total_critical	Final total individuals requiring critical care by the end of the pandemic (assuming China-like age-severity profile)
peak_critical_bed_demand	Number of critical care beds occupied at the peak of the epidemic (i.e. maximum demand/occupancy during the epidemic)
	Note: these are derived from a stochastic, dynamical model rather than analytically as in the Mitigation worksheet and so there is a small difference between the two (typically <1%).

SECTION - 3

161 references for " Evidences of 3 Step treatment protocol for COVID 19'

Vitamin C and Cancer: Medicine or Politics?

Author: Ullica Segerstrale
Date: Jan. 31, 1992
From: Science(Vol. 255, Issue 5044)
Publisher: American Association for the Advancement of Science
Document Type: Book review
Length: 2,055 words
Article Preview :

The author's aim with this book is twofold: to provide a case study of "social construction of science," in line with a current trend in science studies; and to take a swing at the medical establishment, in which regard she steps forth, in the book's final chapter, as an outright spokesperson for alternative medicine.

Richard's strategy is to question the key procedure in the testing of new cancer drugs: the randomized controlled clinical trial. If she can show that there can be no agreement based on factual evidence among proponents and opponents of new therapies, her case would fit right in with the claims of those who see controversies in science as merely a matter of scientists' social or strategic interests, disregarding intellectual commitments, convictions about "good science," standards of proof, and the like. Moreover, the failure of the randomized controlled clinical trial to determine the therapeutic efficacy of new experimental drugs, or of any drug, would serve to undermine the medical experts' monopoly on treatment of cancer patients and open up the possibility for patients to choose freely among therapies, including "alternative" ones.

Richards's choice of case study, Linus Pauling and his fight to get vitamin C accepted as a treatment for cancer, may not quite lend itself to such ambitious aims. The reader who wishes to assess just how well Richards in fact succeeds in proving her point is in for some serious work. Vitamin C and Cancer is an exceedingly well documented, quite complicated case study in which it is sometimes hard to keep track of the sequence and significance of events, despite the author's cross-referencing efforts.

Luckily, the book does not have to be read in such an inquisitory spirit. The case study on its own provides interesting reading and fascinating insights into the world of science and medicine. In fact, the book can be read in several different ways. One can see Pauling as a folk hero, bravely fighting the medical establishment for a fair test of his alternative, easily accessible, and potentially beneficial megavitamin cancer therapy. One can see him as the enfant terrible of established science and medicine, through his various actions testing and challenging the hidden assumptions of established rules and procedures. Or the book might be read as a handbook in scientific Machiavellianism.

The book describes the long-term (about 20 years) collaboration between Pauling and a Scottish doctor, Ewan Cameron, both champions of vitamin C therapy for cancer, albeit with initially rather different rationales. Cameron had written a book on his theoretical views of the cancer process in 1966, explaining the spread of cancer as having to do with the failure of the inhibitor (PHI) of the enzyme hyaluronidase to stop overproduction of the enzyme. This led to the weaking of the "ground substance" surrounding the cells. Cameron believed ascorbic acid to be structurally similar to PHI and speculated that vitamin C may help the body synthesize needed PHI and thus control cancer. He claimed some good observational results from his...

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Kimbarowski JA, Mokrow NJ (1967)
Farbige Ausfällungsreaktion des Harns nach Kimbarowski, als Index der Wirkung von Ascorbinsäure bei Behandlung der Virusgrippe.
Deutsch Gesundheitsw 22:2413-8

Urine Sediment Color Reaction Test According to Kimbarowski (FARK), as an Indication of the Efficacy of Ascorbic Acid in the Treatment of Viral Influenza

By J. A. KIMBAROWSKI and N. J. MOKROW, Moscow

In the domestic as well as the foreign literature one finds well-founded data in support of the concept that - in the presence of infectious diseases – typhus abdominalis, paratyphus, dysentery, scarlet fever, etc. - urine sediment color reaction in the urine represents an objective criterion for determining convalescence and a portent of a relapse or complications. The president of the Academy of Medical Sciences in the USSR emphasized the following on page 36 of his book "Principal Results of Scientific Studies in the Field of Medicine in the USSR in 1959 and 1960": "In order to recognize recovery following typhus abdominalis the results of the sedimentation reaction with $AgNO_5$ are necessary (advisable)."

The urine sediment color reaction test was clinically tested when it was used with a large number of patients and in various fields of medical specialty as well as when it was compared to several other tests used in the course of a disease, and in addition by means of the results of chromatographic and chemical tests. It was confirmed by these means that urine sediment color reaction has clinical and prognostic significance for many diseases, and identifies a distorted nitrogen metabolism or protein metabolism and simultaneously the degree of intoxication.

When observing the course of a number of clinical pictures it is possible in many cases to determine the true condition of a patient by means of the intensity of urine sediment color reaction in the urine and to do so more precisely than is done through the usual laboratory tests (urinstatus, diazoreaction, urochromogen reaction, clinical blood picture, blood sedimentation test, etc.).

Specialists in the field of clinical biochemistry, M. F. Mereshinski and L. S. Tscherkassowa, emphasize the following in their published work "Biochemical Processes as Protective Reactions of the Organism" (1956): "In infections and intoxications following intensified and distorted protein metabolism, insufficiency of the compensatory mechanisms occurs. Generally such insufficiency is not of any specific character and consequently its existence is not determined by any special method. Kimbarowski's urine sediment color reaction test is particularly valuable in such cases. This reaction is especially helpful in determining the overall condition of the compensatory mechanisms, and this reaction has great practical significance . . . We recommend the urine sediment color reaction test for evaluating the general condition of biochemical compensatory mechanisms."

In a published work of A. N. Judkewitsch (1952) "Clinical Significance of the Urine

Sediment Color Reaction Test According to Kimbarowski in Viral Influenza and Seasonal Colds" it is emphasized that in severe cases of the mentioned diseases the intensity of urine sediment color reaction rises. Thus, it turned out that the urine sediment color reaction test is more sensitive than the ESR [erythrocyte sedimentation reaction] and indicates the condition of the patient more precisely than do the clinical blood picture and urinalysis. Available in the literature is useful information from J. A. Kimbarowski concerning urine sediment color reaction's reduction in intensity in the urine when vitamin C is actively supplemented in the form of different foods given to patients suffering from an acute influenza. Kimbarowski points out that because of supplementation with vitamin C the urine becomes less turbid, and bowel movements improve.

The goal of these studies was to clarify the degree of intensity of the urine sediment color reaction in the urine of patients suffering from viral influenza, determine the time required for normalization of the metabolism during recovery based upon the results of the urine sediment color reaction in the urine, and further determine to what extent providing active supplementation of vitamin C to such patients and convalescents affects the metabolic normalization and changes in the intensity of the urine sediment color reaction in the urine.

The studies were conducted with the use of soldiers almost all of whom were of the same age and received the same diet.

The differential diagnosis of severe, moderate, and mild cases of viral influenza that was made was based upon the symptoms, body temperatures, and clinical picture during the period of sickness, and also upon the laboratory results.

The diagnosis of influenza was based mainly on the clinical pictures and epidemiologic data with serological confirmation in a series of cases involving the Type A virus. Observation was done on 130 patients with severe forms of the influenza, 58 with moderate forms, and 26 with mild forms. All of the patients (214) were divided into 2 groups. The 1st group comprised 102 patients (64 with severe forms of the disease, 26 with moderate forms, and 12 with mild forms). During the period of their inpatient treatment and the period of follow-up, ambulatory observation (after clinical improvement and release from the clinic) these subjects were given no supplemental ascorbic acid during a 25 day period, that began with the onset of the disease.

Each of the 112 patients in the 2nd group (65 with severe forms of the influenza, 32 with moderate forms, and 14 with mild forms) received in the same period of time and during their treatment in the clinic and the ambulatory observation period 300 mg of ascorbic acid per day.

It must be noted that the overall number of patients who were followed does not include those who presented with any kind of complications related to the influenza or who displayed any accompanying illnesses, which could have caused a certain degree of intensity of the urine sediment color reaction in the urine.

Ten patients in the 1st group and two in the 2nd group (who were not included in the overall number of 214) suffered complications (bronchopneumonia) on the 6th to the 7th day of illness. During the monitoring of the course of the intensity of the urine sediment color reaction in the urine the anticipated deterioration in the patient's condition was

visible sooner in that the color of the urine sediment had increased. Monitoring of the course of the intensity confirmed this development more unambiguously than did other laboratory/clinical tests (clinical blood picture, ESR, and X-rays).

Many authors refer to this phenomenon in a series of cases of illness.

Thus, it is emphasized in the work published by I. P. Galuschkin (1959): “By showing an increase in intensity the urine sediment color reaction test signals the impending deterioration almost twice as often as do objective clinical and laboratory results or as can be visually determined by the patient’s state of health. The urine sediment color reaction test signals the appearance of complications more often and earlier than do other clinical and laboratory tests.

The urine sediment color reaction test was performed for our patients and convalescents a total of 1926 times during in-patient care and 3424 times after they became out patients, in other words, once each day per patient.

We once again had the opportunity to show that the technically simple method of urine sediment color reaction testing, which also requires very little time, can be easily performed in any clinic and in every out-patient facility. The urine sediment color reaction test results were divided based on intensity into the following categories: within the normal range (negative), questionable, weak positive, positive, strong positive, and highly positive.

The following symptoms were observed in both groups after onset of the illness: chills, strongly impaired state of health, facial hyperemia, severe headaches, and fever of 38 to 39.8 ºC. The clinical blood picture showed in a number of cases an insignificant leucocytosis and rapid blood sedimentation rate, and unremarkable urinalysis, while the urine sediment color reaction in the urine showed various degrees of intensity.

Clinical observations on the 2nd, 3rd, and 4th days produced no significant changes, only drops in temperature down to 37.2 ºC along with profuse sweating and general weakness. During this time frame no negative results were obtained from the urine sediment color reaction in the urine in either the 1st or the 2nd group of patients. The results from both groups during in-patient treatment are summarized in Table 1:

Table 1

Group 1 (102 Patients) no Vitamin-C provided			Group 2 (112 Patients) supplemental provision of 300 mg of vitamin C per day	
Degree of Intensity of urine sediment color reaction in the urine				
Negative	0 % of cases		0 % of cases	
Questionable	9.6%		20.1%	
Weak positive	26.6%		38.4%	
Positive	34.8%		24.2%	
Strong and highly positive	29.0%	63.8% [=34.8+29.0]	17.3%	41.5% [=24.2+17.3]
	100.0%		100.0%	

It can be seen from the results listed in Table 1 that for group 1, which received no vitamin C, the percentage total of the positive through the highly positive range of urine sediment color reaction in the urine is 63.8 %, while for group 2, which received active vitamin C supplementation the percentage was at 41.5 significantly smaller.

Toward the end of the 1st week the patients who were suffering from the weak positive and the positive form of the influenza experienced improvement in their general condition, but their state of health continued to be adversely affected, while headaches began and facial hyperemia disappeared. Body temperatures fell to normal levels.

In this period the intensity of the urine sediment color reaction in the urine showed an obvious tendency to decline: In the 1st group the negative urine sediment color reaction amounted to 36.4 % and the positive 63.6 %, while in the 2nd group the negative urine sediment color reaction rose to 59.7 % and the positive fell to 41.3 %.

In this period the clinical blood picture showed in a number of cases leucopenia and a shift to the left.

Observations of the inpatient treatment on the 8th and 9th day showed significant improvement in the state of health of all patients. In all forms of the illness at normal temperatures in the course of two days normalization of the blood picture, the ESR, and the urinalysis occurred. This applies particularly to patients in the 2nd group with respect to whom clinical convalescence was determined to exist. During this period the patients in the 2nd group were released, or more properly, the convalescents were released, for follow-up ambulatory observation.

The convalescents in the 1st group were released in most cases 2 to 3 days later. The number of complications in this group was greater than that in the 2nd group.

The results of the urine sediment color reaction in the urine during the in-patient treatment as well as during the follow-up ambulatory observations in both the 1st and 2nd groups are summarized in Table 2:

Table 2

	1st Group (102 Cases)				2nd Group (112 Cases)			
Intensity of the FARK	In-patient from 1st to 12th day		Ambulatory Observations (following release)		In-patient from 1st to 9th day		Ambulatory Observations (following release)	
	No. of Observations.	% of cases	No. of Observations.	% of cases	No. of Observations.	% of cases	No. of Observations.	% of cases
Within normal range (negative)	94	10.1	314	19.2	168	16.7	1067	59.4
Questionable	87	9.3	549	33.6	224	22.2	432	24.1
Weak positive	206	22.4	594	36.4	222	22.1	261	15.0
Positive	361	39.7	154	9.4	282	28.0	32	1.5
Strong positive	124	13.5	21	1.4	86	8.5		
Highly positive	46	5.0			26	2.5		
Total	918	100	1632	100	1008	100	1792	100

The summarized results in Table 2 show that where in the 1st group (period of in-patient care up to 12 days) the total number of the negative, questionable, and weak positive urine sediment color reactions amounted to 41.8 %, in the 2nd group, in which vitamin C was actively supplemented, (period of in-patient care up to 9 days) the number for the same categories was higher, representing 61.0 % of the cases. Similar results were also obtained during the subsequent ambulatory observations: In the 1st group the percentage was 52.8 % of the cases while in the second group it was 83.5 %. During the in-patient treatment the total percentage of the positive and highly positive urine sediment color reactions was 58.2 % in the 1st group and only 39.0 % of the cases in the 2nd group. During the further ambulatory observation the total percentage of the positive and highly positive urine sediment color reactions was 10.8 % of the cases, while in the 2nd group no highly positive urine sediment color reactions were observed and the percentage of the positive urine sediment color reactions was only 1.5 % of the cases.

All of this proves that in spite of the treatment rendered (antibiotics, sulfonamide, salicylate preparations, treatment of symptoms and general care) the urine sediment color reaction in the urine made the disturbed (distorted) nitrogen and protein metabolisms discernable in the course of both observed groups and demonstrated the necessity of

including simultaneous supplementation of vitamin C in the complex therapy in order to normalize the metabolism.

Examinations of the 1st group undertaken on the 25th day after the illness began showed the total percentage of negative, questionable, and weak positive urine sediment color reactions as 89.2 % of the cases and the percentage of the positive as 10.8 % versus in the 2nd group, which actively received supplemental vitamin C (300 mg/day for each patient), percentages of 98.5 % and 1.5 %. Upon release from in-patient care, excreted urine of patients in the 1st group contained only trace amounts of ascorbic acid, while the excreted urine of those in the 2nd group contained 0.3 mg/hr. During the ambulatory observation of the patients (up to the 25th day after the illness began) examination of vitamin C content in the urine produced a similar picture: in the first group less than 0.5 mg/hr and in the 2nd group more than 0.9 mg/hr.

These observations showed that persons who have had viral influenza and who now for all practical purposes are healthy require additional saturation of the organism with vitamin C in order to attain full recovery and normalization of the disturbed metabolism.

Conclusions

1. When patients suffering from viral influenza are treated with complex therapy active supplementation of vitamin C (at least 300 mg/day) is required. When the convalescent state begins, the same dosage of active supplementation of vitamin C must be continued for up to 2 weeks.
2. During the course of the illness the urine sediment color reaction test according to Kimbarowski shows the pathological condition of the organism, the distorted nitrogen (protein) metabolism more precisely than do general laboratory/clinical examinations of the blood and urine, and establish improvement of the oxidation-reduction process as a consequence of the application of ascorbic acid.
3. The urine sediment color reaction test is technically easy to perform. Under ambulatory conditions it constitutes an additional criterion for determining recovery following viral influenza. It also signals impending complications sooner than do other tests.

Summary

[Translator's comment:
The original document sent to this translator contains
a summary in English that is adequately translated.]

English Summary by the authors:

The study described in the present paper aimed at ascertaining the degree of intensity of the coloured precipitation reaction of the urine according to Kimbarowski (FARK) in virus grippe patients during a period of 25 days under clinical and ambulant conditions. 214 patients almost all of whom belonged to one and the same age group and received the usual hospital diet were subjected to daily check-ups.

The authors wanted to determine the date of normalization of the metabolism of acutely suffering and recovering patients. They also wanted to detect in how far the active "C"-vitaminization effects a shortening of the duration of illness, an improvement of metabolic processes and changes with regard to the intensity of the coloured precipitation reaction (FARK) in the urine.

For this reason, the authors compared findings obtained during the process of the disease in a group of 102 patients (64 severe, 26 medium and 12 light cases) who had not received any additional doses of vitamine C with findings obtained in a second group of 112 patients (65 severe, 32 medium and 14 light cases of grippe) who received a daily dose of 300 mg ascorbinic acid during their stationary treatment and outpatient control during the same period.

The total number of patients does not cover those suffering from grippe-induced complications or attendant diseases. Moreover, it does not cover 12 patients of the 1st and 2nd group who manifested a bronchopneumonia as a complication on the 6th and 7th day of illness.

It should be emphasized that FARK signalized the impending complications earlier than other laboratory-clinical examinations (clinical blood and urine tests, blood sedimentation-rate test and radioscopy). The grippe diagnostics was mainly based on the clinical picture, epidemiological data, the serological type A of the virus being confirmed in a number of cases.

The present paper describes the patients' state in both groups on the 2nd, 3rd, 4th, 8th and 9th day of illness, as well as their state during the stage of recovery. The intensity of the FARK in the urine is compared with other tests. The respective results have been summarized in two tables.

The authors demonstrate that during the acute illness and upon release from hospital the number of positive and highly positive FARK in the urine was much lower with the patients who had been actively C-vitaminized than with those patients who had not received any additional supply of vitamine C. Most patients who had received vitamine C were released from stationary treatment on the 9th day of illness, while the patients who had undergone any vitamization were released only 2-3 days later, mostly on the 12th day of illness *[HH comment: this is oppostite to the main text, see above]*. These patients manifested complications less frequently than the vitaminized patients. As was proved by the further outpatient observation, the number of positive to highly positive FARK in the urine amounted to 10.8 per cent of the cases in the group without additional C-vitaminizatiuon. In the 2nd group we did not observe any strongly positive FARK. The number of positive FARK came up to only 1.5 per cent.

The dynamic observations induced the authors to draw the following final conclusions.

1. In case of a complex therapy of the virus grippe patients an active "C"-vitaminization (not less than 300 mg/day) is required. After beginning of the recovery stage the active C-vitaminization should be carried through in indicated quantities up to 2 weeks.

2. The coloured precipitation reactions according to Kimbarowski reflect the

pathological state of the organism, the distorted nitrogen (protein) metabolism more exactly than general laboratory-clinical examinations of blood and urine, demonstrating the improvement of the oxidation-reduction process due to the application of ascorbinic acid.

3. The FARK can be carried through very simply, and under outpatient conditions it is an additional criterium of recovery following a virus-grippe. It also signalizes impending complications earlier than other tests.

Contribution to the question of pneumonia treatment with vitamin C
Elisabeth Bohnholtzer
Deutsche Medizinische Wochenschrift 63(26):1001-1003, June 25, 1937.

CONTRIBUTION TO THE QUESTION OF PNEUMONIA TREATMENT WITH VITAMIN C

Vitamin C metabolism of has been subjected to more detailed investigations in recent times. The importance of this vitamin for body balance has been understood in more detail since the amount of ascorbic acid in the organs and fluids of the body and its urinary excretion have been amenable to determination. It has been found that a pronounced vitamin C deficiency exists not only in the ailments named after Skorbut and Möller-Barlow, the terminal states of a vitamin C deficiency, but also in many other disease states, such as hemorrhagic diathesis, bone diseases, dyspepsia, adrenal insufficiency, allergic conditions, intoxications, pregnancy and particularly infectious diseases. A. Hochwald, Prague, has demonstrated — especially for the so-called hyperergic diseases whose histological expression is fibrinous inflammation according to Rössle — that extra consumption and a resulting deficiency of reducing substances arises during the antigen/antibody reaction that takes place in the body, whereby leading to cell damage and the formation of histamine-like substances that are capable of triggering toxic phenomena as severe as anaphylactic shock. As a result of adequately administering such reducing substances, there has been success in preventing this effect and, hence, in favorably modifying the course of the disease. In the way in which Böger and Schröder had success in alleviating the left displacement* of blood protein substances via the long-term administration of vitamin C, Hochwald was able to arrive at the same results following the administration of high doses of ascorbic acid in animal experiments. Simultaneous alleviation of the immunization effect did not take place. Hochwald's experiments mostly extended to modifying anaphylactic shock in guinea pigs and croupous pneumonia in humans via the administration of ascorbic acid.

These investigations and the following personally observed case, likewise, predisposed us to carry out the treatment of fibrinous pneumonia with vitamin C as the sole therapeutic agent.

Despite conventional therapy with Solvochin and Cardiacis, the most severe prostration with typhous muzziness, cyanosis, high-grade dyspnea and life threatening circulatory impairment arose in the aforementioned case. The occurrence of severe nosebleeds induced us to administer vitamin C as tablets in the form of Cebion (Merck). The bleeding soon ceased, general health visibly improved and the pneumonia took a favorable course.

An additional stimulus was provided by the study by J. Gander and W. Niederberrer [sic; Niederberger] (Stans Cantonal Hospital, Switzerland) namely "Vitamin C in the treatment of pneumonia."

In our investigations of vitamin C deficiency or the urinary excretion of ascorbic acid, we made use of the miniature method that had been indicated by Jezler and Niederbeuger [sic; Niederberger] using dichlorophenolindophenol as the indicator.

We proceeded as follows from the therapeutic standpoint: we initially administered 400 or 500 mg ascorbic acid 3 times daily as an intramuscular injection up to defervescence or positive urinary

* [Translator's note: Considering the year, this probably refers to paper or starch block electrophoresis.]

excretion, and then 100 mg 3 times daily per os up to resolution of the pneumonia. Redoxon (Roche) was used in the initial investigations; Later Cebion (Merck) was exclusively used. According to data from the companies, both are the chemically pure sodium salt of l-ascorbic acid. We were not able to establish any difference in the mode of action of the two agents.

In our experience, intramuscular injection was preferred to the intravenous version, since slower absorption apparently ensures better utilization in cases of quantitatively lower excretion.

The worse tolerance of intramuscular injection of ascorbic acid described in the literature might be correlated with the earlier use of pure ascorbic acid, whereas we noted no unpleasantness apart from short-term pain soon after the injection at the injection site upon administration of the sodium salt of ascorbic acid. In regard to other medications, only expectorants and circulatory agents were administered, the latter of which proving to be necessary only to a conspicuously small extent.

Freshly passed urine was tested for ascorbic acid on each occasion prior to initiating treatment. It was not detectable even once in the cases of croupous pneumonia, and the same could also be established, incidentally, in 28 other febrile diseases. The deficit in the urine was thus not specific to croupous pneumonia. After all, it is conspicuous that the seasons of the year for the largest vitamin C deficiency coincide with the times of the most frequent pneumonic diseases.

In order to record the time of the first appearance of ascorbic acid in the urine, the ascorbic acid determination was carried out on the 1st and 2nd days of treatment, namely 3-5 h after each injection; on all the later days, only in the mornings using fresh urine.

Our investigations extended to 16 cases of pneumonia. For comparison purposes, 2 cases of bronchopneumonia and 1 case of chronic pneumonia were intentionally treated in the same way or under the same conditions. No detectable influence of ascorbic acid on the course of these latter diseases could be recorded.

In the treatment of genuine croupous pneumonia, it was found that a positive ascorbic acid balance sheet or, expressed more carefully, urinary excretion, sometimes occurred even after the 1st injection (in 5 cases after 400 mg, and in 2 cases after 500 mg); in the other cases, at least on the 2nd or 3rd day of treatment. The more severe the disease, the longer it took to offset the vitamin C deficiency at the same dosage. The longest recorded time until the appearance of ascorbic acid in the urine was observed occurred in a fatally progressing case of bronchopneumonia; it amounted to 6 days.

The drop in temperature was mostly accompanied by the first excretion. The nature of the defervescence was critical in 8 cases and lytic in 4 cases. In one case, a fever peak (up to 38.5°C) occurred once again after the initial defervescence and the changeover from intramuscular injection to peroral administration. The temperature rise exactly coincided with the negative urinary excretion of ascorbic acid, and it immediately disappeared after the administration of larger intramuscular doses of vitamin C. In lytic defervescence, the excretion of ascorbic acid preceded completely normal temperatures by several days. In the 2 bronchopneumonia and the chronic pneumonia that were utilized for comparison purposes and progressed to death, the fever existed until death; urinary excretion of ascorbic acid occurred shortly beforehand. The last-mentioned fatally progressing cases were to be regarded as desolate from the outset. We give brief medical reports below.

1. Male patient P., 50 years old. Only slight temperatures and expectoration three weeks prior to admission to the hospital; highly febrile disease 8 days prior to the start of the treatment. The patient was in extremely bad general health. Diffuse infiltrations were found in both lung fields. As a consequence of circulatory insufficiency, which could not be alleviated even by means of analeptics, death occurred on the 9th day of the treatment, i.e., on the 17th day of the disease.

2. Female patient K., 73 years old, came to us for treatment on the 5th day of the disease. She was in very bad general health. Myocardiopathy with absolute arrhythmia was present. Apart from fibrinous pneumonia of the lower left lobe together with pleuritis, multiple pneumonic foci were present in all segments of the right lung. Death in the evening of the day of admission as a result of circulatory weakness.

3. Female patient E., 26 years old, had been confined to bed for several weeks. Fever up to 40°C, allegedly for 6 days prior to admission to the hospital. On the 3rd day of treatment, death as a consequence of circulatory insufficiency. The autopsy revealed partially carneous, fibrinous pneumonia of the entire left lung, and fresh pneumonia of the lower right lobe. In the opinion of the pathologist, the process on the left side was certainly already 4 weeks old. Pronounced hypoplasia of the vascular system was also present.

The fever curves of two croupous pneumonia cases treated with ascorbic acid, are reproduced below.

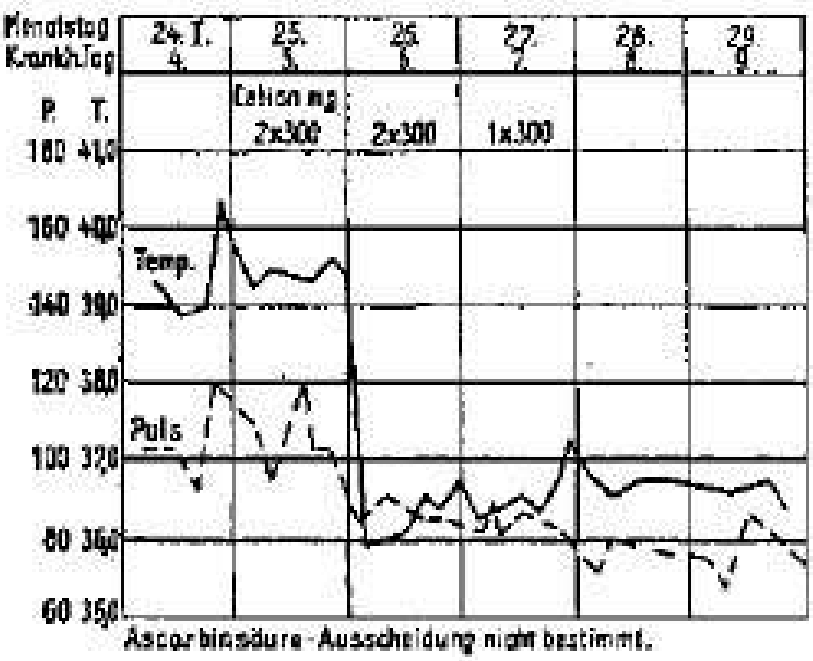

Figure 1

Figure 1 shows the critical drop in temperature on the 1st day following the Cebion treatment, although this first commenced on the 5th day of the disease. Since this was one of our first patients who was being treated in this way, the determination of ascorbic acid in the urine was not yet being carried out.

Figure 2

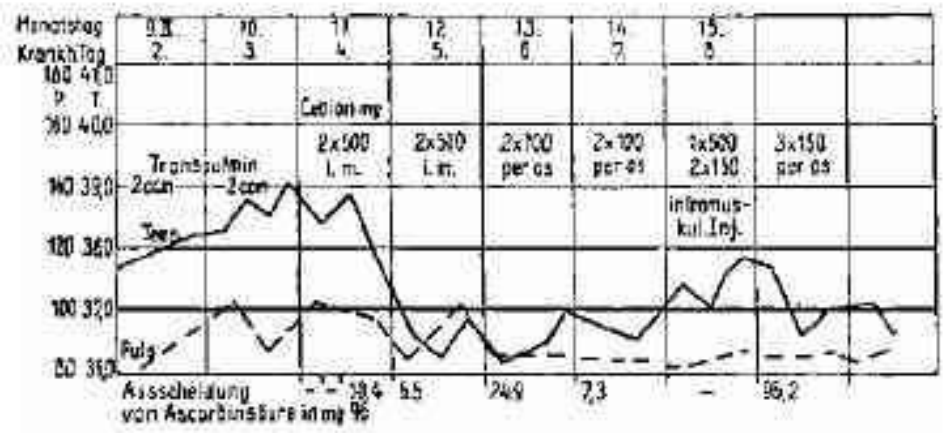

Figure 2 shows the course of fibrinous pneumonia in an 86-year-old female patient following treatment with Cebion. Critical defervescence took place after the appearance of ascorbic acid in the urine. When the excretion became negative again — because the need for ascorbic acid was apparently not being satisfied via per os administration — an increased temperature arose again on the 8th day of the disease and once again reverted to normal following the more adequate administration of vitamin C.

The frequently critical defervescence, which regularly arose without complications (no deliria) almost immediately after resolution of the vitamin C deficiency, is in contrast to the findings of Hochwald and Gander and Niederberger, who mostly observed lytic defervescence even in diseases that had persisted for a longer period of time.

Respiration and the subjective health (prostration, pain, inappetance, dyspnea) generally improved even after the first injection, whereby this was probably partly engendered by the decrease in temperature and certainly also partly as a result of eliminating toxic substances. The pulse rate fell at the same time as the decrease in temperature; the pulse was full and regular. The slight effect of vitamin C in terms of reducing blood pressure did not show any injurious influence on circulation. As has been stated, circulatory agents were required only to a small extent. In contrast to Hochwald, and despite timely defervescence, we were, however, unable to establish any physically or radiologically detectable acceleration of the resolution when the treatment first commenced several days after the beginning of the disease.

A conspicuous aspect in all our patients was the small amount of sputum. Expectorate was sometimes even completely absent, so that a determination of the type of pneumococci could not always be carried out. Simply because of the small number of our investigations, we should therefore like to withhold any opinion as yet in regard to the better or worse ability to influence the individual types. In the same way, for the same reason we would not yet like to go into the globulin/albumin ratios in the blood, the differential blood count and the changes in metabolic balance. Our investigations in this direction continue. An aspect that is also to be emphasized is that, among the 16 cases that were treated with ascorbic acid, absolutely no complications were observed and, particularly, no cases of the development of emphysema were observed.

Summary

Ascorbic acid treatment has a very favorable influence on the course of croupous pneumonia. Immediate suppression is mostly possible in the beginning of the disease; in treatment that commences later, critical or lytic defervescence in two to three days can also generally be achieved even when all the stages of the pneumonia were traversed. The improvement in general health (prostration, dyspnea) is most conspicuous. In contrast to the observations of Hochwald, however, a more rapid resolution of the pneumonia could then no longer be attained.

Metabolic changes due to vitamin treatment have not yet been investigated in greater detail because of the small number of our observations. In the same way, it must be left to a larger number of investigations as to whether, in already advanced stages, significantly more favorable results could not also be attained via a combination of vitamin C and chemotherapeutic agents or, above all, via serum. An aspect that is of importance is that we managed with considerably smaller doses of vitamin C than those indicated by Hochwald, whereby this is not insignificant in light of the currently continuing high price of the preparations.

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Münch Med Wschr 83:2074-7

Research and Clinical Picture.

From the Kantonsspital Stans (Switzerland). (Chief physician: Dr. J. Gander.)

Vitamin C in the treatment of pneumonia.

By J. Gander and W. Niederberger.

During investigations about vitamin C metabolism in older people, we were surprised to learn about the noticeably favorable effects of ascorbic acid administration in a case of inflammation of the lungs, which led us to ask: is there perhaps a disorder in vitamin C metabolism in cases of pneumonia and if this situation is remedied, does it have a favorable effect on the course of the disease?

The following five important personages will speak to the accuracy of such an assumption:

1. The good experience with vitamin C-rich fruits and fruit juices under feverish conditions and cases of pneumonia. Already in use for a very long time, the particularly favorable outcome of this procedure again proves to be very pleasing to well-known diet specialists. “In very general terms, fruit should be made one of the major food groups for patients with fever, to a much greater extent than is now customary”, says von Noorden, for example.

2. The surprisingly large number of vitamin C deficiency diseases that are diagnosed regularly after pneumonia has been overcome (Schroeder, Guldager and Poulsen, Harde and staff).

3. Animal experiments by Stiner, as well as by Heymann, which show that chronically occurring vitamin C deficiency disease surprisingly does not usually result in scurvy, but rather in pneumonia, most often in the central lobe.

4. The noteworthy parallels between cumulative pneumonia mortality and cumulative occurrence of vitamin C deficiency disease. An independent experiment over a period of one year showed us that vitamin C deficiency disease, influenced by a still unknown weather factor, occurs more frequently from October to about the end of May, and especially in December and April, than in the other months. But it is precisely during this time that pneumonia mortality is greater!

Fig. 1. Extent of vitamin C deficiency disease following pneumonia. Comparison with normal cases and scurvy.

Fig. 2. Cumulative occurrence of vitamin C deficiency disease.

Pneumonia mortality (according to Henschen).

5. The increase in pneumonia mortality in old age. Our previous research material showed us that vitamin C deficiency disease in general, among healthy persons, could reach the following values: up to 50 years 0-1000 mg, in older age groups: 1500 to 2500 mg. A comparison with pneumonia mortality in the various age groups shows surprising parallels.

Fig. 3. Extent of vitamin C deficiency disease in persons of various ages among healthy individuals,

Pneumonia cases of death in various age groups
(according to Henschen.)

All of these observations indicated that during the genesis of pneumonia, vitamin C metabolism takes on a very significant meaning and that in cases of pneumonia, likely results in better and quicker recovery under the effects of vitamin C. We therefore began to systematically study the course of pneumonia under the administration of vitamin C, with one of us (G) working primarily on the therapeutic issues and the other (N) concentrating on the methodical issues. We thus proceeded in four stages: we first checked to see whether the administration of vitamin C had a favorable effect in the traditional treatment of pneumonia. This was found to be true. We thereupon began to completely eliminate the existing vitamin C deficit, using the Klein method of Jezler und Niederberger as a means of control, initially within 2-3 days, and finally on the first day of the illness. The results became more and more favorable, so that we finally dared to attempt treatment by eliminating the vitamin C deficiency disease on the first day of the illness, without the administration of other medications.

We currently have observation material from about 15 cases. One typical example from each of the four stages described shall be reproduced here:

1. Stage: usual treatment of pneumonia + administration of vitamin C without determining the absorbing capacity of C and without early application of Redoxon (oxidation reduction) treatment.

C. A. 73-year-old. Pneumonia in the right inferior pulmonary lobe. Strikingly strong toxic phenomena: hectic (flustering) redness, soft arrhythmic pulse, sharp rheumatic pains, pressure sensitivity of the nerve trunks of the right arm, continuous vomiting, dyspnea (shortness of breath) temperatures between 38 and 39^0. Treatment: on the first day of the illness, large doses of Coramin, Digalen, etc., on the second day 4 ccm Solvochin, 10.0 camphor oil and morphine. On the third day of the illness, after significant deterioration of condition, vitamin C in the form of two Redoxon ampoules is given intramuscularly. The usual treatment is maintained, except for Solvochin and morphine; instillation of glucose and 10.0 of calcium Sandoz is also administered. After just 400 mg of vitamin C, the patient felt significantly fresher, the neuritic manifestations abated entirely, the hectic redness disappeared, the vomiting ceased and the pneumonia eased according to the lytic type. A far lesser disintegration of strength was observed during convalescence than would have been expected according to the severity of the clinical picture.

2. Stage: usual pneumonia treatment + gradual elimination of the vitamin C deficiency disease.

N. M. 3-year-old. Pneumonia of the right inferior pulmonary lobe. Temperature 40.5. Appearance poor, pulse coursing. Face cyanotic, extremities cool, moist, motor restlessness. •Treatment: Cardiazol-Chinin 1 ampoules and Redoxon 3 ampoules daily intramuscularly, then 300 mg Rodoxon by mouth, dissolved in sugar water. Temperature remained very high during the first three days. The condition was quite serious. As the vitamin C deficiency disease of 1200 mg was eliminated on the third day, the fever suddenly fell critically to the norm.

3. Stage: usual pneumonia treatment + elimination of the vitamin C deficiency disease on the first day of the disease.

N. E. 20-year-old. Soldier. Lobar pneumonia of the right middle and inferior pulmonary lobes, onset of collapse, temperature 39.5. Pulse weak, extremities cool, facial color cyanotic, appearance tired and suffering, sputum tinged with blood. Treatment: Redoxon 18 ampoules intramuscularly during the course of 8 hours, then 2 tablets of Redoxon every two hours. In addition, 10.0 calcium Sandoz, camphor, Solvochin and Transpulmin administered in the usual way. The urine was checked every three hours for vitamin C excretion. After a total of 2100 mg of vitamin C, given within 8 hours, the vitamin C deficiency disease was eliminated, the temperature immediately dropped critically back to the norm, the pains eased completely without the use of narcotics, the pulse became strong and the patient felt noticeably well. On the day the fever fell, a pleuritic exudate was evident. Puncture resulted in a cloudy liquid, which contained bacteriologically gram-positive streptococcus (enterococcus). The pleural sac had to be opened up and drained. The patient endured this operation under a general feeling of well-being.

4. Stage: Elimination of the vitamin C deficiency disease on the first day of illness without the use of other medications.

B. R. 9-year-old. Patient fell ill with lobar pneumonia of the left inferior pulmonary lobe on August 6, 1936 in a holiday colony. Six hours after the onset of the initial chills and fever, Redoxon medication was started. After taking 1000 mg of Redoxon by mouth, even though the vitamin C deficiency was eliminated and critical defervescence set in, local pulmonary findings showed still massive depression and twanging large and medium-sized bubbly rales. The general condition was so good, that transportation home for the patient could be arranged as early as August 8th. According to reports from the parents, the patient continued to remain without fever.

The preliminary overall results of our studies with vitamin C in cases of pneumonia are: ascorbic acid has a positive influence on the course of the illness, particularly if the vitamin C deficiency disease is eliminated on the first day of the illness. Recovery then almost always sets in with satiation of the organism and the fever subsides, usually critically, back to the norm, as the following graph for the case described above under item 3 shows:

The existing pains disappear, so that the administration of narcotics can be limited. The pulse remains in good tone, side effects are completely lacking. In particular, in the cases we observed, there was never any collapse observed that could have been caused by the blood pressure lowering effect of the vitamin C.

Fig. 4 N.E. 20-year-old. Disease history above under "3rd Stage"

The general condition is always favorably influenced to a noticeable extent, as is the convalescence, which proceeds better and more quickly than in cases of pneumonia which are not treated with vitamin C. Still remaining for some time are the depression, the bronchial breathing and the rales, obviously because the organism is unable to pursue the rapid course of recovery together with the clearing up of the pathological substrate. We have not seen any failures up to now, despite the fact that some of the cases being treated were of a various serious nature.

Vitamin C therefore appears to be a very valuable therapeutic aid for the treatment of pneumonia.

We would nevertheless prefer to view this current information as only preliminary, which inspires further investigation, but is not yet to be interpreted as absolute fact. This is true in the case of pneumonia, because generally known final conclusions are only possible based on extensive material stemming from various cities and countries.

In particular, the issue of whether vitamin C achieves its optimum effect in the treatment of pneumonia alone or in conjunction with calcium, must undergo detailed examination. We got the impression that the combination of vitamin C with calcium further improved the therapeutic effect and helped speed up resorption.

Since pneumonia must be treated under all possible conditions, the apparatus with which the elimination of vitamin C deficiency disease can be determined, both in its structure and handling, must be as easy as possible. For this reason, we selected the procedures of the medical clinic of Basel for our initial investigations (Jezler and Kapp, Jezler and Niederberger), since these were very reliable and at the same time, simple and manageable.

With the Klein method of Jezler/Niederberger, we determined the reduction value of normal urine during the first visit to the patient ill with pneumonia, then applied the vitamin C treatment and after 3 – 5 hours – this is the time during which normal vitamin C metabolism or metabolism that has been returned to normal first begins to show in excretion in the urine – again checked the reduction value of the urine. We were thus able to determine that the recovery, especially the reduction of fever, momentarily always set in at the time the reduction capacity of the urine had doubled, but increased to a minimum of 5 mg percent. Thus, contrary to views still frequently voiced, vitamin C deficiency disease is to be considered eliminated if the reduction capacity of the urine has doubled within 3 – 5 hours after vitamin C application and exceeds a minimum of 5 mg percent.

If we could have carried out the titration ourselves or if we had trained personnel at our disposal, we would have made out all right with the Jezler/Niederberger method. But in any instances where untrained nursing personnel were on hand – and this is almost always the case in the home treatment of pneumonia – and we ourselves were unable to carry out the titration after 3 – 5 hours, difficulties set in and we were forced to work out an even easier procedure. We finally succeeded in

doing this with the help of the tablets of dichlorophenolindophenol “Roche", a blue dye, which is immediately discolored by vitamin C.

20 ccm of 5 mg-percent urine still enables the blue color of the solution of 1 tablet of dichlorphenolindophenol “Roche" to disappear in approx. 50 cm of water. If we were to then place such a dye solution into a beaker or bottle and add 20 ccm of urine from a patient ill with pneumonia, there would for the most part be no discoloration before the vitamin C treatment. As soon as the vitamin C deficiency disease was eliminated, or the reduction value rose to over 5 mg-percent, the blue color disappeared immediately.

We therefore had the principle for the following simple method: a bottle with a cubic capacity of 70-100 ccm (beakers are not as well suited for use in the home of the patient as they are in surgery practice, since they are too breakable) is filled with 50 ccm of water, 1 tablet of dichlorphenolindophenol is added, to which 20 ccm of urine is added after the tablet dissolves and observed to see whether or not discoloring occurs immediately.

ln more than 95 percent, i.e. in all cases where the original reduction value of the urine is below 5 mg-percent, this procedure works just fine. In some cases, however, the original value of the urine is over 5 mg-percent, so that the normal urine already discolors the solution. These cases can also be easily determined, however, by following the above procedure, but by adding the 20 ccm of urine in portions of 5 ccm each to the dye solution instead of adding the 20 ccm all at once. The amount that discolors is divided by 2 and for the next control, instead of the 20 ccm, half of the urine quantity that discolored is used.

Based on all of these experiences and preliminary work, we can now recommend the following procedure for the treatment of pneumonia: Before going to the patient, you should equip yourself with the following utensils:

1. Vitamin C in the form of tablets and ampoules[1]
2. Dichlorphenolindophenol ,”Roche" in tubes of 20 tablets
3. A bottle, as shown in Figure 5

Fig. 5. Bottle for Determining Vitamin C Deficiency Diseases. This is set up as follows: take a medicine bottle with a capacity of 70-100 ccm and a screw-off top, fill it completely with water and then take out 4 times 5 ccm, marking the respective water level on the bottle using an ampoule file [rasp]. It is a good idea to keep a small supply of such bottles on hand.

If pneumonia is diagnosed, then one would assume that the vitamin C deficit at this moment may have already reached values of 1000-2000 mg or more and would from the very start apply high doses of vitamin C. Approx. 500 mg would be in the form of injections and about 300 mg in the form of tablets, which would be ingested in water, fruit syrup, sugar water, etc. The following orders would then be given to the relative or nursing personnel: over the course of the next three

1 For all of our experiments we used Redoxon "Roche", of which one tablet contains 50 mg and one ampoule contains 100 mg of vitamin C. Purchase price: 20 tablets RM 2.27. 6 ampules RM 533.

hours, another 18 tablets or 900 mg of vitamin C are to be given, 3 tablets approx. every half hour. If it becomes impossible to administer these doses due to gastrointestinal upsets (Stepp), it will then be necessary to effect fast saturation by means of 3-4 daily injections of 500 mg.

Approximately 3-4 hours after the visit, the urine must be checked using dichlorphenolindophenol ”Roche". The process of checking the urine is demonstrated at the first visit, so that it will be carried out correctly by the person in charge, by proceeding as follows: take the bottle mentioned under item 3, fill it up to the first mark with spring water and add one tablet of dichlorphenolindophenol. After it dissolves, add 5 ccm portions of urine (lines 2-5 on the bottle!), shake briefly after each addition and look to see whether or not discoloring has occurred. If the color remains the same after 20 ccm of urine, fill up the entire bottle with urine for the next check. But if discoloring occurs beforehand, mark the spot up to which the urine should be filled (= half of the amount of urine which discolored) with a leucoplast and fill up to this point. The relatives/caretakers then receive instructions to carry out the experiment as previously demonstrated after 3-5 hours using fresh urine and to report the results.

Disappearance of the blue color indicates that the vitamin C deficiency disease has been eliminated, while non-disappearance indicates that it still exists. In the latter case, vitamin C is to be offered again. In this case it is important to return to the patient as quickly as possible, re-inject, have the patient take tablets again and carry out the urine test after 3-4 hours. The vitamin C deficiency disease is generally eliminated after the second check. If not, vitamin C is given once again until the urine begins to discolor the blue reagent.

While we were coming close to reaching a specific conclusion through our experiments regarding the treatment of pneumonia with vitamin C, we became aware of the work of Hochwald from the Klinik Nonnenbruch in Prague on the same subject. His starting point was the observation gleaned from an animal experiment that vitamin C possesses anti-allergic properties. Since the croupy form of pneumonia is now included among the allergic diseases based on new views, particularly those represented by the Nürnberg pathologist Lauche, Hochwald began to study the effect of administering vitamin C in more detail. Following an initial report at the Verein deutscher Aerzte (association of German physicians) in Prague on February 7th of this year, the results were promising. The course of pneumonia was able to be shortened and a lytic defervescence achieved from the time of the very first injections. At the same time improvement could be observed in general condition, blood count and X-ray findings.

In the meantime, Hochwald has laid down his experience in a detailed publication entitled “Observations on the Effects of Ascorbic Acid in Croupy Pneumonia” and has kindly allowed us to have a look at the manuscript before it is published. We are thus in a position to reproduce some of his conclusions here.

"Ascorbic acid, injected as early as possible in large doses (individual doses of 0.5 g every 1 ½ hours, where possible until complete defervescence) provided a medicinal benefit in croupy pneumonia, which was expressed in improvement of general condition (prostration, dyspnea, etc.), rapid defervescence, earlier disappearance of local diagnostic findings, normalization of leukocytic blood count, and in suitable cases of urinary diagnostic findings as well."

Venturing out from vastly different starting points and independent of each other, both Hochwald and we arrived at almost the same conclusions in our examination of Vitamin C in cases of pneumonia.

Thanks to the fact that we were in possession of the analysis apparatus for vitamin C deficiency disease developed by the Staehelin-Klinik and not yet publicized at the beginning of our experiments, we had the opportunity to study and clarify the dosing issue in more detail and to also make the procedure available for practitioners who handle the majority of the patients ill will pneumonia. We were then also able to determine that, in general, the high doses as used by Hochwald – up to 5000 mg per the respective total of 10,000-15,000 mg -are never necessary or are only necessary on an exceptional basis, and that one can generally get by on about 1000-2000 mg. The vitamin C therapy for pneumonia will therefore be significantly cheaper and applicable not only for the clinical picture, but also for actual practice.

If we look at the vitamin C therapy for pneumonia a little more closely, then it is basically nothing more than the re-establishment of a physiological state which had become abnormal due to the illness. Elimination of the vitamin C deficiency disease is therefore, strictly speaking, not a medicinal intervention. Even when it is undertaken very quickly, there are no unpleasant side effects to be feared, so that from this standpoint as well there are no obstacles standing in the way of verification.

In conclusion let us emphasize again that the results turn out best when the vitamin C deficiency disease is eliminated on the first day of illness. Special note must be made of this fact during the verification process.

In summary: in cases of pneumonia, elimination of a vitamin C deficiency disease on the first day of illness resulted in such surprisingly favorable results, that it seemed to us that vitamin C represents a valuable enrichment of pneumonia therapy. As much detailed verification as possible is needed, however. To make this possible, a procedure was worked out which allows the elimination of vitamin C deficiency disease on the first day of illness in patients with pneumonia.

J Am Coll Nutr. 1995 Apr;14(2):116-23.

Vitamin C and the common cold: a retrospective analysis of Chalmers' review.

Hemilä H[1], Herman ZS.

Author information

Abstract

In 1975 Thomas Chalmers analyzed the possible effect of vitamin C on the common cold by calculating the average difference in the duration of cold episodes in vitamin C and control groups in seven placebo-controlled studies. He found that episodes were 0.11 +/- 0.24 (SE) days shorter in the vitamin C groups and concluded that there was no valid evidence to indicate that vitamin C is beneficial in the treatment of the common cold. Chalmers' review has been extensively cited in scientific articles and monographs. However, other reviewers have concluded that vitamin C significantly alleviates the symptoms of the common cold. A careful analysis of Chalmers' review reveals serious shortcomings. For example, Chalmers did not consider the amount of vitamin C used in the studies and included in his meta-analysis was a study in which only 0.025-0.05 g/day of vitamin C was administered to the test subjects. For some studies Chalmers used values that are inconsistent with the original published results. Using data from the same studies, we calculated that vitamin C (1-6 g/day) decreased the duration of the cold episodes by 0.93 +/- 0.22 (SE) days; the relative decrease in the episode duration was 21%. The current notion that vitamin C has no effect on the common cold seems to be based in large part on a faulty review written two decades ago.

Comment in

- Vitamin C supplements and disease--counterpoint. [J Am Coll Nutr. 1995]

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Vitamin C intake and susceptibility to pneumonia

HARRI HEMILÄ, PHD

Feeding guinea pigs a diet deficient in vitamin C increases their susceptibility to infections, which may be caused by the effects of the vitamin on T lymphocytes and phagocytes.[1] A few studies suggest that vitamin C intake affects human susceptibility to infections to some as yet unknown extent.[1] In particular four trials involving British males showed an average 30% decrease in common cold incidence in groups given vitamin C, suggesting effects in certain population groups.[2] Controlled trials have consistently found that large dose vitamin C supplementation alleviates the symptoms of the common cold, but the mechanism of this effect is poorly understood.[1-3] Here we assess the relation of vitamin C intake to the incidence of pneumonia by analyzing findings from three controlled trials.

The literature on vitamin C and infectious diseases has already been explored thoroughly[1,2] and all controlled trials that reported the number of pneumonia cases in the study groups were selected for this analysis (Table 1). Fisher's exact test was used to calculate the one-tailed mid-*P* values[4] for each set of data separately. Exact hypothesis test for several 2 × 2 contingency tables[4] was used to calculate an one-tailed mid-*P* value for the combined data of two or three studies.

Three controlled trials have reported the number of pneumonia cases in a vitamin C group and a control group, each trial finding a considerably lower incidence of pneumonia in the group given vitamin C (Table 1).

Glazebrook and Thomson[5] studied schoolboys (15 to 20 years old) in an institution in the UK. No cases of pneumonia occurred in the vitamin C group. Placebo was not used, but because the vitamin was added to the food in the kitchen the placebo effect does not seem relevant. For practical reasons the subjects were not randomly allocated to the study groups, but certain administrative divisions were served vitamin-supplemented food and others remained as controls. A tonsillitis epidemic that affected all divisions uniformly the year before had shown that they could not be considered discrete units.[5]

Kimbarowski and Mokrow[6] in the former Soviet Union investigated military recruits who had acquired influenza A infection. The number of pneumonia cases was significantly smaller in the vitamin C group. Placebo was not used and the allocation method was not described. Nevertheless the distribution of influenza severity was similar in both study groups.

Pitt and Costrini,[7] primarily interested in whether vitamin C affects the common cold, carried out a randomized double blind placebo-controlled trial with military recruits in a training camp in the United States. Pneumonia incidence was substantially lower in the vitamin C group.

Each of these three trials found a ≥80% lower incidence of pneumonia in the vitamin C group. It is highly unlikely that the differences reported between the study groups in favor of the vitamin C groups would have occurred purely by chance ($P = 0.00002$). The study of Pitt and Costrini[7] is the most carefully conducted of the three, but the size of the effect is similar to the others. Thus there is no obvious tendency for the technically superior trial to show a smaller effect. If the Kimbarowski-Mokrow study is excluded from the analysis because it is technically the least satisfactory, there is still a highly significant difference in the pneumonia incidence between the vitamin C and control groups in the remaining two trials ($P = 0.0004$).

The notion that vitamin C intake may effect various infections is an old one.[1, 5, 8, 9] In 1917 Hess[10] concluded from his clinical experience with children that one of the important consequences of vitamin C deficiency was a markedly increased susceptibility to infection, pneumonia being a particular danger. In 1939 Sabin[11] reported about 5 cases of pneumonia in 25 rhesus monkeys deficient in vitamin C whereas no cases were seen in 21 monkeys with adequate vitamin C intake ($P = 0.02$). The controlled trials assessed here suggest that vitamin C intake may affect susceptibility to pneumonia at least in some population groups.

A pertinent question as regards the interpretation of the three pneumonia trials is whether the differences

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TABLE 1. Vitamin C supplementation and the incidence of pneumonia

Study*	Vitamin C Dose (g/Day)	Cases/Total		Difference in Incidence (%)	*P* (1-Tail)
		Vitamin C group	Control group		
Glazebrook and Thomson,[5] 1942	0.05–0.3	0/335	17/1100	−100	0.006
Kimbarowski and Mokrow,[6] 1967	0.3	2/114	10/112	−80	0.009
Pitt and Costrini,[7] 1979	2	1/331	7/343	−85	0.022

* Combined test for all three sets of data: *P* (1-tailed) = 0.00002.

between the study groups result mainly from a marginal deficiency in the control group or the high dose supplementation in the vitamin group. It was proposed previously that the reported decrease in common cold incidence in British males was better explained by a low dietary intake of vitamin C in the control group than by high dose supplements.[2] Glazebrook and Thomson[5] estimated that their subjects obtained only 10 to 15 mg of vitamin C per day. Kimbarowski and Mokrow[6] did not explicitly estimate the dietary intake of their subjects but it seems likely that military recruits in the former Soviet Union also had a low intake. In both trials the vitamin dose administered was rather small, being in the range quite easily obtainable from diet (0.05 to 0.3 g/day). Accordingly the subjects of these two trials may have suffered from a marginal deficiency of vitamin C. Pitt and Costrini[7] did not estimate the dietary intake of their subjects but the whole blood vitamin C level was rather high initially (10 mg/1) and increased by only 36% when high vitamin C doses were administered (2 g/day), indicating the absence of marginal deficiency in the control group. Consequently the high dose supplementation seems to explain the difference between the study groups in this trial. In this respect these three trials do not invite a consistent and straightforward interpretation.

Because of the technical deficiencies in two trials[5,6] and the small number of pneumonia cases in each of the three trials, no firm conclusions can be drawn. Nevertheless the considerably lower pneumonia incidence in the vitamin C groups indicates that further work should be performed to address the question of whether vitamin C affects susceptibility to pneumonia more explicitly.

ACKNOWLEDGMENTS

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s About Micronutrient Supplements in American Academic Medicine

Archives of Internal Medicine 158(20):2187-91 · December 1998 with 344 Reads
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oodwin
niversity of Texas Medical Branch at Galveston

M R Tangum

20th century American academic medicine has resisted the concept that supplementation with
ave health benefits. This resistance is evident in several ways: (1) by the uncritical acceptance of news
belief that vitamin C supplements cause kidney stones; (2) by the angry, scornful tone used in
utrient supplementation in the leading textbooks of medicine; and (3) by ignoring evidence for possible
ient supplement, such as the use of vitamin E for intermittent claudication.

COMMENTARY

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udes About Micronutrient Supplements in American Academic Med

THROUGHOUT THE 20th century American academic medicine has resisted the concept that supplettion with micronutrients have health benefits. This ance is evident in several (1) by the uncritical accepof news of toxicity, such as lief that vitamin C supplecause kidney stones; (2) by gry, scornful tone used in ssions of micronutrient ementation in the leading oks of medicine; and (3) by g evidence for possible effiof a micronutrient supplesuch as the use of vitamin E ermittent claudication.

art of the resistance stems the fact that the potential ts of micronutrients were ced by outsiders, who took nessage directly to the pubd part from the fact that the ot of a deficiency disease did in well with prevailing bioal paradigms, particularly the heory. Similar factors might pected to color the response demic medicine to any alter- treatment.

n *The Crime of Galileo*, histoiorgio de Santillana[1] presents ionist view of the great scienstruggle with the Catholic n. According to de Santillana, o's crime was not his proing a heliocentric universe; it at he wrote in Italian; he comated his revolutionary ideas about astronomy directly to the public. Previous scientists wrote in Latin, limiting their audience to other scholars. Within this small community, controversial ideas could be entertained. Copernicus' proposal of a heliocentric universe 70 years before Galileo's treatises had elicited no attempts at suppression by the church. The 17th-century church represented the intellectual establishment, and Galileo's persecutors included some of the finest minds of his time. Galileo was punished not for writing heresy, not for threatening paradigms, but for bypassing the intellectual establishment and taking his exciting ideas directly to the people. The establishment, threatened not so much by his ideas as by his methods, did what it could to destroy his credibility.

In addition, Galileo did not respect professional boundaries. He was a mathematician, and yet his writings dealt with phenomena considered within the purview of philosophers, a profession of considerably higher status than mathematics.[2] Thus, he was considered a usurper as well as a popularizer. In what follows we argue that the reaction of academic medicine to the concept of micronutrient supplementation can best be understood in light of the foregoing description of Galileo. Our thesis is that throughout much of the 20th century, American academic medicine was resistant to the concept that micronutrient supplementation might prove beneficial, and that the cause of this resistance was similar to that which faced Galileo. This resistance is evident in several ways: (1) by unc of bad news abo supplements; r effects were rarel widely quoted; (2 dismissive tone o about micronutri tion in textbook tone avoided in m troversies; and (3 reaction greeting cacy of a micronu other therapies; in were simply ignor

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Herein we r tiple editions of 2 medical textbook *Medicine*[8] and *Pri Medicine*.[9] Each lished in 12 dif between 1950 an be presumed to lished opinions a sample how m changes over tim

e Center on Aging, The University s Medical Branch, Galveston.

ıtion, race, background diseases, and lifestyle can be mentioned among the underlying factors of kidney very much depends on the diet [25, 34, 35] . In our study, the prevalence of stones was 61.2% for CaOx, for uric acid, and 62% for cysteine stones. ...

P, uric acid and CaOx stones was 62%, the frequency of CaP and CaOx stones was 10.6%, the uric acid Table 2. Frequency of mixed stones by gender [6]. In the study by Altaf et al, the prevalence of s was 37%, and the prevalence of CaOx + CaP stones was 5% [35] , which is close to the results of our highest frequency of uric acid + CaOx stones was seen in men with 27 cases and the male to female ratio 3:1, which is close to the results of a study by Riyadh et al [36]. ...

valence of the stones was seen in the age group 30-39 years (25.8%) and 40-49 years (20.5%), which is ılts of the study by Tadayyon et al [6]. In another study conducted in New York in 2006, the highest d in the age group 18-45 years [35] . In our study, a significant relationship was found between age and nsistent with the results of a study by Antonia Boza [40]. ...

ne Different Compositions in Patients Referred to a Lithotripsy Center in Ilam, West of Iran

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ı Moradi · Milad Azami · Milad Borji

sing the preconceptions in academic medicine on micronutrient supplements, Goodwin and Tangum gave pport the conclusion that there has been systematic bias against the concept that vitamins might be er than the minimum required to avoid classic deficiency diseases [275] . In other papers, Goodwin and eral cases in which an effective method of treatment was erroneously rejected: the rejection seemed to be nderstanding of the physiological mechanism of the effect [276,277]. ...

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S Franceschi

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To assess the antioxidant status in HIV positive children. HIV positive children under the age group of 3-12 years from lower socio-economic strata were chosen for the study (Group 1). The values were compared with normal children (Group 2) not suffering from any disease in the same age group and similar socio-economic strata. The antioxidants chosen for the present study were vitamin A ... [Show full abstract]

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January 2011 · International journal of cancer prevention

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Vitamin C is an acidic molecule with strong reducing activity. It is an essential micronutrient in man, due to the absence of L-gulonolactone oxidase. Vitamin C has several important roles and there are many enzymes utilizing ascorbate as a co-factor. Besides, vitamin C protects human health by scavenging toxic free radicals and other reactive oxygen species (ROS) formed in cell metabolism. On ... [Show full abstract]

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C J Bates

Assay procedures for plasma concentrations of vitamin C, and hence for vitamin C status, currently in use in European population-surveillance laboratories and elsewhere, are based on a wide range of disparate techniques and reactions. The problem of achieving harmonisation between these techniques, and between laboratories, is further complicated by the instability of the vitamin, and the ... [Show full abstract]

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Ewelina Chajduk
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mmonly occurs in chronic heart failure (HF) and is associated with poor prognosis. Neither its causes nor nificance are clearly understood. We aimed to assess iron status and the effect of iron supplementation in the rat dial infarction (MI) HF. Four weeks after induction of MI to induce HF or sham surgery, rats received intravenous iron e) or saline, 4 doses in 1-week intervals. HF alone did not cause anemia, systemic or myocardial ID, but reduced ggesting depleted cardiomyocyte iron stores. Iron therapy increased serum Fe, ferritin and transferrin saturation as epatic iron content in HF rats, but did not increase myocardial ferritin. This was accompanied by: (1) better ntricular (LV) ejection fraction and smaller LV dilation, (2) preservation of function of Ca^{2+} handling proteins in LV 3) reduced level of inflammatory marker, CRP. Furthermore, iron supplementation did not potentiate oxidative stress or cardiomyocyte function, but increased activity of antioxidant defenses (cardiac superoxide dismutase). Despite lack of al ID we found evidence of depleted cardiomyocyte iron stores in the rat model of HF. Furthermore we observed supplementation and confirmed safety of iron supplementation in this setting.

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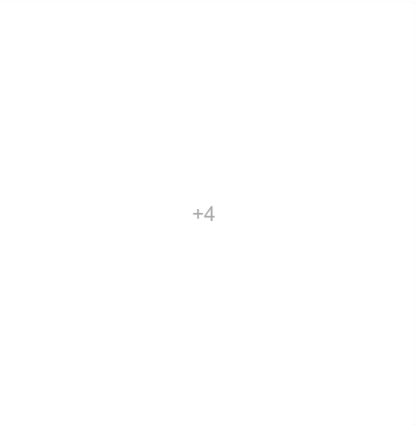
The effect of intravenous iron (Fe) supplementation on echocardiographic…



The effect of intravenous iron (Fe) supplementation on tissues Fe content and…

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Beneficial effects of intravenous iron therapy in a rat model of hea failure with preserved systemic ir status but depleted intracellular cardiac stores

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2018

Aleksandra Paterek[1], Marta Kępska[1], Barbara Sochanowicz[2], Ewelina Chajduk[3], Joanna Kołodziejczyk[1], Halina Polkowska-Motrenko[3], Marcin Kruszewski[2,4,5], Przemysław Leszek[6], Urszula Mackiewicz[1] & Michał Mączewski [1]

Iron deficiency (ID) commonly occurs in chronic heart failure (HF) and is associated with poor pro Neither its causes nor pathophysiological significance are clearly understood. We aimed to asses iron status and the effect of iron supplementation in the rat model of post-myocardial infarction (MI) HF. Four weeks after induction of MI to induce HF or sham surgery, rats received intravenou iron (ferric carboxymaltose) or saline, 4 doses in 1-week intervals. HF alone did not cause anemia systemic or myocardial ID, but reduced myocardial ferritin, suggesting depleted cardiomyocyte stores. Iron therapy increased serum Fe, ferritin and transferrin saturation as well as cardiac and hepatic iron content in HF rats, but did not increase myocardial ferritin. This was accompanied b better preservation of left ventricular (LV) ejection fraction and smaller LV dilation, (2) preservat function of Ca^{2+} handling proteins in LV cardiomyocytes and (3) reduced level of inflammatory m CRP. Furthermore, iron supplementation did not potentiate oxidative stress or have toxic effects

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safety of iron supplementation in this setting.**

Iron is a vital element for the body, especially for metabolically active tissues such as myocardium. It is component of oxygen carrying protein, hemoglobin and of multiple oxidative enzymes and respirat proteins, including those containing Fe-S clusters, involved in cellular metabolism. Dietary iron is abs enterocytes and then secreted into circulation where it is bound to an iron transporting protein, tra which on one hand delivers iron to target cells (by binding to the transferrin receptor-1 [TfR1]), on the o tralizes its free radical generating activity. Iron can be utilized by target cells or stored, bound to ferriti in the liver. Thus transferrin saturation with iron is a good indicator of usable iron pool, while ferritin indicator of total body iron (however, being an acute phase protein, it can be increased in inflammatory

Iron deficiency (ID), occurs in up to 50% of patients with chronic heart failure (HF), both with c anemia and with normal hemoglobin values[1]. Its etiology is likely multifactorial and remains largely u Broadly speaking, ID can be attributed to the factors related to HF per se (e.g. malabsorption due t

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eferences (35)

cused on increasing the concentration of haemoglobin, an oxygen-carrying protein. But neither erythropoietin analogs lobin concentration 6 nor intravenous iron that provided an essential element not only for haemoglobin, but also other rdiac energetics 7 provided unequivocal benefits in human clinical trials, though recent data, including our own work, 8 e of some value here. ...

pendent cardiovascular diseases by myo-inositol trispyrophosphate (ITPP)-enhancement of oxygen delivery by red

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hra El-Hafny-Rahbi · Aleksandra Paterek · Claudine Kieda

stmyocardial infarction heart failure, which had the advantage of identical genetic background, diet as well as the es and concomitant therapies, we demonstrated lack of systemic ID in heart failure. We also did not find signs of , we noticed depleted myocardial iron stores (Paterek et al., 2018) . Similar results were found in rats with ischemic e no alteration of iron status was observed, in particular serum, myocardial and hepatic iron remained unchanged. ...

adigm shift from systemic to cardiomyocyte abnormalities

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Urszula Mackiewicz · Michał Mączewski

he AFFIRM-AHF trial: a randomised, double-blind, placebo-controlled trial comparing the effect of intravenous hospitalisations and mortality in iron-deficient patients admitted for acute heart failure

FAIL

ridget-Anne Kirwan · Stefan D. Anker · Ewa Anita Jankowska

n Heart Failure: Common, Clinically Relevant and Treatable

le

Goran Loncar · Andelka Isakovic · Jerneja Farkas

Project

omega-3 PUFA and the heart

Michał Mączewski · Monika K Duda · Mariusz Marciszek · [...] · Agnieszka Dobrzyn

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Chapter

Anemia and Iron Deficiency in Heart Failure

January 2019

Otmar Pfister

Anemia and iron deficiency (ID) are common co-morbidities in chronic heart failure (CHF) patients and are both independently associated with increased morbidity and mortality. Anemia affects one of three CHF patients and ID is present in half of CHF patients. While the treatment of anemia remains a challenge, ID has become a valid treatment target. ID is diagnosed when ferritin is lower than 100 ... [Show full abstract]

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Iron Deficiency among Pregnant Women Attending Antenatal Clinic at the KNUST Hospital, Kumasi, Ghana

January 2015

Christian Obirikorang · Linda Ahenkorah Fondjo · Samuel Adomako · [...] · Isaac Acheampong

Background: Pregnant women constitute a high risk group for iron deficiency due to increased iron requirements for foetal and maternal tissues growth. This study sought to find out the prevalence of iron deficiency among Ghanaian pregnant women obtaining antenatal care at the University hospital, Kumasi, Ghana. Methods: The study was conducted between January and May, 2013. A total of 180 women, ... [Show full abstract]

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Анемия и железодефицит у больных с хронической сердечной недостаточностью. Anaemia and Iron Deficien...

May 2019 · Kardiologiia

N. T. Vatutin · Gennadiy Taradin · Irina Kanisheva · Victoria Venzheha

В представленном обзоре затронуты вопросы распространенности анемии и железодефицита (ЖД) при ХСН, их влияние на течение и прогноз этого состояния. Сформулировано определение анемии и ЖД на основе оценки различных лабораторных данных. В частности, обсуждается диагностическая значимость определения сывороточного железа, ферритина сыворотки крови, коэффициента насыщения трансферрина, общей ... [Show full abstract]

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Iron deficiency and anaemia in heart failure: Understanding the FAIR-HF trial

November 2010 · European Journal of Heart Failure

José González-Costello · Josep Comin-Colet

Treatment of anaemia in patients with chronic heart failure (CHF) and reduced left ventricular ejection fraction has traditionally focused on erythropoietin-stimulating agents. However, recent studies have shown that treatment with intravenous (IV) iron can improve the symptoms and quality of life in patients with CHF and iron deficiency (ID), with or without anaemia. The management of ID is ... [Show full abstract]

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Article

Iron deficiency in a multi-ethnic Asian population with and without heart failure: Prevalence, clini...

September 2014 · European Journal of Heart Failure

Tee Joo Yeo · Daniel Yeo · Raymond Ching Chiew Wong · [...] · Carolyn S.P. Lam

Aims: Current heart failure (HF) guidelines highlight the importance of iron deficiency (ID) in HF. Whether HF itself or age-related comorbidities contribute to ID is uncertain, and previous data were limited to Western populations. We aimed to study the prevalence, clinical correlates, functional significance and prognosis of ID in HF patients, compared with community-based controls in a ... [Show full abstract]

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MARCH 1974

DCIEM REPORT NO. 74-R-1012

HEALTH PROBLEMS AND VITAMIN C IN CANADIAN NORTHERN MILITARY OPERATIONS

B.H. SABISTON

M.W. RADOMSKI

(Text of Communication presented at the Twenty-Fifth Symposium of the Defence Research Board, Department of National Defence, Canada. Presented 14 November 1973 by B.H. Sabiston)

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DEFENCE RESEARCH BOARD — DEPARTMENT OF NATIONAL DEFENCE — CANADA

ABSTRACT

As part of a continuing study of health problems pertinent to Canadian Northern Military operations, two aspects of Vitamin C have been examined in land element personnel participating on Northern Winter Exercises. This report describes results of an ongoing Vitamin C survey designed to examine both the Vitamin C status of troops and the effects of a daily Vitamin C supplement on the incidence and severity of colds in troops undergoing operational training. Results indicate that a daily 1000 mg supplement of Vitamin C reduced significantly the incidence of colds as assessed on the basis of symptom complexes reported on health survey cards. While the overall incidence of colds was influenced significantly by Vitamin C, both on an individual and a tent group basis, the duration of local cold symptoms was not. In those individuals who contracted a cold, nasal and throat and chest symptoms were observed to persist for similar periods of time in both placebo and Vitamin C supplemented groups. The Vitamin C group, however, did show a significant reduction in the duration of the more constitutional symptoms related to a general feeling of "well-being". The Vitamin C status of individuals was assessed on the basis of whole blood ascorbate levels determined before and after participation on Northern exercises. A significant reduction of whole blood ascorbate was observed post-exercise on three separate serials of Exercise New Viking, the troops of which were supplied with RP-4 field rations. In view of the fact that only a minor reduction of whole blood ascorbate was observed on another serial, the troops of which were supplied with IRP field rations, it is not possible to determine whether the reduction in ascorbate status was a reflection of altered dietary intake or an increased requirement for Vitamin C under the activity and exposure conditions experienced on Northern operations. Further work is required to clarify this situation.

HEALTH PROBLEMS AND VITAMIN C IN CANADIAN NORTHERN MILITARY OPERATIONS

Since the early part of 1972, the Biosciences Division of the Defence and Civil Institute of Environmental Medicine (DCIEM) has been involved in an extensive field program designed to examine some of the health problems pertinent to Canadian Northern Military operations.

Table 1 lists some of the potential health problem areas encountered in a transit military population operating under Arctic or sub-Arctic conditions. These have been divided, somewhat arbitrarily, into two groups: Environmental and Operational.

TABLE 1

POTENTIAL HEALTH PROBLEM AREAS NORTHERN OPERATIONS

ENVIRONMENTAL	OPERATIONAL
Cold Injury	Nutrition
Frostbite	Rations
Trench Foot	Dehydration
Hypothermia	Constipation
Snow Blindness	Tent Eye
Sunburn	Physical Fitness
Cold Sores	Wound Heating
	Upper Respiratory Infection
	Dental

(1) *Environmental* problems are those which arise as a consequence *of direct insult upon the individual by his environment.*

(2) *Operational* problems are those which arise as a consequence of *restrictions placed upon an individual by his environment.*

This report describes results dealing with some problems in the operational category, specifically with regard to rations and Vitamin C, the Vitamin C status of individuals, and the effect of Vitamin C supplementation on symptoms of respiratory distress.

One of the approaches which has been applied throughout the field program has been the administration of a health survey to men taking part in military winter exercises. This survey was established primarily to answer the questions, "does the abrupt introduction of a man into the Northern climate produce any demonstrable change in health pattern? If so, what is the nature of this alteration? "

The majority of health surveys which have investigated environmental factors impinging on health have been concerned with indigenous populations or isolated communities. Data derived from such studies are not applicable directly to transit populations such as members of mobile military forces. Recognition of this fact prompted DCIEM to establish a protocol for obtaining epidemiologic data on military men making periodic excursions into the North. The survey has been restricted to members of the land element for it is these individuals who are exposed most directly to the adverse environment for periods of greater than a few hours.

Table 2 lists the exercises which have been surveyed to date. With one exception (Northern Ramble, May 1972) the field program has utilized men taking part in New Viking training exercises. It is important to recognize the fact that these are *training* exercises and that as such, the men are living under the most "ideal" Arctic conditions in the sense that experienced instructors are with them at all times. Consequently, the men are under constant supervision to ensure that they protect themselves adequately from the environment. Hence, any health problems which arise on such exercises should be taken as a minimal estimate of problems which may arise on more operational missions.

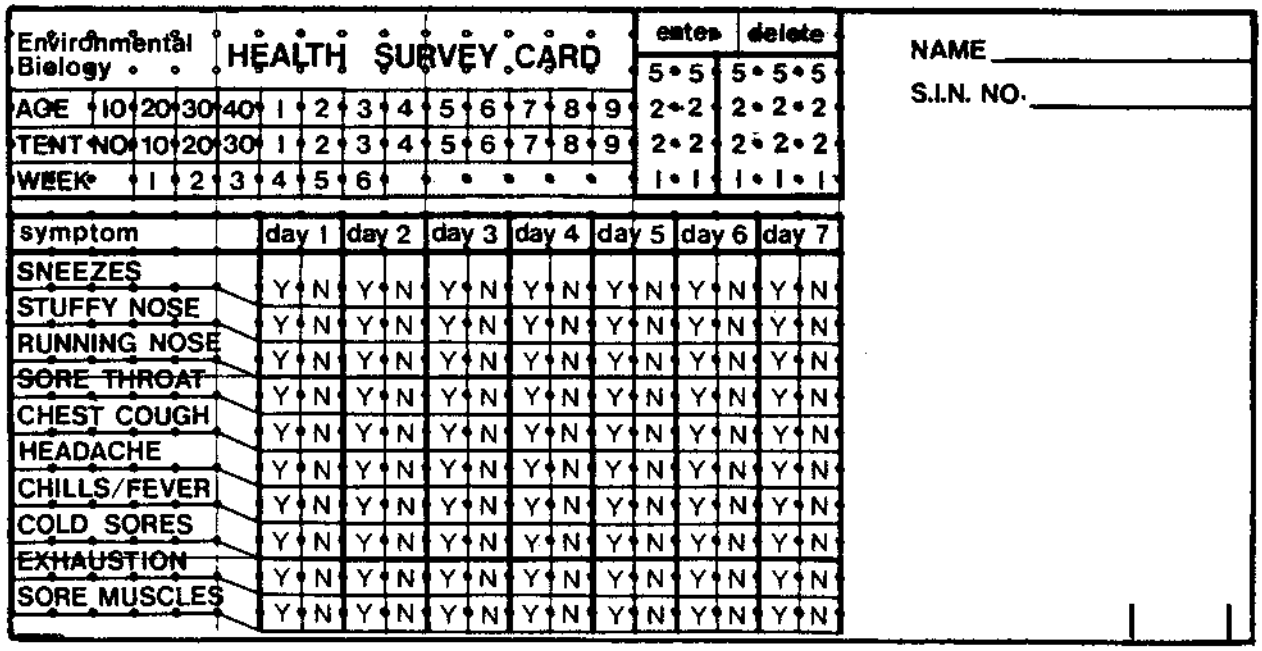
TABLE 2

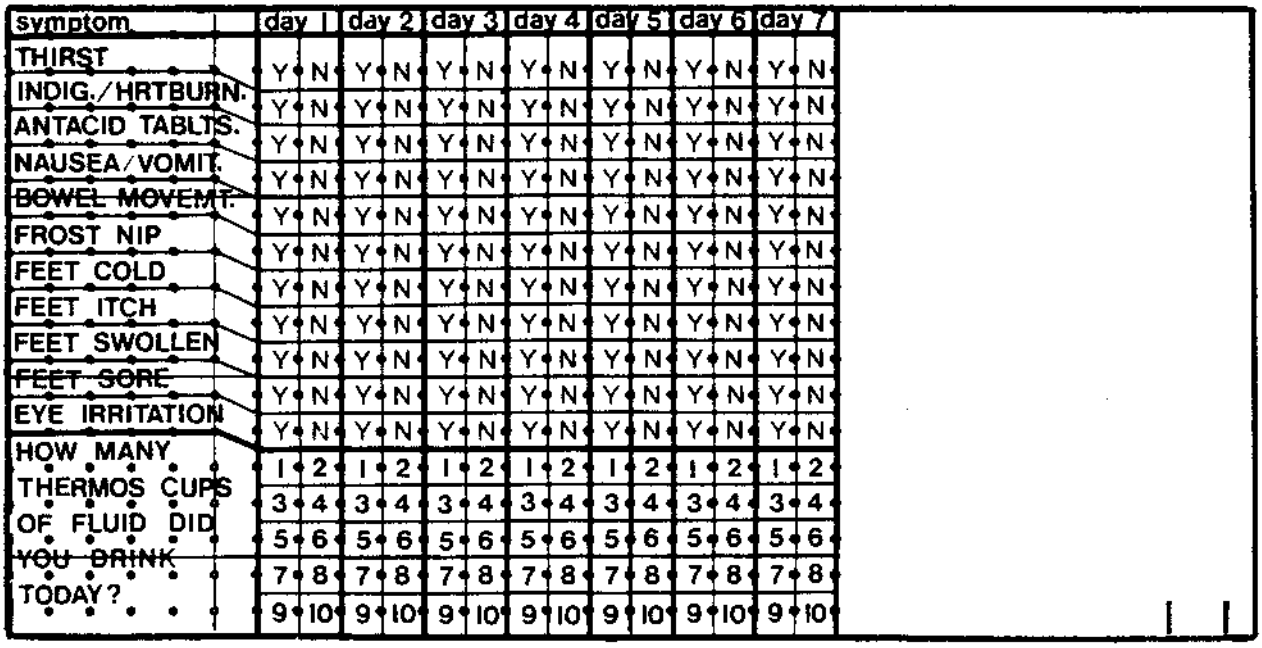
NORTHERN EXERCISES UTILIZED FOR THE INVESTIGATION OF HEALTH PROBLEMS, 1972-73

Exercise	Date	Home CFB	N	Northern Location
New Viking 37	March 1972	Petawawa	70	Coral Harbor
Northern Ramble	May 1972	London	400	Churchill
New Viking 49	December 1972	London	100	Coral Harbor
New Viking 52	January 1973	Gagetown	100	Churchill
New Viking 55	February 1973	Petawawa	100	Frobisher Bay
New Viking 56	March 1973	Calgary	120	Frobisher Bay
New Viking 57	April 1973	Petawawa	100	Frobisher Bay

The health survey card used in the collection of field data is shown in Figure 1. The health survey has been conducted on an individual tent-group basis and extensive use has been made of the tent-group commanders who have been responsible for administering the survey cards on a daily basis. The survey period has extended typically from one week before the exercise to one week after the exercise. Tabulation of the incidence of individual symptoms and symptom complexes has been carried out post-exercise and it has become apparent that, to one degree or another, the incidence of individual symptoms is affected by movement into the North. The most marked alteration in symptoms reported has been noted in symptoms related to the upper respiratory system and it is these symptoms which have been examined in greater detail in DCIEM Vitamin C studies.

IN-FIELD HEALTH SURVEY CARD





An assessment of Vitamin C was undertaken for a number of reasons:

(1) The whole question of Vitamin C and its effect on colds is a topical and debatable issue. It was hoped that some light would be shed on this problem by utilizing a very restricted population of comparable age, typical cold history, common dietary regimen, activity schedule and environmental exposure.

(2) It has been suggested that Vitamin C may play a role in increasing cold tolerance - with particular regard to maintaining peripheral circulation.

(3) Finally, it was determined that the RP-4 rations (1970-71) on which the men were living, apparently provided a maximum of 37–41 mg Vitamin C per day in a single fruit-drink mix. As previous observations suggested that the fruit-drink mix was an unpopular item in the rations and tended to be discarded, it appeared that the individual intake of Vitamin C could be below the recommended daily allowance.

Accordingly, a protocol was established for dispensing tablets of either Vitamin C or placebo to individuals in each tent. Men in each tent group were assigned randomly to either the Vitamin C or placebo group. Extensive use was made again, of tent-group commanders who carried with them the supply of pills for their own tent. Two pill vials were provided for each tent, one containing Vitamin C and one containing placebo. Each vial contained the names of the men who were to receive the respective pills. Pills were dispensed twice a day, once with the morning meal and once with the evening meal. The total dose of Vitamin C received each day was 1000 mg.

At the completion of the exercise the incidence and duration of colds was examined by assessing the presence or absence of a cold on the basis of symptom constellations. In order for a man to be classified as having a cold, he had to have two nasal symptoms in conjunction with a minimum of sore throat or chest cough which persisted for two or more days. As a further restriction, the sore throat or chest cough had to be absent at the time the nasal symptoms began. Frequently, it was found that more constitutional symptoms such as headache, chills and fever, general malaise, nausea or vomiting were indicated at some time during the symptom constellation.

Table 3 indicates that the random allocation of men to the two treatment groups resulted in two well-matched populations with respect to age and typical cold history.

TABLE 3

THE MEAN AGE AND COMMON COLD HISTORY OF MEMBERS OF A SINGLE INFANTRY COMPANY OF 112 MEN ALLOCATED RANDOMLY TO VITAMIN C AND PLACEBO PREPARATIONS

Group	N	Age	Incidence of Usual Spring Cold %
Vitamin C	56	25.3 ± 6.3* (Range 17 - 40)	61.6
Placebo	56	25.4 ± 8.1 (Range 17 47)	60.0

*Mean ± S.D.

Table 4 depicts the frequency of colds assessed in a single infantry company on a Northern Military exercise. The incidence of colds in two other companies participating on the exercise, but not subjected to pill supplementation, was 21.0% and 29.4% respectively.

TABLE 4

INDIVIDUAL INCIDENCE OF COLDS ASSESSED IN A SINGLE INFANTRY COMPANY OF 112 MEN PARTICIPATING ON A NORTHERN MILITARY EXERCISE

Group	N	Frequency	Percent Frequency
Vitamin C	56	6	10.7
Placebo	56	14	25.0
χ^2	3.87		P=0.05

The results indicate that the Vitamin C group experienced significantly fewer colds than the corresponding placebo group. This ameliorating effect of Vitamin C was also reflected in the frequency of colds reported by individual tent groups (Table 5). Of the 14 tent groups involved in this study, nine groups (64.3%) indicated the presence of at least one cold during the exercise period. Of these nine groups, six (66.6%) indicated colds present only in placebo individuals, whereas the remaining three (33.3%) indicated colds present in both placebo and Vitamin C groups. In no case did a tent group indicate the presence of colds in Vitamin C individuals only.

TABLE 5

TENT GROUP INCIDENCE OF COLDS IN AN INFANTRY COMPANY OF 112 MEN PARTICIPATING ON A NORTHERN MILITARY EXERCISE

Number of Tent Groups Reporting One or More Colds Amongst its Members	Number of Tent Groups Indicating Colds Present		
	In Vitamin C Individuals only	In Placebo Individuals only	In Both Vitamin C and Placebo Individuals
9/14	0/9	6/9	3/9
(64.3%)	–	(66.6%)	(33.3%)

The data presented in Table 6 indicate that despite a reduction in the frequency of colds in Vitamin C individuals, the duration of cold symptoms as related to the presence of nasal, throat or chest complaints was not significantly influenced. In other words, if an individual experienced a cold while on Vitamin C, the continued daily intake of 1000 mg/day did not alter the course of the cold with respect to the local symptoms. Examination of the more constitutional symptoms however (Table 7) revealed that the duration of these was significantly reduced in the Vitamin C group. This perhaps is a significant finding for it is these symptoms which are related to the general feeling of "well-being" and it is these symptoms which, in a civilian population, could predispose a person to remain at home. In a military population where refuge cannot be sought easily, it is these symptoms which would tend to reduce a man's level of effectiveness.

TABLE 6

THE MEAN DURATION OF UPPER RESPIRATORY SYMPTOMS REPORTED BY MEN AFFLICTED WITH A COMMON COLD

Group	N	Duration of Symptoms (days)	
		Nasal	Throat/Chest
Vitamin C	6	4.2 ± 3.8*	4.3 ± 3.0
Placebo	14	5.6 ± 2.8	6.0 ± 3.0
P		> 0.4 > 0.5	> 0.2 > 0.3

*Mean ± S.D.

TABLE7

THE MEAN DURATION OF CONSTITUTIONAL SYMPTOMS RELATED TO A FEELING OF WELL-BEING REPORTED BY MEN AFFLICTED WITH A COMMON COLD

Group	N	Duration of Symptoms (days)
Vitamin C	6	0.8 ± 0.8*
Placebo	14	2.4 ± 2.1
		$p < 0.05$

On subsequent exercises an examination of the Vitamin C status of men was carried out by examining the whole-blood ascorbate levels before and immediately after the exercise. Table 8 shows the incidence of altered ascorbate status on four Northern exercises. In all cases, a significant number of men demonstrated a decrease in whole-blood ascorbate, however the magnitude of this decrease (Table 9) was significant on only three of the exercises. Coincidentally, these three exercises were supplied with the RP4 ration while the fourth exercise (Serial 56) received IRP field rations. The IRP ration provides approximately 50–90 mg of Vitamin C per day, about 50% of which is in a single fruit-drink mix and 50% is distributed throughout other ration components.

TABLE 8

INCIDENCE OF ALTERED WHOLE-BLOOD ASCORBATE STATUS OCCURRING ON NORTHERN EXERCISES

Serial	N	% of Individuals Demonstrating a Decrease in Ascorbate	% of Individuals below 0.50 mg% Ascorbate	
			Pre-Exercise	Post-Exercise
NV 49	86	70	4	8
NV 51	29	83	28	41
NV 55	24	46	21	12
NV 56	34	47	32	32

TABLE 9

MEAN WHOLE-BLOOD ASCORBATE STATUS BEFORE AND AFTER PARTICIPATION ON NORTHERN EXERCISES

Serial	N	Pre-Exercise Level mg%	Post-Exercise Mean Change	
			mg%	%
NV 49	86	1.05 ± 0.04*	−0.19 ± 0.04	−18
NV 51	29	0.86 ± 0.07	−0.21 ± 0.04	−24
NV 55	24	0.91 ± 0.10	−0.13 ± 0.06	−14
NV 56	34	0.76 ± 0.05	−0.03 ± 0.06	− 4

*Mean ± S.E.M.

One further point with reference to Table 8 is the rather surprising number of men who demonstrated whole-blood ascorbate levels lower than 0.50 mg%. This value is generally taken to indicate the threshold of a possible sub-clinical scorbutic condition. Two of the four serials examined post-exercise demonstrated a definite shift towards this subclinical scorbutic state, one (Serial 56) remained unchanged and the other (Serial 55) demonstrated a shift in the opposite direction.

In view of the variation in diet and distribution of change in ascorbate status, it is not possible from these data to determine whether the reduction in ascorbate levels, observed post-exercise on three of the four serials, was a consequence of reduced dietary intake of Vitamin C or a reflection of a possible increased requirement for this vitamin under the activity and exposure conditions existing on Northern operations. Clearly, a determination of ascorbate excretion is required before any estimate of requirement under these conditions can be made.

This study is part of a continuing program to assess the nature and incidence of health problems pertinent to Canadian military Northern operations. With regards to Vitamin C and its influence on general body health the data to date suggest that a daily supplement of 1000 mg Vitamin C appears to reduce the overall incidence of colds in transit military populations. It must be appreciated however, that the nature of the military exercise itself represents a marked departure from the "normal" daily routine. Over the period of this study, the men are transported by air into an adverse environment and live in close association with that

environment. Their dietary regimen is altered dramatically with regards both to frequency of meals and nature of food eaten. In view of these factors the results reported here do not necessarily characterize the civilian population in general. Further, insufficient data exist to enable us to determine whether the observed beneficial effect of Vitamin C observed in this study, is prophylactic or therapeutic, although the analysis of colds by tent groups suggests that the effect may be prophylactic. In addition the study was restricted to an examination of the efficacy of a daily 1000 mg dose of Vitamin C, which may represent neither the optimal nor minimal daily supplement required. The whole-blood ascorbate levels of individuals receiving a Vitamin C supplement were increased well above normal (100–150%). In view of the demonstrated decrease in whole-blood ascorbate occurring in non-supplemented men, the optimal dose of Vitamin C may be in a range which is sufficient to prevent such a decrease. Further work is required to clarify this situation.

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13. ABSTRACT

This report describes results of an ongoing Vitamin C survey designed to examine both the Vitamin C status of troops and the effects of a daily Vitamin C supplement on the incidence and severity of colds in troops undergoing operational training. Results indicate that a daily 1000 mg supplement of Vitamin C reduced significantly the incidence of colds as assessed on the basis of symptom complexes reported on health survey cards. While the overall incidence of colds was influenced significantly by Vitamin C, both on an individual and a ten group basis, the duration of local cold symptoms was not. In those individuals who contracted a cold, nasal and throat and chest symptoms were observed to persist for similar periods of time in both placebo and Vitamin C supplemented groups. The Vitamin C group, however, did show a significant reduction in the duration of the more constitutional symptoms related to a general feeling of "well-being". Significant reduction of whole blood ascorbate levels was observed post-exercise on three separate serials of Exercise New Viking. Further work is required to determine whether this reduction in ascorbate status reflects altered dietary intake or an increased requirement for Vitamin C under the activity and exposure conditions experienced on Northern operations.

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HEALTH PROBLEMS AND VITAMIN C IN CANADIAN NORTHERN MILITARY OPERATIONS

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Which Plasma Antioxidants Are Most Related to Fruit and Vegetable Consumption?

Gladys Block,[1] Edward Norkus,[2] Mark Hudes,[1] Shelly Mandel,[1] and Kathy Helzlsouer[3]

Substantial evidence suggests that fruit and vegetable intake reduces the risk of some cancers and other chronic diseases. While a varied diet containing fruits and vegetables may confer benefits greater than those of any single nutrient, it would be useful to have data on the plasma nutrients most influenced by fruit and vegetable intake. The authors examined the correlation between fruit and vegetable intake as measured by the abbreviated CLUE II food frequency questionnaire and several plasma antioxidants. This study includes 116 male subjects aged 35–72 years who were nonsmokers and nonusers of vitamin supplements and who provided blood samples in the CLUE II Study in Washington County, Maryland. Plasma was assayed for ascorbic acid, beta-carotene, beta-cryptoxanthin, and alpha- and gamma-tocopherol. Lipid- and energy-adjusted partial correlation for the relation with fruit and vegetable intake was $r = 0.64$ for ascorbic acid, $r = 0.44$ for beta-carotene, and $r = 0.50$ for beta-cryptoxanthin. While this study does not address efficacy, the stronger association of ascorbic acid with fruit and vegetable intake seen here may imply that ascorbic acid is an important component of the protective effect seen for fruits and vegetables in numerous epidemiologic studies. *Am J Epidemiol* 2001;154:1113–18.

antioxidants; ascorbic acid; biological markers; carotenoids; fruit; questionnaires; vegetables

Numerous studies have found a significant inverse relation between cancer risk and intake of fruits and vegetables (1). Although the consumption of whole foods provides a complex nutrient mix that may confer a benefit superior to that of any particular component, it would be useful to understand which nutrients are most associated with a high intake of fruits and vegetables. A number of studies using food frequency questionnaires (FFQs) have examined the relation between dietary estimates of particular nutrients and the corresponding plasma nutrient levels. Very few, however, have examined the plasma nutrient levels simply in relation to reported intake of foods rather than to estimates of nutrients. In other words, what plasma nutrient levels are most influenced by a diet high in fruits and vegetables? This study examines plasma levels of several antioxidants in relation to intake of fruits and vegetables.

MATERIALS AND METHODS

Subjects were selected from among participants in the Washington County, Maryland, CLUE II Study, a blood collection campaign conducted by the Johns Hopkins Training Center for Epidemiologic Research and the Washington County Health Department. In 1989, CLUE II recruited residents of Washington County and surrounding counties; most samples were obtained in the fall. CLUE II obtained plasma samples, brief personal data, and a brief food frequency questionnaire. More than 30,000 persons from Washington County and surrounding counties provided samples.

Respondents for this study were selected from counties surrounding Washington County. Subjects were men aged 35–72 years (mean, 53 years) who did not smoke and did not take vitamin supplements. Respondents with an estimated energy intake of less than 1,000 kcal were dropped to exclude persons who may have been ill, were dieting, or had completed the questionnaire incorrectly.

The questionnaire used in the CLUE II Study is a 60-item scannable version of the Block/National Cancer Institute (NCI) questionnaire. The questionnaire contained 10 vegetable items and six fruit items (table 1). Collectively, these foods contribute 70.6 percent of the carotenoid intake in the US diet among men in this age range and 57.8 percent of the dietary vitamin C in the United States, on the basis of the Third National Health and Nutrition Examination Survey (G. Block, unpublished data, 1997). Frequency of consumption of these foods was summed to estimate total fruit and vegetable consumption. (The "GRPFRQ" variables produced by the software were used rather than the portion size-related measures; summary "global" questions were not asked in this FFQ.) Questionnaires were analyzed by using the Block/NCI software (2), and estimates were made of usual dietary intake of nutrients and food groups. Subjects

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Abbreviations: FFQ, food frequency questionnaire; FV, fruit and vegetable consumption; Heme, meat intake; NCI, National Cancer Institute.

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TABLE 1. Foods used to rank subjects on fruit and vegetable intake*, Washington County, Maryland, 1989

Fruits and vegetables on the CLUE II questionnaire
Carrots or mixed vegetables containing carrots
Spinach
Broccoli
Sweet potatoes, yams
Tomatoes, tomato juice
Vegetable or tomato soups
Coleslaw, cabbage, sauerkraut
Mustard greens, turnip greens, collards
Green salad
Any other vegetables, including green beans, corn, peas
Oranges
Grapefruit
Orange juice or grapefruit juice
Cantaloupe
Apples, applesauce, pears
Any other fruit, including bananas, fruit cocktail

* These items comprise foods that contribute the following proportions of US nutrient intake of carotenoids: 70.6% (65.4% from the 14 foods excluding "Any other vegetables" and "Any other fruit") and of dietary vitamin C: 57.8% (44.8% from the 14 foods excluding "Any other vegetables" and "Any other fruit"). (Block, unpublished data, 1997).

were included in this analysis if their reported dietary intake placed them in either the top or bottom quintile on both fruit and vegetable consumption (FV) and meat intake (Heme). (Heme was obtained for a different analysis, and those results are reported elsewhere (3).) Subjects were selected in groups of four (HiFV + HiHeme, HiFV + LoHeme, LoFV + HiHeme, and LoFV + LoHeme), matched within each group on age and body weight. A total of 29 subjects were selected for each of the four groups, resulting in a sample of 116 men for these analyses.

Venous blood was drawn in heparinized Vacutainers (Becton, Dickinson, & Co., Franklin Lakes, New Jersey), centrifuged, and processed within a few hours. One aliquot was prepared by using 10 percent metaphosphoric acid to stabilize ascorbic acid. All samples were stored at –70˚C. The long-term stability of these nutrients, when stored at –70˚C to –80˚C, has been examined in numerous studies and found to be acceptable (4–6). Masked duplicate samples were sent to each laboratory and included in the assays. In addition, a single pooled blood sample was divided into multiple aliquots and shipped with samples over the course of the study to permit analyses of laboratory drift. Reproducibility of all assays was excellent.

Plasma was assayed for ascorbate, beta-carotene, beta-cryptoxanthin, and alpha- and gamma-tocopherol by one of the investigators (E. N.). Plasma ascorbate concentration was determined spectrophotometrically by using 2,4-dinitrophenylhydrazine as chromogen (7), which has been shown to correlate highly with high-pressure liquid chromatography methods (8–11). Plasma carotenoids and vitamin E were determined by reversed-phase high-pressure liquid chromatography (12).

Analysis of variance, *t* tests, and Pearson and Spearman correlations were used. Variables were examined for normality and skewness and transformed by using log or square root, as appropriate. Pearson correlations using the transformed variables were almost identical to Spearman correlations, so only the latter are reported here. Statistical analyses were performed using PC-SAS version 6.11 (SAS Institute, Inc., Cary, North Carolina).

RESULTS

The characteristics of the participants in this analysis are shown in table 2. Body weight ranged from 120 to 250 pounds (54.48 to 11.35 kg), and mean frequency of fruit and vegetable intake was 2.9 times per day. Analysis of variance including the meat category, the fruit and vegetable category, and their interaction term indicated that meat consumption and the interaction term were not related to any plasma antioxidant (data not shown). Consequently, all analyses in this report related to plasma antioxidant level consider only the fruit and vegetable intake.

Correlations between frequency of FV and plasma antioxidants are shown in table 3. Both carotenoids and ascorbic acid are highly significantly associated with frequency of consumption of fruits and vegetables. However, the correlation with ascorbic acid is considerably higher than that for the carotenoids, both unadjusted and after adjustment for several covariates. This higher correlation of FV with ascorbic acid remained after standardization of the plasma carotenoids by plasma cholesterol. Plasma alpha-tocopherol is positively associated with FV only after standardization with plasma cholesterol, while gamma-tocopherol is significantly negatively correlated with FV. Partial correlations adjusted for age, education, body weight, energy intake, or fat intake did not change this pattern. After adjustment for age and energy intake, the correlation between fruit and vegetable intake and ascorbic acid was 0.64, while lipid-adjusted total carotenoids reached only 0.44. The highest correlation besides that of ascorbic acid was lipid-adjusted beta-cryptoxanthin (which is found largely in oranges and orange juice), at 0.50.

DISCUSSION

Although numerous investigators have examined the relation between serum antioxidant nutrient levels and estimates of antioxidant intake from food frequency questionnaires, few have reported the correlations between serum antioxidants and fruit and vegetable frequency as opposed to nutrient estimates (13–19). Only two studies were of nonsmokers (16, 17), and the results presented here correspond well to the carotenoid correlations observed in these earlier reports. Campbell et al. (16) recruited 50 male and 49 female nonsmokers aged 18–37 years, selecting only those in the highest or lowest quintile of FV; 29 percent were supplement users. (Smoking lowers plasma beta-carotene and ascorbic acid levels, and supplement use increases them, irrespective of fruit and vegetable intake. Inclusion of subjects with these behaviors makes it difficult to detect a relation between these plasma nutrients and fruit and vegetable intake.) The 153-item Willett FFQ was self-administered and included 35 veg-

TABLE 2. Characteristics of the sample, for 116 men aged 35–72 years, Washington County, Maryland, 1989

	Mean (SD)*	25th percentile	Median (50th percentile)	75th percentile	Range
Age group (% in each category)					
35–44 (19.0)					
45–54 (32.8)					
55–64 (33.6)					
65–74 (6.9)					
Missing (7.8)					
Body weight (pounds)†	182 (24.4)	165	180	195	120–250
Fruit and vegetable frequency (times/day)‡	2.9 (1.9)	1.3	2.6	4.1	0.1–9.5
Ascorbic acid (mg/dl)	1.0 (0.4)	0.76	1.0	1.3	0.2–2.7
Total carotenoids (μg/dl)	80.6 (34.0)	57.7	72.6	98.5	21.3–227
Beta-carotene (μg/dl)	13.5 (11.4)	6.5	10.4	17.3	1.2–75.2
Cryptoxanthin (μg/dl)	11.2 (9.1)	6.7	9.5	13.5	1.6–71.5
Alpha-tocopherol (μg/dl)	0.96 (0.2)	0.81	0.95	1.12	0.46–1.73
Gamma-tocopherol (μg/dl)	0.24 (0.1)	0.17	0.23	0.29	0.04–0.56

* SD, standard deviation.
† 1 pound = 0.454 kg.
‡ Frequency of consumption; does not take serving size into account.

etable items and 24 fruit items. Lipid- and energy-adjusted correlations between total fruit and vegetable intake and the average of two measurements of plasma beta-carotene and cryptoxanthin were 0.45 and 0.47, respectively, for men and women combined. (Results were not reported separately by gender.) Michaud et al. (17) analyzed data from 110 male nonsmokers from the Health Professionals Follow-up Study. The study questionnaire contained 131 food items (including 31 vegetables and 15 fruits). Supplement use was not addressed, but was presumably present for some participants. Plasma carotenoids were adjusted for lipids, body mass index, and age; fruit and vegetable estimates were based on the average of two FFQs and two 1-week diet records. For men, correlations were 0.35 and 0.36 for beta-carotene and cryptoxanthin, respectively. Thus, our results of 0.38 and 0.50 for these two plasma carotenoids are consistent with previous data on nonsmokers.

Other studies of fruit and vegetable intake and plasma nutrients examined correlations with serum carotenoids and included both smokers and supplement users (18, 19). Tucker et al. (18) reported on the relation between total fruit and vegetable intake, as estimated by the 126-item Willett FFQ, in participants in the Framingham Heart Study. Ten percent of the 201 men were smokers, and 11.9 percent used beta-carotene supplements. Among men, after adjustment for energy and other risk factors, correlations were $r = 0.25$ for alpha- and beta-carotene, 0.16 for beta-cryptoxanthin, 0.17 for lycopene, and 0.14 for lutein-zeaxanthin. Resnicow

TABLE 3. Spearman correlations and partial correlations between fruit/vegetable frequency of consumption and several plasma antioxidants for 116 men aged 35–72 years, Washington County, Maryland, 1989

	Ascorbic acid*	Total carotene**	Lipid-adjusted total carotene*	β-carotene**	Lipid-adjusted β-carotene*	Cryptoxanthin*	Lipid-adjusted cryptoxanthin*	α-toc††,‡	Lipid-adjusted α-toc†	Gamma-toc***	Lipid-adjusted gamma-toc†
Unadjusted correlation with fruit and vegetable frequency	0.59	0.34	0.40	0.35	0.38	0.43	0.46	0.06	0.26	−0.25	−0.20
Adjusted for											
Age	0.59	0.37	0.43	0.34	0.36	0.43	0.47	0.03	0.22	−0.26	−0.21
Education	0.58	0.33	0.40	0.35	0.38	0.41	0.45	0.07	0.27	−0.24	−0.18
Body weight	0.61	0.35	0.42	0.36	0.38	0.43	0.47	0.06	0.26	−0.25	−0.20
Dietary energy intake	0.62	0.34	0.41	0.36	0.39	0.44	0.49	0.06	0.28	−0.26	−0.20
Dietary fat intake	0.60	0.34	0.40	0.34	0.37	0.42	0.46	0.05	0.25	−0.24	−0.19
Age and energy intake	0.64	0.37	0.44	0.36	0.38	0.46	0.50	0.03	0.24	−0.28	−0.22

* All correlations in this column, $p < 0.0001$.
** All correlations in this column, $p < 0.001$.
*** All correlations in this column, $p < 0.01$.
† All correlations in this column, $p < 0.05$.
†† All correlations in this column, $p > 0.10$.
‡ α-toc, alpha-tocopherol.

et al. (19) studied fruit and vegetable intake and plasma carotenoids in 775 African-American men and women in Atlanta, Georgia. Smokers and vitamin supplement users were included. A modification of the full-length Block/NCI questionnaire was used, which contained 36 fruit and vegetable items. Correlations were $r = 0.34$ for alpha-carotene, 0.31 for beta-carotene, 0.26 for beta-cryptoxanthin, and 0.21 for lutein. In a subset of 68 persons who completed three 24-hour recalls, correlations between the 36-item fruit and vegetable questionnaire and these serum carotenoids were much higher ($r = 0.52$, 0.46, 0.43, and 0.30, respectively). Other studies have examined serum nutrient relations with individual foods (14, 15) or have conducted small feeding studies with subjects, many of whom were vitamin supplement users (20).

To our knowledge, only one other study has examined both plasma carotenoids and ascorbic acid in relation to fruit and vegetable intake. In France, Drewnowski et al. (13) studied a community-based sample of 837 subjects, of whom 23.1 percent of the women and 41.6 percent of the men were current smokers. Supplement use was not reported. Data were collected by using a dietary history interview. Correlations with energy-adjusted fruit and vegetable intake were $r = 0.36$ for serum beta-carotene and 0.29 for ascorbic acid.

In our study, ascorbic acid was considerably more highly associated with fruit and vegetable intake than were the carotenoids. Thus, it is possible that ascorbic acid is as important as or more important than carotenoids in conferring the protective benefit of fruits and vegetables. Unless studies examine plasma ascorbic acid in addition to other plasma antioxidants, conclusions regarding the active agent may be misleading. Interestingly, both this study and that of Michaud et al. (17) found beta-cryptoxanthin to be more highly correlated with fruit and vegetable intake than was beta-carotene (although others have not observed this (18, 19)). In this context, it should be noted that the major contributors of beta-cryptoxanthin are oranges and orange juice. Thus, if ascorbic acid is high, beta-cryptoxanthin may also be high. Without a measurement of plasma ascorbic acid, it may be difficult to attribute effects to the proper nutrient.

This study does not directly address the potential *efficacy* of ascorbic acid or other nutrients in affecting disease prevention. That would require epidemiologic studies that obtain a wide range of plasma nutrients and precursors of endogenous antioxidant systems. The stronger association of ascorbic acid with fruit and vegetable intake seen here may imply that ascorbic acid is an important component of the protective effect seen for fruits and vegetables in numerous epidemiologic studies. However, it is also possible that ascorbic acid appeared to be more strongly associated than carotenoids because of differences in storage or metabolism or in the difficulties of measurement. Ascorbic acid is water soluble, with major stores in muscle tissue, and the rate of utilization depends on numerous factors, including body weight, smoking, vigorous exercise, exposure to stressors, and, possibly, gender. Carotenoids are lipid soluble, with storage in fatty tissue, and utilization also depends on smoking and body weight, although possibly to a lesser extent. It is possible that had carotenoids been measured in adipose tissue, correlations with fruit and vegetable intake would have been higher.

The inverse association of gamma-tocopherol with fruit and vegetable intake is not well understood. In an unsupplemented diet, vegetable oils and salad dressings are the main sources of both tocopherols, although vegetables do provide some alpha-tocopherol. Supplementation with alpha-tocopherol is known to suppress gamma-tocopherol levels, and these data suggest an inverse relation between alpha- and gamma-tocopherol, even in an unsupplemented diet. Some studies suggest that gamma-tocopherol is a more potent antioxidant than alpha-tocopherol in some assay conditions, but the inverse relation between gamma-tocopherol and fruit and vegetable intake seen here seems inconsistent with a beneficial effect of gamma-tocopherol.

Often, investigators in major studies do not obtain plasma ascorbic acid because of the belief that it is too difficult to process and too labile to be feasible. This study shows that this is not the case. The CLUE II Study obtained blood samples from 32,808 respondents in a period of 6 months. Samples were obtained in multiple sites across Washington County, including temporary interviewing locations such as in mobile trailers. Blood samples were transported to a central site as whole blood, and processing was done centrally, usually within 6 hours of collection. Ascorbic acid is stable in whole blood for several hours (21), and after centrifugation, the processing of samples for ascorbic acid involves only the preparation of one additional tube containing a stabilizing agent (in our case, metaphosphoric acid). Ascorbic acid in plasma prepared in this way has been shown to be stable at –70°C over a period of several years.

In addition, investigators sometimes fail to include ascorbic acid because of the belief that blood levels represent only the previous few hours or that fasting blood is essential. Again, this appears not to be the case. Most participants in this study were not fasting at the time the blood was drawn, and the correlations shown are with dietary estimates from a questionnaire that asked about average intake in the previous year. These data suggest that plasma ascorbic acid is not as labile or as difficult to process in large studies as has been feared and should be included when studies assess antioxidant status.

A strength of this study is that the effect of fruit and vegetable intake on plasma nutrients could be examined without the effect modification by smoking (22, 23) and without confounding by supplement use (24). In addition, it is notable that the plasma correlations shown here are with reported *frequency* of consumption of fruits and vegetables, not with dietary estimates of nutrient intake or with grams of intake estimated using reported portion size. Thus, the observed correlations are not influenced by possible inaccuracies in the nutrient database for carotenoids or by problems with portion size estimation. Furthermore, this approach provides data that are directly relevant to the bulk of epidemiologic literature; that body of literature has typi-

cally been based on frequency rather than on portion-based servings and has tended to find stronger etiologic associations with fruit and vegetable intake rather than with specific nutrient estimates.

While the list of fruits and vegetables on the CLUE II questionnaire is not long (10 vegetable items and six fruit items), it encompasses the major sources of these nutrients in the US diet, including eight of the top 10 sources of carotenoids and seven of the top 10 sources of vitamin C. Not counting the two "any other fruit" and "any other vegetable" items, the remaining 14 items represent more than two thirds of all the mentions of fruits and vegetables in the Third National Health and Nutrition Examination Survey database among men in this age group (Block, unpublished data, 1997). If the "any other…" items are considered, then, of course, the list represents the great majority of all fruits and vegetables consumed in the United States. Eight of the 14 specific foods on the questionnaire are major dark green or deep yellow vegetables or fruits. Thus, while the higher correlation of ascorbic acid with fruit and vegetable intake seen here is with *this particular list* of fruits and vegetables, it should be noted that the list actually encompasses a higher proportion of carotenoids in the US diet (70.6 percent) than of vitamin C (57.8 percent).

As in the study by Campbell et al. (16), subjects were selected for this research by virtue of being either in the upper or the lower quintile of the distribution of frequency of fruit and vegetable intake. This approach tends to result in correlations that are higher than might be observed in studies that include the middle ranges of intake. However, the approach may also make it possible to see relations between intake and plasma most clearly, unobscured by the greater misclassification found in the middle ranges of intake. Estimates at the top and bottom of a frequency-of-consumption distribution are easiest for respondents to report and are reported with less error than estimates in the middle ranges. For example, it is easy and reasonably accurate to say "I eat carrots almost every day" or "I eat carrots only once a year." What is more difficult, and thus measured with more error, is deciding whether carrots are eaten once a month or twice a month. Thus, we believe that our sample selection approach gives a more accurate picture of the plasma nutrients that may be represented by questionnaires asking about fruits and vegetables.

In summary, this study has found that while both carotenoids and ascorbic acid are elevated in those with higher fruit and vegetable intakes, ascorbic acid is considerably more highly correlated with fruit and vegetable intake than are the carotenoids. Thus, it is possible that raising ascorbic acid levels may be an important mechanism by which fruit and vegetable consumption confers protective benefits. The study has also demonstrated the feasibility of obtaining plasma vitamin C measures in large-scale epidemiologic studies. Epidemiologic studies should include measures of plasma or serum ascorbic acid, in addition to other nutrients, to fully understand etiology and mechanisms.

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Ascorbic acid for the common cold. A prophylactic and therapeutic trial.

Karlowski TR, Chalmers TC, Frenkel LD, Kapikian AZ, Lewis TL, Lynch JM.

Abstract

Three hundred eleven employees of the National Institutes of Health volunteered to take 1 gm of ascorbic acid or lactose placebo in capsules three times a day for nine months. At the onset of a cold, the volunteers were given an additional 3 gm daily of either a placebo or ascorbic acid. One hundred ninety volunteers completed the study. Dropouts were defined as those who missed at least one month of drug ingestion. They represented 44% of the placebo group and 34% of those taking ascorbic acid. Analysis of these data showed that ascorbic acid had at best only a minor influence on the duration and severity of colds, and that the effects demonstrated might be explained equally well by a break in the double blind.

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Format: Abstract

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Vitamin C and common cold incidence: a review of studies with subjects under heavy physical stress.

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Author information

Abstract

Several studies have observed an increased risk of respiratory infections in subjects doing heavy physical exercise. Vitamin C has been shown to affect some parts of the immune system, and accordingly it seems biologically conceivable that it could have effects on the increased incidence of respiratory infections caused by heavy physical stress. In this report the results of three placebo-controlled studies that have examined the effect of vitamin C supplementation on common cold incidence in subjects under acute physical stress are analyzed. In one study the subjects were school-children at a skiing camp in the Swiss Alps, in another they were military troops training in Northern Canada, and in the third they were participants in a 90 km running race. In each of the three studies a considerable reduction in common cold incidence in the group supplemented with vitamin C(0.6-1.0 g/day) was found. The pooled rate ratio (RR) of common cold infections in the studies was 0.50 (95% CI: 0.35-0.69) in favour of vitamin C groups. Accordingly, the results of the three studies suggest that vitamin C supplementation may be beneficial for some of the subjects doing heavy exercise who have problems with frequent upper respiratory infections.

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A trial of ascorbic acid in the treatment of the common cold.

D A Tyrrell, J W Craig, T W Meada, and T White

Abstract

A randomised controlled trial was carried out to study the effect of 10 g of ascorbic acid taken during the first 2 1/2 days on the symptoms of the common cold. Altogether 1524 volunteers were recruited from a number of working groups in different parts of the country; 482 developed colds. There was no evidence that upper respiratory or general constitutional symptoms were alleviated by ascorbic acid. Among the men who had any colds at all, significantly fewer on active than on placebo treatment had two or more colds; however, this effect was not seen in women. Ascorbic acid is of no value in the treatment of the common cold; its preventive effect, if any, is not such as to justify advising its general use as a prophylactic measure.

Full text

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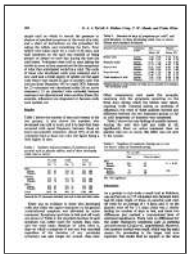

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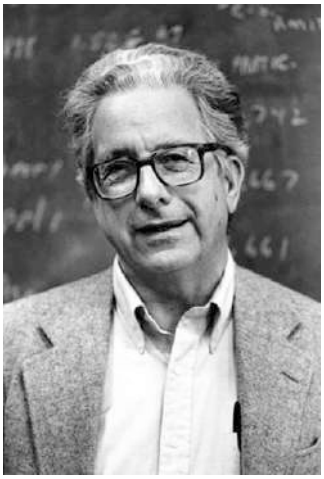
Paul Meier

A Man Behind the Method

Kellyn Betts, MA



Paul Meier. Courtesy of the University of Chicago. Printed with permission.

IN 1951, WHEN PAUL MEIER received his doctorate in mathematics from Princeton University and became one of the first statisticians to enter medical research, potential new medical treatments were evaluated in a very different fashion than they are today. At the time, researchers commonly followed practices such as giving a new remedy to patients they thought might benefit from it and comparing the outcomes with other patients who were not treated. In other situations, patients who stopped taking a new medicine might be counted as controls who had never been exposed to it.

Meier, who died on August 7, 2011, at the age of 87, had a profound impact on how clinical trials now evaluate the efficacy of new drugs and treatment methodologies throughout the world. Meier's "many published works and writings have had a huge influence on the application of statistics to medical research—particularly the design, conduct, and analysis of randomized clinical trials and in the advancement of evidence-based medicine in general," according to the Society of Clinical Trials, which Meier helped found in 1978.[1]

Meier was tireless in his promotion of the now-standard practice of randomly assigning patients enrolled in clinical trials to receive either the conventional remedy or the new treatment being evaluated. This is now considered the most rigorous way to conduct a study and the best way to gather evidence of a new drug or treatment's effectiveness. "Perhaps more than any other U.S. statistician, [Dr. Meier] influenced U.S. drug regulatory agencies, and hence clinical researchers throughout the U.S. and other countries, to insist on the central importance of randomized evidence," said Sir Richard Peto of Oxford University, who was also a leading advocate for randomization, in Meier's *New York Times* obituary.[2] "That strategic decision half a century ago has already saved millions of lives, and those millions should be attributed to Paul," Peto said.

"I defended randomization every chance I got, and I had a fair number of chances," Meier said in a 2003 interview in the journal *Clinical Trials*.[3(p137)] "For a fairly long time randomization was not thought of so highly," he explained. He said that in 2001,

> a very distinguished statistician told me that I had a major influence on the Food and Drug Administration's policies on randomized clinical trials. I don't know how true that was, but if so, it would be something of which I am very proud,

adding that his success in encouraging the use of randomization in clinical trials is the achievement he prized most highly.[3(p137)]

Together with Edward L. Kaplan of the California Radiation Laboratory, Meier also helped formulate what the Society for Clinical Trials terms "our most popular method of estimating survival functions from continuously observed data."[4] Published in the *Journal of the American Statistical Association*[4] in 1958, it went on to become one of the most widely cited articles in the medical literature. At the time of Meier's death, the Kaplan-Meier article had been cited more than 34 000 times. Theodore Karrison, PhD, director of the University of Chicago Department of Health Studies' Biostat Lab and one of Meier's doctoral students, attested to the article's continuing relevance

PAUL MEIER HAD A PROFOUND IMPACT on how clinical trials now evaluate the efficacy of new drugs and treatment methodologies throughout the world. Meier's tireless promotion of the now-standard practice of randomly assigning patients enrolled in clinical trials to receive either the conventional remedy or the new treatment being evaluated helped ensure its current status as the most rigorous way to gather evidence of a new drug or treatment's effectiveness. Meier also helped formulate the Kaplan-Meier estimator, which is now the most popular method of estimating clinical trial participant survival. It is one of the most widely cited articles in the medical literature.

by noting: "If you open up at random a medical journal you're likely to see in at least one of the articles a citation to the Kaplan-Meier paper" (oral communication, December 1, 2011).

Over his long and distinguished career, Meier earned many honors—as well as widespread admiration for being quick on his feet.

> At professional meetings . . . he often astonished me by giving comments from the audience, which, though spontaneous, displayed a depth of reasoning and perfect eloquence, which few others could have matched with any amount of advanced preparation,

recalled Rick Chappell (written communication, November 8, 2011; and oral communication, November 23, 2011), who was Meier's last doctoral student and is now a professor of biostatistics and medical informatics at the University of Wisconsin at Madison.

Through it all, including the stroke in 1995 that robbed him of some of his eloquence, Meier was also a kind and gentle man, according to a statement issued by the Statistics Department at Columbia University,[5] where Meier spent his final years (he also held a joint appointment at Columbia's Mailman School of Public Health). Karrison, Chappell, and Daniel Heitjan, PhD, a professor of biostatistics at the University of Pennsylvania's Perelman School of Medicine, attested that Meier was both widely respected and loved. "He was a person who cared about people . . . and someone you could go to with a problem," Karrison said.

A RELUCTANT BIOSTATISTICIAN

Meier graduated from Oberlin College in 1945 and went on to Princeton University to pursue a doctorate in mathematics, where he studied under the celebrated mathematician John Tukey. Meier's dissertation project involved a statistical problem suggested by William Cochran, the noted statistician who chaired Johns Hopkins University's Department of Biostatistics from 1948 to 1958. At the time, Meier was also very interested in "the notion that randomization could clear away confounders that you did not know about."[3(p133)] As one of a very few mathematicians focusing on medical applications, Meier recognized the potential value of randomization's application in medicine.[3]

After Meier earned his doctorate, he spent one more year at Lehigh University, where he had been teaching since 1948. Tukey recommended that he accept a position at Hopkins with Cochran, who was enthusiastic about Meier's dissertation.

> I was a little nervous because by and large, biostatistics was not a field with a lot of mathematics in it, and I wished more or less to be a mathematician,

Meier said. But when Cochran insisted that going to Hopkins was a good idea, Meier accepted his first position as a statistician.[3]

In those early days, Meier said, "I was looked at with amazement by my medical colleagues," when he brought up the idea of randomization for assessing new medical treatments, he recalled. The physicians would say "'Randomize? We know that this treatment is better than that one,'" he explained. "People who knew and respected me were astounded that I should want to randomize their patients."[3(p133)]

Then Meier became involved with the controversial 1954 Salk

Meier´s Recollections of the Salk Polio Vaccine Trial

The 1954 field trial of Jonas Salk's polio vaccine "was the most elaborate trial that was ever done," Meier recalled. One of the reasons that the trial was so complicated is because polio was very scarce, he explained. "I've not been involved in many trials like that and I've been involved in lots of multicenter studies," he said.[3(p133)]

The situation was further handicapped because the diagnosis of polio is tricky, Meier said. "We need to have the entire country's physicians participate, because we can't look over every case where there's some kind of paralysis. So physicians reported the cases they thought were polio according to the protocol, and we accepted those cases." Meier estimated that "about half those cases were probably not polio at all."[3(p133)]

But the biggest issue, for Meier, emerged during a seminar attended by many of the researchers working on the project, where it became apparent that members of the team were suppressing the data related to some of the test vaccine lots. As soon became clear, the polio virus used in the trial vaccines was not always properly inactivated. Jonas Salk, the vaccine's inventor, "cut out data in order not to show what happened to some lots," Meier charged.[3(p134)] He said that the National Foundation for Infantile Paralysis, which sponsored the study, dropped from its advisory committee scientists who did not agree with how the results were being presented.[3]

The field trial's findings were reported to show the vaccine's effectiveness, over the objections of some of the committee members, Meier said. Soon after, the US Public Health Service reported cases of paralytic polio in children inoculated with the vaccine. The original cases were traced back to lots produced by Cutter Laboratories, of Berkeley, CA, one of six manufacturers licensed to produce the vaccine. However, Meier said that the problem was more widespread. He said:

> I got some data from a physician who was working on this, and we found that not only was Cutter wrong, but there were various other companies that had the same polio virus in their samples, although not as much as the samples from Cutter Laboratories. But because there were so many improperly diagnosed cases out there, and because the other manufacturers went around to various newspapers and threatened to cut their advertising, it was dumped on Cutter. Cutter was responsible because they did things in producing and testing the vaccine they were told not to do.[3(p134)]

Polio Vaccine field trials. The Society for Clinical Trials called the polio vaccine trial "the project that put randomized trials on the map in this country" in part because of the key role Meier played by publishing a critical article in *Science* in 1957.[6] The article reviewed "some aspects of the poliomyelitis vaccine testing program which seem to have important implications for scientists generally."[6(p1067)] It indicted both the National Foundation for Infantile Paralysis and the government for withholding information from the participants. It also faulted the testing program for accepting without scrutiny Salk's assertion that the vaccine was "absolute[ly] safe," and for not employing the expensive and difficult tests that had been suggested to ensure that the final product was free of residual live virus. Meier said that many journals turned his manuscript down and their editors warned him that publishing such an article would limit his career path.[3]

Although Meier was denied tenure at Hopkins, he succeeded in securing an appointment to the University of Chicago in 1957. He stayed there until 1992, and taught at different schools and departments—including the college, graduate school, law school, and medical school—over the years. For more than a decade, he led the Department of Statistics as chair or acting chair.

In 1958, Meier published his highly cited article describing what is now known as the Kaplan-Meier estimator in the *Journal of the American Statistical Association*.[4] Kaplan was also a student of Tukey at Princeton. Working independently, Meier and Kaplan solved a problem that was dogging medical researchers at the time. The issue revolved around the fact that many participants in clinical trials do not participate in the experiment for the same length of time because of the time required to recruit study volunteers. The Kaplan-Meier statistic enables researchers to take into account observable time of survival and death.

Initially, Meier recalled, both he and Kaplan had submitted separate articles. The publication's editor asked them to collaborate to produce one article. "I swallowed hard, and I guess Kaplan swallowed hard as well," Meier said. "We worked quite hard and at one place he solved a problem that I couldn't solve; other cases I solved problems he couldn't."[3(p133)]

LOVE FOR CLINICAL TRIALS

In the subsequent decades, Meier's stature continued to grow, and he was involved in many clinical trials, which he called his "true love." In addition to helping found the Society for Clinical Trials in the 1970s, he wrote some influential articles about the ethics of performing them.[7,8] In his spare time, Meier enjoyed music, particularly folk songs, and played the flute, recalled Chappell, Heitjan, and Karrison. Meier was also a sailor, and he took out his small sailboat, The Salty Dog, in the waters near his summer home near Lake Michigan during his years at the University of Chicago. After Meier moved to New York City in 1992, he sailed in the Hudson River outside Dutchess County, New York.

Over the course of his 50-plus-year career, Meier's facility for explaining statistical concepts to people outside the discipline resulted in calls to testify before the US Congress and popularity with journalists such as Gina Kolata of the *New York Times*, Chappell remembered. It also made him popular with clinicians, such as the University of Chicago medical school students he taught about clinical trials, Karrison said.

Meier's stroke occurred three years after he retired from the University of Chicago in 1992 and moved to Columbia University. There, he held appointments as both the Howard Levene Professor of Statistics in the statistics department and head of the Mailman School of Public Health's biostatistics department, and he remained active professionally for years after his stroke. "He still kept going to meetings," Karrison recalled. Meier "struggled courageously," added Heitjan, who worked closely with him at Columbia (oral communication, November 22, 2012).

Heitjan collaborated with Meier during the Randomized Evaluation of Mechanical Assistance for the Treatment of Congestive

Honors and Awards

Meier was named as a fellow of the American Association for the Advancement of Science, the American Statistical Association, the Institute of Mathematical Statistics, the American Academy of Arts and Sciences, the Royal Statistical Society, and the John Guggenheim Memorial Foundation. He served as president of both the Institute of Mathematical Statistics and the Society for Clinical Trials. He was also elected to senior membership in the National Academy of Sciences' Institute of Medicine.[5,11]

He also held temporary appointments as a National Institutes of Health Special Fellow at the University of London and Imperial College; he was a visiting professor at Harvard University and Jerusalem's Hebrew University; and he was a fellow of Stanford University's Center for Advanced Study in the Behavioral Sciences.[5,11]

Heart Failure (REMATCH) trial, which began in 1998 and ran through 2001 and involved 20 cardiac transplant centers around the country.[9,10] Although this artificial heart trial was relatively small compared with many drug trials, it was one of the most significant device trials ever conducted, Heitjan said. Meier insisted that the trial needed to be randomized and he refused to allow the group carrying it out to cut corners, Heitjan recalled.

> Clinical trials in the device world are often small, single-arm trials [where results are compared with historical controls] . . . in part because a lot of the companies that make devices are small and can't support major trials,

Heitjan explained. The trial was randomized so it could determine whether the devices could extend and improve the quality of recipients' lives sufficiently to justify the expense of implanting them, he said.

It was the first high-profile randomized clinical trial that Heitjan had worked on, and "having Paul around to be my mentor and guide was very important to me." When the two would attend meetings related to the trial, Meier was quiet most of the time

> because it was a little harder for him to communicate and get his point across so he had to choose his battles carefully. He would only speak out at what I considered critical moments,

Heitjan said. Nevertheless it was clear that Meier's understanding of both the technical and political issues in the trial was undiminished, Heitjan said.

Heitjan recalled attending a Society for Clinical Trials meeting with Meier in 1998. One after another, distinguished senior physician–scientists came up to greet Meier, pay homage to him, and testify to how he had opened their eyes to the critical importance of the randomized clinical trial, Heitjan remembered.

"Being with [Meier] lifted you up," Heitjan summarized. Perhaps just as important as his intellect and accomplishments, Meier "was a genuinely good human being," Karrison said. He was a "great and gentle man," Chappell agreed. ■

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Format: Abstract

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Ascorbate recycling in human neutrophils: induction by bacteria.

Wang Y[1], Russo TA, Kwon O, Chanock S, Rumsey SC, Levine M.

Author information

Abstract

Ascorbate (vitamin C) recycling occurs when extracellular ascorbate is oxidized, transported as dehydroascorbic acid, and reduced intracellularly to ascorbate. We investigated microorganism induction of ascorbate recycling in human neutrophils and in microorganisms themselves. Ascorbate recycling was determined by measuring intracellular ascorbate accumulation. Ascorbate recycling in neutrophils was induced by both Gram-positive and Gram-negative pathogenic bacteria, and the fungal pathogen Candida albicans. Induction of recycling resulted in as high as a 30-fold increase in intracellular ascorbate compared with neutrophils not exposed to microorganisms. Recycling occurred at physiologic concentrations of extracellular ascorbate within 20 min, occurred over a 100-fold range of effector/target ratios, and depended on oxidation of extracellular ascorbate to dehydroascorbic acid. Ascorbate recycling did not occur in bacteria nor in C. albicans. Ascorbate did not enter microorganisms, and dehydroascorbic acid entry was less than could be accounted for by diffusion. Because microorganism lysates reduced dehydroascorbic acid to ascorbate, ascorbate recycling was absent because of negligible entry of the substrate dehydroascorbic acid. Because ascorbate recycling occurs in human neutrophils but not in microorganisms, it may represent a eukaryotic defense mechanism against oxidants with possible clinical implications.

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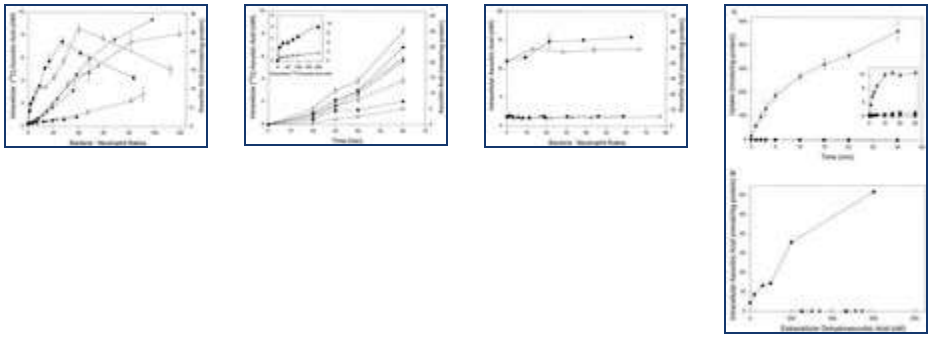

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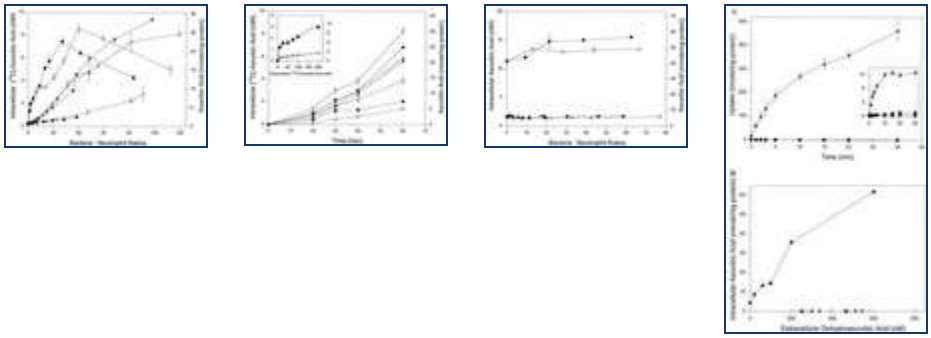

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Ascorbic acid and the common cold

Linus Pauling, Ph.D.

For a number of years I have been interested in the possibility that the state of health of many people could be significantly improved by the ingestion in the optimum amounts of certain substances normally present in the human body, including the vitamins. This interest developed from the work that my associates and I have done on molecular diseases, especially the hemoglobinemias (1). I decided in 1953 that it would be worthwhile to make a study of the extent to which mental diseases could be described as molecular diseases. Work along these lines was carried out in our laboratory in the California Institute of Technology from 1954 to 1964, and was continued in the University of California, San Diego, and (since 1969) in Stanford University. In the course of this period I formulated some ideas about orthomolecular medicine, defined as the preservation of good health and the treatment of disease by varying the concentrations in the human body of substances that are normally present in the body and are required for health (2–4). I also became aware of arguments indicating that the optimum rate of intake of ascorbic acid may be far greater than the recommended daily allowance of this vitamin, which is approximately 50 mg/day. Part of the evidence on this point had been presented especially clearly in the papers of Stone (5–8).

Last year I published a small book, *Vitamin C and the Common Cold*, in which I presented the evidence supporting the conclusion that ascorbic acid ingested in larger amounts than the recommended daily allowance has value in decreasing the incidence and severity of the common cold and related infectious diseases (9).

This opinion is in agreement with a rather widespread popular belief that ascorbic acid has value in providing protection against the common cold. This popular belief has, however, not been generally shared by physicians, authorities on nutrition, and official bodies.

For example, as recently as November 1970, Dr. Philip L. White (10), Secretary of the Council on Foods and Nutrition of the American Medical Association, stated that "Unfortunately, it is still a widespread belief that extra ascorbic acid can not only prevent colds but also lessen the severity and duration of colds and other respiratory infections. Even when consumed at the first sign of a sniffle, large doses of the vitamin are useless." Also, many statements contradicting my conclusions were made by physicians, experts in nutrition, and health officials within a few weeks after the publication of my book. For example, Dr. Charles C. Edwards, United States Food and Drug Commissioner, was reported in the press on December 29, 1970 as having said that the use of ascorbic acid was ridiculous, and that there was no scientific evidence and never have been any meaningful studies indicating that vitamin C is capable of preventing or curing colds. The Editors of *The Medical Letter* published an article in which nearly all my statements were contradicted; for example, it was stated that there had been no controlled trials of the effectiveness of vitamin C, in comparison with a placebo, against upper respiratory infections over a long period and including many hundreds of persons (11).

In fact, there have been several carefully conducted double-blind studies of ascorbic acid and the common cold, carried out by responsible medical investigators. Some of these studies have given results that reject with statistical significance the null hypothesis that ascorbic acid has no more value than a placebo in decreasing the incidence and severity of the common cold when the ascorbic acid is administered regularly to subjects over a period of time beginning before the illness has set in, and the subjects are exposed to cold viruses in the ordinary way (by casual contact with other people). I shall discuss some of these studies in the following paragraphs. The amount of protection against

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Ascorbic Acid and the Common Cold: Evaluation of its Efficacy and Toxicity

PART I

By LINUS PAULING, Ph.D.

Dr. Pauling is President of the Linus Pauling Institute of Science and Medicine, 2700 Sand Hill Road, Menlo Park, Calif. 94025, and Professor Emeritus of Chemistry at Stanford University and the California Institute of Technology.

Brief descriptions are given of the thirteen controlled trials that have been made of ascorbic acid in comparison with a placebo in relation to the common cold, with the ascorbic acid or placebo given to subjects over a period of time and with the subjects in good health at the beginning of the trial and exposed to cold viruses in the ordinary way. The integrated morbidity (amount of illness per person) found in these trials was an average of 36% less for the ascorbic-acid subjects (average intake 1 g per day) than for the placebo subjects. Several investigators have reported that no serious adverse effects of ascorbic acid were observed. So far there is no significant evidence for the various adverse reactions that have been hypothesized. The apparent benefit in health from an increase in intake of ascorbic acid justifies its widespread use.

In a recent article[1] Dykes and Meier discussed some of the clinical data published since 1938 on the efficacy of pharmacologic doses of ascorbic acid in the prevention and treatment of the common cold and both clinical data and data obtained from intact animals that relate to the possible toxicity of ascorbic acid. They pointed out that in several studies the subjects receiving ascorbic acid had less illness than those receiving the placebo, but they criticized most of the studies with respect to some details of design or execution and concluded that there is little convincing evidence of a protective effect large enough to be clinically important. They also stated that many hypothetical adverse reactions to the intake of large amounts of ascorbic acid have been suggested, but that there is little evidence about the possible incidence of such reactions currently available.

The conclusions reached by Dykes and Meier have been widely misrepresented in press releases, newspapers, and magazines. For example, it has been said, on the basis of their paper and another paper published at the same time[2], that "Vitamin C will not prevent or cure the common cold".[3] In fact, their conclusion was that "Until such time as pharmacologic doses of ascorbic acid have been shown to have obvious, important clinical value in the prevention and treatment of the common cold, and to be safe in a large varied population, we cannot advocate its unrestricted use for such purposes." Moreover, some significant studies in this field were not mentioned by Dykes and Meier, and some important aspects of the studies discussed by them were also not mentioned by them. My conclusions, presented below, from the thorough analysis of the existing information, are somewhat different from those of Dykes and Meier.

Dykes and Meier mention that the evaluation of efficacy may be made uncertain by its partial dependence on subjective reports by the patients. The number of colds is especially unreliable because of uncertainty as to whether or not to record as a cold a mild indisposition lasting only one or two days. I consider the average number of days of illness per person (the integrated morbidity[4]) to be the best quantity to use in determining the relative efficacy of ascorbic acid and placebo. This quantity, which can be assessed in a reasonably objective way (by signs recorded by the physician, number of days of absence from school or work, etc.), is emphasized in the following discussion.

COWAN, DIEHL, AND BAKER

In the study by Cowan, Diehl, and Baker[5] 208 students in the University of Minnesota received about 200 mg of vitamin C per day for 28 weeks and 155 students received a placebo. Dr. Cowan has written me that the study was a double-blind one. The average number of days lost from school per person was 1.1 for the ascorbic-acid group and 1.6 for the placebo group, with standard deviations not given. 1fhis measure of the integrated morbidity thus shows 31% (range 26 to 36%) less illness per subject for the ascorbic-acid subjects than for the placebo subjects. The information given in the paper does not permit an accurate calculation to be made of the statistical significance of the rejection of the null hypothesis that ascorbic acid and the placebo have the same effect. I have made the conservative estimate[4] that P is less than 0.02.

Dykes and Meier have criticized this study on several points. I may add that the investigators were at fault in not reporting their observations precisely (rounding off the average number of days of illness and not giving the standard deviations).

FRANZ, SANDS, AND HEYL

Franz, Sands, and Heyl carried out a double-blind study in Dartmouth Medical School with 89 volunteer med-

ical students.[6] They were divided in a random way into four groups, receiving ascorbic acid (205 mg per day), ascorbic acid and a bioflavonoid, a placebo, or the bioflavonoid alone. No effect of the bioflavonoid was observed. The number of colds in the combined ascorbic-acid groups was 14 (for 44 subjects) and that in the placebo groups was 15 (for 45 subjects). The number of colds not cured or improved in 5 days was only 1 for the ascorbic-acid group, much less than the value 8 for the placebo group. The authors state that "those receivin:: ascorbic acid showed more rapid improvement in their colds than those not receiving it .. . statistically significant at the 0.05 level." My estimate of the statistical significance (based on the assumption mentioned in the following paragraph) is P (one-tailed) = 0.01. Dykes and Meier state that I apparently used an erroneous summary result; their treatment of the data gives P (one-tailed) < 0.0283, P (two-tailed) < 0.0566. We all agree that the null hypothestis of equal effect jaf ascorbic acid and placebo is to be rejected.

I have estimated the average number of days of illness per person for the two groups by making the assumption that the distribution function for colds in respect to their duration is the one given by observations made in another investigation.[7] This calculation leads to the conclusion that the integrated morbidity per person was 40% less for the ascorbic-acid subjects than for the placebo subjects.

RITZEL

Ritzel[8] reported observations made in a double-blind study on 279 schoolboys, 15 to 17 years old, on two week-long stays in a ski camp. Half of the subjects (139) received 1 g of ascorbic acid each day, and the other half (140) a placebo. There were 17 colds in the ascorbic-acid subjects •(total days of illness 31) and 31 -colds in the placebo subjects (total days of illness 80). The number of total individual signs and symptoms recorded by the physicians in their daily inspections of the subjects was 42 for the ascorbic-acid subjects and 119 for the placebo subjects. The integrated morbidity is 63% less for the ascorbic-acid group than for the placebo group (average of 61.0% from average days of illness per person and 64.5% from average number of recorded signs and symptoms). The statistical significance of this difference is high, P (one-tailed) < 0.01.

Dykes and Meier criticize Ritzel on several points, and do not mention the results that he reported. One criticism is that he does not give in his tables the total number of colds in each group. They state that "Pauling infers the number of subjects by dividing 'illness days' by 'mean illness days' and concludes that there is a significant difference in proportions of subjects experiencing colds. If his interpretation is correct, the difference is indeed significant."

It is hard for me to understand why Dykes and Meier should suggest that my interpretation might be incorrect. It involves a very simple calculation. Ritzel states (in his Table 1) that the total number of days of illness for the ascorbic-acid subjects was 31. He also states (page 66) that the average number of days per episode of illness was 1.8. The ratio 31/1.8 is 17.2; that is, there were 17 episodes of illness in this group. A similar calculation gives 31 colds for the placebo subjects (80 total days of illness, 2.6 average number of days per episode). It is safe to assume that no subjects had two colds in the same week. With this assumption, the null hypothesis of equal probability of colds for the two groups is rejected at the level P (one-tailed) < 0.015.

Dykes and Meier mention that I give great weight to the Ritzel study. I do give great weight to it, and I find it strange that they should reject it on the basis of trivial complaints, such as their apparent failure to understand the simple calculation described above.

ANDERSON, REID, AND BEATON

In the 1972 double-blind Toronto study[9,10] 407 subjects received ascorbic acid (1 g per day plus 3 g per day for 3 days at the onset of any illness) and 411 subjects received a closely matching placebo. The duration of the study was four months. The number of days confined to house per subject was 30% less for the ascorbic-acid group than for the placebo group, and the number of days off work per subject was 33% less. The authors mention that these differences have high statistical significance (P< 0.001).

Dykes and Meier present these results with little comment, except to state that the observed effect is considerably less than had been predicted by me.[4] This is true; I predicted about twice as much protection, on the basis of the study by Ritzel. I surmise that two effects may be involved in this difference. First, the amount of protection, relative to the placebo subjects, is probably less when the basic intake of ascorbic acid is high (Toronto) than when it is low (Switzerland), and second, the observed protection is probably less in a long test (4 months) than in a short one (one week).

Anderson, Reid, and Beaton reported also a smaller amount (by 40%) of non-respiratory illness in the ascorbic-acid subjects than in the placebo subjects.

ANDERSON, SURANYI, AND BEATON

A second double-blind study, with over 2000 subjects, was also carried out in Toronto.[11] In this very large study there were two placebo groups, one with 285 and the other with 293 subjects, and six ascorbic-acid groups (receiving various amounts), with 275 to 331 subjects. The study continued for three months.

A complication in the analysis of this study is presented by the fact that the results observed for the two placebo groups do not agree with one another. One placebo group had the greatest amount of illness of all eight groups, and the other had the smallest amount. The authors conclude that their observations are compatible with an effect of small magnitude (less than 20%) from both the prophylactic regimen (250 mg, 1 g, or 2 g of ascorbic acid per day) and the therapeutic regimen (4 or 8 g on the first day of illness), with an effect of somewhat greater magnitude from the combined regimen (1 g per day and 4 g on the first day of illness). They state also that there was no evidence of side effects from the 1 g or 2 g of ascorbic acid per day and no evidence of a rebound increase in illness during the month following withdrawal of the daily vitamin supplement.

The authors give the amounts of illness per subject (days of symptoms, days indoors, days off work) relative to the first placebo and relative to tj)e first plus the second (there is sonpe reason to suspect that the second placebo group was not a representative sample of the general population). I have averaged these two sets of values, and have obtained 9% as the average decrease in integrated morbidity of the ascorbic-acid subjects.

WILSON, LOH, AND FOSTER

Some studies involving several hundred students in four boarding schools in Dublin have been reported by Wilson and his collaborators.[12,13,U] As is mentioned by Dykes and Meier, their analysis of prophylactic benefit is much complicated by the subdivision of colds into three somewhat overlapping categories, catarrhal, toxic, and whole. The investigators state that the girls, in two schools were benefited, with statistical significance, by ascorbic acid, and that the boys, in the other two schools, were not. I have not been able to abstract from their papers any reliable value of the integratedmiorbidity for their subjects.

COULEHAN, REISINGER, ROGERS, AND BRADLEY

A double-blind study of 641 children in a Navajo boarding school was carried out over a 14-week period.[15] The younger children received 1 g and the older children 2 g of ascorbic acid (or placebo) per day. The number of days of illness per subject was 28% less for the ascorbic-acid group of younger children than for the placebo group, and 34% less for the older children (weighted average 30%). The statistical significance of this difference is uncertain.

KARLOWSKI ET AL.

The results of a double-blind ninemonths study with 190 employees of the National Institutes of Health have been reported recently by Karlowski, Chalmers, Frenkel, Kapikian, Lewis, and Lynch.[2] The study was well designed and well executed except for the use of a poor placebo, easily distinguished from ascorbic acid by taste. Ascorbic acid, 1 g per day, was taken by 101 subjects (groups C and D, Table 1) of whom 57 (group D) also received an additional 3 g per day for the first five days of any illness, beginning, however, only after the subjects had returned to the pharmacy to have their symptoms and clinical observations recorded and to receive their supplemental capsules. A group (A) of 46 received only placebo capsules, and a group (B) of 43 received daily placebo capsules and ascorbic-acid supplementary capsules.

Table 1

Summary of Results Reported by Karlowski et al.

Group	Number of subjects	Dose*	Average number of colds	Days of illness per cold	Days of illness per person	Decrease relative to A
A	46	P+P	1.41	7.1	1u.01	—
B	43	P+V	1.30	6.5	8.45	16%
C	44	V+P	1.18	6.7	7.91	21%
D	57	V+V	1.33	5.9	7.85	22%

**The first P means daily placebo, the first V daily ascorbic acid (1 g), the second P supplemental placebo, and the second V supplemental ascorbic acid (3 g per day for the first five days of any illness).*

The reported average number of colds and average days of illness per cold are given in Table 1. The product of these (sixth column) is the average number of days of illness per person, which is a measure of the integrated morbidity. The subjects regularly taking 1 g of ascorbic acid per day (group C) had 21% less illness than the control group (A). Nearly the same amount of decreased illness was found for the group taking only supplemental ascorbic acid (B, 16%) and the group taking both daily and supplemental ascorbic acid (D, 22%). The weighted average, 20%, of these three values is the observed decrease in integrated morbidity for all ascorbic-acid subjects relative to the placebo subjects. The statistical significance of this decrease cannot be calculated because the investigators do not give standard deviations of the averages or equivalent information.

Many of the subjects had tasted the contents of their capsules and correctly interpreted the taste. Much of the decreased illness was found in the subjects who learned in this way that they were receiving ascorbic acid. The investigators indicate that much of the apparent protective effect of ascorbic acid might be the result of a psychological effect, the power of suggestion. I doubt, as do some others, that such psychological effects can operate significantly in a large population over periods of several months, and I accept the results of the National Institutes of Health study with about as much confidence as the others.

Karlowski et al. conclude "that ascorbic acid had at best only a minor influence on the duration and severity of colds, and that the effects demonstrated might be explained equally well by a break in the double blind." They also say that "the effects of ascorbic acid on the number of colds seem to be nil," and this statement has been quoted in the AMA press release[3] without the additional information about the number of colds given by Karlowski et al. In fact (Table 1), the group receiving prophylactic ascorbic acid had 16% fewer colds than the control group, and the three ascorbic-acid groups together had 10% fewer. It is not correct to say that the effects seem to be nil.

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Am J Clin Nutr. 1991 Dec;54(6 Suppl):1147S-1152S. doi: 10.1093/ajcn/54.6.1147s.

Ascorbic acid and carnitine biosynthesis.

Rebouche CJ[1].

Author information

Abstract

It has been suggested that early features of scurvy (fatigue and weakness) may be attributed to carnitine deficiency. Ascorbate is a cofactor for two alpha-ketoglutarate-requiring dioxygenase reactions (epsilon-N-trimethyllysine hydroxylase and gamma-butyrobetaine hydroxylase) in the pathway of carnitine biosynthesis. Carnitine concentrations are variably low in some tissues of scorbutic guinea pigs. Ascorbic acid deficiency in guinea pigs resulted in decreased activity of hepatic gamma-butyrobetaine hydroxylase and renal but not hepatic epsilon-N-trimethyllsine hydroxylase when exogenous substrates were provided. It remains unclear whether vitamin C deficiency has a significant impact on the overall rate of carnitine synthesis from endogenous substrates. Nevertheless, results of studies of enzyme preparations and perfused liver in vitro and of scorbutic guinea pigs in vivo provide compelling evidence for participation of ascorbic acid in carnitine biosynthesis.

PMID: 1962562 DOI: 10.1093/ajcn/54.6.1147s

[Indexed for MEDLINE]

Publication type, MeSH terms, Substances

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Ann. Rev. Nutr. 1986. 6:365–406

THE BIOCHEMICAL FUNCTIONS OF ASCORBIC ACID

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CONTENTS

SCOPE OF THIS REVIEW

This review is concerned primarily with functions of ascorbate that have been studied at the level of specific enzymatic reactions using in vitro systems. This approach excludes detailed consideration of many functions that become disturbed in the scorbutic animal if they have not also been studied in cell or organ culture systems or using isolated enzymes. In our final discussion we consider

Can CAM treatments be evidence-based?

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https://doi.org/10.1111/fct.12110

Citation

Louhiala , P & Hemilä , H 2014 , ' Can CAM treatments be evidence-based? ' Focus on Alternative and Complementary Therapies , vol. 19 , no. 2 , pp. 84-89 . DOI: 10.1111/fct.12110

Title:	Can CAM treatments be evidence-based?
Author:	Louhiala, Pekka; Hemilä, Harri
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ISSN:	1465-3753
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Abstract:	In this article, we first take a critical look at the definitions of evidence-based medicine (EBM) and complementary and alternative medicine (CAM). We then explore the question of whether there can be evidence-based forms of CAM. With the help of three examples, we show that EBM and CAM are not opposites, but rather concepts pointing at different dimensions. Each of the three examples is an evidence-based treatment according to three to five randomised, double-blind placebo controlled trials with consistent findings and narrow pooled confidence intervals. The most reasonable interpretation for the existence of evidence-based CAM treatments seems to be that the opposite of CAM is ‘mainstream medicine’, and the demarcation line between CAM and mainstream medicine is not simply defined by the question of whether a treatment works or not. Some effective treatments may belong to the CAM domain for historical reasons and because of preconceptions within mainstream medicine. Therefore, some treatments that currently lie outside mainstream medicine can be evidence-based.
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Biochem Cell Biol. 1990 Oct;68(10):1166-73.

Cellular functions of ascorbic acid.

Padh H[1].

Author information

Abstract

It has long been suspected that ascorbic acid is involved in many cellular reactions. This is evident from the multitude of seemingly unrelated symptoms seen in scurvy. However, until recently, our understanding of its involvement was confined to its role in the synthesis of collagen. Studies in the past few years have unveiled mechanisms of its actions in collagen formation and many other enzymatic reactions. In addition, numerous physiological responses are reportedly affected by ascorbic acid. From the well-characterized enzymatic reactions involving ascorbic acid, it has become clear that in animal cells the ascorbate does not seem to be directly involved in catalytic cycles. Rather its major function seems to keep prosthetic metal ions in their reduced form. The role of ascorbate as a reductant in these enzymatic reactions complements its other antioxidant functions which have been recently appreciated, including that as a scavenger of free radicals. Therefore, it seems that the major function of ascorbate is to protect tissues from harmful oxidative products and to keep certain enzymes in their required reduced forms. However, it remains unclear how the deficiency of ascorbate leads to the pathological symptoms found in scurvy.

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Scottish Medical Journal

Changes in Leucocyte Ascorbic Acid during the Common Cold

R. Hume, Elspeth Weyers

First Published January 1, 1973 | Research Article | Find in PubMed
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Abstract

Leucocyte ascorbic acid was measured in *7* subjects during the common cold. There was a significant fall in L.A.A. to scorbutic levels within *24* hours of the onset of symptoms. By the fifth day the L.A.A. had returned to normal, which coincided with the cessation of symptoms. Absorption studies suggested 1g. ascorbic acid per day as a prophylactic dose and 6g. ascorbic acid per day as a therapeutic dose. The effect of such supplements of ascorbic acid in *4* episodes of the common cold in *3* subjects suggests that the L.A.A. pattern can be changed by this therapy. The implications are discussed.

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Clinical manifestations of ascorbic acid deficiency in man

Robert E. Hodges, M.D., James Hood, M.D., John E. Canham, M.D., Howerde E. Sauberlich, Ph.D., Eugene M. Baker, Ph.D.

The American Journal of Clinical Nutrition, Volume 24, Issue 4, April 1971, Pages 432–443,
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Summary

Six healthy volunteers from the Iowa State Penitentiary at Fort Madison, Iowa, participated in studies of human scurvy. They were hospitalized on the Metabolic Ward of University Hospitals in Iowa City, Iowa, and fed a diet totally devoid of vitamin C.

One of the men withdrew from the study because of personal reasons. The remaining five subjects developed clinical scurvy in 84 to 97 days, manifested by signs and symptoms of fatigue, hemorrhagic phenomena, swollen joints, swollen bleeding gums, follicular hyperkeratosis, muscular aches and pains, and emotional changes.

Urinary ascorbic acid rapidly declined to undetectable levels early in the course of depletion and blood levels progressively became too low to measure accurately. Serum protein abnormalities appeared that consisted primarily of a decrease in albumin and an increase in alpha-2 and gamma globulins. Other changes occurred in serum lipids.

Radioisotopic studies indicated progressive depletion of the body pools during the depletion phase of the study and repletion in proportion to the amount of ascorbic acid administered daily. This study confirms and extends the observations made in our earlier study that the full clinical syndrome does not appear until the normal body pool has been depleted to less than 300 mg.

The minimal amount of ascorbic acid necessary to prevent or cure scurvy appears to be slightly less than 10 mg daily. Once again our observations are in accord with those of the British Medical Research Council. Estimates of the optimal intake of ascorbic acid must be made on the basis of these data plus a knowledge of the biological and physiological variables of mankind.

Topic: albumins, diet, emotions, fatigue, ascorbic acid deficiency, gamma-globulins, gingival hemorrhage, hospitals, university, pain, patients' rooms, scurvy, signs and symptoms, urinary tract, ascorbic acid, lipids, medical research, correctional facilities, phrynoderma

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Multicenter Study PLoS Med, 4 (12), e352 Dec 2007

Clustered Environments and Randomized Genes: A Fundamental Distinction Between Conventional and Genetic Epidemiology

George Davey Smith [1], Debbie A Lawlor, Roger Harbord, Nic Timpson, Ian Day, Shah Ebrahim

Affiliations

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Abstract

Background: In conventional epidemiology confounding of the exposure of interest with lifestyle or socioeconomic factors, and reverse causation whereby disease status influences exposure rather than vice versa, may invalidate causal interpretations of observed associations. Conversely, genetic variants should not be related to the confounding factors that distort associations in conventional observational epidemiological studies. Furthermore, disease onset will not influence genotype. Therefore, it has been suggested that genetic variants that are known to be associated with a modifiable (nongenetic) risk factor can be used to help determine the causal effect of this modifiable risk factor on disease outcomes. This approach, mendelian randomization, is increasingly being applied within epidemiological studies. However, there is debate about the underlying premise that associations between genotypes and disease outcomes are not confounded by other risk factors. We examined the extent to which genetic variants, on the one hand, and nongenetic environmental exposures or phenotypic characteristics on the other, tend to be associated with each other, to assess the degree of confounding that would exist in conventional epidemiological studies compared with mendelian randomization studies.

Methods and findings: We estimated pairwise correlations between nongenetic baseline variables and genetic variables in a cross-sectional study comparing the number of correlations that were statistically significant at the 5%, 1%, and 0.01% level (alpha = 0.05, 0.01, and 0.0001, respectively) with the number expected by chance if all variables were in fact uncorrelated, using a two-sided binomial exact test. We demonstrate that behavioural, socioeconomic, and physiological factors are strongly interrelated, with 45% of all possible pairwise associations between 96 nongenetic characteristics (n = 4,560 correlations) being significant at the $p < 0.01$ level (the ratio of observed to expected significant associations was 45; p-value for difference between observed and expected < 0.000001). Similar findings were observed for other levels of significance. In contrast, genetic variants showed no greater association with each other, or with the 96 behavioural, socioeconomic, and physiological factors, than would be expected by chance.

Conclusions: These data illustrate why observational studies have produced misleading claims regarding potentially causal factors for disease. The findings demonstrate the potential power of a methodology that utilizes genetic variants as indicators of exposure level when studying environmentally modifiable risk factors.

Figures

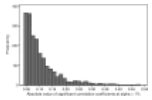

Figure 1. Histogram of Statistically Significant (at...

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intravenous route at an advanced stage of tetanus led to the survival of the animals.

From the above results, it definitely appears that vitamin C can be effectively used as a simple prophylactic and therapeutic tool to combat the neurotoxic effects of tetanus toxin.

Thanks are due to Prof. S. R. MOITRA for his interest in this work.

Eingegangen am 31. Marz 1966

[1] DEY, P. K.: (a) Naturwissenschaften 52, 164 (1964); — (b) Ind. J. Exptl. Biol. (communicated, 1966). — [2] SHERRINGTON, C. S.: The Integrative Action of Nervous System, p. 303, 112. New York: Yale University Press 1906. — *[3]* BROOKS, B. V., D. R. CURTIS, and J. C. ECCLES: Nature 175, 120 (1955)- — *[4]* JUNGBLUT, C. W.: J. Immunol. 33, 203 (1937).

Efficacy of Vitamin C in Counteracting Tetanus Toxin Toxicity

P. K. DRY

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The author has shown [7] that ascorbic acid is most effective as prophylactic and therapeutic agent in nullifying the lethal and convulsive properties of strychnine. He now examined the efficacy of ascorbic acid in counteracting the toxic action of tetanus toxin since SHERRINGTON *[2]* observed that the effects of strychnine poisoning are similar to those appearing in tetanus toxin toxicity and BROOKS et al. [3] confirmed the findings of SHERRINGTON that the action of tetanus toxin in the spinal cord closely resembles that of strychnine. Also, JUNGBLUT *[4]* has shown that the toxin is destroyed *in vitro* by vitamin C.

Adult rats were used in all the experiments. Diet, temp, and space allowed for movement were kept uniform. The gastrocnemius muscle was the site used for the intramuscular administration of toxin.

Group 1. 5 rats were given 2MLD (minimum lethal dose) of tetanus toxin, rhe symptoms of toxicity were then noted. — *Group 2:* 5 rats were given simultaneously 2MLD of toxin and 1 gm/kg of vitamin C intraperitoneally. Then for subsequent three days, vitamin C (l gm/kg) was only administered twice daily i. p. — *Group 3:* 5 rats were administered ascorbic acid l gm/kg twice daily for three days. Then 2MLD of toxin was given, followed again by administration of vitamin C for subsequent three days at the previous dose. — *Group 4:* 5 rats were given 2MLD of toxin. Usally after 16 to 26 hours, local tetanus appeared in the affected leg. When such beginning of symptoms were noted, vitamin C (1 gm/kg) was given i. p. twice daily for 3 days. — *Group 5*: 10 rats were given 2MLD of toxin. After 40 to 47 hours, general tetanic symptoms markedly developed, vitamin C (300 mg) was administered intravenously after anaesthetizeing the animal with Na-thiopental.

Results: Group 1. Following tetanus toxin, local tetanus appeared in 16 to 26 hours. The affected leg was in fixed position and toes were extended. Within 27 to 39 hours, the tail, extremity and hip deviated to the injection side. Both extremities assumed a parallel extended position. In 40 to 47 hours, spasticity of the abdominal and thoracic musculature and flexor muscles of the spine and neck was seen. Tachycardia, dyspnoea, and convulsions were oberved. Death followed in 47 to 65 hours. — *Group 2:* All the animals survived. Only very mild local tetanus were seen at the affected leg after 18 hours. — *Group 3:* All the animals survived. No symptoms of toxicity appeared. — *Group 4:* When the initial symptoms of local tetanus appeared, administration of vitamin C prevented the further spread of the symptoms and they finally survived. — *Group 5:* Administration of vitamin C through

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53. Jahrgang, 1966 Heft 11 (Erstes Juniheft)

intravenous route at an advanced stage of tetanus led to the survival of the animals.

From the above results, it definitely appears that vitamin C can be effectively used as a simple prophylactic and therapeutic tool to combat the neurotoxic effects of tetanus toxin.

Thanks are due to Prof. S. R. MOITRA for his interest in this work.

Eingegangen am 31. Marz 1966

[1] DEY, P. K.: (a) Naturwissenschaften 52, 164 (1964); — (b) Ind. J. Exptl. Biol. (communicated, 1966). — [2] SHERRINGTON, C. S.: The Integrative Action of Nervous System, p. 303, 112. New York: Yale University Press 1906. — *[3]* BROOKS, B. V., D. R. CURTIS, and J. C. ECCLES: Nature 175, 120 (1955)- — *[4]* JUNGBLUT, C. W.: J. Immunol. 33, 203 (1937).

Efficacy of Vitamin C in Counteracting Tetanus Toxin Toxicity

P. K. DRY

Department of Physiology, University College of Science, Calcutta

The author has shown [7] that ascorbic acid is most effective as prophylactic and therapeutic agent in nullifying the lethal and convulsive properties of strychnine. He now examined the efficacy of ascorbic acid in counteracting the toxic action of tetanus toxin since SHERRINGTON *[2]* observed that the effects of strychnine poisoning are similar to those appearing in tetanus toxin toxicity and BROOKS et al. [3] confirmed the findings of SHERRINGTON that the action of tetanus toxin in the spinal cord closely resembles that of strychnine. Also, JUNGBLUT *[4]* has shown that the toxin is destroyed *in vitro* by vitamin C.

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Group 1. 5 rats were given 2MLD (minimum lethal dose) of tetanus toxin, rhe symptoms of toxicity were then noted. — *Group 2:* 5 rats were given simultaneously 2MLD of toxin and 1 gm/kg of vitamin C intraperitoneally. Then for subsequent three days, vitamin C (l gm/kg) was only administered twice daily i. p. — *Group 3:* 5 rats were administered ascorbic acid l gm/kg twice daily for three days. Then 2MLD of toxin was given, followed again by administration of vitamin C for subsequent three days at the previous dose. — *Group 4:* 5 rats were given 2MLD of toxin. Usally after 16 to 26 hours, local tetanus appeared in the affected leg. When such beginning of symptoms were noted, vitamin C (1 gm/kg) was given i. p. twice daily for 3 days. — *Group 5*: 10 rats were given 2MLD of toxin. After 40 to 47 hours, general tetanic symptoms markedly developed, vitamin C (300 mg) was administered intravenously after anaesthetizeing the animal with Na-thiopental.

Results: Group 1. Following tetanus toxin, local tetanus appeared in 16 to 26 hours. The affected leg was in fixed position and toes were extended. Within 27 to 39 hours, the tail, extremity and hip deviated to the injection side. Both extremities assumed a parallel extended position. In 40 to 47 hours, spasticity of the abdominal and thoracic musculature and flexor muscles of the spine and neck was seen. Tachycardia, dyspnoea, and convulsions were oberved. Death followed in 47 to 65 hours. — *Group 2:* All the animals survived. Only very mild local tetanus were seen at the affected leg after 18 hours. — *Group 3:* All the animals survived. No symptoms of toxicity appeared. — *Group 4:* When the initial symptoms of local tetanus appeared, administration of vitamin C prevented the further spread of the symptoms and they finally survived. — *Group 5:* Administration of vitamin C through

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The New England
Journal of Medicine

VOLUME 207 OCTOBER 13, 1932 NUMBER 15

NEW ENGLAND PEDIATRIC SOCIETY

A meeting of the Society was called to order by the President, Dr. Lewis Webb Hill, Boston, at 8:15 P. M., on May 6, 1932 who spoke as follows:

This meeting represents an attempt to arrive at conclusions concerning the rational use of the vitamin preparations in pediatric practice. There is one man whose work on deficiency diseases and allied subjects has been so brilliant and so applicable to the everyday work of each one of us that any such meeting as this could not be complete without his presence—Dr. Alfred Hess of New York.

DIET, NUTRITION AND INFECTION*

BY ALFRED F. HESS, M.D.†

IT is a commonplace that the relationship is intimate between composition of the diet and susceptibility to infection. However, the extent of this relationship and its importance in clinical medicine has only just begun to be realized; in fact we are still uncertain as to the limits of altered susceptibility. From the standpoint of disease, diet, nutrition and resistance to infection should be regarded as an etiologic unit rather than as a triad. In appraising dietaries from this point of view, not only the several vitamins should be considered, but the various inorganic and organic constituents which likewise may be implicated in bacterial infection. It would lead too far afield, however, to consider these various aspects of the subject, so that I shall confine myself to the rôle of some of the vitamins, basing my conclusions mainly on observations made during the past ten to fifteen years in a child-caring institution. As my experience has been concerned chiefly with the antirachitic, antiophthalmic and antiscorbutic vitamins, in other words with vitamins D, A and C, I shall limit my comments to these specific nutritional factors. Furthermore, I shall take into consideration only clinical data, to the exclusion of experiments on animals.

After an experience of several years with the effect of *ultraviolet rays* in the prevention and cure of rickets, an effort was made to lessen the incidence of infection in the institution by means of irradiation with the mercury vapor lamp. As is well-known, respiratory infections constitute one of the last vestiges of institutionalism in hospitals and asylums for children and, during the winter months, plague and torment their foster-parents. Our first attempt, undertaken in 1926[1] with the confidence born of inexperience, was most disappointing. In the course of the winter, in spite of irradiation carried out every other day for a period embracing four months, quite as many infections occurred among the group of infants who were irradiated as among those who lived under the same régime except that they were not irradiated. It may be added that the irradiated group evidenced an initial increase in weight which, however, did not continue during the subsequent months.

Two years later a similar investigation was carried out[2] with the only difference that a carbon arc lamp was used as the source of radiation, as it was thought that these rays might be superior because they more nearly resemble the spectrum of the sun. Again our efforts were fruitless. In spite of systematic exposures to these rays no relative diminution in the incidence of respiratory infections occurred during an observational period of three months.

The following year, 1929, the problem of infection was attacked in a different way[3]. Rickets was prevented by means of the usual doses of cod liver oil, in other words of three teaspoonfuls daily for babies three months or more of age. The diet was composed of full amounts of pasteurized milk, cereals, orange juice, and of vegetables for the older infants. In order to render exposure as infrequent as possible, what was termed "aseptic nursing" was carried out in one ward—physicians, nurses and attendants coming in contact with the infants were required to wear surgical masks which were changed daily; hands were scrubbed thoroughly and frequently; visiting was allowed but once a month and visitors were provided with masks; fondling and petting of infants were prohibited and nurses who had colds or infections were temporarily excluded from service. Once again our attempts at prophylaxis resulted in failure; infections

*Read before the New England Pediatric Society at its meeting, May 6, 1932.

†Hess—Clinical Professor of Pediatrics, University and Bellevue Hospital Medical College. For record and address of author see "This Week's Issue," page 679.

Format: Abstract

Med Microbiol Immunol. 1982;171(2):113-22.

Disorders of neutrophil function in children with recurrent pyogenic infections.

Patrone F, Dallegri F, Bonvini E, Minervini F, Sacchetti C.

Abstract

Ten patients with neutrophil dysfunctions and recurrent pyogenic infections, mainly of the skin middle-ear, and respiratory tract, are described. The most frequently affected functions were chemotaxis and bacterial killing. Pharmacologic restoration of functional defects was tried in all cases. Levamisole was given in two cases and ascorbic acid in the other eight cases. During a follow up of at least 18 months, seven patients showed a complete restoration of neutrophil function and a long-lasting clinical remission. One of the two patients with Chronic Granulomatous Disease has been free from infections for 1 year, despite persistent neutrophil dysfunction, while the other did not display consistent clinical improvement. Another patient, who was given ascorbic acid for a short period only due to non compliance, showed neither laboratory nor clinical improvement.

PMID: 7144693 DOI: 10.1007/bf02124918

[Indexed for MEDLINE]

Publication types, MeSH terms, Substances

LinkOut - more resources

Format: Abstract

Am J Med. 1975 Apr;58(4):532-6.

Effects of ascorbic acid on the common cold. An evaluation of the evidence.

Chalmers TC.

Abstract

Of 14 clinical trials of ascorbic acid in the prevention and treatment of the common cold, the data from 8 were considered well enough gathered to be creditable and to warrant combining for an over-all assessment of efficacy. Differences in mean prorated numbers of colds per year and durations of illness were 0.09 plus or minus 0.06 (plus or minus 1 standard error) and 0.11 plus or minus 0.24, respectively, favoring ascorbic acid over the placebo. These are minor and insignificant differences, but in most studies the severity of symptoms was significantly worse in the patients who received the placebo. In one study lasting 9 months, a large number of the volunteers tasted their capsules and correctly guessed what group they were in. All differences in severity and duration were eliminated by analyzing only the data from those who did not know which drug they were taking. Since there are no data on the long-term toxicity of ascorbic acid when given in doses of 1 g or more per day, it is concluded that the minor benefits of questionable validity are not worth the potential risk, no matter how small that might be.

PMID: 1092164 DOI: 10.1016/0002-9343(75)90127-8

[Indexed for MEDLINE]

Publication types, MeSH terms, Substances

LinkOut - more resources

Format: Abstract

J Appl Physiol. 1976 Aug;41(2):202-5.

Effect of ascorbic acid on rate of heat acclimatization.

Strydom NB, Kotze HF, van der Walt WH, Rogers GG.

Abstract

There is some indication in the literature that ascorbic acid (vitamin C) may reduce the physiological responses to heat stress. Consequently, the effect of ascorbic acid ingestion on heat-strain indicators has been studied on a group of 60 mining recruits undergoing climatic room acclimatization. Of the 60 men, 19 received a daily dose of 250 mg ascorbic acid; 21 a daily dose of 500 mg ascorbic acid; and 20 received a placebo daily. Measurements of rectal temperature, heart rate, and hourly sweat rate were made on all subjects during the 4 h of heat exposure per day for 10 days. The wet bulb temperature was 32.2 degrees C, the dry bulb 33.9 degrees C, the air movement 0.4 m/s, and the work rate 35 W. The results indicate that the rate and degree of acclimatization, as assessed by 4th-h rectal temperature, is enhanced by ascorbic acid supplementation and that no differences in response could be shown between daily dosages of 250 and 500 mg of vitamin C.

PMID: 956103 DOI: 10.1152/jappl.1976.41.2.202

[Indexed for MEDLINE]

Publication types, MeSH terms, Substance

LinkOut - more resources

Format: Abstract

Eur Respir J. 1989 Mar;2(3):229-33.

Effects of vitamin C on airway responsiveness to inhaled histamine in heavy smokers.

Bucca C[1], Rolla G, Caria E, Arossa W, Bugiani M.

Author information

Abstract

Histamine bronchial threshold, the provocation concentration of histamine causing a 25% fall in maximal expiratory flow at 50% of forced vital capacity from the control value (PC25MEF50), was measured in seven heavy smokers and in seven sex- and age-matched nonsmokers before and one hour after ingestion, double-blind, of vitamin C (2 g) or placebo. Smokers had significantly lower baseline values of serum ascorbate, maximal expiratory flow at 50% of forced vital capacity (MEF50) and PC25MEF50: the latter was negatively related to serum ascorbate (r = -0.85; p less than 0.001). Acute treatment with vitamin C produced a significant decrease in PC25MEF50 in smokers (95% confidence limit (CL) from 4.87-3.36 to 2.91-2.01 mg.ml-1; p = 0.017), whilst it had no effect in nonsmokers. A preliminary open study on the effect of prolonged administration of vitamin C (1 g daily) was performed in smokers. One week of treatment produced a further significant decrease in PC25MEF50 (p less than 0.0001). Our results suggest that in heavy smokers histamine bronchial responsiveness may be attenuated by chronic ascorbate deficiency. In these circumstances, acute and short-term treatment with vitamin C may increase the bronchoconstrictive response to inhaled histamine.

PMID: 2731601

[Indexed for MEDLINE]

Publication types, MeSH terms, Substances

LinkOut - more resources

JAMA. 1975 Mar 10;231(10):1073-9.

Ascorbic acid and the common cold. Evaluation of its efficacy and toxicity.

Dykes MH, Meier P.

Abstract

We reviewed the clinical data relating to the efficacy and safety of pharmacologic doses of ascorbic acid in the prevention and treatment of the common cold. Although one study tentatively supports the hypothesis that such doses of ascorbic acid may be efficacious, a second study by the same group did not confirm the significant findings, and no clear, reproducible pattern of efficacy has emerged from the review of all the evidence. Similarly, there is currently little adequate evidence on either the presence or the absence of serious adverse reactions to such doses of ascorbic acid, although many such reactions have been hypothesized. The unrestricted use of ascorbic acid for these purposes cannot be advocated on the basis of the evidence currently available.

PMID: 1089817

[Indexed for MEDLINE]

LinkOut - more resources

Proc Natl Acad Sci U S A. 1993 Jan 1;90(1):317-21.

Glutathione ester delays the onset of scurvy in ascorbate-deficient guinea pigs.

Mårtensson J[1], Han J, Griffith OW, Meister A.

Author information

Abstract

Previous studies showed that administration of ascorbate to glutathione (GSH)-deficient newborn rats and guinea pigs prevented toxicity and mortality and led to increased tissue and mitochondrial GSH levels; ascorbate thus spares GSH. In the present work, we tried to answer the converse question: Does administration of GSH spare ascorbate? Because administered GSH is not well transported into most cells, we gave GSH monoethyl ester (which is readily transported and converted into GSH intracellularly) to guinea pigs fed an ascorbate-deficient diet. We found that treatment with GSH ester significantly delays appearance of the signs of scurvy and that this treatment spares ascorbate; thus, the decrease of tissue levels of ascorbate was delayed. The findings support the conclusions that (i) GSH is essential for the physiological function of ascorbate because it is required in vivo for reduction of dehydroascorbate and (ii) there is metabolic redundancy and overlap of the functions of these antioxidants. The sparing effect of GSH in scurvy may be mediated through an increase in the reduction of dehydroascorbate (which would otherwise be degraded) and to antioxidant effects of GSH that are also produced by ascorbate. Other studies indicate that GSH deficiency in adult mice stimulates ascorbate synthesis in liver. During this work we found that administration of GSH itself is highly toxic to ascorbate-deficient guinea pigs when given in divided i.p. doses totaling 3.75 mmol/kg daily.

PMID: 8419936 PMCID: PMC45651 DOI: 10.1073/pnas.90.1.317

[Indexed for MEDLINE] **Free PMC Article**

Publication types, MeSH terms, Substances, Grant support

LinkOut - more resources

The Effect of Vitamin E on Common Cold Incidence Is Modified by Age, Smoking and Residential Neighborhood

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http://dx.doi.org/10.1080/07315724.2006.10719543

Links to the references are added to this manuscript version.

Fig. 1 is redrawn as a more accurate version at the end of this paper.

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The Effect of Vitamin E on Common Cold Incidence Is Modified by Age, Smoking and Residential Neighborhood

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ABSTRACT

Background: We have previously found a 28% reduction in common cold incidence with 50 mg/day vitamin E supplementation in a subgroup of the Alpha-Tocopherol Beta-Carotene Cancer Prevention (ATBC) Study cohort: older city-dwelling men (≥65 years) who smoked only 5–14 cigarettes/day.

Objective: To carry out more detailed analyses to explore the modification of vitamin E effect by age, smoking, and residential neighborhood.

Methods: We examined the effect of vitamin E on common cold risk in subjects consisting of the placebo and vitamin E arms (n = 14,573) of the ATBC Study, which recruited males aged 50–69 years who smoked ≥5 cigarettes/day at the baseline. The ATBC Study was conducted in southwestern Finland in 1985–1993; the active follow-up lasted for 4.7 years (mean). We modeled common cold risk as a function of age-at-follow-up in the vitamin E arm compared with the placebo arm using linear splines in Poisson regression.

Results: In participants of 72 years or older at follow-up, the effect of vitamin E diverged. Among those smoking 5–14 cigarettes per day at baseline and living in cities, vitamin E reduced common cold risk (RR = 0.54; 95% CI 0.37–0.80), whereas among those smoking more and living away from cities, vitamin E increased common cold risk (RR = 1.58; 1.23–2.01).

Conclusions: Vitamin E may cause beneficial or harmful effects on health depending on various modifying factors. Accordingly, caution should be maintained in public health recommendations on vitamin E supplementation until its effects are better understood.

INTRODUCTION

Animal studies have found that vitamin E may affect susceptibility to and severity of diverse viral and bacterial respiratory infections (1-5). Although several studies found that vitamin E may have beneficial effects on various laboratory measures of the immune system in animals and humans (5,6), harmful effects on the immune system have also been reported (7,8). Two animal studies found positive effects on the immune system with moderate vitamin E doses, but adverse effects with large doses (9,10).

Only a few trials have examined the effect of vitamin E supplementation on clinical infectious disease outcomes, such as respiratory and urinary tract infections (5,11-15) and tuberculosis (16) in human subjects. On the whole, these trials found no unequivocal benefit from vitamin E and, paradoxically, one trial found an increase in the severity of acute respiratory illness with 200 mg per day of vitamin E (12). Three trials examined the effect of vitamin combinations containing vitamin E on respiratory infections; however, no specific conclusions of vitamin E can be drawn of these trials (17-19).

We previously found no overall effect on common cold risk with 50 mg per day of vitamin E in the Alpha-Tocopherol Beta-Carotene Cancer Prevention (ATBC) Study (20). However, in a small subgroup of older city-dwelling men (≥65 years) who smoked only 5–14 cigarettes per day, vitamin E supplementation was associated with a statistically highly significant, but quantitatively modest, reduction in common cold incidence (RR = 0.72; 95% CI: 0.62–0.83) (20). Whether this observation resulted from a physiological effect or emerged by chance from a series of subgroup analyses remained an open question. Since the number of common cold episodes recorded in the ATBC Study was very high, we carried out more detailed analyses to explore the possibility that vitamin E effect is modified by age, smoking, and residential neighborhood.

PARTICIPANTS AND METHODS

Study Participants and Intervention Groups

The design and methods of the ATBC Study examining the effects of vitamin E (*dl*-α-tocopheryl acetate (AT), 50 mg/day) and β-carotene (BC, 20 mg/day) on the incidence of lung cancer and other cancers have already been described in detail (20,21). In brief, the trial participants were recruited in 1985–88 from the total male population aged 50–69 years living in southwestern Finland (n = 290,406). To be eligible, participants had to smoke ≥5 cigarettes per day at entry. The eligible participants (n = 29,133) were randomized to one of four intervention arms and administered placebo, AT, BC, or AT + BC. The planned intervention continued for 5 to 8 years (median 6.1 years) until April 30, 1993, with 3 follow-up visits annually, but because of deaths and drop-outs the active follow-up lasted for 4.7 years (mean). The trial was approved by the institutional review boards of the participating institutions; all participants gave written informed consent. At baseline, prior to randomization, the men completed a questionnaire on their medical and smoking histories and general background characteristics. In the current analysis we excluded participants who were administered β-carotene to avoid any problems caused by potential interaction between vitamin E and β-carotene, so that we restricted ourselves to the placebo and AT arms of the trial (n = 14,573; Table 1).

Outcome Definition and Smoking Status Evaluation during Follow-Up

At each follow-up visit to the local study center, 3 times per year with 4-month intervals (Table 1), the participant was asked "Have you had a common cold since the previous visit, and if so, how many times?" The occurrence of "other upper respiratory tract infection" and "acute bronchitis" was also asked about. The number of colds reported at each follow-up visit was used as the outcome for this study. This outcome, self-reported colds, is based on subjective symptoms and not on any laboratory findings. However, since it is the subjective symptoms that lead a person to seek medical attention and obtain sick-leave, in this respect the subjective outcome is most relevant for public health purposes. The manifestations of the common cold are so typical that self-diagnosis by the patient is usually correct (22). During 69,094 person-years of active follow-up covered by visits to the study centers, 55,770 common cold episodes were recorded.

At each follow-up visit, the participant was asked: "Have you been smoking since the previous visit?" with the following alternative responses provided: 1) no, 2) yes, but now I have quit, 3) yes, continuously (Table 1). In this study we used responses 1) and 3) when exploring the effect of smoking cessation before the follow-up visit.

Statistical Methods

Because we analyzed the modification of vitamin E effect by age, and the ATBC Study lasted for some 6 years, in the current analyses we used the age of participant at the follow-up visit. This is the biological age at the point of time when the outcome for the preceding 4-month period is evaluated.

The number of common cold episodes was modeled using Poisson regression. The risk ratio (RR) and the likelihood ratio-based 95% confidence interval (95% CI) were calculated using the SAS PROC GENMOD program (release 8.1, SAS Institute, Inc., Cary, NC). Linear spline-modeling (23) was carried out for the four groups defined by baseline smoking and residential neighborhood as follows.

First, using a base model containing the mean vitamin E-effect, and a linear trend to adjust for the average reduction in common cold incidence with age, we added ten linear splines to both trial arms at 2 year-intervals starting at 52 years of age-at-follow-up. Thereafter, linear spline terms for the vitamin E arm were added to the same knots, and the statistical significance of the vitamin E—age-at-follow-up interaction was calculated from the change in the –2 × Log(Likelihood) difference. This saturated model was simplified by dropping the knots that had the least effect on the vitamin E spline model, starting with those with the lowest Wald-test χ^2 value. The corresponding knots covering both arms were concurrently dropped out. The models were simplified until all remaining vitamin E arm knots gave a significant contribution to the spline model ($\chi^2 > 4$). Thus, the final model contained knots at the same years for both arms to provide the baseline, and for the vitamin E arm to provide the age-modification. Visually, the final models captured all the main features of the saturated models (graphs for saturated models not shown). The optimized models are described in Table 2 and the corresponding graphs in Fig. 1. Two-tailed *p*-values were used.

We tested the modifying effect of residential neighborhood on the vitamin E effect separately in participants who smoked 5–14 and those who smoked ≥15 cigarettes per day. Based on the appearance of the spline curves (Fig. 1), we restricted this analysis to participants aged ≥62 and ≥65 years at the follow-up visit, respectively, in the light and heavy smokers. First we added a linear trend to adjust for the average reduction in common cold incidence with age, the mean vitamin E-effect, mean effect of residential neighborhood, and a linear spline to the vitamin E arm at 62 or 65 years. To test the role of residential neighborhood, we further added the mean vitamin E effect and a linear spline to the vitamin E arm to the city-dwellers. The change in the –2 × Log(Likelihood) gives χ^2(2 df), which was used to calculate the *p*[2-tail]-value to test the role of residential neighborhood in the vitamin E spline-models.

As to supplementation, the analyses were carried out following the intention-to-treat principle. Compliance with supplementation was high: some 80% of participants took more than 95% of their prescribed capsules during their active participation in the trial; there were no differences in capsule consumption among the intervention groups (21). The outcome was, however, available only for those participants who continued with the trial and participated in the follow-up visits.

Table 1. Baseline Characteristics of Participants, and the Age and Smoking Status at Follow-Up Visits, The ATBC Study 1985–1993; No β-Carotene Participants

Baseline characteristics	No. of participants	
All participants	14,573	(100%)
Baseline age (years)		
50–54	5,275	(36%)
55–59	4,639	(32%)
60–64	3,183	(22%)
65–69	1,476	(10%)
Smoking (cigarettes/day)		
5–14	2,910	(20%)
15–	11,663	(80%)
Age of smoking initiation*		
<21 years	10,842	(74%)
≥21 years	3,727	(26%)
Residential neighborhood during the last 20 years*		
City (>50,000 inhab.)	6,233	(43%)
Town	3,093	(21%)
Village	2,092	(14%)
Countryside	3,153	(22%)

Follow-up visit variables	No. of visits	
All visits	207,284	(100%)
Age at follow-up visit		
50–51	5,265	
52–53	16,603	(8%)
54–55	25,517	(12%)
56–57	29,240	(14%)
58–59	28,127	(14%)
60–61	25,902	(12%)
62–63	22,588	(11%)
64–65	18,685	(9%)
66–67	14,513	(7%)
68–69	10,642	(5%)
70–71	6,485	(3%)
72–73	2,805	(1.5%)
74–77	912	(0.5%)
Smoking since the previous visit		
No	23,032	(11%)
Yes, but quit before current visit	5,817	(3%)
Yes, continuously	178,433	(86%)

* Data on residential neighborhood was missing from 2 participants, and on age at smoking initiation from 4 participants.

Table 2. Optimizing the Spline Models for the Age-Modification of Vitamin E Effect on Common Cold Incidence

Group	Saturated model*	Simple model*
≥15 cigarettes per day living away from cities	χ^2(10 df) = 40.9	χ^2(4 df) = 36.5 p = 0.0000002 knots at 52, 56, 58, 68 yrs
≥15 cigarettes per day living in a city	χ^2(10 df) = 17.3	χ^2(2 df) = 7.8 p = 0.02 knots at 64, 66 yrs
5–14 cigarettes per day living away from cities	χ^2(10 df) = 22.3	χ^2(1 df) = 18.9 p = 0.00002 knot at 56 yrs
5–14 cigarettes per day living in a city	χ^2(10 df) = 46.5	χ^2(2 df) = 38.7 p = 0.000000004 knots at 60, 62 yrs

* The χ^2 measures the improvement in the Poisson model when the knots indicated are added to the vitamin E arm in the simple model.
In the saturated model, 10 knots at 2-year intervals were added, starting at 52 years.

RESULTS

Table 1 shows the distributions for the baseline data for age, smoking level, age of smoking initiation, residential neighborhood, and follow-up data for age and smoking at the follow-up visits. On average, 0.27 common cold episodes were reported at each four-monthly follow-up visit, corresponding to an annual rate of 0.8 cold episodes.

There is no overall effect, with a narrow confidence interval, of vitamin E supplementation in the four groups defined by baseline smoking and residential neighborhood (Table 3). To examine the potential modification of vitamin E effect by age, we constructed linear spline models for the vitamin E effect as a function of age-at-follow-up separately for the four groups defined by baseline smoking and residential neighborhood. These groups show statistically highly significant modification of vitamin E effect by age-at-follow-up, except for city-dwellers smoking ≥15 cigarettes per day (Fig. 1, Table 2).

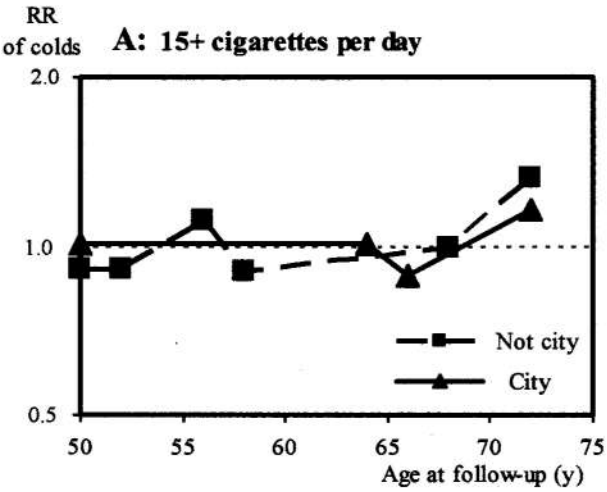
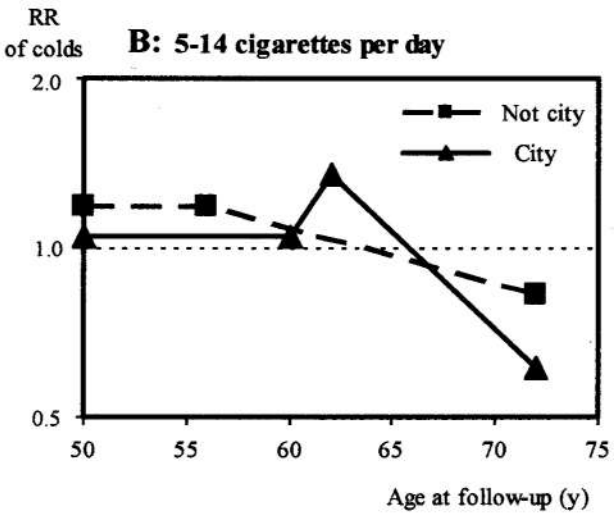


Fig. 1. The effect of vitamin E on the relative risk of common cold as a function of age at follow-up. Participants smoking more (A) and less (B) are further divided into subgroups by residential neighborhood. RR indicates the relative risk of colds between the vitamin E and placebo arms. See Table 2 for the description of the statistical models.
See Fig. 1. redrawn in 2014 at the end of this paper.



Among participants who smoked ≥15 cigarettes per day at baseline, the spline curve of vitamin E effect shows a trend towards harm for old participants (Fig. 1A). Among the heavy smokers living away from cities, there is a peak of increased risk at 56 years of age. Although there is no apparent biological rationale for such a sharp peak in the common cold risk, dropping out the knots at 52, 56, and 58 years would reduce the χ^2 value by 17.9 (3 df; $p = 0.0005$) so that these knots are retained in the spline model.

Among participants who smoked only 5–14 cigarettes per day at baseline, the spline curves suggest slight harm for young participants, but there is an age-dependent trend towards benefit in old participants (Fig. 1B). Among the city-dwellers who smoke less, there is a peak indicating harm at about 62 years of age. Although there is no apparent biological rationale for such a sharp peak here either, omitting the knot at 62 years reduces the χ^2 value by 16.3 (1 df; $p = 0.0001$); therefore both knots are retained in the spline model. The knot at 56 years in the participants smoking less, who live away from cities, remained after the stepwise reduction of the spline model, but there was no meaningful difference compared with spline models with a single knot located at 52, 54 or 58 years.

Because this work was motivated by the effect of vitamin E observed in the subgroup of ≥65 year old city-dwellers who smoked 5–14 cigarettes per day (20) and inclusion of that subgroup in the vitamin E spline model does not provide a test independent of the original finding, we examined whether age is a modifier outside of this small subgroup. When the participants aged ≥65 years at baseline were excluded from the spline model of the city-dwellers who smoked 5–14 cigarettes per day at baseline, the vitamin E spline model was still highly significant (χ^2[2 df] = 12.3, p = 0.002). The other three of the four subgroups test the age-modification of vitamin E effect independently of the original hypothesis-generating subgroup (Table 2).

Among the oldest participants, the effect of vitamin E on common cold incidence substantially diverges in the light and heavy smokers, but the role of residential neighborhood is less evident (Fig. 1). Therefore we tested whether including the residential neighborhood significantly improves the vitamin E spline models at the upper age range. Among participants who smoked 5–14 cigarettes per day there was strong evidence that the age at visit of 62 years or more modifies the vitamin E effect differently in city-dwellers and those who live away from cities (p = 0.018). In contrast, for those who smoked ≥15 cigarettes per day there was weaker evidence that the age at visit of 65 years or more modifies the vitamin E effect differently in the residential neighborhood groups (p = 0.042).

Based on the appearance of the spline curves, certain age-ranges were selected for explicit calculation of the effect estimate of vitamin E supplementation and its confidence interval (Fig. 1, Table 3). Vitamin E supplementation for participants smoking less was associated with a significant increase in the risk of colds at 50–56 years in those who live away from cities, and at 61–63 years in the city-dwellers. For city-dwellers who smoke less, vitamin E supplementation caused a substantial reduction in the risk of colds for participants aged 69 years or more, but the benefit was smaller among participants living away from cities. Among the heavy smokers, vitamin E supplementation significantly increased the risk of colds among the oldest participants (Table 3).

It is noteworthy that among the ≥72 year old participants the greatest benefit was seen in city-dwellers smoking 5–14 cigarettes per day, whereas the greatest harm was seen in the mirror image, i.e., participants living outside cities and smoking ≥15 cigarettes per day (Fig. 1, Table 3). The confidence intervals for the vitamin E effect on these two groups are strikingly different. It is also noteworthy that in both of these groups there is a peak of harm at 62 and 54 years respectively, whereas the remaining two groups do not show comparable peaks for the younger participants.

The preceding analysis is based on defining the subgroups by smoking level at baseline. To explore whether other measures of cigarette smoke exposure would further modify the effect of vitamin E, we analyzed the risk of colds in participants aged ≥72 years by combining the residential neighborhood groups, but keeping the baseline low and heavy smoking groups separate. Among the old participants who smoked heavily at baseline, the vitamin E effect is significantly modified by the age of smoking initiation (Table 4). In these heavy smokers, there was no definite evidence of harm from vitamin E in those who quit smoking before the visit, but the number of quitters is low. Among participants who smoked less at baseline, age of smoking initiation did not modify the vitamin E effect, and smoking cessation did not lead to a greater vitamin E benefit (Table 4).

Table 3. The Effect of Vitamin E Supplementation on the Risk of the Common Cold in Selected Age-Groups by Baseline Smoking and Residential Neighborhood

	≥15 cigarettes per day		**5–14 cigarettes per day**	
	Town, village, or countryside	**City**	**Town, village, or countryside**	**City**
Number of participants:	6,587	5,074	1,751	1,159
All visits (207,270 visits)				
RR	0.98	1.00	1.02	1.02
95% CI	0.95–1.01	0.97–1.03	0.97–1.08	0.96–1.08
Age at visit				
50–56 yrs (62,054 visits)				
RR	1.01	0.98	1.20	1.07
95% CI	0.96–1.05	0.93–1.03	1.08–1.32	0.96–1.20
61–63 yrs (35,182 visits)				
RR	0.93	1.02	0.97	1.30
95% CI	0.87–0.99	0.95–1.10	0.86–1.09	1.13–1.50
69–71 yrs (11,321 visits)				
RR	1.11	1.04	0.80	0.68
95% CI	0.98–1.27	0.90–1.19	0.67–0.96	0.54–0.84
72–77 yrs (3,717 visits)				
RR	1.58	1.35	0.90	0.54
95% CI	1.23–2.01	1.03–1.76	0.63–1.28	0.37–0.80

Table 4. Modification of Vitamin E Effect on Common Cold Risk by Age at Smoking Initiation and by Recent Smoking among Participants Aged 72 Years or More at the Follow-Up Visit

	Risk of colds in the vitamin E arm RR; 95% CI	**Test of interaction** *p*
Baseline smoking ≥15 cigarettes per day		
All in the subgroup (2,513 visits)	1.42; 1.18–1.70	
Age at smoking initiation		
<21 years (1,482 visits)	1.68; 1.34–2.12	0.02
≥21 years (1,031 visits)	1.09; 0.82–1.45	
Smoking at follow-up		
Continued (1,992 visits)	1.48; 1.21–1.80	0.10
Quit (444 visits)	0.96; 0.59–1.55	
Baseline smoking 5–14 cigarettes per day		
All in the subgroup (1,204 visits)	0.71; 0.54–0.91	
Age at smoking initiation		
<21 years (578 visits)	0.67; 0.45–0.98	0.6
≥21 years (626 visits)	0.75; 0.53–1.06	
Smoking at follow-up		
Continued (788 visits)	0.62; 0.45–0.87	0.12
Quit (368 visits)	0.98; 0.61–1.55	

DISCUSSION

In a previous paper we reported a 28% reduction in common cold incidence with vitamin E supplementation in older city-dwelling men who smoked only 5–14 cigarettes per day (20). The present work was carried out to analyze whether the three characteristics specifying the small subgroup, i.e., age, smoking, and residential neighborhood, would cause a more general modification of the vitamin E effect. The current spline model analyses over age-at-follow-up seem to show that the reduction of common cold incidence with vitamin E in the previously identified small subgroup (20) is explained by its physiological effects rather than by a chance occurrence emerging from a series of subgroup analyses.

Age and smoking are plausible modifying factors for the effect of vitamin E on common cold incidence, but a biological rationale for the role of residential neighborhood as a modifying factor is not as apparent. Possibly higher level of air pollution or much more frequent use of public transport with concomitant exposure to infectious agents could explain the observed difference between cities and smaller communities.

Recently, a small trial with 617 elderly participants in long-term care facilities found a slightly lower incidence of colds among participants administered 200 mg per day of vitamin E (RR = 0.83; 95% CI: 0.68–1.01) (13). Another small trial with 652 elderly noninstitutionalized people found a slightly higher incidence of respiratory infection among participants administered 200 mg per day of vitamin E (RR = 1.12; 0.88–1.25), and a statistically significant increase in symptom severity, fever and restriction in activity (12). Although such divergence may result from the small size of the trials, it might also result from biological heterogeneity, as we found both increases and decreases in common cold risk with 50 mg per day of vitamin E supplementation in our current study, depending on the characteristics of the subgroup.

We found quite sharp peaks of increase in common cold risk at 54 and 62 years with vitamin E supplementation in two of our four subgroups (Fig. 1), both highly unlikely to be due to chance, although there is no apparent biological rationale for such peaks. Possibly the peaks may be related to social factors such as retirement, which in Finland occurs usually at about 58 to 60 years; however, retirement does not occur as such a sharp peak as seen in the spline models.

The modification of the vitamin E effect on the common cold risk by age, smoking, and residential neighborhood may be of more general interest as regards the physiological effects of antioxidants. There is evidence indicating that free radical production may be important in the emergence of various chronic diseases such as cancer and cardiovascular diseases (24,25) as well as in the pathogenesis of certain viral and bacterial diseases (26–28). It is sometimes assumed that antioxidants, including vitamin E, might have a consistent unidirectional broad-spectrum benefit on the human system by protecting it against the free radicals (24,25). Our finding that vitamin E supplementation significantly increases or decreases common cold risk depending on the three variables in question is inconsistent with the notion of uniform benefits from antioxidant supplementation.

In the current work we had available a very large number of outcomes (55,770 episodes of the common cold) which rendered it possible to analyze the age-dependence of the vitamin E effect in the four subgroups accurately. With severe diseases such as cancers or cardiovascular diseases, the statistical power is usually too small to permit analyses similar to the current spline models. Still, it is possible that comparable effect-modification occurs in the case of more serious diseases, even though directly extrapolating the particular modifying factors observed in this work to any other diseases is not justified. In a previous analysis of the ATBC Study cohort, we found that the effect of vitamin E on the risk of pneumonia was modified by the age of smoking initiation so that vitamin E reduced pneumonia risk in participants who began smoking at a later age, whereas vitamin E slightly increased the risk among participants who began smoking at an early age (14)

(see also Table 4). Thus, our findings for pneumonia risk also suggest substantial heterogeneity between population groups in the effects of vitamin E supplementation.

A recent meta-analysis focusing on the potential harm of vitamin E supplementation found that, starting from approximately 150 mg/day of vitamin E, there was increased mortality among people supplemented with vitamin E (29). However, it is possible that there is biological heterogeneity between population groups, so that people's characteristics may determine whether vitamin E supplementation caused net benefit or harm. In our current study, the vitamin E dose was 50 mg/day, which is substantially less than the estimated threshold level in the above-mentioned meta-analysis (29); however, our current analyses on common cold incidence and our previous analyses on pneumonia incidence make it seem probable that some population groups are harmed at levels of 50 mg/day, even though the same low dose seems beneficial for other population groups (14,15). Thus, it may be unjustifiable to assume that there is a single threshold level for harmful effects that is valid for the entire population. Another recent review on vitamin E safety concluded that supplements appear harmless for most adults in amounts up to 1 g/day (30), whereas our subgroup analyses indicate harmful effects on restricted population groups at doses as low as 50 mg/day (Tables 3 and 4).

The definition of a common cold episode in our study was based on self-diagnosis, which is usually reliable (22). Although subjective perception of what is classified as a cold varies between participants, such inaccuracy in outcome assessment does not lead to consistent differences between our double-blinded study arms; rather, the inaccuracy renders the differences smaller than they may actually be. Our implicit assumption in this work was that the effect of vitamin E is based on its reported effects on the immune system (5,6), but even if the mechanism of the effect of vitamin E would be on other factors that determine whether a person has subjective symptoms of the common cold, the conclusions of our double-blind trial are not affected. Furthermore, even though a proportion of the self-reported colds may be caused by non-infectious etiology, this does not affect the validity of our observation that this common set of symptoms seems to be affected differently with vitamin E in different subgroups of people.

The modification of the vitamin E effect on common cold risk also bears on the heterogeneity of findings in common cold trials examining vitamin C, the major water-soluble antioxidant, which interacts with lipid-soluble vitamin E (5,31,32). The largest vitamin C trials found no effect on the risk of the common cold; however, low dietary vitamin C intake and acute physical stress were proposed as modifying factors that may explain statistically significant reduction in common cold risk with vitamin C supplementation in several small trials (5,33,34). Thus, it seems possible that these two closely related antioxidants, vitamin E and vitamin C, may affect common cold risk in restricted groups of people, even though there seems to be no overall effect in the general Western population.

The main finding of our study is that vitamin E supplementation may cause benefit or harm to health depending on several modifying factors. It is premature to draw any practical conclusions from our study except that general caution should be maintained in public health recommendations on vitamin E supplementation until the effects of this vitamin are better understood. The possibility that vitamin E may reduce the risk of the ubiquitous common cold infection by half in some groups of elderly people would seem to warrant further study to define more precisely the population groups that might benefit from supplementation.

ACKNOWLEDGMENTS

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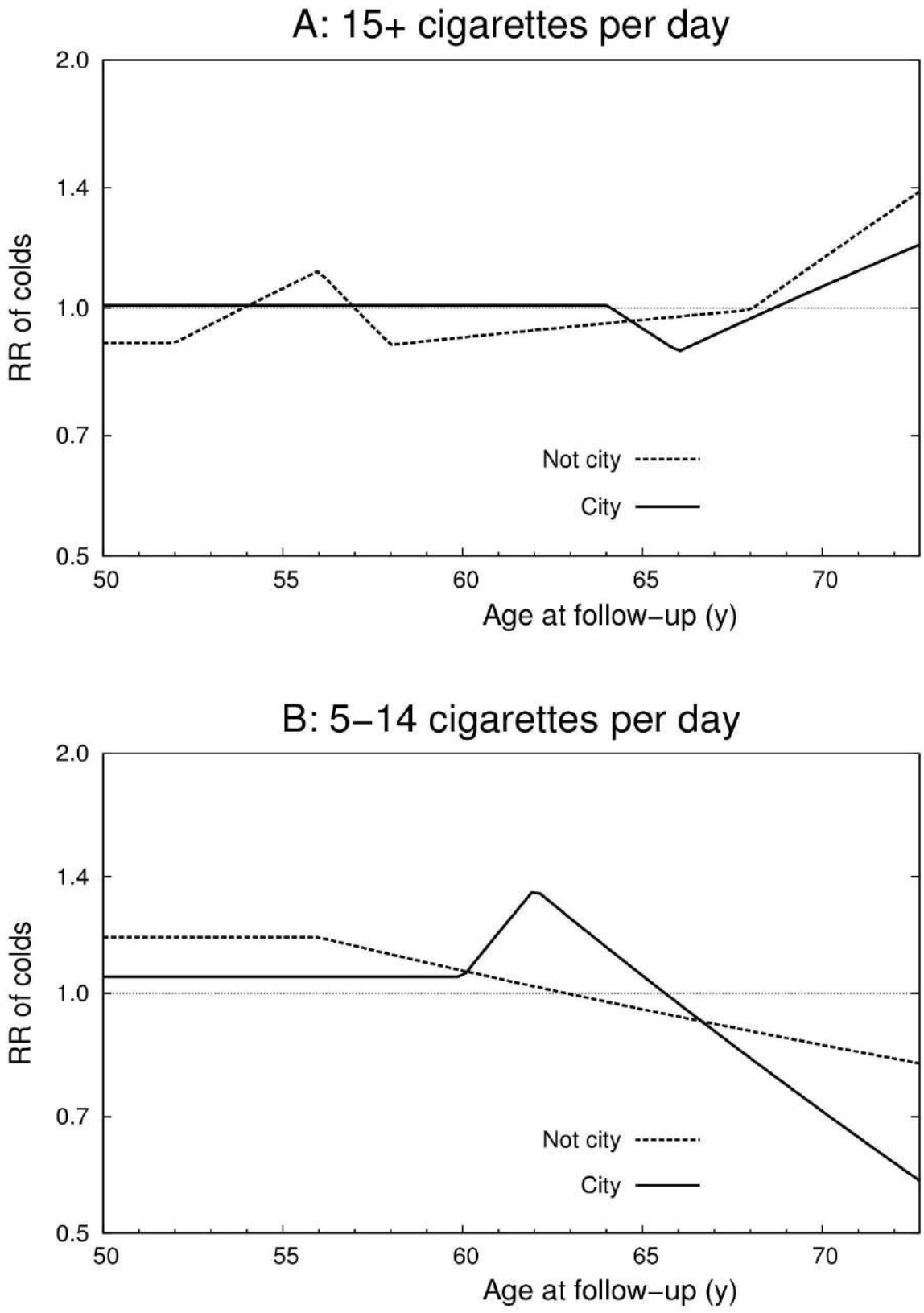

Fig. 1. The effect of vitamin E on the relative risk of common cold as a function of age at follow-up. Participants smoking more (A) and less (B) are further divided into subgroups by residential neighborhood. RR indicates the relative risk of colds between the vitamin E and placebo arms. See Table 2 for the description of the statistical models. These versions were redrawn in 2014.

Format: Abstract

Trop Geogr Med. 1980 Jun;32(2):132-7.

High dose ascorbic acid in Nigerian asthmatics.

Anah CO, Jarike LN, Baig HA.

Abstract

Forty-one asthmatic patients in remission were randomly allocated to two treatment groups in a double-blind trial. One group took 1 g, of ascorbic acid as one effervescent tablet once daily and the second group took a matching placebo. The asthmatics were selected from those attending the Asthma Clinic. One criterion for selection was the increase in exacerbation during the rainy season. These exacerbations were precipitated by respiratory infection. After 14 weeks, an assessment of the severity and rate of attacks showed that those on ascorbic acid suffered less severe and less frequent attacks of asthma during the study period. Plasma ascorbic acid astimations showed a significant rise in the level in those taking ascorbic acid over those on placebo. ($P < 0.01$). Cessation of ascorbic acid in the group taking it increased attack rates. It is concluded that high dose ascorbic acid is probably a good prophylaxis in some bronchial asthmatics.

PMID: 7423602

[Indexed for MEDLINE]

Publication types, MeSH terms, Substance

LinkOut - more resources

Scurvy: historically a plague of the sailor that remains a consideration in the modern intensive care unit.

Holley AD,
Osland E,
Barnes J,
Krishnan A,
Fraser JF

Author information

Internal Medicine Journal, 01 Mar 2011, 41(3):283-285
DOI: 10.1111/j.1445-5994.2010.02413.x PMID: 21426466

Abstract

We report the case of the case of a 56 year old female with sepsis on a background of rheumatoid arthritis and steroid use manifesting with overt clinical features of scurvy. Ascorbic acid assays were able to demonstrate severe deficiency and confirm a diagnosis of scurvy. Clinical resolution of signs and symptoms following commencement of vitamin C replacement was rapid. The intensivist and dietitian need to consider this diagnosis even in the first world setting, particularly in the presence of sepsis, inflammatory conditions, steroid use and importantly malnutrition.

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PMID: 15771570

How Neutrophils Kill Microbes

Anthony W. Segal

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Abstract

Neutrophils provide the first line of defense of the innate immune system by phagocytosing, killing, and digesting bacteria and fungi. Killing was previously believed to be accomplished by oxygen free radicals and other reactive oxygen species generated by the NADPH oxidase, and by oxidized halides produced by myeloperoxidase. We now know this is incorrect. The oxidase pumps electrons into the phagocytic vacuole, thereby inducing a charge across the membrane that must be compensated. The movement of compensating ions produces conditions in the vacuole conducive to microbial killing and digestion by enzymes released into the vacuole from the cytoplasmic granules.

Keywords: bacteria, protease, free radical, microbicidal, ion channel, enzyme

INTRODUCTION

Neutrophils are highly motile phagocytic cells that constitute the first line of defense of the innate immune system. They were first discovered by Elie Metchnikoff when he inserted rose thorns into starfish larvae and found that wandering mesodermal cells accumulated at the puncture site. He showed these cells to be phagocytic and described the larger cells as macrophagocytes, or macrophages, and the smaller as microphagocytes, now known as granulocytes, of which by far the most numerous are the neutrophils.

The ability of these cells to engulf and degrade bacteria was logically assumed to indicate a killing function. A microbicidal function was ascribed to the contents of their abundant cytoplasmic granules that were discharged into the phagocytic vacuole containing the microbe (1) (Figure 1). Attention was then directed toward the characterization of the granules by electron microscopy, fractionation, and biochemical analysis. Several of the purified granule proteins were shown to kill microbes.

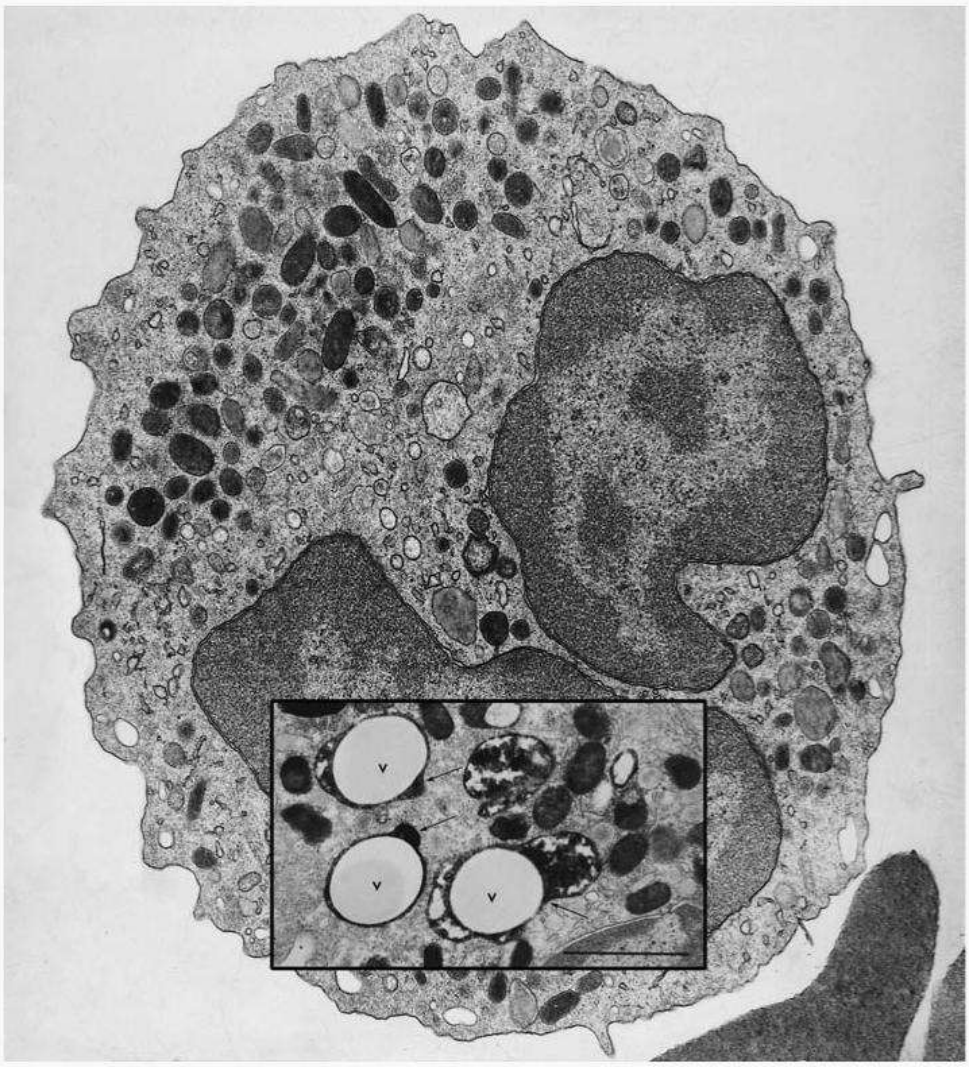

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Figure 1

Transmission electron micrograph of a human neutrophil. Inset is an image taken from a neutrophil 20 s after the phagocytosis of latex particles opsonized with IgG (V, vacuole). The section was stained for myeloperoxidase (MPO) to reveal the electron-dense product in the azurophil granules, some of which can be seen degranulating into the phagocytic vacuole (arrows). Bar = 1 μm. (Figure from 17.)

Parallel with studies into microbicidal activity of the granule contents, investigations were undertaken into the metabolism of phagocytosing neutrophils. The neutrophils demonstrated a significant “extra respiration of phagocytosis,” which was non-mitochondrial and was associated with a dramatic increase in turnover of the hexose monophosphate (HMP) shunt and the production of large amounts of H_2O_2 (2). These metabolic changes were shown to be essential for microbial killing.

In the late 1960s and early 1970s, a number of related discoveries cast a very different perspective on the killing process. Chronic granulomatous disease (CGD), a profound immunodeficiency to bacterial and fungal infections, was associated with failure of these metabolic changes (3). In addition, myeloperoxidase (MPO)-mediated halogenation, which is microbicidal in the test tube, was also defective in these patients (4).

Soon after its discovery in 1969, superoxide dismutase was used to show that activated neutrophils generate superoxide (5) and that this process is lacking in CGD. This important development provided a direct link between free radical chemistry and biology. At the time, most free radical chemistry was conducted by radiation biologists in test tubes, and its application to biology was purely theoretical. This new discovery was thought to prove that the production of free radical reactions in a biological process was toxic enough to kill organic structures as tough as bacteria and fungal spores. Soon these observations were extrapolated to implicate free radical reactions in a host of pathological processes involving neutrophil infiltration and tissue damage.

During the past few years, the pendulum has swung firmly back to implicating a major primary role for the granule proteins in the killing process (6), with a less direct but still facilitating and activating role for the respiratory burst through the NADPH oxidase. This review concentrates on the elucidation of these recent developments in our understanding of the relationship between the oxidase and granule enzyme activation. Because of the breadth of the subject and space limitations, references are made to authoritative reviews where available.

LIMITATIONS TO UNDERSTANDING KILLING SYSTEMS

Neutrophils are essential for resistance to bacterial and fungal infections. Severe neutropaenia invariably leads to infection by a wide range of organisms (7), most of which are not normally pathogenic, even in CGD. This, coupled with the fact that most CGD patients are able to kill most invading microbes most of the time (8), indicates that killing systems of the neutrophil are highly efficient and multilayered. Investigators once considered oxygen-dependent mechanisms essential for killing invading microbes, but such microbes can in fact be killed by other systems (9). In general, research has concentrated on determining those mechanisms involved in killing the most resistant organisms. The advent of gene-targeting technology allows researchers to determine the roles of the different antimicrobial molecules and their functional interrelationships with various microbes. Additionally, most studies have examined the killing of microbes within the phagocytic vacuole. We do not know whether neutrophils are capable of killing organisms extracellularly in vivo, nor the mechanisms involved if they are.

We have derived the bulk of our detailed information from the study of infection in CGD and the role of the oxidase in microbial killing. Because CGD patients can remain free of infection for many years (8), these methods are imprecise because they only measure some components of the lethal systems. Nonetheless, oxygen-dependent, intravacuolar killing provides a clearly defined set of processes, the examination of which has advanced knowledge of important physiological mechanisms.

THE NADPH OXIDASE

The NADPH oxidase plays a pivotal role in microbial killing because its dys-function causes CGD, characterized by a profound predisposition to bacterial and fungal infection (8, 10), and killing is compromised under anaerobic conditions (11).

Detailed reviews of the biochemistry and bioenergetics of this system have recently been undertaken (12, 13), to which I refer readers. A schematic representation of the oxidase is shown in Figure 2.

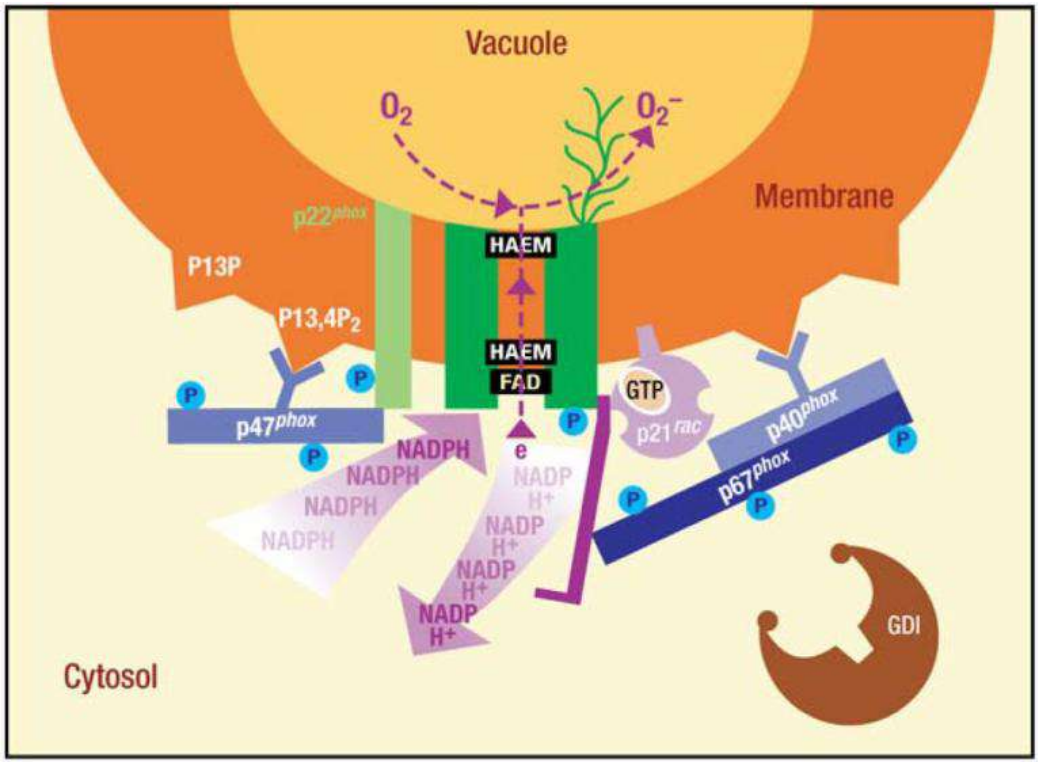

Figure 2

Schematic representation of the NADPH oxidase. Flavocytochrome b_{558} is a heterodimer of gp91^{phox}, which contains the haem- and flavin-binding sites, and p22^{phox}. Electron transport is activated by phosphorylation and translocation to the vacuolar membrane of p47^{phox} and p67^{phox}. p21^{rac}, in the GTP-bound form, is also required (12).

The Electron Transport Chain Through the Membrane

Flavocytochrome b_{558} is the core component of the NADPH oxidase. It is distributed between the plasma membrane and the membrane of the specific granules, and it is incorporated into the wall of the phagocytic vacuole, where it forms a conduit for electrons to be pumped from NADPH in the cytosol onto oxygen in the vacuole.

Flavocytochrome b_{558} is a heterodimer composed of one molecule of p22^{phox} (α-subunit, the product of the *CYBA* gene) and one molecule of gp91^{phox} (β-subunit, *CYBB* gene).

gp91^{phox}

gp91^{phox} contains the entire electron transporting machinery of the flavocytochrome b. It is composed of two major, and very different, domains.

C-Terminus: NADPH and FAD Binding The hydrophilic C-terminal (282–570) portion of gp91^{phox} contains the FAD- and NADPH-binding sites. These have distant, but recognizable homology to the large family of ferredoxin-NADP reductase (FNR) proteins, of which cytochrome P450 reductase, nitric oxide (NO) synthase, and yeast ferric reductase are members. This homology has allowed the construction of a model with the depiction of the FAD- and NADPH-binding sites.

N-Terminus: Haem Coordination The hydrophobic N-terminal half of gp91^{phox}contains six membrane-spanning α helices. Helices III and V each contain two histidine residues appropriately positioned (101:209 and 115:222) to coordinate two haem prosthetic groups perpendicular to the plane of the membrane. These histidine residues are completely conserved among all the NADPH OXIDASE (NOX) family members. Site-directed mutagenesis studies support the proposal that these histidine residues form the axial ligands to the haem groups. The predicted placing of the haem groups (one toward the inner face and one toward the outer face) is consistent with their function to transport electrons from the NADPH (via FAD) on the inside (cytosol) across the membrane to the interior of the phagocytic vacuole where molecular O_2 is reduced to form O_2^- . Biological membranes are ~25 Å thick, and thus at least two redox centers are required to span them to allow electrons to transfer at kinetically significant rates. The haem groups are nonequivalent and have different redox potentials.

The second (120–167) and third (224–257) external loops of gp91^{phox} contain the N-linked glycosylation sites (asparagines 132, 149, and 240).

p22^{phox} p22^{phox} is a 194 amino acid (~21 kDa) protein with a hydrophobic, membrane-spanning N-terminus (1-132). It provides high-affinity binding sites for the cytosolic NADPH oxidase subunits. p47^{phox} binds to a proline-rich domain (151–160) in the cytoplasmic hydrophilic C-terminus and confers stability on gp91^{phox}.

The Activating Proteins in the Cytosol

For electron transport to occur through the flavocytochrome, it must interact with a number of cytosolic proteins that translocate to the membrane of the phagocytic vacuole. This activation depends on a change in the conformation of the flavocytochrome, possibly by displacing the small helix that is predicted in the molecular model to occupy the NADPH-binding site in the inactive state (14) or through the facilitation of electron transfer between the flavin and haem.

Because of their interaction with each other, with lipids, and with phox proteins in the membranes, these cytosolic phox proteins have relatively large numbers of specific interaction domains. Targeting these molecules specifically to that region of the plasma membrane that makes up the wall of the vacuole requires specific local changes, which might include the accumulation of phosphatidylinositol phosphates (PIPs) at this site. Only a small proportion of these cytosolic proteins translocate to the membranes, and these appear to be phosphorylated, as does the flavocytochrome.

p67^{phox} p67^{phox} (NOXA2 from NOX Activator) is a 59,735-Da protein (526 amino acids) with a pI of 6.12. Protein-protein interaction domains include two SH3 domains, two proline-rich regions flanking the central SH3 domain, an N-terminal TPR (tetratricopeptide repeat), and a PB1 domain C-terminal to the central SH3 domain. The TPR domains are thought to bind rac. PB1 domains are known to interact with octicosapeptide motifs, and p67^{phox} binds to p40^{phox} through this domain. p67^{phox} attaches directly to flavocytochrome b_{558}, and at high concentration, in combination with rac or in the form of a p67$^{phox/rac}$ chimera, p67^{phox} is sufficient to induce electron transport.

p47^{phox} p47^{phox} (NOXO2 from NOX Organizer) is a basic protein (pI = 9.6) of molecular weight 44,681 Da (390 amino acids) that is heavily phosphorylated during neutrophil activation. It contains a number of well-defined motifs, including a PX domain (involved in phosphoinositide binding), two SH3 domains (involved in protein-protein interactions), and at least one proline-rich motif (the reciprocal target for SH3 domain interactions). It appears to be an adaptor molecule forming a bridge between p22^{phox} and p67^{phox}, and it also binds to cytoplasmic regions of gp91^{phox}, thereby stabilizing the attachment of p67^{phox} to flavocytochrome b_{558}.It might also directly influence the function of

flavocytochrome b_{558}. The N-terminal regions of $p40^{phox}$ and $p47^{phox}$ contain homologous stretches of 120–130 amino acids that form a structure called the phox homology, or PX domain, which binds to PIPs and directs these proteins to this activated membrane (reviewed in 15).

The two SH3 domains face each other to form a groove in which its C-terminal polybasic region fits. Investigators have suggested that this polybasic region is phosphorylated upon activation, releasing it from its auto-inhibitory role and making the groove accessible to bind the proline-rich tail in the C-terminal portion of $p22^{phox}$.

p40*phox* $p40^{phox}$ was discovered when it copurified with $p67^{phox}$, to which it is tightly bound. It is a protein of 39,039 Da (339 amino acids), strongly homologous with $p47^{phox}$, with an N-terminal PX domain, followed by an SH3 domain. Toward the C-terminus, there is an octicosapeptide repeat (also known as a PC domain) that seems to be involved in the binding of $p40^{phox}$ to $p67^{phox}$. The protein probably functions as a shuttle partner, transporting $p67^{phox}$, which does not contain a PX domain, to the membrane of the phagocytic vacuole by binding to PIPs.

p21rac After the discovery of $p47^{phox}$ and $p67^{phox}$, it became clear that they were not sufficient to reconstitute the active oxidase when combined with membranes. A third protein, a guanosine 5′-triphosphatase (GTP)-dependent factor, was shown to be rac1 or rac2 and was purified from cytosol. The causes of the separation of rac from its complex with guanine nucleotide dissociation inhibitors (GDI) in the cytosol are not known. Rac translocates to the membrane independently from $p67^{phox}$ and $p47^{phox}$. Its guanosine diphosphate (GDP) is probably exchanged for GTP on the membrane through the action of P-Rex1, a 185-kDa guanine nucleotide exchange factor (GEF) that is activated by phosphatidylinositol-3,4,5-trisphosphate and by the $\beta\gamma$ subunits of heterotrimeric G proteins.

Molecular Genetics of CGD

Defects in any one of four genes give rise to the known forms of CGD. *CYBB* (coding for $gp91^{phox}$, NOX2) is located on the X chromosome and accounts for about 65% of cases, almost exclusively in males (except in rare female carriers in whom there is extreme lyonization). The other three genes are all autosomal, with defects in *NCF1* ($p47^{phox}$ or NOXO2 protein), *NCF2* ($p67^{phox}$ or NOXA2), and *CYBA* ($p22^{phox}$), causing approximately 25%, 5%, and 5% of cases, respectively. No instances of CGD have been identified in which a lesion of $p40^{phox}$ is causal.

A small subgroup of CGD patients have what is known as “variant” CGD (16). In these cases there is partial loss of a protein or its function. Often as much as 10%, and up to 30% (H. Malech, personal communication), of normal oxidase activity can be measured.

PRODUCTS OF THE OXIDASE AND THEIR IMPLICATION IN MICROBIAL KILLING

Initiation of NADPH oxidase activity coincides with degranulation, with a lag phase of approximately 20 s (17). It occurs after closure of the vacuole and is limited to the plasma membrane comprising the vacuolar membrane (18). Thus, superoxide cannot be detected on the exterior of a phagocytosing cell (19, 20) unless engulfment is “frustrated” by an overwhelming excess of particles and vacuolar closure becomes impossible.

Because activity of the NADPH oxidase is essential for efficient microbial killing, investigators have focused attention on the products of the oxidase themselves as the lethal agents.

Oxygen radicals and their reaction products, collectively referred to as reactive oxygen species (ROS), are produced as a consequence of NADPH oxidase activity, which pumps superoxide (O_2^-) into the phagocytic vacuole. Because ROS can react with organic molecules, an enormous body of literature has developed that causally links ROS to the death of the microbe.

O_2^- and H_2O_2

The superoxide anion radical has been recognized in chemical systems for many years. Proof of its existence in biology followed the discovery of the enzymatic function of superoxide dismutase, which accelerates the dismutation of $2O_2^- \rightarrow O_2 + O_2^{2-}$ (21). Investigators (5) soon showed that neutrophils produce large amounts of O_2^-, estimated between approximately 1 (22) and 4 (6) M/l in the vacuole. The steady state concentration has been estimated to be in the μM range (22) because dismutation to H_2O_2 (2) is very rapid (23, pp. 60–61) under the prevailing conditions.

Experiments were performed that appeared to demonstrate the killing of microbes by O_2^- generated by xanthine oxidase (24, 25). It is not clear what, if any, ROS other than O_2^- and H_2O_2 (2) are produced in significant quantities in the vacuole.

$HO^\bullet$

O_2^- and H_2O_2 can combine to generate the highly reactive hydroxyl radical ($HO^\bullet$) via the Haber-Weiss reaction. This requires a metal such as iron in the Fenton reaction: $Fe^{2+} + H_2O_2 \rightarrow Fe^{3+} + HO^- + HO^\bullet$. $HO^\bullet$ has been measured in a broken cell preparation (26) and has been implicated as a microbicidal agent (27). These radicals are probably not found in intact cells (28) because lactoferrin, which is unsaturated in neutrophil granules (29, 30), inhibits the generation of $HO^\bullet$ (31) and other free radical reactions (29) by binding free copper and iron. The reaction between HOCl and O_2^- could produce $HO^\bullet$ but does not appear to do so (32).

Cobalt-based radicals could be produced by the Co in cyanocobalamin (33), but a binding protein, transcobalamin 2, present in specific granules, might be there to prevent this from occurring.

Ozone

It has recently been suggested that ozone generated by an antibody-based catalysis is involved in the killing of bacteria within neutrophils (34, 35). Doubt has been subsequently raised, however, on the specificity of the indicator used for ozone, which can apparently also detect O_2^- (36).

Myeloperoxidase-Mediated Halogenation

Myeloperoxidase (MPO) is a di-haem protein composed of two identical heterodimers. Each heterodimer is formed from the post-translational modification of a single polypeptide precursor. The two symmetric halves are linked by disulphide bonds between the two heavy chains. The covalently bound haem has a unique structure and exhibits unusual spectral properties that are responsible for its green color (37). MPO constitutes about 5% of the total neutrophil protein and is present in the cytoplasmic granules at very high concentrations. It makes up about 25% of the granule protein, and this achieves concentrations of about 100 mg/ml (1 mM) in the vacuole.

Investigators thought that this enzyme catalyzes the H_2O_2-dependent oxidation of halides that can react with and kill microbes. Experiments with the MPO-H_2O_2-halide system demonstrated that this enzyme can kill bacteria in the test tube (22, 38-41), and MPO-mediated halogenation has been accepted as an important antimicrobial mechanism for several decades.

A few patients were discovered whose neutrophils lacked MPO and who were also thought to be immunodeficient (42). Recently MPO knockout mice have also shown an undue susceptibility to bacterial and fungal infections (43-45).

Nitric Oxide

Although evidence suggests that neutrophils can induce the synthesis of nitric oxide (NO) synthase during sepsis (46), little evidence implicates the involvement of NO in microbial killing. Even in mice, in the neutrophils of which NO synthase is expressed at much higher levels than in humans, knocking out this molecule has little effect on the killing of microbes for which neutrophils are normally responsible. In contrast, these mice are profoundly susceptible to intracellular organisms such as *S. enterica* and *M. tuberculosis* (47), which classically proliferate within macrophages.

CYTOPLASMIC GRANULES AND THEIR CONTENTS

Researchers have known for almost a century that neutrophils phagocytose and kill microbes. Alexander Fleming discovered and named lysozyme, which he termed "a remarkable bacteriolytic element found in tissues and secretions," including leukocytes (48). He showed that it lysed about two thirds of the bacteria he mixed with it. Researchers subsequently showed that phagocytosis was associated with discharge of the cytoplasmic granules into the vacuole (1) (Figure 1). Attention then focused on microbicidal components within these granules. The first microbicidal granule extract was called phagocytin (49), which was later shown to be composed of an array of cationic antibacterial proteins (50).

Substantial reviews have recently covered this subject (51, 52). Different subsets of granules have been characterized by electron microscopy (53), by various staining techniques, by cell fractionation (54), and by their different functions. There are two predominant types of granules, the azurophil and the specific. They are produced in the promyelocytic and myelocytic stages, and their contents depend on the proteins that are being synthesized at that time as well as on the presence of appropriate signaling peptides (51, 52). The granules also differ in their primary functions, as discussed below.

Azurophil (or Primary) Granules

The azurophils largely contain proteins and peptides directed toward microbial killing and digestion, whereas the specific granules replenish membrane components and help to limit free radical reactions. Azurophil (or primary) granules are the first to be produced. They contain MPO and three predominant neutral proteinases: cathepsin G, elastase, and proteinase 3. Bactericidal/permeability-increasing protein (BPI) was first purified as a factor that permeabilized and killed *E. coli* (55, 56). It has lipopolysaccharide-binding and neutralizing activities (57) and appears to be attached to the granule membrane. Defensins are peptides with molecular weights of 3000–4000 Da, and each contains six disulphide-linked cysteines (58). They exhibit antibacterial activity, but this is inhibited by physiological concentrations of salt. About one third of the total lysozyme (54) is found in these granules.

These granules contain an abundant matrix composed of strongly negatively charged sulphated proteoglycans (59). This matrix strongly binds almost all the peptides and proteins other than lysozyme, which are strongly cationic. This sequestration together with the acidic pH at which the granule interior is maintained (60) keeps these enzymes in a quiescent, inactivated state.

Specific (or Secondary) Granules

Specific granules contain unsaturated (61) lactoferrin, which binds and sequesters iron and copper; transcobalamin II, which binds cyanocobalamin; about two thirds of the lysozyme (54); neutrophil gelatinase-associated lipocalin (62); and a number of membrane proteins also present in the plasma membrane, including flavocytochrome b_{558} of the NADPH oxidase (63).

Gelatinase (or Tertiary) Granules

Some granules contain gelatinase in the absence of lactoferrin, although most of the lactoferrin-containing specific granules also contain gelatinase (64). The designation of granules as "gelatinase granule" refers to granules that contain gelatinase but not lactoferrin; they may represent one end of the spectrum of a single type of granule with the same contents but in differing proportions.

Lysosomes

Lysosomes contain acid hydrolases. The activity of these enzymes appears to fractionate with the azurophil granules. They are, however, released into the phagocytic vacuole much later than the azurophil contents and therefore must be in a distinct compartment (17).

Secretory Vesicles

These endocytic vesicles contain serum albumin (65) and are probably the empty vesicular structures described previously (66). They provide a valuable reservoir of membrane components. Their reassociation with the plasma membrane replenishes that which is consumed during phagocytosis, as well as its component proteins such as complement receptor (67) and flavocytochrome b_{558}.

CONDITIONS IN THE PHAGOCYTIC VACUOLE

One must clearly understand the conditions in the phagocytic vacuole when attempting to define killing mechanisms. A heavily opsonized particle is taken up into the phagocytic vacuole within 20 s (17, 68), and killing is almost immediate (68). The apparent delay in many assays results from a low collision frequency between neutrophils and microbes, which is due to low densities of both, coupled with slow mixing (69) and suboptimal opsonization.

To determine the concentration of the vacuolar contents, one must know the volume of the space between the surface of the organism and the membrane of the phagocytic vacuole. It is certainly very small (17) (Figure 1), and possibly negligible, as has been shown in macrophages (70).

The human neutrophil has numerous granules, the contents of which are released into the vacuole and squeezed onto the surface of the organism in very high concentrations, almost like attaching a limpet mine to a target (17). Researchers have estimated that the granule protein makes up about 40% of the vacuolar volume (22), achieving protein concentrations of about 500 mg/ml (6). It was initially thought that the specific granules degranulated first, followed by the azurophils. These studies were conducted on rabbit neutrophils, and alkaline phosphatase, which we now know to be a marker for membranes, was used as the marker for the specific granules (71). In fact, both of these granule types fuse with the phagocytic vacuole with roughly similar kinetics approximately 20 s after particle uptake (17). The acid hydrolases only enter the vacuole after about 5 min, when the pH has started to fall to levels appropriate for the optimal activity of these enzymes.

Investigators had initially reported that the pH in the vacuole fell to about 6 after 3 min and to 4 after 6 min (72). However, subsequent studies have shown that the NADPH oxidase elevates the pH to about 7.8–8.0 in the first 3 min after phagocytosis, after which it gradually falls to about 7.0 after 10–15 min

(68, 73, 74). The NADPH oxidase consumes 0.2 fmols of O_2 when a particle the size of a bacterium is engulfed. This equates to massive amounts of O_2^-, on the order of 1–4 Mols/l, that are injected into the vacuole.

NEUTRAL PROTEASES ARE ESSENTIAL FOR BACTERIAL AND FUNGAL KILLING

Although the proposal that ROS are toxic to ingested microbes was attractive, it was never adequately tested under the conditions pertaining to the phagocytic vacuole. The opportunity was provided by the development of gene targeting. This technique allowed the production of a mouse model that lacks the major neutrophil proteases: neutrophil elastase (NE) (6, 75), cathepsin G (6), or both enzymes (6, 76, 77) (Figure 3).

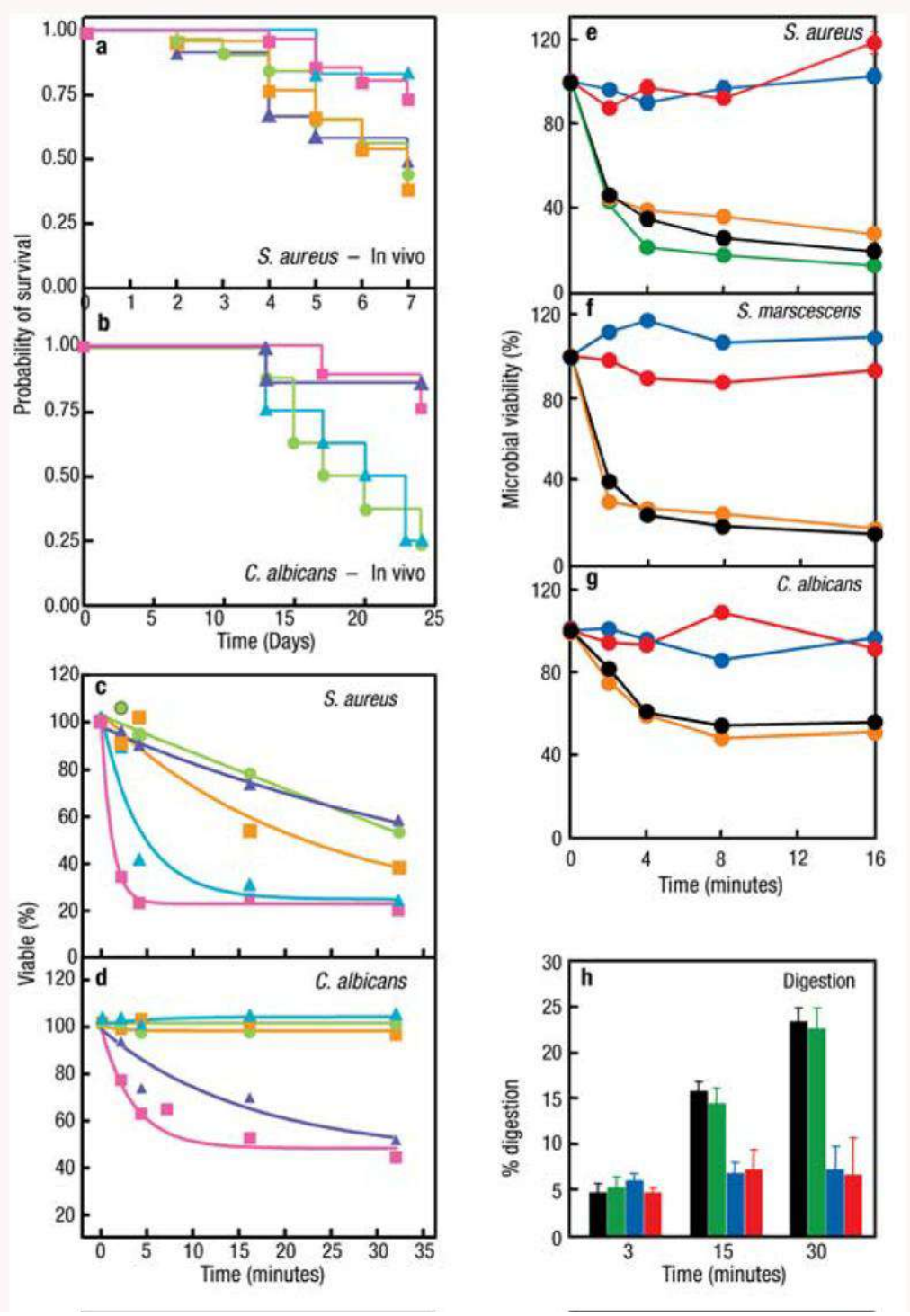

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Figure 3

The neutral proteases elastase and cathepsin G as well as K^+ flux are required for microbial killing and digestion by neutrophils. Cathepsin G, neutrophil elastase (NE), and $p47^{phox}$ (CGD) knockout mice are susceptible to *S. aureus* (*a*) and *C. albicans* (*b*) in vivo, and their neutrophils kill these organisms poorly in the test tube (*c*) and (*d*) (adapted from 6). Inhibition of the BK_{Ca} K^+ channel with specific inhibitors

paxilline (PAX) and iberiotoxin (IBTX) prevents killing of *S. aureus* (*e*), *S. marscescens* (*f*), and *C. albicans* (*g*) by neutrophils, whereas the opener NS1619 and nonspecific inhibitor 4-aminopyridine were without effect. The BK_{Ca} K^+ channel blockers also inhibited digestion of radiolabeled, killed *S. aureus* (*h*) (adapted from 74). Neither the loss of the proteases nor blockage of the BK_{Ca} channel affected phagocytosis, oxidase activity, or iodination.

NE-deficient mice were excessively susceptible to infection with Gram-negative (*K. pneumoniae* and *E. coli*) (75) but not Gram-positive (*S. aureus*) bacteria. NE was also necessary for protection against *C. albicans* (6). Both enzymes were required to kill *A. fumigatus*. The loss of cathepsin G alone was found by others (77) to be without effect on the killing of various of bacteria. The loss of both NE and cathepsin G conferred as profound a defect of bacterial killing as was observed with the CGD mouse model (6).

In these studies on protease-deficient mice, microbial killing was abolished despite a completely normal respiratory burst and normal levels of iodination. This established that ROS and metabolites of the action of MPO generated in the vacuole are not sufficient to kill these bacteria and fungi.

Thus, it was clear that the combination of NADPH oxidase activity and neutral protease enzymes are require for microbial killing to take place. This raises the question of the connection between these two processes.

THE RELATIONSHIP BETWEEN THE NADPH OXIDASE AND KILLING BY GRANULE CONTENTS

Activity of the NADPH Oxidase Alters the Appearance of the Contents of the Phagocytic Vacuole

The activity of the NADPH oxidase alters the appearance of the contents of phagocytic vacuoles in electron micrographs of neutrophils examined soon after they had phagocytosed bacteria (6). In normal cells, the contents of the vacuole had a diffuse, almost ground-glass appearance, with very few intact aggregates of granule contents. By contrast, in CGD cells there was little dispersion, with obvious clumping of the granular contents. This abnormal appearance was also apparent in vacuoles from a patient with variant CGD with 10% of the normal oxidase activity.

These obvious structural differences, coupled with the massive amounts of O_2^- injected into the vacuole and the fact that 10% of this amount of O_2^- in variant CGD (amounting to some 100–400 mMols/l) was insufficient, suggested to researchers that the oxidase was exerting some physico-chemical influence on the granule contents rather than simply producing ROS or substrate for MPO. Segal and colleagues (6) therefore turned their attention to electron transport across the membrane and its consequences for the movement of other ions.

Charge Compensation Across the Vacuolar Wall

The oxidase is electrogenic, transferring electrons, unaccompanied by protons, across the vacuolar membrane (78-81). The vacuolar volume is about 0.2 μm^3, with a membrane surface area of about 1.65 μm^2.In each vacuole, 0.8–2.0 fmols of O_2^- are produced, and thus about 5–10 × 10^8 electrons pass across each μ^2 of membrane. The charge on one electron is 1.6 × 10^{-19} coulombs, so 3–7 × 10^8 charges in one square micron would produce from 4.6 × 10^{-3} to 1.2 × 10^{-2} coulombs/cm^2. With the capacitance of the membrane at approximately 1 microfarad/cm^2 (82), this charge would depolarize the

membrane potential by 4,600–11,700 volts! Depolarization of the membrane to +190 mV shuts down NADPH oxidase activity completely (83). Thus, for significant oxidase activity to occur, the charge must be compensated.

The changes in the vacuolar pH, which is elevated from that of the extracellular medium to 7.8–8.0 (68) despite the release into the vacuole of 500 mg/ml of acidic granule protein contents (6), hold the key to understanding the nature of the compensating ions (Figure 4). These granule contents are maintained at pH 5.0 in the granule by a proton pump (60) and have strong buffering powers. About 400 μmol potassium hydroxide is required per gram of granule protein to elevate the pH from 5.0 to 8.0 (6).

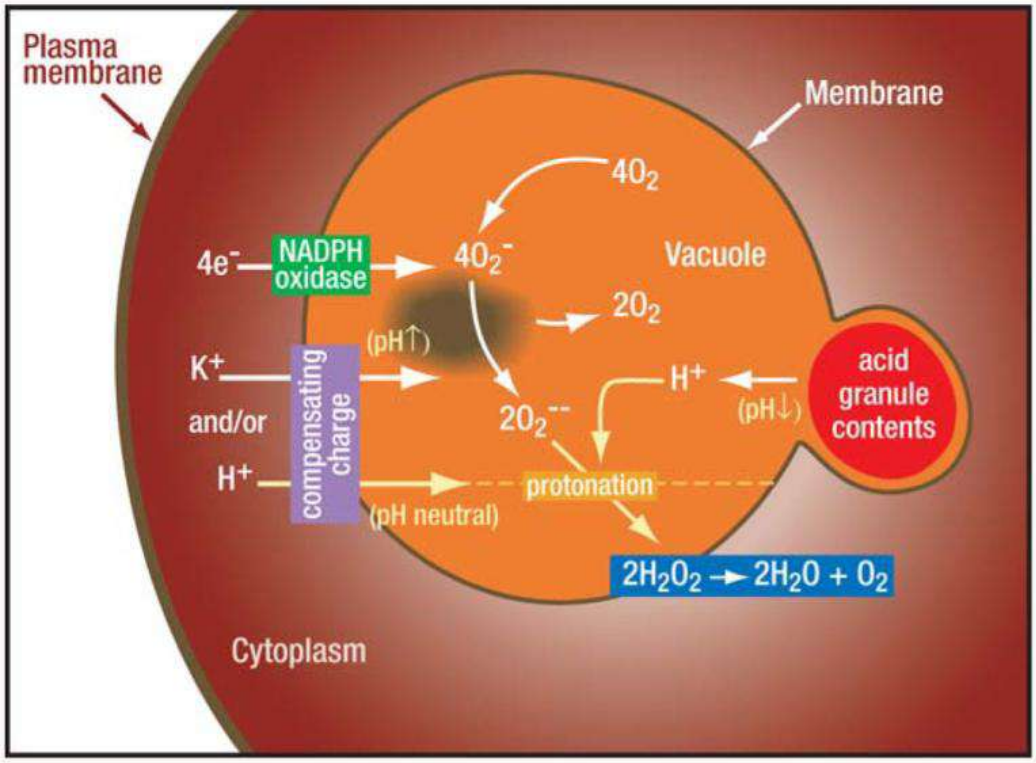

Figure 4

Activity of the NADPH oxidase depolarizes the membrane. The nature of the compensating charge governs the changes in vacuolar pH and tonicity. Electrons are transported across the vacuolar membrane to form O_2^-, which dismutates to O_2^{2-}. O_2^- and O_2^{2-} become protonated to form HO_2 and H_2O_2, thereby consuming protons and elevating the pH in the vacuole despite the entry of acidic granule contents. This process can only occur if part of the charge is compensated by ions other than protons, which in part occurs through the passage of K^+ ions (6, 74).

The vacuole becomes alkaline despite the entry of acidic granule contents, indicating that the O_2^- and O_2^{2-} are consuming protons in the vacuole. This would not happen if each electron passing across the membrane was accompanied by a proton, demonstrating that compensating charges cannot be solely in the form of H^+ from the cytoplasm.

The major cation in the cytoplasm is K^+, which accumulates in the vacuole at concentrations of up to about 600 mM as a consequence of oxidase activity (6). Transport of K^+ ions is markedly diminished when the pH rises above 8.0, indicating that the K^+ channel provides an important self-regulating mechanism for elevating the vacuolar pH while also ensuring that it does not go too high.

K^+ flux only accounts for about 6% of the compensating charge (6). The putative proton channel discussed below does not appear to compensate for all the rest of the charge because its inhibition with Zn^{2+} and Cd^{2+} fails to block the NADPH oxidase (74). Therefore, some other major ion flux must also be involved. As is described below, this is accomplished by the flux of chloride ions through a glycine-gated, strychnine-sensitive channel.

The K^+ Enters the Phagocytic Vacuole Through BK_{Ca} Channels

K^+ enters the vacuole through the large conductance Ca^{2+}-activated K^+ channel (74). Iberiotoxin (IBTX) and paxilline (PAX), both highly selective and potent inhibitors of this channel (84, 85), prevent the alkalinization of the vacuole, confirming the importance of the influx of K^+ into the vacuole on alkalinization of this compartment. The IC_{50} values for this effect were in the region of 10 nM for IBTX and PAX, consistent with their IC_{50} for channel block. In addition, the BK_{Ca}channel opener, NS1619 (86), significantly augmented the rise in pH to supranormal levels. A variety of blockers and openers of other K^+ channels were without effect.

$^{86}Rb^+$ release from activated neutrophils after stimulation with phorbol myristate acetate (PMA) was also induced by NS1619 and even further enhanced by the combination of this opener and PMA. PMA-induced and NS1619-induced efflux were both completely abrogated by IBTX and PAX. The same was found to apply to eosinophils.

BK_{Ca} channels are classically opened by the combination of membrane depolarization and elevated cytosolic Ca^{2+} (87). The same holds true for this channel in neutrophils and eosinophils. Neither depolarizing the membrane nor elevating the cytosolic Ca^{2+} was sufficient to fully open the K^+ channel, whereas the combination of the two caused as much channel opening as did stimulation with PMA. Although PMA stimulation is well known to depolarize the neutrophil plasma membrane (88), it is generally thought not to elevate cytosolic Ca^{2+}. One mechanism by which this might occur is through a drop in pH just beneath the plasma membrane as a consequence of charge separation induced by the oxidase. Corresponding elevations in Ca^{2+} and falls in pH were seen just beneath the plasma membrane in activated cells (74).

Charge Compensation by Protons

Protons remain in the cytoplasm as a result of charge separation, which occurs when the electrons are transported from NADPH across the wall of the phagocytic vacuole. Additional protons are produced in the cytosol by the HMP shunt, which generates NADPH (89), as well as during the production of energy by glycolysis. This proton generation by an active oxidase, estimated to be about 150 mMols/l (90), causes an initial slight fall in cytosolic pH that rapidly returns to normal.

Three mechanisms appear to be associated with the extrusion of these protons, which are extruded in roughly equimolar quantities with the O_2^- that is generated (91, 92). The predominant one is a Na^+/H^+ antiport (93, 94). Its inhibition by the removal of extracellular Na^+ or blockage with amiloride causes acidification of the cytosol upon stimulation of the cells. In addition, both Zn^{2+} and Cd^{2+}-sensitive proton channels (95, 96) and vacuolar (V)-type H^+ pumps, inhibited by bafilomycins (90), are also present.

Investigators generally agree that the charge induced by electron translocation (I_e) through the NADPH oxidase is compensated by proton efflux (78, 83, 97), although the identity of the proposed channel is currently highly contentious. One school of thought holds that protons pass through voltage-gated proton channels that are distinct from any NADPH oxidase component (98). The opposing view is that they pass through flavocytochrome b_{558} of the oxidase, gp91^{phox}, itself (99-101).

One of the hallmarks of the assumption that I_e is largely compensated by proton fluxes is that both Zn^{2+} and Cd^{2+}, known proton channel blockers (98, 102, 103), were also thought to inhibit O_2^- production (83, 97). The discrepancy between the low μM concentrations of these cations that block proton channels and the mM concentrations needed to inhibit cytochrome c reduction was recently explained by the voltage dependence of I_e. Zn^{2+} and Cd^{2+} shift the threshold voltage for activating voltage-gated proton channels into the steeply voltage-dependent region of I_e, thereby attenuating O_2^- production (83).

However, Zn^{2+} and Cd^{22+} inhibition of voltage-gated proton channels do not inhibit the NADPH oxidase: They have no effect on PMA-induced oxygen consumption, the true measure of oxidase activity. Zn^{2+} and Cd^{2+} interfere with the reduction of cytochrome c by accelerating the dismutation of O^{2-} to H_2O_2 (74). In a system in which xanthine-xanthine oxidase generated O_2^- , 3 mM concentrations of these elements induced the dismutation of O_2^- to H_2O_2 at a rate indistinguishable from that catalyzed by superoxide dismutase (1 μg/ml). Zn^{2+}, at concentrations three orders of magnitude greater than those causing almost complete blockage to proton channels, was also without effect on the currents measured in electrophysiological studies performed on neutrophils, eosinophils, or on PMA-induced ^{86}Rb efflux from these cells (74). This does not mean that H^+ movement through proton channels does not compensate some of the charge, but only that the justification hitherto provided is incorrect.

Charge Compensation by Cl^-

We showed that K^+ accounts for only about 5%–10% of the compensation of the total electron transport, and, contrary to the description in a recent critique of our work (104), we never claimed that it was the only compensating ion. More recently, we (J. Ahluwalia, G. Gabella, S. Pope, A. Warley, A. Segal, unpublished) have discovered that that Cl^-, passing through strychnine-sensitive, glycine-activated homomeric channels, compensates about 90% of the charge. These channels were characterized by patch clamping whole cells and isolated phagocytic vacuoles, and by Western blotting. The removal of Cl^- or the blockage of this channel abolished both the respiratory burst and microbial killing. High concentrations of Cl^- and glycine required for the optimal function of these channels are contained within the cytoplasmic granules, which empty into the vacuole. NADPH oxidase activity was lost when the granules were removed and regained when Cl^- was reintroduced into the vacuole. Lysozyme, cathepsin G, and elastase were inactivated by hypertonic Cl^-, the removal of which would be important for their function. These Cl^- fluxes provide a direct couple between the extent of degranulation and oxidase activity required to activate the released enzymes.

The Movement of K^+ into the Vacuole Activates NE and Cathepsin G

The contents of the cytoplasmic azurophil granules are not freely in solution. They are almost exclusively highly cationic proteins that are strongly bound to the highly negatively charged proteoglycans heparin and chondroitin sulphate (59), in which state they are inactive. They are activated in the vacuole both by the elevation in pH described above and by the hypertonic K^+. The latter breaks the charged interaction between the enzymes and the matrix, releasing them in a soluble

form (6) (Figure 5). For these hypertonic conditions to develop, water must be prevented from entering the vacuole in response to the osmotic attraction of the salts. This is achieved by encasing the vacuole in a meshwork of cytoskeletal proteins, including paxillin and vinculin.

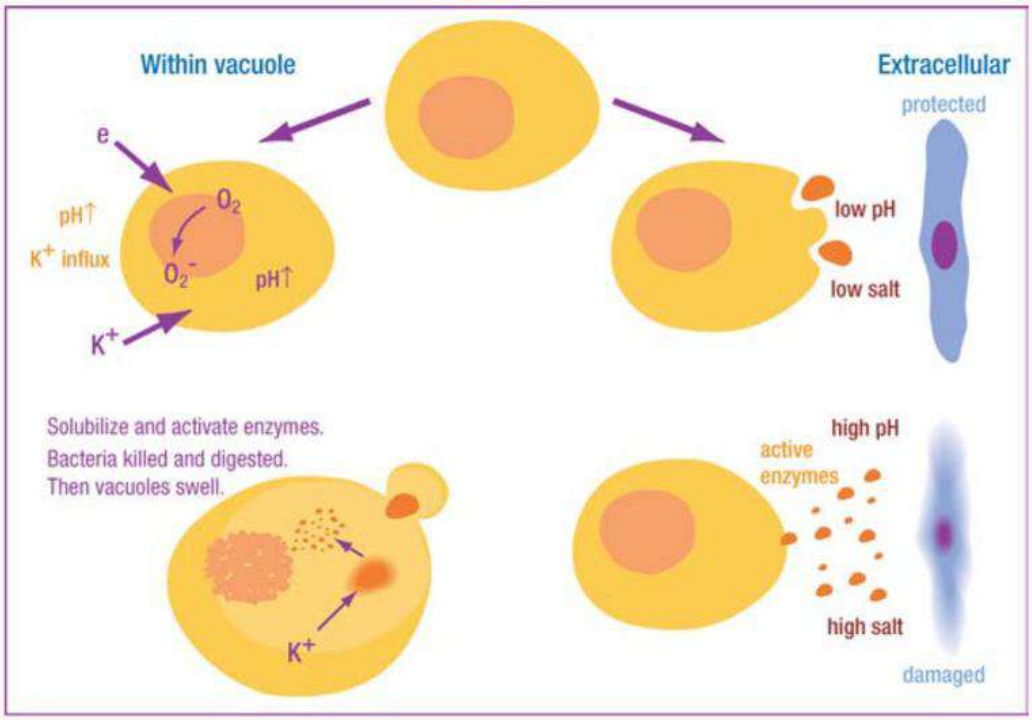

Figure 5

Schematic representation of interaction between NADPH oxidase and granule proteases. Electron transport through flavocytochrome b_{558} consumes protons in the vacuole, elevating pH to a level optimal for neutral proteases, which are also activated by K^+ driven into the vacuole to compensate the charge across the membrane. The hypertonic K^+ solubilizes the cationic granule proteases and peptides by displacing them from the anionic sulphated proteoglycan granule matrix. The requirement for an alkaline, hypertonic environment restricts the toxicity of these proteins to the vacuolar compartment, thereby limiting damage to normal tissues.

The importance of the accumulation of K^+ in the vacuole was shown when this was diminished either with the K^+ ionophore valinomycin (6), or by blocking the BK_{Ca} channel with the specific inhibitors IBTX or PAX (74). In both cases, microbial killing and digestion was almost completely prevented (Figure 3) despite the generation of normal quantities of ROS and normal levels of iodination.

Why Was the Importance of Granule Contents in the Killing Process so Overshadowed by ROS and MPO-Mediated Halogenation?

The theory that microbes are killed within the phagocytic vacuole by ROS had fertile ground on which to develop. The lack of production of O_2^- and H_2O_2 in anaerobic cells and in CGD with impaired killing under these conditions supported this theory (3, 11), as did the concept of toxicity engendered in the name “reactive oxygen species.” Although experiments were performed in support of these ideas,

the conditions under which they were performed in no way reflected the conditions pertaining in the vacuole. They were often done at the wrong pH, and never in the presence of the enormously high concentrations of protein that occur naturally.

O_2^-

Initial studies claimed that killing occurred by O_2^- generated by the reaction of xanthine with xanthine oxidase, but in fact in those experiments the microbes were killed in the absence of the substrate xanthine, and killing was not inhibited by superoxide dismutase (24). In a similar experiment, no killing of bacteria by O_2^- was observed after 15 min (25).

H_2O_2

H_2O_2, which is used as a topical antiseptic (105), is produced by neutrophils and has been thought of as capable of killing microbes within them (106, 107). Supportive evidence was provided by the finding that catalase-negative organisms rarely infect patients with CGD (108). The explanation was that these bacteria generated enough H_2O_2 to catalyze their own MPO-mediated halogenation within the vacuole of the neutrophil (109, 110). In vitro mutagenesis was used to generate strains of *S. aureus* containing varying levels of catalase, and their virulence in mice was found to be inversely proportional to their catalase content (111). Recently, however, doubts have been cast on this theory. Catalase-deficient *A. nidulans* (112) and *S. aureus* (113) are as virulent as the catalase-positive varieties in mouse models of CGD, and the bacteria could never come near to producing the relatively enormous quantities of H_2O_2 generated even by cells from patients with variant CGD.

When glucose oxidase was administered to CGD cells in liposomes, it appeared to correct the killing defect (114, 115). However, no explanation was provided as to how glucose would gain access to the vacuole in adequate amounts to generate sufficient quantities of H_2O_2, and the killing of bacteria in the extracellular medium was not excluded.

MPO

Experiments that demonstrated that the MPO-H_2O_2-halide system can kill bacteria in the test tube (22, 38-41) were conducted under nonphysiological conditions, with relatively low concentrations of MPO (50 μg/ml rather than 100 mgs/ml), at low pH (5.0 rather than 7.8–8.0), and, most important of all, in the absence of the high levels of proteins (approximately 500 mgs/ml) found in the vacuole. When bacteria were exposed to 100 mM H_2O_2 or 1 mM HOCl in the presence of 25 mg/ml granule proteins (technically much more manageable than the experimentally determined 500 mg/ml), killing was almost abolished (116).

Neutrophils clearly iodinate and chlorinate proteins when bacteria are phagocytosed, and this halogenation is dependent on an active NADPH oxidase and MPO (118). However, it is largely the proteins of the neutrophil granule rather than the microbial proteins that are iodinated (116, 119) and chlorinated (120), a highly inefficient system if its primary purpose is to halogenate bacterial proteins. Further indications as to the inefficiency of the proposed system come from the amounts of H_2O_2 generated. It seems highly unlikely that substrate would need to be provided at molar concentrations and that the 100 mM H_2O_2 produced by patients with variant CGD would be insufficient when it is effective at 50 μM in the test tube (38).

A few patients were discovered whose neutrophils lacked MPO who were also thought to be immunodeficient (42), and an MPO knockout mouse was shown to be susceptible to yeast but not bacterial infection (45). However, the advent of automated differential leukocyte counting machines, in

which the identification of neutrophils depended on a peroxidase stain, revealed that about 1 in 2000 of the general population are MPO-deficient without any undue predisposition to infection (121). The neutrophils of birds also lack MPO (122).

One possible function of MPO is to protect the digestive enzymes from oxidative denaturation (123) by removing H_2O_2 from the phagocytic vacuole. MPO has catalase activity (124), but this only functions efficiently if the compound II that accumulates is reduced back to the native enzyme. This reduction can be achieved by the high concentrations of O_2^- in the vacuole with which MPO forms an adduct to produce compound III (125). The impaired microbial killing observed in the MPO knockout mouse (126) could result from oxidative inactivation of antimicrobial proteins by the H_2O_2 that accumulates under these conditions (106).

MPO may also have dual functions, one as a catalase under the conditions pertaining in the vacuole, but another in a microbicidal capacity outside the cell where enzyme and substrate is much more dilute, and the pH, which is generally low at sites of infection and inflammation, is more conducive to halogenation reactions.

CONCLUDING REMARKS AND PERSPECTIVES

The complexity of the NADPH oxidase and its associated ion fluxes might seem excessive for the apparently simple purpose of activating enzymes within the phagosome. These enzymes, however, have the potential to be highly destructive to normal tissues, and yet organs housing the most exuberant inflammation and neutrophil infiltration can undergo resolution and return completely to normal a week or two later. Some of the neutrophil are removed by apoptosis, but many also necrose with the resultant release of their granules. The requirement of the combination of hypertonicity and alkalinity, neither of which occurs naturally in inflammatory foci, for the activation of these enzymes severely limits the toxicity of granules released into the tissues (Figure 5).

The demonstration that ROS and MPO-mediated halogenation are not the primary killing systems they were long believed to be has reopened many questions relating to mechanisms of innate immunity in the neutrophil. The roles of the different granule constituents in the killing and digestion of specific organisms is of interest, as are the consequences of the interaction of ROS with these granule contents on their biophysical, biochemical, and hence antimicrobial properties.

A number of problems still need to be resolved to clarify the mechanisms involved in charge compensation across the vacuolar membrane. These include the relationship between the channels conducting these charges and electron transport through flavocytochrome b_{558} and the mechanisms responsible for activating, regulating, and integrating the fluxes of these different ions.

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PHYSIOLOGICAL REVIEWS

VOL. XIV JULY, 1934 No. 3

THE INFLUENCE OF NUTRITION UPON RESISTANCE TO INFECTION

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The possibility that diet may have some influence upon the incidence, course, and final outcome of infection, is a comparatively recent idea. Since 1900 the idea has gained ground, and quite a body of work has appeared in the literature. The task of reviewing it is not easy for several reasons: in many cases the results are contradictory, in others they may be difficult of interpretation because of many variables. At best the literature is a scattered one. In considering the actual infection, the author has confined himself to infections of bacterial origin, and has not included, for lack of space, much excellent and suggestive work on infections of protozoan and metazoan origin.

In general one may say that the work in this field is in its infancy, but that there is much suggestive work that merits further study.

Vitamin B complex. Petragnani (1921) claimed that pigeons, fed on polished rice, lose their immunity, both natural and acquired, to anthrax, even before symptoms of polyneuritis develop. Corda (1923) believes that this loss of immunity may not be due to deficiency of vitamin B, but may in part be ascribed to underfeeding. Healthy adult pigeons, starved four days, or fed only 10 grams fresh asparagus tips for four days, die within two days after receiving injections of anthrax cultures—i.e., as promptly as do pigeons with polyneuritis. No attention was given to the temperature of the animals, although Pasteur had clearly shown that chilling abolishes the natural resistance of the chicken to anthrax. G. M. Finlay (1923) was able to show that normal animals, whose body temperature is lowered by pyramidon, or in the course of vitamin B deficiency, invariably die if inoculated with pneumococcus, B. coli, or B. enteritidis; whereas they nearly always survive these infec-

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TITLE: **A Survey of the Experience and Impact of Acute Upper Respiratory Tract Infections on People in Six Countries in the 2011/2012 Common Cold and Flu Season**

AUTHORS: *John David Hull, Ian Paul Barton, Jennifer Torgersen, Christine Marie McNeil*

KEYWORDS: Common Cold; Upper Respiratory Tract Infections; Common Cold Survey

JOURNAL NAME: **Open Journal of Respiratory Diseases**, Vol.3 No.4, November 22, 2013

ABSTRACT: Introduction: Acute Upper Respiratory Tract Infections (URTIs) are the most common infectious diseases of humankind. While usually mild and self-limiting, they are characterized by a series of simultaneously occurring symptoms/ signs that are sufficiently disruptive to sufferers' normal activities in which medication is frequently sought. While the literature has many examples of epidemiological studies on these infections, there are few reports on patient experience and impact. This study was designed to investigate these aspects of Common Cold/Flu across six countries. Methods: A minimum of 500 adults aged 18 and older were recruited in each of six countries (Brazil, China, Germany, India, Russia, and the US) using customary survey research sampling techniques. Single 30-minute (online) or 40-minute door-to-door quantitative questionnaires with c. 50 questions were completed with each participant by the global research firm Ipsos. Main Findings: Across countries, incidence and seasonality of infections reported to this study were consistent with published data. There appears to be a need for patient education on the causes and transmission routes of respiratory infections. Getting good quality sleep and being able to continue with daily activities as an infection resolves are significant drivers to therapy. The most common non-prescription therapies reported were multi-ingredient products in line with the simultaneously occurring multi-symptom nature of the condition(s). Conclusions: This study indicated that acute URTIs exert a significant deleterious effect on sufferers. Public health education, possibly best undertaken by Pharmacists has the potential to impact the extent of virus transmission by ensuring that people know the true cause of the infection, how it is transmitted and how best to combat this. The several simultaneously occurring symptoms encourage sufferers to seek multi-ingredient remedies to allow them to continue with normal activities as their infection resolves naturally.

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VOLUME 42, NO. 1 JANUARY, 1942

THE ADMINISTRATION OF VITAMIN C IN A LARGE INSTITUTION AND ITS EFFECT ON GENERAL HEALTH AND RESISTANCE TO INFECTION

BY A. J. GLAZEBROOK, M.R.C.S., L.R.C.P.
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(With 3 Figures in the Text)

CONTENTS

INTRODUCTION

IN any institution, where large numbers of people are supplied with food from central kitchens, the diet usually contains only small amounts of vitamin C. Destruction of this vitamin takes place during overcooking and the reheating of the food while it is awaiting distribution. Fresh fruit and vegetables are rarely supplied.

Crandon, Lund & Dill (1940) concluded that the maximal utilization of vitamin C lies between 30 and 45 mg. daily. Their figures were derived from a study of experimental human scurvy. The 'minimal-optimum' intake of vitamin C for adults has been computed at 25 mg. a day per 10 stones of body weight, and this results in an excretion of 13–15 mg. a day (Abbasy, Harris, Ray & Marrack, 1935; Harris & Abbasy, 1937). The 'minimal-optimum' intake is based on the amount found necessary to prevent a tendency to increased capillary fragility (Gothlin, 1937). Fox (1941) reviewed the results of the experiments of Fox, Dangerfield, Gottlich & Jokl (1940), Crandon *et al.* (1940) and Kellie & Zilva (1939), and concluded that remarkably good health can be maintained on 15 mg. of vitamin C daily, but he remarked on the precarious nature of such meagre supplies.

Certainly large numbers of people live on a diet containing less than the 'minimal-optimum' intake, without apparent ill effect. Investigations by

Orr (1936) and by Crawford & Broadley (1938) indicate that the diet of one-half to three-quarters of the population of Great Britain contains inadequate quantities of vitamin C, the lower figure being obtained by adopting 'minimum' (British Medical Association) standards, and the higher figure by adopting 'minimal-optimum' (League of Nations) standards.

There are, of course, wide variations in the extent to which individuals will tolerate low vitamin C diets. Jennings & Glazebrook (1938) described a man who had taken a scorbutic diet for 40 years before he showed ill effects. On the other hand, children have developed scurvy while receiving generous supplements of vitamin C, such as orange juice, and the condition is cured by giving ascorbic acid parenterally, or in large amounts by mouth (Hess, 1923; Hagmann, 1937; Parsons, 1938).

The requirements of the body for vitamin C vary with several factors. Children require a larger amount per kg. of body weight than do adults (Abbasy *et al.* 1935; Smith, 1938), and it is probable that adolescents also require a greater intake.

The body's requirements are increased if the metabolism is increased (Parsons, 1938). Thus, hard exercise and exposure to cold may precipitate scurvy, and at one time scurvy was considered to be due to damp and exposure. Crandon *et al.* (1940) found an abnormally high level of blood lactate after muscular exercise in their case of experimentally induced human scurvy. The subject was capable of a maximum effort corresponding to that of a man 80 years old. Stewart, Learmonth & Pollock (1941) suggest that ascorbic acid secures a more adequate supply of oxygen to the tissues.

Certain intestinal conditions, by permitting the growth of vitaminolytic bacteria (Kendall & Chinn, 1938), may markedly increase requirements owing to the great destruction of the vitamin and consequent failure of absorption.

Many infective states increase the body's requirements, and this has been shown in tuberculosis by Hasselbach (1936*a*, *b*), Heise & Martin (1936) and by Abbasy, Harris & Ellman (1937); in rheumatoid arthritis by Abbasy, Harris and Ellman (1937) and by Rinehart, Greenberg & Baker (1936); in osteomyelitis by Abbasy, Harris & Hill (1937); in juvenile rheumatism by Abbasy, Hill & Harris (1936). It has been recorded in other infections by Harde, Rothstein & Ratish (1935).

Abbasy & Harris (1937) found a correlation between the erythrocyte sedimentation rate and the excretion of vitamin C in cases of tuberculosis and rheumatoid arthritis. They concluded that the excretion of vitamin C varied inversely with the severity of the condition, probably because of increased utilization in the body. The Groth-Petersons (1939) found that tuberculous patients require a greater intake of ascorbic acid to maintain a normal serum level than do healthy people.

Rinehart, Greenberg, Olney & Choy (1938) found a low level of ascorbic acid in the blood of cases of rheumatism, not only in the acute phase, but also in convalescence and in very low-grade infections.

This increased destruction of vitamin C in febrile illnesses may be incidental to the disordered metabolism, and serve no useful purpose. It seems clear, however, that there is an increased liability to infection in both man and animals in cases of frank scurvy (Hess, 1920; Hamburger & Goldschmidt, 1922-3; Werkman, Nelson & Fulmer, 1924; Grant, 1926; Schmidt-Weyland & Koltzsch, 1928; Grant, 1930; Bloch, 1931; Mackay, 1934; Robertson, 1934).

In cases of so-called 'latent scurvy' the evidence is equivocal. Hess (1917 first suggested that this condition occurs and is analogous to latent tetany. It is thought that this state is a cause of ill-health and may lower resistance to infection (Harris, 1937; Bourne, 1938; Szent-Gyorgyi, 1938). Vitamin C is said to control outbreaks of pneumonia (Funck, 1931), and a deficiency of it to play a part in the production of both acute juvenile rheumatism and rheumatoid arthritis (Rinehart & Mettier, 1934; Rinehart, 1935). Vogl (1937) claimed to have used it successfully in the prophylaxis of post-operative pneumonia. On the other hand, Fox *et al.* (1940) administered vitamin C over a period of 7 months to adult negroes, previously subsisting on a low intake, and found no difference in illness as compared with controls.

The evidence that vitamin C exerts a beneficial effect in cases of actual illness is not clear. Fresh fruits and their juices, particularly lemons and black currants, have long been common household remedies for simple acute infections. Low levels of vitamin C have been found in many illnesses, so low in some instances that the vitamin has been thought to have some specific aetiological significance. Hopes that saturation with the vitamin would cure such diseases have not been realized. While full tissue saturation is probably unnecessary, it would seem desirable to increase the intake of vitamin C during illness.

Otani (1936) and Ormerod & Unkauf (1937) considered that vitamin C improved cases of whooping cough. Gairdner (1938) in a controlled experiment found that the duration of illness in a group receiving vitamin C was shorter than in controls. The difference in the two groups was not a significant one, and he considered that the alleged benefits of vitamin C in whooping cough were unproven.

Beneficial results have been claimed in diphtheria (Bamberger & Wendt, 1935; Bamberger & Zell, 1936; Dieckhoff & Schuler, 1938; Szirmai, 1940). Zilva (1938) found that vitamin C saturation made no difference to the fate of guinea-pigs injected with diphtheria toxin.

An acceleration of healing, or a general improvement, in cases of tuberculosis treated with vitamin C has been claimed by several workers (Radford, de Savitsch & Sweeney, 1937; Albrecht, 1938; Bakhsh & Rabbani, 1939; Warns, 1938; Birkhaug, 1939). Some of these observations were based on controlled experiments. Hurford (1938), on the other hand, saw no significant change after saturation, except in the blood picture of anaemic cases. Erwin, Wright & Doherty (1940) state quite definitely that vitamin C is of no value in the treatment of tuberculosis. This conclusion was arrived at as a result of

their observations upon a series of chronic, or acute broncho-pneumonic, cases, 'unlikely to improve on any known form of treatment'. With such unpromising material, disappointing results would seem to be inevitable.

There is evidence that it is of value in pneumonia, particularly in hastening convalescence, and the claims made do not appear to have been contradicted (Gander & Niederberger, 1936; Vogl, 1937; Bonnholtzer, 1937; Hochwald, 1937; Gunzel & Kroehnert, 1937; Sennewald, 1938; Szirmai, 1940). Szirmai (1940) noted that while tissue saturation is necessary to obtain maximal benefit in pneumonia, cases of typhoid fever and diphtheria were improved by daily supplements of vitamin C without producing saturation.

Estimations of deficiency

Of the various methods of estimating a deficiency of vitamin C in the body, that described by Harris, Abbasy & Yudkin (1936) is the most popular. It is recognized that the excretion of vitamin C in the urine is dependent on the reserve in the body as well as on the amount ingested during the previous few days. Accordingly, a test dose (300–600 mg.) of ascorbic acid is given and the amount excreted in the urine during the following 24 hr. is measured. The procedure is repeated for several days until large amounts of ascorbic acid are excreted. It is recognized that although the amount excreted in the urine of normal people depends on the previous amounts in the diet, this amount cannot be used to measure the degree of saturation of the tissues. Abbasy *et al.* (1935) have found that a daily intake of 90 mg. will result in an excretion of 50 mg. in the urine, but an intake of 15 mg. will result in an excretion of 15 mg. Accordingly, it is considered that any deficiency of vitamin C is best measured in terms of saturation of the tissues (Hess & Benjamin, 1934; Johnson & Zilva, 1934; Harris, Ray & Ward, 1933; Harris & Ray, 1935; Pemberton, 1940). Following the same principle, estimations of vitamin C in the blood have been made and an ascorbic acid tolerance curve devised, following an intravenous injection of 1000 mg. (Farmer & Abt, 1935; Mirsky, Swadesh & Soskin, 1935; Wright, Lilienfield & Maclenathen, 1937; Portnoy & Wilkinson, 1938).

In a large training school under our observation there were some 1500 youths aged 15–20 years. For the most part they were drawn from the lower wage-earning classes, and a large proportion came from Scotland and the North Midlands, where economic conditions are probably below the average for the country. It is a reasonable assumption that the previous dietary of the recruits had been somewhat deficient in vitamin C judged by the standards already quoted.

The diet of the institution allowed over 4000 cal. per student per day. The food distribution was badly managed. Electric ovens were used to reheat the food, and to keep it hot whilst awaiting distribution. Often 8 hr. elapsed between the time the food was cooked and its arrival on the dining tables. The minimum time that heat was applied to the food, including the original cooking and the subsequent reheating, was 2 hr.

The daily ration of potatoes was 12 oz. The vitamin C content of potatoes varies, but this quantity in the raw state should contain approximately 50 mg. A full ration of potatoes, as served on the dining tables, after cooking and reheating, was found to contain, on the average, about 4 mg.

The other vegetables suffered an equal loss, with the exception of turnips, portions of which contained up to 6 mg. The milk was pasteurized, and half a pint of it contained about 1·5 mg. The other cooked foods contributed negligible amounts. The total intake of vitamin C varied from about 10 to 15 mg. per student per day.

Menus for one month

Day and date	Breakfast	Dinner	Tea	Supper
		Week ending 4 December 1937		
Sunday, 28 Nov.	Bacon and egg	Tomato soup Roast pork Cabbage Steamed apple pudding and custard sauce	Assorted pastries	Veal loaf Beetroot
Monday, 29 Nov.	Porridge Smoked fillets	Mulligatawny soup Roast beef Marrowfat peas Suet roll and syrup sauce	Jam, marmalade or syrup	Highland hash Mashed potatoes
Tuesday, 30 Nov.	Bacon and beans	Julienne soup Roast mutton Cabbage Dundee pudding	Doughnuts	Irish stew Doughboys Mashed potatoes
Wednesday, 1 Dec.	Liver and chips	Scotch broth Steak and kidney pie Mashed turnips Prunes and custard	Jam, marmalade or syrup	Fish and crisps
Thursday, 2 Dec.	Bacon and sausage	Pea soup Roast beef Cabbage Sultana roll and custard sauce	Bananas	Bubble and squeak and bacon
Friday, 3 Dec.	Porridge Fried fish	Pea soup Meat pudding Haricot beans Tapioca pudding	Jam, marmalade or syrup	Durham cutlets Marrowfat peas
Saturday, 4 Dec.	Fried sausages	Pot mess Carrots Doughboys Bananas	Tea cakes	Pea soup Cheese
		Week ending 11 December 1937		
Sunday, 5 Dec.	Bacon and egg	Tomato soup Roast mutton Cabbage Bananas and custard	Assorted pastries	Preserved meat Beetroot
Monday, 6 Dec.	Porridge Bloaters	Pea soup Roast beef Marrowfat peas Snowdon pudding	Jam, marmalade or syrup	Cottage pie
Tuesday, 7 Dec.	Fried sausages	Pea soup Beef steak pudding Cabbage Tapioca pudding	Jam, marmalade or syrup	Layer pie

Week ending 11 December 1937 (*continued*)

Day and date	Breakfast	Dinner	Tea	Supper
Wednesday, 8 Dec.	Bacon and liver	Potato soup Ragout of rabbit Marrowfat peas Suet pudding and jam	Assorted pastries	Fish and chips
Thursday, 9 Dec.	Fried or boiled eggs	Pea soup Roast beef Cabbage Apple pudding and custard sauce	Fish paste	Saveloys and pease pudding
Friday, 10 Dec.	Porridge Fried fish	Pea soup Steak and kidney pie Carrots Prunes and custard	Jam, marmalade or syrup	Savoury Mince and haricot beans
Saturday, 11 Dec.	Bacon and sausage	Pott mess Doughboys Butter beans Rice custard	Doughnuts	Salmon Beetroot

Week ending 29 January 1938

Sunday, 23 Jan.	Bacon and egg	Tomato soup Roast pork Cabbage Apple tart and custard	Slab cake	Salmon Beetroot
Monday, 24 Jan.	Fried or boiled eggs	Pea soup Roast beef Marrowfat peas Sultana roll and custard sauce	Jam, marmalade or syrup	Cottage pie
Tuesday, 25 Jan.	Porridge Kippers	Pea soup Steak and kidney pie Cabbage Rice custard	Rock cakes	Fried steak Mashed potatoes
Wednesday, 26 Jan.	Fried sausages	Potato soup Roast beef Turnips Ginger pudding	Jam, marmalade or syrup	Fish and chips
Thursday, 27 Jan.	Bacon and tomatoes	Pea soup Preserved meat Braized onions Durban pudding	Fish paste	Lamb's heart Potatoes
Friday, 28 Jan.	Porridge Fresh fish	Mulligatawny soup Roast mutton Cabbage Prunes and custard	Doughnuts	Bacon and bubble and squeak
Saturday, 29 Jan.	Sausage and egg	Pot mess Doughboys Carrots Bananas	Currant bread	Cheese and sauce

Week ending 18 June 1938

Sunday, 12 June	Bacon and egg	Tomato soup Roast mutton Cabbage Rhubarb tart Custard	Slab cake	Salmon Cucumber
Monday, 13 June	Porridge Kippers	Pea soup Roast beef Marrowfat peas Snowdon pudding and custard sauce	Syrup	Cambridge stew

Week ending 18 June 1938 (*continued*)

Day and Date	Breakfast	Dinner	Tea	Supper
Tuesday, 14 June	Fried eggs	Lancashire hot-pot Doughboys Onions Blanc-mange and prunes	Assorted pastries	Fish and chips
Wednesday, 15 June	Liver and bacon	Pea soup Baked and steamed pies Cabbage Sponge trifle	Bananas	Roast beef Potatoes
Thursday, 16 June	Fried eggs	Stewed rabbits and pork Dumplings Butter beans Macaroni pudding	Lemon curd	Fish and chips
Friday, 17 June	Sausages and gravy	Pea soup Roast mutton Cabbage Durban pudding Custard	Bananas	Lamb's heart Peas
Saturday, 18 June	Porridge Fresh fish	Irish stew Doughboys Haricot beans Rice pudding	Doughnuts	Cheese and pickles

Extra to menu. Tea, sugar, milk, bread, butter and potatoes, cocoa and biscuits: buns at stand easy.

METHODS

For a preliminary survey seventy-seven tests were carried out on otherwise healthy youths by giving them 300 mg. of ascorbic acid, and not one excreted appreciable amounts in his urine. Using the same method on twenty of the administrative staff who had a different dietary, it was found that fifteen excreted a considerable proportion of their test dose. Although it is recognized that other substances in the urine reduce the dye, 2:6-dichlorindophenol, the investigation revealed a difference between the two groups.

Estimations of the resting level of excretion, i.e. the total amount excreted in 24 hr. in the absence of a 'test dose', were also made. The amounts varied between 5·6 and 1·1 mg. with an average of about 2·5 mg. as compared with the normal amount of 13–15 mg.

These preliminary observations, therefore, indicated that the intake of vitamin C was at a very low level. This was to be expected from a consideration of the vitamin C content of the diet, and the probable 'minimal-optimum' requirements of the boys.

Daily excretion levels

Pure ascorbic acid powder was added to the diet of a group of boys numbering 350, whose average age was 16. Initially, 200 mg. per day were given to each boy, 100 mg. being placed in the morning cocoa, and 100 mg. in an evening glass of milk. The mixing was done in bulk in the kitchens before issue. The powder dissolved quickly and easily, and did not alter the appearance or taste of the vehicle.

From time to time samples of milk and cocoa were titrated after issue, in order to ensure that the mixing was properly carried out, and that full doses reached the youths. Figures varying from 78 to 118 mg. per glass were obtained in the case of the milk, and from 58 to 68 mg. per cup in the case of the cocoa. Heating of the cocoa no doubt explained the loss. Together with the amount occurring naturally in the diet, the intake per boy was approximately 200 mg. per day. The daily output of vitamin C was measured in different groups of boys each day, the titration of each sample of urine being carried out immediately after it was passed.

Fig. 1 shows the slow rise in urinary output which occurred. It was not until the 8th day that figures approximating to the resting level of normal adults were obtained, and high figures indicative of saturation point were not noted until the 22nd day. In other words, saturation was not achieved until 22 doses of 200 mg. per day had been given, or a total of some 4000 mg. This figure was probably too high, since it was likely that on occasions the boys under test did not pass all their urine in the Sick Quarters as ordered.

On the 28th day the dosage was reduced to 50 mg. twice a day, and on this dosage excretion continued at a level rather higher than that of a normal adult on optimum intake.

A fresh group of boys was observed, and the initial dosage was increased to 150 mg. twice a day. Figures indicative of saturation were obtained on the 15th day, and subsequently the dose was reduced to 25 mg. twice a day, when an excretion level approximating to the normal adult level was maintained. This is shown in Fig. 2.

A third batch of boys was examined. In this batch all the boys selected were recruits who showed possible clinical evidence of a vitamin C deficiency in the form of a mild gingivo-stomatitis. The ascorbic acid in this case was given in tablet form (Redoxon, Roche Products), in a dosage of 200 mg. once daily. Instead of estimating the vitamin C excretion of individual boys as in the two previous experiments, several were instructed to pass their urine each day and night in the Sick Quarters. The urine specimens were pooled. From the mixed specimens a sample was taken and acidified by the addition of one-ninth the volume of glacial acetic acid. The samples were titrated, and the amount of ascorbic acid per 1500 c.c. of urine recorded and charted (Fig. 3). This chart is very similar in form to Fig. 1. High outputs were observed on the 23rd day; the dose was then reduced to 50 mg. once a day in tablet form.

These charts show that, in order to maintain an optimal excretion level, a daily addition of 50 mg. of ascorbic acid was required.

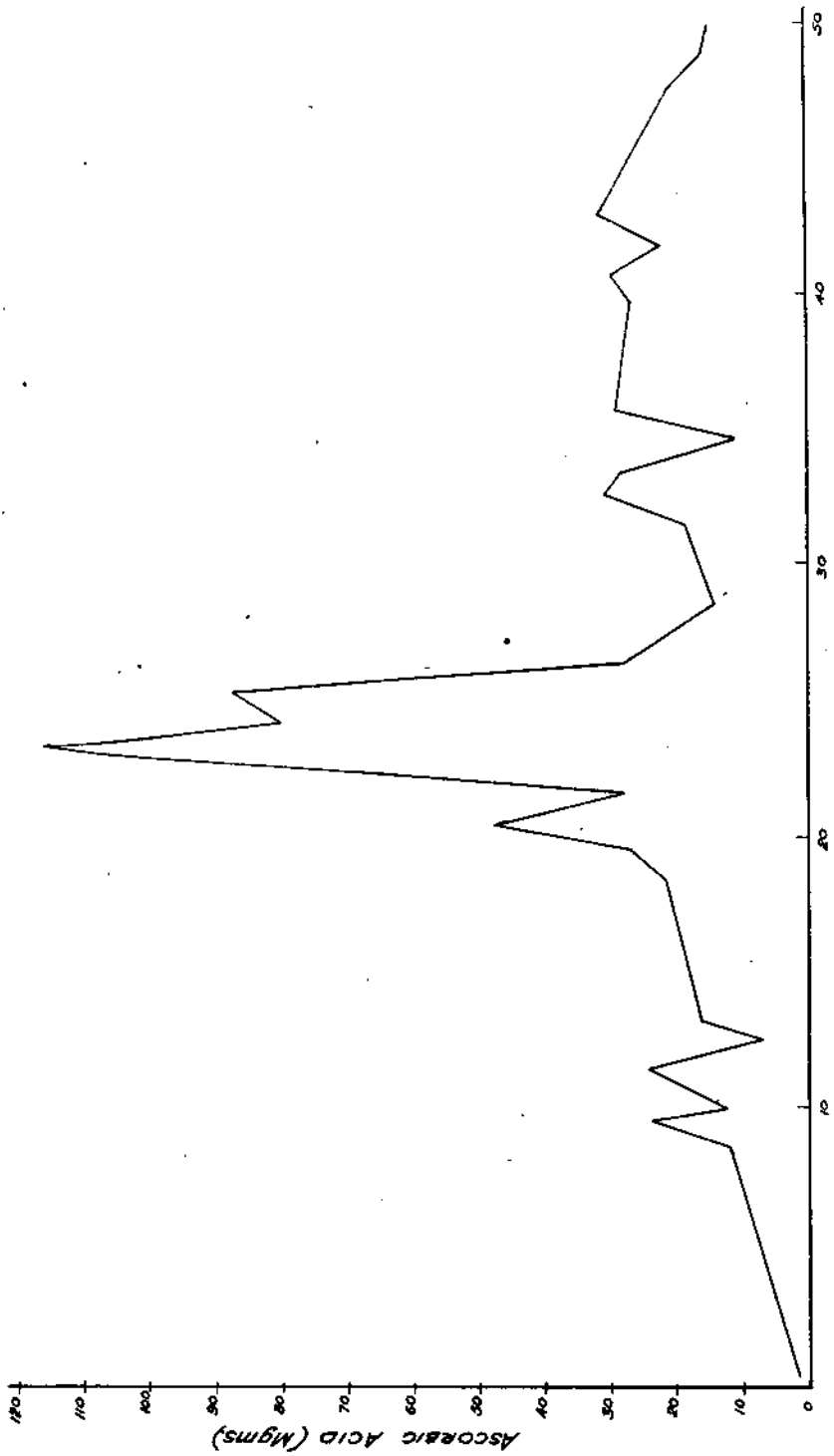

Fig. 1. Daily output of vitamin C in the urine. Group 1.

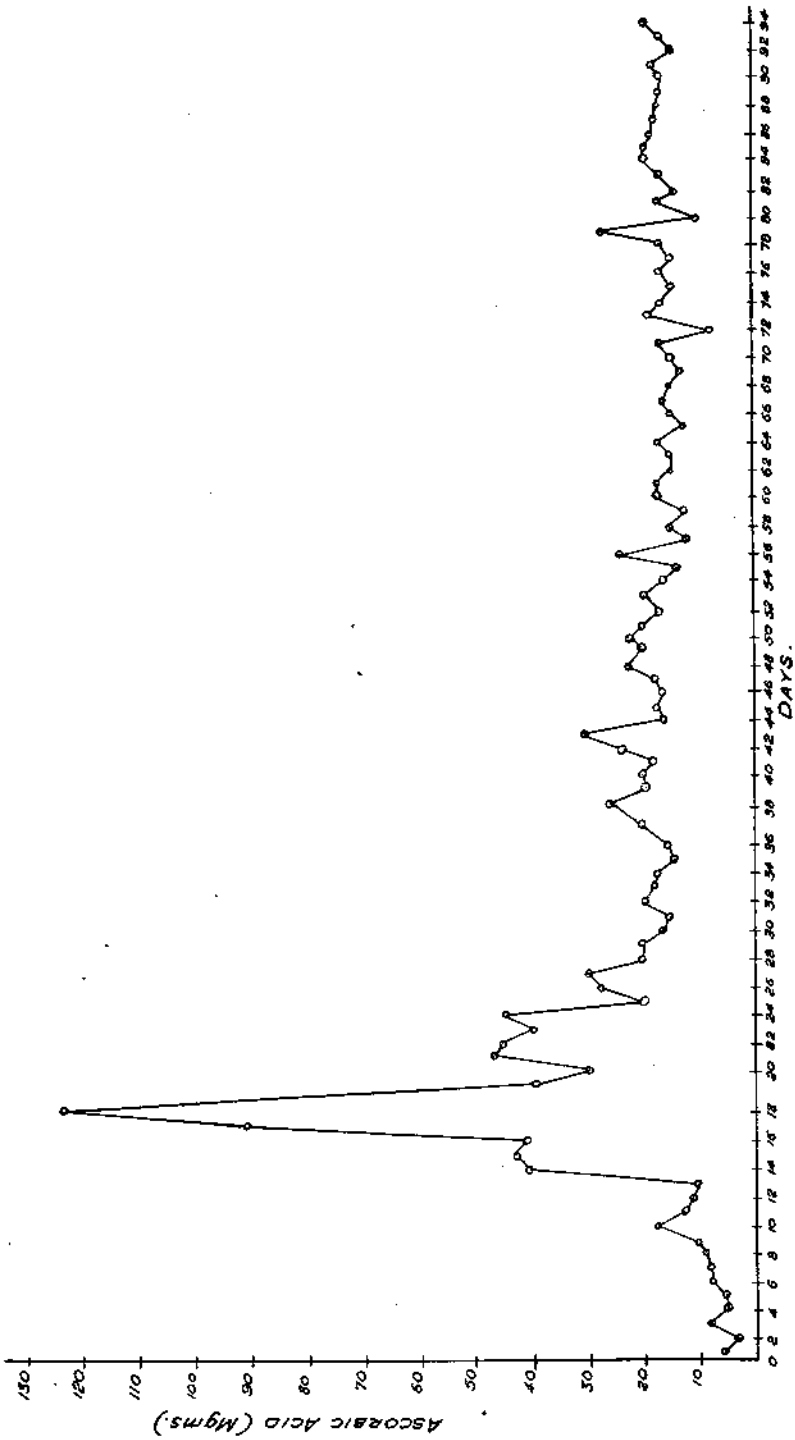

Fig. 2. Daily output of vitamin C in the urine. Group 2.

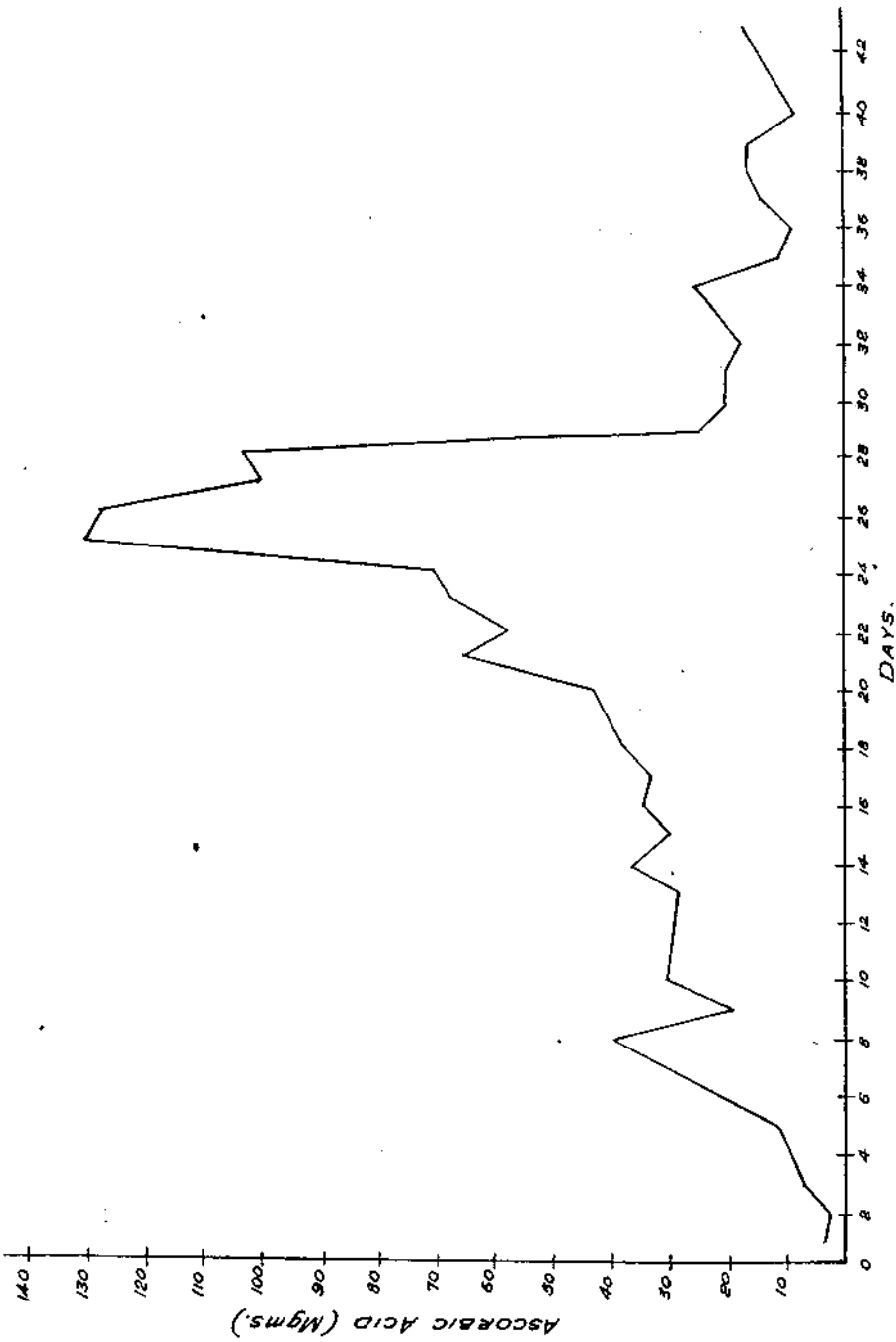

Fig. 3. Daily output of vitamin C in the urine. Group 3.

THE RELATIONSHIP OF VITAMIN C TO RESISTANCE

In the institution, there were some 1500 students whose ages ranged from 15 to 20 years. The establishment was divided into seven groups or divisions for administrative purposes. The youths of one division worked as a unit, and occupied certain tables in the dining hall. To some extent each division occupied particular dormitories, but this separation was not absolute, and there was a fair amount of mixing of divisions in the sleeping quarters. Sleeping and feeding conditions were, of course, the same for all divisions. Careful records had been kept of the incidence of all infections for 1½ years before the observations described here were begun. In the preceding year there had been an epidemic of tonsillitis, which had affected all the divisions uniformly, so that they could not be regarded as separate units within the larger population.

The observations were made by supplying vitamin C in the form of pure ascorbic acid to one or more divisions. This was considered to be the only practical method of carrying out the observations without introducing unnecessary complications. For example, it was not possible to choose boys at random as it would have been impossible to supply them with vitamin C-treated cocoa or milk in the dining room. With the method actually chosen, all that was necessary was to add vitamin C to the supplies of cocoa or milk serving the tables for the appropriate divisions.

Moreover, all of the divisions had a population more or less the same as regards duration of stay in the establishment ('institution age'). Infectious diseases were more common amongst those who had more recently joined the institution. This was known from our previous records of infectious illnesses in the institution (Thomson & Glazebrook, 1942), and in view of these points the method of supplying the vitamin C to a whole division was decided upon.

Many minor infective conditions, such as conjunctivitis, boils, impetigo, etc., were not reviewed, as the number of cases of each disease was small.

The most common infective conditions which occurred were coryza and tonsillitis. The term 'tonsillitis' is used here to be an index of haemolytic streptococcal disease of the nose and throat, and covers all such terms as 'tonsillitis', 'sore throat', 'otitis media', 'pharyngitis' and 'cervical adenitis', as nearly all these cases are of haemolytic streptococcal origin. Throat swabs were taken of large numbers of cases of tonsillitis to determine that the haemolytic streptococcus was the causative organism.

Table 1 shows the number of cases of tonsillitis and common colds recorded in the two groups.

Table 1. *Incidence of tonsillitis and common colds in the two groups*

	Youths on vitamin C (335 youths)	Controls (1100 youths)
Colds	72 = 21·2%	286 = 26%
Tonsillitis	29 = 8·5%	94 = 8·6%

It is obvious, therefore, that vitamin C had no effect on the incidence either of common cold or tonsillitis.

The experiment was complicated, however, by the admission of 250 recruits into the two groups in the middle of the observations, replacing fully trained youths. This was of special interest, as it was known from previous experience that infections were more common amongst those who had more recently entered the institution. This would be true of any institution where infectious diseases were common. The test group admitted relatively more of the recruits into its population. No recruits were admitted during the 3 months preceding the period of the observations.

The recruits were those of group 6 (Thomson & Glazebrook, 1942), and no observations were made until they had been in the institution for a month. During this period the recruits who entered the test divisions were saturated with vitamin C, and it was during this same period that the recruits experienced much of their heavier incidence of disease. After a month had elapsed a record was kept of sixty youths who entered a test division and ninety who entered a control division. There was still a heavier incidence of infectious diseases amongst them as compared with the others who had been in the institution for some time. The duration of the period over which the recruits were observed was about one-half of the duration of the whole investigation. Table 2 shows that there was a greater incidence of disease amongst the recruits as a whole as compared with the others, but no difference in incidence of disease between the two groups of recruits.

The numbers of cases of tonsillitis and common cold which occurred amongst the 250 recruits were not sufficiently great to alter the incidence rates in the two experimental groups.

Table 2. *Incidence of infection amongst recruits*

	Youths on vitamin C (60 youths)	Controls (90 youths)
Colds	17 = 28·3 %	29 = 32·2 %
Tonsillitis	1	7 = 8 %

The next point examined was to see what effect, if any, the vitamin C had on the duration of the illness.

When a youth fell ill he was admitted to Sick Quarters unless his complaint was very mild. In the latter case he was placed on the out-patients list and excused all duties except attendance at school instruction. Most of the cases of common cold and tonsillitis were admitted to Sick Quarters. In analysing the durations of illnesses, observations were restricted to the cases in the Sick Quarters. The number of days spent there was obviously a more reliable index of the duration of illness, since the patient was under constant medical supervision. Frequently when a youth was discharged from the Sick Quarters he was put on the out-patients list, and this 'convalescent period' was neglected. The admission to and discharge from the hospital was not under our control.

The diet in the Sick Quarters was basically similar to that of the healthy boys. It was modified, of course, to suit the needs of the sick, but was prepared in the central kitchens and suffered an equally drastic loss of its vitamin C. When a student from the experimental division fell ill and was admitted to Sick Quarters, his dosage of ascorbic acid was continued there.

In a period of 6 months the average number of days spent in the sick room per boy due to infective conditions was 2·5 in the vitamin-C treated division, and 4·98 in the control division. In a period of 6 weeks, within the period of 6 months, the corresponding figures among the recruits were 3·2 in the vitamin-C treated group, and 4·0 in the control group.

It would appear that the saturation with vitamin C probably had some effect on duration of illnesses, and accordingly an analysis was made of this.

Days ill with common cold

In the vitamin C classes fifty-nine of the seventy-two cases (81·9%) were treated in the Sick Quarters, and the average period of stay was 6·32 days.

Among the controls 253 cases out of 286 (88·5%) were treated in the Sick Quarters, and the average period of stay was 6·4 days.

There was, therefore, no difference in the two groups either in incidence or duration of illness of common cold, and there was no difference in the proportion of total cases admitted to hospital.

Days ill with tonsillitis

The results are shown in Table 3.

Table 3. *Duration of attack of tonsillitis*

Class	Total no. of cases	No. admitted to hospital	Hospital cases expressed as percentage of total	Average stay in hospital	Standard deviation
Vitamin C class	29	18	62	10·05	6·96 (1)
Controls	94	83	88	16·7	11·86 (2)

An analysis showed that a difference as great or greater than that obtained would be expected once in fifty times in a homogeneous population.

Analysis of the more severe illnesses

It has been shown that youths on vitamin C spent 2·5 days in hospital due to infective conditions as compared with 4·98 in the control group. No conclusions were drawn from this observation, and it has been shown above that some of this difference was due to the duration of illness of tonsillitis in the two groups.

Some of this difference, however, was due to the occurrence of acute rheumatism and pneumonia in the control group with no case of either disease in the vitamin C-treated group.

There were seventeen cases of pneumonia and sixteen cases of acute rheumatism among 1100 controls, and no case of either disease among 335 youths having vitamin C. It would appear that the vitamin C exerted a considerable effect on the prevention of these two diseases. Of the sixteen cases of acute rheumatism, eleven were primary attacks, while five were recurrences.

The incidence of the diseases in the various divisions of the institution is shown in Table 4.

Table 4. *Incidence of pneumonia and rheumatism in the various divisions of the institution*

		Number of cases	
	Division	Pneumonia	Rheumatism
Vitamin C divisions	A	0	0
	B	0	0
Control divisions	C	5	3
	D	3	5
	E	2	3
	F	4	3
	G	3	2

Thus, the most marked effect of the vitamin C was to reduce the incidence of two severe illnesses.

Analysis shows that a difference as great or greater than this would be expected once in fifty times in a homogeneous population.

DISCUSSION

In a large institution there was a marked difference between the degree of vitamin C saturation of the students and the teaching staff as determined by a simple 'test-dose' method. The students were given a high calorie diet, which was subjected to prolonged heating. This overcooking resulted in a reduction of the total daily vitamin C intake to a level of 10–15 mg. per head. A daily addition of 50 mg. of ascorbic acid per head was required to maintain an optimal excretion level.

Better management of the food distribution and cooking arrangements might have achieved this result. The potato ration alone, allowing for normal cooking losses, should have supplied at least 25 mg. of vitamin C daily.

Some vitamin loss, of course, is unavoidable when food is cooked for communities in central kitchens. Normally, this can easily be countered by the supply of uncooked fresh or canned foods. In this case, for instance, the reduction of the diet from 4000 cal. to the more reasonable level of 3000 cal. per day, would at this time (1938) have probably offset the cost of an orange a day.

The dietary of the teaching staff included the supply of fresh fruit at each of the main meals. It was prepared in separate kitchens and escaped the overcooking. Nevertheless, judging from a single 'test-dose', 25% of the staff

were 'deficient' in vitamin C, in spite of their adequate intake. Harrison, Mourane & Wormall (1938) similarly found that the method indicated a 'deficiency' in 25% of medical students. The single 'test-dose' is not, of course, a reliable measure when applied to individuals.

The surprisingly large amount of 4000 mg. of vitamin C was required to produce tissue saturation of the youths. Attention has been drawn to the possibilities of experimental error, and many of the factors which increase utilization were present.

The subjects were adolescents. Infections were very common in the institution, and there had been a very severe epidemic of tonsillitis during the preceding session. The experiments were carried out during the winter months. Physical training and games occupied much of the day, and it was found that youths at rest in bed required approximately half the quantity of vitamin C, i.e. 2000 mg., to produce full saturation.

A special group of boys exhibited a mild gingivo-stomatitis, considered to be probably a scorbutic manifestation. Their saturation curve, however, was very similar to that of the other groups. The clinical appearance of this gingivo-stomatitis has been described (Roff & Glazebrook, 1939, 1940). It proved resistant to ordinary methods of dental treatment, and responded only to vitamin C saturation. It would appear that, under exactly similar conditions of suboptimal vitamin C intake, a gingivitis occurs in only a proportion of the cases. This, of course, was known to Lind (1772), who wrote: 'In Haslar Hospital the appearances of the disease [scurvy] were various—the gums were not always affected.'

No differences in the incidences of common cold and tonsillitis were found in two groups of boys, one of which received large doses of vitamin C. It was found, however, that the average duration of illness of the cases of tonsillitis in the control group was much longer than in the vitamin C-treated group. No such difference was found in the cases of common cold.

The period of treatment of cases of tonsillitis and common cold in the Sick Quarters was completely outside our control, and no biased attitudes influenced these durations from which we have drawn our conclusions.

In addition, there were seventeen cases of pneumonia and sixteen cases of rheumatic fever in the control group, with no case of either disease in the vitamin C-treated group. These cases were subjected to special investigations by us (X-rays, etc.) to establish certain criteria for the diagnosis. There was, however, in our opinion a relationship between these conditions.

Rheumatic 'pneumonitis' is a condition which is now recognized to occur not infrequently as a complication of rheumatic fever. The post-mortem appearance and pathology of this pneumonitis have been demonstrated by Hadfield (1938).

In the institution a type of low-grade basal lung consolidation or 'pneumonitis' occurred, and appeared to be related both to rheumatism and vitamin C deficiency. It was characterized on the one hand by its tendency

to progress into rheumatism, and on the other hand by its rapid disappearance when treated with ascorbic acid. This pneumonitis, apart from a vague picture of ill health, gave little clinical evidence of its presence, but it probably predisposed towards the development of acute pneumonia.

It is agreed that cases of rheumatic fever almost invariably give a history of upper respiratory tract infection, usually some 2 weeks previously. Such an infection depletes the reserves of vitamin C, more especially in those individuals whose intake is already at a low and precarious level. When the vitamin C reserves have fallen, it may be that the reaction of the body to an infection with the haemolytic streptococcus is altered. This may help to determine the onset of the syndrome of rheumatism in some cases, even although vitamin C has no specific action upon the established disease. In some cases of pneumonia, too, a similar train of events may occur, and there is much evidence that vitamin C does assist recovery.

Certainly, protracted mild deficiencies of vitamin C produce bone and cartilage changes, the histological and skiagraphical appearances of which have been accurately described (Park, Guild, Jackson & Bond, 1935; Wolbach & Howe, 1926). Ham & Elliott (1936) showed that the epiphyseal changes occurred when the vitamin C intake was sufficient to prevent scurvy although less than the basic requirements. These changes are marked during the period of growth. Under similar circumstances Mouriquand & Edel (1940) have demonstrated osteophytic formation. Rinehart & Mettier (1933, 1934) produced lesions simulating rheumatism in the myocardium of guinea-pigs fed on a scorbutic diet. Wolbach (1936) showed the presence of vitamin C to be essential for the formation of collagen. Swelling of the collagen is the earliest pathological change in rheumatism.

The calcium and vitamin B content of the dietary of the institution could perhaps be criticized, but the only *outstanding* deficiency, according to modern standards, was in vitamin C. As far as this one factor was concerned, the boys were almost certainly worse off, subsisting on the institution diet, than they would have been at home.

SUMMARY

1. The vitamin C in the dietary of an institution was largely destroyed by the methods of cooking and distribution.

2. Some 50 mg. of ascorbic acid per head per day were required to be added to the diet to produce an optimum excretion level.

3. Large doses of ascorbic acid were given to a group of adolescents in the institution over a period of several months. A record was kept of the incidences of infectious diseases in this treated group and in the remainder (controls). The following conclusions were reached:

(*a*) The incidences of common cold and tonsillitis were the same in the two groups.

(*b*) The average duration of illness due to the common cold was the same in the two groups.

(*c*) The duration of illness of tonsillitis was longer in the control group than in the test group.

(*d*) Cases of rheumatic fever and pneumonia occurred in the control group but no case of either disease occurred in the test group.

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SAC

ASCORBIC ACID in Treatment of the Canine Distemper Complex

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A CLINICAL investigation of ascorbic acid as a therapeutic agent in treatment of canine distemper complex was initiated in the author's practice early in 1967. This move was prompted by reading a report that vitamin C had been used clinically, with notable success, in treating 12 cases of distemper complex (canine and feline) in one practice.[1]

Ten years of practice had led me to view skeptically all reports of the type cited. However, experience during those same years had made me aware that the recovery rate among my patients showing signs of CNS disturbance, and treated with the generally accepted therapeutic regimen, was a dismal 5% to 10%. With many of these patients, the prognosis appeared to be hopeless from the first examination. Many others progressed rapidly from showing signs of the distemper complex to a state of chorea followed by death.

With this background in mind, intravenous injection of ascorbic acid (250 mg./cc.), Scorbate® Injection (Burns Pharmaceuticals) was added to the course of treatment given for canine distemper in our practice.

About a year after the investigation was started, John E. Reinert, M.D., a local neurologist and neurosurgeon, became interested in the work and thereafter was associated with the study. Dr. Reinert examined many of the dogs for neurologic impairment and observed their progress after treatment. After assessing the results in dogs, he began using ascorbic acid to

treat some of his own patients, with favorable results.

During the 22 months before this paper was prepared, 67 dogs in which canine distemper had been diagnosed were treated with ascorbic acid and a running summary of their histories was kept.* The following case histories are typical examples.

Case Histories

Case No. 1

This 2-year-old male Miniature Poodle with typical signs of distemper had been under treatment for 10 days. On the eleventh day, convulsions began to occur almost continuously. Within 24 hours, the animal was semicomatose, unable to stand, and stricken with chomping and foaming seizures. During the next five days, while the dog remained in the same condition and failed to respond to treatment, the owner refused permission for euthanasia to be performed.

On the morning of the sixth day following the onset of convulsions, 1,500 mg. of ascorbic acid was given intravenously. Late that afternoon, although mildly incoordinated, the dog was standing, walking in the cage and drinking water.

By the following morning, there were no signs of incoordination and the temperature had dropped from 103 F. to 101.8 F. After a second 1,500-mg. dose of ascorbic acid was injected, the condition continued to improve. The dog drank water and ate several meals of solid food during the day. A third dose of 1,500 mg. ascorbic acid was given the next day, although by that time no signs of distemper were present.

Five days after the beginning of treatment with ascorbic acid, the dog was discharged. Weekly checkups for the next three weeks indicated a complete return to clinical normalcy. When last examined, one and a half years later, the patient was physically sound and in apparent good health.

Case No. 22

A 2½-year-old male Shetland Sheepdog had been treated elsewhere for one month. Throughout that time, this dog's temperature had remained within a range of 103 F. to 104 F. The general condition of the animal upon presentation at our hospital was classified as poor.

In addition to our standard treatment for distemper, a 2,000-mg. intravenous dose of ascorbic acid was given daily for three days. By the second day, the temperature had dropped to 102 F. from 104 F.; on the third day it was 101.6 F.

The patient was discharged on the fifth day. Recovery was uneventful.

Case No. 43

Clinical signs in this 9-month-old male Poodle were convulsions, tremors over the entire body, incoordination, and a temperature of 106.4 F.

Treatment was immediately started with 2,000 mg. ascorbic acid in conjunction with Dilantin® Suspension (Parke-Davis), Sparine® (Wyeth), atropine, and phenobarbital. Within 24 hours, the convulsions had ceased. The temperature was 101 F., and it remained normal throughout the rest of the treatment period.

By the third day, the tremors had disappeared and all medication but ascorbic acid was discontinued. After the fifth day of treatment with ascorbic acid, the patient was discharged, giving every indication of being completely normal.

Case No. 65

When presented, this 2½-year-old male Poodle had been exhibiting signs of hardpad distemper for six weeks. A slight posterior paralysis and mild incoordination were present. The temperature was 103.6 F.

After two daily doses of 2,000 mg. as-

*A tabular summary showing clinical signs, daily temperatures, dosages of ascorbic acid, adjunctive therapy and results for each patient, is available upon request to the editors.

TABLE 1: Recovery Rates among Dogs Treated with Ascorbic Acid* for Canine Distemper Complex

Patient Group	No. Treated	No. Recovered	Recovery Rate
All dogs treated	67	48	71.64%
Cases showing CNS disturbance	16	7	43.75%
Atypical cases with CNS disturbance but no convulsions	4	3	75.00%
Typical cases with convulsions	12	4	33.33%
Cases without CNS disturbance	51	41	80.39%
Typical cases with convulsions and given 3 or fewer doses of ascorbic acid	7	1	14.29%
Typical cases with convulsions and given more than 3 doses of ascorbic acid	5	3	60.00%
Typical cases without convulsions and given more than 3 doses of ascorbic acid	14	11	78.57%

*Sterbate(®) Injection (Burns Pharmaceuticals)

TABLE 2: Dogs Given Massive Doses of Ascorbic Acid over a Three-Day Period

Breed	Sex	Age	Weight	Total Dose*
Poodle—X	M	1 Yr.	16.5 Lb.	45,000 mg.
Terrier—X	F	8 Mo.	13 Lb.	45,000 mg.
Shepherd—X	F	4 Mo.	25 Lb.	45,000 mg.

*5,000 mg. ascorbic acid, Sterbate(®) Injection (Burns Pharmaceuticals) given intravenously three times a day for three days

corbic acid, the temperature was reduced to 101.4 F. After four more days of treatment with ascorbic acid, the patient was discharged.

Two and a half weeks later, the owner requested euthanasia because of a recurrence of the paresis and incoordination which were becoming progressively worse.

Discussion

RECOVERY RATES observed during the investigation are shown in Table 1. As might be expected, treatment beginning at the onset of clinical signs gave more favorable results than treatment delayed until the condition was in an advanced stage. Although relatively few animals exhibited convulsions in conjunction with the typical signs of distemper, the recovery rate for those in this group that were given more than three doses of ascorbic acid was much higher than that for those given fewer doses (60% as compared to 14%).

Temperatures were elevated in most of the 67 dogs at the time of the first examination, but in almost all cases were within normal limits at 24 or 48 hours after treatment was started. During the latter part of the investigation, when hourly temperature charts were kept, many temperatures were found to be normal within 2 to 6 hours

after the first injection of ascorbic acid.

In all instances, the ascorbic acid was administered intravenously at a rapid rate. Some drowsiness, which lasted only a few minutes, was seen in 2 dogs immediately after injection of the vitamin. However, there were no other visible side effects and no toxicity attributable to treatment. To help establish dosage and determine the possible consequence of giving large doses of ascorbic acid, 3 dogs were obtained from a shelter and given 5,000 mg. ascorbic acid three times daily for three days *(Table 2)*. No side effects were seen in any of these dogs. All three were placed in homes, and are doing well to date.

Conclusion

FROM THE results observed in 67 clinical cases of canine distemper complex, it appears that a daily dose of 1,000 mg. to 2,500 mg. of ascorbic acid given intravenously for at least three days is beneficial in the treatment of canine distemper, and that the recovery rate can be markedly improved by including ascorbic acid in the treatment regimen.

During this investigation, ascorbic acid produced a rapid drop in temperature. The recovery rate during a 22-month period was 71.64%. When more than three doses were given, the rate rose to 78.57% for dogs that did not have convulsions. When more than three doses were given to dogs that exhibited convulsions, the recovery rate rose from 14.29% to 60%.

Fully recognizing that this investigation did not constitute a controlled study, but encouraged by the results, the author has presented these observations in the hope that they will be of help to other practitioners and perhaps stimulate additional work in this area. Certainly, more basic research is needed to define the mechanisms involved and to validate the observations reported here.

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Massive Doses of Vitamin C In the Treatment of Viral Diseases

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TREATMENT OF VIRAL DISEASES presents to the physician a perplexing and frequently unrewarding problem, particularly since some 50 different diseases of man are of viral etiology. To date no generally effective therapeutic measures have been devised for treating viral diseases, although some diseases caused by the largest of the known viruses appear to be affected by some chemotherapeutic agents. Therapy with specific antisera is useful as a preventive measure during the incubation period of some viral diseases, but is generally of little value once clinical manifestations of the infection have ensued.[1] Therefore, an effective therapeutic agent that would substantially reduce the morbidity of the majority of viremias would provide the physician with a most valuable adjunct to treatment.

There have been a number of reports in the literature suggesting that infectious disease processes rapidly accelerate vitamin C depletion and greatly increase vitamin C requirement." The role of vitamin C in maintaining stability and tensile strength of connective tissue is well known. This property favors, among other things, the building of a protective barrier against infectious invasion.[4] When ascorbic acid stores are severely depleted during the course of infectious diseases, capillary resistance decreases and susceptibility to the action of certain toxins appears to increase.[2] It has been suggested that means of altering the susceptibility of cells to invasion by viruses could provide a method of controlling as well as preventing infection.[7]

Several investigators have reported employing massive parenteral doses of ascorbic acid in the adjunctive treatment of viral diseases. Klenner[3] has advocated and employed massive doses of intravenous ascorbic acid for many years in the treatment of various viral diseases including measles, mumps, chickenpox, viral pneumonia and viral encephalitis, and has reported remarkable results. Even with doses as high as 65 mg./Kg. Klenner rarely encountered any adverse effects and those were limited to the site of injection. Klenner has administered chemotherapeutic agents along with ascorbic acid to reduce secondary bacterial infection and has recommended the subsequent use of Vitamin BI following infectious diseases involving the nervous system. He further theorizes that the near absence of ascorbic acid in infectious states may be attributed to the vitamin combining with the toxin and/or virus to form a new complex which is easily destroyed by oxidation.

Free from Reaction

McCormick[4] administered ascorbic acid intravenously or intramuscularly in massive repeated doses, 500 to 1000 mg. every four hours. He reported that this approach exhibited a potent chemotherapeutic-like action in acute infectious processes which compared favorably to that of the sulfonamides or antibiotics but with the advantage of complete freedom from toxic or allergic reactions. Baur and Staub[5] reported highly satisfactory results were obtained with daily intravenous infusions of 10 gm. of ascorbic acid in 1000 cc. of isotonic saline solution administered for an average of five days to patients with infectious hepatitis. They have described the action of ascorbic acid as "virucidal." Calleja and Brooks[6] reported that daily intravenous infusion of 5 gms. of ascorbic acid for 24 days resulted in remarkable improvement in a patient with acute hepatitis when other therapeutic measures had proved futile.

Reports from German literature show

that high doses of vitamin C are beneficial in epidemic hepatitis in children. These beneficial effects were clearly observed in 63 cases of epidemic hepatitis treated with high doses of vitamin C in doses of 10 gms. daily for an average of five days given either by rectal infusion or intravenously, or both.[9]

This investigator evaluated a product trademarked Viron-1* as an adjunct in the treatment of a series of cases involving diseases of probable viral etiology. Viron is a preparation for intravenous administration consisting of 2000 mg. of ascorbic acid per dose fortified with certain B-vitamins. I was primarily concerned with patient response to this mode of therapy since time of recovery was of major economic importance to these patients. It has been my past experience that the more intense the patient's symptoms the greater the morbidity and the longer the convalescent period.

The following case histories are representative of this therapeutic regime:

Infectious Hepatitis

A 20-year-old white female hospital medical technician was first seen for the present illness on Nov. 9, 1959. The illness dates back to the spring of 1959 when she began to feel progressively weaker, exhibited malaise, anorexia, slight nausea, when it was discovered that she had an icteric tinge in her serum. She was treated with bed rest for four days and the sub-clinical jaundice disappeared with a return of her icterus index to normal.

Later in November her symptoms of malaise were intensified, she began to lose weight, became progressively weaker, and presented herself for examination. It was decided that she had clinical jaundice of a minor degree; however, the liver was not palpable and her physical examination was essentially normal.

She was hospitalized on Nov. 11 and was seen in consultation by an internist who confirmed the diagnosis of hepatitis, etiology unknown. Her admission laboratory work revealed a urine which was essentially negative, except for the presence of bile. Her heterophile antibody titer was negative; the icterus index was 13.8 units (normal being 4 to 6 for the method used); her hemoglobin level was 7.5 gms., hematocrit reading was 21%, white blood count was 13,000 with 72% polymorphs, 22% lymphocytes, 3% monocytes and 3% eosinophiles. Prothrombin time was 105%- of standard. Occult blood was found in her stool. Other diagnostic procedures including chest x-ray and gastrointestinal series were normal.

The patient was treated with bed rest for three days while confirming laboratory tests, observations and examinations were made. Her icterus index rose to 32.5 on Nov. 14. The patient's temperature remained "low grade" being 99.2-99.4 orally at the highest points. After a period of complete bed rest and high carbohydrate diet, the diagnosis was confirmed by the internist, a second consultant, and this clinician. At no time in her illness did she receive chemotherapeutic agents.

Dramatic Improvement

The administration of Viron-1 was initiated and she received six intravenous 10 cc. injections during the remainder of her hospital stay. Following the second injection of Viron-1 the patient was amazed with her progress and remarked that she had lost the feeling of "being sick." She wanted to go home within 24 hours after Viron-1. injections were initiated, but hospitalization was continued. She was dismissed on Nov. 20, 1959, markedly improved in subjective feeling and dramatically improved clinically.

The patient was seen in my office on Dec. 1, 1959 at which time her white count had dropped to 7,000 with 53 % polymorphs, 37% lymphocytes, 3% monocytes and 4% eosinophiles. Hemoglobin level was 12.8 gms. and her icterus index had dropped to 8.0.

There is no question in the mind of this investigator that the intravenous administration of Viron-1 had a profound therapeutic effect upon this patient. She had obtained minimal benefit from complete bed rest and high carbohydrate diet before the administration of Viron-1. She outwardly

* Viron-l was supplied by Lincoln Laboratories, Inc., Decatur, 111.

exhibited, and freely discussed with the attending physicians, her feeling of well-being following the administration of intravenous Viron-1. An accurate diagnosis of the exact type of hepatitis was impossible. It was assumed to be viral in nature; however, it may well have been a toxic condition. Other than the academics involved, the exact etiology is relative. The important factor to consider is that she responded to Viron-1 in a most satisfactory manner and one cannot but assume that the medication exerted a profound effect upon her progress.

Past experience with hepatitis of various etiologies has given this observer the impression that recovery from hepatitis, regardless of etiology, is extremely slow and painstaking. The rapid and complete response of this patient to Viron-1 has not been observed following classic and accepted therapeutic measures for treating hepatitis. It is difficult to comprehend a set of circumstances that would coincidentally explain the marked and rapid improvement in a patient as sick as this girl. It was certainly the most dramatic recovery from hepatitis that I have ever observed.

Infectious Mononucleosis

A while female, age 36, complained of generalized aching, exhaustion, anorexia and malaise. Her physical condition prior to these symptoms had been normal. Fever, remittent in type, accompanied the symptomatic complaints. A complete blood count revealed large vacuolated lymphocytes. A positive heterophile antibody titer of 1:226 was recorded. A diagnosis of acute infectious mononucleosis was made and intravenous Viron-1 therapy was initiated. Clinical and subjective response to three consecutive daily 10 cc. injections was excellent. Symptoms remitted in one week following beginning of therapy. The overall morbidity was reduced beyond expectation for the diagnosed condition. The medication was well tolerated and no adverse side effects were noted. The rapidity of patient response to Viron-1 was dramatic since full recovery from infectious mononucleosis rarely takes place in less than two to three weeks in my experience.

Virus Pneumonia

A 60-year-old male physician presented himself with a history of excellent health except for his present illness. His symptoms were exhaustion, cough, low grade fever, anorexia, generalized aching and profuse sweating upon exertion. Viral pneumonia—patchy type—of the right upper lobe was found and confirmed by x-ray findings. Treatment consisted of 10 ce. intravenous Viron-1 for three days, bed rest, and ASA Compound. The response was excellent—strength returned on the fourth day and on the fifth day the physician returned to work. The I. V. Viron-1 was well tolerated and no untoward side effects were observed. Viron certainly shortened the expected morbidity for a case of this nature.

Acute Viral Type Pneumonia

A female, age 47, was in excellent general physical condition with exception of chronic bronchiectasis. When first seen for her present illness this woman was completely debilitated. She was confined to her bed and complained of exhaustion, anorexia and generalized chest pain. Temperature elevation ranged from minimal to normal. A diagnosis was made of acute viral type pneumonia with secondary bacterial involvement of sinus and bronchial tree. She was given intravenous Viron-1, 10 cc. injections, on Oct. 26, 27 and SO and Nov. 3, 6, 9,1959. No other medication was utilized. Patient felt better after the second injection of Viron-1 and insisted on continued therapy. Her exhaustion syndrome continued to show remarkable improvement. Progress was continuous and the administration of Viron-1 markedly reduced morbidity as compared to her previous recurrent pneumonias. She tolerated the injections well and no adverse side effects were observed.

Viral Pneumonia and Bronchitis

A male, age 41, was in good physical condition except for the present illness and recurring pain from a herniated lumbosacral disk. He complained of headache, generalized muscular aching and exhaustion. His temperature was 100°-100.4° orally. The diagnosis was acute viral pneumonia and

bronchitis, following acute sinusitis. Injections of intravenous Viron-1, 10 cc., were given on July 14, 15, 16, 1959. The patient was seen for follow-up examination on July 23 and was symptom free. He had experienced marked relief both from sinusitis and viral pneumonia symptoms and had returned to work on fifth day following therapy without my permission. The morbidity period in this case was definitely shortened beyond expectation. Viron-1 was well tolerated by the patient and no side effects were observed.

Generalized Viremia

This male, age 72, was in fair general physical condition. Patient complained of "feeling bad", hoarseness, exhaustion and depression following "influenza." His temperature was normal, but he had a persistent cough. I made a diagnosis of generalized viremia with bronchitis and right recurrent laryngeal neuritis. Viron-1 was given intravenously on Oct. 28, 30 and Nov. 6, 1959. He experienced a relief of symptoms and felt better. Marked improvement in symptoms of viremia were observed. The medication was of questionable benefit to the neuritis. Viron-1 was well tolerated—no untoward side effects were observed.

Summary

In these selected six cases of probable viral infections, Viron-1 promoted prompt patient response. In four of the above mentioned cases improvement was especially rapid and dramatic. The patients were of different groups and conditions treated were varied. Of significant interest is the shortened morbidity period observed when Viron-1 was given either singly or in conjunction with other therapy. No untoward side effects were observed.

Conclusion

In the experience of this investigator daily doses of 2000 mg. of ascorbic acid fortified with B-complex vitamins given intravenously provides a valuable adjunct in the routine management of a variety of acute viral infections. Further investigation is warranted to determine the complete range of viral diseases which can be treated beneficially with this therapeutic adjunct.

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Massive Doses of Vitamin C and the Virus Diseases

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IT has been reported that one of the mold-derived drugs, in addition to being a good antibiotic, is a super-vitamin Conversely, we argue that vitamin C, besides being an essential vitamin, is a super-antibiotic. Vitamin C in vitro, if maintained at body temperature, inactivates certain toxins at an unbelievable rate. Five parts per thousand of vitamin C with toxins and appropriate controls, incubated at 37° C. for 48 hours showed when tested on mice the minimal lethal dose for the control tubes to be 1 16,000 c.c., while that from the mixture of vitamin C and toxin was only 1/1,000 of a c.c. (Klegler, Guggenheim, Warburg, 1938). In this study the loss of vitamin C in toxin broth and ordinary broth controls followed a constant pattern: the loss, however, was always greater in the toxin broth tube. The difference between the rate of disappearance of vitamin C in toxin and ordinary broth was more striking the greater the concentration of vitamin C. It is. therefore, reasonable to conclude that the degree of neutralization in a virus infection will be in proportion to the concentration of the vitamin and the length of time in which it is employed.

Since it has long been known that the virus organism resembles more the toxins and ferments than the common animate causes of disease, it would seem plausible that the detoxication effected by vitamin C is produced by a direct combination of the vitamin with the toxin and/or virus, this followed by the oxidation of the new compound which destroys both the virus and/or toxin and the vitamin. This destruction of the virus by oxidation has been concurred in by many investigators. Since vitamin C is an integral part of the oxidation-reduction system of the body, its function in the role of an antibiotic becomes intelligible. To appreciate the antagonistic properties of vitamin C against the virus organism and the chemical ferments of exotoxin-producing microorganisms, one must forget its present academic status as a factor essential for life. A cow is valuable to the farmer not only for her ability to produce milk, but also as a source of organic fertilizer. Vitamin C, likewise, is important, not only as a detoxifying agent, as a catalyst aiding cellular respiration by acting as a hydrogen transport, as a catalyst in the assimilation of iron, and as a conservator of collagen fibers and bundles in tissues of mesenchymal origin; but, also, because of its function as a reducing agent or the precursor of such a substance. In this latter capacity it fulfills the requirements of an antibiotic. A striking phenomenon of vitamin C is the similarity of response, whether to correct pathologic processes due to a deficiency of this compound, acting as a vitamin; or to destroy the ferments of microorganisms, acting as an antibiotic. Within a few hours after institution of adequate vitamin C therapy to correct an avitaminosis, his-

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tological evidence of bone improvement is obtainable Fibroblasts begin to form normal connective tissue and capillary buds are invading hemorrhagic areas (Youmans, 1941). Similar is its dramatic antibiotic action, the rule being clear evidence of clinical response within a few hours.

The purpose of this paper is to present clinical proof of such action for this vitamin.

Case I is one of premeasles in a ten-months-old baby. The term "premeasles" is adopted to express the syndrome of fever, redness of eyes and throat, catarrh, spasmodic bronchial cough and Koplik spots. Vitamin C, 65 mgm. per Kg. of body weight, was injected intramuscularly every four hours. The fever dropped from 105 to 97.6° F. within 12 hours. All symptoms showed marked clearing. This sudden drop in the fever was thought to be explainable on one of three grounds: 1) Common right drop. 2) Due to the antibiotic action of vitamin C. 3) Even if the vitamin C administration had been continued, possibly a moderate rise would have occurred in the late afternoon of the second day, granting a highly virulent organism and a poorly resisting host. To determine which of these deductions was valid, vitamin C was discontinued for a period of eight hours. At this point the rectal temperature was back up to 103.4. Vitamin C therapy was resumed and instead of the expected 8 P M. climb, the temperature was down to 99.2 (R) eight hours later. The vitamin C injections were continued, the baby made an uneventful recovery and was discharged 60 hours following admission. No measles rash developed. Eighteen months have elapsed since this illness and the child has not had clinical measles. This is not due to the establishment of active immunity but to the lack of a second exposure.

Case 2 confirms the previous case. This case is that of a 22-months-old infant with symptoms identical with that just described. The same medication was followed; the same clinical course followed. Under parental pressure the child was discharged from the hospital within 36 hours, apparently well. Four days later the child's brother and sister broke out with measles, which ran the usual course, having received no specific therapy. Seven days later the 22-months child broke out with measles. This time vitamin C was not given. The case was judged as modified.

The response as observed in measles was characteristic for vitamin C *versus* virus infections. Two cases of virus pneumonia complicated by encephalitis were so unusual that case histories are given.

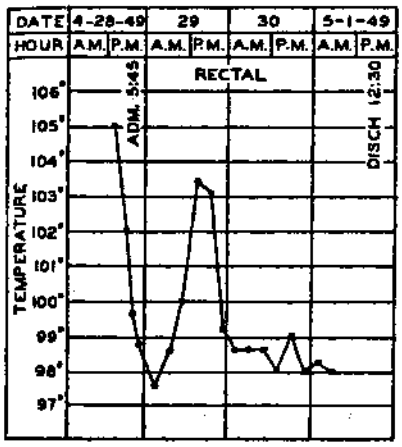
Case 3 is that of a colored woman, aged 28. with history (given by a relative) of chills and fever and chest and head cold for 14 days, severe headache for three days. In stupor when first seen, eye lids closed, a white foam at the mouth which she periodically tried to spit out. Temperature by axilla 106.8. Dehydration was much in evidence, breath sounds diminished to absent, tactile fremitus increased over the entire right lung. The sulfa drugs, penicillin and streptomycin with supportive treatment had been exhausted. Four grams of vitamin C was given intravenously along with 1000 c.c. of 5 per cent dextrose in saline solution. Temperature dropped to 100 (Ax.) within 11 hours. Four hours later, vitamin C was resumed—every two to three hours, in dosage of 2 to 4 grams depending upon the response. After 72 hours the patient was awake, sitting up in bed and taking fluids freely by mouth. There was no fever at this time, nor for the remainder of the time in hospital. Vitamin C was continued for a period of two weeks; the frequency was cut to every 12 hours, two grams at a dose. An interesting complication was deafness; her speech gave a loud, monotonous, bell-sound effect. It was debated whether this was the result of the streptomycin or to the encephalitis. Prostigmin 1:2000, 1 c.c., and vitamin B1, 200 mgm., were given IM twice daily. On the tenth day of treatment the hearing suddenly returned to normal. The x-ray picture of the right lung was one of almost complete consolidation. Although the patient was clinically well of her pneumonia after 72 hours, the x-ray picture was not completely clear until 90 days later.

This phenomenon of Nature clearing the debris after killing out the virus organism was observed in five other cases. The time required was in direct proportion to the degree of pulmonary involvement. There is nothing new about this procedure; Nature merely duplicating a stage in the metamorphosis of the frog in getting rid of its tadpole tail.

Case 4. that of a white baby 19 months old, bothered with a little cold for two weeks, not very sick until the last 24 hours, in which the baby had been "runnings high_fever that could not be_broken with aspirin." Clonic convulsive seizures of the right arm and leg began 12 hours before admission. An undernourished infant, lying rigid in its mother's arms, skin cold to touch, color cadaver-like, eyes closed, grade -2 mucopurulent nasal discharge, throat red. The temperature was 103.8 (R). Breath and heart sounds practically inaudible. Areas of skin over the back presented an appearance similar to that seen in rigor mortis.

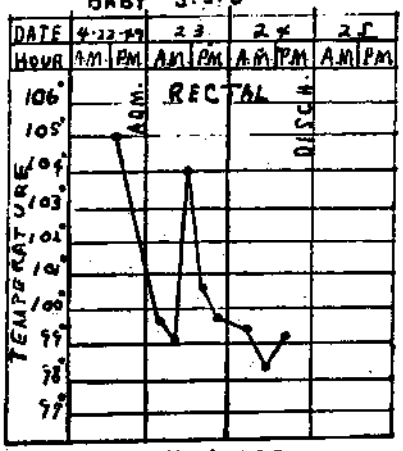
Vitamin C, 1000 mg., was given IM. repeated every four to six hours. At the first injection the baby did not move and the sensation was like that of sticking an orange. To give rapid external heat, mustard plasters were applied to the anterior and posterior chest in a mixture of one part mustard to three parts flour. A croup tent was set up. the vapor carrying compound tincture benzoin; 50

BABY D. S (29753)



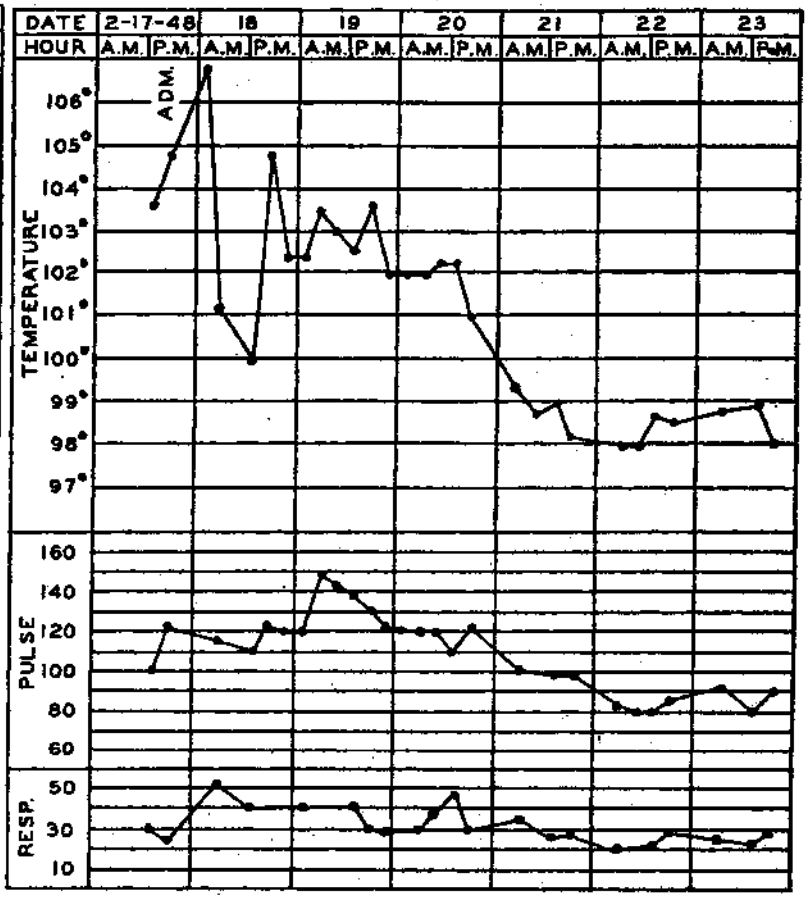
MEASLES

BABY J.S.D



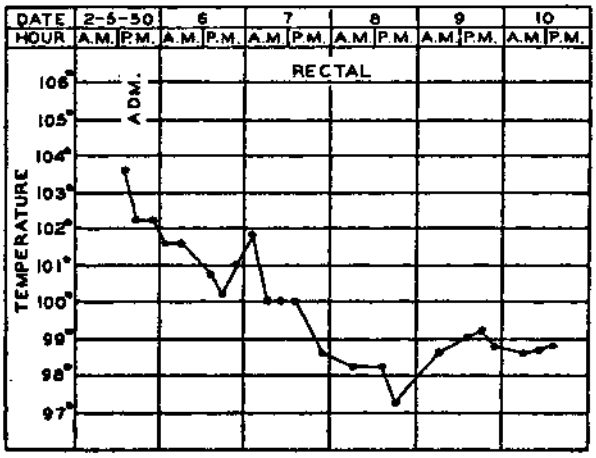
MEASLES

M. H. (26047)



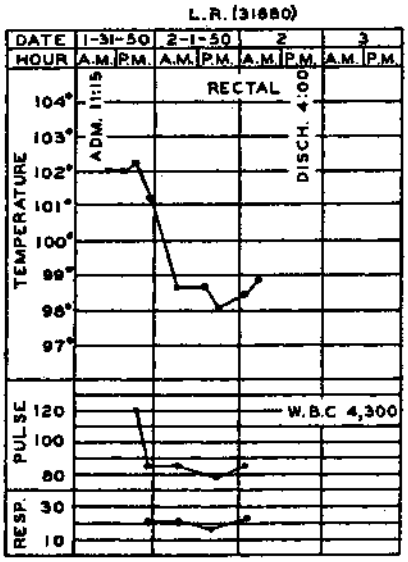
VIRUS PNEUMONIA

BABY W. M. (32219)



VIRUS ENCEPHALITIS-MENINGITIS

L.R. (31880)



VIRUS INFECTION (PULMONARY)

c.c. of 5 per cent dextrose in saline was given under the skin in the scapular areas. Two hours after the first injection of vitamin C the baby drank 240 c.c. of orange juice, the first food of any type taken by the baby in 24 hours. This was repeated $1^{1}/_{2}$ hours later. At this time there was total paralysis of the right arm and leg. Twelve hours after admission the baby moved ks right leg and one hour later grasped a bottle of orange juice with both hands. From this point on the recovery was uneventful. Of secondary importance is the laboratory report of Ascaris lumbricoides ova and hemoglobin 55 per cent.

Cases 5 and 6 are of pulmonary virus infection, (a) in a boy of 14 years, and (b) in a man of 58 years. In the case of the boy the fever curve was of the type showing a fast response to heavy vitamin C injections. The WBC was 4,300, urine sugar ++ Twenty-six grams of vitamin C was given IV to this patient in a 44-hour period.

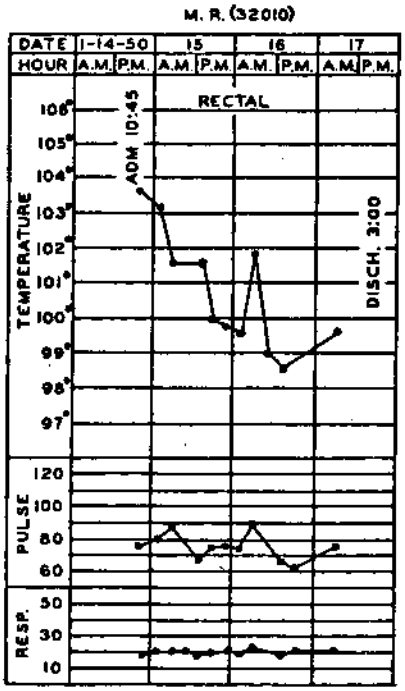
In the case of the man, Case 6, the fever decline was after a modified step-ladder fashion. In this instance the amount of vitamin C injected was less than half of the recommended dose. The WBC was 5,850, admission urine sugar +++. Thirty-one grams of vitamin C was injected intravenously over a period of 60 hours. It is to be noted that the same amount of vitamin C (2 grams every four hours) was given to the boy and to the man, disregarding the factor of body weight. Had the man received four or five grams every four hours, or two grams every two hours, his hospital course would probably have followed the same pattern as that of the boy. A point of great interest was that at subsequent examinations the urine was consistently negative for sugar. The course in these cases emphasizes the necessity of administering massive doses of vitamin C at frequent, regular intervals so as -to maintain the proper level of this antibiotic in the tissues.

The amount of vitamin C for optimal effect will vary greatly with the individual The type of the disease and the degree of toxemia are important guides in determining the dosage. Although the usual dose of vitamin C is calculated on the basis of 65 mgm. per Kg. of body weight, and given every two to four hours by needle, under certain conditions larger single injections can be used to good advantage. Vitamin C given to a child with measles, mumps or chickenpox will abort or modify the attack, depending upon the intensity of the treatment. If the activity of the pathogen is stopped, the development of active immunity will be interrupted. In handling these particular childhood diseases, when uncomplicated, the treatment should be aimed at modification of the infection as the plan of choice. To accomplish this end vitamin C should be increased to 250 mgm. per Kg. of body weight, and the injection given intramuscularly. It will be necessary, at .times, to repeat with half of this amount eight hours later. The vitamin was given in a concentration of 500 mg. per c.c. of solution. Pain was slight and lasted only a few minutes. Procaine, 0.5 to 2 per cent, instilled from a second syringe into the gluteal muscle through a placed needle just before giving the vitamin might solve this problem. The itch of measles and of chickenpox, the occasional vomiting of these illnesses, and the pain of mumps were fully controlled within one hour, when 250 mg./Kg. body weight was used. Instead of repeating waves of macules in chickenpox, and the usual seven to nine days required for crusting, following the heavy modifying injection no new eruptions appeared and crusting was present within six hours. Further clinical studies may prove that the routine use of the higher dose (250 mg./Kg. body wt.) replacing the usual (65 mg./Kg. body wt.) is indicated in all virus infections and the results produced may be even more dramatic.

The greatest value of vitamin C in virus infections does not rest with these lesser kinds of diseases, some of which, e.g. measles, can be modified or prevented by the proper use of immune globulin. The value above all others is its positive action against the virus causing poliomyelitis. A report of this usage was published in the official journal of this association in 1949. Many physicians refuse to employ vitamin C in the amounts suggested, simply because it is counter to their fixed ideas of what is reasonable; but it is not against their reason to try some new product being advertised by an alert drug firm. It is difficult for me to reconcile these two attitudes. On the other hand, many physicians who have been willing to try vitamin C against the virus of poliomyelitis have obtained the same striking results as we reported. Scores of letters from practitioners here in the United States and in Canada could be presented in evidence. In some instances doctors have cured their own children of poliomyelitis by giving vitamin C and in other cases doctors themselves have been cured-

In poliomyelitis vitamin C performs three important functions: 1) It destroys the virus; 2) acting as the dehydrator and diuretic of first choice, it removes the edema fluid from the brain and the cord; 3) it preserves the lining of the central canal and maintains more regular spacing and less crowding of the ependymal cells (Altman). The pressure within the bony vault of the central nervous system resulting from the inflammatory process excited by the virus, acts as a haemostat to cut off the blood supply to the anterior horn cells. This compression of their vessels denies to the horn cells the essentials for function, for life even.

It is of more than academic interest to review



VIRUS PNEUMONIA

the findings of McCormick in 50 confirmed cases of poliomyelitis in and around Toronto, Canada, during the epidemic of 1949. This report is that children of families eating brown bread who came down with poliomyelitis did not develop paralysis; whereas in those families eating white bread many of the children having poliomyelitis did develop paralysis. The point here is that brown bread has 28 times more vitamin B_1 than does white bread Obviously, then, the paralysis which complicates acute poliomyelitis appears to be due to a B_1 avitaminosis. Vitamin C by removing edema fluid relieves from pressure these vessels that supply nutriment to the horn cells, thus allowing the normal complement of vitamin B_1 to reach these cells. In December, 1949, a 5-year-old white girl was brought to my office with paralysis of both lower extremities of 4½ days' duration. The child had been ill for 12 days. There was complete flaccid paralysis of the right leg, 85 per cent paralysis of the left leg. Pain was directed to the knee and to the lumbar back. In hospital the diagnosis of poliomyelitis was confirmed by four consulting physicians. Spinal fluid cells were 82. No medication of any type was given exclusive of vitamin C. Massage was started immediately. The rationale of using early massage had two bases: 1) In the course of general practice patients would give a history of having had poliomyelitis when a child and that their mother rubbed the paralyzed member day and night until function returned. 2) That paralyzed muscle was in profound shock and "artificial respiration" would maintain proper metabolism during .the emergency phase. To the first injection of vitamin C there was definite response. After 96 hours the child was moving both legs. The flexion was slow and deliberate. She was discharged from the hospital at this time, vitamin C being continued by mouth—1000 mg. every two hours with fruit juice for seven days. On the llth day of treatment the child was walking about the house, but her gait was slow and her posture was poor, being bent forward. Vitamin C was discontinued and vitamin B_1 started—10 mg. before meals and bed hour- Carbonated drinks were encouraged for their sugar content and mild stimulating action. Nineteen days after starting treatment there was complete return of sensory and motor function which has persisted to this date.

A boy of eight years was brought to my office with a history of having had "flu" for a week, and four days previously having developed photophobia, conjunctivitis, sore throat, nausea, vomiting and a back-of-the-eyes type headache of such intensity that adult doses of aspirin had no effect. The boy was either rubbing his neck on the left side or holding his head between his hands, begging for something to relieve his pain. The fever was 104.4 (Ax.) He was tender in the lumbar region and he had a drawing sensation referred to the hamstring attachments at the knee. Two grams of vitamin C was given IV while in the office. He was then sent to the local hospital where he received promptly a second injection of 2 grams of tjie vitamin, after which it was given every four hours. Six hours after commencing therapy the neck pain was gone, the headache completely relieved, he could tolerate the ceiling light, his eyes were dry and the redness clearing. Nausea and vomiting had disappeared, the fever was down to 100.6 (Ax.), and he was sitting up in bed in a jovial mood while he drank a carbonated beverage. He was discharged from the hospital after receiving 26 grams of the vitamin in a 48-hour period, clinically well. Vitamin C was continued by mouth, 1500 mg. every two hours with fruit juice for one week, then change was made to vitamin B_1, 25 mg. before meals and bed hour. Vitamin B_1 in these cases should be continued for a period of no less than three months as nerve tissue is slow in recovering from damage.

In using vitamin C as an antibiotic minor complications were occasionally seen. These fall into six groups: 1) Diarrhea in two cases. In each instance the preparation contained sodium bisulfate. The enteritis cleared on giving a preparation of vitamin C not containing this salt. 2) Induration in 42 cases—seen either immediately following the injection (allergy), or delayed. In the latter it was found that the injections were being given too close to the surface. Applications of warm magnesium

sulfate as a. compress gave prompt relief of the pain -and swelling. In two of these cases fluctuation ensued and healing was effected by surgical drainage and the application of compresses. The impression in these two cases was that a vein had been opened by the needle. The exudate was dark and both the slide and culture studies were negative for bacteria. 3) Endothelial irritation in three cases. Acute pain radiated from the site of the injection to the shoulder. In each instance the concentration of the vitamin was one gram to each 5 c.c. solution and the amount given exceeded two grams. After slowing the rate of injection this reaction did not occur. 4) Venous thrombosis in one case. The concentration was 500 mg..per c.c. solution; the total dose 5 c-c. Compressing relieved the pain. The pathology was very similar to that following the use of 50 per cent dextrose solution. 5) Syncope—In maximum doses given IV a sensation of fainting and dyspnea occurred seven times. Five of these patients were over 55 years of age. The disagreeable symptoms were relieved by slowing the speed of the injections. 6) Rash—In three cases a pin-point dermatitis occurred, limited to the face and upper third of the torso, identical to that seen in infants taking orange juice. This did not necessitate discontinuance of therapy and cleared spontaneously several days after vitamin C was stopped.

Calcium, in vivo, duplicates the chemical behavior of vitamin C in many respects. Calcium gluconate and calcium lexulinate were used in conjunction with vitamin C therapy in a small series of pulmonary virus infections and in mild cases of influenza. There was a definite synergistic response. Patients with colds derived most benefit from this combined treatment. Because of its action on cardiac muscle, the use of calcium was limited to adults and the amount injected to two grams per day- One gram administered IV at moderate speed will so slow the heart as in many cases to produce syncope. If the concentration becomes great enough cardiac arrest in a tonically contracted state might result. It is, however, quite possible that, with the proper ionic balance of oalcium and vitamin C in the same solution, larger amounts could be given without side effects. The massive dose schedule limits the usefulness of the calcium ion in virus diseases to that of an adjuvant only.

In all of the cases of virus infection reviewed in this study one laboratory finding stood out as of great significance. On admission to the hospital the first routine urine examination showed some degree of glycosuria. The pattern of the qualitative Benedict's reaction was constant enough to postulate that the higher the reading the more severe was the pathology. Repeat urine sugar studies following vitamin C therapy revealed complete clearing. This was true even though fruit juices were forced to tolerance. This finding confirmed the knowledge that interference with the normal physiology of the adrenal glands, either by the toxins produced by microorganisms or by surgery, has a profound influence on metabolism, especially of the carbohydrates. Adrenalin in the blood stream causes hyperglycemia with resulting glycosuria. Adrenalin acts either by stimulation of the sympathetic nervous system or directly via the blood. This action of adrenalin is via the blood only, because the effect, as demonstrated in experimental animals, is still realized after destruction of the cord and sympathetic plexuses and degeneration of the peripheral post-ganglionic fibers (Evans, 1930). The glycosuria found in these cases was not due to a lowering of the threshold for sugar excretion by the kidney, paralleling a phloridzin diabetes, since the carbohydrate mechanism was associated with a hyperglycemia (Zuelzer, 1901, Metzger, 1902, Paton, 1903). Likewise there was no evidence of kidney damage. Albumin was reported negative and the microscopic examination showed no cells or casts. Apparently this is a condition of artificial diabetes mellitus, which would suggest the answer for the diabetic who loses ability to maintain sugar-insulin balance when embarrassed with an acute infection.

The story of a 7-year-old boy may have a lesson. He has been known to be diabetic since the age of four years. Any incident of infection in this lad produced an alarming interference of his sugar-insulin-diet equilibrium. Recently he contracted measles, and as the disease process developed toward its height the urine sugar curve swung sharply upward. From an occasional dose of 5 units regular insulin his requirement rose to 30 units regular insulin, three times each day, while still running a 3- or 4-plus Benedict's test. (Other forms of insulin proved by trial to be too dangerous.) At the peak of his infection vitamin C was started in a modifying dose of one gram every four hours. His general condition soon improved and in the course of several days he returned to his usual diet-insulin schedule and his usual urine sugar. In patients with diabetes, vitamin C should be discontinued just as soon as the temperature returns to normal. Prolonged use of vitamin C might prove undesirable due to its dehydrating and diuretic powers.

The pathologic process at work here is only compatible with abnormal amounts of adrenalin in the blood stream. It is not a response to an emotional stimulus to the adrenal medulla, since free adrenalin in the circulating blood has a transitory action, being so rapidly oxidized that none gets into the urine. This suggested that the regulator of the adrenalin mechanism had been removed, so that a constant supply of adrenalin would be present in the blood, making possible a concentration sufficiently high to cause constant vasoconstriction.

Ritzmann (1909) found that adrenalin affected carbohydrate metabolism only when this vasoconstriction phase existed. This finding was concurred in by Lusk (1914), who further concluded that ihis action on blood vessels caused asphyxia of the tissues which tended to increase the acidity of the blood and the tissues. This superimposed acidity further promotes the production of .adrenalin hyperglycemia (Peters and Geyelin, 1917). McDannell and Underbill (1919), studying these phenomena in rabbits, found that slight hyperglycemia could be controlled by the administration of sodium carbonate.

The rationale of forcing fruit juices in the old treatment of colds was based on this theory as postulated by Hawley et al. (1936) that a highly alkaline urine would have lower amounts of vitamin C than a highly acid urine; the alkaline ash from the organic acids serving to retain the vitamin C in the blood and tissues where Nature had assigned it to guard against the many enemies of the body—the toxins and ferments of bacteria. As a result of avitaminosis C, liver glycogen is mobilized—glycogenolysis; and further storing of sugar ir the liver is prevented—glycogenesis (Mackenzie, 1917). To further enhance the hyperglycemia this vasoconstriction brings *about* a decrease in the pancreatic secretions by lessening the amount of blood passing through the gland {Mann and McLachlan, 1917).

That the adrenal glands and vitamin C are closely allied in the defense of the body has been proven by experimentation and by autopsy. In normal persons any excess of vitamin C is excreted in the urine. In persons suffering with an acute infection, particularly a virus infection, vitamin C is riot only absent from the urine but is also missing from the blood serum. This is true even when moderate amounts are given intravenously. These observations on serum were made with a Klett-Summerson photoelectric colorimeter using the method described by Mindlin and Butler. The observations on the urine were conducted according to the instructions of Goldsmith and Ellenger. Harde and Benjamin (1934-35) found the vitamin C fraction of the adrenal glands greatly reduced in monkeys killed or paralyzed by the virus of poliomyelitis. Yavorsky, Almoden and King (1934) reported identical findings in humans having died of various infectious agents.

This gives us an important concept of the value of vitamin C in virus diseases. The explanation for the absence of vitamin C in the infectious states is that this agent joins with the toxin and/or virus to form a new compound which is then destroyed by oxidation. Since the body is dependent on food for vitamin C to meet its daily needs, it is obvious that the body tissues would soon be depleted, and we would expect to find evidence of a prescorbutic state in patients who had hypovitaminosis C. In patients seriously ill with a virus invader, the added strain on the capillaries by the application of a tourniquet, even for a few seconds, produced petechial hemorrhages at the site of constriction, bince not all patients thus demonstrated this capillary weakness, all .patients ill with a virus infection were investigated by the aid of a petechiometer. Increased capillary fragility was found to exist in all cases, and the number of petechiae as expressed in centimeters of mercury followed the urine sugar findings. This deficiency syndrome was reversed as the glycosuria cleared, indicating that both were responsive to a proper plasma level for vitamin C.

At this same time the anaerobic conditions in the tissues will be relieved by the catalytic action of vitamin C acting as a gas transport to aid this cellular respiration. The abnormal acidity of the blood and tissues will be removed and abnormal amounts of free adrenalin will disappear from the blood stream. Following this the constriction of the blood vessels will cease, 'allowing the liver and pancreatic tissue to return to nftrmal function. Continuance of frequent injections of properly calculated doses of vitamin C will restore the normal physiology of the body. This is not all of the story.

Lojkin (1937), studying the various phases of the inactivation of crystalline tobacco mosaic virus by 1-ascorbic acid, suggested that the action was not due to reduced vitamin C nor to the irreversibly oxidized dehydroascorbic acid. Lojkin felt that il was due to a specific intermediate product which is formed in the course of the catalytic auto-oxidation of vitamin C, an action stimulated by the presence of copper ions. This intermediate product must .be a peroxide because a peroxide is formed during copper-catalyzed oxidation of vitamin C. This peroxide is decomposed as rapidly as it is formed (Barrow, De Meio, Klemperer, 1935-36). Lyman and associates (1937) confirmed the peroxide theory by observing that the oxygen uptake, beyond that calculated for the reaction ascorbic acid to dehydroascorbic acid, was not due to further oxidation of dehydroascorbic acid to an irreversible oxidation product, because treatment of the oxidized solution with hydrogen sulfide gave complete recovery of the ascorbic acid. These men also found that copper catalysis accelerates not only the reversible oxidation of vitamin C, but also further oxidation of dehydroascorbic acid. This action of the copper ion elucidates the findings that vitamin C in massive, frequent doses works better in the body than in a laboratory test tube.

Hippocrates declared the highest duty of medicine to be to get the patient well. He further declared that, of several remedies physicians should choose the least sensational- Vitamin C would seem to meet both these requirements.

NOTE:

PubMed gives a different
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compared with the one printed:

Massive doses of vitamin C and the virus diseases. South Med Surg. 1951 Apr;**113**(4):101-7. No abstract available.
PMID: 14855098 [PubMed - indexed for MEDLINE]

J Nutr. 1997 May;127(5 Suppl):962S-965S. doi: 10.1093/jn/127.5.962S.

Oxidants and antioxidants in viral diseases: disease mechanisms and metabolic regulation.

Peterhans E[1].

Author information

Abstract

Reactive oxygen and nitrogen metabolites play a complex role in many diseases and in metabolic regulation. Because viruses replicate in living cells, such metabolites influence the growth of viruses in addition to serving as a host defense mechanism. Low levels of reactive oxygen species (ROS) play a role in mitogenic activation, and the early phase of lytic and nonlytic virus infection indeed resembles that of mitogenic cell activation. In addition to these subtle cell-activating effects shared by many viruses, influenza and paramyxoviruses activate a respiratory burst in phagocytic cells. These viruses are toxic when injected in animals. Cells lavaged from the lungs of mice infected with influenza virus are primed for enhanced superoxide generation. Moreover, xanthine oxidase is enhanced and the buffering capacity of small molecular antioxidants is decreased in the lungs, suggesting that infection leads to oxidative stress. The wide array of cytokines produced in the lungs during influenza could contribute to the systemic effects of influenza. Oxidative stress has also been shown in human immunodeficiency virus (HIV) infection in humans. Via activation of NF kappa B, ROS may activate viral replication, but oxidants are believed to contribute also to the loss of CD4 T cells by apoptosis. Antioxidants, together with agents interfering with the harmful effects of cytokines and lipid mediators, may have a role in the treatment of viral diseases. Such agents could not only alleviate disease symptoms but also

decrease the long-term effects of chronic oxidative stress, which have been linked to the development of cancer in some viral infections.

PMID: 9164274 DOI: 10.1093/jn/127.5.962S

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Paul Meier A Man Behind the Method

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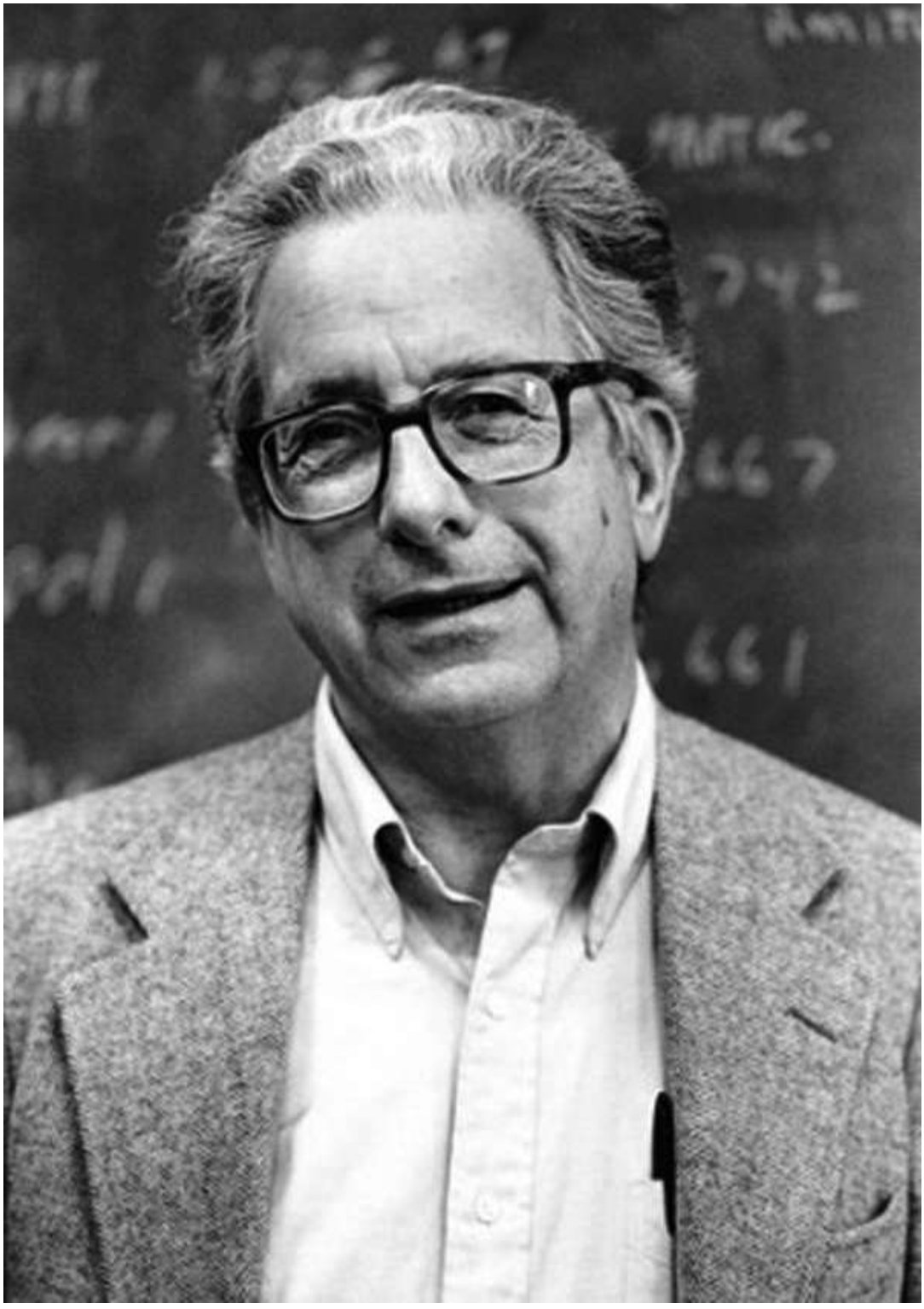

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Paul Meier. Courtesy of the University of Chicago. Printed with permission.

IN 1951, WHEN PAUL MEIER received his doctorate in mathematics from Princeton University and became one of the first statisticians to enter medical research, potential new medical treatments were evaluated in a very different fashion than they are today. At the time, researchers commonly followed practices such as giving a new remedy to patients they thought might benefit from it and comparing the outcomes with other patients who were not treated. In other situations, patients who stopped taking a new medicine might be counted as controls who had never been exposed to it.

PAUL MEIER HAD A PROFOUND IMPACT on how clinical trials now evaluate the efficacy of new drugs and treatment methodologies throughout the world. Meier's tireless promotion of the now-standard practice of randomly assigning patients enrolled in clinical trials to receive either the conventional remedy or the new treatment being evaluated helped ensure its current status as the most rigorous way to gather evidence of a new drug or treatment's effectiveness. Meier also helped formulate the Kaplan-Meier estimator, which is now the most popular method of estimating clinical trial participant survival. It is one of the most widely cited articles in the medical literature.

Meier, who died on August 7, 2011, at the age of 87, had a profound impact on how clinical trials now evaluate the efficacy of new drugs and treatment methodologies throughout the world. Meier's "many published works and writings have had a huge influence on the application of statistics to medical research—particularly the design, conduct, and analysis of randomized clinical trials and in the advancement of evidence-based medicine in general," according to the Society of Clinical Trials, which Meier helped found in 1978.[1]

Meier was tireless in his promotion of the now-standard practice of randomly assigning patients enrolled in clinical trials to receive either the conventional remedy or the new treatment being evaluated. This is now considered the most rigorous way to conduct a study and the best way to gather evidence of a new drug or treatment's effectiveness. "Perhaps more than any other U.S. statistician, [Dr. Meier] influenced U.S. drug regulatory agencies, and hence clinical researchers throughout the U.S. and other countries, to insist on the central importance of randomized evidence," said Sir Richard Peto of Oxford University, who was also a leading advocate for randomization, in Meier's *New York Times* obituary.[2] "That strategic decision half a century ago has already saved millions of lives, and those millions should be attributed to Paul," Peto said.

"I defended randomization every chance I got, and I had a fair number of chances," Meier said in a 2003 interview in the journal *Clinical Trials*.[3](p137) "For a fairly long time randomization was not thought of so highly," he explained. He said that in 2001,

> *a very distinguished statistician told me that I had a major influence on the Food and Drug Administration's policies on randomized clinical trials. I don't know how true that was, but if so, it would be something of which I am very proud,*

adding that his success in encouraging the use of randomization in clinical trials is the achievement he prized most highly.[3](p137)

Together with Edward L. Kaplan of the California Radiation Laboratory, Meier also helped formulate what the Society for Clinical Trials terms "our most popular method of estimating survival functions from continuously observed data."[4] Published in the *Journal of the American Statistical Association*[4] in 1958, it went on to become one of the most widely cited articles in the medical literature. At the time of Meier's death, the Kaplan-Meier article had been cited more than 34000 times. Theodore Karrison, PhD, director of the University of Chicago Department of Health Studies' Biostat Lab and one of Meier's doctoral students, attested to the article's continuing relevance by noting: "If you open up at random a medical journal you're likely to see in at least one of the articles a citation to the Kaplan-Meier paper" (oral communication, December 1, 2011).

Over his long and distinguished career, Meier earned many honors—as well as widespread admiration for being quick on his feet.

> *At professional meetings … he often astonished me by giving comments from the audience, which, though spontaneous, displayed a depth of reasoning and perfect eloquence, which few others could have matched with any amount of advanced preparation,*

recalled Rick Chappell (written communication, November 8, 2011; and oral communication, November 23, 2011), who was Meier's last doctoral student and is now a professor of biostatistics and medical informatics at the University of Wisconsin at Madison.

Through it all, including the stroke in 1995 that robbed him of some of his eloquence, Meier was also a kind and gentle man, according to a statement issued by the Statistics Department at Columbia University,[5] where Meier spent his final years (he also held a joint appointment at Columbia's Mailman School of Public Health). Karrison, Chappell, and Daniel Heitjan, PhD, a professor of biostatistics at the University of Pennsylvania's Perelman School of Medicine, attested that Meier was both widely respected and loved. "He was a person who cared about people … and someone you could go to with a problem," Karrison said.

A RELUCTANT BIOSTATISTICIAN

Meier graduated from Oberlin College in 1945 and went on to Princeton University to pursue a doctorate in mathematics, where he studied under the celebrated mathematician John Tukey. Meier's dissertation project involved a statistical problem suggested by William Cochran, the noted statistician who chaired Johns Hopkins University's Department of Biostatistics from 1948 to 1958. At the time, Meier was also very interested in "the notion that randomization could clear away confounders that you did not know about."[3(p133)] As one of a very few mathematicians focusing on medical applications, Meier recognized the potential value of randomization's application in medicine.[3]

After Meier earned his doctorate, he spent one more year at Lehigh University, where he had been teaching since 1948. Tukey recommended that he accept a position at Hopkins with Cochran, who was enthusiastic about Meier's dissertation.

> *I was a little nervous because by and large, biostatistics was not a field with a lot of mathematics in it, and I wished more or less to be a mathematician,*

Meier said. But when Cochran insisted that going to Hopkins was a good idea, Meier accepted his first position as a statistician.[3]

In those early days, Meier said, "I was looked at with amazement by my medical colleagues," when he brought up the idea of randomization for assessing new medical treatments, he recalled. The physicians would say "'Randomize? We know that this treatment is better than that one,'" he explained. "People who knew and respected me were astounded that I should want to randomize their patients."[3(p133)]

Meier´s Recollections of the Salk Polio Vaccine Trial

The 1954 field trial of Jonas Salk's polio vaccine "was the most elaborate trial that was ever done," Meier recalled. One of the reasons that the trial was so complicated is because polio was very scarce, he explained. "I've not been involved in many trials like that and I've been involved in lots of multicenter studies," he said.[3(p133)]

The situation was further handicapped because the diagnosis of polio is tricky, Meier said. "We need to have the entire country's physicians participate, because we can't look over every case where there's some kind of paralysis. So physicians reported the cases they thought were polio according to the protocol, and we accepted those cases." Meier estimated that "about half those cases were probably not polio at all."[3(p133)]

But the biggest issue, for Meier, emerged during a seminar attended by many of the researchers working on the project, where it became apparent that members of the team were suppressing the data related to some of the test vaccine lots. As soon became clear, the polio virus used in the trial vaccines was not always properly inactivated. Jonas Salk, the vaccine's inventor, "cut out data in order not to show what happened to some lots," Meier charged.[3(p134)] He said that the National Foundation for Infantile Paralysis, which sponsored the study, dropped from its advisory committee scientists who did not agree with how the results were being presented.[3]

The field trial's findings were reported to show the vaccine's effectiveness, over the objections of some of the committee members, Meier said. Soon after, the US Public Health Service reported cases of paralytic polio in children inoculated with the vaccine. The original cases were traced back

to lots produced by Cutter Laboratories, of Berkeley, CA, one of six manufacturers licensed to produce the vaccine. However, Meier said that the problem was more widespread. He said:

> *I got some data from a physician who was working on this, and we found that not only was Cutter wrong, but there were various other companies that had the same polio virus in their samples, although not as much as the samples from Cutter Laboratories. But because there were so many improperly diagnosed cases out there, and because the other manufacturers went around to various newspapers and threatened to cut their advertising, it was dumped on Cutter. Cutter was responsible because they did things in producing and testing the vaccine they were told not to do.*[3(p134)]

Then Meier became involved with the controversial 1954 Salk Polio Vaccine field trials. The Society for Clinical Trials called the polio vaccine trial "the project that put randomized trials on the map in this country" in part because of the key role Meier played by publishing a critical article in *Science* in 1957.[6] The article reviewed "some aspects of the poliomyelitis vaccine testing program which seem to have important implications for scientists generally."[6(p1067)] It indicted both the National Foundation for Infantile Paralysis and the government for withholding information from the participants. It also faulted the testing program for accepting without scrutiny Salk's assertion that the vaccine was "absolute[ly] safe," and for not employing the expensive and difficult tests that had been suggested to ensure that the final product was free of residual live virus. Meier said that many journals turned his manuscript down and their editors warned him that publishing such an article would limit his career path.[3]

Honors and Awards

Meier was named as a fellow of the American Association for the Advancement of Science, the American Statistical Association, the Institute of Mathematical Statistics, the American Academy of Arts and Sciences, the Royal Statistical Society, and the John Guggenheim Memorial Foundation. He served as president of both the Institute of Mathematical Statistics and the Society for Clinical Trials. He was also elected to senior membership in the National Academy of Sciences' Institute of Medicine.[5,11]

He also held temporary appointments as a National Institutes of Health Special Fellow at the University of London and Imperial College; he was a visiting professor at Harvard University and Jerusalem's Hebrew University; and he was a fellow of Stanford University's Center for Advanced Study in the Behavioral Sciences.[5,11]

Although Meier was denied tenure at Hopkins, he succeeded in securing an appointment to the University of Chicago in 1957. He stayed there until 1992, and taught at different schools and departments—including the college, graduate school, law school, and medical school—over the years. For more than a decade, he led the Department of Statistics as chair or acting chair.

In 1958, Meier published his highly cited article describing what is now known as the Kaplan-Meier estimator in the *Journal of the American Statistical Association*.[4] Kaplan was also a student of Tukey at Princeton. Working independently, Meier and Kaplan solved a problem that was dogging medical researchers at the time. The issue revolved around the fact that many participants in clinical trials do not participate in the experiment for the same length of time because of the time required to recruit study volunteers. The Kaplan-Meier statistic enables researchers to take into account observable time of survival and death.

Initially, Meier recalled, both he and Kaplan had submitted separate articles. The publication's editor asked them to collaborate to produce one article. "I swallowed hard, and I guess Kaplan swallowed hard as well," Meier said. "We worked quite hard and at one place he solved a problem that I couldn't solve; other cases I solved problems he couldn't."[3(p133)]

LOVE FOR CLINICAL TRIALS

In the subsequent decades, Meier's stature continued to grow, and he was involved in many clinical trials, which he called his "true love." In addition to helping found the Society for Clinical Trials in the 1970s, he wrote some influential articles about the ethics of performing them.[7,8] In his spare time, Meier enjoyed music, particularly folk songs, and played the flute, recalled Chappell, Heitjan, and Karrison. Meier was also a sailor, and he took out his small sailboat, The Salty Dog, in the waters near his summer home near Lake Michigan during his years at the University of Chicago. After Meier moved to New York City in 1992, he sailed in the Hudson River outside Dutchess County, New York.

Over the course of his 50-plus-year career, Meier's facility for explaining statistical concepts to people outside the discipline resulted in calls to testify before the US Congress and popularity with journalists such as Gina Kolata of the *New York Times*, Chappell remembered. It also made him popular with clinicians, such as the University of Chicago medical school students he taught about clinical trials, Karrison said.

Meier's stroke occurred three years after he retired from the University of Chicago in 1992 and moved to Columbia University. There, he held appointments as both the Howard Levene Professor of Statistics in the statistics department and head of the Mailman School of Public Health's biostatistics department, and he remained active professionally for years after his stroke. "He still kept going to meetings," Karrison recalled. Meier "struggled courageously," added Heitjan, who worked closely with him at Columbia (oral communication, November 22, 2012).

Heitjan collaborated with Meier during the Randomized Evaluation of Mechanical Assistance for the Treatment of Congestive Heart Failure (REMATCH) trial, which began in 1998 and ran through 2001 and involved 20 cardiac transplant centers around the country.[9,10] Although this artificial heart trial was relatively small compared with many drug trials, it was one of the most significant device trials ever conducted, Heitjan said. Meier insisted that the trial needed to be randomized and he refused to allow the group carrying it out to cut corners, Heitjan recalled.

> *Clinical trials in the device world are often small, single-arm trials [where results are compared with historical controls] … in part because a lot of the companies that make devices are small and can't support major trials,*

Heitjan explained. The trial was randomized so it could determine whether the devices could extend and improve the quality of recipients' lives sufficiently to justify the expense of implanting them, he said.

It was the first high-profile randomized clinical trial that Heitjan had worked on, and "having Paul around to be my mentor and guide was very important to me." When the two would attend meetings related to the trial, Meier was quiet most of the time

> *because it was a little harder for him to communicate and get his point across so he had to choose his battles carefully. He would only speak out at what I considered critical moments,*

Heitjan said. Nevertheless it was clear that Meier's understanding of both the technical and political issues in the trial was undiminished, Heitjan said.

Heitjan recalled attending a Society for Clinical Trials meeting with Meier in 1998. One after another, distinguished senior physician–scientists came up to greet Meier, pay homage to him, and testify to how he had opened their eyes to the critical importance of the randomized clinical trial, Heitjan remembered.

"Being with [Meier] lifted you up," Heitjan summarized. Perhaps just as important as his intellect and accomplishments, Meier "was a genuinely good human being," Karrison said. He was a "great and gentle man," Chappell agreed.

Acknowledgments

The author would like to thank Rick Chappell, Daniel Heitjan, and Theodore Karrison for their help in putting together this article.

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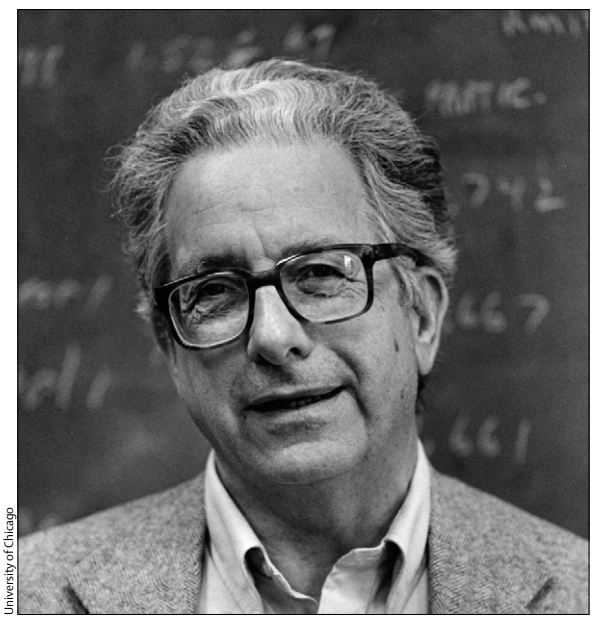

University of Chicago

Paul Meier

Statistician who was a leading proponent of randomised clinical trials and who co-developed a system for estimating survival rates. Born on July 24, 1924, in New York, NY, USA, he died from complications of a stroke in New York on Aug 7, 2011, aged 87 years.

Randomised trials have a prominent place in modern clinical research. Assigning participants in a random way to receive different treatments allows investigators to eliminate bias in their findings. But half a century ago, when Paul Meier was advocating for this approach, his enthusiasm raised eyebrows: "When I said 'randomize' in breast cancer trials I was looked at with amazement by my clinical colleagues", Meier said in a 2004 interview published in the journal *Clinical Trials*. "'Randomize? We know this treatment is better than that one', they said. I said 'Not really…'"

Meier was a leading figure in the generation of statisticians who, during the mid-20th century, helped establish randomisation as a key part of clinical research, says Sir Richard Peto, Professor of Medical Statistics and Epidemiology at the University of Oxford, UK. In doing so, they helped save countless lives. "Perhaps more than any other American statistician, Paul Meier was the one who influenced US drug regulatory agencies, and hence clinical researchers, to insist upon the central importance of randomised evidence", Peto told *The Lancet*.

The son of a chemist and a schoolteacher, Meier graduated from Oberlin College in 1945 with a bachelor's degree in mathematics and physics, before earning a master's in mathematical logic and a doctorate in statistics from Princeton University. After teaching at Lehigh University, he moved to Johns Hopkins University where he began the work that led to one of his major contributions to medical research: the Kaplan-Meier estimator. Meier and Edward Kaplan had independently developed the same elegant method to estimate survival rates, which took appropriate account of the fact that although some patients die at known times, others survive beyond the end of the study. Both submitted the method to the *Journal of the American Statistical Association*, and the editor convinced them to produce a combined paper, which was published in 1958. Kaplan-Meier curves are now widely used in clinical research.

In 1957, Meier moved to the Department of Statistics at the University of Chicago where he remained for 35 years, serving as departmental chairman or acting chairman for more than 10 years. After leaving Chicago, he became Head of Biostatistics at Columbia University. Theodore Karrison, Director of Chicago University's Biostatistics Laboratory, was a student of Meier's who worked with him on multicentre clinical trials and remembers how "Paul was a person who displayed a deep concern for others; he would go out of his way to help people whenever he could, whether it was a struggling student, an individual coping with an illness, or a colleague making a difficult career choice or other decision."

Throughout his career, clinical trials were Meier's "true love", as he put it in the *Clinical Trials* interview. An early and prominent example of his work was his involvement in the US field trials of the Salk polio vaccine in 1954, which Meier, as statistician, ensured included a large number of participants randomly assigned to vaccine or placebo. In doing this, Meier followed in the path of British statistician Sir Austin Bradford Hill, most notably in the well known 1948 Medical Research Council trial of streptomycin in tuberculosis. "Randomisation would probably have been introduced anyway some time around the middle of the century, as it was so essential if moderate differences in treatment efficacy were to be established or refuted reliably", said Peto. "A few investigators had used it or proposed it before Hill did so, but they didn't trigger the avalanche of randomised evidence that Hill triggered and Meier helped propagate."

Meier helped found the Society for Clinical Trials, and was its President in 1986–87. He was also an adviser to the US Food and Drug Administration (FDA), where he could be relied on to demand credible data, says Robert Temple, Deputy Center Director for Clinical Science at the FDA's Center for Drug Evaluation and Research: "I remember Paul as unfailingly polite but quite firm—although I recall no rudeness—and he made his views and disagreements, where necessary, quite visible. He was a powerful force whenever he was present." Meier is survived by his wife of 63 years, Louise Goldstone Meier, and their three daughters and five grandchildren.

Stephen Pincock

Dutch medical association calls halt to euthanasia prosecutions

The Royal Dutch Medical Association wants Justice Minister Winnie Sorgdrager to stop test cases on euthanasia being brought to court, especially those on assisted deaths in neonates. The association's chairwoman, Joke Lanphen, says in the association's magazine, *Medisch Contact*, this week, that she is "very unhappy that juridical clarity has to be obtained at the expense of a few individual doctors' distress".

From this month, the association has introduced new procedures that could form the basis for changes in the law. A crucial move is that a committee of doctors, ethicists, and lawyers has been set up to review selected cases. The association hopes that the results of this project will help them succeed in changing the system to one in which doctors will be subject to the criminal law only when they ignore legal guidelines.

Lanphen refers to the widespread disappointment in medical circles that the way euthanasia is handled in the Dutch legal system—ie, a doctor automatically faces criminal prosecution when he complies with the rules to report non-natural deaths—is inconsistent with the conclusions of all serious reports and discussions that the association has initiated. Because of the attitude of former (Christian Democrat) Justice Minister, Ernst Hirsch Ballin, prosecution officers are holding juridical inquiries into the actions of several doctors. Lanphen wants these inquiries stopped and the charges dismissed. She wants instead talks with Sorgdrager about the minister's suggestion in the evening newspaper *NRC Handelsblad* to create a "medical exception" in the law for doctors who act according to the rules. The effect of the guidelines laid down in law in 1994 on assisted deaths are being examined. The evaluation is expected to be ready in the second half of this year, so that will be the political moment to change the legislators' opinion, says Lanphen.

Marjanke Spanjer

Thomas C Chalmers

Thomas Chalmers, who pioneered the use of randomised control trials (RCTs), died on Dec 27, 1995, aged 78. Despite serious illness he worked with his collaborators world wide almost to the day he died.

I first met Tom 14 years ago, when he was visiting professor at the Harvard School of Public Health, teaching and recruiting young colleagues to projects that critically appraised the existing research. It was hard not to absorb the enthusiasm of this gentleman already at a point in his professional life when many are content to wind down their research career.

Tom Chalmers

A theme running through Tom's scientific life was the posing of challenging questions about the effectiveness of medical practice. He was promoting the use of RCTs at a time when the method was far from accepted in clinical research. A good example of how RCTs can alter long-standing practice based on the observational approach is the 1951 trial that challenged the wisdom of bed rest and diet in the treatment of acute hepatitis.

Tom's lifelong concern was quality of clinical research. For several years he worked on a quality score—still referred to as "Chalmers' quality score"—for assessing trials. Although he did not succeed in validating it, standards of reporting of scientific articles have improved, thanks to his work.

At a time when the issue was largely unrecognised, he published in 1978 a paper critical to our current understanding of the danger of RCTs of inadequate statistical power. In that paper he reviewed 71 "negative" RCTs published in leading medical journals and showed that the vast majority of them could have missed important clinical benefits. This led Tom to become one of the pioneers of the use of meta-analysis in clinical medicine, where he contributed important publications in gastroenterology and cardiology, among others.

In 1992, he introduced the concept of "cumulative meta-analysis". Reviewing RCTs on the treatment of myocardial infarction, he made a strong plea for systematic reviews of clinical trials by showing that medical textbooks often give advice that contradicts results of such reviews.

Amongst all these activities Tom always found time to be generous, supportive, and friendly to many people, especially young colleagues. To me he was a great teacher and an extraordinary example.

Alessandro Liberati

Netherlands seeks heroin for addicts

Will Dutch Health Minister Els Borst-Eilers get permission from Vienna to purchase the 50 kg heroin needed for the planned heroin maintenance programmes? When approved by parliament (see *Lancet* Sept 16, p 761), such pilot programmes will be introduced in Rotterdam and Amsterdam, and perhaps in Arnhem.

In keeping with routine procedure, Borst-Eilers has put in a preliminary request to the UN drugs bureau in Vienna for permission to buy 50 kg heroin, ahead of the formal round, in November, of estimations of need. The Netherlands usually asks for 200g. But there is concern about the dificulties of overcoming objections by the Vienna bureau, known to be conservative and critical. When the Swiss first sought permission in 1993 to obtain heroin for 800 addicts in their maintenance programmes, they had to wait 6 months while every detail of their project was scrutinised.

For the Dutch their first hurdle is to get the Rotterdam and Amsterdam authorities to agree on the design of maintenance programmes. A sticking point is whether to include a "smokeable" form of heroin, especially now that the Swiss have observed complications such as haemoptysis. Making addicts change their habits (to injecting heroin) for the sake of an experiment is thought by some to be unethical.

Marjanke Spanjer

- Published: May 2002

Preventing the common cold with a vitamin C supplement: A double-blind, placebo-controlled survey

- Michael Van Straten[1] &
- Peter Josling B.Sc. Hons.[1]

Advances in Therapy **volume 19**, Article number: 151 (2002) Cite this article

Abstract

One hundred sixty-eight volunteers were randomized to receive a placebo or a vitamin C supplement, two tablets daily, over a 60-day period between November and February. They used a five-point scale to assess their health and recorded any common cold infections and symptoms in a daily diary. Compared with the placebo group, the active-treatment group had significantly fewer colds (37 vs 50, P<.05), fewer days challenged virally (85 vs 178), and a significantly shorter duration of severe symptoms (1.8 vs 3.1 days, P<.03). Consequently, volunteers in the active group were less likely to get a cold and recovered faster if infected. Few side effects occurred with the active treatment, and volunteers reported greatly increased satisfaction with the study supplement compared with any previous form of vitamin C. This well-tolerated vitamin C supplement may prevent the common cold and shorten the duration of symptoms. Volunteers were generally impressed by the protection afforded them during the winter months and the general acceptability

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Recycling of vitamin C by a bystander effect.

Nualart FJ[1], Rivas CI, Montecinos VP, Godoy AS, Guaiquil VH, Golde DW, Vera JC.

Author information

Abstract

Human cells transport dehydroascorbic acid through facilitative glucose transporters, in apparent contradiction with evidence indicating that vitamin C is present in human blood only as ascorbic acid. On the other hand, activated host defense cells undergoing the oxidative burst show increased vitamin C accumulation. We analyzed the role of the oxidative burst and the glucose transporters on vitamin C recycling in an in vitro system consisting of activated host-defense cells co-cultured with human cell lines and primary cells. We asked whether human cells can acquire vitamin C by a "bystander effect" by taking up dehydroascorbic acid generated from extracellular ascorbic acid by neighboring cells undergoing the oxidative burst. As activated cells, we used HL-60 neutrophils and normal human neutrophils activated with phorbol 12 myristate 13-acetate. As bystander cells, we used immortalized cell lines and primary cultures of human epithelial and endothelial cells. Activated cells produced superoxide anions that oxidized extracellular ascorbic acid to dehydroascorbic acid. At the same time, there was a marked increase in vitamin C uptake by the bystander cells that was blocked by superoxide dismutase but not by catalase and was inhibited by the glucose transporter inhibitor cytochalasin B. Only ascorbic acid was accumulated intracellularly by the bystander cells. Glucose partially blocked vitamin C uptake by the bystander cells, although it increased superoxide production by the activated cells. We conclude that the local production of superoxide

anions by activated cells causes the oxidation of extracellular ascorbic acid to dehydroascorbic acid, which is then transported by neighboring cells through the glucose transporters and immediately reduced to ascorbic acid intracellularly. In addition to causing increased intracellular concentrations of ascorbic acid with likely associated enhanced antioxidant defense mechanisms, the bystander effect may allow the recycling of vitamin C in vivo, which may contribute to the low daily requirements of the vitamin in humans.

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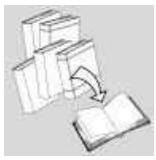
REVIEW



Role of free radicals in viral pathogenesis and mutation

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SUMMARY

Oxygen radicals and nitric oxide (NO) are generated in excess in a diverse array of microbial infections. Emerging concepts in free radical biology are now shedding light on the pathogenesis of various diseases. Free-radical induced pathogenicity in virus infections is of great importance, because evidence suggests that NO and oxygen radicals such as superoxide are key molecules in the pathogenesis of various infectious diseases. Although oxygen radicals and NO have an antimicrobial effect on bacteria and protozoa, they have opposing effects in virus infections such as influenza virus pneumonia and several other neurotropic virus infections. A high output of NO from inducible NO synthase, occurring in a variety of virus infections, produces highly reactive nitrogen oxide species, such as peroxynitrite, via interaction with oxygen radicals and reactive oxygen intermediates. The production of these various reactive species confers the diverse biological functions of NO. The reactive nitrogen species cause oxidative tissue injury and mutagenesis through oxidation and nitration of various biomolecules. The unique biological properties of free radicals are further illustrated by recent evidence showing accelerated viral mutation by NO-induced oxidative stress. NO appears to affect a host's immune response, with immunopathological consequences. For example, NO is reported to suppress type 1 helper T cell-dependent immune responses during infections, leading to type 2 helper T cell-biased immunological host responses. NO-induced immunosuppression may thus contribute to the pathogenesis of virus infections and help expansion of quasispecies population of viral pathogens. This review describes the pathophysiological roles of free radicals in the pathogenesis of viral disease and in viral mutation as related to both nonspecific inflammatory responses and immunological host reactions modulated by NO. Copyright © 2001 John Wiley & Sons, Ltd.

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INTRODUCTION

To date, much attention has been paid to the pathogenic roles of free radicals produced in excess in various pathological settings. Free radical species are potentially reactive because of the physical instability of oxygen- or nitrogen-based unpaired electrons in their orbits, which leads to a number of deleterious pathological consequences *in vivo*. Among a series of free radicals, superoxide anion radical (O_2^-) and nitric oxide (NO) are now considered to be the most biologically relevant elements derived from hosts during microbial infections [1–7]. During the past decade, considerable evidence has revealed unique and diverse biological functions of NO, a gaseous nitrogen-centred inorganic free radical produced endogenously in a number of cells and tissues [8–10]. NO and reactive oxygen species, including O_2^-, hydrogen peroxide (H_2O_2) and hypochlorite anion (OCl^-), are generated by infiltrating phagocytic cells and xanthine oxidase (XO) expressed in inflamed tissues [6,7,11–15]. They are believed to contribute to nonspecific (innate) and immunological host defence as well

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Abbreviations used:
CGD, chronic granulomatous disease; CMV, cytomegalovirus; CTL, cytotoxic T lymphocyte; DTCS, (N-dithiocarboxy)sarcosine; EMCV, encephalomyocarditis virus; ESR, electron spin resonance; GFP, green fluorescent protein; HBV, hepatitis B virus; HIV, human immunodeficiency virus; HNO_2, nitrous acid; H_2O_2, hydrogen peroxide; HSV, herpes simplex virus; IFN, interferon; IL, interleukin; iNOS, inducible nitric oxide synthase; $iNOS^{-/-}$, iNOS deficient (knockout) mouse; L-NMMA, N^{ω}-monomethyl-L-arginine; MMP, matrix metalloproteinase; MPO, myeloperoxidase; NO, nitric oxide; NO^+, nitrosonium cation; NO_2, nitrogen dioxide; N_2O_3, dinitrogen trioxide; O_2^-, superoxide anion radical; OCl^-, hypochlorite anion; ·OH, hydroxyl radical; $ONOO^-$, peroxynitrite; SeV, Sendai virus; SOD, superoxide dismutase; TBE-V, tick-borne encephalitis virus; Th, helper T cell ($CD4^+$); XO, xanthine oxidase

[1–7]. It is now well accepted that the chemical and biological reactivities of NO produced in environments such as inflamed tissues are greatly affected by concomitantly formed oxygen radicals, particularly O_2^-, via the formation of reactive nitrogen oxides such as peroxynitrite ($ONOO^-$) [16–21]. These reactive nitrogen intermediates, rather than NO or O_2^-, seem to be involved in the pathogenesis of various diseases. The pathophysiological action of $ONOO^-$ is particularly important for pathogenesis of virus infection, because $ONOO^-$ is not only a potent oxidant but also a nitrating agent of proteins, nucleic acids and membrane unsaturated lipids [16–18,22,23]. In addition, reactive nitrogen oxides formed endogenously during virus infection have a potential impact on mutagenesis of both the intruding viruses and the hosts, as well as causing host cell and tissue injuries by induction of oxidative stresses.

A major goal in medical microbiology is a general understanding of the mechanisms of host–pathogen interactions, which determine the pathological consequences of infection. An understanding of host–pathogen interactions at the molecular level requires the characterisation of host-derived small radical molecules, which appear to play an important role in the pathogenesis of virus infection. An emerging concept related to free radicals will help us to gain insight into the molecular mechanisms of pathological events occurring as a result of interactions between viruses and hosts [11–15]. In this review, I place particular emphasis on the host response to various virus infections, in view of the pathological consequences, such as oxidative tissue injuries and viral mutations, that result from overproduction of free radicals during virus infection.

INDUCTION OF OXYGEN RADICALS AND PRODUCTION OF NO IN VIRUS INFECTION

It is now well documented that O_2^- and NO production is elevated in inflamed tissues. O_2^- and its related reactive oxygen intermediates are generated by two components of the host response: cellular reactions, mediated by inflammatory phagocytic cells such as neutrophils and macrophages expressing phagocyte NADPH oxidase and humoral responses involving xanthine oxidase (XO). Host reactions occur in response to foreign matter, microorganisms and damage caused by trauma, radiation or ischaemia–reperfusion injury. Because the genetic deficiency of components of an O_2^--generating NADPH oxidase in phagocytic cells gives rise to chronic granulomatous disease (CGD), which is associated with severe chronic bacterial infections, oxygen radical formation is important in antimicrobial actions of the host [24,25]. However, excessive production of O_2^- induces lipid peroxidation, membrane damage, mitochondrial dysfunction and inflammatory and ischaemia–reperfusion injuries [26–28]. A high production of O_2^- is most clearly observed in murine pneumonia caused by influenza A virus, Sendai virus (SeV) and cytomegalovirus (CMV) [11,12,29–31]. Experimental evidence shows that O_2^- contributes to the pathogenesis of viral disease, because inhibitors of O_2^- effectively improve lung pathology and survival in viral pneumonia. Evidence indicates that O_2^- itself is not the molecular species that causes the pathological effects but is a precursor of a more potent oxidant such as hydroxyl radical (·OH) [32,33]. Earlier studies indicated that O_2^- might function as a reducing agent for ferric iron, forming ferrous iron to act as a catalyst for the production of highly reactive ·OH from H_2O_2 [32,33]. Because ·OH was suggested to mediate cell and tissue damage, at the initial stage of our study of viral pathogenesis almost a decade ago we sought to identify ·OH generation in influenza virus-infected mouse lung by electron spin resonance (ESR), but no proof of appreciable ·OH generation was obtained (Akaike *et al.*, unpublished observation).

Of great interest are the similarities in the physiological and pathophysiological effects of O_2^- and NO, such as host defence and oxidative stress, although NO has much more complicated and diverse functions than does O_2^- [8,14,17,18] Both free radicals are often generated concomitantly in inflammatory and infectious sites and from the same cellular origins in the host. For example, rapid and transient production of O_2^- from phagocytes is triggered by appropriate membrane stimulation leading to a respiratory burst in which O_2 is consumed [7]; XO generates constant O_2^- generation together with H_2O_2, depending on the supply of the substrates hypoxanthine/xanthine plus O_2 [11,28–30]. Elevated levels of O_2^- produced by both phagocyte NADPH oxidase and XO occur during virus infections *in vitro* and *in vivo* [29–31,34,35].

In contrast, overproduction of NO is mainly

caused by inducible NO synthase (iNOS), which is usually expressed by inflammatory phagocytic cells and other types of cells (e.g. epithelial and neuronal cells) [1–3,8,9]. iNOS produces a much larger amount of NO (i.e. 10–100 times more) for a longer time than do the other two constitutive enzymes, neuronal NOS and endothelial NOS.

It seems that iNOS is ubiquitously expressed during host responses to viral replication *in vivo*. iNOS expression is observed in human diseases caused by human immunodeficiency virus-1 (HIV-1) and hepatitis B virus (HBV) [36,37]. It is induced in a variety of experimental virus infections in rats and mice, including infections with neuroviruses, such as Borna disease virus, herpes simplex virus type 1 (HSV-1) and rabies virus, and pneumotropic and cardiotropic viruses, such as influenza virus, SeV and coxsackievirus [12–15,38–45]. For example, iNOS is expressed by exudate macrophages and bronchial epithelial cells in lung tissues infected with either influenza virus or SeV in mice; the high output of NO has been clearly identified and quantified by ESR spin trapping with the use of a dithiocarbamate–iron complex [13–15,43–45]. NO–dithiocarbamate–iron adducts with a triplet hyperfine structure of g perpendicular 2.04 are generated (Figure 1). The production of these adducts is completely nullified by pharmacological inhibition of NOS by the use of N^{ω}-monomethyl-L-arginine (L-NMMA) or by genetic disruption of iNOS [43–45], indicating that excessive production of NO is due to localised iNOS expression in the tissues infected with virus.

iNOS induction in virus infection is mediated by proinflammatory cytokines such as interferon-γ (IFN-γ) (Figure 2). IFN-γ is known to be associated with type 1 helper T cell (Th1) responses. In pneumonia induced by influenza virus or SeV, NO production is greatly attenuated in IFN-γ-deficient mice (Akaike *et al.*, unpublished observation). Furthermore, the iNOS-inducing potential in bronchoalveolar lavage fluid in influenza virus pneumonia is attributable solely to IFN-γ, as revealed by an immunoadsorption study using a specific anti-IFN-γ antibody [43]. These results strongly support the suggestion that IFN-γ is a major cytokine inducing iNOS and NO overproduction in the pathogenesis of virus infection.

Downregulation of iNOS expression is also reported for some cytokines, e.g. interleukin (IL)-4, IL-10 and transforming growth factor-β [46–48]. In addition, these suppressor cytokines may reduce NO production indirectly via induction of arginase [49–51], which diminishes the supply of the substrate (L-arginine) for iNOS. Because IL-4 and IL-10 are induced by type 2 helper T cell (Th2) responses, iNOS expression may be regulated by a balance between Th1 and Th2 responses involved in the host immune response to the intruding virus. In fact, in our influenza model, induction of IL-4 seems to be inversely related to INF-γ and iNOS induction in virus-infected lungs, suggesting downregulation by IL-4 of NO overproduction [13]. Induction of arginase 1 mRNA has been identified in virus-infected lung, and the time profile of its induction paralleled the induction of IL-4 (our unpublished observation). Therefore, iNOS expression and the resultant NO biosynthesis seem to undergo elegant regulation by a polarised Th1–Th2 balance (Figure 2).

In some viral diseases, viral replication or viral components directly induce iNOS without mediation by proinflammatory cytokines (Figure 2). iNOS expression in HIV-1 encephalitis is of particular interest in this regard [36]. An envelope glycoprotein of HIV, gp41, triggers iNOS expression in human astrocytes and murine cortical brain cells in culture [52,53]. Thus, NO produced by iNOS may contribute directly to the pathogenesis of HIV-associated dementia and cardiomyopathy as well [36,52–55]. Similarly, the human paramyxovirus respiratory syncytial virus directly upregulates iNOS in human type 2 alveolar epithelial cells (A549 cells) through a pathway independent of proinflammatory cytokines [56]. It is also interesting that double-stranded RNA (dsRNA) formed during viral replication upregulates iNOS in human respiratory epithelial cells by triggering dsRNA-activated protein kinase coupled with nuclear factor-κB and IFN regulatory factor 1 activation [57]. There are therefore two pathways for iNOS induction in virus infections: cytokine-dependent mechanisms and direct upregulation by virus.

VIRUS-INDUCED OXIDATIVE STRESS CAUSED BY FREE RADICALS AND ITS MOLECULAR MECHANISM

NO has antimicrobial activity against bacteria, parasites and fungi [1–7,58–63]. NO itself,

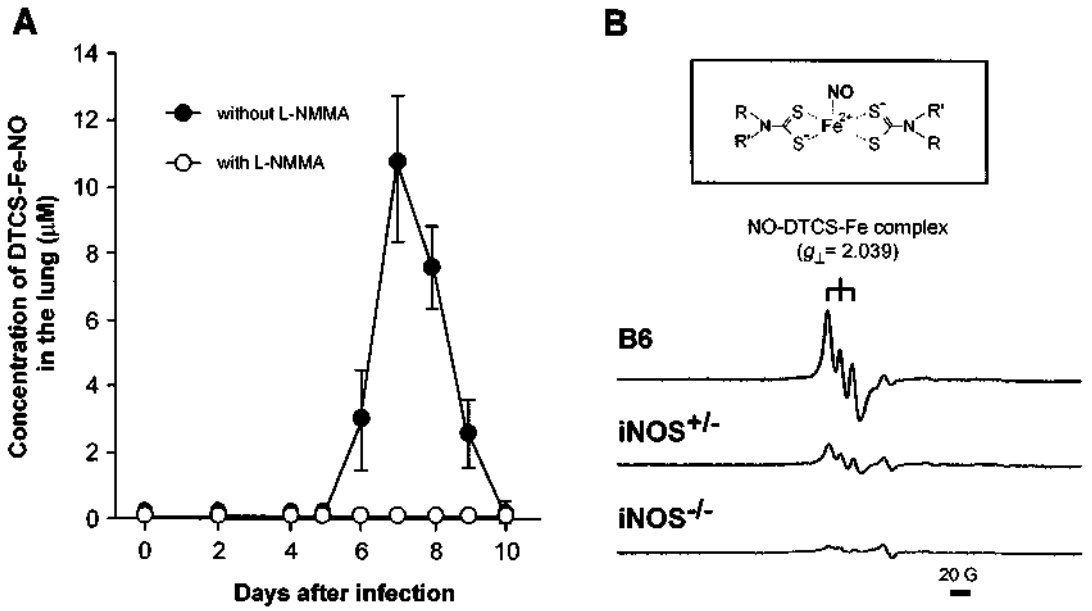

Figure 1. (A) Time profile of NO production in the lung after influenza virus infection. Influenza infection in mice was produced by inhalation of $2 \times LD_{50}$ of influenza A virus. The amount of NO generated in the lung with or without L-NMMA treatment was quantified by ESR spectroscopy (110 K) with (*N*-dithiocarboxy)sarcosine (DTCS)-Fe^{2+} complex as a spin trap. L-NMMA (2 mg/mouse) was given i.p. to mice 2 h before ESR measurement. Data are mean ± SEM ($n = 4$). (B) NO signals as identified by ESR spectroscopy with DTCS-Fe^{2+} complexes in influenza virus-infected lung (7 days after virus infection). Wild-type mice (C57BL/6, B6), iNOS heterozygotes ($iNOS^{+/-}$) and mice deficient in iNOS ($iNOS^{-/-}$) were infected with influenza virus in the same manner as in (A). The chemical structure of the adduct is shown at the top of the figure. Adapted from Akaike *et al.* [12,15] with permission from Blackwell Science and Society for Experimental Biology and Medicine

however, has a limited bactericidal effect, and NO-dependent antimicrobial actions are expressed by other reactive nitrogen oxides such as $ONOO^-$, nitrogen dioxide (NO_2), dinitrogen trioxide (N_2O_3), and nitrosothiols [nitrosonium cation (NO^+) adducts of sulphhydryls] [64–69]. Also, antiviral effects of NO are known for some types of virus, most typically DNA viruses such as murine poxvirus (ectromelia virus) and herpesviruses including HSV and Epstein–Barr virus, and some RNA viruses such as coxsackievirus [58,70–75].

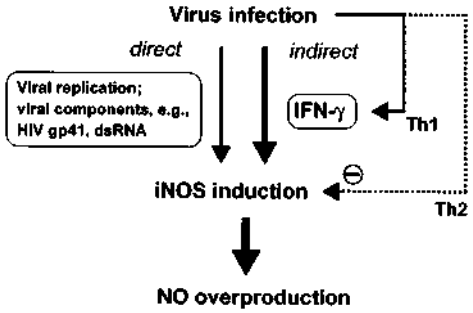

Figure 2. Mechanisms of iNOS induction in viral diseases. In many virus infections, iNOS expression appears to be regulated indirectly via interferon-γ (IFN-γ) induction, which depends on the Th1 response. The host's Th2 response, in contrast, down-regulates iNOS induction. Direct iNOS induction may occur in some cases, such as with respiratory syncytial virus, HIV-1 (gp41), and viral replicative intermediate dsRNA. Modified from Akaike and Maeda [15] with permission from Blackwell Science

Activity of NO against other viruses remains unclear, however. Recent reports suggest that NO has no appreciable antiviral effect on several types of viruses such as ortho- and paramyxovirus, murine vaccinia virus, coronavirus (mouse hepatitis virus), lymphocytic choriomeningitis virus, murine encephalomyocarditis virus (EMCV), tick-born encephalitis virus (TBE-V) and others [76–81]. This lack of antiviral activity of NO has been verified in murine pneumotropic virus infections caused by influenza virus and SeV in a series of our *in vitro* and *in vivo* studies (Akaike *et al.*, unpublished observation) [43,45]. More importantly, antiviral host defence is not impaired by pharmacological interventions resulting in

NOS inhibition or by genetic iNOS deficiency in mice infected with either influenza virus or SeV [43,45]. Such NO inhibition and lack of NO biosynthesis, however, significantly reduce the pathological consequences of various virus infections including viral pneumonia in mice caused by influenza virus, SeV and HSV-1; HSV-1-induced encephalitis in rats; EMCV-induced carditis and diabetes; and murine encephalitis induced by flavivirus (Murray Valley encephalitis virus; TBE-V) [43–45,77,81–85]. It is thus conceivable that NO is not entirely an antiviral molecule, but it can be pathogenetic in various, if not all, virus infections. A similar pathogenicity with a lack of antiviral effect is observed for O_2^- in several experimental models of virus-induced pneumonia including those caused by influenza virus and CMV [11,12,29–31,86].

What are the molecular mechanisms related to the NO- and O_2^--dependent pathogenesis of certain virus infections? Both O_2^- and NO are inert radicals and are much less reactive compared with other naturally occurring oxygen and alkyl radicals [16–18,20,21,32,33,64–69]. Oxidised nitrogen intermediates are formed via pathways mediated by heavy metal ions, molecular oxygen (O_2), O_2^- and peroxidases [e.g. myeloperoxidase (MPO)], and their biological consequences are summarised in Figure 3 [17,18,64,68,69,87–89]. Of the complex chemistry of NO, the most important and biologically relevant reaction is the formation of $ONOO^-$ via a very rapid radical coupling with O_2^- ($NO + O_2^- \rightarrow ONOO^-$: $k = 6.7 \times 10^9\ M^{-1}s^{-1}$) [16–18,20,21]. Although NO can function as an antioxidant, particularly in lipid peroxidation [18], it also has indirect prooxidant activity after conversion to a strong oxidant and is a potent nitrating agent ($ONOO^-$) causing oxidative stress [17]. In addition, although NO and nitrosothiols show strong anti-apoptotic effects [69,89], $ONOO^-$ induces apoptosis, possibly via mitochondrial damage leading to cytochrome *c* release [19,90]. The reaction between NO and O_2^- takes place in virus-infected inflammatory tissues, leading to the formation of $ONOO^-$. $ONOO^-$ nitrates aromatic organic compounds such as tyrosine very effectively, so that nitration of free or protein-bound tyrosine to give 3-nitrotyrosine can serve as a footprint of $ONOO^-$ formed *in vivo* [17,20,21]. Indeed, immunohistochemical analysis with antinitrotyrosine antibody shows positive staining in macrophages and neutrophils infiltrating the alveoli and interstitial tissues, as well as in inflammatory intraalveolar exudate

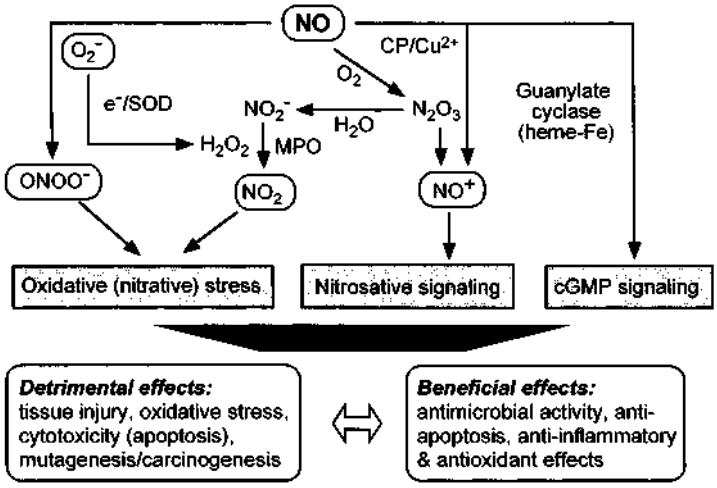

Figure 3. Mechanisms of formation of various reactive nitrogen intermediates from NO and their biological effects. Reactive nitrogen oxides are produced by interactions of NO with molecular oxygen (O_2), active oxygen and oxygen radicals such as O_2^- and H_2O_2 and heavy metals (particularly iron and copper). $ONOO^-$ and NO_2 mediate oxidative and nitrative stresses through oxidation and nitration of various biomolecules including protein, lipid and nucleic acid [16-21]. NO_2 is generated via oxidation of nitrite catalysed by peroxidases such as myeloperoxidase (MPO) (plus H_2O_2) from neutrophils [137]. Ceruloplasmin (CP) and copper ion catalyse one-electron oxidation of NO to form nitrosonium cation (NO^+), which is involved in nitrosative signalling [69,88]. The best known NO-dependent pathway is mediated by cyclic guanosine 3′,5′-monophosphate (cGMP), which is produced by soluble guanylate cyclase activation by NO-heme iron binding in the vicinity of the catalytic site of the enzyme [138]

from virus-infected lung in our experimental models [43,45], which provides indirect evidence of $ONOO^-$ generation during virus infection.

In addition to causing various pathological events in virus infections, such as host cell apoptosis and necrosis, $ONOO^-$ may be involved in NO-induced suppressive effects on immune effector cells such as macrophages and lymphocytes, as described in detail in a later section. We also found that $ONOO^-$ activates matrix metalloproteinases (MMPs), which are involved in extracellular tissue damage and remodelling [91]. Oxidative injury in virus-infected tissues may thus be mediated by $ONOO^-$-induced MMP activation. In fact, remarkable improvements in pathological conditions in the lung and in the survival rate of virus-infected mice were observed with L-NMMA treatment, with the use of the O_2^- scavenger superoxide dismutase (SOD) and the XO inhibitor allopurinol, and when there was a genetic lack of NOS expression [29–31,43,45,77,82,86]. Furthermore, a therapeutic effect on influenza pathogenesis was found with a selenium-containing organic compound, ebselen (unpublished observation), which shows potent $ONOO^-$-scavenging action [92]. These beneficial effects of suppression of $ONOO^-$ generation indicate that $ONOO^-$ could be an important molecular species responsible for the pathogenesis of viral diseases.

It was recently suggested that NO and O_2^- contribute in concert to antimicrobial host defence [3,6,66]. These oxygen and nitrogen reactive intermediates, however, cannot discriminate between exogenous invading pathogens and the hosts themselves, so they function as mediators of nonspecific innate defence against various microbes. Autotoxicity can also occur so that host organisms discard expendable parts. To minimise such self-sacrifice during the elimination of pathogens, a host has primitive tactics, using recruited phagocytes, for physical containment of pathogens in infectious foci (Figure 4, right panel). Most bacteria, for example, can be phagocytosed and confined to septic foci, which are typically abscesses or granulomas. Therefore, chemically reactive NO, O_2^- and $ONOO^-$ can affect bacteria rather selectively; the surrounding normal tissue remains intact. In virus infections, in contrast, free radical mediators cause nonspecific oxidative damage in virus-infected tissue and produce oxidative stress, because virus cannot be confined to limited areas by the nonspecific host defence mediated by phagocytes, NO and O_2^- (Figure 4, left panel) [12–14]. Oxidative stress induced by free radical generation during virus infections may thus cause deleterious events in host–pathogen relationships.

FREE RADICAL-INDUCED VIRAL MUTATION AND ITS POTENTIAL ROLE IN VIRAL EVOLUTION

Among the pathological effects associated with oxidative stress, the mutagenic potential of oxygen radicals and NO for microbial pathogens is highly intriguing. As described in earlier sections, overproduction of NO and oxygen radicals appears to be a common phenomenon in various infections. The resultant reactive molecular species such as $ONOO^-$ nonselectively affect the host's cells and tissues. Obviously, such host defence effectors are originally produced to kill the intruding pathogens, which then suffer oxidative stress because of the host. It may therefore be logical to assume that mutagenesis of various pathogens occurs during infections in biological systems as a result of host defence.

It was previously shown that human leukocytes producing O_2^-, but not leukocytes from patients with CGD, are mutagenic for *Salmonella typhimurium* TA100 [93]. Also, the degree of RNA virus mutation was reported to be increased by chemical mutagens including nitrous acid (HNO_2) [94–97], although the degree of mutation appears to be slight compared with that of spontaneous viral mutation [98]. HNO_2 is an oxidised metabolite that can be formed from N_2O_3 ($N_2O_3 + H_2O \rightarrow 2\ HNO_2$) via reaction of NO_2 and NO during the oxidation reaction of NO by O_2 in biological systems (cf. Figure 3), and it is involved in nitrosylation, oxidation and deamination reactions, at least *in vitro*. However, because of the low pKa (3.3) of HNO_2 and the strong buffering actions of biological fluids, HNO_2 after generation would be neutralised to form NO_2^-, which is much less reactive and is more stable at physiological pH. The chemical reactivity of HNO_2 would thus be greatly limited.

In contrast, as described above, $ONOO^-$ formed via O_2^- and NO generation during infections shows potent nitrating and oxidising potential for many biomolecules including nucleic

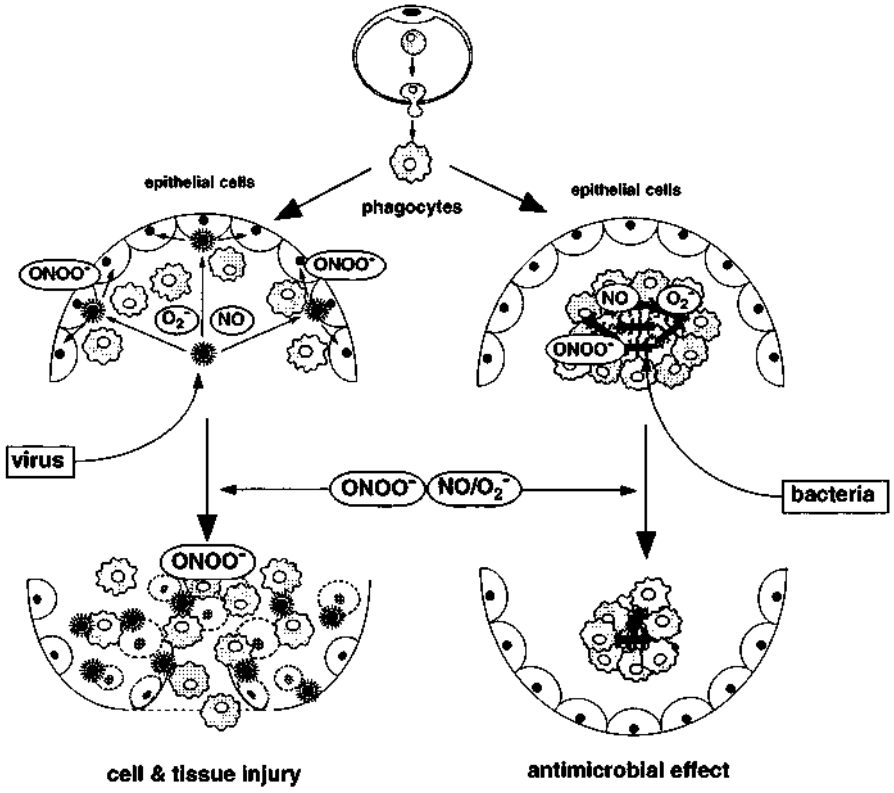

Figure 4. Schematic drawing of the different mechanisms of biological effects of free radicals such as O_2^- and NO, and their product $ONOO^-$, in virus and bacterial infections. Adapted from Akaike *et al.* [12] by copyright permission from Society for Experimental Biology and Medicine

acids [17,18,22,23]. $ONOO^-$ has mutagenic effects on prokaryotic DNA, possibly via nitration of guanine residues of DNA [99]. A typical base substitution caused by $ONOO^-$ is G to T transversion, which is an indirect result of depurination of nitroguanine in DNA [22,23]. A recent study by Wogan's group documented that a high output of NO induced mutations in an endogenous hypoxanthine-guanine phosphoribosyltransferase (*hprt*) gene of murine macrophages expressing iNOS [100]. Genetic analysis of the mutated gene induced by NO indicated that the NO-associated mutational spectrum was similar to that arising spontaneously, but small deletions and insertions were found in the NO-induced mutant gene. The same group showed that mutagenicity is enhanced with NO overproduction *in vivo*, as assessed by mutation of an exogenously expressed *lacZ* by using *lacZ*-containing pUR288 plasmid-transgenic mice [101]. Also important, Ohshima's group reported that p53 is inactivated by $ONOO^-$, which may indirectly increase genetic mutation related to oxidative damage of DNA [102]. Excess production of NO by iNOS induced by inflammatory cytokines, possibly through reactive nitrogen intermediates (particularly $ONOO^-$), caused DNA damage and impaired DNA repair in human cholangiocarcinoma cells, as assessed by the comet assay, suggesting NO-dependent development and progression of cholangiocarcinoma [103].

It has been known for a long time that many naturally occurring mutagens and carcinogens may act as free radical generators [104]. Moreover, oxygen radicals and reactive oxygen species, as endogenous initiators of DNA damage and mutation, are involved in multiple stages of carcinogenesis [105–108]. Free radical species such as O_2^- and NO are thus considered to be potent endogenous mutagens that may be implicated in the pathogenesis of numerous diseases or states involving DNA degeneration, e.g. cancer and aging.

The most striking feature of a virus is its considerable adaptability to various environmental

stresses [109,110]. Viruses containing RNA as their nucleic acid include a number of important pathogens causing various diseases in humans, animals and plants. RNA viruses exist as highly heterogeneous populations called quasispecies, primarily because of the error-prone nature of the replicase of the viruses. In fact, RNA viruses share a high mutation rate, ranging from 10^{-5} to 10^{-3} misincorporation/nucleotide site/round of copying, which is more than 10^4-fold higher than the rate error for DNA viruses [109–112]. The low fidelity of RNA replication is believed to be due to the lack of proofreading and repair functions of RNA polymerase or reverse transcriptase [109,113]. Our recent preliminary study, however, showed that RNA is chemically unstable, so that base modifications via $ONOO^-$-induced oxidation and nitration occur more readily in viral RNA than in eukaryotic DNA (unpublished observation). Thus, the higher incidence of erroneous viral RNA replication may be partly due to RNA's greater susceptibility to oxidative damage compared with DNA.

Only a few reports have explored a possible association between oxidative stress and viral mutation, however. A previous study indicated that oxidative stress augmented the integration of duck HBV DNA into genomic DNA in cells by means of DNA damage and impairment of DNA repair [114]. Although this increased integration is related to proto-oncogene activation induced by hepatitis virus during carcinogenic processes rather than related to viral mutation, it may suggest that oxidative stress causes molecular alteration of viral DNA through mutagenic activities. Beck *et al*. showed that the pathogenicity of coxsackievirus B3 is strongly potentiated *in vivo* in mice fed a selenium-deficient diet [115]. More important, an avirulent strain of the virus is converted to a potent cardiotoxic variant during infection in selenium-depleted animals. The deficiency of selenium may result in an ineffective antioxidant system, e.g. low levels of glutathione peroxidase. The results of similar studies extended to animals deficient in vitamin E and glutathione peroxidase suggest that oxidative stress facilitates selection and generation of virulent mutants [116]. More specifically, the impaired immunological viral clearance related to oxidative stress may cause increased survival of heterogeneous mutants, resulting in the selection of highly pathogenic variants of coxsackievirus [117]. In this context, it is of great interest that NO has an immunosuppressive effect by means of modulation of the T cell immune response during virus infection, as described in the next section of this article.

Many methods are available for estimating viral mutation, including measurement of mutation frequencies of phenotypic variations such as temperature-sensitive growth, plaque morphology, host range and pathogenicity. These criteria, however, cannot be used for accurate and quantitative assessment of viral mutation, because such phenotypic variants often contain multiple base alterations in different genes [118]. Identification of the escape mutant from neutralising antibody is much more reliable for the quantification of viral mutation. For example, escape of a virus from a particular neutralising monoclonal antibody occurs by a single base substitution, leading to a single codon change on the epitope. The frequency of escape mutants thus determined in cultured cells *in vitro* was within the same range, $\sim 10^{-4.5}$, for four different negative-strand RNA viruses: i.e. SeV, vesicular stomatitis virus, Newcastle disease virus and influenza A virus [119,120]. Nevertheless, selection via antibody is not entirely established to be definitive and reproducible, because the frequencies fluctuate greatly, even within a given virus species, depending on the antibodies used for the selection [118]. This selection method has another flaw: it is not used for *in vivo* studies because of the natural immunological selection of the escape mutants during a host's immune response.

We therefore sought to develop a quantitative assay that is applicable to *in vivo* study of mutagenesis [45]. A recombinant SeV was constructed with an exogenous genome, green fluorescent protein (GFP), for the virus. Base substitutions occurring in the GFP in SeV, whether synonymous or non-synonymous, are primarily neutral and do not affect viral replication and clearance of virus from the host. Viral mutation is readily quantified, based on the loss of strong fluorescence caused by GFP gene mutations. This GFP-based assay is convenient and useful for estimating *in vivo* viral mutagenesis. Our recent study thus verifies, for the first time, that oxidative stress induced by a high output of NO accelerates are mutation of the RNA virus [45]. By using the GFP-based mutation analysis and iNOS-deficient

($iNOS^{-/-}$) mice, we clearly showed that oxidative stress induced *in vivo* by NO in wild-type mice remarkably increases and accelerates viral mutation rates compared with the situation in $iNOS^{-/-}$ mice (Figure 5A). The same method used in cultured cells revealed the strong mutagenic potential of $ONOO^-$ (Figure 5B).

This process of accelerated mutation may occur in other virus infections *in vivo*. For example, NO-induced oxidative stress may cause greater heterogeneity of variants of RNA viruses including HIV and influenza virus, leading to rapid viral evolution under selective pressure and to the production of drug-resistant and immunologically tolerant and cell tropism-altered mutants [121]. We now know that NO and O_2^- and hence $ONOO^-$ and other reactive molecular species such as NO_2, OCl^- and H_2O_2 are generated universally as a result of host responses during infections. Therefore, we may expect such chemical mutagenesis in DNA viruses, bacteria and even host cells, although it may not be as effective as that in single-strand RNA viruses.

SUPPRESSIVE EFFECTS OF NO ON IMMUNOLOGICAL RESPONSES DURING VIRUS INFECTION

The effect of oxidative stress on the host immune response is another important facet of viral pathogenesis and mutation. There is growing awareness of the unique immunoregulatory function of NO, which appears to be mediated through cytotoxic or suppressive effects of NO on particular subsets of immune cells [3,122–124]. Th cells, divided into two subsets (Th1 and Th2), protect hosts from intruding viral pathogens via virus-specific Th1 responses, potentiation of $CD8^+$ cytotoxic T lymphocyte (CTL) activity, and B cell proliferation [125,126]. It has been suggested that NO affects the polarised Th1–Th2 response, causing a Th2-biased immunoregulatory balance, via a relatively specific suppressive effect on Th1 subpopulations [122–124]. Such NO-induced immunomodulation occurs during virus infection in mice, as revealed by recent studies of HSV-1 and influenza virus infections [77,127], although such immunoregulatory effects of NO on the Th1–Th2 balance are commonly observed only with specific viruses, not all viruses [76,78]. These biased Th2 responses are clearly demonstrated by using $iNOS^{-/-}$ mice, which show enhanced Th1 immune responses after virus infections [77,127]. NO seems to downregulate the Th1-associated cytokine IFN-γ, which is a major iNOS-inducing cytokine in virus infections as described above, and CTL responses as well, possibly through the suppression of IL-12 production [128–130].

In noncytopathic virus infections CTLs, rather

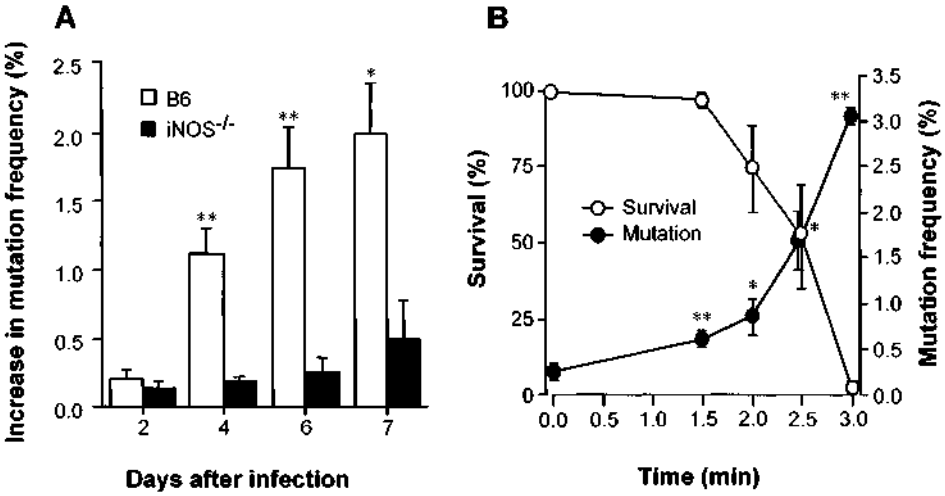

Figure 5. NO-dependent SeV mutation as revealed by genetic mutation of GFP in a recombinant SeV (GFP-constructed SeV, GFP-SeV). (A) The mutation frequency of the virus (GFP-SeV) isolated from the lung of wild-type B6 mice and $iNOS^{-/-}$ mice was quantified by use of the GFP-based mutation assay. (B) Increase in mutation frequency of SeV by $ONOO^-$. GFP-SeV was treated in a constant-flux $ONOO^-$ (0.8 μM) system, and the mutation frequency was determined by the GFP-based mutation assay. Data are mean ± SEM ($n = 4$). *$p < .05$, **$p < .01$, compared with controls or $iNOS^{-/-}$ mice (*t*-test). Adapted from Akaike *et al.* [45] by copyright permission from Federation of American Societies for Experimental Biology

than Th1–Th2 cells, are important for antiviral host defence [125,131]. However, some types of viruses such as influenza virus can be eradicated without the help of CTLs [132]. For influenza virus, a virus-specific Th1 response is more important for antiviral defence than are Th2 responses, because Th2 cells exacerbate pathological lung reactions in influenza pneumonia [133]. In this context, Karupiah *et al*. reported that NO impairs the anti-influenza virus response of the host by suppressing Th1-dependent IFN-γ induction [77]. However, it has now been demonstrated that IFN-γ, a Th1-dependent cytokine, is eventually inefficient in clearance of influenza virus from infectious foci [134]. Our recent experiments using iNOS$^{-/-}$ mice indicate that clearance of virus from lungs infected with either influenza virus or SeV is not affected by a lack of iNOS expression (Akaike *et al*., unpublished observation) [45]. In fact, iNOS$^{-/-}$ mice recuperate from viral pneumonia much better than do wild-type animals, because of reduced levels of oxidative stress in virus-infected tissues [45]. Therefore, not only NO-induced Th1 suppression but also NO-induced oxidative injury may be attributable to pathogenesis of infection with certain viruses that are resistant to the direct antiviral actions of NO.

In addition, NO seems to have profound immunosuppressive and immunopathological effects, most typically in *Mycobacterium avium* and *S. typhimurium* infections [4,135,136], which may be due to NO-induced cytotoxic effects on immune effector cells such as macrophages. Similar immunosuppression by NO is clearly demonstrated with vaccinia virus-infected murine macrophages, which show a loss of antiviral activity because of inhibition of IFN-α/β production by NO [80].

In summary, NO has complex roles in immunological host responses to viruses. The immunosuppression caused by NO may result from NO-induced oxidative stress on professional immune effector cells such as T cells and macrophages. An immunocompromised state of the host caused by NO production not only may enhance the pathogenicity of the virus but also may help the generation and expansion of new mutant viruses by oxidative mutagenesis (Figure 6).

CONCLUSIONS

The pathological consequences of free radical generation during virus infections and the implications for viral pathogenesis and mutation are discussed in terms of current concepts concerning free radicals. It is now recognised more than ever that free radicals, produced primarily as effector molecules of the host defence response, have quite diverse functions in virus infections. Their biological effects are not necessarily beneficial to the virus-infected host; indeed, they are often detrimental. Understanding of the pathophysiological functions of NO and oxygen radicals will provide profound insights into many aspects of infectious diseases.

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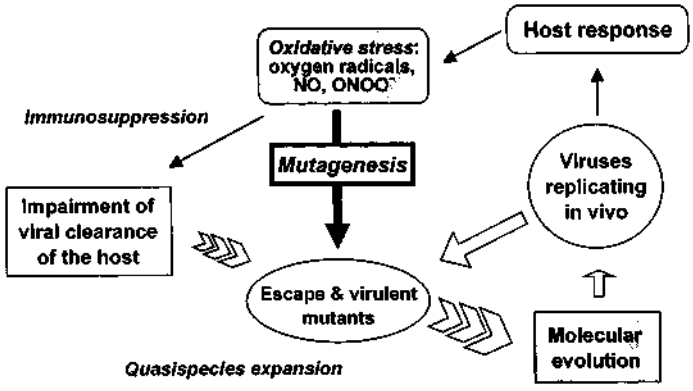

Figure 6. Possible roles of free radicals in viral mutation and evolution. Oxygen radicals and NO-derived reactive nitrogen intermediates, via their potent mutagenic activities, may contribute to the molecular evolution of viruses. NO may also affect viral evolution by inhibiting a host's antiviral immune responses, which may impair clearance of viral mutants

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THE VITAMINS AND RESISTANCE TO INFECTION

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CONTENTS

INTRODUCTION

In many of the investigtions on the relation between the vitamins and resistance to infection rations lacking hi several essentials have been employed, usually in an effort to test the effect-of inadequate human dietaries. Although such investigations have yielded results of practical value, they do not disclose the roles played by the diverse missing substances. More definite information on this question can be obtained from experiments in which diets deficient in one vitamin only are utilized and the following review has been limited, with very few. exceptions, to the discussion of such work. Very numerous papers on this subject have appeared and some no doubt have been overlooked by the author. Wherever possible the investigations have been described in sufficient detail for the reader critically to appraise them. Unfortunately many of the experiments have been carried out on such small numbers of annuals that the results are not statistically significant.

The problem of whether the metabolic changes resulting from the deficiency of a vitamin are .accompanied by changes in the defense mechanism has been attacked by at least four different methods, as follows:

(1) By the determination of any changes in the natural immune bodies or cellular reactions, due to the deficiency.

VITAMIN C

1. Variations in the natural immune bodies or tissue reactions in vitamin C deficiency

(a) Results indicating that these are reduced. Fortenato (1) reported in 1921 that the opsonic index was lower in scorbutic than in normal guinea pigs. In the following year, Leichentritt and Zielaskowski (2) measured the trypanocidal substance in the blood of guinea pigs suffering with scurvy and found that it was reduced. Hojer (3) however criticized the latter's experiments on the grounds that they were carried out on too few animals.

According to Prausnitz and Schilf (4) tuberculous scorbutic guinea pigs show considerably smaller tuberculin reactions, which also dis-

appear more quickly than those in tuberculous guinea pigs subsisting on normal diets. The febrile reaction after the tuberculin injection was also less marked in the scorbutic animals. This reduced skin reactivity was not correlated with a generalized unsusceptibility to tuberculin (5) as the animals with scurvy died more frequently than the normal controls when this substance was injected subcutaneously in large amounts (5 cc.).

In addition, Bieling (6) and also Arkwright and Zilva (7) found that markedly scorbutic guinea pigs gave smaller skin reactions to diphtheria toxin than normal. The former author noted that the necrosis of the skin was slower coming on, and that the subcutaneous oedema was absent or very slight. The latter authors reported that animals on diets which contained suboptimal amounts of vitamin C, but enough to allow a gain in weight of about 25 per cent, still showed large Schick reactions, whereas if this vitamin was further reduced so that a loss of about the same magnitude occurred, the reactions were very small. Scorbutic guinea pigs however are definitely more susceptible to large doses of diphtheria toxin and die earlier than normal animals according to Bieling. A possible clinical application of these findings was provided by Hess (8) in 1932. He had encountered nasal diphtheria very commonly in children with scurvy. The Schick reactions were regularly negative, although the patients showed the bloody mucous nasal discharge which is typical of this disease, and one child apparently died from it. In three cases, virulence tests showed the bacilli to be virulent. The last of these three cases gave no skin reaction to dilutions of from 1/50 to 1/5 M.L.D. of toxin. In his brief review the author does not discuss the possibility of these cases being carriers, already self-immunized. He suggests that in scurvy the pharyngeal mucous membrane loses its immunity to the diphtheria bacilli, whereas the general immunity as reflected by the negative Schick test is still maintained. A simpler explanation however might be that the scorbutic skin does not react in the usual manner to the toxin, although the organism as a whole is not immune to it.

Lawrynowicz (9) suggests that scurvy may so reduce the resistance that a carrier may become the victim of bacteria which it previously carried with impunity. For example, a guinea pig that had been well

for one month after it had been used in a crude test for B. diphtheria was placed on a scorbutic diet. Thirty-seven days later it died. The post-mortem showed the changes found in diphtheritic deaths and the organism was recovered from the spleen.

When Vercellana (10) injected strychnine nitrate or aqueous extracts of poisonous fungi subcutaneously into scorbutic guinea pigs, he found that they were killed more frequently by these substances than controls fed normal diets. The ration of the deficient animals consisted of oats exclusively. Also aleuronat, broth, peptone, cinnabar and other substances, when injected by Dluzewski (11) into the peritoneal cavities of scorbutic animals, did not provoke the normal inflammatory reaction with the outpouring of leucocytes.

(b) Results indicating that these are not reduced. Li contrast to some of the above findings, Lawrynowicz and Bohdanowicz (9). state that they have never established any difference between the Schick reactions of normal and scorbutic guinea pigs.

In 1919, Zilva (12) determined the complement titres in normal and scorbutic guinea pigs and found that they were the same. Four years later, Hamburger and Goldschmidt (13) reported that the complement titres were not lowered in scorbutic children and guinea pigs. In fact, some of the latter animals showed increased complement titres, which were apparently correlated with high albumin concentrations in the serum. Koch and Smith (14) found consistently increased complement titres in a series of twelve scorbutic guinea pigs. When an antiscorbutic was added to the diet, the titres fell, but still remained somewhat higher than they had been before the onset of the scurvy. On the other hand, Bohdanowicz and Lawrynowicz (9) found that complement did not show any constant or characteristic chanjges in guinea pig scurvy.

The phagocytic indices in scorbutic guinea pigs were reported by Werkman et al. (15) to be unaltered.

Hamburger and Goldschmidt (13) also determined the bactericidal titres of the sera of scorbutic and normal guinea pigs and of scorbutic and normal children to the same strain of colon bacillus and found that they were similar, This organism was used because the pyelonephritis which frequently complicates guinea pig scurvy is usually caused by it.

2. Variations in acquired immune bodies due to vitamin C deficiency

(a) Results indicating that these immune bodies are altered. When scorbutic guinea pigs were sensitized to horse serum, or red blood corpuscles, Zolog (16) found that they were much less sensitive to anaphylactic shock than normal diet controls. The minimum lethal dose was three to ten times higher in the animals with scurvy. Sereni (17), on the other hand, reported that scorbutic guinea pigs showed much more severe anaphylactic shock than the control animals. Hurwitz and Wessels (18) went further into the question and found that the uterine muscles of sensitized vitamin C deficient guinea pigs would not react either to the specific antigen or to smooth muscle stimulants, whereas the bronchial muscles of such animals reacted normally. In addition, when Bieling (5) immunized scorbutic guinea pigs with diphtheria toxin, he found that they did not produce as much antitoxin as the adequately fed controls.

(b) Results indicating that these immune bodies are not reduced. Scorbutic and normal guinea pigs produced agglutinins to B. typhosus equally well according to both Zilva (12) and Werkman (15). In addition, the former author stated that amboceptors to the same organism were also produced in normal amounts by guinea pigs on vitamin C deficient diets, and the same findings also held true for the rat. In 1922, Hess (19) reported that the diphtheria antitoxin production in scorbutic guinea pigs was as good as that in normal controls.

Summary of immunological investigations. I. Non-immune animals. In several of these studies conflicting results have been obtained. For example, Werkman reported that the opsonic indices of non-immune scorbutic guinea pigs were as high as those of normal animals, whereas Fortenato found them reduced. And again, Lawrynowicz stated that the presence or absence of scurvy did not affect the size of the Schick reaction in guinea pigs, whereas Bieling and also Arkwright found these reactions considerably reduced when scurvy was present. Other workers reported that tuberculin reactions were also considerably decreased. As the immunological significance of the Schick andituberculin reactions are entirely different, one would infer that the general reactivity of scorbutic skin was depressed. The smaller Schick reactions were not due to any increased antitoxin in the animal, as Bieling

showed that these guinea pigs died more frequently and more quickly after the injection of large amounts of toxin. In fact, scorbutic guinea pigs seem more susceptible to the subcutaneous injections of toxic substances generally, e.g., to tuberculin, strychnine and poisonous fungus extract. Lawrynowicz suggests, on evidence gathered from the study of one animal only, that scurvy so lowers the resistance of a healthy carrier that it may become the prey of bacteria which formerly did not harm it. This sequence of events however might have occurred without the aid of the scurvy-producing diet. Leichentritt found that the substance in the blood which destroyed trypanosomes was reduced in scurvy, and further evidence of the reduced capacity of the scorbutic animal to cope with infections was provided by Dluzewski, who reported that the inflammatory reactions which followed the injection of foreign substances into the peritoneum were much reduced. Two authors stated that the complement titre was unchanged in scurvy, but a similar number of investigators found it increased. One of the latter however did not find it consistently raised, but at least it was never lowered.

II. Immune animals. Comparatively few studies have been carried out on such animals, and many of the results are conflicting.

For instance, Hess found that scorbutic guinea pigs could produce diphtheria antitoxin as well as normal animals, whereas Bieling states that this is not the case. Zilva and Werkman were not able to demonstrate any difference between the amounts of anti-typhoid antibodies produced by guinea pigs and rats lacking vitamin C and those fed adequate diets.

The results of the anaphylaxis experiments are of interest because most of them suggest a reduced activity in the tissues of animals suffering from scurvy, analogous to the lessened skin reactions.

3. Occurrence of spontaneous infections in vitamin C deficiency

(a) Infections indicating a reduced resistance. I. Experimental. In 1932, Suzuki (20) stated that the nasal mucous membrane and glands were atrophied and showed catarrhal inflammation in vitamin C deficient guinea pigs. The crushed oats, autoclaved milk diet that McCarrison (21) fed his guinea pigs is mainly lacking in vitamin C. He

found that the bladders in such animals at postmortem examination were tightly contracted and that the mucous membrane of this organ was congested and necrotic. The duodenum was also intensely congested and punched out ulcers were present in the intestines and sometimes in the stomach. Mackie and Chitre (22) gave their monkeys very small amounts of orange juice, but most of them developed scurvy, and in addition they showed in their large intestines very marked necrotic and ulcerated lesions, which were laden with common intestinal bacteria. These various pathological findings provide possible explanations for some of the frequent secondary infections that occur in cases of human scurvy.

In Höjer's (3) series only about 30 per cent of his severely scorbutic guinea pigs showed infections. This low figure may be partly explained by the fact that they survived for just a few weeks. On the other hand, 50 per cent of the animals with mild scurvy developed infectious lesions, and about 20 per cent of the much longer-lived normal animals showed similar lesions.

In the course of his experiments, Heymann (23) reported that he lost a large number of scorbutic guinea pigs with pneumococcic pneumonia.

II. Clinical—latent scurvy. Even before the onset of definite symptoms of human scurvy, in the so-called period of latent scurvy, the affected individual is particulaly susceptible to infections (24) and if these are contracted they run an unusually severe course.

In 1919, Wiltshire (25) described the occurrence of small conical swellings in the hair follicles of the legs of scorbutic Serbian troops and he also found them during the scurvy season (January and June) in apparently normal individuals. The latter were probably suffering from latent scurvy.

One of the most typical pathological lesions in scurvy is the increased permeability of the blood vessel wall which allows the blood to ooze into the tissues. Gothlin (26) was able to devise a method of measuring the permeability of the cutaneous capillaries. In 1931, he found that 18 per cent of a group of apparently healthy Swedish country school children (11 to 14 years) were suffering from vitamin C undernourishment. Hopkins (27) was able to associate a period of ill

health in boys in a preparatory school with a lack of fresh fruit and vegetables during the winter months. When a little fresh fruit was supplied, the minor ailments and the listlessness disappeared.

In children who are suffering from undiagnosed latent scurvy, vaccination may precipitate acute scorbutic symptoms (28, 29). Abels (29) quotes the case of an anemic, atrophic ten months old child who developed both scurvy and a high prolonged fever after vaccination. This may explain the reluctance of parents in backward regions of Austria towards having their children vaccinated in the winter, when no doubt their diets are partially deficient in this vitamin. In such children, coryza and pharyngitis may be surprisingly severe and may usher in evident scurvy, and skin ulcers and cystitis are also very prevalent. In fact, this author has gone so far as to say that manifest scurvy is always preceded by an infection. Other investigators (30) however have found this sequence of events to occur frequently, but not invariably. The increased metabolism caused by the infection probably accentuates the vitamin deficiency and hastens the appearance of active scurvy.

As in the case of the other deficiency diseases, there seems to be some predisposition to scurvy, as only a certain number of those on a uniformly deficient diet develop it (24b).

Manifest scurvy. Infections are very commonly associated with active scurvy (31), and Von Niedner (31) reported that scorbutic soldiers succumb to the slightest infection. Numerous authors (29, 32) have found respiratory infections, including grippe and pneumonia, to be very common in such individuals. One of these authors, Erdheim (33), stated that such diseases were frequently very grave and persistent in scorbutic children. Tuberculosis was also very prevalent in several series (32b, 34). In one of these, Salle and Rosenberg (34) found that all the deaths (17) in their 461 cases were from tuberculosis and that 9 to 22 per cent of their different groups of scorbutic patients suffered from this disease. They also remarked on the great frequency with which cases of infantile scurvy were complicated by florid tuberculosis. Diphtheria (8, 32b, 34b) and dysentery and typhoid (29, 34a, 35) were also very often encountered by various clinicians in scorbutic individuals. Mackie (22) described an epidemic of dysentery (Shiga) among scorbutic war refugees in the near East, which was almost as

virulent as cholera. Many investigators (32b, 35, 36) have reported that cystopyelitis and nephritis were very common, and that furuncles, paronychia and gun shot wounds (2, 32b, 35, 36) were often very difficult to clear up in scorbutic patients.

In 1927, Funk (37) stated that an epidemic of pneumonia in the Sudan disappeared when antiscorbutic treatment was given to the numerous cases of scurvy which appeared at about the same time. This would suggest that scurvy lowered the resistance to this infection.

Oral infections. If a guinea pig is kept on a completely vitamin C free diet for even two days, marked abnormalities are seen in its teeth (3, 30), and if such a diet is kept up for a few weeks, the teeth may become devitalized. Apical abscesses are prone to appear in such teeth later on. The same processes may occur in man (38), and the resistance to infection may be indirectly lowered by the presence of these bacterial foci. Höjer and Westin (30) also found that although enough vitamin C was given (1.2 minimum protective doses of orange juice) to prevent the appearance of any scorbutic changes in the teeth, except perhaps an uncertain hyperemia in the pulp cavity, the animals were still markedly susceptible to infection.

After analyzing the diets of groups of individuals, Hanke (39) stated that those whose diets were complete suffered from dental caries, gingival irritation or pyorrhoea much less frequently than those whose diets were deficient in either or both vitamin C and vitamin D. The details of the diets were unfortunately not given. Spongy gums, associated with infections, were cleared up by the use of an adequate diet plus 1 pint of orange juice, the juice of a lemon and from one-fourth to one-half a head of lettuce daily. The resistance to other infections, especially to colds, was raised at the same time, and in one individual a long standing osteo-myelitis was also cured. When pyorrhoea was present surgical measures had usually to be combined with the dietetic treatment unless the condition was very mild.

4. Susceptibility to artificially induced infections

(a) Reduced resistance in vitamin C deficient animals. In 1923, Findlay (40) reported that guinea pigs fed on a vimamin C deficient diet died more frequently after mtraperitoneal injections of bacteria than

controls fed on normal diets. The organisms used were B. coli, staphylococcus aureus, streptococcus hemolyticus and pneumococcus.

In the same year, Werkman and his co-workers (15) found that there was a definitely, although not markedly, increased susceptibility to intraperitoneal injections of pneumococci or B. anthracis in scorbutic guinea pigs as compared with controls.

According to Abels (41), guinea pigs with scurvy die after intraperitoneal injection of B. coli, whereas normal animals withstand several times this dose.

B. aertrycke cultures were fed to 2 scorbutic and 2 normal guinea pigs by Grant (42). One of the scorbutic animals died and the three others were killed so that the spread of the bacilli to the various organs and the blood could be determined. Liver, spleen, lung and blood cultures were negative in the normal animals, whereas both the spleen and one of the blood and one of the liver cultures from the scorbutic animals yielded B. aertrycke. These findings would suggest that in scurvy the intestinal wall is more permeable to bacteria.

Schmidt-Weyland and Koltzsch (43) infected normal and scorbutic guinea pigs by either inhalation or feeding, or by the combination of both methods, with a mixture of pneumococci and a fowl cholera pasteurella strain. They found that the animals on the scurvy producing diet were much more susceptible to such infections and that many of them died of pneumonia.

A trypanosome infection was set up in half their scorbutic guinea pigs by Nassau and Scherzer (44). They reported that this procedure hastened the onset of the scurvy, but only slightly decreased the duration of life.

Hojer (3) divided about ninety guinea pigs into several groups which were fed normal, completely vitamin C deficient, and several different partially C deficient diets. Half of each group was infected intramuscularly with probably too large a dose of a low virulent human strain of B. tuberculosis. All of the four severely scorbutic animals showed larger lesions than many of the rest. Only one guinea pig, which was fed the normal diet, showed no evidence of the disease, except for fibrous healing at the site of the subcutaneous injection. The course of the disease did not parallel the degree of scurvy in the partially scorbutic animals, but microscopic examination showed that

the connective tissue reaction to the tuberculous foci at a specified time after infection varied directly with the amount of vitamin C in the diet. The more vitamin C fed, the more adequate was the connective tissue response.

Coulard (45) stated that the tuberculous processes at the site of injection, the enlargement of the glands, and the lesions in the spleen developed much more rapidly in the scorbutic than in the normal guinea pig.

Guinea pigs suffering from slight scurvy were reported by Heymann (23) to be no more susceptible to tuberculosis than normal animals. When however the scurvy was moderately severe, marked loss in weight and early death (73 days) followed infection with a human strain of tuberculosis. Similarly infected guinea pigs fed on a normal diet lived 141 days on the average.

In order to induce intestinal tuberculosis in the guinea pig after the feeding of tuberculous sputum, McConkey (46) found that a partial deficiency of vitamins A, C and D was necessary. However, the lack of vitamin C seemed to be especially important.

Bieling (5) was able to produce a localized chronic tuberculosis in his guinea pigs. These animals were strong and well nourished and remained in such condition for over a year. If, however, they were put on a vitamin C free diet, they seemed particularly susceptible to scurvy and died long before the non-infected controls. These early deaths could be attributed to an activation of the chronic tuberculosis by the scurvy, although the sections showed neither very marked scurvy nor tuberculosis extensive or severe enough to explain the rapid deaths. This increased susceptibility of the tuberculous animal to scurvy was gradually built up, as recently infected animals did not react differently from uninfected ones. If the amount of vitamin C in the diet was reduced but not absent, the same phenomena were observed, but the onset of scurvy and the deaths were delayed. Apparently therefore the development of scurvy is accelerated when tuberculosis is present.

Quite a number of studies on this subject have been carried out by Mouriquand and his collaborators. In 1924, they (5b) showed that a larger percentage of scorbutic than of normal guinea pigs died after the injection of tuberculin. In 1925 (47), they determined the effect

of the injection of fairly large (10 million) and very small numbers (400) of tubercle bacilli into chronic scorbutic and normal guinea pigs. When the massive dose was used, for the first three weeks the deficient animals showed less extensive lesions and less loss in weight than the controls. After this time the scorbutic animals went rapidly down hill and died before the controls. With the smaller dose no initial refractory stage was seen, and the lesions in the animals with scurvy progressed more rapidly and led to earlier death. Two years later, they reported that if after feeding a diet completely deficient hi vitamin C, a ration partially lacking in this factor was given, a chronic scurvy was established which was characterized by a tendency to relapses of the active scurvy, and by great susceptibility to infection with B. tuberculosis. When such an infection was set up, the animals suffering from chronic scurvy lost weight and died after a short time, and there was not the slightest evidence of tissue reaction against the bacilli, even though these were much attenuated. Normal animals similarly infected reacted with "multiple" sclerosis and lived considerably longer.

(6) *Increased resistance due to the addition of vitamin C.* The addition of vitamin C rich lemon juice to an adequate diet favorably influenced the course of tuberculosis in guinea pigs, according to Leichentritt (48), The experiments of Hericourt and Richet (49) may possibly be interpreted as providing further confirmation of the important rdle played by vitamin C in this disease. They found that if dogs were injected with raw meat juice they withstood a tuberculous infection better than similar animals injected with cooked meat juice. The cooking no doubt destroyed the vitamin C, but it may have had other deleterious effects on the meat juice as well. When the diet contained vitamin D, Grant (50) found that increasing the amount of vitamin C seemed to decrease the severity and extent of the tuberculous lesions in the lungs of guinea pigs.

(c) No reduced resistance in vitamin C deficient animals. In some of Grant's (50) other experiments she used diets in which the vitamins were unbalanced and the results were entirely different. For example, she reported that if vitamin D was deficient in the diet, the addition of vitamin C tended to increase the amount of tuberculosis in the

lungs, and the same effect also followed the substitution of vitamin C for vitamin D at the time of inoculation.

In one of their earlier publications (1922), Mouriquand (51) and his co-workers reported that chronic scurvy did not accelerate the course of tuberculosis in the guinea pig. Their later work gave results entirely opposed to those of this early investigation.

Bieling (5a) stated that "transitory milk or hunger scurvy" did not lead to a decreased resistance to infection.

When Jaffe (52) infected the leg bones, muscles or skin with staphylococci and put the guinea pigs on a scorbutogenic diet at the same time, he found that about half of them developed severe infections and that these animals lived longer (42 days) than the uninfected controls, and did not show scorbutic changes at death. If the infections were mild, death from scurvy occurred at about the usual tune (21 to 30 days). If the annuals were on the deficient diet for 10 days before infection, they died abnormally quickly from the scurvy (7 to 12 days). Baj (53) partially confirmed these findings when he reported that the characteristic bone changes of scurvy were less marked in animals infected with staphylococci. He suggested that antiscorbutic substances were formed by the bacteria. He also stated that the infections in scorbutic animals were no more severe than those in controls fed normal diets.

As many mice on a vitamin C deficient diet survived after intraperitoneal injections of mouse typhoid bacilli as mice on a complete diet, according to Hotta's (54) results.

Summary of artificial infection experiments. Relatively few of these investigators have brought forward evidence to the effect that a deficiency of vitamin C does not lead to a lower resistance to infection, and some criticism of their work is possible. For example, Hotta's results were based on one experiment including at the most 32 rats, and the rat is apparently able to synthesize this vitamin, and Mouriquand's numerous later results contradicted his earlier report, which need not therefore be considered further.

Qn the other hand, Findlay, Werkman and also Nassau found that a greater proportion of scorbutic than of normal guinea pigs died after intraperitoneal injections of bacteria or trypanosomes. The last two

authors stated that the reduction in the resistance was not marked. Jaffe infected the legs of guinea pigs that had been on a scurvy producing diet for ten days with staphylococci and found that they died very quickly. As Schmidt-Weyland's method of infection more nearly simulates that occurring in nature, it is probably preferable to those used by the above mentioned authors. Schmidt-Weyland's results showed many more deaths from pneumonia among the scorbutic animals.

The interest in the question of whether scurvy renders an annual particularly susceptible to tuberculosis was possibly engendered by clinical reports to that effect. The guinea pig develops scurvy readily and it is also very susceptible to tuberculosis. It is probably more susceptible to both these conditions than man. Consequently, in most of these experiments the resistance has had to be gauged either by variations in the duration of life or in the extent and nature of the lesions. As the course of tuberculosis in even normal guinea pigs is variable, these criteria are somewhat unsatisfactory. According to Heymann, the susceptibility varies with the severity of the scurvy. Slight scurvy does not affect the resistance, whereas animals suffering from moderately severe scurvy are less resistant and die quickly from tuberculosis. Hojer's experiments, which might have confirmed Heymann's, gave variable results from the point of view of duration of life. Goulard and also Mouriquand found that tuberculosis was fatal more quickly in scorbutic than in normal guinea pigs. When Hojer examined his animals in regard to the extent of the lesions, his results were more consistent, as the markedly scorbutic animals showed the greatest involvement, the normal the least, and in the slightly scorbutic the lesions were variable. Goulard also remarked on the more extensive tuberculosis found in scorbutic animals. Mouriquand noted that guinea pigs affected with chronic scurvy were unable to produce the usual connective tissue reaction to tubercle infection. Hojer also reported that the efficiency with which this reaction took place varied directly with the amount of vitamin C in the diet.

Several authors have provided information on the part played by bacteria in precipitating acute scurvy. Bieling found that animals with chronic tuberculosis were very susceptible to scurvy and Nassau also stated that the presence of a trypanosome infection seemed to

accelerate the onset of scurvy. Jaffe, on the other hand, found that a marked subcutaneous or osseous infection prevented the onset of scurvy and that a mild infection did not affect the course of this avitaminosis.

However, Jaffe's results may possibly have been due to the production of the vitamin by the bacteria. Baj, who suggested the above explanation, also found that the presence of a staphylococcic infection lessened the severity of the scurvy.

From Grant's experiment it would appear that the intestinal mucous, membrane in animals suffering from scurvy is more permeable to bacteria, and McConkey indicates that the intestine in such animals is more susceptible to infection.

Three investigators also have shown that added amounts of vitamin C assist animals on normal diets in their reactions against tuberculosis.

5. *The use of vitamin C in clinical infections*

Numerous reports demonstrating the good effect of vitamin rich diets in clinical tuberculosis have been published, but it is impossible to decide what role vitamin C plays in such treatment. Also, one can not be sure that the good results which Höjer (3) obtained when he fed a series of twenty tuberculous children raw blood serum (50 to 100 cc.) daily for four months were due to the vitamin C contained in that substance. In a later experiment, the same author (30) compared the effect of the addition of vitamin C (one orange daily) or of added carbohydrate (a pastry) on samtorium cases of tuberculosis. The patients were grouped in pairs as closely alike in age, sex, tuberculous involvement, and prognosis as possible. One of each pair received the orange and one the pastry. The sanitorium was in an isolated region where the supply of vegetables and fruit was limited, especially in thd three months of the experiment (March, April and May). The highest mortality from this disease also usually occurred in these three months. Of the cases fed the extra vitamin C, 17 showed better, 3 showed similar, and 1 showed worse results than the controls. The cases were examined regularly by expert clinicians, and although the effects were not easy to evaluate, it appeared that the provision of plenty of vitamin C assisted in the healing of the tuberculous lesions. Woringer and Sala (55) advised generous additions of vitamin C to

whooping cough cases, for although scurvy is very rare in Strassburg, they saw four cases of whooping cough and scurvy together. McConkey (56) reported that the administration of cod liver oil and tomato juice has a favorable effect on intestinal tuberculosis which was secondary to a pulmonary infection. In order to determine whether the vitamin C was of value he gave three patients on normal diets a cod liver oil concentrate alone. No change could be seen until orange juice was added also, when two of them began to show satisfactory improvement. In a second test, he gave two cases irradiated brewer's yeast. Again they did not improve until the orange juice was administered also. The possibility that the good effects were due to the combination of the vitamins can not be ruled out, as none of the patients were given vitamin C alone. Bloch (57) is of the opinion that vitamin A is of more importance than vitamin C in the treatment of tuberculosis, but other authors (31) claim that generous amounts of vitamin C are essential in the treatment of such cases.

Summary. The results which have been published up to date suggest that this factor plays a very important r61e in the combatting of tuberculous infections, but further investigations will be necessary before this can be conclusively settled.

6. The mechanism underlying the decreased resistance in scurvy

According to Höjer (3), the decreased resistance in scurvy is due to the atrophy of the various organs hi the body that protect it against infections. These organs include the lymph nodes, spleen and bone marrow. Findlay (40) had previously ascribed the low resistance which he found in scorbutic animals to the changes that were present hi the bone marrow.

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Role of vitamin C in the function of the vascular endothelium.

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Abstract

SIGNIFICANCE: Vitamin C, or ascorbic acid, has long been known to participate in several important functions in the vascular bed in support of endothelial cells. These functions include increasing the synthesis and deposition of type IV collagen in the basement membrane, stimulating endothelial proliferation, inhibiting apoptosis, scavenging radical species, and sparing endothelial cell-derived nitric oxide to help modulate blood flow. Although ascorbate may not be able to reverse inflammatory vascular diseases such as atherosclerosis, it may well play a role in preventing the endothelial dysfunction that is the earliest sign of many such diseases.

RECENT ADVANCES: Beyond simply preventing scurvy, evidence is mounting that ascorbate is required for optimal function of many dioxygenase enzymes in addition to those involved in collagen synthesis. Several of these enzymes regulate the transcription of proteins involved in endothelial function, proliferation, and survival, including hypoxia-inducible factor-1α and histone and DNA demethylases. More recently, ascorbate has been found to acutely tighten the endothelial permeability barrier and, thus, may modulate access of ascorbate and other molecules into tissues and organs.

CRITICAL ISSUES: The issue of the optimal cellular content of ascorbate remains unresolved, but it appears that low millimolar ascorbate concentrations are normal in most animal tissues, in human leukocytes, and probably in the endothelium. Although there may be little benefit of increasing near maximal cellular ascorbate concentrations in normal people, many diseases and conditions have either systemic or localized cellular ascorbate deficiency as a cause for endothelial dysfunction, including early atherosclerosis, sepsis, smoking, and diabetes.

FUTURE DIRECTIONS: A key focus for future studies of ascorbate and the vascular endothelium will likely be to determine the mechanisms and clinical relevance of ascorbate effects on endothelial function, permeability, and survival in diseases that cause endothelial dysfunction.

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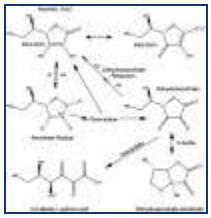
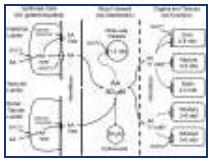
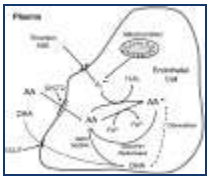
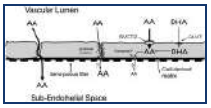
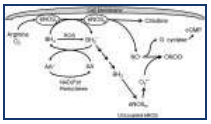


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Scurvy in hospitalized elderly patients

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Abstract

Objectives

The aim of this study was to systematically screen hospitalized elderly patients for clinical symptoms of scurvy and to confirm the diagnosis with biological measures.

Settings

Geriatric acute care ward.

Measurements

Scurvy symptoms (one or more among perifollicular hyperkeratosis, petechiae or bruises, haemorrhagic features caused by venous puncture, severe gingivitis). We compared associated diseases, nutritional status, need for assistance for feeding, serum albumin, transthyretin, B9 and B12 vitamins, iron status and Serum Ascorbic Acid Level (SAAL) and outcome (in-hospital mortality) between scurvy and scurvy free patients.

Results

18 patients with clinical symptoms of scurvy (scurvy group) were identified out of 145 consecutive patients (12%). They were compared to 23 consecutive control patients with no clinical symptoms of scurvy (scurvy-free group). SAAL was significantly lower (1.09 ± 1.06 vs 4.87 ± 4.2 mg.L-1, p<.001) and vitamin C deficiency more frequent (94 vs 30 %, p<.001) in the scurvy group. Moreover, in scurvy group, coronary heart disease (39 vs 9 %, p=.028), need for assistance for feeding (56 vs 13 %, p=.006) and in-hospital deaths (44 vs 9 %, p=.012) were more frequent.

Conclusion

Ninety-four percent of patients with clinical symptoms of scurvy had vitamin C deficiency. Our results suggest that in hospitalized elderly patients, clinical symptoms allow scurvy diagnosis. Scurvy could be a frequent disease in elderly patients admitted to acute geriatric ward.

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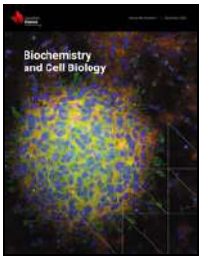

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STUDIES ON ACCLIMATIZATION AND ON THE EFFECT OF ASCORBIC ACID IN MEN EXPOSED TO COLD

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ABSTRACT

This experiment was planned to study the problem of acclimatization in humans and to determine the effect of ascorbic acid in men exposed to cold while being fed a normal or survival ration. Ascorbic acid has greatly improved the resistance of men exposed to cold and fed a survival ration. No beneficial effect was observed when the subjects were fed a normal ration. This difference in response may be due to the fact that the experimental conditions differed somewhat between these two experiments. In any event, the subjects on a restricted food intake were certainly under greater conditions of stress. Evidence of acclimatization was obtained with survival rations but not with normal rations. Some conclusions have been made on the use, by men exposed to cold, of survival rations composed exclusively of carbohydrates. Finally, it is estimated that 2800 calories is the daily requirement for men relatively inactive, wearing only shorts, low shoes, and socks, and exposed to an ambient temperature of 60°F.

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Subgroup analysis of large trials can guide further research: a case study of vitamin E and pneumonia

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Abstract

Background:

Biology is complex and the effects of many interventions may vary between population groups. Subgroup analysis can give estimates for specific populations, but trials are usually too small for such analyses.

Purpose:

To test whether the effect of vitamin E on pneumonia risk is uniform over subgroups defined by smoking and exercise.

Methods:

The Alpha-Tocopherol Beta-Carotene Cancer Prevention Study examined the effects of vitamin E (50 mg per day) and β-carotene (20 mg per day) on lung cancer in 29,133 male smokers aged 50–69 years using a 2 × 2 factorial design. The trial was conducted among the general community in Finland during 1985–1993; the intervention lasted for 6.0 years (median). In the present study, we tested the uniformity of vitamin E effect on the risk of hospital-treated pneumonia (898 cases) by adding a dummy variable to allow each subgroup its own vitamin E effect in a Cox model covering all participants.

Results:

Vitamin E effect was not uniform over eight subgroups defined by baseline smoking (5–19 vs ≥20 cigarettes per day), age of smoking initiation (≤20 vs ≥21 years), and exercise during leisure time (yes vs no). Vitamin E decreased pneumonia risk by 69% (95% CI: 43% to 83%) among participants who had the least exposure to smoking and exercised during leisure time. Vitamin E increased pneumonia risk by 79% (95% CI: 27% to 150%) among those who had the highest exposure to smoking and did not exercise.

Limitations:

Although the evidence of heterogeneity is strong, it is not evident to what extent the estimates of effect or

the limits between the subgroups can be extrapolated to other populations.

Conclusion:

Subgroup analysis of large trials should be encouraged, though caution is needed in the interpretation of findings. The role of vitamin E in susceptibility to pneumonia in physically active nonsmokers warrants further study.

Trial registration:

ClinicalTrials.gov NCT00342992.

Keywords: vitamin E, pneumonia, smoking, leisure time exercise, α-tocopherol, β-carotene, subgroup analysis

Introduction

The size of a controlled trial is usually based on a power calculation, the goal of which is to determine the minimal number of participants needed to test whether an overall difference exists between the intervention and control groups. Such trials are too small to test subgroup differences. Furthermore, carrying out numerous subgroup comparisons leads to the multiple testing problem. Such reasoning is the major cause for discouraging subgroup analyses.[1–5]

The above argument has limitations, however. For example, if a trial collects data on a secondary outcome which are much more numerous than the primary outcome, say lung cancer, subgroup analysis on the secondary outcome, such as the common cold,[6] does not suffer from low statistical power. Furthermore, most controlled trials study the effect of drugs having a specific biochemical target within patients who are narrowly selected, and a large within-trial variation in the effect may be unlikely in such cases. However, it is possible that the within-trial variation in the effect is substantially greater for interventions that have complex and broad effects on the human system, in particular when the effects are studied in heterogeneous populations. Thus, while reasons exist for being cautious about subgroup analysis in general, there are conditions when subgroup analyses may be justified.

Previously, we explored the effect of vitamin E on pneumonia risk among the 29,133 male smokers of the Alpha-Tocopherol Beta-Carotene [ATBC] Study.[7,8] We found significant modification of vitamin E effect by age of smoking initiation, in that the vitamin reduced the risk in those who started smoking at a late age and, within this subgroup, baseline smoking further modified the effect so that the benefit was greatest among those who smoked the least.[9] Since physical activity leads to oxidative stress,[10] we separately hypothesized that vitamin E might reduce pneumonia risk among physically active ATBC Study participants, and found that the vitamin halved the risk in those who exercised during leisure time.[11] These findings indicate that cigarette smoking and exercise might modify the effect of vitamin E on pneumonia risk. However, since several comparisons were made, the multiple testing problem cannot be entirely dismissed. Therefore, in this paper we analyze the subgroup differences in all ATBC Study participants simultaneously.

If there is firm evidence that the effect of vitamin E supplementation on health outcomes of the ATBC participants is heterogeneous, this would imply that subgroup analyses in other large-scale trials on vitamin E, and possibly in large-scale trials on other subjects, should be encouraged rather than discouraged.

Material and methods

Participants

The rationale, design, and methods of the ATBC Study examining the effects of vitamin E (*dl*-α-tocopheryl acetate, AT, 50 mg/day) and β-carotene (BC, 20 mg/day) on the incidence of lung cancer and other cancers have been described in detail.[7–9] The ATBC Study is registered at ClinicalTrials.gov under the identifier NCT00342992.

In brief, to be eligible, male participants aged 50–69 years had to smoke ≥5 cigarettes per day at entry, and those enrolled in the trial (N = 29,133) were randomized to one of four intervention arms and administered placebo, AT, BC, or AT + BC, using a 2 × 2 factorial design. Compared with baseline levels, supplementation increased the serum level of α-tocopherol by 50%.[7,8] The intervention continued for 5 to 8 years until April 1993. The trial was approved by the review boards of the participating institutions and all participants gave written informed consent. Compliance with supplementation was high: some 90% of the subjects took more than 90% of their prescribed capsules during their active participation in the trial.[7,8]

Baseline characteristics

Before randomization at baseline, the participants completed questionnaires on medical and smoking histories and general background characteristics. A detailed dietary history questionnaire was completed that provided data regarding vitamins C and E, and coffee consumption.[12] Age of smoking initiation was not available for seven participants and dietary data for 2,022 participants.

Previously, we found that dichotomization of the age of smoking initiation with the cutoff point at 21 years appropriately captured the variation of the vitamin E effect,[9] and the same cutoff was used in this study. Although smoking is a continuous variable, it is heavily clustered to multiples of 20 (and 10) cigarettes per day. In this study, we dichotomized cigarette smoking to 5–19 cigarettes per day and to ≥20 per day. As we recognized that in both cases dichotomization leads to a loss of information of the continuous variables, we examined the effect of vitamin E in smaller ranges in Tables 2 and 3.

Table 2

The effect of vitamin E on pneumonia incidence in ATBC participants who initiated smoking at ≥21 years, smoked 5–19 cigarettes per day, and exercised during leisure time

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Notes:

[a]The number of participants in the vitamin E and no-vitamin E groups was the same within 8% accuracy in all subgroups shown;
[b]A/B refers to A cases of pneumonia among the vitamin E participants and B cases of pneumonia among the no-vitamin E participants;
[c]The Cox model comparing participants who received vitamin E with those who did not;
[d]Data on diet were missing for 160 participants, which included one case of pneumonia in the vitamin E group and three cases in the no-vitamin E group.

Abbreviations: RR, risk ratio; CI, confidence interval.

Table 3

The effect of vitamin E on pneumonia incidence in ATBC participants who initiated smoking at ≤20 years, smoked ≥20 cigarettes per day, and did not exercise during leisure time

Subgroup	No. of men[a]	Cases of pneumonia[b]	Effect of vitamin E	
			RR (95% CI)[c]	Test for interaction (*P*)
All	6,686	152/115	1.35 (1.06, 1.7)	
β-Carotene supplementation				
No	3,371	89/51	1.79 (1.27, 2.5)	0.02
Yes	3,315	63/64	1.01 (0.71, 1.4)	
Restriction to the no-β-carotene participants:				
No β-carotene	3,371	89/51	1.79 (1.27, 2.5)	
Cigarettes (1/day)				
20–25	2,269	62/36	1.78 (1.18, 2.7)	1.0
26–80	1,102	27/15	1.83 (0.97, 3.5)	
Age of smoking initiation (years)				
6–17	1,616	48/26	1.94 (1.20, 3.1)	0.6
18–20	1,755	41/25	1.64 (1.00, 2.7)	
Age at baseline (years)				
50–59	2,466	55/31	1.84 (1.19, 2.9)	0.8
60–69	905	34/20	1.70 (0.98, 3.0)	
Dietary vitamin E (mg/day)[d]				
<9	1,231	31/22	1.52 (0.88, 2.6)	0.5
≥9	1,909	49/26	1.90 (1.18, 3.1)	
Dietary vitamin C (mg/day)[d]				
<70	1,229	38/22	1.76 (1.04, 3.0)	0.9
≥70	1,911	42/26	1.69 (1.03, 2.8)	
Coffee (mL/day)[d]				
<500	1,188	38/20	1.95 (1.13, 3.4)	0.5
≥500	1,952	42/28	1.56 (0.96, 2.5)	

Notes:

[a]The number of participants in the vitamin E and no-vitamin E groups was the same within 5% accuracy in all subgroups shown;

[b]A/B refers to A cases of pneumonia among the vitamin E participants and B cases of pneumonia among the no-vitamin E participants;

[c]The Cox model comparing participants who received vitamin E with those who did not;

[d]Data on diet were missing for 231 participants, which included nine cases of pneumonia in the vitamin E group and three cases in the no-vitamin E group.

Abbreviations: RR, risk ratio; CI, confidence interval.

The baseline questionnaire on physical activity during leisure time was a modification of that used originally in the Gothenburg study focusing on cardiovascular diseases.[13] The intensity of average physical activity during leisure time over the previous 12 months was enquired about using the following alternatives: 1) light: reading, watching TV, listening to the radio, or going to movies, ie, activities that are not physically demanding; 2) moderate: walking, fishing, hunting, or gardening quite regularly; and 3) heavy: actual physical exercise, such as jogging, skiing, swimming, gymnastics, and court and field sports quite regularly. In the current analyses we combined answers 2) [n = 15,191] and 3) [n = 1,744] to the category "exercise during leisure time". Data on exercise were not available for 14 participants.

Outcome and follow-up time

The events for this study, the first hospital-treated cases of pneumonia after randomization, were ascertained from the national Hospital Discharge Register using the unique personal identification numbers for linkage (see details in Hemilä et al)[9]. Pneumonia cases recorded in the Hospital Discharge Register reflect clinically more severe cases of greater health and economic significance, whereas less severe cases of pneumonia treated as outpatients are not recorded in the Register. Use of the Hospital Discharge Register allowed for the obtaining of information on pneumonia in all study participants irrespective of whether they continued in or had dropped out of the trial.

Follow-up time for each participant began from the day of randomization, and continued until the date of first hospital discharge for pneumonia, death, or the end of the trial, April 30, 1993, whichever came first. The median follow-up time of the participants was 6.0 years, and there was a total of 167,968 person-years of observation.

Statistical methods

We estimated the effect of vitamin E supplementation on pneumonia incidence through Cox models. We calculated the risk ratio (RR) and the 95% confidence interval (CI) of the RR using the PROC PHREG program of the SAS package of programs (release 8.2, SAS Institute, Inc., Cary, NC). No covariates were included in the models analyzing the treatment effects. As to supplementation, we carried out the analyses following the intention-to-treat (ITT) principle.

In Table 1, we compared the trial participants administered vitamin E (AT and AT + BC) with those not receiving vitamin E (the no-vitamin E participants; placebo and BC). Since, in Table 3, we observed that AT and BC supplementations interacted, we restricted further subgroup analyses of Table 3 to the no-BC participants (AT and placebo arms). Because of this interaction, we also re-tested the heterogeneity of Table 1 by restricting to the no-BC participants.

Table 1

The effect of vitamin E on pneumonia incidence by level of cigarette smoke exposure and exercise during leisure time: ATBC Study 1985–1993

Age of smoking initiation (years)	Cigarettes per day at baseline		Effect of vitamin E	
			Exercise during leisure time	
			Yes	No
≥21	5–19	RR[a] (95% CI)[a]	0.31 (0.17, 0.57)	0.85 (0.44, 1.64)
		Cases of pneumonia[b]	14/43	17/19
		No. of men[c]	2,216	1,043
≥21	≥20	RR[a] (95% CI)[a]	0.84 (0.48, 1.46)	0.86 (0.50, 1.49)
		Cases of pneumonia[b]	24/27	24/28
		No. of men[c]	2,445	1,763
≤20	5–19	RR[a] (95% CI)[a]	1.24 (0.87, 1.78)	1.05 (0.71, 1.56)
		Cases of pneumonia[b]	68/56	51/50
		No. of men[c]	4,602	2,688
≤20	≥20	RR[a] (95% CI)[a]	0.88 (0.67, 1.15)	1.35 (1.06, 1.73)
		Cases of pneumonia[b]	97/110	152/115
		No. of men[c]	7,669	6,686

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Notes:

[a]The Cox model comparing participants who received vitamin E with those who did not;
[b]A/B refers to A cases of pneumonia among the vitamin E participants and B cases of pneumonia among the no-vitamin E participants. Data on age of smoking initiation or exercise were missing from two pneumonia cases among the vitamin E participants and from one case among the no-vitamin E participants; these cases are not included in this table;
[c]The number of participants in the vitamin E and no-vitamin E groups was the same within 5% accuracy in each of the eight groups. The uniformity of the vitamin E effect was tested by adding a dummy variable for vitamin E effect in seven groups of the table, allowing each of the eight groups their own vitamin E effect. The regression model was improved by $\chi^2(7\ \text{df}) = 26.6$, $P = 0.0004$, compared to the model with a uniform vitamin E effect. Heterogeneity is mainly caused by the upper-left and lower-right cells: the addition of only these two cells improved the model by $\chi^2(2\ \text{df}) = 23.4$. The difference between the above two models is fully explained by chance: $\chi^2(5\ \text{df}) = 3.2$. The addition of the third-order interaction term, between vitamin E supplementation, age of smoking initiation, cigarettes per day, and leisure time exercise, to the model containing all lower level interaction terms, improved the regression model by $\chi^2(1\ \text{df}) = 5.4$, $P = 0.02$. Since vitamin E and β-carotene supplementations interact in the lower-right cell (see Table 3), we also tested the uniformity of vitamin E effect among the no-β-carotene participants (n = 14,564). Adding a dummy

variable for vitamin E effect in seven groups of the table improved the model by $\chi^2(7\ df) = 22.8$, $P = 0.002$. Adding only the upper-left and lower-right cells improved the model by $\chi^2(2\ df) = 17.8$, indicating that the effect of vitamin E is restricted to the upper-left and lower-right cells. The difference between the two models is fully explained by chance: $\chi^2(5\ df) = 5.0$. Nevertheless, adding the third-order interaction term to a model containing all lower level interactions did not significantly improve the model: $\chi^2(1\ df) = 2.0$, $P = 0.16$. Vitamin E and β-carotene supplementations did not interact in cells of this table other than the lower-right cell.

Abbreviations: RR, risk ratio; CI, confidence interval.

To test the statistical significance of interaction between vitamin E supplementation and potential modifying factors, we first added vitamin E and the modifying factor to the regression model. The statistical significance of the interaction was thereafter calculated from the change in −2 × log (likelihood) when the interaction term for vitamin E supplementation and the modifying factor were added to the model. In our subgroup analyses in Tables 2 and 3, we split the subgroup variables at levels leading to a reasonably similar number of cases in the control groups.

Nelson-Aalen cumulative hazard functions were constructed using the STATA sts program (Release 9, Stata Corp, College Station, TX). Two-tailed *P*-values are presented.

Results

Among all ATBC participants, the cases of pneumonia were identically divided between the vitamin E and no-vitamin E groups: 449 vs 449, corresponding to RR = 1.00 (95% CI: 0.88, 1.14).

We divided the participants into eight subgroups on the basis of age of smoking initiation, level of smoking at the baseline of the trial, and exercise during leisure time (Table 1). We tested the uniformity of the vitamin E effect by adding a dummy variable for vitamin E effect in seven groups of the table, and this significantly improved the Cox model ($P = 0.0004$). The heterogeneity in Table 1 is fully explained by the upper-left and lower-right corners, ie, by the opposite corners of the table. Furthermore, the third-level interaction term between vitamin E supplementation, age of smoking initiation, level of smoking, and exercise was significant when comparing the vitamin E and no-vitamin E participants. Since the effect of vitamin E was restricted to the upper-right and lower-left corners, we analyzed these two groups further.

Among the 2,216 participants who initiated smoking at a late age, smoked less than a pack of cigarettes per day, and exercised during leisure time, vitamin E supplementation reduced pneumonia risk by 69% (upper-left cell in Table 1; Figure 1). The estimated effect of vitamin E in this subgroup was robust in several further subgroup analyses. The effect was not modified by BC supplementation, age, or dietary vitamins C and E (Table 2). Dividing the participants by the age of smoking initiation and baseline smoking also led to compatible effects within the smaller subgroups. Previously, we found that coffee consumption significantly modified the benefit of vitamin E in those who started smoking at a late age.[9] The subgroup differences in Table 2 are in line with the earlier findings, but not significantly.

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Figure 1

Vitamin E and pneumonia risk in ATBC Study participants who started smoking at ≥21 years, smoked 5–19 cigarettes per day, and exercised (n = 2,216). Nelson-Aalen cumulative hazard functions for vitamin E and no-vitamin E groups are shown. Each step indicates one case of pneumonia. For the difference between the two survival curves, the logrank test gives $P = 0.00005$. The survival curves are cut at 7.2 years because the number of participants declines abruptly thereafter (no cases after 6.8 years). At six-year follow-up 576 and 535 participants remained in the vitamin E and the no-vitamin E groups, respectively.

Among the 6,686 participants who initiated smoking at an early age, smoked a pack of cigarettes daily or more, and did not exercise, vitamin E increased pneumonia risk by 35% when compared with the no-vitamin E group (lower-right cell in Table 1). However, in this subgroup the vitamin E effect was modified by BC supplementation so that the harm of vitamin E was restricted to those who were not administered BC (Table 3). Therefore, we restricted the further subgroup analyses of Table 3 to the no-BC participants. Among the no-BC participants, vitamin E increased pneumonia risk by 79%, and this effect was robust in further subgroup analyses (Table 3).

Previously, we hypothesized that the marginally significant 14% increase in pneumonia risk among those ATBC participants who started smoking at an early age (n = 21,657; the four lowest cells in Table 1) might correspond to a more unambiguous harmful effect among low-weight participants, based on an assumption of dose-dependency.14 Then we found that vitamin E increased pneumonia risk in participants weighing less than 60 kg. Unexpectedly, vitamin E also increased pneumonia risk at the opposite end of the weight scale, among those weighing over 100 kg.14 Furthermore, in both groups, harm caused by vitamin E was restricted to those who had a dietary vitamin C intake above the median. Therefore, we examined whether weight and vitamin C intake might modify the effect of supplementation outside of the lower-right corner in Table 1.

Of the low-weight high vitamin C participants, 72% (337 of 468) were outside the lower-right corner of Table 1; in these 337 participants there were 19 pneumonia cases among the vitamin E and eight cases among the no-vitamin E participants (RR = 2.7, 95% CI: 1.18–6.2). Of the overweight high vitamin C participants, 65% (397 of 613) were outside the lower-right corner of Table 1; in these 397 participants there were 10 pneumonia cases among the vitamin E and one case among the no-vitamin E participants ($P = 0.01$, Fisher's test). Consequently, weight and dietary vitamin C appear to modify the effect of vitamin E independent of smoking and exercise.

Discussion

The numbers of pneumonia cases in the ATBC Study were equally distributed between the vitamin E and no-vitamin E participants, indicating a lack of overall effect with great accuracy. However, in this study we have shown that the effect of vitamin E is not uniformly nil over all the ATBC Study population. Depending simultaneously on the two different measures of cigarette smoking and on the level of exercise, vitamin E supplementation decreased, increased or had no effect on the incidence of pneumonia (Table 1).

Among those who had the least exposure to smoking and exercised during leisure time, vitamin E decreased the risk of pneumonia by 69%. This group covers 8% of the ATBC Study participants. The effect estimate was robust in further subgroup analyses (Table 2).

The group that had the highest exposure to smoking and did not exercise covered 23% of the ATBC participants. In this group, vitamin E increased pneumonia risk by 79% in the no-BC participants (Table 3). This effect estimate was also robust in further subgroup analyses, however simultaneous BC supplementation nullified the harmful effects of vitamin E.

In our subgroup analysis focusing on smoking and exercise, 69% of the ATBC participants fell into the six middle groups that were consistent with vitamin E having no effect (Table 1). Nevertheless, it is possible that there are further modifying factors in addition to smoking and physical activity. Previously, we found that coffee drinking modified the effect of vitamin E among those who started smoking at a late age.9 Among those who started smoking at an early age, weight and dietary vitamin C intake modified the vitamin E supplementation effect.14 The current analyses are not inconsistent with these earlier subgroup findings. Thus, it seems possible that vitamin E can affect pneumonia risk in some groups of people depending on six or more modifying factors meaning that the modification is complex and does not follow a simple multiplicative model.

It is often suggested that subgroup findings should be trusted only when they are replicated in other trials. Although such a suggestion seems sound, the heterogeneity we found in the effect of vitamin E on pneumonia suggests that testing a subgroup difference in another sample of people can be all but simple. When the effect of vitamin E may depend simultaneously on six or more modifying factors, the findings for the first-level interactions depend on the selection of participants.

For example, in the whole ATBC Study, baseline smoking did not modify the effect of vitamin E ($P = 0.2$).9 However, Table 1 indicates that baseline smoking modifies the vitamin E effect conditionally on the age of smoking initiation and the level of exercise. This means that depending on the composition of the population, baseline smoking may or may not modify the effect of vitamin E. Similarly, we previously found that vitamin E halved the risk of pneumonia in ATBC participants who exercised during leisure time;11 however, Table 1 indicates that this effect is conditional on low level of exposure to smoking. On the basis of these examples, replication is not a universally valid method for deciding whether the subgroup differences observed in one trial are real or not.

Peto et al argued that “believing that a treatment effect exists in one stratum of patients, even though no overall significant treatment effect exists, is a common error”.4 This comment may be sound with respect to rather small therapeutic trials. However, Table 1 and our previous ATBC Study subgroup analyses6,9,11,14–17 show that there can be strong evidence of vitamin E effect in specific groups of people, even though no overall effect exists. Accordingly, Peto et al’s argument should not be taken as a universal objection to analyzing subgroups in the absence of overall effect.

Several investigators have strongly discouraged subgroup analysis.1–5 However, other authors have considered that a universal denial of subgroup analysis is an exaggerated reaction. Feinstein wanted to “rescue the scientific importance of valid pathophysiologic subgroups from being forgotten or destroyed by excessive vehemence in suggestions that all subgroups are evil”.18 Lagakos noted that “avoiding any presentation of subgroup analysis because of their history of being overinterpreted is a steep price to pay for a problem that can be remedied by more responsible analysis and reporting”.19 Rothwell responded to popular arguments against subgroup analysis and described situations where subgroup analysis seems to be justified.20

Altman considered that biological plausibility is a weak criterion when deciding whether a subgroup finding is likely to be real, since, according to him, physicians seem able to find a biologically plausible explanation for any finding.2 There is much room for speculation at the biochemical level, because the number of genes and their effects is huge, and Altman’s argument can have validity in such a context. However, the number of variables relevant at the population level of biology is much more limited. For example, few factors compare with the importance of smoking as a factor influencing the health of the lungs. Physical activity is also a fundamentally important factor determining health. Smoking affects the metabolism of vitamin E21 and sporadic physical stress causes oxidative stress which is not compensated by an increase in antioxidative enzyme levels, unlike regular physical activity.10 Therefore, both smoking and exercise are plausible modifying factors for the effects of vitamin E supplementation, which increases the credibility of the heterogeneity seen in Table 1.

Previously, two small trials examined the effect of vitamin E on respiratory infections in elderly people, both with less than 700 participants and lasting for about one year. In the first, Meydani et al calculated 13 *P*-values for ITT comparisons between 200 mg/day vitamin E and placebo groups, and only one of them suggested that vitamin E might reduce the incidence of respiratory infections, yet very marginally so ($P = 0.048$).[22] In the second, Graat et al found that 200 mg/day of vitamin E did not influence the incidence of respiratory infections, yet made the symptoms more severe ($P = 0.02$).[23] Because both of these trials are small and there are differences in outcome definitions etc, it is not possible to decide whether their findings are inconsistent or not. Graat et al's findings indicating harmful effects of vitamin E conflict with the wide spread belief that the vitamin is beneficial, or at least not harmful.[24] Therefore, it is not obvious whether Graat et al's findings should be interpreted as a reflection of real harm or as a result of chance. Given the strong evidence of heterogeneity we observed in the effect of vitamin E on pneumonia (Table 1) and on the common cold,[6] it seems plausible that the harmful effects observed by Graat et al are real and are explained by the selection of participants, but do not reflect a universal harmful effect of vitamin E. In this respect, the observed heterogeneity in the ATBC Study can influence the interpretation of smaller trials. Nevertheless, we are skeptical as regards the possibility of extrapolating the effect estimates and the exact limits of the subgroups of Table 1 to other contexts.

Although the division of participants on the basis of baseline physical activity and smoking is sound, both of these factors can change with time. Some participants stopped exercising or smoking over the several-years-long follow-up, yet they remained classified in the same subgroups. This phenomenon can dilute the differences between the subgroups and shift the estimates of effect closer to unity; however, it cannot explain the significant heterogeneity observed when the participants are divided by the baseline measurements. Furthermore, exercise and smoking are correlated with numerous other life style variables and we cannot dismiss the possibility that other life style factors might be behind the heterogeneity observed in Table 1. Nevertheless, this concern does not challenge the evidence indicating that substantial heterogeneity exists across various population groups in the effect of vitamin E on pneumonia risk, even if the real modifying variables might be different from those used for defining the subgroups of Table 1.

The ATBC Study included 29,133 participants which is over 40 times more than the number of participants in the Meydani et al[22] and Graat et al[23] trials. In this respect, a large trial can be considered as a series of smaller trials when there is sound justification for setting the borders between the subgroups. A particular strength of a subgroup analysis of a large trial is that the intervention and outcome definitions are identical over the trial. Therefore, subgroup analysis of a large trial can yield much more valid explanations for the heterogeneity of effect compared with the analysis of the heterogeneity of small trials that have numerous concurrent differences.

For many diseases, recognized risk factors account for at best only a modest fraction of variation in disease risk. Much effort is put into identifying new factors, either environmental or genetic. Our analyses indicate that complex patterns of interaction, perhaps in a context-specific manner, may also contribute to disease risk. Such effects may thus account for some of the unexplained variability of disease risk.

Our subgroup analyses of the respiratory infections of ATBC participants[6,9,14,15] made it also possible to hypothesize that the identified modifying factors might modify the effect of vitamin E on the mortality of these participants. We found that, conditional on a high level of dietary vitamin C intake, age modified the effect of vitamin E on mortality.[16,17] Thus, we could partially extrapolate the modifying factors identified in the subgroup analyses on respiratory infections to an outcome that has a very weak relation to such infections.

Vandenbroucke pointed out that medical science has two divergent goals.[25] First, controlled trials test whether an intervention works or not. Second, most basic medical science emphasizes discovery – searching for the biological mechanisms and causes of diseases, and for explanations in general. This divergence in views is relevant when considering a proper attitude to subgroup analysis. Evidently, great caution must be exercised when proposing a treatment on the basis of unanticipated subgroup findings. On the other hand, subgroup analysis can generate new hypotheses and direct research to new paths, which is the second goal of medical science. Refusing to conduct the subgroup analysis of large trials would lead to an inefficient use of data, the collection of which has required a substantial amount of resources.

Conclusion

The overall effect of vitamin E on pneumonia risk in the ATBC Study implies that there would be no justification for investing further resources into studying the topic because the narrow confidence interval rejects any substantial overall benefits (RR from 0.88 to 1.14). In contrast, our subgroup analysis suggests a path that should be explored: does vitamin E affect the incidence of pneumonia in physically active males who are nonsmokers or who have had only little exposure to smoking?

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Footnotes

Disclosure

The authors report no conflicts of interest in this work.

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Nutrition. 1996 Nov-Dec;12(11-12):804-9.

Vitamin C supplementation and common cold symptoms: problems with inaccurate reviews.

Hemilä H[1].

Author information

Abstract

In 1971, Linus Pauling carried out a meta-analysis of four placebo-controlled trials and concluded that it was highly unlikely that the decrease in the "integrated morbidity of the common cold" in vitamin C groups was caused by chance alone ($P < 0.00003$). Studies carried out since then have consistently found that vitamin C (> or = 1 g/d) alleviates common cold symptoms, indicating that the vitamin does indeed have physiologic effects on colds. However, widespread conviction that the vitamin has no proven effects on the common cold still remains. Three of the most influential reviews drawing this conclusion are considered in the present article. Two of them are cited in the current edition of the RDA nutritional recommendations as evidence that vitamin C is ineffective against colds. In this article, these three reviews are shown to contain serious inaccuracies and shortcomings, making them unreliable sources on the topic. The second purpose is to suggest possible conceptual reasons for the persistent resistance to the notion that vitamin C might have effects on colds. Although placebo-controlled trials have shown that vitamin C does alleviate common cold symptoms, important questions still remain.

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Vitamin C supplementation and respiratory infections: a systematic review.

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Abstract

In this review, the vitamin C trials with military personnel and with other subjects living under conditions comparable to those of military recruits are analyzed to find out whether vitamin C supplementation affects respiratory infections. For this systematic review, we identified seven trials with military personnel, three trials with students in crowded lodgings, and two trials with marathon runners. Eight of these trials were double blind and placebo controlled and seven were randomized. Five small trials found a statistically significant 45 to 91% reduction in common cold incidence in the vitamin C group. These trials were short and the participants were under heavy exertion during the trial. Furthermore, three other trials found a statistically significant 80 to 100% reduction in the incidence of pneumonia in the vitamin C group. The large number of positive findings seems to warrant further consideration of the role of vitamin C in respiratory infections, particularly in military recruits.

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Effect of ascorbic acid on the clinical course of infection-related bronchial asthma and the formation of reactive oxygen metabolites by BAL cells

Schertling M, Winsel K, Müller S, Henning R, Meiske W And Slapke J
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References and Figures are available in the above versions.

From the Berlin-Buch Research Institute for Pulmonary Diseases and Tuberculosis
(Official Director: Dr. P. Luther)

Effect of ascorbic acid on the clinical course of infection-related bronchial asthma and the formation of reactive oxygen metabolites by BAL cells

By MARGIT SCHERTLING, KLAUS WINSEL, STEFAN MÜLLER, RUDOLF HENNING, WOLFGANG MEISKE and JÜRGEN SLAPKE

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Infection-related bronchial asthma, ascorbic acid, antioxidant, peak flow, bronchial hyperreactivity, broncho-alveolar lavage, alveolar differential cell count, chemiluminescence, reactive oxygen metabolites

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List of abbreviations

AM	Alveolar macrophages
BAL	Bronchoalveolar lavage
BHR	Bronchial hyperreactivity
CL	Chemiluminescence
DCC	Differential cell count
ROM	Reactive oxygen metabolites
R_{AW}	Airway resistance (measured by occlusive pressure techniques)

Summary (Authors‘ summary in english)
Possible anti-asthmatic effectiveness of ascorbic acid was checked, in a double blind study, on patients with infection-related bronchial asthma. Basic medication to 29 out-patients was accompanied by three oral doses of 5 g/day of ascorbic acid, as compared to placebo, through 35 days. Testing periods were randomised by cross-over design with seven-day washout periods. The following parameters were investigated and were evaluated:

- Daily asthma symptom score;
- Four measurements per day of expiratory peak flow, throughout the entire study;
- Three checks throughout study of bronchial hyperreactivity, using histamine provocation;
- Broncho-alveolar lavage at the end of testing periods, with determination of alveolar differential cell count and measurement of metabolic activity of broncho-alveolar cells, using chemiluminescence;
- Global assessment of effectiveness and tolerance by doctor and patient.

Ascorbic acid exhibited merely poor broncholytic action. Symptom scores were slightly improved in the course of treatment, and peak flow values were slightly increased, as well. Hence, clinically relevant anti-asthmatic and, more specifically, broncholytic effects were not observed. However, bronchial hyperreactivity was reduced by uptake of ascorbic acid in 52 percent of all asthma patients involved. Alveolar differential cell count in patients with infection-related bronchial asthma was characterised by alveolar lymphocytosis. Chemiluminescence measurements were applied to alveolar macrophages and revealed reduced chemiluminescence response under the impact of ascorbic acid. These findings are likely to support the assumption that ascorbic acid, an anti-oxidant, reduced the buildup of reactive oxygen metabolites in patients with infection-related asthma and thus counteracted the inflammatory pathogenetic mechanism and, consequently, might be conducive to moderate lowering of bronchial hyperreactivity. The use of ascorbic acid for prophylactic medication on patients with bronchial hyperreactivity or mild forms of asthma appears to be a possible option, as a result of this study. Due consideration should be given to contraindications to administration of anti-oxidants, such as purulent infections.

Summary (Translation from German; English translation by original authors above)

The potential anti-asthmatic effectiveness of ascorbic acid was studied in patients with infection-related bronchial asthma. In addition to the basic medication, 29 outpatients were additionally treated for a period of 35 days with 5 g/day of ascorbic acid in comparison to oral placebo in 3 daily doses. The allocation of the testing periods was randomized by cross-over design with 7-day washout periods. The following parameters were investigated and evaluated: daily asthma symptom score, measurement of the expiratory peak flow 4 times per day during the entire course of the study, testing of bronchial reactivity using histamine provocation at 3 time points during the course of the study, broncho-alveolar lavage at the end of the study periods with determination of the alveolar differential cell count and measurement of metabolic activity of the bronchoalveolar cells using chemiluminescence, and global assessment of the efficacy and tolerability by doctor and patient.

Ascorbic acid exhibited a weak broncholytic effect. During treatment, symptom scores were slightly improved and there was also a slight increase in peak flow values. Hence, a clinically relevant anti-asthmatic and in particular, broncholytic effect was not observed. However, bronchial hyperreactivity was reduced by taking ascorbic acid in 52 percent of the asthma patients. The alveolar differential cell count was characterized by alveolar lymphocytosis in patients with infection-related bronchial asthma. Chemiluminescence measurements of alveolar macrophages revealed a reduced chemiluminescence response under the impact of ascorbic acid. These findings suggest that ascorbic acid, as an antioxidant, reduces the formation of reactive oxygen metabolites in patients with infection-related asthma and thus counteracts the inflammatory pathomechanism and consequently might be able to bring about moderate lowering of bronchial hyperreactivity. The use of ascorbic acid as prophylactic medication for patients with bronchial hyperreactivity or mild forms of asthma appears to be a possibility as a result of this study. Due consideration should be given to possible contraindications to administration of antioxidants, e.g., the presence of purulent infections.

Introduction

In the past 40 years, a number of works have been published that deal with the effect of ascorbic acid (4, 29) on the clinical course of bronchial asthma or on the histamine, antigen or metacholine induced bronchospasm, although some of the results that were achieved were contradictory. While in some studies, a protective effect (1, 12, 15, 19, 28, 35) of ascorbic acid on the pharmacodynamic or allergen induced bronchospasm or clinical course of bronchial asthma was established, in other cases, no effect of ascorbic acid (16, 17) could be found. The possible positive effect of ascorbic acid on bronchial asthma could be due to its antioxidative properties (2, 3, 5, 9). Lipid peroxide and reactive oxygen metabolites (ROM) (O_2^-, H_2O_2, OCl^-, OH^-) which can be formed in excess in the lungs under pathological conditions stimulate, e.g., arachidonic acid metabolism and lead to the formation of cyclo-oxygenase and lipoxygenase products which have a bronchoconstrictive effect, such as prostaglandins and leukotrienes (8, 12).

In general, in vivo, various antioxidants (including ascorbic acid) and antioxidant enzymes, so-called radical scavengers protect the lungs from damage due to reactive oxygen metabolites and lipid peroxide (10). In the presence of increased activity of the pulmonary inflammatory cells (e.g., alveolar macrophages, granulocytes) with bronchial asthma, the equilibrium between oxidative and antioxidative capacity in the lungs may be displaced in favor of the oxidative process, such that additional administration of ascorbic acid at a high dose (5 g/day) and over a longer period of time may be expected to provide a therapeutic effect. In the present work, the hypothesis of an anti-asthmatic effect of ascorbic acid is to be tested (6, 7).

Materials and methods

A total of 29 patients with infection-related bronchial asthma (18 men and 11 women from 18 to 60 years of age) were recruited for the double blind crossover study under ambulatory conditions. Inhaled and systemic corticosteroids, renal disease and acute and serious purulent infections were considered to be exclusion criteria. The study was conducted over a period of 35 days. It was divided into a 2-week placebo period, 1-week wash-out test and 2-week ascorbic acid period. The sequence of the test periods was chosen at random (Fig. 1).

For the present study, in addition to the basic medication, a daily dose of 5 g ascorbic acid (Ascorvit containing 500 mg) was defined in comparison to oral placebo in 3 individual doses. Coated tablets from VEB Jenapharm, Clinical Research Division, lot numbers 150485 and 050886 were used. The patients received packages furnished with lot numbers that were coded according to the double blind study conditions. The code was not broken during the study.

During a pre-period of 2 weeks, the starting values for pulmonary function parameters were to be determined under the anti-asthmatic treatment up to that time. At the same time during this period, the patients were to learn how to complete the diary and determine the maximum expiratory peak flow with the peak flow meter.

During the 35-day double blind treatment period, the patients were seen 4 times: on the 8^{th}, 14^{th}, 29^{th} and 35^{th} day after the start of treatment. In the middle of the verum [HH: verum = active intervention] and placebo periods, measurements of bronchial hyperreactivity were performed again and at the end of the test period, a broncho-alveolar lavage with cytological examination and chemiluminescence measurement were performed.

In principle, the efficacy of an anti-asthmatic agent cannot be determined by a single target parameter. Even asthma symptoms are expressed in distinctly different ways. To record the symptoms, the complaints were listed separately in a diary (Table 1).

Each patient was given a peak flow monitor (Vitalograph) at the start of the study to measure the maximum expiratory velocity during the course of the study. The measurement was performed 4 times a day (6 a.m., 9 a.m., 12 noon, and 6 p.m.) by the patients while sitting. The highest value (l/min) out of each of three measurements was noted in the diary.

The measurement of nonspecific BHR was performed on the Bronchoscreen Measuring Station (Jaeger, Wuerzburg/West Germany) under the use of histamine dihydrochloride at a concentration of 1 mg/ml as the pharmacodynamic provocation substance [20]. The advantage of this method is that in contrast to conventional measuring procedures, better quantification of the bronchial reaction can be achieved with a distinct reduction in time needed for the examination. The histamine aerosol administration was performed breath for breath during the inspiratory phase during spontaneous respiration (nebulizer output per breath: 5 µmol). The bronchial reaction was simultaneously determined on the same instrument with the airway resistance method (R_{AW}). As target criteria of the BHR, a 50% increase in respiratory tract resistance (R_{AW}) in comparison to the starting value with simultaneous exceedance of the R_{AW} value of 0.3 kPa/(l · s) post provocation was defined. The following pulmonary function parameters prior to inhalative provocation were valid as exclusion criterion for the examination: $R_{AW} > 0.5$ kPa/(l · s) or $FEV_1 < 80$ % of the target value. Through pre-testing, BHR to a cumulative histamine dose of ≤8 µmol was demonstrated for all 29 patients. To enable a semiquantitative evaluation in the hyperreactivity zone, during the test periods, the threshold dose for the BHR to 1 µmol histamine was determined that corresponds to 40 respirations. The BHR ($PD_{50}R_{AW}$) was defined as positive at a cumulative provocation dose of ≤1 µmol histamine, and negative at >1 µmol histamine.

Broncho-alveolar lavage (BAL): The alveolar macrophages (AM) were obtained under outpatient conditions by broncho-alveolar lavage. The BAL was performed in the medial lobe with a fiber optic bronchoscope under local anesthesia with sterile physiological NaCl solution in individual portions (20 ml 57 times) (18, 20, 21, 31). The rinse fluid was pooled in a siliconized Erlenmeyer flask cooled in ice water, then filtered through a wire sieve (250 µm) and centrifuged at 4°C (500 g, 10 min). The cell sediment was treated for 10 min. at 4°C with 10 ml sterile erythrocyte lysis buffer (pH = 7.4) and then washed twice with phosphate buffered physiologic saline solution (PBS) and set to a cell density of 106 AM/ml PBS.

Cytologic investigations: The total cell count and the proportion of AM in the cell suspension were determined in the cell chamber according to Neubauer using morphological criteria and by an esterase test with α-naphthyl acetate. The cell differentiation was performed after staining the cell suspension with a mixture of equal parts of 1 % aqueous Nile blue chloride and thionine tartaric acid solution according to Feyrter (1 g thionine + 0.5 g tartaric acid/100 ml distilled H_2O) at a 1:1 ratio.

Chemiluminescence (CL) measurement

Measuring technique: The measurement was performed with the liquid scintillation counter Isocap300 (Searle Nuclear Chicago Division, Holland) in out-of-coincidence mode and recycling operating mode. The measuring time per sample was 0.2 min at an interval of approximately 6 min. Polypropylene test tubes (so-called mini vials) were used (measurement temperature 24°C). The work room was completely darkened and equipped with dark room illumination (33).

Reagents: As a medium for the CL measurement was veronal buffered physiological NaCl solution with an adjuvant of albumin, glucose, Ca^{2+} and Mg^{2+} according to information provided by Wulf et al. (34). The yeast cell walls for the stimulation of the AM were isolated from baker's yeast (23). The opsonization of the yeast cell walls was performed with human serum (concentration of the yeast cell wall dispersion 5 mg/1 ml PBS). Luminol (CL intensifier) was brought into solution at a concentration of 6 mg/3 ml PBS with the addition of 24 µl diethylamine by ultrasound treatment. Lucigenin (Cl intensifier) was dissolved in PBS (10.2 mg/2 ml).

Measuring technique: 2 ml veronal buffer, 20 µl Luminal or Lucigenin solution and 100 µl of AM suspension ($1 \cdot 10^5$ AM) were mixed in a measuring tube and pre-incubated for approximately 15 minutes with liquid scintillation counter. Afterwards, the yeast cell wall suspension (500 µg) was added and the CL measurement performed.

The Luminol and Lucigenin intensified CL was measured in parallel for this[1)]. For quantitative analysis of the measurement results, the peak heights (IPM) and areas under the CL curves (IP) were determined within 200 min after stimulation with the yeast cell wall suspension.

For characterization of the pharmacokinetics of ascorbic acid for the therapy regimen used, the daily profile of the serum level of ascorbic acid was determined enzymatically with the L-ascorbic acid color test (Boehringer, Mannheim, West Germany). Global evaluation of efficacy and tolerability were recorded by patient and physician.

The arithmetic mean (x) and the standard deviation (s) were determined for the statistical analysis of the measured variables.

The statistical comparison of the groups was performed with the paired t-test and the Wilcoxon test.

[1)] The Lucigenin intensified chemiluminescence shows the formation of superoxide anion (O_2^-), while the Luminol dependent chemiluminescence is specific for hypohalogenite.

Fig. 1: Schedule for the controlled double blind trial with ascorbic acid/placebo in patients with infection-related bronchial asthma. BHR – bronchial hyperreactivity, BAL – broncho-alveolar lavage

		Test periods				
	Pre-period	Placebo-Verum		Washout period	Verum-Placebo	
Days		8	14	21	29	35
Peak flow diary	4 times a day [over all study]					
Physician consultation	*	*	*		*	*
BHR	*	*			*	
BAL			*			*
Ascorbic acid serum level measurement		*	*		*	*

Note [HH]:
Verum: active treatment, here vitamin C

Table 1: Symptom scores

Analysis of asthmatic symptoms:

0 = no symptoms

1 = mild or brief symptoms that do not require additional use of medication

2 = more severe symptoms that are relieved within 15 minutes by additional medication

3 = more severe symptoms that do not respond adequately to or in a delayed manner to additional medication or require repeated use

Symptoms can include: intermittent dyspnea, wheezing, sensation of tightness in the morning or dry irritating cough

Results

The overall mean peak flow value for all asthmatics was 410 l/min in the placebo phase and 419 l/min in the verum phase. This slight increase of an average of 9 l/min in the ascorbic acid group was statistically not significant and may also not be clinically relevant. A similar impression resulted from the analysis of the symptom scores. The mean in the placebo phase was 0.72 points and under ascorbic acid it was 0.65 points. Consequently, a slight decrease in symptoms could be observed in the treatment period with ascorbic acid.

The investigations on bronchial hyperreactivity were performed at each of 3 time points, in the pre-period, after 8 days and on the 29th day. The course of bronchial hyperreactivity in 23 subjects during the investigation period is presented in Table 2. In 11 asthmatics, no change occurred during both periods. In 12 subjects, bronchial hyperreactivity was detectable during the placebo phase, while in the ascorbic acid phase, a negative reaction was observed. The opposite case did not occur. This asymmetry is significant ($p \leq 0.0003$; test on the basis of the binomial distribution). As a result of this, in 52% of patients with bronchial asthma, bronchial hyperreactivity could be effectively lowered.

The analysis of the bronchial lavage showed that 8 out of 24 patients exhibited an alveolar differential cell count that was commensurate with standards during both test periods. In 5 patients, normalization of the alveolar cell count resulted under ascorbic acid treatment, and in 6 other patients, the alveolar lymphocytes primarily present subsided. In 3 cases, alveolar eosinophilia persisted. Of note, there was considerable lymphocytosis (>28%) in 3 patients during both periods (Table 3).

The results of the CL measurements on AM from the BAL fluid show that under ascorbic acid, a reduction in the chemiluminescence response results with the Lucigenin as well as the Luminol intensification (Table 4).

The difference between the two groups (placebo period, ascorbic acid period) is statistically significant for the peak heights ($p \sim 0.03$).

The changes in the alveolar macrophage activity measured on the basis of the formation of ROM do not correlate or only weakly correlate with the changes in peak flow values and symptom scores ($|r| < 0.04$ in all cases).

In the analysis of the results, more precise characterization of those patients for whom definite therapeutic or hyperreactivity lowering effects could be proven was attempted (Fig. 2). However, the search for responder-typical commonalities was unsuccessful.

The serum level on the 8th day was 13.8–26.8 mg and 10.1–28.4 mg ascorbic acid/l on the 14th day, corresponding to the administration rhythm. As was expected, they were considerably above the normal range for men (Fig. 3).

The evaluation of the tolerability of the test preparation by the physician and the patient did not reveal any relevant differences between the test periods.

Only 1 patient complained of nausea during the ascorbic acid period; another indicated increased sensation of thirst over the entire test period. 3 patients noted temperature increases up to 38.2°C once in the evening on the day of the broncho-alveolar lavage.

Table 2: Course of bronchial hyperreactivity (BHR) with oral ascorbic acid (5 g/day for 35 days) in comparison to placebo (n = 23)
Positive criteria: $PD_{50}R_{AW} \leq 1$ µmol histamine

		BHP in the vitamin C period		
		Positive	Negative	Totals
BHR in the placebo period	Positive	9	12	21
	Negative	0	2	2
	Totals	9	14	23

Table 3: Cell distribution in the broncho-alveolar fluid in patients with infection-related bronchial asthma:
0 = conforms to standards, ↑ = elevated, ↑↑ = strongly elevated
(estimation of results based on normal values according to Hunninghake and Crystal [31])

	Placebo period		Ascorbic acid period	
n	Lymphocytes	Eosinophils	Lymphocytes	Eosinophils
8	0	0	0	0
2	0	(5%) ↑	0	0
3	(15%) ↑	0	0	0
3	(15%) ↑	(5%) ↑	0	(5%) ↑
3	(34%) ↑	(3%) ↑	(53%) ↑↑	0
1	(16%) ↑	(8%) ↑	(14%) ↑	(25%) ↑
1	0	(8%) ↑	(18%) ↑	0
1	(17%) ↑	0	0	(5%) ↑
1	0	0	(53%) ↑↑	0
1	(16%) ↑	0	(26%) ↑	(8%) ↑
24 (Total)				

Table 4: Comparison of the parameter of the chemiluminescence (CL) curves of the alveolar macrophages of patients with infection-related bronchial asthma (n = 24)

	Area under the CL curve	Peak height
	IP 10^{-8} *	IPM 10^{-6} **
	x ± s	x ± s
Placebo period		
Lucigenin	1.78 ± 1.51	2.11 ± 1.93
Luminol	2.17 ± 2.94	2.23 ± 2.77
Ascorbic acid period		
Lucigenin	1.29 ± 0.74	1.41 ± 0.87
Luminol	1.81 ± 1.72	1.91 ± 2.07
Statistics	a:c p ~ 0.08	a:c p ~ 0.03
Wilcoxon test	b:d p ~ 0.09	b:d p ~ 0.03
* IP = impulses		
** IPM = impulses per minute		

Fig. 2: Peak flow course curve of an asthma patient during the entire study

L l/min Days [Tage]

see the German versions for the figure:
As characters: http://www.mv.helsinki.fi/home/hemila/A/Schertling_1990_ch.pdf
As a bitmat: http://www.mv.helsinki.fi/home/hemila/A/Schertling_1990_bm.pdf

Fig. 3: Daily profile of the serum level of ascorbic acid in a male asthmatic.

Ascorbic acid [mg/l]
Intake [Einnahme]

14th day [14. Tage]
8th day [8. Tage]

Normal range for men [Normbereich fur Manner]
Time [h.] [Zeit]

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Discussion

In comparison to the individual studies with ascorbic acid in bronchial asthma to date in which low doses were used over a shorter administration time period (11, 15, 17, 19, 25, 30), for the first time in a complex study a therapeutic effect of ascorbic acid could be proven by including pulmonary function, symptom scores, bronchial hyperreactivity and broncho-alveolar lavage, which is most notably expressed by significant lowering of bronchial hyperreactivity. Bronchial hyperreactivity is an important quantifiable characteristic in asthmatic disease. Hyperreactivity is usually already recognizable before the manifestation of 'clinical asthma' and is consequently causally involved in the pathogenesis of asthma. Nowadays, bronchial hyperreactivity is even considered to be common denominator of all asthma forms (27). The inhaled provocation with histamine has proven to be the established quantitative method for the study of bronchial hyperreactivity (20). A clinically relevant raising of the threshold of bronchial reactivity resulted in 52% of asthmatics, and indeed, in contrast to the placebo period, a hyperreactivity lowering effect could be measured in 11 subjects under ascorbic acid.

An effective reduction in bronchial hyperreactivity must be considered to be a decisive element of asthma prevention measures today (26). At the same time, bronchial hyperreactivity is considered to be the most important determining factor for the course of asthma disease. Pulmonary function studies frequently give varying results depending on external influences, daily rhythm and medication. For this reason, the peak flow value, as a more objective pulmonary function parameter, was measured four times a day and documented in the diary. Relatively rare, selective measurements of pulmonary function parameters by more extensive measuring techniques such as spirometry or body plethysmography, in spite of higher personnel/technical expenditure, do not result in more reliable results than the significantly more frequently measured peak flow value that records the daily variation range of pulmonary function of asthmatics in a more representative manner. The peak flow values and the symptom scores indeed showed a tendency toward improvement during ascorbic acid therapy, but the differences in both test time periods were not significant.

The results of the chemiluminescence measurements on alveolar macrophages demonstrated that under ascorbic acid treatment, a reduced chemiluminescence response resulted. This indicates that ascorbic acid reduces the formation of reactive oxygen metabolites in patients with bronchial asthma and consequently could also have an inhibitory effect on the biosynthesis of cyclo-oxygenase and lipoxygenase products which have a bronchoconstrictive effect. Ascorbic acid probably does not directly reduce the formation of reactive oxygen metabolites e.g., by the NAD(P)H oxidase system of inflammatory cells. The oxygen radicals and toxic oxidants that arise are reduced and are thus rendered innocuous before they can react with the pulmonary cells or the lung tissue. Furthermore, the present study underlines the value of bronchial alveolar lavage in bronchial asthma (13, 24, 32). Statements about the degree of inflammation in infection-related bronchial asthma and the therapeutic effect of anti-asthmatic/allergic acting substances can be made from the alveolar differential cell count (14, 22). From the results, it can be concluded that ascorbic acid at a high dose (5 g/day) is a suitable antioxidant for reduction of radical formation in infection-related bronchial asthma and consequently could favorably affect the clinical course of asthma. This must be further clarified in other comprehensive studies.

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J Virol. 2010 Aug;84(15):7418-26. doi: 10.1128/JVI.02290-09. Epub 2010 Apr 7.

The ABCs of rhinoviruses, wheezing, and asthma.

Gern JE[1].

Author information

Abstract

Human rhinoviruses (HRVs) were discovered as common cold pathogens over 50 years ago. Recent advances in molecular viral diagnostics have led to an appreciation of their role in more-significant respiratory illnesses, including bronchiolitis in infancy, childhood pneumonia, and acute exacerbations of chronic respiratory diseases such as asthma, chronic obstructive lung disease, and cystic fibrosis. Until a few years ago, only two groups of HRVs (A and B) had been recognized. However, full and partial sequencing of HRVs led to the discovery of a third species of HRV (HRV-C) that has distinct structural and biologic features. Risk factors and pathogenic mechanisms for more-severe HRV infections are being defined, and yet fundamental questions persist about mechanisms relating this common pathogen to allergic diseases and asthma. The close relationship between HRV infections and asthma suggests that antiviral treatments could have a major impact on the morbidity associated with this chronic respiratory disease.

PMID: 20375160 PMCID: PMC2897627 DOI: 10.1128/JVI.02290-09

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Eur J Pediatr. 2011 Jan;170(1):59-63. doi: 10.1007/s00431-010-1270-z. Epub 2010 Aug 6.

The effect of vitamin C on upper respiratory infections in adolescent swimmers: a randomized trial.

Constantini NW[1], Dubnov-Raz G, Eyal BB, Berry EM, Cohen AH, Hemilä H.

Author information

Abstract

The risk of upper respiratory infections (URIs) is increased in people who are under heavy physical stress, including recreational and competitive swimmers. Additional treatment options are needed, especially in the younger age group. The aim of this study was to determine whether 1 g/day vitamin C supplementation affects the rate, length, or severity of URIs in adolescent swimmers. We carried out a randomized, double-blind, placebo-controlled trial during three winter months, among 39 competitive young swimmers (mean age 13.8 ± 1.6 years) in Jerusalem, Israel. Vitamin C had no effect on the incidence of URIs (rate ratio = 1.01; 95% confidence interval (CI) = 0.70-1.46). The duration of respiratory infections was 22% shorter in vitamin C group, but the difference was not statistically significant. However, we found a significant interaction between vitamin C effect and sex, so that vitamin C shortened the duration of infections in male swimmers by 47% (95% CI: -80% to -14%), but had no effect on female swimmers (difference in duration: +17%; 95% CI: -38% to +71%). The effect of vitamin C on the severity of URIs was also different between male and female swimmers, so that vitamin C was beneficial for males, but not for females. Our study indicates that vitamin C does not affect the rate of respiratory infections in competitive swimmers. Nevertheless, we found that vitamin C decreased the duration and severity of respiratory infections in male swimmers, but not in females. This finding warrants further research.

PMID: 20689965 DOI: 10.1007/s00431-010-1270-z

[Indexed for MEDLINE]

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Can Med Assoc J. 1974 Jul 6; 111(1): 31–36.

PMCID: PMC1947567
PMID: 4601508

The effect on winter illness of large doses of vitamin C

T. W. Anderson, G. Suranyi, and G. H. Beaton

Abstract

Between December 1972 and February 1973, 2349 volunteers participated in a double-blind trial to assess the effect of large doses of vitamin C on the incidence and severity of winter illness. In addition, records were kept but no tablets taken during March. Subjects were randomly allocated to eight treatment regimens: three prophylactic-only (daily dose 0.25, 1 or 2 g), two therapeutic-only (4 or 8 g on the first day of illness), one combination (1 g daily and 4 g on the first day of illness), and two all-placebo. None of the groups receiving vitamin C showed a difference in sickness experience that was statistically significant from that of the placebo groups, but the results obtained were compatible with an effect of small magnitude from both the prophylactic and therapeutic regimens, and an effect of somewhat greater magnitude from the combination regimen. The combination regimen was associated more with a reduction in severity than frequency of illness, although the extra dosage was limited to the first day of illness. In spite of the eightfold range in daily dose, the three prophylactic-only regimens showed no evidence of a dose-related effect, but the 8 g therapeutic dose was associated with less illness than the 4 g therapeutic dose. There was no evidence of side effects from the 1 and 2 g prophylactic doses of vitamin C, and no evidence of a rebound increase in illness during the month following withdrawal of the daily vitamin supplements. On the basis of this and other studies it is suggested that the optimum daily dose of vitamin C is less than 250 mg, except possibly at the time of acute illness, when a larger daily intake may be beneficial.

Full text

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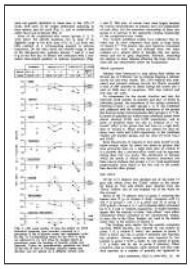

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Am J Clin Nutr. 1979 Aug;32(8):1686-90.

The effects of ascorbic acid and flavonoids on the occurrence of symptoms normally associated with the common cold.

Baird IM, Hughes RE, Wilson HK, Davies JE, Howard AN.

Abstract

A controlled study was made of the effects of natural orange juice, synthetic orange juice, and placebo in the prevention of the common cold; both natural and synthetic orange juices contained 80 mg of ascorbic acid daily. Three-hundred sixty-two healthy normal young adult volunteers, ages 17 to 25 years, were studied for 72 days with 97% of participants completing the trial. There was a 14 to 21% reduction in total symptoms due to the common cold in the supplemented groups that was statistically significant (P less than 0.05). Ascorbic acid supplementation also increased the number of "episode-free" subjects. However, the clinical usefulness of the results does not support prophylactic ascorbic acid supplements in the well-nourished adult. The results in this study with both natural and synthetic orange juice of physiological content of ascorbic acid, are similar to those obtained using a "megadose" of ascorbic acid.

PMID: 463806 DOI: 10.1093/ajcn/32.8.1686

[Indexed for MEDLINE]

Social Studies of Science

The Politics of Therapeutic Evaluation: The Vitamin C and Cancer Controversy

Evelleen Richards

First Published November 1, 1988 | Research Article
https://doi.org/10.1177/030631288018004004

Abstract

This paper reconstructs and analyzes the content and context of the debate over the efficacy of vitamin C in the treatment of cancer, and compares it with medical responses to, and evaluations of, two other cancer drugs — the cytotoxic drug SFU (conventionally used in the treatment of gastro-intestinal cancers) and the `naturally-occurring' (but recombinant DNA-produced) drug interferon. This comparative approach is designed to facilitate the integration of microsociological and structural levels of analysis of the processes by which knowledge claims about therapeutic efficacy are evaluated by the powerful adjudicating medical community. It is argued that the assessment of medical therapies is inherently a social and political process; that the idea of neutral appraisal is a myth; that clinical trials, no matter how rigorous their methodology, inevitably embody the professional values or commitments of the assessors; and that judgements about experimental findings may be structured by wider social interests, such as consumer choice or market forces. It is concluded that the necessarily social character of medical knowledge cannot be eliminated by methodological reform, and that this has important implications for the social implementation of medical therapies and techniques.

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Proc Natl Acad Sci U S A. 1971 Nov; 68(11): 2678–2681.
doi: 10.1073/pnas.68.11.2678
PMCID: PMC389499
PMID: 4941984

The Significance of the Evidence about Ascorbic Acid and the Common Cold

Linus Pauling
Department of Chemistry, Stanford University, Stanford, California 94305

Abstract

Only four independent double-blind studies have been reported of the effect of ascorbic acid regularly ingested in daily amounts more than 100 mg, in comparison with a placebo, in decreasing the incidence and integrated morbidity of the common cold for subjects exposed to cold viruses in the ordinary way and without colds when the test period began. A statistical analysis of these four studies leads to rejection of the null hypothesis that ascorbic acid has no more protective power than the placebo at the 99.86% level of confidence for the incidence of colds and the 99.9978% level of confidence for the integrated morbidity.

Full text

Full text is available as a scanned copy of the original print version. Get a printable copy (PDF file) of the complete article (853K), or click on a page image below to browse page by page. Links to PubMed are also available for Selected References.

2678

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11;**1**(5540):603–606. [PMC free article] [PubMed] [Google Scholar]

Article

January 17, 1977

Therapeutic Effect of Vitamin C
A Co-Twin Control Study

Judy Z. Miller; Walter E. Nance, MD, PhD; James A. Norton, PhD; et al

» Author Affiliations

JAMA. 1977;237(3):248-251. doi:10.1001/jama.1977.03270300052006

Abstract

Three different dosages of vitamin C, dependent on body weight, were administered to 44 school-aged monozygotic twins for five months using a double-blind, co-twin control study design. The mothers recorded daily observations of cold symptoms, and multiple biochemical, anthropometric, and psychological measurements were made at the beginning and end of the study. Paired comparisons showed no significant overall treatment effect on cold symptoms, but the response was not uniform in all subgroups. Treated girls in the youngest two groups had significantly shorter and less severe illness episodes, and an effect on severity was also observed in the youngest group of boys. The seven treated twins in the latter group also grew an average of 1.3 cm more than their untreated co-twins during the five-month period of the study.

(*JAMA* 237:248-251, 1977)

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Precision Oncology News, 2018

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Questionable Survival Benefit of Aspirin Use in Patients With Biliary Tract Cancer

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Questionable Survival Benefit of Aspirin Use in Patients With Biliary Tract Cancer—Reply

JAMA Oncology | *Comment & Response* | *March 19, 2020*

Format: Abstract

Free Radic Biol Med. 2016 Apr;93:84-93. doi: 10.1016/j.freeradbiomed.2015.12.017. Epub 2015 Dec 15.

Therapeutic treatment with ascorbate rescues mice from heat stroke-induced death by attenuating systemic inflammatory response and hypothalamic neuronal damage.

Chang CY[1], Chen JY[2], Chen SH[3], Cheng TJ[4], Lin MT[5], Hu ML[6].

Author information

Abstract

The impact of ascorbate on oxidative stress-related diseases is moderate because of its limited oral bioavailability and rapid clearance. However, recent evidence of the clinical benefit of parenteral vitamin C administration has emerged, especially in critical care. Heatstroke is defined as a form of excessive hyperthermia associated with a systemic inflammatory response that results in multiple organ dysfunctions in which central nervous system disorders such as delirium, convulsions, and coma are predominant. The thermoregulatory, immune, coagulation and tissue injury responses of heatstroke closely resemble those observed during sepsis and are likely mediated by similar cellular mechanisms. This study was performed by using the characteristic high lethality rate and sepsis-mimic systemic inflammatory response of a murine model of heat stroke to test our hypothesis that supra-physiological doses of ascorbate may have therapeutic use in critical care. We demonstrated that parenteral administration of ascorbate abrogated the lethality and thermoregulatory dysfunction in murine model of heat stroke by attenuating heat stroke-induced accelerated systemic inflammatory, coagulation responses and the resultant multiple organ injury, especially in hypothalamus. Overall, our findings support the

hypothesis and notion that supra-physiological doses of ascorbate may have therapeutic use in critical care.

KEYWORDS: Ascorbate; Heat stroke; Systemic inflammatory response

PMID: 26703968 DOI: 10.1016/j.freeradbiomed.2015.12.017

[Indexed for MEDLINE]

Publication type, MeSH terms, Substance

LinkOut - more resources

Format: Abstract

J Infect Dis. 1997 Feb;175(2):237-46.

Perspective: validating surrogate markers--are we being naive?

De Gruttola V[1], Fleming T, Lin DY, Coombs R.

Author information

Abstract

Because of the difficulties in conducting studies of clinical efficacy of new therapies for human immunodeficiency virus infection and other diseases, there is increasing interest in using measures of biologic activity as surrogates for clinical end points. A widely used criterion for evaluating whether such measures are reliable as surrogates requires that the putative surrogate fully captures the "net effect"-the effect aggregated over all mechanisms of action-of the treatment on the clinical end point. The variety of proposed metrics for evaluating the degree to which this criterion is met are subject to misinterpretation because of the multiplicity of mechanisms by which drugs operate. Without detailed understanding of these mechanisms, metrics of "surrogacy" are not directly interpretable. Even when all of the mechanisms are understood, these metrics are associated with a high degree of uncertainty unless either treatment effects are large in moderate-size studies or sample sizes are large in studies of moderately effective treatments.

PMID: 9203643 DOI: 10.1093/infdis/175.2.237

[Indexed for MEDLINE]

Publication types, MeSH terms, Substance, Grant support

LinkOut - more resources

Format: Abstract

Biomarkers. 2013 Aug;18(5):446-54. doi: 10.3109/1354750X.2013.810668.

Variability in oxidative stress biomarkers following a maximal exercise test.

Mullins AL[1], van Rosendal SP, Briskey DR, Fassett RG, Wilson GR, Coombes JS.

Author information

Abstract

The oxidative stress response to maximal exercise may provide useful clinical biomarkers for assessing redox homeostasis. The aim was to determine the between-individual variability in the exercise-induced change in oxidative stress measures and investigate predictors of these responses. Plasma F2-isoprostanes (Isop), protein carbonyls (PCs), glutathione peroxidase (GPX) activity and total antioxidant capacity (TAC) were measured before and after a maximal treadmill exercise test. Exercise produced significant increases in Isop (27.0%), PC (6.2%) and GPX (7.8%). There were large between-individual coefficients of variation: Isop (152%), PC, (240%), GPX (130%) and TAC (243%).

PMID: 23862764 DOI: 10.3109/1354750X.2013.810668

[Indexed for MEDLINE]

MeSH terms, Substances

LinkOut - more resources

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Vitamin C and Cancer: Medicine or Politics?

Author: Ullica Segerstrale
Date: Jan. 31, 1992
From: Science(Vol. 255, Issue 5044)
Publisher: American Association for the Advancement of Science
Document Type: Book review
Length: 2,055 words
Article Preview :

The author's aim with this book is twofold: to provide a case study of "social construction of science," in line with a current trend in science studies; and to take a swing at the medical establishment, in which regard she steps forth, in the book's final chapter, as an outright spokesperson for alternative medicine.

Richard's strategy is to question the key procedure in the testing of new cancer drugs: the randomized controlled clinical trial. If she can show that there can be no agreement based on factual evidence among proponents and opponents of new therapies, her case would fit right in with the claims of those who see controversies in science as merely a matter of scientists' social or strategic interests, disregarding intellectual commitments, convictions about "good science," standards of proof, and the like. Moreover, the failure of the randomized controlled clinical trial to determine the therapeutic efficacy of new experimental drugs, or of any drug, would serve to undermine the medical experts' monopoly on treatment of cancer patients and open up the possibility for patients to choose freely among therapies, including "alternative" ones.

Richards's choice of case study, Linus Pauling and his fight to get vitamin C accepted as a treatment for cancer, may not quite lend itself to such ambitious aims. The reader who wishes to assess just how well Richards in fact succeeds in proving her point is in for some serious work. Vitamin C and Cancer is an exceedingly well documented, quite complicated case study in which it is sometimes hard to keep track of the sequence and significance of events, despite the author's cross-referencing efforts.

Luckily, the book does not have to be read in such an inquisitory spirit. The case study on its own provides interesting reading and fascinating insights into the world of science and medicine. In fact, the book can be read in several different ways. One can see Pauling as a folk hero, bravely fighting the medical establishment for a fair test of his alternative, easily accessible, and potentially beneficial megavitamin cancer therapy. One can see him as the enfant terrible of established science and medicine, through his various actions testing and challenging the hidden assumptions of established rules and procedures. Or the book might be read as a handbook in scientific Machiavellianism.

The book describes the long-term (about 20 years) collaboration between Pauling and a Scottish doctor, Ewan Cameron, both champions of vitamin C therapy for cancer, albeit with initially rather different rationales. Cameron had written a book on his theoretical views of the cancer process in 1966, explaining the spread of cancer as having to do with the failure of the inhibitor (PHI) of the enzyme hyaluronidase to stop overproduction of the enzyme. This led to the weaking of the "ground substance" surrounding the cells. Cameron believed ascorbic acid to be structurally similar to PHI and speculated that vitamin C may help the body synthesize needed PHI and thus control cancer. He claimed some good observational results from his...

Source Citation

Segerstrale, Ullica. "Vitamin C and Cancer: Medicine or Politics?" *Science*, vol. 255, no. 5044, 1992, p. 613+. Accessed 20 Mar. 2020.

Gale Document Number: GALE|A11973053

s About Micronutrient Supplements in American Academic Medicine

Archives of Internal Medicine 158(20):2187-91 · December 1998 with 344 Reads
.20.2187 · Source: PubMed

oodwin
niversity of Texas Medical Branch at Galveston

M R Tangum

20th century American academic medicine has resisted the concept that supplementation with
ave health benefits. This resistance is evident in several ways: (1) by the uncritical acceptance of news
belief that vitamin C supplements cause kidney stones; (2) by the angry, scornful tone used in
utrient supplementation in the leading textbooks of medicine; and (3) by ignoring evidence for possible
ient supplement, such as the use of vitamin E for intermittent claudication.

COMMENTARY

tling Quackery

udes About Micronutrient Supplements in American Academic Med

S. Goodwin, MD; Michael R. Tangum, MD

HROUGHOUT THE 20th century American academic medicine has resisted the concept that supple- tion with micronutrients have health benefits. This ance is evident in several (1) by the uncritical accep- of news of toxicity, such as lief that vitamin C supple- cause kidney stones; (2) by gry, scornful tone used in ssions of micronutrient ementation in the leading oks of medicine; and (3) by g evidence for possible effi- f a micronutrient supple- such as the use of vitamin E rmittent claudication.

art of the resistance stems the fact that the potential ts of micronutrients were ced by outsiders, who took nessage directly to the pub- d part from the fact that the t of a deficiency disease did in well with prevailing bio- al paradigms, particularly the heory. Similar factors might ected to color the response demic medicine to any alter- treatment.

n *The Crime of Galileo*, histo- iorgio de Santillana[1] presents ionist view of the great scien- struggle with the Catholic n. According to de Santillana, o's crime was not his pro- ing a heliocentric universe; it at he wrote in Italian; he com- ated his revolutionary ideas about astronomy directly to the public. Previous scientists wrote in Latin, limiting their audience to other scholars. Within this small community, controversial ideas could be entertained. Copernicus' proposal of a heliocentric universe 70 years before Galileo's treatises had elicited no attempts at suppression by the church. The 17th-century church represented the intellectual establishment, and Galileo's persecutors included some of the finest minds of his time. Galileo was punished not for writing heresy, not for threatening paradigms, but for bypassing the intellectual establishment and taking his exciting ideas directly to the people. The establishment, threatened not so much by his ideas as by his methods, did what it could to destroy his credibility.

In addition, Galileo did not respect professional boundaries. He was a mathematician, and yet his writings dealt with phenomena considered within the purview of philosophers, a profession of considerably higher status than mathematics.[2] Thus, he was considered a usurper as well as a popularizer. In what follows we argue that the reaction of academic medicine to the concept of micronutrient supplementation can best be understood in light of the foregoing description of Galileo. Our thesis is that throughout much of the 20th century, American academic medicine was resistant to the concept that micronutrient supplementation might prove beneficial, and that the cause of this resistance was similar to that which faced Galileo. This resistance is evident in several ways: (1) by unc of bad news abo supplements; r effects were rarel widely quoted; (2 dismissive tone o about micronutri tion in textbook tone avoided in m troversies; and (3 reaction greeting cacy of a micronu other therapies; in were simply ignor

Note that in mentioned above reaction to micro to other therapies bias to be concern or to be skeptical cacy. Bias occur and skepticism a tively. Also note proposing to pro ticular micronutr is indeed efficacio of earlier drafts o concluded that v for megavitami Rather, the vitam one of a series of used to discuss influence medica than those stemm scientific discover

Herein we r tiple editions of 2 medical textbook *Medicine*[8] and *Pri Medicine*.[9] Each lished in 12 dif between 1950 and be presumed to lished opinions a sample how m changes over time

e Center on Aging, The University s Medical Branch, Galveston.

References (79)

ıtion, race, background diseases, and lifestyle can be mentioned among the underlying factors of kidney very much depends on the diet [25, 34, 35] . In our study, the prevalence of stones was 61.2% for CaOx, for uric acid, and 62% for cysteine stones. ...

P, uric acid and CaOx stones was 62%, the frequency of CaP and CaOx stones was 10.6%, the uric acid Table 2. Frequency of mixed stones by gender [6]. In the study by Altaf et al, the prevalence of s was 37%, and the prevalence of CaOx + CaP stones was 5% [35] , which is close to the results of our highest frequency of uric acid + CaOx stones was seen in men with 27 cases and the male to female ratio 3:1, which is close to the results of a study by Riyadh et al [36]. ...

valence of the stones was seen in the age group 30-39 years (25.8%) and 40-49 years (20.5%), which is ılts of the study by Tadayyon et al [6]. In another study conducted in New York in 2006, the highest d in the age group 18-45 years [35] . In our study, a significant relationship was found between age and nsistent with the results of a study by Antonia Boza [40]. ...

ne Different Compositions in Patients Referred to a Lithotripsy Center in Ilam, West of Iran

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ı Moradi · Milad Azami · Milad Borji

sing the preconceptions in academic medicine on micronutrient supplements, Goodwin and Tangum gave pport the conclusion that there has been systematic bias against the concept that vitamins might be er than the minimum required to avoid classic deficiency diseases [275] . In other papers, Goodwin and eral cases in which an effective method of treatment was erroneously rejected: the rejection seemed to be nderstanding of the physiological mechanism of the effect [276,277]. ...

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Mohamad Chaaban · James S Goodwin · Dong Zhang

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Micronutrients and breast cancer

January 1998 · European Journal of Cancer Prevention

S Franceschi

A large part of the epidemiological debate on diet and breast cancer has been dominated by the issue of whether fat, particularly animal fat, increases risk. Lately, the possible protective effect of various dietary constituents has received more attention. Vitamins C and E, and beta-carotene have antioxidant activity and may thus provide a cellular defence against reactive oxygen species that ... [Show full abstract]

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April 1985 · Zhonghua wai ke za zhi [Chinese journal of surgery]

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May 2008 · The Indian Journal of Pediatrics

Aruna Srinivas · Bina F. Dias

To assess the antioxidant status in HIV positive children. HIV positive children under the age group of 3-12 years from lower socio-economic strata were chosen for the study (Group 1). The values were compared with normal children (Group 2) not suffering from any disease in the same age group and similar socio-economic strata. The antioxidants chosen for the present study were vitamin A ... [Show full abstract]

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Metal ions mediated pro-oxidative reactions with vitamin C: Possible implications for treatment of d...

January 2011 · International journal of cancer prevention

John Gruia Ionescu · Borut Poljšak

Vitamin C is an acidic molecule with strong reducing activity. It is an essential micronutrient in man, due to the absence of L-gulonolactone oxidase. Vitamin C has several important roles and there are many enzymes utilizing ascorbate as a co-factor. Besides, vitamin C protects human health by scavenging toxic free radicals and other reactive oxygen species (ROS) formed in cell metabolism. On ... [Show full abstract]

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February 1994 · International Journal for Vitamin and Nutrition Research

C J Bates

Assay procedures for plasma concentrations of vitamin C, and hence for vitamin C status, currently in use in European population-surveillance laboratories and elsewhere, are based on a wide range of disparate techniques and reactions. The problem of achieving harmonisation between these techniques, and between laboratories, is further complicated by the instability of the vitamin, and the ... [Show full abstract]

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:nous iron therapy in a rat model of heart failure with preserved systemic iron status but depleted intracellular cardiac stores

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ımmonly occurs in chronic heart failure (HF) and is associated with poor prognosis. Neither its causes nor ınificance are clearly understood. We aimed to assess iron status and the effect of iron supplementation in the rat dial infarction (MI) HF. Four weeks after induction of MI to induce HF or sham surgery, rats received intravenous iron e) or saline, 4 doses in 1-week intervals. HF alone did not cause anemia, systemic or myocardial ID, but reduced ggesting depleted cardiomyocyte iron stores. Iron therapy increased serum Fe, ferritin and transferrin saturation as epatic iron content in HF rats, but did not increase myocardial ferritin. This was accompanied by: (1) better ntricular (LV) ejection fraction and smaller LV dilation, (2) preservation of function of Ca^{2+} handling proteins in LV 3) reduced level of inflammatory marker, CRP. Furthermore, iron supplementation did not potentiate oxidative stress or cardiomyocyte function, but increased activity of antioxidant defenses (cardiac superoxide dismutase). Despite lack of al ID we found evidence of depleted cardiomyocyte iron stores in the rat model of HF. Furthermore we observed supplementation and confirmed safety of iron supplementation in this setting.

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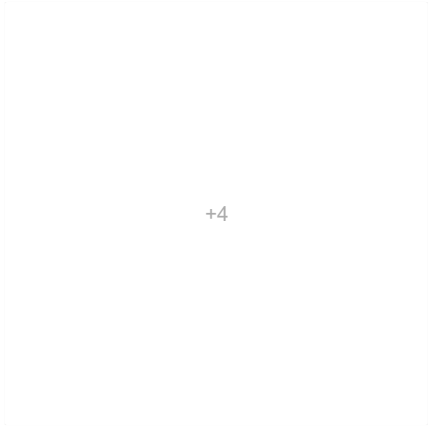

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Beneficial effects of intravenous iron therapy in a rat model of hea failure with preserved systemic ir status but depleted intracellular cardiac stores

018
2018

Aleksandra Paterek[1], Marta Kępska[1], Barbara Sochanowicz[2], Ewelina Chajduk[3], Joanna Kołodziejczyk[1], Halina Polkowska-Motrenko[3], Marcin Kruszewski[2,4,5], Przemysław Leszek[6], Urszula Mackiewicz[1] & Michał Mączewski [1]

Iron deficiency (ID) commonly occurs in chronic heart failure (HF) and is associated with poor pro Neither its causes nor pathophysiological significance are clearly understood. We aimed to asses iron status and the effect of iron supplementation in the rat model of post-myocardial infarction (MI) HF. Four weeks after induction of MI to induce HF or sham surgery, rats received intravenou iron (ferric carboxymaltose) or saline, 4 doses in 1-week intervals. HF alone did not cause anemia systemic or myocardial ID, but reduced myocardial ferritin, suggesting depleted cardiomyocyte stores. Iron therapy increased serum Fe, ferritin and transferrin saturation as well as cardiac and hepatic iron content in HF rats, but did not increase myocardial ferritin. This was accompanied b better preservation of left ventricular (LV) ejection fraction and smaller LV dilation, (2) preservat function of Ca^{2+} handling proteins in LV cardiomyocytes and (3) reduced level of inflammatory m CRP. Furthermore, iron supplementation did not potentiate oxidative stress or have toxic effect

**cardiomyocyte function, but increased activity of antioxidant defenses (cardiac superoxide dism
Despite lack of systemic or myocardial ID we found evidence of depleted cardiomyocyte iron sto
the rat model of HF. Furthermore we observed positive effect of iron supplementation and confi
safety of iron supplementation in this setting.**

Iron is a vital element for the body, especially for metabolically active tissues such as myocardium. It is
component of oxygen carrying protein, hemoglobin and of multiple oxidative enzymes and respirato
proteins, including those containing Fe-S clusters, involved in cellular metabolism. Dietary iron is abs
enterocytes and then secreted into circulation where it is bound to an iron transporting protein, tra
which on one hand delivers iron to target cells (by binding to the transferrin receptor-1 [TfR1]), on the o
tralizes its free radical generating activity. Iron can be utilized by target cells or stored, bound to ferritin
in the liver. Thus transferrin saturation with iron is a good indicator of usable iron pool, while ferritin
indicator of total body iron (however, being an acute phase protein, it can be increased in inflammatory

Iron deficiency (ID), occurs in up to 50% of patients with chronic heart failure (HF), both with co
anemia and with normal hemoglobin values[1]. Its etiology is likely multifactorial and remains largely u
Broadly speaking, ID can be attributed to the factors related to HF per se (e.g. malabsorption due t

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Mackiewicz and Michał Mączewski contributed equally. Correspondence and requests for materials s
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eferences (35)

cused on increasing the concentration of haemoglobin, an oxygen-carrying protein. But neither erythropoietin analogs lobin concentration 6 nor intravenous iron that provided an essential element not only for haemoglobin, but also other rdiac energetics 7 provided unequivocal benefits in human clinical trials, though recent data, including our own work, 8 e of some value here. ...

pendent cardiovascular diseases by myo-inositol trispyrophosphate (ITPP)-enhancement of oxygen delivery by red

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ED

hra El-Hafny-Rahbi · Aleksandra Paterek · Claudine Kieda

stmyocardial infarction heart failure, which had the advantage of identical genetic background, diet as well as the es and concomitant therapies, we demonstrated lack of systemic ID in heart failure. We also did not find signs of , we noticed depleted myocardial iron stores (Paterek et al., 2018) . Similar results were found in rats with ischemic e no alteration of iron status was observed, in particular serum, myocardial and hepatic iron remained unchanged. ...

adigm shift from systemic to cardiomyocyte abnormalities

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Urszula Mackiewicz · Michał Mączewski

he AFFIRM-AHF trial: a randomised, double-blind, placebo-controlled trial comparing the effect of intravenous hospitalisations and mortality in iron-deficient patients admitted for acute heart failure

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ridget-Anne Kirwan · Stefan D. Anker · Ewa Anita Jankowska

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Chapter

Anemia and Iron Deficiency in Heart Failure

January 2019

Otmar Pfister

Anemia and iron deficiency (ID) are common co-morbidities in chronic heart failure (CHF) patients and are both independently associated with increased morbidity and mortality. Anemia affects one of three CHF patients and ID is present in half of CHF patients. While the treatment of anemia remains a challenge, ID has become a valid treatment target. ID is diagnosed when ferritin is lower than 100 ... [Show full abstract]

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Iron Deficiency among Pregnant Women Attending Antenatal Clinic at the KNUST Hospital, Kumasi, Ghana

January 2015

Christian Obirikorang · Linda Ahenkorah Fondjo · Samuel Adomako · [...] · Isaac Acheampong

Background: Pregnant women constitute a high risk group for iron deficiency due to increased iron requirements for foetal and maternal tissues growth. This study sought to find out the prevalence of iron deficiency among Ghanaian pregnant women obtaining antenatal care at the University hospital, Kumasi, Ghana. Methods: The study was conducted between January and May, 2013. A total of 180 women, ... [Show full abstract]

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Анемия и железодефицит у больных с хронической сердечной недостаточностью. Anaemia and Iron Deficien...

May 2019 · Kardiologiia

N. T. Vatutin · Gennadiy Taradin · Irina Kanisheva · Victoria Venzheha

В представленном обзоре затронуты вопросы распространенности анемии и железодефицита (ЖД) при ХСН, их влияние на течение и прогноз этого состояния. Сформулировано определение анемии и ЖД на основе оценки различных лабораторных данных. В частности, обсуждается диагностическая значимость определения сывороточного железа, ферритина сыворотки крови, коэффициента насыщения трансферрина, общей ... [Show full abstract]

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Iron deficiency and anaemia in heart failure: Understanding the FAIR-HF trial

November 2010 · European Journal of Heart Failure

José González-Costello · Josep Comin-Colet

Treatment of anaemia in patients with chronic heart failure (CHF) and reduced left ventricular ejection fraction has traditionally focused on erythropoietin-stimulating agents. However, recent studies have shown that treatment with intravenous (IV) iron can improve the symptoms and quality of life in patients with CHF and iron deficiency (ID), with or without anaemia. The management of ID is ... [Show full abstract]

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Iron deficiency in a multi-ethnic Asian population with and without heart failure: Prevalence, clini...

September 2014 · European Journal of Heart Failure

Tee Joo Yeo · Daniel Yeo · Raymond Ching Chiew Wong · [...] · Carolyn S.P. Lam

Aims: Current heart failure (HF) guidelines highlight the importance of iron deficiency (ID) in HF. Whether HF itself or age-related comorbidities contribute to ID is uncertain, and previous data were limited to Western populations. We aimed to study the prevalence, clinical correlates, functional significance and prognosis of ID in HF patients, compared with community-based controls in a ... [Show full abstract]

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MARCH 1974

DCIEM REPORT NO. 74-R-1012

HEALTH PROBLEMS AND VITAMIN C IN CANADIAN NORTHERN MILITARY OPERATIONS

B.H. SABISTON

M.W. RADOMSKI

(Text of Communication presented at the Twenty-Fifth Symposium of the Defence Research Board, Department of National Defence, Canada. Presented 14 November 1973 by B.H. Sabiston)

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DEFENCE RESEARCH BOARD — DEPARTMENT OF NATIONAL DEFENCE — CANADA

ABSTRACT

As part of a continuing study of health problems pertinent to Canadian Northern Military operations, two aspects of Vitamin C have been examined in land element personnel participating on Northern Winter Exercises. This report describes results of an ongoing Vitamin C survey designed to examine both the Vitamin C status of troops and the effects of a daily Vitamin C supplement on the incidence and severity of colds in troops undergoing operational training. Results indicate that a daily 1000 mg supplement of Vitamin C reduced significantly the incidence of colds as assessed on the basis of symptom complexes reported on health survey cards. While the overall incidence of colds was influenced significantly by Vitamin C, both on an individual and a tent group basis, the duration of local cold symptoms was not. In those individuals who contracted a cold, nasal and throat and chest symptoms were observed to persist for similar periods of time in both placebo and Vitamin C supplemented groups. The Vitamin C group, however, did show a significant reduction in the duration of the more constitutional symptoms related to a general feeling of "well-being". The Vitamin C status of individuals was assessed on the basis of whole blood ascorbate levels determined before and after participation on Northern exercises. A significant reduction of whole blood ascorbate was observed post-exercise on three separate serials of Exercise New Viking, the troops of which were supplied with RP-4 field rations. In view of the fact that only a minor reduction of whole blood ascorbate was observed on another serial, the troops of which were supplied with IRP field rations, it is not possible to determine whether the reduction in ascorbate status was a reflection of altered dietary intake or an increased requirement for Vitamin C under the activity and exposure conditions experienced on Northern operations. Further work is required to clarify this situation.

HEALTH PROBLEMS AND VITAMIN C IN CANADIAN NORTHERN MILITARY OPERATIONS

Since the early part of 1972, the Biosciences Division of the Defence and Civil Institute of Environmental Medicine (DCIEM) has been involved in an extensive field program designed to examine some of the health problems pertinent to Canadian Northern Military operations.

Table 1 lists some of the potential health problem areas encountered in a transit military population operating under Arctic or sub-Arctic conditions. These have been divided, somewhat arbitrarily, into two groups: Environmental and Operational.

TABLE 1

POTENTIAL HEALTH PROBLEM AREAS NORTHERN OPERATIONS

ENVIRONMENTAL	OPERATIONAL
Cold Injury	Nutrition
Frostbite	Rations
Trench Foot	Dehydration
Hypothermia	Constipation
Snow Blindness	Tent Eye
Sunburn	Physical Fitness
Cold Sores	Wound Heating
	Upper Respiratory Infection
	Dental

(1) *Environmental* problems are those which arise as a consequence *of direct insult upon the individual by his environment.*

(2) *Operational* problems are those which arise as a consequence of *restrictions placed upon an individual by his environment.*

This report describes results dealing with some problems in the operational category, specifically with regard to rations and Vitamin C, the Vitamin C status of individuals, and the effect of Vitamin C supplementation on symptoms of respiratory distress.

One of the approaches which has been applied throughout the field program has been the administration of a health survey to men taking part in military winter exercises. This survey was established primarily to answer the questions, "does the abrupt introduction of a man into the Northern climate produce any demonstrable change in health pattern? If so, what is the nature of this alteration? "

The majority of health surveys which have investigated environmental factors impinging on health have been concerned with indigenous populations or isolated communities. Data derived from such studies are not applicable directly to transit populations such as members of mobile military forces. Recognition of this fact prompted DCIEM to establish a protocol for obtaining epidemiologic data on military men making periodic excursions into the North. The survey has been restricted to members of the land element for it is these individuals who are exposed most directly to the adverse environment for periods of greater than a few hours.

Table 2 lists the exercises which have been surveyed to date. With one exception (Northern Ramble, May 1972) the field program has utilized men taking part in New Viking training exercises. It is important to recognize the fact that these are *training* exercises and that as such, the men are living under the most "ideal" Arctic conditions in the sense that experienced instructors are with them at all times. Consequently, the men are under constant supervision to ensure that they protect themselves adequately from the environment. Hence, any health problems which arise on such exercises should be taken as a minimal estimate of problems which may arise on more operational missions.

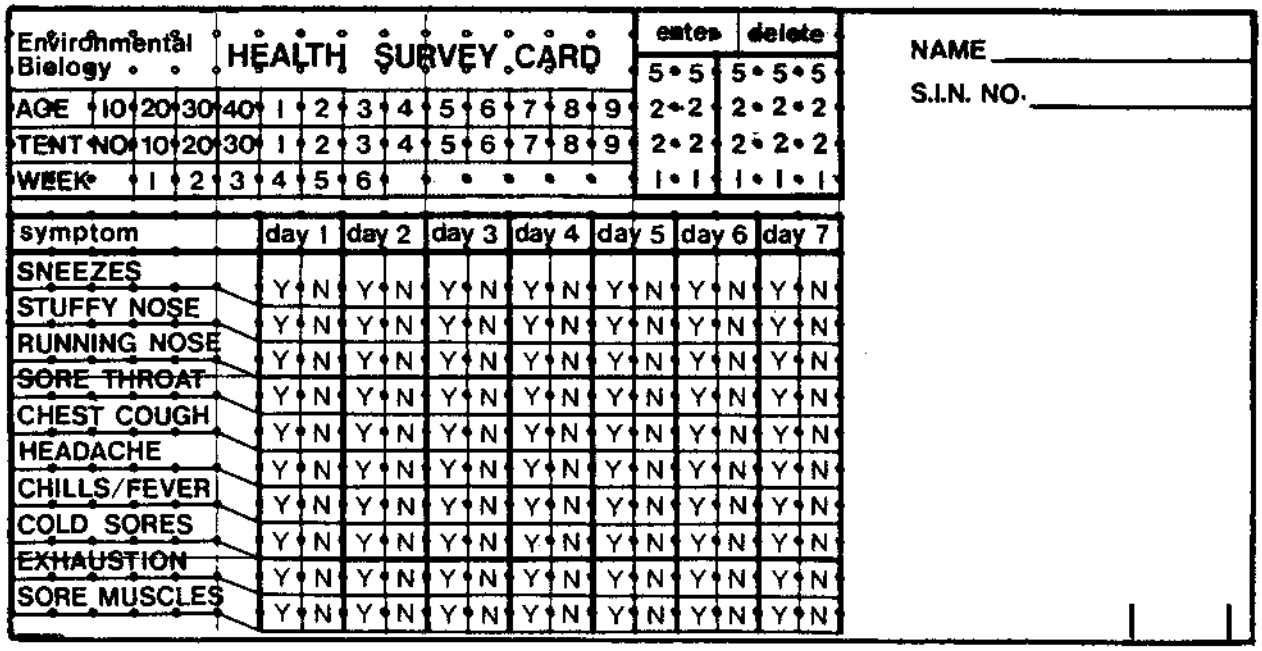
TABLE 2

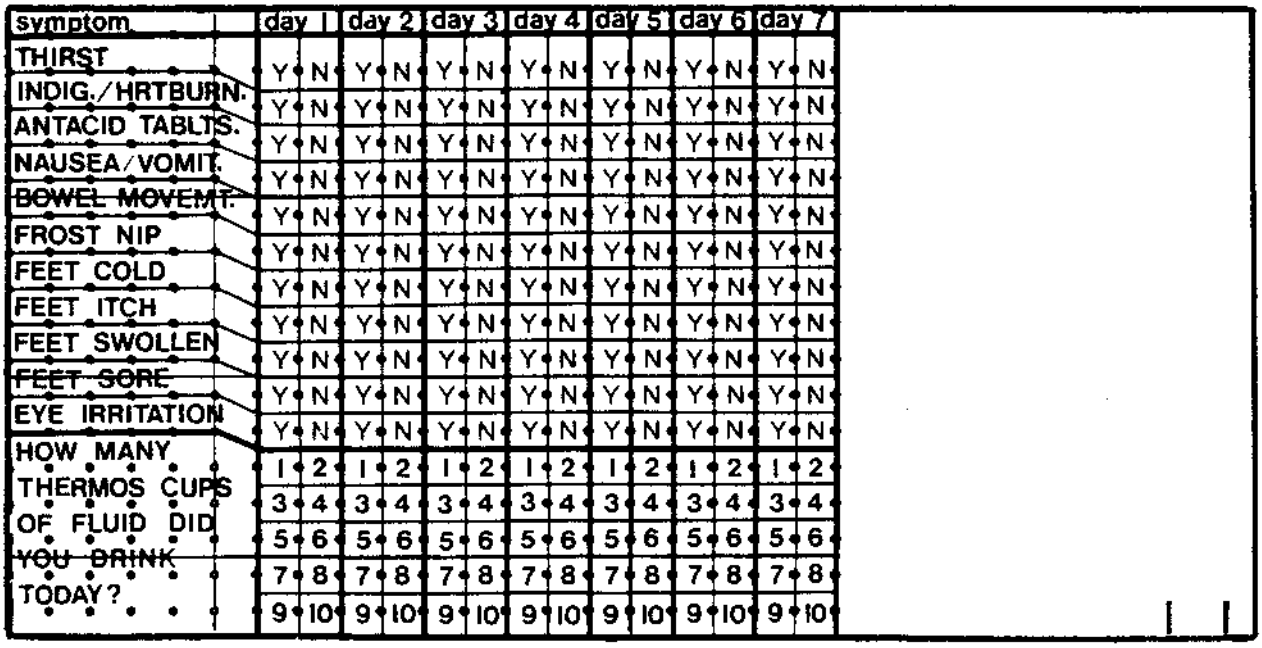
NORTHERN EXERCISES UTILIZED FOR THE INVESTIGATION OF HEALTH PROBLEMS, 1972-73

Exercise	Date	Home CFB	N	Northern Location
New Viking 37	March 1972	Petawawa	70	Coral Harbor
Northern Ramble	May 1972	London	400	Churchill
New Viking 49	December 1972	London	100	Coral Harbor
New Viking 52	January 1973	Gagetown	100	Churchill
New Viking 55	February 1973	Petawawa	100	Frobisher Bay
New Viking 56	March 1973	Calgary	120	Frobisher Bay
New Viking 57	April 1973	Petawawa	100	Frobisher Bay

The health survey card used in the collection of field data is shown in Figure 1. The health survey has been conducted on an individual tent-group basis and extensive use has been made of the tent-group commanders who have been responsible for administering the survey cards on a daily basis. The survey period has extended typically from one week before the exercise to one week after the exercise. Tabulation of the incidence of individual symptoms and symptom complexes has been carried out post-exercise and it has become apparent that, to one degree or another, the incidence of individual symptoms is affected by movement into the North. The most marked alteration in symptoms reported has been noted in symptoms related to the upper respiratory system and it is these symptoms which have been examined in greater detail in DCIEM Vitamin C studies.

IN-FIELD HEALTH SURVEY CARD





An assessment of Vitamin C was undertaken for a number of reasons:

(1) The whole question of Vitamin C and its effect on colds is a topical and debatable issue. It was hoped that some light would be shed on this problem by utilizing a very restricted population of comparable age, typical cold history, common dietary regimen, activity schedule and environmental exposure.

(2) It has been suggested that Vitamin C may play a role in increasing cold tolerance - with particular regard to maintaining peripheral circulation.

(3) Finally, it was determined that the RP-4 rations (1970-71) on which the men were living, apparently provided a maximum of 37–41 mg Vitamin C per day in a single fruit-drink mix. As previous observations suggested that the fruit-drink mix was an unpopular item in the rations and tended to be discarded, it appeared that the individual intake of Vitamin C could be below the recommended daily allowance.

Accordingly, a protocol was established for dispensing tablets of either Vitamin C or placebo to individuals in each tent. Men in each tent group were assigned randomly to either the Vitamin C or placebo group. Extensive use was made again, of tent-group commanders who carried with them the supply of pills for their own tent. Two pill vials were provided for each tent, one containing Vitamin C and one containing placebo. Each vial contained the names of the men who were to receive the respective pills. Pills were dispensed twice a day, once with the morning meal and once with the evening meal. The total dose of Vitamin C received each day was 1000 mg.

At the completion of the exercise the incidence and duration of colds was examined by assessing the presence or absence of a cold on the basis of symptom constellations. In order for a man to be classified as having a cold, he had to have two nasal symptoms in conjunction with a minimum of sore throat or chest cough which persisted for two or more days. As a further restriction, the sore throat or chest cough had to be absent at the time the nasal symptoms began. Frequently, it was found that more constitutional symptoms such as headache, chills and fever, general malaise, nausea or vomiting were indicated at some time during the symptom constellation.

Table 3 indicates that the random allocation of men to the two treatment groups resulted in two well-matched populations with respect to age and typical cold history.

TABLE 3

THE MEAN AGE AND COMMON COLD HISTORY OF MEMBERS OF A SINGLE INFANTRY COMPANY OF 112 MEN ALLOCATED RANDOMLY TO VITAMIN C AND PLACEBO PREPARATIONS

Group	N	Age	Incidence of Usual Spring Cold %
Vitamin C	56	25.3 ± 6.3* (Range 17 - 40)	61.6
Placebo	56	25.4 ± 8.1 (Range 17 47)	60.0

*Mean ± S.D.

Table 4 depicts the frequency of colds assessed in a single infantry company on a Northern Military exercise. The incidence of colds in two other companies participating on the exercise, but not subjected to pill supplementation, was 21.0% and 29.4% respectively.

TABLE 4

INDIVIDUAL INCIDENCE OF COLDS ASSESSED IN A SINGLE INFANTRY COMPANY OF 112 MEN PARTICIPATING ON A NORTHERN MILITARY EXERCISE

Group	N	Frequency	Percent Frequency
Vitamin C	56	6	10.7
Placebo	56	14	25.0
χ^2	3.87		P=0.05

The results indicate that the Vitamin C group experienced significantly fewer colds than the corresponding placebo group. This ameliorating effect of Vitamin C was also reflected in the frequency of colds reported by individual tent groups (Table 5). Of the 14 tent groups involved in this study, nine groups (64.3%) indicated the presence of at least one cold during the exercise period. Of these nine groups, six (66.6%) indicated colds present only in placebo individuals, whereas the remaining three (33.3%) indicated colds present in both placebo and Vitamin C groups. In no case did a tent group indicate the presence of colds in Vitamin C individuals only.

TABLE 5

TENT GROUP INCIDENCE OF COLDS IN AN INFANTRY COMPANY OF 112 MEN PARTICIPATING ON A NORTHERN MILITARY EXERCISE

Number of Tent Groups Reporting One or More Colds Amongst its Members	Number of Tent Groups Indicating Colds Present		
	In Vitamin C Individuals only	In Placebo Individuals only	In Both Vitamin C and Placebo Individuals
9/14	0/9	6/9	3/9
(64.3%)	–	(66.6%)	(33.3%)

The data presented in Table 6 indicate that despite a reduction in the frequency of colds in Vitamin C individuals, the duration of cold symptoms as related to the presence of nasal, throat or chest complaints was not significantly influenced. In other words, if an individual experienced a cold while on Vitamin C, the continued daily intake of 1000 mg/day did not alter the course of the cold with respect to the local symptoms. Examination of the more constitutional symptoms however (Table 7) revealed that the duration of these was significantly reduced in the Vitamin C group. This perhaps is a significant finding for it is these symptoms which are related to the general feeling of "well-being" and it is these symptoms which, in a civilian population, could predispose a person to remain at home. In a military population where refuge cannot be sought easily, it is these symptoms which would tend to reduce a man's level of effectiveness.

TABLE 6

THE MEAN DURATION OF UPPER RESPIRATORY SYMPTOMS REPORTED BY MEN AFFLICTED WITH A COMMON COLD

Group	N	Duration of Symptoms (days)	
		Nasal	Throat/Chest
Vitamin C	6	4.2 ± 3.8*	4.3 ± 3.0
Placebo	14	5.6 ± 2.8	6.0 ± 3.0
P		> 0.4 > 0.5	> 0.2 > 0.3

*Mean ± S.D.

TABLE7

THE MEAN DURATION OF CONSTITUTIONAL SYMPTOMS RELATED TO A FEELING OF WELL-BEING REPORTED BY MEN AFFLICTED WITH A COMMON COLD

Group	N	Duration of Symptoms (days)
Vitamin C	6	0.8 ± 0.8*
Placebo	14	2.4 ± 2.1
		$p < 0.05$

On subsequent exercises an examination of the Vitamin C status of men was carried out by examining the whole-blood ascorbate levels before and immediately after the exercise. Table 8 shows the incidence of altered ascorbate status on four Northern exercises. In all cases, a significant number of men demonstrated a decrease in whole-blood ascorbate, however the magnitude of this decrease (Table 9) was significant on only three of the exercises. Coincidentally, these three exercises were supplied with the RP4 ration while the fourth exercise (Serial 56) received IRP field rations. The IRP ration provides approximately 50–90 mg of Vitamin C per day, about 50% of which is in a single fruit-drink mix and 50% is distributed throughout other ration components.

TABLE 8

INCIDENCE OF ALTERED WHOLE-BLOOD ASCORBATE STATUS OCCURRING ON NORTHERN EXERCISES

Serial	N	% of Individuals Demonstrating a Decrease in Ascorbate	% of Individuals below 0.50 mg% Ascorbate	
			Pre-Exercise	Post-Exercise
NV 49	86	70	4	8
NV 51	29	83	28	41
NV 55	24	46	21	12
NV 56	34	47	32	32

TABLE 9

MEAN WHOLE-BLOOD ASCORBATE STATUS BEFORE AND AFTER PARTICIPATION ON NORTHERN EXERCISES

Serial	N	Pre-Exercise Level mg%	Post-Exercise Mean Change	
			mg%	%
NV 49	86	1.05 ± 0.04*	−0.19 ± 0.04	−18
NV 51	29	0.86 ± 0.07	−0.21 ± 0.04	−24
NV 55	24	0.91 ± 0.10	−0.13 ± 0.06	−14
NV 56	34	0.76 ± 0.05	−0.03 ± 0.06	− 4

*Mean ± S.E.M.

One further point with reference to Table 8 is the rather surprising number of men who demonstrated whole-blood ascorbate levels lower than 0.50 mg%. This value is generally taken to indicate the threshold of a possible sub-clinical scorbutic condition. Two of the four serials examined post-exercise demonstrated a definite shift towards this subclinical scorbutic state, one (Serial 56) remained unchanged and the other (Serial 55) demonstrated a shift in the opposite direction.

In view of the variation in diet and distribution of change in ascorbate status, it is not possible from these data to determine whether the reduction in ascorbate levels, observed post-exercise on three of the four serials, was a consequence of reduced dietary intake of Vitamin C or a reflection of a possible increased requirement for this vitamin under the activity and exposure conditions existing on Northern operations. Clearly, a determination of ascorbate excretion is required before any estimate of requirement under these conditions can be made.

This study is part of a continuing program to assess the nature and incidence of health problems pertinent to Canadian military Northern operations. With regards to Vitamin C and its influence on general body health the data to date suggest that a daily supplement of 1000 mg Vitamin C appears to reduce the overall incidence of colds in transit military populations. It must be appreciated however, that the nature of the military exercise itself represents a marked departure from the "normal" daily routine. Over the period of this study, the men are transported by air into an adverse environment and live in close association with that

environment. Their dietary regimen is altered dramatically with regards both to frequency of meals and nature of food eaten. In view of these factors the results reported here do not necessarily characterize the civilian population in general. Further, insufficient data exist to enable us to determine whether the observed beneficial effect of Vitamin C observed in this study, is prophylactic or therapeutic, although the analysis of colds by tent groups suggests that the effect may be prophylactic. In addition the study was restricted to an examination of the efficacy of a daily 1000 mg dose of Vitamin C, which may represent neither the optimal nor minimal daily supplement required. The whole-blood ascorbate levels of individuals receiving a Vitamin C supplement were increased well above normal (100–150%). In view of the demonstrated decrease in whole-blood ascorbate occurring in non-supplemented men, the optimal dose of Vitamin C may be in a range which is sufficient to prevent such a decrease. Further work is required to clarify this situation.

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12. SPONSORING ACTIVITY

13. ABSTRACT

This report describes results of an ongoing Vitamin C survey designed to examine both the Vitamin C status of troops and the effects of a daily Vitamin C supplement on the incidence and severity of colds in troops undergoing operational training. Results indicate that a daily 1000 mg supplement of Vitamin C reduced significantly the incidence of colds as assessed on the basis of symptom complexes reported on health survey cards. While the overall incidence of colds was influenced significantly by Vitamin C, both on an individual and a ten group basis, the duration of local cold symptoms was not. In those individuals who contracted a cold, nasal and throat and chest symptoms were observed to persist for similar periods of time in both placebo and Vitamin C supplemented groups. The Vitamin C group, however, did show a significant reduction in the duration of the more constitutional symptoms related to a general feeling of "well-being". Significant reduction of whole blood ascorbate levels was observed post-exercise on three separate serials of Exercise New Viking. Further work is required to determine whether this reduction in ascorbate status reflects altered dietary intake or an increased requirement for Vitamin C under the activity and exposure conditions experienced on Northern operations.

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HEALTH PROBLEMS AND VITAMIN C IN CANADIAN NORTHERN MILITARY OPERATIONS

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Which Plasma Antioxidants Are Most Related to Fruit and Vegetable Consumption?

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Substantial evidence suggests that fruit and vegetable intake reduces the risk of some cancers and other chronic diseases. While a varied diet containing fruits and vegetables may confer benefits greater than those of any single nutrient, it would be useful to have data on the plasma nutrients most influenced by fruit and vegetable intake. The authors examined the correlation between fruit and vegetable intake as measured by the abbreviated CLUE II food frequency questionnaire and several plasma antioxidants. This study includes 116 male subjects aged 35–72 years who were nonsmokers and nonusers of vitamin supplements and who provided blood samples in the CLUE II Study in Washington County, Maryland. Plasma was assayed for ascorbic acid, beta-carotene, beta-cryptoxanthin, and alpha- and gamma-tocopherol. Lipid- and energy-adjusted partial correlation for the relation with fruit and vegetable intake was $r = 0.64$ for ascorbic acid, $r = 0.44$ for beta-carotene, and $r = 0.50$ for beta-cryptoxanthin. While this study does not address efficacy, the stronger association of ascorbic acid with fruit and vegetable intake seen here may imply that ascorbic acid is an important component of the protective effect seen for fruits and vegetables in numerous epidemiologic studies. *Am J Epidemiol* 2001;154:1113–18.

antioxidants; ascorbic acid; biological markers; carotenoids; fruit; questionnaires; vegetables

Numerous studies have found a significant inverse relation between cancer risk and intake of fruits and vegetables (1). Although the consumption of whole foods provides a complex nutrient mix that may confer a benefit superior to that of any particular component, it would be useful to understand which nutrients are most associated with a high intake of fruits and vegetables. A number of studies using food frequency questionnaires (FFQs) have examined the relation between dietary estimates of particular nutrients and the corresponding plasma nutrient levels. Very few, however, have examined the plasma nutrient levels simply in relation to reported intake of foods rather than to estimates of nutrients. In other words, what plasma nutrient levels are most influenced by a diet high in fruits and vegetables? This study examines plasma levels of several antioxidants in relation to intake of fruits and vegetables.

MATERIALS AND METHODS

Subjects were selected from among participants in the Washington County, Maryland, CLUE II Study, a blood collection campaign conducted by the Johns Hopkins Training Center for Epidemiologic Research and the Washington County Health Department. In 1989, CLUE II recruited residents of Washington County and surrounding counties; most samples were obtained in the fall. CLUE II obtained plasma samples, brief personal data, and a brief food frequency questionnaire. More than 30,000 persons from Washington County and surrounding counties provided samples.

Respondents for this study were selected from counties surrounding Washington County. Subjects were men aged 35–72 years (mean, 53 years) who did not smoke and did not take vitamin supplements. Respondents with an estimated energy intake of less than 1,000 kcal were dropped to exclude persons who may have been ill, were dieting, or had completed the questionnaire incorrectly.

The questionnaire used in the CLUE II Study is a 60-item scannable version of the Block/National Cancer Institute (NCI) questionnaire. The questionnaire contained 10 vegetable items and six fruit items (table 1). Collectively, these foods contribute 70.6 percent of the carotenoid intake in the US diet among men in this age range and 57.8 percent of the dietary vitamin C in the United States, on the basis of the Third National Health and Nutrition Examination Survey (G. Block, unpublished data, 1997). Frequency of consumption of these foods was summed to estimate total fruit and vegetable consumption. (The "GRPFRQ" variables produced by the software were used rather than the portion size-related measures; summary "global" questions were not asked in this FFQ.) Questionnaires were analyzed by using the Block/NCI software (2), and estimates were made of usual dietary intake of nutrients and food groups. Subjects

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Abbreviations: FFQ, food frequency questionnaire; FV, fruit and vegetable consumption; Heme, meat intake; NCI, National Cancer Institute.

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TABLE 1. Foods used to rank subjects on fruit and vegetable intake*, Washington County, Maryland, 1989

Fruits and vegetables on the CLUE II questionnaire
Carrots or mixed vegetables containing carrots
Spinach
Broccoli
Sweet potatoes, yams
Tomatoes, tomato juice
Vegetable or tomato soups
Coleslaw, cabbage, sauerkraut
Mustard greens, turnip greens, collards
Green salad
Any other vegetables, including green beans, corn, peas
Oranges
Grapefruit
Orange juice or grapefruit juice
Cantaloupe
Apples, applesauce, pears
Any other fruit, including bananas, fruit cocktail

* These items comprise foods that contribute the following proportions of US nutrient intake of carotenoids: 70.6% (65.4% from the 14 foods excluding "Any other vegetables" and "Any other fruit") and of dietary vitamin C: 57.8% (44.8% from the 14 foods excluding "Any other vegetables" and "Any other fruit"). (Block, unpublished data, 1997).

were included in this analysis if their reported dietary intake placed them in either the top or bottom quintile on both fruit and vegetable consumption (FV) and meat intake (Heme). (Heme was obtained for a different analysis, and those results are reported elsewhere (3).) Subjects were selected in groups of four (HiFV + HiHeme, HiFV + LoHeme, LoFV + HiHeme, and LoFV + LoHeme), matched within each group on age and body weight. A total of 29 subjects were selected for each of the four groups, resulting in a sample of 116 men for these analyses.

Venous blood was drawn in heparinized Vacutainers (Becton, Dickinson, & Co., Franklin Lakes, New Jersey), centrifuged, and processed within a few hours. One aliquot was prepared by using 10 percent metaphosphoric acid to stabilize ascorbic acid. All samples were stored at –70˚C. The long-term stability of these nutrients, when stored at –70˚C to –80˚C, has been examined in numerous studies and found to be acceptable (4–6). Masked duplicate samples were sent to each laboratory and included in the assays. In addition, a single pooled blood sample was divided into multiple aliquots and shipped with samples over the course of the study to permit analyses of laboratory drift. Reproducibility of all assays was excellent.

Plasma was assayed for ascorbate, beta-carotene, beta-cryptoxanthin, and alpha- and gamma-tocopherol by one of the investigators (E. N.). Plasma ascorbate concentration was determined spectrophotometrically by using 2,4-dinitrophenylhydrazine as chromogen (7), which has been shown to correlate highly with high-pressure liquid chromatography methods (8–11). Plasma carotenoids and vitamin E were determined by reversed-phase high-pressure liquid chromatography (12).

Analysis of variance, *t* tests, and Pearson and Spearman correlations were used. Variables were examined for normality and skewness and transformed by using log or square root, as appropriate. Pearson correlations using the transformed variables were almost identical to Spearman correlations, so only the latter are reported here. Statistical analyses were performed using PC-SAS version 6.11 (SAS Institute, Inc., Cary, North Carolina).

RESULTS

The characteristics of the participants in this analysis are shown in table 2. Body weight ranged from 120 to 250 pounds (54.48 to 11.35 kg), and mean frequency of fruit and vegetable intake was 2.9 times per day. Analysis of variance including the meat category, the fruit and vegetable category, and their interaction term indicated that meat consumption and the interaction term were not related to any plasma antioxidant (data not shown). Consequently, all analyses in this report related to plasma antioxidant level consider only the fruit and vegetable intake.

Correlations between frequency of FV and plasma antioxidants are shown in table 3. Both carotenoids and ascorbic acid are highly significantly associated with frequency of consumption of fruits and vegetables. However, the correlation with ascorbic acid is considerably higher than that for the carotenoids, both unadjusted and after adjustment for several covariates. This higher correlation of FV with ascorbic acid remained after standardization of the plasma carotenoids by plasma cholesterol. Plasma alpha-tocopherol is positively associated with FV only after standardization with plasma cholesterol, while gamma-tocopherol is significantly negatively correlated with FV. Partial correlations adjusted for age, education, body weight, energy intake, or fat intake did not change this pattern. After adjustment for age and energy intake, the correlation between fruit and vegetable intake and ascorbic acid was 0.64, while lipid-adjusted total carotenoids reached only 0.44. The highest correlation besides that of ascorbic acid was lipid-adjusted beta-cryptoxanthin (which is found largely in oranges and orange juice), at 0.50.

DISCUSSION

Although numerous investigators have examined the relation between serum antioxidant nutrient levels and estimates of antioxidant intake from food frequency questionnaires, few have reported the correlations between serum antioxidants and fruit and vegetable frequency as opposed to nutrient estimates (13–19). Only two studies were of nonsmokers (16, 17), and the results presented here correspond well to the carotenoid correlations observed in these earlier reports. Campbell et al. (16) recruited 50 male and 49 female nonsmokers aged 18–37 years, selecting only those in the highest or lowest quintile of FV; 29 percent were supplement users. (Smoking lowers plasma beta-carotene and ascorbic acid levels, and supplement use increases them, irrespective of fruit and vegetable intake. Inclusion of subjects with these behaviors makes it difficult to detect a relation between these plasma nutrients and fruit and vegetable intake.) The 153-item Willett FFQ was self-administered and included 35 veg-

TABLE 2. Characteristics of the sample, for 116 men aged 35–72 years, Washington County, Maryland, 1989

	Mean (SD)*	25th percentile	Median (50th percentile)	75th percentile	Range
Age group (% in each category)					
35–44 (19.0)					
45–54 (32.8)					
55–64 (33.6)					
65–74 (6.9)					
Missing (7.8)					
Body weight (pounds)†	182 (24.4)	165	180	195	120–250
Fruit and vegetable frequency (times/day)‡	2.9 (1.9)	1.3	2.6	4.1	0.1–9.5
Ascorbic acid (mg/dl)	1.0 (0.4)	0.76	1.0	1.3	0.2–2.7
Total carotenoids (μg/dl)	80.6 (34.0)	57.7	72.6	98.5	21.3–227
Beta-carotene (μg/dl)	13.5 (11.4)	6.5	10.4	17.3	1.2–75.2
Cryptoxanthin (μg/dl)	11.2 (9.1)	6.7	9.5	13.5	1.6–71.5
Alpha-tocopherol (μg/dl)	0.96 (0.2)	0.81	0.95	1.12	0.46–1.73
Gamma-tocopherol (μg/dl)	0.24 (0.1)	0.17	0.23	0.29	0.04–0.56

* SD, standard deviation.
† 1 pound = 0.454 kg.
‡ Frequency of consumption; does not take serving size into account.

etable items and 24 fruit items. Lipid- and energy-adjusted correlations between total fruit and vegetable intake and the average of two measurements of plasma beta-carotene and cryptoxanthin were 0.45 and 0.47, respectively, for men and women combined. (Results were not reported separately by gender.) Michaud et al. (17) analyzed data from 110 male nonsmokers from the Health Professionals Follow-up Study. The study questionnaire contained 131 food items (including 31 vegetables and 15 fruits). Supplement use was not addressed, but was presumably present for some participants. Plasma carotenoids were adjusted for lipids, body mass index, and age; fruit and vegetable estimates were based on the average of two FFQs and two 1-week diet records. For men, correlations were 0.35 and 0.36 for beta-carotene and cryptoxanthin, respectively. Thus, our results of 0.38 and 0.50 for these two plasma carotenoids are consistent with previous data on nonsmokers.

Other studies of fruit and vegetable intake and plasma nutrients examined correlations with serum carotenoids and included both smokers and supplement users (18, 19). Tucker et al. (18) reported on the relation between total fruit and vegetable intake, as estimated by the 126-item Willett FFQ, in participants in the Framingham Heart Study. Ten percent of the 201 men were smokers, and 11.9 percent used beta-carotene supplements. Among men, after adjustment for energy and other risk factors, correlations were $r = 0.25$ for alpha- and beta-carotene, 0.16 for beta-cryptoxanthin, 0.17 for lycopene, and 0.14 for lutein-zeaxanthin. Resnicow

TABLE 3. Spearman correlations and partial correlations between fruit/vegetable frequency of consumption and several plasma antioxidants for 116 men aged 35–72 years, Washington County, Maryland, 1989

	Ascorbic acid*	Total carotene**	Lipid-adjusted total carotene*	β-carotene**	Lipid-adjusted β-carotene*	Cryptoxanthin*	Lipid-adjusted cryptoxanthin*	α-toc††,‡	Lipid-adjusted α-toc†	Gamma-toc***	Lipid-adjusted gamma-toc†
Unadjusted correlation with fruit and vegetable frequency	0.59	0.34	0.40	0.35	0.38	0.43	0.46	0.06	0.26	−0.25	−0.20
Adjusted for											
Age	0.59	0.37	0.43	0.34	0.36	0.43	0.47	0.03	0.22	−0.26	−0.21
Education	0.58	0.33	0.40	0.35	0.38	0.41	0.45	0.07	0.27	−0.24	−0.18
Body weight	0.61	0.35	0.42	0.36	0.38	0.43	0.47	0.06	0.26	−0.25	−0.20
Dietary energy intake	0.62	0.34	0.41	0.36	0.39	0.44	0.49	0.06	0.28	−0.26	−0.20
Dietary fat intake	0.60	0.34	0.40	0.34	0.37	0.42	0.46	0.05	0.25	−0.24	−0.19
Age and energy intake	0.64	0.37	0.44	0.36	0.38	0.46	0.50	0.03	0.24	−0.28	−0.22

* All correlations in this column, $p < 0.0001$.
** All correlations in this column, $p < 0.001$.
*** All correlations in this column, $p < 0.01$.
† All correlations in this column, $p < 0.05$.
†† All correlations in this column, $p > 0.10$.
‡ α-toc, alpha-tocopherol.

et al. (19) studied fruit and vegetable intake and plasma carotenoids in 775 African-American men and women in Atlanta, Georgia. Smokers and vitamin supplement users were included. A modification of the full-length Block/NCI questionnaire was used, which contained 36 fruit and vegetable items. Correlations were $r = 0.34$ for alpha-carotene, 0.31 for beta-carotene, 0.26 for beta-cryptoxanthin, and 0.21 for lutein. In a subset of 68 persons who completed three 24-hour recalls, correlations between the 36-item fruit and vegetable questionnaire and these serum carotenoids were much higher ($r = 0.52$, 0.46, 0.43, and 0.30, respectively). Other studies have examined serum nutrient relations with individual foods (14, 15) or have conducted small feeding studies with subjects, many of whom were vitamin supplement users (20).

To our knowledge, only one other study has examined both plasma carotenoids and ascorbic acid in relation to fruit and vegetable intake. In France, Drewnowski et al. (13) studied a community-based sample of 837 subjects, of whom 23.1 percent of the women and 41.6 percent of the men were current smokers. Supplement use was not reported. Data were collected by using a dietary history interview. Correlations with energy-adjusted fruit and vegetable intake were $r = 0.36$ for serum beta-carotene and 0.29 for ascorbic acid.

In our study, ascorbic acid was considerably more highly associated with fruit and vegetable intake than were the carotenoids. Thus, it is possible that ascorbic acid is as important as or more important than carotenoids in conferring the protective benefit of fruits and vegetables. Unless studies examine plasma ascorbic acid in addition to other plasma antioxidants, conclusions regarding the active agent may be misleading. Interestingly, both this study and that of Michaud et al. (17) found beta-cryptoxanthin to be more highly correlated with fruit and vegetable intake than was beta-carotene (although others have not observed this (18, 19)). In this context, it should be noted that the major contributors of beta-cryptoxanthin are oranges and orange juice. Thus, if ascorbic acid is high, beta-cryptoxanthin may also be high. Without a measurement of plasma ascorbic acid, it may be difficult to attribute effects to the proper nutrient.

This study does not directly address the potential *efficacy* of ascorbic acid or other nutrients in affecting disease prevention. That would require epidemiologic studies that obtain a wide range of plasma nutrients and precursors of endogenous antioxidant systems. The stronger association of ascorbic acid with fruit and vegetable intake seen here may imply that ascorbic acid is an important component of the protective effect seen for fruits and vegetables in numerous epidemiologic studies. However, it is also possible that ascorbic acid appeared to be more strongly associated than carotenoids because of differences in storage or metabolism or in the difficulties of measurement. Ascorbic acid is water soluble, with major stores in muscle tissue, and the rate of utilization depends on numerous factors, including body weight, smoking, vigorous exercise, exposure to stressors, and, possibly, gender. Carotenoids are lipid soluble, with storage in fatty tissue, and utilization also depends on smoking and body weight, although possibly to a lesser extent. It is possible that had carotenoids been measured in adipose tissue, correlations with fruit and vegetable intake would have been higher.

The inverse association of gamma-tocopherol with fruit and vegetable intake is not well understood. In an unsupplemented diet, vegetable oils and salad dressings are the main sources of both tocopherols, although vegetables do provide some alpha-tocopherol. Supplementation with alpha-tocopherol is known to suppress gamma-tocopherol levels, and these data suggest an inverse relation between alpha- and gamma-tocopherol, even in an unsupplemented diet. Some studies suggest that gamma-tocopherol is a more potent antioxidant than alpha-tocopherol in some assay conditions, but the inverse relation between gamma-tocopherol and fruit and vegetable intake seen here seems inconsistent with a beneficial effect of gamma-tocopherol.

Often, investigators in major studies do not obtain plasma ascorbic acid because of the belief that it is too difficult to process and too labile to be feasible. This study shows that this is not the case. The CLUE II Study obtained blood samples from 32,808 respondents in a period of 6 months. Samples were obtained in multiple sites across Washington County, including temporary interviewing locations such as in mobile trailers. Blood samples were transported to a central site as whole blood, and processing was done centrally, usually within 6 hours of collection. Ascorbic acid is stable in whole blood for several hours (21), and after centrifugation, the processing of samples for ascorbic acid involves only the preparation of one additional tube containing a stabilizing agent (in our case, metaphosphoric acid). Ascorbic acid in plasma prepared in this way has been shown to be stable at –70°C over a period of several years.

In addition, investigators sometimes fail to include ascorbic acid because of the belief that blood levels represent only the previous few hours or that fasting blood is essential. Again, this appears not to be the case. Most participants in this study were not fasting at the time the blood was drawn, and the correlations shown are with dietary estimates from a questionnaire that asked about average intake in the previous year. These data suggest that plasma ascorbic acid is not as labile or as difficult to process in large studies as has been feared and should be included when studies assess antioxidant status.

A strength of this study is that the effect of fruit and vegetable intake on plasma nutrients could be examined without the effect modification by smoking (22, 23) and without confounding by supplement use (24). In addition, it is notable that the plasma correlations shown here are with reported *frequency* of consumption of fruits and vegetables, not with dietary estimates of nutrient intake or with grams of intake estimated using reported portion size. Thus, the observed correlations are not influenced by possible inaccuracies in the nutrient database for carotenoids or by problems with portion size estimation. Furthermore, this approach provides data that are directly relevant to the bulk of epidemiologic literature; that body of literature has typi-

cally been based on frequency rather than on portion-based servings and has tended to find stronger etiologic associations with fruit and vegetable intake rather than with specific nutrient estimates.

While the list of fruits and vegetables on the CLUE II questionnaire is not long (10 vegetable items and six fruit items), it encompasses the major sources of these nutrients in the US diet, including eight of the top 10 sources of carotenoids and seven of the top 10 sources of vitamin C. Not counting the two "any other fruit" and "any other vegetable" items, the remaining 14 items represent more than two thirds of all the mentions of fruits and vegetables in the Third National Health and Nutrition Examination Survey database among men in this age group (Block, unpublished data, 1997). If the "any other…" items are considered, then, of course, the list represents the great majority of all fruits and vegetables consumed in the United States. Eight of the 14 specific foods on the questionnaire are major dark green or deep yellow vegetables or fruits. Thus, while the higher correlation of ascorbic acid with fruit and vegetable intake seen here is with *this particular list* of fruits and vegetables, it should be noted that the list actually encompasses a higher proportion of carotenoids in the US diet (70.6 percent) than of vitamin C (57.8 percent).

As in the study by Campbell et al. (16), subjects were selected for this research by virtue of being either in the upper or the lower quintile of the distribution of frequency of fruit and vegetable intake. This approach tends to result in correlations that are higher than might be observed in studies that include the middle ranges of intake. However, the approach may also make it possible to see relations between intake and plasma most clearly, unobscured by the greater misclassification found in the middle ranges of intake. Estimates at the top and bottom of a frequency-of-consumption distribution are easiest for respondents to report and are reported with less error than estimates in the middle ranges. For example, it is easy and reasonably accurate to say "I eat carrots almost every day" or "I eat carrots only once a year." What is more difficult, and thus measured with more error, is deciding whether carrots are eaten once a month or twice a month. Thus, we believe that our sample selection approach gives a more accurate picture of the plasma nutrients that may be represented by questionnaires asking about fruits and vegetables.

In summary, this study has found that while both carotenoids and ascorbic acid are elevated in those with higher fruit and vegetable intakes, ascorbic acid is considerably more highly correlated with fruit and vegetable intake than are the carotenoids. Thus, it is possible that raising ascorbic acid levels may be an important mechanism by which fruit and vegetable consumption confers protective benefits. The study has also demonstrated the feasibility of obtaining plasma vitamin C measures in large-scale epidemiologic studies. Epidemiologic studies should include measures of plasma or serum ascorbic acid, in addition to other nutrients, to fully understand etiology and mechanisms.

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Ascorbic acid for the common cold. A prophylactic and therapeutic trial.

Karlowski TR, Chalmers TC, Frenkel LD, Kapikian AZ, Lewis TL, Lynch JM.

Abstract

Three hundred eleven employees of the National Institutes of Health volunteered to take 1 gm of ascorbic acid or lactose placebo in capsules three times a day for nine months. At the onset of a cold, the volunteers were given an additional 3 gm daily of either a placebo or ascorbic acid. One hundred ninety volunteers completed the study. Dropouts were defined as those who missed at least one month of drug ingestion. They represented 44% of the placebo group and 34% of those taking ascorbic acid. Analysis of these data showed that ascorbic acid had at best only a minor influence on the duration and severity of colds, and that the effects demonstrated might be explained equally well by a break in the double blind.

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LinkOut - more resources

Format: Abstract

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Vitamin C and common cold incidence: a review of studies with subjects under heavy physical stress.

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Author information

Abstract

Several studies have observed an increased risk of respiratory infections in subjects doing heavy physical exercise. Vitamin C has been shown to affect some parts of the immune system, and accordingly it seems biologically conceivable that it could have effects on the increased incidence of respiratory infections caused by heavy physical stress. In this report the results of three placebo-controlled studies that have examined the effect of vitamin C supplementation on common cold incidence in subjects under acute physical stress are analyzed. In one study the subjects were school-children at a skiing camp in the Swiss Alps, in another they were military troops training in Northern Canada, and in the third they were participants in a 90 km running race. In each of the three studies a considerable reduction in common cold incidence in the group supplemented with vitamin C(0.6-1.0 g/day) was found. The pooled rate ratio (RR) of common cold infections in the studies was 0.50 (95% CI: 0.35-0.69) in favour of vitamin C groups. Accordingly, the results of the three studies suggest that vitamin C supplementation may be beneficial for some of the subjects doing heavy exercise who have problems with frequent upper respiratory infections.

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A trial of ascorbic acid in the treatment of the common cold.

D A Tyrrell, J W Craig, T W Meada, and T White

Abstract

A randomised controlled trial was carried out to study the effect of 10 g of ascorbic acid taken during the first 2 1/2 days on the symptoms of the common cold. Altogether 1524 volunteers were recruited from a number of working groups in different parts of the country; 482 developed colds. There was no evidence that upper respiratory or general constitutional symptoms were alleviated by ascorbic acid. Among the men who had any colds at all, significantly fewer on active than on placebo treatment had two or more colds; however, this effect was not seen in women. Ascorbic acid is of no value in the treatment of the common cold; its preventive effect, if any, is not such as to justify advising its general use as a prophylactic measure.

Full text

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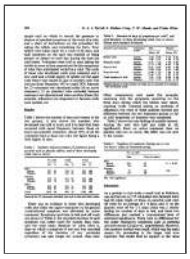

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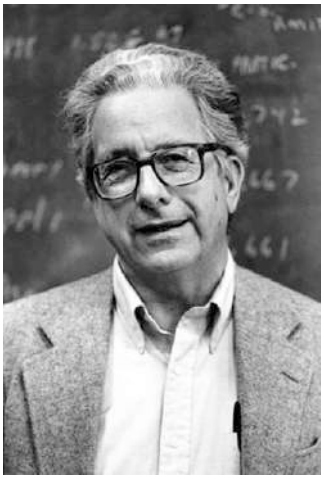
Paul Meier

A Man Behind the Method

Kellyn Betts, MA



Paul Meier. Courtesy of the University of Chicago. Printed with permission.

IN 1951, WHEN PAUL MEIER received his doctorate in mathematics from Princeton University and became one of the first statisticians to enter medical research, potential new medical treatments were evaluated in a very different fashion than they are today. At the time, researchers commonly followed practices such as giving a new remedy to patients they thought might benefit from it and comparing the outcomes with other patients who were not treated. In other situations, patients who stopped taking a new medicine might be counted as controls who had never been exposed to it.

Meier, who died on August 7, 2011, at the age of 87, had a profound impact on how clinical trials now evaluate the efficacy of new drugs and treatment methodologies throughout the world. Meier's "many published works and writings have had a huge influence on the application of statistics to medical research—particularly the design, conduct, and analysis of randomized clinical trials and in the advancement of evidence-based medicine in general," according to the Society of Clinical Trials, which Meier helped found in 1978.[1]

Meier was tireless in his promotion of the now-standard practice of randomly assigning patients enrolled in clinical trials to receive either the conventional remedy or the new treatment being evaluated. This is now considered the most rigorous way to conduct a study and the best way to gather evidence of a new drug or treatment's effectiveness. "Perhaps more than any other U.S. statistician, [Dr. Meier] influenced U.S. drug regulatory agencies, and hence clinical researchers throughout the U.S. and other countries, to insist on the central importance of randomized evidence," said Sir Richard Peto of Oxford University, who was also a leading advocate for randomization, in Meier's *New York Times* obituary.[2] "That strategic decision half a century ago has already saved millions of lives, and those millions should be attributed to Paul," Peto said.

"I defended randomization every chance I got, and I had a fair number of chances," Meier said in a 2003 interview in the journal *Clinical Trials*.[3(p137)] "For a fairly long time randomization was not thought of so highly," he explained. He said that in 2001,

> a very distinguished statistician told me that I had a major influence on the Food and Drug Administration's policies on randomized clinical trials. I don't know how true that was, but if so, it would be something of which I am very proud,

adding that his success in encouraging the use of randomization in clinical trials is the achievement he prized most highly.[3(p137)]

Together with Edward L. Kaplan of the California Radiation Laboratory, Meier also helped formulate what the Society for Clinical Trials terms "our most popular method of estimating survival functions from continuously observed data."[4] Published in the *Journal of the American Statistical Association*[4] in 1958, it went on to become one of the most widely cited articles in the medical literature. At the time of Meier's death, the Kaplan-Meier article had been cited more than 34 000 times. Theodore Karrison, PhD, director of the University of Chicago Department of Health Studies' Biostat Lab and one of Meier's doctoral students, attested to the article's continuing relevance

PAUL MEIER HAD A PROFOUND IMPACT on how clinical trials now evaluate the efficacy of new drugs and treatment methodologies throughout the world. Meier's tireless promotion of the now-standard practice of randomly assigning patients enrolled in clinical trials to receive either the conventional remedy or the new treatment being evaluated helped ensure its current status as the most rigorous way to gather evidence of a new drug or treatment's effectiveness. Meier also helped formulate the Kaplan-Meier estimator, which is now the most popular method of estimating clinical trial participant survival. It is one of the most widely cited articles in the medical literature.

by noting: "If you open up at random a medical journal you're likely to see in at least one of the articles a citation to the Kaplan-Meier paper" (oral communication, December 1, 2011).

Over his long and distinguished career, Meier earned many honors—as well as widespread admiration for being quick on his feet.

> At professional meetings . . . he often astonished me by giving comments from the audience, which, though spontaneous, displayed a depth of reasoning and perfect eloquence, which few others could have matched with any amount of advanced preparation,

recalled Rick Chappell (written communication, November 8, 2011; and oral communication, November 23, 2011), who was Meier's last doctoral student and is now a professor of biostatistics and medical informatics at the University of Wisconsin at Madison.

Through it all, including the stroke in 1995 that robbed him of some of his eloquence, Meier was also a kind and gentle man, according to a statement issued by the Statistics Department at Columbia University,[5] where Meier spent his final years (he also held a joint appointment at Columbia's Mailman School of Public Health). Karrison, Chappell, and Daniel Heitjan, PhD, a professor of biostatistics at the University of Pennsylvania's Perelman School of Medicine, attested that Meier was both widely respected and loved. "He was a person who cared about people . . . and someone you could go to with a problem," Karrison said.

A RELUCTANT BIOSTATISTICIAN

Meier graduated from Oberlin College in 1945 and went on to Princeton University to pursue a doctorate in mathematics, where he studied under the celebrated mathematician John Tukey. Meier's dissertation project involved a statistical problem suggested by William Cochran, the noted statistician who chaired Johns Hopkins University's Department of Biostatistics from 1948 to 1958. At the time, Meier was also very interested in "the notion that randomization could clear away confounders that you did not know about."[3(p133)] As one of a very few mathematicians focusing on medical applications, Meier recognized the potential value of randomization's application in medicine.[3]

After Meier earned his doctorate, he spent one more year at Lehigh University, where he had been teaching since 1948. Tukey recommended that he accept a position at Hopkins with Cochran, who was enthusiastic about Meier's dissertation.

> I was a little nervous because by and large, biostatistics was not a field with a lot of mathematics in it, and I wished more or less to be a mathematician,

Meier said. But when Cochran insisted that going to Hopkins was a good idea, Meier accepted his first position as a statistician.[3]

In those early days, Meier said, "I was looked at with amazement by my medical colleagues," when he brought up the idea of randomization for assessing new medical treatments, he recalled. The physicians would say "'Randomize? We know that this treatment is better than that one,'" he explained. "People who knew and respected me were astounded that I should want to randomize their patients."[3(p133)]

Then Meier became involved with the controversial 1954 Salk

Meier´s Recollections of the Salk Polio Vaccine Trial

The 1954 field trial of Jonas Salk's polio vaccine "was the most elaborate trial that was ever done," Meier recalled. One of the reasons that the trial was so complicated is because polio was very scarce, he explained. "I've not been involved in many trials like that and I've been involved in lots of multicenter studies," he said.[3(p133)]

The situation was further handicapped because the diagnosis of polio is tricky, Meier said. "We need to have the entire country's physicians participate, because we can't look over every case where there's some kind of paralysis. So physicians reported the cases they thought were polio according to the protocol, and we accepted those cases." Meier estimated that "about half those cases were probably not polio at all."[3(p133)]

But the biggest issue, for Meier, emerged during a seminar attended by many of the researchers working on the project, where it became apparent that members of the team were suppressing the data related to some of the test vaccine lots. As soon became clear, the polio virus used in the trial vaccines was not always properly inactivated. Jonas Salk, the vaccine's inventor, "cut out data in order not to show what happened to some lots," Meier charged.[3(p134)] He said that the National Foundation for Infantile Paralysis, which sponsored the study, dropped from its advisory committee scientists who did not agree with how the results were being presented.[3]

The field trial's findings were reported to show the vaccine's effectiveness, over the objections of some of the committee members, Meier said. Soon after, the US Public Health Service reported cases of paralytic polio in children inoculated with the vaccine. The original cases were traced back to lots produced by Cutter Laboratories, of Berkeley, CA, one of six manufacturers licensed to produce the vaccine. However, Meier said that the problem was more widespread. He said:

> I got some data from a physician who was working on this, and we found that not only was Cutter wrong, but there were various other companies that had the same polio virus in their samples, although not as much as the samples from Cutter Laboratories. But because there were so many improperly diagnosed cases out there, and because the other manufacturers went around to various newspapers and threatened to cut their advertising, it was dumped on Cutter. Cutter was responsible because they did things in producing and testing the vaccine they were told not to do.[3(p134)]

Polio Vaccine field trials. The Society for Clinical Trials called the polio vaccine trial "the project that put randomized trials on the map in this country" in part because of the key role Meier played by publishing a critical article in *Science* in 1957.[6] The article reviewed "some aspects of the poliomyelitis vaccine testing program which seem to have important implications for scientists generally."[6(p1067)] It indicted both the National Foundation for Infantile Paralysis and the government for withholding information from the participants. It also faulted the testing program for accepting without scrutiny Salk's assertion that the vaccine was "absolute[ly] safe," and for not employing the expensive and difficult tests that had been suggested to ensure that the final product was free of residual live virus. Meier said that many journals turned his manuscript down and their editors warned him that publishing such an article would limit his career path.[3]

Although Meier was denied tenure at Hopkins, he succeeded in securing an appointment to the University of Chicago in 1957. He stayed there until 1992, and taught at different schools and departments—including the college, graduate school, law school, and medical school—over the years. For more than a decade, he led the Department of Statistics as chair or acting chair.

In 1958, Meier published his highly cited article describing what is now known as the Kaplan-Meier estimator in the *Journal of the American Statistical Association*.[4] Kaplan was also a student of Tukey at Princeton. Working independently, Meier and Kaplan solved a problem that was dogging medical researchers at the time. The issue revolved around the fact that many participants in clinical trials do not participate in the experiment for the same length of time because of the time required to recruit study volunteers. The Kaplan-Meier statistic enables researchers to take into account observable time of survival and death.

Initially, Meier recalled, both he and Kaplan had submitted separate articles. The publication's editor asked them to collaborate to produce one article. "I swallowed hard, and I guess Kaplan swallowed hard as well," Meier said. "We worked quite hard and at one place he solved a problem that I couldn't solve; other cases I solved problems he couldn't."[3(p133)]

LOVE FOR CLINICAL TRIALS

In the subsequent decades, Meier's stature continued to grow, and he was involved in many clinical trials, which he called his "true love." In addition to helping found the Society for Clinical Trials in the 1970s, he wrote some influential articles about the ethics of performing them.[7,8] In his spare time, Meier enjoyed music, particularly folk songs, and played the flute, recalled Chappell, Heitjan, and Karrison. Meier was also a sailor, and he took out his small sailboat, The Salty Dog, in the waters near his summer home near Lake Michigan during his years at the University of Chicago. After Meier moved to New York City in 1992, he sailed in the Hudson River outside Dutchess County, New York.

Over the course of his 50-plus-year career, Meier's facility for explaining statistical concepts to people outside the discipline resulted in calls to testify before the US Congress and popularity with journalists such as Gina Kolata of the *New York Times*, Chappell remembered. It also made him popular with clinicians, such as the University of Chicago medical school students he taught about clinical trials, Karrison said.

Meier's stroke occurred three years after he retired from the University of Chicago in 1992 and moved to Columbia University. There, he held appointments as both the Howard Levene Professor of Statistics in the statistics department and head of the Mailman School of Public Health's biostatistics department, and he remained active professionally for years after his stroke. "He still kept going to meetings," Karrison recalled. Meier "struggled courageously," added Heitjan, who worked closely with him at Columbia (oral communication, November 22, 2012).

Heitjan collaborated with Meier during the Randomized Evaluation of Mechanical Assistance for the Treatment of Congestive

Honors and Awards

Meier was named as a fellow of the American Association for the Advancement of Science, the American Statistical Association, the Institute of Mathematical Statistics, the American Academy of Arts and Sciences, the Royal Statistical Society, and the John Guggenheim Memorial Foundation. He served as president of both the Institute of Mathematical Statistics and the Society for Clinical Trials. He was also elected to senior membership in the National Academy of Sciences' Institute of Medicine.[5,11]

He also held temporary appointments as a National Institutes of Health Special Fellow at the University of London and Imperial College; he was a visiting professor at Harvard University and Jerusalem's Hebrew University; and he was a fellow of Stanford University's Center for Advanced Study in the Behavioral Sciences.[5,11]

Heart Failure (REMATCH) trial, which began in 1998 and ran through 2001 and involved 20 cardiac transplant centers around the country.[9,10] Although this artificial heart trial was relatively small compared with many drug trials, it was one of the most significant device trials ever conducted, Heitjan said. Meier insisted that the trial needed to be randomized and he refused to allow the group carrying it out to cut corners, Heitjan recalled.

> Clinical trials in the device world are often small, single-arm trials [where results are compared with historical controls] . . . in part because a lot of the companies that make devices are small and can't support major trials,

Heitjan explained. The trial was randomized so it could determine whether the devices could extend and improve the quality of recipients' lives sufficiently to justify the expense of implanting them, he said.

It was the first high-profile randomized clinical trial that Heitjan had worked on, and "having Paul around to be my mentor and guide was very important to me." When the two would attend meetings related to the trial, Meier was quiet most of the time

> because it was a little harder for him to communicate and get his point across so he had to choose his battles carefully. He would only speak out at what I considered critical moments,

Heitjan said. Nevertheless it was clear that Meier's understanding of both the technical and political issues in the trial was undiminished, Heitjan said.

Heitjan recalled attending a Society for Clinical Trials meeting with Meier in 1998. One after another, distinguished senior physician–scientists came up to greet Meier, pay homage to him, and testify to how he had opened their eyes to the critical importance of the randomized clinical trial, Heitjan remembered.

"Being with [Meier] lifted you up," Heitjan summarized. Perhaps just as important as his intellect and accomplishments, Meier "was a genuinely good human being," Karrison said. He was a "great and gentle man," Chappell agreed. ■

About the Author

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Format: Abstract

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Ascorbate recycling in human neutrophils: induction by bacteria.

Wang Y[1], Russo TA, Kwon O, Chanock S, Rumsey SC, Levine M.

Author information

Abstract

Ascorbate (vitamin C) recycling occurs when extracellular ascorbate is oxidized, transported as dehydroascorbic acid, and reduced intracellularly to ascorbate. We investigated microorganism induction of ascorbate recycling in human neutrophils and in microorganisms themselves. Ascorbate recycling was determined by measuring intracellular ascorbate accumulation. Ascorbate recycling in neutrophils was induced by both Gram-positive and Gram-negative pathogenic bacteria, and the fungal pathogen Candida albicans. Induction of recycling resulted in as high as a 30-fold increase in intracellular ascorbate compared with neutrophils not exposed to microorganisms. Recycling occurred at physiologic concentrations of extracellular ascorbate within 20 min, occurred over a 100-fold range of effector/target ratios, and depended on oxidation of extracellular ascorbate to dehydroascorbic acid. Ascorbate recycling did not occur in bacteria nor in C. albicans. Ascorbate did not enter microorganisms, and dehydroascorbic acid entry was less than could be accounted for by diffusion. Because microorganism lysates reduced dehydroascorbic acid to ascorbate, ascorbate recycling was absent because of negligible entry of the substrate dehydroascorbic acid. Because ascorbate recycling occurs in human neutrophils but not in microorganisms, it may represent a eukaryotic defense mechanism against oxidants with possible clinical implications.

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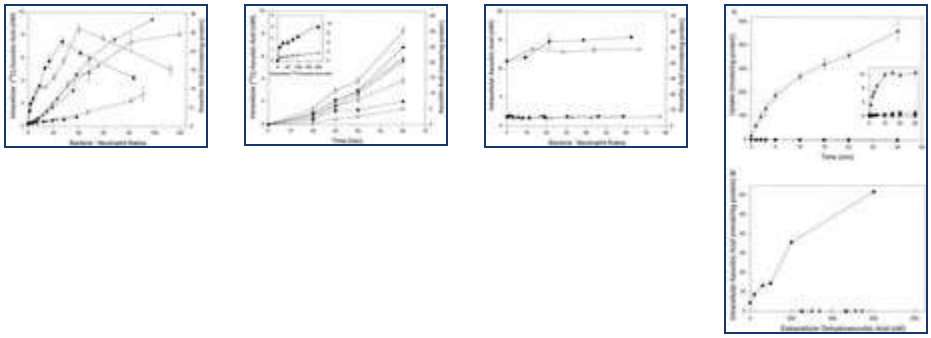

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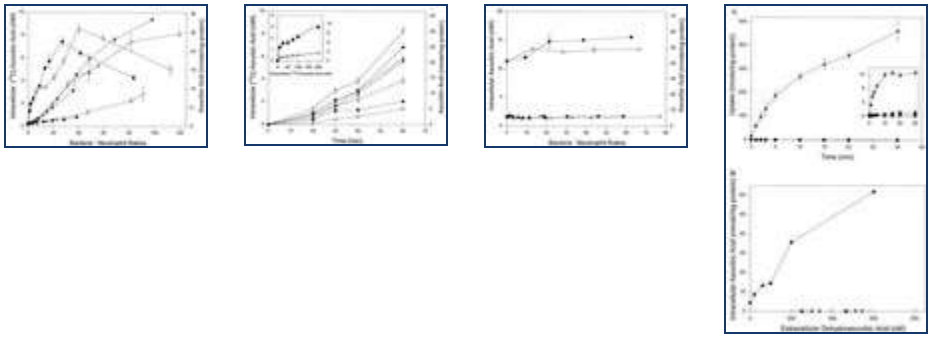

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Ascorbic acid and the common cold

Linus Pauling, Ph.D.

For a number of years I have been interested in the possibility that the state of health of many people could be significantly improved by the ingestion in the optimum amounts of certain substances normally present in the human body, including the vitamins. This interest developed from the work that my associates and I have done on molecular diseases, especially the hemoglobinemias (1). I decided in 1953 that it would be worthwhile to make a study of the extent to which mental diseases could be described as molecular diseases. Work along these lines was carried out in our laboratory in the California Institute of Technology from 1954 to 1964, and was continued in the University of California, San Diego, and (since 1969) in Stanford University. In the course of this period I formulated some ideas about orthomolecular medicine, defined as the preservation of good health and the treatment of disease by varying the concentrations in the human body of substances that are normally present in the body and are required for health (2–4). I also became aware of arguments indicating that the optimum rate of intake of ascorbic acid may be far greater than the recommended daily allowance of this vitamin, which is approximately 50 mg/day. Part of the evidence on this point had been presented especially clearly in the papers of Stone (5–8).

Last year I published a small book, *Vitamin C and the Common Cold*, in which I presented the evidence supporting the conclusion that ascorbic acid ingested in larger amounts than the recommended daily allowance has value in decreasing the incidence and severity of the common cold and related infectious diseases (9).

This opinion is in agreement with a rather widespread popular belief that ascorbic acid has value in providing protection against the common cold. This popular belief has, however, not been generally shared by physicians, authorities on nutrition, and official bodies.

For example, as recently as November 1970, Dr. Philip L. White (10), Secretary of the Council on Foods and Nutrition of the American Medical Association, stated that "Unfortunately, it is still a widespread belief that extra ascorbic acid can not only prevent colds but also lessen the severity and duration of colds and other respiratory infections. Even when consumed at the first sign of a sniffle, large doses of the vitamin are useless." Also, many statements contradicting my conclusions were made by physicians, experts in nutrition, and health officials within a few weeks after the publication of my book. For example, Dr. Charles C. Edwards, United States Food and Drug Commissioner, was reported in the press on December 29, 1970 as having said that the use of ascorbic acid was ridiculous, and that there was no scientific evidence and never have been any meaningful studies indicating that vitamin C is capable of preventing or curing colds. The Editors of *The Medical Letter* published an article in which nearly all my statements were contradicted; for example, it was stated that there had been no controlled trials of the effectiveness of vitamin C, in comparison with a placebo, against upper respiratory infections over a long period and including many hundreds of persons (11).

In fact, there have been several carefully conducted double-blind studies of ascorbic acid and the common cold, carried out by responsible medical investigators. Some of these studies have given results that reject with statistical significance the null hypothesis that ascorbic acid has no more value than a placebo in decreasing the incidence and severity of the common cold when the ascorbic acid is administered regularly to subjects over a period of time beginning before the illness has set in, and the subjects are exposed to cold viruses in the ordinary way (by casual contact with other people). I shall discuss some of these studies in the following paragraphs. The amount of protection against

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Ascorbic Acid and the Common Cold: Evaluation of its Efficacy and Toxicity

PART I

By LINUS PAULING, Ph.D.

Dr. Pauling is President of the Linus Pauling Institute of Science and Medicine, 2700 Sand Hill Road, Menlo Park, Calif. 94025, and Professor Emeritus of Chemistry at Stanford University and the California Institute of Technology.

Brief descriptions are given of the thirteen controlled trials that have been made of ascorbic acid in comparison with a placebo in relation to the common cold, with the ascorbic acid or placebo given to subjects over a period of time and with the subjects in good health at the beginning of the trial and exposed to cold viruses in the ordinary way. The integrated morbidity (amount of illness per person) found in these trials was an average of 36% less for the ascorbic-acid subjects (average intake 1 g per day) than for the placebo subjects. Several investigators have reported that no serious adverse effects of ascorbic acid were observed. So far there is no significant evidence for the various adverse reactions that have been hypothesized. The apparent benefit in health from an increase in intake of ascorbic acid justifies its widespread use.

In a recent article[1] Dykes and Meier discussed some of the clinical data published since 1938 on the efficacy of pharmacologic doses of ascorbic acid in the prevention and treatment of the common cold and both clinical data and data obtained from intact animals that relate to the possible toxicity of ascorbic acid. They pointed out that in several studies the subjects receiving ascorbic acid had less illness than those receiving the placebo, but they criticized most of the studies with respect to some details of design or execution and concluded that there is little convincing evidence of a protective effect large enough to be clinically important. They also stated that many hypothetical adverse reactions to the intake of large amounts of ascorbic acid have been suggested, but that there is little evidence about the possible incidence of such reactions currently available.

The conclusions reached by Dykes and Meier have been widely misrepresented in press releases, newspapers, and magazines. For example, it has been said, on the basis of their paper and another paper published at the same time[2], that "Vitamin C will not prevent or cure the common cold".[3] In fact, their conclusion was that "Until such time as pharmacologic doses of ascorbic acid have been shown to have obvious, important clinical value in the prevention and treatment of the common cold, and to be safe in a large varied population, we cannot advocate its unrestricted use for such purposes." Moreover, some significant studies in this field were not mentioned by Dykes and Meier, and some important aspects of the studies discussed by them were also not mentioned by them. My conclusions, presented below, from the thorough analysis of the existing information, are somewhat different from those of Dykes and Meier.

Dykes and Meier mention that the evaluation of efficacy may be made uncertain by its partial dependence on subjective reports by the patients. The number of colds is especially unreliable because of uncertainty as to whether or not to record as a cold a mild indisposition lasting only one or two days. I consider the average number of days of illness per person (the integrated morbidity[4]) to be the best quantity to use in determining the relative efficacy of ascorbic acid and placebo. This quantity, which can be assessed in a reasonably objective way (by signs recorded by the physician, number of days of absence from school or work, etc.), is emphasized in the following discussion.

COWAN, DIEHL, AND BAKER

In the study by Cowan, Diehl, and Baker[5] 208 students in the University of Minnesota received about 200 mg of vitamin C per day for 28 weeks and 155 students received a placebo. Dr. Cowan has written me that the study was a double-blind one. The average number of days lost from school per person was 1.1 for the ascorbic-acid group and 1.6 for the placebo group, with standard deviations not given. 1fhis measure of the integrated morbidity thus shows 31% (range 26 to 36%) less illness per subject for the ascorbic-acid subjects than for the placebo subjects. The information given in the paper does not permit an accurate calculation to be made of the statistical significance of the rejection of the null hypothesis that ascorbic acid and the placebo have the same effect. I have made the conservative estimate[4] that P is less than 0.02.

Dykes and Meier have criticized this study on several points. I may add that the investigators were at fault in not reporting their observations precisely (rounding off the average number of days of illness and not giving the standard deviations).

FRANZ, SANDS, AND HEYL

Franz, Sands, and Heyl carried out a double-blind study in Dartmouth Medical School with 89 volunteer med-

ical students.[6] They were divided in a random way into four groups, receiving ascorbic acid (205 mg per day), ascorbic acid and a bioflavonoid, a placebo, or the bioflavonoid alone. No effect of the bioflavonoid was observed. The number of colds in the combined ascorbic-acid groups was 14 (for 44 subjects) and that in the placebo groups was 15 (for 45 subjects). The number of colds not cured or improved in 5 days was only 1 for the ascorbic-acid group, much less than the value 8 for the placebo group. The authors state that "those receivin:: ascorbic acid showed more rapid improvement in their colds than those not receiving it .. . statistically significant at the 0.05 level." My estimate of the statistical significance (based on the assumption mentioned in the following paragraph) is P (one-tailed) = 0.01. Dykes and Meier state that I apparently used an erroneous summary result; their treatment of the data gives P (one-tailed) < 0.0283, P (two-tailed) < 0.0566. We all agree that the null hypothestis of equal effect jaf ascorbic acid and placebo is to be rejected.

I have estimated the average number of days of illness per person for the two groups by making the assumption that the distribution function for colds in respect to their duration is the one given by observations made in another investigation.[7] This calculation leads to the conclusion that the integrated morbidity per person was 40% less for the ascorbic-acid subjects than for the placebo subjects.

RITZEL

Ritzel[8] reported observations made in a double-blind study on 279 schoolboys, 15 to 17 years old, on two week-long stays in a ski camp. Half of the subjects (139) received 1 g of ascorbic acid each day, and the other half (140) a placebo. There were 17 colds in the ascorbic-acid subjects •(total days of illness 31) and 31 -colds in the placebo subjects (total days of illness 80). The number of total individual signs and symptoms recorded by the physicians in their daily inspections of the subjects was 42 for the ascorbic-acid subjects and 119 for the placebo subjects. The integrated morbidity is 63% less for the ascorbic-acid group than for the placebo group (average of 61.0% from average days of illness per person and 64.5% from average number of recorded signs and symptoms). The statistical significance of this difference is high, P (one-tailed) < 0.01.

Dykes and Meier criticize Ritzel on several points, and do not mention the results that he reported. One criticism is that he does not give in his tables the total number of colds in each group. They state that "Pauling infers the number of subjects by dividing 'illness days' by 'mean illness days' and concludes that there is a significant difference in proportions of subjects experiencing colds. If his interpretation is correct, the difference is indeed significant."

It is hard for me to understand why Dykes and Meier should suggest that my interpretation might be incorrect. It involves a very simple calculation. Ritzel states (in his Table 1) that the total number of days of illness for the ascorbic-acid subjects was 31. He also states (page 66) that the average number of days per episode of illness was 1.8. The ratio 31/1.8 is 17.2; that is, there were 17 episodes of illness in this group. A similar calculation gives 31 colds for the placebo subjects (80 total days of illness, 2.6 average number of days per episode). It is safe to assume that no subjects had two colds in the same week. With this assumption, the null hypothesis of equal probability of colds for the two groups is rejected at the level P (one-tailed) < 0.015.

Dykes and Meier mention that I give great weight to the Ritzel study. I do give great weight to it, and I find it strange that they should reject it on the basis of trivial complaints, such as their apparent failure to understand the simple calculation described above.

ANDERSON, REID, AND BEATON

In the 1972 double-blind Toronto study[9,10] 407 subjects received ascorbic acid (1 g per day plus 3 g per day for 3 days at the onset of any illness) and 411 subjects received a closely matching placebo. The duration of the study was four months. The number of days confined to house per subject was 30% less for the ascorbic-acid group than for the placebo group, and the number of days off work per subject was 33% less. The authors mention that these differences have high statistical significance (P< 0.001).

Dykes and Meier present these results with little comment, except to state that the observed effect is considerably less than had been predicted by me.[4] This is true; I predicted about twice as much protection, on the basis of the study by Ritzel. I surmise that two effects may be involved in this difference. First, the amount of protection, relative to the placebo subjects, is probably less when the basic intake of ascorbic acid is high (Toronto) than when it is low (Switzerland), and second, the observed protection is probably less in a long test (4 months) than in a short one (one week).

Anderson, Reid, and Beaton reported also a smaller amount (by 40%) of non-respiratory illness in the ascorbic-acid subjects than in the placebo subjects.

ANDERSON, SURANYI, AND BEATON

A second double-blind study, with over 2000 subjects, was also carried out in Toronto.[11] In this very large study there were two placebo groups, one with 285 and the other with 293 subjects, and six ascorbic-acid groups (receiving various amounts), with 275 to 331 subjects. The study continued for three months.

A complication in the analysis of this study is presented by the fact that the results observed for the two placebo groups do not agree with one another. One placebo group had the greatest amount of illness of all eight groups, and the other had the smallest amount. The authors conclude that their observations are compatible with an effect of small magnitude (less than 20%) from both the prophylactic regimen (250 mg, 1 g, or 2 g of ascorbic acid per day) and the therapeutic regimen (4 or 8 g on the first day of illness), with an effect of somewhat greater magnitude from the combined regimen (1 g per day and 4 g on the first day of illness). They state also that there was no evidence of side effects from the 1 g or 2 g of ascorbic acid per day and no evidence of a rebound increase in illness during the month following withdrawal of the daily vitamin supplement.

The authors give the amounts of illness per subject (days of symptoms, days indoors, days off work) relative to the first placebo and relative to tj)e first plus the second (there is sonpe reason to suspect that the second placebo group was not a representative sample of the general population). I have averaged these two sets of values, and have obtained 9% as the average decrease in integrated morbidity of the ascorbic-acid subjects.

WILSON, LOH, AND FOSTER

Some studies involving several hundred students in four boarding schools in Dublin have been reported by Wilson and his collaborators.[12,13,U] As is mentioned by Dykes and Meier, their analysis of prophylactic benefit is much complicated by the subdivision of colds into three somewhat overlapping categories, catarrhal, toxic, and whole. The investigators state that the girls, in two schools were benefited, with statistical significance, by ascorbic acid, and that the boys, in the other two schools, were not. I have not been able to abstract from their papers any reliable value of the integratedmiorbidity for their subjects.

COULEHAN, REISINGER, ROGERS, AND BRADLEY

A double-blind study of 641 children in a Navajo boarding school was carried out over a 14-week period.[15] The younger children received 1 g and the older children 2 g of ascorbic acid (or placebo) per day. The number of days of illness per subject was 28% less for the ascorbic-acid group of younger children than for the placebo group, and 34% less for the older children (weighted average 30%). The statistical significance of this difference is uncertain.

KARLOWSKI ET AL.

The results of a double-blind ninemonths study with 190 employees of the National Institutes of Health have been reported recently by Karlowski, Chalmers, Frenkel, Kapikian, Lewis, and Lynch.[2] The study was well designed and well executed except for the use of a poor placebo, easily distinguished from ascorbic acid by taste. Ascorbic acid, 1 g per day, was taken by 101 subjects (groups C and D, Table 1) of whom 57 (group D) also received an additional 3 g per day for the first five days of any illness, beginning, however, only after the subjects had returned to the pharmacy to have their symptoms and clinical observations recorded and to receive their supplemental capsules. A group (A) of 46 received only placebo capsules, and a group (B) of 43 received daily placebo capsules and ascorbic-acid supplementary capsules.

Table 1

Summary of Results Reported by Karlowski et al.

Group	Number of subjects	Dose*	Average number of colds	Days of illness per cold	Days of illness per person	Decrease relative to A
A	46	P+P	1.41	7.1	1u.01	—
B	43	P+V	1.30	6.5	8.45	16%
C	44	V+P	1.18	6.7	7.91	21%
D	57	V+V	1.33	5.9	7.85	22%

**The first P means daily placebo, the first V daily ascorbic acid (1 g), the second P supplemental placebo, and the second V supplemental ascorbic acid (3 g per day for the first five days of any illness).*

The reported average number of colds and average days of illness per cold are given in Table 1. The product of these (sixth column) is the average number of days of illness per person, which is a measure of the integrated morbidity. The subjects regularly taking 1 g of ascorbic acid per day (group C) had 21% less illness than the control group (A). Nearly the same amount of decreased illness was found for the group taking only supplemental ascorbic acid (B, 16%) and the group taking both daily and supplemental ascorbic acid (D, 22%). The weighted average, 20%, of these three values is the observed decrease in integrated morbidity for all ascorbic-acid subjects relative to the placebo subjects. The statistical significance of this decrease cannot be calculated because the investigators do not give standard deviations of the averages or equivalent information.

Many of the subjects had tasted the contents of their capsules and correctly interpreted the taste. Much of the decreased illness was found in the subjects who learned in this way that they were receiving ascorbic acid. The investigators indicate that much of the apparent protective effect of ascorbic acid might be the result of a psychological effect, the power of suggestion. I doubt, as do some others, that such psychological effects can operate significantly in a large population over periods of several months, and I accept the results of the National Institutes of Health study with about as much confidence as the others.

Karlowski et al. conclude "that ascorbic acid had at best only a minor influence on the duration and severity of colds, and that the effects demonstrated might be explained equally well by a break in the double blind." They also say that "the effects of ascorbic acid on the number of colds seem to be nil," and this statement has been quoted in the AMA press release[3] without the additional information about the number of colds given by Karlowski et al. In fact (Table 1), the group receiving prophylactic ascorbic acid had 16% fewer colds than the control group, and the three ascorbic-acid groups together had 10% fewer. It is not correct to say that the effects seem to be nil.

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Am J Clin Nutr. 1991 Dec;54(6 Suppl):1147S-1152S. doi: 10.1093/ajcn/54.6.1147s.

Ascorbic acid and carnitine biosynthesis.

Rebouche CJ[1].

Author information

Abstract

It has been suggested that early features of scurvy (fatigue and weakness) may be attributed to carnitine deficiency. Ascorbate is a cofactor for two alpha-ketoglutarate-requiring dioxygenase reactions (epsilon-N-trimethyllysine hydroxylase and gamma-butyrobetaine hydroxylase) in the pathway of carnitine biosynthesis. Carnitine concentrations are variably low in some tissues of scorbutic guinea pigs. Ascorbic acid deficiency in guinea pigs resulted in decreased activity of hepatic gamma-butyrobetaine hydroxylase and renal but not hepatic epsilon-N-trimethyllsine hydroxylase when exogenous substrates were provided. It remains unclear whether vitamin C deficiency has a significant impact on the overall rate of carnitine synthesis from endogenous substrates. Nevertheless, results of studies of enzyme preparations and perfused liver in vitro and of scorbutic guinea pigs in vivo provide compelling evidence for participation of ascorbic acid in carnitine biosynthesis.

PMID: 1962562 DOI: 10.1093/ajcn/54.6.1147s

[Indexed for MEDLINE]

Publication type, MeSH terms, Substances

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Ann. Rev. Nutr. 1986. 6:365–406

THE BIOCHEMICAL FUNCTIONS OF ASCORBIC ACID

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CONTENTS

SCOPE OF THIS REVIEW

This review is concerned primarily with functions of ascorbate that have been studied at the level of specific enzymatic reactions using in vitro systems. This approach excludes detailed consideration of many functions that become disturbed in the scorbutic animal if they have not also been studied in cell or organ culture systems or using isolated enzymes. In our final discussion we consider

Can CAM treatments be evidence-based?

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https://doi.org/10.1111/fct.12110

Citation

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Title:	Can CAM treatments be evidence-based?
Author:	Louhiala, Pekka; Hemilä, Harri
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Abstract:	In this article, we first take a critical look at the definitions of evidence-based medicine (EBM) and complementary and alternative medicine (CAM). We then explore the question of whether there can be evidence-based forms of CAM. With the help of three examples, we show that EBM and CAM are not opposites, but rather concepts pointing at different dimensions. Each of the three examples is an evidence-based treatment according to three to five randomised, double-blind placebo controlled trials with consistent findings and narrow pooled confidence intervals. The most reasonable interpretation for the existence of evidence-based CAM treatments seems to be that the opposite of CAM is ‘mainstream medicine’, and the demarcation line between CAM and mainstream medicine is not simply defined by the question of whether a treatment works or not. Some effective treatments may belong to the CAM domain for historical reasons and because of preconceptions within mainstream medicine. Therefore, some treatments that currently lie outside mainstream medicine can be evidence-based.
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Biochem Cell Biol. 1990 Oct;68(10):1166-73.

Cellular functions of ascorbic acid.

Padh H[1].

Author information

Abstract

It has long been suspected that ascorbic acid is involved in many cellular reactions. This is evident from the multitude of seemingly unrelated symptoms seen in scurvy. However, until recently, our understanding of its involvement was confined to its role in the synthesis of collagen. Studies in the past few years have unveiled mechanisms of its actions in collagen formation and many other enzymatic reactions. In addition, numerous physiological responses are reportedly affected by ascorbic acid. From the well-characterized enzymatic reactions involving ascorbic acid, it has become clear that in animal cells the ascorbate does not seem to be directly involved in catalytic cycles. Rather its major function seems to keep prosthetic metal ions in their reduced form. The role of ascorbate as a reductant in these enzymatic reactions complements its other antioxidant functions which have been recently appreciated, including that as a scavenger of free radicals. Therefore, it seems that the major function of ascorbate is to protect tissues from harmful oxidative products and to keep certain enzymes in their required reduced forms. However, it remains unclear how the deficiency of ascorbate leads to the pathological symptoms found in scurvy.

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Scottish Medical Journal

Changes in Leucocyte Ascorbic Acid during the Common Cold

R. Hume, Elspeth Weyers

First Published January 1, 1973 | Research Article | Find in PubMed
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Abstract

Leucocyte ascorbic acid was measured in *7* subjects during the common cold. There was a significant fall in L.A.A. to scorbutic levels within *24* hours of the onset of symptoms. By the fifth day the L.A.A. had returned to normal, which coincided with the cessation of symptoms. Absorption studies suggested 1g. ascorbic acid per day as a prophylactic dose and 6g. ascorbic acid per day as a therapeutic dose. The effect of such supplements of ascorbic acid in *4* episodes of the common cold in *3* subjects suggests that the L.A.A. pattern can be changed by this therapy. The implications are discussed.

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Clinical manifestations of ascorbic acid deficiency in man

Robert E. Hodges, M.D., James Hood, M.D., John E. Canham, M.D., Howerde E. Sauberlich, Ph.D., Eugene M. Baker, Ph.D.

The American Journal of Clinical Nutrition, Volume 24, Issue 4, April 1971, Pages 432–443,
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Summary

Six healthy volunteers from the Iowa State Penitentiary at Fort Madison, Iowa, participated in studies of human scurvy. They were hospitalized on the Metabolic Ward of University Hospitals in Iowa City, Iowa, and fed a diet totally devoid of vitamin C.

One of the men withdrew from the study because of personal reasons. The remaining five subjects developed clinical scurvy in 84 to 97 days, manifested by signs and symptoms of fatigue, hemorrhagic phenomena, swollen joints, swollen bleeding gums, follicular hyperkeratosis, muscular aches and pains, and emotional changes.

Urinary ascorbic acid rapidly declined to undetectable levels early in the course of depletion and blood levels progressively became too low to measure accurately. Serum protein abnormalities appeared that consisted primarily of a decrease in albumin and an increase in alpha-2 and gamma globulins. Other changes occurred in serum lipids.

Radioisotopic studies indicated progressive depletion of the body pools during the depletion phase of the study and repletion in proportion to the amount of ascorbic acid administered daily. This study confirms and extends the observations made in our earlier study that the full clinical syndrome does not appear until the normal body pool has been depleted to less than 300 mg.

The minimal amount of ascorbic acid necessary to prevent or cure scurvy appears to be slightly less than 10 mg daily. Once again our observations are in accord with those of the British Medical Research Council. Estimates of the optimal intake of ascorbic acid must be made on the basis of these data plus a knowledge of the biological and physiological variables of mankind.

Topic: albumins, diet, emotions, fatigue, ascorbic acid deficiency, gamma-globulins, gingival hemorrhage, hospitals, university, pain, patients' rooms, scurvy, signs and symptoms, urinary tract, ascorbic acid, lipids, medical research, correctional facilities, phrynoderma

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Multicenter Study PLoS Med, 4 (12), e352 Dec 2007

Clustered Environments and Randomized Genes: A Fundamental Distinction Between Conventional and Genetic Epidemiology

George Davey Smith [1], Debbie A Lawlor, Roger Harbord, Nic Timpson, Ian Day, Shah Ebrahim

Affiliations

PMID: 18076282 PMCID: PMC2121108 DOI: 10.1371/journal.pmed.0040352

Abstract

Background: In conventional epidemiology confounding of the exposure of interest with lifestyle or socioeconomic factors, and reverse causation whereby disease status influences exposure rather than vice versa, may invalidate causal interpretations of observed associations. Conversely, genetic variants should not be related to the confounding factors that distort associations in conventional observational epidemiological studies. Furthermore, disease onset will not influence genotype. Therefore, it has been suggested that genetic variants that are known to be associated with a modifiable (nongenetic) risk factor can be used to help determine the causal effect of this modifiable risk factor on disease outcomes. This approach, mendelian randomization, is increasingly being applied within epidemiological studies. However, there is debate about the underlying premise that associations between genotypes and disease outcomes are not confounded by other risk factors. We examined the extent to which genetic variants, on the one hand, and nongenetic environmental exposures or phenotypic characteristics on the other, tend to be associated with each other, to assess the degree of confounding that would exist in conventional epidemiological studies compared with mendelian randomization studies.

Methods and findings: We estimated pairwise correlations between nongenetic baseline variables and genetic variables in a cross-sectional study comparing the number of correlations that were statistically significant at the 5%, 1%, and 0.01% level (alpha = 0.05, 0.01, and 0.0001, respectively) with the number expected by chance if all variables were in fact uncorrelated, using a two-sided binomial exact test. We demonstrate that behavioural, socioeconomic, and physiological factors are strongly interrelated, with 45% of all possible pairwise associations between 96 nongenetic characteristics (n = 4,560 correlations) being significant at the $p < 0.01$ level (the ratio of observed to expected significant associations was 45; p-value for difference between observed and expected < 0.000001). Similar findings were observed for other levels of significance. In contrast, genetic variants showed no greater association with each other, or with the 96 behavioural, socioeconomic, and physiological factors, than would be expected by chance.

Conclusions: These data illustrate why observational studies have produced misleading claims regarding potentially causal factors for disease. The findings demonstrate the potential power of a methodology that utilizes genetic variants as indicators of exposure level when studying environmentally modifiable risk factors.

Figures

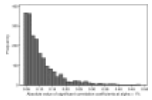

Figure 1. Histogram of Statistically Significant (at...

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intravenous route at an advanced stage of tetanus led to the survival of the animals.

From the above results, it definitely appears that vitamin C can be effectively used as a simple prophylactic and therapeutic tool to combat the neurotoxic effects of tetanus toxin.

Thanks are due to Prof. S. R. MOITRA for his interest in this work.

Eingegangen am 31. Marz 1966

[1] DEY, P. K.: (a) Naturwissenschaften 52, 164 (1964); — (b) Ind. J. Exptl. Biol. (communicated, 1966). — [2] SHERRINGTON, C. S.: The Integrative Action of Nervous System, p. 303, 112. New York: Yale University Press 1906. — *[3]* BROOKS, B. V., D. R. CURTIS, and J. C. ECCLES: Nature 175, 120 (1955)- — *[4]* JUNGBLUT, C. W.: J. Immunol. 33, 203 (1937).

Efficacy of Vitamin C in Counteracting Tetanus Toxin Toxicity

P. K. DRY

Department of Physiology, University College of Science, Calcutta

The author has shown [7] that ascorbic acid is most effective as prophylactic and therapeutic agent in nullifying the lethal and convulsive properties of strychnine. He now examined the efficacy of ascorbic acid in counteracting the toxic action of tetanus toxin since SHERRINGTON *[2]* observed that the effects of strychnine poisoning are similar to those appearing in tetanus toxin toxicity and BROOKS et al. [3] confirmed the findings of SHERRINGTON that the action of tetanus toxin in the spinal cord closely resembles that of strychnine. Also, JUNGBLUT *[4]* has shown that the toxin is destroyed *in vitro* by vitamin C.

Adult rats were used in all the experiments. Diet, temp, and space allowed for movement were kept uniform. The gastrocnemius muscle was the site used for the intramuscular administration of toxin.

Group 1. 5 rats were given 2MLD (minimum lethal dose) of tetanus toxin, rhe symptoms of toxicity were then noted. — *Group 2:* 5 rats were given simultaneously 2MLD of toxin and 1 gm/kg of vitamin C intraperitoneally. Then for subsequent three days, vitamin C (l gm/kg) was only administered twice daily i. p. — *Group 3:* 5 rats were administered ascorbic acid l gm/kg twice daily for three days. Then 2MLD of toxin was given, followed again by administration of vitamin C for subsequent three days at the previous dose. — *Group 4:* 5 rats were given 2MLD of toxin. Usally after 16 to 26 hours, local tetanus appeared in the affected leg. When such beginning of symptoms were noted, vitamin C (1 gm/kg) was given i. p. twice daily for 3 days. — *Group 5*: 10 rats were given 2MLD of toxin. After 40 to 47 hours, general tetanic symptoms markedly developed, vitamin C (300 mg) was administered intravenously after anaesthetizeing the animal with Na-thiopental.

Results: Group 1. Following tetanus toxin, local tetanus appeared in 16 to 26 hours. The affected leg was in fixed position and toes were extended. Within 27 to 39 hours, the tail, extremity and hip deviated to the injection side. Both extremities assumed a parallel extended position. In 40 to 47 hours, spasticity of the abdominal and thoracic musculature and flexor muscles of the spine and neck was seen. Tachycardia, dyspnoea, and convulsions were oberved. Death followed in 47 to 65 hours. — *Group 2:* All the animals survived. Only very mild local tetanus were seen at the affected leg after 18 hours. — *Group 3:* All the animals survived. No symptoms of toxicity appeared. — *Group 4:* When the initial symptoms of local tetanus appeared, administration of vitamin C prevented the further spread of the symptoms and they finally survived. — *Group 5:* Administration of vitamin C through

DIE NATURWISSENSCHAFTEN

53. Jahrgang, 1966 Heft 11 (Erstes Juniheft)

intravenous route at an advanced stage of tetanus led to the survival of the animals.

From the above results, it definitely appears that vitamin C can be effectively used as a simple prophylactic and therapeutic tool to combat the neurotoxic effects of tetanus toxin.

Thanks are due to Prof. S. R. MOITRA for his interest in this work.

Eingegangen am 31. Marz 1966

[1] DEY, P. K.: (a) Naturwissenschaften 52, 164 (1964); — (b) Ind. J. Exptl. Biol. (communicated, 1966). — [2] SHERRINGTON, C. S.: The Integrative Action of Nervous System, p. 303, 112. New York: Yale University Press 1906. — *[3]* BROOKS, B. V., D. R. CURTIS, and J. C. ECCLES: Nature 175, 120 (1955)- — *[4]* JUNGBLUT, C. W.: J. Immunol. 33, 203 (1937).

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Group 1. 5 rats were given 2MLD (minimum lethal dose) of tetanus toxin, rhe symptoms of toxicity were then noted. — *Group 2:* 5 rats were given simultaneously 2MLD of toxin and 1 gm/kg of vitamin C intraperitoneally. Then for subsequent three days, vitamin C (l gm/kg) was only administered twice daily i. p. — *Group 3:* 5 rats were administered ascorbic acid l gm/kg twice daily for three days. Then 2MLD of toxin was given, followed again by administration of vitamin C for subsequent three days at the previous dose. — *Group 4:* 5 rats were given 2MLD of toxin. Usally after 16 to 26 hours, local tetanus appeared in the affected leg. When such beginning of symptoms were noted, vitamin C (1 gm/kg) was given i. p. twice daily for 3 days. — *Group 5*: 10 rats were given 2MLD of toxin. After 40 to 47 hours, general tetanic symptoms markedly developed, vitamin C (300 mg) was administered intravenously after anaesthetizeing the animal with Na-thiopental.

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The New England
Journal of Medicine

VOLUME 207 OCTOBER 13, 1932 NUMBER 15

NEW ENGLAND PEDIATRIC SOCIETY

A meeting of the Society was called to order by the President, Dr. Lewis Webb Hill, Boston, at 8:15 P. M., on May 6, 1932 who spoke as follows:

This meeting represents an attempt to arrive at conclusions concerning the rational use of the vitamin preparations in pediatric practice. There is one man whose work on deficiency diseases and allied subjects has been so brilliant and so applicable to the everyday work of each one of us that any such meeting as this could not be complete without his presence—Dr. Alfred Hess of New York.

DIET, NUTRITION AND INFECTION*

BY ALFRED F. HESS, M.D.†

IT is a commonplace that the relationship is intimate between composition of the diet and susceptibility to infection. However, the extent of this relationship and its importance in clinical medicine has only just begun to be realized; in fact we are still uncertain as to the limits of altered susceptibility. From the standpoint of disease, diet, nutrition and resistance to infection should be regarded as an etiologic unit rather than as a triad. In appraising dietaries from this point of view, not only the several vitamins should be considered, but the various inorganic and organic constituents which likewise may be implicated in bacterial infection. It would lead too far afield, however, to consider these various aspects of the subject, so that I shall confine myself to the rôle of some of the vitamins, basing my conclusions mainly on observations made during the past ten to fifteen years in a child-caring institution. As my experience has been concerned chiefly with the antirachitic, antiophthalmic and antiscorbutic vitamins, in other words with vitamins D, A and C, I shall limit my comments to these specific nutritional factors. Furthermore, I shall take into consideration only clinical data, to the exclusion of experiments on animals.

After an experience of several years with the effect of *ultraviolet rays* in the prevention and cure of rickets, an effort was made to lessen the incidence of infection in the institution by means of irradiation with the mercury vapor lamp. As is well-known, respiratory infections constitute one of the last vestiges of institutionalism in hospitals and asylums for children and, during the winter months, plague and torment their foster-parents. Our first attempt, undertaken in 1926[1] with the confidence born of inexperience, was most disappointing. In the course of the winter, in spite of irradiation carried out every other day for a period embracing four months, quite as many infections occurred among the group of infants who were irradiated as among those who lived under the same régime except that they were not irradiated. It may be added that the irradiated group evidenced an initial increase in weight which, however, did not continue during the subsequent months.

Two years later a similar investigation was carried out[2] with the only difference that a carbon arc lamp was used as the source of radiation, as it was thought that these rays might be superior because they more nearly resemble the spectrum of the sun. Again our efforts were fruitless. In spite of systematic exposures to these rays no relative diminution in the incidence of respiratory infections occurred during an observational period of three months.

The following year, 1929, the problem of infection was attacked in a different way[3]. Rickets was prevented by means of the usual doses of cod liver oil, in other words of three teaspoonfuls daily for babies three months or more of age. The diet was composed of full amounts of pasteurized milk, cereals, orange juice, and of vegetables for the older infants. In order to render exposure as infrequent as possible, what was termed "aseptic nursing" was carried out in one ward—physicians, nurses and attendants coming in contact with the infants were required to wear surgical masks which were changed daily; hands were scrubbed thoroughly and frequently; visiting was allowed but once a month and visitors were provided with masks; fondling and petting of infants were prohibited and nurses who had colds or infections were temporarily excluded from service. Once again our attempts at prophylaxis resulted in failure; infections

*Read before the New England Pediatric Society at its meeting, May 6, 1932.

†Hess—Clinical Professor of Pediatrics, University and Bellevue Hospital Medical College. For record and address of author see "This Week's Issue," page 679.

Format: Abstract

Med Microbiol Immunol. 1982;171(2):113-22.

Disorders of neutrophil function in children with recurrent pyogenic infections.

Patrone F, Dallegri F, Bonvini E, Minervini F, Sacchetti C.

Abstract

Ten patients with neutrophil dysfunctions and recurrent pyogenic infections, mainly of the skin middle-ear, and respiratory tract, are described. The most frequently affected functions were chemotaxis and bacterial killing. Pharmacologic restoration of functional defects was tried in all cases. Levamisole was given in two cases and ascorbic acid in the other eight cases. During a follow up of at least 18 months, seven patients showed a complete restoration of neutrophil function and a long-lasting clinical remission. One of the two patients with Chronic Granulomatous Disease has been free from infections for 1 year, despite persistent neutrophil dysfunction, while the other did not display consistent clinical improvement. Another patient, who was given ascorbic acid for a short period only due to non compliance, showed neither laboratory nor clinical improvement.

PMID: 7144693 DOI: 10.1007/bf02124918

[Indexed for MEDLINE]

Publication types, MeSH terms, Substances

LinkOut - more resources

Format: Abstract

Am J Med. 1975 Apr;58(4):532-6.

Effects of ascorbic acid on the common cold. An evaluation of the evidence.

Chalmers TC.

Abstract

Of 14 clinical trials of ascorbic acid in the prevention and treatment of the common cold, the data from 8 were considered well enough gathered to be creditable and to warrant combining for an over-all assessment of efficacy. Differences in mean prorated numbers of colds per year and durations of illness were 0.09 plus or minus 0.06 (plus or minus 1 standard error) and 0.11 plus or minus 0.24, respectively, favoring ascorbic acid over the placebo. These are minor and insignificant differences, but in most studies the severity of symptoms was significantly worse in the patients who received the placebo. In one study lasting 9 months, a large number of the volunteers tasted their capsules and correctly guessed what group they were in. All differences in severity and duration were eliminated by analyzing only the data from those who did not know which drug they were taking. Since there are no data on the long-term toxicity of ascorbic acid when given in doses of 1 g or more per day, it is concluded that the minor benefits of questionable validity are not worth the potential risk, no matter how small that might be.

PMID: 1092164 DOI: 10.1016/0002-9343(75)90127-8

[Indexed for MEDLINE]

Publication types, MeSH terms, Substances

LinkOut - more resources

Format: Abstract

J Appl Physiol. 1976 Aug;41(2):202-5.

Effect of ascorbic acid on rate of heat acclimatization.

Strydom NB, Kotze HF, van der Walt WH, Rogers GG.

Abstract

There is some indication in the literature that ascorbic acid (vitamin C) may reduce the physiological responses to heat stress. Consequently, the effect of ascorbic acid ingestion on heat-strain indicators has been studied on a group of 60 mining recruits undergoing climatic room acclimatization. Of the 60 men, 19 received a daily dose of 250 mg ascorbic acid; 21 a daily dose of 500 mg ascorbic acid; and 20 received a placebo daily. Measurements of rectal temperature, heart rate, and hourly sweat rate were made on all subjects during the 4 h of heat exposure per day for 10 days. The wet bulb temperature was 32.2 degrees C, the dry bulb 33.9 degrees C, the air movement 0.4 m/s, and the work rate 35 W. The results indicate that the rate and degree of acclimatization, as assessed by 4th-h rectal temperature, is enhanced by ascorbic acid supplementation and that no differences in response could be shown between daily dosages of 250 and 500 mg of vitamin C.

PMID: 956103 DOI: 10.1152/jappl.1976.41.2.202

[Indexed for MEDLINE]

Publication types, MeSH terms, Substance

LinkOut - more resources

Format: Abstract

Eur Respir J. 1989 Mar;2(3):229-33.

Effects of vitamin C on airway responsiveness to inhaled histamine in heavy smokers.

Bucca C[1], Rolla G, Caria E, Arossa W, Bugiani M.

Author information

Abstract

Histamine bronchial threshold, the provocation concentration of histamine causing a 25% fall in maximal expiratory flow at 50% of forced vital capacity from the control value (PC25MEF50), was measured in seven heavy smokers and in seven sex- and age-matched nonsmokers before and one hour after ingestion, double-blind, of vitamin C (2 g) or placebo. Smokers had significantly lower baseline values of serum ascorbate, maximal expiratory flow at 50% of forced vital capacity (MEF50) and PC25MEF50: the latter was negatively related to serum ascorbate (r = -0.85; p less than 0.001). Acute treatment with vitamin C produced a significant decrease in PC25MEF50 in smokers (95% confidence limit (CL) from 4.87-3.36 to 2.91-2.01 mg.ml-1; p = 0.017), whilst it had no effect in nonsmokers. A preliminary open study on the effect of prolonged administration of vitamin C (1 g daily) was performed in smokers. One week of treatment produced a further significant decrease in PC25MEF50 (p less than 0.0001). Our results suggest that in heavy smokers histamine bronchial responsiveness may be attenuated by chronic ascorbate deficiency. In these circumstances, acute and short-term treatment with vitamin C may increase the bronchoconstrictive response to inhaled histamine.

PMID: 2731601

[Indexed for MEDLINE]

Publication types, MeSH terms, Substances

LinkOut - more resources

JAMA. 1975 Mar 10;231(10):1073-9.

Ascorbic acid and the common cold. Evaluation of its efficacy and toxicity.

Dykes MH, Meier P.

Abstract

We reviewed the clinical data relating to the efficacy and safety of pharmacologic doses of ascorbic acid in the prevention and treatment of the common cold. Although one study tentatively supports the hypothesis that such doses of ascorbic acid may be efficacious, a second study by the same group did not confirm the significant findings, and no clear, reproducible pattern of efficacy has emerged from the review of all the evidence. Similarly, there is currently little adequate evidence on either the presence or the absence of serious adverse reactions to such doses of ascorbic acid, although many such reactions have been hypothesized. The unrestricted use of ascorbic acid for these purposes cannot be advocated on the basis of the evidence currently available.

PMID: 1089817

[Indexed for MEDLINE]

LinkOut - more resources

Proc Natl Acad Sci U S A. 1993 Jan 1;90(1):317-21.

Glutathione ester delays the onset of scurvy in ascorbate-deficient guinea pigs.

Mårtensson J[1], Han J, Griffith OW, Meister A.

Author information

Abstract

Previous studies showed that administration of ascorbate to glutathione (GSH)-deficient newborn rats and guinea pigs prevented toxicity and mortality and led to increased tissue and mitochondrial GSH levels; ascorbate thus spares GSH. In the present work, we tried to answer the converse question: Does administration of GSH spare ascorbate? Because administered GSH is not well transported into most cells, we gave GSH monoethyl ester (which is readily transported and converted into GSH intracellularly) to guinea pigs fed an ascorbate-deficient diet. We found that treatment with GSH ester significantly delays appearance of the signs of scurvy and that this treatment spares ascorbate; thus, the decrease of tissue levels of ascorbate was delayed. The findings support the conclusions that (i) GSH is essential for the physiological function of ascorbate because it is required in vivo for reduction of dehydroascorbate and (ii) there is metabolic redundancy and overlap of the functions of these antioxidants. The sparing effect of GSH in scurvy may be mediated through an increase in the reduction of dehydroascorbate (which would otherwise be degraded) and to antioxidant effects of GSH that are also produced by ascorbate. Other studies indicate that GSH deficiency in adult mice stimulates ascorbate synthesis in liver. During this work we found that administration of GSH itself is highly toxic to ascorbate-deficient guinea pigs when given in divided i.p. doses totaling 3.75 mmol/kg daily.

PMID: 8419936 PMCID: PMC45651 DOI: 10.1073/pnas.90.1.317

[Indexed for MEDLINE] **Free PMC Article**

Publication types, MeSH terms, Substances, Grant support

LinkOut - more resources

The Effect of Vitamin E on Common Cold Incidence Is Modified by Age, Smoking and Residential Neighborhood

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http://dx.doi.org/10.1080/07315724.2006.10719543

Links to the references are added to this manuscript version.

Fig. 1 is redrawn as a more accurate version at the end of this paper.

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The Effect of Vitamin E on Common Cold Incidence Is Modified by Age, Smoking and Residential Neighborhood

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ABSTRACT

Background: We have previously found a 28% reduction in common cold incidence with 50 mg/day vitamin E supplementation in a subgroup of the Alpha-Tocopherol Beta-Carotene Cancer Prevention (ATBC) Study cohort: older city-dwelling men (≥65 years) who smoked only 5–14 cigarettes/day.

Objective: To carry out more detailed analyses to explore the modification of vitamin E effect by age, smoking, and residential neighborhood.

Methods: We examined the effect of vitamin E on common cold risk in subjects consisting of the placebo and vitamin E arms (n = 14,573) of the ATBC Study, which recruited males aged 50–69 years who smoked ≥5 cigarettes/day at the baseline. The ATBC Study was conducted in southwestern Finland in 1985–1993; the active follow-up lasted for 4.7 years (mean). We modeled common cold risk as a function of age-at-follow-up in the vitamin E arm compared with the placebo arm using linear splines in Poisson regression.

Results: In participants of 72 years or older at follow-up, the effect of vitamin E diverged. Among those smoking 5–14 cigarettes per day at baseline and living in cities, vitamin E reduced common cold risk (RR = 0.54; 95% CI 0.37–0.80), whereas among those smoking more and living away from cities, vitamin E increased common cold risk (RR = 1.58; 1.23–2.01).

Conclusions: Vitamin E may cause beneficial or harmful effects on health depending on various modifying factors. Accordingly, caution should be maintained in public health recommendations on vitamin E supplementation until its effects are better understood.

INTRODUCTION

Animal studies have found that vitamin E may affect susceptibility to and severity of diverse viral and bacterial respiratory infections (1-5). Although several studies found that vitamin E may have beneficial effects on various laboratory measures of the immune system in animals and humans (5,6), harmful effects on the immune system have also been reported (7,8). Two animal studies found positive effects on the immune system with moderate vitamin E doses, but adverse effects with large doses (9,10).

Only a few trials have examined the effect of vitamin E supplementation on clinical infectious disease outcomes, such as respiratory and urinary tract infections (5,11-15) and tuberculosis (16) in human subjects. On the whole, these trials found no unequivocal benefit from vitamin E and, paradoxically, one trial found an increase in the severity of acute respiratory illness with 200 mg per day of vitamin E (12). Three trials examined the effect of vitamin combinations containing vitamin E on respiratory infections; however, no specific conclusions of vitamin E can be drawn of these trials (17-19).

We previously found no overall effect on common cold risk with 50 mg per day of vitamin E in the Alpha-Tocopherol Beta-Carotene Cancer Prevention (ATBC) Study (20). However, in a small subgroup of older city-dwelling men (≥65 years) who smoked only 5–14 cigarettes per day, vitamin E supplementation was associated with a statistically highly significant, but quantitatively modest, reduction in common cold incidence (RR = 0.72; 95% CI: 0.62–0.83) (20). Whether this observation resulted from a physiological effect or emerged by chance from a series of subgroup analyses remained an open question. Since the number of common cold episodes recorded in the ATBC Study was very high, we carried out more detailed analyses to explore the possibility that vitamin E effect is modified by age, smoking, and residential neighborhood.

PARTICIPANTS AND METHODS

Study Participants and Intervention Groups

The design and methods of the ATBC Study examining the effects of vitamin E (*dl*-α-tocopheryl acetate (AT), 50 mg/day) and β-carotene (BC, 20 mg/day) on the incidence of lung cancer and other cancers have already been described in detail (20,21). In brief, the trial participants were recruited in 1985–88 from the total male population aged 50–69 years living in southwestern Finland (n = 290,406). To be eligible, participants had to smoke ≥5 cigarettes per day at entry. The eligible participants (n = 29,133) were randomized to one of four intervention arms and administered placebo, AT, BC, or AT + BC. The planned intervention continued for 5 to 8 years (median 6.1 years) until April 30, 1993, with 3 follow-up visits annually, but because of deaths and drop-outs the active follow-up lasted for 4.7 years (mean). The trial was approved by the institutional review boards of the participating institutions; all participants gave written informed consent. At baseline, prior to randomization, the men completed a questionnaire on their medical and smoking histories and general background characteristics. In the current analysis we excluded participants who were administered β-carotene to avoid any problems caused by potential interaction between vitamin E and β-carotene, so that we restricted ourselves to the placebo and AT arms of the trial (n = 14,573; Table 1).

Outcome Definition and Smoking Status Evaluation during Follow-Up

At each follow-up visit to the local study center, 3 times per year with 4-month intervals (Table 1), the participant was asked "Have you had a common cold since the previous visit, and if so, how many times?" The occurrence of "other upper respiratory tract infection" and "acute bronchitis" was also asked about. The number of colds reported at each follow-up visit was used as the outcome for this study. This outcome, self-reported colds, is based on subjective symptoms and not on any laboratory findings. However, since it is the subjective symptoms that lead a person to seek medical attention and obtain sick-leave, in this respect the subjective outcome is most relevant for public health purposes. The manifestations of the common cold are so typical that self-diagnosis by the patient is usually correct (22). During 69,094 person-years of active follow-up covered by visits to the study centers, 55,770 common cold episodes were recorded.

At each follow-up visit, the participant was asked: "Have you been smoking since the previous visit?" with the following alternative responses provided: 1) no, 2) yes, but now I have quit, 3) yes, continuously (Table 1). In this study we used responses 1) and 3) when exploring the effect of smoking cessation before the follow-up visit.

Statistical Methods

Because we analyzed the modification of vitamin E effect by age, and the ATBC Study lasted for some 6 years, in the current analyses we used the age of participant at the follow-up visit. This is the biological age at the point of time when the outcome for the preceding 4-month period is evaluated.

The number of common cold episodes was modeled using Poisson regression. The risk ratio (RR) and the likelihood ratio-based 95% confidence interval (95% CI) were calculated using the SAS PROC GENMOD program (release 8.1, SAS Institute, Inc., Cary, NC). Linear spline-modeling (23) was carried out for the four groups defined by baseline smoking and residential neighborhood as follows.

First, using a base model containing the mean vitamin E-effect, and a linear trend to adjust for the average reduction in common cold incidence with age, we added ten linear splines to both trial arms at 2 year-intervals starting at 52 years of age-at-follow-up. Thereafter, linear spline terms for the vitamin E arm were added to the same knots, and the statistical significance of the vitamin E—age-at-follow-up interaction was calculated from the change in the –2 × Log(Likelihood) difference. This saturated model was simplified by dropping the knots that had the least effect on the vitamin E spline model, starting with those with the lowest Wald-test χ^2 value. The corresponding knots covering both arms were concurrently dropped out. The models were simplified until all remaining vitamin E arm knots gave a significant contribution to the spline model ($\chi^2 > 4$). Thus, the final model contained knots at the same years for both arms to provide the baseline, and for the vitamin E arm to provide the age-modification. Visually, the final models captured all the main features of the saturated models (graphs for saturated models not shown). The optimized models are described in Table 2 and the corresponding graphs in Fig. 1. Two-tailed *p*-values were used.

We tested the modifying effect of residential neighborhood on the vitamin E effect separately in participants who smoked 5–14 and those who smoked ≥15 cigarettes per day. Based on the appearance of the spline curves (Fig. 1), we restricted this analysis to participants aged ≥62 and ≥65 years at the follow-up visit, respectively, in the light and heavy smokers. First we added a linear trend to adjust for the average reduction in common cold incidence with age, the mean vitamin E-effect, mean effect of residential neighborhood, and a linear spline to the vitamin E arm at 62 or 65 years. To test the role of residential neighborhood, we further added the mean vitamin E effect and a linear spline to the vitamin E arm to the city-dwellers. The change in the –2 × Log(Likelihood) gives χ^2(2 df), which was used to calculate the *p*[2-tail]-value to test the role of residential neighborhood in the vitamin E spline-models.

As to supplementation, the analyses were carried out following the intention-to-treat principle. Compliance with supplementation was high: some 80% of participants took more than 95% of their prescribed capsules during their active participation in the trial; there were no differences in capsule consumption among the intervention groups (21). The outcome was, however, available only for those participants who continued with the trial and participated in the follow-up visits.

Table 1. Baseline Characteristics of Participants, and the Age and Smoking Status at Follow-Up Visits, The ATBC Study 1985–1993; No β-Carotene Participants

Baseline characteristics	No. of participants	
All participants	14,573	(100%)
Baseline age (years)		
50–54	5,275	(36%)
55–59	4,639	(32%)
60–64	3,183	(22%)
65–69	1,476	(10%)
Smoking (cigarettes/day)		
5–14	2,910	(20%)
15–	11,663	(80%)
Age of smoking initiation*		
<21 years	10,842	(74%)
≥21 years	3,727	(26%)
Residential neighborhood during the last 20 years*		
City (>50,000 inhab.)	6,233	(43%)
Town	3,093	(21%)
Village	2,092	(14%)
Countryside	3,153	(22%)

Follow-up visit variables	No. of visits	
All visits	207,284	(100%)
Age at follow-up visit		
50–51	5,265	
52–53	16,603	(8%)
54–55	25,517	(12%)
56–57	29,240	(14%)
58–59	28,127	(14%)
60–61	25,902	(12%)
62–63	22,588	(11%)
64–65	18,685	(9%)
66–67	14,513	(7%)
68–69	10,642	(5%)
70–71	6,485	(3%)
72–73	2,805	(1.5%)
74–77	912	(0.5%)
Smoking since the previous visit		
No	23,032	(11%)
Yes, but quit before current visit	5,817	(3%)
Yes, continuously	178,433	(86%)

* Data on residential neighborhood was missing from 2 participants, and on age at smoking initiation from 4 participants.

Table 2. Optimizing the Spline Models for the Age-Modification of Vitamin E Effect on Common Cold Incidence

Group	Saturated model*	Simple model*
≥15 cigarettes per day living away from cities	χ^2(10 df) = 40.9	χ^2(4 df) = 36.5 p = 0.0000002 knots at 52, 56, 58, 68 yrs
≥15 cigarettes per day living in a city	χ^2(10 df) = 17.3	χ^2(2 df) = 7.8 p = 0.02 knots at 64, 66 yrs
5–14 cigarettes per day living away from cities	χ^2(10 df) = 22.3	χ^2(1 df) = 18.9 p = 0.00002 knot at 56 yrs
5–14 cigarettes per day living in a city	χ^2(10 df) = 46.5	χ^2(2 df) = 38.7 p = 0.000000004 knots at 60, 62 yrs

* The χ^2 measures the improvement in the Poisson model when the knots indicated are added to the vitamin E arm in the simple model.
In the saturated model, 10 knots at 2-year intervals were added, starting at 52 years.

RESULTS

Table 1 shows the distributions for the baseline data for age, smoking level, age of smoking initiation, residential neighborhood, and follow-up data for age and smoking at the follow-up visits. On average, 0.27 common cold episodes were reported at each four-monthly follow-up visit, corresponding to an annual rate of 0.8 cold episodes.

There is no overall effect, with a narrow confidence interval, of vitamin E supplementation in the four groups defined by baseline smoking and residential neighborhood (Table 3). To examine the potential modification of vitamin E effect by age, we constructed linear spline models for the vitamin E effect as a function of age-at-follow-up separately for the four groups defined by baseline smoking and residential neighborhood. These groups show statistically highly significant modification of vitamin E effect by age-at-follow-up, except for city-dwellers smoking ≥15 cigarettes per day (Fig. 1, Table 2).

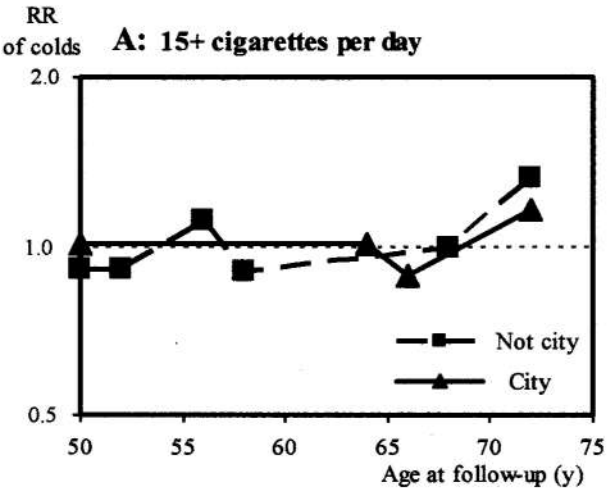
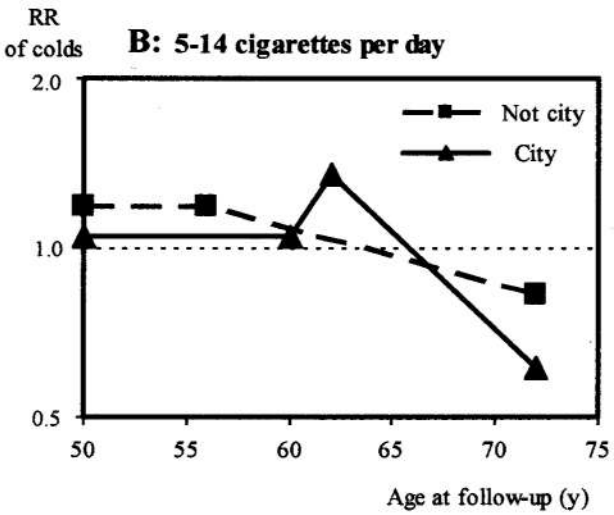


Fig. 1. The effect of vitamin E on the relative risk of common cold as a function of age at follow-up. Participants smoking more (A) and less (B) are further divided into subgroups by residential neighborhood. RR indicates the relative risk of colds between the vitamin E and placebo arms. See Table 2 for the description of the statistical models.
See Fig. 1. redrawn in 2014 at the end of this paper.



Among participants who smoked ≥15 cigarettes per day at baseline, the spline curve of vitamin E effect shows a trend towards harm for old participants (Fig. 1A). Among the heavy smokers living away from cities, there is a peak of increased risk at 56 years of age. Although there is no apparent biological rationale for such a sharp peak in the common cold risk, dropping out the knots at 52, 56, and 58 years would reduce the χ^2 value by 17.9 (3 df; $p = 0.0005$) so that these knots are retained in the spline model.

Among participants who smoked only 5–14 cigarettes per day at baseline, the spline curves suggest slight harm for young participants, but there is an age-dependent trend towards benefit in old participants (Fig. 1B). Among the city-dwellers who smoke less, there is a peak indicating harm at about 62 years of age. Although there is no apparent biological rationale for such a sharp peak here either, omitting the knot at 62 years reduces the χ^2 value by 16.3 (1 df; $p = 0.0001$); therefore both knots are retained in the spline model. The knot at 56 years in the participants smoking less, who live away from cities, remained after the stepwise reduction of the spline model, but there was no meaningful difference compared with spline models with a single knot located at 52, 54 or 58 years.

Because this work was motivated by the effect of vitamin E observed in the subgroup of ≥65 year old city-dwellers who smoked 5–14 cigarettes per day (20) and inclusion of that subgroup in the vitamin E spline model does not provide a test independent of the original finding, we examined whether age is a modifier outside of this small subgroup. When the participants aged ≥65 years at baseline were excluded from the spline model of the city-dwellers who smoked 5–14 cigarettes per day at baseline, the vitamin E spline model was still highly significant (χ^2[2 df] = 12.3, p = 0.002). The other three of the four subgroups test the age-modification of vitamin E effect independently of the original hypothesis-generating subgroup (Table 2).

Among the oldest participants, the effect of vitamin E on common cold incidence substantially diverges in the light and heavy smokers, but the role of residential neighborhood is less evident (Fig. 1). Therefore we tested whether including the residential neighborhood significantly improves the vitamin E spline models at the upper age range. Among participants who smoked 5–14 cigarettes per day there was strong evidence that the age at visit of 62 years or more modifies the vitamin E effect differently in city-dwellers and those who live away from cities (p = 0.018). In contrast, for those who smoked ≥15 cigarettes per day there was weaker evidence that the age at visit of 65 years or more modifies the vitamin E effect differently in the residential neighborhood groups (p = 0.042).

Based on the appearance of the spline curves, certain age-ranges were selected for explicit calculation of the effect estimate of vitamin E supplementation and its confidence interval (Fig. 1, Table 3). Vitamin E supplementation for participants smoking less was associated with a significant increase in the risk of colds at 50–56 years in those who live away from cities, and at 61–63 years in the city-dwellers. For city-dwellers who smoke less, vitamin E supplementation caused a substantial reduction in the risk of colds for participants aged 69 years or more, but the benefit was smaller among participants living away from cities. Among the heavy smokers, vitamin E supplementation significantly increased the risk of colds among the oldest participants (Table 3).

It is noteworthy that among the ≥72 year old participants the greatest benefit was seen in city-dwellers smoking 5–14 cigarettes per day, whereas the greatest harm was seen in the mirror image, i.e., participants living outside cities and smoking ≥15 cigarettes per day (Fig. 1, Table 3). The confidence intervals for the vitamin E effect on these two groups are strikingly different. It is also noteworthy that in both of these groups there is a peak of harm at 62 and 54 years respectively, whereas the remaining two groups do not show comparable peaks for the younger participants.

The preceding analysis is based on defining the subgroups by smoking level at baseline. To explore whether other measures of cigarette smoke exposure would further modify the effect of vitamin E, we analyzed the risk of colds in participants aged ≥72 years by combining the residential neighborhood groups, but keeping the baseline low and heavy smoking groups separate. Among the old participants who smoked heavily at baseline, the vitamin E effect is significantly modified by the age of smoking initiation (Table 4). In these heavy smokers, there was no definite evidence of harm from vitamin E in those who quit smoking before the visit, but the number of quitters is low. Among participants who smoked less at baseline, age of smoking initiation did not modify the vitamin E effect, and smoking cessation did not lead to a greater vitamin E benefit (Table 4).

Table 3. The Effect of Vitamin E Supplementation on the Risk of the Common Cold in Selected Age-Groups by Baseline Smoking and Residential Neighborhood

	≥15 cigarettes per day		**5–14 cigarettes per day**	
	Town, village, or countryside	**City**	**Town, village, or countryside**	**City**
Number of participants:	6,587	5,074	1,751	1,159
All visits (207,270 visits)				
RR	0.98	1.00	1.02	1.02
95% CI	0.95–1.01	0.97–1.03	0.97–1.08	0.96–1.08
Age at visit				
50–56 yrs (62,054 visits)				
RR	1.01	0.98	1.20	1.07
95% CI	0.96–1.05	0.93–1.03	1.08–1.32	0.96–1.20
61–63 yrs (35,182 visits)				
RR	0.93	1.02	0.97	1.30
95% CI	0.87–0.99	0.95–1.10	0.86–1.09	1.13–1.50
69–71 yrs (11,321 visits)				
RR	1.11	1.04	0.80	0.68
95% CI	0.98–1.27	0.90–1.19	0.67–0.96	0.54–0.84
72–77 yrs (3,717 visits)				
RR	1.58	1.35	0.90	0.54
95% CI	1.23–2.01	1.03–1.76	0.63–1.28	0.37–0.80

Table 4. Modification of Vitamin E Effect on Common Cold Risk by Age at Smoking Initiation and by Recent Smoking among Participants Aged 72 Years or More at the Follow-Up Visit

	Risk of colds in the vitamin E arm RR; 95% CI	**Test of interaction** *p*
Baseline smoking ≥15 cigarettes per day		
All in the subgroup (2,513 visits)	1.42; 1.18–1.70	
Age at smoking initiation		
<21 years (1,482 visits)	1.68; 1.34–2.12	0.02
≥21 years (1,031 visits)	1.09; 0.82–1.45	
Smoking at follow-up		
Continued (1,992 visits)	1.48; 1.21–1.80	0.10
Quit (444 visits)	0.96; 0.59–1.55	
Baseline smoking 5–14 cigarettes per day		
All in the subgroup (1,204 visits)	0.71; 0.54–0.91	
Age at smoking initiation		
<21 years (578 visits)	0.67; 0.45–0.98	0.6
≥21 years (626 visits)	0.75; 0.53–1.06	
Smoking at follow-up		
Continued (788 visits)	0.62; 0.45–0.87	0.12
Quit (368 visits)	0.98; 0.61–1.55	

DISCUSSION

In a previous paper we reported a 28% reduction in common cold incidence with vitamin E supplementation in older city-dwelling men who smoked only 5–14 cigarettes per day (20). The present work was carried out to analyze whether the three characteristics specifying the small subgroup, i.e., age, smoking, and residential neighborhood, would cause a more general modification of the vitamin E effect. The current spline model analyses over age-at-follow-up seem to show that the reduction of common cold incidence with vitamin E in the previously identified small subgroup (20) is explained by its physiological effects rather than by a chance occurrence emerging from a series of subgroup analyses.

Age and smoking are plausible modifying factors for the effect of vitamin E on common cold incidence, but a biological rationale for the role of residential neighborhood as a modifying factor is not as apparent. Possibly higher level of air pollution or much more frequent use of public transport with concomitant exposure to infectious agents could explain the observed difference between cities and smaller communities.

Recently, a small trial with 617 elderly participants in long-term care facilities found a slightly lower incidence of colds among participants administered 200 mg per day of vitamin E (RR = 0.83; 95% CI: 0.68–1.01) (13). Another small trial with 652 elderly noninstitutionalized people found a slightly higher incidence of respiratory infection among participants administered 200 mg per day of vitamin E (RR = 1.12; 0.88–1.25), and a statistically significant increase in symptom severity, fever and restriction in activity (12). Although such divergence may result from the small size of the trials, it might also result from biological heterogeneity, as we found both increases and decreases in common cold risk with 50 mg per day of vitamin E supplementation in our current study, depending on the characteristics of the subgroup.

We found quite sharp peaks of increase in common cold risk at 54 and 62 years with vitamin E supplementation in two of our four subgroups (Fig. 1), both highly unlikely to be due to chance, although there is no apparent biological rationale for such peaks. Possibly the peaks may be related to social factors such as retirement, which in Finland occurs usually at about 58 to 60 years; however, retirement does not occur as such a sharp peak as seen in the spline models.

The modification of the vitamin E effect on the common cold risk by age, smoking, and residential neighborhood may be of more general interest as regards the physiological effects of antioxidants. There is evidence indicating that free radical production may be important in the emergence of various chronic diseases such as cancer and cardiovascular diseases (24,25) as well as in the pathogenesis of certain viral and bacterial diseases (26–28). It is sometimes assumed that antioxidants, including vitamin E, might have a consistent unidirectional broad-spectrum benefit on the human system by protecting it against the free radicals (24,25). Our finding that vitamin E supplementation significantly increases or decreases common cold risk depending on the three variables in question is inconsistent with the notion of uniform benefits from antioxidant supplementation.

In the current work we had available a very large number of outcomes (55,770 episodes of the common cold) which rendered it possible to analyze the age-dependence of the vitamin E effect in the four subgroups accurately. With severe diseases such as cancers or cardiovascular diseases, the statistical power is usually too small to permit analyses similar to the current spline models. Still, it is possible that comparable effect-modification occurs in the case of more serious diseases, even though directly extrapolating the particular modifying factors observed in this work to any other diseases is not justified. In a previous analysis of the ATBC Study cohort, we found that the effect of vitamin E on the risk of pneumonia was modified by the age of smoking initiation so that vitamin E reduced pneumonia risk in participants who began smoking at a later age, whereas vitamin E slightly increased the risk among participants who began smoking at an early age (14)

(see also Table 4). Thus, our findings for pneumonia risk also suggest substantial heterogeneity between population groups in the effects of vitamin E supplementation.

A recent meta-analysis focusing on the potential harm of vitamin E supplementation found that, starting from approximately 150 mg/day of vitamin E, there was increased mortality among people supplemented with vitamin E (29). However, it is possible that there is biological heterogeneity between population groups, so that people's characteristics may determine whether vitamin E supplementation caused net benefit or harm. In our current study, the vitamin E dose was 50 mg/day, which is substantially less than the estimated threshold level in the above-mentioned meta-analysis (29); however, our current analyses on common cold incidence and our previous analyses on pneumonia incidence make it seem probable that some population groups are harmed at levels of 50 mg/day, even though the same low dose seems beneficial for other population groups (14,15). Thus, it may be unjustifiable to assume that there is a single threshold level for harmful effects that is valid for the entire population. Another recent review on vitamin E safety concluded that supplements appear harmless for most adults in amounts up to 1 g/day (30), whereas our subgroup analyses indicate harmful effects on restricted population groups at doses as low as 50 mg/day (Tables 3 and 4).

The definition of a common cold episode in our study was based on self-diagnosis, which is usually reliable (22). Although subjective perception of what is classified as a cold varies between participants, such inaccuracy in outcome assessment does not lead to consistent differences between our double-blinded study arms; rather, the inaccuracy renders the differences smaller than they may actually be. Our implicit assumption in this work was that the effect of vitamin E is based on its reported effects on the immune system (5,6), but even if the mechanism of the effect of vitamin E would be on other factors that determine whether a person has subjective symptoms of the common cold, the conclusions of our double-blind trial are not affected. Furthermore, even though a proportion of the self-reported colds may be caused by non-infectious etiology, this does not affect the validity of our observation that this common set of symptoms seems to be affected differently with vitamin E in different subgroups of people.

The modification of the vitamin E effect on common cold risk also bears on the heterogeneity of findings in common cold trials examining vitamin C, the major water-soluble antioxidant, which interacts with lipid-soluble vitamin E (5,31,32). The largest vitamin C trials found no effect on the risk of the common cold; however, low dietary vitamin C intake and acute physical stress were proposed as modifying factors that may explain statistically significant reduction in common cold risk with vitamin C supplementation in several small trials (5,33,34). Thus, it seems possible that these two closely related antioxidants, vitamin E and vitamin C, may affect common cold risk in restricted groups of people, even though there seems to be no overall effect in the general Western population.

The main finding of our study is that vitamin E supplementation may cause benefit or harm to health depending on several modifying factors. It is premature to draw any practical conclusions from our study except that general caution should be maintained in public health recommendations on vitamin E supplementation until the effects of this vitamin are better understood. The possibility that vitamin E may reduce the risk of the ubiquitous common cold infection by half in some groups of elderly people would seem to warrant further study to define more precisely the population groups that might benefit from supplementation.

ACKNOWLEDGMENTS

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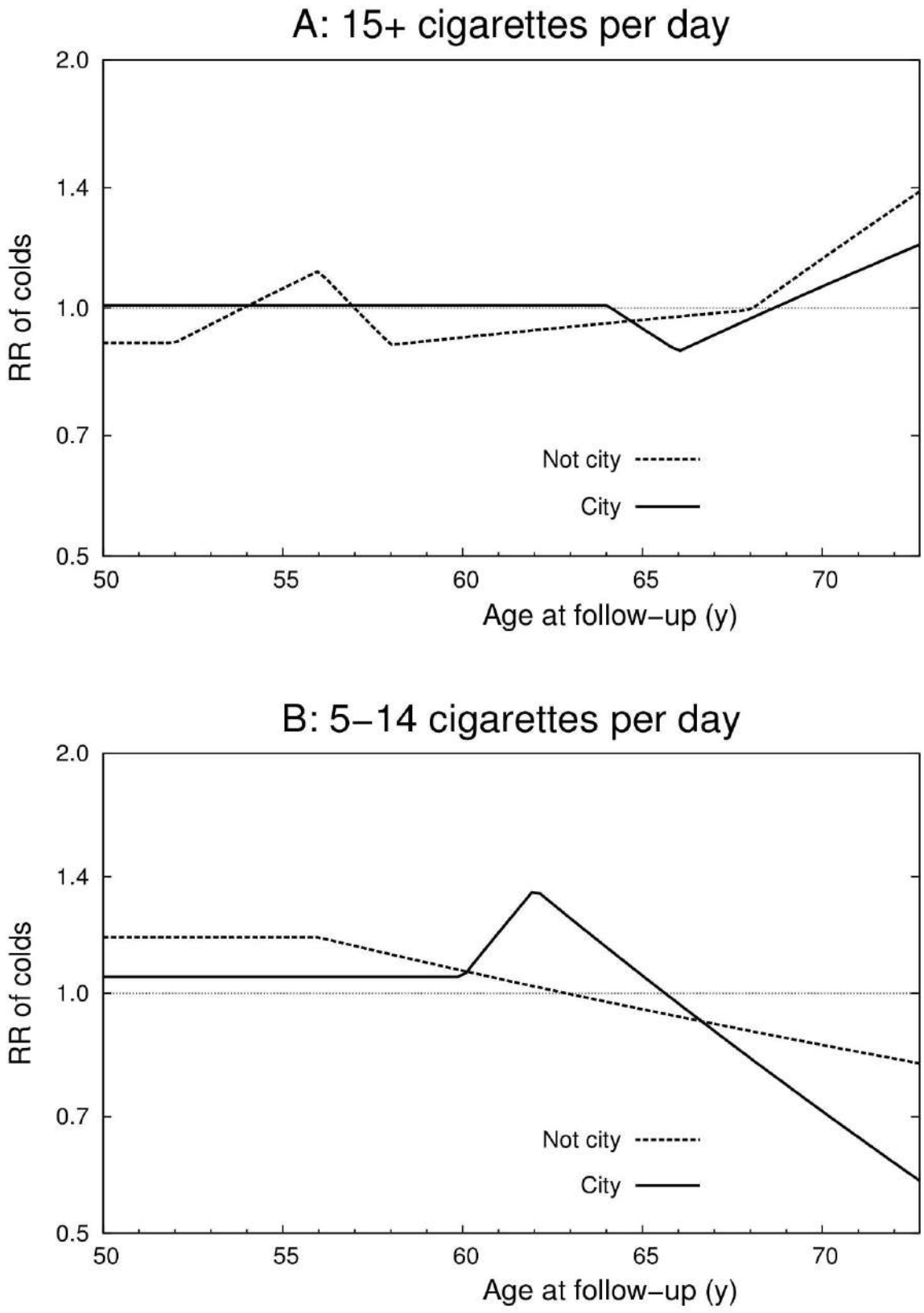

Fig. 1. The effect of vitamin E on the relative risk of common cold as a function of age at follow-up. Participants smoking more (A) and less (B) are further divided into subgroups by residential neighborhood. RR indicates the relative risk of colds between the vitamin E and placebo arms. See Table 2 for the description of the statistical models. These versions were redrawn in 2014.

Format: Abstract

Trop Geogr Med. 1980 Jun;32(2):132-7.

High dose ascorbic acid in Nigerian asthmatics.

Anah CO, Jarike LN, Baig HA.

Abstract

Forty-one asthmatic patients in remission were randomly allocated to two treatment groups in a double-blind trial. One group took 1 g, of ascorbic acid as one effervescent tablet once daily and the second group took a matching placebo. The asthmatics were selected from those attending the Asthma Clinic. One criterion for selection was the increase in exacerbation during the rainy season. These exacerbations were precipitated by respiratory infection. After 14 weeks, an assessment of the severity and rate of attacks showed that those on ascorbic acid suffered less severe and less frequent attacks of asthma during the study period. Plasma ascorbic acid astimations showed a significant rise in the level in those taking ascorbic acid over those on placebo. ($P < 0.01$). Cessation of ascorbic acid in the group taking it increased attack rates. It is concluded that high dose ascorbic acid is probably a good prophylaxis in some bronchial asthmatics.

PMID: 7423602

[Indexed for MEDLINE]

Publication types, MeSH terms, Substance

LinkOut - more resources

Scurvy: historically a plague of the sailor that remains a consideration in the modern intensive care unit.

Holley AD,
Osland E,
Barnes J,
Krishnan A,
Fraser JF

Author information

Internal Medicine Journal, 01 Mar 2011, 41(3):283-285
DOI: 10.1111/j.1445-5994.2010.02413.x PMID: 21426466

Abstract

We report the case of the case of a 56 year old female with sepsis on a background of rheumatoid arthritis and steroid use manifesting with overt clinical features of scurvy. Ascorbic acid assays were able to demonstrate severe deficiency and confirm a diagnosis of scurvy. Clinical resolution of signs and symptoms following commencement of vitamin C replacement was rapid. The intensivist and dietitian need to consider this diagnosis even in the first world setting, particularly in the presence of sepsis, inflammatory conditions, steroid use and importantly malnutrition.

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EMSID: UKMS1203
PMID: 15771570

How Neutrophils Kill Microbes

Anthony W. Segal

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Abstract

Neutrophils provide the first line of defense of the innate immune system by phagocytosing, killing, and digesting bacteria and fungi. Killing was previously believed to be accomplished by oxygen free radicals and other reactive oxygen species generated by the NADPH oxidase, and by oxidized halides produced by myeloperoxidase. We now know this is incorrect. The oxidase pumps electrons into the phagocytic vacuole, thereby inducing a charge across the membrane that must be compensated. The movement of compensating ions produces conditions in the vacuole conducive to microbial killing and digestion by enzymes released into the vacuole from the cytoplasmic granules.

Keywords: bacteria, protease, free radical, microbicidal, ion channel, enzyme

INTRODUCTION

Neutrophils are highly motile phagocytic cells that constitute the first line of defense of the innate immune system. They were first discovered by Elie Metchnikoff when he inserted rose thorns into starfish larvae and found that wandering mesodermal cells accumulated at the puncture site. He showed these cells to be phagocytic and described the larger cells as macrophagocytes, or macrophages, and the smaller as microphagocytes, now known as granulocytes, of which by far the most numerous are the neutrophils.

The ability of these cells to engulf and degrade bacteria was logically assumed to indicate a killing function. A microbicidal function was ascribed to the contents of their abundant cytoplasmic granules that were discharged into the phagocytic vacuole containing the microbe (1) (Figure 1). Attention was then directed toward the characterization of the granules by electron microscopy, fractionation, and biochemical analysis. Several of the purified granule proteins were shown to kill microbes.

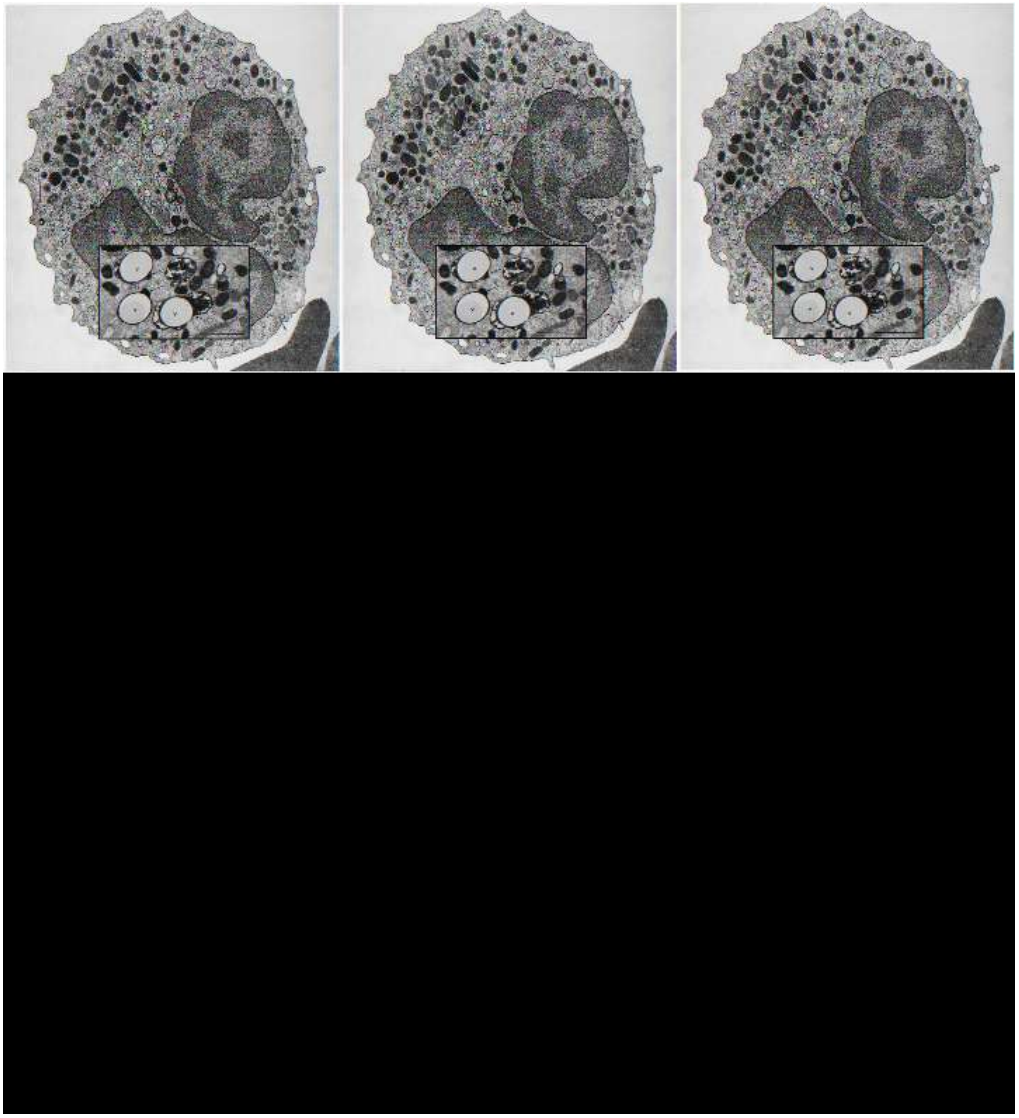

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Figure 1

Transmission electron micrograph of a human neutrophil. Inset is an image taken from a neutrophil 20 s after the phagocytosis of latex particles opsonized with IgG (V, vacuole). The section was stained for myeloperoxidase (MPO) to reveal the electron-dense product in the azurophil granules, some of which can be seen degranulating into the phagocytic vacuole (arrows). Bar = 1 μm. (Figure from 17.)

Parallel with studies into microbicidal activity of the granule contents, investigations were undertaken into the metabolism of phagocytosing neutrophils. The neutrophils demonstrated a significant “extra respiration of phagocytosis,” which was non-mitochondrial and was associated with a dramatic increase in turnover of the hexose monophosphate (HMP) shunt and the production of large amounts of H_2O_2 (2). These metabolic changes were shown to be essential for microbial killing.

In the late 1960s and early 1970s, a number of related discoveries cast a very different perspective on the killing process. Chronic granulomatous disease (CGD), a profound immunodeficiency to bacterial and fungal infections, was associated with failure of these metabolic changes (3). In addition, myeloperoxidase (MPO)-mediated halogenation, which is microbicidal in the test tube, was also defective in these patients (4).

Soon after its discovery in 1969, superoxide dismutase was used to show that activated neutrophils generate superoxide (5) and that this process is lacking in CGD. This important development provided a direct link between free radical chemistry and biology. At the time, most free radical chemistry was conducted by radiation biologists in test tubes, and its application to biology was purely theoretical. This new discovery was thought to prove that the production of free radical reactions in a biological process was toxic enough to kill organic structures as tough as bacteria and fungal spores. Soon these observations were extrapolated to implicate free radical reactions in a host of pathological processes involving neutrophil infiltration and tissue damage.

During the past few years, the pendulum has swung firmly back to implicating a major primary role for the granule proteins in the killing process (6), with a less direct but still facilitating and activating role for the respiratory burst through the NADPH oxidase. This review concentrates on the elucidation of these recent developments in our understanding of the relationship between the oxidase and granule enzyme activation. Because of the breadth of the subject and space limitations, references are made to authoritative reviews where available.

LIMITATIONS TO UNDERSTANDING KILLING SYSTEMS

Neutrophils are essential for resistance to bacterial and fungal infections. Severe neutropaenia invariably leads to infection by a wide range of organisms (7), most of which are not normally pathogenic, even in CGD. This, coupled with the fact that most CGD patients are able to kill most invading microbes most of the time (8), indicates that killing systems of the neutrophil are highly efficient and multilayered. Investigators once considered oxygen-dependent mechanisms essential for killing invading microbes, but such microbes can in fact be killed by other systems (9). In general, research has concentrated on determining those mechanisms involved in killing the most resistant organisms. The advent of gene-targeting technology allows researchers to determine the roles of the different antimicrobial molecules and their functional interrelationships with various microbes. Additionally, most studies have examined the killing of microbes within the phagocytic vacuole. We do not know whether neutrophils are capable of killing organisms extracellularly in vivo, nor the mechanisms involved if they are.

We have derived the bulk of our detailed information from the study of infection in CGD and the role of the oxidase in microbial killing. Because CGD patients can remain free of infection for many years (8), these methods are imprecise because they only measure some components of the lethal systems. Nonetheless, oxygen-dependent, intravacuolar killing provides a clearly defined set of processes, the examination of which has advanced knowledge of important physiological mechanisms.

THE NADPH OXIDASE

The NADPH oxidase plays a pivotal role in microbial killing because its dys-function causes CGD, characterized by a profound predisposition to bacterial and fungal infection (8, 10), and killing is compromised under anaerobic conditions (11).

Detailed reviews of the biochemistry and bioenergetics of this system have recently been undertaken (12, 13), to which I refer readers. A schematic representation of the oxidase is shown in Figure 2.

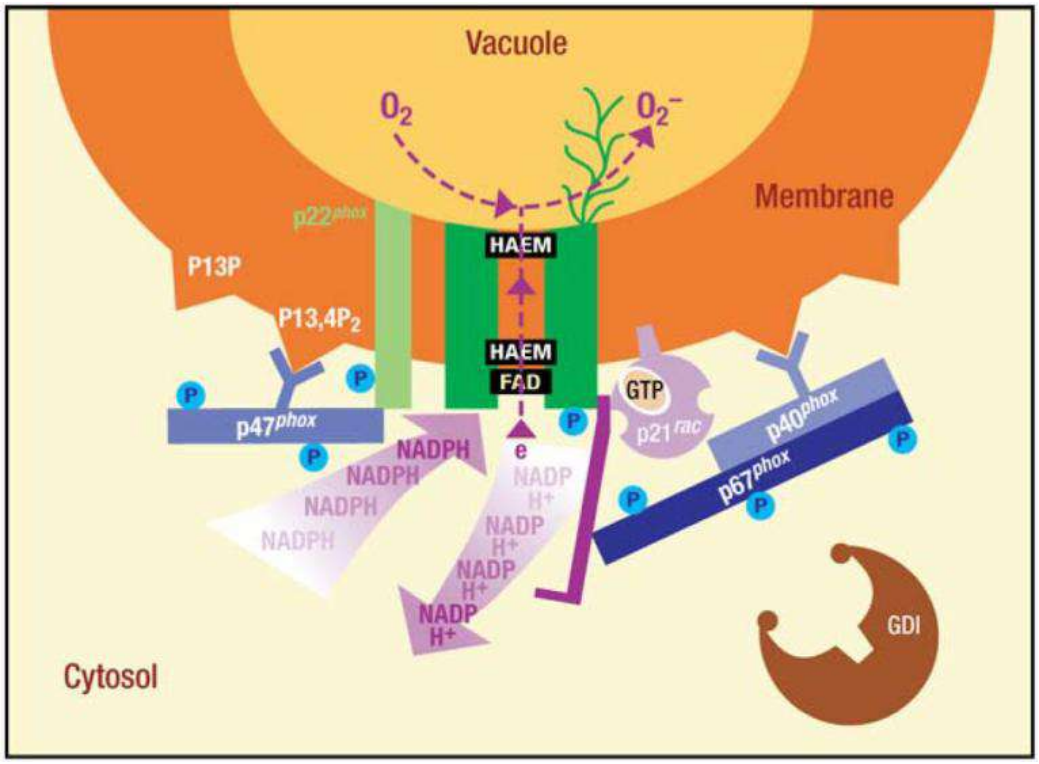

Figure 2

Schematic representation of the NADPH oxidase. Flavocytochrome b_{558} is a heterodimer of gp91^{phox}, which contains the haem- and flavin-binding sites, and p22^{phox}. Electron transport is activated by phosphorylation and translocation to the vacuolar membrane of p47^{phox} and p67^{phox}. p21^{rac}, in the GTP-bound form, is also required (12).

The Electron Transport Chain Through the Membrane

Flavocytochrome b_{558} is the core component of the NADPH oxidase. It is distributed between the plasma membrane and the membrane of the specific granules, and it is incorporated into the wall of the phagocytic vacuole, where it forms a conduit for electrons to be pumped from NADPH in the cytosol onto oxygen in the vacuole.

Flavocytochrome b_{558} is a heterodimer composed of one molecule of p22^{phox} (α-subunit, the product of the *CYBA* gene) and one molecule of gp91^{phox} (β-subunit, *CYBB* gene).

gp91^{phox}

gp91^{phox} contains the entire electron transporting machinery of the flavocytochrome b. It is composed of two major, and very different, domains.

C-Terminus: NADPH and FAD Binding The hydrophilic C-terminal (282–570) portion of gp91^{phox} contains the FAD- and NADPH-binding sites. These have distant, but recognizable homology to the large family of ferredoxin-NADP reductase (FNR) proteins, of which cytochrome P450 reductase, nitric oxide (NO) synthase, and yeast ferric reductase are members. This homology has allowed the construction of a model with the depiction of the FAD- and NADPH-binding sites.

N-Terminus: Haem Coordination The hydrophobic N-terminal half of gp91^{phox}contains six membrane-spanning α helices. Helices III and V each contain two histidine residues appropriately positioned (101:209 and 115:222) to coordinate two haem prosthetic groups perpendicular to the plane of the membrane. These histidine residues are completely conserved among all the NADPH OXIDASE (NOX) family members. Site-directed mutagenesis studies support the proposal that these histidine residues form the axial ligands to the haem groups. The predicted placing of the haem groups (one toward the inner face and one toward the outer face) is consistent with their function to transport electrons from the NADPH (via FAD) on the inside (cytosol) across the membrane to the interior of the phagocytic vacuole where molecular O_2 is reduced to form O_2^- . Biological membranes are ~25 Å thick, and thus at least two redox centers are required to span them to allow electrons to transfer at kinetically significant rates. The haem groups are nonequivalent and have different redox potentials.

The second (120–167) and third (224–257) external loops of gp91^{phox} contain the N-linked glycosylation sites (asparagines 132, 149, and 240).

p22^{phox} p22^{phox} is a 194 amino acid (~21 kDa) protein with a hydrophobic, membrane-spanning N-terminus (1-132). It provides high-affinity binding sites for the cytosolic NADPH oxidase subunits. p47^{phox} binds to a proline-rich domain (151–160) in the cytoplasmic hydrophilic C-terminus and confers stability on gp91^{phox}.

The Activating Proteins in the Cytosol

For electron transport to occur through the flavocytochrome, it must interact with a number of cytosolic proteins that translocate to the membrane of the phagocytic vacuole. This activation depends on a change in the conformation of the flavocytochrome, possibly by displacing the small helix that is predicted in the molecular model to occupy the NADPH-binding site in the inactive state (14) or through the facilitation of electron transfer between the flavin and haem.

Because of their interaction with each other, with lipids, and with phox proteins in the membranes, these cytosolic phox proteins have relatively large numbers of specific interaction domains. Targeting these molecules specifically to that region of the plasma membrane that makes up the wall of the vacuole requires specific local changes, which might include the accumulation of phosphatidylinositol phosphates (PIPs) at this site. Only a small proportion of these cytosolic proteins translocate to the membranes, and these appear to be phosphorylated, as does the flavocytochrome.

p67^{phox} p67^{phox} (NOXA2 from NOX Activator) is a 59,735-Da protein (526 amino acids) with a pI of 6.12. Protein-protein interaction domains include two SH3 domains, two proline-rich regions flanking the central SH3 domain, an N-terminal TPR (tetratricopeptide repeat), and a PB1 domain C-terminal to the central SH3 domain. The TPR domains are thought to bind rac. PB1 domains are known to interact with octicosapeptide motifs, and p67^{phox} binds to p40^{phox} through this domain. p67^{phox} attaches directly to flavocytochrome b_{558}, and at high concentration, in combination with rac or in the form of a p67$^{phox/rac}$ chimera, p67^{phox} is sufficient to induce electron transport.

p47^{phox} p47^{phox} (NOXO2 from NOX Organizer) is a basic protein (pI = 9.6) of molecular weight 44,681 Da (390 amino acids) that is heavily phosphorylated during neutrophil activation. It contains a number of well-defined motifs, including a PX domain (involved in phosphoinositide binding), two SH3 domains (involved in protein-protein interactions), and at least one proline-rich motif (the reciprocal target for SH3 domain interactions). It appears to be an adaptor molecule forming a bridge between p22^{phox} and p67^{phox}, and it also binds to cytoplasmic regions of gp91^{phox}, thereby stabilizing the attachment of p67^{phox} to flavocytochrome b_{558}.It might also directly influence the function of

flavocytochrome b_{558}. The N-terminal regions of $p40^{phox}$ and $p47^{phox}$ contain homologous stretches of 120–130 amino acids that form a structure called the phox homology, or PX domain, which binds to PIPs and directs these proteins to this activated membrane (reviewed in 15).

The two SH3 domains face each other to form a groove in which its C-terminal polybasic region fits. Investigators have suggested that this polybasic region is phosphorylated upon activation, releasing it from its auto-inhibitory role and making the groove accessible to bind the proline-rich tail in the C-terminal portion of $p22^{phox}$.

p40*phox* $p40^{phox}$ was discovered when it copurified with $p67^{phox}$, to which it is tightly bound. It is a protein of 39,039 Da (339 amino acids), strongly homologous with $p47^{phox}$, with an N-terminal PX domain, followed by an SH3 domain. Toward the C-terminus, there is an octicosapeptide repeat (also known as a PC domain) that seems to be involved in the binding of $p40^{phox}$ to $p67^{phox}$. The protein probably functions as a shuttle partner, transporting $p67^{phox}$, which does not contain a PX domain, to the membrane of the phagocytic vacuole by binding to PIPs.

p21rac After the discovery of $p47^{phox}$ and $p67^{phox}$, it became clear that they were not sufficient to reconstitute the active oxidase when combined with membranes. A third protein, a guanosine 5′-triphosphatase (GTP)-dependent factor, was shown to be rac1 or rac2 and was purified from cytosol. The causes of the separation of rac from its complex with guanine nucleotide dissociation inhibitors (GDI) in the cytosol are not known. Rac translocates to the membrane independently from $p67^{phox}$ and $p47^{phox}$. Its guanosine diphosphate (GDP) is probably exchanged for GTP on the membrane through the action of P-Rex1, a 185-kDa guanine nucleotide exchange factor (GEF) that is activated by phosphatidylinositol-3,4,5-trisphosphate and by the $\beta\gamma$ subunits of heterotrimeric G proteins.

Molecular Genetics of CGD

Defects in any one of four genes give rise to the known forms of CGD. *CYBB* (coding for $gp91^{phox}$, NOX2) is located on the X chromosome and accounts for about 65% of cases, almost exclusively in males (except in rare female carriers in whom there is extreme lyonization). The other three genes are all autosomal, with defects in *NCF1* ($p47^{phox}$ or NOXO2 protein), *NCF2* ($p67^{phox}$ or NOXA2), and *CYBA* ($p22^{phox}$), causing approximately 25%, 5%, and 5% of cases, respectively. No instances of CGD have been identified in which a lesion of $p40^{phox}$ is causal.

A small subgroup of CGD patients have what is known as “variant” CGD (16). In these cases there is partial loss of a protein or its function. Often as much as 10%, and up to 30% (H. Malech, personal communication), of normal oxidase activity can be measured.

PRODUCTS OF THE OXIDASE AND THEIR IMPLICATION IN MICROBIAL KILLING

Initiation of NADPH oxidase activity coincides with degranulation, with a lag phase of approximately 20 s (17). It occurs after closure of the vacuole and is limited to the plasma membrane comprising the vacuolar membrane (18). Thus, superoxide cannot be detected on the exterior of a phagocytosing cell (19, 20) unless engulfment is “frustrated” by an overwhelming excess of particles and vacuolar closure becomes impossible.

Because activity of the NADPH oxidase is essential for efficient microbial killing, investigators have focused attention on the products of the oxidase themselves as the lethal agents.

Oxygen radicals and their reaction products, collectively referred to as reactive oxygen species (ROS), are produced as a consequence of NADPH oxidase activity, which pumps superoxide (O_2^-) into the phagocytic vacuole. Because ROS can react with organic molecules, an enormous body of literature has developed that causally links ROS to the death of the microbe.

O_2^- and H_2O_2

The superoxide anion radical has been recognized in chemical systems for many years. Proof of its existence in biology followed the discovery of the enzymatic function of superoxide dismutase, which accelerates the dismutation of $2O_2^- \rightarrow O_2 + O_2^{2-}$ (21). Investigators (5) soon showed that neutrophils produce large amounts of O_2^-, estimated between approximately 1 (22) and 4 (6) M/l in the vacuole. The steady state concentration has been estimated to be in the μM range (22) because dismutation to H_2O_2 (2) is very rapid (23, pp. 60–61) under the prevailing conditions.

Experiments were performed that appeared to demonstrate the killing of microbes by O_2^- generated by xanthine oxidase (24, 25). It is not clear what, if any, ROS other than O_2^- and H_2O_2 (2) are produced in significant quantities in the vacuole.

$HO^\bullet$

O_2^- and H_2O_2 can combine to generate the highly reactive hydroxyl radical ($HO^\bullet$) via the Haber-Weiss reaction. This requires a metal such as iron in the Fenton reaction: $Fe^{2+} + H_2O_2 \rightarrow Fe^{3+} + HO^- + HO^\bullet$. $HO^\bullet$ has been measured in a broken cell preparation (26) and has been implicated as a microbicidal agent (27). These radicals are probably not found in intact cells (28) because lactoferrin, which is unsaturated in neutrophil granules (29, 30), inhibits the generation of $HO^\bullet$ (31) and other free radical reactions (29) by binding free copper and iron. The reaction between HOCl and O_2^- could produce $HO^\bullet$ but does not appear to do so (32).

Cobalt-based radicals could be produced by the Co in cyanocobalamin (33), but a binding protein, transcobalamin 2, present in specific granules, might be there to prevent this from occurring.

Ozone

It has recently been suggested that ozone generated by an antibody-based catalysis is involved in the killing of bacteria within neutrophils (34, 35). Doubt has been subsequently raised, however, on the specificity of the indicator used for ozone, which can apparently also detect O_2^- (36).

Myeloperoxidase-Mediated Halogenation

Myeloperoxidase (MPO) is a di-haem protein composed of two identical heterodimers. Each heterodimer is formed from the post-translational modification of a single polypeptide precursor. The two symmetric halves are linked by disulphide bonds between the two heavy chains. The covalently bound haem has a unique structure and exhibits unusual spectral properties that are responsible for its green color (37). MPO constitutes about 5% of the total neutrophil protein and is present in the cytoplasmic granules at very high concentrations. It makes up about 25% of the granule protein, and this achieves concentrations of about 100 mg/ml (1 mM) in the vacuole.

Investigators thought that this enzyme catalyzes the H_2O_2-dependent oxidation of halides that can react with and kill microbes. Experiments with the MPO-H_2O_2-halide system demonstrated that this enzyme can kill bacteria in the test tube (22, 38-41), and MPO-mediated halogenation has been accepted as an important antimicrobial mechanism for several decades.

A few patients were discovered whose neutrophils lacked MPO and who were also thought to be immunodeficient (42). Recently MPO knockout mice have also shown an undue susceptibility to bacterial and fungal infections (43-45).

Nitric Oxide

Although evidence suggests that neutrophils can induce the synthesis of nitric oxide (NO) synthase during sepsis (46), little evidence implicates the involvement of NO in microbial killing. Even in mice, in the neutrophils of which NO synthase is expressed at much higher levels than in humans, knocking out this molecule has little effect on the killing of microbes for which neutrophils are normally responsible. In contrast, these mice are profoundly susceptible to intracellular organisms such as *S. enterica* and *M. tuberculosis* (47), which classically proliferate within macrophages.

CYTOPLASMIC GRANULES AND THEIR CONTENTS

Researchers have known for almost a century that neutrophils phagocytose and kill microbes. Alexander Fleming discovered and named lysozyme, which he termed "a remarkable bacteriolytic element found in tissues and secretions," including leukocytes (48). He showed that it lysed about two thirds of the bacteria he mixed with it. Researchers subsequently showed that phagocytosis was associated with discharge of the cytoplasmic granules into the vacuole (1) (Figure 1). Attention then focused on microbicidal components within these granules. The first microbicidal granule extract was called phagocytin (49), which was later shown to be composed of an array of cationic antibacterial proteins (50).

Substantial reviews have recently covered this subject (51, 52). Different subsets of granules have been characterized by electron microscopy (53), by various staining techniques, by cell fractionation (54), and by their different functions. There are two predominant types of granules, the azurophil and the specific. They are produced in the promyelocytic and myelocytic stages, and their contents depend on the proteins that are being synthesized at that time as well as on the presence of appropriate signaling peptides (51, 52). The granules also differ in their primary functions, as discussed below.

Azurophil (or Primary) Granules

The azurophils largely contain proteins and peptides directed toward microbial killing and digestion, whereas the specific granules replenish membrane components and help to limit free radical reactions. Azurophil (or primary) granules are the first to be produced. They contain MPO and three predominant neutral proteinases: cathepsin G, elastase, and proteinase 3. Bactericidal/permeability-increasing protein (BPI) was first purified as a factor that permeabilized and killed *E. coli* (55, 56). It has lipopolysaccharide-binding and neutralizing activities (57) and appears to be attached to the granule membrane. Defensins are peptides with molecular weights of 3000–4000 Da, and each contains six disulphide-linked cysteines (58). They exhibit antibacterial activity, but this is inhibited by physiological concentrations of salt. About one third of the total lysozyme (54) is found in these granules.

These granules contain an abundant matrix composed of strongly negatively charged sulphated proteoglycans (59). This matrix strongly binds almost all the peptides and proteins other than lysozyme, which are strongly cationic. This sequestration together with the acidic pH at which the granule interior is maintained (60) keeps these enzymes in a quiescent, inactivated state.

Specific (or Secondary) Granules

Specific granules contain unsaturated (61) lactoferrin, which binds and sequesters iron and copper; transcobalamin II, which binds cyanocobalamin; about two thirds of the lysozyme (54); neutrophil gelatinase-associated lipocalin (62); and a number of membrane proteins also present in the plasma membrane, including flavocytochrome b_{558} of the NADPH oxidase (63).

Gelatinase (or Tertiary) Granules

Some granules contain gelatinase in the absence of lactoferrin, although most of the lactoferrin-containing specific granules also contain gelatinase (64). The designation of granules as "gelatinase granule" refers to granules that contain gelatinase but not lactoferrin; they may represent one end of the spectrum of a single type of granule with the same contents but in differing proportions.

Lysosomes

Lysosomes contain acid hydrolases. The activity of these enzymes appears to fractionate with the azurophil granules. They are, however, released into the phagocytic vacuole much later than the azurophil contents and therefore must be in a distinct compartment (17).

Secretory Vesicles

These endocytic vesicles contain serum albumin (65) and are probably the empty vesicular structures described previously (66). They provide a valuable reservoir of membrane components. Their reassociation with the plasma membrane replenishes that which is consumed during phagocytosis, as well as its component proteins such as complement receptor (67) and flavocytochrome b_{558}.

CONDITIONS IN THE PHAGOCYTIC VACUOLE

One must clearly understand the conditions in the phagocytic vacuole when attempting to define killing mechanisms. A heavily opsonized particle is taken up into the phagocytic vacuole within 20 s (17, 68), and killing is almost immediate (68). The apparent delay in many assays results from a low collision frequency between neutrophils and microbes, which is due to low densities of both, coupled with slow mixing (69) and suboptimal opsonization.

To determine the concentration of the vacuolar contents, one must know the volume of the space between the surface of the organism and the membrane of the phagocytic vacuole. It is certainly very small (17) (Figure 1), and possibly negligible, as has been shown in macrophages (70).

The human neutrophil has numerous granules, the contents of which are released into the vacuole and squeezed onto the surface of the organism in very high concentrations, almost like attaching a limpet mine to a target (17). Researchers have estimated that the granule protein makes up about 40% of the vacuolar volume (22), achieving protein concentrations of about 500 mg/ml (6). It was initially thought that the specific granules degranulated first, followed by the azurophils. These studies were conducted on rabbit neutrophils, and alkaline phosphatase, which we now know to be a marker for membranes, was used as the marker for the specific granules (71). In fact, both of these granule types fuse with the phagocytic vacuole with roughly similar kinetics approximately 20 s after particle uptake (17). The acid hydrolases only enter the vacuole after about 5 min, when the pH has started to fall to levels appropriate for the optimal activity of these enzymes.

Investigators had initially reported that the pH in the vacuole fell to about 6 after 3 min and to 4 after 6 min (72). However, subsequent studies have shown that the NADPH oxidase elevates the pH to about 7.8–8.0 in the first 3 min after phagocytosis, after which it gradually falls to about 7.0 after 10–15 min

(68, 73, 74). The NADPH oxidase consumes 0.2 fmols of O_2 when a particle the size of a bacterium is engulfed. This equates to massive amounts of O_2^-, on the order of 1–4 Mols/l, that are injected into the vacuole.

NEUTRAL PROTEASES ARE ESSENTIAL FOR BACTERIAL AND FUNGAL KILLING

Although the proposal that ROS are toxic to ingested microbes was attractive, it was never adequately tested under the conditions pertaining to the phagocytic vacuole. The opportunity was provided by the development of gene targeting. This technique allowed the production of a mouse model that lacks the major neutrophil proteases: neutrophil elastase (NE) (6, 75), cathepsin G (6), or both enzymes (6, 76, 77) (Figure 3).

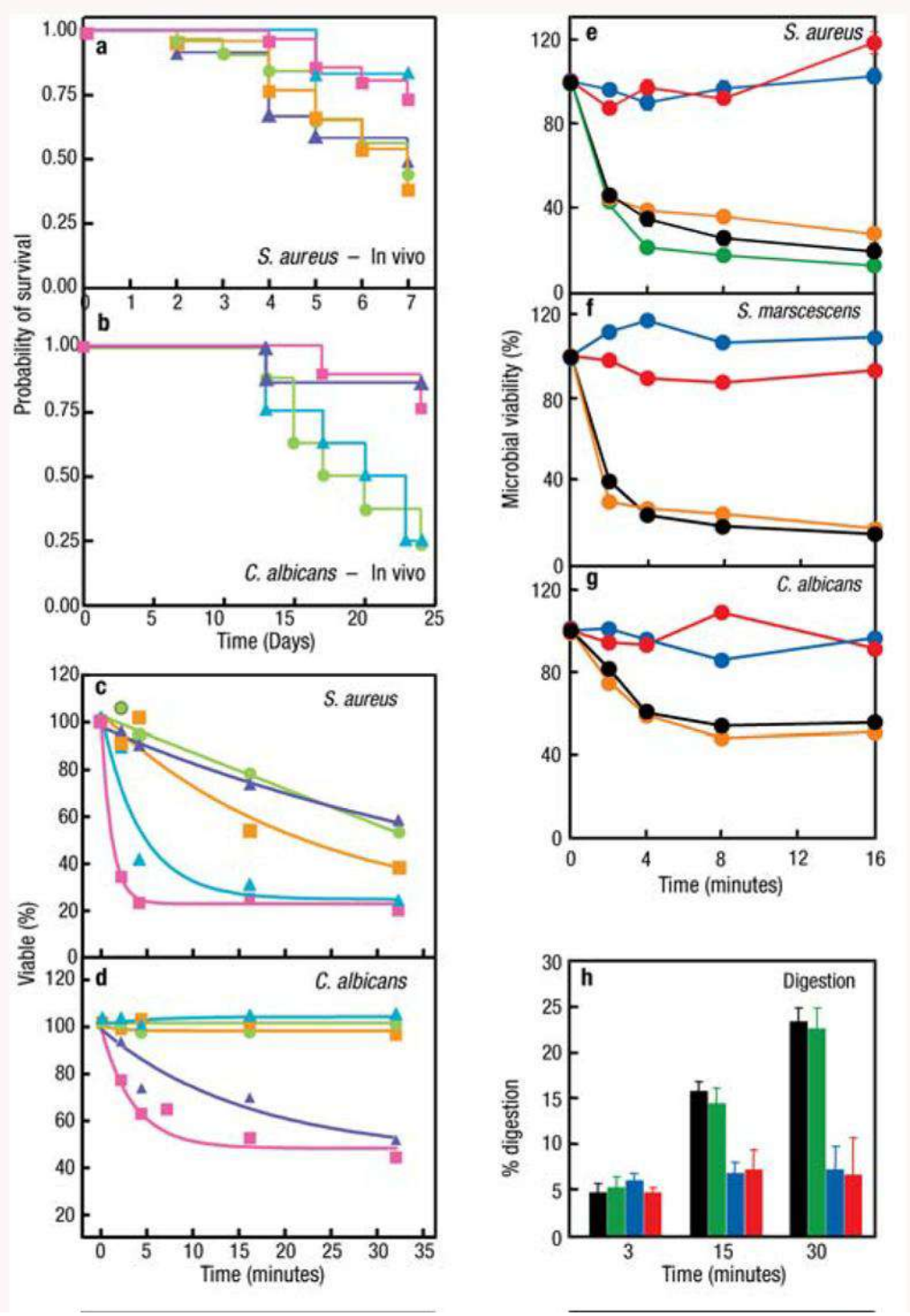

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Figure 3

The neutral proteases elastase and cathepsin G as well as K^+ flux are required for microbial killing and digestion by neutrophils. Cathepsin G, neutrophil elastase (NE), and $p47^{phox}$ (CGD) knockout mice are susceptible to *S. aureus* (*a*) and *C. albicans* (*b*) in vivo, and their neutrophils kill these organisms poorly in the test tube (*c*) and (*d*) (adapted from 6). Inhibition of the BK_{Ca} K^+ channel with specific inhibitors

paxilline (PAX) and iberiotoxin (IBTX) prevents killing of *S. aureus* (*e*), *S. marscescens* (*f*), and *C. albicans* (*g*) by neutrophils, whereas the opener NS1619 and nonspecific inhibitor 4-aminopyridine were without effect. The BK_{Ca} K^+ channel blockers also inhibited digestion of radiolabeled, killed *S. aureus* (*h*) (adapted from 74). Neither the loss of the proteases nor blockage of the BK_{Ca} channel affected phagocytosis, oxidase activity, or iodination.

NE-deficient mice were excessively susceptible to infection with Gram-negative (*K. pneumoniae* and *E. coli*) (75) but not Gram-positive (*S. aureus*) bacteria. NE was also necessary for protection against *C. albicans* (6). Both enzymes were required to kill *A. fumigatus*. The loss of cathepsin G alone was found by others (77) to be without effect on the killing of various of bacteria. The loss of both NE and cathepsin G conferred as profound a defect of bacterial killing as was observed with the CGD mouse model (6).

In these studies on protease-deficient mice, microbial killing was abolished despite a completely normal respiratory burst and normal levels of iodination. This established that ROS and metabolites of the action of MPO generated in the vacuole are not sufficient to kill these bacteria and fungi.

Thus, it was clear that the combination of NADPH oxidase activity and neutral protease enzymes are require for microbial killing to take place. This raises the question of the connection between these two processes.

THE RELATIONSHIP BETWEEN THE NADPH OXIDASE AND KILLING BY GRANULE CONTENTS

Activity of the NADPH Oxidase Alters the Appearance of the Contents of the Phagocytic Vacuole

The activity of the NADPH oxidase alters the appearance of the contents of phagocytic vacuoles in electron micrographs of neutrophils examined soon after they had phagocytosed bacteria (6). In normal cells, the contents of the vacuole had a diffuse, almost ground-glass appearance, with very few intact aggregates of granule contents. By contrast, in CGD cells there was little dispersion, with obvious clumping of the granular contents. This abnormal appearance was also apparent in vacuoles from a patient with variant CGD with 10% of the normal oxidase activity.

These obvious structural differences, coupled with the massive amounts of O_2^- injected into the vacuole and the fact that 10% of this amount of O_2^- in variant CGD (amounting to some 100–400 mMols/l) was insufficient, suggested to researchers that the oxidase was exerting some physico-chemical influence on the granule contents rather than simply producing ROS or substrate for MPO. Segal and colleagues (6) therefore turned their attention to electron transport across the membrane and its consequences for the movement of other ions.

Charge Compensation Across the Vacuolar Wall

The oxidase is electrogenic, transferring electrons, unaccompanied by protons, across the vacuolar membrane (78-81). The vacuolar volume is about 0.2 μm^3, with a membrane surface area of about 1.65 μm^2.In each vacuole, 0.8–2.0 fmols of O_2^- are produced, and thus about 5–10 × 10^8 electrons pass across each μ^2 of membrane. The charge on one electron is 1.6 × 10^{-19} coulombs, so 3–7 × 10^8 charges in one square micron would produce from 4.6 × 10^{-3} to 1.2 × 10^{-2} coulombs/cm^2. With the capacitance of the membrane at approximately 1 microfarad/cm^2 (82), this charge would depolarize the

membrane potential by 4,600–11,700 volts! Depolarization of the membrane to +190 mV shuts down NADPH oxidase activity completely (83). Thus, for significant oxidase activity to occur, the charge must be compensated.

The changes in the vacuolar pH, which is elevated from that of the extracellular medium to 7.8–8.0 (68) despite the release into the vacuole of 500 mg/ml of acidic granule protein contents (6), hold the key to understanding the nature of the compensating ions (Figure 4). These granule contents are maintained at pH 5.0 in the granule by a proton pump (60) and have strong buffering powers. About 400 μmol potassium hydroxide is required per gram of granule protein to elevate the pH from 5.0 to 8.0 (6).

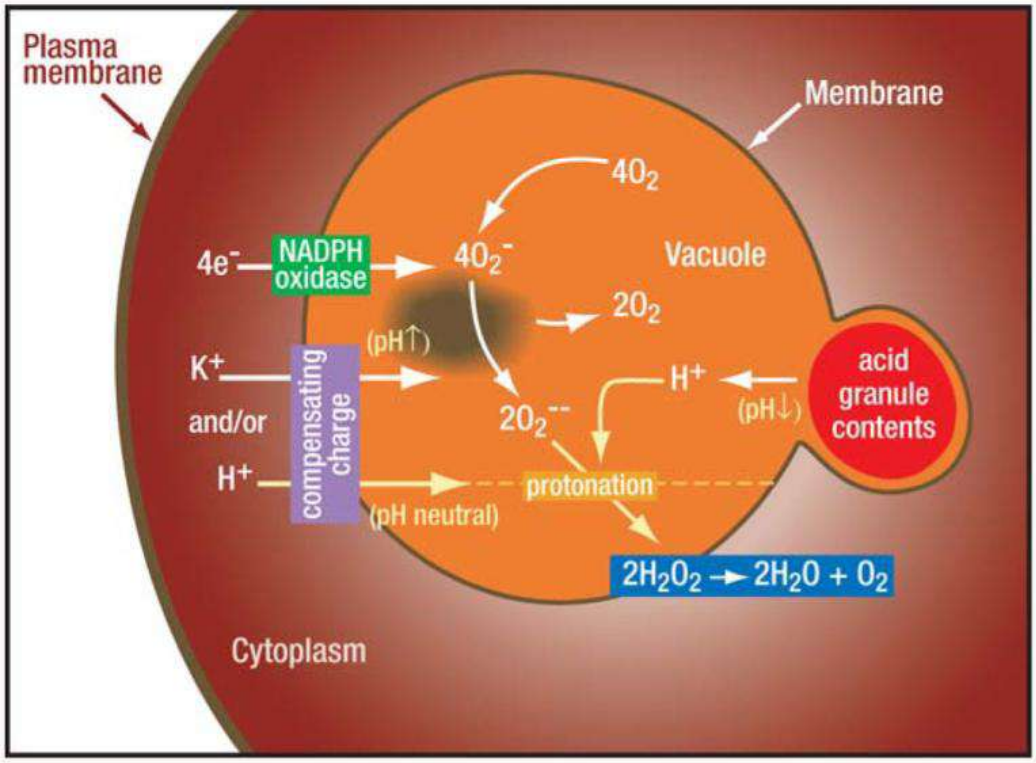

Figure 4

Activity of the NADPH oxidase depolarizes the membrane. The nature of the compensating charge governs the changes in vacuolar pH and tonicity. Electrons are transported across the vacuolar membrane to form O_2^-, which dismutates to O_2^{2-}. O_2^- and O_2^{2-} become protonated to form HO_2 and H_2O_2, thereby consuming protons and elevating the pH in the vacuole despite the entry of acidic granule contents. This process can only occur if part of the charge is compensated by ions other than protons, which in part occurs through the passage of K^+ ions (6, 74).

The vacuole becomes alkaline despite the entry of acidic granule contents, indicating that the O_2^- and O_2^{2-} are consuming protons in the vacuole. This would not happen if each electron passing across the membrane was accompanied by a proton, demonstrating that compensating charges cannot be solely in the form of H^+ from the cytoplasm.

The major cation in the cytoplasm is K^+, which accumulates in the vacuole at concentrations of up to about 600 mM as a consequence of oxidase activity (6). Transport of K^+ ions is markedly diminished when the pH rises above 8.0, indicating that the K^+ channel provides an important self-regulating mechanism for elevating the vacuolar pH while also ensuring that it does not go too high.

K^+ flux only accounts for about 6% of the compensating charge (6). The putative proton channel discussed below does not appear to compensate for all the rest of the charge because its inhibition with Zn^{2+} and Cd^{2+} fails to block the NADPH oxidase (74). Therefore, some other major ion flux must also be involved. As is described below, this is accomplished by the flux of chloride ions through a glycine-gated, strychnine-sensitive channel.

The K^+ Enters the Phagocytic Vacuole Through BK_{Ca} Channels

K^+ enters the vacuole through the large conductance Ca^{2+}-activated K^+ channel (74). Iberiotoxin (IBTX) and paxilline (PAX), both highly selective and potent inhibitors of this channel (84, 85), prevent the alkalinization of the vacuole, confirming the importance of the influx of K^+ into the vacuole on alkalinization of this compartment. The IC_{50} values for this effect were in the region of 10 nM for IBTX and PAX, consistent with their IC_{50} for channel block. In addition, the BK_{Ca}channel opener, NS1619 (86), significantly augmented the rise in pH to supranormal levels. A variety of blockers and openers of other K^+ channels were without effect.

$^{86}Rb^+$ release from activated neutrophils after stimulation with phorbol myristate acetate (PMA) was also induced by NS1619 and even further enhanced by the combination of this opener and PMA. PMA-induced and NS1619-induced efflux were both completely abrogated by IBTX and PAX. The same was found to apply to eosinophils.

BK_{Ca} channels are classically opened by the combination of membrane depolarization and elevated cytosolic Ca^{2+} (87). The same holds true for this channel in neutrophils and eosinophils. Neither depolarizing the membrane nor elevating the cytosolic Ca^{2+} was sufficient to fully open the K^+ channel, whereas the combination of the two caused as much channel opening as did stimulation with PMA. Although PMA stimulation is well known to depolarize the neutrophil plasma membrane (88), it is generally thought not to elevate cytosolic Ca^{2+}. One mechanism by which this might occur is through a drop in pH just beneath the plasma membrane as a consequence of charge separation induced by the oxidase. Corresponding elevations in Ca^{2+} and falls in pH were seen just beneath the plasma membrane in activated cells (74).

Charge Compensation by Protons

Protons remain in the cytoplasm as a result of charge separation, which occurs when the electrons are transported from NADPH across the wall of the phagocytic vacuole. Additional protons are produced in the cytosol by the HMP shunt, which generates NADPH (89), as well as during the production of energy by glycolysis. This proton generation by an active oxidase, estimated to be about 150 mMols/l (90), causes an initial slight fall in cytosolic pH that rapidly returns to normal.

Three mechanisms appear to be associated with the extrusion of these protons, which are extruded in roughly equimolar quantities with the O_2^- that is generated (91, 92). The predominant one is a Na^+/H^+ antiport (93, 94). Its inhibition by the removal of extracellular Na^+ or blockage with amiloride causes acidification of the cytosol upon stimulation of the cells. In addition, both Zn^{2+} and Cd^{2+}-sensitive proton channels (95, 96) and vacuolar (V)-type H^+ pumps, inhibited by bafilomycins (90), are also present.

Investigators generally agree that the charge induced by electron translocation (I_e) through the NADPH oxidase is compensated by proton efflux (78, 83, 97), although the identity of the proposed channel is currently highly contentious. One school of thought holds that protons pass through voltage-gated proton channels that are distinct from any NADPH oxidase component (98). The opposing view is that they pass through flavocytochrome b_{558} of the oxidase, gp91^{phox}, itself (99-101).

One of the hallmarks of the assumption that I_e is largely compensated by proton fluxes is that both Zn^{2+} and Cd^{2+}, known proton channel blockers (98, 102, 103), were also thought to inhibit O_2^- production (83, 97). The discrepancy between the low μM concentrations of these cations that block proton channels and the mM concentrations needed to inhibit cytochrome c reduction was recently explained by the voltage dependence of I_e. Zn^{2+} and Cd^{2+} shift the threshold voltage for activating voltage-gated proton channels into the steeply voltage-dependent region of I_e, thereby attenuating O_2^- production (83).

However, Zn^{2+} and Cd^{22+} inhibition of voltage-gated proton channels do not inhibit the NADPH oxidase: They have no effect on PMA-induced oxygen consumption, the true measure of oxidase activity. Zn^{2+} and Cd^{2+} interfere with the reduction of cytochrome c by accelerating the dismutation of O^{2-} to H_2O_2 (74). In a system in which xanthine-xanthine oxidase generated O_2^- , 3 mM concentrations of these elements induced the dismutation of O_2^- to H_2O_2 at a rate indistinguishable from that catalyzed by superoxide dismutase (1 μg/ml). Zn^{2+}, at concentrations three orders of magnitude greater than those causing almost complete blockage to proton channels, was also without effect on the currents measured in electrophysiological studies performed on neutrophils, eosinophils, or on PMA-induced ^{86}Rb efflux from these cells (74). This does not mean that H^+ movement through proton channels does not compensate some of the charge, but only that the justification hitherto provided is incorrect.

Charge Compensation by Cl^-

We showed that K^+ accounts for only about 5%–10% of the compensation of the total electron transport, and, contrary to the description in a recent critique of our work (104), we never claimed that it was the only compensating ion. More recently, we (J. Ahluwalia, G. Gabella, S. Pope, A. Warley, A. Segal, unpublished) have discovered that that Cl^-, passing through strychnine-sensitive, glycine-activated homomeric channels, compensates about 90% of the charge. These channels were characterized by patch clamping whole cells and isolated phagocytic vacuoles, and by Western blotting. The removal of Cl^- or the blockage of this channel abolished both the respiratory burst and microbial killing. High concentrations of Cl^- and glycine required for the optimal function of these channels are contained within the cytoplasmic granules, which empty into the vacuole. NADPH oxidase activity was lost when the granules were removed and regained when Cl^- was reintroduced into the vacuole. Lysozyme, cathepsin G, and elastase were inactivated by hypertonic Cl^-, the removal of which would be important for their function. These Cl^- fluxes provide a direct couple between the extent of degranulation and oxidase activity required to activate the released enzymes.

The Movement of K^+ into the Vacuole Activates NE and Cathepsin G

The contents of the cytoplasmic azurophil granules are not freely in solution. They are almost exclusively highly cationic proteins that are strongly bound to the highly negatively charged proteoglycans heparin and chondroitin sulphate (59), in which state they are inactive. They are activated in the vacuole both by the elevation in pH described above and by the hypertonic K^+. The latter breaks the charged interaction between the enzymes and the matrix, releasing them in a soluble

form (6) (Figure 5). For these hypertonic conditions to develop, water must be prevented from entering the vacuole in response to the osmotic attraction of the salts. This is achieved by encasing the vacuole in a meshwork of cytoskeletal proteins, including paxillin and vinculin.

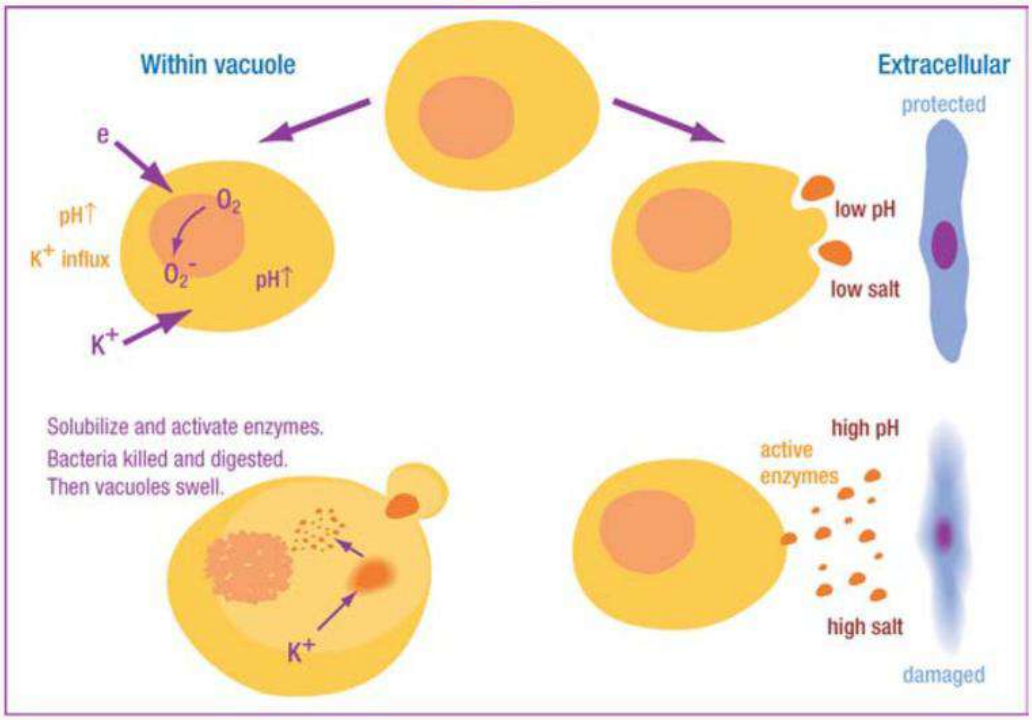

Figure 5

Schematic representation of interaction between NADPH oxidase and granule proteases. Electron transport through flavocytochrome b_{558} consumes protons in the vacuole, elevating pH to a level optimal for neutral proteases, which are also activated by K^+ driven into the vacuole to compensate the charge across the membrane. The hypertonic K^+ solubilizes the cationic granule proteases and peptides by displacing them from the anionic sulphated proteoglycan granule matrix. The requirement for an alkaline, hypertonic environment restricts the toxicity of these proteins to the vacuolar compartment, thereby limiting damage to normal tissues.

The importance of the accumulation of K^+ in the vacuole was shown when this was diminished either with the K^+ ionophore valinomycin (6), or by blocking the BK_{Ca} channel with the specific inhibitors IBTX or PAX (74). In both cases, microbial killing and digestion was almost completely prevented (Figure 3) despite the generation of normal quantities of ROS and normal levels of iodination.

Why Was the Importance of Granule Contents in the Killing Process so Overshadowed by ROS and MPO-Mediated Halogenation?

The theory that microbes are killed within the phagocytic vacuole by ROS had fertile ground on which to develop. The lack of production of O_2^- and H_2O_2 in anaerobic cells and in CGD with impaired killing under these conditions supported this theory (3, 11), as did the concept of toxicity engendered in the name “reactive oxygen species.” Although experiments were performed in support of these ideas,

the conditions under which they were performed in no way reflected the conditions pertaining in the vacuole. They were often done at the wrong pH, and never in the presence of the enormously high concentrations of protein that occur naturally.

O_2^-

Initial studies claimed that killing occurred by O_2^- generated by the reaction of xanthine with xanthine oxidase, but in fact in those experiments the microbes were killed in the absence of the substrate xanthine, and killing was not inhibited by superoxide dismutase (24). In a similar experiment, no killing of bacteria by O_2^- was observed after 15 min (25).

H_2O_2

H_2O_2, which is used as a topical antiseptic (105), is produced by neutrophils and has been thought of as capable of killing microbes within them (106, 107). Supportive evidence was provided by the finding that catalase-negative organisms rarely infect patients with CGD (108). The explanation was that these bacteria generated enough H_2O_2 to catalyze their own MPO-mediated halogenation within the vacuole of the neutrophil (109, 110). In vitro mutagenesis was used to generate strains of *S. aureus* containing varying levels of catalase, and their virulence in mice was found to be inversely proportional to their catalase content (111). Recently, however, doubts have been cast on this theory. Catalase-deficient *A. nidulans* (112) and *S. aureus* (113) are as virulent as the catalase-positive varieties in mouse models of CGD, and the bacteria could never come near to producing the relatively enormous quantities of H_2O_2 generated even by cells from patients with variant CGD.

When glucose oxidase was administered to CGD cells in liposomes, it appeared to correct the killing defect (114, 115). However, no explanation was provided as to how glucose would gain access to the vacuole in adequate amounts to generate sufficient quantities of H_2O_2, and the killing of bacteria in the extracellular medium was not excluded.

MPO

Experiments that demonstrated that the MPO-H_2O_2-halide system can kill bacteria in the test tube (22, 38-41) were conducted under nonphysiological conditions, with relatively low concentrations of MPO (50 μg/ml rather than 100 mgs/ml), at low pH (5.0 rather than 7.8–8.0), and, most important of all, in the absence of the high levels of proteins (approximately 500 mgs/ml) found in the vacuole. When bacteria were exposed to 100 mM H_2O_2 or 1 mM HOCl in the presence of 25 mg/ml granule proteins (technically much more manageable than the experimentally determined 500 mg/ml), killing was almost abolished (116).

Neutrophils clearly iodinate and chlorinate proteins when bacteria are phagocytosed, and this halogenation is dependent on an active NADPH oxidase and MPO (118). However, it is largely the proteins of the neutrophil granule rather than the microbial proteins that are iodinated (116, 119) and chlorinated (120), a highly inefficient system if its primary purpose is to halogenate bacterial proteins. Further indications as to the inefficiency of the proposed system come from the amounts of H_2O_2 generated. It seems highly unlikely that substrate would need to be provided at molar concentrations and that the 100 mM H_2O_2 produced by patients with variant CGD would be insufficient when it is effective at 50 μM in the test tube (38).

A few patients were discovered whose neutrophils lacked MPO who were also thought to be immunodeficient (42), and an MPO knockout mouse was shown to be susceptible to yeast but not bacterial infection (45). However, the advent of automated differential leukocyte counting machines, in

which the identification of neutrophils depended on a peroxidase stain, revealed that about 1 in 2000 of the general population are MPO-deficient without any undue predisposition to infection (121). The neutrophils of birds also lack MPO (122).

One possible function of MPO is to protect the digestive enzymes from oxidative denaturation (123) by removing H_2O_2 from the phagocytic vacuole. MPO has catalase activity (124), but this only functions efficiently if the compound II that accumulates is reduced back to the native enzyme. This reduction can be achieved by the high concentrations of O_2^- in the vacuole with which MPO forms an adduct to produce compound III (125). The impaired microbial killing observed in the MPO knockout mouse (126) could result from oxidative inactivation of antimicrobial proteins by the H_2O_2 that accumulates under these conditions (106).

MPO may also have dual functions, one as a catalase under the conditions pertaining in the vacuole, but another in a microbicidal capacity outside the cell where enzyme and substrate is much more dilute, and the pH, which is generally low at sites of infection and inflammation, is more conducive to halogenation reactions.

CONCLUDING REMARKS AND PERSPECTIVES

The complexity of the NADPH oxidase and its associated ion fluxes might seem excessive for the apparently simple purpose of activating enzymes within the phagosome. These enzymes, however, have the potential to be highly destructive to normal tissues, and yet organs housing the most exuberant inflammation and neutrophil infiltration can undergo resolution and return completely to normal a week or two later. Some of the neutrophil are removed by apoptosis, but many also necrose with the resultant release of their granules. The requirement of the combination of hypertonicity and alkalinity, neither of which occurs naturally in inflammatory foci, for the activation of these enzymes severely limits the toxicity of granules released into the tissues (Figure 5).

The demonstration that ROS and MPO-mediated halogenation are not the primary killing systems they were long believed to be has reopened many questions relating to mechanisms of innate immunity in the neutrophil. The roles of the different granule constituents in the killing and digestion of specific organisms is of interest, as are the consequences of the interaction of ROS with these granule contents on their biophysical, biochemical, and hence antimicrobial properties.

A number of problems still need to be resolved to clarify the mechanisms involved in charge compensation across the vacuolar membrane. These include the relationship between the channels conducting these charges and electron transport through flavocytochrome b_{558} and the mechanisms responsible for activating, regulating, and integrating the fluxes of these different ions.

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PHYSIOLOGICAL REVIEWS

VOL. XIV JULY, 1934 No. 3

THE INFLUENCE OF NUTRITION UPON RESISTANCE TO INFECTION

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The possibility that diet may have some influence upon the incidence, course, and final outcome of infection, is a comparatively recent idea. Since 1900 the idea has gained ground, and quite a body of work has appeared in the literature. The task of reviewing it is not easy for several reasons: in many cases the results are contradictory, in others they may be difficult of interpretation because of many variables. At best the literature is a scattered one. In considering the actual infection, the author has confined himself to infections of bacterial origin, and has not included, for lack of space, much excellent and suggestive work on infections of protozoan and metazoan origin.

In general one may say that the work in this field is in its infancy, but that there is much suggestive work that merits further study.

Vitamin B complex. Petragnani (1921) claimed that pigeons, fed on polished rice, lose their immunity, both natural and acquired, to anthrax, even before symptoms of polyneuritis develop. Corda (1923) believes that this loss of immunity may not be due to deficiency of vitamin B, but may in part be ascribed to underfeeding. Healthy adult pigeons, starved four days, or fed only 10 grams fresh asparagus tips for four days, die within two days after receiving injections of anthrax cultures—i.e., as promptly as do pigeons with polyneuritis. No attention was given to the temperature of the animals, although Pasteur had clearly shown that chilling abolishes the natural resistance of the chicken to anthrax. G. M. Finlay (1923) was able to show that normal animals, whose body temperature is lowered by pyramidon, or in the course of vitamin B deficiency, invariably die if inoculated with pneumococcus, B. coli, or B. enteritidis; whereas they nearly always survive these infec-

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TITLE: **A Survey of the Experience and Impact of Acute Upper Respiratory Tract Infections on People in Six Countries in the 2011/2012 Common Cold and Flu Season**

AUTHORS: *John David Hull, Ian Paul Barton, Jennifer Torgersen, Christine Marie McNeil*

KEYWORDS: Common Cold; Upper Respiratory Tract Infections; Common Cold Survey

JOURNAL NAME: **Open Journal of Respiratory Diseases**, Vol.3 No.4, November 22, 2013

ABSTRACT: Introduction: Acute Upper Respiratory Tract Infections (URTIs) are the most common infectious diseases of humankind. While usually mild and self-limiting, they are characterized by a series of simultaneously occurring symptoms/ signs that are sufficiently disruptive to sufferers' normal activities in which medication is frequently sought. While the literature has many examples of epidemiological studies on these infections, there are few reports on patient experience and impact. This study was designed to investigate these aspects of Common Cold/Flu across six countries. Methods: A minimum of 500 adults aged 18 and older were recruited in each of six countries (Brazil, China, Germany, India, Russia, and the US) using customary survey research sampling techniques. Single 30-minute (online) or 40-minute door-to-door quantitative questionnaires with c. 50 questions were completed with each participant by the global research firm Ipsos. Main Findings: Across countries, incidence and seasonality of infections reported to this study were consistent with published data. There appears to be a need for patient education on the causes and transmission routes of respiratory infections. Getting good quality sleep and being able to continue with daily activities as an infection resolves are significant drivers to therapy. The most common non-prescription therapies reported were multi-ingredient products in line with the simultaneously occurring multi-symptom nature of the condition(s). Conclusions: This study indicated that acute URTIs exert a significant deleterious effect on sufferers. Public health education, possibly best undertaken by Pharmacists has the potential to impact the extent of virus transmission by ensuring that people know the true cause of the infection, how it is transmitted and how best to combat this. The several simultaneously occurring symptoms encourage sufferers to seek multi-ingredient remedies to allow them to continue with normal activities as their infection resolves naturally.

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VOLUME 42, NO. 1 JANUARY, 1942

THE ADMINISTRATION OF VITAMIN C IN A LARGE INSTITUTION AND ITS EFFECT ON GENERAL HEALTH AND RESISTANCE TO INFECTION

BY A. J. GLAZEBROOK, M.R.C.S., L.R.C.P.
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(With 3 Figures in the Text)

CONTENTS

INTRODUCTION

IN any institution, where large numbers of people are supplied with food from central kitchens, the diet usually contains only small amounts of vitamin C. Destruction of this vitamin takes place during overcooking and the reheating of the food while it is awaiting distribution. Fresh fruit and vegetables are rarely supplied.

Crandon, Lund & Dill (1940) concluded that the maximal utilization of vitamin C lies between 30 and 45 mg. daily. Their figures were derived from a study of experimental human scurvy. The 'minimal-optimum' intake of vitamin C for adults has been computed at 25 mg. a day per 10 stones of body weight, and this results in an excretion of 13–15 mg. a day (Abbasy, Harris, Ray & Marrack, 1935; Harris & Abbasy, 1937). The 'minimal-optimum' intake is based on the amount found necessary to prevent a tendency to increased capillary fragility (Gothlin, 1937). Fox (1941) reviewed the results of the experiments of Fox, Dangerfield, Gottlich & Jokl (1940), Crandon *et al.* (1940) and Kellie & Zilva (1939), and concluded that remarkably good health can be maintained on 15 mg. of vitamin C daily, but he remarked on the precarious nature of such meagre supplies.

Certainly large numbers of people live on a diet containing less than the 'minimal-optimum' intake, without apparent ill effect. Investigations by

Orr (1936) and by Crawford & Broadley (1938) indicate that the diet of one-half to three-quarters of the population of Great Britain contains inadequate quantities of vitamin C, the lower figure being obtained by adopting 'minimum' (British Medical Association) standards, and the higher figure by adopting 'minimal-optimum' (League of Nations) standards.

There are, of course, wide variations in the extent to which individuals will tolerate low vitamin C diets. Jennings & Glazebrook (1938) described a man who had taken a scorbutic diet for 40 years before he showed ill effects. On the other hand, children have developed scurvy while receiving generous supplements of vitamin C, such as orange juice, and the condition is cured by giving ascorbic acid parenterally, or in large amounts by mouth (Hess, 1923; Hagmann, 1937; Parsons, 1938).

The requirements of the body for vitamin C vary with several factors. Children require a larger amount per kg. of body weight than do adults (Abbasy *et al.* 1935; Smith, 1938), and it is probable that adolescents also require a greater intake.

The body's requirements are increased if the metabolism is increased (Parsons, 1938). Thus, hard exercise and exposure to cold may precipitate scurvy, and at one time scurvy was considered to be due to damp and exposure. Crandon *et al.* (1940) found an abnormally high level of blood lactate after muscular exercise in their case of experimentally induced human scurvy. The subject was capable of a maximum effort corresponding to that of a man 80 years old. Stewart, Learmonth & Pollock (1941) suggest that ascorbic acid secures a more adequate supply of oxygen to the tissues.

Certain intestinal conditions, by permitting the growth of vitaminolytic bacteria (Kendall & Chinn, 1938), may markedly increase requirements owing to the great destruction of the vitamin and consequent failure of absorption.

Many infective states increase the body's requirements, and this has been shown in tuberculosis by Hasselbach (1936*a*, *b*), Heise & Martin (1936) and by Abbasy, Harris & Ellman (1937); in rheumatoid arthritis by Abbasy, Harris and Ellman (1937) and by Rinehart, Greenberg & Baker (1936); in osteomyelitis by Abbasy, Harris & Hill (1937); in juvenile rheumatism by Abbasy, Hill & Harris (1936). It has been recorded in other infections by Harde, Rothstein & Ratish (1935).

Abbasy & Harris (1937) found a correlation between the erythrocyte sedimentation rate and the excretion of vitamin C in cases of tuberculosis and rheumatoid arthritis. They concluded that the excretion of vitamin C varied inversely with the severity of the condition, probably because of increased utilization in the body. The Groth-Petersons (1939) found that tuberculous patients require a greater intake of ascorbic acid to maintain a normal serum level than do healthy people.

Rinehart, Greenberg, Olney & Choy (1938) found a low level of ascorbic acid in the blood of cases of rheumatism, not only in the acute phase, but also in convalescence and in very low-grade infections.

This increased destruction of vitamin C in febrile illnesses may be incidental to the disordered metabolism, and serve no useful purpose. It seems clear, however, that there is an increased liability to infection in both man and animals in cases of frank scurvy (Hess, 1920; Hamburger & Goldschmidt, 1922-3; Werkman, Nelson & Fulmer, 1924; Grant, 1926; Schmidt-Weyland & Koltzsch, 1928; Grant, 1930; Bloch, 1931; Mackay, 1934; Robertson, 1934).

In cases of so-called 'latent scurvy' the evidence is equivocal. Hess (1917 first suggested that this condition occurs and is analogous to latent tetany. It is thought that this state is a cause of ill-health and may lower resistance to infection (Harris, 1937; Bourne, 1938; Szent-Gyorgyi, 1938). Vitamin C is said to control outbreaks of pneumonia (Funck, 1931), and a deficiency of it to play a part in the production of both acute juvenile rheumatism and rheumatoid arthritis (Rinehart & Mettier, 1934; Rinehart, 1935). Vogl (1937) claimed to have used it successfully in the prophylaxis of post-operative pneumonia. On the other hand, Fox *et al.* (1940) administered vitamin C over a period of 7 months to adult negroes, previously subsisting on a low intake, and found no difference in illness as compared with controls.

The evidence that vitamin C exerts a beneficial effect in cases of actual illness is not clear. Fresh fruits and their juices, particularly lemons and black currants, have long been common household remedies for simple acute infections. Low levels of vitamin C have been found in many illnesses, so low in some instances that the vitamin has been thought to have some specific aetiological significance. Hopes that saturation with the vitamin would cure such diseases have not been realized. While full tissue saturation is probably unnecessary, it would seem desirable to increase the intake of vitamin C during illness.

Otani (1936) and Ormerod & Unkauf (1937) considered that vitamin C improved cases of whooping cough. Gairdner (1938) in a controlled experiment found that the duration of illness in a group receiving vitamin C was shorter than in controls. The difference in the two groups was not a significant one, and he considered that the alleged benefits of vitamin C in whooping cough were unproven.

Beneficial results have been claimed in diphtheria (Bamberger & Wendt, 1935; Bamberger & Zell, 1936; Dieckhoff & Schuler, 1938; Szirmai, 1940). Zilva (1938) found that vitamin C saturation made no difference to the fate of guinea-pigs injected with diphtheria toxin.

An acceleration of healing, or a general improvement, in cases of tuberculosis treated with vitamin C has been claimed by several workers (Radford, de Savitsch & Sweeney, 1937; Albrecht, 1938; Bakhsh & Rabbani, 1939; Warns, 1938; Birkhaug, 1939). Some of these observations were based on controlled experiments. Hurford (1938), on the other hand, saw no significant change after saturation, except in the blood picture of anaemic cases. Erwin, Wright & Doherty (1940) state quite definitely that vitamin C is of no value in the treatment of tuberculosis. This conclusion was arrived at as a result of

their observations upon a series of chronic, or acute broncho-pneumonic, cases, 'unlikely to improve on any known form of treatment'. With such unpromising material, disappointing results would seem to be inevitable.

There is evidence that it is of value in pneumonia, particularly in hastening convalescence, and the claims made do not appear to have been contradicted (Gander & Niederberger, 1936; Vogl, 1937; Bonnholtzer, 1937; Hochwald, 1937; Gunzel & Kroehnert, 1937; Sennewald, 1938; Szirmai, 1940). Szirmai (1940) noted that while tissue saturation is necessary to obtain maximal benefit in pneumonia, cases of typhoid fever and diphtheria were improved by daily supplements of vitamin C without producing saturation.

Estimations of deficiency

Of the various methods of estimating a deficiency of vitamin C in the body, that described by Harris, Abbasy & Yudkin (1936) is the most popular. It is recognized that the excretion of vitamin C in the urine is dependent on the reserve in the body as well as on the amount ingested during the previous few days. Accordingly, a test dose (300–600 mg.) of ascorbic acid is given and the amount excreted in the urine during the following 24 hr. is measured. The procedure is repeated for several days until large amounts of ascorbic acid are excreted. It is recognized that although the amount excreted in the urine of normal people depends on the previous amounts in the diet, this amount cannot be used to measure the degree of saturation of the tissues. Abbasy *et al.* (1935) have found that a daily intake of 90 mg. will result in an excretion of 50 mg. in the urine, but an intake of 15 mg. will result in an excretion of 15 mg. Accordingly, it is considered that any deficiency of vitamin C is best measured in terms of saturation of the tissues (Hess & Benjamin, 1934; Johnson & Zilva, 1934; Harris, Ray & Ward, 1933; Harris & Ray, 1935; Pemberton, 1940). Following the same principle, estimations of vitamin C in the blood have been made and an ascorbic acid tolerance curve devised, following an intravenous injection of 1000 mg. (Farmer & Abt, 1935; Mirsky, Swadesh & Soskin, 1935; Wright, Lilienfield & Maclenathen, 1937; Portnoy & Wilkinson, 1938).

In a large training school under our observation there were some 1500 youths aged 15–20 years. For the most part they were drawn from the lower wage-earning classes, and a large proportion came from Scotland and the North Midlands, where economic conditions are probably below the average for the country. It is a reasonable assumption that the previous dietary of the recruits had been somewhat deficient in vitamin C judged by the standards already quoted.

The diet of the institution allowed over 4000 cal. per student per day. The food distribution was badly managed. Electric ovens were used to reheat the food, and to keep it hot whilst awaiting distribution. Often 8 hr. elapsed between the time the food was cooked and its arrival on the dining tables. The minimum time that heat was applied to the food, including the original cooking and the subsequent reheating, was 2 hr.

The daily ration of potatoes was 12 oz. The vitamin C content of potatoes varies, but this quantity in the raw state should contain approximately 50 mg. A full ration of potatoes, as served on the dining tables, after cooking and reheating, was found to contain, on the average, about 4 mg.

The other vegetables suffered an equal loss, with the exception of turnips, portions of which contained up to 6 mg. The milk was pasteurized, and half a pint of it contained about 1·5 mg. The other cooked foods contributed negligible amounts. The total intake of vitamin C varied from about 10 to 15 mg. per student per day.

Menus for one month

Day and date	Breakfast	Dinner	Tea	Supper
		Week ending 4 December 1937		
Sunday, 28 Nov.	Bacon and egg	Tomato soup Roast pork Cabbage Steamed apple pudding and custard sauce	Assorted pastries	Veal loaf Beetroot
Monday, 29 Nov.	Porridge Smoked fillets	Mulligatawny soup Roast beef Marrowfat peas Suet roll and syrup sauce	Jam, marmalade or syrup	Highland hash Mashed potatoes
Tuesday, 30 Nov.	Bacon and beans	Julienne soup Roast mutton Cabbage Dundee pudding	Doughnuts	Irish stew Doughboys Mashed potatoes
Wednesday, 1 Dec.	Liver and chips	Scotch broth Steak and kidney pie Mashed turnips Prunes and custard	Jam, marmalade or syrup	Fish and crisps
Thursday, 2 Dec.	Bacon and sausage	Pea soup Roast beef Cabbage Sultana roll and custard sauce	Bananas	Bubble and squeak and bacon
Friday, 3 Dec.	Porridge Fried fish	Pea soup Meat pudding Haricot beans Tapioca pudding	Jam, marmalade or syrup	Durham cutlets Marrowfat peas
Saturday, 4 Dec.	Fried sausages	Pot mess Carrots Doughboys Bananas	Tea cakes	Pea soup Cheese
		Week ending 11 December 1937		
Sunday, 5 Dec.	Bacon and egg	Tomato soup Roast mutton Cabbage Bananas and custard	Assorted pastries	Preserved meat Beetroot
Monday, 6 Dec.	Porridge Bloaters	Pea soup Roast beef Marrowfat peas Snowdon pudding	Jam, marmalade or syrup	Cottage pie
Tuesday, 7 Dec.	Fried sausages	Pea soup Beef steak pudding Cabbage Tapioca pudding	Jam, marmalade or syrup	Layer pie

Week ending 11 December 1937 (*continued*)

Day and date	Breakfast	Dinner	Tea	Supper
Wednesday, 8 Dec.	Bacon and liver	Potato soup Ragout of rabbit Marrowfat peas Suet pudding and jam	Assorted pastries	Fish and chips
Thursday, 9 Dec.	Fried or boiled eggs	Pea soup Roast beef Cabbage Apple pudding and custard sauce	Fish paste	Saveloys and pease pudding
Friday, 10 Dec.	Porridge Fried fish	Pea soup Steak and kidney pie Carrots Prunes and custard	Jam, marmalade or syrup	Savoury Mince and haricot beans
Saturday, 11 Dec.	Bacon and sausage	Pott mess Doughboys Butter beans Rice custard	Doughnuts	Salmon Beetroot

Week ending 29 January 1938

Sunday, 23 Jan.	Bacon and egg	Tomato soup Roast pork Cabbage Apple tart and custard	Slab cake	Salmon Beetroot
Monday, 24 Jan.	Fried or boiled eggs	Pea soup Roast beef Marrowfat peas Sultana roll and custard sauce	Jam, marmalade or syrup	Cottage pie
Tuesday, 25 Jan.	Porridge Kippers	Pea soup Steak and kidney pie Cabbage Rice custard	Rock cakes	Fried steak Mashed potatoes
Wednesday, 26 Jan.	Fried sausages	Potato soup Roast beef Turnips Ginger pudding	Jam, marmalade or syrup	Fish and chips
Thursday, 27 Jan.	Bacon and tomatoes	Pea soup Preserved meat Braized onions Durban pudding	Fish paste	Lamb's heart Potatoes
Friday, 28 Jan.	Porridge Fresh fish	Mulligatawny soup Roast mutton Cabbage Prunes and custard	Doughnuts	Bacon and bubble and squeak
Saturday, 29 Jan.	Sausage and egg	Pot mess Doughboys Carrots Bananas	Currant bread	Cheese and sauce

Week ending 18 June 1938

Sunday, 12 June	Bacon and egg	Tomato soup Roast mutton Cabbage Rhubarb tart Custard	Slab cake	Salmon Cucumber
Monday, 13 June	Porridge Kippers	Pea soup Roast beef Marrowfat peas Snowdon pudding and custard sauce	Syrup	Cambridge stew

Week ending 18 June 1938 (*continued*)

Day and Date	Breakfast	Dinner	Tea	Supper
Tuesday, 14 June	Fried eggs	Lancashire hot-pot Doughboys Onions Blanc-mange and prunes	Assorted pastries	Fish and chips
Wednesday, 15 June	Liver and bacon	Pea soup Baked and steamed pies Cabbage Sponge trifle	Bananas	Roast beef Potatoes
Thursday, 16 June	Fried eggs	Stewed rabbits and pork Dumplings Butter beans Macaroni pudding	Lemon curd	Fish and chips
Friday, 17 June	Sausages and gravy	Pea soup Roast mutton Cabbage Durban pudding Custard	Bananas	Lamb's heart Peas
Saturday, 18 June	Porridge Fresh fish	Irish stew Doughboys Haricot beans Rice pudding	Doughnuts	Cheese and pickles

Extra to menu. Tea, sugar, milk, bread, butter and potatoes, cocoa and biscuits: buns at stand easy.

METHODS

For a preliminary survey seventy-seven tests were carried out on otherwise healthy youths by giving them 300 mg. of ascorbic acid, and not one excreted appreciable amounts in his urine. Using the same method on twenty of the administrative staff who had a different dietary, it was found that fifteen excreted a considerable proportion of their test dose. Although it is recognized that other substances in the urine reduce the dye, 2:6-dichlorindophenol, the investigation revealed a difference between the two groups.

Estimations of the resting level of excretion, i.e. the total amount excreted in 24 hr. in the absence of a 'test dose', were also made. The amounts varied between 5·6 and 1·1 mg. with an average of about 2·5 mg. as compared with the normal amount of 13–15 mg.

These preliminary observations, therefore, indicated that the intake of vitamin C was at a very low level. This was to be expected from a consideration of the vitamin C content of the diet, and the probable 'minimal-optimum' requirements of the boys.

Daily excretion levels

Pure ascorbic acid powder was added to the diet of a group of boys numbering 350, whose average age was 16. Initially, 200 mg. per day were given to each boy, 100 mg. being placed in the morning cocoa, and 100 mg. in an evening glass of milk. The mixing was done in bulk in the kitchens before issue. The powder dissolved quickly and easily, and did not alter the appearance or taste of the vehicle.

From time to time samples of milk and cocoa were titrated after issue, in order to ensure that the mixing was properly carried out, and that full doses reached the youths. Figures varying from 78 to 118 mg. per glass were obtained in the case of the milk, and from 58 to 68 mg. per cup in the case of the cocoa. Heating of the cocoa no doubt explained the loss. Together with the amount occurring naturally in the diet, the intake per boy was approximately 200 mg. per day. The daily output of vitamin C was measured in different groups of boys each day, the titration of each sample of urine being carried out immediately after it was passed.

Fig. 1 shows the slow rise in urinary output which occurred. It was not until the 8th day that figures approximating to the resting level of normal adults were obtained, and high figures indicative of saturation point were not noted until the 22nd day. In other words, saturation was not achieved until 22 doses of 200 mg. per day had been given, or a total of some 4000 mg. This figure was probably too high, since it was likely that on occasions the boys under test did not pass all their urine in the Sick Quarters as ordered.

On the 28th day the dosage was reduced to 50 mg. twice a day, and on this dosage excretion continued at a level rather higher than that of a normal adult on optimum intake.

A fresh group of boys was observed, and the initial dosage was increased to 150 mg. twice a day. Figures indicative of saturation were obtained on the 15th day, and subsequently the dose was reduced to 25 mg. twice a day, when an excretion level approximating to the normal adult level was maintained. This is shown in Fig. 2.

A third batch of boys was examined. In this batch all the boys selected were recruits who showed possible clinical evidence of a vitamin C deficiency in the form of a mild gingivo-stomatitis. The ascorbic acid in this case was given in tablet form (Redoxon, Roche Products), in a dosage of 200 mg. once daily. Instead of estimating the vitamin C excretion of individual boys as in the two previous experiments, several were instructed to pass their urine each day and night in the Sick Quarters. The urine specimens were pooled. From the mixed specimens a sample was taken and acidified by the addition of one-ninth the volume of glacial acetic acid. The samples were titrated, and the amount of ascorbic acid per 1500 c.c. of urine recorded and charted (Fig. 3). This chart is very similar in form to Fig. 1. High outputs were observed on the 23rd day; the dose was then reduced to 50 mg. once a day in tablet form.

These charts show that, in order to maintain an optimal excretion level, a daily addition of 50 mg. of ascorbic acid was required.

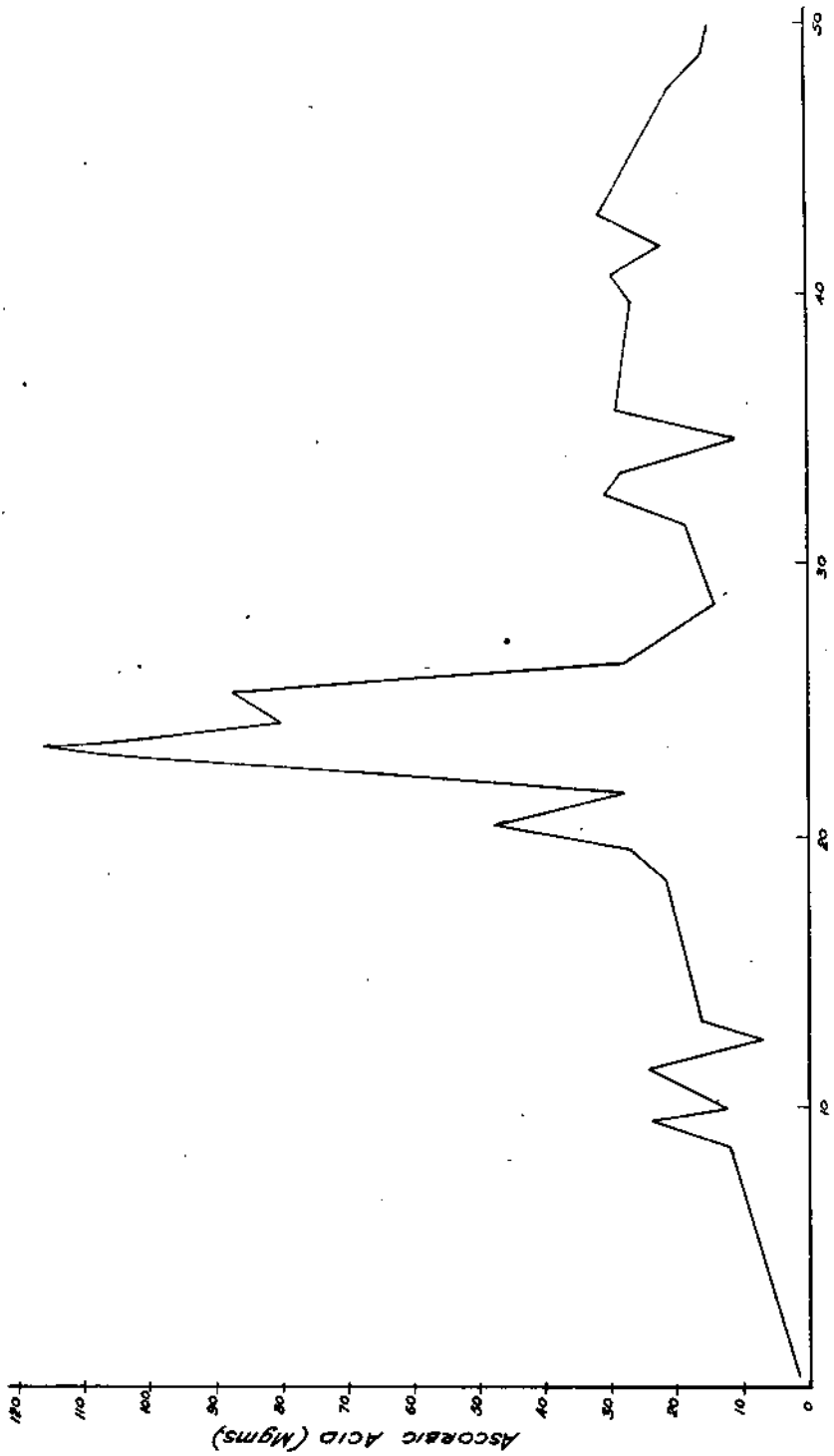

Fig. 1. Daily output of vitamin C in the urine. Group 1.

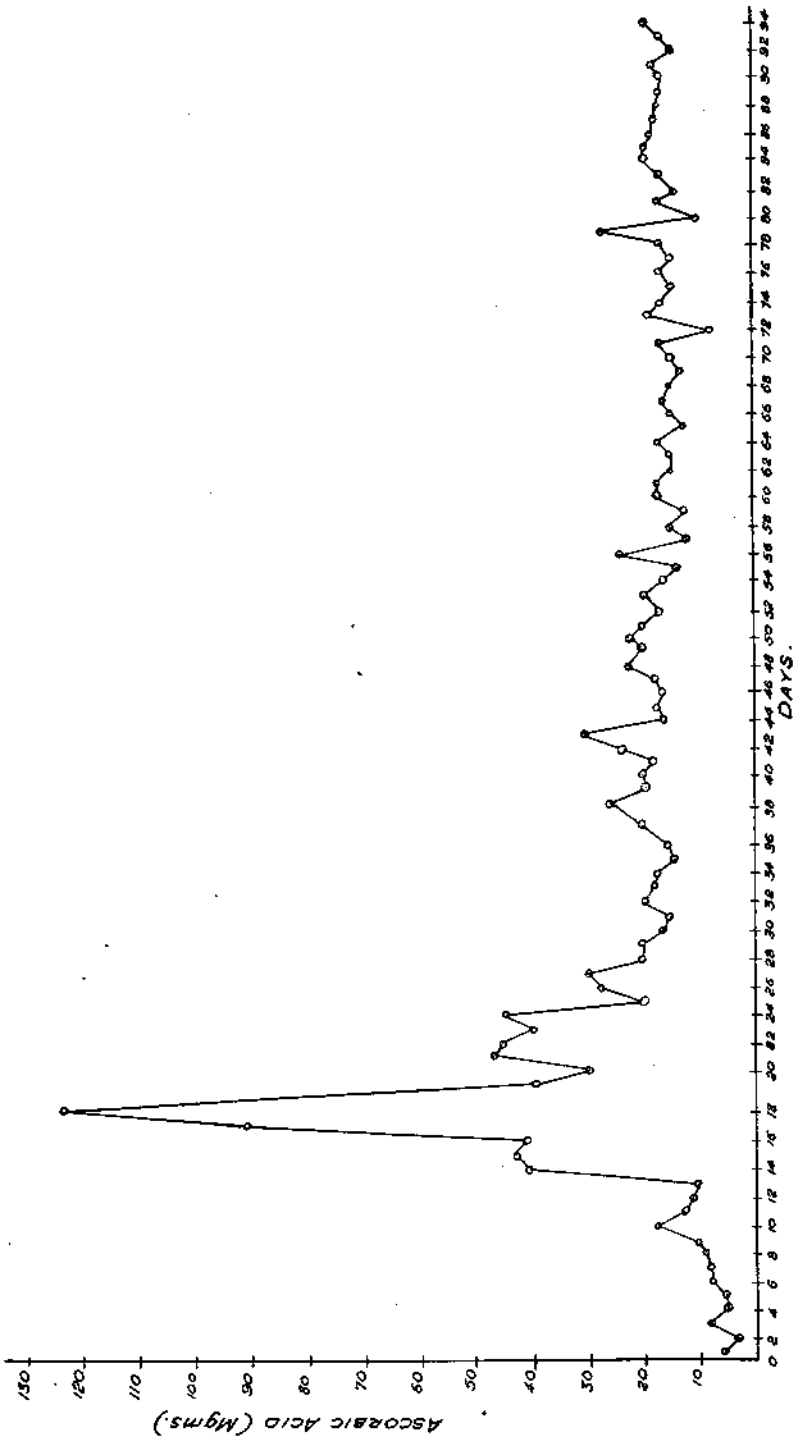

Fig. 2. Daily output of vitamin C in the urine. Group 2.

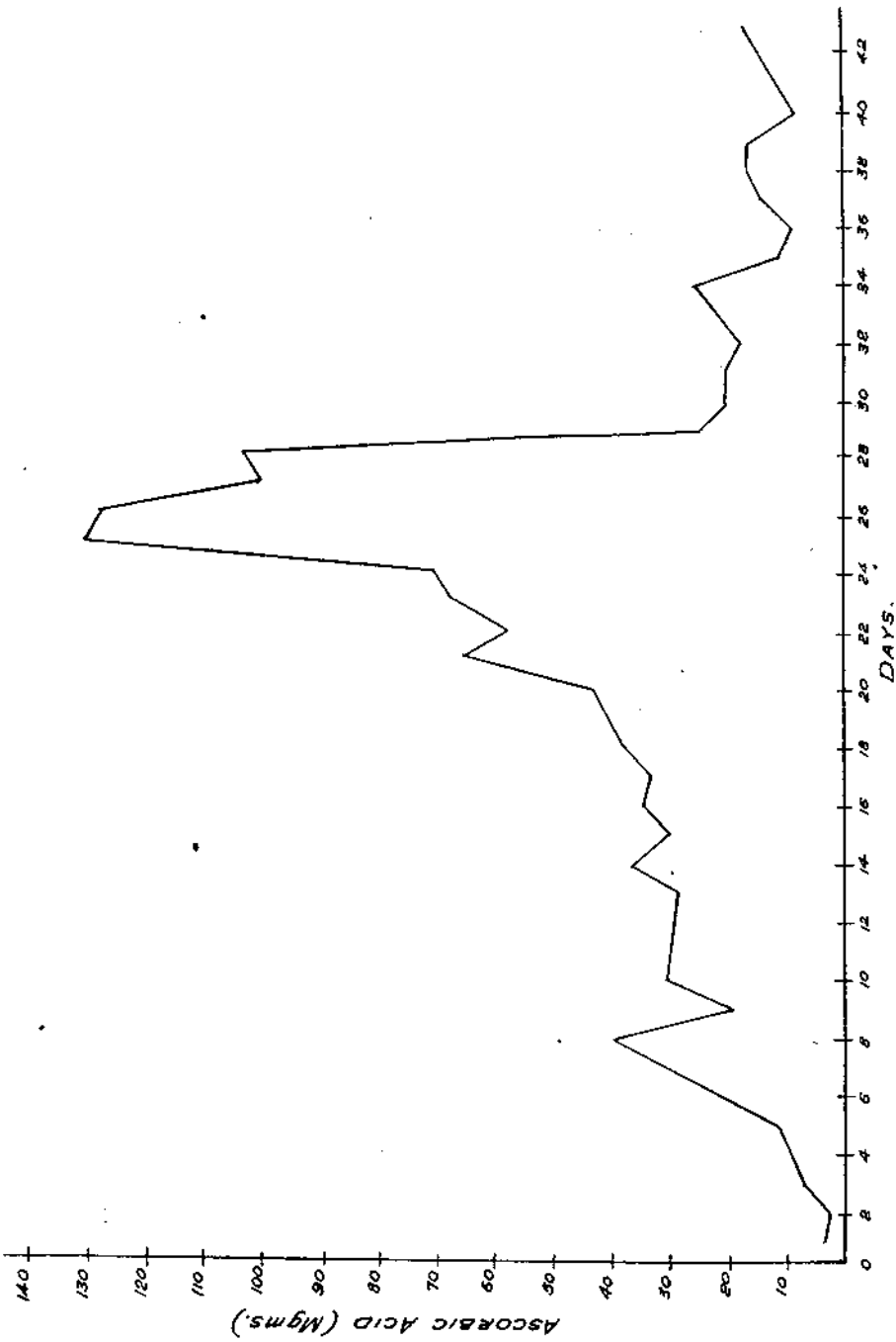

Fig. 3. Daily output of vitamin C in the urine. Group 3.

THE RELATIONSHIP OF VITAMIN C TO RESISTANCE

In the institution, there were some 1500 students whose ages ranged from 15 to 20 years. The establishment was divided into seven groups or divisions for administrative purposes. The youths of one division worked as a unit, and occupied certain tables in the dining hall. To some extent each division occupied particular dormitories, but this separation was not absolute, and there was a fair amount of mixing of divisions in the sleeping quarters. Sleeping and feeding conditions were, of course, the same for all divisions. Careful records had been kept of the incidence of all infections for 1½ years before the observations described here were begun. In the preceding year there had been an epidemic of tonsillitis, which had affected all the divisions uniformly, so that they could not be regarded as separate units within the larger population.

The observations were made by supplying vitamin C in the form of pure ascorbic acid to one or more divisions. This was considered to be the only practical method of carrying out the observations without introducing unnecessary complications. For example, it was not possible to choose boys at random as it would have been impossible to supply them with vitamin C-treated cocoa or milk in the dining room. With the method actually chosen, all that was necessary was to add vitamin C to the supplies of cocoa or milk serving the tables for the appropriate divisions.

Moreover, all of the divisions had a population more or less the same as regards duration of stay in the establishment ('institution age'). Infectious diseases were more common amongst those who had more recently joined the institution. This was known from our previous records of infectious illnesses in the institution (Thomson & Glazebrook, 1942), and in view of these points the method of supplying the vitamin C to a whole division was decided upon.

Many minor infective conditions, such as conjunctivitis, boils, impetigo, etc., were not reviewed, as the number of cases of each disease was small.

The most common infective conditions which occurred were coryza and tonsillitis. The term 'tonsillitis' is used here to be an index of haemolytic streptococcal disease of the nose and throat, and covers all such terms as 'tonsillitis', 'sore throat', 'otitis media', 'pharyngitis' and 'cervical adenitis', as nearly all these cases are of haemolytic streptococcal origin. Throat swabs were taken of large numbers of cases of tonsillitis to determine that the haemolytic streptococcus was the causative organism.

Table 1 shows the number of cases of tonsillitis and common colds recorded in the two groups.

Table 1. *Incidence of tonsillitis and common colds in the two groups*

	Youths on vitamin C (335 youths)	Controls (1100 youths)
Colds	72 = 21·2%	286 = 26%
Tonsillitis	29 = 8·5%	94 = 8·6%

It is obvious, therefore, that vitamin C had no effect on the incidence either of common cold or tonsillitis.

The experiment was complicated, however, by the admission of 250 recruits into the two groups in the middle of the observations, replacing fully trained youths. This was of special interest, as it was known from previous experience that infections were more common amongst those who had more recently entered the institution. This would be true of any institution where infectious diseases were common. The test group admitted relatively more of the recruits into its population. No recruits were admitted during the 3 months preceding the period of the observations.

The recruits were those of group 6 (Thomson & Glazebrook, 1942), and no observations were made until they had been in the institution for a month. During this period the recruits who entered the test divisions were saturated with vitamin C, and it was during this same period that the recruits experienced much of their heavier incidence of disease. After a month had elapsed a record was kept of sixty youths who entered a test division and ninety who entered a control division. There was still a heavier incidence of infectious diseases amongst them as compared with the others who had been in the institution for some time. The duration of the period over which the recruits were observed was about one-half of the duration of the whole investigation. Table 2 shows that there was a greater incidence of disease amongst the recruits as a whole as compared with the others, but no difference in incidence of disease between the two groups of recruits.

The numbers of cases of tonsillitis and common cold which occurred amongst the 250 recruits were not sufficiently great to alter the incidence rates in the two experimental groups.

Table 2. *Incidence of infection amongst recruits*

	Youths on vitamin C (60 youths)	Controls (90 youths)
Colds	17 = 28·3 %	29 = 32·2 %
Tonsillitis	1	7 = 8 %

The next point examined was to see what effect, if any, the vitamin C had on the duration of the illness.

When a youth fell ill he was admitted to Sick Quarters unless his complaint was very mild. In the latter case he was placed on the out-patients list and excused all duties except attendance at school instruction. Most of the cases of common cold and tonsillitis were admitted to Sick Quarters. In analysing the durations of illnesses, observations were restricted to the cases in the Sick Quarters. The number of days spent there was obviously a more reliable index of the duration of illness, since the patient was under constant medical supervision. Frequently when a youth was discharged from the Sick Quarters he was put on the out-patients list, and this 'convalescent period' was neglected. The admission to and discharge from the hospital was not under our control.

The diet in the Sick Quarters was basically similar to that of the healthy boys. It was modified, of course, to suit the needs of the sick, but was prepared in the central kitchens and suffered an equally drastic loss of its vitamin C. When a student from the experimental division fell ill and was admitted to Sick Quarters, his dosage of ascorbic acid was continued there.

In a period of 6 months the average number of days spent in the sick room per boy due to infective conditions was 2·5 in the vitamin-C treated division, and 4·98 in the control division. In a period of 6 weeks, within the period of 6 months, the corresponding figures among the recruits were 3·2 in the vitamin-C treated group, and 4·0 in the control group.

It would appear that the saturation with vitamin C probably had some effect on duration of illnesses, and accordingly an analysis was made of this.

Days ill with common cold

In the vitamin C classes fifty-nine of the seventy-two cases (81·9%) were treated in the Sick Quarters, and the average period of stay was 6·32 days.

Among the controls 253 cases out of 286 (88·5%) were treated in the Sick Quarters, and the average period of stay was 6·4 days.

There was, therefore, no difference in the two groups either in incidence or duration of illness of common cold, and there was no difference in the proportion of total cases admitted to hospital.

Days ill with tonsillitis

The results are shown in Table 3.

Table 3. *Duration of attack of tonsillitis*

Class	Total no. of cases	No. admitted to hospital	Hospital cases expressed as percentage of total	Average stay in hospital	Standard deviation
Vitamin C class	29	18	62	10·05	6·96 (1)
Controls	94	83	88	16·7	11·86 (2)

An analysis showed that a difference as great or greater than that obtained would be expected once in fifty times in a homogeneous population.

Analysis of the more severe illnesses

It has been shown that youths on vitamin C spent 2·5 days in hospital due to infective conditions as compared with 4·98 in the control group. No conclusions were drawn from this observation, and it has been shown above that some of this difference was due to the duration of illness of tonsillitis in the two groups.

Some of this difference, however, was due to the occurrence of acute rheumatism and pneumonia in the control group with no case of either disease in the vitamin C-treated group.

There were seventeen cases of pneumonia and sixteen cases of acute rheumatism among 1100 controls, and no case of either disease among 335 youths having vitamin C. It would appear that the vitamin C exerted a considerable effect on the prevention of these two diseases. Of the sixteen cases of acute rheumatism, eleven were primary attacks, while five were recurrences.

The incidence of the diseases in the various divisions of the institution is shown in Table 4.

Table 4. *Incidence of pneumonia and rheumatism in the various divisions of the institution*

		Number of cases	
	Division	Pneumonia	Rheumatism
Vitamin C divisions	A	0	0
	B	0	0
Control divisions	C	5	3
	D	3	5
	E	2	3
	F	4	3
	G	3	2

Thus, the most marked effect of the vitamin C was to reduce the incidence of two severe illnesses.

Analysis shows that a difference as great or greater than this would be expected once in fifty times in a homogeneous population.

DISCUSSION

In a large institution there was a marked difference between the degree of vitamin C saturation of the students and the teaching staff as determined by a simple 'test-dose' method. The students were given a high calorie diet, which was subjected to prolonged heating. This overcooking resulted in a reduction of the total daily vitamin C intake to a level of 10–15 mg. per head. A daily addition of 50 mg. of ascorbic acid per head was required to maintain an optimal excretion level.

Better management of the food distribution and cooking arrangements might have achieved this result. The potato ration alone, allowing for normal cooking losses, should have supplied at least 25 mg. of vitamin C daily.

Some vitamin loss, of course, is unavoidable when food is cooked for communities in central kitchens. Normally, this can easily be countered by the supply of uncooked fresh or canned foods. In this case, for instance, the reduction of the diet from 4000 cal. to the more reasonable level of 3000 cal. per day, would at this time (1938) have probably offset the cost of an orange a day.

The dietary of the teaching staff included the supply of fresh fruit at each of the main meals. It was prepared in separate kitchens and escaped the overcooking. Nevertheless, judging from a single 'test-dose', 25% of the staff

were 'deficient' in vitamin C, in spite of their adequate intake. Harrison, Mourane & Wormall (1938) similarly found that the method indicated a 'deficiency' in 25% of medical students. The single 'test-dose' is not, of course, a reliable measure when applied to individuals.

The surprisingly large amount of 4000 mg. of vitamin C was required to produce tissue saturation of the youths. Attention has been drawn to the possibilities of experimental error, and many of the factors which increase utilization were present.

The subjects were adolescents. Infections were very common in the institution, and there had been a very severe epidemic of tonsillitis during the preceding session. The experiments were carried out during the winter months. Physical training and games occupied much of the day, and it was found that youths at rest in bed required approximately half the quantity of vitamin C, i.e. 2000 mg., to produce full saturation.

A special group of boys exhibited a mild gingivo-stomatitis, considered to be probably a scorbutic manifestation. Their saturation curve, however, was very similar to that of the other groups. The clinical appearance of this gingivo-stomatitis has been described (Roff & Glazebrook, 1939, 1940). It proved resistant to ordinary methods of dental treatment, and responded only to vitamin C saturation. It would appear that, under exactly similar conditions of suboptimal vitamin C intake, a gingivitis occurs in only a proportion of the cases. This, of course, was known to Lind (1772), who wrote: 'In Haslar Hospital the appearances of the disease [scurvy] were various—the gums were not always affected.'

No differences in the incidences of common cold and tonsillitis were found in two groups of boys, one of which received large doses of vitamin C. It was found, however, that the average duration of illness of the cases of tonsillitis in the control group was much longer than in the vitamin C-treated group. No such difference was found in the cases of common cold.

The period of treatment of cases of tonsillitis and common cold in the Sick Quarters was completely outside our control, and no biased attitudes influenced these durations from which we have drawn our conclusions.

In addition, there were seventeen cases of pneumonia and sixteen cases of rheumatic fever in the control group, with no case of either disease in the vitamin C-treated group. These cases were subjected to special investigations by us (X-rays, etc.) to establish certain criteria for the diagnosis. There was, however, in our opinion a relationship between these conditions.

Rheumatic 'pneumonitis' is a condition which is now recognized to occur not infrequently as a complication of rheumatic fever. The post-mortem appearance and pathology of this pneumonitis have been demonstrated by Hadfield (1938).

In the institution a type of low-grade basal lung consolidation or 'pneumonitis' occurred, and appeared to be related both to rheumatism and vitamin C deficiency. It was characterized on the one hand by its tendency

to progress into rheumatism, and on the other hand by its rapid disappearance when treated with ascorbic acid. This pneumonitis, apart from a vague picture of ill health, gave little clinical evidence of its presence, but it probably predisposed towards the development of acute pneumonia.

It is agreed that cases of rheumatic fever almost invariably give a history of upper respiratory tract infection, usually some 2 weeks previously. Such an infection depletes the reserves of vitamin C, more especially in those individuals whose intake is already at a low and precarious level. When the vitamin C reserves have fallen, it may be that the reaction of the body to an infection with the haemolytic streptococcus is altered. This may help to determine the onset of the syndrome of rheumatism in some cases, even although vitamin C has no specific action upon the established disease. In some cases of pneumonia, too, a similar train of events may occur, and there is much evidence that vitamin C does assist recovery.

Certainly, protracted mild deficiencies of vitamin C produce bone and cartilage changes, the histological and skiagraphical appearances of which have been accurately described (Park, Guild, Jackson & Bond, 1935; Wolbach & Howe, 1926). Ham & Elliott (1936) showed that the epiphyseal changes occurred when the vitamin C intake was sufficient to prevent scurvy although less than the basic requirements. These changes are marked during the period of growth. Under similar circumstances Mouriquand & Edel (1940) have demonstrated osteophytic formation. Rinehart & Mettier (1933, 1934) produced lesions simulating rheumatism in the myocardium of guinea-pigs fed on a scorbutic diet. Wolbach (1936) showed the presence of vitamin C to be essential for the formation of collagen. Swelling of the collagen is the earliest pathological change in rheumatism.

The calcium and vitamin B content of the dietary of the institution could perhaps be criticized, but the only *outstanding* deficiency, according to modern standards, was in vitamin C. As far as this one factor was concerned, the boys were almost certainly worse off, subsisting on the institution diet, than they would have been at home.

SUMMARY

1. The vitamin C in the dietary of an institution was largely destroyed by the methods of cooking and distribution.

2. Some 50 mg. of ascorbic acid per head per day were required to be added to the diet to produce an optimum excretion level.

3. Large doses of ascorbic acid were given to a group of adolescents in the institution over a period of several months. A record was kept of the incidences of infectious diseases in this treated group and in the remainder (controls). The following conclusions were reached:

(*a*) The incidences of common cold and tonsillitis were the same in the two groups.

(*b*) The average duration of illness due to the common cold was the same in the two groups.

(*c*) The duration of illness of tonsillitis was longer in the control group than in the test group.

(*d*) Cases of rheumatic fever and pneumonia occurred in the control group but no case of either disease occurred in the test group.

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SAC

ASCORBIC ACID in Treatment of the Canine Distemper Complex

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A CLINICAL investigation of ascorbic acid as a therapeutic agent in treatment of canine distemper complex was initiated in the author's practice early in 1967. This move was prompted by reading a report that vitamin C had been used clinically, with notable success, in treating 12 cases of distemper complex (canine and feline) in one practice.[1]

Ten years of practice had led me to view skeptically all reports of the type cited. However, experience during those same years had made me aware that the recovery rate among my patients showing signs of CNS disturbance, and treated with the generally accepted therapeutic regimen, was a dismal 5% to 10%. With many of these patients, the prognosis appeared to be hopeless from the first examination. Many others progressed rapidly from showing signs of the distemper complex to a state of chorea followed by death.

With this background in mind, intravenous injection of ascorbic acid (250 mg./cc.), Scorbate® Injection (Burns Pharmaceuticals) was added to the course of treatment given for canine distemper in our practice.

About a year after the investigation was started, John E. Reinert, M.D., a local neurologist and neurosurgeon, became interested in the work and thereafter was associated with the study. Dr. Reinert examined many of the dogs for neurologic impairment and observed their progress after treatment. After assessing the results in dogs, he began using ascorbic acid to

treat some of his own patients, with favorable results.

During the 22 months before this paper was prepared, 67 dogs in which canine distemper had been diagnosed were treated with ascorbic acid and a running summary of their histories was kept.* The following case histories are typical examples.

Case Histories

Case No. 1

This 2-year-old male Miniature Poodle with typical signs of distemper had been under treatment for 10 days. On the eleventh day, convulsions began to occur almost continuously. Within 24 hours, the animal was semicomatose, unable to stand, and stricken with chomping and foaming seizures. During the next five days, while the dog remained in the same condition and failed to respond to treatment, the owner refused permission for euthanasia to be performed.

On the morning of the sixth day following the onset of convulsions, 1,500 mg. of ascorbic acid was given intravenously. Late that afternoon, although mildly incoordinated, the dog was standing, walking in the cage and drinking water.

By the following morning, there were no signs of incoordination and the temperature had dropped from 103 F. to 101.8 F. After a second 1,500-mg. dose of ascorbic acid was injected, the condition continued to improve. The dog drank water and ate several meals of solid food during the day. A third dose of 1,500 mg. ascorbic acid was given the next day, although by that time no signs of distemper were present.

Five days after the beginning of treatment with ascorbic acid, the dog was discharged. Weekly checkups for the next three weeks indicated a complete return to clinical normalcy. When last examined, one and a half years later, the patient was physically sound and in apparent good health.

Case No. 22

A 2½-year-old male Shetland Sheepdog had been treated elsewhere for one month. Throughout that time, this dog's temperature had remained within a range of 103 F. to 104 F. The general condition of the animal upon presentation at our hospital was classified as poor.

In addition to our standard treatment for distemper, a 2,000-mg. intravenous dose of ascorbic acid was given daily for three days. By the second day, the temperature had dropped to 102 F. from 104 F.; on the third day it was 101.6 F.

The patient was discharged on the fifth day. Recovery was uneventful.

Case No. 43

Clinical signs in this 9-month-old male Poodle were convulsions, tremors over the entire body, incoordination, and a temperature of 106.4 F.

Treatment was immediately started with 2,000 mg. ascorbic acid in conjunction with Dilantin® Suspension (Parke-Davis), Sparine® (Wyeth), atropine, and phenobarbital. Within 24 hours, the convulsions had ceased. The temperature was 101 F., and it remained normal throughout the rest of the treatment period.

By the third day, the tremors had disappeared and all medication but ascorbic acid was discontinued. After the fifth day of treatment with ascorbic acid, the patient was discharged, giving every indication of being completely normal.

Case No. 65

When presented, this 2½-year-old male Poodle had been exhibiting signs of hardpad distemper for six weeks. A slight posterior paralysis and mild incoordination were present. The temperature was 103.6 F.

After two daily doses of 2,000 mg. as-

*A tabular summary showing clinical signs, daily temperatures, dosages of ascorbic acid, adjunctive therapy and results for each patient, is available upon request to the editors.

TABLE 1: Recovery Rates among Dogs Treated with Ascorbic Acid* for Canine Distemper Complex

Patient Group	No. Treated	No. Recovered	Recovery Rate
All dogs treated	67	48	71.64%
Cases showing CNS disturbance	16	7	43.75%
Atypical cases with CNS disturbance but no convulsions	4	3	75.00%
Typical cases with convulsions	12	4	33.33%
Cases without CNS disturbance	51	41	80.39%
Typical cases with convulsions and given 3 or fewer doses of ascorbic acid	7	1	14.29%
Typical cases with convulsions and given more than 3 doses of ascorbic acid	5	3	60.00%
Typical cases without convulsions and given more than 3 doses of ascorbic acid	14	11	78.57%

*Sterbate(®) Injection (Burns Pharmaceuticals)

TABLE 2: Dogs Given Massive Doses of Ascorbic Acid over a Three-Day Period

Breed	Sex	Age	Weight	Total Dose*
Poodle—X	M	1 Yr.	16.5 Lb.	45,000 mg.
Terrier—X	F	8 Mo.	13 Lb.	45,000 mg.
Shepherd—X	F	4 Mo.	25 Lb.	45,000 mg.

*5,000 mg. ascorbic acid, Sterbate(®) Injection (Burns Pharmaceuticals) given intravenously three times a day for three days

corbic acid, the temperature was reduced to 101.4 F. After four more days of treatment with ascorbic acid, the patient was discharged.

Two and a half weeks later, the owner requested euthanasia because of a recurrence of the paresis and incoordination which were becoming progressively worse.

Discussion

RECOVERY RATES observed during the investigation are shown in Table 1. As might be expected, treatment beginning at the onset of clinical signs gave more favorable results than treatment delayed until the condition was in an advanced stage. Although relatively few animals exhibited convulsions in conjunction with the typical signs of distemper, the recovery rate for those in this group that were given more than three doses of ascorbic acid was much higher than that for those given fewer doses (60% as compared to 14%).

Temperatures were elevated in most of the 67 dogs at the time of the first examination, but in almost all cases were within normal limits at 24 or 48 hours after treatment was started. During the latter part of the investigation, when hourly temperature charts were kept, many temperatures were found to be normal within 2 to 6 hours

after the first injection of ascorbic acid.

In all instances, the ascorbic acid was administered intravenously at a rapid rate. Some drowsiness, which lasted only a few minutes, was seen in 2 dogs immediately after injection of the vitamin. However, there were no other visible side effects and no toxicity attributable to treatment. To help establish dosage and determine the possible consequence of giving large doses of ascorbic acid, 3 dogs were obtained from a shelter and given 5,000 mg. ascorbic acid three times daily for three days *(Table 2)*. No side effects were seen in any of these dogs. All three were placed in homes, and are doing well to date.

Conclusion

FROM THE results observed in 67 clinical cases of canine distemper complex, it appears that a daily dose of 1,000 mg. to 2,500 mg. of ascorbic acid given intravenously for at least three days is beneficial in the treatment of canine distemper, and that the recovery rate can be markedly improved by including ascorbic acid in the treatment regimen.

During this investigation, ascorbic acid produced a rapid drop in temperature. The recovery rate during a 22-month period was 71.64%. When more than three doses were given, the rate rose to 78.57% for dogs that did not have convulsions. When more than three doses were given to dogs that exhibited convulsions, the recovery rate rose from 14.29% to 60%.

Fully recognizing that this investigation did not constitute a controlled study, but encouraged by the results, the author has presented these observations in the hope that they will be of help to other practitioners and perhaps stimulate additional work in this area. Certainly, more basic research is needed to define the mechanisms involved and to validate the observations reported here.

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Massive Doses of Vitamin C In the Treatment of Viral Diseases

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TREATMENT OF VIRAL DISEASES presents to the physician a perplexing and frequently unrewarding problem, particularly since some 50 different diseases of man are of viral etiology. To date no generally effective therapeutic measures have been devised for treating viral diseases, although some diseases caused by the largest of the known viruses appear to be affected by some chemotherapeutic agents. Therapy with specific antisera is useful as a preventive measure during the incubation period of some viral diseases, but is generally of little value once clinical manifestations of the infection have ensued.[1] Therefore, an effective therapeutic agent that would substantially reduce the morbidity of the majority of viremias would provide the physician with a most valuable adjunct to treatment.

There have been a number of reports in the literature suggesting that infectious disease processes rapidly accelerate vitamin C depletion and greatly increase vitamin C requirement." The role of vitamin C in maintaining stability and tensile strength of connective tissue is well known. This property favors, among other things, the building of a protective barrier against infectious invasion.[4] When ascorbic acid stores are severely depleted during the course of infectious diseases, capillary resistance decreases and susceptibility to the action of certain toxins appears to increase.[2] It has been suggested that means of altering the susceptibility of cells to invasion by viruses could provide a method of controlling as well as preventing infection.[7]

Several investigators have reported employing massive parenteral doses of ascorbic acid in the adjunctive treatment of viral diseases. Klenner[3] has advocated and employed massive doses of intravenous ascorbic acid for many years in the treatment of various viral diseases including measles, mumps, chickenpox, viral pneumonia and viral encephalitis, and has reported remarkable results. Even with doses as high as 65 mg./Kg. Klenner rarely encountered any adverse effects and those were limited to the site of injection. Klenner has administered chemotherapeutic agents along with ascorbic acid to reduce secondary bacterial infection and has recommended the subsequent use of Vitamin BI following infectious diseases involving the nervous system. He further theorizes that the near absence of ascorbic acid in infectious states may be attributed to the vitamin combining with the toxin and/or virus to form a new complex which is easily destroyed by oxidation.

Free from Reaction

McCormick[4] administered ascorbic acid intravenously or intramuscularly in massive repeated doses, 500 to 1000 mg. every four hours. He reported that this approach exhibited a potent chemotherapeutic-like action in acute infectious processes which compared favorably to that of the sulfonamides or antibiotics but with the advantage of complete freedom from toxic or allergic reactions. Baur and Staub[5] reported highly satisfactory results were obtained with daily intravenous infusions of 10 gm. of ascorbic acid in 1000 cc. of isotonic saline solution administered for an average of five days to patients with infectious hepatitis. They have described the action of ascorbic acid as "virucidal." Calleja and Brooks[6] reported that daily intravenous infusion of 5 gms. of ascorbic acid for 24 days resulted in remarkable improvement in a patient with acute hepatitis when other therapeutic measures had proved futile.

Reports from German literature show

that high doses of vitamin C are beneficial in epidemic hepatitis in children. These beneficial effects were clearly observed in 63 cases of epidemic hepatitis treated with high doses of vitamin C in doses of 10 gms. daily for an average of five days given either by rectal infusion or intravenously, or both.[9]

This investigator evaluated a product trademarked Viron-1* as an adjunct in the treatment of a series of cases involving diseases of probable viral etiology. Viron is a preparation for intravenous administration consisting of 2000 mg. of ascorbic acid per dose fortified with certain B-vitamins. I was primarily concerned with patient response to this mode of therapy since time of recovery was of major economic importance to these patients. It has been my past experience that the more intense the patient's symptoms the greater the morbidity and the longer the convalescent period.

The following case histories are representative of this therapeutic regime:

Infectious Hepatitis

A 20-year-old white female hospital medical technician was first seen for the present illness on Nov. 9, 1959. The illness dates back to the spring of 1959 when she began to feel progressively weaker, exhibited malaise, anorexia, slight nausea, when it was discovered that she had an icteric tinge in her serum. She was treated with bed rest for four days and the sub-clinical jaundice disappeared with a return of her icterus index to normal.

Later in November her symptoms of malaise were intensified, she began to lose weight, became progressively weaker, and presented herself for examination. It was decided that she had clinical jaundice of a minor degree; however, the liver was not palpable and her physical examination was essentially normal.

She was hospitalized on Nov. 11 and was seen in consultation by an internist who confirmed the diagnosis of hepatitis, etiology unknown. Her admission laboratory work revealed a urine which was essentially negative, except for the presence of bile. Her heterophile antibody titer was negative; the icterus index was 13.8 units (normal being 4 to 6 for the method used); her hemoglobin level was 7.5 gms., hematocrit reading was 21%, white blood count was 13,000 with 72% polymorphs, 22% lymphocytes, 3% monocytes and 3% eosinophiles. Prothrombin time was 105%- of standard. Occult blood was found in her stool. Other diagnostic procedures including chest x-ray and gastrointestinal series were normal.

The patient was treated with bed rest for three days while confirming laboratory tests, observations and examinations were made. Her icterus index rose to 32.5 on Nov. 14. The patient's temperature remained "low grade" being 99.2-99.4 orally at the highest points. After a period of complete bed rest and high carbohydrate diet, the diagnosis was confirmed by the internist, a second consultant, and this clinician. At no time in her illness did she receive chemotherapeutic agents.

Dramatic Improvement

The administration of Viron-1 was initiated and she received six intravenous 10 cc. injections during the remainder of her hospital stay. Following the second injection of Viron-1 the patient was amazed with her progress and remarked that she had lost the feeling of "being sick." She wanted to go home within 24 hours after Viron-1. injections were initiated, but hospitalization was continued. She was dismissed on Nov. 20, 1959, markedly improved in subjective feeling and dramatically improved clinically.

The patient was seen in my office on Dec. 1, 1959 at which time her white count had dropped to 7,000 with 53 % polymorphs, 37% lymphocytes, 3% monocytes and 4% eosinophiles. Hemoglobin level was 12.8 gms. and her icterus index had dropped to 8.0.

There is no question in the mind of this investigator that the intravenous administration of Viron-1 had a profound therapeutic effect upon this patient. She had obtained minimal benefit from complete bed rest and high carbohydrate diet before the administration of Viron-1. She outwardly

* Viron-l was supplied by Lincoln Laboratories, Inc., Decatur, 111.

exhibited, and freely discussed with the attending physicians, her feeling of well-being following the administration of intravenous Viron-1. An accurate diagnosis of the exact type of hepatitis was impossible. It was assumed to be viral in nature; however, it may well have been a toxic condition. Other than the academics involved, the exact etiology is relative. The important factor to consider is that she responded to Viron-1 in a most satisfactory manner and one cannot but assume that the medication exerted a profound effect upon her progress.

Past experience with hepatitis of various etiologies has given this observer the impression that recovery from hepatitis, regardless of etiology, is extremely slow and painstaking. The rapid and complete response of this patient to Viron-1 has not been observed following classic and accepted therapeutic measures for treating hepatitis. It is difficult to comprehend a set of circumstances that would coincidentally explain the marked and rapid improvement in a patient as sick as this girl. It was certainly the most dramatic recovery from hepatitis that I have ever observed.

Infectious Mononucleosis

A while female, age 36, complained of generalized aching, exhaustion, anorexia and malaise. Her physical condition prior to these symptoms had been normal. Fever, remittent in type, accompanied the symptomatic complaints. A complete blood count revealed large vacuolated lymphocytes. A positive heterophile antibody titer of 1:226 was recorded. A diagnosis of acute infectious mononucleosis was made and intravenous Viron-1 therapy was initiated. Clinical and subjective response to three consecutive daily 10 cc. injections was excellent. Symptoms remitted in one week following beginning of therapy. The overall morbidity was reduced beyond expectation for the diagnosed condition. The medication was well tolerated and no adverse side effects were noted. The rapidity of patient response to Viron-1 was dramatic since full recovery from infectious mononucleosis rarely takes place in less than two to three weeks in my experience.

Virus Pneumonia

A 60-year-old male physician presented himself with a history of excellent health except for his present illness. His symptoms were exhaustion, cough, low grade fever, anorexia, generalized aching and profuse sweating upon exertion. Viral pneumonia—patchy type—of the right upper lobe was found and confirmed by x-ray findings. Treatment consisted of 10 ce. intravenous Viron-1 for three days, bed rest, and ASA Compound. The response was excellent—strength returned on the fourth day and on the fifth day the physician returned to work. The I. V. Viron-1 was well tolerated and no untoward side effects were observed. Viron certainly shortened the expected morbidity for a case of this nature.

Acute Viral Type Pneumonia

A female, age 47, was in excellent general physical condition with exception of chronic bronchiectasis. When first seen for her present illness this woman was completely debilitated. She was confined to her bed and complained of exhaustion, anorexia and generalized chest pain. Temperature elevation ranged from minimal to normal. A diagnosis was made of acute viral type pneumonia with secondary bacterial involvement of sinus and bronchial tree. She was given intravenous Viron-1, 10 cc. injections, on Oct. 26, 27 and SO and Nov. 3, 6, 9,1959. No other medication was utilized. Patient felt better after the second injection of Viron-1 and insisted on continued therapy. Her exhaustion syndrome continued to show remarkable improvement. Progress was continuous and the administration of Viron-1 markedly reduced morbidity as compared to her previous recurrent pneumonias. She tolerated the injections well and no adverse side effects were observed.

Viral Pneumonia and Bronchitis

A male, age 41, was in good physical condition except for the present illness and recurring pain from a herniated lumbosacral disk. He complained of headache, generalized muscular aching and exhaustion. His temperature was 100°-100.4° orally. The diagnosis was acute viral pneumonia and

bronchitis, following acute sinusitis. Injections of intravenous Viron-1, 10 cc., were given on July 14, 15, 16, 1959. The patient was seen for follow-up examination on July 23 and was symptom free. He had experienced marked relief both from sinusitis and viral pneumonia symptoms and had returned to work on fifth day following therapy without my permission. The morbidity period in this case was definitely shortened beyond expectation. Viron-1 was well tolerated by the patient and no side effects were observed.

Generalized Viremia

This male, age 72, was in fair general physical condition. Patient complained of "feeling bad", hoarseness, exhaustion and depression following "influenza." His temperature was normal, but he had a persistent cough. I made a diagnosis of generalized viremia with bronchitis and right recurrent laryngeal neuritis. Viron-1 was given intravenously on Oct. 28, 30 and Nov. 6, 1959. He experienced a relief of symptoms and felt better. Marked improvement in symptoms of viremia were observed. The medication was of questionable benefit to the neuritis. Viron-1 was well tolerated—no untoward side effects were observed.

Summary

In these selected six cases of probable viral infections, Viron-1 promoted prompt patient response. In four of the above mentioned cases improvement was especially rapid and dramatic. The patients were of different groups and conditions treated were varied. Of significant interest is the shortened morbidity period observed when Viron-1 was given either singly or in conjunction with other therapy. No untoward side effects were observed.

Conclusion

In the experience of this investigator daily doses of 2000 mg. of ascorbic acid fortified with B-complex vitamins given intravenously provides a valuable adjunct in the routine management of a variety of acute viral infections. Further investigation is warranted to determine the complete range of viral diseases which can be treated beneficially with this therapeutic adjunct.

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Massive Doses of Vitamin C and the Virus Diseases

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IT has been reported that one of the mold-derived drugs, in addition to being a good antibiotic, is a super-vitamin Conversely, we argue that vitamin C, besides being an essential vitamin, is a super-antibiotic. Vitamin C in vitro, if maintained at body temperature, inactivates certain toxins at an unbelievable rate. Five parts per thousand of vitamin C with toxins and appropriate controls, incubated at 37° C. for 48 hours showed when tested on mice the minimal lethal dose for the control tubes to be 1 16,000 c.c., while that from the mixture of vitamin C and toxin was only 1/1,000 of a c.c. (Klegler, Guggenheim, Warburg, 1938). In this study the loss of vitamin C in toxin broth and ordinary broth controls followed a constant pattern: the loss, however, was always greater in the toxin broth tube. The difference between the rate of disappearance of vitamin C in toxin and ordinary broth was more striking the greater the concentration of vitamin C. It is. therefore, reasonable to conclude that the degree of neutralization in a virus infection will be in proportion to the concentration of the vitamin and the length of time in which it is employed.

Since it has long been known that the virus organism resembles more the toxins and ferments than the common animate causes of disease, it would seem plausible that the detoxication effected by vitamin C is produced by a direct combination of the vitamin with the toxin and/or virus, this followed by the oxidation of the new compound which destroys both the virus and/or toxin and the vitamin. This destruction of the virus by oxidation has been concurred in by many investigators. Since vitamin C is an integral part of the oxidation-reduction system of the body, its function in the role of an antibiotic becomes intelligible. To appreciate the antagonistic properties of vitamin C against the virus organism and the chemical ferments of exotoxin-producing microorganisms, one must forget its present academic status as a factor essential for life. A cow is valuable to the farmer not only for her ability to produce milk, but also as a source of organic fertilizer. Vitamin C, likewise, is important, not only as a detoxifying agent, as a catalyst aiding cellular respiration by acting as a hydrogen transport, as a catalyst in the assimilation of iron, and as a conservator of collagen fibers and bundles in tissues of mesenchymal origin; but, also, because of its function as a reducing agent or the precursor of such a substance. In this latter capacity it fulfills the requirements of an antibiotic. A striking phenomenon of vitamin C is the similarity of response, whether to correct pathologic processes due to a deficiency of this compound, acting as a vitamin; or to destroy the ferments of microorganisms, acting as an antibiotic. Within a few hours after institution of adequate vitamin C therapy to correct an avitaminosis, his-

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tological evidence of bone improvement is obtainable Fibroblasts begin to form normal connective tissue and capillary buds are invading hemorrhagic areas (Youmans, 1941). Similar is its dramatic antibiotic action, the rule being clear evidence of clinical response within a few hours.

The purpose of this paper is to present clinical proof of such action for this vitamin.

Case I is one of premeasles in a ten-months-old baby. The term "premeasles" is adopted to express the syndrome of fever, redness of eyes and throat, catarrh, spasmodic bronchial cough and Koplik spots. Vitamin C, 65 mgm. per Kg. of body weight, was injected intramuscularly every four hours. The fever dropped from 105 to 97.6° F. within 12 hours. All symptoms showed marked clearing. This sudden drop in the fever was thought to be explainable on one of three grounds: 1) Common right drop. 2) Due to the antibiotic action of vitamin C. 3) Even if the vitamin C administration had been continued, possibly a moderate rise would have occurred in the late afternoon of the second day, granting a highly virulent organism and a poorly resisting host. To determine which of these deductions was valid, vitamin C was discontinued for a period of eight hours. At this point the rectal temperature was back up to 103.4. Vitamin C therapy was resumed and instead of the expected 8 P M. climb, the temperature was down to 99.2 (R) eight hours later. The vitamin C injections were continued, the baby made an uneventful recovery and was discharged 60 hours following admission. No measles rash developed. Eighteen months have elapsed since this illness and the child has not had clinical measles. This is not due to the establishment of active immunity but to the lack of a second exposure.

Case 2 confirms the previous case. This case is that of a 22-months-old infant with symptoms identical with that just described. The same medication was followed; the same clinical course followed. Under parental pressure the child was discharged from the hospital within 36 hours, apparently well. Four days later the child's brother and sister broke out with measles, which ran the usual course, having received no specific therapy. Seven days later the 22-months child broke out with measles. This time vitamin C was not given. The case was judged as modified.

The response as observed in measles was characteristic for vitamin C *versus* virus infections. Two cases of virus pneumonia complicated by encephalitis were so unusual that case histories are given.

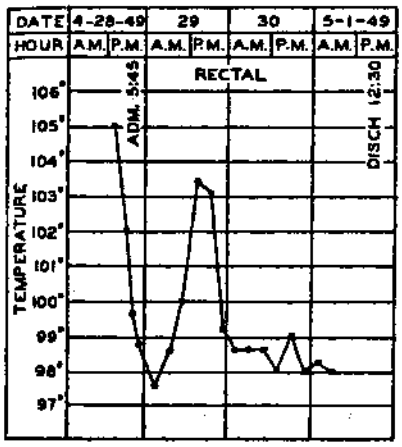
Case 3 is that of a colored woman, aged 28. with history (given by a relative) of chills and fever and chest and head cold for 14 days, severe headache for three days. In stupor when first seen, eye lids closed, a white foam at the mouth which she periodically tried to spit out. Temperature by axilla 106.8. Dehydration was much in evidence, breath sounds diminished to absent, tactile fremitus increased over the entire right lung. The sulfa drugs, penicillin and streptomycin with supportive treatment had been exhausted. Four grams of vitamin C was given intravenously along with 1000 c.c. of 5 per cent dextrose in saline solution. Temperature dropped to 100 (Ax.) within 11 hours. Four hours later, vitamin C was resumed—every two to three hours, in dosage of 2 to 4 grams depending upon the response. After 72 hours the patient was awake, sitting up in bed and taking fluids freely by mouth. There was no fever at this time, nor for the remainder of the time in hospital. Vitamin C was continued for a period of two weeks; the frequency was cut to every 12 hours, two grams at a dose. An interesting complication was deafness; her speech gave a loud, monotonous, bell-sound effect. It was debated whether this was the result of the streptomycin or to the encephalitis. Prostigmin 1:2000, 1 c.c., and vitamin B1, 200 mgm., were given IM twice daily. On the tenth day of treatment the hearing suddenly returned to normal. The x-ray picture of the right lung was one of almost complete consolidation. Although the patient was clinically well of her pneumonia after 72 hours, the x-ray picture was not completely clear until 90 days later.

This phenomenon of Nature clearing the debris after killing out the virus organism was observed in five other cases. The time required was in direct proportion to the degree of pulmonary involvement. There is nothing new about this procedure; Nature merely duplicating a stage in the metamorphosis of the frog in getting rid of its tadpole tail.

Case 4. that of a white baby 19 months old, bothered with a little cold for two weeks, not very sick until the last 24 hours, in which the baby had been "runnings high_fever that could not be_broken with aspirin." Clonic convulsive seizures of the right arm and leg began 12 hours before admission. An undernourished infant, lying rigid in its mother's arms, skin cold to touch, color cadaver-like, eyes closed, grade -2 mucopurulent nasal discharge, throat red. The temperature was 103.8 (R). Breath and heart sounds practically inaudible. Areas of skin over the back presented an appearance similar to that seen in rigor mortis.

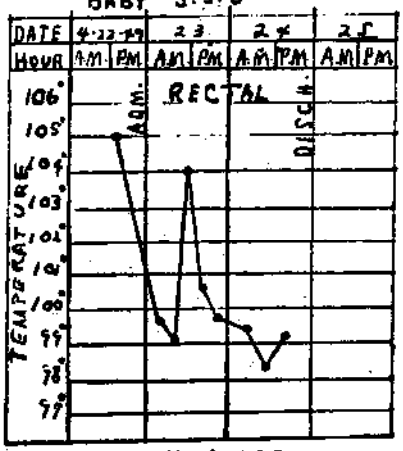
Vitamin C, 1000 mg., was given IM. repeated every four to six hours. At the first injection the baby did not move and the sensation was like that of sticking an orange. To give rapid external heat, mustard plasters were applied to the anterior and posterior chest in a mixture of one part mustard to three parts flour. A croup tent was set up. the vapor carrying compound tincture benzoin; 50

BABY D. S (29753)



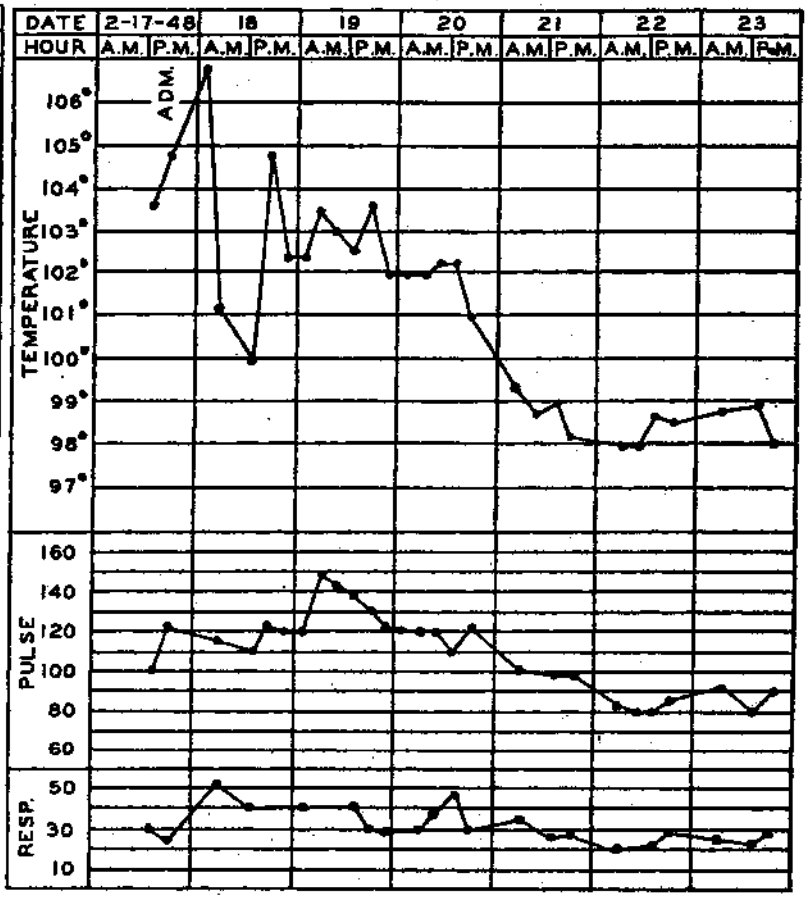
MEASLES

BABY J.S.D



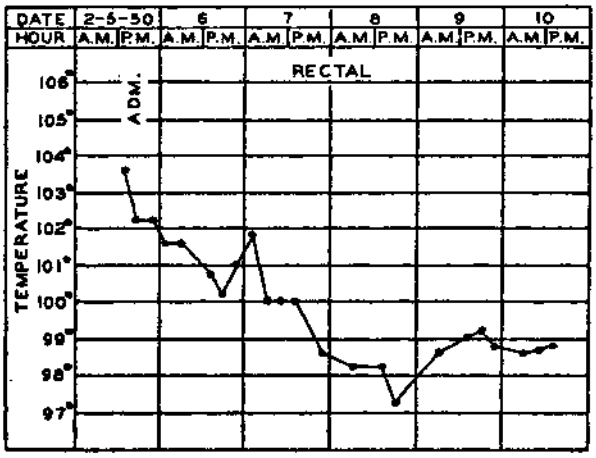
MEASLES

M. H. (26047)



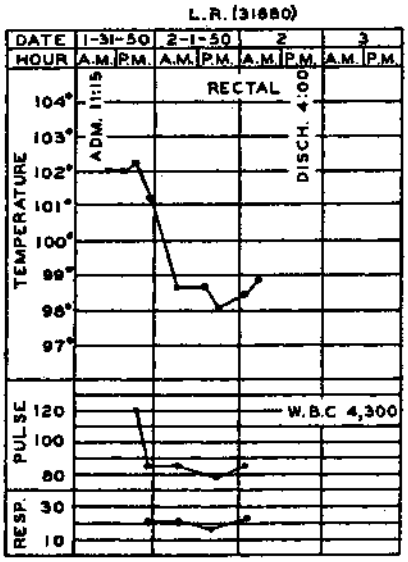
VIRUS PNEUMONIA

BABY W. M. (32219)



VIRUS ENCEPHALITIS-MENINGITIS

L.R. (31880)



VIRUS INFECTION (PULMONARY)

c.c. of 5 per cent dextrose in saline was given under the skin in the scapular areas. Two hours after the first injection of vitamin C the baby drank 240 c.c. of orange juice, the first food of any type taken by the baby in 24 hours. This was repeated $1^{1}/_{2}$ hours later. At this time there was total paralysis of the right arm and leg. Twelve hours after admission the baby moved ks right leg and one hour later grasped a bottle of orange juice with both hands. From this point on the recovery was uneventful. Of secondary importance is the laboratory report of Ascaris lumbricoides ova and hemoglobin 55 per cent.

Cases 5 and 6 are of pulmonary virus infection, (a) in a boy of 14 years, and (b) in a man of 58 years. In the case of the boy the fever curve was of the type showing a fast response to heavy vitamin C injections. The WBC was 4,300, urine sugar ++ Twenty-six grams of vitamin C was given IV to this patient in a 44-hour period.

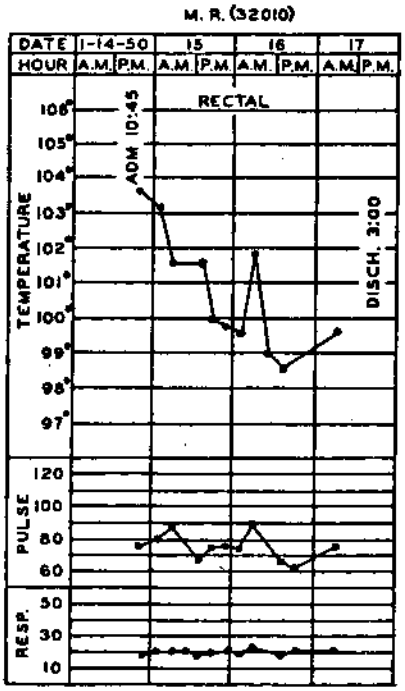
In the case of the man, Case 6, the fever decline was after a modified step-ladder fashion. In this instance the amount of vitamin C injected was less than half of the recommended dose. The WBC was 5,850, admission urine sugar +++. Thirty-one grams of vitamin C was injected intravenously over a period of 60 hours. It is to be noted that the same amount of vitamin C (2 grams every four hours) was given to the boy and to the man, disregarding the factor of body weight. Had the man received four or five grams every four hours, or two grams every two hours, his hospital course would probably have followed the same pattern as that of the boy. A point of great interest was that at subsequent examinations the urine was consistently negative for sugar. The course in these cases emphasizes the necessity of administering massive doses of vitamin C at frequent, regular intervals so as -to maintain the proper level of this antibiotic in the tissues.

The amount of vitamin C for optimal effect will vary greatly with the individual The type of the disease and the degree of toxemia are important guides in determining the dosage. Although the usual dose of vitamin C is calculated on the basis of 65 mgm. per Kg. of body weight, and given every two to four hours by needle, under certain conditions larger single injections can be used to good advantage. Vitamin C given to a child with measles, mumps or chickenpox will abort or modify the attack, depending upon the intensity of the treatment. If the activity of the pathogen is stopped, the development of active immunity will be interrupted. In handling these particular childhood diseases, when uncomplicated, the treatment should be aimed at modification of the infection as the plan of choice. To accomplish this end vitamin C should be increased to 250 mgm. per Kg. of body weight, and the injection given intramuscularly. It will be necessary, at .times, to repeat with half of this amount eight hours later. The vitamin was given in a concentration of 500 mg. per c.c. of solution. Pain was slight and lasted only a few minutes. Procaine, 0.5 to 2 per cent, instilled from a second syringe into the gluteal muscle through a placed needle just before giving the vitamin might solve this problem. The itch of measles and of chickenpox, the occasional vomiting of these illnesses, and the pain of mumps were fully controlled within one hour, when 250 mg./Kg. body weight was used. Instead of repeating waves of macules in chickenpox, and the usual seven to nine days required for crusting, following the heavy modifying injection no new eruptions appeared and crusting was present within six hours. Further clinical studies may prove that the routine use of the higher dose (250 mg./Kg. body wt.) replacing the usual (65 mg./Kg. body wt.) is indicated in all virus infections and the results produced may be even more dramatic.

The greatest value of vitamin C in virus infections does not rest with these lesser kinds of diseases, some of which, e.g. measles, can be modified or prevented by the proper use of immune globulin. The value above all others is its positive action against the virus causing poliomyelitis. A report of this usage was published in the official journal of this association in 1949. Many physicians refuse to employ vitamin C in the amounts suggested, simply because it is counter to their fixed ideas of what is reasonable; but it is not against their reason to try some new product being advertised by an alert drug firm. It is difficult for me to reconcile these two attitudes. On the other hand, many physicians who have been willing to try vitamin C against the virus of poliomyelitis have obtained the same striking results as we reported. Scores of letters from practitioners here in the United States and in Canada could be presented in evidence. In some instances doctors have cured their own children of poliomyelitis by giving vitamin C and in other cases doctors themselves have been cured-

In poliomyelitis vitamin C performs three important functions: 1) It destroys the virus; 2) acting as the dehydrator and diuretic of first choice, it removes the edema fluid from the brain and the cord; 3) it preserves the lining of the central canal and maintains more regular spacing and less crowding of the ependymal cells (Altman). The pressure within the bony vault of the central nervous system resulting from the inflammatory process excited by the virus, acts as a haemostat to cut off the blood supply to the anterior horn cells. This compression of their vessels denies to the horn cells the essentials for function, for life even.

It is of more than academic interest to review



VIRUS PNEUMONIA

the findings of McCormick in 50 confirmed cases of poliomyelitis in and around Toronto, Canada, during the epidemic of 1949. This report is that children of families eating brown bread who came down with poliomyelitis did not develop paralysis; whereas in those families eating white bread many of the children having poliomyelitis did develop paralysis. The point here is that brown bread has 28 times more vitamin B_1 than does white bread Obviously, then, the paralysis which complicates acute poliomyelitis appears to be due to a B_1 avitaminosis. Vitamin C by removing edema fluid relieves from pressure these vessels that supply nutriment to the horn cells, thus allowing the normal complement of vitamin B_1 to reach these cells. In December, 1949, a 5-year-old white girl was brought to my office with paralysis of both lower extremities of 4½ days' duration. The child had been ill for 12 days. There was complete flaccid paralysis of the right leg, 85 per cent paralysis of the left leg. Pain was directed to the knee and to the lumbar back. In hospital the diagnosis of poliomyelitis was confirmed by four consulting physicians. Spinal fluid cells were 82. No medication of any type was given exclusive of vitamin C. Massage was started immediately. The rationale of using early massage had two bases: 1) In the course of general practice patients would give a history of having had poliomyelitis when a child and that their mother rubbed the paralyzed member day and night until function returned. 2) That paralyzed muscle was in profound shock and "artificial respiration" would maintain proper metabolism during .the emergency phase. To the first injection of vitamin C there was definite response. After 96 hours the child was moving both legs. The flexion was slow and deliberate. She was discharged from the hospital at this time, vitamin C being continued by mouth—1000 mg. every two hours with fruit juice for seven days. On the llth day of treatment the child was walking about the house, but her gait was slow and her posture was poor, being bent forward. Vitamin C was discontinued and vitamin B_1 started—10 mg. before meals and bed hour- Carbonated drinks were encouraged for their sugar content and mild stimulating action. Nineteen days after starting treatment there was complete return of sensory and motor function which has persisted to this date.

A boy of eight years was brought to my office with a history of having had "flu" for a week, and four days previously having developed photophobia, conjunctivitis, sore throat, nausea, vomiting and a back-of-the-eyes type headache of such intensity that adult doses of aspirin had no effect. The boy was either rubbing his neck on the left side or holding his head between his hands, begging for something to relieve his pain. The fever was 104.4 (Ax.) He was tender in the lumbar region and he had a drawing sensation referred to the hamstring attachments at the knee. Two grams of vitamin C was given IV while in the office. He was then sent to the local hospital where he received promptly a second injection of 2 grams of tjie vitamin, after which it was given every four hours. Six hours after commencing therapy the neck pain was gone, the headache completely relieved, he could tolerate the ceiling light, his eyes were dry and the redness clearing. Nausea and vomiting had disappeared, the fever was down to 100.6 (Ax.), and he was sitting up in bed in a jovial mood while he drank a carbonated beverage. He was discharged from the hospital after receiving 26 grams of the vitamin in a 48-hour period, clinically well. Vitamin C was continued by mouth, 1500 mg. every two hours with fruit juice for one week, then change was made to vitamin B_1, 25 mg. before meals and bed hour. Vitamin B_1 in these cases should be continued for a period of no less than three months as nerve tissue is slow in recovering from damage.

In using vitamin C as an antibiotic minor complications were occasionally seen. These fall into six groups: 1) Diarrhea in two cases. In each instance the preparation contained sodium bisulfate. The enteritis cleared on giving a preparation of vitamin C not containing this salt. 2) Induration in 42 cases—seen either immediately following the injection (allergy), or delayed. In the latter it was found that the injections were being given too close to the surface. Applications of warm magnesium

sulfate as a. compress gave prompt relief of the pain -and swelling. In two of these cases fluctuation ensued and healing was effected by surgical drainage and the application of compresses. The impression in these two cases was that a vein had been opened by the needle. The exudate was dark and both the slide and culture studies were negative for bacteria. 3) Endothelial irritation in three cases. Acute pain radiated from the site of the injection to the shoulder. In each instance the concentration of the vitamin was one gram to each 5 c.c. solution and the amount given exceeded two grams. After slowing the rate of injection this reaction did not occur. 4) Venous thrombosis in one case. The concentration was 500 mg..per c.c. solution; the total dose 5 c-c. Compressing relieved the pain. The pathology was very similar to that following the use of 50 per cent dextrose solution. 5) Syncope—In maximum doses given IV a sensation of fainting and dyspnea occurred seven times. Five of these patients were over 55 years of age. The disagreeable symptoms were relieved by slowing the speed of the injections. 6) Rash—In three cases a pin-point dermatitis occurred, limited to the face and upper third of the torso, identical to that seen in infants taking orange juice. This did not necessitate discontinuance of therapy and cleared spontaneously several days after vitamin C was stopped.

Calcium, in vivo, duplicates the chemical behavior of vitamin C in many respects. Calcium gluconate and calcium lexulinate were used in conjunction with vitamin C therapy in a small series of pulmonary virus infections and in mild cases of influenza. There was a definite synergistic response. Patients with colds derived most benefit from this combined treatment. Because of its action on cardiac muscle, the use of calcium was limited to adults and the amount injected to two grams per day- One gram administered IV at moderate speed will so slow the heart as in many cases to produce syncope. If the concentration becomes great enough cardiac arrest in a tonically contracted state might result. It is, however, quite possible that, with the proper ionic balance of oalcium and vitamin C in the same solution, larger amounts could be given without side effects. The massive dose schedule limits the usefulness of the calcium ion in virus diseases to that of an adjuvant only.

In all of the cases of virus infection reviewed in this study one laboratory finding stood out as of great significance. On admission to the hospital the first routine urine examination showed some degree of glycosuria. The pattern of the qualitative Benedict's reaction was constant enough to postulate that the higher the reading the more severe was the pathology. Repeat urine sugar studies following vitamin C therapy revealed complete clearing. This was true even though fruit juices were forced to tolerance. This finding confirmed the knowledge that interference with the normal physiology of the adrenal glands, either by the toxins produced by microorganisms or by surgery, has a profound influence on metabolism, especially of the carbohydrates. Adrenalin in the blood stream causes hyperglycemia with resulting glycosuria. Adrenalin acts either by stimulation of the sympathetic nervous system or directly via the blood. This action of adrenalin is via the blood only, because the effect, as demonstrated in experimental animals, is still realized after destruction of the cord and sympathetic plexuses and degeneration of the peripheral post-ganglionic fibers (Evans, 1930). The glycosuria found in these cases was not due to a lowering of the threshold for sugar excretion by the kidney, paralleling a phloridzin diabetes, since the carbohydrate mechanism was associated with a hyperglycemia (Zuelzer, 1901, Metzger, 1902, Paton, 1903). Likewise there was no evidence of kidney damage. Albumin was reported negative and the microscopic examination showed no cells or casts. Apparently this is a condition of artificial diabetes mellitus, which would suggest the answer for the diabetic who loses ability to maintain sugar-insulin balance when embarrassed with an acute infection.

The story of a 7-year-old boy may have a lesson. He has been known to be diabetic since the age of four years. Any incident of infection in this lad produced an alarming interference of his sugar-insulin-diet equilibrium. Recently he contracted measles, and as the disease process developed toward its height the urine sugar curve swung sharply upward. From an occasional dose of 5 units regular insulin his requirement rose to 30 units regular insulin, three times each day, while still running a 3- or 4-plus Benedict's test. (Other forms of insulin proved by trial to be too dangerous.) At the peak of his infection vitamin C was started in a modifying dose of one gram every four hours. His general condition soon improved and in the course of several days he returned to his usual diet-insulin schedule and his usual urine sugar. In patients with diabetes, vitamin C should be discontinued just as soon as the temperature returns to normal. Prolonged use of vitamin C might prove undesirable due to its dehydrating and diuretic powers.

The pathologic process at work here is only compatible with abnormal amounts of adrenalin in the blood stream. It is not a response to an emotional stimulus to the adrenal medulla, since free adrenalin in the circulating blood has a transitory action, being so rapidly oxidized that none gets into the urine. This suggested that the regulator of the adrenalin mechanism had been removed, so that a constant supply of adrenalin would be present in the blood, making possible a concentration sufficiently high to cause constant vasoconstriction.

Ritzmann (1909) found that adrenalin affected carbohydrate metabolism only when this vasoconstriction phase existed. This finding was concurred in by Lusk (1914), who further concluded that ihis action on blood vessels caused asphyxia of the tissues which tended to increase the acidity of the blood and the tissues. This superimposed acidity further promotes the production of .adrenalin hyperglycemia (Peters and Geyelin, 1917). McDannell and Underbill (1919), studying these phenomena in rabbits, found that slight hyperglycemia could be controlled by the administration of sodium carbonate.

The rationale of forcing fruit juices in the old treatment of colds was based on this theory as postulated by Hawley et al. (1936) that a highly alkaline urine would have lower amounts of vitamin C than a highly acid urine; the alkaline ash from the organic acids serving to retain the vitamin C in the blood and tissues where Nature had assigned it to guard against the many enemies of the body—the toxins and ferments of bacteria. As a result of avitaminosis C, liver glycogen is mobilized—glycogenolysis; and further storing of sugar ir the liver is prevented—glycogenesis (Mackenzie, 1917). To further enhance the hyperglycemia this vasoconstriction brings *about* a decrease in the pancreatic secretions by lessening the amount of blood passing through the gland {Mann and McLachlan, 1917).

That the adrenal glands and vitamin C are closely allied in the defense of the body has been proven by experimentation and by autopsy. In normal persons any excess of vitamin C is excreted in the urine. In persons suffering with an acute infection, particularly a virus infection, vitamin C is riot only absent from the urine but is also missing from the blood serum. This is true even when moderate amounts are given intravenously. These observations on serum were made with a Klett-Summerson photoelectric colorimeter using the method described by Mindlin and Butler. The observations on the urine were conducted according to the instructions of Goldsmith and Ellenger. Harde and Benjamin (1934-35) found the vitamin C fraction of the adrenal glands greatly reduced in monkeys killed or paralyzed by the virus of poliomyelitis. Yavorsky, Almoden and King (1934) reported identical findings in humans having died of various infectious agents.

This gives us an important concept of the value of vitamin C in virus diseases. The explanation for the absence of vitamin C in the infectious states is that this agent joins with the toxin and/or virus to form a new compound which is then destroyed by oxidation. Since the body is dependent on food for vitamin C to meet its daily needs, it is obvious that the body tissues would soon be depleted, and we would expect to find evidence of a prescorbutic state in patients who had hypovitaminosis C. In patients seriously ill with a virus invader, the added strain on the capillaries by the application of a tourniquet, even for a few seconds, produced petechial hemorrhages at the site of constriction, bince not all patients thus demonstrated this capillary weakness, all .patients ill with a virus infection were investigated by the aid of a petechiometer. Increased capillary fragility was found to exist in all cases, and the number of petechiae as expressed in centimeters of mercury followed the urine sugar findings. This deficiency syndrome was reversed as the glycosuria cleared, indicating that both were responsive to a proper plasma level for vitamin C.

At this same time the anaerobic conditions in the tissues will be relieved by the catalytic action of vitamin C acting as a gas transport to aid this cellular respiration. The abnormal acidity of the blood and tissues will be removed and abnormal amounts of free adrenalin will disappear from the blood stream. Following this the constriction of the blood vessels will cease, 'allowing the liver and pancreatic tissue to return to nftrmal function. Continuance of frequent injections of properly calculated doses of vitamin C will restore the normal physiology of the body. This is not all of the story.

Lojkin (1937), studying the various phases of the inactivation of crystalline tobacco mosaic virus by 1-ascorbic acid, suggested that the action was not due to reduced vitamin C nor to the irreversibly oxidized dehydroascorbic acid. Lojkin felt that il was due to a specific intermediate product which is formed in the course of the catalytic auto-oxidation of vitamin C, an action stimulated by the presence of copper ions. This intermediate product must .be a peroxide because a peroxide is formed during copper-catalyzed oxidation of vitamin C. This peroxide is decomposed as rapidly as it is formed (Barrow, De Meio, Klemperer, 1935-36). Lyman and associates (1937) confirmed the peroxide theory by observing that the oxygen uptake, beyond that calculated for the reaction ascorbic acid to dehydroascorbic acid, was not due to further oxidation of dehydroascorbic acid to an irreversible oxidation product, because treatment of the oxidized solution with hydrogen sulfide gave complete recovery of the ascorbic acid. These men also found that copper catalysis accelerates not only the reversible oxidation of vitamin C, but also further oxidation of dehydroascorbic acid. This action of the copper ion elucidates the findings that vitamin C in massive, frequent doses works better in the body than in a laboratory test tube.

Hippocrates declared the highest duty of medicine to be to get the patient well. He further declared that, of several remedies physicians should choose the least sensational- Vitamin C would seem to meet both these requirements.

NOTE:

PubMed gives a different
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compared with the one printed:

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J Nutr. 1997 May;127(5 Suppl):962S-965S. doi: 10.1093/jn/127.5.962S.

Oxidants and antioxidants in viral diseases: disease mechanisms and metabolic regulation.

Peterhans E[1].

Author information

Abstract

Reactive oxygen and nitrogen metabolites play a complex role in many diseases and in metabolic regulation. Because viruses replicate in living cells, such metabolites influence the growth of viruses in addition to serving as a host defense mechanism. Low levels of reactive oxygen species (ROS) play a role in mitogenic activation, and the early phase of lytic and nonlytic virus infection indeed resembles that of mitogenic cell activation. In addition to these subtle cell-activating effects shared by many viruses, influenza and paramyxoviruses activate a respiratory burst in phagocytic cells. These viruses are toxic when injected in animals. Cells lavaged from the lungs of mice infected with influenza virus are primed for enhanced superoxide generation. Moreover, xanthine oxidase is enhanced and the buffering capacity of small molecular antioxidants is decreased in the lungs, suggesting that infection leads to oxidative stress. The wide array of cytokines produced in the lungs during influenza could contribute to the systemic effects of influenza. Oxidative stress has also been shown in human immunodeficiency virus (HIV) infection in humans. Via activation of NF kappa B, ROS may activate viral replication, but oxidants are believed to contribute also to the loss of CD4 T cells by apoptosis. Antioxidants, together with agents interfering with the harmful effects of cytokines and lipid mediators, may have a role in the treatment of viral diseases. Such agents could not only alleviate disease symptoms but also

decrease the long-term effects of chronic oxidative stress, which have been linked to the development of cancer in some viral infections.

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Paul Meier A Man Behind the Method

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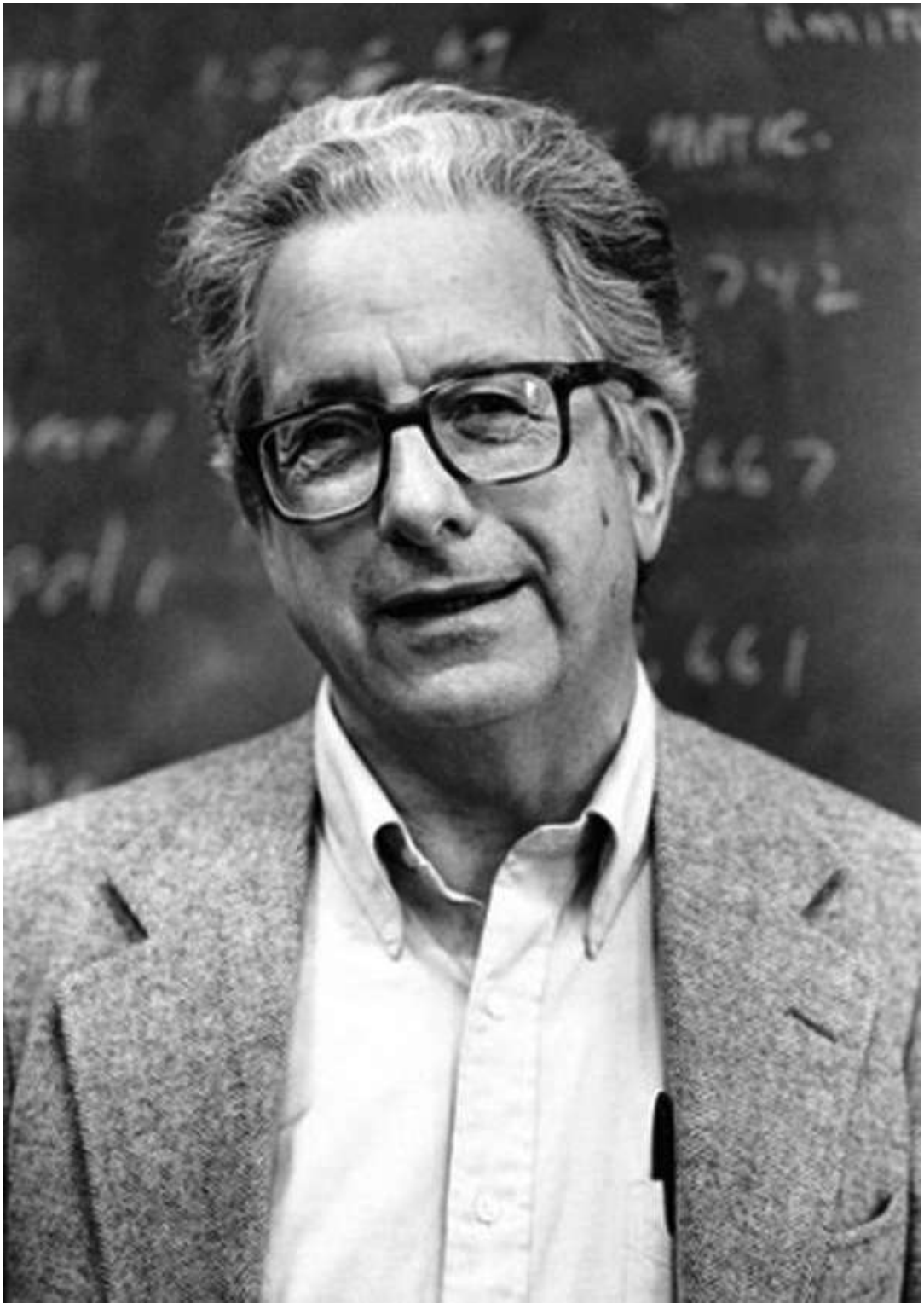

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Paul Meier. Courtesy of the University of Chicago. Printed with permission.

IN 1951, WHEN PAUL MEIER received his doctorate in mathematics from Princeton University and became one of the first statisticians to enter medical research, potential new medical treatments were evaluated in a very different fashion than they are today. At the time, researchers commonly followed practices such as giving a new remedy to patients they thought might benefit from it and comparing the outcomes with other patients who were not treated. In other situations, patients who stopped taking a new medicine might be counted as controls who had never been exposed to it.

PAUL MEIER HAD A PROFOUND IMPACT on how clinical trials now evaluate the efficacy of new drugs and treatment methodologies throughout the world. Meier's tireless promotion of the now-standard practice of randomly assigning patients enrolled in clinical trials to receive either the conventional remedy or the new treatment being evaluated helped ensure its current status as the most rigorous way to gather evidence of a new drug or treatment's effectiveness. Meier also helped formulate the Kaplan-Meier estimator, which is now the most popular method of estimating clinical trial participant survival. It is one of the most widely cited articles in the medical literature.

Meier, who died on August 7, 2011, at the age of 87, had a profound impact on how clinical trials now evaluate the efficacy of new drugs and treatment methodologies throughout the world. Meier's "many published works and writings have had a huge influence on the application of statistics to medical research—particularly the design, conduct, and analysis of randomized clinical trials and in the advancement of evidence-based medicine in general," according to the Society of Clinical Trials, which Meier helped found in 1978.[1]

Meier was tireless in his promotion of the now-standard practice of randomly assigning patients enrolled in clinical trials to receive either the conventional remedy or the new treatment being evaluated. This is now considered the most rigorous way to conduct a study and the best way to gather evidence of a new drug or treatment's effectiveness. "Perhaps more than any other U.S. statistician, [Dr. Meier] influenced U.S. drug regulatory agencies, and hence clinical researchers throughout the U.S. and other countries, to insist on the central importance of randomized evidence," said Sir Richard Peto of Oxford University, who was also a leading advocate for randomization, in Meier's *New York Times* obituary.[2] "That strategic decision half a century ago has already saved millions of lives, and those millions should be attributed to Paul," Peto said.

"I defended randomization every chance I got, and I had a fair number of chances," Meier said in a 2003 interview in the journal *Clinical Trials*.[3](p137) "For a fairly long time randomization was not thought of so highly," he explained. He said that in 2001,

> *a very distinguished statistician told me that I had a major influence on the Food and Drug Administration's policies on randomized clinical trials. I don't know how true that was, but if so, it would be something of which I am very proud,*

adding that his success in encouraging the use of randomization in clinical trials is the achievement he prized most highly.[3](p137)

Together with Edward L. Kaplan of the California Radiation Laboratory, Meier also helped formulate what the Society for Clinical Trials terms "our most popular method of estimating survival functions from continuously observed data."[4] Published in the *Journal of the American Statistical Association*[4] in 1958, it went on to become one of the most widely cited articles in the medical literature. At the time of Meier's death, the Kaplan-Meier article had been cited more than 34000 times. Theodore Karrison, PhD, director of the University of Chicago Department of Health Studies' Biostat Lab and one of Meier's doctoral students, attested to the article's continuing relevance by noting: "If you open up at random a medical journal you're likely to see in at least one of the articles a citation to the Kaplan-Meier paper" (oral communication, December 1, 2011).

Over his long and distinguished career, Meier earned many honors—as well as widespread admiration for being quick on his feet.

> *At professional meetings … he often astonished me by giving comments from the audience, which, though spontaneous, displayed a depth of reasoning and perfect eloquence, which few others could have matched with any amount of advanced preparation,*

recalled Rick Chappell (written communication, November 8, 2011; and oral communication, November 23, 2011), who was Meier's last doctoral student and is now a professor of biostatistics and medical informatics at the University of Wisconsin at Madison.

Through it all, including the stroke in 1995 that robbed him of some of his eloquence, Meier was also a kind and gentle man, according to a statement issued by the Statistics Department at Columbia University,[5] where Meier spent his final years (he also held a joint appointment at Columbia's Mailman School of Public Health). Karrison, Chappell, and Daniel Heitjan, PhD, a professor of biostatistics at the University of Pennsylvania's Perelman School of Medicine, attested that Meier was both widely respected and loved. "He was a person who cared about people … and someone you could go to with a problem," Karrison said.

A RELUCTANT BIOSTATISTICIAN

Meier graduated from Oberlin College in 1945 and went on to Princeton University to pursue a doctorate in mathematics, where he studied under the celebrated mathematician John Tukey. Meier's dissertation project involved a statistical problem suggested by William Cochran, the noted statistician who chaired Johns Hopkins University's Department of Biostatistics from 1948 to 1958. At the time, Meier was also very interested in "the notion that randomization could clear away confounders that you did not know about."[3(p133)] As one of a very few mathematicians focusing on medical applications, Meier recognized the potential value of randomization's application in medicine.[3]

After Meier earned his doctorate, he spent one more year at Lehigh University, where he had been teaching since 1948. Tukey recommended that he accept a position at Hopkins with Cochran, who was enthusiastic about Meier's dissertation.

> *I was a little nervous because by and large, biostatistics was not a field with a lot of mathematics in it, and I wished more or less to be a mathematician,*

Meier said. But when Cochran insisted that going to Hopkins was a good idea, Meier accepted his first position as a statistician.[3]

In those early days, Meier said, "I was looked at with amazement by my medical colleagues," when he brought up the idea of randomization for assessing new medical treatments, he recalled. The physicians would say "'Randomize? We know that this treatment is better than that one,'" he explained. "People who knew and respected me were astounded that I should want to randomize their patients."[3(p133)]

Meier´s Recollections of the Salk Polio Vaccine Trial

The 1954 field trial of Jonas Salk's polio vaccine "was the most elaborate trial that was ever done," Meier recalled. One of the reasons that the trial was so complicated is because polio was very scarce, he explained. "I've not been involved in many trials like that and I've been involved in lots of multicenter studies," he said.[3(p133)]

The situation was further handicapped because the diagnosis of polio is tricky, Meier said. "We need to have the entire country's physicians participate, because we can't look over every case where there's some kind of paralysis. So physicians reported the cases they thought were polio according to the protocol, and we accepted those cases." Meier estimated that "about half those cases were probably not polio at all."[3(p133)]

But the biggest issue, for Meier, emerged during a seminar attended by many of the researchers working on the project, where it became apparent that members of the team were suppressing the data related to some of the test vaccine lots. As soon became clear, the polio virus used in the trial vaccines was not always properly inactivated. Jonas Salk, the vaccine's inventor, "cut out data in order not to show what happened to some lots," Meier charged.[3(p134)] He said that the National Foundation for Infantile Paralysis, which sponsored the study, dropped from its advisory committee scientists who did not agree with how the results were being presented.[3]

The field trial's findings were reported to show the vaccine's effectiveness, over the objections of some of the committee members, Meier said. Soon after, the US Public Health Service reported cases of paralytic polio in children inoculated with the vaccine. The original cases were traced back

to lots produced by Cutter Laboratories, of Berkeley, CA, one of six manufacturers licensed to produce the vaccine. However, Meier said that the problem was more widespread. He said:

> *I got some data from a physician who was working on this, and we found that not only was Cutter wrong, but there were various other companies that had the same polio virus in their samples, although not as much as the samples from Cutter Laboratories. But because there were so many improperly diagnosed cases out there, and because the other manufacturers went around to various newspapers and threatened to cut their advertising, it was dumped on Cutter. Cutter was responsible because they did things in producing and testing the vaccine they were told not to do.*[3(p134)]

Then Meier became involved with the controversial 1954 Salk Polio Vaccine field trials. The Society for Clinical Trials called the polio vaccine trial "the project that put randomized trials on the map in this country" in part because of the key role Meier played by publishing a critical article in *Science* in 1957.[6] The article reviewed "some aspects of the poliomyelitis vaccine testing program which seem to have important implications for scientists generally."[6(p1067)] It indicted both the National Foundation for Infantile Paralysis and the government for withholding information from the participants. It also faulted the testing program for accepting without scrutiny Salk's assertion that the vaccine was "absolute[ly] safe," and for not employing the expensive and difficult tests that had been suggested to ensure that the final product was free of residual live virus. Meier said that many journals turned his manuscript down and their editors warned him that publishing such an article would limit his career path.[3]

Honors and Awards

Meier was named as a fellow of the American Association for the Advancement of Science, the American Statistical Association, the Institute of Mathematical Statistics, the American Academy of Arts and Sciences, the Royal Statistical Society, and the John Guggenheim Memorial Foundation. He served as president of both the Institute of Mathematical Statistics and the Society for Clinical Trials. He was also elected to senior membership in the National Academy of Sciences' Institute of Medicine.[5,11]

He also held temporary appointments as a National Institutes of Health Special Fellow at the University of London and Imperial College; he was a visiting professor at Harvard University and Jerusalem's Hebrew University; and he was a fellow of Stanford University's Center for Advanced Study in the Behavioral Sciences.[5,11]

Although Meier was denied tenure at Hopkins, he succeeded in securing an appointment to the University of Chicago in 1957. He stayed there until 1992, and taught at different schools and departments—including the college, graduate school, law school, and medical school—over the years. For more than a decade, he led the Department of Statistics as chair or acting chair.

In 1958, Meier published his highly cited article describing what is now known as the Kaplan-Meier estimator in the *Journal of the American Statistical Association*.[4] Kaplan was also a student of Tukey at Princeton. Working independently, Meier and Kaplan solved a problem that was dogging medical researchers at the time. The issue revolved around the fact that many participants in clinical trials do not participate in the experiment for the same length of time because of the time required to recruit study volunteers. The Kaplan-Meier statistic enables researchers to take into account observable time of survival and death.

Initially, Meier recalled, both he and Kaplan had submitted separate articles. The publication's editor asked them to collaborate to produce one article. "I swallowed hard, and I guess Kaplan swallowed hard as well," Meier said. "We worked quite hard and at one place he solved a problem that I couldn't solve; other cases I solved problems he couldn't."[3(p133)]

LOVE FOR CLINICAL TRIALS

In the subsequent decades, Meier's stature continued to grow, and he was involved in many clinical trials, which he called his "true love." In addition to helping found the Society for Clinical Trials in the 1970s, he wrote some influential articles about the ethics of performing them.[7,8] In his spare time, Meier enjoyed music, particularly folk songs, and played the flute, recalled Chappell, Heitjan, and Karrison. Meier was also a sailor, and he took out his small sailboat, The Salty Dog, in the waters near his summer home near Lake Michigan during his years at the University of Chicago. After Meier moved to New York City in 1992, he sailed in the Hudson River outside Dutchess County, New York.

Over the course of his 50-plus-year career, Meier's facility for explaining statistical concepts to people outside the discipline resulted in calls to testify before the US Congress and popularity with journalists such as Gina Kolata of the *New York Times*, Chappell remembered. It also made him popular with clinicians, such as the University of Chicago medical school students he taught about clinical trials, Karrison said.

Meier's stroke occurred three years after he retired from the University of Chicago in 1992 and moved to Columbia University. There, he held appointments as both the Howard Levene Professor of Statistics in the statistics department and head of the Mailman School of Public Health's biostatistics department, and he remained active professionally for years after his stroke. "He still kept going to meetings," Karrison recalled. Meier "struggled courageously," added Heitjan, who worked closely with him at Columbia (oral communication, November 22, 2012).

Heitjan collaborated with Meier during the Randomized Evaluation of Mechanical Assistance for the Treatment of Congestive Heart Failure (REMATCH) trial, which began in 1998 and ran through 2001 and involved 20 cardiac transplant centers around the country.[9,10] Although this artificial heart trial was relatively small compared with many drug trials, it was one of the most significant device trials ever conducted, Heitjan said. Meier insisted that the trial needed to be randomized and he refused to allow the group carrying it out to cut corners, Heitjan recalled.

> *Clinical trials in the device world are often small, single-arm trials [where results are compared with historical controls] … in part because a lot of the companies that make devices are small and can't support major trials,*

Heitjan explained. The trial was randomized so it could determine whether the devices could extend and improve the quality of recipients' lives sufficiently to justify the expense of implanting them, he said.

It was the first high-profile randomized clinical trial that Heitjan had worked on, and "having Paul around to be my mentor and guide was very important to me." When the two would attend meetings related to the trial, Meier was quiet most of the time

> *because it was a little harder for him to communicate and get his point across so he had to choose his battles carefully. He would only speak out at what I considered critical moments,*

Heitjan said. Nevertheless it was clear that Meier's understanding of both the technical and political issues in the trial was undiminished, Heitjan said.

Heitjan recalled attending a Society for Clinical Trials meeting with Meier in 1998. One after another, distinguished senior physician–scientists came up to greet Meier, pay homage to him, and testify to how he had opened their eyes to the critical importance of the randomized clinical trial, Heitjan remembered.

"Being with [Meier] lifted you up," Heitjan summarized. Perhaps just as important as his intellect and accomplishments, Meier "was a genuinely good human being," Karrison said. He was a "great and gentle man," Chappell agreed.

Acknowledgments

The author would like to thank Rick Chappell, Daniel Heitjan, and Theodore Karrison for their help in putting together this article.

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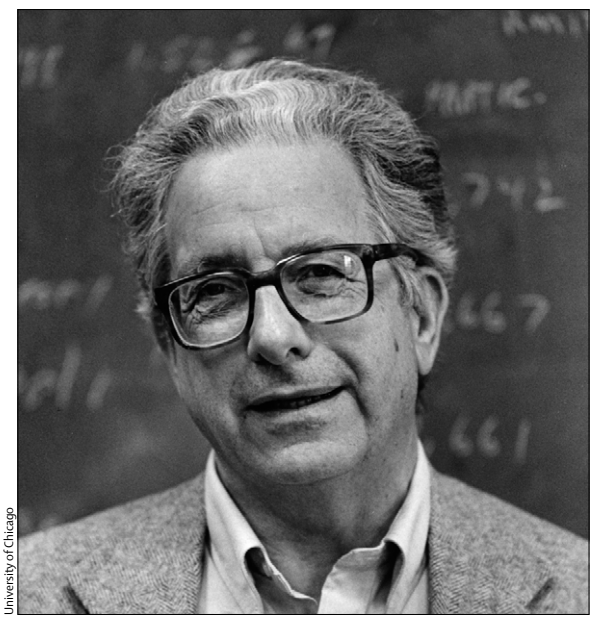

University of Chicago

Paul Meier

Statistician who was a leading proponent of randomised clinical trials and who co-developed a system for estimating survival rates. Born on July 24, 1924, in New York, NY, USA, he died from complications of a stroke in New York on Aug 7, 2011, aged 87 years.

Randomised trials have a prominent place in modern clinical research. Assigning participants in a random way to receive different treatments allows investigators to eliminate bias in their findings. But half a century ago, when Paul Meier was advocating for this approach, his enthusiasm raised eyebrows: "When I said 'randomize' in breast cancer trials I was looked at with amazement by my clinical colleagues", Meier said in a 2004 interview published in the journal *Clinical Trials*. "'Randomize? We know this treatment is better than that one', they said. I said 'Not really…'"

Meier was a leading figure in the generation of statisticians who, during the mid-20th century, helped establish randomisation as a key part of clinical research, says Sir Richard Peto, Professor of Medical Statistics and Epidemiology at the University of Oxford, UK. In doing so, they helped save countless lives. "Perhaps more than any other American statistician, Paul Meier was the one who influenced US drug regulatory agencies, and hence clinical researchers, to insist upon the central importance of randomised evidence", Peto told *The Lancet*.

The son of a chemist and a schoolteacher, Meier graduated from Oberlin College in 1945 with a bachelor's degree in mathematics and physics, before earning a master's in mathematical logic and a doctorate in statistics from Princeton University. After teaching at Lehigh University, he moved to Johns Hopkins University where he began the work that led to one of his major contributions to medical research: the Kaplan-Meier estimator. Meier and Edward Kaplan had independently developed the same elegant method to estimate survival rates, which took appropriate account of the fact that although some patients die at known times, others survive beyond the end of the study. Both submitted the method to the *Journal of the American Statistical Association*, and the editor convinced them to produce a combined paper, which was published in 1958. Kaplan-Meier curves are now widely used in clinical research.

In 1957, Meier moved to the Department of Statistics at the University of Chicago where he remained for 35 years, serving as departmental chairman or acting chairman for more than 10 years. After leaving Chicago, he became Head of Biostatistics at Columbia University. Theodore Karrison, Director of Chicago University's Biostatistics Laboratory, was a student of Meier's who worked with him on multicentre clinical trials and remembers how "Paul was a person who displayed a deep concern for others; he would go out of his way to help people whenever he could, whether it was a struggling student, an individual coping with an illness, or a colleague making a difficult career choice or other decision."

Throughout his career, clinical trials were Meier's "true love", as he put it in the *Clinical Trials* interview. An early and prominent example of his work was his involvement in the US field trials of the Salk polio vaccine in 1954, which Meier, as statistician, ensured included a large number of participants randomly assigned to vaccine or placebo. In doing this, Meier followed in the path of British statistician Sir Austin Bradford Hill, most notably in the well known 1948 Medical Research Council trial of streptomycin in tuberculosis. "Randomisation would probably have been introduced anyway some time around the middle of the century, as it was so essential if moderate differences in treatment efficacy were to be established or refuted reliably", said Peto. "A few investigators had used it or proposed it before Hill did so, but they didn't trigger the avalanche of randomised evidence that Hill triggered and Meier helped propagate."

Meier helped found the Society for Clinical Trials, and was its President in 1986–87. He was also an adviser to the US Food and Drug Administration (FDA), where he could be relied on to demand credible data, says Robert Temple, Deputy Center Director for Clinical Science at the FDA's Center for Drug Evaluation and Research: "I remember Paul as unfailingly polite but quite firm—although I recall no rudeness—and he made his views and disagreements, where necessary, quite visible. He was a powerful force whenever he was present." Meier is survived by his wife of 63 years, Louise Goldstone Meier, and their three daughters and five grandchildren.

Stephen Pincock

Dutch medical association calls halt to euthanasia prosecutions

The Royal Dutch Medical Association wants Justice Minister Winnie Sorgdrager to stop test cases on euthanasia being brought to court, especially those on assisted deaths in neonates. The association's chairwoman, Joke Lanphen, says in the association's magazine, *Medisch Contact*, this week, that she is "very unhappy that juridical clarity has to be obtained at the expense of a few individual doctors' distress".

From this month, the association has introduced new procedures that could form the basis for changes in the law. A crucial move is that a committee of doctors, ethicists, and lawyers has been set up to review selected cases. The association hopes that the results of this project will help them succeed in changing the system to one in which doctors will be subject to the criminal law only when they ignore legal guidelines.

Lanphen refers to the widespread disappointment in medical circles that the way euthanasia is handled in the Dutch legal system—ie, a doctor automatically faces criminal prosecution when he complies with the rules to report non-natural deaths—is inconsistent with the conclusions of all serious reports and discussions that the association has initiated. Because of the attitude of former (Christian Democrat) Justice Minister, Ernst Hirsch Ballin, prosecution officers are holding juridical inquiries into the actions of several doctors. Lanphen wants these inquiries stopped and the charges dismissed. She wants instead talks with Sorgdrager about the minister's suggestion in the evening newspaper *NRC Handelsblad* to create a "medical exception" in the law for doctors who act according to the rules. The effect of the guidelines laid down in law in 1994 on assisted deaths are being examined. The evaluation is expected to be ready in the second half of this year, so that will be the political moment to change the legislators' opinion, says Lanphen.

Marjanke Spanjer

Thomas C Chalmers

Thomas Chalmers, who pioneered the use of randomised control trials (RCTs), died on Dec 27, 1995, aged 78. Despite serious illness he worked with his collaborators world wide almost to the day he died.

I first met Tom 14 years ago, when he was visiting professor at the Harvard School of Public Health, teaching and recruiting young colleagues to projects that critically appraised the existing research. It was hard not to absorb the enthusiasm of this gentleman already at a point in his professional life when many are content to wind down their research career.

Tom Chalmers

A theme running through Tom's scientific life was the posing of challenging questions about the effectiveness of medical practice. He was promoting the use of RCTs at a time when the method was far from accepted in clinical research. A good example of how RCTs can alter long-standing practice based on the observational approach is the 1951 trial that challenged the wisdom of bed rest and diet in the treatment of acute hepatitis.

Tom's lifelong concern was quality of clinical research. For several years he worked on a quality score—still referred to as "Chalmers' quality score"—for assessing trials. Although he did not succeed in validating it, standards of reporting of scientific articles have improved, thanks to his work.

At a time when the issue was largely unrecognised, he published in 1978 a paper critical to our current understanding of the danger of RCTs of inadequate statistical power. In that paper he reviewed 71 "negative" RCTs published in leading medical journals and showed that the vast majority of them could have missed important clinical benefits. This led Tom to become one of the pioneers of the use of meta-analysis in clinical medicine, where he contributed important publications in gastroenterology and cardiology, among others.

In 1992, he introduced the concept of "cumulative meta-analysis". Reviewing RCTs on the treatment of myocardial infarction, he made a strong plea for systematic reviews of clinical trials by showing that medical textbooks often give advice that contradicts results of such reviews.

Amongst all these activities Tom always found time to be generous, supportive, and friendly to many people, especially young colleagues. To me he was a great teacher and an extraordinary example.

Alessandro Liberati

Netherlands seeks heroin for addicts

Will Dutch Health Minister Els Borst-Eilers get permission from Vienna to purchase the 50 kg heroin needed for the planned heroin maintenance programmes? When approved by parliament (see *Lancet* Sept 16, p 761), such pilot programmes will be introduced in Rotterdam and Amsterdam, and perhaps in Arnhem.

In keeping with routine procedure, Borst-Eilers has put in a preliminary request to the UN drugs bureau in Vienna for permission to buy 50 kg heroin, ahead of the formal round, in November, of estimations of need. The Netherlands usually asks for 200g. But there is concern about the dificulties of overcoming objections by the Vienna bureau, known to be conservative and critical. When the Swiss first sought permission in 1993 to obtain heroin for 800 addicts in their maintenance programmes, they had to wait 6 months while every detail of their project was scrutinised.

For the Dutch their first hurdle is to get the Rotterdam and Amsterdam authorities to agree on the design of maintenance programmes. A sticking point is whether to include a "smokeable" form of heroin, especially now that the Swiss have observed complications such as haemoptysis. Making addicts change their habits (to injecting heroin) for the sake of an experiment is thought by some to be unethical.

Marjanke Spanjer

- Published: May 2002

Preventing the common cold with a vitamin C supplement: A double-blind, placebo-controlled survey

- Michael Van Straten[1] &
- Peter Josling B.Sc. Hons.[1]

Advances in Therapy **volume 19**, Article number: 151 (2002) Cite this article

Abstract

One hundred sixty-eight volunteers were randomized to receive a placebo or a vitamin C supplement, two tablets daily, over a 60-day period between November and February. They used a five-point scale to assess their health and recorded any common cold infections and symptoms in a daily diary. Compared with the placebo group, the active-treatment group had significantly fewer colds (37 vs 50, P<.05), fewer days challenged virally (85 vs 178), and a significantly shorter duration of severe symptoms (1.8 vs 3.1 days, P<.03). Consequently, volunteers in the active group were less likely to get a cold and recovered faster if infected. Few side effects occurred with the active treatment, and volunteers reported greatly increased satisfaction with the study supplement compared with any previous form of vitamin C. This well-tolerated vitamin C supplement may prevent the common cold and shorten the duration of symptoms. Volunteers were generally impressed by the protection afforded them during the winter months and the general acceptability

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Recycling of vitamin C by a bystander effect.

Nualart FJ[1], Rivas CI, Montecinos VP, Godoy AS, Guaiquil VH, Golde DW, Vera JC.

Author information

Abstract

Human cells transport dehydroascorbic acid through facilitative glucose transporters, in apparent contradiction with evidence indicating that vitamin C is present in human blood only as ascorbic acid. On the other hand, activated host defense cells undergoing the oxidative burst show increased vitamin C accumulation. We analyzed the role of the oxidative burst and the glucose transporters on vitamin C recycling in an in vitro system consisting of activated host-defense cells co-cultured with human cell lines and primary cells. We asked whether human cells can acquire vitamin C by a "bystander effect" by taking up dehydroascorbic acid generated from extracellular ascorbic acid by neighboring cells undergoing the oxidative burst. As activated cells, we used HL-60 neutrophils and normal human neutrophils activated with phorbol 12 myristate 13-acetate. As bystander cells, we used immortalized cell lines and primary cultures of human epithelial and endothelial cells. Activated cells produced superoxide anions that oxidized extracellular ascorbic acid to dehydroascorbic acid. At the same time, there was a marked increase in vitamin C uptake by the bystander cells that was blocked by superoxide dismutase but not by catalase and was inhibited by the glucose transporter inhibitor cytochalasin B. Only ascorbic acid was accumulated intracellularly by the bystander cells. Glucose partially blocked vitamin C uptake by the bystander cells, although it increased superoxide production by the activated cells. We conclude that the local production of superoxide

anions by activated cells causes the oxidation of extracellular ascorbic acid to dehydroascorbic acid, which is then transported by neighboring cells through the glucose transporters and immediately reduced to ascorbic acid intracellularly. In addition to causing increased intracellular concentrations of ascorbic acid with likely associated enhanced antioxidant defense mechanisms, the bystander effect may allow the recycling of vitamin C in vivo, which may contribute to the low daily requirements of the vitamin in humans.

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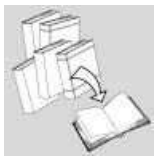
REVIEW



Role of free radicals in viral pathogenesis and mutation

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SUMMARY

Oxygen radicals and nitric oxide (NO) are generated in excess in a diverse array of microbial infections. Emerging concepts in free radical biology are now shedding light on the pathogenesis of various diseases. Free-radical induced pathogenicity in virus infections is of great importance, because evidence suggests that NO and oxygen radicals such as superoxide are key molecules in the pathogenesis of various infectious diseases. Although oxygen radicals and NO have an antimicrobial effect on bacteria and protozoa, they have opposing effects in virus infections such as influenza virus pneumonia and several other neurotropic virus infections. A high output of NO from inducible NO synthase, occurring in a variety of virus infections, produces highly reactive nitrogen oxide species, such as peroxynitrite, via interaction with oxygen radicals and reactive oxygen intermediates. The production of these various reactive species confers the diverse biological functions of NO. The reactive nitrogen species cause oxidative tissue injury and mutagenesis through oxidation and nitration of various biomolecules. The unique biological properties of free radicals are further illustrated by recent evidence showing accelerated viral mutation by NO-induced oxidative stress. NO appears to affect a host's immune response, with immunopathological consequences. For example, NO is reported to suppress type 1 helper T cell-dependent immune responses during infections, leading to type 2 helper T cell-biased immunological host responses. NO-induced immunosuppression may thus contribute to the pathogenesis of virus infections and help expansion of quasispecies population of viral pathogens. This review describes the pathophysiological roles of free radicals in the pathogenesis of viral disease and in viral mutation as related to both nonspecific inflammatory responses and immunological host reactions modulated by NO. Copyright © 2001 John Wiley & Sons, Ltd.

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INTRODUCTION

To date, much attention has been paid to the pathogenic roles of free radicals produced in excess in various pathological settings. Free radical species are potentially reactive because of the physical instability of oxygen- or nitrogen-based unpaired electrons in their orbits, which leads to a number of deleterious pathological consequences *in vivo*. Among a series of free radicals, superoxide anion radical (O_2^-) and nitric oxide (NO) are now considered to be the most biologically relevant elements derived from hosts during microbial infections [1–7]. During the past decade, considerable evidence has revealed unique and diverse biological functions of NO, a gaseous nitrogen-centred inorganic free radical produced endogenously in a number of cells and tissues [8–10]. NO and reactive oxygen species, including O_2^-, hydrogen peroxide (H_2O_2) and hypochlorite anion (OCl^-), are generated by infiltrating phagocytic cells and xanthine oxidase (XO) expressed in inflamed tissues [6,7,11–15]. They are believed to contribute to nonspecific (innate) and immunological host defence as well

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Abbreviations used:
CGD, chronic granulomatous disease; CMV, cytomegalovirus; CTL, cytotoxic T lymphocyte; DTCS, (N-dithiocarboxy)sarcosine; EMCV, encephalomyocarditis virus; ESR, electron spin resonance; GFP, green fluorescent protein; HBV, hepatitis B virus; HIV, human immunodeficiency virus; HNO_2, nitrous acid; H_2O_2, hydrogen peroxide; HSV, herpes simplex virus; IFN, interferon; IL, interleukin; iNOS, inducible nitric oxide synthase; $iNOS^{-/-}$, iNOS deficient (knockout) mouse; L-NMMA, N^{ω}-monomethyl-L-arginine; MMP, matrix metalloproteinase; MPO, myeloperoxidase; NO, nitric oxide; NO^+, nitrosonium cation; NO_2, nitrogen dioxide; N_2O_3, dinitrogen trioxide; O_2^-, superoxide anion radical; OCl^-, hypochlorite anion; ·OH, hydroxyl radical; $ONOO^-$, peroxynitrite; SeV, Sendai virus; SOD, superoxide dismutase; TBE-V, tick-borne encephalitis virus; Th, helper T cell ($CD4^+$); XO, xanthine oxidase

[1–7]. It is now well accepted that the chemical and biological reactivities of NO produced in environments such as inflamed tissues are greatly affected by concomitantly formed oxygen radicals, particularly O_2^-, via the formation of reactive nitrogen oxides such as peroxynitrite ($ONOO^-$) [16–21]. These reactive nitrogen intermediates, rather than NO or O_2^-, seem to be involved in the pathogenesis of various diseases. The pathophysiological action of $ONOO^-$ is particularly important for pathogenesis of virus infection, because $ONOO^-$ is not only a potent oxidant but also a nitrating agent of proteins, nucleic acids and membrane unsaturated lipids [16–18,22,23]. In addition, reactive nitrogen oxides formed endogenously during virus infection have a potential impact on mutagenesis of both the intruding viruses and the hosts, as well as causing host cell and tissue injuries by induction of oxidative stresses.

A major goal in medical microbiology is a general understanding of the mechanisms of host–pathogen interactions, which determine the pathological consequences of infection. An understanding of host–pathogen interactions at the molecular level requires the characterisation of host-derived small radical molecules, which appear to play an important role in the pathogenesis of virus infection. An emerging concept related to free radicals will help us to gain insight into the molecular mechanisms of pathological events occurring as a result of interactions between viruses and hosts [11–15]. In this review, I place particular emphasis on the host response to various virus infections, in view of the pathological consequences, such as oxidative tissue injuries and viral mutations, that result from overproduction of free radicals during virus infection.

INDUCTION OF OXYGEN RADICALS AND PRODUCTION OF NO IN VIRUS INFECTION

It is now well documented that O_2^- and NO production is elevated in inflamed tissues. O_2^- and its related reactive oxygen intermediates are generated by two components of the host response: cellular reactions, mediated by inflammatory phagocytic cells such as neutrophils and macrophages expressing phagocyte NADPH oxidase and humoral responses involving xanthine oxidase (XO). Host reactions occur in response to foreign matter, microorganisms and damage caused by trauma, radiation or ischaemia–reperfusion injury. Because the genetic deficiency of components of an O_2^--generating NADPH oxidase in phagocytic cells gives rise to chronic granulomatous disease (CGD), which is associated with severe chronic bacterial infections, oxygen radical formation is important in antimicrobial actions of the host [24,25]. However, excessive production of O_2^- induces lipid peroxidation, membrane damage, mitochondrial dysfunction and inflammatory and ischaemia–reperfusion injuries [26–28]. A high production of O_2^- is most clearly observed in murine pneumonia caused by influenza A virus, Sendai virus (SeV) and cytomegalovirus (CMV) [11,12,29–31]. Experimental evidence shows that O_2^- contributes to the pathogenesis of viral disease, because inhibitors of O_2^- effectively improve lung pathology and survival in viral pneumonia. Evidence indicates that O_2^- itself is not the molecular species that causes the pathological effects but is a precursor of a more potent oxidant such as hydroxyl radical (·OH) [32,33]. Earlier studies indicated that O_2^- might function as a reducing agent for ferric iron, forming ferrous iron to act as a catalyst for the production of highly reactive ·OH from H_2O_2 [32,33]. Because ·OH was suggested to mediate cell and tissue damage, at the initial stage of our study of viral pathogenesis almost a decade ago we sought to identify ·OH generation in influenza virus-infected mouse lung by electron spin resonance (ESR), but no proof of appreciable ·OH generation was obtained (Akaike *et al.*, unpublished observation).

Of great interest are the similarities in the physiological and pathophysiological effects of O_2^- and NO, such as host defence and oxidative stress, although NO has much more complicated and diverse functions than does O_2^- [8,14,17,18] Both free radicals are often generated concomitantly in inflammatory and infectious sites and from the same cellular origins in the host. For example, rapid and transient production of O_2^- from phagocytes is triggered by appropriate membrane stimulation leading to a respiratory burst in which O_2 is consumed [7]; XO generates constant O_2^- generation together with H_2O_2, depending on the supply of the substrates hypoxanthine/xanthine plus O_2 [11,28–30]. Elevated levels of O_2^- produced by both phagocyte NADPH oxidase and XO occur during virus infections *in vitro* and *in vivo* [29–31,34,35].

In contrast, overproduction of NO is mainly

caused by inducible NO synthase (iNOS), which is usually expressed by inflammatory phagocytic cells and other types of cells (e.g. epithelial and neuronal cells) [1–3,8,9]. iNOS produces a much larger amount of NO (i.e. 10–100 times more) for a longer time than do the other two constitutive enzymes, neuronal NOS and endothelial NOS.

It seems that iNOS is ubiquitously expressed during host responses to viral replication *in vivo*. iNOS expression is observed in human diseases caused by human immunodeficiency virus-1 (HIV-1) and hepatitis B virus (HBV) [36,37]. It is induced in a variety of experimental virus infections in rats and mice, including infections with neuroviruses, such as Borna disease virus, herpes simplex virus type 1 (HSV-1) and rabies virus, and pneumotropic and cardiotropic viruses, such as influenza virus, SeV and coxsackievirus [12–15,38–45]. For example, iNOS is expressed by exudate macrophages and bronchial epithelial cells in lung tissues infected with either influenza virus or SeV in mice; the high output of NO has been clearly identified and quantified by ESR spin trapping with the use of a dithiocarbamate–iron complex [13–15,43–45]. NO–dithiocarbamate–iron adducts with a triplet hyperfine structure of g perpendicular 2.04 are generated (Figure 1). The production of these adducts is completely nullified by pharmacological inhibition of NOS by the use of N^{ω}-monomethyl-L-arginine (L-NMMA) or by genetic disruption of iNOS [43–45], indicating that excessive production of NO is due to localised iNOS expression in the tissues infected with virus.

iNOS induction in virus infection is mediated by proinflammatory cytokines such as interferon-γ (IFN-γ) (Figure 2). IFN-γ is known to be associated with type 1 helper T cell (Th1) responses. In pneumonia induced by influenza virus or SeV, NO production is greatly attenuated in IFN-γ-deficient mice (Akaike *et al.*, unpublished observation). Furthermore, the iNOS-inducing potential in bronchoalveolar lavage fluid in influenza virus pneumonia is attributable solely to IFN-γ, as revealed by an immunoadsorption study using a specific anti-IFN-γ antibody [43]. These results strongly support the suggestion that IFN-γ is a major cytokine inducing iNOS and NO overproduction in the pathogenesis of virus infection.

Downregulation of iNOS expression is also reported for some cytokines, e.g. interleukin (IL)-4, IL-10 and transforming growth factor-β [46–48]. In addition, these suppressor cytokines may reduce NO production indirectly via induction of arginase [49–51], which diminishes the supply of the substrate (L-arginine) for iNOS. Because IL-4 and IL-10 are induced by type 2 helper T cell (Th2) responses, iNOS expression may be regulated by a balance between Th1 and Th2 responses involved in the host immune response to the intruding virus. In fact, in our influenza model, induction of IL-4 seems to be inversely related to INF-γ and iNOS induction in virus-infected lungs, suggesting downregulation by IL-4 of NO overproduction [13]. Induction of arginase 1 mRNA has been identified in virus-infected lung, and the time profile of its induction paralleled the induction of IL-4 (our unpublished observation). Therefore, iNOS expression and the resultant NO biosynthesis seem to undergo elegant regulation by a polarised Th1–Th2 balance (Figure 2).

In some viral diseases, viral replication or viral components directly induce iNOS without mediation by proinflammatory cytokines (Figure 2). iNOS expression in HIV-1 encephalitis is of particular interest in this regard [36]. An envelope glycoprotein of HIV, gp41, triggers iNOS expression in human astrocytes and murine cortical brain cells in culture [52,53]. Thus, NO produced by iNOS may contribute directly to the pathogenesis of HIV-associated dementia and cardiomyopathy as well [36,52–55]. Similarly, the human paramyxovirus respiratory syncytial virus directly upregulates iNOS in human type 2 alveolar epithelial cells (A549 cells) through a pathway independent of proinflammatory cytokines [56]. It is also interesting that double-stranded RNA (dsRNA) formed during viral replication upregulates iNOS in human respiratory epithelial cells by triggering dsRNA-activated protein kinase coupled with nuclear factor-κB and IFN regulatory factor 1 activation [57]. There are therefore two pathways for iNOS induction in virus infections: cytokine-dependent mechanisms and direct upregulation by virus.

VIRUS-INDUCED OXIDATIVE STRESS CAUSED BY FREE RADICALS AND ITS MOLECULAR MECHANISM

NO has antimicrobial activity against bacteria, parasites and fungi [1–7,58–63]. NO itself,

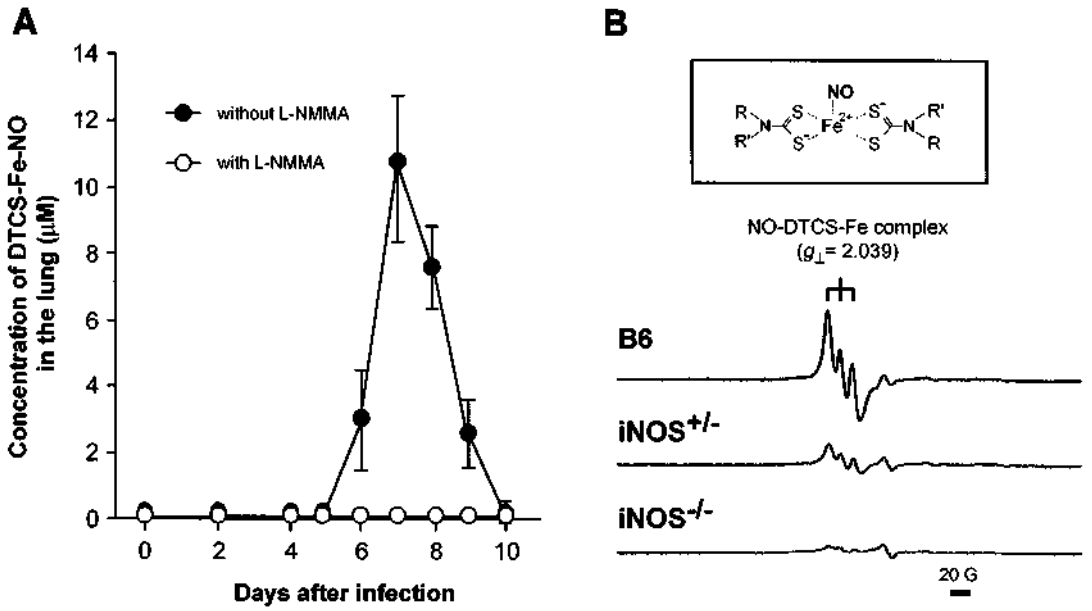

Figure 1. (A) Time profile of NO production in the lung after influenza virus infection. Influenza infection in mice was produced by inhalation of $2 \times LD_{50}$ of influenza A virus. The amount of NO generated in the lung with or without L-NMMA treatment was quantified by ESR spectroscopy (110 K) with (*N*-dithiocarboxy)sarcosine (DTCS)-Fe^{2+} complex as a spin trap. L-NMMA (2 mg/mouse) was given i.p. to mice 2 h before ESR measurement. Data are mean ± SEM ($n = 4$). (B) NO signals as identified by ESR spectroscopy with DTCS-Fe^{2+} complexes in influenza virus-infected lung (7 days after virus infection). Wild-type mice (C57BL/6, B6), iNOS heterozygotes ($iNOS^{+/-}$) and mice deficient in iNOS ($iNOS^{-/-}$) were infected with influenza virus in the same manner as in (A). The chemical structure of the adduct is shown at the top of the figure. Adapted from Akaike *et al.* [12,15] with permission from Blackwell Science and Society for Experimental Biology and Medicine

however, has a limited bactericidal effect, and NO-dependent antimicrobial actions are expressed by other reactive nitrogen oxides such as $ONOO^-$, nitrogen dioxide (NO_2), dinitrogen trioxide (N_2O_3), and nitrosothiols [nitrosonium cation (NO^+) adducts of sulphhydryls] [64–69]. Also, antiviral effects of NO are known for some types of virus, most typically DNA viruses such as murine poxvirus (ectromelia virus) and herpesviruses including HSV and Epstein–Barr virus, and some RNA viruses such as coxsackievirus [58,70–75].

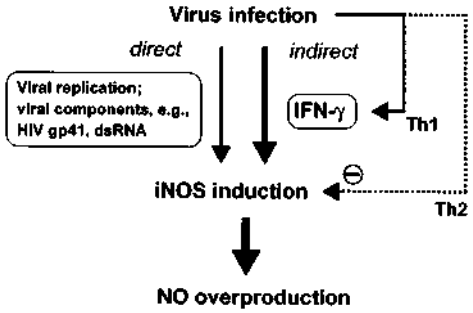

Figure 2. Mechanisms of iNOS induction in viral diseases. In many virus infections, iNOS expression appears to be regulated indirectly via interferon-γ (IFN-γ) induction, which depends on the Th1 response. The host's Th2 response, in contrast, down-regulates iNOS induction. Direct iNOS induction may occur in some cases, such as with respiratory syncytial virus, HIV-1 (gp41), and viral replicative intermediate dsRNA. Modified from Akaike and Maeda [15] with permission from Blackwell Science

Activity of NO against other viruses remains unclear, however. Recent reports suggest that NO has no appreciable antiviral effect on several types of viruses such as ortho- and paramyxovirus, murine vaccinia virus, coronavirus (mouse hepatitis virus), lymphocytic choriomeningitis virus, murine encephalomyocarditis virus (EMCV), tick-born encephalitis virus (TBE-V) and others [76–81]. This lack of antiviral activity of NO has been verified in murine pneumotropic virus infections caused by influenza virus and SeV in a series of our *in vitro* and *in vivo* studies (Akaike *et al.*, unpublished observation) [43,45]. More importantly, antiviral host defence is not impaired by pharmacological interventions resulting in

NOS inhibition or by genetic iNOS deficiency in mice infected with either influenza virus or SeV [43,45]. Such NO inhibition and lack of NO biosynthesis, however, significantly reduce the pathological consequences of various virus infections including viral pneumonia in mice caused by influenza virus, SeV and HSV-1; HSV-1-induced encephalitis in rats; EMCV-induced carditis and diabetes; and murine encephalitis induced by flavivirus (Murray Valley encephalitis virus; TBE-V) [43–45,77,81–85]. It is thus conceivable that NO is not entirely an antiviral molecule, but it can be pathogenetic in various, if not all, virus infections. A similar pathogenicity with a lack of antiviral effect is observed for O_2^- in several experimental models of virus-induced pneumonia including those caused by influenza virus and CMV [11,12,29–31,86].

What are the molecular mechanisms related to the NO- and O_2^--dependent pathogenesis of certain virus infections? Both O_2^- and NO are inert radicals and are much less reactive compared with other naturally occurring oxygen and alkyl radicals [16–18,20,21,32,33,64–69]. Oxidised nitrogen intermediates are formed via pathways mediated by heavy metal ions, molecular oxygen (O_2), O_2^- and peroxidases [e.g. myeloperoxidase (MPO)], and their biological consequences are summarised in Figure 3 [17,18,64,68,69,87–89]. Of the complex chemistry of NO, the most important and biologically relevant reaction is the formation of $ONOO^-$ via a very rapid radical coupling with O_2^- ($NO + O_2^- \rightarrow ONOO^-$: $k = 6.7 \times 10^9\ M^{-1}s^{-1}$) [16–18,20,21]. Although NO can function as an antioxidant, particularly in lipid peroxidation [18], it also has indirect prooxidant activity after conversion to a strong oxidant and is a potent nitrating agent ($ONOO^-$) causing oxidative stress [17]. In addition, although NO and nitrosothiols show strong anti-apoptotic effects [69,89], $ONOO^-$ induces apoptosis, possibly via mitochondrial damage leading to cytochrome *c* release [19,90]. The reaction between NO and O_2^- takes place in virus-infected inflammatory tissues, leading to the formation of $ONOO^-$. $ONOO^-$ nitrates aromatic organic compounds such as tyrosine very effectively, so that nitration of free or protein-bound tyrosine to give 3-nitrotyrosine can serve as a footprint of $ONOO^-$ formed *in vivo* [17,20,21]. Indeed, immunohistochemical analysis with antinitrotyrosine antibody shows positive staining in macrophages and neutrophils infiltrating the alveoli and interstitial tissues, as well as in inflammatory intraalveolar exudate

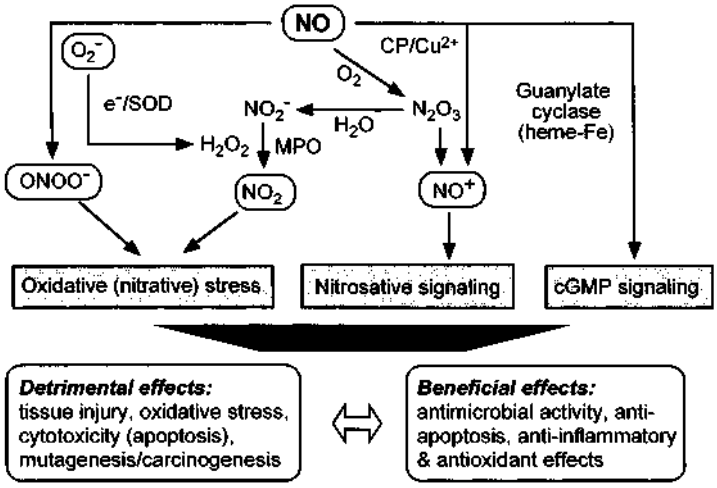

Figure 3. Mechanisms of formation of various reactive nitrogen intermediates from NO and their biological effects. Reactive nitrogen oxides are produced by interactions of NO with molecular oxygen (O_2), active oxygen and oxygen radicals such as O_2^- and H_2O_2 and heavy metals (particularly iron and copper). $ONOO^-$ and NO_2 mediate oxidative and nitrative stresses through oxidation and nitration of various biomolecules including protein, lipid and nucleic acid [16-21]. NO_2 is generated via oxidation of nitrite catalysed by peroxidases such as myeloperoxidase (MPO) (plus H_2O_2) from neutrophils [137]. Ceruloplasmin (CP) and copper ion catalyse one-electron oxidation of NO to form nitrosonium cation (NO^+), which is involved in nitrosative signalling [69,88]. The best known NO-dependent pathway is mediated by cyclic guanosine 3′,5′-monophosphate (cGMP), which is produced by soluble guanylate cyclase activation by NO-heme iron binding in the vicinity of the catalytic site of the enzyme [138]

from virus-infected lung in our experimental models [43,45], which provides indirect evidence of $ONOO^-$ generation during virus infection.

In addition to causing various pathological events in virus infections, such as host cell apoptosis and necrosis, $ONOO^-$ may be involved in NO-induced suppressive effects on immune effector cells such as macrophages and lymphocytes, as described in detail in a later section. We also found that $ONOO^-$ activates matrix metalloproteinases (MMPs), which are involved in extracellular tissue damage and remodelling [91]. Oxidative injury in virus-infected tissues may thus be mediated by $ONOO^-$-induced MMP activation. In fact, remarkable improvements in pathological conditions in the lung and in the survival rate of virus-infected mice were observed with L-NMMA treatment, with the use of the O_2^- scavenger superoxide dismutase (SOD) and the XO inhibitor allopurinol, and when there was a genetic lack of NOS expression [29–31,43,45,77,82,86]. Furthermore, a therapeutic effect on influenza pathogenesis was found with a selenium-containing organic compound, ebselen (unpublished observation), which shows potent $ONOO^-$-scavenging action [92]. These beneficial effects of suppression of $ONOO^-$ generation indicate that $ONOO^-$ could be an important molecular species responsible for the pathogenesis of viral diseases.

It was recently suggested that NO and O_2^- contribute in concert to antimicrobial host defence [3,6,66]. These oxygen and nitrogen reactive intermediates, however, cannot discriminate between exogenous invading pathogens and the hosts themselves, so they function as mediators of nonspecific innate defence against various microbes. Autotoxicity can also occur so that host organisms discard expendable parts. To minimise such self-sacrifice during the elimination of pathogens, a host has primitive tactics, using recruited phagocytes, for physical containment of pathogens in infectious foci (Figure 4, right panel). Most bacteria, for example, can be phagocytosed and confined to septic foci, which are typically abscesses or granulomas. Therefore, chemically reactive NO, O_2^- and $ONOO^-$ can affect bacteria rather selectively; the surrounding normal tissue remains intact. In virus infections, in contrast, free radical mediators cause nonspecific oxidative damage in virus-infected tissue and produce oxidative stress, because virus cannot be confined to limited areas by the nonspecific host defence mediated by phagocytes, NO and O_2^- (Figure 4, left panel) [12–14]. Oxidative stress induced by free radical generation during virus infections may thus cause deleterious events in host–pathogen relationships.

FREE RADICAL-INDUCED VIRAL MUTATION AND ITS POTENTIAL ROLE IN VIRAL EVOLUTION

Among the pathological effects associated with oxidative stress, the mutagenic potential of oxygen radicals and NO for microbial pathogens is highly intriguing. As described in earlier sections, overproduction of NO and oxygen radicals appears to be a common phenomenon in various infections. The resultant reactive molecular species such as $ONOO^-$ nonselectively affect the host's cells and tissues. Obviously, such host defence effectors are originally produced to kill the intruding pathogens, which then suffer oxidative stress because of the host. It may therefore be logical to assume that mutagenesis of various pathogens occurs during infections in biological systems as a result of host defence.

It was previously shown that human leukocytes producing O_2^-, but not leukocytes from patients with CGD, are mutagenic for *Salmonella typhimurium* TA100 [93]. Also, the degree of RNA virus mutation was reported to be increased by chemical mutagens including nitrous acid (HNO_2) [94–97], although the degree of mutation appears to be slight compared with that of spontaneous viral mutation [98]. HNO_2 is an oxidised metabolite that can be formed from N_2O_3 ($N_2O_3 + H_2O \rightarrow 2\ HNO_2$) via reaction of NO_2 and NO during the oxidation reaction of NO by O_2 in biological systems (cf. Figure 3), and it is involved in nitrosylation, oxidation and deamination reactions, at least *in vitro*. However, because of the low pKa (3.3) of HNO_2 and the strong buffering actions of biological fluids, HNO_2 after generation would be neutralised to form NO_2^-, which is much less reactive and is more stable at physiological pH. The chemical reactivity of HNO_2 would thus be greatly limited.

In contrast, as described above, $ONOO^-$ formed via O_2^- and NO generation during infections shows potent nitrating and oxidising potential for many biomolecules including nucleic

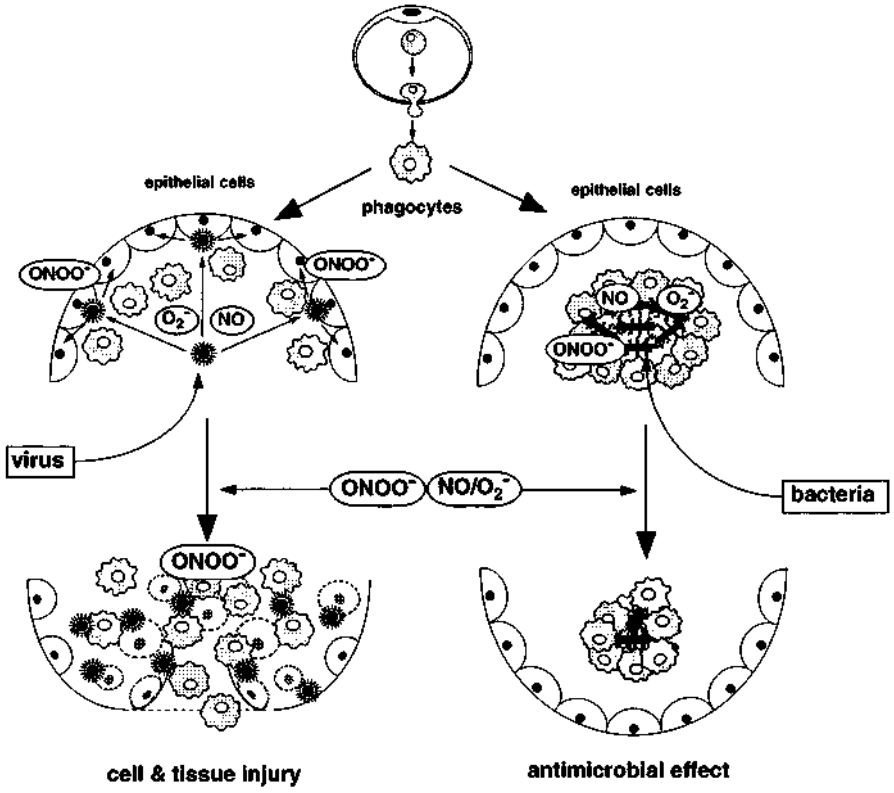

Figure 4. Schematic drawing of the different mechanisms of biological effects of free radicals such as O_2^- and NO, and their product $ONOO^-$, in virus and bacterial infections. Adapted from Akaike *et al.* [12] by copyright permission from Society for Experimental Biology and Medicine

acids [17,18,22,23]. $ONOO^-$ has mutagenic effects on prokaryotic DNA, possibly via nitration of guanine residues of DNA [99]. A typical base substitution caused by $ONOO^-$ is G to T transversion, which is an indirect result of depurination of nitroguanine in DNA [22,23]. A recent study by Wogan's group documented that a high output of NO induced mutations in an endogenous hypoxanthine-guanine phosphoribosyltransferase (*hprt*) gene of murine macrophages expressing iNOS [100]. Genetic analysis of the mutated gene induced by NO indicated that the NO-associated mutational spectrum was similar to that arising spontaneously, but small deletions and insertions were found in the NO-induced mutant gene. The same group showed that mutagenicity is enhanced with NO overproduction *in vivo*, as assessed by mutation of an exogenously expressed *lacZ* by using *lacZ*-containing pUR288 plasmid-transgenic mice [101]. Also important, Ohshima's group reported that p53 is inactivated by $ONOO^-$, which may indirectly increase genetic mutation related to oxidative damage of DNA [102]. Excess production of NO by iNOS induced by inflammatory cytokines, possibly through reactive nitrogen intermediates (particularly $ONOO^-$), caused DNA damage and impaired DNA repair in human cholangiocarcinoma cells, as assessed by the comet assay, suggesting NO-dependent development and progression of cholangiocarcinoma [103].

It has been known for a long time that many naturally occurring mutagens and carcinogens may act as free radical generators [104]. Moreover, oxygen radicals and reactive oxygen species, as endogenous initiators of DNA damage and mutation, are involved in multiple stages of carcinogenesis [105–108]. Free radical species such as O_2^- and NO are thus considered to be potent endogenous mutagens that may be implicated in the pathogenesis of numerous diseases or states involving DNA degeneration, e.g. cancer and aging.

The most striking feature of a virus is its considerable adaptability to various environmental

stresses [109,110]. Viruses containing RNA as their nucleic acid include a number of important pathogens causing various diseases in humans, animals and plants. RNA viruses exist as highly heterogeneous populations called quasispecies, primarily because of the error-prone nature of the replicase of the viruses. In fact, RNA viruses share a high mutation rate, ranging from 10^{-5} to 10^{-3} misincorporation/nucleotide site/round of copying, which is more than 10^4-fold higher than the rate error for DNA viruses [109–112]. The low fidelity of RNA replication is believed to be due to the lack of proofreading and repair functions of RNA polymerase or reverse transcriptase [109,113]. Our recent preliminary study, however, showed that RNA is chemically unstable, so that base modifications via $ONOO^-$-induced oxidation and nitration occur more readily in viral RNA than in eukaryotic DNA (unpublished observation). Thus, the higher incidence of erroneous viral RNA replication may be partly due to RNA's greater susceptibility to oxidative damage compared with DNA.

Only a few reports have explored a possible association between oxidative stress and viral mutation, however. A previous study indicated that oxidative stress augmented the integration of duck HBV DNA into genomic DNA in cells by means of DNA damage and impairment of DNA repair [114]. Although this increased integration is related to proto-oncogene activation induced by hepatitis virus during carcinogenic processes rather than related to viral mutation, it may suggest that oxidative stress causes molecular alteration of viral DNA through mutagenic activities. Beck *et al*. showed that the pathogenicity of coxsackievirus B3 is strongly potentiated *in vivo* in mice fed a selenium-deficient diet [115]. More important, an avirulent strain of the virus is converted to a potent cardiotoxic variant during infection in selenium-depleted animals. The deficiency of selenium may result in an ineffective antioxidant system, e.g. low levels of glutathione peroxidase. The results of similar studies extended to animals deficient in vitamin E and glutathione peroxidase suggest that oxidative stress facilitates selection and generation of virulent mutants [116]. More specifically, the impaired immunological viral clearance related to oxidative stress may cause increased survival of heterogeneous mutants, resulting in the selection of highly pathogenic variants of coxsackievirus [117]. In this context, it is of great interest that NO has an immunosuppressive effect by means of modulation of the T cell immune response during virus infection, as described in the next section of this article.

Many methods are available for estimating viral mutation, including measurement of mutation frequencies of phenotypic variations such as temperature-sensitive growth, plaque morphology, host range and pathogenicity. These criteria, however, cannot be used for accurate and quantitative assessment of viral mutation, because such phenotypic variants often contain multiple base alterations in different genes [118]. Identification of the escape mutant from neutralising antibody is much more reliable for the quantification of viral mutation. For example, escape of a virus from a particular neutralising monoclonal antibody occurs by a single base substitution, leading to a single codon change on the epitope. The frequency of escape mutants thus determined in cultured cells *in vitro* was within the same range, $\sim 10^{-4.5}$, for four different negative-strand RNA viruses: i.e. SeV, vesicular stomatitis virus, Newcastle disease virus and influenza A virus [119,120]. Nevertheless, selection via antibody is not entirely established to be definitive and reproducible, because the frequencies fluctuate greatly, even within a given virus species, depending on the antibodies used for the selection [118]. This selection method has another flaw: it is not used for *in vivo* studies because of the natural immunological selection of the escape mutants during a host's immune response.

We therefore sought to develop a quantitative assay that is applicable to *in vivo* study of mutagenesis [45]. A recombinant SeV was constructed with an exogenous genome, green fluorescent protein (GFP), for the virus. Base substitutions occurring in the GFP in SeV, whether synonymous or non-synonymous, are primarily neutral and do not affect viral replication and clearance of virus from the host. Viral mutation is readily quantified, based on the loss of strong fluorescence caused by GFP gene mutations. This GFP-based assay is convenient and useful for estimating *in vivo* viral mutagenesis. Our recent study thus verifies, for the first time, that oxidative stress induced by a high output of NO accelerates are mutation of the RNA virus [45]. By using the GFP-based mutation analysis and iNOS-deficient

($iNOS^{-/-}$) mice, we clearly showed that oxidative stress induced *in vivo* by NO in wild-type mice remarkably increases and accelerates viral mutation rates compared with the situation in $iNOS^{-/-}$ mice (Figure 5A). The same method used in cultured cells revealed the strong mutagenic potential of $ONOO^-$ (Figure 5B).

This process of accelerated mutation may occur in other virus infections *in vivo*. For example, NO-induced oxidative stress may cause greater heterogeneity of variants of RNA viruses including HIV and influenza virus, leading to rapid viral evolution under selective pressure and to the production of drug-resistant and immunologically tolerant and cell tropism-altered mutants [121]. We now know that NO and O_2^- and hence $ONOO^-$ and other reactive molecular species such as NO_2, OCl^- and H_2O_2 are generated universally as a result of host responses during infections. Therefore, we may expect such chemical mutagenesis in DNA viruses, bacteria and even host cells, although it may not be as effective as that in single-strand RNA viruses.

SUPPRESSIVE EFFECTS OF NO ON IMMUNOLOGICAL RESPONSES DURING VIRUS INFECTION

The effect of oxidative stress on the host immune response is another important facet of viral pathogenesis and mutation. There is growing awareness of the unique immunoregulatory function of NO, which appears to be mediated through cytotoxic or suppressive effects of NO on particular subsets of immune cells [3,122–124]. Th cells, divided into two subsets (Th1 and Th2), protect hosts from intruding viral pathogens via virus-specific Th1 responses, potentiation of $CD8^+$ cytotoxic T lymphocyte (CTL) activity, and B cell proliferation [125,126]. It has been suggested that NO affects the polarised Th1–Th2 response, causing a Th2-biased immunoregulatory balance, via a relatively specific suppressive effect on Th1 subpopulations [122–124]. Such NO-induced immunomodulation occurs during virus infection in mice, as revealed by recent studies of HSV-1 and influenza virus infections [77,127], although such immunoregulatory effects of NO on the Th1–Th2 balance are commonly observed only with specific viruses, not all viruses [76,78]. These biased Th2 responses are clearly demonstrated by using $iNOS^{-/-}$ mice, which show enhanced Th1 immune responses after virus infections [77,127]. NO seems to downregulate the Th1-associated cytokine IFN-γ, which is a major iNOS-inducing cytokine in virus infections as described above, and CTL responses as well, possibly through the suppression of IL-12 production [128–130].

In noncytopathic virus infections CTLs, rather

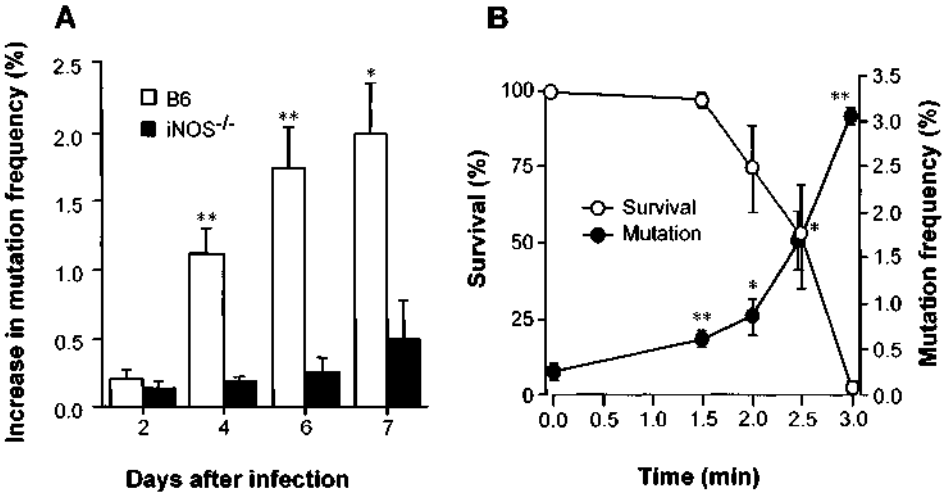

Figure 5. NO-dependent SeV mutation as revealed by genetic mutation of GFP in a recombinant SeV (GFP-constructed SeV, GFP-SeV). (A) The mutation frequency of the virus (GFP-SeV) isolated from the lung of wild-type B6 mice and $iNOS^{-/-}$ mice was quantified by use of the GFP-based mutation assay. (B) Increase in mutation frequency of SeV by $ONOO^-$. GFP-SeV was treated in a constant-flux $ONOO^-$ (0.8 μM) system, and the mutation frequency was determined by the GFP-based mutation assay. Data are mean ± SEM ($n = 4$). *$p < .05$, **$p < .01$, compared with controls or $iNOS^{-/-}$ mice (*t*-test). Adapted from Akaike *et al.* [45] by copyright permission from Federation of American Societies for Experimental Biology

than Th1–Th2 cells, are important for antiviral host defence [125,131]. However, some types of viruses such as influenza virus can be eradicated without the help of CTLs [132]. For influenza virus, a virus-specific Th1 response is more important for antiviral defence than are Th2 responses, because Th2 cells exacerbate pathological lung reactions in influenza pneumonia [133]. In this context, Karupiah *et al*. reported that NO impairs the anti-influenza virus response of the host by suppressing Th1-dependent IFN-γ induction [77]. However, it has now been demonstrated that IFN-γ, a Th1-dependent cytokine, is eventually inefficient in clearance of influenza virus from infectious foci [134]. Our recent experiments using iNOS$^{-/-}$ mice indicate that clearance of virus from lungs infected with either influenza virus or SeV is not affected by a lack of iNOS expression (Akaike *et al*., unpublished observation) [45]. In fact, iNOS$^{-/-}$ mice recuperate from viral pneumonia much better than do wild-type animals, because of reduced levels of oxidative stress in virus-infected tissues [45]. Therefore, not only NO-induced Th1 suppression but also NO-induced oxidative injury may be attributable to pathogenesis of infection with certain viruses that are resistant to the direct antiviral actions of NO.

In addition, NO seems to have profound immunosuppressive and immunopathological effects, most typically in *Mycobacterium avium* and *S. typhimurium* infections [4,135,136], which may be due to NO-induced cytotoxic effects on immune effector cells such as macrophages. Similar immunosuppression by NO is clearly demonstrated with vaccinia virus-infected murine macrophages, which show a loss of antiviral activity because of inhibition of IFN-α/β production by NO [80].

In summary, NO has complex roles in immunological host responses to viruses. The immunosuppression caused by NO may result from NO-induced oxidative stress on professional immune effector cells such as T cells and macrophages. An immunocompromised state of the host caused by NO production not only may enhance the pathogenicity of the virus but also may help the generation and expansion of new mutant viruses by oxidative mutagenesis (Figure 6).

CONCLUSIONS

The pathological consequences of free radical generation during virus infections and the implications for viral pathogenesis and mutation are discussed in terms of current concepts concerning free radicals. It is now recognised more than ever that free radicals, produced primarily as effector molecules of the host defence response, have quite diverse functions in virus infections. Their biological effects are not necessarily beneficial to the virus-infected host; indeed, they are often detrimental. Understanding of the pathophysiological functions of NO and oxygen radicals will provide profound insights into many aspects of infectious diseases.

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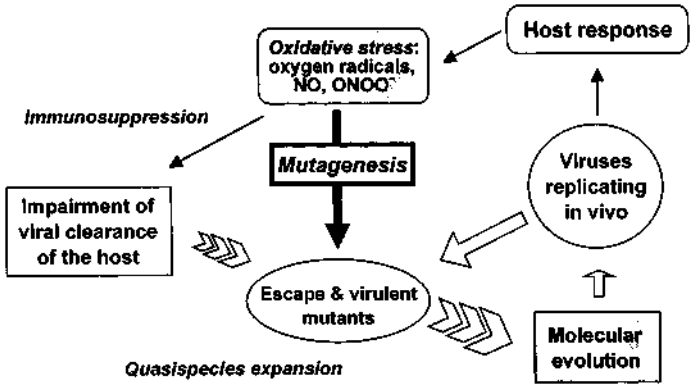

Figure 6. Possible roles of free radicals in viral mutation and evolution. Oxygen radicals and NO-derived reactive nitrogen intermediates, via their potent mutagenic activities, may contribute to the molecular evolution of viruses. NO may also affect viral evolution by inhibiting a host's antiviral immune responses, which may impair clearance of viral mutants

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THE VITAMINS AND RESISTANCE TO INFECTION

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CONTENTS

INTRODUCTION

In many of the investigtions on the relation between the vitamins and resistance to infection rations lacking hi several essentials have been employed, usually in an effort to test the effect-of inadequate human dietaries. Although such investigations have yielded results of practical value, they do not disclose the roles played by the diverse missing substances. More definite information on this question can be obtained from experiments in which diets deficient in one vitamin only are utilized and the following review has been limited, with very few. exceptions, to the discussion of such work. Very numerous papers on this subject have appeared and some no doubt have been overlooked by the author. Wherever possible the investigations have been described in sufficient detail for the reader critically to appraise them. Unfortunately many of the experiments have been carried out on such small numbers of annuals that the results are not statistically significant.

The problem of whether the metabolic changes resulting from the deficiency of a vitamin are .accompanied by changes in the defense mechanism has been attacked by at least four different methods, as follows:

(1) By the determination of any changes in the natural immune bodies or cellular reactions, due to the deficiency.

VITAMIN C

1. Variations in the natural immune bodies or tissue reactions in vitamin C deficiency

(a) Results indicating that these are reduced. Fortenato (1) reported in 1921 that the opsonic index was lower in scorbutic than in normal guinea pigs. In the following year, Leichentritt and Zielaskowski (2) measured the trypanocidal substance in the blood of guinea pigs suffering with scurvy and found that it was reduced. Hojer (3) however criticized the latter's experiments on the grounds that they were carried out on too few animals.

According to Prausnitz and Schilf (4) tuberculous scorbutic guinea pigs show considerably smaller tuberculin reactions, which also dis-

appear more quickly than those in tuberculous guinea pigs subsisting on normal diets. The febrile reaction after the tuberculin injection was also less marked in the scorbutic animals. This reduced skin reactivity was not correlated with a generalized unsusceptibility to tuberculin (5) as the animals with scurvy died more frequently than the normal controls when this substance was injected subcutaneously in large amounts (5 cc.).

In addition, Bieling (6) and also Arkwright and Zilva (7) found that markedly scorbutic guinea pigs gave smaller skin reactions to diphtheria toxin than normal. The former author noted that the necrosis of the skin was slower coming on, and that the subcutaneous oedema was absent or very slight. The latter authors reported that animals on diets which contained suboptimal amounts of vitamin C, but enough to allow a gain in weight of about 25 per cent, still showed large Schick reactions, whereas if this vitamin was further reduced so that a loss of about the same magnitude occurred, the reactions were very small. Scorbutic guinea pigs however are definitely more susceptible to large doses of diphtheria toxin and die earlier than normal animals according to Bieling. A possible clinical application of these findings was provided by Hess (8) in 1932. He had encountered nasal diphtheria very commonly in children with scurvy. The Schick reactions were regularly negative, although the patients showed the bloody mucous nasal discharge which is typical of this disease, and one child apparently died from it. In three cases, virulence tests showed the bacilli to be virulent. The last of these three cases gave no skin reaction to dilutions of from 1/50 to 1/5 M.L.D. of toxin. In his brief review the author does not discuss the possibility of these cases being carriers, already self-immunized. He suggests that in scurvy the pharyngeal mucous membrane loses its immunity to the diphtheria bacilli, whereas the general immunity as reflected by the negative Schick test is still maintained. A simpler explanation however might be that the scorbutic skin does not react in the usual manner to the toxin, although the organism as a whole is not immune to it.

Lawrynowicz (9) suggests that scurvy may so reduce the resistance that a carrier may become the victim of bacteria which it previously carried with impunity. For example, a guinea pig that had been well

for one month after it had been used in a crude test for B. diphtheria was placed on a scorbutic diet. Thirty-seven days later it died. The post-mortem showed the changes found in diphtheritic deaths and the organism was recovered from the spleen.

When Vercellana (10) injected strychnine nitrate or aqueous extracts of poisonous fungi subcutaneously into scorbutic guinea pigs, he found that they were killed more frequently by these substances than controls fed normal diets. The ration of the deficient animals consisted of oats exclusively. Also aleuronat, broth, peptone, cinnabar and other substances, when injected by Dluzewski (11) into the peritoneal cavities of scorbutic animals, did not provoke the normal inflammatory reaction with the outpouring of leucocytes.

(b) Results indicating that these are not reduced. Li contrast to some of the above findings, Lawrynowicz and Bohdanowicz (9). state that they have never established any difference between the Schick reactions of normal and scorbutic guinea pigs.

In 1919, Zilva (12) determined the complement titres in normal and scorbutic guinea pigs and found that they were the same. Four years later, Hamburger and Goldschmidt (13) reported that the complement titres were not lowered in scorbutic children and guinea pigs. In fact, some of the latter animals showed increased complement titres, which were apparently correlated with high albumin concentrations in the serum. Koch and Smith (14) found consistently increased complement titres in a series of twelve scorbutic guinea pigs. When an antiscorbutic was added to the diet, the titres fell, but still remained somewhat higher than they had been before the onset of the scurvy. On the other hand, Bohdanowicz and Lawrynowicz (9) found that complement did not show any constant or characteristic chanjges in guinea pig scurvy.

The phagocytic indices in scorbutic guinea pigs were reported by Werkman et al. (15) to be unaltered.

Hamburger and Goldschmidt (13) also determined the bactericidal titres of the sera of scorbutic and normal guinea pigs and of scorbutic and normal children to the same strain of colon bacillus and found that they were similar, This organism was used because the pyelonephritis which frequently complicates guinea pig scurvy is usually caused by it.

2. Variations in acquired immune bodies due to vitamin C deficiency

(a) Results indicating that these immune bodies are altered. When scorbutic guinea pigs were sensitized to horse serum, or red blood corpuscles, Zolog (16) found that they were much less sensitive to anaphylactic shock than normal diet controls. The minimum lethal dose was three to ten times higher in the animals with scurvy. Sereni (17), on the other hand, reported that scorbutic guinea pigs showed much more severe anaphylactic shock than the control animals. Hurwitz and Wessels (18) went further into the question and found that the uterine muscles of sensitized vitamin C deficient guinea pigs would not react either to the specific antigen or to smooth muscle stimulants, whereas the bronchial muscles of such animals reacted normally. In addition, when Bieling (5) immunized scorbutic guinea pigs with diphtheria toxin, he found that they did not produce as much antitoxin as the adequately fed controls.

(b) Results indicating that these immune bodies are not reduced. Scorbutic and normal guinea pigs produced agglutinins to B. typhosus equally well according to both Zilva (12) and Werkman (15). In addition, the former author stated that amboceptors to the same organism were also produced in normal amounts by guinea pigs on vitamin C deficient diets, and the same findings also held true for the rat. In 1922, Hess (19) reported that the diphtheria antitoxin production in scorbutic guinea pigs was as good as that in normal controls.

Summary of immunological investigations. I. Non-immune animals. In several of these studies conflicting results have been obtained. For example, Werkman reported that the opsonic indices of non-immune scorbutic guinea pigs were as high as those of normal animals, whereas Fortenato found them reduced. And again, Lawrynowicz stated that the presence or absence of scurvy did not affect the size of the Schick reaction in guinea pigs, whereas Bieling and also Arkwright found these reactions considerably reduced when scurvy was present. Other workers reported that tuberculin reactions were also considerably decreased. As the immunological significance of the Schick andituberculin reactions are entirely different, one would infer that the general reactivity of scorbutic skin was depressed. The smaller Schick reactions were not due to any increased antitoxin in the animal, as Bieling

showed that these guinea pigs died more frequently and more quickly after the injection of large amounts of toxin. In fact, scorbutic guinea pigs seem more susceptible to the subcutaneous injections of toxic substances generally, e.g., to tuberculin, strychnine and poisonous fungus extract. Lawrynowicz suggests, on evidence gathered from the study of one animal only, that scurvy so lowers the resistance of a healthy carrier that it may become the prey of bacteria which formerly did not harm it. This sequence of events however might have occurred without the aid of the scurvy-producing diet. Leichentritt found that the substance in the blood which destroyed trypanosomes was reduced in scurvy, and further evidence of the reduced capacity of the scorbutic animal to cope with infections was provided by Dluzewski, who reported that the inflammatory reactions which followed the injection of foreign substances into the peritoneum were much reduced. Two authors stated that the complement titre was unchanged in scurvy, but a similar number of investigators found it increased. One of the latter however did not find it consistently raised, but at least it was never lowered.

II. Immune animals. Comparatively few studies have been carried out on such animals, and many of the results are conflicting.

For instance, Hess found that scorbutic guinea pigs could produce diphtheria antitoxin as well as normal animals, whereas Bieling states that this is not the case. Zilva and Werkman were not able to demonstrate any difference between the amounts of anti-typhoid antibodies produced by guinea pigs and rats lacking vitamin C and those fed adequate diets.

The results of the anaphylaxis experiments are of interest because most of them suggest a reduced activity in the tissues of animals suffering from scurvy, analogous to the lessened skin reactions.

3. Occurrence of spontaneous infections in vitamin C deficiency

(a) Infections indicating a reduced resistance. I. Experimental. In 1932, Suzuki (20) stated that the nasal mucous membrane and glands were atrophied and showed catarrhal inflammation in vitamin C deficient guinea pigs. The crushed oats, autoclaved milk diet that McCarrison (21) fed his guinea pigs is mainly lacking in vitamin C. He

found that the bladders in such animals at postmortem examination were tightly contracted and that the mucous membrane of this organ was congested and necrotic. The duodenum was also intensely congested and punched out ulcers were present in the intestines and sometimes in the stomach. Mackie and Chitre (22) gave their monkeys very small amounts of orange juice, but most of them developed scurvy, and in addition they showed in their large intestines very marked necrotic and ulcerated lesions, which were laden with common intestinal bacteria. These various pathological findings provide possible explanations for some of the frequent secondary infections that occur in cases of human scurvy.

In Höjer's (3) series only about 30 per cent of his severely scorbutic guinea pigs showed infections. This low figure may be partly explained by the fact that they survived for just a few weeks. On the other hand, 50 per cent of the animals with mild scurvy developed infectious lesions, and about 20 per cent of the much longer-lived normal animals showed similar lesions.

In the course of his experiments, Heymann (23) reported that he lost a large number of scorbutic guinea pigs with pneumococcic pneumonia.

II. Clinical—latent scurvy. Even before the onset of definite symptoms of human scurvy, in the so-called period of latent scurvy, the affected individual is particulaly susceptible to infections (24) and if these are contracted they run an unusually severe course.

In 1919, Wiltshire (25) described the occurrence of small conical swellings in the hair follicles of the legs of scorbutic Serbian troops and he also found them during the scurvy season (January and June) in apparently normal individuals. The latter were probably suffering from latent scurvy.

One of the most typical pathological lesions in scurvy is the increased permeability of the blood vessel wall which allows the blood to ooze into the tissues. Gothlin (26) was able to devise a method of measuring the permeability of the cutaneous capillaries. In 1931, he found that 18 per cent of a group of apparently healthy Swedish country school children (11 to 14 years) were suffering from vitamin C undernourishment. Hopkins (27) was able to associate a period of ill

health in boys in a preparatory school with a lack of fresh fruit and vegetables during the winter months. When a little fresh fruit was supplied, the minor ailments and the listlessness disappeared.

In children who are suffering from undiagnosed latent scurvy, vaccination may precipitate acute scorbutic symptoms (28, 29). Abels (29) quotes the case of an anemic, atrophic ten months old child who developed both scurvy and a high prolonged fever after vaccination. This may explain the reluctance of parents in backward regions of Austria towards having their children vaccinated in the winter, when no doubt their diets are partially deficient in this vitamin. In such children, coryza and pharyngitis may be surprisingly severe and may usher in evident scurvy, and skin ulcers and cystitis are also very prevalent. In fact, this author has gone so far as to say that manifest scurvy is always preceded by an infection. Other investigators (30) however have found this sequence of events to occur frequently, but not invariably. The increased metabolism caused by the infection probably accentuates the vitamin deficiency and hastens the appearance of active scurvy.

As in the case of the other deficiency diseases, there seems to be some predisposition to scurvy, as only a certain number of those on a uniformly deficient diet develop it (24b).

Manifest scurvy. Infections are very commonly associated with active scurvy (31), and Von Niedner (31) reported that scorbutic soldiers succumb to the slightest infection. Numerous authors (29, 32) have found respiratory infections, including grippe and pneumonia, to be very common in such individuals. One of these authors, Erdheim (33), stated that such diseases were frequently very grave and persistent in scorbutic children. Tuberculosis was also very prevalent in several series (32b, 34). In one of these, Salle and Rosenberg (34) found that all the deaths (17) in their 461 cases were from tuberculosis and that 9 to 22 per cent of their different groups of scorbutic patients suffered from this disease. They also remarked on the great frequency with which cases of infantile scurvy were complicated by florid tuberculosis. Diphtheria (8, 32b, 34b) and dysentery and typhoid (29, 34a, 35) were also very often encountered by various clinicians in scorbutic individuals. Mackie (22) described an epidemic of dysentery (Shiga) among scorbutic war refugees in the near East, which was almost as

virulent as cholera. Many investigators (32b, 35, 36) have reported that cystopyelitis and nephritis were very common, and that furuncles, paronychia and gun shot wounds (2, 32b, 35, 36) were often very difficult to clear up in scorbutic patients.

In 1927, Funk (37) stated that an epidemic of pneumonia in the Sudan disappeared when antiscorbutic treatment was given to the numerous cases of scurvy which appeared at about the same time. This would suggest that scurvy lowered the resistance to this infection.

Oral infections. If a guinea pig is kept on a completely vitamin C free diet for even two days, marked abnormalities are seen in its teeth (3, 30), and if such a diet is kept up for a few weeks, the teeth may become devitalized. Apical abscesses are prone to appear in such teeth later on. The same processes may occur in man (38), and the resistance to infection may be indirectly lowered by the presence of these bacterial foci. Höjer and Westin (30) also found that although enough vitamin C was given (1.2 minimum protective doses of orange juice) to prevent the appearance of any scorbutic changes in the teeth, except perhaps an uncertain hyperemia in the pulp cavity, the animals were still markedly susceptible to infection.

After analyzing the diets of groups of individuals, Hanke (39) stated that those whose diets were complete suffered from dental caries, gingival irritation or pyorrhoea much less frequently than those whose diets were deficient in either or both vitamin C and vitamin D. The details of the diets were unfortunately not given. Spongy gums, associated with infections, were cleared up by the use of an adequate diet plus 1 pint of orange juice, the juice of a lemon and from one-fourth to one-half a head of lettuce daily. The resistance to other infections, especially to colds, was raised at the same time, and in one individual a long standing osteo-myelitis was also cured. When pyorrhoea was present surgical measures had usually to be combined with the dietetic treatment unless the condition was very mild.

4. Susceptibility to artificially induced infections

(a) Reduced resistance in vitamin C deficient animals. In 1923, Findlay (40) reported that guinea pigs fed on a vimamin C deficient diet died more frequently after mtraperitoneal injections of bacteria than

controls fed on normal diets. The organisms used were B. coli, staphylococcus aureus, streptococcus hemolyticus and pneumococcus.

In the same year, Werkman and his co-workers (15) found that there was a definitely, although not markedly, increased susceptibility to intraperitoneal injections of pneumococci or B. anthracis in scorbutic guinea pigs as compared with controls.

According to Abels (41), guinea pigs with scurvy die after intraperitoneal injection of B. coli, whereas normal animals withstand several times this dose.

B. aertrycke cultures were fed to 2 scorbutic and 2 normal guinea pigs by Grant (42). One of the scorbutic animals died and the three others were killed so that the spread of the bacilli to the various organs and the blood could be determined. Liver, spleen, lung and blood cultures were negative in the normal animals, whereas both the spleen and one of the blood and one of the liver cultures from the scorbutic animals yielded B. aertrycke. These findings would suggest that in scurvy the intestinal wall is more permeable to bacteria.

Schmidt-Weyland and Koltzsch (43) infected normal and scorbutic guinea pigs by either inhalation or feeding, or by the combination of both methods, with a mixture of pneumococci and a fowl cholera pasteurella strain. They found that the animals on the scurvy producing diet were much more susceptible to such infections and that many of them died of pneumonia.

A trypanosome infection was set up in half their scorbutic guinea pigs by Nassau and Scherzer (44). They reported that this procedure hastened the onset of the scurvy, but only slightly decreased the duration of life.

Hojer (3) divided about ninety guinea pigs into several groups which were fed normal, completely vitamin C deficient, and several different partially C deficient diets. Half of each group was infected intramuscularly with probably too large a dose of a low virulent human strain of B. tuberculosis. All of the four severely scorbutic animals showed larger lesions than many of the rest. Only one guinea pig, which was fed the normal diet, showed no evidence of the disease, except for fibrous healing at the site of the subcutaneous injection. The course of the disease did not parallel the degree of scurvy in the partially scorbutic animals, but microscopic examination showed that

the connective tissue reaction to the tuberculous foci at a specified time after infection varied directly with the amount of vitamin C in the diet. The more vitamin C fed, the more adequate was the connective tissue response.

Coulard (45) stated that the tuberculous processes at the site of injection, the enlargement of the glands, and the lesions in the spleen developed much more rapidly in the scorbutic than in the normal guinea pig.

Guinea pigs suffering from slight scurvy were reported by Heymann (23) to be no more susceptible to tuberculosis than normal animals. When however the scurvy was moderately severe, marked loss in weight and early death (73 days) followed infection with a human strain of tuberculosis. Similarly infected guinea pigs fed on a normal diet lived 141 days on the average.

In order to induce intestinal tuberculosis in the guinea pig after the feeding of tuberculous sputum, McConkey (46) found that a partial deficiency of vitamins A, C and D was necessary. However, the lack of vitamin C seemed to be especially important.

Bieling (5) was able to produce a localized chronic tuberculosis in his guinea pigs. These animals were strong and well nourished and remained in such condition for over a year. If, however, they were put on a vitamin C free diet, they seemed particularly susceptible to scurvy and died long before the non-infected controls. These early deaths could be attributed to an activation of the chronic tuberculosis by the scurvy, although the sections showed neither very marked scurvy nor tuberculosis extensive or severe enough to explain the rapid deaths. This increased susceptibility of the tuberculous animal to scurvy was gradually built up, as recently infected animals did not react differently from uninfected ones. If the amount of vitamin C in the diet was reduced but not absent, the same phenomena were observed, but the onset of scurvy and the deaths were delayed. Apparently therefore the development of scurvy is accelerated when tuberculosis is present.

Quite a number of studies on this subject have been carried out by Mouriquand and his collaborators. In 1924, they (5b) showed that a larger percentage of scorbutic than of normal guinea pigs died after the injection of tuberculin. In 1925 (47), they determined the effect

of the injection of fairly large (10 million) and very small numbers (400) of tubercle bacilli into chronic scorbutic and normal guinea pigs. When the massive dose was used, for the first three weeks the deficient animals showed less extensive lesions and less loss in weight than the controls. After this time the scorbutic animals went rapidly down hill and died before the controls. With the smaller dose no initial refractory stage was seen, and the lesions in the animals with scurvy progressed more rapidly and led to earlier death. Two years later, they reported that if after feeding a diet completely deficient hi vitamin C, a ration partially lacking in this factor was given, a chronic scurvy was established which was characterized by a tendency to relapses of the active scurvy, and by great susceptibility to infection with B. tuberculosis. When such an infection was set up, the animals suffering from chronic scurvy lost weight and died after a short time, and there was not the slightest evidence of tissue reaction against the bacilli, even though these were much attenuated. Normal animals similarly infected reacted with "multiple" sclerosis and lived considerably longer.

(6) *Increased resistance due to the addition of vitamin C.* The addition of vitamin C rich lemon juice to an adequate diet favorably influenced the course of tuberculosis in guinea pigs, according to Leichentritt (48), The experiments of Hericourt and Richet (49) may possibly be interpreted as providing further confirmation of the important rdle played by vitamin C in this disease. They found that if dogs were injected with raw meat juice they withstood a tuberculous infection better than similar animals injected with cooked meat juice. The cooking no doubt destroyed the vitamin C, but it may have had other deleterious effects on the meat juice as well. When the diet contained vitamin D, Grant (50) found that increasing the amount of vitamin C seemed to decrease the severity and extent of the tuberculous lesions in the lungs of guinea pigs.

(c) No reduced resistance in vitamin C deficient animals. In some of Grant's (50) other experiments she used diets in which the vitamins were unbalanced and the results were entirely different. For example, she reported that if vitamin D was deficient in the diet, the addition of vitamin C tended to increase the amount of tuberculosis in the

lungs, and the same effect also followed the substitution of vitamin C for vitamin D at the time of inoculation.

In one of their earlier publications (1922), Mouriquand (51) and his co-workers reported that chronic scurvy did not accelerate the course of tuberculosis in the guinea pig. Their later work gave results entirely opposed to those of this early investigation.

Bieling (5a) stated that "transitory milk or hunger scurvy" did not lead to a decreased resistance to infection.

When Jaffe (52) infected the leg bones, muscles or skin with staphylococci and put the guinea pigs on a scorbutogenic diet at the same time, he found that about half of them developed severe infections and that these animals lived longer (42 days) than the uninfected controls, and did not show scorbutic changes at death. If the infections were mild, death from scurvy occurred at about the usual tune (21 to 30 days). If the annuals were on the deficient diet for 10 days before infection, they died abnormally quickly from the scurvy (7 to 12 days). Baj (53) partially confirmed these findings when he reported that the characteristic bone changes of scurvy were less marked in animals infected with staphylococci. He suggested that antiscorbutic substances were formed by the bacteria. He also stated that the infections in scorbutic animals were no more severe than those in controls fed normal diets.

As many mice on a vitamin C deficient diet survived after intraperitoneal injections of mouse typhoid bacilli as mice on a complete diet, according to Hotta's (54) results.

Summary of artificial infection experiments. Relatively few of these investigators have brought forward evidence to the effect that a deficiency of vitamin C does not lead to a lower resistance to infection, and some criticism of their work is possible. For example, Hotta's results were based on one experiment including at the most 32 rats, and the rat is apparently able to synthesize this vitamin, and Mouriquand's numerous later results contradicted his earlier report, which need not therefore be considered further.

Qn the other hand, Findlay, Werkman and also Nassau found that a greater proportion of scorbutic than of normal guinea pigs died after intraperitoneal injections of bacteria or trypanosomes. The last two

authors stated that the reduction in the resistance was not marked. Jaffe infected the legs of guinea pigs that had been on a scurvy producing diet for ten days with staphylococci and found that they died very quickly. As Schmidt-Weyland's method of infection more nearly simulates that occurring in nature, it is probably preferable to those used by the above mentioned authors. Schmidt-Weyland's results showed many more deaths from pneumonia among the scorbutic animals.

The interest in the question of whether scurvy renders an annual particularly susceptible to tuberculosis was possibly engendered by clinical reports to that effect. The guinea pig develops scurvy readily and it is also very susceptible to tuberculosis. It is probably more susceptible to both these conditions than man. Consequently, in most of these experiments the resistance has had to be gauged either by variations in the duration of life or in the extent and nature of the lesions. As the course of tuberculosis in even normal guinea pigs is variable, these criteria are somewhat unsatisfactory. According to Heymann, the susceptibility varies with the severity of the scurvy. Slight scurvy does not affect the resistance, whereas animals suffering from moderately severe scurvy are less resistant and die quickly from tuberculosis. Hojer's experiments, which might have confirmed Heymann's, gave variable results from the point of view of duration of life. Goulard and also Mouriquand found that tuberculosis was fatal more quickly in scorbutic than in normal guinea pigs. When Hojer examined his animals in regard to the extent of the lesions, his results were more consistent, as the markedly scorbutic animals showed the greatest involvement, the normal the least, and in the slightly scorbutic the lesions were variable. Goulard also remarked on the more extensive tuberculosis found in scorbutic animals. Mouriquand noted that guinea pigs affected with chronic scurvy were unable to produce the usual connective tissue reaction to tubercle infection. Hojer also reported that the efficiency with which this reaction took place varied directly with the amount of vitamin C in the diet.

Several authors have provided information on the part played by bacteria in precipitating acute scurvy. Bieling found that animals with chronic tuberculosis were very susceptible to scurvy and Nassau also stated that the presence of a trypanosome infection seemed to

accelerate the onset of scurvy. Jaffe, on the other hand, found that a marked subcutaneous or osseous infection prevented the onset of scurvy and that a mild infection did not affect the course of this avitaminosis.

However, Jaffe's results may possibly have been due to the production of the vitamin by the bacteria. Baj, who suggested the above explanation, also found that the presence of a staphylococcic infection lessened the severity of the scurvy.

From Grant's experiment it would appear that the intestinal mucous, membrane in animals suffering from scurvy is more permeable to bacteria, and McConkey indicates that the intestine in such animals is more susceptible to infection.

Three investigators also have shown that added amounts of vitamin C assist animals on normal diets in their reactions against tuberculosis.

5. *The use of vitamin C in clinical infections*

Numerous reports demonstrating the good effect of vitamin rich diets in clinical tuberculosis have been published, but it is impossible to decide what role vitamin C plays in such treatment. Also, one can not be sure that the good results which Höjer (3) obtained when he fed a series of twenty tuberculous children raw blood serum (50 to 100 cc.) daily for four months were due to the vitamin C contained in that substance. In a later experiment, the same author (30) compared the effect of the addition of vitamin C (one orange daily) or of added carbohydrate (a pastry) on samtorium cases of tuberculosis. The patients were grouped in pairs as closely alike in age, sex, tuberculous involvement, and prognosis as possible. One of each pair received the orange and one the pastry. The sanitorium was in an isolated region where the supply of vegetables and fruit was limited, especially in thd three months of the experiment (March, April and May). The highest mortality from this disease also usually occurred in these three months. Of the cases fed the extra vitamin C, 17 showed better, 3 showed similar, and 1 showed worse results than the controls. The cases were examined regularly by expert clinicians, and although the effects were not easy to evaluate, it appeared that the provision of plenty of vitamin C assisted in the healing of the tuberculous lesions. Woringer and Sala (55) advised generous additions of vitamin C to

whooping cough cases, for although scurvy is very rare in Strassburg, they saw four cases of whooping cough and scurvy together. McConkey (56) reported that the administration of cod liver oil and tomato juice has a favorable effect on intestinal tuberculosis which was secondary to a pulmonary infection. In order to determine whether the vitamin C was of value he gave three patients on normal diets a cod liver oil concentrate alone. No change could be seen until orange juice was added also, when two of them began to show satisfactory improvement. In a second test, he gave two cases irradiated brewer's yeast. Again they did not improve until the orange juice was administered also. The possibility that the good effects were due to the combination of the vitamins can not be ruled out, as none of the patients were given vitamin C alone. Bloch (57) is of the opinion that vitamin A is of more importance than vitamin C in the treatment of tuberculosis, but other authors (31) claim that generous amounts of vitamin C are essential in the treatment of such cases.

Summary. The results which have been published up to date suggest that this factor plays a very important r61e in the combatting of tuberculous infections, but further investigations will be necessary before this can be conclusively settled.

6. The mechanism underlying the decreased resistance in scurvy

According to Höjer (3), the decreased resistance in scurvy is due to the atrophy of the various organs hi the body that protect it against infections. These organs include the lymph nodes, spleen and bone marrow. Findlay (40) had previously ascribed the low resistance which he found in scorbutic animals to the changes that were present hi the bone marrow.

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Role of vitamin C in the function of the vascular endothelium.

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Abstract

SIGNIFICANCE: Vitamin C, or ascorbic acid, has long been known to participate in several important functions in the vascular bed in support of endothelial cells. These functions include increasing the synthesis and deposition of type IV collagen in the basement membrane, stimulating endothelial proliferation, inhibiting apoptosis, scavenging radical species, and sparing endothelial cell-derived nitric oxide to help modulate blood flow. Although ascorbate may not be able to reverse inflammatory vascular diseases such as atherosclerosis, it may well play a role in preventing the endothelial dysfunction that is the earliest sign of many such diseases.

RECENT ADVANCES: Beyond simply preventing scurvy, evidence is mounting that ascorbate is required for optimal function of many dioxygenase enzymes in addition to those involved in collagen synthesis. Several of these enzymes regulate the transcription of proteins involved in endothelial function, proliferation, and survival, including hypoxia-inducible factor-1α and histone and DNA demethylases. More recently, ascorbate has been found to acutely tighten the endothelial permeability barrier and, thus, may modulate access of ascorbate and other molecules into tissues and organs.

CRITICAL ISSUES: The issue of the optimal cellular content of ascorbate remains unresolved, but it appears that low millimolar ascorbate concentrations are normal in most animal tissues, in human leukocytes, and probably in the endothelium. Although there may be little benefit of increasing near maximal cellular ascorbate concentrations in normal people, many diseases and conditions have either systemic or localized cellular ascorbate deficiency as a cause for endothelial dysfunction, including early atherosclerosis, sepsis, smoking, and diabetes.

FUTURE DIRECTIONS: A key focus for future studies of ascorbate and the vascular endothelium will likely be to determine the mechanisms and clinical relevance of ascorbate effects on endothelial function, permeability, and survival in diseases that cause endothelial dysfunction.

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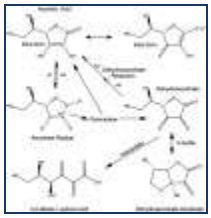
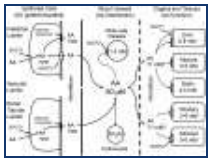
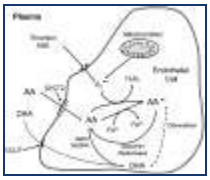
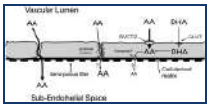
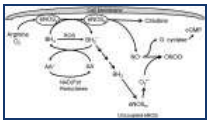


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Scurvy in hospitalized elderly patients

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Abstract

Objectives

The aim of this study was to systematically screen hospitalized elderly patients for clinical symptoms of scurvy and to confirm the diagnosis with biological measures.

Settings

Geriatric acute care ward.

Measurements

Scurvy symptoms (one or more among perifollicular hyperkeratosis, petechiae or bruises, haemorrhagic features caused by venous puncture, severe gingivitis). We compared associated diseases, nutritional status, need for assistance for feeding, serum albumin, transthyretin, B9 and B12 vitamins, iron status and Serum Ascorbic Acid Level (SAAL) and outcome (in-hospital mortality) between scurvy and scurvy free patients.

Results

18 patients with clinical symptoms of scurvy (scurvy group) were identified out of 145 consecutive patients (12%). They were compared to 23 consecutive control patients with no clinical symptoms of scurvy (scurvy-free group). SAAL was significantly lower (1.09 ± 1.06 vs 4.87 ± 4.2 mg.L-1, p<.001) and vitamin C deficiency more frequent (94 vs 30 %, p<.001) in the scurvy group. Moreover, in scurvy group, coronary heart disease (39 vs 9 %, p=.028), need for assistance for feeding (56 vs 13 %, p=.006) and in-hospital deaths (44 vs 9 %, p=.012) were more frequent.

Conclusion

Ninety-four percent of patients with clinical symptoms of scurvy had vitamin C deficiency. Our results suggest that in hospitalized elderly patients, clinical symptoms allow scurvy diagnosis. Scurvy could be a frequent disease in elderly patients admitted to acute geriatric ward.

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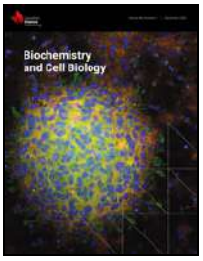

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STUDIES ON ACCLIMATIZATION AND ON THE EFFECT OF ASCORBIC ACID IN MEN EXPOSED TO COLD

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ABSTRACT

This experiment was planned to study the problem of acclimatization in humans and to determine the effect of ascorbic acid in men exposed to cold while being fed a normal or survival ration. Ascorbic acid has greatly improved the resistance of men exposed to cold and fed a survival ration. No beneficial effect was observed when the subjects were fed a normal ration. This difference in response may be due to the fact that the experimental conditions differed somewhat between these two experiments. In any event, the subjects on a restricted food intake were certainly under greater conditions of stress. Evidence of acclimatization was obtained with survival rations but not with normal rations. Some conclusions have been made on the use, by men exposed to cold, of survival rations composed exclusively of carbohydrates. Finally, it is estimated that 2800 calories is the daily requirement for men relatively inactive, wearing only shorts, low shoes, and socks, and exposed to an ambient temperature of 60°F.

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Subgroup analysis of large trials can guide further research: a case study of vitamin E and pneumonia

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Abstract

Background:

Biology is complex and the effects of many interventions may vary between population groups. Subgroup analysis can give estimates for specific populations, but trials are usually too small for such analyses.

Purpose:

To test whether the effect of vitamin E on pneumonia risk is uniform over subgroups defined by smoking and exercise.

Methods:

The Alpha-Tocopherol Beta-Carotene Cancer Prevention Study examined the effects of vitamin E (50 mg per day) and β-carotene (20 mg per day) on lung cancer in 29,133 male smokers aged 50–69 years using a 2 × 2 factorial design. The trial was conducted among the general community in Finland during 1985–1993; the intervention lasted for 6.0 years (median). In the present study, we tested the uniformity of vitamin E effect on the risk of hospital-treated pneumonia (898 cases) by adding a dummy variable to allow each subgroup its own vitamin E effect in a Cox model covering all participants.

Results:

Vitamin E effect was not uniform over eight subgroups defined by baseline smoking (5–19 vs ≥20 cigarettes per day), age of smoking initiation (≤20 vs ≥21 years), and exercise during leisure time (yes vs no). Vitamin E decreased pneumonia risk by 69% (95% CI: 43% to 83%) among participants who had the least exposure to smoking and exercised during leisure time. Vitamin E increased pneumonia risk by 79% (95% CI: 27% to 150%) among those who had the highest exposure to smoking and did not exercise.

Limitations:

Although the evidence of heterogeneity is strong, it is not evident to what extent the estimates of effect or

the limits between the subgroups can be extrapolated to other populations.

Conclusion:

Subgroup analysis of large trials should be encouraged, though caution is needed in the interpretation of findings. The role of vitamin E in susceptibility to pneumonia in physically active nonsmokers warrants further study.

Trial registration:

ClinicalTrials.gov NCT00342992.

Keywords: vitamin E, pneumonia, smoking, leisure time exercise, α-tocopherol, β-carotene, subgroup analysis

Introduction

The size of a controlled trial is usually based on a power calculation, the goal of which is to determine the minimal number of participants needed to test whether an overall difference exists between the intervention and control groups. Such trials are too small to test subgroup differences. Furthermore, carrying out numerous subgroup comparisons leads to the multiple testing problem. Such reasoning is the major cause for discouraging subgroup analyses.[1–5]

The above argument has limitations, however. For example, if a trial collects data on a secondary outcome which are much more numerous than the primary outcome, say lung cancer, subgroup analysis on the secondary outcome, such as the common cold,[6] does not suffer from low statistical power. Furthermore, most controlled trials study the effect of drugs having a specific biochemical target within patients who are narrowly selected, and a large within-trial variation in the effect may be unlikely in such cases. However, it is possible that the within-trial variation in the effect is substantially greater for interventions that have complex and broad effects on the human system, in particular when the effects are studied in heterogeneous populations. Thus, while reasons exist for being cautious about subgroup analysis in general, there are conditions when subgroup analyses may be justified.

Previously, we explored the effect of vitamin E on pneumonia risk among the 29,133 male smokers of the Alpha-Tocopherol Beta-Carotene [ATBC] Study.[7,8] We found significant modification of vitamin E effect by age of smoking initiation, in that the vitamin reduced the risk in those who started smoking at a late age and, within this subgroup, baseline smoking further modified the effect so that the benefit was greatest among those who smoked the least.[9] Since physical activity leads to oxidative stress,[10] we separately hypothesized that vitamin E might reduce pneumonia risk among physically active ATBC Study participants, and found that the vitamin halved the risk in those who exercised during leisure time.[11] These findings indicate that cigarette smoking and exercise might modify the effect of vitamin E on pneumonia risk. However, since several comparisons were made, the multiple testing problem cannot be entirely dismissed. Therefore, in this paper we analyze the subgroup differences in all ATBC Study participants simultaneously.

If there is firm evidence that the effect of vitamin E supplementation on health outcomes of the ATBC participants is heterogeneous, this would imply that subgroup analyses in other large-scale trials on vitamin E, and possibly in large-scale trials on other subjects, should be encouraged rather than discouraged.

Material and methods

Participants

The rationale, design, and methods of the ATBC Study examining the effects of vitamin E (*dl*-α-tocopheryl acetate, AT, 50 mg/day) and β-carotene (BC, 20 mg/day) on the incidence of lung cancer and other cancers have been described in detail.[7–9] The ATBC Study is registered at ClinicalTrials.gov under the identifier NCT00342992.

In brief, to be eligible, male participants aged 50–69 years had to smoke ≥5 cigarettes per day at entry, and those enrolled in the trial (N = 29,133) were randomized to one of four intervention arms and administered placebo, AT, BC, or AT + BC, using a 2 × 2 factorial design. Compared with baseline levels, supplementation increased the serum level of α-tocopherol by 50%.[7,8] The intervention continued for 5 to 8 years until April 1993. The trial was approved by the review boards of the participating institutions and all participants gave written informed consent. Compliance with supplementation was high: some 90% of the subjects took more than 90% of their prescribed capsules during their active participation in the trial.[7,8]

Baseline characteristics

Before randomization at baseline, the participants completed questionnaires on medical and smoking histories and general background characteristics. A detailed dietary history questionnaire was completed that provided data regarding vitamins C and E, and coffee consumption.[12] Age of smoking initiation was not available for seven participants and dietary data for 2,022 participants.

Previously, we found that dichotomization of the age of smoking initiation with the cutoff point at 21 years appropriately captured the variation of the vitamin E effect,[9] and the same cutoff was used in this study. Although smoking is a continuous variable, it is heavily clustered to multiples of 20 (and 10) cigarettes per day. In this study, we dichotomized cigarette smoking to 5–19 cigarettes per day and to ≥20 per day. As we recognized that in both cases dichotomization leads to a loss of information of the continuous variables, we examined the effect of vitamin E in smaller ranges in Tables 2 and 3.

Table 2

The effect of vitamin E on pneumonia incidence in ATBC participants who initiated smoking at ≥21 years, smoked 5–19 cigarettes per day, and exercised during leisure time

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Notes:

[a]The number of participants in the vitamin E and no-vitamin E groups was the same within 8% accuracy in all subgroups shown;
[b]A/B refers to A cases of pneumonia among the vitamin E participants and B cases of pneumonia among the no-vitamin E participants;
[c]The Cox model comparing participants who received vitamin E with those who did not;
[d]Data on diet were missing for 160 participants, which included one case of pneumonia in the vitamin E group and three cases in the no-vitamin E group.

Abbreviations: RR, risk ratio; CI, confidence interval.

Table 3

The effect of vitamin E on pneumonia incidence in ATBC participants who initiated smoking at ≤20 years, smoked ≥20 cigarettes per day, and did not exercise during leisure time

Subgroup	No. of men[a]	Cases of pneumonia[b]	Effect of vitamin E	
			RR (95% CI)[c]	Test for interaction (*P*)
All	6,686	152/115	1.35 (1.06, 1.7)	
β-Carotene supplementation				
No	3,371	89/51	1.79 (1.27, 2.5)	0.02
Yes	3,315	63/64	1.01 (0.71, 1.4)	
Restriction to the no-β-carotene participants:				
No β-carotene	3,371	89/51	1.79 (1.27, 2.5)	
Cigarettes (1/day)				
20–25	2,269	62/36	1.78 (1.18, 2.7)	1.0
26–80	1,102	27/15	1.83 (0.97, 3.5)	
Age of smoking initiation (years)				
6–17	1,616	48/26	1.94 (1.20, 3.1)	0.6
18–20	1,755	41/25	1.64 (1.00, 2.7)	
Age at baseline (years)				
50–59	2,466	55/31	1.84 (1.19, 2.9)	0.8
60–69	905	34/20	1.70 (0.98, 3.0)	
Dietary vitamin E (mg/day)[d]				
<9	1,231	31/22	1.52 (0.88, 2.6)	0.5
≥9	1,909	49/26	1.90 (1.18, 3.1)	
Dietary vitamin C (mg/day)[d]				
<70	1,229	38/22	1.76 (1.04, 3.0)	0.9
≥70	1,911	42/26	1.69 (1.03, 2.8)	
Coffee (mL/day)[d]				
<500	1,188	38/20	1.95 (1.13, 3.4)	0.5
≥500	1,952	42/28	1.56 (0.96, 2.5)	

Notes:

[a]The number of participants in the vitamin E and no-vitamin E groups was the same within 5% accuracy in all subgroups shown;

[b]A/B refers to A cases of pneumonia among the vitamin E participants and B cases of pneumonia among the no-vitamin E participants;

[c]The Cox model comparing participants who received vitamin E with those who did not;

[d]Data on diet were missing for 231 participants, which included nine cases of pneumonia in the vitamin E group and three cases in the no-vitamin E group.

Abbreviations: RR, risk ratio; CI, confidence interval.

The baseline questionnaire on physical activity during leisure time was a modification of that used originally in the Gothenburg study focusing on cardiovascular diseases.[13] The intensity of average physical activity during leisure time over the previous 12 months was enquired about using the following alternatives: 1) light: reading, watching TV, listening to the radio, or going to movies, ie, activities that are not physically demanding; 2) moderate: walking, fishing, hunting, or gardening quite regularly; and 3) heavy: actual physical exercise, such as jogging, skiing, swimming, gymnastics, and court and field sports quite regularly. In the current analyses we combined answers 2) [n = 15,191] and 3) [n = 1,744] to the category "exercise during leisure time". Data on exercise were not available for 14 participants.

Outcome and follow-up time

The events for this study, the first hospital-treated cases of pneumonia after randomization, were ascertained from the national Hospital Discharge Register using the unique personal identification numbers for linkage (see details in Hemilä et al)[9]. Pneumonia cases recorded in the Hospital Discharge Register reflect clinically more severe cases of greater health and economic significance, whereas less severe cases of pneumonia treated as outpatients are not recorded in the Register. Use of the Hospital Discharge Register allowed for the obtaining of information on pneumonia in all study participants irrespective of whether they continued in or had dropped out of the trial.

Follow-up time for each participant began from the day of randomization, and continued until the date of first hospital discharge for pneumonia, death, or the end of the trial, April 30, 1993, whichever came first. The median follow-up time of the participants was 6.0 years, and there was a total of 167,968 person-years of observation.

Statistical methods

We estimated the effect of vitamin E supplementation on pneumonia incidence through Cox models. We calculated the risk ratio (RR) and the 95% confidence interval (CI) of the RR using the PROC PHREG program of the SAS package of programs (release 8.2, SAS Institute, Inc., Cary, NC). No covariates were included in the models analyzing the treatment effects. As to supplementation, we carried out the analyses following the intention-to-treat (ITT) principle.

In Table 1, we compared the trial participants administered vitamin E (AT and AT + BC) with those not receiving vitamin E (the no-vitamin E participants; placebo and BC). Since, in Table 3, we observed that AT and BC supplementations interacted, we restricted further subgroup analyses of Table 3 to the no-BC participants (AT and placebo arms). Because of this interaction, we also re-tested the heterogeneity of Table 1 by restricting to the no-BC participants.

Table 1

The effect of vitamin E on pneumonia incidence by level of cigarette smoke exposure and exercise during leisure time: ATBC Study 1985–1993

Age of smoking initiation (years)	Cigarettes per day at baseline		Effect of vitamin E	
			Exercise during leisure time	
			Yes	No
≥21	5–19	RR[a] (95% CI)[a]	0.31 (0.17, 0.57)	0.85 (0.44, 1.64)
		Cases of pneumonia[b]	14/43	17/19
		No. of men[c]	2,216	1,043
≥21	≥20	RR[a] (95% CI)[a]	0.84 (0.48, 1.46)	0.86 (0.50, 1.49)
		Cases of pneumonia[b]	24/27	24/28
		No. of men[c]	2,445	1,763
≤20	5–19	RR[a] (95% CI)[a]	1.24 (0.87, 1.78)	1.05 (0.71, 1.56)
		Cases of pneumonia[b]	68/56	51/50
		No. of men[c]	4,602	2,688
≤20	≥20	RR[a] (95% CI)[a]	0.88 (0.67, 1.15)	1.35 (1.06, 1.73)
		Cases of pneumonia[b]	97/110	152/115
		No. of men[c]	7,669	6,686

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Notes:

[a]The Cox model comparing participants who received vitamin E with those who did not;
[b]A/B refers to A cases of pneumonia among the vitamin E participants and B cases of pneumonia among the no-vitamin E participants. Data on age of smoking initiation or exercise were missing from two pneumonia cases among the vitamin E participants and from one case among the no-vitamin E participants; these cases are not included in this table;
[c]The number of participants in the vitamin E and no-vitamin E groups was the same within 5% accuracy in each of the eight groups. The uniformity of the vitamin E effect was tested by adding a dummy variable for vitamin E effect in seven groups of the table, allowing each of the eight groups their own vitamin E effect. The regression model was improved by $\chi^2(7\ \text{df}) = 26.6$, $P = 0.0004$, compared to the model with a uniform vitamin E effect. Heterogeneity is mainly caused by the upper-left and lower-right cells: the addition of only these two cells improved the model by $\chi^2(2\ \text{df}) = 23.4$. The difference between the above two models is fully explained by chance: $\chi^2(5\ \text{df}) = 3.2$. The addition of the third-order interaction term, between vitamin E supplementation, age of smoking initiation, cigarettes per day, and leisure time exercise, to the model containing all lower level interaction terms, improved the regression model by $\chi^2(1\ \text{df}) = 5.4$, $P = 0.02$. Since vitamin E and β-carotene supplementations interact in the lower-right cell (see Table 3), we also tested the uniformity of vitamin E effect among the no-β-carotene participants (n = 14,564). Adding a dummy

variable for vitamin E effect in seven groups of the table improved the model by $\chi^2(7\ df) = 22.8$, $P = 0.002$. Adding only the upper-left and lower-right cells improved the model by $\chi^2(2\ df) = 17.8$, indicating that the effect of vitamin E is restricted to the upper-left and lower-right cells. The difference between the two models is fully explained by chance: $\chi^2(5\ df) = 5.0$. Nevertheless, adding the third-order interaction term to a model containing all lower level interactions did not significantly improve the model: $\chi^2(1\ df) = 2.0$, $P = 0.16$. Vitamin E and β-carotene supplementations did not interact in cells of this table other than the lower-right cell.

Abbreviations: RR, risk ratio; CI, confidence interval.

To test the statistical significance of interaction between vitamin E supplementation and potential modifying factors, we first added vitamin E and the modifying factor to the regression model. The statistical significance of the interaction was thereafter calculated from the change in −2 × log (likelihood) when the interaction term for vitamin E supplementation and the modifying factor were added to the model. In our subgroup analyses in Tables 2 and 3, we split the subgroup variables at levels leading to a reasonably similar number of cases in the control groups.

Nelson-Aalen cumulative hazard functions were constructed using the STATA sts program (Release 9, Stata Corp, College Station, TX). Two-tailed *P*-values are presented.

Results

Among all ATBC participants, the cases of pneumonia were identically divided between the vitamin E and no-vitamin E groups: 449 vs 449, corresponding to RR = 1.00 (95% CI: 0.88, 1.14).

We divided the participants into eight subgroups on the basis of age of smoking initiation, level of smoking at the baseline of the trial, and exercise during leisure time (Table 1). We tested the uniformity of the vitamin E effect by adding a dummy variable for vitamin E effect in seven groups of the table, and this significantly improved the Cox model ($P = 0.0004$). The heterogeneity in Table 1 is fully explained by the upper-left and lower-right corners, ie, by the opposite corners of the table. Furthermore, the third-level interaction term between vitamin E supplementation, age of smoking initiation, level of smoking, and exercise was significant when comparing the vitamin E and no-vitamin E participants. Since the effect of vitamin E was restricted to the upper-right and lower-left corners, we analyzed these two groups further.

Among the 2,216 participants who initiated smoking at a late age, smoked less than a pack of cigarettes per day, and exercised during leisure time, vitamin E supplementation reduced pneumonia risk by 69% (upper-left cell in Table 1; Figure 1). The estimated effect of vitamin E in this subgroup was robust in several further subgroup analyses. The effect was not modified by BC supplementation, age, or dietary vitamins C and E (Table 2). Dividing the participants by the age of smoking initiation and baseline smoking also led to compatible effects within the smaller subgroups. Previously, we found that coffee consumption significantly modified the benefit of vitamin E in those who started smoking at a late age.[9] The subgroup differences in Table 2 are in line with the earlier findings, but not significantly.

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Figure 1

Vitamin E and pneumonia risk in ATBC Study participants who started smoking at ≥21 years, smoked 5–19 cigarettes per day, and exercised (n = 2,216). Nelson-Aalen cumulative hazard functions for vitamin E and no-vitamin E groups are shown. Each step indicates one case of pneumonia. For the difference between the two survival curves, the logrank test gives $P = 0.00005$. The survival curves are cut at 7.2 years because the number of participants declines abruptly thereafter (no cases after 6.8 years). At six-year follow-up 576 and 535 participants remained in the vitamin E and the no-vitamin E groups, respectively.

Among the 6,686 participants who initiated smoking at an early age, smoked a pack of cigarettes daily or more, and did not exercise, vitamin E increased pneumonia risk by 35% when compared with the no-vitamin E group (lower-right cell in Table 1). However, in this subgroup the vitamin E effect was modified by BC supplementation so that the harm of vitamin E was restricted to those who were not administered BC (Table 3). Therefore, we restricted the further subgroup analyses of Table 3 to the no-BC participants. Among the no-BC participants, vitamin E increased pneumonia risk by 79%, and this effect was robust in further subgroup analyses (Table 3).

Previously, we hypothesized that the marginally significant 14% increase in pneumonia risk among those ATBC participants who started smoking at an early age (n = 21,657; the four lowest cells in Table 1) might correspond to a more unambiguous harmful effect among low-weight participants, based on an assumption of dose-dependency.14 Then we found that vitamin E increased pneumonia risk in participants weighing less than 60 kg. Unexpectedly, vitamin E also increased pneumonia risk at the opposite end of the weight scale, among those weighing over 100 kg.14 Furthermore, in both groups, harm caused by vitamin E was restricted to those who had a dietary vitamin C intake above the median. Therefore, we examined whether weight and vitamin C intake might modify the effect of supplementation outside of the lower-right corner in Table 1.

Of the low-weight high vitamin C participants, 72% (337 of 468) were outside the lower-right corner of Table 1; in these 337 participants there were 19 pneumonia cases among the vitamin E and eight cases among the no-vitamin E participants (RR = 2.7, 95% CI: 1.18–6.2). Of the overweight high vitamin C participants, 65% (397 of 613) were outside the lower-right corner of Table 1; in these 397 participants there were 10 pneumonia cases among the vitamin E and one case among the no-vitamin E participants ($P = 0.01$, Fisher's test). Consequently, weight and dietary vitamin C appear to modify the effect of vitamin E independent of smoking and exercise.

Discussion

The numbers of pneumonia cases in the ATBC Study were equally distributed between the vitamin E and no-vitamin E participants, indicating a lack of overall effect with great accuracy. However, in this study we have shown that the effect of vitamin E is not uniformly nil over all the ATBC Study population. Depending simultaneously on the two different measures of cigarette smoking and on the level of exercise, vitamin E supplementation decreased, increased or had no effect on the incidence of pneumonia (Table 1).

Among those who had the least exposure to smoking and exercised during leisure time, vitamin E decreased the risk of pneumonia by 69%. This group covers 8% of the ATBC Study participants. The effect estimate was robust in further subgroup analyses (Table 2).

The group that had the highest exposure to smoking and did not exercise covered 23% of the ATBC participants. In this group, vitamin E increased pneumonia risk by 79% in the no-BC participants (Table 3). This effect estimate was also robust in further subgroup analyses, however simultaneous BC supplementation nullified the harmful effects of vitamin E.

In our subgroup analysis focusing on smoking and exercise, 69% of the ATBC participants fell into the six middle groups that were consistent with vitamin E having no effect (Table 1). Nevertheless, it is possible that there are further modifying factors in addition to smoking and physical activity. Previously, we found that coffee drinking modified the effect of vitamin E among those who started smoking at a late age.9 Among those who started smoking at an early age, weight and dietary vitamin C intake modified the vitamin E supplementation effect.14 The current analyses are not inconsistent with these earlier subgroup findings. Thus, it seems possible that vitamin E can affect pneumonia risk in some groups of people depending on six or more modifying factors meaning that the modification is complex and does not follow a simple multiplicative model.

It is often suggested that subgroup findings should be trusted only when they are replicated in other trials. Although such a suggestion seems sound, the heterogeneity we found in the effect of vitamin E on pneumonia suggests that testing a subgroup difference in another sample of people can be all but simple. When the effect of vitamin E may depend simultaneously on six or more modifying factors, the findings for the first-level interactions depend on the selection of participants.

For example, in the whole ATBC Study, baseline smoking did not modify the effect of vitamin E ($P = 0.2$).9 However, Table 1 indicates that baseline smoking modifies the vitamin E effect conditionally on the age of smoking initiation and the level of exercise. This means that depending on the composition of the population, baseline smoking may or may not modify the effect of vitamin E. Similarly, we previously found that vitamin E halved the risk of pneumonia in ATBC participants who exercised during leisure time;11 however, Table 1 indicates that this effect is conditional on low level of exposure to smoking. On the basis of these examples, replication is not a universally valid method for deciding whether the subgroup differences observed in one trial are real or not.

Peto et al argued that “believing that a treatment effect exists in one stratum of patients, even though no overall significant treatment effect exists, is a common error”.4 This comment may be sound with respect to rather small therapeutic trials. However, Table 1 and our previous ATBC Study subgroup analyses6,9,11,14–17 show that there can be strong evidence of vitamin E effect in specific groups of people, even though no overall effect exists. Accordingly, Peto et al’s argument should not be taken as a universal objection to analyzing subgroups in the absence of overall effect.

Several investigators have strongly discouraged subgroup analysis.1–5 However, other authors have considered that a universal denial of subgroup analysis is an exaggerated reaction. Feinstein wanted to “rescue the scientific importance of valid pathophysiologic subgroups from being forgotten or destroyed by excessive vehemence in suggestions that all subgroups are evil”.18 Lagakos noted that “avoiding any presentation of subgroup analysis because of their history of being overinterpreted is a steep price to pay for a problem that can be remedied by more responsible analysis and reporting”.19 Rothwell responded to popular arguments against subgroup analysis and described situations where subgroup analysis seems to be justified.20

Altman considered that biological plausibility is a weak criterion when deciding whether a subgroup finding is likely to be real, since, according to him, physicians seem able to find a biologically plausible explanation for any finding.2 There is much room for speculation at the biochemical level, because the number of genes and their effects is huge, and Altman’s argument can have validity in such a context. However, the number of variables relevant at the population level of biology is much more limited. For example, few factors compare with the importance of smoking as a factor influencing the health of the lungs. Physical activity is also a fundamentally important factor determining health. Smoking affects the metabolism of vitamin E21 and sporadic physical stress causes oxidative stress which is not compensated by an increase in antioxidative enzyme levels, unlike regular physical activity.10 Therefore, both smoking and exercise are plausible modifying factors for the effects of vitamin E supplementation, which increases the credibility of the heterogeneity seen in Table 1.

Previously, two small trials examined the effect of vitamin E on respiratory infections in elderly people, both with less than 700 participants and lasting for about one year. In the first, Meydani et al calculated 13 *P*-values for ITT comparisons between 200 mg/day vitamin E and placebo groups, and only one of them suggested that vitamin E might reduce the incidence of respiratory infections, yet very marginally so ($P = 0.048$).[22] In the second, Graat et al found that 200 mg/day of vitamin E did not influence the incidence of respiratory infections, yet made the symptoms more severe ($P = 0.02$).[23] Because both of these trials are small and there are differences in outcome definitions etc, it is not possible to decide whether their findings are inconsistent or not. Graat et al's findings indicating harmful effects of vitamin E conflict with the wide spread belief that the vitamin is beneficial, or at least not harmful.[24] Therefore, it is not obvious whether Graat et al's findings should be interpreted as a reflection of real harm or as a result of chance. Given the strong evidence of heterogeneity we observed in the effect of vitamin E on pneumonia (Table 1) and on the common cold,[6] it seems plausible that the harmful effects observed by Graat et al are real and are explained by the selection of participants, but do not reflect a universal harmful effect of vitamin E. In this respect, the observed heterogeneity in the ATBC Study can influence the interpretation of smaller trials. Nevertheless, we are skeptical as regards the possibility of extrapolating the effect estimates and the exact limits of the subgroups of Table 1 to other contexts.

Although the division of participants on the basis of baseline physical activity and smoking is sound, both of these factors can change with time. Some participants stopped exercising or smoking over the several-years-long follow-up, yet they remained classified in the same subgroups. This phenomenon can dilute the differences between the subgroups and shift the estimates of effect closer to unity; however, it cannot explain the significant heterogeneity observed when the participants are divided by the baseline measurements. Furthermore, exercise and smoking are correlated with numerous other life style variables and we cannot dismiss the possibility that other life style factors might be behind the heterogeneity observed in Table 1. Nevertheless, this concern does not challenge the evidence indicating that substantial heterogeneity exists across various population groups in the effect of vitamin E on pneumonia risk, even if the real modifying variables might be different from those used for defining the subgroups of Table 1.

The ATBC Study included 29,133 participants which is over 40 times more than the number of participants in the Meydani et al[22] and Graat et al[23] trials. In this respect, a large trial can be considered as a series of smaller trials when there is sound justification for setting the borders between the subgroups. A particular strength of a subgroup analysis of a large trial is that the intervention and outcome definitions are identical over the trial. Therefore, subgroup analysis of a large trial can yield much more valid explanations for the heterogeneity of effect compared with the analysis of the heterogeneity of small trials that have numerous concurrent differences.

For many diseases, recognized risk factors account for at best only a modest fraction of variation in disease risk. Much effort is put into identifying new factors, either environmental or genetic. Our analyses indicate that complex patterns of interaction, perhaps in a context-specific manner, may also contribute to disease risk. Such effects may thus account for some of the unexplained variability of disease risk.

Our subgroup analyses of the respiratory infections of ATBC participants[6,9,14,15] made it also possible to hypothesize that the identified modifying factors might modify the effect of vitamin E on the mortality of these participants. We found that, conditional on a high level of dietary vitamin C intake, age modified the effect of vitamin E on mortality.[16,17] Thus, we could partially extrapolate the modifying factors identified in the subgroup analyses on respiratory infections to an outcome that has a very weak relation to such infections.

Vandenbroucke pointed out that medical science has two divergent goals.[25] First, controlled trials test whether an intervention works or not. Second, most basic medical science emphasizes discovery – searching for the biological mechanisms and causes of diseases, and for explanations in general. This divergence in views is relevant when considering a proper attitude to subgroup analysis. Evidently, great caution must be exercised when proposing a treatment on the basis of unanticipated subgroup findings. On the other hand, subgroup analysis can generate new hypotheses and direct research to new paths, which is the second goal of medical science. Refusing to conduct the subgroup analysis of large trials would lead to an inefficient use of data, the collection of which has required a substantial amount of resources.

Conclusion

The overall effect of vitamin E on pneumonia risk in the ATBC Study implies that there would be no justification for investing further resources into studying the topic because the narrow confidence interval rejects any substantial overall benefits (RR from 0.88 to 1.14). In contrast, our subgroup analysis suggests a path that should be explored: does vitamin E affect the incidence of pneumonia in physically active males who are nonsmokers or who have had only little exposure to smoking?

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Footnotes

Disclosure

The authors report no conflicts of interest in this work.

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Nutrition. 1996 Nov-Dec;12(11-12):804-9.

Vitamin C supplementation and common cold symptoms: problems with inaccurate reviews.

Hemilä H[1].

Author information

Abstract

In 1971, Linus Pauling carried out a meta-analysis of four placebo-controlled trials and concluded that it was highly unlikely that the decrease in the "integrated morbidity of the common cold" in vitamin C groups was caused by chance alone ($P < 0.00003$). Studies carried out since then have consistently found that vitamin C (> or = 1 g/d) alleviates common cold symptoms, indicating that the vitamin does indeed have physiologic effects on colds. However, widespread conviction that the vitamin has no proven effects on the common cold still remains. Three of the most influential reviews drawing this conclusion are considered in the present article. Two of them are cited in the current edition of the RDA nutritional recommendations as evidence that vitamin C is ineffective against colds. In this article, these three reviews are shown to contain serious inaccuracies and shortcomings, making them unreliable sources on the topic. The second purpose is to suggest possible conceptual reasons for the persistent resistance to the notion that vitamin C might have effects on colds. Although placebo-controlled trials have shown that vitamin C does alleviate common cold symptoms, important questions still remain.

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Vitamin C supplementation and respiratory infections: a systematic review.

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Abstract

In this review, the vitamin C trials with military personnel and with other subjects living under conditions comparable to those of military recruits are analyzed to find out whether vitamin C supplementation affects respiratory infections. For this systematic review, we identified seven trials with military personnel, three trials with students in crowded lodgings, and two trials with marathon runners. Eight of these trials were double blind and placebo controlled and seven were randomized. Five small trials found a statistically significant 45 to 91% reduction in common cold incidence in the vitamin C group. These trials were short and the participants were under heavy exertion during the trial. Furthermore, three other trials found a statistically significant 80 to 100% reduction in the incidence of pneumonia in the vitamin C group. The large number of positive findings seems to warrant further consideration of the role of vitamin C in respiratory infections, particularly in military recruits.

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Effect of ascorbic acid on the clinical course of infection-related bronchial asthma and the formation of reactive oxygen metabolites by BAL cells

Schertling M, Winsel K, Müller S, Henning R, Meiske W And Slapke J
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References and Figures are available in the above versions.

From the Berlin-Buch Research Institute for Pulmonary Diseases and Tuberculosis
(Official Director: Dr. P. Luther)

Effect of ascorbic acid on the clinical course of infection-related bronchial asthma and the formation of reactive oxygen metabolites by BAL cells

By MARGIT SCHERTLING, KLAUS WINSEL, STEFAN MÜLLER, RUDOLF HENNING, WOLFGANG MEISKE and JÜRGEN SLAPKE

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Infection-related bronchial asthma, ascorbic acid, antioxidant, peak flow, bronchial hyperreactivity, broncho-alveolar lavage, alveolar differential cell count, chemiluminescence, reactive oxygen metabolites

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List of abbreviations

AM	Alveolar macrophages
BAL	Bronchoalveolar lavage
BHR	Bronchial hyperreactivity
CL	Chemiluminescence
DCC	Differential cell count
ROM	Reactive oxygen metabolites
R_{AW}	Airway resistance (measured by occlusive pressure techniques)

Summary (Authors‘ summary in english)
Possible anti-asthmatic effectiveness of ascorbic acid was checked, in a double blind study, on patients with infection-related bronchial asthma. Basic medication to 29 out-patients was accompanied by three oral doses of 5 g/day of ascorbic acid, as compared to placebo, through 35 days. Testing periods were randomised by cross-over design with seven-day washout periods. The following parameters were investigated and were evaluated:

- Daily asthma symptom score;
- Four measurements per day of expiratory peak flow, throughout the entire study;
- Three checks throughout study of bronchial hyperreactivity, using histamine provocation;
- Broncho-alveolar lavage at the end of testing periods, with determination of alveolar differential cell count and measurement of metabolic activity of broncho-alveolar cells, using chemiluminescence;
- Global assessment of effectiveness and tolerance by doctor and patient.

Ascorbic acid exhibited merely poor broncholytic action. Symptom scores were slightly improved in the course of treatment, and peak flow values were slightly increased, as well. Hence, clinically relevant anti-asthmatic and, more specifically, broncholytic effects were not observed. However, bronchial hyperreactivity was reduced by uptake of ascorbic acid in 52 percent of all asthma patients involved. Alveolar differential cell count in patients with infection-related bronchial asthma was characterised by alveolar lymphocytosis. Chemiluminescence measurements were applied to alveolar macrophages and revealed reduced chemiluminescence response under the impact of ascorbic acid. These findings are likely to support the assumption that ascorbic acid, an anti-oxidant, reduced the buildup of reactive oxygen metabolites in patients with infection-related asthma and thus counteracted the inflammatory pathogenetic mechanism and, consequently, might be conducive to moderate lowering of bronchial hyperreactivity. The use of ascorbic acid for prophylactic medication on patients with bronchial hyperreactivity or mild forms of asthma appears to be a possible option, as a result of this study. Due consideration should be given to contraindications to administration of anti-oxidants, such as purulent infections.

Summary (Translation from German; English translation by original authors above)

The potential anti-asthmatic effectiveness of ascorbic acid was studied in patients with infection-related bronchial asthma. In addition to the basic medication, 29 outpatients were additionally treated for a period of 35 days with 5 g/day of ascorbic acid in comparison to oral placebo in 3 daily doses. The allocation of the testing periods was randomized by cross-over design with 7-day washout periods. The following parameters were investigated and evaluated: daily asthma symptom score, measurement of the expiratory peak flow 4 times per day during the entire course of the study, testing of bronchial reactivity using histamine provocation at 3 time points during the course of the study, broncho-alveolar lavage at the end of the study periods with determination of the alveolar differential cell count and measurement of metabolic activity of the bronchoalveolar cells using chemiluminescence, and global assessment of the efficacy and tolerability by doctor and patient.

Ascorbic acid exhibited a weak broncholytic effect. During treatment, symptom scores were slightly improved and there was also a slight increase in peak flow values. Hence, a clinically relevant anti-asthmatic and in particular, broncholytic effect was not observed. However, bronchial hyperreactivity was reduced by taking ascorbic acid in 52 percent of the asthma patients. The alveolar differential cell count was characterized by alveolar lymphocytosis in patients with infection-related bronchial asthma. Chemiluminescence measurements of alveolar macrophages revealed a reduced chemiluminescence response under the impact of ascorbic acid. These findings suggest that ascorbic acid, as an antioxidant, reduces the formation of reactive oxygen metabolites in patients with infection-related asthma and thus counteracts the inflammatory pathomechanism and consequently might be able to bring about moderate lowering of bronchial hyperreactivity. The use of ascorbic acid as prophylactic medication for patients with bronchial hyperreactivity or mild forms of asthma appears to be a possibility as a result of this study. Due consideration should be given to possible contraindications to administration of antioxidants, e.g., the presence of purulent infections.

Introduction

In the past 40 years, a number of works have been published that deal with the effect of ascorbic acid (4, 29) on the clinical course of bronchial asthma or on the histamine, antigen or metacholine induced bronchospasm, although some of the results that were achieved were contradictory. While in some studies, a protective effect (1, 12, 15, 19, 28, 35) of ascorbic acid on the pharmacodynamic or allergen induced bronchospasm or clinical course of bronchial asthma was established, in other cases, no effect of ascorbic acid (16, 17) could be found. The possible positive effect of ascorbic acid on bronchial asthma could be due to its antioxidative properties (2, 3, 5, 9). Lipid peroxide and reactive oxygen metabolites (ROM) (O_2^-, H_2O_2, OCl^-, OH^-) which can be formed in excess in the lungs under pathological conditions stimulate, e.g., arachidonic acid metabolism and lead to the formation of cyclo-oxygenase and lipoxygenase products which have a bronchoconstrictive effect, such as prostaglandins and leukotrienes (8, 12).

In general, in vivo, various antioxidants (including ascorbic acid) and antioxidant enzymes, so-called radical scavengers protect the lungs from damage due to reactive oxygen metabolites and lipid peroxide (10). In the presence of increased activity of the pulmonary inflammatory cells (e.g., alveolar macrophages, granulocytes) with bronchial asthma, the equilibrium between oxidative and antioxidative capacity in the lungs may be displaced in favor of the oxidative process, such that additional administration of ascorbic acid at a high dose (5 g/day) and over a longer period of time may be expected to provide a therapeutic effect. In the present work, the hypothesis of an anti-asthmatic effect of ascorbic acid is to be tested (6, 7).

Materials and methods

A total of 29 patients with infection-related bronchial asthma (18 men and 11 women from 18 to 60 years of age) were recruited for the double blind crossover study under ambulatory conditions. Inhaled and systemic corticosteroids, renal disease and acute and serious purulent infections were considered to be exclusion criteria. The study was conducted over a period of 35 days. It was divided into a 2-week placebo period, 1-week wash-out test and 2-week ascorbic acid period. The sequence of the test periods was chosen at random (Fig. 1).

For the present study, in addition to the basic medication, a daily dose of 5 g ascorbic acid (Ascorvit containing 500 mg) was defined in comparison to oral placebo in 3 individual doses. Coated tablets from VEB Jenapharm, Clinical Research Division, lot numbers 150485 and 050886 were used. The patients received packages furnished with lot numbers that were coded according to the double blind study conditions. The code was not broken during the study.

During a pre-period of 2 weeks, the starting values for pulmonary function parameters were to be determined under the anti-asthmatic treatment up to that time. At the same time during this period, the patients were to learn how to complete the diary and determine the maximum expiratory peak flow with the peak flow meter.

During the 35-day double blind treatment period, the patients were seen 4 times: on the 8^{th}, 14^{th}, 29^{th} and 35^{th} day after the start of treatment. In the middle of the verum [HH: verum = active intervention] and placebo periods, measurements of bronchial hyperreactivity were performed again and at the end of the test period, a broncho-alveolar lavage with cytological examination and chemiluminescence measurement were performed.

In principle, the efficacy of an anti-asthmatic agent cannot be determined by a single target parameter. Even asthma symptoms are expressed in distinctly different ways. To record the symptoms, the complaints were listed separately in a diary (Table 1).

Each patient was given a peak flow monitor (Vitalograph) at the start of the study to measure the maximum expiratory velocity during the course of the study. The measurement was performed 4 times a day (6 a.m., 9 a.m., 12 noon, and 6 p.m.) by the patients while sitting. The highest value (l/min) out of each of three measurements was noted in the diary.

The measurement of nonspecific BHR was performed on the Bronchoscreen Measuring Station (Jaeger, Wuerzburg/West Germany) under the use of histamine dihydrochloride at a concentration of 1 mg/ml as the pharmacodynamic provocation substance [20]. The advantage of this method is that in contrast to conventional measuring procedures, better quantification of the bronchial reaction can be achieved with a distinct reduction in time needed for the examination. The histamine aerosol administration was performed breath for breath during the inspiratory phase during spontaneous respiration (nebulizer output per breath: 5 µmol). The bronchial reaction was simultaneously determined on the same instrument with the airway resistance method (R_{AW}). As target criteria of the BHR, a 50% increase in respiratory tract resistance (R_{AW}) in comparison to the starting value with simultaneous exceedance of the R_{AW} value of 0.3 kPa/(l · s) post provocation was defined. The following pulmonary function parameters prior to inhalative provocation were valid as exclusion criterion for the examination: $R_{AW} > 0.5$ kPa/(l · s) or $FEV_1 < 80$ % of the target value. Through pre-testing, BHR to a cumulative histamine dose of ≤8 µmol was demonstrated for all 29 patients. To enable a semiquantitative evaluation in the hyperreactivity zone, during the test periods, the threshold dose for the BHR to 1 µmol histamine was determined that corresponds to 40 respirations. The BHR ($PD_{50}R_{AW}$) was defined as positive at a cumulative provocation dose of ≤1 µmol histamine, and negative at >1 µmol histamine.

Broncho-alveolar lavage (BAL): The alveolar macrophages (AM) were obtained under outpatient conditions by broncho-alveolar lavage. The BAL was performed in the medial lobe with a fiber optic bronchoscope under local anesthesia with sterile physiological NaCl solution in individual portions (20 ml 57 times) (18, 20, 21, 31). The rinse fluid was pooled in a siliconized Erlenmeyer flask cooled in ice water, then filtered through a wire sieve (250 µm) and centrifuged at 4°C (500 g, 10 min). The cell sediment was treated for 10 min. at 4°C with 10 ml sterile erythrocyte lysis buffer (pH = 7.4) and then washed twice with phosphate buffered physiologic saline solution (PBS) and set to a cell density of 106 AM/ml PBS.

Cytologic investigations: The total cell count and the proportion of AM in the cell suspension were determined in the cell chamber according to Neubauer using morphological criteria and by an esterase test with α-naphthyl acetate. The cell differentiation was performed after staining the cell suspension with a mixture of equal parts of 1 % aqueous Nile blue chloride and thionine tartaric acid solution according to Feyrter (1 g thionine + 0.5 g tartaric acid/100 ml distilled H_2O) at a 1:1 ratio.

Chemiluminescence (CL) measurement

Measuring technique: The measurement was performed with the liquid scintillation counter Isocap300 (Searle Nuclear Chicago Division, Holland) in out-of-coincidence mode and recycling operating mode. The measuring time per sample was 0.2 min at an interval of approximately 6 min. Polypropylene test tubes (so-called mini vials) were used (measurement temperature 24°C). The work room was completely darkened and equipped with dark room illumination (33).

Reagents: As a medium for the CL measurement was veronal buffered physiological NaCl solution with an adjuvant of albumin, glucose, Ca^{2+} and Mg^{2+} according to information provided by Wulf et al. (34). The yeast cell walls for the stimulation of the AM were isolated from baker's yeast (23). The opsonization of the yeast cell walls was performed with human serum (concentration of the yeast cell wall dispersion 5 mg/1 ml PBS). Luminol (CL intensifier) was brought into solution at a concentration of 6 mg/3 ml PBS with the addition of 24 µl diethylamine by ultrasound treatment. Lucigenin (Cl intensifier) was dissolved in PBS (10.2 mg/2 ml).

Measuring technique: 2 ml veronal buffer, 20 µl Luminal or Lucigenin solution and 100 µl of AM suspension ($1 \cdot 10^5$ AM) were mixed in a measuring tube and pre-incubated for approximately 15 minutes with liquid scintillation counter. Afterwards, the yeast cell wall suspension (500 µg) was added and the CL measurement performed.

The Luminol and Lucigenin intensified CL was measured in parallel for this[1)]. For quantitative analysis of the measurement results, the peak heights (IPM) and areas under the CL curves (IP) were determined within 200 min after stimulation with the yeast cell wall suspension.

For characterization of the pharmacokinetics of ascorbic acid for the therapy regimen used, the daily profile of the serum level of ascorbic acid was determined enzymatically with the L-ascorbic acid color test (Boehringer, Mannheim, West Germany). Global evaluation of efficacy and tolerability were recorded by patient and physician.

The arithmetic mean (x) and the standard deviation (s) were determined for the statistical analysis of the measured variables.

The statistical comparison of the groups was performed with the paired t-test and the Wilcoxon test.

[1)] The Lucigenin intensified chemiluminescence shows the formation of superoxide anion (O_2^-), while the Luminol dependent chemiluminescence is specific for hypohalogenite.

Fig. 1: Schedule for the controlled double blind trial with ascorbic acid/placebo in patients with infection-related bronchial asthma. BHR – bronchial hyperreactivity, BAL – broncho-alveolar lavage

		Test periods				
	Pre-period	Placebo-Verum		Washout period	Verum-Placebo	
Days		8	14	21	29	35
Peak flow diary	4 times a day [over all study]					
Physician consultation	*	*	*		*	*
BHR	*	*			*	
BAL			*			*
Ascorbic acid serum level measurement		*	*		*	*

Note [HH]:
Verum: active treatment, here vitamin C

Table 1: Symptom scores

Analysis of asthmatic symptoms:

0 = no symptoms

1 = mild or brief symptoms that do not require additional use of medication

2 = more severe symptoms that are relieved within 15 minutes by additional medication

3 = more severe symptoms that do not respond adequately to or in a delayed manner to additional medication or require repeated use

Symptoms can include: intermittent dyspnea, wheezing, sensation of tightness in the morning or dry irritating cough

Results

The overall mean peak flow value for all asthmatics was 410 l/min in the placebo phase and 419 l/min in the verum phase. This slight increase of an average of 9 l/min in the ascorbic acid group was statistically not significant and may also not be clinically relevant. A similar impression resulted from the analysis of the symptom scores. The mean in the placebo phase was 0.72 points and under ascorbic acid it was 0.65 points. Consequently, a slight decrease in symptoms could be observed in the treatment period with ascorbic acid.

The investigations on bronchial hyperreactivity were performed at each of 3 time points, in the pre-period, after 8 days and on the 29th day. The course of bronchial hyperreactivity in 23 subjects during the investigation period is presented in Table 2. In 11 asthmatics, no change occurred during both periods. In 12 subjects, bronchial hyperreactivity was detectable during the placebo phase, while in the ascorbic acid phase, a negative reaction was observed. The opposite case did not occur. This asymmetry is significant ($p \leq 0.0003$; test on the basis of the binomial distribution). As a result of this, in 52% of patients with bronchial asthma, bronchial hyperreactivity could be effectively lowered.

The analysis of the bronchial lavage showed that 8 out of 24 patients exhibited an alveolar differential cell count that was commensurate with standards during both test periods. In 5 patients, normalization of the alveolar cell count resulted under ascorbic acid treatment, and in 6 other patients, the alveolar lymphocytes primarily present subsided. In 3 cases, alveolar eosinophilia persisted. Of note, there was considerable lymphocytosis (>28%) in 3 patients during both periods (Table 3).

The results of the CL measurements on AM from the BAL fluid show that under ascorbic acid, a reduction in the chemiluminescence response results with the Lucigenin as well as the Luminol intensification (Table 4).

The difference between the two groups (placebo period, ascorbic acid period) is statistically significant for the peak heights ($p \sim 0.03$).

The changes in the alveolar macrophage activity measured on the basis of the formation of ROM do not correlate or only weakly correlate with the changes in peak flow values and symptom scores ($|r| < 0.04$ in all cases).

In the analysis of the results, more precise characterization of those patients for whom definite therapeutic or hyperreactivity lowering effects could be proven was attempted (Fig. 2). However, the search for responder-typical commonalities was unsuccessful.

The serum level on the 8th day was 13.8–26.8 mg and 10.1–28.4 mg ascorbic acid/l on the 14th day, corresponding to the administration rhythm. As was expected, they were considerably above the normal range for men (Fig. 3).

The evaluation of the tolerability of the test preparation by the physician and the patient did not reveal any relevant differences between the test periods.

Only 1 patient complained of nausea during the ascorbic acid period; another indicated increased sensation of thirst over the entire test period. 3 patients noted temperature increases up to 38.2°C once in the evening on the day of the broncho-alveolar lavage.

Table 2: Course of bronchial hyperreactivity (BHR) with oral ascorbic acid (5 g/day for 35 days) in comparison to placebo (n = 23)
Positive criteria: $PD_{50}R_{AW} \leq 1$ µmol histamine

		BHP in the vitamin C period		
		Positive	Negative	Totals
BHR in the placebo period	Positive	9	12	21
	Negative	0	2	2
	Totals	9	14	23

Table 3: Cell distribution in the broncho-alveolar fluid in patients with infection-related bronchial asthma:
0 = conforms to standards, ↑ = elevated, ↑↑ = strongly elevated
(estimation of results based on normal values according to Hunninghake and Crystal [31])

	Placebo period		Ascorbic acid period	
n	Lymphocytes	Eosinophils	Lymphocytes	Eosinophils
8	0	0	0	0
2	0	(5%) ↑	0	0
3	(15%) ↑	0	0	0
3	(15%) ↑	(5%) ↑	0	(5%) ↑
3	(34%) ↑	(3%) ↑	(53%) ↑↑	0
1	(16%) ↑	(8%) ↑	(14%) ↑	(25%) ↑
1	0	(8%) ↑	(18%) ↑	0
1	(17%) ↑	0	0	(5%) ↑
1	0	0	(53%) ↑↑	0
1	(16%) ↑	0	(26%) ↑	(8%) ↑
24 (Total)				

Table 4: Comparison of the parameter of the chemiluminescence (CL) curves of the alveolar macrophages of patients with infection-related bronchial asthma (n = 24)

	Area under the CL curve	Peak height
	IP 10^{-8} *	IPM 10^{-6} **
	x ± s	x ± s
Placebo period		
Lucigenin	1.78 ± 1.51	2.11 ± 1.93
Luminol	2.17 ± 2.94	2.23 ± 2.77
Ascorbic acid period		
Lucigenin	1.29 ± 0.74	1.41 ± 0.87
Luminol	1.81 ± 1.72	1.91 ± 2.07
Statistics	a:c p ~ 0.08	a:c p ~ 0.03
Wilcoxon test	b:d p ~ 0.09	b:d p ~ 0.03
* IP = impulses		
** IPM = impulses per minute		

Fig. 2: Peak flow course curve of an asthma patient during the entire study

L l/min Days [Tage]

see the German versions for the figure:
As characters: http://www.mv.helsinki.fi/home/hemila/A/Schertling_1990_ch.pdf
As a bitmat: http://www.mv.helsinki.fi/home/hemila/A/Schertling_1990_bm.pdf

Fig. 3: Daily profile of the serum level of ascorbic acid in a male asthmatic.

Ascorbic acid [mg/l]
Intake [Einnahme]

14th day [14. Tage]
8th day [8. Tage]

Normal range for men [Normbereich fur Manner]
Time [h.] [Zeit]

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Discussion

In comparison to the individual studies with ascorbic acid in bronchial asthma to date in which low doses were used over a shorter administration time period (11, 15, 17, 19, 25, 30), for the first time in a complex study a therapeutic effect of ascorbic acid could be proven by including pulmonary function, symptom scores, bronchial hyperreactivity and broncho-alveolar lavage, which is most notably expressed by significant lowering of bronchial hyperreactivity. Bronchial hyperreactivity is an important quantifiable characteristic in asthmatic disease. Hyperreactivity is usually already recognizable before the manifestation of 'clinical asthma' and is consequently causally involved in the pathogenesis of asthma. Nowadays, bronchial hyperreactivity is even considered to be common denominator of all asthma forms (27). The inhaled provocation with histamine has proven to be the established quantitative method for the study of bronchial hyperreactivity (20). A clinically relevant raising of the threshold of bronchial reactivity resulted in 52% of asthmatics, and indeed, in contrast to the placebo period, a hyperreactivity lowering effect could be measured in 11 subjects under ascorbic acid.

An effective reduction in bronchial hyperreactivity must be considered to be a decisive element of asthma prevention measures today (26). At the same time, bronchial hyperreactivity is considered to be the most important determining factor for the course of asthma disease. Pulmonary function studies frequently give varying results depending on external influences, daily rhythm and medication. For this reason, the peak flow value, as a more objective pulmonary function parameter, was measured four times a day and documented in the diary. Relatively rare, selective measurements of pulmonary function parameters by more extensive measuring techniques such as spirometry or body plethysmography, in spite of higher personnel/technical expenditure, do not result in more reliable results than the significantly more frequently measured peak flow value that records the daily variation range of pulmonary function of asthmatics in a more representative manner. The peak flow values and the symptom scores indeed showed a tendency toward improvement during ascorbic acid therapy, but the differences in both test time periods were not significant.

The results of the chemiluminescence measurements on alveolar macrophages demonstrated that under ascorbic acid treatment, a reduced chemiluminescence response resulted. This indicates that ascorbic acid reduces the formation of reactive oxygen metabolites in patients with bronchial asthma and consequently could also have an inhibitory effect on the biosynthesis of cyclo-oxygenase and lipoxygenase products which have a bronchoconstrictive effect. Ascorbic acid probably does not directly reduce the formation of reactive oxygen metabolites e.g., by the NAD(P)H oxidase system of inflammatory cells. The oxygen radicals and toxic oxidants that arise are reduced and are thus rendered innocuous before they can react with the pulmonary cells or the lung tissue. Furthermore, the present study underlines the value of bronchial alveolar lavage in bronchial asthma (13, 24, 32). Statements about the degree of inflammation in infection-related bronchial asthma and the therapeutic effect of anti-asthmatic/allergic acting substances can be made from the alveolar differential cell count (14, 22). From the results, it can be concluded that ascorbic acid at a high dose (5 g/day) is a suitable antioxidant for reduction of radical formation in infection-related bronchial asthma and consequently could favorably affect the clinical course of asthma. This must be further clarified in other comprehensive studies.

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J Virol. 2010 Aug;84(15):7418-26. doi: 10.1128/JVI.02290-09. Epub 2010 Apr 7.

The ABCs of rhinoviruses, wheezing, and asthma.

Gern JE[1].

Author information

Abstract

Human rhinoviruses (HRVs) were discovered as common cold pathogens over 50 years ago. Recent advances in molecular viral diagnostics have led to an appreciation of their role in more-significant respiratory illnesses, including bronchiolitis in infancy, childhood pneumonia, and acute exacerbations of chronic respiratory diseases such as asthma, chronic obstructive lung disease, and cystic fibrosis. Until a few years ago, only two groups of HRVs (A and B) had been recognized. However, full and partial sequencing of HRVs led to the discovery of a third species of HRV (HRV-C) that has distinct structural and biologic features. Risk factors and pathogenic mechanisms for more-severe HRV infections are being defined, and yet fundamental questions persist about mechanisms relating this common pathogen to allergic diseases and asthma. The close relationship between HRV infections and asthma suggests that antiviral treatments could have a major impact on the morbidity associated with this chronic respiratory disease.

PMID: 20375160 PMCID: PMC2897627 DOI: 10.1128/JVI.02290-09

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Eur J Pediatr. 2011 Jan;170(1):59-63. doi: 10.1007/s00431-010-1270-z. Epub 2010 Aug 6.

The effect of vitamin C on upper respiratory infections in adolescent swimmers: a randomized trial.

Constantini NW[1], Dubnov-Raz G, Eyal BB, Berry EM, Cohen AH, Hemilä H.

Author information

Abstract

The risk of upper respiratory infections (URIs) is increased in people who are under heavy physical stress, including recreational and competitive swimmers. Additional treatment options are needed, especially in the younger age group. The aim of this study was to determine whether 1 g/day vitamin C supplementation affects the rate, length, or severity of URIs in adolescent swimmers. We carried out a randomized, double-blind, placebo-controlled trial during three winter months, among 39 competitive young swimmers (mean age 13.8 ± 1.6 years) in Jerusalem, Israel. Vitamin C had no effect on the incidence of URIs (rate ratio = 1.01; 95% confidence interval (CI) = 0.70-1.46). The duration of respiratory infections was 22% shorter in vitamin C group, but the difference was not statistically significant. However, we found a significant interaction between vitamin C effect and sex, so that vitamin C shortened the duration of infections in male swimmers by 47% (95% CI: -80% to -14%), but had no effect on female swimmers (difference in duration: +17%; 95% CI: -38% to +71%). The effect of vitamin C on the severity of URIs was also different between male and female swimmers, so that vitamin C was beneficial for males, but not for females. Our study indicates that vitamin C does not affect the rate of respiratory infections in competitive swimmers. Nevertheless, we found that vitamin C decreased the duration and severity of respiratory infections in male swimmers, but not in females. This finding warrants further research.

PMID: 20689965 DOI: 10.1007/s00431-010-1270-z

[Indexed for MEDLINE]

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Medical knowledge that matters Des connaissances médicales d'envergure

Can Med Assoc J. 1974 Jul 6; 111(1): 31–36.

PMCID: PMC1947567
PMID: 4601508

The effect on winter illness of large doses of vitamin C

T. W. Anderson, G. Suranyi, and G. H. Beaton

Abstract

Between December 1972 and February 1973, 2349 volunteers participated in a double-blind trial to assess the effect of large doses of vitamin C on the incidence and severity of winter illness. In addition, records were kept but no tablets taken during March. Subjects were randomly allocated to eight treatment regimens: three prophylactic-only (daily dose 0.25, 1 or 2 g), two therapeutic-only (4 or 8 g on the first day of illness), one combination (1 g daily and 4 g on the first day of illness), and two all-placebo. None of the groups receiving vitamin C showed a difference in sickness experience that was statistically significant from that of the placebo groups, but the results obtained were compatible with an effect of small magnitude from both the prophylactic and therapeutic regimens, and an effect of somewhat greater magnitude from the combination regimen. The combination regimen was associated more with a reduction in severity than frequency of illness, although the extra dosage was limited to the first day of illness. In spite of the eightfold range in daily dose, the three prophylactic-only regimens showed no evidence of a dose-related effect, but the 8 g therapeutic dose was associated with less illness than the 4 g therapeutic dose. There was no evidence of side effects from the 1 and 2 g prophylactic doses of vitamin C, and no evidence of a rebound increase in illness during the month following withdrawal of the daily vitamin supplements. On the basis of this and other studies it is suggested that the optimum daily dose of vitamin C is less than 250 mg, except possibly at the time of acute illness, when a larger daily intake may be beneficial.

Full text

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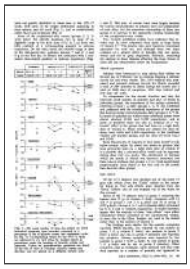

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Am J Clin Nutr. 1979 Aug;32(8):1686-90.

The effects of ascorbic acid and flavonoids on the occurrence of symptoms normally associated with the common cold.

Baird IM, Hughes RE, Wilson HK, Davies JE, Howard AN.

Abstract

A controlled study was made of the effects of natural orange juice, synthetic orange juice, and placebo in the prevention of the common cold; both natural and synthetic orange juices contained 80 mg of ascorbic acid daily. Three-hundred sixty-two healthy normal young adult volunteers, ages 17 to 25 years, were studied for 72 days with 97% of participants completing the trial. There was a 14 to 21% reduction in total symptoms due to the common cold in the supplemented groups that was statistically significant (P less than 0.05). Ascorbic acid supplementation also increased the number of "episode-free" subjects. However, the clinical usefulness of the results does not support prophylactic ascorbic acid supplements in the well-nourished adult. The results in this study with both natural and synthetic orange juice of physiological content of ascorbic acid, are similar to those obtained using a "megadose" of ascorbic acid.

PMID: 463806 DOI: 10.1093/ajcn/32.8.1686

[Indexed for MEDLINE]

Social Studies of Science

The Politics of Therapeutic Evaluation: The Vitamin C and Cancer Controversy

Evelleen Richards

First Published November 1, 1988 | Research Article
https://doi.org/10.1177/030631288018004004

Abstract

This paper reconstructs and analyzes the content and context of the debate over the efficacy of vitamin C in the treatment of cancer, and compares it with medical responses to, and evaluations of, two other cancer drugs — the cytotoxic drug SFU (conventionally used in the treatment of gastro-intestinal cancers) and the `naturally-occurring' (but recombinant DNA-produced) drug interferon. This comparative approach is designed to facilitate the integration of microsociological and structural levels of analysis of the processes by which knowledge claims about therapeutic efficacy are evaluated by the powerful adjudicating medical community. It is argued that the assessment of medical therapies is inherently a social and political process; that the idea of neutral appraisal is a myth; that clinical trials, no matter how rigorous their methodology, inevitably embody the professional values or commitments of the assessors; and that judgements about experimental findings may be structured by wider social interests, such as consumer choice or market forces. It is concluded that the necessarily social character of medical knowledge cannot be eliminated by methodological reform, and that this has important implications for the social implementation of medical therapies and techniques.

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Proc Natl Acad Sci U S A. 1971 Nov; 68(11): 2678–2681.
doi: 10.1073/pnas.68.11.2678
PMCID: PMC389499
PMID: 4941984

The Significance of the Evidence about Ascorbic Acid and the Common Cold

Linus Pauling
Department of Chemistry, Stanford University, Stanford, California 94305

Abstract

Only four independent double-blind studies have been reported of the effect of ascorbic acid regularly ingested in daily amounts more than 100 mg, in comparison with a placebo, in decreasing the incidence and integrated morbidity of the common cold for subjects exposed to cold viruses in the ordinary way and without colds when the test period began. A statistical analysis of these four studies leads to rejection of the null hypothesis that ascorbic acid has no more protective power than the placebo at the 99.86% level of confidence for the incidence of colds and the 99.9978% level of confidence for the integrated morbidity.

Full text

Full text is available as a scanned copy of the original print version. Get a printable copy (PDF file) of the complete article (853K), or click on a page image below to browse page by page. Links to PubMed are also available for Selected References.

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11;**1**(5540):603–606. [PMC free article] [PubMed] [Google Scholar]

Article

January 17, 1977

Therapeutic Effect of Vitamin C
A Co-Twin Control Study

Judy Z. Miller; Walter E. Nance, MD, PhD; James A. Norton, PhD; et al

» Author Affiliations

JAMA. 1977;237(3):248-251. doi:10.1001/jama.1977.03270300052006

Abstract

Three different dosages of vitamin C, dependent on body weight, were administered to 44 school-aged monozygotic twins for five months using a double-blind, co-twin control study design. The mothers recorded daily observations of cold symptoms, and multiple biochemical, anthropometric, and psychological measurements were made at the beginning and end of the study. Paired comparisons showed no significant overall treatment effect on cold symptoms, but the response was not uniform in all subgroups. Treated girls in the youngest two groups had significantly shorter and less severe illness episodes, and an effect on severity was also observed in the youngest group of boys. The seven treated twins in the latter group also grew an average of 1.3 cm more than their untreated co-twins during the five-month period of the study.

(*JAMA* 237:248-251, 1977)

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Precision Oncology News, 2018

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JAMA Oncology | *Comment & Response* | *March 19, 2020*

Format: Abstract

Free Radic Biol Med. 2016 Apr;93:84-93. doi: 10.1016/j.freeradbiomed.2015.12.017. Epub 2015 Dec 15.

Therapeutic treatment with ascorbate rescues mice from heat stroke-induced death by attenuating systemic inflammatory response and hypothalamic neuronal damage.

Chang CY[1], Chen JY[2], Chen SH[3], Cheng TJ[4], Lin MT[5], Hu ML[6].

Author information

Abstract

The impact of ascorbate on oxidative stress-related diseases is moderate because of its limited oral bioavailability and rapid clearance. However, recent evidence of the clinical benefit of parenteral vitamin C administration has emerged, especially in critical care. Heatstroke is defined as a form of excessive hyperthermia associated with a systemic inflammatory response that results in multiple organ dysfunctions in which central nervous system disorders such as delirium, convulsions, and coma are predominant. The thermoregulatory, immune, coagulation and tissue injury responses of heatstroke closely resemble those observed during sepsis and are likely mediated by similar cellular mechanisms. This study was performed by using the characteristic high lethality rate and sepsis-mimic systemic inflammatory response of a murine model of heat stroke to test our hypothesis that supra-physiological doses of ascorbate may have therapeutic use in critical care. We demonstrated that parenteral administration of ascorbate abrogated the lethality and thermoregulatory dysfunction in murine model of heat stroke by attenuating heat stroke-induced accelerated systemic inflammatory, coagulation responses and the resultant multiple organ injury, especially in hypothalamus. Overall, our findings support the

hypothesis and notion that supra-physiological doses of ascorbate may have therapeutic use in critical care.

KEYWORDS: Ascorbate; Heat stroke; Systemic inflammatory response

PMID: 26703968 DOI: 10.1016/j.freeradbiomed.2015.12.017

[Indexed for MEDLINE]

Publication type, MeSH terms, Substance

LinkOut - more resources

Format: Abstract

J Infect Dis. 1997 Feb;175(2):237-46.

Perspective: validating surrogate markers--are we being naive?

De Gruttola V[1], Fleming T, Lin DY, Coombs R.

Author information

Abstract

Because of the difficulties in conducting studies of clinical efficacy of new therapies for human immunodeficiency virus infection and other diseases, there is increasing interest in using measures of biologic activity as surrogates for clinical end points. A widely used criterion for evaluating whether such measures are reliable as surrogates requires that the putative surrogate fully captures the "net effect"-the effect aggregated over all mechanisms of action-of the treatment on the clinical end point. The variety of proposed metrics for evaluating the degree to which this criterion is met are subject to misinterpretation because of the multiplicity of mechanisms by which drugs operate. Without detailed understanding of these mechanisms, metrics of "surrogacy" are not directly interpretable. Even when all of the mechanisms are understood, these metrics are associated with a high degree of uncertainty unless either treatment effects are large in moderate-size studies or sample sizes are large in studies of moderately effective treatments.

PMID: 9203643 DOI: 10.1093/infdis/175.2.237

[Indexed for MEDLINE]

Publication types, MeSH terms, Substance, Grant support

LinkOut - more resources

Format: Abstract

Biomarkers. 2013 Aug;18(5):446-54. doi: 10.3109/1354750X.2013.810668.

Variability in oxidative stress biomarkers following a maximal exercise test.

Mullins AL[1], van Rosendal SP, Briskey DR, Fassett RG, Wilson GR, Coombes JS.

Author information

Abstract

The oxidative stress response to maximal exercise may provide useful clinical biomarkers for assessing redox homeostasis. The aim was to determine the between-individual variability in the exercise-induced change in oxidative stress measures and investigate predictors of these responses. Plasma F2-isoprostanes (Isop), protein carbonyls (PCs), glutathione peroxidase (GPX) activity and total antioxidant capacity (TAC) were measured before and after a maximal treadmill exercise test. Exercise produced significant increases in Isop (27.0%), PC (6.2%) and GPX (7.8%). There were large between-individual coefficients of variation: Isop (152%), PC, (240%), GPX (130%) and TAC (243%).

PMID: 23862764 DOI: 10.3109/1354750X.2013.810668

[Indexed for MEDLINE]

MeSH terms, Substances

LinkOut - more resources

Virus Pneumonia and Its Treatment With Vitamin C

FRED R. KLENNER, M.D., Reidsville, North Carolina

VIRUS PNEUMONIA (primary atypical pneumonia, non-specific pneumonitis, epidemic non-bacterial pneumonia, desseminated focal pneumonia, viral pneumonia) has been accepted as an entity and has been under observation in this country and abroad for the past twelve years. No bacteriological studies have confirmed the etiology of this disease other than by negative findings. The sputum shows the usual flora of gram-positive and gram-negative organisms. In 1938, Reimann reported that a filterable infectious agent was recovered from the nasopharynx of one and from the blood of another out of a series of eight cases, but not sufficient evidence could be found to determine such as the causative factor. It must be closely allied to the virus causing influenza, because in the first twenty-four to thirty-six hours it is very commonly thought to be that type of infection. Horsfall and his co-workers at the Rockefeller Institute have cultured an organism, which they have designated Streptococcus MG, from a large percentage of their patients with primary atypical pneumonia. The exact role of this bacterium is not known, but it is seldom found except in persons ill of this disease. Since it is not present in all cases, it is not the primary cause, but only a characteristic secondary invader or associate. The disease also resembles psittacosis in many respects and since penicillin might be of value in such cases it is of great importance to establish the diagnosis quickly.

The onset of this type of virus infection is always gradual. Like all virus diseases there is a wide variation of the prodromal symptoms. There might be none; there might be the classical generalized malaise. This disease is highly contagious, and our observations over a five-year period point to a definite incubation period of from five to fourteen days. We have also noted that the longer the incubation period the milder the infection: the shorter the incubation period the more severe is the infection. This must be interpreted in the first instance as either a mildly virulent organism or a high degree of resistance or immunity on the part of the host and in the second instance as a very virulent organism or no immunity at all on the part of the host. In some instances, however, the patient will have a slight attack with apparent recovery due either to good resistance against a weak virus or good response to treatment only to be followed in seven to ten days by a return of symptoms in a more severe form and producing a critically ill patient. This type of case cannot be classified as a fourteen-day incubation period, but rather it is one in which the virus was only attenuated or else there has been the factor of a second infection.

Read by Title to the Tri-State Medical Association of the Carolinas and Virginia, meeting at Charleston, February 9th and 10th.

The chief complaint, however, will always be one of sudden onset, since the patient begins his concept of his illness from the time he first experienced waves of chilly sensations or a frank chill alternating with hot spells and associated with burning in the nose, a sore throat, hoarseness, a bad taste in his mouth, moderate vertigo, nausea and grade-two type frontal headache. This picture will then develop to the point where severe frontal headache is noted along with a feeling of weakness in the lower extremities so marked that the patient complains of a dragging sensation when moving about in bed. This weakness persists for some days after clearing of all symptoms and negative chesi films. The patient can hardly support his body weight without the feeling of buckling at the knees. Added to the above might be substernal pain or generalized tightness in the chest with varying degrees of tracheo-bronchitis. The fever is usually found during this phase to be about 102° F. After pulmonary involvement of as much as 6 by 8 cm. areas have been reached the fever will be up to 103 and 104° F. in adults and up to 105° F. in infants and early childhood. Dry hacking cough is a most constant factor especially after the second day of illness. Occasionally this cough is paroxysmal, and if the invasion is severe enough it will in the final clearing stage of the disease be thick, tenacious, brownish-gray — even blood-streaked. This disease shows remarkable versatility in that it will vary its symptoms and signs to fit with that of a mild cold on one hand to a very serious medical complexity on the other. It suggests sometimes that more than one bacteriologic unit is involved. The pulse will be increased in a very definite ratio to the toxic effect of the virus. If the invasion is mild the pulse rate will be normal even though the fever may be recorded at 103° F. If, however, the invasion is severe, meaning that physical findings approximating those of a lobar pneumonia (with or without a definite complicating encephalitis or meningitis) are present, or with an accompanying pleurisy, then the pulse rate will be rapid and will follow the temperature curve. Sweating is common and it is usually very profuse. Cyanosis and dyspnea occurred only in those patients that had at least as much as a lobe of lung involvement and where the fever continued to climb to a 104° F. each night.

The physical findings are limited to the head and chest. There is marked rhinitis with swelling of the turbinates. The accessory nasal sinuses are involved; the frentals being the chief offenders. The tonsil bed is not remarkable but the lymphoid tissue on the posterior pharyngeal wall is thickened and edematous and scarlet in color. The vocal cords appear like those seen in any simple laryngitis. In the lungs diminished breath sounds with moist and dry rales (sometimes very coarse) are usually the only evidence of disease. When there are extensive areas of consolidation the usual dullness to percussion, tubular breathing and pectoriloquy are present.

The laboratory findings are of little importance. The" white blood count and differential are nearly always within normal limits. A 6500 white count is typical regardless of the lung pathology. The sedimentation rate will be normal except in very-acute cases, with cerebral symptoms. The sputum examination is valuable only in its negative findings.

Chemotherapy may be tried where x-ray facilities are not convenient or not obtainable. If sulfonamides and/or penicillin are given for twenty-four to thirty-six hours without response both should be discontinued and treatment for virus infection instituted. In our age it requires some measure of boldness to discontinue these important drugs so early especially with the patient still running a fever of from 102 to 104° F. In this case boldness counts.

There is no constant x-ray picture to be found in virus pneumonia, but some evidence of pneumonitis will nearly always be present regardless of the physical signs—even when the physical signs are absent. The chest film will show anything from extensive consolidation to a patchy and sometimes fleecy infiltration suggestive of tuberculosis. This patchy form will be scattered in all diameters of the lung fields. Plates taken daily or every second to third day will often show the pneumonic process clearing in some areas while new areas are developing at other points. The disease begins as an infiltrative process starting at the hilus, and then, by a peribronchial route gradually spreading to the interbrdnchial regions. Usually there will be an involvement of several segments of lung comprising several lobes. These isolated segments soon become confluent, giving the film a smoky appearance. This process may go on to involvement of an entire lobe and in many respects look like a lobar pneumonia. The marked difference lies in the fact that even when the density is massive a streaky background can always be seen; the shadow in virus pneumonia is never entirely solid. Resolution, either spontaneous or from some method of treatment, may give positive x-ray films days and even weeks after there has been a complete clinical response.

The treatment of virus infections, including frank virus pneumonia, has been for the most part without specific recommendations. Oppenheimer in 56 cases employed x-rays in doses from 35r to 90r which he states relieved cough and shortened the course of the disease. Offutt employed 100r doses daily or every other day, depending on the severity and response, alternating front and back or alternating sides if both lungs were involved. None in his series of twelve cases received over four treatments. Both men report surprising uniformity in the disappearance of fever and symptoms after one or two exposures. No unfavorable reactions occurred in either series. Aminophyllin in doses of three grains every four hours has been given with varying results in the belief that it improved the circulation through the lung fields. We have employed the drug in smaller doses when there was evidence that the patient had a coexisting coronary impairment. Since this was given along with the drug of our choice, ascorbic acid, this paper cannot evaluate its merits. Multiple transfusions from multiple donors and blood from patients convalescing from virus pneumonia have also been used.

The purpose of this paper is to outline a new and different form of treatment for this type of virus infection which in 42 cases over a five-year period has given excellent results. The treatment has dojuble merit due to the simplicity of its schedule. The remedy used was vitamin C (ascorbic acid) given in massive doses. Since it is common knowledge that there are definite individual variations in absorption of vitamin C from the intestinal tract and under certain pathological conditions still greater variations in the absorption factors the I. V. and I. M. routes were used. When a diagnosis of virus pneumonia was entertained the patient was given 1000 mg. vitamin C intravenously every six to twelve hours. If it was by chance that a diagnosis was established in the home the usual initial dose was 500 mg. given in the gluteal muscle. Subsequent injections were given I. V. because the injection was thus made painless and the response was faster. In infants and very small children, however, 500 mg. I. M. every six to twelve hours was the method of choice. From three to seven injections gave complete clinical and x-ray response in all of our cases. The series comprised types of cases from very slight consolidation to those resembling lobar pneumonia. Two cases were complicated by cerebral manifestations. Vitamin C was also given by mouth in one-third of this series but there was no outstanding difference in the response. The dosage was from 100 to 500 mg., depending on the age of the pa-

tient, and it was given every four to six hours. In almost every case the patient felt better within an hour after the first injection and noted a very definite change after two hours. Nausea was relieved by the first injection as was the headache. The heat regulating center showed a quick response and it was the rule to find a drop of 2° F. several hours after the first 1000 mg. Penicillin was given in conjunction with ascorbic acid in five cases. It was our observation that penicillin had some retarding effect on the action of vitamin C, since the response was not so rapid and in one case the results were not obtained until the penicillin was discontinued.

Supportive treatment was given by forcing fluids, particularly fruit juices, to tolerance. Soda-water was given to adults in the amount of four glasses in 24 hours, each glass containing one teaspoonful sodium bicarbonate. Infants and children were given this alkaline drink in proportion to age. The rationale of bicarbonate of soda is based on the findings of Hawley and others that the amount of vitamin C excreted in the urine may vary according to the acid:alkali content of the diet, a highly alkaline urine having lower amounts of vitamin C than a highly acid urine. Codeine sulfate and aspirin were given by mouth. In adults the dose was codeine 0.5 grain, aspirin 10 grains given every six hours. Infants and children according to age. Some few patients complained of severe chest pain and some others of a constricting sensation that they described as cutting off their breath. These symptoms were relieved by employing either Numotizine as a plaster or the old-fashioned mustard plaster. The mustard plaster was made up with cold water and was applied cold for a period of about IS minutes. The proportions used were one part mustard and two parts flour. The amount of flour used in preparing the plaster for children was according to age but in no instance was the ratio greater than one to six. In childhood an expiratory grunt was taken as an index to use plasters. Oxygen inhalation was not employed even though cyanosis existed in twelve cases of the series; an additional injection of 500 mg. of vitamin C was given with almost spontaneous alleviation of the distressing condition. In two cases codeine sulfate was given in one grain amounts because of the weight of the patient. Diet was forced even though there was no desire to eat.

It is difficult to evaluate the role played by vitamin C against the virus organism. We have seen ascorbic acid give response in other types of virus infections but not sufficient evidence is on hand to state that it is a virus killer. It has been shown histologically that vitamin C regulates the intercellular substance of the capillary wall. In the human body its chief function is concerned with the formation of colloidal intercellular substances. The intercellular substances which appear to be regulated by vitamin C are of mesencyhmal origin—this means the collogen of all fibrous tissue structure, all non-epithelial cement substances including the intercellular substance of the capillary wall. Gothlin found increased capillary fragility in individuals with blood levels of 1 mg. of vitamin C per liter or less. It must be remembered too, however, that ascorbic acid has been reported to function as a respiratory catalyst, aiding cellular respiration by acting as a hydrogen transport.

Finally we consider the case of the liver in that the saturation of the blood plasma with vitamin C betters the detoxifying powers of this organ. It has been known that fever, toxemia and specific bacteria do act on the vitamin C concentration of the blood plasma with a lowering effect. Could it be that, by maintaining a high blood level of this vitamin, all body tissue is allowed to return to normal in spite of the existing fever and the presence of the specific organism, and that, acting as a respiratory catalyst, it enables the body to build up adequate resistance to the invader?

SUMMARY

Virus pneumonia is a true clinical entity. Although it gives symptoms similar to influenza in the early stage of illness the virus has not been identified. The onset is gradual and has an incubation period of five to fourteen days. The usual beginning is a hanging-on cold or generalized malaise. The chief symptoms, although not all are necessarily present each time, are chilly sensations or a single frank chill, followed with hot spells, burning in the nose, sore throat, hoarseness, bad taste in mouth, nausea, frontal headache, dry cough at first—later productive in the clearing phase of the disease—sweating, and this is usually profuse, normal pulse unless complicated with cerebral symptoms, pleurisy or a condition approximating lobar pneumonia when it will be rapid. Fever is from 100 to 104° F. The physical findings are inflammation of the turbihates and accessory nasal sinuses, hypertrophy of the lymphoid tissue on the posterior pharyngeal wall. Breath sounds are diminished and moist and dry rales are sometimes present. In extensive consolidation dullness to percussion, tubular breathing and pectoriloquy are found. The laboratory findings show the blood picture within normal limits; the sputum is negative. Sulfonamides and penicillin are good diagnostic aids since they have no effect on the disease. The x-ray findings can be anything from negative films through pneumonitis on to frank consolidation. Vitamin C in doses of 1000 mg. every six to twelve hours for three to seven injections has been specific in the experience of the author. X-ray in

To Page **46**

VIRUS PNEUMONIA—From P. 38

doses from 35 to 100r daily, or every second to third day, for not more than four exposures, aminophyllin and transfusions from convalescing or multiple donors have some usefulness as adjuvants in some cases.

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Health Benefits

In the eighteenth century, seasoned sailors found that by sucking on lemons they could avoid scurvy. When the lemon's key nutrient was formally identified in 1928, it was named ascorbic acid for its anti-scurvy, or antiscorbutic, action. Today ascorbic acid is widely known as Vitamin C. The health benefits of Vitamin C are abundant and varied, but it's probably best known as a cell protector, immunity booster, and powerful antioxidant. The body's ligaments, tendons, and collagen (a protein found in connective tissues) rely on the presence of Vitamin C to stay strong and healthy. Like all antioxidants, Vitamin C counters the effects of cell-damaging molecules called free radicals. As an added benefit, it even helps the body recycle other antioxidants. For certain conditions, Vitamin C is best taken with other antioxidants, such as Vitamin E, flavonoids, and carotenoids.

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Format: Abstract

Allergy Asthma Clin Immunol. 2013 Nov 26;9(1):46. doi: 10.1186/1710-1492-9-46.

Vitamin C and common cold-induced asthma: a systematic review and statistical analysis.

Hemilä H[1].

Author information

Abstract

BACKGROUND: Asthma exacerbations are often induced by the common cold, which, in turn, can be alleviated by vitamin C.

OBJECTIVE: To investigate whether vitamin C administration influences common cold-induced asthma.

METHODS: Systematic review and statistical analysis of the identified trials. Medline, Scopus and Cochrane Central were searched for studies that give information on the effects of vitamin C on common cold-induced asthma. All clinically relevant outcomes related to asthma were included in this review. The estimates of vitamin C effect and their confidence intervals [CI] were calculated for the included studies.

RESULTS: Three studies that were relevant for examining the role of vitamin C on common cold-induced asthma were identified. The three studies had a total of 79 participants. Two studies were randomized double-blind placebo-controlled trials. A study in Nigeria on asthmatics whose asthma attacks were precipitated by respiratory infections found that 1 g/day vitamin C decreased the occurrence of asthma attacks by 78% (95% CI: 19% to 94%). A cross-over study in former East-Germany on patients who had infection-related asthma found that 5 g/day vitamin C decreased the proportion of participants who had bronchial hypersensitivity to histamine by 52 percentage points (95% CI: 25 to 71). The third study did not use a placebo. Administration of a single dose of 1 gram of vitamin C to Italian non-asthmatic common cold patients increased the provocative concentration of histamine (PC20) 3.2-fold (95% CI: 2.0 to 5.1), but the vitamin C effect was significantly less when the same participants did not suffer from the common cold.

CONCLUSIONS: The three reviewed studies differed substantially in their methods, settings and outcomes. Each of them found benefits from the administration of vitamin C; either against asthma attacks or against bronchial hypersensitivity, the latter of which is a characteristic of asthma. Given the evidence suggesting that vitamin C alleviates common cold symptoms and the findings of this systematic review, it may be reasonable for asthmatic patients to test vitamin C on an individual basis, if they have exacerbations of asthma caused by respiratory infections. More research on the role of vitamin C on common cold-induced asthma is needed.

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LETTER TO THE EDITOR | VOLUME 102, ISSUE 4, P625-626, APRIL 01, 2008

Vitamin C and sex differences in respiratory tract infections

Harri Hemilä

Open Archive • Published: January 29, 2008 • DOI: https://doi.org/10.1016/j.rmed.2007.12.011

In their systematic review of sex differences in respiratory tract infections (RTIs), Falagas et al. concluded that males develop RTIs more frequently than females, in particular lower RTIs, and the course of the infection is often more severe in males than in females.[1]

In 1997, I reported a meta-analysis of British trials on vitamin C and the common cold which gives a complementary viewpoint on sex differences in RTIs.[2] In four trials with males, vitamin C supplementation reduced common cold incidence by 30% (95% CI: −40% to −19%), but had no effect in four trials with females (estimate −5%; 95% CI: −14% to +4%). The divergence in the confidence intervals suggests different effects on males and females. Three studies reported data for both males and females and the largest of these, by Baird et al.,[3] found highly significant interaction between sex and vitamin C effect on common cold incidence (Table 1). The two smaller trials had wide confidence intervals that overlapped between males and females.[2] Furthermore, in four trials with British males, vitamin C reduced recurrent colds during the study period by 46% (−60% to −26%), but had no effect on females.[2] In particular, Tyrrell et al.[4] found that therapeutic vitamin C during the first cold episode reduced subsequent colds in males by 40% (−63% to −3%),[2] but not in females (−7%; −45% to +54%). The Baird et al.[3] and Tyrrell et al.[4] studies were randomised placebo-controlled double-blind trials and their findings cannot be dismissed on methodological grounds.

Table 1 Interaction between sex and the effect of vitamin C on common cold incidence in British students (Baird et al., 1979).[3]

	Vitamin C		Placebo		RR (95% CI)
	Participants	No. of colds	Participants	No. of colds	
Males	133	184	61	135	0.63 (0.50–0.78)
Females	105	199	51	78	1.24 (0.95–1.61)

These data are from Refs. 2 and 3. The statistical significance of interaction was calculated from the change in −2×log(likelihood) when the interaction term was added to the model (STATA program Poisson).

Open table in a new tab

Because large-scale trials give no evidence that high-dose vitamin C supplementation (⩾1 g/day) decreases common cold incidence,[2] the findings with British males call for special explanations. Several surveys had reported low dietary vitamin C intake in the UK and thus the benefit of supplementation may be explained by treating marginal deficiency.[2] This explanation is consistent with the estimated low daily vitamin C intake in Baird's study, 50 mg/day, and the particularly low dosage of vitamin C supplementation, 80 mg/day.[3] Usually plasma and leucocyte vitamin C concentrations are lower in males than in females although it is not clear to what extent this is due to dietary and physiological differences between the sexes.[2] Concluding from the British studies,[2, 3, 4] it seems that

sex differences in RTIs may be generated by variations in dietary vitamin C intakes, in addition to the factors mentioned by Falagas et al.[1]

Furthermore, in a recent Cochrane review we identified three prophylactic vitamin C trials and each of them reported an 80% or greater decrease in pneumonia incidence in the vitamin C group.[5] All these trials examined males only and the incidence of pneumonia was particularly high. The benefit of vitamin C supplementation seemed to be explained by marginal deficiency and by increased requirement caused by heavy exertion.[5]

It is obvious that the findings of the common cold trials with British males[2] and pneumonia trials with males[5] cannot be extrapolated to the general population of the western countries. Nevertheless, further vitamin C trials are warranted among males with low dietary vitamin C intake.

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Cochrane Database Syst Rev. 2007; (⟨http://dx.doi.org/10.1002/14651858.CD005532.pub2⟩): CD005532

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Publication History

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Identification

DOI: https://doi.org/10.1016/j.rmed.2007.12.011

Tables

Table 1 : Interaction between sex and the effect of vitamin C on common cold incidence in British students (Baird et al., 1979).3

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Format: Abstract

Med J Aust. 1981 Oct 17;2(8):411-2.

Vitamin C and the common cold: using identical twins as controls.

Carr AB, Einstein R, Lai LY, Martin NG, Starmer GA.

Abstract

We analysed self-reported cold data for 95 pairs of identical twins who took part in a double-blind trial of vitamin C tablets. One member of each twin pair took vitamin C and the other took a well matched placebo each day for 100 days. Vitamin C had no significant effect except for shortening the average duration of cold episodes by 19%.

PMID: 7033746

[Indexed for MEDLINE]

Publication types, MeSH terms, Substance

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Format: Abstract

Cochrane Database Syst Rev. 2013 Jan 31;(1):CD000980. doi: 10.1002/14651858.CD000980.pub4.

Vitamin C for preventing and treating the common cold.

Hemilä H[1], Chalker E.

Author information

Abstract

BACKGROUND: Vitamin C (ascorbic acid) for preventing and treating the common cold has been a subject of controversy for 70 years.

OBJECTIVES: To find out whether vitamin C reduces the incidence, the duration or severity of the common cold when used either as a continuous regular supplementation every day or as a therapy at the onset of cold symptoms.

SEARCH METHODS: We searched CENTRAL 2012, Issue 11, MEDLINE (1966 to November week 3, 2012), EMBASE (1990 to November 2012), CINAHL (January 2010 to November 2012), LILACS (January 2010 to November 2012) and Web of Science (January 2010 to November 2012). We also searched the U.S. National Institutes of Health trials register and WHO ICTRP on 29 November 2012.

SELECTION CRITERIA: We excluded trials which used less than 0.2 g per day of vitamin C and trials without a placebo comparison. We restricted our review to placebo-controlled trials.

DATA COLLECTION AND ANALYSIS: Two review authors independently extracted data. We assessed 'incidence' of colds during regular supplementation as the proportion of participants experiencing one or more colds during the study period. 'Duration' was the mean number of days of illness of cold episodes.

MAIN RESULTS: Twenty-nine trial comparisons involving 11,306 participants contributed to the meta-analysis on the risk ratio (RR) of developing a cold whilst taking vitamin C regularly over the study period. In the general community trials involving 10,708 participants, the pooled RR was 0.97 (95% confidence interval (CI) 0.94 to 1.00). Five trials involving a total of 598 marathon runners, skiers and soldiers on subarctic exercises yielded a pooled RR of 0.48 (95% CI 0.35 to 0.64).Thirty-one comparisons examined the effect of regular vitamin C on common cold duration (9745 episodes). In adults the duration of colds was reduced by 8% (3% to 12%) and in children

by 14% (7% to 21%). In children, 1 to 2 g/day vitamin C shortened colds by 18%. The severity of colds was also reduced by regular vitamin C administration.Seven comparisons examined the effect of therapeutic vitamin C (3249 episodes). No consistent effect of vitamin C was seen on the duration or severity of colds in the therapeutic trials.The majority of included trials were randomised, double-blind trials. The exclusion of trials that were either not randomised or not double-blind had no effect on the conclusions.

AUTHORS' CONCLUSIONS: The failure of vitamin C supplementation to reduce the incidence of colds in the general population indicates that routine vitamin C supplementation is not justified, yet vitamin C may be useful for people exposed to brief periods of severe physical exercise. Regular supplementation trials have shown that vitamin C reduces the duration of colds, but this was not replicated in the few therapeutic trials that have been carried out. Nevertheless, given the consistent effect of vitamin C on the duration and severity of colds in the regular supplementation studies, and the low cost and safety, it may be worthwhile for common cold patients to test on an individual basis whether therapeutic vitamin C is beneficial for them. Further therapeutic RCTs are warranted.

Update of

Vitamin C for preventing and treating the common cold. [Cochrane Database Syst Rev. 2007]

PMID: 23440782 DOI: 10.1002/14651858.CD000980.pub4

[Indexed for MEDLINE]

Publication types, MeSH terms, Substance

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Format: Abstract

Cochrane Database Syst Rev. 2013 Aug 8;(8):CD005532. doi: 10.1002/14651858.CD005532.pub3.

Vitamin C for preventing and treating pneumonia.

Hemilä H[1], Louhiala P.

Author information

Abstract

BACKGROUND: Pneumonia is one of the most common serious infections, causing two million deaths annually among young children in low-income countries. In high-income countries pneumonia is most significantly a problem of the elderly.

OBJECTIVES: To assess the prophylactic and therapeutic effects of vitamin C on pneumonia.

SEARCH METHODS: We searched CENTRAL 2013, Issue 3, MEDLINE (1950 to March week 4, 2013), EMBASE (1974 to April 2013) and Web of Science (1955 to April 2013).

SELECTION CRITERIA: To assess the therapeutic effects of vitamin C, we selected placebo-controlled trials. To assess prophylactic effects, we selected controlled trials with or without a placebo.

DATA COLLECTION AND ANALYSIS: Two review authors independently read the trial reports and extracted data.

MAIN RESULTS: We identified three prophylactic trials which recorded 37 cases of community-acquired pneumonia in 2335 people. Only one was satisfactorily randomised, double-blind and placebo-controlled. Two trials examined military recruits and the third studied boys from "lower wage-earning classes" attending a boarding school in the UK during World War II. Each of these three trials found a statistically significant (80% or

greater) reduction in pneumonia incidence in the vitamin C group. We identified two therapeutic trials involving 197 community-acquired pneumonia patients. Only one was satisfactorily randomised, double-blind and placebo-controlled. That trial studied elderly patients in the UK and found lower mortality and reduced severity in the vitamin C group; however, the benefit was restricted to the most ill patients. The other therapeutic trial studied adults with a wide age range in the former Soviet Union and found a dose-dependent reduction in the duration of pneumonia with two vitamin C doses. We identified one prophylactic trial recording 13 cases of hospital-acquired pneumonia in 37 severely burned patients; one-day administration of vitamin C had no effect on pneumonia incidence. The identified studies are clinically heterogeneous which limits their comparability. The included studies did not find adverse effects of vitamin C.

AUTHORS' CONCLUSIONS: The prophylactic use of vitamin C to prevent pneumonia should be further investigated in populations who have a high incidence of pneumonia, especially if dietary vitamin C intake is low. Similarly, the therapeutic effects of vitamin C should be studied, especially in patients with low plasma vitamin C levels. The current evidence is too weak to advocate prophylactic use of vitamin C to prevent pneumonia in the general population. Nevertheless, therapeutic vitamin C supplementation may be reasonable for pneumonia patients who have low vitamin C plasma levels because its cost and risks are low.

Update of

Vitamin C for preventing and treating pneumonia. [Cochrane Database Syst Rev. 2007]

PMID: 23925826 DOI: 10.1002/14651858.CD005532.pub3

[Indexed for MEDLINE]

Publication types, MeSH terms, Substances

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Clinical Trial J Infect Dis, 173 (6), 1502-5 Jun 1996

Vitamin C for the Treatment of Recurrent Furunculosis in Patients With Imparied Neutrophil Functions

R Levy [1], O Shriker, A Porath, K Riesenberg, F Schlaeffer

Affiliations

PMID: 8648230 DOI: 10.1093/infdis/173.6.1502

Abstract

The effect of vitamin C treatment on 23 patients with a history of recurrent furunculosis with negative nasal cultures was studied. Neutrophil functions (chemotaxis, phagocytosis, or superoxide generation) of 12 patients were significantly lower than those of the matched controls. In this group, treatment with vitamin C (1 g/day) caused a dramatic clinical response as well as a significant improvement of neutrophil functions, reaching values similar to those of the controls. Two patients remained vitamin C-dependent. In the patients with normal neutrophil functions, vitamin C treatment neither affected neutrophil activity nor caused a clinical response. Therefore, patients suffering from recurrent furunculosis with defective neutrophil functions may be treated successfully with vitamin C, contributing to both neutrophil function recovery and a dramatic clinical response.

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Vitamin C pharmacokinetics in healthy volunteers: evidence for a recommended dietary allowance.

M Levine, C Conry-Cantilena, Y Wang, R W Welch, P W Washko, K R Dhariwal, J B Park, A Lazarev, J F Graumlich, J King, and L R Cantilena

National Institute of Diabetes and Digestive and Kidney Diseases, National Institutes of Health, Bethesda, MD 20892-1372, USA.

Abstract

Determinants of the recommended dietary allowance (RDA) for vitamin C include the relationship between vitamin C dose and steady-state plasma concentration, bioavailability, urinary excretion, cell concentration, and potential adverse effects. Because current data are inadequate, an in-hospital depletion-repletion study was conducted. Seven healthy volunteers were hospitalized for 4-6 months and consumed a diet containing <5 mg of vitamin C daily. Steady-state plasma and tissue concentrations were determined at seven daily doses of vitamin C from 30 to 2500 mg. Vitamin C steady-state plasma concentrations as a function of dose displayed sigmoid kinetics. The steep portion of the curve occurred between the 30- and 100-mg daily dose, the current RDA of 60 mg daily was on the lower third of the curve, the first dose beyond the sigmoid portion of the curve was 200 mg daily, and complete plasma saturation occurred at 1000 mg daily. Neutrophils, monocytes, and lymphocytes saturated at 100 mg daily and contained concentrations at least 14-fold higher than plasma. Bioavailability was complete for 200 mg of vitamin C as a single dose. No vitamin C was excreted in urine of six of seven volunteers until the 100-mg dose. At single doses of 500 mg and higher, bioavailability declined and the absorbed amount was excreted. Oxalate and urate excretion were elevated at 1000 mg of vitamin C daily compared to lower doses. Based on these data and Institute of Medicine criteria, the current RDA of 60 mg daily should be increased to 200 mg daily, which can be obtained from fruits and vegetables. Safe doses of vitamin C are less than 1000 mg daily, and vitamin C daily doses above 400 mg have no evident value.

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Vitamin C supplementation reduces the incidence of postrace symptoms of upper-respiratory-tract infection in ultramarathon runners.

Peters EM[1], Goetzsche JM, Grobbelaar B, Noakes TD.

Author information

Abstract

This study determined whether daily supplementation with 600 mg vitamin C would reduce the incidence of symptoms of upper-respiratory-tract (URT) infections after participation in a competitive ultramarathon race (> 42 km). Ultramarathon runners with age-matched controls were randomly divided into placebo and experimental (vitamin C-supplemented) groups. Symptoms of URT infections were monitored for 14 d after the race. Sixty-eight percent of the runners in the placebo group reported the development of symptoms of URT infection after the race; this was significantly more ($P < 0.01$) than that reported by the vitamin C-supplemented group (33%). The duration and severity of symptoms of URT infections reported in the vitamin C-supplemented nonrunning control group was also significantly less than in the nonrunning control group receiving the placebo ($P < 0.05$). This study provides evidence that vitamin C supplementation may enhance resistance to the postrace URT infections that occur commonly in competitive ultramarathon runners and may reduce the severity of such infections in those who are sedentary.

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Prophylactic vitamin C: misplaced zeal. [Am J Clin Nutr. 1994]

PMID: 8185726 DOI: 10.1093/ajcn/57.2.170

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Nutrients. 2014 Jul 9;6(7):2572-83. doi: 10.3390/nu6072572.

Vitamin C supplementation slightly improves physical activity levels and reduces cold incidence in men with marginal vitamin C status: a randomized controlled trial.

Johnston CS[1], Barkyoumb GM[2], Schumacher SS[3].

Author information

Abstract

The early indications of vitamin C deficiency are unremarkable (fatigue, malaise, depression) and may manifest as a reduced desire to be physically active; moreover, hypovitaminosis C may be associated with increased cold duration and severity. This study examined the impact of vitamin C on physical activity and respiratory tract infections during the peak of the cold season. Healthy non-smoking adult men (18-35 years; BMI < 34 kg/m2; plasma vitamin C < 45 μmol/L) received either 1000 mg of vitamin C daily (n = 15) or placebo (n = 13) in a randomized, double-blind, eight-week trial. All participants completed the Wisconsin Upper Respiratory Symptom Survey-21 daily and the Godin Leisure-Time Exercise Questionnaire weekly. In the final two weeks of the trial, the physical activity score rose modestly for the vitamin C group vs. placebo after adjusting for baseline values: +39.6% (95% CI [-4.5,83.7]; p = 0.10). The number of participants reporting cold episodes was 7 and 11 for the vitamin C and placebo groups respectively during the eight-week trial (RR = 0.55; 95% CI [0.33,0.94]; p = 0.04) and cold duration was reduced 59% in the vitamin C versus placebo groups (-3.2 days; 95% CI [-7.0,0.6]; p = 0.06). These data suggest measurable health advantages associated with vitamin C supplementation in a population with adequate-to-low vitamin C status.

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Medical Hypotheses
Volume 7, Issue 11, November 1981, Pages 1359-1376

Vitamin C, TITRATING TO BOWEL TOLERANCE, ANASCORBEMIA, and ACUTE INDUCED SCURVY

Robert F. Cathcart

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https://doi.org/10.1016/0306-9877(81)90126-2

Abstract

A method of utilizing vitamin C in amouts just short of the doses which produce diarrhea is described (TITRATING TO BOWEL TOLERANCE). The amount of oral ascorbic acid tolerated by a patient without producing diarrhea increases somewhat proportionately to the stress or toxicity of his disease. Bowel tolerance doses of ascorbic acid ameliorate the acute symptoms of many diseases. Lesser doses often have little effect on acute symptoms but assist the body in handling the stress of disease and may reduce the morbidity of the disease. However, if doses of ascorbate are not provided to satisfy this potential draw on the nutrient, first local tissues involved in the disease, then the blood, and then the body in general become deplete of ascorbate (ANASCORBEMIA and ACUTE INDUCED SCURVY). The patient is thereby put at risk for complications of metabolic processes known to be dependent upon ascorbate.

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Volume 116, Issue 9
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Vitamin E and the risk of pneumonia: using the I^2 statistic to quantify heterogeneity within a controlled trial

Harri Hemilä (a1)

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Abstract

Analyses in nutritional epidemiology usually assume a uniform effect of a nutrient. Previously, four subgroups of the Alpha-Tocopherol, Beta-Carotene Cancer Prevention (ATBC) Study of Finnish male smokers aged 50–69 years were identified in which vitamin E supplementation either significantly increased or decreased the risk of pneumonia. The purpose of this present study was to quantify the level of true heterogeneity in the effect of vitamin E on pneumonia incidence using the I^2 statistic. The I^2 value estimates the percentage of total variation across studies that is explained by true differences in the treatment effect rather than by chance, with a range from 0 to 100 %. The I^2 statistic for the effect of vitamin E supplementation on pneumonia risk for five subgroups of the ATBC population was 89 % (95 % CI 78, 95 %), indicating that essentially all heterogeneity was true variation in vitamin E effect instead of chance variation. The I^2 statistic for heterogeneity in vitamin E effects on pneumonia risk was 92 % (95 % CI 80, 97 %) for three other ATBC subgroups defined by smoking level and leisure-time exercise level. Vitamin E decreased pneumonia risk by 69 % among participants who had the least exposure to smoking and exercised during leisure time (7·6 % of the ATBC participants), and vitamin E increased pneumonia risk

by 68 % among those who had the highest exposure to smoking and did not exercise (22 % of the ATBC participants). These findings refute there being a uniform effect of vitamin E supplementation on the risk of pneumonia.

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Vitamin E Supplementation May Transiently Increase Tuberculosis Risk in Males Who Smoke Heavily and Have High Dietary Vitamin C Intake

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Abstract

Vitamin E and beta-carotene affect the immune function and might influence the predisposition of man to infections. To examine whether vitamin E or beta-carotene supplementation affects tuberculosis risk, we analysed data of the Alpha-Tocopherol Beta-Carotene Cancer Prevention (ATBC)Study, a randomised controlled trial which examined the effects of vitamin E (50 mg/d) and beta-carotene (20 mg/d) on lung cancer. The trial was conducted in the general community in Finland in 1985-93; the intervention lasted for 6.1 years (median). The ATBC Study cohort consists of 29,023 males aged 50-69 years, smoking at baseline, with no tuberculosis diagnosis prior to randomisation. Vitamin E supplementation had no overall effect on the incidence of tuberculosis (risk ratio (RR) = 1.18; 95% CI 0.87, 1.59) nor had beta-carotene (RR = 1.07; 95% CI 0.80, 1.45). Nevertheless, dietary vitamin C intake significantly modified the vitamin E effect. Among participants who obtained 90 mg/d or more of vitamin Cin foods (n 13,502), vitamin E supplementation increased tuberculosis risk by 72 (95% CI 4, 185)%. This effect was restricted to participants who smoked heavily. Finally, in participants not supplemented with vitamin E, dietary vitamin C had a negative association with tuberculosis risk so that the adjusted risk was 60 (95% CI 16, 81)% lower in the highest intake quartile compared with the lowest. Our finding that vitamin E seemed to transiently increase the risk of tuberculosis in those who smoked heavily and had high dietary vitamin C intake should increase caution towards vitamin E supplementation for improving the immune system.

Comment in

Vitamin E supplementation may transiently increase tuberculosis risk in males who smoke heavily and have high dietary vitamin C intake--comments by Hernández-Garduño.
Hernández-Garduño E. Hernández-Garduño E. Br J Nutr. 2009 Jan;101(1):145; discussion 146-7. doi: 10.1017/S0007114508994411. Epub 2008 Jun 23. Br J Nutr. 2009. PMID: 18570687 No abstract available.

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Council Report

Vitamin Preparations as Dietary Supplements and as Therapeutic Agents

Council on Scientific Affairs

Healthy adult men and healthy adult nonpregnant, nonlactating women consuming a usual, varied diet do not need vitamin supplements. Infants may need dietary supplements at given times, as may pregnant and lactating women. Occasionally, vitamin supplements may be useful for people with unusual lifestyles or modified diets, including certain weight reduction regimens and strict vegetarian diets. Vitamins in therapeutic amounts may be indicated for the treatment of deficiency states, for pathologic conditions in which absorption and utilization of vitamins are reduced or requirements increased, and for certain nonnutritional disease processes. The decision to employ vitamin preparations in therapeutic amounts clearly rests with the physician. The importance of medical supervision when such amounts are administered is emphasized. Therapeutic vitamin mixtures should be so labeled and should not be used as dietary supplements.

(*JAMA* 1987;57:1929-1936)

VITAMIN preparations are used extensively in the practice of medicine and are valuable when used properly. It is important that a clear distinction be made between vitamins as dietary supplements and vitamins as therapeutic agents. It is also important for the practitioner to understand the usefulness and the limitations of given vitamin preparations in given clinical situations. Vitamins are essential organic substances whose usual source is food. They are required by man in amounts ranging from micrograms to milligrams per day. There are four fat-soluble vitamins (A, D, E, and K) and nine water-soluble vitamins (thiamine, riboflavin, niacin, pantothenic acid, folic acid, biotin, and vitamins B_6, B_{12}, and C), and all are essential for the normal growth, development, and maintenance of the human organism.

The Advisory Panel on Vitamin Preparations as Dietary Supplements and as Therapeutic Agents of the Council on Scientific Affairs has reviewed the indications for administration of vitamins, the composition and dosage of vitamin preparations, and the hazards of excessive intakes of vitamins and adopted the following statement. This statement updates one made on this subject by the Council on Foods and Nutrition in 1959.[1]

DEFINITIONS

Recommended Dietary Allowances (RDA)

The *RDA* are "the levels of intake of essential nutrients considered, in the judgment of the Committee on Dietary Allowances of the Food and Nutrition Board on the basis of available scientific knowledge, to be adequate to meet the known nutritional needs of practically all healthy persons."[2] (The abbreviation RDA is used for both the singular and plural of the term in accordance with National Academy of Sciences usage.[2]) The RDA are not requirements for an individual, but recommendations for the daily amounts of nutrients that *populations* should consume over a period of time to protect all members of that population. With exception of the allowances for energy, RDA are estimated to exceed the requirements of most individuals to ensure that the needs of nearly all members of a population will be met. In this country, RDA are set approximately 2 SDs above the mean requirement and will therefore encompass the needs of 97% of the population. Allowances are established for a wide range of age, weight, and sex groups and for pregnancy and lactation. The 1980 RDA for vitamins are shown in Table 1.

From the Council of Scientific Affairs, American Medical Association, Chicago.

This report was submitted to the AMA House of Delegates in June 1985 as an informational report.

This report is not intended to be construed or to serve as a standard of medical care. Standards of medical care are determined on the basis of all of the facts and circumstances involved rn an individual case and are subject to change as scientific knowledge and technology advance and patterns of practice evolve. This report reflects the views of the scientific literature as of November 1986.

Reprint requests to Council on Scientific Affairs, American Medical Association, 535 N Dearborn St, Chicago, IL 60610 (William R. Hendee, PhD).

Misuses of Vitamins

The FDA has estimated that 40% of the adult population uses vitamin and mineral supplements on a daily basis.[35] Ascorbic acid (vitamin C), either alone or in combination with other nutrients, was the most widely consumed nutrient (90.6%) of supplement users. Even among 2000 registered nurses surveyed, 38% were taking multiple vitamin supplements daily, 23% were using high dosages of ascorbic acid, the most common being 500 mg daily, 15% took 400 IU of vitamin E daily, and 4% took 10000 IU of vitamin A daily.[36]

With such widespread use of vitamins by the American public, there is ample opportunity for misuse. Misuse of vitamins is considered any application of a vitamin or vitamins in a dose that is inappropriate or for a purpose that has no basis in established scientific practice. The rationales are often based on myths, or distortions of experimental studies in laboratory animals. Some vitamins, such as A, E, C, and B_6, are abused more commonly than others.[37] Some persons have taken large doses of multivitamins in the belief that vitamins combat the chronic degenerative diseases or extend life. No objective benefits, however, have been demonstrated.

Some of the most frequently encountered examples of vitamin misuse include the following: Vitamin E has been taken in large quantities in pursuit of rejuvenation, increased libido, and improved sexual performance. Under the rubric of "orthomolecular psychiatry," large doses of niacin have been given for the treatment of a variety of mental disorders without measurable effect. Large doses of vitamin B_6 have been promoted for the treatment of carpal tunnel syndrome, premenstrual tension, and mental disorders, without established benefit.[37] One of the most widely misused vitamins is ascorbic acid. There is no reliable evidence that large doses of ascorbic _acid prevent colds or shorten their duration.[38]

Public health nutrition will be served best by the insistence on a scientifically sound basis for vitamin supplementation and therapy. All health practitioners should emphasize repeatedly that properly selected diets are the primary basis for good nutrition.

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Vitamin E and the risk of pneumonia: using the I^2 statistic to quantify heterogeneity within a controlled trial

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Abstract

Analyses in nutritional epidemiology usually assume a uniform effect of a nutrient. Previously, four subgroups of the Alpha-Tocopherol, Beta-Carotene Cancer Prevention (ATBC) Study of Finnish male smokers aged 50–69 years were identified in which vitamin E supplementation either significantly increased or decreased the risk of pneumonia. The purpose of this present study was to quantify the level of true heterogeneity in the effect of vitamin E on pneumonia incidence using the I^2 statistic. The I^2 value estimates the percentage of total variation across studies that is explained by true differences in the treatment effect rather than by chance, with a range from 0 to 100 %. The I^2 statistic for the effect of vitamin E supplementation on pneumonia risk for five subgroups of the ATBC population was 89 % (95 % CI 78, 95 %), indicating that essentially all heterogeneity was true variation in vitamin E effect instead of chance variation. The I^2 statistic for heterogeneity in vitamin E effects on pneumonia risk was 92 % (95 % CI 80, 97 %) for three other ATBC subgroups defined by smoking level and leisure-time exercise level. Vitamin E decreased pneumonia risk by 69 % among participants who had the least exposure to smoking and exercised during leisure time (7·6 % of the ATBC participants), and vitamin E increased pneumonia risk by 68 % among those who had the highest exposure to smoking and did not exercise (22 % of the ATBC participants). These findings refute there being a uniform effect of vitamin E supplementation on the risk of pneumonia.

Key words: Antioxidants: Dietary supplements: Effect modifiers (epidemiology): Population characteristics: Respiratory tract infections

The effect of vitamin E supplementation on mortality has been studied in numerous randomised trials, the results of which have been pooled in several meta-analyses[1–3]. Usually meta-analyses calculate a single estimate of effect, such as a 4 % increase in mortality by vitamin E[1]. The calculation of a single estimate is based on the assumption that there is a uniform size of effect that is informative for all the included trials, and also applies to populations not included in the analysed trials.

Biology is complex, and it is possible that the effect of vitamin E on health outcomes depends on various characteristics of people and on their lifestyles. Therefore, a single universal estimate of vitamin E effect might be substantially misleading for some population groups. We found in our previous analyses of the Alpha-Tocopherol, Beta-Carotene Cancer Prevention (ATBC) Study on Finnish male smokers that the effects of vitamin E supplementation were modified as follows: the risk of common cold by age, smoking and residential neighbourhood[4], the risk of tuberculosis by vitamin C intake[5] and mortality by age and vitamin C intake[6]. These findings challenge the notion that the health effects of vitamin E are uniform over the entire ATBC Study population. However, a quantitative estimation of the true within-trial heterogeneity in vitamin E effects has not been carried out previously.

The I^2 statistic was developed for the quantification of true heterogeneity between multiple controlled trials included in a meta-analysis[7,8]. The I^2 value estimates the percentage of total variation across different studies, which is explained by true variation in the treatment effect rather than by chance variation. The range of the I^2 scale is from 0 to 100 %, and a value greater than about 75 % indicates a high level of true treatment heterogeneity[8]. To our knowledge, the I^2 statistic has not been used previously to quantify the level of true heterogeneity between the subgroups of a single randomised trial.

Vitamin E is an antioxidant and it influences the immune system[9,10]. Therefore, it might influence infections of the lungs exposed to O_2 and airborne oxidants. In our previous analyses of the ATBC Study data, the effect of vitamin E on pneumonia incidence differed from the null effect for several subgroups, which were identified by different types of reasoning: by the level of smoking, physical activity, weight and dietary vitamin C intake[11–15]. The goal of this study was to quantify the level of true heterogeneity in the effect of vitamin E on pneumonia risk

Abbreviations: AT, DL-α-tocopheryl acetate; ATBC, Alpha-Tocopherol, Beta-Carotene Cancer Prevention; BC, β-carotene.

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over the identified ATBC Study subgroups by using the I^2 statistic.

Methods

Participants

The rationale, design and methods of the ATBC Study, to examine the effects of vitamin E (DL-α-tocopheryl acetate, AT, 50 mg/d) and β-carotene (BC, 20 mg/d) on the incidence of lung cancer and other cancers and the primary findings, have been described in detail[16,17]. The ATBC Study is registered at ClinicalTrials.gov under the identifier NCT00342992. In brief, males aged 50–69 years who smoked ≥5 cigarettes/d at entry (*n* 29 133) were randomised into one of four intervention arms – placebo, AT, BC or AT + BC – according to a 2 × 2 factorial design. Supplementation with vitamin E in the form of DL-α-tocopheryl-acetate increased the mean serum levels of α-tocopherol by 50 % compared with baseline[17]. The intervention continued for 5–8 years until April 1993. The trial was approved by the review boards of the participating institutions, and all participants gave their written informed consent. Compliance with supplementation was high: 90 % of the subjects took >90 % of their prescribed capsules during their active participation in the trial[17].

Baseline characteristics

Before randomisation, the participants completed questionnaires on medical and smoking histories and general background characteristics[11,12,16,17]. The baseline questionnaire enquired about the intensity of leisure-time physical activity in terms of the following three alternatives: (1) light: reading, watching TV, listening to the radio or going to movies; (2) moderate: walking, fishing, hunting or gardening quite regularly; and (3) heavy: actual physical exercise such as jogging, skiing, swimming, gymnastics and court and field sports quite regularly. In the current analysis, 'exercise during leisure time' combines positive responses to alternatives (2) (*n* 15 191) and (3) (*n* 1744).

Outcome and follow-up time

The outcome of this study, the first hospital-treated case of pneumonia after randomisation, was ascertained from the national Hospital Discharge Register using the volunteer's unique personal identification number, given to all Finnish residents, for linkage[11]. Follow-up time began from the day of randomisation and continued until the date of the first hospital discharge for pneumonia, death or the end of the trial, whichever came first. There was a total of 167 968 person-years of observation (median follow-up 5·8 years).

Statistical methods

The effect of vitamin E supplementation on pneumonia incidence was estimated by Cox's proportional hazards models. The trial participants to whom vitamin E alone or in combination with BC were administered (AT and AT + BC) were compared with the no-vitamin E supplement groups (placebo and BC). The exceptions were subgroup 3 in Fig. 1 and 2 and subgroup A in Fig. 3, for which the comparison was restricted to no-BC participants because of the significant interaction between AT and BC[15]. We calculated the risk ratio (RR) and the 95 % CI of the RR using the PROC PHREG program of the SAS package of programs (release 9.4; SAS Institute Inc.). Forest plots were constructed using the metagen and forest programs of the R program package; the I^2 statistic with its 95 % CI and the Cochran Q test-based χ^2 values for heterogeneity were calculated[18]. To test the statistical significance of interaction between vitamin E supplementation and the set of subgroups, vitamin E and the subgroups were first added to the Cox's model. The statistical significance of the interaction was thereafter calculated from the change in −2 × log (likelihood) when the vitamin E subgroup interaction terms were added to the model.

Results

The ATBC Study included males aged 50–69 years who smoked ≥5 cigarettes/d at entry. Further characteristics of the participants have been described previously[11–17]. There were 898 pneumonia cases during the follow-up period corresponding to an average rate of 5·3 pneumonia cases per 1000 person-years. Among all 29 133 ATBC participants, the pneumonia cases were identically distributed between the vitamin E and no-vitamin E groups, 449 *v.* 449, corresponding to the average effect of vitamin E supplementation of RR 1·00 (95 % CI 0·88, 1·14).

To quantify the level of heterogeneity in vitamin E effect, the ATBC participants were divided into six subgroups on the basis of previous findings (Fig. 1). The primary cut-off point for the subgroups was the age at which the participant initiated smoking (≤20 *v.* ≥21 years), which significantly modified the effect of vitamin E in the first series of subgroup analyses[11]. The second-level subgroups 1 and 2 were formed by the subject's body weight and dietary vitamin C intake[14], and subgroups 3 and 6 were formed by the level of cigarette smoking at baseline and the level of exercise at leisure time at baseline[15]. The participants who did not fall into these second-level subgroups were classified as 'the rest', and they comprised subgroups 4 and 5. A forest plot of the six subgroups is shown in Fig. 2. The number of pneumonia cases in the six subgroups is shown in the online Supplementary Table S1.

Essentially all heterogeneity over the six subgroups was true variation in the vitamin E effect rather than chance variation: $I^2 = 87$ % (95 % CI 73, 93 %) (Fig. 2).

In subgroup 6, vitamin E supplementation decreased the risk of pneumonia by 69 % (95 % CI 44, 87 %; *n* 2216). This group included people who started smoking at a later age (≥21 years), smoked just 5–19 cigarettes/d at study entry and carried out leisure-time exercise[15]. This subgroup in which vitamin E was beneficial covered 7·6 % of the ATBC participants.

The three groups – 1, 2 and 3 – for which vitamin E increased pneumonia risk by 209 % (95 % CI 45, 560 %; *n* 468), 134 % (95 % CI 7, 408 %; *n* 1328) and by 68 % (95 % CI 18, 140 %;

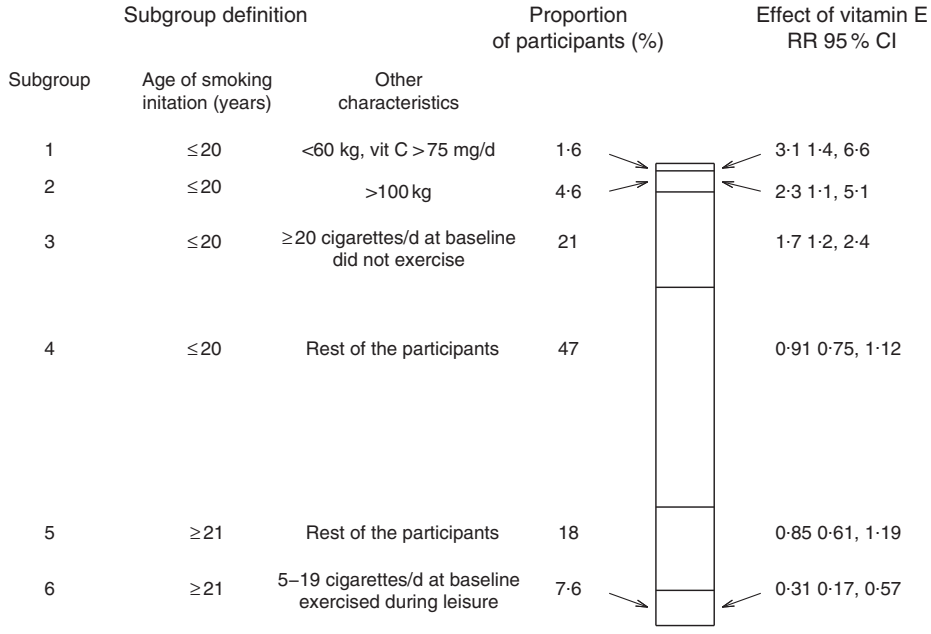

Fig. 1. Proportion of participants and the effect of vitamin E on the incidence of pneumonia in the Alpha-Tocopherol, Beta-Carotene Cancer Prevention Study, 1985–1993. The left-hand side shows the proportion of participants in six subgroups. The right-hand side shows the effect of vitamin E supplementation on the risk of pneumonia for the same subgroups. Group 3 shows the estimate of vitamin E effect based on the no-β-carotene participants, because vitamin E and β-carotene had a significant interaction in that subgroup[15]. Groups 1 and 2 had 60 and 289 participants, respectively, overlapping with group 3. In Fig. 1 and 2, the overlapping participants are included in groups 1 and 2, so that these two subgroups are consistent with the study of Hemilä & Kaprio[14]. RR, risk ratio.

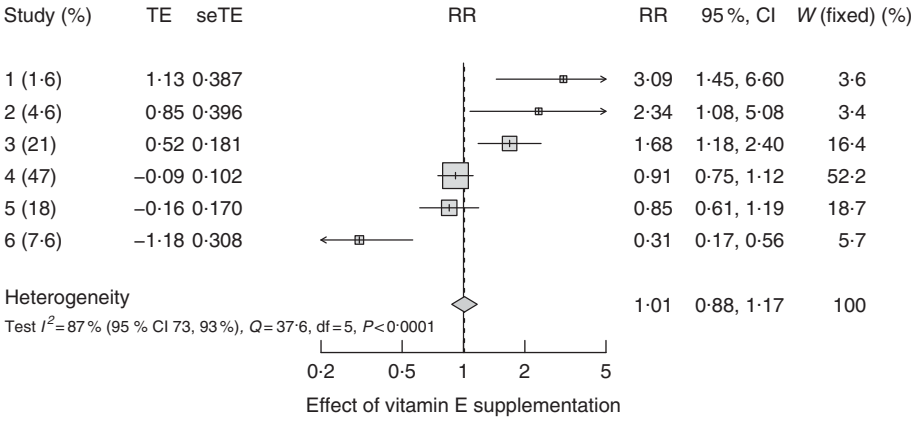

Fig. 2. A forest plot of six subgroups on vitamin E and the incidence of pneumonia in the Alpha-Tocopherol, Beta-Carotene Cancer Prevention (ATBC) Study, 1985–1993. The subgroups of Fig. 1 are shown in the same order in this forest plot. The percentage shown after group identification indicates the proportion of ATBC Study participants falling in that subgroup. On the right-hand side, the vertical line indicates the no-vitamin E level. The horizontal lines indicate the 95 % CI for the vitamin E effect, and the squares at the centre of the horizontal lines indicate the point estimates of the effects in those particular groups. The sizes of the squares indicate the relative weights of the groups. The Cochran Q test $\chi^2 = 37{\cdot}6$ (5 df) corresponds to $P = 10^{-6}$. The two 'rest of the participants' groups 4 and 5 are redundant, and when they are combined to a single 'rest of the participants' group (4 + 5) the I^2 increases to 89 % (95 % CI 78, 95 %) with $\chi^2 = 37{\cdot}5$ (4 df) corresponding to $P = 10^{-7}$ (see the online Supplementary Fig. S1). RR, risk ratio; TE, treatment effect on the logarithmic scale; seTE, standard error of TE.

n 3022), respectively, included males who started smoking at a younger age (≤20 years). In addition, these participants had low body weight and vitamin C intakes above the median (group 1), high body weight (group 2), smoked ≥20 cigarettes/d at study entry and did not carry out leisure-time exercise (group 3)[14,15]. In all, these three subgroups in which vitamin E was harmful covered 28 % of the ATBC participants.

Vitamin E supplementation did not influence pneumonia risk among the rest of the participants (groups 4 and 5). These two subgroups covered 66 % of the ATBC study participants. In Fig. 1 and 2, these two groups are shown separately to illustrate the background of the subgroup division. However, maintaining the two 'rest of the participants' groups separately is redundant, as both of them are consistent with no effect. When these two groups were combined, the heterogeneity over the remaining five subgroups increased to $I^2 = 89$ % (95 % CI 78, 95 %) (online Supplementary Fig. S1). When the five subgroups were allowed independent vitamin E effects in the Cox's regression model, the statistical model was improved by $\chi^2 = 42{\cdot}3$ (4 df) corresponding to $P = 10^{-8}$.

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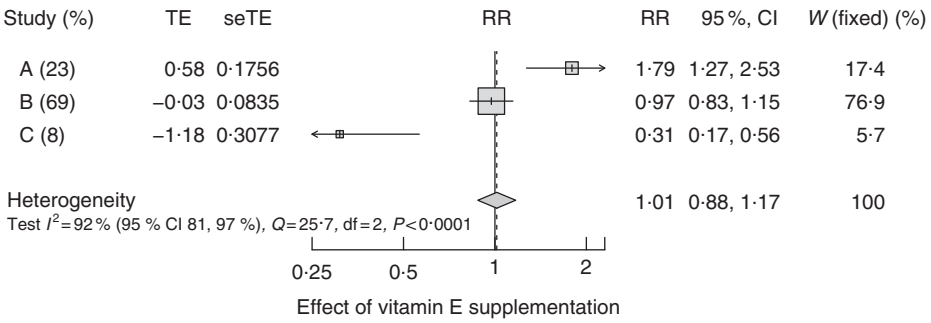

Fig. 3. A forest plot of three subgroups on vitamin E and the incidence of pneumonia in the Alpha-Tocopherol, Beta-Carotene Cancer Prevention (ATBC) Study, 1985–1993. Group A in this forest plot includes participants who started smoking at ≤20 years of age and smoked ≥20 cigarettes/d at study entry and did not carry out leisure-time exercise (23·0 % of the ATBC participants). Group C includes males who started smoking at ≥21 years of age and smoked 5–19 cigarettes/d at study entry and carried out leisure-time exercise (7·6 %). Group B includes all the other participants (69·4 %). The estimate of effect shown for subgroup 3 is based on the no-β-carotene participants only, as vitamin E and β-carotene had a significant interaction in that subgroup; see Hemilä & Kaprio[15] for the origin of these three subgroups. In the forest plot on the right-hand side, the vertical line indicates the placebo level. The Cochran Q heterogeneity test $\chi^2 = 25{\cdot}7$ (2 df) corresponds to $P = 10^{-5}$. When the analysis was restricted to the no-β-carotene participants (n 14 573), then $I^2 = 88$ % (95 % CI 65, 96 %; $P = 0{\cdot}0003$) (see the online Supplementary Fig. S2). RR, risk ratio; TE, treatment effect on the logarithmic scale; seTE, standard error of TE.

When small subgroups are formed, the balance of the baseline variables might be compromised. The uppermost subgroup 1 was small with only 468 participants – that is, only 1·6 % of all ATBC Study participants (Fig. 1 and 2). Nevertheless, the baseline differences in relevant variables between the vitamin E and no-vitamin E participants in this subgroup were close to zero with narrow CI. Furthermore, inclusion of baseline variables in the Cox's model did not substantially change the estimate of vitamin E effect (online Supplementary Table S2). Thus, the difference in pneumonia occurrence between the vitamin E and the no-vitamin E participants in subgroup 1 cannot be explained by an imbalance in relevant baseline variables. The other groups, 2, 3 and 6, in which vitamin E significantly affected pneumonia risk are much larger, and a baseline imbalance is of even less concern.

A simplified analysis with only three subgroups was also carried out (Fig. 3). This division was based on the age at initiating smoking, the level of cigarette smoking at baseline and the level of leisure-time exercise at baseline[15]. Group A had the highest smoking levels without leisure-time exercise. Group C had the lowest levels of smoking with active leisure-time exercise. Thus, the characteristics of group C are the opposite of group A. The effects of vitamin E also point to the opposite directions in these two subgroups. Group B includes participants who did not belong to group A or C. The I^2 statistic for heterogeneity in this set of three subgroups was 92 % (95 % CI 81, 97 %), indicating that essentially all the heterogeneity in this subgroup division was a true variation of the vitamin E effect and not chance fluctuation. When the three subgroups were allowed independent vitamin E effects in the Cox's regression model, the statistical model improved by $\chi^2 = 28{\cdot}7$ (2 df) corresponding to $P = 10^{-6}$.

Discussion

The number of pneumonia cases in the ATBC Study was evenly distributed between the vitamin E and the no-vitamin E participants, indicating no overall average effect with great accuracy. Nevertheless, within the ATBC Study population, there was a high level of true heterogeneity for the effect of vitamin E on pneumonia risk as shown in the present study. Not only the I^2 point estimates but also the entire 95 % CI ranges of the I^2 were above the 75 % level, which has been judged as the threshold for high level of true heterogeneity[8]. This indicates that the overall average zero effect is not applicable for all ATBC participants. It follows, therefore, that there cannot be a uniform vitamin E supplementation effect on pneumonia risk over the Western male population, as Finnish males of the ATBC Study form a subgroup of Western males.

All the variables used to define the subgroups of Fig. 1 have a biological rationale: smoking has an influence on vitamin E metabolism[19], vitamins C and E interact[19,20] and sporadic physical activity causes oxidative stress[21] against which antioxidant vitamin E may protect. Finally, the dose–effect relationship is a basic concept in pharmacology. Consequently, the effects of a fixed vitamin E dose may depend on body weight as the dose per body weight varies[14].

When the modification of vitamin E effect is complex and defined by half a dozen or more variables, there is no unambiguous way to form subgroups that are distinguished by different sizes of the vitamin E effect. Pragmatic cut-off limits are used in Fig. 1–3; yet, it is unreasonable from the biological perspective to assume exact cut-off points. Nevertheless, the main issue in this study is not the specific locations of the cut-off points, but the finding of the very high level of true heterogeneity in the vitamin E effect over the 29 133 ATBC participants.

The level of true heterogeneity of vitamin E effect depends on the combination of the sizes of the vitamin E effects for the subgroups and the sizes of the subgroups themselves. Thus, the estimate of $I^2 = 92$ % in Fig. 3 is not a characteristic of vitamin E but it is generated by the combination of the specific subgroup sizes and the effects of vitamin E within the particular subgroups of the ATBC Study cohort.

The high level of true heterogeneity in the effect of vitamin E on pneumonia has important implications. First, it provides a strong argument against the opinion that subgroup analyses of

randomised trials should be strongly discouraged because they can lead to false-positive findings due to the multiple comparison problem[22–25]. Altman stated that biological plausibility is a weak criterion when deciding whether a subgroup finding is likely to be real, as in his view 'doctors seem able to find a biologically plausible explanation for any finding'[22]. Although there is much room for speculation at the molecular level of biology because the number of genes and proteins is huge, the number of variables relevant at the population level of biology is much more limited. Few variables are as important at the population level as smoking, which modified the effect of vitamin E (Fig. 1–3).

Many trials are small and they do not have the statistical power to analyse subgroup differences. For example, one study on vitamin E and respiratory infections included 652 participants who were followed-up for 788 person-years[26], and another study included 617 participants followed-up for 540 person-years[27]. In contrast, the ATBC Study included 29 133 participants followed-up for 168 000 person-years. Consequently, the ATBC Study, when analysed as subgroups, may be considered to be a large series of small studies covering a wide range of population groups with different characteristics. A large, randomised trial has consistent treatment and outcome definitions. Therefore, a subgroup analysis of a large trial is much more informative than a comparison of a series of small trials with slightly varying interventions and outcome definitions, even when the total number of participants in the latter might be the same. Although the multiple comparison problem is a relevant concern in subgroup analysis of small studies, it is not a reasonable explanation for the narrow CI of the I^2 statistic found in the present subgroup analysis (Fig. 2 and 3).

Biology is complex and it is unlikely that the belief in a uniform treatment effect is usually justified. The groups of people in whom a treatment is either most or least effective can be found only by comparing the effects on different groups of people. Feinstein wanted to 'rescue the scientific importance of valid pathophysiologic subgroups from being forgotten or destroyed by excessive vehemence in suggestions that all subgroups are evil'[28] and Lagakos commented that 'avoiding any presentation of subgroup analysis because of their history of being over-interpreted is a steep price to pay for a problem that can be remedied by more responsible analysis and reporting'[29]. Given the long-term commitment of study participants and the resources invested, it might even be considered as an ethical duty of the researchers to analyse large trials extensively rather than simply calculating a single overall average effect. Nevertheless, it is also important to carry out subgroup analysis with caution and not over-interpret the findings.

The second implication of the high level of true heterogeneity within the ATBC Study cohort concerns the pooling of diverse randomised trials in meta-analyses. Calculation of a pooled estimate of effect is based on the assumption that there is a uniform effect that is informative. However, small studies have wide CI and may not reveal heterogeneity even if the biological effect does differ between the studied populations. On the other hand, large studies may include people who vary substantially in their characteristics and in the effects of treatments; yet, the overall average effect may camouflage substantial variations between subpopulations as shown in Fig. 1–3. Therefore, the pooled estimates of meta-analyses can be spuriously precise and may suffer from ecological fallacy, which means that study-level analysis can lead to different conclusions than corresponding individual-level analysis[30,31]. Analyses of the ATBC Study also found evidence that the effect of vitamin E on mortality was heterogeneous[6,32]. Therefore, the averages calculated in meta-analyses, such as the 4 % increase in mortality for vitamin E supplementation[1], may not be valid for many population groups.

The third implication of the heterogeneity in vitamin E effects is that cohort studies on nutrition and health may often be misleading. In cohort studies, confounders are adjusted to allow the calculation of a single estimate of effect over the study population. For example, in their cohort study with male US health professionals between 40 and 75 years of age, Merchant *et al.*[33] reported no association between daily vitamin E intake and community-acquired pneumonia. However, when several variables modify the effect of vitamin E on pneumonia risk (Fig. 1–3), it is evident that the effects of vitamin E should be investigated separately in subpopulations defined by those modifier variables, instead of calculating a single average effect adjusting for those variables as if they were confounders. Large trials such as the ATBC Study can give accurate effect estimates for subgroups as shown by the current study. However, similar subgroup analyses in cohort studies are much more challenging or impossible because of the close associations between dietary variables with each other and with numerous other lifestyle factors[34].

Finally, vitamin E supplementation has been proposed for improving the immune system[35]. However, in the ATBC Study, 28 % of males had an increased risk of pneumonia because of vitamin E administration (Fig. 1). In addition, the combination of vitamin E supplementation and a high level of dietary vitamin C intake increased the risk of tuberculosis by 72 % (95 % CI 4, 185 %)[5], and vitamin E increased the risk of common cold in a subpopulation of the participants[4]. Thus, even though subgroup 6 of Fig. 1 indicates that some people may benefit from vitamin E by gaining protection against infection, there is evidence of harm in some other people. Given the current limited understanding about who might benefit, vitamin E should not be suggested for the general population for improving the immune system.

Although the 69 % reduction in the risk of pneumonia is a substantial effect in subgroup 6 (Fig. 1), given the pneumonia rate of about six cases/1000 person-years, approximately 250 people would need vitamin E supplementation for 1 year to prevent one episode of pneumonia in males in that subgroup. Community-acquired pneumonia in middle-aged people is usually cured quite rapidly by antibiotics and rarely leads to long-term or permanent sequelae; thus, the practical significance of vitamin E is not clear even in this subgroup. Furthermore, the ATBC Study participants were mostly born in the 1920s and 1930s and lived through the WWII years. Therefore, the estimate of effect calculated for the 7·6 % subgroup of the ATBC Study cohort should not be generalised to current middle-aged males in Western countries.

In conclusion, the I^2 statistic may be a useful measure when analysing within-trial heterogeneity in large, randomised trials. The numerical estimates of vitamin E effect in the analysed subgroups of the present study are much less essential than the high level of true heterogeneity over the entire ATBC Study cohort. When an effect is heterogeneous, great caution should be exercised in the extrapolation of the effect estimates to other contexts. The high level of true heterogeneity found in the current study indicates that the uniform effect estimates calculated in meta-analyses and cohort studies on vitamin E may often be misleading. There seems to be a need for further research on vitamin E for non-smoking, middle-aged and older males who exercise in their leisure time.

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The author had full access to all the data in this study, and the author takes full responsibility for the accuracy of the data analyses.

A table showing the number of pneumonia cases in the subgroups of Fig. 1, a table comparing the baseline balance of vitamin E and no-vitamin E groups of subgroup 1 of Fig. 1 and two additional forest plots are shown in the online Supplementary File.

There are no conflicts of interest.

Supplementary material

For supplementary material/s referred to in this article, please visit http://dx.doi.org/doi:10.1017/S0007114516003408

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December 19, 1942

VITAMINS FOR THE PREVENTION OF COLDS

DONALD W. COWAN, M.D.; HAROLD S. DIEHL, M.D.; A. B. BAKER, M.D.

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JAMA. 1942;120(16):1268-1271. doi:10.1001/jama.1942.02830510006002

Abstract

Repeated studies have shown that both animals and man have a decreased resistance to infections of various kinds when suffering from vitamin deficiencies. Apparently this may be true for each of the better known vitamins. On the other hand, it has not been shown by adequately controlled experiments that the addition of any of the vitamins to a reasonably adequate diet produces increased resistance to infections of the upper respiratory tract, the millions of dollars' worth of vitamin preparations which are sold each year for this alleged purpose notwithstanding.

Most of the studies of vitamins for the prevention of colds have been limited to vitamin A alone or to vitamins A and D as contained in cod liver oil. The experiments with vitamin A have resulted almost uniformly in negative results, while cod liver oil has been reported by a number of authors to reduce the severity and by some

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Am J Epidemiol, 154 (12), 1113-8 2001 Dec 15

Which Plasma Antioxidants Are Most Related to Fruit and Vegetable Consumption?

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Affiliations

PMID: 11744516 DOI: 10.1093/aje/154.12.1113

Abstract

Substantial evidence suggests that fruit and vegetable intake reduces the risk of some cancers and other chronic diseases. While a varied diet containing fruits and vegetables may confer benefits greater than those of any single nutrient, it would be useful to have data on the plasma nutrients most influenced by fruit and vegetable intake. The authors examined the correlation between fruit and vegetable intake as measured by the abbreviated CLUE II food frequency questionnaire and several plasma antioxidants. This study includes 116 male subjects aged 35-72 years who were nonsmokers and nonusers of vitamin supplements and who provided blood samples in the CLUE II Study in Washington County, Maryland. Plasma was assayed for ascorbic acid, beta-carotene, beta-cryptoxanthin, and alpha- and gamma-tocopherol. Lipid- and energy-adjusted partial correlation for the relation with fruit and vegetable intake was r = 0.64 for ascorbic acid, r = 0.44 for beta-carotene, and r = 0.50 for beta-cryptoxanthin. While this study does not address efficacy, the stronger association of ascorbic acid with fruit and vegetable intake seen here may imply that ascorbic acid is an important component of the protective effect seen for fruits and vegetables in numerous epidemiologic studies.

Can Med Assoc J. 1975 Apr 5; 112(7): 823–826. PMCID: PMC1958969
PMID: 1091343

Winter illness and vitamin C: the effect of relatively low doses.

T. W. Anderson, G. H. Beaton, P. Corey, and L. Spero

Abstract

After their random -llocation to one of three treatment aroups, 622 volunteers received either vitamin C or placebo in a maintenance dose of 500 mg once weekly and a therapeutic dose of 1500 mg daily on the 1st day and 1000 mg on the next 4 days of any illness. Two forms of vitamin C were employed: a sustained-release capsule containing ascorbic acid and a regular tabet containing a mixture of sodium and calcium ascorbate. In the 448 subjects who completed an average of 15 weeks in the study of total of 635 episodes of illness were recroded. Respiratory symptoms were recorded on at least 1 day in 92 per cent of these episodes. There were no consistent or significant differences in the sickness experience of the subjects receiving the sustained-release vitamin capsules compared to those receiving the vitamin tablets, but subjects in both vitamin groups experienced less severe illness than subjects in the placebo group, with approximately 25 per cent fewer days spent indoors because of the illness (P smaller than 0.05). These results are compatible with the belief that supplementary vitamin C can reduce the burden of winter illness, but the intake need not be as high as has sometimes been claimed.

Full text

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Virus Pneumonia and Its Treatment With Vitamin C

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VIRUS PNEUMONIA (primary atypical pneumonia, non-specific pneumonitis, epidemic non-bacterial pneumonia, desseminated focal pneumonia, viral pneumonia) has been accepted as an entity and has been under observation in this country and abroad for the past twelve years. No bacteriological studies have confirmed the etiology of this disease other than by negative findings. The sputum shows the usual flora of gram-positive and gram-negative organisms. In 1938, Reimann reported that a filterable infectious agent was recovered from the nasopharynx of one and from the blood of another out of a series of eight cases, but not sufficient evidence could be found to determine such as the causative factor. It must be closely allied to the virus causing influenza, because in the first twenty-four to thirty-six hours it is very commonly thought to be that type of infection. Horsfall and his co-workers at the Rockefeller Institute have cultured an organism, which they have designated Streptococcus MG, from a large percentage of their patients with primary atypical pneumonia. The exact role of this bacterium is not known, but it is seldom found except in persons ill of this disease. Since it is not present in all cases, it is not the primary cause, but only a characteristic secondary invader or associate. The disease also resembles psittacosis in many respects and since penicillin might be of value in such cases it is of great importance to establish the diagnosis quickly.

The onset of this type of virus infection is always gradual. Like all virus diseases there is a wide variation of the prodromal symptoms. There might be none; there might be the classical generalized malaise. This disease is highly contagious, and our observations over a five-year period point to a definite incubation period of from five to fourteen days. We have also noted that the longer the incubation period the milder the infection: the shorter the incubation period the more severe is the infection. This must be interpreted in the first instance as either a mildly virulent organism or a high degree of resistance or immunity on the part of the host and in the second instance as a very virulent organism or no immunity at all on the part of the host. In some instances, however, the patient will have a slight attack with apparent recovery due either to good resistance against a weak virus or good response to treatment only to be followed in seven to ten days by a return of symptoms in a more severe form and producing a critically ill patient. This type of case cannot be classified as a fourteen-day incubation period, but rather it is one in which the virus was only attenuated or else there has been the factor of a second infection.

Read by Title to the Tri-State Medical Association of the Carolinas and Virginia, meeting at Charleston, February 9th and 10th.

The chief complaint, however, will always be one of sudden onset, since the patient begins his concept of his illness from the time he first experienced waves of chilly sensations or a frank chill alternating with hot spells and associated with burning in the nose, a sore throat, hoarseness, a bad taste in his mouth, moderate vertigo, nausea and grade-two type frontal headache. This picture will then develop to the point where severe frontal headache is noted along with a feeling of weakness in the lower extremities so marked that the patient complains of a dragging sensation when moving about in bed. This weakness persists for some days after clearing of all symptoms and negative chesi films. The patient can hardly support his body weight without the feeling of buckling at the knees. Added to the above might be substernal pain or generalized tightness in the chest with varying degrees of tracheo-bronchitis. The fever is usually found during this phase to be about 102° F. After pulmonary involvement of as much as 6 by 8 cm. areas have been reached the fever will be up to 103 and 104° F. in adults and up to 105° F. in infants and early childhood. Dry hacking cough is a most constant factor especially after the second day of illness. Occasionally this cough is paroxysmal, and if the invasion is severe enough it will in the final clearing stage of the disease be thick, tenacious, brownish-gray — even blood-streaked. This disease shows remarkable versatility in that it will vary its symptoms and signs to fit with that of a mild cold on one hand to a very serious medical complexity on the other. It suggests sometimes that more than one bacteriologic unit is involved. The pulse will be increased in a very definite ratio to the toxic effect of the virus. If the invasion is mild the pulse rate will be normal even though the fever may be recorded at 103° F. If, however, the invasion is severe, meaning that physical findings approximating those of a lobar pneumonia (with or without a definite complicating encephalitis or meningitis) are present, or with an accompanying pleurisy, then the pulse rate will be rapid and will follow the temperature curve. Sweating is common and it is usually very profuse. Cyanosis and dyspnea occurred only in those patients that had at least as much as a lobe of lung involvement and where the fever continued to climb to a 104° F. each night.

The physical findings are limited to the head and chest. There is marked rhinitis with swelling of the turbinates. The accessory nasal sinuses are involved; the frentals being the chief offenders. The tonsil bed is not remarkable but the lymphoid tissue on the posterior pharyngeal wall is thickened and edematous and scarlet in color. The vocal cords appear like those seen in any simple laryngitis. In the lungs diminished breath sounds with moist and dry rales (sometimes very coarse) are usually the only evidence of disease. When there are extensive areas of consolidation the usual dullness to percussion, tubular breathing and pectoriloquy are present.

The laboratory findings are of little importance. The" white blood count and differential are nearly always within normal limits. A 6500 white count is typical regardless of the lung pathology. The sedimentation rate will be normal except in very-acute cases, with cerebral symptoms. The sputum examination is valuable only in its negative findings.

Chemotherapy may be tried where x-ray facilities are not convenient or not obtainable. If sulfonamides and/or penicillin are given for twenty-four to thirty-six hours without response both should be discontinued and treatment for virus infection instituted. In our age it requires some measure of boldness to discontinue these important drugs so early especially with the patient still running a fever of from 102 to 104° F. In this case boldness counts.

There is no constant x-ray picture to be found in virus pneumonia, but some evidence of pneumonitis will nearly always be present regardless of the physical signs—even when the physical signs are absent. The chest film will show anything from extensive consolidation to a patchy and sometimes fleecy infiltration suggestive of tuberculosis. This patchy form will be scattered in all diameters of the lung fields. Plates taken daily or every second to third day will often show the pneumonic process clearing in some areas while new areas are developing at other points. The disease begins as an infiltrative process starting at the hilus, and then, by a peribronchial route gradually spreading to the interbrdnchial regions. Usually there will be an involvement of several segments of lung comprising several lobes. These isolated segments soon become confluent, giving the film a smoky appearance. This process may go on to involvement of an entire lobe and in many respects look like a lobar pneumonia. The marked difference lies in the fact that even when the density is massive a streaky background can always be seen; the shadow in virus pneumonia is never entirely solid. Resolution, either spontaneous or from some method of treatment, may give positive x-ray films days and even weeks after there has been a complete clinical response.

The treatment of virus infections, including frank virus pneumonia, has been for the most part without specific recommendations. Oppenheimer in 56 cases employed x-rays in doses from 35r to 90r which he states relieved cough and shortened the course of the disease. Offutt employed 100r doses daily or every other day, depending on the severity and response, alternating front and back or alternating sides if both lungs were involved. None in his series of twelve cases received over four treatments. Both men report surprising uniformity in the disappearance of fever and symptoms after one or two exposures. No unfavorable reactions occurred in either series. Aminophyllin in doses of three grains every four hours has been given with varying results in the belief that it improved the circulation through the lung fields. We have employed the drug in smaller doses when there was evidence that the patient had a coexisting coronary impairment. Since this was given along with the drug of our choice, ascorbic acid, this paper cannot evaluate its merits. Multiple transfusions from multiple donors and blood from patients convalescing from virus pneumonia have also been used.

The purpose of this paper is to outline a new and different form of treatment for this type of virus infection which in 42 cases over a five-year period has given excellent results. The treatment has dojuble merit due to the simplicity of its schedule. The remedy used was vitamin C (ascorbic acid) given in massive doses. Since it is common knowledge that there are definite individual variations in absorption of vitamin C from the intestinal tract and under certain pathological conditions still greater variations in the absorption factors the I. V. and I. M. routes were used. When a diagnosis of virus pneumonia was entertained the patient was given 1000 mg. vitamin C intravenously every six to twelve hours. If it was by chance that a diagnosis was established in the home the usual initial dose was 500 mg. given in the gluteal muscle. Subsequent injections were given I. V. because the injection was thus made painless and the response was faster. In infants and very small children, however, 500 mg. I. M. every six to twelve hours was the method of choice. From three to seven injections gave complete clinical and x-ray response in all of our cases. The series comprised types of cases from very slight consolidation to those resembling lobar pneumonia. Two cases were complicated by cerebral manifestations. Vitamin C was also given by mouth in one-third of this series but there was no outstanding difference in the response. The dosage was from 100 to 500 mg., depending on the age of the pa-

tient, and it was given every four to six hours. In almost every case the patient felt better within an hour after the first injection and noted a very definite change after two hours. Nausea was relieved by the first injection as was the headache. The heat regulating center showed a quick response and it was the rule to find a drop of 2° F. several hours after the first 1000 mg. Penicillin was given in conjunction with ascorbic acid in five cases. It was our observation that penicillin had some retarding effect on the action of vitamin C, since the response was not so rapid and in one case the results were not obtained until the penicillin was discontinued.

Supportive treatment was given by forcing fluids, particularly fruit juices, to tolerance. Soda-water was given to adults in the amount of four glasses in 24 hours, each glass containing one teaspoonful sodium bicarbonate. Infants and children were given this alkaline drink in proportion to age. The rationale of bicarbonate of soda is based on the findings of Hawley and others that the amount of vitamin C excreted in the urine may vary according to the acid:alkali content of the diet, a highly alkaline urine having lower amounts of vitamin C than a highly acid urine. Codeine sulfate and aspirin were given by mouth. In adults the dose was codeine 0.5 grain, aspirin 10 grains given every six hours. Infants and children according to age. Some few patients complained of severe chest pain and some others of a constricting sensation that they described as cutting off their breath. These symptoms were relieved by employing either Numotizine as a plaster or the old-fashioned mustard plaster. The mustard plaster was made up with cold water and was applied cold for a period of about IS minutes. The proportions used were one part mustard and two parts flour. The amount of flour used in preparing the plaster for children was according to age but in no instance was the ratio greater than one to six. In childhood an expiratory grunt was taken as an index to use plasters. Oxygen inhalation was not employed even though cyanosis existed in twelve cases of the series; an additional injection of 500 mg. of vitamin C was given with almost spontaneous alleviation of the distressing condition. In two cases codeine sulfate was given in one grain amounts because of the weight of the patient. Diet was forced even though there was no desire to eat.

It is difficult to evaluate the role played by vitamin C against the virus organism. We have seen ascorbic acid give response in other types of virus infections but not sufficient evidence is on hand to state that it is a virus killer. It has been shown histologically that vitamin C regulates the intercellular substance of the capillary wall. In the human body its chief function is concerned with the formation of colloidal intercellular substances. The intercellular substances which appear to be regulated by vitamin C are of mesencyhmal origin—this means the collogen of all fibrous tissue structure, all non-epithelial cement substances including the intercellular substance of the capillary wall. Gothlin found increased capillary fragility in individuals with blood levels of 1 mg. of vitamin C per liter or less. It must be remembered too, however, that ascorbic acid has been reported to function as a respiratory catalyst, aiding cellular respiration by acting as a hydrogen transport.

Finally we consider the case of the liver in that the saturation of the blood plasma with vitamin C betters the detoxifying powers of this organ. It has been known that fever, toxemia and specific bacteria do act on the vitamin C concentration of the blood plasma with a lowering effect. Could it be that, by maintaining a high blood level of this vitamin, all body tissue is allowed to return to normal in spite of the existing fever and the presence of the specific organism, and that, acting as a respiratory catalyst, it enables the body to build up adequate resistance to the invader?

SUMMARY

Virus pneumonia is a true clinical entity. Although it gives symptoms similar to influenza in the early stage of illness the virus has not been identified. The onset is gradual and has an incubation period of five to fourteen days. The usual beginning is a hanging-on cold or generalized malaise. The chief symptoms, although not all are necessarily present each time, are chilly sensations or a single frank chill, followed with hot spells, burning in the nose, sore throat, hoarseness, bad taste in mouth, nausea, frontal headache, dry cough at first—later productive in the clearing phase of the disease—sweating, and this is usually profuse, normal pulse unless complicated with cerebral symptoms, pleurisy or a condition approximating lobar pneumonia when it will be rapid. Fever is from 100 to 104° F. The physical findings are inflammation of the turbihates and accessory nasal sinuses, hypertrophy of the lymphoid tissue on the posterior pharyngeal wall. Breath sounds are diminished and moist and dry rales are sometimes present. In extensive consolidation dullness to percussion, tubular breathing and pectoriloquy are found. The laboratory findings show the blood picture within normal limits; the sputum is negative. Sulfonamides and penicillin are good diagnostic aids since they have no effect on the disease. The x-ray findings can be anything from negative films through pneumonitis on to frank consolidation. Vitamin C in doses of 1000 mg. every six to twelve hours for three to seven injections has been specific in the experience of the author. X-ray in

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VIRUS PNEUMONIA—From P. 38

doses from 35 to 100r daily, or every second to third day, for not more than four exposures, aminophyllin and transfusions from convalescing or multiple donors have some usefulness as adjuvants in some cases.

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Health Benefits

In the eighteenth century, seasoned sailors found that by sucking on lemons they could avoid scurvy. When the lemon's key nutrient was formally identified in 1928, it was named ascorbic acid for its anti-scurvy, or antiscorbutic, action. Today ascorbic acid is widely known as Vitamin C. The health benefits of Vitamin C are abundant and varied, but it's probably best known as a cell protector, immunity booster, and powerful antioxidant. The body's ligaments, tendons, and collagen (a protein found in connective tissues) rely on the presence of Vitamin C to stay strong and healthy. Like all antioxidants, Vitamin C counters the effects of cell-damaging molecules called free radicals. As an added benefit, it even helps the body recycle other antioxidants. For certain conditions, Vitamin C is best taken with other antioxidants, such as Vitamin E, flavonoids, and carotenoids.

*These statements have not been evaluated by the Food and Drug Administration. This article is not intended to diagnose, treat, cure or prevent any disease.

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Format: Abstract

Allergy Asthma Clin Immunol. 2013 Nov 26;9(1):46. doi: 10.1186/1710-1492-9-46.

Vitamin C and common cold-induced asthma: a systematic review and statistical analysis.

Hemilä H[1].

Author information

Abstract

BACKGROUND: Asthma exacerbations are often induced by the common cold, which, in turn, can be alleviated by vitamin C.

OBJECTIVE: To investigate whether vitamin C administration influences common cold-induced asthma.

METHODS: Systematic review and statistical analysis of the identified trials. Medline, Scopus and Cochrane Central were searched for studies that give information on the effects of vitamin C on common cold-induced asthma. All clinically relevant outcomes related to asthma were included in this review. The estimates of vitamin C effect and their confidence intervals [CI] were calculated for the included studies.

RESULTS: Three studies that were relevant for examining the role of vitamin C on common cold-induced asthma were identified. The three studies had a total of 79 participants. Two studies were randomized double-blind placebo-controlled trials. A study in Nigeria on asthmatics whose asthma attacks were precipitated by respiratory infections found that 1 g/day vitamin C decreased the occurrence of asthma attacks by 78% (95% CI: 19% to 94%). A cross-over study in former East-Germany on patients who had infection-related asthma found that 5 g/day vitamin C decreased the proportion of participants who had bronchial hypersensitivity to histamine by 52 percentage points (95% CI: 25 to 71). The third study did not use a placebo. Administration of a single dose of 1 gram of vitamin C to Italian non-asthmatic common cold patients increased the provocative concentration of histamine (PC20) 3.2-fold (95% CI: 2.0 to 5.1), but the vitamin C effect was significantly less when the same participants did not suffer from the common cold.

CONCLUSIONS: The three reviewed studies differed substantially in their methods, settings and outcomes. Each of them found benefits from the administration of vitamin C; either against asthma attacks or against bronchial hypersensitivity, the latter of which is a characteristic of asthma. Given the evidence suggesting that vitamin C alleviates common cold symptoms and the findings of this systematic review, it may be reasonable for asthmatic patients to test vitamin C on an individual basis, if they have exacerbations of asthma caused by respiratory infections. More research on the role of vitamin C on common cold-induced asthma is needed.

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LETTER TO THE EDITOR | VOLUME 102, ISSUE 4, P625-626, APRIL 01, 2008

Vitamin C and sex differences in respiratory tract infections

Harri Hemilä

Open Archive • Published: January 29, 2008 • DOI: https://doi.org/10.1016/j.rmed.2007.12.011

In their systematic review of sex differences in respiratory tract infections (RTIs), Falagas et al. concluded that males develop RTIs more frequently than females, in particular lower RTIs, and the course of the infection is often more severe in males than in females.[1]

In 1997, I reported a meta-analysis of British trials on vitamin C and the common cold which gives a complementary viewpoint on sex differences in RTIs.[2] In four trials with males, vitamin C supplementation reduced common cold incidence by 30% (95% CI: −40% to −19%), but had no effect in four trials with females (estimate −5%; 95% CI: −14% to +4%). The divergence in the confidence intervals suggests different effects on males and females. Three studies reported data for both males and females and the largest of these, by Baird et al.,[3] found highly significant interaction between sex and vitamin C effect on common cold incidence (Table 1). The two smaller trials had wide confidence intervals that overlapped between males and females.[2] Furthermore, in four trials with British males, vitamin C reduced recurrent colds during the study period by 46% (−60% to −26%), but had no effect on females.[2] In particular, Tyrrell et al.[4] found that therapeutic vitamin C during the first cold episode reduced subsequent colds in males by 40% (−63% to −3%),[2] but not in females (−7%; −45% to +54%). The Baird et al.[3] and Tyrrell et al.[4] studies were randomised placebo-controlled double-blind trials and their findings cannot be dismissed on methodological grounds.

Table 1 Interaction between sex and the effect of vitamin C on common cold incidence in British students (Baird et al., 1979).[3]

	Vitamin C		Placebo		RR (95% CI)
	Participants	No. of colds	Participants	No. of colds	
Males	133	184	61	135	0.63 (0.50–0.78)
Females	105	199	51	78	1.24 (0.95–1.61)

These data are from Refs. 2 and 3. The statistical significance of interaction was calculated from the change in −2×log(likelihood) when the interaction term was added to the model (STATA program Poisson).

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Because large-scale trials give no evidence that high-dose vitamin C supplementation (⩾1 g/day) decreases common cold incidence,[2] the findings with British males call for special explanations. Several surveys had reported low dietary vitamin C intake in the UK and thus the benefit of supplementation may be explained by treating marginal deficiency.[2] This explanation is consistent with the estimated low daily vitamin C intake in Baird's study, 50 mg/day, and the particularly low dosage of vitamin C supplementation, 80 mg/day.[3] Usually plasma and leucocyte vitamin C concentrations are lower in males than in females although it is not clear to what extent this is due to dietary and physiological differences between the sexes.[2] Concluding from the British studies,[2, 3, 4] it seems that

sex differences in RTIs may be generated by variations in dietary vitamin C intakes, in addition to the factors mentioned by Falagas et al.[1]

Furthermore, in a recent Cochrane review we identified three prophylactic vitamin C trials and each of them reported an 80% or greater decrease in pneumonia incidence in the vitamin C group.[5] All these trials examined males only and the incidence of pneumonia was particularly high. The benefit of vitamin C supplementation seemed to be explained by marginal deficiency and by increased requirement caused by heavy exertion.[5]

It is obvious that the findings of the common cold trials with British males[2] and pneumonia trials with males[5] cannot be extrapolated to the general population of the western countries. Nevertheless, further vitamin C trials are warranted among males with low dietary vitamin C intake.

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Cochrane Database Syst Rev. 2007; (⟨http://dx.doi.org/10.1002/14651858.CD005532.pub2⟩): CD005532

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Tables

Table 1 : Interaction between sex and the effect of vitamin C on common cold incidence in British students (Baird et al., 1979).3

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Med J Aust. 1981 Oct 17;2(8):411-2.

Vitamin C and the common cold: using identical twins as controls.

Carr AB, Einstein R, Lai LY, Martin NG, Starmer GA.

Abstract

We analysed self-reported cold data for 95 pairs of identical twins who took part in a double-blind trial of vitamin C tablets. One member of each twin pair took vitamin C and the other took a well matched placebo each day for 100 days. Vitamin C had no significant effect except for shortening the average duration of cold episodes by 19%.

PMID: 7033746

[Indexed for MEDLINE]

Publication types, MeSH terms, Substance

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Format: Abstract

Cochrane Database Syst Rev. 2013 Jan 31;(1):CD000980. doi: 10.1002/14651858.CD000980.pub4.

Vitamin C for preventing and treating the common cold.

Hemilä H[1], Chalker E.

Author information

Abstract

BACKGROUND: Vitamin C (ascorbic acid) for preventing and treating the common cold has been a subject of controversy for 70 years.

OBJECTIVES: To find out whether vitamin C reduces the incidence, the duration or severity of the common cold when used either as a continuous regular supplementation every day or as a therapy at the onset of cold symptoms.

SEARCH METHODS: We searched CENTRAL 2012, Issue 11, MEDLINE (1966 to November week 3, 2012), EMBASE (1990 to November 2012), CINAHL (January 2010 to November 2012), LILACS (January 2010 to November 2012) and Web of Science (January 2010 to November 2012). We also searched the U.S. National Institutes of Health trials register and WHO ICTRP on 29 November 2012.

SELECTION CRITERIA: We excluded trials which used less than 0.2 g per day of vitamin C and trials without a placebo comparison. We restricted our review to placebo-controlled trials.

DATA COLLECTION AND ANALYSIS: Two review authors independently extracted data. We assessed 'incidence' of colds during regular supplementation as the proportion of participants experiencing one or more colds during the study period. 'Duration' was the mean number of days of illness of cold episodes.

MAIN RESULTS: Twenty-nine trial comparisons involving 11,306 participants contributed to the meta-analysis on the risk ratio (RR) of developing a cold whilst taking vitamin C regularly over the study period. In the general community trials involving 10,708 participants, the pooled RR was 0.97 (95% confidence interval (CI) 0.94 to 1.00). Five trials involving a total of 598 marathon runners, skiers and soldiers on subarctic exercises yielded a pooled RR of 0.48 (95% CI 0.35 to 0.64).Thirty-one comparisons examined the effect of regular vitamin C on common cold duration (9745 episodes). In adults the duration of colds was reduced by 8% (3% to 12%) and in children

by 14% (7% to 21%). In children, 1 to 2 g/day vitamin C shortened colds by 18%. The severity of colds was also reduced by regular vitamin C administration.Seven comparisons examined the effect of therapeutic vitamin C (3249 episodes). No consistent effect of vitamin C was seen on the duration or severity of colds in the therapeutic trials.The majority of included trials were randomised, double-blind trials. The exclusion of trials that were either not randomised or not double-blind had no effect on the conclusions.

AUTHORS' CONCLUSIONS: The failure of vitamin C supplementation to reduce the incidence of colds in the general population indicates that routine vitamin C supplementation is not justified, yet vitamin C may be useful for people exposed to brief periods of severe physical exercise. Regular supplementation trials have shown that vitamin C reduces the duration of colds, but this was not replicated in the few therapeutic trials that have been carried out. Nevertheless, given the consistent effect of vitamin C on the duration and severity of colds in the regular supplementation studies, and the low cost and safety, it may be worthwhile for common cold patients to test on an individual basis whether therapeutic vitamin C is beneficial for them. Further therapeutic RCTs are warranted.

Update of

Vitamin C for preventing and treating the common cold. [Cochrane Database Syst Rev. 2007]

PMID: 23440782 DOI: 10.1002/14651858.CD000980.pub4

[Indexed for MEDLINE]

Publication types, MeSH terms, Substance

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Format: Abstract

Cochrane Database Syst Rev. 2013 Aug 8;(8):CD005532. doi: 10.1002/14651858.CD005532.pub3.

Vitamin C for preventing and treating pneumonia.

Hemilä H[1], Louhiala P.

Author information

Abstract

BACKGROUND: Pneumonia is one of the most common serious infections, causing two million deaths annually among young children in low-income countries. In high-income countries pneumonia is most significantly a problem of the elderly.

OBJECTIVES: To assess the prophylactic and therapeutic effects of vitamin C on pneumonia.

SEARCH METHODS: We searched CENTRAL 2013, Issue 3, MEDLINE (1950 to March week 4, 2013), EMBASE (1974 to April 2013) and Web of Science (1955 to April 2013).

SELECTION CRITERIA: To assess the therapeutic effects of vitamin C, we selected placebo-controlled trials. To assess prophylactic effects, we selected controlled trials with or without a placebo.

DATA COLLECTION AND ANALYSIS: Two review authors independently read the trial reports and extracted data.

MAIN RESULTS: We identified three prophylactic trials which recorded 37 cases of community-acquired pneumonia in 2335 people. Only one was satisfactorily randomised, double-blind and placebo-controlled. Two trials examined military recruits and the third studied boys from "lower wage-earning classes" attending a boarding school in the UK during World War II. Each of these three trials found a statistically significant (80% or

greater) reduction in pneumonia incidence in the vitamin C group. We identified two therapeutic trials involving 197 community-acquired pneumonia patients. Only one was satisfactorily randomised, double-blind and placebo-controlled. That trial studied elderly patients in the UK and found lower mortality and reduced severity in the vitamin C group; however, the benefit was restricted to the most ill patients. The other therapeutic trial studied adults with a wide age range in the former Soviet Union and found a dose-dependent reduction in the duration of pneumonia with two vitamin C doses. We identified one prophylactic trial recording 13 cases of hospital-acquired pneumonia in 37 severely burned patients; one-day administration of vitamin C had no effect on pneumonia incidence. The identified studies are clinically heterogeneous which limits their comparability. The included studies did not find adverse effects of vitamin C.

AUTHORS' CONCLUSIONS: The prophylactic use of vitamin C to prevent pneumonia should be further investigated in populations who have a high incidence of pneumonia, especially if dietary vitamin C intake is low. Similarly, the therapeutic effects of vitamin C should be studied, especially in patients with low plasma vitamin C levels. The current evidence is too weak to advocate prophylactic use of vitamin C to prevent pneumonia in the general population. Nevertheless, therapeutic vitamin C supplementation may be reasonable for pneumonia patients who have low vitamin C plasma levels because its cost and risks are low.

Update of

Vitamin C for preventing and treating pneumonia. [Cochrane Database Syst Rev. 2007]

PMID: 23925826 DOI: 10.1002/14651858.CD005532.pub3

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Publication types, MeSH terms, Substances

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Clinical Trial J Infect Dis, 173 (6), 1502-5 Jun 1996

Vitamin C for the Treatment of Recurrent Furunculosis in Patients With Imparied Neutrophil Functions

R Levy [1], O Shriker, A Porath, K Riesenberg, F Schlaeffer

Affiliations

PMID: 8648230 DOI: 10.1093/infdis/173.6.1502

Abstract

The effect of vitamin C treatment on 23 patients with a history of recurrent furunculosis with negative nasal cultures was studied. Neutrophil functions (chemotaxis, phagocytosis, or superoxide generation) of 12 patients were significantly lower than those of the matched controls. In this group, treatment with vitamin C (1 g/day) caused a dramatic clinical response as well as a significant improvement of neutrophil functions, reaching values similar to those of the controls. Two patients remained vitamin C-dependent. In the patients with normal neutrophil functions, vitamin C treatment neither affected neutrophil activity nor caused a clinical response. Therefore, patients suffering from recurrent furunculosis with defective neutrophil functions may be treated successfully with vitamin C, contributing to both neutrophil function recovery and a dramatic clinical response.

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Proc Natl Acad Sci U S A. 1996 Apr 16; 93(8): 3704–3709.
doi: 10.1073/pnas.93.8.3704
PMCID: PMC39676
PMID: 8623000

Vitamin C pharmacokinetics in healthy volunteers: evidence for a recommended dietary allowance.

M Levine, C Conry-Cantilena, Y Wang, R W Welch, P W Washko, K R Dhariwal, J B Park, A Lazarev, J F Graumlich, J King, and L R Cantilena

National Institute of Diabetes and Digestive and Kidney Diseases, National Institutes of Health, Bethesda, MD 20892-1372, USA.

Abstract

Determinants of the recommended dietary allowance (RDA) for vitamin C include the relationship between vitamin C dose and steady-state plasma concentration, bioavailability, urinary excretion, cell concentration, and potential adverse effects. Because current data are inadequate, an in-hospital depletion-repletion study was conducted. Seven healthy volunteers were hospitalized for 4-6 months and consumed a diet containing <5 mg of vitamin C daily. Steady-state plasma and tissue concentrations were determined at seven daily doses of vitamin C from 30 to 2500 mg. Vitamin C steady-state plasma concentrations as a function of dose displayed sigmoid kinetics. The steep portion of the curve occurred between the 30- and 100-mg daily dose, the current RDA of 60 mg daily was on the lower third of the curve, the first dose beyond the sigmoid portion of the curve was 200 mg daily, and complete plasma saturation occurred at 1000 mg daily. Neutrophils, monocytes, and lymphocytes saturated at 100 mg daily and contained concentrations at least 14-fold higher than plasma. Bioavailability was complete for 200 mg of vitamin C as a single dose. No vitamin C was excreted in urine of six of seven volunteers until the 100-mg dose. At single doses of 500 mg and higher, bioavailability declined and the absorbed amount was excreted. Oxalate and urate excretion were elevated at 1000 mg of vitamin C daily compared to lower doses. Based on these data and Institute of Medicine criteria, the current RDA of 60 mg daily should be increased to 200 mg daily, which can be obtained from fruits and vegetables. Safe doses of vitamin C are less than 1000 mg daily, and vitamin C daily doses above 400 mg have no evident value.

Full text

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Am J Clin Nutr. 1993 Feb;57(2):170-4.

Vitamin C supplementation reduces the incidence of postrace symptoms of upper-respiratory-tract infection in ultramarathon runners.

Peters EM[1], Goetzsche JM, Grobbelaar B, Noakes TD.

Author information

Abstract

This study determined whether daily supplementation with 600 mg vitamin C would reduce the incidence of symptoms of upper-respiratory-tract (URT) infections after participation in a competitive ultramarathon race (> 42 km). Ultramarathon runners with age-matched controls were randomly divided into placebo and experimental (vitamin C-supplemented) groups. Symptoms of URT infections were monitored for 14 d after the race. Sixty-eight percent of the runners in the placebo group reported the development of symptoms of URT infection after the race; this was significantly more ($P < 0.01$) than that reported by the vitamin C-supplemented group (33%). The duration and severity of symptoms of URT infections reported in the vitamin C-supplemented nonrunning control group was also significantly less than in the nonrunning control group receiving the placebo ($P < 0.05$). This study provides evidence that vitamin C supplementation may enhance resistance to the postrace URT infections that occur commonly in competitive ultramarathon runners and may reduce the severity of such infections in those who are sedentary.

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Prophylactic vitamin C: misplaced zeal. [Am J Clin Nutr. 1994]

PMID: 8185726 DOI: 10.1093/ajcn/57.2.170

[Indexed for MEDLINE]

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Nutrients. 2014 Jul 9;6(7):2572-83. doi: 10.3390/nu6072572.

Vitamin C supplementation slightly improves physical activity levels and reduces cold incidence in men with marginal vitamin C status: a randomized controlled trial.

Johnston CS[1], Barkyoumb GM[2], Schumacher SS[3].

Author information

Abstract

The early indications of vitamin C deficiency are unremarkable (fatigue, malaise, depression) and may manifest as a reduced desire to be physically active; moreover, hypovitaminosis C may be associated with increased cold duration and severity. This study examined the impact of vitamin C on physical activity and respiratory tract infections during the peak of the cold season. Healthy non-smoking adult men (18-35 years; BMI < 34 kg/m2; plasma vitamin C < 45 μmol/L) received either 1000 mg of vitamin C daily (n = 15) or placebo (n = 13) in a randomized, double-blind, eight-week trial. All participants completed the Wisconsin Upper Respiratory Symptom Survey-21 daily and the Godin Leisure-Time Exercise Questionnaire weekly. In the final two weeks of the trial, the physical activity score rose modestly for the vitamin C group vs. placebo after adjusting for baseline values: +39.6% (95% CI [-4.5,83.7]; p = 0.10). The number of participants reporting cold episodes was 7 and 11 for the vitamin C and placebo groups respectively during the eight-week trial (RR = 0.55; 95% CI [0.33,0.94]; p = 0.04) and cold duration was reduced 59% in the vitamin C versus placebo groups (-3.2 days; 95% CI [-7.0,0.6]; p = 0.06). These data suggest measurable health advantages associated with vitamin C supplementation in a population with adequate-to-low vitamin C status.

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Medical Hypotheses
Volume 7, Issue 11, November 1981, Pages 1359-1376

Vitamin C, TITRATING TO BOWEL TOLERANCE, ANASCORBEMIA, and ACUTE INDUCED SCURVY

Robert F. Cathcart

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https://doi.org/10.1016/0306-9877(81)90126-2

Abstract

A method of utilizing vitamin C in amouts just short of the doses which produce diarrhea is described (TITRATING TO BOWEL TOLERANCE). The amount of oral ascorbic acid tolerated by a patient without producing diarrhea increases somewhat proportionately to the stress or toxicity of his disease. Bowel tolerance doses of ascorbic acid ameliorate the acute symptoms of many diseases. Lesser doses often have little effect on acute symptoms but assist the body in handling the stress of disease and may reduce the morbidity of the disease. However, if doses of ascorbate are not provided to satisfy this potential draw on the nutrient, first local tissues involved in the disease, then the blood, and then the body in general become deplete of ascorbate (ANASCORBEMIA and ACUTE INDUCED SCURVY). The patient is thereby put at risk for complications of metabolic processes known to be dependent upon ascorbate.

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Vitamin E and the risk of pneumonia: using the I^2 statistic to quantify heterogeneity within a controlled trial

Harri Hemilä (a1)

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Abstract

Analyses in nutritional epidemiology usually assume a uniform effect of a nutrient. Previously, four subgroups of the Alpha-Tocopherol, Beta-Carotene Cancer Prevention (ATBC) Study of Finnish male smokers aged 50–69 years were identified in which vitamin E supplementation either significantly increased or decreased the risk of pneumonia. The purpose of this present study was to quantify the level of true heterogeneity in the effect of vitamin E on pneumonia incidence using the I^2 statistic. The I^2 value estimates the percentage of total variation across studies that is explained by true differences in the treatment effect rather than by chance, with a range from 0 to 100 %. The I^2 statistic for the effect of vitamin E supplementation on pneumonia risk for five subgroups of the ATBC population was 89 % (95 % CI 78, 95 %), indicating that essentially all heterogeneity was true variation in vitamin E effect instead of chance variation. The I^2 statistic for heterogeneity in vitamin E effects on pneumonia risk was 92 % (95 % CI 80, 97 %) for three other ATBC subgroups defined by smoking level and leisure-time exercise level. Vitamin E decreased pneumonia risk by 69 % among participants who had the least exposure to smoking and exercised during leisure time (7·6 % of the ATBC participants), and vitamin E increased pneumonia risk

by 68 % among those who had the highest exposure to smoking and did not exercise (22 % of the ATBC participants). These findings refute there being a uniform effect of vitamin E supplementation on the risk of pneumonia.

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Vitamin E Supplementation May Transiently Increase Tuberculosis Risk in Males Who Smoke Heavily and Have High Dietary Vitamin C Intake

Harri Hemilä [1], Jaakko Kaprio

Affiliations

PMID: 18279551 DOI: 10.1017/S0007114508923709

Abstract

Vitamin E and beta-carotene affect the immune function and might influence the predisposition of man to infections. To examine whether vitamin E or beta-carotene supplementation affects tuberculosis risk, we analysed data of the Alpha-Tocopherol Beta-Carotene Cancer Prevention (ATBC)Study, a randomised controlled trial which examined the effects of vitamin E (50 mg/d) and beta-carotene (20 mg/d) on lung cancer. The trial was conducted in the general community in Finland in 1985-93; the intervention lasted for 6.1 years (median). The ATBC Study cohort consists of 29,023 males aged 50-69 years, smoking at baseline, with no tuberculosis diagnosis prior to randomisation. Vitamin E supplementation had no overall effect on the incidence of tuberculosis (risk ratio (RR) = 1.18; 95% CI 0.87, 1.59) nor had beta-carotene (RR = 1.07; 95% CI 0.80, 1.45). Nevertheless, dietary vitamin C intake significantly modified the vitamin E effect. Among participants who obtained 90 mg/d or more of vitamin Cin foods (n 13,502), vitamin E supplementation increased tuberculosis risk by 72 (95% CI 4, 185)%. This effect was restricted to participants who smoked heavily. Finally, in participants not supplemented with vitamin E, dietary vitamin C had a negative association with tuberculosis risk so that the adjusted risk was 60 (95% CI 16, 81)% lower in the highest intake quartile compared with the lowest. Our finding that vitamin E seemed to transiently increase the risk of tuberculosis in those who smoked heavily and had high dietary vitamin C intake should increase caution towards vitamin E supplementation for improving the immune system.

Comment in

Vitamin E supplementation may transiently increase tuberculosis risk in males who smoke heavily and have high dietary vitamin C intake--comments by Hernández-Garduño.
Hernández-Garduño E. Hernández-Garduño E. Br J Nutr. 2009 Jan;101(1):145; discussion 146-7. doi: 10.1017/S0007114508994411. Epub 2008 Jun 23. Br J Nutr. 2009. PMID: 18570687 No abstract available.

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Am J Epidemiol, 154 (12), 1113-8 2001 Dec 15

Which Plasma Antioxidants Are Most Related to Fruit and Vegetable Consumption?

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Abstract

Substantial evidence suggests that fruit and vegetable intake reduces the risk of some cancers and other chronic diseases. While a varied diet containing fruits and vegetables may confer benefits greater than those of any single nutrient, it would be useful to have data on the plasma nutrients most influenced by fruit and vegetable intake. The authors examined the correlation between fruit and vegetable intake as measured by the abbreviated CLUE II food frequency questionnaire and several plasma antioxidants. This study includes 116 male subjects aged 35-72 years who were nonsmokers and nonusers of vitamin supplements and who provided blood samples in the CLUE II Study in Washington County, Maryland. Plasma was assayed for ascorbic acid, beta-carotene, beta-cryptoxanthin, and alpha- and gamma-tocopherol. Lipid- and energy-adjusted partial correlation for the relation with fruit and vegetable intake was r = 0.64 for ascorbic acid, r = 0.44 for beta-carotene, and r = 0.50 for beta-cryptoxanthin. While this study does not address efficacy, the stronger association of ascorbic acid with fruit and vegetable intake seen here may imply that ascorbic acid is an important component of the protective effect seen for fruits and vegetables in numerous epidemiologic studies.

Can Med Assoc J. 1975 Apr 5; 112(7): 823–826. PMCID: PMC1958969
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Winter illness and vitamin C: the effect of relatively low doses.

T. W. Anderson, G. H. Beaton, P. Corey, and L. Spero

Abstract

After their random -llocation to one of three treatment aroups, 622 volunteers received either vitamin C or placebo in a maintenance dose of 500 mg once weekly and a therapeutic dose of 1500 mg daily on the 1st day and 1000 mg on the next 4 days of any illness. Two forms of vitamin C were employed: a sustained-release capsule containing ascorbic acid and a regular tabet containing a mixture of sodium and calcium ascorbate. In the 448 subjects who completed an average of 15 weeks in the study of total of 635 episodes of illness were recroded. Respiratory symptoms were recorded on at least 1 day in 92 per cent of these episodes. There were no consistent or significant differences in the sickness experience of the subjects receiving the sustained-release vitamin capsules compared to those receiving the vitamin tablets, but subjects in both vitamin groups experienced less severe illness than subjects in the placebo group, with approximately 25 per cent fewer days spent indoors because of the illness (P smaller than 0.05). These results are compatible with the belief that supplementary vitamin C can reduce the burden of winter illness, but the intake need not be as high as has sometimes been claimed.

Full text

Full text is available as a scanned copy of the original print version. Get a printable copy (PDF file) of the **complete article** (864K), or click on a page image below to browse page by page. Links to PubMed are also available for **Selected References**.


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