Vitamin C and Cancer: Medicine or Politics?

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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.

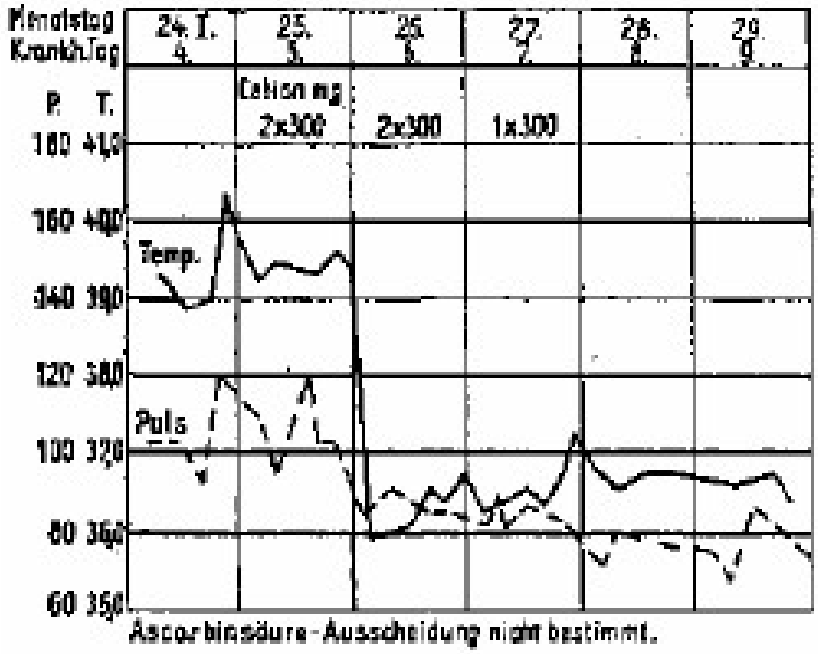

Figure 1

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

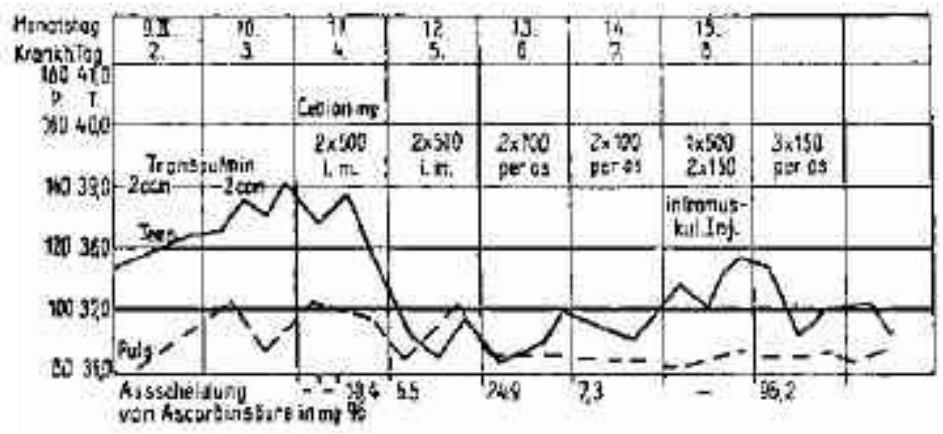

Figure 2 shows the course of fibrinous pneumonia in an 86-year-old female patient following treatment with Cebion. Critical defervescence took place after the appearance of ascorbic acid in the urine. When the excretion became negative again — because the need for ascorbic acid was apparently not being 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.

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Vitamin C and the common cold: a retrospective analysis of Chalmers' review.

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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

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Vitamin C intake and susceptibility to pneumonia

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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.

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s About Micronutrient Supplements in American Academic Medicine

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niversity of Texas Medical Branch at Galveston

M R Tangum

20th century American academic medicine has resisted the concept that supplementation with
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belief that vitamin C supplements cause kidney stones; (2) by the angry, scornful tone used in
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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

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 micronut 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 an be presumed to lished opinions a sample how m changes over tim

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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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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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Antioxidants in HIV positive children

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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Plasma vitamin C assays: a European experience. EC FLAIR Concerted Action No. 10: Micronutrient Meas...

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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enous iron therapy in a rat model of heart failure with preserved systemic iron status but depleted intracellular cardiac stores

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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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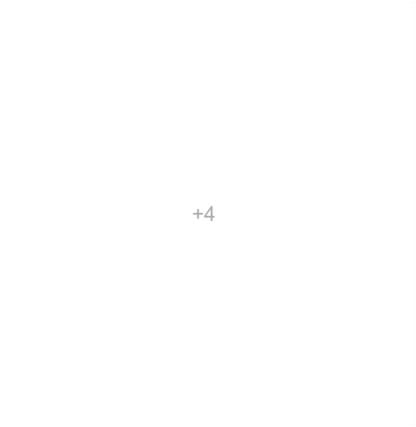
The effect of intravenous iron (Fe) supplementation on echocardiographic…



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

**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 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 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

[1]Department of Clinical Physiology, Centre of Postgraduate Medical Education, Warsaw, Poland. [2]C Radiobiology and Biological Dosimetry, Institute of Nuclear Chemistry and Technology, Warsaw, Poland. [3]La of Nuclear Analytical Methods, Institute of Nuclear Chemistry and Technology, Warsaw, Poland. [4]Depar Molecular Biology and Translational Research, Institute of Rural Health, Lublin, Poland. [5]Department o Biology and Translational Research, Faculty of Medicine, University of Information Technology and Mana Rzeszów, Poland. [6]Heart Failure and Transplantology Department, Institute of Cardiology, Warsaw, Polanc Mackiewicz and Michał Mączewski contributed equally. Correspondence and requests for materials s addressed to M.M. (email: michal.maczewski@cmkp.edu.pl)

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

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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.

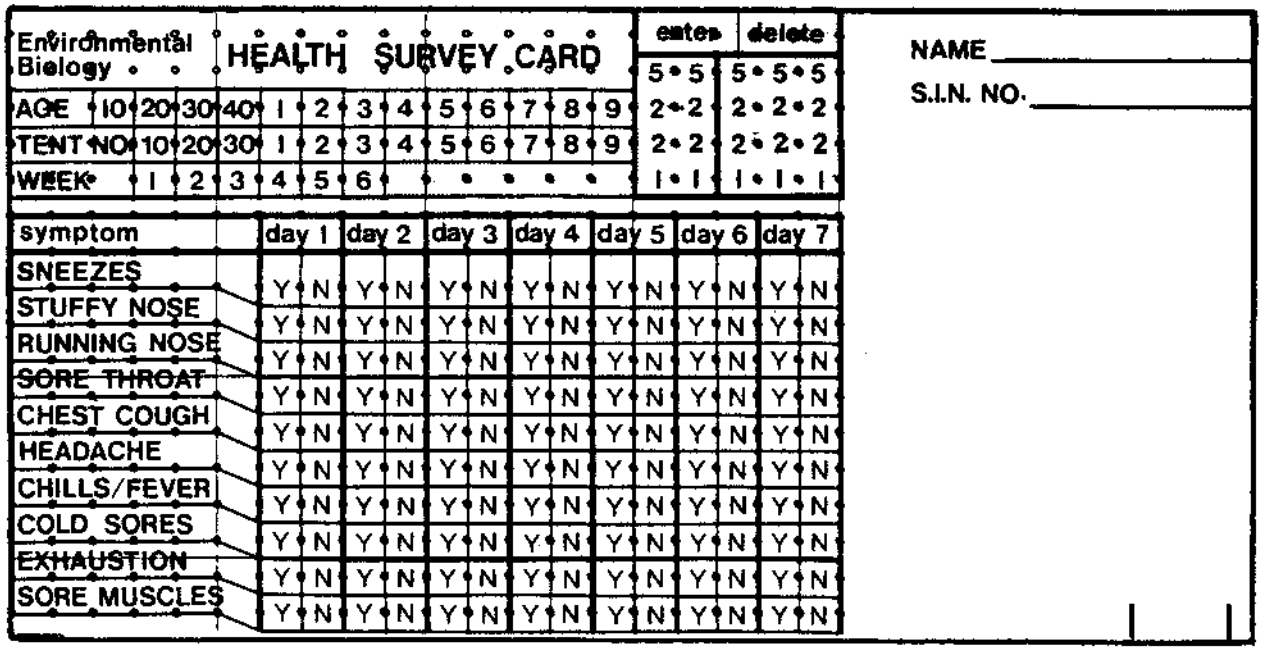
TABLE 2

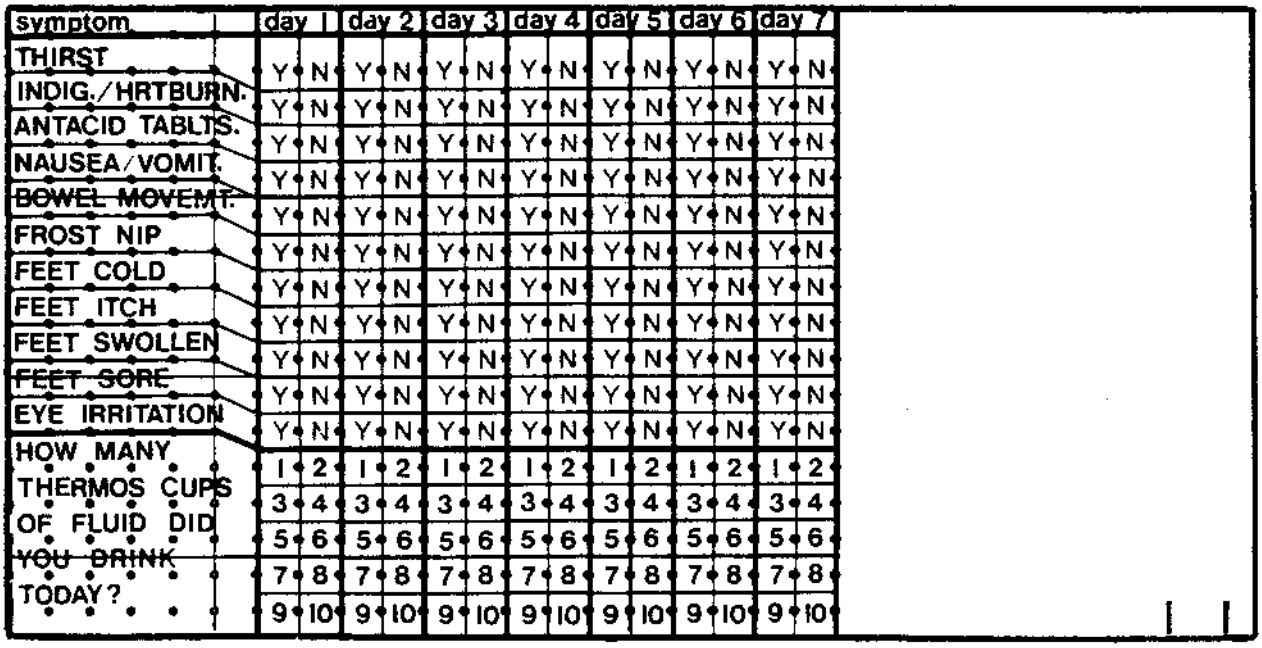
NORTHERN EXERCISES UTILIZED FOR THE INVESTIGATION OF HEALTH PROBLEMS, 1972-73

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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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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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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JAMA. 1975 Mar 10;231(10):1038-42.

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.

PMID: 163386

[Indexed for MEDLINE]

LinkOut - more resources

Format: Abstract

Int J Sports Med. 1996 Jul;17(5):379-83.

Vitamin C and common cold incidence: a review of studies with subjects under heavy physical stress.

Hemilä H[1].

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.

PMID: 8858411 DOI: 10.1055/s-2007-972864

[Indexed for MEDLINE]

Publication type, MeSH terms, Substance

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Br J Prev Soc Med. 1977 Sep; 31(3): 189–191. PMCID: PMC479021
doi: 10.1136/jech.31.3.189 PMID: 338079

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

Full text is available as a scanned copy of the original print version. Get a printable copy (PDF file) of the **complete article** (487K), 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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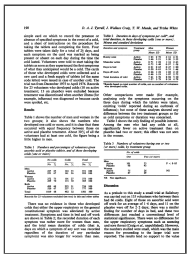

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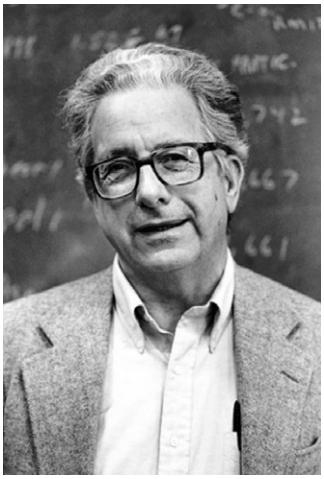
Paul Meier

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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Proc Natl Acad Sci U S A. 1997 Dec 9;94(25):13816-9.

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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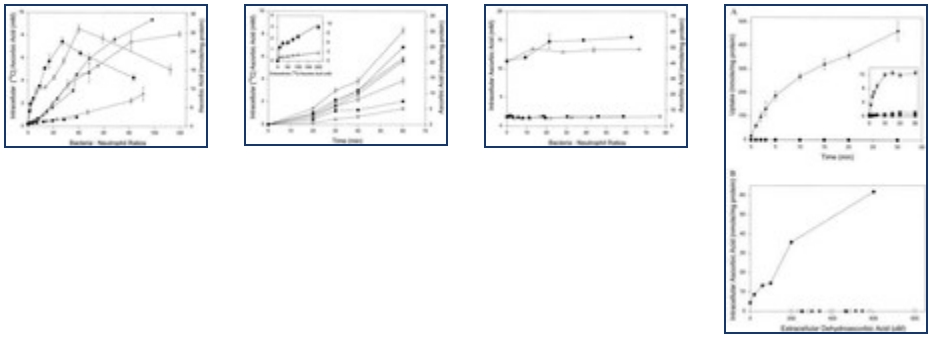

Publication types, MeSH terms, Substance

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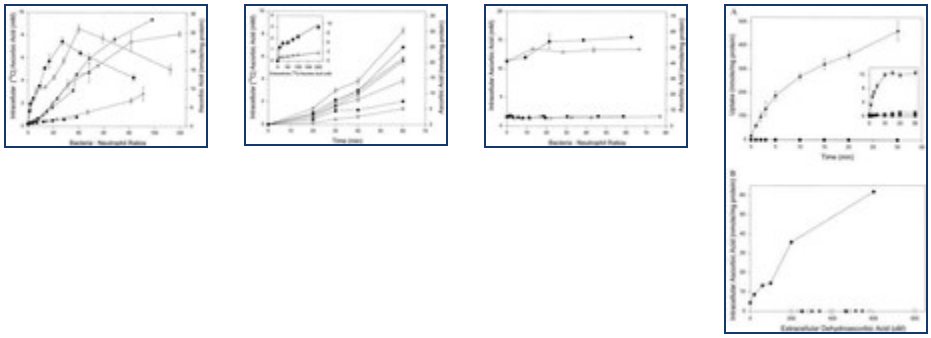

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Ascorbic acid and the common cold

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.

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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.

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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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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.

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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

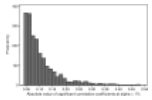

Figure 1. Histogram of Statistically Significant (at...

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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)

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[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.

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

Format: Abstract

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

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The Effect of Vitamin E on Common Cold Incidence Is Modified by Age, Smoking and Residential Neighborhood

Harri Hemilä, Jarmo Virtamo, Demetrius Albanes and Jaakko Kaprio

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http://www.ncbi.nlm.nih.gov/pubmed/16943455
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).

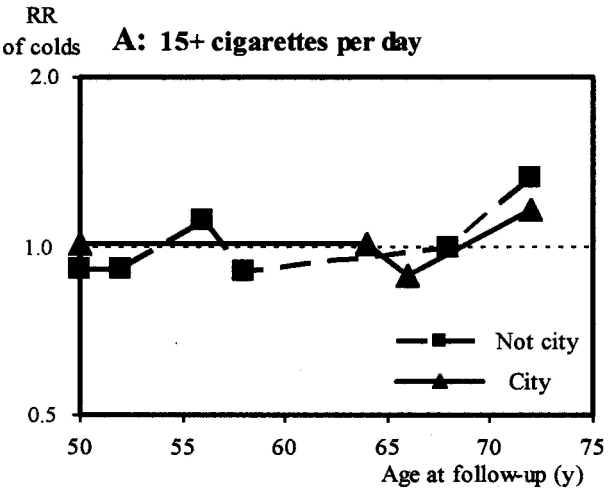
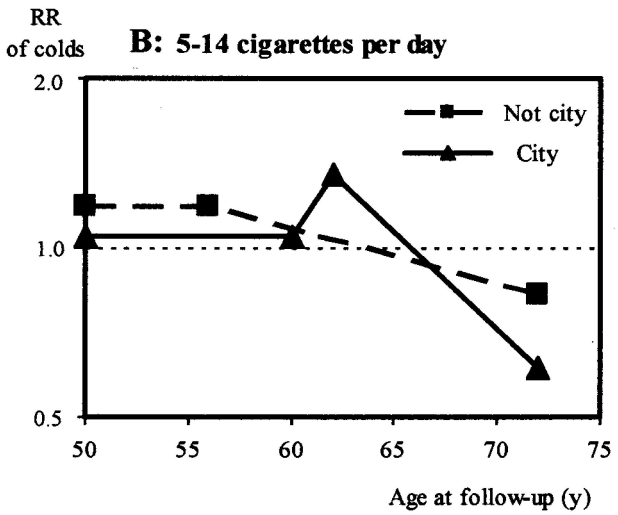


Fig. 1. The effect of vitamin E on the relative risk of common cold as a function of age at follow-up. Participants smoking more (A) and less (B) are further divided into subgroups by residential neighborhood. RR indicates the relative risk of colds between the vitamin E and placebo arms. See Table 2 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.

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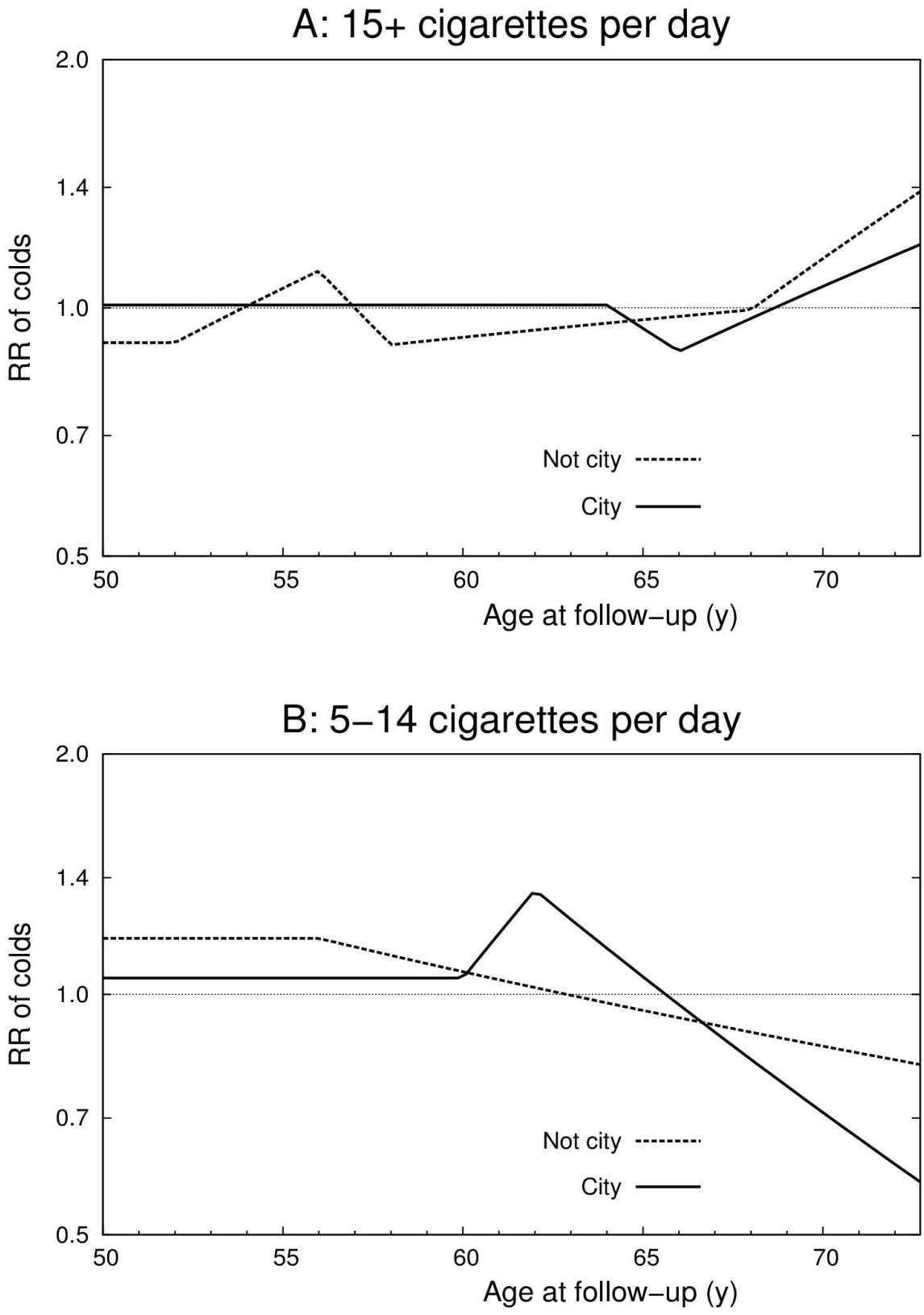

Fig. 1. The effect of vitamin E on the relative risk of common cold as a function of age at follow-up. Participants smoking more (A) and less (B) are further divided into subgroups by residential neighborhood. RR indicates the relative risk of colds between the vitamin E and placebo arms. See Table 2 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]

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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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.

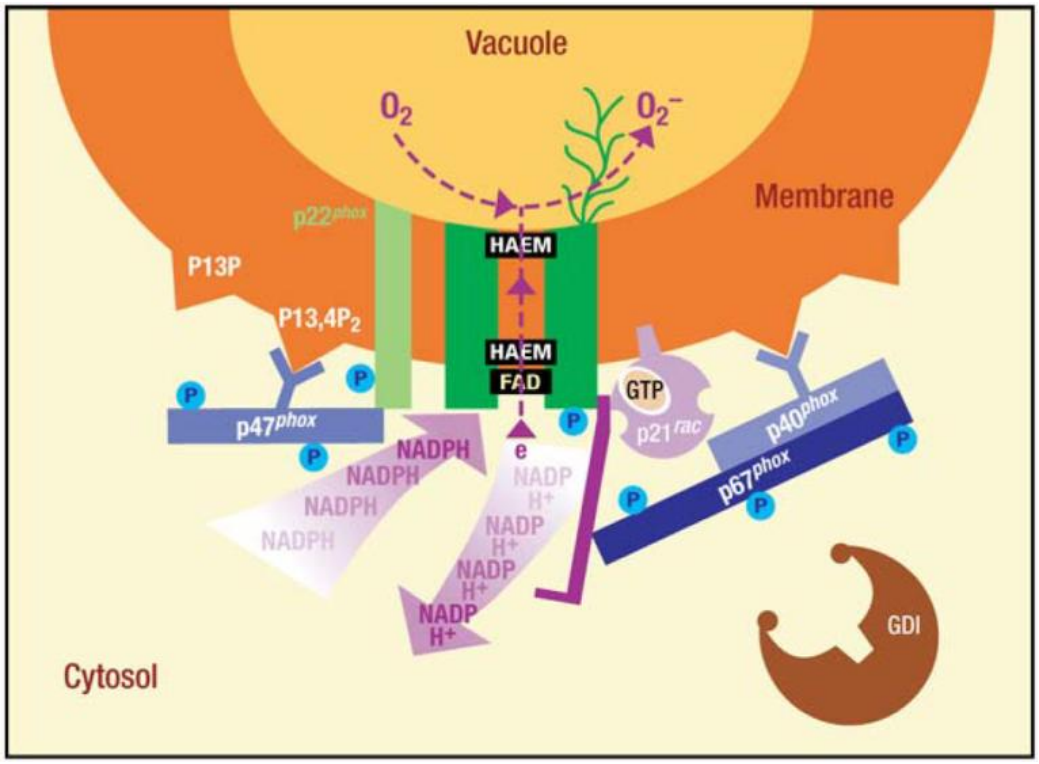

Figure 2

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).

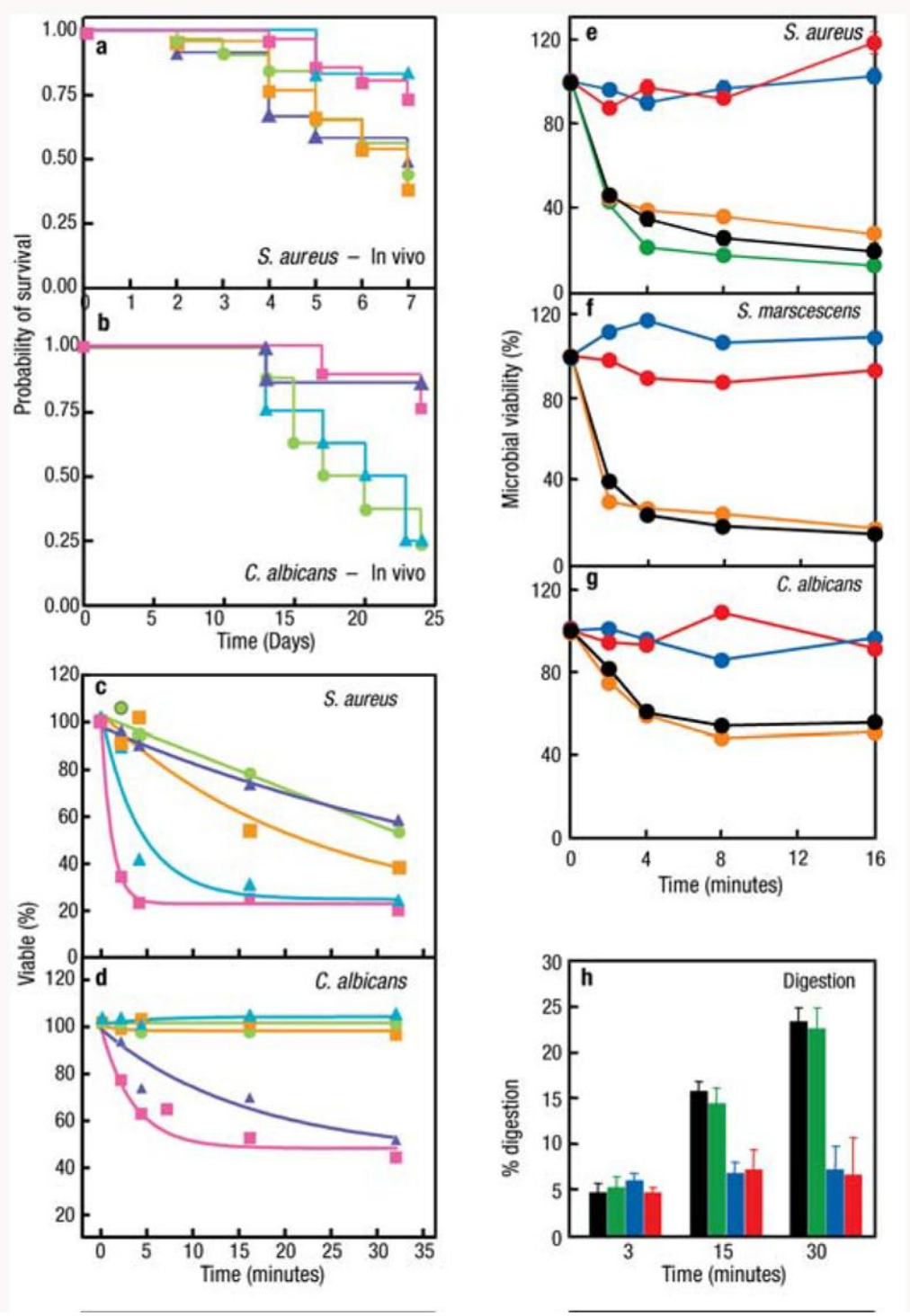

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Figure 3

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).

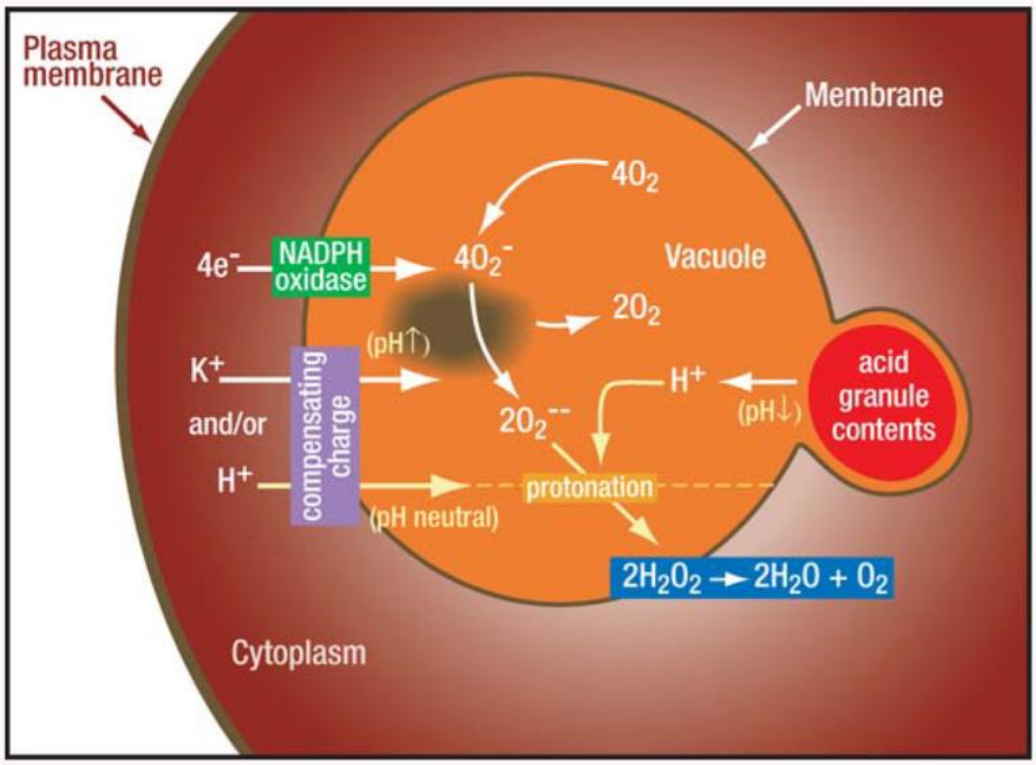

Figure 4

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.

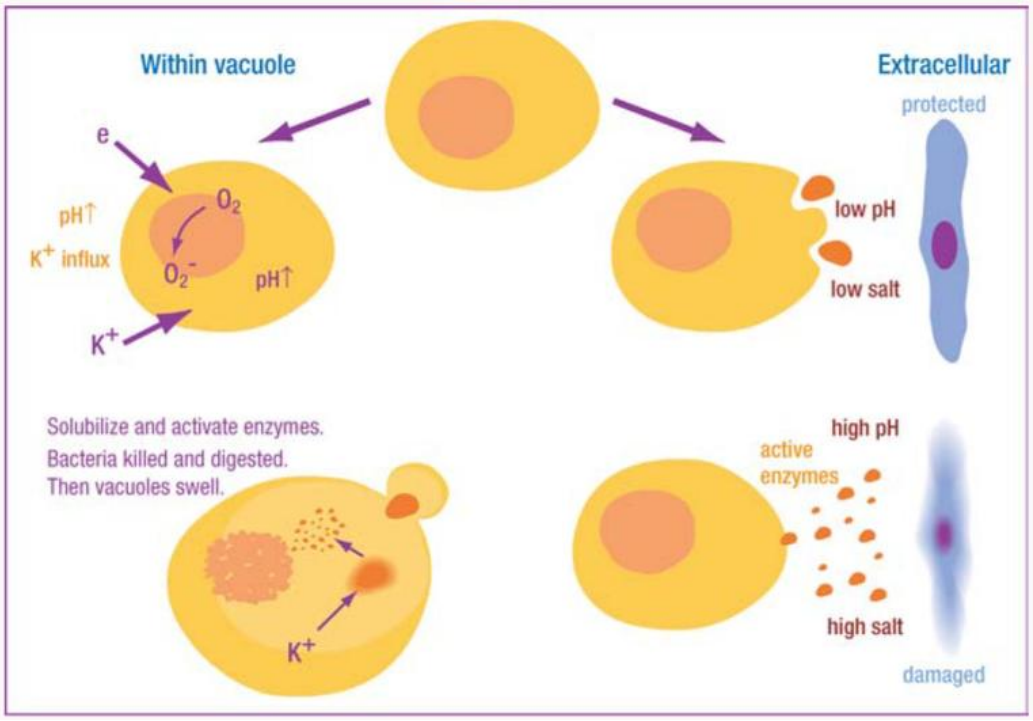

Figure 5

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 0_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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R. Eccles, "Is the Common Cold a Clinical Entity or a Cultural Concept?" Rhinology, Vol. 51, No. 1, 2013, pp. 3-8.

has been cited by the following article:

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.

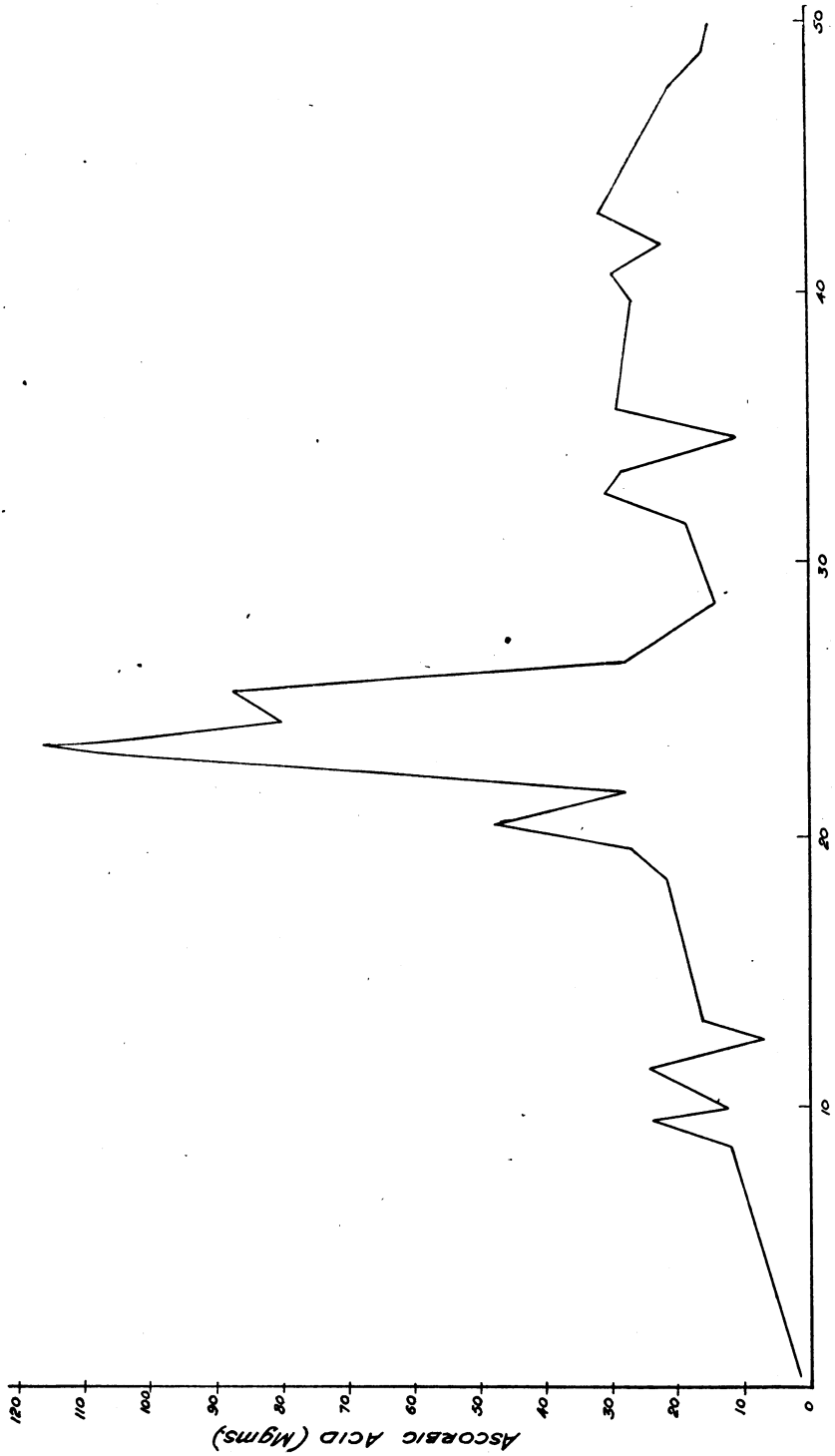

Fig. 1. Daily output of vitamin C in the urine. Group 1.

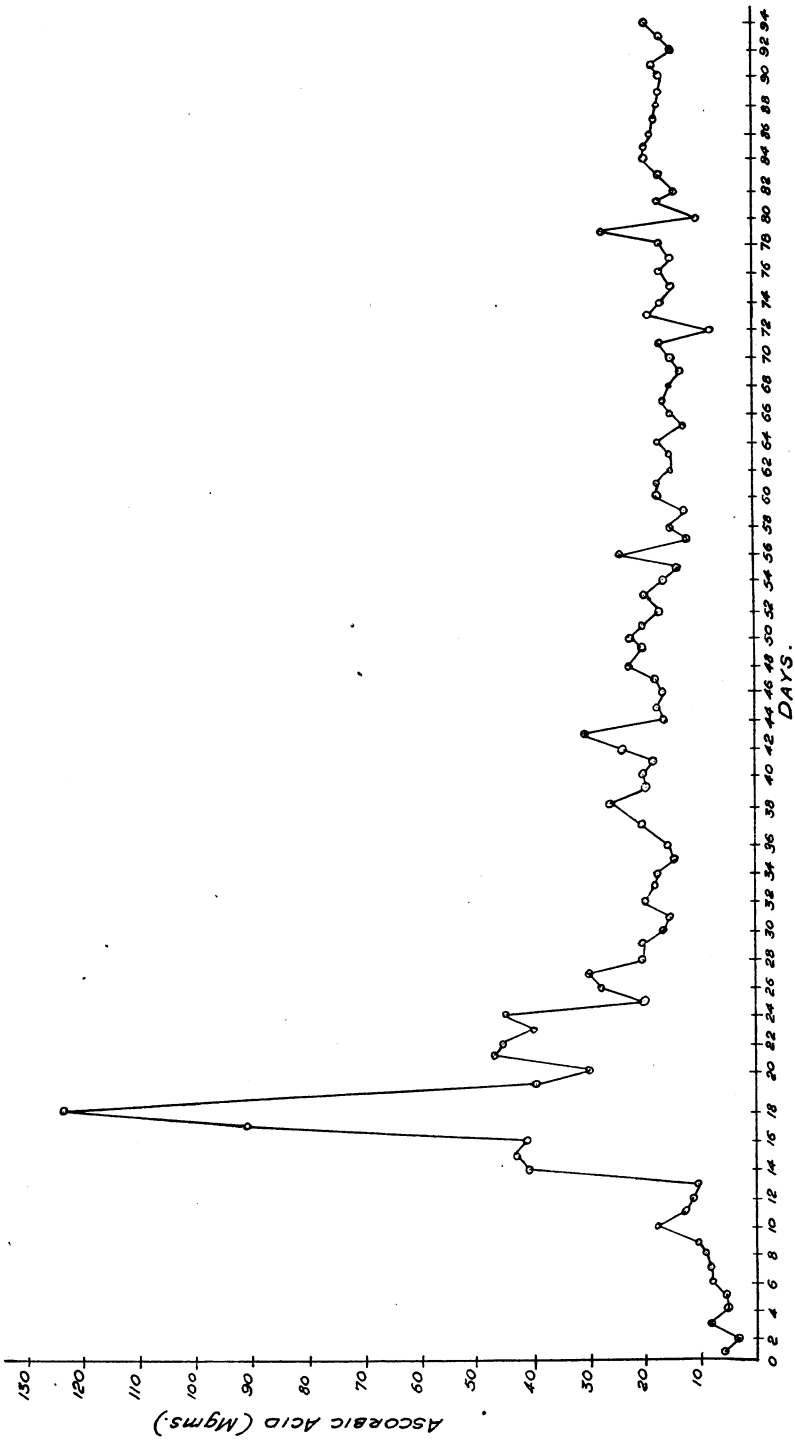

Fig. 2. Daily output of vitamin C in the urine. Group 2.

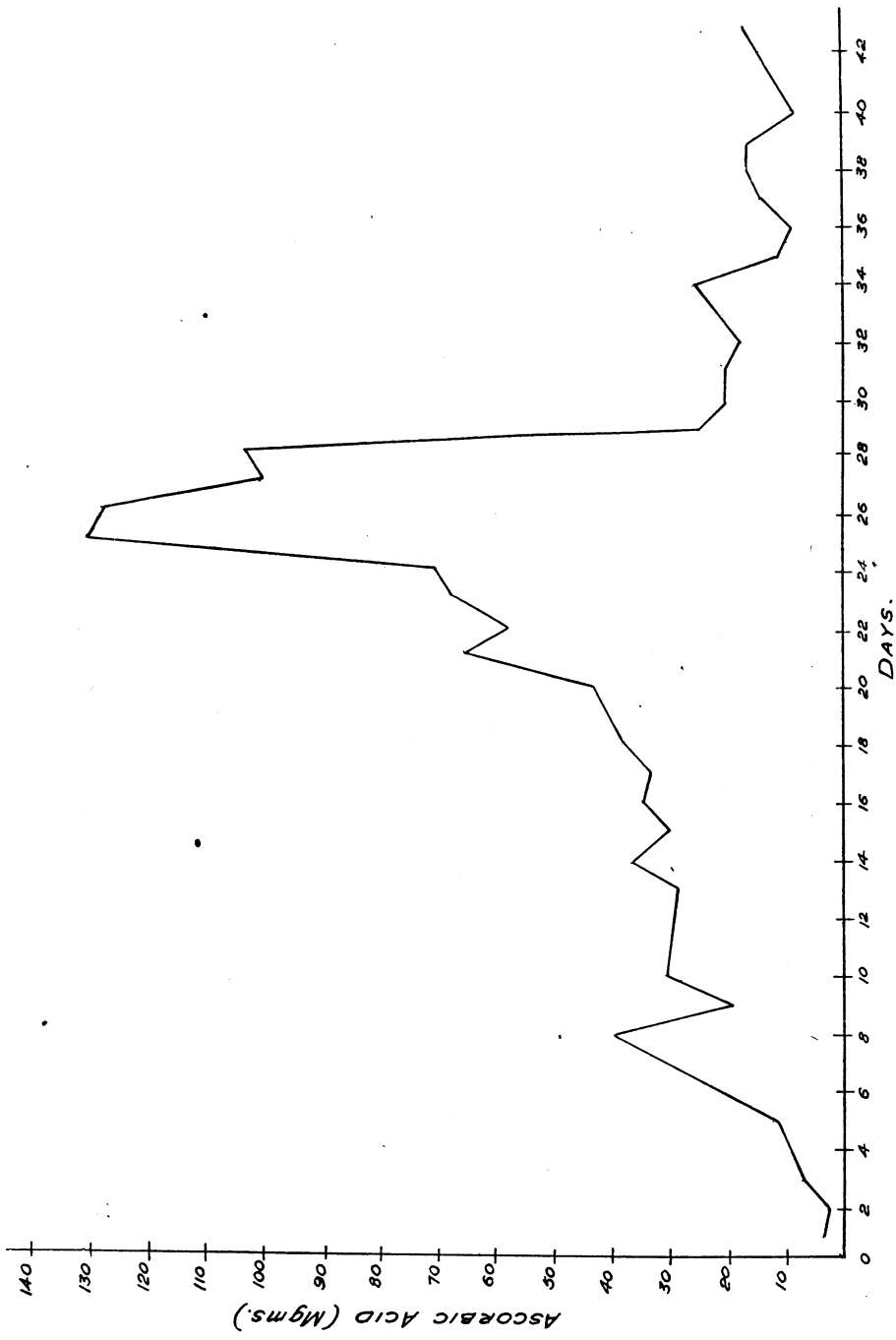

Fig. 3. Daily output of vitamin C in the urine. Group 3.

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 $1\frac{1}{2}$ 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%

*Scorbate® 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, Scorbate® 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.

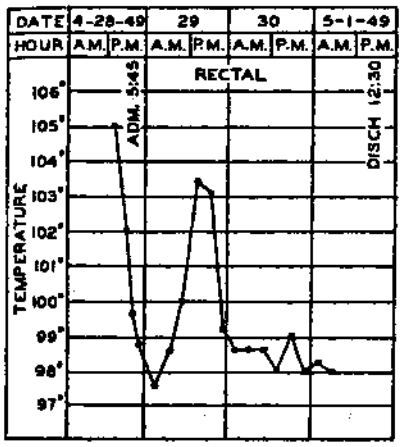
Case 3 is that of a colored woman, aged 28. with history (given by a relative) of chills and fever and chest and head cold for 14 days, severe headache for three days. In stupor when first seen, eye lids closed, a white foam at the mouth which she periodically tried to spit out. Temperature by axilla 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

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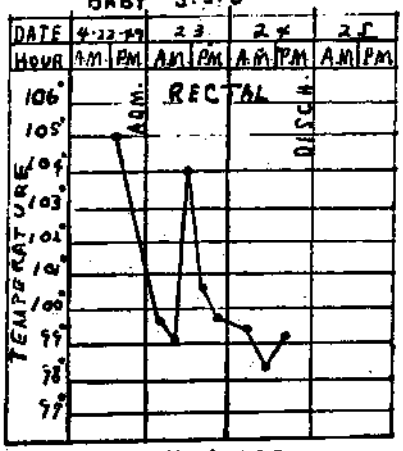

MEASLES

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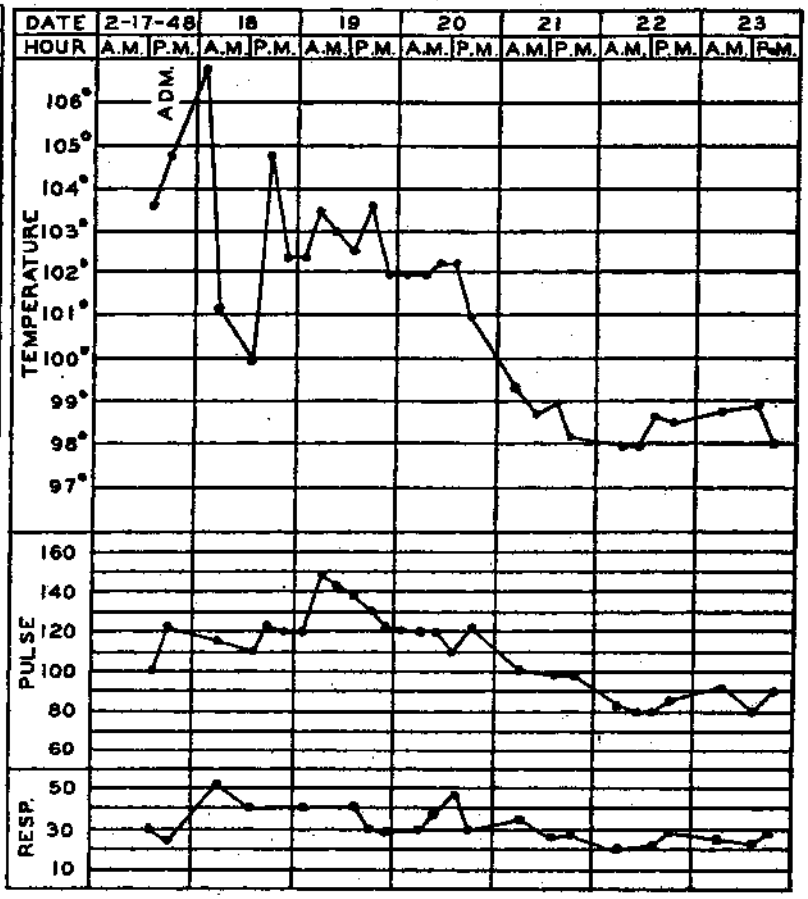

VIRUS PNEUMONIA

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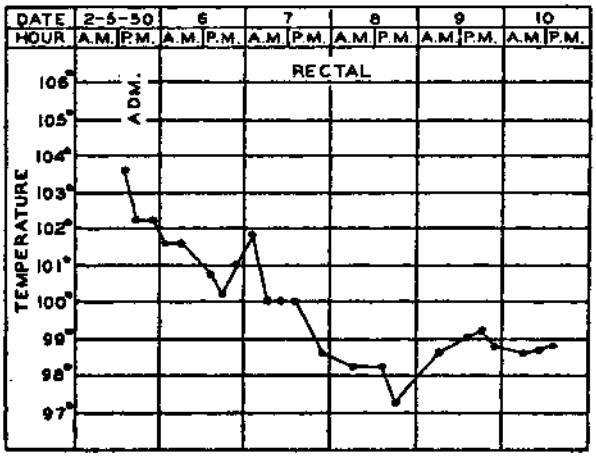
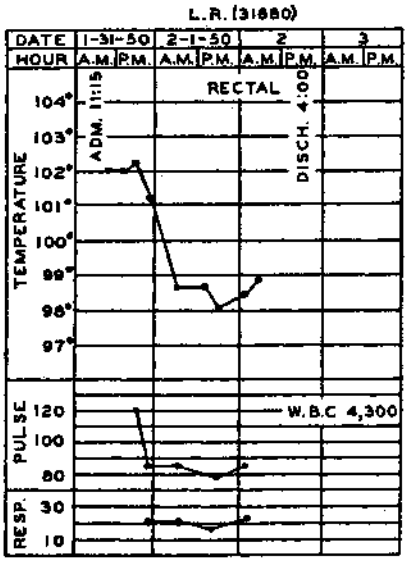


VIRUS ENCEPHALITIS-MENINGITIS

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.

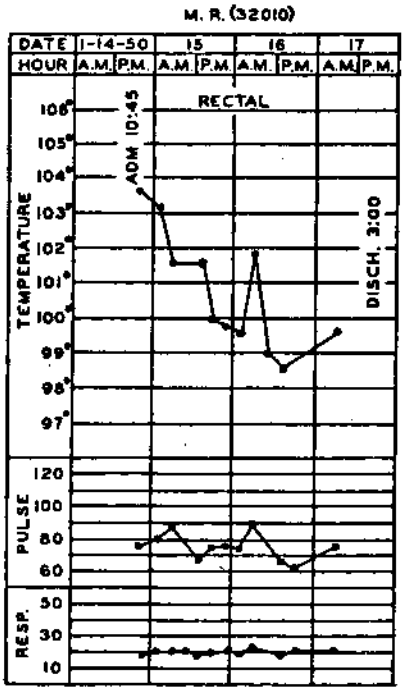
In the case of the man, Case 6, the fever decline was after a modified step-ladder fashion. In this instance the amount of vitamin C injected was less than half of the recommended dose. The WBC was 5,850, admission urine sugar +++. Thirty-one grams of vitamin C was injected intravenously over a period 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:

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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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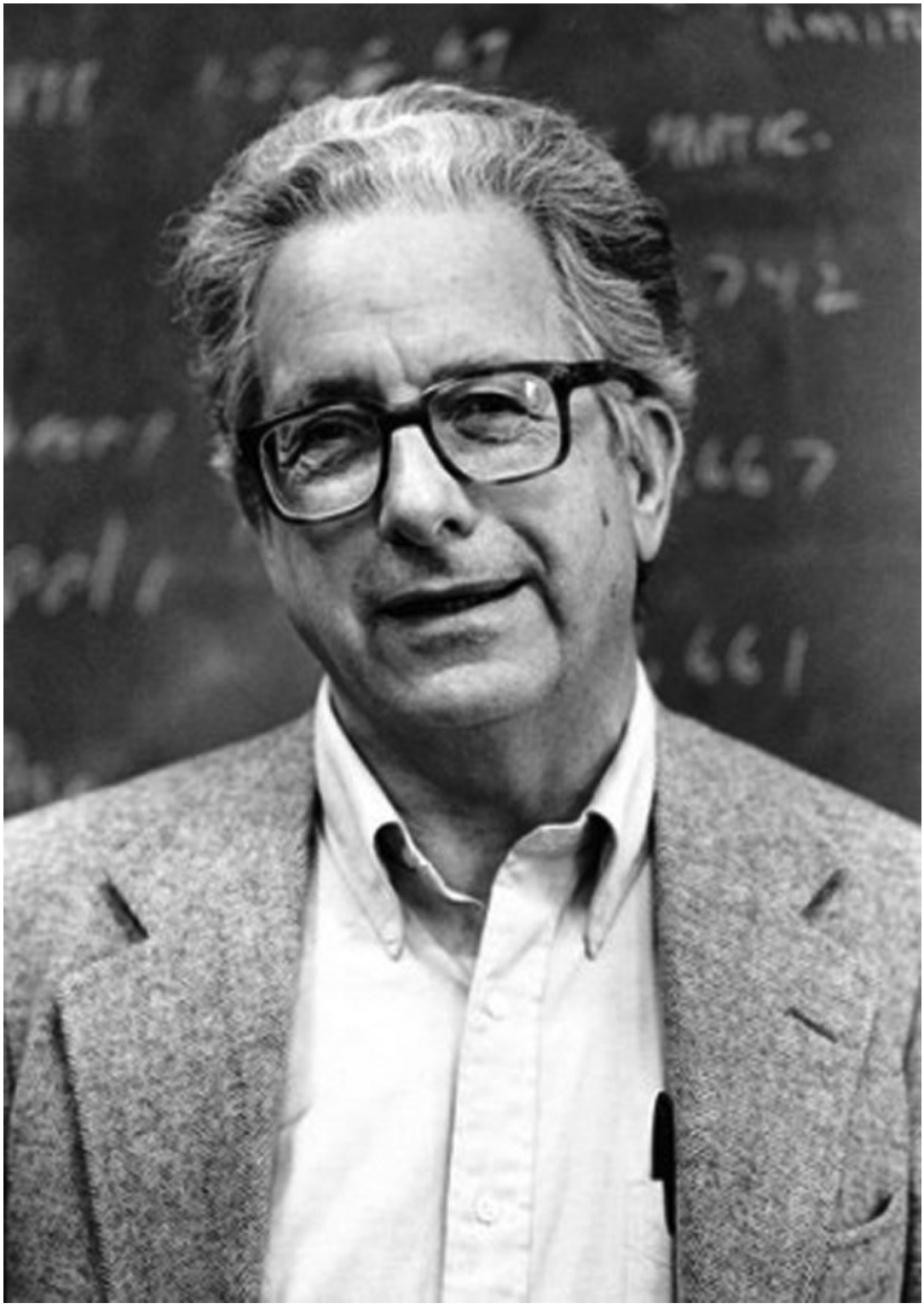

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Paul Meier. Courtesy of the University of Chicago. Printed with permission.

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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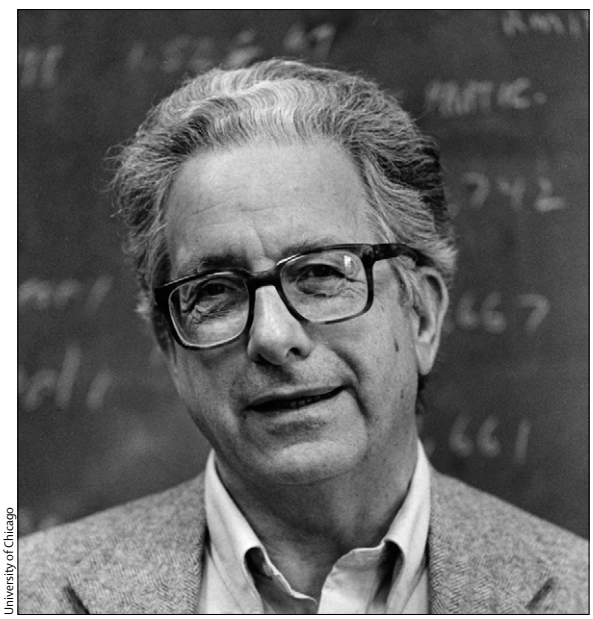

University of Chicago

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.

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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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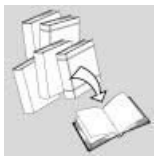
REVIEW



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,

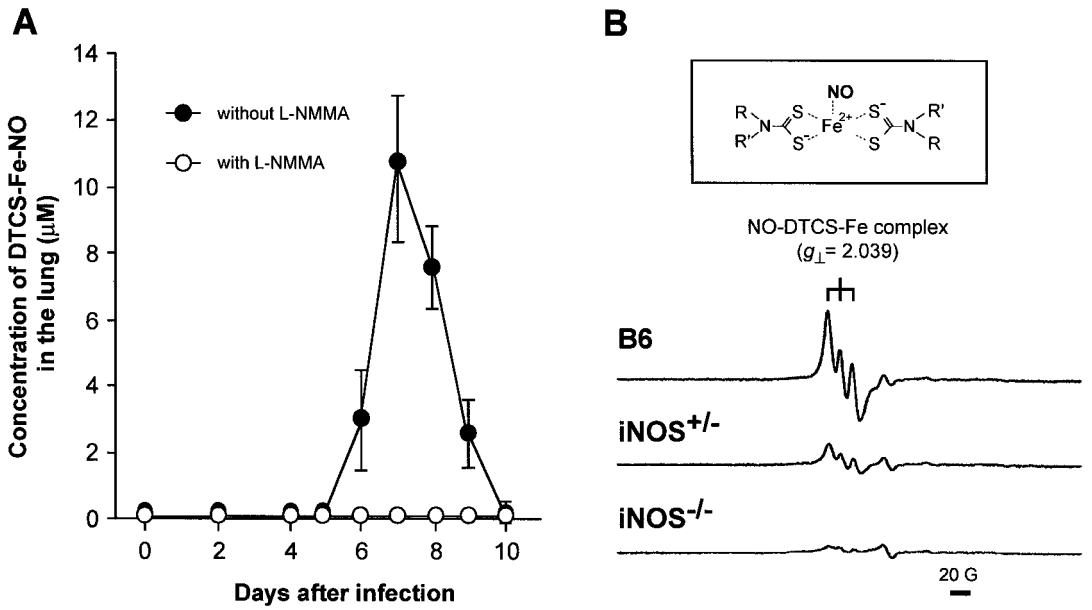

Figure 1. (A) Time profile of NO production in the lung after influenza virus infection. Influenza infection in mice was produced by inhalation of $2 \times LD_{50}$ of influenza A virus. The amount of NO generated in the lung with or without L-NMMA treatment was quantified by ESR spectroscopy (110 K) 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].

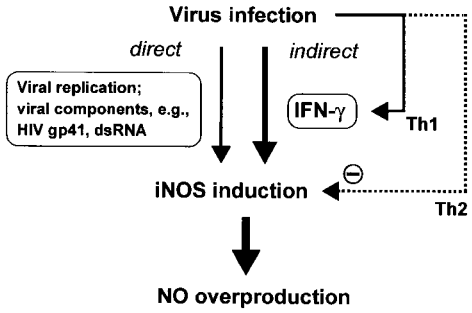

Figure 2. Mechanisms of iNOS induction in viral diseases. In many virus infections, iNOS expression appears to be regulated indirectly via interferon-γ (IFN-γ) induction, which depends on the Th1 response. The host's Th2 response, in contrast, down-regulates iNOS induction. Direct iNOS induction may 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

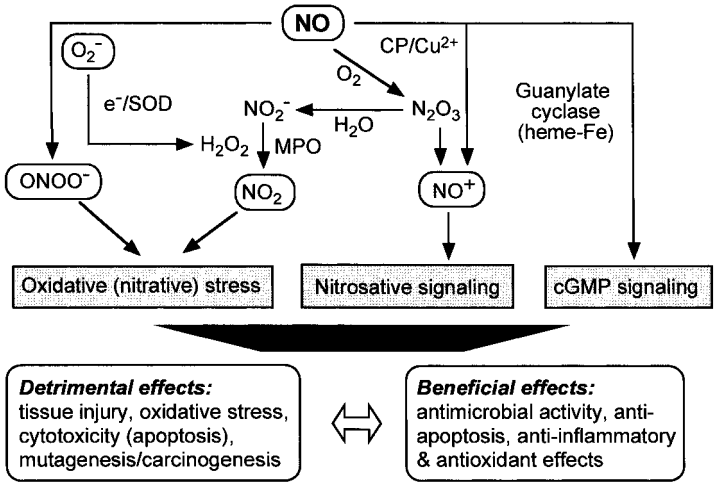

Figure 3. Mechanisms of formation of various reactive nitrogen intermediates from NO and their biological effects. Reactive nitrogen oxides are produced by interactions of NO with molecular oxygen (O_2), active oxygen and oxygen radicals such as O_2^- and H_2O_2 and heavy metals (particularly iron and 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

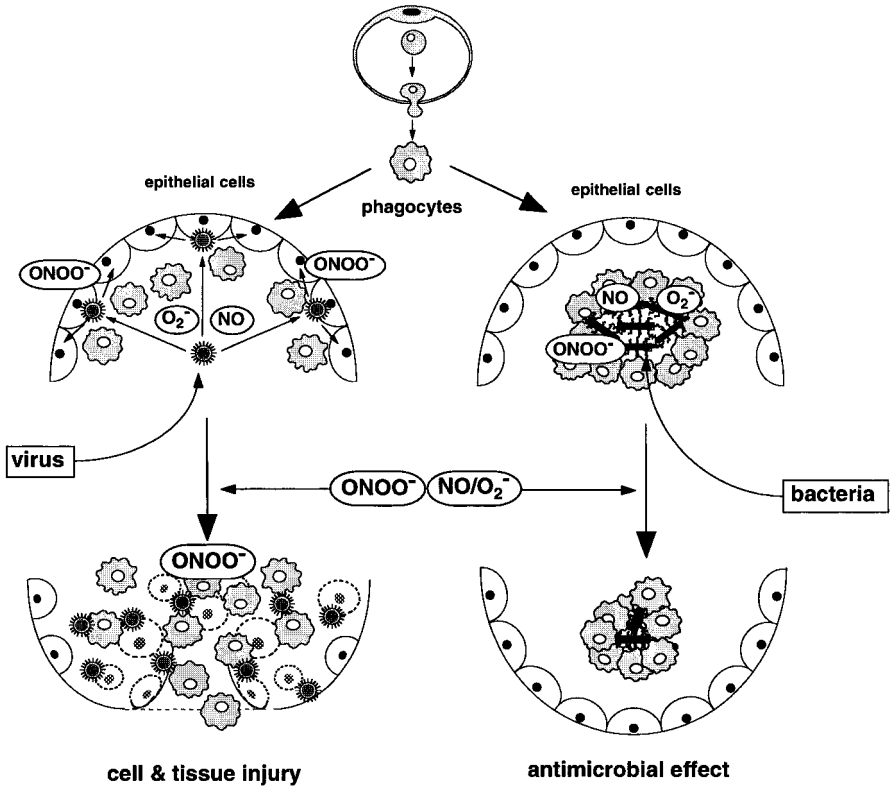

Figure 4. Schematic drawing of the different mechanisms of biological effects of free radicals such as O_2^- and NO, and their product $ONOO^-$, in virus and bacterial infections. Adapted from Akaike *et al.* [12] by copyright permission from Society for Experimental Biology and Medicine

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

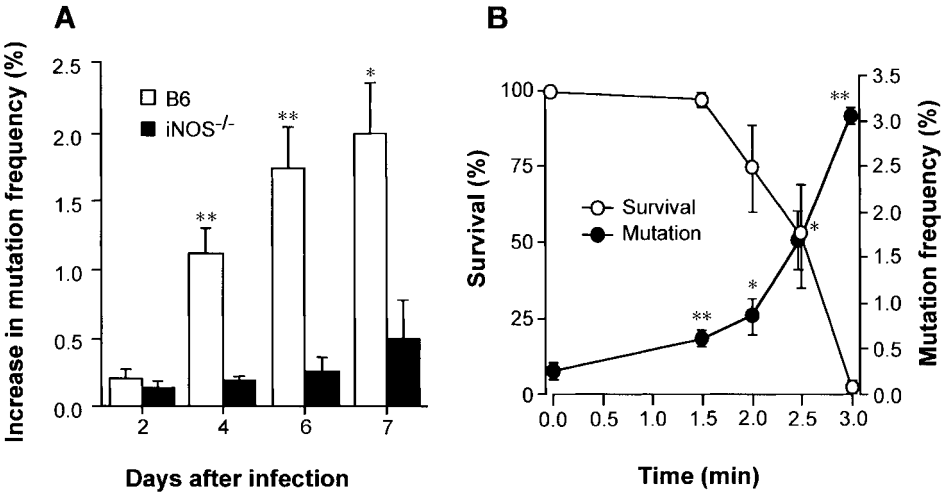

Figure 5. NO-dependent SeV mutation as revealed by genetic mutation of GFP in a recombinant SeV (GFP-constructed SeV, GFP-SeV). (A) The mutation frequency of the virus (GFP-SeV) isolated from the lung of wild-type B6 mice and $iNOS^{-/-}$ mice was quantified by use of the GFP-based mutation assay. (B) 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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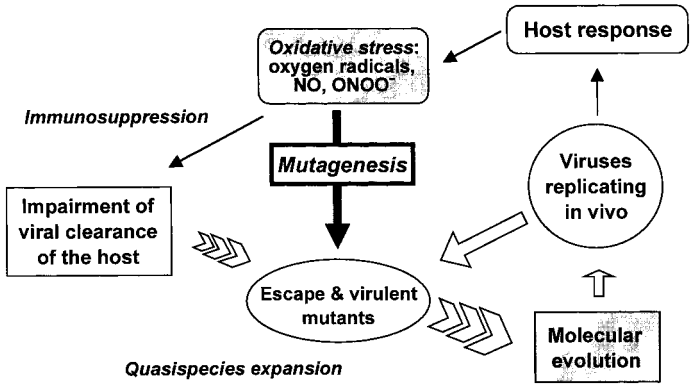

Figure 6. Possible roles of free radicals in viral mutation and evolution. Oxygen radicals and NO-derived reactive nitrogen intermediates, via their potent mutagenic activities, may contribute to the molecular evolution of viruses. NO may also affect viral evolution by inhibiting a host's antiviral immune 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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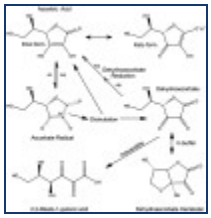
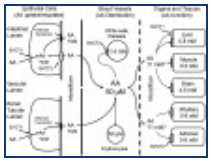
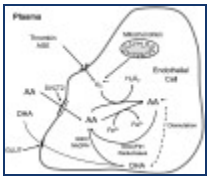
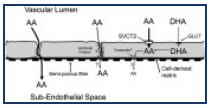
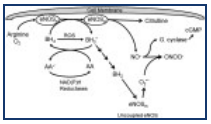


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Scurvy in hospitalized elderly patients

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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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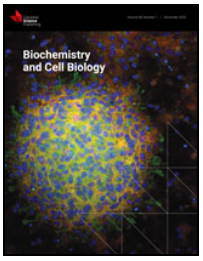

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STUDIES ON ACCLIMATIZATION AND ON THE EFFECT OF ASCORBIC ACID IN MEN EXPOSED TO COLD

J. LeBlanc, , M. Stewart, , G. Marier, and , M. G. Whillans

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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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Vitamin C supplementation and common cold symptoms: problems with inaccurate reviews.

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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

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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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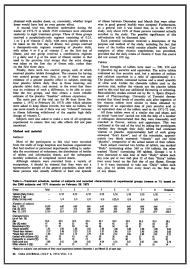

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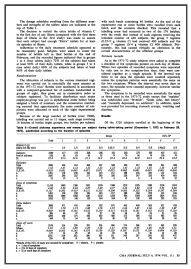

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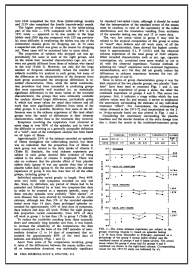

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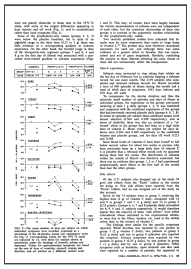

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Am J Clin Nutr. 1979 Aug;32(8):1686-90.

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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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

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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

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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.

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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

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 n 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 an be presumed to lished opinions a sample how m changes over tim

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]. ...

ıe Length of Stay in the ICU: A Meta-Analysis

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ı Chalker

l Outcome of Sinonasal Tumors in a Nigerian Tertiary Hospital – 6-year Review

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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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[Effect of high-dose vitamin C on the formation of experimental renal stones in the rat]

April 1985 · Zhonghua wai ke za zhi [Chinese journal of surgery]

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Antioxidants in HIV positive children

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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Plasma vitamin C assays: a European experience. EC FLAIR Concerted Action No. 10: Micronutrient Meas...

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

) Scientific Reports 8(1) · December 2018 *with* 114 Reads
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Ewelina Chajduk
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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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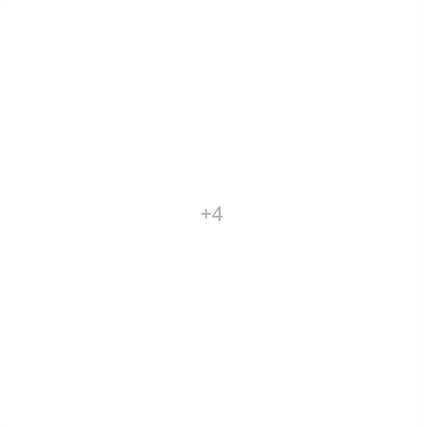
The effect of intravenous iron (Fe) supplementation on echocardiographic…



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 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

[1]Department of Clinical Physiology, Centre of Postgraduate Medical Education, Warsaw, Poland. [2]C
Radiobiology and Biological Dosimetry, Institute of Nuclear Chemistry and Technology, Warsaw, Poland. [3]La
of Nuclear Analytical Methods, Institute of Nuclear Chemistry and Technology, Warsaw, Poland. [4]Depar
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Rzeszów, Poland. [6]Heart Failure and Transplantology Department, Institute of Cardiology, Warsaw, Polanc
Mackiewicz and Michał Mączewski contributed equally. Correspondence and requests for materials s
addressed to M.M. (email: michal.maczewski@cmkp.edu.pl)

EPORTS | (2018) 8:15758 | DOI:10.1038/s41598-018-33277-2

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

le

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

OL

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

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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.

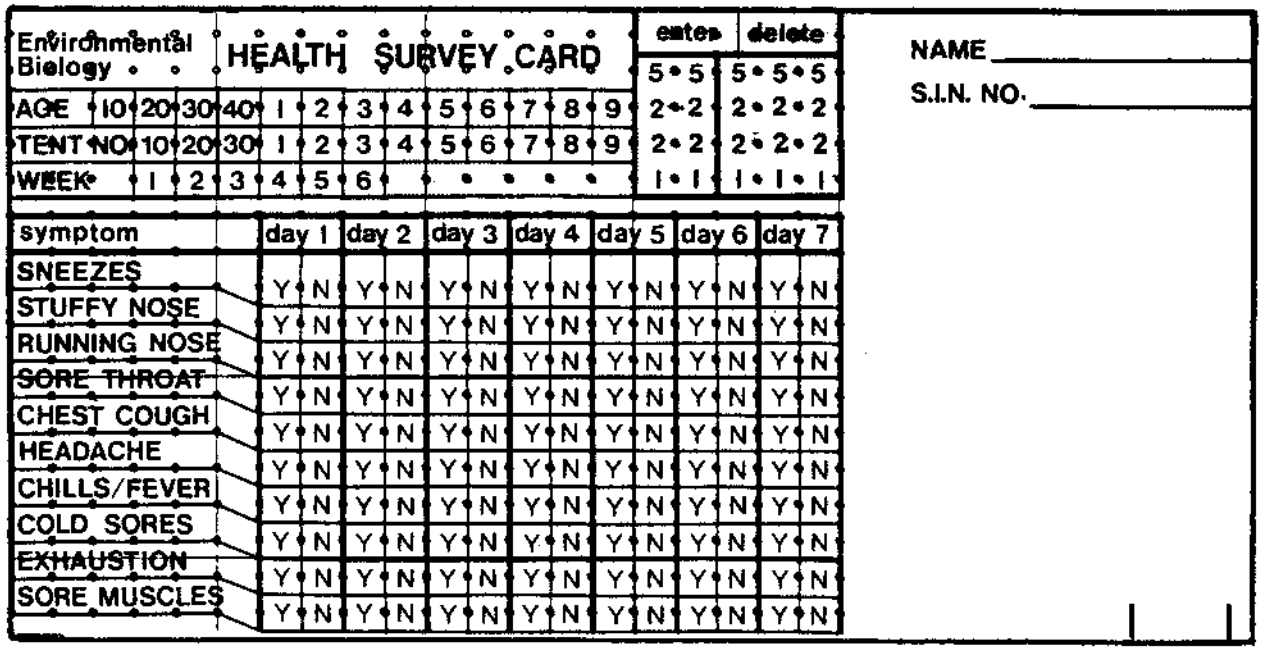
TABLE 2

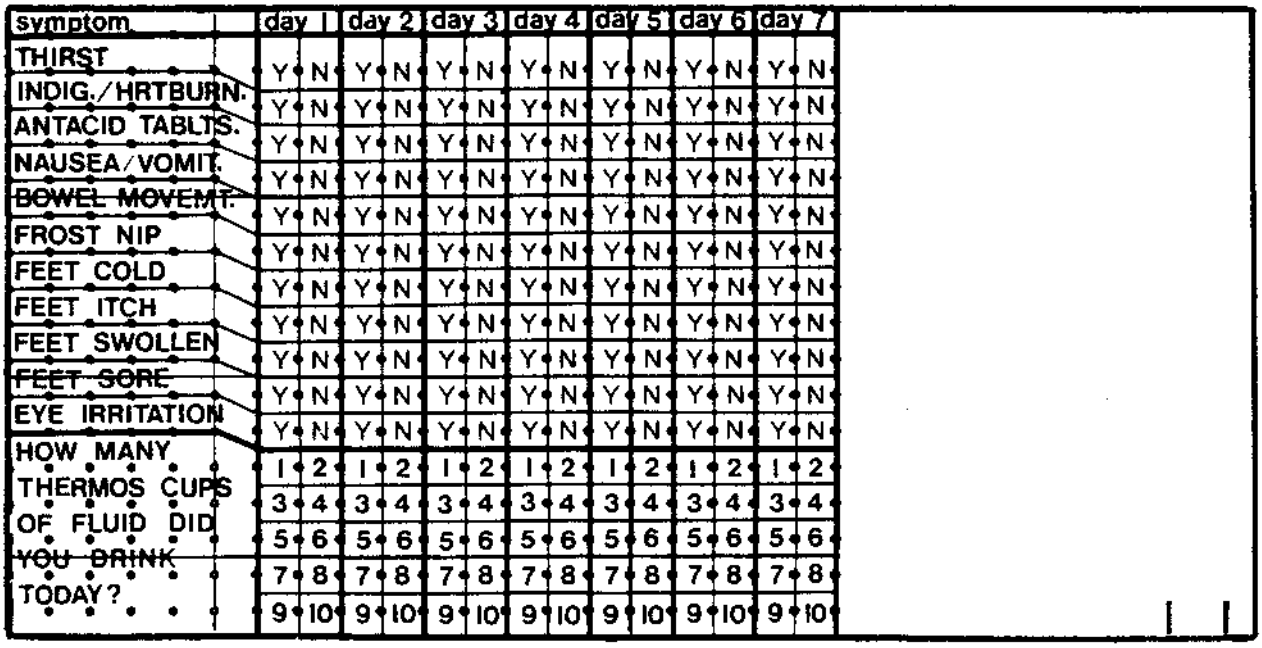
NORTHERN EXERCISES UTILIZED FOR THE INVESTIGATION OF HEALTH PROBLEMS, 1972-73

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.

PMID: 163386

[Indexed for MEDLINE]

LinkOut - more resources

Format: Abstract

Int J Sports Med. 1996 Jul;17(5):379-83.

Vitamin C and common cold incidence: a review of studies with subjects under heavy physical stress.

Hemilä H[1].

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

Full text is available as a scanned copy of the original print version. Get a printable copy (PDF file) of the **complete article** (487K), 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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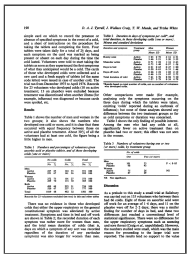

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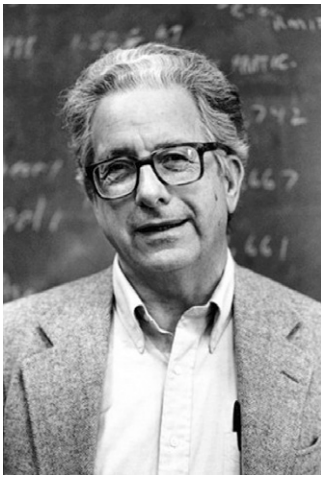
Paul Meier

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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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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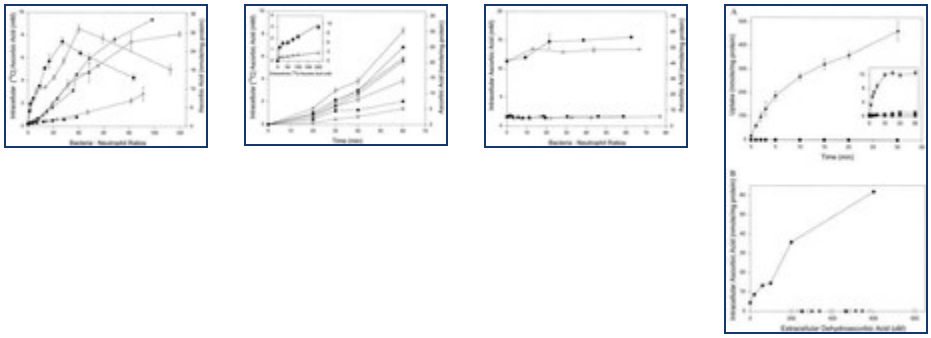

Publication types, MeSH terms, Substance

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Proc Natl Acad Sci U S A. 1997 Dec 9;94(25):13816-9.

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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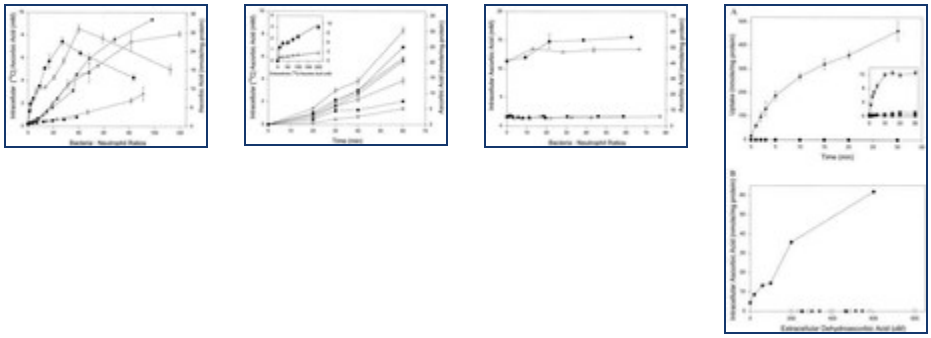

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Ascorbic acid and the common cold

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.

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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.

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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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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.
Subject:	3142 Public health care science, environmental and occupational health ALTERNATIVE MEDICINE vitamin C Common Cold Complementary medicine Evidence based medicine Asthma zinc lozenges
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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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Publication types, MeSH terms, Substances, Grant support

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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
https://doi.org/10.1177/003693307301800102

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,
https://doi.org/10.1093/ajcn/24.4.432
Published: 01 April 1971

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

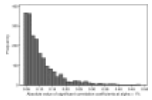

Figure 1. Histogram of Statistically Significant (at...

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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

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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.

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DIE NATURWISSENSCHAFTEN

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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

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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

Format: Abstract

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

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The Effect of Vitamin E on Common Cold Incidence Is Modified by Age, Smoking and Residential Neighborhood

Harri Hemilä, Jarmo Virtamo, Demetrius Albanes and Jaakko Kaprio

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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.

Journal of the American College of Nutrition, Vol. 25, No. 4, 332-339 (2006)
Published by the American College of Nutrition

The Effect of Vitamin E on Common Cold Incidence Is Modified by Age, Smoking and Residential Neighborhood

Harri Hemilä, MD, PhD, Jarmo Virtamo, MD, PhD, Demetrius Albanes, MD and Jaakko Kaprio, MD, PhD

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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).

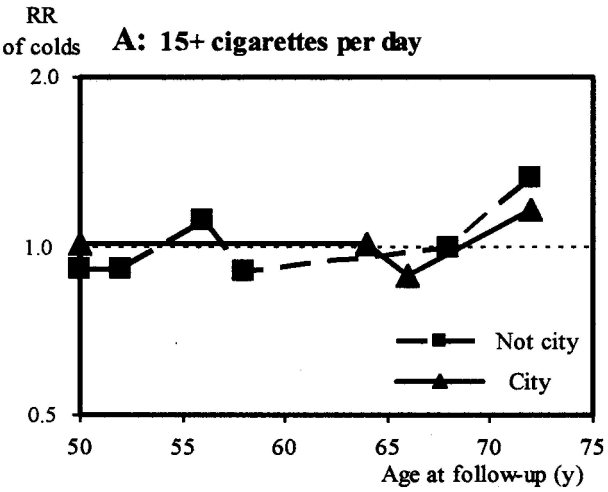
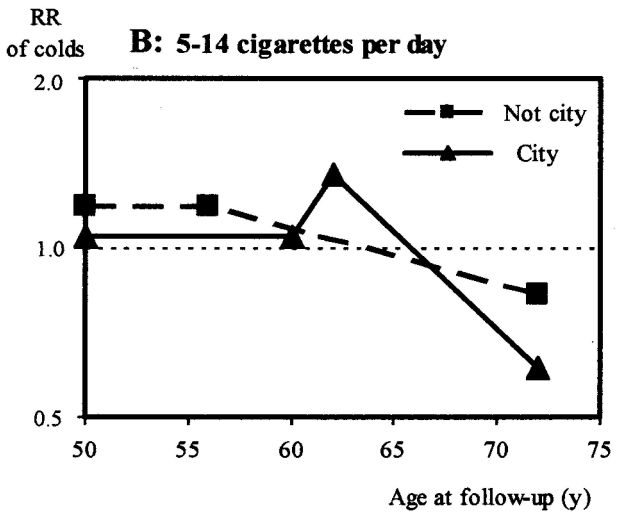


Fig. 1. The effect of vitamin E on the relative risk of common cold as a function of age at follow-up. Participants smoking more (A) and less (B) are further divided into subgroups by residential neighborhood. RR indicates the relative risk of colds between the vitamin E and placebo arms. See Table 2 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.

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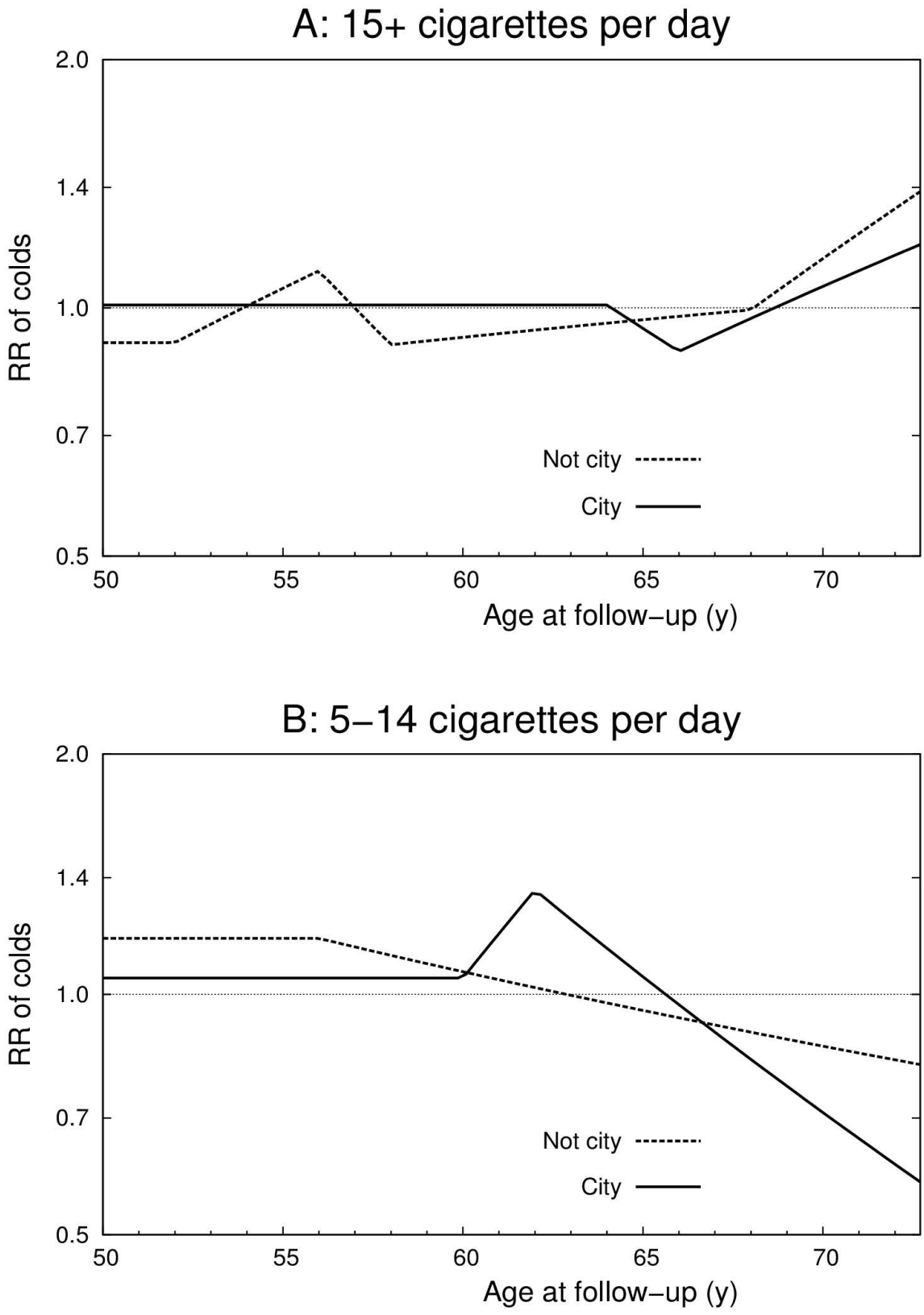

Fig. 1. The effect of vitamin E on the relative risk of common cold as a function of age at follow-up. Participants smoking more (A) and less (B) are further divided into subgroups by residential neighborhood. RR indicates the relative risk of colds between the vitamin E and placebo arms. See Table 2 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.

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[Indexed for MEDLINE]

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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How Neutrophils Kill Microbes

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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.

Open in a separate window

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.

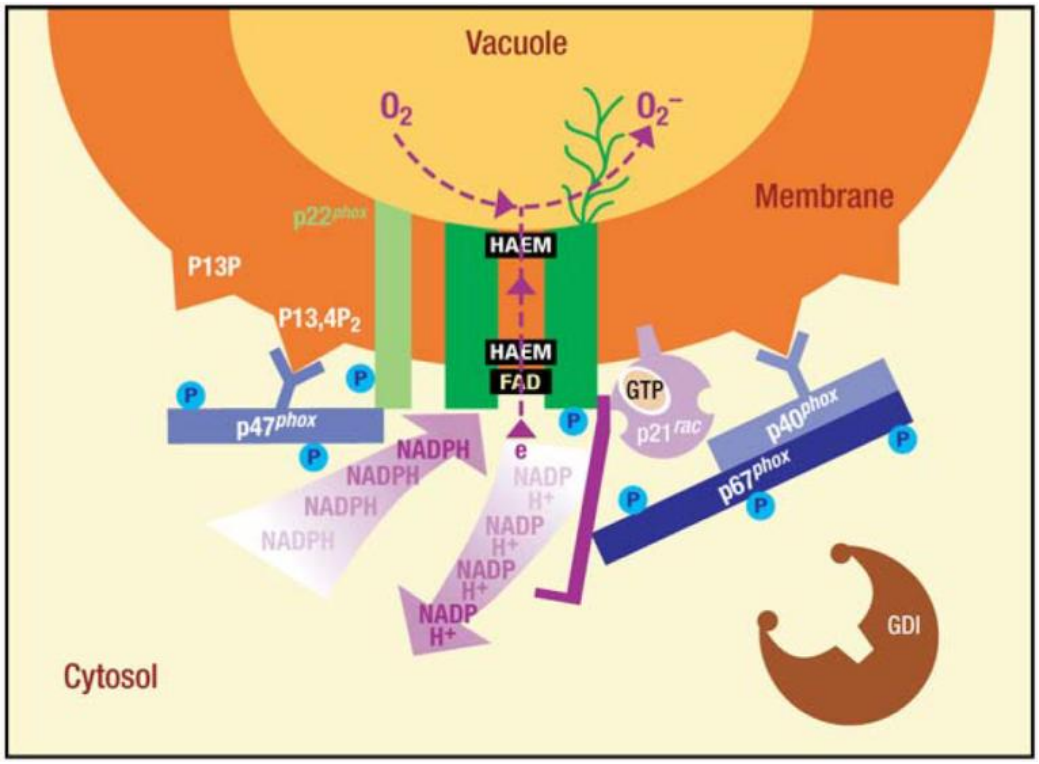

Figure 2

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).

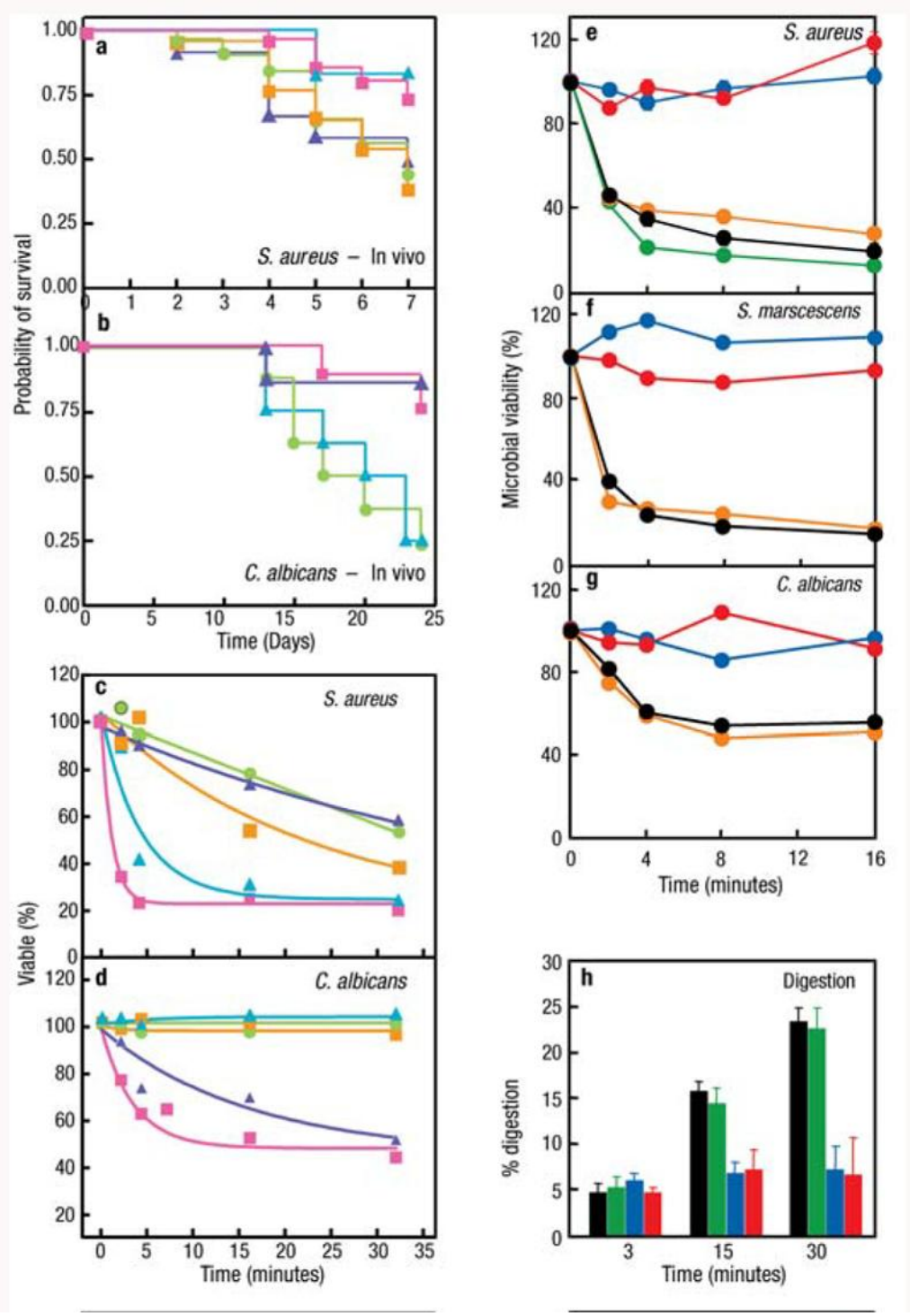

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Figure 3

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).

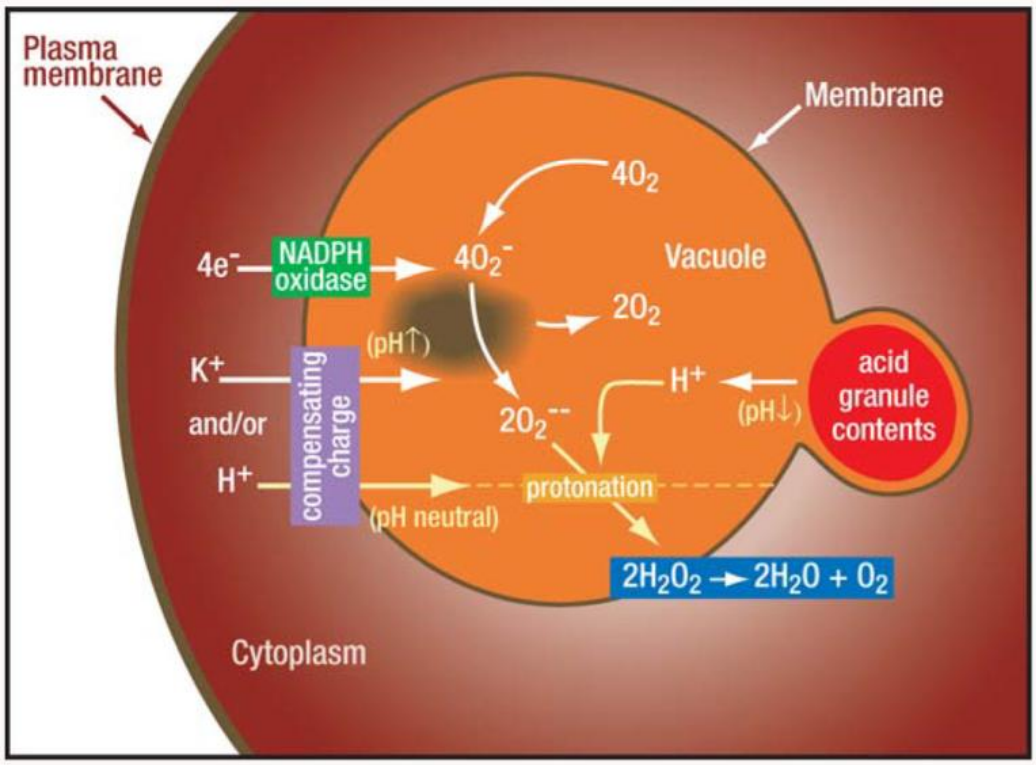

Figure 4

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.

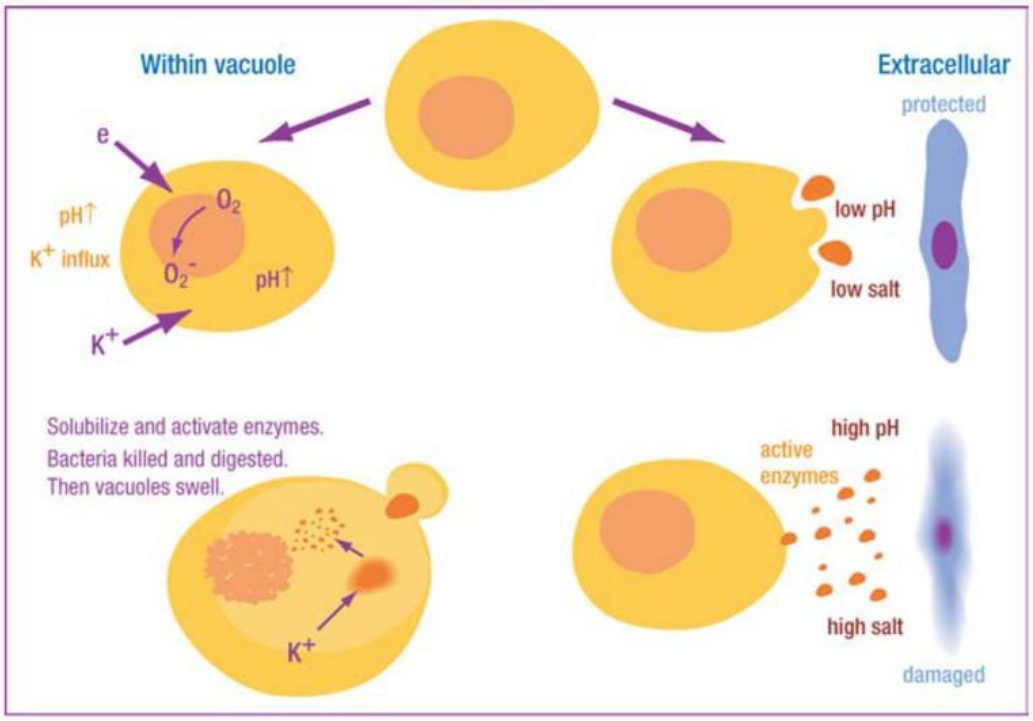

Figure 5

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 0_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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R. Eccles, "Is the Common Cold a Clinical Entity or a Cultural Concept?" Rhinology, Vol. 51, No. 1, 2013, pp. 3-8.

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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.

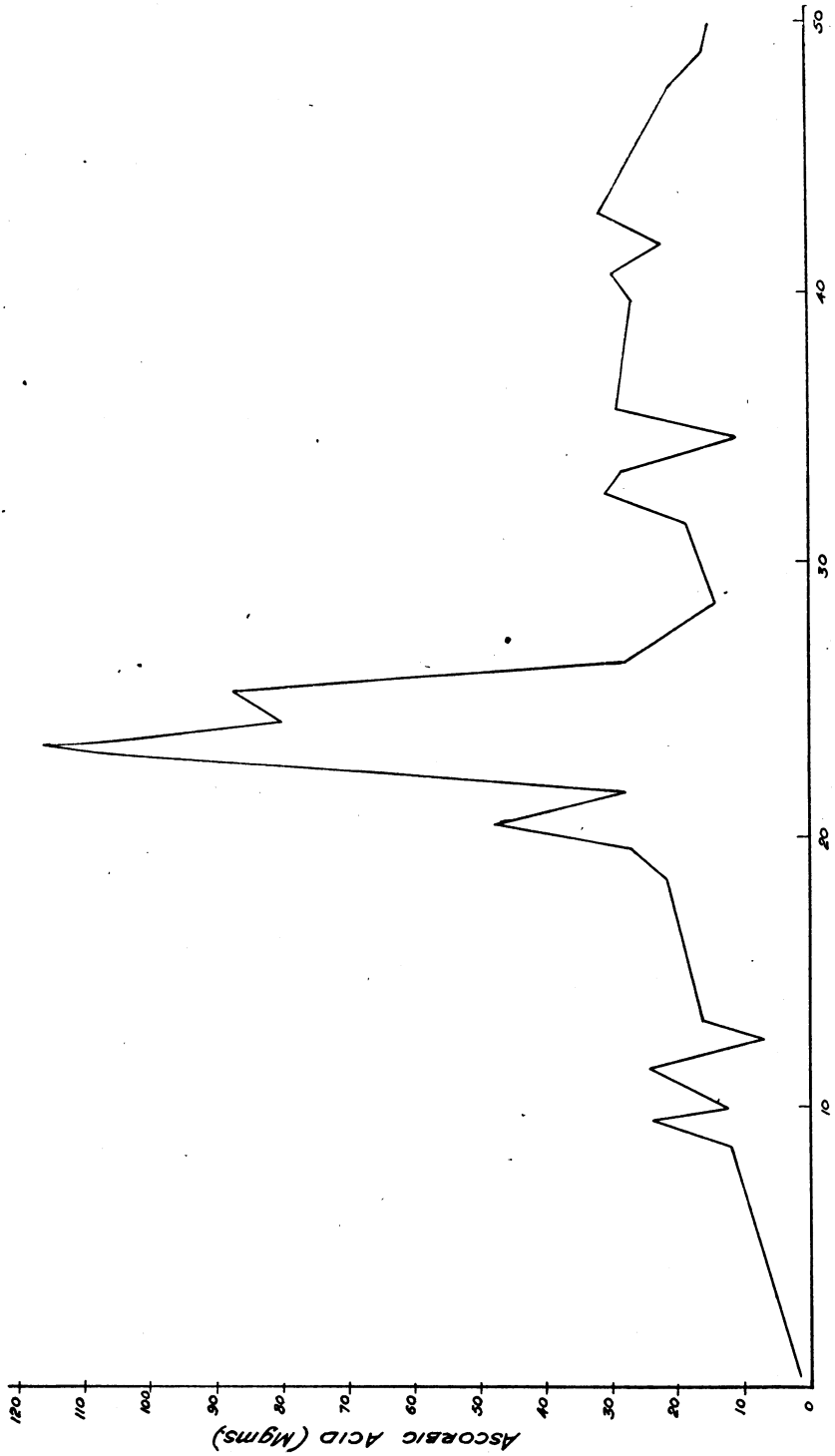

Fig. 1. Daily output of vitamin C in the urine. Group 1.

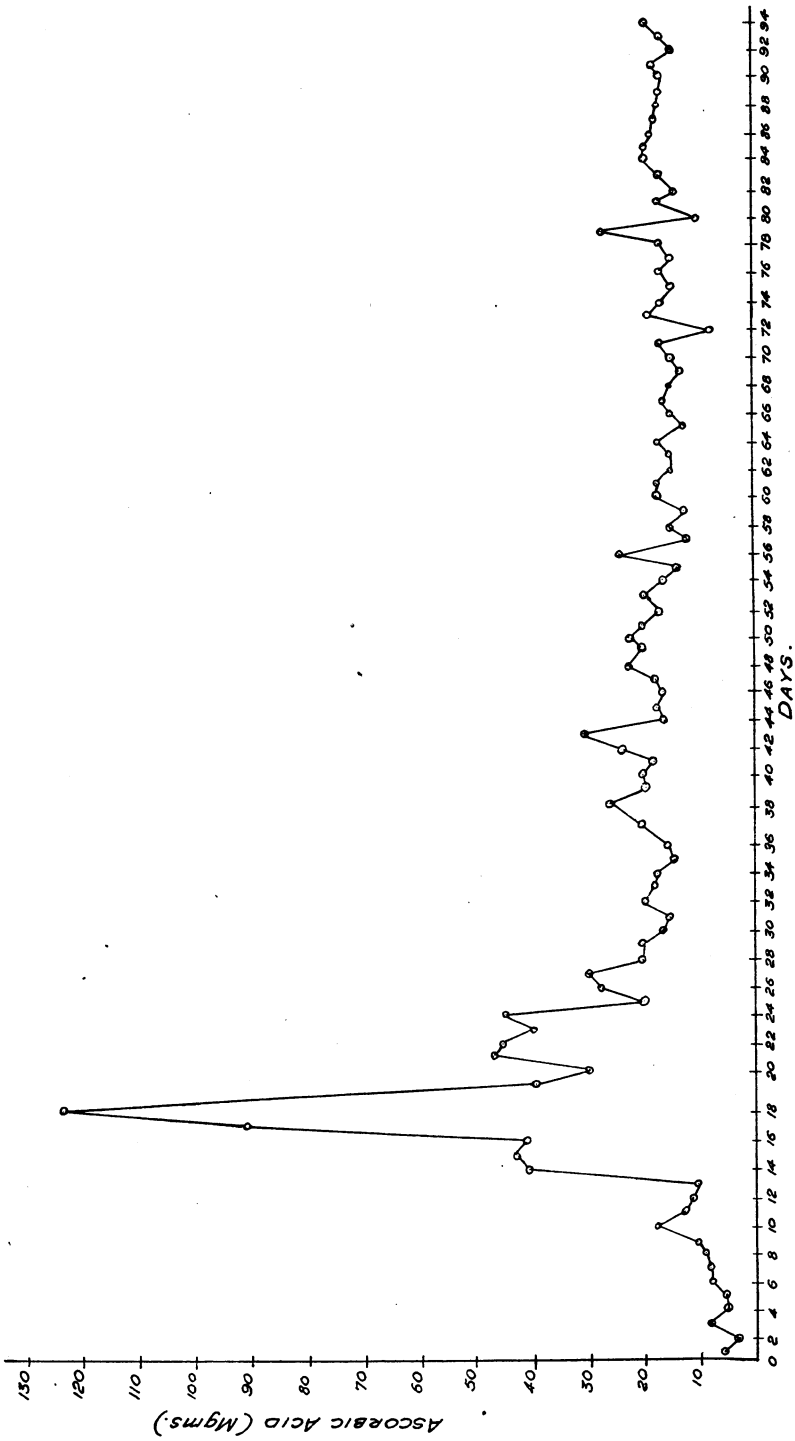

Fig. 2. Daily output of vitamin C in the urine. Group 2.

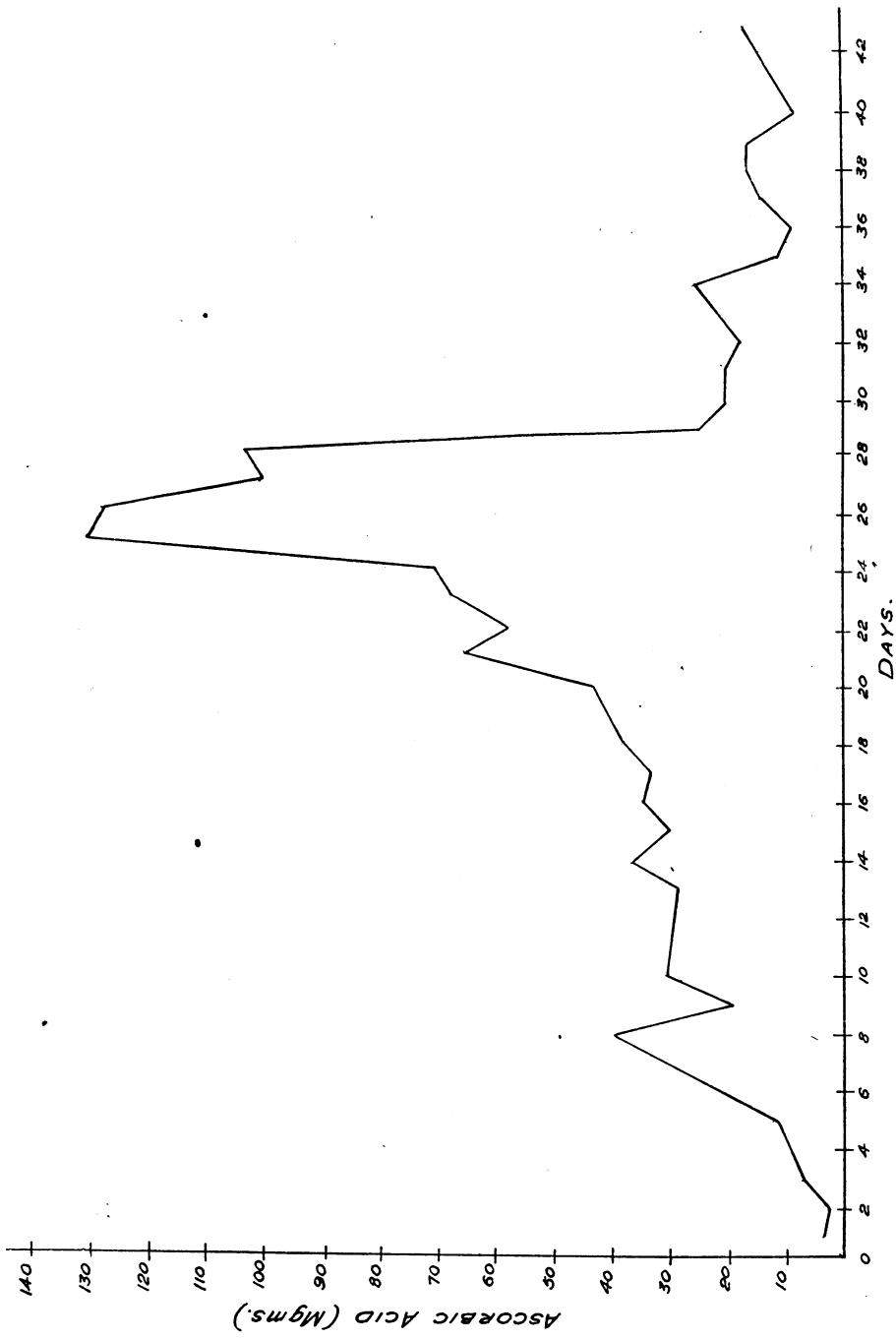

Fig. 3. Daily output of vitamin C in the urine. Group 3.

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 $1\frac{1}{2}$ 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%

*Scorbate® 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, Scorbate® 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

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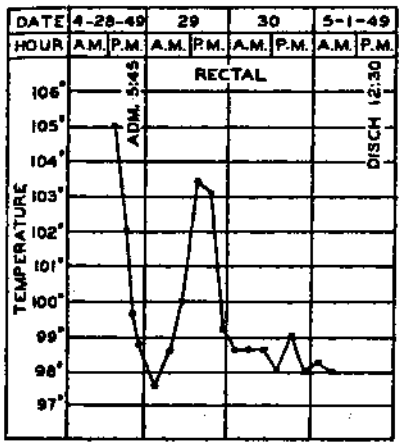

MEASLES

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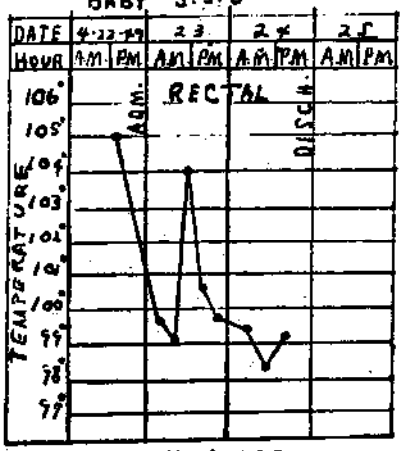

MEASLES

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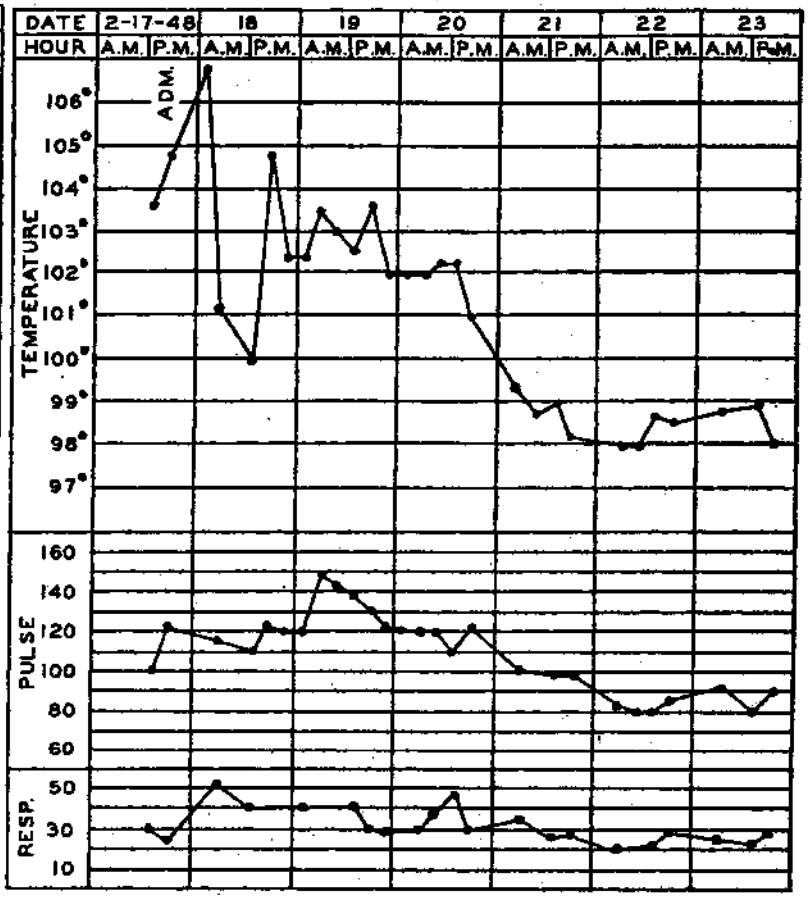

VIRUS PNEUMONIA

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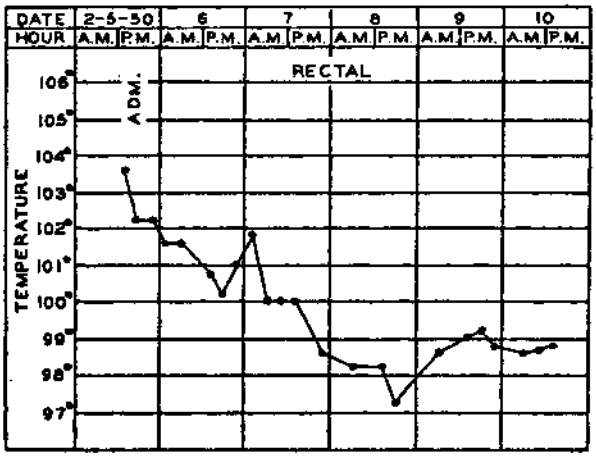
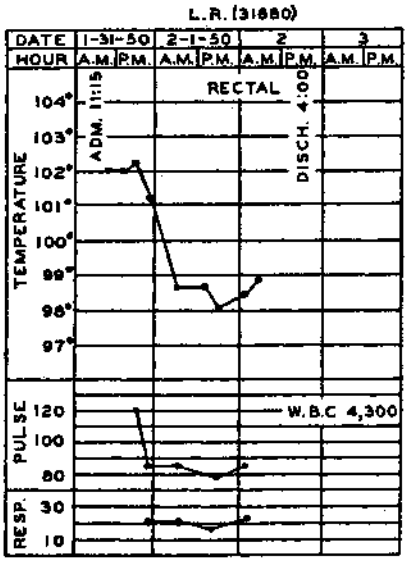


VIRUS ENCEPHALITIS-MENINGITIS

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.

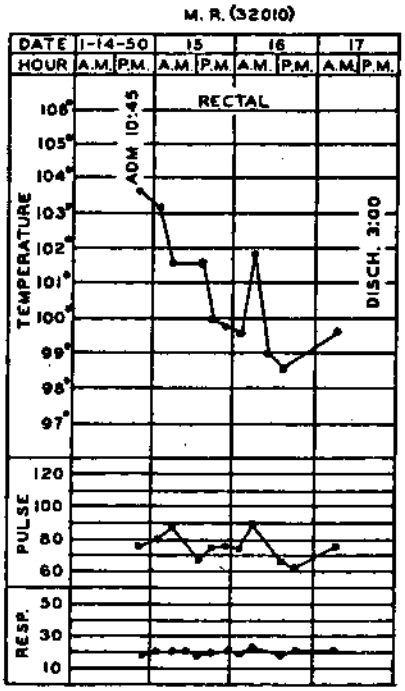
In the case of the man, Case 6, the fever decline was after a modified step-ladder fashion. In this instance the amount of vitamin C injected was less than half of the recommended dose. The WBC was 5,850, admission urine sugar +++. Thirty-one grams of vitamin C was injected intravenously over a period 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
Volume -number
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.

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Paul Meier A Man Behind the Method

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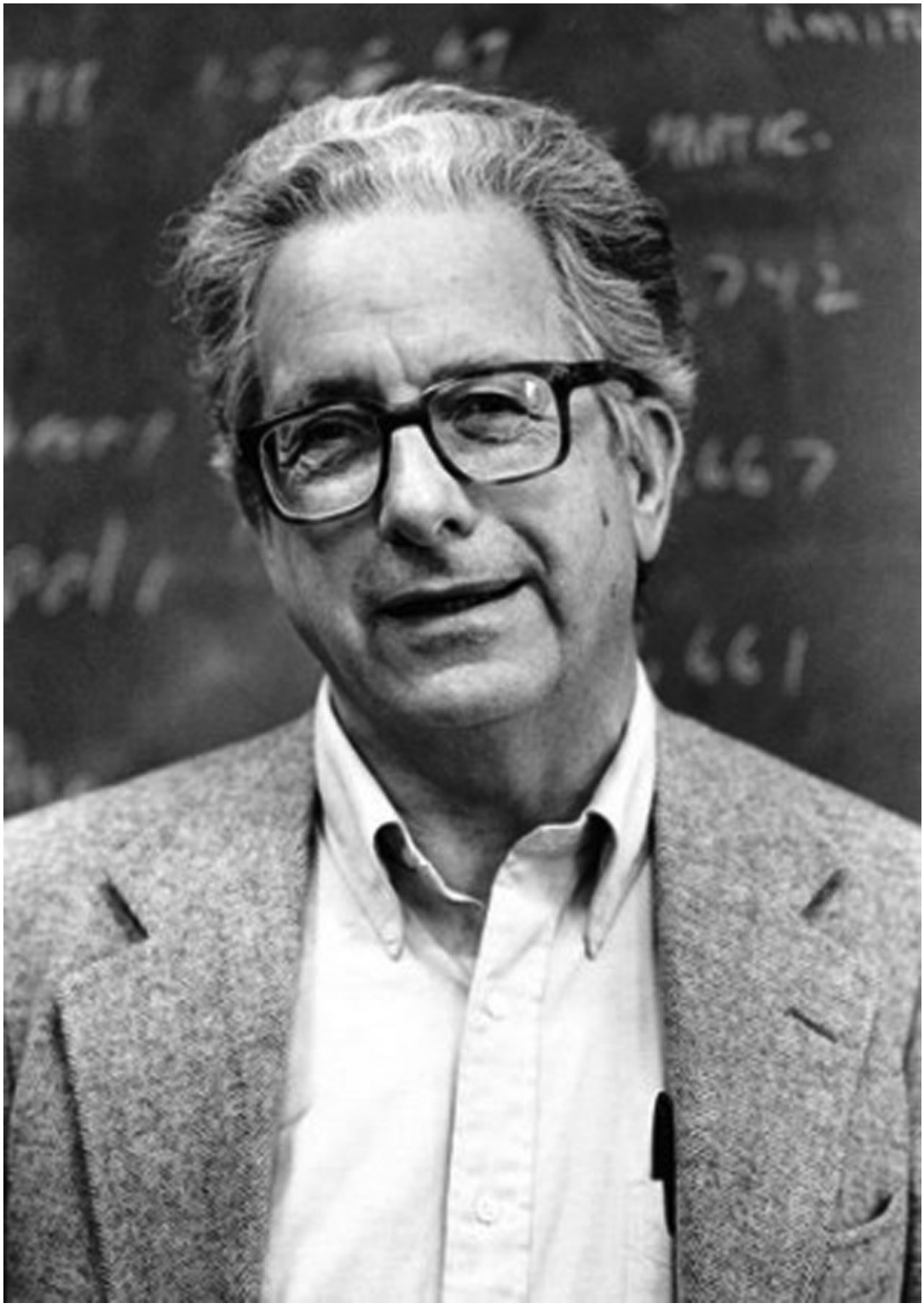

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Paul Meier. Courtesy of the University of Chicago. Printed with permission.

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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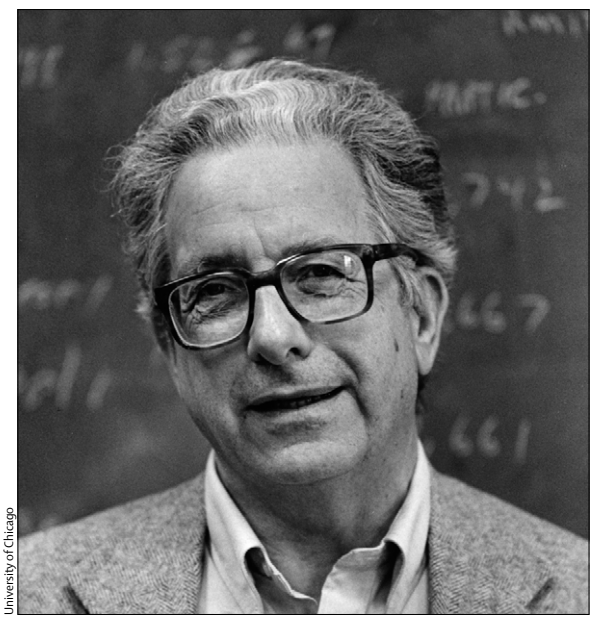

University of Chicago

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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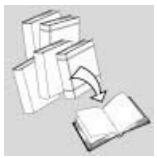

Role of free radicals in viral pathogenesis and mutation

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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,

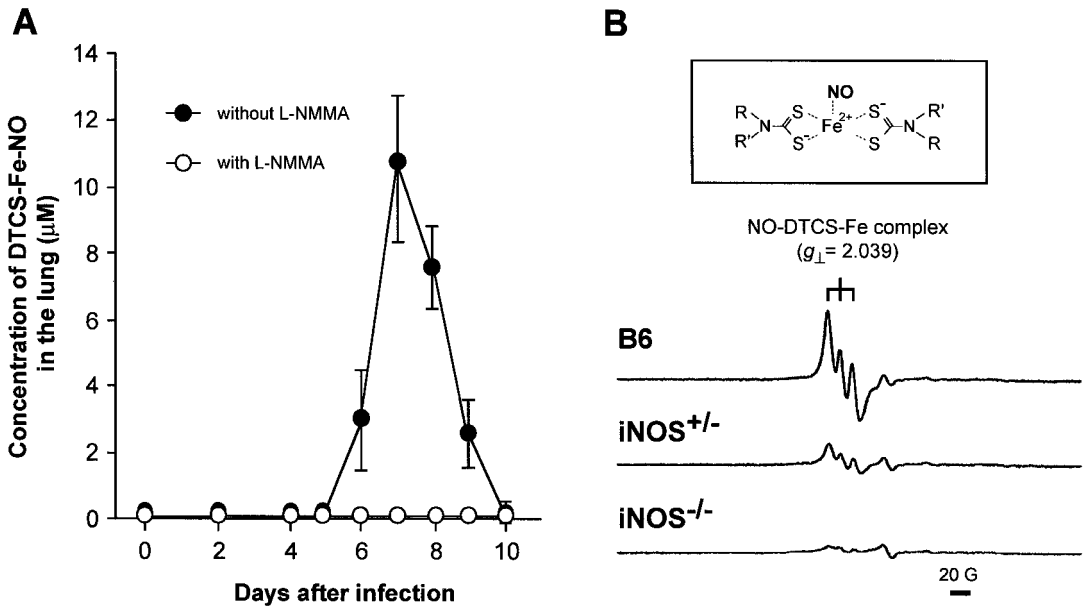

Figure 1. (A) Time profile of NO production in the lung after influenza virus infection. Influenza infection in mice was produced by inhalation of $2 \times LD_{50}$ of influenza A virus. The amount of NO generated in the lung with or without L-NMMA treatment was quantified by ESR spectroscopy (110 K) 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].

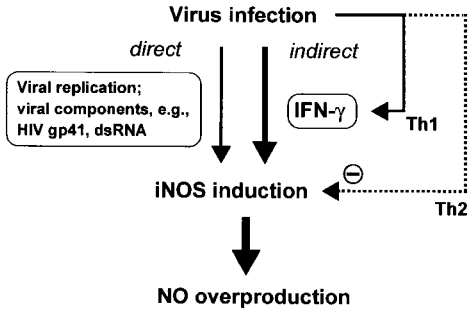

Figure 2. Mechanisms of iNOS induction in viral diseases. In many virus infections, iNOS expression appears to be regulated indirectly via interferon-γ (IFN-γ) induction, which depends on the Th1 response. The host's Th2 response, in contrast, down-regulates iNOS induction. Direct iNOS induction may 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

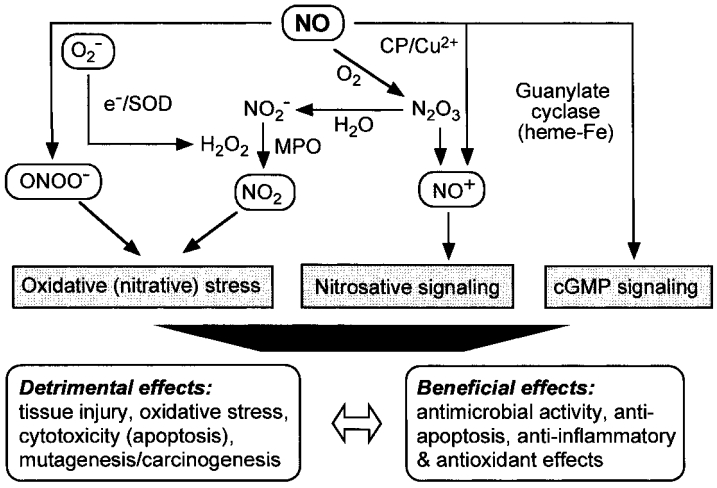

Figure 3. Mechanisms of formation of various reactive nitrogen intermediates from NO and their biological effects. Reactive nitrogen oxides are produced by interactions of NO with molecular oxygen (O_2), active oxygen and oxygen radicals such as O_2^- and H_2O_2 and heavy metals (particularly iron and 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

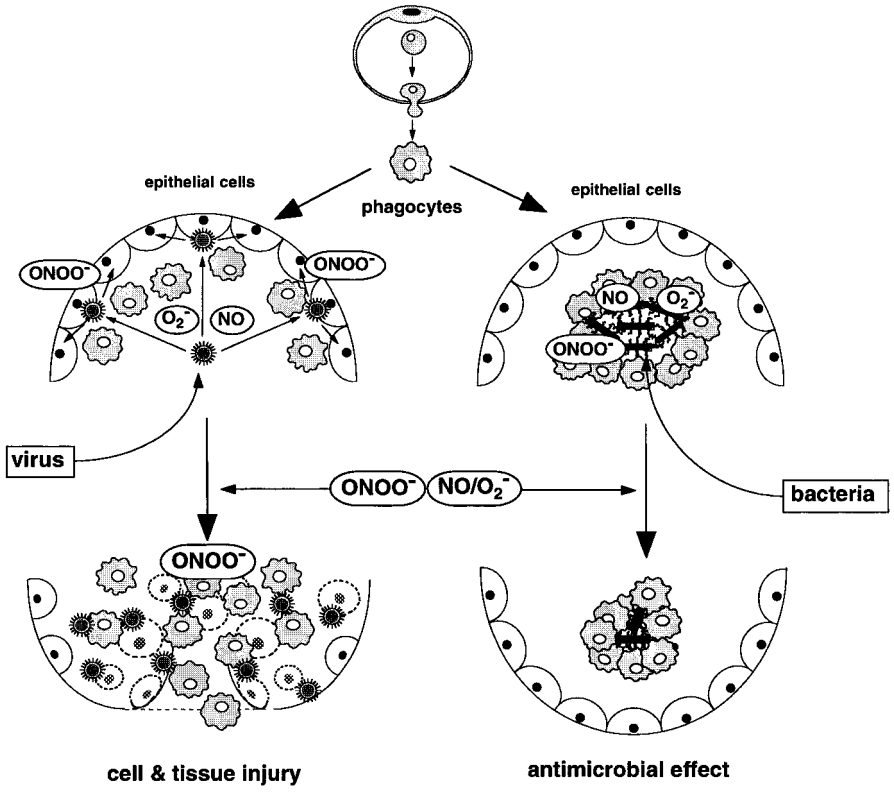

Figure 4. Schematic drawing of the different mechanisms of biological effects of free radicals such as O_2^- and NO, and their product $ONOO^-$, in virus and bacterial infections. Adapted from Akaike *et al.* [12] by copyright permission from Society for Experimental Biology and Medicine

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

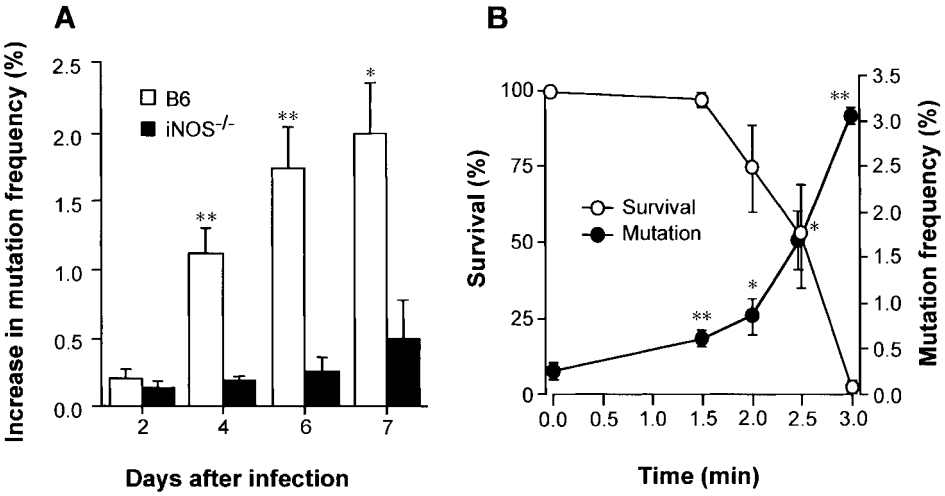

Figure 5. NO-dependent SeV mutation as revealed by genetic mutation of GFP in a recombinant SeV (GFP-constructed SeV, GFP-SeV). (A) The mutation frequency of the virus (GFP-SeV) isolated from the lung of wild-type B6 mice and $iNOS^{-/-}$ mice was quantified by use of the GFP-based mutation assay. (B) 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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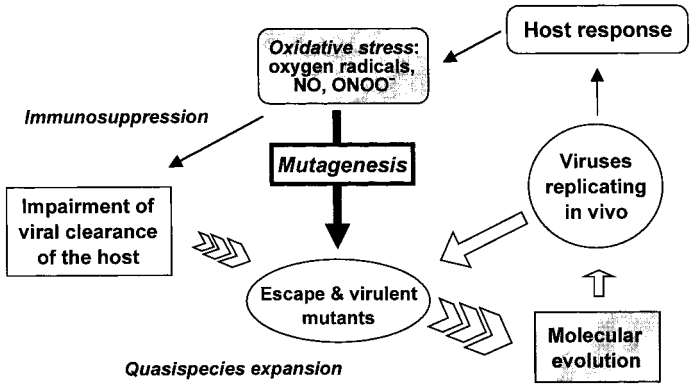

Figure 6. Possible roles of free radicals in viral mutation and evolution. Oxygen radicals and NO-derived reactive nitrogen intermediates, via their potent mutagenic activities, may contribute to the molecular evolution of viruses. NO may also affect viral evolution by inhibiting a host's antiviral immune 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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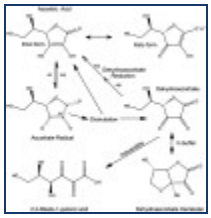
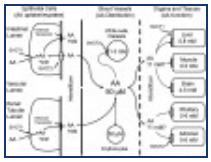
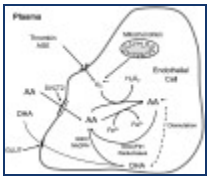
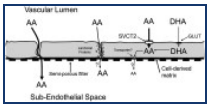
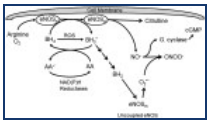


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Scurvy in hospitalized elderly patients

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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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Key words

- Scurvy
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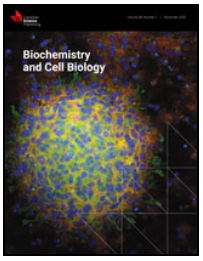

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STUDIES ON ACCLIMATIZATION AND ON THE EFFECT OF ASCORBIC ACID IN MEN EXPOSED TO COLD

J. LeBlanc, , M. Stewart, , G. Marier, and , M. G. Whillans

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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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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

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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.

References are not copied here:
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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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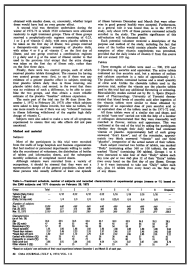

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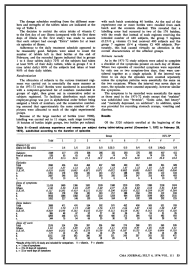

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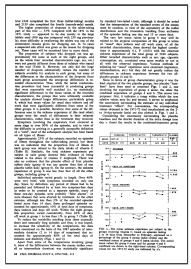

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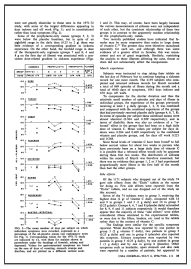

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Am J Clin Nutr. 1979 Aug;32(8):1686-90.

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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May 1996

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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

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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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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

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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Vitamin C

VITAMIN C *

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).

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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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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Sex differences in the incidence and severity of respiratory tract infections

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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]

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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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

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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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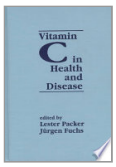

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doi: 10.1073/pnas.93.8.3704
PMCID: PMC39676
PMID: 8623000

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.

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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.

Comment in

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.

PMID: 25010554 PMCID: PMC4113757 DOI: 10.3390/nu6072572

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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**
14 November 2016 , pp. 1530-1536

Vitamin E and the risk of pneumonia: using the I^2 statistic to quantify heterogeneity within a controlled trial

Harri Hemilä (a1)

DOI: https://doi.org/10.1017/S0007114516003408
Published online by Cambridge University Press: 26 October 2016

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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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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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 %;

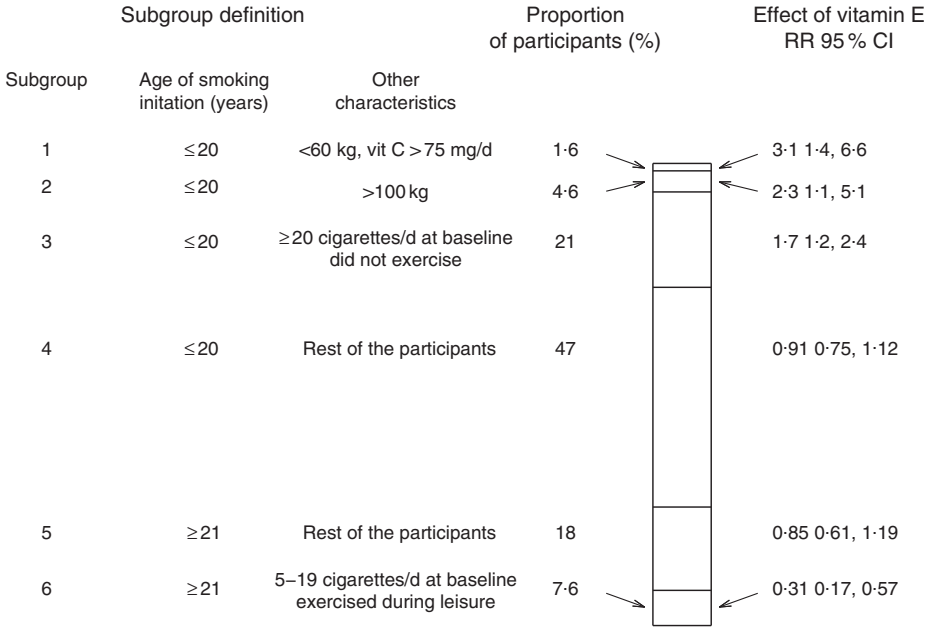

Fig. 1. Proportion of participants and the effect of vitamin E on the incidence of pneumonia in the Alpha-Tocopherol, Beta-Carotene Cancer Prevention Study, 1985–1993. The left-hand side shows the proportion of participants in six subgroups. The right-hand side shows the effect of vitamin E supplementation 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.

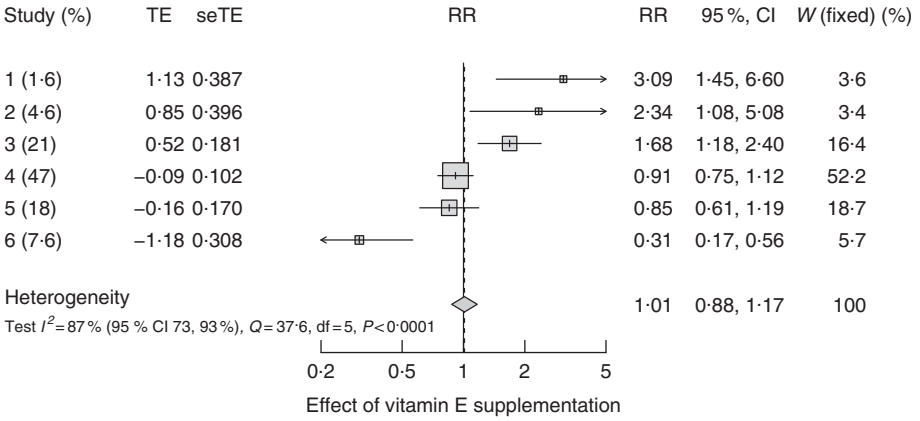

Fig. 2. A forest plot of six subgroups on vitamin E and the incidence of pneumonia in the Alpha-Tocopherol, Beta-Carotene Cancer Prevention (ATBC) Study, 1985–1993. The subgroups of Fig. 1 are shown in the same order in this forest plot. The percentage shown after group identification indicates the proportion 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 $P = 10^{-8}$.

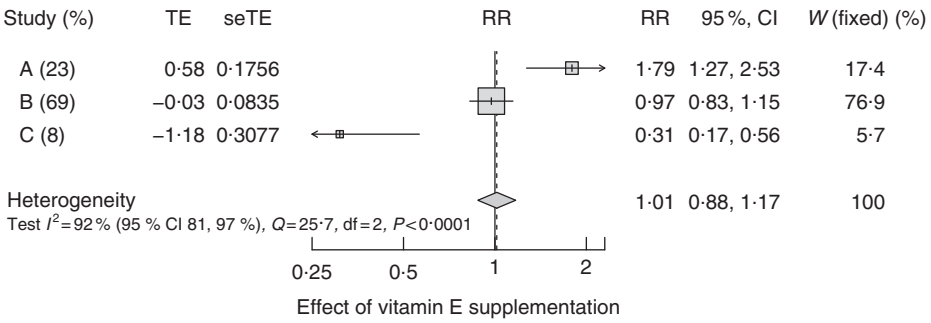

Fig. 3. A forest plot of three subgroups on vitamin E and the incidence of pneumonia in the Alpha-Tocopherol, Beta-Carotene Cancer Prevention (ATBC) Study, 1985–1993. Group A in this forest plot includes participants who started smoking at ≤20 years of age and smoked ≥20 cigarettes/d at study entry and 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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VITAMINS FOR THE PREVENTION OF COLDS

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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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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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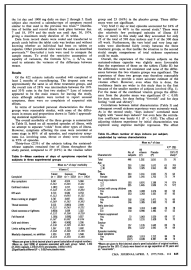
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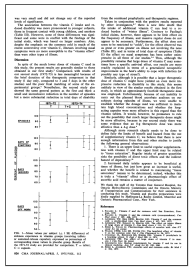
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Virus Pneumonia and Its Treatment With Vitamin C

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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

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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Vitamin C

VITAMIN C *

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).

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

Published online: January 29, 2008

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]

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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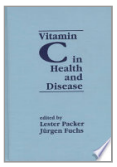

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Vitamin C in Health and Disease

By Lester Packer

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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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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.

Comment in

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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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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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
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

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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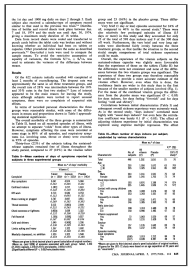
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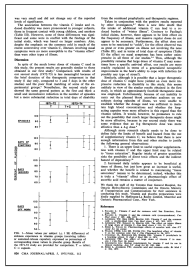
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