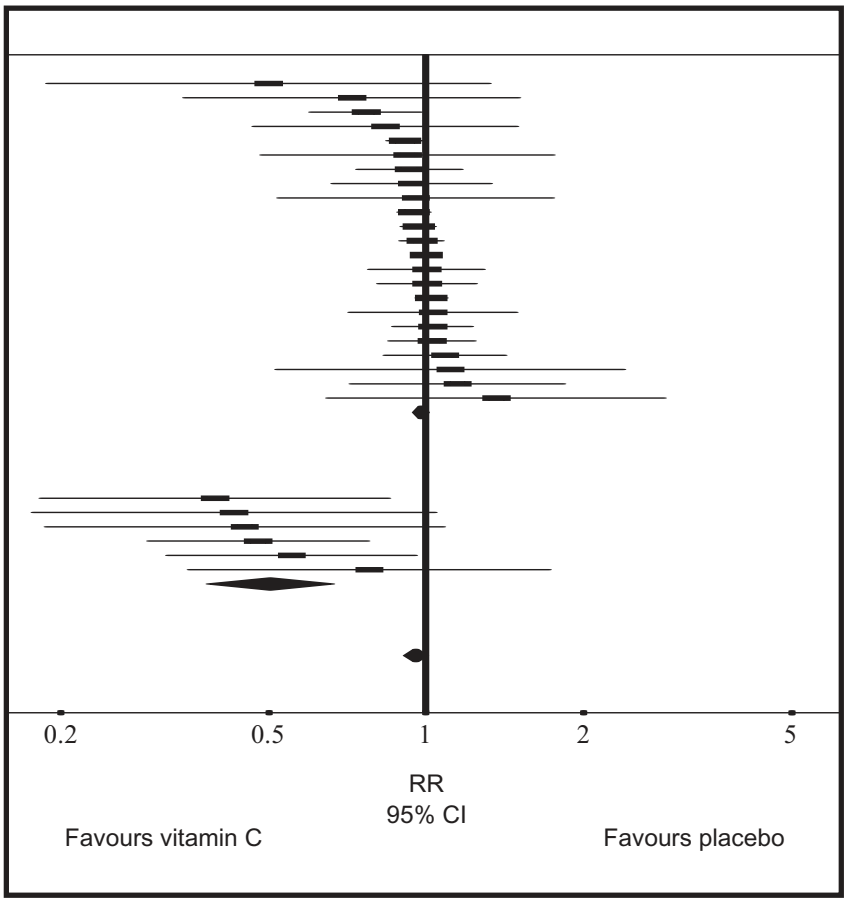
Do vitamins C and E affect respiratory infections ?

Harri Hemilä



Helsinki 2006

Do vitamins C and E affect respiratory infections ?

Harri Hemilä

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ACADEMIC DISSERTATION
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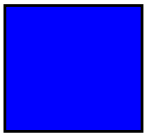

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Cover: Meta-analysis of the effect of vitamin C supplementation on common cold incidence. The lower subgroup of 6 trials consists of studies with participants under short term exposure to severe physical stress and/or cold, see p 48. The fifth trial from the top of the lower subgroup is the Ritzel trial (1961; Table 3; pp 13, 35-6, 43-5). The two diamond shapes below the two subgroups, and the third diamond shape below all the trials indicate the 95% confidence intervals for the respective pooled results. RR = 0.5 corresponds to 50% reduction in common cold incidence with vitamin C supplementation, and RR = 1.0 indicates no difference between vitamin C and the placebo. This graph is Fig. 1 from Douglas, Hemilä, *et al.* (2004); see also Douglas & Hemilä (2005) for a summary of the meta-analysis.

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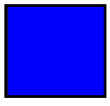


... Since then I never pay any attention to anything by ‘experts.’
I calculate everything myself.
I’ll never make that mistake again, reading the experts’ opinions.
Of course, you only live one life, and you make all your mistakes,
and learn what not to do, and that’s the end of you.

Richard Feynman, 1985
"Surely You're Joking, Mr. Feynman"

Have no respect whatsoever for authority;
forget who said it and instead look at what he starts with, where he ends up,
and ask yourself, "is it reasonable?"

Richard Feynman, 1988
"What Do You Care What Other People Think?"

When an old and distinguished person speaks to you, listen to him carefully and with respect - but do not believe him. Never put your trust in anything but your own intellect. Your elder, no matter whether he has grey hair or has lost his hair, no matter whether he is a Nobel Laureate, may be wrong. The world progresses, year by year, century by century, as the members of the younger generation find out what was wrong among the things that their elders said. So you must always be skeptical - always think for yourself.
There are, of course, exceptional circumstances: when you are taking an examination, it is smart to answer the questions not by saying what you think is right, but rather what you think the professor thinks is right.

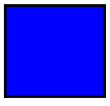
Linus Pauling, 1955
Advice to Students

I wish to propose for the reader’s favourable consideration a doctrine which may, I fear, appear wildly paradoxical and subversive. The doctrine in question is this: that it is undesirable to believe a proposition when there is no ground whatever for supposing it true, I must, of course, admit that if such an opinion became common it would completely transform our social life and our political system; since both are at present faultless, this must weight against it. I am also aware (what is more serious) that it would tend to diminish the incomes of clairvoyants, bookmakers, bishops and others who live on the irrational hopes of those who have done nothing to deserve good fortune here or hereafter.

Bertrand Russell, 1928
Sceptical Essays

… Where I have been necessarily led, in this disagreeable part of the work, to criticise the sentiments of eminent and learned authors, I have not done it with a malignant view of depreciating their labours, or their names; but from a regard to truth, and to the good of mankind. I hope such motives will, to the candid, and to the most judicious, be a sufficient apology for the liberties I have assumed.

James Lind, 1753
A Treatise of the Scurvy



LIST OF ORIGINAL PUBLICATIONS

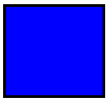
I **Hemilä H** (**1996**) Vitamin C, the placebo effect, and the common cold:
a case study of how preconceptions influence the analysis of results.
Journal of Clinical Epidemiology 49:1079-1084 http://dx.doi.org/10.1016/0895-4356(96)00189-8
and reply to comments 49:1087 http://dx.doi.org/10.1016/0895-4356(96)00191-6

II **Hemilä H** (**1997**) Vitamin C intake and susceptibility to the common cold.
British Journal of Nutrition 77:59-72 [will be available as a digitilized text]
and reply to comments 78:861-866 http://dx.doi.org/10.1079/BJN19970201

III **Hemilä H, Kaprio J, Albanes D, Heinonen OP, Virtamo J** (**2002**) Vitamin C, vitamin E and beta-carotene in relation to common cold incidence in male smokers.
Epidemiology 13:32-37 http://dx.doi.org/10.1097/00001648-200201000-00006

IV **Hemilä H, Kaprio J, Pietinen P, Albanes D, Heinonen OP** (**1999**) Vitamin C and other compounds in vitamin C rich food in relation to risk of tuberculosis in male smokers.
American Journal of Epidemiology 150:632-641
http://aje.oxfordjournals.org/cgi/content/abstract/150/6/632

V **Hemilä H, Virtamo J, Albanes D, Kaprio J** (**2004**) Vitamin E and beta-carotene supplementation and hospital-treated pneumonia in male smokers.
Chest 125:557-565 http://dx.doi.org/10.1378/chest.125.2.557



ABSTRACT

Deficiency of vitamin C causes a specific deficiency disease, scurvy, which led to the deaths of numerous seamen in the Age of Sail because they had no access to fruit or vegetables during long voyages. Nowadays scurvy is very rare in the Western countries. Vitamin E is found in such a great variety of foods and is stored in the body for such long periods that deficiency of this vitamin hardly ever results from poor diet.

Since a large number of studies found that vitamins C and E affect the immune system, it has been suggested that these vitamins might affect infectious diseases. Numerous animal studies found that vitamins C and E reduce the incidence and severity of various viral and bacterial infections. These findings justify research into whether these two vitamins affect the incidence and severity of respiratory infections in humans.

The earlier medical literature contains suggestions that vitamin C may be beneficial against respiratory infections, but this topic achieved wider popular interest only after 1970 when Linus Pauling, a dual Nobel Laureate, wrote a book in which he concluded that large doses of vitamin C prevent and alleviate colds. Pauling's proposal led to a large series of new placebo-controlled trials. It is shown in this thesis that these new trials consistently found that regular vitamin C supplementation shortens the duration and alleviates the symptoms of the common cold, partly confirming Pauling's hypothesis. The new trials did not find reduction of common cold incidence in the ordinary Western population with vitamin C supplementation. Nevertheless, in this thesis it is shown that there may be sub-populations, such as people undergoing heavy acute physical stress and young males with low dietary vitamin C intakes, in which regular vitamin C supplementation may reduce the incidence of the common cold.

While the trials carried out since 1970 have shown that vitamin C alleviates common cold symptoms, it is strange that major medical textbooks state that this vitamin has no effect on colds. In this thesis it is shown that the most influential reviews on vitamin C and the common cold cited in the major textbooks contain numerous erroneous statements, and that they even present data that are inconsistent with the original study reports. Consequently, the negative statements in the medical textbooks are based on biased reviews. It is also shown that the most influential vitamin C common cold trial carried out at the National Institutes of Health in the USA and published in *JAMA* in 1975 was erroneously analyzed.

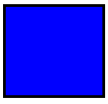
Overall, the analyses of the vitamin C common cold trials indicate that further therapeutic trials examining the effect of vitamin C on the common cold are warranted, in particular among children. Proper understanding of the restricted subgroups or particular living conditions in which daily vitamin C supplementation might be beneficial also requires further study.

The second part of this thesis consists of analyses of the ATBC (Alpha-Tocopherol, Beta-Carotene Cancer Prevention) Study with 29,133 male smokers. The current analysis focuses on the question of whether vitamin E might affect respiratory infections.

In the ATBC Study, vitamin E supplementation had no overall effect on the incidence of the common cold or pneumonia, but in both cases there was substantial modification of the vitamin E effect, suggesting benefit in restricted sub-populations. Common cold incidence was reduced by vitamin E in a small group of elderly city-dwellers who smoked 5-14 cigarettes per day. Although the effect was statistically highly significant, it was small and the assessment of its practical importance requires further study. The incidence of pneumonia was reduced among participants who started cigarette smoking at later ages. This effect was also statistically highly significant, but further work is needed to evaluate its practical significance. Vitamin E supplementation had no effect on the risk of tuberculosis.

The intake level of vitamin C in diet was estimated at the baseline of the ATBC Study. It was found that the level of vitamin C in diet had no association with the risk of the common cold or tuberculosis.

A major finding in this thesis is heterogeneity in the effects of daily vitamin C and vitamin E supplementation. While there is no evidence that ordinary people might benefit from supplementation of these two vitamins, the heterogeneity in effects indicates that further study should be carried out to identify and characterize the population groups or living conditions in which these vitamins might be beneficial.



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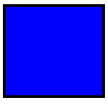

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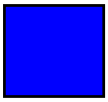
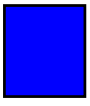
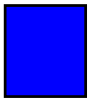


ABBREVIATIONS

Abbreviations that are used only in one chapter and defined within the same chapter are not listed here except when the term may need more explicit definition.

ATBC Study	The Alpha-Tocopherol, Beta-Carotene Cancer Prevention Study with 29,133 participants (p 52; ATBC 1994a,b)
CI	Confidence Interval; a measure of the accuracy of a result
DB	double blind
EBM	Evidence-Based Medicine
FDA	US Food and Drug Administration; regulates prescription and non-prescription drugs, and the labeling of products under its jurisdiction
FNB	Food and Nutrition Board of the NRC; sets up committees to establish RDA levels; NRC: National Research Council; the principal operating agency of the US National Academy of Sciences, administered jointly by the Academy and the Institute of Medicine (the latter established in 1970 by the US National Academy of Sciences to secure the services of eminent members of appropriate professions in the examination of policy matters pertaining to public health)
NIH	National Institutes of Health of the USA
NNT	Number Needed to Treat; how many people need to be treated to produce benefit for a single person
RCT	Randomized Controlled Trial
RDA	Recommended Dietary Allowance; the dietary intake level sufficient to meet the nutrient requirement of nearly all healthy individuals (FNB 1989a, 1994a, 2000)
OR	Odds Ratio; an approximation of the RR
RR	Risk Ratio; the ratio of risk in two populations; *e.g.*, if 10% of the study population and 40% of the control population contract a disease, then RR = 0.25





INTRODUCTION

Overview of vitamin C

History of vitamin C and its deficiency, scurvy, is fascinating and it is briefly summarized in **Appendix 1**.

Scurvy, the deficiency of vitamin C, was a serious disease in particular of sailors in the Age of Sail. James Lind (1753) wrote the classic monograph *A Treatise of the Scurvy* in which he reported a systematic review of all the earlier literature. In the literature before Lind, the clinical definition of scurvy had become ambiguous and in many cases the name was used for diseases unrelated to our current definition. Lind's own clinical definition was based on "putrid gums, swelled legs, and spots, accompanying each other, and in their progress usually attended with rigid tendons in the ham … observed in no other distemper".

In 1747, Lind (1753) carried out the first well described controlled trial in medicine, on HMS Salisbury. Lind treated two patients with scurvy with 2 oranges and 1 lemon per day, and to the other groups of scorbutic patients he administered other supposed anti-scurvy remedies. The patients administered oranges and lemons recovered rapidly whereas the other patients did not. In addition to providing direct evidence that oranges and lemons cured scurvy, Lind's trial was a milestone in medical research methodology. For example, Hampton (2002) commented that "the elegant trial of the use of oranges and lemons for the treatment of scurvy was hardly bettered until the trial of streptomycin for tuberculosis designed by Austin Bradford Hill [in 1948]."

In spite of his own trial showing that fruit cured scurvy, Lind was convinced that lack of fruit and vegetables was not the primary etiological cause of scurvy, and proposed that the primary cause was moisture of air (see Appendix 1). It seems that the correct explanation of the etiology of scurvy was first proposed in 1734 by John Bachstrom, a physician in Holland who proposed that scurvy was caused by the absence of fresh vegetable food from the diet for a considerable time. About a century later, John Elliotson (1831), professor of medicine in London, also proposed that scurvy was caused by the deficiency of some substances in diet. However, the concept of necessary minor constituents of diet started to be accepted in main-line medicine only in the early twentieth century, in particular, because of the work of Sir Frederick Hopkins who was awarded the 1929 Nobel Prize for his discovery of the growth-stimulating substances, which he called 'accessory food factors', that were later named vitamins (NF 2005a).

Although there was strong evidence that fruit cured scurvy, there was no biological rationalization for fruit, and a large number of false theories about scurvy prevailed long after Lind's 1747 trial (**Table 1**). Since vitamin C is synthesized by all mammals with the few exceptions of primates, the guinea pig and fruit-eating bats, a practical animal model for scurvy was not easy to find. Holst and Frölich (1907) were able to produce scurvy in guinea pigs and thereby an animal model for vitamin C deficiency was identified; however, see footnote to Table 1. Vitamin C was first isolated by Albert Szent-Györgyi, who was awarded the Nobel Prize in Medicine or Physiology in 1937 partly for identifying vitamin C (NF 2005b). The chemical structure of vitamin C was solved by Haworth, who was awarded the Nobel Prize in Chemistry in 1937 (NF 2005c).

Typical symptoms of classical scurvy include swollen and bleeding gums, dropping teeth, and poor healing of wounds (Hess 1920; Kalaja 1939; Crandon *et al.* 1940; Peters *et al.* 1948; Krebs 1953; Hodges *et al.* 1971; Carpenter 1986; Forsius 1997; Harvie 2002; Bown 2003). Since these symptoms are explained by the participation of vitamin C in the synthesis of collagen, major textbooks of biochemistry mention only the role of vitamin C in proline hydroxylation (*e.g.*, Berg *et al.* 2002). Vitamin C, however, also participates in the enzymatic synthesis of dopamine, carnitine, and a number of neuroendocrine peptides (Englard & Seifter 1986; Levine 1986; Hughes 1988; Padh 1990; Hemilä & Antila 1993b; Rebouche 1991; Rice 2000). In addition, vitamin C participates in the transformation of cholesterol into bile acids (Ginter 1973, 1989; Hemilä 1990d, 1992c, 1993). The survival time of vitamin C deficient guinea pigs is extended by carnitine (Jones & Hughes 1982) and glutathione (Mårtensson *et al.* 1993), indicating that scurvy is not explained simply by the defects in collagen hydroxylation. Furthermore, vitamin C is a powerful reducing agent, antioxidant, and reacts with oxidants produced by phagocytes, through which it may affect the functions of the immune system (Hemilä *et al.* 1984; Hemilä 1992a,b, 1997a, 2003a). Thus the notion presented in the textbooks that vitamin C participates only in the hydroxylation of proline in collagen is grossly oversimplified and misleading.

A number of early animal studies indicated that vitamin C may affect susceptibility to infection (Perla & Marmorston 1937). After James Lind's treatise on scurvy, the next English treatise was written by Alfred Hess (1920; Darby & Woodruff 1962; Wiedemann 1993), a pediatrician in New York. In various parts of his monograph, Hess noted the increased risk of infection, in particular pneumonia, in vitamin C deficiency. A decade later, in a major medical journal, Hess (1932) commented that in "infantile scurvy … a lack of the antiscorbutic factor which leads to scurvy, at the same time predisposes to infections [particularly of the respiratory tract]. … Similar susceptibility to infections goes hand in hand with adult scurvy." Such opinions did not leave traces in mainstream medicine and, according to the current widespread consensus, vitamin C has relevance only in preventing and curing classical scurvy.

The current world production of vitamin C is about 100,000,000 kg per year, *i.e.*, 15 grams per year per each inhabitant of the globe (Hancock & Viola 2002; Baier 2004). Approximately half of the vitamin C produced is used in vitamin supplements and pharmaceutical preparations. A survey of female physicians in the USA found that 18% of

Table 1. Erroneous theories of scurvy by eminent people maintained after James Lind's controlled trial in 1747, which showed that citrus fruit cured scurvy

Person and his position	Theory of scurvy
James Lind (1716-1794) [1] Carried out the first systematic review and the first well documented controlled trial	Caused by moisture, prevented and cured by dry air [1]
Sir John Pringle (1707-1787) President of the Royal Society Physician to King George III	Caused by putrefaction, correctable by foods fermenting to yield carbon dioxide (wort of malt)
Sir Robert Christison (1797-1882) President of the British Medical Association, Physician to Queen Victoria	Caused by protein deficiency
Lord Lister (1827-1912) President of the Royal Society, Surgeon to Queen Victoria	Caused by ptomaine intoxication (substances in spoiled food)
Jean-Antoine Villemin (1827-1892) Member of the Academy of Medicine, Paris	Caused by a contagious miasm
William Hammond (1828-1900) US Army Surgeon-General	Caused by deficiency of potassium and/or iron
Elmer McCollum (1879-1967) [2] The most important US nutrition scientist in the early 1900s	Caused by constipation and cured by laxatives [2]

Modified of Carpenter (1986) Table 10.4

[1] See Appendix 1.

[2] McCollum discovered vitamin A and was "one of the giants of nutritional biochemistry" (Simoni *et al*. 2002). For the work of McCollum, see McCollum (1953, 1967), Rider (1970), Day (1974, 1979, 1997), Schneider (1986), Carpenter (2003b), JHBSPH (2005).

Each year the E.V. McCollum Award is given by the American Society of Clinical Nutrition to a clinical investigator currently perceived as a major creative force, actively generating new concepts in nutrition, and personally seeing to the execution of studies testing the validity of these concepts (ASCN 2005). In 1972, the McCollum award was given to Victor Herbert (see pp 62-4 of this thesis), and in 1990 to Ranjit K. Chandra (see pp 16-8 of this thesis).

In 1917, McCollum and Pitz published the results of a series of experiments with guinea pigs in a paper which was recently categorized as a "classic paper" by the *Journal of Biological Chemistry* (Simoni *et al*. 2002).

McCollum and Pitz (1917) stated in the *Journal of Biological Chemistry* paper that "the experimental data presented in this paper form a conclusive line of evidence which proves that scurvy in the guinea pig is not a deficiency disease in the sense in which Holst, Funk, Hess and others have regarded it … The efficiency of orange juice as an antiscorbutic may well be accounted for by its content of sodium and potassium citrates, both of which possess laxative properties" (p 234), "chart 7 offers definite and convincing evidence that scurvy is in reality the sequel to retention of feces in the cecum" (p 235), "we are inclined to attribute the beneficial effects of orange juice to its laxative action" (p 237), "the observations reported in this paper furnish definite support for the idea that scurvy in the guinea pig is not the result of the deficiency of a specific protective substance… it becomes necessary to offer a new interpretation as to the etiology of experimental scurvy in the guinea pig. Our interpretation, that the first cause of the disease is associated with the retention of feces … is we believe supported by adequate experimental data" (pp 238-9). "The significance of this interpretation is far reaching. It removes from the list one of the syndromes (scurvy) which has long been generally accepted as being due to dietary deficiency" (p 239). "This fact, together with convincing evidence that scurvy is not in reality a deficiency disease in the sense of being caused by a lack of specific protective substance, warrants an attitude of skepticism regarding the validity of the 'vitamine' theory of the etiology of such other diseases as pellagra, rickets, etc., which have been attributed to specific dietary deficiency" (p 239-40). "… There is therefore no reason whatever why we should assume as Voegtlin, Goldberger, Funk, and other have done that pellagra is due to a lack of a specific unidentified dietary factor, a 'vitamine' " (p 241).

McCollum (1917) repeated his conclusions in JAMA: "Scurvy in the guinea-pig is the result of the retention of feces… I am inclined to attribute the protective power of orange juice as an antiscorbutic to its content of certain salts of citric acid, rather than to the presence of an unidentified organic substance of the class of the so-called vitamins" (p 1385).

them were regularly using vitamin C supplements (Frank *et al.* 2000), and about 30% of the general US adult population takes vitamin C supplements (FNB 2000). This high level consumption of vitamin C by people's own initiative makes the health effects of supplementation of considerable public health interest, be they positive or negative.

Overview of vitamin E

The history of vitamin E is much less dramatic than that of vitamin C (Mason 1977; Raacke 1983; Ames 1984). Vitamin E deficiency caused sterility in male and female rats (Evans & Bishop 1922), and vitamin E was isolated from wheat germ oil using the 'ability of rats to bear litters' as the biological test. Its chemical name, tocopherol, was derived from tocos ('childbirth' in Greek) and phero ('bringing' birth in Greek).

Because of the ubiquitous distribution of vitamin E in grain, vegetable oil and animal fat, vitamin E deficiency is virtually nonexistent as a result of poor diet (FNB 2000). Vitamin E deficiency can occur because of malabsorption of dietary vitamin E resulting from various gastrointestinal disorders that interfere with the digestion or absorption of lipids. In humans, vitamin E deficiency causes various neurological symptoms such as ataxia and myopathy (Sokol 1993).

Vitamin E is a fat-soluble antioxidant that prevents the propagation of free-radical reactions, which seems to be its main biochemical function (FNB 2000). In particular, vitamin E protects polyunsaturated fatty acids within membrane phospholipids and in plasma lipoproteins. Vitamin E also inhibits protein kinase C activity, but the physiological significance of this is not clear (FNB 2000; Ricciarelli *et al.* 2001; Azzi *et al.* 2002).

The first report suggesting that vitamin E might affect the severity of infections was published in the 1940s (Sabin & Duffy 1940; p 131), but wider interest in its possible effects on the immune system and susceptibility to infection was initiated in the 1970s with studies on chickens (Tengerdy *et al.* 1972; Nockels 1979).

A survey of female physicians in the USA found that 16% were regularly using vitamin E supplements (Frank *et al.* 2000), and about 25% of the general US adult population take vitamin E supplements (FNB 2000). This high proportion of people using vitamin E supplements makes the health effects of such supplementation of considerable public health interest irrespective of whether the effects are beneficial or harmful.

REVIEW OF THE LITERATURE

Infections studied in the present work

The common cold

The term 'the common cold' does not denote any precisely defined disease, but this illness is personally familiar to practically everybody. Typically the symptoms of the common cold consist of some combination of nasal discharge and obstruction, sore throat, cough, lethargy, and malaise, with or without fever. On average, young children have 6 colds per year, and the incidence decreases with age so that elderly people have about one per year (Monto 1994). The common cold is the leading cause of acute morbidity and of visits to a physician in the Western countries, and a major cause of absenteeism from work and school (Gwaltney 2005).

The common cold is usually caused by respiratory viruses (rhino, corona, adeno, parainfluenza, influenza, respiratory syncytial), which overall have some 200 serotypes (Gwaltney 2005; Puhakka 1999; Mäkelä *et al.* 1998; West 2002; Heikkinen & Järvinen 2003). Thus, the term 'common cold' does not refer to a single entity but to a group of diseases caused by numerous unrelated etiological agents. The most frequent agent causing the common cold is rhinovirus, which is found in some 30-50% of sufferers. In subjects with cold symptoms, the etiology remains undefined in some third of subjects even when extensive virological tests are used. It is not clear to what extent this latter group is explained by the low sensitivity of the tests, unidentified viruses, or similar symptoms arising from non-viral etiology. Different respiratory viruses have different symptom profiles, but the patterns are not consistent enough to validate etiological conclusions from the patients' symptoms.

Although the great majority of common cold episodes are caused by the respiratory virus group, the symptom-based definition of the 'common cold' also covers some diseases caused by other viruses (varicella, measles, rubella, cytomegalo, Epstein-Barr) and some bacterial infections. For example, since streptococcal pharyngitis cannot be differentiated from viral pharyngitis on clinical grounds, it can also be included within the broad definition of the common cold. Symptoms of illnesses caused by *Mycoplasma pneumoniae* and *Chlamydia pneumoniae* may also be similar to the symptoms caused by the respiratory viruses.

The large number of etiological agents, the mild character of the disease, and the high cost of the virological tests (*e.g.*, $ 700 per patient in Mäkelä *et al.* 1998) mean that a functional everyday definition of the 'common cold' cannot be based on laboratory tests, but must be based on symptoms. A chest x-ray has no relevance in excluding pneumonia when the symptoms are mild and the patient is not seriously ill. The manifestations of the common cold are so typical that self-diagnosis by the patient is usually correct. Hay fever and vasomotor rhinitis may give similar nasal symptoms, but the recurrent and chronic nature of these diseases is soon recognized by the patient and easily diagnosed by the physician from the patient's history (Gwaltney 2005).

In several common cold trials an explicit definition of the common cold has been used for logistic reasons; for example, based on the duration and the set of symptoms to yield an explicitly defined outcome. Such limits are, however, biologically arbitrary. There is no exact minimum duration or combination of symptoms which is meaningful when drawing a conclusion as to whether the symptoms should be explained by a viral infection or by allergic or mechanical irritation of nasal airways or throat.

The use of antibiotics for a typical acute common cold episode is useless since the vast majority of colds are caused by viruses (Arroll & Kenealy 2005). Nevertheless, about half of common cold patients in the USA received antibiotics according to some surveys (Mainous *et al.* 1996; Gonzales *et al.* 1997). In this respect, the alternative treatment options for the common cold are of substantial public health interest (Turow 1997).

Pneumonia

Pneumonia denotes infection of the lungs, *i.e.*, an infection defined by an anatomical region. 'Community-acquired pneumonia' means pneumonia cases occurring at home, and 'hospital acquired (nosocomial) pneumonia' means pneumonia cases initiating in hospitals. The practical reason for this division is that the spectrum of etiological agents causing these two types of pneumonia is different, and consequently the selection of antibiotics and prognosis is different.

Pneumonia can be caused by bacteria, viruses, *Rickettsia*, fungi, or parasites, and close to 100 species in these groups have been identified as etiological agents (Donowitz & Mandell 2005). The majority of diagnosed pneumonia cases are caused by bacteria, the most common being *Streptococcus pneumoniae* which causes 15-40% of the acute community-acquired pneumonia (Fine 2003). In various studies, viruses caused about 8-18% of community-acquired pneumonia episodes while the etiological agent remained unidentified in 20-45% of cases. In the case of pneumonia which is not severe, it is impractical to try to identify the etiological agent. Different bacteria generally have differences in the sets of symptoms which are relevant in choosing a proper antibiotic as the susceptibility of bacteria to antibiotics varies. Pneumonia caused by fungi and parasites is rare and usually affects subjects with serious background diseases.

The suspicion that a patient has pneumonia usually arises when the general condition of the subject has been declining in association with respiratory symptoms such as cough, chest pain, and difficulty in breathing, often associated with fever. Diagnosis of pneumonia is usually based on new infiltrates in a chest x-ray, but high resolution computed tomography can sometimes identify pulmonary changes caused

by pneumonia even when the chest x-ray is normal (Syrjälä *et al.* 1998).

The risk of pneumonia is elevated in young children and the aged (Graham 1990). It has been estimated that in developing countries pneumonia causes annually about 2 million deaths in children under 5 (Graham 1990; Jones *et al.* 2003; Rudan *et al.* 2004). In Finland, the annual rate of pneumonia-related hospital treatment is 2.7 per 1,000 men and 1.1 per 1,000 women in the age group of 15-64 years, and 26 per 1,000 men and 18 per 1,000 women among people aged ≥65 years (Säynäjäkangas *et al.* 1997a,b; Säynäjäkangas 1999).

Tuberculosis

In contrast to the common cold and pneumonia, tuberculosis has an exact etiological definition as a disease caused by *Mycobacterium tuberculosis* (Fitzgerald & Haas 2005). Nevertheless, it is important to differentiate tuberculosis infection from tuberculosis disease. Infection implies seeding of some anatomic focus with the bacterium, which may or may not cause clinical disease. It has been estimated that one third of world's population is infected with *M. tuberculosis* (Corbett *et al.* 2003). Tuberculosis infection can be detected by the tuberculin test (Mantoux) in countries where BCG (Bacille Calmette-Guerin) vaccination is not used, and consequently it is not a practicable method in Finland where children are generally BCG vaccinated.

A latent tuberculosis infection can be activated into disease by various factors affecting the immune system such as aging, chronic diseases, and malnutrition. The most common form of tuberculosis disease is pulmonary tuberculosis, *i.e.*, a specific type of pneumonia, but the tuberculosis bacterium can also affect various other organs, in which case the term 'extrapulmonary tuberculosis' is used. The best method of diagnosing tuberculosis disease is to culture or stain the bacteria, or to detect the DNA by the polymerase chain reaction (PCR) method. Pulmonary tuberculosis can usually be identified by typical changes in the chest x-ray.

It has been estimated that tuberculosis causes the death of some 2 million people per year in the developing countries (Corbett *et al.* 2003). In Finland, tuberculosis was a major problem in the early twentieth century, but its incidence dramatically decreased towards the end of the century. In 1940 there were 7,842 deaths caused by tuberculosis in Finland, but in 1995 there were only 135. In 1995 there were 561 new cases of tuberculosis, 391 cases being pulmonary tuberculosis and 170 cases extrapulmonary tuberculosis (Härö 1998).

Vitamin C and infections

Effects on the immune system

In the intensive search for specific molecules participating in the defense against viruses and bacteria, vitamin C has not been particularly interesting as it is not specifically and firmly linked to any single immunological mechanism. Still, it is possible that as an efficient reducing agent vitamin C has non-specific effects on diverse parts of the immune system (Hemilä 1992a, 1997a, 2003a).

Upon activation, phagocytes release a set of oxidizing agents intended to kill viruses and bacteria. Many of these oxidants appear to be harmful to the host cells, and in some cases the oxidizing agents produced during viral and bacterial infections seem to play a substantial role in the pathogenesis (Goode & Webster 1993; Akaike *et al.* 1998; Peterhans 1997). Vitamin C is an efficient water-soluble antioxidant and may protect host cells against the oxidants released by phagocytes, for example (Hemilä *et al.* 1984; Frei *et al.* 1989; Hemilä 1990a, 1992a,b, 1997a). Phagocytes have a specific transport system by which the oxidized form of vitamin C (dehydroascorbic acid) is imported into the cells, where reduced vitamin C is regenerated (Wang *et al.* 1997; Nualart *et al.* 2003). If the major role of vitamin C in the immune system is that of a physiological antioxidant protecting various host cells against oxidative stress during an infection, it could have important effects in certain conditions even though the mechanisms are apparently non-specific.

A number of authors have reviewed the literature on vitamin C and the immune system (Bourne 1949; Thomas & Holt 1978; Gross & Newberne 1980; Beisel 1982; Leibovitz & Siegel 1981; Cunningham-Rundles *et al.* 1993; Jariwalla & Harakeh 1996; Hemilä 1992a, 1997a). Overall, more than 100 studies have examined the effect of vitamin C on various parts of the immune system (Hemilä 1997a). The concentration of vitamin C in phagocytes and lymphocytes is far higher than in plasma, indicating that it may have functional roles in these immune system cells. In various experimental settings, vitamin C has affected random migration and chemotaxis of phagocytes (Goetzl *et al.* 1974), transformation of influenza virus-infected lymphocytes (Manzella & Roberts 1979), production of interferon (Siegel 1974), and replication of viruses (Atherton *et al.* 1978; Bissell *et al.* 1980; Harakeh *et al.* 1990). In guinea pigs, vitamin C deficiency suppressed the development of tuberculin skin reactivity (Mueller & Kies 1962; Zweiman *et al.* 1966). Recently, vitamin C was reported to affect the gene expression of monocyte adhesion molecules (Rayment *et al.* 2003). In some studies it affected complement and immunoglobulin levels, but the literature is quite inconsistent on these topics.

Although the great majority of studies found effects suggesting benefits from vitamin C on the immune system, one study with 5 subjects found that 2 g/day vitamin C impaired *in vitro* bactericidal activity of leukocytes when using *E. coli* as the test organism (Shilotri & Bhat 1977). The significance of this solitary report is unclear.

Even if vitamin C had a role in the immune system, it is possible that the level of vitamin C intake is important only in particular conditions. For example, if vitamin C intake is important only in the low range of intakes, it is possible that the variation in intake does not affect the immune system in

the ordinary Western population. Still, it might be a limiting factor in populations with low vitamin C intakes.

If the fundamental question is whether vitamin C intake affects the incidence and severity of infections, then laboratory measures of the immune system are only surrogate markers. Since there are quite a few examples where the effect on a clinical outcome diverged from the effect on a surrogate end point (Fleming & DeMets 1996; DeGruttola *et al.* 1997), the effects on surrogate end points should be considered cautiously. Neither are the physiological mechanisms usually crucial in epidemiological reasoning when considering potential cause and effect relationships, since what is biologically plausible depends upon the biological knowledge of the day and in many cases this understanding is quite limited and changes with time (Hill 1965). Nevertheless, the extensive literature on the effects of vitamin C on the immune system provides a meaningful conceptual framework to consider whether this vitamin might have effects on infections.

Vitamin C metabolism during infections

In guinea pigs, tuberculosis and other infections lead to a decrease in vitamin C concentration in the adrenals and urine (Abbasy *et al.* 1937a,b; Harris *et al.* 1937; Birkhaug 1938). In rats, *Trypanosoma* infection decreased the concentration of reduced vitamin C in spleen and adrenals, and increased the proportion of vitamin C in the oxidized dehydroascorbic acid form (Nyden 1948). In mice, influenza A infection resulted in a decrease in vitamin C concentration in bronchoalveolar lavage fluid, concomitant with an increase in dehydroascorbic acid (Buffinton *et al.* 1992). In macaque monkeys, malaria caused a reduced vitamin C level in plasma (McKee & Geiman 1946). In cows, intramammary infusion of *E. coli* (Weiss *et al.* 2004) and clinical mastitis (Kleczkowski *et al.* 2005) decreased plasma vitamin C concentration. Furthermore, bacterial toxins cause loss of vitamin C from many tissues (Harde 1934; Lyman & King 1936; Harris *et al.* 1937; Torrance 1940; Garcia *et al.* 1990; Rojas *et al.* 1996; Benito & Bosch 1997; Yamaguchi *et al.* 1997; Armour *et al.* 2001; Victor *et al.* 2002), but one study found an increase in vitamin C level in the livers of mice administered endotoxin (Jeffries 1965).

A large number of studies with human subjects found that vitamin C levels decrease in plasma, leucocytes and urine during various infections, including pneumonia and tuberculosis (Leppo 1939; Banerjee *et al.* 1940; Sweany *et al.* 1941; Sinha *et al.* 1984; Tanzer & Özalp 1993; Hunt *et al.* 1994; Galley *et al.* 1996, 1997; Pfitzenmeyer *et al.* 1997; Plit *et al.* 1998; Bakaev *et al.* 2004; for further refs., see Hemilä 1997a; Hemilä & Douglas 1999). Moreover, several reports noted that more severe forms of tuberculosis are more often associated with lower vitamin C levels than milder forms of the disease (Hemilä 1997a).

In the common cold infections, plasma, leukocyte and urine vitamin C levels decrease (for refs., see Hemilä 1992a, 1997a). Hume and Weyers (1973; see also Pauling 1973) reported that vitamin C level in leukocytes was reduced to half when their subjects contracted a cold, but the level returned to the original level in about a week after the episode. Vitamin C supplementation (6 g/day) essentially abolished the fall in leukocyte vitamin C level caused by colds (**Fig. 1**).

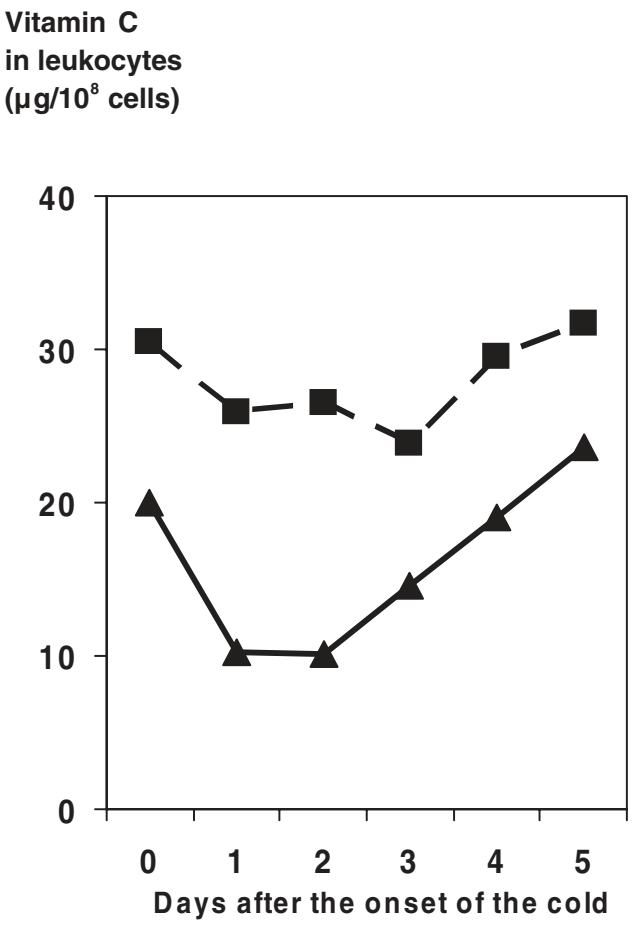


Fig. 1. The effect of the common cold infection on leucocyte vitamin C level. In 7 control participants, the vitamin C level in leucocytes (µg/10^8 cells) is indicated by triangles. Vitamin C level is indicated by squares in 3 vitamin C participants supplemented regularly with 1 g/day and, after the onset of the cold, with 6 g/day for 3 days, and thereafter with 1 g/day again. Day 0 indicates pre-cold level. The figure is based on tables I and III of Hume & Weyers (1973).

Although low vitamin C levels in patients with infections may partly be explained by low dietary intakes, there are several studies in which the dietary intake of vitamin C was comparable between the patients and healthy controls, indicating that low dietary intake cannot be the only cause of low vitamin C levels in the patients with infections (Hemilä 1997a). Increased levels of dehydroascorbic acid have also been reported in patients with various infections, consistent with the notion that vitamin C may be oxidized during infections (Banerjee & Belavady 1953; Chakrabarti & Banerjee 1955).

Endotoxin administration reduced plasma vitamin C concentration in human subjects (Pleiner *et al.* 2002, 2003), and vitamin C supplementation abolished the exercise-induced increase in plasma endotoxin level (Ashton *et al.* 2003).

Infections in animals

When you can measure what you are speaking about, and express it in numbers, you know something about it; but when you cannot measure it, when you cannot express it in numbers, your knowledge of it is of a meager and unsatisfactory kind.

Lord Kelvin (1883)

Since primates and the guinea pig have lost their capability to synthesize vitamin C (Burns 1957; Pauling 1970b; Chatterjee 1973; Sato & Udenfriend 1978; Nishikimi & Yagi 1996; Nandi *et al.* 1997; Smirnoff 2001), these animals are of primary interest when considering the possible effects of low vitamin C intake on susceptibility to infection.

In the early studies on vitamin C deficiency in the guinea pig, bacteria were so often found in histological sections to indicate the infectious nature of scurvy to some authors. Hess (1920 pp 133-4) concluded that such results show merely that the tissues of scorbutic animals frequently harbor bacteria, but "There is no doubt that invasion of the bloodstream does occur readily in the course of scurvy, but this takes place generally after the disease has developed and must be regarded as a secondary phenomenon and therefore unessential from an etiological standpoint. Indeed one of the striking and important symptoms of scurvy is the marked susceptibility to infection" (p 32). Summarizing autopsy findings of experimental scurvy in the guinea pig, Hess also noted that "Pneumonia is met with very frequently and constitutes a common terminal infection" (p 123) and "The superficial lymphatic glands are frequently palpable in scurvy, especially those in the inguinal region… It has seemed to us attributable less to the nutritional condition than to the infections which so frequently complicate the disorder" (p 141).

A large number of studies have reported that guinea pigs fed a vitamin C deficient diet have low resistance to various infections (for review, see Höjer 1924; Clausen 1934; Robertson 1934; Perla & Marmorston 1937; Birkhaug 1938; McCullough 1938; Scrimshaw *et al.* 1968; Hemilä 1997a, 1998). Most of the early animal studies did not examine the effect of pure vitamin C, the 'vitamin C group' being administered oranges or other fruit or vegetables containing vitamin C. Even though the positive findings in such studies are consistent with the notion that vitamin C intake may affect susceptibility to infection, the effect may be caused by other substances in fruit and vegetables as well (see Paper IV). The studies in which pure vitamin C was administered to the 'vitamin C group' are thus presented separately (**Appendix 2**) from those in which the 'vitamin C group' was administered fruit or vegetables as the source of the vitamin (**Appendix 3**). Studies in which animals were administered diphtheria toxin, tetanus toxin, or endotoxin are included in the tables, since these toxins are essential components in the pathogenesis of the bacteria concerned. A meta-analytic summary of the animal studies with pure vitamin C is shown in **Table 2**.

The studies in Appendix 2 are ordered with respect to the importance of the animal model to human beings so that those with primates are first, followed by guinea pigs, and thereafter studies with animals producing vitamin C, first mammals and then birds. At the end of Appendix 2 there are studies with fish, which are incapable of synthesizing vitamin C, yet in evolutionary terms they are most distant from humans.

In a series of studies with rhesus monkeys infected with poliovirus, Jungeblut (1937a,b, 1939) found significant benefit from vitamin C in several settings, but there was considerable variation in the results. In a small study, Albert Sabin (1939) found no effect using vitamin C. The studies by Jungeblut and Sabin differ in the method of virus inoculation, and Sabin administered oranges to his control monkeys, whereas Jungeblut does not describe the basal diet; these are some examples of potential factors explaining the inconsistency in their findings. With marmosets, Murphy *et al.* (1974) found that vitamin C increased resistance against experimental parainfluenza infection. Several studies with guinea pigs found that vitamin C protected against *M. tuberculosis* and *M. bovis*, and against various other micro-organisms (Appendix 2).

Rabbits, cats and mice synthesize vitamin C and thus cannot be used to study the effects of vitamin C deficiency, but the effects of supplementation can be studied in these species (Appendix 2). Three studies with rabbits found protection against pneumococcus using vitamin C, and cats administered vitamin C recovered faster from rhinotracheitis. Several studies with mice found benefits from vitamin C supplementation.

Davelaar and Bos (1992) found substantial protection in chickens against infectious bronchitis virus (IBV). This is an interesting report because IBV belongs to the family of coronaviruses which includes the recently identified SARS (severe acute respiratory syndrome) coronavirus (Hemilä 2003b). Vitamin C also protected chickens against *Salmonella* and *E. coli* infections (Appendix 2). Finally, a few studies found that vitamin C supplementation improved the resistance of fish to various infections.

Appendix 2 lists 148 separate animal studies with vitamin C, 86 of which found a statistically highly significant benefit, $P \leq 0.01$, on at least one infectious disease outcome, while 58 studies reported benefit at a level of $P \leq 0.001$ (Table 2). Of 100 studies with mammals, 58 found highly significant benefit, $P \leq 0.01$, from vitamin C on some infectious disease outcome.

Although many of the studies in Appendix 2 are old, it seems unlikely that administering a fixed dose of vitamin C and evaluating mortality were meaningfully different in the older days compared with modern methods. Furthermore,

97 of the papers were published in the 1970s or later, and 56 of these newer papers reported a statistically highly significant effect, $P \leq 0.01$, on at least one infectious disease outcome (Appendix 2). Furthermore, among the non-significant recent studies, Bourke *et al.* (1980) reported over 100% longer median survival time in vitamin C supplemented mice infected with malaria, but the number of animals was not reported, making any statistical interpretation impossible. In mice infected with pneumococcus, Esposito (1986) found no effect on mortality; nevertheless, vitamin C significantly enhanced the clearance of pneumococci from the lungs. Thus, there is no apparent pattern that the newer studies would have yielded less positive results than the older. In most of the studies in Appendix 2, statistical analy-

Table 2. Meta-analysis of the studies on animal infections: effects of vitamins C and E

Vitamin C studies (for the studies, see Appendix 2)	Number of studies		
		Benefit in any infectious disease outcome with	
Category	Total in the category	P[1-t] ≤ 0.01	P[1-t] ≤ 0.001
All studies	148	86	58
Published ≥1970	97	56	39
Mammals	100	58	40
Monkeys or guinea pigs	49	25	15

Vitamin E studies (for the studies, see Appendix 4)	Number of studies		
		Benefit in any infectious disease outcome with	
Category	Total in the category	P[1-t] ≤ 0.01	P[1-t] ≤ 0.001
All studies	70	37	20
Published ≥1970	69	37	20
Mammals	44	28	13

A 'study' is defined here operationally as the group of findings with a stated number of animals in Appendices 2 and 4.

Appendices 2 and 4 are based on extensive literature searches. Studies in which the number of events is very small are excluded, since they are uninformative. A few studies used different levels of vitamins C or E, the lowest being compared with the highest in most cases in the appendices, but in some cases different vitamin groups are combined and, when pertinent, different levels of intake are shown separately against the lowest level.

In some studies, different amounts of bacteria or different time-points, etc. were used and in these cases the selection of results shown in Appendices 2 and 4 involved subjective considerations, but the goal was to present the findings as objectively as possible given the complexity of some publications. A few reports stated in their text that vitamin C or E had no effect on a relevant outcome but no explicit data was mentioned preventing inclusion of those studies in the Appendices.

ses of the results are missing or inappropriate in the original papers, and in this respect the statistical analyses in Appendix 2 are essentially all novel.

Matsumoto *et al.* (1992) examined the effect of 2-octadecylascorbic acid in parallel with ascorbic acid, but only the results with the latter are presented in Appendix 2. Nevertheless, assuming that the effect of vitamin C on infections is based on the antioxidant effect, the results with the derivative are also of interest. When 2-octadecylascorbic acid was administered subcutaneously in *E. coli* infected rats, only 2 of 8 rats produced renal scarring of the two highest scores (2 or 3), in contrast to all 8 control rats (mid-P = 0.002). Similarly with pyelonephritis caused in rats by *Serratia marcensces,* subcutaneous administration of the derivative resulted in none of 8 rats producing renal scarring of the two highest scores (2 or 3), in contrast to 7 of the 8 control rats (mid-P = 0.000,4).

Nonaka *et al.* (1990) studied the effect of 2-octadecylascorbic acid on mouse endotoxemia. None of the mice administered the vitamin C derivative died (0/20) whereas 45% (9/20) of the control mice died (mid-P = 0.000,3).

Appendix 3 lists studies in which vitamin C group was administered either fruit or vegetables as the source of vitamin C. Albert Sabin (1939) found that rhesus monkeys fed vitamin C deficient diet had a high mortality due to infections, in particular pneumonia. In a number of studies using guinea pigs, fruit or vegetables protected against various infections better than a deficient diet. Although vitamin C is an important substance in fruit and vegetables, it is also possible that the differences are caused by substances other than vitamin C (see Paper IV). In this respect, no specific conclusion can be drawn from Appendix 3 as to the importance of vitamin C *per se,* although these studies are consistent with the notion of benefits from higher levels of vitamin C intake.

In addition to the animal studies yielding quantitative data on the effect of vitamin C on infections (Appendices 2 and 3), a few studies reported qualitative findings on its role on infections. Russell *et al.* (1944) found that caseous foci caused by tuberculosis were considerably larger in vitamin C deficient guinea pigs than in animals administered lettuce as a source of vitamin C. Tuberculous lesions in the spleen were also substantially larger and less well demarcated in vitamin C deficient guinea pigs than in normal animals. Höjer (1924) infected guinea pigs with tuberculosis bacilli and, compared with normally nourished animals, he found that in animals that had scurvy, tuberculous foci in muscles, lymph-glands and the spleen were not surrounded or penetrated by any connective tissue, and the necrotic parts passed into non-tubercular tissue without such demarcation. Meyer and Meyer (1944) reported that abscesses caused by staphylococcal infection remained soft and undemarcated in vitamin C deficient guinea pigs, whereas they were hard and button-like in animals administered pure vitamin C. In an uncontrolled study, Hans Zinsser *et al.* (1931) found that the infection caused by *Rickettsiae* was very severe in vitamin C deficient guinea pigs, so that particularly high concentrations of *Rickettsiae* were found in pleural and peritoneal exudates. Witt *et al.* (1988) reported that an unexpected outbreak of pneumococcal infections in guinea pigs was associated with a diet unintentionally low in vitamin C. Nungester *et al.* (1951) did not find any difference in the clearance of streptococci from the lungs of rats and guinea pigs, or in the occurrence of pneumonia by vitamin C level administered.

Rawal *et al.* (1974) reported that survival of mice infected with *Pseudomonas aeruginosa* was increased by vitamin C supplementation in a dose-dependent fashion. Senatuite and Biziulevicius (1986) reported that in rats infected with *Trichinella spiralis* the average number of muscle larvae after 3 weeks was 40% lower in the vitamin C administered group. Chaiyotwittayakun *et al.* (2002) induced mastitis in cows using intramammary infusion of endotoxin, and vitamin C reduced the fall in milk production caused by endotoxin. Goldschmidt (1991) reported that in rhesus monkeys vitamin C administration dropped oral *Actinomyces viscosus* counts by six orders of magnitude.

Two case-series were reported describing the therapeutic benefit of vitamin C on dogs afflicted by the canine distemper virus. Belfield (1967) described a series of 10 dogs that appeared to benefit from 1 – 2 g/day of vitamin C injected *i.v.* over 3 consecutive days. Leveque (1969) had some ten years of veterinary practice and with the pessimistic observation that only 5-10% of dogs recovered from canine distemper with signs of central nervous system (CNS) disturbance, he became interested in Belfield's case series. In a series of 16 dogs showing CNS disturbance that were treated with vitamin C, the proportion of dogs that recovered was 44% (95% CI: 20% to 70%; based on 7/16)(Leveque 1969).

Although the great majority of studies found benefits with higher vitamin C intake on various infections, a study on cryptosporidiosis in mice found more oocysts and a higher infection score in the vitamin C supplemented group (Leitch & He 1999).

Mortality and severity of infection in animals are hard outcomes, rather than surrogates such as various laboratory measures of the immune system. In this respect the animal studies with actual infections are more interesting from the human perspective than studies on laboratory measures of the human immune system. It is noteworthy that dramatic effects have been reported in numerous animal species ranging from rhesus monkey to fish, and with a great diversity of infecting organisms. Still, it is not clear to what degree the animal studies can be extrapolated to human subjects. It would seem unlikely that human beings qualitatively differ from the other animal species with respect to the general role of vitamin C on infections. Therefore, the fundamental question in human beings is not whether vitamin C affects susceptibility to and severity of infections, but rather the relevant questions seem to be focused on estimation: *e.g.*, what the population groups that might get some benefit from higher vitamin C intakes are, what the dose-dependency relation between intake and the effects on infections is, and how great the proper levels of intake might be.

Vitamin E and infections

Effects on the immune system

Vitamin E is a lipophilic antioxidant which may affect the immune system by protecting against reactive oxygen species much the same way as vitamin C.

The extensive literature on vitamin E and the immune system has been reviewed by several authors (Nockels 1979; Tengerdy 1989; Meydani *et al.* 1995; Moriguchi & Muraga 2000; Serafini 2000). A recent review of this topic contained about 100 references (Moriguchi & Muraga 2000).

Some studies have indicated a complex dose-response relationship between the amount of vitamin E and its immunological effects. In mice, lymphocyte proliferation caused by concanavalin A and phytohemagglutinin increased with medium dose vitamin E supplementation, but was suppressed by high dose supplementation, indicating that large doses may cause harmful effects on the immune system (Yasunaga *et al.* 1982; Bendich *et al.* 1986).

The effects of vitamin E supplementation on the immune system have been variable in human studies. Although some found apparent benefit on the immune system from vitamin E supplementation (Meydani *et al.* 1997; Serafini 2000), some other studies found none (Goodwin & Garry 1983, 1988; Payette *et al.* 1990; Waart *et al.* 1997) and, moreover, a few studies found reduced bactericidal activity of phagocytes in subjects administered large doses of vitamin E (Baehner *et al.* 1977; Prasad 1980; Kaul *et al.* 2000).

Infections in animals

A number of animal studies found that vitamin E supplementation affects resistance to various infections (**Appendix 4**). As regards human respiratory infections analyzed in this thesis, the protection of mice against *S. pneumoniae* (Heinzerling *et al.* 1974a), influenza virus (Hayek *et al.* 1997; Han *et al.* 2000), and coxsackie virus B3 infection (Beck *et al.* 1994, 2003), and lambs against *Chlamydia* (Stephens *et al.* 1979) with vitamin E supplementation are of most prominent interest.

Two animal studies compared different doses of vitamin E, finding that medium dose vitamin E increased resistance, but the groups with large vitamin E doses had higher mortality than those with medium doses (Heinzerling *et al.* 1974a; Peck & Alexander 1991b). Consequently, these two studies suggest dose dependency, so that excessive doses of vitamin E may become harmful, as was also observed in two immunological studies (Yasunaga *et al.* 1982; Bendich *et al.* 1986).

Appendix 4 lists 70 separate animal studies with vitamin E, 37 of which found a statistically highly significant benefit, $P \leq 0.01$, on at least one infectious disease outcome, while 20 studies reported benefit even at a level of $P \leq 0.001$ (Appendix 4). Of 44 studies with mammals, 28 found highly significant benefit, $P \leq 0.01$, from vitamin E on some infectious disease outcome.

The studies discussed above used pure vitamin E. In addition, wheat germ oil, which is a rich source of vitamin E, affected the development of fatal paralysis in young mice infected with vesicular stomatitis virus (Sabin & Duffy 1949; **Appendix 5**); this study was the first to indicate that vitamin E intake may affect infections. Vitamin E was also studied in combination with other vitamins and minerals, but it is not clear what the role of vitamin E is in these combinations (Appendix 5).

In addition to the controlled studies listed in Appendices 4 and 5, the deficiency of vitamin E rendered rats and hamsters susceptible to *M. leprae*, to which these animals are normally resistant, but there was no parallel control group (Mason & Bergel 1955). In a study with rats, vitamin E did not protect against liver damage caused by *E. coli* endotoxin (Rücker *et al.* 1997). In rat models of chronic portal hypertension and common bile-duct ligation, the combination of vitamin E and C led to reduction in the bacterial colonization of the liver and spleen (Schimpl *et al.* 1997). In rats, vitamin E administration reduced the degenerative changes in kidney glomeruli caused by *E. coli* endotoxin (Coskun *et al.* 2005).

The animal studies in Appendix 4 mostly found positive effects on animal infections with vitamin E supplementation, but a few studies found that a high intake of vitamin E is harmful in animal models of malaria, so that animals with low intakes survived longer (Godfrey 1957; Eaton *et al.* 1976; Eckman *et al.* 1976; Friedman 1979; Taylor *et al.* 1997). In hamsters infected with *Leishmania donovani* vitamin E increased the parasite counts (Garg *et al.* 2004). The combination of vitamins E, C and A, and selenium led to increased number of *Trichinella spiralis* worms in intestines and muscles of rats (El-Sheikh *et al.* 1999). Wahli *et al.* (1998) found that vitamin E increased the incidence of VHS (viral hemorrhagic septicemia) virus in rainbow trout administered low vitamin C levels, but not in those administered high vitamin C levels (Appendix 4).

Interaction between vitamins C and E

Vitamin C and vitamin E are both antioxidants and protect against reactive oxygen species. These substances are of parallel interest as water-soluble vitamin C regenerates lipid-soluble vitamin E *in vitro* (Packer *et al.* 1979; Sharma & Buettner 1993; Wijesunara & Berger 1994).

There is much evidence indicating that vitamins C and E may also have a physiologically relevant interaction. In guinea pigs, vitamin C deficiency led to reduced levels of vitamin E in the liver, kidney, spleen, and lungs (Kanazawa *et al.* 1981; Hruba *et al.* 1982; Bendich *et al.* 1984). In inherently scorbutic rats, vitamin C deficiency led to reduced vitamin E levels in the liver, kidney, and heart (Tanaka *et al.* 1997). In guinea pigs administered oxidized frying oil, large doses of vitamin C increased the level of vitamin E in liver, kidney, heart, lung, and spleen (Liu & Lee 1997). In normal rats, vitamin C supplementation increased plasma vitamin E level (Chen *et al.* 1980). Nevertheless, in one

study, vitamin C deficiency did not affect the plasma vitamin E level in guinea pigs (Hruba *et al.* 1982), and in another, excessive doses of vitamin C reduced plasma vitamin E levels (Chen & Chang 1979).

In studies with human subjects, vitamin C supplementation increased plasma lipid standardized α-tocopherol (Hamilton *et al.* 2000). Vitamin C supplementation also led to a higher level of vitamin E in plasma in participants who were administered 800 mg/day of vitamin E than in participants administered vitamin E alone (Baker *et al.* 1996).

In normal rats and guinea pigs, vitamin E supplementation increased the plasma vitamin C level (Chen *et al.* 1980; Bendich *et al.* 1984). In inherently scorbutic rats, a deficiency of vitamin E led to lower levels of vitamin C in the plasma, liver, kidney, and heart (Tanaka *et al.* 1997). In rats, vitamin E deficiency did not affect the plasma vitamin C level, but the muscle vitamin C level was significantly increased (Gohil *et al.* 1986).

In a study with human subjects, vitamin E supplementation increased the plasma level of vitamin C (Hamilton *et al.* 2000). In smokers, but not in nonsmokers, vitamin E disappearance in plasma was inversely related to vitamin C levels (Bruno *et al*. 2005)

A recent study found that a combined deficiency of vitamins C and E in guinea pigs produced a clinical picture different from scurvy. Many of these doubly deficient animals had paralysis of their limbs, and there was evidence of oxidative damage in the central nervous system (Hill *et al.* 2003). In endotoxin-treated guinea pigs, simultaneous supplementation of vitamins C and E led to higher levels of vitamin E in the liver than vitamin E supplementation alone (Cadenas *et al.* 1998). Wahli *et al.* (1998) found an interaction between vitamins C and E in their effects on the susceptibility to infection of rainbow trout (pp 118, 129).

Accordingly, there is experimental data indicating that vitamin C and vitamin E have a physiological interaction, but its significance is poorly understood both in experimental animals and human beings.

Infections in humans

Vitamin C and the common cold

Before the 1970s a few physicians had concluded from their own observations that vitamin C administration would be beneficial against the common cold (*e.g.*, Ruskin 1938; Markwell 1947; Bessel-Lorck 1959; Regnier 1968). The authors of the influential Sheffield trial examining the effects of vitamin C deprivation of human subjects concluded that common cold episodes "lasted longer in the deprived group" (Bartley, Krebs & O'Brien 1953 p 43). Some other early controlled trials also indicated that vitamin C intake may affect the common cold, but the results of various trials were not quite consistent and the early positive findings did not affect mainstream medicine.

The notion that vitamin C might be of benefit against colds achieved wide popularity in the 1970s when Linus Pauling wrote a best-selling book entitled *Vitamin C and the Common Cold* in which he claimed that supplementation would prevent and alleviate common cold episodes (Pauling 1970a, 1971c,d, 1976a; Marinacci 1995 pp 249-51). In 1971, Pauling's book received the Phi Beta Kappa award for the best scientific book of the year in the USA (PBK 2005). Pauling's book made million of Americans interested in vitamin C supplements and, in the academic world, it led to the initiation of dozens of new trials intended to find out whether Pauling was right or wrong (**Fig. 2**). To understand why Pauling's book could have such an enormous impact on the vitamin C field, his personal background must be briefly reviewed.

Pauling is widely considered the greatest chemist of the previous century (Greenberg 1984; Lipscomb 1994; Pauling 1994; Rich 1994; Dunitz 1996, 1997; McConnell 1996; Mason 1997; NLM 2005a; OSU 2005a,b,c,d; Nakamura & Csikszentmihalyi 2001; UC 2005). "In 1931, at only thirty years old, the Oregon-raised Linus Pauling knew he was the world's best chemist. Ten years later the rest of the world's chemists agreed" (Watson 1999, 2001). "Pauling helped to transform chemistry from a largely phenomenological subject to one based firmly on structural and quantum mechanical principles" (Perutz 1994). "The extrapolation from physics to chemistry and the articulation of chemistry as an independent subject was the handiwork of a single individual. Linus Pauling ranks … as one of the great thinkers and visionaries of the millennium… Chemistry grew and prospered simply by proving time and again that Pauling was correct in just about all his conjectures, for he projected with unerring accuracy into future with only 0.01% of today's structural information" (Desiraju 2000).

Pauling was also one of the founders of molecular biology (Gray 1949; Judson 1979; Pauling 1986b, 1993; Kay 1989; Crick 1992; Perutz 1994; Rich 1995; Mason 1997; Morgan 1998; Edison 2001; Eisenberg 2003; UC 2005; Gruber & Lupas 2003). Before any protein structures had been solved, Pauling conceived, for example, that "A molecular explanation of biological specificity results from a detailed complementariness in structure of two molecules … The significance of the hydrogen bond for physiology is greater than that of any other structural feature," and "Enzymes are molecules that are complementary to the activated complexes of the reactions that they catalyze." Pauling discovered the basic structural features of proteins, α-helix and β-sheet, and invented the concept of the "molecular evolutionary clock." In one of his major papers, Pauling *et al.* (1949) showed that hemoglobin from patients suffering from sickle cell anemia had a different electric charge from that from healthy individuals, and this report laid the groundwork for establishing the field of molecular medicine (Azar 1996; Strasser 1999, 2002; Eaton 2003). Pauling received the Nobel Prize in Chemistry in 1954 (Pauling 1954; NF 2005e).

At the end of the Second World War, Pauling served on a presidential committee formed to plot the path of science in the post-war period. The resulting Vannevar Bush Report

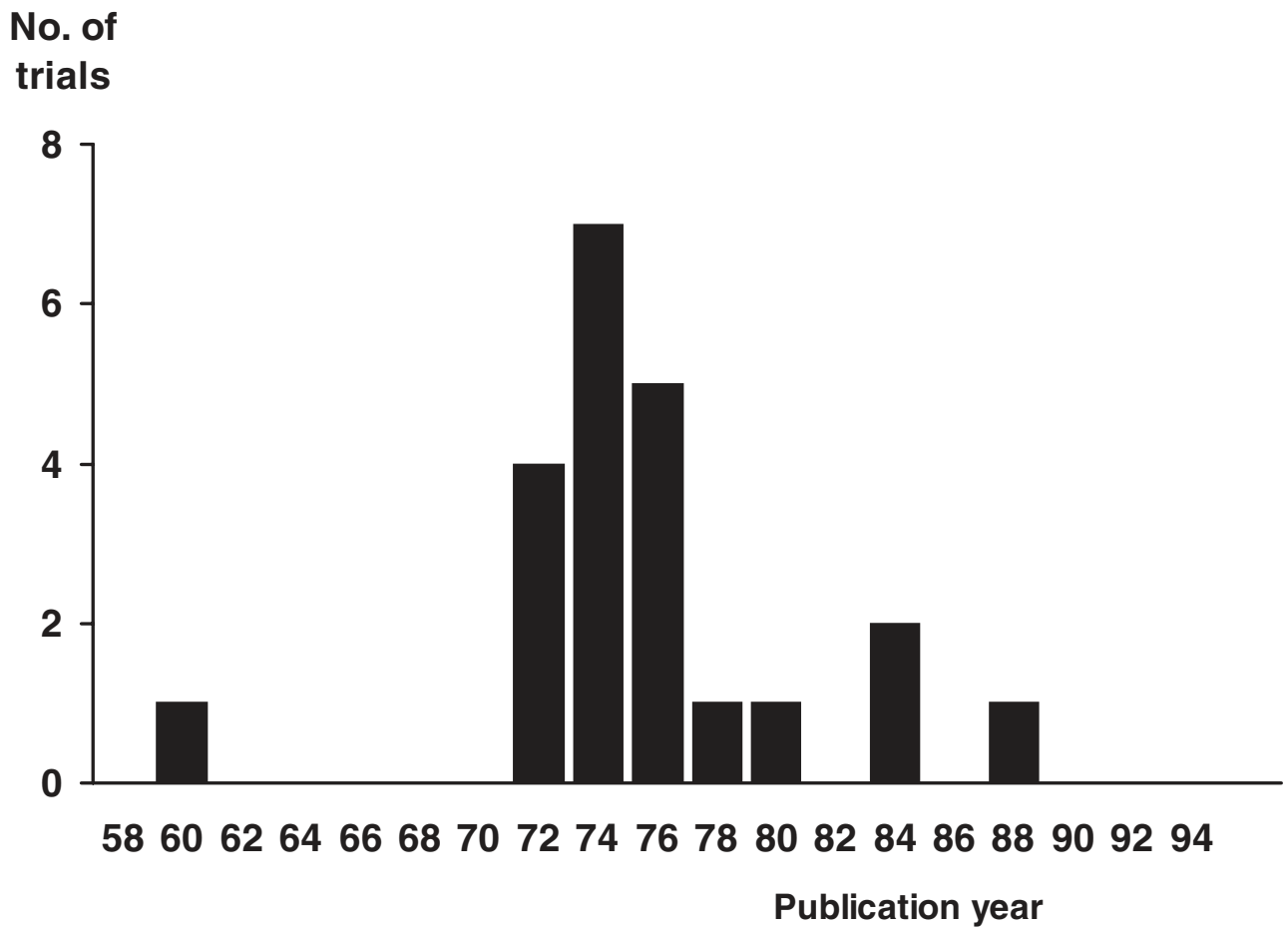


Fig. 2. Placebo-controlled studies in which ≥1 g/day of vitamin C was administered to the participants regularly over the trial period. Regular supplementation refers here to initiating supplementation with healthy people and continuing over the occurring common cold episodes. The number of studies published over two consecutive years is combined and plotted for the first of the two years. For the list of references to these trials, see Hemilä (1992a, 1994a). This figure is reproduced from Hemilä (1997b) *International Journal for Vitamin and Nutrition Research* 67:329-335 with permission.

led to the expansion of the National Institutes of Health (NIH), allowing for extramural research funding, and the creation of the National Science Foundation (Paradowski 1996). In 1946 Pauling joined the Emergency Committee of Atomic Scientists, chaired by Albert Einstein, whose most important task was to make people in all countries aware of the transformation of the world brought about by the development of the atomic bomb. During 1958, Pauling sent a copy of the petition opposing nuclear weapons testing, with endorsement by 11,021 scientists from 49 countries including 40 members of the US National Academy of Sciences, 216 members of the Soviet Academy of Sciences and 36 Nobel Laureates, to Dag Hammarskjöld, the Secretary-General of the United Nations. Public opinion worldwide led to test-ban negotiations in late 1958. On the same day (10th Oct 1963) that the limited test ban treaty signed by the USA, UK, and the USSR went into effect, it was announced that Linus Pauling would be awarded the Nobel Peace Prize for 1962 (Pauling 1963; Kreisler 1983; Mason 1997; Jolly 2002; NF 2005f). Because Pauling was the best-known American scientist publicly arguing for disarmament, he began to come under attack from right-wing political groups. Because of the pressures of McCarthyism on government officials, Pauling encountered difficulties in obtaining a passport. For example, he was without a passport when his Nobel Prize in Chemistry was announced in 1954, but the Undersecretary of State overruled the Passport Division, enabling him to travel to Sweden to receive his award (Allison 1960; Paradowski 1996; Mason 1997; Nye 1999).

With such a particularly strong background in science and high-level competence in politics, it is obvious that Pauling's 1970 book *Vitamin C and the Common Cold* reached extensive readerships in both lay and academic circles and had great impact. Because of Pauling's book, the demand for vitamin C substantially increased. In the USA, the production of vitamin C increased from 8.9 million pounds in 1969 to 11.7 in 1971, corresponding to an increase of 39% in two years and an annual growth rate of 18% (CMR 1972a), in contrast to the annual growth rate of about 6% in the 1960s (CMR 1972c). About 5.6 million pounds of vitamin C were also imported to the US between January and November 1971, up about 160% from the amount imported during the same period in 1970 (CMR 1972b). In the USA, Pauling personified the issue of vita-

min C (Apple 1996). The US Food and Drug Administration (FDA) was alarmed by this increasing interest in the consumption of large doses of vitamins, and proposed that over-the-counter sales be restricted to relatively low-potency products, with 'megadoses' requiring a physician's prescription. The health food industry felt threatened and it encouraged customers to protest. Members of the US Congress received more mail on this issue than any other, except for the Vietnam War, and the FDA had to give up their attempt. Why, it was asked, did FDA threaten the sales of vitamins, and do nothing comparable about tobacco and alcohol, whose ill effects in excess were not debatable (Apple 1996).

In addition to his book intended for lay people, Pauling (1971a) carried out a meta-analysis of 4 placebo-controlled trials, which was one of the very first meta-analyses in medicine. The results of 3 of the 4 trials are shown in **Table 3**. The fourth trial by Wilson *et al.* (1973a,b,c) was available to Pauling only as an abstract (Wilson & Loh 1969) and the final report was complicated with 48 significance tests, with several outcomes in various subgroups, so that it does not provide unambiguous data (Kinlen & Peto 1973). In his meta-analysis, Pauling combined the P-values of the 4 trials by Fisher's method, concluding that it was highly unlikely that all the reported effects on common cold morbidity from vitamin C supplementation could be explained by chance alone. Pauling (1971a) also pointed out that, out of these 4 trials, the greatest benefit was found by Ritzel (1961; Brubacher 1989), who used the largest dose, 1 g/day. Pauling thus concluded that gram doses of vitamin C daily would prevent colds in the general population. Pauling (1971b) also carried out a second meta-analysis in which he focused on the two best early trials (Cowan *et al.* 1942; Ritzel 1961; Table 3).

Pauling analyzed previously published trials but did not carry out any experimental work on the common cold himself. His book and other activities, however, led to the initiation of several new trials. Before the 1970s, there was only one trial which used ≥1 g/day of vitamin C regularly over the trial (Ritzel 1961), but within a decade of Pauling's book about 20 new trials using such high doses regularly over the trial had appeared (Fig. 2). However, the interest in vitamin C and colds disappeared in the middle of the 1970s. This waning interest was caused by the publication of two negative reviews in wide-circulation journals (Chalmers 1975; Dykes & Meier 1975), and by a particularly influential trial, carried out at the National Institutes of Health (NIH) and published in *JAMA*, which concluded that the apparent benefits of vitamin C were simply explained by the placebo effect (Karlowski *et al.* 1975). Thus all these three important negative papers were published the same year. Furthermore, the Karlowski trial (1975) and the Dykes and Meier review (1975) were published in the same issue of *JAMA*, and Thomas Chalmers was both the principal investigator of the Karlowski, Chalmers, *et al.* trial (1975; Chalmers 1996) and the author of the other review published in the same year (Chalmers 1975). The importance of these three papers is reflected, for example, by the comment in the *TIME* magazine: "Three reports now cast a further shadow on Pauling's theory … that large doses of vitamin C would prevent or cure the common cold" (Anonymous 1975 [see Pauling 1975]). Only a few trials on vitamin C and the common cold were initiated after the middle of the 1970s, after the publication of these three influential papers (Fig. 2).

Since this large set of new trials was carried out, there has been general consensus that vitamin C has no effects on colds, and the three papers published in 1975 are the most usual references for such negative statements. For example, based on these three papers, vitamin C was stated to be useless for colds in major textbooks on infectious diseases (Gwaltney 1979, 1985, 1990, 1995; Liu 1989; Dick *et al.* 1992, 1998; Cherry 1987, 1992, 1998), in *Cecil Textbook of Medicine* (Kapikian 1985; Hendley 1996, 2000), various textbooks on nutrition (Halsted 1993; Thurnham *et al.* 2000; Hamilton & Whitney 1982, 1994; Whitney & Rolfes 1993; Shils *et al.* 1994), and the US nutritional recommendations (FNB 1980, 1989a).

The conclusion that vitamin C is useless for colds has also reached the clinicians. For example, a survey of general practitioners in the Netherlands revealed that 47% of respondents considered that homeopathy is efficacious in the treatment of the common cold, whereas only 20% of the respondents considered that vitamin C was (Knipschild *et al.* 1990). On a scale from 0 to 10, with the high scores corresponding to stronger belief in efficacy, homeopathy for colds received a mean score of 4.7, whereas vitamin C for colds received a mean of only 2.5.

In the UK, 52% of 86 general practitioner trainees considered that homeopathy was useful, whereas only 7% considered large doses of vitamins useful, although no specific indications for usage were presented for either therapy (Reilly 1983). In the USA, 21% of 348 pediatricians considered that homeopathy may be effective, and 21% considered that high-dose antioxidant vitamins may be so, but again no indications for usage were presented (Sikand & Laken 1998). Accordingly, the use of vitamin C to treat the common cold comes unambiguously under the Eisenberg *et al.* notion (1993; Dalen 1998) that "medical interventions not taught widely at [US] medical schools or generally available at [US] hospitals" are unconventional or alternative medicine (Barrett & Herbert 1994; Turow 1997; Goodwin & Goodwin 1984; Goodwin & Tangum 1998; Hemilä 2000a,b).

Vitamin C and infections other than the common cold

The effect of vitamin C on the common cold was not studied for any particular biological reason, but because of the publicity aroused by Linus Pauling on the topic (Fig. 2). The common cold is a ubiquitous ailment with high incidence and is therefore easy to research. Nevertheless, a large number of animal studies have indicated that vitamin C intake may affect a wide variety of infections caused by viruses, bacteria, and protozoa (Table 2).

In the early twentieth century, several authors suggested that low intake of vitamin C may decrease resistance to in-

Table 3. Results of three major early vitamin C and common cold trials

Trial	Treatment: Vit C	Treatment: Placebo	Difference	P [1]
Ritzel (1961)				
Schoolchildren				
Skiing camp				
in Swiss Alps				
1 g/d vit C				
1 week				
Participants	139	140		
The common cold [2]				
All cases	17	31	-45%	0.015
Duration of colds:				
Mean (d)	1.8	2.6	-31%	<0.05
Days per group	31	80	-61%	
Constitutional Symptoms during the common cold [3]				
Cases	8	21	-62%	0.006
Days per group	9	48	-81%	
Cowan *et al*. (1942)				
Schoolchildren				
USA				
0.2 g/d vit C				
4 months				
Participants	208	155		
Cold episodes				
Mean per person	1.9	2.2	-14%	0.003
SD [4]	1.01	1.00		
SE	0.07	0.08		
Days lost from school				
Mean per person	1.1	1.6	-31%	0.000,3
SD [4]	1.1	1.6		
Franz *et al.* (1956)				
Schoolchildren				
USA				
0.2 g/d vit C				
4 months				
Participants [5]	44	45		
Common cold episodes	14	15		
Colds cured or improved in 5 d	13	8	+74%	0.012

[1] One-tailed mid-P-value was calculated by the current author. The P-value for the duration of colds was calculated by Ritzel.

[2] The number of episodes not being published, it is inferred from the total number of days per group and the average duration (Pauling 1971a, 1976b; pp 43-5).

[3] General malaise, headache, muscle ache, abdominal pain, vomiting, diarrhoea.

[4] Cowan *et al.* (1942): the SD values for the number of 'cold episodes per person' were calculated from the reported SE values. Cowan *et al*. did not report the SD or SE for 'days lost from school per person.' In most trials, the SD for common cold duration has been about 70% of the mean duration of colds (Douglas, Hemilä, *et al.* 2004). Here the P-value for 'days lost from school per person' is calculated on the assumption that both SD values are equal to the mean.

[5] The Franz *et al.* trial (1956) had a 2x2 factorial design and the bioflavonoid and no-bioflavonoid groups are combined here. This data is from their tables 1 to 4. Their summary table 5 erroneously records 4 'improved or cured,' for the bioflavonoid group whereas their table 4 gives 5 (= 1 + 4).

fections other than the common cold (Hess 1917, 1920, 1932; Clausen 1934, 1935; Robertson 1934; Perla & Marmorston 1937). Thomas Barlow, who defined the disease infantile scurvy, pointed out that in infantile scurvy "If the cachexia is very profound the supervention of bronchitis, pleuro-pneumonia, severe diarrhoea, or an intercurrent exanthem may bring about a fatal issue" (1894). Casimir Funk, who coined the term 'vitamin[e]' (1912), stated that an epidemic of pneumonia in the Sudan disappeared when an antiscorbutic, vitamin C containing, treatment was given to the numerous cases of scurvy which appeared at about the same time (cited in Robertson 1934).

Hess (1920 p 88) summarized a large series of autopsy findings on scorbutic patients: "Pneumonia, lobular or lobar, is one of the most frequent complications [of scurvy] and causes of death. Active tuberculosis is a not uncommon secondary manifestation" and the histopathological findings: "Secondary pneumonias, usually broncho-pneumonic in type, are of common occurrence, and in many [scurvy] epidemics constitute the prevailing cause of death. Tuberculosis lesions are also frequently present, and are stated to assume fresh activity as a result of the nutritional disorder (p 99). In the chapter on the prognosis of scurvy, Hess commented: "An important factor in the prognosis of scurvy … is the marked susceptibility to infection. Even latent or subacute scurvy causes peculiar susceptibility to diphtheria (especially the nasal type), or coryza, bronchitis, and pneumonia. A perusal of the literature shows that this susceptibility was noted by the older authors in relation to adults" (p 227).

Three exhaustive searches of the old literature on trials about vitamin C and infections have been published, but the data of the original publications were not quantitatively analyzed in any of the reviews (Stone 1972; Briggs 1984; Levy 2002). The findings of the published controlled trials were recently analyzed (Hemilä 1997a). The few trials related to the incidence of tuberculosis and pneumonia are briefly analyzed in the experimental section of this thesis because these topics are covered by a few controlled trials and because these topics are the subject of the analytic epidemiological work of this thesis. Therapeutic studies with vitamin C are briefly commented on here.

There have been suggestions by German and US physicians that vitamin C might have therapeutic benefits for pneumonia patients (McCormick 1951; Klenner 1971; see also Hemilä & Douglas 1999; Hemilä & Louhiala 2005). Gander and Niederberger (1936) concluded from a series of 15 cases that "The general condition is always favorably influenced [by vitamin C] 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". Referring to seven German papers, Glazebrook and Thomson (1942) commented that "there is evidence that [vitamin C] is of value in pneumonia, particularly in hastening convalescence, and the claims made do not appear to have been contradicted." Benefit of intravenous vitamin C was reported in a series of over 40 cases (Klenner 1948, 1951), and in 3 cases of viral pneumonia (Dalton 1962). Large-dose oral vitamin C was also claimed beneficial for patients with viral pneumonia (Cathcart 1981; Luberoff 1978). In one of his last texts, Albert Szent-Györgyi (1978) mentioned a personal experience: "Last year I collected a rather unfortunate personal experience. I broke down with pneumonia which I could not shake off for months, until I discovered that the quantities of ascorbic acid which I took (one gram daily) had become insufficient at my age (84 years). When I went up from one gram to eight, my troubles were over."

However, only one randomized trial has examined the effect of vitamin C treatment on lower respiratory tract infections. This was carried out in the UK with elderly patients suffering from pneumonia (n = 17) and acute bronchitis (n = 40) (Hunt *et al.* 1994). Therapeutic vitamin C caused a statistically significant reduction in the score of respiratory symptoms in patients who were most severely ill when admitted to hospital, and a decrease bordering on statistical significance in all patients (**Table 4**). Furthermore, there was a substantially lower mortality among the vitamin C group compared with the placebo group. The effect of vitamin C operated over and above the normal medication, mainly antibiotics and cough medicines, to which all patients of this double-blind trial were exposed.

Several controlled trials reported that vitamin C caused therapeutic benefit to patients with tuberculosis (Hemilä 1997a). All of these trials are old and technically more or less deficient. In some reports there is no data allowing the calculation of the P-value corresponding to the reported differences. Still, several of the therapeutic tuberculosis trials reported statistically significant differences in favor of the vitamin C groups, but it is likely that the differences are caused at least in part by the placebo effect and/or biases between the study groups.

The limited and fragmentary data on the potential therapeutic effects of vitamin C on other infections have been summarized elsewhere (*e.g.*, Stone 1972; Briggs 1984; Levy 2002; Hemilä 1997a, 1998), and only two particularly interesting trials are mentioned here (**Table 5**).

Since a study with rats found that vitamin C protected against tetanus toxin even after the tetanus symptoms appeared (Dey 1966; p 112), the study by Jahan *et al.* (1984) in Bangladesh reporting that vitamin C reduced mortality caused by tetanus infection is particularly interesting (Table 5). The allocation method is not described and a placebo was not used. However, it does not appear reasonable to assume that such a difference between study groups would arise from the placebo effect, which overall is negligible (Hrobjartsson & Gøtzsche 2001, 2004). The patients of the trial received conventional therapy upon which the effect of vitamin C supplementation was tested.

Another dramatic benefit in a vitamin C supplemented group was found by Terezhalmy *et al.* (1978) who investigated the effect of vitamin C combined with an equal amount of bioflavonoids on herpes labialis (Table 5). Pain and time before healing were shorter in the vitamin C+bioflavonoid group, and the formation of vesicles was significantly reduced. Furthermore, Terezhalmy *et al.* (1978) reported that

Table 4. Therapeutic effect of 0.2 g/day of vitamin C on elderly hospitalized patients with lower respiratory tract infections (Hunt *et al.* 1994)

Patients and Outcomes	Treatment		
	Vit C	Placebo	P [1]
No. of patients	28	29	
Decrease in 'total respiratory clinical score' in 4 weeks (scale 3 to 10)			
Mean	3.43	2.31	0.026
SD	1.77	2.44	
Deaths	1	5	0.059
Subgroup of patients most severely ill when admitted to hospital (initial score 8 or 9)			
No. of patients	12	15	
Decrease in 'total respiratory clinical score' in 4 weeks			
Mean	4.25	1.87	0.015
SD	2.14	3.00	

[1] One-tailed P-value. The mid-P-value was calculated for mortality. The difference in the change in clinical score was calculated using the t-test; according to the original authors, the 'clinical score' was approximately normally distributed. A score value of 10 corresponds to death.

when vitamin C supplementation was initiated within 24 hours of the onset of the symptoms, 6 out of 26 patients (23%) developed herpes vesicles, whereas with delayed initiation of supplementation 8 out of 12 patients (67%) did so. It is unlikely that such a difference is caused by chance (mid-P[2-t] = 0.003), indicating that the rapidity of initiation of vitamin C supplementation may be important in achieving a positive effect. Terezhalmy's trial was double-blind and placebo-controlled, but the method of allocation of participants to the study groups was not described. Because vitamin C was administered with an equal amount of bioflavonoids, this comparison is not specific to vitamin C; however, there is no compelling data indicating that bioflavonoids could affect infections.

Vitamin E

In the case of vitamin E, the focus on surrogates is a substantially greater problem than with vitamin C. There are several dozen immunological studies about the effects of vitamin E, and proposals that supplementation might be useful for people are mainly based on extrapolations from the immunological studies. Although a large number of animal studies found some benefit from vitamin E on various infections (Appendix 4), the number of animal studies with vitamin E yielding highly significant benefits is substantially lower than for vitamin C (Table 2). Moreover, only a few trials with human subjects have been carried out to examine the effects of vitamin E supplementation.

Most of the vitamin E trials with human subjects have been small and of short duration, which increases the risk of false negative conclusions. In doses of 200 and 400 mg/day, vitamin E did not affect the incidence of respiratory and urinary tract infections among patients in a chronic care facility (Harman & Miller 1986). In two rather small trials, the risk of respiratory and urinary tract infection was not affected by the combination of 15 mg/day of vitamin E with 6 mg/day of β-carotene and 120 mg/day of vitamin C in subjects aged ≥65 years (Girodon *et al.* 1997, 1999). In addition, 30 mg/day of vitamin E taken with other vitamins had no impact on unspecified infections among non-institutionalized subjects aged 60 years or more (Chavance *et al.* 1993). A small trial of people aged ≥65 years reported 30% fewer unspecified infections among participants administered 60-800 mg/day of vitamin E, but the difference was not statistically significant (Meydani *et al.* 1997). A trial with 652 subjects aged ≥60 years found no effect on the incidence of respiratory infections with 200 mg/day of vitamin E, but the illness duration was unexpectedly increased by vitamin E (Graat *et al.* 2002). A recent vitamin E trial involving 617 elderly participants observed a slightly lower common cold incidence in the group administered 200 mg/day of vitamin E (RR = 0.83; 95% CI: 0.68-1.01) (Meydani *et al.* 2004).

Combination of vitamins C and E had no effect on the incidence of nosocomial pneumonia in critically ill surgical patients (RR = 0.79; 95% CI: 0.53-1.20), but days of mechanical ventilation (-0.9; -0.6 to -1.2) and days of stay in the intensive care unit (-1.2; -0.8 to -1.5) were significantly reduced in the antioxidant group (Nathens *et al.* 2002). In adults aged ≥45 years, vitamin and mineral supplement containing 60 mg/day vitamin E reduced the incidence of all infections by 41% (P < 0.001), but the role of vitamin E *per se* remains unclear (Barringer *et al.* 2003). A recent trial examining the effect of a multivitamin-multimineral supplement containing 10 mg/day of vitamin E and 60 mg/day of vitamin C found no effect on infections in elderly people (Avenell *et al.* 2005 [see Hemilä 2005g]).

A few small trials found that vitamin E supplementation reduced the severity of hepatitis B and C infections (Herbay *et al.* 1997; Ramrakhiani & Neuschwander 1997; Andreone *et al.* 2001), except one (Nguyen *et al.* 1998).

The majority of the vitamin E trials have thus been negative. Moreover, an often-cited trial which examined the ef-

Table 5. Therapeutic effect of vitamin C on non-respiratory infections

Tetanus (Jahan *et al*. 1984)	Treatment			
1 g/d vit C	Vit C	Control	Reduction in mortality	P [1]
All patients	58	59		
All deaths	10	42	75%	0.000,000,002
Age subgroups				
1 – 12 years				
Patients	31	31		
Deaths	0	23	100%	0.000,000,000,1
13– 30 years				
Patients	27	28		
Deaths	10	19	45%	0.013

Herpes labialis (Terezhalmy *et al*. 1978)	Treatment		
	Vit C	Placebo	Reduction in outcome
0.6 g/d vit C			
Patients	19	10	
Duration of pain (d)			
Mean	1.7	3.5	51% 0.000,000,2
SD	0.6	0.8	
Time before healing (d)			
Mean	4.2	9.7	56% 0.000,000,2
SD	1.7	2.8	
1.0 g/d vit C			
Patients	19	10	
Duration of pain (d)			
Mean	1.3	3.5	63% 0.000,000,003
SD	0.6	0.8	
Time before healing (d)			
Mean	4.4	9.7	54% 0.000,4
SD	3.9	2.8	
0.6-1.0 g/d vit C			
Patients	38	10	
Vesicle development			
No. of patients	14	10	63% 0.000,2

[1] One-tailed P-value. The mid-P-value was calculated for dichotomous data and the t-test was used for continuous data.

fects of a supplement containing vitamin E (44 mg/day) along with other micronutrients on infections in elderly subjects by Ranjit K Chandra (1992) was recently shown to contain fabricated data (Shenkin et al. 2002; Carpenter *et al.* 2003; Roberts & Sternberg 2003; White 2004; Meguid 2005; Payne 2005). Chandra was the recipient of the year 1990 McCollum Award of the American Society for Clinical Nutrition (ASCN 2005); see footnote to Table 1.

Furthermore, sometimes papers on vitamin E and infections are grossly biased. In the recent vitamin E paper, Meydani *et al.* (2004) cited the above-mentioned paper with fabricated data (Chandra 1992), without noting its problems. Meydani *et al.* also cited a retrospective study of elderly subjects who were asked about their recollection of preceding infections which were correlated with plasma vitamin E levels (Chavance *et al.* 1985, 1989), although this type of experimental design has a high risk of recall bias, and plasma vitamin E has no meaningful correlation with vitamin E intake (FNB 2000 p 210). In contrast to these poor-quality studies, Meydani *et al.* failed to cite a randomized placebo controlled double-blind trial that found no overall effect of vitamin E on the incidence of colds in 21,796 participants (III; Hemilä & Kaprio 2004), which is a very large trial compared with the 617 participants in the Meydani trial (2004). Meydani *et al.* also failed to cite the controlled trial by Chavance *et al.* (1993) that was motivated by their above-mentioned retrospective study, but which produced negative results. Meydani *et al.* (2004) thus cited poor-quality studies when the results were consistent with their own preconceptions, but failed to cite better quality trials if the results were inconsistent with those preconceptions.

AIMS OF THE PRESENT THESIS

The primary purpose of this thesis was to determine whether vitamin C has effects on respiratory infections. This thesis was initiated with two different lines of approach: (1) meta-analyses of published controlled trials on vitamin C and respiratory infections, and (2) analysis of the baseline dietary vitamin C intake of the ATBC Study in relation to respiratory infections during the follow-up. The main purpose of the ATBC Study was to examine the effect of β-carotene and vitamin E supplementation on the incidence of lung cancer (ATBC 1994a,b; Virtamo *et al.* 1987). Since vitamin C and vitamin E are both antioxidants which have a chemical and physiological interaction (see pp 10-1), the scope of this thesis was later expanded to cover (3) the effects of vitamin E supplementation on respiratory infections.

Although β-carotene was the supplementation of primary interest in the ATBC Study when the trial was initiated in 1985, it was not of interest in the current thesis, even though in most cases the β-carotene findings were analyzed in parallel with the vitamin E findings in Papers III to V.

Because the analyses of the previously published vitamin C trials, and the analyses of the ATBC Study are quite dissimilar activities, they are discussed under separate categories.

CURRENT STUDIES AND THEIR FINDINGS

A) Analyses and meta-analyses of trials on vitamin C and respiratory infections

Methods

Searches for the trials

Independently of the present author, the earlier literature on vitamin C-common cold trials was thoroughly searched for by Briggs (1984) and Kleijnen *et al.* (1989). Kleijnen *et al.* (1989; Kleijnen & Knipschild 1992) identified over 60 controlled trials on vitamin C and the common cold, and published the bibliography. Briggs (1984) published an extensive bibliography covering all infections. Although some authors urge that the literature search should be explicitly described to show that the search strategy is valid (*e.g.*, Kleijnen & Knipschild 1992), in many cases a simple explicit search phrase may yield grossly misleading results. For example, a 'pneumonia' search of Medline does not identify any of the three vitamin C trials that reported the number of pneumonia cases in the study groups (Glazebrook & Thomson 1942; Kimbarowski & Mokrow 1967; Pitt & Costrini 1979). The oldest trial is not included in the Medline database whereas the two more recent ones are, yet pneumonia is not mentioned as a keyword in either of them despite being an important secondary outcome in both trials. Consequently, an explicit search strategy is no evidence that a search has a meaningful coverage of relevant publications.

The literature searches for trials on vitamin C and infections by the current author were composed of several searches using various strategies in different databases (Medline, Web of Science, Embase, and Chemical Abstracts). In some cases the search phrases were rather loose, *e.g.*, 'vitamin C' alone, whereas some other searches were limited by additional phrases such as 'infection' or 'immune.' Papers citing the older seminal papers were sought in the citation data base (Web of Science). Thus, no single or simple explicit search strategy was used for this thesis. The reference-lists of relevant articles, reviews, and books were also perused. As to the trials on vitamin C and the common cold, Kleijnen *et al.* (1989) missed a few old trials, and a few trials on vitamin C and the common cold have been published since Kleijnen's bibliography (**Table 6**).

Statistical methods

The statistical methods used are mostly standard methods described in textbooks of medical statistics (see Altman 1991; Feinstein 2002). The mid-P method (Lancaster 1961; Berry & Armitage 1995), used to analyze the 2x2 tables, is not described in the ordinary medical statistics textbooks, but is described by Rothman (1986 pp 138-9, 161-2, 203-5), Rothman and Greenland (1998 pp 222-4), and Clayton and Hills (1993). Since the mid-P value is extensively used in this thesis, the rationalization for this concept is explained in **Table 7**.

One-tailed P-values are mostly used in the analyses of studies in this text, since the questions in the present thesis are whether vitamin C or vitamin E supplementation decreases the incidence or severity of infections or not, and these questions are unidirectional. When pertinent, the 2-tailed P-value is given, and in such a case the 2-tails are explicitly mentioned (P[2-t]). If the 1-tailed P-value is higher than about 0.98, there is reason to assume harm in the treatment group of the particular experiment.

Table 6. Vitamin C common cold trials not included in the Kleijnen *et al.* bibliography (1989)

Published before 1989
Niemi-1951
Boines-1956
Masek-1974
Carson-1975b
Briggs-1984
Mink-1988
Published in 1989 or later
Shult-1990
Peters-1993
Peters-1996
Moolla-1996
Himmelstein-1998
Gorton-1999
Audera-2001
Van Straten-2002
Sasazuki-2006

Table 7. Rationalization of the mid-P-value

Let us assume that we observe results forming the following 2x2 table:

1	1
1	1

In the Fisher exact test, we calculate the probability of all tables that have the same fixed margins (3 tables in this case):

		Probability of the table:
2	0	
0	2	P[up] = 1/6
1	1	
1	1	P[obs] = 4/6
0	2	
2	0	P[low] = 1/6

The lower 1-tailed 'Fisher P' adds the probabilities of the observed table (P[obs]) and the probabilities of the tables of the lower tail. Thus here,
Fisher P[1-t] = 4/6 + 1/6 = 5/6 = 0.833.

The lower 1-tailed 'mid-P' adds half of the probability of the observed table (½ x P[obs]) and the probabilities of the tables of the lower tail.
Thus here,
mid-P[1-t] = 2/6 + 1/6 = 3/6 = 0.5.

With the 'observed table' having 1 observation in each cell, there is no evidence of any difference from the null effect, and we should expect the corresponding P[1-t] to be exactly 0.5. In this respect the mid-P corresponds to scaling the P-value so that P[1-t] = 0.5 corresponds to the null effect. Thus the mid-P modification is consistent with the t-test, which also yields P[1-t] = 0.5 if there is no difference between the groups, so that their means are equal.

The 1-tailed mid-P-values never add up to a 2-tailed mid-P-value above 1. In contrast, the 1-tailed Fisher P-values may add up to the 2-tailed Fisher P-value above 1 as in the case above (Fisher P[2-t] = 2 x 0.833 = 1.67); however, P values above 1 are inconsistent with the concept of probability.

The most influential trial on vitamin C and the common cold: Karlowski *et al.* (1975)

In the middle of the 1970s, Karlowski, Chalmers, *et al.* (1975) published a vitamin C common cold trial which received widespread attention for several reasons. First, the trial was carried out at the NIH. Since the participants in the trial were NIH employees, the social background of this particular trial was highly significant. Secondly, the principal investigator of the trial was Thomas Chalmers, who was an eminent clinical trialist (see p 36). Third, the trial was published in *JAMA*, a respected journal with a particularly wide circulation. Finally, the same issue of *JAMA* contained a review of vitamin C and the common cold, which concluded that it has no effects on colds (Dykes & Meier 1975; see pp 42-5).

Technically, the Karlowski trial is not among the best. Karlowski's placebo capsules contained lactose which is sweet, whereas ascorbic acid was used in the vitamin C capsules, so that some of the participants may have identified their treatment by taste. In fact, the authors concluded from their trial that the results were explained by the placebo effect: "The effects demonstrated might be explained equally well by a break in the double blind" (Karlowski *et al.* 1975).

The Karlowski trial lasted for 9 months and included 4 treatment arms (**Table 8**). Each participant received 2 kinds of capsule: prophylactic (each day) and therapeutic (5 days during a cold). Ascorbic acid (3 g/day) was used in the active capsules and lactose in the placebo capsules, a different combination being administered to each of the 4 study arms. The mean duration of cold episodes in each study arm is shown in Table 8. After the Karlowski trial was concluded, the participants were asked in a questionnaire which capsules they thought they had been administered. In the case of prophylactic capsules there was strong bias in favor of correct answers ($P < 10^{-6}$). In contrast, there was no evidence of bias in the case of therapeutic capsules (P[2-t] = 0.3). After finding that several participants had correctly identified their treatment, Karlowski *et al.* carried out a subgroup analysis in which they divided participants into those who remained blinded and to those who were unblinded after the trial was concluded. In this subgroup analysis, Karlowski was able to show that all the benefit of vitamin C was seen in the 'unblinded' group, whereas there was no effect from vitamin C on the participants who remained 'blinded' (Table 8). The authors thus concluded that vitamin C supplementation has no physiological effect, and the apparent benefit was simply caused by 'the placebo effect.' Thus, the Karlowski trial seemed to provide direct evidence of the placebo-effect in action. In 1996 Thomas Chalmers stated that he was "more proud of [the Karlowski trial] than almost any other that I have published."

Several consecutive editions of two major textbooks on infectious diseases referred to the Karlowski trial in no uncertain terms: "Many participants correctly surmised from the taste of the contents of the capsules used whether they were receiving vitamin C or a placebo" (Gwaltney 1979, 1985, 1990, 1995), and "It is most probable that the reported benefits are a result of statistical artifacts and placebo effect due to poor study design rather than specific pharmacologic drug effects" (Cherry 1987, 1992, 1998).

The Karlowski trial was also alluded to in two recent editions of the *Cecil Textbook of Medicine*: "Because the

Table 8. Results and subgroup analysis of the Karlowski *et al.* trial (1975)

Study Group	Vitamin C dose		All subjects		Subgroup analysis			
					'Blinded subjects'		'Unblinded subjects'	
	Prophylaxis (g/d)	Therapy (g/d)	Duration of colds (d)	No. of colds	Duration of colds (d)	No. of colds	Duration of colds (d)	No. of colds
#0	0	0	7.1	65	6.3	30	8.6	16
#1	0	3	6.5	56	6.7	18	4.7	15
#2	3	0	6.7	52	6.4	14	7.0	8
#3	3	3	5.9	76	6.5	30	4.8	13

subjective symptoms of a cold disappear in 7 days without intervention, a variety of actually ineffective treatments have been reported to be effective due to inadequate blinding of placebo recipients. One example of this phenomenon was a study of large dose of vitamin C to prevent colds, in which many placebo recipients dropped out of the study because they could tell by tasting the medication that they were not receiving the vitamin C" (Hendley 1996, 2000). Although the Karlowski trial had stronger evidence of divergence in dropping out than the 5 largest vitamin C common cold trials, the difference between the vitamin C and placebo groups was nevertheless not statistically significant in the Karlowski trial (**Table 9**), and in this respect the comment is misleading.

A US Food and Nutrition Board monograph referred to the Karlowski trial when stating that vitamin C has no worthwhile effects on colds (FNB 1989b). Another recent monograph by the Food and Nutrition Board also briefly discusses the role of vitamin C on the common cold, stating that in the Karlowski trial "the break in the double-blind study may have been due to the curiosity of the scientist participants" (FNB 2002).

Karlowski's trial has been cited in major reviews of common cold therapy (Coulehan 1979; Sperber & Hayden 1988; Spiers 2002), in *BMJ* (Editorial 1976), and in a recent review on echinacea and the common cold when proposing that the effects of echinacea in placebo-controlled trials are caused by the placebo effect (Caruso & Gwaltney 2005 [see Hemilä 2005a]).

Because the notion of 'breaking blindness' is technically a highly relevant issue in the field of controlled clinical trials, the Karlowski trial has been cited by several clinical trialists. In a paper discussing frauds and errors in medical research, clinical trialist DeMets (1997) wrote of the Karlowski trial: "Since patients in the study were employees of the NIH, they had either direct or indirect access to laboratories, and were easily able to break the double blind … Patients were asked if they had, in fact, used their own resources in the laboratories to break the blind." This is, however, erroneous, since Karlowski *et al.* (1975) did not report evidence that their participants actually examined the contents of their capsules. The participants were simply asked to "guess which substance they had been taking." The same comments on the Karlowski trial were reiterated in a more recent paper by DeMets (1999).

A popular textbook *Fundamentals of Clinical Trials* used the Karlowski trial as an example of unsatisfactory blinding causing false conclusions (Friedman *et al.* 1998 p 83): "A trial of the possible benefits of ascorbic acid in the common cold started out as a double-blind study. However, it soon became apparent that many of the participants, most of whom were medical staff, discovered whether they were on ascorbic acid or placebo. Since evaluation of severity and duration of colds depended on the participants' reporting of their symptoms, this unblinding was important. Among those participants who claimed not to know the identity of the treatment, ascorbic acid showed no benefit over placebo. In contrast, among participants who knew or suspected what they were on, ascorbic acid did better than placebo. Therefore preconceived notions about the benefit of a treatment, coupled with a subjective response variable, may have yielded biased reporting. Only the alertness of the investigators prevented them from arriving at probably false conclusions."

The Karlowski trial was also cited in a recent paper in *JAMA* discussing the blinding of trials (Devereaux *et al.* 2001), and in recent papers by specialists in controlled trials commenting on the question of breaking of blindness in blinded trials (Bang *et al.* 2004 [see Hemilä 2005b]; Chow & Shao 2004 [see Hemilä 2006a]; Forder *et al.* 2005 [see Hemilä 2005d]; Rees *et al.* 2005; Walter *et al.* 2005).

The Karlowski trial was cited as an example of placebo effect in action by the CONSORT group, an international group of clinical trialists, statisticians, epidemiologists, and

Table 9. Drop-outs in the major vitamin C common cold trials

	Vitamin C group		Placebo group		
	No. of participants		No. of participants		Difference in drop-out proportion
Trial [1]	Initiated	Concluded	Initiated	Concluded	P[2-t]
Karlowski-1975 (regular capsules)	153	101	158	89	0.063
Anderson-1972 [2]	500	407	500	411	0.7
Anderson-1974 [3]	1,320	860	880	578	0.8
Pitt-1979	429	331	432	343	0.4
Ludvigsson-1977b [4]	321	304	321	311	0.2
Elwood-1976 [5]	449	339	474	349	0.5

[1] These trials are the 6 largest in terms of the total number of episodes that administered ≥1 g/day vitamin C regularly during the trial (see Table 10; Paper II; Douglas, Hemilä et al. 2004).

[2] Randomization was in pairs. The participants at start totalled 1,000, but the distribution into groups is not stated; here it is assumed that the groups were initially of equal size.

[3] In the Anderson et al. trial (1974), the placebo group refers to arms #4 and #6 (see Table 16), and the vitamin C group refers to arms #1, #2, and #3, which were administered ≥1 g/day vitamin C regularly during the trial.

[4] In all, 642 initiated the trial; "every class was divided at random into two groups." A total of 615 completed the trial, so that 27 children had dropped out. Side-effects were the reason for 1 child to drop out from each group, but the distribution of the remaining drop-outs (27 – 2 = 25) is not reported. Here it is assumed that the size of the groups was initially the same.

[5] Dropping out because of 'moving away' was 5, and records of 2 participants were lost, but the distribution of these 7 participants between the treatment groups is not reported; it is assumed here that 3 of these were in the vitamin C group, and 4 in the placebo group. Dropping out because of 'inadequate co-operation' was 107 in the vitamin C group and 121 in the placebo group.

biomedical editors, who suggested improvements in reporting clinical trials (Begg *et al.* 1996). Consecutive editions of the *Cochrane Reviewers' Handbook* also cite the Karlowski trial, stating that "There is evidence that participants who are aware of their assignment status report more symptoms, leading to biased results" (Mulrow & Oxman 1994 s 6.4; Clarke & Oxman 2002 ss 6.4 and 6.6; Alderson *et al.* 2004 ss 6.4 and 6.6; Higgins & Green 2005 ss 6.4 and 6.6). The Karlowski trial was also referred to in a popular textbook of epidemiology as an example of important controlled trials (Rothman 1986 p 54; Rothman & Greenland 1996 p 70).

The 'placebo-explanation' of the Karlowski-results was forcefully emphasized in a concurrent and influential review by Thomas Chalmers, the principal investigator of the Karlowski trial (Chalmers 1975, 1996; see pp 36-8).

Furthermore, the Karlowski trial was also cited in a text discussing vitamin C and the common cold by Stephen Barrett (1995, 2005; see p 64), a prominent and influential crusader against health quackery.

There are several vitamin C common cold trials that are considerably larger, and that do not suffer from the shortcoming that vitamin C could be distinguished from the placebo by taste, 5 larger trials confirming that their vitamin C and placebo tablets were indistinguishable (**Table 10**). However, these larger and better trials are much less frequently cited than the Karlowski trial. For example, none of them are cited in the major textbooks of infectious diseases (Gwaltney 1979, 1985, 1990, 1995, 2000, 2005; Cherry 1987, 1992, 1998), even though these 5 trials together recorded 27 times as many common cold episodes as the Karlowski trial (Table 10).

Table 10 shows that, excluding the citations in Hemilä's papers, the Karlowski trial (1975) has been cited 13 times per 100 recorded common cold episodes (33/249), whereas the 5 largest trials together have been cited 0.2 times per 100 recorded cold episodes (14/7,167). Thus, per recorded episode, the Karlowski trial has had 65 times as great an impact as the 5 largest trials combined, even though the latter trials used valid placebos, unlike Karlowski. In this respect, the great impact of the Karlowski trial seems to be a good example of the Mathew effect in science (Merton 1968), in that famous scientists in famous institutions are cited because of their social context, irrespective of the quality of their work. Citation bias has also been documented in several other topics (Gøtzsche 1987; Ravnskov 1992; Kjaergard & Gluud 2002).

Table 10. Citations of the major vitamin C common cold trials

Trial [2]	Total no. of cold episodes in the trial	Citations from 1991 to 2002 [1]: Total citations	Citations from 1991 to 2002 [1]: Excluding citations by Hemilä	Constituent of the placebo
Karlowski-1975	249	43	33	Lactose
Anderson-1972	1,170	12	4	Citric acid [3]
Anderson-1974 [4]	2,182	9	4	Lactose and citric acid [3]
Pitt-1979	1,219	12	3	Citric acid [3]
Ludvigsson-1977b	1,279	11	2	not stated [3]
Elwood-1976	1,317	9	1	not stated [3]
Total episodes excluding Karlowski-1975	7,167	Total citations excluding Karlowski-1975	14	

[1] ISI Web of Science search.
[2] These trials are the 6 largest administering ≥1 g/day vitamin C regularly during the trial (see Paper II; Douglas, Hemilä, et al. 2004). All were randomized, double-blind, and placebo-controlled.
[3] The authors state that placebo and vitamin C tablets looked and tasted the same.
[4] The number of episodes is based on the total of arms #1, #2, and #3, which were administered ≥1 g/day vitamin C regularly during the trial, and arms #4 and #6, which were administered placebo (see Table 16).

Paper I: Re-analysis of the Karlowski *et al.* trial (1975)

Problems with Karlowski's 'placebo explanation'

The authors of the Karlowski trial concluded that the observed benefit in vitamin C groups was explained by the placebo effect (Table 8). Since the 'placebo explanation' is such a spectacular conclusion to a double-blind placebo-controlled trial, and since this is by far the most influential vitamin C common cold trial (Table 10), it was considered worthwhile to determine whether Karlowski's 'placebo explanation' is actually consistent with the reported differences between the study groups.

Karlowski *et al.* (1975) asked participants after the trial to guess the type of capsule they were given, and Karlowski used a 'correct answer' as a surrogate for actually 'knowing,' without considering that many answers were correct purely by guesswork (**Table 11**). There was strong statistical evidence that participants gave a correct answer to the question on the *prophylactic* capsules more often than expected, but there was no evidence that participants identified the *therapeutic* capsules more often than by guessing (Table 11). However, prophylactic capsules had considerably less effect (-0.48 days; see Paper I) on the duration of colds than the therapeutic capsules (-0.73 days; P = 0.05; see Paper I). The greater benefit from the therapeutic capsules is inconsistent with the 'placebo explanation,' because there is no statistical evidence that therapeutic capsules were identified by the participants, and at most only 11 (8%) participants identified the therapeutic capsules (Table 11).

Furthermore, Karlowski e*t al.* did not describe how they divided participants into the two subgroups of 'blinded' and 'unblinded.' The sum of these two subgroups does not match with data for all participants, although the authors presented the subgroups as if they were complementary. Table 8 shows that the total number of common cold episodes in the placebo group (#0) was 65. The 'blinded' placebo subgroup contained 30 episodes, and the 'unblinded' placebo subgroup contained 16 episodes, with a total of 46. Thus, there were 19 common cold episodes (= 65 - 46) in the placebo group affecting participants who were neither 'blinded' nor 'unblinded.' In total, there were 105 common cold episodes (42% of all) missing from Karlowski's subgroup analysis (Table 8). The maximum effect of vitamin C on common cold duration can be calculated for the 'missing group' (-1.4 days/episode; difference between 6 g/day and 0 g/day groups; see Paper I); thus it is even greater than the effect in the entire study population (-1.2 days/episode [=7.1 – 5.9]; Table 8). Karlowski *et al.* did not mention the exclusion of the 105 episodes from their subgroup analysis, nor did they offer their rationalization for the greater than average benefit in those participants who were neither 'blinded' nor 'unblinded.'

A number of further problems with the 'placebo explanation' are discussed in Paper I. Consequently, the 'placebo effect' explanation is inconsistent with the data reported by Karlowski *et al.* (1975).

An alternative explanation of the bias in 'knowing': Inference from subjective observations

There has been a persistent popular belief, long predating Pauling's book (1970a), that vitamin C is beneficial against the common cold. Consequently, it is possible that several participants inferred the vitamin C treatment from the subjective feeling that their cold symptoms were milder than they expected. Similarly, the impression that the symptoms were prolonged may have led a participant to infer that the placebo was being given, not vitamin C.

Vitamin C has substantially reduced the duration or severity of colds in several placebo-controlled trials (up to 20-50%; **Fig. 3**), so that it would be no surprise if vitamin C could be correctly distinguished from placebo by some people on the basis of subjective observation. Moreover, one double-blind placebo-controlled trial was initiated to examine the therapeutic effects of 6 g/day of vitamin C on common cold symptoms, but the trial was discontinued because the participants receiving the vitamin could be identified by their clinical progress (Asfora 1977). With such positive reports it is quite possible that correct identification of capsules in the Karlowski trial was partly caused by the physiological effects of vitamin C.

In fact, the 'knowledge inferred from subjective observation' explanation is directly supported by the data from a parallel publication of the Karlowski trial (Lewis *et al.* 1975). Among participants who had not tasted their prophylactic placebo capsules, those who had colds during the trial tended to suspect that they were being given placebo (15 of 18), whereas those who did not have colds tended to suspect they were being given vitamin C (6 of 8) (mid-P[2-t] = 0.004). A similar inference can apparently be drawn from the duration or severity of colds, but this was not considered by the authors. The alternative explanation is briefly mentioned but not properly analyzed in the *JAMA* paper (Karlowski *et al.* 1975) or in the parallel study report (Lewis *et al.* 1975), and the 'placebo explanation' is strongly favored in both papers. However, the alternative explanation is not mentioned at all in Chalmers' review (1975).

The re-analysis of the Karlowski trial (I) was criticized by Thomas Chalmers (1996), but the criticism seems not to be valid (I; Hemilä 1996c), except for Chalmers' statement that they used capsules in their trial, and not tablets as erroneously stated in Paper I. Unfortunately Chalmers died while Paper I and the accompanying commentaries were being published (Feinstein 1996).

Inference of treatment by subjective observation in the Pitt and Costrini trial (1979)

The possibility that certain of Karlowski's participants may have correctly inferred their treatment from subjective observation is supported by the findings of Pitt and Costrini (1979) in a randomized double-blind placebo-controlled trial with Marine recruits in the USA. In this trial, "The placebo tablets were formulated from citric acid and were indistin-

Table 11. Subjective deduction of the treatment in the double-blind trials by Karlowski *et al*. (1975) and Pitt & Costrini (1979)

	Karlowski *et al*. (1975)		Pitt & Costrini (1979)
The published figures	Prophylactic	Therapeutic	
No. of participants	190	132	674
Vitamin C group	101	70	331
Placebo group	89	62	343
Did not guess	88	79	358 (53%) [1]
Guessed	102	53	316
Participants giving an answer:			
Correct answer			
Vitamin C group	40	20	89 (27%) [1]
Placebo group	39	12	89 (26%) [1]
Guessed incorrectly			
Vitamin C group	12	11	66 (20%) [1]
Placebo group	11	10	72 (21%) [1]
Both groups			
Correct answer	79	32	178
Guessed incorrectly	23	21	138
Assuming pure guessing, χ^2-test for the distribution of correct and wrong answers P[2-t]	<0.000,1	0.3	0.013
Inference from the published figures			
'Correct answers' are composed of:	79	32	178
Pure guess (assumed the same as the wrong guesses)	23	21	138
Really knew	56	11	40
Of correct answers	71% (56/79)	34% (11/32)	22% (40/178)
Of all participants	29% (56/190)	8.3% (11/132)	5.9% (40/674)

[1] Pitt & Costrini (1979) published the percentages indicated and the corresponding numbers of participants was calculated from these.

guishable in appearance and taste from the vitamin C tablets." After the trial, participants were asked which pill they thought they were taking, and the percentage of participants giving various answers was reported; the number of participants corresponding to the percentage is calculated in Table 11. There is a statistically significant difference in the number of participants who gave the correct answer (n = 178) compared with those giving an incorrect answer (n = 138). Correct identification may be caused by the positive or negative effects of vitamin C, but the authors found no difference in adverse events between the study groups. Instead they reported a statistically significant, but clinically minor effect on common cold severity (P = 0.012), and an 85% lower incidence of pneumonia in the vitamin C group (see pp 50-1). Pitt and Costrini's data suggests that some 6% of all participants (40/674) inferred their treatment by subjective observation (Table 11).

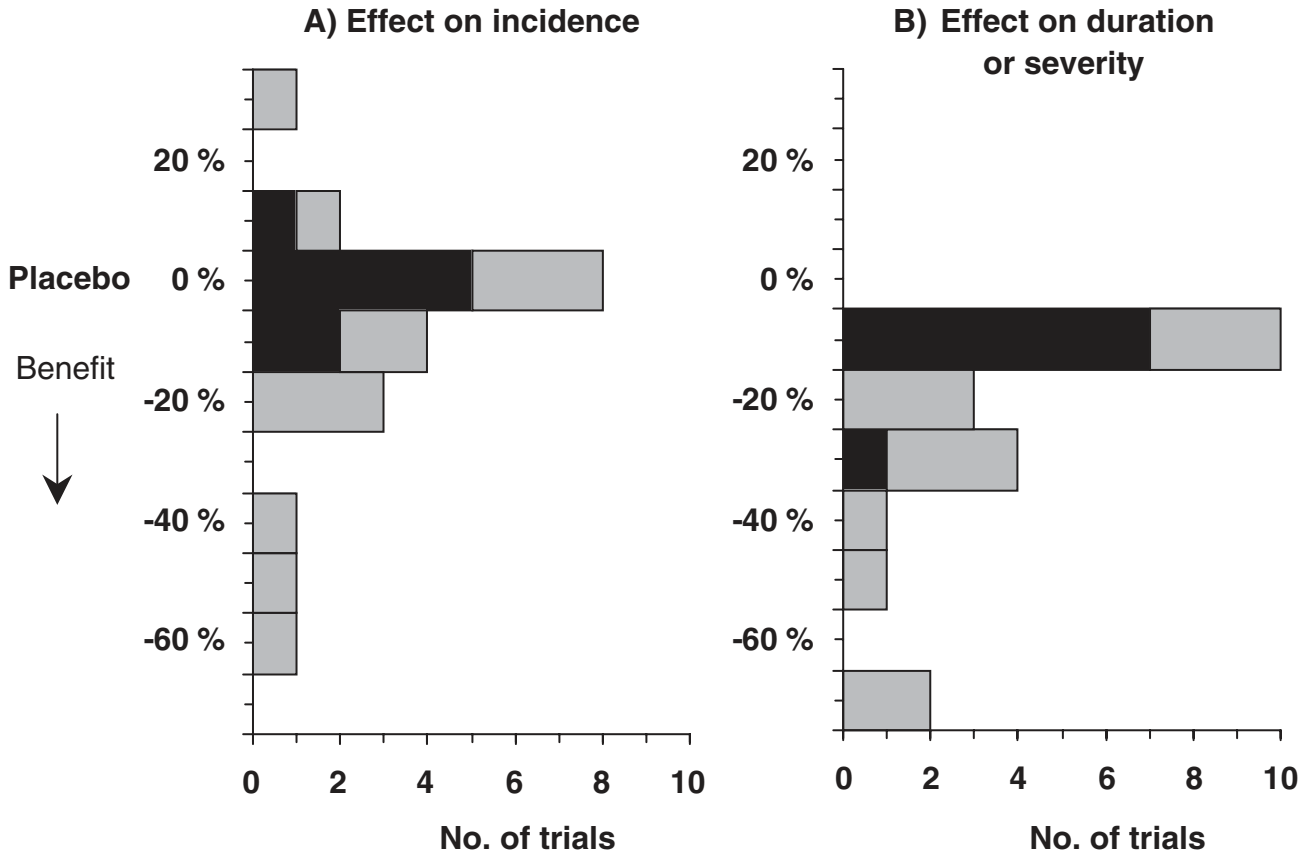


Fig. 3. Vitamin C supplementation does not affect the incidence of the common cold (A), but does decrease its duration and alleviate its symptoms (B). The vertical scale shows the relative effect of vitamin C compared with the placebo. The horizontal bar indicates the number of trials reporting the effect falling within the vertical limits of the bar. Black bars indicate trials with ≥400 participants. All trials used 1-4 g/day of vitamin C, all were placebo-controlled, and all except one were double-blind. For details and original references, see Hemilä (1994a) *Scandinavian Journal of Infectious Diseases* 26:1-6 from which the figure is reproduced with permission.

Meta-analysis: methodological considerations

Meta-analyses on vitamin C and the common cold: from Linus Pauling and Thomas Chalmers to the Cochrane Collaboration

In the 1970s, three meta-analyses on vitamin C and the common cold were published (Pauling 1971a,b; Chalmers 1975). In fact, these three papers were among the first few meta-analyses carried out in medicine. However, the conclusions of these three meta-analyses diverged substantially.

Linus Pauling (1971a; see pp 13, 35-6) combined the P-values derived from 4 placebo-controlled trials by the Fisher method, concluding that there was strong evidence that vitamin C decreased the 'incidence of colds' (P = 0.0014), and the 'integrated morbidity' due to colds (P = 0.000,022). In a second meta-analysis, Pauling (1971b) focused on 'days of illness per person' in the best two trials (Cowan *et al.* 1942; Ritzel 1961; Table 3). Combining the P-values by the Fisher method led him to conclude that "The null hypothesis of equal effectiveness of ascorbic acid and placebo is rejected at the level P less than 0.001."

Thomas Chalmers (1975; see pp 36-8) carried out a meta-analysis of 8 placebo-controlled trials, calculating the unweighted average of the treatment effect. According to his calculation, colds in vitamin C groups were 0.11 ± 0.24 (SE) days shorter, and the incidence of colds in vitamin C groups was 0.09 ± 0.06 (SE) episodes less per year, neither of which is a statistically or clinically significant difference. This meta-analysis of vitamin C and the common cold was Thomas Chalmers' second, his first being published in the 1960s (Grace *et al.* 1966).

In the late 1980s, a fourth meta-analysis on vitamin C and the common cold was published, but neither combined P-values nor pooled estimates were calculated (Kleijnen *et al.* 1989; see pp 38-41). In the 1990s, a series of meta-analyses which focused on various different questions related to the possible effects of vitamin C on the common cold was published (Hemilä 1990a,b, 1992a, 1994a, 1995a, 1996a,b, 1997a,b, 1999a). Since all 21 trials with regular ≥1 g/day doses had found that vitamin C was better than placebo when measuring the severity or duration of colds, the sign-test was used to calculate the probability that all 21 trials would find vitamin C to be better than placebo (P = 2exp(-21) = 0.000,000,5; Fig. 3; Hemilä 1994a). A Cochrane Review on vitamin C and the common cold also appeared in the late 1990s (Douglas *et al.* 1998; see pp 40-2).

Because meta-analysis has been extensively used as a method of analyzing the potential effect of vitamin C on the common cold, the opportunities provided by this method as well as some of its limitations are considered here, in particular since Paper II of the current thesis covers 3 meta-analyses of vitamin C and the common cold incidence with different objectives.

The purpose of meta-analysis

In general, 'meta-analysis' denotes systematic and thorough investigation of scientific literature on a specific topic, and combining the results of 'close enough' studies by statistical formulae, but there is substantial difference of opinion as to how people see the coverage of the term 'meta-analysis.' The term was coined by Gene Glass to describe the process of synthesizing results from separate but similar studies (Mann 1990).

Interestingly, the origin of the meta-analytic approach was connected to vitamin C (Hampton 2002; Milne & Chalmers 2004), since in his treatise on vitamin C deficiency, James Lind carried out a systematic search of all the older literature and wrote that "As it is no easy matter to root out old prejudices, or to overturn opinions which have acquired an establishment by time, custom and great authorities; it became therefore requisite for this purpose, to exhibit a full and impartial view of what has hitherto been published on the scurvy; and that in a chronological order, by which the sources of those mistakes may be detected. Indeed, before this subject could be set in a clear and proper light, it was necessary to remove a great deal of rubbish" (1753 p 7; see Appendix 1).

Typically, meta-analysis is used (1) to increase statistical power for primary end points and for subgroups, or (2) to improve estimates of the size of the effect, or/and to (3) resolve uncertainty when reports disagree (Sacks *et al.* 1987). The purpose of increasing statistical power emerges from the problem that a large proportion of controlled trials are so small that they cannot provide meaningful evidence for the effectiveness or otherwise of therapy, simply because the confidence intervals (CI) are very wide. This problem of low statistical power was illustrated by Freiman, Chalmers, *et al.* (1978) who analyzed 71 'negative' trials (P > 0.05 for the difference of interest), showing that 50 of them could have missed a 50% benefit because the trials were simply too small. Thus, meta-analysis can be used to enhance the use of data from small studies with ambiguous results by combining the results of several to test whether there is any overall evidence of effect, and to estimate its magnitude. This is the most common use of meta-analysis.

When the optimism on the fruitful opportunities provided by meta-analysis was high, Chalmers' group believed that "A quantitative synthesis of the data in similar randomized controlled trials can potentially be more useful to the practicing physician than a traditional narrative review article, but such a synthesis must be properly performed to warrant serious attention" (Sacks *et al.* 1987). Thomas Chalmers also commented that "Meta-analysis is the wave of the future. The days of the expert supposedly putting the state of the field into a review article are numbered" (Mann 1990).

Some examples of problems with meta-analysis

The most severe problems of meta-analysis are related to the experimental similarity of the studies that are combined and their validity. 'Combining apples and oranges' has been

commonly used as a metaphor to describe this problem, but often the meta-analytic mixtures are so heterogeneous that 'combining rotten fruits' might sometimes be a more appropriate way to describe the problem (Feinstein 1995).

Improper consideration of the experimental features of trials is illustrated by an early, often-cited meta-analysis that combined the findings of 6 small randomized trials examining the value of anticoagulants in acute myocardial infarction (Chalmers *et al.* 1977); this was Thomas Chalmers' third meta-analysis. Of the 6 randomized trials included, 2 contained no criteria for the diagnosis of myocardial infarction, and in 3 others the published definitions were so inexact that the patient populations could not be reproducibly identified (Goldman & Feinstein 1979). The treatments also varied from heparin alone to heparin with warfarin or warfarin derivatives and even warfarin with optional heparin, but the modes of action of heparin and warfarin are different and it should not be assumed that these treatments are pharmacologically equivalent enough to be combined in a meta-analysis (Goldman & Feinstein 1979).

In another example of the lack of properly considering the experimental aspects of controlled trials included in meta-analyses, Bailar (1995; MacArthur *et al.* 1995) discussed one meta-analysis by the Chalmers group of 6 trials dealing with the effects of diethylstilbestrol on the outcome of pregnancy (Goldstein *et al.* 1989). One of the 6 trials did not deal with diethylstilbestrol at all, and 3 others were methodologically flawed enough to destroy any credibility in their reported findings. Two studies appeared to have had adequate methodological strength, but one dealt with a series of pregnant women from the general populations, while the other was limited to pregnant women who had diabetes. It seems questionable, at best, to pool results from these last two studies without some thoughtful discussion (Bailar 1995). Furthermore, although Goldstein *et al.* had excluded one trial based on the probable non-alternate assignment of treatment as well as inconsistencies in the text, Bailar identified the same problems in 3 of 5 trials that Goldstein *et al.* did include. Considering the various shortcomings, Bailar concluded that the 'typical odds ratio' calculated by Goldstein *et al.* (1989) was meaningless.

In a further meta-analysis by the Chalmers group, 9 trials with 744 participants were combined to form a pooled estimate of antibiotic prophylaxis for recurrent acute otitis media (Williams *et al.* 1993). "Of those [nine] studies, 2 were on special populations, Alaskan Eskimos and asthmatics involving 388 children. They are nonrepresentative groups of the general population because of differences in severity and pathogenesis. Williams *et al.* also included 3 small crossover trials which used sulfisoxazole (a drug not recommended for extended use). Excluding those studies, there were only 4 RCTs with a combined population of 235 patients [in contrast to Williams' 744 patients]. In that group, the rate difference was only 0.067 [episodes per month; in contrast to Williams' estimate of 0.11]" (Cantekin 1994, 1998).

In addition to the problems in the Goldstein *et al.* meta-analysis (1989), Bailar (1995) also pointed out serious shortcomings in 4 other meta-analyses. Klein (2000) pointed out severe flaws in 4 meta-analyses comparing psychotherapy with pharmacotherapy. Indeed, there are numerous examples of unreliable meta-analyses.

The concept of meta-analysis seems to imply objectivity, but the selection criteria can vary substantially when different research groups carry out meta-analysis of the 'same' topic. In a 'meta-meta-analysis' Katerndahl and Lawler (1999) analyzed 23 meta-analyses that had examined the value of cholesterol reduction in coronary heart disease, finding substantial variation in the meta-analyses and their conclusions. Similarly, Prins and Buller (1996) discussed the divergent findings in 4 meta-analyses on the preferred dosage of aminoglycosides and concluded that "The physician can only follow the conclusion of the meta-analysis most closely in accordance with his or her own beliefs." The divergent and incompatible conclusions of the first three meta-analyses on vitamin C and the common cold were mentioned above (p 28).

Although many meta-analyses of controlled trials are problematic, the problems are even greater in meta-analyses of non-experimental studies, since there may be consistent biases in the studies included. A meta-analysis on the association between chlorination of drinking water and cancer risk by the Chalmers group (Morris *et al.* 1992) included such severely biased studies that, independently, Bailar (1995) and Shapiro (1997) pointed out various problems. Also, Cantor (1994) commented that "A recent meta-analysis [by Morris *et al.*] … has probably confused the situation. This exercise may have been premature since most of the input data came from studies with (1) inadequate control of confounding and other sources of bias, and (2) highly limited estimates of historical exposure to drinking water contaminants."

A meta-analysis of the association between alcohol consumption and breast cancer by the Chalmers group (Longnecker *et al.* 1988) included studies that had such severe methodological defects, that the meta-analysis was considered seriously misleading by Shapiro (1994, 1997; see also Rosenberg 1989), who published both the initial association which led to the series of studies, and finally the large study finding a null result. Because of the large variety of potential biases in non-experimental studies, Shapiro (1997) considered that "The meta-analysis of non-randomized observational studies resembles the attempt of a quadriplegic person to climb Mount Everest unaided."

The main limitations and challenges of meta-analysis are related to the experimental issues. The statistical methods available for combining P-values or data on individual studies are well established (*e.g.*, Greenland 1987, 1998; Laird & Mosteller 1990; Fleiss 1993; Higgins & Green 2005 ss 8.6 to 8.8), but there are examples of demonstrably invalid methods of analysis even in some of the influential meta-analyses. In one meta-analysis, the average case fatality percentage was calculated for 6 trials, without using any weight, even though the number of participants in the 6 trials varied from 53 to 1,427; *i.e.*, close to 30 fold (Chalmers *et al.* 1977). In fact, since the large trials found a consider-

ably smaller benefit than the small trials, combining the actual data instead of percentages led to a substantially smaller difference (by 30%) between the pooled study groups (Goldman & Feinstein 1979). The Chalmers (1977) meta-analysis was cited in a recent systematic review comparing randomized and non-randomized trials (Kunz & Oxman 1998), without noting its severe methodological problems, yet Oxman was an editor of the previous edition of the *Cochrane Reviewers' Handbook*, which commented that "interpretation of results is dependent upon the validity of the included studies" (Clarke & Oxman 2002 s 6).

Lack of basic arithmetic is also seen in a meta-analysis of vitamin C and the common cold in which treatment effects on 'duration in days' were averaged without considering either the differences in the size of the trials or the large variations in the cold duration in the control groups (Chalmers 1975; see pp 36-8). Sometimes meta-analysts are not familiar with the standard methods either; for example, in his vitamin C common cold meta-analysis, Chalmers (1975) claimed that in the earlier meta-analysis on vitamin C and the common cold, Pauling (1971a) had "averaged 'p' values from the different studies." However, in his statistical analysis Pauling used the well-established Fisher method of calculating the combined P-value from several independent P-values (1938; Sokal & Rohlf 1981; Laird & Mosteller 1990), which cannot be described as naive 'averaging.' Furthermore, Chalmers himself used the same Fisher method two years after his criticism (Chalmers *et al.* 1977).

Because of many published meta-analyses were not properly performed, several experts have been rather skeptical as to its usefulness (Meinert 1989; Spitzer 1991; Feinstein 1995; Feinstein & Horwitz 1997; Eysenck 1994; Bailar 1995, 1997a,b, 1999; Shapiro 1994, 1997). Bailar (1995) commented that "Meta-analysis has been seized with enthusiasm by many scientists not trained in statistics and cognate sciences, and it is clear in conversations that many of them have utterly unrealistic views about its scope and power." Shapiro (1997) noted that "I think there is something profoundly amiss in the uncritical way in which the epidemiologists, and indeed the medical profession as a whole, have allowed themselves to be seduced by the numerological abradacabra of meta-analysis." Meinert (1989) commented that "There are no easy, inexpensive answers to complex questions and attempts to substitute small trials and meta-analysis for large trials is illusionary and detrimental to both medicine and clinical trials." In an editorial in a major journal, Bailar (1997a) further commented that "In my own review of selected meta-analyses, problems were so frequent and so serious … that it was difficult to trust the overall 'best estimates' … I still prefer conventional narrative reviews of the literature, a type of summary familiar to readers of the countless review articles on important medical issues." Although such strong comments can be understood against the background of the severe errors in some of the products by meta-analysts, 'meta-analysis' as a method will not disappear. As a statistical tool it has inherent strengths and weaknesses which should be understood by those carrying out such analyses, and by readers of the conclusions of meta-analyses. Furthermore, meta-analyses have provided a large number of conclusions that have been consistent with later findings.

The political and social consequences of meta-analysis have also aroused concern. In particular, the Evidence-Based Medicine (EBM) movement puts great weight on the semi-official meta-analyses of the Cochrane Collaboration (EBMWG 1992; Chalmers *et al.* 1992; Editorial 1992; Sackett 1994; Bero & Rennie 1995; Hill 2000; Cochrane 2005a). Feinstein and Horwitz (1997) concluded a thorough critique with the comment that "The threat of official, corporate, or private abuse will always remain, whenever any collection of information has been prominently heralded as the 'best available evidence.' A new form of dogmatic authoritarianism may then be revived in modern medicine, but the pronouncements will come from Cochranian Oxford rather than Galenic Rome." Shapiro (1994) was worried that "Government departments will continue to make public health decisions, often misguided ones, based on the results of meta-analyses." Bailar (1995) commented that "A traditional narrative review can do much more than estimate parameters, and the additions are critical to the progress of science. Meta-analysis … is a poor tool for developing new concepts, new hypotheses, and new methods of study… meta-analysis has never been promoted as an alternative to thoughtful but unstructured reading, but it may nevertheless carry the seeds of a diminished respect for and a diminished role for simple browsing through the primary literature."

Quality of studies included

The internal validity of the studies included in a meta-analysis is a relevant concern. For example, substantial baseline differences in the treatment groups were found in therapeutic trials that used 'non-random' assignment of participants to the treatment groups, and 'unblinded randomization' led to substantial baseline differences, whereas 'blinded randomization' led to relatively similar baseline variable levels (Chalmers *et al.* 1983). Because of such severe problems with non-randomized studies, the advocates of EBM have suggested that "If you find that the study was not randomized, we'd suggest that you stop reading it and go on to the next article" (Sackett *et al.* 1997 p 94).

If such an opinion became common it would completely transform current medicine, since probably not even extensive literature searches would reveal randomized trials supporting the widespread beliefs that smoking, high-level alcohol usage, and overweight increase the risk of poor health. It is also inappropriate to require that therapeutic conclusions should be based simply on randomized trials. For example, Sir Austin Bradford Hill, who designed the first modern randomized controlled trial (Doll 1992, 1998; Yoshioka 1998; Hampton 2002; Armitage 2003), commented that "Any belief that the controlled trial is the only way [to study therapeutic efficacy] would mean not that the pendulum had swung too far but that it had come right off its hook" (Hill 1966).

In any case, because of the problems related to the study quality, Chalmers *et al.* (1981) proposed a quality scale to assess the validity of trials. About two dozen further 'quality scales' have since been devised. The scoring systems, however, have various shortcomings (Higgins & Green 2005 ss 6.7 to 6.11). Scoring is based on whether something was reported rather than whether it was done appropriately in the study. For example, if the original investigators explicitly stated criteria for the diagnosis of 'congestive heart failure' the trial is given 'quality points' because of the explicit definition. However, if 'congestive heart failure' is defined as 'use of digitalis' the evidence is of poor scientific quality and is clinically silly, but still gets the 'quality score points' because of the explicit definition (Feinstein 1995). A recent survey requesting the technical features directly from the investigators found that in many cases randomization and allocation concealment were appropriate although they were not properly described in the study reports, so that Hill *et al.* (2002) concluded that it is likely to be inappropriate to characterize the quality of randomized controlled trials as 'good' or 'poor' on the basis of the published report.

Furthermore, many scores also contain items that are not directly related to validity, such as whether a power calculation was done (related to precision and not validity) or whether the inclusion and exclusion criteria were clearly described (related to applicability and not validity) (Higgins & Green 2005 s 6.7). In a recent comparison, the summary quality scores were not significantly associated with treatment effects, indicating that the relevant methodological aspects should be assessed individually (Jüni *et al.* 1999). In another recent meta-analysis of 276 trials, double blinding and allocation concealment, two quality measures that are frequently used in meta-analyses, were not associated with treatment effect (Balk *et al.* 2002).

It has been argued that quality scoring is based on subjective assignment of points based on features of the studies, and quality scoring submerges important information by combining disparate study features into a single score (Greenland 1998). "It also introduces an unnecessary and somewhat arbitrary subjective element into the analysis via the scoring scheme. Quality scoring can and should be replaced by direct categorical and regression analyses of the impact of each quality item. Such item-specific analyses let the data, rather than the investigator, indicate the importance of each item in determining the estimated effect."

Shapiro (1997) commented that "Who are these meta-analysts, sitting on high, to decide for the rest of us what is and is not good quality, and then to measure it? Quality is best evaluated qualitatively: as opposed to meta-analysis, in any adequate qualitative review, we require that the author should give reasons for judging the quality of any given study as good or bad in transparent and easily comprehensible language. It is then up to the reader to decide whether he agrees or disagrees."

It is desirable to use a placebo in controlled trials to increase their internal validity. However, a recent meta-analysis of studies comparing a placebo group to a no-treatment group found that there was no placebo effect in studies with binary outcomes and, among studies with continuous outcomes, only those that measured pain showed evidence of the placebo effect (Hrobjartsson & Gøtzsche 2001, 2004). Consequently, lack of a placebo should not lead to the mechanical exclusion of a trial from a meta-analysis, since the relevance of the placebo depends on the topic.

Because of the various problems of 'quality scores,' the current version of *Cochrane Reviewers' Handbook* suggests that "Reviewers should avoid the use of 'quality scores' and undue reliance on detailed quality assessments. It is not supported by empirical evidence, it can be time-consuming, and it is potentially misleading" (Higgins & Green 2005 s 6.11). Thus, it is not reasonable to employ a rigid mechanical algorithm to discard 'low quality score' studies from meta-analysis. The features related to validity should rather be considered case by case because the relevant features depend on the particular scientific question. One type of 'quality scale' was used in the fourth meta-analysis on vitamin C and the common cold for selecting 'high quality' trials for deeper analysis (Kleijnen *et al.* 1989; see pp 38-41). Also, one kind of 'quality scale' was used in a recent review on echinacea and the common cold when selecting two 'best' trials on which the conclusions were based (Caruso & Gwaltney 2005 [see Hemilä 2005a]).

Although randomization is a feasible method of allocating participants in most controlled trials, it seems that the problems caused by the lack of randomization have been grossly exaggerated. For example, Thomas Chalmers' much-cited classical study (1983) suggesting that 'blinded random allocation' leads to smaller treatment effects than 'non-random assignment' was itself severely biased. The group of 'blinded randomization' trials contained 9 trials about beta-blockers and 0 trials about coronary care units. In contrast, the group of 'non-random assignment' trials contained 1 trial about beta-blockers and 11 trials about coronary care units. With such extremely biased distribution of study topics between 'random' and 'non-random' allocation groups, it is not reasonable to assume that the method of allocation is the only reason for the difference between the findings in the two groups, even though Chalmers *et al.* (1983) did so. For example, they presented the 'results of trials in terms of case-fatality rates' by the method of allocation without stratifying by the topic of the trials; there are probably substantial base-line differences between the participants in beta-blocker trials and coronary care unit trials. Some other tables in Chalmers *et al.* (1983) are also misleading as pointed out earlier (Gillman & Runyan 1984).

In spite of the severe methodological shortcomings, the Chalmers *et al.* paper (1983) has been extensively cited, *e.g.*, by EBM proponents when claiming that "Studies in which treatment is allocated by any method other than randomization tend to show larger (and frequently false-positive) treatment effects than do randomized trials" (Guyatt, Sackett, Cook 1993), "Less rigorous studies tend to overestimate the effectiveness of therapeutic and preventive interventions" (Oxman *et al.* 1994), and "Because the potential for bias is much greater in cohort and case-control studies than in RCTs, recommendations from overviews

combining observational studies will be much weaker" (Guyatt, Sackett, *et al.* 1995). Thus, in this case the EBM advocates did not read critically the paper they cited, although they emphasize the importance of critical reading elsewhere (*e.g.*, Sackett *et al.* 1997 pp 79-156).

The Chalmers 1983 paper was also cited in a recent systematic review comparing randomized and non-randomized trials drawing the conclusion "direction of bias: overestimation of effect" (Kunz & Oxman 1998) which makes no sense considering the extremely biased distribution of study topics between the 'blinded randomization' and 'non-random assignment' groups mentioned above. Furthermore, the Chalmers 1983 paper was cited in the *Cochrane Reviewers' Handbook* without paying attention to its lack of validity (Clarke & Oxman 2002 ss 4.2 and 6.3), although the *Handbook* does comment that "Interpretation of results is dependent upon the validity of the included studies" (Clarke & Oxman 2002 s 6), and a guideline-paper for readers of reviews also stated that "Authors will come to correct conclusions only if they accurately assess the validity of the primary studies on which the review is based" (Oxman & Guyatt 1988).

A recent comparison of randomized controlled trials with observational studies on 19 different treatments found that the estimates of treatment effects from the controlled trials and the observational studies were similar. In only 2 of the 19 analyses did the combined magnitude of the treatment effect in observational studies lie outside the 95% CI of the pooled estimate of the controlled trials (Benson & Hartz 2000). Another analysis of 5 clinical topics also found that the average results of observational studies were remarkably similar to those of controlled trials (Concato *et al.* 2000). Both of these two analyses were motivated by the overemphasis on randomization by EBM advocates. Furthermore, a recent analysis of a large set of studies focusing on cirrhosis and hepatitis saw no difference between non-randomized studies and randomized trials in the '20-year survival of conclusions' derived from these studies (Poynard *et al.* 2002).

Cumulative meta-analysis

The various potential limitations of the experimental data should make a meta-analyst cautious in drawing conclusions, but sometimes the conclusions are extraordinarily comprehensive considering the kind of small trials on which they are based.

A particularly bold general proposal related to drawing conclusions from meta-analyses made by the Chalmers group was that meta-analyses should be updated with each new trial so that when, or if, the combined P-value becomes statistically significant at a chosen level, the treatment should be considered proven efficacious, and further trials may be considered even unethical. This approach was called 'cumulative meta-analysis' (Antman *et al.* 1992; Lau *et al.* 1992), a concept considered to be among the most important contributions to medicine by Thomas Chalmers (Ian Chalmers 1996; Liberati 1996). Using cumulative meta-analysis, the Chalmers group showed that the effect of administering intravenous magnesium in acute myocardial infarction reached $P[2\text{-}t] < 0.05$ in 1989, and $P[2\text{-}t] < 0.001$ in 1990 with a cumulative OR of 0.44 (95% CI: 0.27 – 0.71), and they concluded that the evidence for the benefit of magnesium was persuasive (Antman *et al.* 1992; Lau *et al.* 1992). However, a large trial with 58,050 patients carried out thereafter showed that mortality in the first 5 weeks after myocardial infarction was, paradoxically, slightly higher in the magnesium group (+6%; 95% CI: 0% to +12%) (ISIS-4 1995; Egger & Smith 1995). The 'cumulative meta-analysis' thus led to a completely false conclusion.

Nevertheless, it is of interest that, following the reasoning of 'cumulative meta-analysis', if Chalmers (1975; pp 36-8) had restricted his meta-analysis of vitamin C and the common cold to double-blind placebo-controlled trials in which≥ 2 g/day of vitamin C was regularly administered to participants, he might have found powerful evidence by 1975 from 5 trials that vitamin C alleviates the symptoms and/or reduces the duration of colds during supplementation ($P = 0.000{,}002$; Hemilä 1996a). Using the approach of Chalmers' 'cumulative meta-analysis,' the addition of 3 trials that were carried out after 1975 led to $P = 0.000{,}000{,}02$ by 1996 (Hemilä 1996a). No trials with this selection criterion have been published since 1996.

Publication bias

One concern in meta-analyses of small studies is the possibility of 'publication bias' which indicates that studies with 'negative' findings tend to remain unpublished more often than those with 'positive' findings (Chalmers *et al.* 1990). In such cases, meta-analytical conclusions from the published trials may be too optimistic. However, in an analysis of 487 research projects approved by the Oxford Research Ethics Committee, there was evidence of publication bias in the case of observational and laboratory-based experimental studies (odds ratio [OR] 3.8 for the comparison of positive vs. negative results to be published), whereas there was no evidence of publication bias in the case of randomized clinical trials (OR 0.8) (Easterbrook *et al.* 1991). Thus, although publication bias is probably of concern in certain conditions, and must be kept in mind in considering the findings of a meta-analysis, its role should not be exaggerated.

Meta-analysis versus large single trials

In addition to the meta-analysis of intravenous magnesium in acute myocardial infarction, there are several other examples of misleading conclusions from meta-analyses. LeLorier *et al.* (1997) compared the results of 12 large trials with meta-analyses published earlier on the same topic, finding that the meta-analyses would have led to the adoption of an inefficient treatment in 32% of cases, and to the rejection of a useful treatment in 33% of cases.

When large trials were compared with other large trials on the same topic, agreement among them was approximately as low as that reported by LeLorier *et al.* between meta-analyses and large trials, and thus Furukawa *et al.* (2000) concluded that taking large randomized trials as the 'gold standard' can be problematic, and there is "no substitute for clear and hard thinking for a study, be it meta-analysis or a mega-trial." Horwitz (1987), who analyzed 36 topics on which controlled trials led to discrepant results, also considered that the 'gold standard' status of randomized trials is misleading as the results depend substantially on the settings, such as clinical heterogeneity among patients enrolled in the trials, varying protocols across the trials, etc. When Poynard *et al.* (2002) compared the 'survival of conclusions' from meta-analyses with the conclusions from non-randomized studies and randomized trials, they found that meta-analysis was conclusively the poorest of the three for producing viable conclusions. It seems possible that meta-analysis is used on average less on topics on which large studies have shown clearly negative or positive findings, and in this respect the poor 'survival of conclusions' may reflect the topics that are studied better than the method *per se*.

The relevance of the biology of the topic under consideration

"By means centering, I refer to the tendency to consider that the essence of science lies in its procedures rather than in its problems, questions, or goals …
If means-centering philosophies were extreme, and if they were quite consistent, there would be no way to distinguish between an important meta-analysis and an unimportant one.
There could be only technically well-prosecuted meta-analyses and technically poorly prosecuted meta-analyses.
Using only methodological criteria, the most trivial research could demand as much respect as the most fruitful one."

Modification of Maslow (1954)

'Meta-analysis' as a tool is quite easy to understand and apparently powerful, and this has led some people to carry out meta-analyses without making themselves familiar with the actual biology of the topic. Yusuf (1997) was worried that "Sometimes individuals with only limited knowledge of the pharmacologic aspects of treatment, the biology of disease, or clinical circumstances that relate to the specific question may perform meta-analyses leading to an analysis with little clinical relevance." Bailar (1995) commented that "A good meta-analysis requires at least as much as to do a good original article. To proceed without this level of understanding is likely to lead to serious difficulty. The 'job shop' that turns its skills to the formulaic meta-analysis of a sequence of unrelated topics is asking for trouble – and may often get it. Meta-analysis must not be routinized."

A good example of this procedure as a mechanical endeavor detached from biology is carrying out meta-analyses on homeopathy, which is not a reasonable scientific topic for obvious reasons (Vandenbroucke 1997, 1998a; Vandenbroucke & de Craen 2001). A meta-analysis of homeopathic trials by Kleijnen *et al.* (1991) did not refer to any laboratory studies, although a series of papers published in *Nature* only a few years before would have provided relevant background in the form of laboratory experiments (Metzger & Dreskin 1988). In their meta-analysis, Kleijnen *et al.* (1991) commented that one homeopathic "trial of very high quality was … initiated by the French Ministry of Social Affairs and performed by a group consisting of regular and homeopathic researchers. After the earlier publication of several trials in which homeopathy was shown to decrease the time of recovery of bowel movements after abdominal surgery this hypothesis was retested in a rigorous trial… No differences at all were found" between the treatment groups (Mayaux *et al.* 1988). This is substantially similar to the 'positive' findings in the laboratory study on homeopathy, which could not be repeated in four other laboratories (Metzger & Dreskin 1988; Seagrave 1988; Bonini *et al.* 1988; Hirst *et al.* 1993), and not even in the original laboratory when the editor of *Nature* came to visit (Maddox *et al.* 1988).

Nevertheless, the number of 'good quality' homeopathy trials with 'positive results' was so large that Kleijnen *et al.* (1991) concluded that "The evidence presented in this review would probably be sufficient for establishing homeopathy as a regular treatment for certain indications" without specifying what these were. In this case, the thoughtful narrative review on this topic by Vandenroucke & de Craen (2001) is substantially more useful than the meta-analytic list of homeopathic trials with their 'quality scores' by Kleijnen *et al.* (1991).

In another meta-analysis, Kleijnen *et al.* (1989; pp 38-41) focused on vitamin C and the common cold. This meta-analysis contains no reference to any papers related to the immune system studies (some 100 listed by Hemilä 1997a) or to any animal studies (see Table 2 and Appendices 2 and 3) that would provide important background for considering whether this is a reasonable biological issue rather than a topic for mechanical 'job shop' pooling. Neither did Chalmers (1975; pp 36-8) refer to any immune system studies or animal studies in his meta-analysis of vitamin C and the common cold; however, he did mention the Hume and Weyers study (1973) reporting that vitamin C in leukocytes drops sharply on the first day of the common cold (Fig. 1), and such a dramatic change in vitamin C metabolism does provide one way to rationalize the question of whether large doses of vitamin C might have therapeutic effects on colds. Pauling (1971a,b; pp 35-6) did not supply any direct reference to biological studies in his meta-analyses, merely referring to a book which discussed the biological background (Pauling 1970a).

Heterogeneity of studies

Much of the consideration of meta-analysis is focused on the experimental features of the studies included. Another important issue is the question of the goals of conducting meta-analysis. The notions of 'exploratory' and 'analytical' meta-analysis were proposed to emphasize the great variation in the possible goals (Anello & Fleiss 1995), the former referring to combining small trials of variable quality, and the latter to combining large well-conducted trials intended to shed more light on the precision of the estimate and on more powerful exploration of subgroup differences. Although such dichotomizing of goals may be too extreme, the notion that meaningful goals of meta-analysis differ substantially case by case should be understood.

One particular issue related to the goals of meta-analysis is how to handle large variations in the results of the trials under consideration. Analysis of heterogeneity can be the most important function of meta-analysis, often more important than computing the fictional average effect of a heterogeneous mixture. A common problem in meta-analysis is the failure to investigate the sources of heterogeneity appropriately. Although overzealous inspection of the data in hand may lead to false conclusions, a sensible investigation of the sources of heterogeneity may increase both the scientific and clinical relevance of the results of meta-analysis (Thompson 1994; Greenland 1998). One problem in the statistical consideration of heterogeneity has been the low power of standard tests of heterogeneity leading to the false conclusion that the data is homogeneous, but a more sensitive test has been developed recently (Higgins *et al.* 2003).

Non-comparability of the vitamin C trials

One particular problem in the meta-analysis of vitamin C trials arises from the fundamental difference between vitamin C and ordinary drugs such as antibiotics. It is possible to select a control group which has no intake of an ordinary drug, rendering the interpretation of results relatively simple. It is impossible, however, to select control subjects who have no vitamin C intake, and no vitamin C in their system. Accordingly, all vitamin C trials compare two different intake levels, the lower level being obtained from the diet, and usually not estimated at all, which hampers the comparison of different trials and the generalization of their results.

Since the intake of vitamin C in the diet, and the supplementary dosage have both varied substantially between trials (**Table 12**), the comparison of vitamin C trials is sometimes quite problematic. For example, while there is some 50-fold difference in the vitamin C intake in the diet by the control group between the Glazebrook and Thomson (1942) and Peters *et al.* (1993) trials, both are 'control groups' of vitamin C trials.

The vitamin dosage in the 'vitamin C group' has also varied dramatically (Table 12). At the extreme, Karlowski *et al.* (1975) administered up to 6 g/day of vitamin C to their subjects, whereas Cowan *et al.* (1942) administered 25 mg/day as their lowest supplementary dose. There is thus up to a 240-fold difference between the lowest and highest vitamin C supplementary dose used in these trials, yet in many cases such studies are simply labeled 'vitamin C trial' without paying any attention to the dosage.

Furthermore, the *placebo group* of Peters *et al.* trial (1993) received some 500 mg/day of vitamin C in their diet, whereas the *vitamin C group* in Baird *et al.* trial (1979) received only 130 mg/day (diet and supplement together). Thus, vitamin C intake in the placebo group of the former trial was 4 times higher than the vitamin C intake in the vitamin C group of the latter trial.

A further problem in vitamin C doses is the addition of vitamin C to the placebo group in some studies with the apparent rationalization of excluding the possibility that any observed effects of large doses might be explained by incidentally treating marginal deficiency. For example, Carr *et al.* (1981) administered 70 mg/day and Miller *et al.* (1977) 50 mg/day to their placebo subjects. When comparing these 'placebo-group' vitamin C doses to the 80 mg/day of vitamin C administered to the 'vitamin C group' of the Baird *et al.* trial (1979) or 25-50 mg/day in the Cowan *et al.* trial

Table 12. Variation in vitamin C intake in diet and supplementary doses in common cold trials

Trial Country Participants	Vitamin C in diet (mg/d)	Vitamin C supplement (mg/d)
Glazebrook-1942 UK Schoolboys	10-15	50-300
Peters-1993 South Africa Marathon runners	500	600
Baird-1979 UK Students	50	80
Cowan-1942 USA schoolchildren	?	25-50
Karlowski-1975 USA Adults	?	3,000-6,000

(1942), it is clear that the comparison of 'vitamin C trials' can be very complex, and extrapolation of the findings of a single trial is limited irrespective of the methodological quality of the particular trial.

The great variation in the vitamin C doses in diet and in supplements probably explains part of the variation in the results of the trials. An interesting example of the possible modification of supplement effect through diet was reported by Anderson *et al.* (1972, 1975), who found that vitamin C was more beneficial for participants who consumed low amounts of orange juice, which is an important source of vitamin C (**Table 13**).

The intake of vitamin C in the diet, and the supplementary dosages are rarely considered in discussions of vitamin C trials. For example, they are not considered in the meta-analyses by Chalmers (1975; pp 36-8) and Kleijnen *et al.* (1989; pp 38-41). Dykes and Meier (1975; pp 42-5) listed the supplementary vitamin C doses used in the trials, but did not consider the variation in dietary intake.

Table 13. Subgroup differences in the Anderson *et al.* trials (1972, 1975)

	Effect on 'total days indoors'	
Subgroups	(1972) Regular supplement	(1975) Therapeutic supplement
Daily juice		
0-3 oz	-48%	-33%
≥4 oz	-22%	-22%
Contact with young children		
Yes	-46%	-40%
No	-17%	-13%
Usual colds		
≥2	-43%	
0-1	-13%	

Anderson *et al*. (1972) administered 1 g/day over the whole study period and 3 g/day extra for 3 days during cold episodes. Anderson *et al*. (1975) administered 1.5 g/day on the first day of the cold and 1 g/day for the following 4 days. In the latter trial participants were also regularly administered 0.5 g once a week (i.e., 0.07 g/day) on a rationalization that remains obscure.

Major meta-analyses and reviews on vitamin C and the common cold

Pauling's meta-analyses (1971a,b)

In his first meta-analysis in the *Proceedings of the National Academy of Sciences*, Linus Pauling analyzed the findings of 4 placebo-controlled trials in which at least 0.1 g/day of vitamin C was administered regularly to the study group (1971a). In his second meta-analysis in the *American Journal of Clinical Nutrition*, Pauling focused on the best 2 of the 4 trials (1971b; Cowan *et al.* 1942; Ritzel 1961; Table 3).

Among the 4 trials included in Pauling's meta-analysis (1971a; see Pauling 1972; p 28), the largest dose was used by Ritzel (1961), and Pauling based his quantitative estimations on this trial. Ritzel found that the common cold symptoms in the vitamin C group were 31% shorter and the number of colds 45% lower in the vitamin C group. Pauling also calculated the combination of duration and incidence, 'integrated morbidity' referring to the total sickness days per person during the trial, and this was reduced by 61% in the Ritzel trial (Table 3). Pauling (1971a) then modeled the dose-dependency of vitamin C effect with exponential formulas for which he took constants from the Ritzel trial. Pauling assumed that the main problem in his estimation was inaccuracy caused by 'experimental error,' although he did note that "The values are, of course, expected to depend somewhat on the nature of the population and environment." However, even with these explicit reservations he was far too optimistic. He could not imagine how great the variations in the results would be in the forthcoming trials. Neither did he consider the possibility that the effects observed by Ritzel may have been caused at least in part by low dietary vitamin C intake, in which case a smaller dose might have produced a similar benefit, and in such a case modeling the vitamin C effect as a function of the supplementary dose would be completely erroneous. Pauling attributed the difference between the study groups entirely to the large dose given to the treatment group. Furthermore, Ritzel carried out his trial with schoolchildren in a skiing school in the Swiss Alps, children who are not a good representative selection of the general population even though technically the trial was good as it was randomized, double-blind and placebo-controlled. Thus, when Pauling extrapolated the results of Ritzel to all people (*i.e.*, to children at school and adults), he took a bold step and went wrong (Hemilä 1997b).

Pauling (1971a,b) put much weight on the 'integrated morbidity' outcome and summarized the findings of trials by this outcome in his later texts as well (1976a,b,c, 1986a). This measure led Pauling to adhere strongly to the idea of regular supplementation. However, this is not a good combined measure, since the effects on incidence and duration/severity have quite different patterns (Fig. 3), and it is thus more to the point to analyze these two outcomes separately. Thus, Pauling was qualitatively correct in his conclusion that vitamin C does affect the duration and severity of colds, and probably the incidence of colds in certain specific conditions, but he was greatly over-optimistic.

It is worth noting that the Ritzel trial (1961) falls to the group of 6 trials with participants under heavy acute physical and/or cold stress that consistently found reduction in common cold incidence (p 48; graph on the cover; Douglas & Hemilä 2005). Thus, it was not a misjudgment by Pauling to put the greatest weight on this trial, but his error was to extrapolate the findings to the general population. The other trial on which Pauling put great weight was the Cowan *et al.* trial (1942; Table 3) which was carried out with schoolchildren during the war years and probably the dietary vitamin C intake was low and in this respect the benefit may be explained by the correction of marginal deficiency as in the UK studies with schoolboys and male students (pp 46-7; Paper II).

As regards the errors in Pauling's quantitative conclusions, it should obviously be taken into account that essentially all of the trials available today were carried out after Pauling worked on the topic and, even more importantly, were carried out precisely because Pauling popularized the topic (Fig. 2). Without bold conjectures, progress in science is slow or non-existent, and in this respect the accuracy of Pauling's extrapolation from the single placebo-controlled trial using regular 1 g/day supplementation available to him in the early 1970s is of secondary concern. Furthermore, Pauling's own view of science was that an occasional mistake, even when published, was not as bad as lowering one's sights to less challenging research (Lipscomb 1994).

Chalmers' meta-analysis (1975)

Thomas Chalmers was an eminent pioneer in controlled trials, and one of the early promoters of meta-analysis (Dickersin 1996; Huth 1996; Liberati 1996; Maclure 1996; NLM 2005d). Greenhouse (1996) commented that Chalmers was the most influential non-statistician to affect the acceptance and direct the course of the randomized, controlled clinical trial among clinical investigators, at least in the USA. Chalmers was a founding member of the Society for Clinical Trials, and president of that society in 1984 (Knatterud 1996). "In leadership roles at the US Veterans Administration and National Institutes of Health, Chalmers' intellectual and administrative support of randomized trials helped lead to their establishment as a prime source of therapeutic evidence" (Feinstein 1996). "Tom Chalmers' name is synonymous with randomized, controlled trial and meta-analysis… Many of the medical advances discovered in the past several decades through the use of randomized, controlled trials can in large measure be attributed to his tireless advocacy" (Lau 1996). It was even commented that Thomas Chalmers "contributed more than anyone else to the general field of clinical science" (Ian Chalmers 1996).

A volume of the *Annals of the New York Academy of Sciences* was dedicated "To Thomas C. Chalmers - A Meta-Analyst for All Seasons" (Warren & Mosteller 1993), and a book entitled *Systematic Reviews* was dedicated to "Thomas C Chalmers in appreciation of his many pioneering contributions to the science of reviewing health research, and in particular, for the first clear demonstration of the dangers of relying on traditional reviews of research to guide clinical practice" (Ian Chalmers & Altman 1995). At each annual Cochrane Colloquium "The Thomas C Chalmers MD Award" is given for the best oral or poster presentation at the Colloquium (Cochrane 2005b).

Because of this personal background, a meta-analysis on vitamin C and the common cold by Thomas Chalmers obviously became highly influential, and found its place in major medical textbooks as soon as the ink on the paper had dried.

For his meta-analysis in the *American Journal of Medicine*, Chalmers (1975) selected 8 placebo-controlled trials and presented their results in a table. Chalmers calculated that the common cold episodes were on average 0.11 ± 0.24 (SE) days shorter in the vitamin C groups than the placebo groups. Even if real, a 0.11 day (= 2.6 hours) decrease in the duration of colds would have no clinical relevance, and the great variation in the results indicates that the slight difference is not real. Apparently, because of the presentation of the published results in a table, and the clear-cut negative findings, Chalmers' meta-analysis met ready approval.

Chalmers' meta-analysis has been frequently cited as evidence that vitamin C has been shown to be worthless for colds. For example, it was cited in the US nutritional recommendations (FNB 1980, 1989a), major textbooks of infectious diseases (Gwaltney 1979, 1985, 1990, 1995; Liu 1989; Dick *et al.* 1992, 1998), and numerous textbooks on nutrition (Halsted 1993; Thurnham *et al.* 2000; Hamilton & Whitney 1982, 1994; Whitney & Rolfes 1993; Shils *et al.* 1994).

The American Medical Association based its official statement that "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" wholly on Chalmers' review (AMA 1987 p 1934). *Lancet* also referred to Chalmers' meta-analysis in stating that vitamin C has no worthwhile effect on the common cold (Editorial 1979 [see Pauling 1979]).

Chalmers' meta-analysis was cited in several major reviews of common cold therapy (Hirsch & Swartz 1980; Anderson *et al.* 1983; Sperber & Hayden 1988; Steele 1988; Lorber 1996), and it was also cited by Finnish authorities on nutrition (Aro 1985a,b, 1990a) in stating that vitamin C is useless for colds.

Chalmers' meta-analysis was recently shown to contain a large number of serious errors (Hemilä & Herman 1995). In some cases the data was inconsistent with the original published data, there were errors in calculation, the selection of trials was not consistent, and in some trials a clinically less meaningful outcome was selected. **Table 14** shows the data for common cold duration that Chalmers presented in his main table (one trial did not provide data for common cold duration and is not listed in Table 14: Franz *et al.* 1956; see Table 3). The figures which are erroneous or misleading in Chalmers' table are underlined in Table 14 (for details see Hemilä & Herman 1995).

Chalmers did not consider the dose of vitamin C used in the trials. At the extreme, Karlowski *et al.* (1975) administered up to 6 g/day of vitamin C to their subjects, whereas

Cowan *et al.* (1942) administered 25 mg/day as their lowest supplementary dose (Table 12), meaning an up to 240-fold difference between the lowest and highest vitamin C doses used; however, these two trials were presented side-by-side by Chalmers (1975) in his table without noting this difference (Table 14). Furthermore, Chalmers justified his meta-analysis by referring to Pauling's 1970 book, but Chalmers included in his table a trial in which only 0.025 - 0.05 g/day of the vitamin was used (Cowan *et al.* 1942). This latter trial obviously does not serve as a meaningful test of Pauling's hypothesis that ≥1 g/day might be beneficial against colds.

In the case of the Anderson *et al.* trial (1972) Chalmers did not justify his outcome selection. He showed the effect of vitamin C on days 'symptoms present' per episode which was reduced by 5% (P = 0.15) in his table, but did not mention the effect on days 'confined to house' per common cold episode which was reduced by 21% (P = 0.007), even though the latter outcome would seem more relevant for public health considerations since it is a measure of how much these infections cause actual functional limitations, whereas a runny nose is simply a minor nuisance.

Furthermore, there are severe arithmetical problems in Chalmers' meta-analysis. In his table, the shortest cold duration in a placebo group is 1.0 days and the longest 6.3 days (Table 14). Chalmers calculated the mean for the absolute difference in duration without paying any attention to the great variation in the control group duration. For example, if a 6-day cold is shortened by 1 day, it is not equivalent to a 1-day cold being shortened by the same amount, although both differences are equal in absolute units. Furthermore, a 6-day cold can be shortened by 2 days, but a 1-day cold cannot. Thus the outcome scales of such trials are incompatible in absolute units. Consequently, it seems much more reasonable to calculate the relative effect of vitamin C, so that a 6-day cold shortened by 2 days and a 1-day cold shortened by 0.33 days both correspond to an equivalent 33% reduction. Calculating the relative effect corresponds to normalization of all control groups to an episode duration of one unit or 100%.

Another shortcoming in Chalmers analysis was not considering the size of the trials. For example, Anderson *et al.* (1972) recorded 1,170 episodes, whereas Karlowski *et al.* (1975) recorded only 249 (Table 10). In calculating a pooled estimate, the former trial should obviously be given much greater weight than the latter trial. Lack of weighting by study size leads to an inappropriate significance of small inaccurate trials in the calculation of the summary estimate.

A further arithmetical problem in Chalmers' meta-analysis relates to the presentation of papers that contain more than one trial. In the case of the Cowan *et al.* paper (1942), Chalmers correctly presents the two reported trials separately (Table 14). However, in the case of the Coulehan *et al.* paper (1974), Chalmers combines the two reported trials together, one with 1 g/day and another with 2 g/day of vitamin C. This combination of Coulehan trials gives more emphasis in his table to the two trials by Cowan *et al.* which administered only 0.2 g/day and 0.025-0.05 g/day. Further-

Table 14. Ascorbic acid and the common cold:
'Reasonably well-controlled studies' according to Chalmers (1975)

	Ascorbic acid		Placebo		
Trial	No. of subjects	Mean duration (days)	No. of subjects	Mean duration (days)	Difference in duration (days)
Anderson-1972	407	3.96	411	4.18	0.22
Anderson-1974	583	3.28	578	3.18	-0.10
Coulehan-1974	321	4.71	320	5.92	1.21
Wilson-1973	158	2.65	144	2.79	0.14
Karlowski-1974	101	6.80	89	6.30	0.50
Cowan-1942	233	1.10	194	1.60	0.50
Cowan-1942	227	1.70	120	1.00	-0.70
				Mean:	0.11 ± 0.24 (SE)
Ritzel-1961	139	1.35	140	1.95	0.60

Erroneous and misleading numerical values are indicated by underlining.
Chalmers also listed the incidence of colds, but these are left out to save space.
This table is reproduced from Hemilä & Herman (1995) *Journal of the American College of Nutrition* 14:116-23 with permission.

more, when the unweighted mean effect is calculated, the two small-dose trials of Cowan *et al.* are accorded a weighting factor of two, while the two large-dose trials of Coulehan *et al.* get a weighting factor of only one.

The trials known to Chalmers which had used ≥1 g/day of vitamin C, *i.e.*, that were testing Pauling's proposal, were reanalyzed and the common cold episodes were calculated as 0.93 ± 0.22 (SE) (unweighted mean; P = 0.005) days shorter in the vitamin C groups (Hemilä & Herman 1995). Thus, an estimate of more than 8 times Chalmers' estimate was obtained by employing correct values and keeping to trials which used doses as high as Pauling had suggested. Furthermore, a more useful measure of effect from the same trials is a 21.2% ± 3.0% (SE) reduction of symptom duration. As expected, the relative effect leads to a more accurate estimate of effect as indicated by the smaller P-value (P = 0.000,5).

Chalmers review (1975) is no longer cited in the recent editions of major textbooks on infectious diseases (Liu 1994; Gwaltney 2000, 2005), but it is still cited in the current US nutritional recommendations (FNB 2000 p 127). Also, the Chalmers review (1975) was cited in a recent review on echinacea and the common cold when proposing that the effects of echinacea in placebo-controlled trials are caused by the placebo effect (Caruso & Gwaltney 2005 [see Hemilä 2005a]).

Kleijnen's meta-analysis (1989)

Kleijnen *et al.* (1989) carried out a thorough search of the literature on vitamin C and the common cold and published a biography of the controlled trials identified. Only a few old trials are missing from Kleijnen's bibliography, and a few have been published since (Table 6). Kleijnen also published a table presenting the findings of major trials.

Kleijnen's meta-analysis (1989) was published in Dutch with a translation into English in his thesis. This meta-analysis is of particular interest since Kleijnen became the director of the Centre for Reviews and Dissemination (CRD, York, UK) which writes abstracts of meta-analyses for the Database of Abstracts of Reviews of Effectiveness (DARE). Prior to joining CRD Kleijnen was the Director of the Dutch Cochrane Centre. Also, Kleijnen is one of the authors of the book entitled *Systematic Reviews to Support Evidence-Based Medicine* (Khan *et al.* 2003). Furthermore, Kleijnen's 1989 meta-analysis of vitamin C and the common cold was used as an example in a paper on systematic reviews in *BMJ* (Knipschild 1994), which was later republished as part of a book (Ian Chalmers & Altman 1995 pp 9-16). Furthermore, the Kleijnen *et al.* paper (1989) is cited in one of these DARE abstracts (Anonymous 2005). A meta-analysis on vitamin C and the common cold by an expert on systematic reviews is highly important. Finally, the selection of 11 'high scoring' trials by Kleijnen *et al.* (1989) was directly used in the 1998 version of the Cochrane Review on the same topic (Douglas *et al.* 1998; see next section).

It has been argued that quality scores are at best useless and at worst misleading (Greenland 1994); however, Kleijnen *et al.* used a scoring system to select trials for further analysis. For example, Kleijnen gave 1 point for trials that had over 200 participants, and 1 point for trials that lasted over 3 months. When the outcome of interest is a 'common cold episode,' the number of episodes is of primary interest because it is directly related to the precision of the results. Duration of the trial and the number of participants, used in the Kleijnen scoring system, are not directly relevant in this respect since there are considerable variations in the incidence of colds in the published trials, long duration or a large number of participants does not always lead to a large number of episodes.

At the extreme, Kleijnen's scoring system led to the inclusion of the Coulehan *et al.* trial (1974), which recorded only 75 cold episodes, and the exclusion of the Elwood *et al.* trial (1976), which recorded 1,317 episodes, the excluded trial recording 17 times as many episodes as that included. Furthermore, Kleijnen explicitly commented that "We think that randomization and blinding are most important." However, the Coulehan trial (1974) used allocation by alphabetic order, not randomization, whereas the excluded Elwood trial (1976) used randomization. The Elwood trial was a double-blind placebo-controlled trial and there are no obvious methodological reasons to exclude it.

Furthermore, Kleijnen wrote that "1 point was given if the placebo had been described". Karlowski *et al.* (1975) described that "This study was designed during the summer and rushed into operation to take advantage of the rise in upper respiratory infections expected to occur in the fall. There was no time to design, test, and have manufactured a placebo that would be indistinguishable from ascorbic acid" (p 1041) … "Some of the volunteers had tasted their capsules and professed to know whether they were taking the ascorbic acid or the placebo [consisting of lactose which is sweet and not acidic]" (p 1041). In contrast, Elwood *et al.* (1976) described that their tablets "contained either 1 g ascorbic acid in an effervescent base or a matching placebo" indicating that their vitamin C and placebo tablets were indistinguishable. Although we have good reason to assume that the placebo control was substantially better in the Elwood trial, Kleijnen included in his further analysis the Karlowski trial but not the Elwood trial.

Kleijnen *et al.* (1989) calculated the point scores for each trial, 11 trials receiving 8 points or more. These 11 trials were included in the further analysis and their results were presented in a table in Kleijnen's paper (listed in **Table 15**).

The 11 'high score' trials in Kleijnen's table recorded overall 9,201 common cold episodes. The largest trial in Kleijnen's table was the Anderson *et al.* trial (1974), which recorded 3,590 episodes in all. This trial, however, was very complicated. Anderson had 8 study arms, 2 of which were administered a placebo. Recollection of previous colds considerably differed between the 2 placebo groups, indicating that there were problems with allocation of participants to these study arms (**Table 16**). In the case of recollection of 'usual days indoors' placebo group #6 showed the shortest colds among all 8 arms and there is strong evidence that this group is inconsistent with the 6 vitamin C groups in

Table 15. The major vitamin C common cold trials according to Kleijnen *et al.* (1989)

Trial	Total No. of episodes	Allocation	Blindness [1]
Anderson-1972	1,170	random	DB
Anderson-1974	3,590	random	DB
Coulehan-1974	75	alternately [2]	DB
Anderson-1975 [3]	635	random	DB
Karlowski-1975 [4]	249	random	DB
Coulehan-1976	196	random	DB
Ludvigsson-1977b	1,279	random	DB
Miller-1977	412	random	DB
Pitt-1979	1,219	random	DB
Carr-1981	292	random	DB
Bancalari-1984	84	random	DB
Total	9,201 episodes in all trials of this table		
Total	5,611 episodes if Anderson-1974 is excluded (see text)		

[1] DB, double-blind trial.
[2] "All children were assigned alternately, from an alphabetic listing by classroom, to one of two study groups… [by] a pharmacist, not otherwise involved in this investigation."
[3] Therapeutic trial; 1.5 g/day of vitamin C was administered on the first day and 1 g/day for the following 4 days (i.e., a total of 5 days). Once a week the vitamin C group was administered 0.5 g of vitamin C (i.e., on average 71 mg/day), for which the rationalization is obscure.
[4] Therapeutic (3 g/day for 5 days) and regular supplementation (3 g/day each day) trial with a 2x2 factorial design (see Table 8).

this baseline variable (P[2-t] = 0.000,02; Table 16). The recollection of 'usual days off work' was also peculiarly low in placebo group #6. Furthermore, the proportion of participants who reported 'contact with children' differed significantly between the 2 placebo groups. When the expected benefit of vitamin C supplementation is of a magnitude of 10-30%, this kind of baseline bias seriously hampers the analysis of the results. Furthermore, during the trial, placebo group #6 had significantly lower 'total days of symptoms' per participant compared with placebo group #4 (P[2-t] = 0.005; Table 16). In fact, Anderson (1974) pointed out in their discussion that the 2 placebo groups were divergent, indicating that not all of the groups were well matched. Moreover, Anderson reported that there was a labeling error in 2 batches of bottles out of the 176 batches, but they changed participants between 2 study arms and considered the labeling error was thus compensated for. There were also 1,171 dropouts among the original 3,520 participants (*i.e.*, 33%) which also decreases the validity of this particular trial. Kleijnen (1989) paid no attention to the various shortcomings of the Anderson (1974) trial, but calculated the point scores mechanically. With an 8-arm trial with explicit evidence of bias between 2 placebo arms, a 33% dropout proportion, and errors in labeling it is not clear that one should simply look at the high 'Kleijnen scores.' It seems that Anderson was too ambitious in his 1974 trial.

If we exclude Anderson's trial (1974) referring to the shortcomings discussed above, there are 5,611 common cold episodes remaining in Kleijnen's 'high score' trials, *i.e.*, a reduction by 39% in the number of cold episodes.

Kleijnen excluded 27 placebo-controlled trials from further analysis because they got low 'Kleijnen scores' in the arbitrary scoring system (listed in **Table 17**). Overall these trials contain 8,579 common cold episodes, thus nearly as many as the 'high score trials.' 11 of the trials excluded are randomized and double-blind trials recording 3,740 common cold episodes (Table 17). Kleijnen thus excluded a large number of randomized and double-blind trials from his further analysis, but included the Coulehan trial (1974) that was non-randomized and the Anderson trial (1974) which had evidence of various problems. Although the number of episodes is highly relevant to the precision of the results, Kleijnen included 2 trials that had less than 100 episodes (Table 15) but excluded 7 randomized double-blind placebo-controlled trials that had over 100 episodes (Table 17). The final sets of included and excluded trials in Kleijnen's review are good examples of the problems that result from mechanical 'quality scoring.'

Furthermore, Kleijnen shows the 11 'high scoring' trials in a table with subjective comments, but the presentation suffers from several shortcomings (**Table 18**). The subjective comments are also in some cases demonstrably erroneous. Kleijnen states that Pitt and Costrini (1979) found 'no difference' in the severity of colds between the study groups. In fact, they reported an average severity score of 1.87 in the vitamin C group and 1.97 in the placebo group and tested the difference: χ^2 (15 df) = 27.8 (P = 0.012). Although a 5% difference in severity is a clinically minor finding, the statistically significant difference suggests a real biological effect. It is possible that this effect is greater under dissimilar conditions and in this respect a subjective comment of 'no difference' is misleading.

In case of the complicated Anderson *et al.* trial (1974), Kleijnen comments that there was 'no difference' in common cold duration or severity. In fact, Anderson remarked that there seemed to be "a consistent dose-related effect associated with the 4 and 8 g therapeutic-only regimens [groups #7 and #8] … group #8 (8 g/day on the first day of illness) experienced a larger number of one-day 'false-alarm' or 'aborted' episodes than any other group" (**Table 19**). Groups #7 and #8 are well balanced with respect to the recollection of previous colds (Table 16), and the incidence of colds during the trial was nearly identical (Table 19). In this

Table 16. The allocation problem in the Anderson *et al.* trial (1974)

Study Arm	Dose [1] (g/d)	Recollection at baseline: Usual days indoors (days/cold)	Recollection at baseline: Usual days off work (days/cold)	Recollection at baseline: Contact with children	Outcome during the trial: Total days of symptoms	Outcome during the trial: Free of sickness
#6 Placebo	0/0	1.97 [2]	1.56 [2]	46% (202/440) [3]	4.16 [4]	25.6% (75/293) [4]
#1 Vitamin	1/4	2.46	2.07	35%		
#2 Vitamin	1/0	2.23	1.72	43%		
#3 Vitamin	2/0	2.33	1.79	42%		
#5 Vitamin	0.25/0	2.46	2.12	38%		
#7 Vitamin	0/4	2.46	1.95	37%		
#8 Vitamin	0/8	2.46	2.01	41%		
#4 Placebo	0/0	2.57 [2]	2.07	38% (167/440) [3]	5.40 [4]	18.2% (52/285) [4]
Difference between two placebo groups (#6 and #4)		-23% (1.97/2.57)	-25% (1.56/2.07)	+21% (.46/.38)	-23% (4.16/5.40)	+41% (.256/.182)

[1] "A/B" dosage indicates that the regular dose over the entire study period was A, and B indicates the therapeutic dose the participant took on only the first day of the cold episode.

[2] Based on the central limit theorem (Altman 1991 p 154) we may assume that the mean values of the 6 vitamin groups follow the normal distribution. Thus the mean 'usual days indoors' is 2.40 ± 0.10 (SD) for the set of 6 vitamin groups, so that in this distribution the placebo group #6 level (1.97) corresponds to Standard Normal deviate z = 4.3 [=0.43/0.10] and corresponds to P[2-t] = 0.000,02, while the group #4 level (2.57) corresponds to z = 1.7 and P[2-t] = 0.09.
For the 6 vitamin groups, the mean 'usual days off work' is 1.94 ± 0.16 (SD). Assuming this corresponds to the normal distribution, the group #6 level (1.56) corresponds to z = 2.37 and P[2-t] = 0.018.

[3] The number of participants is inferred from the reported percentage. Comparison of placebo groups #6 and #4: mid-P[2-t] = 0.017.

[4] For the placebo groups, the 'total days of symptoms' during the trial follow-up: 4.16 (SD 4.53; N = 293) and 5.40 (SD 6.00; N = 285) vary significantly (P[2-t] = 0.005; t-test), between arms #6 and #4. There is also a statistically significant difference in the outcome 'free of sickness during the trial' between the 2 placebo groups (mid-P[2-t] = 0.027).

respect the 6.6% difference in favor of the higher dosage is interesting as regards the possible therapeutic effects of vitamin C. Thus, it is misleading to state, as Kleijnen does, that 'no differences' were observed in the Anderson trial (1974).

The Cochrane Collaboration meta-analysis (1998)

In the late 1990s, a Cochrane Review of the role of vitamin C for preventing and treating the common cold was published (Douglas *et al.* 1998). This analysis was based on the combination of trials selected by Kleijnen *et al.* (1989) and Hemilä (1992a). Cochrane systematic reviews are particularly important as they are a kind of official review within the populistic EBM movement, which has various conceptual and practical shortcomings (Feinstein & Horwitz 1997; Charlton 1997; Miles *et al.* 1997, 2003; Black 1998; Charlton & Miles, 1998; Hampton 1997, 2002; Tonelli 1998; Vandenbroucke 1998b; Berk & Janet 1999; Goodman 1999; Norman 1999, 2003; Rees 2000; Williams & Garner 2002; Louhiala & Hemilä 2005a,b,c). In some recent reviews on the common cold, the Cochrane 1998 review was the only reference to vitamin C trials (Scott & Orzano 2001; West 2002; Heikkinen & Järvinen 2003). The Cochrane Review was also used in commenting on the role of vitamin C on the common cold in editorials of *BMJ* (Bender 2002) and *Epidemiology* (Spiers 2002). It was also cited in a recent report on the use of complementary and alternative medicine in the USA (HPDP 2005).

The severe problems in the Kleijnen *et al.* selection of 'high score' trials (1989) are discussed in the preceding section. When describing the Kleijnen set, the Cochrane 1998 review stated that several of the trials were "rejected by Kleijnen because they were not double-blind randomized controlled trials" and "Kleijnen adopted double-blind randomized controlled trials as his standard for inclusion."

Table 17. Placebo-controlled trials on 'vitamin C and the common cold' excluded from Kleijnen's meta-analysis (1989) because of 'low quality scores'

Trial	Total no. of episodes	Allocation [1]	Blindness [1]
Cowan-1942	736	alternately	SB
Glazebrook-1942	358	[2]	SB
Bergquist-1943	(551 subj)	alternately	SB
Dahlberg-1944	307	alternately	DB
Brown-1945	298	alternately	SB
Scheunert-1949	(1,066 subj)	?	? [3]
Cowan-1950	790	?	DB
Bartley-1953	62	?	DB
Franz-1956	29	alternately	DB
Tebrock-1956	1,915	alternately	DB
Ritzel-1961	48	random	DB
Walker-1967	36	?	SB
Abbott-1968	270	random	DB
Liljefors-1972	22	cross-over [4]	DB
Charleston-1973	124	?	SB
Elliott-1973	(70 subj)	random	DB
Schwartz-1973	21	?	DB
Wilson-1973a,b,c	(230 subj)	random	DB
Sabiston-1974	20	random	DB
Carson-1974	386	random	DB
Clegg-1975	141	?	DB
Elwood-1976	1,317	random	DB
Asfora-1977	(133)	alternately	DB [5]
Elwood-1977	264	random	DB
Ludvigsson-1977a	236 [6]	random	DB
Tyrrell-1977	603	random	DB
Baird-1979	596	random	DB
Total [7]	8,579 episodes in all trials in this table		
Total [7]	3,740 episodes in double-blind randomized trials in this table		

[1] Method of allocation into study groups: 'random' refers to the description by the authors; in most cases the method of randomization is not described; 'alternately' refers to allocating participants sequentially to different study groups on their arrival or on the basis of odd and even identity numbers; '?', allocation method not described.

Blindness: DB, double blind: the person evaluating the status of the blinded participant is unaware of the treatment. In many cases the participants self-evaluated their illness status; SB, single blind: explicit comment only on the blindness of the participant, and no explicit comment on the blinding of the observer.

[2] In the Glazebrook & Thomson trial, for practical reasons the school-children in a boarding school were not randomly allocated into the study groups, but certain administrative groups occupying the same tables in the dining room were served vitamin C supplemented food while the others remained as controls. The authors argued that a tonsillitis epidemic affected all the administrative groups uniformly the year before, indicating that the dining tables could not be considered as isolated units. Vitamin C was added to food in the kitchen which functionally corresponds to a placebo control design since the addition of vitamin C is not apparent to the consumer of the food in the dining room.

[3] In Scheunert's trial, the term 'placebo' is not used. Study arm #1 was administered 20 mg/day while arm #2 was administered 50 mg/day vitamin C. In several trials the 'placebo groups' were administered a small dose of vitamin C; e.g., Carr et al. (1981) administered 70 mg/day, and Miller et al. (1977) administered 50 mg/day. In this respect, Scheunert's 20 mg and 50 mg vitamin C arms correspond to placebo groups compared with the 100 mg/day (#3) and 300 mg/day (#4) arms.

[4] The method of allocation into study groups was not described, but participants were given vitamin C and placebo using a cross-over setting.

[5] The trial was initiated as a double-blind trial, but was terminated when it seemed obvious on clinical grounds who were receiving the 'active' substance and who were receiving the placebo. The trial was thereafter continued as an open trial.

[6] Ludvigsson et al. (1977a,b) reported two trials in a single paper, but only the larger one was included in Kleijnen's set of 'high score' trials.

[7] The Bergquist (1943), Scheunert (1949), Elliott (1973), Wilson et al. (1973), and Asfora (1977) trials are not included in the sum because the number of episodes was not stated.

Table 18. Major shortcomings of Kleijnen's meta-analysis (1989)

1. Selection of trials is based on an arbitrary and illogical scoring system
2. No P-values extracted from papers or calculated by Kleijnen himself
3. No calculation of effect measures (RR or percentage benefit)
4. No pooling of data from comparable trials
5. No consideration of what might explain differences between the trial results
6. No consideration of vitamin C intake in diet
7. No consideration of vitamin C supplementary doses
8. No partition of regular supplementation trials from therapeutic supplementation trials
9. No consideration of biological plausibility (i.e., immune system effects, animal studies, changes in vitamin C metabolism during colds, etc.)

Table 19. Therapeutic vitamin C in the Anderson *et al.* trial (1974)

		Common cold episodes			
Dose of vitamin C on only the first day of illness (arm)	Episodes per person	No. of 1-day colds	No. of ≥2 day colds	Percentage of colds being shorter (95% CI)	P
4 g/d (#7)	1.52	164 (39%)	253	0.0% (Reference)	
8 g/d (#8)	1.58	222 (46%)	261	6.6% (0.2%, 13.1%)	0.023

Data are from table II in Anderson *et al*. (1974). For the baseline comparability of these two groups, see Table 16.

In fact, 11 of the trials excluded by Kleijnen were double-blind randomized controlled trials (Table 17), while the Coulehan trial (1974) was not randomized. Thus the selection criteria for the Kleijnen set of trials, based on an arbitrary and illogical scoring system, was not properly considered in the 1998 Cochrane Review.

The purpose of the Hemilä meta-analysis (1992a) was to test the narrow and specific question of whether Pauling's hypothesis (1970a), that regular gram doses of vitamin C would prevent and alleviate colds, is correct or not. To test Pauling's hypothesis, the selection of trials was restricted to those published *after* Pauling published his hypothesis, in which participants were regularly administered ≥1g/day vitamin C. This restriction by Hemilä (1992a) is meaningful in testing Pauling's hypothesis, but in a general analysis of the effects of vitamin C on respiratory infections this selection has no rationale. For example, it is possible that vitamin C intake affects respiratory infections in the low range of intakes and under such conditions doses less than 1 g/day are interesting, even though they do not test Pauling's hypothesis. There are also therapeutic trials which should be taken into consideration instead of being restricted only to regular dose trials. Finally, there are trials published before Pauling's book (1970a), which do not test Pauling's hypothesis, but may be relevant in summing up all published trials.

A new version of the Cochrane Review was recently prepared (Douglas, Hemilä, *et al.* 2004; Douglas & Hemilä 2005), and this 2004 Cochrane Review covers all published placebo-controlled trials using at least 200 mg/day of vitamin C, not being restricted to the selections by Kleijnen *et al.* (1989) and Hemilä (1992a).

The Dykes and Meier review (1975)

In 1975 *JAMA* published a review of vitamin C and the common cold by Michael Dykes and Paul Meier in the same issue as the Karlowski *et al.* trial (1975). Meier is an eminent statistician, the second author of the widely used Kaplan-Meier survival analysis method (1958), and he was elected statistician of the year in the USA in 1985 (ASA 2005). This review in a wide circulation medical journal has thus been highly influential.

The Dykes and Meier review (1975) has been extensively cited as evidence that vitamin C is worthless for colds; for example, in the US nutritional recommendations (FNB 1980, 1989a), *Cecil Textbook of Medicine* (Kapikian 1985), and reviews and commentaries of common cold therapy (Editorial 1976; Hirsch & Swartz 1980; Anderson *et al.* 1983; Lowenstein & Parrino 1987; Spiers 2002). Dykes and Meier's review (1975) was also referred to in the popular textbook *Modern Epidemiology* as an example of important topics that have been examined by controlled trials (Rothman 1986 p 54; Rothman & Greenland 1998 p 70).

The Dykes and Meier review (1975) contains several shortcomings (Hemilä 1996a). The authors state that "Material will only be considered that was published in the scientific literature and was, therefore, subjected to both the careful editorial peer review and the critical scrutiny of the general scientific community that are inherent in that process." It is puzzling that a statistician uses 'publication in a journal' as a surrogate for good quality, instead of critically reading the papers for himself. For example, Dykes and Meier (1975 p 1976) uncritically accepted the 'placebo effect' explanation of the Karlowski *et al.* trial (1975): "When the analysis is restricted to those subjects who did not know their treatment, no appreciable differences were found in any of the reported indices." However, critical reading of the Karlowski paper would have revealed to Dykes and Meier that 42% of common cold episodes were missing from the subgroup analysis without any justification, and there are many further problems with the 'placebo effect' explanation (Paper I; see p 25). Apparently because of their blind faith in this explanation, Dykes and Meier (1975) did not present the actual results of the Karlowski *et al.* (1975) trial.

Compared with the placebo group, 3 g/day of vitamin C decreased the duration of colds by 6% to 9%, whereas 6 g/day decreased it by 17% (P = 0.025), suggesting dose dependency up to 6 g/day (Hemilä 1999a).

Dykes and Meier justified their review by referring to Pauling's papers (1971a,b). Furthermore, they stated that "Pauling gave great weight to the 1961 study of schoolchildren in a skiing camp in Swiss Alps by Ritzel." With such an explicit background it is odd that they did not present any of Ritzel's data (Table 3). Dykes and Meier (1975) claimed that Ritzel's "presentation of the data is confused and unclear in a number of respects." In fact, Ritzel (1961) listed the duration of colds in his text, and in table form showed the total days of colds per group, and the number of cases and total days of 'constitutional symptoms' per group, which are extracted in the present Table 3. Thus, Dykes and Meier did not mention that there was a 31% decrease in the mean duration of episodes, a 45% decrease in the incidence of colds, and a 61% decrease in the total number of days of colds in the group administered 1 g/day of vitamin C. Nor did they mention that there was a 62% decrease in the number of children with 'constitutional symptoms,' and an 81% decrease in the total number of days with constitutional symptoms in vitamin C group. Dykes and Meier merely commented that the difference in common cold incidence in the two groups was only marginally significant (P[2-t] = 0.04), which seems to be intentional camouflaging of the actual results. It is quite possible that some readers of *JAMA* might have considered that a 61% reduction in total number of sickness days, or an 81% reduction in days with constitutional symptoms might be worth serious considerations when the results arise from a randomized double-blind placebo-controlled trial with children, even though a one-week trial in a skiing camp does not allow any direct extrapolation to ordinary schoolchildren. Thus, concealing the actual results prevented a critically minded reader from drawing his or her own conclusions.

Dykes and Meier (1975) presented the results of the Anderson *et al.* trial (1972), but did not calculate any estimates of effect, such as percentage difference in favor of the vitamin C group, which hampers the reader in considering the practical significance of the findings. Dykes and Meier wrote of the Anderson 1972 trial: "The estimated effect is considerably less than that predicted by Pauling for the dose level." Anderson had reported a 30% decrease (P = 0.000,5) in the 'total number of days confined to house' per participant. Dykes and Meier's comment seems irrelevant as many readers might consider that with an inexpensive nutrient which costs pennies per gram and is safe in large doses, even such moderate benefits might be worthy of exploitation irrespective of how they compare with Pauling's predictions. Moreover, on biological grounds one would expect the benefit of supplementation to be greater for subjects with a low dietary vitamin C intake, and in this respect Anderson's subgroup finding that supplementation was substantially more beneficial for those participants that had low intake of fruit juices is highly interesting (Table 13). Thus Dykes and Meier were not consistent in their review when they uncritically accepted Karlowski's subgroup analysis which was based on the exclusion of 42% of cold episodes without any rationalization, and rejecting Anderson's subgroup analysis, which is biologically well rationalized.

Dykes and Meier comment on the Anderson *et al.* trial (1974) that "A question is raised whether one of two control groups may have been biased, but the data given are not sufficient to evaluate this point." This is quite a puzzling comment by a statistician, since the discrepancy between the 2 placebo groups in the Anderson 1974 trial can be seen in several simple calculations (Table 16), and accordingly the data *are* sufficient to evaluate this point, in contrast to Dykes and Meier's statement.

Dykes and Meier (1975) explicitly commented on the Coulehan *et al.* trial (1974) with Navajo schoolchildren that "Because the data required for an appropriate analysis are not presented, the statistical significance of the differences reported cannot be considered to have been established." In fact, Coulehan *et al.* (1974) reported in their table 4 that 44% (143/321) of school children administered vitamin C were 'never ill on active surveillance,' while only 29% (93/320) of those administered placebo were 'never ill' (**Table 20**). Since it is highly unlikely that such a difference between the vitamin C and placebo groups would arise purely by chance (P = 0.000,03; Table 20), in contrast to Dykes and Meier's claim in their review, important elements of Coulehan's results were presented and can be re-analyzed by a statistically oriented reader. When such highly significant differences between vitamin C and placebo groups are reported, it is grossly misleading to state in a review that significant differences "cannot be considered to have been established."

Furthermore, Dykes and Meier are not consistent in their text. They accuse Coulehan *et al.* (1974) of failure to present relevant data (partly true), whereas they themselves fail to present the data Coulehan did report (*e.g.*, Table 20) in their own review. Coulehan *et al.* (1974) also found that the duration of colds was 12% and 29% shorter in children administered 1 and 2 g/day of vitamin C respectively, suggesting dose dependency up to 2 g/day, but this data was also missing from the Dykes and Meier review. Finally, Dykes and Meier failed to present any results of the Ritzel (1961) and Karlowski *et al.* (1975) trials, thereby preventing readers from drawing any independent conclusions about them.

Pauling (1976b) considered that "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." Pauling thus wrote a manuscript in which he presented the results of the relevant studies, his own interpretations, and some criticism of Dykes and Meier's argument. For example, commenting on the Ritzel trial, Dykes and Meier (1975 p 1075) stated that "Pauling infers the number of subjects [with colds] 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." To which Pauling replied that "It is hard

Table 20. Prophylactic benefit of vitamin C in large trials

Trial	Free of illness during the trial / Persons in group: Vitamin C	Free of illness during the trial / Persons in group: Placebo	Proportion of participants getting benefit (95% CI)	P [1]
Coulehan-1974 [2]				
641 Navajo schoolchildren				
Duration 3 months				
Alternative allocation, DB				
Children never ill				
on active surveillance				
by a medically trained clerk				
or the school nurse				
All children [2]	143/321 (44%)	93/320 (29%)	16% (8%, 23%)	0.000,03
Low grades				
1 g/d vit C				
Boys	31/81 (38%)	18/87 (21%)	18% (4%, 31%)	0.007
Girls	30/109 (28%)	12/105 (11%)	16% (5%, 26%)	0.002
High grades				
2 g/d vit C				
Boys	40/62 (64%)	34/61 (56%)	9% (-8%, 26%)	0.2
Girls	42/69 (61%)	29/67 (43%)	18% (1%, 34%)	0.021
Anderson-1972				
818 adults in Canada				
with colds usually in winter				
Duration 3 months				
Randomized, DB				
1 g/d vit C				
Free of any illness				
during the trial	105/407 (26%)	76/411 (18%)	7.3% (1.6%, 13%)	0.006
No ‘days confined to house’	232/407 (57%)	195/411 (47%)	9.5% (2.7%, 17%)	0.003
No ‘days off work’	275/407 (67%)	243/411 (59%)	8.4% (1.8%, 15%)	0.006

[1] One-tailed mid-P-value. The CI was calculated using the CIA (confidence interval analysis, BMJ) program.

[2] In the Coulehan trial, the number of children ‘free of illness’ in the placebo group does not match in the original paper: 18 + 12 + 34 + 29 = 93, and not the 92 published by Coulehan; in this table N = 93 is used as the sum of children ‘free of illness’ in the placebo group since it matches with the subgroup data. DB, double-blind.

The outcome in Coulehan’s table 4 is erroneously called as ‘No. of days without sickness observed per total in group’ whereas the actual data is ‘Number of children without sickness observed per total in group’ (Coulehan 1995).

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 [see current Table 3]), 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" (Pauling 1976b).

Pauling submitted his manuscript to *JAMA*, but his paper was rejected even after Pauling twice made revisions to meet the suggestions of the referees, and the manuscript was finally published in a minor journal (1976b,c; see also: 1976a pp 133-5; 1986a pp 231-2). The rejection of Pauling's review was quite a strange policy by *JAMA*, since the readers were thereby prevented from seeing the other side of an important scientific controversy.

Truswell's mini-review (1986)

A. Steward Truswell is an eminent nutritionist who was a co-editor of a major textbook of nutrition (Davidson *et al.* 1975, 1979), and the author of a popular book *ABC of Nutrition*, which is currently printed as the fourth edition (Truswell 2003). Truswell (1986) reviewed the vitamin C trials in a letter to the *New England Journal of Medicine*. The main text of the letter was only half a column long, but a journal with great prestige and a wide circulation makes the statements in Truswell's mini-review influential and worthy of a critical look. This mini-review was cited in Truswell's own book on nutrition (2003 p 64) as the only reference to the topic of vitamin C and the common cold, and in a Finnish book chapter stating that vitamin C has no effect on colds (Aro 1994 [see Hemilä 1995a]).

Truswell did not present any figures or P-values of the original reports, merely providing subjective comments about the trials. Neither did he make any effort to rationalize the great variations in the reported results. For example, on pharmacological grounds it is highly plausible that the effect depends on the dose. Truswell, however, listed the Karlowski *et al.* trial (1975) in which vitamin C dose was up to 6 g/day and the Dahlberg *et al.* trial (1944) in which the dose was only 0.05 g/day side-by-side without mentioning that there was a 120-fold discrepancy in vitamin C dosage. Obviously, such trials are not equivalent.

At the end of his mini-review, Truswell stated that "In another five combined trials there appeared to be slight amelioration of symptoms, which was not statistically significant." In fact, the 5 papers cited by Truswell contained 6 trials and not 5 (**Table 21**; Hemilä 1996a). Furthermore, all 6 trials reported a statistically significant benefit in at least one of the outcomes (Table 21). Thus Truswell's statement is gravely misleading, even though the 5 papers did contain some other outcomes in which the benefit was not significant statistically. Some further problems in Truswell's mini-review are discussed elsewhere (Hemilä 1996a).

Table 21. 'Five' trials in which the amelioration of common cold symptoms by vitamin C was not statistically significant according to Truswell (1986)

Trial	No. of subjects	Vitamin C dose (g/d)	The effect of vitamin C (%)	P[2-t]	Outcome
Karlowski-1975	103	6	-17	0.047	Duration of symptoms
Pitt-1979	674	2	-5	0.023	Severity of symptoms
Ludvigsson-1977a [1]	158	1	-39	0.003	Duration of symptoms
Ludvigsson-1977b [1]	615	1	-14	0.016	Absence from school
Carr-1981	190	1	-19	<0.05	Duration of symptoms
Wilson-1973 [2]	128	0.2	-45	0.035	Intensity of symptoms

All 6 trials were placebo-controlled and the effect of vitamin C refers to the difference between the vitamin C and placebo groups. The exact P values were calculated where appropriate data was available. This table is reproduced from Hemilä (1996) *Nutrition* 12:804-9 with permission.

[1] Ludvigsson *et al*. (1977) reported the results of 2 separate trials in the same paper.

[2] 'Whole colds' among girls administered 0.2 g/day of vitamin C; for details, see the reference.

Paper II: Meta-analyses of trials on vitamin C and common cold incidence

Based on previously published trials, Pauling (1970a, 1971a,b) concluded that regular high dose vitamin C supplementation considerably decreases the incidence of the common cold. To test Pauling's hypothesis, a meta-analysis was carried out to analyze placebo-controlled trials in which large vitamin C doses (≥1 g/day) were regularly administered to participants. A second selection criterion required that ≥200 common cold episodes be recorded in the trials. Small trials with negative results may remain unpublished, leading to publication bias, whereas large-scale trials are probably published irrespective of the results. 7 trials satisfying the selection criteria were identified, but the Anderson *et al.* trial (1974) was excluded because of the significant divergence in the placebo arms (Table 16). The largest 5 of the 6 trials are listed in Table 10. Since all 6 trials were published after 1970 they explicitly tested Pauling's hypothesis. Since the 6 trials were carried out in 5 different Western countries, using schoolchildren, adults and military recruits as participants, they cover a wide range of populations in the Western countries.

None of these 6 major trials found a significant difference between vitamin C and placebo groups in the incidence of colds. Pooling the results of the 6 trials did not reveal any difference between the vitamin and placebo groups with a narrow confidence interval (pooled RR = 0.99; 95% CI: 0.93-1.04). Thus these 6 large-scale trials suggest firm rejection of Pauling's general hypothesis that regular high dose vitamin C supplementation markedly decreases the incidence of common cold episodes across all population groups in Western countries.

Nevertheless, even though these trials found no overall benefit from vitamin C, Anderson *et al.* (1972) found a statistically significant decrease in the incidence of 'throat colds' (-21%, P < 0.01) and Elwood *et al.* (1976) in the incidence of 'chest colds' (-18%, P = 0.015), neither finding any effect on the incidence of 'simple colds', however (nose running or sneezing). Thus, concluding from these two large-scale trials, it is still possible that vitamin C supplementation has some limited effects on incidence, depending on the type of cold.

Quite a few technically satisfactory small trials found that vitamin C group had a significantly lower incidence of colds than the placebo group and explorative meta-analyses of the common cold trials were therefore carried out on the hypothesis that vitamin C supplementation may affect common cold susceptibility in specific groups of people.

Assuming that any potential effects of vitamin C supplementation may be most conspicuous in subjects with a particularly low dietary vitamin C intake, it is possible that supplementation would have effects in populations with low intakes. Unfortunately, since few trials have estimated the vitamin C intake of the participants, a surrogate for low vitamin C intake was used, namely, trials that were carried out in the UK. Several studies over a long period of time reported low vitamin C intake in the UK of 30-60 mg/day (II). By comparison, the median vitamin C intake in the USA is about 100 mg/day (FNB 2000 pp 416-9).

In 4 trials with UK females, vitamin C supplementation had no marked effect on common cold incidence (pooled RR = 0.95; 95% CI: 0.86-1.04). However, in 4 trials with UK males, a statistically highly significant but quantitatively modest reduction in common cold incidence was found in vitamin C groups (pooled RR = 0.70; 95% CI: 0.60-0.81; P = 0.000,001). These 4 trials with UK males indicate that vitamin C may have physiological effects on susceptibility to the common cold under specific conditions, but the effect seems quantitatively meaningful only in limited groups of people and is not large.

Furthermore, 4 trials with UK males reported the number of people with ≥2 colds during the study period and a significant reduction in the number of men with recurrent colds was found in the vitamin C groups (pooled RR = 0.54; 95% CI: 0.40-0.74; P = 0.000,05). **Table 22** shows the proportion of participants benefiting from vitamin C supplementation when the outcome is ≥2 colds during the trial. Table 22 also includes the results of the recent UK trial by van Straten and Joslin (2002), which reported 37 common cold episodes in the vitamin C group (N = 84), 50 in the placebo group (N = 84) and, for the difference in incidence, the authors calculated the "Student's t-distribution .375" corresponding to "P<.05." In fact, t(166 df) = 0.375 corresponds to P[2-t] = 0.71 which is not smaller than 0.05. Furthermore, the t-test is inappropriate for analyzing incidence data. Analysis of this incidence data with the normal approximation of Poisson distribution (see Paper II) yields RR = 0.74, 95% CI: 0.48-1.13; P[2-t] = 0.17. Nevertheless, despite the errors in statistical analysis, van Straten and Joslin (2002) reported a highly significant reduction in the number of participants who had 2 colds during the trial (Table 22). The van Straten and Joslin (2002) trial consisted mainly of women and they did not present the results separately for sexes.

Methodologically, the trials in Table 22 diverge substantially. Charleston and Clegg (1972), Elwood *et al.* (1976), and van Straten and Joslin (2002) administered 1 g/day vitamin C. Clegg and Macdonald (1975) administered 1 g/day ascorbic acid to one group and 1 g/day D-isoascorbic acid (erythorbic acid) to another, and the two groups are combined in Table 22 based on the fact that the D-isomer is an equivalent antioxidant, although it does not cure scurvy as efficiently as the L-isomer. Baird *et al.* (1979) administered only 80 mg/day of vitamin C. All the trials administered vitamin C over the whole study period, except Tyrrell *et al.* (1977), which administered vitamin C therapeutically at 4 g/day for only 2.5 days during the first common cold episode, so that therapeutic treatment seemed to reduce the occurrence of later episodes.

Paper II was commented on (Bates 1997; Schorah 1997), but none of the criticism seems to be valid (II; Hemilä 1997d).

Table 22. Prophylactic effect of vitamin C on recurrent colds in the UK trials

Trial	Duration (weeks)	Persons with ≥2 colds / All persons in the group		Proportion of participants getting benefit (95% CI)		P [1]
		Vitamin C	Placebo			
Males						
Charleston-1972	15	2/24 (8%)	17/30 (57%)	48%	(27%, 69%)	0.000,1
Baird-1979	10	21/133 (16%)	23/61 (38%)	22%	(8%, 35%)	0.000,6
Tyrrell-1977	16	23/351 (7%)	43/392 (11%)	4.4%	(0.4%, 9%)	0.018
Clegg-1975	14	18/89 (20%)	9/47 (19%)	-1%	(-15%, 13%)	0.5
Females						
Clegg-1975	14	8/52 (15%)	10/23 (43%)	28%	(6%, 51%)	0.007
Charleston-1972	15	10/23 (43%)	9/13 (69%)	25%	(-7%, 58%)	0.08
Tyrrell-1977	16	26/373 (7%)	29/385 (8%)	1%	(-3%, 4%)	0.4
Elwood-1976	14	196/339 (58%)	201/349 (58%)	0%	(-7%, 7%)	0.5
Baird-1979	10	19/105 (18%)	7/51 (14%)	-4%	(-16%, 8%)	0.7
Both genders						
van Straten-2002 27 men, 141 women	9	2/84 (2%)	16/84 (19%)	17%	(8%, 26%)	0.000,2

[1] One-tailed mid-P. The CI was calculated using the CIA (confidence interval analysis, BMJ) program.

Other findings from the controlled trials on vitamin C

Common cold severity and duration

High dose vitamin C supplementation administered regularly throughout the trial period has consistently reduced common cold severity and duration (Hemilä 1990a,b, 1992a, 1994a, 1995a, 1996a, 1997a,b, 1999a,b, 2003a). The practical importance of this effect is, however, unsettled. Certain subgroup differences have been identified when comparing different trials and subgroups within individual trials (Hemilä 1997a, 1999a; Table 13).

In the published trials, there is a trend for trials with children to reveal greater benefit than adult trials (Hemilä 1999a). In 18 trials with adults administered ≥0.2 g/day of vitamin C regularly over the trial, the duration of colds was reduced by 8.0% (95% CI: 3.0% to 13.1%) in the vitamin C groups, whereas in 12 trials with children administered ≥0.2 g/day of vitamin C regularly over the trial, the duration of colds was reduced by 13.6% (5.6% to 21.6%) (Douglas, Hemilä, *et al.* 2004; Douglas & Hemilä 2005).

There is also a trend for trials with ≥2 g/day of vitamin C to yield a greater benefit than those with 1 g/day supplementation (Hemilä 1999a), and dose-dependency was also seen in two trials (Coulehan *et al.* 1974, Karlowski *et al.* 1975; see Hemilä 1999a). Assuming dose-dependency, the effect of the same dose should be greater in children than adults, because children weigh on average substantially less. Comparison of adult trials with exactly 1 g/day of vitamin C with child trials with exactly 2 g/day of vitamin C reveals a big difference in the point estimates of the benefit (**Table 23**). The two trials with children are small and the confidence interval of the pooled estimate wide, but this comparison would seem to warrant further study of children in particular.

The great majority of published trials have examined the effect of regular vitamin C supplementation. However, if the main effect of vitamin C is the alleviation of cold symptoms, it would appear more reasonable to administer vitamin C therapeutically, starting immediately after the first symptoms. However, only a few therapeutic trials have been carried out and they have substantial variations in their settings which hampers their interpretation (Hemilä 1999a). Elwood *et al.* (1977) and Tyrrell *et al.* (1977) administered 3-4 g/day of vitamin C for only 3 days (total 10 g in both) when the participants contracted colds, and Audera *et al.* (2001) administered 1 or 3 g/day of vitamin C for 3 days (total 3 or 9 g). These 3 trials found no benefit on common cold duration with vitamin C, but Tyrrell *et al.* did find an unexpected reduction in the number of men with recurrent colds (Table 22). On the basis of the consistent benefit in regular supplementation trials (*e.g.*, Fig. 3; Table 23), we may hypothesize that the duration of supplementation might be an important variable modifying the effect of vitamin C supplementation. A 5-day therapeutic trial by Anderson *et al.* (1975) found a reduction in 'days spent indoors per subject' because of illness by 25% (P = 0.024; vitamin C 1.20 days [SD 1.78], placebo 1.61 days [SD 2.46]) among the vitamin C participants (1-1.5 g/day). Using 5-day therapeutic supplementation of 3 g/day of vitamin C, Karlowski *et al.* (1975) found that colds were 0.73 days (10%) shorter (P = 0.05; Paper I). These two therapeutic trials with 5-day supplementation are thus consistent with the possibility that 3 days might be too short a time for vitamin C to produce unambiguous benefits, and further therapeutic trials should obviously use longer than 3-day supplementation.

Furthermore, it is possible that the rapidity of initiation of vitamin C supplementation may affect the effect. Asfora (1977) gave the same subjects either vitamin C (6 g/day for 5 days) or other medications (aspirin, etc.) during different common cold episodes, but not in a double-blind fashion. When treatment started within 24 hours of the onset of symptoms, the mean duration of vitamin C treated colds was 3.6 days, whereas the duration was 6.9 days with the other medications (-48%; t(88 df)=3.5; P = 0.000,4). However, if vitamin C supplementation was initiated later than 24 hours from the onset of symptoms, there was no meaningful benefit. A similar effect by time of supplement initiation was reported by Terezhalmy *et al.* (1978) when treating herpes labialis with a combination of vitamin C and bioflavonoids (see pp 15-6). Evidently, further therapeutic trials should use rapid initiation of supplementation.

Common cold incidence

Although vitamin C has not shown any consistent effect on the common cold incidence (II; Fig. 3), there are findings indicating that it may have preventive effects on the common cold in particular conditions or among restricted groups of people.

It seems that people under heavy acute physical stress may find benefit with prophylactic vitamin C supplementation (Hemilä & Antila 1993a; Hemilä 1996b). Combining the results of 3 short-term randomized placebo-controlled trials with marathon runners (Peters *et al.* 1993), Canadian soldiers in short-term winter exercises (Sabiston & Radomski 1975), and school-children in a skiing camp in the Swiss Alps (Ritzel 1961) yielded a pooled RR = 0.50 (95% CI 0.35-0.69; P = 0.000,03) lower incidence of colds in vitamin C groups than the placebo groups (Hemilä 1996b). Following Thomas Chalmers' concept of cumulative meta-analysis (p 32), 3 newer trials with marathon runners (Peters *et al.* 1996; Moolla 1996; Himmelstein 1998) were added to the 1996 meta-analysis of physically stressed subjects. The estimated effect did not change, but the confidence interval became slightly narrower, and the corresponding P-value somewhat smaller: RR = 0.50 (95% CI: 0.38-0.66; P = 0.000,000,3) (Douglas, Hemilä, *et al.* 2004; Douglas & Hemilä 2005; graph on the cover). Thus 6 studies with participants under heavy acute physical stress with or without a cold environment have shown substantial reduction in common cold incidence but most of the trials were carried out with marathon runners and the finding should

Table 23. The effect of regular vitamin C supplementation on common cold duration

Trial [1]	Vitamin C group			Placebo group			Decrease in Duration (95% CI)
	No of Episodes	Duration of colds		No. of Episodes	Duration of colds		
		Mean	SD		Mean	SD	
Adults with 1 g/d							
Anderson-1974 [2,3]	414	3.35	3.35	437	3.52	3.52	-5% (-18%, +8%)
Clegg-1975	68	7.2	3.1	73	7.6	3.0	-5% (-18%, +8%)
Briggs-1984 [3]	125	3.1	3.1	121	3.3	3.3	-6% (-30%, +18%)
Elwood-1976	627	5.97	5.73	690	6.38	6.33	-6% (-16%, +4%)
Charleston-1972	44	3.54	1.93	80	4.2	0.82	-16% (-30%, -1%)
van Straten-2002	37	1.8	2.98	50	3.1	4.65	-42% (-93%, +10%)
					Pooled estimate [4]		-8% (-14%, -2%) (P = 0.003)
Children with 2 g/d							
Bancalari-1984	38	3.4	2.78	46	4.5	2.92	-24% (-51%, +3%)
Coulehan-1974 [3]	16	4.44	4.44	17	6.29	6.29	-29% (-88%, +30%)
					Pooled estimate [4]		-25% (-50%, -0.1%) (P = 0.025)

[1] The Carr et al. trial (1981) used 1 g/day of vitamin C, but is excluded since the participants had an age range from 14 to 64 years with a mean of 25 years; thus, it is a mixture of children and adults. The Himmelstein et al. trial (1998) with adults used 1 g/day, but is excluded because of exceptionally high drop-out rates (up to 75%). The Sabiston and Radomski trial (1974) is excluded because the participants were military recruits whose living conditions were quite different from those of ordinary adults.

[2] The Anderson et al. (1974) comparison here denotes vitamin C group #2 (1 g/day) and placebo group #4 (see Table 16).

[3] Where the SD was not reported, the SD was estimated by the mean, since the average for the ratio SD per mean was on average 0.7 in the trials that reported SD (Douglas, Hemilä, et al. 2004). Thus, using a ratio of 1.0 leads to a somewhat smaller than average weight to the trials that did not report SD.

[4] The pooled estimate was calculated using the RevMan program (Cochrane Collaboration).

therefore be extrapolated with great caution. In any case, these findings definitely warrant further study with physically stressed subjects.

Staying free of illness during the study period is also an important measure of preventive effect. Anderson *et al.* (1972) found about 8% increase in the proportion of participants who were 'not ill during the trial', 'not confined to the house', and 'not off work' in the vitamin C group (Table 20). Accordingly, about 1 participant in 12 benefited from vitamin C supplementation in this particular setting (NNT = 12). It is noteworthy, however, that participants in this Canadian trial were asked not to enroll to the trial unless they normally experienced at least one cold in the wintertime, and in this respect the subjects do not represent the average population.

Coulehan *et al.* (1974) studied Navajo schoolchildren and found a 16% higher proportion of children in the vitamin C group who were 'never ill on active surveillance' by a medically trained clerk or the school nurse (Table 20). This indicates that about 1 of 6 schoolchildren benefited from vitamin C in this particular setting (NNT = 6).

Although the Anderson *et al.* (1972) and Coulehan *et al.* (1974) trials found statistically significant prophylactic benefit from regular ≥1 g/day vitamin C, it is not apparent how these two trials should be considered in the wider context. When a large number of trials are carried out, some find statistically significant differences purely by chance. However, only 8 common cold trials have been carried out with ≥400 participants administered ≥1 g/day vitamin C regularly (Fig. 3; Hemilä 1992a) and in this respect the P-values calculated in Table 20 are not easily explained by the multiple comparison problem. Although these two trials support the notion that limited sub-populations might benefit from regular vitamin C supplementation, the proportions of 8% and 16% cannot be directly extrapolated to any other populations. They would, however, seem to justify further study.

Vitamin C and respiratory infections other than the common cold

Tuberculosis

Two old studies of young and middle aged African Americans in the USA reported a lower incidence of tuberculosis in subjects with a higher intake of vitamin C (**Table 24**). In a controlled trial, Downes (1950) administered other nutrients along with vitamin C, and any effects may be explained by the other nutrients. In a cohort study, Getz *et al.* (1951) correlated tuberculosis incidence with plasma vitamin C level, but any association with dietary intake could be caused by other dietary or non-dietary factors associated with vitamin C intake levels.

Pneumonia

Three controlled trials reported the number of pneumonia cases among participants on vitamin C supplementation (**Table 25**). Each of these 3 trials reported at least an 80% lower incidence of pneumonia in the vitamin C group, the difference being statistically significant in each case. The combined test leads to a very small P-value. The latest of these trials was a randomized double-blind placebo-controlled trial with Marine recruits in the USA (Pitt & Costrini

Table 24. Vitamin C intake and the incidence of tuberculosis

	Cases / Total			
	Vitamin C			
Study	High	Low	Difference in incidence	P [1]
Downes-1950 Controlled trial Vitamin C dose 0.02-0.37 g/d other vitamins also	1/644	10/1096	-83%	0.026
Getz-1951 Observational study Plasma vitamin C limit 34 µmol/l (6 mg/l)	0/117	27/896	-100%	0.017

[1] One-tailed mid-P

1979), whereas placebo was not used by Kimbarowski and Mokrow (1967). Glazebrook and Thomson (1942) carried out a large-scale trial with male students (15-20 years) in a boarding school in Scotland. No placebo was used; however, vitamin C was added to the food in the kitchen, so that the placebo effect does not seem a relevant concern. Furthermore, a recent meta-analysis found that placebo was powerless in studies measuring binary outcomes (Hrobjartsson & Gøtzsche 2001, 2004), and it would seem highly unlikely that a placebo would cause a substantial effect on such a hard outcome as pneumonia.

Since dietary vitamin C intake was low in the 2 older trials, the benefit of higher vitamin C intake might be explained by correction of marginal deficiency. However, in the Pitt and Costrini trial (1979) the dietary vitamin C intake was quite high. It is worth noting that 2 of these trials used military recruits (Pitt & Costrini 1979; Kimbarowski & Mokrow 1967), and the third used young males who were accommodated in crowded lodgings (Glazebrook & Thomson 1942). In each of the 3 trials, the incidence of pneumonia was very high (Table 25) when compared, for example, with the incidence of hospital-treated pneumonia in the ATBC Study cohort, which was about 5 cases per 1,000 person years (Paper V), so that it seems obvious that the findings in these 3 trials cannot be extrapolated to the general population. In any case, the studies in Table 25 justify examination of the role of vitamin C on people with high physical stress and/or who live in crowded accommodation (Hemilä 2004).

Table 25. Vitamin C supplementation and the incidence of pneumonia

Trial	Vitamin C dose (g/d)	Pneumonia Cases / Total: Vitamin C group	Pneumonia Cases / Total: Control group	Difference in incidence	P [1]	Pneumonia incidence in the control group (1/1000 person years)
Glazebrook-1942	0.05-0.3	0/335	17/1100	-100%	0.006	30
Pitt-1979	2	1/331	7/343	-85%	0.022	120
Kimbarowski-1967	0.3	2/114	10/112	-80%	0.009	9% of the patients

Combined test for all three sets of data: mid-P[1-t] = 0.000,02 (Hemilä 1997c).
This table is reproduced from Hemilä (1997c) *Pediatric Infectious Disease Journal* 16:836-7 with permission, except that the incidence of pneumonia in the control group was added to this table.
[1] One-tailed mid-P

B) Analyses of the ATBC Study

Participants and Methods

Participants

The rationale of the design and methods of the ATBC Study examining the effects of α-tocopherol (AT, 50 mg/day) and β-carotene (BC, 20 mg/day) on the incidence of lung and other cancers has been described in detail (Virtamo *et al.* 1987; ATBC 1994a,b). In brief, the trial participants were recruited from the total male population aged 50-69 years living in Southwestern Finland, starting in 1985. To be eligible, participants had to smoke ≥5 cigarettes per day at entry. The eligible participants in the trial (N = 29,133) were randomized to one of four intervention arms and administered a placebo, AT, BC, or AT+BC, using a 2x2 factorial design. The active intervention continued for 5 to 8 years (median 6.1 years) until April 30, 1993.

Baseline data and dietary assessment

At baseline prior to randomization the men completed questionnaires on their general background characteristics, including medical and smoking histories, and various social characteristics. At the first baseline visit, the participants were given a separate, detailed dietary history questionnaire for completion at home (Pietinen *et al.* 1988), and the questionnaire was returned at the second visit two weeks later. The questionnaire included a colour picture booklet and questions about portion sizes for 276 common foods and mixed dishes and the usual frequency of their consumption over the previous year. Data from this questionnaire was used to calculate the dietary intake of vitamin C, vitamin E and other relevant nutrients (Ovaskainen *et al.* 1996).

The validity of the dietary history questionnaire was assessed by comparing it with food consumption records of 190 participants for 12 separate 2-day periods distributed evenly over a period of 6 months. To evaluate the degree of similarity between the results of these two methods, the nutrient intake distributions were divided into quintiles (Pietinen *et al.* 1988). Classified by the dietary history questionnaire, 74-76% of participants classified by food consumption records fell into the same vitamin C intake quintile or into the within-one-quintile category. Among participants who belonged to the lowest vitamin C quintile using the dietary history questionnaire, 46-54% fell into the lowest quintile and 77-79% into the lowest two quintiles, whereas only 3-6% fell into the highest quintile when categorized on the basis of the food consumption record.

Outcomes of the ATBC Study analyses

There were 3 follow-up visits annually in the ATBC Study, at each of which the participants were asked about the number of common cold episodes since the preceding follow-up visit (III). The occurrence of tuberculosis (IV) and pneumonia (V) among the ATBC Study participants was identified in the National Hospital Discharge Register (Keskimäki & Aro 1991). The outcomes are described in detail in the original Papers (III-V).

Statistical methods

The statistical methods used in the original Papers (III-V) are described in detail in the papers and are not reiterated here. One-tailed P-values are mostly used in the analyses of studies in this text, since the particular questions in the present thesis are whether higher vitamin C intake in the diet or vitamin E supplementation decreases the incidence or severity of infections or not, and these questions are unidirectional. Where pertinent, the 2-tailed P-value is shown, and in such a case the 2-tails are mentioned (P[2-t]). If the 1-tailed P-value is higher than about 0.98, there is reason to assume harm in the treatment group of the particular study.

Paper III: The common cold in the ATBC Study cohort

The meta-analysis of the trials with UK males found a statistically significant reduction in common cold incidence with vitamin C supplementation, and it was hypothesized that this effect is explained by the particularly low dietary vitamin C intake in the UK (Paper II).

To test the hypothesis that low dietary vitamin C intake increases the incidence of colds, the relation between common cold incidence and vitamin C intake was analyzed in the ATBC Study participants, all of whom were male smokers aged 50 to 69 years at baseline. The analysis of the role of vitamin C intake was restricted to the placebo arm to avoid any possible modifications by vitamin E and β-carotene supplementation. The cumulative common cold incidence over 4 years was used as the outcome. On average, there were 0.8 self-reported colds per person-year during the 4-year follow-up.

Low vitamin C intake was not associated with an increased incidence of colds; in fact, the incidence was slightly lower than for participants with high vitamin C intake. Similarly, the incidence of colds was slightly lower among participants with low dietary intake of vitamin E, and participants with low intake of fruit and vegetables.

In the ATBC Study, no overall effect on the common cold incidence was caused by vitamin E supplementation (RR = 0.99; 95% CI: 0.98-1.01). Nevertheless, in subgroup analysis, vitamin E supplementation was associated with a marginally significant reduction in common cold incidence in participants aged ≥65 years (RR = 0.95; 0.90-1.00), but had no effect on the younger participants (RR = 1.00; 0.98-1.01).

Exploratory analyses among participants aged ≥65 revealed that smoking and residential neighborhood both modified the effect of vitamin E. Combining these two factors revealed that vitamin E had no effect in the group of elderly participants who lived out of cities and/or smoked ≥15 cigarettes per day (RR = 0.99; 0.94-1.05), but among city-dwellers who smoked 5-14 cigarettes per day (n = 261) the risk of colds was reduced by vitamin E supplementation (RR = 0.72; 0.62-0.83; P = 0.000,003).

Paper IV: Tuberculosis in the ATBC Study cohort

Two studies reported a lower incidence of tuberculosis in a group with higher vitamin C intake level (Table 24) and several studies with guinea pigs found that vitamin C was beneficial against tuberculosis (Appendices 2 and 3), which prompted research on whether dietary vitamin C intake is associated with the incidence of tuberculosis. The occurrence of hospital-treated tuberculosis among the ATBC Study participants was retrieved from the National Hospital Discharge Register, restricted to participants with baseline dietary data (167 cases).

In a crude analysis, a statistically highly significant inverse association between dietary vitamin C intake and the incidence of tuberculosis was found (P = 0.003), but after adjusting for age and smoking, which are often the only confounders adjusted for in epidemiological studies, the association became weaker but remained statistically significant (P = 0.01). However, the association became non-significant when body mass index, residential neighborhood and marital status were also added to the statistical model (P = 0.1). However, the point estimate of tuberculosis risk still remained 30% lower in the highest vitamin C intake quartile group compared with the lowest.

The remaining association between vitamin C and tuberculosis might be explained by residual confounding, but another alternative is an actual biological effect, so that the small number of cases would render the estimate inaccurate and non-significant. Therefore, further analyses were carried out to explore whether vitamin C intake could be more explicitly rejected as a cause of the 30% lower risk of tuberculosis in the high vitamin C intake group.

Fruit, vegetables and berries were the main source of vitamin C in the ATBC Study participants and the sum of their intake was calculated for further analysis (henceforth FRUVEBE). Dietary vitamin C intake and FRUVEBE are highly correlated (r = 0.9). The risk of tuberculosis was decreased with increasing FRUVEBE and this association remained statistically significant after adjusting for all available confounders (P = 0.01).

To determine whether the weak association between vitamin C and tuberculosis could be explained as a statistical artifact arising from other substances in fruit, vegetables and berries, FRUVEBE was modeled by linear regression as a function of dietary vitamin C, and the residuals of FRUVEBE were calculated (FRUVEBE-RES). As designed, the FRUVEBE-RES has no correlation with dietary vitamin C intake, so that it can be used to determine whether there are vitamin C independent effects on tuberculosis caused by fruit, vegetables and berries. FRUVEBE-RES had a significant association with tuberculosis risk when adjusted for all available confounders (P = 0.002), *i.e.*, even stronger than FRUVEBE, indicating that the effect of FRUVEBE on tuberculosis risk is caused by substances other than vitamin C.

Table 26 shows the risk of tuberculosis in vitamin C quartiles stratified by the median of FRUVEBE-RES. Variation in vitamin C intake has no association with tuberculosis risk in participants who consumed fruit, vegetables and berries rich in vitamin C (residual below median) which means a smaller than average amount of other substances of fruit, vegetables, and berries. Within the two highest vitamin C quartiles, tuberculosis risk was less than half in participants who consumed fruit, vegetables and berries poor in vitamin C (residual above median), compared with participants who consumed fruit, vegetables and berries rich in vitamin C (residual below median). This stratified analysis provides strong evidence against vitamin C as the substance in fruit, vegetables, and berries that affects the tuberculosis risk of the ATBC Study participants.

The vitamin E arm of the ATBC Study had a slightly higher incidence of tuberculosis (RR = 1.23; 95% CI: 0.80-1.90) than the placebo arm.

Table 26. Relative risk of tuberculosis by vitamin C intake and by the residual of fruit, vegetable and berry consumption, the ATBC Study

	Vitamin C intake quartile				
Vitamin C in diet (mg/d)	1.	2.	3.	4.	Test for vitamin C
Range:	5-65	66-89	90-120	121-534	trend [1]
Median:	52	78	103	148	P[1-t]
HIGH residual group [vitamin C poor food]					
Cases of tuberculosis	27	20	11	8	
Adjusted RR [1]	1.09	0.90	0.52	0.41	0.002
95% CI	0.64-1.86	0.50-1.60	0.26-1.05	0.18-0.90	
Fruit, vegetable, berry median (g/d)	110	190	260	390	
LOW residual group [vitamin C rich food]					
Cases of tuberculosis	27	24	29	21	
Adjusted RR [1]	1.00	1.00	1.31	1.03	0.7
95% CI	ref.	0.58-1.75	0.77-2.22	0.58-1.84	
Fruit, vegetable, berry median (g/d)	60	120	180	280	
Comparison between HIGH and LOW groups					
Adjusted RR [1]	1.06	0.90	0.39	0.43	
95% CI	0.62-1.82	0.49-1.63	0.19-0.79	0.18-0.97	

Participants (N = 26,975) were divided by the median of FRUVEBE-RES (-2.62 g/day) into those with high residual intake (HIGH) and low residual intake (LOW). The total number of tuberculosis cases in this cohort was 167.

[1] The RR and CI were calculated using the proportional hazards regression model adjusting for age, smoking, BMI, marital status, and residential neighbourhood (see Paper IV). The test for trend was carried out for square root transformed vitamin C intake.

Paper V: Pneumonia in the ATBC Study cohort

In three controlled trials, vitamin C supplementation reduced the incidence of pneumonia (Table 25). Vitamin C and vitamin E are both antioxidants and appear to have a physiological interaction (see pp 10-1), which prompted investigation of the effect of vitamin E supplementation on the risk of pneumonia in the ATBC Study cohort. Several animal studies also found that vitamin E affects the risk of respiratory infection (Appendix 4). The first occurrence of hospital-treated pneumonia of the ATBC Study participants was retrieved from the National Hospital Discharge Register (898 cases).

Vitamin E supplementation had no overall effect on the incidence of pneumonia (RR = 1.00; 95% CI: 0.88-1.15). Nevertheless, the age of smoking initiation was a highly significant modifying factor for vitamin E supplementation. Vitamin E decreased the risk of pneumonia (RR = 0.65; 0.49-0.87; P = 0.002) among participants who had initiated smoking at a later age (at ≥21 years; N = 7,469 with 196 cases of pneumonia).

Further exploratory subgroup analyses of supplementary vitamin E effects were carried out among participants who initiated smoking at a later age to find out whether other factors might modify the vitamin E effect within this subgroup. Vitamin E had a greater effect on participants who smoked less than a packet of cigarettes per day at baseline (RR = 0.47; 0.30-0.73; P = 0.000,4) than on participants smoking more (RR = 0.85; 0.57-1.25). The recent smoking history just prior to the occurrence of pneumonia was also analyzed in relation to the effect of vitamin E supplementation. Among participants who had quit smoking, the occurrence of pneumonia was significantly lower in the vitamin E group than the no-vitamin E group (OR = 0.21; 0.07-0.60; P = 0.002), whereas among participants who continued smoking prior to pneumonia, the vitamin E group did not differ from the no-vitamin E group (OR = 0.80; CI: 0.53-1.19).

Since vitamin E has been thought to protect against oxidative stress caused by heavy exercise, we analyzed its effect among participants who had a heavy or moderately heavy job, and among participants who were carrying out heavy or moderate exercise during their leisure. In the ATBC Study participants, vitamin E supplementation had no effect on pneumonia incidence among participants with moderate or heavy physical activity on the job (RR = 1.28; 0.78-2.10). However, among participants with moderate or heavy exercise during leisure, vitamin E reduced the risk of pneumonia by half (**Table 27**). Since these findings in differently defined physical activity groups appear discordant, we determined whether the kind of job might modify the vitamin E effect among participants carrying out leisure time exercise. We found a significant interaction between physical activity on job and during leisure (Table 27). Vitamin E supplementation affected the risk of pneumonia among those participants who had a light or very light job, whereas it had no effect among those who had a heavy or moderately heavy job. Its effect was also modified by baseline age and age of smoking initiation. While the number of cases is quite small and these modifications should be considered cautiously, these potential modifications should be contemplated if further study is carried out on the role of vitamin E on physically stressed subjects.

Table 27. Relative risk of hospital-treated pneumonia by vitamin E supplementation in participants who did moderate or heavy exercise during leisure, the ATBC Study, 3-year follow-up

Subgroup	No. of subjects	Intervention				RR (95% CI) [1]	Test for interaction P[2-t]
		Vitamin E		No vitamin E			
		No. of cases	Rate (1/1,000 person-years)	No. of cases	Rate (1/1,000 person-years)		
All in the group	9,570	22	1.5	43	3.0	0.50 (0.30-0.84)	
Physical activity on job							
Light or very light	5,467	6	0.7	25	3.1	0.24 (0.10-0.59)	0.020
Moderate or heavy	4,103	16	2.6	18	3.0	0.86 (0.43-1.68)	
Age at baseline (years)							
<60	8,322	21	1.7	31	2.5	0.67 (0.38-1.17)	0.013
≥60	1,248	1	0.5	12	6.6	0.08 (0.01-0.61)	
Age of smoking initiation (years)							
≤20	6,839	19	1.9	25	2.5	0.76 (0.42-1.38)	0.013
≥21	2,729	3	0.7	18	4.5	0.16 (0.04-0.54)	
Cigarettes (1/d)							
5-19	3,321	6	1.2	17	3.4	0.35 (0.14-0.89)	0.3
≥20	6,247	16	1.7	26	2.8	0.60 (0.32-1.12)	
Coffee (ml)							
<300	1,071	7	4.4	8	5.0	0.88 (0.31-2.41)	0.2
≥300	7,927	14	1.2	33	2.8	0.42 (0.22-0.78)	
Vitamin E in diet (mg/d)							
<11.4 (median)	4,500	13	1.9	24	3.6	0.53 (0.27-1.04)	0.8
≥11.4	4,498	8	1.2	17	2.5	0.47 (0.20-1.08)	
Vitamin C in diet (mg/d)							
<99.2 (median)	4,500	11	1.6	24	3.6	0.45 (0.22-0.91)	0.6
≥99.2	4,498	10	1.5	17	2.5	0.58 (0.26-1.27)	
Marital status							
Married	8,149	16	1.3	32	2.7	0.49 (0.27-0.90)	0.8
Not married	1,421	6	2.8	11	5.2	0.54 (0.20-1.47)	
Residential area							
City (>50,000)	3,980	11	1.8	18	3.1	0.60 (0.28-1.27)	0.5
Small neighborhood	5,589	11	1.3	25	3.0	0.43 (0.21-0.88)	

[1] Proportional hazards regression model comparing participants who received vitamin E with those who did not. No covariates were included in the model. Data was missing for coffee consumption, vitamin C and E intake in diet (n = 572), age at smoking initiation (n = 2) and residential area (n = 1).

DISCUSSION

The effect of vitamin C on respiratory infections

The role of vitamin C in infections was studied quite extensively in the early twentieth century and studies with guinea pigs led to extensive reviews indicating that vitamin C may affect susceptibility to and severity of infections (Clausen 1934; Robertson 1934; Perla & Marmorston 1937). A number of clinicians concluded that vitamin C deficiency seemed to increase susceptibility to infections (*e.g.*, Barlow 1894; Hess 1932; reviewed by Stone 1972; Briggs 1984; Levy 2002). Although a few studies with human participants reported favorable results, there were various technical deficiencies in these studies. Nevertheless, the topic of vitamin C and infections was not dismissed because of carefully conducted trials showing that vitamin C lacked any effect on infections. Rather, there appear to be two major reasons for the general disregard of the early studies. Antibiotics were introduced in the 1940s and, because of their highly specific effects on microbes, they were obviously much more practicable drugs for patients with infections than vitamin C. A second reason for the rejection of the topic apparently was the oversimplified notion that the physiological purpose of vitamin C is just to prevent scurvy. Apparently it was not reasonable to assume that a substance that participates in the synthesis of connective tissue proteins might affect infections. However, the biochemistry of vitamin C is much more complex than basic textbooks suggest. Although it is not a specific agent against any infection, it may have moderate effects on general resistance to infection in human beings as indicated by the large number of animal studies (Table 2 and Appendices 2 and 3).

In the 1970s, interest in vitamin C and infections, in particular the common cold, reappeared as an academic topic largely because of Linus Pauling's activity (Fig. 2). However, interest evaporated after the middle of the 1970s mainly because of the influential reviews by Chalmers (1975) and Dykes and Meier (1975), and the controlled trial by Karlowski *et al.* (1975). The two reviews were however erroneous, as detailed elsewhere in this text (see pp 36-45), and the Karlowski *et al.* (1975) trial was inappropriately analyzed (Paper I; see p 25).

Problems in planning and analyzing the common cold trials

Although a large number of trials was published on the vitamin C and common cold relationship, rather less understanding was generated compared with the time and effort invested. Most of the literature related to vitamin C and the common cold is the product of investigators with only a transient interest in the topic.

Lack of carefully formulated questions has been a problem typical of the published trials. In most cases a group of authors carried out a single trial without a proper look at those already published. The major exceptions to this problem are Anderson, who carried out 3 large trials (Anderson *et al.* 1972, 1974, 1975), and Clegg, who carried out 2 trials (Charleston & Clegg 1972; Clegg & Macdonald 1975). Both of these authors changed their settings on the basis of their earlier trials. Coulehan *et al.* (1974, 1976) carried out 2 trials, but the second does not consider the dose-response tendency observed in the first.

In most cases the question in the trials has been oversimplified; whether Pauling was wrong or not, or whether vitamin C has any effects on colds or not. When there already is a large set of trials with conflicting results, it is difficult to see that any single new trial could provide meaningful progress without explicitly formulated questions based on the older findings. Hypotheses could be generated to explain the older conflicting results and such hypotheses could be tested in a new trial, but in most cases no such hypotheses were formulated by the authors before initiating their own trial. With the existence of a large set of published trials, the meta-analytical approach, extensively used in this thesis, may be useful.

The problem of planning individual trials is seen in several aspects of the published trials. Pauling (1970a) suggested that vitamin C would substantially reduce the incidence of colds. The large-scale trial by Anderson *et al.* (1972) showed that vitamin C had no meaningful preventive effects in the general population, but did find a statistically highly significant reduction in common cold severity. After such a finding it would appear reasonable to plan new trials explicitly to test the possible therapeutic effect of vitamin C. Essentially all of the later trials (see Hemilä 1992a, 1994a, 1999a; Douglas, Hemilä, *et al.* 2004), however, used regular supplementation without any hypothesis about why the subjects or experimental conditions in these new trials might yield a different result on common cold incidence compared with the Anderson trial (1972). Consequently, there is a gross shortage of therapeutic trials.

A dose-response relation was observed in schoolchildren by Coulehan *et al.* (1974), who reported that 2 g/day of vitamin C shortened colds by 29%, whereas 1 g/day shortened colds by only 12%. Karlowski *et al.* (1975) found that 6 g/day was about twice as effective as 3 g/day (Table 8). Although these two trials do not establish dose-dependency, they should have justified further trials to investigate whether there actually is dose-dependency in the high dose region. Essentially all later trials, however, employed only a single dose, in most cases only 1 g/day (Hemilä 1992a, 1999a). Thus, here also many authors did not look at the previous literature to formulate proper questions for their own trials.

Most of the vitamin C trials did not appropriately consider the type of subject that might be meaningful. In this respect, the Anderson (1972) trial is one of the positive exceptions, since Anderson selected subjects who had usually at least one cold during winter. This kind of selection seems

sound as the role of vitamin C appears to be quite small, and there is no point in examining the effect of vitamin C on people that usually have no problems with colds in winter. It seems that most authors were simply enrolling subjects who were easy to reach, rather than subjects for whom there was a sound biological rationalization.

Thus, problems in planning and analysis of trials have led to a considerable waste of man-years of work on the subject of vitamin C and the common cold. Detailed description of the randomization method, double-blindness and other technical features are no substitutes for a meaningful biological question, even though the technical features are relevant for the validity of the trial. However, some of the recent trials are of limited use because of severe technical deficiencies.

In one recent trial, the 'control group' data was collected in 1990 with one group of students, whereas the 'vitamin C' group data was collected in 1991 with a different group, and, moreover, the protocols of data collection differed between 1990 and 1991 (Gorton & Jarvis 1999). Given the variability of cold experience from year to year, the claim by the authors that the observed differences were attributable to vitamin C must be disputed.

Another recent trial (Himmelstein *et al.* 1998) started with 52 marathon runners in two groups, but 42% (22 of 52) of the vitamin C group and 75% (38 of 52) of the placebo group dropped out during the trial. With such an extreme and significantly divergent drop-out rate (mid-P[2-t] = 0.002), and such a small number of participants in the treatment groups, it makes no sense to draw any conclusions from the differences in the incidence or severity of upper respiratory tract infections between them, although the authors have done so.

The conclusions of the Karlowski et al. *trial (1975)*

The Karlowski *et al.* (1975) trial is by far the most influential of the trials on vitamin C and the common cold (Table 10). Karlowski *et al.* concluded that the apparent benefit of vitamin C in their double-blind placebo-controlled trial was explained, paradoxically, by the placebo effect. In this thesis it is shown that the 'placebo effect' explanation is inconsistent with Karlowski's own data (Paper I; see p 25). The rejection of Karlowski's 'placebo explanation' is important since it validates the use of Karlowski's results in the quantitative estimation of the effects of vitamin C. Karlowski's results are important in three particular respects.

First, the comparison of the therapeutic and prophylactic methods of supplementation in the same trial provides an estimate of their relative effects. From Karlowski's results it appears that with a fixed dose, proper therapeutic supplementation can yield approximately as great a benefit as prophylactic supplementation (I). Most of the published trials have used regular supplementation, and Karlowski's results suggest that the effects of appropriate therapeutic supplementation might be similar.

Second, Karlowski's results suggest that there is linear dose-dependency, even with high intakes of up to 6 g/day (I; Table 8; Hemilä 1999a), and it seems possible that still higher doses might produce even greater effects. The largest dose (6 g/day) decreased the duration of colds by 17% and crude linear extrapolation suggests that 18 g/day might shorten common cold duration by half. Even though such a crude extrapolation must be regarded cautiously, it is noteworthy that this dose estimate is of the same magnitude as the dosage proposed by a few physicians who used vitamin C in the treatment of the common cold: Bee (1980) proposed 10-15 g/day for treating colds, and Cathcart (1981; Luberoff 1978) suggested that the optimum dose might be over 30 g/day. Thus, it appears possible that the vitamin C doses used in the common cold trials (highest doses 6 g/day for adults and 2 g/day for children) have not been large enough to reveal the maximum effects of supplementation.

Third, the observed effect is quite small in Karlowski's study when considering the fairly large doses used (up to 6 g/day) compared with the effect in several other trials with smaller doses (Hemilä 1992a, 1999a). One possible explanation of the relatively small benefit is the character of the participants used in the Karlowski trial. Anderson *et al.* (1972, 1975) found that vitamin C supplementation was much more beneficial for subjects with a low daily intake of fruit juice, which is a major source of vitamin C (Table 13). Participants in the Karlowski trial were NIH employees and it seems probable that such medically-aware people have on average a much healthier diet than the general population and eat more fruit and fruit juices. Thus, Karlowski's study with employees of the NIH may underestimate the potential benefits of similar vitamin C doses for subjects with a diet which is more typical of the general population or for subjects with a poor diet.

Karlowski *et al.* (1975) considered that the effect, even if real, has no clinical importance, and concluded that "It does not seem worthwhile to take two capsules or tablets three times a day for the rest of one's life to achieve such a small and equivocal benefit." In this conclusion, they missed several important findings in their own trial, which are discussed above. First, they could have noted that three times a day during a cold episode is much more feasible and much less expensive than 'three times a day for the rest of one's life,' and their own results indicated that the effect of therapeutic supplementation may be even greater than that of regular supplementation. Second, as there was a linear dose-dependency in their own results, they could have noted that therapeutic doses larger than 6 g/day might have decreased the duration of episodes by over 17%. Third, since they cited the Anderson *et al.* paper (1972), reporting that vitamin C supplementation is much more beneficial for subjects with low fruit juice intake, they could have noted that their own subjects were probably not the kind of people that would derive the greatest benefit from vitamin C supplementation. It would seem more relevant to ask what the subgroups that would benefit most from vitamin C supplementation are, rather than to ask whether a fixed numerical value found with NIH employees is great enough to validate regular vitamin C supplementation for the general population.

Published trials suggest that it would be worthwhile to carry out further trials to assess the effects of large therapeutic vitamin C doses on the symptoms of the common cold. Furthermore, the regular supplementation trials have on average found a considerably greater benefit for children than for adults, whereas none of the published therapeutic trials used children as subjects (Hemilä 1999a; Douglas, Hemilä, *et al.* 2004). Thus, it would seem worthwhile to carry out well-planned therapeutic trials to obtain better quantitative estimates of the optimum doses and maximum therapeutic effects, and to better understand the potential differences between various groups of people. Furthermore, although a tablet is practical and the most common form of administering vitamin C, it is worth noting that administering vitamin C powder directly into the nose has also been suggested (Gotzsche 1989).

Findings from meta-analyses

Pauling (1970a, 1971a,b) concluded from earlier trials that high-dose vitamin C supplementation would substantially reduce the incidence of colds in the general Western population; however, in this thesis it is shown that the 6 largest trials suggest a firm rejection of Pauling's hypothesis (Paper II). Nevertheless, 2 of these large-scale trials found a statistically significant but small reduction in the incidence of throat and chest colds (Anderson *et al.* 1972; Elwood *et al.* 1976). Furthermore, a few trials found a reduction in common cold incidence in subjects under heavy acute physical stress during the trial period (Hemilä 1996b; Douglas & Hemilä 2005; graph on the cover). It is also noteworthy that in 2 trials 8% and 16% of participants remained free of illness because of vitamin C supplementation (Table 20) and, in 2 other vitamin C trials, 6% and 29% of participants could infer their treatment by subjective observations (Table 11). Thus, it appears possible that regular vitamin C supplementation has beneficial effects in particular conditions and/or among particular sub-populations.

People with low dietary intake of vitamin C might show the most conspicuous effects of supplementation, assuming that the level of vitamin C intake is important. Low levels of vitamin C intake were observed in a number of British surveys, indicating that the average intake was fairly low; in this thesis the common cold studies carried out in the UK were analyzed on the assumption that the vitamin C intake in diet was low in all the UK trials (II).

Pooling the results of the UK trials with males yielded an estimated 30% decrease in the number of common cold episodes, and a 46% decrease in the number of males with recurrent infections with vitamin C supplementation (II). Nevertheless, these estimates should be interpreted as crude approximations. If vitamin C intake does have an effect on the incidence of colds, there is obviously no single estimate for the effect that is generally applicable. For example, the effect of supplementation obviously decreases with an increase in the dietary vitamin C intake, and it is also possible that there are other factors modifying the quantitative effects of vitamin C intake in a group of subjects. For example, a substantial difference between the sexes was found in the UK studies (II), although a recent trial found a significant effect from vitamin C on recurrent colds in a population consisting mainly of women (van Straten & Joslin 2002; Table 22).

Meta-analysis of 3 controlled trials found a substantially lower incidence of pneumonia in vitamin C supplementation groups than the control groups (Table 25; Hemilä 1997c). A recent cohort study found no association between vitamin C intake and community-acquired pneumonia in middle-aged men in the USA (Merchant *et al.* 2004). There are, however, substantial differences in this cohort study from the 3 trials of Table 25. Merchant *et al.* investigated male US health professionals aged 40-75 years, a selection which meant a population which has a much greater than average interest in factors that affect health and whose working conditions are quite sedentary. In Merchant's cohort, the median vitamin C intake of the lowest quintile was 153 mg/day, whereas the overall median of the average US population is about 100 mg/day (FNB 2000 pp 416-9). In contrast, of the 3 trials in Table 25, Glazebrook and Thomson (1942) estimated that the diet of their participants contained only 10-15 mg/day of vitamin C, and the Kimbarowski and Mokrow trial (1967) was carried out with military recruits in the former Soviet Union whose diet was apparently not rich in vitamin C. Furthermore, 2 of the trials in Table 25 were composed of military recruits (Kimbarowski & Mokrow 1967; Pitt & Costrini 1979), while the third investigated schoolboys in a boarding school (Glazebrook & Thomson 1942). Thus the living conditions of these participants were quite different from middle aged US health professionals. This divergence in social background is also seen in the difference in the incidence of pneumonia. Merchant *et al.* (2004) recorded 3 pneumonia cases per 1,000 person years, which is close to that in the ATBC Study cohort with 5 cases per 1,000 person years. In contrast, Glazebrook and Thomson (1942) and Pitt and Costrini (1979) recorded 30 and 120 pneumonia cases per 1,000 person years in their control groups respectively, *i.e.*, 10-40 times higher incidence than in Merchant's cohort. Thus, even though the Merchant *et al.* (2004) cohort study suggests that the level of vitamin C intake does not affect the risk of pneumonia in sedentary middle aged populations when the intake range starts from some 150 mg/day, the findings cannot be extrapolated to substantially different population groups.

It is noteworthy that heavy physical stress is a prominent feature of 2 of the military recruit trials that found some benefit from vitamin C on pneumonia (Table 25; Hemilä 1997c), a feature apparent in a subgroup of trials that observed a reduction of common cold incidence with vitamin C supplementation (Hemilä 1996b; Douglas, Hemilä, *et al.* 2004; Douglas & Hemilä 2005; graph on the cover). Thus, it would seem worthwhile to research the possible preventive role of vitamin C against pneumonia in more detail; for example, among military recruits who have a high rate of pneumonia (Hemilä 2004).

Findings from the ATBC Study analyses

The relationship between dietary vitamin C intake and the common cold incidence was analyzed in the ATBC Study cohort (Paper III), motivated by the positive findings in the UK trials on the common cold in men, and on the interpretation that the benefit in those trials is explained by low dietary vitamin C intake. Low vitamin C intake was not associated with a greater risk of colds, but with a slightly lower risk. This association in an unanticipated direction may be explained by residual confounding by life-style variables as it seems unlikely that such an association might be explained by the actual biological effects of low vitamin C intake. Nevertheless, it is noteworthy that the participants in the 4 UK male trials included in the meta-analysis were schoolchildren or students (II), whereas all participants in the ATBC Study were ≥50 years old (III). Thus, it remains a possibility that low vitamin C intake might affect susceptibility to the common cold only in younger people, or that there may be other reasons that explain the possible benefit of vitamin C supplementation among the UK males.

Another recent smaller cohort study also found no association between dietary vitamin C intake and common cold incidence (Takkouche *et al.* 2002).

A series of studies with guinea pigs found that pure vitamin C, and fruit and vegetables as a source of vitamin C, affected the risk of tuberculosis (Appendices 2 and 3). Two older studies of young and middle-aged African Americans reported a lower incidence of tuberculosis among participants with higher vitamin C intake levels, but the comparison is not specific to vitamin C (Table 24). In the ATBC Study participants, vitamin C intake had a statistically highly significant association with the risk of tuberculosis in an unadjusted analysis, but adding all available confounders rendered the association non-significant (IV). Furthermore, it was shown that the association between vitamin C intake and the risk of tuberculosis was only a statistical artifact caused by the strong correlation between vitamin C intake and the total fruit, vegetable, and berry intake. The importance of vitamin C intake as a risk factor for tuberculosis was thereby rejected in the ATBC Study cohort, but since the role of vitamin C may vary between populations, this negative result should not be directly extrapolated to populations that substantially differ from the ATBC Study participants.

The presence of substances other than vitamin C in fruit, vegetables, and berries that may reduce the risk of tuberculosis by some 60% is interesting, but the identity of the substance(s) is unknown. This finding of the ATBC Study cohort may indicate that the benefits found in the animal studies of Appendix 3 may at least partly be caused by substances other than vitamin C in fruit and vegetables.

Fundamental concepts on the health effects of vitamin C

As to ascorbic acid, right from the beginning I felt that the medical profession misled the public. If you don't take ascorbic acid with your food you get scurvy, so the medical profession said that if you don't get scurvy you are all right. I think that this is a very grave error. Scurvy is not the first sign of the deficiency but a premortal syndrome, and for full health you need much more, very much more. I am taking, myself, about 1 g/day. This does not mean that this is really the optimum dose because we do not know what full health really means and how much ascorbic acid you need for it. What I can tell you is that one can take any amount of ascorbic acid without the least danger.

Albert Szent-Györgyi
(cited by Pauling 1976a, 1986a)

Scurvy is a definite clinical end point, and preventing scurvy was the basis of the US RDA nutritional recommendations for vitamin C from the 1940s until the 10th edition (FNB 1989a, 1994a). Approximately 10 mg/day of vitamin C prevents overt symptoms of scurvy, and the RDA of the 10th edition was arbitrarily set at 60 mg/day to 'provide an adequate margin of safety' against scurvy (FNB 1989a p 118). "This level of intake will prevent signs of scurvy for at least 4 weeks" in the fictional condition that a person completely stopped getting vitamin C.

Scurvy is an acute severe disease resulting from particularly low vitamin C intake but the lack of a frank deficiency *per se* does not necessarily reflect optimal metabolism. Thus, while the goal of the early RDA was to protect against overt scurvy, the goal could be to obtain optimal amounts of nutrients (Bourne 1949; Pauling 1968, 1970b, 1974; Hughes 1981; Hemilä 1984, 1985, 1986, 1987, 1989, 1990c, 1991; Levine 1986; Levine *et al.* 1995; Ginter 1989; Eaton *et al.* 1996). There were no studies described in the RDA recommendations which would suggest that 60 mg/day is any better than 10 mg/day as regards any health status outcome in the long term (FNB 1989a pp 115-24). In the 10th edition of the RDA, this lack of concern with optimal intakes was explicitly recognized: "RDAs are not necessarily optimal levels of intake" (FNB 1989a p 8).

In the 10th edition (FNB 1989a p 1) the "RDAs are defined as the levels of intake of essential nutrients that … are … adequate to meet the nutrient needs of practically all healthy persons." However, 'nutrient need' is a vague concept and was not defined in any more detail in the RDA recommendations. The concept of 'nutrient need' gives the false impression that exact amounts of vitamins are required daily, so that larger amounts could not have any active physiological effects, simply providing passive reserves, such that 60 mg/day of vitamin C would only cause 'reserves' large enough to prevent signs of scurvy for 4 weeks. Such reasoning clearly demonstrates the lack of consideration of the principles of biochemical reaction kinetics, as the rates of biochemical reactions change smoothly with increasing concentrations. The physiological purpose of vitamin C is not to be 'in reserve against scurvy' but to participate in chemical reactions in the body, and the rates of these reactions depend on concentrations, which depend on the levels

of intake. Consequently, an exact level of 'nutrient need' sharply distinguishing a frank deficiency from 'normal health' is not a sound concept (Hemilä 1984, 1985, 1991).

Furthermore, mathematical modeling of nutrient dose-response relationships in animals does not identify any parameter with 'nutrient need', the dose-response relations following the saturation type of functions (Morgan *et al.* 1975; Schulz 1987, 1991), as expected on biochemical grounds (Pauling 1968). In contrast to the 'nutrient need,' the concept of 'optimal intake' seems to be consistent with biochemical knowledge (Pauling 1968; Hemilä 1984, 1985, 1986, 1987, 1991).

Some early authors on vitamin C and infectious diseases commented on the question of optimal intake. King (1936) commented that "The fact that there is a wide zone of vitamin deficiency between scurvy and optimum health is of more interest in relation to human health than the problem of clinical scurvy," and from his own study on the effects of vitamin C on diphtheria toxin on guinea pigs he concluded that "It is evident from the data presented that the level of vitamin C intake for optimum *in vivo* detoxification of diphtheria toxin is considerably greater than that necessary to protect from scurvy or to show a favorable growth rate" (Sigal & King 1937; p 109). Perla (1937) and Perla and Marmorston (1937 p 686) asked: "Is one certain of the optimal vitamin requirements? Can the criteria of growth, progressive increase in weight and absence of clinical evidences of deficiency be accepted as adequate? Is it not possible that all these may be present and still, under a given stress, such as invasion with micro-organisms or injections of poisons, the apparent optimal amounts do not prove to be so?"

With the disappearance of deficiency diseases as a serious problem in the Western countries, and the increasing interest in the role of nutrition in affecting chronic diseases (FNB 1994a,b; Lachance & Langseth 1994), "A new paradigm for determining RDAs was indicated" (King 1996). After the 10th edition of the RDA, the approach of the nutritional recommendations was extensively revised. In the most recent US recommendations (FNB 2000) the possibility of affecting chronic diseases with vitamin C was extensively discussed. In these latest recommendations, the length of the vitamin C chapter is 91 pages (FNB 2000 pp 95-185), in contrast to the 10 pages of the 10th edition of RDA (FNB 1989a pp 115-24). Although the latest recommendations thoroughly discuss the epidemiological literature relating vitamin C intake with chronic diseases, the authors conclude that there are no appropriate studies that would yield data about what might be proper doses for preventing the chronic diseases. Instead, the latest vitamin C recommendations consider that "In the absence of other data, maximal neutrophil concentration with minimal urinary loss appears to be the best biomarker at the present time" (FNB 2000 p 140). This end point is, however, a surrogate and there is no evidence cited that vitamin C level in neutrophils has any relation to some clinical disease end point, so that the maximal level in these cells would correspond to optimal intake level in the long term (FNB 2000 pp 95-185). However, there are quite a few examples in which the effect on a surrogate marker diverged from the effect on a clinical outcome (Fleming & DeMets 1996; DeGruttola *et al.* 1997).

A further important restriction of both the early and the current RDAs is that they apply specifically to 'healthy persons' (FNB 1989a p 1) and 'apparently healthy individuals' (FNB 2000 p 23). Consequently, the RDA levels are *not* intended to provide reference levels of intake for people who are sick, for example, with the common cold or more severe infections.

While the nutritional recommendations have great importance in nutrition politics, they cannot be used as a basis for claiming that amounts in excess of RDA levels are known to be useless. The early RDA recommendations used an acute clinical outcome that is irrelevant when considering the possible long-term effects of vitamin C intake, and the new recommendations use a surrogate that has no known validity as regards its long-term health effects.

Kunkel (1996; Kunkel & Thompson 1988) criticized the lack of appropriate philosophical considerations behind the nutritional recommendations: "The reasoning, however, can become circular and unsound when the considered moral judgments, moral principles and relevant background are not independent sources of information. If they are mixed or, for example, an intuition is mistaken for a scientific conclusion, the reasoning can be flawed."

The safety of vitamin C

Often the benefits of vitamin C observed in randomized double-blind placebo-controlled trials are disregarded, although at the same time authors may exaggerate the potential harm caused by vitamin C even when it is purely anecdotal (*e.g.*, Olson & Hodges 1987; Herbert 1993).

In a casual survey of 20 physician colleagues, Goodwin and Tangum (1998) found that all of them were aware that high-dose vitamin C ingestion can cause kidney stones. Goodwin and Tangum were, however, interested in where this common 'knowledge' comes from and they combed the medical literature without finding any articles in refereed journals reporting instances of high-dose vitamin C causing kidney stones. Review articles cited book chapters that in turn cited abstracts, letters, and other review articles. Goodwin and Tangum concluded that nowhere in the trail of citations was there any fundamental information on whether or how frequently high-dose vitamin C supplementation might lead to kidney stones. The authors simply stated that vitamin C may cause kidney stones, and as proof they cited other authors who had said the same thing. Thus, this description reveals a typical urban legend; a story that is retold, yet no-one confirms that the story is true.

The anecdote of vitamin C and kidney stones is mentioned in a major textbook of pharmacology: "…risks of megadose treatment ... include formation of kidney stones" (Marcus & Coulston 2001). The statement that vitamin C may cause kidney stones has been reiterated in the Finnish literature without any references (Aro 1985a,b, 1994), and in the Nordic Nutritional Recommendations, also without any references (NNR 2004 p 310).

When reviewing the health effects of vitamin C, Olson and Hodges (1987) and Herbert (1993) claimed that "Large intakes of vitamin C may reduce insulin production." This statement was based on a paper published in 1946. Levey and Suter (1946) reported that vitamin C potentiates the diabetogenic action of alloxan in rats, whose blood-sugar level was determined 3 days after injecting alloxan, or alloxan with vitamin C. Hyperglycemia was observed in 50% of the rats treated with alloxan and vitamin C, in contrast to 17% of the rats treated with alloxan alone. Nevertheless, the authors concluded from their own previous work that "ascorbic acid alone does not produce hyperglycemia" (Levey & Suter 1946). Thus, this old experiment with alloxan-treated rats was the basis for Olson and Hodges (1987) and Herbert (1993) to state that large doses of vitamin C alone may reduce insulin production in human subjects.

Olson and Hodges (1987) and Herbert (1993) stated that "Large intakes of vitamin C may interrupt pregnancies." This suggestion was based on a brief Russian paper published in 1964. Twenty women whose menstruation was delayed by 10-15 days were given 6 g/day of vitamin C, and 16 of them started to menstruate within 3 days (Samborskaya & Ferdman 1964). Pauling (1976a) wrote a letter to the authors inquiring whether any actual direct test of pregnancy was carried out, but he received only a copy of the publication by way of reply. Thus, there was no evidence that the women were pregnant to begin with. Possibly the women just had irregular menstruation, yet this report was valid enough for Olson and Hodges (1987) and Herbert (1993) to conclude that vitamin C may cause miscarriages.

Olson and Hodges (1987) and Herbert (1993) also argued that "Large intakes of vitamin C may lower plasma vitamin B12 levels." This claim was originally made by Herbert himself (Herbert & Jacob 1974), however, it was shown afterwards that the apparent breakdown of vitamin B12 was due to methodological shortcomings (Newmark *et al.* 1979; Marcus 1981), and the vitamin B12 level was not decreased in patients administered as much as 4 g/day of vitamin C for 11 months or more (Afroz *et al.* 1975), or in children administered gram-doses of vitamin C for years (Ekvall *et al.* 1981). However, these papers were not cited by Olson and Hodges (1987) or Herbert (1993).

In extreme cases, suggestions about vitamin C toxicity have been based on double-speculation. Herbert (1993) stated that (1) vitamin C might cause elevated iron levels, and (2) elevated iron levels might cause increased risk of coronary heart disease. However, (1) in order to quantify the effect of vitamin C supplementation on iron status, Cook *et al.* (1984) administered 2 g/day of vitamin C to 9 subjects for 2 years without finding indications of iron accumulation, and (2) several studies with different types of settings were unable to corroborate the hypothesis that raised iron levels increase the risk of coronary heart disease (Bendich & Langseth 1995; Hemilä & Paunio 1997). In contrast to ordinary people, patients suffering from actual iron overload may derive harm from vitamin C; however, its harmful effect on these particular patients and the rationale of treating such patients with the combination of vitamin C and desferrioxamine has been known for a long time (Nienhuis 1981).

Rivers (1987) reviewed 74 publications dealing with the possible toxicity of vitamin C and concluded that "Large quantities of ascorbic acid will not result in calcium-oxalate stones, increased uric acid excretion, impaired vitamin B12 status, iron overload, systemic conditioning, or increased mutagenic activity in healthy individuals." In another review, Marks (1989) concluded that "A large number of adverse reactions have been alleged to occur with the use of large doses of ascorbic acid, but almost without exception further study has demonstrated that the allegations are without foundation ... an overview of all the information shows that the safe daily level is at least 100 times the RDA." The RDA level for vitamin C was 60 mg/day at that time. Hathcock (1997) stated that "Many hypothetical adverse effects of high intakes of vitamin C have been cited for decades. Most, with the exception of mild and transient gastrointestinal effects, seem to have little or no known factual basis." Several other reviews have also concluded that vitamin C is safe in doses around 1 g/day (Hanck 1982; Bendich & Langseth 1995; Diplock 1995; Hathcock *et al.* 2005). The recent US nutritional recommendations suggest that the safe range of vitamin C intake goes to 2 g/day for adults, but the basis for this upper limit of 'safe doses' is the appearance of loose bowels (FNB 2000 pp 155-65), which, however, is quite a trivial adverse effect and disappears quite quickly with a change to lower intake levels.

There are a few reports of severe harm caused by high-dose vitamin C administration. Nevertheless, the death of a 68-year old African American man was not attributed to intravenous injection of 80 grams of vitamin C on 2 consecutive days *per se*, but to his coincident glucose-6-phosphate deficiency (Campbell *et al.* 1975). Such isolated instances have no public health relevance. In a recent pharmacokinetic study participants were administered up to 100 grams of vitamin C within a few hours indicating the safety of such a large dose *per se* in healthy people (Padayatty *et al.* 2004).

There is much evidence indicating that vitamin C metabolism changes during infections and this may affect the relationship between doses and adverse effects (Fig. 1; see pp 6-7). It has been reported that people with serious infections can ingest over 50 g/day of vitamin C without gastric problems (Luberoff 1978; Cathcart 1981).

Problems with statements by experts

The status of an 'expert' implies that an individual is thoroughly familiar with the particular field. Unfortunately, in the vitamin C field, the track record of many experts is poor.

Olson, Hodges, and Herbert (see the preceding section) were all members of the first committee preparing the 10th edition of the US RDA nutritional recommendations, which plays a central role in US governmental policy, and indirectly affects policy in numerous other counties. James A. Olson was also an editor of a major textbook on nutrition (Shils,

Olson, *et al.* 1994, 1999). The review of vitamin C by Olson and Hodges (1987), which contained the anecdotal stories of vitamin C toxicity (see the previous section), was planned as the vitamin C section of the 10th edition of the RDA recommendations (Olson 1986; Pellett 1988). The US National Academy of Sciences decided, however, not to publish the RDA draft by the first committee because of an impasse resulting from scientific differences of opinion between the first committee and the scientific reviewers appointed by the National Academy of Sciences (Press 1985; Marshall 1985). One reason for the disagreements was the proposal that the RDA level for vitamin C recommendation be lowered from 60 mg/day to 40 mg/day for men and 30 mg/day for women. The second RDA committee kept the vitamin C level at 60 mg/day for both men and women (FNB 1989a).

Victor Herbert, who reiterated the anecdotal stories of vitamin C toxicity (see the previous section) in one of his papers (Herbert 1993), was also a member of the committee preparing the 9th edition of the US RDA nutritional recommendations (FNB 1980). Herbert received the 1972 McCollum Award for outstanding scientific accomplishment in the field of nutrition (Anonymous 1973; ASCN 2005; see footnote to Table 1), and was the president of the American Society for Clinical Nutrition in 1980-1981. In 1984 Herbert received the FDA Commissioner's Special Citation Award for "outstanding and consistent contributions against the proliferation of nutrition quackery to the American consumer" (Halsted 2003; Oransky 2003). In his reminiscences Pauling described an acrimonious exchange of letters with Victor Herbert who rejected the results of all placebo-controlled trials on vitamin C and the common cold without any reasonable argument. Pauling commented that "I finally became sufficiently irritated by this fellow that I decided I ought to do something about it. So I sat down one summer and in two months wrote a book, *Vitamin C and the Common Cold* " (Marinacci 1995 pp 248-51). In this respect Herbert was, paradoxically, indirectly behind the increase in the popular enthusiasm for vitamin C supplementation that resulted from Pauling's book. The author of this current thesis pointed out that Herbert's conclusions (1993), that there is no reliable data to show that vitamin C supplementation may provide any benefit and that vitamin C supplements may instead be highly harmful, were based on a grossly biased selection of references (Hemilä 1994b).

Stephen Barrett (2005) has written several books on nutritional quackery (Barrett 1980; Barrett & Herbert 1994), and received the FDA Commissioner's Special Citation in 1984 for "outstanding and consistent contributions against the proliferation of nutrition quackery to the American consumer." Barrett also received the 2001 Distinguished Service to Health Education Award from the American Association for Health Education. However, Barrett's presentation of facts related to the findings from studies on vitamin C and the common cold have been markedly biased. In a comment on Barrett's paper (1995) claiming there is no evidence that vitamin C might affect colds, Hemilä (1995b) pointed out that "Anderson *et al.* (1972) found that vitamin C supplementation (1-4 g/day) decreased the 'numbers of days confined to house' per subject by 48% in subjects with a low dietary intake of fruit juices [see Table 13]. Barrett's claim that at best there is only a slight reduction in symptoms appears grossly misleading considering the published results." Stephen Barrett replied to this that "Anderson's first study found … a 30% difference." In a subsequent letter Edgar Villchur (1995) pointed out that "Barrett's reply in the same issue challenges Hemilä's reporting accuracy, but Hemilä is correct … Barrett, however, doesn't say he is citing a different part of the Anderson data, and thus makes it seem that Hemilä has either misread or misrepresented Anderson." In a reply to this accusation, Stephen Barrett conceded that "Villchur is correct that Hemilä and I referred to different figures."

Thomas Chalmers, Paul Meier, A. Steward Truswell, and Jos Kleijnen are experts in controlled trials, medical statistics, nutrition, and systematic reviews, yet their reviews on vitamin C and the common cold contain lots of factual errors and misleading statements (see pp 36-45). The Chalmers (1975) and Dykes and Meier (1975) reviews were cited in major textbooks of infectious diseases and in the US RDA recommendations, although some of the numerous shortcomings of both reviews should have been apparent to any expert even superficially familiar with the original study reports.

In the most recent US nutritional recommendations (FNB 2000 pp 126-7), Chalmers' review (1975) and the Hemilä and Herman criticism (1995) of Chalmers' review are cited in the same paragraph without mentioning that the latter paper shows that the former review is invalid. Thus, the experts writing the vitamin C chapter did not read or understand the two papers cited to see that they were incompatible. Although the most recent recommendations extensively discuss the observational studies related to the possibility that vitamin C intake might affect chronic diseases, the recommendations ignore some 40 placebo-controlled trials that have examined the preventive effect of vitamin C on colds (FNB 2000 pp 117, 127; Tables 6, 15, 17). Only 4 controlled trials are cited in the US recommendations (Peters *et al.* 1993; Coulehan *et al.* 1976; Miller *et al.* 1977; Ludvigsson *et al.* 1977). Since the Ludvigsson *et al.* trial (1977) is the only one of the 6 largest (Table 10) mentioned in the recommendations, 83% (5/6) of the largest common cold trials are simply ignored, and some 90% of all the placebo-controlled trials on vitamin C and the common cold are ignored in the current US nutritional recommendations.

Even in the case of the Ludvigsson paper (1977), the study findings are disregarded in the recommendations (FNB 2000 p 127). Ludvigsson *et al.* (1977) found a 14% reduction ($P = 0.008$) in the duration of 'absence from school because of upper respiratory tract infection' in the main trial, and a 39% reduction ($P = 0.002$) in the duration of 'upper respiratory tract infection' in the smaller trial, but these findings are not mentioned in the recommendations. Two large trials reporting that vitamin C supplementation significantly increased the proportion of participants who remained free of illness during supplementation (Table 20) are not cited in the recommendations either, or any of the UK trials that found a significant proportion of participants benefiting when the outcome is ≥2 colds during the trial (Table 22).

Neither were any of the 3 trials that found a significant reduction in pneumonia incidence by vitamin C supplementation (Table 25) mentioned in the recommendations. It is not clear to what extent the poor coverage of literature is caused by lack of knowledge of the studies, and how much by an intentional decision to discard studies that do not fit with a preconception that vitamin C is useless for colds and other respiratory infections.

In systematic reviews it is essential to cover the published studies widely and analyze the results objectively (Ian Chalmers & Altman 1995; Higgins & Green 2005), but this was not done in the most recent US nutritional recommendations (FNB 2000). Although the common cold trials do not allow explicit conclusions to be drawn about what might be the best doses of vitamin C intake, the published trials do show that large doses affect common cold severity and duration in large groups of people, and the incidence of colds in some people. Objective discussion of the common cold trials also provides justification to carry out further study, and to use susceptibility to and severity of infections as potential outcomes of interest in considering the 'optimal intake levels' instead of focusing only on cancers and cardiovascular diseases, and using 'maximal neutrophil concentration with minimal urinary loss' as a surrogate with no established validity against clinically relevant outcomes.

The problems of expert reliability in nutritional recommendations are also seen in other vitamin C issues. In the 10th edition of RDA, comments on vitamin C and cholesterol metabolism were based on 2 trials that did not use placebo-control (FNB 1989a p 120-1), and 3 placebo-controlled studies which found a significant decrease in elevated cholesterol levels with vitamin C supplementation were disregarded (Hemilä 1992c, 1993).

Similar problems are faced in the UK nutritional recommendations (DH 1991 [see Hemilä 1997d]), where no mention is made of the possible role of vitamin C on the common cold, although some 40 placebo-controlled trials have been carried out. Furthermore, one of the trials cited in the UK recommendations did report that the geometric mean duration of colds was 6.4 days in vitamin C-deprived subjects and 3.3 days in non-deprived subjects. The authors concluded that "Such evidence as there is, however, definitely confirms the hypothesis that the absence of vitamin C tended to cause colds to last longer" (Bartley, Krebs & O'Brien 1953 p 43), but the authors of the UK recommendations disregarded this (DH 1991). The brief discussion of the vitamin C and cholesterol issue referred to a single uncontrolled intervention study, and disregarded 11 published placebo-controlled trials (DH 1991; Hemilä 1992c, 1997d).

In Finland, an authoritative monograph on antioxidants based its comments on vitamin C and the common cold on one observational study and one trial with marathon runners (Aro 1994). Observational studies are substantially less reliable than controlled trials, whereas there are some 40 published placebo-controlled trials on vitamin C and the common cold, and findings with marathon runners cannot be generalized to the normal population (Hemilä 1995a). A brief Finnish review of the vitamin C and cholesterol relationship (Aro 1990b) claimed that one cited trial was placebo-controlled whereas it was not (Hemilä 1990d), and 3 placebo-controlled trials that found a statistically significant effect on plasma cholesterol level with vitamin C supplementation were disregarded (Hemilä 1990d, 1992c, 1993). Obviously, such biased presentations were the motivation for the concept of 'systematic reviews' (Ian Chalmers & Altman 1995; Higgins & Green 2005).

In the most recent editions of Mandell *et al.*'s textbook of infectious diseases (Gwaltney 2000, 2005), Chalmers' review (1975) is no longer cited; however, comments on vitamin C and the common cold are based on 2 small trials with artificially inoculated colds (Walker *et al.* 1967; Schwartz *et al.* 1973). These 2 trials recorded only 36 and 21 common cold episodes respectively, whereas none of the 5 large-scale trials recording over 1,000 natural common cold episodes per trial (Table 14) is cited (Gwaltney 2000, 2005). In a classic paper, Thomas Chalmers pointed out that negative findings from small trials may correspond to the type II error in statistics and concluded that "Concern for the probability of missing an important therapeutic improvement because of small sample sizes deserves more attention in the planning of clinical trials" (Freiman *et al.* 1979). Thus, drawing conclusions from trials with 36 and 21 common cold episodes may lead to a false negative conclusion. Furthermore, it is possible that natural colds differ from artificially induced colds. The rate of the former is about 1 per year or 0.02 per week for adults, whereas the rate of the latter was 0.4 to 1.0 per week (Walker *et al.* 1967; Schwartz *et al.* 1973), indicating that the exposure level to viruses was orders of magnitude higher in the artificially inoculated colds, which may affect the role of vitamin C.

Pauling (1971b, 1976a pp 121-38, 1986a pp 225-36) also provided several examples of how the authorities in nutrition misinterpreted and ignored the findings of published trials. Evidently, a personal problem for Pauling was that "Medical experts have a long history of resisting scientific innovations from what they define as 'the outside' " (Barber 1961).

The poor quality of expert comments on vitamin C and the common cold is puzzling. Goodwin and Tangum (1998) provided several examples to support the conclusion that there has been systematic bias against the concept that vitamins might be beneficial in levels higher than the minimum required to avoid classic deficiency diseases: "Throughout much of the 20th century, American academic medicine was resistant to the concept that micronutrient supplementation might prove beneficial. This resistance is evident in several ways: (1) by uncritical acceptance of bad news about micronutrient supplements; reports of toxic effects were rarely questioned and widely quoted; (2) by the scornful, dismissive tone of the discussions about micronutrient supplementation in textbooks of medicine, a tone avoided in most medical controversies; and (3) by the skeptical reaction greeting any claim of efficacy of a micronutrient, relative to other therapies; indeed, most claims were simply ignored."

Bias against vitamin C was also documented by Richards (1988, 1991; Galloway 1991; Huxtable 1992; Segerstråle

1992) who compared the attitudes and arguments of physicians to three putative cancer medicines: 5-fluorouracil, interferon, and vitamin C.

The evaluation of the potential effectiveness of a therapeutic method usually depends greatly on the possibility of biologically rationalizing the method. Goodwin and Goodwin (1981, 1984) reviewed several cases in which an effective method of treatment was erroneously rejected due to a lack of understanding of the physiological mechanism of the effect. They designated this problem 'the tomato effect', since the tomato was considered poisonous in the USA in the 1700s because several other plants in the same family were poisonous: "The tomato effect in medicine occurs when an efficacious treatment for a certain disease is ignored because it does not 'make sense' in the light of accepted theories of disease mechanism and drug action." Thus, the question of evaluating a new method of therapy is not just whether a moderate effect is reproducible in controlled trials, but substantially depends on conceptual issues related to the biological explanations (Vandenbroucke 1998a; Vandenbroucke & de Craen 2001). It is possible, for example, that the claim in the biochemistry textbooks that vitamin C participates in the hydroxylation of proline in collagen (Berg *et al.* 2002) leads to a misleading impression of the mechanism of vitamin C action, as the effects of vitamin C on other biochemical reactions (Englard & Seifter 1986; Padh 1990) and on the immune system (Hemilä 1997a, 2003a) are not mentioned at all. Some further possible conceptual reasons for the bias against the effects of vitamin C on common cold have been discussed elsewhere (Hemilä 1996a, 1997a).

The effect of vitamin E on respiratory infections

Findings from the ATBC Study analyses

Suggestions that vitamin E supplementation might help prevent infections have mainly been based on immunological studies. However, immune system markers are only surrogates of susceptibility to infection and it is not clear whether such immune effects have any actual relevance to clinical infections in human subjects. Nevertheless, several animal studies indicated that vitamin E may affect infections (Table 2; Appendices 4 and 5).

Only a few trials have previously examined the effects of vitamin E supplementation on clinical infections in human subjects. Most of the trials found no benefit with vitamin E, and moreover, one trial found an increase in the duration of respiratory infections with vitamin E supplementation (Graat *et al.* 2002).

In this work, we found that vitamin E supplementation had no overall effect on common cold risk among the middle-aged male smokers of the ATBC Study (III). Nevertheless, in a small subgroup of the ATBC Study participants, ≥65 year old city-dwellers who smoked less, vitamin E supplemented participants had a 28% lower risk of colds than the non-vitamin E participants. It was not clear whether this difference resulted from a physiological effect, or emerged by chance from the series of subgroup analyses. Nevertheless, even if real, a 28% effect would correspond to some 4 people taking vitamin E supplement for a year in order to prevent 1 common cold episode (NNT = 4), which appears to be quite a low cost-benefit ratio. Still, the statistically highly significant effect on a universal ailment in the narrow subgroup justified more detailed analyses to explore the modification of vitamin E effect by age, smoking, and residential neighborhood (Hemilä *et al.* submitted).

Likewise, vitamin E supplementation had no overall effect on the risk of pneumonia in the ATBC Study (Paper V). Nevertheless, various measures of smoking seemed to modify its effect on pneumonia risk. Among participants who started to smoke late in life, vitamin E lowered the risk of pneumonia by 35%. Furthermore, within this subgroup of participants starting to smoke late in life, those who smoked less than a pack per day at the baseline of the trial seemed to get greater benefit from vitamin E than those who smoked more at the baseline. Furthermore, within the subgroup of participants starting to smoke late in life, the difference in pneumonia risk between vitamin E participants and no-vitamin E participants was substantially greater among those who had quit smoking prior to the occurrence of pneumonia than among participants who continued smoking until its occurrence. Thus three different measures of cigarette smoking appeared to modify the effect of vitamin E supplementation on pneumonia risk, so that in each case less exposure to cigarette smoking was associated with greater benefit from vitamin E supplementation. Extrapolation would suggest that the effect of vitamin E might be greatest among people who have no life-time exposure to cigarette smoking. Since all ATBC Study participants were selected as smokers at the baseline, the effect of vitamin E on the risk of pneumonia in life-long non-smokers could not be examined in this work.

Although a 35% reduction in the risk of pneumonia is a substantial effect, it is not clear whether it has practical relevance even if we assume that the finding is valid. At a pneumonia rate of 5 episodes per 1,000 person-years, a reduction of 35% corresponds to 600 people needing vitamin E supplementation for a year to prevent 1 episode of community-acquired pneumonia (NNT = 600), which, after all, is usually cured quite rapidly by antibiotics and rarely leads to long-term or permanent sequelae.

A recent cohort study found no association between vitamin E intake and community-acquired pneumonia among middle-aged men in the USA (Merchant *et al.* 2004).

Because it has been suggested that moderate physical activity improves the immune system and reduces the risk of respiratory infections (Nieman 1994, 2000; Woods *et al.* 1999), we analyzed the relationship between physical activity and the incidence of the common cold in the ATBC Study cohort. No association between physical activity on the job or during leisure with common cold incidence was found (Hemilä *et al.* 2003). Neither was any association found between physical activity on the job, or during lei-

sure, and the incidence of pneumonia (Hemilä *et al.* 2006).

Since heavy physical activity causes oxidative stress in the body, it was suggested that antioxidants, in particular vitamin E, might be beneficial for people carrying out heavy exercise during leisure (Packer 1997; Sen 2001). Vitamin E did not reduce the common cold risk among ATBC Study participants with a heavy job, or participants doing strenuous exercise (Hemilä *et al.* 2003).

Vitamin E supplementation did not affect the pneumonia risk among ATBC Study participants with heavy or moderately heavy job. However, it did reduce the risk of pneumonia by 50% among participants doing heavy or moderate exercise during leisure (Table 27; Hemilä *et al.* 2006). Given the latter finding, we explored the possible role of adaptation to physical activity with the following rationale. The body adapts to physical stress arising from regular workload, there being substantial experimental evidence indicating that the activities of antioxidant enzymes increase in response to exercise in both animals and humans (Ji 1999; Powers *et al.* 1999; Sen 2001). In contrast, light or very light jobs leave people unadapted to oxidative stress caused by sporadic heavy leisure time exercise. Consequently, leisure time exercise could generate severe episodic oxidative stress in participants having a light or very light job. Consistent with this rationalization, we found that vitamin E had a great effect on participants carrying out leisure time exercise who had a light or very light job, whereas it had no effect on participants doing leisure time exercise who had a heavy or moderately heavy job. Still, it is noteworthy that the rate of pneumonia is not increased in the no-vitamin E participants who had a light or very light job, compared with no-vitamin E participants who had heavy or moderately heavy job (Table 27). This is inconsistent with the notion that poor adaptation to oxidative stress increases the risk of pneumonia, so that vitamin E would reduce an elevated risk towards the level of adapted participants. Nevertheless, although the pattern of findings is not quite consistent with the oxidative stress adaptation hypothesis, the subgroup finding arises from large randomized groups. The effect of vitamin E on the participants carrying out leisure time exercise was also modified by baseline age, and by age of smoking initiation (Table 27); however, the number of pneumonia cases is small and the subgroup differences should be considered cautiously.

The risk of tuberculosis was slightly higher in the vitamin E arm compared with the placebo arm of the ATBC Study, but the difference was non-significant (IV). Nevertheless, this finding is inconsistent with substantial benefits of vitamin E supplementation affecting the risk of tuberculosis.

Recommended intake of vitamin E

Overt vitamin E deficiency symptoms have never been described in normal individuals consuming diets low in vitamin E because it is found in such a great variety of foods, and is stored in the body for such long periods (FNB 2000). Thus there is no known minimum intake level that prevents vitamin E deficiency in the same sense as 10 mg/day of vitamin C prevents scurvy.

The most recent US recommendation of vitamin E intake uses 'hydrogen peroxide-induced hemolysis' as the outcome on which the adequacy of vitamin E is assessed, based on the argument that "In the absence of other scientifically sound data, hydrogen peroxide-induced hemolysis is the best marker at the present time" (FNB 2000 p 232). The plasma concentration of 12 μmol/l α-tocopherol was chosen as the minimal level, since this concentration is associated with normal *in vitro* hydrogen peroxide-induced hemolysis. "Based on NHANES III data, more than 95% of the population surveyed would have plasma concentrations greater than 12 μmol/l, thus indicating that the American public is not vitamin E deficient by this criterion" (FNB 2000 p 233). Since a study with subjects who had been on a controlled vitamin E diet for over 6 years found 12 μmol/l to correspond to a dietary vitamin E intake level of 12 mg/day (pp 232-6), the RDA level was set at 15 mg/day to provide an arbitrary margin of safety.

However, this kind of argument does not seem reasonable. 'Hydrogen peroxide-induced hemolysis' is a surrogate marker and there is no evidence cited that the percentage of hemolysis would meaningfully correlate with any clinically relevant outcome (FNB 2000). The correlation between dietary vitamin E intake and plasma α-tocopherol concentration is also very weak or nonexistent in freely living people. One study "reported that plasma α-tocopherol concentrations were not associated with dietary intake, whereas some others report that associations seen were largely due to vitamin E supplement intake … [and] … plasma α-tocopherol concentrations in NHANES III did not correlate with the 24-hour dietary recall data" (FNB 2000 p 210). Thus, there is no evidence that among freely living people vitamin E intake might meaningfully correlate with plasma α-tocopherol level and, moreover, there is no evidence that the percentage of hemolysis might correlate with any clinically relevant outcome.

In any case, the current vitamin E recommendation for both men and women was set at 15 mg/day (FNB 2000 p 237). Consequently, in the USA, 90% of men and 99% of women aged 19-30 years have a vitamin E intake lower than 15 mg/day (FNB 2000 pp 422-3). However, there is no evidence cited that this 90-99% of young adults with an intake lower than the RDA level might suffer any harmful effect on health because of their 'low' intake (FNB 2000). Thus the level of vitamin E recommendation is arbitrary to an extreme degree.

In the previous RDA recommendation, the recommended vitamin E intake was based on the following argument: "The allowance … is therefore based primarily on customary intakes from U.S. food sources. … the subcommittee has established an arbitrary but practical allowance for male adults of 10 mg of alpha-tocopherol equivalents per day. Because women are generally smaller, their allowance is 8 mg/day" (FNB 1989a p 103). At these older RDA levels, only 25% of US men and 50% of women aged 19-30 years would get less than the recommended level (FNB 2000 pp 422-3).

The safety of vitamin E

A number of reviewers have concluded that vitamin E is safe at high doses and most of the suggested harmful effects are not based on valid evidence (Bendich & Machlin 1988, 1993; Marks 1989; Kappus & Diplock 1992; Diplock 1995; Hathcock *et al.* 2005 [see Hemilä 2005e]).

In a review discussing the potentially adverse effects of vitamin E, Diplock (1995) commented that "It is strange to observe that the most frequently cited observations in the literature are those that appear to have the least scientific rigor" concluding that "Regarding controlled, double-blinded studies of vitamin E toxicity in humans, several reports exist that also confirm that vitamin E has very low toxicity and no consistent adverse effects have been reported." Bendich and Machlin (1988, 1993) also concluded that "The majority of side effects was reported in letters to the editor as individual case reports or uncontrolled studies. Most of the side effects have not been observed in the larger, well-controlled studies … vitamin E supplementation at high doses is not associated with any clinically relevant adverse effects."

The most recent US nutritional recommendations consider that for vitamin E, "The Tolerable Upper Intake Level (UL)", which means "the highest level of daily nutrient intake that is likely to pose no risk of adverse health effects in almost all individuals" is 1,000 mg/day (FNB 2000 p 249-59). Furthermore, according to the same monograph, "clinical trials of doses above the UL should not be discouraged" (FNB 2000 p 249).

Evidently, the lack of adverse effects from such high doses does not indicate that such doses are beneficial. The current recommended level of intake is 15 mg/day in the USA as mentioned above; however, even this low level of recommended intake is based on highly dubious argument as mentioned above, and there is no evidence of ill effects even if the intake level is still lower, such as 8-10 mg/day, which were the previous RDA levels of vitamin E (FNB 1989a p 103).

In the ATBC Study, vitamin E supplementation was associated with a higher risk of subarachnoid hemorrhage (P[2-t] = 0.07), but a lower risk of cerebral infarction (P[2-t] = 0.03) (Leppälä *et al.* 2000a). In a subgroup analysis, the increase in the risk of subarachnoid hemorrhage was restricted to ATBC Study participants who had high systolic blood pressure (RR = 2.4; P[2-t] = 0.03; Leppälä *et al.* 2000b), but there was no effect of vitamin E on the risk of subarachnoid hemorrhage in male smokers with low blood pressure. Statistical interaction between vitamin E and systolic blood pressure was, however, non-significant (P[2-t] = 0.17). Furthermore, given the multiple comparison condition (one of several outcomes and one of three blood pressure subgroups) the P-value for increased risk (0.03) is easily explained by chance. Consequently, it is not clear how far this potential harm in male smokers with high blood pressure can be extrapolated to other population groups. In the ATBC Study there was no difference in mortality between the vitamin E and no-vitamin E groups (+2%; 95% CI: -5% to +9%; P[2-t] = 0.6) (ATBC 1994a), indicating no net harm or benefit from vitamin E supplementation (50 mg/day) to middle-aged male smokers.

A recent trial with 652 subjects aged ≥60 years found greater severity of respiratory infections among participants supplemented with vitamin E (200 mg/day). The presence of fever during respiratory episodes was more common (P[2-t] = 0.009) and the total illness duration was longer (P[2-t] = 0.02) among the vitamin E supplemented participants (Graat *et al.* 2002).

In a recent meta-analysis focusing on the potentially harmful effects of vitamin E supplementation, Miller *et al.* (2005) estimated that doses larger than 150 mg/day of vitamin E might increase mortality. However, their assumption of a precise threshold level valid for all people may be a gross oversimplification. It is possible that there is biological heterogeneity between population groups, so that people's characteristics would determine whether vitamin E supplementation causes net benefit or harm (Hemilä 2005f,g). For example, in the ATBC Study cohort, the effect of vitamin E on the risk of pneumonia was significantly modified (P[2-t] = 0.000,7) by the age of smoking initiation, so that vitamin E was beneficial to participants who initiated smoking at later ages (RR = 0.65), but harmful to those who initiated smoking at earlier ages (RR = 1.14) (Paper V). In the ATBC Study, the vitamin E dose was 50 mg/day, which is substantially less than the threshold of 150 mg/day estimated by Miller *et al.* (2005). However, these subgroup differences observed in the ATBC Study cohort suggest that some population groups suffer ill effects at the low dosage of 50 mg/day, but the same dose seems beneficial to others.

Thus, although there is evidence indicating that doses up to 1 g/day of vitamin E do not cause adverse effects to the ordinary population, there is new data suggesting that much lower doses, 50-200 mg/day of vitamin E, may be harmful to particular restricted population groups. Obviously, the harm associated with low doses should not be extrapolated directly outside of these population groups.

Possible heterogeneity in the effects of vitamins C and E

A common feature of the discussions of the physiological effects of vitamins C and E has been the implicit assumption that their effects are similar in all people. If such an assumption was valid, it would allow extrapolation of any study findings widely, given that the trial is well conducted and so large that the results are accurate. The validity of this assumption, that the effects of these vitamins are similar over the population, is thus of fundamental importance.

For example, Pauling (1971a; pp 35-6) assumed that the findings of the Ritzel trial (1961) could be directly extrapolated to the general population without considering that the positive effects of vitamin C found in the trial may be valid, but not appropriate for extrapolation because of the special conditions of a trial with schoolchildren in a skiing camp.

Similar careless extrapolation from the findings of a single small trial (N = 617) was recently carried out by Hamer and Meydani (2004), who calculated that "Given that 34 million elderly people live in nursing homes, this would translate into more than 5 million fewer elderly nursing home residents contracting upper respiratory tract infections in a year," with their estimate of the effect being based on a marginally significant finding of RR = 0.84 (95% CI: 0.69-1.00; Meydani *et al.* 2004), whereas a much larger trial (N = 21,796) found that vitamin E supplementation had no overall effect on common cold incidence (Paper III; Hemilä & Kaprio 2004). Another recent example of this implicit assumption of similar effects is seen in the meta-analysis of the potentially deleterious effect of vitamin E supplementation by Miller *et al.* (2005 [see Hemilä 2005f]), which was based on the assumption that there is a universal threshold level, so that higher intakes of vitamin E would progressively increase the risk of harm in all people equally. Thus, the implicit assumption of similar effects in all people is common to many considerations of the effects of vitamins C and E.

An important finding in the current thesis was that the effects of daily vitamin C and E supplementation on the incidence of the common cold and pneumonia seem to be modified by various factors, so that the effects of these two vitamins vary between different population groups. Consequently, the assumption of similar effects in all people seems not to be valid.

If the effects of daily vitamin C and E supplementation vary substantially between different subpopulations, the heterogeneity of the effect evidently means a need for careful consideration of goals when planning new trials on these vitamins. Assuming heterogeneity, further trials should try to identify and characterize the population groups or living conditions in which these vitamins might be beneficial, rather than re-examining the effects on ordinary Western people for whom the studies already available have not found any meaningful overall benefits from daily supplementation.

CONCLUSIONS

1. A large number of studies with various animal species, and with a large diversity of infectious agents have found that vitamins C and E affect the incidence and severity of infections.

2. The potential effect of vitamin C on the common cold has been a controversial topic for several decades. It has been shown in this thesis that since several major reviews on vitamin C and the common cold present the findings of original trial results erroneously, the conclusions of the reviews are unjustified. Two of these flawed reviews were used as references in major textbooks on infectious diseases and in the US RDA nutritional recommendations as support for the claim that vitamin C has no effect on the common cold.

3. The most influential vitamin C common cold trial, carried out by Karlowski *et al.* (1975), was re-analyzed in this thesis. It was shown that the original conclusions of the authors were inconsistent with the published data. The Karlowski trial found, consistently with a large number of other placebo-controlled trials, that regular vitamin C supplementation shortens the duration of common cold episodes. However, if the main effect of vitamin C is the alleviation of cold symptoms, it would appear more reasonable to administer it therapeutically, starting immediately after the first symptoms, rather than regularly. Nevertheless, only a few therapeutic trials have been carried out, and none were conducted with children, for whom the regular vitamin C supplementation trials found substantially greater benefit. Consequently, further therapeutic trials are needed to examine whether vitamin C supplementation starting without delay after the first common cold symptoms might substantially reduce the duration and symptoms of colds.

4. Meta-analysis of the six largest vitamin C-common cold trials showed that the great majority of people in Western countries cannot lower common cold incidence by vitamin C supplementation. Still, vitamin C may affect common cold risk in certain restricted groups of people, such as those with heavy acute physical stress and/or cold stress, and young males with low dietary vitamin C intakes, and these findings warrant further research.

5. In an analysis of the ATBC Study cohort of middle-aged male smokers, the intake level of vitamin C in diet was not associated with the incidence of the common cold or tuberculosis.

6. Vitamin E supplementation had no overall effect on the risk of the common cold, pneumonia and tuberculosis in the ATBC Study. However, in the cases of the common cold and pneumonia there was strong evidence indicating that the effect of vitamin E supplementation differed between subgroups, so that certain restricted groups of people seemed to derive benefit from supplementation. Vitamin E reduced common cold incidence in a small group of elderly city-dwellers who smoked 5-14 cigarettes per day, and pneumonia incidence in participants who initiated cigarette smoking late in life. Although both of these effects were statistically highly significant, they were rather small. The assessment of the practical importance of these findings requires further study.

7. The trials analyzed in this thesis suggest that there is no evidence that ordinary Western people would derive benefit from daily supplementation of vitamin C or vitamin E as regards the incidence of respiratory infections. Nevertheless, in both cases, there was substantial heterogeneity in the effects between population groups. It is possible that restricted population groups might experience benefit from daily supplements and further study is warranted to investigate this possibility.

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The primary inspiration of this thesis, focusing initially on vitamin C and infections, arose from the texts of Linus Pauling. Its purpose was not to prove that Pauling was in some simple way right or wrong, although the findings do allow some conclusions about the degree to which Pauling drew correct or incorrect conclusions from the small amount of data that he had available in the early 1970s. Pauling died in 1994 (see pp 11-3).

A great part of this thesis consists of statistical analyses of the data set of the large-scale ATBC Study, which was made available to the author by Professor Olli P. Heinonen, its principal investigator and the head of the Department of Public Health. I am also grateful to Professor Heinonen for providing working space and research facilities at the department and for his support for my work. Unfortunately, Professor Heinonen died in 2001 (Huttunen & Virtamo 2001; Jaakkola & Paunio 2002).

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If no source for a fulltext version of the publication was identified, or if a fulltext was identified but not freely available, the PubMed identification number is provided if an abstract is available for the entry (http://www.ncbi.nlm.nih.gov/entrez/query.fcgi). The actual www-address of the PubMed abstract is so long that they are mostly identified with the ID number after "pubmed". For example, pubmed 163386 refers to the PubMed abstract: http://www.ncbi.nlm.nih.gov/entrez/query.fcgi?cmd=Retrieve&db=pubmed&dopt=Abstract&list_uids=163386&query_hl=20 which corresponds to the Karlowski *et al.* (1975) paper. Additional digital documents closely related to the formal reference are listed after the reference in square brackets, when relevant documents were identified. Lack of both full-text link and PubMed identification number mostly indicates that there is no abstract in PubMed available for the reference. For the references to the studies of birds and fishes in Appendices 2 to 5 no links are provided since they are most marginal in this thesis.

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Appendix 1. A brief history of vitamin C and its deficiency, scurvy

We were all hearty seamen, no cold did we fear
And we have from all sickness entirely kept clear
Thanks be to the Captain he has proved so good
Amongst all the Islands to give us fresh food.

Song of James Cook's Sailors
(Kodicek & Young 1969)

Scurvy, vitamin C deficiency, was a serious occupational disease of sailors in the Age of Sail. It has been estimated that over two million sailors perished from scurvy (Carpenter 1986; Harvie 2002; Bown 2003).

Vasco da Gama began his expedition to India in 1497 and when his ships arrived on southeast coast of Africa, most of the crew were afflicted with scurvy. Da Gama recorded that "Many of our men fell ill here, their feet and hands swelling, and their gums growing over their teeth so that they could not eat." As they sailed farther up the east coast of Africa, they met local traders, who traded them fresh oranges, and within 6 days of eating them, the crew recovered. Although da Gama recorded that "It pleased God in his mercy that ... all our sick recovered their health for the air of this place is very good," the crew were convinced that the oranges that they had eaten were powerful curatives, because they particularly asked for them the next time scurvy appeared (Carpenter 1986 pp 1-3). It is thought that scurvy was the cause of the deaths of 100 of Vasco da Gamas 160 men (Harvie 2002 pp 12).

The English sea captain Sir Richard Hawkins stated in 1622 that "In 20 years, since that I have used the sea, I dare take upon me to give accompt of 10,000 men consumed with scurvy" emphasizing the magnitude of the problem. Hawkins "wished that some learned man would write of it, for it is the plague of the sea, and the spoil of mariners." In the 1780s, twenty-one British warships were stationed in the West Indies, which had become the major theatre for naval battles involving France, Spain, and England during the War of American Independence. Sir Gilbert Blane, who was the personal physician to the admiral, counted that, during his first year in the West Indies, out of 12,019 mariners only 60 died from enemy action, whereas 1,518 perished from disease, with cases of scurvy outnumbering all other illnesses combined (Bown 2003 pp 199-225; Carpenter 1986 pp 91-7), corresponding to a mortality rate for scurvy of about 1 per 10 person-years.

When Commodore George Anson set out with 8 ships and 1,854 sailors to the South Seas in 1740, he returned in 1744 with only 1 ship and 188 men; the great majority of the rest died of scurvy (Gordon 1984; Carpenter 1986 pp 46-51; Watt 1998). In contrast, during James Cook's second voyage towards the South Pole and round the World, from 1772 to 1775, he lost no men to scurvy: "… the Resolution performed a voyage of three years and eighteen days, through all climates … with the loss of one man only by disease, and who died of a complicated and lingering illness, without any mixture of scurvy. Two others were unfortunately drowned, and one killed by a fall; so that of the whole number with which I set out from England I lost only four" (Cook 1776). After this voyage, Cook was honored with the Copley Medal of the Royal Society, Cook's most prestigious award. This was not for his navigational discoveries, but for his success in maintaining a long sea voyage without a death from scurvy among the men in his crew (Chick 1953; Kodicek & Young 1969). Still, Cook could not receive the medal in person because he had already left on yet another voyage, in which he was to be killed by natives on a beach in Hawaii.

Paradoxically, Cook's success in preventing scurvy, far from hastening the cure of the disease, instead delayed the identification of the actual cure (Lloyd 1961). Cook was primarily a navigator and explorer and not a dietician, and he did not examine specifically which of the numerous anti-scurvy measures was the actual reason for its absence. Cook's experience could thus not be used to exclude useless treatments, and false explanations for the cause and treatment of scurvy prevailed for a long period in spite of his success in keeping his own sailors free of it (Table 1).

Strong observational evidence had shown that fruit had been useful for treating and preventing scurvy since Vasco da Gama's voyage, and Hawkins (1622) commented that "That which I have seene most fruitfull for this sicknesse, is sower oranges and lemmons." The benefit of fruit was, however, forgotten for a long period and was rediscovered only in the 1700s.

James Lind (1753) carried out a systematic review of all the earlier literature on scurvy and wrote the classic monograph *A Treatise of the Scurvy*. At the end of his treatise, Lind wrote a brief summary of each of the earlier publications on scurvy ("Bibliotheca scorbutica"; Lind 1753 pp 249-354). In the literature before Lind, the clinical definition of scurvy had become highly ambiguous and, for example, one of the earlier authors had stated "As this case cannot properly be referred to any other disease, it may justly be deemed scorbutic" (p 36). Because of the imprecise definitions of scurvy, and in many cases using the name for unrelated diseases as we define it nowadays, Lind discarded most of the earlier texts by eminent authors. Working for years as a surgeon on Navy ships, Lind had substantial personal experience with scorbutic patients, and his own clinical definition was based on "putrid gums, swelled legs, and spots, accom-

panying each other, and in their progress usually attended with rigid tendons in the ham, are observed in no other distemper" (p 53). In contrast to most earlier authors on scurvy, Lind's line of exploring the nature of the disease was empirical: "I shall propose nothing dictated merely from theory; but shall confirm all by experience and facts, the surest and most unerring guides" (p 144).

In 1747, Lind carried out the first well described controlled trial in medicine, on HMS Salisbury (1753 pp 145-8). In this trial, Lind kept "12 patients in the scurvy … their cases were as similar as I could have them," in the same quarters; and he saw to it that they all had the same diet. Groups of 2 men were then allocated to 6 different daily treatments for a period of 14 days. One group was administered 2 oranges and 1 lemon per day for 6 days only, when the supply was exhausted. Other groups were administered vinegar, sea-water, and other supposed anti-scurvy remedies. From this trial, Lind concluded that "The most sudden and visible good effects were perceived from the use of the oranges and lemons; one of those who had taken them, being at the end of six days fit for duty" (p 146).

Lind's trial was a milestone in medical research methodology (Dudley 1953; Thomas 1969, 1997; Hughes 1975; McBride 1991; Dunn 1997; Friedman *et al.* 1998 p 1; Manchester 1998a; Sutton 2003; Currie 2003; Milne & Chalmers 2004). Hampton (2002) stated that "The elegant trial of the use of oranges and lemons for the treatment of scurvy was hardly bettered until the trial of streptomycin for tuberculosis designed by Austin Bradford Hill [in 1948]."

Thus, Lind's trial provided further empirical evidence that citrus fruits could cure scurvy. However, most current authors refer to this trial out of context, claiming that it proved the essential role of fruit in preventing scurvy, and establishing a way to understand deficiency diseases (Carpenter 1986; Bartholomew 2002a,b). The trial is described in just 3 pages of a book of some 350 pages. Lind himself did not put as much weight on his trial as the current commentators retrospectively do. In fact, he drew his own conclusions, which were completely false, about the etiology of scurvy from observational data.

Lind was convinced that lack of fruit and vegetables was not the primary etiological cause of scurvy, and argued at length for this conclusion. "Before determining what are the true causes of scurvy being so often epidemic at sea, it may not be amiss to remark what they are not, although commonly accused" (1753 p 71). "Others have supposed such to be the constitution of the human body, that health and life cannot be preserved long, without the use of green herbage, vegetables, and fruits; and that a long abstinence from these, is alone the cause of scurvy. But if this were truly the case, we must have had the scurvy very accurately described by the ancients; whose chief study seems to have been the art of war; and whose manner of besieging towns was generally by a blockage, till they had forced a surrender by famine. Now, as they held out many months, sometimes years, without a supply of vegetables; we should, no doubt, have heard of many dying of the scurvy" (pp 73-4). "There are persons everywhere, who, from choice, eat few or no green vegetables; and some countries are deprived of the use of them for five or six months of the year; as is the case of many parts in the highlands of Scotland, Newfoundland, etc., where, however, the scurvy is not a usual malady" (p 74). Lind also describes his own experience while on board HMS Salisbury where he did not observe a correlation between the consumption of greens and the occurrence of scurvy, concluding that "although it is a certain and experienced truth, that the use of greens and vegetables is effectual in preventing the disease, and extremely beneficial in the cure … yet there are unquestionably to be found at sea, other strong sources of [scurvy]; which we shall hereafter distinguish by the name of the *predisposing causes* to it." (p 76; Lind's italics).

Thereafter Lind extensively describes his own notions of the etiology of scurvy concluding that "I am certain it will be allowed, by all who have had an opportunity of making observations on this disease at sea, or will attentively consider the situation of seamen there, that the *principal and main predisposing cause* to it, is a manifest and obvious quality of the air, *viz.* its *moisture*" (pp 84-5; Lind's italics; Martini 2004). Lind also argued "I will venture to affirm, that, without any one exception, *scurvy is unknown in dry places*" (p 98), "The lazy and indolent, and those of a sedentary life … are most subject to scurvy; while hard labourers … keep entirely free. … Those that are of a cheerful and contented disposition, are less liable to it, than others of a discontented and melancholy mind" (p 105). Finally, when discussing "the cure of the disease, and its symptoms" in the latter part of his treatise, Lind stated "All mankind have not the benefit of a pure wholesome air, warm dry lodgings, with proper conveniences to guard against the inclemency of different weather and seasons… Experience shews, that the cure of the adventitious scurvy is very simple, *viz*. a pure dry air, with the use of green herbage or wholesome vegetables, almost of any sort; which for the most part prove effectual… Thus a free and pure country-air, with such moderate exercise as at the same time conduces to the agreeable amusement of the mind, is requisite" (pp 178-9). "And by all faithful and accurate observations made on this disease, moisture is experienced to be the principal and main predisposing cause to it" (p 206). Although proposing 'moisture' explicitly as the principal cause of scurvy, Lind did consider that diet may have importance as an 'occasional cause' of scurvy, *i.e.*, secondary to moisture.

The correct explanation of the etiology of scurvy was proposed in 1734, *i.e.*, before Lind's trial and monograph, by John Bachstrom, a physician in Holland who claimed that the cause of scurvy was the absence of fresh vegetable food from the diet for a considerable time (Carpenter 1986 pp 44-5; Lind 1753 pp 314-7). Carpenter comments that "Bachstrom's treatise seems to the modern reader a straightforward argument and one that deserved at least a serious consideration. But to the contemporary main-line physician it was not impressive because it dealt with a single disease in isolation and did nothing toward establishing a view of the nature of 'disease' in general – an ideal which the medical profession had as its goals, by analogy with the universal laws being developed by the physicists at that time. In

other words, Bachstrom was ‘a mere empirick’." Lind explicitly disagreed with Bachstrom’s proposal that "a long abstinence from fruits and vegetables is alone the cause of scurvy" (1753 p 73).

One hundred years later, John Elliotson (1831), professor of medicine in London, proposed specifically that "scurvy is disease purely *chemical*. The body, structure, and functions are not in the least in fault; in one sense, each part of the system is ready to perform all its functions, but one of the external things necessary for its doing so is taken away. In the case of *suffocation*, the body is not at all in fault, but it suffers from a want of fresh air; so in scurvy, the functions are all right, but the food which the body by nature requires is withheld from it… The case of scurvy is exactly like the case of impending suffocation – the body would be in good health if not deprived of its proper external supply." A few years later, in 1842, George Budd also suggested that scurvy was a deficiency disease: "From this we must infer, that the ill effects of a diet consisting of sugar, starch, oil, fat, do not result from want of protein only but from want of other principles also requisite for the support of the body. Perhaps the deficiency of each principle shows itself in a particular way" (Hughes 1973; Carter 1977; Carpenter 1986 pp 98-9, 249-51; Cook 2004). These were visionary considerations far ahead of their time.

The concept of necessary minor constituents started to be accepted in main-line medicine over half a century after Elliotson and Budd published their arguments, in the early twentieth century. In 1906, Sir Frederick Hopkins wrote that "The animal body is adjusted to live either upon plant tissues or the tissues of other animals, and these contain countless substances other than the proteins, carbohydrates, and fats. Physiological evolution, I believe, has made some of these well-nigh as essential as are the basal constituents of diet… The field is almost unexplored; only is it certain that there are many minor factors in all diets of which the body takes account. In diseases such as rickets, and particularly in scurvy, we have had for long years knowledge of a dietetic factor; but though we know how to benefit these conditions empirically, the real errors in the diet are to this day quite obscure. They are, however, certainly of the kind which comprises these minimal qualitative factors that I am considering." Hopkins was awarded the 1929 Nobel Prize in Medicine or Physiology for his discovery of the growth-stimulating vitamins, which he called ‘accessory food factors’ and in 1931 he became the president of the Royal Society (Hopkins 1906, 1929; Harris 1947a,b; Needham 1962a,b; Kamminga 1997; Carpenter 2003b, 2004; NF 2005a).

In 1912, Casimir Funk assembled all the various strands of work supporting the deficiency theory of disease, concluding that "The diseases mentioned above [in the title of the paper] present certain general characters which justify their inclusion in one group, called deficiency diseases. They were considered for years either as intoxications by food or as infectious diseases, and twenty years of experimental work were necessary to show that diseases occur which are caused by a deficiency of some essential substances in the food. Although this view is not yet generally accepted, there is now sufficient evidence to convince everybody of its truth, if the trouble be taken to follow step by step the development of our knowledge on this subject. This article is written with the intention of giving a summary of the modern investigations … there is perhaps no other subject in medicine where so many contradictory and inexact statements were made, which instead of advancing the research retarded it by leading investigators in a wrong direction." Subsequent research confirmed most of Funk’s opinions and vindicated most of the arguments he provided in their support. Since his paper, additional work on the deficiency diseases can be thought of as elaboration of an existing theory (Carter 1977); however, see footnote to Table 1. Funk (1912) also coined the term ‘vitamine’ (‘vital’ substances that were chemically ‘amines’), but the letter ‘e’ was later dropped out when it was found that not all these vital substances were ‘amines’, so that the term for later use became ‘vitamin’ (Rosenfeld 1997; Carpenter 2004).

Nevertheless, as to the actual cure of scurvy, it took several decades after Lind’s trial in 1747 before citrus fruits were properly utilized in preventing it. Sir John Pringle believed that scurvy was caused by putrefaction, his own theory being that ‘wort of malt’ was its cure and, because Sir John happened to be the president of the Royal Society, ‘wort of malt’ was a more respectable remedy for scurvy than lemons long after Lind’s controlled trial (Table 1). In 1778, Pringle resigned his position at the Royal Society, and in 1795, Sir Gilbert Blane, a follower of Lind, was able to persuade the British Admiralty to issue a daily ration of lemon juice to all sailors, which virtually eliminated scurvy aboard Navy ships. It seems this defeat of vitamin C deficiency was a major reason why the British Navy was able to protect the country against Napoleon’s invasion and, in particular, why Nelson was able to beat the French and Spanish fleets at the Battle of Trafalgar in 1805 when their mariners were suffering from scurvy. Never before, and never since, has vitamin C as a chemical substance had such a crucial role in global politics (Bown 2003 pp 227-55).

Although scurvy caused its greatest evils on the long sea voyages, it has also been a problem on land, sometimes called the ‘land scurvy’ (Lind 1753 pp 52-63; Hess 1920 pp 1-22; Lorenz 1953, 1957; Wilson 1975; Carpenter 1986 pp 98-132; Hughes 1990; Bollet 1992; Harvie 2002 pp 225-34). French explorer Jacques Cartier had winter on the Saint Lawrence River in 1535-6 since his ships were frozen in the ice, and most of his crew got scurvy. Local indians taught them to prepare juice of white cedar and "after drinking it two or three times, they recovered health and strength and were cured of all the diseases they had ever had" (Carpenter 1986 pp 7-12; Martini 2002). Juice made of pine needles was used also during the siege of Leningrad in the Second World War to prevent scurvy (Shishkin 1943). Land scurvy has been a problem in various circumstances. In the American Civil War of 1861-1865, 7,000 Union army deaths were directly attributed to scurvy, and another 45,000 deaths from dysentery and diarrhea followed from severe scurvy. In the California Gold Rush, some 10,000 men died from scurvy, half of them succumbing in the first two winters alone. In the

Irish Famine, caused by the failure of the potato crops in the late 1840s, approximately one million people died of scurvy and other diseases. Moreover, in the late 1800s, scurvy became a pediatric problem when children were administered heated milk and artificial foods which did not contain vitamin C; this form of scurvy was called 'the Barlow's disease' (Barlow 1894; Ylppö 1929; Aspin 1993; Rajakumar 2001; Carpenter 1986 pp 158-72, 2003a). In Scandinavia, 'land scurvy' was explicitly described in the 1500s, being largely associated with wars (Olaus Magnus Gothus 1555). Nowadays, 'land scurvy' is a problem in the refugee camps, where its prevalence has been up to 44% at the upper extreme (in Somalia in 1985; WHO 1999), and in Afghanistan (Ahmad 2002). In the developed world, severe vitamin C deficiency is currently rare; nevertheless, because its clinical features are no longer familiar, even frank scurvy may remain undiagnosed (Sherlock & Rothschild 1967; Reuler *et al.* 1985; Scully *et al.* 1986; Fain *et al.* 1998; Hirschmann & Raugi 1999; Weinstein *et al.* 2001; Akikusa *et al.* 2003; Bingham *et al.* 2003; DeLuna 2003; Pimentel 2003).

Although there was strong observational evidence even before Lind's trial that citrus fruits were beneficial for scurvy, there was no biological rationalization for fruit, and a large number of false theories about scurvy prevailed long after Lind's controlled trial (Table 1). Since vitamin C is synthesized by all mammals with the few exceptions of primates, the guinea pig and fruit-eating bats, a reasonable animal model for scurvy was not easy to find. Holst and Frölich (1907; Johnson 1954) were able to produce scurvy in guinea pigs by administering them a diet deficient in fruit, whereby a suitable animal model for vitamin C deficiency was identified. Since then, the guinea pig has been the most important animal model for studies examining the physiological effects of vitamin C. Carpenter (1986 p 173, 2003a) considered that the Holst and Frölich paper has been the most important in the whole history of vitamin C and scurvy; however, see footnote to Table 1.

There were a few systematic efforts to isolate vitamin C, but it was first isolated by chance by Albert Szent-Györgyi (1933, 1963, 1971; Bendiner 1982; Hughes 1983; Edsall 1986; Straub 1987; Grazer 1988; NLM 2005b), who had initially considered that "Vitamins were, to my mind, theoretically uninteresting. 'Vitamin' means that one has to eat it. What one has to eat is the first concern of the chef, not the scientist." Nevertheless, in 1928, while working in Frederick Hopkin's laboratory, Szent-Györgyi isolated a sugar-like molecule from adrenals and citrus fruits. Since he did not know much about the substance, he proposed the name 'ignose' ('ignorant' plus '-ose' which is the suffix for sugar), but the editor of the Biochemical Journal did not like jokes and rejected the name. Thereafter Szent-Györgyi proposed 'Godnose' ('God knows' the purpose of the substance), but the fate of this second proposal was the same, and the substance was finally named 'hexuronic acid' since it has 6 carbon atoms and is acidic. In 1932, when Szent-Györgyi showed that the substance cured scurvy in guinea pigs, the substance was renamed 'ascorbic acid' ('scorbutus' is scurvy in Latin). Szent-Györgyi spent the next several years "preaching vitamin C" (as he put it) all over Europe, suggesting that it might be valuable as a preventive or cure for the common cold and other illnesses. He attempted to interest some of the British biochemists in running some clinical trials, but they considered the idea crankish and refused to consider it. Vitamin C proved disappointing as a miracle cure, however, and Szent-Györgyi eventually got back to his basic research in other areas (NLM 2005b). The Nobel Prize in Medicine or Physiology was awarded to Szent-Györgyi in 1937 for identifying vitamin C and for studies on energy metabolism (Szent-Györgyi 1937; Simola 1937; Krebs 1970; Manchester 1998b; NF 2005b). In parallel with Szent-Györgyi's work, Charles King identified vitamin C at nearly the same time and this led to disagreements over who was first (King 1953, 1968, 1979; Szent-Györgyi 1938; Jukes 1988; Stare & Stare 1988; NLM 2005c).

The chemical structure of vitamin C was solved by Sir Norman Haworth, who was awarded the Nobel Prize in Chemistry in 1937 (NF 2005c). In parallel with Haworth's vitamin C synthesis, Tadeus Reichstein developed a more practical method of synthesizing vitamin C, which became commercially useful, and patents allowed Reichstein to amass considerable financial rewards. Although many people were surprised that Reichstein did not receive the Nobel Prize for the synthesis of vitamin C, he received the Nobel Prize in Medicine or Physiology in 1951 for isolating and identifying cortisone (Rothschild 1999; NF 2005d). The Reichstein synthesis of vitamin C has been used to produce it for decades, but currently there is a change to synthetic processes involving genetically modified microbes. The current world production of vitamin C is about 100,000,000 kg per year (Hancock & Viola 2002; Baier 2004).

Appendix 2. The effect of vitamin C on animal infections: Administration of pure vitamin C to the 'vitamin C group'

Study Animal Infection (Source of data)	Treatment		P [1]
Jungeblut-1937a Rhesus monkey Intracerebral inoculation of poliovirus Tables II and III Natural vitamin C	Vit C	Cont	
5 mg/d			
Animal no.	33	38	
Paralysis	17	36	.000,02
10-50 mg/d			
Animal no.	19	38	
Paralysis	16	36	.12
100-700 mg/d			
Animal no.	10	38	
Paralysis	10	36	.7
Jungeblut-1937b Rhesus monkey Infection with poliovirus	Vit C	Cont	
Intracerebral inoculation (Table II) Synthetic vit C			
5-100 mg/d			
Animal no.	101	98	
Paralysis	90	93	.07
Natural vit C (2.5% impurities)			
5-100 mg/d			
Animal no.	181	98	
Paralysis	123	93	<.000,001
Intranasal inoculation (Table IV) Natural vit C (2.5% impurities)			
5-25 mg/d			
Animal no.	20	10	
Paralysis	19	10	.5
50-100 mg/d			
Animal no.	10	5	
Paralysis	1	5	.001
Sabin-1939 Rhesus monkey Intranasal inoculation of poliovirus Table I Natural vit C	Vit C	Cont	
Series I Vit C adequate diet			
5 mg/d			
Animal no.	6	10	
Paralysis	5	8	.5
100 mg/d			
Animal no.	10	10	
Paralysis	9	8	.7
Series II Vit C inadequate diet			
Animal no.	7	5	
Paralysis	7	4	.8
Jungeblut-1939 Rhesus monkey Intranasal inoculation of poliovirus Natural or synthetic vit C *10-500 mg/d*	Vit C	Cont	
Infected nasally by droplets (Table IV)			
Animal no.	56	20	
Paralysis	23	15	.006
Infected nasally by pressure (Table IV)			
Animal no.	50	20	
Paralysis	49	20	.4

[1] The 1-tailed P-value. In the case of dichotomous data, the mid-P-value was calculated by the current author (see Table 7). The survival data, and the recovery time in Edwards (1968) and Naresh (2002), and the number of tuberculin doses in Stein & Klein (1936) were analyzed using the log-rank test. The score data, and the bacterial count data of Wang et al. (2000), was analyzed using the Wilcoxon test with normal approximation. Continuous data was analyzed using the t-test. In the case of survival data and score data, the number in parenthesis (n) indicates the number of animals with the same outcome value; '+' indicates censoring.

Murphy-1974	Vit C	Cont	
Marmoset			
Intranasal inoculation			
of parainfluenza virus			
Animal no.	14	7	
Signs of infection			
within 3 d	7	7	.015
Mortality	5	4	.2
Mean duration of sickness			
in survivors (d)	9	20	

Steinbach-1936	Vit C	Cont	
Guinea pig			
Infected with *M. bovis*			
Tuberculin injection			
Animal no.	3	3	
Mortality	0	3	.025
Tuberculin injection			
Animal no.	8	8	
No. of sequential	9+;	5+;9;	.008
tuberculin doses	15+(4);	15(4);	
before death	17;	17+;	
(animals in pairs)	18+(2)	18	

Birkhaug-1938, 1939,a,b	Vit C	Cont	
Guinea pig			
Infected with *M. bovis*			
subcutaneous inoculation			
to the left groin			
Animal no.	12	12	
Weight change in 49 d			
Mean (g)	+12	−40	.005
SD (g)	35	52	
Size of tuberculin [2]			
reaction at 51 d			
Mean (mm^3)	575	4168	<.000,001
SD	228	1429	
t(log-size)=12.9			
Central necrosis			
in tuberculin			
reaction ≥140 mm^3	3	12	.000,1
Overall score of			
tuberculous	1(7);	2(7);	.000,3
involvement	2(5)	3(5)	
(scale 0 to 4)			

Size of lymph glands:			
Left superficial inguinal [2]			
Mean (mm^3)	281	1885	.000,02
SD	252	1759	
t(log-size)=5.07			
Left tracheobronchial			
Mean (mm^3)	319	1212	.000,005
SD	183	514	
Periportal			
Mean (mm^3)	554	1548	<.000,001
SD	236	436	
Total size of lymph glands			
Mean (% of body wgt)	1.21	2.37	<.000,001
SD	0.139	0.49	
Size of spleen			
Mean (mm^3)	1421	2764	.001
SD	524	1220	
Extensive caseonecrotic			
lesions (no. of animals):			
Lymph glands			
Left deep inguinal	4	11	.003
Tracheobronchial	3	7	.063
Liver	3	9	.011
Spleen	5	9	.063
Tuberculous pneumonia	2	5	.11

Kleimenhagen-1941 [3]	Vit C	Cont	
Guinea pig			
Infected with			
M. tuberculosis			
Young animals			
Animal no.	6	8	
Survival time (d)	68;75;	68;69;	.018
	182;	70;77;	
	190;	81;92;	
	197;	101;	
	259	107	
Old animals			
Animal no.	9	9	
Survival time (d)	50;69;	43;46;	.020
	103;	54;58;	
	138;	81;114;	
	156+(5)	122;	
		136;	
		156+	

[2] Birkhaug (1939a,b) listed the data for the size of tuberculin reaction and the size of the left superficial inguinal lymph nodes per animal, and these were log-transformed before the t-test by the current author. Birkhaug (1939b) listed mean and SD for the total size of lymphoid glands and the size of the spleen; however, since he calculated the SD using 'n' as the denominator, the SD was corrected here to 'sample SD' using 'n-1' as the denominator.

[3] Kleimenhagen (1941): survival data for young guinea pigs is from his table 6, and for old guinea pigs from his table 13. The current vitamin C group is his group III (5 mg/day) and the control group is his group I (0.5 mg/day).

Steinbach-1941 [4]	Vit C	Cont	
Guinea pig			
Infected with *M. bovis*			
Animal no.	13	6	
Score of tuberculous involvement (scale 0 to 4)	1(10); 2;3; 4	1; 2(3); 4(2)	.015

Gangadharam-1953 [5]	Vit C	Cont	
Guinea pig			
Infected with *M. tuberculosis*			
Animal no.	4	4	
Mean survival (d)	68	64	
Mean weight change (g)	−21	−70	
Pathological lesions: (scale 0 to 4)			
Lungs, Mean	1.0	1.5	
Liver, Mean	2.0	2.5	
Spleen, Mean	1.0	1.5	

Boyden-1956 [6]	Vit C	Cont	
Guinea pig			
Infected with *M. tuberculosis*			
Animal no.	10	10	
Survival time (d)	17;21; 43;44; 45;55; 68;78; 100; 120+	13;21; 22(2); 24;26; 27;29; 30;35	.000,5

McCullough-1938	Vit C	Cont	
Guinea pig			
Subcutaneous inj. of *Fusobacterium necrophorum*			
Animal no.	50	50	
Lesions at the site of injection	0	31	<.000,001
Abscesses containing *F. necrophorum*	0	16	.000,002
Animal no.	10	10	
Abscesses containing *F. necrophorum* after 2 weeks	0	8	.000,2

Karel-1941	Vit C	Cont	
Guinea pig			
Infection with group C streptococcus			
Animal no.	30	30	
Mortality in 2 d	18	16	.7
Sulfanilamide groups			
Animal no.	30	30	
Mortality in 14 d	15	12	.8

Jones-1943	Vit C	Cont	
Guinea pig			
Wound infection with β-hemolytic *Streptococcus*			
Animal no.	6	14	
Mortality	1	10	.020

Peck-1991a [7]	Vit C	Cont	
Guinea pig			
Infected with *E. coli* and *S. aureus*			
Animal no.	13	15	
Mortality	7	11	.2

Nelson-1992	Vit C	Cont	
Guinea pig			
Burned animals, *S. aureus* inj.			
Animal no.	9	6	
Bacteria in abscess (no.)			
Mean	270	380	.2
SD	183	249	

Sjunnesson-2001	Vit C	Cont	
Guinea pig			
Infected with *Helicobacter pylori*			
Animal no.	12	14	
H. pylori isolated after 5 weeks	5	6	.5
Gastritis score (scale 0 to 3)			
Mean	0.66	0.93	.3
SD	0.91	1.02	

[4] Steinbach & Klein (1941): data is from their table 3. The current vitamin C group is their group III (5 and 10 mg/day), and the control group is their group IV.

[5] Gangadharam & Sirsi (1953): the current vitamin C group is their group D, and the control group is their group C.

[6] Boyden & Andersen (1956): the survival times were measured by the current author from their fig. 1. The current vitamin C group is their group 4 (hay-corn-beet + 140 mg/week vitamin C), and the control group is their group 1 (hay-corn-beet).

[7] Peck & Alexander (1991a): data is from their table III. The current vitamin C group is the combination of their two low vitamin E groups (1× and 3×RDA) administered high vitamin C (25×RDA) and the current control group is the combination of their two lower vitamin E groups administered low vitamin C (1×RDA).

Rogers-1983	Vit C	Cont	
Guinea pig			
Infected with			
Candida albicans			
Fig. 1 (1×10exp[+5]) [8]			
Vit C 4 weeks			
Animal no.	5	5	
Log (no. of *Candida* per kidney pair)			
Mean	1.78	2.41	.036
SD	0.60	0.39	
Vit C 6 weeks			
Animal no.	5	5	
Log (no. of *Candida* per kidney pair)			
Mean	1.87	2.61	.010
SD	0.43	0.41	
Vit C 8 weeks			
Animal no.	5	5	
Log (no. of *Candida* per kidney pair)			
Mean	1.87	2.80	.004
SD	0.43	0.46	
Fig. 2 (2×10exp[+6]) [9]			
Animal no.	6	6	
Log (no. of *Candida* per kidney pair)			
Mean	3.63	4.70	.010
SD	0.65	0.69	

Perla-1937	Vit C	Cont	
Guinea pig			
Infected with			
Trypanosoma brucei			
–protozoa (sleeping sickness)			
1. Experiment			
vit C for 6 weeks before infection			
Animal no.	12	12	
Mortality	2	12	.000,02
2. Experiment			
vit C for 11 weeks before infection			
Animal no.	7	7	
Mortality	7	5	.9
3. Experiment			
vit C for 6 weeks before infection			
Animal no.	10	10	
Mortality in 64 d	0	7	.000,8

Sadun-1951	Vit C	Cont	
Guinea pig			
Infected with			
Entamoeba histolytica			
–protozoa (amebiasis)			
Animal no.	14	10	
Size of spleen			
Mean (% of body wgt) (t = 5.314) [10]	0.15	0.37	.000,02
Animal no.	48	43	
Lesions in cecal pouch caused by infection	32	37	.017
Mortality	13	37	<.000,001

Banic-1975	Vit C	Cont	
Guinea pig			
Infected with rabies virus			
Animal no.	48	50	
Mortality	17	35	.000,4

Greenwald-1935 [11]	Vit C	Cont	
Guinea pig			
Diphtheria toxin inj.			
Animal no.	8	8	
Survival time (d)	3;5;6; 9;9+(2); 20;22	3; 4(6);5	.002

Jungeblut-1935	Vit C	Cont	
Guinea pig			
Diphtheria toxin inj.			
Series II (Table III) [12]			
Animal no.	8	22	
Mortality	4	22	.002
Series III (Table IV) [13]			
Animal no.	10	13	
Local reaction to toxin (scale 0 to 5)	0;2(5); 4;5(3)	4(4); 5(9)	.006

Swanson-1936	Vit C	Cont	
Guinea pig			
Diphtheria toxin inj.			
Animal no.	5	5	
Broken incisors	0	5	.002

Sigal-1937	Vit C	Cont	
Guinea pig			
Diphtheria toxin inj.			
Table 3			
Animal no.	13	14	
Weight change in 18 d [14]			
Mean (g)	+31	-68	.003
SD (g)	86	75	
Mortality in 23 d	1	7	.012

Zilva-1937 [15]	Vit C	Cont	
Guinea pig			
Diphtheria toxin inj.			
Animal no.	12	3	
Survival time (d)	2;3.5; 4(2); 4.5(2); 5.5(2); 9+(3); 10+	2; 3.5; 4.5	.031

King-1940 [16]	Vit C	Cont	
Guinea pig			
Diphtheria toxin inj.			
Animal no.	12	12	
Injury to the odontoblasts and dentin	0	12	<.000,001

Fuller-1971 [17]	Vit C	Cont	
Guinea pig			
Endotoxin inj.			
7-9 week old animals			
Animal no.	10	12	
Shock	3	12	.000,4
Survival time (h)	6; 48+(9)	1(11); 6	.000,002
14-16 week old animals			
Animal no.	10	14	
Shock	3	14	.000,2
Mortality in 48 h	3	10	.031

Kuenzig-1980 [18]	Vit C	Cont	
Guinea pig			
Endotoxin inj.			
Animal no.	10	14	
Signs of physical stress, such as ocular exudate and piloerection	1	7	.028

[8] Rogers et al. (1983): data for 1×10exp(+5) inoculum is from their fig. 1. Vitamin C, 20 mg/day, was administered for 4, 6 or 8 weeks before infection in separate groups, and the number of Candida in the kidneys was determined 3 days after infection. The mean and SD for the Log (no. of Candida) were measured by the current author from their fig. 1. The number of animals was 5-8 per group, but the exact number is not reported. The current calculation is based on the conservative assumption that the groups consisted of 5 animals.

[9] Rogers et al. (1983): data for 2×10exp(+6) inoculum is from their fig. 2. Vitamin C, 100 mg/day, was administered before the inoculation, and the number of Candida was determined 3 days after infection. The mean and SD for the Log(no. of Candida) were measured from their fig. 2. The number of animals was 6-8 per group, and the present calculation is based on the conservative assumption that the groups consisted of 6 animals.

[10] Sadun et al. (1951) calculated the t-test yielding the t-value in the parentheses but the SD-values for the size of the spleen were not reported.

[11] Greenwald & Harde (1935): the current vitamin C group is the combination of two 4-animal groups administered 10-20 mg/day. The control group is the combination of the corresponding two 4-animal control groups: in their first control group all animals died on the 4th day, whereas in their second control group, the animals died at "3 to 5 days" and it is here assumed that the survival times are 3, 4, 4, and 5 days in the latter group.

[12] Jungeblut & Zwemer (1935): the diphtheria toxin dose in series II was twice the minimal lethal dose (2×MLD). The current vitamin C group consists of 8 guinea pigs administered 25 to 200 mg of the vitamin. The current control group refers to table I of the original paper, but not all the control animals were concurrent with the vitamin-treated animals.

[13] Jungeblut & Zwemer (1935): 0.02×MLD was injected intracutaneously, the score being recorded 3 days after the injection. Vitamin C doses were 10 to 100 mg/day for 6 days.

[14] Sigal & King (1937): data is from their table 3 with 0.6×MLD of diphtheria toxin. The range of weights on the 18th day was reported as from 436 to 608 g in the high vitamin C group and from 340 to 490 g in the low vitamin C group. The SD was estimated in the current table conservatively as half of the range, i.e., 86 and 75 g respectively. In the control group n=13 at 18th day.

[15] Zilva (1937): the current vitamin C group is the combination of his groups D and E with a mixed diet supplemented with 100 mg/day of vitamin C, and the control group is his group B with a mixed diet without additional vitamin C; all these animals were administered 1×MLD of diphtheria toxin. When survival time of an animal was presented as a range, such as 3-4 days, the mean of the range (3.5) was used in the log-rank test and is shown in the current table.

[16] King et al. (1940): the current vitamin C group is the combination of their groups 2 to 7, and the control group is the combination of their groups 9 to 14. The authors state that ‘two or more animals were taken from each group for examination of the teeth" and here the calculation of the P-value has been based on the conservative assumption that each group consists of exactly 2 animals.

[17] Fuller et al. (1971) administered E. coli lipopolysaccharide B. The methods are not described in detail, but reference is made to Schlumberger (1959), which indicates that 4 mg/day of pure vitamin C was added to the vitamin C supplemented group.

[18] Kuenzig et al. (1980) administered sublethal doses of E. coli endotoxin. In their brief abstract, only the percentage of animals with physical distress is indicated: 57%, 43%, and 10% in the low, normal and high vitamin C level groups respectively. The lower estimate of the number of animals was inferred from these percentages: 4/7, 3/7, and 1/10 respectively. The low and normal groups have been combined into the control group of the current table (i.e., 7/14).

Naresh-2002	Vit C	Cont	
Cow			
Treatment of mastitis			
<u>Clinical mastitis</u> [19]			
Animal no.	12	6	
Recovery time (d)	4(10); 5(2)	6(5); 15	.000,02
<u>Subclinical mastitis</u> [19]			
Animal no.	12	6	
Recovery time (d)	5(8); 6(2); 15+(2)	15+(6)	.001

Hamdy-1967 [20]	Vit C	Cont	
Lamb			
Spontaneous pneumonia			
Animal no.	44	92	
Mortality before slaughter	14	12	.993
Pneumonia in slaughtered lambs	3	48	<.000,001

Dwenger-1994 [21]	Vit C	Cont	
Sheep			
Endotoxin inj.			
Animal no.	8	8	
Effect of vitamin C on:			
Pulmonary arterial pressure			.000,05
Arterial O_2 saturation			.005
Arterio-to-venous O_2 pressure difference			.000,3

Büsing-1939 [22]	Vit C	Cont	
Rabbit			
Intravenous inj.			
S. pneumoniae (Table 1)			
Animal no.	5	5	
Survival time (h)	61;71; 72; 89(2)	30(2); 45(2); 61	.003
S. aureus (Table 4)			
Animal no.	12	12	
Survival time (d)	21; 24+(11)	14(2); 15(2); 19;24+(7)	.026

Locke-1937	Vit C	Cont	
Rabbit			
Infected with *S. pneumoniae*			
Animal no.	11	12	
Positive blood culture after 30 min	4	9	.042

Locke-1939 [23]	Vit C	Cont	
Rabbit			
Infected with *S. pneumoniae*			
Animal no.	11	10	
Mortality	5	10	.005

Fukuda-1963 [24]	Vit C	Cont	
Rabbit			
Endotoxin inj.			
Liver glycogen depletion			
<u>Normal rabbits</u>			
Animal no.	5	5	
Liver glycogen			
Mean (% of liver wgt)	2.04	0.70	.000,5
SD	0.54	0.25	
<u>Adrenalectomized rabbits</u>			
Animal no.	5	6	
Liver glycogen			
Mean (% of liver wgt)	2.02	0.70	.001
SD	0.67	0.32	

Edwards-1968	Vit C	Cont	
Cat			
Duration of rhinotracheitis (feline herpes virus)			
<u>Tylosin-treatment</u>			
Animal no.	15	19	
Recovery time (d)	4(6); 5(3); 6(2); 7(2); 9;36	4;7(6); 8;9(5); 10(2); 11(2); 14(2)	.005
<u>No tylosin-treatment</u> (vit C alone)			
Animal no.	26		
Recovery time, Median (d)	4.5		

[19] Naresh et al. (2002) administered antibiotics to all cows with clinical mastitis, so that the effect of vitamin C was over and above that of antibiotics. For subclinical mastites, tables 2 and 3 are inconsistent, the latter being consistent with the text.

[20] Hamdy et al. (1967): vitamin C was administered at birth and twice per week for 4-5 weeks.

[21] Dwenger et al. (1994) administered E. coli endotoxin. This is a cross-over trial with 8 sheep. The P-values were calculated by the original authors from the comparisons between several time points. In the current table, the P-values presented are those calculated in the time-range of 2 to 4 h after endotoxin administration.

[22] Büsing (1939): the survival times were measured by the current author from their fig. 1 for S. pneumoniae, and their fig. 4 for S. aureus with the kinin and non-kinin groups being combined.

[23] Locke (1939): data is from table 2. Current vitamin C group is his group 6, and current control group his group 5.

[24] Fukuda et al. (1963): the liver glycogen percentages were measured from the data points of fig. 1.

Matsumoto-1992	Vit C	Cont	
Rat			
Experimental			
E. coli pyelonephritis			
Oral vit C			
Animal no.	8	8	
Score of renal scarring	1(6);	1(2);	.030
(scale 0 to 3)	2(2)	2(6)	
Subcutaneous vit C			
Animal no.	8	8	
Score of renal scarring	0(2);1;	2(3);	.036
(scale 0 to 3)	2(3);	3(5)	
	3(2)		

Miyagatani-1998 [25]	Vit C	Cont	
Rat			
Experimental peritonitis			
Animal no.	27	28	
Mortality in 2 d	6	14	.019

Armour-2001 [26]	Vit C	Cont	
Rat			
Experimental peritonitis			
Vit C 7.5 mg/100 g			
Animal no.	5	16	
Mortality in 1 d	0	4	.16
BP at 1 d			
Mean (mmHg)	115.6	90.1	.000,5
SD	7.6	3.3	
Density of perfused capillaries			
Mean (1/mm)	23.9	16.8	.010
SD	3.9	2.2	
Vit C 76 mg/100 g			
Animal no.	7	16	
Mortality in 1 d	1	4	.3
BP at 1 d			
Mean (mmHg)	111	90.1	.008
SD	12	3.3	
Density of perfused capillaries			
Mean (1/mm)	27	16.8	.003
SD	4.5	2.2	

Kastenbauer-2002 [27]	Vit C	Cont	
Rat			
Experimental			
S. pneumonia meningitis			
Animal no.	4	10	
Median intracranial pressure (mmHg)	24	22	.5
Urate treated			
Animal no.	6	8	
Median intracranial pressure (mmHg)	13	25	<.05 [28]

[25] Miyagatani et al. (1998): rats were made septic by cecal ligation and puncture (CLP).

[26] Armour et al. (2001): rats were made septic by cecal ligation and perforation (CLP). Blood pressure (BP) values were measured from their fig 5; there were 4-10 rats per group; the SE values for the groups were reported and here the SE value is transformed to SD assuming conservatively that there were 4 rats per group. Capillary density values were measured from their fig. 6; there were 4-7 rats per group; the SE values for the groups were reported and here the SE value is transformed to SD assuming conservatively that there were 4 rats per group. For the high dose (76 mg/100 g) group, the results are described in text, and BP is based on n = 4, and capillary density on n = 5.

[27] Kastenbauer et al. (2002): all animals were treated with ceftriaxone, so that the possible effect of vitamin C was over and above the effect of the antibiotic.

[28] Kastenbauer et al. (2002) calculated the P-value using a non-parametric test.

Tyml-2005 [29]	Vit C	Cont	
Rat			
Experimental peritonitis			
Vit C 6 h after CLP [30]			
Animal no.	9	9	
BP at 1 d			
Mean (mmHg)	101	97	.3
SD	15	11	
Density of perfused capillaries at 1 d			
Mean (1/mm)	19.5	11.3	.003
SD	4.2	3.5	
Vit C 1 h after CLP [31]			
Animal no.	7	19	
Mortality in 2 d	0	4	.13
Rectal temp at 2 d			
Mean (°C)	33.9	37.8	.000,1
SD	0.23	1.34	
Change in BP in 2 d			
Mean (mmHg)	17	28	.15
SD	17	20	
Density of perfused capillaries at 2 d			
Mean (1/mm)	18.0	10.4	.019
SD	5.0	4.6	
Vit C 24 h after CLP [32]			
Animal no.	20	19	
Mortality in 2 d	6	4	.7
Rectal temp at 2 d			
Mean (°C)	35.1	37.8	.003
SD	0.90	1.34	
Change in BP in 2 d			
Mean (mmHg)	18	28	.12
SD	6	20	
Density of perfused capillaries at 2 d			
Mean (1/mm)	16.1	10.4	.017
SD	2.1	4.6	

Dey-1966 [33]	Vit C	Cont	
Rat			
Tetanus toxin inj.			
Vit C before or simultaneously with toxin			
Animal no.	10	5	
Mortality	0	5	.000,2
Vit C after signs of tetanus appeared			
Animal no.	15	5	
Mortality	0	5	.000,04

Feng-2004 [34]	Vit C	Cont	
Rat			
Endotoxin inj.			
Animal no.	6	6	
Lung weight at 4 h			
Mean (mg)	170	318	.000,004
SD	29.4	31.8	

Kanter-2005 [35]	Vit C	Cont	
Rat			
Endotoxin inj.			
Animal no.	10	10	
Diameter of glomeruli			
Mean (µm)	70.13	43.84	<.000,001
SD	2.15	1.23	
Vitamin A groups			
Animal no.	10	10	
Diameter of glomeruli			
Mean (µm)	77.94	48.13	<.000,001
SD	3.46	1.26	

[29] Tyml et al. (2005): rats were made septic by cecal ligation and perforation (CLP).

[30] Tyml et al. (2005): vitamin C 6 hours after CLP. Blood pressure (BP) values were measured from their Fig. 2; there were 9-24 rats per group; SE values for the groups were reported and here the SE value is transformed to SD assuming conservatively that there were 9 rats per group. Capillary density values were measured from their Fig. 3; there were 6 rats per group.

[31] Tyml et al. (2005): vitamin C was administered 1 hour after CLP. Rectal temperature was measured for 5 - 8 rats per group and the SE values for the groups were reported in table 1; the SE value is transformed to SD assuming conservatively that there were 5 rats per group. Blood pressure (BP) changes were measured from Fig. 4; there were 7-14 rats per group; SE values for the groups were reported and here the SE value is transformed to SD assuming conservatively that there were 7 rats per group. Capillary density values were measured from their Fig. 5; there were 5 rats per group.

[32] Tyml et al. (2005): vitamin C was administered 24 hours after CLP. See footnote 31.

[33] Dey (1966): 2×MLD of tetanus toxin was administered.

[34] Feng et al. (2005): K. pneumoniae lipopolysaccharide.

[35] Kanter et al. (2005): E. coli lipopolysaccharide. Statistical analysis inappropriate in the original paper, see Hemilä (2006b).

Zhang-1997 [36]	Vit C	Cont	
Gerbil			
Infected with *H. pylori*			
Strain MCP37B			
Animal no.	6	6	
Log(CFU)			
Mean	3.30	5.15	.000,007
SD	0.32	0.48	
Strain ATCC43504			
Animal no.	6	6	
Log(CFU)			
Mean	3.07	4.96	.000,007
SD	0.56	0.16	

Garg-2004 [37]	Vit C	Cont	
Hamster			
Inoculation of *Leishmania*			
-protozoa			
Prophylactic vit C			
250 mg/kg			
Animal no.	20	20	
Parasite count			
Mean	5.0	37.2	<.000,001
SD	2.5	17.4	
Mortality in 90 d	0	20	<.000,001
100 mg/kg			
Animal no.	30	30	
Parasite count			
Mean	8.6	36.2	<.000,001
SD	3.7	9.8	
Mortality in 90 d	0	30	<.000,001
50 mg/kg			
Animal no.	30	30	
Parasite count			
Mean	6.0	38.5	<.000,001
SD	1.2	6.3	
25 mg/kg			
Animal no.	20	20	
Parasite count			
Mean	55.4	56.1	.4
SD	7.6	8.7	
Continuous vit C			
250 mg/kg			
Animal no.	20	20	
Parasite count			
Mean	37.0	37.0	.5
SD	14.1	17.2	

Hastings-1976	Vit C	Cont	
Mouse			
Inoculation of			
M. leprae to foot pads			
Table 1: 6 months follow-up			
0.45% vit C in feed			
Animal no.	6	6	
No. of bacteria in			
foot pad (10exp(+4))			
Mean	4.44	13.5	.003
SD	1.45	6.2	
0.15% vit C in feed			
Animal no.	6	6	
No. of bacteria in			
foot pad (10exp(+4))			
Mean	5.21	13.5	.005
SD	1.27	6.2	
0.05% vit C in feed			
Animal no.	4	6	
No. of bacteria in			
foot pad (10exp(+4))			
Mean	7.88	13.5	.058
SD	0.90	6.2	

Esposito-1986 [38]	Vit C	Cont	
Mouse			
Infected with			
S. pneumoniae			
Animal no.	50	50	
Survival time (d)	2;	2(4);	.2
	3(9);	3(10);	
	4(11);	4(11);	
	5(4);	5(3);	
	5+(25)	5+(22)	

Fang-1990 [39]	Vit C	Cont	
Mouse			
Burned animals,			
infected with			
Ps. aeruginosa			
Low+Med vit C			
Animal no.	59	26	
Mortality	42	18	.6
High vit C			
Animal no.	30	26	
Mortality	27	18	.96

[36] Zhang et al. (1997): data was measured by the current author from their fig. 2. CFU, colony forming units in stomach.

[37] Garg et al. (2002): The parasite counts were calculated from their Fig. 1A. The number of animals is not quite clear. The materials and methods state that "For each individual experiment a batch of 30 hamsters was equally divided into 3 groups [prophylactic vit C, continued vit C, control]" but it is not explicitly stated whether an "experiment" indicates Fig 1 or part of it, e.g. Fig. 1A. Here it is assumed that e.g. Fig. 1A (250 mg/day) consists of "an experiment" which was replicated twice according to the legend of Fig. 1. Mortality is described in text [p 688]: "animals given vitamin C prophylactically …survived until the day the experiment was terminated i.e. on day 90 p.i. [post infection], while the animals of the other experimental groups died between day 50 and day 60 p.i.

[38] Esposito (1986): the survival times were measured by the current author from their fig. 1.

[39] Fang et al. (1990): data is for the group administered vitamin C on a single day at the time of infection, 1 day after the burn.

Gallin-1979 [40]	Vit C	Cont	
Mouse			
Infected with *C. albicans*			
Normal mouse			
Animal no.	16	19	
Survival time (d)	3;4; 5(2); 5.5(5); 6.5; 7.5; 8+(5)	2.5; 3(2); 3.5(3); 4(2); 5(3);5.5; 6;6.5; 7;7.5; 8(2);8+	.025
CHS mouse			
Animal no.	16	19	
Survival time (d)	1;1.5(6); 2(5); 2.5(4)	0.5(16); 1(3)	<.000,001

Wang-2000 [41]	Vit C	Cont	
Mouse			
Infected with *Helicobacter pylori*			
Animal no.	10	10	
Bacterial count Log (CFU)	0.7(4); 1.3;2.2; 2.3;2.4; 3.0;3.7	3.2(2); 3.5(3); 3.6;3.7; 4.0;4.2; 4.3	.000,5
Inflammation score (scale 0 to 3)	1.2; 1.3(4); 1.5(2); 1.6(2); 1.7	1.7(2); 1.8(2); 2.0(3); 2.3(2); 2.7	.000,1

Wu-2003 [42]	Vit C	Cont	
Mouse			
Experimental peritonitis			
Animal no.	8	8	
BP at 6 h			
Mean (mmHg)	69.0	61.6	.023
SD	4.9	8.2	

Wu-2004 [43]	Vit C	Cont	
Mouse			
Experimental sepsis			
Vit C 0.5 h before CLP			
Animal no.	29	29	
Survival time (h)	18(7); 24(6); 24+(16)	18(20); 24(6); 24+(3)	.000,04
BP at 6 h			
Mean (mmHg)	63	41	.000,003
SD	4	4	
Vit C 3 h after CLP			
Animal no.	6	6	
BP at 6 h			
Mean (mmHg)	39	40	.3
SD	10	5	

Seah-1974 [44]	Vit C	Cont	
Mouse			
Infected with *Toxoplasma gondii* –protozoa			
A: Vit C 8 mg/d			
Animal no.	12	24	
Survival time (d)	5(3); 6(4); 7(4); 9	3; 4(15); 5(8)	.000,001
B: Vit C 16 mg/d			
Animal no.	12	24	
Survival time (d)	4(3); 5(4); 6(3); 8;9	3; 4(15); 5(8)	.000,5

Bourke-1980	Vit C	Cont
Mouse		
Infected with *Plasmodium berghei* –protozoa (malaria)		
0.5 g vit C /kg body wgt		
Animal no.	?	?
Survival time, Median (d)	18	9
1 g vit C /kg body wgt		
Animal no.	?	?
Survival time, Median (d)	21	9

[40] Gallin et al. (1979): the survival times were measured by the current author from their fig. 1. CHS; Chediac Higashi Syndrome mouse model.

[41] Wang (2000): Half of the animals from each group were sacrificed 1 day after the cessation of treatment and the other half were sacrificed 10 days after the cessation of treatment. The animals sacrificed at different times are combined in this table. The data for this table was measured from Figs 1 and 2 of the paper. Detection limit of bacterial growth was 0.7 Log units. CFU, colony forming units per ml of stomach homogenate.

[42] Wu et al. (2003): mice were made septic by cecal ligation and puncture (CLP). Blood pressure (BP) values were measured from their Fig. 5.

[43] Wu et al. (2004): mice were made septic by cecal ligation and puncture (CLP). Blood pressure (BP) values were measured from their Fig. 5 for early vitamin C and from their Fig. 8 for late vitamin C; in both cases there were 6 animals per group.

[44] Seah (1974) reported the mean and SD/SE for four groups; two were administered vitamin C (groups A and B) and two were controls (groups C and D). The individual survival times were calculated from the published mean and SD (for groups A and B) or SE (groups C and D) values; thereafter the control group data have been combined in the current table.

Büller Souto-1939 [45]	Vit C	Cont	
Mouse			
Clostridial toxins			
1×LD50			
Animal no.	125	62	
Mortality in 2 d	27	29	.000,3
2×LD50			
Animal no.	130	68	
Mortality in 2 d	78	63	<.000,001

Hill-1955 [46]	Vit C	Cont	
Chicken			
Infected with *Salmonella*			
Table 5			
Animal no.	40	40	
Mortality	7	20	.002

Little-1971	Vit C	Cont	
Chicken			
Infected with *Eimeria* –protozoa (coccidiosis)			
Table 3			
Animal no.	80	80	
Mortality	26	20	.8
Table 4			
Animal no.	80	80	
Mortality	56	48	.9

Gross-1988a	Vit C	Cont	
Chicken			
Infected with *E. coli*			
Table 1			
Animal no.	36	33	
Pericarditis or dead	4	23	<.000,001
Table 3:			
0.33-0.44 g vit C/kg feed			
Animal no.	26	13	
No. infected	13	12	.005

Gross-1988b [47]	Vit C	Cont	
Chicken			
Infected with *E. coli*			
Experiment 1			
Animal no.	22	21	
Mortality	3	11	.005

Gross-1992	Vit C	Cont	
Chicken			
Experiment 4			
Infected with *Mycoplasma gallisepticum,* Newcastle disease virus, and *E. coli*			
Animal no.	26	42	
Pericarditis or dead	9	32	.000,5
Experiment 5			
Infected with *M. gallisepticum,* and Newcastle disease virus, 0.1 g vit C/kg feed			
Animal no.	5	5	
Lesion score, Mean (scale 0 to 2)	0.52	1.50	<.05 [48]
Experiment 6			
Infected with *E. coli* 0.1 g vit C/kg feed			
Animal no.	8	8	
Lesion score, Mean (scale 0 to 4)	0.50	2.90	<.05 [48]

[45] Büller Souto & Lima (1939) data was extracted by Clemetson (2002) to his table 1 and the current figures are from the latter source. Toxins of four Clostridium species (C. welchii, C. septicum, C. oedematiens, C. histolyticum) were administered intramuscularly to different groups of mice and the groups are combined to this table. LD50; dose leading to the death of 50% of animals.

[46] Hill et al. (1955): data is from their table 5. There were 40 birds per treatment group, and the mortality was calculated from the percentages in their table 5. The current vitamin C group is their 'basal diet+vitamins+0.1%C' and the control group is 'basal diet+vitamins–C' (excluding vitamin C).

[47] Gross (1988b): the results of HA and LA lines of chickens in their fig. 1 are combined. Comparison is between 0.4 g vitamin C/kg feed and no supplementation.

[48] Gross (1992) himself calculated the P-values for the lesion scores.

Davelaar-1992 [49]	Vit C	Cont	
Chicken			
Infected with avian coronavirus			
(infectious bronchitis virus)			
Experiment 1			
0.33 g vit C/kg feed			
Animal no.	20	20	
Table 1			
14 d: air sacculitis			
no. of animals	0	12	.000,02
Table 2 [50]			
10 d: tracheal lesion score	0(4);	1;2(5);	.000,02
(scale 0 to 3)	1(8);	3(9)	
	2(3)		
Experiment 2			
0.3 g vit C/kg feed			
Animal no.	24	24	
Table 3			
14 d: air sac lesion score	0(16);	0(9);	.009
(scale 0,1,2,4)	1(3);	1(3);	
	2(5)	2(7);	
		4(5)	
Table 4			
10 d: tracheal lesion score	0(5);	0(2);	.000,07
(scale 0 to 3)	1(18);	1(7);	
	2	2(15);	

Amakye-2000	Vit C	Cont	
Chicken			
Infected with			
infectious bursal disease virus			
Animal no.	30	30	
Clinical signs	0	30	<.000,01
Mortality	0	9	.000,5

Hanzlik-1936 [51]	Vit C	Cont	
Pigeon			
Diphteria toxin inj.			
Animal no.	6	24	
Mortality	1	15	.032

Durve-1982	Vit C	Cont	
Catfish			
Infected with			
Edwardsiella tarda			
Temperature 23° C			
Animal no.	20	20	
Mortality	4	20	<.000,1
Temperature 33° C			
Animal no.	20	20	
Mortality	2	10	.004

Li-1985 [52]	Vit C	Cont	
Catfish			
Infected with			
Edwardsiella ictaluri			
Table 3			
Animal no.	20	20	
Mortality	0	20	<.000,1

Liu-1989 [53]	Vit C	Cont	
Catfish			
Infected with *E. ictaluri*			
2 g vit C/kg feed			
Animal no.	300	60	
Log(LD50)			
(10exp[+5] cells/fish)		5.0	0.007
Vit C (g/kg feed)			
0.1	5.2		
0.5	5.8		
1	6.8		
2	7.1		
4	7.1		

Li-1993 [54]	Vit C	Cont	
Catfish			
Infected with *E. ictaluri*			
Table 5: Experiment 2			
0.1 g vit C/kg feed			
Animal no.	250	250	
Mortality	151	195	<.000,1
2 g vit C/kg feed			
Animal no.	250	250	
Mortality	175	195	.020

[49] Davelaar & Bos (1992) reported the mean and confidence limits of the score values. The original score values per animal were inferred by the current author from the published data. In the control groups, the maximum harm caused by infection to the trachea was seen at 10 days and to the air sacs at 14 days, which are thus selected as times for examining the effect of vitamin C in this table. In Table 2 for both groups n=15.

[50] Davelaar & Bos (1992): In table 2 for both groups n = 15.

[51] Hanzlik & Terada (1936): their vitamin C groups 2 and 3 have been combined (n = 3 for both groups), and compared with the control groups administered 9 µl and 10 µl of diphtheria toxin (n = 12 for both groups). The mortality in the lower dose control group (9 µl) was 50%, indicating that 6 of 12 pigeons died. The mortality in the higher dose control group (10 µl) was 80%, which does not produce an integer number of deaths, and the mortality is conservatively rounded downwards (9 of 12). These two control groups were collapsed to obtain the ratio 15/24 for the control group of the current table.

[52] Li & Lovell (1985): data is at 8 days after infection; the current vitamin C group is fish administered 3 g vitamin C/kg feed, and the control group is fish administered no vitamin C.

[53] Liu et al. (1989): the P-value was calculated by the current author as the test of slope in linear regression of Log(LD50) as a function of the square root of vitamin C dosage.

[54] Li et al. (1993): the cumulative mortality was calculated from the mortality percentage and the total number of fish at the start (n = 50 in 5 aquaria per diet).

Duncan-1993, 1994	Vit C	Cont	
Catfish			
Spontaneous infection			
by *Flexibacter columnaris*			
0.2 vs. 0.02 g vit C/kg feed			
Low folic acid			
Animal no.	240	240	
Total mortality	88	110	.021
Mortality caused by			
F. columnaris	10	13	.3
Medium folic acid			
Animal no.	240	240	
Total mortality	30	121	<.000,1
Mortality caused by			
F. columnaris	1	17	<.000,1
High folic acid			
Animal no.	240	240	
Total mortality	22	20	.6
Mortality caused by			
F. columnaris	4	1	.9
Experimental infection			
with *E. ictaluri*			
Low folic acid			
Animal no.	24	24	
Mortality	18	21	.15
Medium folic acid			
Animal no.	24	24	
Mortality	9	15	.048
High folic acid			
Animal no.	24	24	
Mortality	7	7	.5

Lim-2000	Vit C	Cont	
Catfish			
Infected with			
E. ictaluri			
50 mg vit C/kg feed			
30 mg iron/kg feed			
Animal no.	60	60	
Mortality	30	40	.034
300 mg iron/kg feed			
Animal no.	60	60	
Mortality	20	49	<.000,1

Wahli-1986	Vit C	Cont	
Rainbow-trout			
Infected with			
Ichthyphthirius multifiliis			
–protozoa			
8800 tomites per fish			
Animal no.	50	50	
Mortality	1	26	<.000,1
11500 tomites per fish			
Animal no.	50	50	
Mortality	8	50	<.000,1

Navarre-1989	Vit C	Cont	
Rainbow-trout			
Infected with			
Vibro anguillarum			
Fig 3			
2×10exp(+4) bacteria/ml [55]			
Animal no.	?	?	
Mortality percentage			
vit C g/kg feed			
0.0		55%	0.005
0.1	46%		
0.5	21%		
1.0	1%		
2.0	1%		
1.4×10exp(+6) bacteria/ml [55]			
Animal no.	?	?	
Mortality percentage			
vit C g/kg feed			
0.0		100%	0.005
0.1	84%		
0.5	36%		
1.0	8%		
2.0	4%		
2.8×10exp(+8) bacteria/ml			
Animal no.	?	?	
Mortality percentage			
vit C g/kg feed			
0.0		100%	.5
2.0	100%		

Wahli-1995	Vit C	Cont	
Rainbow trout			
Infected with *I. multifiliis*			
–protozoa			
9 600 theronts/fish			
Animal no.	40	40	
Mortality	11	40	<.000,1
20 000 theronts/fish			
Animal no.	40	40	
Mortality	29	40	.000,2

[55] Navarre et al. (1989): the P-value was calculated by the current author as the test of slope in linear regression of mortality percentage as a function of the square root of vitamin C dosage.

Wahli-1998 [56]	Vit C	Cont	
Rainbow trout			
Infected with VHS virus			
Low vit E			
Animal no.	50	50	
Mortality	10	13	.2
High vit E			
Animal no.	50	50	
Mortality	2	29	<.000,1
Infected with *Yersinia ruckeri*			
Low vit E			
Animal no.	50	50	
Mortality	20	48	<.000,1
High vit E			
Animal no.	50	50	
Mortality	18	16	.6
Infected with *I. multifiliis* –protozoa			
Low vit E			
Animal no.	50	50	
Mortality	3	21	<.000,1
High vit E			
Animal no.	50	50	
Mortality	4	1	.9

Hardie-1991 [57]	Vit C	Cont	
Atlantic salmon			
Infected with *Aeromonas salmonicida*			
Animal no.	25	25	
Mortality	2	23	<.000,1

Waagbo-1992, 1993 [58]	Vit C	Cont	
Atlantic salmon			
Infected with *A. salmonicida*			
Animal no.	50	50	
Mortality	6	10	.14

Lygren-1999 [59]	Vit C	Cont	
Atlantic salmon			
Infected with infectious salmon anemia virus			
Animal no.	90	90	
Mortality in 43 d	84	90	.007
Fe added to feed			
Animal no.	90	90	
Mortality in 43 d	82	90	.002

Bell-1984 [60]	Vit C	Cont	
Sockeye salmon			
Infected with *Renibacter salmoninarum*			
High Zn and Mn			
Animal no.	30	30	
Median survival time (d)	79	78	.5
Low Zn and Mn			
Animal no.	30	30	
Median survival time (d)	75	90	>.999

Sobhana-2002	Vit C	Cont	
Hamilton			
Infected with *Aeromonas hydrophila*			
Animal no.	10	10	
Mortality	2	7	.019

Martins-1998 [61]	Vit C	Cont	
Pacu-fish			
Infected with *Anacanthorus penilabiatus* –protozoa			
Animal no.	30	10	
No. of parasites in gills			
Mean	20.4	42.5	.003
SD	13.1	35.1	

[56] Wahli et al. (1998): the current vitamin C group is fish administered 2 g vitamin C/kg feed. The low vitamin E group was not administered additional vitamin E; the high vitamin E group was administered 0.8 g vitamin E/kg feed. VHS, viral hemorrhagic septicemia virus.

[57] Hardie et al. (1991): data is from their fig. 5: the current vitamin C group is fish administered 2.75 g vitamin C/kg feed.

[58] Waagbo et al. (1993): data is from their fig. 2: the current vitamin C group is fish administered 4 g vitamin C/kg feed, and the control group was administered 40 mg vitamin C/kg feed. The cumulative mortality at 55 days was calculated from the mortality percentage and the total number of fish at the start (n = 50).

[59] Lygren et al. (1999): data from their table 5 with 43 days follow-up. The cumulative mortality at 43 days was calculated from the mortality percentage and the total number of fish at the start (n = 90).

[60] Bell et al. (1984): data for groups inoculated with bacterial dosage 5.69×10exp(+5) is presented in the current table. The survival times were measured by the current author from their fig. 3. High Zn and Mn comparison means comparison of groups 1 and 3, and low Zn and Mn comparison means comparison of groups 4 and 6.

[61] Martins et al. (1998): the current vitamin C group is the combination of their 50-200 mg/kg feed groups.

Appendix 3. The effect of vitamin C on animal infections: Difference between the groups not specific to vitamin C

Study Animal Infection	Treatment		P [1]
Sabin-1939 Rhesus monkey Occurrence of spontaneous infections	Vit C (oranges)	Cont	
Animal no.	21	25	
Mortality due to			
pneumonia	0	5	.020
all infections	1	9	.006
Honjo-1969 Macaque monkey Experimental Shigellosis	Vit C (sweet potatoes)	Cont	
Animal no.	4	7	
Bloody stool	0	3	.10
McConkey-1933 [2] Guinea pig Infected with *M. tuberculosis* by oral inoculation	Vit C (tomato juice)	Cont	
Experiment I			
Animal no.	5	10	
Intestinal tract TB at autopsy	1	7	.053
Experiment II			
Animal no.	5	16	
Intestinal tract TB at autopsy	0	15	.000,2
	Vit C (cabbage)	Cont	
Experiment II			
Animal no.	10	16	
Intestinal tract TB at autopsy	5	15	.010
De Savitsch-1934 [3] Guinea pig Subcutaneous inoculation of *M. tuberculosis*	Vit C (orange juice)	Cont	
Animal no.	11	12	
TB in the spleen score (scale 0 to 4)	0(6); 1(2); 2(2);3	0;1(2); 2(5); 3(4)	.008
TB in the liver score (scale 0 to 4)	0(9); 1(2);	0(6); 1(2); 2(3);3	.038
TB in the lungs score=0 (scale 0 to 4)	8	3	.016
Greene-1936 [4] Guinea pig Infected with *M. tuberculosis*	Vit C (orange juice)	Cont	
Table 3:			
Intraperitoneal inoculation			
Animal no.	12	12	
Survival time, Median (d)	81	60.5	.042
Table 7:			
Subcutaneous inoculation			
Animal no.	16	15	
Survival time, Median (d)	152	80	.000,6
Table 9:			
Subcutaneous inoculation			
Animal no.	9	12	
Survival time, Median (d)	67	46	.016
Table 15:			
Oral administration			
Animal no.	17	17	
Mortality in 150 d	1	8	.005
Table 20:			
Oral administration			
Animal no.	12	11	
Survival time, Median (d)	171	107	.011
Table 22:			
Sputum fed animals			
Animal no.	17	14	
Survival time, Median (d)	168	121	.002

[1] The 1-tailed P-value. In the case of dichotomous data, the mid-P-value was calculated by the current author (see Table 7). The survival data was analyzed using the log-rank test. In the case of survival and score data, the number in parenthesis (n) indicates the number of animals with the same outcome value; '+' indicates censoring. The score data was analyzed using the Wilcoxon test with normal approximation.

[2] McConkey & Smith (1933): TB, tuberculosis. Experiment I: comparison of groups E and G. Experiment II: comparison of groups I and K (tomato juice) and M (cabbage leaves).

[3] De Savitsch et al. (1934): TB, tuberculosis.

[4] Greene et al. (1936) reported the survival data of their guinea pigs, but the data is not reproduced here; the median survival time was estimated using the Kaplan-Meier method. Since the survival of uninoculated vitamin C deficient guinea pigs was over 200 days, the shorter survival time in infected vitamin C deficient guinea pigs is not caused by vitamin C deficiency per se.

Boyden-1955 [5]	Vit C	Cont	
Guinea pig	(fresh		
Infected with	alfalfa,		
M. tuberculosis	grass)		
Animal no.	10	10	
Survival time (d)	53(3);	20;21;	<.000,1
	61;67;	22(2);	
	74(2);	25(2);	
	99+(3)	29;32;	
		36;50	

Findlay-1923	Vit C	Cont	
Guinea pig	(orange		
Infected with	juice)		
S. pneumoniae			
Intraperitoneal inj. [6]			
(Table II: 1-2×10exp[+9])			
Animal no.	16	8	
Survival time, Median (d)	6.5	2	<.000,1
Subcutaneous inj.			
Table III			
Animal no.	8	4	
Mortality	3	4	.036

Werkman-1924	Vit C	Cont	
Guinea pig	(lemon		
	juice)		
S. pneumoniae (Table 1)			
Animal no.	8	8	
Mortality in 3 d	1	4	.077
S. pneumoniae			
or *Bacillus anthracis*			
(Table 4) [7]			
Animal no.	43	34	
Mortality in 4 d	23	28	.005

Takahashi-1935	Vit C	Cont	
Guinea pig	(juice of		
Infected with	Daikon		
Staphylococcus	-radish)		
Table 7			
Animal no.	6	11	
Osteomyelitis in the ribs	0	9	.002
Table 8			
2 or 3 week vit C deficiency			
in the control group			
Animal no.	9	18	
Osteomyelitis			
in the ribs	0	18	<.000,1
in the long bones	0	8	.010

Taylor-1937 [8]	Vit C	Cont	
Guinea pig	(orange		
Intradermal inj. of	juice)		
S. haemolyticus			
Animal no.	7	10	
Bacteria stained			
in the mitral valve	0	7	.003

Grant-1926	Vit C	Cont	
Guinea pig	(orange		
Salmonella typhimurium	juice)		
(B. aertrycke)			
Spontaneus infection			
caused by intestinal bacteria			
(Table 1: cod-liver oil)			
Animal no.	12	20	
Infected	0	10	.002
Inoculation by mouth			
(Table 2: cod-liver oil)			
Animal no.	4	6	
Infection of spleen	0	6	.003

Höjer-1924 [9]	Vit C	Cont	
Guinea pig	(orange		
Spontaneous	juice)		
acute infections			
Animal no.	45	72	
Signs of infections	9	34	.002
Animal no.	40	24	
Mortality due to infections	8	13	.004

Schmid-Weyland-1928	Vit C	Cont	
Guinea pig	(lemon		
Inhalation of fowl cholera	juice)		
(*Pasteurella*)			
and *S. pneumoniae*			
Table 4: series III			
Animal no.	10	10	
Infected with fowl cholera	0	8	.000,2

Karel-1941	Vit C	Cont	
Guinea pig	(green		
Infection with group C	food)		
streptococcus			
Animal no.	30	20	
Mortality in 2 d	16	9	.7
Sulfanilamide groups			
Animal no.	30	20	
Mortality in 14 d	12	12	.091

King-1935 Guinea pig Diphtheria toxin inj.	Vit C (orange juice)	Cont	
Table 1			
Animal no.	15	15	
Mortality in 26 d	5	10	.043
Subcutaneous hemorrhagic necrosis at the site of injection	0	15	<.000,1
Figure 1: B-groups			
Animal no.	5	5	
Mortality	0	5	.002
Subcutaneous hemorrhagic necrosis at the site of injection	0	5	.002

Schultz-1936 Guinea pig Infection with group C streptococcus	Vit C (orange juice, cabbage)	Cont	
Control group: 3 ml/week orange juice			
Animal no.	26	6	
Mortality	7	6	.001
Control group: 6-9 ml/week orange juice			
Animal no.	26	17	
Mortality	7	9	.051

[5] Boyden & Anderson (1955): the survival times were measured by the current author from their fig. 1.

[6] Findlay (1923) reported the survival data of his guinea pigs but the data is not reproduced here; the median survival time was estimated using the Kaplan-Meier method.

[7] Werkman et al. (1924): data is from their table 4 which "summarized the results of 4 final series of experiments using the pneumococcus or B. anthracis."

[8] Taylor (1937): the current vitamin C group is the combination of groups 3 (normal diet) and 5 (scorbutic diet before infection and normal diet after infection), and the control group is the combination of groups 2 (scurvy) and 4 (subacute scurvy).

[9] Höjer (1924 pp 37-38, 120): the current vitamin C group consists of his animals with a ‘complete diet’, and the control group of his guinea pigs with marginal scurvy (‘scorbut mitior’).

Appendix 4. The effect of vitamin E on animal infections: Administration of pure vitamin E to the 'vitamin E group'

Study Animal Infection	Treatment		P [1]
Smith-1984 [2]	Vit E	Cont	
Cows			
Spontaneous mastitis			
Animal no.	41	39	
No. of quarters:	164	156	
Clinical mastitis	43	60	.010
Weiss-1997 [3]	Vit E	Cont	
Cows			
Spontaneous mastitis			
Animal no.	19	22	
No. of quarters	76	88	
Infected quarters	9	28	.002
Clinical mastitis	2	22	.000,02
Stephens-1979	Vit E	Cont	
Lamb			
Inoculated with *Chlamydia*			
Animal no.	10	10	
Chlamydia isolated in lungs at 11 d	0	4	.022
Weight gain in 11 d (kg) [4]			
Mean	2.32	1.37	.023
SD	0.66	1.23	
Teige-1978 [5]	Vit E	Cont	
Pig			
Treponema hyodysenteriae			
Table 3			
Animal no.	12	12	
Days before diarrhea	8(3); 9(3); 10(2); 11; 13(2); 30+	3(2); 4; 8(3); 10(2); 11;15; 17;30+	.4
Teige-1982 [6]	Vit E	Cont	
Pig			
T. hyodysenteriae			
Table 3			
Animal no.	12	12	
Days before diarrhea	6(6); 7;8(2); 9;11; 12;	6(3); 7;8; 9(2); 10;12; 22+(3)	.029
Peck-1991a [7]	Vit E	Cont	
Guinea pig			
Infected with *E. coli* and *S. aureus*			
Medium dose vit E			
Animal no.	15	15	
Mortality	7(46%)	14	.004
High dose vit E			
Animal no.	14	15	
Mortality	10(71%)	14	.08

[1] The 1-tailed P-value. The mid-P-value was calculated for dichotomous data by the current author (see Table 7), and the t-test was used for continuous data. The survival data and the days before diarrhoea were analyzed using the log-rank test. In the case of survival and score data, the number in parenthesis (n) indicates the number of animals with the same outcome value; '+' indicates censoring.

[2] Smith et al. (1984): data on the incidence of mastitis is from their table 3: the no-selenium and selenium groups are combined in this table.

[3] Weiss et al. (1997): data on the incidence of mastitis: the current vitamin E group is treatment 3 (2,000 IU/day vitamin E during lactation) and the control group is treatment 1 (100 IU/day vitamin E). The number of quarters with mastitis has been calculated from the published percentages.

[4] Stephens et al. (1979) used the t-test to calculate that $P < 0.05$. The measure of variation in their table apparently thus refers to "SD" and not "SE" as stated in the paper.

[5] Teige (1978): the no-selenium and selenium groups are combined in this table.

[6] Teige (1982): the no-selenium and selenium groups are combined in this table.

[7] Peck & Alexander (1991a): data is from their table III. The groups of the current table are the combinations of the two low vitamin C groups (1× and 5×RDA). Current high vitamin E refers to 9×RDA and medium vitamin E to 3×RDA.

Tvedten-1973 [8]	Vit E	Cont	
Rat			
Infected with *Mycoplasma pulmonis*			
Animal no.	5	5	
Mycoplasma in the lungs	0	4	.012

Powell-1989 [9]	Vit E	Cont	
Rat			
Experimental peritonitis			
Animal no.	10	10	
Survival time (d)	1(2); 1.5(3) 3;4;7+(3)	0.5(5); 1(5)	.000,1

Powell-1991 [10]	Vit E	Cont	
Rat			
Experimental peritonitis			
Animal no.	10	32	
Survival time (d)	1(2); 1.5(3); 3;3+(4)	1(30); 1.5;3+	.000,03

Soybir-2002 [11]	Vit E	Cont	
Rat			
Experimental peritonitis			
Animal no.	28	30	
Mortality	9	16	.057

Özlü -1999 [12]	Vit E	Cont	
Rat			
Injection of bacterial suspension to trachea			
Animal no.	10	10	
CFU in lavage at 3 d			
Mean	208	4849	.002
SD	358	4798	

Yoshikawa-1982 [13]	Vit E	Cont	
Rat			
Endotoxin inj.			
Rat model of DIC			
Animal no.	10	12	
Glomeruli with fibrin deposits (% of glomeruli)			
Mean	4.2%	75.2%	<.000,001
SD	1.5%	9.7%	
Fibrinogen			
Mean (mg/ml)	0.37	<0.2	
SD	0.10		
Fibrin degradation products			
Mean (µg/ml)	5.1	43.1	<.000,001
SD	1.0	5.4	

Yoshikawa-1984 [14]	Vit E	Cont	
Rat			
Endotoxin inj.			
Rat model of DIC			
Animal no.	18	19	
Glomeruli with fibrin deposits (% of glomeruli)			
Mean	40.2%	57.3%	.000,002
SD	9.1%	9.3%	
Fibrinogen			
Mean (mg/ml)	0.62	0.37	.000,002
SD	0.11	0.16	
Fibrin degradation products			
Mean (µg/ml)	10.1	13.8	.028
SD	5.2	6.1	

McKechnie-1986 [15]	Vit E	Cont	
Rat			
Endotoxin inj.			
Animal no.	15	15	
Survival time (d)	1(7); 4+(8)	1(13); 3(2)	.000,6

Kunimoto-1987 [16]	Vit E	Cont	
Rat			
Endotoxin inj.			
Animal no.	20	30	
Survival time (h)	3(3); 6(8); 12(2); 24+(7)	3(8); 6(14); 12(2); 24; 24+(5)	.062

[8] Tvedten et al. (1973): data is from their table 4: the current vitamin E group is conventionally maintained 'vitamins A and E supplemented' group and the control group is their 'vitamin E deficient' group.

[9] Powell et al (1989): sepsis caused by cecal ligation and puncture (CLP). Three rats in vitamin E group remained alive 1 week after the study was completed. Thus, there was 1 death between 3 and 7 days.

[10] Powell et al. (1991): sepsis caused by cecal ligation and puncture (CLP). The current vitamin E group is the PRE-AT group with 3 days pretreatment prior to CLP.

[11] Soybir et al. (2002): the current vitamin E group consists of group 5 (vitamin E) and group 7 (antibiotics and vitamin E) and the control group consists of group 1 (control) and group 6 (antibiotics).

[12] Özlü et al. (1999): 0.1 ml of a suspension containing 6 bacterial species was instilled into the tracheal lumen by trans-tracheal injection. P-value calculated using Mann-Whitney U-test by the original authors. CFU, colony forming units.

[13] Yoshikawa et al. (1982) administered E. coli lipopolysaccharide B. DIC, disseminated intravascular coagulation.

[14] Yoshikawa et al. (1984) administered E. coli lipopolysaccharide B. DIC, disseminated intravascular coagulation.

[15] McKechnie et al. (1986) administered E. coli lipopolysaccharide.

[16] Kunimoto et al. (1987) administered E. coli lipopolysaccharide. The highest vitamin E group has been compared with the group pretreated with saline.

Ashorobi-1995 [17]	Vit E	Cont	
Rat			
Endotoxin inj.			
Animal no.	40	40	
Survival time (h)	5(13); 12(2); 24(3); 48; 48+(21)	5(29); 24(4); 48+(7)	.000,3
Indomethacin treated			
Animal no.	20	20	.004
Survival time (h)	5(2); 48+(18)	5(5); 12(4); 24; 48+(10)	

Suntres-1996 [18]	Vit E	Cont	
Rat			
Endotoxin inj.			
Lung edema			
Vit E 2 h after endotoxin			
Animal no.	5	5	
Lung weight increase in 1 d			
Mean	31%	61%	.021
SD	12%	25%	
Vit E 4 h after endotoxin			
Animal no.	5	5	
Lung weight increase in 1 d			
Mean	33%	62%	.005
SD	12%	15%	

Suntres-1998 [19]	Vit E	Cont	
Rat			
Endotoxin inj.			
Lung edema			
Animal no.	5	5	
Lung weight increase in 2 h			
Mean	18%	37%	.034
SD	9%	18%	

Heinzerling-1974a [20]	Vit E	Cont	
Mouse			
Infected with *S. pneumoniae*			
Nonimmunized mice			
Medium vit E			
Animal no.	20	20	
Mortality	7(35%)	16	.003
High vit E			
Animal no.	30	20	
Mortality	16(53%)	16	.031
Immunized mice			
Medium vit E			
Animal no.	20	20	
Mortality	6(30%)	15	.003
High vit E			
Animal no.	30	20	
Mortality	16(53%)	15	.07

Marubayashi-1989 [21]	Vit E	Cont	
Mouse			
Endotoxin inj.			
Animal no.	35	12	
Survival time (d)	1(3); 2(17); 2+(15)	1(3); 2(9)	.002

Fang-1990 [22]	Vit E	Cont	
Mouse			
Burned animals, infected with *Ps. aeruginosa*			
Animal no.	51	25	
Mortality	23	21	.000,7

[17] Ashorobi et al. (1995) administered E. coli lipopolysaccharide. The current vitamin E group is the combination of groups VII and VIII and the control group is the combination of groups III and IV. The current indomethacin control group is group VI, and the indomethacin+vitamin E group is group XII.

[18] Suntres et al. (1996) administered E. coli lipopolysaccharide. The current control group is their liposome group. Data is measured from their Fig. 1.

[19] Suntres et al. (1998) administered E. coli lipopolysaccharide. The current control group is their 'plain liposome group'.

[20] Heinzerling et al. (1974a): data for nonimmunized mice is from their fig. 1. The current control group is the combination of 0 and 60 mg/kg, the vitamin E(medium) group of 120 and 180 mg/kg, and the vitamin E(high) group of the 240, 300 and 360 mg/kg diet groups. Data for immunized mice is from their fig. 2 with the same collapse of categories.

[21] Marubayashi et al. (1989) administered E. coli lipopolysaccharide.

[22] Fang et al. (1990): data is for their group administered vitamin E on 4 consecutive days starting 2 days preceding the burn. The current vitamin E group is the combination of their MED and HI groups.

Peck-1991b	Vit E	Cont	
Mouse			
Burned animals, infected with *Ps. aeruginosa*			
Low vit E			
Animal no.	30	30	
Mortality	20	21	.4
High+Med vit E			
Animal no.	60	30	
Mortality	51	21	.94

Beck-1994 [23]	Vit E	Cont	
Mouse			
Infected with coxcackievirus			
Strain CVB3/20			
Lard diet			
Animal no.	5	5	
Virus detectable in myocardium at 14 d	0	5	.002
Menhadden oil diet			
Animal no.	5	5	
Virus detectable in myocardium at 14 d	0	5	.002
Strain CVB3/0			
Lard diet			
Animal no.	5	5	
Virus detectable in myocardium at 14 d	0	5	.002
Menhadden oil diet			
Animal no.	5	5	
Virus detectable in myocardium at 14 d	0	5	.002

Beck-2003	Vit E	Cont	
Mouse			
Infected with coxcackievirus			
Se deficient			
Animal no.	4	4	
Log(cardiac virus titre) [24]			
Mean	5.6	9.5	.007
SD	1.3	1.9	
Log(liver virus titre) [24]			
Mean	4.6	7.9	.006
SD	1.6	0.8	

Hayek-1997	Vit E	Cont	
Mouse			
Infected with influenza virus			
Young mice			
Animal no.	9	7	
Log(virus titre) in the lungs at 5 d			
Mean	2.95	3.50	.024
SD	0.53	0.46	
Animal no.	5	6	
Log(virus titre) in the lungs at 7 d			
Mean	0.88	0.91	.5
SD	0.70	0.73	
Old mice			
Animal no.	6	4	
Log(virus titre) in the lungs at 5 d			
Mean	2.35	3.61	.001
SD	0.43	0.45	
Animal no.	6	5	
Log(virus titre) in the lungs at 7 d			
Mean	1.05	2.08	.024
SD	0.73	0.75	

Han-2000	Vit E	Cont	
Mouse			
Infected with influenza virus			
Animal no.	5	5	
Weight loss in 5 d			
Mean (g)	1.84	6.78	.009
SD	2.03	3.11	

Sugino-1987 [25]	Vit E	Cont	
Mouse			
Endotoxin inj.			
Animal no.	35	21	
Survival time (d)	1(3); 2(17); 2+(15)	1(12); 2(9);	.000,003

[23] Beck et al. (1994): There were 5 or 10 animals per group. Here it is assumed conservatively that there were 5 animals per group.

[24] Beck et al. (2003) published the cardiac and liver virus titers for 4 mice per treatment group in their table 1. It is not stated whether these are the same mice as the 5 mice used for histopathologic analysis. In the current table, the titers were log-transformed before the t-test. One of the 4 liver virus titers was "not detectable" which was replaced with 10 units, which is the lowest reported titer in their table.

[25] Sugino et al. (1987) administered E. coli endotoxin.

Mayorga-2004 [26]	Vit E	Cont	
Mouse			
Endotoxin inj.			
Fig. 3 vit E 22 h before LPS			
4 mg LPS/kg			
Animal no.	10	10	
No. viable embryos			
Mean	15.4	9.5	.000,5
SD	4.0	2.5	
10 mg LPS/kg			
Animal no.	10	10	
No. viable embryos			
Mean	18.6	7.2	<.000,001
SD	4.6	2.2	
Fig. 4 vit E 5 d before LPS			
4 mg LPS/kg			
Animal no.	10	10	
No. viable embryos			
Mean	19.0	9.5	<.000,001
SD	1.0	2.6	
10 mg LPS/kg			
Animal no.	10	10	
No. viable embryos			
Mean	13.7	7.1	.000,2
SD	2.6	3.8	

Sell-1997	Vit E	Cont	
Turkey			
Infected with *E. coli*			
Table 3			
Animal no.	23	24	
Mortality	7	3	.92

Zhu-2003	Vit E	Cont	
Turkey			
Infected with			
Listeria monocytogenesis			
Experiment 1			
Animal no.	25	24	
Days of cloacal	1(8);	1(2);	.026
swabs becoming	4(16);	4(20);	
clean of *Listeria*	4+	4(2)+	

Huff-2004 [27]	Vit E	Cont	
Turkey			
Infected with *E. coli*			
Animal no.	60	60	
Mortality	0	8	.002
Isolation of *E. coli*			
from the liver	0	10	.000,4

Hill-1955 [28]	Vit E	Cont	
Chicken			
Infected with *Salmonella*			
Animal no.	40	40	
Mortality	7	15	.026

Heinzerling-1974b [29]	Vit E	Cont	
Chicken			
Infected with *E. coli*			
Animal no.	151	78	
Mortality	7	22	<.000,001

Tengerdy-1975 [30]	Vit E	Cont	
Chicken			
Infected with *E. coli*			
Animal no.	30	30	
Mortality	2	12	.000,2

Tengerdy-1977 [31]	Vit E	Cont	
Chicken			
Infected with *E. coli*			
Animal no.	42	39	
Mortality	10	19	.011

Likoff-1981 [32]	Vit E	Cont	
Chicken			
Infected with *E. coli*			
Animal no.	22	22	
Mortality in 2 d	8	18	.002
Aspirin groups			
Animal no.	22	22	
Mortality in 2 d	0	9	.000,4

[26] Mayorga et al. (2004) administered S. typhimurium lipopolysaccharide (LPS).

[27] Huff et al. (2004): data is from their figs. 2 and 4. There were 60 birds per group, and the mortality was calculated from the percentages in the figures.

[28] Hill et al. (1992): data is from their table 5. There were 40 birds per treatment group, and the mortality was calculated from the percentages in their table. The current vitamin E group is their group with 'basal diet+vitamins' and the current control group is their group with 'basal diet+vitamins–E' (vitamin E excluded).

[29] Heinzerling et al. (1974b): data is from their table 1. Trials 1 and 2 are combined, and two vitamin E groups (150 and 300 mg/kg diet) are combined to the current vitamin E group.

[30] Tengerdy & Nockels (1975): data is from their Table 3. Mortality is calculated from mortality percentage and the total number of chicken (n=30).

[31] Tengerdy & Brown (1977): data on experiment 1 (table 4: groups IE2 vs. IC) and experiment 2 (table 6: groups IE2 vs. IC) has been combined in the current table.

[32] Likoff et al. (1981) reported the mortality percentage in their table 5. The number of chickens per group is inferred from their table 1 footnote.

Downs-2000 [33]	Vit E	Cont	
Chicken			
Experimental *E. coli* cellulites			
Animal no.	200	200	
Chickens with cellulites lesions	84	102	.036

Yang-2000	Vit E	Cont	
Chicken			
Infected with *E. coli*			
HAS-chicks			
Animal no.	50	50	
Weight loss in 1 d			
Mean	6.4%	8.5%	.027
SD	5.7	5.0	
LAS-chicks			
Animal no.	50	50	
Weight loss in 1 d			
Mean	4.4%	3.5%	.8
SD	6.4	5.0	

Schildknecht-1979	Vit E	Cont	
Chicken			
Infected with *Histomonas meleagridis* –protozoa (histomoniasis)			
Animal no.	24	24	
Mortality	19	20	.4
Ipronidazole-treatment			
Animal no.	18	18	
Mortality	7	11	.11

Allen-2002 [34]	Vit E	Cont	
Chicken			
Infected with *Eimeria maxima* -protozoa			
Trial 1: strain ESS			
Animal no.	15	15	
Intestinal lesion score			
Mean	1.87	2.80	.015
SD	1.05	1.16	
Trial 2: strain Guelph			
Animal no.	10	10	
Intestinal lesion score			
Mean	3.6	3.3	.7
SD	1.27	0.63	

Furones-1992 [35]	Vit E	Cont	
Rainbow trout			
Infected with *Y. ruckeri*			
Animal no.	90	90	
Mortality	28	39	.047

[33] Downs et al. (2000): data was measured by the current author from their fig. 1, which gives the percentage of broilers with lesions. Since there were 800 broilers in the study with 4 study groups, it is assumed that there were 200 broilers per group.

[34] Allen et al. (2002): the current vitamin E groups are their 153 and 200 ppm groups, and control groups are their 13.2 and 13 ppm groups in their trials 1 and 2 respectively.

[35] Furones et al. (1992): data is for injected fish: the number of diseased fish was calculated from the mortality percentage.

Wahli-1998 [36]	Vit E	Cont	
Rainbow trout			
Infected with VHS virus			
Low vit C			
Animal no.	50	50	
Mortality	29	13	.9993
High vit C			
Animal no.	50	50	
Mortality	2	10	.008
Infected with *Y. ruckeri*			
Low vit C			
Animal no.	50	50	
Mortality	16	48	<.000,1
High vit C			
Animal no.	50	50	
Mortality	18	20	.4
Infected with *I. multifiliis* –protozoa			
Low vit C			
Animal no.	50	50	
Mortality	1	21	<.000,1
High vit C			
Animal no.	50	50	
Mortality	4	3	.6

Thorarinsson-1994 [37]	Vit E	Cont	
Chinook salmon			
Infected with *R. salmoninarum*			
Low selenium			
Animal no.	150	150	
Mortality	4	46	<.000,1
High selenium			
Animal no.	150	150	
Mortality	0	5	.015

Ito-1999	Vit E	Cont	
Yellowtail-fish			
Infected with the bacterium causing fish jaundice			
Animal no.	10	10	
Mortality	4	0	.98

[36] Wahli et al. (1998): the current vitamin E group refers to fish administered 0.8 g vitamin E/kg feed. Low vitamin C groups were not administered any additional vitamin C; high vitamin C groups were administered 2 g vitamin C/kg feed. VHS, viral hemorrhagic septicemia virus.

[37] Thorarinsson et al. (1994): data was measured by the current author from their fig. 3. The cumulative mortality at 324 days was calculated from the mortality percentage and the total number of fish at the start (n = 150).

Appendix 5. The effect of vitamin E on animal infections: Difference between the groups not specific to vitamin E

Study Animal Infection	Treatment		P [1]
Sabin-1940 [2] Mice Infected with VSV virus at 4 weeks of age	Vit E (wheat germ oil)	Cont	
<u>Table I</u> Effect of maternal diet Animal no. Fatal paralysis	15 4	15 8	 .08
<u>Table II</u> Prematurely weaned mice Animal no. Fatal paralysis	16 6	15 12	 .011
Teige-1977 Pig Experimental swine dysentery	Vit E+Se	Cont	
Animal no. Days before diarrhea	8 8;10; 11;12; 13; 14(2); 25+	8 7(3); 8(3); 11; 12	 .004
Teige-1978 Pig *T. hyodysenteriae* Table 2	Vit E+Se	Cont	
Animal no. Days before diarrhea	6 15;22; 23;30; 30+(2)	6 5;7; 10;15; 19;20	 .002
<u>Cod liver oil groups</u> Animal no. Days before diarrhea	6 30+(6)	6 6;8;9; 13;17; 21	 .000,3
Broner-1989 [3] Rabbitt Experimental *E. coli* sepsis Moxolactam-treatment	Vit E+	Cont	
Animal no. Mortality	12 9	12 8	 .6
Bennett-1999 Rat Infected with *E. coli* Ciprofloxacin-treatment	Vit E+A	Cont	
Animal no. Renal inflammation	8 1	8 8	 .000,4
Umar-1999 Rabbit Infected with *T. brucei*	Vit E+C	Cont	
Animal no. Log(parasites in blood/ml) SD	5 7.9 0.2	5 7.9 0.1	 .5
Sjunnesson-2001 [4] Guinea pig Infected with *H. pylori*	Vit E+	Cont	
Animal no. Gastritis score (scale 0 to 3) Mean SD	12 0.33 0.66	14 0.93 1.02	 .047

[1] The 1-tailed mid-P-value was calculated by the current author (see Table 7). The days before diarrhoea were analyzed using the log-rank test. Continuous data was analyzed using the t-test. The number in parenthesis (n) indicates the number of animals with the same outcome value; '+' indicates censoring.

[2] Sabin and Duffy (1940): data on maternal diet is from their table I, and on prematurely weaned mice from their table II. The 'control group' was administered casein, yeast, cod liver oil, and the 'vitamin E group' was administered wheat germ oil in addition to the basic diet. VSV, vesicular stomatitis virus.

[3] Broner et al. (1989) administered vitamin E along with superoxide dismutase, sodium thiosulphate, sodium diethylthiocarbamate, and desferoxamine to the antioxidant group.

[4] Sjunnesson et al. (2001): data was measured by the current author from their fig. 2. The current vitamin E group refers to diet 3: vitamins A, C and E, and selenium supplementation.