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REVIEW

Vitamin C and immunity: an assessment of the evidence

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SUMMARY

The high concentration of ascorbate in leucocytes and its rapid expenditure during infection and phagocytosis suggests a role for the vitamin in the immune process. Evidence published to date shows an involvement in the migration and phagocytosis by macrophages and leucocytes, as well as the induction and expression of delayed hypersensitivity. Its effect on antibody production and complement levels is controversial but probably minimal. This study suggests there is room for further investigation into the effect of ascorbate on immunity, particularly with defined populations, but cautions the use of megadose therapy.

INTRODUCTION

During the last 30 years the recommended 10 mg daily intake of ascorbate, a regimen based upon scurvy prevention, has been questioned by a number of authors. Recently, the challenge has been led by L. Pauling, both through the medical literature (Pauling, 1970a) and the lay press (Pauling, 1970b). The latter advocates a daily intake of 2 g for an adult, an estimate based upon the work of Bourne (1949) showing that gorillas eat approximately 4·5 g/day, and the study of Salaman & Stubbs (1961), who found that the rat, an animal which does not require dietary ascorbate, synthesizes 26–59 mg/kg/day, equivalent to 2–4 g/day for an adult human.

This essentially empirical argument has stimulated a series of clinical trials, many on a large scale and many of which have been ill-conceived and poorly controlled (Chalmers, 1975). Taken together with the fact that vitamin C has been proposed as a remedy for an array of seemingly unrelated human diseases (influenza, cancer, artherosclerosis, arthritis), it is hardly surprising that the overall reaction of the scientific community has been one of scepticism.

Controversy of this nature unfortunately serves to cloud what may well be an important medical issue. In summarizing the evidence pertinent to vitamin C and immunity, the review below will, hopefully, avoid adding needlessly to this controversy.

While it would appear that there is, as yet, no justification for the consumption of megadoses of ascorbate over an extended period, the available data does suggest an important role for this vitamin in certain manifestations of the immune response, particularly those involving leucocyte mobility. Furthermore, the rapid consumption of ascorbate by leucocytes during infection, and the depression in leucocyte ascorbate which appears to accompany a variety of situations associated with depressed immunological function, suggest that further, more detailed studies on the role of vitamin C in the immune response are warranted.

CLINICAL TRIALS

There is general agreement that ascorbate supplementation is ineffective in reducing the incidence of

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recently, however, Stankova *et al.* (1975) reported that neutrophils from scorbutic guinea pigs kill *S. aureus* and produce hydrogen peroxide as readily as normal neutrophils. These authors presented evidence that contaminating erythrocytes may have suppressed phagocytosis in Nungester's experiments—such contamination of peritoneal exudates from scorbutic guinea pigs is frequent and results from increased vascular fragility in these animals.

Goetzl *et al.* (1974), using tissue concentrations of ascorbate *in vitro*, found that ascorbate increased random migration of neutrophils, as well as migration induced by kallikrein or the complement component C5a. Identical effects were observed with monocytes and eosinophils. Sandler, Gallen & Vaughan (1975) demonstrated that ascorbate supplementation increases endotoxin-induced migration of leucocytes. The increased migration appears to result from increased HMP activity (Goetzl *et al.*, 1974); the ascorbate-stimulated neutrophils, as in other studies, showed no evidence of increased erythrophagocytosis.

Like the polymorphonuclear phagocytes, both peritoneal and alveolar macrophages concentrate ascorbate (Glick & Hosoda, 1965; De Chatelet *et al.*, 1974). The importance of adequate levels of ascorbate for normal macrophage function is indicated by the observations of Garguly, Durieux & Waldman (1976) on scorbutic guinea pigs; these animals exhibit a numerically and functionally (depressed migration) deficient population of macrophages in the peritoneal cavity. Macrophages from the scorbutic guinea pigs were also smaller than normal, but there was no defect in their ability to phagocytose *S. aureus*. Likewise, Mueller & Evans (cited by Kies *et al.*, 1964) were unable to demonstrate impaired phagocytosis in peritoneal exudate cells from scorbutic guinea pigs; however, these results were offered only as 'unpublished data' and are therefore difficult to assess. A defect in macrophage function in scorbutic guinea pigs was, however, noted by Kaw & Zaidi (1969), who reported that macrophages from these animals failed to aggregate in the lesions of experimental pulmonary silicosis.

The addition of ascorbate to cultures of normal macrophages has been found to increase motility (Goetzl *et al.*, 1974; Sandler, Gallen & Vaughan, 1975), as well as cyclic GMP levels (Sandler *et al.*, 1975) and HMP shunt activity (Cooper, McCall & De Chatelet, 1971). The effect of exogenous ascorbate on the phagocytic capacity of normal mouse macrophages has been studied by the authors (Fig. 1)—ascorbate supplementation increased phagocytic activity in a dose-dependent fashion.

(c) *Delayed-type hypersensitivity*

The first indication of a role for ascorbate in delayed-type hypersensitivity (DTH) reactions was provided by Mueller & Kies (1962), who demonstrated depressed responses to the mycobacteria in Freund's complete adjuvant in scorbutic guinea pigs—reversal of this energy was produced by dietary ascorbate supplementation. This group also reported that the induction of experimental autoimmune encephalomyelitis (EAE) in scorbutic guinea pigs by the injection of CNS extract in Freund's adjuvant was substantially suppressed relative to normal animals (Mueller *et al.*, 1962). The authors considered that the leucopenia induced by ascorbate deprivation was unimportant in this context, because similar depletion caused by X-rays did not suppress the development of EAE, and they further showed that the immunological defect in the scorbutic animals was not restricted to depressed reactivity to the adjuvant (Kies, Mueller & Alvord, 1964).

In an extensive study, Zweiman, Schoenwetter & Hildreth (1966b) established that scorbutic guinea-pigs, anergic to mycobacteria, possessed sensitized lumphocytes which could transfer DTH to normal animals. In contrast, sensitized lymphocytes from normal animals were ineffective in transferring DTH to scorbutic recipients. Subsequently, it was shown that proliferative responses of lymphocytes from the scorbutic animals above to phytohaemagglutinin (PHA) and old tuberculin *in vitro* were normal (Zweiman, Besdire & Hildreth, 1966a). These authors also noted that the inflammatory response of these scorbutic animals to a non-specific irritant was depressed (Zweiman *et al.*, 1966b), and suggested that the failure to manifest DTM may reflect a defective migration of recruited cells to the site of challenge, rather than a central defect in lymphocyte function. This suggestion is compatible with the observations above on defective macrophage migration in the scorbutic state, and may also reflect defects in the microvasculature which result from ascorbate deprivation. However, although such defects in the effector

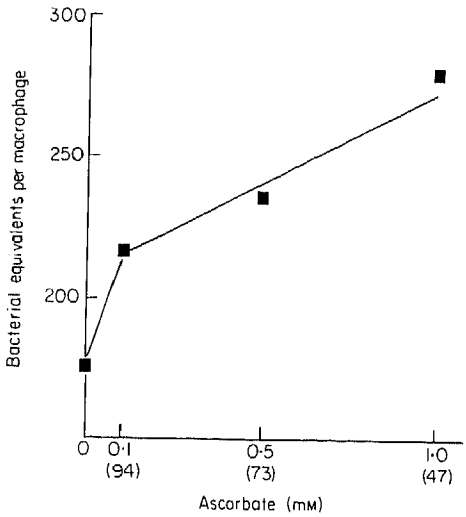

FIG. 1. Phagocytic activity of peritoneal macrophages after incubation with media supplemented with ascorbate. Monolayer cultures of mouse peritoneal macrophages were incubated for 20 hr with the concentration of ascorbate shown. Media containing fresh ascorbate was then added and after 1 hr their ability to phagocytose heat-killed radiolabelled *Pseudomonas aeruginosa* was measured. Results are the mean of three cultures and show the amount of radioactivity, in bacterial equivalents, accumulated after 2 hr incubation in media containing $18{\cdot}8 \times 10^7$ bacteria per ml. Details of the method used have been published (Thomas, Holt & Keast, 1974). Some cell death occurred during the incubation and the percentage viability of the cultures is shown in parentheses.

stage of the reaction are likely to contribute to the failure of scorbutic animals to develop DTH, they do not appear to be the sole cause, as animals returned to normal diets after sensitization still fail to respond (Zweiman *et al.*, 1966b). The failure of scorbutic animals to develop EAE in the model described above was also suggested to stem from a defect in induction, as opposed to expression, of DTH; this conclusion was based upon the author's successful demonstration of positive skin reactions in scorbutic animals which had been sensitized prior to vitamin C deprivation (Kies *et al.*, 1964).

In a more recent study, Kalden & Guthy (1972) have clearly demonstrated that adequate dietary ascorbate is a prerequite for normal skin graft rejection.

(d) *Immediate hypersensitivity*

Some evidence indicates that ascorbate may play a role in the immediate hypersensitivity reactions. Exposure of leucocytes from allergic individuals to specific allergens *in vitro* depressed their ascorbate uptake (Wilson, Loh & Watters, 1975). This finding is not open to clear interpretation, and likewise the reported ability of ascorbate to potentiate antihistamine(s) in their protective action in anaphylaxis (Csaba & Toth, 1971) could be due to several unphysiological mechanisms. It is, however, possible that these effects are related to the fact that mast cells contain extremely high levels of ascorbate, approximately three times as much as macrophages (Glick & Hosoda, 1965).

Kumar & Axelrod (1969) compared the capacity of scorbutic and normal guinea pigs, with similar titres of antibody to diphtheria toxoid, to mount Arthus-type reactions following challenge with this antigen. Skin reactions in the scorbutic groups were markedly depressed. However, a similar reduction in the reaction of scorbutic guinea pigs to the non-specific irritant, histamine, was also observed by these workers. This latter observation parallels that of Zweiman *et al.* (1966b) mentioned above.

(c) *Antibody*

The early literature on vitamin C and immunity suggests an important role for ascorbate in the humoral immune response, as the addition of ascorbate to immunizing doses of antigen appeared to increase antibody production (reviewed by Bourne, 1949) and deprivation apparently reduced the response (Long,

1950). The latter author claimed that while scorbutic guinea pigs mounted normal primary responses, their secondary responses were depressed thirty-fold. However, these results have been challenged by more recent workers. Kumar & Axelrod (1969) repeated the study of Long (1950), which involved measuring primary and secondary responses to diphtheria toxoid in normal and scorbutic guinea pigs, under more controlled conditions and employing more sophisticated methodology. Kumar & Axelrod (1969) failed to detect any deficiency in either the primary or the secondary responses of the scorbutic animals. They pointed out, however, that their study differed from that of Long (1950) in two important respects. In contrast to Long, they used a highly purified diet, which induced a severe deficiency state; Long's diet induced only mild ascorbate deficiency, but was also shown to induce methionine deficiency and result in abnormally low sulphydryl levels in tissues. Secondly, the two groups employed different techniques to assess serum antibody levels, which other workers have shown to correlate poorly (Stavitsky, 1954). The observations of Kumar & Axelrod are in accord with those of Simola & Brunius (1933), who found no defect in the ability of scorbutic guinea pigs to respond to sheep red cells.

Murphy *et al.* (1974) reported that ascorbate supplementation did not affect antibody responses in marmosets challenged with parainfluenza virus. However, they also observed a reduction in symptoms in the ascorbate-supplemented group, which may indicate depressed multiplication of the virus and, as a consequence, reduced stimulation of the immune response.

(f) *Complement*

Studies on the relationship between ascorbate and complement have produced contradictory results. Marsh (1936) claimed that complement titres in scorbutic guinea pigs were reduced, and Ecker *et al.* (1938) and Ecker & Pillemer (1939) claimed that if these animals were given graded doses of ascorbate, the larger doses of this vitamin were paralleled by increases in the complement titre. The apparent conclusiveness of these studies was, however, challenged by other authors. Zilva (1936) and Kodicek & Traub (1943) found no significant alteration in complement levels in scorbutic guinea pigs, while Simola & Brunius (1933) found only a slight effect. Maccolini (1939) reached similar conclusions, and further claimed that ascorbate supplementation had no effect on complement titres.

In man, the weight of evidence does not support a role for ascorbate in maintaining complement levels. Crandon, Lund & Dill (1940) and Spink, Mitchelson & Dahl (1941) found that complement titres did not alter in human scurvy, and Deeny, Murdoch & Rogan (1943), in a study of eighty patients with acute infections, found no relationship between blood vitamin C levels and complement titre. However, an association of high blood vitamin C and complement levels has been reported (Chu & Chow, 1938).

Recently, it has been shown that ascorbate can repair oxidative changes induced in (canine) complement components both *in vivo* and *in vitro* (Boyer, Wyde & Brer, 1975), but the relevance of this observation to man is obscure.

Hughes (1977), in pointing to the central role of ascorbate in collagen production, observed that a collagen-like amino acid sequence was also characteristic of the C1q subcomponent of complement (Reid, 1974), and suggested that at least some of the so-called 'extra-antiscorbutic' involvement of ascorbate may prove to be explicable in terms of a mechanism related to its mode of action in preventing classical scurvy. On face value, this suggestion would not appear to be compatible with the evidence above from the early literature on complement activity during ascorbate deficiency.

(g) *Interferon*

Three recent reports implicate ascorbate in interferon activity. Siegel (1974) reported that mice fed on an ascorbate-supplemented diet displayed augmented levels of circulating interferon after stimulation with murine leukaemia virus, and in a later communication (Siegel, 1975) demonstrated a similar phenomenon *in vitro* employing cultures of murine L cells and embryonic fibroblasts stimulated with polynucleotides. Subsequently, Dahl & Degre (1976) obtained comparable results with human embryonic fibroblasts stimulated with Newcastle disease virus or polynucleotides, but were unable to demonstrate a stimulatory effect of ascorbate on human lymphoblastoid cell lines. The authors noted that human fibroblasts and leucocytes produce at least two distinctly different species of interferon, and suggested

that the divergent effects of ascorbate in this context may reflect differences in the production pattern or release mechanisms of this lymphokine in different cell types.

Dahl & Degre (1976) also observed that leucocyte interferon assayed in lung fibroblasts titrated 0·2–0·3 $\log_{10}$ units higher in the presence of 5·0 μg ascorbate than in the absence of the latter.

DELETERIOUS EFFECTS OF ASCORBATE SUPPLEMENTATION

Birkaug (1939) reported that if he injected guinea pigs with tubercle bacilli and administered 10 mg/day ascorbate to the animals, there was a significant reduction in the tuberculin reaction in these animals in relation to controls. Heise & Steenken (1939) were unable to confirm this observation, but Steinbach & Klein (1941) found that administration of ascorbate to tuberculous guinea pigs increased their tolerance to repeated large doses of tuberculin. Subsequently, Long, Miles & Perry (1951) demonstrated depressed DTH development in guinea pigs maintained for prolonged periods on ascorbate supplementation (20 mg thrice weekly).

A factor which may have contributed to these results was recently suggested by Hughes (1977)—that ascorbate catabolism becomes geared to the rapid rate of breakdown necessary to accommodate the high tissue levels produced by supplementation. The consequences of such an eventuality would obviously vary considerably between different ascorbate regimens, but it is conceivable that if accelerated catabolism induced by megatherapy is not matched by subsequent vitamin intake, ascorbate deficiency may ensue. This effect has been observed both in man and experimental animals (Rhead & Schrauzer, 1971; Sorenson, Devine & Rivers, 1974). It is also possible that transient deficiency may occur in experimental situations during regular ascorbate supplementation, notably in situations where several days elapse between subsequent megadoses of the vitamin.

CONCLUSIONS

Research on the effects of vitamin C on host defence has, regrettably, proceeded in a piece-meal fashion over the last 30 years. As a result, the literature in this field is bedevilled by controversy and lack of confirmation, and indeed it is difficult to reach a concensus on many of the proposed roles for ascorbate in the immunological processes. Studies employing experimental animals have been particularly confusing; not only do models featuring deprivation as opposed to supplementation often appear to yield divergent results, but in numerous situations opposite results have been obtained in different laboratories examining essentially similar questions.

On the positive side, animal studies have yielded evidence that ascorbate is involved in leucocyte migration and phagocytosis, the induction and expression of hypersensitivity, and perhaps interferon production. However, there is a singular lack of evidence in support of a role for this vitamin *per se* in defence against infection.

Studies in man, while still controversial, nevertheless serve to place a number of these observations in perspective. Firstly, there is ample evidence that ascorbate supplementation is ineffective in reducing the incidence of cold and winter illness, but there is also general agreement that it may be effective in reducing (albeit modestly) symptoms—this suggestion has been borne out by one study in non-human primates. The exact role of ascorbate in this context has yet to be defined, but the high levels of this vitamin in cells of the immune series, its rapid expenditure during infection and phagocytosis and the claimed relationship between severity of illness and leucocyte ascorbate levels provide avenues for future investigation.

It is doubtful, at this stage, whether further studies involving the scorbutic state in animals would provide significant insight into the role of ascorbate in host defence. Severe ascorbate deficiency is no longer an important problem, and the participation of this vitamin in immunological mechanisms in this situation is likely to be qualitatively and quantitively different to that in the more mild deficiency states encountered clinically. A more realistic approach would appear to involve investigating the role of ascorbate in defined groups known to manifest varying degrees of deficiency, e.g. the aged, the insti-

tutionalized, patients on immunosuppressive drugs and pregnant women. A pointer to such studies is provided by Chretien & Garagusi (1973), who demonstrated that defective neutrophil function in patients undergoing steroid therapy was corrected by ascorbate supplementation.

Future clinical trials on the use of vitamin C in the modification of cold symptoms may also benefit from the use of such defined groups, and in view of the claimed relationship between leucocyte ascorbate and severity of illness, should also include the measurement of levels of this vitamin in the subjects under test. The design of such trials should also take due consideration of the possible side effects of prolonged megatherapy.

It would also appear that the dosages employed in most clinical trials are unnecessarily high, as it has been shown that only a small proportion of an administered megadose is incorporated into the body ascorbate pool (Hodges *et al.*, 1971) and recently a daily supplement of 100 mg was shown to produce essentially the same concentration of leucocyte ascorbate as a daily megadose of 1·0 (Hughes, 1977).

The possible long-term risks of vitamin C megatherapy are only beginning to become apparent. Apart from earlier suggestions of interference with hypersensitivity mechanisms, there have been suggestions of hypovitaminosis after withdrawal of supplementation (Rhead & Schrauzer, 1971), effects on the foetus resulting from maternal megatherapy (Cochrane, 1965; Hughes, 1977), familial-associated disturbances in oxalate production (Briggs, Garcia-Webb & Davies, 1973; Briggs, 1976), enhancement of metal toxicity (Hughes, 1977; Blackstone, Hurley & Hughes, 1974; Murray & Hughes, 1976), gastrointestinal disturbances (Hume & Weyers, 1973), depressed detoxification of dietary cyanide (Basu, 1977) and in drug metabolism (Houston & Levy, 1975; Basu, 1977). Finally, there have been recent indications that ascorbate metabolites have mutagenic properties (Stick *et al.*, 1976). As considerable amounts of the latter are ingested daily in foodstuffs treated with ascorbate during processing (Hughes, 1977), a detailed characterization of their formation and activity would appear to be long overdue.

In summary, there is evidence for a positive role for ascorbate in some aspects of host defence, but no justification for prolonged or even short-term megatherapy. The self-prescribing public should be made aware that a daily intake of 100–150 mg is sufficient to attain tissue saturation, and be further informed of the potential dangers which may accompany the intake of megadoses over long periods.

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