THE EFFECT OF AN EXCESS OF VITAMIN C ON THE NATURAL RESISTANCE OF MICE AND GUINEA PIGS TO TRYpanosome INFECTIONS.¹

BY

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The interest in the importance of vitamins in the diet on the bodily well-being and the accumulation of evidence of the intimate dependence of natural resistance to infection on dietary factors has led investigators to test the value of an excess of such factors on resistance. Much of such work has been disappointing. It may be that a deficiency of a factor essential to normal cellular metabolism results in a depression of the organism to abnormal stimuli as infection, toxemia and the like. It does not necessarily follow that an excess above the bodily requirements would increase the natural resistance of the host above the normal.

However, in the case of vitamin C, sufficient evidence has accumulated to suggest the importance of ascorbic acid in oxidation reduction processes of cellular metabolism and it is conceivable that an increase in its availability might raise the capacity of the tissues to 'destroy toxic substances introduced into the body. For a review on the role of vitamin C in resistance, see Perla and Marmorston (1).

Previous work of other investigators has shown that a moderate excess of ascorbic acid may raise the natural resistance of guinea pigs to diphtheria toxin (Harde (2), Harde and Phillippe (3), Greenwald and Harde (4), Jungeblut and Zwemer (5)). In in vitro experiments, both diphtheria toxin and poliomyelitis virus may be neutralized (Jungeblut (6)). Jungeblut (7) succeeded in increasing the natural resistance of monkeys to poliomyelitis. Of a total of 62 treated monkeys, 19 survived without paralysis, but of 38 untreated controls, 36 succumbed. Vitamin C in amounts greater than 50 milligrams a day were found to be ineffective. The clinical use of large quantities of ascorbic acid in the treatment of diphtheria either alone or as a supplement to specific serum therapy has not yielded encour-
aging results (Bamberger and Wendt (8)). Otto (9) states that ascorbic acid at best tended to modify to some degree the hemorrhagic tendency, particularly if a thrombopenia was present; Steinbach and Klein (10) report that an excess of ascorbic acid given to tuberculous animals decreased their sensitivity to tuberculin. Solomonica (11) has found that vitamin C in excess may enhance the resistance of guinea pigs to anaphylactic shock, particularly if the vitamin C is administered prior to sensitization. The literature on the effect of an excess of ascorbic acid-containing foods in human infections as tuberculosis is of little value. It is suggested, though as yet not well established, that an excess of vitamin C raises the resistance of animals to intestinal tuberculosis (12). Therapeutically, in tuberculous animals, great excess of vitamin C administered for Jong periods is apparently of little value in modifying the severity of the disease (Heise and Martin (13)).

It must be emphasized that a relative insufficiency of vitamin C increases the susceptibility of an animal to the effects of an infection, and further, that in the presence of chronic infection, the need of vitamin C is definitely increased (1).

**EXPERIMENTAL.**

*Trypanosoma equiperdum* infection in mice.

An excess of vitamin C had no influence in modifying the course of an infection with *Trypanosoma equiperdum* in mice. The mice were on a complete and adequate diet consisting of rolled oats, hominy, meat scrap, dried milk, salt, with additions daily of fresh milk, yeast, cod liver oil and wheat germ. Fresh greens were added twice a week. The animals thrive and gain rapidly on this diet. The mice were bred from a strain obtained from the Rockefeller Institute for Medical Research and were free from latent infections, such as *Eperythrozoon coccoides*, *Bartonella muris* and *Klossiella muris*. The strain of *Trypanosoma equiperdum* originally carried in rats was passed through guinea pigs for several transfers, to separate it from *Bartonella muris*, as this latter organism is destroyed in the guinea pig. The vitamin C was administered as daily intraperitoneal injections of a solution of synthetic ascorbic acid in amounts of 1 mg. per day per mouse, during a period of 14 clays prior to the injection of trypanosomes, and continued thereafter. In preliminary experiments, the minimal effective dose of trypanosomes was determined necessary to produce an infection in the normal animal. Ten to 30
times this amount was used in the experiment to ensure uniform takes. The experiments were repeated three times on different occasions, using 20 mice for each test including equal numbers of controls in each experiment. The course and the duration of the infection was studied.

No modification of the infection was observed in any of the experiments as a result of injections of ascorbic acid. The resistance of the mice was not modified by an excess availability of ascorbic acid.

These experiments, though suggesting that the resistance of mice to a protozoan infection cannot be altered by an excess of vitamin C parenterally administered, were done in an animal known to produce its own vitamin C. It is well established that scurvy cannot be produced in rats or mice deprived completely of vitamin C during as long a period as 3 generations (Simmonds (14)). It was felt that since the rats and mice are capable of producing their own vitamin C, studies on the effect of an excess of this factor on resistance could not be interpreted.

Trypanosoma brucei infection in guinea pigs.

In another series of experiments, therefore, the effect of repeated injections of ascorbic acid on trypanosonie infection in guinea pigs was studied. The guinea pigs were of a uniform stock, raised in the laboratory for more than 10 years on a uniform adequate diet of oats, alfalfa hay, greens ad lib, and water. The ascorbic acid was injected daily intraperitoneally in amounts of 10 mg, dissolved in physiological saline and brought up to a pH of 7.5 just prior to use. The injections were continued during a period of 42 days prior to the injection of trypanosomines and thereafter to the death of the animal. In the first experiment, 12 guinea pigs, 3 to 4 months of age, were treated with ascorbic acid, and 12 of the same age were used as controls. All received 30,000 trypanosomes intraperitoneally.

Of the treated pigs, only 2 developed infections, and died within 25 and 30 days, and 10 escaped infection. Of the controls all succumbed.

In a second experiment, the same daily quantities of vitamin C were given during a period of 77 days prior to the injection of Try-
panosoma brucei Thirty thousand trypanosomes were used as the infecting dose. Of the 7 guinea pigs treated, all succumbed to the infection within a period of 11 to 27 days (average 19 days). Of the 7 controls, 5 succumbed and 2 escaped infection. The duration of the infection varied from 19 to 31 days, an average of 26 days. The interval between injection of the trypanosomes arid the appearance of organisms in the bloodstream was 7 to 9 clays in the control and 5 to 7 days in the vitamin C-treated guinea pigs. The treated animals lost considerably in weight. Apparently, the continued injection of 10 mg. of vitamin C for a long period may be deleterious and toxic and may lower the resistance of the host.

In view of the results of the earlier experiments, the effect of an excess of vitamin C on resistance to infection with *Trypanosoma brucei* in guinea pigs was again tried. To ensure an adequate vitamin C content in the diet of the control animals, all the guinea pigs received a minimum of 1 mg. of ascorbic acid intraperitoneally, administered each day. Twenty-one guinea pigs, 3 weeks old, were placed on this regimen in addition to the usual stock diet. When they were 2 months old, they were utilized in the experiment in the following manner. Eleven guinea pigs were continued on the same dietary and vitamin C regimen. Ten guinea pigs were given daily 10 mg. of synthetic ascorbic acid intraperitoneally. After a period of 42 days, all the guinea pigs received 50,000 trypanosomes intraperitoneally. The strain of *Trypanosoma brucei* had been carried for several passages in rats and only one passage in guinea pigs to diminish the virulence for guinea pigs. The vitamin C administration was continued to the end of the experiment.

All the animals became infected. Of the 10 guinea pigs given the excess of vitamin C for 42 days, the average duration of life was 76.3 days, but no guinea pig of this group died before 64 days after injection of the trypanosomes. Of the 10 guinea pigs given an adequate amount of vitamin C (1 mg. per day above the content in the food), the average duration of life was 56.1 days. However, in this group, 3 animals were dead in less than 80 days and 7 in less than 60 days. Whereas 70 per cent of the controls had died within 60 days of the injection of the trypanosomes, none of the guinea pigs that were receiving 10 mg. of ascorbic acid had died up to that time.

The ascorbic acid was introduced into the peritoneal cavity where the trypanosomes were subsequently placed. The question may be raised that some non-specific changes in the peritoneal lining may have been induced by the material containing 10 mg. of vitamin C
and not called forth, or only to a less extent, by that containing only 1 mg. This is not likely since 110 manifest evidence of irritation, or inflammation was produced by the intraperitoneal injection of vitamin C. If the degree of permeability of the peritoneum were in some manner altered by the larger amounts of vitamin G, one might expect some delay in the appearance of the trypanosomes in the blood; but such was not the case. The severity of the infection as manifested by the height of the number count was unaffected, and the incubation period between introduction of the trypanosomes and their appearance in the circulation was the same in both groups. The capacity of the animals to survive the infection induced was modified and therefore the duration of life was greater in those animals receiving vitamin C in amounts of 10 mg. per day.

It is evident, therefore, that a moderate excess of vitamin C administered for a period of 6 weeks prior to infection and throughout its course raised the natural resistance to an induced *Trypanosoma brucei* infection in animals of known non-scurvy stock in which the C content was rigidly controlled. In general, animals under such conditions may either develop abortive infections, or if a fatal one supervenes may live considerably longer than control animals. This effect was not consistently obtained for when the vitamin C was administered in similar amounts for a longer period prior to infection no favorable effect on the course of the disease was observed. At present we are unable to explain this discrepancy. It may be that after prolonged administration by the intraperitoneal route large amounts of vitamin C may have a deleterious effect.

DISCUSSION.

The importance of vitamin C in resistance is secondary to its essential role in the maintenance of normal cellular metabolism \(^3\) (see review of Perla and Marmorston (1)). By its withdrawal cellular respiration is promptly inhibited and the threshold level of tolerance to poisons, infections or other types of injury is necessarily depressed. It is apparent that an excess of ascorbic acid may raise the threshold level of tolerance, but such effect by the nature of the chemical processes involved is necessarily limited in its possibilities. Since it has been shown that a latent scurvy may be converted into clinical disease in the presence of a mild infection, it is apparent that the physiological need for vitamin C must be greater in conditions in which the

\(^3\) For the chemistry of vitamin 0 mid its function in cell physiology see King (19) and Bourne (20).
rate of cellular metabolism is increased. In the presence of infection, therefore, an increase in the intake of vitamin C may be of considerable importance.

It has been suggested by the work of Harde (2), Harde and Philippe (3), Greenwald and Harde (4), Jungeblut and his coworkers (5, 6), Kligler (21) and others that vitamin C may have a direct detoxifying effect on bacterial toxins, and according to Kligler (21) and Gagyi (22) that its value in the treatment of infectious diseases may be dependent on the inhibitory action of vitamin C on bacterial growth as well as toxin formation. It would appear that the action \textit{in vivo} of vitamin C either as a direct bactericidal or detoxifying agent is problematic. It is doubtful whether such a high degree of saturation of the cells of the body with vitamin C could be attained, as is apparently necessary for a bactericidal action comparable to the \textit{in vitro} experiments. Not only is this improbable clue to the rapidity with which vitamin C is excreted in the urine when administered in excess, but vitamin C in very large amounts may prove deleterious. It is more probable that it exerts its influence independently of any direct bactericidal effect by its role in oxidation reduction processes in cellular respiration.\footnote{The influence of vitamin C in resistance is dependent in part on its importance in the production of intercellular cement substances. Its absence inhibits reparative processes secondary to injury in connective tissue and bone (Wolbaeh and Howe (23)). Ascorbic acid may accelerate the rate of proliferation of monocytes (Baker (24)). It is essential also, in the respiration of erythrocytes.}

Granting the importance of vitamin C in tissue respiration, are we certain of what constitutes the optimal vitamin requirement? Can the criteria of growth, progressive weight increase and the absence of clinical evidences of deficiency be accepted as adequate? It is possible for all these to be present and still in the event of a given stress, such as invasion with microorganisms, or injections with poisons, the apparent optimal requirements for normal conditions prove to be inadequate.

In view of these facts, the least we may demand of experiments on the effects of an excess of a vitamin on resistance is rigid control of the stock from which the experimental animals are drawn. The animals used should be of the same stock, the history of which is known. The dietary regimen of the mother prior to the birth of the young, as well as the experimental animal since birth, should be under control and adequate in vitamin content. A relative deficiency during the early weeks after birth may cause a permanent impairment.
iii resistance of the host regardless of subsequent normal diet. A rise in natural resistance of a group of animals given a vitamin in excess above a control group, for a short period, may not indicate an increase above the normal optimum, but may suggest that a relative deficiency of the vitamin had previously existed in both groups. Unless such precautions are used, it is difficult to apply the implications, from experimental evidence in animals of unknown source, of the effects of a temporary excess of a vitamin on natural resistance directly to human infection. We believe we have at least observed these essential precautions.

SUMMARY.

An excess of vitamin C administered for a period of 2 weeks prior to infection and throughout its course had no influence on the natural resistance of mice to *Trypanosoma equiperdum* infection.

A moderate excess of vitamin C administered for a period of 6 weeks prior to infection and throughout its course raised the natural resistance to *Trypanosoma brucei* infection in guinea pigs of known stock in which the dietary content of vitamin C had been rigidly controlled. These animals either developed abortive infections or if a fatal one supervened lived considerably longer than control animals. This result, however, was not consistently obtained for when ascorbic acid was administered in the same daily quantities for a longer period of time prior to the injection of the trypanosomes, a favorable effect on the course of the disease was not observed.

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