

General Review

ROLE OF VITAMIN C IN RESISTANCE

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NEW YORK

INTRODUCTION

Though the importance of vitamin C in the maintenance of resistance to infection ¹ has gathered support from numerous experimental and clinical observations in the past two decades, it is only in recent years that the problem has been approached systematically. In a recent review on the role of the adrenal glands in resistance, we emphasized that the mechanism of natural resistance is dependent on the maintenance of normal cellular metabolism and that procedures that impair the processes of oxidation and reduction depress the resistance of the somatic cells to all abnormal stimuli whether these are marked variations in temperatures or poisons, toxins or infectious agents. We further stressed that when the physiologic action of the adrenal cortical hormone is better understood, and when the chemical nature of the life-prolonging hormone of this gland is identified and the relation of the hormone to oxidation-reduction systems and cevitamic acid (introduced as ascorbic acid) is determined, a new approach to the problem of the mechanism of natural resistance in the body may be available.

The stimulus to this conception and to work in this field was greatly enhanced by the recent work on the close relationship between adrenal cortical function and cellular oxidation. In studying oxidation systems in plant life, Szent-Györgyi - succeeded in isolating hexuronic acid from cabbage and other plants. He found large amounts of this strongly reducing substance in the adrenal cortices of animals. In other tissues, it probably does not occur in comparable amounts. From its chemical properties this compound is apparently important in certain oxidation-reduction systems of cellular metabolism. Independently, Svirbely and

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1. For recent reviews on the general subject of diet and resistance, see E. C. Robertson (Medicine 13:123, 1934) and S. W. Clausen (Physiol. Rev. 14:309, 1934).

2. Szent-Györgyi, A.: Biochem. J. 22:1387, 1928.

Szent-Györgyi³ and Waugh and King⁴ identified hexuronic acid with the antiscorbutic vitamin C, and King- isolated the same compound from orange and lemon juice concentrates.

This work opened a new field in the physiology of the adrenal cortex in relation to the utilization of vitamin C. Cevitamic acid is not identical with the life-prolonging hormone of the adrenal cortex, and more recent studies have failed to substantiate the theory that the adrenal cortex is essential to the production and physiologic activity of vitamin C. Nevertheless, there is probably a close interrelationship between the vitamin and the adrenal cortical hormone in their influence on certain anaerobic oxidation-reduction systems in cellular metabolism.

The availability of crystalline synthetic vitamin C offered an excellent opportunity for studying its relation to resistance and bodily functions. Its importance in the treatment of certain hemorrhagic diatheses has been suggested by Szent-Györgyi⁵ and others. That an excess of vitamin C in the diet will appreciably raise the natural resistance of the organism is intimated by recent work in this field, but much remains to be established.⁶

3. Svirbely, L. J., and Szent-Györgyi, A.: *Biochem. J.* 26:865, 1932.

4. Waugh, W. A., and King, C. G.: *Science* 76:630, 1932.

5. Szent-Györgyi, A.: *Verhandl. d. Gesellsch. f. Verdauungs- u. Stoffwechselkr.* 12:49, 1934.

6. Recently St. Rusznyak and Szent-Györgyi (*Nature*, London 138:27, 1936) described the isolation of a new vitamin factor (P) which is intimately associated with cevitamic acid. Previous work had led to the suggestion that cevitamic acid is accompanied in the cell by a substance of related activity and similar importance. In the absence of vitamin C, the symptoms of scurvy conceal the evidence of the deficiency of this factor. It was previously found that in certain pathologic conditions, such as the vascular type of hemorrhagic purpura, in which there are increased permeability and fragility of the capillary walls, cevitamic acid is ineffective. Such a condition can be readily cured, however, by the administration of extracts of Hungarian red pepper. These extracts are also effective in cases in which the capillary walls show permeability to protein only.

When fractioned, the active principle was found to be almost entirely flavonol. When used intravenously in the treatment of hemorrhagic purpura in amounts of 40 mg. a day, the spontaneous bleeding and evidence of capillary fragility ceased within two weeks.

The vitaminoid nature of the phenylbenzo-7-pyrone dyes was demonstrated in a subsequent report by St. Rusznyak and Szent-Györgyi (*ibid.* 138:798, 1936). A group of animals was given a scurvy-producing diet and another group the same diet plus 1 mg. daily of "citrin," the crystalline flavone fraction (vitamin P) of lemon juice. The animals receiving the scurvy-producing diet alone died in twenty-eight days with progressive loss in weight whereas the animals given this diet plus flavone lived, on an average, forty-four days. Both groups of animals showed signs of scurvy, with fragility of the bones, looseness of the teeth and swelling of the joints, but the number of hemorrhages in the flavone-fed animals was

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II. EFFECT OF DEFICIENCY OF VITAMIN C ON THE FORMATION
OF NATURAL ANTIBODIES

In a comprehensive study of the influence of deficient nutrition on the production of complement and amboceptor, Zilva⁷ did not detect any difference in the content of natural antibodies in normal guinea-pigs fed an unrestricted mixed diet and those fed a scorbutic diet.

Koch and Smith⁸ followed the variation in the titer of complement during the development and cure of scurvy in the guinea-pig. Twelve guinea-pigs were fed a diet of autoclaved soy bean meal, dried yeast, salt, cod liver oil and dried boiled cabbage. On this diet scurvy developed in from thirteen to sixteen days. The substitution of fresh cabbage for the dried boiled cabbage was sufficient to bring about normal growth. The titer of the complement was determined twice during the period prior to the dietary experiment, once during the height of scurvy and once after a period of starvation, when the animals had lost 20 per cent of their weight. The observers noted that during the period of scurvy the titer of the complement was appreciably increased and that during convalescence it fell but was slightly higher than normal. Starvation resulted in definite lowering of the titer. They suggested that there is an obscure connection between the complement of the serum and the natural effort of the body to compensate for the physiologic disturbance incident to scurvy. This hypothesis does not seem tenable.

Hamburger and Goldschmidt⁹ observed no appreciable difference in titer of the complement between normal and scorbutic guinea-pigs or children. A slight increase in the complement of scorbutic subjects may be due to an increase in the protein content of the blood. The titers of the bacteriolysin and amboceptor of the serum of scorbutic animals were the same as those found for the normal animals. In testing for bacteriolysins, they employed Langer's method of mixing 0.1 cc. of serum with 0.5 cc. of a culture of *Bacterium coli* on cover

strikingly less than the number in the other group. Vitamin P thus seems to have a marked and somewhat specific influence on the capillary system.

Experimental scurvy is apparently caused by the combined lack of vitamins C and P. Pure avitaminosis P presents no clinical symptoms, but the withholding of vitamin P in avitaminosis C will greatly modify this pathologic condition.

The authors, in a subsequent note (*ibid.* 139:326, 1937), suggested that the therapeutic effects observed after administration of "citrin" in septic conditions and conditions accompanied by polyarthritis and endocarditis in man are partly due to its vitamin P content. The data on which this assertion is based have apparently not been published as yet.

7. Zilva, S. S.: *Biochem. J.* 13:172, 1919.

8. Koch, M. L., and Smith, A. H.: *Proc. Soc. Exper. Biol. & Med.* 21:366, 1924.

9. Hamburger, R., and Goldschmidt, L.: *Jahrb. f. Kinderh.* 100:210, 1923.

slips in Petri dishes. After four hours' incubation of the culture, agar was poured into the dishes. The culture was then kept at 37 C. for twenty-four hours, and thereafter the colonies were counted. The use of *Bact. coli* was suggested because scorbutic infants frequently have pyelonephritis. Similar results were reported by Werkman, Nelson and Fulmer¹⁰ from a study of the opsonic indexes of normal and scorbutic guinea-pigs to *Bacterium typhosum* and *Staphylococcus albus*.

Summary.—The natural antibodies are not depressed in scurvy, but complement may be increased.

III. EFFECT OF DEFICIENCY OF VITAMIN C ON THE FORMATION OF SPECIFIC ANTIBODIES

Scorbutic guinea-pigs showed no change in their capacity to form agglutinin against *Bact. typhosum* (Zilva⁷). Similar observations have been reported by Werkman, Nelson and Fulmer.¹⁰

Starvation and undernutrition disturbed the formation of antitoxin to diphtheria toxin in immature rats and guinea-pigs but had no effect on the development of immunity to *Streptococcus erysipelatis* in rabbits (Bieling¹¹).

Bieling used 6 young rabbits, to 3 of which he gave a diet of oats, cloves and turnips, and to 3, only hay and a little clover. The animals were kept on these diets for about six weeks and were then given subcutaneous injections of increasing amounts (0.5, 0.7, 1, 1.5 and 2 cc.) of a culture of *Str. erysipelatis*. Seven days after the last injection, the immune body content of the serum was determined by means of protection tests, in mice. The tests were repeated two and three weeks later. The titer of immune bodies in the serum as thus determined was perhaps slightly higher for the starved than for the well fed rabbits.^{11a} The differences were not statistically significant.

In subsequent studies Bieling observed that rats fed on a diet free from vitamin C did not produce antitoxin in sufficient concentration following injection of tetanus toxin to protect them against a dose of tetanus poison which the immunized well nourished rats all survived. Deficiency of vitamin C cannot be established in the rat, as this animal is capable of producing its own vitamin C. The depression in antitoxin was probably associated with deficiency in other dietary factors.^{11b}

In another short experiment he fed guinea-pigs a scorbutic diet and on the fourth and ninth days after the onset of the experiment gave these animals, together with an equal number of controls, injections of formaldehydized tetanus toxin. Twenty-one days after the first immunizing injection, all the animals received a mixture of tetanus

10. Werkman, C. H.; Nelson, V. E., and Fulmer, E. I.: *J. Infect. Dis.* **34**: 447, 1924.

11. Bieling, R.: (a) *Ztschr. f. Hyg. u. Infektionskr.* 104:631, 1925; (b) **104**: 518, 1925; (c) 106:188, 1926.

toxin and antitoxin. Two of the scorbutic guinea-pigs presented tetanus, but none of the controls did. Similar tests were performed on scorbutic guinea-pigs with formaldehydized diphtheria toxin, administered subcutaneously at the same intervals. Controls (immunized normally fed guinea-pigs) which received injections of the toxin-antitoxin mixture twenty-one days after the first immunizing injection survived, but 4 of the 6 immunized scorbutic guinea-pigs died.

In unpublished experiments, we observed no depression in the hemolysin-forming capacity of rats fed for two months on a diet deficient only in vitamin C.

Summary.—Deficiency in vitamin C does not interfere with the formation of immune antibodies, although it does impair the effectiveness of immunization to tetanus or diphtheria toxin.

IV. EFFECT OF DEFICIENCY OF VITAMIN C ON SKIN SENSITIZATION AND ANAPHYLAXIS

Sensitivity to Diphtheria Toxin.—Alterations in the inflammatory response of the skin to diphtheria toxin have been observed in scorbutic guinea-pigs. Arkwright and Zilva,¹² however, who observed diminution in intracutaneous reactions to diphtheria toxin, attributed this to loss in weight on the part of the animals and not specifically to scurvy.

Guinea-pigs fed a diet of water and bran and very little cabbage gave much smaller reactions to injected fractions of the minimal lethal dose of diphtheria toxin than normally fed animals. Animals that grew fairly well gave prominent reactions, but those that lost weight gave slight ones. This effect was the same whether the decrease in weight was due to diminished intake of a normal diet or to a diet deficient in vitamin C. If the animals lost more than 50 Gm. in from five to six days, the inflammatory reaction was less than half the size observed in the animals of fair growth, and the cellular exudate in the local lesion was less pronounced.

Bieling^{1b} observed increased sensitivity of scorbutic guinea-pigs to diphtheria toxin, although the local skin reactions to intracutaneous or subcutaneous injections were definitely less than those in normally fed guinea-pigs.

The guinea-pigs were led a diet of autoclaved milk and oats for seventeen days to produce a scorbutic state. The intracutaneous tests were made with various dilutions of diphtheria toxin and subsequently with toxin-antitoxin mixtures. In control experiments, he fed animals a diet of fresh turnips in small amounts, fresh hay and water, which he considered an adequate normal diet, and found after a period of nine days that animals that had lost weight on small amounts of this normal diet showed slightly less edema about the areas of injection but a skin reactivity that was otherwise the same as in the normal animals.

12. Arkwright, J. A, and Zilva, S. S.: J. Path. & Bact. 27:346, 1924.

Bieling's experiments were performed on very small numbers of animals. The diet certainly was deficient in more than vitamin C. We believe that this criticism applies to many of the reports in this field, for it has been assumed that if the diet produced scurvy in the guinea-pig no essential factor other than vitamin C was absent. In most instances, unless special precautions were taken, the diet, while it was scurvy-producing, was really free from all vitamins.

Sensitivity to Tuberculin.—Similarly, the skin reactions to intracutaneous injections of tuberculin in scorbutic tuberculous animals are definitely diminished as compared with those in normally fed tuberculous animals (Bieling^{11b}). Underfed animals demonstrate a similar depression in skin reactivity. Though this indicates that the vascular reaction is diminished in hunger, it is not an index of the effect of the bacterial poison on any other tissue cells of the body. Bieling injected tubercle bacilli into 10 guinea-pigs, and ten weeks later placed some of these animals on a scorbutic diet and some on normal fodder. When he then injected tuberculin subcutaneously, the animals fed a scorbutic diet during a period of a few days succumbed more readily than the controls.

The observation that scorbutic tuberculous guinea-pigs react less to intracutaneous injections of tuberculin and show diminished skin reactions as compared with nonscorbutic tuberculous animals was confirmed by Prausnitz and Schilf.¹³

Sensitivity to Drugs.—Frei¹⁴ reported that he was able to sensitize the skin of guinea-pigs to neoarsphenamine, but that animals on summer fodder were more resistant than those on winter fodder. The experiments with paraphenyldiamine showed a similar variation in resistance. Sulzberger and Oser¹⁵ attributed this to a varying content of vitamin C in the diet.

They fed animals a basic diet of dried autoclaved milk, oat flakes, the whites of eggs, cod liver oil and dried hay. They had observed that animals fed such a diet lost weight after from ten to fifteen days, showed clinical signs of scurvy and died in from three to four weeks. After a period of ten days on this basic diet the animals in the experiment under consideration were divided into five groups. To the diets of the different groups cevitamic acid was added in amounts varying from 0.25 to 2 mg. Ten days later all the animals were given intracutaneous injections of a 0.15 per cent solution of neoarsphenamine in saline solution—0.1 cc. to each. None of the animals showed an immediate reaction, but some showed a flare up from six to ten days later. On the twenty-eighth day, or eighteen days after the first injection, each animal was given a second injection of neoarsphenamine in the opposite flank. Within twenty-four hours the sensitized animals showed a severe local reaction that progressed in severity.

13. Prausnitz, C., and Schilf, F.: . Deutsche med. Wchnschr. 50:102, 1924.

14. Frei, W.: Klin. Wchnschr. 7:1026, 1928.

15. Sulzberger, M. B., and Oser, B. L.: Proc. Soc. Exper. Biol. & Med. 32: 716, 1934.

Hypersensitivity was evident in most of the guinea-pigs that received 1.5 mg. or less of cevitamic acid per day as a supplement to their diet. Scurvy, however, occurred only in animals that received 0.5 mg. or less of cevitamic acid per day. Of 9 animals given 2 mg., 6 showed no reaction. An occasional cachectic animal among those that received the smallest amounts of cevitamic acid failed to react.

This experiment is extremely interesting and was well controlled. According to Chapman and Morrell,¹⁶ however, when animals were fed on a diet of a grain mixture and carrots, no sensitization to neoarsphenamine could be achieved. If 2 mg. of cevitamic acid was added to the diet of each, daily severe reactions were obtained with the sensitizing and the provocative doses. It is hard to reconcile these divergent findings, though the conditions of the experiments of Chapman and Morrell did not duplicate in detail those of Sulzberger and Oser.¹⁵

Sensitivity to Poison Ivy.—Recently Simon observed that a dietary deficiency in vitamin C inhibited the development of hypersensitiveness to poison ivy in guinea-pigs.¹⁷

He divided 24 guinea-pigs into six groups of 4 animals each. Four of the groups received diets deficient, respectively, in vitamin A, vitamin B, vitamin C and vitamin D ; two groups were given a full diet.¹⁸ Sixteen days after the onset of the experiment, 5 animals in the first four groups were dead. At this time, each of the surviving animals in the first five groups was given a cutaneous application of an extract of poison ivy.¹⁹ Ten days later, all the animals were tested by cutaneous applications of the extract in dilutions of 1: 10, 1:40 and 1: 100. Readings made on the following day showed well developed sensitivity in all the prepared groups except those given a diet deficient in vitamin C. No other dietary deficiency influenced the result.

Anaphylaxis.—As early as 1911, Lesne and Dreyfus²⁰ observed that starvation in rabbits diminishes their response to anaphylaxis. Rabbits were given injections of egg white intravenously and for fifteen days thereafter were fed a normal diet. Four days prior to the administration of the shocking dose, the animals were placed on a diet exclusively of water. The period of starvation tended to suppress the anaphylactic

16. Chapman, C. W., and Morrell, C. A.: Proc. Soc. Exper. Biol. & Med. 32:813, 1935.

17. Simon, F. A.: J. Immunol. 30:275, 1936.

18. The diet deficient in vitamin A consisted of casein, corn starch, dried brewers' yeast, salt mixture, autoclaved alfalfa and distilled water. The diet deficient in vitamin B contained casein, corn starch, butter fat, autoclaved yeast, cod liver oil, salt mixture, alfalfa and water. The diet deficient in vitamin C consisted of ground whole oats, heated skim milk powder, butter fat, table salt, autoclaved alfalfa and distilled water. The one deficient in vitamin D consisted of ground yellow corn, ground wheat, wheat gluten, gelatin, calcium carbonate, sodium chloride and distilled water. The full diet contained hay, oats, carrots, lettuce, cabbage and water.

19. A 10 per cent acetone extract of *Rhus toxicodendron* radicans.

20. Lesne, E., and Dreyfus, L.: Compt. rend. Soc. de biol. 71:153, 1911.

reaction. Similar results were observed by Konstansoff²¹ in guinea-pigs sensitized to horse serum, egg white or cow's milk. According to Pierret and Crampton,²² starvation of as short a period as thirty-six hours prevented anaphylactic shock in guinea-pigs sensitized subcutaneously to horse serum if the period of starvation immediately preceded the injection of the shocking dose. Zolog²³ could not confirm this observation, but in a carefully controlled series of experiments he noted that guinea-pigs fed a diet deficient in vitamin C prior to sensitization were less sensitive to the reinjected antigen than the controls.

The basic diet was dried grass and oats. The controls received fresh grass and beet root in addition. The guinea-pigs were fed the diet for thirty days prior to the first injection of the antigen—horse serum in some of the experiments, and in others, red cells of the horse. Fifteen days after sensitization, they were given reinjections of the antigens and those that had received the scurvy-producing diet survived greater shocking doses than the controls. They survived five times the control amount of red blood cells, and in one experiment, ten times the control amount of horse serum.

Zolog controlled the factor of inanition by duplicating the conditions of experiments of Pierret and Crampton²² but observed no depression in reaction in animals starved for a short period prior to the injection of the shocking dose. He concluded that the hyposensitivity to anaphylaxis is greater the longer the period during which the guinea-pig is on the scurvy-producing diet and the younger the animal.

It is interesting, however, that the interval of time during which the animals are fed the scurvy-producing diet is important in determining the variations in sensitivity. Sartori,²⁴ for example, observed that if guinea-pigs are fed on a diet deficient in vitamin C for a period of five days prior to the injection of the sensitizing dose they are more sensitive to anaphylactic shock than are normally fed animals. If, however, the vitamin-deficient diet is instituted after sensitization has occurred, no effect is observed on the subsequently induced anaphylaxis. The avitaminosis in guinea-pigs may shorten the period of anaphylactic incubation to as little as five days instead of a normal period of fifteen days. However, at the end of thirty-three days, the animals are still as sensitive as in the early periods. This work was confirmed by the experiments of Sereni,²⁵ who fed his guinea-pigs a scurvy-producing diet of bread, oats and milk for thirteen days prior to sensitization. As early as the eleventh day the animals with scurvy were killed by a second injection, but normally fed animals tested on the same day

21. Konstansoff, S.: *Compt. rend. Soc. de biol.* 72:263, 1912.

22. Pierret, A., and Crampton, C. W.: *Compt. rend. Soc. de biol.* 89:1282, 1923.

23. Zolog, M.: *Compt. rend. Soc. de biol.* 91:215, 1924.

24. Sartori, C.: *Pathologica* 17:160, 1925.

25. Sereni, E.: *Boll. d. Soc. ital. di biol. sper.* 2:254, 1927.

survived. When tested on the twentieth day with the same shocking doses, both scorbutic and normal animals succumbed. He attributed this discrepancy to the fact that animals with scurvy produce antibodies at a slower rate than do normal animals. He did not, however, offer any proof of this assumption. Guinea-pigs fed for some time on a scurvy-producing diet and passively sensitized survived an intracardiac injection of serum administered twenty-four hours later. The guinea-pigs fed a normal diet and similarly treated succumbed. He concluded, however, that the scorbutic animals were more sensitive than the controls to anaphylaxis.

Hurwitz and Wessels²⁶ observed that guinea-pigs fed for four weeks on a diet of rolled oats, milk and bran and then sensitized with horse serum showed a modification in the response of the sensitized uteri examined *in vitro* three weeks later, but not in the response of the bronchi.

The uterine segments were suspended in Locke's solution, and the bronchial muscle reaction was studied by bronchial perfusion of the sensitized lungs. The uterine muscle strips from those animals that had been fed the scurvy-producing diet failed to respond either to antigen or to smooth muscle stimulants. The introduction of the same substance into the bronchi (histamine, posterior pituitary, barium or horse serum) caused bronchial constriction in both the vitamin-deficient and the normally fed animals.

Summary.—In animals deficient in vitamin C there is a decrease in sensitivity of the skin, as indicated by diminished reactions to injections of diphtheria toxin and to injections of tuberculin. This diminution in skin sensitivity is, however, associated with greater susceptibility of the animals to the toxic effects of parenteral injections of these poisons. Skin reactivity to drugs, such as arsphenamine, may be elicited in guinea-pigs previously treated by injections of small amounts of such drugs if these animals are fed a diet deficient in vitamin C but not completely depleted of this factor. In animals whose avitaminosis is sufficient to cause clinical manifestation of scurvy the skin is no longer reactive.

This is also true of skin hypersensitiveness to poison ivy. A short period of vitamin C deficiency prior to the administration of the sensitizing dose of protein antigenic substances increases the anaphylactic response to the subsequent injection of the shocking dose and shortens the period of incubation of anaphylactic sensitization. Extreme scurvy or periods of starvation may under certain conditions impair the anaphylactic response. There is diminution in the smooth muscle response of the uterus in the scorbutic sensitized animal to smooth muscle stimulants and antigen.

26. Hurwitz, S. H., and Wessels, A. L.: Proc. Soc. Exper. Biol. & Med. 29:122, 1931.

In general, subclinical scurvy may be associated with increased sensitivity of the skin to poisons and tuberculin and increased susceptibility of the body to anaphylaxis, but frank scurvy may be accompanied by suppression of the skin reaction and depression of the general susceptibility to anaphylaxis.

V. EFFECT OF DEFICIENCY OF VITAMIN C ON NATURAL
RESISTANCE TO POISONS AND TOXINS

Vercellana²⁷ observed that guinea-pigs deficient in vitamins were less resistant to a number of drugs and poisons than were normally fed animals.

The animals did not survive injections of barium carbonate, atropine, alcohol, spartein, nicotine, quinine, epinephrine and the toxin of *Bacterium dysenteriae* (Shiga) in amounts that were readily tolerated by normal animals. Scorbutic guinea-pigs were killed by an amount of strychnine nitrate equal to a third of the minimal lethal dose for normal guinea-pigs. In part, this was due to under-nutrition, as animals starved for two or three days prior to the injection of the drug also showed a drop in resistance, but to a lesser degree than that observed in the vitamin-deficient group. Similarly, the minimal lethal dose of a fungus poison (aqueous extract of *Russula nigrigans*) administered subcutaneously to scorbutic guinea-pigs was reduced to less than a sixth of the corresponding dose for normally fed guinea-pigs. The normal animals were killed by 4 cc. of a concentrated fungus solution; the vitamin-deficient animals, by less than 1 cc. Animals starved for two or three days also died following the injection of 1 cc. of the fungus solution.

Here, obviously, the drop in natural resistance was largely a matter of general undernutrition.

Latent scurvy in infants is associated with a relatively high incidence of nasal diphtheria, according to Hess.²⁸ This drop in local resistance occurs even though the blood of the infants contains sufficient anti-toxin to afford protection; negative Schick tests were obtained in spite of clinical diphtheria. Hess²⁸ explained this apparent paradox by stating that the lack of antiscorbutic vitamin exerts a local effect on the mucous membrane which diminishes its immunity, but at the same time does not act to lower systemic immunity.

Significant of the effect of a dietary deficiency of vitamin C on resistance to a bacterial toxin are the results of the carefully controlled studies of King and Menten.²⁹ They found that there is a wide zone of vitamin C deficiency without the appearance of frank scurvy in which

27. Vercellana, G.: *Ann. d'ig.* 38:364. 1928.

28. Hess, A. F.: *Scurvy: Past and Present*, Philadelphia, T. B. Lippincott Company, 1920.

29. King, C. G., and Menten, M. L.: *J. Nutrition* 10:129, 1935.

the physical processes are subnormal and the animal is more sensitive to injury from bacterial toxin.

In their studies, the diphtheria toxin was diluted in sterile saline solution and administered subcutaneously. Determinations of tissue respiration were made on 100 mg. of liver and kidney suspended in Locke's solution, by means of the Barcroft-Warburg constant volume manometric apparatus. The oxygen consumption from air was recorded at thirty minute intervals over a period of two hours. Determinations of vitamin C were made on the tissues by a titration method using trichloro-acetic acid and 2-6 dichlorophenolindophenol. They observed that single injections of amounts of diphtheria toxin containing 1.5 or 2 M. L. D. produced extensive lesions. The survival time of 5 animals receiving 5 mg. of pure vitamin C daily prior to the injection of the toxin was greater than that of 4 animals depleted of vitamin C until mild indications of scurvy were present. With smaller repeated doses of toxin (0.1, 0.3 and 0.5 M. L. D.) the differences were more striking. The injection of toxin into normal guinea-pigs produced degeneration and hemorrhage of the adrenal cortex. Similar effects occurred as a result of vitamin C deficiency alone. The vitamin C content of the tissues was unaltered in animals given injections of the toxin. Guinea-pigs receiving vitamin C daily—some at an abundant, protective level (0.5 mg. cevitamic acid) and some at a subprotective level (0.25 mg.)—were given subcutaneous injections of standardized diphtheria toxin in amounts of 0.1, 0.3, 0.5 and 1 M. L. D. In animals in which the reserves of vitamin C were partially depleted without their showing external signs of scurvy the time of survival following the injection of lethal amounts of toxin was shortened about 50 per cent and the loss in weight was more severe. Hemorrhage and necrosis at the sites of injection were more marked in the animals suffering with latent scurvy. The decrease in the oxygen consumption of the hepatic and renal tissue after injection of the toxin was in the range of from 5 to 15 per cent.

These studies indicate that latent scurvy in guinea-pigs is associated with increased sensitivity to a bacterial toxin parenterally administered.

In subsequent studies, Menten and King³⁰ observed the production of diffuse hyperplastic arteriosclerosis and degeneration in various tissues in guinea-pigs maintained on diets deficient in vitamin C and given injections of sublethal amounts of diphtheria toxin. Such animals also had hydropic degeneration of the islets of Langerhans, associated with hyperglycemia and low tolerance to sugar. With recovery, the pancreatic lesions disappeared.

In the medium-sized arterioles of the liver, lung, spleen and kidney were found connective tissue proliferation of the media and swelling of the internal elastic lamella, with degenerative changes in the smaller vessels. The smooth muscle showed marked swelling, pyknosis and necrosis. This was associated with loss of resiliency and tone in the elastic fibers, induced by compensatory hyperplasia of the connective tissue of the arteries. In larger vessels the changes began in the media, with gradual complete involvement and thinning of the vessels. No

30. Menten, M. L., and King, C. G.: *J. Nutrition* 10:141, 1935.

compensatory fibrosis was found, but nodules of cartilage such as are seen in old guinea-pigs occurred.

In guinea-pigs each of which was given 0.25 mg. of cevitamic acid daily and 0.1 M.L.D. of diphtheria toxin on the fourteenth, seventeenth, twenty-first and twenty-fifth days, no such changes occurred. However, diffuse hyperplastic arteriosclerosis was produced with 0.3 M.L.D. regardless of the level of the vitamin C in the diet. This condition cannot be produced in such animals as the rat, which synthesizes its own vitamin C. The vitamin C content of smooth muscle is comparatively low, and it can readily be reduced in amount. Degenerative lesions of the islets of Langerhans can readily be produced with other organisms, such as *Bacterium enteritidis* (Gaertner). The lesions are restricted to the beta cells, the nuclei of which show vacuolation and pyknosis. The same amount of toxin is required to produce lesions of the islets as to produce vascular lesions in the presence of relative vitamin C deficiency.

Wolbach and Howe³¹ showed that in the guinea-pig an adequate intake of vitamin C is indispensable for the formation and the integrity of collagen. Menten and King³⁰ suggested that the metabolism and respiration of connective tissue intimately adjusted to such relationship, and that perhaps this accounts for the unique vascular response to a variety of infectious agents injected into guinea-pigs with latent scurvy.

Summary.—Latent scurvy in animals and man is associated with marked increase in their susceptibility to drugs and bacterial toxins. The vascular injury produced by the toxins of certain bacteria, such as *Corynebacterium diphtheriae*, is more pronounced in the presence of deficiency of cevitamic acid. Such injury is associated with decrease in tissue respiration.

VI. EFFECT OF DEFICIENCY OF VITAMIN C ON NATURAL RESISTANCE TO SPONTANEOUS INFECTIONS

Observations of spontaneous infections in experimental animals with latent or manifest scurvy are scattered. Theobald Smith³² noted in guinea-pigs epizootics of dysentery and of pneumococcic pneumonia and pleurisy that occurred at the times of the year when green food was given in minimal amounts. Such epizootics have been interpreted by others as due to latent scurvy in the guinea-pigs.

Guinea-pigs fed a diet of crushed oats and autoclaved milk, which is deficient in vitamin C, show severe changes in the gastro-intestinal tract, such as congestion, hemorrhage, ecchymoses, punched-out ulcers, particularly of the duodenum, and thinning of the walls of the large

31. Wolbach, S. B., and Howe, P. R.: *Arch. Path.* 1:1, 1926.

32. Smith, T.: *J. M. Research* 29:291, 1913.

intestine. Degenerative changes are found in the myenteric plexus, and necrotic changes, in the cellular elements of the mucous membranes. These changes in the intestines, McCarrison³³ pointed out, often occur before other signs of scurvy are manifest and permit bacterial invasion to occur.

Höjer³⁴ maintained that guinea-pigs fed a diet deficient in vitamin C readily contract infections. Of 189 animals with scurvy, 60 suffered from acute infections, mainly of the upper respiratory tract, and from pneumonia, septicemia and enterocolitis. Animals with latent scurvy showed about twice as many infections leading to death as those on a complete dietary. Of 72 animals fed an insufficient antiscorbutic diet, 47 per cent showed signs of infection as compared with 20 per cent of the controls. Of 62 animals fed an absolutely scorbutic diet, 29 per cent showed signs of acute infections. However, the life span was twice as long in guinea-pigs fed a relatively deficient antiscorbutic diet as in those fed an absolutely scorbutic diet.

Clinical observations on the association of latent and manifest scurvy with infections are numerous. Hess³⁵ stated that the scorbutic state even in the absence of frank symptoms of scurvy lowers the resistance of the subject to such infections as diphtheria.

During the World War, latent and manifest scurvy occurred among the soldiers of the Turkish and Russian armies. Coincident with the appearance of scorbutic manifestations there was apparent increase in acute infections. Typhoid fever in patients with scurvy ran a severe course. The high incidence of dysentery and malaria was in part attributed to the undernutrition of the soldiers. Latent scurvy became manifest scurvy with the onset of infection. In general, there was noticeable increase in the hemorrhagic character of the exanthems, which Salle and Rosenberg³⁶ and others attributed to concomitant latent scurvy. Patients with tuberculosis who acquired scurvy readily succumbed. Tuberculosis in persons with latent scurvy was rapidly fatal. The incidence of tuberculosis among the undernourished Turkish soldiers was considerable. An attempt to arrive at an accurate analysis of the factors underlying the statistics on the incidence of infections during and after the war and their relationship to undernutrition is hazardous. There is little doubt that infectious diseases increased among soldiers exposed to the conditions of crowding, herding, poor sanitation and undernutrition. The civilian population as well suffered from infections in increasing numbers. The new generation showed

33. McCarrison, R.: *Indian J. M. Research* 7:167, 1919.

34. Höjer, J. A.: *Acta paediat. (supp.)* 3:8, 1924.

35. Hess, A. F.: *New England J. Med.* 207:637, 1932.

36. Salle, V., and Rosenberg, M.: *Ergebn. d. inn. Med. u. Kinderh.* 19:31, 1921.

increased susceptibility to many infections, such as tuberculosis, for many years after the war. But it is impossible to attribute this to the deficiency in any one dietary factor, though it is readily conceded that undernutrition contributed greatly to the depression in natural resistance.

Rosenbund³⁷ observed that the diminished resistance to infection in infants with scurvy, as determined by the high incidence of transient infections, is not rapidly altered by curing the scurvy. For several months after antiscorbutic treatment is administered, intercurrent infections are as frequent as in the scorbutic state. Infants with latent scurvy not only are subject to more frequent infection but may present manifest scurvy following a minor infection, such as vaccination against smallpox. Stern³⁸ observed in a series of 23 infants with latent scurvy severe reactions following injections of cowpox lymph. Fever developed, with hemorrhages at the sites of vaccination.

Patients with scurvy who die generally present at autopsy evidences of severe infection. Schagen³⁹ reported that in half the autopsies on a series of scorbutic children active tuberculosis was observed. Pneumonia, grip, otitis, furunculosis and pyuria are common in scorbutic children (Reyher⁴⁰). Similar observations were reported by Dubois,⁴¹ who noted the frequency of infections of the upper respiratory and of the urinary tracts. The administration of vitamins, particularly vitamin C, was followed by the disappearance of many of the infections.

Höjer³⁴ discussed at length the relation of scurvy to tuberculosis. Tuberculosis may influence latent scurvy in many ways. The diminished appetite may lead to insufficient consumption of vitamin C. The intestinal tract may change so that the consumed dose is not absorbed. Scorbutic atrophy of the vascular wall may be associated with hemorrhage in the region of the tuberculous focus. The resistance of the body to tuberculosis is lessened during the course of scurvy in patients, as is evidenced by numerous observations. In the northern provinces of Sweden, fresh vegetables are as rare as tuberculosis is frequent (Höjer). Höjer's observations led him to suggest that vitamin C in fully protective amounts should be given to persons suffering with tuberculosis, particularly children. His observations on the therapeutic value of vitamins in a part of Sweden where fresh vegetables are scarce suggested the importance, apparently, of adequate vitamin C in the diet of tuberculous patients. These therapeutic tests were made in 70 selected cases of tuberculosis of equal severity; half of the patients were given

37. Rosenbund, R.: *Ztschr. f. Kinderh.* 34:333, 1922.

38. Stern, R.: *Ztschr. f. Kinderh.* 36:32, 1923.

39. Schagen, B.: *Jahrb. f. Kinderh.* 104:225, 1924.

40. Reyher, P.: *Arch. f. Kinderh.* 76:215, 1925.

41. Dubois, R.: *Nourrisson* 19:337, 1931.

orange juice as a dietary supplement. Höjer³⁴ believed that the patients treated with orange juice improved.

The frequent association of scurvy and spontaneous infection led to the erroneous conclusion that scurvy was due to an infection, and, as late as the war, "organisms" were isolated from the tissues of scorbutic animals that were held responsible in part for the changes observed in scurvy. The endemic occurrence of scurvy, the presence of acute infections in association with the onset of the disease in the subjects and the frequent occurrence of scurvy with fever suggested the dependence of scurvy on infection. Though in man scurvy frequently becomes manifest in association with infection, in guinea-pigs, in a high percentage, infection does not accompany scurvy. When infection occurs it is merely a concomitant manifestation due to the lowered resistance of the host with latent or manifest scurvy. In animals the infections that appear spontaneously do so as a rule late in the disease. There is little doubt that in animals and man the scorbutic state is associated with increased susceptibility to spontaneous infection. What part is due solely to a deficiency specifically of vitamin C, what part to general undernutrition and what part to concomitant deficiencies of other dietary factors is not well established.

In this connection, the studies of Finkle⁴² are of interest. He reconsidered the question of the role of vitamin C deficiency in the etiology of pathologic conditions other than scurvy. Using the method of Harris, Ray and Ward,⁴³ he confirmed their observations that in normal persons from 0.03 to 0.05 mg. of cevitamic acid was excreted per cubic centimeter of urine during the day.^{42a} He studied the excretion of vitamin C before and after intravenous administration of 100 mg. of cevitamic acid to each of 127 patients suffering from a variety of conditions and to a number of normal controls. He observed that in about from two to three hours after the intravenous administration of vitamin C the cevitamic acid excreted in the urine rises to levels from five to six times as high as the preinjection levels. In cases in which the preinjection output of vitamin C in the urine fell much below 0.03 mg., the total output for the twenty-four hour period was considerably below the normal level. In these cases, the intravenous administration of vitamin C was not followed by a rise in the vitamin C excreted in the urine during the twenty-four hour period.

The excretion and utilization of vitamin C were studied by this method in the following conditions: purpura haemorrhagica, hemophilia,

42. Finkle, P.: (a) *Proc. Soc. Exper. Biol. & Med.* 32:1163, 1934; (b) *Vitamin C Saturation Levels in the Body in Normal and in Various Pathological Conditions*, to be published.

43. Harris, L. J.; Ray, S. N., and Ward, A.: *Biochem. J.* 27:2011, 1933.

acute lupus erythematosus, acute glomerulonephritis, hypertension, acute rheumatic fever, rheumatoid arthritis, jaundice due to various conditions, Addison's disease (a few instances) and ulcerative colitis. He observed that of 14 patients with rheumatic fever, 5 showed a considerable increase in the excretion of vitamin C following the injection of cevitamic acid, even some whose preinjection level was lower than normal. However, most of the patients in this group showed a daily excretion of vitamin C below the normal level.⁴⁴ Two patients showed an excretion above this level.

Of 24 patients with rheumatoid arthritis, only 4 showed a normal curve of excretion of vitamin C in the urine. The remaining ones either had excretion levels below the normal or no rise following intravenous administration of cevitamic acid. He also observed a consistent disturbance in the metabolism of vitamin C in lupus erythematosus. In the 5 cases studied the excretion level was low even with adequate intakes of orange juice and did not respond to intravenous injections of vitamin C.

From his studies Finkle⁴² concluded that a large percentage of a hospital population have an excretion of vitamin C below that of normal persons and suffer from varying degrees of subclinical scurvy, for the intravenous injection of vitamin C results in little or no rise in the cevitamic acid excreted in the urine. This degree of subclinical scurvy accompanies a variety of pathologic conditions. Finkle does not believe that deficiency of vitamin C has any causal relationship to any condition other than scurvy.

He is unable to explain, however, the disturbed metabolism of vitamin C that accompanies lupus erythematosus and rheumatoid arthritis.

VIII. EFFECT OF DEFICIENCY OF VITAMIN C ON NATURAL RESISTANCE TO EXPERIMENTALLY INDUCED INFECTIONS

Acute Infections with Staphylococcus.—Jaffe⁴⁵ made the interesting observation that guinea-pigs fed on a scurvy-producing diet and acquiring acute suppurative osteomyelitis did not manifest the cardinal symptoms of scurvy.

Twelve guinea-pigs were fed a diet free from vitamin C. Scurvy developed in all of them in from thirteen to eighteen days. The wrists became tender and swollen; the teeth became loose; scorbutic changes occurred at the costochondral junctions; there was cessation of the formation of new marrow, with diminution in the number of osteoblasts in the zone of calcification; rarefaction

44. Since marked increases in output followed injection of ascorbic acid, the author believes there is no disturbance of vitamin C metabolism in these cases.

45. Jaffe, H. L.: J. Infect. Dis. 40:502, 1927.

of the existing cortex and irregularities of the spongy portion occurred; there were absorption of cartilage columns and formation of a zone of fragmented bone distal to the costochondral junction; fractures and hemorrhages were frequently observed in this area; the marrow of the shaft in this vicinity became filled with a loosely constructed gelatinous fibrillar tissue (*Gerüstmark*).

These animals served as controls. Eleven other guinea-pigs were placed on the same diet and infected simultaneously with an eighteen hour broth culture of *Staphylococcus pyogenes-aureus*, which was injected directly into the marrow cavity of the tibia, muscles of the leg and skin. Six of the animals showed severe local infection with sepsis of varying degrees and died between the nineteenth and the fortieth day without any symptoms of scurvy. The bones were brittle because of osteoporosis; the liver was fatty; the spleen, large. Five animals died of scurvy between the twenty-first and the thirtieth day. In none of these was there severe local infection or sepsis.

In another experiment, Jaffe injected cultures of *Staphylococcus aureus* into guinea-pigs ten days after the scorbutic diet was instituted. The animals died in from seven to twelve days, much sooner than the controls. He further observed that repeated administration of *Staphylococcus* vaccine to guinea-pigs prior to placing them on a scurvy-producing diet did not in any degree retard the development of scurvy.⁴⁶ These experiments suggested that the protective effect of staphylococcal infection in preventing the occurrence of scorbutic symptoms was probably due to the production of vitamin C by the bacterial growth. (The capacity of bacteria to produce vitamins A and B was demonstrated by Burrows and Jorstad.⁴⁷)

Baj⁴⁸ confirmed Jaffe's observations. An infection with *Staph. albus* does not arrest the phenomena of scurvy if the infection is induced when these are already present. If, however, the infection is induced on the same day that the animals are placed on the deficient diet, the manifestations of scurvy may be averted.

The basic diet used was the same as in Jaffe's experiments. Four groups of 12 guinea-pigs each were used. One group was placed on a scurvy-producing diet and observed. A second group was placed on the same diet and a culture of *Staph. albus* injected on the same day. A third group was fed a normal diet and similarly infected. A fourth group was infected after systemic scurvy was present. With the basic diet, scurvy appeared in from sixteen to eighteen days and proved fatal in from thirty to thirty-five days. In the animals that were infected at the beginning of the experiment suppuration and sepsis occurred, but

46. The diet used in Jaffe's experiments consisted of soy bean flour, 50 parts ; rolled oats, 29; dried milk powder, 10; brewers' yeast, 4; butter fat, 5, and calcium carbonate and sodium chloride 1 part. The soy bean flour, oats and milk were autoclaved. In addition, the animals received hay for roughage and distilled water ad libitum.

47. Burrows, M. T., and Jorstad, L. H.: *Am. J. Physiol.* 77:38, 1926.

48. Baj, J. L.: *Chir. d. org. di movimento* 14:477, 1929.

the bones showed no changes in the cartilaginous zone indicative of decalcification or of medullary hemorrhages or sclerosis. The infection, however, was more severe in the scorbutic animals than in the normally fed animals.

In other types of infection, such as that with *Trypanosoma Brucei*, a greater need for vitamin C is evident, for infections of this type may hasten the onset of scurvy (Nassau and Scherzer⁴⁹).

Pneumococcus.—Though guinea-pigs lacking vitamin C revealed no change in their ability to produce specific agglutinins and though there was no difference from the normal in their phagocytic mechanism as estimated by the opsonic index, their natural resistance to pneumococcal infection was lowered, according to Werkman, Nelson and Fulman.¹⁰ Unfortunately, the data are limited. A virulent strain of *Pneumococcus* was injected intraperitoneally into 9 scorbutic guinea-pigs and 8 controls. There was a slight difference in the duration of life in the two groups. The scurvy-producing diet of ground oats, wheat and autoclaved milk was deficient in other vitamins as well as C. A culture of the organism employed was not used, but an emulsion of livers from infected mice. The evidence does not warrant the conclusion.

Schmidt-Weyland and Koltzsch,⁵⁰ who observed spontaneous infections of scorbutic guinea-pigs with the pasteurella of fowl cholera and pneumococci, studied the incidence of induced infections with these organisms under experimentally controlled conditions.

They fed cultures of these bacteria to the experimental animals or sprayed the cultures into cages and subsequently exposed normal and scorbutic animals to infection from this source. The blood and various organs were cultured at death or at the termination of the experiment.

Of 40 normally fed guinea-pigs exposed to the cultures, only 5 died. Of 58 uninfected scorbutic animals not subjected to exposure to the cultures, 31 showed sepsis from fowl cholera and 12 sepsis from pneumococcal infection. Of 33 scorbutic guinea-pigs exposed to the spray of pneumococci and fowl cholera organisms, 27 showed sepsis from fowl cholera and 3 sepsis from pneumococcal infection. Of 5 fed the cultures by mouth, only 1 contracted pneumococcal infection. Of 9 given the mixed culture by mouth and exposed to spraying, 6 contracted fowl cholera.

Apparently, the incidence of spontaneous pasteurella or pneumococcal infection in scorbutic guinea-pigs was as high as the incidence in those in which the cultures were administered by spray or by feeding. The presence of sepsis was established by cultivation of the infecting organisms from the tissues at death. The data are confusing and difficult to evaluate. It is apparent that the incidence of spontaneous infections

49. Nassau, E., and Scherzer, M.: *Klin. Wchnschr.* 3:314, 1924.

50. Schmidt-Weyland, P., and Koltzsch, W.: *Ztschr. f. Hyg. u. Infektionskr.* 108:199, 1928.

was greater in the scorbutic guinea-pigs than in the normally fed guinea-pigs. But the effort to determine quantitatively the degree of natural resistance of scorbutic animals to induced infections under controlled conditions failed, since the animals used were already carriers of the organisms employed.

Bacterium Coll.—Cognizant of the difficulties of studying induced acute infectious diseases in scorbutic guinea-pigs, Findlay⁵¹ produced chronic scurvy by supplementing the basic diet with inadequate amounts of orange juice. Thus, to a diet of bran, oats and autoclaved milk, he added 2 cc. per day of orange juice. The chronic scurvy that resulted showed a major difference from acute scurvy in the changes in the marrow. The framework of the marrow persisted, but there were areas of gelatinous degeneration, consisting of homogeneous interstitial ground substance and containing blood pigment, which replaced the hematopoietic tissue. No atrophy of the lymph glands, thymus, lymphatic tissue or spleen occurred. Little difference in antibody formation as determined by serum reaction was observed.

Findlay⁵¹ observed the effect of chronic scurvy on the natural resistance to infections induced with *Pneumococcus*, *Staph. aureus*, *Streptococcus haemolyticus* and *Bact. coli*. In these experiments he made an effort to estimate the quantity of bacterial material necessary to produce infection, the duration of life and the character of the cellular reaction to the infection.

The animals were fed the scorbutic diet during a period of six weeks. Then they were infected with a particular organism. In the case of *Pneumococcus*, 6 scorbutic animals died in an average period of from two to three days, while 6 normally fed animals died in from six to eight days, of the same dose of infecting organisms. In another experiment, 4 animals with scurvy died in from five to eight days after injection of smaller quantities of pneumococci, while 4 of 8 control guinea-pigs survived, and 4 died in eight days. Similar differences in the minimal lethal dose of *Str. haemolyticus* and *Bact. coli* were observed.

The guinea-pigs with chronic scurvy were killed by a quantity of bacteria estimated to be about two thirds of the quantity required to kill the normal animals. He attributed their decreased natural resistance to the degeneration of the hematopoietic system. However, no change in the blood count was observed except a very slight reduction in the number of red cells and leukocytes.

Though the number of animals in each group studied was small, the results in all the experiments were consistent.

Bacterium Aertryke.—From a study of the effect of variations in the calcium, vitamin C and vitamin D ratio in the diet on the perinea-

51. Findlay, G. M.: J. Path. & Bact. 26:1, 1923.

bility of the intestinal wall to bacteria, Grant⁵² concluded that an adequate balance of these three dietary constituents is necessary to maintain the normal resistance of the intestines to the migration of bacteria into the blood stream. She believed that either an excess of vitamin D with a deficiency of vitamin C or the reverse may alter the permeability of the intestinal tract to bacteria. Similar effects of deficiency of both C and D or an excess of calcium were noted.

It is probable that an excess of cod liver oil in the diet may inhibit the utilization of vitamin C or exaggerate the effects of a scorbutic diet. The injury to the intestine by a diet deficient in vitamin C was pointed out by McCarrison,³³ who suggested that in scurvy alteration in the intercellular cement substance alters the permeability of the mucous membranes. This has never been demonstrated experimentally, however.

In her experiments, Grant⁵² used a basic diet of rolled oats, timothy hay and sodium chloride. This basal diet in itself induced scurvy in 20 guinea-pigs, but in no case was there evidence of spontaneous infection with *Bact. aertryke*. When 3 cc. of cod liver oil was added daily for two weeks, 10 of 20 animals became spontaneously infected. In 12 guinea-pigs in which 1.5 cc. of orange juice was added to the basic diet daily no infection developed. Of 10 animals to the diet of which calcium lactate was added, only an occasional one became infected. When both calcium lactate and cod liver oil were added to the diet, about half became spontaneously infected. When calcium lactate and linseed oil were added, none became infected.

In a second series of experiments guinea-pigs were placed on these diets and 0.5 cc. of a culture of *Bact. aertryke* was administered orally. In those groups fed a normal diet of hay, oats, salt and cabbage plus an excess of cod liver oil, positive cultures from the organs were obtained, whereas of those fed a normal diet alone, none became infected, nor did those animals given a diet containing an excess of cod liver oil plus an excess of orange juice. The diets were all begun two weeks prior to inoculation.

Apparently, an excess of cod liver oil in an animal with an intestinal wall injured by a deficiency of vitamin C increased the permeability of the wall to bacteria, and this increase was not due to the excess of fat in the cod liver oil since similar quantities of linseed oil did not have the same effect.

In infections induced with *Bact. aertryke* in animals fed normal diets, an excess of cod liver oil seemed to increase the permeability of the intestine to the organism. If simultaneously an excess of orange juice was given, the deleterious effect was not manifest. It is difficult to interpret the significance of these experiments or to evaluate the results. The conditions are too complex.

Chronic Infections with Mycobacterium Tuberculosis.—A number of articles have appeared on the relation of scurvy to experimental tuber-

52. Grant, A. H.: J. Infect. Dis. 39:502, 1926.

culosis in animals. Mouriquand, Michel and Bertoye ⁵³ stated that tuberculosis in guinea-pigs did not modify the evolution of scurvy nor did chronic scurvy seem to accelerate the development of experimental tuberculosis. The coexistence of this infection did not increase the intensity of the scorbutic phenomena. In subsequent papers ⁵⁴ in which the data are given in more detail, these investigators corroborated their earlier findings.

Of 15 guinea-pigs infected with human tubercle bacilli, 2 fed a scurvy-producing diet died in from thirty to thirty-four days with signs of severe scurvy and tuberculosis of moderate severity; 6 were fed insufficient quantities of the antiscorbutic factors furnished by dried barley and 10 cc. of sterilized lemon juice; 7 were placed on an adequate diet and given 10 cc. of fresh lemon juice daily and 10 Gm. of fresh barley. In the second and third groups, 2 guinea-pigs died accidentally on the eighth day, and 10 died in the period from the thirty-fifth to the forty-seventh day, presenting tuberculous lesions of similar intensity; these 10 included 5 from each of the two groups.

In a second series of 16 guinea-pigs infected as before, 4 were fed kennel rations, 6 barley, grass and fresh lemon juice, and the rest a diet containing an inadequate amount of antiscorbutic factors. On the sixty-first and sixty-third day they killed 3 animals from each of the three groups. The lesions were of the same intensity in all. The others died in the period from the eighty-first to the one hundred and forty-fourth day with lesions varying in intensity according to the duration of life and regardless of the diet.

In a third series, guinea-pigs were infected with the same strain of organisms and given the same diets as before. The inoculations were not made, however, until the twenty-eighth day after the diet was started. There was no difference in longevity whether the animals were fed fresh lemon juice, sterilized lemon juice or kennel rations.

These observers believed that there was no essential difference in the course of the chronic scurvy or in that of the tuberculosis which could be ascribed to the fact that both were present. Though their experiments are suggestive, the number of animals in any one group was too small to allow one to evaluate their results.

Coulard ⁵⁵ observed that guinea-pigs deprived of vitamin C and inoculated with extremely small doses of tubercle bacilli were more severely affected than normally fed animals similarly infected. The appearance of the local lesion and of adenopathy was hastened, and the extension of the lesions to the spleen and viscera was more rapid.

Incident to an extended study on the effect of vitiated air on experimental tuberculosis, Sewall, Lurie, Stoifer, Silver and Woo ⁵⁶

53. Mouriquand, C.; Michel, P., and Bertoye, P.: *Compt. rend. Soc. de biol.* 87:854, 1922.

54. Mouriquand, C.; Michel, P., and Bertoye, P.: *Compt. rend. Soc. de biol.* 88:1043, 1923.

55. Coulard, E.: *Presse med.* 31:611, 1923.

56. Sewall, H.; Lurie, M. B.; Stoifer, I.; Silver, R., and Woo, C. T.: *Am. Rev. Tuberc.* 15:328, 1927.

observed the effect of an inadequate diet on the incidence of tuberculosis in groups of guinea-pigs exposed to infected cage mates.

They divided 96 guinea-pigs into groups of 6 each. To the cage of each group they added a guinea-pig inoculated subcutaneously with 0.1 mg. of a bovine strain of tubercle bacilli. Half of the animals were fed on a vitamin-deficient diet of rolled oats, dry rye bread, timothy hay and water. The normally fed guinea-pigs when exposed to the animals with open lesions contracted tuberculosis in from three to four months. In the animals fed a diet deficient in vitamin C the interval between exposure to the tuberculous animals and death from tuberculosis was the same as in the normally fed animals.

In this series a high mortality from intercurrent pneumonia occurred, particularly in the chronically deficient group. Thus, from the tables furnished one learns that 17 died in three months, 22 in from four to five months, 34 in from eight to twelve months and 19 in from twelve to sixteen months, making a total of 92 deaths from pneumonia in the experimental period of sixteen months.

Only 5 guinea-pigs contracted tuberculosis spontaneously, and these died in from four to sixteen months after exposure. On the other hand, of the animals fed a full diet and exposed to cage mates with open tuberculous lesions, 6 acquired fatal tuberculous infection during the same period. The mortality from spontaneously acquired tuberculosis was 10 per cent as compared with 5 per cent in the group fed a diet low in vitamin C.

This type of experiment is valuable, for it simulates more closely human conditions of exposure to open tuberculosis in the community. It is regrettable that the experimental epidemiological approach is not more frequently used in studies on variations in natural resistance to such diseases as tuberculosis.⁵⁷

An analysis of the data of Sewall and his co-workers⁵⁶ reveals certain possible sources of error. The high incidence of intercurrent pneumonic infections in both groups, particularly high in those fed the vitamin-deficient diet, tends to vitiate the experimental findings. These animals died within the first half of the year, reducing the average length of exposure of this group. Jaffe⁴⁵ and later Baj⁴⁸ showed that certain infections may inhibit scorbutic manifestations owing to the fact that some bacteria are apparently capable of producing vitamin C. The chronic pneumonic infections in guinea-pigs may serve a similar purpose. The authors have further committed an error in including among their controls all the vitamin-deficient animals that had apparently escaped tuberculous infection in the experiment. Most of these

57. The method of utilizing the experimental epidemiological approach to problems in host susceptibility to infection has been investigated by S. Flexner (J. Exper. Med. 36:9, 1922), H. L. Amoss (ibid. 36:25 and 45, 1922), L. T. Webster (ibid. 36:71, 1922; 37:21, 33, 231, 269 and 781, 1923; 38:33 and 45, 1923; 39:879, 1924), I. W. Pritchett (ibid. 41:195, 1925) and W. W. C. Topley (J. Hyg. 19:350, 1920; 20:103, 1921; 21:20, 226, 237 and 243, 1922) in mouse typhoid and rabbit snuffles, and by D. Perla (J. Exper. Med. 45:209, 1927) and M. Lurie (ibid. 51:729, 743 and 769, 1930) in tuberculosis.

controls had already been exposed for more than ten months to contact with guinea-pigs having active tuberculosis. It appears from the tables that practically all the controls were from this source, though this is not clearly stated. This is obviously an unfair control, for animals already fed deficient diets for a year cannot be assumed to become normal when restored to a normal diet. In fact, the authors stated that in spite of a full diet 6 or 8 of the controls within two years presented keratitis and other manifestations of vitamin deficiency. Finally, the significant observation made incidentally in the text of their work that the males in the group on the deficient diet showed no evidence of fighting among themselves whereas the normally fed animals had severe lesions on their backs due to conflict suggests that in the deficiently fed animals there was a general decrease in their interest in the environment. Such apathetic members would be less likely to express interest in an ulceration of their tuberculous cage mate, which was the source of infection, and their chances of infection would therefore be less than those of the robust animals fed on a normal diet.

For these reasons the findings of Sewall and his co-workers⁵⁶ cannot be accepted as throwing light on the problem of the relation of chronic insufficiency of vitamin C or of vitamins in general to natural resistance to infection.

Recently the role of chronic deficiency of vitamin C in the pathogenesis of tuberculosis in the guinea-pig was studied by Greene, Steiner and Kramer.⁵⁸

The diet partially deficient in vitamin C consisted of a mixture called sobee (soy bean flour, olive oil, arrowroot starch, dicalcium chloride, dextrimaltose), 76 per cent; one termed alacta (butterfat, protein, lactose, salts), 20 per cent, brewers' yeast, 3 per cent, and cod liver oil, 1 per cent. For inoculation, the Saranac Laboratory strains of *Myco. tuberculosis* H₃₇ and R₁ were used. Guinea-pigs fed diets completely deficient in vitamin C died within from four to five weeks. When the diet was supplemented with small amounts of an antiscorbutic substance, the animals were maintained in a state of chronic scurvy for several months. Chronic deficiency of vitamin C combined with progressive tuberculous infection caused significant shortening of the period of survival. Chronic scurvy did not cause development of generalized tuberculosis in animals infected with the relatively avirulent R₁ strain. When infection with strain H₃₇ or with bacilli in sputum from tuberculous patients was obtained by the subcutaneous or the enteric route, the infection became generalized more rapidly in the animals that were chronically deficient in vitamin C than in the normally fed guinea-pigs.

Intestinal lesions were apparently more frequent in the vitamin-deficient group than in the control group. It is interesting that the

58. Greene, M. R.; Steiner, M., and Kramer, B.: *Am. Rev. Tuberc.* 33:585, 1936.

tuberculous intestinal lesions were more extensive when sputum was fed than when pure cultures of tubercle bacilli were administered.

However, guinea-pigs infected with tubercle bacilli and allowed to become acutely scorbutic showed tuberculosis in no greater degree than guinea-pigs given a normal diet.

The evidence for these conclusions is too detailed to recount. Though sufficient numbers of animals were employed in each group to permit the observation of significant variations, the application of the methods of statistical analysis to such small groups appears unjustifiable. From the data presented, the differences in the extent of the lesions in various groups, though objectively stated, cannot be quantitatively estimated. The data do not seem to justify the conclusion that generalized tuberculosis developed more rapidly in the chronically scorbutic animals than in the controls.

Heise and Martin⁵⁹ studied the excretion of vitamin C in 44 cases of minimal, moderately advanced and far advanced human tuberculosis. The method of Harris, Ray and Ward⁴³ for the estimation of vitamin C in urine by titration against dichlorophenolindophenol was used. It was roughly found that the more advanced and active the tuberculosis, the less was the daily excretion of cevitamic acid.

In another group of cases, 55 mg. of cevitamic acid in the form of 4 ounces (118 cc.) of orange juice was administered daily for two weeks. The increase in the cevitamic acid excreted was noted. The increase was considered positive if it exceeded 16 mg. daily. In all of the patients with inactive tuberculosis, it was positive; in 80 per cent of those with active disease, it was negative. The authors stated (erroneously) that the amounts of cevitamic acid required daily by normal healthy persons vary from 15 to 20 mg. whereas the amounts required daily to bring tuberculous patients into equilibrium range from 55 to 138 mg. and in some instances as high as 200 mg. Apparently, an increased requirement of the vitamin is manifested in tuberculosis, and the daily excretion seems to parallel the activity of the disease.

Trypanosoma Brucei.—In infection with *T. Brucei* in guinea-pigs, the symptoms of scurvy occur at an earlier period in animals fed a vitamin-deficient diet than in uninfected animals similarly fed (Nassau and Scherzer⁴⁹). Five guinea-pigs infected with *T. Brucei* and then placed on a vitamin-free diet showed signs of scurvy within from ten to eleven days and died within from eighteen to twenty-two days. Four control uninfected guinea-pigs similarly fed did not begin to show scorbutic manifestations until the eighteenth day and died within twenty-

59. Heise, H., and Martin, G. J.: Proc. Soc. Exper. Biol. & Med. 34:642, 1936.

three days. In *T. Brucei* infections there is a greater demand for vitamin C, and apparently this organism cannot synthesize vitamin C. No essential difference in the severity of the infection in the scorbutic animals as determined by trypanosomal counts was indicated, though the duration of life was shortened by the deficiency of vitamin C in the diet. The details of the experiment in this regard are not clearly indicated. The animals with scurvy lost markedly in weight during the infection; the normally feel infected guinea-pigs did not.

Typhus Fever.—Guinea-pigs and rats subjected to vitamin deficiency and then, when symptoms appeared, inoculated with typhus virus exhibited clinical pictures which indicated far more severe infection than that observed in normal animals after inoculation (Zinsser, Ruiz Castaneda and Seastone⁶⁰). There were also wider distribution and greater concentration of rickettsiae, amounting in the pleural and peritoneal exudates almost to cultural proportions.

These experiments were carried out in an effort to obtain large amounts of rickettsiae suitable for immunologic studies. Thirteen guinea-pigs were fed a diet of rolled oats, skimmed milk (autoclaved for one hour) and water during a period of from fourteen to twenty days and then inoculated intraperitoneally with tunica vaginalis from animals suffering with Mexican typhus. In many of the guinea-pigs the disease ran a severe course, and rickettsiae were recovered in abundance from tunica and peritoneum. The animals, of course, were scorbutic, but the authors do not attribute the result to a lack of any one vitamin since all were absent from the diet.

A small number of rats on a similar vitamin-free diet for fourteen days likewise showed marked susceptibility to induction of typhus. Rickettsiae were recovered from visceral scrapings and were found in the spleen, liver, blood, pia mater and endocardium in normally fed rats. The infection was very mild.

These experiments demonstrate the drop in natural resistance that follows complete vitamin deficiency but do not indicate the specific effect of deprivation of vitamin C. Vitamin deficiency is one of the important factors responsible for the high mortality from typhus historically observed during periods of war and famine (Zinsser and others⁶⁰).

Fungus Diseases.—Guinea-pigs were fed a diet deficient in vitamin C, consisting of alfalfa, rolled oats, purified casein and salt, during a period sufficient to produce early manifestations of scurvy. They

60. Zinsser, H.; Ruiz Castaneda, M., and Seastone, C. V., Jr.: J. Exper. Med. 53:333, 1931.

were then given intratracheally and per os daily cultures of *Monilia albicans*. Although the cells of *M. albicans* were found in the lung as long as seven days after inoculation, their presence was only sometimes associated with pathologic changes. The fungus was not limited to scorbutic animals but was equally prominent in normal animals. Apparently, deficiency in vitamin C does not alter the susceptibility of the guinea-pig to infection by *M. albicans*.

Rheumatic Fever and Scurvy.—In the guinea-pig chronic scurvy plus superimposed infection with beta streptococci and to a lesser extent chronic scurvy alone produce arthropathy with pathologic similarities to rheumatic fever and rheumatoid arthritis, according to Rinehart, Connor and Mettier.^{61a} These workers stated that lesions produced in the heart valves by combined scurvy and infection were similar to those of rheumatic fever. From their observations they suggested that sub-clinical degrees of scurvy may predispose to rheumatic infection in human beings.

In their experimental studies on guinea-pigs the diet used consisted of skimmed milk, ground oats and bran, butterfat, dried yeast, cod liver oil, sodium chloride and ferrous lactate. As the infecting agent, they used a strain of hemolytic streptococci (obtained from a guinea-pig) which produced subacute lymphadenitis. The animals were placed on the diet deficient in vitamin C for a period of twenty-two days prior to the injection of the bacteria. Definite arthropathy was found in the scorbutic animals, but more definite arthropathy, in the infected scorbutic animals. In the guinea-pigs with chronic scurvy, eosinophilic hyaline material was present in the joint spaces and especially in the periarticular subsynovial layers.

In other studies they noted that infection in guinea-pigs maintained on an adequate diet usually produced no significant lesions in the heart valves. When such lesions occurred, they were of an exudative rather than proliferative type. In uncomplicated scurvy, definite atrophic -and degenerative changes occurred in the collagenic stroma of the heart valves. In scurvy with added infection (beta streptococci), striking lesions of a combined degenerative and proliferative character developed in the heart valves with considerable frequency. These lesions, they believed, closely resembled the endocarditis of acute rheumatic fever. Further, degenerative and proliferative lesions of the heart muscle and pericardium occurred in the experimental animals subjected to combined scurvy and infection, similar in type to the Aschoff reaction. The authors did not insist that organisms other than the beta streptococcus may not, in the presence of scurvy, produce similar lesions. They called attention, however, to the fact that a basic lesion in both conditions is a degenerative change occurring in collagen. Lesions resembling the "fibrinoid degeneration" of Klinger were seen in various sites in animals subjected to combined scurvy and infection.

Though not questioning the presence of infection as a factor in rheumatic fever, Rinehart, Connor, and Mettier⁶¹ suggested that there

61. (a) Rinehart, J. F.; Connor, C. L., and Mettier, S. R.: *J. Exper. Med.* 59:97, 1934. (b) Rinehart, J. F., and Mettier, S. R.: *Am. J. Path.* 9:932, 1933.

is a considerable amount of evidence that some factor in addition to simple infection contributes to the development of this disease. Experimentally, infection alone or scurvy alone did not produce significant lesions, but when these were combined, striking lesions were produced, particularly of the heart valves.

To further the argument that a condition of undernutrition in vitamin C may be a necessary background for the development of rheumatic fever, Rinehart and his collaborators have presented epidemiological and clinical data that are suggestive of this contention and intriguing in their implications. They ^{62a} have reported that the blood plasma cevitamic acid (reduced) of patients with active rheumatoid arthritis is regularly low if the patients have not been maintained on a diet with a supplement high in vitamin C. The low level rises in response to extra supplements of vitamin C. But in many this rise is refractory; that is, the intake required to maintain an adequate level of vitamin C in the plasma is much above the average requirement for normal persons. The investigators can offer no explanation for this.

In 21 cases of acute rheumatic fever, they ^{62b} found the level of the reduced cevitamic acid in the blood plasma to be uniformly lowered.

Rinehart, Connor and Mettier^{61a} pointed out that the debilitated state of the prerheumatic child has been emphasized clinically and that rheumatic fever is a disease of the poor and undernourished. Campbell and Warner ⁶³ and Swift ⁶⁴ found that rheumatic fever is from fifteen to twenty times more frequent in the laboring classes than in the middle and upper classes and most frequent among poor children of elementary school age living in the city. Gothlin,⁶⁵ using reduced capillary strength as an index of latent scurvy, found evidence of undernutrition in vitamin C in 18 per cent of the school children in the provinces of Upland, Sweden, during August and May, a time when rheumatic fever is at its height. In Norrbotten, in the arctic, Falk, Gedda and Gothlin ^{GG} observed a similar incidence of latent scurvy in presumably healthy children. Dalldorf,⁶⁷ using capillary resistance tests, found that mild

62. (a) Rinehart, J. F.; Greenberg, L. D., and Baker, F.: Proc. Soc. Exper. Biol. & Med. 35:347, 1936. (b) Rinehart, J. F.; Greenberg, L. D., and Christie, A. U.: *ibid.* 35:350, 1936.

63. Campbell, M., and Warner, E. C.: *Lancet* 1:61, 1930.

64. Swift, H. F.: *Am. Heart J.* 6:625, 1931.

65. Göthlin, G. F.: *Skandinav. Arch. f. Physiol.* 61:225, 1931.

66. Falk, G.; Gedda, K. C., and Gothlin, G. F.: *Upsala läkaref. forh.* 38:11, 1932.

67. Dalldorf, G.: *Am. J. Dis. Child.* 46:794, 1933.

degrees of deficiency in vitamin C may constitute a problem of importance to the public health since it occurs in as high as from 35 to 65 per cent of the school population. Children from 5 to 14 years of age require an intake of vitamin C twice that of adults. It is a matter of common knowledge that latent scurvy and rheumatic fever occur late in winter and early in spring, during the period of diminished availability of vitamin C. The vitamin C content of winter milk is very low.

The geographic distribution of rheumatic fever has been carefully studied, and its absence in the tropics, where scurvy is indeed a rare disease, is conceded by numerous observers. Seegal and Seegal⁶⁸ in a study of the geographic distribution of rheumatic fever in Canada and the United States showed that the incidence diminishes as the tropics are approached. In an experience with 150,000 patients in the true tropics Clark⁶⁹ observed no rheumatic fever, mitral stenosis or chorea.

That diet is of importance in the predisposition to rheumatic fever is suggested by the fact, reported by van Breemen,⁷⁰ that this disease is uncommon in Holland, where the dietary is presumably rich in foods containing vitamin C.

The long and sterile search for an etiologic agent of rheumatic fever has led into the treacherous fields of endless controversy. The reproduction of the disease in animals has been simulated repeatedly, but there is no agreement concerning the etiology. It may well be that the work of Rinehart and his associates⁷¹ indicates a possible explanation of the difficulties hitherto encountered.

However, it may be stated at this point that from the point of view of comparative pathology the lesions produced by Rinehart and his associates⁶¹ were not identical with those observed in rheumatic fever in human beings, nor was the disease similar in its clinical course to that termed rheumatic fever. Their work, however, adds support to the contention that chronic scurvy enhances the allergic response of the body to antigenic substances. Such manifestations of myocardial and joint reactions in guinea-pigs and rabbits are nonspecific and are observed in a large variety of allergic and toxic states in these animals.

It would be worth while to carry their suggestion into the field of preventive medicine and gauge the effect of adequate vitamin C in the diet of the children in a community on the incidence of rheumatic infection over a period of ten years.

68. Seegal, D., and Seegal, B. C.: *J. A. M. A.* **89:11**, 1927.

69. Clark, J. T.: *J. Trop. Med.* 33.: 249, 1930.

70. van Breemen, J.: *M. J. & Rec.* 128:469, 1928.

71. Rinehart and others.⁶¹

Schultz,⁷² in a recent study on the utilization of cevitamic acid in rheumatic fever, made careful determinations of the excretion of vitamin C in the urine according to a quantitative method devised by Sendroy and Schultz.⁷³ He was unable, however, to detect any evidence of a deficiency of this acid in patients with rheumatic fever. Of the 13 patients studied at the height of their infection, 8 were found to have relatively deficient, 4 normal and 1 above normal excretion of cevitamic acid.

Sendroy and Schultz felt that the urinary excretion of cevitamic acid was a fair index of the availability of vitamin C, since it is dependent on the relative state of saturation of the tissues and the quantity absorbed from the ingested food. If the intake can be kept constant by the ingestion and absorption of a fixed dose of cevitamic acid, the results may be reliably interpreted as a measure of the state of saturation of the tissue.

In 5 of the patients examined, the results were unfortunately invalidated by vomiting. In 8 patients there was apparent deficiency of cevitamic acid; in only 2 could this be ascribed to the diet. Schultz did not feel that this evidence supported the contention that deficiency in cevitamic acid is a predisposing factor in the causation of rheumatic fever.

In subsequent studies he reported the use of cevitamic acid in the prevention of rheumatic activity and in the treatment of rheumatic infection. He had observed increased capillary permeability in many children suffering from rheumatic fever. Though this test may imply the presence of subclinical scurvy, the condition is found in other febrile states as well as in rheumatic infections. He admitted, however, that this increase in permeability was an expression of dietary deficiency rather than of a rheumatic state, because no characteristic change in the capillary permeability accompanied the onset or disappearance of signs of rheumatic activity. The frequency of change of capillary permeability coincident with rheumatic carditis and other febrile states has been reported by Stephan,⁷⁴ Cutter and Marquardt⁷⁵ and Dalldorf.⁶⁷

Of 56 patients between the ages of 4 and 19 in whom attacks of rheumatic fever had occurred in previous years, 28 were given cevitamic acid in amounts of 100 mg. daily. The control group was given the same amount of lactose by mouth. This quantity of cevitamic acid, Schultz

72. Schultz, M.P.: *J. Clin. Investigation* 15:385, 1935.

73. Sendroy, J., and Schultz, M. P.: *J. Clin. Investigation* 15:369, 1936.

74. Stephan, R.: *Berl. klin. Wchnschr.* -58:317, 1921.

75. Cutter, I. S., and Marquardt, G. H.: *Proc. Soc. Exper. Biol. & Med.* 28:113, 1930.

believed, was sufficient to protect against scurvy, in view of the estimated minimal daily requirement of from 50 to 60 mg. for children (Falk, Gedda and Gothlin⁶⁶).

The capillary fragility was determined at frequent intervals by means of a suction method similar to that described by Johnson. By this method the effects on the surface of the skin of negative pressures within a suction cup at intervals of 10 mm. of mercury are determined. The reading which is recorded is the lowest negative pressure at which capillary hemorrhages appear.

Though the patients of the one group were treated with cevitamic acid over a period of a year, no apparent differences were observed in either of the two groups. There was, however, definite improvement in capillary permeability in the group treated with cevitamic acid.

In a second series of 20 patients with active rheumatic fever, cevitamic acid was used as a therapeutic agent; 17 patients each received 250 mg. of cevitamic acid daily by mouth or intravenously for from one to five months, and 7, 200 cc. of orange juice by mouth daily.

Though the diet was supplemented further with yeast and cod liver oil, none of these measures exerted a demonstrable effect on the clinical picture of the fever. Schultz⁷² concluded that the clinical manifestations of acute rheumatic fever are not demonstrably affected by additions of vitamin C as cevitamic acid or as orange juice to the dietary or by intravenous injections of cevitamic acid over several months. He did not believe that deficiency in cevitamic acid is a necessary factor in the etiology of rheumatic fever, or that adequate intake of this acid over several months prevents the occurrence of rheumatic fever in patients who otherwise would be expected to have the disease.

Incidental to a consideration of the question of the role of a deficiency of vitamin C in the etiology of a number of pathologic conditions other than scurvy, Finkle^{42b} observed the excretion of this vitamin before and after intravenous injections of the vitamin in 14 patients with rheumatic fever. Though most of the patients showed before the injections low levels of excretion of the vitamin in the urine, in 5 there was a considerable increase in the output of vitamin C following the injections of cevitamic acid. He believes that there was no disturbance of the metabolism of this vitamin in these patients. It is of interest that of 24 patients with rheumatoid arthritis, only 4 showed a normal curve of excretion of vitamin C in the urine. The remainder of the group either had excretion levels below the normal or showed no rise following the administration of cevitamic acid.

We do not feel that the clinical experiments reported by Schultz,⁷² Sendroy and Schultz⁷³ and Finkle⁴² definitely negate the hypothesis of Rinehart and his co-workers^{61a} that vitamin C is a factor in the

causation of rheumatic fever. There are several fallacies in their deductions :

1. It is admitted by them that a large number of patients with latent or active rheumatic fever present evidences of sub-clinical scurvy, as determined by capillary fragility tests and by diminished excretion of cevitamic acid. The fact that the rheumatic patient is capable of utilizing vitamin C as the normal person is, as determined by studies, seems to be totally irrelevant since patients suffering from severe scurvy are also capable of utilizing vitamin C.

2. In the second place, the administration of cevitamic acid or of orange juice in amounts hardly twice the minimal protective antiscorbutic dose does not seem to us to insure an adequate excess either in the prophylaxis or in the treatment of rheumatic infection. For, in spite of the disappearance of symptoms of subclinical scurvy, can one be certain of what constitutes the optimal physiologic requirement of the body? This particularly applies to the problems of resistance to infection.

3. The failure of the administration of cevitamic acid either to decrease the incidence of recurrences in latent rheumatic fever or to cure rheumatic infection when it is already present does not, in our opinion, in itself negate the hypothesis that deficiency of vitamin C may be an etiologic factor in rheumatic fever. The contribution of Rinehart and his associates⁷¹ is not that rheumatic fever is caused by deficiency of vitamin C but that an organism such as a streptococcus, which may be the immediate bacterial moment of the disease, can gain a foothold in persons who have had a prolonged relative insufficiency of this vitamin. It is possible that, once a rheumatic infection is established, the restoration of normal availability of vitamin C will not affect the carditis or prevent recurrences of the infection which is entrenched in the organism.

4. Finally, one must not lose sight of the fact that prolonged insufficiency of a vitamin may irreparably injure the organism to a point at which restoration of the normal state may never occur in the life span of the organism. This has been demonstrated in the case of vitamin A by Sherman and MacLeod,⁷⁶ who observed that relative deficiency of vitamin A early in the life of an animal may severely injure the mechanism of resistance and that this injury will persist for many years after the dietary deficiency has been rectified.

76. Sherman, H. C, and MacLeod, F. L.: J. Am. Chem. Soc. 47:1658, 1925.

The ideal clinical experiment to test the validity of Rinehart's hypothesis would be to compare the incidence of rheumatic fever in children raised from birth on a diet adequate in vitamin C with the normal incidence of the disease in the community.

Summary.—Certain bacteria are apparently capable of synthesizing vitamin C. Subacute infection with staphylococci may inhibit the occurrence of scurvy in animals fed a diet deficient in vitamin C.

The experimental data are confusing as to the effect of deficiency of vitamin C in the diet on natural resistance to subsequently induced or coexisting acute bacterial infection. Much of the work does not bear critical analysis. The conclusions are often unsubstantiated by the experimental evidence offered. The conditions of the experiments reported are frequently too involved and complicated.

Though it is probable that the incidence of spontaneous infection with the pasteurella of fowl cholera may be greater in scorbutic than in normally fed guinea-pigs, it is not definitely established that scurvy lowers the natural resistance to this infection. There is a suggestion that scorbutic guinea-pigs succumb to acute infection with *Str. haemolyticus*, *Bact. coli*, *Staph. aureus* or *Pneumococcus* in shorter periods of time and required slightly lower minimal quantities of these organisms to become fatally infected than normally fed animals. The differences, however, with the exception of *Pneumococcus*, are not statistically significant.

Under certain conditions, vitamin C deficiency increases the permeability of the intestinal wall to bacteria, such as *Bact. aertryke*.

The evidence as to the effect of deficiency of vitamin C in the diet on the natural resistance of guinea-pigs to subsequently induced or coexistent tuberculous infection is contradictory. It seems a priori impossible to establish significant differences in the duration of a chronic infection induced in an animal with acute scurvy.

It is reported that guinea-pigs fed a diet inadequate in vitamin C and subsequently exposed to cage mates with open tuberculous lesions (skin ulcerations) show no greater incidence of contact infection than do normally fed animals. The data and conditions of the experiments, however, are such that they throw no light on the important problem of the effect of chronic scurvy on the susceptibility of an animal to tuberculous infection.

In the guinea-pig with chronic scurvy, infection with certain organisms, such as the beta streptococcus, induces arthropathy with endocardial and myocardial lesions resembling those of rheumatic fever in human beings. It is suggested that undernutrition in vitamin C lowers the natural resistance of man to rheumatic infection and that such a nutritional factor may play a significant etiologic role in this disease.

In brief, complete deficiency of vitamin C in the diet may lower natural resistance to subsequently induced acute infection. The experimental evidence, however, is still inadequate and requires further confirmation.

It has not been unequivocally established what effect chronic insufficiency of vitamin C in the diet has on natural resistance to a subsequently induced chronic infection, such as tuberculosis.

Chronic insufficiency of vitamin C in the diet lowers the natural resistance of the guinea-pig to a subsequently induced infection with the beta streptococcus, and a condition simulating rheumatic infection may result.

(To be concluded)

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Volume 23
1937

PUBLISHERS
AMERICAN MEDICAL ASSOCIATION
CHICAGO, ILL.