General Review

ROLE OF VITAMIN C IN RESISTANCE

DAVID PERLA, M.D.
AND
JESSIE MARMORSTON, M.D.
NEW YORK

(Concluded from page 575)

VIII. EFFECT OF INSUFFICIENCY OF VITAMIN C ON ACQUIRED RESISTANCE TO EXPERIMENTALLY INDUCED INFECTION

Mycobacterium Tuberculosis.—The effect of chronic latent scurvy on the course of an existing infection with Myco. tuberculosis has been investigated.

The acquired resistance of tuberculous guinea-pigs that were well fed except in regard to vitamin C was in general considerably less than that of normally fed infected animals of almost any age, according to Bieling.[77] It was not the undernutrition itself, he maintained, which was the factor in early death but the absence of vitamin C in the diet, for the depression in resistance was observed even if the guinea-pig remained constant in weight. In guinea-pigs with chronic tuberculosis the effects of withdrawal of vitamin C were more pronounced than in the uninfected. He observed no disturbance in the consumption of oxygen in scorbutic animals as determined by the Benedict apparatus.

Many of his guinea-pigs had chronic tuberculosis of two years' duration. Within from five to nine days after such animals were placed on a scorbutic diet, they died. It seemed unlikely that death occurring in so short a period was the result of a break in the mechanism of resistance with reactivation of the tuberculous process. At autopsy no evidence of fresh miliary dissemination of tubercles was noted, so that the tuberculous infection did not apparently flare up under the influence of the deficient diet. There was no sudden increase in the virulence of the tubercle bacilli. The possibility of the effect of toxemia either from the tuberculous process or from the undernutrition was excluded, as no symptoms of this were present. Chronically tuberculous guinea-pigs died before any symptoms of scurvy developed when such animals were placed on a diet deficient in vitamin C. These observations may explain in part the increased mortality

77. Bieling, R.: (*a*) Ztschr. f. Hyg. u. Infektionskr. **101**:442, 1923; (*b*) **102**:568, 1924.

from tuberculous infection in human beings subjected to conditions of undernutrition.

It is regrettable that the data on which these deductions are based are so limited and so inadequately presented. They are concerned chiefly with the effect of a scorbutic diet on the course of a tuberculous infection induced with small quantities of virulent tubercle bacilli some time prior to the change in diet. The infection existed for periods of from one to two years prior to the alteration in diet. Apparently, an animal suffering with chronic tuberculous infection is more susceptible to effects of deprivation of vitamin C in the diet than a noninfected animal similarly treated.

In other experiments, Bieling [77b] injected tubercle bacilli into guinea-pigs and three weeks later altered the diet of the animals from a normal one of hay, turnips, fresh grass and oats to one of oats and autoclaved milk. Another group were put on the scurvy-producing diet after six weeks of tuberculous infection. Animals for which the diet was changed had a shorter duration of life than those for which it was not altered.

The increased sensitivity to avitaminosis was more apparent after six than after three weeks. None of the animals, when they died, showed widespread tuberculosis. The experiments were restricted to small numbers of guinea-pigs, and the diets were deficient in other vitamins as well as in vitamin C. Bieling [77b] suggested that the occurrence of scurvy even in mild degree may constitute a menace to the health of the chronically tuberculous. This conclusion seems justified.

Heymann [78] studied the effect of a diet deficient in vitamin C on the course of an induced infection with tubercle bacilli in guinea-pigs. The infection was initiated before the diet was altered. The infecting dose in all instances was 0.00001 mg. of a human strain of tubercle bacilli. The diet consisted of oats, hay and water. A small but inadequate quantity of lemon juice was added to the diet. In guinea-pigs infected with tubercle bacilli from three to four weeks prior to the change in diet no change in the course of the tuberculosis occurred, nor did evidence of scurvy develop. When in a second experiment the quantity of lemon juice was half the amount and other conditions as in the first experiment, the animals soon lost weight, showed signs of scurvy and died within an average period of approximately two months after the beginning of the experiment as compared with four and a half months in the control group. No influence on the character of the tuberculous process was noted, but there was an influence on the tempo of the infection. No tuberculosis of bones or joints was observed.

In these experiments of Heymann,[78] though the diet appears adequate in respects other than that of vitamin C, the numbers of animals

78. Heymann, B.: Klin. Wchnschr. **5**:59, 1926.

used in the various groups are not mentioned and it is difficult, therefore, to evaluate his data. Apparently, however, deficiency of vitamin C in the diet of the tuberculous animal hastens the fatal issue.

McConkey and Smith [79] studied the effect of adequate amounts of vitamin C and D in the diet on the course of intestinal tuberculosis in guinea-pigs. They fed tuberculous sputum to 72 guinea-pigs daily for periods ranging from six weeks to four months. Of 37 that were maintained on a diet partially deficient in vitamin C, 26 acquired intestinal tuberculosis. Of the other 35 animals, on a diet that was supplemented by an adequate amount of vitamin C, only 2 showed tuberculous ulcers in the intestines. From these data the conclusion seems justified that an adequate supply of vitamin C protects the tuberculous guinea-pig against ulcerative intestinal tuberculosis and that mere ingestion of tubercle bacilli by the guinea-pig is not the sole factor in the production of intestinal tuberculosis. The course of the general disease is not modified.

Analogous experiences in human beings have not been collected. Apparently, deficiency in vitamin C leads to alterations in the permeability of the intestinal mucosa, as was indicated from the earlier observations of McCarrison.[33] The high incidence of tuberculous enteritis, particularly in women, at institutions such as Montefiore Hospital, where diets adequate in vitamin C are given, suggests that perhaps in man another mechanism is operative. This problem is now being studied in the tuberculosis division. Many of the patients enter the country sanatorium early in their pulmonary disease, and intestinal tuberculosis develops while they are under observation. It may, however, be that functional digestive disturbances interfere with adequate absorption of vitamin C.

Summary.—If guinea-pigs are fed a diet containing insufficient quantities of vitamin C but not completely depleted of this factor, a chronic infection of relatively low virulence may run a more rapid and severe course in them than in normally fed animals. Guinea-pigs that have suffered with tuberculous infection of a low grade for long periods of time may when placed on a diet deficient in vitamin C die rapidly, before manifestations of scurvy appear. Their death may be due either to sudden progression of the tuberculosis (Heymann [78]) or to toxemia induced by the avitaminosis. Undernutrition itself is not responsible for this drop in resistance.

An insufficient quantity of vitamin C in the diet increases the incidence of intestinal tuberculosis in guinea-pigs previously inoculated with virulent tubercle bacilli and subsequently fed tuberculous material.

79. McConkey, M., and Smith, D. T.: J. Exper. Med. **58**:503, 1933.

These experiments are concerned more with disturbances in *acquired* resistance to tuberculous infection.

Apparently, the acquired resistance of the guinea-pig established by an induced infection with Myco. tuberculosis of low virulence is lowered if the diet is subsequently depleted partially or completely of vitamin C. The tempo of the disease increases, or the animals die of acute toxemia.

IX. EFFECT OF EXCESS OF VITAMIN C ON NATURAL RESISTANCE TO TOXINS, SPONTANEOUS INFECTIONS AND INDUCED INFECTIONS

The growing interest in the importance of vitamins in the diet in the bodily well-being, and the accumulation of evidence of the intimate dependence of natural resistance to infection on dietary factors, have led investigators to test the value of an excess of such factors on resistance. Much of this work has been disappointing. It may be that deficiency of a factor essential to normal cellular metabolism results in depression of the organism to abnormal stimuli, as infection, toxemia and the like. It does not necessarily follow that an excess above the bodily requirements increases the natural resistance of the host. That is, there is probably an optimal effect of any nutritional factor, and an excessive intake may not result in raising the optimal effect. However, in the case of vitamin C, sufficient evidence has accumulated to suggest the importance of cevitamic acid in oxidation-reduction processes in cellular metabolism, and it is conceivable that increase in its availability raises the capacity of the tissues to destroy toxic substances introduced into the body. On the other hand, certain dangers in experiments of this type should be emphasized. The animals used should be of the same stock, the history of which should be known. The dietary regimen of the mother as well as of the experimental animal since birth should be under control and adequate in vitamin content. Relative deficiency during the early months of life may cause permanent impairment in resistance regardless of a subsequent normal diet. A rise in the natural resistance of a group of animals given a vitamin in excess, above that of a control group, may not indicate an increase above the normal optimum but may suggest that a relative deficiency of the vitamin had previously existed in both groups. With these points in mind, it is difficult to evaluate the data in this field. Is one certain of the optimal vitamin requirements? Can the criteria of growth, progressive increase in weight and absence of clinical evidences of deficiency be accepted as adequate? Is it not possible that all these may be present and still, under a given stress, such as invasion with micro-organisms or injections of poisons, the apparent optimal amounts do not prove to be so?

For these reasons it is difficult to apply the implications of experimental evidence of effects of a temporary excess of a vitamin on resistance in animals of unknown source directly to human infection. In view of these remarks, the least one may demand of experiments of this type is, first, rigid control of the stock from which the experimental animals are drawn; second, the use of a strain of animals raised under ideal laboratory conditions, with a constant complete dietary for more than one generation; third, adequate controls in sufficient numbers to eliminate chance variations; fourth, statistically significant differences between the results in the group given the excess vitamin and the controls.

Infections: Tuberculosis.—Heise and Martin [80] showed experimentally in guinea-pigs that the injection of a great excess of crystalline vitamin C for a period of five months did not alter the course of induced tuberculosis.

They gave each of 10 guinea-pigs a daily intraperitoneal injection of 20 *mg.* of cevitamic acid during a period of one week prior to and five months subsequent to a subcutaneous injection of 300,000 virulent human tubercle bacilli (H_{37}). Five controls were given the tuberculous infection at the same time. The total amount of cevitamic acid given to each animal was 30 Gm. No detrimental effects of the daily injections were observed. When one of the controls died, all the remaining animals were killed and autopsies made. No significant differences in the dissemination and severity of the disease in the two groups were noted.

Leichtentritt[81] attempted to increase the resistance of guinea-pigs to tuberculosis by giving them an excess of vitamin C.

The normal dietary of the guinea-pigs consisted of oats, dried milk and lemon juice (0.5 cc.) daily. In two preliminary experiments in which the animals were infected by means of sputum from a tuberculous patient, she observed a slight beneficial effect from an excess of lemon juice in the diet on the longevity of the infected animals.

In another experiment, 11 guinea-pigs were infected with the tubercle bacilli contained in sputum. Five of these each received 20 cc. of lemon juice daily in addition to the diet, for seventy-three days. Four received 20 cc. of hydrous wool fat as a control. The differences in longevity in the two groups were slightly in favor of those given additions of lemon juice. Using cerebrospinal fluid obtained from a patient with tuberculous meningitis as a source of the infecting organisms, she made a similar experiment in which 2 guinea-pigs received supplements of lemon juice and 5 received lemon juice heated to 40 C, in vacuo. Those receiving fresh lemon juice lived twice as long as the controls.

These experiments yielded data which, while suggestive, are of little value. The source of infection could not be standardized, and the

80. Heise, F. H., and Martin, G. J.: Proc. Soc. Exper. Biol. & Med. **35**:337, 1936.

81. Leichtentritt, B.: Ztschr. f. Hyg. u. Infektionskr. **102**:388, 1924.

animals were too few for one to consider that the error of chance variation had been eliminated.

In an additional experiment, 8 guinea-pigs were each infected with 0.0025 mg. of tubercle bacilli (human) subcutaneously. Of these, 6 were given daily dietary supplements of 15 cc. of lemon juice and 2 were fed an adequate diet. The two controls died at a little over two months after infection. Of the guinea-pigs fed excessive supplements of lemon juice, 2 died at four and a half and six and a half months, respectively, and the remaining 4 were killed after a period of about seven months. The latter had gained in weight and showed little tuberculosis. (The strain of tubercle bacilli used was not very virulent.)

Apparently, an excess of vitamin C may increase the resistance of guinea-pigs -to tuberculous infection.

The use of high vitamin supplements to the diet has been recommended in the treatment of intestinal tuberculosis. McConkey [82] treated 128 patients with intestinal tuberculosis complicating pulmonary disease. Of these, 50 were given ultraviolet radiation; 50 were given cod liver oil and tomato juice in liberal quantities, and 28 were treated palliatively. Of those given heliotherapy, 60 per cent improved, while 68 per cent of those receiving cod liver oil and tomato juice improved. Of the control group, only 3.5 per cent improved. When, after an unstated period, the patients were traced, 71 per cent of the untreated group, 24 per cent of those given heliotherapy and only 10 per cent of those treated with vitamins were dead.

These encouraging results are difficult to evaluate. The diagnosis of intestinal tuberculosis is not easily made on the basis of symptoms. Even with the aid of roentgenograms, the diagnosis may be deceptive (Schwatt and Steinbach [83]).

Frimodt-Moller,[84] in India, gave 25 persons suffering from intestinal tuberculosis diets high in vitamins, with beneficial effects. Brown and Sampson stated that cod liver oil and tomato juice in abundance had cured many patients of intestinal tuberculosis.[84a]

Steinbach and Rosenblatt[85] fed diets high in vitamins (tomato juice and cod liver oil) to patients with advanced pulmonary disease during a period of two years without obtaining any evidence of healing of intestinal complications. Such therapeutic tests are hardly a fair indication of the usefulness of a high vitamin C content in the diet.

The evidence is only suggestive that an excess of vitamin C may increase the natural resistance of the host to tuberculosis. Neither

82. McConkey, M.: Am. Rev. Tuberc. **21**:627, 1930.
83. Schwatt, H., and Steinbach, M. M.: Am. Rev. Tuberc. **8**:9, 1923.
84. Frimodt-Moller, C.: Indian M. Gaz. **65**:692, 1930.
84a. Brown, L., and Sampson, H.: Intestinal Tuberculosis: Its Importance, Diagnosis and Treatment, Philadelphia, Lea & Febiger, 1930.
85. Steinbach, M. M., and Rosenblatt, M. B.: Am. Rev. Tuberc. **31**:35, 1935.

the experimental nor the clinical observations are unequivocal. It is possible that an excess may decrease the incidence of intestinal tuberculosis in tuberculous guinea-pigs.

Diphtheria Toxin.—Independently, Jungeblut and Zwemer[86] and Greenwald and Harde [87] studied the effects of an excess of vitamin C as cevitamic acid on diphtheria toxin in vivo and in vitro. They had the opportunity of utilizing crystalline vitamin C.

Jungeblut and Swemer [86] added to 2 M. L. D. of diphtheria toxin varying amounts of vitamin C from 0.05 to 100 mg. at a p_H of from 6.6 to 6.8. After allowing the mixtures to stand half an hour at room temperature, they injected them subcutaneously into 37 guinea-pigs. They found that from 0.5 to 5 mg. of vitamin C detoxified the toxin. Greater or lesser amounts did not. Of 22 controls given the same amounts, all died within from twenty-four to seventy-two hours showing symptoms of diphtheritic intoxication. In a second experiment, the effects of vitamin C in vivo were tried. They gave each of 14 guinea-pigs 2 M. L. D. of diphtheria toxin subcutaneously and immediately afterward injected vitamin C in acid solution in amounts varying from 1 to 200 mg. Half the guinea-pigs died, and half survived 2 M. L. D. Amounts of from 5 to 25 mg. of vitamin C were protective. Excessive amounts were toxic. In a third experiment, 13 guinea-pigs were given daily subcutaneous injections of vitamin C in varying amounts. Each was then given 4 injections of diphtheria toxin in amounts of 1/50, 1/100, 1/200, 1/500 M. L. D. intracutaneously on two sides of the abdomen. Readings of the degrees of local inflammation were made every day for five days. The extent and the degree of redness as well as the intensity of necrosis were recorded. The stored vitamin C inhibited or suppressed the local reaction to diphtheria toxin.

Jungeblut and Zwemer concluded that vitamin C inactivates diphtheria toxin and helps to protect guinea-pigs against the fatal outcome of diphtheritic intoxications. Guinea-pigs treated with suitable amounts of this substance are rendered temporarily negative or definitely less sensitive to small doses of the toxin, as determined in intracutaneous tests. They believed that the results suggest that vitamin C plays an important part in the mechanism of natural resistance to diphtheria toxin.

Similar findings were reported at an earlier date by Harde [88] and by Harde and Philippe.[89] Appearing at the same time was the report of Greenwald and Harde [87] on the effect of feeding an excess of vitamin C on the resistance to diphtheria toxin.

They fed 4 guinea-pigs an excess of spinach for ten days, and then injected 1 M. L. D. of toxin into each, and all died. Each of 5 guinea-pigs was given

86. Jungeblut, C. W., and Zwemer, R. L.: Proc. Soc. Exper. Biol. & Med. **32**:1229, 1935.

87. Greenwald, C. K., and Harde, E.: Proc. Soc. Exper. Biol. & Med. **32**: 1157, 1935.

88. Harde, E.: Compt. rend. Acad. d. sc. **199**:618, 1934.

89. Harde, E., and Philippe, M.: Compt. rend. Acad. d. sc. **199**:738, 1934.

subcutaneously 10 mg. of vitamin C for three days and then 1 M. L. D. of toxin. All died. Each of 4 guinea-pigs was given 1 M. L. D. of toxin and then injections of vitamin C. They lost weight, but 2 survived, and 4 controls died. From five to six weeks later, the survivors were readily killed by the same amount of toxin, indicating that no immunity had been established. The investigators observed no deleterious effects of vitamin C on antitoxic potency or the potency of toxin-antitoxin mixtures in vitro.

They incubated 150 mg. of vitamin C and 500 M. L. D. of toxin at p_H 4 to 4.4 at room temperature for eighteen hours. The toxin was definitely inactivated, and from 1½ to 2 M. L. D. were no longer fatal.

They concluded that vitamin C under certain conditions increases the resistance of guinea-pigs to 1 M. L. D. of a standardized diphtheria toxin, and that diphtheria toxin kept in contact with vitamin C for one hour before injection is less toxic.

This is consistent with the observations of Lyman and King,[90] who noted a marked loss of vitamin C in the adrenal glands, pancreas and kidneys of guinea-pigs that had received injections of diphtheria toxin. In 39 animals given toxin, the decrease in vitamin C in these organs varied from 21 to 38 per cent as compared with 39 controls given similar injections. The concentration of vitamin C was estimated by titration with 2,6-dichlorophenolindophenol. Apparently, the physiologic need of vitamin C in guinea-pigs given diphtheria toxin is greatly increased.

The observation that guinea-pigs having a high storage of vitamin C are less sensitive to diphtheria toxin than are those having a low storage of this vitamin may be important in relation to the pathogenesis of diphtheria infection.

Kligler[91] attempted to ascertain the effect of vitamin C on the production of toxin by Corynebacterium diphtheriae in vitro. He studied the influence of the vitamin in the culture medium on the toxicity of filtrates of cultures of a toxin-producing strain. A sterile solution of vitamin C was added to the culture medium in concentrations ranging from 0.05 to 1.25 per cent. The flasks containing the vitamin, as well as corresponding controls, were inoculated with C. diphtheriae and incubated for forty-eight hours at 30 C. The toxicity of the filtrates was determined by intracutaneous injections into guinea-pigs.

The vitamin C added to the culture medium rapidly disappeared on incubation at from 30 to 37 C. When the medium was inoculated with C. diphtheriae immediately after the vitamin was added, the vitamin disappeared more slowly. Even small residual amounts of the vitamin (from 20 to 40 mg. per hundred cubic centimeters) are sufficient to

90. Lyman, C. M., and King, C. G.: J. Pharmacol. & Exper. Therap. **56**:209, 1936.

91. Kligler, I. J.: Nature, London **138**:291, 1936.

inhibit production of toxin. The same strain of C. diphtheriae was found to produce a potent toxin in the flask without vitamin C and little or no toxin in the one containing the same medium plus cevitamic acid in the concentration of 0.05 per cent.

Kligler [91] suggested that the varying toxic character of a diphtheritic infection may depend as much on the tissue saturation with vitamin C as on the toxigenic properties of the infecting strain. He further suggested that the mildness of diphtheritic infection in tropical and subtropical countries is in some way related to the quantitative differences in the concentration of this vitamin.

Bamberger and Wendt[92] observed some beneficial effect in severe diphtheria with circulatory disturbances from the combined use of adrenal cortical hormone and vitamin C. The use of vitamin C alone they found to be without effect. Of 8 patients treated with combined adrenal cortical hormone and cevitamic acid, 5 seemed definitely improved.

The clinical use of large quantities of cevitamic acid as a supplement to specific serum in the treatment of diphtheria has yielded unencouraging results. Otto[93] stated that the cevitamic acid at best tends to modify to some degree the hemorrhagic tendency, particularly when thrombopenia is present.

He [93] treated a series of 50 patients with diphtheritic antitoxin alone and compared the results with those obtained in 42 patients treated with antitoxin and cevitamic acid. In the patients treated with serum alone the membrane disappeared in an average period of six days and the fever dropped to normal in an average period of 7.5 days. Practically identical with these results were those in the group given large doses of cevitamic acid in addition to the antitoxin. The number of patients with severe complications was the same in both groups, and 2 patients in each group died. The cevitamic acid was given intravenously, in amounts as high as 500 mg. a day, and also as a 10 per cent spray directly to the membranous areas.

Anaphylattic Shock.—Vitamin C may have a protective effect against anaphylactic shock in guinea-pigs, which is more marked when the vitamin C has been administered prior to sensitization than when it has been given after sensitization (Salomonica [94]).

Salomonica[94] utilized 91 guinea-pigs. Each was sensitized by an intra-abdominal injection of 1 cc. of a 1: 100 dilution of horse serum and shocked twenty-one days later by an intravenous injection of 0.5 cc. of a 1: 10 dilution of horse serum. This amount had been found to kill the sensitized animal. In all, eight groups of animals were used. One group of 17 animals was sensitized and shocked and received no treatment with vitamin C, All succumbed. In a

92. Bamberger, P., and Wendt, L.: Klin. Wchnschr. **14**:846, 1935.
93. Otto, H.: Klin. Wchnschr. **15**:1510, 1936.
94. Salomonica, B.: J. Immunol. **31**:209, 1936.

second group of 9, the guinea-pigs received crystalline vitamin C in amounts varying from 1 to 10 mg. a day during a period of three weeks prior to and after administration of the sensitizing dose. All of these survived. The third group received vitamin C in the same amounts during a period of three weeks prior to sensitization; of 17 guinea-pigs, 14 survived. In a fourth group the vitamin C was given during three weeks after the sensitizing dose; of 16 guinea-pigs, 4 survived. In a fifth group the vitamin C was administered only during the last two weeks of the period of sensitization, and of 9 animals, none survived. In a sixth group 11 animals received the vitamin C during the last week of the period of sensitization and, of these animals, 3 survived. In a seventh group the vitamin C was given in a single dose from one to four hours prior to the injection of the shocking dose; of 7 guinea-pigs, 1 survived. Finally, the cevitamic acid was administered simultaneously with the shocking dose of horse serum, and 2 of 3 guinea-pigs were protected when 10 mg. of the vitamin C was used. In all cases, a crystalline product of vitamin C diluted in distilled water was employed.

The results support the conclusion that vitamin C administered to guinea-pigs prior to sensitization protects such animals against anaphylactic shock. The administration of vitamin C during sensitization has much less effect. When the vitamin is administered with the shocking dose, some protective value is observed. Salomonica [94] suggested that the protective action of vitamin C against anaphylaxis is dependent on its effect on the endocrine glands. He argued that hyperfunction of the thyroid gland increases and diminished function tends to decrease allergic phenomena. In guinea-pigs removal of the thyroid gland had a protective effect against anaphylactic shock, according to Lanzenberg and Kepinow [95] and Kreitmair,[96] who observed that the lethal effect of thyroxine can be cpmpletely checked by cevitamic acid. This antagonistic effect of vitamin C might explain in part, he believed, its protective action against anaphylactic shock. That cevitamic acid tends to depress hyperactivity of the thyroid gland is suggested by the work of Marine and Baumann.[97]

Tuberculin Shock.—In a recent communication Steinbach and Klein[98] reported the effect of crystalline vitamin C on the tolerance of tuberculous guinea-pigs to tuberculin. In preliminary experiments they observed that cevitamic acid mixed with skin test doses of tuberculin in vitro prior to intracutaneous inoculation of those doses in tuberculous guinea-pigs failed to inactivate the tuberculin. Prolonged treatment of *7* tuberculous guinea-pigs with cevitamic acid prior to

95. Lanzenberg, A., and Kepinow, L.: Compt. rend. Soc. de biol. **86**:204 and 906, 1922.

96. Kreitmair, H.: Arch. f. exper. Path. u. Pharmakol. **176**:326, 1934.

97. Marine, D.; Baumann, E. J., and Rosen, S. H.: Proc. Soc. Exper. Biol. & Med. **31**:870, 1934.

98. Steinbach, M. M., and Klein, S. J.: Proc. Soc. Exper. Biol. & Med. **35**: 151, 1936.

infection with tubercle bacilli failed to reduce the reactivity of the skin to tuberculin. They further observed that the vitamin was unable to inactivate tuberculin either in vitro or in vivo when the tuberculin was used in doses large enough to cause death of control tuberculous guinea-pigs. In the tests in vivo, the vitamin C was injected intravenously just prior to the injection of tuberculin.

They did observe, however, increased tolerance to tuberculin in tuberculous animals treated for prolonged periods with injections of cevitamic acid.

In two individual experiments 22 animals were used. Each was given a subcutaneous injection of 0.001 mg. of a bovine strain of the tubercle bacillus (B_1). From the eighteenth day after the injection of the bacilli, half the animals each received a subcutaneous injection of 5 mg. of cevitamic acid daily during the entire experimental period. Twenty-three days after infection, each of the guinea-pigs received 2 injections of old tuberculin weekly in increasing amounts of from 50 to 1,600 mg. administered subcutaneously. Whenever an animal of one group died, a guinea-pig of the other group was killed for the purpose of comparing the extent of the disease. After the eighteenth injection of tuberculin, all the untreated animals died of tuberculin shock, and only one of the vitamin-treated group.

They found that practically every one of the animals given cevitamic acid survived injection of an amount of tuberculin which killed the untreated tuberculous guinea-pig. They also noted that the untreated animals showed more extensive tuberculosis than did the animals treated with cevitamic acid. They further compared the cortices of the adrenal glands of the animals that died of tuberculin shock with those of the treated surviving guinea-pigs, by means of the silver nitrate reaction. There was no reduction of the silver nitrate (i. e., no cevitamic acid) in the adrenal glands of the shocked animals, but some was present in those treated animals which survived the shock.

They concluded that an excess of cevitamic acid given to tuberculous animals decreased their sensitivity to tuberculin shock. It may be mentioned that the cevitamic acid in the adrenal cortex of the animal dying of an infection or of a debilitating process is rapidly exhausted in the processes leading to death. The reaction is further dependent on the presence of a peroxidase enzyme, which is likewise destroyed with the death of the animal. The comparison of this reaction in animals that die of tuberculin shock with that in animals killed for control observations may not be valid.

It will be recalled that Sulzberger and Oser[15] observed hypersensitiveness to neoarsphenamine in guinea-pigs given less than 0.2 mg. of cevitamic acid per day, but when the animals were given from 1 to 5 mg. a day their capacity for allergic response to neoarsphenamine

disappeared. The investigators concluded justifiably that this hypersensitiveness was due to a relative state of insufficiency of vitamin C. This suggests that certain experiments on the effect of what is interpreted as an excess of vitamin C on resistance to toxins, infection and anaphylactic shock may be open to criticism. It is possible that the control animals employed in such studies may really have been suffering from a relative insufficiency of vitamin C at the time of the onset of the experiment,, and that the experimental animals that received additions of vitamin C in the diet represented those fed a diet physiologically adequate in vitamin C and not containing necessarily an excess of the vitamin.

Poliomyelitis.—Jungeblut" observed that substances neutralizing the virus of poliomyelitis were present in serum, human tears, placenta, the urine of pregnant women, extracts of adrenal gland, extract of adrenal cortex, epinephrine and serum containing diphtheritic antitoxin. He then studied the effect of vitamin C in neutralizing the virus of poliomyelitis.

To portions of the supernatant fluid of a 10 per cent emulsion of spinal cord from a monkey infected with poliomyelitis he added graded amounts of a solution of crystalline vitamin C varying from 0.05 to 50 mg. Then 0.1 cc. quantities of these mixtures were injected into 30 monkeys, respectively. Inactivation was obtained with 1, 5, 10 and 50 mg. Amounts smaller than 0.1 and larger than 10 mg. were not constantly effective, and 50 and 100 mg. were toxic.

He concluded that multiple paralytic doses of the virus of poliomyelitis when mixed with very small amounts of crystalline vitamin C are rendered noninfectious, as determined by intracerebral injection of such mixtures into monkeys (Macacus rhesus).

Recently Jungeblut[100a] reported before the American Bacteriological Society experiments in vivo on the action of vitamin C in preventing poliomyelitis in monkeys. By injecting small doses of vitamin C subcutaneously in monkeys, beginning with the day on which the animals were inoculated with the virus, he found that the treated animals were more resistant than the controls.

He divided 30 monkeys into four groups. Three groups were given subcutaneous injections of varying amounts of crystalline vitamin C from the day of injection daily for two and a half weeks. The fourth group was left untreated, as controls. The monkeys treated with 5 mg. of vitamin C survived without showing any signs of poliomyelitis. In 10 monkeys which had had no treatment with vitamin C but which had received the same dose of virus, poliomyelitis developed.

99. Jungeblut, C. W.: J. Exper. Med. **62**:517, 1935.

100. Jungeblut, C. W.: (*a*) J. Bact. **31**:34, 1936; (*b*) J. Exper. Med. **65**: 127, 1937.

Subsequently Jungeblut[100b] extended these experiments and confirmed his earlier results.

A group of 34 rhesus monkeys, weighing between 2,000 and 3,000 Gm. each, were inoculated intracerebrally with 0.1 cc. of a 10 per cent suspension of the virus. Following the inoculation, 9 animals were treated with daily injections of from 700 to 100 mg. each of crystalline natural vitamin C, 16 with from 50 to 10 mg. and 9 with 5 mg., during a period of two weeks—34 tested monkeys in all. Six of the treated monkeys survived without showing paralysis; 2 of these had received from 50 to 10 mg., while 4 had received 5 mg. Of 19 untreated monkeys simultaneously infected with the same amount of virus, all showed paralysis.

In a second group, 13 monkeys were given intracerebral injections of 0.05 cc. of the virus. Following the injection, 1 animal was treated with 25 mg. and 5 animals with 5 mg. of vitamin C daily for a period of two weeks; 7 were untreated. One animal that had received 5 mg. daily survived without evidence of paralysis. Of the untreated monkeys, all presented paralysis.

In a third experiment, 22 monkeys were infected intracerebrally with 0.01 cc. of the virus. Of this group, 1 was treated with 100 mg., 2 with 50 to 10 mg. and 19 with 5 mg. of vitamin C daily during a period of two weeks. Of this group 1 that received 10 mg. and 11 that had received 5 mg. survived without showing paralysis. Of 12 untreated control monkeys similarly infected with the same dose, 2 failed to show paralysis, and 10 subsequently had paralysis.

In summarizing his results, Jungeblut[100b] stated that of a total of 62 treated monkeys, 19 survived without paralysis; 43 succumbed to the disease. Of a total of 38 untreated controls, only 2 failed to succumb to paralysis. All monkeys treated with vitamin C in doses of from 700 to 100 mg. presented paralysis; 3 of 19 monkeys that received 50 to 10 mg. survived without paralysis, while nearly half of the animals receiving 5 mg. escaped this disease.

The use of vitamin C prophylactically against subsequent intracerebral infection failed to protect monkeys in any instance. Of 2 control animals that were given crystalline carotene in amounts of 100 mg. for two weeks and two that received 2 Gm. of vitamin B in the form of powdered yeast, following injection of the virus, all succumbed with paralysis. The addition of vitamin C directly to normal nonneutralizing monkey serum gave irregular results.

Jungeblut[100b] is of the belief that the susceptibility of certain children and adults to the virus of poliomyelitis is dependent in part on a partial deficiency of vitamin C in the diet. The majority of children and most adults fail to contract poliomyelitis when exposed to the infectious agent. In some instances, this is due to immunity acquired in a paralyzing attack, and this immunity may or may not be associated with circulating antibodies. The more important type of protection is represented by natural resistance to the-disease. It is believed by some epidemiologists that the widespread resistance to the virus is due to the immunizing effect of subclinical or abortive attacks of the disease. Subclinical immunization in monkey and man, however, has not been

demonstrated, and the viricidal substances that are found in normal human and animal tissues, serum or other body fluids are probably not of an antibody character, according to Jungeblut, but resemble agents of a vitamin-like or hormonal nature.

Since immunization by means of killed virus had proved inadequate and that by means of live virus dangerous, Jungeblut suggested that prophylaxis and treatment of infantile paralysis must depend on enhancing the natural resistance of the host. From his results, he concluded that this might be accomplished by supplying the exposed or infected persons with optimal amounts of vitamin C.

It is known that the minimum requirement of an adult guinea-pig in health is from 1 to 2 mg. of cevitamic acid per day and that the need of vitamin C in the presence of infection is considerably greater.[101] Five milligrams of vitamin C daily, the dosage utilized in many of the experiments reported in the literature, may not provide an excess of this substance.

The problem of the effect of an excess of vitamin C on the natural resistance of man and animals, such as the guinea-pig or the monkey, requires further investigation. Animals utilized in such experiments should be of a stock known to be constant, one maintained under controlled conditions, on an adequate standard diet containing cevitamic acid in a physiologically adequate amount from the day of birth. Comparisons of the resistance of such animals to infection may then be made with that of animals given a sufficient excess above this minimal adequate amount for different intervals of time. In this way it can be determined to what degree the natural resistance to infection may be raised above the normal level by the administration of vitamin C.

Virus of Herpes.—Holden and Resnick [102] studied the effect of vitamin C on inactivation of the virus of herpes in experiments in vitro. They observed that cevitamic acid failed to inactivate "w" virus, which is immunologically related to the virus of herpes, when the p_H of the virus-vitamin mixture fell within the rnage of p_H 5 to p_H 8. When *pn* was less than 5, the cevitamic acid inactivated the virus. It was apparent that the action of the cevitamic acid on the "w" virus is nonspecific and due entirely to the effect of p_H.

Trypanosoma Equip erdum.—An excess of vitamin C had no influence in modifying the course of infection with T. equiperdum in mice (Perla[103]).

101. It is further known that animals fed a relatively inadequate amount cf vitamin C and then given in addition an infection may be thrown into a more advanced state of vitamin C insufficiency and even frank scurvy.

102 Holden, M., and Resnick, R.: J. Immunol. **31**:455, 1936.

103. Perla, D.: To be published.

The mice were on a complete and adequate diet, consisting of rolled oats, hominy, meat scrap, dried milk and salt, with daily additions of fresh milk, yeast, cod liver oil and wheat germ. Fresh greens were added twice a week. Mice thrive and gain rapidly on such a diet. The mice had been bred from a strain originally of the Rockefeller Institute for Medical Research and were free from latent infections. The strain of T. equiperdum had been carried in guinea-pigs for several passages to separate it from Bartonella muris, as this organism is destroyed in the guinea-pig. The mice were given daily intraperitoneal injections of a solution of synthetic cevitamic acid in amounts of 1 mg. per day per mouse during a period of fourteen days prior to the injection of trypanosomes and thereafter. The minimal effective dose of trypanosomes necessary to produce an infection in the normal animal had been determined in preliminary experiments. From ten to thirty times this amount was used to insure uniform "takes." The experiments with vitamin C were repeated three times on different occasions, an equal number of controls being used in each experiment. The course of the infection and the duration of life were studied in all the experiments.

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

These experiments, though suggesting that the resistance of mice to a protozoan infection cannot be altered by an excess of vitamin C parenterally administered, were made in a species known to produce its own vitamin C. It is well established that scurvy cannot be established in rats or mice on diets deprived completely of vitamin C during as long a period as three generations (Simmonds [104]).[105] It was felt that since rats and mice are capable of producing their own vitamin C the results of studies of the effect of an excess of this factor on their resistance could not be interpreted.

In another series of experiments, therefore, Perla [103] studied the effect of repeated injections of cevitamic acid on trypanosomal infection in guinea-pigs.

The guinea-pigs were of a uniform stock, which had been raised in the laboratory for more than ten years, on a uniform adequate diet of oats, alfalfa hay, greens ad libitum and water. Cevitamic acid was injected daily, intraperitoneally, in amounts of 10 mg. dissolved in physiologic solution of sodium chloride, brought up to a p_H of 7.5, during a period of forty-two days prior to the injection of trypanosomes and thereafter until the death of the animal. In the first experiment, 12 guinea-pigs were treated with cevitamic acid, and 12 were con-

104. Simmonds, N.: Am. J. Hyg. (supp.) **4**:1, 1924.

105. No difference in rate of growth or reproductive capacity is observed in rats fed a diet deficient in vitamin C. G. A. Hartwell (Biochem. J. **24**:967, 1930), H. T. Parsons (J. Biochem. **44**:587, 1920), and S. Lepkovsky and M. T. Nelson (J. Biol. Chem. **59**:91, 1924) found the livers of rats fed a scurvy-producing diet rich in antiscorbutic substance. The -livers of rats of a second generation raised on a diet free from vitamin C were still equally rich in vitamin C. Rats fed a diet deficient in vitamin C showed no decrease in their capacity to produce immune antibodies.[103]

trols of the same age (from 6 to 7 weeks). Each animal received 30,000 trypanosomes intraperitoneally. Of the treated guinea-pigs, only 2 became infected; they died within twenty-five and thirty days. Ten escaped infection. Of the untreated ones, all succumbed.

In a second experiment, the same daily quantities of vitamin C were given during a period of seventy-seven days prior to the injection of T. Brucei. The infecting dose was 30,000 trypanosomes. Of the 7 guinea-pigs treated, all succumbed to the infection within a period of from eleven to twenty-seven days (average, nineteen days). Of the 7 controls, 6 succumbed and 1 survived. The duration of the infection varied from nineteen to thirty-one days (average, twenty-six days). The interval between the injection of the trypanosomes and the appearance of organisms in the blood stream was nine days in the control and from five to seven days in the vitamin C-treated guinea-pigs. The treated animals lost, considerably in weight. These animals were from 4 to 5 months of age. The difference in the two groups is not significant.

In the third experiment, still in progress, the conditions were more carefully controlled. A group of 24 guinea-pigs was removed from stock at the age of 5 weeks, and each animal was given 1 mg. of cevitamic acid daily intraperitoneally. After a short period, 12 of the guinea-pigs received 10 mg. daily, and the others, as controls, continued to receive 1 mg. daily. The diet was adequate as in the previous experiments.

At the end of forty days of treatment each animal was given an injection of 50,000 trypanosomes (Trypanosoma Brucei), and the injections of vitamin C were continued. (Use of individual syringes and needles in administration of vitamin C after infection is important, to avoid cross-infections.) A manifest infection developed in all animals. After a period of six weeks 10 of the controls had died, whereas only 2 of the 12 that had received 10 mg. a day had died. The rest, at the time of writing, either showed remission or had a very mild infection.

It was thought that this type of experiment eliminated the objections as to the uncertainty of the dosage of vitamin C in the controls. The protective effect of an excess of vitamin C on resistance was more pronounced in the young animals.

In moderate excess cevitamic acid raises the natural resistance of the guinea-pig to a trypanosomal infection.

Summary.—A moderate excess of cevitamic acid may increase the natural resistance of guinea-pigs to diphtheria toxin and tuberculin and to anaphylactic shock. Both diphtheritic toxin and the virus of poliomyelitis may be neutralized by this acid in vitro. A moderate excess of vitamin C, parenterally introduced, raises the resistance of monkeys to the virus of poliomyelitis and of guinea-pigs to T. Brucei. Large quantities of cevitamic acid may be toxic to guinea-pigs.

The literature on the effect of an excess of vitamin C containing foods on human infections such as tuberculosis is of little worth. It is suggested, though it is not as yet well established, that an excess of vitamin C raises the resistance of animals to intestinal tuberculosis. Therapeutically, in tuberculous animals a great excess of vitamin C administered for long periods is apparently of little value in modifying the severity of the general disease.

X. IMPORTANCE OF VITAMIN C IN MAINTENANCE OF NORMAL TOOTH GROWTH

The importance of the carious tooth as a portal of entry for bacterial infection has been well established. In recent years the role of dietary factors in the maintenance of normal tooth structure and growth has been stressed. The earliest and most characteristic changes caused by vitamin C deficiency are found in the teeth and gums (Dalldorf and Zall,[106] Höjer and Westin,[107] Wolbach and Howe[108]).

Zilva and Wells [10!)] were the first to stress the fact that scurvy even in the mildest form produces marked changes in the structure of the teeth. Fibroid degeneration takes place in the tooth pulp. When the librosis is complete, no cellular elements, no trace of cellular organization, no cement substance is seen. The nerve cells, blood vessels and odontoblasts all share in the fibroid degeneration and are no longer recognizable. The irregular osteoid condition of the dentin is well marked, and the refractive appearance of the dentin is due to a hemorrhagic condition of dentinal fibrils. The change starts first in the odontoblastic cells at the top of the pulp, working down toward the apex, and is followed by engorgement of blood vessels and hemorrhage, leading to complete fibroid degeneration.

The teeth are affected as early as the seventh day in guinea-pigs placed on a diet of oats, bran and autoclaved milk. When scorbutic conditions during life are so slight as to be almost unrecognizable the condition of the teeth may be severe. The hemorrhagic phenomena in muscle and .periosteum and the changes at the epiphyses of the long-bones and ribs appear at a much later period.

Robb and his co-workers [110] studied the pathogenesis of the changes in the pulp in greater detail. In the normal guinea-pig, the odontoblastic layer of cells is of the tall columnar type, and the inner surface of the dentin is smooth. The pulp contains a fine fibrillar connective tissue stroma and is well supplied with blood vessels. In scurvy, the odontoblasts lose their columnar form, and some of them are enclosed in osteodentin. The odontoblasts secrete osteodentin rapidly, and the osteodentin soon completely encloses the pulp cavity. It is thick, and the inner surface is thrown into folds; there is hyperemia of the pulp, and small hemorrhages are present. There is increased elimination of

106. Dalldorf, G., and Zall, C.: J. Exper. Med. **52**:57, 1930.

107. Hojer, A., and Westin, G.: Dental Cosmos **67**:1, 1925.

108. Wolbach, S. B., and Howe, P. R.: Proc. Soc. Exper. Biol. & Med. **22**:400, 1924. Wolbach and Howe.[31]

109. Zilva, S. S., and Wells, F. M.: Proc. Roy. Soc., London, s.B **90**:505, 1919.

110. Robb, E. F.; Medes, G.; McClendon, J. F.; Graham, M., and Murphy, I. J.: T. Dent. Research **3**:39, 1921.

calcium in the scorbutic state, and the teeth rapidly lose calcium and cementin. The osteodentin eventually fills the pulp cavity. Robb and his co-workers [1TO] estimated that in scurvy the osteodentin of the pulp cavity is a hundred times greater than the normal. The foregoing observations are not entirely accurate.

The changes in the jawbones and teeth were further investigated by Höjer and Westin[107] and the reconstruction of the pathogenesis modified:

In the jawbone, the calcified bone becomes porous through a process which starts in the marrow spaces and the bone canals. New bone is formed on the outer surfaces of the jawbone. This bone is deficient in collagen and inferior in quality.

In the teeth, the first positive sign of scurvy, according to Höjer and Westin,[107] is a gradual change and disappearance of the odontoblastic layer. There is amorphous calcification of the predentin, with disappearance of Tomes' canals in this layer. The dentin that was calcified at the onset of the scurvy becomes porous through a process which starts from the protoplasm content of Tomes' canals and results in their dilatation and confluence. A new formation of pulp bone instead of dentin occurs. This bone has a spongy structure and an appearance distinctly corresponding with that of the new-formed, inferior bone of the outer surfaces of the jawbone. Dilatation of the pulpal vessels and hemorrhages occur. Subsequently, hydropic degeneration of the pulp develops with necrosis. Finally, after new formation of bone in the pulp has stopped, atrophy and resorption of this bone, of the dentin and of the pulp occur. Large cystic areas filled with fluid may replace the disappearing pulp.

In latent scurvy, the process is similar but less pronounced. If an insufficient quantity of antiscorbutic substances is given in the diet, irregular dentin is formed, which has Tomes' canals. In the lingual part of the pulp there are symmetrically arranged ridges of pulp bone without dental canals.

Höjer and Westin[107] observed an overproduction of osteodentin such as was described by Robb and his associates [106] in those instances in which as much as 80 per cent of the minimal protective quantities of vitamin C was given in the diet. In such instances, no pulp bone was laid down, all the newly formed hard tissue consisting of osteodentin.

Wolbach and Howe,[108] in a careful review of the pathologic changes in the teeth in complete scurvy, offered interpretations differing in some respects from those of Höjer and Westin.[107] They observed that in complete scurvy in guinea-pigs there is no formation of osteodentin or pulp bone, but that these represent regenerative processes due to the presence of small amounts of antiscorbutic substance. The earliest

changes noted when all vitamin C is withdrawn from the diet occur in from seven to twelve days as a separation of the odontoblast layer from the dentin by a narrow margin, occasional irregular calcium deposits in the odontogenic zone (predentin) and irregularities in the odontoblast layer. The individual cells become smaller and stain more deeply. The blood vessels are engorged. In the predentin zone there are occasional deposits of basic-staining granular material, interpreted as calcium salts, while between the processes of the odontoblasts continued formation of dentin can be inferred because of the presence of globules of hyaline material interpreted as a matrix of spherites. After from twelve to fourteen days, complete separation of the odontoblasts from the dentin occurs. There is rupture of the processes of the odontoblasts, and the spaces separating the odontoblasts and the dentin contain no stainable material. Such spaces appear first as vacuoles, and it is probable that an accumulation of liquid material causes the separation. The odontoblasts exhibit changes in size and increased density of staining. Changes in the pulp occur as edema and granular deposits between the connective tissue. In absolute scurvy, the pulp is shrunken, freed from dentin and apparently floating in a liquid material. Within twenty-four hours after the administration of orange juice or vitamin C a zone of new dentin forms on the separated odontoblasts, and within three days of treatment the new dentin completely fills the space between the odontoblast layer and the dentin layer. It appears irregular in contour, following the irregular surface of the odontoblasts. This Wolbach and Howe [108] interpreted as a jelling of the defective material. They hypothecated that in scurvy the odontoblasts continue to produce a liquid material. This theory accounts for the edematous appearance of the fibrous tissue-like structure in bones called *Gerüstmark.* The theory is supported by the promptness and the volume of the matrix formation following antiscorbutic treatment.

These observations have been confirmed in detail by Hanke,[111] who in a recent monograph pictorially reviewed the changes in progressive scurvy. In addition, he studied the reparative process in guinea-pigs fed a scorbutic diet for twenty-one days and then given vitamin C as orange juice in amounts of 5 cc. a day for twenty days. Essentially, the reparative process involved partial restoration to the normal state. With additions of vitamin C, a network of calcified organic matter appeared in the pulp. Calcification was most pronounced in the regions contiguous to the original dentin. Spicules of calcified organic matter radiated toward the center of the pulp. At two days, odontoblasts were not in evidence, and the pulp was still very abnormal, containing many vacuoles and no normal pulp cells. At five days, the radiating network of

111. Hanke, M. T.: J. Am. Dent. A. **17**:957, 1930.

calcified organic matter had advanced from all sides almost to the center of the pulp chamber. Cells resembling odontoblasts were seen growing over all the surfaces of the abnormal calcine deposits. At ten days, the pulp chamber had become almost a solid mass of calcine organic matter. At twenty days, the calcified organic matter in the pulp chamber had acquired an appearance similar to that of imperfectly formed dentin. Odontoblasts appeared at the apical end, and tubules were apparent in the imperfectly formed dentin at this end.

In those instances in which the diet had contained vitamin C in small but insufficient amounts for twenty-one days, the addition of adequate amounts of vitamin C was followed by a restoration of the tooth structure practically to the normal. Thus, after five days of treatment, odontoblasts appeared in large numbers and distributed themselves along the inner margins of the imperfectly formed dentin. New tubules appeared in the imperfectly formed dentin contiguous to the odontoblasts, and the pulp was firmer and more fibrotic in structure. After ten days of treatment, the newly formed tubules extended to the zone where the original predentin had become amorphously calcified with the onset of scurvy. The amorphously calcified predentin that marked the region of the original injury was vague, but the pulp still exhibited many wide spaces, about some of which the odontoblasts were distorted. Between the spaces, the cells of the pulp appeared to be normal.

The observations on the changes in the teeth in scurvy indicate the interesting fact that even with subclinical scurvy the transformation of the pulp may go beyond the point where complete restoration to normal can occur, regardless of treatment. That is, some phase of the injury leaves permanent distortion. In milder scurvy the restoration may proceed further but apparently is not complete.

Hanke[111] believed that the incidence of dental caries could be checked by a diet rich in vitamin C, but this has not been established either clinically or experimentally.

There is some evidence that gingivitis may be perceptibly improved by an excess of vitamin C, for it is likely that this condition indicates relative absence of vitamin C.

It is established that vitamin C is important in normal tooth growth and tooth structure and in the maintenance of a healthy condition of the gums. Indirectly, therefore, it must be important in the natural resistance of the pulp and peridental tissue to focal infection.

XT. INTERPRETATION OF THE ROLE OF VITAMIN C IN RESISTANCE

Physiologic Importance of Vitamin C in Formation of Intercellular Cement.—Vitamin C is physiologically important in oxidation-reduction systems in the body. Its properties were described chemically by Szent-Györgyi,[2] who isolated a reducing substance from the adrenal cortex

and found an identical principle in cabbage and orange juice. His interest was directed to the substance in the course of a study of peroxidase systems in plants. The reducing, principle that he isolated he believed to be a hexuronic acid and later demonstrated its anti-scorbutic properties and its identity with vitamin C.[3] The structure was subsequently found to be a diketo-acid showing tautomeric change (Haworth, Hirst and Reynolds,[112]) and its synthesis from xylosone was described by Reichenstein, Grussner and Oppenauer.[113] For a complete review of the recent advances in the chemistry of cevitamic acid, the reader is referred to the excellent survey by King.[114]

A deficiency of vitamin C leads to a change in the intercellular cement substance (Wolbach and Howe [108]). Through the careful studies of Aschoff and Koch,[115] Höjer and Westin,[107] and finally Wolbach and Howe, the essential effects have been established. Wolbach and Howe have contributed the most plausible explanation of the changes seen in vitamin C deficiency from their excellent work on guinea-pigs. They observed the sequence of events in the absolute scorbutic state in the incisor teeth and the bones of growing guinea-pigs and they studied the process of repair of the soft tissues and bone following injury in scorbutic guinea-pigs. In scurvy, there is an inability of the supporting structures to produce and maintain intercellular substances. Direct proof of this conclusion has been obtained in the study of teeth in regard to the dentin, in the study of growth and repair of bone in regard to the bone matrix and in the study of repair of soft tissue in regard to the collagen of connective tissues.

The failure of capillary formation is due to the failure of endothelial cells to form cement substances. The proliferative power of epidermis, endothelium, fibroblasts and osteoblasts is not diminished in scorbutus. The osteoblasts, in spite of morphologic change, preserve their chemical potentialities and produce a bone matrix. The odontoblasts continue to produce a liquid material which lacks jelling properties. A failure of the cement substance in blood vessels accounts for the hemorrhage. This failure of the cell to produce intercellular substance in scurvy is due to absence of an agent common to all supporting tissues which is responsible for the setting or jelling of a liquid product.

This explanation helps to clarify much of the pathologic picture. It is possible that changes in the resistance of scorbutic animals to infection

112. Haworth, W. N.; Hirst, E. L., and Reynolds, R. J. W.: J. Soc. Chem. Indust. **52**:482, 1933.

113. Reichenstein, T.; Grussner, A., and Oppenauer, R.: Helvet. chim. acta **17**: 570, 1934.

114. King, C. D.: Physiol. Rev. **16**:238, 1936.

115. Aschoff, L., and Koch, W.: Veroffentl. a. d. Geb. d. Kreigs- u. Konstitutionspath. **1**:4, 1919.

are in part attributable to the altered permeability of membranes resulting from this change. That is, there is a breakdown in the normal protective capacity at the site of bacterial invasion of the body. This also suggests a decrease in the resistance of the tissue in general to injury of any type.

Physiologic Importance of Vitamin C in Cellular Respiration.—With the recent advance in knowledge of the chemistry of vitamin C, it becomes apparent that this vitamin is essential in reduction-oxidation processes in all cells of the body. Though existing in higher concentration within the adrenal cortex, it is found in the ovaries, brain, liver (Bessey and King[116]), pituitary gland, pancreas and kidney, and large amounts are present in the thymus (von Euler and Klussmann [117]). Its presence in association with cholesterol or glutathione may be of significance. Since the adrenal gland is a tissue of extremely high metabolic activity, it would of necessity utilize in its physiologic processes large amounts of so important a reducing substance as cevitamic acid. The utilization of the vitamin elsewhere in the body or the capacity of certain animals to manufacture it may not be dependent specifically on the adrenal cortex. The possibility of a specific relationship was suggested by the fact that vitamin C and the adrenal hormones are concerned with oxidation-reduction systems in animal metabolism, that adrenalectomized animals present hemorrhagic phenomena suggestive of those in scorbutic animals, and that cevitamic acid occurs in large amounts in the cortex. A specific interrelationship has not, however, been demonstrated.

Svirbely and Kendall[118] studied the influence of cortical hormone on the nitrogen metabolism of adrenalectomized dogs maintained on a vitamin C-free diet. No changes in nitrogen metabolism occurred under these circumstances. In the absence of the adrenal cortical hormone in adrenalectomized animals, the nitrogen metabolism tends to become negative (Sandberg and Perla[119]). Svirbely and Kendall observed no changes suggestive of scurvy in adrenalectomized dogs maintained on a vitamin C-free diet but given extract of adrenal cortex. No mention is made of a detailed histologic examination of bones.

Nevertheless, Lockwood and Hartman [120] and Lockwood, Swan and Hartman [121] have observed that an extract of adrenal cortex relatively

116. Bessey, O. A., and King, C. G.: J. Biol. Chem. **103**:687, 1933.

117. von Euler, H., and Klussmann, E.: Ztschr. f. physiol. Chem. **217**:167, 1933.

118. Svirbely, L. J., and Kendall, E. C.: Am. J. Physiol. **116**:187, 1936.

119. Sandberg, M., and Perla, D.: J. Biol. Chem. **113**:35, 1936.

120. Lockwood, J. E., and Hartman, F. A.: Endocrinology **17**:501, 1933.

121. Lockwood, J. E.; Swan, D. R., and Hartman, F. A.: Am. J. Physiol. **117**:553, 1936.

free from vitamin C ameliorated the symptoms of scurvy. Control extracts of liver offered no protection. They noted that a definite amount of cortical extract afforded optimum protection, and greater amounts did not increase the effect. They expressed the belief that the substance is not vitamin C, present as a contaminant, but that cortical hormone or some unknown substance from the adrenal cortex delays the onset of scurvy.

In another connection, Schour and Rogoff [122] studied the histologic changes in the teeth of rats at various intervals after adrenalectomy. Slight changes were noted in the character of the predentin. They suggested that these changes are associated with a disturbance of calcium metabolism.

Perla and Sandberg [123] reported a series of experiments undertaken to determine whether an animal such as the rat, normally capable of producing its own vitamin C, shows impairment of this capacity in the absence of the adrenal glands. They observed microscopic changes in the teeth due to adrenalectomy somewhat similar to the changes noted by Schour and Rogoff.[122] These changes consisted in disappearance of the predentin zone or condensation and calcification of this zone, irregularity of the odontoblast layer, and vacuolation and pyknosis of the nuclei. Occasionally, metaplasia of the dentin with formation of islands of osteoid tissue occurred.

Adrenalectomy in the rat was found to be unassociated with a significant decrease in the vitamin C stored in the liver or in the vitamin C excreted in the urine.[124] Further, the dental changes were found in animals regardless of the vitamin C content of the food: they may not, therefore, be considered an expression of vitamin C deficiency but rather are due to a metabolic disturbance produced by the removal of the adrenal gland.

The changes in the teeth of adrenalectomized rats, though at first suggestive of early changes seen in scurvy in the guinea-pig, are apparently not an expression of an impairment of the ability of the rat either to utilize or to synthesize vitamin C, nor are they an expression of an inhibition of cellular respiration per se. This is probably true, since repeated injections of methyl cyanide, a drug that inhibits cellular oxidation, into normal rats does not produce alterations of this type. The tooth changes are not an expression of a disturbance in calcium metabolism, since chemically the disturbance in calcium metabolism after

122. Schour, I., and Rogoff, J. M.: Am. J. Physiol. **115**:334, 1936.

123. Perla, D., and Sandberg, M.: Arch. Path. **23**:372, 1937.

124. Nevertheless, they did observe that prolonged maintenance of rats not subject to operation on a scorbutic diet results in condensation and calcification of the epiphyses of the long bones, with complete cessation of growth of the cartilaginous columns. No changes in the teeth of these animals were observed.

adrenalectomy is too slight to be of significance in tooth metabolism (Sandberg and Perla[125]). It was further observed that the decrease in natural resistance to histamine poisoning following adrenalectomy in the rat cannot be influenced by injections of an excess of cevitamic acid (Perla[126]). From these observations, Perla and Sandberg[123] concluded that the adrenal gland is probably not essential in the production or the utilization of vitamin C. A specific interrelationship is not present.

From the work of Bourne [127] and Giroud, Leblond and Giroux,[128] it is probable that almost every cell in the body contains traces of vitamin C. Bourne [129] suggested that vitamin C forms an oxidation-reduction system with glutathione, and it is likely that it is important in cellular respiration. It is thus nonspecifically associated with the production of hormones in the endocrine glands.

It is now well established that vitamin C is important as an agent for the transportation of hydrogen between unidentified metabolites and other carriers by way of two or more oxidase enzyme systems. It is therefore essential in cellular respiration.

The importance of vitamin C in resistance is secondary, then, to its essential role in the maintenance of normal cellular metabolism. By its withdrawal, cellular respiration is promptly inhibited, and the threshold of tolerance to poisons, infections or other types of injury is necessarily depressed. It is possible that an excess of cevitamic acid may raise the threshold of tolerance, but such effect, by the nature of the chemical processes involved, would be limited in its possibilities. However, since it has been shown that latent scurvy may be converted into clinical disease in the presence of mild infection, it is apparent that the physiologic need for vitamin C must be greater in conditions in which the rate of cellular metabolism is increased. In the presence of infection, therefore, an increase in the intake of vitamin C may be of importance.

Detoxifying and Bactericidal Action of Vitamin C.—It has been suggested by the work of Harde [88] and Harde and Philippe,[89] Jungeblut and Zwemer,[86] Kligler [91] and others that vitamin C may have a direct detoxifying effect on bacterial toxins. According to Kligler [91] and Gagyi,[130] its value in the treatment of infectious diseases may be

125. Sandberg, M., and Perla, D.: J. Biol. Chem. **113:35,** 1936.
126. Perla, D.: Proc. Soc. Exper. Biol. & Med. **35**:390, 1936.
127. Bourne, G.: Nature, London **131**:874, 1933.
128. Giroud, A.; Leblond, C. P., and Giroux, M.: Compt. rend. Acad. d. sc. **198**:850, 1934.
129. Bourne, G.: Physiol. Rev. **16**:442, 1936.
130. Gagyi, J.: Klin. Wchnschr. **15**:190, 1936.

dependent on its inhibitory action on bacterial growth as well as on toxin formation.

The action of vitamin C in neutralizing diphtheria toxin in vitro may be dependent on its acidifying effect as well as on its action as a reducing agent. Its bactericidal action in vitro, according to Gagyi,[130] is due to its reducing properties. Gagyi [130] determined the bactericidal power of a 2 to 5 per cent vitamin C solution on suspensions of various micro-organisms. Dilute suspensions of cultures of C. diphtheriae, C. pseudodiphtheriae, Diplococcus pneumoniae, Micrococcus catarrhalis, organisms of the typhoid-paratyphoid-colon-dysentery group and Myco. tuberculosis were placed in a 2 per cent saline solution of cevitamic acid and incubated-for various periods at 37 C. The organisms were then subcultured to determine their viability, and the quantity of vitamin C destroyed in the solution was estimated by titration.

The diphtheria organisms and those of the typhoid-paratyphoid group were extremely sensitive to the effects of vitamin C and were rapidly destroyed when exposed to solutions of this vitamin. Pneumococcus and Str. haemolyticus were more sensitive and more easily destroyed than Staphylococcus. The viability of tubercle bacilli was unaffected even after seventy hours' exposure to a 5 per cent solution of vitamin C. The destruction of the vitamin C by such bacterial suspensions may be due to the formation of alkali by the bacteria as well as to the metabolic utilization of the vitamin by the bacteria (Gagyi [130]).

Whether the action of vitamin C in vivo is due either to a direct bactericidal or to a detoxifying effect is problematic. It is doubtful whether one could secure such a high degree of saturation of the cells of the body with vitamin C as is apparently necessary for bactericidal action comparable with that attained in vitro. Not only is it improbable owing to the rapidity with which vitamin C is excreted in the urine when administered in excess, but vitamin C in very large amounts would probably prove deleterious in human beings as it does in animals. It is very unlikely, therefore, that the activity of vitamin C in resistance is dependent on any direct bactericidal action. It is far more probable that it exerts its influence independently by its effect on oxidation-reduction processes in cellular metabolism.

Influence of Vitamin C on Hematopoiesis, on Growth of Monocytes and on Response to Injury.—Alterations in natural resistance in scurvy are probably independent of any interference in the production of immune antibodies or in the availability of so-called natural antibodies as opsonins, bacteriolysins and complement. However, some reduction in the phagocytic power of the leukocytes was observed with respect to Bact. coli and Myco. tuberculosis by Lawrynowicz.[131]

131. Lawrynowicz, A.: J. de physiol. et path. gen. **29**:270, 1931.

Findlay [132] observed late leukopenia in chronic scurvy in guinea-pigs and noted degenerative changes in the marrow. The leukoblastic reaction to infection is feeble in scorbutic guinea-pigs infected with pneumococci. In scurvy there is a relative increase in the number of lymphocytes, which decreases before death, according to Maxia.[133] The Arneth index is turned to the left. The reduction in the leukoblastic reaction of the organism in scurvy was observed by Lawrynowicz.[131] The intraperitoneal injection of irritating substances, such as aleuronat and peptone, into scorbutic animals called forth an exudate which was not as rich in leukocytes as that obtained in the normal guinea-pig.

Though some investigators postulate that the diminished resistance to infection in scorbutic states is secondary to these minor changes in leukoblastic response and slight alteration in opsonic power, it is probable that these are merely indications of an altered cellular activity of a more fundamental character.

Further evidence of alterations in the activity of the marrow is furnished by Mettier and Chew,[134] who observed in guinea-pigs fed a diet deficient in vitamin C a progressive profound anemia accompanied by definite cytologic changes in the marrow indicative of retarded production of red blood cells. The red cell count dropped to 2,500,000 per cubic millimeter in fifteen days, and the hemoglobin to 5.5 Gm., from a normal level of 12 Gm. per hundred cubic centimeters. The reticulocytes increased terminally to 5 and 10 per cent, from a normal of 1 per cent. The marrow of these animals showed almost complete disappearance of fat cells, marked decrease in the number of mature erythrocytes and increase in the number of normoblasts.

With the administration of orange juice, the reticulocytes rose from 12 to 20 per cent, and the anemia promptly disappeared. Sections of the marrow removed from these animals at the height of the reticulocyte response showed marked cellularity. However, in contrast to the marrow before treatment, the marrow after treatment showed marked increase in mitotic figures.

That cevitamic acid functions as one of the regulators of erythrocyte metabolism seems probable from the experiments of Baron and Baron.[135] It had been discovered by Waltner and Waltner[136] and Mascherpa [137] that cobalt salts, when given to animals, produced poly-

132. Findlay, G. M.: J. Path. & Bact. **24**:446, 1921.

133. Maxia, C.: Scritti biol. **5**:35, 1930.

134. Mettier, S. R., and Chew, W. B.: Proc. Soc. Exper. Biol. & Med. **29**: 11, 1931.

135. Baron, A. G., and Baron, E.. S.; Proc. Soc. Exper. Biol. & Med. **35:** 407, 1936.

136. Waltner, K., and Waltner, K.: Klin. Wchnschr. **8**:313, 1929.

137. Mascherpa, P.: Arch. ital. de biol. **82**:112, 1930.

cythemia, as determined by the increase in the number of red ceils and hemoglobin in the peripheral blood. Mascherpa [137] noted in dogs with polycythemia due to cobalt increased activity of the marrow, Baron and Baron,[135] by daily subcutaneous injection of 0.01 Gm. of cobalt sulfate, induced, within six to seven days, definite polycythemia with appearance of reticulocytes and erythroblasts in the circulating blood. The occurrence of these young cells in the blood was associated with an increase in the consumption of oxygen by the red cells to ten times that observed in controls, in which erythroblasts were not present in the circulating blood.

When cevitamic acid was injected intravenously into rabbits in amounts of 60 mg. a day simultaneously with cobalt sulfate, polycythemia failed to appear. When cevitamic acid was withdrawn, polycythemia appeared at the end of six or seven days. When it was injected after the production of polycythemia, the concentration of hemoglobin and the number of red cells decreased temporarily.

Baron and Baron[135] expressed the belief that the polycythemia described was due to inhibition of the respiratory function of immature red blood cells by cobalt, and that for this reason the cells appeared in the general circulation as mature nonrespirational cells, being replaced in the marrow by new cells. Thus, the addition of cobalt sulfate in vitro to suspensions of red cells from animals with polycythemia of this type was followed by marked inhibition in respiration, an effect not observed in the red cells of normal rabbits.

Cevitamic acid seems definitely to stimulate normal respiration of the red cells and acts therefore in maintaining the level of red cells in the circulating blood. This effect has been reported also in certain instances of anemia.

That cevitamic acid may accelerate the proliferation of monocytes is indicated by the experiments of Baker.[135] It may be that the influence of vitamin C on resistance in certain infections is in part explainable on this basis.

Baker [13S] studied the effect of additions of cevitamic acid on the growth of monocytes in tissue culture. The medium contained blood serum, dextrose, Witte's peptone, cystine, hemin, insulin, thyroxin, vitamins A, B and D and salts. The cevitamic acid solution was preserved with glutathione, which inhibits the oxidation of vitamin C. The glutathione-cevitamic acid mixture was added in sufficient quantity to make a total concentration of cevitamic acid in the medium of from 0.05 to 2.5 mg. per hundred cubic centimeters. In concentrations of from 0.1 to 0.5 mg. of the vitamin per hundred cubic centimeters, the cells showed less granulation than normal cells, and proliferation was

138. Baker, L.: Compt. rend, Soc, de biol. **121**:427, 1936.

much more rapid Tbis effect, however, was not manifest until the end of the second week. If greater concentrations were used, the cells remained transparent and showed no increase in rate of growth. Glutathione alone had little or no effect. A solution of cevitamic acid alone, prepared just prior to use, had marked stimulative action. Baker[138] expressed the belief that cevitamic acid is one of the substances necessary for multiplication of monocytes in vitro.

The scorbutic state interferes with the reparative processes following injury to bone or soft tissues, according to Wolbach and Howe.[139] They fed 12 guinea-pigs for ten days a diet deficient in vitamin C. Simple incisions were made in tissues and bone and the processes of repair observed. In one group, orange juice was added to the diet. In the other, the deficient diet was maintained. The sequences of repair as studied at two, four, six and nine days showed that, though the epidermis grew normally, defective granulation tissue formed in the subcutaneous wound. The wounds remained ulcerated in many cases. There was no difference in the mononuclear inflammatory response, in the foreign body giant cell reaction of in the proliferation of muscle nuclei in the scorbutic animals as compared with the controls. Healing of the wound, however, proceeded by avascular organization. Fibroblasts grew in and produced no collagen or only traces of it, though fibroglia fibrils formed. In the scorbutic guinea-pigs, the injury to the bone was not followed by new bone formation as in the normally fed animals. The gap was filled with fibroblast-like cells without a trace of collagen and unaccompanied by capillaries. When orange juice was given, new bone was readily formed in a fibrin-like matrix in immediate contact with the cut bone. In scurvy, there is complete absence of repair of bone, without the formation of collagen or bone matrix.

The effect of avitaminosis on regenerative processes following experimental injuries is of considerable importance in this discussion, since the mechanism of resistance is intimately linked to the mechanism of repair of injury. Ishido [140] observed that in guinea-pigs and rats maintained on vitamin-free diets, wound healing is definitely delayed as compared with that in animals fed a normal, adequate diet or animals partially starved on a normal diet. The adherence of the wound is poor, and improper new formation of connective tissue occurs. This leads to collection of fluid in the floor of the wound with cavity formation and necrosis, and secondary infection is frequent. Saitta [141] likewise observed that guinea-pigs fed a scorbutic diet during a period of sixteen days prior to injury showed impairment of cicatrization.

139. Wolbach, S. B., and Howe, P. R.: Am. J. Path. **9**:275, 1933.
140. Ishido, B.: Virchows Arch. f. path. Anat. **240**:241, 1922.
141. Saitta, S.: Scritti biol. **4**:301, 1924,

These findings are in accord with the more accurate observations of Wolbach and Howe,[139] previously cited, on the failure of proper repair in scorbutic animals, due to a defect in the formation of intercellular cement substance. Similar observations were reported by Lauber.[142]

However, a diet rich in vitamin C had little effect on the rate of healing of experimentally produced wounds of guinea-pigs, according to Lauber,[142] though he admitted that he did not standardize the quantity of vitamin C used. Saitta[141] suggested that an application of vitamin C to wounds stimulated healing.

These experiments on wound repair suggest the importance of vitamin C in the feparative process subsequent to injury and inflammation.

SUMMARY

The scorbutic state is associated with a drop in natural resistance to spontaneous infection and to induced bacterial infection. The production of natural or immune antibodies is unaffected, with the possible exception of that of opsonins. An interference with the capacity for immunization to tetanus and diphtheria toxin may occur in scurvy. Skin sensitivity to diphtheria toxin and tuberculin is diminished, but this is associated with greater susceptibility of the animal to the toxic effect of parenteral injections of these poisons. Skin reactivity to drugs such as arsphenamine occurs in guinea-pigs fed a diet partially deficient in vitamin C. When the avitaminosis is sufficient to cause clinical manifestations of scurvy, the skin is no longer reactive.

A short period of vitamin C deficiency prior to the administration of the sensitizing dose of protein antigen increases the anaphylactic response to a shocking dose subsequently injected and shortens the period of incubation of anaphylactic sensitization. Extreme scurvy or periods of starvation may, under certain conditions, impair the response to anaphylaxis.

Experimentally, it has been observed that the natural resistance of scorbutic guinea-pigs to tuberculosis and to infections with the colon group of organisms is lowered. Chronic staphylococcic infections tend to inhibit the deleterious effects of scurvy, apparently owing to the fact that such bacteria are capable of producing cevitamic acid in their growth.

By its profound influence on tooth growth and structure and gingival conditions, a deficiency of vitamin C may lead to increased susceptibility of the gums to infection and lower resistance of the tooth pulp to injury. In this way, the opportunity for the creation of dental foci of infection is greatly increased. Since such dental injury occurs very

142. Lauber, H. J.: Beitr. z. klin. Chir. **157**:244, 1933.

early in clinical scurvy and in so-called latent scurvy, the influence of vitamin C deficiency in infection is greater than is at first apparent.

Clinically, the scorhutic state and latent scurvy are associated with marked increase in the incidence of infections of the respiratory tract, of tuberculosis and of pyelitis in infants. The latent scorbutic state is often converted into frank scurvy by infections, and under such conditions hemorrhagic phenomena are frequent. This suggests that during the course of infections a greater demand is made on available vitamin C because of increase in the rate of tissue respiration.

The possible importance of vitamin C deficiency in susceptibility to rheumatic infections has been suggested, and experimental lesions said to be analogous to lesions observed in rheumatic endocarditis, myocarditis and arthritis have been produced by injecting cultures of several organisms into guinea-pigs suffering with latent scurvy.

An excess of cevitamic acid increases the resistance of guinea-pigs to diphtheria toxin, to tuberculin, to anaphylaxis and to trypanosoma infections and of monkeys to the virus of poliomyelitis. Its value experimentally and clinically in the treatment of tuberculosis in guinea-pigs and in man is not established, though it is possible that an excess of the vitamin may modify the incidence of intestinal complications. The prophylactic or therapeutic value of an excess of vitamin C needs further confirmation through experimentation. It seems logical that in the course of infectious diseases the increased rate of cellular oxidation would demand a greater amount of cevitamic acid than is needed normally.

The influence of vitamin C on resistance is dependent in part on its importance in the production of intercellular cement substance. However, the wide distribution of vitamin C in the body, its chemical properties and its influence on tissue respiration suggest that its role in natural resistance to infection is dependent on its physiologic importance in oxidation-reduction processes in cellular metabolism.

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