bic splitting metabolism under aerobic conditions, was not dependent upon the rate of respiration but upon the concentration of oxygen.

8889 P

Effect of Crystalline Vitamin C (Ascorbic Acid) on Tolerance to Tuberculin.

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One of the characteristics of the reaction of tuberculous animals to a large dose of tuberculin is congestion and capillary hemorrhage in all tissues, particularly in those containing tubercles. This is attributed to altered cell permeability and capillary dilatation. Since vitamin C has been shown to decrease capillary fragility (Dalldorf[1]), and has been employed with encouraging therapeutic results in certain types of hemorrhage (Willstaedt[2]), we were led to study this substance in relation to tuberculin intoxication. It is of interest to note that about 10 years before the discovery of the anti-infectious and antitoxic action of vitamin C (Harde,[3] Jungeblut and Zwemer,[4] King and Menten,[5] Jungeblut[6]), Bieling[7] had already found that a dose of tuberculin which killed only one of 2 tuberculous guinea pigs maintained on a normal diet, sufficed to kill 2 scorbutic tuberculous guinea pigs.

The effect of crystalline vitamin C (l-ascorbic acid*) on tuberculin was investigated in tuberculous guinea pigs fed a normal diet which included an abundance of fresh lettuce as a natural source of vitamin C. Ascorbic acid mixed *in vitro* with skin test doses of tuberculin prior to intracutaneous inoculation in tuberculous guinea pigs failed to inactivate the tuberculin in tests on 12 animals. Nor did prolonged pretreatment of 7 tuberculous guinea pigs with ascorbic acid reduce the reactivity of the skin to small doses of

1 Dalldorf, G., *J. Am. Med. Assn.*, 1935, **104**, 1701.

2 Willstaedt, H., *Klin. Woch.*, 1935, **14**, 1705.

3 Harde, E., *Compt. rend. Acad. Sci.*, 1934, **199**, 618.

4 Jungeblut, C. W., and Zwemer, R. L., PROC. SOC. EXP. BIOL. AND MED., 1935, **32**, 1229.

5 King, C. G., and Menten, M. L., *J. Nutrit.*, 1935, **10**, 129.

6 Jungeblut, C. W., *J. Exp. Med.*, 1935, **62**, 517.

7 Bieling, R., *Zeit. f. Hyg.*, 1925, **104**, 518.

* We are indebted to Merck & Co. for a generous supply of crystalline vitamin C (Cebione; Cevitamic Acid Merck).

tuberculin. We were also unable to determine any ability of the vitamin to inactivate tuberculin either *in vitro* or *in vivo* when the tuberculin was used in a dose large enough to cause death of control tuberculous guinea pigs. The *in vitro* test was performed by injecting 6 tuberculous guinea pigs with tuberculin previously incubated with vitamin C; 6 control tuberculous animals were injected with tuberculin alone. Four animals died of tuberculin shock in each group. Two experiments were performed on the effect of injecting ascorbic acid intravenously in doses of from 0.5 to 75 mg. into tuberculous guinea pigs just prior to inoculation with a large dose of tuberculin, lethal for control tuberculous guinea pigs. In both experiments all 12 animals receiving ascorbic acid followed by 250 or 500 mg. of tuberculin died of typical tuberculin shock. Another experiment was tried in which 4 tuberculous guinea pigs were injected subcutaneously with 5 mg. of ascorbic acid daily for one month. These guinea pigs, together with 4 non-treated tuberculous controls, were then injected subcutaneously with 450 mg. of tuberculin. Only 2 of the control guinea pigs died, whereas all 4 ascorbic acid treated animals died. Thus, in none of these experiments with lethal doses of tuberculin had any tuberculin-inactivating power of ascorbic acid been demonstrated. When, however, the tuberculin was injected in somewhat smaller but repeated doses, large enough in the aggregate to be fatal for control tuberculous animals, it was found that daily administration of ascorbic acid usually resulted in survival of the animal.

This observation was first made in a preliminary experiment on 6 guinea pigs infected subcutaneously with 0.001 mg. of bovine tubercle bacilli. Two months after infection the animals were divided into 2 groups. Three guinea pigs were injected subcutaneously with 5 mg. of ascorbic acid daily throughout the course of the experiment; the other 3 guinea pigs served as controls. During this same time both groups received injections of Old Tuberculin† twice weekly according to the following schedule: Four 100 mg. doses intracutaneously, then subcutaneous injections of 200 mg., 200 mg., 225 mg., 250 mg., 250 mg., and 250 mg. One control guinea pig died after the first subcutaneous injection of tuberculin, another died after the fifth, and the third control animal died after the sixth subcutaneous injection. All 3 vitamin C treated animals survived the entire series of tuberculin injections and, furthermore, proved able to tolerate several additional 250 mg. doses of tuberculin.

† Koch's Old Tuberculin was obtained through the courtesy of the Bureau of Laboratories, Department of Health, New York City.

The increased tuberculin tolerance of vitamin C treated animals was again convincingly demonstrated in a subsequent experiment. Sixteen male albino guinea pigs were infected subcutaneously with 0.001 mg. of a bovine strain of tubercle bacillus (B1). Eighteen days later, the guinea pigs were grouped in pairs on a basis of comparable weight, one animal of each pair receiving subcutaneous injection of 5 mg. of ascorbic acid every day, and the other animal serving as control. The ascorbic acid was dissolved in sterile distilled water just prior to injection. One guinea pig had died of intercurrent infection 5 days after inoculation with tubercle bacilli, leaving an unmated animal in the ascorbic acid treated group. All guinea pigs received 2 injections of Old Tuberculin weekly, starting 23 days after infection. The schedule of tuberculin doses (in milligrams) was as follows: (1) 50, (2) 100, (3) 150, (4) 200, (5) 250, (6) 300, (7) 400, (8) 550, (9) 700, (10) 700, (11) 900, (12) 900, (13) 1100, (14) 1100, (15) 1300, (16) 1300, (17) 1600, (18) 1600 mg. The first dose of tuberculin was given intracutaneously, all others subcutaneously. Whenever an animal died, its mate in the other group was killed for purposes of comparing the extent of the disease and the vitamin C content of the adrenal.

The first death occurred in a control animal following the ninth injection of tuberculin. At the 15th injection, 4 additional deaths had occurred, all of which were in the control group. At the 17th injection the first of the vitamin C treated animals died. This animal showed a non-tuberculous peritonitis at autopsy. At the 18th injection, the last control animal died. Thus, there was only one death in the vitamin-injected group as compared to 6 deaths in the control group. The unmated guinea pig which had been included in the vitamin C treated group had also survived the entire series of tuberculin injections. This animal proved able to survive additional tuberculin injections of 1600, 2000, and 2500 mg. It was apparent, therefore, that the animals receiving ascorbic acid daily were better able to tolerate repeated large doses of tuberculin.

The adrenal glands of the animals in this experiment were roughly estimated for vitamin C content by treating the sliced organ with 0.4% silver nitrate for one-half hour, and examining for visible blackening in the cortex. All the ascorbic acid treated animals showed the presence of reducing substances at autopsy, with the exception of the one animal which had succumbed spontaneously. On the other hand, of the 6 control guinea pigs which had died of tuberculin shock, 5 failed to reveal any trace of reducing substance in the adrenal, and the sixth gave a much weaker reaction than did the paired animal of the treated group. The one control animal

which did not succumb to tuberculin showed an appreciable degree of blackening in the adrenal. These observations suggested that tuberculin death was associated with depletion of vitamin C, whereas those animals that survived tuberculin shock always showed vitamin C in the adrenal.

Details of the autopsy findings will be reported elsewhere. We note here that in 6 of the 7 pairs of autopsies, the control animal showed more extensive tuberculosis than did the corresponding ascorbic acid treated animal. The precise mechanism for the observed effect of vitamin C on tuberculosis and tuberculin tolerance remains to be clarified. No evidence was obtained for a direct inactivation of tuberculin by vitamin C. Nevertheless, the results suggest that vitamin C may prove of value in tuberculosis by combatting the prolonged toxemia of the disease. We are at present testing this hypothesis in both clinical and animal experiments.

Summary. Daily injection of crystalline vitamin C increased the tolerance of tuberculous guinea pigs to repeated large doses of tuberculin.

8890 C

Effect of Ether Anesthesia and Amytal Anesthesia on the Erythrocytic Findings in Control and Splenectomized Dogs.

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In a study of the influence of ether anesthesia and amytal anesthesia on the blood of the dog it was shown by Searles and Essex[1] that following ether anesthesia there was usually a marked increase in the erythrocyte count, the value for the hemoglobin and the hematocrit determinations. When amytal was used as the anesthetic there was usually a decided decrease in the erythrocyte count, the value for the hemoglobin and the hematocrit readings when compared with these findings before the induction of anesthesia. Since it is a common observation that the spleen of the dog is usually markedly dilated following amytal anesthesia, the decrease in the

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1 Searles, P. W., and Essex, H. E., *Proc. Staff Meet. Mayo Clinic*, 1936, **11**, 481.