Effects of Prolonged High Dosage with Ascorbic Acid.* (19623)

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In recent years relatively large doses of ascorbic acid have been prescribed in the treatment of a variety of diseases including hypertension and allergic conditions. It has been generally assumed that daily doses of one gram are innocuous although there have been reports that vagotonic symptoms occur in children and that animals receiving large quantities of ascorbic acid may lose weight(1).

Wilson and Lubschez(2) have reported preliminary data suggesting that possibly the ingestion of 500 to 1000 mg ascorbic acid per day for a long period might not be innocuous. These investigators found a paradoxical decrease in the ascorbic acid of the white blood cells after a time on high dosage of the vitamin and suggest that their results might arise from the organism becoming too efficient in the disposal of ascorbic acid.

Accordingly, it was felt desirable to investigate further the effect of prolonged high dosage with ascorbic acid. One woman and 3 men were each given 1000 mg/day for 3 months. Measurements were made at appropriate intervals of the ascorbic acid concentration in the serum, the concentration in the white blood cells plus platelets, the ascorbic acid tolerance curve, and the urinary output of the vitamin.

Methods and procedure. All analyses were performed at least in duplicate. The white blood cells plus platelets from 0.1 ml of finger blood were analyzed as previously described (3). The serum ascorbic acid was measured using 0.01 ml samples. The original procedure(4) was altered as follows: (a) charcoal was omitted from the precipitating acid; (b) the dinitrophenyl hydrazine reagent was prepared as follows: to a 2.2% dinitrophenyl hydrazine solution in 10 N H_2SO_4 is added 5 volumes of 5% thiourea solution and 5 volume % of 0.6% $CuSO_4 \cdot 5H_2O$ solution. The reagent is satisfactory for use at least a week if held at 4°C. It is prepared from the more stable stock solutions; and (3) the incubation time was increased from 3 to 4 hours at 38°C. The copper, which is easier to use, substitutes for charcoal in converting ascorbic acid to dehydroascorbic acid. This change gives results which average 0.1 mg % higher than with charcoal, a difference of no consequence in the present study. The urine was preserved with 0.1 N acetic acid and analyzed in the same manner as serum except the use of charcoal was retained as it has been found necessary to remove the urinary pigments. A single urine sample analyzed with dichlorophenol indophenol gave a value of 7% less than with dinitrophenyl hydrazine. This may indicate some conversion to dehydroascorbic acid prior to analysis, and suggests an advantage in the use of dinitrophenyl hydrazine which measures both ascorbic and dehydroascorbic acids. The 1000 mg daily supplement of ascorbic acid was administered in 3 divided doses (with meals). The 100 mg pills used were analyzed and found to be within 2% of the stated value.

Results and conclusion. The data for the 4 subjects were so concordant, they have been averaged together and treated as replicates. There is clearly no progressive change in either the serum or white cell level, tolerance curve, or urinary excretion (Table I). The averages obscure the fact that the individual tolerance curves differed a little in shape. However, the individual character of the curves remained essentially unchanged throughout. There seems, therefore, to be no reason to believe that the prolonged high dosage of ascorbic acid had any qualitative or quantitative effect on the manner of disposal of excess acid by the body. Furthermore, in these four

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TABLE I. The Ascorbic Acid of Urine, White Cells, and Serum of 4 Subjects Receiving 1000 mg Ascorbic Acid per Day.

Day of exp.	Urine, mg/day	White cells,* mg %	Serum after 400 mg test dose (mg %) 0	1 hr	3 hr	5 hr	7 hr
0		27 ± 2†	1.22 ± .09	1.78 ± .13	1.97 ± .11	1.80 ± .06	1.63 ± .04
5	817‡ ± 35†	28 ± 3	1.81 ± .09	2.45 ± .09	2.39 ± .08	2.14 ± .11	1.94 ± .04
21	804 ± 25	30 ± 1	1.79 ± .06	2.28 ± .11	2.35 ± .05	2.19 ± .11	2.03 ± .04
39	714§ ± 35	28 ± 1	1.82 ± .13				
55		28 ± 2	1.54 ± .08				
67		31 ± 1	1.93 ± .15	2.45 ± .17	2.48 ± .20	2.19 ± .24	2.09 ± .26
98	822 ± 47	28 ± 1	1.64 ± .10	2.24 ± .99	2.14 ± .08	1.83 ± .13	1.76 ± .06

* White blood cells + platelets. † ± Stand. error. ‡ 3rd day of exp. § 43rd day of exp.

persons, at least, no harmful effects whatever were observed during the 3 months with 1000 mg daily intake.

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α-Aminosulfonic Acids and Viral Propagation.* (19624)

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In the case of methionine(1-3) and tryptophane(4), it has been shown that metabolites as small as these amino acids are involved in the propagation of animal viruses and that analogs of these compounds will inhibit viral multiplication. A number of interesting analogs of amino acids have been prepared by McIlwain. *In vitro* these α-aminosulfonic acids are potent inhibitors of bacterial growth (5). Further α-aminophenylmethanesulfonic acid and α-aminoisobutanesulfonic acid which resemble in structure phenylalanine and valine have been reported to inhibit the multiplication of vaccinia virus in tissue culture(6). An analog of glycine, α-aminomethanesulfonic acid, will inhibit the propagation of a bacteriophage of *B. coli*(7). The mode of action of these compounds to the present has not been clearly explained, but there is evidence to show that they interfere with amino acid metabolism.

Three α-aminosulfonic acids reminiscent in structure of tyrosine or phenylalanine have been synthesized and tested as inhibitors of viral propagation in various biological systems. In the following, the results of these studies are reported, and their significance is discussed.

Virus and tissue. The PR8 strain of Type A influenza virus was selected for those studies in which the Warburg flask culture or the embryonate egg was employed. After isolation from man, this virus had undergone 7 passages in ferrets, 593 passages in mice, and 131 passages in eggs. For the experiments performed with mice, the WS strain of Type A influenza virus was used. This virus was cultured for several years in tissue culture and has undergone 99 passages in mice(8). The host cells for the Warburg culture were those of the chorioallantoic membrane and were

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