

Nutritional Studies with Isoascorbic Acid in the Guinea Pig

JAMES S. REIFF and
ALFRED H. FREE
Miles-Ames Research
Laboratory, Elkhart, Ind.

The antiscorbutic activity of isoascorbic acid in guinea pigs has been studied. As much as 250 mg. per day did not support guinea pigs fed a vitamin C-deficient diet. Administration of isoascorbic acid tended to slow down the development of acute vitamin C deficiency. In vitamin C-depleted animals isoascorbic acid had no therapeutic effect, whereas such animals responded to ascorbic acid. Animals maintained on a suboptimal intake of ascorbic acid showed some response to isoascorbic acid. These observations suggest that isoascorbic acid has a protective effect on ascorbic acid within the body, but no significant antiscorbutic activity per se.

CONCURRENT WITH THE synthesis and establishment of the structure of ascorbic acid, about 25 years ago, there appeared a number of chemically related compounds. A particularly interesting compound in this group is isoascorbic acid, which has been studied by several investigators for its antiscorbutic activity. Yourga, Esselen, and Fellers (8) in 1944 reported isoascorbic acid as having one twentieth the antiscorbutic activity of ascorbic acid. This figure has been frequently cited in considering the nutritional relationship between the two acids. Isoascorbic acid is a potent reducing agent and has been suggested as an antioxidant in foods. The present paper describes nutritional studies of the antiscorbutic activity of isoascorbic acid in guinea pigs.

The structural formulas of ascorbic and isoascorbic acids are identical, with the exception of the configuration about the fifth carbon atom. In this presentation, we use the term isoascorbic acid for the material, *Chemical Abstracts* names it *d*-araboascorbic acid.

Methods

The primary means of determining nutritional responses is by measurement of the growth curves of young guinea pigs which is a type of study employed in many investigations similar to this one. The guinea pigs were maintained in individual cages with a supply of food and water available at all times. The scorbutic diet employed was Rockland rabbit ration. A preliminary experiment indicated that this diet was comparable to the ascorbic acid-deficient diet supplied commercially by Nutritional Biochemicals, Inc., and to the synthetic diet used by Sherman, LaMer, and Campbell (7). In all subsequent experiments Rockland rabbit ration was used as the deficient diet. The control diet employed to show optimal growth was freshly milled Rockland guinea pig diet (ascorbic acid forti-

fied). In all the experiments the diet supplements were prepared fresh daily and administered by stomach tube daily. The duration of each experiment was not less than 50 days, and over 200 pigs were employed over a 12-month period. A group of animals in each instance involved five guinea pigs and, throughout this report, average weight responses for the group are indicated.

Results

Figure 1, bottom, shows the average weight response curve of five guinea pigs maintained on the deficient diet. These animals rapidly decreased in weight and by the end of four weeks all were dead. Clear indications of scurvy were observed at 3 weeks, which corresponded with the classical picture of scurvy as described by Cohen and Mendel (7). In Figure 1 are also shown the average weight response curve of the group maintained on the Rockland guinea pig control diet, and the average weight response curve of a group on the deficient diet plus a daily supplement of 10 mg. of ascorbic acid. The animals with the supplement show a normal weight response, indicating the adequacy of the deficient diet with respect to all nutrients except vitamin C.

The growth curves in Figure 1, third from top, compare the average weight response of animals on the deficient diet with similar groups which were given daily supplements of isoascorbic acid. A supplement of 2 mg. per day of isoascorbic acid slowed the weight loss of the group and prolonged life. However, all the pigs lost weight and died with typical signs of scurvy. The use of 10, 100, and 250 mg. per day supplements of isoascorbic acid had a small increasing effect in slowing weight loss and prolonging the life of the animals on the deficient diet. Even with such massive doses of isoascorbic acid, typical signs of ascorbic acid deficiency developed. In contrast,

supplements of ascorbic acid as small as 1 mg. per day had a considerably different effect, and as indicated in Figure 1, 10 mg. of ascorbic acid per day gave optimal growth with the deficient diet.

Figure 1, second from top, shows the effect of various amounts of isoascorbic acid supplements on animals maintained on a suboptimal intake of 0.3 mg. of ascorbic acid. This experiment was carried out, because results of the preceding study demonstrated a protective effect of isoascorbic acid, even though the compound did not prevent signs of acute ascorbic acid deprivation. The deficient diet alone resulted in rapid loss of weight. The broken line shows the weight response curve of a group of animals receiving 0.3 mg. of ascorbic acid per day. This amount of vitamin C did not provide adequate nutrition and by the end of 6 weeks all the pigs were dead. Addition of 1 mg. of isoascorbic acid had little effect, the average weight curve being somewhat lower than the curve of the animals on 0.3 mg. of ascorbic acid alone. Supplements of 10 and 100 mg. of isoascorbic acid had an influence in slowing weight loss, but were not sufficient to prevent scurvy and death.

A third type of study involved determination of the therapeutic properties of isoascorbic acid (Figure 1, top). Groups of animals were kept on the deficient diet for 2 weeks with no supplements of any kind. At the end of this time after definite weight loss had occurred, daily supplements of isoascorbic acid and ascorbic acid were started. As indicated, the groups receiving 1, 10, and 100 mg. of isoascorbic acid continued to lose weight and the animals died. A very slight difference was observed between groups on 1, 10, and 100 mg. of isoascorbic acid. Also shown are the growth curves of deficient animals given supplements of ascorbic acid following the depletion period; 0.1 mg. of ascorbic acid had no effect, whereas 1 and 10 mg. had a profound therapeutic influence.

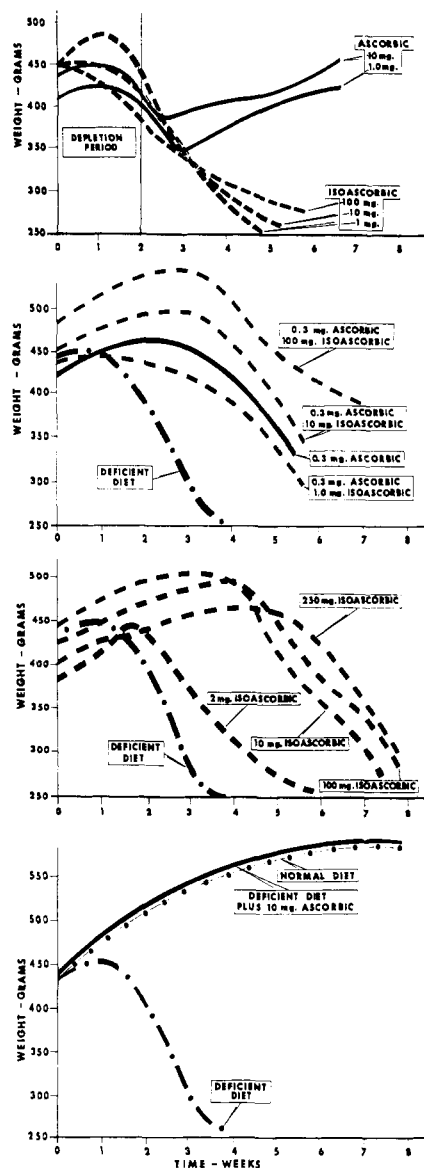


Figure 1. Weight response of guinea pigs on deficient diets

Discussion

Throughout the literature isoascorbic acid has been described as having one twentieth the antiscorbutic activity of ascorbic acid. As early as 1933, Dalmer and Moll (2) reported isoascorbic acid to possess one twentieth the antiscorbutic activity of ascorbic acid when given parenterally and one fortieth the antiscorbutic activity of ascorbic acid when given orally. The following year Demole (3) stated that he had found that the antiscorbutic activity of isoascorbic acid in guinea pigs was "... about 1/20 of the activity of 1-ascorbic acid, in agreement with the results of Dalmer and Moll." Also in 1934 Ohle, Erlbach, and Carls (6) indicated that they had supplied isoascorbic acid to von Euler, who had found that it was antiscorbutic, in agreement with Dalmer and Moll. Yourga, Esselen, and Fellers (8) assayed the anti-

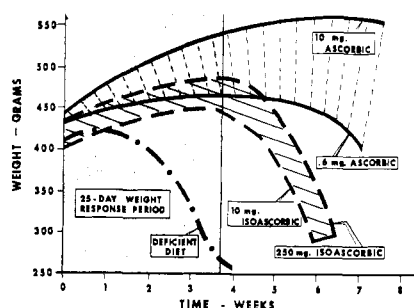


Figure 2. Composite weight response curves of animals receiving varying ascorbic acid and isoascorbic acid supplements

Shortcomings of a 25-day bioassay are emphasized by the difference in the curves in the shaded (25 days) and those observed over longer periods

scorbutic activity of isoascorbic acid using the 25-day assay procedure of Dunker, Fellers, and Esselen (4). They also reported that isoascorbic acid apparently had one twentieth the antiscorbutic activity of ascorbic acid.

New Concept of Isoascorbic Acid Activity

Only a limited amount of experimental data appears in the literature and experiments which do appear were not carried on for more than 25 days.

As Figure 2 indicates, guinea pigs supplemented with isoascorbic acid gained weight for approximately 4 weeks, but then began to lose weight and later died of scurvy. It appears that isoascorbic acid, being a potent reducing agent, protects the ascorbic acid stored in the body tissues, but, with the exhaustion of ascorbic acid reserves, it no longer produces a beneficial effect. This fact is more clearly indicated in Figure 1, top, which compares the response of a group of depleted guinea pigs receiving ascorbic acid with the response of depleted guinea pigs receiving isoascorbic acid. The two compounds have entirely different effects. One can conclude that with ascorbic acid depletion, isoascorbic acid is therapeutically ineffective. One milligram of ascorbic acid per day reversed the depletion, whereas 100 times this amount of isoascorbic acid had no beneficial effect in the depleted animals. These observations do not support the concept proposed by earlier workers (8) that isoascorbic acid has one twentieth the antiscorbutic activity of ascorbic acid.

From Figure 2 one can better visualize the shortcomings of a 25-day bioassay such as those appearing in the literature. The range of weight response curves for supplements of ascorbic acid ranging from 0.6 to 10 mg. per day is indicated by the area shaded with vertical dashed

lines. The weight response curves for isoascorbic acid are shown by the area shaded with horizontal lines, whereas the weight response curve for the deficient diet alone is shown by the single line. During the first 25 days of supplementation, the protective period, isoascorbic acid maintains a weight increase, as does ascorbic acid. Interpretation of the weight responses during this interval could easily lead to the conclusion that isoascorbic acid has one twentieth the antiscorbutic activity exhibited by the ascorbic acid. However, if the weight curves beyond 25 days are noted, it is apparent that with isoascorbic acid weight is rapidly lost and scurvy ensues. Little difference is observed between the growth curves of 10 and 250 mg. of isoascorbic acid. Ascorbic acid has a distinctly different effect and with amounts ranging from 0.6 to 10 mg. weight increases and with the larger intake weight response coincides with that seen with an optimal diet.

Little work has been recorded on the use of isoascorbic acid in man. Ikeuchi (5) in Japan observed the retention of ascorbic acid and isoascorbic acid when administered to healthy subjects and vitamin C-deficient human subjects. Deficient subjects retained a large proportion of administered vitamin C, whereas a large portion of administered isoascorbic acid was excreted. These results led Ikeuchi to state "... its (isoascorbic acid) antiscorbutic activity in the vitamin C-deficient human body is trifling compared with that of ascorbic acid."

Isoascorbic acid has been used as an antioxidant in foods. The results of this study indicate that it may not only protect ascorbic acid in foods against oxidation, but may also protect ascorbic acid within the body.

Literature Cited

- (1) Cohen B., Mendel, L. B., *J. Biol. Chem.* **35**, 425-53 (1918).
- (2) Dalmer, O., Moll, T. H., *Z. physiol. Chem.* **222**, 116-20 (1933).
- (3) Demole, V., *Biochem. J.* **28**, 770-3 (1934).
- (4) Dunker, C. F., Fellers, C. R., Esselen, W. B., Jr., *Food Research* **7**, 260-6 (1942).
- (5) Ikeuchi, M., *Vitaminology* **1**, 192-9 (1955).
- (6) Ohle, H., Erlbach, H., Carls, H., *Ber. deut. chem. Ges.* **67B**, 324-32 (1934).
- (7) Sherman, H. C., LaMer, U. K., Campbell, H. L., *J. Am. Chem. Soc.* **44**, 165-72 (1922).
- (8) Yourga, F. J., Esselen, W. B., Jr., Fellers, C. R., *Food Research* **9**, 188-96 (1944).

Received for review August 18, 1958. Accepted October 30, 1958. Division of Biological Chemistry, 132nd Meeting, ACS, New York, N. Y., September 1957.