

Hypercholesterolemia due to Ascorbic Acid (39263)

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Recently it has become fashionable to ingest large amounts of ascorbic acid (vitamin C) in the hope of decreasing the frequency or the severity of upper respiratory infections collectively known as "colds." In comparison to the search for respiratory benefit, little effort has been expended in the search for side effects of this practice.

Ascorbic acid seems to inhibit the absorption of copper from the intestinal tracts of animals (1). Such inhibition may be harmful according to the zinc/copper hypothesis (2-6) regarding the etiology of ischemic heart disease, the leading cause of death in the United States (7). This hypothesis states that high ratios of zinc to copper are associated with hypercholesterolemia and high risk of heart disease. Consequently it was decided to test the hypothesis that ascorbic acid fed to rats would produce hypercholesterolemia.

Methods. Twenty male rats of the Carworth strain weighing approximately 55 g were fed demineralized water and a diet based upon 62% sucrose and 20% egg white (by weight), which contains all nutrients known to be essential for the rat. Half the animals were fed the diet supplemented with 150 mg ascorbic acid/kg of diet.

The diet was similar to that previously described (2) with the following exceptions. Coconut oil (Teklad Mills, Madison, Wisconsin)¹ was substituted for corn oil except in the vitamin ADE mix. The amount of biotin was increased to 2.00 mg/kg of diet. Finely ground, reagent grade $\text{Zn}(\text{C}_2\text{H}_3\text{O}_2)_2 \cdot 2\text{H}_2\text{O}$ (zinc acetate, 44.0 mg) and $\text{CuSO}_4 \cdot 5\text{H}_2\text{O}$ (cupric sulfate, 1.246 mg) were added to each kilogram of diet to give zinc and

copper concentrations of 14.9 and 1.03 mg/kg, respectively.

The amounts of zinc and copper in the diet plus these added amounts are sufficient to produce adequate growth (2, 8) and hematocrits (2, 9). The diet resembles the average diet of people in the United States as it is high in saturated fat and sucrose and has a high ratio (14.5:1) of zinc to copper (3, 4).

The rats were housed as described previously (10) except that extreme filtration of the air was assumed to be unnecessary in a town without heavy industry.

Cholesterol in plasma was measured by a fluorometric method (11). Comparison of means was done by the Student's *t* test (12).

Results. After 150 days the mean (SE) concentration of cholesterol in the plasma of rats fed ascorbic acid was 129 (6.3) mg/dl. This concentration was significantly higher ($t = 2.67$, $P < 0.02$) than the 109 (4.0) mg/dl of the rats fed no ascorbic acid (Fig. 1). The mean (SE) weight of the rats fed ascorbic acid was 430 (9.7) g and was not significantly lower than the 436 (15.4) g of the rats fed no ascorbic acid.

Discussion. A relationship between the metabolism of cholesterol and the amount of ascorbic acid ingested by animals has been sought for almost a quarter of a century. Most of the experiments have been done with guinea pigs either deficient or marginally insufficient in ascorbic acid. Generally (13-26), but not invariably (13, 16, 18, 27), extra ascorbic acid increased the destruction or decreased the accumulation of cholesterol. The experiments with animals not requiring exogenous ascorbic acid usually have been designed to determine whether an abnormally high concentration of cholesterol in plasma could be decreased by ascorbic acid (17, 21-23).

This experiment was designed to determine whether ascorbic acid in the diet would cause an increase in the concentration of

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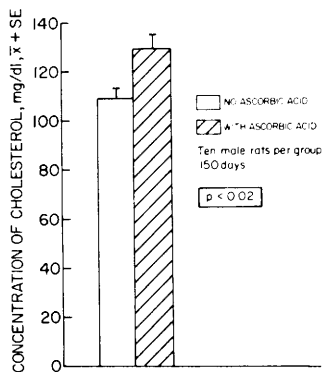


FIG. 1. The concentration of cholesterol in plasma.

cholesterol in the plasma of normal animals. Rats do not require exogenous ascorbic acid and may be comparable to people who consume an amount of ascorbic acid similar to that of the Recommended Dietary Allowance in the United States (45 mg) (28). In an early experiment (16), hypercholesterolemia was produced acutely in normal rats with ascorbic acid.

The amount of ascorbic acid in this experiment can be compared to the amount of ascorbic acid ingested in capsules by humans in two ways. The sum of the weights of the nutrients (excluding water) consumed daily by a 70-kg man is about 550 g (29). If the average man ingested capsules containing ascorbic acid so that the concentration of capsular ascorbic acid in his diet was the same as in the diet of the rats in this experiment, he would ingest 82 mg in excess of that in his diet. If the amount of ascorbic acid ingested daily by the rats is multiplied by the ratio of the weight of the average man to that of the average rat, the result is 630 mg in excess of the amount of ascorbic acid in the normal human diet.

Both the estimates, 82 and 630 mg, are maximal estimates because the human diet includes non-nutritive substances such as fiber and because small animals generally need more of an active substance per unit weight than do large animals (30). However, both estimates are below the amounts of ascorbic acid ingested by those in pursuit of respiratory benefit (31, 32).

Epidemiologic evidence relating the metabolism of cholesterol or the risk of ischemic heart disease to exposure to ascorbic

acid is conflicting. No cases of scurvy were found among 40,000 people recently examined for nutritional disorders in the United States (33). In 1970 there were 666,665 deaths due to ischemic heart disease and 3 deaths due to ascorbic acid deficiency in the United States (7). It seems unlikely that if ischemic heart disease and scurvy share a common etiology as has been suggested (26, 34) the diseases should be distributed so unequally.

In the United States, classification of business and professional men into three groups in ascending order of the mean concentration of cholesterol in serum revealed a similar ascent in the mean daily intake of ascorbic acid (35). In England and Wales a negative correlation was found between the daily intake of ascorbic acid and the standardized mortality ratio due to ischemic heart disease (36). In Kenya, among people the majority of whom would be considered to have a low concentration of cholesterol in serum in comparison with people from the United States, a positive correlation between serum cholesterol and plasma ascorbate was found (37). No such relationship was found in the United States (38) or Wales (39). These data lead to the conclusion that a study of ascorbic acid ingestion under conditions of dietary control will be necessary to determine a relationship between the metabolism of ascorbic acid and cholesterol in man.

It is not surprising that studies of the consumption of relatively large amounts of ascorbic acid without control of other variables have resulted in contradictions. Both increases (34, 40, 41) and a decrease (42) in the concentration of cholesterol have been attributed to ascorbic acid. In none of these studies (34, 40-42) was the effect statistically significant, if in the former study (34) patients were not grouped selectively. In a similar study of patients with hypercholesterolemia the mean concentrations of cholesterol in plasma before and after ingestion of ascorbic acid were virtually identical (43). In other studies an effect attributed to ascorbic acid could have been due to a change in dietary fat (22, 23). In studies in which no data were given the authors stated that they found no effect of ascorbic acid ingestion on

the concentration of cholesterol in serum (32, 44).² If the direction of the response to ascorbic acid were known in each of these latter experiments, data could be combined with other experiments (34, 42, 45, 46) to measure the probability of the number of increases (or decreases) in the concentration of cholesterol (47).

The addition of ascorbic acid to the diets of men under conditions that approximate the control of other dietary factors has shown ascorbic acid to be associated with an increase in the concentration of cholesterol in serum. The increase found by Anderson *et al.* (45) was not statistically significant. In a study of experimental scurvy, Hodges *et al.* (46) found a significant decrease in the concentration of cholesterol in serum during a period of depletion of ascorbic acid and a significant increase during repletion. Apparently the decrease began before the onset of scurvy and the increase continued after scurvy had been cured.

The mechanism by which ascorbic acid produced the hypercholesterolemia in this experiment is not certain. No data have been found regarding an effect of ascorbic acid upon the intestinal absorption of zinc, but Evans (1) has reviewed the work of several authors which suggests that ascorbic acid depresses the intestinal absorption of copper. It is possible that a decreased absorption of copper relative to zinc resulted in an increased ratio of zinc to copper absorbed. An increase in the ratio of zinc to copper ingested by rats has resulted in hypercholesterolemia (2). Such a mechanism would be consonant with the zinc/copper hypothesis regarding the etiology of ischemic heart disease (2–6) and with the observation that the increase in the concentration of cholesterol in serum was greater to a highly significant degree when human scurvy was treated with oral doses of ascorbic acid compared to intramuscular doses (44).

Conclusions. Rats do not require exogenous ascorbic acid and are comparable to people who consume amounts of ascorbic

acid approximating the Recommended Dietary Allowance in the United States. Addition of ascorbic acid to the diet of rats produced hypercholesterolemia in confirmation of a prediction based upon previous observations that a high ratio of ingested zinc to copper produced hypercholesterolemia. These results were consonant with data on men fed ascorbic acid under reasonable dietary control.

Summary. Rats fed a purified diet containing ascorbic acid developed hypercholesterolemia. Because rats do not require exogenous ascorbic acid, they may be comparable to humans who supplement their diets with ascorbic acid in capsular form. The amount of ascorbic acid in this experiment was equivalent to 82 to 630 mg of capsular ascorbic acid ingested by an average man and was well below the amount ingested by those in search of respiratory benefit. The data are consonant with those on humans consuming controlled diets.

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² Response by Dr. Anderson to a question about Ref. 31 at the Western Hemisphere Nutrition Congress, IV.

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