EXPERIMENTAL NUTRITION

ASCORBIC ACID AND THE CATABOLISM OF CHOLESTEROL

Guinea pigs with a latent, chronic ascorbic acid deficiency were injected with 26-^{14}C-cholesterol and the conversion of the labeled cholesterol into bile acids was estimated by following the output of $^{14}CO_2$ for several weeks. Deficient guinea pigs accumulated serum and tissue cholesterol and had a lower output of $^{14}CO_2$ than did the controls. In addition, the turnover time of the labeled cholesterol was less than that of contols. From these, as well as previous results, it was concluded that adequate tissue concentrations of ascorbic acid were necessary for conversion of cholesterol to bile acids.

Key Words: cholesterol catabolism, ascorbic acid, bile acids

It is well documented that scorbutic guinea pigs show aberrations in their normal patterns of lipid metabolism that are prevented by supplementation with ascorbic acid.[1] Of particular interest is a hypercholesteremia often seen in guinea pigs deficient in ascorbic acid[2] as well as an increase in total body cholesterol.[3] There appears to be little evidence that biosynthesis of cholesterol is increased sufficiently to account entirely for the accumulation,[1] so the possibility that cholesterol degradation may be impaired must be considered. Until recently, relatively few studies on the role of ascorbic acid in the overall catabolism of cholesterol have been conducted, even though it is acknowledged that the vitamin is involved in the transformation of cholesterol to the adrenal steroid hormones.[4]

Guchhait et al.[5] have investigated the metabolism of 4-^{14}C-cholesterol in scorbutic guinea pigs and found that less of the steroid label appeared in gallbladder bile acids of severely deficient animals than in the bile acids of controls. In the intestine and liver of deficient animals, the radioactivity of the nonsaponifiable fraction of the lipid extract (mainly cholesterol) was higher than that of the controls. In vitro studies with liver mitochondria also showed that the radioactivity of bile acids in the ascorbic acid-deficient animals was less than that of controls. It was suggested, therefore, that conversion of cholesterol to bile acids must have been inhibited by the deficiency of ascorbic acid.

More recently, Ginter and his group have investigated this problem further. According to them, most previous studies have involved guinea pigs completely deficient in ascorbic acid. Since these animals refuse to eat, have severely retarded growth, and may develop internal hemorrhage leading to anemia, such a complex condition is produced that it is difficult to interpret. The authors felt that such an acute ascorbic acid deficiency was unrealistic, especially in regard to ascorbic acid deficiency in a human population, so they developed what they call a chronic, latent, hypovitaminosis C in their guinea pigs. The animals are fed the deficient diet for 14 days, which is sufficient time to decrease tissue levels of ascorbic acid, but food intake and growth remain normal. The experimental animals are then maintained on 0.5 mg. ascorbic acid daily, whereas controls are fed 10 mg. of the vitamin per day. Male guinea pigs on this regimen for several months maintained their weight equivalent to that of controls, but accumulated significantly higher concentrations of cholesterol in their livers.[6]

Since earlier experiments had shown that there was no difference in the rate of cho-

lesterol synthesis between the chronically deficient animals and their controls, Ginter et al.[7] also felt that cholesterol under-utilization may be a cause of the increased cholesterol concentrations, so they studied catabolism of 4-^{14}C-cholesterol by injecting it into chronically-deficient guinea pigs and their controls. Cholesterol in liver and plasma and fecal excretion of the radioactivity as bile acids and as neutral sterols were determined over a 20-day period. Chronically deficient guinea pigs had higher liver and plasma cholesterol concentrations than did controls, and deficient animals excreted less radioactivity as bile acids. Excretion of neutral sterol radioactivity was similar for both groups. Gallbladder bile radioactivity was slightly depressed in deficient animals, whereas labeling of neutral sterols in the bile was nearly the same for both groups of animals.

An additional experiment, in which 26-^{14}C-cholesterol was injected and the respiratory output of radioactive CO_2 was measured over a period of ten days, indicated that the animals with chronic hypovitaminosis C eliminated less $^{14}CO_2$ than did controls. Since an early step in conversion of cholesterol to bile acids is cleavage and oxidation of the terminal isopropyl group of the cholesterol side chain, the depressed output of $^{14}CO_2$ by the chronically deficient guinea pigs was consistent with the view that ascorbic acid is involved in cholesterol catabolism.

In a later publication,[8] Ginter presented some results on cholesterol turnover to bile acids in control and deficient animals. Chronic hypovitaminosis C was developed over a three-month period as previously described. All animals were then given an intraperitoneal injection of 26-^{14}C-cholesterol (emulsified with Tween 20 in saline). Three guinea pigs from each group were put into metabolism cages for a 24-hour collection of $^{14}CO_2$. Ascorbic acid in liver and spleen was determined, as was total cholesterol from liver and plasma. Radioactivity of the lipid extracts was also measured (according to the author, radioactivity of the extract was identical to that of the precipitated digitonide, so only the extracts were analyzed). Similar determinations were made from successive groups of three or four deficient or control guinea pigs sacrificed at intervals of one, three, five, seven, nine and 11 weeks after being given the labeled cholesterol. The rate of bile acid formation from cholesterol was estimated as the ratio of $^{14}CO_2$ radioactivity expired during 24 hours to the specific activity of the total liver cholesterol.

Although no data were given, the author stated that the deficient guinea pigs ate and grew normally. Tissue ascorbic acid of the chronically-deficient groups, represented by the liver and spleen, was reduced to about one-fourth that of controls (liver and spleen in controls had 8.2 mg. and 21.6 mg. ascorbic acid per 100 g. of organ respectively, whereas deficient animals had 1.6 mg. and 4.7 mg. ascorbic acid per 100 g. organ). Serum and liver cholesterol increased significantly in the deficient animals (serum, 218 mg. cholesterol per 100 ml.; liver, 443 mg. cholesterol per 100 g.), as compared with controls (serum, 126 mg. cholesterol per 100 ml.; liver 359 mg. cholesterol per 100 g.).

The decrease in $^{14}CO_2$ output was accompanied by a similar decrease in specific activity of liver cholesterol, so ratios for the two groups of animals remained constant throughout the experiment. From the rate of $^{14}CO_2$ production, a turnover time for the conversion of cholesterol to bile acids was calculated and it indicated that there was less conversion in the hypovitaminosis-C animals (8.3 mg. bile acids per 24 hours per 500 g. B.W.) than in controls (11.3 mg. bile acids per 24 hours per 500 g. B.W.). A significant negative correlation ($p<0.001$) was found between the concentration of ascorbic acid in liver and the concentration of cholesterol in serum and liver.

As a result of these and previous studies, the author has proposed that the cholesterol concentration in the serum and liver of guinea pigs is controlled by the rate of cho-

lesterol conversion to bile acids and that adequate tissue levels of ascorbic acid are essential for the transformation to occur. Inasmuch as major reactions in the conversion of cholesterol to bile acids are hydroxylations of various positions on the sterol nucleus,[9] and a generally-accepted function of ascorbic acid is in promoting a variety of reactions of this type, it is suggested that ascorbic acid may be necessary for the hydroxylations in the formation of bile acids from cholesterol.

This hypothesis for the function of ascorbic acid in the catabolism of cholesterol is attractive, but more studies will be required before such a conclusion may be finally drawn. For example, how much of the $^{14}CO_2$ may be recycled and either retained in the deficient animals or excreted as something other than $^{14}CO_2$? In vitro studies with perfused livers or liver mitochondria are needed to provide a more direct measurement of conversion of cholesterol to bile acids. An effect of supplemental ascorbic acid or, perhaps, other antioxidants on the conversion will ultimately have to be demonstrated.

However, these papers by Ginter and his group are important because they have demonstrated the usefulness of a guinea-pig model in which a chronic hypovitaminosis C may be maintained without all the side effects exhibited by the acutely deficient animal. In addition, their studies have focused attention on the possible relationship between cholesterol catabolism and ascorbic acid. It remains to be seen whether this information has any application to problems of hypercholesteremia in man. Ginter et al.[10] have reported that a daily supplementation of 300 mg. of ascorbic acid for seven weeks to subjects with a seasonal deficit of ascorbic acid produced a modest decrease in serum cholesterol levels. This observation awaits confirmation.

Shortly after completion of the above part of this review, a paper by Ginter et al.[11] appeared showing the same data, in an expanded form, as that presented in an earlier experiment.[8] Cholesterol analyses of 15 tissues are presented for control and hypovitaminosis-C guinea pigs (only serum, liver, and, possibly, skin showed significant differences) as well as vitamin-C content of liver and spleen at each weekly interval in which samples were obtained. In addition, die-away curves, after injection of 26-^{14}C-cholesterol, for $^{14}CO_2$ excretion, urinary ^{14}C excretion, and liver specific activity are presented for the 11-week experimental period.

Inasmuch as the basic data were the same as those published in the shorter paper,[8] the conclusion remained the same, that is, the rate of cholesterol catabolism is slowed down in latent vitamin C deficiency, which then caused the accumulation of cholesterol in blood and liver of the hypovitaminosis-C guinea pigs. □

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