Cholesterol Metabolism in Scorbutic Guinea Pigs

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By use of isotopically labeled acetate, Bloch et al. (1-4) have shown that acetic acid is the most important source of carbon and hydrogen for cholesterol synthesis. Banerjee and Deb (5) and Oosterling and Long (6) have reported a lowering of adrenal cholesterol in scurvy. Belavady and Banerjee (7) have shown that during the development of scurvy, guinea pigs excreted in the urine a lesser amount of injected PABA1 in the acetylated form than did normal controls. The decrease in adrenal cholesterol in scurvy has been attributed to the diminished acetylation in the body. But it has also been observed that although the cholesterol content of the adrenals, spleen, and lungs decreased, that of the testes and small intestine increased, and that there was no change in the cholesterol content of liver and kidney. Becker et al. (8) have reported that the liver and adrenals of scorbutic guinea pigs that have been fed labeled acetate contain cholesterol with higher specific activity, indicating that the tissues of scorbutic guinea pigs synthesize more cholesterol from administered acetate. Lahiri and Banerjee (9) have shown that the total body cholesterol is significantly increased in scorbutic guinea pigs. They have further shown that CoA activity in scurvy is not significantly different from that of the normal controls, and this possibly indicates that the diminished acetylation and abnormal cholesterol metabolism in scurvy are not ascribable to an alteration in the CoA activity.

Hotta and Chaikoff (10) have reported that the high rate of incorporation of acetate into cholesterol by livers of alloxan-diabetic rats is decreased by insulin. Mukherjee and Sadhu (11 14) have observed a relationship between cholesterol synthesis and decreased oxidation of acetate and acetoacetate in hypothyroidism (11), nephrosis (12), chronic poisoning by malonate and arsenite (13), and alloxan diabetes (14). They have suggested that continued depression of acetate and acetoacetate oxidation in these cases might result in channeling these metabolites in the direction of cholesterol synthesis.

Insulin has been shown to increase the acetylation of injected PABA in normal rabbits (15) and to raise the lowered acetylation activity of alloxan diabetic rat to normal (16). In view of the observations of Banerjee et al. that scurvy is associated with the decreased insulin content of the pancreas (17) and with marked disturbance in carbohydrate metabolism (18, 19), it was of interest to study cholesterol metabolism in scorbutic guinea pigs which have been under prolonged treatment with insulin.

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1 The abbreviations used are: PABA, p-aminobenzoic acid; CoA, coenzyme A.

Materials and Methods

Female guinea pigs weighing from 250 to 300 gm. were fed with green grass, soaked gram, and scorbutic diet (20) for 5 to 6 days. The animals that grew well on this diet were selected and divided into several groups, each group consisting of one normal, one scorbutic, and one insulin-treated scorbutic guinea pig. The animals in each group were fed equal amounts of scorbutic diet. The normal control was fed 5 mg. of ascorbic acid daily. All the animals were fed 2 drops of a concentrate of vitamins A and D twice a week. Insulin (Lilly), 0.1 unit per 100 gm. of body weight per day, was injected subcutaneously into the animal receiving hormone treatment, the amount of insulin being increased to 0.3 unit per 100 gm. of body weight per day at the beginning of the second week. On the day of the experiment insulin injection was stopped.

Estimation of Total Body Cholesterol—Six groups of animals were studied for total body cholesterol content. During the 4th week of the regime with the scorbutic diet, the animals were fasted overnight and then killed. The gastrointestinal tract was washed, the fur was plucked off, and the whole body was weighed and then minced and dried at 85° for 16 hours. The dried sample was powdered and extracted, over a 24 hour period, with redistilled acetone in a 1 liter Soxhlet apparatus. The total cholesterol content of the extract was estimated by the method of Sobel and Mayer (21). Results are shown in Table I.

Estimation of Cholesterol Content of Tissues—Nine groups of animals were studied for tissue cholesterol content. When the guinea pigs had developed severe scurvy, in the 4th week of the scorbutic regime, they were fasted overnight and killed the next morning by decapitation. Blood was collected from the neck vein, and total and ester cholesterol contents were estimated by the method of Sobel and Mayer (21). Tissues were removed from the body, washed off, and finely minced with a glass mortar and pestle. An aliquot of the finely ground tissue was thinly spread over a previously weighed, circular piece of filter paper, 8 cm. in diameter. The filter paper with its sample of tissue was again weighed to obtain the weight of the tissue and was then dried in an electric oven at 60° for 8 hours. The dried sample was then extracted with redistilled acetone in a continuous extractor for 6 hours, and the extract was estimated for total and ester cholesterol according to the method of Sobel and Mayer (21). Tissues studied by this procedure were the adrenals, intestine, kidney, liver, and spleen. Results are shown in Table II.

Acetylation Studies—Eight groups of animals were used for urinary acetylation studies. When the animals had developed severe scurvy, intraperitoneal injections of PABA (1 mg. dissolved in 1 ml. of sterile water) were administered to each guineapig.
pig daily, for 3 consecutive days; the urine excreted by each animal was collected daily in a flask containing 5 ml. of 4 N hydrochloric acid. Total and acetylated PABA excreted in the urine were estimated by the method of Bratton and Marshall (22). The amounts of acetylated PABA are expressed as per cent of the total PABA excreted in the urine. Results are shown in Table III.

Estimation of Total and Reduced CoA Content of Tissues—Seven groups of animals were studied for total and reduced CoA content of liver, kidney, and adrenals. Differential estimation of total and reduced CoA was accomplished by the method of Boxer et al. (23). When the animals had become severely scorbutic in the 4th week of the scorbutic regime, they were killed; tissues were quickly removed, chilled, and homogenized with ice-cold water in an atmosphere of carbon dioxide to prevent air oxidation. Alkylation of the sulfhydryl form of CoA by iodoacetate was performed according to the method described by Boxer et al. (23). The assay of the total CoA and the disulfide form was performed by the sulfanilamide acetylation method of Kaplan and Lipmann (24). The results were expressed in Lipmann units per gm. of fresh tissue.

The CoA used as the standard of reference was prepared in the laboratory from rabbit liver by the method of Kaplan and Lipmann (24). The potency and purity of this CoA were not known, hence the absolute values for CoA in the test samples were subject to some error. But it could be safely assumed that the relative values serve satisfactorily for comparison. In each assay run, a standard curve with reference CoA was prepared. At CoA saturation, approximately 65 per cent of the added sulfanilamide was acetylated.

Adenosine triphosphate was prepared as dibarium salt from rabbit muscles by the method of Dounce et al. (25). On analysis...
**RESULTS**

The total body cholesterol content of scorbutic guinea pigs increased significantly in comparison to that of normal controls. Prolonged treatment with insulin lowered the cholesterol value of the scorbutic animals to the normal level (Table I). In the scorbutic guinea pigs, the cholesterol content of the adrenals and spleen decreased and that of the intestine increased significantly; there was no change in the cholesterol content of blood, liver, and kidney. Prolonged insulin treatment of the scorbutic animals lowered the cholesterol content of the intestine to the normal level, but it had no effect on the cholesterol content of other tissues studied (Table II). Severely scorbutic guinea pigs excreted in the urine a lesser amount of injected PABA in the acetylated form than did the normal controls (Table III). Treatment with insulin raised the acetylation to the normal level. Total CoA content of liver, kidney, and adrenal did not change, but the reduced CoA content of liver and kidney tended to increase in ascorbic acid-deficient animals; treatment with insulin had no effect on either the total or the reduced CoA content of tissues (Table IV).

**DISCUSSION**

Except for the formation of adrenal steroids, the effect of vitamin C deficiency on the catabolic transformation of cholesterol is not yet known. Scurvy is usually accompanied by greatly increased excretion of adrenocortical steroids (26, 27). In spite of this increased catabolic activity, cholesterol accumulates in the body, which fact possibly indicates its increased synthesis in vitamin C deficiency. It has been shown previously (19) that the amount of citric acid that accumulates in the tissues of scorbutic guinea pigs is brought down to almost normal levels when insulin is injected into the animals. Insulin treatment also lowers the total body cholesterol of scorbutic guinea pigs to normal level. The increased cholesterologenesis in scurvy, therefore, might be due to the utilization of an increased pool of acetate which is not burned through the tricarboxylic acid cycle. As isotopic tracer methods were not employed, the data presented here cannot throw any light on the pattern of cholesterol metabolism as affected by vitamin C deficiency in various tissues.

In spite of an increased ability for cholesterol formation, the scorbutic animals showed decreased capacity to acetylate injected PABA. The degree of acetylation of foreign amines as a measure of the degree of incorporation of acetate into cholesterol has, however, been questioned (28). The observed effects of insulin in increasing acetylation of PABA and in lowering total body cholesterol value in scurvy also suggest absence of interference between acetylation and cholesterol formation. Total and reduced CoA content of tissues did not change in scurvy. This shows that diminished acetylation is not likely to be the result of an alteration of CoA activity.

**SUMMARY**

1. Total body cholesterol content increased in scorbutic guinea pigs in comparison with normal controls. Prolonged treatment of the scorbutic animals with insulin lowered the cholesterol content to the normal level.

2. Although both total and ester cholesterol content of adrenal and spleen diminished in scorbutic guinea pigs, cholesterol content of intestine increased and there was no change in that of blood, liver, and kidney. Insulin treatment of the scorbutic animals had no effect on the cholesterol content of tissues studied, except that of the intestine, which was lowered to the normal level.

3. Scorbutic guinea pigs showed a decreased ability to acetylate injected p-aminobenzoic acid, in comparison with normal controls. Treatment with insulin raised the acetylation to normal level.

4. Total coenzyme A content of liver, kidney, and adrenal was not altered in the normal, scorbutic, or the insulin-treated, scorbutic guinea pigs. Reduced coenzyme A content of liver and kidney tended to increase in scorbutic guinea pigs, but that of the adrenal was not altered.

5. It has been suggested that depression of acetate oxidation may be responsible for the increased cholesterol content in scurvy.
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