Coenzyme A Activity in Tissues of Normal and Scorbutic Guinea Pigs. (22336)

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By using labelled acetate Bloch *et al.*(1-4) showed that cholesterol is formed in the body from acetate. Adrenal cholesterol diminished in scurvy(5). Scorbutic guinea pigs excreted a lesser amount of injected p-aminobenzoic acid (PABA) in the acetylated form(6). Diminished adrenal cholesterol in scorbutic animals seemed possibly due to diminished acetylation in the body. But it was also observed(6) that while cholesterol content of adrenals, spleen and lungs diminished, that of testes and small intestine increased and there was no change in the cholesterol content of liver and kidney(6). It was, therefore, not clear if the total synthesis of cholesterol by a scorbutic guinea pig was altered. Becker *et al.*(7) reported that liver and adrenals of scorbutic guinea pigs fed labelled acetate contained cholesterol with higher specific activity, which indicated that adrenal glands of scorbutic guinea pigs synthesized more cholesterol from administered acetate. It was, therefore, difficult to explain why scorbutic guinea pigs showed diminished acetylation reaction. Decreased acetylation of PABA might be due to diminished coenzyme A (Co A) activity of tissues. The present investigation was undertaken to study Co A activity of tissues of scorbutic and paired fed normal guinea pigs. Cholesterol content of the whole body of the animals was also determined.

Methods. Production of scurvy. Selection of guinea pigs and feeding a scorbutic diet, with or without the supplement of ascorbic acid by the paired feeding technic, was the same as described previously(5). *Studies with urine.* Acetylation studies were carried out as described previously(6). Results are given in Table I. *Adenosinetriphosphate* (ATP) was prepared as dibarium salt by the method of Dounce *et al.*(8) from rabbit muscle. Before use, the barium salt was converted into potassium-ATP. *Preparation and aging of apoenzyme from pigeon liver.* Apoenzyme was prepared from pigeon liver and aged by the method of Kaplan and Lipmann (9).

TABLE I. Percentage of Acetylated PABA Excreted in Urine by Guinea Pigs. Average of 3 days excretion.

Normal (5)	25.0 ± 2.4*	Scorbutic (5)	17.4 ± 2.5	t: 2.2

* Stand. error.
Figures in parentheses indicate No. of animals.

Results. Studies with tissues. Scorbutic animals were sacrificed on the 25th day of experiment along with their paired fed controls and Co A activity of liver, kidney and brain was determined by the method of Kaplan and Lipmann(9). Dry weights of the tissues were determined and Co A activity was expressed on dry weight basis. No Co A preparation of known potency was available. Crude preparation of Co A was obtained by the method of Kaplan and Lipmann(9) from rabbit liver and was used as the reference Co A. Activity-concentration curve was prepared with this reference Co A for each batch of extract of apoenzyme over a wide range including the saturation point. Half of the maximum activity was taken as unit. From this standard curve the Co A contents of the test samples were obtained directly. Results are given in Table II.

Estimation of total body cholesterol. Animals were killed, gastrointestinal tracts washed, whole body minced, weighed, dried

TABLE II. Coenzyme A Contents of Tissues of Normal and Scorbutic Guinea Pigs. Lipmann units of Co A/g dry tissue.

	Liver	Kidney	Brain
Normal (9)	959 ± 58	420 ± 35	294 ± 27
Scorbutic (9)	1007 ± 54	500 ± 40	301 ± 21

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TABLE III. Total Body Cholesterol of Normal and Scorbutic Guinea Pigs. mg/100 g fresh tissue.

Normal (8)	219 ± 5.7	Scorbutic (8)	269 ± 9.8	t: 4.4

at 85°C for 16 hours, powdered and extracted with acetone in a Soxhlet apparatus for 24 hours. Cholesterol was estimated in aliquots of extracts by the method of Sobel and Mayer (11). Results are given in Table III.

Discussion. Scorbutic guinea pigs showed decreased acetylation of administered PABA. Co A activity of tissues of scorbutic guinea pigs did not change. As the paired feeding technic was employed the consumption of pantothenic acid was similar in both the groups of animals. The decreased excretion of injected PABA in acetylated form by scorbutic guinea pigs indicated that ascorbic acid was in some way concerned in acetylation reactions in the body. A significant increase in the whole body cholesterol of scorbutic guinea pigs was observed. This might be due to either increased synthesis or decreased utilization of cholesterol in the body of a scorbutic guinea pig. The high rate of incorporation of acetate into cholesterol by the livers of alloxan-diabetic rats is reduced by insulin(12). Recent observations indicate that in insulin insufficiency and also in vit. C deficiency the oxidation of acetate in the tricarboxylic acid cycle is hampered(13-15). Vit. C deficiency in turn decreases the insulin content of pancreas(16). Thus acetate pool which is not burned through tricarboxylic acid cycle might be utilized in the increased formation of cholesterol. The increased cholesterogenesis in the scorbutic guinea pigs, therefore, might be due to diminished insulin synthesis.

Summary. Acute scurvy was produced in guinea pigs by paired feeding technic. Urinary excretion of injected PABA in the acetylated form was estimated in scorbutic and paired fed normal guinea pigs. Scorbutic animals excreted decreased amount of acetylated PABA. Co A activity of liver, kidney and brain of scorbutic and paired fed control guinea pigs was determined. Co A activity which was present in the tissues of normal guinea pigs did not change in scurvy. Total body cholesterol of scorbutic guinea pigs increased significantly. The implications of the results obtained have been discussed.

1. Bloch, K., and Rittenberg, D., *J. Biol. Chem.*, 1942, v145, 625.
2. ———, *ibid.*, 1945, v159, 45.
3. Bloch, K., *ibid.*, 1944, v155, 255.
4. Little, H. N., and Bloch, K., *ibid.*, 1950, v183, 33.
5. Banerjee, S., and Deb, C., *ibid.*, 1951, v190, 177.
6. Belavady, B., and Banerjee, S., *ibid.*, 1954, v209, 641.
7. Becker, R. R., Burch, A. B., Salmon, L. L., Venkitasubramanian, T. A., and King, C. G., *J. Am. Chem. Soc.*, 1953, v75, 2020.
8. Dounce, A. L., Rothstein, A., Beyer, G. T., Meier, R., and Freer, R. M., *J. Biol. Chem.*, 1948, v174, 361.
9. Kaplan, N. O., and Lipmann, F., *ibid.*, 1948, v174, 37.
10. Bratton, A. C., and Marshall, E. K., Jr., *ibid.*, 1939, v128, 537.
11. Sobel, A. E., and Mayer, A. M., *ibid.*, 1945, v157, 255.
12. Hotta, S., and Chaikoff, I. L., *ibid.*, 1952, v198, 895.
13. Beatty, C. H., and West, E. S., *ibid.*, 1955, v215, 661.
14. Takeda, Y., and Hara, M., *ibid.*, 1955, v214, 657.
15. Foster, J. M., and Villee, C. A., *ibid.*, 1954, v211, 797.
16. Banerjee, S., and Ghosh, N. C., *ibid.*, 1947, v168, 207.

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