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A Possible Role of Pantothenic Acid in Diabetic Cholesterologenesi

By S. MOOKERJEA* AND D. P. SADHU

Department of Biochemistry, Nagpur University, Nagpur, India and Department of Physiology, Bengal Veterinary College, Calcutta, India

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The mechanism of cholesterol synthesis in diabetes has been studied by Hotta & Chaikoff (1952), who reported a tenfold increase in the conversion of labelled acetate into cholesterol in liver slices from alloxan-diabetic rats. The investigation of Brady & Gurin (1950) shows that in diabetes lack of insulin prevents conversion of the short-chain into long-chain fatty acids and therefore results in accumulation of the short-chain acids as the fat depots become depleted. Their investigation indicates that the accumulation of these substances accentuates the formation of cholesterol in diabetes as an alternative path of acetate metabolism. The relationship between the capacity of tissues to form acetoacetate and cholesterol synthesis has also been stressed (Gurin & Brady, 1951). Another observation (Van Bruggen, Yamada, Huchens & West, 1954), however, shows that although in the intact rat fatty acid synthesis from $[1-^{14}\text{C}]$ acetate is impaired in alloxan diabetes, the incorporation of labelled acetate into liver, gut, carcass and skin cholesterol is unchanged, suggesting unaltered cholesterologenesi.

Our previous findings indicate a relationship between decreased oxidation of acetate and acetoacetate and increased cholesterol synthesis in hypothyroidism (Mookerjea & Sadhu, 1954a, 1955b), nephrosis (Sadhu & Mookerjea, 1955) and chronic poisoning by malonate and arsenite (Mookerjea & Sadhu, 1955c), but similar studies in diabetes (Mookerjea & Sadhu, 1956) appear to show a less clear picture. There is a small rise of cholesterol only in a prolonged state of diabetes of 6-7 weeks. Liver slices, however, show decreased

oxidation of acetate and a considerable increase in ketogenesis. This, together with ketonuria and ketonaemia, the characteristic features of diabetes, shows that the availability of acetate and acetoacetate, the potential precursors of cholesterol, is increased in diabetes, but the less obvious rise of cholesterol indicates that they are not completely used for cholesterol synthesis.

The role of pantothenic acid in cholesterol synthesis is well established. Pantothenic acid deficiency has been shown to cause a decrease in tissue cholesterol due to lack of coenzyme A (Klein & Lipmann, 1953; Mookerjea & Sadhu, 1954b). The interesting observations by Migicovsky & Greenberg (1954) show the importance of relative requirement of both acetate and coenzyme A for cholesterol synthesis. They have shown that even in small amounts coenzyme A behaves as an inhibitor of cholesterol synthesis in the presence of relatively low acetate concentrations. When the acetate concentration is raised, the same amount of coenzyme A increases cholesterol formation. It appears from this that both these factors, namely availability of coenzyme A and size of acetate pool, are necessary for the synthesis of cholesterol.

The observations of Ralli & Dumm (1953), showing a great rise of adrenal cholesterol when excess of pantothenate is fed, further prompted us to investigate the importance of adequate levels of pantothenic acid for diabetic cholesterologenesi.

EXPERIMENTAL

Forty-eight male albino rats weighing 100-136 g. were fed with stock laboratory diet containing 70% of whole-wheat flour, 17% of whole-milk powder, 6% of groundnut oil, 2.5% of dried brewer's yeast, 2.5% of crude casein and 2% of salt mixture. Two drops of cod-liver oil was fed to each animal twice weekly. The average food intake/day/animal

* Present address: Post-doctorate Fellow, Atlantic Regional Laboratory, National Research Council, Halifax, N.S., Canada.

was approximately 7-10 g., which did not show any marked variation during the course of the experiment.

Animals of series I served as controls. Series II animals were fed daily with excess of calcium pantothenate (4 mg./10 g. of the stock laboratory diet). Series III and IV animals were rendered diabetic by the subcutaneous injection of alloxan, 200 mg./kg. body wt. Series III remained as control diabetic animals, and the diabetic animals of series IV were fed with excess of pantothenate at a similar rate to series II animals.

Excretion of sugar (Benedict, 1911) and of acetoacetate (Edson, 1935) in urine was followed during the course of the experiment. Changes in body weights of the animals were also noted.

Animals were killed after the experiment had continued for 6-7 weeks. Free and ester cholesterol of different tissues were determined according to the method of Sobel & Mayer (1945), as modified by Mookerjee & Sadhu (1955a) for tissue estimations.

RESULTS

Series II animals showed a greater increase in body weight than the animals of series I (Fig. 1). The fall of body weight due to prolonged diabetes (series III animals) was counteracted by excess of dietary pantothenate (series IV).

Excretion of sugar and acetoacetate was absent in series I and II animals. The sugar-excretion curves of series III and IV animals are similar. Series III animals excreted considerable amounts of acetoacetate. In the latter part of the experiment the curve shows a slight decline, while series IV animals show a sharp decline of acetoacetate excretion after an initial phase of high excretion.

Table 1 shows a definite rise of cholesterol in liver and adrenal tissues of series II animals compared with the controls (series I). The ester fraction increased more than the free cholesterol.

The cholesterol values of series III showed a similar rise to that obtained before (Mookerjee & Sadhu, 1956). The ester fractions of kidney, liver, adrenal and blood of the diabetic animals show a definite increase and the free fractions of liver and blood show a small rise.

Both free and ester cholesterol values in liver, adrenals and blood of series IV animals show a

Fig. 1. Growth curve and 24 hr. excretion of sugar and acetoacetate. ●, Series I (control); x, series II (fed on excess of pantothenate); ○, series III (diabetic); Δ, series IV (diabetic fed on excess of pantothenate). Each point on the curve represents the average value of the twelve animals in each group. Excretion of sugar was absent or negligible in series I and II. Excretion of acetoacetate was absent in series I and II.

Table 1. *Effect of excess of dietary pantothenic acid on the level of cholesterol in blood and tissues of normal and diabetic rats*

Average values of free and ester cholesterol in blood, liver, kidney and adrenal in mg./100 ml. or 100 g. wet wt. $\pm$ S.E. Each group consisted of twelve animals.

Group	Cholesterol							
	Blood		Liver		Kidney		Adrenal	
	Free	Ester	Free	Ester	Free	Ester	Free	Ester
I (Control)	46 $\pm$ 3	60 $\pm$ 4	232 $\pm$ 8	84 $\pm$ 7	354 $\pm$ 12	98 $\pm$ 4	4987 $\pm$ 54	1082 $\pm$ 22
II (Fed on excess of pantothenate)	50 $\pm$ 4	62 $\pm$ 7	248 $\pm$ 9	128 $\pm$ 5	350 $\pm$ 14	102 $\pm$ 8	6849 $\pm$ 62	2123 $\pm$ 32
III (Diabetic)	58 $\pm$ 6	88 $\pm$ 4	282 $\pm$ 13	122 $\pm$ 8	360 $\pm$ 16	124 $\pm$ 11	5247 $\pm$ 52	1214 $\pm$ 34
IV (Diabetic, fed on excess of pantothenate)	68 $\pm$ 8	108 $\pm$ 12	338 $\pm$ 16	145 $\pm$ 9	332 $\pm$ 18	104 $\pm$ 12	7456 $\pm$ 84	2524 $\pm$ 42

pronounced rise in these animals, compared with the animals of series III. Kidney tissue does not show any definite rise, but free cholesterol shows a small fall.

DISCUSSION

The present study attempts to elaborate a possible role of pantothenic acid in diabetic cholesterogenesis. In the liver of rats deficient in pantothenic acid, Klein & Lipmann (1953) showed a parallelism between the fall of coenzyme A level and decline of cholesterol synthesis. It appears from the study of Migicovsky & Greenberg (1954) that coenzyme A behaves as an activating factor for cholesterol synthesis, provided that the proper concentrations of acetate and cofactor are present. In untreated diabetes, when fatty acid synthesis is impaired (Brady & Gurin, 1950), unoxidized acetate and over-produced acetoacetate partially take the route of cholesterol synthesis. Their use in this metabolic pathway is, however, incomplete, as indicated by the less-pronounced rise of cholesterol in diabetes (Mookerjea & Sadhu, 1956) compared with the increased excretion of acetoacetate.

If an adequate concentration of coenzyme A is made available in diabetes, synthesis of cholesterol from its potential precursors may be further accelerated. This may be a plausible explanation for the results obtained in the present study. Animals of series IV, where excess of pantothenic acid feeding has been superimposed on diabetes, show a very sharp rise of cholesterol compared with the rise of cholesterol in animals which are either only diabetic (series III) or fed only with excess of pantothenate (series II).

The excretion of acetoacetate by series IV animals shows a sharp decline after a preliminary rise. This fact lends further support to our interpretation. Feeding of excess of pantothenic acid possibly diverts the acetoacetate which would otherwise be excreted towards further cholesterol synthesis.

Another conclusion from the present study is that the weight loss in diabetes is prevented by feeding excess of pantothenate. This fact may stimulate further research on the role and importance of pantothenic acid in diabetes.

SUMMARY

1. Free and ester cholesterol were determined in different tissues of normal rats (series I), rats fed

with excess of pantothenate (series II), diabetic rats (series III) and diabetic rats fed on excess of pantothenate (series IV).

2. Series II and III animals showed small rises of cholesterol in some tissues. These rises are additive, as shown by the cholesterol levels in tissues of animals in series IV, where excess of pantothenate feeding was superimposed on diabetes.

3. Sugar and acetoacetate excretion by animals of series IV declined sharply after a preliminary rise. The growth curves of the animals also show that feeding of pantothenate prevents the weight loss of diabetic animals.

4. The availability of adequate amounts of pantothenic acid possibly guides the metabolism of unoxidized acetate and over-produced acetoacetate towards increased synthesis of cholesterol in diabetes.

5. The importance of both the availability of coenzyme A and size of acetate pool for the synthesis of cholesterol is discussed.

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