Lipid Metabolism in the Diabetic Rat

II. CHOLESTEROL TURNOVER STUDIES*

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Although hypercholesterolemia is often associated with diabetes, we found normal or slightly depressed cholesterologenesis in the chronic diabetic rat (1), and a pronounced depression in cholesterologenesis in acute alloxan diabetic and pancreactomized rats (2). In the animals with acute diabetes, a pronounced hypercholesterolemia was present. In an attempt to explain these findings, an investigation of the rate of cholesterol turnover in the diabetic animal has been made. Cholesterol turnover in the normal rat has been studied by various investigators, but absolute figures have not as yet been established as is indicated by the reported half-lives of 40 hours to 6 days for liver cholesterol (3). To the best of our knowledge, information on cholesterol turnover of the diabetic is unavailable in the literature.

Control, acute alloxan diabetic, and pancreactomized rats were treated by injection with acetate-1-C\textsuperscript{14} to label the body lipids, and were then killed at various time intervals. The rate of disappearance of the labeled cholesterol was measured to obtain information on the rate of turnover of cholesterol in the carcass, skin, gut, liver, and serum. It was found that the diabetic rats had depressed turnover times in liver and gut cholesterol fractions, and it is suggested that this depression may be related to the observed hypercholesterolemia of these animals.

EXPERIMENTAL

Male, Sprague Dawley rats weighing about 200 g were used in this study. The techniques of preparing alloxan diabetic and pancreactomized animals and the feeding regime of these animals have been described previously (2). The diabetic animals, with fasting blood sugars exceeding 165 mg per 100 ml, were used 5 to 7 weeks after the onset of diabetes.

One hour after the last meal, each rat was given an intraperitoneal injection of from 30 to 60 \( \mu \)c of acetate-1-C\textsuperscript{14}, which was prepared in this laboratory and which contained 0.19 mc per mg. After this injection, the animal was placed in a cage hood assembly (4) to minimize radioactive contamination of the laboratory. Animals were killed at various time intervals up to 28 days after the injection of acetate.

The procedures for the isolation and assay of the lipid fractions were the same as those used in the previous study (2). The fatty acids were determined gravimetrically and the cholesterol colorimetrically. Both the fatty acid and sterol fractions were radioassayed as infinitely thin samples, using a D47 Micromil Geiger counter (Nuclear-Chicago). The lipid specific activities are expressed as counts per minute per mg and were corrected to a standard dose of 60 \( \mu \)c and to the equivalent activity of infinitely thick BaCO\textsubscript{3} plates.

RESULTS

Information on the turnover of cholesterol in the control, alloxan diabetic, and pancreactomized rats is graphically presented in Figs. 1 and 2 in which the tissue cholesterol specific activities are plotted as a function of time. Best fitting lines were drawn according to the method of least squares (5). In a number of cases of nonlinearity, the decay curves were divided by graphic inspection into two components, and the turnover of cholesterol of that particular tissue was interpreted as being composed of two rate components. Used in this study were 30 control, 22 alloxan diabetic, and 17 pancreactomized animals. Since serum was not available from all animals, data on serum cholesterol shown in Fig. 2 represents the use of 14 control, 12 alloxan, and 15 pancreactomized animals.

In Table I there are presented four parameters of cholesterol turnover. The half-life values (\( t_1/2 \)) were calculated from the slopes of the curves and tested for statistical differences by the \( t \) test. Differences were considered to be significant if the \( p \) value was 0.05 or less. The turnover time is defined as the time required for the turnover of an amount of material equal to the pool size and is calculated as \( T = \frac{1}{T_1/2} \). The percentage of turnover represents the fraction of a given pool which is turned over per day (\( k = 1/T_1/2 \)), and the turnover rate is the milligrams of cholesterol turned over per day per 100 g of tissue weight (6).

It is to be noted that the diabetic animals have zero time cholesterol specific activities less than those of the normal because of the defect in cholesterologenesis previously described (2). Regardless of the starting point, preparations having the same turnover should exhibit decay curves of the same slope. In the following discussion, the curves of Figs. 1 and 2 will be used to indicate the trend of the decay as well as to illustrate the cases where simple decay curves are not present.

In reference to Fig. 1, it is seen that the specific activities of the skin cholesterol fractions of the diabetics increased above the zero time level. Although a similar trend seemed to be present in the control group, statistical treatment revealed nonsignificant deviation from a single line. As shown in Table I, the turnover times of the skin fractions of all three preparations did not differ after the initial rise.

Carcass cholesterol of the control group had a half-time of 11.9 days which was significantly greater than the half-times of the diabetic carcass cholesterol fraction. The diabetic preparations
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had similar carcass cholesterol turnover times. It is recognized that the carcass fraction consists of a mixture of metabolically diverse tissues such as bone, muscle, connective tissue, adrenals, and kidneys. We cannot at this time identify the role of the various tissues in the total turnover effect described here. In other studies, we have shown that the adrenals are very active whereas muscle fibers have a low order of activity in regard to sterol metabolism.

The control and alloxan gut cholesterol decay curves are biphasic; the control gut shows an initial rapid phase followed by a slow phase with a long half-life. From the half-life values, it is seen that the turnover rates of cholesterol of the initial phases of the three preparations do not differ greatly from each other.

The biphasic turnover curve of the control liver contains an initial component, indicating an extremely rapid decay, which is lacking in the diabetic liver. The half-lives of all three liver cholesterol fractions are different from each other and both diabetic liver fractions have longer cholesterol half-lives than do the controls.

An early rise in the serum cholesterol specific activity of the alloxan preparation similar to that of skin is shown in Fig. 2. The subsequent linear decay curves reveal similar turnover times in all preparations. The serum cholesterol studies were carried out for a period of 7 days.

DISCUSSION

It is clear from the data presented above that cholesterol turnover in the rat is a complex process involving various systems and multiple reaction rates. The present studies do not presume to have explored each aspect of these complex systems.

Let us first consider the initial rises in specific activities of the skin cholesterol of the diabetic animals. After acetate-1-C\textsuperscript{14} is injected, the time course of C\textsubscript{14}O\textsubscript{2} excretion indicates that the labeled acetate is essentially completely metabolized in less than an hour (7). The skin cholesterol formed in this period then should have a maximum specific activity in the first few hours after acetate injection. On this basis, the increase of specific
<table>
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<th>Tissue</th>
<th>Half-life</th>
<th>Turnover time&lt;sup&gt;b&lt;/sup&gt;</th>
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<sup>a</sup> Half-life -=  time required for specific activity to fall by one-half.

<sup>b</sup> Turnover time -=  time required for the turnover of an amount of material equal to the pool size.

<sup>c</sup> Percentage of turnover per day, the fraction of the pool turned over per day.

<sup>d</sup> Turnover rate, milligrams of material turned over per day per 100 g of tissue weight.

<sup>e</sup> The significance of differences between mean values has been tested by the $t$ test. The $p$ values that bracket respective groups indicate significant differences if 0.05 or less.

<sup>f</sup> Values of slow component in biphasic curves.
activity after a few days cannot be attributed to a continued synthesis from acetate. Delayed increases in specific activities in the testis, spleen, kidney, and lung of normal rats after multiple acetate administration were observed by Landon and Greenberg (8). It is possible that during the first two days, labeled cholesterol of high specific activity was being transported to and deposited in the skin. It may also be considered that other radioactive substances were being converted to radioactive cholesterol in situ. Possible sources of high specific activity cholesterol are the liver and the gut, both of which have cholesterol specific activities much higher than that of the skin. However, in relating this high specific activity of gut and liver cholesterol to the delayed increase of skin cholesterol specific activity, one must bear in mind the role of blood cholesterol as an immediate precursor of skin cholesterol. The specific activities of serum cholesterol were found to be higher than those of the skin cholesterol, and in addition, the serum of the alloxan-treated animals showed the same delayed increase of cholesterol specific activity as did the skin tissue of the alloxan-treated animals.

No gross differences were observed in liver cholesterol turnover between the alloxan and pancreatectomized rats, so that these two diabetic preparations may be considered to have similar cholesterol catabolic activities.

The effect of diabetes upon cholesterol metabolism in the rat is definitely shown by our findings to be a decreased turnover of cholesterol in the liver tissues of both alloxan and pancreatectomized rats. As previously stated, the specific activity-time relations of the liver cholesterol of the control group could not be fitted into a straight line due to the presence of an early rapid phase which may well represent metabolism of approximately 90% of all the radioactive cholesterol. This rapid phase is lacking in the diabetic livers resulting in net slower turnover rates.

We believe these findings to indicate that a major defect in lipid metabolism in the diabetic liver tissue is a decreased ability to catabolize cholesterol. This is contrasted with the theory of an increased synthesis of cholesterol by the diabetic liver (9). Cagan et al. (10) suggested a defect in utilization of cholesterol by the diabetic from their findings of plasma cholesterol levels twice that of normal in diabetic rats on a high fat diet, but these workers did not localize such a defect.

In the presence of a decreased turnover of liver cholesterol and an increase in plasma cholesterol, one might expect to find an increase in liver cholesterol. We found no accumulation of cholesterol in the liver but did find a decreased rate of cholesterol synthesis in this tissue. Thus, in the diabetic liver, there may be a decreased synthesis coupled with a decreased destruction of cholesterol resulting in no accumulation of cholesterol.

CONCLUSIONS

The rates of cholesterol turnover in the carcass, skin, gut, liver, and serum of alloxan diabetic, pancreatectomized, and control rats were studied with acetate-1-C14 as the radioactive tracer.

The alloxan and pancreatectomized animals did not differ grossly from each other in the measurements of cholesterol turnover and are thus believed to metabolize cholesterol by similar mechanisms. The diabetic rats differed from the controls in having decreased rates of cholesterol turnover in the liver. It is concluded that there may be a defect in the mechanism of cholesterol degradation in the diabetic rat.

REFERENCES

4. Van Bruggen, J. T., Nucleonics, 10, 64 (1952).
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