ENDOGENOUS CHOLESTEROL METABOLISM IN THE RAT STUDIED WITH C\(^{14}\)-LABELED ACETATE\(^*\)

BY ERWIN J. LANDON\(^t\) AND DAVID M. GREENBERG

(From the Department of Physiological Chemistry, University of California School of Medicine, Berkeley, California)

(Received for publication, January 25, 1954)

In previous investigations rates of cholesterol metabolism in the intact rat were determined by measuring the rate of incorporation of an isotope into tissue cholesterol. Karp and Stetten (1) employed D\(_2\)O maintained at a constant level in the body fluids. Pihl, Bloch, and Anker (2) used C\(^{14}\)-labeled acetate and took the specific activity of acetate in excreted acetylated foreign amines as a measure of the acetate specific activity at the site of cholesterol synthesis.

In the present study a different method was used: The rate of disappearance of previously labeled cholesterol from the tissues of the intact animal was measured. The pools of tissue cholesterol were labeled by administering C\(^{14}\)-labeled acetate. After sufficient time had elapsed so that radioactive precursors could be presumed to be negligible, the disappearance and redistribution of labeled cholesterol were determined in the various tissues. The results give a more clearly defined picture of the rôle of the individual body tissues in the synthesis, exchange, and catabolism of cholesterol. A faster turnover rate in the rat than has previously been reported also is indicated.

EXPERIMENTAL

The experimental animals were adult male rats of the Long-Evans strain. They were raised on Purina laboratory chow diet, the standard feed in our laboratory, which contains 0.075 per cent cholesterol. The "white diet," used for nutrition of breeder animals, contains 0.006 per cent cholesterol. This was considered adequately free of cholesterol, so, prior to doing the experiments, the animals employed were placed on this diet. It contains about 10 per cent more protein than the standard stock diet.

The experimental groups were made up of four to eight rats, of uniform weight and age, and often of litter mates. One large experimental group

\(^*\) Aided by research grants from the American Heart Association and the Life Insurance Medical Research Fund. Prepared from a thesis submitted by E. J. Landon to the University of California for the degree of Doctor of Philosophy, June, 1953.

\(^t\) Fellow of the American Heart Association.

\(^1\) Manufactured by the Simonsen Laboratories, Gilroy, California.
oi thirty-two rats was also employed. The animals comprising an experimental group were injected intraperitoneally with uniform doses of sodium acetate-\(^{14}\)C. The injected dose usually was of the order of 1 \(\mu\)c. per 100 gm. of rat, given three times every 4 hours. 0 hour was 4 hours after

![Fig. 1. Specific activity of total serum cholesterol in a representative bile fistula rat (six experiments) following one injection of \(^{14}\)C-labeled acetate. 0 hour is 4 hours after the injection. To each serum aliquot 5 mg. of carrier cholesterol were added. The values of the ordinates are not corrected for dilution by carrier.](image1)

![Fig. 2. Specific activity of total (●) and free (▲) serum cholesterol in six control rats following one injection of \(^{14}\)C-labeled acetate. 0 hour is 4 hours after the injection. To each serum aliquot 5 mg. of carrier cholesterol were added. The values of the ordinates are not corrected for dilution by carrier.](image2)
the last injection; the animals were sacrificed at this and subsequent suitable time intervals.

The radioactivity of tissue cholesterol was determined by its isolation and counting as the digitonide. The specific activities are reported in counts per minute per gram of dry tissue.

**Fig. 3.** Specific activity of liver cholesterol in rats with a bile fistula or ligated bile ducts. The rats were given three intraperitoneal injections of C\(^{14}\)-labeled acetate. At least 36 hours elapsed after operation for bile fistula or ligation of bile ducts. Each point represents one animal. 0 hour is 4 hours after the last injection. Data plotted by method of least squares.

**Fig. 4.** Specific activity of tissue cholesterol in thirty-two control rats. The rats were given three intraperitoneal injections of C\(^{14}\)-labeled acetate. Each point represents two to ten animals. 0 hour is 4 hours after the last injection.
per minute per mg. of cholesterol digitonide. Adrenal cholesterol was isolated with the addition of 5 mg. of carrier to each pair of adrenals. This proved to be justified, since negligible variation in cholesterol content occurred in 67 per cent of the adrenals analyzed. Repeated pyridine splitting and reprecipitation of the digitonide from all our tissues (3), purification of the cholesterol from skin and liver by dibromide precipitation (4), or repeated recrystallization of liver cholesterol did not alter the count.

Blood samples were obtained by heart puncture. For multiple determinations of serum radioactive cholesterol in an individual rat, the animal was given an intraperitoneal injection of 70 µc. of the acetate, and 5 mg. of carrier cholesterol were added to each serum aliquot.

To aid in the interpretation the results were plotted as the logarithm of the specific activity against time. From the data the turnover time (mean time of existence) was calculated from the equation \( t_t = 1.441t_t \), where \( t_t \) is the turnover time and \( t_t \) the half life.

Figs. 1 to 7 and Tables I and II represent a different experimental group of animals with the following exceptions: The bile duct-ligated rats are the same in Figs. 3, 5, and 6, and the bile fistula rats are the same in Figs. 3 and 6.
Results

The graphic data showed three distinct patterns. Fig. 1 shows the typical linear plot of radioactivity of serum cholesterol found in the rat with a bile fistula. Fig. 2 shows that in six control rats there occurs a second peak in the plasma radioactivity curve between 20 and 50 hours. This pattern holds for both free and total cholesterol.

Fig. 3 shows again a linear plot for liver in rats with a bile fistula or ligated bile duct. In our first control groups, the other tissues of which are presented in Figs. 5 and 6, a sharp secondary rise in liver cholesterol specific activity was observed. To examine this effect more precisely a larger control group of thirty-two animals was tested (Fig. 4). There is no secondary peak, but a plateau in the semilog plot is evident.

The other tissues in Figs. 5 and 6 show a third pattern. The peak of radioactivity in these tissues occurs more than 16 hours after the last acetate injection.

The specific activity of intestinal cholesterol, as seen in Fig. 4, averages higher than and, in general, tends to parallel that of the liver. This is also true when a bile fistula is present. There was a great variation in the individual animals, the specific activity of the intestinal cholesterol being lower than that of the liver in some instances.

The turnover time of liver cholesterol, calculated from Fig. 3, in the animals with ligated bile ducts is 41.5 hours and 33.6 hours in those with bile
fistulas. The turnover time of adrenal cholesterol, calculated from Fig. 6, is rapid and of the same order as that of the liver. Similar calculations from the data of Fig. 5 for other tissues show a generally much slower turnover rate.

**DISCUSSION**

It is assumed that during the time the changes in specific activities of tissue cholesterol were being studied the precursors of cholesterol were of negligible specific activity. The validity of our interpretation of the findings reported above depends in good part upon this assumption. Gould et al. (5) have demonstrated that, 4 hours after an acetate injection, the free acetate has been almost completely metabolized to other substances. Squalene, a postulated intermediate between acetate and cholesterol, is present in minute quantity only (6). The amount of \( \Delta^7 \)-cholest-tenol in rat tissues, except for the skin, is only about 1 per cent of the cholesterol (7). If it is an obligatory precursor of cholesterol, it would have to have a turnover time about 100 times faster than that of cholesterol. The specific activity of the digitonide measured in our experiments included \( \Delta^7 \)-cholestenol. On purification by dibromide precipitation of the liver and skin cholesterol this compound was removed (7), but no detectable change in specific activity occurred. This agrees with the results of Schwenk and Werthessen (8) who found that, 4 hours after an injection of \( \mathrm{C}^{14} \)-labeled acetate in the intact rat, purification by dibromide precipitation did not alter the specific activity of tissue cholesterol. In view of the high percentage of \( \Delta^7 \)-cholest-tenol present in rat skin (9) these observations suggest that there may be a rapid interconversion between cholesterol and \( \Delta^7 \)-cholestenol.

It is observed in Figs. 2 and 4 that in normal animals in which bile is secreted into the intestine secondary peaks or interruptions in the decline of specific activity occurred in serum, intestine, and liver. The simplest explanation of this is that radioactive bile cholesterol is secreted into the intestine and reabsorbed at a later time. When bile was not secreted into the intestine, this does not occur (Figs. 1 and 3). The specific activity of bile cholesterol collected over various periods of time was higher than or about the same as that in the liver at the time of sacrifice (Table I). It was also found that all the secretion of radioactive cholesterol into the bile occurred within 6 hours after the acetate injection. This cholesterol is significantly radioactive when reabsorbed some hours later. The bile cholesterol secreted subsequently does not contribute any radioactivity.

The true specific activities of cholesterol in liver and serum were usually somewhat lower in the serum than in the liver (Table II). Serum cholesterol turnover, as calculated from data on bile fistula rats (Fig. 1), averages about the same as that in the liver.
The radioactivity of cholesterol in the spleen, lung, testis, kidney, and adrenal rose to a peak reached more than 16 hours after the last acetate injection. At this time significant amounts of radioactive non-steroid pre-

<table>
<thead>
<tr>
<th>Experiment</th>
<th>Period of bile collection</th>
<th>Bile cholesterol</th>
<th>Liver cholesterol*</th>
</tr>
</thead>
<tbody>
<tr>
<td>A</td>
<td>From 1st acetate injection to sacrifice at 0 hr.†</td>
<td>90</td>
<td>75</td>
</tr>
<tr>
<td></td>
<td>“ “ “ “ “ “ “ “ 36 hrs.</td>
<td>69.3</td>
<td>60</td>
</tr>
<tr>
<td>B</td>
<td>1st to 3rd injection</td>
<td>19.2</td>
<td></td>
</tr>
<tr>
<td></td>
<td>3rd injection to 20 hrs.</td>
<td>13.6</td>
<td>9.2†</td>
</tr>
</tbody>
</table>

* Measured at times of sacrifice indicated.
† 0 hour refers to 4 hours after last acetate injection.
† Time 10 hours.

The figures are in counts per minute per mg. of digitonide.

<table>
<thead>
<tr>
<th>Group No.</th>
<th>Time hrs.</th>
<th>Liver c.p.m. per mg. digitonide</th>
<th>Serum* c.p.m. per mg. digitonide</th>
<th>Skin†</th>
</tr>
</thead>
<tbody>
<tr>
<td>I</td>
<td>0</td>
<td>139</td>
<td>56.7</td>
<td>12.4</td>
</tr>
<tr>
<td></td>
<td>12</td>
<td>67.7</td>
<td>20.3</td>
<td>10.8</td>
</tr>
<tr>
<td></td>
<td>24</td>
<td>24.8</td>
<td>25.6</td>
<td>10.6</td>
</tr>
<tr>
<td></td>
<td>36</td>
<td>45.0</td>
<td>28.0</td>
<td>17.3</td>
</tr>
<tr>
<td></td>
<td>62</td>
<td>20.2</td>
<td>12.0</td>
<td>12.4</td>
</tr>
<tr>
<td>II</td>
<td>16.5</td>
<td>78.6</td>
<td>50</td>
<td></td>
</tr>
<tr>
<td></td>
<td>38</td>
<td>43.5</td>
<td>23</td>
<td></td>
</tr>
<tr>
<td></td>
<td>38</td>
<td>34.7</td>
<td>22</td>
<td></td>
</tr>
<tr>
<td></td>
<td>65</td>
<td>30.1</td>
<td>24</td>
<td></td>
</tr>
<tr>
<td>III</td>
<td>1</td>
<td>76.2</td>
<td>50.8</td>
<td></td>
</tr>
<tr>
<td></td>
<td>25</td>
<td>25.4</td>
<td>17.0</td>
<td></td>
</tr>
<tr>
<td></td>
<td>33.5</td>
<td>15.2</td>
<td>12.0</td>
<td></td>
</tr>
<tr>
<td></td>
<td>51</td>
<td>14.9</td>
<td>17.5</td>
<td></td>
</tr>
</tbody>
</table>

* Cholesterol isolated from 3 to 5 cc. of serum with carrier and the true specific activity calculated.
† In skin the digitonide counted contains about 30 per cent of Δ^7-cholestenol (9).
the liver cholesterol is higher than that of the above tissues, as is required for this concept to be valid.

The very rapid turnover rate found in the liver was not anticipated, since prior reported values for the rate of total synthesis of cholesterol for rat liver were 8 days (2) and 4 days (1). This study emphasizes the quantitative importance of the endogenous cholesterol metabolism.

The greater variability in specific activity in the intestine is not surprising, since the turnover here may represent mostly the transfer of the cholesterol to the intestinal lymphatics (10). Srere et al. (11) reported that the kidney and testis in the eviscerated rat are able to convert acetate to cholesterol. The results under the more physiologic conditions presented above, however, suggest that formation of cholesterol by the tissues studied, other than liver and intestine, is not a major metabolic process.

According to Brady (12), testis slices converted labeled acetate more efficiently to testosterone than to cholesterol. By implication, the idea was presented that cholesterol is not a precursor of testosterone. If, however, the testis normally does not primarily manufacture its own cholesterol, the concept of cholesterol as a primary precursor of testosterone is not ruled out.

Hechter (13) found that perfused beef adrenals exhibited a relatively poor synthesis of cholesterol or corticosteroids from acetate, but were extremely efficient in the synthesis of corticosteroids from cholesterol. This is entirely consistent with the finding that the adrenal cholesterol is not primarily synthesized in the gland but is derived from plasma cholesterol.

If tissues exchange cholesterol with the surrounding plasma but do not catabolize it, they would have a relatively slower turnover rate. Spleen, lung, and kidney may very well fit such a criterion. If there is, in addition, active tissue catabolism of cholesterol, this apparent turnover time will be shortened. Since the uptake is from the plasma, no matter what the true turnover time is, the apparent time can never exceed that of the plasma cholesterol or the liver with which plasma cholesterol equilibrates. This limiting case of active cholesterol catabolism matches the findings in the adrenal, the cholesterol of which appears to have about the same turnover time as that of the liver. This provides further support to the concept that adrenal cholesterol is the precursor of steroid hormones.

Since adrenal cholesterol turnover was found to be rapid, but was not more precisely determinable by this experimental approach, it was decided to measure the turnover rate in the adrenalectomized animal. If adrenal catabolism of cholesterol in hormone synthesis accounts for a major part of the turnover in vivo, the removal of the gland should slow this process greatly. Since it was a screening experiment, the testis which
also makes a steroid hormone was removed as well. The result was a very marked slowing of the cholesterol turnover in the liver. This was attributable to either the adrenals or testis. The experiment was repeated in castrated male rats. The results are shown in Fig. 7. Very marked slowing of the turnover in liver and intestine is evident. Since the low specific activity of cholesterol of the testis in the control animals precludes this organ as a major site of catabolism, the effect can be attributed to the absence of male sex hormone. We have, therefore, not only demonstrated that cholesterol metabolism is very rapid in the intact rat, but have preliminary evidence that the turnover of cholesterol is profoundly affected,

Fig. 7. Specific activity of intestine (△) and liver (○) cholesterol in castrated male rats. The rats were given three intraperitoneal injections of C14-labeled acetate. Injection at least 72 hours after castration. Each point represents two animals. 0 hour is 4 hours after the last injection. Data plotted by method of least squares.

either directly by the male hormone or as a consequence of metabolic changes that are induced by the hormone.

The adrenal cholesterol of these castrated animals plotted unequivocally the same turnover time as the liver. No gross change was observed in the cholesterol content of these livers during the experimental period after castration. Liver slices of castrated male rats converted C14-acetate to cholesterol at least as efficiently as the controls. The pattern in other tissues remained unchanged from the controls (Fig. 5).

Skin sterol digitonide had a very low specific activity (Table II). It is known that synthesis of cholesterol can occur in the skin (11). To what extent labeling is due to blood transport and to what extent to synthesis cannot be calculated from our figures. Because of the large amount of cholesterol in the skin and the large mass of the organ, even low specific activities may signify a great amount of cholesterol metabolism.
SUMMARY

1. The dynamic endogenous metabolism of cholesterol in vivo has been studied by measuring the disappearance of previously labeled tissue cholesterol in rats maintained on a diet free of cholesterol.

2. Important sites of cholesterol synthesis are liver, intestine, and perhaps skin.

3. The large scale catabolism of cholesterol must be largely confined to some of the tissues with a maximal turnover rate. These are liver, intestine, and adrenal. Because of its extremely high cholesterol content the skin may also play a significant rôle.

4. The turnover time of liver cholesterol was calculated to be about 40 hours.

5. It is suggested that the cholesterol of the testis, spleen, kidney, and lung is primarily derived from the plasma.

6. This also appears true for adrenal cholesterol, and its turnover time is equal to or faster than that of the liver.

7. Castration results in an apparent marked slowing of the turnover time in liver and intestine.

8. The most significant fact obtained in this work is that the endogenous cholesterol metabolism is far greater in scope than heretofore reported.

BIBLIOGRAPHY

ENDOGENOUS CHOLESTEROL METABOLISM IN THE RAT STUDIED WITH C$^{14}$-LABELED ACETATE
Erwin J. Landon and David M. Greenberg


Access the most updated version of this article at http://www.jbc.org/content/209/2/493.citation

Alerts:
- When this article is cited
- When a correction for this article is posted

Click here to choose from all of JBC’s e-mail alerts

This article cites 0 references, 0 of which can be accessed free at http://www.jbc.org/content/209/2/493.citation.full.html#ref-list-1