The Origin of Cholesterol in Liver, Small Intestine, Adrenal Gland, and Testis of the Rat: Dietary versus Endogenous Contributions

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All mammalian tissues so far examined, with the exception of adult brain, are capable of incorporating acetate carbon into cholesterol (1-3). It has been estimated that man synthesizes in the neighborhood of 1.5 gm. of cholesterol per day, a quantity apparently exceeding that ingested when an ordinary mixed diet is fed (6). According to Chevalier (7), the laboratory rat synthesizes about 43 mg. of cholesterol daily, which is also in excess of that normally ingested by this animal. The significance of synthetic cholesterol was emphasized in a recent report (8) in which it was estimated that as much as 75 per cent of plasma cholesterol is of endogenous origin in rats fed an ordinary diet, and that the endogenous contribution to plasma cholesterol could not be completely suppressed even when the rats received, for 6 weeks, a diet containing 2.0 per cent cholesterol. It would thus appear that endogenous sources must contribute significantly to the cholesterol found in most tissues of animals fed laboratory diets.

The present report deals with the relative contributions of dietary and endogenous cholesterol to the composition of cholesterol in four tissues: liver, small intestine, adrenal gland, and testis of rats fed diets containing 0.05 and 2.0 per cent cholesterol. The small intestine and liver are of interest because they are concerned in large measure with the handling of absorbed cholesterol; the two glandular tissues, because they participate in the conversion of cholesterol to steroid hormones.

EXPERIMENTAL

The rats used in this study were fed, from the time of weaning, a diet practically devoid of cholesterol; not more than 0.006 per cent. The preparation of the two diets containing cholesterol-4-C14 and the treatment of the rats have been described elsewhere (8).

The rats had access to food and water until they were killed. All the rats dealt with here ate well throughout the course of the experiment. The food intake of each rat, measured daily, averaged about 15 gm. At necropsy, food was found in the stomachs of all rats.

At the end of the experiment the rats were bled by heart puncture, and the various tissues were excised. The small intestine was ligated just below the level of the entrance of the bile duct and just above the cecum. The intestine was cut above the upper ligature, and stripped of extraneous tissue. It was next cut just above the lower ligature, and its lumen was washed with 15 ml. of a 0.9 per cent solution of NaCl. The loop of intestine was opened and blotted with filter paper. The mucosa was removed with the aid of a blunt scalpel. The remainder of the intestinal wall is referred to as serosa.

Extraction of Lipides—Each tissue was frozen immediately after its removal from the animal, and stored until analyzed. It was minced and homogenized, and treated first with 25 volumes of a 3:1 alcohol-ether mixture at 60° for 2 hours, and next with 15 volumes of the same solvent at the same temperature for 1 hour. The residue was washed four times, each time with 15-ml. portions of ethyl ether. The volume of the combined extracts was reduced until approximately 50 per cent water was present. The aqueous, lipid-containing concentrate was extracted five times, each time with 15 to 20 ml. of petroleum ether.

Separation and Determination of Free and of Esterified Cholesterol—A modified Sperry-Webb procedure (9) was used for the separation of free and esterified cholesterol in all tissues except testes. Both the free and the esterified cholesterol of testes were separated by the more precise silicic acid-column method of Fillerup and Mead (10), as modified by La Roche (11). The values obtained with this method were identical with those obtained by the Sperry-Webb method.

The colorimetric determination of each cholesterol fraction, the free and the esterified, as well as the determination of their C14 contents, has been described elsewhere (8).

Typical values for the specific activities of free and ester cholesterol in liver, adrenal, and testes are shown in Table I. Because of the good agreement in the specific activities of the two cholesterol fractions of each tissue at each time interval, all calculations dealt with below are based on free cholesterol.

RESULTS

The plasma results for these rats have already been reported (8).

In the rats fed the 2.0 per cent cholesterol diet, there were no significant increases in the cholesterol contents of plasma, small intestine, adrenal, and testes. In the liver, however, the cholesterol content rose from 2.5 to 25 mg. per gm. The increased amounts of cholesterol in the liver were predominantly in the esterified form.

Maximal values for specific activities of all tissues, except the testes, were found at the end of 2 weeks in the rats fed the 0.05 per cent cholesterol diet (Table II). In rats that received the 2.0 per cent cholesterol diet, maximal incorporation of dietary
Changes with time of feeding in specific activities of 1000 c.p.m. per mg. diet was 5500 c.p.m. per mg.; that in the high cholesterol diet, both diets were observed at the end of 3 weeks.

As follows:

The specific activity of a tissue's cholesterol have been attained. Where SA expresses the specific activity of the cholesterol of a given tissue. This calculation for the endogenous contribution is valid only at equilibrium, i.e., when maximal values for the specific activity of a tissue's cholesterol have been attained.

**DISCUSSION**

1. Origin of Cholesterol in Terms of Dietary and Endogenous Contributions—In an earlier study (8), we dealt with the relative contribution of diet and synthesis to the cholesterol composition of serum in rats fed diets containing 0.05 and 2 per cent cholesterol. These diets provided each rat, daily, with about 7.5 and 300 mg., respectively, of cholesterol. In the rats fed the low cholesterol diet, the bulk of plasma cholesterol was of endogenous origin. When the high cholesterol diet was fed for 6 weeks, the endogenous contribution to plasma cholesterol did not exceed 18 per cent. Table I shows that the composition of the cholesterol in small intestine, liver, and adrenal gland also responded to the cholesterol content of the diet, the endogenous contribution being high when the diet contained little cholesterol, and low when the diet was rich in cholesterol. As in the case of plasma, it was not possible to suppress completely an endogenous contribution to the cholesterol of these three tissues even when large amounts of cholesterol were fed for long periods.

The extent of depression in the endogenous contribution induced by cholesterol feeding was not so pronounced in the small intestine as in the liver. Thus, by the time the 2.0 per cent cholesterol diet had been fed for 6 weeks, a maximum of 12 per cent (average) of the liver's cholesterol was of endogenous origin, whereas in the mucosa, a maximum of 21 per cent (average) was contributed by endogenous sources; the corresponding figure for serum was 20 per cent (Table II).

Our present findings in the intact animal with respect to dietary and endogenous contributions to liver cholesterol were not unexpected in view of earlier findings with surviving liver slices (12-15). But our findings with the small intestine were surprising because Gould et al. (6, 13) in experiments with the intestinal mucosa of the dog and Cox et al. (15) in experiments with monkeys failed to detect a depression in cholesterol synthesis from acetate after the feeding of diets rich in cholesterol.

Among the tissues studied, the testis is unique with respect to the origin of its cholesterol. Regardless of whether the diet contained 0.05 or 2 per cent cholesterol, testicular cholesterol was found to be largely of endogenous origin; about 90 per cent in the case of the low cholesterol diet and about 60 per cent in the experiment with the 2.0 per cent cholesterol diet.

2. Relation of Tissue Cholesterol to Plasma Cholesterol—So far, we have arbitrarily considered the origin of tissue cholesterol in terms of dietary and endogenous components. The endogenous component of cholesterol in a given tissue may arise in situ from smaller components or it may have been transported to that tissue by plasma. In the discussion that follows we shall make two assumptions: (a) that the liver not only processes dietary cholesterol but, in addition, is the principal source of the synthetic component of plasma cholesterol; and (b) that tissues

**TABLE I**

Typical values obtained for specific activities of free and esterified cholesterol

<table>
<thead>
<tr>
<th>Cholesterol content of diet*</th>
<th>Weeks fed</th>
<th>Tissue</th>
<th>Specific activity of cholesterol</th>
<th>Free c.p.m./mg.</th>
<th>Esterified c.p.m./mg.</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>%</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>0.05</td>
<td>3</td>
<td>Liver</td>
<td></td>
<td>1580</td>
<td>1550</td>
</tr>
<tr>
<td>0.05</td>
<td>5</td>
<td>Liver</td>
<td></td>
<td>1500</td>
<td>1430</td>
</tr>
<tr>
<td>0.05</td>
<td>6</td>
<td>Adrenal</td>
<td></td>
<td>1720</td>
<td>1820</td>
</tr>
<tr>
<td>0.05</td>
<td>3</td>
<td>Testis</td>
<td></td>
<td>560</td>
<td>580</td>
</tr>
<tr>
<td>0.05</td>
<td>4</td>
<td>Testis</td>
<td></td>
<td>760</td>
<td>750</td>
</tr>
<tr>
<td>2.0</td>
<td>2</td>
<td>Liver</td>
<td></td>
<td>730</td>
<td>730</td>
</tr>
<tr>
<td>2.0</td>
<td>4</td>
<td>Liver</td>
<td></td>
<td>870</td>
<td>840</td>
</tr>
<tr>
<td>2.0</td>
<td>6</td>
<td>Liver</td>
<td></td>
<td>890</td>
<td>860</td>
</tr>
</tbody>
</table>

* The specific activity of the cholesterol in the low cholesterol diet was 5500 c.p.m. per mg.; that in the high cholesterol diet, 1000 c.p.m. per mg.

**TABLE II**

Changes with time of feeding in specific activities of tissue cholesterol in rats fed diets containing 0.05 and 2.0 per cent cholesterol

<table>
<thead>
<tr>
<th>Cholesterol content of diet*</th>
<th>Weeks fed</th>
<th>Liver</th>
<th>Small intestine</th>
<th>Adrenal</th>
<th>Testis</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td>Muco sa</td>
<td>Scro sa</td>
<td></td>
</tr>
<tr>
<td>%</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>0.05</td>
<td>2</td>
<td>1480</td>
<td>± 675</td>
<td>1100</td>
<td>± 110</td>
</tr>
<tr>
<td>0.05</td>
<td>3</td>
<td>1480</td>
<td>± 110</td>
<td>1100</td>
<td>± 220</td>
</tr>
<tr>
<td>0.05</td>
<td>4</td>
<td>1650</td>
<td>± 165</td>
<td></td>
<td></td>
</tr>
<tr>
<td>0.05</td>
<td>5</td>
<td>1540</td>
<td>± 165</td>
<td></td>
<td></td>
</tr>
<tr>
<td>0.05</td>
<td>6</td>
<td>1500</td>
<td>± 220</td>
<td>1210</td>
<td>± 220</td>
</tr>
<tr>
<td>2.0</td>
<td>2</td>
<td>730</td>
<td>± 20</td>
<td>650</td>
<td>± 40</td>
</tr>
<tr>
<td>2.0</td>
<td>3</td>
<td>750</td>
<td>± 40</td>
<td></td>
<td></td>
</tr>
<tr>
<td>2.0</td>
<td>4</td>
<td>840</td>
<td>± 60</td>
<td></td>
<td></td>
</tr>
<tr>
<td>2.0</td>
<td>5</td>
<td>850</td>
<td>± 30</td>
<td></td>
<td></td>
</tr>
<tr>
<td>2.0</td>
<td>6</td>
<td>870</td>
<td>± 20</td>
<td>790</td>
<td>± 80</td>
</tr>
</tbody>
</table>

* See footnote Table I.
† The figures denote average and standard deviation.

Each value was calculated from averages of separately determined measurements in 3 or 4 rats fed the diets for 6 weeks. All values are given as per cent.

**TABLE III**

Origin of tissue cholesterol with respect to dietary and endogenous sources

Each value was calculated from averages of separately determined measurements in 3 or 4 rats fed the diets for 6 weeks. All values are given as per cent.

<table>
<thead>
<tr>
<th>Cholesterol content of diet</th>
<th>Liver</th>
<th>Small intestine</th>
<th>Adrenal</th>
<th>Testis</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Muco sa</td>
<td>Scro sa</td>
<td></td>
</tr>
<tr>
<td>Diet</td>
<td></td>
<td>Diet</td>
<td>Endo- generous</td>
<td>Diet Endo- generous</td>
</tr>
<tr>
<td>0.05</td>
<td>29</td>
<td>71</td>
<td>19</td>
<td>81</td>
</tr>
<tr>
<td>2.0</td>
<td>88</td>
<td>12</td>
<td>79</td>
<td>21</td>
</tr>
</tbody>
</table>
glands' cholesterol in the intact animal. Landon and Greenberg had already been attained at the end of 3 or 4 weeks, the close relation between the specific activities of plasma and adrenal gland cholesterol shown there for all groups of rats indicates that are capable of incorporating acetate carbon into cholesterol, but such observations provide no information on the origin of the not the major source of adrenal gland cholesterol in the intact (23) expressed the view that cholesterol synthesized in rat, and this view is supported by the data in Table IV. The liver and plasma may be looked upon as a single entity in so far as the metabolism of cholesterol is concerned (22).

Surviving slices of both the adrenal gland (2) and testis (1) are capable of incorporating acetate carbon into cholesterol, but such observations provide no information on the origin of the glands' cholesterol in the intact animal. Landon and Greenberg (23) expressed the view that cholesterol synthesized in situ is not the major source of adrenal gland cholesterol in the intact rat, and this view is supported by the data in Table IV. The close relation between the specific activities of plasma and adrenal gland cholesterol shown there for all groups of rats indicates that most of adrenal gland cholesterol is derived from plasma.

Even though maximal specific activities of tecticular cholesterol had already been attained at the end of 3 or 4 weeks, the feeding of the two cholesterol diets was continued for 6 weeks, and the calculations (Table IV) for the contributions in situ were based on the results obtained at 6 weeks. The data in Table IV show that, regardless of the cholesterol content of the diet, most of the cholesterol present in the testis is formed in situ. These findings may be explained either by the presence of a large, inactive pool of cholesterol in the testis or by a constant synthesis of cholesterol in that tissue, which dilutes the incoming plasma cholesterol.

**SUMMARY**

1. For periods up to 6 weeks, rats were fed diets containing either 0.05 or 2.0 per cent cholesterol labeled with cholesterol-4-COOC in these animals the relative contribution of dietary and endogenous cholesterol to the composition of the cholesterol in liver, mucosa and serosa of the small intestine, adrenal gland, and testis was determined.

2. In the rats fed the low cholesterol diet, endogenous sources contributed about 70 to 80 per cent of the cholesterol in liver, small intestine, and adrenal gland. In the rats fed the high cholesterol diet, the corresponding values ranged from 10 to 30 per cent.

3. An endogenous contribution of cholesterol in liver, small intestine, and adrenal gland was not completely suppressed by the prolonged feeding of very large amounts of cholesterol.

4. Regardless of the cholesterol content of the diet, most of the cholesterol in testis is of endogenous origin.

5. The relation between plasma and tissue cholesterol is discussed.

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