SUPPRESSION OF HEPATIC CHOLESTEROL SYNTHESIS IN THE RAT BY CHOLESTEROL FEEDING*

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Gould and Taylor (1, 2), working with slices and with intact animals, have shown that addition of cholesterol to the diet of the dog or the rabbit causes a depression of synthesis of cholesterol in the liver. Using the slice technique, we have investigated this phenomenon in the rat, with and without destruction of the thyroid gland by means of radioactive iodine.

EXPERIMENTAL

Two dozen Sprague-Dawley male rats, 5 weeks old, were used. Half were given intraperitoneal injections of 500 µc. of I¹³¹ each. After 11 weeks on a diet of Purina laboratory chow, blood was withdrawn from the tail of each rat for determination of protein-bound iodine on pooled samples by the method of Barker (3). 2 weeks later 1 per cent of cholesterol was added to the diet of six of the normal rats and six of the rats which had received radioactive iodine. The cholesterol was added by dissolving it in ether and allowing the solution to dry after being poured over the pellets of food. 1 day to 5 weeks later, rats were killed by fours, one from each group. Prior to decapitation, blood was drawn from each rat for determination of total serum cholesterol by the revised Schoenheimer-Sperry method (4). Liver slices were cut, and duplicate incubations for each rat were carried out under the conditions described in Table I, in the presence of C¹⁴-carboxyl-labeled sodium acetate. After 3 hours, the slices from each vessel were sealed in a test-tube with 2.5 cc. of 15 per cent KOH in 95 per cent ethanol. After hydrolysis for 16 hours in boiling water, 2.5 cc. of water were added to each tube, and cholesterol was extracted for 3 hours with petroleum ether (b.p. 30–60°) in a liquid-liquid continuous extractor. The extract from each tube was evaporated to dryness, and the residue was dissolved in 3 cc. of 1:1 acetone-absolute ethanol. The solutions were filtered, and the cholesterol was precipitated by addition of an excess of a 0.5 per cent solution of digitonin in 50 per cent ethanol.

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After at least 3 hours, the precipitates were filtered on Whatman No. 50 filter paper and counted, as previously described for BaCO₃ (5). The results were corrected for self-absorption to a thickness of 4 mg. spread over the 3.7 sq. cm. area (approximately the amount of cholesterol digitonide usually obtained from 0.5 gm. of slices from a normal rat liver). After being counted, the samples were dried over P₂O₅, scraped from the filter paper, and weighed.

As a test of the efficacy of this method of recovery of cholesterol from tissue, quadruplicate analyses were carried out on a hydrolysate of liver from a rat which had been given a dose of C¹⁴-acetate intraperitoneally. The 3 hour extract was treated as usual, and the extraction was continued for an additional 4 hours with fresh petroleum ether. Carrier cholesterol (1 mg.) was added to the second extract, and samples for counting were prepared for comparison. The radioactivity of the cholesterol digitonide from the initial 3 hour extraction was as follows: 562, 561, 544, 561 net c.p.m.; from the additional 4 hour extraction, 5, 11, 6, 6 net c.p.m. This experiment indicates that the 3 hour extraction used for removal of cholesterol from the hydrolysates of the slices probably removes about 98 per cent of the cholesterol and is reproducible within a range of about 4 per cent.

At the time the slices were cut, an additional 1 gm. sample of liver tissue was homogenized and brought to a volume of 5 cc. with water. Aliquots were taken for determination of total nitrogen by the method of Ma and Zuazaga (6) and of total cholesterol by the Schoenheimer-Sperry method (4). Although Table I and Figs. 1 to 3 show concentrations of liver cholesterol in mg. per 100 gm. for comparison with the values for serum, the figures were actually calculated per 3.2 gm. of nitrogen, which is the average value that we obtained for 100 gm. of wet tissue. This method of calculation eliminates variations which would otherwise be introduced in the weighing of wet tissue.

In the calculation of the percentage of acetate carbon incorporated into cholesterol, a correction was likewise applied, based on the weight of cholesterol digitonide actually counted and the cholesterol to nitrogen ratio of the liver from which it was derived. This correction eliminates errors from the weighing of the slices prior to incubation and from the impossibility of quantitatively removing the tissue from the vessel after incubation.

Results

In Table I are summarized the serum and liver cholesterol levels of each of the four groups, the percentage of the added radioactive carbon recovered as cholesterol, the relative isotope concentration of acetate added and cholesterol recovered, and the protein-bound iodine of the serum.
**Table I**

Dependence of Hepatic Cholesterol Synthesis on Diet, Thyroid Activity, and Serum and Liver Cholesterol Levels

<table>
<thead>
<tr>
<th>Thyroid</th>
<th>Diet</th>
<th>Protein-bound Iodine</th>
<th>Average Body Weight</th>
<th>Cholesterol Conversion</th>
<th>Relative Isotope Concentration*</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>% per cent gm.</td>
<td>mg. per 100 cc.</td>
<td>mg. per 100 gm.</td>
<td>per cent</td>
</tr>
<tr>
<td>Normal</td>
<td>Normal</td>
<td>3.6 359</td>
<td>62 ± 7</td>
<td>248 ± 5</td>
<td>0.64 ± 0.12</td>
</tr>
<tr>
<td></td>
<td>High</td>
<td>2.8 340</td>
<td>79 ± 4</td>
<td>649 ± 87</td>
<td>0.042 ± 0.013</td>
</tr>
<tr>
<td>Damaged</td>
<td>Normal</td>
<td>0.5 243</td>
<td>88 ± 5</td>
<td>215 ± 8</td>
<td>1.04 ± 0.15</td>
</tr>
<tr>
<td></td>
<td>High</td>
<td>0.8 245</td>
<td>118 ± 8</td>
<td>704 ± 100</td>
<td>0.036 ± 0.009</td>
</tr>
</tbody>
</table>

Each vessel contained 3.7 cc. of Krebs-Ringer phosphate solution, 2.5 mg. of C¹⁴-carboxyl-labeled sodium acetate (272,000 c.p.m. if counted at a thickness of 4 mg. spread over an area of 3.7 sq. cm.), and 0.5 gm. of liver slices, approximately 0.5 mm. in thickness. Temperature 37°; pH 7.4; time of incubation 3 hours; shaking rate 120 oscillations per minute; atmosphere 100 per cent oxygen.

* (Counts per minute per mg. of cholesterol recovered X 100)/(counts per minute per mg. of sodium acetate added).

† Standard error of the mean. Each figure represents an average from six rats. Each incubation was carried out in duplicate.

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**Fig. 1**

**Fig. 2**

**Fig. 1 and 2.** Relation between cholesterol content of serum and liver in rats with normal and with damaged thyroid glands, respectively.
It appears that, under our experimental conditions, feeding cholesterol alone to a normal rat produced only a minimal rise in the concentration of blood serum cholesterol, although the concentration of liver cholesterol rose considerably. The serum cholesterol of the rats with severely damaged thyroid glands rose slightly on a normal diet and to a greater extent with cholesterol feeding. The percentage of the added acetate converted to cholesterol was depressed to about 7 per cent of the control value in the cholesterol-fed animals and to an even greater extent in those fed cholesterol after thyroid damage. When the results are expressed in terms of relative isotope concentrations, the effect of cholesterol feeding appears still greater.

Figs. 1 and 2 show the relation between the cholesterol levels of blood serum and liver. The concentration of cholesterol in the serum remained nearly constant over a rather wide range of concentrations in the liver, both in the normal animals and in those with damaged thyroids.

In Fig. 3 the logarithm of the percentage conversion of acetate to cholesterol for each incubation is plotted against the cholesterol content of the liver. Despite a considerable scatter of the points, an inverse relationship is apparent. Since the rats with highest liver cholesterol levels had been on the diet for the longest periods, a time factor as well as the concentration of cholesterol may have contributed to the production of these large effects.

**DISCUSSION**

Maloof, Dobyns, and Vickery (7) have demonstrated the effectiveness of the method used in these experiments for production of damage to the
thyroid glands of rats. They found that 48 days after administration of 300 μe. of I¹³¹ the thyroid was virtually obliterated and replaced by scar tissue. The lowered rate of weight gain and the reduced concentration of protein-bound iodine in the blood, as shown in Table I, provide further evidence of hypothyroidism in our animals.

The relative constancy of the concentration of serum cholesterol, despite large differences in the cholesterol content of the liver, suggests the existence of a homeostatic mechanism. The liver appears to act as a buffer for the serum cholesterol. If large amounts of cholesterol are absorbed from the intestinal tract, most of it is quickly removed from the blood by the liver. As the cholesterol content of the liver rises, synthesis is depressed, and the rise in total body cholesterol is thereby also minimized.

SUMMARY

1. Rats fed a diet to which had been added 1 per cent of cholesterol showed a large rise in the concentration of liver cholesterol, but only a minimal rise in the concentration of serum cholesterol.

2. The serum cholesterol concentration of rats with damaged thyroid glands was slightly higher than that of the controls, even on a normal diet. After cholesterol feeding, the rise was greater than in the animals with normal thyroid glands. The liver cholesterol rose at least as much after cholesterol feeding as in the rats with normal thyroids.

3. The action of the liver could be viewed as that of a buffer for the serum cholesterol.

4. The rate of hepatic cholesterol synthesis appeared to depend on the liver's cholesterol content and to be very sensitive to it.

5. There was no evidence that inhibition of cholesterol synthesis by dietary cholesterol is mediated through the thyroid gland; synthesis was inhibited to at least as great an extent in animals which had received radioactive iodine as in normal rats.

BIBLIOGRAPHY

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