Observations Concerning the Metabolism of Cholesterol in the Hypo- and Hyperthyroid Rat

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(With the technical assistance of Eichi Shibata, B.S.)

The administration of thiouracil induced a rise and administration of thyroid substance a decrease of the plasma cholesterol concentration in rats. The concentration and daily output of bile cholesterol was markedly reduced in hypothyroid rats and markedly increased in hyperthyroid rats. Evidence is presented which suggests that the rate of synthesis of cholesterol is significantly increased in the hyperthyroid rat and decreased in the hypothyroid rat.

AN INVERSE relationship between the plasma cholesterol concentration and the level of thyroid activity has long been recognized. Although this relationship is not quantitative, in general, hyperthyroidism is associated with a decrease, and hypothyroidism with an increase, in the plasma cholesterol concentration. The underlying mechanism has not as yet been clarified.

Preliminary studies from this laboratory have shown a disturbance of biliary excretion of cholesterol in rats with deranged thyroid activity. The striking increase in concentration and daily output of cholesterol that was found in the bile of rats with induced hyperthyroidism suggested that this state was associated with an increased rate of hepatic synthesis of cholesterol. Conversely, the markedly decreased biliary cholesterol output in hypothyroid rats suggested a decreased rate of hepatic synthesis of cholesterol. The present studies were undertaken to test the validity of this hypothesis.

Methods

Two separate series of rats were fed either thiouracil or powdered thyroid substance in their diet.

The first series of 8 week old male rats (Long-Evans) consisted of (a) animals fed 0.25 per cent thiouracil in their food for 33 days, (b) rats fed 0.12 per cent powdered thyroid substance in their diet for 18 days, and (c) control rats maintained on ordinary stock diet. The second series of 13 week old rats was followed for 42 days. It consisted of (a) rats maintained on 0.3 per cent thiouracil, (b) rats maintained on 0.3 per cent thyroid substance, and (c) control rats. The results obtained on the plasma and bile contents of cholesterol were essentially similar in the two series and accordingly have been grouped together.

Cannulation of the bile duct, 24-hour collection and analysis of bile for cholesterol were accomplished according to previously described methods. Food was withheld from all rats during such bile collections. Data concerning rat bile were accepted only if autopsy examination indicated that there had been neither rupture nor obstruction of the bile duct.

Data on the rate of accumulation of cholesterol in plasma in the experimental animals were obtained by analyses of the plasma for cholesterol before and 24 hours after biliary obstruction.

Study of the rate of disappearance of cholesterol from plasma was accomplished in both the hypo- and hyperthyroid rat by the measurement of the plasma cholesterol in each rat immediately, 1, 3, 6, 12, and 24 hours after the intravenous injection of 2 cc. of hypercholesteremic rat plasma (950 mg. per 100 cc.). The latter was prepared as described previously.

Results

A. Production of Hypo- and Hyperthyroidism in Rats

The administration of thiouracil and powdered thyroid substance was found, as already described, to produce hypo- and hyperthyroidism, respectively, in our rats. The weight gain was greatest in the control and least in

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the hyperthyroid rats (table 1). No untoward manifestations other than retarded growth and decreased activity were observed in the rats receiving thiouracil. The rats given thyroid content of 41 rats made hyperthyroid fell from 50.4 to 40 mg. per 100 cc. No significant change occurred in the plasma of 29 control rats. The decreased total cholesterol concentration in

**TABLE 1.—The Changes in Average Plasma Cholesterol Concentration after Production of Hypo- and Hyperthyroidism**

<table>
<thead>
<tr>
<th>Type of Rat</th>
<th>No. of Rats</th>
<th>Weight (Gm.)</th>
<th>Plasma Cholesterol Concentration (mg./100 cc.)</th>
<th>Before Medication</th>
<th>After Medication</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Before</td>
<td>After</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Control</td>
<td>29</td>
<td>215</td>
<td>262</td>
<td>54</td>
<td>5.9</td>
</tr>
<tr>
<td>Hypothyroid</td>
<td>45</td>
<td>201</td>
<td>227</td>
<td>47.3</td>
<td>4.5</td>
</tr>
<tr>
<td>Hyperthyroid</td>
<td>41</td>
<td>196</td>
<td>212</td>
<td>54</td>
<td>7.2</td>
</tr>
</tbody>
</table>

* S.E.M. = standard error of the mean.

**TABLE 2.—The Average Concentration and Daily Output of Bile Cholesterol in Hypo- and Hyperthyroid Rats**

<table>
<thead>
<tr>
<th>Type of Rat</th>
<th>No. of Rats</th>
<th>Bile Volume (cc./24 hrs.)</th>
<th>Cholesterol Concentration (mg./100 cc.)</th>
<th>Content (mg./24 hrs.)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>16</td>
<td>13.2</td>
<td>20.7</td>
<td>2.7</td>
</tr>
<tr>
<td>Hypothyroid</td>
<td>17</td>
<td>12</td>
<td>9.8</td>
<td>1.2</td>
</tr>
<tr>
<td>Hyperthyroid</td>
<td>17</td>
<td>13.3</td>
<td>42.1</td>
<td>5.6</td>
</tr>
</tbody>
</table>

* S.E.M. = standard error of the mean.

substance exhibited hyperactivity, marked tremor, tachycardia, and poor weight gain, despite a significant polyphagia.

Histologic examination of the thyroid glands from representative examples of each group revealed changes closely resembling those described by Karp and Stetten.

**B. Changes in the Plasma Cholesterol Concentration of Hypo- and Hyperthyroid Rats**

Both the free and esterified cholesterol content of plasma rose (table 1) in those rats made hypothyroid. The average plasma total cholesterol content in 45 rats thus rose from 47.3 mg. per 100 cc. before thiouracil administration to 67.2 mg. per 100 cc. Conversely, (table 1), the average plasma total cholesterol the hyperthyroid rats was due largely to a decrease in the ester fraction.

**C. Changes in Excretion of Biliary Cholesterol in Hypo- and Hyperthyroid Rats**

In strong contrast to the change observed in plasma, the cholesterol content of the bile of the hypothyroid rat was significantly reduced. Thus, the average daily biliary excretion of cholesterol in 17 hypothyroid rats was 1.1 mg., compared with the average excretion of 2.6 mg. found in 16 control animals (table 2). The diminution observed in the hypothyroid rats was due to a reduction in biliary concentration of cholesterol (table 2), the volume of bile being approximately unchanged.

On the other hand, 17 hyperthyroid rats
were found to excrete an average of 5.6 mg. of cholesterol in their bile per day (table 2). This increase in excretion was found also to be due primarily to an increase in the biliary concentration of cholesterol of the control rats increased approximately 72 mg. per 100 cc. in 24 hours (47.5 to 119 mg. per 100 cc.). (See table 3.) The average increase in the hypothyroid rats was considerably less, being approximately 49 mg. per 100 cc. (65.6 to 115 mg. per 100 cc.). On the other hand, an average increase of 109 mg. per 100 cc. (37.6 to 147 mg. per 100 cc.) occurred in the plasma cholesterol content of the hyperthyroid rats. These results, of course, suggested (a) that the liver of the hyperthyroid rat was either manufacturing or discharging cholesterol into plasma at a far faster rate, or (b) that there was a marked decrease in the rate of destruction or excretion of the cholesterol in plasma. Exactly converse mechanisms were suggested to occur in the hypothyroid rat.

### Table 3.—Changes in Average Plasma Cholesterol Concentration after Bile Duct Ligation in Hypo- and Hyperthyroid Rats

<table>
<thead>
<tr>
<th>Type of Rat</th>
<th>No. of Rats</th>
<th>Average Plasma Cholesterol Concentration (mg./100 cc.)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Before Ligation 24 Hours After Ligation Net Increase After</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Total Free Ester Total Free Ester Ligation, Total</td>
</tr>
<tr>
<td>Control</td>
<td>11</td>
<td>47.5 6.7 40.8 119 49.3 69.7 72</td>
</tr>
<tr>
<td>Hypothyroid</td>
<td>19</td>
<td>65.6 12.3 53.3 115 46.2 68.8 49</td>
</tr>
<tr>
<td>Hyperthyroid</td>
<td>21</td>
<td>37.6 7.1 30.5 147 78.6 68.4 100</td>
</tr>
</tbody>
</table>

*S.E.M. = standard error of the mean.

### Table 4.—Rate of Disappearance of Injected Cholesterol in Hypo- and Hyperthyroid Rats

| Type of Rat | No. of Rats | Immediately After Injection 1 Hour After Injection 3 Hours After Injection 6 Hours After Injection 12 Hours After Injection |
|-------------|-------------|--------------------------------------------------|--------------------------------------------------|--------------------------------------------------|--------------------------------------------------|
| Control     | 5           | 114 (S.E. Mean: ±5.4) 113† (S.E. Mean: ±3.4) 69.6 (S.E. Mean: ±3.5) 33 (S.E. Mean: ±3.5) 9 (S.E. Mean: ±3.5) |
| Hyperthyroid| 7           | 137 (S.E. Mean: ±7) 103 (S.E. Mean: ±6.5) 58.4 (S.E. Mean: ±4.1) 6 (S.E. Mean: ±4.1) −18 (S.E. Mean: ±7.4) |
| Hypothyroid | 6           | 127.6 (S.E. Mean: ±8.4) 118 (S.E. Mean: ±6.3) 90 (S.E. Mean: ±6.3) 51.5 (S.E. Mean: ±6.3) 10.8 (S.E. Mean: ±4.4) |

* Hypercholesteremia was induced by intravenous injection of hypercholesteremic rat plasma.† These increments represent the plasma cholesterol (mg. per 100 cc.) in excess of the preinjection values. † Number of rats too small to permit calculation of the standard error.

D. Changes in Plasma Cholesterol Content after Biliary Obstruction in Hypo- and Hyperthyroid Rats

Following the induction of biliary obstruction, the liver of the rat apparently discharges excess cholesterol at a constant rate into the plasma. Therefore, it was thought desirable to study the rate of such discharge in both the hypo- and hyperthyroid animal. Biliary obstruction was produced in 11 control, 19 hypo-, and 21 hyperthyroid rats. The average plasma concentration of cholesterol rather than in the total volume of bile excreted.
E. The Rate of Disappearance of Parenterally Administered Cholesterol in Hypo- and Hyperthyroid Rats

In view of the above results, it appeared necessary to determine whether a change in the rate of destruction or excretion of plasma cholesterol occurred in the hypo- and hyperthyroid animal. Therefore, excess cholesterol (approximately 19 mg.) in the form of hypercholesteremic rat plasma was given intravenously to rats with deranged and normal thyroid activity and the rate of its disappearance from plasma was studied. It was found (see table 4) that following such injection an immediate hypercholesteremia of not very different degree occurred in the eu-, hypo-, and hyperthyroid rats. However, a far faster rate of disappearance of this injected cholesterol was observed in the hyperthyroid rat. Thus, although no significant change had occurred in the plasma cholesterol concentration of both the eu- and hypothyroid rats one hour after injection, the cholesterol content had fallen 30 mg. per 100 cc. in the hyperthyroid rat. Moreover, after six hours, the abnormal cholesterol content had disappeared almost completely from the blood of the hyperthyroid rat, although a similar disappearance was not observed in the control and hypothyroid rats until some time between 12 and 24 hours after injection. These results indicated that the hyperthyroid rat was able to rid his plasma far more quickly of excess cholesterol than either the eu- or hypothyroid animal. The results also suggested strongly that the previously observed abnormally rapid rate of cholesterol accumulation in the blood of the hyperthyroid rat, after biliary obstruction, could only be due to increased hepatic discharge of cholesterol into plasma.

Discussion

The foregoing studies demonstrate that hyperthyroidism induces a consistent decrease, and hypothyroidism, a consistent increase in the plasma cholesterol concentration of the rat. When the duration of the drug administration is more prolonged, the magnitude of such changes may be considerably greater than those observed here. The decreased plasma concentration observed in the hyperthyroid state might conceivably follow a more rapid rate of destruction, excretion, or tissue deposition of cholesterol, or it might result from a depressed rate of cholesterol synthesis. The converse possibilities are to be considered in the hypothyroid state.

Besides these expected changes in the plasma content of cholesterol of rats suffering from deranged thyroid activity, we also have found other evidences of disturbed cholesterol metabolism. Thus, the biliary excretion of cholesterol was found increased in hyper- and decreased in hypothyroidism despite variations in the opposite direction of the plasma cholesterol in these two states. Moreover, the amount of cholesterol excreted in the bile of many of the hyperthyroid rats was great enough to suggest the possibility that this type of rat must synthesize cholesterol at a more rapid rate than the eu- or hypothyroid rat.

This latter possibility was verified in the subsequent experiments in which the hyperthyroid rat was found to accumulate cholesterol in his plasma more rapidly after biliary obstruction than the eu- or hypothyroid rat. Moreover, this process of accumulation was not found due to a diminished rate of elimination or destruction of plasma cholesterol. This last finding, of course, suggested that the excess accumulation was due to an increase in rate of cholesterol synthesis. This increased rate undoubtedly occurs in the liver, in view of the fact that previous studies from this laboratory have demonstrated that the liver is the chief, if not the sole, source of plasma cholesterol. A decreased rate, on the other hand, occurs in the hypothyroid state. The data obtained in these studies are consistent with the observations of Karp and Stetten on the rate of deuterium incorporation into the hepatic steroid content of rats with deranged thyroid metabolism.

Whether increased intestinal excretion or increased destruction of cholesterol precedes, accompanies, or follows the increased rate of hepatic manufacture of cholesterol in hyperthyroidism cannot be determined at this time with certainty. It should be pointed out, however, that the existence of the relatively low
blood cholesterol in this state and the reverse in hypothyroidism, suggests that the initial change may be one involving the rate of elimination or destruction of cholesterol from the blood and tissues of the animal.

The reciprocal relationship observed between the biliary output of cholesterol and the plasma content of the same substance in the two states of thyroid derangement, of course, has intrigued us. It might be considered, at first glance, that excessive biliary excretion of cholesterol observed in hyperthyroidism might be responsible for the low plasma cholesterol; however, other studies of ours\(^5\) have indicated that the excretion of biliary cholesterol bears no relation to the plasma level of cholesterol.

The increased cholesterol content of bile in hyperthyroid rats may be a reflection, in some manner, of the already demonstrated increased rate of hepatic synthesis or discharge of cholesterol. The converse, of course, might be true in hypothyroidism. Studies relating to this are now in progress.

**SUMMARY**

The administration of thiouracil induced a significant rise and thyroid substance a significant decrease of the plasma cholesterol concentration in rats.

The concentration and daily output of cholesterol was considerably reduced in the bile of hypothyroid rats and markedly increased in the bile of hyperthyroid rats.

The hyperthyroid rat was found to accumulate cholesterol in his plasma after biliary obstruction much more rapidly than the euthyroid hypothyroid rat. This accumulation was found to be due to a far more rapid rate of manufacture or discharge of cholesterol by the liver of the hyperthyroid rat. The converse was found in the hypothyroid rat.

**ACKNOWLEDGMENTS**

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