Changes in Excretion of Intestinal Cholesterol and Sterol Digitonides in Hyper- and Hypothyroidism

By Meyer Friedman, M.D., Sanford O. Byers, Ph.D., and Ray H. Rosenman, M.D.

RECENTLY, a series of studies1–2 from this laboratory demonstrated that the source of the plasma cholesterol was the liver. Even more interesting perhaps, was the observation4 that the rate of cholesterol synthesis by the liver could be gaged by estimation of the daily biliary cholesterol. Thus, a means was provided by which rather exact information concerning the rate of cholesterol synthesis could be obtained in the intact animal.

At the time that these observations were being made, associated studies of hyper- and hypothyroid rats revealed some interesting results. First, it was discovered2 that the biliary excretion of cholesterol was markedly increased and decreased in hyper- and hypothyroid rats, respectively. Secondly, it was found5 that cholesterol accumulated in the plasma after biliary obstruction far more rapidly in the hyperthyroid rat than it did in the plasma of the eu- or hypothyroid rat. These two findings, when considered in the light of the earlier observations described above, seemed to permit but one conclusion, namely that a marked increase in the rate of hepatic synthesis of cholesterol is present in the hypothyroid rat and the converse in the hyperthyroid rat.

Despite this increased rate of synthesis of cholesterol in the hyperthyroid state (and the opposite in the hypothyroid state), no increase has been observed in the concentration of cholesterol either in the tissues6 or in the blood of the hyperthyroid animal.5 Therefore, it would seem likely that the rate of cholesterol excretion and/or destruction must also be more rapid in the hyperthyroid state (and retarded, on the other hand, in the hypothyroid state). The results of the present study do indeed indicate that the rate of excretion of cholesterol is increased in the hyperthyroid rat.

Methods

(1) Physiologic. A series of 29 male rats (Long Evans strain), approximately 12 weeks old, was divided into three groups. Each group was fed a basic laboratory diet of Purina laboratory chow. However, group I (nine rats) also was given desiccated thyroid substance* in the diet (0.3 per cent of basic diet). Group II (10 rats) was given thiouracil* in the diet (0.3 per cent of basic diet). Group III (10 rats) received only the basic diet and served as the controls. This type of feeding resulted in the production of marked hyperthyroidism in group I and hypothyroidism in group II, as previously shown.6

Eight weeks after this type of feeding had been instituted, each group of rats was placed in Bollman rat cages, supplied a sterol free diet,6 and the stools of each rat were collected separately for 72 hours. Rats of groups I and II continued to receive thyroid substance and thiouracil, respectively, during the collection. At the end of the collection period, the animals were killed, and the intestinal contents were collected and added to the stool specimen.

* We are grateful to Dr. Kenneth C. Kohlstaedt of the Eli Lilly Co., Indianapolis, for generous supplies of the thyroid substance and the thiouracil used in these studies.
(2) Chemical. Because a large portion of cholesterol in the intestine is changed to coprosterol, the stools were assayed not only for total cholesterol but also for all sterols precipitated by digitonin. In this manner, an estimate could be obtained of the actual amount of cholesterol originally excreted as such into the intestine.

The stools, together with the expressed intestinal contents of each rat, were placed in individual containers and dried at 60 C. for 48 hours. Thus, all sterol percentages are expressed as milligrams per 100 Gm. of fecal material so collected and dried.

The total dry collection from each animal was ground in a mortar until powdered. Samples of approximately 0.25 Gm. were weighed in Erlenmeyer flasks of 400 ml. volume and the sterols extracted by refluxing with 50 ml. of 1:1 alcohol-acetone mixture for three hours. The extract then was filtered into a 50 ml. volumetric flask, the residue being washed three times with 5 ml. portions of alcohol-acetone. If necessary, the filtrate was partially evaporated between washings to accommodate the volume of wash fluid.

Five ml. portions of the 50 ml. volume of extract were hydrolyzed at 40 C. in a sand bath in a sealed container by incubating for 40 minutes with 5 drops of 5 per cent potassium hydroxide according to the method of Schoenheimer and Sperry. The hydrolyzate was neutralized with 10 per cent acetic acid, using 1 drop in excess and then precipitated with 7 ml. of a clear solution of digitonin in alcohol as described in a previous paper. After standing overnight, the precipitate was transferred quantitatively and filtered through a weighed, sintered porcelain crucible of 1 ml. capacity, washed with a saturated solution of cholesterol digitonide and, finally, once with ether. The value for total digitonin-precipitable sterols was calculated from the precipitate weight and known weight of cholesterol precipitated by a given weight of our digitonin, assuming that noncholesterol sterols precipitated with digitonin in the same ratio by weight as cholesterol.

The entire crucible plus the precipitate then was placed in a test tube (20 by 150 mm.) and the precipitate dissolved in acetic anhydride–dioxane mixture. The acetic anhydride–dioxane solution of sterol digitonide was transferred quantitatively to a colorimeter tube using fresh acetic anhydride–dioxane as wash fluid, and the cholesterol color developed with sulfuric acid. Exact details of these latter procedures have been given in a previous paper. The weight of the noncholesterol sterols was determined by subtracting the assayed weight of cholesterol in the digitonin precipitate from the weight of the total sterol.

<table>
<thead>
<tr>
<th>Table 1.—Intestinal Excretion of Cholesterol and Sterol Digitonides</th>
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<td><strong>Type of Rat</strong></td>
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<td>Hyperthyroid</td>
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<td>Control</td>
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* Range of values
† Standard error of the mean

Results

As table 1 demonstrates, the hyperthyroid rat excretes considerably more cholesterol than the normal rat. Furthermore, his noncholesterol sterol excretion is significantly greater than that of the normal rat. Since this latter contains the coprosterol of the stool, which is believed to be derived from cholesterol, it can be seen that the hyperthyroid rat is excreting considerably more cholesterol than the normal rat. Moreover, this cholesterol is coming from endogenous sources since the animal received no sterol in his food during the feces collection period.

On the other hand, the hypothyroid rat was found to excrete considerably less noncholesterol sterol (see table 1) than the normal or
hyperthyroid rat. His actual cholesterol excretion, however, was not markedly reduced. This may be due to the fact that the general sluggishness of his intestinal activity allowed far more time for the conversion of cholesterol to coprosterol. It is of interest that the actual amount of cholesterol and sterol per gram of stool is not reduced but rather that the amount of stool itself is considerably reduced.

**DISCUSSION**

The discovery of the marked increase in biliary cholesterol excretion in the hyperthyroid rat, and the converse in the hypothyroid rat, appeared to us to present possible clues, not only about the state of cholesterol metabolism in these two opposing situations, but also about the significance and relationship of bile cholesterol to hepatic synthesis of cholesterol. Subsequent studies have confirmed this earlier belief, for one study has indicated that the rate of cholesterol synthesis is increased in the hyperthyroid rat. A later study, in turn, made it clear that the rate of biliary cholesterol excretion may be employed as a measure of the rate of hepatic synthesis of cholesterol.

Although our observations suggested an increased rate of cholesterol synthesis in hyperthyroidism (and the reverse in hypothyroidism), little information was gained about the rate of cholesterol excretion in disturbed thyroid states. The present study, however, demonstrates a marked increase in intestinal excretion of cholesterol and sterol digitonides in the hyperthyroid state. The hypothyroid rat, on the other hand, was found to have considerably less fecal sterol than the euthyroid rat, and possibly less unchanged cholesterol. These changes in the content of fecal cholesterol and sterol considerably exceed the differences which might be expected to occur as a result of the previously observed changes in the biliary excretion of cholesterol in these two states.

The magnitude of the increased intestinal excretion of cholesterol and sterols (derived in part from cholesterol) in the hyperthyroid rat, when conjoined with that amount of cholesterol excreted in its bile, further attests to the presence of an increased rate of cholesterol synthesis in this type of thyroid derangement.

Thus, if no abnormal decrease has been found in the tissue cholesterol and only a moderate one in the plasma of the hyperthyroid rat, despite the increased intestinal loss of cholesterol, then an increase in the rate of cholesterol synthesis would seem likely. As demonstrated previously, the chief site of this increased synthesis of plasma cholesterol is the liver.

Despite the increase in the rate of hepatic synthesis of cholesterol in the hyperthyroid rat, its plasma cholesterol concentration tends to be lowered. This last finding may be due to the fact that the increased intestinal excretion is relatively greater than the increased rate of hepatic synthesis. It is conceivable, moreover, that the increase in hepatic synthesis of cholesterol represents the response of the liver to a plasma being deprived more rapidly than normal of its cholesterol through an increased intestinal excretion and possibly, too, by an increased rate of destruction in the tissues of the body.

**SUMMARY**

The intestinal excretion of cholesterol and noncholesterol sterol was found to be increased in the hyperthyroid rat. The converse was found in respect to noncholesterol sterol in the hypothyroid rat. These findings furnish confirmation of the previously observed increased rate of hepatic synthesis of cholesterol in the hyperthyroid rat and the decreased rate of such synthesis in the hypothyroid rat.

**ACKNOWLEDGMENTS**

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