# Effect of Thyroidectomy on Conversion of Cholesterol into Bile Acids[1] (37364)

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In earlier studies concerned with the pathogenesis of hypercholesterolemia occurring in the hypothyroid rat (1, 2), we found that this hypercholesterolemia took place despite an impaired rate of cholesterol synthesis. This finding suggested that the elevation in serum cholesterol observed in these animals could only be due to either an increased absorption of cholesterol or a retardation in the rate of excretion or catabolism of that cholesterol which had been synthesized or absorbed. Subsequent studies (3, 4) indicated that the rates both of excretion and catabolism of cholesterol were impaired in the hypothyroid rat. Similar findings subsequently were observed by other investigators (5, 6).

Since cholesterol is catabolized in the rat to bile acids (7), it is highly probable that the impaired rate of catabolism of cholesterol noted in the hypothyroid rat (4) might be due to the diminished capability of such an animal to convert cholesterol into bile acids. In order to determine this point, we administered serum containing labelled cholesterol to hypothyroid rats and studied the biliary excretion of both bile acids and cholesterol in these animals.

*Methods.* Twelve of 17 young adult male rats (Long–Evans strain, weighing between 250–300 g) were thyroidectomized. Six of these 12 thyroidectomized (T) rats were injected intraperitoneally daily with 2 mg of thyroid substance.[2] All rats were fed 30 ml/day of a liquid diet consisting of evaporated milk (250 ml), glucose (80 g), Kaopectate suspension (30 ml), vegetable oil (12 ml), iodized NaCl (4 g), egg white powder (18 g), Poly-Vi-Sol suspension (0.3 ml) and $H_2O$ (180 ml). This diet contained 70 mg of cholesterol/100 ml. It is comprised of 70% water, 6% protein, 3.6% fat, 18% carbohydrate and 1.9% ash; on a dry basis, the diet is 19.7% protein, 12% fat, 62% carbohydrate and 6.4% ash.

One week following the thyroidectomy, after a prior starvation period of 13 hr, all rats were anesthetized with ether, their bile ducts cannulated as previously described (8) and their bile was collected. Immediately after each rat was cannulated it was injected intravenously with 0.6 ml of serum containing lipoprotein cholesterol labelled with cholesterol-1$\alpha$,2$\alpha$-$^3$H. Blood samples were taken immediately, and 6 hr after the injection of the labelled lipoprotein.

Bile secreted 0–3 hr and 3–6 hr was collected separately. This was done because we found in preliminary experiments (also see Table I) that the total body pool of bile acids [*i.e.*, 15–20 mg of bile acids (5)] was excreted during the first 3 hr of collection. Therefore, the subsequent 3-hr biliary collection allowed the determination of the secretion rate of newly formed bile acid after all bile acids in the enterohepatic circulation had been excreted and possibly before the normal rate of synthesis of bile acids was too greatly accelerated by cessation of feedback regulation.

The rats were killed at the end of 6 hr, their livers perfused with normal saline and then removed. Cholesterol was determined quantitatively in each serum, bile and liver sample and the specific radioactivity of digitonin-precipitable sterols was also determined according to previously described methods (9). Bile acids were determined quantitatively by the method of Talalay (10) as

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[2] Thyroid powder, Armour Laboratories, Kankakee, IL.

TABLE I. Biliary Secretion of Bile Acids and Cholesterol in Thyroidectomized Rats.

| | Thyroidectomized rats | Thyroidectomized rats given thyroid extract | Control intact rats |
|---|---|---|---|
| Number of rats | 6 | 6 | 5 |
| Average weight (g) | 276 | 270 | 271 |
| Volume of bile (ml) | | | |
| 0–3 hr | 1.5 | 1.95 | 2.0 |
| SE mean | ±0.11 | ±0.15 | ±0.09 |
| 3–6 hr | 1.4 | 1.95 | 2.0 |
| SE mean | ±0.17 | ±0.14 | ±0.18 |
| Total (6 hr) | 2.9 | 3.90 | 4.0 |
| SE mean | ±0.24 | ±0.26 | ±0.23 |
| Bile acids | | | |
| (1) mg/ml bile | | | |
| 0–3 hr | 13.2 | 14.0 | 14.2 |
| SE mean | ±0.72 | ±1.5 | ±1.12 |
| 3–6 hr | 12.8 | 11.8 | 12.1 |
| SE mean | ±2.7 | ±1.7 | ±1.9 |
| (2) mg in total bile | | | |
| 0–3 hr | 20.1 | 26.5 | 28.4 |
| SE mean | ±1.8 | ±3.7 | ±2.7 |
| 3–6 hr | 17.9 | 22.5 | 27.0 |
| SE mean | ±4.2 | ±2.4 | ±3.3 |
| Total (6 hr) | 38.0 | 49.0 | 55.4 |
| SE mean | ±4.4 | ±5.1 | ±3.6 |
| (3) DPM $^3H \times 10^3$/ml bile (in bile acids) | | | |
| 0–3 hr | 10.2 | 11.63 | 12.76 |
| SE mean | ±1.6 | ±1.13 | ±2.6 |
| 3–6 hr | 11.45 | 11.26 | 17.37 |
| SE mean | ±1.2 | ±1.3 | ±9.9 |
| (4) DPM $^3H \times 10^3$ in total bile (in bile acids) | | | |
| 0–3 hr | 14.14 | 21.76 | 25.18 |
| SE mean | ±1.4 | ±2.1 | ±4.6 |
| 3–6 hr | 15.86 | 21.36 | 34.42 |
| SE mean | ±2.3 | ±1.59 | ±3.85 |
| Total (6 hr) | 30.0 | 43.12 | 59.60 |
| SE mean | ±3.4 | ±2.5 | ±8.4 |
| Bile cholesterol | | | |
| (1) mg/ml bile | | | |
| 0–3 hr | 0.15 | 0.19 | 0.22 |
| SE mean | ±0.05 | ±0.02 | ±1.4 |
| 3–6 hr | 0.12 | 0.22 | 0.21 |
| SE mean | ±0.02 | ±0.04 | ±2.0 |
| (2) mg in total bile | | | |
| 0–3 hr | 0.225 | 0.361 | 0.44 |
| SE mean | ±0.02 | ±0.03 | ±0.03 |

TABLE I (continued)

| | Thyroidectomized rats | Thyroidectomized rats given thyroid extract | Control intact rats |
|---|---|---|---|
| 3–6 hr | 0.168 | 0.43 | 0.42 |
| SE mean | ±0.01 | ±0.02 | ±0.03 |
| Total (6 hr) | 0.393 | 0.791 | 0.86 |
| SE mean | ±0.02 | ±0.01 | ±0.04 |
| (3) DPM $^3H \times 10^3$ in total bile (in cholesterol) | | | |
| 0–3 hr | 2.43 | 3.08 | 4.13 |
| SE mean | ±0.29 | ±0.15 | ±0.45 |
| 3–6 hr | 2.69 | 3.76 | 4.93 |
| SE mean | ±0.25 | ±0.30 | ±0.69 |
| Total (6 hr) | 5.12 | 6.84 | 9.06 |
| SE mean | ±0.50 | ±0.42 | ±1.1 |

modified by Admirand and Small (11). Radioactivity in bile cholesterol and bile acids was determined after partition of the alkaline bile sample between polar and nonpolar solvents by extraction with chloroform/methanol, 2/1 according to the method of Folch *et al.* (12).

The serum containing labelled lipoprotein cholesterol was produced by injecting intraperitoneally into each of 3 normal rats 0.5 mCi of cholesterol-1, 2-$^3H$ dissolved in 1, 2-propanediol and bleeding them 48 hr later. Employing thin-layer chromatography analysis as described by Chedid *et al.* (13), Folch *et al.* (12) and Parker and Peterson (14), 25% of the radioactivity of the pooled serum sample was found in its cholesterol and 75% in its cholesterol esters.

*Results.* The average serum cholesterol (111 mg/100 ml) of the 6 T rats was significantly greater ($p < 0.001$) than that (56 mg/100 ml) of the 5 control rats and also significantly greater ($p < 0.001$) than the average serum cholesterol (64 mg/100 ml) of the 6 treated T rats. However, the average serum cholesterol of the treated T rats was not significantly different from that of the control rats.

The injected labeled lipoprotein cholesterol did not disappear as rapidly from the serum of the T rats as it appeared to do in the treated T and control intact rats. Thus, 6 hr after injection of the labeled cholesterol, the DPM of the cholesterol present in 1 ml of serum was $3.3 \times 10^4$ in the T rats and only $2.54 \times 10^4$ and $2.62 \times 10^4$ in the treated T and control rats, respectively. This difference between the value found in the T rats and those of the other 2 groups was statistically significant ($p < 0.03$). However, it is possible that this observed difference in tracer disappearance may be attributable to the approximate 100% difference in plasma cholesterol pool size.

Despite this observed difference in disappearance rate of injected labeled cholesterol from the serum of the T rats, analysis of the DPM $^3H$ content of the total liver cholesterol of the T rats 6 hr after injection was $2.22 \times 10^5$—a finding essentially the same as that ($2.06 \times 10^5$ DPM $^3H$) found in the control rats. Also the average cholesterol content of the livers of the T rats (3.62 mg/g liver) was significantly greater ($p < 0.02$) than that (2.11 mg/g liver) found in the control rats.

Inspection of Table I reveals that thyroidectomy significantly altered both bile acid and cholesterol metabolism as reflected by the changes observed in biliary excretion. The data in Table I also indicate that administration of thyroid extract either totally or almost totally corrected these alterations.

Perhaps the most obvious change observed in biliary dynamics after thyroidectomy was the significant reduction ($p < 0.01$) in the flow of bile whether measured during the

first or the second 3-hr intervals. The administration of thyroid extract prevented this decline in flow.

Although the concentration of bile acids in the bile was essentially the same in all 3 groups of rats whether measured during the first or second interval of collection, because of the above volume changes, the T rats both during the first and second 3-hr collection periods excreted significantly less ($p < 0.05$) bile acids (see Table I) than did the control rats. Those T rats given thyroid extract, however, excreted about as much bile acids (49 mg/6 hr) as the control rats (55.5 mg/6 hr).

Similarly, as Table I illustrates, the T rats excreted significantly less ($p < 0.05$) labeled bile acids than the control rats during both bile collection periods. The decrease in this particular hepatic function was so marked that even though the bile volume of the T rats was markedly reduced, nevertheless, as Table I indicates, the concentration of labeled bile acids also was less in the bile collected from the T rats during the second 3-hr collection. The administration of thyroid extract, however, to T rats appeared to shift towards normal (see Table I, Part 4) their excretion of labeled bile acids in the total bile, despite an equivalence in DPM per ml, since the treated rats show a normal volume of bile while T rats show diminished volume.

As earlier observed (4), the total amount of bile cholesterol excreted by the T rats during both collection periods (see Table I) was significantly ($p < 0.05$) less than that excreted by the control rats. Again, the administration of thyroid extract normalized the biliary excretion of cholesterol. The bile of the T rats also was found to contain less of the labeled cholesterol than the bile of the other 2 groups.

*Discussion.* The present results indicate quite clearly that the hypothyroid rat not only excretes less cholesterol in its bile than the normal rat but, far more important from a quantitative standpoint, it also is markedly deficient in excreting total bile acids whether this function is measured at the time that the animal's liver is still receiving bile acids reabsorbed from the intestinal tract or after such reabsorption essentially ceases.

The cause of the reduced biliary secretion of bile observed in the T rat appears to be due primarily to the relative inability of this animal to convert cholesterol readily into bile acids. Certainly the observed failure of the T rat to excrete as much $^3$H-labeled total bile acids as the control rat after both types of animal had been injected with $^3$H-labeled cholesterol points to this conclusion, although receipt of tracer cholesterol by the liver of T rats was possibly diminished by the larger plasma cholesterol pool size in these animals.

It is probably this specific relative failure of the T rat to convert cholesterol into bile acids that is chiefly responsible for the hypercholesterolemia observed in this animal. We believe this is so for several reasons: First, the already observed decrease in biliary (4) or intestinal (2) secretion of cholesterol in the T rat is not of sufficient magnitude to account for the hypercholesterolemia whereas the decrease in the rate of catabolic conversion of cholesterol to bile acids observed in the present study is of sufficient quantity to account for the excess of cholesterol present in the serum. Second, the accumulation of cholesterol occurring in the serum of the T rat cannot be due to any increase in rate of synthesis of cholesterol because the latter actually is markedly reduced (1) nor can it be due to any change in the amount of cholesterol absorbed because this is not changed (15) in the T rat. Third, the hypercholesterolemia of the T rat does not appear to be due to the slight retardation in the rate of exit of cholesterol from its plasma if only because the livers of the T rats 6 hr after the injection of the labeled cholesterol contained approximately the same amount of this labeled cholesterol as the livers of the control rats. Finally, decreased catabolic transformation of cholesterol into bile acids appears to be the chief cause of the hypercholesterolemia in the T rats because when such catabolism is increased experimentally in the normal rat, either by excess administration of thyroid hormone (4, 16) or by interference with the reabsorption of bile acids (17), the serum

cholesterol concentration promptly diminishes to subnormal levels.

*Summary.* Analyses of the bile of thyroidectomized rat following the injection of labeled cholesterol indicated that this type of animal exhibited a defect in its ability to catabolize cholesterol into bile acids. It is suggest that this decreased catabolic transformation of cholesterol is the chief cause of the hypercholesterolemia observed after thyroidectomy.

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