Biliary Excretion of Cholic Acid and Cholesterol in Hyper-, Hypo-, and Euthyroid Rats.* (20320)

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Previous studies from this laboratory(1,2) have indicated that cholic acid and cholesterol excretion in the bile can be followed serially for a period of over 14 days. The ratios of excretion of these constituents remained quite constant. The isolation of labeled cholic acid from the bile of animals previously given deuterium-labeled(3) or tritium-labeled(4) cholesterol is evidence for the consideration of cholic acid as a metabolic degradation product of cholesterol. Because of the well-known effect of changes in thyroid activity on blood cholesterol(5), it was of interest to ascertain the effects of altered states of thyroid activity on the biliary output of cholic acid and cholesterol.

Recent studies on the relation of thyroid function to cholesterol by Rosenman, Byers, *et al.*(6-10) have led these authors to conclude that the metabolism of cholesterol is increased in hyperthyroidism and decreased in hypothyroidism. In their experiments the biliary excretion of cholesterol was determined in experimental hyper- and hypothyroidism (8-10) upon bile collected for 24 hours following cannulation of the bile duct. During this period we have found the amount of bile secreted and its composition to be disturbed and erratic. The present work details the effects of altered states of thyroid activity on the serial analysis of the cholic acid-cholesterol inter-relationship in bile collected over a period extensive enough to allow hepatic physiology time to recover from operative trauma.

Experimental. All animals used in this work were young male Wistar rats ranging in weight from 220-260 g. They all were fed a standard synthetic diet containing 3% fat and 18% casein(11)† throughout the experimental period. Following cannulation of the bile duct, animals were given 1% NaCl to drink *ad libitum*. The animals were divided into 3 main groups. The control (euthyroid) group was treated as outlined. Hyperthyroid rats were prepared by feeding 100 mg thyroid (Parke, Davis USP) per day for 10-15 days preceding bile duct cannulation. Following operation, the thyroid was given as 0.33% of the diet. These animals failed to gain weight in spite of increased food intake and had an increased pulse rate and generalized tremors. Hypothyroid rats were prepared by administering propyl thiouracil,‡ as 0.5% of the diet, for 18-21 days preoperatively and were maintained on the same dosage following cannulation. These animals lost little weight in spite of a markedly diminished food consumption.

Cannulation of the common bile duct was accomplished by following the technic described by Fisher and Vars(1). In this, a polyethylene tube (PE 50) with an internal diameter of 0.023 inch, is inserted into the duct at the junction of its proximal and middle thirds. The tube is brought through the body wall and threaded subcutaneously to emerge on the dorsal mid-line at the base of the tail, and be inserted into a latex bag. This bag is changed every 24 hours, the bile volume measured and aliquots taken for analysis of cholic acid by method of Irvin, Johnston, and Kopala(12) and of cholesterol by method of Foldes and Wilson(13).

Results. The data presented were obtained

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	%
† Diet—Casein	18
Sucrose	73
Cod liver oil	3
Celluflour	2
Jones salt Mixture No. 12	4

This diet was supplemented with menadione and adequate B vitamins.

‡ Furnished through the courtesy of Lederle, Inc.

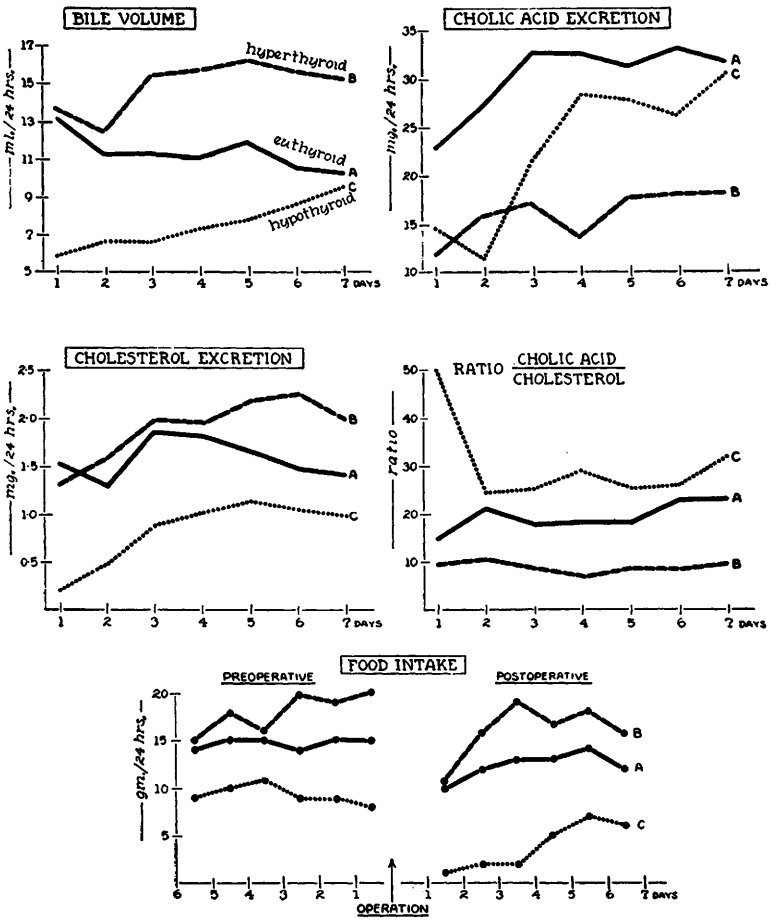

FIG. 1. Average daily biliary data, and food intake of hyper-, hypo-, and euthyroid rats.

over a bile collection period of 7 days. No animal was included in the series that was not stabilized as to food and water intake and volume of bile produced for a period of at least one day in excess of the 7 reported here.

The results are presented in graphic form in Fig. 1. The animals are divided into Group A, euthyroid control; Group B, hyperthyroid; and Group C, hypothyroid. Each group represents 10 animals. The average results were as follows (all averages except those for food intake are for the 7-day period postoperatively):

1. Volume—A:11.3 ml, B:14.9 ml, C:7.5 ml.

2. Cholic acid excretion — A:30.3 mg, B:16.0 mg, C:23.0 mg.

3. Cholesterol excretion — A:1.58 mg, B:1.89 mg, C:0.82 mg.

4. Cholic acid/cholesterol ratio—A:19.2, B:8.5, C:28.1.

5. Food intake—The animals failed to eat on the day of operation. Average figures reported are for 6 days preceding operation, and for second to seventh day postoperative. Preoperative—A:15 g, B:18 g, C:9 g. Postoperative—A:12 g, B:16 g, C:4 g.

As can be seen in Fig. 1, the data from the first 2 days' bile collection differ from those collected over the subsequent 5 days. In the hypothyroid group, for instance, the average daily cholic acid excretion for the first 2 days was 13.1 mg, for the subsequent 5 days the average output was 27.0 mg; the average daily cholesterol excretion for this group for the first 2 days was 0.38 mg, and for the next 5 days was 1.0 mg.

Discussion. These data indicate that the

amount of cholesterol excreted in the bile is a reflection of the level of thyroid activity, and as such, are in agreement with the findings of Rosenman, Friedman, and Byers(9). The absolute values for biliary cholesterol excretion in the present report, especially those in the hyperthyroid rat, are, however, considerably lower.

The excretion of cholic acid was found to be below control levels in both the hypo- and hyperthyroid animals, although a greater amount was excreted in the hypothyroid animals than in the hyperthyroid group. There was a gradual rise in the cholic acid output in the hypothyroid rats, reflecting perhaps the longer time needed by the hepatic excretory mechanisms of the propyl thiouracil-treated animals to return to normal. It is possible that the cholic acid excretion would return to control values in time.

It is of interest to note that following the first postoperative day, the cholic acid/cholesterol ratio for each of the 3 experimental groups remains quite constant and quite distinct.

Byers and coworkers have found that the cholesterol(14) and the cholic acid(15) output in the bile are uniformly higher on the first day following cannulation than during subsequent 24-hour periods. Our data, on the contrary, indicate that the biliary levels for these 2 substances rise following cannulation and reach a fairly constant plateau on the third or fourth postoperative day. It is evident from this, and from a study of the serial food intake, that the bile-cannulated animal does not approach a state of functional equilibrium until 3 or 4 days after cannulation.

Summary. 1. Hyper- and hypothyroid rats have been prepared and, along with euthyroid control rats, have been subjected to cannulation of the common bile duct and total daily bile collection for 7 days following cannulation. 2. Analysis of this bile for cholesterol and cholic acid revealed that the excretion of cholesterol is a reflection of the level of thyroid activity, and that cholic acid excretion is lowered in both hyper- and hypothyroidism. 3. After the third postoperative day, the ratio of cholic acid to cholesterol excreted in the bile was found to be relatively constant for each type of animal. 4. Cholic acid and cholesterol excretion in the bile was suppressed in both the hyper- and hypothyroid rats in the immediate postoperative period. In 3 or 4 days following cannulation, these levels reached a plateau.

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