and specificity would apply to the *in vitro* method reported by Schizume and associates (9).

The dose range for submaximal responses was 100 fold in the method using intact frogs. With the isolated preparations the range was only 2 fold. A long range for submaximal responses is considered desirable for a bioassay since it permits comparison between standard and unknown even though their potencies may vary considerably.

As a measure of the variability of the response to a given dose, the mean coefficient of variation was 3.4 times greater in the method using isolated frog skin.

With respect to the regression line, the slope obtained with the *in vitro* method was greater than that with the *in vivo* method. However the variance of error of the latter method was considerably less than that of the former. Thus, lambda, the precision index, was greater for the *in vitro* method, and approximately 2.4 times as many determinations would have to be carried out using this method in order to obtain an error of 10% or less.

Conclusions. It is concluded that the method employing intact frogs is superior to that in which strips of frog skin are used for assay of melanophore stimulating hormone.

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Received January 22, 1957. P.S.E.B.M., 1957, v94.

Biliary Excretion of Bile Acids and Cholesterol in Bile Fistula Rats. Bile Acids and Steroids.*† (23018)

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It has been demonstrated that bile acids are the main metabolic end products of cholesterol in the rat (Byers and Biggs(1), Bergström(2), Chaikoff and Dauben(3)). Rat bile contains taurocholic and taurochenodesoxycholic acid (Bergström and Sjövall(4)). Several authors have studied excretion of bile acids and cholesterol in the bile duct cannulated rat. Friedman, Byers and Michaelis (5) found that excretion of cholic acid and cholesterol in bile gradually decreased during the first 2 days following cannulation. Thompson and Vars(6a,b) however, studying the influence of altered thyroid activity upon excretion of cholic acid found that the biliary level of this substance rose following cannulation and reached a fairly constant plateau on third or fourth postoperative day. Recently Wysock, Portman and Mann(7) reported studies on nutritional effects upon biliary excretion of cholic acid and dihydroxycholanic acids. Their results, however, were based entirely upon determinations of

* Preliminary report was read at meeting of the Swedish Biochem. Soc., Uppsala, Jan. 27, 1956(10).

† This work has been supported by grant from Medical Faculty, University of Lund.

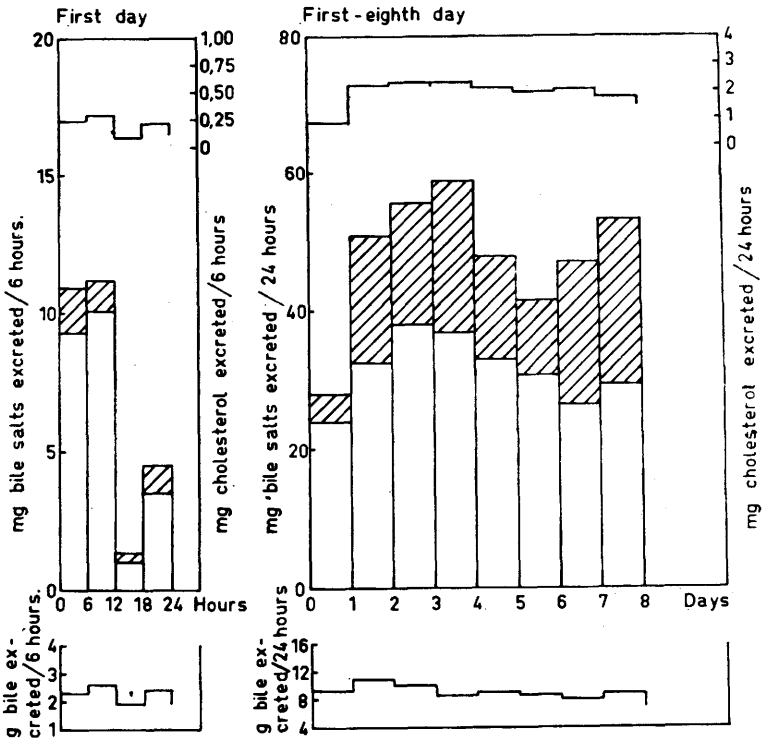

FIG. 1. Bile fistula rat 15 (230 g). Excretion of sodium taurocholate □, sodium taurochenodesoxycholate ▨ and cholesterol during 8 days. Values are expressed as mg/6 hr during the first day and as mg/24 hr during the following days.

bile acids in bile collected during the first 24 hours after operation.

The aim of the present investigation was to undertake a detailed study of excretion of taurocholic and taurochenodesoxycholic acid and cholesterol in the bile duct-cannulated rat. The methods developed by Sjövall(8) and Sjövall and Eriksson(9) make a direct, specific and quantitative microdetermination of both these acids possible.

Methods. White female or male rats of the institute stock, (weight: 200-300 g), were fed a diet consisting mainly of oats and white bread prior to operation. The common bile duct was cannulated and the animals were then kept in restraining cages during the experiment. Following cannulation, the animals were given white bread and 0.9% NaCl *ad libitum*. Bile was collected for various time intervals, usually for 6 hours, during the first day and later in 24 hour portions. Total time of collection ranged from one to 2 weeks. After collection, the bile was weighed and made up to definite volume with ethanol. Taurocholic and taurochenodesoxycholic acids were determined as their sodium salts by the methods developed by Sjövall(8). For paper chromatographic separation of the 2 acids, the ascending method with 70% formic acid as stationary and isoamylacetate heptane 85:15 as moving phase was used. Equilibration prior to running (15-20 hours), the chromatograms were found unnecessary. Seventy % formic acid gave more distinct spots than the 70% acetic acid previously used.‡ *Cholesterol in bile* was determined by a modification of the method of Abell *et al.*(11) for determination of total cholesterol in serum. One ml samples of the 24 hour bile diluted to 50 ml with ethanol, were measured into 12 ml glass stoppered centrifuge tubes. One ml ethanol and 1 ml 33% KOH was added to each tube. The stoppered tubes were kept in water bath at 60°C for 45 min-

‡ To be published.

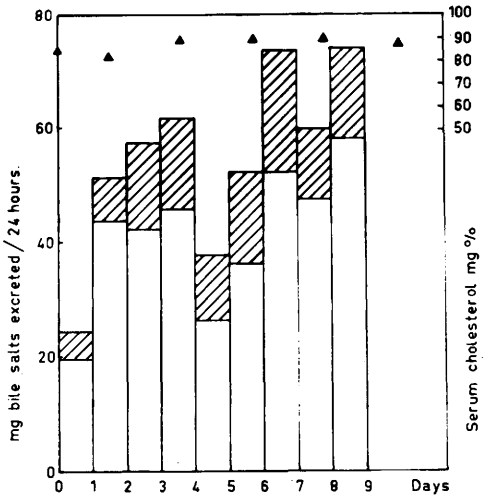

FIG. 2. Bile fistula rat 20 (290 g). Total serum cholesterol determined prior to operation and at various intervals after operation. Sodium taurocholate □ sodium taurochenodesoxycholate ▨.

utes. After cooling to room temperature 2 ml of water and 5 ml of petroleum ether were added and the tubes shaken vigorously for 1 minute. After 2 clear layers had formed 3 ml of petroleum ether was pipetted off and another 3 ml of petroleum ether added and the extraction procedure repeated in the same manner. The petroleum ether was evaporated to dryness in nitrogen atmosphere. Standards were run through the procedure along with the samples. The colour reaction used was a modified Tscgufaeff reaction as described by Dam *et al.*(12). The procedure described gave a 95-105% recovery of added cholesterol in the region 12-60 μg added to sample containing about 60 μg. *Total serum cholesterol* was determined according to Abell *et al.*(11) using the colour reaction described above. Blood samples (100-150 μl) were obtained from a cut on the tail. The determination was performed using 50 μl of serum.

Results. In Fig. 1 a typical excretion pattern is shown. During the first 18-24 hours after operation there is always a fall in excretion of taurocholic and taurochenodesoxycholic acid. A minimum generally occurs between 12 and 18 hours after operation. Following this minimum there is a rapid increase in amount excreted, and a fairly constant level is reached by the second or third day. During subsequent days total excretion of bile acids ranges between 40 and 60 mg/day. The major part, approximately 75%, is taurocholic acid. Excretion of cholesterol during the first day was about 1 mg and increased during the following days to about 2 mg/day. Amount of bile excreted usually decreases somewhat after the first day of collection (10-15 g/day). Fig. 2 shows the excretion pattern of a second rat. This Figure also shows that serum cholesterol remains practically unchanged during the time of experiment (9 days).

Thompson and Vars(6) in their work on biliary excretion of cholic acid in rats with modified thyroid activity noted low and erratic levels of cholic acid during the first day following cannulation. Cholic acid level then reached a fairly constant plateau. Their values on cholic acid are in agreement with ours. The low level of cholic acid was ascribed to hepatic injury at operation and plateau values were thought to represent normal formation of cholic acid in the intact animal. Friedman and Byers(5) noted decreasing values during the first 2 days after operation but did not follow excretion beyond 2 days. In our experiments we found a constant excretion pattern consisting of essentially 2 parts; during the first 12 or 24 hours following cannulation we observed a fall in excretion of both taurocholic and taurochenodesoxycholic acid. The amount of bile acids excreted in this time interval obviously represents excretion of preformed bile acids present in the enterohepatic circulation. From Fig. 1-3 it is possible to obtain an approximate value of the taurocholic acid pool. The amount of sodium taurocholate excreted during the first 18 hours varies between 6.1 and 8.8 with a mean value of 7.2 mg/100 g bodyweight. In another investigation under way, we determined the size of the taurocholic acid pool in the rat by using isotope dilution *in vitro* and *in vivo*. Preliminary data indicate the size of the pool to be about 12 mg sodium taurocholate in a rat weighing 200 g.§

Lindstedt and Norman(13) have estimated

§ To be published.

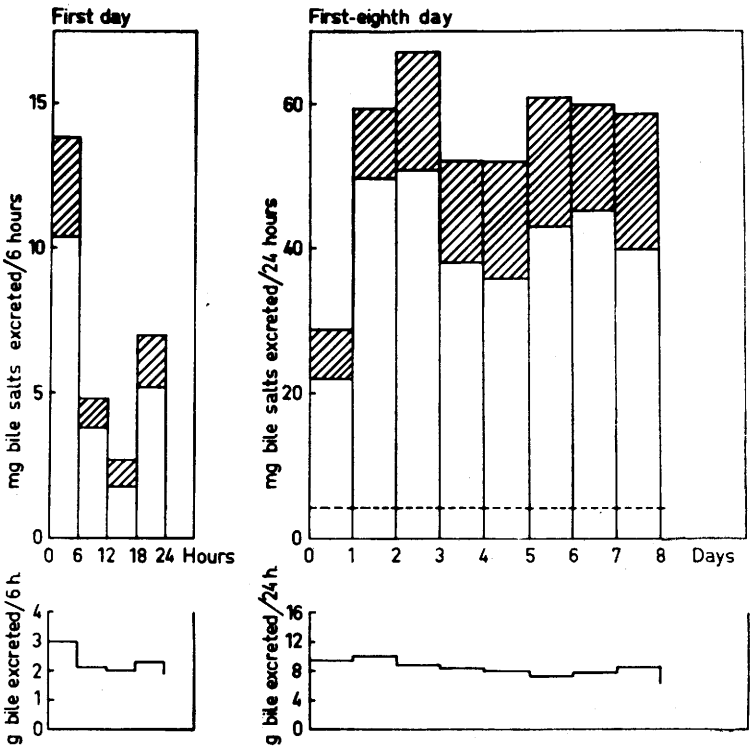

FIG. 3. Bile fistula rat 14 (260 g). Excretion of sodium taurocholate □ and sodium taurochenodesoxycholate ▨. Values are expressed as mg/6 hr during the first day and as mg/24 hr during following days. Broken line shows magnitude of taurocholic acid synthesis in the normal rat without a fistula.

the half lives of bile acids in the intact rat to be about 2.5 days. Using the preliminary figure of bile acid pool of 12 mg in a 200 g rat, the daily synthesis of taurocholic acid would be 3.2 mg. This means a synthesis of 0.12 mg/hour. Using this figure one obtains a value of 2.2 mg for synthesis during the first 18 hours assuming unchanged rate of synthesis. Subtracting this value from 14.4 mg one obtains 12.2 mg sodium taurocholate, which would thus represent the amount present in the enterohepatic circulation. The amount in a 200 g rat would consequently be 12.2 mg, a figure in good agreement with the value for the pool cited above. The second part of the excretion curve (comprising excretion from the second day and onwards) thus does not represent the normal formation of bile acids in the rat but rather the maximal capacity of the liver for bile acid production. This capacity is fully made use of when bile acids are continually withdrawn in a fistula and the portal blood does not carry any bile acids back to the liver. In Fig. 3 the normal synthesis of taurocholic acid compared to the large synthesis which occurs after preformed bile acids have left the enterohepatic circulation is shown. The amount of bile excreted in the bile fistula rat is thus increased 10-20 fold, as a result of the broken enterohepatic circulation.

Our values for excretion of cholesterol in bile agree with those found by Friedman and Byers(5). In spite of the large synthesis of bile acids in fistula animals serum cholesterol remained unaffected. Apparently, the effects of these metabolic changes in cholesterol-bile acid metabolism are mainly limited to the liver in these experiments.

Summary. 1. A detailed analysis of excretion of taurocholic taurochenodesoxycholic acid and cholesterol in bile duct cannulated rats has been undertaken. 2. A constant excretion pattern consisting of 2 parts

was observed: A) Excretion of preformed bile acids present in the enterohepatic circulation during 12 hours following cannulation; B) An increased formation of bile acids resulting in a 10-20 fold increased synthesis of bile acids (40-70 mg/day), as compared to synthesis of bile acids in the intact animal (3-4 mg/day), is present from the second or third day.

The author is indebted to Professor S. Bergström and Dr. J. Sjövall for advice and facilities put at my disposal.

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Received January 23, 1957. P.S.E.B.M., 1957, v94.

Influence of Thyroid Activity on Excretion of Bile Acids and Cholesterol in the Rat.*† (23019)

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It has long been realized that a relationship exists between plasma cholesterol concentration and thyroid activity(1). In general, hyperthyroidism is associated with decreased plasma cholesterol level and hypothyroidism with elevated concentration. Rosenman, Friedman and Byers(2) found biliary cholesterol in rats, increased in hyperthyroidism and decreased in hypothyroidism. On the basis of turn-over studies, Rosenman *et al.* (3) also concluded that thyroid hormone enhances cholesterol destruction. Marx, Gustin and Levi(4) measuring incorporation of D_2O into cholesterol found cholesterol synthesis increased in thyroxine-injected and decreased in thyroidectomized rats. In recent years the direct importance of bile acid metabolism for quantitative aspects of metabolism of cholesterol has been stressed. Thus, work by Siperstein *et al.*(5) and by Bergström and Norman(6) has indicated that bile acids are the main metabolic end product of cholesterol. Thompson and Vars(7) studied the effects of altered thyroid activity on excretion of cholic acid and cholesterol in bile fistula rats. They expected cholic acid output to be high in the hyperthyroid state and low in the hyperthyroid state. However, they found lower values of cholic acid both in the hyperthyroid and the hypothyroid group as compared to control animals. In their experiments they did not determine taurochenodesoxycholic acid, the second bile acid of quantitative importance in the rat (Bergström and Sjövall(8)).

We have recently investigated excretion of cholesterol, taurocholic and taurochenodesoxycholic acid in normal bile fistula rats(9).

* Preliminary report was read at meeting of Norwegian Biochem. Soc., Oslo, June 4, 1956.

† This work has been supported by grant from the Medical Faculty, University of Lund.