Biochemical site of regulation of bile acid biosynthesis in the rat

SARAH SHEFER, SUSAN HAUSER, IHOR BEKERSKY, and ERWIN H. MOSBACH

Department of Laboratory Diagnosis, Public Health Research Institute of the City of New York, Inc., and the Bureau of Laboratories, New York City Department of Health, New York 10016

ABSTRACT The production of bile salts by rat liver is regulated by a feedback mechanism, but it is not known which enzyme controls endogenous bile acid synthesis. In order to demonstrate the biochemical site of this control mechanism, bile fistula rats were infused intravenously with ^{14}C-labeled bile acid precursors, and bile acid biosynthesis was inhibited as required by intraduodenal infusion of sodium taurocholate.

The infusion of taurocholate (11–14 mg/100 g of rat per hr) inhibited the incorporation of acetate-1-^{14}C, mevalonolactone-2-^{14}C, and cholesterol-4-^{14}C into bile acids by approximately 90%. In contrast, the incorporation of 7α-hydroxycholesterol-4-^{14}C into bile acids was reduced by less than 10% during taurocholate infusion.

These results indicate that the regulation of bile acid biosynthesis is exerted via cholesterol 7α-hydroxylase provided that hepatic cholesterol synthesis is adequate.

SUPPLEMENTARY KEY WORDS in vivo bile acid biosynthesis · enterohepatic circulation · cholesterol 7α-hydroxylase

In mammals, the bile acids appear to be derived exclusively from cholesterol (1). The biosynthesis of bile acids takes place in the liver and is regulated homeostatically by the amount of bile acid returning to the liver via the enterohepatic circulation (2). Biosynthetic control of bile acid formation could be exerted, theoretically, at any of the numerous steps between acetate and cholanoate. However, the results of in vitro studies have provided strong suggestive evidence that the major, and perhaps sole rate-determining step of bile acid synthesis is the 7α-hydroxylation of cholesterol (3, 4). The present experiments were carried out to demonstrate the rate-controlling function of cholesterol 7α-hydroxylase in an in vivo system.

METHODS

Experimental Animals

Male Charles River rats, 250–400 g, fasted overnight, were anesthetized with Diabutal (Diamond Laboratories, Inc., Des Moines, Iowa). Cannulas were inserted into the bile duct, duodenum, and femoral vein. The animals were placed in restraining cages and given unrestricted access to water. The duodenal cannula was connected to an infusion pump (Harvard Apparatus Co., Inc., Millis, Mass.) which delivered 1.22 ml/hr of a commercially available liquid diet (Liquid diet No. 116 E.C.; General Biochemicals, Chagrin Falls, Ohio). This diet was diluted to supply a 300 g rat with approximately 24 kcal/day. The cholesterol content of the diet was 0.016 mg/ml. It was observed that during the experimental period serum concentrations of Na^+ and K^+ remained within normal limits.

The venous cannula was connected to a second infusion pump which delivered the radioactive precursors dissolved in isotonic saline at a constant rate, 0.624 ml/hr. The water-insoluble precursors, cholesterol, and 7α-hydroxycholesterol were "solubilized" in the saline with

Address requests for reprints to Dr. E. H. Mosbach, Public Health Research Institute, 455 First Avenue, New York, N. Y. 10016.

Abbreviations: GLC, gas–liquid chromatography; TLC, thin-layer chromatography.

Systematic names of the sterols and bile acids referred to in the text by their trivial names are as follows: cholesterol, cholest-5-en-3β-ol; 7α-hydroxycholesterol, cholest-5-en-3β,7α-diol; cholic acid, 3α,7α,12α-trihydroxy-5β-cholanoic acid; deoxycholic acid, 3α,12α-dihydroxy-5β-cholanoic acid; chenodeoxycholic acid, 3α,7α-dihydroxy-5β-cholanoic acid; α-muricholic acid, 3α,6β,7α-trihydroxy-5β-cholanoic acid; β-muricholic acid, 3α,6β,7β-trihydroxy-5β-cholanoic acid.

Tween 20 (5). The final concentration of Tween 20 was 0.05%, and that of the precursors 1–10 μg/ml. Bile was collected by a fraction collector at 6-hr intervals. Bile volume ranged from 1 to 1.5 ml/rat per hr.

Sodium Taurocholate

The bile salt was purchased from Calbiochem, Los Angeles, Calif. After drying in a vacuum oven at 60°C for 48 hr, the sample was of adequate purity as shown previously (2). Further purification of the sodium taurocholate (treatment with Darco G-60 followed by two crystallizations from 80% alcohol–ether), had no detectable effect on its physiological properties. The sodium taurocholate was dissolved in the liquid diet as required and infused via the duodenal cannula at rates ranging from 11 to 14 mg/100 g of rat per hr. The bile was analyzed for cholesterol, cholanoic acids, and radioactivity as described previously (2).

During periods of taurocholate infusion, the amounts of endogenous taurocholate could not be measured directly, but were calculated by assuming that the specific radioactivity of chenodeoxycholate and endogenous cholate were equal both before and during the experimental manipulations. The validity of this assumption is suggested, but not proven, by the data shown in Table 1. The amounts of α- and β-muricholic acid were calculated from the known specific radioactivity of taurochenodeoxycholate since these 6β-hydroxylated derivatives are known to be derived directly from chenodeoxycholate (6). The assumptions involved in these calculations have been discussed in a previous publication (2).

Labeled Compounds

Sodium acetate-1-^{14}C was purchased from New England Nuclear Corp. (Boston, Mass.), and was used without further purification. The radioactive purity of D,L-mevalonolactone-2-^{14}C (purchased from Amersham-Searle, Arlington Heights, Ill.) was shown to be greater than 98% by TLC.[1] Cholesterol-4-^{14}C (New England Nuclear Corp.) was shown to be 98% pure by TLC (7). It contained less than 0.1% of 7α-hydroxycholesterol. 7α-Hydroxycholesterol-4-^{14}C was prepared from cholesterol 4-^{14}C (8). It was shown to be 98–99% pure by TLC (4) and contained less than 0.5% cholesterol-4-^{14}C.

EXPERIMENTAL DESIGN

While the pathway from acetate to bile acids involves many individual chemical reactions, only a few are likely to be rate-limiting. In order to locate such steps, the incorporation of different labeled precursors into bile salts was measured in bile fistula rats (interrupted enterohepatic circulation). The effect of restoring the enterohepatic circulation by infusion of sodium taurocholate was then studied either in the same rat or in a second animal. It was reasoned that if rats with either intact or interrupted enterohepatic circulation convert a given precursor to bile acids at the same or similar rates, it cannot have been acted upon by a rate-limiting enzyme. Conversely, if a precursor is acted upon by a rate-limiting enzyme, then its rate of incorporation into bile acid would be smaller in a rat with an intact enterohepatic circulation than in an animal with biliary diversion. For example, if the rate-controlling enzyme were HMG-CoA reductase, the rate of incorporation of mevalonate into bile acids would be unaffected by the state of the enterohepatic circulation, while the incorporation of acetate should proceed faster in bile fistula rats than in animals in which the enterohepatic circulation had been restored by intraduodenal infusion of sodium taurocholate.

TABLE 1* SPECIFIC RADIOACTIVITIES OF TAUROCHENODEOXYCHOLATE AND TAUROCHOLATE 60 hr FOLLOWING INTERRUPTION OF ENTEROHEPATIC CIRCULATION†

Precursor	Rat No.	Specific Radioactivity‡ NaTCD	Specific Radioactivity‡ NaTCA
		dpm/mg	
Acetate-1-^{14}C	62	49,500	50,600
	63	58,800	56,200
D,L-Mevalonolactone-2-^{14}C	110	80,300	80,900
	75	122,000	125,100
Cholesterol-4-^{14}C	96	135,700	134,200
	89	145,900	147,200
7α-Hydroxycholesterol-4-^{14}C	93	4,900	4,640
	108	1,500	1,640
	115	10,450	10,620

* The following abbreviations are used in this table: NaTCA, sodium taurocholate; NaTCD, sodium taurochenodeoxycholate.

† Bile fistulas were established, and the rats were infused with different labeled precursors. Specific radioactivities of biliary bile acids were determined either 60 hr following surgery, or 60 hr after bile salt infusion had been discontinued. The specific radioactivities of the two primary bile acids were equal within the precision of measurement.

‡ Not corrected for difference in molecular weight between NaTCD and NaTCA.

RESULTS

Fig. 1 shows production of endogenous total bile salts and the incorporation of radioactivity from acetate-1-^{14}C into total bile salts during an experiment lasting 156 hr. 72 hr following surgery the animal had attained a fairly constant rate of acetate incorporation and bile acid production. Intraduodenal infusion of sodium taurocholate was then begun at a rate of 11.3 mg/100 g of rat per hr and was continued for 36 hr. In accord with our previously published data (2), bile acid production decreased

[1] Louw, A. I., and E. H. Mosbach. Unpublished observations.

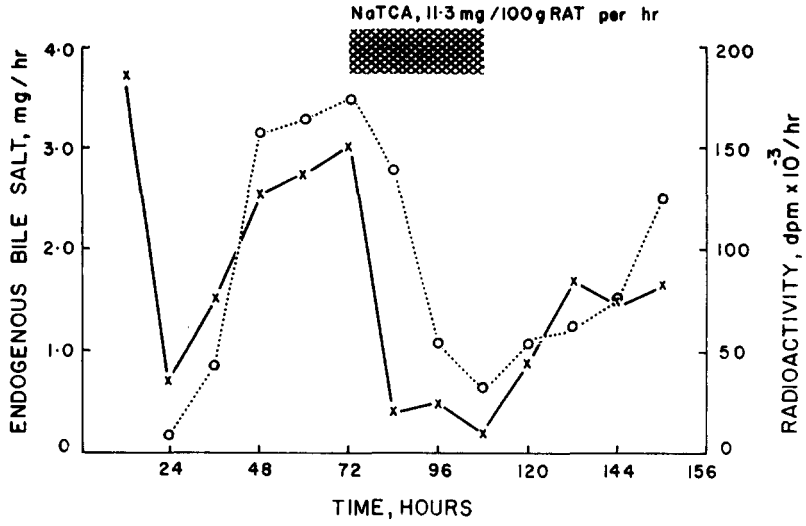

FIG. 1. Effect of taurocholate infusion on bile salt production and on the incorporation of acetate-1-^{14}C into biliary bile salts. Rat 63, weight 265 g, taurocholate pool 15.3 mg/100 g of rat. Intravenous infusion of acetate-1-^{14}C (5 μCi [0.205 mg]/hr) begun 12 hr after biliary diversion.

NaTCA, intraduodenal infusion of sodium taurocholate (11.3 mg/100 g of rat per hr) for 36 hr, begun 72 hr after surgery. Taurocholate infusion inhibited bile salt production by more than 80%. ×—×, total endogenous bile salt, mg/hr; O--O, radioactivity in total bile salts, dpm × 10^{-3}/hr.

by more than 80% when calculated either on the basis of acetate incorporation or by weight. When the infusion of taurocholate was discontinued, the inhibition of hepatic bile acid synthesis was released, and bile acid production tended to return to the values typical of bile fistula rats. Entirely analogous results were obtained in 11 additional experiments.

The time course of the inhibition was studied in more detail in eight rats. The bile was analyzed for taurochenodeoxycholate at hourly intervals following the start of the sodium taurocholate infusion. Three typical experiments are illustrated in Fig. 2. In all three animals, the concentration of biliary taurochenodeoxycholate was decreased by at least 70% at the end of 6 hr, and 90% at the end of 12 hr. However, the inhibition could not be demonstrated consistently at the end of 1 hr. Some animals (rat 110) actually exhibited an increase, others (rat 75) a decrease, and a third group (rat 64) was unchanged.

The release of the inhibition when sodium taurocholate infusion was discontinued was not equally rapid. As shown in Fig. 3, which is typical of a series of five experiments, usually a period of at least 48 hr was required for bile salt excretion to return to the maximal value observed in bile fistula rats. Taurocholate reached its maximum more slowly than taurochenodeoxycholate plus tauromurichcholates.

Fig. 4 illustrates one of five experiments in which D,L-mevalonolactone-2-^{14}C was employed as the labeled precursor. Bile acid production was allowed to approach a maximal value as determined by radioactivity and GLC data. Thus at the end of 72 hr, the total bile salt production was 2.28 mg/hr, and radioactivity in total bile salts was 93,500 dpm/hr. Although this is not shown in the figure, the radioactivity was distributed to an approximately equal extent between taurocholate and taurochenodeoxycholate plus its metabolites, the tauromurichcholates. Taurocholate infusion at the rate of 14 mg/100 g of rat per hr was started at the end of 72 hr and continued for 36 hr. This quantity of taurocholate inhibited the incorporation of mevalonate into bile salts by 87% based on radioactivity data, and by 98% on a weight basis.

Fig. 5 illustrates the inhibitory effect of sodium taurocholate on the incorporation of cholesterol-4-^{14}C into bile salts. Two rats of identical weight (310 g) were infused intravenously with identical amounts of cholesterol-4-^{14}C (1.01×10^6 dpm [1.97 μg]/hr) during the experimental period (60 hr). The control animal was not infused with taurocholate and incorporated 5×10^5 dpm/hr of cholesterol-4-^{14}C into total bile salt. The experimental animal, infused with taurocholate (14 mg/100 g of rat per hr), beginning 12 hr after surgery, incorporated only 67,000 dpm/hr into total bile salt.

Fig. 6 illustrates a different experiment with cholesterol-4-^{14}C as a precursor in which the experimental animal was used as its own control. After maximal incorporation of radioactivity into total bile salt (5×10^5 dpm/hr) had been reached (84 hr following surgery) sodium taurocholate was infused at a rate of 14 mg/100 g of rat per hr for 36 hr. Incorporation of cholesterol-4-^{14}C into total bile salt was inhibited by 93% based on radioactivity and

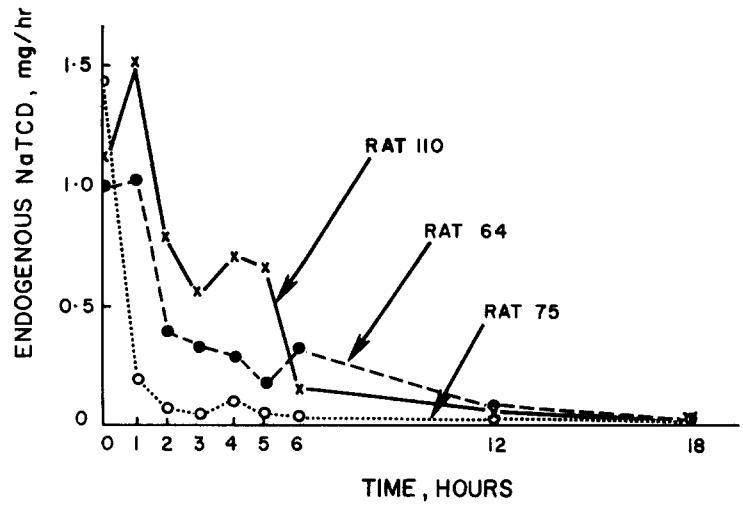

FIG. 2. Time course of inhibition of taurochenodeoxycholate production in three bile fistula rats infused with inhibitory amounts of taurocholate. Bile fistula rats were prepared and maintained for a period of 48 hr or until bile salt production had reached a maximal value. Sodium taurocholate was infused at a rate of 14 mg/hr, and biliary taurochenodeoxycholate was measured at intervals, as indicated. These experiments were selected because they were typical of the inhibitory response. NaTCD, sodium taurochenodeoxycholate.

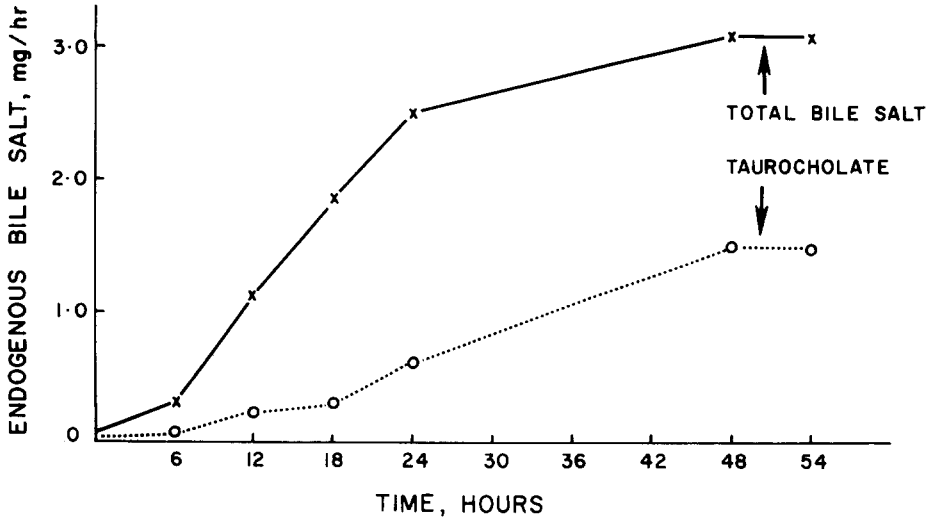

FIG. 3. Rate of restoration of maximal bile salt production in a bile fistula rat previously inhibited by taurocholate infusion. Rat 74, weight 230 g; bile salt biosynthesis had been inhibited for 84 hr by infusion of sodium taurocholate (13 mg/100 g of rat per hr) previous to period shown in graph. This slow rate of return to maximal bile salt synthesis was typical of the animals studied in these experiments. The difference between total bile salt and taurocholate excretion represents taurochenodeoxycholate plus tauromuricholates.

by 97% on a weight basis. The experiments shown in Figs. 5 and 6 are representative of a total of 12 such studies.

An experiment in which 7α-hydroxycholesterol-4-^{14}C was used as the labeled precursor is shown in Fig. 7. Following excretion of the pool (taurocholate pool 16.1 mg/100 g of rat; total bile salt pool 17.4 mg/100 g of rat), endogenous bile salt production was maintained at an inhibited level for 60 hr by infusing sodium taurocholate intraduodenally at a rate of 14 mg/100 g of rat per hr. No taurocholate was infused during the subsequent 72 hr. When the inhibition was thus released, the quantity of bile acid produced rose from 0.09 mg/hr to nearly 5 mg/hr. The incorporation of radioactivity into total bile salt remained fairly constant during the entire experimental period; average incorporation of ^{14}C from 7α-hydroxycholesterol was 2970 dpm/hr during taurocholate infusion and 3350 dpm/hr after taurocholate infusion was discontinued.

Fig. 8 illustrates data from an additional experiment

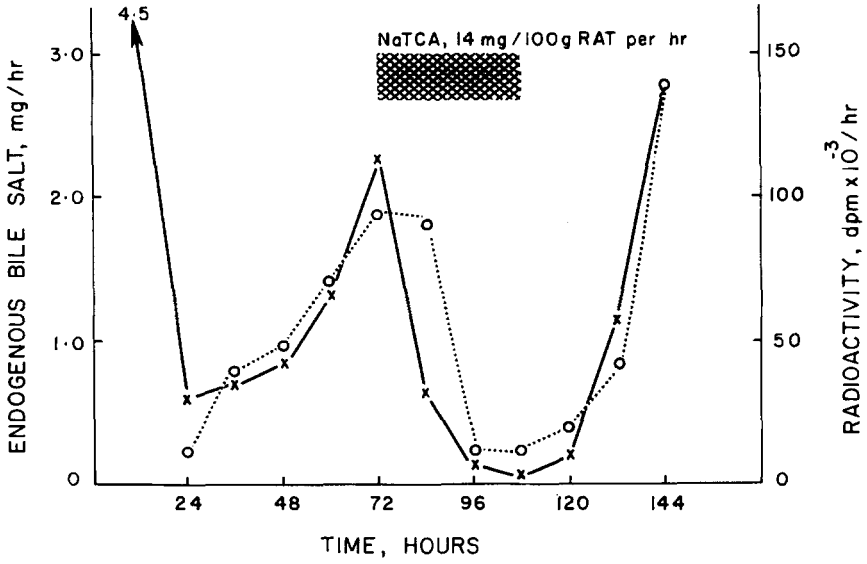

FIG. 4. Effect of taurocholate infusion on bile salt production and on the incorporation of D,L-mevalonolactone-2-^{14}C into biliary bile salts. Rat 111, weight 360 g, taurocholate pool 13.8 mg/100 g of rat. Intravenous infusion of D,L-mevalonolactone-2-^{14}C (L-mevalonolactone-2-^{14}C, 708,000 dpm [5 μg]/hr) begun 12 hr after biliary diversion.

NaTCA, intraduodenal infusion of sodium taurocholate (14 mg/100 g of rat per hr) for 36 hr, begun 72 hr after surgery. Sodium taurocholate infusion produced approximately 87% inhibition in the incorporation of ^{14}C into total bile salts, and 98% inhibition in production of endogenous total bile salts. ×—×, total bile salts in bile, mg/hr; O----O, radioactivity in total bile salt, dpm × 10^{-3}/hr.

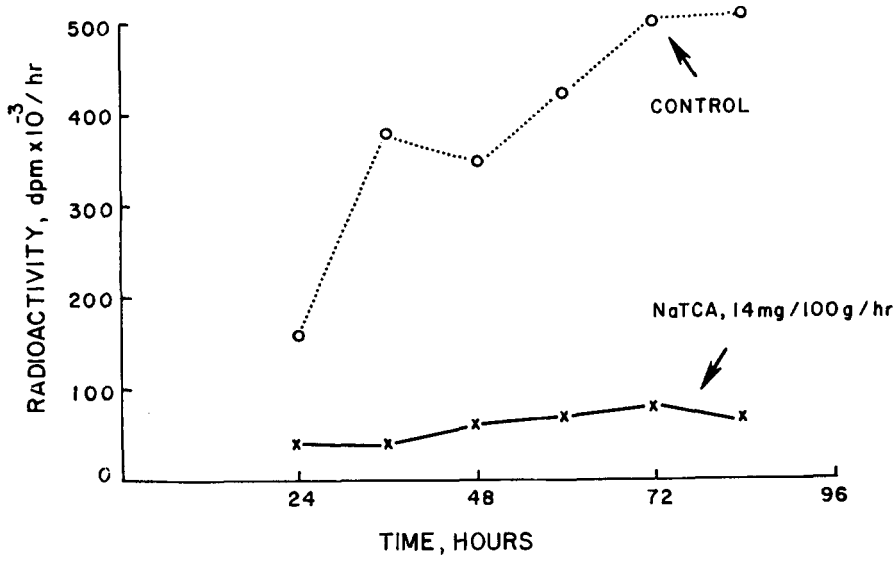

FIG. 5. Effect of intraduodenal infusion of sodium taurocholate (14 mg/100 g of rat per hr) on incorporation of cholesterol-4-^{14}C into biliary total bile salts. Both animals received cholesterol-4-^{14}C (1.01 × 10^6 dpm [1.97 μg]/hr) by intravenous infusion starting 12 hr after surgery. Rat 112 was given intraduodenal infusion of sodium taurocholate starting 12 hr after surgery. ×—×, radioactivity in biliary bile salts, dpm × 10^{-3}/hr; rat 112, experimental, weight 310 g, taurocholate pool 19.3 mg/100 g of rat. O---O, radioactivity in biliary bile salts, dpm × 10^{-3}/hr, rat 113, control, weight 310 g, taurocholate pool, 17.3 mg/100 g of rat. The infusion of taurocholate inhibited incorporation of ^{14}C from cholesterol into total bile salt by approximately 87%.

with 7α-hydroxycholesterol-4-^{14}C. In this study the animal was allowed to attain maximal bile acid synthesis (at the end of 84 hr) at which point sodium taurocholate was infused at the rate of 14 mg/100 g of rat per hr for the subsequent 36 hr. While the sodium taurocholate inhibited bile acid biosynthesis (total bile salt excretion fell from 2.4 mg/hr to 0.08 mg/hr), the incorporation of radioactivity remained unchanged throughout the ex-

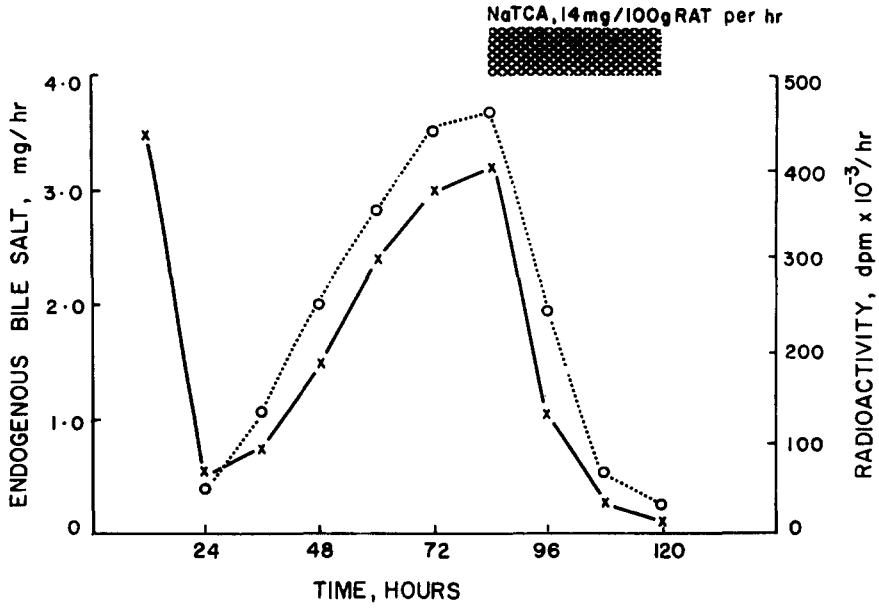

FIG. 6. Effect of taurocholate infusion on bile salt production and on the incorporation of cholesterol-4-^{14}C into biliary bile salts. Rat 89, weight 285 g, taurocholate pool 14.2 mg/100 g of rat. Intravenous infusion of cholesterol-4-^{14}C (658,000 dpm [2 μg]/hr) begun 12 hr after biliary diversion.

NaTCA, intraduodenal infusion of sodium taurocholate (14 mg/100 g of rat per hr) for 36 hr, begun 72 hr after surgery. Sodium taurocholate infusion produced approximately 93% inhibition in the incorporation of ^{14}C into total bile salts, and 97% inhibition in production of endogenous total bile salts. ×—×, total bile salts in bile, mg/hr; O---O, radioactivity in total bile salt, dpm × 10^{-3}/hr.

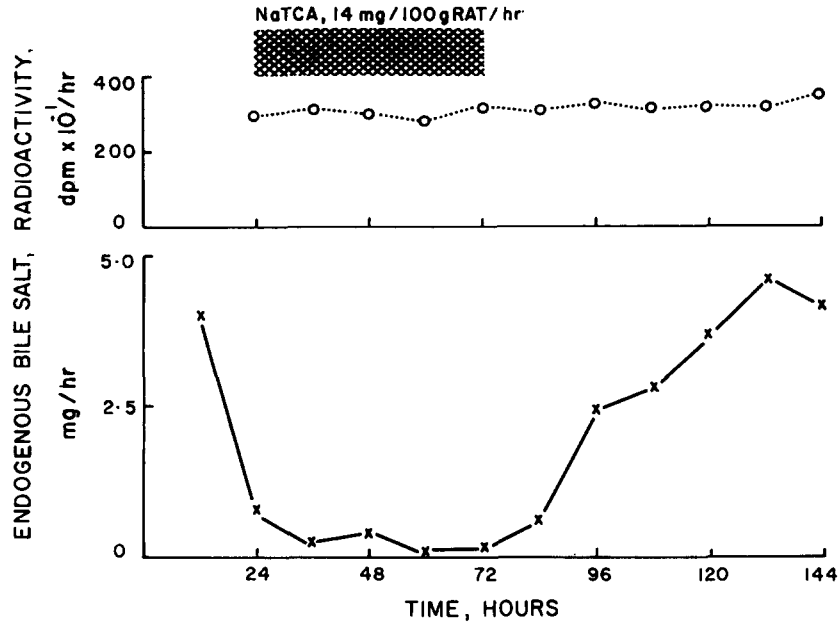

FIG. 7. Effect of taurocholate infusion on bile salt production and on the incorporation of 7α-hydroxycholesterol-4-^{14}C into biliary bile salts. Rat 107, weight 280 g, taurocholate pool 16.1 mg/100 g of rat. Intravenous infusion of 7α-hydroxycholesterol (10,420 dpm [7 μg]/hr) begun 12 hr after biliary diversion.

NaTCA, intraduodenal infusion of sodium taurocholate (14 mg/100 g of rat per hr) for 60 hr begun 12 hr after surgery. The infused bile salt prevented the typical increase in bile salt production usually observed in bile fistula rats (see Fig. 3). This inhibition was maintained as long as the sodium taurocholate was infused. However, the incorporation of 7α-hydroxycholesterol-4-^{14}C into bile salt was inhibited only 11% during taurocholate infusion. ×—×, total bile salts in bile, mg/hr; O---O, radioactivity in total bile salts, dpm × 10^{-1}/hr.

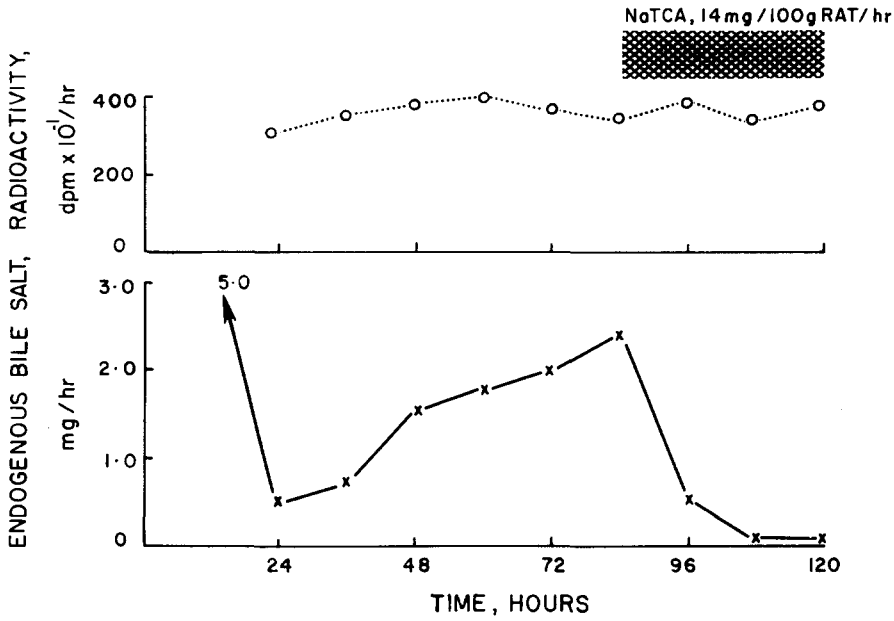

FIG. 8. Effect of taurocholate infusion on bile salt production and on the incorporation of 7α-hydroxycholesterol-4-^{14}C into biliary bile salts. Rat 108, weight 280 g, taurocholate pool, 18.6 mg/100 g of rat Intravenous infusion of 7α-hydroxycholesterol-4-^{14}C (10,420 dpm [7 μg]/hr) begun 12 hr after biliary diversion.

NaTCA, intraduodenal infusion of sodium taurocholate (14 mg/100 g of rat per hr) begun 84 hr after surgery and continued for 36 hr. Sodium taurocholate inhibited endogenous bile salt production by 96.5%, but had no effect on the incorporation of 7α-hydroxycholesterol-4-^{14}C into bile salt.

periment. While this is not apparent from the figure, it should be noted that the incorporation of radioactivity into neither taurochenodeoxycholate nor taurocholate was affected. The data obtained in these and eight additional experiments clearly indicate that the transformation of 7α-hydroxycholesterol into bile salt is not affected by the quantity of bile salt returning to the liver via the enterohepatic circulation.

The results of all experiments are summarized in Table 2. The data in this table were obtained by measuring the percentage change in the amounts of bile salts (taurochenodeoxycholate plus metabolites and taurocholate) secreted before and 36 hr after, infusion of inhibitory amounts of Na taurocholate. The corresponding percentage changes in the incorporation of the four radioactive precursors are shown in adjacent columns. These data again illustrate that the incorporation of three of these precursors (acetate-1-^{14}C, D,L-mevalonolactone-2-^{14}C, and cholesterol-4-^{14}C) into bile acids is strongly inhibited in the presence of a circulating bile acid pool. In contrast, 7α-hydroxycholesterol-4-^{14}C is incorporated into bile salts to approximately the same extent in rats with intact or interrupted enterohepatic circulation. The maximal inhibition observed in the 10 experiments averaged less than 10% suggesting that the incorporation of 7α-hydroxycholesterol into bile salts was largely independent of the presence of the bile acid pool.

DISCUSSION

These experiments confirm our previous observation (2) that hepatic bile acid synthesis is regulated by the amount of bile acid returning to the liver via the enterohepatic circulation. The results of the present experiments further suggest that negative feedback control of bile acid biosynthesis from *cholesterol* is exerted via the enzyme which catalyzes the 7α-hydroxylation of cholesterol. These data are in accord with earlier in vitro studies from this and other laboratories (3, 4) implicating cholesterol 7α-hydroxylase as the rate-controlling enzyme of bile acid synthesis.

The present experiments do not enable us to decide whether there are additional rate-controlling steps of bile salt formation preceding the 7α-hydroxylation of cholesterol. The formation of bile acids would seem to depend upon an adequate supply of liver cholesterol so that an inhibition of cholesterol biosynthesis should be associated with a reduction of bile acid synthesis. Since the rate-controlling enzyme of hepatic cholesterol biosynthesis appears to be HMG-CoA reductase (9, 10), factors reducing the activity of this enzyme, for example fasting (11), should also tend to reduce bile acid production. However, attempts to demonstrate that the activity of HMG-CoA reductase is controlled by bile acids in vivo or in vitro have not been conclusive (3, 12).

There was a slight inhibition in the incorporation of

TABLE 2* DECREASE IN BILE ACID SYNTHESIS AND IN INCORPORATION OF LABELED PRECURSORS INTO BILE ACIDS IN RESPONSE TO INHIBITORY AMOUNTS OF TAUROCHOLATE†

Tracer	No. of Experiments	Maximal Percentage Decrease in NaTCD + TαM + TβM‡		Maximal Percentage Decrease in Endogeneous NaTCA‡	
		By Weight	By Radioactivity	By Weight	By Radioactivity
Acetate-1-^{14}C	12	95.0 (1.6)§	90.1 (1.9)	97.3 (1.5)	92.6 (1.6)
D,L-Mevalonolactone-2-^{14}C	5	96.9 (0.6)	91.2 (4.6)	97.5 (0.8)	91.8 (2.9)
Cholesterol-4-^{14}C	12	96.5 (1.0)	88.9 (5.1)	98.9 (0.5)	96.9 (1.4)
7α-Hydroxycholesterol-4-^{14}C	10	96.5 (1.1)	9.5 (3.0)	96.9 (1.4)	9.6 (2.3)

* The following abbreviations are used in this table: NaTCD, sodium taurochenodeoxycholate; NaTCA, sodium taurocholate; TαM, sodium tauro-α-muricholate; TβM, sodium tauro-β-muricholate.

† Bile fistula rats were infused with a labeled precursor, i.v., 12 hr after surgery. Bile acid biosynthesis was inhibited by intraduodenal infusion of NaTCA (11–14 mg/100 g of rat per hr). Bile acid synthesis in the noninhibited state was measured either before NaTCA infusion when the rat was producing maximal amounts of bile acids, or 48 hr after NaTCA infusion had been discontinued.

‡ Measured after 36–48 hr of TC infusion.

§ Standard deviation.

7α-hydroxycholesterol into bile acids in rats receiving intraduodenal infusion of sodium taurocholate. Therefore, it seems possible that one or more additional steps on the pathway following the synthesis of 7α-hydroxycholesterol are affected to some extent by the circulating bile acid pool. Since the inhibition observed was small, it is presumed that such steps are not of major importance in the regulation of bile acid synthesis. One such additional site of regulation may be located at the mitochondrial enzyme system which cleaves the cholesterol side chain (13).

In any case, it seems likely that once 7α-hydroxycholesterol has been formed, the *quantity* of bile acid produced by the liver cells is no longer under control of the circulating bile acid pool. However, the *type* of bile acid produced that is, the cholic acid–chenodeoxycholic acid ratio, seems to be regulated, at least in part, by the enzyme which catalyzes the 12α-hydroxylation of 7α-hydroxycholest-4-en-3-one (14). This conclusion is also based on experiments dealing with the effect of thyroid hormone on bile composition (15). As the present time, it is not possible to interpret the biological role of the mitochondrial system which oxidizes the side chain of cholesterol since this system has also been shown to be sensitive to hormonal influence (13).

These experiments give no information on the mechanism whereby bile salts regulate the activity of cholesterol 7α-hydroxylase. It seems likely, however, that the synthesis and degradation of a protein are involved. It has been calculated that the half-lifetime for the breakdown of the 7α-hydroxylase in bile fistula rats is 2–3 hr (16). Our experiments dealing with the rate of inhibition of the 7α-hydroxylase during bile salt infusion (Fig. 2) appear to be in accord with this conclusion. However, the rate of biosynthesis of the enzyme appears to be much slower, at least on the basis of the time interval required for bile fistula rats to attain maximal rates of bile acid biosynthesis (Fig. 4).

This work was supported in part by Research Grants AM-05222 and HE-10894 from the National Institutes of Health.

Manuscript received 16 February 1970; accepted 30 April 1970.

REFERENCES

1. Kritchevsky, D. 1958. Cholesterol. John Wiley & Sons, Inc., New York. 120.
2. Shefer, S., S. Hauser, I. Bekersky, and E. H. Mosbach. 1969. *J. Lipid Res.* **10:** 646.
3. Danielsson, H., K. Einarsson, and G. Johansson. 1967. *Eur. J. Biochem.* **2:** 44.
4. Shefer, S., S. Hauser, and E. H. Mosbach. 1968. *J. Lipid Res.* **9:** 328.
5. Siperstein, M. D., and I. L. Chaikoff. 1952. *J. Biol. Chem.* **198:** 93.
6. Voigt, W., P. J. Thomas, and S. L. Hsia. 1968. *J. Biol. Chem.* **243:** 3493.
7. Waldi, D. 1965. *In* Thin-Layer Chromatography. E. Stahl, editor. Springer-Verlag K.G., Berlin. 252.
8. Fieser, L. F., J. E. Herz, M. W. Klohs, M. A. Romero, and T. Utne. 1952. *J. Amer. Chem. Soc.* **74:** 3309.
9. Bucher, N. L. R., P. Overath, and F. Lynen. 1960. *Biochim. Biophys. Acta.* **40:** 491.
10. Hamprecht, B. 1969. *Naturwissenschaften.* **56:** 398.
11. Hamprecht, B., C. Nüssler, and F. Lynen. 1969. *FEBS Letters.* **4:** 117.
12. Back, P., B. Hamprecht, and F. Lynen. 1969. *Arch. Biochem. Biophys.* **133:** 11.
13. Mitropoulos, K. A., and N. B. Myant. 1965. *J. Biochem. (Tokyo).* **94:** 594.
14. Einarsson, K. 1968. *Eur. J. Biochem.* **5:** 101.
15. Eriksson, S. 1957. *Proc. Soc. Exp. Biol. Med.* **94:** 578.
16. Einarsson, K., and G. Johansson. 1968. *FEBS Letters.* **1:** 219.