Examination of bile acid negative feedback regulation in rats

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Abstract Recent data obtained using cultured rat hepatocytes showed that bile acids do not inhibit bile acid synthesis, whereas cholesterol concentrations vary in parallel with bile acid synthesis (Davis et al. (1983. *J. Biol. Chem.* **258:** 4079–4082). This led us to re-evaluate in vivo experiments upon which the consensus that bile acid synthesis is primarily regulated by bile acid "negative feedback" is based. Infusion of taurocholate into either the jugular vein or duodenum of bile-diverted rats stimulated biliary cholesterol secretion and bile flow, but it did not inhibit bile acid synthesis. The lack of an inhibitory effect was evident using several different infusion rates of taurocholate. Even at the greatest rate of taurocholate infusion (25 μmol/(100 g · hr)) there was no significant inhibition of bile acid synthesis. In contrast, infusing mevinolin (1 mg/hr), a potent competitive inhibitor of HMG-CoA reductase, almost completely inhibited bile acid synthesis and biliary cholesterol secretion. Since mevinolin did not affect bile flow, these results cannot be ascribed to bile secretory failure. Thus, while these studies suggest that taurocholate may not regulate bile acid synthesis directly via negative feedback, cholesterol is likely to act as a positive effector of bile acid synthesis. —**Davis, R. A., C. A. Musso, M. Malone-McNeal, G. R. Lattier, P. M. Hyde, J. Archambault-Schexnayder, and M. Straka.** Examination of bile acid negative feedback regulation in rats. *J. Lipid Res.* 1988. **29:** 202–211.

Supplementary key words bile acid synthesis • negative feedback regulation • hepatotoxicity • cholesterol availability

The catabolism of cholesterol to bile acids is the predominant pathway through which cholesterol is eliminated from the mammalian body (1). Since biliary cholesterol secretion is dependent on bile acid secretion (2, 3), additional cholesterol is secreted in bile (mainly in the form of bile acid-phospholipid mixed micelles). Thus, the total amount of cholesterol which is removed via the biliary system is the sum of that which is catabolized to bile acids plus the amount transported across the canaliculus in the form of biliary free cholesterol.

The factors and mechanisms responsible for regulating bile acid synthesis are poorly understood. In some biochemistry text books there is implicit and often explicit reference to the existence of "negative feedback regulation" of bile acid synthesis (4, 5). However, because of the inherent amphipathic nature of bile acids, it is difficult to experimentally show that bile acids inhibit bile acid synthesis via a physiologic mechanism that is free from toxic effects. Bile acid "negative feedback regulation" was developed to account for the stimulation of bile acid synthesis that occurs in response to diversion of bile components from the enterohepatic circulation. There is no doubt that diversion of bile components via bile duct cannulation and by cholestyramine feeding results in increased synthesis of bile acids (6–9). However, attempts to reconstruct the enterohepatic circulation by infusing bile acids into bile-diverted rats have led to discrepant results in regard to their ability to suppress bile acid synthesis in a specific manner (10–15).

The development of in vitro hepatocyte models afforded the opportunity to examine the direct effects of bile acids on bile acid synthesis. Botham et al. (16) showed that bile acids at concentrations similar to those found in portal blood did not inhibit bile acid synthesis by suspended hepatocytes. Recently, we found that bile acids at concentrations that were up to 50 times their concentration in portal blood had no effect on bile acid synthesis by cultured rat hepatocytes (17). However, bile acid synthesis was sensitive to changes in the availability of cellular cholesterol (altered by changes in its synthesis and by uptake of specific lipoprotein particles) (18). Similar results showing that bile acids do not affect bile acid synthesis by

Abbreviations: TLC, thin-layer chromatography; GLC, gas–liquid chromatography; HMG-CoA reductase, 3-hydroxy-3-methylglutaryl CoA reductase; DME, Dulbecco's modified Eagle's medium. The term bile acids is used to signify both bile acids and bile salts.

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rat hepatocytes were subsequently reported by others (19).

A potential problem with data obtained with cultured cells is relating findings to the in vivo situation. Because of the fundamental role that bile acid synthesis plays in regard to cholesterol homeostasis, it is essential to determine whether or not bile acids regulate bile acid synthesis. This information is required to focus efforts toward gaining an understanding of the regulation of bile acid synthesis. Therefore, we have extended our studies to the bile-diverted rat model. Our results show that infusion of taurocholate, at rates used by others (12–14), does *not* inhibit bile acid synthesis in bile-diverted rats.

MATERIALS AND METHODS

Glucose-6-phosphate, NADP, bile acid standards, β-mercapto-ethylamine, glucose-6-phosphate dehydrogenase, 3α-steroid dehydrogenase, buffers, and taurocholate were obtained from Sigma Chemical Co., St. Louis, MO. The taurocholate was found to be 100% conjugated (TLC) and to be 98% cholate (GLC) (18). TLC plates were purchased from Analtech, Newark, DE. Solvents were obtained from Baker Chemicals, Phillipsburg, NJ. Sterol standards were obtained from Steraloids, Wilton, NH. Radioisotopes were obtained from New England Nuclear, Boston, MA.

Male Sprague-Dawley rats weighing 250–350 g were purchased from Holtzman, Madison, WI. Rats were fed a standard chow diet ad libitum and drinking water. Rats were exposed to two different light cycles: light from either 0700–1900 hr (control light) or 1600–0400 hr (dark-adapted light) for at least a week prior to use. Rats were used between 0830 and 0900 hr.

Measurement of biliary bile acid secretion

Jugular vein infusions. Jugular vein catheters (using PE-50 tubing) and bile duct catheters (using PE-10 tubing) were placed as described in detail (20). Rats were placed in Bollman restraining cages and infused with a sterile solution containing 5% dextrose in 0.5% saline. At the time indicated (see legends) taurocholate was added to the infusate at a concentration appropriate for the bile acid flux to be as indicated in legends. The infusion rate was constant (1 ml/hr). Rats had free access to 2% $NaHCO_3$ in drinking water (to maintain electrolyte balance) and rat chow throughout the entire experiment. Bile was collected in hourly samples using a fraction collector.

Intestinal infusions. Rats were treated as described above, except no catheter was placed into the jugular vein. Instead, a catheter (PE-10) was inserted into the distal bile duct. A solution was infused into the bile duct at a rate of 3 ml/hr. During the control periods, the rats received 20% Dulbecco's modified Eagle's medium (DME) containing 5% dextrose. During the taurocholate infusion periods, rats received taurocholate dissolved in 5% dextrose and 20% DME at a concentration such that at an infusion rate of 3 ml/hr the amount of bile acid infused into the duodenum was equal to that described in the legends.

The taurocholate solutions contained a known amount of [24-^{14}C]taurocholate. The amount of taurocholate infused was accurately determined by infusing the same taurocholate solution directly into a duplicate fraction collector. The configuration of the infusion pump (Sage Instruments, White Plains, NY) was such that two syringes were driven by the same gear driver. In other words, the flow out of both syringes had to be the same. By determining the volume of and amount of [24-^{14}C]taurocholate in the "dummy" fraction collector, we insured the accuracy of the amount of taurocholate infused into each rat. The results showed that within an hour of changing the infusion solutions, the flow of the infusate became stable. The recovery of [24-^{14}C]taurocholate in bile was determined for each hourly point. Except for the first hour of infusion, the recovery in bile of [24-^{14}C]taurocholate was not significantly different from 100% (data not shown).

In some experiments, instead of infusing rats with [24-^{14}C]taurocholate, rats were infused with [2-^{14}C]mevalonic acid (1 μCi/ml) (see Figs. 1 and 2). In these experiments, during the taurocholate infusion period, no [24-^{14}C]taurocholate was added. Bile in these experiments was extracted with hexane and the hexane extracts were separated by TLC (20) in order to quantitate the amount of [^{14}C]cholesterol. Preliminary experiments showed that using the above procedure, the recovery from bile of [4-^{14}C]cholesterol standard was quantitative (97%). The amount of ^{14}C-labeled bile acids was quantitated following extraction of bile with $CHCl_3$–MeOH 1:2 (v/v) and separation on glass fiber plates as described (17). The values of ^{14}C-labeled bile acids reported are the combined total of all bile acids. Preliminary experiments showed that the recovery of [24-^{14}C]taurocholate standard from bile was quantitative (98%).

Bile flow was determined by weight. Bile acid concentrations were determined using the 3α-steroid dehydrogenase assay as described in detail (20). Cholesterol concentrations in bile were determined by GLC as previously described (18).

Statistical analysis

All values are reported as the mean and the standard deviation of the mean (triplicate assays for each data point). The numbers of individual rats are reported in each legend. Differences of means were determined to be statistically significant using Student's t test. Linear regression analysis was performed on a Hewlett-Packard calculator. Statistical analysis of individual time points (taurocholate infusion studies) was performed via two-way analysis of variance with repeated measures design

using an Apple II computer and a statistical program. Values of $P < 0.05$ were considered to be significant.

RESULTS

Effect of intravenously administered taurocholate on bile acid synthesis

In the first series of experiments, we determined the effect of intravenously administered taurocholate on the synthesis and secretion of de novo synthesized ^{14}C-labeled bile acids. Bile-diverted rats were infused with [2-^{14}C]mevalonic acid in order to label the cholesterol pool. After the secretion rate of ^{14}C-labeled bile acids became stable (48 hr, **Fig. 1**), taurocholate was infused at a rate of 12 μmol/(100 g · hr) for 24 hr. After this time, the rats were infused with saline. During the taurocholate infusion period there was no significant inhibition in the secretion of ^{14}C-labeled bile acids (Fig. 1).

Bile acid secretion is known to augment biliary cholesterol secretion (2, 3). As shown in **Fig. 2,** the secretion

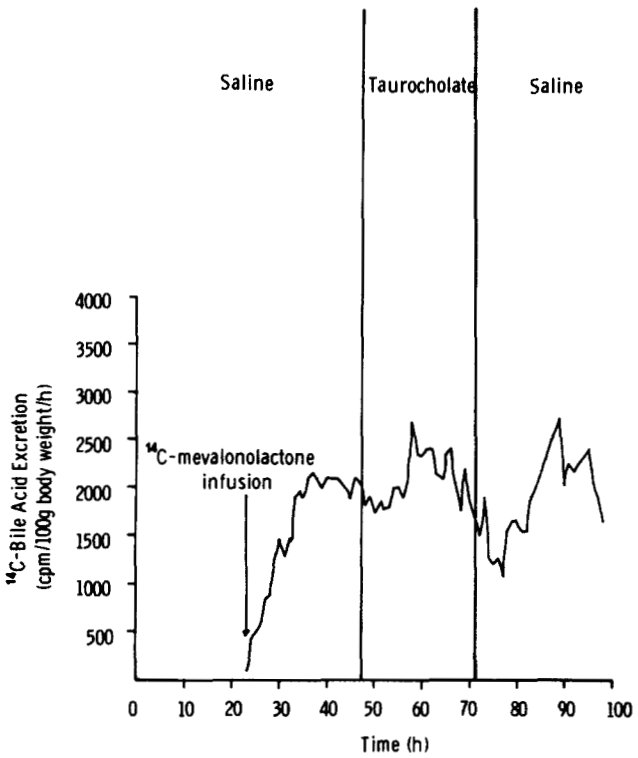

Fig. 1. Effect of taurocholate on the synthesis of ^{14}C-labeled bile acids. Rat number 1, Table 1 was infused with glucose/saline for 48 hr. At hour 20, the infusate was changed to one that contained [2-^{14}C]mevalonic acid (1 μCi/ml). At hour 48, the infusate was changed to one that contained [2-^{14}C]mevalonic acid and taurocholate so that the delivery rate was 12 μmol/(100 g · hr). At hour 72, the infusate was changed to one that contained [2-^{14}C]mevalonic acid, but no taurocholate. Bile secreted into a bile duct catheter was collected in hourly samples using a fraction collector. The amount of ^{14}C-labeled bile acids secreted was determined for each sample as described in Methods.

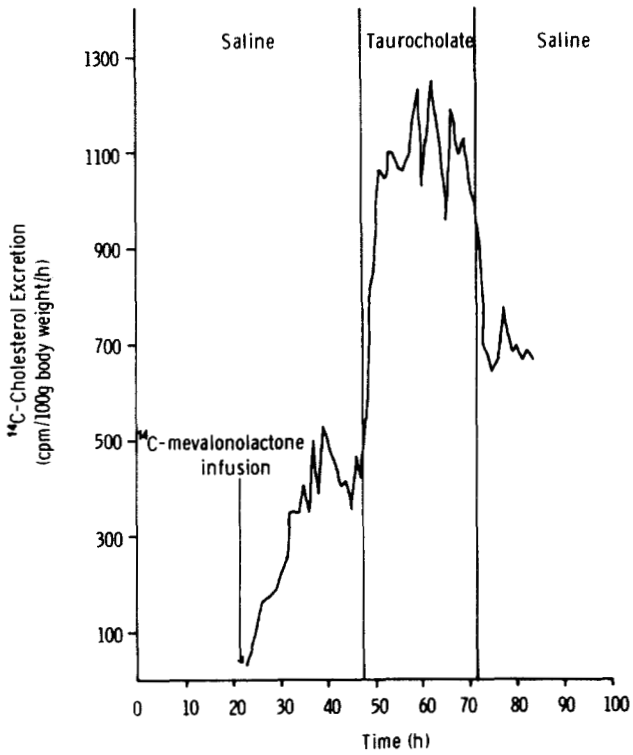

Fig. 2. Effect of taurocholate on the secretion of [^{14}C]cholesterol. The hourly secretion of biliary [^{14}C]cholesterol produced in the experiment outlined in Fig. 1 was quantitated as described in Methods. During the taurocholate infusion period there was significantly more [^{14}C]cholesterol secreted.

of ^{14}C-labeled biliary cholesterol was increased during the period of taurocholate infusion. Cessation of taurocholate infusion caused ^{14}C-labeled cholesterol secretion to return toward rates encountered before the taurocholate was infused. In control rats not infused with taurocholate, the secretion of ^{14}C-labeled bile acids and cholesterol remained stable from hours 36–90, indicating the changes were caused by the taurocholate.

It is possible that these results reflect changes in the labeling of biliary cholesterol/bile acid precursor pools. Therefore, we also performed similar experiments and quantitated bile acid mass secretion. In these studies bile-diverted rats were infused intravenously with [24-^{14}C]taurocholate. This allowed us to determine accurately the amount of secreted biliary bile acid that was contributed by de novo synthesis (i.e., the total amount secreted – the amount infused).

The amount of [24-^{14}C]taurocholate that was recovered in bile was slightly variable but was almost quantitative (about 100%, data not shown). When we compared the amount of [24-^{14}C]taurocholate recovered in bile with the amount infused (obtained using a "dummy" syringe, see Methods section) there were slight (<15%) differences. These differences were random. Since the infusion rate was constant (as demonstrated by the "dummy" syringe),

it is likely that the variation in recovery was biological in nature. Therefore, the data presented in this report were derived as the difference between the total amount of bile acid secreted (for each hourly time point) and the amount of [24-^{14}C]taurocholate recovered in bile.

Subtraction of the amount of [24-^{14}C]taurocholate recovered in bile from the total amount of bile acid secreted showed that taurocholate infusion (12 μmol/(100 g · hr) did not significantly inhibit the secretion of de novo synthesized bile acids (**Fig. 3**). This experiment was repeated seven times and a summary of the data and statistical analysis is presented in **Table 1.** Because the bile acid secretion was variable, we would not be able to detect small differences (<25%) in bile acid secretion. However, as shown in Fig. 3 and summarized in Table 1, there clearly was no significant decrease or increase of the secretion of de novo synthesized bile acids during the taurocholate infusion period. In the summary (Table 1) mean values are reported. We also analyzed (two-way analysis of variance with repeated measures design) individual hourly points during the taurochoate infusion period and found that there was no significant difference in the rate of bile acid secreted. Furthermore, cessation of the taurocholate infusion also did not alter the rate of secretion of de novo synthesized bile acids.

In contrast to there being no effect on bile acid synthesis during the period of taurocholate infusion, the secretion of cholesterol (mass) was significantly increased (+40%, **Fig. 4**). We also calculated our data using 24-hr periods to avoid possible influences of diurnal variation in a manner similar to Pries et al. (14). We obtained similar results (i.e., taurocholate did not significantly inhibit bile acid secretion).

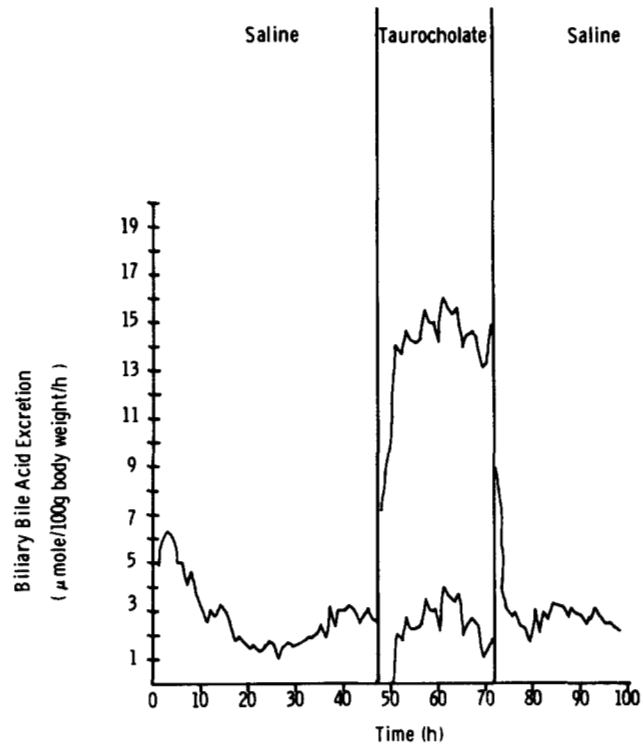

Fig. 3. Effect of intravenous taurocholate on bile acid synthesis. Rat number 2, Table 1, was infused, via the jugular vein, with glucose/saline for 48 hr. The infusate was then changed to one that contained [24-^{14}C]taurocholate so that the rate of delivery was 12 μmol/(100 g · hr) for 24 hr. The infusate was then changed to one that contained glucose/saline only. Bile was collected in hourly samples and the amount of bile acids was quantitated using 3α-steroid dehydrogenase as described in Methods. For the taurocholate infusion period, the top line is total amount of bile acids secreted, whereas the bottom line is the difference between the total amount secreted and the amount of [24-^{14}C]taurocholate recovered in bile. During the taurocholate infusion period, bile acid synthesis was not significantly inhibited.

TABLE 1. Effect of taurocholate infusion on bile acid synthesis[a]

Experiment Number	Taurocholate Infusion (μmol/(100 g · hr))	Bile Acid Excretion (μmol/(100 g · hr))		
		Pre-Infusion (12 hr before taurocholate)	Infusion Period (12 hr after start)	Post-Infusion (12 hr after taurocholate)
1[b]	12 μmol i.v. for 24 hr	2.9 ± 0.8	3.3 ± 0.8	2.3 ± 0.4
2[b]	12 μmol i.v. for 24 hr	2.6 ± 0.7	2.4 ± 1.2	2.4 ± 0.4
3[b]	12 μmol i.v. for 36 hr	5.7 ± 1.3	4.6 ± 3.0	2.6 ± 0.9
4[b]	14.4 μmol i.v. for 40 hr	3.4 ± 1.4	3.6 ± 1.5	5.4 ± 1.2
5[b]	11 μmol i.v. for 34 hr	3.7 ± 0.7	4.2 ± 1.1	5.8 ± 1.0
6[b]	12.5 μmol i.v. for 32 hr	3.7 ± 1.4	7.1 ± 1.8	6.5 ± 1.3
7[c]	25 μmol i.v. for 12 hr	5.2 ± 1.8	3.7 ± 1.6[d]	4.6 ± 1.2
8[e]	20 μmol i.d. for 22 hr	4.7 ± 1.2	6.3 ± 1.7	5.2 ± 1.3
9[e]	20 μmol i.d. for 23 hr	4.1 ± 0.8	3.7 ± 1.7	4.7 ± 1.0
10[e]	20 μmol i.d. for 22 hr	3.6 ± 1.3	2.9 ± 1.6	2.6 ± 1.4

[a] All values are the mean ± SD of each hourly point for the time period listed; i.v., intravenously; i.d., intraduodenally.

[b] Rats with bile duct cannula and jugular vein catheter were infused first with glucose/saline for a total of 48 hr, then with taurocholate (for the time indicated), and then with glucose/saline.

[c] Rats with bile duct cannula and jugular vein catheter were infused with glucose/saline, then with taurocholate (5 hr with 5 μmol/(100 g · hr), 5 hr with 10 μmol/(100 g · hr), 5 hr with 15 μmol/(100 g · hr), 5 hr with 20 μmol/(100 g · hr), and 12 hr with 25 μmol/(100 g · hr), after which the infusate was changed to glucose/saline for 24 hr. During all taurocholate infusion periods, bile acid synthesis was not significantly inhibited.

[d] Corresponds to the period of infusing 25 μmol/(100 g · hr).

[e] Rats with two bile duct cannulae (one distal, one proximal) were infused (distal) with glucose/saline for 48 hr, then with taurocholate 20 μmol/(100 g · hr) (for the time indicated) and then with glucose/saline for 24 hr.

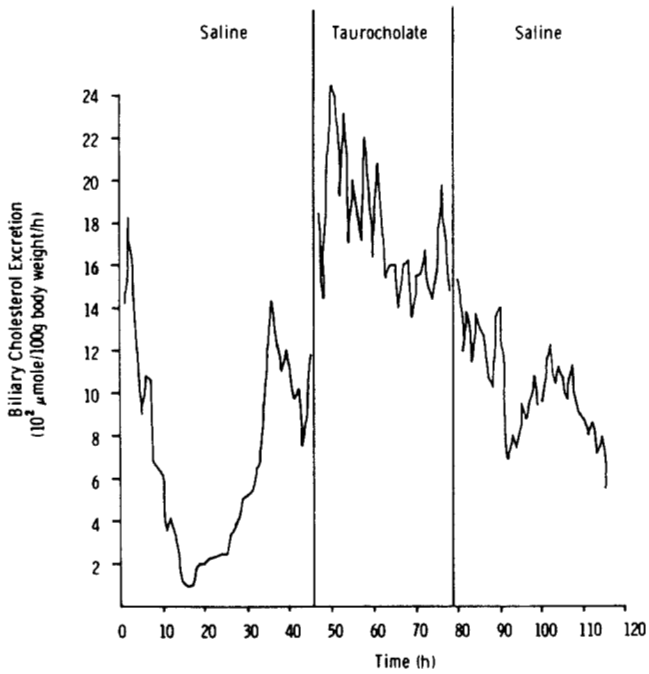

Fig. 4. Effect of intravenous taurocholate on biliary cholesterol secretion. Rat number 6, Table 1, was infused with glucose/saline for 46 hr, after which the infusate was changed to one that contained [24-^{14}C]taurocholate so that the rate of delivery was 12.5 μmol/(100 g · hr). After 32 hr the infusate was changed to one that contained glucose/saline only. Bile was collected in hourly samples and the amount of cholesterol was determined by GLC as described in Methods. Statistical analysis of the data obtained during the taurocholate infusion period showed that except for hours 65 and 69 there was significantly more cholesterol secreted.

Bile acid secretion also stimulates bile flow (21, 22). Bile flow is a sensitive parameter of hepatic function (23). To insure that the rates of taurocholate infusion were not too great (toxic), we determined bile flow. As shown in **Fig. 5,** intravenous infusion of taurocholate rapidly stimulated bile flow. Furthermore, cessation of taurocholate infusion was associated with a rapid return of bile flow toward pre-infusion rates (Fig. 5). Thus, the liver responded in an expected physiologic manner to taurocholate infusion (at least in regard to biliary cholesterol secretion and bile flow). In some experiments the taurocholate infusion was sustained for longer periods (up to 40 hr). In all experiments, intravenous infusion of taurocholate did not significantly inhibit bile acid synthesis (Table 1).

Effect of intestinal infusion of taurocholate on bile acid synthesis

We also examined the effect of intestinal infusion of taurocholate on biliary bile acid secretion. Taurocholate was infused into the duodenum at a rate of 20 μmol/(100 g · hr), which is slightly greater than that used by Shefer et al. (12) in similarly designed experiments. As shown in **Fig. 6,** even at this high rate of taurocholate infusion, the secretion of de novo synthesized bile acids was not affected. In contrast to the lack of an effect of taurocholate infusion on biliary bile acid secretion, both biliary cholesterol secretion (**Fig. 7**) and bile flow (**Fig. 8**) were stimulated. Cessation of the taurocholate infusion rapidly returned biliary cholesterol secretion and bile flow toward rates observed during the pre-infusion periods (Figs. 7 and 8). This experiment was repeated a total of three times and similar results were obtained (Table 1), i.e., taurocholate did not significantly inhibit bile acid synthesis, whereas it did stimulate both biliary cholesterol secretion and bile flow.

In another set of experiments, bile-diverted rats were infused intraduodenally with and without taurocholate (20 μmol/(100 g · hr)) begun within 1 hr of bile diversion. Bile acid synthesis was determined in hourly samples between 24 and 36 hr after the beginning of the experiment. There was no significant difference in bile acid synthesis between both groups (in μmol/(100 g · hr): 5.1 ± 1.2, control; 5.4 ± 0.9, taurocholate infused; n = three rats in each group). Thus, taurocholate infusion into the duodenum, either during bile diversion or 24 hr after bile diversion, did not significantly affect the synthesis of bile acids.

Effect of mevinolin on biliary bile acid secretion

Previous studies using cultured rat hepatocytes showed that mevinolin, a competitive inhibitor of HMG-CoA

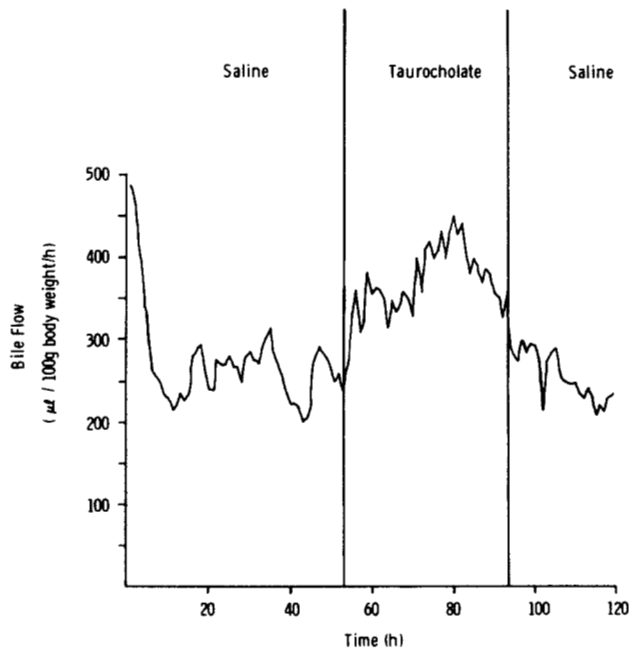

Fig. 5. Effect of intravenous taurocholate on bile flow. Rat number 4, Table 1, was infused, via the jugular vein, with glucose/saline for 52 hr, after which the infusate was changed to one that contained [24-^{14}C]taurocholate that was delivered at a rate of 14.4 μmol/(100 g · hr). After 40 hr, the infusate was changed to one that contained glucose/saline only. Bile collected in hourly samples using a fraction collector was weighed. Bile flow was quantitated assuming a density of bile of 1 g/ml. Bile flow was significantly increased during the taurocholate infusion period at hours 56, 57–63, and 69–90.

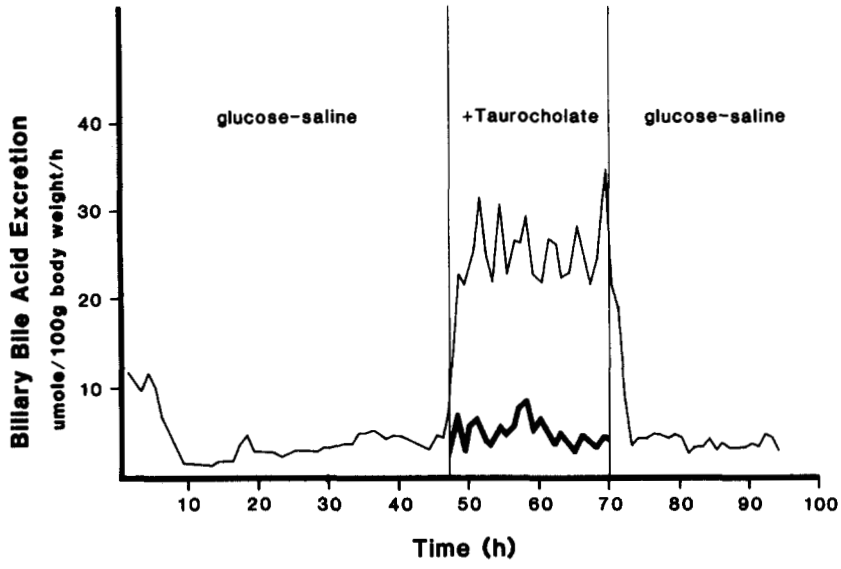

Fig. 6. Effect of taurocholate infused into the duodenum on bile acid synthesis. Rat number 8, Table 1, was infused with a solution containing 20% DME in glucose/saline for 48 hr, after which the infusate was changed to one that contained [24-^{14}C]taurocholate so that the rate of delivery was 20 μmol/(100 g · hr). After 23 hr the infusate was changed back to the original one. Bile acid content in bile samples collected every hour using a fraction collector was quantitated by 3α-steroid dehydrogenase. During the taurocholate infusion period, the top line represents the total amount of bile acid secreted, whereas the bottom line represents the difference between the total amount secreted and the amount of [24-^{14}C]taurocholate secreted in bile. Statistical analysis showed that during the taurocholate infusion period bile acid synthesis was unchanged.

reductase (24), inhibited bile acid synthesis by cultured rat hepatocytes (17, 18). We examined the possibility that mevinolin might also inhibit bile acid synthesis in vivo.

Intravenous infusion of mevinolin (1 mg/hr) did not affect biliary bile acid secretion until after the first 8 hr (**Fig. 9**). From hours 8 until the end of the experiment, biliary bile acid secretion was dramatically reduced by mevinolin infusion.

Mevinolin infusion also reduced the secretion of biliary cholesterol (**Fig. 10**). However, unlike the reduction of

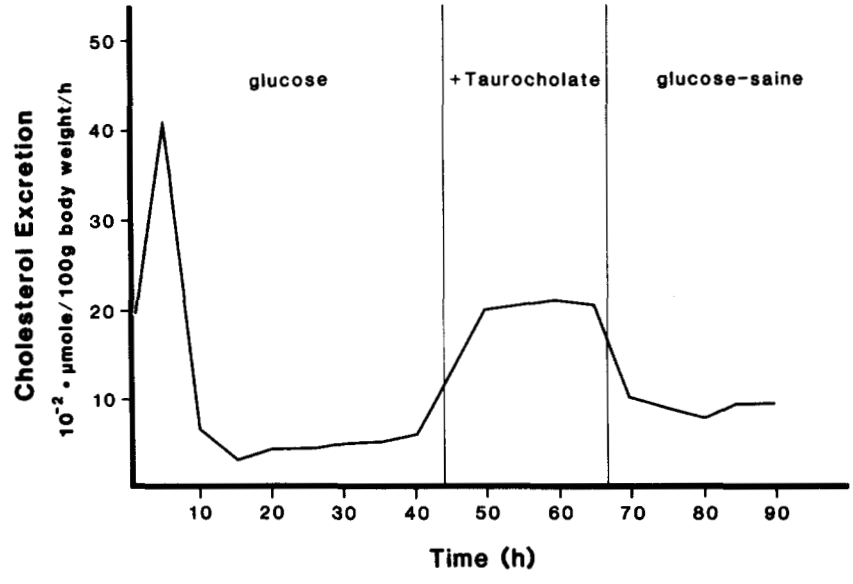

Fig. 7. Effect of taurocholate infused into the intestine on biliary cholesterol secretion. Rat number 9, Table 1, was infused as in Fig. 6; however, the taurocholate solution was infused during hours 44–66. The cholesterol content of bile was quantitated in every fifth hour sample. During the taurocholate infusion period biliary cholesterol secretion was significantly increased.

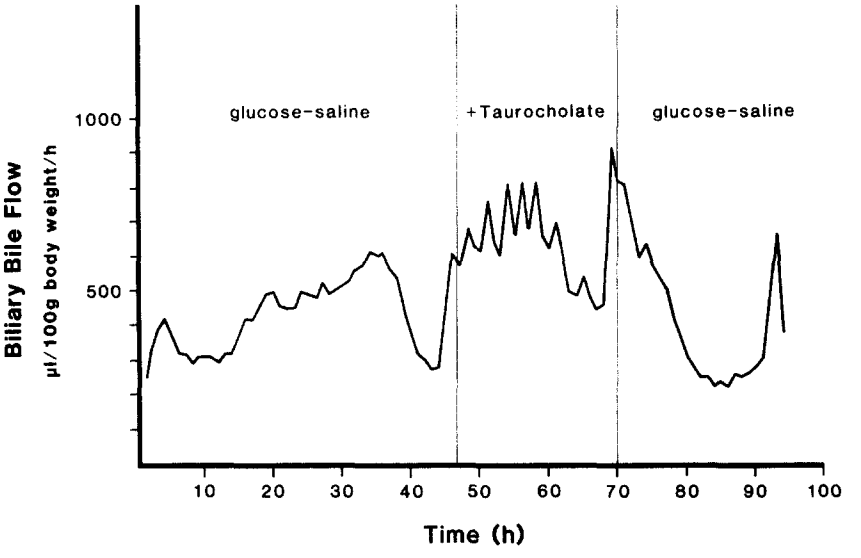

Fig. 8. Effect of infusing taurocholate into the duodenum on bile flow. Rat number 8, Table 1, was treated as described in Fig. 6. Hourly bile flow was quantitated as described in Fig. 5. During the taurocholate infusion period bile flow was significantly increased at hours 49, 52, 54, 56, 58, and 69.

bile acid secretion which occurred after 8 hr of mevinolin infusion (Fig. 9), biliary cholesterol secretion was reduced within 3 hr.

In marked contrast to the reduction in secretion of bile acid and cholesterol, mevinolin did not inhibit bile flow consistently (**Fig. 11**). Thus, the reduction in biliary secretion of cholesterol and bile acid was specific and not caused by bile secretory failure (toxicity).

DISCUSSION

We chose to examine the effects of taurocholate for several reasons. It is the predominant bile acid in the rat. Also, compared to its unconjugated derivative cholic acid, it is the most potent inhibitor of bile acid synthesis (14). Moreover, since it is the most commonly used bile acid to examine bile acid "negative feedback regulation" (10–15)

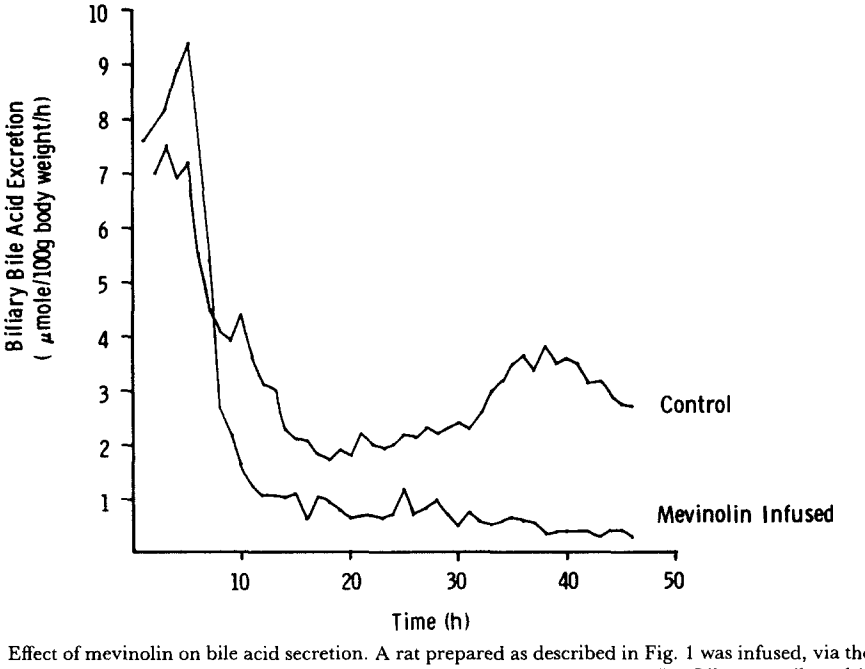

Fig. 9. Effect of mevinolin on bile acid secretion. A rat prepared as described in Fig. 1 was infused, via the jugular vein, with glucose/saline containing mevinolin so that the delivery rate was 1 mg/hr. Bile was collected in hourly samples and bile acid content was determined as described in Fig. 3. The control rat was infused with glucose/saline only. Bile acid secretion was inhibited in every bile sample from hour 9 until the end of the experiment.

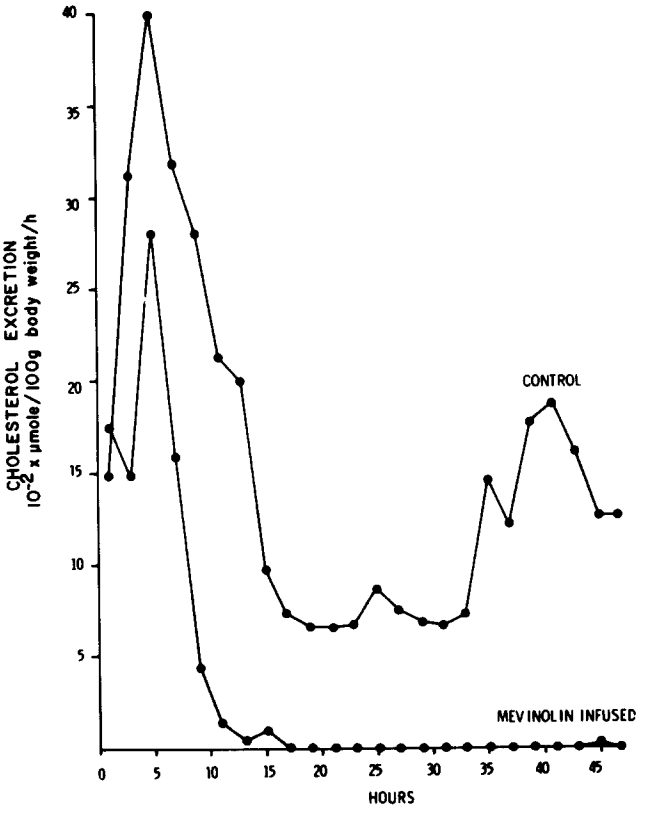

Fig. 10. Effect of mevinolin on biliary cholesterol secretion. The amount of biliary cholesterol in hourly samples of bile secreted by a rat treated as described in Fig. 9 was determined as in Fig. 4. Biliary cholesterol secretion was significantly decreased in bile samples from hour 3 until the end of the experiment.

we could readily compare our results. In our experiments the infusion rate of taurocholate was as great or greater than that used previously to show "negative feedback" regulation (10–14). In addition, our experimental protocols included two different procedures used by others to show bile acid "negative feedback" regulation (10–14): infusion into the duodenum and infusion into the jugular vein. Furthermore, bile acid synthesis was determined by both mass quantitation and the incorporation of ^{14}C-labeled mevalonic acid.

In our experiments bile acid synthesis was quantitated as the difference between the total amount of bile acid secreted/hour time point and the amount of [24-^{14}C]taurocholate secreted in bile. This assumes that de novo synthesized bile acids are quantitatively secreted into bile. If this is not so, bile acid synthesis would be underestimated during the taurocholate infusion period. Furthermore, if the recovery in bile of infused taurocholate is less than quantitative, again one would obtain falsely reduced rates of bile acid synthesis. In other words, this experimental design is more likely to yield minimal estimates of bile acid synthesis rather than overestimates. However, since in all our experiments the recovery in bile of infused [24-^{14}C]taurocholate was quantitative, it is likely that our estimated rates of bile acid synthesis are accurate.

Experiments examining the effect of bile acids on bile acid synthesis have yielded discrepant results. Several years ago, Shefer et al. (12) addressed the reason for this inconsistency. They concluded that the reason why some could not observe inhibition is that they did not infuse enough taurocholate. Shefer et al. (12, 13) found that when taurocholate was infused into the duodenum of bile-diverted rats at rates greater than 10 mg/(100 g · hr) (18.6 µmol/(100 g · hr)) bile acid synthesis was inhibited by 90%. Significant 70% inhibitions were achieved within 6 hr of infusing the taurocholate (13). In contrast, when Wilson, Bentley, and Crowley (15) infused taurocholate into the duodenum of bile-diverted rats, no inhibition of bile acid synthesis was observed. The reason for this apparent difference in response is not clear, but might be related to the methods used. Shefer et al. (13) used an isotopic determination of endogenous bile acid synthesis and also determined the effect of taurocholate infusion on the mass secretion of chenodeoxycholate. Their calculations used in isotopic studies were based upon the assumption that "the specific radioactivity of chenodeoxycholic and endogenous cholate were equal . . ." (13). Subsequent experimental findings by Mitropoulos et al. (25) showed that in bile-diverted rats the specific radioactivities of cholate and chenodeoxycholate were variable and different from each other, suggesting that the assumption of Shefer et al. (13) was not valid. Wilson et al. (15) used mass quantitation, methods similar to those used by us.

In our studies, we infused taurocholate into the duodenum at a rate (20 µmol/(100 g · hr)) slightly greater than that used by Shefer et al. (13). Although we could demon-

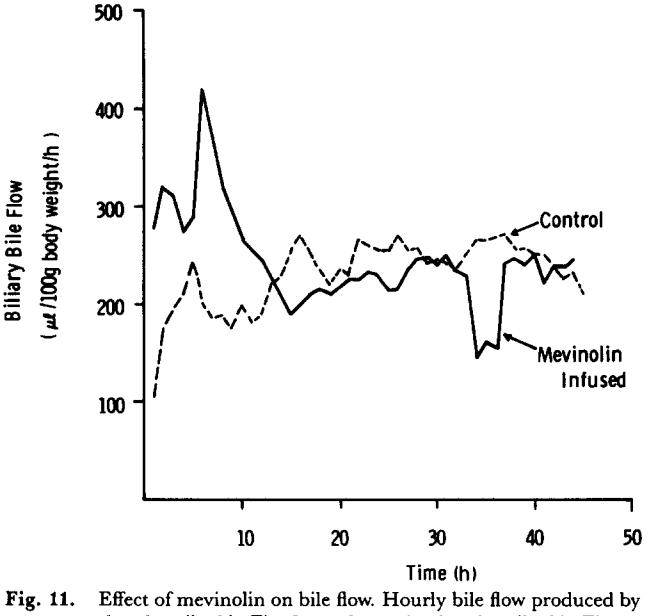

Fig. 11. Effect of mevinolin on bile flow. Hourly bile flow produced by a rat treated as described in Fig. 9 was determined as described in Fig. 5. Mevinolin decreased bile flow only during hours 33–35.

strate increased bile flow and biliary cholesterol secretion (Figs. 8 and 9), we could not demonstrate inhibition of bile acid synthesis (Fig. 8 and Table 1).

To our knowledge there is only one published report describing the effect of intravenously administered taurocholate on bile acid synthesis. In this report, Pries et al. (14) showed a linear relationship between the inhibition of bile acid synthesis and the rate of taurocholate infusion. Maximal (>90%) inhibition was obtained by Pries et al. (14) at a taurocholate infusion rate of 12 μmol/(100 g · hr). They proposed two possible mechanisms to account for their results: inhibition of gene expression and interaction with 7α-hydroxylase. Their data showing a linear response to bile acids is inconsistent with genetic repression (threshold response), but is consistent with bile acids interacting with 7α-hydroxylase, membranes and cofactors. Studies by Schwartz and Margolis (26) show that cholic acid did not interfere with the in vitro production of 7α-hydroxycholesterol by rat liver microsomes.

The results of Pries et al. (additive inhibition (14)) are inconsistent with the results of Shefer et al. (threshold inhibition (12)) and our results (no inhibition). In our jugular vein infusion studies, no inhibition of bile acid synthesis was evident even when the infusion rate was increased up to 25 μmol/(100 g · hr). The difference between our results and those of Pries et al. (14) cannot be the taurocholate used. In an attempt to gain an understanding for our differences, we used the taurocholate used by Pries et al. (kindly provided by Dr. W. C. Duane) for our experiments and obtained no inhibition (Table 1, rat 6).

The only time we were able to obtain a significant inhibition of bile acid synthesis (by infusing bile acids into either the duodenum or the jugular vein) was when the rats became sick as evidenced by dramatically reduced bile flow (50% inhibition) and subsequent death (within 6 hr) (data not shown). It is possible that the high rates of bile acid infusion required to demonstrate inhibition of bile acid synthesis was associated with and/or was the result of hepatotoxicity. At high infusion rates, taurocholate is known to cause toxicity, impair hepatic function, and inhibit biliary bile acid secretion (27). The sensitivity of rats toward taurocholate toxicity is variable and is greater in rats treated with ethinylestradiol (27). Therefore, there are data available showing that infusing taurocholate at high enough rates into rats can inhibit bile acid synthesis. However, we question whether this is a physiologic regulatory response or the result of established toxic effects of bile salts.

The findings by Shefer et al. (12) of no increase in biliary cholesterol secretion (during taurocholate infusion) and also that only 59–72% of the taurocholate infused was recovered in bile might be due to a dose of taurocholate great enough to impair hepatic function. In their studies showing inhibition of bile acid synthesis by taurocholate, Pries et al. (14) also reported variable recoveries of intravenously infused taurocholate (67–120%). In order for an inhibition to be physiologically relevant, it should be shown to be specific and not associated with a generalized impairment. In addition, the inhibition should be demonstrable by a concentration and/or rate of supply of an inhibitor that is physiologic and the inhibition should be reversible in a manner temporally similar to that which occurs in vivo.

It is difficult (if not impossible) to accurately quantitate the amount of bile acid which fluxes through the liver of a rat. Experimentally, the maximum rate of sustained bile acid secretion via infusion studies is 20–25 μmol/(100 g body weight · hr) (Table 1). In our experiments, higher rates of infusion resulted in hepatotoxicity. Using an external catheterized "extracorporeal bile duct rat model," Weis and Barth (28) reported an excretion rate of 30.5 μmol/(100 g · hr) (fasting) to 48 μmol/(100 g · hr) (feeding) for short (5 min) periods of time. Interpretation of these experiments is complicated by the possible trauma of surgical manipulation. Defining the actual hepatic flux of bile acids must await new noninvasive techniques. Attempts in our laboratory to infuse taurocholate into the duodenum of bile fistula rats at rates equal to those reported by Weis and Barth (28) (i.e., 30 μmol/(100 g · hr) (16 rats) showed that all the animals exhibited respiratory failure, hematuria, and death (data not shown). At the present time it is not known whether or not a flux rate of bile acids as high as that reported by Weis and Barth (28) can inhibit bile acid synthesis without causing toxicity. Nevertheless, our data presented in this report clearly show that at rates of bile acid flux previously shown to inhibit bile acid synthesis, we were unable to observe significant inhibition of bile acid synthesis.

In contrast, when infused intravenously, mevinolin, a potent inhibitor of HMG-CoA reductase, inhibited both the secretion of cholesterol and of bile acids. These results obtained in vivo are in complete agreement with previous results obtained using in vitro rat hepatocyte models (16–19). Moreover, these results are consistent with the hypothesis that cholesterol availability, at least in part, is an important determinant of bile acid synthesis.

While the data obtained using both cultured rat hepatocytes (16–19) and these in vivo studies argue against bile acids acting directly on the liver to induce negative feedback regulation, they are not inconsistent with regulation of bile acid synthesis via the enterohepatic circulation. It is clear that bile acid diversion increases, whereas bile acid feeding decreases bile acid synthesis (29–31). The data obtained in our study lead us to conclude that the mechanism via which the enterohepatic circulation regulates bile acid synthesis does not involve a direct inhibitory affect of bile acids on the liver. The elucidation of the actual effector and its possible interaction with the process determining hepatic cholesterol availability remain to be determined.

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