Effect of Sodium Taurolithocholate on Bile Flow and Bile Acid Excretion

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ABSTRACT Sodium taurolithocholate and sodium taurocholenate were infused intravenously into rats and hamsters. Each bile acid salt was given alone or in combination with varying amounts of a primary bile salt, either sodium taurocholate or sodium taurochenodeoxycholate. Bile flow, total bile acid salt excretion, and the excretion of sodium taurolithocholate were quantitatively determined. In addition, mannitol excretion in bile was determined at various flow rates.

Sodium taurolithocholate was found to be rapidly excreted in bile in concentrations greater than its aqueous solubility. When the endogenous excretion rate of bile salt or the infusion of primary bile salt was less than the molar amount of administered sodium taurolithocholate, cholestasis always occurred. Increasing molar amounts of primary bile salt prevented cholestasis and enhanced the excretion rate of sodium taurolithocholate.

Infusion of sodium taurocholenate, a nonhemolytic bile salt, caused an effect on bile flow and bile acid salt excretion qualitatively similar to sodium taurolithocholate.

The induction of cholestasis can be attributed to the physical properties of these poorly water soluble bile salts. The reduction in bile flow could not be shown to be related to water reabsorption from the biliary tree since there was no increase in mannitol concentration in bile during cholestasis. Reduction in bile flow may be related to obstruction of segments of the biliary tree by precipitates of sodium taurolithocholate and possibly to a decrease in water entry into the biliary tree during infusion of this bile acid salt.

INTRODUCTION

It is generally accepted that the bile acid salts are major determinants of bile flow. This view is based, in part, on the knowledge that glycine and (or) taurine conjugates of chenodeoxycholic,[1] deoxycholic, and cholic acids are present in much higher concentrations in the bile than in the plasma of many mammalian species (1), and that infusions of some of these compounds cause an increase in bile flow and bile acid excretion (2, 3).

Preliminary studies indicated (4) that in contrast to the choleretic effect of these compounds, a structurally related bile acid salt, sodium taurolithocholate, caused a decrease in bile flow and bile acid salt excretion. This cholestatic effect appeared to be dependent on the rate of excretion of other bile acid salts.

Since lithocholic acid is occasionally detectable in normal human bile (5), accounts for as much as 32% of the normal fecal bile acids (6), and is present in increased amounts in the feces of some patients with cirrhosis (7), it seems cogent to

[1] Systematic names of the bile acids referred to are: taurolithocholic acid, 3α-hydroxy-5β-cholanoyl taurine; taurochenodeoxycholic acid, 3α, 7α,-dihydroxy-5β-cholanoyl taurine; taurodeoxycholic acid, 3α, 12α,-dihydroxy-5β-cholanoyl taurine; taurocholic acid, 3α, 7α, 12α-trihydroxy-5β-cholanoyl taurine; taurocholenic acid, 3β-hydroxy-5-cholenoyl taurine; muricholic acids, 3α, 6β, 7α-trihydroxy-5β-cholanoic acid, 3α, 6β, 7β-trihydroxy-5β-cholanoic acid.

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attempt to define circumstances under which cholestasis might be expected to occur and the possible alterations in bile formation determining this response.

METHODS

Materials. Lithocholic acid (Nutritional Biochemicals Corporation, Cleveland, Ohio) was twice recrystallized and the taurine conjugate prepared by the mixed anhydride method of Norman (8). The final product was recrystallized and was chromatographically homogeneous. Sodium taurolithocholate-24-^{14}C was similarly prepared from lithocholic acid-24-^{14}C (New England Nuclear Corp., Boston, Mass.). Recrystallization to constant specific activity was done before (32.7 μc/mmole) and after taurine conjugation (31.8 μc/mmole).

3β-hydroxy-5-cholenic acid was obtained from Mann Research Labs. Inc., New York. Several minor impurities were detected by chromatography and were removed by recrystallization from dimethylformamide-water. The melting point of the chromatographically homogeneous material was 234.5–235.5°C (recorded 236–237°C [9]). The taurine conjugate was prepared (8) and the final product recrystallized from hot ethanol. The material was chromatographically homogeneous and identical in R_f with taurolithocholic acid in the solvent systems used. However, the two compounds could be distinguished chromatographically by use of the silver nitrate impregnation technique (10) for retardation of unsaturated steroids. When *n*-butanol–acetic acid-water (10:1:1) were used the R_f for taurolithocholate was 0.48 and for taurocholenate 0.41.

Sodium taurocholenate was added in excess to 1.0 ml of H_2O and the concentration in the supernatant determined after incubation at 37°C for 2 hr. Analysis in triplicate gave a value of 2.7 ± 0.1 μmoles/ml.

The hemolytic activity of sodium taurolithocholate and sodium taurocholenate was tested with saline-washed human erythrocytes. 3 mg of each compound, dissolved in 0.1 ml of propylene glycol, was added to 2.0 ml of erythrocytes suspended in saline and incubated at 37°C for 20 min. Complete hemolysis occurred in the tube containing sodium taurolithocholate, and no spectrophotometrically detectable hemolysis occurred in the tube containing sodium taurocholenate. These findings confirm those of Berliner and Schoenheimer (11) who used the sodium salt of the unconjugated bile acid.

Sodium taurocholate and sodium taurochenodeoxycholate were obtained from Maybridge Chemical Co., North Tintagel, Cornwall, England.

Mannitol-^{14}C was obtained from Nuclear-Chicago Corporation, Chicago, Ill. and mannitol-^{3}H from New England Nuclear Corp.

For intravenous infusion the bile acid salts were dissolved in 10% human serum albumin, prepared by dilution of a heat-stabilized preparation (25% salt-poor human serum albumin, Hyland Laboratories, Los Angeles, Calif.) with a solution containing 5% glucose and 0.45% sodium chloride. Sodium taurocholate (0.2–6 mmoles/liter and sodium taurochenodeoxycholate (0.4–16 mmoles/liter) dissolved at room temperature. Sodium taurolithocholate and sodium taurocholenate (0.2–9 mmoles/liter) required heating to 60°C to obtain a clear solution when the higher concentrations were used. The solution then remained clear for several hours at room temperature but developed turbidity when stored at 10°C. Clarity of the solution was restored by warming to 60°C.

The infusion rates expressed in the text were calculated by multiplying the weighed concentration of the bile acid salt by the rate of fluid delivery from syringes that had been calibrated previously. Sodium taurolithocholate-^{14}C infusion rate was determined directly by estimation of the delivery rate into a volumetric flask.

Analyses. Chromatographic analyses were done with thin-layer techniques with silica gel G or H and solvent systems appropriate for the particular bile acid as outlined by Hofmann (12) and given in detail previously (13).

Total bile acids were quantitatively estimated with hydroxysteroid dehydrogenase obtained from *Pseudomonas testeroni* (Worthington Biochemical Corp., Freehold, N. J.). The preparation and use of this enzyme for steroid analysis has been described in detail by Talalay (14). For these studies the enzyme was prepared by sonic disruption of 1 g of dried cells in 100 ml of water for 20 min. A Biosonik II ultrasonicator (Will Scientific, Inc., Rochester, N. Y.) with probe intensity setting of 70 has been found satisfactory. After centrifugation for 20 min at 18,000 *g* at 10°C, the supernatant was decanted and ammonium sulfate added to a final concentration of 60%. The precipitate was collected by centrifugation as described above and the supernatant discarded. The material was stored at −20°C, and approximately one-sixth of the amount was suspended in 30 ml of water just before use. An active preparation was always obtained.

The enzyme was used for quantitative bile acid analysis by Iwata and Yamasaki (15). The amount of bile salt present is determined by the amount of nicotinamide adenine dinucleotide, reduced form, [NADH] generated during the oxidation of the hydroxyl group at C-3 to a ketone. Hydrazine hydrate traps the ketone formed and thus aids in bringing the reaction to completion. The incubation mixture contained 1.0 ml of enzyme solution, 0.5 ml of 1 M hydrazine hydrate, 0.25 ml of 5 mM NADP, and 2.0 ml of 0.1 M potassium phosphate buffer, pH 9.4.

10 μl of either bile or bile acid standard (0.15 or 0.30 μmole/10 μl of methanol) was added, and the net increase in absorbance when a bile and reagent blank was used was determined at 340 mμ after 30 min of incubation at room temperature. When crystalline, chromatographically homogeneous deoxycholic acid (generously provided by Dr. Alan F. Hofmann) was used mean recovery for 61 determinations was 88% ± 0.85 SEM. This value is consistent with the reported values of 88–102% (14) when a variety of conjugated and unconjugated bile acids were used. Mean recovery of 3β-hydroxy-5-cholenic acid, not previously analyzed by this method, was 97 ± 2% in three determinations. Addition of bile acid standard to bile did not alter the per cent recovered.

Sodium taurolithocholate-^{14}C and its metabolites in bile were determined by liquid scintillation spectrometry using techniques previously described (13). The ^{14}C in bile was calculated as μmoles using the specific activity of the infused sodium taurolithocholate. Mannitol in serum and bile was determined by adding 50-μl aliquots to 1 ml of hyamine and then adding 10 ml of toluene-containing scintillant (16). The greater quenching that initially occurred in bile samples disappeared within 24 hr as the samples become colorless and is therefore probably related to bilirubin. The same efficiency of counting was then obtained for bile and serum samples. Minimum counting rates for mannitol were greater than 40 times the background, and less than 3% of the ^{14}C counts were found in the tritium channel.

Animal procedures. Male hamsters (95–138 g, body weight) and male and female Wistar rats (250–380 g, body weight) were used. During intraperitoneal pentobarbital anesthesia a polyethylene cannula was inserted in the common bile duct, and bile was collected quantitatively into tared tubes. Since the hamster has a gall bladder, cholecystectomy was necessary to obtain complete collections of bile. Bile acid salt infusions were given through an indwelling intravenous cannula. In addition, it was possible to insert an indwelling polyethylene femoral arterial cannula into the rat to facilitate collections of blood. In studies with mannitol, bilateral renal pedicle ligation was done before intravenous injection of the compound. After surgery, the animals were placed in restraining cages and offered food and water.

Experimental design. It was decided that the cholestatic effect of sodium taurolithocholate should be demonstrated in a manner requiring the least amount of experimental manipulation. Accordingly, sodium taurolithocholate was infused immediately after surgery before significant depletion of the endogenous bile salt pool and before possible secondary effects of surgery such as malnutrition and infection. The infusion was usually continued until a distinct fall in bile flow occurred. Continuing the infusion beyond this effect prolonged the recovery phase unduly. Since a reduction in bile flow could occur from inadvertant mechanical obstruction of the cannula, it was also considered important to allow for a spontaneous return of bile flow.

The prevention of cholestasis by combined bile salt infusion was usually attempted on the day after surgery. Occasionally, a third and fourth infusion were given to the same animal either to reproduce the cholestatic effect or to show that the same amount of sodium taurolithocholate would not cause cholestasis provided that sufficient primary bile salt was also given. This general plan is shown in detail in the animals comprising Tables I and II. Combined bile salt infusions given immediately after surgery (A-51, A-75, Table III) had the same effect on bile flow and bile acid excretion as when given on subsequent days.

While these studies were in progress, it was found that the metabolism of sodium taurolithocholate in the hamster was different from that of the rat. By infusion of the ^{14}C isotope it became possible to quantitate sodium taurolithocholate excretion in a species in which detectable amounts of muricholic acids were not produced from sodium taurolithocholate. The experimental design used in the hamsters was similar to that employed in the rat with the exception that the various infusions were usually given on the day of surgery. In only about a third of the ham-

TABLE I

*Effect of Sodium Taurolithocholate on Bile Flow and Bile Acid Excretion of Male Wistar Rats**

Animal No. and wt....	A-58, 237 g			A-65, 291 g			A-66, 350 g		
Elapsed time	Infusion rate T-Lith	Bile flow	Bile A excretion	Infusion rate T-Lith	Bile flow	Bile A excretion	Infusion rate T-Lith	Bile flow	Bile A excretion
min	*μmole/min*	*mg/min*	*μmole/min*	*μmole/min*	*mg/min*	*μmole/min*	*μmole/min*	*mg/min*	*μmole/min*
30		9.3	0.13		11.3	0.28		16.2	0.37
45	0.12	7.5	0.12	0.6	11.1	0.22	0.6	8.1	0.17
60	0.12	6.3	0.11	0.6	6.4	0.14	0.6	2.4	0.05
90	0.12	4.6	0.11	0.6	1.3	0.04		1.7	0.04
120		3.3	0.08		1.3	0.02		2.6	0.06
150		4.4	0.11		1.3	0.02		4.5	0.15
180	0.30	3.0	0.08		1.5	0.04		4.5	0.15
210	0.30	0.7	0.03		1.5	0.04		4.4	0.20
240		2.4	0.06		1.5	0.09		4.3	0.23
270		3.2	0.06		1.8	0.13		4.4	0.23
285		4.1	0.07		2.1	0.37		10.1	0.49
315					4.9	0.23		10.3	0.46
‡		8.6	0.05		9.8	0.12		10.9	0.05

T-Lith, sodium taurolithocholate.

* Infusion of sodium taurolithocholate (Infusion rate T-Lith) 1–2 hr after bile duct cannulation.

‡ Bile collections of 13–18 hr duration.

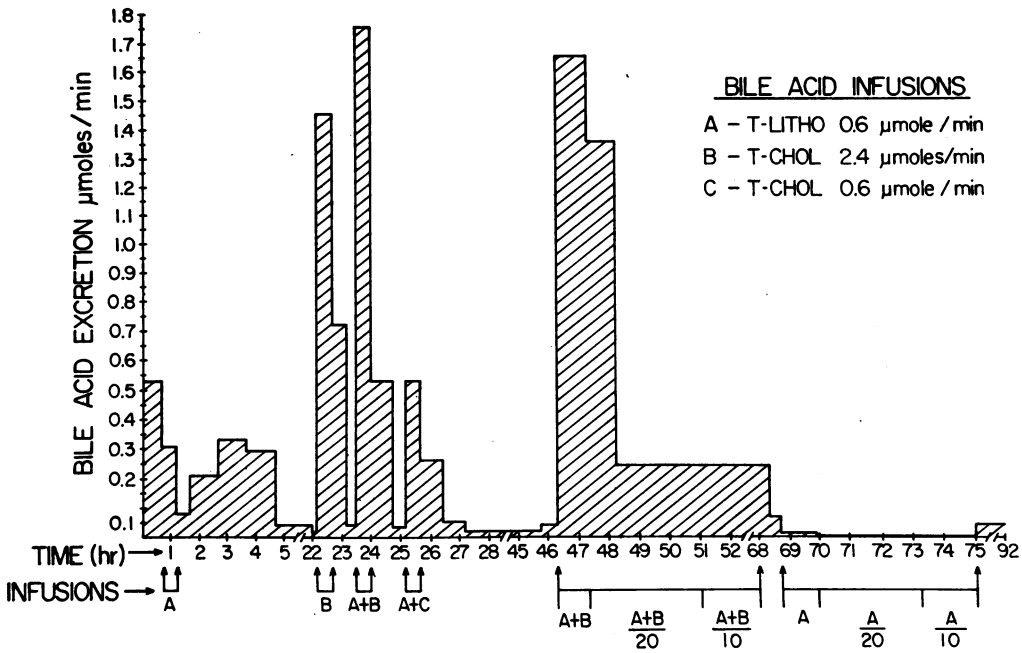

FIGURE 1 Relationship of total bile salt excretion (255 g ♂ Wistar rat) to infusions of various mixtures of sodium taurocholate (*T-Chol* [*B* and *C*]) and sodium taurolithocholate (*T-Lith* [*A*]) during a 4 day period. Sodium taurocholate (0.12–2.4 µmoles/min) and sodium taurolithocholate (0.03–0.6 µmole/min) were infused alone or in combination. Continuous infusion of sodium taurolithocholate at varying rates together with sodium taurocholate for 21.5 hr had no apparent delterious effect on bile salt excretion. Infusion of sodium taurolithocholate alone at the same rates caused a prompt and continuous suppression of bile salt excretion. Even after this prolonged cholestasis, some return to excretory function occurred before the study was terminated.

TABLE II

Modification of Effect of Sodium Taurolithocholate on Bile Flow and Bile Acid Excretion by Simultaneous Infusion of Sodium Taurocholate

	Animal No.....A-58				A-65				A-66	
	Infusion rate*				Infusion rate*					
Elapsed time	T-C	T-Lith	Bile flow	Bile A excretion	T-C	T-Lith	Bile flow	Bile A excretion	Bile flow	Bile A excretion
min	*µmole/min*		*mg/min*	*µmole/min*	*µmole/min*		*mg/min*	*µmole/min*	*mg/min*	*µmole/min*
Control‡			14.9	0.06			9.7	0.04	15.4	0.04
30	0.8	0.3	26.4	0.95	0.3	0.3	20.1	0.55	20.0	0.41
45	0.8	0.3	25.9	0.99	0.3§	0.3§	20.4	0.60	14.0	0.23
60	0.8	0.3	25.3	1.06			13.2	0.32	14.0	0.32
90			15.1	0.41			9.4	0.08	11.2	0.06
120			14.5	0.06			6.9	0.04	10.8	0.05
150							6.0	0.04	12.8	0.03
180					—	0.3	3.7	0.01	4.2	0.01
195					—	0.3§	2.6	0.004‖	2.1	0.003‖
18–22 hr			15.3	0.04			4.7	0.02	8.0	0.02

* Sodium taurocholate (T-C) and taurolithocholate (T-Lith) given 19–24 hr after duct cannulation.
§ Applies only to animal A-65.
‖ Bile collected was colorless.
‡ Mean of two to four periods of 15 min.

sters was a technically successful cholecystectomy and bile duct and vein cannulation achieved.

Bile salt infusion rates did not exceed 0.2 ml/min. By addition of either sodium taurocholate or sodium taurochenodeoxycholate to the infusion mixtures, the concentration of other constituents was kept constant. Also the volumes of fluid administered to each animal during single and combined bile salt infusions were the same. Between bile salt infusions the animals were maintained on 5% dextrose in 0.45% NaCl given at the rate of 0.1–0.2 ml/min.

RESULTS

Effect of sodium taurolithocholate on bile flow and bile acid excretion. Infusion of sodium taurolithocholate at rates varying from 0.12 to 0.6 μmole/min caused a prompt fall in bile flow and bile salt excretion (Table I). The cholestatic effect appeared to be greater with the higher infusion rates. Since there was negligible depletion of the bile salt pool (17), bile salt excretion usually

TABLE III

Relationship of the Cholestatic Effect of Sodium Taurolithocholate to Excretion and Infusion Rates of Other Bile Acid Salts in Rats

Expt. No.	Infusion rate		Infusion length	Elapsed time§	Bile acid excretion		Bile flow		Molar ratio**	Net effect‡‡ (−)/(+)
	T-C*	T-Lith‡			Control‖	Infusion¶	Control‖	Infusion¶		
	μmoles/min		*min*	*hr*	*μmoles/min*		*mg/min*			
A-75	—	1.20	45	22	0.04	0.003	6.4	4.1	0.03	(−)
A-74	—	0.60	60	68	0.04	0.02	10.6	4.4	0.07	(−)
A-52	—	0.30	30	20	0.02	0.01	9.9	4.6	0.07	(−)
A-66	—	0.30	30	25	0.03	0.01	12.8	4.2	0.10	(−)
A-65	—	0.30	45	23	0.04	0.008	6.0	3.3	0.13	(−)
A-63	—	0.30	30	24	0.04	0.02	14.5	4.1	0.13	(−)
A-57	—	0.30	60	22	0.04	0.005	5.5	2.0	0.13	(−)
A-71	—	1.20	45	1.8	0.40	0.27	20.0	6.3	0.33	(−)
A-58	—	0.30	60	3	0.11	0.06	4.4	1.9	0.37	(−)
A-65	—	0.60	60	2	0.28	0.11	11.3	5.0	0.47	(−)
A-74	—	0.60	30	1	0.30	0.19	18.3	10.8	0.50	(−)
A-66	—	0.60	30	1	0.37	0.11	16.2	5.3	0.62	(−)
A-52	—	0.30	30	1.5	0.20	0.06	4.6	0.8	0.67	(−)
A-57	—	0.30	60	3.5	0.22	0.11	10.4	3.9	0.70	(−)
A-74	0.6	0.60	30	25	0.03	0.82	13.1	21.2	1.05	(+)
A-58	—	0.12	60	1.8	0.13	0.11	9.3	5.8	1.08	(−)
A-66	0.3	0.30	30	23	0.04	0.41	15.4	20.0	1.11	(+)
A-65	0.3	0.30	45	21	0.04	0.57	9.7	20.2	1.11	(+)
A-63	—	0.30	60	1	0.44	0.23	15.9	10.2	1.13	(−)
A-71	—	0.30	45	1	0.40	0.69	20.0	24.0	1.33	(+)
A-56	—	0.12	60	2	0.18	0.27	15.9	15.8	1.50	(+)
A-51	0.3	0.30	30	1	0.30	0.68	10.1	17.0	2.00	(+)
A-75	2.4	1.20	45	1.5	0.30	2.65	25.0	42.9	2.25	(+)
A-57	—	0.12	60	1.5	0.21	0.19	11.3	8.0	2.33	(−)
A-58	0.8	0.30	60	24	0.06	0.99	14.9	26.0	2.87	(+)
A-53	0.2	0.10	60	24	0.10	0.31	10.1	13.2	3.00	(+)
A-74	2.4	0.60	30	24	0.03	1.90	13.9	28.9	4.05	(+)
A-74	2.4	0.60	60	47	0.05	1.58	15.4	24.5	4.08	(+)
A-63	1.2	0.30	75	21	0.04	0.89	15.0	19.0	4.13	(+)
A-58	1.2	0.30	60	48	0.04	0.94	15.0	23.0	4.13	(+)
A-56	0.5	0.10	60	22	0.05	0.44	7.3	10.7	5.50	(+)

* Sodium taurocholate.

‡ Sodium taurolithocholate.

§ Elapsed time, time from bile duct cannulation to beginning of infusion.

‖ Control, bile flow and bile acid excretion before infusion of bile acid salts.

¶ Infusion, mean bile flow and bile acid excretion during period of infusion.

** Molar ratio, (control bile acid excretion rate + T-C infusion rate)/(T-Lith infusion rate).

‡‡ Net effect, decrease (−) or increase (+) in bile flow and (or) bile acid excretion during infusion.

returned to levels approximate to those before sodium taurolithocholate infusion. After overnight collections of bile, these animals received repeat infusions of sodium taurolithocholate together with sodium taurocholate (Table II). It is apparent that sodium taurolithocholate does not prevent the choleretic effect of simultaneously administered sodium taurocholate when the latter is given in equimolar or greater amounts. However, subsequent infusion of the same amount of sodium taurocholate alone again caused a marked reduction in bile flow and bile acid excretion (A-65, A-66).

This relationship was studied further over a 4 day period (Fig. 1). Infusions of sodium taurolithocholate at rates varying from 0.03 to 0.6 μmole/min were given alone or in combination with sodium taurocholate varying from 0.12 to 2.4 μmoles/min. Infusion of both bile salts at varying rates gave a proportional response in bile salt excretion. Even when sodium taurolithocholate was infused continuously for 21.5 hr, bile salt excretion remained appropriate to the simultaneously infused sodium taurocholate. However, infusion of sodium taurolithocholate alone at the same rates for 6.25 hr caused a prompt and sustained suppression of bile salt excretion. After this period, some return in bile salt excretion occurred overnight before the study was terminated. Bile flow varied together with bile salt excretion throughout the study.

A summary of the results obtained from 31 infusions of sodium taurolithocholate into 12 rats is shown in Table III. The data are ranked according to the ratio of the molar amount of nontaurolithocholate bile salts to infused sodium taurolithocholate. The nontaurolithocholate amount represents the endogenous bile salt excretion before infusion of sodium taurolithocholate plus the rate of sodium taurocholate administration. Infusion of 0.3–1.2 μmoles/min of sodium taurolithocholate always caused cholestasis when the molar ratio was less than 1. Infusion of similar amounts of sodium taurolithocholate did not usually cause cholestasis when the molar ratio was between 1 and 3. Above a molar ratio of 3, no instances of decreased bile flow or bile salt excretion occurred.

Provided that sufficient sodium taurocholate is given, neither the time nor duration of sodium taurolithocholate infusion appears to determine the effect on bile flow or bile salt excretion.

Preliminary studies in hamsters indicated that sodium taurolithocholate could cause a decrease in bile flow and bile salt excretion. In this species, sodium taurochenodeoxycholate is the only identified metabolite of sodium taurolithocholate, and both compounds together account for 94% of the total radioactivity found in bile after administration of radioactive sodium taurolithocholate (12). In the study shown in Fig. 2, an infusion of sodium taurochenodeoxycholate was given at 1.6 μmoles/min until a steady excretion rate in bile was obtained. Then, in addition, an infusion of sodium taurolithocholate was given. The isotope rapidly

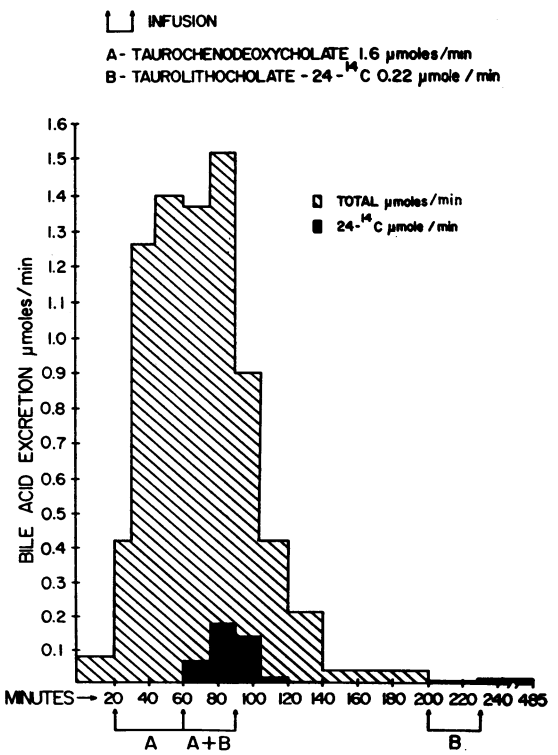

FIGURE 2 Total bile salt excretion and 24-^{14}C excretion in a 113 g ♂ golden hamster receiving sodium taurochenodeoxycholate and sodium taurolithocholate-24-^{14}C. Two infusions of sodium taurolithocholate (*B*) were given. The first infusion was given when total bile salt excretion was augmented by the administration of sodium taurochenodeoxycholate (*A*). No fall in total bile salt excretion occurred, and the isotope was rapidly and completely excreted in bile. The second infusion was given after return of total bile salt excretion to basal levels. Prompt and prolonged suppression of bile salt excretion occurred.

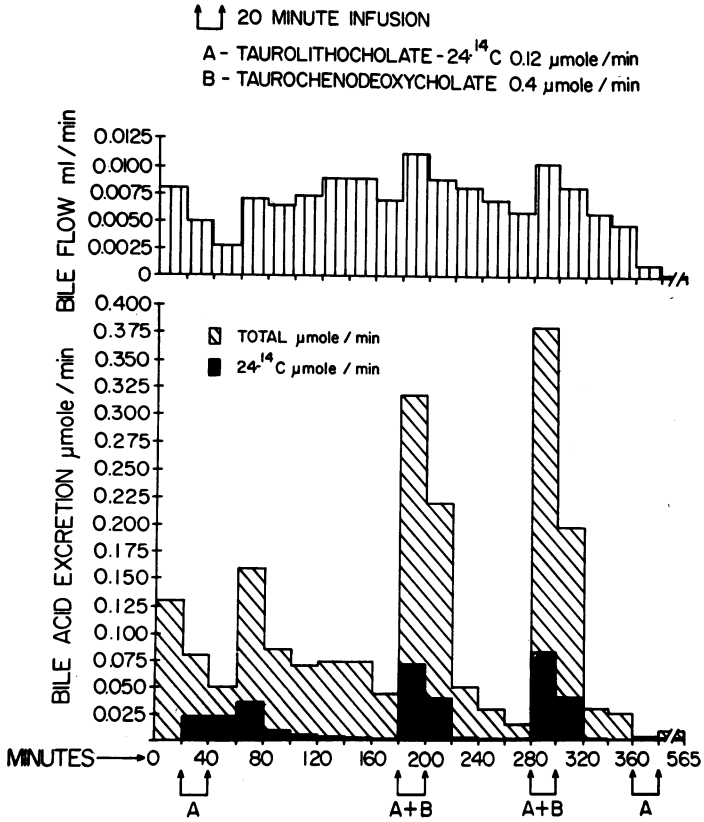

FIGURE 3 Bile flow, total bile salt excretion, and 24-^{14}C excretion in a 138 g ♂ golden hamster receiving sodium taurochenodeoxycholate and sodium taurolithocholate-24-^{14}C. Sodium taurolithocholate was administered on four occasions either alone (A) or in combination with sodium taurochenodeoxycholate ($A+B$). The rate of excretion of the isotope was greater during combined bile acid salt infusions. Sodium taurolithocholate given alone suppressed total bile salt excretion. The effect was greatest in the last infusion when basal bile salt excretion was less than that which occurred at the beginning of the study.

appeared in bile, and more than half the administered dose was excreted during the infusion. After discontinuance of the bile salt infusions, bile salt excretion returned to basal levels. When sodium taurolithocholate was given again, a marked fall in bile salt excretion occurred, and little of the administered compound was excreted before the study was terminated. The reproducibility of the cholestatic effect is shown in Fig. 3. In this study, sodium taurolithocholate was given on four occa-

TABLE IV

Excretion of Sodium Taurolithocholate-^{14}C and Its Metabolite in Hamster Bile

Hamster	Infusion No.	Infusion rate		% ^{14}C recovered in bile		% metabolized to T-Cheno-^{14}C		Concentration of taurolithocholate	
		T-Cheno*	T-Lith‡	Infusion	Post-infusion	Infusion	Post-infusion	Infusion§	Post-infusion¶
		µmoles/min						*µmoles/ml*	
Fig. 2	1	1.6	0.22	63	41	24	38	8.0	6.2
	2	—	0.22	0.4	11	15	83	0.4	0.2
Fig. 3	1	—	0.12	23	77	15	46	4.0	4.2
	2	0.4	0.12	72	36	22	24	4.9	3.6
	3	0.4	0.12	83	26	23	20	6.2	3.8
	4	—	0.12	0.3	18	49	74	0.12	0.75

* T-Cheno, sodium taurochenodeoxycholate.

‡ T-Lith, sodium taurolithocholate.

§ Concentration of sodium taurolithocholate in bile obtained during last 15 min of infusion.

¶ Concentration of sodium taurolithocholate in bile obtained during first postinfusion period.

sions. During two infusions sodium taurochenodeoxycholate was given in addition. A cholestatic effect occurred during the first and fourth infusion when sodium taurolithocholate was given alone. The duration of the cholestatic effect was greater during the last infusion when the rate of endogenous bile salt excretion was less than that which occurred at the beginning of the study.

The proportion of the isotope excreted as the metabolite, sodium taurochenodeoxycholate, is shown in Table IV. During combined bile acid infusions more than half the administered isotope is excreted during the infusion mostly as sodium taurolithocholate. The occurrence of cholestasis diminishes the rate of excretion of sodium taurolithocholate and increases the proportion recovered as sodium taurochenodeoxycholate.

The maximum concentration of sodium taurolithocholate in bile during the various infusions are also shown in Table IV. It is apparent that the decreased excretion rates of sodium taurolithocholate when given alone is attributable in part to a decrease in concentration in bile. The higher endogenous bile salt excretion during the initial infusion of sodium taurolithocholate to the animal shown in Fig. 3 probably accounts for the greater concentration of sodium taurolithocholate in bile than when given subsequently (fourth infusion).

A summary of the results obtained from 17 infusions of sodium taurolithocholate into 8 hamsters is shown in Table V. The data are ranked as explained previously. The cholestatic effect of sodium taurolithocholate is evident when the molar amounts of nontaurolithocholate bile salts yield a molar ratio of 1.5 or less. When the molar was greater than 2, no instance of decreased bile flow or bile salt excretion occurred.

Effect of sodium taurocholate and sodium taurolithocholate on mannitol excretion in bile. Bile salt infusions were given to animals with ligated renal pedicles 2 hr after the intravenous injection

TABLE V

Relationship of the Cholestatic Effect of Sodium Taurolithocholate to Excretion and Infusion Rates of Other Bile Acid Salts in Hamsters

Expt. No.	Infusion rate		Infusion length	Elapsed time§	Bile acid excretion		Bile flow		Molar ratio**	Net effect‡‡ (−)/(+)
	T-C*	T-Lith‡			Control‖	Infusion¶	Control‖	Infusion¶		
	μmoles/min		*min*	*hr*	*μmoles/min*		*mg/min*			
H-7	—	0.12	20	7.6	0.03	0.006	6.1	1.2	0.25	(−)
H-8	—	0.22	30	4.0	0.05	0.10	7.0	1.5	0.25	(−)
H-15	—	0.30	30	3.5	0.09	0.002	5.0	0.6	0.30	(−)
H-6	—	0.12	20	7.2	0.04	0.004	7.0	1.8	0.33	(−)
H-6	—	0.12	20	2.0	0.12	0.04	7.1	3.0	1.00	(−)
H-7	—	0.12	20	1.0	0.13	0.08	8.2	5.3	1.08	(−)
H-3	0.2	0.20	56	2.0	0.08	0.05	5.4	3.1	1.40	(−)
H-4	0.2	0.20	55	2.0	0.10	0.04	6.1	3.0	1.50	(−)
H-15	0.6	0.30	30	2.0	0.06	0.64	5.0	9.7	2.20	(+)
H-1	0.2	0.12	45	2.0	0.09	0.30	6.0	10.0	2.42	(+)
H-7	0.4	0.12	20	5.3	0.02	0.38	6.1	11.1	3.50	(+)
H-6	0.4	0.12	20	4.0	0.04	0.42	5.1	8.3	3.67	(+)
H-6	0.4	0.12	20	6.0	0.04	0.47	7.0	10.0	3.67	(+)
H-7	0.4	0.12	20	3.6	0.05	0.32	7.1	11.3	3.75	(+)
H-4	0.4	0.12	55	2.0	0.10	0.41	7.0	12.4	4.17	(+)
H-8	1.6	0.22	30	2.0	0.08	1.43	5.1	9.5	7.64	(+)
H-53	1.6	0.20	30	1.0	0.15	1.52	9.7	17.0	8.75	(+)

* Sodium taurocholate or sodium taurochenodeoxycholate.
‡ Sodium taurolithocholate.
§ Time from bile duct cannulation to beginning of infusion.
‖ Control, bile flow and bile acid excretion before infusion of bile acid salts.
¶ Infusion, mean bile flow and bile acid excretion during period of infusion.
** Molar ratio, (control bile acid excretion + T-C infusion rate)/(T-Lith infusion rate).
‡‡ Net effect, decrease (−) or increase (+) in bile flow and bile acid excretion during infusion.

TABLE VI

Effect of Sodium Taurolithocholate and(or) Sodium Taurocholate on Mannitol Excretion Rate in Bile of Hamster and Rat

	309 g male Wistar rat						107 g male hamster				
	Infusion rate*			Mannitol			Infusion rate				
Elapsed time	T-C	T-Lith	Bile flow	Bile	Ratio	Bile A excretion	T-C	T-Lith	Bile flow	Mannitol bile	Bile A excretion
min	*μmoles/min*		*mg/min*	*cpm/min*	*bile/serum*	*μmoles/min*	*μmoles/min*		*mg/min*	*cpm/min*	*μmole/min*
20	—	—	13.3	1319	1.13	0.17	—	—	5.5	81	0.20
40	1.6	—	22.0	2014		1.10	—	—	5.3	82	0.12
60	3.2	—	31.9	2688	1.14	2.20	—	—	5.1	76	0.10
80	3.2	—	38.0	3049	1.10	2.86	—	—	5.0	76	0.08
100	3.2	—	39.5	3288		3.49	0.4	0.2	8.4	114	0.22
120	—	—	30.1	2281	1.05	2.46	—	—	5.9	77	0.11
140	—	—	18.2	1481	1.20	0.47	—	—	5.8	81	0.07
160	1.6	0.45	25.0	1725		1.20	—	0.2	2.0	26	0.02
180	—	—	19.3	1317	1.05	1.12	—	—	4.3	59	0.08
200	—	—	17.5	1178	1.18	0.71	—	—	5.1	73	0.09
220	—	0.45	8.9	647		0.16	—	—	4.7	52	0.05
240	—	—	0.7	49		0.01	—	—	5.0	58	0.04
260	—	—	0.1	8	1.18	0.02	—	—	5.0	71	0.03
310	—	—	0.2	11		0.08					
1210‡	—	—	8.0	506		0.08					
1220	—	—	9.6	523	1.20	0.02					

* Infusion of sodium taurocholate (T-C) and(or) sodium taurolithocholate (T-Lith).
‡ 15 hr bile collection.

of mannitol. Administration of sodium taurocholate alone or in combination with sodium taurolithocholate caused an increase in bile flow and a parallel increase in mannitol excretion (Table VI). Sodium taurolithocholate infusion caused a typical fall in bile flow together with a decrease in mannitol excretion. Since blood specimens could be obtained from the rat it was possible to correlate the bile-to-plasma concentration of mannitol during the entire range of bile flow and mannitol excretion rates that occurred during the 20 hr of study. When the method of least squares was used the the correlation coefficient was found to be 0.95. The effect of sodium taurolithocholate on mannitol excretion in bile was determined in two additional studies in rats. In both studies the excretion rate of mannitol decreased as cholestasis occurred. At no time was there an increase in

TABLE VII

Effect of Sodium Taurocholenate on Bile Flow and Bile Acid Excretion of Male Wistar Rats

Experiment No.	DR-6		DR-7		DR-8	
Rat weight	255 g		253 g		260 g	
Elapsed time	Infusion rate taurocholenate	Bile flow	Bile A excretion	Bile flow	Bile A excretion	Bile flow	A Bile excretion
min	*μmole/min*	*mg/min*	*μmole/min*	*mg/min*	*μmole/min*	*mg/min*	*μmole/min*
Control*	—	15.4	0.35	13.0	0.42	14.5	0.42
15	0.6	12.9	0.27	11.5	0.37	13.8	0.39
30	0.6	5.9	0.14	9.5	0.26	4.5	0.18
45	0.6‡	3.3	0.04	6.3	0.07	1.4	0.04
60	0.6‡	8.6	0.04	5.8	0.04	1.4	0.04
75	—	8.6	0.10	5.3	0.06	3.5	0.08
105	—	18.9	0.56	5.4	0.09	4.7	0.16
135	—	20.9	0.52	7.9	0.23	11.0	0.53
165	—	19.1	0.31	12.7	0.44	11.2	0.49

* Two periods of 15 min each beginning 1–1.5 hr after bile duct cannulation.
‡ Applies only to animals DR-7 and DR-8.

mannitol concentration in bile above the levels before the infusion of sodium taurolithocholate.

Blood samples could not be obtained from the hamster. However, the mean concentration of mannitol in bile during the study was 13.8 cpm/mg of bile (1.32 SD) indicating little variation between bile flow and mannitol excretion rate. Some of the variation is presumably attributable to a slowly falling plasma mannitol concentration during the several hours of the study.

Effect of sodium taurocholenate on bile flow and bile acid excretion. Infusion of sodium taurocholenate into rats at 0.6 μmole/min for 30–60 min caused a decrease in bile flow and bile salt excretion (Table VII). The effect appears quite similar to that induced by sodium taurolithocholate with a tendency toward an increase in bile salt concentration in bile during the recovery phase and also several periods in which bile salt excretion exceeds preinfusion levels. The failure of sodium taurocholenate to alter significantly the choleretic effect of sodium taurocholate was studied in the hamster (Table VIII). Infusion of taurocholenate at 0.1 μmole/min during an infusion of sodium taurocholate at 0.2 μmole/min caused an increase in total bile acid excretion. In contrast, infusion of sodium taurocholenate at the same rate when bile acid excretion had returned to a lower endogenous level caused a prompt marked suppression of bile flow and bile acid excretion.

TABLE VIII

Effect of Sodium Taurocholenate on Bile Flow and Bile Acid Excretion of the Hamster

Elapsed time	Infusion rate*		Bile flow	Bile A excretion
	T-C	T-Chn		
min	*μmole/min*		*mg/min*	*μmole/min*
30	—	—	4.4	0.08
40	0.2	—	6.2	0.20
70	0.2	—	6.2	0.22
100	0.2	0.1	5.7	0.30
130	0.2	—	5.8	0.23
145	0.2	—	5.5	0.21
160	—	—	5.1	0.09
190	—	0.1	3.5	0.06
200	—	0.1	0.7	0.02
250	—	—	1.0	0.02
301	—	—	1.6	0.04
340	—	—	2.0	0.06
1330‡	—	—	3.6	0.02

* Infusion of sodium taurocholate (T-C) and(or)sodium taurocholenate (T-Chn) into 122 g hamster beginning 2 hr after bile duct cannulation.

‡ Overnight bile collection.

Chromatographic analysis of bile obtained from each of the animals during the infusion showed the appearance of a compound identical in R_f with the infused sodium taurocholenate. When silica gel G plates sprayed with silver nitrate were used it was possible to exclude the presence of detectable sodium taurolithocholate.

DISCUSSION

The cholestatic effect of sodium taurolithocholate in both the rat and the hamster is dependent on the excretion rate of the normal primary bile salts. Since these bile salts are of similar structure, differing only in the number of hydroxyl groups, it is reasonable to think that they may share a common pathway in their transfer from plasma to bile. If the maximum capacity of this transfer pathway is less than the infusion rate of the bile salts, then the rate of excretion of each bile salt would be dependent in part on their proportion in the infusion mixture. An increase in the proportion of sodium taurocholate or taurochenodeoxycholate would therefore decrease the rate of excretion of sodium taurolithocholate. The infusion rates used in these studies are for the most part less than the reported maximum rate for bile salt excretion of 1.6 μmoles/min per 100 g of body weight (18). Under these circumstances the isotope studies in the hamsters indicate that an increase in the proportion of primary bile salt enhances the excretion rate of sodium taurolithocholate. It appears therefore that the prevention of cholestasis by primary bile salt is related to an increase rather than a decrease in sodium taurolithocholate excretion.

The increase in sodium taurolithocholate excretion during combined bile salt infusions is attributable to an increase in flow rate and an increase in sodium taurolithocholate concentration in bile. Since the maximum solubility of sodium and potassium taurolithocholate in water at 40°C is 2.5 mmoles/liter (19), it is apparent that under certain circumstances there is greater solubility in bile. This value represents the maximum concentration of a molecular solution. However, it is known that the bile salts form micellar solutions

of much greater total concentration. The temperature at which the sodium and potassium salts of lithocholic acid and other monohydroxy bile acids form micellar solutions is much higher than body temperature (20). Addition of sodium taurocholate to an aqueous suspension of sodium taurolithocholate or sodium lithocholate can result in a clear solution of mixed micellar composition. The temperature necessary to form a mixed micellar solution varies with the molar ratio of sodium taurocholate and approaches the critical micelle temperature of pure sodium taurocholate ($< 0°C$ [20]). Thus the high concentration of sodium taurolithocholate in bile and the enhanced excretion rate during combined bile salt infusions is most reasonably attributed to the formation of a mixed micellar solution.

Cholestasis is prevented in the hamster when the molar ratio of primary bile salt is greater than 2. In the rat lesser amounts of the primary bile salt can prevent cholestasis. Although a number of variables may affect this apparent difference (21), the extent of conversion to other bile acids salts may be the most significant. The rat metabolizes sodium taurolithocholate to a variety of dihydroxy and trihydroxy bile salts (13) consistent with the known occurrence of muricholic acids in this species (22). It is apparent from the studies with hamsters that synthesis of muricholic acids is not necessary to induce cholestasis. Nor is there any reason to suspect that the synthesis of these more water soluble di- and trihydroxy derivatives should have a deleterious effect on liver function. The findings that during recovery from cholestasis in the hamster there is an increased conversion of taurolithocholate to taurochenodeoxycholate, and that the molar amounts of primary bile salt needed to prevent cholestasis in the rat may be less than in the hamster, suggest that conversion to more water soluble compounds protects against the deleterious effects of sodium taurolithocholate.

It is more difficult to define precisely and to document the exact sequence of events causing a reduction in bile flow and bile salt excretion. Probably the simplest explanation is that in the absence of sufficient primary bile acid salt, sodium taurolithocholate precipitates in segments of the biliary tree causing obstruction to flow and bile salt excretion. Although the bile occasionally became turbid after taurolithocholate infusion, and heavy precipitates were noted after freezing and thawing of the bile, no consistent data were obtained. On some occasions a colorless bile was obtained that may represent the nonbile salt–dependent fraction of bile analogous to that found in the dog (23). Admixture of this nonbile salt–dependent fraction at a site distal to the excretion of sodium taurolithocholate (24) could account for the very low concentration of sodium taurolithocholate found in collected bile. Considering that one obtains only the free-flowing bile, the concentration of sodium taurolithocholate would not be expected to exceed saturation. During recovery from cholestasis, precipitates within the biliary tree might be solubilized by the continued excretion of primary bile salts. Thus, although it is difficult to document the occurrence of intrahepatic obstruction, the occasional changes seen in the physical properties of the bile together with the high concentration of sodium taurolithocholate that does occur during combined bile salt infusions suggest that the induction of cholestasis is related to the physical properties of the bile salt.

Several possibilities exist as to the events leading to the precipitation of sodium taurolithocholate in the biliary tree. Sperber has suggested (25) that the active transport of bile salt from the liver cell to the canaliculus obligates a flow of water to maintain an isoosmotic relationship with surrounding tissues. The failure of bile flow to increase in response to sodium taurolithocholate transport would indicate that there is no increase in the osmotic gradient. The lack of an increase in the osmotic gradient could indicate that the bile salt precipitates immediately upon transfer across the cell membrane. Alternatively, sodium taurolithocholate could form part of a mixed micelle structure without actually increasing the total number of molecules in solution. The latter possibility would only explain a failure of bile flow to increase but would not explain an actual decrease in flow. A decrease in flow could occur if the total number of molecules and molecular aggregates in solution actually decreased. Such an event might occur if sodium taurolithocholate either lowered the bile salt concentration at which micelle formation occurs or caused further aggregation of the micelles into even larger complexes. The possibility of such mechanisms must await

more data on the physical properties of mixed micelles (26).

A decrease in bile flow as a result of sodium taurolithocholate infusion could also be attributed to reabsorption of water from the biliary tree. Schanker and Hogben (27) have shown that the bile-to-plasma concentration ratio for mannitol in the rat is approximately 1, indicating little, if any, water reabsorption from the biliary tree. The same results were found in these studies. In addition, mannitol excretion rate was found to parallel water flow during choleresis and cholestasis. Since the bile-to-plasma concentration ratio did not increase during the development of cholestasis, no evidence for water reabsorption was obtained. However, since mannitol could be freely diffusible across the biliary epithelium under these circumstances, it is possible that water reabsorption did occur and was not detected by this technique.

Although the physical properties of sodium taurolithocholate can explain the initial events in cholestasis, consideration should be given to the marked hemolytic and inflammatory properties that occur (11, 28). The mechanisms for these effects are not precisely known, but presumably the configuration of the molecule disrupts the membrane leading to liberation of hemoglobin from erythrocytes and inflammation in tissues. Effects of this type might account for the inability to rapidly reverse the cholestasis by infusion of sodium taurocholate. The trapping of sodium taurolithocholate within the biliary tree could have deleterious effects on the surrounding cells that temporarily alter their transport activity.

The monohydroxy, poorly water soluble, conjugated bile salt, sodium taurocholenate, is useful in evaluating the physiologic properties of sodium taurolithocholate in inducing cholestasis. This compound has a steroid ring configuration identical with cholesterol, and either compound protects against the hemolysis caused by lithocholate when added to a hemolytic system in vitro (11). Sodium taurocholenate was found to induce cholestasis which in all respects resembled the effect of sodium taurolithocholate. Although this compound could be metabolized to a variety of other compounds, the absence of detectable taurolithocholate indicates that the effect is not mediated via formation of this possible metabolite.

In conclusion, it seems reasonable to attribute the initial events of sodium taurolithocholate-induced cholestasis to the poor water solubility of this compound. The exact mechanisms by which cholestasis occurs is not clear. Hopefully, as knowledge of micelle structure and bile formation increases, a precise explanation will be found.

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