Effects of Taurodihydrofusidate, a Bile Salt Analogue, on Bile Formation and Biliary Lipid Secretion in the Rhesus Monkey

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ABSTRACT Bile salts play a major role in bile formation and biliary lipid secretion. Sodium taurodihydrofusidate (TDHF), a derivative of the antibiotic fusidic acid, closely resembles bile salts in terms of structure, micellar characteristics, and capacity to solubilize otherwise insoluble lipids. We have therefore studied the biliary secretion of this bile salt analogue and its influence on bile formation and biliary lipid secretion in primates.

Alert, unanesthetized female rhesus monkeys prepared with a total biliary fistula were allowed to reach a steady bile salt secretion rate before each study. In three animals (group I), [^{14}C]TDHF was infused intravenously. Most of the compound was secreted rapidly in bile chemically unchanged. The biliary secretion of this drug produced a twofold increase in bile flow; however, the bile salt output was markedly reduced during the infusion. In spite of this reduction, the phospholipid output remained essentially unchanged whereas the cholesterol output increased almost twofold.

In five other animals (group II), the effect of TDHF on the bile salt secretion was further investigated by an intravenous infusion of [^{14}C]taurocholate followed by a combined infusion of [^{14}C]taurocholate and TDHF. When TDHF was added to the infusate, a reduction in the [^{14}C]taurocholate output and a progressive rise in the plasma [^{14}C]taurocholate concentration were observed in each animal.

An analysis of the data in both groups indicates that (*a*) the most likely explanation to account for the decreased bile salt output is that the bile salt analogue, TDHF, interfered with bile salt secretion into the biliary canaliculi; (*b*) TDHF induces a greater secretion of biliary water than was observed with bile salts, an effect consistent with a stimulation of the bile salt-independent canalicular flow; (*c*) at similar 3α-hydroxysteroid secretion rates TDHF caused a significant increase in cholesterol secretion compared to that induced by bile salt. This finding suggests that TDHF affects cholesterol metabolism or secretion in a way distinct from bile salts. Thus, the solubilization of biliary lipids in mixed micelles, although essential, is only one of the factors which determine their secretion into bile.

INTRODUCTION

Bile salts undergo an enterohepatic circulation. Released from the gallbladder during a meal, they are absorbed from the intestine, returned to the liver by the portal vein, and resecreted in bile together with newly synthetized bile salts. Since the capacity of the liver to synthetize new bile salts is limited (1), efficient transport systems both in the intestine and in the liver are required to maintain a normal bile salt pool. Under normal circumstances, these requirements are remarkably well fullfilled. By a combination of passive absorption over the whole length of the intestine and active transport in the distal ileum, approximately 97% of bile salts entering the intestine each day are returned to the

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liver (1, 2). Bile salts in the portal blood are so efficiently extracted and secreted in bile by the liver that their concentration in systemic blood remains at very low levels (3).

In the course of their enterohepatic circulation, bile salts play a major role in the transport of various compounds. In the intestine, they facilitate lipid- and fat-soluble vitamin absorption. In bile, a close correlation has been demonstrated between the excretion of water (4), inorganic electrolytes (5), phospholipids, and cholesterol (6–8) and bile salt secretion rate. The nature of these relations, especially in bile, remains to a large extent poorly understood. Whereas, the effect of bile salt transport on bile and inorganic electrolytes secretion can be duplicated by various organic compounds transferred into bile (9), its influence on the biliary secretion of phospholipids and cholesterol, two highly water-insoluble lipids, has been regarded as unique. It has been postulated that this process occurs through incorporation of these two lipids into bile salt micelles (7, 10). Dehydrocholate, a synthetic bile salt which does not form micelles at physiologic concentration (11), does not stimulate cholesterol and phospholipid secretion in bile (7, 8). This finding has been used as evidence in favor of the micellar theory of biliary lipid transport. However, the extent to which phospholipid and cholesterol secretion in bile is dependent upon the physical properties of bile salts is unknown. An insight into that problem might be provided by drugs with similar physicochemical properties.

The sodium salt of fusidic acid, a steroid antibiotic produced by fermentation of the fungus *Fusidium coccineum* (12), closely resembles bile salts in terms of micellar characteristics and capacity to solubilize otherwise insoluble lipids (13). This antibiotic has also been shown to be excreted mainly in bile (12). In a subsequent study, the physicochemical properties of taurine and glycine conjugates of fusidic acid and other derivatives were investigated (14). One of these, taurodihydrofusidate (TDHF),[1] which does not exhibit any significant antibacterial activity (14), showed certain structural similarities with taurocholate (Fig. 1). It was also found to possess micellar characteristics similar to those of taurocholate (14) and, in the presence of lecithin, to solubilize as much cholesterol as taurocholate.[2] These similarities prompted us to investigate if this bile salt analogue would also mimic the physiologic effects of bile salts.

In this paper we report the effects of TDHF administration in rhesus monkeys. Specifically, we (*a*) describe how TDHF is metabolized and secreted by the liver, (*b*) examine the influence of TDHF on bile flow, (*c*) characterize its effect on bile salt, phospholipid, and cholesterol secretion into bile, and (*d*) compare these effects with those induced by bile salts.

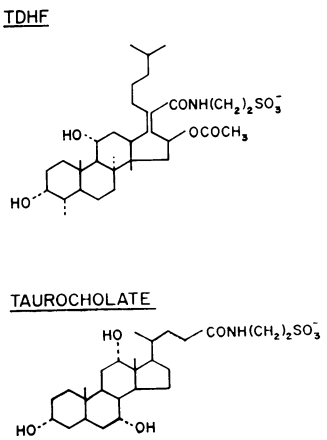

FIGURE 1 Chemical configuration of TDHF and taurocholate. Note that these two steroids possess a hydroxyl group in the 3α position.

METHODS

Materials

Chemicals. Sodium tauro-24,25-dihydrofusidate[3] (TDHF) (Fig. 1) differs structurally from the parent compound, sodium fusidate, by its conjugation with taurine and saturation of the C24–C25 double bond. Its purity was greater than 99% when checked by thin-layer chromatography (solvent system, isoamylacetate : propionic acid : *n*-propanol : water, 40 : 30 : 20 : 10, vol/vol) (15). Sodium taurocholate was purchased (Calbiochem, San Diego, Calif.) and was at least 98% pure by thin-layer chromatography and proton titration.

Isotopes. [^{14}C]TDHF[3] (sp act 0.061–0.121 μCi/mg) was biosynthetized from [^{14}C]mevalonate and its radiochemical purity, estimated by thin-layer chromatography, varied between 94 and 98%. [24-^{14}C]Sodium taurocholate (sp act 4.11 μCi/μmol) was purchased (Mallinckrodt Chemical Works, St. Louis, Mo.) and found to be 98% pure by thin-layer chromatography.

Experimental design

Animal model. The primate model and the electronic apparatus used in these studies have been described previously (16). Briefly, female rhesus monkeys, weighing 4–5 kg, were trained to sit in restraining chairs for periods of 1–2 wk before surgery. Under sodium pentobarbital (Nembutal, Abbott Laboratories, North Chicago, Ill.) anesthesia (20 mg), the cystic duct and the distal part of the common bile duct were ligated and T-tubes were implanted in the proximal part of the common bile duct and in the duodenum and exteriorized through the abdominal wall. A period of 3 wk was allowed for recovery from surgery. Before and between experiments, the enterohepatic circulation of bile

[1] *Abbreviations used in this paper:* CMC, critical micellar concentration; TDHF, taurodihydrofusidate; T_m, transport maximum.

[2] Carey, M. C., and D. M. Small. Unpublished observations.

[3] A generous gift of Drs. W. O. Godtfredsen and W. von Daehne, Leo Pharmaceutical Products, Ballerup, Denmark.

salts was preserved through an electronic stream splitter which returned 95% of the bile to the animal while the remainder was collected and used for various analyses.

In order to be able to recognize changes in biliary flow and lipid outputs which might have been induced by feeding, animals were fed 60 g of Purina Monkey Chow (Ralston Purina Co., St. Louis, Mo.) twice daily, at regular intervals, and fasted between meals except for ad libitum access to water. Before study, the enterohepatic circulation of bile salts was interrupted by a complete collection of bile. The events following such an interruption have been well characterized in the rhesus monkey (1, 2, 6). After an initial period of 4–5 h during which the bile salt pool is washed out, the bile salt secretion rate reaches a low value corresponding to the bile salt synthetic rate under normal conditions. Over the next 24–48 h bile salt synthesis increases to reach a constant maximum rate by 48 h. All studies were carried out on the 4th day after the interruption of the enterohepatic circulation of bile salts, at a time when a steady state had been reached. Each animal served as its own control.

Intravenous infusion of TDHF (group I). Intravenous infusions of TDHF were carried out in three animals. Each animal was studied twice, giving a total of six experiments. A 0.63% TDHF solution (2–4 μCi of [^{14}C]TDHF mixed with carrier) was prepared in 0.15 M saline and filtered through a 0.22-μm filter (Millipore Corp., Bedford, Mass.) with a microsyringe filter holder (XX30 025 00, Millipore Corp.). After a 3-h infusion of 0.15 M saline (control period) at a rate of 22.14 ml/h (Harvard pump model 600-000, Harvard Apparatus Co., Inc., Millis, Mass.) through a femoral vein catheter, TDHF was infused at a rate of 200 μmol/h (corresponding to a volume of 22.14 ml/h) for the next 4 h. By using a fraction collector (Buckler Instruments Div., Searle Analytic Inc., Fort Lee, N. J.), hourly collections of bile were obtained during the 24 h following the start of the experiment and bile volume determined in calibrated graduated centrifuge tubes (10–15-ml capacity). Blood samples were withdrawn from a peripheral vein in the middle of a bile collection period and plasma separated by centrifugation. Urine was recovered separately from feces and collected over 24 h. All specimens were stored at − 15°C until analyzed. At the conclusion of the experiment, the enterohepatic circulation of the animal was reestablished. A minimum of 2 wk was allowed between experiments in the same animal.

Intravenous infusion of taurocholate and TDHF (group II). Five studies were performed in five different animals. The various solutions used in these studies were filtered as described above and the infusion rate kept constant during the experiment (22.14 ml/h). After a 2-h infusion of 0.15 M NaCl, sodium taurocholate (0.48% solution in 0.15 M saline containing 3 μCi of [^{14}C]taurocholate) was administered intravenously at a rate of 200 μmol/h for 4 h. During the following 4 h, taurocholate and TDHF were infused simultaneously each at a rate of 200 μmol/h (1.11% solution in 0.15 M saline containing 3 μCi of [^{14}C]taurocholate). In three animals, this combined infusion was followed by an additional infusion of taurocholate at a rate of 400 μmol/h for 4 h (0.96% solution in 0.15 M NaCl with 6 μCi of [^{14}C]taurocholate). Samples were collected and processed as in the previous group of experiments.

Laboratory procedures

Radioactivity determination. ^{14}C activity in bile, plasma, and urine was determined in a liquid scintillation spectrometer (model LS-250, Beckman Instruments, Inc., Fullerton, Calif.). All biological samples were prepared by mixing an aliquot of 100 μl with 1 ml of Hyamine hydroxide (New England Nuclear, Boston, Mass.), 2 ml of methanol, and 15 ml of Liquifluor (New England Nuclear). The bile samples after the addition of Hyamine hydroxide and methanol were bleached under an ultraviolet lamp (Black Ray B-100A, Ultra-Violet Products, Inc., San Gabriel, Calif.) for 4 h. In order to achieve complete dissolution, the plasma and Hyamine hydroxide mixture was heated at 56°C for 3–5 h. Correction for quenching was made with an internal standard.

Thin-layer chromatography. A suitable aliquot of bile was spotted on a 0.25-mm silica gel G (Brinkmann Instruments, Inc., Westbury, N. Y.) plate and developed in isoamylacetate : propionic acid : *n*-propranol : water (40 : 30 : 20 : 10, vol/vol) (15). The plate was then sprayed with a 50% sulfuric acid solution and heated at 80°C for 10–15 min. This technique revealed bile salts as yellow bands, cholesterol as a pink band, and TDHF and related compounds as purple bands. These purple bands were transferred to scintillation vials and eluted in 1 ml of Hyamine hydroxide and 2 ml of methanol. The radioactivity in each band was then determined as described above.

Biliary lipid determination. Biliary phospholipids were measured directly in bile as inorganic phosphorus by the method of Bartlett (17). Cholesterol was determined with Carr and Drekter's modification (18) of the original method of Abell et al. (19). Total bile salt concentration in the absence of TDHF was measured enzymatically by the 3α-hydroxysteroid dehydrogenase method of Talalay (20) as modified by Admirand and Small (21). Bile salt concentration in samples containing TDHF was determined with this enzymatic technique after corrections for the amount of TDHF present (see following section).

RESULTS

Bile salt determination in the presence of TDHF in bile

As shown in Fig. 1, TDHF possesses a 3α-hydroxy group which, in bile salts, is the structural requirement for their determination by the 3α-hydroxysteroid dehydrogenase method (20). Fig. 2 indicates that TDHF can act as a substrate for this enzyme. In the presence of 3α-hydroxysteroid dehydrogenase and NAD, methanolic

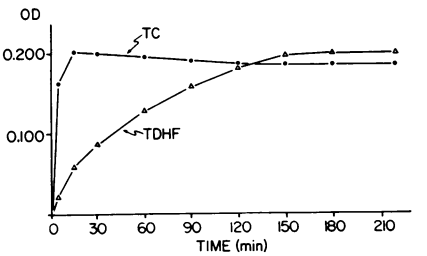

FIGURE 2 Enzymatic assay of TDHF. Graph shows the curves of optical densities (OD) vs. time for methanolic solutions of TDHF (open triangles) and taurocholate (closed circles) of identical molarities (12 mM). The peaks in optical densities for each curve are similar but attained after different intervals of time.

solutions of TDHF and taurocholate of identical molarity lead to the formation of similar amounts of NADH, represented by the peak in optical densities. However, the time required for the completion of the reaction is quite different for these two substrates. A peak is attained in 15 min with taurocholate whereas in the case of TDHF it is reached only after 150 min.

The assay of a bile sample, with a bile salt concentration of 14 mM is illustrated on Fig. 3. Also represented on this figure for comparison is the assay of the same bile in which, by dilution with water and addition of TDHF, the bile salt and TDHF concentrations were each adjusted to 7 mM, thus bringing the total concentrations of 3α-hydroxysteroids to 14 mM. The peaks in optical densities for these two samples, although reached after different intervals of time, have similar values. This enzymatic assay can thus measure the total 3α-hydroxysteroids (bile salts and TDHF), provided enough time is allowed for the completion of the reaction. Therefore, for *each* bile sample, the reaction was carried out until a peak or a plateau was obtained. The bile salt concentration in such a mixture was obtained by subtracting the TDHF concentration measured by radioactivity from the total 3α-hydroxysteroid concentration determined with the enzymatic assay.

Recovery of TDHF in bile and in urine

In six experiments performed on three monkeys (group I), during which [^{14}C]TDHF was infused intravenously at a rate of 200 μmol/h for 4 h, 67.5% (±SEM 4.4) of the radioactivity was recovered in the bile and 24.3% (±SEM 3.6) in the urine during the 24 h following the start of the infusion (Table I). During the 2nd day after the infusion, an additional 2% was recovered in the urine and bile, and 6% was unaccounted for. Fig. 4 shows the cumulative excretion of TDHF in bile, expressed as a percent of the infused dose. As indicated by the occurrence of a plateau, most of the biliary excretion of TDHF occurred in the first 6 h after the start of the infusion, 88% of the biliary radioactivity having been recovered over that period of time.

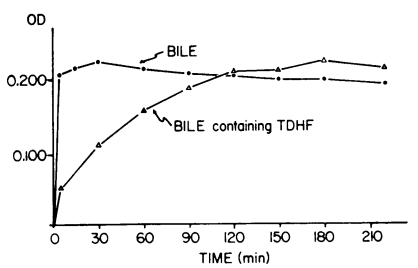

FIGURE 3 Enzymatic assay of bile containing TDHF. Graph shows the curves of optical densities (OD) vs. time for a bile sample (bile salt concentration, 14 mM) (closed circles) and for the same bile (open triangles) where, by dilution and addition of TDHF, the 3α-hydroxysteroid concentration was adjusted to 14 mM (bile salts, 7 mM; TDHF, 7 mM). The peaks in optical densities for each curve are similar but attained after different intervals of time.

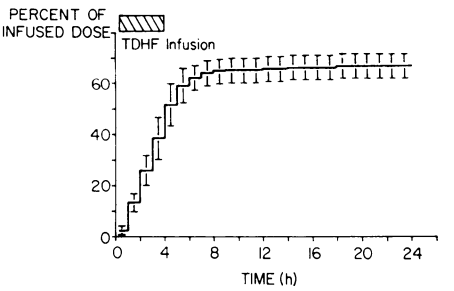

FIGURE 4 Cumulative excretion of TDHF in bile after a 4-h intravenous infusion of TDHF. Values correspond to the mean±SEM of six experiments in three monkeys.

Thin-layer chromatography of bile during TDHF infusion

Aliquots of bile obtained during the middle of the infusion, in each experiment, were examined by thin-layer chromatography. A representative plate is shown schematically in Fig. 5. In addition to bile salts and

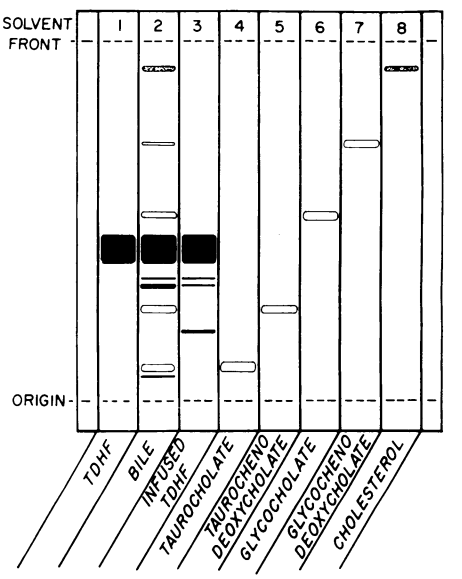

FIGURE 5 Schematic thin-layer chromatogram of bile obtained during TDHF infusion in a representative experiment. Bile salts were revealed as yellow bands (open spots), TDHF and related compounds as purple bands (solid spots), and cholesterol as a pink band (stippled spots). In addition to a large purple band (lane 2), corresponding to authentic TDHF (lane 1), the bile chromatogram showed three smaller purple bands; two of these have R_f values similar to two small bands contained in the infused TDHF (lane 3).

TABLE I
TDHF Infusion (Group I)

Monkey	TDHF recovery in bile*	TDHF recovery in urine*	Biliary radio-activity as TDHF	Bile-to-plasma concen-tration ratio of TDHF‡	Bile flow‡	TDHF output‡	3α-Hydroxysteroid output‡	Bile salt output‡	Phospho-lipid output‡	Cholesterol output‡
	% of dose	*% of dose*	*%*		*ml/h*	*μmol/h*	*μmol/h*	*μmol/h*	*μmol/h*	*μmol/h*
IV*c*										
Control	—	—	—	—	3.6	—	29.9	29.9	5.80	0.65
TDHF	60.6	32.8	91.2	99	6.3	71.4	80.8	9.4	5.77	1.36
IV*d*										
Control	—	—	—	—	5.4	—	101.7	101.7	24.78	2.53
TDHF	88.6	10.1	92.1	889	7.9	119.4	184.9	65.6	26.22	5.75
V*b*										
Control	—	—	—	—	2.0	—	55.5	55.5	11.80	1.27
TDHF	63.5	29.1	88.0	82	5.7	62.5	60.0	−2.5	14.16	2.28
V*c*										
Control	—	—	—	—	3.8	—	104.5	104.5	14.10	1.52
TDHF	59.0	19.6	90.3	202	6.1	67.5	90.7	23.2	11.21	2.14
VII*a*										
Control	—	—	—	—	3.4	—	85.8	85.8	19.81	2.40
TDHF	68.8	22.0	93.9	370	5.3	82.6	106.8	24.2	17.33	4.10
VII*b*										
Control	—	—	—	—	2.5	—	21.1	21.1	5.05	2.36
TDHF	64.2	32.1	91.6	236	3.5	70.0	89.0	19.0	27.54	5.59
Mean±SEM§										
Control	—	—	—	—	3.5 ±0.3	—	66.4 ±8.5	66.4 ±8.5	13.6 ±1.8	1.79 ±0.18
TDHF	67.5 ±4.4	24.3 ±3.6	91.2 ±0.80	313 ±122.8	5.8 ±0.2	78.9 ±3.5	102.0 ±7.2	23.1 ±3.8	17.0 ±1.4	3.54 ±0.31
P value‖	—	—	—	—	$P < 0.01$	—	$0.1 > P > 0.05$	$P < 0.02$	NS	$P < 0.02$

* During the 24 h following the start of the infusion.
‡ Values for the control period correspond to the mean of all measurements in each animal. Values for the TDHF infusion correspond to the mean of all measurements in each animal during the 6 h following the start of the infusion.
§ Mean±SEM of all values in all animals.
‖ Determined by Studnet's *t* test.

cholesterol, the bile chromatogram revealed a large purple band, corresponding to authentic TDHF, plus three smaller and more polar purple bands. The TDHF solution infused contained small amounts of impurities, as shown by the three small purple bands in the corresponding lane. Two of these impurities had R_f values similar to the small purple bands found in bile. The number of these smaller bands in bile obtained from different experiments varied between two and four. In bile, TDHF accounted for 91.2% (±SEM 0.8) of the radioactivity present in these various bands (Table I), the remaining radioactivity was accounted for within the smaller bands.

Uptake and biliary excretion of TDHF

In Fig. 6, TDHF biliary secretion and plasma concentration, calculated from the radioactivity of bile or plasma and the specific activity of the infused TDHF, is shown during and after TDHF infusion into group I animals. After a rapid increase in the first 2 h of the infusion, a relatively constant output is reached during the

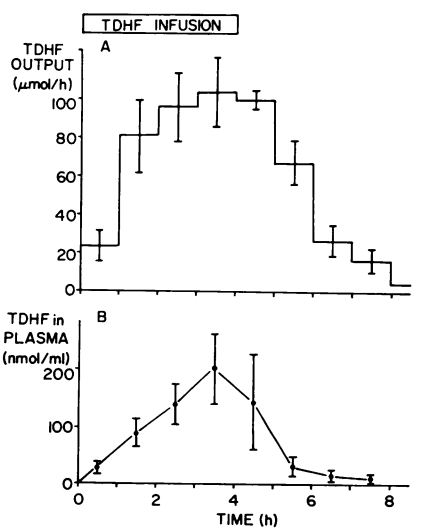

FIGURE 6 TDHF biliary output (A) and plasma concentration (B) during and after a 4-h intravenous infusion of TDHF. Values correspond to the mean±SEM of six experiments in three monkeys.

following 3 h. This plateau averaged approximately half the infusion rate. In spite of a constant rate of administration, the plasma concentration of TDHF increased at a steady rate during the duration of the infusion. After the cessation of the infusion the plasma concentration fell. Biliary concentrations of TDHF were 82–889 times greater than in plasma (Table I).

Effect of TDHF on bile flow

The TDHF infusion in group I led, in each experiment, to an increase in bile flow (Table I). The mean bile flow during the control period was 3.5 ml/h (±SEM 0.3) as compared to 5.8 ml/h (±SEM 0.2) during the 6 h following the start of the TDHF infusion ($P < 0.01$). During this period, there was a significant correlation between bile flow and the output of 3α-hydroxysteroids determined enzymatically ($r = 0.609$, $P < 0.001$). In Fig. 7, bile flow values in these animals are plotted against the corresponding 3α-hydroxysteroid outputs (closed circles). These values include only those where a relatively constant output of TDHF were observed and where presumably a steady state was achieved. Infusion of taurocholate at a rate of 200 μmol/h in group II also produced a significant increase in bile flow (control period, 2.7 ml/h ±SEM 0.5; 4-h taurocholate infusion, 5.3 ml/h ±SEM 0.1; $P < 0.01$). A linear relationship was found between bile flow and 3α-hydroxysteroid output determined enzymatically in this group ($y = 0.0139x + 2.58$, $r = 0.845$, $P < 0.001$). Bile flow and 3α-hydroxysteroid output values before and during the taurocholate infusion in this group are plotted on

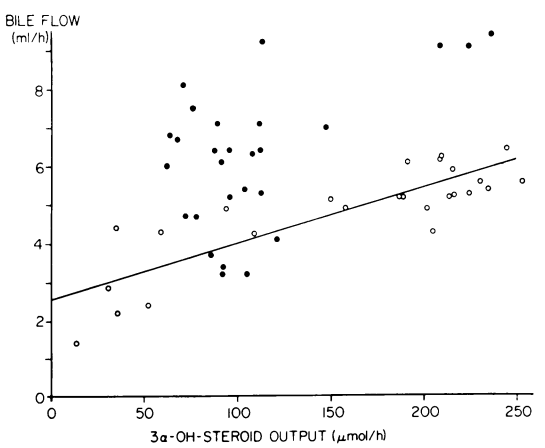

FIGURE 7 Relationship between bile flow and 3α-hydroxysteroid output in animals infused with TDHF (closed circles) and taurocholate (open circles). Closed circles represent only values where a relatively constant output of TDHF in bile was obtained. Open circles and the calculated regression line (method of least mean squares) correspond to all values obtained before and during the taurocholate infusion.

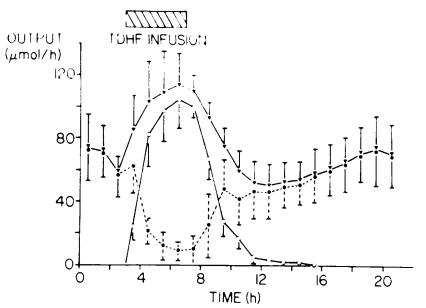

FIGURE 8 Bile salt output before, during, and after a TDHF infusion. Values represent the mean±SEM of six experiments in three animals. Closed triangles correspond to the 3α-hydroxysteroid output measured with the enzymatic assay. Open circles correspond to the TDHF output measured by radioactivity. Closed circles correspond to the bile salt output calculated by difference.

Fig. 7 (open circles), together with the corresponding regression line. These values include all those obtained during the control period and the 4-h taurocholate infusion. The 3α-hydroxysteroids in these animals consisted only of bile salts. Except for one experiment in group I (corresponding to the five closed circles below the regression line) a clear separation between the two groups is achieved: for comparable outputs of 3α-hydroxysteroids, the bile flow is greater in the animals receiving TDHF.

Effect of TDHF on bile salt output

Group I. The effect of TDHF on the bile salt output in these animals is illustrated on Fig. 8. The 3α-hydroxysteroid output measured by the enzymatic technique is represented by the closed triangles and thus corresponds to the sum of the TDHF plus bile salt outputs. The TDHF output measured by radioactivity determination is represented by the open circles. The bile salt output, calculated by difference between these two, is represented by the closed circles. Although variations in the bile salt output during the control period were observed between animals and between experiments in the same animal (Table I), a similar change in the bile salt output was found in every animal during the infusion of TDHF. During the 6 h following the start of the infusion, the bile salt output was markedly reduced (control), 66.4 μmol/h ±SEM 8.5; TDHF infusion, 23.1 μmol/h SEM 3.8; $P < 0.02$). During this period, the 3α-hydroxysteroid output increased moderately ($0.1 > P > 0.05$), and this increase was accounted for entirely by the TDHF secretion. After cessation of the infusion, the bile salt output returned progressively to control values, thus excluding the possibility that the changes observed were the result of an unsteady state.

Group II. The [^{14}C]taurocholate output in these animals was calculated from the ^{14}C content of bile and

TABLE II

[^{14}C]Taurocholate Plasma Concentrations before, during, and after a TDHF Infusion

Monkey	Time, *h*...	Infusion: [^{14}C]Taurocholate, 200 μmol/h				Infusion: [^{14}C]Taurocholate, 200 μmol/h, + TDHF, 200 μmol/h				Infusion: [^{14}C]Taurocholate, 400 μmol/h			
		1	2	3	4	5	6	7	8	9	10	11	12
		nmol/ml											
VII*b*		1.5	0	1.5	1.5	3.8	19.8	56.3	53.3	—	—	—	—
IX		24.5	38.7	24.5	40.0	59.5	127.8	173.3	189.8	—	—	—	—
X		—	80.7	—	53.4	96.0	104.7	153.8	273.7	219.2	200.7	172.3	195.2
XI		21.6	19.4	23.7	22.4	29.7	31.4	31.9	40.7	53.4	47.8	47.5	59.1
XII		45.3	20.9	27.9	23.9	54.6	68.9	86.9	113.6	128.8	78.7	79.1	98.7
Mean		23.2	31.9	19.4	28.2	48.7	70.5	100.4	134.2	—	—	—	—
±SEM		±9.0	±13.6	±6.0	±8.8	±15.4	±20.7	±27.4	±43.7	—	—	—	—

the specific activity of the infused taurocholate. During the infusion of [^{14}C]taurocholate alone, the output of [^{14}C]taurocholate in bile reached a plateau after the 1st h of infusion. In Fig. 9, the mean [^{14}C]taurocholate output during the following 3 h, for each animal, is compared with the mean [^{14}C]taurocholate output during the 4-h combined infusion of [^{14}C]taurocholate and TDHF. In each animal, a decrease in the [^{14}C]taurocholate output was observed when TDHF was added to the infusate. The mean output during [^{14}C]taurocholate infusion was 161.6 μmol/h (±SEM 5.8) compared to 141.7 μmol/h (±6.5) during the TDHF and [^{14}C]taurocholate infusion ($P < 0.05$). The [^{14}C]taurocholate plasma concentration (Table II), while almost constant during the infusion of [^{14}C]taurocholate alone, rose continuously during the 4 h when [^{14}C]taurocholate and TDHF were administered simultaneously. In three animals of this group the combined administration of [^{14}C]taurocholate and TDHF was followed by a [^{14}C]taurocholate infusion at a rate of 400 μmol/h. During this period biliary [^{14}C]taurocholate output rose to 303.9 μmol/h (±SEM 13.6) whereas the plasma concentration remained relatively constant (Table II).

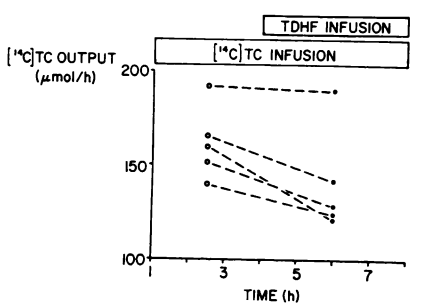

FIGURE 9 Effect of TDHF on the biliary output of [^{14}C]-taurocholeate (TC) during a constant intravenous infusion (200 μmol/h) of [^{14}C]taurocholate. Open circles correspond to the mean value, in each animal, for the last 3 h during the infusion of [^{14}C]taurocholate alone. Closed circles correspond to the mean value, in each animal, for the 4-h combined infusion of TDHF and [^{14}C]taurocholate.

Effect of TDHF on the phospholipid and cholesterol secretion

Animals infused with TDHF alone (group I) presented an insignificant increase in the phospholipid output (control period, 13.6 μmol/h ±SEM 1.8; TDHF infusion, 17.0 μmol/h ±SEM 1.4; NS) but a twofold increase in the cholesterol output (control period, 1.79 μmol/h ±SEM 0.18; TDHF infusion, 3.54 μmol/h ±SEM 0.31; $P < 0.02$) (Table I). However, since the secretion rate of 3α-hydroxysteroid was different in control and TDHF periods (66.5±8.5 vs. 102.0±7.2 μmol/h, Table I) it is important to compare phospholipid and cholesterol secretion rates at a similar secretion rate of 3α-hydroxysteroid. Table III compares the secretion rate of phospholipids and cholesterol at similar

TABLE III

Phospholipid and Cholesterol Secretion Rates at Comparable 3α-Hydroxysteroid Secretion Rates

	Secretion rates: 3α-Hydroxysteroid	Phospholipid	Cholesterol
	μmol/h		
Bile salt	99.1±8.7*	20.1±1.5	2.21±0.18
		NS‡	$P < 0.001$§
"TDHF"‖	102.0±7.2	17.0±1.4	3.54±0.31

* Mean±SEM.
‡ No significant difference.
§ Significant by Student's *t* test.
‖ The mean secretion rate was 77% TDHF and its minor metabolites and about 23% endogenous bile salts.

secretion rates of bile acids (99.1±8.7 μmol/h) and total 3α-hydroxysteroid during the TDHF period (102.0 ±7.2 μmol/h). While there is no significant difference in phospholipid secretion ($0.2 > P > 0.1$), cholesterol secretion is significantly enhanced during the period of TDHF secretion ($P < 0.001$).

DISCUSSION

These studies were undertaken as an extension of previous work (13, 14) in which we have characterized the physical properties of fusidic acid and some of its derivatives. Herein, we have investigated how one of these derivatives, TDHF, is metabolized and excreted in the rhesus monkey and determined its effect on bile formation and biliary lipid secretion.

In any study of a drug, it is most important to determine its metabolic stability in vivo. Chemical alterations of the parent compound may result in metabolites with different properties. Bile collected from patients given fusidic acid contained, in addition to unchanged fusidic acid, at least seven other metabolites (12). Such an extensive metabolism has also been observed in rats (22) and monkeys[4] infused with 3-acetoxyl fusidate. On the other hand, we have shown that the taurine conjugate of dihydrofusidic acid is much more resistant to metabolic alteration since 91% of TDHF was excreted unchanged in bile. No further attempts were made to identify the other minor compounds revealed by thin-layer chromatography, although two of these represent impurities contained in the infused preparation. The finding that conjugation of this steroid with taurine almost entirely prevents it metabolism is similar to a recent observation by Young and Hanson (23). Using a perfused liver preparation, they have shown that dehydrocholate, which in various species is largely metabolized to many different compounds (24–26), is excreted unaltered in bile when conjugated with glycine.

TDHF was excreted rapidly in bile since 88% of the biliary excretion occurred in the first 6 h after the start of the infusion. This rapid excretion in bile was paralleled by a prompt fall in TDHF plasma concentration in the 2 h following the cessation of the infusion (Fig. 6). In humans, after a single oral dose of 500 mg, plasma concentration of fusidic acid or its biologically active metabolites remained elevated for 8 h, and even after 24 h considerable amounts could be detected (12). This suggests that conjugation of this steroid with taurine accelerates its clearance by the liver. A similar observation has been reported by O'Maille et al. in dogs, where the plasma disappearance of taurocholate after a bolus injection was more rapid than that of unconjugated cholate (27). A looser binding to plasma proteins, increased uptake by the hepatocytes, reduced transit time in liver cells in the absence of any biotransformation, or greater affinity for the canalicular transport mechanism could, individually or together, account for the more rapid clearance of these two types of steroids when conjugated.

During the infusion, high biliary concentrations of TDHF were achieved with bile-to-plasma concentration ratio ranging from 82 to 889. In spite of an infusion rate of 200 μmol/h, the maximum biliary output was approximately 100 μmol/h. Such a difference between the amounts infused and excreted into bile could result at least partly from excretory pathways other than bile, such as urine, or from tissue distribution. However, the plasma concentration of TDHF rose steadily during the whole length of the infusion period whereas the biliary secretion reached a plateau after the 2nd h. (Fig. 6). These findings, that is, a concentration gradient between bile and plasma and a steady output in the face of increasing plasma concentration (28), suggest that TDHF is probably transferred into bile by an active transport system and that the apparent biliary transport maximum (T_m) has been reached by an infusion rate of 200 μmol/h. This apparent T_m would correspond to a biliary output of approximately 100 μmol/h. A stronger argument to establish that a true T_m for TDHF had really been reached would, however, have required various infusion rates instead of a single one as carried out in this study. Although no T_m value for bile salt have been reported in rhesus monkeys, its value is greater than 800 μmol/h (2). The T_m of taurocholate in dogs (29) is equal to 8.5 μmol/min per kg. If a similar value for a 4.5 kg monkey is assumed, the T_m for taurocholate would be equal to 2,295 μmol/h in this species, a value far exceeding the apparent T_m of TDHF. The T_m of other organic anions secreted in bile such as bilirubin (30) or sulfobromophthalein (28, 31) is also much lower than that of bile salts. The capacity of the liver to transport bile salts surpasses that of any organic compound and this remains true for TDHF.

Current views on the sites and mechanisms of bile formation hold that bile salts are responsible for an important fraction of the biliary water secreted in the canaliculi (4). This effect is thought to be the result of an osmotic filtration induced by the high concentration of bile salts achieved in the canalicular lumen (32). The increase in bile flow associated with the biliary excretion of TDHF in high amounts and a return to control values as the TDHF output fell would suggest a similar mechanism for the choleretic effect of TDHF. However, as shown in Fig. 7, the bile production in response to a given 3α-hydroxysteroid output was greater in most studies during TDHF infusion than during taurocholate infusion. Previous studies have documented

[4] Beaudoin, M., J. C. Montet, M. C. Carey, and D. M. Small. Unpublished observations.

the fact that various organic anions secreted in bile differ in their choleretic properties. For example, ioglycamide, a biliary contrast medium, and sulfobromophthalein were found to be more potent, on a molar basis, than taurocholate (9). Hoenig and Preisig suggested that these differences could be accounted for by the aggregation of bile salts with phospholipids and cholesterol into mixed micelles (9). Their osmotic activity as part of such micelles and presumably their choleretic potency should be less than that of nonassociating drugs.

No information is available on the osmotic properties of simple and mixed bile salt or TDHF micelles. However, systematic physicochemical studies on the critical micellar concentration (CMC), micellar mass, and degree of counterion binding of several fusidane detergents including TDHF suggest that these parameters are very similar to those of the common free and conjugated bile salts in both water and 0.15 M NaCl solutions (13, 14).[5] In view of the fact that the osmotic activity of a detergent solution is directly dependent on CMC, aggregation number, and the percentage of counterions bound by micelles, it would be very surprising if the osmotic activity of equimolar solutions of bile salts and TDHF differed significantly. This would suggest that part of the secretion of water induced by TDHF appears to be independent of its osmotic effect. Recent studies have indicated that a significant fraction of the canalicular bile flow is produced independently of the bile salt secretion (33–35). A plausible explanation for the greater choleretic effect of TDHF would be a stimulation of this bile salt-independent canalicular flow. Such an effect has already been postulated to account for the pronounced choleresis produced by dehydrocholate metabolites (26) and an organic acid, SC 2644 (8).

The influence of TDHF on the bile salt output in bile was investigated in two different situations. In animals of group I, the bile salt output reflects bile salt *synthesis* in the liver cell and *secretion* into the canalicular lumen. It was reduced by 66% during the TDHF infusion. Since the bile salt output in these experiments was calculated by difference between the 3α-hydroxysteroid output and the TDHF output, underestimation of the former or overestimation of the latter could give a falsely low value for the bile salt output. 9% of the radioactivity excreted in bile was associated with compounds other than TDHF. We have not determined if these compounds were measured by the 3α-hydroxysteroid dehydrogenase since each one was present in minute amounts; if they were not, this would lower the 3α-hydroxysteroid output by a value insufficient to account for the marked reduction in the bile salt output. This reduced output, in that given experimental situation, can therefore be attributed to an inhibition of either bile salt synthesis or secretion. In animals of group II, we have looked at the transport of labeled taurocholate from blood to bile, before, during, and after a TDHF infusion. The taurocholate output in this group reflects *hepatic uptake* and *secretion* into bile. During TDHF infusion, plasma concentrations of taurocholate rose progressively and taurocholate output into bile fell. These findings can be attributed to an impairment of either bile salt uptake or secretion.

In view of the findings in both groups of experiments, the only explanation for the effects of TDHF on the bile salt output is an impairment of bile salt *secretion.* An abnormality in the uptake of bile salts does not explain the result in the first group since the enterohepatic circulation was completely interrupted. Further, the fact that, in this group, the bile salt output returned rapidly to near control values as soon as the TDHF output fell makes it unlikely that synthesis was involved; change in bile salt synthesis rate in the monkey occurs over a much longer period of time (1). In group II, an impairment of synthesis cannot account for the reduction of the labeled taurocholate output. Further, the elevation of the plasma taurocholate during the TDHF infusion is consistent with a block in secretion with a subsequent intracellular accumulation and decreased uptake. In addition, the reduction in the bile salt output for a similar infusion rate of TDHF was more pronounced in animals who had initially a low bile salt secretion (group I) than in those who had a high one (group II); this is consistent with a competitive inhibition between TDHF and bile salt for a common carrier which is partially reversed by increasing the amount of the bile salts presented to this carrier. Thus, an impairment of bile salt secretion by TDHF remains the single and most likely explanation which could account for all of the findings although our studies do not completely exclude the possibility that TDHF might interfere simultaneously at several steps in the transport and metabolism of bile salts in the liver. For instance, a combined defect in uptake and synthesis could explain the effects of TDHF.

It has been previously documented that there is a close correlation between bile salt secretion and both phospholipid and cholesterol secretion (6-8). TDHF secretion also affects phospholipid and cholesterol secretion. During the 6-h period following the start of the TDHF infusion, the mean 3α-hydroxysteroid secretion rate was 102±7.2 μmol/h, of which only 23.1±3.4 μmol/h was bile salt. Thus, during this period about 77% of the 3α-hydroxysteroid output was TDHF and its minor metabolites. When compared to a similar secretion rate of bile salts during the control period (99.1±8.7 μmol/h), TDHF appearance in bile was accompanied by a significant increase in cholesterol secretion, but no in-

[5] Carey, M. C., and D. M. Small. Unpublished observations.

crease in phospholipid secretion (Table III). Thus, for a given rate of 3α-hydroxysteroid secretion, cholesterol secretion is enhanced by TDHF.

Aside from stimulating phospholipid and cholesterol secretion into bile, bile salts also exert a profound influence on the intrahepatic metabolism of these lipids. Lecithin synthesis in the liver has been shown to be dependent on bile salt flux through the liver (36, 37), while hydroxymethylglutaryl-CoA reductase, the rate-limiting enzyme in cholesterol synthesis, was depressed by bile salt feeding (38, 39). These metabolic effects of bile salts may, to a certain extent, determine the availability of phospholipids and cholesterol for biliary secretion and the relative proportion of these three lipids in bile. In the absence of any significant difference in the lipid-solubilizing capacity of TDHF and bile salts, the peculiar pattern of cholesterol secretion observed with TDHF suggests that this steroid influences the intrahepatic metabolism or secretion of cholesterol in a manner distinct from endogenous bile salts.

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