Effects of monosulfate esters of taurochenodeoxycholate on bile flow and biliary lipids in hamsters[1]

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Abstract The effect of the 3α- and 7α-monosulfate esters of taurochenodeoxycholate on bile flow and biliary lipids was compared to the effect of unsulfated taurochenodeoxycholate. Test bile salts were infused directly into the portal circulation through a catheter introduced into the splenic pulp. Recovery of unsulfated and sulfated bile salts was complete; no biotransformation of any of the administered compounds was noted. Equivalent choleresis was noted in response to administration of each of the test bile salts. Of particular interest, the biliary cholesterol and phospholipid content was tightly linked to biliary bile salt monosulfates; the slope of the line describing the relationship between bile salts and lipids was similar to that for the unsulfated bile salt. The critical micellar concentration of the 3α- and 7α-monosulfate esters was 19 mM and 18 mM, respectively. Sulfation of taurochenodeoxycholate, therefore, does not impair its bile secretory function. Despite a higher critical micellar concentration, biliary lipid excretion with monosulfate esters is equivalent to that seen with unsulfated bile salt. The role of hydrophobic/hydrophilic balance in the promotion of biliary lipid excretion may need to be redefined.—**Stevens, R. D., L. Lack, R. H. Collins, W. C. Meyers, Jr., and P. G. Killenberg.** Effects of monosulfate esters of taurochendeoxycholate on bile flow and biliary lipids in hamsters. *J. Lipid Res.* 1989. **30:** 673–679.

Supplementary key words bile salts • bile salt monosulfate esters • choleresis • bile flow • biliary phospholipid • biliary cholesterol

Several mammalian species including humans synthesize sulfate esters of conjugated bile salts in response to cholestasis (1–3). The proportion of the bile salt pool that is excreted into the bile as sulfate esters rises from about 6% in normal humans to over 50% in patients with chronic cholestatic illnesses (4). During both intra- and extrahepatic cholestasis, taurochenodeoxycholate conjugates are the major constituents of the bile salt pool.

Known consequences of sulfation of conjugated bile salts include increased renal clearance of bile salts (5) and interruption of the enterohepatic circulation of the bile salt pool due to decreased ileal reabsorption (6). These changes in the metabolism of the bile salts may be attributed to an increase in the electrostatic charge conferred by the addition of the sulfate group. The resultant increase in urinary and fecal loss of bile salt sulfates due to increased negative charge is reflected in a decrease in the size of the bile salt pool (7). Sulfate esterification of an hydroxyl group on the A or B ring of a bile salt also changes the hydrophobic/hydrophilic balance of the bile salt and should raise the critical micellar concentration (CMC) above that for the unsulfated bile salt (8). Because CMC is thought to be an important reflection of the ability of a given bile salt to promote biliary excretion of lipids, increased biliary excretion of bile salt sulfates might be expected to result in diminished biliary cholesterol and phospholipid excretion.

Recent work by Yousef and colleagues (9) has shown that biliary excretion of completely sulfated, unconjugated bile acids does not promote biliary excretion of cholesterol or phospholipid. However, these studies may not be relevant to an understanding of the role of bile salt sulfation in clinical cholestasis because in humans almost all of the bile salts in bile are conjugated with glycine or taurine (10). Furthermore the di- and tri-sulfated bile acids used in these studies are uncommon in urine or bile, even during cholestasis (1, 11). Monosulfated conjugated bile salts predominate in cholestatic animals and patients (12). Therefore, the physiological significance of these studies is not clear.

Abbreviations: TCDC, taurochenodeoxycholate; TCDC-3-SO_4, TCDC-7-SO_4, 3α- and 7α-sulfate esters of taurochenodeoxycholate; TC, taurocholate; TUDC, tauroursodeoxycholate; CMC, critical micellar concentration; TLC, thin-layer chromatography; HPLC, high performance liquid chromatography.

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In the present work, we studied the effect of the monosulfate esters of taurochenodeoxycholic acid on bile flow and biliary lipid excretion in conscious hamsters. The biliary behavior or taurochenodeoxycholate-3α sulfate (TCDC-3-SO_4) or taurochenodeoxycholate-7α sulfate (TCDC-7-SO_4) was compared to that of unsulfated taurochenodeoxycholate (TCDC). As part of the investigation, the critical micellar concentration (CMC) of the monosulfate isomers was measured, and the relationship between CMC of the sulfated bile salts and biliary lipid excretion was compared to the relationship previously reported for nonsulfated bile salts (13).

METHODS

Materials

Chenodeoxycholic, cholic, and ursodeoxycholic acids were obtained from Aldrich (Milwaukee, WI). Taurine and [1,2-^{14}C]taurine, purchased from Sigma (St. Louis, MO) and DuPont (Boston, MA), respectively, were used to synthesize radiolabeled conjugated bile salts. Taurochenodeoxycholate (TCDC), taurocholate (TC), and tauroursodeoxycholate (TUDC) were synthesized by the method of Lack et al (14). The 3α-sulfate ester of taurochenodeoxycholate was prepared and purified by the methods of Parmentier and Eyssen (15). The 7α-sulfate ester of taurochenodeoxycholate was prepared by the method of Tserng and Klein (16) and purified by the methods cited above (15). The purity of the synthetic preparation was assessed by TLC (15) and HPLC (17) and quantitated by 3α-hydroxysteroid dehydrogenase (18). The sulfate esters were solvolyzed (15) prior to enzyme assay. The chromatographic purity (HPLC) of the sulfate esters was greater than 97%. The specific activity of the synthetic radiolabeled bile salt was typically 7 μCi/mmol. Orange OT was synthesized by the method of Abu-Hamdiyyah and Mysels (19). We are indebted to Alan F. Hofmann for several detailed suggestions regarding the preparation of Orange OT.

Bile analysis

Bile volume was determined gravimetrically. ^{14}C Radioactivity was measured by liquid scintillation counting in Ultrafluor, (National Diagnostics, Somerville, NJ) and, after correcting for quenching, was used to measure the biliary content of TCDC, TCDC-3-SO_4, and TCDC-7-SO_4. Bile was diluted (2:1, v/v) with 2-propanol and photochemically bleached for 24 h using a grow light, (GRO LUX F-40 GRO, Sylvania). The bleached samples were analyzed for biliary lipids after centrifugation at 3000*g*. Cholesterol was determined by a cholesterol oxidase method (Boehringer Mannheim, Indianapolis, IN). Bile acids were measured enzymatically as indicated previously (18). Phosphatidylcholine was measured by a choline oxidase method, (Wako Pure Chemical Industries, Osaka, Japan) which has been previously validated for use with bile (20). Lipid phosphorus was measured by the method of Bartlett (21) and phospholipids were qualitatively analyzed by TLC using the methods of Gilfillan et al. (22) and Skipski et al. (23).

Biotransformation of the administered bile salts was assessed by TLC (15) and HPLC (17). In addition, radiochromatograms were created by counting the effluent from the HPLC column.

Determination of the critical micellar concentration

Critical micellar concentration of TCDC, TCDC-3-SO_4, TCDC-7-SO_4, TUDC, and TC was determined according to the method of Roda, Hofmann, and Mysels (24). Individual bile salts and excess Orange OT dye were incubated at 25°C for 72 h in 0.01 M sodium phosphate, 0.14 M NaCl, pH 7.2. Solubilization of the Orange OT dye was estimated by absorbance at 483 nm of the filtrate after the incubation medium was passed through a Millex-GV 0.22-μm filter unit (Millipore, Bedford, MA).

Animal model

Male golden Syrian hamsters (90–160 g) were purchased from Charles River Laboratories, (Wilmington, MA), fed Purina Rodent Chow ad lib. (Purina, St. Louis, MO), and maintained in hanging cages with a 12-hr light/dark cycle until the time of surgery. Animals were anesthetized with ether. A PE-10 catheter was inserted into the pulp of the spleen and held in place with two 4/0 black silk ligatures. The free end of the catheter was brought out through the skin incision and utilized for perfusion into the portal circulation. A second incision was made in the right subcostal area. The common bile duct was identified and ligated with a single 4/0 black silk suture. The common bile duct above the ligature was opened on its ventral surface and a PE-10 catheter was inserted into the lumen of the common bile duct and secured with two 4/0 black silk ligatures. The free end of this catheter was brought out through the right subcostal incision. The length of the catheter was adjusted to 30 cm from the skin incision. Finally, the gallbladder was identified, grasped with a hemostat, and ligated at its base with a single 4/0 black silk suture. The dome of the gallbladder was then excised. Following operation, each animal was placed in a restraining cage. Hydration was maintained by perfusion into the splenic pulp catheter of a dextrose and salt solution (2.5 g dextrose and 0.45 g NaCl/100 ml water) at a rate of 0.8 ml per h.

The total bile output was collected overnight. On the morning after surgery, bile output was measured for three successive 30-min periods following which animals re-

ceived infusion of one of the test compounds (TCDC, TCDC-3-SO_4 or TCDC-7-SO_4) into the intrasplenic catheter. Thereafter, bile was collected in 30-min periods for the next 7 h and in 60-min periods for an additional 3 h, a total of 10 h of collection after the start of the test infusion. Test infusions consisted of 285 μmol/kg body weight of the test compounds dissolved in 2.4 ml of 0.9% NaCl in water and administered over 3 h at the rate of 0.8 ml per h.

Statistics

Parametric data were analyzed using Student's *t*-test (25). When multiple comparisons were made in any series, the correction of Bonferroni was used (26). Non-parametric data were analyzed by the Wilcoxon Signed Rank test (27). The relationship between variables is described by regression to a line by the method of least squares (25). Except as otherwise noted, variation about means in the text and figures is given as ± 1 standard error of the mean (25).

RESULTS

Characteristics of the model

Overnight biliary drainage results in depletion of the endogenous bile salt pool. During this time, 165 ± 41 μmol bile salts/kg body weight were measured in the bile. Gurantz and Hofmann (13) have estimated the hamster's total bile salt pool to be 190 μmol/kg body weight. The duration of the biliary drainage (14–16 h) is insufficient for induction of significant new synthesis of the bile salts (28).

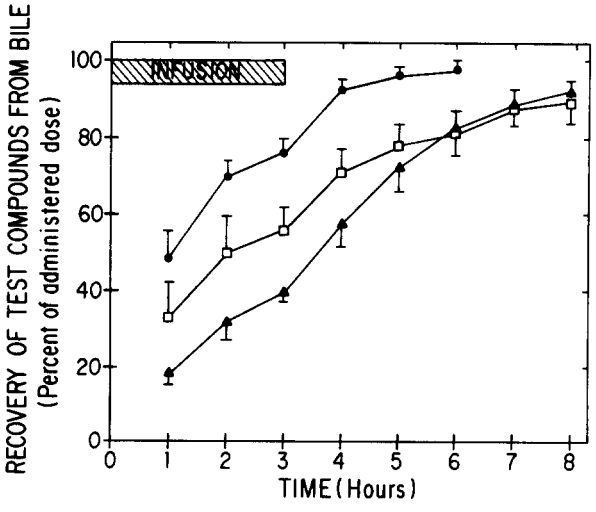

Fig. 1. Cumulative recovery of test compounds from bile. Data points represent the mean ± 1 SEM for n animals. Test compounds: TCDC (□), n = 8; TCDC-3-SO_4 (●), n = 6; TCDC-7-SO_4 (▲), n = 8.

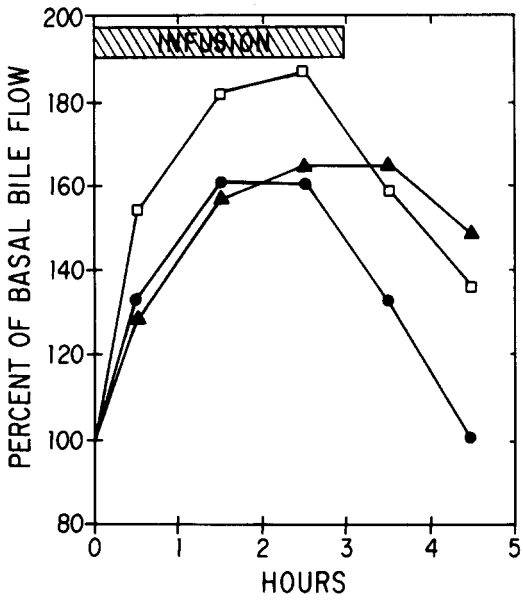

Fig. 2. Increase in bile flow in response to test substances. Data points represent means of experiments in n animals as described in Fig. 1.

The rate of bile salt excretion is low the morning after overnight drainage: 0.022 ± 0.003 μmol/min per kg. Greater than 95% of the bile salt in bile at that time is taurocholate.

Recovery of test compounds

Infusion directly into the portal circulation results in efficient hepatocellular uptake of TCDC, TCDC-3-SO_4, and TCDC-7-SO_4. Cumulative recovery of radioactivity from bile during the 8 h after administration was: 89.2 ± 4.9% for TCDC, 98.3 ± 1.7% for TCDC-3-SO_4, and 92.8 ± 1.9% for TCDC-7-SO_4 (**Fig. 1**). Urine collected for 24 h after administration of ^{14}C-labeled compounds contained no radioactivity above background. The speed at which the compounds were cleared in the bile differed; by the end of the 3-h infusion 77 ± 3.5% of the TCDC-3-SO_4 administered had already been excreted into bile compared to 56 ± 6.5% for TCDC and 40 ± 1.9% for TCDC-7-SO_4 ($P < 0.001$).

Analysis of bile showed no evidence of biotransformation of the administered compounds. Bile secreted after infusion of [^{14}C]TCDC, [^{14}C]TCDC-3-SO_4, or [^{14}C]TCDC-7-SO_4 was analyzed by TLC (15). Radioactivity above background was found only in scrapings obtained from areas on the plate that corresponded to the R_f of the infused compound that was chromatographed in an adjacent lane. Furthermore, HPLC analysis of bile after infusion of either the monosulfate esters or unsulfated TCDC demonstrated that the infused compound accounted for greater than 95% of the bile salts in bile. Thus, there is no evidence of further metabolism of any of the test compounds.

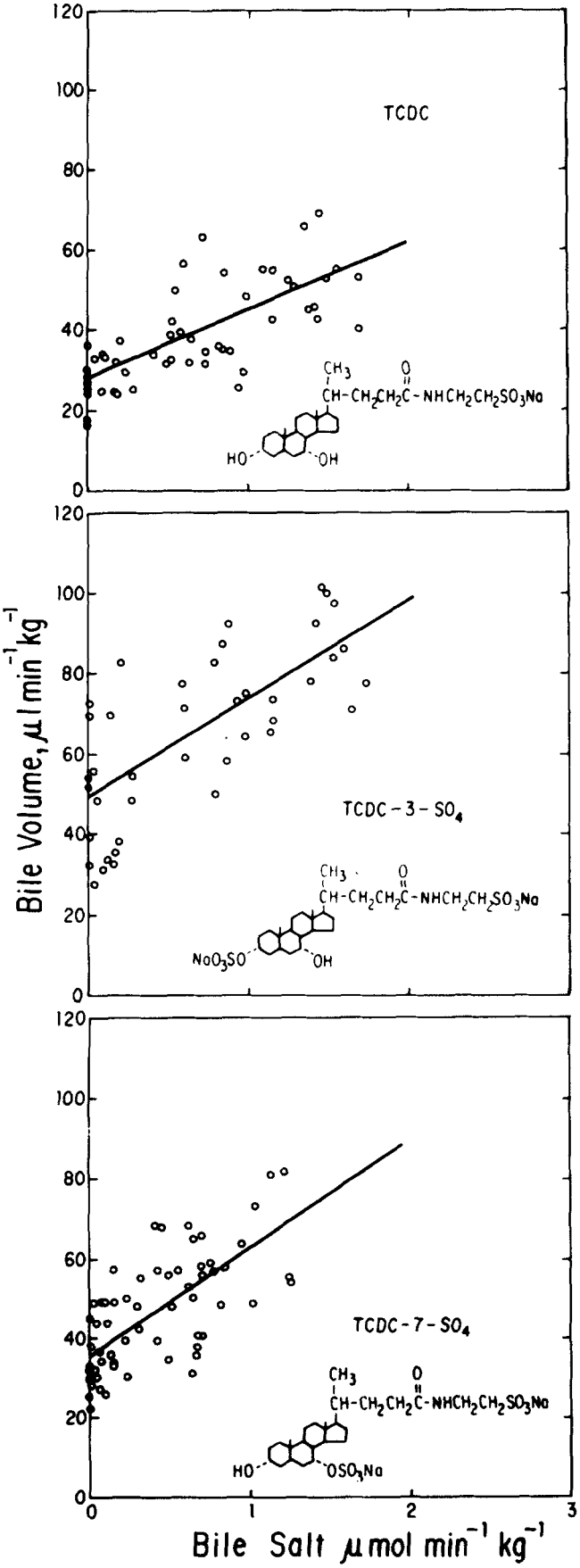

Fig. 3. Relationship between biliary excretion of test bile salt and bile volume. See text for details.

Choleresis

Bile flow increased following administration of TCDC, TCDC-3-SO_4, and TCDC-7-SO_4(**Fig. 2**). The volume response to each of the compounds was similar; comparison of the areas under each of the curves indicated no significant difference ($P<0.50$). The volume of bile flow was proportional to bile salt excretion for each of the compounds (**Fig. 3**). The slopes (± SE) of the lines describing the relationship between bile volume and biliary excretion of the test substances were: TCDC, 17.0 ± 2.0 μl/μmol, r = 0.75; TCDC-3-SO_4, 24.3 ± 3.8 μl/μmol, r = 0.72; and TCDC-7-SO_4, 25.4 ± 3.5 μl/μmol, r = 0.66. These slopes are similar to those published for the hamster by Gurantz and Hofmann (13) using a variety of nonsulfated bile salts.

Biliary lipid excretion

Portal administration of monosulfated and unsulfated taurochenodeoxycholate resulted also in an increase in phospholipid and cholesterol excretion (**Fig. 4**). Biliary excretion of both phospholipid and cholesterol are tightly coupled to biliary excretion of each of the administered bile salts; the linkage between bile salt excretion and excretion of phospholipid or cholesterol was similar for each of the monosulfate esters and the nonsulfated bile salt. The ratio of phospholipid to cholesterol in the bile at the peak excretion of cholesterol varied from 9.0 ± 2.1 following TCDC to 4.2 ± 0.7 with TCDC-3-SO_4, and 4.7 ± 1.1 with TCDC-7-SO_4, ($P<0.01$). The differences in ratio are the result of a higher phospholipid flux following TCDC, 0.23 ± 0.05 μmol/min per kg versus TCDC-3-SO_4, 0.13 ± 0.02 μmol/min per kg and TCDC-7-SO_4, 0.15 ± 0.06 μmol/min per kg ($P<0.01$) with no difference in cholesterol: TCDC, 0.027 ± 0.006 μmol/min per kg, TCDC-3-SO_4, 0.034 ± 0.007 μmol/min per kg, and TCDC-7-SO_4, 0.031 ± 0.009 μmol/min per kg. Lecithin was the only phospholipid detected following TLC of bile of these animals; phospholipid estimates by choline oxidase agreed with those determined by phosphate release.

CMC as a determinant of bile salt-biliary lipid linkage

Using the Orange OT solubilization method, we determined the CMC of TCDC to be 2.1 mM; TUDC, 4 mM; TC, 9 μmM; TCDC-3-SO_4, 19 mM; and TCDC-7-SO_4, 18 mM (**Fig. 5**).

A plot of △ biliary lipid/△ biliary bile salt versus CMC for the three nonsulfated bile salts studied (**Fig. 6**) conformed to the negative hyperbolic relationship ori-

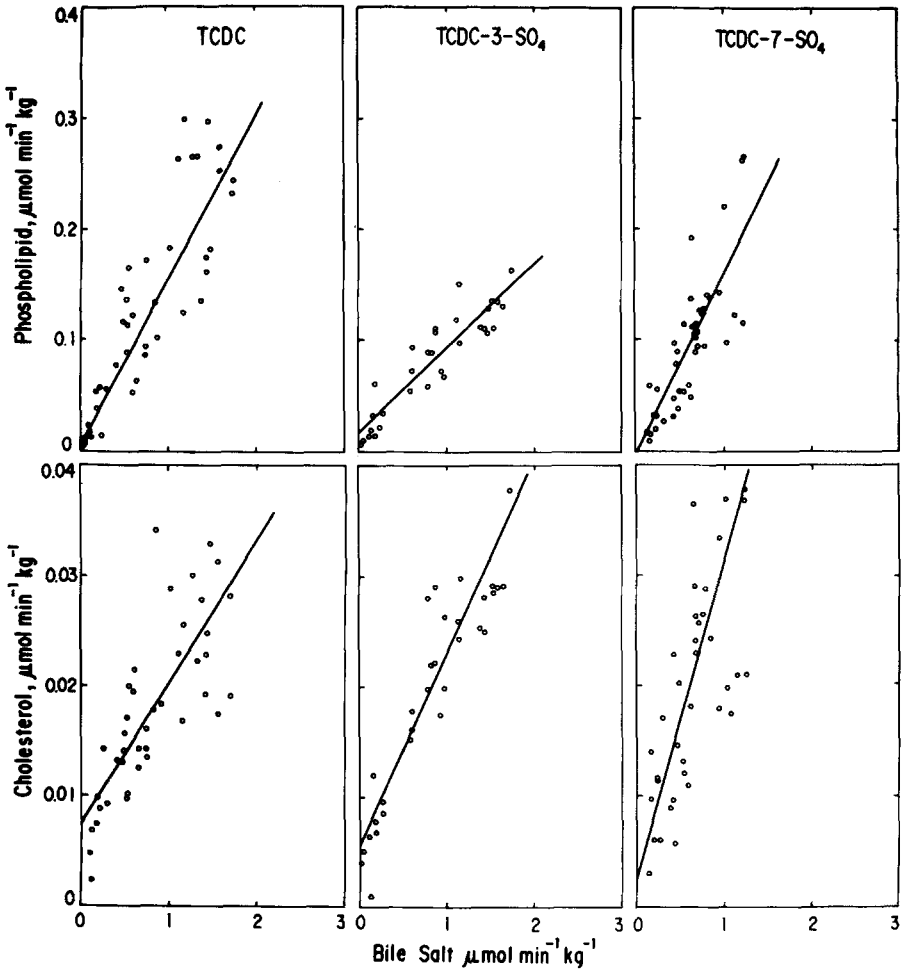

Fig. 4. Relationship between biliary excretion of test bile salt and biliary phospholipids (top) and cholesterol (bottom). See text for details.

ginally described in the extensive series of studies of Gurantz and Hofmann (13). When the same data were plotted for TCDC-3-SO_4 and TCDC-7-SO_4 it was apparent that biliary lipid flux with these compounds was higher than would be predicted by the aforementioned curve.

DISCUSSION

The animal model used in these experiments has several advantages over other models in which bile acids are administered into a systemic vein while the animal is under general anesthesia. The first advantage is that direct infusion of sulfated and nonsulfated bile salts into the portal circulation results in prompt and efficient hepatic extraction; there is no systemic distribution of the infused material.

In addition, we found no evidence of hepatic or systemic toxicity. No animals died during or within 12 h of the infusion of test bile salts. In all animals there was a prompt increase in bile flow following initiation of the infusions. The animals continued to make urine. There was no visual evidence of hemoglobinuria nor of hemoglobinemia in serum obtained postinfusion from some of the animals.

Gurantz and Hofmann (13) have described a model in which bile salts are infused into the small intestine of hamsters. Bile salts absorbed through the intestinal lumen enter the portal circulation and are efficiently taken up and excreted into the bile (13). Of interest, the slope of the line describing bile volume as a reflection of bile salt excretion in our experiments is similar to that obtained by Gurantz and Hofmann. There are two differences between our model and that of Gurantz and Hofmann. First, we studied animals in an awakened state 16 h after

surgery, thus minimizing any effect of anesthesia. Second, overnight biliary drainage depleted the endogenous bile salt pool allowing us to study infusion of a test bile salt free of influence from endogenous bile salts.

Using this model, we report for the first time the biliary secretory response to monosulfate esters of TCDC. Of interest, under the present experimental conditions the monosulfated bile salts exhibit choleretic properties that are similar to those of nonsulfated TCDC. Others have suggested the presence of different transport systems for biliary secretion of sulfated and nonsulfated bile salts in the rat (29, 30). Our data obtained in hamsters do not shed further light on this interesting question.

The present studies demonstrate that biliary excretion of phospholipid and cholesterol are similar during biliary secretion of sulfated and nonsulfated TCDC. This finding would not be predicted from the expected change in hydrophobic/hydrophilic balance that follows sulfation. Diminished biliary excretion of lipid, expecially phospholipid, would be predicted from the previous in vitro studies of Armstrong and Carey (8).

The present data do reflect a slight decrease in phospholipid to cholesterol ratio in bile after infusion of TCDC monosulfates. The apparent decrease in phospholipid excretion is smaller than would be predicted by the

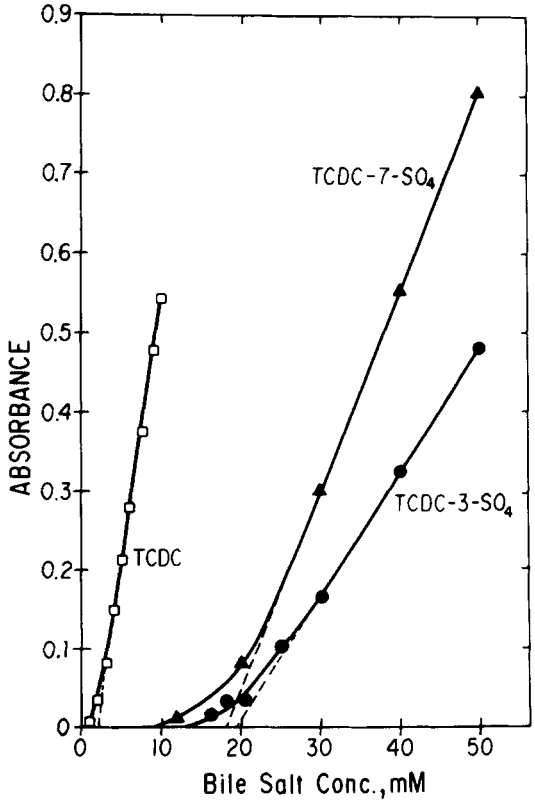

Fig. 5. Determination of critical micellar concentration (CMC) of taurochenodeoxycholate and its monosulfate esters. See text for details.

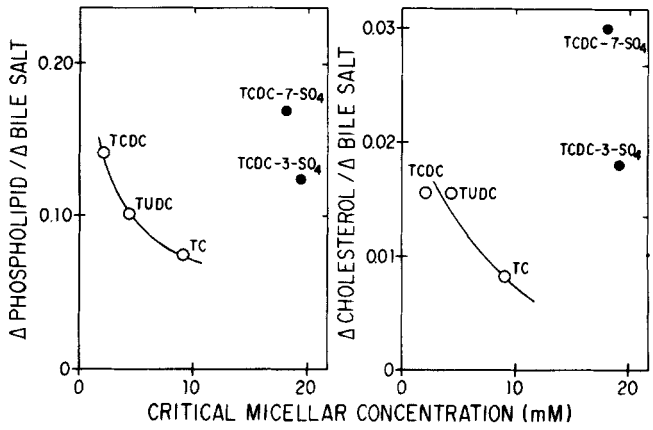

Fig. 6. Relationship between critical micellar concentration and the slope of the lines relating biliary lipid to biliary bile salt excretion: Δphospholipid/Δ bile salt excretion shown on the left, Δ cholesterol/Δ bile salt excretion shown on the right. Data points represent those shown in Fig. 4 plus results of similar experiments in four animals given TUDC and four animals given TC. The solid line is the curvilinear regression of the data for the unsulfated bile salts shown.

increased hydrophilicity. The most striking findings of the present work are the slopes of those lines that describe biliary lipid excretion as a function of excretion of TCDC monosulfates (Fig. 4). As noted in Fig. 6, these slopes are greater than would be predicted by the CMC of the monosulfates (13).

The data presented here offer an important new insight into the biological impact of bile salt sulfation. It would appear that the formation of monosulfate esters of taurochenodeoxycholate in response to cholestasis may not only protect the liver, but, unlike the multi-sulfated bile salts TCDC-3-SO_4 and TCDC-7-SO_4, retain the ability to promote biliary excretion of cholesterol and phospholipid. Therefore, in the presence of partial cholestasis, biliary lipid excretion should be preserved despite sulfation of the bile salt pool. The formation of monosulfate esters of taurochenodeoxycholate may be considered to be a particularly beneficial adaptation to cholestasis which acts to prevent hepatocellular damage from bile salt retention without sacrifice of important physiologic function.

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