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Effects of Acute Administration of Taurocholic and Taurochenodeoxycholic Acid on Biliary Lipid Excretion in the Rat[1] (39173)

DAVID L. EATON[2] AND CURTIS D. KLAASSEN[3]

Department of Pharmacology, University of Kansas Medical Center, Kansas City, Kansas 66103

Admirand and Small (1) presented *in vitro* evidence that mixed bile salt micelles are important for the biliary solubilization of lecithin and cholesterol. The quantitative relationship of bile salt excretion to lipid excretion in bile has been shown *in vivo* in the dog (2), rat (3), and human (4) as well. These studies showed that increases in excretion rates of taurocholic acid, which readily forms micelles, lead to corresponding increases in bile cholesterol and phospholipid excretion rates, whereas increases in excretion rates of taurodehydrocholate, which does not form micelles, were not followed by an increase in lipid excretion; thus, bile lipid excretion is dependent in part on the micelle-forming ability of the bile salt present.

Cholesterol gallstones are formed from bile supersaturated with cholesterol (1); thus, any factor that inhibits or promotes micelle formation may affect the solubility of cholesterol in bile. Clinical evidence accumulated over the past several years has shown that prolonged oral administration of chenodeoxycholic acid dissolves gallstones *in situ*, whereas the same administration of cholate has no effect on gallstone size (5, 6). The mechanism by which chenodeoxycholate but not cholate accomplishes this is not known, although it has been suggested that chenodeoxycholate causes a decreased rate of cholesterol output in relation to bile acid and phospholipid output (7). The present study was undertaken to determine if there is any acute difference in cholesterol and phospholipid excretion when rats are infused with either taurochenodeoxycholate or taurocholate.

Materials and Methods. Animals. Sprague–Dawley rats (180–240 g) were used in all studies. Rats were anesthetized with ether prior to surgery. A PE-50 cannula was secured in the left femoral vein. A PE-10 cannula was secured in the common bile duct and allowed to exit through the abdominal wall. Rats were then placed in a Bollman type restraining cage (Stoelting Company, Chicago, Illinois), and the femoral cannula was attached to a 5-ml syringe in a Harvard infusion pump. Glucose (5%) in water was infused at 1.13 ml/hr until the desired time for bile acid infusion.

Experimental design. Sodium taurocholate or sodium taurochenodeoxycholate (CALBIOCHEM, A grade) were diluted to a concentration of 25 mg/ml in a solution of 1% albumin in normal saline. Serial dilutions of this bile salt solution were made to a final concentration of 0.78 mg/ml. Approximately 15 hr after bile duct cannulation, the lowest concentration of bile salt solution was infused at 1.13 ml/hr for 2 hr. Bile was not collected for the first half hour. Three one-half-hr bile samples were then collected in preweighed 3-ml tubes. The same procedure was followed with each subsequent bile acid solution until a maximal infusion rate of 4.3 μmoles/min/kg was obtained (using the 25 mg/ml bile salt solution). Bile flow was determined gravimetrically, assuming a sp gr of 1.0 for bile.

Phospholipid assay. The phosphorous assay of Fiske and SubbaRow as revised by Bartlett (8) was followed. Five milliliters of 10 *N* H_2SO_4 was added to a 25 μl sample of bile or phosphorous standard (Sigma Chemical Company, 20 μg P_i/ml) in an 8-ml test tube. The samples were heated in an oven at 150° for over 3 hr. The tubes were cooled briefly, 0.1 ml of 30% H_2O_2 was added, and the samples were returned to the oven for

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1½ hr. The tubes were then removed from the oven and cooled, and 0.2 ml of 0.4% ammonium molybdate was added. The samples were mixed vigorously and heated in a boiling water bath for 10 min. The optical density was recorded at 780 nm on a Beckman Model 25 spectrophotometer. Bile phosphorus concentration was then determined from a standard curve.

Cholesterol assay. A gas–liquid chromatographic procedure for measurement of cholesterol similar to that described by Vanlerenberghe and Cassaigne (9) as modified by Hardison and Apter (3) was used. One hundred microliters of bile in 0.7 ml 80% ethanol was extracted twice with 3.0 ml petroleum ether.

The first 3 ml of petroleum ether contained 2.0 μg/ml of 5α cholestane (Stereoloids, Incorporation) as an internal standard; the second petroleum ether extraction contained no internal standard. The combined extracts were evaporated to dryness and 50 μl of a 9:3:1 solution of pyridine: hexamethylsilane: trimethylchlorosilane (Applied Science Laboratories Incorporation) was added just prior to gas chromatographic analysis. All assays were run on a Varian aerograph series 1400 gas chromatograph. The glass column was ⅛ in. by 3 ft long packed with 1.5% QF-1 coated on a solid support of chromosorb W (AW-DMCS) 80–100 mesh. Gas and temperature settings were: hydrogen; 20; air, 300; helium, as a carrier, 37 ml/min; injector, 245; detector, 295; and column, 200°. Cholesterol concentrations were determined from a standard curve obtained by plotting the ratios of internal standard/cholesterol against cholesterol concentration in μg/ml.

Bile acid assay. An enzymatic assay similar to that described by Talalay (10) as modified by Paumgartner *et al.* (11) was employed. Fifty microliters of bile was diluted with 0.45 ml methanol. Fifty microliters of this solution was added to 1.0 ml of a reaction mixture prepared by dissolving 33 mg NAD (Sigma Chemical Company) and 14 mg hydroxysteroid dehydrogenase (0.61 units/mg, Worthington Biochemical Corporation) in 120 ml of a 1 *M* glycine buffer, pH 9.4, containing 0.521 g EDTA and 5.0 ml 64% hydrazine. The reaction was allowed to proceed at room temperature for 1 hr. Optical density at 340 nm was measured against an appropriate blank. Standards ranging from 7.5 μmoles/ml to 60.0 μmoles/ml of sodium taurocholate were used to construct a standard curve.

Thin layer chromatography. Qualitative thin layer separation of bile conjugates was done using the TLC method for bile acid conjugates as described by Hoffman (12).

Results. Figure 1 shows the effects on bile composition of depletion of the bile acid pool by interruption of the enterohepatic circulation, and the biliary response to various infusion rates of taurocholate (TCH) and taurochenodeoxycholate (TCD). The results of a control experiment in which bile was collected at 6-hr intervals for a period of time equivalent to the length of the infusion experiments are also shown in Figure 1. All three major bile constituents and bile flow reached a basal excretory rate before the infusion of TCH or TCD was started. This basal excretory rate remained constant thereafter in the control

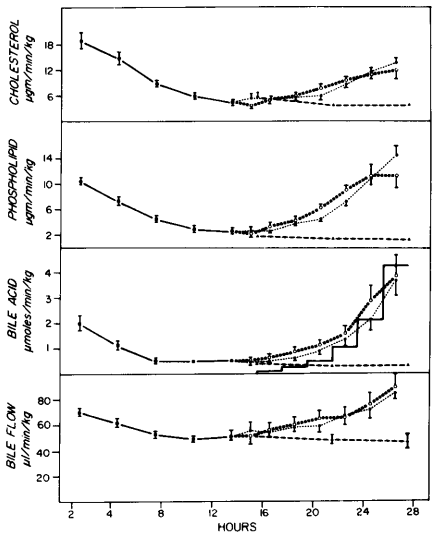

FIG. 1. Effect of bile acid depletion and infusion of TCD and TCH on biliary cholesterol, phospholipid, and bile acid excretion rates, and on bile flow. The solid squares represent values obtained during the depletion of bile acids (n = 11). The open triangles represent a control group of three rats, while open circles indicate results obtained during TCH infusion (n = 5), and closed circles during TCD infusion (n = 6). The stepped lines in the bile acid portion of the figure indicate the rate of infusion of bile acids. Brackets indicate the standard error of the mean.

experiment. Qualitative thin layer chromatography showed that the intensity of the spot corresponding to TCH decreased during the depletion period, while there was no TCD present until infusion of TCD. No increase in the TCH fraction of bile acid excretion was evident during TCD infusion; therefore, the increase in total bile acids measured during TCD infusion is due to an increase in TCD excretion.

The graph at the top of Figure 1 shows that the cholesterol excretion rate fell to 28% of its initial rate in 15 hr, at which time the infusion of bile acids was initiated. Cholesterol excretion increased with each infusion rate of bile acid, but at the highest bile acid infusion rate, cholesterol excretion was only 66% of the initial excretion rate.

The phospholipid excretion rate had declined to 35% of its initial rate by the time bile acid infusion was started. Phospholipid excretion increased with each subsequent infusion of bile acid, attaining an excretion rate 125% of the initial rate at the highest bile acid infusion rate.

The bile acid excretion rate dropped to 25% of its initial rate in 8 hr and maintained this basal excretion rate until bile acid infusion was begun. The bile acid excretion rate increased with increased infusion rates of bile acids, but excretion of total bile acid was less than that infused at the higher infusion rates. At the highest infusion rate bile acid excretion was about 200% of the initial endogenous excretion rate.

Bile flow also corresponds in part to the bile acid excretion rate; however, at low levels of bile acid excretion, bile flow still continued at about 70% of its initial rate.

Statistical analysis using Student's t test ($P < 0.05$) showed no significant difference in the effects of TCH and TCD on bile acid excretion, phospholipid excretion, cholesterol excretion, or bile flow.

Figure 2 illustrates the relationship of phospholipid to bile acid excretion rates. Phospholipid excretion measured during bile acid depletion and that during infusion of either bile acid is similar. After bile acid depletion cholesterol also increased with the infusion of bile acids (Fig. 3). Cholesterol excretion is much less during infusion than during depletion. A plot of cholesterol

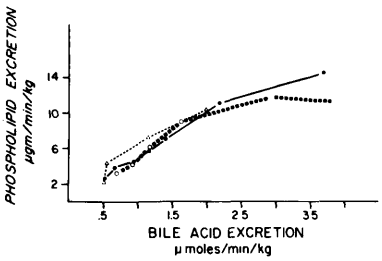

FIG. 2. Phospholipid excretion versus bile acid excretion. The open triangles represent values obtained during the depletion of bile acid pools (n = 11), while closed circles indicate results obtained during TCD infusion (n = 6), and the open circles during TCH infusion (n = 5).

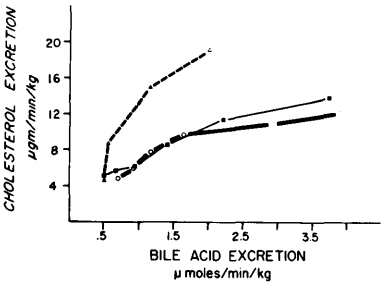

FIG. 3. Cholesterol excretion versus bile acid excretion. Open triangles represent cholesterol values obtained during depletion of the bile acid pool (n = 11), solid squares represent values obtained during TCD infusion (n = 6), and open circles during TCH infusion (n = 5).

versus phospholipid excretion (Fig. 4) yields a linear relationship during bile acid infusion, but again a distinct difference can be seen in the cholesterol levels measured during depletion and infusion.

Infusions of increasing concentrations of bile acids resulted in corresponding increases in cholesterol and phospholipid excretion up to a bile acid excretion rate of about 2 μmoles/min/kg (Figs. 2 and 3). Above this rate, a plateau in cholesterol and phospholipid excretion rates was reached.

Infusion rates of TCD above 4.3 μmoles/min/kg gave erratic results; six of nine rats infused with 8.6 μmoles/min/kg died before the experiment was completed. Results of all nine rats infused with TCH at this rate indicated that there was no significant increase in phospholipid or cholesterol excretion when the bile acid excretion rate

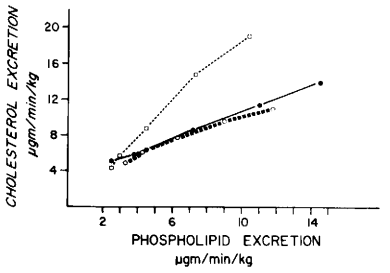

FIG. 4. Cholesterol excretion versus phospholipid excretion. Open squares represent depletion values (n = 11) while solid circles represent values obtained during TCD infusion (n = 6), and open circles during TCH infusion (n = 5).

was increased. TCD infusion above about 3 μmoles/min/kg caused severe hemolysis, and this was presumed to be the cause of death in rats infused with higher concentrations of TCD. Hemolysis was noted during TCH infusion only at the 8.6 μmole/min/kg rate.

Discussion. The results of this study show that there is no significant difference in biliary lipid excretion when rats are infused with either taurochenodeoxycholate or taurocholate. Although this study indicates little difference between the short-term effects of these two bile acids, this does not rule out the possibility of a relative decrease in cholesterol excretion produced by chronic administration of chenodeoxycholate, as proposed by Northfield *et al.* (7)

Our data on cholesterol levels during depletion and infusion of bile acids suggest that there are factors in addition to bile acid excretion which effect the cholesterol excretion rate. This difference in cholesterol excretion is not reflected in the excretion rates of biliary phospholipids during bile acid depletion or infusion. The mechanism of this difference in cholesterol excretion remains unexplained, though several may be proposed. There may be a substance other than bile acids within the enterohepatic circulation which stimulates biliary cholesterol excretion. The presence of this bile component would be dependent upon an intact enterohepatic circulation. However, Adler and Ockner reported a decreased biliary cholesterol output 48 hr after two-thirds of the biliary tree was obstructed, while bile acid output was normal or elevated (13). The enterohepatic circulation was intact up to the point of bile collection. These data suggest that a component of bile other than bile acids probably is not responsible for the relative elevation in cholesterol levels observed during bile acid depletion. Dissociation of cholesterol excretion in bile from that of bile acid and phospholipid excretion has also been shown in the dog (14) and in man (15).

Gregory *et al.* (16) suggest that bile acids have a dual role in cholesterol excretion. Their data indicate that bile acids promote the synthesis of cholesterol destined for biliary excretion, and facilitate the transport of cholesterol and phospholipid from the microsomes to the bile canaliculli. Our data can be explained with a modification of this hypothesis. Cholesterol excreted in the bile reaches the bile in two ways: from facilitated transport with bile acids and from simple diffusion from a cholesterol pool derived from bile acid-stimulated cholesterol synthesis. The contribution from the latter is dependent upon a diffusion gradient which is slowly produced and maintained in the presence of bile acids. Upon interruption of the enterohepatic circulation the bile acid levels fall, and a subsequent fall in cholesterol from both sources occurs. After a period of time, approximately 15 hr in the rat, the cholesterol diffusion gradient is lowered to a point where little diffusion occurs. At this point, the only cholesterol excreted is that which is transported with the bile acids that are newly synthesized in the liver. When bile acids are then infused into the systemic circulation, the cholesterol excretion rate increases with an increase in bile acid excretion via the facilitated transport system. However, the contribution from simple diffusion remains insignificant for a period of time until the diffusion gradient can be built up. Though much work remains to substantiate this hypothesis, it may explain the difference in cholesterol excretion rates observed in this experiment.

Summary. Comparison of the effects of biliary lipid excretion produced by infusion of taurochenodeoxycholate and taurocholate showed no significant difference when

the bile acids were infused for a relatively short period of time. Cholesterol excretion rates measured during depletion of the bile acid pool were significantly higher than cholesterol excretion rates measured during infusion of bile acids at various rates. These data indicate that there is some mechanism in addition to bile acid excretion that is responsible for biliary excretion of cholesterol when the enterohepatic circulation is intact.

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