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Regulation of Biliary Protein Secretion in Dogs (42648)

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Abstract. The biliary secretion of protein in response to bile acids and other agents known to increase bile flow was examined in a chronic bile fistula dog model. Infusion of 25, 50, or 75 μmole/kg/hr sodium taurocholate after 3 hr of bile fistulization increased biliary protein output significantly by 52, 86, and 108% respectively compared to preinfusion values. A proportionate increase in biliary albumin output during taurocholate choleresis was demonstrated. Protein outputs during bile fistulization without taurocholate replacement were unchanged. The non-micelle-forming bile acid dehydrocholate markedly increased bile flow but did not change protein output. Similarly, the hormonal choleretics glucagon and secretin caused significant decreases in biliary protein concentration but no change in protein output. These data indicate a correlation between biliary protein secretion and bile acid-dependent bile flow. It is likely that regulation of certain proteins is dependent on the micelle-forming properties of bile acids.

Although many studies have examined the secretion of lipids into bile, less is known about regulation of biliary protein. Biliary proteins comprise a wide spectrum of hepatocellular lysosomal and plasma membrane enzymes, as well as immunoglobins, hormones, transport proteins, and plasma-derived proteins (1). Albumin and immunoglobin A are present in large amounts, although their proportions may vary according to species (2, 3). The precise roles of the proteins in bile have not been demonstrated, but recent work suggested that they may participate in hepatic lipid metabolism (4, 5), removal of circulating immune complexes (6), and transport of heavy metals (7).

Considering the large number of proteins present in bile and their various possible roles, it is likely that multiple mechanisms of excretion are operative. There is evidence to suggest that plasma proteins could arrive in bile via transcellular or paracellular pathways (1). Transcellular transport after endocytosis may be a mechanism for biliary excretion of polymeric IgA (8), albumin (9), insulin (10), or other proteins. Ceruloplasmin may be transported across the bile duct epithelium from the peribiliary capillaries (11). Hepatocyte-derived proteins such as 5'nucleotidase or alkaline phosphodiesterase I may be released into bile from the canalicular membrane after solubilization by bile acids, perhaps initially in the form of small vesicles (12).

Although bile acids stimulate bile flow and biliary lipid secretion, what happens to biliary proteins during this process remains unclear. Proteins in plasma are important in the transport of bile acids and lipids. However, no correlation between bile flow, bile acid output, and total protein secretion was seen in chronic rat bile fistula model (13). In contrast, certain proteins increased with bile acid infusion in an isolated perfused rat liver model (12). These studies were not performed under physiological conditions. The present study was designed to examine further the control of biliary protein secretion, in particular the relationship between protein and bile acids or other choleretics during taurocholate stabilization in awake, bile fistula dogs.

Materials and Methods. Chronic bile fistulas were prepared in seven female mongrel dogs weighing 12.5–20.0 kg by cholecystectomy, ligation of the lesser pancreatic duct, and insertion of a modified Thomas cannula (14) into the duodenum. Experiments were performed on healthy, conscious dogs no sooner than 3 weeks following surgery, and no more than two experiments a week. After an 18-h fast, the duodenal cannula was

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opened and a No. 6 F ureteral catheter was inserted 5–6 cm into the common bile duct and brought out through a cork used to occlude the duodenal fistula. Bile samples were collected in 15-min intervals, bile volume was measured to the nearest 0.1 ml in graduated tubes, and aliquots were immediately frozen at −20°C until assay. During experiments, the dogs received a continuous intravenous infusion of 0.9% sodium chloride at 50 ml/hr delivered by a calibrated peristaltic infusion pump (Harvard Apparatus, Dover, MA). Choleretic compounds were infused in saline, and control (saline) studies were performed using matching volumes.

Analysis. Bile acid concentration was determined by a 3-α-hydroxysteroid dehydrogenase method (15) with sodium taurocholate as the standard. Biliary lipids were extracted using the method of Folch *et al.* (16) and total phospholipids were quantified by a standard assay of lipid phosphorus (17). Bile was assayed for total protein using a modified Lowry (18) technique. Aliquots (10 μl) of bile were precipitated with 1.0 ml of 6% trichloracetic acid (TCA). Samples were centrifuged at 1700 *g* for 30 min and the supernatant was aspirated. The TCA-precipitable proteins underwent alkaline hydrolysis under fluorescent lights overnight to reduce any remaining pigments which might absorb at 660 nm (19). Total protein was then measured by the Lowry method with bovine serum albumin (Fraction V, Sigma) as the standard. To verify results obtained by this modification of the Lowry method albumin recovery experiments were performed as well as amino acid analyses (Beckman 121 amino acid analyzer) on selected bile samples. Either 0, 20, or 40 μg of dog serum albumin (Fraction V, Sigma) was added to aliquot of dog bile after which proteins were precipitated with TCA and measured with the modified Lowry technique. In addition, samples of bile with low and high bile acids were assayed for total protein using amino acid analysis on a Beckman 121 amino acid analyzer by a standard method.

Biliary canine albumin was determined using the "Laurell" rocket electrophoretic technique (20), with anti-dog albumin antibodies (Miles Laboratories, Inc.). Whole bile samples were diluted (1:1 to 1:2) with Tris barbiturate buffer (pH 8.6) and electrophoresed on a 10% Agarose gel containing 50–60 μl of anti-dog albumin. Gels were run at 2 V cm^{-1} overnight. The height of the rocket was considered proportional to the concentration of albumin in the sample. Purified dog serum albumin (Sigma) standards of known concentration in saline were run with each gel. Immunoglobulin A (IgA) was assayed in a similar fashion. The antibody (Pel-Freez Biological) was specific to the α chain of canine IgA. Canine IgA (Miles) was used as standard.

Statistical comparisons were made between preinfusion and experimental periods and between experimental periods and corresponding periods of control experiments using the *t* test for paired values (21). *P* values less than 0.05 were considered significant. Results are expressed as means ± standard errors of the mean.

Experimental design. The effect of bile acids on total protein output was studied after bile had been drained for 3 hr from cannulated dogs without bile acid replacement. This duration was arbitrarily selected to reduce the pool of existing bile acids and other solutes. Following the third hour of open drainage either sodium taurocholate (99 TLC, Calbiochem, La Jolla, CA), having a critical micellar concentration of 2.27 μM, or sodium dehydrocholic acid (Sigma, St. Louis, MO), a non-micelle-forming bile acid, was infused intravenously for 3 hr in incremented doses of 25, 50, and 75 μmole/kg/hr at concentrations to maintain infusion rates at 50 ml/hr. A bile aliquot was obtained for assay from the last 15-min interval of the initial 3-hr open drainage (preinfusion). Similarly bile aliquots from the last 15-min interval of each hourly incremental bile acid dosage (25, 50, and 75 μmole/kg/hr) were obtained. In previous studies these periods reflected early steady state bile lipid output for each infused bile acid.

Additional bile fistula control studies were performed using the same dogs on different days in order to investigate whether changes in protein secretion could be simply a function of time. In these experiments bile acids were never infused throughout the 6 hr of

study. Bile was assayed during fistulization at times corresponding to those of bile acid infusion periods.

The effects of two hormones known to generate increased bile flow during stabilized bile acid secretion through independent mechanisms were also studied: glucagon, (Litty, Indianapolis, IN) thought to be a bile salt-"independent" canalicular stimulant (22) and secretin (KabiVitrum AB, Stockholm, Sweden), also a bile salt-independent stimulant which appears to act at a separate site (22). Following 3 hr of open drainage, glucagon, 1.8 μg/kg/hr, or secretin 2, U/kg/hr, was infused through a peripheral vein for 2 hr. Bile was assayed from 15-min aliquots obtained just prior to (preinfusion) and at the end of the hormone infusion (postinfusion) period.

Results. In an attempt to validate the modified Lowry technique for protein determinations in bile, amino acid analysis was performed on bile samples obtained from the preinfusion period and from the 50 μmole/kg/hr taurocholate infusion period in five dogs and compared to the Lowry results (Fig. 1). Total protein output by amino acid analysis increased from 1.27 ± 0.6 mg/kg/hr preinfusion to 3.10 ± .82 mg/kg/hr after taurocholate. Lowry determinations showed a similar increase, although values by this assay were 30–50% higher than amino acid analysis. For additional validation, known amounts of dog albumin were added to dog bile and a greater than a 95% recovery of added albumin found using the modified Lowry assay (Table I).

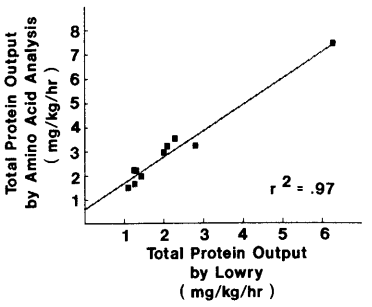

FIG. 1. Correlation of total protein output as measured by amino acid analysis (ordinate) and the modified Lowry technique (abscissa).

TABLE I. ALBUMIN RECOVERY AFTER TCA PRECIPITATION

Added dog albumin (μg)	Protein (Lowry) (μg/10 μl)	% Recovery	n
0.0	36.2 ± 3.0		9
20	55.1 ± 1.7	94.5	10
40	75.1 ± 2.7	97.3	9

Note. Values are means ± SEM.

Bile acids. After 3 hr of bile drainage without bile acid replacement, bile acid concentrations in both taurocholate-infused (T) and saline-infused (S) groups were small (21 ± 5 and 22 ± 8 m*M*, respectively) (Table II). With intravenous sodium taurocholate administration bile acid concentration rose significantly with each increment to 113 ± 14 m*M* at 75 μmole/kg/hr taurocholate infusion. Bile acid output also increased incrementally from 10.1 ± 1.7 μmole/kg/hr preinfusion to 90.8 ± 8.2 μmole/kg/hr at 75 μmole/kg/hr taurocholate infusion. When no bile acids were replaced, bile acid concentration decreased slightly but not significantly from 22 ± 8 m*M* preinfusion to 11 ± 3 m*M* at the end of 6 hr. Bile acid output during the same time period did decrease significantly, from 6.7 ± 1.5 to 4.1 ± 1.1 μmole/kg/hr.

Total protein. Total protein concentration did not change significantly during taurocholate administration. A small but significant increase was seen during bile acid depletion, from 3.9 ± 0.5 mg/ml preinfusion to 5.6 ± 0.5 mg/ml at the end of 6 hr (Table II). With each increment in taurocholate infusion total protein output increased significantly compared both to preinfusion periods and to corresponding periods without bile acid replacement. At 75 μmole/kg/hr taurocholate infusion, protein output was 4.1 ± 0.6 mg/kg/hr, representing a 108% rise over the preinfusion value.

Phospholipid. Biliary phospholipid output increased from 2.31 ± .46 μmole/kg/hr before taurocholate infusion to 11.8 ± 1.5 μmole/kg/hr after 75 μmole/kg/hr infusion (Table II). Phospholipid output decreased during corresponding saline infusion studies

TABLE II. BILIARY FLOW AND CONCENTRATIONS OF COMPONENTS DURING SODIUM TAUROCHOLATE INFUSION OR SALINE

		Preinfusion	25 μmole/kg/hr	50 μmole/kg/hr	75 μmole/kg/hr
Bile flow (ml/kg/hr)	T	0.52 ± .04	0.73 ± .04*,**	0.77 ± .08*,**	0.86 ± .08*,**
	S	0.54 ± .04	0.49 ± .02	0.28 ± .06*	0.37 ± .04*
Bile acid conc. (m*M*)	T	21 ± 5	46 ± 2*,**	81 ± 15*,**	113 ± 14*,**
	S	22 ± 8	10 ± 2	11 ± 2	11 ± 3
Phospholipid conc. (m*M*)	T	3.4 ± 0.8	5.2 ± 0.6	10.0 ± 1.6	11.5 ± 1.6
	S	2.3 ± 0.4	1.3 ± 0.2	1.2 ± 0.2	1.6 ± 0.3
Total protein conc. (mg/ml)	T	3.8 ± 0.6	4.2 ± 0.6	5.2 ± 0.6	5.0 ± 0.7
	S	3.9 ± 0.5	4.5 ± 0.7	5.4 ± 0.4*	5.6 ± 0.5*

Note. T, taurocholate infusion; S, saline infusion (bile fistula).
$n = 7$; values are means ± SEM.
* $P < 0.05$ compared to preinfusion; ** $P < 0.05$ compared to corresponding no bile acid period.

from 1.58 ± .24 to 0.71 ± 0.08 μmole/kg/hr. Figure 2 depicts the increases in total protein, phospholipid, and bile acids in response to taurocholate infusion.

Albumin and IgA. Albumin concentrations did not change significantly during taurocholate infusion (Table III) but did increase during saline control studies. Albumin output increased from 230 ± 50 to 725 ± 180 μg/kg/hr during bile acid infusion and did not change during saline studies, 240 ± 50 to 290 ± 50 μg/kg/hr. Biliary albumin represented 12 to 18% of total protein output during all periods of taurocholate infusion. IgA did not change significantly during taurocholate infusion: 560 ± 150 μg/kg/hr preinfusion; 750 ± 140 after 75 μmole/kg/hr; 850 ± 120 saline control.

Dehydrocholate. Dehydrocholic acid infused at the same rate as sodium taurocholate increased bile flow significantly from 500 ± 65 μl/kg/hr preinfusion to 1700 ± 65 μl/kg/hr at 75 μmole/kg/hr dehydrocholic acid infusion (Table IV). Dehydrocholic acid stimulated bile flow significantly more than taurocholate. Despite this, dehydrocholate had no effect on phospholipid output (1.44 ± .33 μmole/kg/hr preinfusion to 1.74 ± .27 μmole/kg/hr at 75 μmole/kg/hr dehydrocholate infusion). Total protein concentration decreased significantly with increasing dosages of dehydrocholic acid infusion (5.2 ± 0.9 mg/ml preinfusion to 1.3 ± 0.3 mg/ml at 75 μmole/kg/hr). However, total protein output did not change during dehydrocholic acid infusion.

Hormone stimulation. Both glucagon and secretin stimulated bile flow, decreased bile acid concentration, and had no effect on bile acid output (Table V). Total protein concentration decreased from 6.2 ± 1.3 to 3.5 ± 0.3 mg/ml with glucagon, and from 4.8 ± 0.6 to 2.8 ± 0.4 mg/ml with secretin. Total protein output was unchanged by either infusion.

Discussion. This study demonstrates a correlation between biliary protein and bile acid secretion in awake, fasted dogs. Biliary total protein output increased with increasing infusions of sodium taurocholate after 3 hr of biliary fistulization. No change in biliary protein output occurred during saline infusion studies. Biliary albumin, one protein abundant in bile, clearly increased with taurocholate infusion although this protein alone did not explain the magnitude of the increase in total protein and IgA did not change. Dehydrocholic acid, glucagon, and secretin, all potent choleretics which act by different mechanisms (22), had no effect on biliary total protein output in these studies. Therefore, the correlation between biliary protein output and bile acids is not simply a

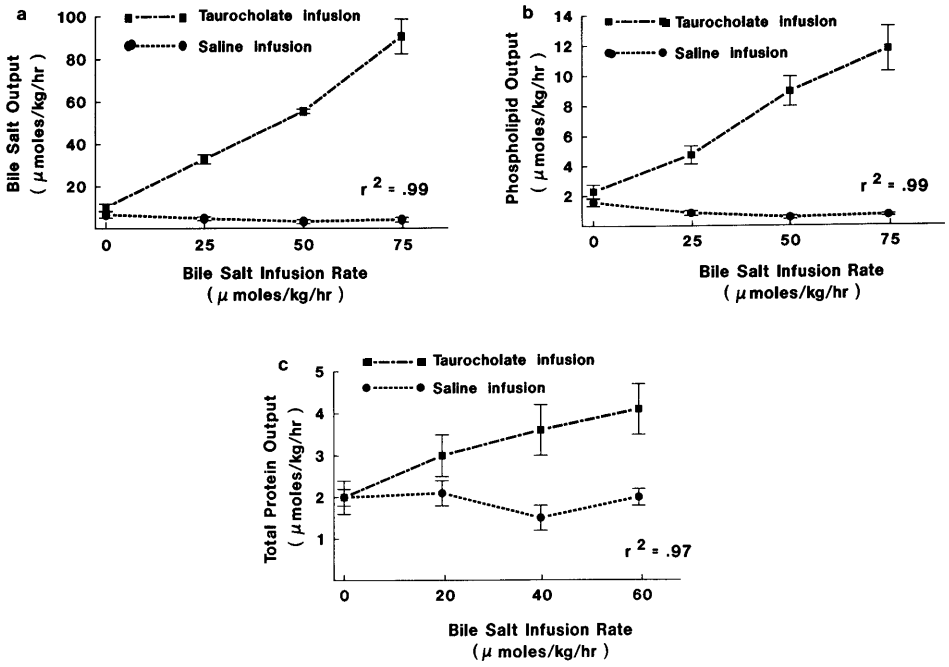

FIG. 2. Outputs of three major biliary components as a function of bile salt infusion rate: (a) bile salt; (b) phospholipid; and (c) total protein.

nonspecific one related to an increase in bile flow and biliary washout.

Protein concentrations were measured by three methods, each of which has advantages and drawbacks. The Lowry method for total protein yields slightly different results for various proteins and may be influenced by other bile components (1). We found that precipitation of protein with TCA followed by photodecomposition of remaining pigment made this a simple, reproducible assay that correlated well with amino acid analysis (Fig. 1). We attempted to validate the technique with two additional studies. An excellent correlation was obtained when known amounts of bovine serum albumin were added to bile. Furthermore, when multiple samples representing a wide range of bile salt concentrations were assayed by both the modified Lowry and the amino acid analysis techniques, again a very good correlation was obtained, although the values obtained with the modified Lowry technique were consistently higher. The fact that this technique is

TABLE III. BILIARY ALBUMIN CONCENTRATION AND OUTPUT DURING TAUROCHOLATE INFUSION OR SALINE

		Preinfusion	25 μmole/kg/hr	50 μmole/kg/hr	75 μmole/kg/hr
Albumin Conc.					
(mg/ml)	T	0.61 ± 0.19	0.63 ± 0.16	0.85 ± 0.14	0.81 ± 0.15
	S	0.48 ± 0.11	0.59 ± 0.15	0.83 ± 0.14*	0.80 ± 0.16*
Albumin Output					
(mg/kg/hr)	T	0.23 ± 0.05	0.38 ± 0.09*	0.62 ± 0.11*	0.72 ± 0.17*
	S	0.24 ± 0.05	0.28 ± 0.07	0.26 ± 0.07	0.29 ± 0.05

Note. $n = 7$; values are means ± SEM.
* $P < 0.05$ compared to preinfusion.

TABLE IV. DEHYDROCHOLIC ACID INFUSION

	Preinfusion	25 μmole/kg/hr	50 μmole/kg/hr	75 μmole/kg/hr
Bile flow (μl/kg/hr)	500 ± 65	925 ± 0.3*	1310 ± 65*	1700 ± 65*
Phospholipid conc. (m*M*)	2.0 ± 0.4	1.9 ± 0.3	1.2 ± 0.2	0.8 ± 0.1*
Phospholipid output (μmole/kg/hr)	1.44 ± 0.33	2.10 ± 0.24	1.96 ± 0.30	1.74 ± 0.27
Total protein conc. (mg/ml)	5.2 ± 0.9	2.7 ± 0.6*	1.7 ± 0.3*	1.3 ± 0.3*
Total protein output (mg/kg/hr)	2.56 ± 0.32	2.34 ± 0.34	2.17 ± 0.41	2.17 ± 0.47

Note. $n = 7$; values are means ± SEM.
* $P < 0.05$ compared to preinfusion.

dependent in part on what proteins are present and that it has been standardized to bovine serum albumin may explain why the slope of the correlation line is greater than 1. The immunoelectrophoretic techniques employed were useful for measurement of individual proteins and correlated well with previously published canine data (3).

Conflicting previous work on bile acid regulation of biliary protein in animals may be a function of differing methodologies or interpretations. In one reported study in dogs (23), a decrease in biliary protein concentration was found after taurocholate. Since bile volume increased markedly in that study, it is possible that protein output actually increased. In another canine study (24), no relationship was found between biliary protein and sodium taurocholate during 12 hr of bile fistulization but little data on protein secretion was reported. In the intact rat with bile fistulas, no correlation between protein and bile acid output was found (25). However, in the isolated perfused rat liver protein output increased with the addition of taurocholate or glycodeoxycholate to the perfusate but not with taurodehydrocholate or saline (12).

Most of the above studies were performed acutely and in several studies it was unclear whether bile acid secretion was stabilized. In the present study, bile acids were returned to the system after 3 hr of acute bile acid depletion to simulate an intact enterohepatic circulation. Although precise data on the physiological range of bile salt delivery to the liver are not available, an estimate can be calculated from portal blood flow and bile salt data obtained by Pries *et al.* (26). They found portal bile salt concentrations ranged between 3 and 235 μ*M*. With hepatic blood flow varying from 1 to 4 ml/g/min this would result in a very wide physiological

TABLE V. GLUCAGON AND SECRETIN INFUSIONS

	Glucagon (1.8 μg/kg/min)		Secretin (2 U/kg/hr)	
	Preinfusion	Postinfusion	Preinfusion	Postinfusion
Bile flow (ml/kg/hr)	0.41 ± 0.02	0.62 ± 0.04*	0.52 ± 0.04	0.73 ± 0.09*
Bile acid Conc. (m*M*)	27 ± 7	11 ± 2*	24 ± 4	9 ± 1*
Bile acid output (μmole/kg/hr)	11.0 ± 2.6	6.2 ± 0.9	12.7 ± 3.0	6.0 ± 0.9
Total protein conc. (mg/ml)	6.2 ± 1.3	3.5 ± 0.3*	4.8 ± 0.6	2.8 ± 0.4*
Total protein output (mg/kg/hr)	2.5 ± 0.6	2.0 ± 0.3	2.4 ± 0.3	2.0 ± 0.3

Note. $n = 7$; values are means ± SEM.
* $P < 0.05$.

range of hepatic bile salt delivery rates varying from 2.5 to 740 μmole/kg/hr. The infusion rates used in this study are well within this range. Therefore, it is unlikely that the protein changes seen were a result of a toxic effect of the bile acids as had been previously suggested when higher dosages of bile acids were used (27). Other toxic effects of taurocholate, such as hemolysis, have not been found in this model with infusion rates less than 2 mmole/hr (R. S. Jones, personal communication).

Previous studies have suggested that biliary levels of certain canalicular membrane proteins and exogenous proteins increase with taurocholate administration (12, 28, 29). Biliary secretion of albumin, however, did not seem to be influenced by bile acids. In the present study performed with physiological bile salt secretion rates, there was a clear correlation between biliary protein output and bile acid choleresis. Biliary total protein and albumin increased considerably with taurocholate, a micelle-forming bile acid, but not with dehydrocholic acid, which does not form micelles despite causing a brisk choleresis. This suggests that the increase in protein secretion is somehow dependent on the colligative properties of the bile acid. Regulation of hepatic lipid metabolism has been suggested as a functional role for biliary proteins (4, 5). The finding of intact apoproteins in human bile (5) and the suggested existence of protein lipid complexes in bile (30, 31) might support this theory, but the present study does not provide evidence regarding the functional role of biliary protein.

The present studies were performed using dogs with indwelling duodenal cannulas because of the experience using this model in our laboratory for studies of biliary secretion (32, 33). The dogs remain healthy and free of infection. Initial fistulization was performed in an attempt to create a dynamic system in which biliary protein outputs reflected ongoing secretory processes. It was interesting that a relatively constant output of protein was seen throughout bile fistulization, despite decreasing concentrations of bile acids, which suggests that a fraction of protein secretion was relatively independent of bile acid secretion. It is also interesting to note that in the intact rat with a chronic bile fistula, a similar small increase seemed to be occurring (25). This question and the possibility that proteins may be a driving force for biliary secretion remain to be investigated.

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