Biliary Excretion of Lecithin and Cholesterol in the Dog

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ABSTRACT The biliary excretion rates of bile acid, lecithin, and cholesterol were measured in unanesthetized dogs after interruption of enterohepatic circulation and during infusions of sodium taurocholate, sodium glycocholate, sodium dehydrocholate, SC2644 (a bicyclic organic acid with high choleretic potency), and secretin. Both lecithin output and cholesterol output were directly related to bile acid excretion rate. The curves describing these relationships were concave downward. Molar concentration ratios of lecithin-to-bile acid declined gradually from approximately 0.4 to 0.2 as bile acid output increased from approximately 1 to 70 μmoles/min. Cholesterol-to-lecithin molar ratios were highest (0.05–0.15) at very low rates of bile acid excretion, but descended rapidly to a plateau (0.03–0.04) which was constant over the entire range of bile acid excretion rates from 10 to 70 μmoles/min.

Similar lipid excretion patterns were observed during glycocholate infusion, but secretin-induced choleresis and dehydrocholate-induced choleresis were unaccompanied by any increments in lecithin or cholesterol excretion and SC2644 (which caused a marked increase in canalicular bile production as measured by erythritol clearance) caused a depression of lipid excretion.

The data are consistent with the view that lecithin moves passively from cell membranes to intracanalicular micelles, that transport of cholesterol is coupled to lecithin transport, and that there is also a small amount of independent passive transport of cholesterol from membranes to micelles. A model developed on these assumptions has been shown to behave in a fashion consistent with the entire range of these observations.

INTRODUCTION

Two highly insoluble lipids, cholesterol and lecithin, are present in bile in appreciable concentrations and are normally kept in aqueous solution by virtue of incorporation into mixed micelles with the conjugated bile acids (1, 2). That the bile acids may actually be responsible for the biliary excretion of these lipids in the first place has been suggested by a variety of studies in several species (3–10). In general, it appears that increased excretion rates of bile acid are associated with increased absolute biliary concentrations and rates of excretion of lecithin and cholesterol but with reduced concentrations of the two lipids relative to the concentration of bile acid. In fact, the studies of Heath, Caple, and Redding (10) in the sheep and of Hardison and Francis (9) in the rat appear to have demonstrated absolute maximal rates of lipid excretion at the higher rates of bile acid excretion. Conversely, low rates of bile acid excretion are associated with the highest concentrations of lecithin and cholesterol relative to bile acid concentration, a condition most likely to predispose susceptible individuals to the risk of cholesterol precipitation (7, 8).

In an effort to delineate further the mechanisms of biliary lipid excretion the following study was undertaken in unanesthetized dogs.

METHODS

Female mongrel dogs (weights 17–20 kg) were prepared beforehand by cholecystectomy and installation of a Thomas cannula in the duodenum (11, 12). On the day of study the bile duct and one or both of the lateral saphenous veins were catheterized, initial samples of blood and bile were obtained, and i.v. infusions (e.g., saline, or solutions containing taurocholate, ^{14}C-labeled erythritol, or ^{14}C-labeled sucrose) were started (12). Approximately 30–60 min elapsed from the time of bile duct catheterization to the time the preparation was complete and the dog was upright in a sling. The anticholinergic drug pipenzolate methylbromide[1] was administered i.v. (0.5 mg/kg initially followed by 0.1 mg/kg every 20 min) in order to minimize fluctuations in bile flow (13).

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[1] Pipenzolate methylbromide (Piptal) was generously supplied by Dr. Murray Finkelstein of Lakeside Laboratories, Milwaukee, Wis.

TABLE I

Experimental Protocols

	A	B	C	D	E	F	G	H	I	J	K
I	T(8)	0	T(8)	T(8)	T(8)	T(8)	T(8)	T(8)	T(35)	0	0
II	T(35)	0	T(35)	T(8) + Se	T(8) + Sc	T(8) + D(64)	T(8) + D(64)	T(8)	T(35) + Sc	0	0
III	T(70)	T(8)	T(70)				T(8)	T(8)		Sc	0
IV	D(32)		G(35)				T(8) + Sc				
V	D(64)		G(70)								

Symbols: T( ), G( ), D( ) = infusions of sodium taurocholate, glycocholate, and dehydrocholate, respectively. Number shown in parentheses is approximate infusion rate in μmoles/min. Actual infusion rate was calculated in each case by calibration of pump speed and, in the case of taurocholate and glycocholate, measurement of bile acid concentration in infusion. Se, infusion of secretin at 4U/min; Sc, infusion of SC2644 at 11 μmoles/min; 0, no bile acid, secretin or SC2644 infusion.

Tracer infusions: ^{14}C-labeled erythritol was infused throughout each experiment except that in protocol G ^{14}C-labeled sucrose was infused and in protocol K no isotope was infused.

Timing: Bile collection periods are indicated by Roman numerals at left. In each case 30–60 minutes elapsed from the time of bile duct catheterization until the time tracer infusion was started and 90 min elapsed between initiation of tracer infusion and start of first bile collection, except that in protocol K (no tracer) 3 hr elapsed from the time of bile duct catheterization until the beginning of the first collection. Initial bile acid infusion (if any) was started at the same time as the tracer except in protocol I where it was started 60 min later. Actual bile collection periods lasted from 10 min (flows > 0.5 ml/min) to 30 min (flows < 0.09 ml/min). Whenever bile acid infusion rate was changed (A, B, C) 20–25 minutes elapsed before starting the next collection period. Whenever a new bile acid was infused (A and C) there was a 30 min hiatus without infusion before the new bile acid was started followed by another 25 min of equilibration on the new infusion. Whenever secretin (D) or SC2644 (E, G, I, J) were infused or when dehydrocholate was superimposed upon a taurocholate infusion (F, G) a 20 min equilibration period was allowed before starting a new bile collection. In protocols H and K and in the first two periods of B and J consecutive bile collections were obtained without interruption. Blood specimens for measurement of ^{14}C activity were obtained 2 min after starting each bile collection period except in protocol K.

11 different protocols were employed (Table I). Two typical experiments are shown in Table II.

Chromatography. Thin-layer chromatography was performed on the bile acid preparations used for infusion and on selected specimens of bile using silica gel G and the solvent systems described by Hofmann for conjugated and unconjugated bile acids (14). The bile specimens were prepared by shaking 0.2 ml of bile with 0.4 ml of chloroform-methanol (1:1) and 0.4 ml of water. The aqueous phase was subjected to chromatography for conjugated bile acids and the organic phase for unconjugated bile acids.

Analytical procedures. ^{14}C activity in plasma was estimated as described previously (12). Bile specimens were decolorized with sodium hypochlorite (Chlorox) as described (12) except that Cab-O-Sil was not used, and 50 μl of 30% H_2O_2 was added to remove unreacted sodium hypochlorite before final dilution and addition of Bray's solution.

Biliary cholesterol concentration was measured by the method of Abell, Levy, Brodie, and Kendall (15). Biliary phospholipid was extracted in chloroform-methanol (2:1) and measured by the Bartlett procedure (16). Total bile acid concentration was determined by the hydroxysteroid dehydrogenase method of Talalay (17) as modified by Admirand and Small (18) using purified enzyme (STDHP—Worthington Biochemical Corp., Freehold, N. J.).

Materials. The sodium taurocholate employed for infusion (Mann Research Labs, Inc., New York) was an impure ox-bile preparation which contained 74% bile acid by weight and which was shown by thin-layer chromatography to contain a small amount of glycocholate, traces of taurine and glycine conjugates of dihydroxy bile acids, and traces of unconjugated cholic acid. The sodium glycocholate (ICN Nutritional Biochemicals Div., Irvine, Calif.) contained 61% bile acid by weight and although glycocholate was found to be the main constituent, it was heavily contaminated with glycine conjugated dihydroxy bile acids and with unconjugated cholic acid and contained traces of taurine conjugated di- and trihydroxy bile acids. Sodium dehydrocholate was obtained as a 20% solution (Decholin; Ames Co., Div. Miles Laboratories, Inc., Elkhart, Ind.) and appeared to be chromatographically pure. All bile acids were diluted to a concentration of approximately 20 mM in 5% dextrose and water and administered at the rates shown in Table I. Actual rates of infusion in each experiment were subsequently calculated from calibrated pump speed and infusion concentration. The latter was measured in the case of glycocholate and taurocholate and based upon the dilution of manufacturer's stated concentration in the case of dehydrocholate.

Secretin (GIH Laboratories, Stockholm) was diluted to a concentration of 10 U/ml in normal saline and administered at 4 U/min.

SC2644[2] (Fig. 1) was mixed with a small volume of water, dissolved by titration with 1 N NaOH to approximately pH 10 and diluted to a final concentration of 0.9%. Dextrose was added to a concentration of 5%. It was administered at 11 μmoles/min.

^{14}C-labeled erythritol and ^{14}C-labeled sucrose (both from Amersham/Searle Corp., Arlington Heights, Ill.) were dissolved and administered as described previously (12).

RESULTS

Relationships of biliary lipid excretions to taurocholate excretions. In the typical study shown in Table II so-

[2] SC2644 was generously provided by Donald L. Cook of G. D. Searle and Co., Skokie, Ill.

dium taurocholate was infused at three different rates. In each of the three states the measured excretion rate of bile acid was approximately equal to the rate of infusion, and the bile flow, erythritol clearance, lecithin excretion rate, and cholesterol excretion rate all increased with each increment in bile acid excretion rate. The absolute biliary concentrations of bile acid also increased and the relative concentrations of lecithin and cholesterol (expressed as per cent of the sum of molar concentrations of bile acid, cholesterol, and lecithin) declined. There was a fall in the molar ratio of lecithin-to-bile acid at the highest rate of infusion. The cholesterol-to-lecithin ratio showed no consistent trend and averaged 0.038.

In another study on the same dog (Table II) no bile acid was infused during the first two collection periods and then sodium taurocholate was infused at 8.8 μmoles/min. During the first two periods the rate of endogenous bile acid excretion was low, as were the bile flow, erythritol clearance, and lipid excretion rates. Despite the low absolute biliary concentration of cholesterol its relative concentration was much higher than the relative concentration observed during taurocholate infusion. The cholesterol-to-lecithin ratio (average 0.109) was higher than the ratios (0.03 to 0.04) found at all higher rates of bile acid excretion. When sodium taurocholate was then infused at 8.8 μmoles/min, the flow and composition of bile approached those observed in the first period of the previous study.

The lipid excretion data obtained from multiple studies on each dog during various rates of taurocholate infusion (or without bile acid infusion) are plotted in Figs. 2–6. The following characteristics were generally apparent: (*a*) Lecithin output was related to bile acid output by an ascending curve which was concave downward and appeared to pass through the origin. (*b*) The relationship of cholesterol output to bile acid output was qualitatively very similar to that described above except that the data obtained at the lowest rates of bile acid excretion in three of the dogs (Figs. 3, 5, and 6) suggested either that the true curve describing the relationship might have a positive intercept along the vertical axis or might have an abrupt change in shape near the origin. (*c*) Consistent with the relationship described in (*a*), the molar ratio of lecithin-to-bile acid declined gradually as bile acid output increased. (*d*) The cholesterol-to-lecithin ratio appeared to diminish rapidly toward a plateau of 0.031–0.042 as bile acid excretion rates rose to 8 μmoles/min or higher. This plateau was quite constant over a very wide range of bile acid excretion rates in each dog. At very low rates of bile acid excretion much higher cholesterol-to-lecithin ratios were observed except in one dog (dog Ad, Fig. 4) in which they were only slightly higher. No observations were made at the very low bile acid excretion rates in dog Ha (Fig. 2).

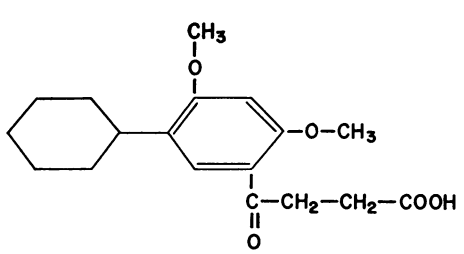

FIGURE 1 Structure of SC2644.

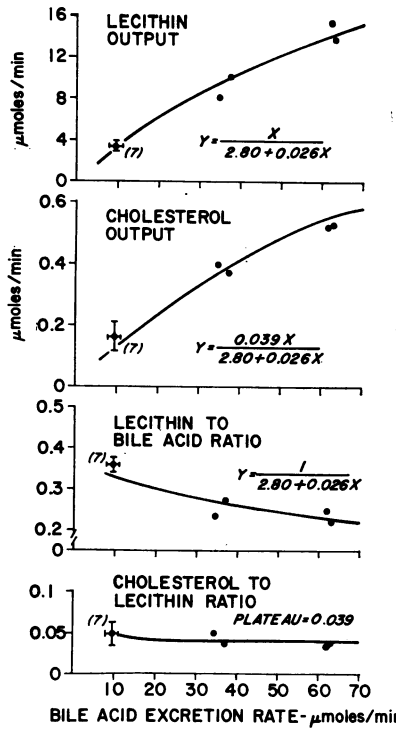

FIGURE 2 Effect of biliary excretion rate of bile acid upon outputs of lecithin and cholesterol and upon biliary molar concentration ratios of lecithin-to-bile acid and cholesterol-to-lecithin in dog Ha during sodium taurocholate infusion. The group of observations obtained during the lowest rate of infusion (*circa* 8 μmoles/min) has been pooled, and the means, standard deviations, and number of observations (in parentheses) are shown for this group. At rates above 20 μmoles/min individual data points are shown. The lowest curve was fitted by eye and the other curves were fitted as described in the text.

Curve fitting. Curves were fitted arbitrarily in the following manner: the curve for cholesterol-to-lecithin ratio (C/L) [3] was fitted by eye and a numerical value

[3] *Abbreviations used in this paper:* BA, bile acid; C, cholesterol; CMC, critical micellar concentration; L, lethicin; R, ratio.

TABLE II

Two Experiments Showing Effects of Various Bile Acid

Bile salt infusion	Infusion rate	Time* of infusion		Time* of collection		Bile flow	Erythritol clearance	Bile acid Conc.
		Start	End	Start	End			
	μmole/min	*min*		*min*		*ml/min*	*ml/min*	*mM*
Taurocholate	7.4	−12	111	91	111	0.12	0.15	63.5
Taurocholate	30.9	111	150	135	150	0.29	0.30	99.3
Taurocholate	61.4	150	180	170	180	0.52	0.48	105.7
Dehydrocholate§	31.4	210	250	235	250	0.64	0.62	(29.8)‡
Dehydrocholate§	62.3	250	280	270	280	0.77	0.85	(32.9)‡
None	—	—	—	105	165	0.06	0.10	13.4
None	—	—	—	165	225	0.06	0.10	12.9
Taurocholate	8.8	226	291	261	291	0.12	0.16	76.7

* Zero time is arbitrarily defined as the time erythritol infusion was started, usually 30–60 min after catheterization of the common bile duct.

‡ Expressed as per cent of the sum of the molar concentrations of bile acid + lecithin + cholesterol.

§ During dehydrocholate infusion there was an appreciable biliary output of metabolites measurable by the 3-hydroxysteroid dehydrogenase method. These measurements, and the values derived therefrom, are given in parentheses since they obviously must underestimate the total output of dehydrocholate and its metabolites (and hence lead to overestimation of the moles per cent of lecithin and cholesterol and of the lecithin : bile acid ratios).

(R) for the ratio represented by the plateau was calculated as the average of all of the C/L values at bile acid excretion rates above 20 μmoles/min. The curve relating the lecithin-to-bile acid ratio (L/BA) to the bile acid excretion rate was fitted by an equation of the form $y = 1/(A + Bx)$.[4] These values of A and B were then used to plot the curve for lecithin excretion rate *vs.* bile acid excretion rate according to the equation $y = x/(A + Bx)$. Finally, the curve relating cholesterol output to bile acid output was plotted as $y = Rx/(A + Bx)$.

Except in the case of cholesterol output at the lowest rates of bile acid excretion (Figs. 3, 5, and 6) this procedure appeared to yield curves which fitted the data quite well. Other types of curves could probably have been fitted to the data just as well, but the particular hyperbolas were chosen for reasons which will be mentioned in the Discussion.

Relationship of biliary lipid excretion to glycocholate and dehydrocholate excretion. The infusion of sodium glycocholate (Table III) at two different rates (35 and 69 μmoles/min) resulted in excretion rates of lecithin and cholesterol similar to those observed at comparable rates of sodium taurocholate infusion. In most instances the bile flows and erythritol clearances were somewhat higher with glycocholate than the taurocholate. As noted under Methods the "glycocholate" employed in these studies was impure and contained appreciable amounts of unconjugated cholic acid. During infusion of this material none of the unconjugated bile acids appeared as such in the bile, but appreciable quantities of taurocholate and some taurine conjugated dihydroxy bile acids were present. However, the major bile acids excreted were glycine conjugates in contrast to the dominant excretion of taurine conjugates when "taurocholate" was infused.

The effect of infusion of sodium dehydrocholate is illustrated by the study shown in Table II, and the data for five dogs are shown in Table III. The infusion of dehydrocholate at 32 and 64 μmoles/min was associated with appreciably higher rates of bile flow and with higher erythritol clearances than those observed during infusion of the natural bile salts at similar rates. The excretion rates of cholesterol and lecithin were very low at both rates of dehydrocholate infusion. The fact that they actually appeared to diminish during the more rapid infusion may not be significant, however, since this could have been due to progressive depletion of endogenous and previously administered natural bile acids.

Effect of secretin, SC2644, and dehydrocholate on biliary lipid excretion during constant infusion of sodium taurocholate. When sodium taurocholate was infused at a constant rate of approximately 7 μmoles/min (Table IV) the addition of secretin at 4 U/min caused an av-

[4] Based on least squares fit to a linear transform using a computer program available through General Electric Information Systems in BASIC language. In carrying out this fitting the mean coordinates of L/BA and BA output in the absence of bile acid infusion and the mean coordinates at low taurocholate infusion (*circa* 8 μmoles/min) were each treated as a single data point.

Infusions on Bile Flow and Biliary Lipid Excretion (Dog Po)

Bile acid		Lecithin			Cholesterol			Lecithin: bile acid molar ratio	Cholesterol: lecithin molar ratio
Moles‡ per cent	Output	Conc.	Moles‡ per cent	Output	Conc.	Moles‡ per cent	Output		
	μmole/min	mM		μmole/min	mM		μmole/min		
73.4	7.6	22.1	25.5	2.6	0.94	1.1	0.11	0.35	0.043
72.5	28.8	36.5	26.6	10.6	1.18	0.9	0.34	0.37	0.032
80.0	55.0	25.5	19.3	13.3	1.01	0.8	0.52	0.24	0.040
(98.8)	(19.1)	0.7	(1.1)	0.4	0.09	(0.2)	0.06	(0.01)	0.138
(99.4)	(25.3)	0.3	(0.5)	0.2	0.08	(0.1)	0.06	(0.01)	0.256
71.1	0.9	5.7	26.3	0.3	0.57	2.6	0.03	0.37	0.100
71.9	0.8	4.5	25.1	0.3	0.53	3.0	0.03	0.35	0.118
74.8	8.9	25.2	24.6	2.9	0.70	0.7	0.08	0.33	0.028

erage increment of 0.24 ml/min in bile flow. In three of the four dogs there was only a small increment in erythritol clearance (average 0.05 ml/min) but in the fourth dog (Ad) there was an appreciable increment of 0.14 ml/min. (The unusual effect of secretin on the erythritol clearance in this single dog was confirmed in a nearly identical subsequent study and was borne out by demonstration of an identical effect of secretin on the mannitol clearance. This is the only dog in which we have seen this phenomenon.) In none of the dogs was any change apparent in the excretion rates of bile salt, lecithin, or cholesterol.

The addition of SC2644 at 11 μmoles/min during a taurocholate infusion of 8 μmoles/min (Table V) caused marked increases in flow (average 0.41 ml/min), in erythritol clearance (mean increase 0.31 ml/min), and in sucrose clearance (mean increase 0.04 ml/min). In every instance there was a *decrease* in the excretion

TABLE III

Effect of Infusions of Taurocholate (T), Glycocholate (G), and Dehydrocholate (D) at Comparable Rates

	Bile acid infusion rate			Bile flow			Erythritol clearance			Bile acid excretion rate			Lecithin excretion rate			Cholesterol excretion rate		
Dog	T*	G	D	T*	G	D	T*	G	D	T*	G	D‡	T*	G	D	T*	G	D
	μmole/min			ml/min			ml/min			μmole/min			μmole/min			μmole/min		
Ha	35.0	37.0	32.0	0.24	0.31	0.51	0.44	0.44	0.59	36.0	34.2	(20.3)	9.1	9.1	0.8	0.38	0.30	0.09
	69.1	70.3	66.0	0.40	0.66	0.60	0.55	0.77	0.78	62.4	65.5	(28.6)	14.6	13.7	0.3	0.52	0.42	0.07
La	32.1	34.0	32.7	0.31	0.37	0.49	0.34	0.42	0.51	32.5	33.5	(18.4)	8.1	9.1	0.7	0.27	0.29	0.04
	63.4	68.5	63.8	0.52	0.48	0.63	0.52	0.51	0.71	56.0	51.9	(28.5)	11.6	10.6	0.2	0.43	0.45	0.04
Ad	34.9	36.8	33.2	0.28	0.57	0.66	0.28	0.42	0.59	28.6	38.5	(18.8)	9.6	13.2	1.6	0.27	0.29	0.08
	68.7	72.5	65.3	0.48	0.63	0.93	0.44	0.61	0.80	55.1	57.1	(26.9)	13.6	14.8	0.5	0.49	0.47	0.06
Po	32.7	35.6	31.4	0.34	0.50	0.64	0.38	0.48	0.62	30.0	35.8	(19.1)	10.8	10.5	0.4	0.36	0.56	0.06
	62.8	66.1	62.3	0.56	0.70	0.77	0.55	0.74	0.85	56.9	56.7	(25.3)	14.1	12.7	0.2	0.51	0.42	0.06
Tu	42.7	38.4	36.6	0.40	0.37	0.65	0.39	0.36	0.65	38.6	28.3	(22.3)	10.7	7.5	0.6	0.44	0.31	0.10
	84.6	75.7	72.8	0.61	0.56	0.74	0.56	0.58	0.79	69.2	67.0	(33.5)	15.7	14.0	0.3	0.68	0.76	0.10

* All taurocholate data are averages of two separate studies.

‡ See footnote to Table II. Measured bile acid output values during dehydrocholate infusion include only the 3-hydroxysteroid dehydrogenase metabolites and are therefore shown in parentheses.

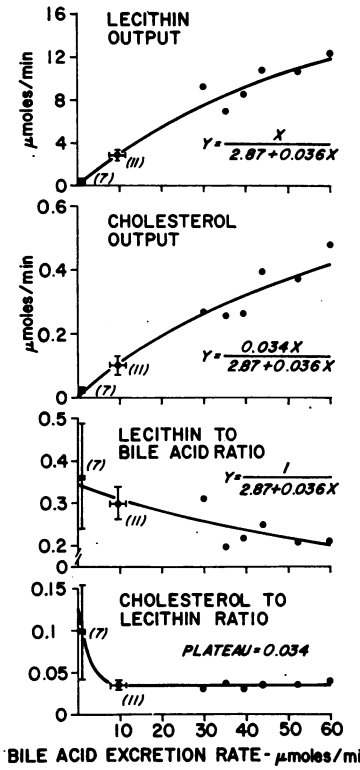

FIGURE 3 Effect of biliary excretion rate of bile acid upon outputs of lecithin and cholesterol and upon ratios of lecithin-to-bile acid and cholesterol-to-lecithin in dog La. Data were obtained in the absence of bile acid infusion and during sodium taurocholate infusion at various rates. Means, standard deviations, and number of observations (parentheses) are shown for the group of observations obtained in the absence of bile acid infusion and for the group obtained at the lowest rate of taurocholate infusion (*circa* 8 μmoles/min). Individual data points are plotted for all higher rates. The lowest curve was fitted by eye and the other curves were fitted as described in the text.

rates of both lecithin and cholesterol. The average decrement in lecithin excretion was 1.1 μmoles/min or 32%, and the decrement in cholesterol excretion was 0.03 μmoles/min or 34% when SC2644 was administered.

When sodium taurocholate was infused at 41 μmoles/min (Table VI) the addition of SC2644 at 11 μmoles/min caused increments in bile flow (average 0.30 ml/min) and erythritol clearance (average 0.31 ml/min) similar to those produced at the lower rate of taurocholate infusion, but caused no consistent changes in lecithin or cholesterol excretion. However, the basal rates of lipid excretion were high enough so that it would have been difficult to detect absolute decrements of the magnitude observed in the preceding experiments.

The addition of sodium dehydrocholate at 64 μmoles/min (Table V) during a taurocholate infusion of 8 μmoles/min caused increases in bile flow, erythritol clearance, and sucrose clearance averaging 0.55, 0.49, and 0.08 ml/min, respectively. Lecithin output decreased in each case (average decrement 1.1 μmoles/min or 32%) but cholesterol output showed little or no change.

TABLE IV

Effect of Secretin during Constant Infusion at Taurocholate at 7 μmoles/min

Dog	Condition	Flow	Erythritol clearance	Bile acid output	Lecithin output	Cholesterol output
		ml/min			*μmole/min*	
Ha	Control	0.07	0.18	9.0	3.4	0.13
	Secretin	0.38	0.22	8.1	4.0	0.10
La	Control	0.11	0.16	10.5	3.6	0.18
	Secretin	0.28	0.20	9.7	3.4	0.14
Ad	Control	0.21	0.13	5.9	2.4	0.16
	Secretin	0.50	0.27	7.1	3.0	0.20
Po	Control	0.11	0.15	8.4	3.2	0.13
	Secretin	0.27	0.22	8.1	3.0	0.12

Effect of SC2644 at low bile acid excretion rates. When SC2644 was administered approximately 4 hr after interruption of enterohepatic circulation to dogs receiving no bile acid infusion (Table VII) there was no obvious effect on total endogenous bile acid excretion

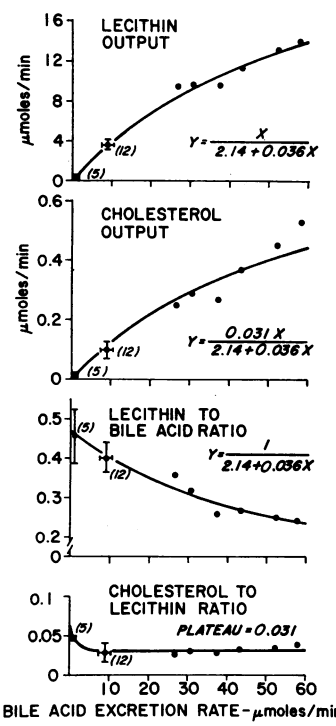

FIGURE 4 Effect of biliary excretion rate of bile acid upon outputs of lecithin and cholesterol and upon ratios of lecithin-to-bile acid and cholesterol-to-lecithin in dog Ad. See legend of Fig. 3 for details.

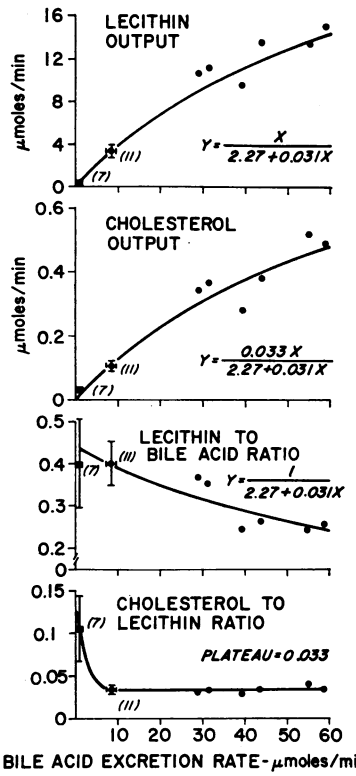

FIGURE 5 Effect of biliary excretion rate of bile acid upon outputs of lecithin and cholesterol and upon ratios of lecithin-to-bile acid and cholesterol-to-lecithin in dog Po. See legend of Fig. 3 for details.

rate but there was a diminution in the excretion rates of both lecithin and cholesterol in every dog. It should be noted that the effect of SC2644 on bile flow (average increase 0.28 ml/min) and on erythritol clearance (average increase 0.25 ml/min) were very similar to those observed during bile acid infusion of 8 (Table V) and 41 (Table VI) µmoles/min.

Observations on possible effects of time on bile composition. Three consecutive 30 min collections of bile during constant taurocholate infusion at 8 µmoles/min (Table VIII) showed remarkably constant outputs and concentration ratios. During total interruption of enterohepatic circulation (also in Table VIII) consecutive 60-min collections showed considerably greater variation. However, in two of three dogs there was no consistent trend with the passage of time. In one experiment on the second dog (La) there was a sixfold decrement in endogenous bile acid excretion rate and the changes in cholesterol and lecithin outputs and concentration ratios were consistent with those predicted during deliberate manipulation of bile acid output (Fig. 3). The trend in this experiment could therefore be attributed to the effects of delayed depletion of the enterohepatic circulating bile acid pool. The studies shown in Table VIII thus provide no evidence for alterations in bile composition attributable to temporal changes in hepatic lipid metabolism during the relatively brief total times required to execute any of the experiments shown in the preceding tables.

Initial "common duct" bile specimens. At the beginning of each study 3–6 ml of bile drained from the common bile duct within the first few minutes after catheterization. Most of this bile had probably been stored in the bile ducts for some time before catheterization and, as noted in earlier studies (19), it tended to have a high concentration of bile acid. The average composition of all "common duct" bile specimens is shown in Table IX. Although the absolute concentrations of bile acids and lipids were very high in most instances the ratios of these constituents were well within the range encountered in flowing hepatic bile at intermediate rates of bile acid excretion (Figs. 2–6).

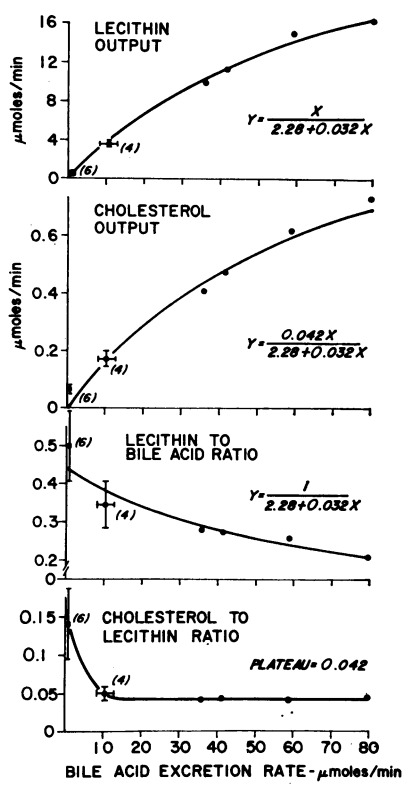

FIGURE 6 Effect of biliary excretion rate of bile acid upon outputs of lecithin and cholesterol and upon ratios of lecithin-to-bile acid and cholesterol-to-lecithin in dog Tu. See legend of Fig. 3 for details.

TABLE V

*Effect of Dehydrocholate and SC2644 during Constant Sodium Taurocholate Infusion at 8 μmoles/min**

Dog	Condition	Bile flow	Erythritol clearance	Sucrose clearance	Bile acid output‡	Lechithin output‡	Cholesterol output	Suc:eryth clearance ratio	Estimated $\sigma_{sucrose}$§
			ml/min			*μmole/min*			
Ha*	Control	0.06	0.13	—	7.2	2.8	0.08	—	—
	SC2644	0.56	0.44	—	6.9	1.3	0.04	—	—
La	Control	0.09	0.19	0.029	10.8	3.0	0.09	0.15	
	Dehydrocholate	0.57	0.68	0.079	—	1.5	0.06	0.12	0.88
	Control	0.09	0.14	0.028	8.0	2.6	0.08	0.20	
	SC2644	0.46	0.40	0.063	8.5	1.8	0.06	0.16	0.84
Ad	Control	0.16	0.18	0.044	10.6	4.4	0.12	0.24	
	Dehydrocholate	0.74	0.58	0.150	—	3.3	0.12	0.26	0.74
	Control	0.15	0.19	0.060	10.6	4.0	0.10	0.32	
	SC2644	0.42	0.46	0.098	11.1	2.8	0.08	0.21	0.79
Po	Control	0.13	0.14	0.048	7.2	2.8	0.10	0.34	
	Dehydrocholate	0.72	0.72	0.131	—	1.8	0.10	0.18	0.82
	Control	0.17	0.14	0.048	9.9	4.1	0.12	0.34	
	SC2644	0.52	0.53	0.096	8.9	1.7	0.07	0.18	0.82

* With the exception of dog Ha each bile flow and output value is the average of two determinations made on different days. The erythritol clearance was estimated on one of the two days and the sucrose clearance on the other. All control values shown were those obtained immediately before the administration of the choleretic agent indicated in the row that follows. Sodium dehydrocholate was administered at 64 μmoles/min and SC2644 at 11 μmoles/min.

‡ Total bile acid outputs are not reported in the dehydrocholate studies because chemical measurement of concentration only included 3-hydroxysteroid metabolites (see footnote to Table II). Since taurocholate was infused throughout these studies the output of taurocholate is assumed to have been at least equal to infusion rate.

§ Reflection coefficient for sucrose ($\sigma_{sucrose}$) was estimated as 1 minus the sucrose:erythritol clearance ratio (see text).

DISCUSSION

Dependence of biliary lipid excretion upon excretion of micelle-forming bile acids

The biliary excretion rates of lecithin and of cholesterol in these dogs, as in the isolated dog liver (5) and in all other species studied previously (6–8, 10), appeared to be intimately dependent upon the biliary excretion rate of the bile acids. That this phenomenon is attributable exclusively to the ability of the bile acids to form micelles and not to the effect of bile acids on bile flow is demonstrated by the fact that three other

TABLE VI

*Effect of SC2644 during Constant Sodium Taurocholate Infusion at 41 μmoles/min**

Dog	Condition	Bile flow	Erythritol clearance	Bile acid output	Lecithin output	Cholesterol output
		ml/min			*μmole/min*	
La	Control	0.30	0.34	41.7	9.8	0.33
	SC2644‡	0.64	0.67	43.1	7.0	0.20
Ad	Control	0.38	0.40	40.2	10.5	0.32
	SC2644‡	0.61	0.68	44.3	11.5	0.30
Po	Control	0.40	0.41	41.4	10.5	0.33
	SC2644‡	0.73	0.72	47.4	9.4	0.27

* Each number is the average of two determinations made on different days.

‡ SC2644 was infused at 11 μmoles/min.

TABLE VII

Effect of SC2644 in the Absence of Bile Acid Infusion

Dog	Condition	Bile flow	Erythritol clearance	Bile acid output	Lecithin output	Cholesterol output
		ml/min			*μmole/min*	
La	Control	0.04	0.15	1.03	0.30	0.026
	Control	0.06	0.11	0.37	0.08	0.016
	SC2644*	0.35	0.28	0.37	0.02	0.004
Ad	Control	0.06	0.07	0.92	0.34	0.018
	Control	0.07	0.08	0.62	0.28	0.015
	SC2644*	0.29	0.24	0.92	0.12	0.011
Po	Control	0.07	0.11	1.44	0.81	0.037
	Control	0.06	0.09	1.25	0.58	0.030
	SC2644*	0.41	0.39	1.03	0.15	0.004
Tu	Control	0.09	0.12	1.43	0.63	0.059
	Control	0.16	0.14	1.14	0.47	0.054
	SC2644*	0.43	0.50	.94	0.11	0.014

* Infused at 11 μmoles/min after two consecutive control periods during which the enterohepatic pool became depleted. Approximately 4 hr had elapsed from the time bile duct was catheterized to the time SC2644 was started (protocol J).

potent choleretic agents failed to enhance (or actually reduced) lipid excretion.

(*a*) *Secretin.* Secretin, which acts primarily by promoting fluid secretion by the bile ducts or ductules (20–22) had no effect on lipid excretion (Table IV).

(*b*) *Dehydrocholate.* Dehydrocholate infusion failed to cause any increase in lipid excretion rates (Tables II, III, and V). This is similar to the findings of Hardison and Francis (9) in rats. The choleretic action of dehydrocholate, like that of the natural bile acids, can be attributed to an increase in the rate of canalicular fluid production as indicated by the increment in erythritol clearance. Dehydrocholate differs from the natural bile acids in that it does not form micelles. However, dehydrocholate is converted to a variety of metabolites before biliary excretion (23). In the present studies, for example, the excretion rate of 3-hydroxylated metabolites (as measured by the hydroxysteroid dehydrogenase reaction) was equal to about one-half of the infusion rate of dehydrocholate (Tables II, III, and V). Since most of the dehydrocholate metabolites, like the parent compound, do not form micelles (23), it is reasonable to assume that lack of micelle formation is responsible for the failure of dehydrocholate to enhance lipid excretion. However, interpretation is complicated by the fact that a small fraction of administered dehydrocholate is actually converted to cholic acid (23). It is possible that if this did not occur there might have been more obvious decrements in lipid excretion such as those observed with SC2644.

(*c*) *SC2644.* This potent choleretic compound also failed to enhance lipid excretion rates (Tables V, VI, and VII). The mechanism responsible for its choleretic effect is unknown, but it is germane to this discussion to examine the evidence regarding its behavior and site of action. Like dehydrocholate, SC2644 apparently caused increased canalicular fluid output as indicated by the increase in erythritol clearance. On either a molar or weight basis it is obviously a far more potent choleretic agent than dehydrocholate in the dog and is more potent than any of the chemically related organic acids studied by Gunter, Kim, Magee, Ralston, and Ivy (24). In fact even on the assumption that it was all secreted into the bile canaliculi, the response to the small dose employed was too great and too prolonged (persisting in these studies for at least 3 hr after cessation of about 30 min of infusion at 11 μmoles/min) to be readily explicable on the basis of an osmotic effect of SC2644 per se. Insofar as the quantity (1 — sucrose clearance/erythritol clearance) may be used as a rough estimate of the reflection coefficient for sucrose ($\sigma_{sucrose}$) at high flows (12, 25) it would appear that this parameter of passive membrane permeability was identical whether choleresis was produced by SC2644 or dehydrocholate (Table V). Moreover, a given dose of SC2644 appeared to produce a nearly identical increment in bile flow and erythritol clearance at three widely different rates of basal flow and bile acid secretion rate (compare Tables V, VI, and VII). Stimulation of "bile acid independent" (12, 26, 27) inorganic solute and water transport into the canaliculi would thus appear to be the most logical tentative explanation for the effect of this agent. Therefore, bile acid secreted into the canalicular lumen would be diluted as a result of SC2644 administration.

Evidence that lipid entry depends upon the micellar state of bile acids in the canaliculi

Bile acids might affect the output of lipids by two possible mechanisms. First, lecithin and cholesterol might be "entrained" at some stage during bile acid transport before actual entry into the canaliculi. Or second, the presence of bile acid micelles within the canalicular lumen might serve to trap lipids which entered via the membrane surrounding the canalicular lumen. The latter possibility is supported by the fact that SC2644 actually *reduced* lipid output during the course of a constant slow infusion of taurocholate (Table V) and that SC2644 caused a marked reduction in lecithin and cholesterol excretion when administered during the course of the very low bile acid excretion rates that accompanied interrupted enterohepatic circulation (Table VII). It is postulated that canalicular fluid produced in response to SC2644 diluted the intracanalicular bile acids toward their critical micellar concentration so that a larger proportion of the total bile acid was in the dissociated state and a smaller proportion was available for solubilization of lipids. In other words, the present results are consistent with the view that the rate of biliary excretion of lipids is a function of the solubilizing capacity of the bile acids within the canalicular lumen rather than the total bile acid excretion rate.

Quantitative relationships between lecithin excretion and bile acid excretion

The present studies do not indicate whether biliary lipids are originally derived directly from the canalicular membranes as has been suggested by Small (28) or whether their origin is intrahepatic. In either case it is possible to conceive of a model based on passive lipid movement which is consistent with most of the present observations. The solubilities of lecithin and cholesterol in water are exceedingly small. Nonetheless it can be assumed that individual lipid molecules will escape transiently into the canalicular aqueous phase from either the membrane or the bile acid micelles and then return to either at random. The net movement of a lipid species between membrane phase and micellar phase should thus be a function of the relative tendency

TABLE VIII

Sequential Bile Collections with and without

Dog	Taurocholate infusion rate	Bile flow			Erythritol clearance			Bile acid output		
		1	2	3	1	2	3	1	2	3
	μmole/min		*ml/min*			*ml/min*			*μmole/min*	
Ha	8.1	0.10	0.11	0.11	0.16	0.16	0.14	10.3	9.2	8.2
La	7.9	0.10	0.12	0.12	0.12	0.16	0.13	8.4	9.0	9.3
	0	0.02	0.04	—	0.06	0.07	—	0.84	0.77	—
	0	0.03	0.02	0.03	—	—	—	2.59	1.13	0.44
Ad	7.8	0.12	0.15	0.14	0.16	0.19	0.17	8.0	9.8	8.2
	0	0.14	0.10	—	0.10	0.08	—	1.04	0.61	—
	0	0.06	0.05	0.04	—	—	—	1.33	1.17	1.14
Po	7.6	0.13	0.18	0.14	0.18	0.22	0.22	8.0	9.6	8.0
	0	0.06	0.06	—	0.10	0.10	—	0.85	0.80	—
	0	0.06	0.06	0.05	—	—	—	0.69	1.08	0.48

* Protocols H, B, and K (Table I).

of lipid to escape from either phase. In the case of lecithin one could assume, for example, that the escaping tendency from membrane to lumen had some fixed value P_1 and that the escaping tendency from the micelles P_2, was related to the concentration of lecithin within the micelles. The latter assumption is based upon the finding of Mysels (29) that the concentration of lipid soluble dye in the aqueous phase of a micellar solution is directly proportional to the ratio of dye to solubilizer in the micellar phase. The relationships between bile acid output and lecithin output could then be predicted as follows: P_1, escaping tendency of lecithin from membrane; P_2, escaping tendency of lecithin from micelles; L, biliary lecithin concentration; B, biliary micellar bile acid concentration; J_L, net lecithin excretion rate into bile; J_B, net bile acid excretion rate into bile; K_1, K_2, arbitrary constants (K_1 is analogous to the partition constant between aqueous and micellar phase described by Mysels, and K_2 is analogous to a diffusion constant across the region separating membrane from micelles).

Assuming that escaping tendency from micelles is proportional to the ratio of lecithin-to-bile acid in the micelles:

$$P_2 = K_1(L/B).$$

($B \approx$ total bile acid concentration where the latter greatly exceeds the critical micellar concentration.)

$$L/B = J_L/J_B$$

$$\therefore \quad P_2 = K_1(J_L/J_B).$$

Assuming net lecithin flux is proportional to the difference between escaping tendencies:

$$J_L = K_2(P_1 - P_2)$$

$$J_L = K_2(P_1 - K_1(J_L/J_B)).$$

Rearranging:

$$J_L = \frac{J_B}{K_1/P_1 + (1/K_2P_1)J_B}, \tag{1}$$

also

$$J_L/J_B = L/B = \frac{1}{K_1/P_1 + (1/K_2P_1)J_B}. \tag{2}$$

There are a number of obvious oversimplifications in this approach, but the model predicts a hyperbolic ascending relationship between lecithin excretion and bile acid excretion and a declining value of lecithin-to-bile acid ratio at increasing rates of bile acid excretion. On this basis hyperbolic functions of the form predicted by equation 2 were fitted to the data for lecithin-to-bile acid ratio, and the constants derived from the fitting procedure (K_1/P_1 and $1/K_2P_1$) were then introduced into equation 1 to obtain the curves for lecithin excretion rates. It is evident that a reasonable fit was obtained in every case (Figs. 2–6) so that at least the data were consistent with the model described. It should be noted that Heath et al. (10) and Hardison and Francis (9) observed apparent "plateaus" or maximal lecithin excretion rates under conditions of bile acid loading in sheep and rats, respectively. Such a phenomenon would be predicted from equation 1 even though plateaus were not actually achieved in

*Constant Infusions of Sodium Taurocholate**

Lecithin output			Cholesterol output			Lecithin: bile acid molar ratio			Cholesterol: lecithin molar ratio		
1	2	3	1	2	3	1	2	3	1	2	3
	μmole/min			*μmole/min*							
3.5	3.5	2.8	0.21	0.22	0.19	0.34	0.38	0.34	0.060	0.063	0.068
2.3	2.6	2.5	0.09	0.10	0.10	0.28	0.29	0.27	0.038	0.038	0.038
0.29	0.47	—	0.024	0.033	—	0.35	0.61	—	0.084	0.070	—
0.72	0.36	0.21	0.028	0.019	0.029	0.28	0.32	0.48	0.038	0.054	0.140
3.1	3.8	3.1	0.08	0.09	0.09	0.39	0.39	0.38	0.026	0.024	0.020
0.42	0.32	—	0.017	0.014	—	0.39	0.54	—	0.042	0.042	—
0.70	0.52	0.42	0.024	0.025	0.022	0.53	0.45	0.37	0.034	0.041	0.052
3.3	3.9	3.4	0.09	0.12	0.10	0.41	0.41	0.43	0.029	0.031	0.028
0.31	0.28	—	0.031	0.033	—	0.37	0.35	—	0.100	0.118	—
0.23	0.20	0.25	0.033	0.026	0.034	0.33	0.18	0.52	0.148	0.133	0.136

the dog. In equation 1, as $J_B \rightarrow \infty$ the predicted value of J_L would be K_2P_1 or roughly 30 μmoles/min in the present animals. However, this value would actually be unattainable because of the limitation imposed by a transport maximum for bile acids of the order of 150 μmoles/min (30) in dogs of this size.

Relationship of cholesterol excretion to bile acid and lecithin excretion, evidence for cholesterol-lecithin coupling

The output of cholesterol, like that of lecithin appears to depend upon the bile acid excretion rate (Figs. 2–6). However, the fact that the cholesterol-to-lecithin ratio was approximately constant over a wide range of bile acid excretion rates and a wide range of outputs of cholesterol and lecithin is consistent with only the following two possibilities: (*a*) That there is a fortuitous relationship between the factors which determine the independent rates of movement of the two lipids. For example, if the rate of cholesterol excretion (J_c) were described by the following equation (analogous to equation 1):

$$J_c = \frac{J_B}{K'_1/P'_1 + (1/K'_2P'_1)J_B},$$

TABLE IX

Composition of Initial Bile Drained from Common Duct

Dog		Bile acid	Lecithin	Cholesterol	L/BA ratio	C/L ratio	Moles per cent		
							BA	L	C
			μmole/liter						
Ha	Mean	244.6	86.8	2.90	0.36	0.035	73.2	25.9	0.86
(N = 5)	SD	24.2	18.7	0.69	0.10	0.010	4.8	4.9	0.15
La	Mean	193.3	51.9	2.03	0.27	0.039	77.9	21.2	0.83
(N = 12)	SD	39.9	8.0	0.61	0.04	0.008	2.6	2.5	0.22
Ad	Mean	95.6	26.9	0.94	0.29	0.036	77.2	22.1	0.78
(N = 11)	SD	21.5	5.5	0.18	0.06	0.010	3.3	3.3	0.19
Po	Mean	102.3	35.2	1.13	0.35	0.032	73.8	25.4	0.82
(N = 12)	SD	13.0	6.2	0.26	0.05	0.006	2.8	2.7	0.18
Tu	Mean	119.7	46.2	1.84	0.39	0.041	71.6	27.4	1.09
(N = 6)	SD	26.2	13.2	0.56	0.10	0.009	4.8	4.8	0.18

then the ratio of cholesterol-to-lecithin would be constant for all values of J_B only if $K_1K_2 = K'_1K'_2$. (*b*) That there is an association or coupling between the two lipid species which determines their relative rates of movement into the bile canaliculi. For example, the association between cholesterol and lecithin in their region of origin (e.g., in canalicular membrane) might be such that an average of one molecule of cholesterol would accompany every 25–30 molecules of lecithin into the canaliculi. In this view cholesterol and lecithin might make the transition from membrane to intracanalicular micelle in the form of dimers or other complexes. Since the constants K_1 and K_2 (or K'_1 and K'_2) describe such different properties of the molecule, the relationships required by the first possibility appear to be unlikely and the second possibility—that of coupling between lecithin and cholesterol movement—appears to provide the more attractive explanation for the constancy of the cholesterol-to-lecithin ratio. Because of this viewpoint the curves which were fitted to the cholesterol output vs. bile acid output data (Figs. 2–6) were calculated simply by multiplying the equation for the lecithin output vs. bile acid output curve by a fixed value equal to the value of the cholesterol-to-lecithin "plateau."

High cholesterol-to-lecithin ratios at very low bile acid excretion rates, evidence for cholesterol entry independent of lecithin

A separate mechanism must be invoked to explain the high cholesterol-to-lecithin ratios observed during total interruption of the enterohepatic circulation. It is possible that it is caused by a slow but continuous secretion of cholesterol independent of bile acid output. It is also possible that there is slow passive movement of cholesterol into the canaliculi *in response* to bile acid secretion but independent of the coupled lecithin-cholesterol movement suggested in the preceding paragraph. The fact that cholesterol excretion dropped markedly when SC2644 was administered in the absence of bile acid infusion (Table VII) appears to support the latter possibility. Although SC2644 did not consistently alter total bile acid excretion rate the marked enhancement of canalicular fluid production (indicated by an average increment in erythritol clearance of 0.25 ml/min) can be estimated to have reduced the canalicular bile acid concentration to the range of 1.5–2.5 mmoles/liter. The critical micellar concentration (CMC) of bile acids in whole bile has not been measured in the dog, but these values are close to the range of the CMC reported by Tamesue and Juniper for whole human bile (31) and it may be assumed that an appreciable fraction of the bile acid was in the dissociated state. The resultant reduction in cholesterol excretion suggests that the entry of cholesterol even at very low rates of bile acid output is still dependent upon the availability of bile acid micelles and is not an independent transport process.

The following modification of the model proposed for lecithin excretion would suffice to explain the data describing cholesterol output and cholesterol-to-lecithin ratios. If it is assumed that entry of cholesterol independent of lecithin obeys the same rules as those described in the derivation of equation 1 then total cholesterol output would be:

$$J_c = \frac{J_B}{K'_1/P'_1 + (1/K'_2P'_1)J_B} + \frac{RJ_B}{K_1/P_1 + (1/K_2P_1)J_B}, \quad (4)$$

where P_1, K_1, and K_2 are the constants described previously for lecithin, P'_1, K'_1, and K'_2 are the analogous constants for cholesterol and R is the coupling ratio which describes the average number of moles of cholesterol which accompany each mole of lecithin.

The cholesterol-to-lecithin ratio would then be:

$$C/L = \frac{K_1/P_1 + (1/K_2P_1)J_B}{K'_1/P'_1 + (1/K'_2P'_1)J_B} + R \quad (5)$$

Equations 4 and 5 have been plotted in the appropriate panels of Fig. 7 after substituting the following values: $K_1P_1 = 2.5$, $1/K_2P_1 = 0.03$, $R = 0.04$, $K'P'_1 = 5$, $1/K'_2P'_1 = 30$. The first three are average values based upon the results of the curve fitting already described. The last two are arbitrary choices although it is not unreasonable to suppose that the tendency for cholesterol to escape from the membrane might be much less than that for lecithin (i.e., $P'_1 \ll P_1$) from which it would be likely that $1/K'_2P'_1 \gg 1/K_2P_1$, and that K'_1, the constant included in the escaping tendency of cholesterol from micelles, might also be low enough so that K'_1/P'_1 would be of the same order of magnitude as K_1/P_1.

It may be seen from Fig. 7, on which equations 1 and 2 have also been plotted, that the hypothetical model, including the provision for a dual mechanism of cholesterol excretion, now provides an adequate description of the entire range of data shown in Figs. 2–6.

Possible role of changes in lipid synthetic rates

Previous studies have demonstrated a positive effect of bile acid on the synthetic rates of hepatic lecithin (32, 33). In the present acute studies, however, there were no consistent changes in biliary lipid excretion with time (Table VIII) during either totally inter-

rupted enterohepatic circulation or constant bile acid infusion. Thus it appears improbable that there was sufficient time for changes in the rates of lipid synthesis to contribute to any of the relationships observed.

"History" of bile found in the common bile duct deduced from its composition

In the cholecystectomized dog the common bile duct provides a reservoir which usually contains 4–6 ml of bile. In the fasting state the bile first aspirated after catheterization (so called "common duct bile") is often indistinguishable in composition from highly concentrated gallbladder bile (19). This is consistent with the known reabsorptive function of the ducts (12). Thus in the present studies, the absolute concentrations of bile acids, lecithin, and cholesterol were often very high in common duct bile (Table IX). The relative concentrations of these constituents, when compared with the other data obtained, provide some indication of the average bile acid excretion rates prevailing at the time the "common duct" specimens were produced by the liver. For example, the mean lecithin-to-bile acid ratios of 0.27 to 0.39 would suggest that this bile was produced at average bile acid excretion rates of roughly 10–30 μmoles/min (based on Figs. 2–6). Thus the bulk of the lipid in this bile was probably excreted during periods of appreciable enterohepatic bile acid circulation. Analysis of a single sample of common duct bile (or, in the intact animal, of gallbladder bile) obviously provides no clue to the range of composition of hepatic bile which may be secreted at various times and under various circumstances in the same animal.

Implications

It is well recognized that the dog is not susceptible to cholesterol gallstone formation. All of the present data lie well within the solubility zone for cholesterol when plotted on the triangular coordinates described by Admirand and Small (18). Nevertheless, an unanesthetized dog may be studied repeatedly and provides a reproducible means of examining the relationship between biliary bile acid and lipid excretion. Our evidence suggests that the micellar state of the bile acids within the canaliculi controls lecithin and cholesterol excretion, and is therefore consistent with the view that these lipids enter via the membranes immediately surrounding the canaliculi. Small (28) has suggested that the biliary lipids may actually represent solubilized membrane constituents, and that the formation of bile which is supersaturated with respect to cholesterol may occur by incorporation of associated cholesterol and lecithin into micelles in a high cholesterol-to-lecithin ratio which is ultimately inconsistent with micellar stability. This possibility is strongly supported by our evidence for cholesterol-lecithin coupling. Although obviously no threat to the dog, such coupling could, in other species and under other circumstances, lead to the formation of unstable micelles.

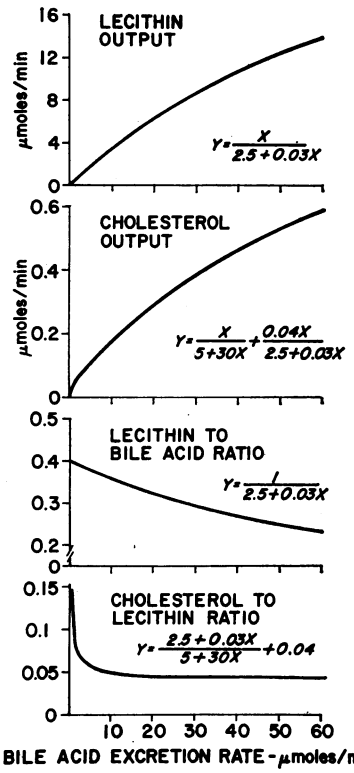

FIGURE 7 Hypothetical curves describing effect of biliary excretion rate of bile acid upon outputs of lecithin and cholesterol and upon ratios of lecithin-to-bile acid and cholesterol-to-lecithin. The derivation of these curves was based on the assumptions that lecithin moves passively from cell membranes to intercanalicular micelles, that transport of cholesterol is coupled to lecithin transport, and that there is also a small amount of independent passive transport of cholesterol from membranes to micelles (see text).

The finding that a very small amount of cholesterol may enter the bile independent of lecithin is interesting, but this process also appears to depend upon the availability of bile acid micelles within the canaliculi. It seems highly unlikely that cholesterol entering in this fashion could ever lead to the production of a bile which was supersaturated with respect to cholesterol.

If biliary lipid excretion is the result of solubilization of canalicular membrane it may perhaps be regarded as an unnecessary (and sometimes unfortunate) by-product of the biliary secretion of powerful detergent compounds necessary for digestion. Our results suggest that the avidity with which the membrane *retains* its lipid constituents could be the major determinant of biliary lipid composition.

Note added in proof. Since the preparation of this manuscript Hardison and Apter (Hardison, W. G. M., and J. T. Apter. 1972. Micellar theory of biliary cholesterol excretion. *Amer. J. Physiol.* **222**: 61) have reported biliary lipid excretion patterns in the rat which are similar in many respects to those described in the present paper and which expand upon the results published in the earlier abstract of Hardison and Francis (9).

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REFERENCES

1. Isaksson, B. 1954. On dissolving power of lecithin and bile salt for cholesterol in human bladder bile. *Acta Soc. Med. Upsal.* **59**: 296.
2. Bourgès, M., D. M. Small, and D. G. Dervichian. 1967. Biophysics of lipid associations. III. The quaternary systems lecithin-bile salt-cholesterol-water. *Biochim. Biophys. Acta.* **144**: 189.
3. Kay, R. E., and C. Entenman. 1961. Stimulation of taurocholic acid synthesis and biliary excretion of lipids. *Amer. J. Physiol.* **200**: 855.
4. Entenman, C., R. J. Holloway, M. L. Albright, and G. F. Leong. 1968. Bile acids and lipid metabolism. I. Stimulation of bile lipid excretion by various bile acids. *Proc. Soc. Exp. Biol. Med.* **127**: 1003.
5. Swell, L., C. C. Bell, Jr., and C. Entenman. 1968. Bile acids and lipid metabolism. III. Influence of bile acids on phospholipid in liver and bile of the isolated perfused dog liver. *Biochim. Biophys. Acta.* **164**: 278.
6. Swell, L., C. Entenman, G. F. Leong, and R. J. Holloway. 1968. Bile acids and lipid metabolism. IV. Influence of bile acids on biliary and liver organelle phospholipids and cholesterol. *Amer. J. Physiol.* **215**: 1390.
7. Dowling, R. H., and D. M. Small. 1968. The effect of controlled interruptions of the enterohepatic circulation on the composition of bile in the rhesus monkey. *J. Clin. Invest.* **47**: 26a.
8. Nilsson, S., and T. Scherstén. 1969. Importance of bile acids for phospholipid secretion into human bile. *Gastroenterology.* **57**: 525.
9. Hardison, W. G. M., and T. I. Francis. 1969. The mechanism of cholesterol and phospholipid excretion in bile. *Gastroenterology.* **56**: 1164.
10. Heath, T., I. W. Caple, and P. M. Redding. 1970. Effect of the enterohepatic circulation of bile salts on the flow of bile and its content of bile salts and lipids in sheep. *Quart. J. Exp. Physiol.* **55**: 93.
11. Thomas, J. E. 1941. An improved cannula for gastric and intestinal fistulas. *Proc. Soc. Exp. Biol. Med.* **46**: 260.
12. Wheeler, H. O., E. D. Ross, and S. E. Bradley. 1968. Canalicular bile production in dogs. *Amer. J. Physiol.* **214**: 866.
13. Preisig, R., H. L. Cooper, and H. O. Wheeler. 1962. The relationship between taurocholate secretion rate and bile production in the unanesthetized dog during cholinergic blockade and during secretin administration. *J. Clin. Invest.* **41**: 1152.
14. Hofmann, A. F. 1962. Thin-layer adsorption chromatography of free and conjugated bile acids on silicic acid. *J. Lipid Res.* **3**: 127.
15. Abell, L. L., B. B. Levy, B. B. Brodie, and F. E. Kendall. 1952. A simplified method for the estimation of total cholesterol in serum and demonstration of its specificity. *J. Biol. Chem.* **195**: 357.
16. Bartlett, G. R. 1959. Phosphorus assay in column chromatography. *J. Biol. Chem.* **234**: 466.
17. Talalay, P. 1960. Enzymic analysis of steroid hormones. *Methods Biochem. Anal.* **8**: 119.
18. Admirand, W. H., and D. M. Small, 1968. The physicochemical basis of cholesterol gallstone formation in man. *J. Clin. Invest.* **47**: 1043.
19. Wheeler, H. O., and O. L. Ramos. 1960. Determinants of the flow and composition of bile in the unanesthetized dog during constant infusion of sodium taurocholate. *J. Clin. Invest.* **39**: 161.
20. Wheeler, H. O., and P. L. Mancusi-Ungaro. 1966. Role of bile ducts during secretin choleresis in dogs. *Amer. J. Physiol.* **210**: 1153.
21. Ó'Máille, E. R. L., T. G. Richards, and A. H. Short. 1966. Factors determining the maximal rate of organic anion secretion by the liver and further evidence on the hepatic site of action of the hormone secretin. *J. Physiol. (London).* **186**: 424.
22. Forker, E. L. 1967. Two sites of bile formation as determined by mannitol and erythritol clearance in the guinea pig. *J. Clin. Invest.* **46**: 1189.
23. Soloway. R. D., A. F. Hofmann, P. J. Thomas, and L. J. Schoenfield. 1971. Dehydrocholic acid: hepatic metabolism and effect of biliary secretion in man. *Gastroenterology.* **60**: 754.
24. Gunter, M. J., K. S. Kim, D. F. Magee, H. Ralston, and A. C. Ivy. 1950. The choleretic potencies of some synthetic compounds. *J. Pharmacol. Exp. Ther.* **99**: 465.
25. Forker, E. L. 1969. The effect of estrogen on bile formation in the rat. *J. Clin. Invest.* **48**: 654.
26. Boyer, J. L., and G. Klatskin. 1970. Canalicular bile flow and bile secretory pressure. Evidence for a non-bile salt dependent fraction in the isolated perfused rat liver. *Gastroenterology.* **59**: 853.
27. Erlinger, S., D. Dhumeaux, P. Berthelot, and M. Dumont. 1970. Effect of inhibitors of sodium transport on bile formation in the rabbit. *Amer. J. Physiol.* **219**: 416.
28. Small, D. M. 1970. The formation of gallstones. *Advan. Intern. Med.* **16**: 243.
29. Mysels, K. J. 1969. Contribution of micelles to the transport of a water-insoluble substance through a membrane. *Advan. Chem. Ser. No. 86.* 24.
30. Ó'Máille, E. R. L., T. G. Richards, and A. H. Short. 1965. Acute taurine depletion and maximal rates of hepatic conjugation and secretion of cholic acid in the dog. *J. Physiol (London).* **180**: 67.
31. Tamesue, N., and K. Juniper, Jr. 1967. Concentrations of bile salts at the critical micellar concentration of human gallbladder bile. *Gastroenterology.* **52**: 473.
32. Nilsson, S., and T. Scherstén. 1970. Influence of bile acids on the synthesis of biliary phospholipids in man. *Eur. J. Clin. Invest.* **1**: 109.
33. Balint, J. A., D. A. Beeler, E. C. Kyriakides, and D. H. Treble. 1971. The effect of bile salts upon lecithin synthesis. *J. Lab. Clin. Med.* **77**: 122.