Selective composition of biliary phosphatidylcholines is affected by secretion rate but not by bile acid hydrophobicity

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Abstract Little is known about the mechanisms of: *1)* biliary phosphatidylcholine (PC) secretion by the hepatocyte, *2)* selectivity for biliary 1-palmitoyl-2-linoleoyl-PC (PLPC) secretion, and *3)* exclusion of 1-stearoyl-2-arachidonyl-PC (SAPC) from bile. The experiments were designed to determine, in rats, whether selectivity (for PLPC and against SAPC) is influenced by bile acid hydrophobicity or secretion rate. We examined the effects of bile acid depletion and of ileal infusion of taurocholic acid, tauroursodeoxycholic acid, and taurochenodeoxycholic acid. Compared to bile acid depletion, infusion of each bile acid caused PLPC to decrease from 59% of bile PC to 48%, and SAPC to increase from 2.6% to 5%. Bile acid hydrophobicity had no effect on PC selectivity, but selectivity decreased to a moderate degree as total PC secretion increased. To determine whether selectivity is for preformed molecular species, we used a new method to isotopically label four species of hepatic PC. This was done by intravenous injection of PLPC and SAPC labeled in the linoleate (^{14}C) and arachidonate (^{3}H) moieties. Assuming rapid mixing of each PC species in the hepatocyte as supported by the specific activity data, bile SAPC and SLPC were derived entirely from hepatic preformed SAPC and SLPC; bile PLPC was from both preformed PLPC (55%) and an unlabeled input (45%, probably direct secretion of newly synthesized PLPC). In conclusion, the selective composition of bile PC is not related to bile acid hydrophobicity, but is partially lost as secretion increases within the physiologic range.—**Shamburek, R. D., and C. C. Schwartz.** Selective composition of biliary phosphatidylcholines is affected by secretion rate but not by bile acid hydrophobicity. *J. Lipid Res.* 1993. **34:** 1833–1842.

Supplementary key words bile lipids • cholic acid • chenodeoxycholic acid • ursodeoxycholic acid

Phospholipid is secreted by hepatocytes into the bile canalicular lumen where it is a major component of both vesicles and mixed micelles and serves a crucial function in cholesterol secretion (1). About 95% of biliary phospholipid is phosphatidylcholine (PC). There are numerous molecular species of PC present in rat and human bile. Most species, including 1-stearoyl-2-arachidonyl-PC (SAPC), comprise a small fraction (< 3%) of total bile PC whereas 1-palmitoyl-2-linoleoyl-PC (PLPC) comprises about 55% (2, 3). In contrast, only 50% of hepatic phospholipid is PC and the major species of PC in rat liver is SAPC (25–30%); PLPC comprises only 20% (2). Thus, the rat and human hepatocyte selectively recruits PLPC for secretion in bile and effectively excludes SAPC and other phospholipids from bile.

Relatively little is known about the intrahepatic mechanisms involved in biliary PC secretion. A fundamental observation has been that bile PC secretion increases as micelle-forming bile acid secretion increases (4). Recent studies have shown that PC initially appears in the canalicular lumen as a unilamellar vesicle (5). An intracellular vesicular transport mechanism has been proposed, but colchicine inhibits biliary PC secretion only at high bile acid secretion rates (6, 7). Vesiculation of PC from the canalicular membrane, stimulated by detergent action of bile acids, is another possible but unproved mechanism for PC secretion (8). The composition of PC in canalicular membranes is similar to that in other hepatocyte membranes (9) but microenvironments of PLPC could exist. Regarding the selectivity for PLPC and against SAPC, PCs containing palmitate at the *sn*-1 position are the major species synthesized de novo via the CDP-choline pathway (10) and secreted in bile (2, 3), and bile acids rapidly stimulate PC synthesis (11, 12). Thus, it is tempting to speculate that bile PLPC is derived from a small, newly synthesized source in the hepatocyte and there is at least one investigation that lends support to this specula-

Abbreviations: HDL, high density lipoproteins; rHDL, reconstituted rat HDL; C-M, chloroform-methanol; TLC, thin-layer chromatography; PC, phosphatidylcholine; PLPC, 1-palmitoyl-2-linoleoyl-PC; SLPC, 1-stearoyl-2-linoleoyl-PC; SAPC, 1-stearoyl-2-arachidonyl-PC; PAPC, 1-palmitoyl-2-arachidonyl-PC; POPC, 1-palmitoyl-2-oleoyl-PC; SOPC, 1-stearoyl-2-oleoyl-PC; LLPC, dilinoleoyl-PC; PDPC, 1-palmitoyl-2-docosahexaenoyl-PC; SDPC, 1-stearoyl-2-docosahexaenoyl-PC; TC, taurocholic acid; TUDC, tauroursodeoxycholic acid; TCDC, taurochenodeoxycholic acid.

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tion (13). However, the results of several others have shown that bile PCs are derived from preformed PCs (14, 15). It has been proposed that the selective composition of bile PC reflects, in part, the hydrophobic-hydrophilic balance of bile acid constituents; hydrophobic bile acids stimulate secretion of arachidonic- and stearic-rich PCs, implying a physical-chemical mechanism for selectivity (16–18).

The experiments described herein were designed to determine whether selectivity for biliary PLPC and against SAPC could be acutely altered by infusion of bile acids ranging widely in hydrophobicity or by rapid depletion of bile acids. We purposely avoided chronic bile acid administration because of its wide range of possible effects on PC and fatty acid metabolism, many of which could be indirect. To determine whether selectivity is altered for preformed molecular species, we used a novel method to label plasma and hepatic preformed PCs (19). The results showed that, with and without bile acids, biliary PLPC was largely derived from hepatic preformed PLPC but partially from newly synthesized (unlabeled) PLPC as well. Selectivity was greatest at low secretion rates that occurred with bile acid depletion; when biliary PC secretion was in the physiologic range, selectivity was partially lost. Of most importance, bile acid hydrophobicity had no acute effect on selectivity for PLPC or against SAPC.

EXPERIMENTAL PROCEDURES

Sprague-Dawley male rats (Harlan Sprague-Dawley, Inc.), 280–325 g, were used in all experiments except for validation studies of bile acid depletion and intestinal infusion experiments in which 270–340 g rats were used. Rats were maintained at 22°C with 12-h light cycles (dark 6:00 PM to 6:00 AM) for at least 1 week prior to the experiments; they were fed Purina Rodent Lab chow ad libitum.

Surgical procedures

Unless otherwise noted, rats were fasted (except for H_2O) for 10 h prior to surgery. Surgery was started under ether anesthesia at approximately 8:00 AM. A PE-50 cannula (Intramedic, Clay Adams) was placed in the jugular vein and intravenous pentobarbital was titrated to maintain anesthesia for the remainder of the experiment. The standard solution of 5% dextrose, 0.45% sodium chloride, and 0.0084% sodium bicarbonate was infused by vein at 0.5 ml/h. The bile duct was cannulated with 0.012 × 0.025-inch silicone (Baxter Scientific Products). PE-50 tubing was inserted through a puncture site in the ileum 20 cm proximal to the cecum and threaded distally for 5 cm. The tubing was sutured to the intestinal wall at the puncture site. Standard solution was infused at 1.0 ml/h into the ileum 15 cm proximal to the cecum. The rat was observed for 1 h to assure patency of the bile cannula, and then a bile acid (or no bile acid) was added to the standard solution infusing the ileum. Either taurocholic acid (Sigma Chemical Co.), 30 μmol/100 g · h; tauroursodeoxycholic acid (Sigma), 30 μmol/100 g · h; taurochenodeoxycholic acid (Sigma), 12 μmol/100 g · h; or standard solution with no bile acid was infused. These rates of bile acid infusion did not cause elevation of serum transaminases (20, 21).

Validation studies: intestinal infusion of bile acids and depletion of endogenous bile acids

Preliminary studies using a constant duodenal or ileal infusion of [24-^{14}C]taurocholate (DuPont, New England Nuclear) were done to determine the time necessary to reach maximal and constant biliary secretion of ^{14}C-activity. In three rats, PE-50 tubing was threaded 4 cm into the duodenum via a gastric puncture. In three rats, the tubing was placed in the ileum. One hour after placement of the intestinal tubing and bile cannula, an infusion was started of standard solution containing ^{14}C-taurocholic acid (0.014 μCi/h and 30 μmol/100 g · h) at a rate of 1.0 ml/h. All bile was collected every 30 min for 7–8 h and ^{14}C-activity was determined.

Studies were carried out to determine the length of time required to acutely deplete the bile acid pool (to minimize the amount of endogenous bile acids during the final experiments). Fourteen rats were given 0.132μCi of [24-^{14}C]TC by gavage. After 24 h (n = 2), 48 h (n = 7), or 72 h (n = 1), a bile duct cannula was placed after a 10-h fast; or, after 48 h (n = 4) a cannula was placed without prior fasting. Bile was collected at 30 min increments for 8 h and the ^{14}C-activity was determined in each collection.

Preparation of rHDL containing labeled PLPC and SAPC

HDL ($1.050 \leq d \leq 1.210$ g/ml) was isolated from rat plasma in a Beckman Model L5-50 ultracentrifuge using a type 40 rotor at 4°C and 40,000 rpm for 24 h at each density. The HDL was dialyzed against 0.9% NaCl and 0.01% EDTA, pH 7.4. About 1 ml of HDL was extracted with 20 ml 95% ethanol–ethyl ether 3:2 (v/v) (22). 1-Palmitoyl-2-[^{14}C]linoleoyl-PC ([1-^{14}C]linoleoyl-PC) and, in most experiments, 1-stearoyl-2-[^{3}H]arachidonyl-PC ([5,6,8,9,11,12,14,15-^{3}H(N)]arachidonyl) (both from DuPont-New England Nuclear Research Products) in 0.25 ml ethanol were added to the extracted HDL. The solvent was evaporated under N_2 at room temperature and 5 ml 0.01% EDTA, pH 7.4, was added. After gentle vortexing at 40°C under N_2, the lipid mixture was sonicated 6 times in 0.5-min bursts. rHDL was then isolated by ultracentrifugation at $1.050 \leq d \leq 1.210$ g/ml and dialyzed as above. The rHDL was filtered (0.2 μm; Gelman Sciences) before analysis and administration.

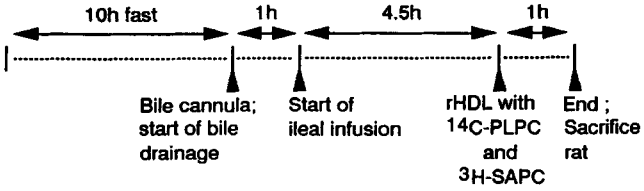

Fig. 1. Final experimental protocol.

About 50% of each isotope added to extracted HDL was present in the rHDL recovered after filtration. On agarose gel electrophoresis (23), all ^{3}H- and ^{14}C-radioactivity migrated with rHDL and parallel to native rat HDL. Of the total radioactivity in rHDL, greater than 95% of ^{3}H-activity and 97% of ^{14}C-activity were found in SAPC and PLPC, respectively, by HPLC.

Experimental design (Fig. 1)

Based on results of the validation studies, rats were fasted for 10 h before surgery for the final experiments which were carried out for 1 h starting 5.5 h after the start of bile drainage. Ileal infusion of individual bile acid (or no bile acid) was started 1 h after the start of bile drainage; isotopic PC in rHDL was given 5.5 h after the start of drainage; the rat was killed 6.5 h after the start of drainage (Fig. 1). An exception was made in three rats infused with TC, administered only [^{14}C]PLPC in rHDL and killed 2 h later, 7.5 h after the start of drainage. rHDL containing 0.16 μCi [^{14}C]PLPC and, in most rats, 0.46 μCi [^{3}H]SAPC was administered in 0.2 ml of 0.9% NaCl over 4 min via the jugular vein catheter which was then rinsed 3 times with 0.1 ml of 0.9% NaCl over 1 min. All bile was collected from the cannula by gravity drainage in tared tubes on ice at 30-min increments. The volume (1 g assumed to equal 1 ml) of each 30-min collection was determined immediately, then the bile was extracted in 20 volumes of C-M 2:1. At the time of killing, the liver was quickly removed, rinsed of blood with chilled 0.9% NaCl via the portal vein, weighed, cut into 3-4-mm cubes, rinsed again, and extracted with C-M 2:1.

Analytic procedures

After extraction of samples in 20 volumes C-M 2:1 (v/v), the methanol and chloroform phases were separated by addition of water (24). The mass of individual bile acids in an aliquot of the methanol phase of bile was determined by HPLC (20, 21). Internal standard (progesterone, 0.1 μmol; Sigma) was added, and the solvent was removed under N_2. The sterols were resuspended in mobile phase (pH 4.2) and injected onto an Ultrasphere ODS 5 μ column (4.6 × 250 mm) with a flow of 1 ml/min; detection was at 195 nm. Neutral lipids were separated from phospholipids by silicic acid column chromatography of the chloroform phase (25).

Individual classes of phospholipids were isolated by TLC on 150A silica-coated plates (Whatman, LK5 series) using $CHCl_3$-MeOH-H_2O-NH_4OH (58%) 60:30:3:1 (v/v/v/v). Plates were sprayed with 0.05% 2,7-dichlorofluorescin in isopropyl alcohol. Phospholipids were identified based on standards applied to adjacent lanes and, except for PC, scraped into scintillation vials for determination of radioactivity. PC was eluted from the silica using $CHCl_3$-MeOH-H_2O-acetic acid 50:39:10:1 (v/v/v/v) and the dichlorofluorescin was removed from the eluate with 4 N NH_4OH. Total phospholipid and PC mass were determined using KH_2PO_4 and egg PC as standards (26). Corrections for 15% loss during isolation of PC were made as described previously (19).

The PC, to which dilauroyl-PC was added as interval standard, was derivatized and individual molecular species were isolated by reverse phase HPLC using a semi-preparative 10 × 250 mm Ultrasphere ODS 5 μ column (Beckman Instruments) at 48°C modified from the descriptions of Patton and Robins (27) and Kito et al. (28). Briefly, PC was hydrolyzed with phospholipase C; the diacylglycerols were reacted with 3,5-dinitrobenzoyl chloride at 25°C for 16 h to form dinitrobenzoyl diacylglycerol derivatives. The solvent was removed and the derivatives, in 0.2 ml CH_3CN, were injected on the HPLC system using a 0.5-ml loop rinsed twice with 0.1 ml CH_3CN. The mobile phase was MeOH-CH_3CN-isopropanol-water 47.5:41:10:1.5 (v/v/v/v) pumped at 2.0 ml/min; detection was at 254 nm. Collections from HPLC (each peak and trough between peaks) were dried and radioactivity was measured by scintillation counting. Identities of peaks were verified based on the relative retention time of standards (PLPC, PAPC, POPC, SAPC, SLPC, SOPC, LLPC, OLPC, PDPC, and SDPC; Avanti Polar Lipids, Inc. Birmingham, AL). The mass of

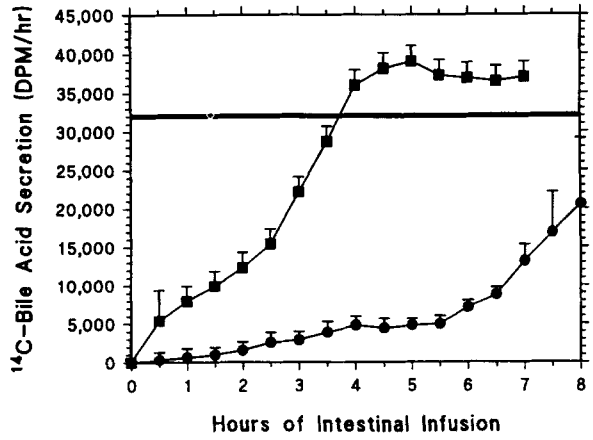

Fig. 2. Validation studies: biliary secretion of ^{14}C-activity during intestinal infusion of [^{14}C]taurocholate. Rats were fasted for 10 h before placement of a bile cannula. After 1 h of bile drainage, ileal (■; n = 3) or duodenal (●; n = 3) infusion of [^{14}C]TC, 0.014 μCi/h (32,000 dpm/h; 30 μmol/100 g · h) was started (time zero); the thick horizontal line shows the rate and duration of [^{14}C]TC infusion. Bile was collected for 30-min periods and ^{14}C-activity was measured; bars indicate 1 SE.

each species was determined by the internal standard method (HP 3396 Series II Integrator).

^{14}C- and ^{3}H-radioactivities were quantitated in a Model 1217 Rackbeta Liquid Scintillation Counter (LKB Wallac). When silica from TLC was transferred to scintillation vials directly, 1 ml MeOH was added, then 10 ml of scintillant. The scintillant for radioisotope counting ^{14}C-labeled bile acid was EcoLite (ICN Biomedicals) and for neutral lipids and phospholipids toluene–Liquifluor–MeOH 96:4:3 (v/v/v) was used. Liquifluor was from DuPont-New England Nuclear.

Values for each group represent the mean ± SE. Statistical comparison between groups was done by Student's two-tailed *t*-test. Hydrophobicity index for bile acids was calculated as described by Heuman (29).

RESULTS

Validation studies: intestinal infusion of bile acids and depletion of endogenous bile acids

As shown in **Fig. 2,** infusion of [^{14}C]taurocholic acid (30 μmol/100 g · h) into the ileum resulted in maximal and constant biliary secretion of ^{14}C after 4 h. Bile flow also reached a maximum at 4 h and then remained constant. By the eighth hour of duodenal infusion, biliary secretion of ^{14}C remained below the expected plateau.

To determine the time required to acutely deplete endogenous bile acids, the pool was prelabeled with [^{14}C]TC by gavage 24 h, 48 h, or 72 h prior to bile cannula insertion and the start of drainage. Of the ^{14}C-labeled bile acids present at the start of drainage in rats fasted for 10 h, 82% ± 4% (n = 10) had been secreted by 5.5 h. Prelabeling by 24 h (75% ± 4%; n = 2), 48 h (84% ± 5%; n = 7), or 72 h (80%; n = 1) did not effect the degree of acute depletion. This calculation was based on the assumption that 20% of the bile acid pool was lost each 24 h prior to the start of drainage (for example, 48 h after prelabeling with [^{14}C]TC, 64% of the ^{14}C would remain in the rat and this value was normalized to 100% for calculation of percent depletion). In four rats prelabeled by 48 h but not fasted prior to drainage, only 63% ± 6% was secreted by 5.5 h.

Effect of bile acid depletion and infusion on bile flow, bile acids, and biliary PC

Bile collected during 0.5–1.0 h of drainage (before bile acid infusion) represents the physiologic rates associated with endogenous bile acid secretion. Compared to the endogenous rates (**Table 1**), ileal infusion of each bile acid (TC, TUDC, and TCDC) caused bile flow and PC secretion to increase slightly, but with no change in total bile acid secretion. The plateau for bile flow was reached after 4 h of infusion of each bile acid and there was no subse-

TABLE 1. Biliary secretion and PC composition in rats with bile drainage

Variable		Endogenous (n = 12)	Endogenous Depletion: No Bile Acid (n = 6)	TC (n = 4)	TUDC (n = 3)	TCDC (n = 3)
Time of depletion (h)		0.5–1.0	5.5–6.5	5.5–6.5	5.5–6.5	5.5–6.5
Bile flow (ml/h)		0.85 ± 0.03	0.88 ± 0.03	1.26 ± 0.06[a,b]	1.32 ± 0.07[a,b]	1.02 ± 0.02[a,b,c,d]
Total bile acid (μmol/h)		49.01 ± 3.28[a]	7.65 ± 0.73	53.22 ± 3.78[a]	55.97 ± 1.44[a]	41.52 ± 3.45[a]
Total bile PC (μmol/h)		5.20 ± 0.40[a]	1.92 ± 0.19	6.48 ± 0.79[a]	6.95 ± 0.64[a,b]	6.94 ± 0.94[a]
Molecular species of PC[e]	Liver[f] (n = 6)	Bile				
	%			%		
LLPC	1.9 ± 0.2	2.0 ± 0.1	1.3 ± 0.3	1.4 ± 0.5	1.0 ± 0.1[b]	1.3 ± 0.3
PDPC	6.9 ± 0.4	4.3 ± 0.4[a]	3.1 ± 0.3	4.5 ± 0.9	3.9 ± 0.2	4.2 ± 0.7
PAPC	23.0 ± 0.5	20.7 ± 0.9	18.9 ± 0.6	25.8 ± 1.1[a,b]	25.7 ± 0.2[a,b]	22.2 ± 0.4[a,c,d]
PLPC	20.5 ± 0.4	52.0 ± 0.7[a]	58.8 ± 1.2	48.0 ± 1.2[a,b]	47.0 ± 0.8[a,b]	50.1 ± 0.6[a,b]
SAPC	27.4 ± 0.7	4.0 ± 0.2[a]	2.6 ± 0.2	4.6 ± 0.5[a]	5.9 ± 0.8[a,b]	4.5 ± 0.3[a]
POPC	5.7 ± 0.1	9.6 ± 0.4	9.8 ± 0.8	8.9 ± 0.6	7.5 ± 1.0	9.1 ± 0.4
SLPC	11.7 ± 0.4	6.1 ± 0.3[a]	4.5 ± 0.3	5.7 ± 0.7	7.9 ± 0.9[a]	7.4 ± 0.4[a]
SDPC	3.0 ± 0.2	1.3 ± 0.4	1.0 ± 0.3	1.1 ± 0.4	1.1 ± 0.4	1.2 ± 0.3

In all rats, bile was externally drained and collected for 6.5 h via a common duct cannula starting upon insertion of cannula. Results are from bile collected during the interval encompassed by Time of depletion. The No Bile Acid group had only standard solution infused into the ileum, whereas the bile acid shown was added to the solution in the other three groups. In the Endogenous group, results are from bile collected for 30 min starting after 30 min of depletion, ending at the start of ileal infusion. Results are mean ± SE; (n), number of rats.

[a] $P < 0.05$ versus No Bile Acid.
[b] $P < 0.05$ versus Endogenous.
[c] $P < 0.05$ versus TC.
[d] $P < 0.05$ versus TUDC.
[e] Composition; minor species of PC not included (<5% of total) so that the eight species shown comprise 100%.
[f] Livers from the No Bile Acid group (all others were virtually identical).

quent decline. In all bile samples, PC comprised >94% of total phospholipid. Without infusion of bile acids, PC secretion decreased from the endogenous rate of 5.2 μmol/h to 1.9 μmol/h between 5.5 and 6.5 h of depletion (drainage) and total bile acid secretion fell 84% to 7.6 μmol/h from its endogenous rate of 49.0 μmol/h. The latter fall was similar to the 82% depletion found in our validation studies by a different approach.

Fig. 3 shows the secretion rates of individual bile acids in each experimental group. The major bile acids in endogenous and no bile acid groups were cholic and muricholic acids as expected. The infused bile acid became the predominant species by 5.5–6.5 h of drainage in the other three groups (95% ± 0.1 TC with TC infusion, 84% ± 1.0 TUDC with TUDC infusion, and 70% ± 1.7 TCDC with TCDC infusion). As shown in Fig. 3, the total bile acid hydrophobicity index varied over a wide range among the groups.

The percent composition of bile PC molecular species in each group is shown in Table 1. Shown for comparison is the composition of liver PC. Numerous minor species (each <1.5%) comprised about 5% of total PC and are excluded from Table 1. Bile PC in all groups revealed marked selectivity for PLPC and against SAPC. Modest degrees of selectivity for POPC and against SLPC, PDPC, and SDPC were also observed.

Two important results shown in Table 1 were the nearly identical degree of selectivity for biliary PC species between the endogenous, TC, TUDC, and TCDC groups of

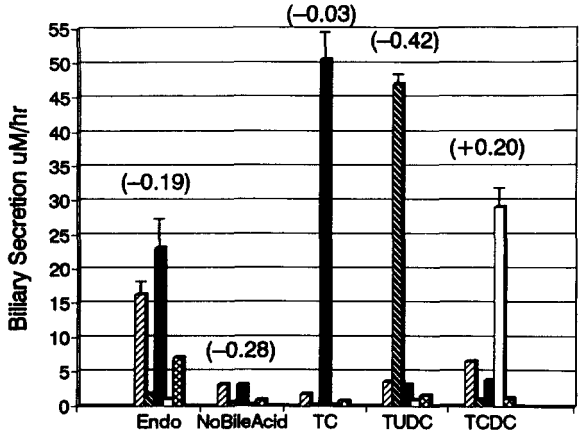

Fig. 3. Biliary secretion rate of individual bile acids in each group of rats. Total bile acid hydrophobicity index for each group is above the bars. Secretion rates in the endogenous (Endo) group were determined from bile collected 0.5–1.0 h after the start of bile drainage; rates were determined 5.5–6.5 h after the start of drainage (depletion) in the other four groups, infused via the ileum with either no bile acid, TC, TUDC, or TCDC starting after 1 h of drainage; (▨) α + β muricholate; (▩) ursodeoxycholate; (■) cholate; (□) chenodeoxycholate; (▧) deoxycholate. All conjugates of each bile acid are combined; taurine conjugates comprised >95% of each bile acid except for the bile acid corresponding to that infused, in which taurine comprised >99%. Bile acids other than the five shown comprised <1% of the total. One SE is shown at the top of bars for selected bile acids. The SEs for all other bile acids were <1.0 μmol/h.

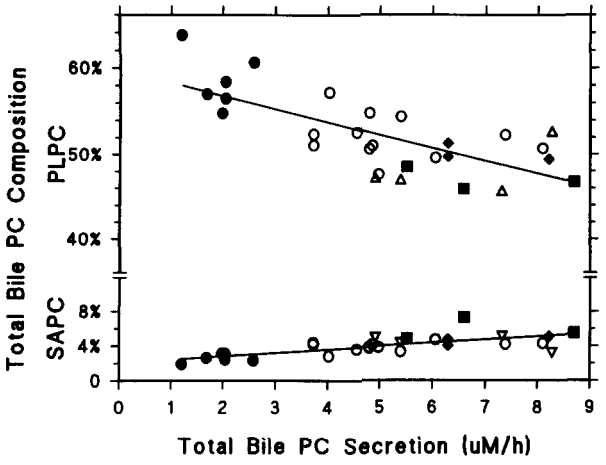

Fig. 4. Relation between total bile PC secretion rate and the % of bile PC comprised by PLPC and SAPC. Individual data for all rats in each group (see Table 1) are shown; (●) no bile acid; (○) endogenous; (△) TC infusion; (♦) TCDC infusion; (■) TUDC infusion. Note change in the y-axis between % SAPC and % PLPC. For PLPC, $y = -1.5(x)+59.7$; $r = -0.72$ ($P < 0.001$), $T = -5.3$ and $n = 28$. For SAPC, $y = 0.4(x)+2.1$; $r = 0.69$ ($P < 0.001$), $T = 4.90$ and $n = 28$.

rats, and the even greater degree of selectivity in the no bile acid group compared to all other groups; i.e., there was partial loss of selectivity for PLPC and against SAPC at the higher PC secretion rates induced by endogenous and exogenous bile acids. Regarding the nearly identical degree of selectivity between the endogenous and bile acid groups, an exception was slightly more (2–5%) PLPC in the endogenous compared to TC, TUDC, and TCDC groups, but there were no differences in PLPC among the latter three groups. There were also several statistically significant differences between the percent PAPC in the endogenous and bile acid groups; however, the magnitudes of the differences were small and they all varied by a small degree (≤3%) around the hepatic PAPC value of 23%. There was no relation between total bile acid hydrophobicity index and the percent that any species comprised of bile PC among all five groups.

Regarding the partial loss of selectivity at the higher PC secretion rates induced by bile acids, PLPC (58.8%) was significantly higher and SAPC (2.6%) significantly lower when the no bile acid group was compared to each of the other four groups (Table 1). SLPC was also more efficiently excluded from bile PC in the no bile acid group (4.5%) compared to endogenous, TUDC, and TCDC groups. The influence of total PC secretion on selectivity for PLPC and against SAPC is shown in **Fig. 4;** as total secretion increased, the percent of bile PC comprised by PLPC significantly decreased and the percent comprised by SAPC increased. To further examine the partial loss of selectivity, **Table 2** shows the absolute (total) secretion rates of five major PC species during 5.5–6.5 h of endogenous depletion with and without bile acid infusion. SAPC secretion during infusion of each bile acid increased

TABLE 2. Total (upper) and bile acid-induced (lower) secretion rates of five major species of biliary PC

Molecular Species of Bile PC	Total Secretion: No Bile Acid (n = 6)	TC (n = 4)		TUDC (n = 3)		TCDC (n = 3)	
	μmol/h	μmol/h	[Ratio][a]	μmol/h	[Ratio][a]	μmol/h	[Ratio][a]
PAPC	0.34 ± 0.04	1.58 ± 0.24[b]	[4.6]	1.68 ± 0.19[b]	[4.9]	1.46 ± 0.20[b]	[4.3]
SAPC	0.05 ± 0.01	0.29 ± 0.03[b]	[6.0]	0.40 ± 0.06[b]	[8.2]	0.31 ± 0.05[b]	[6.4]
PLPC	1.07 ± 0.10	2.96 ± 0.42[b]	[2.8]	3.10 ± 0.26[b]	[2.9]	3.30 ± 0.42[b]	[3.1]
SLPC	0.08 ± 0.01	0.34 ± 0.04[b]	[4.3]	0.51 ± 0.07[b]	[6.4]	0.48 ± 0.08[b]	[6.0]
POPC	0.18 ± 0.02	0.55 ± 0.05[b]	[3.1]	0.49 ± 0.02[b]	[2.7]	0.60 ± 0.13[b]	[3.3]
		Bile Acid-Induced Secretion					
		μmol/h[c]	Comp.[d] %	μmol/h[c]	Comp.[d] %	μmol/h[c]	Comp.[d] %
PAPC		1.24	28.6	1.34	28.0	1.12	23.5
SAPC		0.24	5.5	0.35	7.3	0.26	5.5
PLPC		1.89	43.5	2.03	42.5	2.23	46.8
SLPC		0.26	6.0	0.43	9.0	0.40	8.4
POPC		0.37	8.5	0.31	6.5	0.42	8.8

Results are from bile collected between 5.5 and 6.5 h of endogenous bile acid depletion. Corresponding values for the bile PC secretion rate and composition are shown in Table 1. Values are given as mean ± SE.

[a][Ratio] was calculated from the total secretion rate during infusion of bile acid divided by the rate during infusion of no bile acid.

[b]Difference between bile acid and no bile acid was significant at $P < 0.025$.

[c]Total secretion rate during infusion of bile acid minus the rate during infusion of no bile acid.

[d]Composition of the biliary PC induced by bile acids (LLPC, SDPC, and PDPC were included so that the eight species comprise 100%).

6- to 8-fold relative to SAPC secretion during infusion of no bile acid. PAPC and SLPC secretion increased 4- to 6-fold, and PLPC and POPC increased the least, about 3-fold. The lower part of Table 2 shows the secretion induced by TC, TUDC, and TCDC. The composition of induced molecular species reveals a further decrease in the percent comprised by PLPC and increase in SAPC when compared to Table 1.

Mass and radioactivity in molecular species of hepatic PC

Liver total PC content was 17.4 ± 1.1 μmol/g wet weight (n = 6) in rats infused with no bile acid, virtually identical to the TC, TUDC, and TCDC groups. Liver PC composition in the no bile acid group is shown in Table 1 and was identical for all three bile acid groups. One hour after intravenous administration of rHDL containing [^{14}C]linoleate-PLPC and [^{3}H]arachidonate-SAPC, about one-third of each dose was found in the liver of all rats; 88–93% of ^{3}H and ^{14}C in the liver were found in PC. Only 1%–1.5% of ^{3}H- and ^{14}C-doses were in hepatic neutral lipid suggesting very little free [^{14}C]linoleate or free [^{3}H]arachidonate was formed during the hour. The specific activities (dpm/μmol) of molecular species are shown in **Table 3.** The ^{3}H-specific activity of hepatic PAPC in rats infused with no bile acid was not significantly different than hepatic PAPC in rats infused with exogenous bile acid. The same was true for hepatic [^{3}H]SAPC, [^{14}C]PLPC, and [^{14}C]SLPC. In TC-infused rats administered [^{14}C]linoleate-PLPC, liver [^{14}C]PLPC specific activities at 60 and 120 min were similar; liver [^{14}C]SLPC specific activities were also similar.

Radioactivity in molecular species of biliary PC

During the hour after intravenous administration of rHDL containing [^{3}H]SAPC and [^{14}C]PLPC, only 0.1%–0.6% of the ^{3}H- and ^{14}C-doses were secreted in bile (**Table 4**); over 95% of the ^{3}H-activity in bile was in PAPC and SAPC; over 92% of the ^{14}C-activity was in PLPC and SLPC. Thus, as only [^{3}H]SAPC and [^{14}C]PLPC were administered and nearly all the hepatic and biliary ^{3}H- and ^{14}C-activities were in specific PCs ([^{3}H]PAPC, [^{3}H]SAPC; [^{14}C]SLPC, [^{14}C]PLPC) at 1 h, biliary secretion of [^{3}H]- and [^{14}C]PCs represent secretion of hepatic preformed PCs. Compared to the secretion of ^{3}H-activity in PAPC of rats infused with no bile acid, secretion of [^{3}H]PAPC (preformed PAPC) increased 7.3- and 6.2-fold during TUDC and TCDC infusion, respectively (Table 4). This increase was about 45% greater than that seen for PAPC mass (4.9- and 4.3-fold, Table 2). Secretion of [^{3}H]SAPC and of [^{14}C]SLPC increased 6- to 7-fold, similar to the increase in secretion of their masses during TUDC and TCDC infusion. During infusion of all three bile acids, secretion of [^{14}C]PLPC (preformed PLPC) increased slightly less (10–32%) than the increase in PLPC mass.

The specific activities (dpm/μmol) of bile PC molecular species are shown in Table 3. Bile [^{3}H]PAPC specific ac-

TABLE 3. Specific activities (dpm/μmol) of PAPC, SAPC, PLPC, and SLPC in liver and bile after administration of rHDL containing [^{3}H]SAPC (0.46 μCi) and [^{14}C]PLPC (0.16 μCi)

Molecular Species	No Bile Acid (n = 6) 60 Min[a]	TC (n = 4) 60 Min[a]	TC (n = 3) 120 Min[a]	TUDC (n = 3) 60 Min[a]	TCDC (n = 3) 60 Min[a]
			^{3}H-dpm/μmol[b]		
PAPC					
Liver	1491 ± 259	NA	NA	1835 ± 107	1814 ± 234
Bile	708 ± 125[c]			1413 ± 76[c,d]	1482 ± 230[d]
100 × bile ÷ liver	47%			77%	82%
SAPC					
Liver	2244 ± 413	NA	NA	2495 ± 203	2515 ± 309
Bile	2421 ± 575			2244 ± 323	2613 ± 512
100 × bile ÷ liver	108%			90%	104%
			^{14}C-dpm/μmol[e]		
PLPC					
Liver	1091 ± 92	1193 ± 94	984 ± 69	1321 ± 70	1041 ± 161
Bile	698 ± 68[c]	555 ± 68[c]	551 ± 49[c]	645 ± 41[c]	730 ± 66
100 × bile ÷ liver	64%	47%	56%	49%	70%
SLPC					
Liver	1515 ± 151	1543 ± 145	1335 ± 127	1664 ± 121	1258 ± 186
Bile	1474 ± 132	1109 ± 115	1390 ± 209	1783 ± 394	1396 ± 160
100 × bile ÷ liver	97%	72%	104%	107%	111%

Rats were administered the isotopes 5.5 h after the start of bile drainage and were killed either 60 or 120 min later. The liver was flushed with saline, minced, washed, and extracted. The bile specific activity is from the sample collected for 30 min immediately before killing, the mid-time of which was 15 min less than the time after isotope shown. In the 120-min TC group (three rats), bile PLPC specific activities were 426 ± 65 at 45 min and 560 ± 109 at 75 min. Values are given as mean ± SE; NA, not administered [^{3}H]SAPC.

[a]Time after isotope.

[b]Rats were administered rHDL containing [^{3}H]SAPC. The ^{3}H-specific activities of hepatic and biliary PLPC, SLPC, LLPC, PDPC, and POPC, as well as minor species, were all less than 400 dpm/μmol; most were less than 200 dpm/μmol.

[c]$P < 0.025$ versus corresponding liver molecular species and group.

[d]$P < 0.025$ versus bile PAPC in the No Bile Acid group.

[e]Rats were administered rHDL containing [^{14}C]PLPC. The ^{14}C-specific activities of hepatic and biliary PAPC, SAPC, PDPC, POPC, and minor species except LLPC were less than 200 dpm/μmol. LLPC specific activities were 500–3,000 dpm/μmol.

tivity was higher ($P < 0.025$) in both TUDC and TCDC groups than in the no bile acid group, as anticipated from above; bile [^{14}C]PLPC specific activity was slightly lower (NS) in the TC and TUDC groups than in the no bile acid group. When comparing bile and liver in Table 3, it must be kept in mind that bile values were from the sample collected during the 30 min preceding killing the rats and extraction of the liver; the midpoint of the bile sample was

TABLE 4. Percent of dose secreted in bile PC molecular species during the hour after intravenous administration of rHDL containing [^{3}H]SAPC and [^{14}C]PLPC

Molecular Species of Bile PC	No Bile Acid (n = 6)	TC (n = 4)	TUDC (n = 3)	TCDC (n = 3)
	Percent of ^{3}H-Dose Secreted [Ratio][a]			
PAPC	0.053 ± 0.012	ND	0.385 ± 0.059[b] [7.3]	0.331 ± 0.068[b] [6.2]
SAPC	0.026 ± 0.008	ND	0.178 ± 0.020[b] [6.9]	0.155 ± 0.028[b] [6.0]
	Percent of ^{14}C-Dose Secreted [Ratio][a]			
PLPC	0.159 ± 0.037	0.299 ± 0.019[b] [1.9]	0.342 ± 0.023[b] [2.1]	0.450 ± 0.020[b,c] [2.8]
SLPC	0.020 ± 0.004	0.068 ± 0.009[b] [3.4]	0.139 ± 0.033[b] [6.9]	0.143 ± 0.010[b,c] [7.1]

Results are from bile collected between 5.5 and 6.5 h of endogenous bile acid depletion which corresponds to the hour after administration of rHDL. Less than 0.005% of the ^{3}H-dose was secreted in PLPC and SLPC and less than 0.005% of the ^{14}C-dose was secreted in PAPC and SAPC. Values are given as mean ± SE.

[a][Ratio] was calculated from the percent of ^{3}H-dose (or ^{14}C-dose) secreted in bile during ileal infusion of bile acid divided by the percent of ^{3}H-dose (or ^{14}C-dose) secreted during infusion of no bile acid.

[b]$P < 0.01$ versus No Bile Acid group.

[c]$P < 0.005$ versus TC group.

15 min less than 'time after isotope.' Nevertheless, it is important to note that *1)* in no group did the specific activity of a species of bile PC significantly exceed that of the liver; and *2)* specific activities of [^{14}C]PLPC and [^{14}C]SLPC did not increase between 60 and 120 min. The [^{3}H]SAPC specific activity in bile was essentially identical to that in liver of each group. The same was observed for [^{14}C]SLPC. The [^{14}C]PLPC specific activity in bile was only 47%–70% of that in liver of all groups (significant in all except the TCDC group, $P = 0.12$). The [^{3}H]PAPC specific activity in bile was only 47% of [^{3}H]PAPC in liver with no bile acid infusion, increasing to about 80% with TUDC and TCDC.

DISCUSSION

In these short-term experiments in rats, acute depletion of bile acids resulted in low secretion rates of bile phospholipid that was nearly all (>94%) PC that was comprised of 58.8% PLPC but only 2.6% SAPC. This was in stark contrast to the composition of hepatic phospholipid; only 50% was PC and the PC was comprised of 20.5% PLPC and 27.4% SAPC. There were lower degrees of selectivity for bile POPC and against PDPC, SLPC, and SDPC. Although a high degree of selectivity for PLPC and against SAPC was maintained with normal bile acid and PC secretion rates (endogenous group), it was slightly less pronounced. Selectivity decreased further to about 48% PLPC and 5% SAPC when endogenous bile acids were replaced by either TC, TUDC, or TCDC. The partial loss of selectivity was not due to changes in hepatic PC composition or, as discussed below, to obvious changes in metabolism of hepatic preformed PC. For PLPC and SAPC there was an inverse relation between the degree of selectivity and total PC secretion rate.

The basic mechanism generating the selective composition of bile PC was not identified, but the experiments clearly showed that it is not related to bile acid hydrophobicity. We were able to change the hydrophobicity index over a wide range by depleting endogenous bile acids and infusing either TC, TUDC, or TCDC. If bile acids generated PC selectivity directly, such as by solubilizing certain PCs from the canalicular or other membrane, this physical-chemical effect would have been very apparent. The lack of any acute effect of bile acid hydrophobicity on bile PC composition is in general agreement with Alvaro et al. (3) who found that the composition of endogenous bile acids varied widely among animal species but bile PC composition varied to a limited extent. Thus, hydrophobicity may influence the degree to which bile acids stimulate total PC secretion (30) but does not regulate either the type of PC secreted or appearance of PC in unilamellar vesicles in the canalicular lumen (5). This raises the possibility that a common messenger mediates the stimulation of PC secretion by bile acids.

The experimental model proved effective in rats at achieving, within 5 h, about 82% depletion of endogenous bile acids and enrichment of bile with a single bile acid. With endogenous depletion and ileal infusion of TC, 95% of biliary total bile acids was TC; 84% was TUDC with TUDC infusion. TCDC was infused at a lower rate yet 70% enrichment was achieved. From group to group, total bile acid hydrophobicity index in vivo varied over extremes not reported previously, from +0.20 (TCDC infusion) to −0.42 (TUDC infusion). The essential features of the experimental model were: *1)* 10 h of fasting prior to the start of *2)* complete bile drainage with *3)* ileal infusion of an exogenous bile acid (or no bile acid). Our bile acid infusion rates, delivered into the mid-ileum, induced biliary PC secretion rates within the physiologic range without impairment of bile flow or hemolysis.

The partial loss of selectivity for bile PCs as secretion increased could have occurred acutely due to a change in the preformed or newly synthesized source of a species. We used a new approach to study the former possibility by specifically labeling preformed hepatic PLPC, SLPC, PAPC, and SAPC. rHDL containing [^{3}H]SAPC ([^{3}H]arachidonate) and [^{14}C]PLPC ([^{14}C]linoleate) was administered by vein; the plasma [^{3}H]SAPC and [^{14}C]PLPC are hydrolyzed by phospholipase A_1 and then the labeled 2-acyl-lysoPCs are taken up by the liver (about one-third of plasma PC per h) and reacylated mostly to [^{3}H]PAPC, [^{3}H]SAPC, [^{14}C]SLPC and [^{14}C]PLPC (19). There was no evidence that TC, TUDC, or TCDC infusion or that bile acid depletion affected the hydrolysis or hepatic uptake of PC from plasma. More importantly, 1 h after administration only a trace (<2%) of ^{3}H- and ^{14}C-doses were found in hepatic lipids other than the specific PCs, indicating that insignificant ^{3}H- or ^{14}C-activity entered de novo PC synthesis. The isotopes subsequently appeared in bile only in the specific PCs (SAPC and PAPC for ^{3}H; PLPC and SLPC for ^{14}C) indicating that some preformed species are normally secreted in bile. Compared to bile acid-depleted rats, each bile acid infusion stimulated secretion of preformed (^{3}H) and total SAPC mass to the same degree (about 7-fold); secretion of preformed (^{14}C) and total PLPC mass were both stimulated 2- to 3-fold but total mass increased slightly more. This latter trend suggests that bile acids stimulate secretion of unlabeled (possibly newly synthesized) PLPC slightly more than preformed PLPC.

The relationships between specific activities (dpm/μmol) of bile and liver PCs 1–2 h after intravenous administration of labeled PC, shown in Table 3, add qualitative information and fuel speculation about PC metabolism. In all groups of rats, dpm/μmol of each of the four specifically labeled PCs in bile was about the same as ([^{3}H]SAPC and [^{14}C]SLPC) or lower than ([^{3}H]PAPC

and [^{14}C]PLPC) the corresponding species in the liver. The specific activities in bile and liver changed minimally from 1 h to 2 h as also observed previously (19). The 15-min lag between midtime collection of bile and killing the rats and extraction of liver did not alter these relationships as shown by the 2-h experiments. The specific activities of plasma PCs (19; R. D. Shamburek and C. C. Schwartz, unpublished observations) did not influence the bile-liver relationships; dpm/μmol of [^{3}H]SAPC and [^{14}C]PLPC in plasma were over 20-fold higher than in liver and bile, but dpm/μmol of [^{3}H]PAPC and [^{14}C]SLPC in plasma were lower than in liver and bile. These relationships led to three generalizations. First, plasma PCs are probably not transported directly to bile. Second, within a 1-h time frame there is rapid mixing of each preformed species throughout its anatomic locations in the hepatocyte. Other investigators have shown that flip-flop and mixing of PC is rapid ($t_{1/2}$ < 2 h) between membranes of the hepatocyte (14, 31–33) and that hepatic microsomal PC can exchange completely in 1 h or less (34, 35). As an alternative explanation there could be slow distribution of tracer between multiple hepatic pools of a species such as PLPC; the observed relationships would then be fortuitous. However, kinetic analysis suggests it may not be necessary to invoke a complex system of multiple hepatic pools of preformed PLPC (see below). Third, as [^{14}C]PLPC specific activity in bile was 47%–70% of that in liver of all groups, a component (47%–70%) of bile PLPC was probably derived from the hepatic preformed PLPC pool and the remainder from unlabeled hepatic input (no pool), some of which was secreted directly into bile without mixing in hepatic preformed PLPC. The unlabeled hepatic input could originate from synthesis via the CDP-choline pathway as *sn*-1 palmitate species (especially PLPC) are the major products of this pathway (10, 12). PAPC specific activities in bile and liver followed relationships similar to those of PLPC, also raising the possibility that sorting of PC ultimately destined for bile starts near the site of PC synthesis from CDP-choline in the endoplasmic reticulum. Alternatively, the unlabeled PLPC (or PAPC) in bile could originate from a hepatic preformed pool to which tracer was very slowly distributed from other hepatic PLPC pools, or from direct secretion of PLPC produced by other pathways such as methylation of PLPE, base exchange, or transacylation. However, these pathways do not preferentially make PLPC. Very large components (nearly 100%) of biliary SLPC and SAPC were derived from hepatic preformed SLPC and SAPC in each group. A recently undertaken multicompartmental analysis (R. D. Shamburek and C. C. Schwartz, unpublished observations) of data from TC-infused rats, injected with [^{14}C]PLPC and [^{3}H]glycerol and sampled from 0.5 h to 3 h, has provided support for the three generalizations; one pool of hepatic preformed PLPC, one of SLPC, and direct biliary secretion of a portion of de novo synthesized PLPC were adequate to fit all radioactivity data.

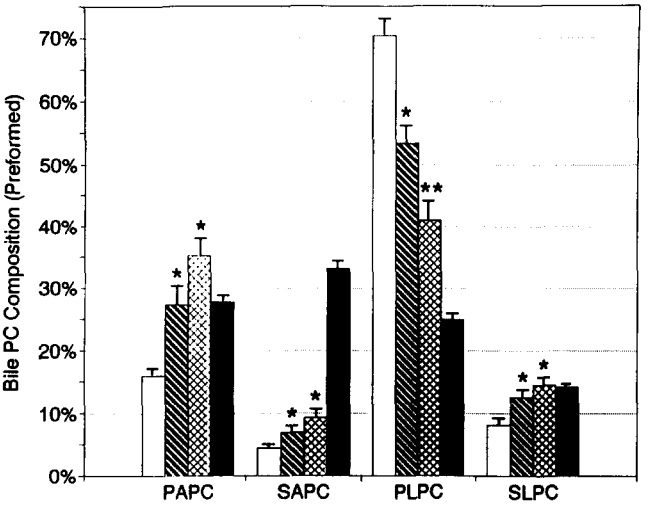

Fig. 5. Mass composition of preformed components (hypothetical) of bile PAPC, SAPC, PLPC, and SLPC secreted during bile acid depletion (no bile acid (□) n = 6) and infusion of TCDC (▨, n = 3) and TUDC (▩, n = 3). Mass composition of hepatic PAPC, SAPC, PLPC, and SLPC (other species excluded) shown for comparison (■, n = 12). Only hepatic PAPC, SAPC, PLPC, and SLPC were primarily labeled and could be used to calculate the component of a bile PC species that was preformed. The secretion of a preformed component was calculated from the absolute secretion rate (Table 2) of a PC species multiplied by the portion that was preformed (Table 3, bile/liver specific activity ratio at 45–60 min), assuming rapid mixing of the PC species in the hepatocyte. If biliary secretion from hepatic preformed PCs was nonselective, the composition of preformed components of bile PCs shown would equal the composition of hepatic PCs shown. *, $P < 0.05$ vs. No Bile Acid group; **, $P < 0.05$ vs. TCDC group.

The secretion rates of preformed components (hypothetical) of bile PLPC, SAPC, PAPC, and SLPC were calculated as described in the legend to **Fig. 5;** their composition is compared to the hepatic mass composition of these four species in Fig. 5. At low rates of PC secretion in bile acid-depleted rats, there was high degree of selectivity: *1)* for biliary secretion of hepatic preformed PLPC and *2)* against secretion of hepatic preformed SAPC. When PC secretion increased with TCDC or TUDC infusion, the composition of bile PCs secreted from preformed sources was closer to the composition of PCs in the liver. Thus, the partial loss of selectivity as bile PC secretion increased was largely due to loss of selectivity for/against the hepatic preformed source of each species. There were no significant differences between TUDC and TCDC groups in selectivity for/against preformed PCs even though TUDC and TCDC varied to extremes of the hydrophobicity index.

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REFERENCES

1. Robins, S. J., and M. J. Armstrong. 1976. Biliary lecithin secretion. II. Effects of dietary choline and biliary lecithin synthesis. *Gastroenterology.* **70:** 397–402.
2. Kawamoto, T., T. Akino, M. Nakamura, and M. Mori. 1980. Metabolism of individual molecular species of phosphatidylcholine in the liver subcellular membranes and bile: origin of bile phosphatidylcholine. *Biochim. Biophys. Acta.* **619:** 35–47.
3. Alvaro, D., A. Cantafora, A. F. Attili, S. G. Corradini, C. De Luca, G. Minervini, A. Di Biase, and M. Angelico. 1986. Relationships between bile salt hydrophilicity and phospholipid composition in bile of various animal species. *Comp. Biochem. Physiol.* **83B:** 551–554.
4. Swell, L., C. C. Bell, Jr., and C. Entenman. 1968. Bile acids and lipid metabolism. III. Influence of bile acids on phospholipids in liver and bile of the isolated perfused dog liver. *Biochim. Biophys. Acta.* **164:** 278–284.
5. Cohen, D. E., L. S. Leighton, and M. C. Carey. 1992. Bile salt hydrophobicity controls vesicle secretion rates and transformations in native bile. *Am. J. Physiol.* **263:** G386–G395.
6. Stein, O., L. Sanger, and Y. Stein. 1974. Colchicine-induced inhibition of lipoprotein and protein secretion into the serum and lack of interference with secretion of biliary phospholipids and cholesterol by rat liver in vivo. *J. Cell Biol.* **62:** 90–103.
7. Crawford, J. M., C. A. Berken, and J. L. Gollan. 1988. Role of the hepatocyte microtubular system in the excretion of bile salts and biliary lipid: implications for intracellular vesicular transport. *J. Lipid Res.* **29:** 144–156.
8. Rahman, K., T. G. Hammond, P. J. Lowe, S. G. Barnwell, B. Clark, and R. Coleman. 1986. Control of biliary phospholipid secretion: effect of continuous and discontinuous infusion of taurocholate on biliary phospholipid secretion. *Biochem. J.* **234:** 421–427.
9. Meier, P. J., E. S. Sztul, A. Reuben, and J. L. Boyer. 1984. Structural and functional polarity of canalicular and basolateral plasma membrane vesicles isolated in high yield from rat liver. *J. Cell Biol.* **98:** 991–1000.
10. Samborski, R. W., N. D. Ridgway, and D. E. Vance. 1990. Evidence that only newly made phosphatidylethanolamine is methylated to phosphatidylcholine and that phosphatidylethanolamine is not significantly deacylated-reacylated in rat hepatocytes. *J. Biol. Chem.* **265:** 18322–18329.
11. Nilsson, S., and T. Schersten. 1970. Influence of bile acids on the synthesis of biliary phospholipids in man. *Eur. J. Clin. Invest.* **1:** 109–111.
12. 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:** 122–133.
13. Schersten, T., A. Gottfries, S. Nilsson, and B. Samuelsson. 1967. Incorporation of plasma free fatty acids into bile lipids in man. *Life Sci.* **6:** 1775–1780.
14. Curstedt, T. 1974. Biosynthesis of molecular species of phosphatidylcholines in bile, liver and plasma of rats given [1,1-$^2H_2$]ethanol. *Biochim. Biophys. Acta.* **369:** 196–208.
15. Robins, S. J., and H. Brunengraber. 1982. Origin of biliary cholesterol and lecithin in the rat: contribution of new synthesis and preformed hepatic stores. *J. Lipid Res.* **23:** 604–608.
16. van Berg Henegouwen, G. P., S. D. J. van der Werf, and A. T. Ruben. 1987. Fatty acid composition of phospholipids in bile in man: promoting effect of deoxycholate on arachidonate. *Clin. Chim. Acta.* **165:** 27–37.
17. Salvioli, G., M. Romani, P. Loria, N. Carulli, and J. M. Pradelli. 1985. Effect of acute administration of bile acids on fatty acid composition of biliary phosphatidylcholine in man. *J. Hepatol.* **1:** 291–300.
18. Berr, F., E. Schreiber, and U. Frick. 1992. Interrelationships of bile acid and phospholipid fatty acid species with cholesterol saturation of duodenal bile in health and gallstone disease. *Hepatology.* **16:** 71–81.
19. Scagnelli, G. P., P. S. Cooper, J. M. VandenBroek, W. F. Berman, and C. C. Schwartz. 1991. Plasma 1-palmitoyl-2-linoleoyl phosphatidylcholine: evidence for extensive phospholipase A_1 hydrolysis and hepatic metabolism of the products. *J. Biol. Chem.* **266:** 18002–18011.
20. Heuman, D. M., C. R. Hernandez, P. B. Hylemon, W. M. Kubaska, C. Hartman, and Z. R. Vlahcevic. 1988. Regulation of bile acid synthesis. I. Effects of conjugated ursodeoxycholate and cholate on bile acid synthesis in chronic bile fistula rat. *Hepatology.* **8:** 358–365.
21. Heuman, D. M., A. S. Mills, J. McCall, P. B. Hylemon, W. M. Pandak, and Z. R. Vlahcevic. 1991. Conjugates of ursodeoxycholate protect against cholestasis and hepatocellular necrosis caused by more hydrophobic bile salts: in vivo studies in the rat. *Gastroenterology.* **100:** 203–211.
22. Scanu, A. M., and C. Edelstein. 1971. Solubility in aqueous solution of ethanol of the small molecular weight peptides of the serum very low density and high density lipoproteins: relevance to the recovery problem during delipidation of serum lipoproteins. *Anal. Biochem.* **44:** 576–588.
23. Noble, R. P. 1968. Electrophoretic separation of plasma lipoproteins in agarose gel. *J. Lipid Res.* **9:** 693–700.
24. Folch, J., M. Lees, and G. H. Sloane Stanley. 1957. A simple method for the isolation and purification of total lipids from animal tissues. *J. Biol. Chem.* **226:** 497–509.
25. Hirsch, J., and E. H. Ahrens, Jr. 1958. The separation of complex lipid mixtures by the use of silicic acid chromatography. *J. Biol. Chem.* **233:** 311–320.
26. Bartlett, G. R. 1959. Phosphorus assay in column chromatography. *J. Biol. Chem.* **234:** 466–468.
27. Patton, G. M., and S. J. Robins. 1987. HPLC of molecular species of glycerophospholipids in studies of lipoproteins and lipid transport. *In* Chromatography of Lipids in Biomedical Research and Clinical Diagnosis. A. Kuksis, editor. Elsevier, Amsterdam. 311–347.
28. Kito, M., H. Takamura, H. Narita, and R. Urade. 1985. A sensitive method for quantitative analysis of phospholipid molecular species by high-performance liquid chromatography. *J. Biochem.* **98:** 327–331.
29. Heuman, D. M. 1989. Quantitative estimation of the hydrophilic-hydrophobic balance of mixed bile salt solutions. *J. Lipid Res.* **30:** 719–730.
30. Alvaro, D., M. Angelico, A. Cantafora, A. Di Biase, A. De Santis, F. Bracci, G. Minervini, S. G. Corradini, A. F. Attili, and L. Capocaccia. 1986. Biliary secretion of phosphatidylcholine and its molecular species in cholecystectomized T-tube patients: effects of bile acid hydrophilicity. *Biochem. Med. Metab. Biol.* **36:** 125–135.
31. Kaplan, M. R., and R. D. Simoni. 1985. Intracellular transport of phosphatidylcholine to the plasma membrane. *J. Cell Biol.* **101:** 441–445.
32. Yaffe, M. P., and E. P. Kennedy. 1983. Intracellular phospholipid movement and the role of phospholipid transfer proteins in animal cells. *Biochemistry.* **22:** 1497–1507.
33. Cronholm, T., T. Curstedt, and J. Sjövall. 1983. Origin of biliary phosphatidylcholines studied by coenzyme labelling with [1,1-$^2H_2$]ethanol. *Biochim. Biophys. Acta.* **753:** 276–279.
34. Van Den Besselaar, A. M. H. P., B. De Kruijff, H. Van Den Bosch, and L. L. M. Van Deenen. 1978. Phosphatidylcholine mobility in liver microsomal membranes. *Biochim. Biophys. Acta.* **510:** 242–255.
35. Zilversmit, D. B., and M. E. Hughes. 1977. Extensive exchange of rat liver microsomal phospholipids. *Biochim. Biophys. Acta.* **469:** 99–110.