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Studies on fat digestion, absorption, and transport in the suckling rat. III. Composition of bile and evidence for enterohepatic circulation of bile salts

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Abstract Biliary secretions from suckling rats (10–15 days old) were characterized: bile flow rate was 197.3 μl/hr per 100 g; bile salt pool size was 19.0 μmol/100 g; secretion rate was 14.5 μmol/hr per 100 g; synthesis rate was 12.0 μmol/day; and the daily turnover frequency was 18.3. Phospholipid and cholesterol secretion rates were 2.3 μmol/hr per 100 g and 0.17 μmol/hr per 100 g, respectively. The bile salt concentration in portal plasma was 0.17 μmol/ml. The fatty acid composition of biliary phosphatidylcholine and ethanolamine, as well as the stereospecific distribution of fatty acids in the former, were similar to that found in phospholipids from adult rat bile. Compositional analysis of bile acids showed greater than 98% taurine conjugates consisting of approximately 54% cholic acid, 40% β-muricholic acid, 2% chenodeoxycholic acid, and 1% each of deoxycholic and hyodeoxycholic acids. Through the use of intestinal and liver perfusion experiments, we have obtained evidence for enterohepatic circulation of taurocholate in neonatal rats. The level of bile salts found in intestinal contents (49.5 μmol/g), and the biliary phospholipid concentration (11.8 mM) both exceed adult values (6–10 μmol/g and 6.3 mM, respectively) and may be important for the utilization of the large amount of milk triacylglycerols ingested during the suckling period.—**Staggers, J. E., S. C. Frost, and M. A. Wells.** Studies on fat digestion, absorption, and transport in the suckling rat. III. Composition of bile and evidence for enterohepatic circulation of bile salts. *J. Lipid Res.* 1982. **23:** 1143–1151.

Supplementary key words neonatal rat • milk lipids • intestine • lymph • bile salts • phospholipids • cholesterol • triacylglycerols • liver perfusion

The mechanisms of fat digestion and absorption in neonates have not been extensively studied (1, 2). The suckling rat is a useful model in which to study the utilization of large amounts of dietary fat because of the high fat content of rat milk (1, 3). The reported low concentration of bile salts in human neonates (4), and the assumed low levels in rats (1, 2), raised questions as to the importance of bile salts in fat digestion in neonatal rats. It has been shown that neonatal rats are capable of synthesizing taurocholate, and that most of the body bile salt is present in the gastrointestinal tract (5). However, the actual concentrations of bile salts in bile and the intestinal tract have not been reported. It has been reported that rats, like other neonates, lack the ileal system for active transport of bile salts (6). Although the extent of passive transport in neonates is unknown, both passive and active transport contribute to bile salt absorption from the intestinal tract of the adult rat (7).

In this study we report *1)* the concentration of bile salts in the bile, portal blood, and intestinal tract, *2)* characterization of the type of bile acids present, and *3)* the content of cholesterol and the content and fatty acid composition of phospholipids (PL) in suckling rat bile. In addition, we present evidence for the enterohepatic circulation of bile salts in the suckling rat.

METHODS

Sprague-Dawley rats were obtained from a breeding colony maintained by the Division of Animal Resources, College of Medicine. At 2 days after birth litters were adjusted to ten pups. Dams had free access to a standard laboratory chow diet and tap water. Most experiments were carried out with 13- to 14-day-old animals, although some used other ages between 10 and 15 days. In a few experiments 7-week-old adult females (135 g) and males (195 g) were used. Suckling animals were used within 30 min after removal from their mother. All surgical procedures were carried out under pentobarbital anesthesia (40 mg/kg body weight, i.p.). Bile

Abbreviations: ANS, 8-anilino 1-naphthalene sulfonic acid; PL, phospholipid; TG, triacylglycerol; PC, phosphatidylcholine; PE, phosphatidylethanolamine; B:P, bile salt to phospholipid molar ratio; TMS, trimethylsilyl; BF, bile flow; BS, bile salts. Trivial names of bile acids refer to hydroxy-substituted 5β-cholanic acids: chenodeoxycholic, 3α,7α; cholic, 3α,7α,12α; deoxycholic, 3α,12α; hyocholic, 3α,6α,7α; hyodeoxycholic, 3α,6α; α-muricholic, 3α,6β,7α; β-muricholic, 3α,6β,7β.

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fistulas were performed, with the aid of a dissecting microscope, below the junction of the hepatic ducts and above the pancreatic ducts (8). Polyethylene tubing (PE 10, Clay Adams) was used as the catheter, and we found it advantageous to use cyanoacrylate glue (9) to hold the catheter in place. The incision was covered by gauze pads saturated with warm physiological saline and the animals were kept in a warm box at 30–35°C.

For washout experiments (10), bile was collected from individual animals into tared, chilled tubes while the animals were maintained under light anesthesia. Bile was collected in 30- or 60-min intervals for a total of 4–5 hr. Bile flow rates were calculated as the weight of bile collected per hr. In some experiments involving compositional studies, bile was collected into a single tube for 4–5 hr and bile from several animals was pooled. Bile samples were diluted with 5 volumes of methanol and centrifuged for 10 min at 1,500 rpm to remove precipitated proteins, stored at 4°C, and analyzed within 2 days. In some cases the glycine and taurine conjugates were separated by solvent extraction as described by Levin, Johnston, and Boyle (11).

For the analysis of intestinal contents, a segment of the small intestine from the point of entrance of the common duct to the cecum was removed, and the contents were recovered by gently squeezing. In the case of neonates this segment was 30 cm, in adult females 65 cm, and in adult males 100 cm. The contents were heated (12) and homogenized, and weighed aliquots were extracted by adding 25 volumes of chloroform–methanol 2:1 (v/v). Phase separation was induced by adding 0.2 volume of physiological saline to the extract. Bile salts were analyzed in the aqueous methanolic phase. In other experiments the entire tract was divided into three equal segments (termed upper, middle, and lower) and the contents of each segment were removed by carefully lavaging with 5 ml of ice-cold physiological saline. The saline suspension was sonicated for 3 min at one-fourth maximal output, using a Branson Sonifer model W140 (Heat Systems-Ultrasonics, Inc., Plainview, NY). For the determination of bilirubin, a portion of the sample was diluted with an equal volume of methanol and centrifuged at 1,500 rpm for 5 min. Another portion was analyzed for bile salts after extraction as described above. Portal blood was obtained from animals as described previously (3), and extracted as described above for intestinal contents.

In order to study the enterohepatic circulation of bile salts, bile salts were perfused through the intestinal tract of bile fistula animals. After establishing the fistula, the small intestine was cut just below the entrance of the common duct and above the cecum. This segment of intestine was lavaged with 10 ml of physiological saline, and the ends were connected to short pieces of PE-100 polyethylene tubing. The animal was then placed in a warm box and the polyethylene tubing was connected to a pump that circulated perfusion buffer at 30°C through the intestine at a flow rate of 60 ml/hr per 100 g body weight. The perfusion fluid (13) contained: 2 mM $CaCl_2$; 40 mM $NaHCO_3$; 85 mM NaCl; 6 mM KCl; 28 mM glucose; and various concentrations of sodium taurocholate. When appropriate, [^{14}C]taurocholate was also present. The perfusion fluid (50 ml) was recirculated for the 4–5 hr duration of the experiment, and the bile was collected for 1-hr intervals as described above.

An additional experiment was performed with 10-day-old rats in which 0.05 μCi of [^{14}C]taurocholate in 50 μl of rat milk was given by stomach tube and 2–3 hr later a bile fistula was established for bile collection. After collecting bile for 2.5 hr, the animal was killed, portal blood was collected, and the liver, intestines, and stomach were removed. Bile was diluted with 5 volumes of methanol and, after centrifugation to remove precipitated protein, an aliquot of the supernatant was counted. Plasma was prepared from portal blood and an aliquot was counted. Stomachs, intestines, and livers were homogenized in 5 ml of physiological saline and 2 ml of methanol, and an aliquot was counted. Samples were counted in Beta Phase (WestChem Products, San Diego, CA) in a Beckman LS-7500 scintillation counter with automatic quench and efficiency correction.

Secretion of bile salts by suckling rat liver was also investigated using perfused livers containing a bile duct fistula. After preparation of the fistula, single-pass liver perfusions were carried out in situ as previously described (14). The perfusion buffer was modified Krebs-Henseleit solution containing 5 mM glucose, no albumin, and various concentrations of sodium taurocholate. When appropriate, [^{14}C]taurocholate was also present. Bile was collected in 30-min intervals into tared, chilled tubes, and extracted for analysis as described above.

Bile salts were measured by the enzymatic assay of Turley and Dietschy (15). In the case of portal plasma and intestinal contents, partial purification of bile salts by solvent extraction (11) did not give significantly different results from those obtained using methanolic extracts directly. Phospholipid phosphorus was determined as described previously (3). Total bilirubin was determined using the van den Bergh reaction as described by Malloy and Evelyn (16). Cholesterol was determined by gas–liquid chromatography on a Hewlett-Packard model 402 gas chromatograph equipped with a Hewlett-Packard model 3380A integrator. Glass columns (20 in, i.d. ⅛ in) were filled with 3% JXR on 100/120 mesh Gas-Chrom Q (Applied Science Laboratories, State College, PA). Separations were carried out at

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230°C. Cholesterol was quantified by the use of an internal stigmasterol standard. Bile acid composition was determined by gas–liquid chromatography of the TMS methyl esters using 1% HiEff 8 BP on Gas-Chrom Q 100–120 mesh (Applied Science Laboratories, State College, PA) at 230°C (17). Bile acid methyl esters were prepared using diazomethane and separated by thin-layer chromatography on silica gel G using chloroform–acetone–methanol 70:25:5 (v/v/v) (18). The methyl esters were visualized with 0.1% ANS in water (19) and eluted with acetone (18). Biliary phosphatidylcholine (PC) and phosphatidylethanolamine (PE) were purified by preparative thin-layer chromatography on silica gel G-coated glass plates, using the solvent system chloroform–methanol–water 95:35:4 (v/v/v). After detection with ANS, the lipid was extracted from the gel and fatty acid methyl esters were prepared and determined as previously described (4). The fatty acid composition at the *sn*-1 and *sn*-2 positions of PC was determined as described (20).

Conjugated bile salts were obtained from Calbiochem (La Jolla, CA). 3 α-Hydroxysteroid dehydrogenase (*Ps. testosteroni*, EC 1.1.1.50) was from Millipore Corp (Freehold, NJ); stigmasterol was a gift from Dr. H. W. Kircher (University of Arizona); and bile acid standards were obtained from Supelco (Bellefonte, PA). The [^{14}C]taurocholic acid (52 mCi/mmol) was obtained from New England Nuclear (Boston, MA) and shown to be 91.5% pure in the solvent system butanol–acetic acid–water 10:1:1 (v/v/v) on silica gel G thin-layer plates. All other solvents and chemicals used were of reagent grade or better. Group mean statistical comparisons were carried out using Student's *t* test. Nonlinear regression analysis was carried out using the program of Duggleby (21).

It was found that the concentration of bile salts in bile from drained suckling animals (washout experiments) showed a first order decrease with time. The composite data from six animals are shown in **Fig. 1.** The line in Fig. 1 was calculated from a nonlinear regression analysis of the data. The equation used was

$$(BS_t - BS_s) = BS_0 \, EXP(-kt) \qquad \textit{Eq. 1)}$$

where BS_t = observed bile salt output at time t; BS_s = basal bile salt output at infinite time, i.e., synthesis rate by the liver; BS_0 = bile salt output at t = 0 − BS_s, i.e., initial secretion rate − basal secretion rate; k = rate constant; t = time. A separate fit was carried out for each animal. The bile salt pool size was calculated by summing the difference between bile salt output and BS_s at all time points for which bile salt output was greater than BS_s. Data for phospholipid output (not shown) also showed a first order decay, which could be

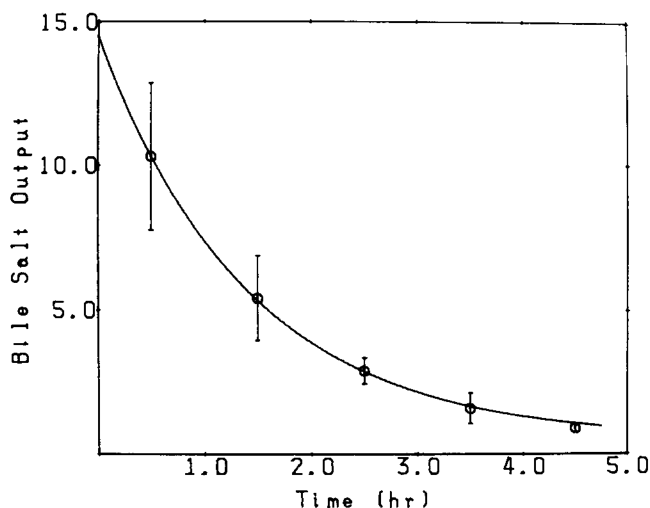

Fig. 1. Bile salt output (μmol/hr per 100 g) as a function of time during washout experiments. The data represent the mean ± SD (bars) for six 14-day-old rats. The line was calculated from equation 1 and the data in Table 1.

fit to

$$PL_t = PL_0 \, EXP(-kt) \qquad \textit{Eq. 2)}$$

where PL_t = phospholipid output at time t; PL_0 = phospholipid output at t = 0; and the other symbols are as in equation *1*. In the case of phospholipid output, it was not necessary to include a term for infinite time. Bile flow also showed a first order decay which could be fit to

$$BF_t = BF_0 \, EXP(-kt) \qquad \textit{Eq. 3)}$$

where BF_t = bile flow at time t; BF_0 = bile flow at t = 0; and the other symbols are as in equation *1*. In the case of cholesterol output, there was no significant change in concentration over the 4–5 hr period studied. The small amount of bile from suckling animals precluded measurement of the density and therefore for calculation of concentrations we have assumed a density of 1.00 g/ml. Although the published value for the density of adult rat bile is 1.011 (22), the error introduced by this assumption is probably negligible.

RESULTS

In vivo studies

The bile salt concentration in the intestinal contents of 14-day-old rats was 49.5 ± 8.7 μmol/g (pooled samples, n = 4). In the adult female this value was 10.3 μmol/g (n = 1), and in the male it was 5.9 ± 1.2 μmol/g (n = 2).

We chose to collect bile using animals in the fed-state under pentobarbital anesthesia as an approach that would allow collection of bile whose properties were as

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TABLE 1. Biliary secretion in the suckling rat[a]

	14 Days Old	Adult	
		Female	Male
Bile flow (μl/hr per 100 g)	197.3 ± 20.6	292 ± 34	452 ± 25
Bile salts			
Pool size (μmol/100 g)	19.0 ± 5.4		
Synthesis rate (μmol/day)	12.0 ± 2.2		
Secretion rate (μmol/hr per 100 g)	14.5 ± 3.8	17.9 ± 1.8	15.1 ± 2.8
Concentration in bile (mM)	73.5 ± 19.3	61.3 ± 6.2	33.5 ± 6.2
Turnover frequency[b]	18.3 ± 4.8		
Phospholipid			
Secretion rate (μmol/hr per 100 g)	2.3 ± 0.6	1.9 ± 0.3	2.8 ± 0.1
Concentration in bile (mM)	11.8 ± 3.1	6.5 ± 1.0	6.2 ± 0.2
Cholesterol			
Secretion rate (μmol/hr per 100 g)	0.17 ± 0.08	0.20 ± 0.01	0.33 ± 0.01
Concentration in bile (mM)	0.86 ± 0.41	0.68 ± 0.03	0.73 ± 0.02

[a] Data for 14-day-old rats represent the average for six animals ± SD. Animals were drained as described in the text and the data represent extrapolation to zero time by nonlinear regression analysis. The average weight of the rats used was 36.5 ± 2.9 g. Data for adult animals are for 7-week-old females (n = 2) and males (n = 2), 135 g and 195 g, respectively, and represent bile collected during the first 30 min after cannulation.

[b] Calculated by dividing the cumulative amount of bile salts secreted during 24 hr (secretion rate) by the pool size (24).

close as possible to that secreted under normal conditions. This approach was taken since it is difficult to maintain suckling animals for long periods of time in the absence of the mother due to problems of feeding and maintenance of proper fluid intake. In addition, suckling rats do not eliminate wastes without stimulation from the mother. Exactly the same conditions were used for adults in order to provide data for direct comparison.

The results of biliary washout experiments for 14-day-old rats are presented in **Table 1.** The data are presented in terms of output per 100 g body weight, as well as mM concentration of components in bile. Table 1 also contains data for adult animals under anesthesia. In these adults 5 hr was not sufficient to effect washout and therefore the values reported are for the first 30 min after cannulation. Except for a lower flow rate, the characteristics of biliary secretions in the suckling are rather similar to those of adults. Esterified cholesterol was below detectable limits, in agreement with reports for adult rat fistula bile (17, 23). The characteristics of biliary secretion in adults under anesthesia are significantly different from those obtained for conscious adults of the same age and strain (24).

It has been shown in previous studies (25, 26) that there is a direct relationship between the output of phospholipid in bile and the bile salt output in bile. That such a relationship also exists for suckling bile can be seen from **Fig. 2,** in which phospholipid output is plotted against bile salt output. These data represent the various time points for the six washout experiments. The line in Fig. 2 was calculated from a nonlinear regression analysis of

$$PL = K_1BS/(K_2 + BS) \qquad Eq. 4)$$

where PL = phospholipid output and BS = bile salt output. The values of the constants are $K_1 = 4.98 \pm 0.63$, which would represent the maximum PL putput, and $K_2 = 15.16 \pm 3.0$, which represents the bile salt output in bile at which half maximal PL output occurs.

Table 2 presents the fatty acid composition of biliary

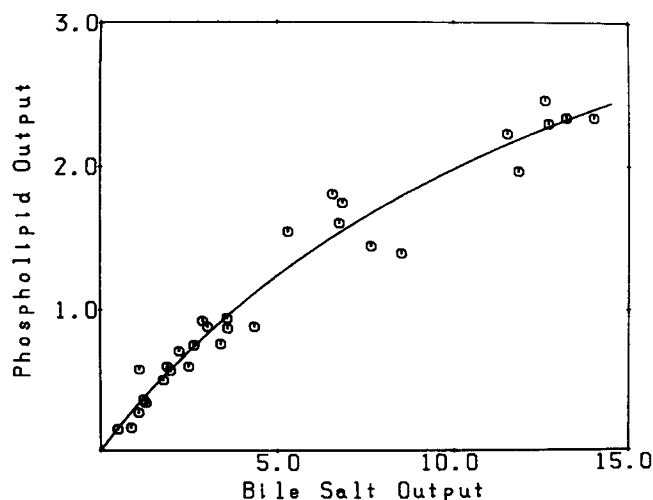

Fig. 2. Phospholipid output (μmol/hr per 100 g) as a function of bile salt output (μmol/hr per 100 g). The data represent the data from the six animals shown in Fig. 1. The solid line was calculated according to equation 4.

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PC, PE, and stereospecific analysis of PC. In suckling rat bile, PC and PE represent more than 95% of the total phospholipid, and the ratio of PC/PE is 15.1. Suckling rat bile PC has a fatty acid composition rather similar to that of PC from adult bile (27), except for a higher content of polyunsaturated fatty acids (20:4 (n − 6), 22:5 (n − 3), and 22:6 (n − 3)), 18.4% as compared to 10.4% in adults. Compared to PC, suckling biliary PE has a higher proportion of 14:0 and 18:1 and a lower proportion of 18:2 (n − 6). Fatty acid analysis of biliary PC isolated from pooled bile samples of 10-day and 18-day-old suckling rats was not significantly different from that shown for the 14-day-old animal. The distribution of fatty acids between the *sn*-1 and *sn*-2 positions of biliary PC is also similar to that reported for adult (27), with 90.7% (suckling) vs. 95.3% (adult) of *sn*-1 fatty acids being saturated and 84.6% (suckling) vs. 82.0% (adult) of *sn*-2 fatty acids having two or more double bonds.

TABLE 2. Fatty acid composition of biliary phospholipids

Fatty Acid	Mol %[a]			
	PC[b]	PC(*sn* − 1)[c]	PC(*sn* − 2)[c]	PE[d]
10:0	0.14 (0.07)			
12:0	0.11 (0.03)			
14:0	1.67 (0.59)	2.65	0.54	6.66 (0.28)
16:0	42.30 (0.41)	79.40	5.56	36.22 (1.71)
16:1	1.08 (0.07)	2.47	0.64	1.10 (0.12)
18:0	3.32 (0.15)	8.65	0.57	6.88 (0.36)
18:1	4.86 (0.30)	2.86	7.19	16.78 (0.73)
18:2 (n − 6)	27.73 (0.84)	2.89	46.33	9.76 (0.31)
18:3 (n − 3)	0.18 (0.03)	0.13	0.13	0.94 (0.67)
20:0	0.19 (0.02)	0.26	0.69	1.00 (0.22)
20:4 (n − 6)	13.53 (1.09)	0.42	28.04	15.80 (0.36)
22:5 (n − 3)	0.47 (0.22)	0.08	0.33	0.46 (0.21)
22:6 (n − 3)	4.42 (0.46)	0.19	9.77	4.42 (2.12)

[a] Mean value with standard deviation in parentheses for bile samples from 14-day-old rats.
[b] Phosphatidylcholine.
[c] Fatty acids at *sn*-1 or *sn*-2 position of phosphatidylcholine.
[d] Phosphatidylethanolamine.

TABLE 3. Bile acid composition (%) of suckling rat bile and portal plasma

Bile Acid	Bile[a]	Portal Plasma[b]	Adult Bile[c]
α-Muricholic[d]	0.47 ± 0.27	0.60	trace
Cholic	53.92 ± 0.89	56.12	78.88
Hyocholic[d]	0.41 ± 0.09	0.13	4.97
Deoxycholic	1.21 ± 0.25	0.57	5.06
Chenodeoxycholic	2.08 ± 0.22	0.77	6.87
β-Muricholic	40.49 ± 0.71	39.87	3.07
Hyodeoxycholic[d]	1.42 ± 0.19	1.94	1.15

[a] Pooled samples (n = 3).
[b] Pooled sample (n = 1).
[c] Pooled sample adult males (n = 1).
[d] Tentative identification based on GLC elution time of TMS methyl ester relative to cholic acid and mobility of methyl ester on TLC.

By solvent extraction methods, suckling bile and portal plasma were found to contain more than 98% taurine conjugated bile salts with no detectable amounts of unconjugated bile acids. Such a high preponderance of taurine conjugated bile salts is a common feature of fetal and neonatal bile salts (28). The bile acid compositions of bile and portal plasma are presented in **Table 3** along with data for adult bile. The bile acid composition for neonates differs significantly from that of adults, but is similar to that reported for adult germ-free rat bile (18). This may arise from the fact that the litters are maintained in laminar flow hoods to prevent respiratory tract infections, which are chronic problems in this desert climate.

The mean concentration of bile salts in neonatal portal plasma (170 ± 50 μM, n = 6) exceeds the values for adults (female = 30.7 μM; male = 19.4 μM). The values for adults are somewhat lower than previously reported (60 μM) (29). The high content of bile salts in plasma, as well as the washout experiments ($t_{1/2}$ = 1 hr), strongly suggest that intestinal bile salt reabsorption occurs to an appreciable extent in the suckling rat. Additional support for this suggestion comes from experiments in which the amounts of bile salts and bilirubin were measured in the contents of intestinal segments. The ratio of bile salt/bilirubin was as follows: upper segment, 540 ± 92; middle segment, 439 ± 50; lower segment, 18.5 ± 0.2 (n = 3). There was no significant difference between the ratio for the upper and middle segments, but the ratio in the lower segment differed significantly from the other two ($P < 0.001$). Assuming that no metabolism of bilirubin occurs in the suckling small intestine and that the isolation procedure does not result in degradation of bilirubin, we can use it as a nonabsorbable marker to estimate the amount of bile salts absorbed. Comparing the ratio in the upper segment to that in the lower segment, one can estimate that >95% of the bile salt in the upper segment was absorbed. This is probably a lower limit value because of the assumptions concerning recovery of bilirubin.

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TABLE 4. Biliary secretion from intestinally perfused suckling rats[a]

	Taurocholate in Perfusion Medium		
	0[b]	1[c]	5[c]
		mM	
Bile flow (μl/hr per 100 g)	72.0 ± 14.3	184.6 ± 27.9[d,e]	215.9 ± 32.9[d,e]
Bile salts			
Secretion rate (μmol/hr per 100 g)	0.5 ± 0.1	2.4 ± 1.0[d,f]	5.3 ± 1.6[d,f]
Concentration in bile (mM)	8.2 ± 1.3	13.1 ± 5.2	24.6 ± 7.6
Phospholipid			
Secretion rate (μmol/hr per 100 g)	0.15 ± 0.04	0.76 ± 0.07[d,f]	1.54 ± 0.11[d,f]
Concentration in bile (mM)	2.1 ± 0.6	4.1 ± 0.4	7.1 ± 0.5
Cholesterol			
Secretion rate (μmol/hr per 100 g)	n.d.	0.13 ± 0.06	0.15 ± 0.05
Concentration in bile (mM)	—	0.70 ± 0.32	0.70 ± 0.23

[a] Bile was collected via a fistula in hourly samples during the 2nd through 5th hours of perfusion. There was not a significant variation in output during this time period and the data represent the mean ± SD.
[b] Average of two animals.
[c] Average of four animals.
[d] P vs. 0 taurocholate <0.01.
[e] 1 and 5 mM not significantly different and not different than drained animals $P > 0.5$ (Table 1).
[f] P 1 mM vs. 5 mM <0.01.

Intestinal perfusion studies

In order to more fully document enterohepatic circulation of bile salts we perfused the intestine with various concentrations of bile salts and measured the output in bile. The results of these experiments are presented in **Table 4.** There are several points to note. *1*) The secretion rate of bile salts from the liver (basal synthesis rate) in the absence of intestinal bile salts is quite low and is identical to the value calculated from the washout experiments (0.5 μmol/hr per 100 g). *2*) In the presence of either 1 or 5 mM taurocholate in the perfusion buffer, there is substantial bile salt output from the liver. We were not able to reach the values calculated from the washout experiments, since at 10 mM taurocholate the intestine was apparently severely damaged as judged by the accumulation of cellular material in the perfusate. We did not attempt to add TG lipolysis products or PL to the perfusion buffer, which may affect uptake of bile salts from the intestine. Furthermore, we did not investigate the effect of a physiological mixture of bile salts on bile salt uptake. *3*) Although these experiments clearly establish the existence of enterohepatic circulation of bile salts, it should be noted that the bile produced during these perfusions had a lower B:P ratio (~3.3) than control bile (~6.0).

Liver perfusion studies

Further characterization of bile salt secretion was investigated via liver perfusion experiments. Initially we used recirculating perfusions with 0.3 mM taurocholate. However over the 3-hr duration of the experiment, bile flow decreased 56%, bile salt output decreased 62%, and PL output decreased 68% compared to the initial 30-min values. These decreases were due to a 72% decrease in the concentration of taurocholate in the perfusate during the experiment as a result of uptake and secretion of bile salt into bile. Therefore, it was necessary to carry out single-pass perfusions in order to keep the taurocholate concentration constant during the experiment. Under these conditions there was not a significant change in output or bile flow over the 3-hr period.

As suggested above, bile salt and PL output decreased rapidly in livers perfused without bile salts. Thus after 30 min, bile salt output was 10.8 and the PL output was 1.2 μmol/hr per 100 g, while after 60 min these values were 1.0 and 0.17 μmol/hr per 100 g, respectively. In marked contrast, the inclusion of bile salt in the perfusion buffer caused substantial and constant output of both bile salt and PL as can be seen from the data in **Table 5.** In the case of the perfused liver, bile flow, bile salt output, and the B:P ratio (8.4–12.5) were higher than in the intact animal, although the concentration of bile salt in the bile was not different from that found in intact animals. The PL content of bile secreted during liver perfusion experiments was lower than in intact animals, but the fatty acid compositions of the PC and PE were not significantly different from those reported in Table 2. The liver had a very high affinity for bile salts in the perfusion buffer. Thus at 0.0375 mM bile salt in the perfusion buffer, the liver removed 77.5% of the bile salts present in the perfusion buffer in a single

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TABLE 5. Biliary secretion during neonatal liver perfusion[a]

	Taurocholate in Perfusion Media			
	0.0375	0.075	0.150	0.300
	mM			
Bile flow (μl/hr per 100 g)	184.2 ± 12.0	320.6 ± 26.6	341.8 ± 23.0	313.0 ± 16.8
Bile salts				
Secretion rate (μmol/hr per 100 g)	9.1 ± 0.9	19.2 ± 0.3	22.6 ± 2.1	21.2 ± 0.9
Concentration in bile (mM)	49.9 ± 5.1	59.9 ± 0.9	66.1 ± 6.1	67.7 ± 2.8
Phospholipid				
Secretion rate (μmol/hr per 100 g)	1.1 ± 0.2	1.7 ± 0.1	1.8 ±0.3	1.7 ± 0.2
Concentration in bile (mM)	6.0 ± 0.9	5.3 ± 0.3	5.3 ± 0.9	5.4 ± 0.7
% Secreted[b]	77.5 ± 8.0	81.7 ± 1.3	48.0 ± 4.5	22.5 ± 0.9
$[BS_b]/[BS_p]$[c]	1330 ± 135	800 ± 12	440 ± 40	225 ± 9

[a] Bile was collected for 30-min intervals via a fistula. There was not a significant variation in output during the 3 hr of collection and the data represent the mean ± SD.
[b] (Bile salt output/bile salt input) × 100.
[c] Ratio of bile salt concentration in bile to that in perfusion media.

pass and secreted bile salts into bile at a concentration 1,330-fold greater than found in the perfusion buffer. At 0.15 mM, which is near the physiological concentration in suckling rats, the liver removed 48% in a single pass and effected a 440-fold concentration increase in the secreted bile. In livers perfused with 0.6 mM bile salt, bile flow, and bile salt and PL output were decreased compared to the values shown in Table 5, apparently due to damage to the liver.

^{14}[C]Taurocholate studies

Further evidence for enterohepatic circulation of bile salts in suckling rats was obtained using radioactive taurocholate (**Table 6**). When radioactive taurocholate was present in the intestinal perfusion buffer, the specific activity of biliary bile salts approached ~75% of that in the perfusion buffer. These data show that even though only 2–4% of total infused bile salts are recycled during intestinal perfusion (Table 4), at least 75% of the bile salts secreted in the bile were derived from infused bile salts. The small amount of total bile salts extracted from the perfusion buffer is a result of the fact that the perfusion buffer contained 20–100 times the total bile salt pool of the animal and was passed through the intestine at a rate far in excess of normal intestinal transit. When radioactive taurocholate was present in the liver perfusion buffer, the specific activity of bile salt in the bile reached a value of ~90% of that in the perfusion buffer. Both of these sets of data support the conclusions regarding enterohepatic circulation reached using nonradioactive taurocholate.

In order to demonstrate that enterohepatic circulation of bile salts also occurs in younger animals, we fed labeled taurocholate to 10-day-old animals by gastric intubation, and measured the distribution of radioactivity in the gastrointestinal tract, portal plasma, liver, and bile. It was necessary to do the experiment this way since we were unable to successfully carry out either washout experiments or intestinal or liver perfusion experiments in these younger animals. The data presented in **Table 7** demonstrate that enterohepatic circulation

TABLE 6. Secretion of radioactive taurocholate in neonatal bile

Perfusion Buffer Bile Salt	Specific Activity of Bile Salt: Buffer	Bile
mM	*μCi/mmol*	
Intestinal perfusion experiments[a]		
5.0	1.15	0.83 ± 0.14
Liver perfusion experiments[b]		
0.0375	7.66	6.92 ± 0.12
0.075	7.44	6.65 ± 0.46
0.150	6.72	6.08 ± 0.13
0.300	6.65	6.06 ± 0.12

[a] Two animals were used for each sample and bile was collected during the 2nd and 3rd hr was analyzed (number of samples = 2).
[b] Each experiment represents a single animal. Bile was collected in 30-min intervals during the 3-hr perfusion and each sample was analyzed.

TABLE 7. Distribution of orally administered taurocholate in 10-day-old rats

Sample	% Recovered Radioactivity[a]
GI tract	75.1 ± 0.9
Bile	23.3 ± 0.5
Portal blood	0.9 ± 0.1
Liver	0.7 ± 0.6

[a] The data are reported as percent recovered radioactivity in each sample. The average recovery of the administered dose was 101.9 ± 9.8% (n = 3).

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of bile salts is established in the rat at least by 10 days of age.

DISCUSSION

The present study provides an extensive analysis of the secretion and composition of suckling rat bile. Synthesis and secretion of biliary lipids in the 14-day-old rat appear to reflect adult patterns. Although the fatty acid composition of PL was similar to that of adults, the composition of bile acids was considerably different. At the present time the significance of this difference is unknown since it may result from environmental factors. The unique aspects of the suckling rat relate to the concentration of biliary components in intestinal contents and portal plasma, and the presence of extensive enterohepatic circulation of bile salts in the absence of the ileal active transport system.

The concentration of bile salts in intestinal contents is more than adequate to support the activity of non-specific lipase. However, the physical milieu within the suckling small intestine makes it difficult to actually define the meaning of concentration. The very high content of bile salts and PL from bile and TG and their hydrolysis products from milk (3) produce a highly viscous fluid, in which there is nevertheless efficient lipolysis, and from which efficient absorption occurs. The extreme differences between the situation in the suckling and adult intestine make it impossible to interpret our current results in terms of models developed for fat digestion in adults.

The high PL level in intestinal contents may be related to utilization of the TG-rich milk diet of the suckling rat (1, 3, 30). Luminal PL has been shown to facilitate lymphatic chylomicron TG release from mucosal enterocytes in adult rats (31–36). In this regard it should be noted that PL from suckling rat lymph contain a high proportion of polyunsaturated fatty acids (3). Since milk TG contain a very low proportion of polyunsaturated fatty acids (3), it would appear that biliary and/or milk PL must contribute a major proportion of lymph PL. A 13- to 14-day-old rat consumes about 9–10 ml of milk/day (37), which would contribute $\sim$5.5 μmol of PL per day. During the same time, bile would contribute $\sim$21 μmol of PL. These calculations indicate that 80% of the PL entering the small intestine is derived from bile and suggest that biliary PL could be a major source of lymphatic PL. It is interesting to note that the polyunsaturated fatty acids of biliary PL are not found in the free fatty acid pool of the intestinal luminal contents nor in the TG of lymph, but are restricted to the PL of lymph (3). The mechanisms controlling distribution of fatty acids between TG and PL remain unknown.

In the adult rat the distal ileum is the major site of bile salt uptake via both active transport and passive diffusion mechanisms, although net uptake via micelle-mediated ionic diffusion occurs in the jejunum (7). Using everted ileal rings, Little and Lester (6) have concluded that the mechanism for active transport is undeveloped in the rat before 15 days. Our data, obtained by a variety of in vivo and in vitro methods, clearly establish that efficient enterohepatic circulation of bile salts occurs in the suckling rat at least by day 10. The absorptive mechanism(s) remains unknown, but the ileum is clearly an important site of uptake.

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