Fetal Bile Salt Metabolism

II. HEPATIC EXCRETION OF ENDOGENOUS BILE SALT AND OF A TAUROCHOLATE LOAD

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A B S T R A C T Bile salt metabolism was studied in fetal dogs 1 wk before term. The size and distribution of the fetal bile salt pool were measured, and individual bile salts were identified. The hepatic excretion of endogenous bile salts was studied in bile fistula fetuses, and the capacity of this excretory mechanism was investigated by the i.v. infusion of a load of sodium taurocholate-^{14}C up to 20 times the endogenous pool size.

The total fetal bile salt pool was 30.9±2.7 μmoles, of which two-thirds was in the fetal gallbladder. Expressed on a body weight basis, this was equal to approximately one-half the estimated pool size in the adult dog (119.2±11.3 vs. 247.5±33.1 μmoles/kg body wt). Measurable quantities of bile salt were found in small bowel (6.0±1.8 μmoles), large bowel (1.1±0.3 μmoles), liver (1.2±0.5 μmoles), and plasma (0.1±0.03 μmoles). Plasma bile salt levels were significantly greater in fetal than in maternal plasma (1.01±0.24 μg/ml vs. 0.36±0.06 μg/ml; $P < 0.05$).

Fetal hepatic bile salt excretion showed a fall over the period of study from 2.04±0.34 to 0.30±0.07 μmoles/hr. The maximal endogenous bile salt concentration in fetal hepatic bile was 18.7±1.5 μmoles/ml. The concentration in fetal gallbladder bile was 73.9±8.6 μmoles/ml; and, in those studies in which hepatic and gallbladder bile could be compared directly, the gallbladder appeared to concentrate bile four- to fivefold.

Taurocholate, taurochenodeoxycholate, and taurodeoxycholate were present in fetal bile, but no free bile salts were identified. The presence of deoxycholate was confirmed by thin-layer chromatography and gas liquid chromatography, and the absence of microorganisms in fetal gut suggests that it was probably transferred from the maternal circulation.

After infusion of a taurocholate load, fetal hepatic bile salt excretion increased 30-fold, so that 85–95% of the dose was excreted by the fetal liver during the period of observation. Placental transfer accounted for less than 5% of the dose. Fetal bile volume increased 15-fold on average, while bile salt concentrations increased two- to threefold.

It is concluded that bile salt is taken up, conjugated, and excreted by the fetal liver with remarkable efficiency. The excreted material is either stored and concentrated in the fetal gallbladder or released into the intestine and reabsorbed to be reexcreted in bile.

INTRODUCTION

The physiologic importance of bile salts in the adult is well established, and their synthesis, excretion, enterohepatic circulation, and physiologic function have been extensively studied (1–4). Until recently, however, little was known about bile salt metabolism during early mammalian development, except for information derived from limited observations on the bile salts in the gallbladder bile and intestine of the near-term fetus and early neonate (5–9). A recent study in fetal dogs in which tracer doses of cholate-^{14}C were used indicated that the fetal liver has a remarkably mature and ef-

ficient mechanism for the uptake, conjugation, and excretion of small amounts of bile salt (10).

In the present study, certain aspects of the *endogenous* fetal bile salt pool—size, distribution, constituent bile salt, extent of enterohepatic circulation—were characterized; and quantitative data on the fetal hepatic bile salt excretory mechanism, including its capacity to excrete a large exogenous load, were collected. The results establish that the near-term fetus has a bile salt pool approximately half the size of that of the adult expressed on a body weight basis. The constituent bile salts of the fetus include deoxycholate, a secondary bile salt, which may be transferred to the fetus from the maternal circulation. The fetal liver responds to a taurocholate load with a 30-fold rise in bile salt output. Thus, the fetus, like the adult, has an immense reserve capacity for the excretion of bile salt.

METHODS

Surgical preparation

Studies were carried out with pedigreed beagles 1–3 yr old. Fetal experiments were undertaken in dogs with pregnancies of 56–58 days (63 days total gestation). All animals were worm-free and had been vaccinated against canine distemper and hepatitis.

Details of the surgical procedures used in fetal experiments have been previously described (10, 11). The methods employed avoided the loss of amniotic fluid during operation, and allowed continuous monitoring of the fetal electrocardiogram (ECG)[1] and blood pressure. In brief, under halothane anesthesia, a "marsupializing" incision was first made in the right subcostal region of the fetus. The gallbladder was aspirated, the cystic duct was tied, and the common bile duct was cannulated (polyethylene catheter, I.D. 0.011 inches, O.D. 0.024 inches); thereafter, fetal and uterine incisions were closed. Through a second "marsupializing" incision, the fetal jugular vein (polyvinyl tubing, I.D. 0.034 inches, O.D. 0.050 inches) and carotid artery (polyvinyl tubing, I.D. 0.023 inches, O.D. 0.038 inches) were cannulated. The second incision was closed, and the fetus was left lying free in the amniotic cavity. ECG electrodes were implanted into the forelegs and one hind leg of the fetus. The maternal cystic duct was ligated, and the common bile duct was cannulated (polyvinyl catheter, I.D. 0.053 inches, O.D. 0.085 inches) before closing the maternal incision.

Operative manipulation in nonpregnant adult dogs was confined to a laparotomy with ligation of the cystic duct, aspiration of the gallbladder, and cannulation of the common bile duct.

Experimental design

Endogenous hepatic bile salt excretion. All studies were carried out under light halothane anesthesia. In *fetal* experiments, half-hourly collections of fetal and maternal hepatic bile were obtained over periods up to 24 hr. At the end of

[1] *Abbreviations used in this paper:* ECG, electrocardiogram; FD$_{1-5}$, fetal dogs, numbers 1–5; GLC, gas-liquid chromatography; TLC, thin-layer chromatography.

TABLE I
Details of Fetuses Infused with Taurocholate Load

Experiment	Infusion					Duration of experiment
	Dose	Specific activity	Volume	Infusion time	Maximal infusion rate	
	μmoles	*dpm/μmole*	*ml*	*hr*	*μmoles/hr*	*hr*
FD$_1$	290	—	5.0	5.5	180.0	10.0
FD$_2$	343	—	6.2	7.0	72.0	24.0
FD$_3$	412	4643	8.0	7.0	180.0	13.0
FD$_4$	412	5250	8.0	7.0	180.0	10.5
FD$_5$	551	3024	5.0	6.0	144.0	10.0

the end experimental period, 8–15 ml of heparinized blood was collected from the fetus by aortic puncture, and the plasma was immediately separated by centrifugation. The fetus was sacrificed, and the abdominal and thoracic viscera together with samples of amniotic fluid and placenta were kept for analysis. Plasma, gallbladder bile, liver, and intestines were obtained from the litter mates of the operative fetus. Samples of maternal plasma, gallbladder bile, liver, kidney, and urine were likewise kept for analysis. Specimens were stored at $-15°C$ until analyzed. In nonpregnant *adult* experiment, hepatic bile was collected at half-hourly intervals over 6–14 hr, and samples of peripheral plasma were obtained. At the conclusion of the experimental period, the dog was sacrificed; as above, samples of gallbladder bile, liver, kidneys, and urine were kept for analysis.

Excretion of a taurocholate load. Five fetuses (FD$_{1-5}$) weighing 263±29 (mean ±SE) were infused with large amounts of sodium taurocholate (Table I). Doses were prepared by dissolving sodium taurocholate (Calbiochem, Los Angeles, Calif.) in 0.9% sodium chloride to form a 60 mM solution, which was titrated to pH 7.4 with 0.1 N sodium hydroxide. The purity of the taurocholate was established by thin-layer chromatography (TLC) in two systems: system I, butanol : water : acetic acid (85 : 10 : 5), and system II, chloroform : methanol : water : acetic acid (65 : 20 : 10 : 5); by gas-liquid chromatography (GLC) using methods described below; and by titration with hydrochloric acid. Sodium taurocholate-^{14}C (SA 2.50–3.12 × 10^4 dpm/μg) was prepared biosynthetically (10) or synthetically (12) from sodium cholate-^{14}C (New England Nuclear Corp., Boston, Mass.) and in FD$_{3-5}$ it was added to each dose, to give a final specific activity of 3024–5250 × 10^3 dpm/μmole (Table I).

The rates of infusion and total infusion time were varied in each fetus in order to assess fetal excretory capacity. The total amount of taurocholate infused into the fetal jugular vein varied from 290–551 μmoles, over 5.5–7 hr, and the duration of study was 10–24 hr (Table I). Maximal infusion rates did not exceed 180 μmoles/hr. Fetal blood samples were obtained throughout the study (FD$_{3-5}$ only) from an indwelling carotid arterial catheter. Fetal bile was collected at half-hourly intervals, and half-hourly collections of maternal bile were obtained. Maternal blood was taken by multiple venipunctures from the external jugular vein. Fetal and maternal ECG's were monitored throughout, and at the conclusion of study, mother and fetus were autopsied, and tissues were obtained for analysis.

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Analytical procedures

Radioactivity in bile, plasma, urine, and amniotic fluid was assayed in a Packard Tricarb liquid scintillation spectrometer (Packard Instrument Co., Inc., Downers, Grove, Ill.) as previously described (13). Solid tissues were weighed, portions were homogenized, and radioactivity was assayed by established methods (14).

Fetal arterial blood samples were analyzed for pH, P_{CO_2} and P_{CO_2} using an Instrumentation Laboratory pH/Gas Analyzer, Model 113 (Instrumentation Laboratory, Inc., Lexington, Mass.).

Total bile salt concentrations in bile samples were measured enzymatically by a modification (15) of the method of Talalay (16). Individual bile salts in fetal and maternal bile were initially identified by TLC on silica gel G (17, 18). The following solvent systems were used: systems I and II (see above); system III, isoamyl acetate: propionic acid: n-propanol: water (4:3:2:1); system IV, iso-octane: ethyl acetate: acetic acid (10:10:2); and system V, benzene: dioxane: acetic acid (75:20:2). Samples of fetal bile were hydrolyzed in 5 vol of 1 N NaOH at 3 atmospheres and 250°C over 3 hr. Fetal bile was run in systems IV and V before hydrolysis to detect the presence of any unconjugated bile salts.

Plasma and biliary bile salts were extracted, identified, and quantitated by GLC through a modification of the method of Sandberg, Sjövall, Sjövall, and Turner (19). Radiochemically pure taurocholate-^{14}C was used an an internal standard in order to monitor recovery. Methylated trifluoroacetate derivatives of bile salt were chromatographed on a Packard gas chromatograph (7400 series; Packard Instrument Co., Inc.) using 1% QF1 on Gas Chrom Q and a hydrogen flame ionization detector (conditions: helium carrier gas with flow rate 60 ml/min, inlet temperature 230°C, column temperature 210–220°C, detector temperature 240°C).

Samples of fetal tissues and gut contents were prepared for GLC by refluxing 2 ml of an aqueous homogenate in 1 N NaOH and 90% ethanol for 1 hr and, after cooling, extracting it three times with 50 ml of petroleum ether (20). The petroleum ether phase was backwashed with 10 ml of 1 N NaOH in 50% ethanol. The aqueous phase was placed on Amberlyst A-26 columns (Rohm and Haas Co., Philadelphia, Pa.), and subsequent extraction and purification was the same as for plasma and bile samples.

The intestinal contents of four fetuses were cultured aerobically and anaerobically after sampling under strictly sterile conditions.

RESULTS

Endogenous fetal bile salt

Identification of bile salts in fetal bile. Taurocholate, taurochenodeoxycholate, and taurodeoxycholate were identified by TLC and GLC in both hepatic and gallbladder bile samples obtained from 13 fetuses. Two bands with Rf values identical to those obtained with taurocholate and taurochenodeoxycholate (and/or taurodeoxycholate) standards were demonstrated by TLC in systems I–III when unsaponified extracts of bile were applied to the thin layer (Fig. 1A). There was no demonstrable unconjugated fetal bile salt; this finding was confirmed by the single band observed at the

origin on TLC of the unsaponified material in Systems IV and V (Fig. 1B). Bile extracts were chromatographed in systems IV and V after rigorous saponification. In system IV, which satisfactorily separates chenodeoxycholate from deoxycholate, three distinct bands corresponding to those of cholate, chenodeoxycholate, and deoxycholate standards were demonstrated (Fig. 1C). In system V, two bands were demonstrated corresponding to those of the cholate standard and the coincident chenodeoxycholate and deoxycholate standards. The methylated trifluoroacetate derivatives of saponified fetal bile salt showed GLC peaks with retention times identical to cholate, chenodeoxycholate, and deoxycholate standards (Fig. 1D). An additional unidentified major peak with a retention time similar, but not identical, to lithocholate was consistently present.

The relative amounts of taurocholate, taurochenodeoxycholate, and taurodeoxycholate in fetal bile were determined by GLC of the saponified methylated trifluoroacetate derivatives. Some variation was observed, but the proportions of cholate to chenodeoxycholate to deoxycholate were approximately 3:1:1 in most specimens of both gallbladder and hepatic bile.

Bile salt concentration in fetal bile. Gallbladder bile was collected from 39 fetuses. The bile salt concentration in the gallbladder was 29.4–173.3 μmoles/ml (73.9 ±8.6 μmoles/ml) (mean ±SE), and the volume of bile was 0.15–0.58 ml. The total bile salt content of the fetal gallbladder varied from 5.1–37.9 μmoles (17.4±1.7 μmoles); there was no relationship between fetal weight and gallbladder bile salt content. For comparison, the bile salt concentrations in the gallbladder bile of 11 adults were 72.9–295.0 μmoles/ml (177.5±20.7 μmoles/ml), and the bile salt content was 729–3360 μmoles (2244±346 μmoles).

Gallbladder bile salt concentration was compared with maximal hepatic bile salt concentration in 13 fetuses with external biliary drainage. The average fetal gallbladder bile salt concentration (73.9±8.6 μmoles/ml) was four to five times greater than the average maximal fetal hepatic bile salt concentration (18.7±1.5 μmoles/ml). This compares with an average 15-fold difference observed in eight adults with biliary drainage.

Rate of excretion of endogenous bile salt after cannulation of the fetal bile duct. Data were obtained from 13 fetuses weighing 257±13.4 g for periods of biliary drainage ranging from 2.7–24 hr. As can be seen in Fig. 2, in 11 of 13 studies, the initial rate of excretion of bile salt was the highest observed, and subsequent rates diminished toward or to low plateau values. The average initial excretion rate was 2.04 ±0.34 μmoles/hr, the average maximal observed rate

TLC   GLC
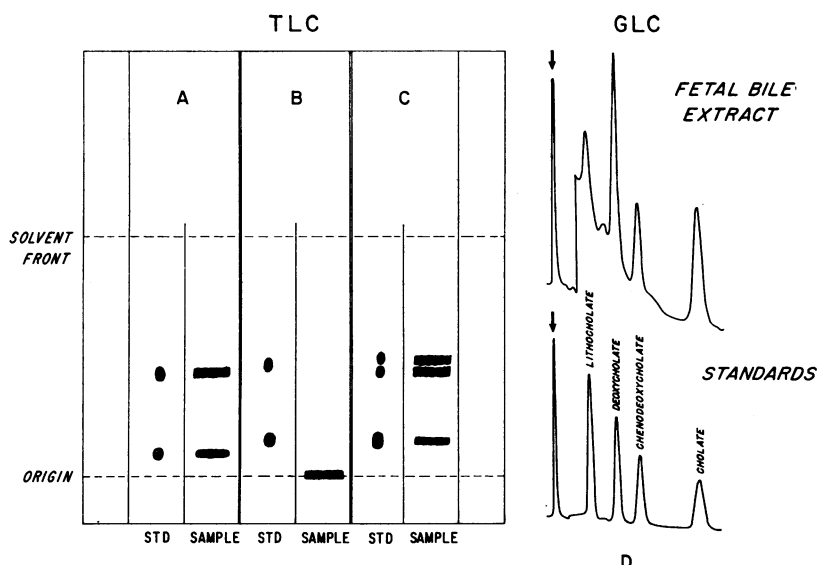
FIGURE 1 (A) TLC of 10 μl of unsaponified fetal bile on silica gel G over 1 hr in system I, showing a band (Rf = 0.09) corresponding to taurocholic acid, and a second band (Rf = 0.44) corresponding to taurodeoxycholic acid/taurochenodeoxycholic acid standards. (B) TLC of unsaponified fetal bile in system V. The single band at the origin indicates that no unconjugated bile salt is present. (C) TLC of rigorously saponified fetal bile in system IV. Three distinct bands, corresponding to cholic acid (Rf = 0.14), chenodeoxycholic acid (Rf = 0.43), and deoxycholic acid (Rf = 0.49) are present. (D) GLC tracing of methylated trifluoroacetic acid derivatives of saponified fetal bile salts (above) and bile acid standards (below). Peaks with retention times identical with cholic acid, chenodeoxycholic acid, and deoxycholic acid are present in saponified fetal bile. The arrow indicates the cholanate internal standard. The large peak with a retention time similar to lithocholate and the small peak with a retention time between those for lithocholate and deoxycholate have not been identified.

was 2.07±0.33 μmoles/hr, and the average final rate 0.39±0.07 μmoles/hr. In one fetus observed for 24 hr, excretion remained constant at 0.1 μmoles/hr for the last 8 hr of study. Bile volumes did not decrease to the same extent as bile salt output, and in several studies bile flow remained virtually constant. Initial bile flow rates averaged 0.12±0.01 ml/hr and final flow rates averaged 0.09±0.01 ml/hr.

Fig. 3 provides comparable data for 13 (nonpregnant) adult animals weighing 12.37±0.79 kg with external biliary drainage. The over-all adult pattern of excretion was more variable than that of the fetus. In some studies, peak excretion rates were observed initially, while in others initial rates were low and rose during the period of observation. The average initial, maximal, and final bile salt excretion rates were 68.5 ±10.7, 85.7±14.2, and 33.9±10.5 μmoles/hr, respectively. In a single study continued for 24 hr, the final excretion rate equalled 3 μmoles/hr.

Tissue bile salts. Fetal plasma bile salt concentrations, which in 14 fetuses averaged 1.01±0.24 μg/ml,

were significantly higher by the *t* test (*P* < 0.05) than those found in nine nonpregnant adult animals (average 0.36±0.06 μg/ml). Assuming a fetal plasma vol-
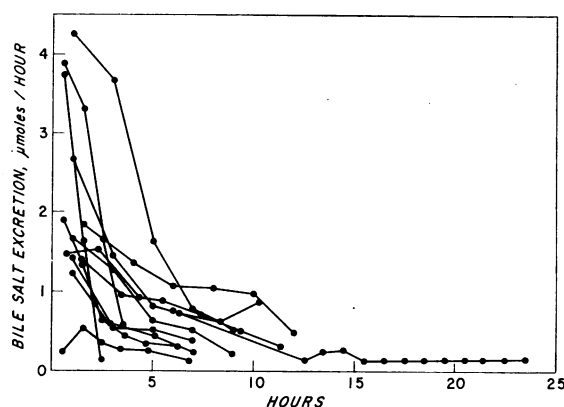

FIGURE 2 Hepatic excretion of endogenous bile salt in 13 fetuses after the establishment of the external biliary fistula. Bile salt excretion falls toward a plateau in a manner analogous to the "washout" curve seen in adult animals (21, 22).
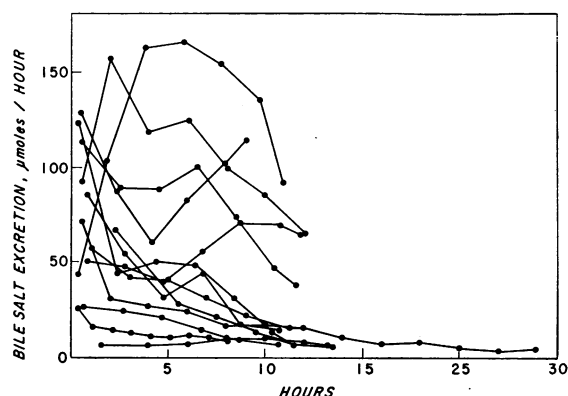
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FIGURE 3 Hepatic excretion of endogenous bile salt in 13 *adult* dogs over periods up to 24 hr.

ume of 9% of body weight,[3] it was calculated that fetal plasma contained 0.03–0.2 μmole of bile salt. Cholate, chenodeoxycholate, and deoxycholate were identified by GLC in both fetal and adult plasma. Although specimens varied, the relative proportions were approximately 2 : 1 : 1.

The bile salt content of fetal small bowel, colon, and liver was measured in five fetuses. Small bowel contained 6.0±1.8 μmoles, colon contained 1.1±0.3 μmoles, and liver contained 1.2±0.5 μmoles. The bile salt content of fetal kidney, spleen, lung, heart, and placenta was below measurable levels.

Fetal bile salt pool. The total bile salt pool, estimated in 15 fetuses weighing 260±11 g, was 30.0±2.7 μmoles, of which 71±4% was in the gallbladder, while 29±4% was estimated to be in the enterohepatic circulation. In five instances, the pool was considered to be the sum of the bile salt contents of the gallbladder, bowel, liver, and plasma, all of which were measured directly. In the remaining 10 fetuses, the gallbladder pool was removed, the cystic duct was tied, the common bile duct was cannulated, and hepatic bile salt excretion was measured for 2.7–13 hr. The "washout" curve of tissue bile salt was considered to be analagous to that found in adult animals (21, 22), and was taken to represent the part of the bile salt pool outside the gallbladder at the time biliary drainage was established. For these 10 fetuses, the total bile salt pool was estimated by adding gallbladder pool plus bile salt excreted by the liver. The two methods for estimating bile salt pool gave similar results (34.0±4.1 and 29.3±3.6 μmoles for direct estimated and "washout" method, respectively) ($P > 0.2$).

For comparison, the total bile salt pool measured by the "washout" method in eight nonpregnant adults

[3] Novy, M. J., G. J. Piasecki, and B. T. Jackson. Unpublished data.

weighing 12.40±1.2 kg, was 2945±348 μmoles, of which 74±8% was in the gallbladder while 26±8% was estimated to be in the enterohepatic circulation. The adult pool size, expressed relative to body weight, was twice that of the fetus (247.2±33.1 vs. 119.2±11.3 μmoles/kg body weight) ($P < 0.01$).

Fetal excretion of an exogenous bile salt load

Effects of experimental manipulation. Preliminary studies were performed in order to observe the effects of increasing taurocholate infusion rates on certain parameters of fetal homeostasis. When taurocholate was infused into a fetus at a rate of 500 μmoles/hr for 60 min there was frank intravascular hemolysis together with marked ECG changes. Therefore, for the present study maximal infusion rates were kept well below this level. Fetuses remained in excellent condition throughout the experiments, and heart rates, blood pressure levels, and ECG tracings remained essentially unaltered. Measurements of arterial P_{CO_2}, P_{O_2}, and pH showed values consistent with those reported in earlier studies (23). Multiple blood sampling caused fetal hematocrits to fall by no more than 8%.

Distribution and excretion of taurocholate load. Five fetuses were infused with a taurocholate load (FD_{1-5}, Tables I, II). In three of these, radioactive tracer (taurocholate-^{14}C) was added to the taurocholate load so that changes in plasma bile salt concentration could be measured conveniently (FD_{3-5}, Fig. 4). Plasma levels rose with increasing infusion rates and reached maximum concentrations of up to 1124 μg/ml by the end of the infusion. Thus, concentrations approximately 300
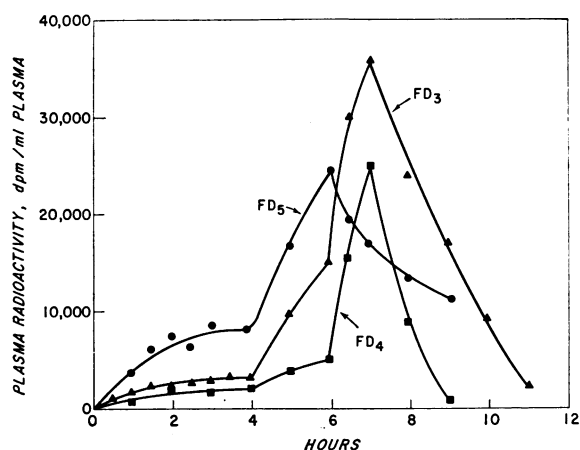

FIGURE 4 Plasma levels of radioactivity with the infusion of a taurocholate load labeled with radioactive tracer (taurocholate-^{14}C) in three fetuses (FD_{3-5}); FD_3 and FD_4 were infused for 7 hr, FD_5 for 6 hr. At the end of infusion less than 10% of the taurocholate load remained in the plasma compartment. Ordinate is corrected for weight and dose.

TABLE II
Fetal Infusion of Taurocholate Load

Experiment	Bile salt (BS) excretion rates		Bile flow rates		Hepatic BS conc.		Gall bladder BS conc.	Maximum BS plasma BS conc.	Fetal BS excretion at end of infusion	Final distribution of radiolabel				
	Initial	Max	Initial	Max	Initial	Max				Fetal bile	Fetal liver	Fetal plasma	Placental transfer	Total
	μmoles/hr		ml/hr		μmoles/ml		μmoles/ml	μg/ml	% of dose	% of dose				
FD₁	1.3	43.1	0.07	0.85	18.0	50.7	71.5	—	30.1	—*	—	—	—	—
FD₂	1.1	39.5	0.08	1.36	15.5	42.2	29.4	—	26.7	—*	—	—	—	—
FD₃	3.3	41.1	0.15	1.77	22.1	30.8	42.5	1123.8	49.3	93.6	0.0	0.0	4.5	98.1
FD₄	2.0	61.5	0.17	2.02	12.9	39.9	56.5	430.2	64.2	93.2	0.0	0.0	1.6	94.8
FD₅	1.5	73.6	0.07	2.16	19.9	82.8	—	650.6	64.1	92.3	1.6	2.1	0.6	96.6
Mean	1.8	51.8	0.11	1.63	17.7	49.3	50.0	734.9	46.9	93.0	0.5	0.7	2.3	96.5

* In FD₁ 87.3% of the dose, and in FD₂ 91.3% of the dose, was excreted into fetal bile as measured enzymatically. FD₁ and FD₂ did not receive radiolabeled taurocholate.

times the highest normal value observed were reached (Table II). At the end of infusion, however, the plasma compartment contained only 9.9, 6.6, and 6.7% (40.1, 28.1, and 35.8 μmoles) of the infused dose of taurocholate-¹⁴C in FD₃, FD₄, and FD₅, respectively. Plasma concentrations fell abruptly after terminating the infusion and in FD₃ and FD₄ reached normal levels by the end of the study.

Fetal biliary excretion of exogenous taurocholate began within the first 20 min and increased during infusion (Fig. 5). The maximal excretory rate observed varied from 39.5 to 73.6 μmoles/hr (i.e., up to 5 μmoles/kg body wt per min). In four fetuses, the maximal excretion rate occurred after the end of infusion while plasma levels were falling (Fig. 5). In

one (FD₅), maximum excretion occurred during infusion. In this latter study, and in one other (FD₂), the rate of biliary excretion of taurocholate-¹⁴C diminished before the end of infusion.

Bile salt excretion increased on average 30-fold (1.8–51.8 μmoles/kg); this was largely mediated by a 15-fold increase in bile volume (0.11–1.63 ml/hr) (Table II). During periods of increasing bile salt output, there was a close correlation between bile salt excretion and bile volume (Table III). Bile salt concentrations also increased (Table II) but to a much lesser extent (two- to threefold) than volume. This increase occurred within the 1st hr; thereafter bile salt concentrations remained relatively steady. Thus, over most of the range of increasing bile salt output, bile volume was the sole determinant of the increase. The slopes and intercepts of the regression equations of bile volume and bile salt output differed among the five fetuses (Table III). Furthermore, during the phase of decreasing bile salt excretion, the relationship between bile volume and bile salt output in each fetus altered. A representative example of this effect is illustrated in
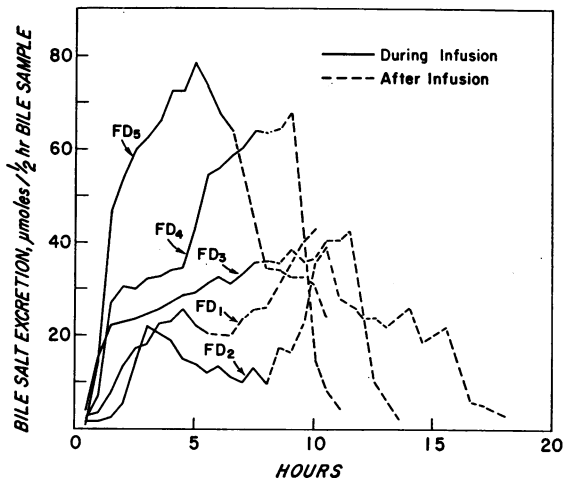

FIGURE 5 Fetal biliary excretion of an infused taurocholate load during and after completion of infusion. In FD₂ and FD₅ excretion rates fell for a period before the end of the infusion. The maximal excretion rate was on average 30-fold greater than the initial excretion of endogenous bile salt.

TABLE III
Fetal Infusion of Taurocholate Load

Experiment	Regression equation*	Correlation coefficient r
FD₁	$y = 0.02x + 0.09$	0.95
FD₂	$y = 0.03x + 0.15$	0.87
FD₃	$y = 0.04x - 0.09$	0.98
FD₄	$y = 0.03x + 0.07$	0.99
FD₅	$y = 0.01x + 0.06$	0.98

* Bile salt output μmoles/hr (x) on bile volume ml/hr (y) during *increasing* bile salt excretion.
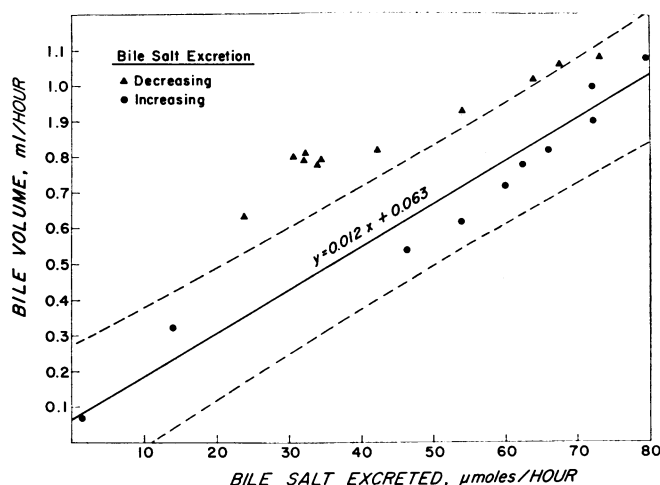
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FIGURE 6 · Representative graph of the relation between bile volume and bile salt output during the excretion of a taurocholate load (FD₅). Values obtained during *decreasing* bile salt excretion lie above the 95% confidence limits of the calculated regression line (method of least mean squares) for values obtained while bile salt excretion is *increasing*.

Fig. 6, where "decreasing" values can be seen to lie outside the 95% confidence limits of the regression line for "increasing" values. The position of the "decreasing" values *above* the "increasing" regression line shows that bile salt concentrations are appreciably lower during this period. This was found in all fetuses studied (FD₂₋₅) during the return of bile salt excretion to resting levels.

Of the total infused dose, 87.3–93.6% appeared in fetal bile by the end of the study. The excreted radioactive material was identified as taurocholate-¹⁴C by TLC. Placental transfer to the maternal circulation accounted for a small proportion of the infused taurocholate-¹⁴C (0.6–4.5% of the dose) (Table II). Small amounts of radioactivity were found in the fetal liver at the end of the study. Total recovery of radioactivity was 95–98% of the dose.

DISCUSSION

The results of the present study together with those published previously characterize the main outlines of bile salt transport and metabolism in the near-term fetal dog. Bile salt is effectively cleared from the fetal plasma by the fetal liver. It was shown previously that less than 5% of tracer amounts of cholate-¹⁴C administered i.v. accumulated in fetal plasma, the bulk of the remainder being cleared from the circulation by the fetal liver (10). Fetal to maternal transfer did not appear to play a significant role in plasma clearance of cholate-¹⁴C. The present finding that virtually all endogenous fetal bile salt is confined within the liver, biliary tree, and intes-

tine also suggests effective fetal hepatic plasma clearance. When taurocholate was infused at rates up to six times the total fetal content of endogenous bile salt per hour, the amounts accumulating in plasma never exceeded 10% of the infused load, and the bulk of the infused material was rapidly removed from the circulation by the fetal liver. Hepatic uptake of bile salt is thus highly developed in the dog fetus near term.

Bile salt is conjugated with taurine before excretion into fetal bile. Tracer amounts of cholate-¹⁴C administered i.v. were. recovered from fetal bile as taurocholate-¹⁴C (10). Similarly, each of the endogenous bile salts identified in fetal hepatic and gallbladder bile was in the form of the taurine conjugate. Bile salt conjugation in fetal dogs, therefore, resembles that in the adult which also excretes taurine conjugates primarily. This contrasts with the primate, however, in which fetal and adult bile salt conjugation differ. The fetal monkey, like the fetal dog, excretes predominantly taurine conjugates, while approximately equal amounts of taurine and glycine conjugates are found in the bile of the adult (24). Studies in newborn infants suggest inadequate hepatic conversion of *p*-aminobenzoate to *p*-aminohippurate (25). The low level of glycine conjugated bile salt in fetal monkey bile may, therefore, result from deficient enzymatic formation of glycine conjugates by immature primate liver. There was no attempt to assess the maximal rate of fetal hepatic conjugation in the present study.

Bile salt is secreted by the fetal liver into the bile ducts and gallbladder. After 10 hr, 15% of tracer cholate-¹⁴C administered i.v. to fetal dogs was recovered

from the duodenum and jejunum; however, entry into the intestine was eliminated by biliary diversion (10). In the present study, up to one-third of endogenous fetal bile salt was in the small bowel. The intact fetal biliary tree, therefore, is open and bile enters the fetal intestine as well as the gallbladder. When the gallbladder was emptied and fetal biliary drainage was instituted, bile salt excretion fell progressively from initial high levels. It is conceivable that this pattern of excretion was due to the diminution of either maternal to fetal placental transfer or fetal synthesis of bile salt coincident with fetal biliary cannulation. Its resemblance to adult excretion patterns (21, 22), however, suggests as the most likely explanation the "washout" of bile salt from loci outside the gallbladder (i.e., from the intestine, plasma, and liver) from which we may infer the reabsorption of the bile salt from the fetal gut under normal circumstances. In addition, fetal intestinal absorption of bile salt has been confirmed by direct study (26). The near-term dog fetus thus shows evidence of a functioning enterohepatic circulation of bile salt.

After drainage for several hours fetal bile salt excretion tended toward low plateau levels. Present technical limitations prevent the observation of fetal bile flow for prolonged periods, but excretion remained constant at 0.1 μmoles/hr for the last 8 hr of the longest study, and it is reasonable to assume that this figure represents a "basal" excretory rate. Indirect evidence suggests that during periods of basal fetal excretion, the excreted bile salt may have been transferred from mother to fetus and/or synthesized by the fetal liver. In support of the former possibility was the identification of deoxycholate in fetal bile by TLC and GLC (identification has been confirmed in preliminary studies by mass spectroscopy).[3] Deoxycholate is a "secondary" bile salt derived from the intestinal bacterial 7-dehydroxylation of cholate (2). Although parasitic infestation has been reported, the fetal dog intestine is thought to be bacteria-free (27), as was confirmed experimentally in the present study. Barring direct fetal synthesis or the presence of undetected microorganisms in fetal intestine, deoxycholate must originate in the maternal circulation which implies maternal to fetal transfer of at least this specific bile salt. However, even if the deoxycholate in fetal bile did indeed originate in the mother, it may have crossed the placenta at an earlier period of gestation and persisted within the fetus. The fact that the mother may well not serve as a continuing source of bile salt for fetal excretion is suggested by the finding that fetal plasma bile salt concentrations are slightly higher than adult levels, and that cholate-^{14}C and taurocholate-^{14}C infused into the maternal circulation showed minimal accumulation

in fetal bile.[4] The observed basal fetal bile salt excretion, therefore, may in part or whole represent fetal hepatic synthesis. Assuming an average fetal pool of 30.9 μmoles, a basal excretory rate of 0.1 μmoles/hr could indicate synthesis of 8% of pool size per day. The exact origin of fetal bile salt, however, remains to be demonstrated definitively.

Several observations suggest that the dog fetus has not fully completed the development of adult hepatobiliary function during the late stage of gestation near term. Evidence was developed previously of a functional hepatic bypass with the escape of bile absorbed from the intestine into the systemic circulation (10). This may explain the present finding that mean values for fetal plasma bile salt concentrations were higher than those of adults (1.02 vs. 0.36 μg/ml, respectively). In addition, the average bile salt concentration in fetal gallbladder bile was 73.9 μmoles/ml, compared with 177.5 μmoles/ml in the adult. Similarly, the average fetal gallbladder bile salt concentration was only four to five times the average maximal fetal hepatic bile salt concentration observed after biliary diversion, while a 15-fold difference was noted in adults. This suggests that the fetal gallbladder concentrates bile less effectively than the adult, but alternative explanations (e.g., longer average retention of the bile in the adult gallbladder; partial emptying of stratified dilute bile by the adult gallbladder) cannot be ruled out. Fetal bile salt pool size expressed in relation to body weight averaged less than half that of the adult. Adult pool size was estimated only approximately by summing the quantity of bile salt in the gallbladder and that "washed out" during the period of study. Although there was good agreement between fetal pool size estimated by washout and by direct measurement, in five of the adults (Fig. 3) washout appeared to be incomplete. Adult pool size may, therefore, have been underestimated, and the difference between fetus and adult was possibly greater than the comparison of estimated pool size suggests.

The maximal rate of fetal bile salt excretion in response to loading equaled 5 μmoles/kg body wt per min. This represents an increase of approximately 30-fold over average initial rates after bile duct catheterization (Table II) and 200-fold over the average minimal rate. The quantities of bile salt necessary for comparative adult studies were not available. Ó'Máille reports maximal rates of 8.5 μmoles/kg body wt per min in anesthetized adult dogs infused with taurocholate (28). Differences in technique make comparison difficult. High rates of fetal infusion were associated with ECG abnormalities and intravascular hemolysis in preliminary studies. Moreover, in four fetuses (FD$_{1-4}$) maximal excretion

[3] Klein, P. D., R. A. Smallwood, B. T. Jackson, and R. Lester. Manuscript in preparation.

[4] Smallwood, R. A., G. J. Piasecki, B. T. Jackson, and R. Lester. Unpublished data.

rates occurred only after the end of infusion as plasma bile salt concentrations were falling, and in FD₂ (Fig. 5), there appeared to be overt inhibition of excretion during the later stages of infusion. "Maximal values" in both the fetus and adult are, therefore, dictated by the toxicity of the infused bile salt rather than saturation of an excretory mechanism, but it is clear that the fetal liver has immense reserve capacity for the excretion of bile salt.

The relation between fetal bile volume and bile salt excretion varied with the conditions of study. After catheterization of the fetal bile duct, bile volumes remained relatively constant (mean initial flow 0.12 ml/hr; mean final flow 0.09 ml/hr) while bile salt excretion rates fell fivefold. At low excretory rates, therefore, fetal bile flow appeared to be controlled by factors independent of bile salt excretion (29). In the adults dog, bile flow is reported to diminish with bile salt excretion as the pool is exhausted (30), and in the present study a threefold drop in adult bile salt excretion was accompanied by a twofold drop in flow rate. However, the difference between fetus and adult may have been the result of anesthesia, and the operative manipulation of the fetus at the time of study. These artifacts may have stimulated the release of secretin (31), or other choleretic factors (32), producing a dilute "basal" bile.

Bile volume was closely correlated with bile salt excretion at the high rates induced by taurocholate loading. A divergence was noted, however, between volumes associated with rising rates of excretion, and those with diminishing excretion. For a given bile salt output, bile volume was consistently greater with diminishing rates of excretion (after cessation of loading) than with increasing rates. This phenomenon clearly cannot be ascribed to differences in the osmotic "pull" of equal quantities of biliary taurocholate. Conceivably, prolonged bile salt excretion at high rates modified cannalicular or biliary water flow through the release of secretin or through "toxic" effects on the fetal hepatocytes, biliary epithelium, or their vasculature. Alternatively, the osmotic effect of high levels of sodium taurocholate in the plasma may have been sufficient to increase biliary water reabsorption and depress bile flow during the period of increasing excretion in FD₃ and FD₄. During the period of diminishing excretion, plasma taurocholate concentrations may have been lower in FD₃₋₄ (Figs. 4, 5), perhaps resulting in less water reabsorption and greater bile flow. Reabsorption of bile water due to the osmotic pressure exerted by plasma taurocholate, however, does not appear to be an adequate explanation for the similar bile volume differences observed in FD₅ (Fig. 6), in which plasma concentrations remained high during the phase of decreasing excretion (Figs. 4, 5). The explanation for the "hysteresis loop" of bile salt-bile volume relationships, therefore, remains unresolved.

From the results of the present and preceding investigations, it can be concluded that bile salt metabolism in the near-term fetal dog resembles, but is not identical to the adult pattern. Fetal plasma clearance and conjugation are effective, and the fetal liver has immense excretory reserve. On the other hand, the small bile salt pool, the apparent limited capacity of the gallbladder to concentrate bile, and the evidence of a functional hepatic bypass all suggest a degree of fetal "immaturity" as compared with the adult. The characterization of fetal intestinal absorption of bile salt, the evaluation of the rate of control of fetal bile salt synthesis, and the extension of these studies to newborn animals await future study.

ACKNOWLEDGMENTS

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