Postnatal Physiologic Hypercholemia in Both Premature and Full-Term Infants

S. BARNES, G. BERKOWITZ, B. I. HIRSCHOWITZ, D. WIRTSCHAFTER, and G. CASSADY, *Comprehensive Cancer Center and Department of Biochemistry, Division of Perinatal Medicine, Department of Pediatrics, Division of Gastroenterology, Department of Medicine, University of Alabama in Birmingham, Birmingham, Alabama 35294*

ABSTRACT Previous studies have shown that bile salt concentrations in human blood taken from the placenta at birth of term infants are in the range found in adults. A ^{125}I-radioimmunoassay procedure and capillary gas liquid chromatography-mass spectrometry have been used in this investigation to measure serum bile salt concentrations in premature and normal term infants. It was found that the serum bile salt concentration in samples taken at birth in premature infants were also similar to that of adults. In the week after birth the serum bile salt concentration rose four- to sevenfold in each of the infant groups. The increase was independent of gestational age and the "health" of the child. A similar increase was observed in term infants. Thus, hypercholemia is physiologic in newborn infants. In conjunction with other abnormalities of the enterohepatic circulation of bile salts there are profound implications in the newborn for the metabolism and excretion of those endogenous and exogenous substances that are dependent on the secretion of bile salt by the liver. In addition, speculations concerning the role of parenteral nutrition in the induction of cholestasis in premature infants should be made with caution.

INTRODUCTION

The role of bile salts in physiologic and pathologic states in newborn infants is an area of increasing interest to clinicians (1–3). Data from studies in human neonates and in fetal and neonatal animals have suggested that the enterohepatic circulation of bile salts is not fully developed at birth. Although both term and premature human infants are capable of bile acid synthesis and excretion, the rate of synthesis and the pool size are decreased in comparison to that in adults (4, 5), being lower in premature infants. It has been suggested that the reduced pool size could be a result of immaturity of the active transport system in the ileum from studies in newborn rats (6, 7) and human infants (8).

In the fetal state much of the toxic, lipophilic anions in the blood, such as bilirubin, are removed by the placenta. Inefficient hepatic uptake, conjugation and excretion of bile salts has been demonstrated in the fetal monkey (9). In other species, such as the dog (10), fetal hepatic function is more mature. Such studies suggest that the maturity of the hepatic excretory function at birth correlates with the amount of excretory function provided by the placenta (2).

In the newborn human it has not been established (11) whether the transport processes in the liver at the sinusoidal and canalicular membranes are as efficient in removing bile salts from the blood as those observed in adults (12), although in a recent study of the effects of parenteral nutrition in the newborn it was reported that the serum concentration of cholyl conjugates as measured by radioimmunoassay was markedly elevated (13).

In this study we have measured the bile salt concentration in sera of newborn infants, both premature and full term, during the first week of postnatal life, using a sensitive radioimmunoassay (14) and capillary gas chromatography-mass spectrometry. The data reveal that there is a four- to sevenfold rise during this period over values measured in blood at birth. The maximum values were reached by the fourth day. These data are consistent with the concept that hepatic transport mechanisms are immature in both premature and full-term newborn human infants.

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Address reprint requests to Dr. Barnes. Dr. Berkowitz's present address is Department of Pediatrics, University of South Carolina School of Medicine, Columbia, S. C.

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METHODS

Patient studies. The study was approved by the Investigational Review Board of the University of Alabama in Birmingham and permission was obtained from a parent before samples were collected from infants.

Blood samples (0.5 ml) were collected from infants of <2,100 g birthweight admitted to the Regional Newborn Intensive Care Unit, University of Alabama Hospitals, either by heel puncture or from indwelling arterial catheters at random over the first postnatal week. Because infants in the immediate postnatal period were feeding at least every 4 h or were receiving parenteral nutrition only, the classification into fasting and postprandial samples has not been made. Any postprandial changes in serum bile salt concentration would also be affected by the inefficient enterohepatic circulation (4–8) that might blunt such changes, as is found in adults with ileal dysfunction (15). Nevertheless, in a subsequent part of the study where serum bile salt concentrations were determined by capillary gas-liquid chromatography-mass spectrometry, the nutritional status of the infants is recorded (Table III). Infants classified as "sick" were those with respiratory disease requiring ventilation, who suffered perinatal asphyxia (Apgar score < 5 at 5 min), or with proven systemic infection. Low birthweight infants who were of appropriate weight for gestational age were divided into three groups, <1 kg, 1–1.50 kg, 1.51–2.1 kg. Infants who were small for gestational age were placed in a fourth group regardless of birthweight (16). Blood samples were also obtained from the placenta or umbilical cords of newborns and repeated by heel puncture on the third postnatal day at the time of phenylketonuria testing for the term infants. Whole blood samples were centrifuged at 11,500 rpm for 10 min, and the separated sera were stored at −20°C until analyzed.

Bilirubin measurements. Bilirubin, direct and indirect, was measured on 20-μl samples by a microspectrophotometric method (17).

Measurement of serum bile salts. Serum bile salt concentrations were measured by radioimmunoassay (RIA)[1] using the tracer cholylglycylhistamine-^{125}I prepared as previously described (14). Sera (10 μl) were diluted with bile salt-free serum prepared by treatment of pooled adult serum with charcoal and Dowex-1 resin (14) (Dow-Corning Co., Midland, Mich.). Sodium taurocholate was used as the bile salt standard since it had the greatest affinity of the normal bile salts for the antibody. The cross-reactivities with other bile salts previously reported for this antibody (14) were confirmed, namely taurocholate (100%), taurochenodeoxycholate (6%), glycocholate (33%), and glycochenodeoxycholate (70%). The taurine conjugate of 3α, 7α, 12α-trihydroxy-5β-cholestanoic acid which was isolated from alligator bile (courtesy of Dr. E. Jacobson, University of Florida) was found to cross-react with the antibody identically to taurocholate. This confirmed the degeneracy of immunological recognition of the side chain of bile salt conjugates as noted previously for glycine and taurine conjugates (18) and meant that the C_{27} bile salts would be detected if present in the sera. Serum bile salt concentrations were also measured using a different and commercially available antibody (Becton Dickinson bile acid RIA kit, Becton, Dickinson & Co., Rutherford, N. J.). The cross-reactivities for the major conjugated bile salts were taurocholate (100%), taurochenodeoxycholate (118%), glycocholate (41%), and glycochenodeoxycholate (100%). Serum bile salt concentrations were additionally measured by the fluorimetric 3-hydroxysteroid dehydrogenase procedure (19), using a 3-hydroxysteroid dehydrogenase from Sigma Chemical Co., St. Louis, Mo. (type H8879), which involved preliminary extraction of the serum with the resin XAD-7.

Gas-liquid chromatography. 23-Nordeoxycholic acid (2 nmol) was added as an internal standard to each of the samples to be analyzed and to blanks and mixtures of conjugated bile salts (0.2-2 nmol each). The serum extracts were dissolved in ice-cold 1 ml 1 M-NaOH and extracted with ice-cold ether (2 × 3 ml) to remove triglycerides and cholesterol and cholesterol esters without hydrolysis. Any ether remaining in the aqueous phase was removed by blowing nitrogen over the samples while warming them. They were then placed in 5-ml reactivials (Reliance Glassworks, Denville, Ill.), sealed with a cap containing a teflon liner and heated to 110°C overnight to hydrolyze the bile salt conjugates. Each hydrolysate was diluted with 9 ml distilled water and extracted with XAD-7 resin (19). The methanol eluates were evaporated to dryness and treated with 1 ml 2,2′-dimethoxypropane and 100 μl of 1 M-HCl (20) to cause solvolysis and formation of methyl esters. After overnight reaction the reagents were evaporated at room temperature. The residue was then treated with 100 μl of the silylation fluid (pyridine:hexamethyldisilazane:trimethylchlorosilane, 9:3:2, by volume) (21) at 50°C for 1 h, to form the trimethylsilyl ethers. Separation of the derivatives from the reagents was achieved by partition between *n*-hexane and water (22). The *n*-hexane phase was evaporated to dryness in a capillary concentration tube and redissolved in 20 μl toluene before injection into the gas chromatograph.

Mass spectral analyses were performed on a Hewlett-Packard 5985 gas chromatograph-mass spectrometer-computer system. A 24-m × 0.2-mm i.d. fused silica capillary column wall-coated with silicone phase (Hewlett-Packard Co., Palo Alto, Calif.) was used. The helium flow rate was 2 ml/min, the column effluent being directly passed into the mass spectrometer. Samples (0.2–2.0 μl) were injected in the splitless mode; the initial oven temperature was 60°C and the purge flow was interrupted for 1 min immediately after injection, after which it was returned to 60 ml/min. The oven temperature was programmed as follows: after 1 min 60–270° at 30°C/min and then 270° isothermally.

Analyses were carried out in the selected ion monitoring mode where only four m/z values were investigated at any one time, and in the scanning mode. In the former technique the unique fragment ion of m/z of 194.1 [D ring + side chain] was used to measure the internal standard, 23-nordeoxycholate. The C_{24} dihydroxy bile salts were quantified using m/z values of 255.3 [M-(3× TMSiOH + side chain)] and 370.4 [M-(2× TMSiOH)] and the C_{24}-trihydroxy bile salts using m/z values of 253.3 [M-(3× TMSiOH + S.C.)] and 368.4 [M-3× TMSiOH]. 3β-hydroxy-5-cholenoate was monitored using its unique ion at an m/z value of 331.3 [M-129]. In addition, the diagnostic fragment ions of m/z values of 412.3 [M-2× TMSiOH] and 410.3 [M-3× TMSiOH] for the C_{27} di- and trihydroxy bile salts, respectively, were monitored.

Quantitative measurements were carried out by computer integration of peaks observed for the particular fragment ions for each bile salt. These values were compared to the area of the 23-nordeoxycholate peak [m/z 194.1] in each unknown sample and related to a standard response curve for the bile salt derivatives in known mixtures.

Statistical methods. Analysis of variance and Duncan's multiple range test were used to compare the effect of time of sampling on serum bile acid concentrations between the low birthweight and normal term infants. Within-group comparisons were made using a paired Student's *t* test (23).

[1] *Abbreviation used in this paper:* RIA, radioimmunoassay.

RESULTS

Premature infants. Sera were collected over the first postnatal week from 53 infants of birthweight <2,100 g. Their mean birthweight was 1,290 g with a mean gestational age of 30.7 wk. The mean serum bile salt concentrations by day of this group are shown in Fig. 1. A significant upward trend from 9.4 μM at birth to 38.3 μM on day 4 was observed ($P < 0.05$, Duncan's multiple range test). Classification of the infants into three birth weight categories, <1,000 g, 1,000–1,500 g, and 1,501–2,100 g and small for gestational age, did not reveal any effect of body weight (Duncan's multiple range tests), (Table I). A significant increase in concentration was also demonstrated in those infants who had had samples taken on postnatal day 1 and days 2 or 3 ($n = 15$, $P = 0.017$, paired Student's t test). There was no difference between healthy and ill babies ($P = 0.25$, analysis of variance) (Table II). Mean serum total bilirubin concentrations rose from 3.3±0.5 mg/dl on day 1 to 6.8±0.6 mg/dl on day 4. There was no relationship between serum bilirubin and bile salt concentrations, except for the first postnatal day (Fig. 2).

Term infants. Blood samples taken at birth and on the third postnatal day were collected on eight full term infants with uncomplicated hospital courses. The mean birthweight of this group was 3,141 g. Mean serum bile salt concentration was 5.0 μM at birth; by the third postnatal day it had risen to 33 μM ($P = 0.046$, paired Student's t test). Including sera taken randomly from a further 23 term infants on the third postnatal day the mean bile salt concentration was 22 μM, not significantly different from that in low birthweight infants (28 μM) taken at the same time.

Using the upper limit (mean+ 2 SD) of normal for serum bile salts measured by our radioimmunoassay in normal fasting adults, 8 μM (14), as a criterion for qualitative comparison, five-sevenths (71%) of samples from low birthweight infants were in the adult range at birth compared with seven-fifteenths (47%) on day 1.

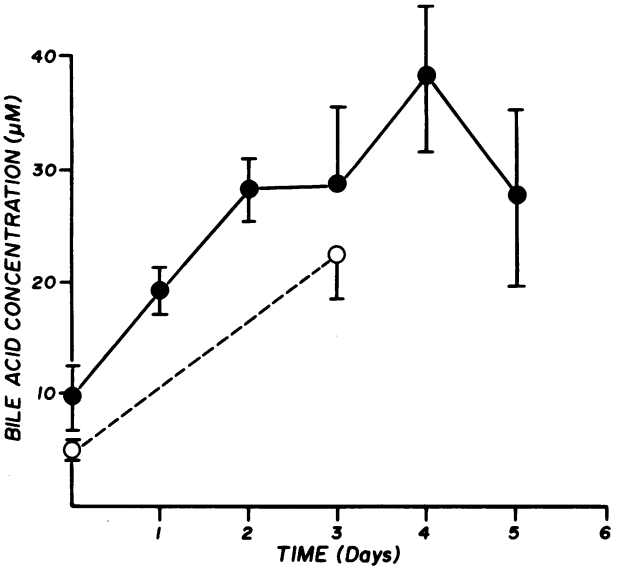

FIGURE 1 Effect of age (in days) on the serum bile salt concentration measured by radioimmunoassay in newborn infants, (○) normal term; (●) premature. Data given is mean±SE.

No normal levels were found on days 2 or 3 in these children. Blood taken at birth from seven-eighths (87%) of the term infants had adult levels of bile salts. On the third postnatal day only two-eighteenths (11%) of the term babies had normal adult levels of serum bile salts. Combining data from each of the infant groups a steady rise in the serum bile salt concentration with time after birth was observed (Fig. 1).

Verification that the radioimmunoassay procedure was indeed measuring bile salts was provided in several ways. Firstly, a second radioimmunoassay procedure using a solid-phase assay and a different antibody was used on 36 sera taken sequentially. A good correlation between the two procedures was observed ($r = 0.91, n = 36, P < 0.001$); each serum that gave a value in the normal range for the first assay (14)

TABLE I

Serum Bile Salt Concentrations (Micromolars) by Weight and Postnatal Age*

Group	Cord blood	Day 1	Day 2	Day 3	Day 4	Day 5–7
<1,000 g	9.4±2.7 (7)‡	20.7±5.2 (8)	36.3±10.5 (3)	29.5±9.5 (2)	41.5±6.5 (2)	18.2±3.8 (4)
1,000–1,500 g	—	17.2±1.7 (10)	25.6±4.9 (8)	29.6±7.9 (5)	38.7±6.8 (7)	39.3±8.3 (7)
1,500–2,100 g	—	17.8±7.3 (5)	31.2±4.9 (5)	22.8±5.0 (4)	35.5±5.1 (2)	14.4±2.4 (2)
SGA§	—	20.8±4.6 (9)	23.2±2.6 (5)	30.7±11.2 (5)	—	24.5±11.4 (6)
Term infants	5.0±1.0 (8)			22.3±4.1 (31)		

* Mean±SE, number of subjects in parentheses.
‡ Combined data from low birthweight group.
§ SGA, small for gestational age.

TABLE II

Paired Comparison of Serum Bile Salt Concentrations (Micromolars) with Respect to Days after Birth and Health of Premature Infants*

Group	Day 1	Day 3
Healthy	18.8±4.3 (9)	33.9±6.4 (9)
Ill	15.1±2.7 (6)	24.3±3.5 (6)

* Mean±SE, number of subjects in parentheses.
Analysis of variance showed that there is an effect due to time ($P = 0.001$), but no effect due to group ($P = 0.395$).

did so for the solid-phase assay. Secondly, a strong, positive correlation was also observed between data obtained using the radioimmunoassay (14) and the 3-hydroxysteroid dehydrogenase methods ($r = 0.88$, $n = 20$, $P < 0.001$) on 20 of the 36 sera for which sufficient serum (200 μl) was available. Thirdly, the immunoreactive substances were recovered from serum by XAD-7 extraction in high yield (80–92%), strongly suggesting that they were not proteins and were both water and lipid soluble.

GLC-Mass spectrometry. These studies (Table III) were performed on a further four sera from premature infants (gestational age 27–29 wk) and five from term infants, obtained 72 h after birth. In sera from term infants the major bile salts were chenodeoxycholate and cholate as confirmed by their identical retention times (Fig. 3) and fragmentation patterns to authentic standards of these bile salts (Fig. 4 and Table IV). No deoxycholate could be detected despite monitoring for its most prominent ion at m/z 255.2 [M-(2× TMSiOH + S.C.)]. Small amounts of 3β-hydroxy-5-cholenoate were detected in one preterm infant using the unique ion for this bile salt at m/z value of 331.3 [M-129]. Trace amounts of a material with the retention time appropriate to a C_{27} bile salt was detected at an m/z value of 410.3, i.e, probably a trihydroxy bile salt. However, it was not possible to obtain a mass spectrum in order to verify this.

Quantitative measurements using the selected ion mode produced bile salt concentrations in these sera which were significantly correlated to the RIA values (Table III). In sera from two of the premature infants the concentration of chenodeoxycholate was >20 μM. In the other two premature infants and in each of the term infants the concentration ranged from 8.2 to 14.6 μM. In each of the premature infants the concentration of chenodeoxycholate substantially exceeded that of cholate, whereas in sera from term children it was only 40% higher. In two of the premature infants cholate was present in only small amounts, although an unidentified trihydroxy bile salt with a retention time greater than that of cholate was detected. Lack of sample precluded the obtaining of a complete mass spectrum.

DISCUSSION

In this study we have shown that serum bile salt concentrations in cord blood from both premature and term infants are similar to those in adults, but rise sharply in the first postnatal week. These observations suggest that hepatic excretory function for bile salts is immature in the newborn. A cholestatic state with respect to bile salts may in fact by physiologic in the newborn period, similar to that observed for bilirubin. It is not possible to say whether this is due to deficien-

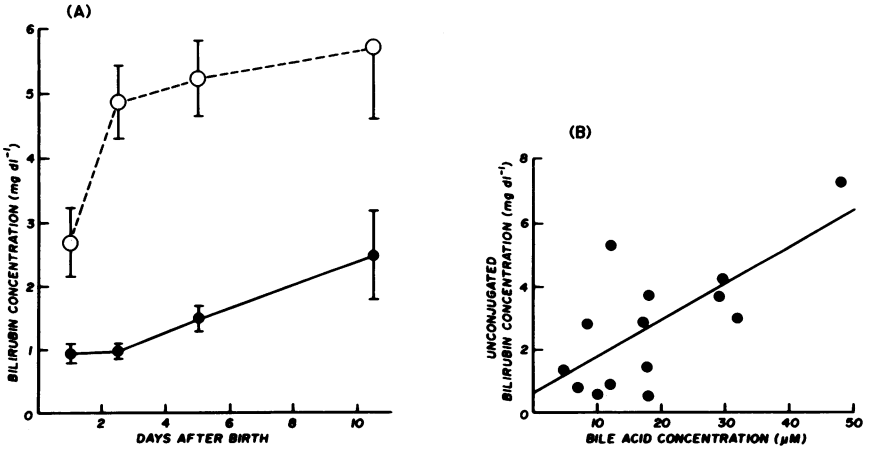

FIGURE 2 (A) Change in serum direct acting (●) and nondirect acting (○) bilirubin concentration over the first 10 d after birth in premature infants. Data given as mean±SE. (B) Relationship between serum nondirect acting bilirubin and bile salt (BS) concentrations during the first day after birth in premature infants. Regression line bilirubin = 0.115 × BS + 0.62, correlation coefficient, $r = 0.69$, $n = 14$, $P = <0.01$.

TABLE III

Clinical and Biochemical Data on Infants and Their Sera Used for GLC-MS Studies

	Number	Sex	Feeding status	Gestational age	RIA value*‡	C‡	CDC‡
				wk			
Preterm	1	M	Parenterally	28	29	4.4	24.9
	2	M	Parenterally	29	33.5	0.6	14.6
	3	M	Parenterally	28	21.9	0.7	8.0
	4	F	Fed, 1–2 h pp.	27	41	7.3	23.0
				Mean	30.9		
				±SEM	4.1		
Term	1	M	2–3 h pp.(20 cal)	39	11	3.2	7.1
	2	F	3 h pp. (20 cal)	38	26	7.2	9.8
	3	F	1 h pp. (20 cal)	39	27	14.3	12.7
	4	M	2–3 h pp. (20 cal)	37	18	17.6	10.8
	5	M	Breast fed	41	17	8.8	8.5
				Mean	24.5		
				±SEM	4.0		

* Micromolars relative to taurocholate.

‡ Regression relationship between RIA value (y) and total bile salt (x) (excluding preterm infants 2 and 3), y = 6.9 + 0.96x. This intercept was not significantly different from zero (t = 1.01); the slope had a standard error of 0.30, i.e. was significantly different from zero. C, cholate; CDC, chenodeoxycholate.

cies in sinusoidal or canalicular transport processes. Indeed, it raises the question as to whether the accumulation of bilirubin in the newborn is not only due to inadequate conjugation, but also impaired hepatic clearance.

Bile salt concentrations in blood taken from the newborn at birth have been previously shown to be similar to those from the mother and other normal fasting adults using gas-liquid chromatographic analysis (24, 25). In the present study the serum bile salt concentrations in blood taken from normal term infants 72 h after birth were markedly higher than in adults, whereas in blood taken from these infants at birth, they were within the adult range of concentration in seven-eighths tested. In a study on the effect of prolonged parenteral nutrition it was found that serum bile salt concentrations in young infants were seven times higher than adults using a RIA for cholyl conjugates (13). Our study confirms this data, and in addition demonstrates that the serum bile salt concentration only rises after birth.

The latter observation has been recently confirmed (26), using specific RIA for cholate and chenodeoxycholate conjugates; the concentrations of these bile salts start rising in the sera of term infants 1 h after birth. It was also shown that the hypercholemia persists for at least 6 mo in term infants before the serum bile salt concentration approaches that of adult levels (26, 27). Whether systematic differences exist between the premature and term infants in their ability to remove bile salts from blood beyond the study period remains to be answered. Part of the increased serum bile salt concentration could be due to stimulation of the enterohepatic circulation by feeding. However, systematic pre- and postfeeding samples were not obtained in this

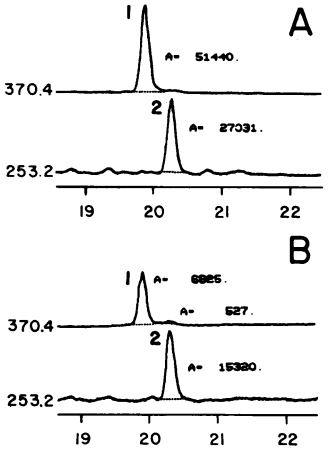

FIGURE 3 Single ion monitoring-capillary gas liquid chromatography of serum bile salts (partial chromatogram-time in minutes is the horizontal axis) from premature (A, 1) and term (B, 2) infants. The integrated areas of each peak are noted (1, chenodeoxycholate and 2, cholate). In A the standard 23-nordeoxycholate peak (monitored at m/z 194.1) had area of 125,228 and in B, 241,823. Serum equivalents of 1.1 μl for A and 0.62 μl for B were injected into the gas chromatograph for this analysis.

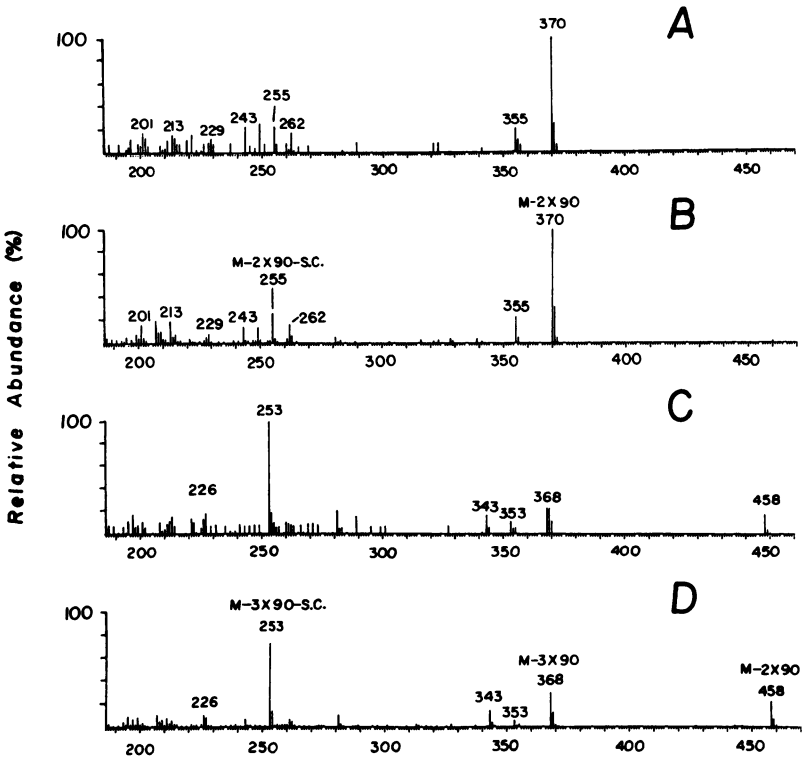

FIGURE 4 Partial (m/z range 185-470) mass spectra of authentic trimethylsilyl ether methyl ester derivatives of chenodeoxycholate (B) and cholate (D) compared to mass spectra obtained for compounds (A and C) detected by single ion monitoring (see Fig. 3).

study so it was not possible to assess this effect. Nonetheless, the two premature infants who had not been fed orally before sample collection had raised concentrations of chenodeoxycholate, but not cholate (an unidentified trihydroxy-bile salt was present). In addition, it has been suggested that the elevated serum bile salt concentrations in premature infants could be the result of shunting via the ductus venosus (28). However, the elevation was also present in term infants as shown by this study and others (13, 26, 27) and per-

TABLE IV

Relative Intensities of Fragment Ions of Authentic Standard and Unknown Bile Salts*

Fragment ion	Relative intensity		Fragment ion	Relative intensity	
	Authentic cholate‡	Unknown*		Authentic chenodeoxycholate‡	Unknown*
	%			%	
226 M-(3x TMSiOH + C-17 + C-16 + S.C.)	14	12	201 M-(2x TMSiOH + Ring A + S.C.)	18	18
253 M-(3x TMSiOH + S.C.)	100	100	213 M-(2x TMSiOH + Ring D + S.C.)	20	17
281 M-(3x TMSiOH + C-22 – C-24)	14	19	229 M-(2x TMSiOH + C-17 + C-16 + S.C.)	10	11
343 M-(2x TMSiOH + S.C)	19	17	243 C-3–C-7	15	21
368 M-(3x TMSiOH)	38	22	249 M-(Ring A + Ring B)	14	24
458 M-(2x TMSiOH)	29	18	255 M-(2x TMSiOH + S.C.)	23	22
			262 M-(Ring A + C-6 + C-19)	17	16
			355 M-(2x TMSiOH + CH_3)	23	22
			370 M-(2x TMSiOH)	100	100

* From premature infant 4. The spectra from this sample were corrected for contribution from the column bleed.
‡ As the trimethylsilyl ether methylester derivatives.

sisted for at least 6 mo (26, 27). This suggests that shunting of blood past the liver cannot be the explanation and that deficiency in the transport processes is probably occurring.

It appears then that the hepatic insufficiency with respect to bile salts in the newborn also exists in the fetal state, but is compensated for via the placental circulation (2). Since hepatic bile salt synthesis is also depressed in the neonates (5), as is small intestinal transport of bile salts (6–8), the degree of hepatic insufficiency is greater than would be interpreted from comparison to concentrations in adults with hepatobiliary disease.

The presence of bile salts in the serum samples was confirmed using a commercially available antibody and by two alternative analytical techniques, namely the 3-hydroxysteroid dehydrogenase assay and capillary gas-liquid chromatography-mass spectrometry. Correlation between values obtained by the different assay methods was highly significant, in spite of the varying specificities afforded by each method.

Capillary gas-liquid chromatography-mass spectrometry demonstrated that chenodeoxycholate and cholate were the major bile salts in sera from 72-h-old infants. The markedly greater proportion of chenodeoxycholate to cholate in the premature infants' sera may be a reflection of the more efficient clearance of conjugated cholates over conjugated chenodeoxycholates, as observed in adults (29). Even at birth in normal term infants the proportion of chenodeoxycholate in the fetal serum is higher than that in the mother's serum (25). No deoxycholate could be detected in the sera of either group of infants. This finding is consistent with previous capillary gas-liquid chromatography analysis of serum bile salts in newborn infants (25). Even though deoxycholate is present in maternal blood (25) little if any placental transfer from the mother to the fetus occurs, as shown in studies in sheep (30). 3β-hydroxy-5-cholenoate was detected in small amounts in one preterm infant who had been fed orally. This bile salt is found in large amounts in amniotic fluid between weeks 32 and 37 (31) and in the meconium (32). Since it is almost always found as its 3β-sulfate ester, intestinal absorption would be expected to be minimal.

The unidentified trihydroxy bile salt observed in sera from the two premature infants who had low concentrations of cholate could be hyocholate (3α, 6α, 7α-tri-hydroxy-5β-cholan-24-oate). This bile salt has been recently discovered in large quantities in the meconium of term infants (33) and has been found in urine of adults with and without cholestasis (34–37). Studies in adults with intrahepatic cholestasis indicate that chenodeoxycholate is the precursor of hyocholate (38). If so, it appears that in these two infants that synthesis of cholate has been inhibited; chenodeoxycholate is the primary bile salt and is 6α-hydroxylated to hyocholate. Hyocholate could, therefore, be termed a primary bile salt, i.e., synthesized in the liver (no bacterial modification) or a secondary bile salt (the 6α-hydroxyl group is added after formation of chenodeoxycholate).

C_{27} bile salts, previously found in certain cholestasis syndromes (39, 40), were essentially absent suggesting that the enzymes involved in the oxidative cleavage of the terminal three carbons of the side chain are present in adequate quantities at this stage of fetal development. No evidence was obtained for the presence of C_{20} and C_{21} bile acids, recently found in meconium (41).

The serum bile salt concentration in blood has been used as a measure of cholestasis in the neonates, potentially caused by prolonged parenteral nutrition and by hepatobiliary disease (11, 13, 28). Such studies could be profoundly affected by the various facets of the immature enterohepatic circulation of bile salts and could lead to erroneous interpretation of the effect of treatment regimes in these infants. Any cholestasis should be interpreted against a background of physiologic hypercholemia, low hepatic bile salt synthesis (4, 5), and potentially poor intestinal reabsorption (6–8).

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