Bile salt sulfotransferase: alterations during maturation and non-inducibility during substrate ingestion

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Abstract Development of the capacity for hepatic biotransformation of potentially toxic, endogenous compounds such as lithocholate may be dependent on induction by substrate or hormonal modulation. Our aim was to observe the ontogeny of hepatic sulfotransferase (ST) activity, a presumed detoxification pathway, and to determine the effect of substrate ingestion and cortisone administration on ST activity. Pregnant rats were fed a standard chow diet containing lithocholate; the maternal diet was continued during the suckling and weaning phase of the pups. Liver cytosol and serum were obtained from dams and from pups at weekly intervals from fetal life through 4 weeks of age. In controls, there was a progressive increase in hepatic ST activity from 6.2 ± 2.9 pmol/mg protein per min, (mean ± SEM) in fetal liver, 18.1 ± 3.9 at 1 week, and 33.6 ± 7.2 at 2 weeks to a peak of 56.4 ± 11.8 at 3 weeks of age. In female rats older than 4 weeks of age, ST activity in hepatic cytosol was threefold higher than in males. There was a decline to adult levels (9.2 ± 2.4 in males and 39.4 ± 4.3 in females) at 56 days of life. Cortisone acetate administration had no effect on enzyme activity in pups except those 3 weeks old or older in which there was a precocious decrease in enzyme activity to adult levels. The administration of lithocholate caused a dose-related postnatal alteration of intrahepatic bile ducts manifest as cholangitis with ductular proliferation; hepatocytes were spared. There was a concomitant age- and dose-related elevation in lithocholate (predominately unsulfated) in serum. Despite these marked structural and functional alterations, there were no significant increases in ST at any age following substrate ingestion. We conclude that age- and sex-related differences in overall sulfotransferase activity are unaltered by substrate ingestion.—**Balistreri, W. F., L. Zimmer, F. J. Suchy, and K. E. Bove.** Bile salt sulfotransferase: alterations during maturation and non-inducibility during substrate ingestion. *J. Lipid Res.* 1984. **25:** 228–235.

Supplementary key words lithocholic acid

The administration of lithocholic acid, a monohydroxy secondary bile acid, induces liver damage in a variety of species (1–3). Sulfation of lithocholate via sulfotransferase (ST) activity creates a polar product which is less readily reabsorbed after biliary secretion or reflux into serum and is, therefore, rapidly eliminated in feces or urine (4–8). This pathway of sulfation of potentially hepatotoxic bile acids may serve to prevent the initiation or promulgation of hepatic injury.

We have measured the end products of bile salt ST activity (i.e., sulfated conjugates of lithocholate) and detected them in serum in only minute amounts in both normal adults and throughout development in both humans and rats (9, 10). This included the period of "physiologic" cholestasis in infants and in developing rats, characterized by an elevation of the primary serum bile acids. However, serum levels of sulfated lithocholate will rise in the presence of cholestatic liver injury (11). The factors responsible for elevation of serum levels of sulfated lithocholate and the site, regulation, and efficiency of sulfation as a detoxification process are unknown.

It is our hypothesis that the ability to sulfate is a genetically determined, species-specific characteristic which may be modulated by various hormones. It is possible that an impaired ability to sulfate could create an enhanced susceptibility to initiation or perpetuation of hepatic damage by toxic bile acids. The purpose of the present study was to define age- and sex-related differences in the overall activity of ST in hepatic cytosol and to define the effect of hormone administration and substrate ingestion on the inducibility of this enzyme.

METHODS

Experimental animals

Dated pregnant, female rats (obtained from Charles River Breeding Laboratories, Wilmington, MA) were killed on the 21st day of a 22-day gestation to obtain fetal liver and serum. The liver tissue from two or three fetuses in each of six litters was pooled to attain tissue sufficient

Abbreviations: ST, sulfotransferase; GLC, glycolithocholate; PAPS, 3′-phosphoadenosine-5′-phosphosulfate; LCC, unsulfated conjugates of lithocholate; SLCC, sulfated conjugates of lithocholate.

for analysis. A second group of pregnant rats was allowed to deliver in our laboratory, and six to eight pups were then killed at 1, 2, 3, or 4 weeks of age. Those older than 3 weeks of age were divided by sex to determine the effects of gonadal hormones on ST activity. The maternal serum and liver were also obtained at the time of cesarean section. Another group, consisting of maternal and newborn rats, received an intraperitoneal injection of either cortisone acetate (Merck, Sharp & Dohme, West Point, PA), 10 mg/100 g body weight, or vehicle for 5 days prior to being killed. In order to study the effect of substrate ingestion, a group of similar-aged animals was studied following the maternal ingestion, throughout gestation, of standard rat chow to which unconjugated lithocholate (ICN/Nutritional Biochemicals, Cleveland, OH) had been added in various concentrations (2.5%, 0.5%, and 0.0% by weight).

Materials

The chemicals used in this study were: 3′-phosphoadenosine-5′-phosphosulfate (P-L Biochemicals, Inc., Milwaukee, WI), [^{35}S]-PAPS (3′-phosphoadenosine-5′ phosphosulfate; ~3.8 Ci/mmol; New England Nuclear Corporation, Boston, MA), glycolithocholate (Calbiochem, LaJolla, CA), [24-^{14}C]glycolithocholic acid; ~650 mCi/mol (courtesy of Dr. L. J. Chen), AH-Sepharose 4B (Pharmacia, Piscataway, NJ), 1-ethyl-3-(3-dimethylaminepropyl) carbodiimide (Sigma Chemical Company, St. Louis, MO). All reagents used in preparation of buffers were of analytical grade.

Tissue preparation

The livers were quickly excised from all animals following cervical dislocation. For preparation of cytosol the tissue was immediately homogenized in an ice-cold solution of 0.25 M sucrose containing 0.05 mM Tris-HCl (pH 7.5), 1.0 mM EDTA, and 1.0 mM dithiothreitol at a ratio of 1 to 5 weight:volume using a Potter-Elvehjem homogenizer with a Teflon pestle. The clear supernatant, prepared by centrifuging the homogenate at 100,000 *g* for 60 min at 4°C, was assayed for sulfotransferase activity. The recovery of cytosolic protein was comparable at all ages, ranging from 52 mg/g liver at day 1 to 74 mg/g in adults.

Sulfotransferase assay

ST activity was analyzed using a modification of the method of Chen (12, 13). AH-Sepharose 4B, bound to glycolithocholate (GLC) as a substrate, was prepared and [^{35}S]-PAPS (+cold PAPS) served as the sulfate donor. The reaction mixture contained 10 μmol of sodium phosphate (pH 6.5), 100 nmol of magnesium chloride, 10 nmol of PAPS, 4×10^5 counts per min of [^{35}S]-PAPS, and 25 nmol of glycolithocholate as GLC-Sepharose (approximately 0.3 μmol of GLC was bound per 1.0 ml of Sepharose as estimated by [24-^{14}C]glycolithocholate) in a total volume of 0.2 ml. The reaction was carried out in a liquid scintillation counting vial and was initiated by adding 25 μl of the cytosol. After incubation for 10 min at 37°C, the reaction was terminated by placing the vial in boiling water for 30 sec. Upon cooling, 1.0 ml of water was added to the reaction mixture and the mixture was centrifuged. The clear supernatant was removed by careful suction and discarded. The precipitated Sepharose was then washed with 3.0 ml of 0.1 N NaCl three times. Radioactivity in the washed gel was counted to estimate the bound fraction; protein concentration was estimated by the method of Lowry et al. (14) with bovine serum albumin as standard.

Serum bile acid assays

Unsulfated conjugates of lithocholate (LCC) were measured by a specific radioimmunoassay (antibody supplied by Drs. A. Roda and C. Colombo) (15); sulfated conjugates of lithocholate (SLCC) were also measured by specific radioimmunoassay (SLCC-RIA, Abbott Laboratories) (11).

Breast milk bile acid assays

In order to estimate the concentration and chemical form of lithocholate ingested by the pups, breast milk was obtained from several animals by suction approximately 30 min after intraperitoneal injection of 1.0–1.5 units of synthetic oxytocin (Pitocin, Parke-Davis). Lithocholate concentration was analyzed by radioimmunoassay for unsulfated (LCC) and sulfated lithocholate (SLCC) concentration performed on breast milk samples obtained during the fourth week of life of the pups.

Histology

Liver tissue, obtained from all animals at the time of killing was immediately fixed in formalin and subsequently graded without knowledge of the dose of lithocholate received.

Statistical analysis

For each segment of the study, the ST assays (control and experimental group) were performed simultaneously in order to avoid intraassay variability due to different batches of bound substrate. Mean and standard error were calculated by standard methods. Statistical significance was obtained using confidence intervals as determined by analysis of variance and Student's *t*-test (16).

RESULTS

Ontogeny of hepatic sulfotransferase

Fetal levels of ST were low (**Table 1**), however, there was a progressive rise during the first 3 weeks of life.

The incremental increases in ST activity were significant at each weekly interval ($P < 0.002$). At 21 days of age, a peak level of approximately 56 pmol/mg protein per min was reached. In 4-week-old animals, the levels declined; however, a significant sex difference was not apparent. In adult males (>56 days of age), however, the ST level was threefold less than in female animals ($P < 0.001$). Maternal levels of ST (36.2 ± 3.8) were similar to those of nonpregnant control females.

Sulfotransferase activity following administration of cortisone acetate

Cortisone acetate, at the dose chosen, had no effect on ST activity in fetal, 1-week, or 2-week-old pups (**Table 2**). However, an effect of corticosteroid injection (i.e., precocious decline) was evident at 3 and 4 weeks of age.

Sulfotransferase activity following lithocholate administration

Following the ingestion of lithocholate, the levels of ST were not significantly increased (**Fig. 1**) in any age group. In fact, the only significant change was a decrease in the 3-week-old animals from mothers who had received 0.5% lithocholate in their diet ($P < 0.05$).

Serum bile acid changes during lithocholate administration

In all control sera, LCC was present in low concentrations; there was, however, no detectable SLCC at any age (**Fig. 2**). Following the ingestion of lithocholate, there was a dose- and time-related elevation in both unsulfated and sulfated lithocholate levels. In all treated groups, the sulfated fraction comprised a small (mean of 11%) fraction of the total lithocholate in serum.

TABLE 1. Normal ontogeny of sulfotransferase activity

Age Group	Sulfotransferase Activity (pmol/mg protein per min) Mean ± SEM
Fetal (21st day)[a]	6.2 ± 3.9
One week	18.1 ± 3.9
Two weeks	33.6 ± 7.2
Three weeks	56.4 ± 11.8
Four weeks	
Male	25.4 ± 9.1[b]
Female	25.8 ± 5.0
Adult	
Male	9.2 ± 2.4[b,c]
Female	39.4 ± 4.3[c]
Maternal	36.2 ± 3.8

[a] $P < 0.002$ for each incremental increase in ST activity (i.e., fetal vs 1 week, 1 week vs 2 weeks, etc.).
[b] $P < 0.001$ for 4-week-old male vs adult male.
[c] $P < 0.001$ for adult male vs adult female.

TABLE 2. Effect of cortisone acetate[a] on sulfotransferase activity

Age Group	Sulfotransferase Activity (pmol/mg protein per min) Mean ± SEM: Control	Sulfotransferase Activity (pmol/mg protein per min) Mean ± SEM: Treated	P Value (vs Control)
Fetal (21st day)[b]	3.0 ± 1.4	2.9 ± 1.4	NS
One week	23.0 ± 5.0	17.0 ± 2.6	NS
Two weeks	34.0 ± 11.4	37.0 ± 6.5	NS
Three weeks	43.0 ± 5.4	30.0 ± 4.5	<0.005
Four weeks	34.0 ± 7.0	22.0 ± 9.6	<0.05

[a] Ten mg/100 g body weight, daily for 5 days.
[b] Administration of cortisone to mother from gestational day 16 to 20; killed on day 21.

Lithocholate concentration in breast milk

Immunoreactive SLCC was elevated in the milk of mothers who had received a high concentration of lithocholate in their diet (857.8 μg/dl) compared to controls (79.4 μg/dl); in addition, there was a marked increase in LCC (148.6 μg/dl vs 4.1 μg/dl). Ninety-five percent of lithocholate was sulfated in controls, while in the lithocholate-treated mothers, the percent decreased slightly to 85%.

Liver histology

The administration of lithocholate consistently caused an age- and dose-related, diffuse, progressive intrahepatic bile duct injury manifest in all 2-week-old pups as mild ductular proliferation and periductular inflammatory reaction (**Fig. 3**). At 3 to 5 weeks after birth, degeneration of bile duct epithelium and accumulation of amorphous crystalline material in the bile ducts were present (**Fig. 4**). In several older animals, an intense obliterative cholangitis was superimposed (**Fig. 5**). Focal periportal parenchymal necrosis was occasionally present, but hepatocytes were generally normal in appearance. Canalicular bile stasis was never seen. There were no distal extrahepatic large bile duct lesions noted up to 4 weeks postnatal. Despite severe maternal intrahepatic bile duct damage similar to that described above, the simultaneously obtained fetal and 1- or 2-day-old livers showed no histological alteration.

DISCUSSION

Our data demonstrate an age-related increase in ST activity in the developing Sprague-Dawley rat; peak activity occurred at approximately the time of weaning, and values then declined to adult levels after 4 weeks of age. In rats older than 4 weeks, the ST activity was threefold higher in hepatic cytosol obtained from the female. This pattern is not unique to lithocholate ST or to the rat;

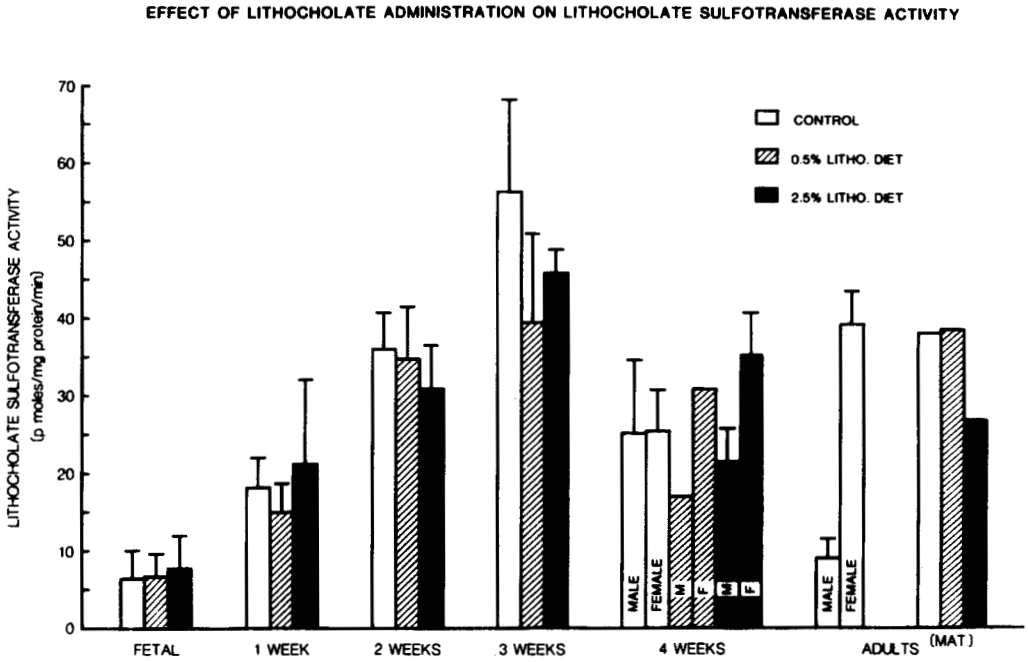

Fig. 1. The progressive rise in ST activity with age, with peak values at 3 weeks, is depicted. The lack of effect of administration of various concentrations of lithocholate on LST activity at any age is apparent (n = 6 or more in each age except maternal (mat) animals where n = 4).

low bile salt ST levels in early life have been found in guinea pigs and humans (5, 6). Rat phenol ST activity increased more than sixfold between 1 and 70 days of age with a decline to adult levels after 12 weeks (17); hepatic UDP-GT and androsterone ST undergo a postnatal rise with a sex-based difference visible after 30 days; however, the genetic expression is inherited as a single dominant trait (18). In the CDR-Fisher rat, total cortisol ST activity rises progressively (in parallel) in both sexes up to 4 weeks of age; following sexual maturation, en-

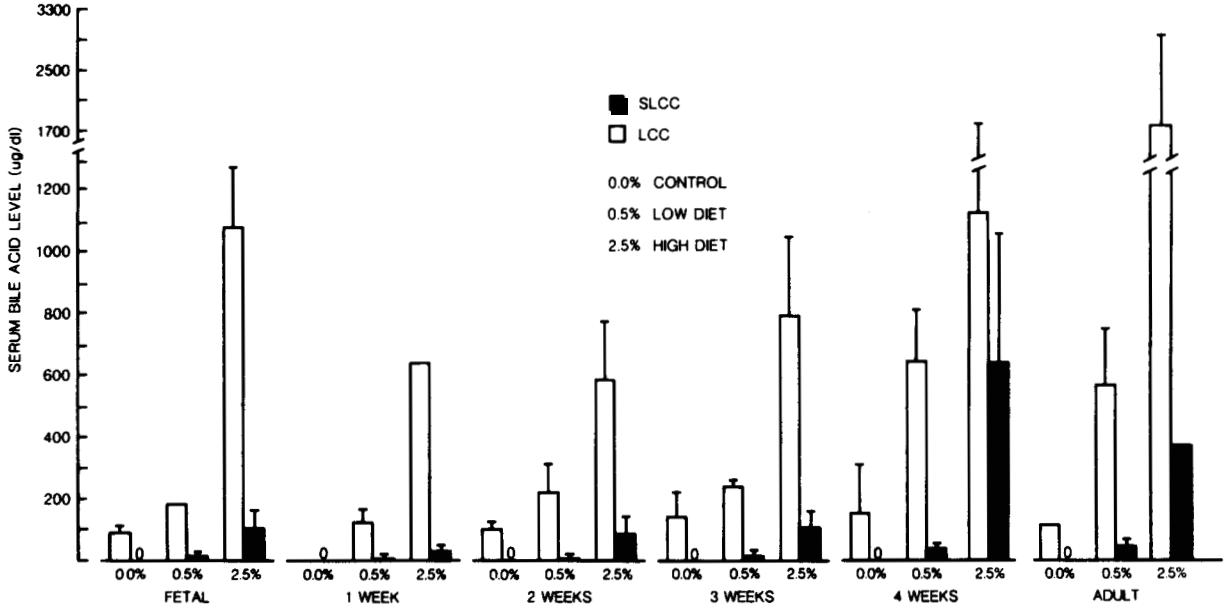

Fig. 2. The alterations in serum bile acid (LCC, unsulfated lithocholate conjugates, SLCC, sulfated lithocholate conjugates) levels in control and in pups whose mothers were ingesting various concentrations of lithocholate (0.5%, low diet or 2.5%, high diet). In controls, LCC was present in low concentrations; SLCC was undetectable. There was a dose- and time-related elevation in SLCC and LCC in the experimental group; the nonsulfated fraction predominated (n = 6 or more in each group except adult animals where n = 4).

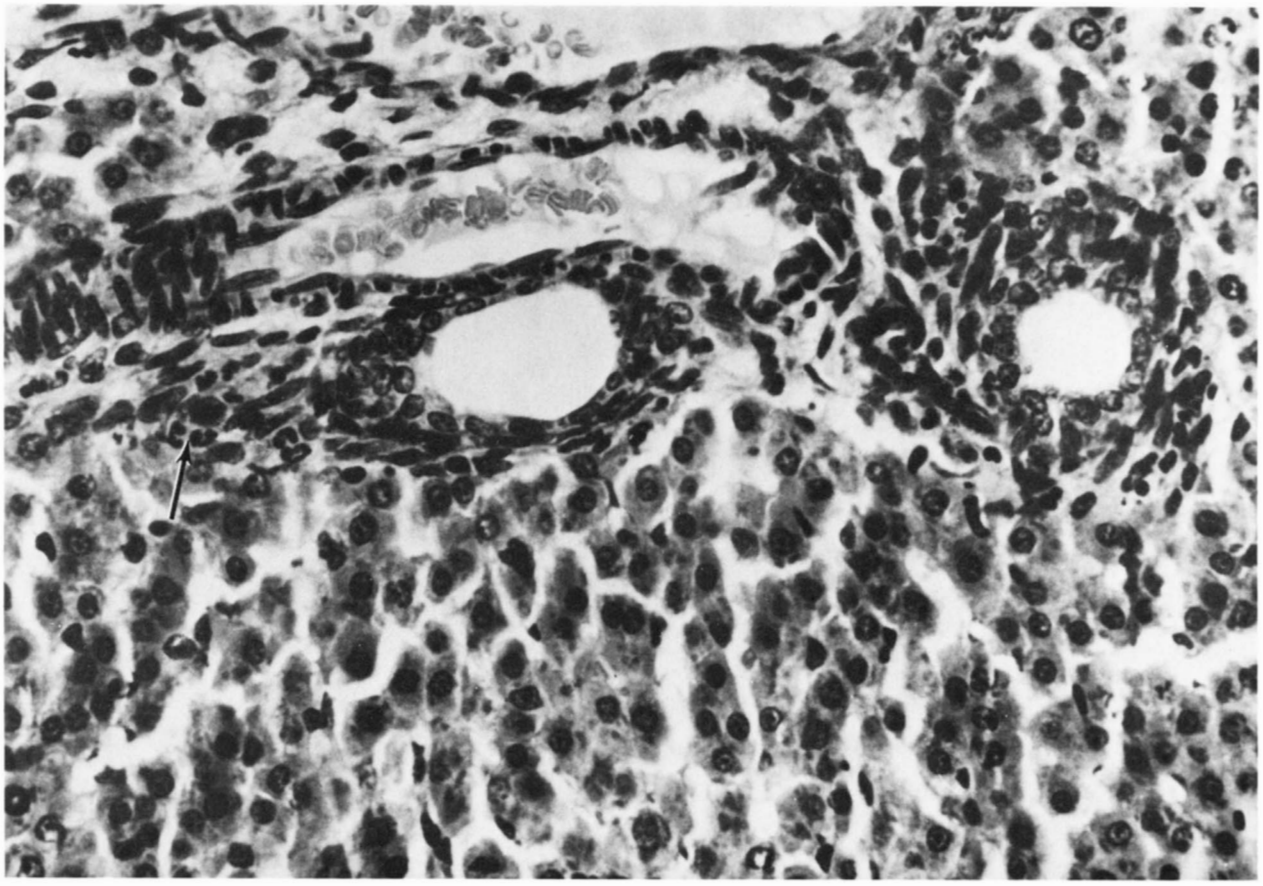

Fig. 3. Small bile ducts of this 2-week-old pup (maternal exposure to 2.5% dietary lithocholate) are slightly dilated; the epithelial cells are flattened and slightly crowded. Periductular inflammation is sparse, but definite, and includes neutrophils (arrow), as well as nuclear debris. (Hematoxylin and eosin × 500)

zymatic activity decreases in males, while enzymatic activity in females continued to increase (19). Total cortisol ST activity in adult males was approximately 15% of that found in adult females.

The factors that modulate the development of ST activity are poorly understood. It is unlikely, however, that endogenous inhibitors of ST were responsible for the observed alterations in enzyme activity since the high specific activity of the sulfate donor (PAPS) allowed the use of dilute supernatant concentrations.

Corticosteroid administration induced a precocious decline in ST activity to normal adult levels after 3 weeks of age but had no effect on the initial development of activity. Our observation of a relationship of ST activity in older rats to sex suggests an additional effect of gonadal hormones; however, we have not examined this issue. Hammerman et al. (20) have previously reported that bile salt ST activity was threefold higher in female rat liver. There are sex-related differences in enzymatic sulfation of steroid hormones by rat liver homogenates; males have approximately ⅓ of the activity of female rats (19, 21).

There are multiple forms of steroid ST in mammals, each with different substrate specificity and each modulated by disparate hormonal input (21, 22). Sex-related differences in bile acid ST may be related to the presence of two isoenzymes, one of which is stimulated by estrogen and accounts for the majority of the activity found in cytosol from adult female rats (23, 24). The existence of multiple enzymes involved in sulfation is further suggested by studies that show difference in substrate specificity in early life in the hamster liver (25). It is conceivable that the postnatal increase in ST activity noted in our study may be due, in part, to the effect of gonadal hormones ingested with breast milk.

Our studies also demonstrate a dose-related toxicity associated with lithocholate ingestion. The maternal liver was consistently injured by ingestion of the hepatotoxic compound. The histologic alterations in younger animals suggest an age- and dose-related effect. This finding is corroborated by the serum bile acid levels which show a parallel increase. Despite marked injury of intrahepatic bile ducts, presumably due to lithocholate, there was

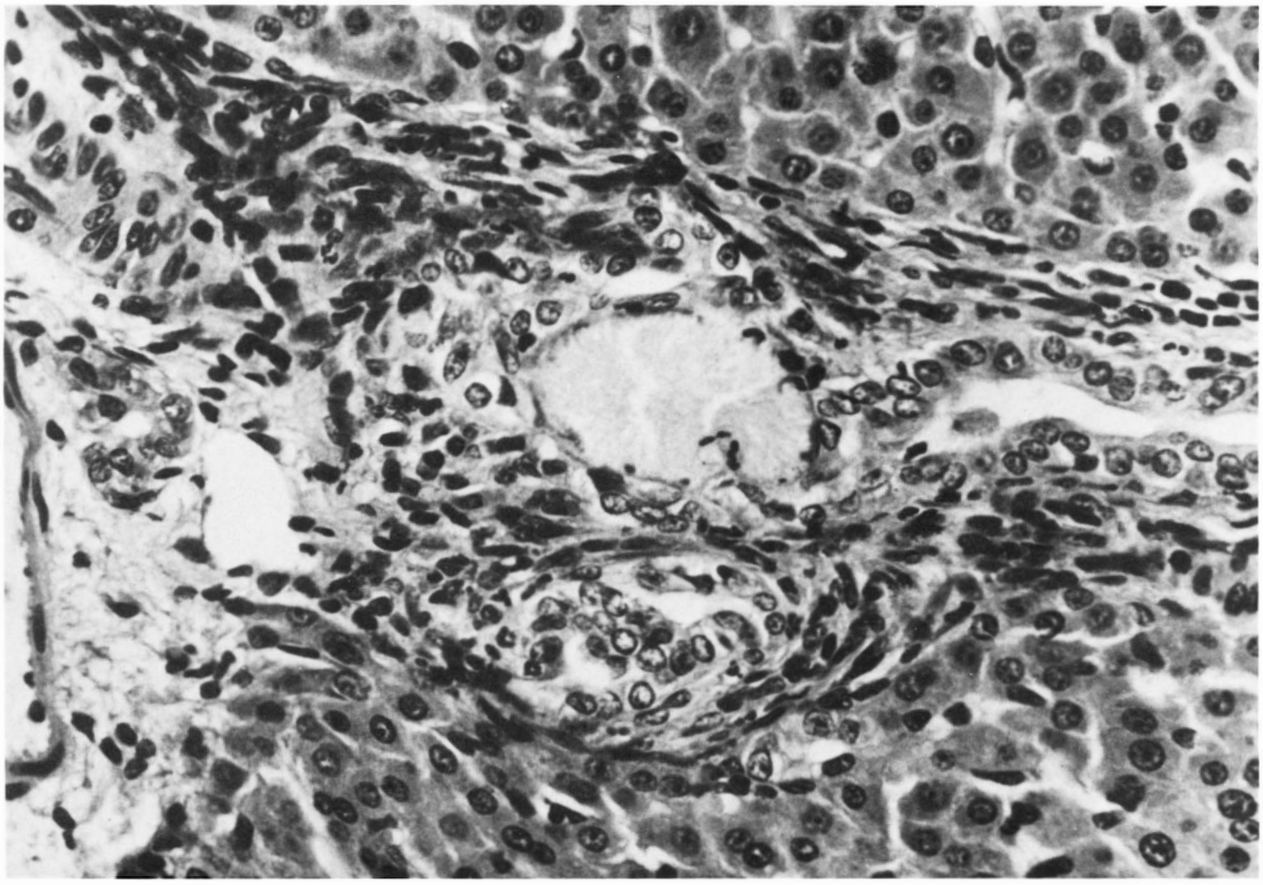

Fig. 4. The number of small bile ducts per portal area of this 3-week-old pup (2.5% dietary lithocholate) are increased and the epithelium is hyperplastic. Periductular infiltrate of lymphocytic/monocytic character is modest. One ductule with degenerating epithelium is obstructed by an isotropic crystalloid. (Hematoxylin and eosin × 500)

no apparent increase in the postulated detoxification pathway.

There was also a consistent progressive postnatal increase in serum bile acid levels as well as in the incidence of intrahepatic bile duct lesions in pups suckled by dams ingesting lithocholate-laden rat chow. The mammary gland apparently was not an effective barrier to monohydroxy bile acids since there were high concentrations of lithocholate in the expressed milk. Continued exposure to this hepatotoxin induced postnatal liver injury. In animals 3–4 weeks of age, ingestion of lithocholate present in rat chow as well as in maternal feces was likely important in perpetuating the toxic effect.

Another striking finding in the present study is that, despite severe maternal bile duct injury, the simultaneously obtained fetal livers were relatively free of histologic damage. This sparing may be due to either: *1*) placental factors or *2*) factors inherent to the fetal liver. Placental transfer may protect the fetal liver by unknown mechanisms. Recent studies suggest that, in sheep, the placenta acts as an excretory route for fetal chenodeoxycholate (26). Membrane composition and physicochemical characteristics (such as membrane fluidity) in early life differ from that of adult tissue, possibly accounting for an inherent decrease in susceptibility to lithocholate toxicity. Cholestasis induced by lithocholate is associated with an increased synthesis of microsomal cholesterol which is then incorporated into the bile canalicular membrane (27), thereby altering the chemical, structural, and functional integrity of the bile canalicular membranes. Yousef et al. (28) were able to lessen the degree of bile stasis as well as the increase in membrane cholesterol by inhibiting protein and/or cholesterol synthesis prior to intoxication. It is possible that the diminished susceptibility to toxicity seen in fetal rats in our study is due in part to these effects (i.e., low levels of microsomal protein or cholesterol synthesis or altered binding of lithocholate to cytosolic proteins).

Regardless of the mechanism, these histologic findings are in contrast to observations made in other species. Administration of lithocholate (or its precursor, chenodeoxycholate) to pregnant hamsters, rhesus monkeys, or baboons did produce hepatic necrosis and bile duct proliferation in the fetus (29–31). Examination by others of

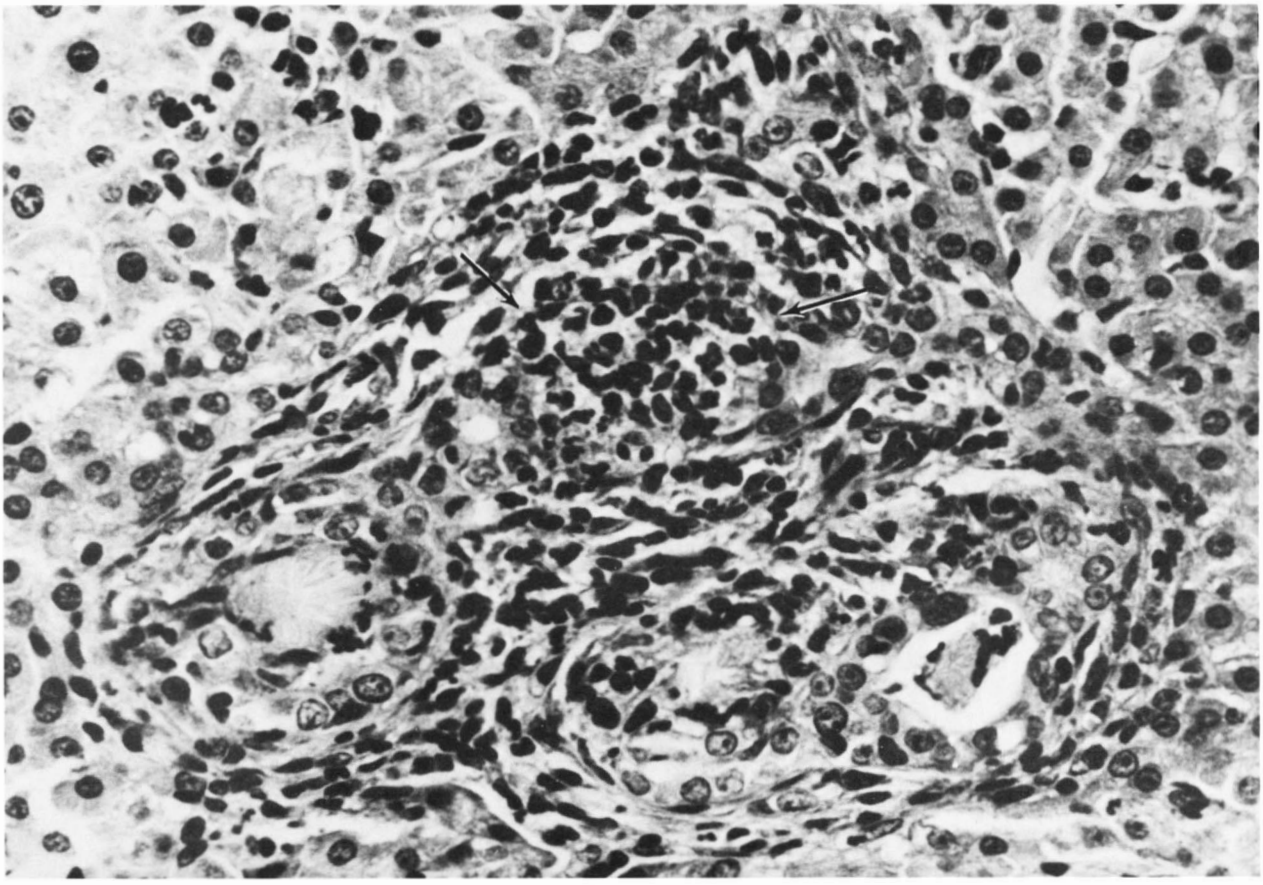

Fig. 5. Bile ductular proliferation with intraductular crystalloid is florid at 4 weeks. Intense focal inflammatory reaction (arrows) occurs at the site of destroyed ductal segment which could be identified in adjacent tissue sections. (Hematoxylin and eosin × 500)

the effect of mechanical cholestasis (e.g., bile duct ligation) (32) or potentially cholestatic agents (ethinyl estradiol) (33) on ST has given variable results; the response may be species-specific, isoenzyme-specific, bile acid/conjugate-specific, or related to the type of injury. Preliminary observations suggest that induction of bile salt ST does not occur in human liver in clinical conditions associated with cholestasis (34).

The ability of the human liver to sulfate lithocholate shows a high degree of variability. This appears to be a genetically predetermined characteristic which is not induced by substrate ingestion. This hypothesis is supported by studies which show that, following the administration of chenodeoxycholate for gallstone dissolution, there was no increase in sulfation capacity. This may have predisposed the patients to the hepatic toxicity evidenced by transaminase elevation in these patients (35). There is also experimental data that suggest a species difference in the ability to sulfate and, hence, in the predisposition to hepatotoxicity. Dose-related biochemical and morphological hepatotoxicity in the rhesus monkey following chenodeoxycholate administration is apparently related to an inherent inability to sulfate the end product of chenodeoxycholate metabolism, lithocholate (36); a similar phenomenon occurs in the baboon (37).

Our studies suggest that bile salt sulfotransferase activity is predetermined and is probably modulated by hormonal input but is unresponsive to substrate. In the face of excessive toxin exposure, there is significant and progressive injury of the intrahepatic bile ducts.

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REFERENCES

1. Palmer, R. H. 1972. Bile acids, liver injury and liver disease. *Arch. Intern. Med.* **130:** 606–617.

2. Dyrska, H., T. Chen, G. Salen, and E. H. Mosbach. 1975. Toxicity of chenodeoxycholic acid in the rhesus monkey. *Gastroenterology.* **69:** 333–337.
3. Miyai, K., W. W. Mayr, and A. L. Richardson. 1975. Acute cholestasis induced by lithocholic acid in the rat: a freeze-fracture replica and thin section study. *Lab. Invest.* **32:** 527–535.
4. Stiehl, A., D. L. Earnest, and W. H. Admirand. 1975. Sulphation and renal excretion of bile salts in patients with cirrhosis of the liver. *Gastroenterology.* **68:** 534–544.
5. Chen, L. J., R. J. Bolt, and W. H. Admirand. 1977. Enzymatic sulfation of bile salts: partial purification and characterization of an enzyme from rat liver that catalyzes the sulfation of bile salts. *Biochim. Biophys. Acta.* **480:** 219–227.
6. Chen, L. J., M. M. Thaler, R. J. Bolt, and M. S. Golbus. 1978. Enzymatic sulfation of bile salts. III. Enzymatic sulfation of taurolithocholate in human and guinea pig fetuses and adults. *Life Sci.* **22:** 1817–1820.
7. Chen, L. J. 1982. Bile salt sulfotransferase in guinea pig liver. *Biochim. Biophys. Acta.* **717:** 316–321.
8. Cowen, A. E., M. G. Korman, A. F. Hofmann, O. W. Cass, and S. B. Coffin. 1975. Metabolism of lithocholate in healthy man. II. Enterohepatic circulation. *Gastroenterology.* **69:** 67–76.
9. Suchy, F. J., W. F. Balistreri, J. E. Heubi, J. E. Searcy, and R. S. Levin. 1981. Physiologic cholestasis: elevation of the primary serum bile acid concentrations in normal infants. *Gastroenterology.* **80:** 1037–1041.
10. Belknap, W. M., W. F. Balistreri, F. J. Suchy, and P. C. Miller. 1981. Physiologic cholestasis. II. Serum bile acid levels reflect the development of the enterohepatic circulation in rats. *Hepatology.* **1:** 613–616.
11. Balistreri, W. F., F. J. Suchy, M. K. Farrell, and J. E. Heubi. 1981. Pathologic versus physiologic cholestasis: elevated serum concentration of a secondary bile acid in the presence of hepatobiliary disease. *J. Pediatr.* **98:** 399–402.
12. Chen, L. J. 1980. A simple method for the assay of bile salt sulfotransferase using glycolithocholate coupled to sepharose. *Anal. Biochem.* **105:** 170–173.
13. Chen, L. J. 1981. Bile salt sulfotransferase. *Methods Enzymol.* **77:** 213–218.
14. Lowry, O. H., N. J. Rosebrough, A. L. Farr, and R. J. Randall. 1951. Protein measurement with the Folin phenol reagent. *J. Biol. Chem.* **193:** 265–275.
15. Roda, A., E. Roda, D. Festi, R. Aldini, G. Mazzella, C. Sama, and L. Barbara. 1978. A radioimmunoassay for lithocholic acid conjugates in human serum and liver tissue. *Steroids.* **32:** 13–24.
16. Sokal, R. R., and F. J. Rohlf. 1969. Biometry. W. H. Freeman and Co., San Francisco. 223–226.
17. Maus, T. P., R. K. Pearson, R. J. Anderson, L. C. Woodson, C. Reiter, and R. M. Weinshilboum. 1982. Rat phenol sulfotransferase assay procedure, developmental changes, and glucocorticoid regulation. *Biochem. Pharmacol.* **31:** 849–856.
18. Matsui, M., and H. K. Watanabe. 1982. Developmental alteration of hepatic UDP-glucuronosyltransferase and sulphotransferase towards androsterone and 4-nitrophenol in Wistar rats. *Biochem. J.* **204:** 441–447.
19. Singer, S. S., D. Giera, J. Johnson, and S. Sylvester. 1976. Enzymatic sulfation of steroids. I. The enzymatic basis for the sex difference in cortisol sulfation by rat liver preparations. *Endocrinology.* **98:** 963–973.
20. Hammerman, K. J., L. J. Chen, A. Fernandez-Corugedo, and D. L. Earnest. 1978. Sex differences in hepatic sulfation of taurolithocholate in the rat. *Gastroenterology.* **75:** 1021–1025.
21. Singer, S. S., M. J. Federspiel, J. Green, W. G. Lewis, V. Martin, K. R. Witt, and J. Tappel. 1982. Enzymatic sulfation of steroids. XV. Studies differentiating between rat liver androgen, estrogen, bile acid, glucocorticoid and phenol sulfotransferases. *Biochim. Biophys. Acta.* **700:** 110–117.
22. Barnes, S., and J. G. Spenney. 1982. Evidence for heterogeneity of hepatic bile salt sulfotransferases in female hamsters and rats. *Biochim. Biophys. Acta.* **704:** 353–360.
23. Kane, R. E., L. J. Chen, and M. M. Thaler. 1981. Bile salt sulfotransferase (BSS) in rat liver: identification of two isoenzymes and influence of gonadal hormones. *Gastroenterology.* **80:** 1337.
24. Kane, R. E., L. J. Chen, and M. M. Thaler. 1982. Sex distribution and substrate specificity of rat hepatic bile salt sulfotransferase. *Gastroenterology.* **82:** 1231.
25. Jordan, J., and P. Killenberg. 1980. Development of enzymatic conjugation and sulfation of bile acids in hamster liver. *Am. J. Physiol.* **232:** G429–G433.
26. Sewell, R. B., K. J. Hardy, R. A. Smallwood, and N. E. Hoffman. 1982. Fetal bile salt metabolism: placental transfer of dihydroxy bile salts in sheep. *Am. J. Physiol.* **243:** G172–G175.
27. Yousef, I. M., M. Lewettes, B. Tuchweber, and C. C. Roy. 1981. The role of microsomes in the pathogenesis of lithocholate cholestasis. *Gastroenterology.* **80:** 1355.
28. Yousef, I. M., B. Tuchweber, A. Weber, D. Lee, and C. C. Roy. 1983. Prevention of lithocholate-induced cholestasis by cyclohexiamide—an inhibitor of protein synthesis. *Life Sci.* **33:** 103–107.
29. Palmer, A. K., and R. Heywood. 1974. Pathological changes in the rhesus fetus associated with the oral administration of chenodeoxycholic acid. *Toxicology.* **2:** 239–246.
30. Silverberg, M., L. Solomon, and J. C. Ehrlich. 1971. The hepatotoxic effect of lithocholic acid in the newborn hamster. *Gastroenterology.* **60:** 753.
31. McSherry, C. K., K. P. Morrissey, R. L. Swarm, P. S. May, W. H. Niemann, and F. Glenn. 1976. Chenodeoxycholic acid-induced liver injury in pregnant and neonatal baboons. *Ann. Surg.* **184:** 490–499.
32. Barnes, S., P. G. Burhol, R. Zander, and B. I. Hirschowitz. 1979. The effect of bile duct ligation on hepatic bile acid sulfotransferase activity in the hamster. *Biochem. Med.* **22:** 165–174.
33. Kirkpatrick, R. B., and P. G. Killenberg. 1980. Effects of ethinyl estradiol on enzymes catalyzing bile acid conjugation and sulfation. *J. Lipid Res.* **21:** 895–901.
34. Loof, L., and B. Wengle. 1982. Enzymatic sulphation of bile salts in man: bile salt sulphotransferase activity in percutaneous liver biopsy specimens from patients with liver disease. *Scand. J. Gastroenterol.* **17:** 69–76.
35. Marks, J. W., S. O. Sue, B. J. Pearlman, G. G. Bonorris, P. Varady, J. M. Lachin, and L. J. Schoenfield. 1981. Sulfation of lithocholate as a possible modifier of chenodeoxycholic acid-induced elevations of serum transaminase in patients with gallstones. *J. Clin. Invest.* **68:** 1190–1196.
36. Gadacz, T. R., R. N. Allan, E. Mack, and A. F. Hofmann. 1976. Impaired lithocholate sulfation in the rhesus monkey: a possible mechanism for chenodeoxycholate toxicity. *Gastroenterology.* **70:** 1125–1129.
37. Palmer, R. H., and C. K. McSherry. 1982. Lithocholate metabolism in baboons fed chenodeoxycholate. *J. Lab. Clin. Med.* **99:** 533–538.