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Effect of Chenodeoxycholic Acid Feeding during Gestation in the Rat on Bile Acid Metabolism and Liver Morphology[1] (41811)

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Abstract. Chenodeoxycholic acid (CDCA) was fed to pregnant rats at the 0.25% level in the diet from Day 11 of gestation to delivery in order to evaluate the effects on (1) maternal tissue bile acid composition, (2) neonatal tissue bile acid composition and cholesterol-7α-hydroxylase activity, and (3) maternal, neonatal, and postnatal liver morphology. Feeding CDCA increased maternal lithocholic acid while significantly decreasing deoxycholic acid, cholic acid, and total bile acids. Feeding CDCA resulted in a significantly higher chenodeoxycholic acid pool in the neonates while neonatal plasma cholesterol and the 7α-hydroxylation of cholesterol was not significantly affected. Morphological examination of maternal, neonatal, and postnatal rat liver revealed no significant hepatotoxicity. This investigation has shown that (a) neonates of CDCA fed dams have a significantly greater pool of CDCA, suggesting maternal-to-fetal transfer of dihydroxy bile acids, (b) neonatal cholesterol-7α-hydroxylase activity and total tissue bile acid pools are not significantly altered by increased pool of CDCA, and (c) no hepatotoxic effects on maternal, neonatal, and postnatal livers were evident with gestational feeding of CDCA at the 0.25% level in the rat.

The metabolism of cholesterol is known to be dependent upon a number of microsomal hydroxylations, including 7α-hydroxylation, the first step in the catabolic conversion of free cholesterol into bile acids (1). Impaired 7α-hydroxylation of cholesterol can lead to progressive accumulation of cholesterol in the liver and tissues (1). The activity of cholesterol-7α-hydroxylase, a monooxygenase involving cytochrome *P*-450 (2–4), is believed to be regulated by the concentrations of bile acids and cholesterol in the liver (1). The retention of bile acids in the intestine preventing significant enterohepatic recirculation by the administration of cholestyramine induces the activity of cholesterol-7α-hydroxylase (5).

Chenodeoxycholic acid (CDCA) has been shown to inhibit the activity of hepatic cholesterol-7α-hydroxylase in many species, including the adult rat (6, 7) and in neonatal guinea pigs (8). Feeding of CDCA to pregnant baboons has been reported to induce focal hepatic injury in both the pregnant and the neonatal baboon (9). CDCA, deoxycholic acid, and lithocholic acid of maternal origin have been found in the fetus of several species in significant amounts in tracer studies (10, 11). In humans treated with chenodeoxycholic acid for dissolving gallstones, transient evidence of hepatic injury has been noted (12), as well as ultrastructural evidence of increased intrahepatic cholestasis (13).

The aims of the present study were to evaluate the effects of CDCA fed to pregnant rats on maternal and neonatal bile acid metabolism. We investigated the effect of CDCA during gestation on (1) maternal bile acid pool size and composition, (2) neonatal bile acid pool size, composition, and activity of hepatic cholesterol-7α-hydroxylase, and (3) the extent, if any, of maternal, neonatal and postnatal hepatotoxicity.

Materials and Methods. *Chemicals.* 4-^{14}C-Cholesterol was obtained from New England Nuclear (Boston, Mass.), diluted with unlabeled cholesterol (99+%, Sigma Chemical Co., St. Louis, Mo.), purified by thin-layer chromatography (TLC) on preparative (500 μm thick) thin-layer plates (Analabs, Inc., North Haven, Conn.), and developed in benzene:ethyl acetate (2:3, v/v). The specific activity of the purified labeled cholesterol was 2.95×10^{12} dpm/mole. Nicotinamide, EDTA,

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sucrose, and mercaptoethanol were obtained from Sigma Chemical Company. NADPH was obtained from Boehringer-Mannheim (Indianapolis, Ind.). Bile acid standards for gas–liquid chromatography (GLC), 7α-hydroxycholesterol, 7β-hydroxycholesterol, and 7β-ketocholesterol were all obtained from Steraloids (Wilton, N.H.). All solvents were of reagent grade and used as supplied.

Diet and animal preparation. Stock rat diet (Ralston–Purina Company, St. Louis, Mo.) supplemented with 0.25% CDCA was prepared by ICN Nutritional Biochemicals (Cleveland, Ohio) in pelleted form. Timed pregnant (11 days) Sprague–Dawley rats were obtained from Harlan Industries, Inc. (Indianapolis, Ind.). The pregnant rats were randomly assigned to one of two groups and fed either stock diet or 0.25% CDCA-supplemented stock diet. Three rats from each treatment group were further randomly selected to be sacrificed on Day 19 of gestation, the pregnant rats being fed either control stock diet or 0.25% CDCA-supplemented diet from Day 11 to Day 19. These rats were anesthetized with diethyl ether and exsanguinated by cardiac puncture. Blood samples were obtained for plasma cholesterol determinations. Portions of liver were taken for light microscopy evaluation. The gastrointestinal tract, including the remaining liver, was removed and frozen until analyzed for composition of tissue bile acids. The remaining pregnant rats were fed either stock diet (5) or 0.25% CDCA-supplemented diet (4) from Day 11 of gestation to delivery on Days 21–23. After delivery, neonates were decapitated and exsanguinated. Neonates from each litter were selected for the following determinations. Neonatal blood samples were pooled for plasma cholesterol determinations (5 to 10 neonates per pool). Neonatal carcasses were frozen, and their gastrointestinal tracts later removed and pooled (3 to 7 neonates per pool) for analysis of tissue bile acids. The liver from selected neonates was rapidly excised for assay of cholesterol-7α-hydroxylase (4 to 5 neonates per pool). Portions of neonatal liver were taken for light and electron microscopy evaluation. At delivery, selected rat neonates were left to nurse with dams on stock diet (previously on either stock or 0.25% CDCA supplemented diet) until weaning. These postnatal rats then remained on stock diet until sacrifice at 7 weeks by decapitation and exsanguination. Portions of liver were taken for light and electron microscopy.

Hepatic cholesterol-7α-hydroxylase assay. Hepatic microsomal cholesterol-7α-hydroxylase activity was assayed as described previously (14) based on the methods of Mitropoulos and Balasubramaniam (15) and Shefer *et al.* (16). Liver removed from the neonates was immediately placed in a chilled solution of 250 m*M* sucrose, 2.5 m*M* EDTA, and 75 m*M* nicotinamide. All subsequent operations were carried out on ice or at 0–4°C unless otherwise specified. A 20% (w/v) liver homogenate was prepared by homogenizing the tissue in 0.1 *M* phosphate buffer, pH 7.4, containing 5 m*M* $MgCl_2$, 1 m*M* EDTA, and 30 m*M* nicotinamide, with a Potter–Elvehjem homogenizer equipped with a Teflon pestle. Liver microsomes were obtained as described by Mitropoulos and Balasubramaniam (15). The 105,000*g* microsomal pellet was resuspended in 0.1 *M* phosphate buffer, pH 7.4, containing 5 m*M* $MgCl_2$, 30 m*M* nicotinamide, and 10 m*M* mercaptoethanol in a volume equal to that from which the pellet was obtained. For the cholesterol-7α-hydroxylase assay, 1.5 ml of the microsomal suspension was added to a tube containing 260,000 dpm of 4-^{14}C-cholesterol "solubilized" in 0.7 mg of Cutscum (detergent: isooctylphenoxy-polyoxyethylene-ethanol, Fisher Scientific Company, Cincinnati, Ohio) suspended in 1 ml of the microsomal assay buffer. The assay was started by adding 8.0 μmole of NADPH and incubating the tubes at 37°C in a shaker bath for 30 min. Each assay was carried out in duplicate with a boiled enzyme blank to correct for autooxidation of cholesterol. The assay was stopped by the addition of 20 ml of chloroform:methanol (2:1), 2.5 ml water and 50 μg each of "carrier" 7α-hydroxycholesterol, 7β-hydroxycholesterol, and 7-ketocholesterol. The lipids were extracted and separated on TLC on preparative (500 μm thick) thin-layer plates and developed in benzene:ethyl acetate (2:3, v/v). The appropriate bands, visualized with 2,7 dichlorofluoroscein, were scraped directly into scintillation vials. Ten milliliters of Aquasol (New England Nuclear) scintillation solution was added and the radioactivity was assayed in a Packard liquid scintillation coun-

ter (Downers Grove, Ill.) with automatic external standard for quench correction. Microsomal protein was measured by the Hartree (17) modification of the Lowry procedure and cholesterol-7α-hydroxylase activity was expressed as picomoles of 7α-hydroxycholesterol formed/mg protein · min.

Analysis of neonatal bile acid pools. The composition of tissue bile acids was determined as described previously (14). The entire gastrointestinal tract, including the liver, was saponified with 1 *N* ethanolic NaOH for 1 hr at 110°C. The neutral sterols were extracted out with two 50-ml portions of petroleum ether. The aqueous extract was further saponified for 3 hr at 110 in 2.8 *N* ethanolic NaOH, acidified to pH 3 with concentrated HCl and the bile acids then extracted with choloroform:methanol (2:1, v/v) and chloroform. The bile acids were methylated with freshly prepared diazomethane and purified from fatty acids by TLC on preparative thin-layer plates, as described by Grundy *et al.* (18). The purified bile acid methyl esters were then quantitated as their methyl ester trifluoroacetates by GLC using hyocholic acid as an internal standard (19). A Hewlett-Packard Model 402 (Palo Alto, Calif.) gas–liquid chromatograph equipped with a 1% QF-1 column (Gas-Chrom Q, 100/120 mesh) was used. The operating temperatures were: column, 215°C; injector, 235°C; and detector, 250°C. Nitrogen was used as the carrier gas at a flow rate of 50 ml/min.

Analysis of maternal bile acids. The composition of tissue bile acids was determined as for the neonates except as briefly noted below. The entire gastrointestinal tract, including liver, was homogenized in 2 vol of 0.9% NaCl in an Osterizer blender (Oster Corporation, Milwaukee, Wisc.). A weighed aliquot of the homogenate was saponified, acidified, and extracted as described previously. The bile acids were methylated with freshly prepared diazomethane and purified from fatty acid methyl esters by TLC as mentioned previously. The purified bile acid methyl esters were acetylated using 1 ml of a mixture of acetic acid, acetic anhydride, perchloric acid (5:5:0.050, v/v/v) at room temperature for 1 hr (20). After adding 8 ml of 20% NaCl, the bile acid methyl ester acetates were extracted with diethyl ether (5 ml × 3) and quantitated by GLC using a Hewlett-Packard Model 402 gas–liquid chromatograph equipped with a 1% OV-225 column (21). The operating temperatures were: column, 240°C; injector, 235°C; and detector, 250°C. Nitrogen was used as the carrier gas at a flow rate of 50 ml/min and hyocholic acid as the internal standard.

Analysis of plasma cholesterol. Plasma cholesterol was analyzed by the Lipid Research Clinics method (22) using a Technicon II Autoanalyzer (Technicon Corporation, Tarrytown, N.Y.).

Light and electron microscopy. Liver slices were fixed in 10% buffered neutral Formalin for light microscopy and stained with hematoxylin and eosin. For electron microscopy, cubes (1.0 mm) of liver tissue collected at necropsy were immediately fixed in 3.0% glutaraldehyde in 0.1 *M* sodium cacodylate, trimmed into 0.5-mm cubes, washed twice in cacodylate buffer, and postfixed in 1.33% osmium tetroxide in 0.1 *M* cacodylate buffer at pH 7.4. Tissues were stained *en bloc* with 2% uranyl acetate, dehydrated through ascending concentrations of ethyl alcohol, transferred to propylene oxide, and embedded according to the method of Luft (23) in Epon 812. Sections were cut at 600–900 Å on an LKB ultramicrotome and mounted on 300-mesh copper grids. The sections were then stained with uranyl acetate and lead citrate and examined with a Philips 201 electron microscope.

Statistical analysis. Control and experimental values were compared using the Student's *t* test for unpaired means.

Results. *Maternal bile acid metabolism.* Feeding 0.25% CDCA for 8 days during gestation slightly increased, though not significantly, the plasma cholesterol (mg%, control: 93.3 ± 15.1; CDCA: 107.3 ± 2.1). Body weight (g) was not affected by CDCA feeding (control: 295.0 ± 2.3; CDCA: 285.6 ± 6.3). Table I lists the bile acid composition and pool size (μg/100 g body wt) in 19-day pregnant control and CDCA fed rats. Overall, the total bile acids were significantly decreased ($P < 0.05$) with CDCA feeding. Lithocholic acid, a major biotransformation metabolite of CDCA, was significantly increased ($P < 0.05$), although CDCA itself, as well as the metabolites α-muricholic acid, hyodeoxycholic acid, and the minor metabolite β-muricholic acid, were not affected significantly by CDCA feeding. Cholic

TABLE I. BILE ACID SIZE AND COMPOSITION IN CONTROL- AND CDCA-FED RATS (FOR 8 DAYS) PREGNANT 19 DAYS[a]

Group	Bile acid (μg/100 g body wt)				
	Lithocholic	3β,12α-Dihydroxycholanoic	Deoxycholic	Chenodeoxycholic	Hydeoxycholic
Control	620.8 ± 114.5	153.8 ± 125.0	1775.4 ± 271.2	2207.5 ± 88.3	853.8 ± 452.1
CDCA	1090.4 ± 123.8[b]	55.9 ± 44.4	615.2 ± 178.5[b]	2151.3 ± 95.5	852.1 ± 70.1

	Cholic	α-Muricholic	β-Muricholic	Total bile acids
Control	3933.7 ± 442.6	1073.8 ± 74.0	228.3 ± 78.6	10830.5 ± 1088.9
CDCA	947.4 ± 331.3[b]	1175.2 ± 358.4	125.3 ± 64.7	7012.9 ± 557.7[b]

[a] Each value represents a mean of three animals ± SEM.
[b] $P < 0.05$.

acid, the major bile acid in adult rats, and its principal biotransformation metabolite, deoxycholic acid, were both significantly decreased ($P < 0.05$). Cholic acid, CDCA and deoxycholic acid were the major bile acids in the control group, while CDCA, α-muricholic, lithocholic, and cholic acids were the major bile acids in the CDCA-treatment group. A very significant decrease ($P < 0.002$) in the ratio of cholic acid and its metabolites (3β,12α-dihydroxycholanoic acid; deoxycholic acid) to CDCA and its metabolites (lithocholic acid; hyodeoxycholic acid; α- and β-muricholic acid) to less than one was found in CDCA fed rats 19-days pregnant (0.3 ± 0.1) when compared to controls (1.2 ± 0.0). Likewise, a significant decrease ($P < 0.02$) in the primary bile acid ratio of cholic acid to CDCA to less than one was found with gestational CDCA feeding (0.4 ± 0.2) when compared to controls (1.8 ± 0.3).

Neonatal bile acid metabolism. Neonatal rat plasma cholesterol was not influenced by maternal CDCA feeding as shown in Table II. Likewise, the neonatal 7α-hydroxylation of cholesterol was not significantly inhibited by feeding of CDCA at the 0.25% level to pregnant rats. Examination of the neonatal bile acid pool composition and size (μg/100 g body wt) did reveal a significant increase in CDCA in those neonatal pools from CDCA treated dams (control: 222 ± 32; CDCA: 613 ± 173; $P < 0.05$, Fig. 1). Cholic acid, CDCA and the ketoacids were the major bile acids in neonates of both control and CDCA fed dams. Total tissue bile acids were not significantly affected in the neonates by maternal CDCA feeding. The ratio of cholic acid and its metabolites (3β,12α-dihydroxycholanoic acid; deoxycholic acid; 3α,12β-dihydroxycholanoic acid) to CDCA and its metabolites (lithocholic acid, hyodeoxycholic acid) was somewhat reduced in neonates from CDCA fed dams (1.7 ± 0.7) compared to control (3.1 ± 0.6), though not statistically significant. Likewise, the primary bile acid ratio of cholic acid to CDCA was also somewhat reduced with CDCA treatment (1.9 ± 0.8) compared to control (3.5 ± 0.4) though not statistically significant. Both of these bile acid ratios remained above one with treatment.

TABLE II. PLASMA CHOLESTEROL AND HEPATIC CHOLESTEROL-7α-HYDROXYLASE ACTIVITY IN CONTROL AND CDCA FED NEONATAL RAT POOLS[a]

Group	Plasma cholesterol (mg %)	Cholesterol-7α-hydroxylase activity (pmol/mg/min)
Control	51.4 ± 8.1 (5)[b]	2.48 ± 0.24 (3)
CDCA	51.5 ± 16.2 (4)	1.65 ± 0.31 (3)

[a] Results are means ± SEM.
[b] (*N*) Represents total number of neonatal rat pools.

Maternal, neonatal, and postnatal liver morphology. Light microscopic examination of liver from rats 19 days pregnant revealed no effects due to 8 days of CDCA feeding at the 0.25% level in the diet. Figure 2 shows no pathology in the portal tract area of the liver in a CDCA-treated pregnant rat. There was no evidence of significant bile duct prolifer-

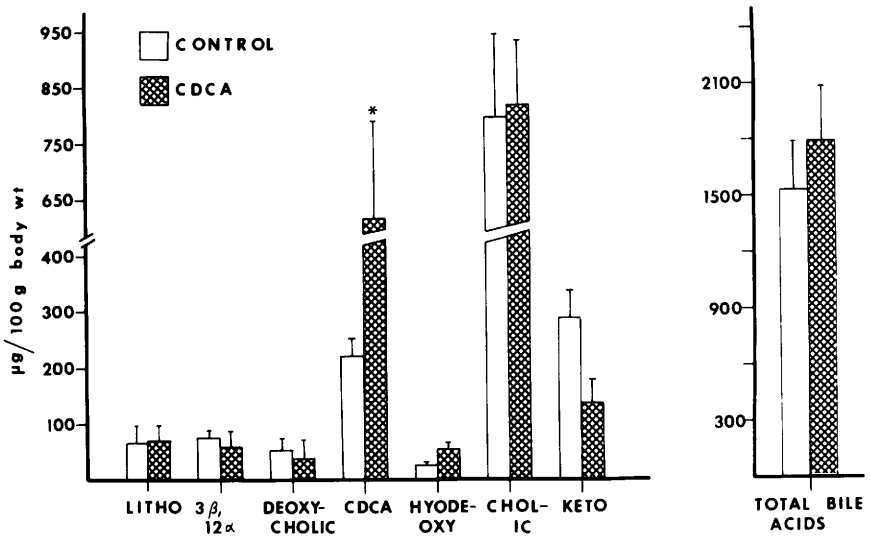

FIG. 1. Neonatal bile acid pool size and composition from control and CDCA fed rats. Each determination represents the mean of neonatal tissue pools (five control and four CDCA pools), the bars denote ± SEM. CDCA (*) in neonates from CDCA-treated dams was significantly elevated at $P < 0.05$. Bile acid abbreviations: LITHO, lithocholic acid; 3β,12α, 3β,12α-dihydroxycholanoic acid; DEOXYCHOLIC, deoxycholic acid; CDCA, chenodeoxycholic acid; HYODEOXY, hyodeoxycholic acid; CHOLIC, cholic acid; KETO, 7-keto-lithocholic acid and 3-keto,7α-hydroxycholanoic acid.

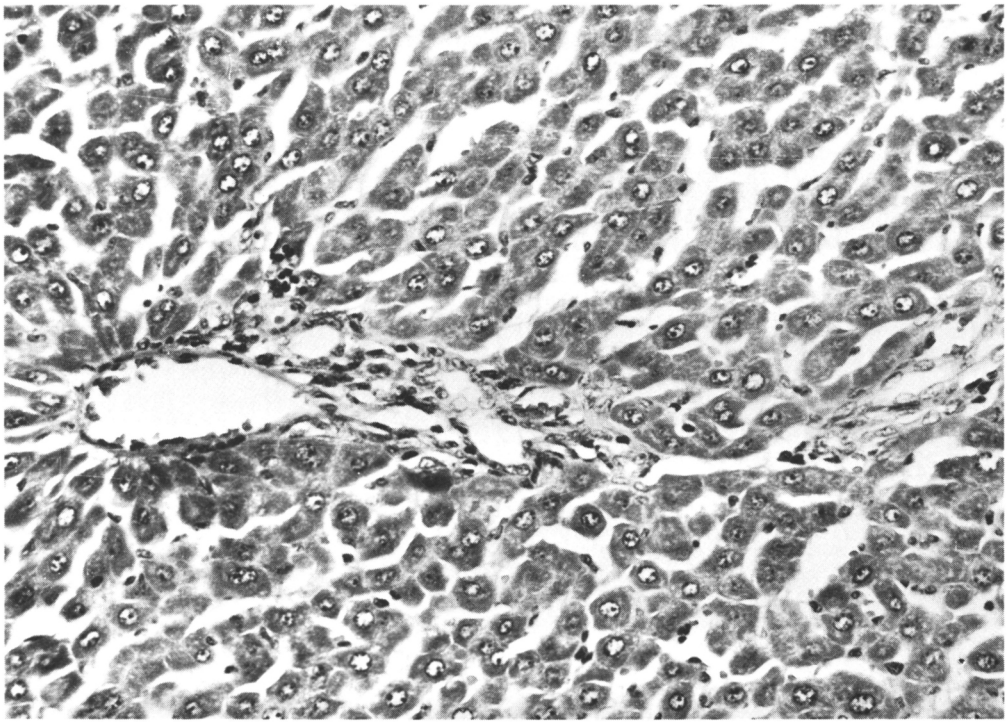

FIG. 2. Portal tract area of maternal rat liver from CDCA-fed rat with structure appearing within normal ranges. H and E, ×275.

ation or inflammation. Light microscopic examination of neonatal liver in the portal tract area again did not reveal any signs of hepatotoxicity (Fig. 3) with maternal CDCA feeding. Ultrastructural examination of the hepatocytes from the control group of neonatal rats showed the usual morphology for developing rat liver (24, 24) with normal appearing mitochondria, microbodies, a few lysosomes, an occasional autophagic vacuole, prominent Golgi, rough endoplasmic reticulum, and slightly dilated developing bile canaliculi with few microvilli (Fig. 4). Hepatocytes of neonates from rats treated with CDCA did not reveal any striking differences in ultrastructural morphology (Fig. 5). There did seem to be, however, an indication of a slight increase in the number of vesiculated electron-dense lysosomes and small secretion saccules near bile canaliculi, these lysosomes possibly containing bile pigment. Ultrastructural examination of hepatocytes of 7-week-old rats previously exposed (Fig. 6) during gestation to maternal CDCA feeding did not reveal any hepatotoxicity when compared to the control group.

Discussion. The results of the present investigation show that feeding of CDCA during gestation influences bile acid pools, metabolism, and hepatic morphology differently in the pregnant and neonatal rat when compared to studies reported in the adult nonpregnant rat (6, 7, 26–31). Bile acid biosynthesis is known to begin late in gestation, increase progressively after birth, and reach adult levels by weaning in mammals (32, 33). Both fetal and neonatal bile acids can originate from either their own synthesis or from maternal transfer across the placenta (34, 35). Taurocholate secretion into the gut begins between Days 19 and 21 of gestation in the fetal rat and increases along with synthesis progressively during late gestation and the first few days after birth (10, 11). With increasing cholate synthesis, CDCA synthesis proportionately decreases with increasing gestational age (11). The early predominance of taurochenodeoxycholate in the rat fetus has been used to support the view that dihydroxy-bile salts diffuse across the placenta more rapidly than trihydroxy-bile salts (10). At birth, rat neonates

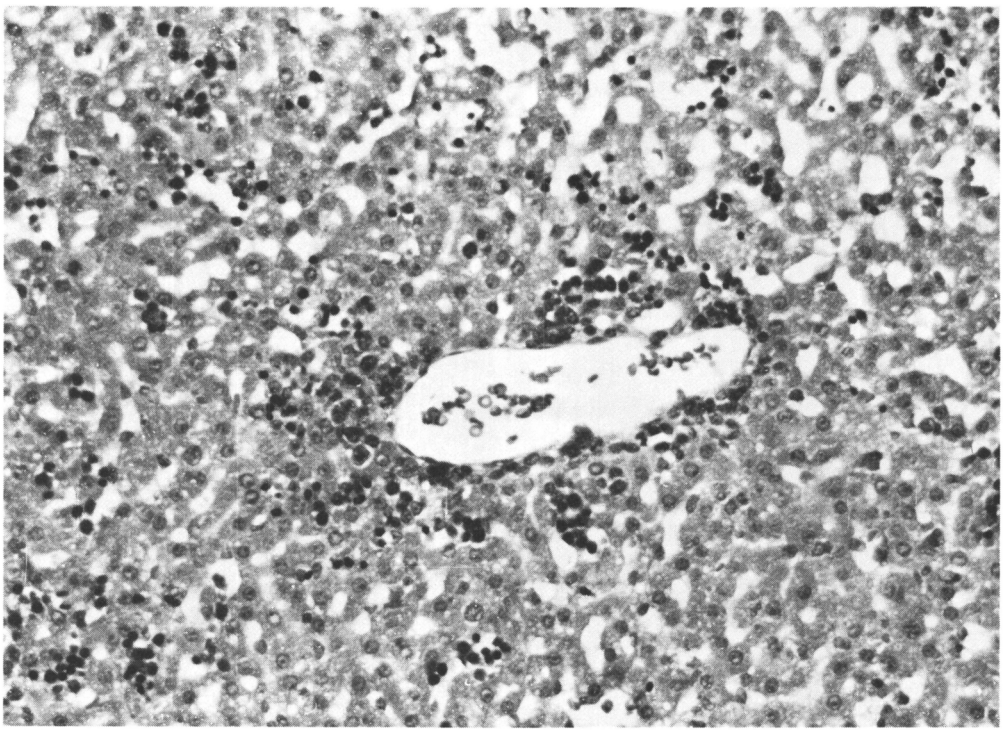

FIG. 3. Portal tract area of neonatal rat liver from CDCA-fed dam with structure appearing within normal ranges. H and E ×275.

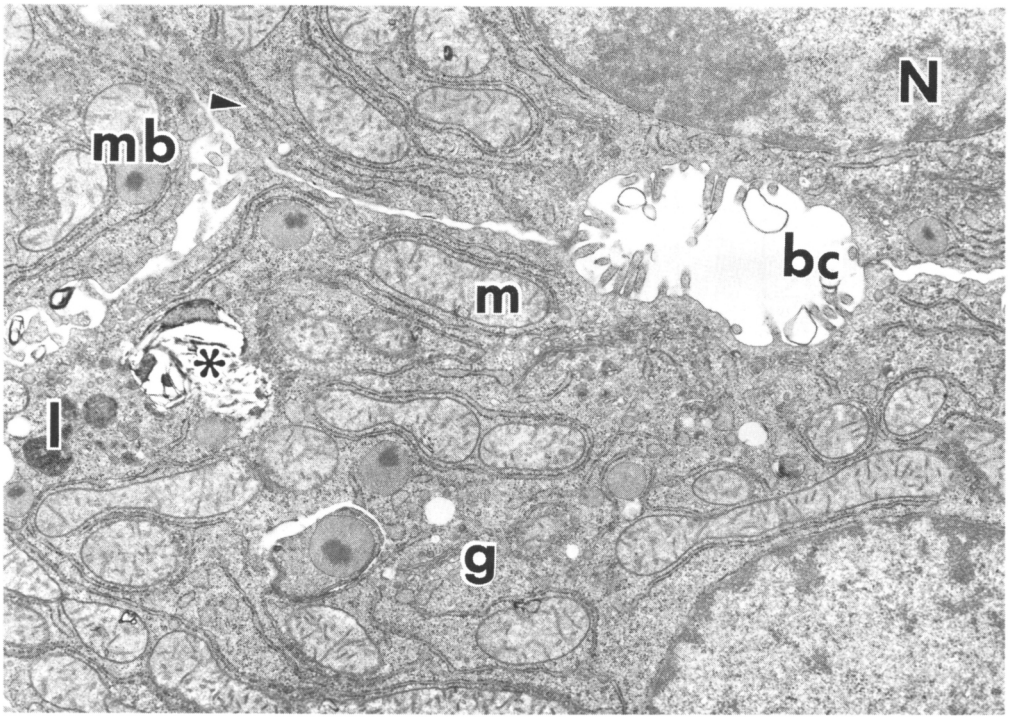

FIG. 4. Hepatocytes of neonatal rat from a control-fed dam with normal appearing mitochondria (m), microbodies (mb), lysosomes (l), Golgi (g), rough endoplasmic reticulum (arrowhead), nucleus (N) and a slightly dilated developing bile canaliculus (bc) with few microvilli. Autophagic vacuole (*) noted. ×12,250.

have been shown to have a biliary bile acid pool of 75% cholic acid and 4% CDCA (11). The development of bile acid synthesis and secretion in the rat is consistent with development and maturation of bile canaliculi during late fetal and early neonatal periods (24, 25). In the present investigation, the significantly greater bile acid pool of CDCA found in neonates of CDCA fed dams suggests significant maternal to fetal transfer of the dihydroxy-bile acid, CDCA.

The 7α-hydroxylation of cholesterol is the major rate limiting step in the overall conversion of cholesterol into cholic acid and CDCA (36). Danielsson and Johansson (6) reported an inhibition of both the 7α-hydroxylation of cholesterol and the 12α-hydroxylation of 7α-hydroxy-4-cholesten-3-one and 5β-cholestane-3α,7α-diol with feeding of 1% CDCA for 2 weeks in the adult rat. After 3 weeks of feeding, a virtual absence of cholic and deoxycholic acid was reported in the bile acid pool. Danielsson (26) also reported inhibition of cholesterol-7α-hydroxylase activity with CDCA feeding after just 3 to 7 days in the adult rat. Shefer *et al.* (27) reported no inhibition of cholesterol-7α-hydroxylase with 7 days of 1% CDCA feeding, while in a later study (28) did report inhibition with 250 mg/rat/day feeding of CDCA. A drop in cholic acid content but no change in biliary lithocholic acid was also found at 250 mg/rat/day along with hepatic inflammation and ultrastructural changes. Recently Shefer *et al.* (7) have reported a reduction of cholesterol-7α-hydroxylase activity in the adult male rat by feeding 1% CDCA, utilizing an assay with a microsomal preparation free of endogenous cholesterol. In our study, feeding of 0.25% CDCA to pregnant rats did not cause a significant inhibition of cholesterol-7α-hydroxylase activity in the neonatal rat, even though CDCA levels were significantly elevated (34% of total bile acid pool) in the neonate. A lack of inhibition of the 7α-hydroxylation of cholesterol may indicate continued synthesis of bile acids. Lack of inhibition is also supported by the lack of change in total neonatal bile

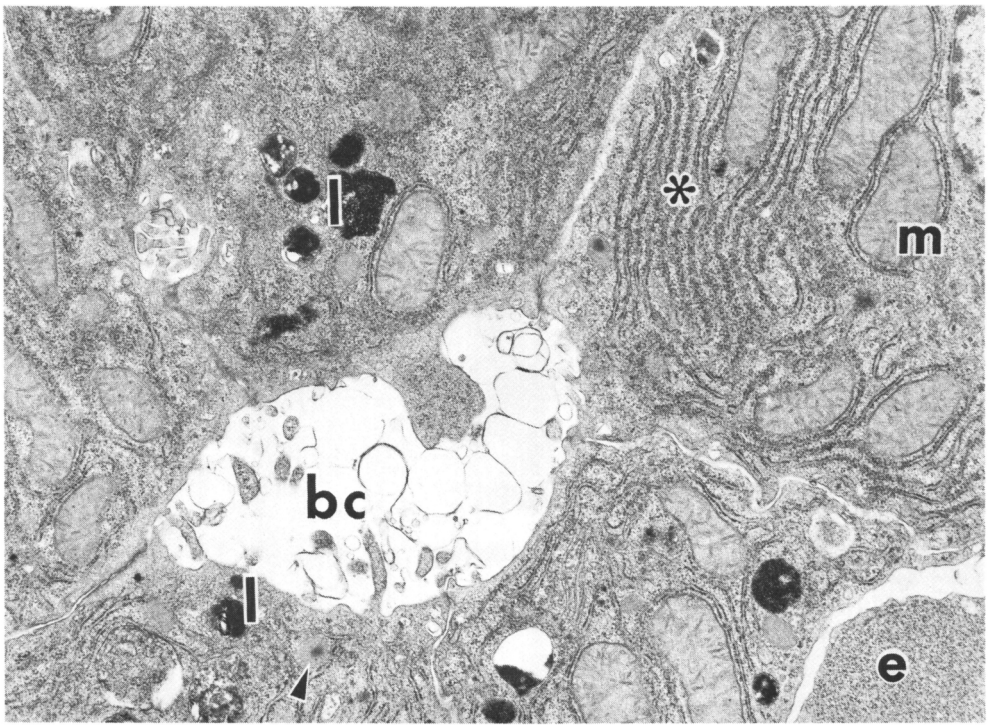

FIG. 5. Hepatocytes of neonatal rat from a CDCA-fed dam with several vesiculated electron-dense lysosomes (l) near a slightly dilated bile canaliculus (bc) with sparse microvilli. Mitochondria (m), microbodies (arrowhead), and prominent rough endoplasmic reticulum (*) noted. Erythroblast (e) in space of Disse. ×12,250.

acids (Fig. 1) with gestational CDCA feeding. These findings suggest that the liver cholesterol-7α-hydroxylase may have been insensitive to feedback inhibition by the elevated CDCA levels in the neonatal rat liver. It is also possible, however, that the dose of 0.25% CDCA was just insufficient to cause significant feedback inhibition since most studies in the adult rat that have demonstrated inhibition of cholesterol-7α-hydroxylase are at the 1% level of CDCA in the diet (6, 7, 26, 28). In contrast to the neonate, in the pregnant rat of the present study the significant reduction in the total bile acid pool as well as in the cholic and deoxycholic acid pools (Table I) with 8 days of 0.25% CDCA feeding might suggest feedback inhibition on cholesterol-7α-hydroxylase even though the enzyme activity was not measured. Alternatively, a possible increase in fecal excretion of bile acids might account for the reduction in the total bile acid pool in the CDCA treated rats. The plasma cholesterol levels of both the pregnant rat and neonate (Table II) were not significantly affected by gestational CDCA feeding. This correlates with a study (37) reported for the adult rat in which 0.5% taurocholate plus 0.16% cholesterol-supplemented stock diet elevated both cholesterol absorption and liver and plasma cholesterol in comparison to no changes in rats on either 0.16% cholesterol-supplemented stock diet or a 0.5% CDCA plus 0.16% cholesterol-supplemented stock diet.

Cholic acid and CDCA are the main bile acids formed from cholesterol in most mammals, including rat and man (38). Few variations in the ratio of cholic acid to CDCA in normal healthy animals of the same species have been reported. The normal ratio in the rat has variously been reported as 4:1, 3:1 (39, 40) or, more simply, as one and above (41). The ratio of cholic acid to CDCA has been reported decreased in cases of cirrhosis and severe hepatic injury (40). The mechanism of reversal of the bile acid ratio (less than one) is not understood. A predominance of CDCA

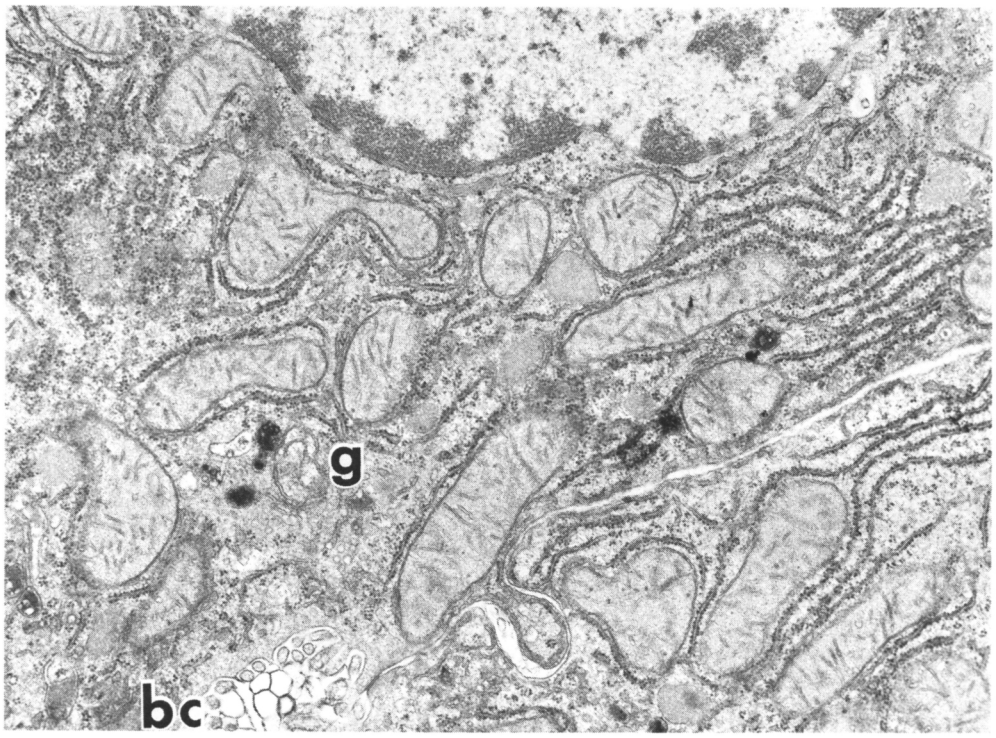

FIG. 6. Hepatocytes of 7-week-old postnatal rat from CDCA-fed dam with normal appearing organelles. Bile canaliculus (bc) and Golgi (g) noted. ×17,500.

has been correlated to poor hepatic parenchymal cell function or biochemical evidence of hepatic injury (39). In our investigation, the primary bile acid ratio (cholic/CDCA) in the neonates remained above one with gestational CDCA feeding, indicating the probable retention of good parenchymal cell function in the neonate as well as our *in vitro* demonstration of a lack of cholesterol-7α-hydroxylase inhibition. In the pregnant rat, however, a significant decrease to less than one was found within the cholic/CDCA ratio with CDCA feeding. Although this generally indicates a possibility of poor parenchymal cell function (39, 40), studies by Weber *et al.* (42) in North American Indians with pediatric familial cholestasis indicated that a ratio of greater than one was correlated with ultrastructural evidence of intrahepatic cholestasis as evidenced by marked accumulation of actin within pericanalicular ectoplasm. A more probable explanation for the decrease in the cholic acid/CDCA ratio in CDCA-fed animals would be an overall decrease in endogenous cholic acid and CDCA counterbalanced by the intake of CDCA resulting in a net decrease in the ratio. This is supported by the decrease in the biliary pool of cholic acid in the pregnant rat with CDCA feeding, but a lack of a difference in the biliary levels of CDCA (Table I).

With decreases in the primary trihydroxy–dihydroxy bile acid ratio, the patterns of secondary bile acid metabolites have been reported to similarly change with relatively more lithocholic acid and relatively less deoxycholic acid being formed (38, 43). Likewise in our study, lithocholic acid, derived from CDCA by the 7α-dehydroxylation of CDCA by intestinal bacteria, was significantly increased while deoxycholic acid, a metabolite of cholic acid, was significantly decreased in the bile acid pool of the pregnant rat with gestational feeding of CDCA (Table I). As would be expected, the ratio of cholic acid and its metabolites to CDCA and its metabolites also was decreased significantly in the pregnant rat with CDCA feeding. Both lithocholic acid and its conjugates and CDCA and its conjugates have been reported to induce intrahepatic choles-

tasis and hepatic injury in experimental animals (29–31, 44–46). Hepatic injury reported in the rat by high doses of CDCA has consisted of cellular necrosis, inflammation, ductular cell proliferation, fibrosis, and ultrastructural changes of dilated bile canaliculi, reduplication of the canalicular membrane, loss or swelling of canalicular microvilli, distended Golgi vesicles, whorling of mitochondria, an increase in dense bodies near the canaliculus, swollen sinusoidal microvilli, and sinusoidal plasma membrane indentations with associated cytoplasmic vacuoles (7, 30, 44, 46). In our investigation, there was no histological evidence of necrosis, inflammation, or bile duct proliferation in the livers from the CDCA-treated pregnant rats or from the livers of neonates and 7-week-old postnatal rats from CDCA-treated dams. Examination of the fine structure of neonatal and postnatal hepatocytes also failed to reveal any significant evidence of hepatotoxicity with gestational CDCA feeding. Although the increased levels of lithocholic acid in the treated pregnant rat might make their livers suspect for hepatic biochemical injury, the histologic patterns were well within normal limits. The sulfation of bile acids, increased both by pregnancy and by feeding CDCA (47, 48), has been suggested as having a protective effect on the rat from hepatotoxicity. However, sulfated lithocholic acid conjugated with glycine is cholestatic in rats and induces ultrastructural changes in hepatocytes (46). Sulfated lithocholic acid conjugated with taurine, however, is not cholestatic in rats (46). In guinea pigs, increasing the availability of dietary taurine prevented cholestasis induced by sulfated lithocholic acid (conjugated almost exclusively with glycine) as well as preventing morphological changes (49). Thus, effective taurine conjugation might play the greater role in protecting the maternal, neonatal, and postnatal livers from CDCA induced injury in the rat.

In summary, the results described in this study have indicated in neonates of CDCA-fed dams a significant maternal to fetal transfer of the dihydroxy bile acid CDCA, the levels of which, although elevated, failed to induce a feedback inhibition on neonatal cholesterol-7α-hydroxylase or on total bile acid pools. CDCA-fed to pregnant rats for 8 days reduced the total bile acid pools in the mother as well as reducing the pools of cholic acid and its metabolite deoxycholic acid. CDCA feeding also increased the pools of lithocholic acid, a metabolite of CDCA in the pregnant rat. A decreased cholic acid/CDCA ratio in the CDCA-fed pregnant rat suggests probable inhibition of cholesterol-7α-hydroxylase along with increased fecal excretion of cholic acid. Such inhibition would most likely result in decreased endogenous cholic acid and CDCA. Endogenous CDCA decreases would be counterbalanced with CDCA intake in the diet, leading to a decreased cholic acid/CDCA ratio. Thus, it appears that cholesterol-7α-hydroxylase in the pregnant rat may have been sensitive to feedback inhibition by CDCA feeding and by increased biliary lithocholic acid levels. Maternal, neonatal, and postnatal rat liver was protected from hepatotoxicity with gestational CDCA feeding at the 0.25% level most probably by the predominance of taurine conjugation reported for the rat as well as high levels of sulfation (46, 49).

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1. Parke DV. The endoplasmic reticulum: Its role in physiological functions and pathological situations. In: Jenner P, Testa B, eds. Concepts in Drug Metabolism. New York, Dekker, Part B, pp1–52, 1981.
2. Shefer S, Hauser S, Bekersky I, Mosbach EH. Biochemical site of regulation of bile acid synthesis in the rat. J Lipid Res **11**:404–411, 1970.
3. Myant NB, Mitropoulos KA. Cholesterol-7α-hydroxylase. J Lipid Res **18**:135–153, 1977.
4. Mellon WS, Witiak DJ, Feller DR. Cholesterol-7α-hydroxylase—evidence for a distinct hepatic microsomal enzyme system. Biochem Pharmacol **27**:1055–1062, 1978.
5. Mason JI, Boyd GS. The suppressive effect of the catatoxic steroid, pregnonolone-16α-carbonitrile, on liver microsomal cholesterol-7α-hydroxylase. Steroids **31**:849–854, 1978.
6. Danielsson H, Johannson G. Effects of long term feeding of chenodeoxycholic acid on biosynthesis and metabolism of bile acids in the rat. Gastroenterology **67**:126–134, 1974.
7. Shefer S, Zaki FG, Salen G. Early morphologic and enzymatic changes in livers of rats treated with chenodeoxycholic and ursodeoxycholic acids. Hepatology **3**:201–208, 1983.
8. Hassan AS, Yunker RL, Sprinkle DJ, Subbiah MTR.

Chronic suppression of cholesterol-7α-hydroxylase by dietary chenodeoxycholic acid in neonatal guinea pigs: Its effect on subsequent bile acid metabolism in the adult. J Nutr **113**:986–995, 1983.

9. McSherry CK, Morrissey KP, Swarm RL, May PS, Niemann WH, Glenn F. Chenodeoxycholic acid induced liver injury in pregnant and neonatal baboons. Ann Surg **184**:490–499, 1976.
10. Lester R, Little JM, Greco R, Piasecki GJ, Jackson BT. Fetal bile salt formation. Pediatr Res **6**:375, Abstr, 1972.
11. Little JM, Richey JE, Van Thiel DH, Lester RJ: Taurocholate pool size and distribution in the fetal rat. J Clin Invest **63**:1042–1049, 1979.
12. Albers JJ, Grundy SM, Cleary PA, Small DM, Lackin JM, Schoenfield, LJ. National Cooperative Gallstone Study. The effect of chenodeoxycholic acid on lipoproteins and apolipoproteins. Gastroenterology **82**:638–646, 1982.
13. Phillips MJ, Fisher RL, Anderson PW, Lan SP, Lackin JM, Boyer JL *et al.* Ultrastructural evidence of intrahepatic cholestasis before and after chenodeoxycholic acid therapy in patients with cholelithiasis: The National Cooperative Gallstone Study. Hepatology **3**:209–220, 1983.
14. Hassan AS, Gallon LS, Zimmer LA, Balistreri WF, Subbiah MTR. Persistent enhancement of bile acid synthesis in guinea pigs following stimulation of cholesterol catabolism in neonatal life. Steroids **38**:477–484, 1981.
15. Mitropoulos KA, Balasubramaniam S. Cholesterol-7α-hydroxylase in rat liver microsomal preparations. Biochem J **128**:1–9, 1972.
16. Shefer S, Hauser S, Mosbach EH. 7α-hydroxylation of cholesterol by rat liver microsomes. J Lipid Res **9**:328–333, 1968.
17. Hartree EF. Determination of protein: A modification of the Lowry method that gives a linear photometric response. Anal Biochem **48**:422–427, 1972.
18. Grundy SM, Ahrens EH, Mittinen TA. Quantitative isolation and gas–liquid chromatographic analysis of total fecal bile acids. J Lipid Res **6**:397–410, 1965.
19. Subbiah MTR, Tyler NE, Buscglia MD, Marai L. Estimation of bile acid excretion in man: Comparison of isotopic turnover and fecal excretion methods. J Lipid Res **17**:78–86, 1976.
20. Parmentier GG, Janssen GA, Eggermont EA, Eyssen HJ. C_{27} bile acids in infants with coprostanic acidemia and occurrence of a 3α,7α,12α-trihydroxy-5β-C_{29} dicarboxylic bile acid as a major component in their serum. Eur J Biochem **102**:173–183, 1979.
21. Yousef IM, Fisher MM, Myher JJ, Kuksis A. Superior gas–liquid chromatography of methyl cholanoate acetates on cyanopropylphenylsiloxane liquid phases. Anal Biochem **75**:538–544, 1976.
22. Lipid Research Clinics Program Manual of Laboratory Operations. Washington, DC, Vol 1, DHEW Publication No 75-628, 1974.
23. Luft JH. Improvements in epoxy resin embedding methods. J Biophys Biochem Cytol **9**:409–414, 1961.
24. Wood R. An electron microscopy study of developing bile canaliculi in the rat. Anat Rec **151**:507–530, 1965.
25. De Wolf-Peeters C, De Vos R, Desmet V. Electron microscopy and histochemistry of canalicular differentiation in fetal and neonatal rat liver. Tissue Cell **4**:379–388, 1972.
26. Danielsson H. Influence of dietary bile acids on formation of bile acids in rat. Steroids **22**:667–676, 1973.
27. Shefer S, Hauser S, Lapar V, *et al.* Regulatory effects of sterols and bile acids on hepatic 3-hydroxy-3-methyl glutaryl CoA reductase and cholesterol-7α-hydroxylase in the rat. J Lipid Res **14**:573–580, 1973.
28. Shefer S, Zaki G, Salen G. Early feeding of chenodeoxycholic and ursodeoxycholic acids on hepatic morphology and cholesterol and bile acid metabolism in the rat. Clin Res **30**:269, Abstr, 1982.
29. Fisher MM, Magnusson R, Miyai K. Bile acid metabolism in mammals. I. Bile induced intrahepatic cholestasis. Lab Invest **25**:88–91, 1971.
30. Miyai K, Price UM, Fischer MM. Bile acid metabolism in mammals. Ultrastructural studies on the intrahepatic cholestasis induced by lithocholic and chenodeoxycholic acid in the rat. Lab Invest **24**:292–302, 1971.
31. Kakis G, Yousef IM. Pathogenesis of lithocholate- and taurolithocholate-induced intrahepatic cholestasis in rats. Gastroenterology **75**:595–607, 1978.
32. Croizat B, Lambiotte M. Les acides biliares au cours de la vie embryonnaire: date d'apparition, nature, evolution et origine. Arch Sci Physiol **25**:303–326, 1971.
33. Li JR, Dinh DM, Ellefson RD, Subbiah MTR: Sterol and bile acid metabolism during development. 3. Occurrence of neonatal hypercholesterolemia in guinea pig and its possible relation to bile acid pool. Metabolism **28**:151–156, 1979.
34. Back P, Walter K. Developmental pattern of bile acid metabolism as revealed by bile acid analysis of meconium. Gastroenterology **78**:671–676, 1980.
35. Hassan AS, Subbiah MTR. Bile acids in the fetal rat: Effect of maternal bile duct ligation. Steroids **36**:709–715, 1980.
36. Shefer S, Hauser S, Bekersky I, Mosbach EH. Biochemical site of regulation of bile acid synthesis in the rat. J Lipid Res **11**:404–411, 1970.
37. Raicht RF, Cohen B, Mosbach EH. Effects of sodium taurochenodeoxycholate and sodium taurocholate on cholesterol absorption in the rat. Gastroenterology **67**:1155–1161, 1974.
38. Danielsson H, Einarsson K. Formation and metabolism of bile acids. In: Bittar EE, Bittar N, eds, The Biological Basis of Medicine. London, Academic Press, Vol 5:pp279–315, 1968.
39. Carey JB. The serum trihydroxy dihydroxy bile acid ratio in liver and biliary tract disease. J Clin Invest **37**:1494–1503, 1958.

40. Strand O. Effects of D- and L-triiodothyronine and of propyl thiouracil on the production of bile acids in the rat. J Lipid Res **4**:305–311, 1963.
41. Javitt NB. Bile acids and hepatobiliary disease. In: Schiff L, Schiff ER, eds. Diseases of the Liver. Philadelphia, Lippincott, 5th ed, p139, 1982.
42. Weber AM, Tuchweber B, Lepage G, Yousef I, Roy CC, Morin CL. Serum bile acid profile and liver ultrastructure in familial and other pediatric cholestatic syndromes. Gastroenterology **79**:1129, 1980.
43. Sandberg DH. Bile acid concentrations in serum during pregnancy and childhood. Pediatr Res **4**:262–267, 1970.
44. Carey JB, Wilson ID, Onsted G, Zaki FG. Role of 12α-hydroxylase deficiency in continuing liver injury. J Clin Invest **46**:1042–1043, Abstr, 1967.
45. King JE, Schoenfield L. Cholestasis induced by sodium taurolithocholate in isolated hamster liver. J Clin Invest **50**:2305–2312, 1971.
46. Yousef IM, Tuchweber B, Vonk RJ, Masse P, Audet M, Roy CC. Lithocholate cholestasis—sulfated glycolithocholate-induced intrahepatic cholestasis in rats. Gastroenterology **80**:233–241, 1981.
47. Chen L, Thaler MM. Development and regulation of hepatic bile salt sulfotransferase (BSS) before and after sexual maturation in rats. Gastroenterology **78**:1303, Abstr, 1980.
48. Laatikainen TJ, Lehtonen PJ, Hesso AE. Fetal sulfated and non-sulfated bile acids in intrahepatic cholestasis of pregnancy. J Lab Clin Med **92**:185–193, 1978.
49. Dorvil NP, Yousef IM, Tuchweber B, Roy CC. Taurine prevents cholestasis induced by lithocholic acid sulfate in guinea pigs. Amer J Clin Nutr **37**:221–232, 1983.

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