Effects of bile salt supplementation on biliary secretion in estrogen-treated rats

X.M. Zhao and J.-C. Montet

INSERM, Marseille, France

The aim of the present study was to determine whether bile acid feeding to rats can reverse ethinyl estradiol-induced cholestasis. Animals received ethinyl estradiol (2 mg/kg/day) for 6 days or were coinfused with estrogen plus various bile acids (60 mg/kg/day). Cholestasis could be significantly prevented by tauroursodeoxycholic acid, was partly corrected by ursodeoxycholic acid, and was unchanged by chenodeoxycholic acid. Total bile salt secretion was increased in every group. The secretion of the major primary bile acids (cholic acid and β-muricholic acid) was restored to a large extent in rats supplemented with tauroursodeoxycholate but not in chenodeoxycholate-fed rats. In the former group, the canalicular transport of taurocholate and the bile salt pool size were identical with those of control rats. The hydrophilic–hydrophobic balance of the administered bile salt species appears to be an essential factor in the restoration of bile secretion, the more hydrophilic bile salt having the more hepatoprotective effect.

Keywords: bile salts; estrogen; cholestasis; cholesterol

Introduction

Estrogens are implicated in intrahepatic cholestasis of pregnancy and in oral contraceptive-induced cholestasis. Such disorders are encountered with an increase in biliary cholesterol, thus augmenting the risk of acquiring cholesterol gallstones.[1]

In rats, ethinyl estradiol (EE) administration causes reversible intrahepatic cholestasis.[2] EE-induced bile secretory failure is partly associated with decreased bile acid-dependent flow,[3] decreased bile acid synthesis,[4] and decreased taurocholate uptake by hepatocyte.[5] These data support the concept that the disorders in biliary secretion of bile salts would constitute an essential underlying mechanism in EE cholestasis.

Since taurocholate reverses cholestasis induced by another steroid, taurolithocholate, by facilitating its biliary excretion into mixed micelles,[6] it was hypothesized that because of their micellar properties bile salts could modify the cholestatic effect of estrogens.[7] The data of Bonazzi et al.[8] have shown that this was not always the case and that chenodeoxycholate, a hydrophobic bile salt that efficiently solubilizes insoluble compounds[9,10] was ineffective in preventing the reduction of biliary secretion in EE-treated rats. The relations between this estrogen, the hepatocyte membranes, and bile salts are poorly understood; however, it can be conceived that the hydrophilic–hydrophobic balance of bile salts plays a crucial role in the excretion of the hormone by the hepatocyte and in the prevention of the cytotoxicity induced by EE treatment. This rationale is founded on the large diversity of physical properties of bile salts[9-16] as well as their behavior toward membranes.[17,18] To gain information on this possibility, the comparative effect of chenodeoxycholic acid (CDC), ursodeoxycholic acid (UDC), and tauroursodeoxycholic acid (TUDC) on biliary secretion was assessed in EE-treated rats.

Materials and methods

Materials

Ethinyl estradiol (EE) (17α-ethinyl-△-1,3,5-estratriene-3,17β-diol) was purchased from Sigma Chemical Co. (St. Louis, MO, USA), and was 99% pure. EE was dissolved in propylene glycol (2 mg/mL). Ursodeoxycholic acid (UDC) (3α,7β-dihydroxy-5β-cholanoic acid) was a generous gift of Roussel-Uclaf Laboratories, Romainville, France. Chenodeoxycholic acid (CDC), (3α,7α-dihydroxy-5β-cholanoic acid) was from Calbiochem (Los Angeles, CA, USA). The bile acids were neutralized with NaOH and dissolved in carbon-

Address reprint requests to J.-C. Montet, INSERM, 46 Boulevard de la Gaye, 13009 Marseille, France.
Supported in part by grants from the Caisse Régionale d'Assurance Maladie du Sud-Est and the Fondation pour la Recherche Médicale.
Received January 8, 1990; accepted March 9, 1990.

ate–bicarbonate buffer 0.05 M, pH 9. Tauroursodeoxycholate (TUDC) and taurocholate (TC) were from Calbiochem. They were solubilized in normal saline. All the bile salts used were better than 98% pure. Taurine (>99%) was purchased from Calbiochem.

Animal treatment

Male Sprague–Dawley rats weighing 200–250 g at the beginning of the experiment were obtained from IFFA-CREDO, France. The animals were kept in an air-conditioned room lighted 12 h a day. They had free access to food and water (with 1% taurine). The chow diet, from U.A.R. Villemoisson, France, contained the following: proteins 26.7%, lipids 5.7%, carbohydrate 56.5%, fibers 4.5%, mineral mix 6%, and vitamin mix 0.5%. Rats (n = 62) were distributed into 5 groups and received a specific treatment for 6 days as follows: Group I, control animals; group II, rats were given a daily subcutaneous injection of EE, 2 mg/kg/day; group III, rats were treated with both EE and UDC; group IV, rats were given EE + TUDC; group V, rats received EE + CDC. Each bile salt was given at a 60 mg/kg/day dose by gavage.

On the 7th day animals were weighed then operated on 12 h after receiving the last dose. The proximal bile duct and a femoral vein were cannulated with PE-10 and Venocath 18 catheters respectively, under light ether anesthesia. While bile was being collected, normal saline was infused intravenously (1 mL/h) by extracorporeal pump to replenish body fluids. The animals were held in restraining cages with body temperature maintained at 37–38°C by a heating lamp. Bile was collected according to three protocols:

1. After 30 min equilibration, bile was collected at 1-h intervals for 3 h and the biliary lipids were measured.
2. Total collection of the bile for 18 h allowed us to estimate the bile salt pool size as described.[19]
3. After 18 h of biliary drainage, rats were given a continuous intravenous infusion of TC (6 μmol/min/kg). The bile salt contents were determined in 20-min samples for 100 min. The largest value of TC output was compared in groups I, II, IV, and V.

Biliary lipid determination

Free cholesterol was analyzed by the cholesterol oxydase method[20] using a Boehringer Mannheim kit. Phospholipid content was determined by the method of Amic et al.[21] Total bile salts were determined enzymatically using a 3α-hydroxysteroid dehydrogenase (Worthington Biochemical Corporation, Freehold, NJ, USA).[22] The ratio between tauroconjugated bile salts and glycoconjugated bile salts was obtained after thin-layer chromatographic separation (solvent butanol–acetic acid–water 100:10:10, v/v/v) and by reverse-phase high-performance liquid chromatography using the C18-bonded stationary phase and acetonitrile–water–tetrabutylammonium phosphate (35:65:1) as the mobile phase. Conjugated bile salts were hydrolyzed by cholylglycine hydrolase (Sigma Chemical Co.) at 37°C for 1 h, acidified with citrate/phosphate buffer (pH 2.6), and extracted with ethyl ether. The bile acids were analyzed by gas–liquid chromatography (Intersmat IGC 120 apparatus) on a fused-silica WCOT capillary column SE-30 (25 m × 0.25 mm i.d.) as their methyl ester–trimethylsilyl ether derivatives. The column temperature was 240°C, the carrier gas was hydrogen (1.3 bar at 25°C, leak 50 mL min^{-1}). The chromatograph was equipped with a flame ionization detector and a split/splitless injector. The individual bile acids were also separated by thin-layer chromatography with the following solvent systems: chloroform–acetic acid–cyclohexane–methanol (80:10:10:2.5 v/v/v/v) and isooctane–ethyl acetate–acetic acid–*n*-butanol (30:5:3:3 v/v/v/v). Spots were visualized and quantitatively evaluated with a CAMAG scanner photodensimeter coupled with a Delsi Enica 10 integrator.

Student's *t* test was used to assess differences between groups. *P* values lower than .05 were considered to be significant.

Results

Body weight loss was 8% in groups II and V, no change was observed in groups III and IV, and body weight gain was about 12% in group I.

Biliary flow and biliary lipid composition

The biliary flow rates and the lipid outputs of EE-treated rats and of rats receiving a diet supplemented with bile salts are presented in *Table 1*. Treatment with EE resulted in a large decrease in bile flow. This EE cholestatic effect could be significantly corrected by the addition of UDC and TUDC (groups III and IV, respectively) but not by CDC (group V). In rats receiving TUDC, choleresis recovered a normal level. EE administration strongly depressed bile salt output. The daily ingestion of UDC or CDC restored normal bile salt secretion, whereas TUDC administration in the same conditions significantly increased bile salt secretion when compared to controls.

The biliary secretion of phospholipids and cholesterol reduced by EE treatment was restored by UDC, CDC, and TUDC.

The molar percentage of cholesterol (*Table 2*) was increased in EE-treated rats during the first hour. A larger increase occurred during biliary drainage (2d and 3d hour). In rats supplemented with bile salts, the percentage of biliary cholesterol was not significantly different from that of control rats.

Bile acid pattern

Biliary bile acids were predominantly conjugated with taurine. The molar ratio tauroconjugated bile salts/glycoconjugated bile salts was larger than 9 in every group. The bile acid pattern of rat bile of each group is illustrated in *Table 3*.

Table 1 Bile flow and biliary lipid outputs in rats submitted to different treatments[a]

Experimental groups	Bile flow (mL/h/kg)	Biliary lipid outputs (μmol/h/kg)		
		Bile salts	Phospholipids	Cholesterol
I Control	4.04 ± 0.17	96.93 ± 7.37	17.28 ± 1.85	1.74 ± 0.14
II EE	2.18 ± 0.15*	43.60 ± 1.96*	12.73 ± 0.74*	1.01 ± 0.05*
III EE + UDC	3.03 ± 0.23*†	98.87 ± 7.60†	24.76 ± 1.74†	1.51 ± 0.19†
IV EE + TUDC	3.88 ± 0.23†	130.41 ± 12.15*†	23.42 ± 2.47†	1.84 ± 0.09†
V EE + CDC	2.53 ± 0.07*	92.62 ± 10.17†	21.57 ± 1.70†	1.45 ± 0.15†

[a] Data represent the means ± SEM during the first hour bile collection. There were 6 rats in groups IV and V and 9 in groups I, II, and III.
*Differs from group I, $p < .05$.
† Differs from group II, $p < .05$.

Table 2 Cholesterol content of hepatic bile during biliary drainage[a]

Experimental groups	Cholesterol (mol%)		
	1st hour	2d hour	3d hour
I Control	1.49 ± 0.12	1.53 ± 0.15	1.47 ± 0.16
II EE	1.77 ± 0.11	2.03 ± 0.23*	2.43 ± 0.29*
III EE + UDC	1.21 ± 0.17†	1.48 ± 0.20†	1.81 ± 0.13†
IV EE + TUDC	1.25 ± 0.12†	1.30 ± 0.17†	1.45 ± 0.28†
V EE + CDC	1.27 ± 0.15†	1.30 ± 0.24†	1.20 ± 0.09†

[a] Values represent the means ± SEM.
* Differs from group I, $p < .05$.
† Differs from group II, $p < .05$.

The primary bile acids C and β-MC represented about 90% of total bile acids in control rats.

Ethinyl estradiol caused a marked increase in the relative amount of 6 hydroxylated bile acids. In these animals, the percentage of C decreased, whereas that of β-MC increased when compared with control rats. CDC and secondary bile acids were minor components and never exceeded 6% of total bile acids.

In EE-treated rats supplemented with bile salts, the infused bile salt accounted for 35–43% of total bile salts, thereby reducing the percentage of each primary bile salt to 20–29%.

The percentage of each bile salt species combined with the bile salt output allowed us to evaluate the output of each molecular species. In *Table 4* are presented the output of the major species present in the various groups. This table shows that

- TUDC completely restored the β-MC output when it was depressed by EE.

Table 3 Effect of EE associated with bile salts on biliary bile acid composition[a]

Experimental groups	C	β-MC	CDC	HDC	UDC	DC
I Control	55 ± 1.9	35 ± 1.0	4 ± 0.1	2 ± 0.1	<1	1 ± 0.3
II EE	34 ± 1.0	49 ± 1.4	6 ± 0.2	4 ± 0.3	<1	4 ± 0.2
III EE + UDC	29 ± 0.8	26 ± 0.8	5 ± 0.1	<1	35 ± 0.8	2 ± 0.2
IV EE + TUDC	28 ± 0.8	23 ± 0.7	2 ± 0.1	2 ± 0.2	43 ±1.2	1 ± 0.1
V EE + CDC	20 ± 0.6	23 ± 0.5	42 ± 1.2	5 ± 0.1	5 ± 0.7	4 ± 0.1

[a] Values represent the mean percentages of each bile acid ± SEM. C, Cholic acid; β-MC, β-muricholic acid; CDC, chenodeoxycholic acid; HDC, hyodeoxycholic acid; UDC, ursodeoxycholic acid; DC, deoxycholic acid.

Table 4 Biliary bile acid output in rats submitted to different treatments[a]

Experimental groups	C	β-MC	CDC	UDC
I Control	53.31 ± 4.05	33.93 ± 2.58	3.88 ± 0.29	1.00 ± 0.06
II EE	14.82 ± 0.66*	21.36 ± 0.96*	2.71 ± 0.16	0.45 ± 0.03
III EE + UDC	28.98 ± 1.99*†	25.99 ± 1.79*†	5.00 ± 0.34	34.98 ± 2.41
IV EE + TUDC	36.51 ± 3.50*†	29.99 ± 2.88†	2.61 ± 0.25	56.08 ± 5.38
V EE + CDC	18.52 ± 2.03*	21.30 ± 2.34*	38.90 ± 4.27	4.63 ± 0.51

[a] Values are μmol/h/kg and represent the means ± SEM.
* Differs from group I, $p < .05$.
† Differs from group II, $p < .05$. See *Table 3* for abbreviations.

Table 5 Bile salt pool size and maximal TC output[a]

Experimental groups	Number of rats	Bile salt pool (μmol/kg)	Maximal TC output (μmol/min/kg)
I Control	8	540.39 ± 33.22	5.43 ± 0.29
II EE	7	425.74 ± 17.84*	4.34 ± 0.33*
IV EE + TUDC	4	551.50 ± 18.85†	5.70 ± 0.25†
V EE + CDC	4	503.73 ± 57.80	4.51 ± 0.02*

[a] Bile salt pool size was estimated by collecting bile during 18 h. Maximal TC output was obtained during infusion of 6 μmol/min/kg of TC. The maximum output was achieved in the third 20-min period in the different groups.
* Differs from group I, $p < .05$.
† Differs from group II, $p < .05$.

- TUDC efficiently improved the C output, which recovered a level equal to 66% of that of control animals.
- CDC did not significantly modify the strong decrease in β-MC and C outputs provoked by EE.
- UDC partially improved the biliary secretion of β-MC and C acids.
- Finally, the biliary output of TUDC was 1.6-fold larger after TUDC administration than after UDC administration.

On the other hand, supplementation of EE-treated rats with TUDC allowed them to recover a bile salt pool and a transhepatic TC transport (*Table 5*) similar to those of control animals. Conversely, rats given EE + CDC exhibited a maximal TC output that was not significantly different from that of EE-treated rats (group II).

Discussion

Each bile salt species is able to specifically determine the expression of some enzymes such as hydroxymethyl CoA reductase and 7α-hydroxylase.[23-25] Thus, CDC is a more potent inhibitor than C, which is itself more potent than UDC.[22] On the other hand, the transport systems that ensure intrahepatocytic transfer of bile salts from the sinusoidal membrane to the canalicular membrane can be differently affected depending on the bile salt type. This is illustrated by the large range of maximal bile acid secretory rates[26,27] where TUDC has the highest value and TCDC the lowest.[27] Last, each bile salt type has a specific apparent choleretic activity.[26,27]

This led us to test in this work whether very hydrophilic bile salts such as UDC and TUDC and a very hydrophobic one, CDC, had a specific action on cholestasis induced by EE. This cholestasis is due, at least in part, to the alteration by EE of mechanisms that are implicated in the bile salt pathways in the hepatocyte. A noncompetitive inhibition of 7α-hydroxylase by EE has been described[4] as well as a noncompetitive inhibition of the taurocholate uptake by the sinusoidal membrane,[5] and a reduction in the conjugated bile salt secretion across the canalicular membrane.[28]

The present studies clearly show that the hydrophilic–hydrophobic balance of bile salts plays an essential role in the recovery of a normal biliary secretion, TUDC, the most hydrophilic species, being the most effective.

The most evident manifestation of this restoration occurs at the level of the choleretic fraction which depends on the bile salt secretion. UDC and TUDC partially correct cholestasis due to EE administration. The larger the bile salt output, the more effective the correction. UDC, which is moderately absorbed in the small intestine due to its high precipitation pH,[29] partly attains the liver and, consequently, induces a limited choleresis. Conversely, TUDC is well absorbed by the intestinal cells. This is correlated with a TUDC biliary output 1.6-fold larger than that obtained with unconjugated UDC (*Table 4*). The bile flow, however, is lower than the control values, which reflects a lack of correction of the bile acid independent flow. In contrast, CDC does not improve the EE-induced cholestasis. The bile flow in this case remains near that of rats receiving exclusively EE. This underscores two points: CDC does not restore the normal secretion of endogenous bile salts (*Table 4*), and CDC has weak choleretic properties. This confirms the results of Baumgartner et al.[27] who describe the absence of choleretic power of TCDC in the rat.

The outputs of endogenous primary bile salts, essentially represented by C and β-MC are improved by TUDC, whereas they are not by CDC (*Table 4*). The recovery of a quasi-normal bile salt secretion is proof of the reestablishment of a normal activity of the hepatocytes in relation to the transport and the synthesis of C and β-MC acids. Thus, the hindrance introduced by the estrogen has been suppressed by TUDC. This is confirmed by the results presented in *Table 5* where it is shown that TUDC treatment restores the capacity of the hepatocyte to transport bile salts from the sinusoidal membranes to the canaliculi.

As an explanation of the various effects of the bile salts on the cell function, it is conceivable that bile salts might differently modify the physicochemical properties of the lipid bilayer of membranes of hepatocytes. Indeed, It has been shown that bile salts modified the internal order of the membranes and induced structural changes of large unilamellar vesicles and that these changes were dependent on the bile salt

type.[30] Moreover, Cabral et al.[31] recently demonstrated that the hydrophobicity of the bile acid was important in determining its rate of transbilayer movement. Changes in membrane elasticity could serve as a starting point to influence the activity of integral proteins such as 7α-hydroxylase. The level of fluidity of the microenvironment of the enzyme may be an important regulatory factor and may constitute a membrane-protecting mechanism in cellular disfunctions induced by ethinylestradiol. To be effective, the bile salt molecule has to possess a hydrophobic hemisphere to bind to membrane lipids. So, the highly hydrophilic sodium dehydrocholate, a nonmicelle-forming bile salt[9] that does not interact with phospholipid membranes, is unable to reverse estrogen–induced cholestasis.[7]

Our findings on the hepatocyte protection by hydrophilic UDC species may be linked to results of clinical trials that showed an improvement of liver function tests in patients with primary biliary cirrhosis[32,33] during UDC treatment.

Rats treated with EE and supplemented with bile salts (CDC, UDC, or TUDC) have a total bile salt output (endogenous plus exogenous) that approaches or even exceeds that of control rats. This results in stimulation of the biliary secretion of lecithin and cholesterol (*Table 1*) and, in every case, reduction in the cholesterol mole percent (*Table 2*). This fact is important for women exposed to contraceptive steroid hormones, in whom the risk to develop biliary stones is large.

In conclusion, we have shown that TUDC can reverse EE-induced cholestasis in rats and that this ability is strongly linked to the hydrophilic characteristics of this bile salt. It would be worthwhile to further investigate the protective effect of TUDC against cholestatic disorders in humans.

Acknowledgments

We thank C. Juste and D. Montet for their help in chromatographic studies.

References

1 Kern, F. and Everson G. (1987). Contraceptive steroids increase cholesterol in bile: mechanisms of action. *J. Lipid Res.* **28,** 828–839

2 Gumucio, J. and Valdivieso, V. (1971). Studies on the mechanisms of ethinyl estradiol impairment of bile flow and bile salt excretion in the rat. *Gastroenterology* **61,** 339–344

3 Stramentinoli, G., Gualano, M., Rovagnati, P., and Di Padova, C. (1979). Influence of *S*-adenosyl-L-methionine on irreversible binding of ethynylestradiol to rat liver microsomes and its implication in bile secretion. *Biochem. Pharmacol.* **28,** 981–984

4 Davis, R.A., Elliot, T.S., Lattier, G.R., Showalter, R.B., and Kern, F. (1986). Regulation of bile acid synthesis via direct effects on the microsomal membrane. *Biochemistry* **25,** 1632–1636

5 Berr, F., Simon, F.R., and Reichen, J. (1984). Ethynylestradiol impairs bile salt uptake and Na-K pump function of rat hepatocytes. *Am. J. Physiol.* **247,** G437–443

6 Layden, J.J. and Boyer, J.L. (1977). Taurolithocholate-induced cholestasis: taurocholate, but not dehydrocholate, reverses cholestasis and bile canalicular membrane injury. *Gastroenterology* **73,** 120–128

7 Adinolfi, L.E., Utili, R., Gaeta, G.B., Abernathy, C.O., and Zimmerman, H.J. (1984). Cholestasis induced by estradiol-17β-D-glucuronide: mechanisms and prevention by sodium taurocholate. *Hepatology* **4,** 30–37

8 Bonazzi, P., Novelli, G., and Galeazzi, R. (1986). Effect of chenodeoxycholic acid on the alterations of biliary lipid secretion induced by ethynylestradiol in the rat. *Br. J. Exp. Pathol.* **67,** 341–348

9 Small, D.M. (1971). The physical chemistry of cholanic acids. In *The Bile Acids*, Vol. 1 (P.P. Nair and D. Kritchevsky, eds.), pp. 249–356, Plenum, New York

10 Carey, M.C., Montet, J.C., Phillips, M.C., Armstrong, M.J., and Mazer, N.A. (1981). Thermodynamic and molecular basis for dissimilar cholesterol-solubilizing capacities by micellar solutions of bile salts: cases of sodium chenodeoxycholate and sodium ursodeoxycholate and their glycine and taurine conjugates. *Biochemistry* **20,** 3637–3648

11 Lindheimer, M., Montet, J.C., Molenat, J., Bontemps, R., and Brun, B. (1981). Ionic self-diffusion of various bile salts. *J. Chim. Phys.* **78,** 447–455

12 Armstrong, M.J. and Carey, M.C. (1982). The hydrophobic-hydrophilic balance of bile salts. Inverse correlation between reverse-phase high performance liquid chromatographic mobilities and micellar cholesterol-solubilizing capacities. *J. Lipid Res.* **23,** 70–80

13 Lindheimer, M., Montet, J.C., Bontemps, R., Rouvière, J., and Brun, B. (1983). Self-diffusion study of bile salt-monoolein micelles. Determination of the intermicellar bile salt concentration. *J. Chim. Phys.* **80,** 315–323

14 Hofmann, A.F. and Roda, A. (1984). Physicochemical properties of bile acids and their relationship to biological properties: an overview of the problem. *J. Lipid Res.* **25,** 1477–1489

15 Montet, J.C., Parquet, M., Sacquet, E., Montet, A.M., Infante, R., and Amic, J. (1987). β-muricholic acid: potentiometric and cholesterol-dissolving properties. *Biochim. Biophys. Acta* **918,** 1–10

16 Montet, J.C., Bégué, J.P., and Montet, A.M. (1988). Colloïdal properties of 6-hydroxylated bile salts. *Gastroenterology* **94,** 572

17 Carey, M.C. and Cohen, D.E. (1987). Biliary transport of cholesterol in vesicles, micelles and liquid crystals. In *Bile Acids and the Liver* (G. Paumgartner, A. Stiehl, and W. Gerok, eds.), pp. 287–300, MTP Press, Lancaster, UK

18 Coleman, R., Rahman, K., Bellringer, M.E., Kan, K., and Hamlin, S. (1989). Secretion of biliary lipids and its control. In *Trends in Bile Acid Research*. (G. Paumgartner, A. Stiehl, and W. Gerok, eds.), pp. 161–175, MTP Press, Lancaster, UK

19 Dowling, R.H., Mack, E., and Small, D.M. (1970). Effects of controlled interruption of the enterohepatic circulation of bile salts by biliary diversion and by ileal resection on bile salt secretion, synthesis and pool size in the Rhesus monkey. *J. Clin. Invest.* **49,** 232–242

20 Fromm, H., Amin, P., Klein, H., and Kupke, I. (1980). Use of a simple enzymatic assay for cholesterol analysis in human bile. *J. Lipid Res.* **21,** 259–261

21 Amic, J., Lairon, D., and Hauton, J. (1972). Technique de dosage automatique de l'orthophosphate de grande fiabilité. *Clin. Chim. Acta* **40,** 107–114

22 Domingo, N., Amic, J., and Hauton, J. (1972). Dosage automatique des sels biliaires conjugués de la bile par la 3-α-hydroxystéroïde déshydrogénase. *Clin. Chim. Acta* **37,** 399–404

23 Spady, D.K. and Dietschy, J.M., (1987). Hepatic bile acid synthesis: studies on regulation utilizing rats whose total body pool of cholesterol is uniformly labelled with ^{3}H. In *Bile Acids and the Liver*. (G. Paumgartner, A. Stiehl, and W. Gerok, eds.), pp. 15–24, MTP Press, Lancaster, UK

24 Heuman, D.M., Hernandez, C.R., Hylemon, P.B., Kubaska, W.M., Hartmann, C., and Vlahcevic, Z.R. (1988). Regulation of bile acid synthesis, I: effects of conjugated ursodeoxycholate and cholate on bile acid synthesis in the chronic bile fistula rat. *Hepatology* **8,** 358–365

25 Heuman, D.M., Vlahcevic, Z.R., Bailey, M.L., and Hylemon, P.B. (1988). Regulation of bile acid synthesis, II: effect of bile acid feeding on enzymes regulating hepatic cholesterol and bile acid synthesis in the rat. *Hepatology* **8,** 892–897

26 Zouboulis-Vafiadis, I., Dumont, M., and Erlinger, S. (1982). Conjugation is rate limiting in hepatic transport of ursodeoxycholate in the rat. *Am. J. Physiol.* **243,** G208–313

27 Baumgartner, U., Schölmerich, J., Leible, P., Farthmann, E.H., and Gerok, W. (1988). Maximal bile acid secretory rate, bile flow, phospholipid and cholesterol output during liver perfusion with various bile acids. *Hepatology* **8,** 1358

28 Tarao, K., Olinger, E., Ostrow, J.D., and Ballistreri, W.F. (1982). Impaired bile acid efflux from hepatocytes isolated from the liver of rats with cholestasis. *Am. J. Physiol.* **243,** G253–258

29 Igimi, H. and Carey, M.C. (1980). pH–solubility relations of chenodeoxycholic and ursodeoxycholic acids: physical–chemical basis for dissimilar solution and membrane phenomena. *J. Lipid Res.* **21,** 72–90

30 Schubert, R. and Schmidt, K.-H. (1988). Structural changes in vesicle membranes and mixed micelles of various lipid compositions after binding of different bile salts. *Biochemistry* **27,** 8787–8794

31 Cabral, D.J., Small, D.M., Lilly, H.S., and Hamilton, J.A. (1987). Transbilayer movement of bile acids in model membranes. *Biochemistry* **26,** 1801–1804

32 Poupon, R., Poupon, R.E., Calmus, Y., Chrétien, Y., Ballet, F., and Darnis, F. (1987). Is ursodeoxycholic acid an effective treatment for primary biliary cirrhosis? *Lancet* **1,** 834–836

33 Raedsch, R., Stiehl, A., and Kommerel, B. (1989). Effects of ursodeoxycholic acid in primary biliary cirrhosis and primary sclerosing cholangitis. In *Trends in Bile Acid Research.* (G. Paumgartner, A. Stiehl, and W. Gerok, eds.), pp. 367–371, MTP Press, Lancaster, UK