Enterohepatic circulation in hamsters with an extracorporeal bile duct

Michael Fuchs, Jürgen Scheibner, Erwin Hörmann, Gisela Tauber, and Eduard F. Stange[1]

Department of Internal Medicine II, University of Ulm, Ulm, Germany

Abstract The present study describes a novel technique for investigations of the enterohepatic circulation in the hamster with an extracorporeal bile duct that allows long-term bile collection in the free-moving animal. The animals recovered for 7 days after the operation before the external loop was cut and bile was collected over a period of 78 h. Under these optimal conditions, initial bile flow (651 ± 89 μl per 100 g · h^{-1}) and the secretion rates of biliary lipids were several-fold higher than reported in an earlier study using the acute fistula hamster. Biliary cholesterol secretion amounted to 369 ± 32 nmol per 100 g · h^{-1}, phospholipid secretion was 2.6 ± 0.3 μmol per 100 g · h^{-1}, and total bile acid secretion was 31.9 ± 2.2 μmol per 100 g · h^{-1}. A clear-cut diurnal rhythm was demonstrated for bile flow and all biliary constituents. After 9 h the depletion of the bile acid pool was complete and cholic acid synthesis derepressed 1.4-fold from a basal rate of 818 nmol per 100 g · h^{-1}, whereas the derepression of chenodeoxycholic acid synthesis was even less pronounced. Biliary cholesterol output increased 2.2-fold, but the phospholipid secretion was constant during the full experiment. It may be concluded that the technique of an extracorporeal bile duct in the free-moving animal allows studies of bile secretion under optimal conditions. Most likely the bile secretion rates given above approach the physiological rates in the hamster. —**Fuchs, M., J. Scheibner, E. Hörmann, G. Tauber, and E. F. Stange.** Enterohepatic circulation in hamsters with an extracorporeal bile duct. *J. Lipid Res.* 1992. **33:** 1383–1392.

Supplementary key words bile acid synthesis • bile fistula • biliary cholesterol • diurnal rhythm

The degradation of cholesterol to bile acids is the key pathway for cholesterol elimination from the mammalian body. Bile acids secreted by the liver are largely reabsorbed in the distal small intestine and recycled to the liver where the more hydrophobic species suppress bile acid synthesis (1, 2). The details of this enterohepatic circulation have been worked out in several animal species including the rat (3), dog (4), pig (5), baboon (6), and monkey (7).

Until recently most of the studies were undertaken using the rat fitted with an acute bile fistula (8–10). However, it could be demonstrated in the rat that after an appropriate recovery period with an extracorporeal bile duct, the rate of bile acid secretion is many-fold higher than previously appreciated (2, 3). Anesthesia, the stress of restraint, and postoperative pain, as well as fasting, apparently affected the results adversely (11–14). Finally, in recent studies from this laboratory on the regulation of bile acid synthesis in the rat fitted with an extracorporeal bile duct (EBD) (2), the sensitivity of the hepatic feedback inhibition was much less than originally observed in the acute bile fistula animal (15). Thus, the extracorporeal bile duct model permits studies under more physiological conditions, but it is still restricted to the rat (2, 3, 13, 16) or large, expensive animal species such as monkeys (7) and pigs (5).

Unfortunately, there are major differences between cholesterol metabolism in the rat and other mammals such as humans. In contrast, the hamster appears to be a more adequate model because it carries more of its plasma cholesterol in the low density lipoprotein (LDL) fraction than the rat (17), and the organ distribution of cholesterol synthesis in hamsters is more comparable to that in humans than in rats (17). As a consequence of the lower capacity of hepatic cholesterol synthesis, the hamster, unlike the rat, regulates LDL transport in response to dietary intake of cholesterol or saturated fatty acids (18), to variations in bile acid pool size (19, 20), or to bile acid administration (21, 22).

Humans and hamsters secrete taurine- as well as glycine-conjugated bile acids. Taurine is primarily of dietary origin in these two species (23) and the glycine to taurine ratio (G/T ratio) is about 2–3:1 (24–26). In contrast, the rat actively synthesizes taurine, and bile acids

Abbreviations: HPLC, high performance liquid chromatography; aufs, absorbance units full scale; dpm, disintegrations per minute; EBD, extracorporeal bile duct; LDL, low density lipoproteins; TCA, taurocholic acid; CA, cholic acid; CDCA, chenodeoxycholic acid; DCA, deoxycholic acid; LCA, lithocholic acid; AP, alkaline phosphatase; ALAT, alanine leucine aminotransferase; γ-GT, γ-glutamyl-transferase.

[1]To whom correspondence should be addressed at: Department of Internal Medicine, Division of Gastroenterology, Medical University of Lübeck, Ratzeburger Allee 160, D-2400 Lübeck, Germany.

are nearly uniformly taurine-conjugated (27, 28). Furthermore, the bile acid species in hamsters and humans are similar (26). Another common feature of hamsters and humans in contrast to the rat is that both do not 6-hydroxylate chenodeoxycholate (CDCA) or ursodeoxycholate (UDCA) during hepatic passage (29). In addition, the hamster has a gallbladder and may be induced to produce lithogenic bile in response to various dietary or pharmacological manipulations (30–33).

The aim of the present study was to investigate the enterohepatic circulation in the hamster under optimal conditions. We therefore developed a new animal model that allows for long-term bile collection in the free-moving and unrestrained hamster. The model permitted the measurement of bile acid synthesis and biliary lipid secretion under more physiological conditions than previously possible.

MATERIALS AND METHODS

Materials

[24-^{14}C]taurocholic acid (57.7 mCi/mmol) was obtained from Amersham Corp. (Braunschweig, Germany) and [26-^{14}C]cholesterol (59 mCi/mmol) was from NEN (Dreieich, Germany). 3α-Hydroxysteroid dehydrogenase [E.C. 1.1.1.50] was purchased from Sigma Chemical GmbH (Munich, Germany). All chemicals used were of the highest grade available commercially.

Animals and diet

Female Golden Syrian hamsters (*Mesocricetus aureatus*) weighing 110–130 g were obtained from the Zentralinstitut für Versuchstierzucht, Hannover, Germany. The animals were kept under temperature-controlled conditions with a fixed light cycling from 6:00 AM to 6:00 PM. The hamsters had free access to Altromin Standard chow 1314 (Altromin GmbH, Lage, Germany) and water. The cholesterol content of the diet was 0.24 mg per g (34).

Surgical procedures

The animals were anesthetized with fluothane (ICI Pharma, Plankstadt, Germany) and the abdomen was shaved and covered with a polyethylene foil. Body temperature was monitored (neoLab Laborbedarf, Heidelberg, Germany) and maintained at 36°–38°C with an infrared lamp and a heating pad. Sterile techniques were used throughout.

The abdominal cavity was opened by a longitudinal incision along the linea alba up to the distal processus xiphoideus. The proximal common bile duct was dissected from the surrounding tissues of the hepatoduodenal ligament and ligated proximal to the entry of the pancreatic ducts with 5-0 Mersilene (Ethicon, Norderstedt, Germany). The tip of a silicon catheter (33 cm, ID 1 mm, OD 1.8 mm, D & N, Berlin, Germany) was inserted into the duodenum close to the papilla Vateri and was fixed by a purse-string suture and a plate of tygon to seal off the insertion site (**Fig. 1**).

Next, the gallbladder was punctured and drained. The other end of the silicon catheter was tunneled through a perforated glass bead (OD 3 mm) and fixed in the gallbladder close to the cystic duct by 3-0 Mersilene leaving a minimal residual lumen required for adequate flow (Fig. 1). The loop was then exteriorized by tunneling the catheter subcutaneously to the neck. The abdominal cavity was closed in two suture layers using 4-0 Prolene and the animal was placed in a harness to protect the loop.

Animal maintenance and experimental procedures

During a postoperative period of 7 days, the animals had free access to chow and water. The hamsters were placed in individual metabolic cages; food consumption and body weight of the animals were monitored daily.

In regenerated hamsters the enterohepatic circulation was interrupted without anesthesia. To determine initial secretion rates, bile was drained into a plastic vial attached directly to the biliary catheter. After 20 min both ends of the catheter were connected to a PE 58-tubing (Clay Adams, Parsippany, NJ) filled with physiological saline. An infusate containing glucose, amino acids, and electrolytes (15) was infused via the intraduodenal catheter at a rate of 1 ml per hour for 78 h using a precision pump (B. Braun, Melsungen, Germany). The infusate was supplemented with 19.6 μmol sodium bicarbonate per 100 g body weight $\cdot$ h^{-1} which exactly substituted for the loss of biliary bicarbonate as determined in the series of animals represented in Table 1. Bile was collected in 3-h intervals after the catheter was filled using a fraction collector (Colora, Lorch, Germany) which was placed below the level of the animal cage so that bile was draining freely.

All infusion experiments were started during the light cycle between 10:00 AM and noon. In a preliminary experiment to estimate the time necessary for the elimination of the intestinal bile acid pool, a bolus of 2 μCi[^{14}C]TCA was given intraduodenally to a series of animals immediately after interruption of the enterohepatic circulation

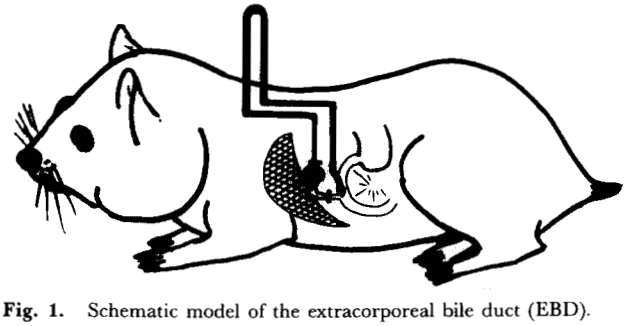

Fig. 1. Schematic model of the extracorporeal bile duct (EBD).

and bile was collected for 24 h. Another group of 11 hamsters was taken into the full bile secretion experiment for 78 h. To measure intestinal absorption and hepatic secretion capacity, 0.09 μCi[^{14}C]TCA per 100 g $\cdot$ h^{-1} was infused continuously. Bile fractions were analyzed for biliary lipids and radioactivity as described below. Throughout the experiments the animals were carefully monitored for appearance, activity, food intake, and body weight.

In a separate series of animals, the bile acid pool size was determined in control hamsters and in 7-day regenerated animals with an extracorporeal bile duct. The animals were exsanguinated under chloralhydrate (4%) intraperitoneal anesthesia and the small and large bowel, gallbladder, extrahepatic bile ducts, and catheter were totally recovered for bile acid extraction performed according to Turley and Dietschy (35).

To estimate the bile acid composition, gallbladder bile was obtained from control animals. In a group of regenerated hamsters with excluded gallbladder, the loop was cut and hepatic bile was collected for 20 min. The same data were used to calculate the initial bile acid secretion rates.

In controls and in animals after the regeneration period or the infusion experiment, the abdominal aorta was punctured (with animals under chloralhydrate anesthesia) and alkaline phosphatase (AP), alanine leucine amino transferase (ALAT), and γ-glutamyl-transferase (γ-GT) activity in serum were determined using commercial kits (Merck, Darmstadt, Germany).

Biochemical analyses

The bile flow was measured gravimetrically and the secreted ^{14}C dpm was determined with a liquid scintillation counter (Rackbeta, LKB, Freiburg, Germany).

[^{14}C]cholesterol was added as internal standard and bile acids as well as biliary cholesterol were extracted from 100 μl of bile with 800 μl isopropanol according to Tietz et al. (36). Biliary cholesterol and bile acids were then separated with 200 μl methanol plus 600 μl petroleum ether.

For the determination of conjugated bile acids, methanol was evaporated and the residue was dissolved with the HPLC solvent. Individual bile acids were then separated by ion pair-HPLC. The equipment consisted of a Waters (Eschborn, Germany) pump M 600, autosampler WISP 712, gradient controller M 600, and ultraviolet absorbance detector UV 490. The column used was a Bondapak C_{18}-rp column (300 mm length $\times$ 3.9 mm ID, 10 μm particle size). The mobile phase consisted of a solvent gradient (solvent A: water–acetonitrile 70:30 (v/v), 7.5 mM/l tetrabutylammoniumhydrogensulfate; solvent B: water–acetonitrile 40:60 (v/v), 7.5 mM/l tetrabutylammonium hydrogensulfate) (37) at a flow rate of 1.5 ml per min. The effluent was scanned at 200 nm and 0.24 absorbance units full scale (aufs). ^{14}C dpm were measured by online liquid scintillation counting (Ramona 5-LS, Raytest, Straubenhardt, Germany) with a dynamic efficiency for ^{14}C of 70%. The mean recovery of [^{14}C]TCA was 95%.

Biliary cholesterol in the evaporated petroleum ether extract was saponified according to Abell et al. (38) using 500 μl ethanolic KOH. Free cholesterol was then extracted with 1 ml hexane and the solvent was evaporated. The residue was dissolved in 500 μl of the HPLC solvent consisting of isopropanol–acetonitrile–water 60:30:10 (v/v/v). The mean recovery of [^{14}C]cholesterol was 87%. Biliary cholesterol was detected under isocratic conditions with the equipment described above. Operating wavelength was 212 nm and 0.24 aufs at a flow rate of 1 ml per min using two Novapak C_{18} rp-columns in series (75 and 150 mm $\times$ 3.9 mm ID, 3 μm particle size, Waters). The dynamic efficiency for ^{14}C was 67%.

Phospholipids were estimated by an enzymatic method using a commercial kit from Merck (Darmstadt, Germany). Electrolytes were determined by a conventional automated technique.

Calculations and statistics

The rates of cholate and chenodeoxycholate synthesis were considered to be identical to their secretion rates after depletion of the enterohepatic pool. The overall intestinal absorption and hepatic secretion rate was determined by the relation of secreted to infused ^{14}C dpm per 100 g $\cdot$ h^{-1}. Data represent the mean $\pm$ 1 SEM. Significance was determined by means of Student's *t*-test for unpaired samples.

RESULTS

Food intake and body weight

In an initial series of studies, the extracorporeal bile duct model was validated with respect to food intake and body weight during the experiment. The food intake of hamsters (n = 12) with an extracorporeal bile duct rapidly increased from a postoperative 1.4 $\pm$ 0.2 g per day to 9.2 $\pm$ 0.6 g per day at day 5 after the operation, which is close to the controls (9.8 $\pm$ 0.5 g per day, n = 12). During the infusion experiment, food intake averaged 8.5 g $\pm$ 0.5 (n = 11). During the first 2 days after surgery the animals lost a mean of 9.1 $\pm$ 0.6% of their body weight. Later during the experiment it was constant until the end of the infusion period.

Liver enzymes and histology

As indicated in **Table 1**, the animals with an extracorporeal bile duct showed similar serum activities of various liver enzymes as control animals both after the regeneration and infusion period. In particular, alkaline phosphatase, γ-GT, and ALAT serum activities were normal. Thus, there was no biochemical evidence for cholestasis or parenchymal injury. Also, there was no evidence for

TABLE 1. Liver enzymes

Serum Biochemical Parameters	Control	EBD	EBD Plus Infusion
AP (U/l)	204 ± 10	152 ± 33	134 ± 12
γ-GT (U/l)	2.4 ± 0.4	<2	<2
ALAT (U/l)	37 ± 3	28 ± 4	22 ± 3

Alkaline phosphatase (AP), alanine leucine amino transferase (ALAT), and γ-glutamyl-transferase (γ-GT) activities were determined in controls (n = 12), hamsters regenerated for 7 days with an extracorporeal bile duct (EBD) (n = 6), and hamsters with an EBD plus a control infusion for another 78 h (n = 11). Values represent the means ± SEM.

liver damage or cholestasis in histological sections prepared after the experiment (not shown).

Bile acid pool size and bile acid composition

The total bile acid pool size amounted to 22.7 ± 1.5 μmol per 100 g in controls, with 6% localized to the gallbladder, and expanded to 38.6 ± 2.8 μmol per 100 g in hamsters with an extracorporeal bile duct ($P < 0.01$). However, this expansion was mostly due to bile in the extracorporeal catheter. When only the organ content was calculated in the EBD hamsters similar to controls, this increase in bile acid pool size was not significant (27.6 ± 2.4 μmol per 100 g; $P > 0.05$).

Bile acid composition determined in gallbladder bile of controls (**Fig. 2**, panel A) was not significantly different from the composition of hepatic bile of hamsters with an extracorporeal enterohepatic circulation (Fig. 2, panel B). The major bile acid was cholate (73.8 vs. 75%), followed by chenodeoxycholate (13.7 vs. 13%) and deoxycholate (9.9 vs. 9.8%), while only a small percentage was lithocholate (2.6 vs. 2.3%). In controls and in animals with an extracorporeal bile duct, the glycine to taurine ratio of the four major bile acids was similar.

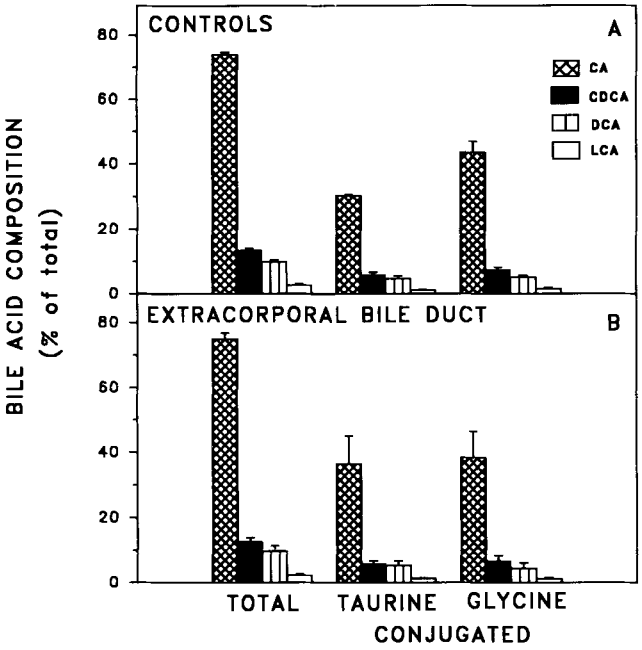

Fig. 2. Bile acid composition. Total bile acids as well as taurine and glycine conjugates were determined in gallbladder bile of controls (panel A, n = 12) and hepatic bile of 7 day regenerated EBD hamsters (panel B, n = 6), respectively, Values represent means ± SEM.

Biliary excretion

In order to estimate the normal secretion rate of bile constituents in the hamster, bile was collected for the first 20 min after cutting the loop in animals with an extracorporeal enterohepatic circulation. The initial rate of bile flow was 651 ± 89 μl per h and decreased rapidly to 271 μl per h during the subsequent 3 h (**Fig. 3**, panel A). Thereafter, there was a slight decline to approximately 220 μl per h with a subtle diurnal variation peaking at 42 and 66 h.

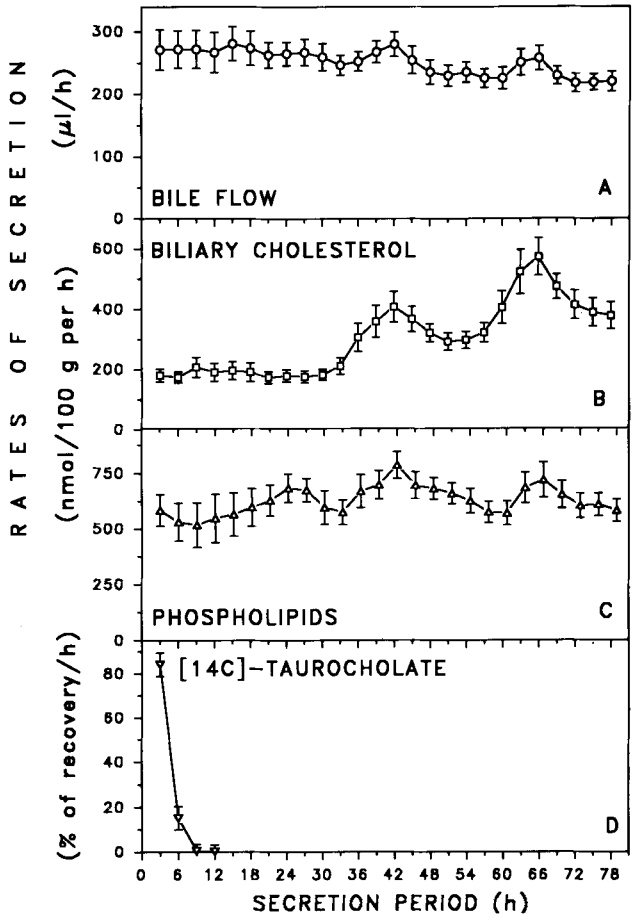

Fig. 3. Bile flow, biliary cholesterol, phospholipid and [^{14}C]TCA secretion. After 7 days of regeneration the enterohepatic circulation was interrupted in a series of 11 animals and bile flow, biliary cholesterol, and phospholipid secretion were determined during the following 78 h (panels A-C). In another group of 6 hamsters, 2 μCi [^{14}C]TCA was given intraduodenally as a bolus (panel D). Bile was collected in 3-h intervals. Values represent the means ± SEM.

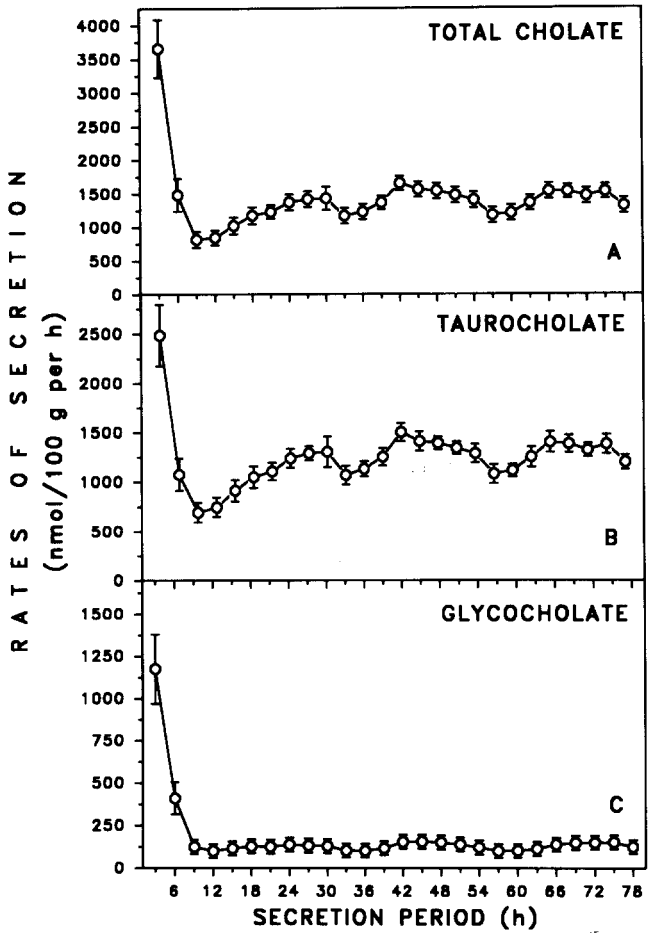

Fig. 4. Cholate secretion after interruption of the enterohepatic circulation. Total cholate secretion (panel A), taurocholate secretion (panel B), and glycocholate secretion (panel C) in hamsters during a 78-h period. Experimental setup as described in the legend of Fig. 3. Values represent the means ± SEM of 11 animals.

The initial cholesterol secretion rate averaged 369 ± 32 nmol per 100 g·h^{-1} and decreased to 181 nmol per 100 g·h^{-1} (Fig. 3, panel B). During the further course of the experiment a conspicuous diurnal rhythm was observed coinciding with the much less pronounced peaks in bile flow. The final peak secretion actually exceeded the initial secretion rate.

The initial drop in phospholipid secretion was even more dramatic than that of cholesterol with a more than 5-fold decrease from an initial 2630 ± 257 nmol per 100 g·h^{-1} hour (Fig. 3, panel C). During the experimental period the secretion was more stable but again exhibited diurnal secretion peaks.

In a preliminary series of experiments it could be shown that 99% of labeled bile acid pool was depleted within 9 h after interrupting the enterohepatic circulation (Fig. 3, panel D). Overall, 99.5% of the intraduodenally infused [^{14}C]TCA was secreted by the liver during the 78-h period of collection. Thus, the bile acids secreted at later time points were newly synthesized in the liver.

Immediately after bile diversion the total cholate secretion (**Fig. 4,** panel A) decreased rapidly from an initial 23920 ± 5690 nmol per 100 g·h^{-1} (**Table 2**) to a basal rate of 818 nmol per 100 g·h^{-1} at 9 h. Bile acid synthesis maximally derepressed 2-fold between 9 and 42 h. If the diurnal rhythm is taken into account, cholate synthesis derepressed 1.4-fold between 9 and 57 h.

Taurocholate secretion (Fig. 4, panel B) showed a comparable initial decrease after the enterohepatic circulation was interrupted. Similar to total cholate, the taurocholate synthesis rate derepressed 1.6-fold between 9 and 57 h. After pool depletion glycocholate secretion (Fig. 4, panel C) represented 15% of total cholate and the absolute rates were constant later during the experiment. In contrast to taurocholate, no derepression of glycocholate synthesis occurred.

Total chenodeoxycholate secretion (**Fig. 5,** panel A) dropped from an initial rate of 4080 ± 730 nmol per 100 g·h^{-1} (Table 2) to 191 nmol per 100 g·h^{-1} 9 h after the start of the infusion experiment. During the following period of bile diversion, chenodeoxycholate exhibited a similar but less pronounced diurnal rhythm as cholate. In contrast to cholate, chenodeoxycholate synthesis showed only minimal derepression over 78 h. As depicted in Fig. 5 (panel B), taurochenodeoxycholate decreased to a basal secretion rate of 162 nmol per 100 g·h^{-1} at 9 h and thereafter the course of secretion was similar to that of total chenodeoxycholate. Glycochenodeoxycholate (Fig. 5, panel C), as glycocholate, represented 15% of the total chenodeoxycholate secretion after bile acid pool depletion without any evidence for derepression.

TABLE 2. Initial bile acid secretion

	Bile Acid Output			
	CA	CDCA	DCA	LCA
	nmol per 100 g per h			
Total	23920 ± 5690	4080 ± 730	3110 ± 1100	730 ± 188
Taurine conjugated	11640 ± 2890	1900 ± 250	1700 ± 500	380 ± 64
Glycine conjugated	12280 ± 2800	2180 ± 480	1410 ± 600	350 ± 124

Seven days after fitting the hamsters with an extracorporeal bile duct, the enterohepatic circulation was interrupted and bile was collected during the initial 20 min. Values represent the means ± SEM of six animals.

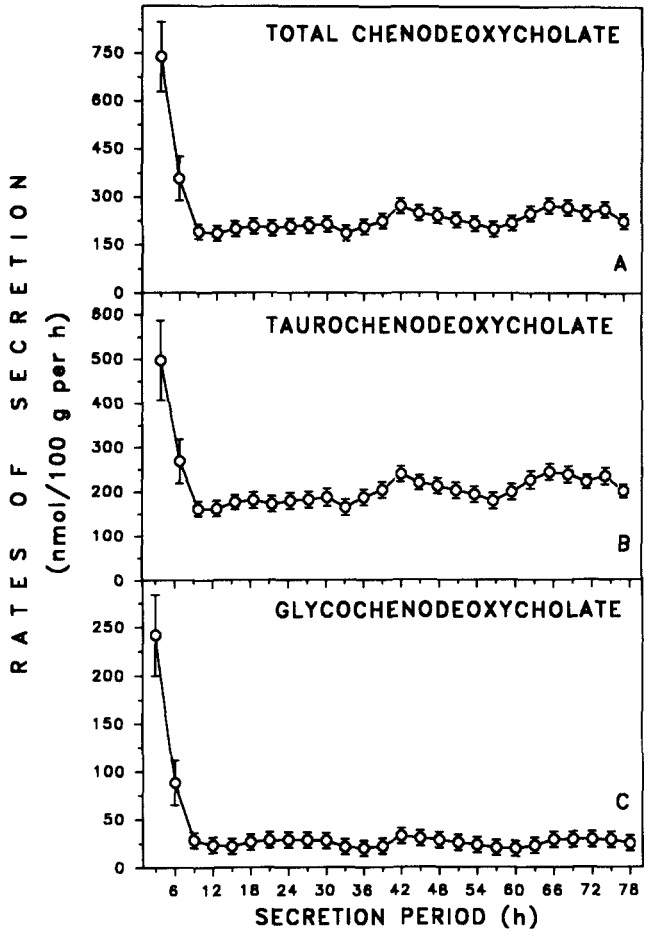

Fig. 5. Chenodeoxycholate secretion after interruption of the enterohepatic circulation. Total chenodeoxycholate secretion (panel A), taurochenodeoxycholate secretion (panel B), and glycochenodeoxy cholate secretion (panel C) in hamsters during a 78-h period. Experimental setup as described in the legend of Fig. 3. Values represent the means ± SEM of 11 animals.

The deoxycholic acid pool was largely (98%) secreted 9 h after the interruption of the enterohepatic circulation (**Fig. 6**, panels A–C) and was hardly detectable thereafter.

DISCUSSION

Recent improvements of the techniques applied to study the enterohepatic circulation in rat and other species have considerably enhanced the understanding of biliary physiology. The extracorporeal bile duct in the restrained (16) or unrestrained (3) rat has permitted a closer approximation of normal secretion and synthesis rates of biliary lipids than was previously feasible in the acute bile fistula animal immediately post surgery. Because of the similarities to human biliary physiology and cholesterol metabolism, we now developed a new animal model that allows long-term bile collection as well as reversible interruption of the enterohepatic circulation in the free-moving unanesthetized hamster.

The validity of this hamster model was tested by various metabolic and biochemical parameters. Five days after the operation and later during the regeneration period, as well as during the infusion period of another 78 h, the animals were in an adequate nutritional state, fed normally, and maintained their weight. To exclude liver damage, several biochemical parameters were checked and liver histology was taken. Sensitive indicators of cholestasis such as the alkaline phosphatase and γ-GT serum activities or liver histology were normal. In a few failures with occlusion of the biliary catheter these enzyme activities were elevated several-fold. In contrast to other work in the field (3, 13), the application of antibiotics after surgery proved unnecessary.

In the present model of the extracorporeal bile duct, hepatic bile drained via the cystic duct and recirculated into the small bowel, i.e., the animals were functionally

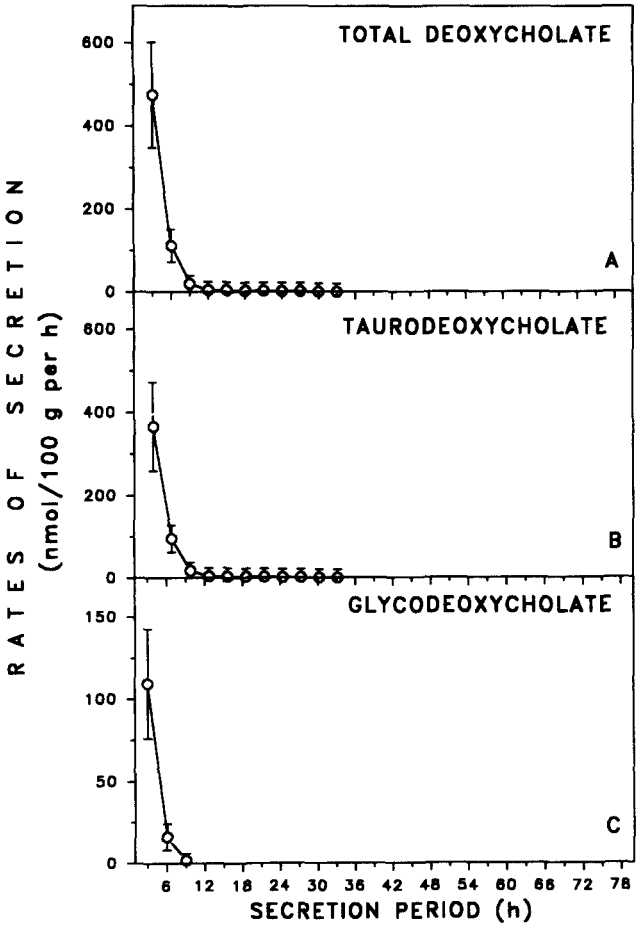

Fig. 6. Deoxycholate secretion after interruption of the enterohepatic circulation. Total deoxycholate secretion (panel A), taurodeoxycholate secretion (panel B), and glycodeoxycholate secretion (panel C) in hamsters during a 78-h period. Experimental setup as described in the legend of Fig. 3. Values represent the means ± SEM of 11 animals.

cholecystectomized. As demonstrated previously, a cholecystectomy has little (39) or no (40, 41) effect on bile acid pool size in the hamster. Similarly, after 7 days of regeneration, the organ content of the animals fitted with an extracorporeal bile duct added up to a near normal bile acid pool size. Thus, the intestinal bile acid content was comparable in intact controls and EBD hamsters. However, when the catheter content of bile was taken into account, total pool size was increased compared to intact controls.

Biliary lipid composition in the present hamsters amounted to 91.4 mol% bile acids, 7.5 mol% phospholipids, and 1.1 mol% biliary cholesterol and was comparable with previous work (42). Both in intact and EBD hamsters the predominant bile acid was CA, followed by CDCA and DCA. LCA was present only in small amounts. In contrast to observations of others (22, 25, 43–45), ursodeoxycholic acid could not be detected in the present study so that the bile acid pattern is comparable to that reported by Kuroki and Hoshita (46). Similar to the results in cholecystectomized hamsters, there was no increase in deoxycholic acid (40, 41). Another point of interest is the bile acid conjugation pattern. Depending on the hamster strain used, glycine to taurine ratios of 1.8 to 2.5 were reported for female or male hamsters (25, 26, 44, 47), whereas in the present strain and that used by Yanaura and Iizuka (43) the initial conjugation ratio was close to unity.

The studies dealing with secretion rates of bile constituents in hamsters used anesthetized animals (34, 45, 48–53) with the exception of Stevens et al. (54) who measured bile acid secretion rate after an overnight bile depletion. In the present investigation, bile flow and initial rates of biliary lipids were determined within the first 20 min after interrupting the enterohepatic circulation to avoid the rapid bile acid depletion thereafter. Compared to others who collected bile during the first hour in acute bile fistula hamsters (48, 53), bile flow was 2- to 6-fold higher in the EBD hamster.

In the present regenerated hamsters, the biliary cholesterol secretion rate was 2- to 20-times higher than in the anesthetized hamsters (45, 52). This may be related initially to the high bile acid secretion rate, but the increment of cholesterol secretion exceeds that of bile acid secretion at least during the further course of the experiment. Turley, Spady, and Dietschy (55) demonstrated in the hamster that biliary cholesterol output is dissociated from hepatic cholesterol synthesis. They also showed that only a small percentage (2–5%) of biliary cholesterol is derived from newly synthesized cholesterol (55). It will be interesting to determine to what extent the increase and diurnal variation of biliary cholesterol is accounted for by newly synthesized cholesterol.

Available data about biliary phospholipid secretion rates in hamsters fitted with an acute bile fistula (53, 56) also showed low values when compared with EBD hamsters. In contrast to biliary cholesterol secretion, phospholipids were more stable during the infusion period, but still exhibited a distinct diurnal variation similar to the other biliary lipids.

Compared to the literature (51, 53) the total bile acid output rate in EBD hamsters was 5- to 16-fold higher. Only Wheeler (49) reported a similar bile acid secretion rate during a 4-h period of bile depletion. Long-term bile depletion studies in small animals were problematic because the lost bicarbonate was usually not replaced (54). The finding in the EBD hamster is comparable to that in the EBD rats where the initial bile acid secretion rate was several-fold higher (2) compared to freshly operated animals (9, 10, 15, 57). However, acute bile fistula animals were not run in parallel and it cannot be completely excluded that the high rates of bile and biliary lipid secretion are due to the specific animal strain used. It is striking that even in this EBD model the derepression of bile acid synthesis relative to the nadir at 9 h is less than in the rat (2). Possibly, bile acid formation in the hamster is not under the same tight feedback control (1, 2). Also, in contrast to rats (2, 15, 58), cholate and chenodeoxycholate exhibited exactly the same diurnal rhythm with coordinated peaks during the day.

It seems unlikely that the extremely high output rates of bile acids and phospholipids and, to a lesser extent, that of cholesterol and bile flow were caused by prior obstructive cholestasis and subsequent overflow when the system was opened. First, one would expect parallel changes in bile volume and the different biliary lipids which was clearly not the case. Second, the biochemical parameters of cholestasis were negative and histological cholestasis could be excluded. Third, deoxycholate in bile did not decrease as might be expected during cholestasis. Fourth, there was no immediate overflow of bile after cutting the loop. Fifth, the bile flow was exactly in proportion to the rate of bile acid secretion as determined in hamsters with a taurocholate infusion (M. Fuchs, J. Scheibner, E. Hörmann, G. Tauber, and E. F. Stange, unpublished data). Finally, the high rate of secretion was not due to the pool expansion in EBD hamsters since intestinal bile acid content subserving reabsorption and hepatic secretion was comparable to controls.

Using the bile acid pool size and total bile acid secretion rate, the calculated cycling frequency of the bile acid pool was 34 times per day which is approximately 2-fold higher than in the rat (2). After interrupting the enterohepatic circulation, the bile acid pool was nearly completely (99%) depleted within 9 h, which is in good agreement with the time course of deoxycholate secretion, and only trace amounts were detectable after 9 h. An intestinal absorption rate of 99.5% supports an intact function of the small bowel and the liver after 7 days of regeneration in contrast to previous studies (34, 54) using acute bile fistula hamsters.

A final point to be made is the conspicuous change in

the proportion of glycine to taurine conjugates during the drainage period. There was a shift in the bile acid conjugation pattern towards the taurine conjugates so that at the end approximately 90% of cholate and chenodeoxycholate were conjugated with taurine. In one previous study (54) more than 90% of secreted bile acids were taurine-conjugated after 16 h of bile diversion. A decrease in the G/T ratio over the course of the infusion of either natural or synthetic bile acids has been reported by Une et al. (59). However, Gurantz and Hofmann (60) showed that the taurine pool was rapidly depleted and bile acids became conjugated predominantly with glycine. Possibly, the depletion of the hepatic taurine pool depends on the strain of hamsters used.

It may be concluded that the present model of an extracorporeal bile duct in the hamster yields rates of bile flow and biliary lipid secretion considerably higher than reported previously in the anesthetized animal, and it is suggested that these rates approximate the physiological flux more accurately. Indeed, when calculated on a body weight basis, these rates are quite comparable in the rat (2) and hamster. These higher rates of transhepatic bile acid flux should be taken into account during investigations of feedback regulation of bile acid synthesis.

The excellent technical assistance of Mrs. C. Engstler, Mrs. E. Preiss, and Mrs. R. Barnikel is gratefully acknowledged. This study was supported by the Deutsche Forschungsgemeinschaft (Sta 172/3-4).

Manuscript received 18 February 1992 and in revised form 19 May 1992.

REFERENCES

1. Heuman, D. M., P. B. Hylemon, and Z. R. Vlahcevic. 1989. Regulation of bile acid synthesis. III. Correlation between biliary bile salt hydrophobicity index and the activities of enzymes regulating cholesterol and bile acid synthesis in the rat. *J. Lipid Res.* **30:** 1161–1171.
2. Stange, E. F., J. Scheibner, and H. Ditschuneit. 1989. Role of primary and secondary bile acids as feedback inhibitors of bile acid synthesis in the rat in vivo. *J. Clin. Invest.* **84:** 173–180.
3. Kuipers, F., R. Havinga, H. Bosschieter, G. P. Toorop, F. R. Hindriks, and R. J. Vonk. 1985. Enterohepatic circulation in the rat. *Gastroenterology.* **88:** 403–411.
4. Scott, R. B., S. M. Strasberg, T. Y. El-Sharkawy, and N. E. Diamant. 1983. Regulation of the fasting enterohepatic circulation of bile acids by migrating myoelectric complex in dogs. *J. Clin. Invest.* **71:** 644–654.
5. Legrand-Defretin, V., C. Juste, T. Corring, and A. Rerat. 1986. Enterohepatic circulation of bile acids in pigs: diurnal pattern and effect of a reentrant biliary fistula. *Am. J. Physiol.* **250:** G295–G301.
6. Redinger, R. N., J. W. Hawkins, and D. M. Grace. 1984. The economy of the enterohepatic circulation of bile acids in the baboon. 1. Studies of controlled enterohepatic circulation of bile acids. *J. Lipid Res.* **25:** 428–436.
7. Dowling, R. H., E. Mack, D. M. Small, and J. Picott. 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.
8. Shefer, S., S. Hauser, I. Bekersky, and E. H. Mosbach. 1969. Feedback regulation of bile acid biosynthesis in the rat. *J. Lipid Res.* **10:** 646–655.
9. Davis, R. A., C. A. Musso, M. Malone-McNeal, G. R. Lattier, P. M. Hyde, J. Archambault-Schexnayder, and M. Straka. 1988. Examination of bile acid negative feedback regulation in rats. *J. Lipid Res.* **29:** 202–211.
10. Pandak, W. M., Y. C. Li, J. Y. L. Chiang, E. J. Studer, E. C. Gurley, D. M. Heuman, Z. R. Vlahcevic, and P. B. Hylemon. 1991. Regulation of cholesterol 7α-hydroxylase mRNA and transcriptional activity by taurocholate and cholesterol in the chronic biliary diverted rat. *J. Biol. Chem.* **266:** 3416–3421.
11. Ruckebusch, Y. 1981. Motor functions of the intestine. *Adv. Vet. Sci.* **25:** 345–369.
12. de Matteis, F. 1969. Liver cholesterol metabolism following trauma. Evidence for increased rate of cholesterol synthesis and breakdown. *Biochim. Biophys. Acta.* **187:** 422–434.
13. Vonk, R. J., A. B. D. Van Doorn, and J. H. Strubbe. 1978. Bile secretion and bile composition in the freely moving, unanaesthetized rat with a permanent biliary drainage: influence of food intake on bile flow. *Clin. Sci. Mol. Med.* **55:** 253–259.
14. Weis, H. J., and J. M. Dietschy. 1975. The interaction of various control mechanisms in determining the rate of hepatic cholesterogenesis in the rat. *Biochim. Biophys. Acta.* **389:** 315–324.
15. Stange, E. F., J. Scheibner, C. Lutz, and H. Ditschuneit. 1988. Feedback regulation of bile acid synthesis in the rat by dietary vs. intravenous cholate or taurocholate. *Hepatology.* **8:** 879–886.
16. Weis, E. E., and C. A. Barth. 1978. The extracorporeal bile duct: a new model for determination of bile flow and bile composition in the intact rat. *J. Lipid Res.* **19:** 856–862.
17. Spady, D. K., and J. M. Dietschy. 1983. Sterol synthesis in vivo in 18 tissues of the squirrel monkey, guinea pig, rabbit, hamster and rat. *J. Lipid Res.* **24:** 303–315.
18. Spady, D. K., and J. M. Dietschy. 1985. Dietary saturated triacylglycerols suppress hepatic low density lipoprotein receptor activity in the hamster. *Proc. Natl. Acad. Sci. USA.* **82:** 4526–4530.
19. Spady, D. K., D. W. Bilheimer, and J. M. Dietschy. 1983. Rates of receptor-dependent and -independent low density lipoprotein uptake in the hamster. *Proc. Natl. Acad. Sci. USA.* **80:** 3499–3503.
20. Spady, D. K., S. D. Turley, and J. M. Dietschy. 1985. Rates of low density lipoprotein uptake and cholesterol synthesis are regulated independently in the liver. *J. Lipid Res.* **26:** 465–472.
21. Spady, D. K., E. F. Stange, L. E. Bilhartz, and J. M. Dietschy. 1986. Bile acids regulate hepatic low density lipoprotein receptor activity in the hamster by altering cholesterol flux across the liver. *Proc. Natl. Acad. Sci.* **83:** 1–5.
22. Malavolti, M., H. Fromm, S. Ceryak, and I. M. Roberts. 1987. Modulation of low density lipoprotein receptor activity by bile acids: differential effects of chenodeoxycholic and ursodeoxycholic acids in the hamster. *J. Lipid Res.* **28:** 1281–1295.
23. Gaull, G. E., H. Pesantes-Morales, and E. Charles. 1985. Taurine in human nutrition: overview. *Prog. Clin. Biol. Res.* **179:** 3–21.
24. Sjövall, J. 1960. Bile acids in man under normal and pathological conditions. *Clin. Chim. Acta.* **5:** 33–41.
25. Bellentani, S., M. Pecorari, P. Cordoma, P. Marchegiano, F. Manenti, E. Bosisio, E. De Fabiani, and G. Galli. 1987. Taurine increases bile acid pool size and reduces bile saturation index in the hamster. *J. Lipid Res.* **28:** 1021–1027.

26. Kuroki, S., S. Muramoto, T. Kuramoto, and T. Hoshita. 1983. Sex differences in gallbladder bile acid composition and hepatic steroid 12α-hydroxylase activity in hamsters. *J. Lipid Res.* **24:** 1543–1549.
27. Vessey, D. A. 1978. The biochemical basis for the conjugation of bile acids with either glycine or taurine. *Biochem. J.* **174:** 621–626.
28. Hardison, W. G. M., and J. H. Proffitt. 1977. Influence of hepatic taurine concentration on bile acid conjugation with taurine. *Am. J. Physiol.* **232:** E75–E79.
29. Bachrach, W. H., and A. F. Hofmann. 1982. Ursodeoxycholic acid in the treatment of cholesterol cholelithiasis. Part I. *Dig. Dis. Sci.* **27:** 737–761.
30. Cohen, B. I., T. Setoguchi, E. H. Mosbach, C. K. McSherry, R. J. Stenger, S. Kuroki, and R. D. Soloway. 1987. An animal model of pigment cholelithiasis. *Am. J. Surg.* **153:** 130–138.
31. Pearlman, B. J., G. G. Bonorris, M. J. Phillips, A. Chung, S. Vimadalal, J. M. Marks, and L. J. Schoenfield. 1979. Cholesterol gallstone formation and prevention by chenodeoxycholic and ursodeoxycholic acids. *Gastroenterology.* **77:** 634–641.
32. Dam, H., and F. Christensen. 1952. Alimentary production of gallstones in hamsters. *Acta Pathol. Microbiol. Scand.* **30:** 236–242.
33. Handelsman, B., G. Bonorris, J. W. Marks, and L. J. Schoenfield. 1982. Enrichment of bile with tauroursodeoxycholic acid and biliary cholesterol saturation in hamsters. *Am. J. Physiol.* **243:** G424–G427.
34. Berr, F., F. Stellaard, A. Goetz, C. Hammer, and G. Paumgartner. 1988. Ethinylestradiol stimulates a biliary cholesterol-phospholipid consecretion mechanism in the hamster. *Hepatology.* **8:** 619–624.
35. Turley, S. D., and J. M. Dietschy. 1978. Re-evaluation of the 3α-hydroxysteroid dehydrogenase assay for total bile acids in bile. *J. Lipid Res.* **19:** 924–928.
36. Tietz, P. S., J. L. Thistle, L. J. Miller, and N. F. Larusso. 1984. Development and validation of a method for measuring the glycine and taurine conjugates of bile acids in bile by high-performance liquid chromatography. *J. Chromatogr.* **336:** 249–257.
37. Swobodnik, W., U. Klüppelberg, J. G. Wechsler, M. Volz, G. Normandin, and H. Ditschuneit. 1985. Rapid and accurate reversed-phase high-performance liquid chromatographic determination of conjugated bile acids in human bile for routine clinical applications. *J. Chromatogr.* **339:** 263–271.
38. Abell, L. L., B. B. Levy, B. B. Brudie, and F. E. Kendall. 1958. Standard Methods of Clinical Chemistry, Academic Press, New York. 26.
39. Bergman, F., and W. Van der Linden. 1973. Bile acid pool size in hamsters during gallstone formation and after cholecystectomy. *Z. Ernaehrungswiss.* **13:** 37–42.
40. Beher, W. T., S. Stradnieks, G. J. Lin, and W. L. Konde. 1977. Comparative studies of the effects of fasting on bile salt pool sizes of hamsters and rats. *Steroids.* **29:** 695–701.
41. Beher, W. T., G. J. Lin, S. Stradnieks, W. N. Konde, and K. S. Rajan. 1977. The effects of cholecystectomy on the bile salt pool of the Syrian hamster. *Digestion.* **15:** 338–347.
42. Kuroki, S., E. H. Mosbach, B. I. Cohen, R. J. Stenger, and C. K. McSherry. 1987. 7-Methyl bile acids: 7β-methylcholic acid inhibits bacterial 7-dehydroxylation of cholic acid and chenodeoxycholic acid in the hamster. *J. Lipid Res.* **28:** 856–863.
43. Yanaura, S., and A. Iizuka. 1981. Changes in bile composition during gallstone formation in hamsters. *J. Pharm. Dyn.* **4:** 820–822.
44. Bellentani, S., E. Bosisio, M. Pecorari, E. De Fabiani, P. Cordoma, M. Crestani, and F. Manenti. 1987. Effect of tauroursodeoxycholate feeding, with or without taurine supplementation on hepatic bile acids and cholesterol metabolism in the hamster. *Pharmacol. Res. Commun.* **19:** 327–339.
45. De Fabiani, E., M. Crestani, B. Malavasi, M. del Puppo, F. Farina, C. Armocida, S. Bellentani, G. Quack, and E. Bosisio. 1989. The effect of etofibrate on cholesterol and bile acid metabolism in the hamster. *Pharmacol. Res.* **21:** 567–576.
46. Kuroki, S., and T. Hoshita. 1983. Effect of bile acid feeding on hepatic steroid 12α-hydroxylase activity in hamsters. *Lipids.* **18:** 789–794.
47. Saito, T. 1987. The preventive effect of vitamin E on gallstone formation. A study of biliary cholesterol and bile acids in vitamin E-deficient hamsters. *Arch. Jpn. Chir.* **56:** 247–261.
48. Reyes, H., and F. Kern. 1979. Effect of pregnancy on bile flow and biliary lipids in the hamster. *Gastroenterology.* **76:** 144–150.
49. Wheeler, H. O. 1973. Biliary excretion of bile acids, lecithin and cholesterol in hamsters with gallstones. *Gastroenterology.* **65:** 92–103.
50. Robins, S. J., and J. Fasulo. 1973. Mechanism of lithogenic bile production: studies in the hamster fed an essential fatty acid-deficient diet. *Gastroenterology.* **65:** 104–114.
51. Rovagnati, P., C. Di Padova, F. Di Padova, L. Munari, and R. Tritapepe. 1982. Effect of a concomitant treatment with ethynylestradiol and ascorbic acid on bile secretion in female hamsters. *Methods Find. Exp. Clin. Pharmacol.* **4:** 313–316.
52. Delage, Y., M. Dumont, and S. Erlinger. 1976. Effect of glycodihydrofusidate on sulfobromophthalein transport maximum in the hamster. *Am. J. Physiol.* **231:** 1875–1878.
53. Malavolti, M., S. Ceryak, and H. Fromm. 1987. Modulation of bile secretion by hepatic low-density lipoprotein uptake and by chenodeoxycholic acid and ursodeoxycholic acid treatment in the hamster. *Gastroenterology.* **93:** 1104–1115.
54. Stevens, R. D., L. Lack, R. H. Collins, W. C. Meyers, and P. G. Killenberg. 1989. Effects of monosulfate esters of taurochenodeoxycholate on bile flow and biliary lipids in hamsters. *J. Lipid Res.* **30:** 673–679.
55. Turley, S. D., D. K. Spady, and J. M. Dietschy. 1983. Alteration of the degree of biliary cholesterol saturation in the hamster and rat by manipulation of the pools of preformed and newly synthesized cholesterol. *Gastroenterology.* **84:** 253–264.
56. Di Padova, C., E. Bosisio, G. Cighetti, P. Rovagnati, M. Mazzocchi, C. Colombo, and R. Tritapepe. 1982. 3-Hydroxy-3-methylglutaric acid (HMGA) reduces dietary cholesterol induction of saturated bile in hamster. *Life Sci.* **30:** 1907–1914.
57. Beher, W. T., G. D. Baker, and D. G. Penney. 1962. A comparative study of the effects of bile acids and cholesterol metabolism in the mouse, rat, hamster and guinea pig. *Nutrition.* **79:** 523–530.
58. Duane, W. C., M. L. Gilberstadt, and D. M. Wiegand. 1979. Diurnal rhythms of bile acid production in the rat. *Am. J. Physiol.* **236:** R175–R179.
59. Une, M., A. K. Singhal, C. K. McSherry, P. May-Donath, and E. H. Mosbach. 1985. Metabolism of 3α,7α-dihydroxy-7β-methyl-5β-cholanoic acid and 3α,7β-dihydroxy-7α-methyl-5β-cholanoic acid in hamster. *Biochim. Biophys. Acta.* **833:** 196–202.
60. Gurantz, D., and A. F. Hofmann. 1984. Influence of bile acid structure on bile flow and biliary lipid secretion in the hamster. *Am. J. Physiol.* **247:** G736–G748.