The extracorporeal bile duct: a new model for determination of bile flow and bile composition in the intact rat

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Abstract A new model is described which allows measurement of bile flow and sampling of bile in the intact rat with a physiologically functioning sphincter of Oddi. A number of metabolic parameters have been followed to show that animals with such an "extracorporeal bile duct" (EBD) behave as intact controls. Especially, there was no difference in the increase of body weight or hepatic fatty acid and cholesterol synthesis between EBD animals and intact controls. The amount of bile salts circulating through the biliary tract amounted to 30.5 ± 1.5 μmol·100 g body $wt^{-1} \cdot hr^{-1}$, when diurnal variations were averaged. Animals adapted to a restricted feeding regimen showed a significant increase of bile flow and of biliary bile salt and cholesterol excretion during feeding (10 AM–3 PM); these parameters reached their maximum 3 hours after onset of food intake.

Supplementary key words bile pressure · bile salts · cholesterol · enterohepatic circulation of steroids

For the purpose of intestinal lipid absorption, a variable flux of bile salts and cholesterol takes place from the liver into the duodenum via the biliary tract. Although the bulk of these steroids is salvaged by reabsorption in the lower intestine, they constitute—by leaving temporarily the "milieu interne" of the organism—a challenge to the animal's capability to maintain homeostasis of its steroid pool. Hence, for an understanding of cholesterol and bile salt metabolism, an analysis of bile flow and steroid concentration in the bile at short intervals in vivo is desirable.

Several methods have been developed that quantitate the amount of steroids passing through the enterohepatic circulation. Grundy and Metzger (1) and Brunner et al. (2) described a method that by differential sampling of duodenal content allows the determination of hepatic secretion in man. The model designed by Campbell et al. (3) makes an accurate determination of bile constituents possible at any given moment. However it neglects the physiological function of the sphincter of Oddi for bile flux, because this possible control point is by-passed in their model. Moreover, the fact that it can be applied only to expensive laboratory animals, i.e., monkeys, makes it impractical for most laboratories. Another method developed by Klauda, McGovern, and Quackenbush (4) allows sampling of bile in a rat model, but determination of bile flow, and hence steroid flux through the enterohepatic circulation, is impossible.

We describe herein a new model that allows measurement of bile flow and sampling of bile in the intact rat with a physiologically functioning sphincter of Oddi. Furthermore, data on diurnal changes of bile flow, of bile composition, and of pressure in the biliary tract are reported.

MATERIALS AND METHODS

Materials

Polyvinylchloride tubes (ID 0.5 mm, OD 1.0 mm) used for the extracorporeal bile duct (EBD) were purchased from Braun Melsungen (Melsungen, West Germany). Pieces of silicone rubber tubing for injection of air bubbles into the EBD were taken from infusion devices (Pfrimmer R 71, Erlangen, West Germany).

Animals

Male Sprague-Dawley rats (SPF) weighing 100–120 g were purchased from the Süddeutsche Versuchstieranstalt (Tuttlingen, West Germany). They were fed a standard rat chow (Altromin R 15, Altrogge, Laage-Lippe, West Germany) and had free access to

Abbreviations: EBD, extracorporeal bile duct; HMG-CoA reductase, mevalonate:NADP oxidoreductase (acylating CoA) (E.C. 1.1.1.34); cholesterol 7α-hydroxylase (E.C. 1.14.-.-); SPF, specifically pathogen free.

water. The animals generally were fed ad libitum; in some experiments they had restricted food access from 10 AM to 3 PM. The rats were kept in a room artificially illuminated from 6 AM to 6 PM. The temperature in the room was kept at approximately 23°C. The animals were adjusted to this environment for at least 8 days before surgery. Rats were kept in groups of six per cage during this period of adaptation.

Insertion of the extracorporeal bile duct

A polyvinylchloride tube was pretreated as follows. The end of a 7-cm piece was drawn out at 80°C, resulting in a 2-cm point with an outer diameter of about 0.5 mm (**Fig. 1a**). The tubes were subsequently sterilized in 8% aqueous formaldehyde overnight and washed extensively with sterile saline before use.

Rats fasted overnight and weighing 150–170 g were anesthetized by intraperitoneal injection of Nembutal (54 mg per kg body weight). The abdomen of the animal was shaved and then covered with a polyethylene foil. The abdominal cavity was opened by a transverse skin incision and a subsequent longitudinal incision of the muscle layer. After pushing away the intestine, the common bile duct was cannulated 10 mm from the liver margin. For this purpose the duct was opened transversely with scissors. The extended and subsequently beveled end of a tube was slipped into the duct through this opening in the direction of the duodenum and fixed with a silk ligature. A second catheter was entered through the same aperture, pointing to the liver, and was secured likewise. An additional ligature at the crossing of the tubes minimized loosening of the catheters (for further details see Fig. 1b). Both catheters were exteriorized through a stab wound in the lower right flank. The tubes were also crossed in the stab wound and fixed there with a wound clamp. The free ends of both tubes were connected to allow bile circulation; for this purpose tubing with an inner diameter of 1.0 mm was used. A wadded steel wire mosquito net was wrapped around the animal's trunk and served to fix the extracorporeal bile duct on the rats neck.

An 18-cm wooden fork fixed to the mosquito net ring formed a mobile restraining cage (Fig. 1c). This restraining device prevented the rat from turning around in the cage. Thus, the animal was not able to gnaw on the tubings that served to measure bile pressure and bile flow (see below).

After surgery the rats were placed in individual cages (Makrolon, 40 × 25 × 15 cm, Ehret, Emmendingen, West Germany). Two weeks after surgery less than 5% of the operated animals had a bacterial infection in the EBD. These animals were not included in the experiments.

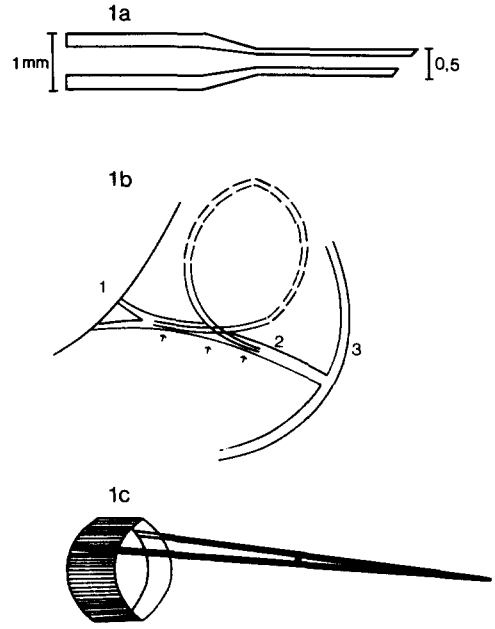

Fig. 1. Experimental details of EBD. *a*) The extended and beveled end of a polyvinylchloride tube for slipping into the bile duct. This end has the dimensions as given in the picture. *b*) The EBD in situ. The numbers indicate 1, the liver margin; 2, the common bile duct; 3, the duodenum. The position of the EBD in the bile duct is schematically drawn. The three arrows indicate sites of silk ligature. *c*) The mobile restraining cage that allows free animal movement in the cage. It is composed of a wadded steel wire mosquito net ring and an 18-cm wooden fork held together with tape.

Flow measurement

Eight to ten days after its insertion the EBD was lengthened by a bubble flow meter. It consisted of a 2-cm piece of smooth silicone rubber tubing connected to a 40-cm polyvinylchloride tube (ID 0.6 mm, OD 1.6 mm). The silicone rubber was readily perforated with a needle for the withdrawal of 50-μl aliquots for determination of bile salt and cholesterol concentrations. It served also for the measurement of speed of bile flow. For this purpose, the 40-cm tube was inserted in a photometer in such a way that the air bubble crossed the light path (578 nm) causing a mark on a recorder. The air bubble (volume ca. 0.4 μl) had to cross the light path four times after having completed a distance representing a total defined volume (about 80 μl). The speed of flow was calculated from the time span between the marks of the recorder. The complete bubble flow meter had a volume of 1000 μl. Because of an eventual transitory perturbation of bile acid homeostasis by the expansion of the extrahepatic biliary volume, physiological parameters were not monitored earlier than 36 hr after insertion of this tubing. Steroid flux through the biliary tract is defined as the product of bile volume per hr (in ml/hr) and the concentration of the steroid (in μmol/ml).

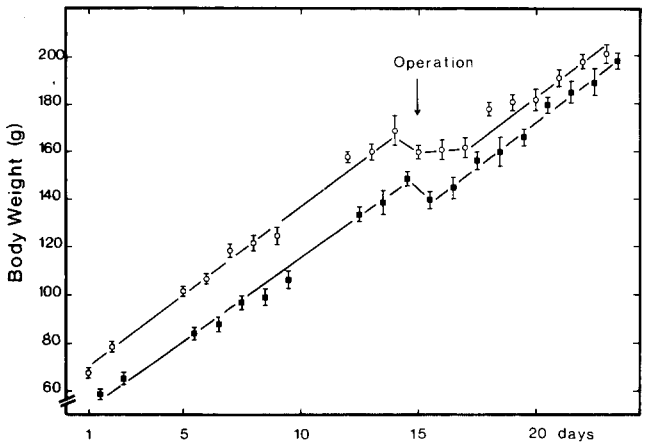

Fig. 2. Increase of body weight of EBD animals and intact controls. The body weight of animals carrying an EBD (circles) and of intact controls (squares) is shown. Means ± SD of seven animals in each group are given. The rats were fed ad libitum up to 16 hr before the insertion of EBD during which both groups were starved. The arrow marks the time of surgery.

The flow of bile in five typically prepared EBD tubings was monitored at pressures of 2, 5, 10, 15, 20, and 25 cm of water; there was a linear rise of bile flow between a pressure of 2 cm of water, where flow was 41 ± 1.3 mg/5 min, and 25 cm of water where it amounted to 1219 ± 63 mg/5 min ($\bar{x}$ ± SD) (data not shown).

Bile pressure

The pressure in the bile system was recorded by connecting a Statham element (CP37B, Statham, Oxnard, CA) to the EBD. A Hellige multiplier and recorder (Hellige, Freiburg, West Germany) served to trace it on paper. The system was gauged with distilled water. The response was linear over a range of pressure from 0 to 40 cm of water.

Biochemical parameters

Serum α-amylase (E.C.3.2.1.1.) was measured as described by Close and Street (5). The normal range was between 400 and 1300 "Street-Close units" (equivalents to 2.4–8.1 U/ml; $\bar{x}$ ± SD). Sorbitol dehydrogenase (E.C.1.1.1.14) activity was measured as described by Gerlach and Hiby (6). We found a normal range of 0–1.6 U/ml serum. Alkaline phosphatase (E.C.3.1.3.1) was measured as described by Bessey, Lowry, and Brock (7). In our hands the normal range was 50–135 mU/ml serum. Bile acids in bile were measured as described by Koss, Mayer, and Haindl (8) with 3α-hydroxysteroid-dehydrogenase (E.C.1.1.1.54). Cholesterol in bile was determined enzymatically (cholesterol: oxygen oxidoreductase (E.C.1.1.3.6)) as described by Röschlau, Bernt, and Gruber (9) as modified by Roda et al. (10). [1-^{14}C]-Acetate incorporation was measured as described by Hamprecht et al. (11).

All statistical values were expressed as mean ± SEM unless otherwise noted.

RESULTS

Validity of model

Several metabolic parameters were compared to make certain that the EBD did not impair the nutritional or metabolic state of the animals. The body weights of animals carrying an EBD and of intact controls were followed over a 3-week period. There was a linear increase in weight during the preoperative period (**Fig. 2**). The steady increase during the preoperative period was interrupted at the time of operation for one day in the intact controls and for 3 days in the EBD group due to the preoperative overnight fast in the former and to the operative trauma in the latter (see Fig. 2). Subsequently both groups gained weight and there was no difference in the rate of weight gain between EBD animals and intact controls.

Fourteen days after the operation the hepatic synthesis of cholesterol and fatty acids, as measured by [1-^{14}C]acetate incorporation by a liver homogenate, was comparable in EBD and control animals (**Table 1**). In each EBD animal several clinical–chemical parameters were determined routinely. Eight percent of 70 EBD animals had an elevated serum sorbitol dehydrogenase activity and 33% had an elevated serum alkaline phosphatase activity. The data obtained from such animals apparently suffering from liver cell damage or cholestasis due to occasional improper insertion of the tubing were not included in the evaluation of the experiments reported here. No rise of α-amylase above control values was observed. Liver sections of five EBD animals and three intact controls showed no difference in histological appearance, i.e., no signs of biliary obstruction.

At the beginning of our experiments, pressure in the bile system was measured by an additional side arm, attached to the EBD. A vertical glass tube served to monitor the pressure in the system. This method was not used further because the bile pressure showed oscillations over a range of 10 cm of water that were so rapid that a quantification without a recorder seemed impossible. Therefore we decided to monitor the bile pressure with a Statham element. Quantitative data were calculated from tracings obtained with multiplier set at a high integrating time constant. There appeared to exist a diurnal rhythm of bile pressure which fell to 10.8 ± 1.3 cm of water at 10 AM and rose to 14.5 ± 1.5 cm of water at 5 PM in animals with

TABLE 1. Rate of synthesis of cholesterol and fatty acids in EBD animals and in intact controls

Time	Cholesterol		Fatty Acids	
	Controls	EBD	Controls	EBD
	μatom [1-^{14}C]acetate carbon · g wet wt^{-1} · hr^{-1}			
10 AM	0.169 ± 0.015 (5)	0.197 ± 0.06 (2)	0.120 ± 0.014 (5)	0.096 ± 0.008 (2)
3 PM	0.230 ± 0.044 (5)	0.187 ± 0.066 (5)	0.062 ± 0.004 (5)	0.086 ± 0.009 (5)
9 PM	0.582 ± 0.008 (5)	0.605 ± 0.110 (5)	0.223 ± 0.045 (5)	0.267 ± 0.044 (5)
Midnight	0.475 ± 0.128 (3)	0.684 ± 0.115 (4)	0.267 ± 0.044 (3)	0.167 ± 0.009 (4)

Cholesterol and fatty acid synthesis was measured as incorporation of ^{14}C from [1-^{14}C]acetate into sterol digitonides and fatty acids in the liver homogenates obtained from animals killed at the times indicated. Data are given as mean ± SEM. Number of animals in parentheses.

restricted food access from 10 AM to 3 PM (n = 13). Rat liver in vivo (12) as well as in the isolated perfused state (13) produces maximal biliary secretory pressures of 17 cm of water.[1] It is noteworthy that our bile pressures ranged between 11 and 14.5 cm of water and never reached this maximal secretory pressure.

Diurnal changes of steroid flux through the biliary tract

When animals had free access to food, a diurnal rhythm of bile flow and bile salt flux was observed with an acrophase of both parameters around midnight (**Fig. 3**). These changes of bile salt flux were mainly caused by the diurnal changes of bile flow, because bile salt concentration did not change markedly over the day (**Table 2**).

Because the changes observed were possibly linked to eating, animals were adapted to a regimen with a short period of feeding restricted to the light phase. A steep rise of bile flow and biliary flux of bile salts (**Fig. 4**) and cholesterol (**Fig. 5**) was observed during this short eating period. The maxima of all three parameters were reached within 3 hr after food presentation and they declined to basal values within 3 hr after food withdrawal.

When the data were integrated over the day, a bile flow of 0.90 ± 0.03 ml·100 g body wt^{-1}·hr^{-1} and a bile salt flux of 30.5 ± 1.5 μmol·100 g body wt^{-1}·hr^{-1} were observed in rats fed ad libitum. In animals with restricted food access, the corresponding values were 0.84 ± 0.06 ml·100 g body wt^{-1}·hr^{-1} and 35.9 ± 1.4 μmol·100 g body wt^{-1}·hr^{-1}, respectively.

Cholesterol flux was 0.196 ± 0.020 μmol·100 g body wt^{-1}·hr^{-1} under conditions of restricted food access. This equals 2.3 mg cholesterol per 100 g body wt being excreted from the liver to the intestine per day.

[1] Maximal secretory pressure has been defined by these investigators (13) as a steady-state pressure reached within 5 min after cannulation of the bile duct.

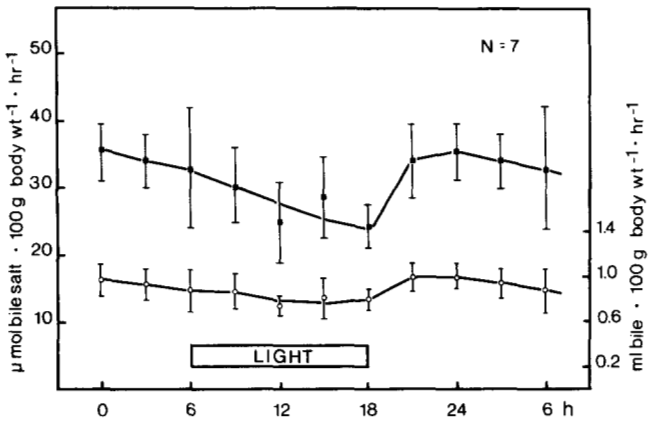

Fig. 3. Diurnal variation of bile flow and bile salt flux in animals fed ad libitum. Bile flow and bile salt concentration at the times given on the abscissa were measured 10–14 days after insertion of EBD. The rats were maintained in conditions of regulated lighting from 6 AM to 6 PM. They had free access to food and water. Bile salt flux equals (bile flow) × (bile salt concentration) and is expressed as μmol bile salt·100 g body wt^{-1}·hr^{-1}. Bile flow is indicated as circles, bile salt flux as squares (mean ± SEM).

DISCUSSION

The experimental model presented here allows for a direct analysis of bile composition and flow in a small

TABLE 2. Diurnal changes of bile salt concentration in bile of animals fed ad libitum (A) or from 10 AM to 3 PM (B)

	A	B
	μmol bile salt × ml^{-1}	
6 AM	34.4 ± 2.4	50.4 ± 4.4
9 AM	34.2 ± 2.7	47.3 ± 3.8
10 AM	n.d.	45.3 ± 3.0
12 AM	34.5 ± 2.9	39.7 ± 2.4
3 PM	37.2 ± 3.9	39.8 ± 3.9
6 PM	32.3 ± 3.2	41.2 ± 2.5
9 PM	35.7 ± 2.9	n.d.
Midnight	36.5 ± 2.0	43.6 ± 3.9
3 AM	35.6 ± 2.8	n.d.

Bile salt concentrations were determined in bile samples obtained from the EBD 2 weeks after its insertion. No more than three bile samples were withdrawn per day in a given animal. For animals fed ad libitum (A; n = 7) see Fig. 3, for animals with restricted food access (B; n = 9) see Fig. 4. n.d., not determined.

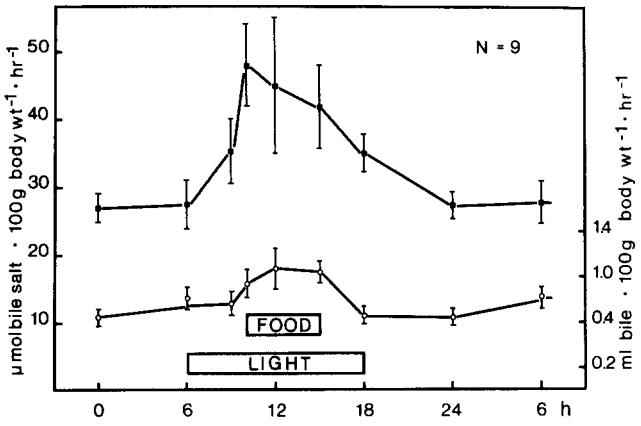

Fig. 4. Diurnal variation of bile flow and bile salt flux in animals with restricted food access. Three weeks before measurements the animals were maintained in conditions of regulated lighting (6 AM to 6 PM) and restricted feeding from 10 AM to 3 PM. They had free access to water. For further details see Fig. 3.

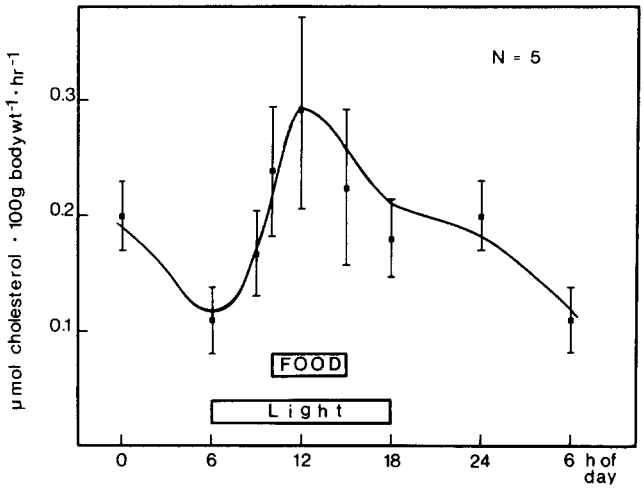

Fig. 5. Diurnal variation of flux of biliary cholesterol in animals with restricted food access. The cholesterol flux was measured 10–14 days after insertion of EBD. Three weeks before measurements the animals were maintained in conditions of regulated lighting (6 AM to 6 PM) and under restricted feeding (10 AM to 3 PM). They had free access to water. For calculation of flux see Fig. 3. Means ± SEM are given.

laboratory animal. Because it consists merely of a tube inserted into the bile duct, excretion and flow of bile take place under physiological conditions, namely, the sphincter of Oddi controls the output of bile into the duodenum and maintains pressure in the biliary tract as in the intact animal.

The rise of body weight and the rate of hepatic lipid synthesis of EBD animals were found to be in the normal range. This is taken as evidence that the nutritional state and lipid metabolism in these animals were normal. This conclusion is particularly justified as rates of hepatic fatty acid (14) and cholesterol synthesis (15–17) are very sensitive to a great variety of stimuli, e.g., nutritional and hormonal alterations. Moreover, the finding of an unaltered diurnal pattern of cholesterol synthesis substantiates our contention that cholesterol and bile salt metabolism in EBD animals is normal and that this model is suitable for studies of the regulation of cholesterol synthesis.

Magnitude of enterohepatic circulation of steroids

Data obtained by this model allow a calculation of the amount of steroids passing through the enterohepatic circulation. Several attempts have been made to estimate the flux of bile salts passing through the biliary tract of the rat. The reported figures range between 5 mg (18) and 10 mg (19) of bile salt per 100 g body weight per hr. Whereas Van Belle (18) based the estimate on bile acid pool size and the number of circulations, Shefer et al. (19) derived their figure from the infused amount of bile salt required to keep bile salt synthesis in an inhibited state. One has to keep in mind that the latter type of estimate gives a minimal figure.

From our direct estimates we have calculated that a greater amount of bile salts passes through the enteropehatic circulation in vivo. It was equivalent to 16.5 mg of sodium taurocholate · 100 g body $wt^{-1} \cdot hr^{-1}$ in animals fed ad libitum and 19.4 mg · 100 g body $wt^{-1} \cdot hr^{-1}$ in animals with restricted access to food. It is noteworthy that in both groups of animals the bile salt flux was similar. This supports the view that the method gives reproducible results. If our method underestimates bile flow because of the resistance inherent to the EBD, the discrepancy between earlier estimates of daily bile salt flux and ours becomes even greater. Our finding of a bile salt flux that was about 60% higher than values reported by earlier investigations is of relevance for studies on the regulation of cholesterol synthesis by bile salts.[2]

Diurnal changes of bile parameters

The data presented here show a diurnal rise of bile salt flux through the biliary tract during the early dark phase (Fig. 3). Because rats eat 80% of their daily ration during the dark period (20, 21), it is tempting to speculate that the above phenomenon is due to increased consumption of food. This assumption seems very probable, as feeding is known to be the most potent stimulator of bile flow (22) and enhances biliary bile salt output (23). A steep rise of bile salt (Fig. 4) and cholesterol (Fig. 5) flux and bile flow was observed, indeed, during a 5-hr feeding period, even if restricted to the light phase. These findings show that ingestion of food is of importance for the syn-

[2] Weis, E. E., and C. A. Barth. Unpublished data.

chronization of diurnal changes in bile flow in the intact animal.

The rise of biliary steroid output coincident with food intake may help to clarify the cause of diurnal changes of hepatic cholesterol (24, 25) and bile salt (26) synthesis reported by numerous investigators. The parallel rise of biliary steroid excretion (documented here) and of HMG-CoA reductase (27, 28) and cholesterol-7α-hydroxylase (29) activity may be causally linked, because a depletion of hepatic steroid pools has regularly been found to cause a rise of these enzymes catalyzing cholesterol and bile salt synthesis (30–32).

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