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ACUTE EFFECTS OF PENTOBARBITAL-ANAESTHESIA ON BILE SECRETION

FOLKERT KUIPERS, TJALLING DIJKSTRA, RICK HAVINGA, WILMA VAN ASSELT and ROEL J. VONK

Department of Pediatrics, State University Groningen, Bloemsingel 10, 9712 ZK Groningen, The Netherlands

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Abstract—Male Wistar rats were equipped with permanent catheters in the bile duct and the duodenum under ether anaesthesia, at least seven days before the experiments. By this technique, the enterohepatic circulation can be interrupted for bile collection without direct surgical intervention.

^{14}C-Pentobarbital (26.6 μmole/100 g body wt) was injected intraperitoneally immediately before interruption of the enterohepatic circulation (NBD, Non-Bile Diverted) or after eight days of bile diversion (BD, Bile Diverted). In NBD rats, bile flow and biliary bile acid excretion were significantly reduced during the first hour after pentobarbital administration when compared to unanaesthetized controls, but markedly increased thereafter. Pentobarbital treatment slightly decreased biliary bile acid excretion in BD rats, but caused a 60% increase in bile flow. Within four hours 22.3 ± 0.4% and 26.0 ± 2.7% of the injected radioactivity was excreted into bile in NBD and BD rats, respectively. The calculated osmotic activity of pentobarbital and its metabolites was 47.8 ± 5.2 μl/μmole in NBD rats and 37.8 ± 1.3 μl/μmole in BD rats. Consequently, pentobarbital treatment affected the bile acid independent fraction of bile flow (BAIF). The calculated BAIF was 2.68 μl/min/100 g body wt in unanaesthetized animals, but 4.27 μl/min/100 g body wt in pentobarbital treated NBD rats. Corresponding values for BD rats were 1.70 and 2.38 μl/min/100 g body wt.

It is concluded that pentobarbital anaesthesia affects bile production in the rat by direct and indirect means. Firstly, pentobarbital and its metabolites are rapidly excreted into bile and exert a significant choleretic effect, thereby increasing the BAIF. Secondly, pentobarbital anaesthesia retards the exhaustion of the intestinal bile acid pool, which leads to secondary changes in the biliary excretion process.

Most experiments in which biliary excretion processes are studied, are performed on restrained or anaesthetized animals. Little is known about effects of stress or anaesthesia on hepatic function. Pentobarbital is a commonly applied anaesthetic agent in hepatic transport studies. It has been reported not to affect bile formation in the bile fistula rat [1–3]. A slight stimulatory effect on bile flow was observed in studies with isolated perfused rat livers [4]. On the other hand, the choleretic action of phenobarbital, another oxybarbiturate, is well documented: acute and chronic phenobarbital administration increased bile flow in the rat by stimulating the bile acid-independent fraction of bile flow [1, 5, 6].

In this study, we investigated acute effects of pentobarbital administration on bile flow and biliary bile acid excretion *in vivo*, using rats equipped with permanent catheters in the bile duct and duodenum. This experimental design allows sampling of bile without intervention of anaesthetics and surgery [7]. Pentobarbital was administered either immediately before interruption of the enterohepatic circulation (EHC), thus in the presence of the endogenous bile acid pool, or after eight days of bile diversion.

MATERIALS AND METHODS

Sodium pentobarbital (PB) was purchased from Brocaceff (Maarssen, The Netherlands). Unlabelled pentobarbital was used to dilute 5-(ring-2-^{14}C) pentobarbital (52 mCi/mmole, New England Nuclear Corp., Boston, MA) to the required specific activity.

Animals. Male Wistar rats of 275–350 g were equipped with permanent silicon catheters (Silastic®, Dow Corning, Midlands, MI, i.d. = 0.5 mm, o.d. = 0.98 mm) in the bile duct and duodenum. These catheters were tunneled subcutaneously to the animal's head, fixed to the skull and shortcircuited with a piece of polyethylene tubing. This resulted in an intact enterohepatic circulation that could be interrupted without surgery. A detailed description of this technique and the validity of this animal model is given elsewhere [7]. A number of animals were also provided with a permanent heartcatheter, which allows repeated sampling of blood without manipulating the rats [8]. After surgery, the rats had a recovery period of at least seven days. In all cases, the rats regained their preoperative weights within four days. Food and water intake then had become normal. The rats were housed in plexiglass cages (25 × 25 × 30 cm) with a light regime of 12 hr dark and 12 hr light.

Experimental procedures. All experiments were started at 1100 hr to exclude effects of circadian variations [9]. During the experiments, rats were kept in metabolic cages which allow separate collection of urine and faeces. PB or the vehicle was administered to rats either immediately before interruption of the EHC by disconnecting bile duct and duodenum catheter (N = 7) or after eight days of bile diversion (N = 5).

The rats were injected intraperitoneally with 0.1 ml/100 g body wt of a 6% pentobarbital solution (26.6 μmole/100 g) or with the same volume of sterile saline. ^{14}C-pentobarbital was added to the PB solution to give a specific activity of 55.5 μCi/g, or 333 μCi/g when radioactivity in bile was subsequently analyzed. PB induced anaesthesia within 2–4 min which lasted for 75 to 120 min.

During anaesthesia, rectal temperature was maintained between 36.5 and 37.5° with a heating pad to exclude temperature effects. Immediately after injection, bile collection was started in 15-min intervals for 4 hr and in a single sample from 4–24 hr, into preweighed test tubes. For this purpose the bile duct catheter was connected to a polyethylene tubing which led the bile to a fraction collector outside the cage. The dead space of the tubing system was always taken into account [7]. In the bile diverted rats two 15 min-bile samples were taken before injection.

Twenty-four hour urine production was collected in all experiments. In two rats of each group blood samples (0.25 ml) were taken before and 1, 2, 3 and 4 hr after PB administration. Blood was transferred to heparinized test tubes and centrifuged (10 min, 5000 rpm). Plasma was stored at −20° until analyzed.

Analyses. The concentration of 3α-hydroxy bile acids in bile and plasma was determined with an enzymatic assay (Sterognost-Flu®, Nyegaard & Co., Oslo, Norway). Biliary cholesterol was measured after lipid extraction [10] by the method of Gamble *et al.* [11]. Bile and urine samples, decolorized with H_2O_2, were analyzed for ^{14}C-radioactivity in an ISOCAP scintillation counter after addition of 5 ml of Hydrocount scintillation fluid (J. T. Baker Chemicals B.V., Deventer, The Netherlands).

The metabolites of PB and unchanged drug in bile were separated by thin-layer chromatography on precoated silica plates (60 F 254, Merck, Darmstadt, F.R.G.) using the solvent system isopropanol–ammonia 30%–chloroform (45:10:45, v/v) [12]. For this purpose 0.25 ml of bile was added to 1.0 ml of 1.0 M acetate buffer (pH 5.5) and extracted twice with 5 ml of ethylacetate. The pooled extracts were concentrated by evaporation under nitrogen and analyzed. Radioactivity was located by an automatic chromatogram scanner (Berthold, Wildbad, F.R.G., model LB 2722).

Statistical analyses. Data are presented as means ± S.E.M. Student's *t*-test was used for statistical evaluation of differences between groups. Analysis of variance was applied to evaluate differences within a group.

Linear regression analysis was performed by the method of least squares.

RESULTS

Effects of pentobarbital on bile flow and biliary bile acid excretion rate

Figure 1 shows bile flow (A), and biliary bile acid (B) and cholesterol excretion (C) immediately after interruption of the EHC (NBD group) in control and PB treated rats. In the controls, bile flow and bile acid excretion remained constant during the first hour after interruption, but rapidly declined thereafter, which reflects the exhaustion of the endogenous bile acid pool [7]. Bile flow and bile acid excretion rate in the PB-group, measured 30 min before PB administration, were similar to that of control rats. After induction of anaesthesia, however, they were both significantly depressed during the first hour, but significantly enhanced during the third and fourth hour of the experiment. Biliary cholesterol excretion showed a similar pattern. Total bile acid output during 4 hr was 74.8 ± 4.6 μmole/100 g body wt in controls and 71.7 ± 5.5 μmole/100 g body wt in PB treated rats. Four-hours cholesterol output was 1.0 ± 0.3 and 1.3 ± 0.2 μmole/100 g body wt, respectively.

Plasma bile acid concentration, measured in two rats of each group, gradually declined from 15 to 20 μmole/l before the interruption to almost non-detectable levels within 4 hr.

The relationship between bile flow and bile acid excretion rate (Fig. 2) was described by a straight line for anaesthetized and unanaesthetized rats. The bile acid-independent fraction of bile flow (BAIF), obtained by extrapolating to zero bile acid excretion,

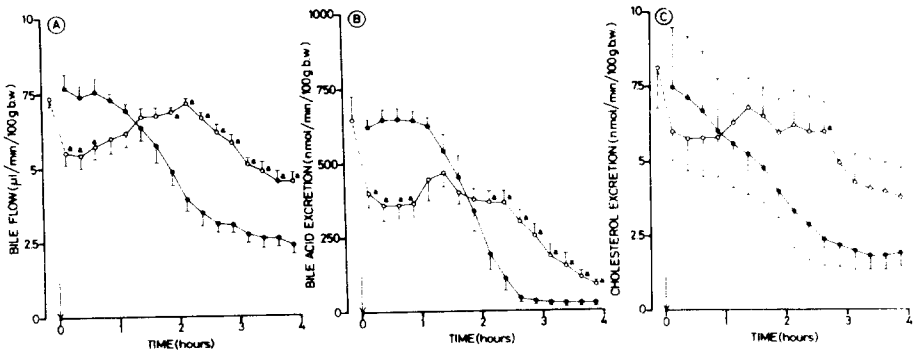

Fig. 1. Bile flow (A), biliary bile acid excretion (B) and biliary cholesterol excretion (C) in control (●, N = 7) and pentobarbital treated rats (○, N = 7), during 4 hr after interruption of the enterohepatic circulation. In the pentobarbital group an extra bile sample was taken 30 min before pentobarbital administration at time = 0 hr. For this purpose the connection between bile duct and duodenumcatheter was interrupted for ± 2 min. The arrow indicates the time of injection. Mean values ± S.E.M. a = significant differences between the groups, $P < 0.05$.

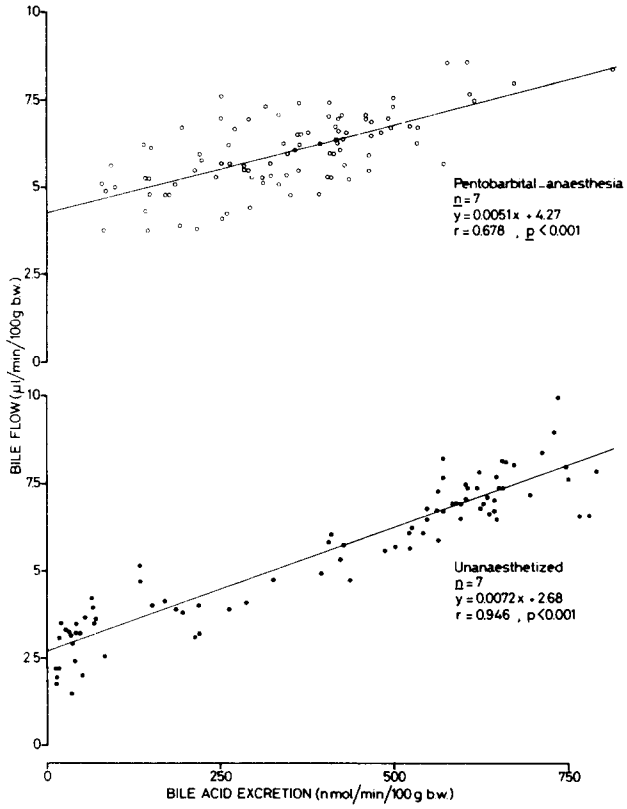

Fig. 2. Relation between bile flow and biliary bile acid excretion in control (●, N = 7) and pentobarbital-treated rats (○, N = 7), during 3 hr after interruption of the enterohepatic circulation.

was 4.27 µl/min/100 g body wt in treated animals and 2.68 µl/min/100 g body wt in controls. The slope of the regression line was larger in the control group, 7.2 µl/µmole vs 5.1 µl/µmole.

Pentobarbital administration to rats with an eight days biliary drainage (BD group) resulted in a 60% increase in bile production compared to pre-injection values (Fig. 3A). In contrast, biliary bile acid excretion was slightly but significantly decreased during the first 90 min after PB injection, but normalized thereafter (Fig. 3B). Plasma bile acid concentrations remained below measurable levels during this experiment.

Analysis of the relation between bile flow and bile acid excretion rate in the BD rats (Fig. 4) resulted in values for the BAIF of 1.70 and 2.38 µl/min/100 g for control and PB treated rats, respectively. The corresponding slopes were 19.5 and 24.7 µl/µmole.

Excretion of ^{14}C-pentobarbital

To evaluate the interference of pentobarbital and/or its metabolites with the biliary excretion process, the biliary excretion of ^{14}C-pentobarbital was studied in four out of the seven NBD rats included in the study and in the five BD rats. Figure 5 shows the biliary excretion of ^{14}C-pentobarbital and its metabolites during 4 hr after injection. The recovery in bile over this period was 22.3 ± 0.4% in NBD rats and 26.0 ± 2.7% in BD rats. In addition, from 4 to 24 hr after injection 8.8 ± 0.7% and 7.8 ± 1.9% of the injected dose was excreted in bile.

Twenty-four hours urinary excretion was

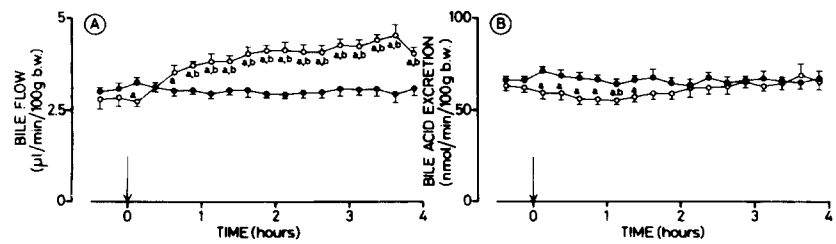

Fig. 3. Bile flow (A) and biliary bile acid excretion (B) before and after intraperitoneal administration of pentobarbital (○, N = 5) or saline (●, N = 5) to rats with an 8-days bile drainage. The arrow indicates the time of injection. Means values ± S.E.M. a = significant differences between the groups, $P < 0.05$. b = significantly different from preinjection values, $P < 0.05$.

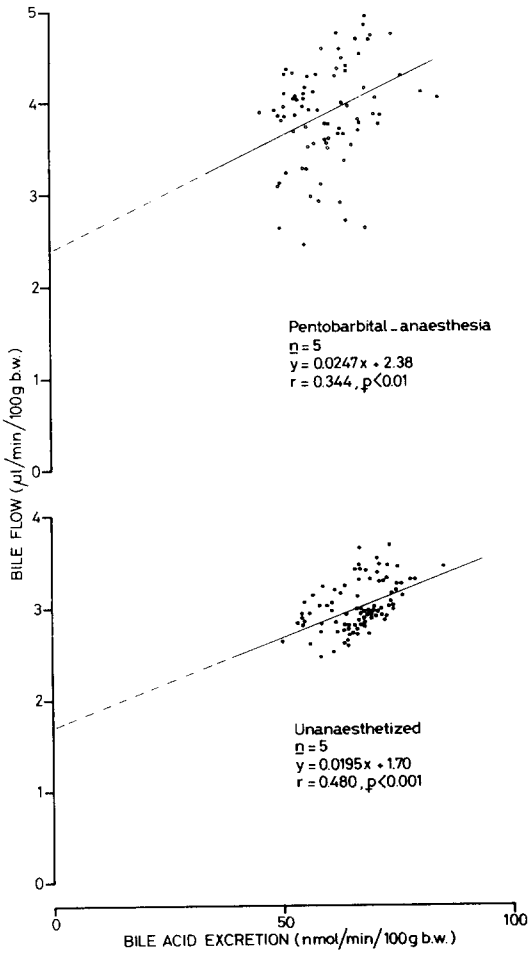

Fig. 4. Relation between bile flow and biliary bile acid excretion in rats with a biliary drainage for eight days. ○ = pentobarbital-treated rats, N = 5, ● = control rat, N = 5.

51.6 ± 3.6% and 60.2 ± 6.5% in NBD and BD rats, respectively. Thin-layer chromatography revealed that approximately 30% of the radioactivity in bile migrated with PB ($R_f = 0.60$), the remaining being transformed to three polar metabolites (R_f values 0.26, 0.21 and 0.0, respectively). Using this technique no quantitative or qualitative differences in PB metabolism were observed between NBD and BD rats. Also, no change in the distribution of activity was found when bile collected at different periods after PB injection was analyzed.

If one assumes that the difference in bile flow (Δ bile flow) between PB treated and control rats is only caused by different bile acid output rates together with the excretion of PB and metabolites into bile, one can calculate the osmotic activity of the latter compounds (Fig. 6, see legend for procedure). After correction for the difference in bile acid output rates between control and treated rats, the resulting Δ bile flow was negative in the first 15-min interval after injection; therefore the procedure was not applicable in this interval. In the BD rats the apparent osmotic activity of PB and its metabolites stabilized after 30 min at a level ranging from 31.7 to 50.6 μl/μmole

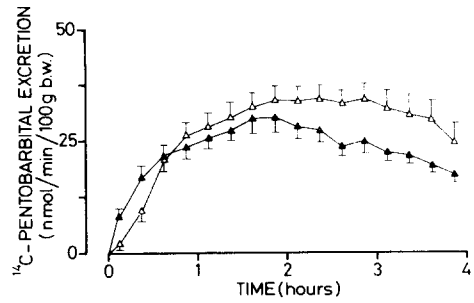

Fig. 5. Biliary excretion of ^{14}C-pentobarbital or metabolites after intraperitoneal injection. ▲ = NBD rats, N = 4. △ = BD rats, N = 5. Mean values ± S.E.M.

(37.8 ± 1.3, N = 14). In NBD rats its value showed larger variations in the same time interval (47.8 ± 5.2 μl/μmole, range 12.5–71.2) and also tended to be higher after recovery from anaesthesia.

DISCUSSION

Our results show that the induction of anaesthesia by pentobarbital (PB) is associated with distinct effects on bile flow and biliary bile acid excretion in the rat. A marked net stimulatory effect on bile flow was found, both in rats with an intact bile acid pool at the time of injection (NBD) and in rats with a long-term bile drainage (BD). Contradictory results on this subject have been reported in the literature so far. A slight stimulatory effect on bile flow was

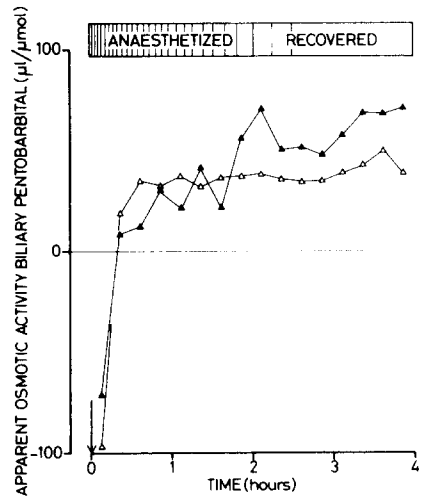

Fig. 6. The apparent osmotic activity of ^{14}C-pentobarbital and its metabolites excreted into bile during exhaustion of the endogenous bile acid pool (▲) and after eight days of bile diversion (△). The apparent osmotic activity was calculated by dividing the difference in bile flow between control and pentobarbital-treated rats in a given time interval by the amount of pentobarbital and metabolites excreted in that interval. The difference in bile flow was corrected for the difference in bile acid output between control and treated rats. For this purpose the osmotic activity of bile acids was assumed to be 7.2 μl/μmole in rats with an intact bile acid pool (see Fig. 2) and 19.5 μl/μmole in bile diverted rats (see Fig. 4).

found by Bizard *et al.* [4], who administered PB to isolated perfused rat livers. In the study of Buttar *et al.* [13], intravenous PB injection also increased bile flow of bile fistula-rats under ether anaesthesia. However, in the latter study biliary bile acid excretion rate was not determined. On the other hand, Cooper *et al.* [2] noticed no effects of PB administration on bile flow and bile acid output in rats after three days of bile drainage. Similarly, the absence of an effect on bile flow has been reported in a number of other studies in the rat [1, 3, 14].

In NBD rats, bile flow and biliary bile acid output were significantly reduced in the early stages of the experiment, but enhanced during the third and fourth hour after PB administration.

If the initial decrease in bile acid output was caused by inhibition of hepatic uptake or biliary excretion of bile acids by PB or its metabolites, considerable amounts of bile acids should remain in the plasma compartment, assuming that the hepatic storage capacity for bile acids is low and the liver and plasma can be considered as one compartment. The biliary bile acid excretion during anaesthesia was decreased with 16 μmole/100 g body wt in the first hour, when compared to the unanaesthetized controls. With a plasma volume of 5.45 ml/100 g body wt [15] this would lead to a plasma concentration of 2.9 mmole/l. Actually, the concentration in PB-treated rats was similar to that of untreated rats and never exceeded 20 μmole/l. Furthermore, total bile acid output over 4 hr was not altered by PB treatment. These results indicate that the influx of bile acids from the intestine must have been drastically reduced in the NBD rats during anaesthesia. It is known that PB depresses intestinal mobility [16] and that more than 90% of a rat's bile acid pool is present in the intestinal lumen [17]. PB anaesthesia thus retards the depletion this pool. The observed changes in the excretion pattern of cholesterol may be secondary to the changes in bile acid output [18, 19]. In BD rats, the intestinal bile acid pool was exhausted before experiments were started. They therefore secreted only newly synthetized bile acids. In this situation, PB caused a slight fall in bile acid output. This might have been a temperature effect. In similar experiments, in which the rats' body temperature was not regulated, we found a 50% decrease in bile acid output within 1 hr. Rectal temperature decreased from 37.2 to 33.5° over this period. It is therefore possible that minor changes in body temperature, which are perhaps not adequately monitored by rectal temperature, are responsible for the observed changes in bile acid output.

The stimulating effect of PB on bile flow in our study is most likely explained by osmotic effects. Approximately one-fourth of the administered dose was excreted into bile within 4 hr. This agrees with the results of Klaassen [20] and Buttar *et al.* [13] who found 28 and 14.6% of the dose, respectively, to be excreted into bile within 6 hr after intravenous injection. In contrast, Ossenberg *et al.* [21] reported a biliary recovery of only 1.5% in 4 hr. As described earlier [13, 20], PB was extensively metabolized before biliary excretion. No qualitative or quantitative differences in metabolism were observed between NBD and BD rats. However, the apparent osmotic activity of excreted molecules differed between the groups and also seemed to change in time for the NBD rats (Fig. 6). These observations are difficult to explain, but may be due to differences in bile composition in these situations, which may also be responsible for the observed difference in the apparent osmotic activity of bile acids (see below).

In the classical analysis, proposed by Erlinger [22], the slope of the regression line describing the relationship between bile flow and bile acid excretion is taken as indicator of the osmotic activity of bile acids, and the intercept with ordinate reflects the bile acid-independent fraction of bile flow (BAIF). As shown in Figs 2 and 4, PB treatment increased the value of the BAIF, both in NBD and in BD rats. This is most likely a consequence of the excretion of osmotically active PB (and metabolite) molecules. The difference in the slopes of the regression lines between anaesthetized and control rats are presumably caused by the fact that the excretion of PB, and thereby the amount of associated fluid, was not the same for all the points included in this analysis. In NBD rats, the PB excretion was low when bile acid output was maximal and increased with decreasing bile acid output rates. This results in a smaller slope of the regression line. Similar phenomena occurred in BD rats.

It should be stressed that the BAIF of the unanaesthetized rats in the present study is 2 to 3 times lower than values reported in the literature for rats [see 23]. Because most studies on this subject are performed with PB anaesthetized animals, overestimation of the BAIF due to PB treatment should be considered.

The relationship between bile flow and bile acid excretion differed markedly between NBD and BD rats, which is in agreement with the observations of Balabaud *et al.* [24]. The estimated BAIF was larger in NBD than in BD rats. In contrast, the apparent osmotic activity of bile acids was almost three times larger in the BD rats (19.4 vs 7.2 μl/μmole). The physiological mechanism for the latter finding is unclear. The biliary bile acid concentration was above the critical micellar concentration in these studies. The presence of bile acids as monomers in bile, which would create a higher osmotic gradient than micelles, is therefore unlikely. It is possible, however, that at lower bile acid concentrations the formed micelles are smaller. Differences in biliary bile acid composition may contribute to the observed variation in osmotic activity, however, cholic acid and β-muricholic acid were quantitatively the most important bile acids in both situations. Furthermore, it has been observed in all species studied, that each μmol of exogenous administered bile acid increases bile flow approximately to the same extent [25]. The only fundamental difference between the biliary bile acids of NBD and BD rats seems to be their origin: in NBD rats they originate mainly from the portal blood, while in the BD rats they are synthetized in the hepatocyte. Whether this is the cause of the observed difference in their osmotic activity remains to be established.

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