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EFFECT OF CHENODEOXYCHOLATE FEEDING UPON THE BILIARY OUTPUT OF PLASMA MEMBRANE ENZYMES IN THE RAT

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Abstract—In model experiments using human erythrocytes, glycochenodeoxycholate caused extensive membrane damage (as judged by release of membrane phospholipid and acetylcholinesterase and by cell lysis) at approximately 10-fold lower concentrations than glycocholate. Chenodeoxycholate feeding had no effect upon the total protein, bile salt or phospholipid concentration of rat bile, although evidence is presented to suggest an expansion of the bile salt pool occurred. Rats fed chenodeoxycholate showed a dose-dependent enrichment of this bile acid in bile; this occurred mainly at the expense of cholate. Chenodeoxycholate feeding resulted in an increased biliary output of the plasma membrane enzymes alkaline phosphatase and 5′-nucleotidase; the hepatic activities of these enzymes were also increased. In contrast, the biliary output and hepatic activities of two other plasma membrane enzymes, alkaline phosphodiesterase I and L-leucine-β-naphthylamidase, were unaffected by chenodeoxycholate feeding. A greater proportion of all four plasma membrane enzymes studied existed in bile of chenodeoxycholate-fed rats in a "soluble" form (as judged by their remaining in the supernatant on centrifugation of bile). These results are discussed in relation to the origin of plasma membrane enzymes in bile and to the potential toxicity of chenodeoxycholate and its conjugates to the membranes of the hepatobiliary system.

The naturally occurring bile acid chenodeoxycholate is used clinically at doses of 14–15 mg/kg per day for up to one year for the dissolution *in vivo* of small radiolucent gallstones [for reviews see 1–5]. A consequence of chenodeoxycholate administration is that the amount of chenodeoxycholate in human bile increases from 25–30% to 75–90%; this occurs at the expense of the other major human bile acids, deoxycholate and cholate [6, 7].

Several plasma membrane and lysosomal marker enzymes have been identified in the biles of several mammalian species [8–11]. Plasma membrane enzymes have been shown to be released from isolated hepatocytes before the onset of lysis by treatment with bile salts [12]. These results, together with the observation of Mullock *et al.* [13] that rat bile 5′-nucleotidase is immunologically identical with the liver enzyme, have led to the suggestion that plasma membrane enzymes occur in bile as a result of bile salt attack on the plasma membrane in the region of the bile canaliculus [10, 14].

In the experiments of Billington *et al.* [12] using isolated hepatocytes, the dihydroxy bile salt glycodeoxycholate released significant amounts of plasma membrane marker enzymes at approximately 10-fold lower concentrations than the trihydroxy bile salts, glycocholate and taurocholate. In other model experiments using human erythrocytes [15], erythrocyte ghosts [16] and rat liver plasma membranes [17], deoxycholate and its conjugates were shown to be more membrane damaging than cholate and its conjugates. It may be postulated, therefore, that if chenodeoxycholate possesses similar membrane damaging properties to its structural isomer, deoxycholate, then enrichment of the biliary bile acid pool with chenodeoxycholate will manifest itself in a more membrane damaging bile. In addition, if plasma membrane marker enzymes arise in bile as a result of bile salt attack at the bile canaliculi, then a more membrane damaging bile may lead to increased output of these enzymes. In this paper we report the effect of chenodeoxycholate feeding upon the biliary output of bile acids and of plasma membrane enzymes in the rat. Although the rat does not possess a gallbladder (whose function is to concentrate and store bile), a similar profile of plasma membrane enzymes occurs in the bile of both rat and man [8]. A preliminary account of some of this work has appeared [18].

MATERIALS AND METHODS

Materials. Female, Wistar rats weighing approximately 250 g were used throughout. Prior to the experiments, animals were maintained on a standard laboratory diet in a constant temperature environment (22°) and under a constant 12 hr light cycle. Chenodeoxycholate was administered by gastric intubation for 14 days at two dosages: 90 mg/kg per day (roughly equivalent to the therapeutic dose in man on the basis of body surface area [19]) and 300 mg/kg per day. The bile acid was dissolved in a final volume of 1 ml and control animals were

similarly intubated with 1 ml of saline. No weight loss was observed in control rats and those fed 90 mg/kg per day; rats fed 300 mg/kg per day lost approximately 10% of their body weight over the 14 day feeding period and developed diarrhoea. Diarrhoea is the most commonly reported side effect of chenodeoxycholate administration in man [20].

All bile duct cannulations were performed with PP10 tubing (Portex Ltd., Hythe, Kent, U.K.) between 09:00–12:00 hr while the animals were under pentobarbitone (Sagatal) anaesthesia. Bile was collected for 2 hr on ice; a 0–2 hr collection period was used since changes in the composition of rat bile owing to interruption of the enterohepatic circulation are minimal over this time period [21, 22]. At the end of the bile collection, the animal was sacrificed and a portion of the liver was homogenised in 10 vol of ice-cold 1 mM $NaHCO_3$, pH 7.4 in a tightly fitting Potter–Elvehjem homogeniser. Bile and liver homogenates were stored at −20° until analysed.

In some experiments, bile was collected for up to 30 hr. In these experiments, the cannula was exteriorised at the back of the animal near to the tail and the animals were kept in restriction cages with free access to food and water. Bile was collected in 6 hr fractions on ice. Aliquots of these bile samples were centrifuged at 150,000 *g* for 60 min at 4° in a swinging-bucket type rotor. Uncentrifuged bile samples and their supernatants were stored at −20° until analysed.

Human blood was obtained through courtesy of the Merseyside Blood Transfusion Service and was used within 10 days of donation. Bile salts (sodium salts, A grade) were obtained from Calbiochem, Bishops Stortford, Herts, U.K. Hydroxysteroid dehydrogenase (grade 11 from *Pseudomonas testosteroni*), cholyglycine hydrolase (partially purified lyophilised powder from *Clostridium perfringens*) and other fine chemicals were from Sigma (London) Chemical Co., Poole, Dorset, U.K. Other reagents were from B.D.H., Poole, Dorset, U.K. and were of the highest grade available.

Membrane damaging properties of bile salts. The membrane damaging properties of glycocholate and glycochenodeoxycholate were assayed by determining the release of haemoglobin, phospholipid and acetylcholinesterase (E.C. 3.1.1.7) from human erythrocytes by treatment with increasing concentrations of bile salt. Blood samples were centrifuged at 2500 *g* for 10 min and the plasma and buffy coat removed by aspiration. The packed erythrocytes were washed with 3 × 5 vol of 0.154 M NaCl, 1.5 mM Hepes [4-(2-hydroxyethyl)-1-piperazine-ethanesulphonic acid], pH 7.4 and resuspended in an equal volume of this buffer to give an approximate phospholipid concentration of 2 mM. One volume of the erythrocyte suspension was incubated at 37° for 10 min with 3 vol of 0.14 M NaCl, 15 mM Hepes, pH 7.4 containing appropriate concentrations of either bile salt. Incubations were terminated by centrifugation at 14,000 *g* for 1 min and the percentage haemolysis was determined by comparing the A_{525} of appropriate dilutions of the supernatant (with water) with that of an uncentrifuged incubation completely haemolysed by dilution with 24 vol of water.

Phospholipid release was determined by assaying lipid extracts [23] of supernatants for phospholipid phosphorus essentially by the method of Bartlett [24], except that samples were digested with 72% (v/v) $HClO_4$. Acetylcholinesterase was assayed at 20° as described previously [25].

Enzyme assays. Alkaline phosphatase (E.C. 3.1.3.1), alkaline phosphodiesterase I (E.C. 3.1.4.1), 5′-nucleotidase (E.C. 3.1.5.1), L-leucine-β-naphthylamidase (E.C. 3.4.11.1) and lactate dehydrogenase (E.C. 1.1.1.27) were assayed in bile and liver homogenates from the same animals as described previously [10]. All enzyme assays were performed at 37° except for lactate dehydrogenase which was at 20°

Protein was determined by the method of Lowry *et al.* [26] with bovine serum albumin as standard. Total biliary bile salt concentrations were determined using 3α-hydroxysteroid dehydrogenase (E.C. 1.1.1.50) [10].

Gas liquid chromatography of biliary bile salts. The bile acids were analysed as trifluoroacetate derivatives of their methyl esters essentially as described by Klaassen [27]. Bile (0.1 ml, approximately 3 μmoles of total bile salts) was incubated at 37° for 1 hr in a final volume of 1 ml of 0.2 M phosphate buffer, pH 5.8 containing 2.4 U of cholyglycine hydrolase (E.C. 3.5.1.24). After acidification with 1 ml of 6 M HCl, the free bile acids were recovered by extracing with 2 × 5 ml of diethyl ether. The combined extracts were evaporated to dryness and the bile acids were methylated with ethereal diazomethane. Trifluoroacetates of the bile acid methyl esters were prepared by addition of 0.2 ml of trifluoroacetic anhydride; the tubes were capped and incubated at 37° for 30 min. Following evaporation to dryness, the residue was redissolved in 200 μl of ethyl acetate prior to injection onto the column.

A Hewlett-Packard 5720A gas chromatograph equipped with a flame ionisation detector was used. The column (2.1 m × 4 mm) was 3% QF-1 on chromosorb W HP (80–100 mesh). Operating conditions were as follows: injection temperature, 280°; detector temperature, 295°; column temperature, 240°; carrier gas (N_2) flow rate, 50 ml/min. Peaks were identified by reference to known standards and quantified by triangulation.

Polyacrylamide gel electrophoresis of bile proteins. Prior to electrophoresis, biliary proteins were delipidated since this gave better resolution of the polypeptide bands. Protein was precipitated from bile by addition of 4 vol of ethanol:diethylether (3:1). The precipitate was washed twice with ethanol, dried under N_2 and dissolved at 37° overnight in 8 M urea, 4% (w/v) sodium dodecylsulphate, 1% (w/v) β-mercaptoethanol, 10 mM phosphate, pH 7.0 containing 0.1% (w/v) bromophenyl blue as tracking dye. Samples (equivalent to approximately 100 μg of biliary protein) were electrophoresed for 5 hr at 80 mA in flat sheets of 7.25% (w/v) polyacrylamide equilibrated in 0.1 M phosphate buffer, pH 7.0 containing 0.1% (w/v) sodium dodecyl sulphate. Bovine serum albumin, pepsin and trypsin were used to calibrate the gels according to molecular weight. Gels were stained with Coomassie Blue and traced

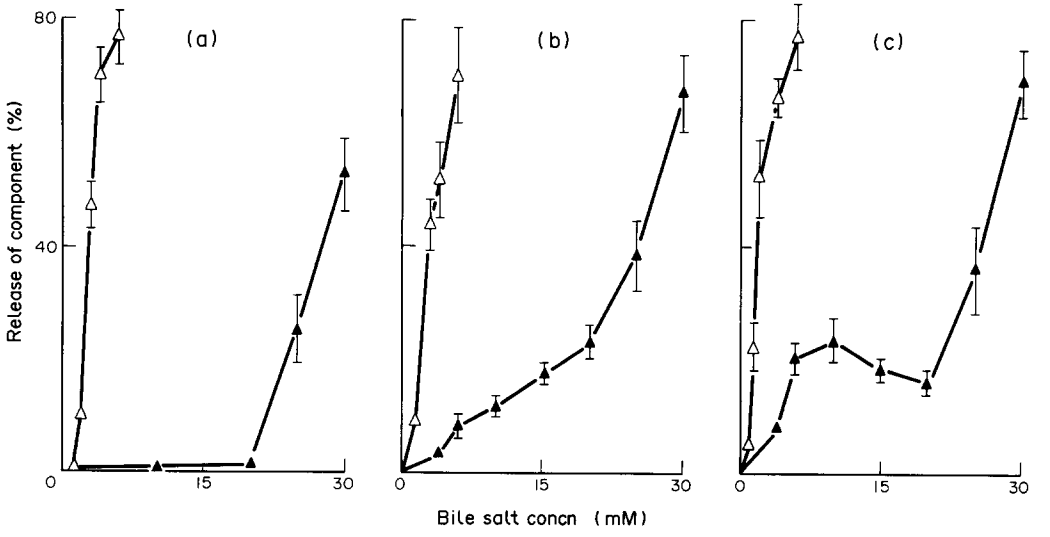

Fig. 1. The release of (a) haemoglobin, (b) phospholipid and (c) acetylcholinesterase from human erythrocytes by glycocholate and glycochenodeoxycholate. Human erythrocytes were incubated with glycocholate (▲) or glycochenodeoxycholate (△) at 37° for 10 min. Values are means for 5 observations ± S.E.M.

by densitometry at 570 nm using a Beckman DU8 spectrophotometer.

RESULTS

Membrane-damaging properties of glycochenodeoxycholate and glycocholate. Three parameters, namely erythrocyte lysis and the release of membrane phospholipid and acetylcholinesterase, were used to assess the membrane-damaging properties of the bile salts. In all cases, glycochenodeoxycholate caused extensive membrane damage at approximately 10-fold lower concentrations than glycocholate. Thus, glycochenodeoxycholate caused extensive lysis of human erythrocytes at approximately 2–3 mM whilst glycocholate concentrations in excess of 20 mM were required to produce the same effect (Fig. 1a). Similarly, glycochenodeoxycholate released significant amounts of membrane phospholipid (Fig. 1b) and acetylcholinesterase (Fig. 1c) at approximately 10-fold lower concentrations than glycocholate.

Biliary output of protein, phospholipid and bile salts and bile flow rate. Both doses of chenodeoxycholate had no significant effect upon the total protein, bile salt or phospholipid concentration of 0–2 hr bile (Table 1). Although a small decrease in bile flow rate was observed at the lower dose of 90 mg/kg per day, this was not seen at the higher dose of 300 mg/kg per day (Table 1), suggesting that chenodeoxycholate-feeding does not appreciably affect bile flow rate. We can offer no explanation for this apparent decrease in bile flow at the lower dose of 90 mg/kg per day. Although a linear relationship exists between bile salt secretion and bile flow [28], no increase was observed in the bile salt concentration of bile at this dose.

In experiments where bile was collected for 30 hr, the fall in bile salt output due to interruption of the enterohepatic circulation was slower in rats fed chenodeoxycholate (300 mg/kg per day) than in control rats (Fig. 2). It is possible to estimate the bile salt pool size from the data in Fig. 2 as the total bile salt output from the time of cannulation until the lowest output minus the contribution due to basal hepatic synthesis [29]. Assuming the lowest output

Table 1. Effect of chenodeyxocholate feeding on total biliary output

	Control	90 mg/kg per day	300 mg/kg per day
Protein (mg/ml)	11.3 ± 1.9 (13)	12.6 ± 2.5 (9)	10.7 ± 2.3 (11)
Bile salts (mM)	28.7 ± 2.1 (9)	28.1 ± 2.1 (7)	32.2 ± 4.4 (7)
Phospholipid (mM)	4.16 ± 0.41 (6)	3.99 ± 0.51 (4)	4.32 ± 1.06 (4)
Bile flow rate (ml/hr)	1.11 ± 0.09 (10)	0.83 ± 0.04* (7)	1.10 ± 0.10 (9)

Bile was collected for 2 hr from control and chenodeoxycholate-fed rats.
Values are means ± S.E.M. for the number of observations given in parentheses.
Significant differences from controls ($P < 0.05$) are indicated by *.

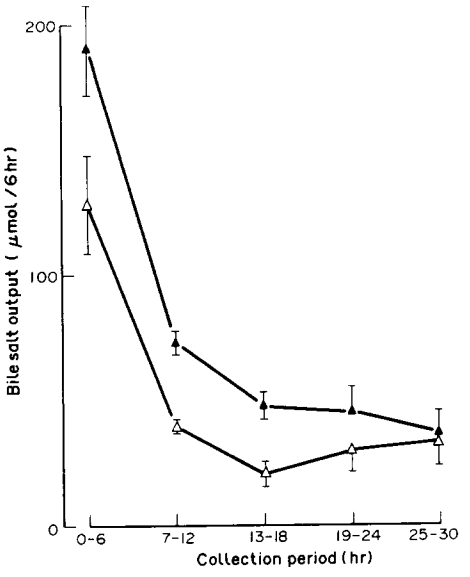

Fig. 2. Biliary bile salt output in control and chenodeoxycholate-fed rats. Bile was collected from control (△) and chenodeoxycholate-fed (▲) rats (300 mg/kg per day) in 6 hr aliquots for 30 hr. Values are means for 3 observations ± S.E.M.

to be in the 13–18 hr fraction, it can be calculated that the bile salt pool in control rats was 50.7 μmoles/100 g body wt and in rats fed chenodeoxycholate was 67.3 μmoles/100 g body wt. Thus, in rats fed chenodeoxycholate, the bile salt pool was increased by approximately one-third.

Biliary bile acid composition. Administration of chenodeoxycholate at 300 mg/kg per day doubled the proportion of chenodeoxycholate in bile such that it became the major bile acid; this occurred at the expense of the other primary bile acid, cholate (Table 2). (Although our GLC system failed to separate cholate and β-muricholate, cholate occurs in rat bile at approximately 10-fold higher concentrations than β-muricholate [30], and therefore, it is unlikely that our combined peak consists almost entirely of cholate). Ursodeoxycholate was only present in control bile in trace amounts (<1%) but represented 4% of the bile acid pool of chenodeoxycholate-fed rats. The proportion of lithocholate was approximately doubled whilst deoxycholate was unaffected by chenodeoxycholate-feeding.

These effects were produced in a dose-dependent relationship in that similar, but less striking changes in biliary bile acid composition were produced by the lower dose of 90 mg/kg per day (Table 2).

Biliary output and hepatic activities of plasma membrane enzymes. The activities of some plasma membrane marker enzymes in bile from chenodeoxycholate-fed rats were markedly increased (Table 3). Alkaline phosphatase and 5′-nucleotidase were increased 3-fold in rats given 300 mg/kg per day; 5′-nucleotidase was also significantly increased in rats given the lower dose of 90 mg/kg per day. In contrast, alkaline phosphodiesterase I activity in bile was unaffected whilst a small but insignificant increase was seen with L-leucine-β-naphthylamidase. Lactate dehydrogenase was absent from the biles of both control and chenodeoxycholate-fed rats (results not shown), showing that plasma membrane enzymes were released into bile in the absence of gross cellular damage.

Alkaline phosphatase and 5′-nucleotidase activities were also increased in the liver of rats fed chenodeoxycholate at the higher dose of 300 mg/kg per day (Table 3). The increase in hepatic alkaline phosphatase mirrored that of the biliary enzyme; thus, its activity in liver was increased by approximately 3-fold. However, hepatic 5′-nucleotidase was increased by only 60% compared to a 3-fold enrichment in its biliary output. Hepatic alkaline phosphodiesterase I and L-leucine-β-naphthylamidase were unaffected by chenodeoxycholate feeding.

Centrifugation of bile. Approximately 20% of the biliary activity of the four plasma membrane marker enzymes studied were sedimented on the centrifugation of bile from control rats (Table 4). It is possible that this represents small plasma membrane fragments or vesicles; indeed, it has been suggested that some plasma membrane material may be released into bile in the form of vesicles from the microvilli of the bile canaliculus [22]. On centrifugation of bile from rats fed chenodeoxycholate at 300 mg/kg per day, significantly less of the plasma membrane enzymes were sedimented; in fact, only L-leucine-β-naphthylamidase was sedimented to any appreciable extent (Table 4).

Polyacrylamide gel electrophoresis of bile proteins.

Table 2. Biliary bile acid composition of control and chenodeoxycholate-fed rats

	Control (N = 8)	90 mg/kg per day (N = 4)	300 mg/kg per day (N = 7)
Lithocholate	2.9 ± 0.3	3.5 ± 0.1	5.1 ± 0.5***
Deoxycholate	4.3 ± 1.0	5.3 ± 0.4	4.0 ± 0.6
Chenodeoxycholate	37.4 ± 2.6	50.4 ± 4.6*	77.0 ± 2.3***
Ursodeoxycholate	trace	1.9 ± 0.3	4.2 ± 0.9
α-Muricholate	1.9 ± 0.3	4.3 ± 0.6**	3.2 ± 0.8
Cholate + β-muricholate	53.5 ± 3.4	34.4 ± 4.3**	6.5 ± 1.2***

Bile was collected for 2 hr from control and chenodeoxycholate-fed rats.

Values presented are the amounts of individual bile acids expressed as a percentage of the total. Values are means ± S.E.M. for the number of observations given; significant differences from controls are indicated by * ($P < 0.05$), ** ($P < 0.01$) and *** ($P < 0.001$).

Table 3. Plasma membrane enzymes in bile and liver from chenodeoxycholate-fed rats

	Activity in bile (μmole/hr/ml of bile)			Activity in liver (μmole/hr/mg of protein)		
	Control	90 mg/kg per day	300 mg/kg per day	Control	90 mg/kg per day	300 mg/kg per day
Alkaline phosphatase	0.126 ± 0.011	0.160 ± 0.011	0.366 ± 0.049*	0.027 ± 0.002	0.031 ± 0.004	0.075 ± 0.015*
Alkaline phospodiesterase I	3.19 ± 0.41	2.97 ± 0.17	2.93 ± 0.45	4.32 ± 0.16	4.33 ± 0.12	3.95 ± 0.38
L-Leucine-β-naphthylamidase	0.42 ± 0.17	0.55 ± 0.06	0.55 ± 0.08	0.41 ± 0.02	0.43 ± 0.03	0.37 ± 0.04
5'-Nucleotidase	2.46 ± 0.26	5.28 ± 0.43*	6.87 ± 0.67*	0.53 ± 0.08	0.75 ± 0.11	0.84 ± 0.10*

Enzyme activities were assayed in bile collected for 2 hr and in liver homogenates (10% w/v in 1 mM $NaHCO_3$, pH 7.4) from control and chenodeoxycholate-fed rats. Values are means ± S.E.M. for 6–10 observations. Significant differences from controls ($P < 0.05$) are indicated by*.

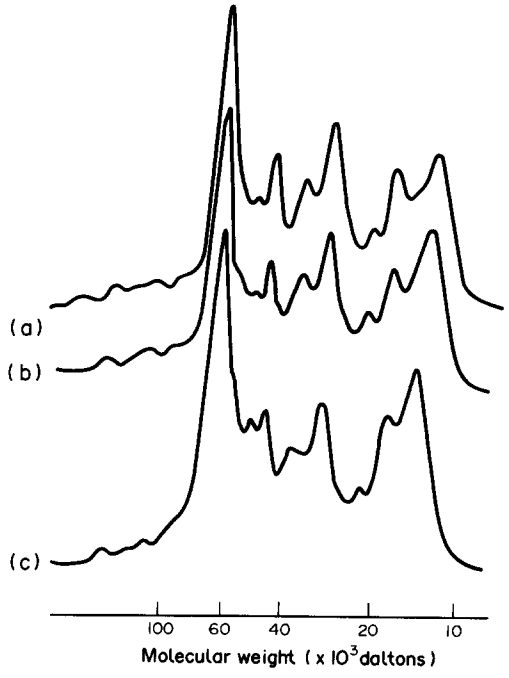

Fig. 3. Polyacrylamide gel electrophoresis of rat bile proteins. Bile was collected from control and chenodeoxycholate-fed rats for 2 hr: (a) control, (b) 90 mg/kg per day, (c) 300 mg/kg per day. Typical densitometric scans from 4 experiments are shown.

Figure 3 shows typical densitometric scans of bile proteins from control and chenodeoxycholate-fed rats. Visual inspection indicates that chenodeoxycholate feeding caused no major alteration of the polypeptide profile; this was confirmed by quantification of peak areas (results not shown). Polymeric IgA occurs in high concentrations in rat bile [31] and the most densely staining band at molecular weight approximately 60,000 probably corresponds to IgA heavy chains (reported molecular weight: 64,000).

DISCUSSION

Chenodeoxycholate is used as an effective means of radiolucent gallstone dissolution *in vivo* (see introduction for references). It is conjugated to either glycine or taurine in the liver and secreted into bile such that it becomes the major biliary bile acid. Chenodeoxycholate reduces the cholesterol saturation of supersaturated bile, allowing cholesterol gallstones to redissolve. Its principle action is to suppress the activity of HMG-CoA reductase, the rate limiting enzyme of cholesterol biosynthesis [32, 33], thereby decreasing cholesterol secretion into bile. At doses of 1 g/day or greater, chenodeoxycholate also expands the bile salt pool size in man [34]. However, it is now generally agreed that the efficacy of chenodeoxycholate is related to its effects on biliary cholesterol secretion, rather than to any effects on biliary bile salt or phospholipid secretion [35].

Chenodeoxycholate feeding caused essentially similar changes in the biliary bile acid composition

Table 4. Centrifugation of bile

	Alkaline phosphatase	Alkaline phosphodiesterase I	L-Leucine-β-naphthylamidase	5′-Nucleotidase
Control	82.4 ± 8.0	80.8 ± 4.3	77.8 ± 4.4	86.3 ± 6.5
Chenodeoxycholate-fed	94.6 ± 2.7	102.2 ± 3.4	83.2 ± 7.4	99.7 ± 5.7

Bile was collected for 6 hr from control and chenodeoxycholate-fed (300 mg/kg per day) rats. Aliquots of bile were centrifuged at 4° at 150,000 *g* for 60 min. Values presented are the activity remaining in the supernatant expressed as a percentage of the total activity in the original, uncentrifuged bile sample; values are means ± S.E.M. for 3 observations.

to that seen in man. At 300 mg/kg per day, chenodeoxycholate feeding increased the proportion of this bile acid to approximately 75%; in man, the proportion of chenodeoxycholate in bile has been reported to increase in direct proportion to dose until it amounts to 75–90% of biliary bile acids [6, 7]. This chenodeoxycholate enrichment of the biliary bile acids occurred almost entirely at the expense of cholate. Whilst only trace amounts of ursodeoxycholate (the 7β-epimer of chenodeoxycholate) were detected in control bile, this bile acid occurred in appreciable quantities in chenodeoxycholate-fed rats; Froom *et al.* [36] have shown that ursodeoxycholate is formed by bacterial oxidation of chenodeoxycholate in the intestine to 3-hydroxy-7-ketocholanic acid, which is then reduced in the liver to both chenodeoxycholate and ursodeoxycholate. The increase in lithocholate was similar to that reported in man [34] and much less than that seen in rhesus monkeys (10–15% of total bile acids at doses of 100 mg/kg per day [37, 38]). Chenodeoxycholate is known to be hepatotoxic in rhesus monkeys owing to impaired lithocholate sulphation [39], allowing lithocholate to accumulate and exert its toxic effects [40].

In earlier model experiments, both phospholipid [10] and protein [12] were shown to protect intact cells against bile salt induced enzyme release. However, since biliary phospholipid and protein were unaffected by chenodeoxycholate feeding (Table 1), it is doubtful if this could explain the increased biliary output of alkaline phosphatase and 5′-nucleotidase. Similarly, the increased biliary output of these enzymes cannot be explained by changes in the total bile salt concentration of bile (Table 1). The most plausible explanation for the increased biliary output of alkaline phosphatase and 5′-nucleotidase lies in the chenodeoxycholate-induced shift of the bile acid profile away from one containing substantial amounts of trihydroxy bile acids to one containing almost entirely chenodeoxycholate (a dihydroxy bile acid). Since chenodeoxycholate conjugates cause extensive membrane damage at approximately 10-fold lower concentrations than those of cholate (see Fig. 1), this has produced a more membrane-damaging bile which has manifested itself in the increased release of alkaline phosphatase and 5′-nucleotidase from the canalicular membrane. This in turn is probably related to the physicochemical properties of the major bile salts. Chenodeoxycholate conjugates have a critical micellar concentration in isotonic saline of approximately 3.5 mM whilst the critical micellar concentration for cholate conjugates is approximately 8.5 mM (Stenhouse and Billington, unpublished work). It is possible, therefore, that biliary enzyme output is inversely related to the critical micellar concentration of the major bile salts in bile. In support of this, glycodeoxycholate and taurochenodeoxycholate, when added to the perfusion medium of isolated rat livers, stimulated the biliary output of plasma membrane enzymes at lower concentrations than taurocholate, whilst the non-micelle forming bile salt analogue, taurodehydrocholate, failed to stimulate biliary enzyme output [41, 42]. An increased biliary excretion of alkaline phosphatase has also been reported by Hatoff and Hardison [43] on infusion of bile salts into rats. In addition, infusion of the chromone, 6,8-diethyl-5-hydroxy-4-oxo-4H-1-benzopyran-2-carboxylic acid, a molecule possessing detergent activity which is excreted via bile, increased the biliary output of alkaline phosphatase and 5′-nucleotidase in dogs [44]. Thus, although the quantitatively major proportion of biliary protein arises as a result of specific transport mechanisms via endocytic vesicles [31], our results lend further support to the suggestion that bile salts are instrumental in the release of plasma membrane enzymes into bile [10, 14].

The biliary output of alkaline phosphodiesterase I and L-leucine-β-naphthylamidase was unaffected by chenodeoxycholate feeding. This is surprising because these enzymes, like alkaline phosphatase and 5′-nucleotidase, are thought to be intrinsic, ecto-orientated proteins of the hepatocyte plasma membrane [45]. However, it is noteworthy that in control animals, the ratio of the biliary activity of alkaline phosphatase and 5′-nucleotidase to that in liver is approximately 5-fold higher than the corresponding ratio for alkaline phosphodiesterase I and L-leucine-β-naphthylamidase (see Table 3). It is possible, therefore, that alkaline phosphodiesterase I and L-leucine-β-naphthylamidase are embedded in the membrane in such a manner as to render them less accessible to detergent extraction than alkaline phosphatase and 5′-nucleotidase.

In addition to increasing the biliary output of alkaline phosphatase and 5′-nucleotidase, chenodeoxycholate feeding increased the activity of these enzymes in liver. Hepatic alkaline phosphatase is induced several-fold in experimental cholestasis [46] and this has been attributed to bile salts since their concentration within cholestatic liver increases approximately 5-fold [47] and bile salts induce alkaline phosphatase in cultured rat hepatocytes [48]. However, it is doubtful whether the small increase in the bile salt pool can totally explain the increase in hepatic alkaline phosphatase and 5′-nucleotidase

on chenodeoxycholate feeding. An alternative explanation for the increased hepatic activity of these enzymes is that it simply represents increased biosynthetic repair to the canalicular membrane in the face of increased biliary output. This is in agreement with Coleman and his co-workers [10, 14] who proposed that following bile salt-induced release of membrane components into bile, the canalicular membrane is maintained by continuous biosynthetic repair.

Godfrey *et al.* [22] have reported that substantial amounts of alkaline phosphodiesterase I and 5'-nucleotidase are sedimented on centrifugation of rat bile collected between 0 and 10 hr. These authors have suggested that this sedimented material represents small vesicles pinched off the tips of the microvilli of the bile canaliculus and that this occurs at low bile salt concentrations [see 25]. The results presented in Table 4 agree with those of Godfrey *et al.* [22] in that approximately 20% of all plasma membrane enzymes studied were sedimented on centrifugation of control bile collected between 0 and 6 hr. However, on centrifugation of bile collected over a similar time period from rats fed chenodeoxycholate, significantly less of all plasma membrane enzymes were sedimented. This can be explained in terms of the enrichment of chenodeoxycholate in the bile of chenodeoxycholate-fed rats. Since chenodeoxycholate conjugates are more membrane damaging than those of cholate, a bile rich in chenodeoxycholate would be expected to be more effective in "solubilising" particulate material than a bile rich in cholate. In agreement with this, Wenham *et al.* [49] have reported that the addition of up to 10 mM glycochenodeoxycholate to human "T"-tube bile increased the recovery of alkaline phosphatase in the supernatant after centrifugation at 150,000 *g* for 60 min.

No evidence of extensive damage to the membranes of the hepatobiliary system was detected. Thus, plasma membrane enzymes were released into the bile of control and chenodeoxycholate-fed rats in the absence of the cytosolic marker enzyme lactate dehydrogenase. In addition, chenodeoxycholate feeding had no effect on the total protein content or the polypeptide profile of bile. Thus, whilst chenodeoxycholate and its conjugates are potentially toxic to the membranes of the hepatobiliary system (as judged from model experiments using human erythrocytes and perfused rat liver [42]), it appears to be well tolerated *in vivo* and only manifests itself in the increased biliary output of some plasma membrane enzymes.

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