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Control of biliary phospholipid secretion

Effect of continuous and discontinuous infusion of taurocholate on biliary phospholipid secretion

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A major determinant of biliary lipid secretion is bile-salt secretion. Taurocholate (TC), a micelle-forming bile salt, was infused continuously at different rates in both isolated perfused livers and biliary-fistula rats. In both of these systems, infusion of TC brought about an elevated secretion of phosphatidylcholine for the duration of the TC infusion period. Initial phospholipid/bile-salt ratios in the bile were higher in the whole-animal model than in isolated livers, but at the higher infusion rates both secreted approx. 6 mol of phospholipid for every 100 mol of bile salt. The secretion of phospholipid, which was maintained even at high rates of bile-salt infusion, suggest a continuous and regulated phospholipid supply and secretion mechanism. In contrast, however, multiple short pulses of TC to the perfused liver, which brought about relatively equal biliary bile-salt output pulses, did not bring about equal phospholipid outputs, since the phospholipid peak size declined with each bile-salt pulse. These experiments taken together suggest either that a threshold (intracellular) bile-salt concentration may be required to 'switch-on' the phospholipid supply and that it may need to be maintained for continuous bilary phospholipid supply to the canalicular membrane.

INTRODUCTION

Phospholipids are important components of most animal biles (Coleman *et al.*, 1979), and abnormalities in the amount or concentration of phospholipids may contribute to the aetiology of cholesterol gallstones (Heuman *et al.*, 1980; Ahlberg *et al.*, 1981). Relatively more interest has been shown in the processes which control bile-salt and cholesterol secretion than in those which control phospholipid secretion into bile.

At physiological rates of output, the secretion of phospholipid into bile depends on the secretion of bile salts (Wheeler, 1972; Hardison & Apter, 1972; references cited in Lowe *et al.* 1984). The characteristics of bile-salt-associated lipid secretion are related to the physical (detergent) properties of the bile salt; non-micelle-forming bile-salt analogues do not stimulate biliary lipid secretion (Hardison & Apter, 1972; Barnwell *et al.*, 1984).

In bile, biliary lipids are often associated with bile salts as mixed micelles; increasing the output of bile salts into bile thus results in an increase in its content of mixed micelles. The site of interaction of bile salts with lipids to generate the mixed micelles has been proposed variously as (i) within the cell (see Forker, 1977; Reuben *et al.*, 1982), (ii) during passage of the bile salt through the canaliculus membrane (Small, 1970) and (iii) subsequent to the entry of the bile salt into the bile canaliculus (Coleman *et al.*, 1977). Rapid kinetic studies of the sequence of appearance of materials into rat bile have shown that bile salts are secreted before phospholipids and cholesterol (Lowe *et al.*, 1984). Mixed-micelle formation thus occurs subsequent to the entry of bile salts into the canaliculus, the third of the above mechanisms. Coleman *et al.* (1977) have suggested that the biliary lipids are solubilized from the membrane of the bile canaliculus by the detergent action of the bile salts, either directly or subsequent to the shedding of small vesicles, probably from specific regions of the membrane enriched in biliary lipid (Lowe *et al.*, 1984). In order that the canaliculus membrane be kept in a state of good repair, a continuous supply of lipid would then be required (Coleman *et al.*, 1977). Colchicine, a drug that affects microtubular function and vesicle movement within cells, reduces the biliary output of phospholipid and cholesterol into bile to a much greater extent than it reduces bile-salt output (Barnwell *et al.*, 1984); this has led to the suggestion that bile salts are transported across the hepatocyte as discrete molecules, but that lipid is supplied to the membrane in vesicular form and it is this process that is interfered with by colchicine (Barnwell *et al.*, 1984; Lowe *et al.*, 1984).

The present experiments further investigate the supply of phospholipid to support the biliary outflow, with a view to gaining a better understanding of the control of this lipid supply. These studies have included both continuous bile-salt secretion of increasing intensity, in an attempt to stress and saturate the system, and also short-term pulse experiments to determine the characteristics of the phospholipid output after the bile-salt output. These studies have used both the biliary-fistula rat, in which lipid can be supplied to the liver by the blood, and the isolated perfused liver, which has no access to circulating plasma lipoproteins.

Abbreviation used: TC, taurocholate.

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MATERIALS AND METHODS

Materials

The following fine chemicals were used: taurocholate (TC) (Calbiochem–Behring Corp., Bishops Stortford, Herts., U.K. > 95% pure), heparin (C.P. Pharmaceuticals, London EC1, U.K.), Sagatal (May and Baker, Dagenham, Essex, U.K.). All other reagents were from Fisons Ltd., Loughborough, Leics., U.K., and were of the highest grade available. Plastic cannula tubing (PP10, PP30) was obtained from Portex, Hythe, Kent, U.K.

Isolated perfused livers: recycling perfusion

Male Wistar rats, weighing 310–340 g, were used throughout. Before the experiments, animals were maintained on a standard laboratory diet in a constant-temperature environment (22 °C) and under a constant 12 h-light/12 h-dark cycle. All bile-duct cannulations were performed with 500 mm of PP10 tubing between 09:00 and 12:00 h while the animals were under pentobarbitone (Sagatal) anaesthesia; their livers were isolated *in situ* (Hems *et al.*, 1966). Liver anoxia was minimized (5–10 s) by commencing recycling perfusion immediately at a constant flow rate of 16 ml·min^{-1} with 150 ml of Krebs–Ringer bicarbonate buffer, pH 7.4 (Krebs & Henseleit, 1932); this buffer also contained 2 mM-$CaCl_2$, 5 mM-glucose, 1% (w/v) bovine serum albumin, a physiological amino acid mixture (see Barnwell *et al.*, 1983) and 40% (v/v) of packed human red blood cells (see Lowe *et al.*, 1984). This solution was recycled, gassed continuously with O_2/CO_2 (19:1) and maintained at 37±0.5 °C within a thermostatically controlled cabinet similar to that recommended by Collins & Skibba (1980). For the initial 90 min of the perfusion, the isolated liver received bile-salt-free perfusion medium; during this period the output of both bile salts and lipids declined to the levels indicated by the 0 min in Figs. 1(*a*)–(*d*) (below). The perfusion medium was then changed to fresh medium to replace substrates used by the liver. At the same time, infusion of TC (dissolved in red-cell-free and albumin-free perfusion medium) into the perfusion line was initiated at a rate of 15 ml/h and continued for 90 min. TC infusion rates were in the range 1–4 μmol/min per rat, equivalent to 3.13–12.5 μmol/min per kg body wt. Bile was collected in preweighed tubes at 5 min intervals during the 0–20 min period after commencing TC infusion and then at 10 min intervals for the 20–90 min period. The volumes of bile samples were determined gravimetrically by assuming a density of 1 g·ml^{-1}; the bile was stored at −20 °C until required for analysis.

Isolated perfused livers: 'single pass' short-pulse experiments

The livers were isolated and initially perfused as described above. For the first 90 min of the perfusion the isolated liver received bile-salt-free perfusion medium; during this period the output of bile salts and lipids declined to the levels indicated by the 0–5 min sample in Fig. 3 (below). After 90 min the liver was supplied with fresh perfusion medium, a conversion made to a 'single pass' perfusion, and a basal bile sample collected (0–5 min); then 5000 nmol of TC (20 μmol/kg body wt.), containing 0.1 μCi of [^{14}C]TC, were infused into the hepatic portal cannula in 60 s. Collections were made at 1 min intervals in preweighed tubes. Subsequently a second and third pulse of TC was injected at 15 min and 25 min and bile collected on ice and frozen at −20 °C until required for analysis. The concentration of TC in the 1 min samples was determined by comparing the radioactivity in the bile with the specific radioactivity of the TC injected into the liver.

Bile-fistula rat

In these experiments male Sprague–Dawley rats weighing 250±20 g were used throughout. All surgical procedures were performed while the rats were anaesthetized with pentobarbitone (Sagatal) diluted in 0.9% NaCl to 20 mg·ml^{-1} and injected intravenously via the tail vein at a dose volume of 3–4 ml/kg body wt.

TC dissolved in saline was infused via a femoral vein cannulated with PP10 tubing. The bile duct was also cannulated with PP10 tubing; this operation was performed as the last of the series. Bile was collected on ice and stored at −20 °C until required for analysis. TC was continuously infused at doses between 0 and 15 μmol/min per kg body wt. in a saline volume of 2 ml/h. The animals were maintained under anaesthesia throughout the experiment and killed at the end of the experiment by intravenous injection of pentobarbitone.

Assays

Total bile salts were assayed with 3α-hydroxysteroid dehydrogenase (EC 1.1.1.50) (Coleman *et al.*, 1979). In the short-pulse experiments, bile salts were assayed by comparing the radioactivity in the bile with the specific radioactivity of the TC injected into the liver.

Phospholipid present in the bile was determined by the method of Bartlett (1959) after lipid extraction by the method of Bligh & Dyer (1959).

Phospholipid t.l.c. was performed by the method of Skipski *et al.* (1964) on lipid extracts prepared by the method of Bligh & Dyer (1959). The chromatography was performed on 170 nmol portions of phospholipid.

RESULTS

The effect of TC on biliary lipid secretion was studied by infusing TC in isolated perfused livers and biliary-fistula rats. The range of TC infusion varied from 3 to 20 μmol/min per kg body wt.

Isolated perfused livers: continuous perfusion

The effect of continuous TC infusion into the isolated perfused rat liver system is shown in Fig. 1. The rate of biliary secretion of phospholipid and bile salt increased with increasing rates of taurocholate infusion (Figs. 1*a*–1*d*). A plateau was reached for phospholipid and bile-salt secretion rate when TC was infused at 3.1, 4.7 and 6.3 μmol/min per kg body wt. respectively; maximum phospholipid response was observed at 4.7 and 6.3 μmol/min per kg body wt. (Figs. 1*a*–1*c*). When TC was infused at 12.5 μmol/min per kg body wt., both the phospholipid and bile-salt secretion rate reached a maximum and then declined (Fig. 1*d*). Thus the maximum rate of output of bile salt under steady-state conditions was 7.5 μmol/min per kg body wt. (Fig. 1*c*). At the higher rate of TC infusion this maximum secretion rate was also achieved (Fig. 1*d*); the secretion rate subsequently declined (Fig. 1*d*), and this paralleled a

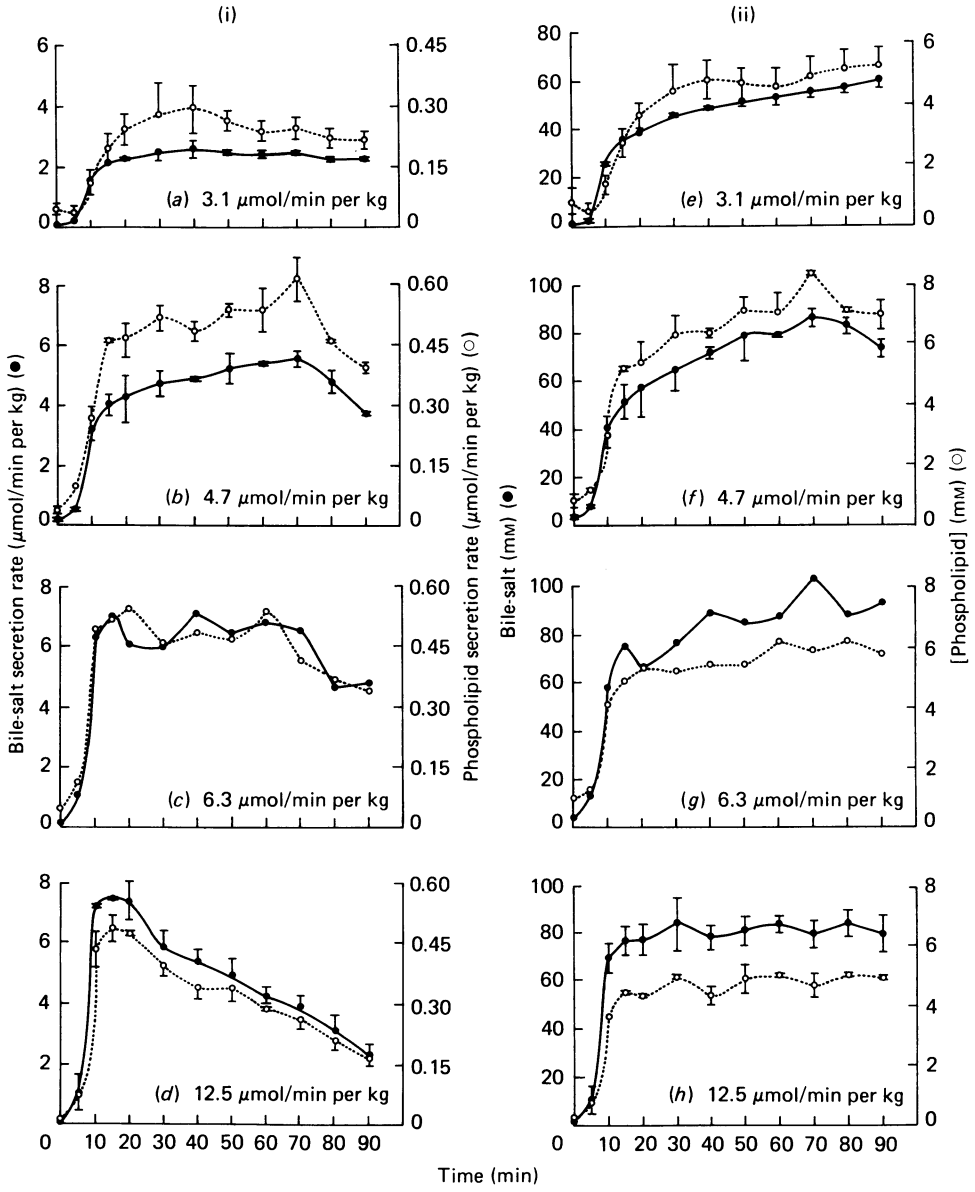

Fig. 1. Effect of TC infusion on the biliary output (i) and concentration (ii) of phospholipid (○) and bile salt (●) in the isolated liver

Liver isolation, perfusion, TC infusion and bile collection are described in the Materials and methods section. Values at 0 min indicate the basal biliary levels of phospholipid and bile salt (after 90 min liver isolation) before commencing the TC infusion at $t = 0$. TC was infused at four different rates: 3.1 (*a*,*e*), 4.7 (*b*,*f*), 6.3 (*c*,*g*) and 12.5 (*d*,*h*) μmol/min per kg body wt. respectively. For '3.1 μmol/min per kg body wt.', values represent means ± S.E.M., $n = 3$; for '4.7' and '12.5 μmol/min per kg body wt.', values represent means ± range, $n = 2$; for '6.3 μmol/min per kg body wt.', $n = 1$.

progressive decline in bile flow (results not shown). The maximum rate of phospholipid secretion under steady-state conditions was 0.48–0.50 μmol/min per kg/body wt. (Figs. 1*b*, 1*c* and 1*d*), and it appears, therefore, that the isolated livers are capable of supplying lipid to the canalicular membrane in response even to high inputs of bile salts.

The concentration of biliary bile salt achieved under steady-state conditions was 80–90 mM (Figs. 1*f* and 1*g*); similar concentrations were achieved during the subsequent fall in secretion rate (Fig. 1*h*). The biliary phospholipid concentration under steady-state conditions was 5–7 mM (Figs. 1*f* and 1*g*) and approx. 5 mM (Fig. 1*h*) as the secretion rate declined (Fig. 1*d*). Virtually all (90–95%) the phospholipid present was identified as phosphatidylcholine by t.l.c. against authentic standards (results not shown).

Biliary-fistula rat: continuous infusion

The effect of infusing TC in the biliary-fistula rat on the biliary phospholipids is shown in Fig. 2. As in the isolated liver, the rate of biliary bile salt and phospholipid output increased with increasing TC infusion rates (Figs. 2*a*–2c). Under steady-state conditions the maximum rate of bile-salt secretion was 6.1 μmol/min per kg body wt. (Fig. 2*b*). At a TC infusion rate of 15 μmol/min per kg, the bile-salt secretion rate rose to 9.4 μmol/min per kg body wt. (Fig. 2*c*), but then subsequently fell (Fig. 2*c*). The

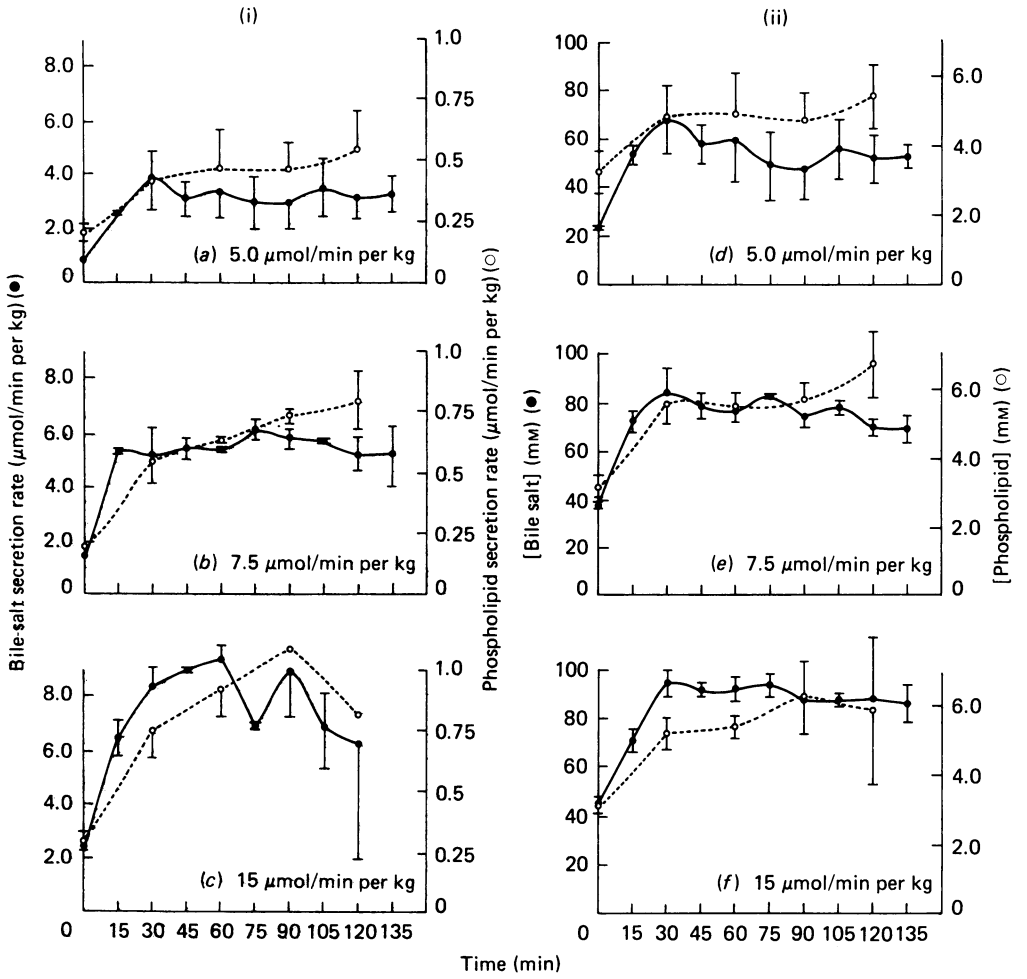

Fig. 2. Effect of TC infusion on the biliary output (i) and concentration (ii) of phospholipid (○) and bile salt (●) in the biliary-fistula rat

TC infusion and bile collection were performed as described in the Materials and methods section. Values at 0 min represent the biliary values of phospholipid and bile-salt pre-infused with TC. TC was infused at three different rates: 5.0 (*a*,*d*), 7.5 (*b*,*e*) and 15.0 (*c*,*f*) μmol/min per kg body wt. respectively. Values are means ± S.E.M. ($n = 3$).

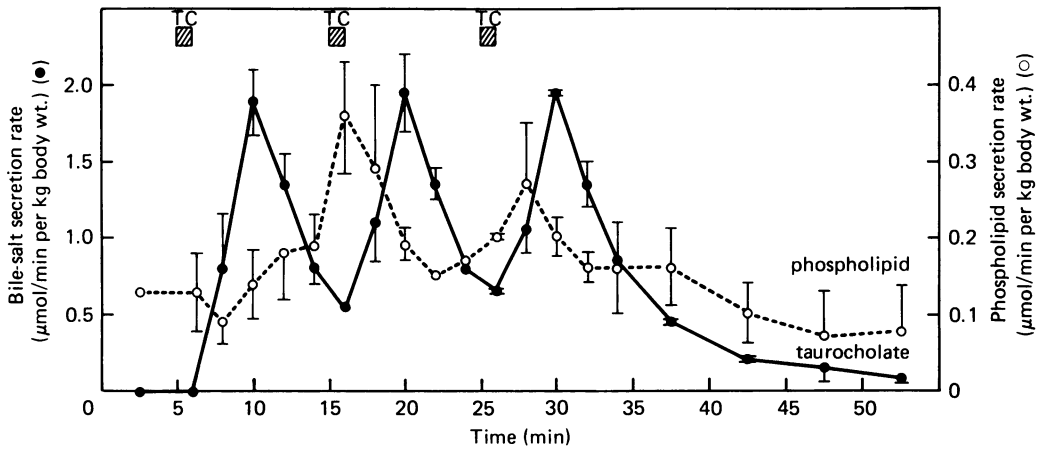

Fig. 3. Effect of multiple TC pulses on the biliary secretion of phospholipid and bile salt in the isolated liver

For details of liver isolation, perfusion and bile collections, see the Materials and methods section. Values at 2½ min indicate the basal levels of phospholipid and bile-salt output after 90 min of liver isolation. The hatched square (▨) indicates the duration of the TC infusion pulse (20 μmol/kg body wt.). Symbols: ●, bile-salt output (left axis); ○, phospholipid output (right axis). Values are means ± S.E.M. ($n = 3$).

rate of biliary phospholipid output under steady-state conditions was 0.79 μmol/min per kg body wt. (Fig. 2*b*), and this rose to 1.10 μmol/min per kg body wt. (Fig. 2*c*).

The maximum bile-salt concentration achieved under steady-state conditions was approx. 95 mM (Figs. 2*e* and 2*f*). The maximum biliary phospholipid concentration under steady-state conditions was 5.5 mM (Fig. 2*e*) and reached 6 mM at one point. (Fig. 2*f*). Thus in the biliary-fistula rat the livers can maintain the phospholipid output into bile in response to increasing bile-salt secretion.

In both the isolated perfused liver and biliary-fistula rat there appears to be a dose-dependent output of bile salts and of phospholipid into bile on TC infusions (Figs. 1 and 2). At intermediate TC infusions the biliary phospholipid concentration reaches a plateau; this is also evidenced at the highest infusion rate of TC (Figs. 1*g*, 1*h* and Figs 2*e* and 2*f*). Under steady-state conditions the ratio of phospholipid/bile salt stayed constant, again confirming that biliary phospholipid output keeps pace with biliary bile-salt output.

'Single-pass' short-pulse experiments

Fig. 3 shows the effect of multiple 'single-pass' short pulses of TC in the isolated perfused liver on biliary phospholipid and bile-salt output. The peak secretion of bile salt occurred in the 10 min sample (4 min after the TC pulse), when approx. 2.0 μmol/min per kg body wt. of bile salt was secreted. The peak secretion of phospholipid occurred in the 16 min sample (10 min after the TC pulse) and was secreted at a rate of 0.40 μmol/min per kg body wt. This result confirms previous work showing that bile salts enter the canalicular lumen first, followed by lipids (Lowe *et al.*, 1984). With subsequent pulses of TC the peak bile-salt secretion was maintained, but phospholipid output declined, however, such that, after the third pulse of TC, phospholipid output was reaching its basal level. It thus appears that the response of phospholipid output in accompanying bile-salt output, which is seen under continuous bile-salt infusion (Figs. 1 and 2) is not seen when infusion is discontinuous (Fig. 3).

DISCUSSION

Bile-salt secretion

Depending upon the individual bile-salt species, up to 90% of the bile acids are extracted from the blood perfusing the liver during a single passage (O'Maille *et al.*, 1967). This is accomplished by a very efficient transcellular active transport mechanism for bile acids. The transport mechanism across the sinusoidal membrane under normal conditions is believed to operate far below saturation (Reichen & Paumgartner, 1976), and it is the transport of bile salts across the canalicular membrane into bile which is probably the rate-limiting step in the whole transcellular process (Gonzalez *et al.*, 1979).

The maximum rates of TC secretion in the present experiments were 7.5 μmol/min/kg body wt. and 9.4 μmol/min per kg body wt. and agree fairly well with data in the literature (9.6 μmol/min per kg body wt., Kitani *et al.*, 1984; 12–14.8 μmol/min per kg body wt., O'Maille & Hofmann, 1981) or which can be extracted from it by calculation [6.6 μmol/min per kg body wt. (Herz *et al.*, 1975); 9.2 μmol/min per kg body wt. (Kitani & Kanai, 1982); 10.5 μmol/min per kg body wt. (Reichen & Le, 1983)]. The biliary TC concentrations reached in both the perfused livers and the biliary-fistula rats were 80–90 mM and are comparable with, or greater than, previous values [72 mM (Herz *et al.*, 1975); 83 mM (Kitani & Kanai, 1982; by calculation)].

No necrotic lesions were observed in biliary-fistula rats infused with TC at rates below 15 μmol/min per kg body wt. (T. G. Hammond, unpublished work). Hardison *et al.* (1981) found that there were no noteworthy alterations in general subcellular morphology, and only a few scattered dead cells, in rats at high bile-salt infusion rates, and such focal-cell necrosis was the only feature in the perfused livers used by Miyai *et al.* (1971).

Phospholipid secretion

Rates of phospholipid secretion in general paralleled those of bile-salt secretion, thus as the rate of bile salt secretion increased, so did the rates of phospholipid secretion and, at high rates of bile-salt infusion, the decline in phospholipid secretion paralleled the decline of bile-salt secretion.

The maximum rates of phospholipid secretion were 0.5 and 0.8–1.0 μmol/min per kg body wt. for the isolated liver and the biliary-fistula rat respectively. These values are higher than those (0.016 μmol/min per kg body wt.) that can be calculated from the data of Eaton & Klaasen (1976); if one assumes that Hardison & Apter (1972) related their values to 100 g body wt., as in their earlier papers, then their data for biliary-fistula rats become approx. 0.8 μmol/min per kg body wt. At high TC infusion rates the phospholipid secretion was maintained at 5–7 mM in both preparations. The phospholipid/bile-salt molar ratio in the perfused liver was 0.10 during the initial stage of TC infusion at low rates and decreased to 0.06 at higher infusion rates. In the biliary-fistula rat it was 0.08–0.14 in the initial stages and fell to 0.06 in the later stages. The data of Hardison & Apter (1972) yield values on calculation of 0.1–0.17 at lower bile-salt infusion rates and values of approx. 0.06 at higher infusion rates. The data of Wheeler & King (1972), who used dogs, also show that the phospholipid/bile-salt ratio falls between early and late stages of the perfusion as the infusion rate of bile salts increased.

In the present experiments, as in those of Hardison & Apter (1972) it is particularly interesting that the ratio remains essentially constant for an extended time, even at the highest rates of bile-salt output, indicating a constant relationship between continuous bile-salt secretion and continuous phospholipid secretion.

Origin of the biliary lipids

The Introduction outlined the sequence of bile-salt and lipid entry into bile, indicating that lipid was brought continuously to the canalicular membrane by some form of vesicle transport and suggested that the lipid was then removed from specific microdomains in the membrane by bile-salt solubilization. Furthermore, the phospholipid composition of bile does not reflect the general composition of the bile-canalicular membrane (Evans *et al.*, 1976); bile contains 80–95% phosphatidylcholine (Swell *et al.*, 1968*a*,*b*), whereas the membrane contains only 33% phosphatidylcholine, together with 25% sphingomyelin and 40% other lipids (Kremmer *et al.*, 1976). Therefore it appears that the phosphatidylcholine

may be coming from an intracellular pool of lipids specifically destined for biliary lipid output.

Yousef *et al.* (1975), Gregory *et al.* (1975), Kawamoto *et al.* (1980) and Robins & Brunengraber (1982) have identified an intracellular pool of lipid which may be specifically destined for the bile canaliculus membrane and thence to the bile; this pool may only represent 5% of the total liver pool of phosphatidylcholine. Schersten (1973) and Gregory *et al.* (1975) have suggested that increased bile salt throughout might increase the rate of hepatic synthesis of phosphatidylcholine; however, those experiments represented much longer exposure to TC than the ones reported in the present paper. Apstein & Roxbury (1985) have, however, noted a regulation of total liver phosphatidylcholine content during ampicillin administration and have suggested that the rate of loss of biliary phosphatidylcholine may regulate its synthesis.

Cronholm *et al.* (1983) have shown that much of the biliary phosphatidylcholine is derived not from synthesis *de novo* but from a more general pool in the liver; likewise only 16–20% of biliary cholesterol appears to be derived from newly synthesized material (Schwartz *et al.*, 1978; Green, 1983). The origin of this general pool could be an equilibration between all cellular membranes in the cell (as suggested also for cholesterol; see Green, 1983).

In the present experiments the supply of phospholipid to bile was maintained over an extended period not only in the bile-fistula rat, which has access to circulating lipoproteins, but in the isolated perfused liver, which does not. This would indicate that the pool of phosphatidylcholine, both preformed and newly synthesized, destined for the bile is appreciable and can be maintained. The indication that the supply of lipid is not readily exhausted is perhaps well made by the observation that, even after 2 h of liver perfusion in the absence of circulating lipoproteins, the phospholipids of bile still comprise almost entirely phosphatidylcholine. There was no appreciable content of other phospholipids, which would represent a more general dissolution of the canalicular membrane if supplies of biliary-directed phosphatidylcholine had been exhausted.

In the biliary-fistula rat the rate of secretion of phospholipid, and hence the phospholipid bile-salt ratios, were higher than in the isolated livers, in low and intermediate bile-salt infusion rates. There are many possibilities for this: (i) age, strain and nutritional status of the animals used: (ii) the effects of a mixed pool of bile salts (animal) versus a single bile-salt species (isolated liver); it is interesting in this connection that the phospholipid/bile-salt ratio changes towards the same value, 0.06, in the biliary-fistula animals as the proportion of TC rises at the higher infusion rates; (iii) a contribution from plasma lipoproteins to the biliary-fistula animals either directly (see the suggestion by Casu *et al.*, 1981) or subsequent to lysosomal processing.

Control of delivery of phospholipids

Irrespective of whether continuous output of biliary phospholipids involves increased synthesis or redistribution, it must also involve a continuous delivery to the canalicular membrane.

It is clear that the rate of delivery is controlled in some way by the bile-salt thoughput in the system, since the rate of phospholipid secretion into bile increases as the bile-salt infusion rate is increased. The higher biliary phospholipid/bile-salt ratios at low bile-salt infusion rates may represent a phospholipid delivery rate that can accommodate a relatively low bile-salt secretory rate and which increases to keep pace with the bile-salt output, maintaining a steady ratio of approx. 0.06. The maximum rate of supply to the canalicular membrane occurs before the cholestatic phase and, when the secretion of bile salt and phospholipid falls, it implies that the bile-salt supply must then down-regulate the phospholipid synthesis and supply to (and fusion with) the membrane, since the phospholipid/bile-salt ratio then remains at 0.06.

The differences in phospholipid secretion rates (or concentration) of individual animals, in the present experiments, which are hidden in the variance of the means, and the differences of rates which can be seen for individual animals in the experiments of Hardison & Apter (1972), may indicate that the rate of supply shows differences from animal to animal.

The actual point of interaction of the bile salt in controlling the rate of phospholipid supply is unknown. However, there appears to be an increased occurrence of vesicles in the pericanalicular region during intense bile-salt transport (Boyer *et al.*, 1979; Jones *et al.*, 1979, 1980; Goldsmith *et al.*, 1983), and this may represent the acceleration in vesicle transport; since vesicle delivery can be interfered with by colchicine (see Barnwell *et al.*, 1984), this effect suggests that the cytoskeleton or the cytoskeletal–vesicle interaction may be the key regulatory site which responds to the passage of bile salts through the cell.

Response of phospholipid output to pulses of bile salts

In pulse experiments with TC (Fig. 3) the entry of bile salts into bile is seen to precede the output of phospholipid: this result confirms and extends the results of Lowe *et al.* (1984), and the implications of this are outlined in the Introduction.

These experiments, however, also reveal a new phenomenon: the output of phospholipid declined progressively with succeeding pulses of TC. This conflicts strongly with the results of continuous infusion, where the phospholipid output is maintained.

In the present experiments where the exposure to TC is of short duration, it is possible that the intracellular signal that initiates the movement of phospholipid to the bile-canalicular membrane is not fully activated. There is a substantial output of phospholipid after the bile-salt pulse, and this may represent biliary-type phospholipid already present in the bile-canalicular membrane or a number of vesicles remaining in the pericanalicular cytoplasm subsequent to the depletion of the endogenous bile-salt pool. Subsequent secretory peaks are decreased and may reflect an inability to bring up lipid to the membrane. A second effect of the low intracellular concentrations of TC may be to reduce phospholipid synthesis, which will ultimately reduce the availability of phospholipid for transport. In experiments with continuous TC infusion it is clear that the signal for phospholipid transport is continuously switched on, ensuring a continuous supply of phospholipid, whereas in the short-pulse experiments it is possible that a threshold of intracellular bile-salt concentration may need to be exceeded and maintained to retain the signal. The short pulse may result in the rapid decay of this signal, and thus subsequent amounts of phospholipid vesicles would be progressively reduced.

The actual point of interaction between bile salts and phospholipid transport has yet to be identified. In hepatocytes, bile salts can be bound to various cytosolic proteins and to organelles, leaving only very small amounts free in the cytosol (Strange, 1981). Whether one of these interactions is the critical signal, or whether a certain concentration of free bile salts is needed to form a fresh interaction to act as the signal, is not known.

The continuous infusion of bile salts appears to be necessary for the activation of phospholipid delivery and secretion. The key to the control of this may lie in judicious interruption of the signal.

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