The effects of colchicine on secretion into bile of bile salts, phospholipids, cholesterol and plasma membrane enzymes: bile salts are secreted unaccompanied by phospholipids and cholesterol

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Colchicine, a drug which interferes with microtubular function, has no effect on the secretion of taurodehydrocholate into bile; it is therefore suggested that bile salts are unlikely to be packaged in vesicles during cellular transit from sinusoidal to canalicular membranes. Colchicine greatly reduces the secretion of phospholipid and cholesterol into bile; it is suggested that this is due to an interruption in the supply of vesicles bringing lipids to repair the canalicular membrane during bile salt output. In the absence of the protective effect of a continuous supply of repair vesicles, micelle-forming bile salts damage the canalicular membrane; the increased concentration of plasma membrane enzymes in bile and the increased aspartate aminotransferase activity in plasma and bile are evidence of this damage. Damage to the canalicular membrane may also be an explanation for the reduction in taurocholate transport and the taurocholate-induced cholestasis which are seen with colchicine-treated livers. Such membrane damage is not observed in colchicine-treated livers during the secretion of the non-micelle forming bile salt, taurodehydrocholate.

A considerable number of studies have indicated that the output into bile of phospholipids, cholesterol and plasma membrane enzymes is determined by the biliary output of bile salts. Coleman et al. (1977) proposed that their presence in bile was due to the detergent action of bile salts upon the membrane of the bile canaliculus and that the integrity of this membrane was maintained by continuous biosynthetic repair. Model systems utilizing erythrocytes (Coleman & Holdsworth, 1976; Billington et al., 1980) and hepatocytes (Billington et al., 1980) have shown that micellar concentrations of bile salts were able to remove plasma membrane materials without causing cell lysis. Subsequently it has been shown by Barnwell et al. (1983a,b), using isolated perfused livers, that plasma membrane enzymes and lipids are released into bile when the biliary concentration of taurocholate exceeds its critical micellar concentration, whereas the bile salt analogue taurodehydrocholate, which does not form micelles, did not release biliary lipids and plasma membrane enzymes.

The microtubule-disrupting agent, colchicine, interferes with the movement of secretory vesicles within hepatocytes. It has been found to prevent the normal release into blood of very low density lipoproteins (Orci et al., 1973; Le Marchand et al., 1973; Stein et al., 1974; Reaven & Reaven, 1980), albumin (Le Marchand et al., 1974; Redman et al., 1975), fibrinogen (Feldman et al., 1975) and clotting factors V and VII (Grätzl & Schwab, 1976). The material originally destined for secretion then accumulated within a proliferation of Golgi-derived vesicles. Barnwell & Coleman (1983) have recently demonstrated that a significant proportion of the accumulated albumin and fibrinogen are then mistakenly released into bile.

In addition to an involvement in sinusoidal secretory events the microtubular network is also implicated in the specific movement of materials to the bile canalicular pole of the hepatocyte. The movement of endocytotic vesicles containing dimeric IgA to the canaliculus (Mullock & Hinton, 1981; Goldman et al., 1983) has been shown to be interrupted by colchicine and vinblastine (Mullock et al., 1980; Godfrey et al., 1982; Barnwell & Coleman, 1983).

In the studies to be reported here the use of the isolated perfused liver has allowed the endogenous bile salt pool to be rapidly removed and then to be completely replaced with either a micelle-forming bile salt (taurocholate) or a non-micelle-forming bile salt analogue (taurodehydrocholate). These

studies therefore yield information on several aspects of bile secretion, including both the mechanisms of bile salt transport and the mechanisms of supply of phospholipids, cholesterol and plasma membrane enzymes to the bile canaliculus.

Materials and methods

Materials

Fine chemicals were obtained as follows: taurocholate (Calbiochem–Behring Corp., Bishop's Stortford, Herts., U.K.), [^{14}C]taurine (Amersham International, Amersham, Bucks., U.K.), heparin (Weddel Pharmaceuticals, London EC1, U.K.), Sagatal (May and Baker, Dagenham, Essex, U.K.), and colchicine and other fine chemicals (Sigma Chemical Co., Poole, Dorset, U.K.).

Assay kits for cholesterol and for aspartate aminotransferase were obtained from the Boehringer Corp. (London), Lewes, E. Sussex, U.K. and plastic cannula tubing (PP10 and PP50) was obtained from Portex, Hythe, Kent, U.K. High performance t.l.c. (HPTLC) plates (Kieselgel 60, Merck) were supplied by BDH (Atherstone, Warwicks., U.K.).

Isolated perfused liver

Male Wistar rats weighing about 250 g were used throughout; these had been allowed free access to a standard laboratory diet and were maintained under a constant-light cycle. The animals were anaesthetized with pentobarbitone (Sagatal) and their bile ducts cannulated with PP10 tubing. After 20 min the liver was initially flushed free of (rat) blood by perfusion *in situ* with Krebs–Ringer bicarbonate buffer pH 7.4, and then switched to a recycling perfusion medium [under conditions of constant flow (18 ml/min) and with a perfusion pressure of approx. 250 mm of water] of 100 ml of Krebs–Ringer bicarbonate buffer, pH 7.4 containing 2 mM-Ca^{2+}, 5 mM-glucose, 1% (w/v) bovine serum albumin, a physiological amino acid mixture (Seglen, 1976; but with 0.44 mM-ornithine, 0.09 mM-alanine and 0.1 mM-arginine) and 10% (v/v) washed human red cells. This medium was maintained at $37 \pm 1°C$ and gassed continuously with O$_2$/CO$_2$ (19:1). After 100 min this perfusion medium was changed to replace substrates used by the liver. This procedure is based on that of Barnwell *et al.* (1983*a*). At the end of the experiment, at 190 min, the liver was again flushed free of red cells with Ca^{2+}-free Krebs–Ringer bicarbonate buffer, pH 7.4. The liver was then excised and homogenized in 0.14 M-NaCl/0.015 M-Hepes [4-(2-hydroxyethyl)-1-piperazine-ethanesulphonic acid], pH 7.4, at 3 ml/g, in a tightly fitting Potter–Elvehjem homogenizer.

Samples of the perfusion fluid were obtained at 100 min and 190 min, and were centrifuged at 400 g for 5 min to remove erythrocytes.

Colchicine treatment

A stock solution of colchicine, 2 mg/ml in 0.15 M-NaCl, was freshly prepared for each day of experiments and kept in the dark. Initial experiments (not reported here), using a range of doses between 0.001 and 0.2 mg, allowed the selection of two suitable dose levels for the experiments, 0.04 mg (0.1 μmol) and 0.1 mg (0.25 μmol), subsequently referred to as low dose and high dose respectively. Thus 20 μl (low dose) or 50 μl (high dose) of the stock solution were added with complete mixing to the perfusion fluid at $t = 40$ min, i.e. about 10 min after the liver had been isolated.

Bile salt additions

The bile salts (either taurocholate or taurodehydrocholate) were added to the liver in the new perfusion medium at 100 min; 20 μmol of bile salt dissolved in 1 ml of Krebs–Ringer bicarbonate were added to 100 ml of perfusion medium.

Bile collection

Bile samples were taken and collected in preweighed tubes on ice; collections were made at $t = 20$, 40, 70, 100, 130, 160 and 190 min. The amount of bile was then determined gravimetrically assuming a density of 1 g/ml. All bile samples, homogenates and samples of centrifuged perfusion fluid were stored at $-20°C$ till required for analysis; no deterioration of biliary components or enzyme activities was observed during the period of storage.

Enzyme assays

All determinations were carried out at 37°C under conditions in which only enzyme concentration was the limiting factor. The assays carried out were: 5'-nucleotidase (EC 3.1.3.5) and phosphodiesterase 1 (EC 3.1.4.1) as described in Godfrey *et al.* (1981), and aspartate aminotransferase (EC 2.6.1.1) as described in Bergmeyer *et al.* (1978).

Chemical determinations

Phospholipid present in bile was determined by the method of Bartlett (1959) after lipid extraction by the method of Bligh & Dyer (1959). The amount of (free) cholesterol in bile was estimated by an assay kit (Boehringer) based on cholesterol oxidase; the H$_2$O$_2$ formed in the conversion of cholesterol to 4-cholesten-3-one is used to produce a coloured dye with absorbance at 490 nm. Allowance was made for background absorbance at 490 nm and the method was calibrated with cholesterol standards. Bile salt concentrations were

determined with hydroxysteroid dehydrogenase (EC 1.1.1.150) according to Coleman *et al.* (1979). This method does not measure dehydrocholate and its conjugates; the concentration of taurodehydrocholate in bile was therefore determined by comparing the radioactivity in bile with the specific radioactivity of the taurodehydrocholate added (1 ml of 20 mM-taurodehydrocholate contained 0.1 µCi of [^{14}C]taurodehydrocholate).

Preparation of [^{14}C]taurodehydrocholate

This was obtained by conjugation *in vivo* of [^{14}C]taurine with administered dehydrocholate in rats in which the bile salt pool had been fully depleted. Male Wistar rats (250 g) were anaesthetized with Sagatal and their bile ducts cannulated with PP10 tubing. They were placed in restriction cages overnight with free access to food and water (16 h) to allow depletion of the endogenous bile salt pool (see Godfrey *et al.*, 1981). The following morning the rats were anaesthetized with Sagatal and maintained at 37°C with a thermostatically controlled heating blanket. The femoral vein was cannulated with PP50 tubing and [^{14}C]taurine (25 µCi) dissolved in 0.5 ml of 0.15 M-NaCl was administered to the rat via the cannula and followed by 0.5 ml of saline alone. After 30 min 20 µmol of dehydrocholate in 1 ml of 0.15 M-NaCl, pH 7.4, was slowly introduced into the femoral cannula over a 5 min period. Bile was collected as a series of 15 min samples and its radioactivity assessed. The first two samples (0–15 and 15–30 min after dehydrocholate administration) were found to contain 2 µCi of ^{14}C, and silicic acid t.l.c. in a butan-1-ol/acetic acid/water (10 : 1 : 1, by vol.) system (Gänshirt *et al.*, 1960) showed that at least 95% of the ^{14}C radioactivity, identified by comparison with authentic samples, was present as taurodehydrocholate.

Subsequent t.l.c. of bile, collected from both control and colchicine-treated livers which had been perfused with [^{14}C]taurodehydrocholate, showed that at least 90% of the ^{14}C radioactivity was present as [^{14}C]taurodehydrocholate. These biles were also analysed by using hydroxysteroid dehydrogenase (Coleman *et al.*, 1979) with which taurodehydrocholate does not react: during the peak excretion of ^{14}C-labelled bile salts the level of bile salts assayed by hydroxysteroid dehydrogenase did not increase above background, indicating that all radioactivity was likely to be present as taurodehydrocholate.

To use the [^{14}C]taurodehydrocholate-containing bile as a source of [^{14}C]taurodehydrocholate for perfusion, bile equivalent to 0.1 µCi was added to unlabelled taurodehydrocholate in Krebs–Ringer bicarbonate and the final concentration was adjusted to 20 mM with respect to total taurodehydrocholate.

Results

Biliary composition during the initial phase of liver isolation (20–100 min)

In control livers there is a parallel decline in the amount of bile salts, phospholipids, cholesterol, 5'-nucleotidase and phosphodiesterase 1. Bile flow also declined to an amount probably largely represented by bile-salt-independent flow. These phenomena are probably all dependent, directly or indirectly, upon the depletion of the endogenous bile salt pool, since biliary output of all components can be re-established upon perfusion of the liver with bile salts (Figs. 1, 2 and 3).
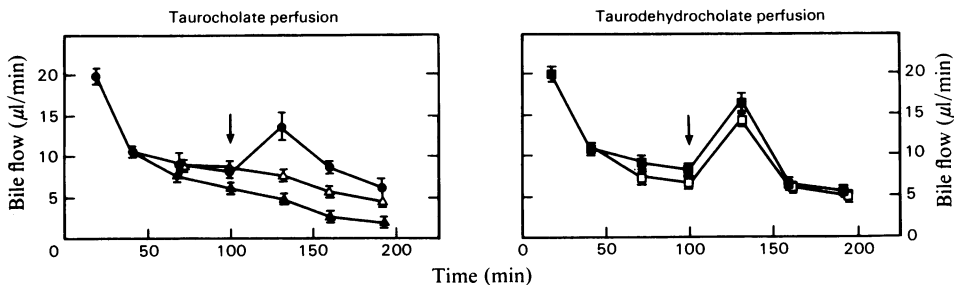

Fig. 1. *Effect of bile salt perfusion on bile flow in control and colchicine-treated isolated perfused livers*
Liver isolation, perfusion, bile collection and colchicine treatment are detailed in the Materials and methods section. Arrows indicate the replacement of perfusion fluid with fresh fluid containing bile salts. Symbols: ●, controls (no colchicine), taurocholate (20 µmol) added at $t = 100$ min; △, colchicine-treated livers (low-dose, 0.1 µmol), taurocholate added at $t = 100$ min; ▲, colchicine-treated livers (high-dose, 0.25 µmol), taurocholate added at $t = 100$ min; ■, controls (no colchicine), taurodehydrocholate (20 µmol) added at $t = 100$ min; □, colchicine-treated livers (0.25 µmol), taurodehydrocholate added at $t = 100$ min. Values are means±S.E.M. of duplicate or triplicate determinations; $n = 20$ in controls prior to $t = 100$ min, for other values $n = 3$.

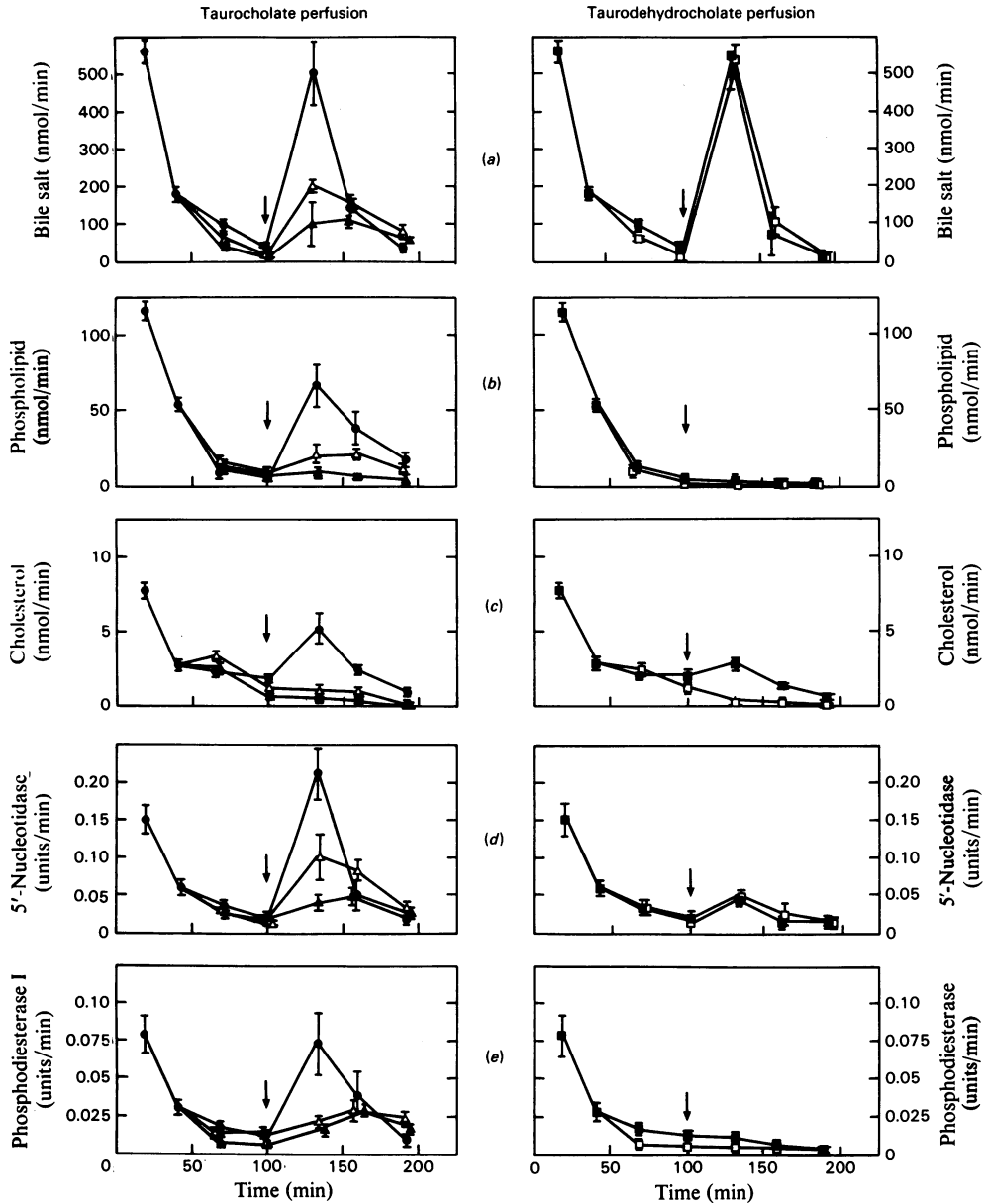

Fig. 2. *Effect of bile salt perfusion on the amount of various materials secreted into bile by control and colchicine treated livers*
For experimental details see the legend to Fig. 1 and the Materials and methods section. In this Figure the output of (a) bile salts, (b) phospholipids and (c) cholesterol into bile are expressed in nmol/min after first determining the total amount of the parameter present in a given bile sample and then dividing this by the number of minutes in the collection period. Enzyme units are μmol of substrate hydrolysed/h at 37°C. Thus the outputs of (d) 5'-nucleotidase and (e) alkaline phosphodiesterase 1 are expressed as units per min of bile collection, found by dividing the total enzyme activity present in a given bile sample by the number of minutes taken to collect the sample. Values are means $\pm$ S.E.M. of duplicate or triplicate determinations (see Fig. 1). Symbols: ●, controls (no colchicine), taurocholate (20 μmol) added at $t = 100$ min (arrows); △, colchicine-treated livers (low dose, 0.1 μmol), taurocholate added at $t = 100$ min; ▲, colchicine-treated livers (high dose, 0.25 μmol), taurocholate added at $t = 100$ min; ■, controls (no colchicine), taurocholate (20 μmol) added at $t = 100$ min; □, colchicine-treated livers (0.25 μmol) with taurodehydrocholate added at $t = 100$ min.
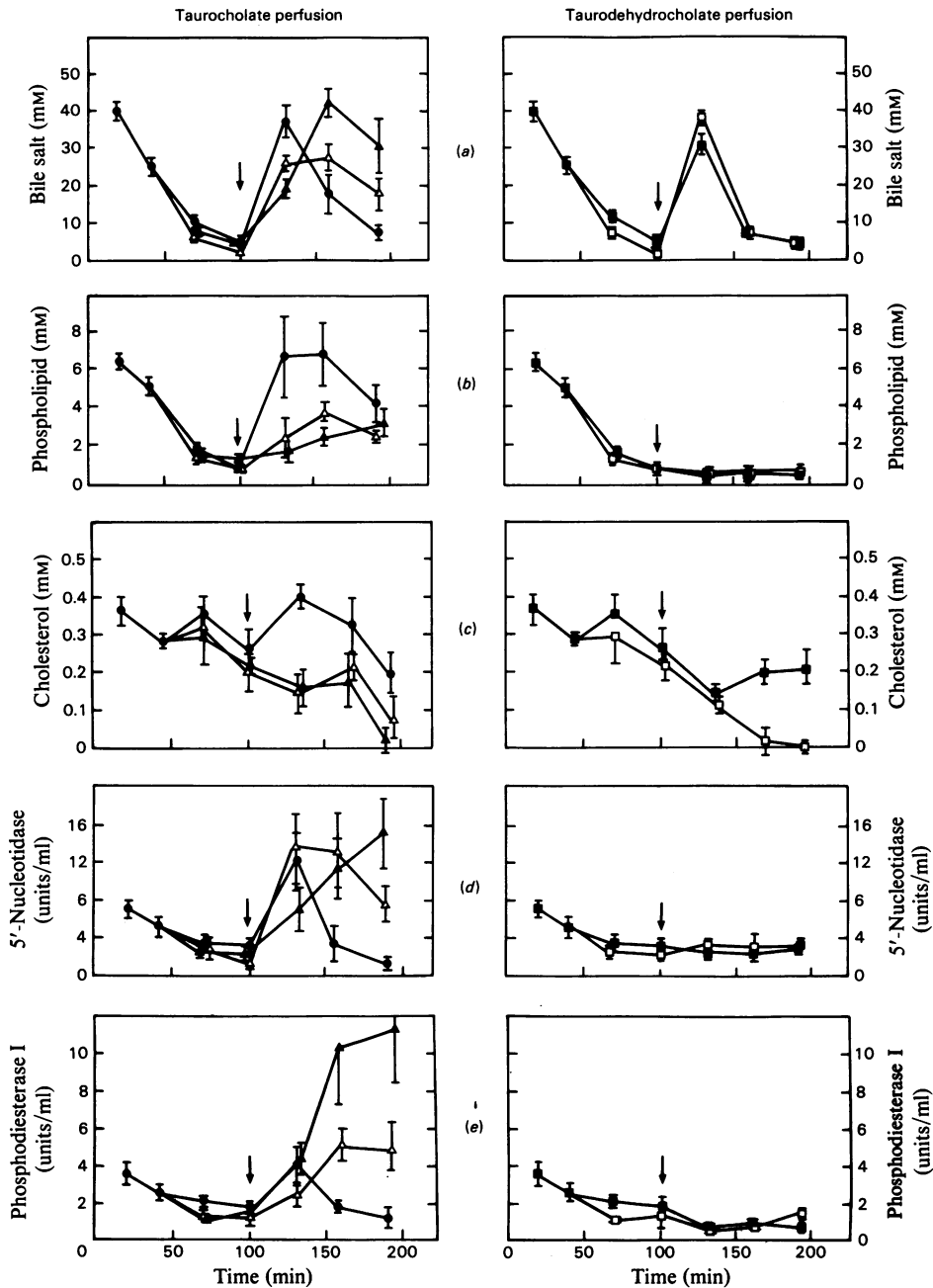
Fig. 3. *Effect of bile salt perfusion on the concentration of various materials secreted into bile by control and colchicine-treated livers*

For experimental details see the legend to Fig. 1 and the Materials and methods section. The concentrations of (*a*) bile salts, (*b*) phospholipids and (*c*) cholesterol are all expressed as mM. The concentration of the plasma membrane enzyme activities (*d*) 5'-nucleotidase and (*e*) alkaline phosphodiesterase 1 present in the samples are shown as units (derived as in the legend to Fig. 2) present per ml of bile. Symbols: ●, controls (no colchicine), taurocholate (20 μmol) added at *t* = 100 min (arrows); △, colchicine-treated livers (low dose, 0.1 μmol), taurocholate added at *t* = 100 min; ▲, colchicine treated livers (high dose, 0.25 μmol), taurocholate added at *t* = 100 min; ■, controls (no colchicine), taurodehydrocholate (20 μmol) added at *t* = 100 min; □, colchicine-treated livers (0.25 μmol), tauro-dehydrocholate added at *t* = 100 min.

Pre-treatment of the livers with colchicine had little effect upon the phenomena seen during depletion of the bile salt pool, except that the total amounts of all the above parameters were marginally, but not significantly, less (Figs. 1, 2 and 3).

Biliary composition during bile-salt-promoted output (100–190 min)

Bile flow. Addition of taurocholate to the perfusion medium at 100 min brought about an increase in bile flow in the controls but no increase in the colchicine-treated livers. Bile flow continued to decline in both high- and low-dose treated livers with the reduction in flow being greatest in the high-dose liver (Fig. 1).

In contrast, perfusion with taurodehydrocholate at 100 min brought about an increase in bile flow, the stimulation being almost identical for both control and colchicine-treated livers (Fig. 1).

Bile salts. Perfusion of control livers with taurocholate brought about a substantial increase in amount and concentration of bile salt in the bile. In the colchicine-treated livers the amount of bile salt secreted was reduced, but the concentrations achieved from the high-colchicine-dosed livers were comparable with the controls, although peak concentration occurred at a later time point (160 min).

Perfusion with taurodehydrocholate produced a substantial increase in biliary bile salt concentration; the amount of taurodehydrocholate put out at each time point was identical for both the colchicine-treated livers and controls (Figs. 2a and 3a).

Recoveries of perfused bile salts in bile during the time course of the experiment (100–190 min) were: control, taurocholate $93.0 \pm 11.2\%$; control, taurodehydrocholate $94.7 \pm 5.7\%$; high-dose colchicine, taurocholate, $43.6 \pm 8.9\%$; high-dose colchicine, taurodehydrocholate $99.3 \pm 6.6\%$ (means $\pm$ s.d. $n = 3$–8).

Biliary phospholipid and cholesterol. Following taurocholate perfusion an increase in biliary phospholipid and cholesterol secretion was observed in the controls; concentrations similar to those at initial isolation ($t = 20$ min) were achieved. This increase was, however, absent from the colchicine-treated livers; total amounts were greatly reduced and different from the pattern with bile salts. The concentrations achieved were substantially less than in the controls (Figs. 2b and 2c, 3b and 3c).

Taurodehydrocholate did not promote phospholipid or cholesterol output from either controls or colchicine-treated livers. At low levels of bile salt and during taurodehydrocholate perfusion of controls, cholesterol, unlike phospholipid, continued

to be secreted (Figs. 2b and 2c, 3b and 3c) (see also Wagner et al., 1976).

Biliary 5'-nucleotidase and phosphodiesterase 1. After taurocholate perfusion there was substantial increase both in amounts and in concentration of the enzymes in the bile of controls. Whilst the amount of enzyme put out in the bile of colchicine-treated livers, after taurocholate perfusion, was less than that of controls and related to the dose of colchicine, the concentrations of the enzymes in the bile were, however, maintained and even exceeded those in the controls. At the higher dose of colchicine the greatest concentrations of 5'-nucleotidase and phosphodiesterase 1 reached in the bile were delayed compared with controls (Figs. 2d and 2e, 3d and 3e).

Perfusion with taurodehydrocholate had little effect upon 5'-nucleotidase or phosphodiesterase 1 release either in controls or in colchicine-treated livers (Figs. 2d and 2e, 3d and 3e).

Aspartate aminotransferase in perfusates and biles

Perfusates were analysed for aspartate aminotransferase as an indication of cell damage. In the initial period (20–100 min) there was little evidence of cell damage from either control rats or from rats treated with either dose of colchicine (0.060%, 0.060% and 0.061% of total liver activity respectively).

Perfusion of control and colchicine-treated livers with taurodehydrocholate brought about a further small leakage (0.12%, 0.103%) as did perfusion of control livers with taurocholate (0.14%) but these increases were not significant. There was, however, a somewhat greater, but more variable, leakage from colchicine-treated livers during taurocholate perfusion.

Leakage of aspartate aminotransferase into bile was very low in both control and colchicine-treated livers in the initial stages of perfusion. It was increased slightly during perfusion with taurodehydrocholate and more so during perfusion with taurocholate. In the case of the highest dose of colchicine the rate of leakage, though still small overall, had increased significantly to give a value over four times that of the control (Table 1).

Homogenate enzyme activities

After the termination of perfusion liver homogenates were prepared and analysed for their activities of 5'-nucleotidase, phosphodiesterase 1 and aspartate aminotransferase. No significant differences were found in any of the above parameters, either between colchicine-treated livers and controls, or between livers perfused with the different bile salts and their controls (results not shown).

Table 1. *Aspartate aminotransferase activities in perfusates and biles of control and colchicine-treated livers*

Results are expressed as means ±s.D. (perfusates and biles, n = 3–8). Total liver activity = 141 ±50 mmol/h (n = 10). For other conditions see the Materials and methods section.

Percentage of liver activity in:

Treatment	Bile salt	Perfusate 20–100min	100–190min	Bile 20–100min	100–190min
Control	Taurocholate	0.062±0.010	0.14±0.05	0.0001±0.00005	0.0038±0.0002
Low-dose colchicine	Taurocholate	0.060±0.005	0.73±0.40	0.0002±0.0003	0.0147±0.0047
High-dose colchicine	Taurocholate	0.060±0.010	0.76±0.33	0.00025±0.0002	0.0158±0.0015
Control	Taurodehydrocholate	0.062±0.010	0.12±0.06	0.0001±0.00005	0.0039±0.0005
High-dose colchicine	Taurodehydrocholate	0.061±0.008	0.103±0.033	0.00025±0.0002	0.0130±0.0031

$P<0.1$ (perfusate); $P<0.05$, $P<0.02$, $P<0.001$ (bile)

Discussion

Effect of colchicine on bile salt transport: mechanism of bile salt transport into bile

In previous studies, colchicine administration has been shown to cause reductions in bile flow and bile salt output to varying extents; no consistent explanation for the effect of colchicine upon bile salt output has emerged. However, on closer inspection of the data it appears that the variability of the effects may be related to the amounts of bile salts presented to the liver. Thus Stein *et al.* (1974) reported that colchicine had little effect upon bile flow, whereas Dubin *et al.* (1980) found that although colchicine had no effect upon basal bile flow it reduced the biliary clearance of, and choleretic response to, infused taurocholate. Fukumoto *et al.* (1982) and Kacich *et al.* (1983) have shown that although small amounts of taurocholate are cleared normally by colchicine-treated livers, they lack the capacity to transport larger doses of this bile salt. Gregory *et al.* (1978) have shown a reduced output of bile salts under conditions of continuous taurocholate infusion, and Erlinger *et al.* (1980) and Berken *et al.* (1983) have shown that colchicine causes a reduction of bile salt output after a pulse of taurocholate. Reichen *et al.* (1981) have suggested from work with isolated hepatocytes that colchicine is a non-competitive inhibitor of taurocholate transport.

In the present experiments it is clear that colchicine-treated livers do not transport taurocholate as efficiently as do controls. However, in view of the lack of reduction in concentration of taurocholate in the bile (Fig. 3b), the effect of colchicine on the secretion of the bile salt cannot be simple.

Further evidence against a simple effect of colchicine on bile salt transport comes from the data on taurodehydrocholate secretion. Taurodehydrocholate transport and secretion are unaffected by colchicine, although it has been demonstrated that this bile salt is transported overall by the same system as taurocholate (O'Maille & Richards, 1976). That taurodehydrocholate is unaffected by colchicine is also shown by the preliminary results of Berken *et al.* (1983).

These results provide support for the view that bile salts are transported across the hepatocyte as discrete molecules, rather than by vesicular transport. Vesicular transport has been suggested to explain the increased numbers of vesicles in the pericanalicular cytoplasm during enhanced taurocholate (Jones *et al.*, 1979) and dehydrocholate (Boyer *et al.*, 1979) secretion. Vesicular transport of taurodehydrocholate, however, would be expected to be blocked by colchicine; this is not seen in the present results. Transport of bile salts across the hepatocyte therefore probably occurs in the cytosol

in solution (Strange, 1981) with transport across the canalicular membrane being driven by the electrochemical gradient (Erlinger, 1981; Inoue *et al.*, 1982).

Mechanisms of biliary lipid secretion

Normal bile contains mixed micelles of bile salts, phospholipids and cholesterol; perfusion of the liver with micelle-forming bile salts normally brings about an increase in biliary lipid secretion (controls, Figs. 2*b* and 2*c*).

Mixed micelle formation may not be obligatory in bile salt transport, however, since taurodehydrocholate, a non-micelle-forming bile salt, does not increase phospholipid and cholesterol secretion (Figs. 2*b* and 2*c*, controls). Moreover, the effects of colchicine on livers perfused with taurocholate demonstrate that high concentrations of this micelle-forming bile salt can be secreted without accompanying lipids (Figs. 3*b* and 3*c*) or with the lipids greatly reduced in proportion to bile salts (Gregory *et al.*, 1978; Erlinger *et al.*, 1980). That bile salts might be secreted independently of lipids was tentatively proposed by Apter & Hardison (1970).

It has been suggested that bile salts may solubilize material from the bile canalicular membrane, that this effect operates after the bile salts have been pumped into the bile canalicular lumen and that continuous repair of the membrane will be needed if the membrane is not to become extensively damaged (Coleman *et al.*, 1977).

There have been several suggestions as to the origin of biliary lipid; these include material derived from an extracellular pool [(high-density lipoproteins) Casu *et al.* (1981)], or from an intracellular pool (Yousef *et al.*, 1975; Gregory *et al.*, 1975; Kawamoto *et al.*, 1980; Robins & Brunengraber, 1982). Transport of this intracellular lipid to the canaliculus membrane has been tentatively suggested to be via transport proteins (Coleman *et al.*, 1977) (but for which direct evidence of involvement has to be found), or in some form of vesicle (Erlinger, 1981).

Such repair vesicles would migrate to and fuse with the bile canaliculus membrane and this vesicle traffic might be expected to be influenced by colchicine. It is therefore of great interest that we have shown that the output of phospholipid and cholesterol into bile is strongly inhibited by colchicine.

The vesicles observed near to the bile canaliculus (Jones *et al.*, 1979; Boyer *et al.*, 1979) during enhanced bile salt secretion may therefore be repair vesicles *en route* to the membrane. Their particular abundance during (tauro)dehydrocholate secretion might be explained by a queuing phenomenon whereby, since lipid is not removed

from the membrane, the oncoming vesicles approach but then do not fuse with the membrane.

If the movements of such phospholipid and cholesterol containing repair vesicles are prevented by the effects of colchicine on microtubules, then continued output of micelle-forming bile salts in the absence of movements of repair vesicles might be expected to result in damage to the bile canaliculus membrane. In control livers the perfusion with taurocholate causes only small amounts of cell damage (Table 1) whereas when the livers have been pretreated with colchicine prior to taurocholate perfusion then the amount of damage is increased substantially. Since this damage is not observed in colchicine-treated livers perfused with taurodehydrocholate it points to the effect of the micelle-forming bile salt upon the membrane rather than an effect of colchicine *per se*.

A symptom of the general effect on the membrane of bile salts is the appearance of plasma membrane enzymes in bile during the secretion of micelle-forming bile salts (Barnwell *et al.*, 1983*a,b*). This effect can be seen in the present experiments in Figs. 2(*d*) and 2(*e*) and 3(*d*) and 3(*e*). Following colchicine treatment, the total amount of these enzymes declines after taurocholate perfusion, but their concentration in bile rises. This suggests that, as the overall composition of the plasma membrane changes, the relative extent of removal of the enzymes increases, ultimately resulting in a weakened membrane, which may then subsequently lose its integrity.

Biliary lipid composition differs considerably from that of the bile canaliculus membrane (Yousef *et al.*, 1975; Gregory *et al.*, 1975). Membrane repair vesicles would therefore have to be specifically enriched in biliary-type lipids. It is possible that fusion of such vesicles with the membrane results in domains of biliary type lipid which are preferentially extracted by bile salts. The remainder of the membrane may then be of low fluidity and thus less susceptible to damage by bile salts (Lowe & Coleman, 1981, 1982). In colchicine-treated livers, which may not have a continuous supply of membrane repair vesicles, the removal of biliary-type lipid would slow down and eventually the more resistant lipid would be attacked, resulting in the greater extent of cell damage mentioned earlier.

Due to the lack of supply of biliary-type lipid, and the subsequent processing of the residual membrane, other properties of the membrane may alter, such as the activities of the bile salt transport system itself and of bile-salt-independent flow, thereby providing an explanation for the bile-salt-induced cholestasis seen in colchicine-treated livers perfused with taurocholate, but not with taurodehydrocholate.

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