Biochem. J. (1983) **210**, 549–557
Printed in Great Britain

Biliary protein output by isolated perfused rat livers

Effects of bile salts

Stephen G. BARNWELL, Phillip P. GODFREY, Phillip J. LOWE and Roger COLEMAN
Department of Biochemistry, University of Birmingham, P.O. Box 363, Birmingham B15 2TT, U.K.

(*Received 17 August 1982/Accepted 21 October 1982*)

The output of proteins into bile was studied by using isolated perfused rat livers. Replacement of rat blood with defined perfusion media deprived the liver of rat serum proteins (albumin, immunoglobulin A) and resulted in a rapid decline in the amounts of these proteins in bile. When bovine serum albumin was incorporated into the perfusion medium it appeared in bile within 20 min and the amount in the bile was determined by the concentration of the protein in the perfusion medium. The use of a defined perfusion medium also deprived the livers of bile salts and the amounts of these, and of plasma-membrane enzymes [5'-nucleotidase (EC 3.1.3.5) and phosphodiesterase I], in bile declined rapidly. Introduction of micelle-forming bile salts (taurocholate or glycodeoxycholate) to the perfusion medium 80 min after liver isolation markedly increased the output of plasma-membrane enzymes but had no effect on the other proteins. The magnitude of this response was dependent on the bile salt used and its concentration in bile; there was little effect on plasma-membrane enzyme output until the critical micellar concentration of the bile salt had been exceeded in the bile. A bile salt analogue, taurodehydrocholate, which does not form micelles, did not produce the enhanced output of plasma-membrane enzymes. This work supports the view that the output of plasma-membrane enzymes in bile is a consequence of bile salt output and also provides evidence for mechanisms by which serum proteins enter the bile.

The source of many rat biliary proteins is the circulating blood; with the exception of the immunoglobulin A-secretory component and haptoglobin–haemoglobin complexes these proteins are present in a level approx. 0.1% of their serum concentration (Hinton *et al.*, 1980). The mechanism thought to be responsible for immunoglobulin A and haptoglobulin transport from blood to bile is discussed by Mullock & Hinton (1981); the other serum proteins may enter bile by less specific mechanisms.

Some biliary proteins have been suggested to be derived from hepatocyte plasma membranes by the action of bile salts (Holdsworth & Coleman, 1975; Coleman *et al.*, 1977). When isolated rat hepatocytes were exposed to bile salts, plasma-membrane enzymes were removed without gross damage to the cells (Billington *et al.*, 1980). Infusion of taurocholate in intact animals has indicated an increase in the biliary output of the plasma-membrane enzymes 5'-nucleotidase, alkaline phosphatase and alkaline phosphodiesterase I (Javitt, 1965; M. N. Eakins, personal communication; Bode *et al.*, 1973; Layden & Poppe, 1977; Reichen *et al.*, 1979). In the present study we have investigated the output of a considerable number of serum and hepatocyte proteins in the bile produced by isolated perfused rat livers and the influence of several bile salts (taurocholate, glycodeoxycholate and a non-micelle-forming analogue, taurodehydrocholate) on such protein outputs. Isolated perfused livers have been used, with considerable success, for the investigation of other aspects of bile secretion and the advantages of this preparation in contrast with whole animals in the context of these experiments are (i) the function of the liver alone is investigated, (ii) blood is replaced by a well-defined perfusion medium whose composition (additions and subtractions) can be strictly controlled and (iii) the endogenous bile salt and serum protein pools accessible to the liver can be depleted very quickly.

Materials and methods

Materials

Antisera to rat immunoglobulin A, rat serum albumin and bovine serum albumin were obtained from Nordic Immunological Laboratories, Maidenhead, Berks., U.K. The bile salts were obtained from

Calbiochem–Behring Corporation, Bishops Stortford, Herts., U.K. Hydroxysteroid dehydrogenase (grade II from *Pseudomonas testosteroni*) and other fine chemicals were from Sigma (London) Chemical Co., Poole, Dorset, U.K. Sagatal was obtained from May and Baker, Dagenham, Essex, U.K., cannulation tubing PP10 was made by Portex, Hythe, Kent, U.K., and heparin was made by Weddel Pharmaceuticals, London, U.K.

Methods

Isolated perfused livers. Male Wistar rats, weighing 250–300g, were used throughout; these had been maintained on a standard laboratory diet and under a constant light cycle. Bile-duct cannulations were performed with PP10 tubing while the rats were under pentobarbital (Sagatal) anaesthesia; bile was collected on ice throughout the experiment. At 20min after cannulation, 2500 units of heparin were injected into the inferior vena cava and the liver was then isolated. The operative procedure used was based on that used for the isolation of hepatocytes by Berry & Friend (1969) with modifications by Seglen (1976). To minimize anoxia, perfusion of the liver was commenced immediately with Krebs–Ringer bicarbonate buffer, pH 7.4 (Krebs & Henseleit, 1932); this buffer was calcium-free and was used to flush the liver free of blood. Once the liver had cleared and was perfusing evenly the perfusion medium was changed to a recycling perfusion with 100ml of Krebs–Ringer bicarbonate buffer, pH 7.4, containing 2mM-$CaCl_2$, 10mM-glucose, 1% (w/v) bovine serum albumin, a physiological amino acid mixture (Seglen, 1976) (but with 0.44mM-ornithine, 0.09mM-alanine and 0.1mM-arginine) and 10% (v/v) of packed human red blood cells. This solution was gassed continuously with O_2/CO_2 (19:1) and maintained at 37±0.5°C (Brauer *et al.*, 1954). All perfusions were carried out in a thermostatically controlled cabinet similar to that recommended by Collins & Skibba (1980) for perfusions *in situ*.

Subsequent to the two initial 20min bile samples, the collection period was increased to 30min for the remainder of the experiment. At 100min after initial cannulation (i.e. 80min after isolation) the perfusion medium was changed to replenish substrates used by the liver. It was at this point, when the bile salt concentration in the collected bile had fallen to approx. 2mM, that bile salts were introduced into the new perfusion medium.

Bile samples were obtained throughout the experiment and bile collection was terminated at 190min after cannulation. Samples of the perfusate were taken at 100min and 190min for testing for evidence of liver cell damage; the sample of perfusate was first centrifuged at 400*g* for 5min to remove erythrocytes. At 190min the perfusion medium was replaced with a calcium-free Krebs–Ringer bicarbonate buffer, pH 7.4, to remove all traces of erythrocytes from the liver. The liver was then excised and homogenized in an equal volume of 0.14M-NaCl/0.015M-Hepes [4-(2-hydroxyethyl)-1-piperazine-ethanesulphonic acid], pH 7.4, using a tightly fitting Potter–Elvehjem homogenizer. Bile samples, centrifuged perfusate and liver homogenates were stored at −20°C until required; there was no appreciable deterioration of activity etc. in the time scale of the analytical period.

Bile salt additions. At 100min, bile salts (dissolved in a minimal volume of Krebs–Ringer bicarbonate buffer, pH 7.4) were added to the perfusion medium to rapidly bring the concentration to the level shown. Subsequently (100–190min) bile salts were infused continuously into the perfusion medium by an infusion pump at the rate shown. The total amount of bile salt added over the period is given in Table 1.

Assays. Bile, perfusates and homogenates were assayed for 5′-nucleotidase (EC 3.1.3.5), phosphodiesterase I (EC 3.1.4.1) and lactate dehydrogenase (EC 1.1.1.27) as described by Godfrey *et al.* (1981). Aspartate aminotransferase (EC 2.6.1.1) was assayed as described by Karman (1955). All determinations except lactate dehydrogenase (20°C) were carried out at 37°C. The enzymes were not inhibited by bile salts at the concentrations used.

Total bile salt concentrations in bile were determined by using hydroxysteroid dehydrogenase (EC 1.1.1.50) as described by Coleman *et al.* (1979). Protein was estimated by the method of Lowry *et al.* (1951) with bovine serum albumin as standard. Immunoglobulin A, rat serum albumin and bovine serum albumin in bile were determined by quantitative radial immunodiffusion by the method of Mancini *et al.* (1965) with specific antisera and using authentic rat serum albumin and bovine serum albumin for standardization; immunoglobulin A, for which no standard was available, is expressed in arbitrary units, relating to the diameter of the precipitation zone.

Critical micellar concentrations of bile salts. These were determined by measuring the increase in

Table 1. *Amounts of bile salts added to perfusion fluid*
Abbreviations: TC, taurocholate; GDOC, glycodeoxycholate; TDHC, taurodehydrocholate.

	Added at 100min (μmol)	Concn. at 100min (μM)	Infused 100–190min (μmol)	Total added (μmol)
High TC	20	200	36	56
Low TC	0	0	36	36
High GDOC	3	30	5.5	8.5
Low GDOC	0	0	5.5	5.5
High TDHC	20	200	36	56

1-anilinonaphthalene-8-sulphonate fluorescence that occurs when the fluorescent probe molecule is taken up into a bile salt micelle. The fluorescence of 10μM-1-anilinonaphthalene-8-sulphonate in 15mM-Hepes/140mM-NaCl, pH 7.4, was measured by using a Perkin–Elmer 203 fluorimeter (exciting wavelength 365nm, detecting wavelength 485nm) for several different concentrations of bile salt. The critical micellar concentration (or range of concentration) is the point where the fluorescence starts to increase from the basal level.

Results and discussion

Perfusion of the livers with bile salt-free media during the period 20–100min resulted in a decline in bile flow and bile salt concentration and in the output of total biliary protein, immunoglobulin A, rat serum albumin and the plasma-membrane enzymes 5′-nucleotidase and phosphodiesterase I (Figs. 1, 2 and 5). Further perfusion with bile salt-free media (control), over the period 100–190min, caused these parameters to decline further.

The general good health of the livers over both of these time periods, however, is indicated by the relatively low amounts of cytosolic enzymes appearing in the bile and the perfusate during this time. The amounts of lactate dehydrogenase and aspartate aminotransferase appearing in bile before 100min are <0.007% of total liver activity and this rate of appearance does not increase in the subsequent 100–190min period. The amount of lactate dehydrogenase in the perfusate is apparently substantial (approx. 5% of total liver activity), but most of this is likely to be derived from erythrocyte lysis in the perfusion medium rather than from liver cell damage. This is supported by the results with aspartate aminotransferase, which does not occur in red blood cells (S. Barnwell, unpublished work) and which occurs in the perfusate only to the extent of 0.5% of total liver activity in both the period 20–100min and 100–190min (Table 2). A further indication of the general good health of the livers within these experiments is their ability to produce a marked choleresis when bile salts are added to the perfusion fluid (Fig. 1). Moreover, the biles produced contain bile salts at high concentration, a feat the requires energy expenditure from integrated metabolism (Fig. 1). Thus the decline in all parameters noted above (in the controls) is likely to be due to the depletion or exhaustion of transportable materials in the perfusion medium and in the liver rather than a decline of liver metabolism in itself.

Bile salt concentration, bile salt output and bile flow

The initial biliary bile salt concentration in these control animals is approx. 40mM and falls to approx. 2mM at about 100min: at this point a biliary

Table 2. *Enzyme content of perfusion media and bile as a percentage of total liver activity*

Values represent (total units in fluid in time stated)/(total units in liver) and are expressed as percentages. Values are means (±S.E.M.) for numbers of observations indicated: when $n = 2$ the values given are means and ranges. For meaning of high and low bile salt concentrations see the legends to Fig. 1 and Table 1. Abbreviations: DH, dehydrogenase; AT, aminotransferase; PD, phosphodiesterase; TC, taurocholate; GDOC, glycodeoxycholate; TDHC, taurodehydrocholate.

	Enzyme content (%)							
	0–100min control	100–190min						
		Control	High TC	Low TC	High GDOC	Low GDOC	High TDHC
Lactate DH (bile)	0.0034 ± 0.001 $n = 7$	0.0002 ± 0.00002 $n = 2$	0.00086 ± 0.00006 $n = 2$	0.001 ± 0.0007 $n = 2$	0.002 ± 0.001 $n = 3$	0.0005 ± 0.0001 $n = 2$	0.0002 ± 0.0001 $n = 2$
Aspartate AT (bile)	0.0065 ± 0.0008 $n = 2$	0.0054 ± 0.0002 $n = 2$	0.013 ± 0.002 $n = 2$	0.007 ± 0.003 $n = 2$	0.006 ± 0.001 $n = 2$	0.0075 ± 0.003 $n = 2$	0.009 ± 0.002 $n = 3$
5′-Nucleotidase (bile)	0.099 ± 0.013 $n = 17$	0.029 ± 0.011 $n = 4$	0.487 ± 0.067 $n = 4$	0.076 ± 0.001 $n = 2$	0.276 ± 0.047 $n = 4$	0.033 ± 0.006 $n = 2$	0.031 ± 0.002 $n = 4$
Alkaline PD (bile)	0.024 ± 0.003 $n = 17$	0.011 ± 0.004 $n = 4$	0.87 ± 0.003 $n = 4$	0.015 ± 0.004 $n = 2$	0.051 ± 0.011 $n = 4$	0.014 ± 0.005 $n = 2$	0.014 ± 0.006 $n = 4$
Lactate DH (perfusate)	4.75 ± 1.36 $n = 9$	0.46 ± 0.13 $n = 2$	3.46 ± 1.69 $n = 3$	2.25 ± 1.25 $n = 2$	2.19 ± 1.10 $n = 4$	0.79 ± 0.53 $n = 2$	3.38 ± 0.38 $n = 4$
Aspartate AT (perfusate)	0.43 ± 0.06 $n = 9$	0.45 ± 0.25 $n = 2$	0.61 ± 0.13 $n = 3$	0.46 ± 0.11 $n = 2$	0.42 ± 0.31 $n = 3$	0.51 ± 0.22 $n = 2$	0.64 ± 0.16 $n = 3$

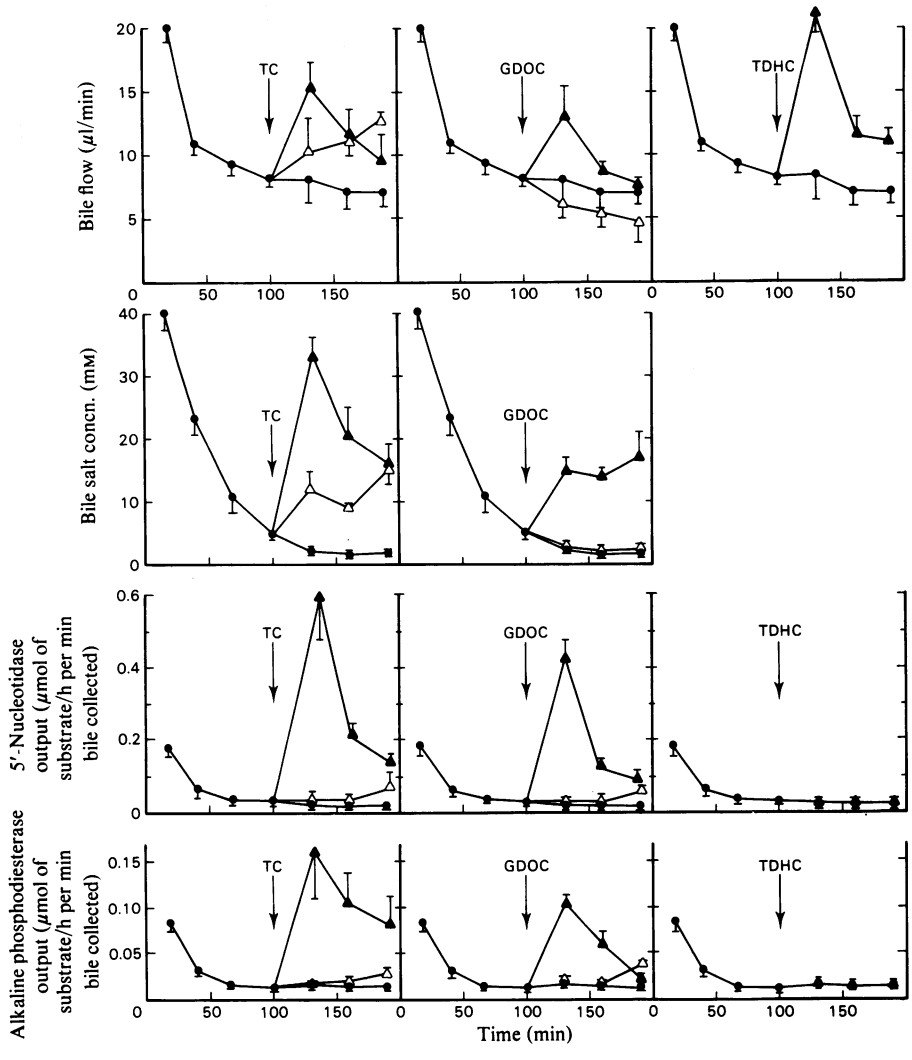

Fig. 1. *Output of biliary components as a function of time and introduction of bile salts*

Bile ducts were cannulated at $t = 0$. Isolation and perfusion were commenced at 20 min. The perfusion fluid was renewed and bile salts were introduced at 100 min (indicated by the arrow). Bile was collected between the time intervals shown and the analyses are recorded as 0–20 min at 20 min etc. Output is obtained by dividing the total amount collected in the time interval over the number of minutes in the collection period. Abbreviations: TC, taurocholate; GDOC, glycodeoxycholate; TDHC, taurodehydrocholate. Symbols: ●, control (no added bile salt); ▲, high bile salt level (bile salt added in substantial amount at 100 min and continuously added to perfusion medium from 100 min; see Table 1); △, low bile salt level (bile salt added continuously to perfusion medium from 100 min, initial substantial addition omitted; see Table 1). Values are means ± S.E.M. ($n = 17$ for controls; $n = 4$ for bile salt additions) of duplicate determinations.

bile salt concentration of approx. 2 mM is maintained, presumably by bile salt synthesis. In bile-fistula rats (Godfrey *et al.*, 1981) the biliary bile salt concentration takes considerably longer to fall to minimal values (i.e. 12–24 h), presumably due both to a return of bile salts from intestinal contents and also to the possibility of a more favourable time for the induction of enhanced levels of the enzymes of bile salt synthesis, notably cholesterol 7α-hydroxylase. Thus the lowest levels of biliary bile salt concentration in bile-fistula animals are 6–8 mM, and subsequently rise (Godfrey *et al.*, 1981).

The addition of bile salts to the perfusion medium at 100 min brought about an increase both in bile salt concentration and bile flow. At the high taurocholate addition, the initial concentration

reached in the bile was 40mM and a substantial concentration was maintained by slow infusion. Infusion alone (i.e. without an initial pulse of taurocholate) achieved a biliary bile salt concentration of approx. 10mM for the first hour and the concentration subsequently rose to 16mM; bile flow rate was also highest at this point (Fig. 1). Glycodeoxycholate addition caused the biliary bile salt concentration to rise to about 15mM, at which point it remained fairly constant. Bile flow was initially elevated, then declined to control levels. Infusion of glycodeoxycholate without an initial pulse gave biliary bile salt levels that differed little from the controls except in the period 160–190min; the slow infusion did not elevate the bile flow rate (Fig. 1). Taurodehydrocholate is not itself measured by the hydroxysteroid dehydrogenase assay, owing to its possession of an oxo group at position 3. It did, however, produce a large stimulation of bile flow that declined after the initial high addition to the perfusate was transported. The bile produced contained metabolites of taurodehydrocholate, some of which were detected by the hydroxysteroid dehydrogenase assay (results not shown).

In all of these experiments with added bile salts, general liver cell damage remained low, since cytosolic enzyme loss into bile or perfusate remained close to that lost in the period 20–100min (maximum 0.014% in bile and 0.64% in perfusate for aspartate aminotransferase) and bore little relationship to the bile salt used or its concentration (Table 2).

Output of immunoglobulin A and rat serum albumin

The biliary output of immunoglobulin A declined with time of perfusion to 100min and, in the control, this decline continued such that immunoglobulin A had virtually disappeared from bile by 190min. The output of rat serum albumin declined more rapidly (Fig. 2). The origin of both of these biliary proteins is probably the serum (Mullock *et al.*, 1978) and the time taken to reduce the biliary levels probably represents the time taken to clear the system (i.e. residual amounts of serum in the perfusion fluid, and material in transit in the liver). It is interesting that it appears to take a different amount of time for the two proteins, which may be cleared into the bile by two distinct mechanisms, i.e. a receptor-mediated vesicular transport (immunoglobulin A) and a less specific mechanism (rat serum albumin).

A further point of interest in relation to rat albumin is that the levels do appear to drop virtually to zero in bile, even though the liver can presumably synthesize albumin during the course of the experiment. Albumin is normally discharged at the sinusoidal face of the cell and not presumably at the canalicular face to any extent, since the rat serum albumin concentration in bile would not decline so completely (Fig. 2).

In experiments in which the effects of colchicine or vinblastine (inhibitors of microtubular function) were studied for their effects on biliary protein output in bile-fistula rats (Godfrey *et al.*, 1982) it was observed that, whereas immunoglobulin A output was greatly reduced, biliary output of rat serum albumin was enhanced; in this case the interruption of normal vesicular traffic to the sinusoidal face of the hepatocyte may either have resulted in an abnormal release of material at the canaliculus or an abnormal permeability of the tight junction to the protein.

When bile salts were administered at 100min (i.e. 80min after isolation) (Fig. 2) they had no effect on residual immunoglobulin A and rat serum albumin output, relative to the control, either at high or low levels of administration (results not shown).

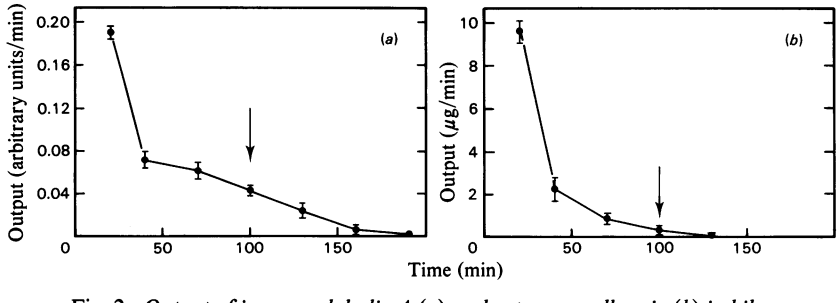

Fig. 2. *Output of immunoglobulin A (a) and rat serum albumin (b) in bile*

For conditions see the legend to Fig. 1 and the Materials and methods section. In both (*a*) and (*b*) results are from experiments with no added bile salt. In some experiments where bile salts were added at 100min (arrow) there was no change in the rate of output of either protein (results not shown). Values are means ± S.E.M. ($n = 8$–10) of duplicate determinations.

Output of the exogenous protein, bovine serum albumin

Although bovine serum albumin is foreign to rat bile it begins to appear in rat bile about 20min after perfusion of the liver with bovine serum albumin-containing media (Fig. 3). If perfusion is instituted with a medium devoid of bovine serum albumin and bovine serum albumin is then added, its appearance in bile also begins to occur at about 20min (results not shown). This time scale of appearance is similar to that for other exogenous proteins (Grant *et al.*, 1980; Thomas, 1980). The output of bovine serum albumin reaches its maximum about 90min after introduction in the perfusion fluid and then showed minor fluctuation that differs little between control and bile salt-administered experiments (Fig. 3*a*). Bile salt infusion had no effect on bovine serum albumin output (results not shown).

Since it is unlikely that the liver will contain receptors for a protein to which it normally is not exposed, clearance into the bile may represent a more general mechanism [e.g. a slow clearance through tight junctions (Dive & Heremans, 1974), loss through fenestrated endothelium at the head of the bile ducts and non-specific fluid pinocytosis through ductular cells (see Thomas, 1980; Jones *et al.*, 1980)]. Such mechanisms are supported indirectly by experiments with increased bovine serum albumin concentrations in the perfusion medium (Fig. 3*b*); the increase in biliary output is approximately proportional to the increase in bovine serum albumin concentration in the perfusion medium. A specific receptor-mediated mechanism for bovine serum albumin transport is much less likely since receptors for this exogenous protein would have been saturated at the high concentration of bovine serum albumin normally employed in the perfusion medium (1%, w/v) and thus a further increase in bovine serum albumin concentration in the perfusion medium would not be expected to increase the output of bovine serum albumin in the bile.

Output of 5'-nucleotidase and phosphodiesterase I

The output of both enzymes declines on liver perfusion and reaches minimal levels by about 70min; in the control the output remains low and essentially constant over the period 100–190min (Fig. 1). This pattern is more reminiscent of the pattern of bile salt concentration decline than of the decline in the concentration of the serum-derived proteins, immunoglobulin A and rat serum albumin (Fig. 2).

Introduction of high concentrations of taurocholate or glycodeoxycholate into the perfusion medium brought about a rapid and marked increase in the biliary output of both enzymes, which rose to well above even the initial output (Fig. 1). In the period 100–190min the total output of 5'-nucleotidase was 16 times (9.6 times) and that of phosphodiesterase 7.9 times (4.6 times) that of the control, for the administration of taurocholate (or glycodeoxycholate). (The differences in amount between the two bile salts may only be due to the different concentrations of the bile salts employed.) This increase in enzyme output is unique to bile since the levels of the two enzymes in the perfusion medium showed essentially little quantitative or systematic change from the control or original during the period of bile salt administration (results not shown). The output of 5'-nucleotidase into bile is 37 times that of aspartate aminotransferase in the same period (Table 2). The biliary output of 5'-nucleotidase induced by the flux of bile salts represents approx. 0.5% of the total liver activity being lost in the space of 90min (Table 2); this compares with 2.5% lost per day by a bile-fistula rat (Godfrey *et al.*, 1981). Administration of taurocholate to bile-fistula rats

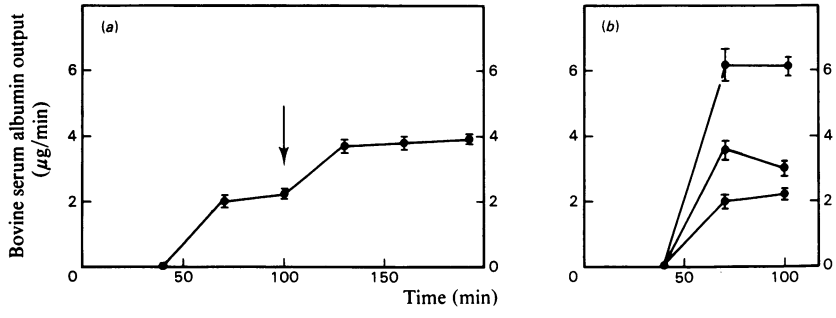

Fig. 3. *Output of bovine serum albumin (BSA) added to perfusion fluid*

Bile ducts were cannulated at $t = 0$. Isolation and perfusion were commenced at 20min and perfusion was established at approx. 22min with medium containing (*a*) 1% (w/v) bovine serum albumin and (*b*) 1%, 2% or 3% bovine serum albumin. Values are means ± S.E.M. (1%, $n = 17$; 2%, $n = 4$; 3%, $n = 4$) of duplicate determinations. The arrow indicates when bile salts (or saline) were introduced; there was no difference between bile salt and saline experiments.

has been shown to cause an increase in the biliary output of 5'-nucleotidase (Javitt, 1965; M. N. Eakins, personal communication; Layden & Poppe, 1977; Reichen *et al.*, 1979) and alkaline phosphatase (Reichen, 1979), and, in humans (Bode *et al.*, 1973), of alkaline phosphodiesterase (Layden & Poppe, 1977), and leucylnaphthylamidase (Reichen *et al.*, 1979).

At lower levels of administration of both bile salts the output of both enzymes was only slightly greater than the controls, except in the period 160–190 min; this correlates with a modest rise in bile salt concentration (Fig. 1). At the lower levels of bile salt administration the concentrations of bile salts in the bile are close to the critical micellar range for the particular bile salt but with the higher bile salt administration the concentrations reached in the bile are considerably in excess.

The relationship of plasma-membrane enzyme output to the critical micellar concentration range of the bile salt was investigated further and greatly supported by experiments that involved the stepwise increase in biliary bile salt concentration by stepwise increase in the amount of taurocholate added to the perfusion fluid at 100 min. It can be seen from Fig. 4 that substantial release of plasma-membrane enzymes does not occur until the concentration of bile salts in bile exceeds 10 mM, which is in the critical micellar concentration range (approx. 8 mM) for this bile salt.

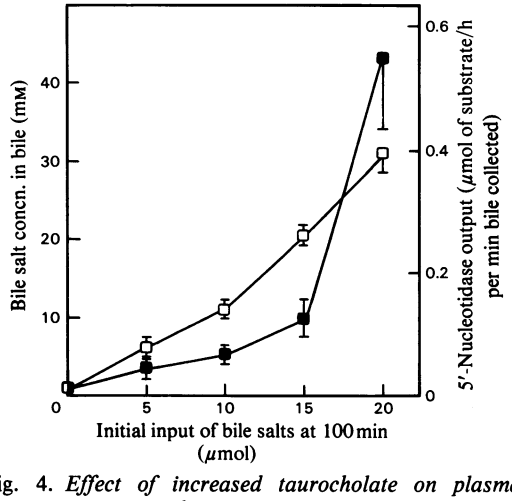

Fig. 4. *Effect of increased taurocholate on plasma-membrane enzyme output*

Bile ducts were cannulated at $t = 0$. Isolation and perfusion were established with bile salt free medium at $t = 20$. At 100 min the perfusion medium was renewed with one containing the following total amounts of taurocholate (5, 10, 15 and 20 μmol) as indicated and slow infusion of taurocholate (24 μmol/h) was commenced at 100 min in all cases. Symbols: ■, 5'-nucleotidase; □, taurocholate. Values are means ± S.E.M. of two to four experiments containing duplicate determinations.

Taurodehydrocholate is a bile salt analogue that does not form micelles; this material, even though put out into bile (along with its metabolites), appears to be unable to enhance the output of plasma-membrane enzymes (Fig. 1) and highlights important differences in the physical properties of the bile salts, which probably determine the perturbation of plasma-membrane structure and subsequent output of plasma-membrane components into the bile. In humans, Bode *et al.* (1973) have shown that dehydrocholate caused only a small elevation of biliary alkaline phosphatase activity compared with taurocholate.

Total protein output

Total protein output into bile declined rapidly after liver isolation, followed by a more gradual decrease in output up to 100 min. Perfusion with taurocholate or glycocholate caused a small increase in the output of protein, which was far less marked in livers presented with taurodehydrocholate, and was not seen in the controls (Fig. 5).

As rat blood is a major source of biliary proteins (Mullock *et al.*, 1978) removal of this source of proteins would be likely to produce a rapid reduction in the amount of protein in bile and, presumably, by 100 min the proteins in the control bile would then be largely derived from the exogenous protein in the perfusion fluid (bovine serum albumin) and from hepatocytes. The most abundant protein from hepatocytes is probably secretory component (see Mullock *et al.*, 1980).

The increase in protein output observed after taurocholate and glycodeoxycholate administration must be partly due to the increased output of plasma-membrane protein (especially at 130 min), as little or no increase was seen in rats treated with taurodehydrocholate or in control rats. The total amount of protein ascribable to plasma-membrane components is, however, relatively small and thus when the bile salt pool of bile-fistula rats declines there is little decline in the overall protein content of the bile (Kakis & Youssef, 1978; Godfrey *et al.*, 1981), since this is largely made up of serum-derived proteins.

Mechanisms of protein output into bile

There are clearly several mechanisms involved in the total protein output in bile. Polymeric immunoglobulin A is removed from blood at the sinusoidal surface of the hepatocyte by a receptor (secretory component) and endocytic vesicles carry the immunoglobulin A secretory complex to the biliary pool of the cell; the mechanism by which the vesicles fuse with the canaliculus membrane is unknown and the concentration of immunoglobulin A in bile is

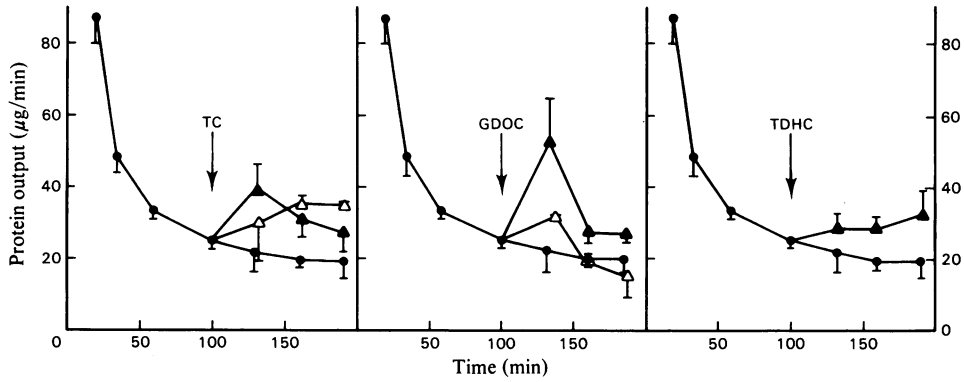

Fig. 5. *Output of protein in bile as a function of time and introduction of bile salts*
The conditions of the experiment, abbreviations and symbols are as described in the legend to Fig. 1. Values are means ± S.E.M. ($n = 17$ for controls, $n = 4$ for bile salt additions) of duplicate determinations.

higher than that in blood. If the immunoglobulin A supply from the blood is denied, secretory component is still found in bile, presumably due to loss from continuing, but empty, vesicle traffic. Similar mechanisms to that for immunoglobulin A and secretory component are probably involved with the transport of haemoglobin–haptoglobin (Mullock & Hinton, 1981).

For other serum proteins, exemplified by rat serum albumin, the concentration gradient is in the direction of blood to bile. In the present experiments the concentration in bile is approx. 2% of that in plasma, a gradient of approx. 50:1. A similar gradient (40:1) is also seen with the exogenous protein bovine serum albumin at all three concentration levels used and suggests that a similar mechanism is operating. This mechanism is unlikely to involve a receptor, since the system shows no saturation kinetics. It remains to be seen whether the mechanism is paracellular, i.e. via a small amount of leakage through tight junctions (see Dive & Heremans, 1974), or a non-specific fluid pinocytosis through ductular cells (see Thomas, 1980; Jones *et al.*, 1980). Such mechanisms would show similar kinetic properties but will require further experimental approaches to resolve them.

Lysosomal enzymes are present in bile (De Duve & Wattiaux, 1966; Toyoda *et al.*, 1977; Mullock *et al.*, 1978; La Russo & Fowler, 1979) and these occur in the absence of cytosolic proteins (Godfrey *et al.*, 1981); this output must therefore represent a specific loss of lysosomal contents. Acidic polysaccharides and Triton WR 1339 have been shown to be eliminated in bile (Kagawa & Tomizawa, 1980; La Russo *et al.*, 1981); these are normally carried in lysosomes.

The presence of plasma-membrane enzymes in bile, in the absence of lytic cell damage, indicates a phenomenon specifically related to the plasma membrane. The present experiments show a good correlation of plasma-membrane enzyme output with bile salt output in that the output of the enzymes declines as bile salt concentration declines and is restored by high biliary bile salt levels (which do not alter the output of other proteins). The correlation with bile salt concentration changes is more marked than can be seen with bile-fistula rats (Godfrey *et al.*, 1981), probably due to the ability of bile-fistula rats to maintain a modest bile salt output due to enhanced biosynthesis (see above).

Presentation of bile salts to the plasma membrane of intact cells results in specific pre-lytic release of plasma-membrane material; the initial release of this material from hepatocytes (Billington *et al.*, 1980) and erythrocytes (Billington & Coleman, 1978) is largely in a sedimentable form, and, in the case of erythrocytes, was shown to be in the form of small vesicles. At higher bile salt concentrations presented to the cells an increasing amount of the material, both proteins and lipids, was in solubilized form (Billington & Coleman, 1978). Sedimentable material containing plasma-membrane enzymes occurs in bile (Godfrey *et al.*, 1981) but its physical nature at different bile salt concentrations remains to be established.

We thank the M.R.C. for financial assistance. S. G. B. is in receipt of an M.R.C. Studentship.

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