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Further evaluation of the interrelationship between the hepatocellular transport of bile acids and endocytosed proteins

M.C. Herrera, M.Y. El-Mir, M.J. Monte, F. Perez-Barriocanal and J.J.G. Marin
Department of Physiology and Pharmacology, University of Salamanca, 37007-Salamanca, Spain

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Summary. Experiments on the relationship between the hepatocellular transport of endogenous or exogenously loaded bile acids (sodium taurocholate, TC, 0.5 μmol/min/100 g body wt) and horseradish peroxidase (HRP) or immunoglobulin A (IgA) (0.5 mg/100 g body wt) were carried out on anaesthetized Wistar rats. The time course of HRP excretion into bile (acceleration in the secretory peak), but not the total amount of HRP output, was affected by TC infusion. Administration of HRP was found to have no stimulatory effect on either spontaneous or TC-induced bile flow, bile acid, lecithin or cholesterol output. Spontaneous bile acid output was increased (25 and 67%, respectively) in rats that were treated for 12-h fasting or by oral administration of TC (45 mg/100 g body wt, every 12 h, for 2 days). These manoeuvres did not change the inability of HRP and IgA to increase bile acid output. Exogenous TC load had no stimulatory effect on the hepatocellular transport of endogenous bile acid pool, that was labelled by a combination of fasting and oral administration of ^{14}C-glycocholic acid 12 h before the experiments. Therefore, exogenous bile acid load-induced stimulation of transcytosis had no effect on endogenous bile acid output. Moreover, bile secretion of both endogenous and exogenously loaded bile acids is unaffected by the administration of proteins, irrespective of whether they are endocytosed by a receptor or non-receptor mediated process.

Keywords: bile acids, bile proteins, horseradish peroxidase, immunoglobulin A

Proteins are able to enter hepatocytes dissolved in extracellular fluid either after sequestration by invagination of the cell membrane during fluid-phase endocytosis, or bound to specific receptors located on this membrane during receptor-mediated endocytosis. Non-specific adsorption onto the plasma membrane is an unusual method of hepatocyte internalization of plasma-derived proteins. Once within the cell, two pathways may be followed by the protein-containing vesicles, either directly toward the basolateral or canalicular membrane for exocytosis or indirectly after being processed by a more complex route involving interaction(s) with other intracellular compartment(s) (Silverstein *et al.* 1977; Coleman 1987). The traffic of proteins from plasma to bile has been

Correspondence: Jose Juan Garcia Marin, Departamento de Fisiologia y Farmacologia, Facultad de Farmacia, Aptdo 449, Campo Charro s/n, 37080-Salamanca, Spain.

reported to occur mainly across the hepatocytes via a vesicular transport mechanism. This requires an intact and functioning microtubular network (Kacich *et al.* 1983; Crawford *et al.* 1988; Pfaff *et al.* 1957).

Several studies using cytochemistry and autoradiography have confirmed the existence of a vesicular pathway for secretion of both horseradish peroxidase (HRP) (Renston *et al.* 1980b) and immunoglobulin A (Renston *et al.* 1980a) into bile. HRP is a glycoprotein of molecular weight 40 kDa that has been used as a marker for fluid-phase endocytosis by the liver for several reasons, namely (i) HRP is not naturally present in bile; (ii) it is easy to determine because of its enzymatic activity; (iii) the transcytosis pathway accounts for a substantial fraction of its blood-to-bile transport (Lake *et al.* 1985). Although a small amount of HRP is classically assumed to reach the bile through the paracellular pathway, resulting in an early peak, the protein is mainly taken up by the hepatocytes at the sinusoidal membrane. Part of it is catabolized and the rest is destined for biliary secretion. Recently, it has been postulated that, while there may be a contribution from the paracellular pathway, HRP secretion into bile occurs mainly via two vesicle-mediated processes which have different properties as far as their time courses and sensitivity to colchicine are concerned (Hayakawa *et al.* 1990). Immunoglobulin A (IgA) belongs to the group of proteins directed to bile after receptor-mediated endocytosis. At the sinusoidal membrane of the hepatocytes IgA binds to a glycoprotein which is also present on the plasma membrane of other epithelial cells. Once internalized in endocytic vesicles, this receptor migrates together with IgA within the cell, and part of it—the so-called "secretory component"—is directed toward the canaliculi, where it is released into the bile (Mullock *et al.* 1980; Mullock & Hinton 1981).

A considerable body of evidence has established that bile acids are able to modulate blood-to-bile transfer of proteins after either fluid-phase or receptor-mediated endocytosis (for review see Coleman 1987). Moreover, under certain circumstances bile acids themselves may use the vesicular-mediated pathway across the hepatocyte (Lamri *et al.* 1988). However, the exact mechanism of the interaction between the vesicular secretory pathways for bile acids and endocytosed proteins is not yet clear. The aim of the present study was to gain information on three aspects of this interaction. Firstly, we studied the time-dependence of the acceleration of transcytosis by an exogenous load of bile acids. Secondly, we investigated the possibility that this stimulation might also affect endogenous bile acid mobilization toward the canaliculi. Finally, we attempted to discover whether endocytosed proteins (either by a fluid-phase or receptor-mediated mechanism) might have any effect on the biliary secretion of bile acids, either under basal conditions or after modifying the hepatic disposal of these compounds.

Materials and methods

Chemicals

Horseradish peroxidase, human immunoglobulin A, taurocholic acid, and 3α-hydroxysteroid dehydrogenase were purchased from Sigma Chemical Co. (St Louis, Mo). Labelled glycocholic acid was from New England Nuclear (DuPont, Itisa, Madrid, Spain). Enzymes and substrates used in plasma glucose determination and in bile lipid measurements were obtained from Biomerieux (Valladolid, Spain). All other chemicals were from Merck (Darmstadt, Germany) or Boehringer (Mannheim, Germany).

Animals

Male Wistar CF rats (200–250 g) (Faculty of Pharmacy, Salamanca, Spain) were used. The animals were fed commercial pelleted rat food (Panlab, Madrid, Spain) and water *ad libitum*. Lighting was controlled by a timer that permitted light between 0800 and 2000 h. Experimental groups comprised, in addi-

tion to untreated control rats, fasted rats 12 h before the experiments and animals that were treated by oral administration of sodium taurocholate (45 mg/100 g body wt., dissolved in 0.5 ml of 150 mM NaCl), every 12 h (at 1000 and 2200 h) for 2 days before collecting bile samples (experiments were carried out at 1600 h, approximately). Trace amounts of ^{14}C-glycocholic acid ($\approx 1.8 \times 10^6$ d.p.m./100 g body wt, specific activity 43.0 mCi/mmol) dissolved in 0.5 ml of 150 mM NaCl were given orally to some rats, after which the animals were fasted until the time of the experiment (12 h later), to reduce faecal loss of radioactivity.

Bile secretion studies

The rats were anaesthetized by i.p. administration (5 mg/100 g body wt.) of sodium pentobarbital (Claudio Barcia SA, Madrid, Spain). Rectal temperature was maintained throughout the experiments at 37.0±0.5°C by means of a digital temperature control system. The animals were surgically prepared as described previously (Marin & Esteller 1984) for intravenous administration and for sampling bile and blood. Bile was collected through a catheter (No. 1 ID 0.3 mm and OD 0.7 mm, Biotrol Pharma, Paris, France) placed in the common bile duct. Immediately after cannulation, the animals were left for an equilibration period of 30 min. Following this, bile was collected in preweighed vials. Some rats received sodium taurocholate by jugular infusion (0.5 μmol/min/100 g body wt, dissolved at 150 mM NaCl) using a peristaltic pump (Microperpex, LKB Instruments, Broma, Sweden) at an infusion rate of 50 μl/min, from min 40. For each experiment, HRP and IgA were freshly dissolved in 150 mM NaCl and given as a bolus (0.5 mg/100 g body wt, injected over a 30-s interval into the left jugular vein) at min 60 or100 as indicated in the Results.

Analytical procedures

Bile flow was determined gravimetrically assuming a density of 1.0 g/ml, and bile flow was expressed as microlitres per min per 100 grams of body weight (μl/min/100 g body wt). Total bile acid concentrations in bile were measured by an enzymatic technique (Talalay 1960) using 3α-hydroxysteroid dehydrogenase. HRP concentrations in bile were determined spectrophotometrically measuring the peroxidase activity by the method of Putter and Becker (1983). Viability of the preparations was confirmed by measuring the microhaematocrit and glycaemia at both the beginning and end of the experimental period. No significant modification was found in any of the treated groups. Glycaemia was determined enzymatically (Kunst *et al.* 1984) using glucose oxidase and peroxidase. Total lipids were extracted by the method of Folch *et al.* (1957). Bile lecithin molecules were hydrolysed by phospholipase D and the choline released was measured by the TRINDER reaction (Gurantz *et al.* 1981; Takayama *et al.* 1977). Biliary cholesterol concentrations were determined according to three coupled enzymatic reactions catalysed by cholesterol esterase, cholesterol oxidase and peroxidase, respectively (Bolton *et al.* 1980). Radioactivity in bile samples due to the presence of ^{14}C-glycocholic acid was measured in a liquid scintillation counter (LS-1800-Beckman, Beckman Instruments España SA, Madrid, Spain). Ready Safe Scintillation Cocktail, also from Beckman, was used as the scintillant. Thin-layer chromatography was carried out to confirm the purity of the radioactive bile acid both in the commercial preparation and in bile samples. Silica gel 60 F_{254} plates (Merck) were used in conjunction with a solvent system of the following composition: iso-amyl acetate/propionic acid/1-propanol/water (4:3:2:1, by vol.) (Hofmann 1962). The recovery was >85% of deposited radioactivity and the purity was found to be always >95%.

Statistical analysis

Results are expressed as means±s.e. To calculate the statistical significance of differ-

ences among groups, the Bonferroni method of multiple-range testing was used, except for single comparisons of two means, when Student's *t*-test was used. Statistical significance within a group was calculated by comparing, with a paired *t*-test, data obtained before and after protein injection or the starting of TC infusion. Statistical analyses were made on a Macintosh computer (Apple Computer, Inc., Cupertino, Ca) with programs supplied by Apple Computer, Inc.

Results

Time-dependence of TC-induced acceleration of HRP output

Figure 1a shows that HRP output into bile was observed shortly after injection of the protein into the left jugular vein. The maximal secretory peak was observed to occur 30 min after injection. Determination of peroxidase activity in bile samples collected for 100 min indicated that cumulated HRP output (Fig. 2) was only ~0.1% of the injected dose. The shape of the curve (bile HRP output *vs* time) was sharper and the time required to reach the secretory peak was shorter under TC infusion (Fig. 1b and c). This acceleration differed depending on the time when HRP was administered after starting TC infusion. Thus, when injected at min 60 (i.e. 20 min after starting TC infusion) the HRP peak appeared in bile 5 min earlier. The acceleration was greater (10 min earlier or secretory peak at 20 min after HRP injection) if HRP was injected 60 min after starting TC infusion. However, TC infusion, regardless of the HRP injection time after starting TC infusion, had no effect on cumulative HRP output up to 100 min after HRP administration (Fig. 2).

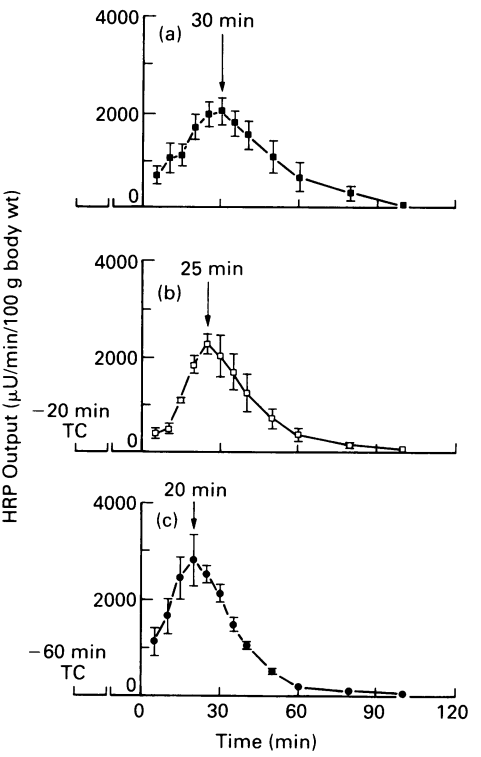

Fig. 1. Time course of biliary HRP output after bolus administration (0.5 mg/100 g body wt) to a, rats receiving no bile acid infusion ($n=6$) or during the infusion of sodium taurocholate (0.5 μmol/min/100 g body wt). HRP bolus was given at b, min 60 ($n=4$) or c, min 100 ($n=5$), i.e. 20 or 60 min after starting taurocholate infusion, respectively. Arrows indicate delay time from bolus administration to peak secretion in bile. Values are means ± s.e.

Effect of HRP on bile formation

The administration of HRP was found to have no significant effect on spontaneous bile flow or bile acid output (Fig. 3). Neither basal lecithin nor cholesterol output was affected by HRP administration (Table 1). Neither did HRP show any ability to stimulate TC-induced bile flow (Fig. 4a) or the biliary output of bile acids (Fig. 4b), lecithin or cholesterol (Table 1). An absence of effect of the HRP on TC-induced bile formation was observed regardless of whether HRP was injected at 20 or 60 min after starting TC infusion. We wondered whether the load of intrahepatic stores for bile acids could change the response to TC infusion or to HRP administration. Prolonged exposure of the

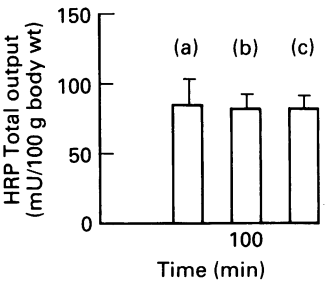

Fig. 2. Cumulative biliary HRP output after bolus administration (0.5 mg/100 g body wt) to rats receiving a, no bile acid infusion ($n=6$) or during the infusion of sodium taurocholate (0.5 μmol/min/100 g body wt). HRP bolus was given at b, min 60 ($n=4$) or c, min 100 ($n=5$), i.e. 20 or 60 min after starting taurocholate infusion, respectively. Values are means ± s.e.

liver to an increased bile acid flux has been reported to increase the maximum secretory rate of these compounds (Adler *et al.* 1977; Watkins & Klaassen 1981). Although induction of new transport sites in the canalicular membrane could allow for this effect (Simon *et al.* 1982), an activation of transcellular pathway might also be involved in an adaptive regulation of bile acid secretion. Therefore, we considered it to be of interest to carry out experiments, similar to those described above, on rats treated in one of the following ways: either after fasting for 12 h (to reduce the amount of bile acids within the extrahepatic fraction of the enterohepatic circulation) or after receiving TC orally for 2 days, (to load the bile acid pool). These treatments led to a significant (both $P<0.05$) increase in spontaneous bile acid output, as calculated from the first 20 min bile sample. This value was 154 ± 13 nmol/min/100 g body wt in untreated rats ($n=13$). In fasted and TC-fed animals the values were 193 ± 11 ($n=17$) and 258 ± 29 ($n=12$) nmol/min/100 g body wt, respectively. These results obtained from these groups in the experiments with TC infusion (Table 2) pointed to an absence of a stimulatory effect of HRP on bile acid output, even under these conditions. Artifactual

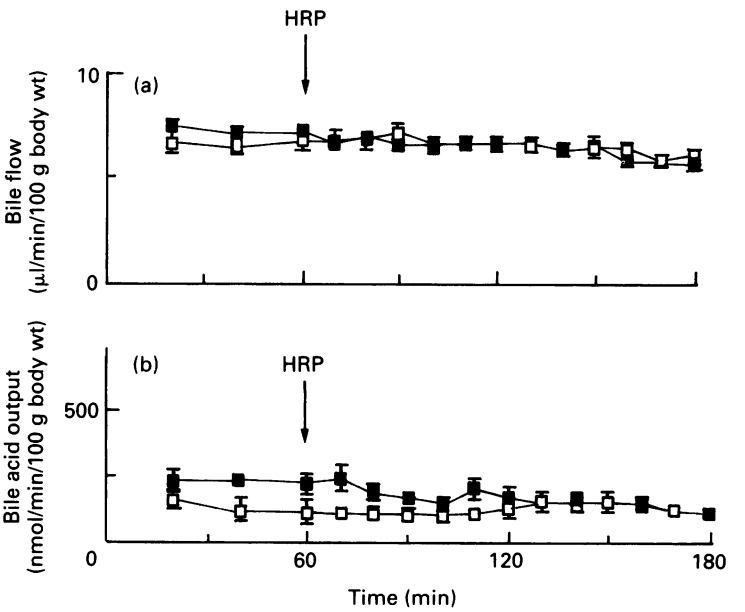

Fig. 3. a, Spontaneous bile flow and b, bile acid output in □, control ($n=8$) and ■, HRP (0.5 mg/100 g body wt) treated rats ($n=7$). Values are means ± s.e. No significant effect of HRP administration was found.

Table 1. Effect of HRP administration on taurocholate-induced biliary lipid output

Taurocholate	HRP	Injection time (min)	nmol/min/100 g body wt		n
			Cholesterol	Lecithin	
No	No	—	2.31±0.07	17.5±0.7	12
No	Yes	60	2.30±0.10	16.6±0.4	12
Yes	No	—	3.69±0.24	46.5±4.8	8
Yes	Yes	60	4.20±0.42	41.0±8.1	4
Yes	Yes	100	4.05±0.19	49.6±3.9	7

Values are means±s.e. from average values obtained from three 20-min samples collected after HRP administration. Some rats received taurocholate infusion (0.5 μmol/min/100 g body wt) from min 40 and HRP bolus (0.5 mg/100 g body wt) at the time indicated. No significant effects of HRP administration were found.

results due to changes in 3α-hydroxysteroid dehydrogenase activity were ruled out because in preliminary assays we confirmed that the presence of HRP has no effect on the analytical method used to measure bile acid concentrations in bile samples.

Effect of IgA or an exogenous TC load on endogenous bile acid secretion

To test the existence of differences in the bile acid secretory response to proteins internalized by the hepatocyte following different mechanisms, i.e. receptor and non-receptor mediated endocytosis, we studied the effect of IgA administration on 3α-hydroxy bile acid output. Immunoglobulin A from human serum was used because it is capable both of binding to plasma membrane receptors of rat hepatocytes (Fisher *et al.* 1979) and of being secreted into bile in a similiar way as rat IgA (Gebhardt 1983). The results indicated that, like HRP, IgA had no effect on bile acid output (Fig. 5b). To confirm this another set of experiments was undertaken with 12-h fasted rats receiving an oral dose of ^{14}C-glycocholic acid (^{14}C-GC) 12 h before the experiments. In this group no increase in bile radioactivity was observed after IgA administration (data not shown). The endogenous bile acid pool was labelled in the same way with ^{14}C-GC, and the effect of TC infusion was studied 12 h later. As shown in Fig. 6, TC infusion was not able to accelerate the mobilization of radioactivity output into bile. These results are in agreement with those obtained with TC infusion in rats whose hepatic bile acid disposal was artificially increased (by fasting or TC-feeding), where no increase in bile acid output was found by comparison with untreated animals (Table 2 and Fig. 4b).

Discussion

The ability of bile acids to stimulate HRP output into bile has been well documented in rats (Lorenzini *et al.* 1986) and in isolated perfused rat livers (Hayakawa *et al.* 1990). Our results confirm and extend these findings, adding some useful information about the time dependence of this effect. The response of the liver to TC infusion was reflected in an acceleration in the HRP traffic toward the canaliculus but not an enhancement in net protein excretion. It is also notable that when HRP was administered, once bile acid output had reached a steady

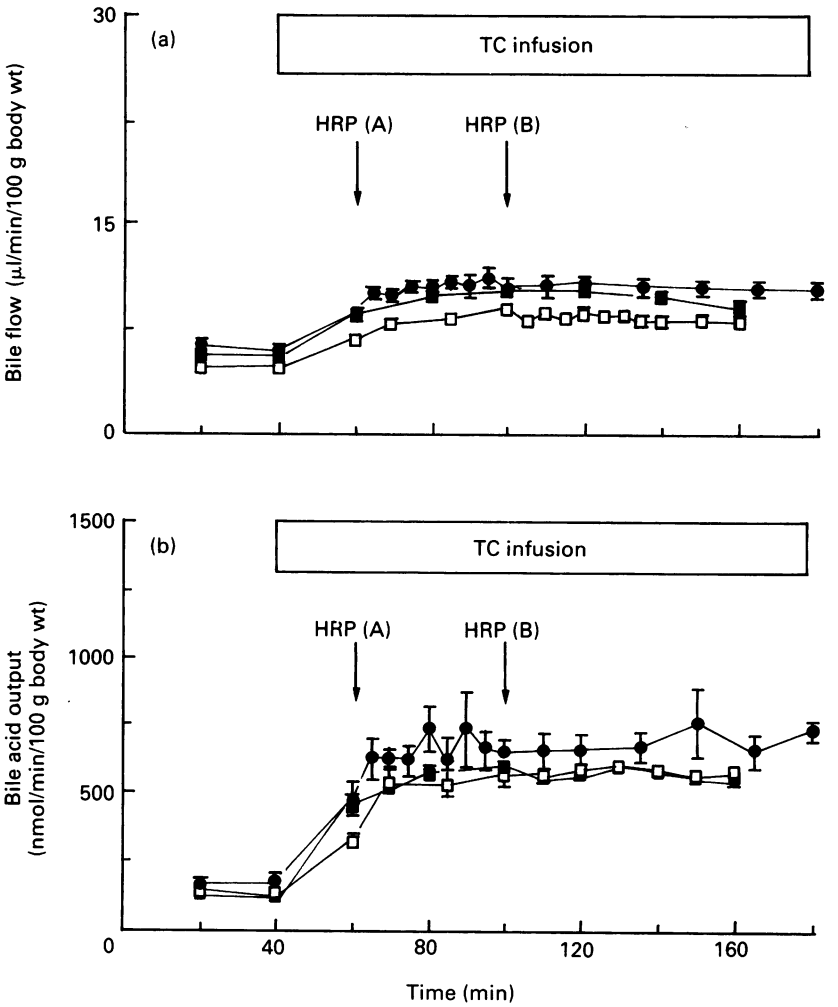

Fig. 4. Effect of HRP administration (0.5 mg/100 g body wt.) on taurocholate infusion (0.5 μmol/min/100 g body wt)-induced a, bile flow and b, bile acid output. ■, Control rats ($n=9$) did not receive HRP. HRP bolus was given at □, min 60 ($n=4$) or ●, min 100 ($n=8$), i.e. 20 or 60 min after starting taurocholate infusion, respectively. Values are means ± s.e. No significant differences were found when groups shown in the same panel were compared.

state, the acceleration was greater than if the protein was injected during the rising of bile acid output after starting TC infusion. This suggests dependence on a sufficiently high intracellular bile acid concentration or the existence of time-dependence of the interaction between secretory events. Among possible explanations for the mechanism(s) underlying this effect, a faster transcytosis of intracellular vesicles and a faster bile acid-induced stimulation of membrane fusion events in the pericanalicular area (Sakisaka *et al.* 1988) may be considered. The absence of a TC-induced increase in the net secretion of HRP suggests that recruitment of a larger number of vesicles can be ruled out. However, caution is required before reaching this conclusion because, although our results

Table 2. Effect of changes in hepatic bile acid pool disposal on bile flow and bile acid output in rats receiving taurocholate infusion and HRP bolus

Treatment	HRP injection time (min)	Bile flow (μl/min/100 g body wt)	Bile acid (nmol/min/100 g body wt)	*n*
Taurocholate-fed	No	10.63±0.98	695.9±65.4	7
Taurocholate-fed	60	9.47±0.96	629.9±35.3	5
Taurocholate-fed	100	10.57±0.80	715.9±98.1	7
Fasted	No	9.30±1.01	754.1±50.3	6
Fasted	60	10.56±0.68	719.5±23.7	6
Fasted	100	9.64±0.83	711.5±45.3	6

Values are means±s.e. from average values obtained from three 20-min samples collected after HRP administration. Rats received taurocholate infusion (0.5 μmol/min/100 g body wt) from min 40 and HRP bolus (0.5 mg/100 g body wt) at the time indicated. No significant effects of HRP were observed as compared to experimental groups with the same treatment (12 h fasting or taurocholate-feeding for 2 days, at an oral dose of 45 mg/100 g body wt every 12 h) with or without HRP administration.

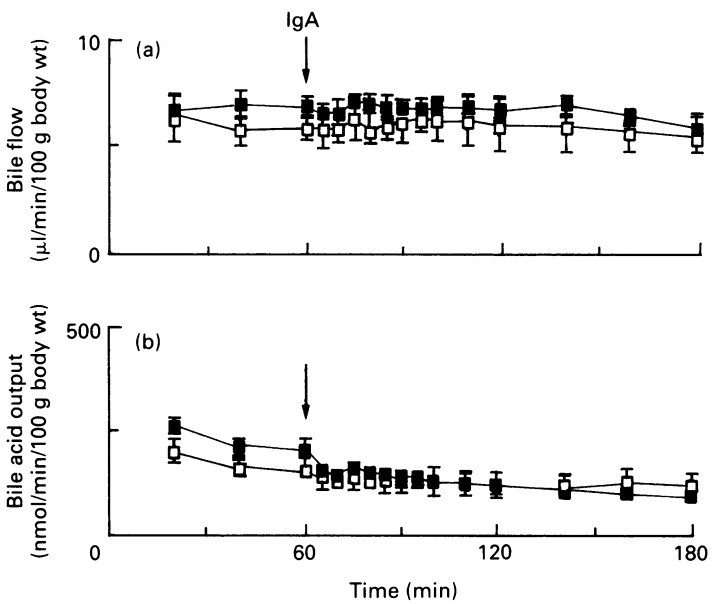

Fig. 5. a, Spontaneous bile flow and b, bile acid output in □, control ($n=8$) and ■, immunoglobulin A (0.5 mg/100 g body wt) treated rats ($n=4$). Values are means±s.e. No significant effect of immunoglobulin A administration was found.

which indicate that taurocholate is unable to stimulate the net biliary secretion of fluid-phase markers agree with those obtained by other authors (Lake *et al.* 1985; Scharschmidt *et al.* 1986), other groups have reported a slight (Lorenzini *et al.* 1986) or marked (Hayakawa *et al.* 1990) enhancement in HRP output under bile acid infusion. Although at first sight it appears difficult to reconcile these different findings, there are

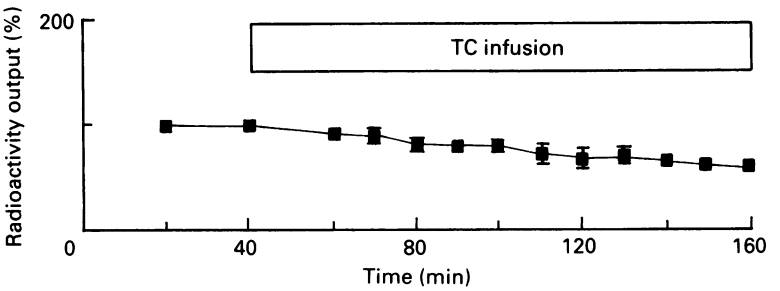

Fig. 6. Biliary ^{14}C-glycocholic acid output in rats receiving taurocholate infusion (0.5 μmol/min/100 g body wt). The animals ($n=4$) were given ^{14}C-glycocholic acid orally 12 h before carrying out the experiments. Values are means ± s.e.

important differences in the experimental model and design as well as in the amount of HRP used in these studies that could be responsible for the diversity of the results. Moreover, when considering the biliary output of this protein it should be kept in mind that unseen differences in the response of the diversion of HRP toward the degradative pathway could alter the results obtained. For instance, this effect seems to play an important role in the dissociation of biliary response to valproic acid administration to rats. This short-chain fatty acid induces an acceleration of biliary HRP output together with a reduction in cumulative secretion (Jezequel *et al.* 1986). Moreover, Bellringer *et al.* (1988) have demonstrated that valproic acid modifies protein transfer across the hepatocyte by acting on the microtubular–vesicular system. In this respect it is interesting to note that valproic acid induces an impairment in acid phosphatase, serum albumin, phospholipids, IgA and HRP secretion into bile, while it has no effect on spontaneous bile acid secretion (Bellringer *et al.* 1988). This would support the existence of a hepatocellular transport route for bile acids which is independent of microtubules (Kacich *et al.* 1983). Another route, which is colchicine sensitive, has been proposed for bile acid load (Barnwell *et al.* 1984; Crawford *et al.* 1988; Dubin *et al.* 1980; Gregory *et al.* 1978). Autoradiography (Suchy *et al.* 1983) and immunohistochemistry (Lamri *et al.* 1988) have confirmed the participation of smooth endoplasmic reticulum and the Golgi apparatus in the vectorial pathway of bile acids from the sinusoidal membrane toward the canaliculus. Our data on the insensitivity of endogenous bile acid secretion to transcytosis activation by an exogenous load of taurocholate provide additional functional evidence to support the existence of two different and probably separate secretory pathways for bile acids. Although the cytoskeleton-dependent pathway is suggested to play a role only as 'complementary pathway', the exact quantitative contribution of the two processes in basal conditions is unclear. Thus, an impairment in spontaneous bile acid secretion and, hence, the cholestasis that follows the administration of some drugs such as cyclosporin A, has been suggested to be due, in part, to an inhibition of the normal vesicular pathway (Roman *et al.* 1990).

Because the integrity of the cytoskeleton is important for the normal biliary transfer of endocytosed proteins as well as bile acids during bile acid load, many studies have been performed to elucidate the effect of microfilament inhibitors, such as phalloidin (Dubin *et al.* 1980; Rahman *et al.* 1986), and microtubule disruptors, such as colchicine (Barnwell *et al.* 1984; Dubin *et al.* 1980; Gregory *et al.* 1978; Lowe *et al.* 1984), on the biliary secretion. However, little attention has been devoted to the sensitivity of the

vesicular routes to the compounds being transported. There is evidence to suggest the existence of a vesicular traffic which is independent of the presence of the protein to be transported (Kloppel *et al.* 1987). Thus, the 'secretory component' is present in bile collected from isolated rat livers, even when they are perfused with IgA-free media. This does not necessarily mean that the *rate* of this traffic is insensitive to the carried compounds; thus, the rate of fluid-phase endocytosis of ^{14}C-sucrose has been reported to be accelerated in sucrose-loaded rats (LeSage *et al.* 1990). However, in this model the liver is exposed for a prolonged period to a large dose of sucrose which causes hypertrophy of the bile hepatocyte vacuolar apparatus. Therefore, an interesting question arises as to whether acute exposure to potentially endocytosed proteins is able to modify the rate of the transport. If this were the case, does an indirect stimulation of the transcytosis for other users of the vesicular pathways, namely, bile acids, exist? Our results from acute experiments to determine the effect of HRP or human IgA administration on bile acid, lecithin and cholesterol output, suggest that the answer to this question is probably negative. This is in agreement with results obtained by LeSage *et al.* (1990) who found no change in bile acid output despite the expansion of the vacuolar apparatus in sucrose-loaded rats. These findings may be interpreted as being due to the absence of a protein-induced activation of the vesicular pathway or to the existence of different routes for vesicles carrying either bile acids or endocytosed proteins. There is evidence to suggest that the vesicular routes associated with HRP or IgA, contrary to that for bile acids, are independent of the Golgi apparatus (Hashieh *et al.* 1989), which may support the latter possibility.

Bile acids enhance the biliary excretion of lecithin and cholesterol (Crawford *et al.* 1988; Rahman *et al.* 1986; Rahman & Coleman 1987) by a mechanism which is not yet well defined but in which the movement of intracellular vesicles and their subsequent fusion with the canalicular membrane may also be involved (Crawford *et al.* 1988; Reuben & Allen 1986). Our data indicate that neither the time-course of spontaneous or TC-induced biliary lipid excretion (data not shown) nor their net output are modified by HRP administration. This is in agreement with the insensitivity of the transcellular vesicular pathway to the presence of HRP.

In summary, the present results confirm the endocytosed proteins, but not endogenous bile acids, are sensitive to transcytotic stimulation by exogenous bile acid load. By contrast, both endogenously and exogenously loaded bile acid outputs into bile are insensitive to the administration of proteins that are endocytosed by receptor or non-receptor mediated processes.

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References

Adler R.D., Wannagat F.J. & Ockner R. (1977) Bile secretion in selective biliary obstruction. Adaptation of taurocholate transport maximum to increased secretory load in the rat. *Gastroenterology* **73**, 129–136.

Barnwell S.G., Lowe P.J. & Coleman R. (1984) 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. *Biochem. J.* **220**, 723–731.

Bellringer M.E., Rahman K. & Coleman R.

(1988) Sodium valproate inhibits the movement of secretory vesicles in rat hepatocytes. *Biochem. J.* **249**, 513–519.

BOLTON C.H., NICHOLS J.S. & HEATON K.W. (1980) Estimation of cholesterol in bile: Assessment of an enzymatic method. *Clin. Chim. Acta* **105**, 225–230.

COLEMAN R. (1987) Biochemistry of bile secretion. *Biochem. J.* **244**, 249–261.

CRAWFORD J.M., BERKEN C.A. & GOLLAN J.L. (1988) Role of the hepatocyte microtubular system in the excretion of bile salts and biliary lipids: Implications for intracellular vesicular transport. *J. Lipid Res.* **29**, 144–156.

DUBIN M., MAURICE M., FELDMANN G. & ERLINGER S. (1980) Influence of colchicine and phalloidine on bile secretion and hepatic ultrastructure in the rat. Possible interaction between microtubules and microfilaments. *Gastroenterology* **79**, 646–654.

FISHER M.M., NAGY B., BAZIN H. & UNDERDOWN B.J. (1979) Biliary transport of IgA: Role of secretory component. *Proc. Natl Acad Sci. USA* **76**, 2008–2012.

FOLCH J., LEES M. & SLOANE-STANLEY G.H. (1957) A simple method for the isolation and purification of total lipids from animals tissues. *J. Biol. Chem.* **226**, 497–509.

GEBHARDT R. (1983) Primary culture of rat hepatocytes as a model system of canalicular development, biliary secretion and intrahepatic cholestasis. III Properties of biliary transport of immunoglobulin A revealed by immunofluorescence. *Gastroenterology* **84**, 1462–1470.

GREGORY D.H., VLAHCEVIC Z.R., PRUGH M.F. & SWELL L. (1978) Mechanism of secretion of biliary lipids: role of microtubular system in hepatocellular transport of biliary lipids in the rat. *Gastroenterology* **74**, 93–100.

GURANTZ D., LAKER M.F. & HOFMANN A.F. (1981) Enzymatic measurement of choline-containing phospholipids in bile. *J. Lipid Res.* **22**, 373–376.

HASHIEH I.A., REMY L., MATHIEU S. & GEROLAMI A. (1989) The effect of monensin on the transport of horseradish peroxidase into intracellular lumina in cultured rat hepatocytes. *Hepatology* **10**, 61–65.

HAYAKAWA T., CHENG O., MA A. & BOYER J.L. (1990) Taurocholate stimulates transcytotic vesicular pathways labeled by horseradish peroxidase in the isolated perfused rat liver. *Gastroenterology* **99**, 216–228.

HOFMANN, A.F. (1962) Thin-layer adsorption chromatography of free and conjugated bile acids on silicic acid. *J. Lipid Res.* **3**, 127–128.

JEZEQUEL A.M., MACARRI G., RINALDESI M.L., VENTURINI C., LORENZINI I. & ORLANDI F. (1986) The fate of electron opaque tracers (horseradish peroxidase and lanthanum chloride) during valproic acid-induced choleresis. *Liver* **6**, 341–349.

KACICH R.L., RENSTON R.H. & JONES A.L. (1983) Effects of cytochalasin D and colchicine on the uptake, translocation and biliary secretion of horseradish peroxidase and [^{14}C] sodium taurocholate in the rat. *Gastroenterology* **85**, 385–394.

KLOPPEL T.M., HOOPS T.C., GASKIN D. & LE M. (1987) Uncoupling of the secretory pathway for IgA and secretory component by cholestasis. *Am. J. Physiol.* **253**, G232–G240.

KUNST A., DRAEGER B. & ZIEGENHORN J. (1984) Colorimetric methods with glucose oxidase and peroxidase. In *Methods of Enzymatic Analysis*. Volume 6, 3rd ed. Eds. H.U. Bergmeyer, J. Bergmeyer & M. Grabl. Weinheim: Verlag Chemie, pp.175–185.

LAKE J.R., LICKO V., VAN DYKE R.W. & SCHARSCHMIDT B.F. (1985) Biliary secretion of fluid-phase markers by the isolated perfused rat liver. Role of transcellular vesicular transport. *J. Clin. Invest.* **76**, 676–684.

LAMRI Y., RODA A., DUMONT M., FELDMANN M. & ERLINGER S. (1988) Immunoperoxidase localization of bile salts in rat liver cells. Evidence for a role of the Golgi apparatus in bile salt transport. *Gastroenterology* **82**, 1278–1282.

LESAGE G.D., ROBERTSON W.E. & WAUMGART M.A. (1990) Demonstration of vesicular-dependent bile flow on the sucrose-loaded rat. *Gastroenterology* **99**, 478–487.

LORENZINI I., SAKISACA S., MEIER P.J. & BOYER J.L. (1986) Demonstration of a transcellular vesicle pathway for biliary excretion of inulin in the rat liver. *Gastroenterology* **91**, 1278–1288.

LOWE P.J., BARNWELL S.G. & COLEMAN R. (1984) Rapid kinetic analysis of bile salt-dependent secretion of phospholipid, cholesterol and plasma membrane enzymes into rat. *Biochem. J.* **222**, 631–637.

MARIN J.J.G. & ESTELLER A. (1984) Biliary interrelationship between phospholipid, bilirubin and taurocholate in the anaesthetized rat. *Clin. Sci.* **67**, 499–504.

MULLOCK B.M. & HINTON R.H. (1981) Transport of proteins from blood to bile. *Trends. Biochem. Soc.* **6**, 188–190.

MULLOCK B.M., HINTON R.H., DOBROTA M., PEPPARD J. & ORLANS E. (1980) Distribution of secretory component in hepatocytes and its mode of transfer into bile. *Biochem. J.* **190**, 819–826.

PFAFF E., JAESCHKE N. & KRELL H. (1957) Quantitative estimation of transcellular and paracellular pathways of biliary sucrose in isolated perfused rat liver. *Biochem. J.* **241**, 635–640.

PUTTER J. & BECKER R. (1983) Peroxidase. In *Methods of Enzymatic Analysis*, Volume 3, 3rd ed. Eds H.U. Bergmeyer, J. Bergmeyer & M. Grabl. Weinheim: Verlag Chemie, pp. 286–293.

RAHMAN K. & COLEMAN R. (1987) Biliary phospholipid secretion and its control. Effect of taurodehydrocholate. *Biochem. J.* **245**, 531–536.

RAHMAN K., HAMMOND T.G., LOWE P.J., BARNWELL S.G., CLARK B. & COLEMAN R. (1986) Control of biliary phospholipid secretion. Effect of continuous and discontinuous infusion of taurocholate on biliary phospholipid secretion. *Biochem. J.* **234**, 421–427.

RENSTON R.H., JONES A.L., CHRISTIANSEN W.D., HRADEN G.T. & UNDERDOWN B.J. (1980a) Evidence for a vesicular transport mechanism in hepatocytes for biliary secretion of immunoglobulin A. *Science* **208**, 1276–1278.

RENSTON R.H., MALONEY A.G., JONES A.L., HRADEH G.T., WONG K.Y. & GOLDFINE I.D. (1980b) Bile secretory apparatus: evidence for a vesicular transport mechanism for proteins in the rat, using horseradish peroxidase and ^{125}I-insulin. *Gastroenterology* **78**, 1373–1388.

REUBEN A. & ALLEN R.M. (1986) Intrahepatic sources of biliary-like micelles. *Biochim. Biophys. Acta* **876**, 1–12.

ROMAN I.D., MONTE M.J., GONZALEZ-BUITRAGO J.M., ESTELLER A. & JIMENEZ R. (1990) Inhibition of hepatocytary vesicular transport by cyclosporin A in the rat: Relationship with cholestasis and hyperbilirubinemia. *Hepatology* **12**, 83–91.

SAKISAKA S., NG O.C. & BOYER J.L. (1988) Tubulovesicular transcytotic pathway in isolated rat hepatocyte couplets in culture. *Gastroenterology* **95**, 793–804.

SCHARSCHMIDT B.F., LAKE J.R., RENNER E.L., LICKO V. & VAN DYKE R.W. (1986) Fluid phase endocytosis by cultured rat hepatocytes and perfused rat liver: Implication for plasma membrane turnover and vesicular trafficking of fluid phase markers. *Proc. Natl Acad. Sci. USA* **83**, 9488–9492.

SILVERSTEIN S.C., STEINMAN R.M. & COHN Z.A. (1977) Endocytosis. *Ann. Rev. Biochem.* **46**, 669–722.

SIMON F.R., SUTHERLAND E.D. & GONZALEZ M. (1982) Regulation of bile salts transport in the liver. Evidence that increased maximum bile salts secretory capacity is due to increased cholic acid receptors. *J. Clin. Invest.* **70**, 401–411.

SUCHY F.J., BALISTRERI W.F., HUNG J., MILLER P. & GARFIELD D. (1983) Intracellular bile acid transport in rat liver visualized by electron microscope autoradiography using a bile acid analog. *Am. J. Physiol.* **245**, G681–G689.

TAKAYAMA M., ITOH S., NAGASAKI T. & TANIMUZI I. (1977) A new enzymatic method for determination of serum choline-containing phospholipid. *Clin. Chim. Acta* **79**, 93–98.

TALALAY P. (1960) Enzymatic analysis of steroid hormones. *Meth. Biochem. Anal.* **8**, 119–143.

WATKINS J.B. & KLAASSEN C.D. (1981) Effect of repeated oral administration of taurocholate on hepatic excretory function in the rat. *J. Pharmacol. Exp.* **218**, 182–187.