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Transcytosis and paracellular movements of horseradish peroxidase across liver parenchymal tissue from blood to bile

Effects of α-naphthylisothiocyanate and colchicine

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The pathways for the entry of horseradish peroxidase (HRP) into bile have been investigated using the isolated perfused rat liver operating under one-pass conditions. Following a 1 min one-pass infusion of HRP, two peaks of HRP activity were noted in the bile. The first, at 5–7 min post-infusion, correlated with the biliary secretion of the [^{3}H]methoxyinulin which was infused simultaneously with the HRP. The second peak of HRP activity occurred at 20–25 min, and correlated with the biliary secretion of ^{125}I-IgA, which was also infused simultaneously with the HRP. If the isolated livers were perfused with a medium containing 2.5 μM-colchicine, the biliary secretion of IgA and the second secretion peak of HRP were inhibited by 60%. If rats were pretreated for 12 h with α-naphthylisothiocyanate (25 mg/100 g body wt.) prior to liver isolation, the biliary secretion of [^{3}H]methoxyinulin and the first secretion peak of HRP were increased. Taken together, these results suggest that HRP enters the bile via two routes. The faster route, which was increased by α-naphthylisothiocyanate and correlated with [^{3}H]methoxyinulin entry into bile, was probably paracellular, involving diffusion across tight junctions. The slower route, which was inhibited by colchicine and correlated with the secretion of IgA, was probably due to transcytosis, possibly within IgA and other transport vesicles.

In addition to bile acids, lipids and bilirubin, bile contains many proteins (Mullock *et al.*, 1978; La Russo, 1984). The bulk of these proteins are derived from the blood plasma (Dive *et al.*, 1974; Dive & Heremans, 1974; Mullock *et al.*, 1978), although several are derived from the hepatocyte (Holdsworth & Coleman, 1975; La Russo & Fowler, 1979; Mullock & Hinton, 1981). For some of the proteins there exists a receptor-mediated vesicle transport pathway; consequently, the concentration of these proteins in the bile is higher than the concentration in the blood plasma [Mullock & Hinton, 1981 (a review including much early work); Renston *et al.*, 1980*a*; Smith *et al.*, 1981; Godfrey *et al.*, 1982; Limet *et al.*, 1982; Goldman *et al.*, 1983; Schiff *et al.*, 1984). For the other plasma-derived proteins the mode of transfer is less specific; the concentration of protein in the bile is far less than the concentration in the blood plasma. Two parallel pathways may be involved: first, the bulk transfer of unmodified plasma, and second, a pathway involving molecular sieving favouring lower M_r proteins (Dive *et al.*, 1974; Dive & Heremans, 1974; Thomas, 1980; Thomas *et al.*, 1982).

The most studied receptor-mediated transcytosis is that of IgA. IgA transcytosis does not involve the lysosomes (Mullock & Hinton, 1981) and is therefore unlike the transcytosis of many other proteins, the majority of which are taken up into lysosomes and degraded [e.g. epidermal growth factor (St Hilaire *et al.*, 1981; Burwen *et al.*, 1984) and various asialoglycoproteins (Stockert *et al.*, 1980; Thomas & Zamcheck, 1983; Schiff *et al.*, 1984)]. Many of the degradation products from lysosomes are found in the bile (St Hilaire *et al.*, 1981; Schiff *et al.*, 1984; Burwen *et al.*, 1984) together with lysosomal enzymes (La Russo & Fowler, 1979; Godfrey *et al.*, 1981; Sewell *et al.*,

Abbreviations used: HRP, horseradish peroxidase; ANIT, α-naphthylisothiocyanate.

1982; La Russo *et al.*, 1982), suggesting exocytosis of lysosomes from the biliary pole of the hepatocyte.

Non-specific transcytosis has been most studied with horseradish peroxidase (HRP), although other proteins have been used (Graham *et al.*, 1966; Grant *et al.*, 1984). HRP can enter bile in an intact form following transcytosis (Matter *et al.*, 1969; Renston *et al.*, 1980*b*, 1983; Kacich *et al.*, 1981; Bajwa & Fujimoto, 1983; Okanoue *et al.*, 1984), but the majority of HRP goes to the lysosomes, presumably for degradation (Matter *et al.*, 1969; Creamers & Jacques, 1971), as in many other cell types (Steinman & Cohn, 1972; Storrie *et al.*, 1984).

Microtubule-affecting drugs disrupt various vesicle-mediated transport pathways. The secretion of newly synthesized protein from the sinusoidal pole of the cell is inhibited by colchicine, vinblastine and vincristine (Orci *et al.*, 1973; Crane & Miller, 1974; Le Marchand *et al.*, 1974; Stein *et al.*, 1974; Redman *et al.*, 1975; Feldmann *et al.*, 1975; Banerjee *et al.*, 1976; Stein & Stein, 1977; Jeanrenaud *et al.*, 1977). The biliary secretion of lipids (Gregory *et al.*, 1978; Erlinger *et al.*, 1981; Barnwell *et al.*, 1984) and some proteins, for example IgA (Mullock *et al.*, 1980; Godfrey *et al.*, 1982; Goldman *et al.*, 1983), is reduced by colchicine and/or vinblastine. The biliary secretion of newly synthesized albumin (Mullock *et al.*, 1980; Godfrey *et al.*, 1982) and fibrinogen (Barnwell & Coleman, 1983) is, however, increased by colchicine. The non-specific transcytosis of protein is either inhibited [HRP (Kacich *et al.*, 1983; Bajwa & Fujimoto, 1983)] or unaffected [bovine serum albumin (Barnwell & Coleman, 1983)] by colchicine.

The parallel pathway to transcytosis is thought to involve a paracellular route, possibly due to a limited permeability of the hepatocyte tight junctions. This pathway is normally probed with lower M_r molecules such as sucrose (342) and inulin (5000) (Bradley & Herz, 1978; Boyer *et al.*, 1979; Jaeschke *et al.*, 1983; Krell *et al.*, 1984; Accatino *et al.*, 1984; Tavaloni, 1984). However, several studies indicate the possibility of junctional diffusion of certain circulating plasma proteins (Dive & Heremans, 1974; Thomas, 1980; Thomas *et al.*, 1982; Barnwell *et al.*, 1983).

In the present study we have used two techniques to study the non-specific entry of horseradish peroxidase into bile. In the first, HRP was injected into the circulation of bile fistula rats; in the second, HRP was infused for 1 min into isolated, one-pass perfused, rat livers. We have made use of drugs which selectively perturb each of the two parallel pathways; colchicine, which disrupts microtubules and hence vesicle transport, and ANIT, which has previously been shown to increase the permeability of the hepatocyte tight junctions to sucrose, inulin and phosphate (Krell *et al.*, 1982; Jaeschke *et al.*, 1983). The appearance of enzymically active HRP in bile was determined at frequent intervals, together with the appearance of several other proteins, including both endogenous and exogenous rat proteins, and [^{3}H]methoxyinulin.

Materials and methods

Materials

Antisera to rat IgA, rat serum albumin, rat fibrinogen and bovine serum albumin were purchased from Nordic Immunological Laboratories, Maidenhead, Berks., U.K. Sagatal was obtained from May and Baker, Dagenham, Essex, U.K. Cannulation tubing PP10 was manufactured by Portex, Hythe, Kent, U.K. and heparin was made by Weddel Pharmaceuticals, London EC1, U.K. HRP, colchicine, ANIT and other fine chemicals were obtained from Sigma. [^{3}H]Methoxyinulin was obtained from New England Nuclear and ^{125}I from Amersham International.

Bile fistula rats

Male Wistar rats, weighing approx. 250g, were used throughout; these had been maintained on a standard laboratory diet and under a constant light cycle. Bile duct cannulations were performed whilst each rat was under pentobarbital (Sagatal) anaesthesia, using 500mm of PP10 tubing. After cannulation, bile was collected for 30min, then 25mg of horseradish peroxidase together with 200mg of bovine serum albumin and 50μCi of [^{3}H]inulin (dissolved in approx. 1ml of sterile saline) was injected into the inferior vena cava. Bile was subsequently collected in 2×5min, then 8×10min, samples. Bile was collected on ice, weighed, and stored at -20°C until assayed. At the end of the experiment the animal was killed and the liver removed and homogenized in iso-osmotic saline, with a tight fitting Potter–Elvehjem homogenizer.

ANIT, finely dispersed in olive oil at a concentration of 0.2g/ml, was administered to the rats at a dose of 25mg/100g body wt. by stomach tube whilst under light ether anaesthesia. Controls received an equal amount of olive oil. The rats were treated with ANIT 12h prior to bile duct cannulation or liver perfusion, this duration of dosing having been previously shown to alter significantly several biliary secretory parameters (Krell *et al.*, 1982).

Isolated perfused rat livers

Following bile duct cannulation as above, the rat

liver was isolated *in situ* according to Hems *et al.* (1966). Liver anoxia was minimized (5–10s) by commencing perfusion immediately, at a constant flow rate of 15–17ml/min, with 150ml of Krebs–Ringer bicarbonate buffer, pH7.4 (Krebs & Henseleit, 1932); this buffer also contained 2mM-$CaCl_2$, 5mM-glucose, 1% (w/v) bovine serum albumin, a physiological amino acid mixture (see Barnwell *et al.*, 1983) and 20% (v/v) packed human red blood cells. This solution was recycled, gassed continuously with O_2/CO_2 (19:1) and maintained at 37 ± 0.5°C within a thermostatically controlled cabinet similar to that recommended by Collins & Skibba (1980).

After an initial 60min of perfusion, the liver was converted to a one-pass perfusion of fresh medium. At the beginning of this 5min period, 25mg of HRP, together with either 8μCi of ^{125}I-IgA or 50μCi of [^{3}H]inulin (in a volume of 1ml of Krebs–Ringer bicarbonate buffer), was slowly infused into the perfusion line just prior to the portal vein cannula. The infusion was carried out over a period of 1min. The subsequent 4min of one-pass perfusion removed any excess compound not taken up by the liver. Bile samples were collected throughout as 2×30min samples prior to infusion, and 5×2min followed by 10×5min samples during and after infusion of the components.

Colchicine, when used, was added to the perfusion medium at a final (perfusion fluid) concentration of 2.5μM, 60min prior to the administration of HRP and ^{125}I-IgA. ANIT was given to the rats 12h previously as detailed above.

The health of the livers was monitored by analysing the extent of leakage into the perfusate and bile of the cytosolic hepatocyte enzyme aspartate aminotransferase. In no case was the leakage of this enzyme increased by any of the treatments described.

Specific protein determinations

HRP in bile was assayed according to Steinman & Cohn (1972) by observing the change in absorbance. One unit of enzyme activity was taken to be equivalent to a change of one absorbance unit per minute. The dimeric IgA which was infused into the isolated perfused livers was determined by counting the trichloroacetic acid-precipitable ^{125}I radioactivity in the bile. The monoclonal rat dimeric IgA (kindly given by Dr. J. Peppard, Institute of Cancer Research, Belmont, Surrey, U.K. and Dr. J. P. Luzio, Addenbrookes Hospital, Cambridge, U.K.) was prepared and labelled with ^{125}I as in Mullock *et al.* (1979).

Rat secretory IgA, rat serum albumin, rat fibrinogen and bovine serum albumin in bile were determined by quantitative radial immunodiffusion by the method of Mancini *et al.* (1965) with specific antisera. Authentic rat serum albumin, rat fibrinogen and bovine serum albumin were used for standardization; secretory IgA was expressed in arbitrary units relating to the diameter of the precipitation zone.

Aspartate aminotransferase (EC 2.6.1.1) was assayed by using an assay kit supplied by the Boehringer Corp., which is based on the method of Bergmeyer *et al.* (1978).

Results

Preliminary studies in the bile fistula rat

Following the injection of 25mg of HRP into the circulation of anaesthetized rats, HRP activity was detected in the bile (Fig. 1, control). HRP activity

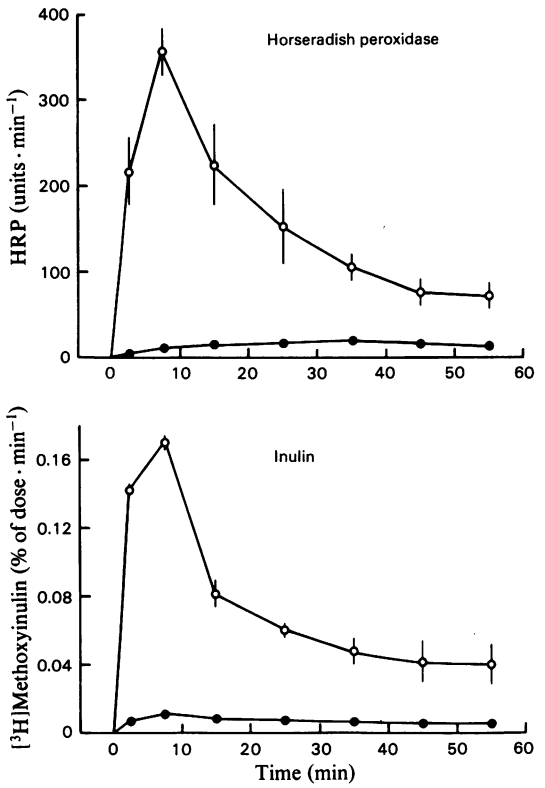

Fig. 1. *Effect of pretreatment with ANIT on biliary secretion of HRP and [^{3}H]methoxyinulin following intravenous injection*

For details of animal preparation and assay procedures see the Materials and methods section. HRP and [^{3}H]methoxyinulin were injected at time zero. The values on the graphs are plotted at the midpoint of each collection period, and are means for three separate experiments ± S.E.M. ●, Biliary secretion rate of component from control rats; ○, biliary secretion rate of component from ANIT-pretreated rats.

in the bile was detectable in the first sample after injection (0–5 min), but the rate of HRP appearance did not reach a peak until the 30–40 min sample. Thereafter the activity in the bile decreased.

In rats which had been pretreated for 12 h with ANIT, HRP secretion was extensive in the 0–5 min sample, the peak secretion of HRP occurring in the 5–10 min sample post-injection (Fig. 1, ANIT). This peak of HRP activity was approx. 17-fold higher than the peak of HRP activity in the control rats, in addition to occurring at an earlier time.

In addition to HRP, [^{3}H]methoxyinulin was also injected into the circulation of control and ANIT-pretreated rats. Radioactivity was detected in the bile of control rats in the 0–5 min sample, and reached a peak rate of appearance in the 5–10 min sample (Fig. 1, control). After 10 min, the secretion rate of the inulin into the bile was lower, but then remained constant for the duration of the experiment. Inulin appearance in the bile of ANIT-pretreated rats reached a peak in the 5–10 min sample postinjection, as in controls (Fig. 1, ANIT). The rate of secretion of inulin was, however, approx. 17-fold greater than in the control rats. The rate of secretion in the ANIT-pretreated rats rapidly decreased after 10 min, but remained at levels above those of the controls.

In addition to studying the appearance in bile of the administered compounds HRP and inulin, the secretion of immunoglobulin A, which is continuously supplied to the liver from the blood supply of the rat, was also monitored. The rate of secretion of IgA into bile in control rats was 4.35 ± 0.11 units/min. The secretion of IgA in ANIT-pretreated rats was 4.65 ± 0.19 units/min, and was not significantly different from the controls.

Taking into account the volume of the bile duct cannula (31 μl), the mean bile flow of 20 μl/min, and the volume of the biliary tree (approx. 35 μl, from Olson & Fujimoto, 1979, and based on data in Lowe *et al.*, 1984), there is a delay in these experiments of about 3.5 min between the time a solute enters the bile canaliculi and the time it is seen in the sample tube. Peak inulin appearance in bile occurs in the 5–10 min bile sample; thus the transit time for inulin from the liver sinusoids to the bile canaliculi is thus likely to be between 1.5 and 6.5 min. This short time effectively negates the possibility of the transfer of inulin to bile by a transcytotic vesicular mechanism, at least at this early time point; the rapidity of the transfer of inulin to bile supports the use of this compound as a probe for the paracellular pathway of solute transport to bile.

Since, in ANIT-pretreated rats, maximal HRP entry into bile occurs in the same sample as maximal inulin entry, this suggests that the main mechanism of HRP entry into bile in ANIT-pretreated rats is via the paracellular route. However, due to the continued supply of HRP from the recirculating blood supply, the data obtainable from bile fistula rats preclude the drawing of any conclusions on HRP transport across control rat livers. Consequently, the technique of the isolated perfused rat liver, operating under one-pass perfusion conditions, was then used to circumvent this problem.

Transport of HRP across the isolated perfused rat liver

Infusions (duration 1 min) containing 25 mg of horseradish peroxidase, plus either 8 μCi of ^{125}I-IgA or 50 μCi of [^{3}H]methoxyinulin, were administered to isolated perfused rat livers which were operating under one-pass perfusion conditions. Horseradish peroxidase activity was first detected in the bile in the 2–4 min sample post-infusion, subsequently reaching peaks in secretion rate in the 4–6 min and in the 20–25 min bile samples (Fig. 2).

The secretion rate of ^{125}I-IgA, as shown by the trichloroacetic acid-precipitable ^{125}I radioactivity, reached a peak in the 20–25 min sample; the profile of IgA secretion was thus very similar to that of the second output of HRP. [^{3}H]Inulin appearance in bile was maximal in the 6–8 min sample, similar to the first peak in HRP activity. The appearance of [^{3}H]inulin in bile did not fall to zero, but declined to a level of 7% of the peak value in the 55–60 min sample.

When colchicine, at a concentration of 2.5 μM, was added to the perfusion medium 60 min prior to the HRP and IgA infusion, the rate of output of both HRP and IgA was reduced; IgA secretion, and the second peak of HRP secretion, were inhibited by 60% (Fig. 3). The first peak of HRP appearance was increased by colchicine, although this was not statistically significant.

Pretreatment of rats with ANIT for 12 h prior to liver isolation altered the pattern of HRP appearance in the bile in a different manner from that of colchicine. The initial peak in HRP secretion rate was increased approx. 6-fold; the second peak was also slightly increased, but this was probably due to an additive effect of the slow decline in HRP secretion from the first peak (Fig. 4). The appearance of inulin in bile was also increased, by a factor of 2, by the ANIT pretreatment.

The use of the isolated rat liver operating under one-pass perfusion conditions allowed a much closer estimation of the transit times of HRP, inulin and IgA from blood to bile. The mean bile flow in these experiments was 12.8 ± 0.5 μl/min in both control and ANIT-treated livers, and the

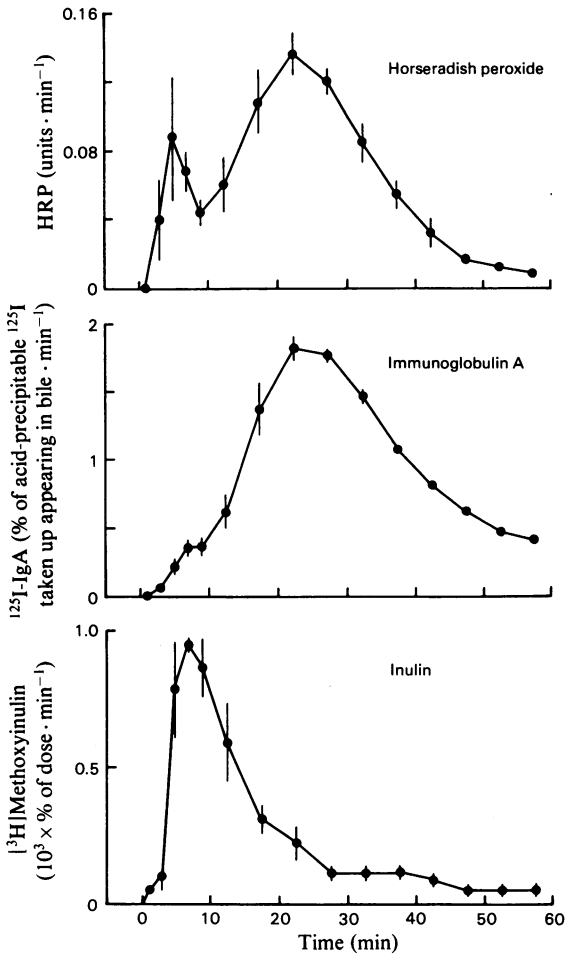

Fig. 2. *Biliary secretion of HRP, ^{125}I-IgA and [^{3}H]methoxyinulin following 1 min infusion into isolated perfused rat livers*

For details of liver perfusion and assay procedures see the Materials and methods section. HRP, IgA and inulin were infused from 0 to 1 min. The values on the graphs are plotted at the midpoint of each collection period, and are means for five experiments (HRP), two experiments (IgA) or three experiments (inulin), ±S.E.M. for HRP and inulin. The error bars on the IgA curve represent the two separate experiments.

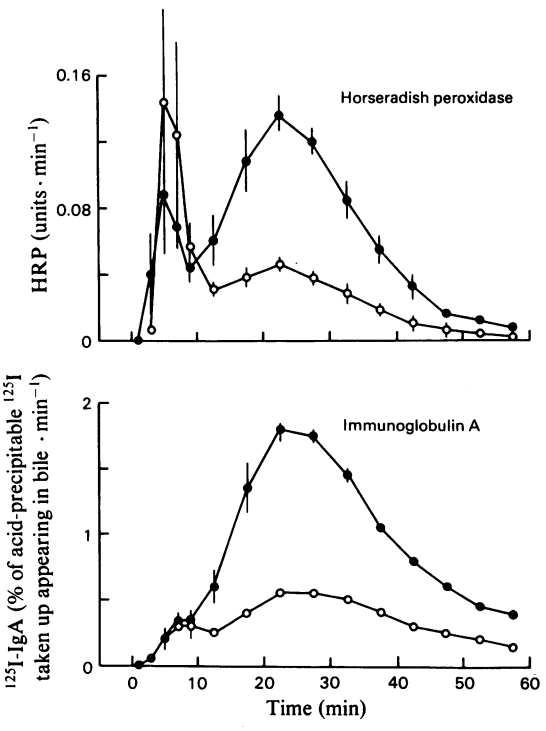

Fig. 3. *Effect of colchicine on the biliary secretion of HRP and ^{125}I-IgA from isolated perfused rat livers*

For details see the Materials and methods section and the legend to Fig. 2. ●, Control, as in Fig. 2; ○, colchicine-perfused livers, ±S.E.M., where $n = 3$.

dead volume in the cannula and biliary tree was about 66 μl; thus there would have been a delay of about 5 min between solute entering the bile canaliculi and the solute reaching the bile collection vessel. The mean transit time for inulin and the first output of HRP to reach the bile canaliculi from the blood would thus have been about 2 min. The average time for IgA and the second output of HRP to reach the bile canaliculi, since maximum secretion occurred in the 20–25 min bile sample, was thus approx. 17 min.

Discussion

Transcytotic entry of HRP into bile

In the control isolated perfused rat livers, the mean transit time for both IgA and the bulk of the HRP entering bile was 17 min. This time is shorter than the 35–40 min seen in previous studies which, in the main, have used bile fistula rats (Matter *et al.*, 1969; Limet *et al.*, 1982; Kacich *et al.*, 1983; Renston *et al.*, 1983; Okanoue *et al.*, 1984; Schiff *et al.*, 1984; and also in Fig. 1, control). The shorter time is probably due to the very brief (1 min) exposure of the livers to IgA and HRP in isolated, one-pass perfused livers, compared with the time taken to clear the proteins from the circulation of bile fistula rats.

As judged from the similarity in the time of entry into bile, the transcytotic movement of HRP and IgA from blood to bile could occur within the same population of vesicles. Further evidence for this comes from the similarity of the inhibitory effect of colchicine on IgA secretion and the second secretion peak of HRP. However, it must be born in mind that IgA is not the only protein to traverse the hepatocyte by transcytosis; haptoglobin (Mul-

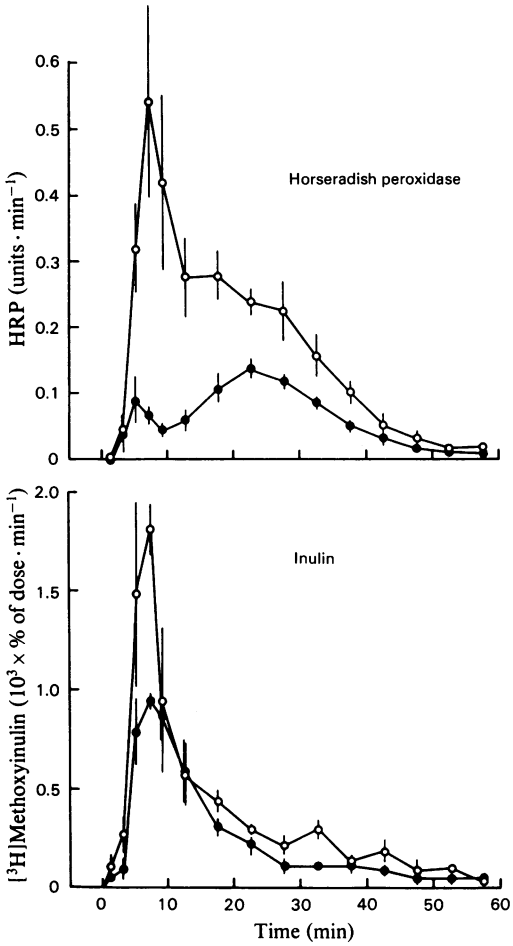

Fig. 4. *Effect of ANIT on the biliary secretion of HRP and [³H]methoxyinulin from isolated perfused rat livers*
For details see the Materials and methods section and the legend to Fig. 2. ●, Control, as in Fig. 2; ○, perfused livers from ANIT-pretreated rats, ±S.E.M., where $n = 5$ (HRP) or $n = 3$ (inulin).

lock & Hinton, 1981), insulin (Renston *et al.*, 1980) and enterokinase (Grant *et al.*, 1984) also enter the bile via transcytosis; thus HRP could share the same vesicles as any one or more of these proteins, or be transported from blood to bile in vesicles independent of the receptor-mediated pathway. Support for the receptor-mediated uptake of ligand together with HRP as a fluid-phase marker into the same endosomal compartment, however, comes from studies by Rodewald & Abrahamson (1982), who studied IgG–ferritin and HRP uptake, and Gonatas *et al.* (1984) who studied the uptake of wheatgerm agglutinin–ferritin and HRP.

The majority of the HRP which is taken up is probably transported to the lysosomes (see the introduction). Therefore, in terms of volume of fluid endocytosed, IgA must traverse the hepatocyte along a minor pathway, assuming that HRP is a true fluid-phase marker. If all the HRP and IgA taken up arrives in the same endosomal compartment, then sorting of the endosomal contents must take place, as IgA does not seem to enter lysosomes (Mullock & Hinton, 1981; Schiff *et al.*, 1984). Sorting of endosomal contents also occurs with other receptor-mediated uptake and transport processes, for example various asialoglycoproteins destined for the lysosomes; some mis-sorting appears to take place however, with a small proportion of the asialoglycoproteins entering bile in an intact form (Schiff *et al.*, 1984). This could be an analogous process to the entry of enzymically active HRP into bile.

Paracellular entry of HRP into bile

Although previous studies have shown a transcytotic vesicle transport system for HRP into bile in normal rats (see the introduction), the present studies have indicated that the protein can also enter bile by a second, more rapid pathway. The kinetics of output into bile of [³H]methoxyinulin and the first output peak of HRP are similar; this suggests that the two compounds enter bile by the same pathway. The transit time for inulin and the first secretion peak of HRP of only about 2min, given these these compounds cannot pass freely through the hepatocyte plasma membranes, precludes the possibility of transhepatocyte transfer to bile in this time scale. The lack of any inhibitory effect of colchicine upon the first secretion peak of HRP into the bile also supports this contention. Thus inulin, and the first portion of the HRP secretion, probably enter bile via the paracellular route. Since in the isolated perfused liver the bile duct cells are not perfused (these normally receive arterial blood only; Jones *et al.*, 1980) the route in this case is probably paracellular around the hepatocytes, via tight junctional diffusion.

The use of ANIT pretreatment provides further evidence for a tight junctional diffusion route of entry of HRP into bile. ANIT, which increases the permeability of the hepatocyte tight junctions to sucrose, inulin and phosphate (Krell *et al.*, 1982; Jaeschke *et al.*, 1983) increased the secretion of HRP into bile at 5–7min in both the isolated perfused liver, and in the bile fistula rat. The lack of effect of ANIT on the biliary secretion of IgA can be taken as evidence that there was no effect on this particular transcytotic pathway. The pretreatment of rats with ANIT increased the secretion of inulin into bile in both the bile fistula rat and isolated perfused rat livers, confirming the action of this compound on the permeability of the hepatocyte tight junctions.

The pathways for the entry of HRP, IgA and inulin are summarized in Fig. 5, together with the

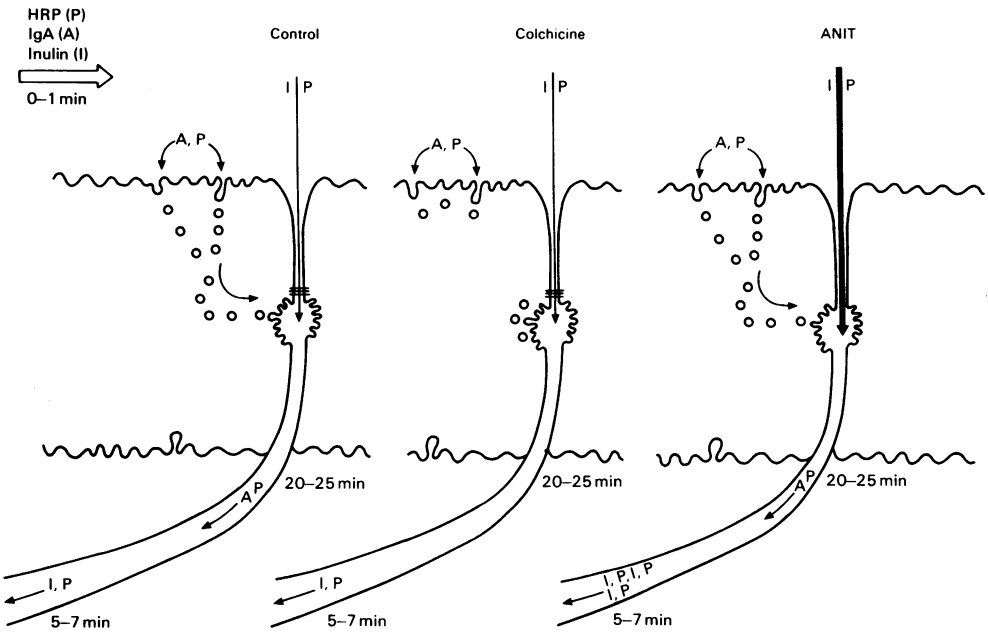

Fig. 5. *Possible pathways involved in the passage of HRP, IgA and inulin into bile*
IgA (A) enters the bile via receptor-mediated transcytosis, together with a proportion of the HRP (P). The mean total transit time for this process is 20–25 min. Inulin (I), and some of the HRP, enters bile via tight junctional diffusion, with a mean total transit time of 5–7 min. Colchicine inhibits the transcytotic mechanism, lowering the biliary secretion of IgA and the second output of HRP. ANIT increases the permeability of the tight junctions to inulin and HRP, increasing the secretion of these components at 5–7 min.

likely actions of colchicine on transcytosis, and of ANIT on tight junctional permeability.

Relevance to the steady state secretion of proteins into bile

Under steady-state or pseudo-steady-state conditions, as with the bile fistula rat, the secretion of HRP is complex; normally, a balance will exist between the transcytotic and paracellular pathways. Since HRP remains in the circulation of the rat for a substantial time, the biliary secretion of HRP achieves higher levels than from isolated one-pass perfused livers (20 versus 0.14 units/min in the bile, respectively, from Figs. 1 and 2, controls). Due to the continuous supply of HRP from the blood, a peak of secretion was not seen at 5–7 min in control rats.

The balance between the two pathways can be disturbed by pretreatment of rats with ANIT, or by treating the isolated livers with colchicine. ANIT increases the secretion of HRP into bile via the paracellular route, whereas colchicine decreases the transcytotic entry of HRP into bile. Colchicine also seems to increase slightly the paracellular secretion of HRP, an effect which can be seen in the work of Kacich *et al.* (1983) who used the bile fistula rat, where the peak secretion of HRP following an intravenous injection occurred at an earlier time than in controls. Colchicine also increases the biliary secretion of intravenously administered sucrose and mannitol, compounds thought to enter bile via tight junctional diffusion (Bajwa & Fujimoto, 1983).

In addition to compounds which can cause intrahepatic cholestasis, such as colchicine and ANIT, extrahepatic cholestasis also alters the permeability of tight junctions to solutes. High biliary pressure, without the complete cessation of bile flow, increases the tight junctional permeability to erythritol and inulin (Accatino *et al.*, 1977), whereas bile duct ligation increases the permeability of tight junctions to HRP (Metz *et al.*, 1977). Thus the balance between the transcytotic and paracellular entry into bile of proteins may be altered in some cases of liver disease, including both intrahepatic and extrahepatic cholestasis.

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