Investigation of endosomal compartments involved in endocytosis and transcytosis of polymeric immunoglobulin A by subcellular fractionation of perfused isolated rat liver

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1. A gamma camera was used to monitor continuously the uptake of radiolabelled polymeric immunoglobulin A (pIgA) into the rat body after intravenous injection. Uptake into liver was fast but, since the peak of liver labelling occurred only after 9–15 min, it was not sufficiently rapid to constitute a pulse dose. A perfused, isolated rat liver system was therefore established which could be given a single pass dose of pIgA; a variety of tests showed such livers remained viable for at least 3 h and could be subsequently fractionated on Ficoll and Nycodenz gradients with normal distributions of marker enzymes. 2. Subcellular fractionation at different times after a single pass dose of pIgA showed that whilst pIgA appeared sequentially in sinusoidal plasma membrane, light endosomes, dense endosomes, very dense endosomes and lysosomes as *in vivo*, the predominance of pIgA in the light endosome compartment disappeared much earlier than after injection *in vivo* of pIgA, presumably because this compartment was not being continuously loaded over the first 10–15 min. The time course of appearance of label in bile was unchanged. A large excess of unlabelled asialofetuin did not change these patterns, indicating that the asialoglycoprotein receptor was not involved. 3. Low doses of the microtubule agent colchicine reduced the proportion of pIgA reaching the bile, but subcellular fractionation of treated liver showed that distribution of label amongst liver fractions was little changed, although the overall liver pIgA content had increased. This would suggest that pIgA did not remain in the common compartment which could have supplied bile or lysosomes but rather flowed out of it as rapidly as in untreated liver but towards those compartments supplying the lysosomes. 4. Experiments with nocodazole, which reversibly disrupts microtubules, showed that very little of the pIgA taken into an inhibited liver appeared in the bile after nocodazole was removed 30 min later, even though a second dose of pIgA, given after nocodazole removal, appeared in bile with a normal time course. The first dose of pIgA must therefore have passed beyond the compartments competent to supply the bile before nocodazole was removed. Such compartments were undamaged since the second dose of pIgA appeared in bile normally. We therefore conclude that the bulk of pIgA must be supplied to the bile from light or dense endosomes rather than from very dense endosomes and lysosomes.

INTRODUCTION

The biochemical dissection of endocytic pathways requires the identification and purification of endocytic compartments (for reviews see Pastan & Willingham, 1985; Mullock *et al.*, 1987), the quantification of ligand transfer through such compartments (Thilo, 1985) and the reconstitution of transfer events between them *in vitro* (Davey *et al.*, 1985; Gruenberg & Howell, 1986; Braell, 1987). In rat liver, the ligands pIgA and asialofetuin (ASF) are endocytosed by hepatocytes, initially into a common endocytic compartment. A sorting process then occurs, much of the pIgA being transferred across the cell and secreted into bile, whereas ASF is found subsequently in dense endosomes and lysosomes where it is degraded. The endocytic pathways followed by these ligands have been investigated both by immunoelectron microscopy (Geuze *et al.*, 1984) and subcellular fractionation (Mullock *et al.*, 1983, 1987; Kindberg *et al.*, 1984; Hoppe *et al.*, 1985; Limet *et al.*,

1985; Branch *et al.*, 1987). Rapid subcellular fractionation using vertical density-gradient centrifugation has identified different endocytic compartments in rat liver and shown the passage of ligands through these after loading *in situ* by intravenous injection of radiolabelled ligands (Branch *et al.*, 1987). It is difficult to quantify endocytic pathways after loading *in situ* because of the recycling of blood and hence labelled ligand through the organ, which allows the possibility of continuous re-loading of early endocytic compartments.

In the present experiments the rate of uptake of intravenously injected radiolabelled pIgA into the liver *in situ* was measured using a whole body scan technique. A perfused isolated rat liver system was established and after single pass loading with radiolabelled pIgA (Lowe *et al.*, 1985a,b) endocytic compartments were identified by subcellular fractionation. The use of a perfused isolated liver system ensures that there is no interference by other organs with respect to uptake or metabolism of injected pIgA, allows the direct use of controlled

Abbreviations used: pIgA, polymeric immunoglobulin A; ASF, asialofetuin.

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concentrations of potential inhibitors of endocytic pathways, enables the investigation of the effects of one ligand on the endocytosis of another and makes possible the quantification of the relative importance of different endocytic pathways entered by a ligand.

EXPERIMENTAL

Monoclonal rat anti-(horseradish peroxidase) pIgA (Peppard *et al.*, 1984) and ASF (type I; Sigma) were radiolabelled as described by Mullock *et al.* (1983). Ligands were administered to anaesthetized rats and bile collections made as previously described (Mullock & Hinton, 1979; Branch *et al.*, 1987).

Whole body scans of rats were conducted after intravenous injection of 10 μ g of ^{123}I -labelled pIgA, since ^{123}I provided better image resolution than ^{125}I or ^{131}I . Rats were placed prone on a small field of view IGE gamma camera interfaced to a Nodecrest mobile computer. Frames (1 min) were collected for 2 h and hepatic pIgA uptake and clearance curves were obtained by drawing a 'region of interest' within the liver. Data was decay, but not background, corrected.

Isolated rat livers were perfused with recycling at 37 °C and 13 ml/min by a modification of the method of Lowe *et al.* (1985b). Perfusion medium was 200 ml Dulbecco's Modified Eagle Medium containing 25 mM-glucose with 33% (v/v) washed human erythrocytes, 3% (w/v) bovine serum albumin, 2000 heparin units/l, 60 mg of penicillin/l, 100 mg of streptomycin/l. Taurocholate (2 mM) was continuously infused at 40 μ mol/h. Perfusion medium was gassed with air/CO₂ (19:1) maintaining pH at 7.30–7.40, pCO₂ 4–5 kPa and pO₂ 5–7 kPa.

Radiolabelled pIgA (typically 1 μ g; 10⁷ d.p.m.), and where indicated, ASF, were injected over 1 min and the perfusate drained for the next 4 min before re-establishing the normal recycling perfusion. The rate of bile flow and appearance of radiolabel in bile and perfusate were monitored. Inhibitors were added to the perfusion medium 1 h before pIgA injection. Inhibitor concentrations are expressed as μ M in red cell-free perfusate.

At stated times after the beginning of the pIgA injection, perfused livers were flushed with 100 ml of cold, buffered iso-osmotic sucrose, homogenized, fractionated and assayed, as previously described (Branch *et al.*, 1987). Refractive index measurements were made on all gradients to identify the positions of the various subcellular fractions; density values were measured with a density meter and related to refractive index measurements on standard gradients of each type.

RESULTS

The uptake of radiolabelled pIgA into the liver of bile duct-cannulated rats was monitored continuously using a gamma scanner after intravenous injection of the radiolabelled ligand (Fig. 1). Although uptake of pIgA into the liver was rapid, the peak of isotope concentration within the liver did not occur until 9–15 min after injection. This was followed by a slow decline in radioactivity. In contrast the appearance of radiolabel in the bile peaked between 40 and 60 min after injection, in agreement with previous studies (Orlans *et al.*, 1978; Goldman *et al.*, 1983). The results demonstrated the difficulty of investigating sequential endocytic com-

Fig. 1. Uptake of intravenously injected ^{123}I -pIgA into rat liver

(a) Whole body scan of rat 30 min after intravenous injection. (b) Uptake of ^{123}I into the right upper quadrant of rat liver as a % of maximum uptake (computer-drawn line) and appearance of ^{123}I in bile as a % of maximum appearance in a 10 min collection period (●).

partments involved in pIgA transcytosis by fractionating the liver of rats after intravenous injection of the ligand, since the liver is clearly not receiving a pulse dose.

A perfused, isolated rat liver system was established to overcome this difficulty. Perfused livers remained viable over a period of at least 3 h. During this time O₂ consumption averaged 3 μ mol/min per g, similar to reported values *in vitro* of 2–2.6 μ mol/min per g (Sugano *et al.*, 1978; Van Dyke *et al.*, 1983). Bile flow averaged 1.4 μ l/min per g of liver in the range of values 0.4–1.7 μ l/min per g of liver ($n = 8$) obtained with bile fistula rats. Bile enzyme content was, for alkaline phosphatase, 11–22 units/l (normal bile 20 units/l), for 5'-nucleotidase, 28–80 units/l (normal bile 150 units/l), and for alanine transferase, 27–63 units/l (normal bile 5 units/l). No significant alteration in the bile content or perfusate content of these enzymes was observed between the start and end of experiments. After 3 h perfusion histological examination showed no differences from normal rat liver. The ability of perfused rat liver to transport radiolabelled pIgA from perfusate to bile was examined in a recycling mode and compared with the transfer from blood to bile after intravenous injection

Fig. 2. Appearance in bile of ^{125}I -pIgA after single-pass perfusion of isolated rat liver

Two single passes of ^{125}I -pIgA (approx. $1\ \mu\text{g}$) were given at times marked (↓) in the absence (○) or presence (●) of 2 mg of ASF. A representative experiment is shown.

into a rat. Transfer was dependent on perfusate volume and over a 3 h period was $> 70\%$ of that occurring in the rat when perfusion volumes were 100 ml or less, but dropped to 20–30% with perfusion volumes of $> 250\ \text{ml}$.

Single-pass perfusion of ^{125}I -pIgA resulted in secretion into the bile with a maximal peak in the 20–30 min sample (Fig. 2), in agreement with previous studies (Lowe *et al.*, 1985a,b). A second pass of ^{125}I -pIgA given 1 h after the first resulted in equivalent secretion into the bile with a similar time course. The presence of 2 mg of ASF, representing a 10-fold excess of expected ASF uptake (Schiff *et al.*, 1986b) during a single pass, had no effect on ^{125}I -pIgA ($1\ \mu\text{g}$) uptake or transfer into bile, indicating that observed pIgA uptake and transcytosis was mediated by the pIgA receptor and not due to uptake on asialoglycoprotein receptors. The uptake of ^{125}I -pIgA by the liver in a single pass was 7% of the radiolabel given. After giving two single-pass perfusions of ^{125}I -pIgA with a 1 h interval (Fig. 2) 21% of the radiolabel taken up by the liver was secreted into bile 2 h after the first single pass; 62% was in the perfusate and 18% remained with the liver. Most (88%) of the pIgA in bile was trichloroacetic acid-precipitable. By comparison, when the isolated perfused liver was loaded with ^{125}I -ASF in a single pass 34% of the radiolabel given was taken up. After giving two single-pass perfusions of ^{125}I -ASF with a 1 h interval, 3% of the radiolabel was found in bile 2 h after the first single pass, 93% in the perfusate and 4% in the liver; 45% of the ASF in the bile was trichloroacetic acid-precipitable.

Subcellular fractionation using vertical density-gradient centrifugation on Ficoll and Nycodenz gradients of isolated rat liver after 2 h perfusion showed that distribution of the marker enzymes succinate dehydrogenase, β -N-acetylglucosaminidase and 5'-nucleotidase, was the same (results not shown) as that observed when fractionating liver from freshly killed rats (Branch *et al.*, 1987). Homogenization and fractionation of perfused, isolated livers at different times after single-pass perfusion of ^{125}I -pIgA using techniques described previously (Branch *et al.*, 1987), allowed the identification of endosomal compartments involved in pIgA endocytosis. The use of isopycnic centrifugation on 1–22%

(w/v) Ficoll and 0.25 M-sucrose/45% Nycodenz gradients in vertical rotors (Fig. 3) showed that, in agreement with data obtained by loading livers with ^{125}I -pIgA *in situ* (Branch *et al.*, 1987), the ligand was initially associated with blood sinusoidal plasma membrane and light endosomes. However, in the perfused liver the predominance of label in these compartments lasted for only the first few min; after 10–15 min the predominant peak on the Ficoll gradient was that at the bottom of the gradient. The ^{125}I -pIgA remaining in the liver subsequently became increasingly associated with the lysosomal position on 0.25 M-sucrose/45% (w/v) Nycodenz gradients (Fig. 3). The identification of blood sinusoidal plasma membrane [Ficoll, refractive index (20 °C, white light) 1.3550–1.3599], light endosome (Ficoll, refractive index 1.3600–1.3660), dense endosome (Ficoll, refractive index 1.3661–1.3729), and lysosome (Nycodenz, refractive index 1.3785–tube bottom) positions on the gradients was made by comparison with data obtained previously after loading livers with radiolabelled ligands *in situ* and measurement of marker enzymes (Branch *et al.*, 1987). In the present experiments it was noted that much of the radioactivity sedimenting to the bottom of the Ficoll gradient at earlier time points was not associated with lysosomes on the Nycodenz gradient (e.g. Figs. 3d and 3k) indicating that a third, very dense, endosome compartment appears to exist in hepatocytes. Recentrifugation of the peak from the bottom of the Ficoll gradient on a Nycodenz gradient has indeed shown that this endosome compartment can be separated. Further analysis of the subcellular fractionation data was conducted to investigate whether a clear sequential time course of loading endocytic compartments and transcytosis into bile could be established. It was found that whereas labelling of the blood sinusoidal plasma membrane preceded loading of light and dense, then very dense, endocytic compartments and lysosomes, it was not possible to establish whether light, dense, or very dense, endosomes were the precursor compartments for pIgA secreted into bile (Fig. 4).

It has been reported that agents causing disruption of microtubules have no effect on pIgA uptake by rat liver but inhibit transcytosis (Mullock *et al.*, 1980; Goldman *et al.*, 1983; Gebhardt, 1984; Lowe *et al.*, 1985a,b). Fractionation of rat livers following intravenous injection of ^{125}I -pIgA after colchicine treatment of the rat failed to show any difference in subcellular distribution of the ligand compared to normal rats (Branch *et al.*, 1987), but in that study, colchicine concentrations used were 300-fold greater than those required to disrupt microtubules during mitosis of cultured cells *in vitro* (Dustin, 1984). In the present study a variety of agents capable of affecting microtubule function were investigated for their effects on ^{125}I -pIgA transcytosis in the isolated perfused rat liver system. All of these agents affected bile production (cf. Gregory *et al.*, 1978) as well as ^{125}I -pIgA transcytosis but the dose required for half-maximal inhibition of transcytosis was always lower than that required for half-maximal inhibition of bile flow (Table 1). Moreover the concentration of lumicolchicine, which has no specific effect on microtubule function, required to inhibit transcytosis was very much greater than that of colchicine. The latter caused inhibition of transcytosis resulting in an increased association of ^{125}I -pIgA with the liver (Fig. 5) at concentrations similar to those needed to inhibit mitosis in cell cultures *in vitro* (Dustin, 1984). At

Fig. 4. Time course of appearance of ^{125}I -pIgA in different subcellular compartments after single-pass perfusion of isolated rat liver

Radioactivity appearing in bile was measured directly and in subcellular compartments calculated from the density gradient data shown in Fig. 3 as described in the text. ●, Bile; ▲, plasma membrane; ■, light endosome; △, dense endosome; □, very dense endosome; ○, lysosome.

higher colchicine concentrations, both liver and bile pIgA contents were reduced.

Since colchicine inhibited transcytosis it might be expected that ^{125}I -pIgA would accumulate in endocytic compartments on the transcytic pathway. When colchicine-treated, perfused, isolated rat livers were fractionated after single-pass perfusion with ^{125}I -pIgA it was found that the distribution of label on the Ficoll gradients was the same as that observed when fractionating normal perfused rat livers (Fig. 3). These results were consistent with colchicine introducing a block on transfer of ^{125}I -pIgA from endosomes to bile without affecting its passage through endosomal compartments on the route to lysosomes. The slightly lower proportion of radiolabel associated with lysosomes on Nycodenz gradients 60 min after single-pass perfusion (Fig. 3n) was consistent with previous reports of colchicine inhibition of ligand transfer from late endo-

Table 1. Effects of microtubule disruption on pIgA transcytosis and bile flow

Perfused, isolated rat livers were pre-treated for 1 h with agents affecting microtubule function and after single-pass loading of ^{125}I -pIgA the appearance in bile of ^{125}I -pIgA was measured over a period of 1 h. For each agent at least six livers were treated, each with a different concentration of agent, and the amount necessary to give 50% inhibition of transcytosis was estimated. Inhibition of bile flow caused by this concentration of the agent was also recorded. Taxol at $15\ \mu\text{M}$ caused only 35% inhibition of transcytosis with 25% inhibition of bile flow.

Agent	Drug concentration required for 50% inhibition of pIgA transcytosis (μM)	Bile flow inhibition (%)
Colchicine	0.5	22
Lumicolchicine	> 6	0
Nocodazole	11.5	32
Vinblastine	0.6	34
Taxol	> 15	≥ 25

somal compartments to lysosomes (Berg *et al.*, 1985; Branch *et al.*, 1987).

During experiments investigating inhibition of transcytosis by microtubule disruption, it was found that nocodazole could act as a short-acting inhibitor and when removed from the perfusion system a second pass of pIgA was almost normally transcytosed to bile (Fig. 6a). In this experiment the second dose of pIgA was labelled with ^{131}I so that the distributions of the two doses could be distinguished. Although this second dose appeared in bile in a normal manner, there was no second peak of the original ^{125}I -pIgA, as might have been expected as the liver recovered from nocodazole inhibition. It would appear then that by the time the nocodazole inhibition was removed, the original pIgA had largely passed to compartments which did not supply the bile. When the liver was fractionated 8 min after the second dose of pIgA (^{131}I -labelled), i.e. 68 min after the first dose of pIgA (^{125}I -labelled), the distribution of both pIgA doses on a Ficoll gradient (Fig. 6b) were normal for their respective times after injection (cf. Figs. 3c and 3g). At this time, $^{125}\text{I}/^{131}\text{I}$ counts ratios in the

Fig. 3. Distribution of ^{125}I -pIgA after isopycnic centrifugation on Ficoll and Nycodenz gradients at increasing times after single-pass perfusion of isolated rat livers

Livers were homogenized 2–60 min after the beginning of a single-pass perfusion of ligand and post-mitochondrial supernatants were loaded on to 1–22% (w/v) Ficoll (a–g) or 0.25 M-sucrose/45% (w/v) Nycodenz (h–n) gradients (....., density) (○, β -N-acetylglucosaminidase activity). Representative gradients of normal livers (●) or colchicine ($1.6\ \mu\text{M}$)-treated livers (▲) at different times are shown. Arrows indicate the limits of each region summed for presentation in Fig. 4. For the Ficoll gradients, these are (1) sinusoidal plasma membrane, density range 1.053–1.066, (2) light endosome, density range 1.067–1.083, (3) dense endosome, density range 1.084–1.100 and (4) very dense endosome and lysosome, density range 1.101 to bottom of tube. On the Nycodenz gradients, region (5) lysosomes had a density range from the bottom of the tube to 1.144. A broken line is drawn at this density to facilitate comparison of radioactivities in the lysosomal region. The time of homogenization, total radioactivity (c.p.m.) loaded on to each gradient and recoveries for Ficoll and Nycodenz gradients were as follows: (a, h) 2 min (410360, 101%, 86%); (b, i) 4 min (371645, 102%, 88%); (c, j) 8 min (●, 383520, 99%, 90%; ▲, 264900, 101%, 90%); (d, k) 15 min (●, 107470, 98%, 84%; ▲, 285650, 96%, 85%); (e, l) 30 min (106080, 102%, 122%); (f, m) 45 min (139710, 98%, 88%); (g, n) 60 min (●, 141320, 97%, 86%; ▲, 47525, 93%, 83%).

Fig. 5. Effect of colchicine on pIgA transcytosis

Isolated rat livers were perfused with different concentrations of colchicine for 1 h before two single-pass perfusions with ^{125}I -pIgA with a 1 h interval between the two. After a further 1 h, the total radioactivity appearing in the bile (●) and remaining in the liver (□) was measured.

various fractions were, light endosomes 0.4, dense endosomes 0.4, very dense endosomes 0.9, lysosomes 2.3, compared with a bile ratio of approx. 500. By 30 min, when ^{131}I -pIgA secretion into bile was reaching a peak, the ratios for the same fractions were 0.5, 0.6, 0.5 and 1.4, respectively compared with a bile ratio of 3. At 1 h the ratios were 0.4, 0.6, 0.6 and 0.9 respectively and the bile ratio 1. By this time the majority of ^{131}I -pIgA still associated with liver was in very dense endosomes and lysosomes. The results conclusively showed that the bulk of pIgA appearing in bile could not be reaching it via very dense endosomes or lysosomes since little of the ^{125}I -pIgA loaded into this compartment during the nocodazole inhibition subsequently reaches the bile when the inhibition ceases. It was not, however, possible to distinguish between light and dense endosomes as a source of bile pIgA.

DISCUSSION

Rapid subcellular fractionation using vertical density gradients has been used previously to identify sequential endocytic compartments involved in the endocytosis of ligands taken up by rat liver *in situ* (Branch *et al.*, 1987). Such an approach to dissecting endocytic pathways is dependent on achieving a single pulse uptake of labelled ligand into the pathway. In the present study, whole body scanning showed that loading of radiolabelled pIgA *in situ* was inadequate to achieve such uptake into liver. Intravenous injection of a single dose of radiolabelled pIgA resulted in continued loading of early endocytic compartments even when transcytosed pIgA was beginning to enter the bile, an observation consistent with data obtained by subcellular fractionation (Branch *et al.*, 1987). Thus, in the present study, subcellular fractionation of perfused, isolated rat livers at different

Fig. 6. Effect of short-acting agent nocodazole on transcytosis and subcellular distribution of pIgA

(a) An isolated rat liver was perfused with $20\text{ }\mu\text{M}$ -nocodazole for 1 h before single-pass perfusion of ^{125}I -pIgA (↓). After a further 30 min the perfusion medium was replaced with fresh nocodazole-free medium (↓) and a subsequent single-pass perfusion with ^{131}I -pIgA carried out 30 min later (↓). The appearance of ^{125}I (Δ) and ^{131}I (●) in bile was monitored and compared with a control liver given two ^{125}I -pIgA single-pass perfusions (○). (b) Distribution of radioactivity on a Ficoll gradient after homogenizing an isolated perfused rat liver treated with nocodazole as in (a). Liver was homogenized 8 min after the single-pass perfusion with ^{131}I -pIgA and the post-mitochondrial supernatant loaded on to a 1–22% (w/v) Ficoll gradient (----, density) and centrifuged as in Fig. 3. Radioactivity loaded was ^{125}I (Δ), 39850 c.p.m., 97% recovery; ^{131}I (●), 108900 c.p.m., 87% recovery. Representative experiments are shown in both (a) and (b).

times after a single pass of ^{125}I -pIgA showed a more rapid transfer of label from light and dense endosomes to very dense endosomes and lysosomes than that observed after loading of liver (Branch *et al.*, 1987) *in situ*. This was in contrast with data obtained with ^{125}I -ASF (not shown) which showed a similar time course of labelling endocytic compartments as after loading *in situ*. The difference in pIgA and ASF labelling of endocytic compartments *in situ* and in perfused liver may be explained partly by reported differences in the affinities of the receptors for the two ligands, the pIgA receptor having a K_a of 10^8 – 10^9 M^{-1} (Brandtzaeg, 1981) the ASF receptor a K_a of 10^9 – 10^{10} M^{-1} (Steer & Ashwell, 1986). It is also probable that the two ligands have differential access into and through the Space of Disse. Transport of macromolecules across this compartment is complex since it does not contain a well-stirred layer (Tomlinson, 1986; Weisiger *et al.*, 1986), and it is known that whereas plasma components enter it, erythrocytes do not (Coleman, 1987). Furthermore, Schiff *et al.* (1983) have shown that access of molecules of $M_r > 100\,000$ to

receptors on the hepatocyte surface is kinetically impaired. Indeed they conclude that it is the sieving effect, not the direct rate of receptor coupling, that limits the rate at which both human and rat IgA clear from the circulation (Schiff *et al.*, 1986a).

In the present study it was found that maintenance of liver viability to achieve good pIgA transcytosis was dependent on a variety of factors. The most important were perfusing with tissue culture medium and 33 % (v/v) erythrocytes to maintain good oxygenation, avoiding toxic levels of O₂, using 3 % (w/v) bovine serum albumin to maintain oncotic pressure and establishing perfusion at a constant pressure of 5–7 cm of perfusate. Taurocholate infusion was necessary to maintain normal volumes of bile flow. pIgA transcytosis was a sensitive indicator of viability. In a recycling mode it was possible to achieve transcytosis of radiolabelled pIgA to levels of > 70 % of those observed *in situ* after intravenous injection. However it should be noted that during perfusion the liver received only 1 µg of radiolabelled pIgA whereas this was diluted by serum pIgA (possibly > 100 µg/ml; Orlans *et al.*, 1978) after intravenous injection. The quantitative values obtained for the relative importance of the transcytic route and endocytic route to lysosomes for endocytosed ¹²⁵I-pIgA in isolated perfused rat liver broadly agree with estimates made on both cultured hepatocytes and the liver *in vivo* (Schneider, 1982). Clearly the ease of quantifying endocytic routes in the isolated perfused liver would make it an ideal system to address the question of the effect of antigen complexed to the pIgA on the relative importance of the two endocytic routes for pIgA. It is widely thought that pIgA contributes to immune defences when present in secretions, but an additional proposal is that it can help clear unwanted antigens from the blood stream (Bienenstock & Befus, 1983). Experiments employing the anti-(horseradish peroxidase) monoclonal pIgA used here have shown that antigen (horseradish peroxidase) in the blood can be cleared to bile when the monoclonal pIgA is present in the circulation (Peppard *et al.*, 1984).

Subcellular fractionation of perfused isolated rat livers at different times after single pass loading with ¹²⁵I-pIgA suggested the presence of three endosomal compartments (light, dense and very dense) in hepatocytes. The time course of pIgA passage through these compartments was more rapid than that observed when fractionating liver after intravenous injection of this ligand (Branch *et al.*, 1987) for the reasons described above. In agreement with previous studies it was found that colchicine and other agents capable of microtubule disruption inhibited transcytosis of pIgA. The present experiments showed that they had no effect on the passage of pIgA to very dense endosomes. Indeed the block on one endocytic route (transcytosis) resulted in the ligand proceeding along the other. Such pharmacological modulation of endocytic pathways could prove useful in directing drugs to specific areas of the cell. It has been shown previously in cultured hepatocytes that colchicine results in the clustering of endocytic vesicles containing pIgA near the cell periphery (Goldman *et al.*, 1983). The present data plus that obtained from fractionating livers that were labelled *in situ* is consistent with pIgA first entering a light endosomal compartment then a dense endosomal compartment near the periphery of the cell. In other cell types morphological studies have described such com-

partments as peripheral or early endosomes (Hopkins, 1986). pIgA is next associated with very dense endosomes (possibly equivalent to deep-lying or late endosomes in other cell types) on the endocytic pathway to lysosomes. Whether transfer to lysosomes is necessary for degradation of pIgA or whether degradation commences in late endosomes as suggested for asialoglycoproteins (Kindberg *et al.*, 1987) was not investigated. It is not clear whether pIgA is transcytosed to the bile canalicular surface of the hepatocyte from light or dense endosomes. Even the use of pIgA labelled with different isotopes and a short-acting drug capable of microtubule disruption has been unable to distinguish between these possibilities. Neither in the present study nor in previous work fractionating liver after loading *in situ* (Branch *et al.*, 1987) were specific transfer vesicles mediating movement between endosomes and bile canalicular plasma membrane identified, though these have been proposed from morphological observations (Geuze *et al.*, 1984). Such transfer vesicles may have the same density in Ficoll and Nycodenz as light or dense endosomes, or they may be transient, representing only a small proportion of endocytic vesicles at any one time. Alternatively they may be continuous with other endosomal elements not broken up in the present homogenization protocol.

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