A two-dimensional electrophoretic analysis of the proteins and glycoproteins of liver plasma membrane domains and endosomes

Implications for endocytosis and transcytosis

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1. Polypeptides of liver plasma membrane fractions enriched in three surface domains of hepatocytes, blood–sinusoidal, lateral and bile canalicular, were analysed by isoelectric focusing (IEF) and non-equilibrium pH gel electrophoresis (NEPHGE) across a wide pH range, followed by SDS/PAGE. The overall Coomassie Blue-stained polypeptide patterns in the fractions were different. Lateral plasma membrane fractions contained a characteristically higher number of polypeptides focusing at the basic pH range, whereas few basic polypeptides were present in sinusoidal plasma membrane fractions. The glycoproteins in these plasma membrane fractions stained by a lectin overlay technique with radioiodinated concanavalin A, wheat-germ agglutinin and a slug lectin, were also different. 2. The polypeptides and glycoproteins of ‘early’ and ‘late’ endosome fractions were also compared by two-dimensional electrophoresis. Their composition was shown by Coomassie Blue staining, lectin overlay staining and in membranes metabolically labelled with [³⁵S]methionine to be generally similar. The glycoproteins of sinusoidal plasma membranes and early and late endosomes were generally similar, but major differences in polypeptides of molecular mass 20–50 kDa, pI 7.5–8.5, in plasma membranes and endosomes were demonstrated, with a specific population of basic (pI 8–9) low-molecular-mass polypeptides being present at highest levels in ‘late’ endosomal fractions (shown by Coomassie Blue staining). 3. Analysis of the distribution of three specific membrane glycoproteins identified by using immunoblotting techniques showed that the asialoglycoprotein and the divalent-cation-sensitive mannose 6-phosphate receptors were present in sinusoidal plasma membrane and in early and late endocytic fractions; they were not detected in canalicular plasma membrane fractions. In contrast, 5′-nucleotidase was detected in all fractions examined. The role of the endocytic compartment in regulating trafficking pathways between the plasma membrane domains of the hepatocyte is discussed.

INTRODUCTION

It is a characteristic property of polarized animal cells that they generate and maintain a functionally and structurally segmented surface membrane. In liver, the blood-facing baso–lateral surface of the hepatocyte is constructed of morphologically distinctive sinusoidal and lateral plasma membrane domains, and is separated by tight junctions from the bile canalicular plasma membrane comprising the apical surface. The biochemical properties of plasma membrane fractions originating from these three surface domains have been reviewed (Evans, 1980; Evans & Enrich, 1989).

Approaches towards elucidating the biogenetic mechanisms giving rise to these functionally distinctive surface membrane domains entail an analysis of exocytic as well as endocytic processes (Evans, 1980; Bartles *et al.*, 1987; Shears *et al.*, 1988; Evans & Enrich, 1989). The development of techniques for the preparation of hepatic endosome fractions (Kindberg *et al.*, 1984; Quintart *et al.*, 1984; Evans & Flint, 1985; Baenziger & Fiete, 1986; Bergeron *et al.*, 1986; Belcher *et al.*, 1987; Mullock *et al.*, 1987) can now allow a biochemical analysis of the protein compositional inter-relationships between the plasma membrane domains and the membranes comprising the endocytic compartment. Furthermore, since ‘early’ and ‘late’ endocytic membranes can be separated by centrifugation in density gradients on the basis of the time-based location of internalized

radioactive ligands (Evans & Flint, 1985), the involvement of these two sub-compartments in endocytic trafficking routes in a polarized epithelial cell is now open to investigation. In the present work, we have carried out a comprehensive two-dimensional electrophoretic analysis of the proteins and glycoproteins in the three major liver plasma membrane domains, as well as in endocytic subfractions, and we discuss the results in the context of the endocytic and transcytotic ligand-trafficking routes which are operational in liver tissue.

EXPERIMENTAL

Preparation of the subcellular fractions

Male Sprague–Dawley rats (200–250 g body weight) were used to prepare, from liver homogenates, plasma membranes originating from the blood–sinusoidal, lateral and bile canalicular domains, as fully described by Wisher & Evans (1975). ‘Early’ and ‘late’ endocytic fractions were prepared as described by Evans & Flint (1985) and Saermark *et al.* (1985). The protein content of the fractions was determined by the Bradford (1976) procedure.

Two-dimensional electrophoresis

The O’Farrell *et al.* (1977) procedure was used with modifications. In isoelectrofocusing (IEF) gels, membrane fractions

Abbreviations used: IEF, isoelectric focusing; NEPHGE, non-equilibrium pH gel electrophoresis; NP-40, Nonidet P-40; G-protein, GTP-binding protein.

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(400 µg of protein for Coomassie Blue staining, 200 µg for lectin overlays) were solubilized in 2% SDS/0.5 mM-MgCl₂/50 mM-Tris/HCl (pH 6.8) by boiling for 2 min, and were diluted with 2 vol. of freshly prepared 9.5 M-urea/2% ampholine (pH 3.5–10 and 5–4), 5% (w/v) mercaptoethanol/8% Nonidet P-40 (NP-40). IEF was carried out in rod-gels, without pre-running, at 300 V for 20 h, followed by 400 V for 1.5 h. The gels were then equilibrated in 0.065 M-Tris/HCl (pH 6.8)/2.3% SDS/5% mercaptoethanol/10% glycerol. For non-equilibrium pH gel electrophoresis (NEPHGE), subcellular fractions were solubilized in a lysis buffer containing 9.5 M-urea, 2% NP-40, 2% ampholines (pH 3.5–10) and 5% (w/v) mercaptoethanol and loaded on to the rod-gels; the samples were then overlaid with 8 M-urea and a mixture of 0.8% ampholines (pH 5–7 and pH 3.5–10). The gels were electrophoresed for 4 h at 400 V without pre-running, with the cathode at the bottom and the anode at the top (i.e. the polarity was the reverse of that used for IEF). After equilibration in a buffer (see above), the rods were stored at –70 °C or used immediately for electrophoresis in the second dimension in 8% or 10% polyacrylamide gels (Laemmli, 1970).

Two-phase partitioning of subcellular fractions

Plasma membranes or endosomes were solubilized at 4 °C in 10 mM-Tris/HCl (pH 7.4)/150 mM-NaCl containing 2% pre-condensed Triton X-114 (1 mg of protein/ml of buffer) (Bordier, 1981). After clarification by centrifugation (Eppendorf; 10 min), the samples (approx. 70–80% of the proteins were soluble) were centrifuged at 6000 *g*_{av} at 20 °C for 3 min to yield two phases. The upper (aqueous) phase was transferred to another tube and re-extracted at 4 °C with 1% Triton X-114, and the centrifugation step was repeated. The lower (detergent) phase was also washed in 10 mM-Tris/HCl (pH 7.4)/150 mM-NaCl/2% Triton-X114. The pooled aqueous and detergent phases were lyophilized and analysed by two-dimensional electrophoresis as described above.

Identification of polypeptides, glycoproteins and antigens

Polypeptides resolved by two-dimensional electrophoresis as described above were visualized by staining with Coomassie Blue. For analysis of small amounts of protein (as in the detergent phases after partitioning of hydrophilic and hydrophobic proteins), fractions were prepared from the livers of rats which had been injected via the tail vein with 3 mCi of [³⁵S]methionine 3 h previously. ³⁵S-labelled polypeptides were visualized by fluorography of gels dried on to filter paper (Bonner & Laskey, 1974).

To identify the glycoproteins, the polypeptides resolved by two-dimensional electrophoresis were transferred to nitrocellulose sheets at 20 V for 16 h (Bio-rad Trans-Blot cell). The sheets were immersed in 4% BSA in phosphate-buffered saline (0.15 M-NaCl/5 mM-sodium phosphate, pH 7.4) for 2 h at room temperature, and then overlaid for 2 h with approx. 1 × 10⁶ c.p.m. of iodinated lectins. Concanavalin A and wheat-germ agglutinin were iodinated by chloramine-T (Hunter & Greenwood, 1962). *Limax flavus* (slug) lectin was iodinated using the Iodogen reagent (Pierce). Iodinations were carried out at room temperature using 100 µg of lectins dissolved in 100–200 µl of phosphate-buffered saline, pH 7.2, in the presence of 40 mg of *N*-acetylglucosamine/ml (with wheat-germ agglutinin or slug lectin) or methyl D-mannoside (concanavalin A) and 0.5 mCi of Na¹²⁵I. Iodinated lectins were separated from unreacted iodine and the inhibitory saccharides were separated by gel filtration in Sephadex G-25 or Bio-gel P-10 columns (Burrige, 1978). With concanavalin A and slug lectin, nitrocellulose sheets were then washed with 200 µl of the lectins in 15 ml of phosphate-buffered saline, pH 7.4, containing 1% BSA, 0.5 mM-CaCl₂, 0.5 mM-

MgCl₂ and 0.5 mM-MnCl₂ for 1 h (Burrige, 1978). With wheat-germ agglutinin, the nitrocellulose sheets were incubated with 20 µl of lectin (1 µg approx.) in 15 ml of phosphate-buffered saline (pH 7.4)/1% BSA for 1 h (Glass *et al.*, 1981). After extensive washing of the nitrocellulose sheets in phosphate-buffered saline, pH 7.4, the sheets were air-dried and exposed for autoradiography using Kodak X-Omat AR5 films. Control experiments were also carried out in which the incubation of nitrocellulose sheets with the iodinated lectin were carried out in the presence of an excess of inhibitory saccharides.

Specific glycoproteins in the subcellular fractions were also identified by using antibodies after electrophoresis and transfer to nitrocellulose sheets as described above. Rabbit polyclonal antibodies recognizing the 43 kDa subunit of the rat asialoglycoprotein receptor (Enrich & Evans, 1989), 5'-nucleotidase (donated by Dr. Paul Luzio, Department of Clinical Biochemistry, University Medical School, Addenbrooke's Hospital, Cambridge) (Baillyes *et al.*, 1982) and cation-sensitive mannose 6-phosphate receptor (donated by Dr. K. von Figura, Biochemistry Department, Göttingen University, Germany) were used. After exposure to ¹²⁵I-Protein A and extensive washing, autoradiographs were prepared as described above.

RESULTS AND DISCUSSION

The present study shows that the different functions attributed to the three major plasma membrane domains of the hepatocyte (Evans, 1980; Evans & Enrich, 1989) are underlined by the presence in these membranes of distinctive polypeptide components. The two-dimensional separation of the major polypeptide constituents, identified by direct and indirect staining methods as well as by metabolic labelling, in the plasma membrane subfractions can now be compared directly with those present in membranes of endosome fractions. Such comparisons are prompted by the view that the compartmentation of the proteins in the sinusoidal plasma membrane, endocytic membranes and the canalicular plasma membrane is dynamic and specific, and is governed to a large extent by the participation of these membranes in endocytic and transcytotic trafficking. These processes account for the transfer by hepatocytes of specific proteins and bile acids between the blood and bile spaces (Renston *et al.*, 1980; Schiff *et al.*, 1984). Different functions carried out at the lateral plasma membrane, e.g. intercellular adhesion and communication, would also require different proteins (Evans & Enrich, 1989).

The subcellular fractionation approach adopted used liver homogenates, for optimal separation of membranes and organelles was obtained with liver tissue-derived fractions. The data are interpreted in the context of hepatocyte architecture and endocytic and transcytotic trafficking, but the contribution of non-parenchymal cells to the fractions deserves comment. Stereological approaches have shown that non-parenchymal cells can contribute up to 23% of the plasma membrane surface area and up to 40% of the pinocytotic surface area (Blouin *et al.*, 1977). The routine use of filtered homogenates has been shown to exclude from liver tissue homogenates a high proportion of non-parenchymal cells (van Berkel & Koster, 1977). The overall similarity of domain-identified plasma membranes and endosomes prepared from liver tissue and isolated hepatocytes (Wisher & Evans, 1977; Baenziger & Fiete, 1986) also adds confidence to the view that one is dealing with predominantly hepatocyte membranes. Finally, an endothelial marker, i.e. a 30 kDa mannose-binding-protein receptor (Steer & Clarenberg, 1979; Petell & Doyle, 1985; Jones, 1987), was not identified by the lectin-binding studies reported in this paper. The enzymic properties of the subcellular fractions studied in the present work have been

documented (Wisher & Evans, 1975; Saermark *et al.*, 1985; Evans & Flint, 1985). All of the fractions were shown to be relatively free of the conventional markers for lysosomes, mitochondria and nuclear membranes, and the contamination of the endosomal fractions by Golgi components was acceptably low. The extent of cross-contamination of fractions of similar buoyant densities, e.g. late endosomes and bile canalicular plasma membranes, was low, and is addressed and confirmed in the present work and in other studies using the same fractions (Ali & Evans, 1990).

Polypeptides and glycoproteins of plasma membranes

Fig. 1 compares the polypeptides resolved by IEF and NEPHGE across the pH range 3.7–9.0 in the blood sinusoidal, lateral and bile canalicular plasma membranes. Although polypeptides with similar molecular mass/pI co-ordinates were present, the fractions were compositionally distinctive. For example, the lateral plasma membranes contained greater numbers of basic polypeptides resolved by the NEPHGE technique within the pH range 7–9, but fewer polypeptides focusing at the acidic region of the gels. A major band with molecular mass/pI co-ordinates of 50–55 kDa/4.2–4.8 (Table 1, polypeptide 1), shown to be non-glycosylated (see Fig. 4), probably corresponded to the cytokeratins studied by Franke *et al.* (1981), although these were reported to focus on the 5.4–6.4 pH range under the conditions employed, or to intermediate filaments attached to desmosomes previously reported to abound as peripheral polypeptides in liver plasma membranes (Hubbard & Palade, 1977). Similarly, polypeptides corresponding to actin and tubulin were identified (Fig. 1) in the three fractions, according to the literature co-ordinates reported for these cytoskeletal components (Table 1).

Fig. 1. Resolution in two dimensions of the polypeptides in sinusoidal (a), canalicular (b) and lateral (c) plasma membrane fractions stained with Coomassie Blue

Numbered polypeptides are as described in Table 1. The area for comparison with early endosomes in Fig. 5 is bracketed.

Table 1. Molecular mass and pI co-ordinates used to identify polypeptides separated by IEF and NEPHGE in plasma membrane and endosomal fractions shown in Figs. 1 and 3–7

Poly-peptide	Identity	Molecular mass (kDa)	pI	Reference
1	Cytokeratins/intermediate filaments	55–47	4.2–4.8	Franke <i>et al.</i> (1981)
2	Actin	45	5.0	Chu & Doyle (1985)
3	Tubulin	55	4.6	Bravo (1984)
4	Asialoglycoprotein receptor (RHL-1)	43	4.0–6.0	Fig. 2
5	5'-Nucleotidase	69	4.8–6.3	Fig. 2
6	Mannose 6-phosphate receptor	46	3.6–4.2	Fig. 2
7	Calmodulin-binding protein	115	4.3	Enrich <i>et al.</i> (1988)
8	Albumin	68	6.4	Tracy & Young (1984)

By employing antibodies to the purified proteins to establish their co-ordinates in liver membranes, it was possible to study the distribution in the subcellular fractions of the asialoglycoprotein and divalent cation-sensitive mannose-6-phosphate receptors (Bleekemolen *et al.*, 1988) as well as that of 5'-nucleotidase (Baillyes *et al.*, 1982) (Fig. 2). Although corresponding polypeptides were identified in Coomassie Blue-stained gels (Fig. 1), more information on their compartmentation in liver membranes was obtained in the lectin overlay studies (see below).

The glycoprotein composition of the plasma membrane domains (Enrich & Gahmberg, 1985) was studied in the two-dimensional gels by using a lectin overlay technique. Radioiodinated concanavalin A, which identifies mannose and glucose moieties, bound to a large number of acidic polypeptides of between 40 and 200 kDa in the plasma membrane fractions (Fig. 3). A smaller number of glycoproteins was visualized when the gels were examined by using wheat-germ agglutinin (Fig. 4), which binds to *N*-acetylglucosamine and sialic acid residues. Comparison of the glycoproteins labelled by wheat-germ agglutinin in the three plasma membrane domains again indicated that different populations were present. A number of the

Fig. 2. Identification in two-dimensional gel electrophoresis of molecular mass and pI co-ordinates by immunoblotting

Antibodies used were to 5'-nucleotidase of a canalicular plasma membrane fraction (a), to divalent-cation-dependent mannose 6-phosphate receptor in liver endosomes (b), and to the asialoglycoprotein receptor in endosomes (c).

Fig. 3. Resolution in two dimensions of the glycoproteins in sinusoidal (a), canalicular (b) and lateral (c) plasma membranes stained with radio-iodinated concanavalin A

Numbered polypeptides are as described in Table 1.

glycoproteins identified by wheat-germ agglutinin were present in the 110–114 kDa range in bile canalicular plasma membrane fractions (Fig. 4); many enzymes of this size are located at the bile canalicular membrane (Meier, 1987). The lectins also allowed the distributions of the asialoglycoprotein and mannose-6-phosphate receptors and of 5'-nucleotidase to be assessed. The two receptors and the enzyme were identified according to their coordinates in sinusoidal plasma membranes, but only 5'-nucleotidase was present in the bile canalicular plasma membranes.

Proteins and glycoproteins of endocytic membranes

A comparison of the major Coomassie Blue-stained polypeptides resolved across the pH range 3.7–9.4 in early and late endosomes is shown in Fig. 5. The results indicated a close correspondence of the major polypeptide composition of early and late endosomes. In Fig. 5, polypeptides corresponding to the 115 kDa calmodulin-binding protein (which is postulated to be endosome-specific; Enrich *et al.*, 1988), the asialoglycoprotein

Fig. 4. Resolution in two dimensions of glycoproteins in sinusoidal (a), canalicular (b) and lateral (c) plasma membranes stained with radio-iodinated wheat-germ agglutinin

Numbered polypeptides are as described in Table 1. The arrow points to a group of acidic glycoproteins of 110–114 kDa, pI 3.5–4.8, which are present in high amounts in canalicular plasma membranes. In these lectin-stained gels, the position of non-glycosylated polypeptides can be deduced, as illustrated by polypeptide 1 (Table 1). The brackets indicate corresponding polypeptides.

receptor and 5'-nucleotidase are shown. Comparison of the major polypeptides of endocytic membranes in Fig. 5 with sinusoidal plasma membranes (Fig. 1) indicated a general similarity, especially in the polypeptides resolved across the pH range 3.7–6.7. However, a number of basic polypeptides resolved by NEPHGE in the pH range 7.8–8.5 and of molecular mass 20–50 kDa were present in endosomes, these being especially enriched in late endosomes (Fig. 5). It was noted that these were absent from sinusoidal plasma membranes (Fig. 1). These basic polypeptides were different to those present in lateral plasma membranes (Fig. 1).

The glycoproteins labelled by concanavalin A and wheat-germ agglutinin in early and late endocytic vesicles were very similar (Figs. 6a and 6b); these fractions also contained a similar glycoprotein profile to that of sinusoidal plasma membranes (Fig. 3). Undoubtedly many of these common glycoproteins, also shown by slug lectin labelling (see Fig. 8), correspond to constituents that recycle between the sinusoidal plasma membrane and the membranes comprising the endocytic networks.

Fig. 5. Resolution in two dimensions of the polypeptides in early (a) and late (b) endosomes stained with Coomassie Blue

Numbered polypeptides are as described in Table 1. The bracketed area is for comparison with the area in Fig. 1.

The asialoglycoprotein and mannose-6-phosphate receptors were clearly delineated in the sinusoidal plasma membranes as well as in early and late endocytic vesicles, where they appeared to be present in similar amounts. These receptors now identified in the endosome fractions probably correspond to the intracellular 'silent' pools identified in livers by others (Stoorvogel *et al.*, 1989). The position of a 115 kDa, pI 4.3, calmodulin-binding protein in endosomes (Enrich *et al.*, 1988) and its identification as a glycoprotein is shown. A major glycoprotein identified in the subcellular fractions was 5'-nucleotidase, which showed charge heterogeneity, especially when stained with the lectins.

Fig. 6. Resolution in two dimensions of the glycoproteins in early (a and c) and late (b and d) endosomes stained with radio-iodinated concanavalin A (a and b) and wheat-germ agglutinin (c and d)

Numbered polypeptides are as described in Table 1.

5'-Nucleotidase is a glycoprotein rich in mannose residues, as shown by its sensitivity to endoglycosidase H (Baron & Luzio, 1987), and this may account for its intense labelling by concanavalin A. The chromatic series of bands of varying molecular mass may result from the removal of sialic acid (Chu & Doyle, 1985) that could occur during recycling of the enzyme between the plasma membrane and the endocytic compartment (van den Bosch *et al.*, 1988). Charge heterogeneity was also a feature of the asialoglycoprotein receptor stained by concanavalin A. Similar results were obtained after staining with the slug lectin (see Fig. 8) that identifies sialic acid.

Properties of the polypeptides of sinusoidal plasma membranes and endosomes

To compare the distributions of integral and peripheral membrane polypeptides in sinusoidal plasma membranes and early endosomes, these fractions were partitioned into aqueous and Triton X-114 phases and then separated by two-dimensional electrophoresis (Fig. 7). The polypeptides separated into the aqueous or detergent phases were clearly different. Although many polypeptides stained by Coomassie Blue corresponded in the aqueous phases of sinusoidal plasma membranes and endocytic vesicles, it was noted, for example, that albumin (Table 1) was a major constituent in the former but a minor one in the latter. The low albumin content of endosomes was indicative of the relative absence of exocytic (Golgi) vesicles from these fractions. The Triton X-114-soluble polypeptides, owing to the effects of the detergent on the separation, proved more difficult to analyse by IEF, but the patterns obtained using membrane fractions metabolically labelled with [35 S]methionine (Fig. 7) indicated that a similar general population of integral polypeptides was present in sinusoidal plasma membranes and endosomal vesicles. The band corresponding in position to 5'-nucleotidase behaved anomalously in the two-phase separation, with most of this protein appearing in the aqueous phase and a minor component being identified in the endosomal Triton X-114

Fig. 7. Comparison of the polypeptides in sinusoidal plasma membranes (a and b) and early endosomes (c and d) partitioning into the aqueous phase (a and c) or the Triton X-114 phase (b and d)

In (a) and (c), polypeptides were stained with Coomassie Blue; in (b) and (d), polypeptides were identified by fluorography of [35 S]methionine-labelled polypeptides. The brackets indicate corresponding polypeptides. Numbered polypeptides are as described in Table 1.

phase. One explanation for this behaviour is that, although the technique separates many integral proteins into the detergent phase, exceptions have been noted, e.g. acetylcholine receptors (Maher & Singer, 1985) and chromaffin granule ATPases (Percy *et al.*, 1985). A second explanation is that proteins anchored in the membranes by a phosphoinositide tail, of which 5'-nucleotidase is one (Bailey *et al.*, 1990), may be released from the membrane by endogenous phospholipase C activity.

Endocytic pathways in relation to hepatocyte surface polarity

The analyses of the proteins and glycoproteins of liver plasma membrane domains and the endocytic compartment now reported can allow the compartmentation of various constituents to be discussed in the context of endocytic pathways in liver tissues. The compositional relationships between sinusoidal plasma membranes, early and late endosomes and canalicular plasma membranes is already evident from the data presented. The relationships between the membrane components were also illustrated independently by using a slug lectin that stains sialoglycoproteins (Hedman *et al.*, 1986) to compare the relevant membrane fractions. As shown in Fig. 8, the sialoglycoproteins of sinusoidal and early and late endosomes were generally similar, but those of canalicular plasma membranes were different. 5'-Nucleotidase was present in all of the fractions, but the asialoglycoprotein receptor was absent from canalicular plasma membranes, again indicating the selectivity in transfer of components from late endosomes to the bile canalicular plasma membrane.

The endocytic compartment is complex in its subcellular location (Hopkins, 1986; Enrich & Evans, 1989), in terms of the kinetics of movement of ligands through its vacuolar components and with respect to its interaction with other intracellular membranes. The designation early and late applied to the endosome subfractions studied in this work is a kinetic one based on the uptake by liver of various ligands administered into the portal vein and recovered first (at 2–5 min) and later (at 10–15 min) in a population of vesicles that were separated by centrifugation in sucrose gradients, followed by isopycnic

Nycodenz gradients. Although many ligands, e.g. prolactin, insulin and asialotransferrin, were demonstrated after internalization to pass sequentially from early to late endosome fractions (Evans & Flint, 1985), further studies using transferrin have shown that this ligand was internalized predominantly into an early endocytic fraction (H. Goldenberg & W. H. Evans, unpublished work). It was shown using cultured cells that transferrin was endocytosed primarily into an early, or peripheral, endosome fraction (Schmid *et al.*, 1988), and the situation thus appears to be paralleled in liver. The involvement of late endosomes in the transcytotic route was demonstrated by studies in which internalized ^{125}I -labelled polymeric immunoglobulin A was recovered in early and late liver endosomes (P. Tabona & W. H. Evans, unpublished work). Also, using antibodies to the secretory component (the polymeric IgA receptor), this was shown to be present as a 110 kDa polypeptide in sinusoidal plasma membranes and in early and late endosomes; in bile canalicular plasma membranes, a 32 kDa breakdown product of the secretory component was detected by the antibodies (N. Ali & W. H. Evans, unpublished work). Thus the two endosome fractions used in the present work contain components of the endocytic compartment participating in endocytosis and transcytosis. However, it cannot be excluded that other membranes comprising the endocytic networks may not have been recovered by the methods used, or that other specific populations of endocytic vesicles trafficking to lysosomes or the bile canalicular membrane, or recycling to the sinusoidal plasma membrane domain, were not adequately separated by the separation methods used.

A major conclusion emerging from the detailed two-dimensional analysis of the proteins of endocytic membranes is the close overall similarity of the major polypeptides present in early and late endosome fractions which were identified using Coomassie Blue staining and the lectin overlays. This is in agreement with the results presented by Baenziger & Fiete (1986) who were unable to demonstrate differences between the polypeptides resolved in two dimensions in two liver endosomal fractions. Studies on the enzyme activities present in the early and late endosomes have indicated an increased recovery and specific activity of 5'-nucleotidase and alkaline phosphodiesterase in late endosomes (Evans & Flint, 1985; Saermark *et al.*, 1985; Quintart *et al.*, 1989). Quantitative differences in the activity of inositol phosphatases and GTP-binding protein (G-protein) content were also found (Shears *et al.*, 1988; Ali *et al.*, 1989).

Although the majority of the polypeptides, especially those identified by dye staining and radio-iodinated lectins, which were resolved across the pI range 3.7–6.5 were similar in early and late endosomes, it was noted (Fig. 5) that late endosomes contained higher relative amounts of low-molecular-mass basic polypeptides in the 20–50 kDa and pI 8–9 range. These results indicate that compositional differences exist between various sub-compartments of the endocytic apparatus, and the polypeptides identified that were absent from sinusoidal plasma membranes (c.f. Fig. 1) may include low-molecular-mass G-proteins that are candidates for roles in the regulation of endocytic trafficking (Mayorga *et al.*, 1989). Nevertheless, the overall results contrast with those of Schmid *et al.* (1988), in which Chinese hamster ovary cell endosomes, corresponding to early and late fractions and separated by free-flow electrophoresis, were shown to be very different after vectorial iodination using endocytosed lactoperoxidase and analysis by two-dimensional electrophoresis. Two explanations for the different results may be advanced. First, the major constituents of two endosome fractions were examined comprehensively in the present work, whereas a subpopulation of endosomal proteins iodinated at the luminal aspect of the membrane were studied by Schmid *et al.*

Fig. 8. Resolution in two dimensions of the polypeptides in sinusoidal plasma membranes (a), early endosomes (b), canalicular plasma membranes (c) and late endosomes (d) stained using a slug lectin

Numbered polypeptides are as described in Table 1.

(1988). Although purification of liver endosomes using free-flow electrophoresis has been reported (Debanne *et al.*, 1982; Evans & Flint, 1985; Fuchs *et al.*, 1989), this method did not separate early and late endosomal populations. A second and more fundamental explanation for the different results obtained is that cultured cells and liver cells differ in their degree of participation in endocytosis. Chinese hamster ovary cells are unpolarized, whereas hepatocytes are polarized epithelial cells that are actively involved in the constitutive endocytosis of ligands from the blood, especially those, e.g. polymeric IgA and possibly bile acids, that undergo transcytosis and release into bile. Thus in liver tissue the endocytic compartment participates in the constant transport of materials from blood to bile.

Lectin overlay studies showed similar glycoprotein profiles in early and late endosomes, especially the asialoglycoprotein receptor (polypeptide 4). This result contrasts with those presented by Mueller & Hubbard (1986) and Evans & Flint (1985) that point to the presence of asialoglycoprotein-receptor-enriched and -deficient endocytic structures in liver. The reasons for these discrepancies are not clear, except that the methodology used to assess receptor numbers differed.

Two models of endocytosis have been proposed (Helenius *et al.*, 1986). Although these concern non-polarized cells in which endocytosis may or may not be triggered by formation at the cell surface of receptor-ligand complexes, whereas endocytosis in liver tissue proceeds constantly as demonstrated by transcytosis of secretory component by liver occurring in the absence of polymeric IgA (Mullock *et al.*, 1980), the plausibility of these models in the light of the present data warrants brief comment. The general similarity between the major polypeptide constituents of pI 3.7–7.5 at the sinusoidal plasma membrane domain, where circulating ligands bind to their respective receptors (but not the lateral and canalicular plasma membrane domains) and endosomes, suggests that there is bulk transfer, especially of a large number of glycoproteins, into an early endocytic membrane that may develop into a late endocytic membrane, and involving mainly quantitative alterations in the major proteins and glycoproteins. However, only a small and specific number of proteins were transferred onwards from the endocytic compartment to the bile canalicular plasma membrane. In the present work, comparison of endosomes with lysosomes was not made, but specific LIMPS (lysosomal integral membrane proteins) have been shown to be common to endosomes, plasma membranes and lysosomes (Lippincott-Schwartz & Fambrough, 1987).

In summary, the data do not lead to a clear distinction being drawn between the 'maturation' and 'shuttle' models used to explain functional relationships between the early and late stages of endocytosis. Indeed, such models in themselves may be oversimplifications of the endocytic process, but the present data favour the view that a 'maturation' model is more likely to be operational in tissue such as liver, in which the transcytosis component of endocytosis is extensive.

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