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Isolation and characterization of canalicular and basolateral plasma membrane fractions from human liver

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A method is described for the isolation of subfractions from human liver plasma membranes, enriched in canalicular domains (cLPM) and basolateral domains (blLPM), respectively, and the results are compared to those obtained with rat liver. The studies were performed in 18 human livers. The cLPM (isolated at densities 1.103–1.127 for human and 1.036–1.127 for rat cLPM) from human as well as rat liver showed a lower density than the blLPM (1.141–1.161 for human and 1.151–1.172 for rat blLPM). Human and rat blLPM were characterized by increased levels of (Na^+/K^+)-ATPase (relative enrichment 33 and 21, respectively). Both human and rat cLPM showed high specific activities of leucine aminopeptidase; relative enrichment factors were 42 and 31, respectively. Mg^{2+}-ATPase and alkaline phosphatase, specific canalicular enzymes in rat liver, were only slightly enriched in the cLPM of human liver, which indicates that these enzymes are not suitable as marker enzymes for human liver cLPM. Both cLPM and blLPM of human and rat origin were only slightly contaminated with mitochondria, lysosomes, Golgi membranes and endoplasmic reticulum. Total recoveries of cLPM and blLPM were 0.02 mg protein/g liver each for the human membrane preparations, compared to 0.07 and 0.16 mg protein/g liver for the membranes prepared from rat liver. Analysis of membrane fluidity revealed that the human liver cLPM were more rigid than blLPM (mean difference in fluorescence polarization P_{DPH} 0.024). They contained more cholesterol (0.43 vs. 0.30 μmol/mg protein) and phospholipids (0.54 vs. 0.39 μmol/mg protein, respectively), which was compatible to rat liver plasma membrane fractions. This study shows that besides similarities, there are several differences between human and rat liver plasma membrane fractions.

Introduction

The hepatocyte is a polarized cell. Canalicular and basolateral liver plasma membrane fractions (cLPM and blLPM, respectively) of rat liver show many differences with respect to membrane lipid composition and fluidity, presence of membrane enzymes, receptor activities and transport processes that are involved in cellular function and bile formation [1]. Little is known about these aspects of human liver plasma membranes. In order to study the plasma membrane related phenomena in the human liver, it is essential to prepare separated canalicular and basolateral plasma membrane fractions. Besides studies on function and composition of normal liver membranes, the possibility to isolate purified membrane preparations from human liver may increase the insight into alterations in membrane structure and function induced by liver disease.

Several methods have been described for the separate isolation of basolateral and canalicular membranes from rat liver [1–8]. However, no procedure has been published so far for the isolation of both types of membrane from human liver.

We describe a method for the simultaneous isolation of plasma membrane fractions enriched in canalic-

Abbreviations: LPM, liver plasma membranes; blLPM, basolateral domains of LPM; cLPM, canalicular domains of LPM; DPH, diphenylhexatriene; P_{DPH}, fluorescence polarization; RE, relative enrichment of enzyme activities.

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ular and basolateral domains, respectively, from human livers. This isolation procedure was adapted from the method described for rat livers by Meier et al. [8]. Membrane fragments were characterized both enzymatically and chemically, and the results were compared to those obtained with rat livers. A preliminary report of this work has been presented [9].

Materials and Methods

Human livers

Human livers were obtained from 11 kidney donors (4 female, 7 male, age 5–68 years) and 7 multi-organ donors of whom part of the liver was used for transplantation (1 female and 6 male, age 4–46 years). Livers were perfused with UW (University of Wisconsin) solution [10] and stored in this buffer at 4°C until the start of the isolation procedure, which was performed within 36 h. Use of the tissue was approved by the Medical-Ethical Committee of the Groningen University Hospital.

Animals

Male Wistar rats weighing 200–250 g were used. Rats were maintained in a light and temperature controlled room. Lights were on from 6 a.m. to 6 p.m. Water and pellet food (RMH-B, Hope Farms, The Netherlands) were available ad libitum. Fed animals were killed by decapitation between 8 and 9 a.m.

Isolation of plasma membranes from rat liver

The procedure used was based on the one described by Meier et al. [8] for Sprague-Dawley rats and modified by us for use with Wistar rats. All steps in the isolation procedure were performed at 0–4°C. Rat livers (weighing 10–12 g) were cut into small pieces, washed in 1.0 mM $NaHCO_3$ (pH 7.4) and homogenized in a loose dounce (Braun, Melsungen, F.R.G.). The homogenate was diluted to 10% (w/v) with the bicarbonate solution and filtered as described elsewhere [8]. After centrifugation ($500 \times g$ for 5 min + $1000 \times g$ for 10 min), the pellet was resuspended in 1.0 mM $NaHCO_3$ and recentrifuged at $1000 \times g$ for 10 min. This crude nuclear pellet was resuspended in 4.5 vol. of 56.0% sucrose (w/w) and stirred for 15 min to disrupt membrane aggregates. Samples of 15 ml of this suspension were transferred to centrifuge tubes (Hitachi 40 PA). The suspension was overlaid with 10 ml 43.0% sucrose, followed by 6 ml 36.5% sucrose. The tubes were filled with 0.25 M sucrose and centrifuged for 120 min at 22000 rpm ($65000 \times g$) in a swing-out rotor (type SRP 28) in an LKB 2331 ultracentrifuge. The plasma membranes were recovered from the 36.5–43.0% interface and washed [8]. The mixed plasma membranes were resuspended in 2 ml 0.25 M sucrose and extensively fragmented in a tight dounce homogenizer (Braun, Melsungen, F.R.G.) by 50 up and down strokes. The plasma membranes were subsequently stored in liquid nitrogen for maximally 1 week, until subfractionation. After rapid thawing at 37°C, membranes were dispersed with a tight dounce as described above and layered on top of a three-step sucrose gradient in Beckman Ultra-Clear™ ultracentrifuge tubes (14 × 89 mm). The gradient consisted of 3.5 ml 38.0% sucrose, 3.5 ml 34.0% sucrose, and 2.5 ml 29.0% sucrose. Tubes were filled to the top with 0.25 M sucrose and centrifuged for 180 min in the Beckman SW 41 rotor at 40000 rpm ($196000 \times g$). The band recovered from the top of the 29% sucrose layer represents the canalicular membranes, whereas the basolateral membranes are recovered from the 34/38% interface. The membranes were diluted in 0.25 M sucrose and harvested by centrifugation at $75000 \times g$ for 90 min in the SRP 28 rotor.

Isolation of plasma membranes from human liver

The procedure for the isolation of plasma membranes from human liver was adapted from that for rat liver. All procedures were performed at 0–4°C.

Pieces of human liver were taken randomly and cut by scissors into small pieces (less than 5 mm). Vascular and connective tissue were removed as extensively as possible. Up to 100 g of liver material in 1.0 mM $NaHCO_3$ (pH 7.4) was homogenized in a thyristor (Ultra-Turrax, type TP 18-10, Janke & Kunkel, Staufen i. Breisgau F.R.G.), for 15 s. The homogenate was filtered through a sieve to remove large pieces of remaining connective tissue. Subsequently, the homogenate was dounced by seven strokes in a loose dounce. The homogenate was filtered through cheesecloth and diluted to a 10% (w/v) suspension with 1.0 mM $NaHCO_3$. The homogenate was centrifuged twice as described above for the rat material, and the final pellet was taken up in 4.5 volumes of 56.0% sucrose. After stirring for 15 min, the suspension was transferred into Hitachi 40 PA ultracentrifuge tubes (up to 20 ml). The suspension was overlayered with 3 ml 44.0%, 3 ml 40.0%, 5 ml 36.0% and 3 ml of 30.0% sucrose. Tubes were filled with 0.25 M sucrose. The tubes were centrifuged for 2 h at 22000 rpm at 4°C. The band enriched in plasma membranes, floating on the 30/36% interface (see Results), was recovered and washed as described [8]. The plasma membranes were dounced in a tight dounce (50 up and down strokes), and stored in liquid nitrogen for maximally 6 months until separation into canalicular and basolateral fractions. Storage under these conditions did not induce changes in enzyme activities. After quick thawing at 37°C, membranes were dounced again and layered on top of a four-step sucrose gradient consisting of 2.5 ml each of 36.0%, 32.0%, 29.0% and 24.0% sucrose. Tubes were filled with 0.25 M sucrose and centrifuged for 180

min in the Beckman SW 41 rotor at 40000 rpm. The bands recovered from the top of the 29% layer (canalicular membranes) and from the top of the 36% layer (basolateral membranes) were diluted in 0.25 M sucrose and harvested by centrifugation as described for the rat membranes.

Electron microscopy

Membrane pellets were fixed overnight at 4°C in 2% glutaraldehyde in 0.1 M phosphate buffer (pH 7.38). Pellets were rinsed in phosphate buffer containing 6.8% sucrose and postfixed in 1% OsO_4 and 1.5% $K_4Fe(CN)_6$ in phosphate buffer. Samples were dehydrated in graded ethanol solutions and embedded in Epon 812. Sections of 50 nm were made using a Reichert ultramicrotome and stained with uranyl acetate and lead acetate [11]. Samples were examined with an Akashi 002A electron microscope at 60 kV.

Enzymatic characterization of plasma membranes

(Na^+/K^+)-ATPase, Mg^{2+}-ATPase [12], leucine aminopeptidase [13] and alkaline phosphatase [14] were determined as described. To quantify the presence of intracellular organelles the following marker enzymes were determined: glucose-6-phosphatase for endoplasmic reticulum [15], succinate cytochrome *c* reductase for mitochondria [16], galactosyltransferase for Golgi membranes [17] and acid phosphatase for lysosomes [18]. The last procedure was slightly adapted: to stop the enzymatic reaction, 1 vol. of the reagens mixture was transferred into 1 vol. 2.0 M NaOH instead of Tris base.

Chemical determinations

Protein was determined by the method of Lowry et al. [19], using bovine serum albumin (Sigma) as standard. Membrane lipids were extracted (total recovery > 95%) [20] and cholesterol [21] and total phospholipid were determined [22]. Fluorescence polarization of diphenylhexatriene (DPH), a measure for membrane fluidity, was determined at 25°C as described previously [23].

Results

Isolation of plasma membranes

To characterize the isolated membrane fractions, several plasma membrane marker enzymes were determined, viz. (Na^+/K^+)-ATPase (a blLPM enzyme in rat liver), Mg^{2+}-ATPase, leucine aminopeptidase, alkaline phosphatase (cLPM enzymes in rat liver). Succinate cytochrome *c* reductase, glucose-6-phosphatase, galactosyltransferase and acid phosphatase activities were determined to establish contamination with mitochondria, endoplasmic reticulum, Golgi membranes and lysosomes, respectively. The activities of these enzymes were determined in homogenates of Wistar rat and human livers; they showed values in the same order of magnitude (Table I).

TABLE I

Characterization of liver homogenates of Wistar rats and humans

Liver homogenates were prepared and enzyme activities were determined as described in the text. Data are expressed as means ± S.D. for the number of livers indicated in parentheses; specific activities as μmol (mg protein)$^{-1}$ h^{-1}, except for galactosyltransferase, where units are nmol (mg protein)$^{-1}$ h^{-1}.

	Rat	Human
Plasma membrane marker enzymes		
(Na^+/K^+)-ATPase	0.88 ± 0.13 (6)	0.48 ± 0.14 (12)
Mg^{2+}-ATPase	2.37 ± 0.18 (6)	0.95 ± 0.35 (12)
Alkaline phosphatase	0.31 ± 0.08 (6)	1.86 ± 0.73 (12)
Leucine aminopeptidase	0.52 ± 0.04 (4)	0.83 ± 0.38 (12)
Marker enzymes for intracellular organelles		
Acid phosphatase	4.43 ± 0.32 (6)	2.35 ± 1.12 (3)
Glucose-6-phosphatase	4.78 ± 0.50 (6)	2.79 ± 0.87 (10)
Succinate cyt. *c* reductase	3.68 ± 0.25 (6)	1.52 ± 0.64 (10)
Galactosyltransferase	7.9 ± 1.1 (3)	16.6 ± 1.6 (6)

Plasma membranes were isolated from rat liver as described in Materials and Methods. This method applied to the human livers resulted in preliminary experiments in membranes with a considerable contamination with mitochondria (up to 6-fold enrichment of succinate cytochrome *c* reductase). Therefore, we adapted the procedure for the use with human material. As a first step, the nuclear pellet was centrifuged on a sucrose gradient from 30 to 45%, and fractions were analyzed for the presence of plasma membranes ((Na^+/K^+)-ATPase and leucine aminopeptidase activity), mitochondria (succinate cytochrome *c* reductase) and endoplasmic reticulum (glucose-6-phosphatase). Results are shown in Fig. 1. Purified plasma membranes, only slightly contaminated with mitochondria, can be obtained by using the fraction between densities 1.131 and 1.161 (30 and 36% sucrose, respectively). The plasma membranes isolated in this way from nine human livers were characterized by high relative enrichments (specific enzyme activity in the membrane preparation relative to that of the total homogenate) for the plasma membrane enzymes, whereas mitochondria, endoplasmic reticulum, Golgi and lysosomes were only slightly enriched or not at all (Table II). Recoveries of the enzymes measured were 83–120%, indicating that no major loss or stimulation of enzyme activity occurred during the isolation procedure. The recovery of plasma membranes from human livers was 0.27 ± 0.08 mg protein/g liver (mean ± S.E.; $n = 9$ livers) and was significantly lower than from rat liver (1.32 ± 0.25; $n = 6$), due to the altered isolation procedure. Besides the nine preparations with relatively high enrichments in plasma membrane markers used for

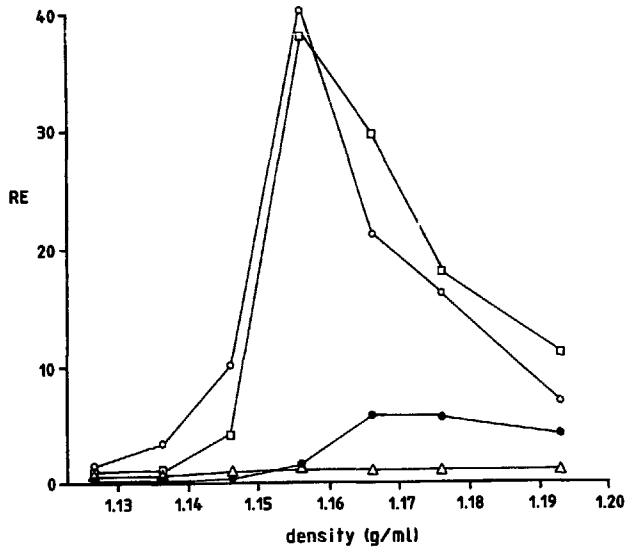


Fig. 1. Fractionation of human liver membranes. Fractionation of nuclear pellet of human liver homogenate on a sucrose gradient. The membranes were taken up in 56% sucrose as described in Materials and Methods, overlaid with the sucrose gradient and centrifuged at 65000× *g* for 2 h. Relative enrichments of the plasma membrane enzyme (Na^+/K^+)-ATPase (○), the canalicular enzyme leucine aminopeptidase (□), the mitochondrial enzyme succinate cytochrome *c* reductase (●) and glucose-6-phosphatase (△), an enzyme of endoplasmic reticulum, are shown at the different sucrose densities (typical experiment).

further analysis, in one additional preparation only a small enrichment in plasma membranes was found (RE = 4 for (Na^+/K^+)-ATPase); this preparation was not used for further studies.

Separation of canalicular and basolateral plasma membrane fragments

The purified plasma membranes from both human and rat liver were dounced tightly and subjected to isopycnic ultracentrifugation on a sucrose gradient at 196000 × *g*. (Na^+/K^+)-ATPase and leucine aminopeptidase, alkaline phosphatase and Mg^{2+}-ATPase were used as the plasma membrane marker enzymes for rat liver (Fig. 2a–d, open symbols), to investigate the location of blLPM and cLPM in the gradient.

(Na^+/K^+)-ATPase, a blLPM enzyme in rat liver [1], was located at higher sucrose densities in both rat and human liver (Fig. 2a).

Enzyme histochemical investigations [24] and immunological studies [25] revealed that the leucine aminopeptidase activity in human liver is predominantly present at the canaliculair pole of the hepatocyte. The peak activity of leucine aminopeptidase in human material was located at low sucrose densities (Fig. 2b), at a position similar to that observed for rat canalicular membranes, which were characterized by high activities of leucine aminopeptidase, alkaline phosphatase and Mg^{2+}-ATPase (Fig. 2b,c,d). In the subfractions of human LPM shown in Fig. 2c, peak activities of alkaline phosphatase were present at a density slightly different from that of leucine aminopeptidase, whereas peak activities for these two enzymes were located at the same density for rat LPM.

Mg^{2+}-ATPase was only slightly enriched in canalicular membranes of human origin, in contrast to those of rats (Fig. 2d). Mitochondrial membranes were predominantly present at the higher sucrose-densities (Fig. 2e). No enrichment in endoplasmic reticulum was observed in the fractions (Fig. 2f).

Based on the results shown in Fig. 2, a discontinuous gradient was developed for the separation of basolateral and canalicular membrane fractions. For the rat, the cLPM were recovered from the 29% sucrose layer (density 1.036–1.127) and the blLPM were recovered from the 34/38% sucrose interface (density 1.151–1.172). By this procedure we obtained membrane preparations enriched in canalicular and basolateral plasma membrane fractions, respectively. Contamination with subcellular membranes was only small, as indicated by low relative enrichments for the appropriate marker enzymes (Table III). For the human membranes, we chose to isolate canalicular membranes from the 24/29% sucrose interface (density 1.103–1.127), in order to get canalicular material only slightly contaminated with basolateral membrane fragments. We decided not to include material of densities less than 1.103 g/ml in the human canalicular fraction; this fraction contained membranous material with very low activities of the enzymes tested. The blLPM were collected from the 32/36% sucrose interface (density 1.141–1.161), i.e., a fraction with a high activity of the plasma membrane enzyme (Na^+/K^+)-ATPase, with minimal mitochondrial contamination (Table III). In Table IV the recoveries of protein and marker enzymes in plasma membranes, cLPM and blLPM are shown for the human liver. In the final preparation approx. 0.02% of the homogenate protein was recovered in the basolateral and the canalicular fractions. In cLPM 0.6 % of the canalicular enzyme leucine aminopeptidase was recovered, whereas about 0.7% of the (Na^+/K^+)-ATPase was recovered in the blLPM. Recoveries of the canalicular and basolateral membrane fragments from the human liver were approx. 0.02 mg protein per g liver. For the rat about 0.07 and 0.16 mg protein, respectively, were recovered per g liver.

Electron microscopy of the final membrane pellets (Fig. 3) showed the presence of vesicles (cLPM) and vesicles plus membrane sheets (blLPM). No morphologic evidence for contamination with subcellular organelles was seen nor were any intact bile canaliculi observed.

The membrane fragments were characterized with respect to membrane fluidity and lipid composition (Table V). The human and rat cLPM contained larger amounts of phospholipid and cholesterol than the re-

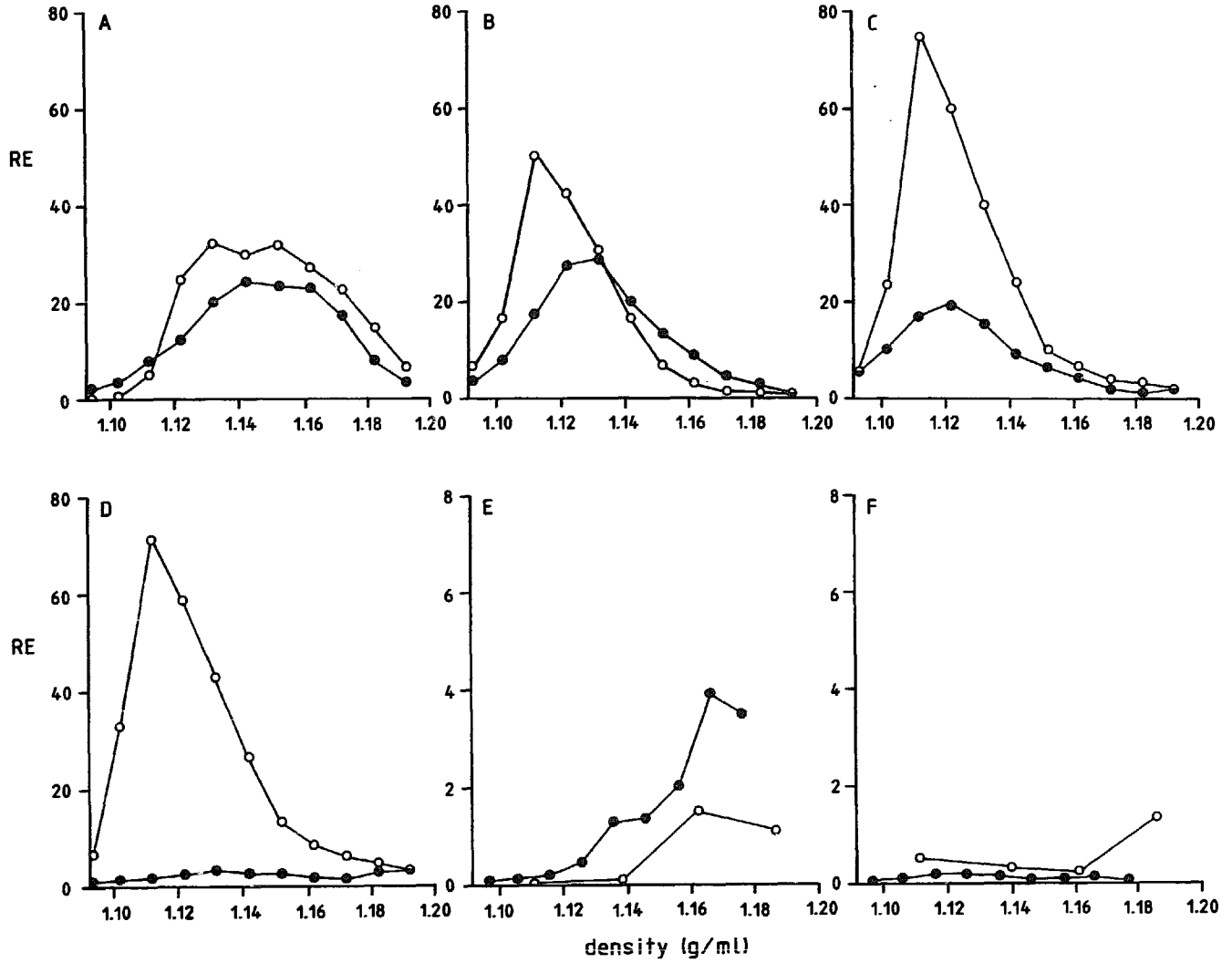


Fig. 2. Separation of basolateral and canalicular plasma membrane fractions from human and rat liver. Purified plasma membranes were dounced tightly and subjected to isopycnic centrifugation on a sucrose gradient for 3 h at 196000× g. The relative enrichments of several marker enzymes are indicated at the different sucrose densities. (a) (Na^+/K^+)-ATPase; (b) leucine aminopeptidase; (c) alkaline phosphatase; (d) Mg^{2+}-ATPase; (e) succinate cytochrome *c* reductase; (f) glucose-6-phosphatase (○ rat, ● human liver plasma membranes). Note the different ordinate-scales of e and f. Based on three experiments we decided to consider the membranes of the densities 1.103–1.127 as canalicular membranes and those of densities 1.141–1.161 as basolateral ones (human liver). For the rat the basolateral and canalicular membranes were isolated from densities of 1.151–1.172 and 1.036–1.127, respectively.

TABLE II

Characterization of plasma membranes of rat and human livers

The plasma membrane fraction was recovered from a discontinuous sucrose gradient at the 36.5/43% interface (rat) and the 30/36% interface (human), respectively. Data are presented as relative enrichments (ratio of specific activities in membranes to specific activities in the homogenate) ±S.D. Number of different livers used in parentheses.

	Rat		I	an	
(Na^+/K^+)-ATPase	15	±3 (6)	22	±10	(8)
Mg^{2+}-ATPase	15	±3 (6)	2	± 1	(8)
Alkaline phosphatase	6	±2 (6)	5	± 4	(9)
Leucine aminopeptidase	6	±3 (4)	17	± 7	(9)
Acid phosphatase	1.2	±0.4 (6)	1.5	± 0.7	(3)
Glucose-6-phosphatase	0.7	±0.3 (6)	1.0	± 1.1	(9)
Succinate cyt. *c* reductase	1.0	±0.2 (6)	1.5	± 1.6	(9)
Galactosyltransferase	0.9	±0.9 (3)	1.2	± 0.8	(5)

spective blLPM. Membrane fluidity of blLPM was higher than that of cLPM for both human and rat material, as demonstrated by the lower value of the fluorescence polarization P.

Discussion

Although several methods have been developed for the isolation of canalicular and basolateral membranes from rat liver, no method for the simultaneous isolation of these membrane fractions from human liver has been published so far. Yet, since hepatic transport functions and bile formation may show large species variations, it is important to verify observations made in rat liver plasma membrane preparations for the human situation. Therefore, we developed an isolation

TABLE III

Enzymatic characteristics of canalicular and basolateral plasma membrane fractions

Membrane fractions were isolated as described in the text. Data are expressed in relative enrichments (ratio of specific activities in membranes to specific activities in the homogenate) ±S.D. Number of different livers used in parentheses; n.d. = not determined.

	Canalicular		Basolateral	
	rat	human	rat	human
(Na^+/K^+)-ATPase	6 ± 7 (6)	6 ± 4 (5)	21 ±5 (6)	33 ±18 (5)
Mg^{2+}-ATPase	57 ±21 (6)	4 ± 2 (4)	9 ±2 (6)	2 ± 1 (5)
Alkaline phosphatase	48 ±27 (6)	8 ± 5 (5)	9 ±3 (6)	3 ± 2 (5)
Leucine aminopeptidase	31 ± 9 (4)	42 ±23 (5)	5 ±2 (4)	18 ±12 (5)
Acid phosphatase	3.7 ± 1.2 (6)	3.0 ± 1.3 (3)	0.9 ±0.1 (6)	2.0 ± 1.1 (3)
Glucose-6-phosphatase	0.49± 0.04 (6)	0.41± 0.09 (4)	0.24±0.05 (6)	0.24± 0.05 (4)
Succinate cytochrome *c* reductase	0.05± 0.04 (6)	0.43± 0.42 (3)	1.52±0.48 (6)	1.58± 1.23 (3)
Galactosyltransferase	n.d.	1.5 ± 0.9 (4)	n.d.	1.0 ± 1.1 (4)

TABLE IV

Recoveries of protein and enzyme activities in human plasma membrane fractions

Membrane fractions were isolated as described in the text. Recoveries are expressed as percentage (±S.E.) of the total homogenate for 3–9 (plasma membranes) or 3–5 (canalicular and basolateral subfractions) preparations.

	Plasma membranes	Canalicular fraction	Basolateral fraction
Protein	0.23±0.07	0.019±0.013	0.021±0.014
(Na^+/K^+)-ATPase	4.2 ±0.6	0.03 ±0.01	0.65 ±0.40
Leucine aminopeptidase	3.7 ±0.8	0.59 ±0.32	0.22 ±0.09
Acid phosphatase	0.61±0.48	0.12 ±0.10	0.05 ±0.04
Glucose-6-phosphatase	0.17±0.08	0.013±0.007	0.004±0.002
Succinate cytochrome *c* reductase	0.39±0.19	0.009±0.007	0.028±0.011
Galactosyltransferase	0.36±0.17	0.04 ±0.02	0.05 ±0.04

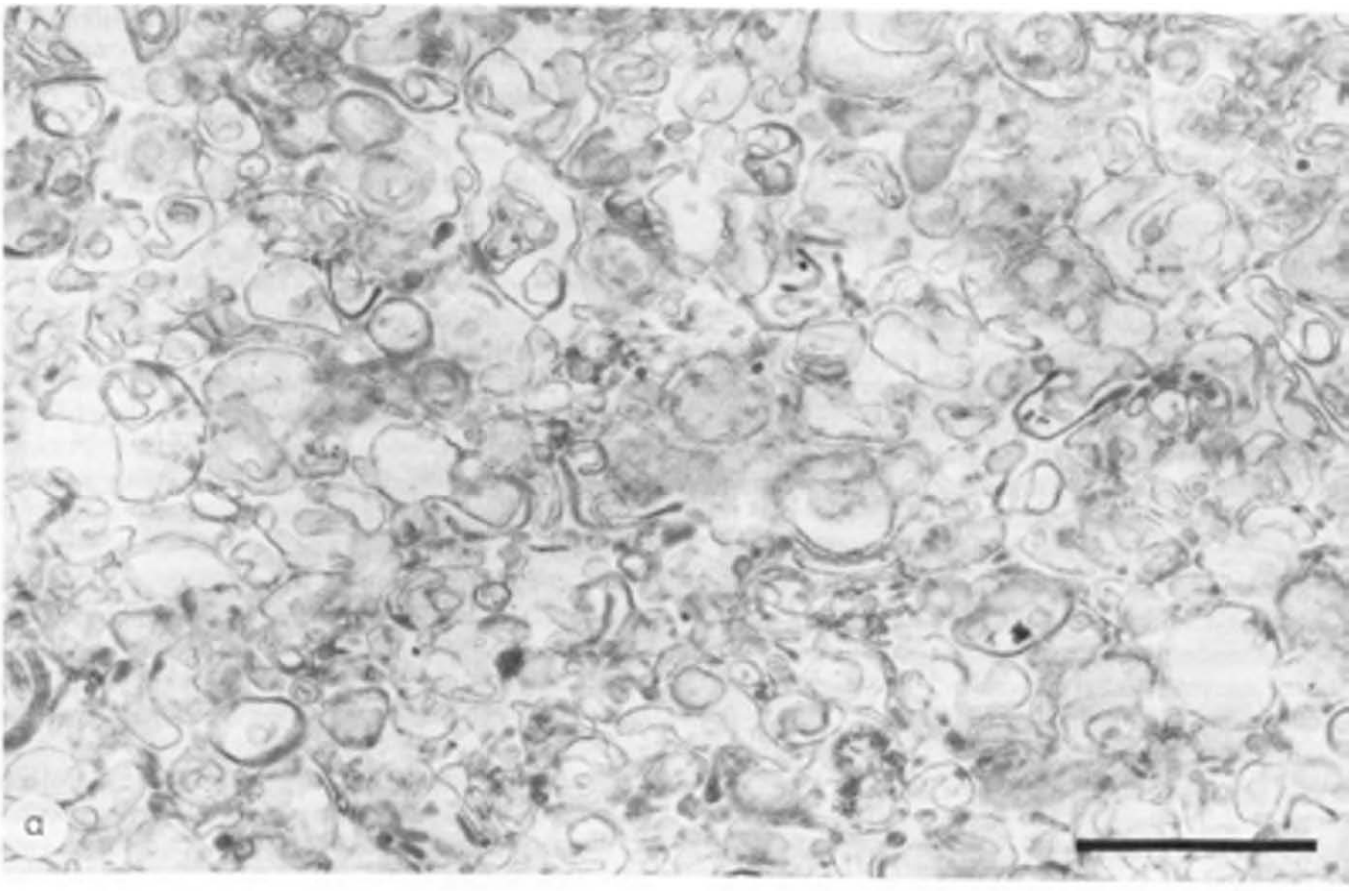
procedure for the plasma membrane fragments from human liver.

Activities of the marker enzymes in homogenates of human and rat liver were found to differ to some extent. Especially the alkaline phosphatase activity was higher in human liver homogenates. This may be caused by the fact that in human liver this enzyme is located both at the canalicular pole of the hepatocyte and at the surface membrane of endothelial cells, whereas in rat livers it is localized mainly at the canalicular side of the hepatocytes [26].

By adapting the isolation procedure for total plasma membranes described for rat liver [8], enrichments in the same order of magnitude were obtained for human and rat liver plasma membranes (Table II). However, due to this adaptation, the total yield of sufficiently pure LPM was relatively low in the human material. The membranes were only slightly contaminated with intracellular organellar membranes, as indicated by the low relative enrichments of the specific enzymes for mitochondria, endoplasmic reticulum, Golgi and lysosomes, and were enriched in both basolateral and canalicular marker enzymes. Variations in the human plasma membrane preparations were larger than those in rat LPM, as is shown by the large standard deviations in Table II, which may be explained by interindividual differences in age, diet, sex etc. No correlation between enzyme activities and age or sex was observed.

It has been shown for rat liver that a number of enzymes, e.g., leucine aminopeptidase, alkaline phosphatase and Mg^{2+}-ATPase, are specifically located at the canalicular pole of the cells [1]. In the human liver, however, the situation appears to be different. Leucine aminopeptidase, which is a specific canalicular enzyme in man [24,25], was highly enriched in canalicular membranes. In each membrane preparation the enzyme activity was higher in the canalicular than the basolateral fraction: ratio cLPM/blLPM of 2.9 ± 1.3 (mean ±

Fig. 3. Electron micrographs of human canalicular (a) and basolateral (b) plasma membrane fractions. Membrane fractions were isolated as described, pelleted (200000× *g* for 45 min), fixed in glutaraldehyde and prepared for electron microscopy as described. The figures show membrane vesicles (a,b) and sheets of membranes (b). No gross contamination with subcellular organelles was observed. Bar 1 μm×30000.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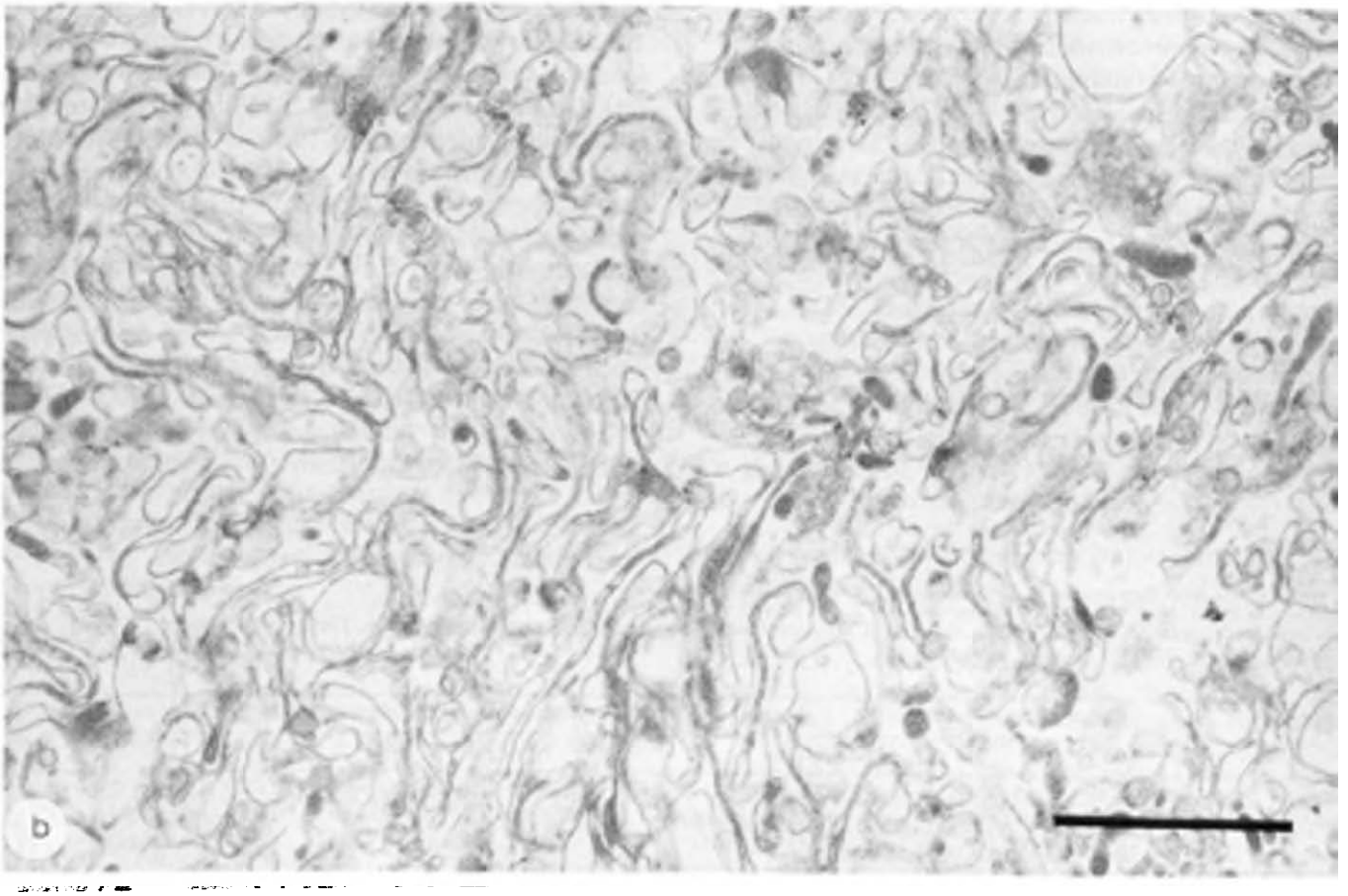
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S.D., $n = 5$), indicating a significant difference ($P < 0.025$). Alkaline phosphatase is not only present at the canalicular domain of the human hepatocyte plasma membrane, but also in endothelial plasma membranes, as was demonstrated by enzyme histochemical investigations [24,27,28]. The enrichment of this enzyme in our putative canalicular membrane fraction with a mean density slightly different from that of leucine aminopeptidase (Fig. 2) might indicate contamination with endothelial membranes. Alternatively, subpopulations of canalicular membranes more strongly enriched in alkaline phosphatase might have slightly lower densities than those more enriched in leucine aminopeptidase. The enzyme activity ratio cLPM/blLPM for alkaline phosphatase was 2.4 ± 0.4, indicating higher activity of this enzyme in the canalicular fraction. Mg^{2+}-ATPase, too, was predominantly enriched in the canalicular membrane preparations (ratio cLPM/blLPM of 2.9 ± 1.5), but to a much smaller extent than leucine aminopeptidase. This observation indicates that the former enzyme is also present in other parts of the cell besides the canalicular membrane, or that it is released from the membranes during the isolation procedure.

At densities below 1.107 g/ml no activity of (Na^+/K^+)-ATPase was present in rat material, indicating that pure canalicular membranes were devoid of any activity of (Na^+/K^+)-ATPase (Fig. 2a). This observation is in accordance with other studies [1,29]. Since the yield of this pure fraction is very low, we decided to isolate a less pure canalicular fraction (density up to 1.127), with some contamination with blLPM. Human membranes did contain some (Na^+/K^+)-ATPase activity even at the lower sucrose densities. This indicates either activity of the enzyme in canalicular membranes, as was suggested by cytochemical experiments [30], or contamination of the canalicular fraction with basolateral membrane fragments of low density. Alternatively, contamination with plasma membranes from nonparenchymal liver cells cannot be excluded.

Enrichments with the plasma membrane marker enzyme (Na^+/K^+)-ATPase in the basolateral membrane fractions were comparable for human and rat liver. In both human and rat livers (Na^+/K^+)-ATPase has a higher specific activity at the basolateral than at the canalicular pole of the cell. Contamination with intracellular organelles was similar in both rat and human preparations.

Human blLPM isolated by the described method contain more canalicular membranes than rat basolateral membranes, as demonstrated by the higher relative enrichments for leucine aminopeptidase (18 and 5, respectively, see Table III). This is probably due to the differences in the isolation procedure: the densities used to isolate human blLPM are closer to the densities of the cLPM than in the procedure used for rat LPM, to avoid contamination with mitochondria. Human blLPM [31], isolated by using a modification of the method described for rat liver [7], were enriched in marker enzymes to an extent similar to our blLPM preparations. These membranes, however, were not tested for the presence of the canalicular marker enzyme leucine aminopeptidase. The values observed for enrichment of plasma membrane markers and enzymes of subcellular organelles in our human liver preparations are compatible with those reported for rat liver membrane isolations [2,6–8].

The membrane phospholipid and cholesterol concentrations show similar distributions in human and rat membrane preparations: canalicular membranes contain significantly ($P < 0.05$) larger amounts of both lipid species (ratio cLPM/blLPM 1.38 ± 0.29 and 1.56 ± 0.40, respectively; mean $\pm$ S.D., $n = 4$), which is in accordance with data presented for rat LPM [1]. The molar ratio of cholesterol to phospholipid was similar for the canalicular fractions (0.87 for man vs. 0.86 for rat), whereas this ratio for blLPM was higher in the human liver preparations (0.79 vs. 0.51). This difference between the human and rat blLPM preparations may partly be caused by the fact that in the isolation procedure used for the human preparations more contamination of blLPM with cLPM occurs. For rat liver, the cholesterol-phospholipid ratio of rat canalicular membranes has been reported to be higher [8,32], identical [33] or lower [4] than for blLPM, probably depending on the isolation procedure used.

The membrane fluidity was lower in the canalicular than basolateral membranes for human preparations

TABLE V

Lipid composition and fluorescence polarization of membrane preparations

Membranes were isolated as described in the text. Cholesterol and phospholipid in μmol/mg protein. Data $\pm$ S.D. Number of livers used in parentheses.

	Canalicular membranes		Basolateral membranes	
	rat	human	rat	human
Cholesterol	0.49 ±0.15 (5)	0.43 ±0.11 (4)	0.19 ±0.03 (5)	0.30 ±0.13 (4)
Phospholipid	0.64 ±0.26 (5)	0.54 ±0.15 (4)	0.38 ±0.07 (5)	0.39 ±0.04 (4)
P_{DPH}	0.268 ± 0.006 (4)	0.289 ± 0.016 (4)	0.252 ± 0.018 (4)	0.265 ± 0.026 (4)

(ratio of P_{DPH} values cLPM/blLPM 1.10 ± 0.07; mean ± S.D., $n = 4$). Similar observations for rat liver plasma membranes have been published [34–36]. Since inner cell membranes are more fluid than plasma membranes [34,37], contamination of plasma membrane preparations with these inner membranes may increase the apparent plasma membrane fluidity. In accordance with this, we observed a tendency for an increased membrane fluidity in rat LPM preparations with the highest mitochondrial contamination (data not shown). Therefore, it is essential that, when one compares plasma membrane preparations from patients with different hepatic disorders [38], the total contamination with intracellular membranes is determined for every membrane preparation to avoid the possibility that differences observed are due to differences in contamination instead of to intrinsic alterations in the plasma membranes. Whether the observed differences in membrane fluidity between cLPM and blLPM are an intrinsic aspect of the plasma membrane fragments, or due to more contamination of blLPM with mitochondrial membranes, remains to be established.

Our results show that plasma membrane fractions specifically enriched in canalicular and basolateral membrane fragments can be isolated separately from human livers. A specific isolation procedure is required as compared to rat liver. The fractions obtained are comparable to those from rat liver, with respect to membrane fluidity, lipid content and the amount of contamination with intracellular organelles. The distribution of some membrane marker enzymes, however, is different from that in rat livers.

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