A New Method for the Rapid Isolation of Basolateral Plasma Membrane Vesicles from Rat Liver

CHARACTERIZATION, VALIDATION, AND BILE ACID TRANSPORT STUDIES

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Bennett L. Blitzter and Carol B. Donovan
From the Liver Study Unit, Division of Digestive Diseases, Department of Internal Medicine, University of Cincinnati College of Medicine, Cincinnati, Ohio 45267 and Liver Study Unit, Department of Medicine, Yale University School of Medicine, New Haven, Connecticut 06510

Basolateral plasma membrane vesicles were prepared from rat liver by a new technique using self-generating Percoll gradients. The method is rapid (total spin time of 2.5 h) and protein yields were high (0.64 mg/g of liver). Transmission electron microscopy studies and measurements of marker enzyme activities indicated that the preparation was highly enriched in basolateral membranes and substantially free of contamination by canaliculal membranes or subcellular organelles. High total recoveries for protein yield and marker enzyme activities during the fractionation procedure indicated that enzymatic activity was neither lost (inactivation) nor increased (activation). Thus, the pattern of marker enzyme activities found in the membrane preparation truly reflected substantial enrichment in membranes from the basolateral surface. Analysis of freeze-fracture electron micrographs suggested that approximately 75% of the vesicles were oriented "right-side-out.

In order to assess the functional properties of the vesicles, the uptake of [3H]taurocholate was studied. In the presence of a Na+ gradient, taurocholate uptake was markedly stimulated and the bile acid was transiently accumulated at a concentration 1.5- to 2-fold higher than that at equilibrium ("overshoot"). In the absence of a gradient but in the presence of equimolar Na+ inside and outside of the vesicle, taurocholate uptake was faster than in the absence of Na+. These findings support a direct co-transport mechanism for the uptake of taurocholate and Na+. Kinetic studies demonstrated that Na+-dependent taurocholate uptake was saturable with a Vm, of 36.5 nmol/mg-min and a Km, of 5.36 mmol/mg-protein min-1.

The high yield, enzymatic profile and retention of transport properties suggest that this membrane preparation is well suited for studies of basolateral transport.

The isolation of plasma membrane vesicles has provided an important tool for studying the driving forces underlying ion and solute transport processes in a wide variety of epithelia (1). In contrast to more intact preparations (e.g., whole animal, isolated perfused organ, or isolated cell), membrane vesicles permit the experimental manipulation of the composition of solutions on both sides of the plasma membrane and are not affected by intracellular metabolic processes.

Recently, plasma membrane vesicles from rat liver have been used in studies of basolateral amino acid and bile acid transport (2-6). However, problems with the membrane vesicle preparations utilized in these studies included: 1) substantial contamination of basolateral (sinusoidal and lateral) membranes with apical (canaliculal) membranes and subcellular organelles (2-4, 6) or subcellular organelles (2, 3) or 2) failure to display sodium-dependent "overshoots" (5) perhaps due to prolonged spin times and consequent loss of transport properties. Other proposed methods have been based on the use of zonal rotors which also involve prolonged spin times and require expensive equipment (7).

Recently, self-generating Percoll gradients have been used to separate basolateral from apical plasma membranes isolated from the small intestine (8) and kidney (9). The advantages of this technique include: 1) rapidity; 2) high protein yields; 3) high degree of purification; 4) the use of relatively inexpensive equipment; and 5) retention of membrane transport properties. In the present study, we describe a new method for the isolation of basolateral liver plasma vesicles based on modifications of the Percoll gradient methods reported for the intestine and kidney. The relative purity of the preparation was assessed by measuring activities and total recoveries of various marker enzymes of the plasma membrane and subcellular organelles and from transmission and freeze-fracture electron microscope studies. Transport studies with taurocholate, the major bile acid in the rat, were performed in order to assess the functional integrity of the vesicles.

**MATERIALS AND METHODS**

**Animals**—Male Sprague-Dawley rats (Charles River Breeding Laboratories, Inc., Wilmington, MA) were maintained in a constant temperature environment (22 °C) with alternate 12-h light and dark cycles and fed Purina lab chow (Ralston Purina, St. Louis, MO) ad libitum. Body weights were between 200 and 250 g. All animals were killed in the fed state between 8:00 and 9:00 a.m.

**Preparation of Rat Liver Basolateral Membrane Vesicles (Fig. 1)**—After pentobarbital sodium anesthesia (5 mg/100 g body weight intraperitoneally) and heparin treatment (500 IU/100 g body weight intravenously via the iliac vein), a 16-gauge cannula (Cathlon IV, Jelco Industries, Raritan, NJ) was placed in the portal vein, and the thoracic portions of the vena cava and aorta were transected. Ice-cold saline (9.5%) was then infused until the liver blanched. The liver was
removed, weighed, and minced in approximately 20 ml of ice-cold Buffer A (10 mM Tris-HCl, pH 7.6, 0.25 M sucrose, 0.1 mM phenylmethylsulfonyl fluoride). After making up to 40 ml with additional Buffer A, homogenization on ice was performed in a loose Dounce homogenizer (Kontes Glass, Vineland, NJ) with 15 up-down strokes of the pestle. Further homogenization was performed with 5 up-down strokes of a motor-driven Teflon glass homogenizer (5431-A295, Arthur H. Thomas) operating at 860 rpm (under no-load conditions). The homogenate was then made up to a volume equal to 20 times the liver wt. with Buffer A and filtered through gauze.

FIG. 1. Flow scheme for the preparation of basolateral plasma membrane vesicles from rat liver.

Membrane Marker Enzyme Activities—The homogenate and membrane fractions were diluted with distilled water immediately prior to the performance of enzyme assays in order to hypotonically burst the vesicles to ensure full access of substrates to both sides of the membranes. Na,K-ATPase and Mg2+-ATPase activities were determined by recording spectrophotometric methods (10). Adenylate cyclase activity was measured by the method of Salomon et al. (11), alkaline phosphatase activity by the method of Pekary et al. (12) as modified by Keele et al. (13), and NADPH-cytochrome c reductase and succinate-cytochrome c reductase according to the methods of Sottocasa et al. (14) and Tisdale (15), respectively. Acid phosphatase was determined by the method of Rothstein and Blum (16). Galactosyl transferase was measured by the method of Fleischer and Smigel (17) using N-acetylglucosamine as the acceptor. Na,K-ATPase activity was determined on the day the membrane fractions were prepared. All other enzymes were assayed after overnight storage at 4 °C with the exception of adenylyl cyclase and galactosyl transferase which were measured after storage at −70 °C for periods of up to 3 weeks. Protein was measured according to the method of Lowry et al. (18) using bovine serum albumin as standard.

Transport of [3H]taurocholate by Basolateral Membrane Vesicles—Twenty microliters of basolateral membrane suspension (IL) containing 40–70 μg of protein were preincubated in a test tube in a 37 °C water bath. Uptake of [3H]taurocholate was initiated by adding 80 μl of incubation buffer (NaCl or KCl, 125 mM; sucrose, 50 mM; HEPES-KOH, 10 mM; pH 7.5) containing varying amounts of [3H]taurocholate and unlabeled bile acid. The final concentrations in the mixture were thus NaCl or KCl, 100 mM; sucrose, 100 mM; HEPES-KOH, 10 mM; and [3H]taurocholate, 1–300 μM. The tube was mixed vigorously and returned to the water bath for varying times. Timed uptakes were terminated by the rapid addition of 3.5 ml of ice-cold stop solution ([NaCl or KCl, 100 mM; sucrose, 100 mM; HEPES-KOH, 10 mM; pH 7.5] and then immediate vacuum filtration through 0.45-μm Millipore filters (HAWP). The test tube was rinsed with an additional 3.5 ml of stop solution and the contents filtered. Finally, the filter was washed twice with 3.5 ml of stop solution. Millipore filters were added to minivials, rendered transparent with 6 ml of Ready-Solv HP (Beckman Instruments, Fullerton, CA), and counted on a Beckman LS 6800 scintillation counter. All uptakes were corrected for a blank in which 3.5 ml of ice-cold stop solution was added to the vesicles prior to the addition of radiolabeled bile acid. In order
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to reduce nonspecific adherence of the bile acid to the filter, the filters were saturated with an excess of cold taurocholate by prefitering with 3 ml of 1 mM sodium taurocholate. In addition, all incubation solutions were prefiltered through 0.22-μm filters prior to use. Preliminary studies demonstrated that basolateral membranes frozen at -70°C for periods up to 3 weeks retained full transport properties.

Chemicals—Phenylmethylsulfonyl fluoride, Percoll, ouabain, HEPES, and enzymatic substrates were obtained from Sigma. [14C] UDP-galactose (302 mCi/mmol) was from New England Nuclear. [3H]Taurocholic acid (3.4-6.6 Ci/mmol) was purchased from New England Nuclear and was >95% pure by thin layer chromatography. Unlabeled sodium taurocholate was obtained from Calbiochem.

RESULTS

Transmission and Freeze-Fracture Electron Microscopy—Transmission electron microscopy (Fig. 2) demonstrated that the final pellet (II,) contained predominantly small closed membrane vesicles with diameters in the range of 0.4-0.6 μm. Morphologic evidence of contamination with subcellular organelles was minimal. Rare mitochondrial membranes and occasional Golgi cisternae were seen. Desmosomes and intact bile canaliculi were not observed. As previously reported for the isolation of intestinal membranes (8), residual Percoll particles could be identified as electron-dense spheres (approximate diameter 0.01-0.02 μm) either free or adherent to membranous structures.

Freeze-fracture electron micrographs (Fig. 2, inset) revealed predominantly highly particulate membrane vesicles with diameters of 0.4-0.6 μm. Assessments of the relative particle densities of convex and concave vesicles (Table I) suggested that 72% of the vesicles were oriented "right-side-out."

Protein Yield and Marker Enzyme Activities (Table II)—The protein yield of the basolateral membrane fraction (II,) was 0.64 ± 0.19 mg/g of liver. Determinations of the activities of various marker enzymes localized to the plasma membrane or subcellular organelles suggested that the membrane vesicles arose primarily from the basolateral portion of the surface membrane. Na+,K+-ATPase activity, a marker of the basolateral surface of the hepatocyte (19, 20), was 28-fold higher in Fraction II, than in the homogenate. Although adenylate cyclase has not been definitively localized by cytochemical techniques, this enzyme is presumed to be localized to the basolateral surface of the hepatocyte (21). Basal activity of this enzyme was enriched 16-fold in II, compared to homogenate. Maximal stimulation of activity by $3 \times 10^{-7}$ M glucagon required the addition of GTP in II, but not in homogenate. Stimulation index (glucagon-stimulated activity/basal activity) was 10.9 ± 2.4 in the membrane fraction. Fluoride-stimulated activity was 11-fold higher in II, than in the homogenate.

Fig. 2. Transmission electron micrograph of basolateral liver plasma membrane vesicles. The preparation contains mostly closed membrane vesicles (V) 0.4-0.6 μm in diameter. Occasional Golgi cisternae (G) were present. Small electron dense spheres (0.01-0.02 μm) either free or adherent to membranous structures are thought to represent residual Percoll. (Bar, 0.5 μm; original magnification, x 25,000). Inset, freeze-fracture electron micrograph showing predominantly highly particulate convex membrane vesicles. (Bar, 0.5 μm; original magnification x 42,000).
membrane vesicles and was transiently accumulated at a concentration 1.5- to 2-fold higher than that at equilibrium. This "overshoot" generally occurred at 5-10 s, and equilibrium was reached in 2-3 min. In contrast, in the presence of an inwardly directed 100 mM KCl gradient, the initial velocity of uptake (measured at 5 s) was only one-third of the velocity in the presence of a Na⁺ gradient. Equilibrium was approached slowly and no overshoot was observed. When the concentration of Na⁺ inside the vesicle was equal to that in the medium (100 mM) again no overshoot was observed. However, the initial velocity of uptake and approach to equilibrium during the first 20 s were significantly faster than in the presence of a K⁺ gradient.

Vesicle uptake of taurocholate at equilibrium (3 min) was inversely proportional to the final osmolality of the incubation medium (Fig. 4). In addition to the observation of the Na⁺-dependent overshoots, this osmotic sensitivity implies actual transport into the vesicle and cannot be accounted for by binding to the membrane (1). The large intercept, however, indicates a significant binding component as has been observed in all previous studies of bile acid transport by membrane vesicles (4-6, 22-24).

Table I

| Vesicle     | Particulate | Smooth |
|-------------|-------------|--------|
| Convex      | 53 (a)      | 28 (b) |
| Concave     | 7 (c)       | 36 (d) |
| Total       | 60          | 64     |

In contrast, activities of enzyme markers for the canalicular portion of the surface membrane were only modestly enriched. Alkaline phosphatase activity was only 5-fold higher in 11, than in homogenate. MP-ATPase was enriched to a similar pattern of marker enzyme activities in the basolateral membrane fraction. The derived kinetic parameters included a taurocholate concentration that was one-third of the velocity in the presence of a Na⁺ gradient. Equilibrium was approached slowly and no overshoot was observed. When the concentration of Na⁺ inside the vesicle was equal to that in the medium (100 mM) again no overshoot was observed. However, the initial velocity of uptake and approach to equilibrium during the first 20 s were significantly faster than in the presence of a K⁺ gradient.

### Kinetics of Na⁺-dependent Taurocholate Uptake (Fig. 5)

To study the kinetics of taurocholate uptake, the vesicles were incubated with varying concentrations of [3H]taurocholate (final concentration 1-300 μM). Na⁺-dependent taurocholate uptake was calculated as the difference between uptake measured in the presence of an inwardly directed Na⁺ gradient and uptake in the presence of a K⁺ gradient. A weighted least squares fit of the individual data points was then performed by a previously described computer program (25). The relationship of Na⁺-dependent taurocholate uptake to taurocholate concentration was well described by a rectangular hyperbola. The derived kinetic parameters included a taurocholate Kₘ of 36.5 ± 3.9 (S.E.) μM and a Vₘₜₐₓ of 5.36 ± 0.19 nmol mg⁻¹ protein min⁻¹.

### DISCUSSION

In the present study, we have described and validated a new Percoll gradient method for the preparation of rat liver basolateral plasma membrane vesicles suitable for transport studies. The method does not require an ultracentrifuge or a...
Values given are the means ± S.D. for the protein yield and total marker enzyme activity contained in each fraction and expressed as a percentage of total homogenate protein or activity. Protein contents of fractions I, II, and III could not be determined due to the presence of Percoll. ND, not detectable.

| Fraction | Protein (10) | Na⁺,K⁺-ATPase (6) | Alkaline phosphatase (9) | Mg⁺-ATPase (6) | Succinate-cytochrome c reductase (10) | NADPH-cytochrome c reductase (7) | Acid phosphatase (6) | Fraction |
|----------|--------------|-------------------|------------------------|---------------|----------------------------------|---------------------------------|---------------------|----------|
| H        | 100          | 100               | 100                    | 100           | 100                              | 100                             | 100                  | H        |
| S₁       | 59.4 ± 6.1   | 17.6 ± 13.5       | 39.2 ± 6.3             | 67.8 ± 8.1    | 13.0 ± 7.2                       | 71.6 ± 8.2                      | 72.6 ± 7.0           | S₁       |
| P₁       | 38.5 ± 1.3   | 81.9 ± 56.0       | 67.9 ± 13.6            | 613 ± 21.7    | 74.9 ± 50.6                      | 27.3 ± 3.8                      | 36.7 ± 5.5           | P₁       |
| S₂       | 46.8 ± 2.8   | ND                | 20.6 ± 2.6             | 45.8 ± 8.9    | 7.4 ± 2.2                        | 52.7 ± 9.9                      | 52.3 ± 16.7          | S₂       |
| P₂a      | 9.8 ± 0.8    | 22.3 ± 7.9        | 15.9 ± 4.5             | 14.8 ± 3.7    | 3.5 ± 1.5                        | 22.9 ± 4.0                     | 16.8 ± 1.9           | P₂a      |
| P₂b      | 1.3 ± 0.3    | 1.4 ± 1.0         | 3.6 ± 1.9              | 1.5 ± 0.7     | 0.7 ± 0.4                        | 2.7 ± 0.3                      | 1.3 ± 0.8            | P₂b      |
| I        | 4.3 ± 3.4    | 2.5 ± 0.4         | 2.1 ± 0.6              | 0.2 ± 0.1     | 1.1 ± 0.4                        | 1.3 ± 0.5                      | I        |
| II       | 10.8 ± 3.7   | 4.6 ± 2.3         | 6.4 ± 1.6              | 0.2 ± 0.1     | 3.4 ± 1.0                        | 2.3 ± 1.3                      | II       |
| III      | ND           | 13.3 ± 3.8        | 7.2 ± 2.1              | 3.7 ± 1.3     | 17.6 ± 3.9                       | 14.1 ± 5.9                     | III      |
| Iₚ        | 0.52 ± 0.11  | 1.1 ± 0.7         | 1.3 ± 0.7              | 1.0 ± 0.4     | 0.1 ± 0.1                        | 0.5 ± 0.1                      | Iₚ       |
| Iₚ        | 0.21 ± 0.14  | 2.2 ± 2.4         | 1.2 ± 0.6              | 0.8 ± 0.6     | 0.05 ± 0.4                       | 0.3 ± 0.2                      | Iₚ       |
| IIₚ       | 0.88 ± 0.18  | 3.6 ± 3.6         | 2.1 ± 1.7              | 1.9 ± 1.2     | 0.2 ± 0.1                        | 1.5 ± 0.6                      | IIₚ      |
| IIIₚ      | 1.60 ± 0.33  | 1.3 ± 1.3         | 4.9 ± 1.5              | 3.6 ± 1.9     | 0.4 ± 0.2                        | 4.4 ± 1.5                      | IIIₚ     |
| IIₚ       | 3.45 ± 0.44  | 2.0 ± 1.2         | 2.4 ± 0.4              | 3.1 ± 1.5     | 1.6 ± 0.5                        | 10.1 ± 0.7                     | IIₚ      |
| IIIₚ      | 0.54 ± 0.26  | 3.4 ± 3.3         | 2.9 ± 0.4              | 2.2 ± 0.7     | 0.07 ± 0.06                      | 1.0 ± 0.9                      | IIIₚ     |
| IIₚ       | 0.26 ± 0.10  | 5.0 ± 2.6         | 1.7 ± 0.6              | 1.7 ± 0.9     | 0.05 ± 0.06                      | 0.5 ± 0.2                      | IIₚ      |

Total recovered activity*: 76% 83% 104% 97% 71% 83% 66%

* (Iₚ + Iₚ + IIₚ + IIIₚ + IIₚ + IIₚ)/P₂a.

Fig. 4. Relationship of taurocholate uptake to medium osmolality. Equilibrium uptake (3 min) of [³H]taurocholate was determined under Na⁺ gradient conditions in a medium in which the osmolality was altered with varying concentrations of sucrose. The individual data points (n = 14) were fitted to a straight line by linear regression analysis. Symbols represent means ± S.D. at each osmolality.

Zonal rotor, and total spin time is only 2.5 h. Starting with two rat livers, the protein yield in the final basolateral membrane pellet (IIₚ) ranged from 12-16 mg. Since the transport experiments required only 40-70 µg of protein per uptake, this technique provides enough membrane for the performance of approximately 170-400 individual uptake measurements.

Judging from transmission electron microscopy studies and measurements of marker enzyme activities, the final membrane pellet (IIₚ) was highly enriched in basolateral membranes. The activity of Na⁺,K⁺-ATPase, an enzyme marker for the basolateral domain of the hepatocyte surface membrane (19, 20), was 28-fold higher in IIₚ than in the starting homogenate. While adenylate cyclase has not been defini-
Fig. 5. Kinetics of Na⁺-dependent taurocholate uptake. Vesicles were incubated with varying concentrations of [³H]taurocholate under the same conditions given in the legend to Fig. 3. The initial velocity of Na⁺-dependent uptake was calculated as the difference between uptake in the presence of a Na⁺ gradient (measured at 5 s) and uptake in the presence of a K⁺ gradient. A weighted least squares fit of the individual data points \( n = 102 \) was performed on a computer yielding the depicted hyperbola and a \( K_m \) of 36.5 ± 3.9 (S.E.) \( \mu M \) and a \( V_{max} \) of 5.36 ± 0.19 nmol mg⁻¹ protein min⁻¹. Symbols represent means ± S.E. of the experimental points at each concentration.

In general, the pattern of marker enzyme enrichments confirmed the qualitative conclusions reached from transmission electron microscopy. Although the homogenization steps employed in this method would be expected to disrupt the normal morphology of the intact canalculus and, therefore, render it difficult to recognize membranes from the apical surface in electron micrographs, the adjoining desmosomes were not observed in the final preparation. Electron microscopy also confirmed the lack of significant contamination by subcellular organelles. Furthermore, the GTP requirement for full hormonal stimulation of adenylate cyclase also suggests that the preparation is relatively free of cytosolic contamination (32). Freeze-fracture studies suggested that approximately 75% of the vesicles were oriented “right-side-out.” This estimate is similar to earlier determinations in less purified membrane preparations (74% (4) and 70% (33)).

As recently shown with other liver plasma membrane preparations, the uptake of [³H]taurocholate into the vesicles was markedly stimulated by a Na⁺ gradient, as manifested by a 3-fold higher initial velocity of uptake in the presence of a Na⁺ gradient as compared to that in the presence of a K⁺ gradient. In addition, an “overshoot” was observed implying “uphill” transport of the bile acid in the presence of a Na⁺ gradient (34). The demonstration of an overshoot suggests that the vesicles are relatively “tight” and, therefore, able to transiently maintain ion gradients across the membrane.

When Na⁺ was present in equal concentrations inside and outside of the vesicles, no overshoot was observed. However, the initial velocity of taurocholate uptake and the approach to equilibrium was faster than in the absence of Na⁺. This finding strongly supports a direct co-transport mechanism for the uptake of taurocholate and Na⁺. Presumably, in the absence of Na⁺ but in the absence of a Na⁺ gradient, the bile acid enters the vesicle via carrier-mediated facilitated transport, a process expected to be more rapid than simple diffusion in the absence of Na⁺. Under these conditions, no overshoot is observed due to the lack of a Na⁺ gradient to provide the energy for “uphill” transport.

The kinetic studies demonstrated that Na⁺-dependent taurocholate uptake was saturable over a physiologic range of bile acid concentrations. The \( K_m \) of 36.5 ± 3.9 \( \mu M \) is similar to that found in isolated hepatocyte studies (35). Since such factors as unstirred layers, cell and lobular geometries, and metabolism may significantly alter observed transport rates in various in vitro preparations, caution must be used in comparing maximal velocities. Nevertheless, the \( V_{max} \) of 5.36 ± 0.19 nmol mg⁻¹ protein min⁻¹ found for the uptake of taurocholate by basolateral membrane vesicles prepared by itself do not result in significant losses (inactivation) or increases (activation) in total enzymatic activity. For this reason, total recovery experiments were performed in order to construct a “balance sheet” for protein yield and the activities of 6 marker enzymes in 16 fractions (Table III). The high recoveries suggest that the pattern of marker enzyme activities found truly reflects a high degree of enrichment in basolateral surface membranes and a lack of significant contamination by subcellular organelles.

Based on electron microscopic morphometric studies (31), the basolateral and canalicular domains comprise 87 and 13% of the surface membrane, respectively. Assuming similar proportions in the starting homogenate and given the 28-fold enrichment in the basolateral marker Na⁺,K⁺-ATPase and the 5-fold enrichment in the canalicular marker alkaline phosphatase, it may be calculated that 97–98% of the surface membranes in the preparation arise from the basolateral surface.

In contrast to the striking enrichments for basolateral marker enzyme activities, the membrane preparation was only modestly enriched in marker enzymes of the canalicular surface. Alkaline phosphatase, which has been cytochemically localized to the bile canaliculus (19), was enriched only 5-fold. Mg²⁺-ATPase, another canalicular marker (20), was enriched 7-fold. The relatively greater enrichment for the latter enzyme may be due to its presence in mitochondria (28) in addition to canalicular membranes.

Based on the relative specific activities of succinate-cytochrome c reductase, NADPH-cytochrome c reductase, and acid phosphatase, the preparation was not enriched in mitochondria, endoplasmic reticulum, or lysosomes. However, galactosyl transferase, a Golgi marker, was enriched 8-fold. Alternatively, adenylate cyclase activity is presumed to be concentrated along the basal or basolateral surface. Alkaline phosphatase, which has been cytochemically localized to the bile canaliculus (19), was enriched only 5-fold. Mg²⁺-ATPase, another canalicular marker (20), was enriched 7-fold. The relatively greater enrichment for the latter enzyme may be due to its presence in mitochondria (28) in addition to canalicular membranes.

Based on the relative specific activities of succinate-cytochrome c reductase, NADPH-cytochrome c reductase, and acid phosphatase, the preparation was not enriched in mitochondria, endoplasmic reticulum, or lysosomes. However, galactosyl transferase, a Golgi marker, was enriched 8-fold. Although basal and glucagon-stimulated activities were enriched 16- and 12-fold, respectively, the enrichment ratios were less than for Na⁺,K⁺-ATPase. Conceivably, incomplete rupture of the vesicles may have prevented free access of substrates to their binding sites resulting in submaximal enzymatic activity (26). Alternatively, adenylate cyclase may not be restricted to the basolateral surface (27).

In contrast to the striking enrichments for basolateral marker enzyme activities, the membrane preparation was only modestly enriched in marker enzymes of the canalicular surface. Alkaline phosphatase, which has been cytochemically localized to the bile canaliculus (19), was enriched only 5-fold. Mg²⁺-ATPase, another canalicular marker (20), was enriched 7-fold. The relatively greater enrichment for the latter enzyme may be due to its presence in mitochondria (28) in addition to canalicular membranes.

Based on the relative specific activities of succinate-cytochrome c reductase, NADPH-cytochrome c reductase, and acid phosphatase, the preparation was not enriched in mitochondria, endoplasmic reticulum, or lysosomes. However, galactosyl transferase, a Golgi marker, was enriched 8-fold. Although there has been controversy over whether this enzyme is also localized to the plasma membrane (29, 30), Golgi cisternae were occasionally seen in transmission electron micrographs of the membrane preparation, implying some contamination by this organelle. However, since Golgi protein represents less than 1% of total liver cell protein, the 8-fold enrichment for galactosyl transferase suggests that Golgi membranes account for less than 8% of the final membrane preparation.

Valid inferences from marker enzyme activities regarding the composition of a membrane preparation can only be made if the various steps of the subcellular fractionation process.
the present method is within an order of magnitude of that reported for isolated hepatocytes (35) or the isolated perfused rat liver (36) when these are expressed per milligram of membrane protein.

Isolation of basolateral membrane vesicles by the method described herein has many advantages. The preparation is performing the adenylate cyclase assays, to E. Cardell for preparing the transmission electron micrographs, and to Sharon Driscoll for expert secretarial assistance.

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