Isolation and characterization of renal cortical membranes using an aqueous two-phase partition technique

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The aqueous two-phase partition technique is a simple, rapid and inexpensive method for the fractionation of membrane preparations. Aqueous two-phase partitioning separates according to surface properties such as charge and hydrophobicity, making it complementary to established centrifugation techniques, which separate on the basis of density. Although aqueous two-phase partitioning has been successfully applied to animal tissues, there are limited data on the functional properties of the isolated membranes. We have applied the aqueous two-phase partition technique to rat renal brush-border membrane vesicles and

sheets. Our aim was to remove organelle contamination while maintaining the functional properties of the membranes. Evidence from marker enzyme analysis and electron microscopy supports the conclusion that renal brush-border membranes are fractionated separate from the mitochondria and endoplasmic reticulum. This separation procedure did not alter the Na⁺-D-glucose symporter and Na⁺-H⁺ antiporter activity in the fractionated preparation increased to the same extent as did the enrichment of enzyme markers for brush-border membranes.

INTRODUCTION

Centrifugation techniques, which separate according to size and density, are a mainstay of protocols for the isolation of cellular organelles. Techniques such as aqueous two-phase partition and free-flow electrophoresis, which separate according to charge and hydrophobicity, have been applied to the fractionation of cellular organelles (Hannig and Heidrich, 1977; Morré and Morré, 1989). The combination of separation techniques is especially powerful: it resolves otherwise insurmountable problems such as the isolation of endosomes from cultured cells (Marsh et al., 1987; Schmidt et al., 1988).

There are very little data on the functional properties of mammalian membranes fractionated according to charge (Mircheff et al., 1984; Morré and Morré, 1989). Although there are a rich variety of potential applications (Morré et al., 1989), aqueous two-phase partition may be especially beneficial for the preparation of cellular organelles such as endosomes, which remain poorly defined due to difficulties in isolation (Hammond et al., 1993). Before applying charge separation to such poorly defined cellular organelles, much can be learnt about the utility of the technique from fractionation of a renal cortical membrane fraction with well-documented enzymic compartmentation and functional properties. Brush-border membranes are ideal for this purpose.

MATERIALS AND METHODS

Animals

We harvested kidneys from pentobarbital-anaesthetized male Sprague–Dawley rats (200–250 g; from Sasco, Omaha, NE, U.S.A.).

Preparation of renal cortical brush-border membrane vesicles

Brush-border membrane vesicles were prepared by bivalent ion precipitation as described previously (Hammond et al., 1985;

Kleinzeller, 1988; Hammond, 1990). Briefly, we placed the harvested kidneys into ice-cold buffer (300 mM mannitol and 12 mM Hepes, adjusted to pH 7.4 with Tris); each kidney was decapsulated and the cortex was dissected free from the medulla and hilum. We minced the cortex with scissors and then homogenized it with ten strokes of a 15 ml tight-fitting Potter–Elvehjem Teflon/glass homogenizer. This step was followed by Polytron homogenization (Brinkman Kinematica CH-6010) and addition of 100 µl of 1 M MgCl₂/10 ml of homogenate. We centrifuged the resulting cortical homogenate at 1700 g for 10 min, then removed the supernatant and centrifuged it at 14500 g for 20 min. The resultant pellet of brush-border membrane vesicles was resuspended in a small volume of buffer. The last two centrifugation steps were repeated one more time.

Preparation of renal cortical membrane sheets

Renal cortical membranes sheets were prepared using the same method as for brush-border membrane vesicles, except that we did not add magnesium to the homogenate or use a Polytron homogenization step.

Aqueous two-phase partition fractionation

For aqueous two-phase partitioning, we prepared stock solutions of 20% (w/w) dextran T-500 (Pharmacia) and 40% (w/w) poly(ethylene glycol) 3350 (PEG) (Union Carbide) in double-distilled water. A 6.0% (w/w) two-phase system prepared by adding 4.80 g of 20% dextran to 2.40 g of 40% PEG in a 30 ml Pyrex tube on ice, after which we added 0.32 ml of 0.2 M potassium phosphate at pH 7.2; the total weight of the contents was made up to 14 g with distilled water. Other weight ratios were prepared by varying the amounts of dextran and PEG added to the system, as previously described (Morré and Morré, 1989). We resuspended the membranes to be separated in a minimum volume of buffer, added the membranes and buffer (total volume < 2 ml) to the two-phase system, and brought the

Abbreviations used: PEG, poly(ethylene glycol); BCECF, 2',7'-bis-(2-carboxyethyl)-5(6)-carboxyfluorescein.

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final weight up to 16 g with buffer. The pyrex tube was covered with Parafilm, and then inverted vigorously 40 times in the cold (9 °C). We centrifuged the resultant mixture at 1000 *g* for 5 min at 4 °C to separate the phases, and the upper phase was removed using a Pasteur pipette and diluted 10-fold with buffer. The membranes were collected from the upper phase by centrifugation (20000 *g* for 30 min).

Brush-border membrane vesicle transport studies

We prepared brush-border membrane vesicles by bivalent ion precipitation with and without aqueous two-phase partition. The primary homogenate from a single set of rat kidneys was split, with aliquots of the same homogenate used for preparation of brush-border membrane vesicles with and without aqueous two-phase partition. Freshly prepared membrane vesicles were used. Uptake of D-[³H]glucose by isolated brush-border membrane vesicles was measured by rapid filtration techniques as described previously (Hammond, 1990; Hammond et al., 1985). In brief, we suspended aliquots of brush-border membrane vesicles in a medium containing 100 mM sucrose, 100 mM NaCl, 5 mM Hepes (pH adjusted with Tris to 7.4) and 0.05 mM D-[³H]glucose (final concentrations). Uptake was terminated at various times by addition of ice-cold 135 mM NaCl and 5 mM Hepes (pH adjusted to 7.4 with Tris). We measured the uptake of solute at 37 °C in duplicate for each time point. The filters were dissolved in 5 ml of Optifluor and radioactivity was determined using a Packard 3330 liquid scintillation spectrometer.

Na⁺-H⁺ antiporter assays

To measure Na⁺-H⁺ antiporter activity, we prepared brush-border membrane vesicles in 100 mM mannitol and 5 mM Mes buffer titrated to pH 6.4. This ensured that the internal pH of the vesicles was fixed at this value. We loaded both brush-border membrane vesicles and two-phase 6.4% upper-phase membranes, prepared in parallel from the same kidneys, with pH-sensitive fluorophore by incubating in 10 µm BCECF/AM on ice for 60 min.

Preliminary experiments were performed to verify that the pH-sensitive fluorophore BCECF/AM was cleaved and loaded into brush-border membrane vesicles. Vesicles were incubated on ice in a cuvette. Cleavage of the non-fluorescent substrate BCECF-AM to give the highly fluorescent product BCECF was monitored by the appearance of fluorescence at 530 nm in a spectrofluorimeter. The rate of appearance of fluorescence in arbitrary units ($n = 4$, means $\pm$ S.D.; * $P < 0.05$ compared with before probe by ANOVA and Scheffe's post hoc comparison) was: 0.34 ± 0.16 (before probe), $1.35 \pm 0.47^*$ (2 min), $3.90 \pm 0.64^*$ (20 min), $5.78 \pm 1.05^*$ (40 min) and $6.04 \pm 1.05^*$ (60 min). Serial addition of anti-fluorescein antibodies reduced the fluorescence by $< 5\%$ in each case. Following vesicles lysis with Triton X-100, fluorescence returned to the levels observed before BCECF/AM addition, demonstrating that the antibodies could quench the fluorescence. To test whether the cleaved probe was retained for the duration of the experiment, we examined the rate of probe leakage. Vesicles were loaded with BCECF and placed in a spectrofluorimeter in the presence of anti-fluorescein antibodies. The fluorescence in arbitrary units ($n = 4$, means $\pm$ S.D.; * $P < 0.05$ compared with before probe by ANOVA and Scheffe's post hoc comparison) was 0.79 ± 0.50 (before probe), $6.16 \pm 0.38^*$ (vesicles loaded for 60 min then washed twice), $4.50 \pm 0.55^*$ (20 min leak), $3.64 \pm 1.10^*$ (40 min leak), $3.27 \pm 1.23^*$ (60 min leak). Hence we showed that the pH-sensitive fluorophore BCECF/AM is cleaved, loaded into brush-border membrane vesicles and retained for the period of study.

To measure Na⁺-H⁺ antiporter activity, we added 25 µl of BCECF-loaded membrane vesicles, with an internal pH of 6.4, to 3 ml of 100 mM mannitol and 5 mM Hepes buffer adjusted to pH 8.0 with Tris in a disposable plastic fluorimeter cuvette (Hammond et al., 1985; Mahnensmith and Aronson, 1985). The cuvette was placed in an Aminco Bowman 8000C spectrofluorimeter, with excitation at 488 nm emission and measured at 530 nm. Once a stable fluorescence baseline was established, we added sodium gluconate to the cuvette to give a final Na⁺ concentration of 0, 22 or 33 mM. The brightness of the fluorescence emission was collected 120 times/s for more than 30 s.

Enzyme assays

Protein was measured using a modification of the Bradford method (Peterson, 1977). The activity of K⁺-stimulated, ouabain-inhibited, nitrophenyl phosphatase, a basolateral marker enzyme that co-localizes with Na⁺-K⁺-ATPase, was assayed by measuring cleavage of *p*-nitrophenyl phosphate (Emmelot and Bos, 1966; Kashiwamata et al., 1979). γ -Glutamyl transpeptidase was assayed by the kinetic cleavage of glutamyl *p*-nitroanilide (Tsao and Curthoys, 1980). Alkaline phosphatase activity was measured by cleavage of *p*-nitrophenyl phosphate at pH 10.3 (Bessey et al., 1946). Assay of succinyl dehydrogenase, a mitochondrial marker, was based on succinate 2-(*p*-iodophenyl)-3-(*p*-nitrophenyl)-5-phenyltetrazolium reductase (Green and Narahara, 1980). The activity of acid phosphatase, a lysosomal marker, was measured by hydrolysis of *p*-nitrophenyl phosphate at pH 4.8 (Andersch and Szczypinske, 1967). Glucose-6-phosphatase was assayed in fresh membrane preparations (Nordlie and Arion, 1966) using Elon's reagent (Harris and Popat, 1954) for secondary phosphate analysis.

Electron microscopy

Samples of membrane vesicles were fixed for electron microscopy with 2% glutaraldehyde in phosphate-buffered saline. We transferred the samples to 1% osmium tetroxide in 0.05 M sodium phosphate (pH 7.2) for several hours. The samples were dehydrated in an acetone series and embedded in Epon (Morré and Morré, 1989; Morré et al., 1989). Lead-stained thin sections were examined and photographed with a Phillips EM/200 electron microscope.

Statistics

We performed statistical analyses of enzyme marker activities, and radioactivity uptakes, by one-way analysis of variance (ANOVA), and Scheffe's post hoc comparison. We expressed data as means $\pm$ S.E.M. Multiple observations on the same sample were averaged and entered as $n = 1$.

RESULTS

We compared the activities of compartment-specific enzyme markers before and after serial aqueous two-phase partition fractionation of renal membrane sheets. The activity of γ -glutamyl transpeptidase (units/litre per mg of protein; means $\pm$ S.E.M.; $n = 4$) in renal membrane sheets (7177 ± 397) was significantly increased in the two-phase membrane fractions 6.4% upper phase (11188 ± 996), 6.0% upper phase (13726 ± 503) and 5.8% upper phase (10001 ± 1657), and reduced in the 5.8% lower phase (2155 ± 186) and in the cortical homogenate (1452 ± 246). The activity of alkaline phosphatase (Sigma) (units/ml per mg of protein; mean $\pm$ S.E.M.; $n = 4$) in

Table 1 Two-phase partition fractionation of rat renal cortex brush-border membrane vesicles: evidence from marker enzymes

The Table shows a comparison of the absolute activities of compartment-specific enzyme markers in fractions prepared by established centrifugation techniques or by serial two-phase partition fractionation. All values are means $\pm$ S.E.M. ($n = 4$). $P < 0.05$ versus brush-border membrane vesicles.

Enzyme activity and origin	Established centrifugation techniques		Serial two-phase partition fractionation						
	Cortical homogenate	Brush-border membrane vesicles	6.8% upper phase	6.6% upper phase	6.4% upper phase	6.2% upper phase	6.0% upper phase	5.8% upper phase	5.8% lower phase
γ -Glutamyl transpeptidase (brush border) (units/litre per mg of protein)	1452 $\pm$ 246*	9475 $\pm$ 445	12438 $\pm$ 1223*	13213 $\pm$ 500*	10757 $\pm$ 736*	13013 $\pm$ 2193*	11255 $\pm$ 763*	6497 $\pm$ 1042*	2662 $\pm$ 239*
Alkaline phosphatase (brush border) (Sigma units/ml per mg of protein)	1.7 $\pm$ 0.3*	13.1 $\pm$ 3.6	26.9 $\pm$ 1.7*	29.1 $\pm$ 3.7*	16.6 $\pm$ 2.5*	19.1 $\pm$ 2.8*	19.8 $\pm$ 2.0*	15.5 $\pm$ 2.5	7.3 $\pm$ 0.5*
K ⁺ -stimulated ouabain-inhibited nitrophenyl phosphatase (basolateral) (μ g of <i>p</i> -nitrophenol/mg of protein)	0.15 $\pm$ 0.03	0.14 $\pm$ 0.04	0.59 $\pm$ 0.05*	0.68 $\pm$ 0.02*	0.73 $\pm$ 0.05*	0.45 $\pm$ 0.08*	0.39 $\pm$ 0.07*	0.47 $\pm$ 0.03*	0.12 $\pm$ 0.02
Succinyl dehydrogenase (mitochondrial) (munits/h per mg of protein)	0.349 $\pm$ 0.028	0.263 $\pm$ 0.068	0.038 $\pm$ 0.007*	0.028 $\pm$ 0.004*	0.012 $\pm$ 0.004*	0.001 $\pm$ 0.001*	0.001 $\pm$ 0.001*	0.006 $\pm$ 0.006*	0.459 $\pm$ 0.044*
Acid phosphatase (lysosomal) (Sigma units/ml per mg of protein)	1.05 $\pm$ 0.13*	3.38 $\pm$ 0.23	1.32 $\pm$ 0.23*	0.96 $\pm$ 0.03*	0.74 $\pm$ 0.02*	0.71 $\pm$ 0.04*	0.71 $\pm$ 0.01*	0.63 $\pm$ 0.01*	0.52 $\pm$ 0.01*

renal membrane sheets (6.35 ± 1.31) was significantly increased in the two-phase membrane fractions 6.4% upper phase (18.28 ± 1.53), 6.0% upper phase (15.13 ± 0.59) and 5.8% upper phase (12.28 ± 0.90), and reduced in the 5.8% lower phase (3.17 ± 0.29) and in the cortical homogenate (1.69 ± 0.30). The activity of the basolateral marker K⁺-stimulated, ouabain-inhibited, nitrophenyl phosphatase (μ g of *p*-nitrophenol/mg of protein; mean $\pm$ S.E.M.; $n = 4$), in renal membrane sheets (0.09 ± 0.01) was significantly greater in the two-phase membrane fractions 6.4% upper phase (0.21 ± 0.04), 6.0% upper phase (0.53 ± 0.37), 5.8% upper phase (0.12 ± 0.02) and cortical homogenate (0.15 ± 0.03), and decreased in the 5.8% lower phase (0.05 ± 0.01). Compared with both renal membranes sheets (0.205 ± 0.016) and cortical homogenate (0.349 ± 0.028), the mitochondrial marker succinyl dehydrogenase (munits/litre per mg of protein; mean $\pm$ S.E.M.; $n = 4$) showed depletion in all of the upper phases (6.4%, 0.031 ± 0.008 ; 6.0%, 0.054 ± 0.023 ; 5.8%, 0.043 ± 0.053) and enrichment in the 5.8% lower phase (0.605 ± 0.183).

Having demonstrated that two-phase aqueous partition increased the enrichment of brush-border markers in membrane sheets, we then turned our attention to brush-border membrane vesicles. Compartment-specific enzyme markers were compared in brush-border membrane vesicle preparations before and after two-phase partition fractionation (Table 1). Compared with brush-border membrane vesicles prepared by Mg²⁺ precipitation, the brush-border markers γ -glutamyl transpeptidase and alkaline phosphatase showed greater activity in the 6.8% upper, 6.6% upper, 6.4% and upper, 6.2% upper and 6.0% upper membrane fractions from two-phase aqueous partition. There was a reduction in these brush-border markers in the 5.8% lower fraction. Compared with mixed renal membranes, the basolateral marker K⁺-stimulated ouabain-inhibited nitrophenyl phosphatase was increased in all of the upper phases from 6.8% to 5.8%, whereas the mitochondrial marker succinyl dehydrogenase was depleted

in all of the upper phases and enriched in the 5.8% lower phase. A lysosomal marker, acid phosphatase, showed no significant partition phase preference. The activity of the endoplasmic reticulum enzyme glucose-6-phosphatase increased significantly ($P < 0.05$ by paired *t*-test) in magnesium precipitation brush-border membrane vesicles (0.22 ± 0.07 μ mol of phosphate/ml per mg of protein) compared with cortical homogenate (0.13 ± 0.03 μ mol/ml per mg) (not shown in Table). Following a single 6.0% aqueous two-phase partition, there was no detectable glucose-6-phosphatase in the upper phase (< 0.01 μ mol of phosphate/ml per mg of protein).

When observed by electron microscopy, the original Mg²⁺-precipitated brush-border membrane vesicle preparation was a mixture of numerous microvilli, with a few mitochondria and other cellular organelles such as endoplasmic reticulum (Figure 1a). The dominant membrane type in the 6.8% upper fraction was brush-border membrane vesicles (Figure 1b), with no other organelles manifest. In the 5.8% lower phase, the dominant membrane types were mitochondria and endoplasmic reticulum (Figure 1c).

A comparison of Na⁺-dependent D-glucose uptake in Mg²⁺-precipitated brush border membrane vesicles with and without a single 6.0% aqueous two-phase separation (Table 2) showed the two-phase-purified membranes to have significantly increased Na⁺-dependent D-glucose uptake per mg of protein at 15 and 30 s than the control vesicles that had not undergone two-phase purification. This initial linear portion of the D-glucose uptake curve was $> 75\%$ Na⁺-dependent. The uptake after 60 min of equilibration also increased in the two-phase-purified membranes.

To study H⁺ fluxes such as the Na⁺-H⁺ antiporter, we loaded a probe with pH-dependent fluorescence emission (BCECF) into brush-border membrane vesicles by incubation in an uncharged non-fluorescent precursor (see preliminary data in the Materials and methods section). Brush-border membrane vesicles cleave

Figure 1 Electron micrographs of brush-border membrane vesicles following aqueous two-phase partition fractionation

(a) Total starting brush-border membrane vesicles fraction prior to aqueous two-phase partition. m = mitochondria. Arrows denote microvilli. (b) A 6.8% upper phase enriched in microvilli; (c) 5.8% lower phase consisting of mitochondria and membrane contaminants. Scale bar = 0.5 μm .

and load the fluorophore BCECF/AM, and retain the cleaved fluorescent form of the probe far beyond the period of the study. Measurement of $\text{Na}^+\text{-H}^+$ antiporter activity in brush-border membrane vesicles prepared by Mg^{2+} precipitation and loaded with BCECF/AM with and without a single 6.0% aqueous two-phase partition showed that the two-phase-purified membranes had more $\text{Na}^+\text{-H}^+$ antiporter activity per mg of protein than the control vesicles that had not undergone two-phase purification (Figure 2; representative of $n = 3$). $\text{Na}^+\text{-H}^+$ antiporter activity was measured as the Na^+ -dependent collapse of a preformed pH gradient.

DISCUSSION

Aqueous two-phase partitioning is a rapid, facile, inexpensive method for purifying plasma membranes from a variety of cultured cell lines and epithelia (Albertsson et al., 1982; Morré

and Morré, 1989). As it relies on surface properties such as charge and hydrophobicity (Morré and Morré, 1989), the aqueous two-phase partition technique is complementary to centrifugation techniques, which rely on size and density. The ability of aqueous two-phase partitioning to fractionate brush-border membranes depends on differences in the partition properties of the brush-border membranes and of contaminants such as mitochondria and endoplasmic reticulum (Morré and Morré, 1989). Evidence from marker enzyme analysis and electron microscopy support the conclusion that renal brush-border membranes are fractionated separate from mitochondria and endoplasmic reticulum.

Enrichment of brush-border membrane enzymic markers by two-phase partitioning was comparable to that obtained with other techniques. Compared with renal membranes, duodenal membranes routinely have relatively high enrichments (Shirazi-Beechey et al., 1990). Repeating the centrifugation steps during

Table 2 D-Glucose uptake in brush-border membrane vesicles prepared by two different techniques

The uptake of [^3H]glucose is shown for membrane vesicles prepared by centrifugation with and without a single 6.0% aqueous two-phase partition step. Na^+ -dependent glucose uptake is shown for the initial linear part of the uptake curve at 15 and 30 s, as well as for a more prolonged 60 min uptake. Results are means $\pm$ S.E.M., in units of pmol of radioactive D-[^3H]glucose per μg of protein ($n = 4$). * $P < 0.05$ centrifugation versus two-phase partition.

Cation present in the incubation	Uptake time	D-Glucose uptake (pmol/ μg of protein)	
		Centrifugation	Two-phase partition
Na^+	15 s	0.87 ± 0.50	3.07 ± 1.73
Na^+	30 s	0.87 ± 0.47	$3.30 \pm 2.01^*$
K^+	30 s	0.25 ± 0.14	$0.78 \pm 0.40^*$
Na^+	60 min	0.34 ± 0.17	$1.60 \pm 0.80^*$

Figure 2 Na^+ - H^+ antiporter activity in brush-border membrane vesicles without (a) and with (b) aqueous two-phase partitioning

The final concentration of sodium in the cuvette (33, 22 or 0 mM) is shown adjacent to each curve.

isolation of renal membranes improves the enrichments but reduces the yield of membranes (Hammond et al., 1985; Debiec and Lorenc, 1988; Kleinzeller, 1988; Hammond, 1990). The strength of the two-phase partition technique is that it can be applied to fractionation problems which remain difficult by centrifugation, such as isolation of the heterogeneous renal endosomal compartments (Hammond et al., 1993).

Basolateral membranes are a minor contaminant of brush-border membrane vesicles prepared by Mg^{2+} precipitation and

centrifugation (Hammond et al., 1985; Kleinzeller 1988; Hammond, 1990). However, the small quantity of basolateral membranes present appears to co-purify with brush borders during two-phase partition fractionation: improved enrichment in brush-border markers following aqueous partition does not reflect any change in the basolateral membrane content.

The utility of aqueous two-phase partitioning depends on the preservation of the functional properties of the membranes of interest. Glucose uptake on the initial linear portion of the uptake curve was greater in membrane vesicles prepared by two-phase partition fractionation than in membrane vesicles prepared by Mg^{2+} precipitation alone. Although this linear portion of the uptake curve has been a benchmark index of transport activity, it does not examine the peak overshoot of uptake (5–10 min into the curve) or the equilibrium (achieved at 4–5 h) (Turner and Moran, 1982a,b; Malathi and Preisner, 1983). Uptake was still relatively greater in membrane aliquots fractionated by two-phase partition than in controls when measurements were performed following 60 min of glucose uptake.

Na^+ - H^+ antiporter activity is a physiologically important functional property of membrane vesicles (Mahnensmith and Aronson, 1985). Study of H^+ fluxes in membrane vesicles has been impeded by the lack of a pH-sensitive probe that is easily retained in membrane vesicles after loading (Verkman and Ives, 1986). The pH-sensitive probe BCECF was selected to study H^+ fluxes in the current study, as its large charge gives it high intravesicular retention (Musgrove et al., 1986). Other currently available pH-sensitive probes were unsuitable to study Na^+ - H^+ antiporter activity in this study: Acridine Orange will not detect small or inwardly directed H^+ fluxes (Verkman and Ives, 1986), and there is poor retention of carboxyfluorescein or 4-methylumbelliferol (Musgrove et al., 1986). Using BCECF, we demonstrated that, like Na^+ -dependent D-glucose uptake, Na^+ - H^+ antiporter activity increased in two-phase-partitioned membranes in proportion to the enrichment of marker enzymes.

The aqueous two-phase partition technique is a simple, rapid, inexpensive method for the fractionation of renal membrane preparations. Following aqueous two-phase partition, brush-border membrane vesicles show increased Na^+ -dependent transport functions, Na^+ -dependent D-glucose uptake and Na^+ - H^+ antiporter activity in proportion to their marker enzyme enrichment. Therefore membranes purified by aqueous two-phase partitioning appear to be suitable for the study of many physiological transport functions.

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REFERENCES

- Albertsson, P. A., Andersson, B., Larsson, C. K. and Akerlund, H. E. (1982) Methods Biochem. Anal. **28**, 115–150
- Andersson, M. A. and Szczypinske, A. J. (1967) Am. J. Clin. Pathol. **17**, 571–574
- Bessey, O. A., Lowry, O. H. and Brock, M. J. (1946) J. Biol. Chem. **164**, 321–329
- Debiec, H. and Lorenc, R. (1988) Biochem. J. **255**, 185–191
- Emmelot, P. and Bos C. J. (1966) Biochim. Biophys. Acta **121**, 375–385
- Green, J. D. and Narahara, H. T. (1980) J. Histochem. Cytochem. **28**, 408–412
- Hammond, T. G. (1990) Kidney Int. **38**, 5–11
- Hammond, T. G., Yusufi, A. N. K., Knox, F. G. and Dousa, T. P. (1985) J. Clin. Invest. **75**, 1983–1989
- Hammond, T. G., Makewski, R. R., Morré, D. J., Schell, K. and Morrissey, L. W. (1993) Cytometry, in the press

- Hannig, K. and Heidrich, H. G. (1977) in *Cell Separation Methods* (Bloemendal, H., ed.), pp. 95–116, North-Holland Publishing Co., Amsterdam
- Harris, W. D. and Popat, P. (1954) *J. Am. Oil Chem. Soc.* **31**, 124–137
- Kashiwamata, S., Goto, S., Semba, R. K. and Suzuki F. N. (1979) *J. Biol. Chem.* **254**, 4577–4584
- Kleinzeller, A. (1988) in *Renal Physiology: People and Ideas* (Gottschalk, C. W., Berliner, R. W. and Giebisch, G. H., eds.), pp. 131–157, American Physiological Society, Washington, DC
- Mahnensmith, R. L. and Aronson, P. S. (1985) *Circ. Res.* **57**, 773–788
- Malathi, P. and Preiser, H. (1983) *Biochim. Biophys. Acta* **735**, 314–324
- Marsh, M., Schmid, S., Kern, H., Harms, E., Male, P., Mellman, I. and Helenius, A. (1987) *J. Cell Biol.* **104**, 875–886
- Mircheff, A. K., Ives, H. E., Yee, V. J. and Warnock, D. G. (1984) *Am. J. Physiol.* **246**, F853–F858
- Morré, D. J. and Morré, D. M. (1989) *BioTechniques* **7**, 946–958
- Morré, D. M., Morré, D. J., Snyder, T., Li, W., Erb, A. and Sammons, D. W. (1989) in *Separation Using Aqueous Phase Systems in Cell Biology and Biotechnology* (Fisher, D. and Sutherland, I., eds.), pp. 113–118, Plenum Press, New York
- Musgrove, E., Rugg, C. and Hedley, D. (1986) *Cytometry* **7**, 347–355
- Nordlie, R. C. and Arion, W. J. (1966) *Methods Enzymol.* **9**, 619–625
- Peterson, G. L. (1977) *Anal. Biochem.* **83**, 346–356
- Schmidt, S. L., Fuchs, R., Male, P. and Mellman, I. (1988) *Cell* **52**, 73–83
- Shirazi-Beechey, S. P., Davies, A. G., Tebbutt, K., Dyer, J., Ellis, A., Taylor, C. J., Faricough, P. and Beechet, R. B. (1990) *Gastroenterology* **98**, 676–685
- Tsao, B. and Curthoys, N. P. (1980) *J. Biol. Chem.* **255**, 7708–7711
- Turner, J. R. and Moran, A. (1982a) *J. Membr. Biol.* **67**, 73–80
- Turner, J. R. and Moran, A. (1982b) *J. Membr. Biol.* **70**, 37–45
- Verkman, A. S. and Ives, H. E. (1986) *Biochemistry* **25**, 2876–2882