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Renal Cortical Brush-Border and Basolateral Membranes: Cholesterol and Phospholipid Composition and Relative Turnover

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Summary. A new procedure for the rapid isolation of renal cortical brush-border and basolateral membranes from the same homogenate is described. Brush-border membranes isolated using Mg^{2+}-EGTA precipitation were enriched 18-fold for leucine aminopeptidase and had a recovery of 32.5%. Basolateral membrane fractions were isolated using a discontinuous sucrose gradient and showed an enrichment of 10.7-fold and recovery of 12.8% using (Na^+,K^+)-ATPase as a marker enzyme. Lipid analysis using two-dimensional TLC separation of phospholipids and gas liquid chromatography for cholesterol showed marked differences in the lipid composition of the brush-border and basolateral membranes. The brush-border membrane had increased sphingomyelin, phosphatidylserine, ethanolamine plasmalogens, and an increased cholesterol-to-phospholipid and sphingomyelin-to-phosphatidylcholine ratio compared to the basolateral membrane. The relative turnover of total membrane and individual phospholipid species using a double isotope ratio method was carried out. Phospholipids were labeled with either phosphorus 32 and 33 or acetate (^3H, 1-^{14}C). The relative turnover of phospholipid species and cholesterol differed strikingly. Phosphatidylcholine showed a high turnover, phosphatidylethanolamine and phosphatidylinositol had intermediate values and sphingomyelin, phosphatidylserine and cholesterol had low relative turnover rates. The order of phospholipid class relative turnover was independent of the labeled precursor used. The brush-border membrane had a significantly reduced relative turnover rate for total membrane phospholipids, sphingomyelin and cholesterol compared to the basolateral membrane. These data show marked differences in the lipid composition and relative turnover rates of the phospholipid species of the brush-border and basolateral membranes. They provide a biochemical basis for the recently reported differences in brush-border and basolateral membrane fluidity and suggest independent cellular regulation of brush-border and basolateral membrane lipids.

Key Words brush-border membranes · basolateral membranes · phospholipids · cholesterol · turnover

Introduction

The primary function of epithelial tissue is to provide and regulate the unidirectional transport of various compounds across cells. This is accomplished in part by having a polarized surface membrane separated by tight junctions into apical (or brush-border) and basolateral membranes. In renal proximal tubule cells these two membrane segments are known to be functionally different in respect to transport processes [3, 22], hormone receptors [34], and electrical resistance [19]. How epithelial cells achieve and maintain this functional polarity is unknown, although most efforts have been directed at the processes involved with intracellular sorting of membrane proteins [18]. Since integral membrane proteins associate with hydrophobic areas of the lipid bilayer, another possibility might be by varying the lipid composition of the two membrane segments. Thus, differences in lipid composition of plasma membrane domains could in effect select for or against specific proteins.

It has recently been reported that renal cortical brush-border and basolateral membranes differ in membrane fluidity [24, 25] as measured by both fluorescent polarization and electron spin resonance. The structural basis for the observed differences has not been determined. The major determinants of membrane fluidity include the cholesterol-to-phospholipid ratio, the sphingomyelin-to-phosphatidylcholine ratio and the degree of fatty acid saturation [13, 32]. Therefore, the brush-border and basolateral membrane should differ in one or more of these parameters.

The present studies were undertaken to determine the lipid composition of renal cortical brush-border and basolateral membranes. Also the relative turnover of both plasma membrane domains and their individual lipid components was measured. The results indicate that the lipid composition and relative turnover of the brush-border and basolateral membranes are markedly different.

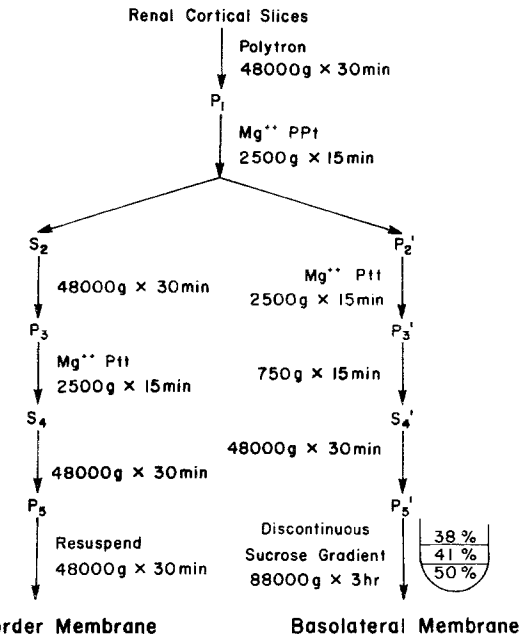

Fig. Simultaneous preparation of brush-border and basolateral membranes from the same cortical homogenate. Details are described in the Materials and Methods section

Materials and Methods

Animals and Diet

Male Sprague-Dawley (King Animal Labs, Inc., Oregon, Wisconsin; and Sasco Labs, Omaha, Nebraska) weighing 180 to 220 g were fed standard rat chow. The animals were fasted overnight prior to the i.p. injection of radioactive compounds, and diets were readministered 2 hr after this injection.

Preparation and Characterization of Membranes

Rat renal cortical brush-border membranes (BBM) and basolateral membranes (BLM) were prepared simultaneously from the same cortical homogenate of individual rats. Brush-border membranes were prepared using the method of Biber et al. [5] with minor modifications. The procedure entailed rapid decapsulization and removal of thin cortical slices in chilled buffer (300 mM mannitol, 5 mM EGTA, 18 mM Tris-HCl, 0.1 mM PMSF, at pH 7.4). All subsequent steps were carried out at 4°C in a cold room, and a fixed angle Sorvall SW34 rotor was used for all centrifugation steps except the ultracentrifugation procedure. The figure is a summary of the procedure. The slices from two kidneys were homogenized using a polytron (Brinkman Co., PT 2000) in 15 ml of buffer for two 45-sec periods separated by 15 sec. The polytron setting was 0.5 with a powerstat (The Superior Electric Co.) setting of 90. An aliquot of homogenate was saved, 21 ml of distilled water was added, and the solution was centrifuged at 48,000 × *g* for 30 min using a Sorvall SW34 rotor. The resulting pellet was resuspended using a loose Dounce glass homogenizer (10 strokes) in buffer, and Mg^{2+} precipitation (15 mmol/liter) was carried out for 20 min by adding 21 ml of distilled water and 0.54 ml of a 1 molar Mg^{2+} solution. The solution was vigorously shaken at 0, 10 and 20 min and then centrifuged for 15 min at 2,445 × *g*. The pellet (P_2) was saved for basolateral membrane isolation while the supernatant was centrifuged at 48,000 × *g* for 30 min to obtain the crude BBM (P_3). The P_3 was resuspended using a Potter-Elvehem Teflon® glass homogenizer in 30 ml of 0.5 N buffer, taken through the Mg^{2+} precipitation process again and washed free of Mg^{2+} using the Potter-Elvehem in 0.5 N buffer. This solution was centrifuged at 48,000 × *g* for 30 min to obtain the final BBM fraction which was resuspended in 1 to 2 ml of buffer.

The basolateral membrane fraction was isolated from the initial Mg^{2+} precipitation pellet (P_2). This pellet was resuspended in 15 ml of buffer using a loose Dounce glass homogenizer (10 strokes) and diluted to 36.54 ml using 21 ml of distilled water and 0.54 ml of a 1 molar Mg^{2+} solution. After 20 min with vigorous shaking at 0, 10 and 20 min the solution is centrifuged at 2,445 × *g* for 15 min to yield $P_{3'}$. $P_{3'}$ is resuspended to 15 ml buffer with a loose Dounce glass homogenizer (10 strokes) and centrifuged at 755 × *g* for 15 min. The resulting supernatant is centrifuged for 30 min at 48,000 × *g* to yield $P_{5'}$. The pellet $P_{5'}$ is resuspended in 19 ml of 50% sucrose using a Potter-Elvehem, 5 strokes. This solution is overlayed with a discontinuous sucrose gradient using 41% (5 ml) and 38% (12 ml) in cellulose-acetate tubes and centrifuged at 88,000 × *g* (5°C) for three hours in a Beckman model L8-70 ultracentrifuge using a swinging bucket SW 27 rotor. The top layer of the discontinuous gradient (38%) is harvested and washed in 1 mM bicarbonate (48,000 × *g* for 30 min), yielding the BLM fraction. This fraction is also stored in 1 to 2 ml of buffer at −30°C.

Enzyme and Protein Measurements

Protein was measured according to Lowry et al. [26] using BSA as a standard. Determinations were carried out on alkaline phosphatase, succinic dehydrogenase, (Na^+,K^+)-ATPase, KCN-resistant NADH dehydrogenase and acid phosphatase as previously reported from our laboratory [33]. Leucine aminopeptidase, TPNH-cytochrome *c* reductase and glucosaminidase were determined as described by Hasse et al. [20], Baron and Tephyl [4] and Scalera et al [30], respectively.

Lipid Determinations

Lipids from approximately 1 mg of membrane protein were extracted in 6 ml of chloroform/methanol (1 : 2 vol/vol) according to the Bligh and Dyer procedure [7]. To each membrane sample 27.5 μg of coprosterol was added as an internal standard for cholesterol recovery and quantification [14]. Total lipid phosphorus was determined on an aliquot of the total lipid extract according to Ames and Dubin [1]. Individual phospholipid species and plasmalogens were separated by two-dimensional TLC on Kesigel® silica gel 60 plates using the technique of Esko and Raetz [17]. The first phase is chloroform/methanol/acetic acid 65 : 25 : 10 vol/vol and the second phase is chloroform/methanol/formic acid 65 : 25 : 10 vol/vol. Plasmalogens were separated from their parent compounds by spraying with 10 mM $HgCl_2$ in between phases. Plates are dried only 30 to 45 min and then scraped to minimize oxidation. Individual phospholipid species were identified using brief exposure to iodine vapor, sprayed with a fine mist of distilled water and scraped off the plates. A

Table 1. Specific activities, enrichment factors and recoveries of marker enzymes obtained for brush-border and basolateral membrane fractions from rat kidney cortex[a]

	Brush-border membrane					Basolateral membrane				
	n	Homogenate spec. act.[b]	Spec. act.	Enrichment	% Recovery	Spec. act.	Enrichment	% Recovery	*n*	Total recovery
Leucine aminopeptidase	8	5.11 + 0.7	92.4 + 8.7	16.8 + 0.9	32.5 + 1.1	7.2 + 1.9	1.4 + 0.1	2.6 ± 0.5	6	92.1 ± 2.8
Alkaline phosphatase	8	62.1 + 10.4	779.8 + 81.1	13.7 + 1.1	27.4 + 3.1	119.1 + 12.0	2.1 + 0.2	2.7 ± 0.3	—	—
(Na^+,K^+)-ATPase	6	10.9 + 0.8	15.6 + 1.1	1.4 + 0.1	2.4 + 0.1	112.6 + 8.3	10.7 + 1.1	12.8 ± 1.0	5	63.6 ± 3.1
Succinic dehydrogenase	6	26.1 + 1.1	5.38 + 0.44	0.21 + 0.02	0.44 + 0.02	54.38 + 3.26	2.11 ± 0.16	2.72 ± 0.25	5	102.8 ± 4.1
KCN-resistant NADH dehydrogenase	6	58.1 + 2.7	11.47 + 1.89	0.21 + 0.04	0.48 + 0.15	132.6 ± 4.69	2.29 ± 0.06	3.03 ± 0.32	6	85.0 ± 8.1
TPNH-cytochrome *c* reductase	8	0.94 + 0.07	0.41 + 0.05	0.44 + 0.05	0.84 + 0.12	2.49 ± 0.25	2.65 ± 0.17	3.30 ± 0.57	—	—
Acid phosphatase	6	4.3 + 0.2	18.2 + 3.5	4.2 + 0.7	7.6 + 0.9	3.8 + 0.7	0.9 + 0.2	1.3 ± 0.1	4	88.4 ± 5.4
Glucosaminidase	5	10.6 + 0.6	3.1 + 0.2	0.28 + 0.02	0.53 + 0.04	8.6 + 0.3	0.83 ± 0.06	1.2 ± 0.14	—	—
Protein	8	—	—	—	1.90 + 0.20	—	—	1.28 ± 0.22	6	87.9 ± 5.7

[a] All values are mean + SE; *n* = number of determinations.
[b] Specific activity is reported as μmol/mg protein/hr.

Bligh and Dyer extraction is carried out after centrifugation at 3,000 × *g* for 20 min and careful decanting to leave almost all of the gel behind. The small amount of transferred gel goes into the upper phase during the extraction and therefore does not interfere with the phosphate determination. Inorganic phosphate determinations were carried out on an aliquot according to Ames and Dubin [1]. Individual phospholipids are reported as a percent of the total recovered phospholipid species.

Plasmalogens are reported as the percent of the parent compound they compose. A serum lipid standard was purchased from Supelco (4- 6430, Bellefonte, Pa.) and used to quantitate recovery of total and individual phospholipid species.

Relative Turnover Procedure

The measurement of BBM and BLM total phosphorus and individual phospholipids relative turnover was carried out according to Lee et al. [23]. The advantages of this method in our studies include identifying two points on the decay curve from one sample and the ability to compare BBM and BLM lipid synthesized from a common pool of isotopes. Phosphorus 32 (1 mCi) and 33 (0.5 mCi) and 1-^{14}C (0.3 mCi) and ^{3}H (1 mCi) acetate (purchased from New England Nuclear) were used in separate experiments. The initial injection was 72 hr prior to membrane isolation and was either ^{33}P or 1-^{14}C acetate. The second i.p. injection was 4 hr prior to membrane isolation and consisted of ^{32}P or ^{3}H acetate. Preliminary data in our laboratory indicated that maximal BBM and BLM total lipid phosphorus specific activity occurred 4 hr after an i.p. injection of ^{32}P. To determine the relative turnover rate, the dpm's of the second isotope (^{32}P, ^{3}H acetate) is divided by the dpm's of the first isotope (^{33}P, 1-^{14}C acetate) according to Lee et al. [23].

Statistical Analysis

A two-tailed student's *t*-test was used to compare differences between brush-border and basolateral membranes. Values were considered significantly different if the *P* value was <0.05. All values are reported as the mean ± SD unless otherwise noted.

Results

Isolation of Plasma Membrane Fractions

The specific activities, enrichment factors and recoveries of marker enzymes for BBM, BLM and intracellular organelles are shown in Table 1. Judged by its marker enzymes, leucine aminopeptidase and alkaline phosphatase, the brush-border fraction has excellent enrichment (16.8-fold and 13.7-fold) and recovery (32.5% and 27.4%, respectively). Enzyme markers of intracellular organelle contamination show minimal activity with the exception of acid phosphatase (4.2 ×). However, previous work [5] has clearly shown that acid phosphatase is not an appropriate marker for lysosomal contamination. Since glucosaminidase is reported to be a more specific marker [10] of the lysosomal fraction, it was also measured. Its activity is deenriched in the brush-border fraction, suggesting minimal lysosomal contamination.

Since sample preparation is known to affect the specific activity of (Na^+,K^+)-ATPase, we first sought to maximize the specific activity of both the homogenate and BLM fractions. Specific activities, enrichment factors and percent recoveries of samples prepared using a different scheme were compared (Table 2). Overnight freezing (−30°C) of samples prior to assay resulted in maximal homogenate and BLM specific activities. Compared to the freshly isolated samples, specific activity of the homogenate and BLM increased 243 and 138 percent, respectively. This disproportionate increase in homogenate (Na^+,K^+)-ATPase specific activity indicates the values measured in fresh and fresh-frozen samples are inappropriately low. In agreement with

Table 2. Effect of sample preparation on the specific activity, enrichment and recovery of (Na^+,K^+)-ATPase[a]

	Homogenate	Basolateral fraction		
	Spec. act.[b]	Spec. act.	Enrichment	% Recovery
Fresh[c]	4.8 ± 0.6	93.4 ± 3.9	20.8 ± 3.2	23.7 ± 1.6
Fresh Frozen[d]	6.9 ± 0.9	94.0 ± 5.5	14.0 ± 1.4	14.1 ± 0.8
Fresh with Triton[e]	11.0 ± 0.6	89.2 ± 3.8	8.2 ± 0.6	9.6 ± 0.7
Frozen for 16 hr[f]	11.7 ± 1.1	129.3 ± 6.8	11.2 ± 0.7	13.1 ± 1.3

[a] All the values are the mean ± SE, $n = 4$. All assays were carried out on the same samples prepared in the four different ways listed.
[b] Specific activity in μmol/hr/mg protein.
[c] Activity measured just after isolation.
[d] Frozen for 15 to 30 min at −30°C just prior to assay.
[e] An equal volume of 0.2% Triton in 1 mM bicarbonate was added to the sample which was then stored on ice for 30 min prior to the assay.
[f] Frozen at −30°C for 16 hr prior to the assay.

Table 3. Brush-border and basolateral membrane cholesterol and total phospholipids[a]

	Cholesterol (nM/mg protein)	Phospholipids (nM/mg protein)	Ratio cholesterol phospholipid
Brush-border	552 ± 9.5	546 ± 13.2	0.97 ± 0.02
Basolateral	337 ± 15.9	712 ± 37.0	0.48 ± 0.02
P value	<0.01	<0.01	<0.01

[a] All values are the mean ± SE; $n = 8$. Significance was determined using a nonpaired 2-tailed student's *t*-test.

this is our finding of greater than 100% recovery for (Na^+,K^+)-ATPase in fresh isolated samples (*data not shown*). Therefore, the significantly increased enrichment and recoveries (Table 2) found when fresh and fresh-frozen samples are assayed are due primarily to a substantial underestimation of the homogenate specific activity of (Na^+,K^+)-ATPase.

Values reported in Table 1 are on samples frozen overnight prior to assay. The BLM fraction showed only a twofold enrichment of both the mitochondrial membrane enzyme marker, succinic dehydrogenase, and endoplasmic reticulum membranes as determined by the marker enzymes, TPNH-cytochrome *c* reductase and KCN-resistant NADH dehydrogenase. Brush-border contamination of the basolateral fraction, as measured by leucine aminopeptidase, was only 1.4-fold. Leucine aminopeptidase is a more specific marker [28] than alkaline phosphatase of brush-border contamination, as alkaline phosphatase is probably also located in the basolateral membrane [37].

A difference in the enrichment of BLM (Na^+,K^+)-ATPase was noted when different sources of Sprague-Dawley rats were used. The technique was developed using Sprague-Dawley rats purchased from the King vendor. Using these animals, we consistently saw enrichments of BLM (Na^+,K^+)-ATPase in the 10- to 12-fold range. This was also observed using Fisher rats (*unpublished observations*). However, the enrichment of (Na^+,K^+)-ATPase is only seven- to ninefold using Sprague-Dawley rats from either the Sasco or Charles River vendors (*unpublished observations*). The reason for this difference is not known.

Total recovery of individual marker enzymes and protein is also shown in Table 1. Protein and marker enzymes were recovered to a similar extent and neither activation nor inhibition was noted for any marker enzyme. As observed before [5] total recovery of the (Na^+,K^+)-ATPase was low. Thus our enrichment and recovery of (Na^+,K^+)-ATPase in the BLM may be an underestimate.

Lipid Characterization of Plasma Membrane Fractions

The individual phospholipid composition of a known lipid standard was measured using our procedure. The total recovery was 77.6 ± 2.4 percent with all individual phospholipid classes recovered to the same extent. Carrying out a Bligh and Dyer extraction of the scraped gel eliminates contamination from gel phosphate and allows quantification of minor phospholipid components with minimal variability. A 2 × 2 cm area of gel carried through the entire process does not read significantly different

Table 4. Individual phospholipid composition of the brush-border and basolateral membrane fractions[a]

	Brush border	Basolateral	*P* value
Lysophosphatidylcholine	0.68 ± 0.05	0.27 ± 0.06	<0.01
Sphingomyelin	34.5 ± 1.1	13.4 ± 0.7	<0.01
Phosphatidylcholine	16.2 ± 0.6	38.4 ± 1.6	<0.01
Percent plasmalogen	0.4 ± 0.05	0.5 ± 0.1	NS
Phosphatidylethanolamine	30.0 ± 0.9	32.3 ± 0.9	NS
Percent plasmalogen	22.8 ± 3.2	11.5 ± 2.1	<0.05
Phosphatidylinositol	1.75 ± 0.12	4.31 ± 0.49	<0.01
Phosphatidylserine	16.8 ± 0.9	7.5 ± 0.3	<0.01
Sphingomyelin phosphatidylcholine ratio	2.14 ± 0.1	0.35 ± 0.02	<0.01

[a] All values are the mean ± SE; $n = 8$ and 6 for the BBM and BLM, respectively. Significance was determined by using a nonpaired, 2-tailed student's *t*-test. Percent plasmalogen is the percent of the parent compound representing plasmalogens ($n = 3$).

from a standard blank essay sample (0.020 to 0.030 OD units).

Table 3 shows the cholesterol and phospholipid composition of brush-border and basolateral membrane fractions. The brush-border fraction has a much higher content of cholesterol (552 *vs.* 337 nm/mg protein, $P < 0.01$) and a significantly reduced total phospholipid content (546 *vs.* 712 nm/mg protein). Therefore, the cholesterol-to-phospholipid ratio is markedly different with the brush-border membrane approaching unity and the basolateral membrane only one-half that value.

As the total phospholipid content varied between the two segments of the plasma membrane, we next quantitated individual phospholipid classes. These data are shown in Table 4. The membranes differed in all phospholipid classes. In particular, the brush-border membrane has an increased content of sphingomyelin, phosphatidylserine, ethanolamine plasmalogen and a reduced amount of phosphatidylcholine and phosphatidylinositol. The sphingomyelin-to-phosphatidylcholine ratio was very high in the brush-border membrane (2.1) especially when compared to the basolateral membrane (0.35).

Lipid Turnover

We next sought to determine the factor or factors accounting for the observed differences in lipid composition. Since membrane lipids are in a dynamic state of flux, one way to alter their composition would be to alter the turnover rate of their individual lipid components. To evaluate this question, the relative turnover rates of both the brush-border and basolateral membranes and their individual lipid components were measured using a double-isotope technique. The data obtained using phosphorus 32 and 33 are shown in Table 5. The basolateral membrane had a relative turnover rate greater than twice that of the brush-border membrane (4.56 *vs.* 2.24). However, when individual phospholipids were compared, sphingomyelin was the only phospholipid class showing a difference between the two membrane fractions. Its relative turnover rate in the brush-border fraction was significantly reduced. This reduced rate and the high content of sphingomyelin in the brush-border membrane primarily account for the reduced relative turnover of the brush-border membrane.

Table 5. Relative turnover rates of brush-border and basolateral membranes and individual phospholipids using phosphorus 32 and 33[a]

	Brush-border membrane	Basolateral membrane	*P* value
Total lipid phosphorus	2.24 ± 0.08	4.56 ± 0.10	<0.01
Sphingomyelin	0.33 ± 0.03	0.53 ± 0.03	<0.01
Phosphatidylcholine	11.23 ± 0.47	11.62 ± 0.90	NS
Phosphatidyl-ethanolamine	3.76 ± 0.03	3.09 ± 0.28	NS
Phosphatidylserine	0.43 ± 0.03	0.52 ± 0.07	NS
Phosphatidylinositol	3.28 ± 0.30	3.15 ± 0.32	NS

[a] All values are reported as the mean ± SD; $n = 3$ and significance was determined using a paired, 2-tailed student's *t*-test.

It should also be noted that individual phospholipid species showed relative turnover rates that differed markedly. Phosphatidylcholine showed a high turnover rate, phosphatidylethanolamine and phosphatidylinositol had intermediate rates and phosphatidylserine and sphingomyelin had very slow relative turnover rates. This has been previously documented in hepatic microsomal and plasma membranes [23].

To verify these results, a different label was employed. Acetate (1-^{14}C and ^{3}H) was used, and the

Table 6. Relative turnover rates of brush-border and basolateral membranes and individual phospholipids using 1-^{14}C and ^{3}H acetate[a]

	Brush-border membrane	Basolateral membrane	*P* Value
Total phospholipids	0.85 ± 0.02	1.20 ± 0.04	<0.01
Sphingomyelin	0.24 ± 0.04	0.36 ± 0.01	<0.05
Phosphatidylcholine	2.61 ± 0.30	2.28 ± 0.14	NS
Phosphatidyl-ethanolamine	0.95 ± 0.07	0.83 ± 0.02	NS
Phosphatidylserine	0.55 ± 0.05	0.62 ± 0.06	<0.05
Phosphatidylinositol	1.10 ± 1.01	0.98 ± 0.06	NS

[a] All values are reported as the mean ± SD; $n = 3$ and significance was judged using a paired, 2-tailed student's *t*-test. All values were standardized by multiplying the following ratio: $\frac{\text{Sample homogenate ratio}}{\text{Mean homogenate ratio}}$ to correct for unequal homogenate labeling pools.

data are shown in Table 6. To correct for possible differences in injection volumes and pool sizes between animals, individual homogenate ratios were divided by the mean homogenate ratio, and the resulting number was used as a correction factor. The general pattern of relative turnover was remarkably similar to and confirms the data observed using phosphorus 32 and 33. Again, the brush-border membrane had a reduced relative turnover rate, as did BBM sphingomyelin. Phosphatidylserine also showed a slight but significantly reduced relative turnover rate in the brush-border fraction in these paired studies. Although the magnitude of the turnover ratio was reduced with acetate labeling, individual phospholipid species had an identical order of relative turnover as when phosphorus 32 and 33 were used. This reduced magnitude may be due to the delay in labeling of phospholipid molecules using acetate which first has to be incorporated into fatty acids. Also, acetate can only be used to measure the relative turnover of nonessential fatty acids, and therefore may not reflect the turnover of essential fatty acids or their polyenoic products.

Since acetate is also incorporated into cholesterol, we were able to quantitate the relative turnover of cholesterol. The brush-border membrane had a small but significantly reduced relative turnover (1.39 ± 0.18 *vs.* 1.55 ± 0.15, $P < 0.05$) of cholesterol when compared to the basolateral membrane.

Discussion

Isolation of renal cortical plasma membranes has primarily focused on obtaining either the brush-border or basolateral fraction. The BBM fraction can be recovered in high yield and with excellent enrichment using divalent cation (Mg^{2+}, Ca^{2+}) precipitation methods, as it is the only major cellular membrane that does not bind either divalent ions. Multiple techniques also exist for the isolation of renal cortical basolateral membranes. The use of a self-orienting Percoll gradient was developed for the rapid isolation of intestinal BLM [15, 30] and has been adapted for renal cortical tissue [29]. This technique results in a BLM fraction with good enrichment and recovery for its marker enzyme (Na^+,K^+)-ATPase.

However, a method for the rapid and simultaneous isolation of both BBM and BLM from the same cortical homogenate has been difficult to develop. Taylor et al. [34] reported a procedure that allowed the simultaneous isolation of BBM and BLM fractions. However, they used Ca^{2+} precipitation and the technique required an overnight dialysis of the crude BLM fraction to allow dissociation of the Ca^{2+} prior to the final centrifugation step. The effect of this prolonged exposure to Ca^{2+} on membrane phospholipid and protein composition has not been determined. Free-flow electrophoresis is another technique that can be used to isolate both the BBM and BLM [9]. However, the apparatus is very expensive. The use of Percoll gradients to obtain both membrane fractions has also met with difficulty. Sacktor et al. [29] reported equal enrichment of both their luminal and basolateral marker enzymes in the brush-border fraction indicating heavy cross-contamination of their brush-border fraction with basolateral membranes. Mamelok et al. [27] reported enrichment of only seven- and fivefold for (Na^+,K^+)-ATPase and alkaline phosphatase in his BLM and BBM fractions, respectively, and did not report on the cross-contamination with other cellular organelles. Boumendil-Podevin and Podevin [10] recently reported a Percoll separation technique giving excellent enrichments and recovery of both the BBM and BLM fractions. However, cross-contamination with the endoplasmic reticulum was not reported. This is of particular importance in studies evaluating synthesis of plasma membrane constituents such as phospholipids and proteins which are synthesized in the endoplasmic reticulum and then moved to the surface membrane. Contamination with the endoplasmic reticulum could therefore cause major errors in interpretation.

Because previous reports of BLM isolation have either used different methods to prepare samples for analysis of (Na^+,K^+)-ATPase or not commented on the preparation, we systematically examined a number of methods. We found that sample preparation has a major influence on specific activi-

ties, enrichment and thus apparent recovery of (Na^+,K^+)-ATPase. Clearly, although overnight freezing leads to loss of total activity, the highest homogenate and BLM (Na^+,K^+)-ATPase activities were obtained. Therefore, sample preparation is important and may result in falsely high enrichments and percent recoveries when the BLM marker (Na^+,K^+)-ATPase is used.

The procedure described in this paper has several advantages. First, it enables the rapid and simultaneous isolation of highly enriched brush-border and basolateral membrane fractions with excellent recovery from the same cortical homogenate. Contamination of either fraction by other intracellular organelles is minimal and should not cause problems with interpretation of the data on lipid synthesis. Therefore, direct comparisons between BBM and BLM isolated from the same kidney can be made. Also, variations in substrate pool size for incorporation into the BBM and BLM are eliminated. This is highly desirable when one is using radiolabeled isotopes to measure simultaneous membrane biosynthesis.

Secondly, using Mg^{2+} precipitation in the presence of 5 mM EGTA may decrease the *in vitro* activation of calcium-dependent phospholipase as has been proposed in rabbit intestinal brush-border vesicles by Hauser et al. [21]. They reported abnormally high concentrations of lysophospholipids in membranes using Ca^{2+} precipitation. We found very low levels of lysophosphatidylcholine (Table 4) in both of our surface membrane fractions and no other lysophospholipids could be detected.

Thirdly, BBM vesicles isolated using the Mg^{2+}/EGTA precipitation method have previously [5] been shown to have improved transport ability when compared to vesicles isolated using the Ca^{2+} precipitation method. This could result from lower levels of lysophospholipids or perhaps decreased protein destruction caused by activation of Ca^{2+}-dependent proteases known to be present in renal cortical tissue [38].

The differences in lipid composition between the BBM and BLM provide a biochemical basis for the observed differences in brush-border and basolateral membrane fluorescence recently reported [24, 25] in both human and canine cortical plasma membrane fractions. The three major factors controlling membrane lipid fluidity are the cholesterol-to-phospholipid ratio, the sphingomyelin-to-phosphatidylcholine ratio and the degree of fatty acid saturation [13, 32]. Increases in any one of these factors decrease membrane fluidity [11, 13, 32]. Therefore, the high cholesterol-to-phospholipid ratio and sphingomyelin-to-phosphatidylcholine ratio of the BBM provide a molecular basis for the physically measured differences between BBM and BLM fluidity. The differences between the two membranes in ethanolamine plasmalogens may also play an important role in determining their respective membrane fluidity [16].

The physiologic importance of this lipid polarity is unknown. However, Chapelle and Gilles-Baillien [12] have recently reported that other epithelial cells share this asymmetric distribution of membrane lipid components. They reported that BBM from intestinal mucosa has an increased content of sphingomyelin and an increased cholesterol-to-phospholipid ratio when compared to BLM from the same tissue. Also, Schwertz et al. [31] have recently published data on renal cortical BBM phospholipid composition which are in close agreement with ours. However, both our data and Schwertz' data disagree with the lipid composition of brush-border membranes reported previously [8]. The reason for this discrepancy is not known but could relate to species differences (pig *vs.* rat) or the method of membrane isolation.

How the cell regulates the lipid composition of the brush-border and basolateral membrane independently is unknown. It is known that plasma membranes of eukaryotic cells in general have the highest content of cholesterol and sphingomyelin of any cellular membrane [36]. Also, cholesterol seems to partition into membranes with high sphingomyelin content [36]. Since sphingomyelin is believed to be synthesized from phosphatidylcholine at the plasma membrane [35], an asymmetric distribution of phosphatidylcholine/ceramide phosphocholinetransferase, could lead to increased brush-border sphingomyelin content. This in turn could result in a high content of cholesterol. We are presently investigating this possibility. The precursor product relationship between phosphatidylcholine and sphingomyelin may also explain the low relative turnover ratio observed for sphingomyelin.

To determine the factor or factors controlling this altered lipid composition, relative turnover studies were carried out. Although this method does not provide the absolute half-time ($t_{1/2}$) it permits comparison of different lipid components in the same membrane and between different membranes without having to analyze multiple time points in many different animals. However, this technique involves a number of assumptions which have been discussed previously by Arias et al. for proteins [2]. They include the following: The isotope is not metabolized into other compounds that would be measured; the synthesis rates are the same at the time both isotopes are administered; the labeled compound is in the process of isotopic decay; and the labeled compounds follow exponential

decay kinetics. Applying this type of study to phospholipids is difficult because of the differences in synthesis and degradation rates of individual membrane lipid components. For example, when both labels of one phospholipid class, i.e. phosphatidylcholine, may be in isotopic decay, this may not be true for another class of phospholipids, e.g. sphingomyelin. Therefore, we have met only the first two criteria for all the phospholipids. However, we feel the data can be used to compare the relative turnover rates of phospholipid classes between the brush-border and basolateral membranes.

The relative turnover data (Tables 5 and 6) presented in this paper indicate that individual phospholipid classes turnover at different rates. Whether this is due to altered synthesis or degradation cannot be determined from the data given. Also, in these studies plasmalogens were not separated from the phospholipids and could therefore alter the relative turnover rates of individual phospholipid classes. This may be of particular importance for ethanolamine plasmalogens since they are distributed asymmetrically and may have a slower rate of turnover than phosphatidylethanolamine [6].

These data do, however, suggest that phospholipids of different classes do not turn over as a unit within the membrane. The fact that the order of relative turnover of phospholipid classes did not change when either the polar headgroup (^{32}P) or fatty acid portion (acetate) was labeled suggests that individual phospholipid molecules are primarily degraded as a unit rather than by the selective removal or exchange of either fatty acids or the polar head groups.

The difference in relative turnover between the BBM and BLM is due to the different phospholipid compositions of the two membranes. The slow turnover of the brush-border membrane is primarily due to its high (51.3%) content of phospholipids with the slowest turnover: sphingomyelin and phosphatidylserine. The BLM, on the other hand, has a much higher content (70.7%) of the more rapid turning over phospholipids, phosphatidylcholine and phosphatidylethanolamine. Thus the slower turnover in the BBM compared to BLM is due to both an increase in sphingomyelin content as well as its strikingly slow turnover. The reason for the slower relative turnover of brush-border sphingomyelin is difficult to know, as the second label had not reached the isotopic decay phase. Therefore, either a change in synthesis or degradation could have caused the observed findings.

In summary, the data indicate that the lipid composition of the renal brush-border and basolateral membranes are quite different. Although the relative turnover of phospholipid species in each membrane differ only for sphingomyelin, the individual phospholipid classes are synthesized and degraded at markedly different rates.

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