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ISOLATION OF BASOLATERAL AND BRUSH-BORDER MEMBRANES FROM THE RABBIT KIDNEY CORTEX

VESICLE INTEGRITY AND MEMBRANE SIDEDNESS OF THE BASOLATERAL FRACTION

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A rapid and reproducible method has been developed for the simultaneous isolation of basolateral and brush-border membranes from the rabbit renal cortex. The basolateral membrane preparation was enriched 25-fold in $(Na^+ + K^+)$-ATPase and the brush-border membrane fraction was enriched 12-fold in alkaline phosphatase, whereas the amount of cross-contamination was low. Contamination of these preparations by mitochondria and lysosomes was minimal as indicated by the low specific activities of enzyme markers, i.e., succinate dehydrogenase and acid phosphatase. The basolateral fraction consisted of 35–50% sealed vesicles, as demonstrated by detergent (sodium dodecyl sulfate) activation of $(Na^+ + K^+)$-ATPase activity and [^{3}H]ouabain binding. The sidedness of the basolateral membranes was estimated from the latency of ouabain-sensitive $(Na^+ + K^+)$-ATPase activity assayed in the presence of gramicidin, which renders the vesicles permeable to Na^+ and K^+. These studies suggest that nearly 90% of the vesicles are in a right-side-out orientation.

Introduction

Renal and intestinal plasma membrane vesicles prepared by various methods have proven useful in the study of membrane phenomena at the luminal and antiluminal membrane surfaces. Such studies, however, rely on the isolation of these epithelial membranes and on their homogeneity with respect to tightness and orientation of the sealed vesicles. A variety of methods have been reported for isolating either brush-border membranes [1–4] or basolateral membranes [5–9], but there are few techniques for the simultaneous preparation of both types of membrane from a common, partly purified, membrane fraction [10–12]. Also, in contrast to brush-border preparations which consist mainly of sealed vesicles that have retained a right-side-out orientation, controversy persists regarding the orientation of the membrane of the basolateral vesicle relative to the orientation [9–11] of the same membrane in vivo.

In the present investigation, a procedure for isolating simultaneously basolateral and brush-border membranes, under identical conditions from the rabbit renal cortex is described. In addition, the present paper presents strong evidence that the basolateral plasma-membrane vesicles obtained by our procedure are sealed and mostly (90%) oriented right-side-out.

Abbreviations: Hepes, *N*-2-hydroxyethylpiperazine-*N'*-2-ethanesulfonic acid; SDS, sodium dodecyl sulfate; PMSF, phenylmethylsulfonyl fluoride.

Materials and Methods

Isolation of basolateral and brush-border membrane fractions

White male rabbits (1.5–2 kg in weight) were anesthetized with sodium pentobarbital. The kidneys were placed on a buffered sucrose medium (0.25 M sucrose/0.1 mM PMSF/2 mM Tris-Hepes (pH 7.4)). All subsequent steps were performed at 0°C. The kidneys were decapsulated, the cortex was dissected out, and minced.

Membrane fractions were prepared according to the diagram depicted in Fig. 1. The present method represented a modification of the technique of Scalera et al. [6] and Sacktor et al. [13] for the isolation of basolateral membranes from the rat kidney cortex, using a self-orienting Percoll gradient.

A partially purified membrane fraction (P_3), obtained after two centrifugation, contained predominantly basolateral and brush-border membranes (Table I). When this partially purified membrane fraction was subjected to centrifugation on a Percoll gradient, three major bands of turbidity were obtained (Fig. 1). The basolateral membrane (fraction F_1) formed an upper band in the Percoll gradient which is centered on the Pharmacia blue marker bead (density = 1.037 g/ml).

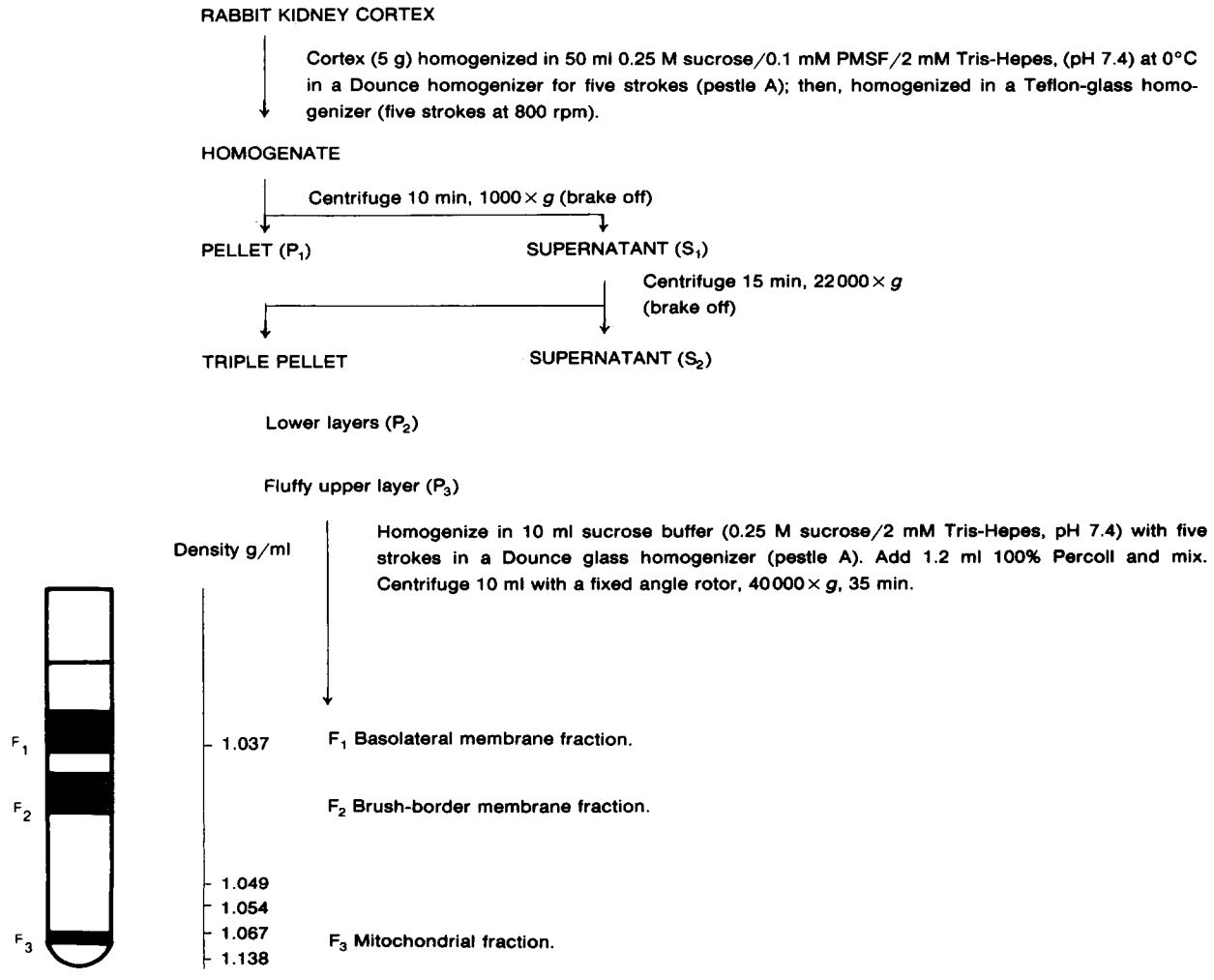

Fig. 1. Purification diagram for the preparation of basolateral and brush-border membranes.

TABLE I

ENZYMATIC ACTIVITIES OF FRACTIONS IN THE PURIFICATION OF THE RENAL CORTEX BASOLATERAL AND BRUSH-BORDER MEMBRANES.

Specific activities are expressed as μmol/h per mg protein. Number of preparations shown in parentheses. Recovery = recovery totalled over the entire fractionation procedure.

	Homogenate	$P_1 - P_2 + S_2$	P_3	F_1	F_2	Relative specific activity (enrichment)			
						P_3/H	F_1/H	F_2/H	
Protein (mg)	1154.1 ± 90.8 (7)	1073.4 ± 143.2	98.9 ± 33.4	5.51 ± 1.80	8.89 ± 1.46				
Recovery									(101.3 ± 11.4%)
$(Na^+ + K^+)$-ATPase									
Specific activity	2.33 ± 0.69 (7)	1.98 ± 0.43	6.07 ± 1.91	57.17 ± 10.93	3.55 ± 0.26	2.60	24.53	1.52	
Total activity	2680 ± 777	2128 ± 552	600 ± 284	309 ± 98	32.7 ± 6.78				
Recovery									(102.7 ± 14.0%)
Succinate dehydrogenase									
Specific activity	3.13 ± 0.66 (6)	3.34 ± 0.80	2.30 ± 0.49	0.93 ± 0.66	2.06 ± 0.68	0.73	0.29	0.66	
Total activity	3613 ± 700	3640 ± 712	244 ± 113	4.92 ± 2.8	17.82 ± 4.48				
Recovery									(108.1 ± 9.8%)
Alkaline phosphatase									
Specific activity	6.9 ± 1.90 (6)	5.25 ± 1.19	12.75 ± 7.23	13.53 ± 6.80	81.8 ± 19.83	1.85	1.96	11.85	
Total activity	8108 ± 2371	5770 ± 1779	1482 ± 1057	76 ± 43	770 ± 269				
Recovery									(88.5 ± 10.8%)
Acid phosphatase									
Specific activity	1.93 ± 0.75 (5)	2.07 ± 0.56	2.61 ± 1.28	3.12 ± 1.06	3.51 ± 0.97	1.35	1.61	1.82	
Total activity	2253 ± 1104	2227 ± 896	278 ± 54	16 ± 8	30.46 ± 5.57				
Recovery									(116.1 ± 14.27%)

The brush-border membranes (fraction F_2) formed a second discernable band having an extrapolated density of 1.041 g/ml. The mitochondrial fraction (F_3) comprised the lower band in the Percoll gradient banding at a density of 1.067 g/ml.

After centrifugation in the gradient, the membrane fractions were aspirated, diluted with 5 vol. 85 mM KCl/85 mM sucrose/2 mM Tris-Hepes (pH 7.4). Percoll was removed from each fraction by centrifugation at $60000 \times g$ for 15 min in an MSE high-speed 25, fixed-angle rotor. After centrifugation, Percoll had formed a glassy pellet on the sloping wall of the tube. The membranes were recovered at the bottom of the tube by adding 200–400 μl of a medium containing 100 mM KCl/100 mM sucrose/2 mM Tris-Hepes (pH 7.4).

Enzyme assays

Alkaline phosphatase was measured as in previous studies [14]. Succinate dehydrogenase was assayed using the method of Pennington [15], as modified [16]. Acid phosphatase was measured by Trouet's method [17].

For ATPase assays, fractions (3–50 μg protein in 10 μl) were added to 750 μl of temperature-equilibrated (37°C) assay medium containing 150 mM NaCl, 10 mM KCl, 8 mM Na_2ATP, 8 mM $MgCl_2$, 1 mM H_4EDTA, 30 mM Tris-HCl and [γ-^{32}P]ATP in tracer amounts, with or without 1 mM ouabain. The pH of these solutions was adjusted to 7.4 by Tris. After incubation for 10–30 min at 37°C the reaction was stopped by addition of 400 μl of a 10% activated charcoal suspension in 1 M HCl and by placement on ice. Charcoal was used to absorb non-cleaved ATP [18,19] and the liberated ^{32}P was separated by centrifugation for 5 min at 5000 rpm in a refrigerated centrifuge. The radioactivity of the supernatants was determined by liquid scintillation counting.

For membrane sidedness studies, basolateral membrane samples (usually 50 to 100 μg in 10 μl) were preincubated for 20 min at 37°C with either 800 μl of a medium comprising 100 mM KCl/100 mM sucrose/2 mM Tris-Hepes (control) or with 800 μl of the same medium containing 10–60 μg of SDS (SDS-activated sample). After preincubation, the reaction was started by the addition of 40-μl aliquots of the control and SDS-activated samples to 750 μl of temperature-equilibrated (37°C) assay medium. Incubations were carried out for 20 min at 37°C. ATPase activity was measured as noted above, except that assay media were modified by replacing 50 mM NaCl by 100 mM sucrose. The presence of sucrose increased the blank values (incubations performed without tissue fractions) about 5-fold. Protein was determined by the method of Lowry et al. [20].

Ouabain binding

Binding of ouabain by basolateral membranes was measured by a Millipore filtration technique. The basolateral samples (50–100 μg protein in 10 μl) were preincubated for 20 min at 37°C with or without SDS exactly as noted above for ATPase assays. Aliquots (20 μl containing 2–5 μg protein) of control and SDS-activated samples were then incubated for 20 min at 37°C in 150 μl medium comprising 150 mM NaCl/8 mM Na_2ATP/8 mM $MgCl_2$/20 mM Tris-Hepes (pH 7.4)/75 nM [^{3}H]ouabain ($2 \cdot 10^6$ cpm/ml). Incubations were terminated by the addition of 0.8 ml of an ice-cold solution comprising 150 mM NaCl/2 mM Tris-Hepes (pH 7.4) (wash buffer), followed by filtration through Millipore filters (HAMK, 0.45 μM) and by washing the filters with 5 ml of cold wash buffer. The radioactivity remaining in the filters was measured in 5 ml aquasol using a SL 4000 Intertechnique liquid scintillation counter. The results are expressed as 'specific binding', i.e., total binding of [^{3}H]ouabain minus binding obtained in the presence of 100 μM unlabelled ouabain measured in parallel experiments.

Chemicals

The [γ-^{32}P]ATP (2–10 Ci/mmol) and [^{3}H]ouabain (10–20 Ci/mmol) were obtained from New England Nuclear. Percoll and density marker beads were from Pharmacia (Uppsala, Sweden), and SDS was from BDH Chemicals Ltd. (Poole, U.K.). Ouabain, Na_2ATP (A-3377), phenylmethylsulfonyl fluoride and gramicidin were from Sigma Chemical Co. (St. Louis, MO, U.S.A.). As gramicidin was added to 90% ethanol, control suspensions received equivalent volumes of ethanol. The final concentration of ethanol was 2.4%.

Statistical analysis was performed by the group t-test. All results are expressed as mean ± S.D.

Results

Purity of the membrane preparations

The procedure outlined in Fig. 1 was developed to isolate simultaneously the antiluminal and the luminal membranes of rabbit renal cortex.

The purity of the membrane preparations was assessed by the activities of marker enzymes. The relevant data are summarized in Table I.

The band containing basolateral membranes (F_1) was enriched 25-fold in ($Na^+ + K^+$)-ATPase over the homogenate. The purity of the basolateral fraction was also evaluated by assays of other marker enzymes. The enrichment factor for succinate dehydrogenase, a mitochondrial marker, was only 0.3. There was some contamination by acid phosphatase (lysosomal marker), and alkaline phosphatase (brush-border marker) with enrichment factors for 1.6 and 1.9, respectively. However, yields for acid phosphatase and alkaline phosphatase activities in the F_1 fraction averaged 0.7 and 0.9% of their respective homogenate values, in contrast to 12% for the yield of ($Na^+ + K^+$)-ATPase.

The band containing brush-border membranes (F_2) was enriched 12-fold in alkaline phosphatase and comprised 9.5% of the total homogenate activity. By contrast, yields for succinate dehydrogenase, ($Na^+ + K^+$)-ATPase and acid phosphatase averaged 0.5, 1.7 and 1.2% of their respectively homogenate values.

The band containing mitochondria (F_3) was not analyzed in detail; the enrichment factor for succinate dehydrogenase was about 4-fold.

Estimate of orientation of basolateral membrane vesicles

The estimate of sidedness exploits the facts that ATP and ouabain diffuse poorly across the membrane and have opposite sidedness of action [21,22]. Ouabain binds only on the external surface of the membrane, whereas the ATP catalytic site is on the cytoplasmic surface. Thus, in sealed, right-side-out vesicles, no ATPase activity would be measured, whereas this activity would be expressed in inside-out-vesicles but would be unaffected by ouabain. On the other hand, ouabain-sensitive ($Na^+ + K^+$)-ATPase activity should be fully expressed in sheets or leaky vesicles.

As shown in Fig. 2, the latent activity of ($Na^+ + K^+$)-ATPase can be expressed when the basolateral membrane vesicles were rendered permeable to ATP by treatment with SDS. The specific activity of ($Na^+ + K^+$)-ATPase was increased approx. 60%, for SDS-treated membrane protein ratios ranging from 0.32 to 0.43, while at higher ratios SDS decreased the specific activity. On the other hand, SDS caused a gradual decrease of the specific activity of the ATPase, as measured in the presence of ouabain (Fig. 2).

The percentage of sealed vesicles was estimated from the degree of detergent activation (Table II). For example, it can be calculated from experiment 1 in Table II that approx. 59% ((38.6/65.4) × 100) of the vesicles were leaky. The difference from 100% gives the percentage of sealed vesicles (41%). The orientation of the basolateral vesicles was estimated from the degree of ouabain inhibition of ATPase in controls and SDS-pretreated membranes. Total ATPase in control membranes re-

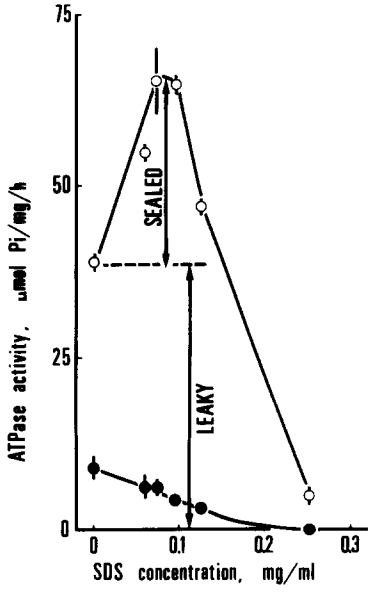

Fig. 2. The concentration dependence of the activation of ATPase by SDS. The basolateral membranes (0.23 mg/ml) were pretreated for 20 min at 37°C with inreasing concentrations of SDS. Subsequently, the ATPase activity was assayed in absence of ouabain, or in the presence of, 1 mM ouabain. The ($Na^+ + K^+$)-ATPase values (○) were obtained by subtracting the values obtained in the presence of ouabain (●) from the total ATPase values. Means ± S.D., $n = 4$.

TABLE II

ESTIMATION OF LEAKINESS AND MEMBRANE ORIENTATION OF BASOLATERAL MEMBRANE VESICLES

Total and $(Na^+ + K^+)$-ATPase activities are expressed as μmol P_i produced/h per mg protein, means $\pm$ S.D. $n = 4$.

Expt.		Control	SDS [a]	Leaky [b]	Vesicles	
					Inside-out [c] (%)	Right-side-out (%)
1	Total ATPase	45.0 $\pm$ 2.10	72.1 $\pm$ 1.26			
	$(Na^+ + K^+)$-ATPase	38.6 $\pm$ 2.12	65.4 $\pm$ 1.29	59	4	37
2	Total ATPase	59.8 $\pm$ 1.79	81.2 $\pm$ 3.07			
	$(Na^+ + K^+)$-ATPase	52.9 $\pm$ 1.97	74.3 $\pm$ 2.82	71	2	27
3	Total ATPase	75.2 $\pm$ 1.06	100.9 $\pm$ 1.91			
	$(Na^+ + K^+)$-ATPase	66.9 $\pm$ 1.07	92.7 $\pm$ 3.36	72	2	26

[a] Measured after preincubation for 20 min at 37°C with SDS (0.4 mg/ml protein).

[b] Percentage of leaky vesicles = [$(Na^+ + K^+)$-ATPase activity (control)/$(Na^+ + K^+)$-ATPase activity (SDS-pretreated)] $\times$ 100.

[c] See text for estimation of membrane sidedness.

flects the activity of both ouabain-sensitive and ouabain-insensitive ATPase present in leaky and inside-out vesicles. Since $(Na^+ + K^+)$-ATPase represented 91% ((65.4/72.1) $\times$ 100) of the total activity of the basolateral fraction, the total ATPase of inside-out vesicles would be 45.0 minus 42.4 ((38.6/91) $\times$ 100) i.e., the total ATPase of leaky vesicles. This difference (2.5), which represents 3.6% of the total ATPase, estimated the sealed inside-out vesicles. From the difference in the amount of sealed vesicles (41%) it can be estimated that 37% of the plasma-membrane preparations consisted of sealed right-side-out vesicles. From the three experiments in Table II, it can be estimated that, of the population of sealed vesicles, 92% were right-side-out and 8% inside-out.

If the inside-out vesicles respond to external ATP by generating an electrochemical gradient, it would be expected that ATP hydrolysis would be decreased, thereby minimizing the number of inside-out vesicles actually present in the preparation. That this is the case is summarized in Table III. Treatment of basolateral vesicles with gramicidin, an ionophore which induces equilibration of cations, increased the ATPase activity measured in the presence of ouabain by 40% ($P < 0.05$) and 60% ($P < 0.01$), compared to controls and SDS-treated membranes, respectively. The degree of detergent activation of gramicidin-treated vesicles (25%) gives an estimate of the right-side-out vesicles. On the other hand, the decrease in the ATPase activity measured in the presence of ouabain in SDS-treated samples as compared to gramicidin-treated samples (3.5%) is a measure of the sealed inside-out plasma membrane vesicles. Thus, the remaining activity (71.5%) estimated the leaky vesicles. From these experiments it can be estimated that 88% of the vesicles are oriented right-side-out whereas 12% are oriented inside-out, corresponding to a 50% increase in the amount of inside-out vesicles as compared to experiments performed without gramicidin (Table II).

[^{3}H]Ouabain binding was used as a further

TABLE III

THE EFFECT OF GRAMICIDIN AND SDS ON ATPase ACTIVITY IN THREE DIFFERENT PREPARATIONS OF BASOLATERAL MEMBRANES

Basolateral membranes were preatred with either SDS (1 μg/2.5 μg membrane protein) or gramicidin (1 μg/2.5 μg membrane protein) for 20 min at 37°C. The maximal value for total ATPase in the different membrane preparations averaged 81.94 μmol P_i/mg per h. This value is designated as having a relatively activity of 100%.

Pretreatment	ATPase activity (% of maximal)	
	no ouabain	plus ouabain
None, control	66.33 $\pm$ 7.37	6.15 $\pm$ 1.63
Gramicidin	76.35 $\pm$ 5.14	8.57 $\pm$ 0.60
SDS	100	5.22 $\pm$ 1.13

approach for the quantification of sealed versus leaky vesicles. In these experiments Na^+, Mg^{2+} and ATP were used to support ouabain binding. Under these conditions, it would be expected that ouabain should not bind to sealed vesicles, regardless of the orientation of the vesicles, since ouabain binds at the external or plasma side of the membrane if ATP is present on the opposite membrane face [23]. Thus, ouabain will bind only to leaky or broken vesicles, while the latent binding capacity of both types of sealed vesicle would be expressed by pretreatment of the basolateral fraction with SDS, which makes the vesicles leaky for ATP and ouabain. With three different membrane preparations it was found that SDS increased [^{3}H]ouabain binding by about 2-fold (from 7.16 to 13.87 pmol/mg per 20 min). This enhanced activity (48.3%) estimated the sealed vesicles. By difference from 100%, approx. 52% of the vesicles were estimated to be leaky. On the other hand, quantitation of sealed versus leaky vesicles using the latency of $(Na^+ + K^+)$-ATPase gives a higher percentage of leaky vesicles ($65.36 \pm 8.15\%$). For each paired experiment, this percentage of leaky vesicles was significantly higher ($P < 0.05$) by the criterion of ATPase assay than by the criterion of [^{3}H]ouabain binding.

Discussion

The present results demonstrate that centrifugation of partially purified membranes from the rabbit renal cortex on a Percoll gradient permits not only the obtainment of basolateral membranes, as recently reported [5,6,13], but also of brush-border membranes. This was achieved by increasing the density of the Percoll gradient and by using centrifuge tubes with a high length/volume ratio. With these modifications, the resolution capacity of the gradient is greatly increased.

The main advantages of this method are its simplicity and its rapidity (requiring less than 3 h for completion). In one run on a Percoll gradient, both types of membrane form discernable bands that can be recovered immediately from the gradient, at the top, without requiring the fractionation of the gradients by displacement and collection of the fractions of interest.

For the basolateral membrane fraction (fraction 1), the enhancement of the specific activity of $(Na^+ + K^+)$-ATPase averaged 25, which is at the upper range (11–22) of enhancement obtained by other methods (7–10), as well as with the range (7–20) of purification obtained with a procedure which used a self-generating Percoll gradient [5,6,11,13]. In terms of enzyme recoveries, the yield of the basolateral membranes (12%) is in the upper range of the yields (5–13%) already reported [5–11]. Moreover, other marker enzymes tested as contaminants, succinodehydrogenase, alkaline phosphatase and acid phosphatase, had only 0.13, 0.9 and 0.7% of their homogenate activities represented in the F_1 fraction. These values are approx. 3-times lower than those reported by Sacktor et al. [13] for basolateral membranes obtained by a method using a Percoll gradient.

The brush-border membrane (fraction 2) is enriched 12-times in the luminal marker alkaline phosphatase. This fraction is slightly contaminated by mitochondria, basolateral membranes and lysosomes as indicated by low activities of marker enzymes.

Recently, Sacktor et al. [13] and Mamelok et al. [24] reported isolation of both basolateral and brush-border membranes on the same gradient of Percoll. The Brush-border fraction obtained by Sacktor et al. [13], however, showed substantial cross contamination with basolateral membranes, since the preparation was enriched approximately to the same degree in luminal (4.5-fold) and in basolateral marker (4-fold).

The specific activities of $(Na^+ + K^+)$-ATPase in the basolateral membrane fraction and of alkaline phosphatase in the brush-border membrane fraction obtained by Mamelok et al. [24] were increased only 7- and 5-fold, respectively, over the homogenate values. Moreover, data on cross-contamination and contamination by mitochondria and lysosomes were not reported.

Results presented in this paper demonstrate that the basolateral membrane preparation consists, in part, of sealed vesicles which are mostly (90%) in a right-side-out orientation.

In the present study, two approaches were used to quantity the amount of sealed vesicles. The method using the latency of [^{3}H]ouabain binding indicates that nearly 50% of the membrane pre-

paration consisted of sealed vesicles, whereas this percentage averaged only 35% using the latency of $(Na^+ + K^+)$-ATPase. Using these two approaches, Forbush [25] recently observed a similar difference in the estimation of sealed versus leaky vesicles in different membrane populations isolated from the outer medulla of the dog kidney. The possibility exists that the method using the latency of $(Na^+ + K^+)$-ATPase underestimated the percentage of sealed vesicles, if some external ATP enters the interior space of right-side-out vesicles. Accordingly intravesicular ATP would be rapidly hydrolyzed, due to the relatively high concentration of ATPase, before binding of [^{3}H]ouabain could occur to the outside of right-side-out vesicles.

The membrane sidedness has been evaluated by measurement of the ouabain-sensitive $(Na^+ + K^+)$-ATPase activity with and without detergent pretreatment. With this method, it was found that 92% of the vesicles are sealed and right-side-out, whereas only 8% of the vesicles are sealed and inside-out. It is shown here, however, that this approach underestimates by about 50% the percentage of inside-out vesicles. Indeed, by the criterion of ATPase assay, the ATPase activity of inside-out vesicles would be limited by the generation of Na^+ gradient [26]. In the presence of gramicidin, an ionophore that induces alkali ion permeability, the ouabain-insensitive ATPase of the basolateral preparation is significantly enhanced. From the gramicidin experiments, it can be estimated as an upper limit that 12% of the vesicles are sealed and inside-out.

Kinsella et al. [10] have previously shown that basolateral membrane vesicles isolated from the cortex of the dog kidney by a method involving selective precipitation by Ca^{2+} and differential and sucrose density centrifugations, were mostly (77%) right-side-out. By contrast, Del Castillo and Robinson [11] recently reported that the basolateral membrane fraction isolated from the guinea-pig intestine by differential and Percoll centrifugations consisted of 40% of unsealed vesicles and 60% of sealed and inside-out vesicles. Such a conclusion was based on a 60% enhancement by detergent in both $(Na^+ + K^+)$-ATPase activity and [^{3}H]ouabain binding. Taken alone, however, the increment in $(Na^+ + K^+)$-ATPase activity or [^{3}H]ouabain binding gives only an estimate of the percentage of sealed vesicles, and cannot be used as an index of membrane sidedness. Recalculation of the data from Del Castillo and Robinson [11] taking into account the degree of ouabain inactivation of the ATPase, indicated that their preparation contained approx. 44% unsealed vesicles, 37% sealed and right-side-out vesicles and only 19% sealed and inside-out-vesicles. Also, that the activation by detergent of [^{3}H]ouabain binding supported by ATP, Mg^{2+} and Na^+ cannot be equated, as proposed [11], to the latent binding capacity of inside-out vesicles, is supported by recent studies [25] using basolateral membranes isolated from dog kidney. In these experiments, Forbush [25] demonstrated that the increment of $(Na^+ + K^+)$-ATPase activity and ouabain binding exposed by detergent were unaffected by trypsin which degraded $(Na^+ + K^+)$-ATPase only at the cytoplasmic face of the plasma membrane [27], indicating that most (95%) of the vesicles were oriented right-side-out.

In summary, a rapid and reproducible method is described for isolating simultaneous, under identical conditions, luminal and antiluminal membranes from the rabbit renal cortex. The sidedness of the basolateral preparation has been characterized and found to be mostly right-side-out, and is thus especially suitable for identifying secondary active transport pathways.

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