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Differential properties of brush-border membrane vesicles from early and late proximal tubules of rat kidney

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Abstract

We describe the preparation and properties of BBM vesicles (BBMV) from the superficial and juxtamedullary rat renal cortex using Ca^{2+}-precipitation method and/or by density gradient centrifugation. BBMV were characterized by the presence of BBM marker enzymes as distributed along microdissected proximal convoluted tubule and proximal straight tubules from superficial and juxtamedullary cortex. In tubules from both superficial and juxtamedullary cortex, the activities of γ-glutamyltransferase and leucine aminopeptidase were 5–10 times higher in proximal straight tubules than in proximal convoluted tubule. The alkaline phosphatase was higher in proximal convoluted tubules than in straight tubules from superficial cortex, but it was lower in proximal convoluted than straight tubules from the juxtamedullary cortex. The Na^+/P_i cotransport had higher V_{max} and lower K_m in BBMV from superficial cortex than from BBMV from juxtamedullary tissue. BBMV from superficial cortex separated on Percoll gradient showed a high activity of alkaline phosphatase and low activities of γ-glutamyltransferase and leucine aminopeptidase. Conversely, BBM from juxtamedullary cortex separated into a major peak with very high activities of γ-glutamyltransferase and leucine aminopeptidase, and lesser activity of alkaline phosphatase. These distinct BBMV fractions showed diverse Na^+/P_i cotransport properties and BBM marker enzyme distributions. Thus, using the outlined methodology it is feasible to prepare BBMV derived predominantly from proximal convoluted tubules or from proximal straight tubules located in either superficial or deep cortical nephrons.

Key words: Proximal tubule; Brush-border membrane; Sodium cotransport; Phosphate transport; (Superficial rat renal cortex); (Juxtamedullary rat renal cortex)

1. Introduction

Major subsegments of proximal tubules of mammalian nephron differ in ultrastructure [1], function [2], as well as in biochemical and metabolic properties [3,4]. The biochemical and ultrastructural basis and the functional significance of this axial heterogeneity of proximal tubules has not yet been clarified. Concerning the reabsorption lumen-to-interstitium transport of solutes across tubular epithelium, the transport properties of luminal brush-border membrane (BBM) play an important and often thermodynamically determining role [5] in the uptake of solutes from tubular fluid; many ultrafiltered via the solute-specific secondary active Na^+-gradient-energized cotransport systems [5]. The proximal tubules originating from glomeruli of superficial (outer) layers of cortex of mammalian kidney, differ in their properties from those originating in deep (juxtamedullary) glomeruli. Yet, in the great majority of studies of transport properties of BBM using BBM vesicles (BBMV) methodology were or are conducted on BBMV simply prepared from the cortical tissue.

In previous studies we [6,7] and other investigators [8–10,34,35] observed that BBMV isolated from different zones of kidney parenchyma, e.g., cortex, and outer stripe of red medulla (which contain proximal straight tubule) do differ in the transport properties [6–10]. Moreover, Na^+-coupled cotransport systems in BBMV isolated from distinct renal tissue zones differ in regu-

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lation by hormones [7,8,11] or by metabolic stimuli [6,8].

In the present study we investigated how BBMV prepared from the superficial cortex (BBMV-superficial cortical tissue) and those prepared from the juxtamedullary renal tissue zone, i.e., a tissue consisting of juxtamedullary cortex and outer stripe of outer medulla [7] of rat kidney (BBMV-juxtamedullary tissue), differ in some basic parameters, such specific activities in BBM enzymes, polypeptide composition, which could be characteristic to distinguish the major between subsegments, i.e., early proximal convoluted portions and late ndash; mainly straight portions of proximal straight tubules of nephrons originating either in glomeruli located in the outer – i.e., superficial – cortex or from glomeruli located in the juxtamedullary cortex.

Further, we explored the possibility that BBMV initially isolated by preparative approaches currently most frequently employed, i.e., by divalentions (Ca^{2+}, Mg^{2+}, or Mn^{2+}) precipitation procedure [12], and subsequently separated by differential and density gradient centrifugation [13], may be enriched predominantly in BBMV originating from proximal convoluted tubule and proximal straight tubule of superficial nephrons and those originating in deep (juxtamedullary) nephrons.

Collectively, the results of our present studies indicate that in preparation of BBMV from the superficial cortex the BBMV from proximal convoluted tubule segments indeed predominate, whereas the BBMV prepared from juxtamedullary tissue are enriched mainly in BBM from segments of proximal straight tubules. Furthermore, similar or even more distinct separation could be achieved by combining the cation precipitation method with subsequent density gradient centrifugation procedure.

2. Materials and methods

Experiments were performed on kidneys removed under ether anesthesia from adult male albino rats weighing 250 g, maintained on regular ad libitum intake of normal (0.7%) P_i diet and ad libitum drinking water.

Dissection of renal tissue

Brush-border membrane (BBMV) vesicles were prepared either from superficial cortex (BBMV-superficial cortical tissue) or from juxtamedullary zone of the renal cortex plus adjacent medulla (BBMV-juxtamedullary tissue). The cortical part of the rat kidney was cut by razor blade in the middle of the cortical thickness (Fig. 1) between the kidney surface and the corticomedullary junction, i.e., superficial cortex denoted 'superficial cortical tissue' [7]. The juxtamedullary part

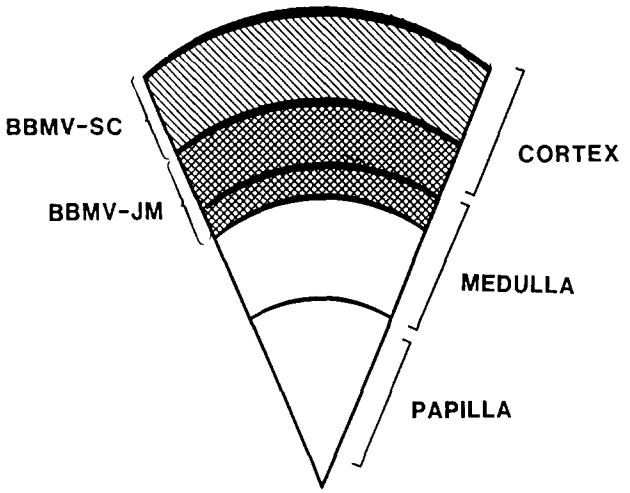

Fig. 1. Scheme of macroscopic subdivisions of rat kidney parenchyma employed as a starting material for preparation of BBMV from *superficial cortex* (hatched;, BBMV-superficial cortical tissue) or from inner cortex plus outer stripe of outer medulla, juxtamedullary zone (cross-hatched, BBMV-juxtamedullary tissue).

of the cortex was carefully separated from superficial cortex. The outer cortical zone, 'superfical cortical tissue', was used as a starting material. Since, in rat kidney nephrons straight portions of the proximal tubules dip beyond the cortical medullary junction [14] into the outermost layer of adjacent medulla (outer stripe of outer medulla), this tissue was included to juxtamedullary cortical tissue (Fig. 1). In further text, we refer to this renal tissue zone as 'juxtamedullary tissue.'

Preparation of BBMV by the Ca^{2+}-precipitation technique

For preparation of membrane vesicles we employed the same Ca_i^{2+}-precipitation method, as in our preceding study on transport heterogeneity in membranes from two kidney zones [7]. The superficial cortical or juxtamedullary tissues were homogenized and BBMV were prepared by Ca^{2+}-precipitation technique as in our previous studies [6,7,15]. BBMV from the superficial cortical tissue are referred to as 'BBMV-superfical cortical tissue' and those from juxtamedullary tissue as 'BBMV-juxtamedullary tissue' [7,11]. When compared, BBMV-superfical cortical tissue and BBMV-juxtamedullary tissues were always prepared simultaneously from the same kidneys and also analyzed simultaneously.

Preparation of specific BBMV by differential and gradient centrifugation

In experiments where BBMV from superfical cortical tissue and juxtamedullary tissue were isolated by differential centrifugation combined with density cen-

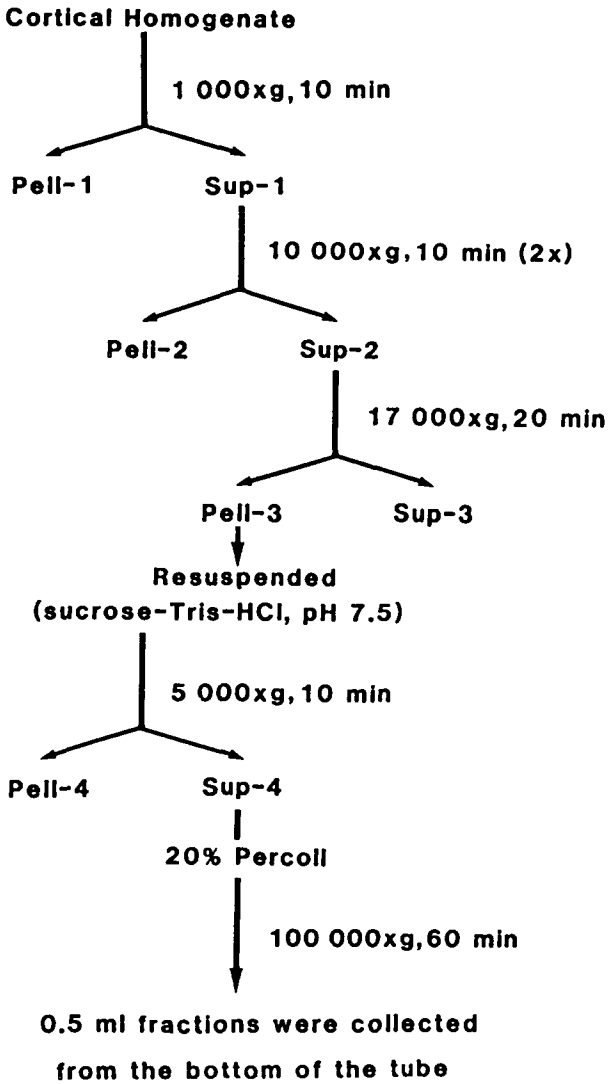

Fig. 2. Fractionation scheme for preparation of BBMV from rat kidney by combination of differential and density gradient (Percoll) centrifugation procedure. The starting material, cortical homogenate, was either tissue from the superficial cortex (for BBMV-superficial cortical tissue) or juxtamedullary zone tissue (for BBMV-juxtamedullary tissue), as defined on the scheme in Fig. 1.

trifugation in the Percoll-gradient, the superfical cortical tissue and juxtamedullary tissues were first homogenized by a Polytron homogenizer in a medium containing 250 mM sucrose and 10 mM Tris-HCl (pH 7.4) by 5 s bursts, repeated four times with 10 s intervals. BBMV fractions were then isolated by differential centrifugation according to the following procedure described in principle by Hittleman et al. [13,16] and outlined on the scheme in Fig. 2.

First, the homogenate (10% wt/vol) was centrifuged at $1000 \times g$ for 10 min. The pellet was washed once, and the resulting supernatants (Sup-1) were centrifuged at $10000 \times g$ for 10 min. The $10000 \times g$ pellet was washed once and this step was repeated one more time and the combined supernatants (Sup-2) were centrifuged at $17000 \times g$ for 20 min. The final pellet (Pell-3) was suspended in 5–6 ml of a medium containing 250 mM sucrose, 10 μM Tris-HCl (pH 7.4) and treated with 15 strokes of a tight-fitting Teflon-glass homogenizer. The resulting suspension was aspirated 3 times through a 22-gauge needle and centrifuged at $5000 \times g$ for 10 min to remove any remaining aggregates.

The remaining suspension of BBM fractions (Sup-4) were further separated by Percoll gradient centrifugation as outlined by Mamelok et al. [13]. A 5 ml suspension of the Sup-4 membrane fraction, prepared as described above, was mixed with 8 ml of Percoll (Sigma) and 27 ml of buffer to give a final Percoll concentration of 20% (v/v). 38 ml of the membrane-Percoll mixture was ultracentrifuged in a Beckman 60 Ti Beckman angle-rotor and the density-gradient fractions formed were recovered from the bottom of the cuvettes by immersing a stainless steel capillary tube connected through a peristaltic pump, and 0.5 ml fractions were sequentially collected. The densities of the Percoll-gradient fractions, total of about 45 fractions of densities ranging from 1.08 to 1.03 (Fig. 3, upper panel), were calculated from the refractive indexes which were measured with a Bausch and Lomb refractometer. The superfical cortical and the juxtamedullary tissue were processed simultaneously in parallel from the same kidneys, to allow direct comparison.

First, the BBM enzyme activities, as well as protein contents were determined in the individual fractions. Then, to measure uptake of solutes, fractions were pooled from the two major zones (Fig. 3, upper panel) of gradient designated in preliminary experiments as *'zone A'*, i.e., gradient fractions Nos. 13–18 (in the higher density range) and *'zone B'*, i.e., gradient fractions Nos. 22–28 in the lower density range (Fig. 3). Percoll was washed out from the fractions by resuspension and BBMV were collected by subsequent recentrifugation in a medium containing 300 mM mannitol buffered with 5 mM Tris-Hepes to pH 8.5.

The uptake of solutes by BBMVs prepared either by the Ca^{2+}-precipitation method, or by the differential and gradient centrifugation method was measured by the rapid filtration technique as described in our earlier studies [6–8,15].

Enzyme activities of alkaline phosphatase, γ-glutamyltransferase, leucine aminopeptidase and maltase were determined (in triplicates) using colorimetric methods used and described in our previous studies [6,7,15]. Activities of Na^+/K^+-ATPase and succinate dehydrogenase were assayed as described in our previous reports [17–19]. The protein content in homogenates and fractions of homogenates was determined, as in our previous studies [6,7] by Lowry's method [20].

Studies on microdissected subsegments of proximal tubules in proximal convoluted tubules and in proximal straight tubules

The rat kidney was prepared for microdissection of tubule segments in a way outlined in detail in our previous studies [21,22]. Proximal convoluted tubules and proximal straight tubule segments dissected either from superfical cortical tissue or from juxtamedullary tissue were identified by established criteria, mainly by typical ultra structural appearance and attachment to adjacent nephron segments [21–23]. Accumulated collected samples of specific segments, proximal convoluted tubules and proximal straight tubules were frozen on solid CO_2 and stored at −80°C prior to determination of enzyme activities and/or protein content. Samples of proximal convoluted tubules or proximal straight tubules were thawed and homogenized in Radnotti microhomogenizers [24] and aliquots of homogenates were used for determination of protein content and enzyme activities of the four BBM enzymes: alkaline phosphatase, γ-glutamyltransferase, leucine aminopeptidase, and maltase.

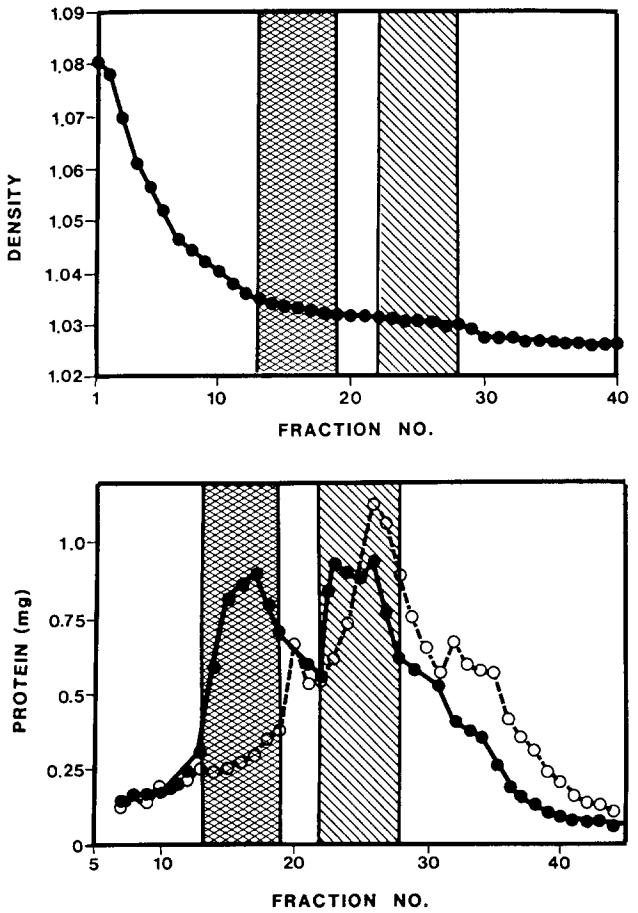

Fig. 3. Upper panel: Profile of the Percoll-density gradient used for the final step in the preparation procedure for BBMV portrayed in Fig. 2. Fractions 13–18 are designated in the text as '*zone A*' (cross-hatched), and fractions 22–28, as 'zone B' (hatched). Fractions from these two regions of the gradient are depicted, in all subsequent figures, as 'zone A,' or 'zone B'. Lower panel: Distribution of protein in BBMV fractions separated on Percoll-density gradient. ○———○, BBMV preparations from superficial cortical tissue (BBMV-superficial cortical tissue); ●———●, preparation from the juxtamedullary tissue (BBMV-juxtamedullary tissue).

The activities of enzymes were determined using colorimetric methods similar to those used for assays of the membrane fractions, except that the volumes were proportionally scaled down at least 10 times, to allow for use of a small quantity of microdissected tubule homogenate material [24–26]. All four enzyme activities, alkaline phosphatase, γ-glutamyltransferase, leucine aminopeptidase, and maltase were always assayed simultaneously in homogenates from proximal convoluted tubules and proximal straight tubules from both zones, i.e., superfical cortical tissue zone and juxtamedullary zone, obtained by dissection of the tissue from the same kidney. The enzyme activities in samples of microdissected tubules were determined in duplicates or triplicates.

Analysis of BBM for protein composition

The protein composition of BBMV, prepared either by Ca^{2+}-precipitation method or by gradient centrifugation method were evaluated by sodium dodecyl sulfate (SDS)-polyacrylamide gel electrophoresis (SDS-PAGE), in principle according to the method of Fairbanks et al. [32] and Laemmli [33] on slab gels. All samples, BBMV and standards for apparent molecular weight (M_r) were boiled for 3 min in a solution containing 1% SDS, 30% sucrose, 40 mM dithiothreitol, 1 mM EDTA, 1 mM Tris-HCl (pH 8.0) and 0.05% Bromophenol blue tracking dye. Samples (40 μl) were applied on top of gel plate and subjected to electrophoresis on a vertical straight (18 cm × 18 cm) slab gel (7% polyacrylamide, 1.5 mm thickness) and run in a Bio-Rad apparatus. The electrophoretic running buffer (pH = 7.5) was composed of 40 mM Tris-base, 20 mM Na^+ acetate, 2 mM EDTA and 0.2% SDS. Electrophoresis was run at a constant current of 100 mA. For polypeptide analysis gels were fixed in 30% isopropanol and stained for proteins by Coomassie blue [37]. The density of hands-on stained gels were evaluated by Cliniscan (Helena Labs) densitometer.

Unless specified otherwise, all experiments described in the Results section were repeated at least two or three times to document reproducibility. When appropriate, statistical evaluation was used and conducted employing Student's *t*-test.

Materials

[^{32}P]P_i and L-[^{3}H]proline were purchased from New England Nuclear, Percoll, colorimetric substrates for enzyme assays and other biochemicals, all of the highest purity grades available, were purchased from Sigma, St. Louis, MO, or other standard suppliers.

3. Results

BBM activities in subsegments of microdissected proximal tubules

The activities of BBM enzymes determined in proximal convoluted tubules or proximal straight tubules microdissected from either superficial cortical tissue or juxtamedullary tissues, defined in Fig. 1 are summarized in Table 1. Proximal convoluted tubules differed from proximal straight tubules most distinctly in activities of γ-glutamyltransferase. The activity of this enzyme in proximal straight tubules was markedly higher than in proximal convoluted tubules (Table 1) and this difference was greater pronounced in proximal straight tubule and proximal convoluted tubule segments dissected from juxtamedullary tissue (13 ×) than in segments from superfical cortical tissue (5 ×). Similarly, the activity of leucine aminopeptidase was distinctly greater (about 3 ×) in proximal straight tubule than in proximal convoluted tubule, both in superficial cortical tissue and in juxtamedullary tissue. The activity of alkaline phosphatase was slightly lower in proximal straight tubules than in proximal convoluted tubules in superficial cortex, but controversely, higher in proximal straight tubules than in proximal convoluted tubules of juxtamedullary tissue. The opposite pattern was found for maltase (Table 1): in the superfical cortical tissue, the activity was higher in proximal straight tubules than in proximal convoluted tubules, wherease the maltase activites were higher in proximal convoluted tubules than in proximal straight tubules in juxtamedullary tissue. These results show that quantitatively distinct and consistent differences exist in BBM enzyme activites between proximal convoluted tubules and proximal straight tubules, namely in the case of γ-glutamyltransferase, and leucine aminopeptidase in both superfical cortical and juxtamedullary tissue, but activites of alkaline phosphatase and maltase have a different pattern between juxtamedullary and superfical cortical tissue.

Studies on BBMV-superfical cortical tissue and BBMV-juxtamedullary tissue prepared by Ca^{2+}-precipitation method

The activities of γ-glutamyltransferase, leucine aminopeptidase and alkaline phosphatase (Table 2) were increased in BBMV compared to homogenate (enrichment) more than 10 times both in BBMV-super-

Table 1
The activities of BBM enzymes in microdissected subsegments of rat renal proximal tubules

	γ-Glutamyl transferase	Leucine aminopeptidase	Alkaline phosphatase	Maltase
(A) Superficial cortex				
Proximal convoluted tubule	19.5 ± 2.0 * (2)	0.78 ± 0.28 (2)	9.9 ± 2.2 (2)	48.4 ± 3.4 (2)
Proximal straight tubule	107.0 ± 3.10 (3) (5.5 ×) **	2.51 ± 1.03 (3) (3.2 ×)	6.9 ± 0.7 (3) (0.7 ×)	76.0 ± 7.0 (2) (1.6 ×)
(B) Juxtamedullary tissue				
Proximal convoluted tubule	18.9 ± 5.2 (2)	1.96 ± 0.56 (3)	6.1 ± 1.9 (2)	85.0 ± 2.0 (2)
Proximal straight tubule	252.0 ± 73.0 (3) (13.3 ×) **	5.89 ± 2.2 (3) (3.0 ×)	15.3 ± 1.0 (3) (2.5 ×)	48.0 ± 1.0 (2) (0.56 ×)

The proximal (convoluted tubules or proximal straight tubules were microdissected either from the (A) superficial cortex ('superficial cortical tissue') or from the (B) juxtamedullary cortex plus outer stripe of the red (outer) medulla, i.e., 'juxtamedullary tissue' (for details, see Materials and methods; also Fig. 1). The specific activities of enzymes are expressed as pmol/mg protein per 1 h.
* Mean ± S.E. from two or three separate experiments. Activities in each of the experiments were measured in duplicate samples.
** In parentheses is the ratio of specific activity in proximal straight tubules to proximal convoluted tubules.

Table 2
Specific activities of BBM enzymes in cortical homogenate (CH) and fractions of brush-border membrane vesicles (BBMV) prepared from superficial cortical (SC) tissue and juxtamedullary-medullary tissue, i.e., juxtamedullary tissue (JM) of rat kidney

	Superficial cortex (SC-tissue)			Juxtamed.-medullary zone (JM-tissue)		
	CH	BBMV-SC	BBMV-SC/CH ratio [a]	CH	BBMV-JM	BBMV-JM/CH ratio [a]
Alkaline phosphatase	7.25 ± 0.6 *	107.0 ± 7.0	14.7 ×	6.77 ± 0.3	88 ± 3 **	13.0 ×
γ-Glutamyl transferase	33 ± 2	495 ± 13	15.0 ×	59 ± 2 **	1623 ± 43 **	27.5 ×
Leucine aminopeptidase	1.1 ± 0.2	14.5 ± 0.8	14.5 ×	2.93 ± 0.2	37 ± 0.8 **	12.6 ×
Maltase	56 ± 2	291 ± 13	5.2 ×	48 ± 3	194 ± 9 **	4.0 ×

The specific activities are expressed as μmoles/mg protein per 1 h (for further details, see Materials and methods).
* Mean ± S.E. from four experiments.
** Significantly different ($P < 0.05$ or higher degree of significance, t-test) from corresponding preparation from superficial cortex.
[a] 'Enrichment' ratio of specific activity in BBMV to cortical homogenate (CH).

fical cortical tissue and BBMV-juxtamedullary tissue; the enrichment of maltase in the same preparation and was lower (about 5 ×) in both types of tissue preparations (Table 2). The pattern in specific activities of the four BBM enzymes were distinctly different between BBMV-superficial cortical tissue and BBMV-juxtamedullary tissue. Specific activities of γ-glutamyltransferase (3.3 ×) and of leucine aminopeptidase (2.5 ×) are higher in BBMV-juxtamedullary tissue compared to BBMV-superficial cortical tissue, whereas, the activities of alkaline phosphatase and maltase were slightly higher in BBMV-superficial cortical tissue compared to BBMV-juxtamedullary tissue (Table 2). BBMV-superfical cortical tissue and BBMV-juxtamedullary tissue differed markedly in relative ratios of some key BBM enzymes, namely alkaline phosphatase and γ-glutamyltransferase. In relative terms, in BBMV-superficial cortical tissue, the ratio of alkaline phosphatase activity to γ-glutamyltransferase activity was 2.1, whereas, in BBMV-juxtamedullary tissue, it was 0.5. Conversely, the ratio of γ-glutamyltransferase to alkaline phosphatase in BBMV-superficial cortical tissue was 0.45 but it was 1.8 in BBMV-juxtamedullary tissue (Table 2).

Table 4
Kinetics of Na^+-gradient-dependent uptake of P_i and L-proline in BBMV-superficial cortical tissue and BBMV-juxtamedullary tissue

Fraction	Experiment No.	Transport of [^{32}P]P_i	
		app K_m (μM)	app V_{max} (pmol/5 s per mg protein)
BBMV-SC	1	58.2	1493
	2	76.5	1873
	mean ± S.E.	67.3 ± 9.1	1683 ± 190
BBMV-JM	1	33.5	735
	2	41.7	905
	mean ± S.E.	37.6 ± 4.1	820 ± 85
Fraction	Experiment No.	Transport of L-[^{3}H]proline	
		app K_m (μM)	app V_{max} (pmol/5 s per mg protein)
BBMV-SC	1	97.8	787
	2	90.0	1010
	mean ± S.E.	93.9 ± 3.9	898.0 ± 0111
BBMV-JM	1	43.6	559
	2	43.0	714
	mean ± S.E.	43.3 ± 0.3	636 ± 77

The initial uptake was measured in at 5 s interval, apparent K_m and V_{max} were determined by double-reciprocal Lineweaver-Burk plot.

Transport properties of BBMV-superficial cortical tissue and BBMV-juxtamedullary tissue prepared by Ca^{2+}-precipitation method

As observed in our preceding studies [7,11], the rate of Na^+-gradient-dependent uptake of P_i by BBMV-superficial cortical tissue was markedly higher than BBMV-juxtamedullary tissue when measured in the concentrative uphill phase (30 s) (Table 3). The kinetic features of the transport were examined measuring the uptake in 5 s period when the uptake is linearly proportional to elapsed [27,28]. The apparent K_m (app K_m) values and apparent V_{max} (app V_{max}) values were determined from Burke double-reciprocal plots (Figs. 4A, 4B, Table 4). The Na + -gradient-dependent uptake of [^{32}P]P_i and the app V_{max} is about twice as high in BBMV-superficial cortical tissue than in BBMV-juxtamedullary tissue, whereas the apparent K_m is about twice as low as in BBMV-juxtamedullary tissue, indicating higher affinity of Na^+-cotransporter for P_i in BBMV-juxtamedullary tissue than in BBMV-superficial cortical tissue (Fig. 4A). The similar pattern of differences between BBMV-superficial cortical tissue and BBMV-juxtamedullary tissue was found for L-proline (Fig. 4B) although the V_{max} was only slightly (+29%) higher in BBMV-superficial cortical tissue

Table 3
Comparison of Na^+-gradient-dependent uptake of P_i and L-proline by BBMV-juxtamedullary tissue, BBMV-superficial cortical tissue in isotonic medium (300 mosM) and BBMV-superficial cortical tissue in hypertonic medium (650 mosM)

	Uptake of [^{32}P]P_i (pmol/mg protein)		
	BBMV-SC (300 mosM)	BBMV-SC (650 mosM)	BBMV-JM (300 mosM)
30 s	2067 ± 264 *	1209 ± 129 **	1036 ± 202 **
120 min	1114 ± 84	832 ± 39 **	575 ± 62 **
Δ% overshoot	+84 ± 12	+44 ± 8	+52 ± 8
	Uptake of L-[^{3}H]proline-(pmol/mg protein)		
	BBMV-SC (300 mosM)	BBMV-SC (650 mosM)	BBMV-JM (300 mosM)
15 s	311 ± 14	156 ± 12 **	318 ± 58 ‡
120 min	49 ± 3	28 ± 2 **	33 ± 4
Δ% overshoot	+543 ± 63	+495 ± 76	+889 ± 147

The uptake under the three conditions was measured simultaneously.
* Mean ± S.E. of four experiments.
** Significantly different from corresponding values of BBMV-superficial cortical tissue (300 mosM); *t*-test.
‡ Significantly different from BBMV-superficial cortical tissue (650 mosM); *t*-test.

than in BBMV-juxtamedullary tissue (Fig. 4B, Table 4).

As observed also in previous experiments [7], the equilibrium uptake of P_i and namely of L-proline, at the 120 min period, is significantly lower in BBMV-juxtamedullary tissue (Table 3). We examined whether a decrease of intravesicular volume by shrinking of BBMV in a hyperosmolar medium (650 mosM) would influence transport rates in both types of BBMV. BBMV-juxtamedullary tissue and BBMV-superficial cortical tissue were either equilibrated by incubation in a hypertonic medium (650 mosM), by addition of sucrose to the standard medium and incubation for 1 hr at room temperature, and compared to BBMV incubated similarly in an isoosmolar (300 mosM) medium; then the transport parameters were measured simultaneously.

The incubation in hyperosmolar medium decreased the uptake of [^{32}P]P_i and L-[^{3}H]proline at all points of the time-course of the uptake, including the equilibrium (Fig. 5). These comparisons indicate that intravesicular volume of both BBMV-juxtamedullary tissue and BBMV-superficial cortical tissue decreased when incubated in hyperosmolar medium. Incubation of BBMV-superficial cortical tissue in osmolality of 650 mosM results in a decrease of the equilibrium uptake of

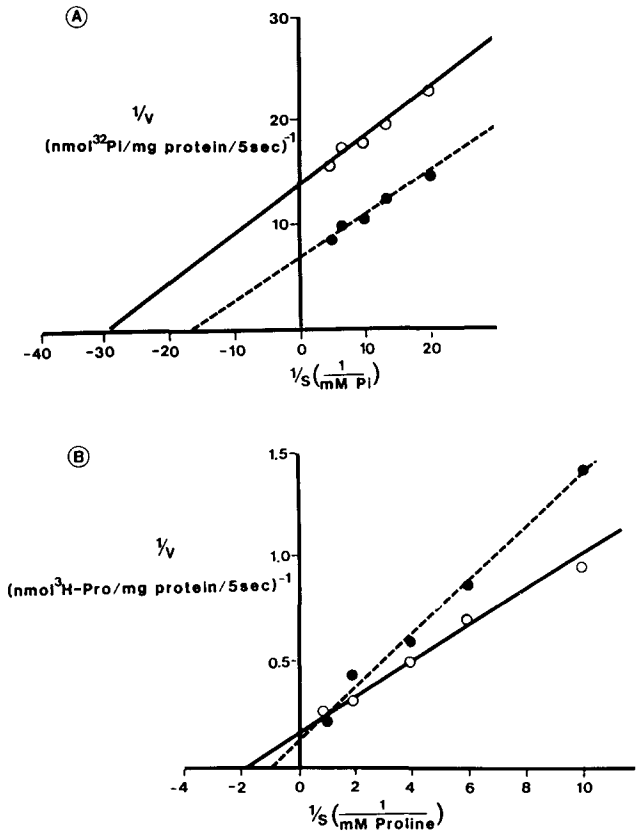

Fig. 4. Kinetics of the Na^+-gradient-dependent uptake of [^{32}P]P_i upper panel (A), and of L-[^{3}H]proline, lower panel (B). Solid lines (○———○) denote BBMV-juxtamedullary tissue and dotted lines (●———●) denotes BBMV-superficial cortical tissue.

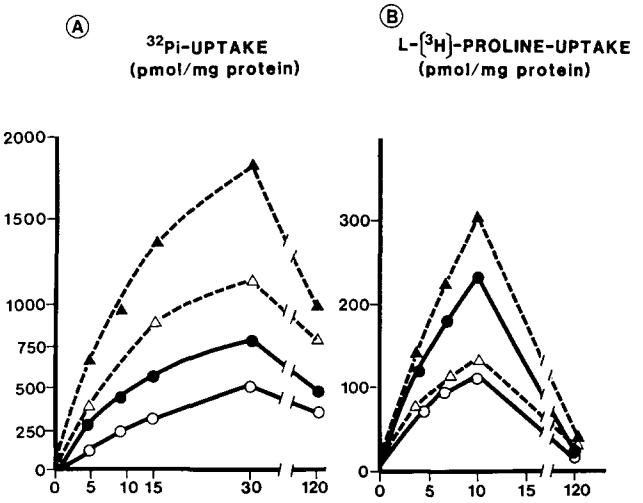

Fig. 5. The time-course of Na^+-gradient-dependent [^{32}P]P_i uptake and of L-[^{3}H]proline uptake by BBMV-superficial cortical tissue and BBMV-juxtamedullary tissue pre-equilibrated in either isotonic (300 mosM) or in hypertonic (650 mosM) media. (▲———▲) denote BBMV-superficial cortical tissue; (●———●) BBMV-juxtamedullary tissue; (△———△) denote uptake by BBMV-superficial cortical tissue and (○———○) pre-equilibrated hypertonic (650 m OsM) medium. Each point is the mean of two experiments based on three or four replicate samples.

L-[^{3}H]proline so that it is indistinguishable from the uptake of BBMV-juxtamedullary tissue in isoosmotic (300 mosM) medium (Fig. 5). Thus, BBMV-juxtamedullary tissue and hypertonically treated BBMV-superficial cortical tissue have the same intravesicular volume. Under conditions of the same intravesicular volume, the Na^+-gradient-dependent concentrative uptake of [^{32}P]P_i at 30 s only was slightly (+20%) higher in BBMV-superficial cortical tissue than in BBMV-juxtamedullary tissue. Moreover, the concentrative Na^+-gradient-dependent uptake of L-[^{3}H]proline was markedly higher in BBMV-juxtamedullary tissue than in BBMV-superficial cortical tissue when intravesicular volume was equalized (Table 3). The diffusional uptake of $^{22}Na^+$ in BBMV-superficial cortical tissue [6,7,15] was also reduced at equilibrium by pre-transport hyperosmotic treatment. The uptake of $^{22}Na^+$ in the initial uphill phase (30 s) was comparable in BBMV-superficial cortical tissue in hyperosmotic (650 mosM) medium to BBMV-juxtamedullary tissue at isotonic (300 mosM) medium (data not shown).

BBMV-superficial cortical tissue and BBMV-juxtamedullary tissue were analyzed by SDS-polyacrylamide gel electrophoresis. BBMV-superficial cortical tissue differed considerably from BBMV-juxtamedullary tissue in profile of composition of polypeptides separated under dissociating conditions in SDS-PAGE systems. While some major protein bands were common for both BBMV-superficial cortical tissue and BBMV-juxtamedullary tissue, some major differences

were obvious even in a simple one-dimensional SDS-PAGE system, as is evident from inspection of electrophoreograms, as well as on densitometric scans of the slab-gel SDS-PAGE (Fig. 6).

BBMV prepared by differential centrifugation combined with Percoll-density gradient centrifugation

BBMV fractions were prepared according to a fractionation scheme first described in principle by Mamelok et al. [13] for the whole cortex; we analyzed BBMV from the two zones of kidney tissue which were a source for preparation of BBMV-superficial cortical tissue and BBMV-juxtamedullary tissue by Ca^{2+}-precipitation method (see above). Proteins in BBMV from juxtamedullary tissue resolved with two major protein peaks in zones A and B, while BBMV from superficial cortical tissue showed mainly one major peak in 'zone B' (Fig. 3).

In the following text the enzyme activities are presented in the figures as total activities per fraction (specific activity times; protein content). BBMV prepared this way from superficial cortical tissue showed major peak of alkaline phosphatase activity (Fig. 7) between Percoll-gradient fractions 22–28 ('zone B'),

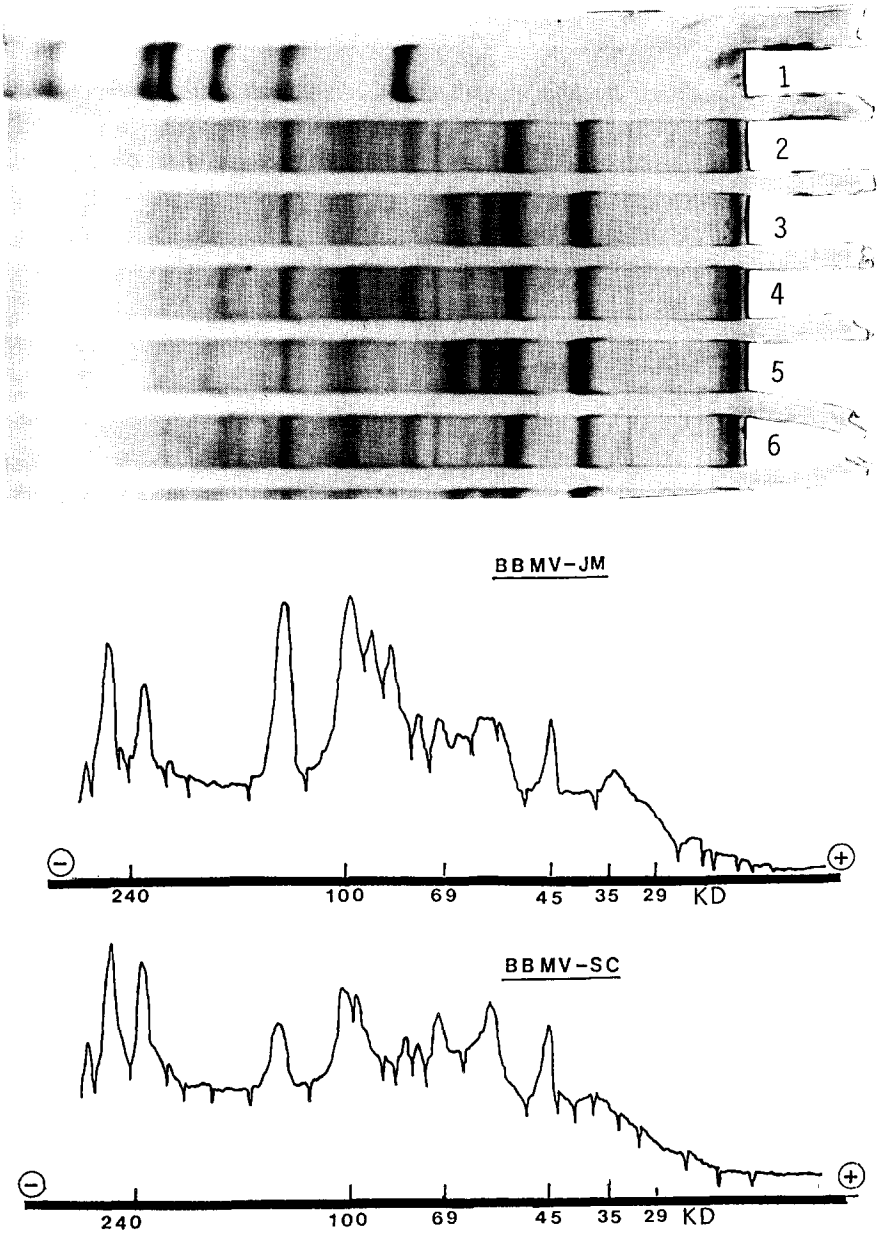

Fig. 6. The SDS-PAGE polypeptide profile of BBMV-superficial cortical tissue and BBMV-juxtamedullary tissue prepared by Ca^{2+}-precipitation method. Upper panel: Photograph of lanes on slab gel stained with Coomassie blue. Lane 1: molecular mass standards: 68, 46, 35, 29, 24 and 12 kDa; Lanes 2 and 4: BBMV-superficial cortical tissue; Lanes 3 and 5: BBMV-juxtamedullary tissue. The most pronounced difference in protein bands pattern in seen in the range 70–200 kDa. Lower panel: Densitometric scan of the same preparation.

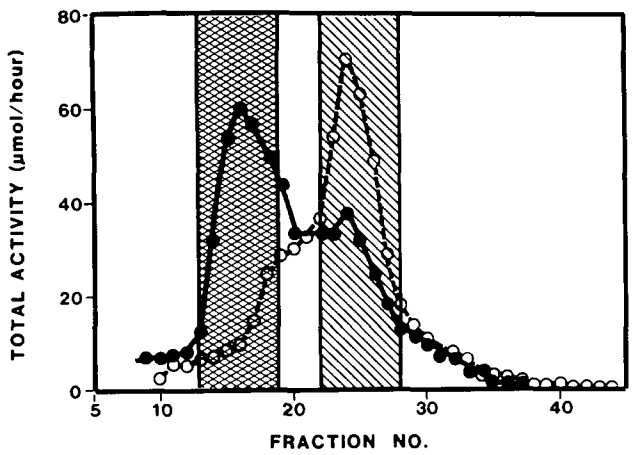

Fig. 7. The Percoll gradient distribution of activities of the alkaline phosphatase in BBMV prepared by the centrifugation method. This and following enzyme profiles are depicting representative experiments repeated at least five times. Ordinate: the total activity of alkaline phosphatase; abcissa: Percoll gradient fractions collected in 'zone A' and 'zone B', as defined in Fig. 3. (●———●) denotes BBMV preparation from juxtamedullary tissue; (○———○) denotes BBMV preparation from superficial cortical tissue (BBMV-superficial cortical tissue), as defined in Fig. 1. The profile of distribution of total activities of other BBM enzymes (Figs. 8–10) are described in a similar way. The BBM enzyme profiles are data of representative experiment, repeated 5–7 times.

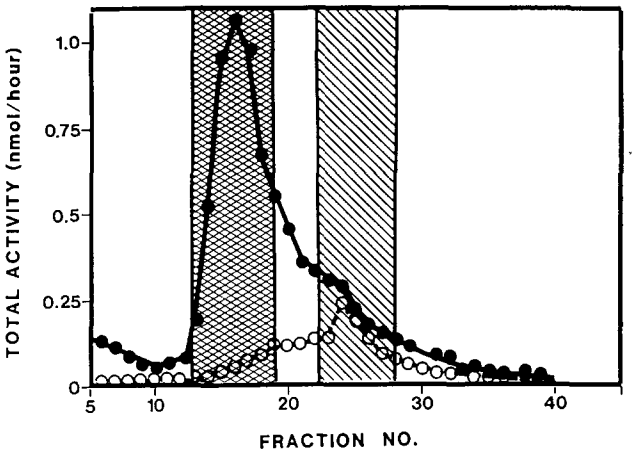

Fig. 8. Percoll-gradient distribution of γ-glutamyltransferase total activity (γ-glutamyltransferase) of the BBMV-superficial cortical tissue (●———●) preparation and BBMV-juxtamedullary tissue (○———○) preparation; the symbols are identical as in Fig. 7.

whereas analogous preparation from juxtamedullary tissue showed a major peak at higher density ('zone A') (fraction 13–18) and smaller peak of activity in 'zone B', at the same density range as major alkaline phosphatase peak from BBMV-superficial cortical tissue (Fig. 7).

The activity of γ-glutamyltransferase (Fig. 8) from superficial cortical tissue showed a small but distinct peak in 'zone B', γ-glutamyltransferase from juxtamedullary tissue showed a very prominent peak of high activity in 'zone A'; in addition, there was a small shoulder elevation in γ-glutamyltransferase activity in 'zone B', coincident with the aforementioned small γ-glutamyltransferase peak from superficial cortical tissue (Fig. 8). The activity of leucine aminopeptidase showed essentially the same (Fig. 9) profile as that of γ-glutamyltransferase. Finally, maltase in BBMV-superficial cortical tissue showed a distinct peak in 'zone B', similar as alkaline phosphatase in superficial cortical tissue preparations (fractions 22–28) (Fig. 10). On the other hand, in membranes from juxtamedullary tissue, only a wide peak of maltase activity was spread between fractions 12 up to 29 (Fig. 10).

Enrichment of enzyme activities occuring during 'zone A' and 'zone B' from superfical cortical tissue cortex and juxtamedullary tissue cortex are shown in Table 5. In BBMV-juxtamedullary tissue, all the enzymes showed the highest enrichment in 'zone A', where enrichment was about 2 × higher than in

Table 5
Activities of BBM enzymes in Percoll-gradient subfractions of BBMV-juxtamedullary tissue and BBMV-superficial cortical tissue

	γ-Glutamyl transferase		Alkaline phosphatase		Leucine amino-peptidase		Maltase	
	BBMV-SC	BBMV-JM	BBMV-SC	BBMV-JM	BBMV-SC	BBMV-JM	BBMV-SC	BBMV-JM
(A) Specific activity								
Cortical homogenate	22 *	72	5.4	5.1	1.1	2.6	33.5	29.6
P_3-pellet	66	350	18.5	25.4	4.2	13.1	148	151
'zone A'	197	1401	47.4	81.2	8.6	81.2	–	702
	(8.9 ×) **	(19.4 ×)	(8.8 ×)	(15.9 ×)	(7.8 ×)	(18.5 ×)		(23.7 ×)
'zone B'	194	401	67.2	51.5	8.3	10.2	329	
	(8.8 ×)	(5.6 ×)	(12.4 ×)	(10.1 ×)	(17.5 ×)	(3.9 ×)		(9.8 ×)
(B) Total activity								
'zone A'	53	1069	24	60	4.7	33.6	76	230
'zone B'	244	304	70	38	10.5	9.8	319	205

A = specific activities (μmol/mg protein per 1 h) B = total activities (pmol/h). 'zone A' denotes band of Percoll-gradient between fractions 13 and 19 and 'zone B', a band including fractions 22–27; for details, see Figs. 1, 5–8. Pellet P_3 is a membrane fraction obtained by differential centrifugation of the homogenate according to scheme in the Materials and methods.

* Activities of enzymes.

** In parentheses the enrichment compared to cortical homogenate of tissue pooled from kidney of three or four rats is given.

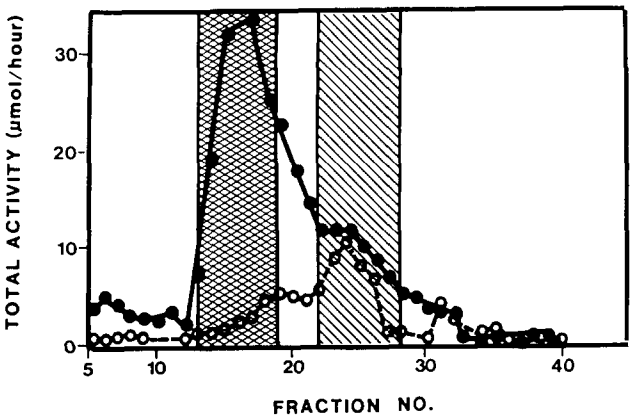

Fig. 9. Percoll gradient distribution of total activity of leucine aminopeptidase BBMV-juxtamedullary tissue (●———●) and BBMV-superficial cortical tissue (○———○). For other details, see legend to Fig. 7.

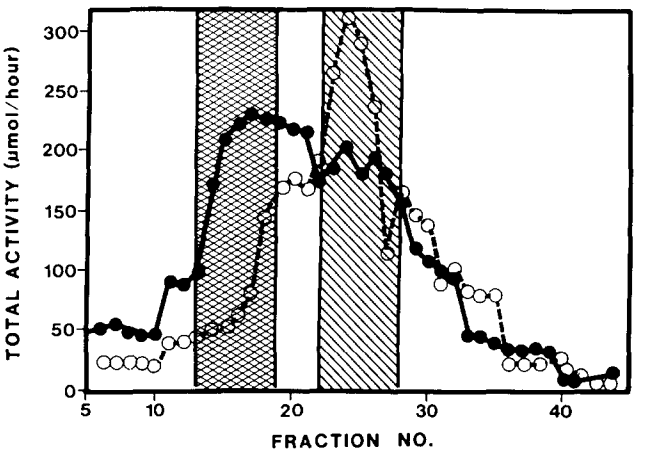

Fig. 10. Percoll-gradient distribution of total activity of maltase BBMV-superficial cortical tissue (○———○) and BBMV-juxtamedullary tissue (●———●). For details, see legend to Fig. 7.

BBMV-superficial cortical tissue preparations (Table 5). Conversely, enrichment in 'zone B' was somehow higher for BBMV-superficial cortical tissue than in BBMV-juxtamedullary tissue preparation (Table 5). Finally, since the juxtamedullary cortex and outer stripe of red medullary tissue contains high activity of Na^+/K^+-ATPase, we determined the activity of this enzyme compared to γ-glutamyltransferase in zones A and B from juxtamedullary tissue (Fig. 11). The results shown on Fig. 11 indicate that by centrifugation in Percoll-gradient, the activity of Na^+/K^+-ATPase remains in lower density range as a distinct peak in and adjacent to 'zone B' and is separated completely from distinct peak of γ-glutamyltransferase in 'zone A' (Fig. 11).

The transport properties and protein composition were distinct in all four preparations

We examined the ability of BBMV subfractions separated via Percoll-gradient centrifugation method for Na^+-gradient-dependent uptake of $[^{32}P]P_i$ and L-[^{3}H]proline. For this purpose, fractions from 'zone A' [13–18] and from 'zone B' [22–28] were pooled, Percoll removed by centrifugation (see Methods), and transport was measured in two ways. First we determined uptake of solutes in the presence of Na^+-gradient [$Na_o^+ > Na_i^+$ in the uphill, concentrative phase (15–30 s) and after equilibration period of 120 min (Table 6).

To determine basic quantitative properties in terms of kinetic parameters, us determined $[^{32}P]P_i$ and L-[^{3}H]proline transport by BBMV in the initial uptake phase (5 s) (Fig. 12). In all 4 major preparations separated on Percoll-gradient, i.e., BBMV-juxtamedullary tissue and BBMV-superficial cortical tissue from the two major 'zone A' and 'zone B', the early uphill uptake of $[^{32}P]P_i$ or L-[^{3}H]proline exceeded the uptake at equilibrium - the 'overshoot'. This indicates that all four fractions contained vesicular structures capable of concentrating the solute above the level in the incubation media. The equilibrium uptake of both solutes by BBMV-juxtamedullary tissue of 'zone A' was about 50% lower than in BBMV-juxtamedullary tissue, 'zone B', or BBMV-superficial cortical tissue in both 'zone A' or 'zone B'. This difference is similar, but more

Table 6
The uptake of $[^{32}P]P_i$ and L-[^{3}H]proline in subfractions of BBMV-superficial cortical tissue and BBMV-juxtamedullary tissue separated by centrifugation in Percoll-gradient

	$[^{32}P]P_i$ uptake			L-[^{3}H]proline		
	30 s	120 min	Δ%	15 s	120 min	Δ%
BBMV-SC						
'zone A'	1021 ± 179 *	338 ± 99	+256 ± 92	126 ± 24	68 ± 8	+116 ± 37
'zone B'	2069 ± 357	370 ± 82	+539 ± 133	165 ± 20	59 ± 4	+181 ± 51
BBMV-JM						
'zone A'	499 ± 86	182 ± 25	+174 ± 36	70 ± 10	31 ± 6	+131 ± 28
'zone B'	1010 ± 155 **	303 ± 78	+280 ± 56	71 ± 4	50 ± 6 **	54 ± 23

For details, see Materials and methods. The uptake is expressed in pmol/mg protein.
* Mean ± S.E. of five experiments.
** Significantly different from corresponding values of 'zone A', *t*-test.

expressed than differences in equilibrium uptake of L-[^{3}H]proline between BBMV-superficial cortical tissue and BBMV-juxtamedullary tissue prepared by the Ca^{2+}-precipitation method (Tables 3 and 6).

The kinetic parameters for Na^+-dependent transport of P_i and L-[^{3}H] proline were established in two preparations which showed, based on the preceding results, most prominent differences in composition and function, that means BBMV-juxtamedullary tissue, 'zone A' and BBMV-superficial cortical tissue, 'zone B'. This analysis indicates that with respect to Na^+-gradient uptake of phosphate in membranes from BBMV-superficial cortical tissue, 'zone B', had more than a six-fold higher capacity (apparent V_{max}) for P_i transport. On the other hand, under the same conditions, the P_i transport system in BBMV-juxtamedullary tissue, 'zone A' has more than twice higher affinity (apparent K_m) for P_i than BBMV-superficial cortical tissue, 'zone B' (Fig. 12A).

The analogous properties of the Na^+-gradient transport system for L-[^{3}H]proline between the two zones are documented by kinetic analysis. As for the P_i, the transport system for L-[^{3}H]proline showed several-fold higher app. V_{max} in BBMV-superficial cortical tissue, 'zone B' and about 50% less affinity (app. K_m) in BBMV-juxtamedullary tissue, 'zone A' from the same kidney (Fig. 12B).

Finally, the electrophoretic pattern on SDS-PAGE of the 4 Percoll-gradient isolated subfractions have been compared in the same experimental setting. Protein analysis showed various differences with some similarities among the zones from both sources of BBM tissues (Fig. 13). However, protein patterns of BBMV in 'zone B' of both superficial cortical tissue and juxtamedullary tissue were almost identical, suggesting that these vesicles might be originating from analogous subsegments of nephrons in different cortical regions. In this case, they may be from proximal convoluted tubules of superficial cortical tissue and juxtamedullary tissue, respectively. In contrast to above, the vesicles in 'zone A' both from superficial cortical tissue and juxtamedullary tissue showed more differences than similarities. Nevertheless, the pattern of proteins was very close to the pattern obtained in BBMV-juxtamedullary tissue of Ca^{2+}-precipitation method (Fig. 6). This comparison suggests that the vesicles from superficial cortical tissue may be originated from S_2 or early S_3 subsegments of proximal

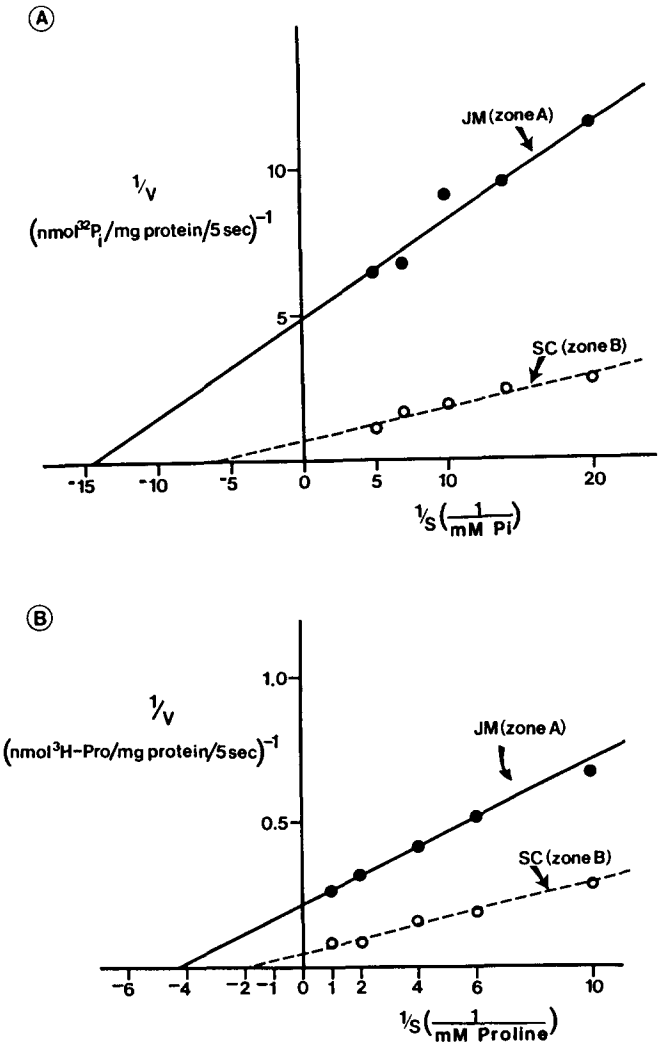

Fig. 12. Lineweaver-Burk double-reciprocal plot of the initial uptakes of [^{32}P]P_i (upper panel, A) and of L-[^{3}H]proline (lower panel, B) by BBMV from superficial cortical tissue or juxtamedullary tissue prepared by the centrifugation method. From each preparation BBMV were collected from pooled fractions of Percoll gradient 'zone A' for BBMV-juxtamedullary tissue ('zone A') and from 'zone B' as defined for BBMV-superficial cortical tissue ('zone B').

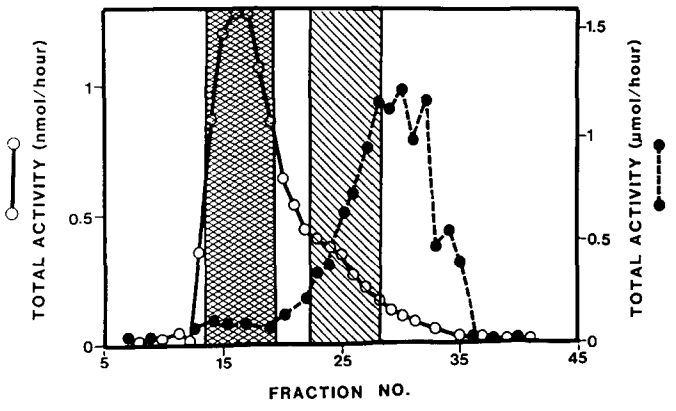

Fig. 11. The separation of total activities of γ-glutamyltransferase (○———○) and Na^+/K^+-ATPase (●———●) in final 36 fractions on Percoll-gradient of BBMV from the juxtamedullary tissue (BBMV-juxtamedullary tissue). Symbols are analogous to those in Figs. 7–10.

convoluted tubules from outer cortical tissue, while the vesicles in 'zone A' from juxtamedullary tissue predominantly originating from proximal straight tubules (mostly S_3 subsegments) of deep cortical nephrons.

4. Discussion

The heterogeneity of properties of BBMV prepared from different basically cortical, parts of kidney, tissue containing proximal tubules became evident in recent studies [6–11]. In the present study, using several independent methodologic strategies, we addressed the question from which subsegments of proximal tubules, namely proximal convoluted tubules and proximal straight tubules, the different BBMV preparations may originate. Differences in transport properties of BBMV prepared from superficial cortical tissue and juxtamedullary tissue observed in our previous study of rat kidney [11] prompted us to analyze from this mammalian species BBMV by more refined fractionation. Based on careful morphometric study on the rat kidney by Pfaller and Rittinger [14], it may be expected that BBMV from superficial cortical tissue may contain predominantly proximal convoluted tubules (namely the S_1 segment) and, to a lesser degree, early portions of proximal straight tubules (subsegments of late S_2, and early S_3 segments). The juxtamdedullary cortex plus the outer stripe of red medulla, i.e., the juxtamedullary tissue (Fig. 1), is expected to be particularly rich in proximal straight tubules and to contain also proximal convoluted tubule (S_1 and S_2) of nephrons originating from juxtamedullary glomeruli.

Results of our comprehensive BBM enzyme analysis of microdissected tubules (Table 1), some of which are in basic agreement with reports by Endou et al. [25] from the rabbit nephron and Sudo and Tanabe [26] do document that specific activities of γ-glutamyltransferase and leucine aminopeptidase are particularly high in proximal straight tubule namely in proximal straight tubule miscrodissected from juxtamedullary tissue and hence, representing mainly the end-portions of S_3 subsegments [14]. In contravention to the findings on mice [29–31], the distribution of alkaline phosphatase in rat kidney is such that the enzyme is not limited to S_1 segments, but that its activity is only slightly lower in proximal straight tubules than in proximal convoluted tubules in the superficial cortical tissue. Moreover, in juxtamedullary tissue, the alkaline phosphatase activity in proximal straight tubules is even higher than in proximal convoluted tubules. Hence, our findings of the distribution of alkaline phosphatase activity in superficial cortical tissue and juxtamedullary tissue subsegments of the rat kidney is in excellent agreement with a careful microhistochemical study by Schmidt and Dubach [36]. The difference of alkaline phosphatase distribution in rat from the findings in mouse [29–31] are most likely due to species difference. The distribution activity of maltase is virtually opposite to that of alkaline phosphatase (Table 1).

Based on the relationship between BBM enzymes in microdissected subsegments of proximal tubules it ap-

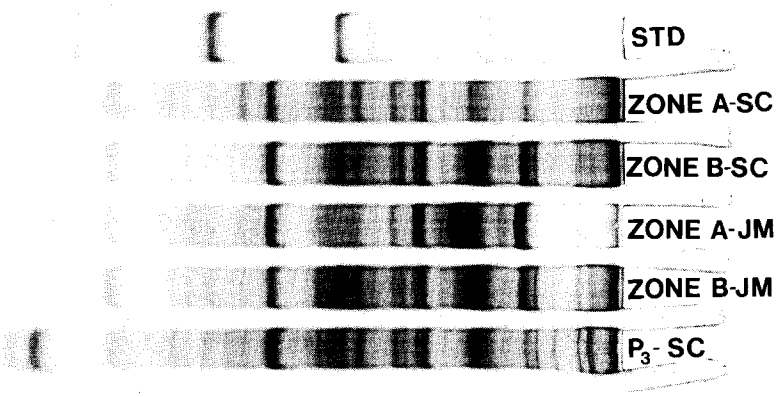

Fig. 13. SDS-PAGE slab gel polypeptide profiles of BBMV-superficial cortical tissue and BBMV-juxtamedullary tissue from 'zone A' and 'zone B' of Percoll gradient prepared by the differential-density gradient centrifugation procedure, and intermediate step P_3-pellet (Fig. 2) STD, molecular mass standards 20–340 kDa; P_3-juxtamedullary tissue, P_3 fraction from juxtamedullary tissue; P_3-superficial cortical tissue, P_3 fraction from superficial cortical tissue; zone B-juxtamedullary tissue, BBMV from juxtamedullary tissue in 'zone B' of gradient; zone A-juxtamedullary tissue, BBMV from juxtamedullary tissue in 'zone A' of gradient; zone A-superficial cortical tissue, BBMV from superficial cortical tissue in 'zone A' of gradients.

pears that, at least for the rat kidney, γ-glutamyltransferase and leucine aminopeptidase can be considered as quite specific markers for proximal straight tubule, whereas, the alkaline phosphatase activity distribution may be used perhaps only when considered in relationship to γ-glutamyltransferase and leucine aminopeptidase in the same preparation. Therefore, at least in approximative terms, the ratio of activities of γ-glutamyltransferase and/or leucine aminopeptidase to alkaline phosphatase may be reasonably employed for identification of BBM fractionated by various separation techniques.

Our preceding study described some differences in the BBM transport properties between BBMV-superficial cortical tissue and BBMV-juxtamedullary tissue [7]. Now, the comparison of specific activities of BBM enzymes reveals a marked difference between these two BBM preparations (Table 2). The activities of γ-glutamyltransferase and leucine aminopeptidase are several-fold higher in BBMV-juxtamedullary tissue than in BBMV-superficial cortical tissue; conversely, the activities of alkaline phosphatase and maltase are somehow lower (Table 2). These comparisons, which are in basic agreement with studies of microdissected tubules do suggest that BBMV-juxtamedullary tissue are derived predominantly from proximal straight tubule segments, whereas, BBMV-superficial cortical tissue predominantly from proximal convoluted tubules.

When BBMV are prepared from the whole cortex by Percoll-gradient centrifugation, as described by Mamelok et al. [13], it was possible to identify separate but sometimes overlapping peaks of fractions enriched in alkaline phosphatase and in γ-glutamyltransferase, respectively. However, when we applied the same procedure separately to superficial cortical tissue and juxtamedullary tissue, respectively, a very distinct pattern of separation of the enzyme activities emerge (Figs. 7–10). Membranes sedimenting in fractions of higher density [13,16], operationally called 'zone A,' show sharp peak of leucine aminopeptidase and γ-glutamyltransferase of very high specific activity in BBM fractions from BBM fractions from juxtamedullary tissue preparation (Figs. 8,9). These peaks coincide with peak of alkaline phosphatase (Fig. 7); moreover, smaller peaks of the three enzymes also appears in 'zone B' (Figs. 7–9).

Concerning the juxtamedullary tissue preparation when expressed in relative terms, the ratio of γ-glutamyltransferase or leucine aminopeptidase to alkaline phosphatase is high in 'zone A,' whereas conversely in 'zone B,' the activity of alkaline phosphatase is relatively higher than γ-glutamyltransferase and leucine aminopeptidase (Figs. 7–9). Taken together with the results on microdissected tubules (Table 1) and on BBMV-juxtamedullary tissue prepared by calcium precipitation (Table 2), these results suggest that BBMV sedimenting in 'zone A' from juxtamedullary tissue are from luminal BBM of proximal straight tubule segments, and those in 'zone B' are probably derived from proximal convoluted tubules originating from deep juxtamedullary nephrons. Furthermore, this fraction of membranes is virtually free of basolateral membranes which are known to contain abundant Na^+/K^+-ATPase, namely in the outer medullary area (Fig. 11).

In preparation from superficial cortical tissue, the most prominent features are peaks of alkaline phosphatase (Fig. 7) and maltase (Fig. 10) in 'zone B,' where are also located small but recognizable peaks of leucine aminopeptidase and γ-glutamyltransferase (Figs. 8, 9). It seems, therefore, that BBMV from superficial cortical tissue in 'zone B' is composed mainly of membranes from proximal convoluted tubules of the superficial nephrons and, unlike with juxtamedullary tissue, no distinct peaks of BBM enzyme activities are detected in 'zone A' of the Percoll-gradient from the superficial cortical tissue.

Although interpretation of these results should be considered with considerable caution, it appears using the Percoll-gradient centrifugation procedure when starting from superficial cortical tissue, it is possible to separate the BBMV mainly from proximal convoluted tubules of outer cortical nephrons. Furthermore, by starting from juxtamedullary tissue, it is possible to BBMV from proximal straight tubules which are sedimenting in 'zone A' and, as a minor fraction, BBMV from proximal convoluted tubules which originate in deep nephrons. In addition, at least starting from juxtamedullary tissue, by centrifugation in Percoll-gradient it will be also possible to separate BBMV of proximal straight tubules from membrane particles containing Na^+/K^+-ATPase (Fig. 11). It seems likely that the peak in 'zone B' represents Na^+/K^+-ATPase which is derived in part from admixture of basolateral membranes from the thick medullary ascending Limbs of Henle's loops which are abundant in outer medullary tissue [14].

Comparison of the transport properties examined in different BBMV preparations, described above, deserves several comments. With respect of BBMV prepared by Ca^{2+}-precipitation method, the interpretation is complicated by the differences in the apparent intravesicular volumes (Ref. 11, Table 2); such difference, i.e., greater intravesicular volume of BBMV-superficial cortical tissue was also observed independently in the study by Loghman-Adham [35]. From the parameters determined in the studies of transport by BBMV solute uptake with use of rapid filtration technique (uphill concentrative phase, peak phase, efflux, and equilibrium phase) it is evident (Fig. 5) that the solute uptake parameter least affected by the differences in the intravesicular volume is the 'initial rate' of uphill uptake (Fig. 5), measured in short intervals (5 s)

when the uptake remains linear with the elapsed time [28]. This analysis of the 'initial uptakes' (Fig. 4, Table 3) indicates quite clearly that BBMV-superficial cortical tissue which originate predominantly from proximal convoluted tubules, have higher maximum capacity (V_{max}) and lower affinity (K_m) for P_i as compared with BBMV-juxtamedullary tissue, mainly from proximal straight tubules, which have higher affinity (lower K_m) and lower transport capacity (V_{max}) for P_i (Fig. 4). Higher affinity (K_m) in BBMV-juxtamedullary tissue, apparently derived mainly from proximal straight tubules applies also for the transport system for L-proline (Fig. 4B) albeit app V_{max} for the initial uptake of L-proline is similar between BBMV-superficial cortical tissue and BBMV-juxtamedullary tissue (Table 4, Fig. 4). These results are similar to differences in kinetic parameters for Na^+/P_i cotransport between BBMV-superficial cortical tissue and BBMV-juxtamedullary tissue found in studies by others conducted on rat kidneys [34,35].

Moreover, M. Levi [34] determined apparent density of Na^+/P_i cotransporters in BBMV from superficial and juxtamedullary cortex with use of P_i-protectable [^{14}C]phosphonoformic acid (PFA) binding [38,39]. These data indicate that higher V_{maxP_i} in BBMV-superficial cortical tissue is, at least in part, due to higher number of Na^+/P_i cotransporters, whereas BBMV-juxtamedullary tissue have lower number of Na^+/P_i cotransporters with higher affinity [34]. Further, higher V_{max} in BBMV-superficial cortical tissue is also contributed to by greater fluidity of BBMV-superficial cortical tissue [34].

Agreement of the present results with these studies [34,35] is even more significant since the pattern of results is similar when BBMV-superficial cortical tissue and BBMV-juxtamedullary tissue were prepared by either Mg^{2+}-precipitation [34] or Ca^{2+}-precipitation (Ref. [35] and this study) methods. These results are also basically similar to studies of D-glucose transport by BBMV from different zones of rabbit kidney to that described by Turner and Moran [10] thus suggesting that transport systems of BBM in proximal straight tubule have higher affinity for solutes, but lower maximum transport capacity (V_{max}). The higher affinity and lower V_{max} in BBM from proximal straight tubules appears to be a general feature of all so far examined Na^+-cotransports for various solutes.

The question of how different intravesicular volume of BBMV relates to transport rate in the latter time intervals of the concentrative phase, the peak and efflux phase remains to be analyzed in detail. It should be noted that any change of volume of sphere changes as a cubic function of diameter, whereas the surface changes as a quadratic function of the diameter of vesicles assuming, for the sake of argument, that vesicles are perfect spheres. Accordingly, the changes in volume has far lesser impact upon the change in surface are, i.e., the site of transporting membrane [37]. Nevertheless, we attempted to equalize the intravesicular volumes by exposing BBMV-superficial cortical tissue to hypertonic medium which decreased extravesicular volume of BBMV-superficial cortical tissue to a size comparable to that of BBMV-juxtamedullary tissue in isotonic medium (Fig. 5, Table 3). This procedure decreased the extent of difference in P_i and L-proline uptakes between BBMV-juxtamedullary tissue and BBMV-superficial cortical tissue (Table 3, Fig. 5). However, interpretation of these results remains to be guarded, since it is not yet known whether exposure to hypertonic medium may, in addition to decrease in apparent intravesicular volume also change other properties of the BBM, in particular, mutual localization of different structural components of BBM within the plane of the membrane.

Measurements of the Na^+-gradient-dependent uptake of P_i and L-proline in BBMV from fractions separated by Percoll-gradient (Table 6) provide first indication that these membranes contain vesicular structures capable of concentrative uptake, since in all the four preparations, the uptake at the initial phase showed 'overshoot', i.e., uptake hihgher than at the equilibrium (Table 6). Kinetic analysis of the transport was done in BBMV from 'zone B' of superficial cortical tissue which most probably represents other vesicles originating from superficial cortical proximal convoluted tubules and BBMV from 'zone A' of juxtamedullary tissue which, in turn, very likely originate from proximal straight tubules mainly of deep nephrons, at least as judged according to the BBM enzyme distributions (Tables 1 and 5). The results indicate that the capacity (V_{max}) for the Na^+-dependent transport of P_i was much ($6\times$) higher in BBMV-'zone B' from superficial cortical tissue compared to BBMV-'zone A' from juxtamedullary tissue and that conversely, the affinity for P_i (K_m) was much ($25\times$) lower in BBMV-'zone B' from superficial cortical tissue than in BBMV-'zone A' from 3 M-tissue (Fig. 12). Such prominent differences in kinetic parameters in Na^+/P_i cotransport were not found when BBMV were prepared only by Ca^+-precipitation method from superficial cortical tissue and juxtamedullary tissue, respectively (Table 4). These results further stress that specific types of BBMV can be isolated and purified BBM vesicles by procedure (Fig. 1) which includes Percoll-density gradient centrifugation. Similar differences were also obtained in kinetic parameters of Na^+-dependent transport of L-proline in BBMV-'zone B' from superficial cortical tissue and BBMV-'zone A' from juxtamedullary tissue (Fig. 12b). Taken together, these results indicate that it is feasible to separate with use of the outlined Percoll-gradient procedure centrifugation (Fig. 2) sub-populations of BBM vesicles show-

ing more specific functional properties than BBMV prepared from superficial cortical tissue and juxtamedullary tissue by Ca^{2+}-precipitation method.

As further characterization of structural properties of the subpopulations of BBMV prepared by Percoll gradient method, the analysis of SDS-PAGE protein pattern, showed very distinct pattern of protein composition among all four subfractions of BBMV (Fig. 13). The protein patterns of BBMV in 'zone B' of both superficial cortical tissue and juxtamedullary tissue were very similar, thus indicating that these membrane vesicles may originate from similar subsegments of proximal tubules, but from nephrons which originate either in superficial cortical tissue or in juxtamedullary tissue cortical zones. The BBM enzyme pattern suggests that these vesicles (Table 5) originate from early proximal subsegments (mostly S_1) of both superfical cortical tissue and juxtamedullary tissue zones of the cortex. On the other hand, the protein pattern in BBMV in 'zone A' showed more differences than similarities if prepared from superfical cortical tissue and juxtamedullary tissue (Fig. 13). We would surmise that BBMV from superficial cortical tissue 'zone A' are mostly from S_2 or early S_3 segments from outer cortical region, whereas BBMV 'zone A' from juxtamedullary tissue are predominantly from proximal straight tubule, mostly S_3 subsegments of deep cortical and outer medullary regions. The SDS-PAGE protein pattern of BBMV-'zone B' was also distinctly different than that of BBMV-'zone A' from both superficial cortical tissue and juxtamedullary tissue. The transport properties (Table 6) and kinetic analysis (Fig. 12) seem to be in agreement with these findings as well as with earlier observations that the capacity of the P_i reabsorption along the proximal tubule is maximal in the early subsegments (S_1) of the outer cortical region and decreases along the length of nephron and reaches minimum in S_3 subsegments of the proximal straight tubule, whereas affinity for Na^+-dependent transport of P_i is highest at the most distal parts of proximal straight tubule. Taken all together, our findings demonstrate that with use of Percoll gradient isolation procedure (Fig. 2) we achieved separation, at least in part, of distinct sub-population of BBMV from outer (superficial cortical tissue) and deep (juxtamedullary tissue) cortical regions of rat kidney which have specific structural and functional properties which is superior to preparation of BBMV from tissue zones by divalent cation precipitation method.

In conclusion, the present studies outline strategy for isolation and study of partially purified BBMV from proximal convoluted tubules and proximal straight tubules of rat kidney. Further developments will be needed to design methods for isolation of BBMV-from proximal convoluted tubules and proximal straight tubules of superficial and juxtamedullary nephrons.

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6. References

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