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TRANSPORT OF *p*-AMINOHIPPURIC ACID, URIC ACID AND GLUCOSE IN HIGHLY PURIFIED RABBIT RENAL BRUSH BORDER MEMBRANES

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Summary

A procedure for preparing highly purified brush border membranes from rabbit kidney cortex using differential and density gradient centrifugation is described. Brush border membranes prepared by this procedure were substantially free of basal-lateral membranes, mitochondria, endoplasmic reticulum and nuclear material as evidenced by an enrichment factor of less than 0.3 for $(Na^+ + K^+)$-ATPase, succinate dehydrogenase, NADPH-cytochrome *c* reductase and DNA. Alkaline phosphatase was enriched ten fold indicating that the membranes were enriched at least 30 fold with respect to other cellular organelles. The yield of brush border membranes was 20%.

Transport of D-glucose by the membranes was identical to that previously reported except that the Arrhenius plot for temperature dependence of transport was curvilinear (E_A = 11.3—37.6 kcal/mol) rather than biphasic. Transport of *p*-aminohippuric acid and uric acid were increased by the presence of NaCl, either gradient or preequilibrated. However, no overshoot was obtained in the presence of a NaCl gradient, and KCl and LiCl also produced equivalent stimulation of transport suggesting a nonspecific ionic strength effect. Uptakes of *p*-aminohippuric acid and uric acid were not saturable, and were increased markedly by reducing the pH from 7.5 to 5.6. Probenecid (1 mM) reduced *p*-aminohippuric acid and uric acid (50 μM) uptake by 49% and 21%, respectively. We conclude that the uptake of uric acid and *p*-aminohippuric acid by renal brush border membranes of the rabbit occurs primarily by a simple solubility-diffusion mechanism.

Abbreviation: Hepes, *N*-2-hydroxyethylpiperazine-*N'*-2-ethanesulfonic acid.

Introduction

While the transport of sugars [1–3] and amino acids [4–6] in renal brush border membranes has been exhaustively studied, little work has yet been reported on the transport of organic acids in these membranes [7]. The authors initially set out to study organic acid transport in renal brush border membranes utilizing previously published techniques for the preparation of the membranes. However, in our hands none of the published procedures for isolating renal membranes by differential and density gradient centrifugation [8–10] consistently produced a reasonable yield of highly purified brush border membranes. Furthermore, the equipment required to perform free-flow electrophoresis, as developed by Heidrich et al. [11], was not readily available to us. Thus, prior to beginning our studies on organic acid transport, it was required to develop a procedure for preparing highly purified renal brush border membranes in good yield. We report here on the procedures we have devised for the isolation of purified renal brush border membranes, and, in addition, on initial experiments examining the transport of glucose, *p*-aminohippuric acid and uric acid in these membranes.

Methods and Materials

Preparation of brush border membranes. The strategy we have developed for the purification of renal brush border membranes relies on extending and modifying previous work of Mircheff and Wright [12] in which preliminary separation of brush border and basal-lateral membranes is obtained with differential centrifugation and subsequent purification utilizes density gradient centrifugation. We have also incorporated a step using Ca^{2+} precipitation as originally devised by Schmitz et al. [13] and subsequently used by Kessler et al. [14]. Marker distributions were evaluated at each step and a balance sheet maintained to identify activation or inactivation of markers during the isolation procedure.

Rabbits were killed with sodium pentobarbital (50 mg/kg). The kidneys were exposed and perfused via the renal arteries with buffer C (250 mM sorbitol, 12.5 mM NaCl, 0.5 mM disodium EDTA, in 5 mM histidine/imidazole buffer, pH 7.5) warmed to 37°C. All further procedures were carried out at 0–4°C. The renal cortices were dissected free from medullary regions with a razor blade. The cortices were put through a tissue press having a 1.5 mm pore size, and the tissue was then placed in glass test tubes containing buffer C (1 g tissue/8 ml buffer per tube). Homogenization was carried out with a Polytron homogenizer (Brinkmann Instruments) operated at maximum power for 20 s with 15 strokes (the test tubes moved up and down). During the period in which this isolation procedure was being developed, we experimented with other homogenization methods including the use of Dounce and glass-Teflon homogenizers and nitrogen cavitation. Use of the Polytron homogenizer provided for maximum disruption of the tissue in an isotonic buffer, and was also exceedingly rapid.

The initial homogenate was placed in 50-ml polypropylene test tubes (20–30 ml/tube) and centrifuged at 1500 × *g* in a Sorvall RC-2B centrifuge using the

SS-34 rotor. The supernatant (S_1) was removed and centrifuged at 110 000 × *g* for 30 min in a Beckman L2-65 ultracentrifuge using the Type 30 rotor. The supernatant (S_2) was decanted and the light-colored upper pellet (P_2-upper) was separated from the small underlying dark pellet (P_2-lower) with a spatula. P_2-upper was suspended in 8 ml of buffer C and homogenized with a glass-Teflon homogenizer (ten strokes at 700 rev./min). To nine parts of the homogenized tissue was added one part of 100 mM $CaCl_2$ in buffer C minus EDTA. The suspension was stirred for 20 min and layered on a discontinuous sorbitol density gradient. The density gradients were formed in 1 inch × $3\frac{1}{2}$ inch polyallomer tubes and consisted of 10 ml of 40% (w/v), 10 ml of 30%, 5 ml of 25% and 5 ml of 20% sorbitol in 5 mM histidine/imidazole buffer, pH 7.5, containing 12.5 mM NaCl. The density gradients were centrifuged at 18 000 × *g* for 15 min in the Beckman L2-65 ultracentrifuge using the SW-27 swinging bucket rotor. The gradients were fractionated as indicated in Fig. 1, using a Pasteur pipet. The brush border membranes remained in layer G_2 (20% and 25% sorbitol). To 10 ml of layer G_2 was added 25 ml of 300 mM mannitol in

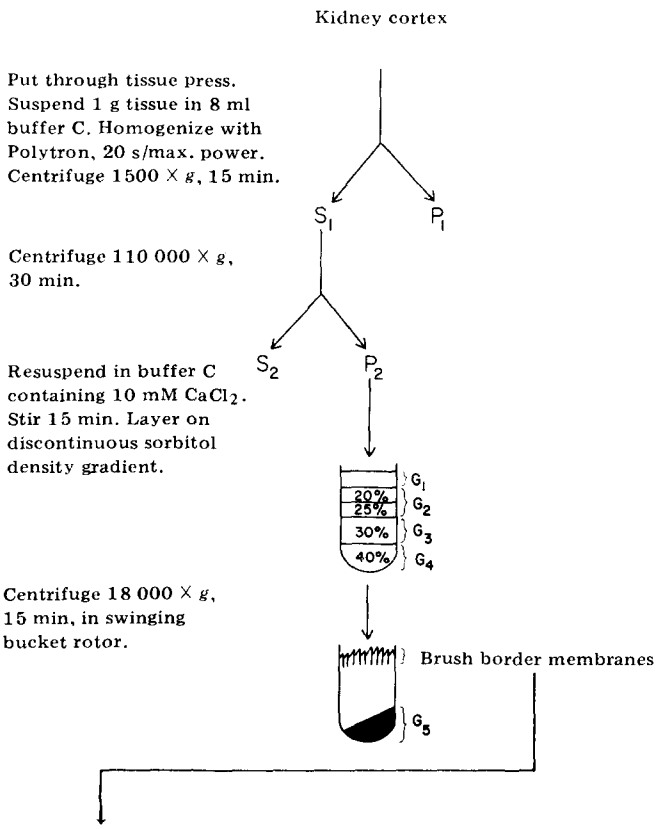


Fig. 1. Flow chart for the preparation of purified renal brush border membranes as described in the text.

1 mM Tris/Hepes buffer (1 mM Hepes brought to pH 7.5 with Tris). The brush border membranes were then pelleted by centrifugation at 50 000 × *g* for 45 min in the Sorvall RC-2B centrifuge. The small brush border membrane pellets were resuspended in a small volume (about 3 ml for the membranes from one rabbit) of the mannitol/Tris/Hepes buffer and stored at 0—4°C until use. The final brush border membrane concentration was 8—10 mg protein/ml of suspension.

Assays for membrane markers. Protein was measured by the BioRad protein assay (BioRad Laboratories). $(Na^+ + K^+)$-ATPase was determined by the method of Fujita et al. [15] with phosphate liberated determined by the method of Fiske and SubbaRow [16]. Trehalase was determined by the method of Dahlqvist [17] and succinate dehydrogenase by the method of Pennington [18]. NADPH-cytochrome *c* reductase was determined as described by Sottocasa et al. [19] and DNA by the procedure of Croft and Lubran [20].

Transport experiments. Uptake into renal brush border membranes was measured by a Millipore filtration technique. In most experiments, we added 50 μl of the brush border membrane suspension to 100 μl of uptake buffer in a 6 ml polypropylene test tube. Unless otherwise stated, the final composition of the uptake buffer was 100 mM mannitol, 100 mM NaCl in 1 mM Tris/Hepes, pH 7.5. The tube was shaken gently until the uptake was terminated by addition of 850 μl of ice-cold stop buffer consisting of 154 mM NaCl in 1 mM Tris/Hepes, pH 7.5 [1]. The suspension was rapidly filtered through a 0.45 μm HAWP Millipore filter. The stopping and filtration procedures took less than 10 s. The filter was then placed in 10 ml of Aquasol (New England Nuclear) and counted by liquid scintillation spectrometry.

Unless otherwise stated, the concentrations of *p*-aminohippuric acid, uric acid, and D- and L-glucose were 50 μM (isotope plus carrier). A blank was prepared in each experiment by adding membranes to a tube already containing substrate and cold stop buffer and filtering immediately. This blank value was subtracted from all uptake data.

Materials. *p*-Amino[*glycyl*-1-^{14}C]hippuric acid, 49.4 Ci/mol, D-[U-^{14}C]-glucose, 313 Ci/mol, and L-[1-^{3}H]glucose, 17.5 Ci/mmol, were obtained from New England Nuclear. [2-^{14}C]Uric acid, 57 Ci/mol, was obtained from Amersham Searle. All other chemicals were of the highest grade commercially available.

Results

Brush border membrane preparation

For following the distribution of cellular components throughout the purification procedure, we used markers whose location has been established by prior investigators. In particular, $(Na^+ + K^+)$-ATPase is presumed to be localized on the basal-lateral membranes [11] whereas alkaline phosphatase and trehalase [21] are found on the brush border membranes. The distribution of the various markers at each stage of membrane isolation is shown in Table I. We obtained a yield of alkaline phosphatase and trehalase of 20% and 14%, respectively, with an enrichment of ten and seven fold with respect to protein. The lesser enrichment of trehalase is due to the fact that the total recovery of

TABLE I

MARKER DISTRIBUTIONS DURING PURIFICATION OF RABBIT RENAL BRUSH BORDER MEMBRANES

Since the amount of tissue used in each membrane preparation varied, data are normalized to a constant value for the amount of each marker in the initial homogenate. All data are given as the mean of at least three determinations ± S.E. followed in parentheses by percent of amount in the initial homogenate. Units for $(Na^+ + K^+)$-ATPase, alkaline phosphatase and trehalase are μmol of substrate hydrolyzed/h. Units for succinate dehydrogenase are ΔE_{490}/h. Units for NADPH-cytochrome *c* reductase are ΔE_{550}/ min. Enrichment is the specific activity (units/mg protein) of the marker in the final brush border membranes (BBM) divided by the specific activity of the marker in the initial homogenate.

Fraction	Protein		$(Na^+ + K^+)$-ATPase		Alkaline phosphatase		Trehalase		Succinate dehydrogenase		NADPH-cytochrome *c* reductase		DNA	
	mg	(%)	Units	(%)	Units	(%)	Units	(%)	Units	(%)	Units	(%)	mg	(%)
Initial homogenate	2401		5661		1756		413		744		59.1		51.7	
P_1	1319 ± 24	(54.9)	3320 ± 326	(58.6)	806 ± 104	(45.9)	135 ± 27	(32.7)	678 ± 64	(91.1)	29.5 ± 2.1	(49.9)	33.9 ± 2.6	(65.6)
S_1	1182 ± 44	(49.2)	1566 ± 170	(27.7)	1008 ± 74	(57.4)	267 ± 43	(64.6)	84.3 ± 7.7	(11.3)	30.1 ± 2.5	(50.9)	16.3 ± 1.4	(31.5)
P_2-upper	333 ± 53	(13.9)	1183 ± 235	(20.9)	905 ± 25	(51.5)	189 ± 19	(45.8)	34.3 ± 7.0	(4.6)	5.7 ± 0.8	(9.6)	10.6 ± 0.9	(20.5)
P_2-lower	34.9 ± 3.3	(1.4)	32.4 ± 6.0	(0.6)	23.3 ± 8.4	(1.3)	13.2 ± 2.7	(3.2)	9.3 ± 2.5	(1.2)	1.2 ± 0.1	(2.0)	1.0 ± 0.1	(1.9)
S_2	678 ± 55	(28.2)	22.6 ± 5.0	(0.4)	102 ± 3.2	(5.8)	5.9 ± 3.5	(1.4)	9.1 ± 3.5	(1.2)	9.4 ± 1.0	(15.9)	2.4 ± 1.4	(4.6)
G_1	20.2 ± 3.2	(0.8)	1.6 ± 1.1	(0.1)	36.0 ± 5.6	(2.0)	6.8 ± 1.4	(1.6)	0.4 ± 0.1	(0.1)	N.D. *		N.D.	
G_2	67.2 ± 1.2	(2.8)	0.6 ± 0.2	(0.01)	333 ± 91	(19.0)	84.8 ± 5.3	(20.5)	2.7 ± 1.0	(0.4)	0.8 ± 0.2	(1.4)	1.1 ± 0.6	(2.1)
G_3	9.6 ± 4.8	(0.4)	12.5 ± 11.9	(0.2)	61.5 ± 13	(3.5)	12.4 ± 1.0	(3.0)	0.6 ± 0.2	(0.1)	0.1 ± 0.1	(0.2)	0.9 ± 0.4	(1.7)
G_4	4.9 ± 2.5	(0.2)	9.5 ± 1.0	(0.2)	32.3 ± 7.2	(1.8)	3.6 ± 1.8	(0.9)	0.8 ± 0.2	(0.1)	0.1 ± 0.1	(0.3)	0.9 ± 0.5	(1.7)
G_5	216 ± 8.3	(9.0)	1158 ± 327	(20.0)	302 ± 79	(17.2)	73.8 ± 3.7	(0.9)	26.4 ± 6.8	(3.6)	6.3 ± 0.3	(10.6)	7.6 ± 0.7	(14.7)
BBM	49.5 ± 4.3	(2.1)	13.1 ± 2.1	(0.1)	358 ± 47	(20.4)	59.2 ± 9.1	(14.3)	0.7 ± 0.4	(0.1)	0.2 ± 0.1	(0.4)	0.3 ± 0.1	(0.6)
Recovery	97%		78%		97%		76%		97%		76%		86%	
Yield	2%		0.2%		20%		14%		0.1%		0.3%		0.6%	
Enrichment	—		<0.2		10		7		<0.1		<0.2		<0.3	

* N.D. = Not determined.

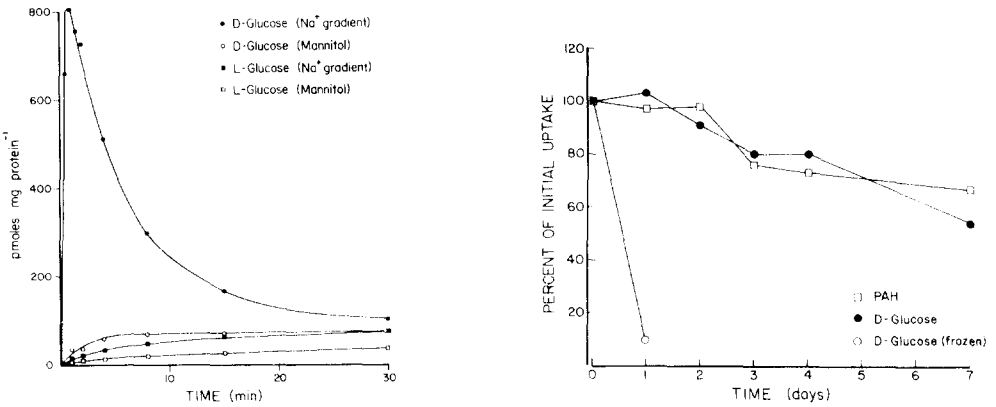


Fig. 2. The time course of the transport of D- and L-glucose in the presence and absence of a Na^+ gradient. The external medium was either 100 mM NaCl, 100 mM mannitol, or 300 mM mannitol, in 1 mM Tris/Hepes, pH 7.5. The uptake of D- and L-glucose (50 μM) were followed simultaneously by the use of double-label counting.

Fig. 3. The time course of viability of renal brush border membranes with respect to transport activity. The 1 min uptake of D-glucose and *p*-aminohippuric acid was measured under Na^+ gradient conditions. Membranes were stored at 0–4°C except for one aliquot which was frozen and stored at −20°C overnight.

trehalase throughout the isolation procedure was only 76% as compared to 97% for alkaline phosphatase, presumably because of inactivation of the trehalase. Brush border membranes prepared by this procedure are substantially free of contamination by basal-lateral membranes, mitochondria, nuclei and endoplasmic reticulum as judged by the fact that ($Na^+ + K^+$)-ATPase, succinate dehydrogenase, DNA and NADPH-cytochrome *c* reductase enrichments are all less than 0.3 (Table I), i.e. the brush border membranes are enriched at least 30 fold with respect to the other subcellular organelles.

As shown in Fig. 2, these membranes contain a highly selective system for the sodium-dependent transport of D-glucose. In this typical experiment, the initial rate of D-glucose uptake (at 50 μM medium D-glucose) in the presence of an initial gradient of 100 mM NaCl was 1320 pmol · mg^{-1} protein · min^{-1} compared with 31.2 pmol · mg^{-1} protein · min^{-1} in the absence of sodium. This difference in initial rates of D-glucose uptake in the presence and absence of sodium is virtually the same as reported by Aronson and Sacktor [1], i.e. greater than 40-fold. At 30 s we obtained a D-/L-glucose ratio of 85 which dropped gradually to 1.4 by 30 min. The transport of L-glucose, while small, was consistently doubled by the presence of sodium, lithium or potassium (see also Table I, Ref. 3).

As shown in Fig. 3, brush border membranes prepared by our procedure retain their capacity to transport D-glucose and *p*-aminohippuric acid (and presumably other substrates) for an extended period of time. After 48 h, the D-glucose transport was 91% and the *p*-aminohippuric acid transport 98% of the transport rates immediately after membrane preparation. Even after 7 days, 54% of the D-glucose and 67% of the *p*-aminohippuric acid transport activity remained. These data suggest that it should be possible to utilize such membranes for transport experiments for at least several days after they are

prepared. Freezing the membranes at −20°C overnight reduced the transport rate of D-glucose to less than 10% of the non-frozen tissue, thus the membranes cannot be stored in this manner to be used in transport studies.

Time course of uric acid and p-aminohippuric acid transport

In initial experiments (data not shown) we determined that uric acid uptake into renal brush border membranes is inversely proportional to medium osmolarity, indicating that uptake occurs into an osmotically reactive space. Similar data have been presented for *p*-aminohippuric acid uptake into brush border membranes [7]. Shown in Fig. 4 is the time course of uptake of uric acid and *p*-aminohippuric acid into renal brush border membranes. In the absence of any salt, the uptake of uric acid was exceedingly slow (0.9 ± 0.6 pmol · mg^{-1} protein · min^{-1}, mean ± S.E.). LiCl, KCl and NaCl (100 mM, external) facilitated uptake of uric acid to the same extent increasing the initial uptake rate to about 15 pmol · mg^{-1} protein · min^{-1}. In a single experiment (not shown) in which the membranes were preequilibrated with 100 mM NaCl, there was no difference between uric acid uptake into the preequilibrated membranes and uric acid uptake under Na^+ gradient conditions. Uptake of uric acid in the presence of a NaSCN gradient was slightly slower than with LiCl, NaCl or KCl, and uptake with a gradient of choline chloride was about 50% of that with LiCl, NaCl or KCl.

Similar data were obtained for the effects of various salts on the uptake of *p*-aminohippuric acid into renal brush border membranes. In the absence of any salt, the initial rate of *p*-aminohippuric acid uptake was 2.7 ± 0.3 pmol · mg^{-1} protein · min^{-1}. Gradients of 100 mM KCl, LiCl and NaCl increased the initial uptake rate to 24.8 ± 6.6, 27.7 ± 7.6 and 33.0 ± 6.6 pmol · mg^{-1} protein · min^{-1}, respectively. Similar gradients of NaSCN and choline chloride increased the initial rate of *p*-aminohippuric acid uptake to 24.8 ± 2.1 and 13.4 ± 5.9 pmol · mg^{-1} protein · min^{-1}. When the membranes were preequilibrated

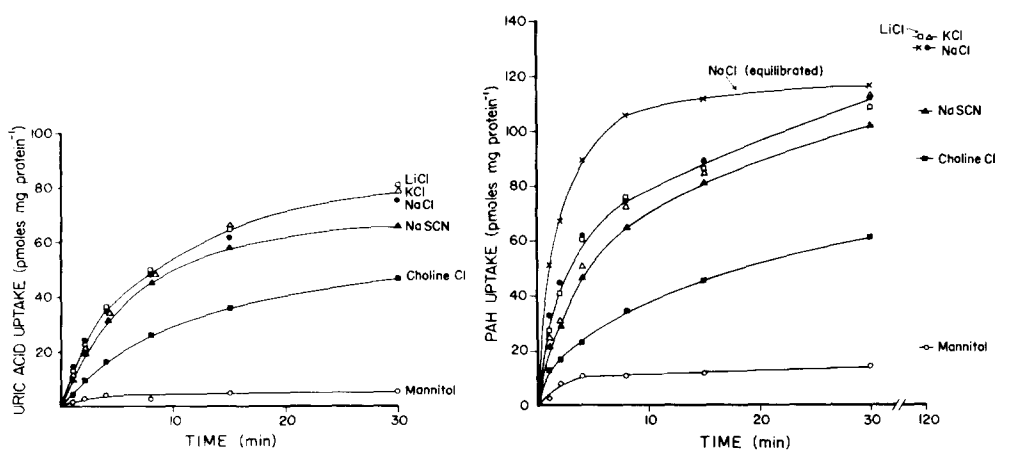


Fig. 4. The time course of the uptake of uric acid and *p*-aminohippuric acid (50 μM) into brush border membranes. Media contained 100 mM salt and 100 mM mannitol in 1 mM Tris/Hepes, pH 7.5. NaCl equilibrated indicates that the membranes were preequilibrated with NaCl-containing buffer prior to beginning the uptake. Data are the mean of three experiments.

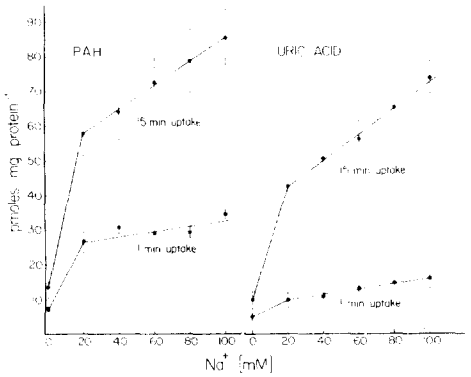


Fig. 5. Uptake of uric acid and *p*-aminohippuric acid in the presence of varying concentrations of NaCl. NaCl was varied by isoosmotic replacement with mannitol. Data are the mean of at least three experiments ± S.E.

with 100 mM NaCl, the uptake of *p*-aminohippuric acid was even more rapid than under gradient conditions, indicating that the main requirement for the facilitation of *p*-aminohippuric acid transport is the presence of NaCl rather than a NaCl gradient. Even after 16 h (data not shown), uptake in the absence of a salt is only 15% of that in the presence of NaCl suggesting that these membranes may be exceedingly tight to substrates in the absence of salt.

Effect of NaCl concentration on uric acid and p-aminohippuric acid uptake

In Fig. 5 are shown data for the NaCl dependence of the transport of uric acid and *p*-aminohippuric acid. The curves for both compounds are similar and of a biphasic nature, with a strictly linear NaCl dependence from 20 mM to

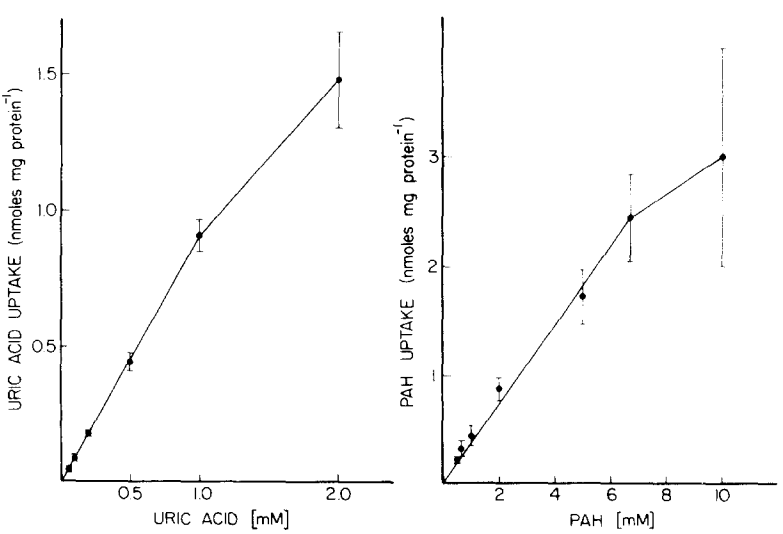


Fig. 6. Effect of varying the uric acid and *p*-aminohippuric acid concentrations on the uptake of uric acid and *p*-aminohippuric acid. Data were obtained as described in the text using a 6 min time point for uric acid and a 1 min time point for *p*-aminohippuric acid. Data shown are for Na^+-dependent uptake (uptake in the presence of 100 mM NaCl minus uptake in the absence of NaCl).

100 mM. These data differ from those for the NaCl dependence of glucose uptake in brush border membranes in which at least partial saturation of the effect of NaCl has been reported [1,3]. However, the data are quite similar to those reported by Fass et al. [5] for the effect of NaCl on L-alanine transport in renal brush border membranes.

Effect of uric acid and p-aminohippuric acid concentration

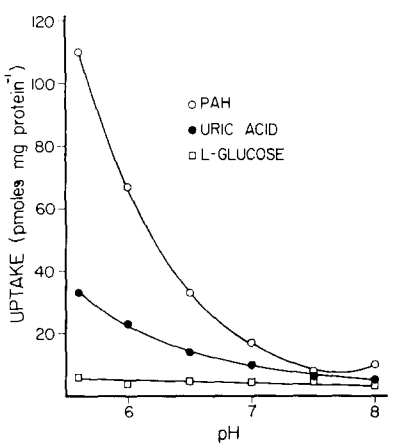
Shown in Fig. 6 are the curves for the concentration dependence of the uric acid and *p*-aminohippuric acid uptake. No evidence for saturation of the transport of either compound is seen in this data. The uptake of uric acid could not be studied at higher concentrations due to its limited solubility under the conditions of these experiments.

Effect of pH on the uptake of uric acid, p-aminohippuric acid and L-glucose

Shown in Fig. 7 are the effects of varying the pH on the uptake of *p*-aminohippuric acid, uric acid and L-glucose by brush border membranes. Decreasing the pH from 7.5 to 5.6 increased the *p*-aminohippuric acid uptake from 8 pmol · mg^{-1} protein · min^{-1} to 110 pmol · mg^{-1} protein · min^{-1}, and uric acid uptake from 7 pmol · mg^{-1} protein · min^{-1} to 33 pmol · mg^{-1} protein · min^{-1}. The uptake of L-glucose was unaffected by variations in pH.

Effects of temperature on the uptake of uric acid, p-aminohippuric acid and glucose

Shown in Fig. 8 are the results of studies of the temperature dependence of



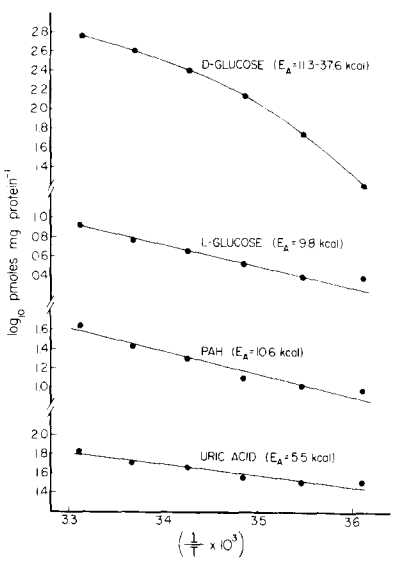


Fig. 7. Effect of pH on the uptake of uric acid, *p*-aminohippuric acid and L-glucose. Uptake buffer was 300 mM mannitol in Tris/Hepes. The data are mean values for 1 min uptakes from two experiments done in duplicate.

Fig. 8. Temperature dependence of the uptake of *p*-aminohippuric acid, uric acid, and D- and L-glucose into brush border membranes. Uptake was determined under Na^+ gradient conditions as described using the following time points: D- and L-glucose, 15 s; *p*-aminohippuric acid, 1 min, and uric acid, 6 min.

TABLE II

EFFECTS OF DRUGS ON THE UPTAKE OF 50 μM URIC ACID, *p*-AMINOHIPPURIC ACID, D-GLUCOSE, AND L-GLUCOSE BY BRUSH BORDER MEMBRANES

Uptake was measured at 1 min for *p*-aminohippuric acid, D-glucose and L-glucose, and at 6 min for uric acid. Drug concentrations were 1 mM. Uptake is given as pmol · mg^{-1} protein. Data are mean of three experiments ± S.E. *P* determined by Student's *t*-test for paired data. n.s., not significant.

Drug	Uric acid		*p*-Aminohippuric acid		D-Glucose		L-Glucose	
	Uptake	% change from control	Uptake	% change from control	Uptake	% change from control	Uptake	% change from control
Control	50.4 ± 4.3	—	33.5 ± 5.7	—	921 ± 177	—	17.5 ± 3.6	—
Probenecid	39.9 ± 3.9	−21 ($P < 0.025$)	17.0 ± 3.9	−49 ($P < 0.005$)	820 ± 147	−11 ($P < 0.025$)	16.3 ± 3.2	−7 (n.s.)
Phenylbutazone	51.1 ± 10.3	+1 (n.s.)	15.5 ± 3.0	−54 ($P < 0.005$)	882 ± 155	−4 (n.s.)	14.7 ± 3.8	−16 (n.s.)
Sulfinpyrazone	27.9 ± 1.6	−45 ($P < 0.025$)	14.7 ± 3.4	−56 ($P < 0.01$)	803 ± 145	−13 ($P < 0.01$)	15.1 ± 3.2	−14 ($P < 0.05$)
Ethacrynic acid	30.9 ± 0.6	−39 ($P < 0.01$)	12.4 ± 3.5	−63 ($P < 0.05$)	681 ± 107	−26 ($P < 0.025$)	15.6 ± 3.5	−11 ($P < 0.05$)
Pyrazinoic acid	53.4 ± 6.0	+6 (n.s.)	34.5 ± 9.1	+3 (n.s.)	829 ± 95	−10 ($P < 0.025$)	15.7 ± 2.8	−10 (n.s.)
Sodium salicylate	49.5 ± 5.8	−2 (n.s.)	38.8 ± 9.3	+16 (n.s.)	837 ± 137	−9 (n.s.)	17.1 ± 4.4	−3 (n.s.)
Phlorizin	41.2 ± 3.7	−18 ($P < 0.05$)	20.5 ± 6.0	−39 ($P < 0.05$)	32 ± 2	−97 ($P < 0.005$)	15.1 ± 3.8	−14 (n.s.)
Ouabain	51.0 ± 4.6	+1 (n.s.)	32.5 ± 6.9	−3 (n.s.)	923 ± 166	+2 (n.s.)	15.5 ± 3.4	−11 (n.s.)

the uptake of D- and L-glucose, uric acid and *p*-aminohippuric acid. The Arrhenius plots for the uptake of uric acid, *p*-aminohippuric acid and L-glucose were linear and gave apparent activation energies of 5.5, 10.6, and 9.8 kcal/mol, respectively. Similar data for uric acid and *p*-aminohippuric acid have not been reported previously; however Kinne et al. [2] reported a value of 5.8 kcal/mol for the uptake of L-glucose in renal brush border membranes prepared from the rat. These same investigators also reported [3] that the Arrhenius plot for D-glucose transport in the rat membranes was biphasic with apparent activation energies of 5.5 and 17.5 kcal/mol. Our data, in contrast, show a curvilinear effect with the apparent activation energy varying from 11.3 to 37.6 kcal/mol.

Effects of various drugs on the uptake of uric acid, p-aminohippuric acid and glucose

Shown in Table II are the effects of several drugs on the uptake of uric acid, *p*-aminohippuric acid and D- and L-glucose. The drugs have all been shown in a prior publication to affect the renal transport of uric acid [22]. Probenecid inhibited uric acid uptake by 21% and *p*-aminohippuric acid uptake by 49%, with only a slight effect on the transport of D-glucose (—11%). These data differ significantly from those of Berner and Kinne [7] who obtained only 16% inhibition of the transport of 10 μM *p*-aminohippuric acid by 0.5 mM probenecid, in brush border membranes prepared from rat kidney, but are in agreement with in vivo data in the rabbit reported by Foulkes [23]. Sulfinpyrazone and ethacrynic acid also caused significant inhibition of *p*-aminohippuric acid and uric acid uptake, with slight effects on the transport of D-glucose. Phlorizin inhibited D-glucose transport by 97% and also caused significant inhibition of the transport of uric acid and *p*-aminohippuric acid.

Discussion

We have devised a preparation of rabbit renal cortical brush border membranes for these studies of uric acid and *p*-aminohippuric acid transport that is worthy of comment. The tissue was homogenized in such a fashion that low speed centrifugation separated most of the brush borders from basal-lateral membranes, mitochondria, nuclei and endoplasmic reticulum. The brush borders were then separated from the soluble proteins and light microsomes by centrifugation at 110 000 $\times g$ for 30 min, and then were purified using a combination of a calcium precipitation procedure and density gradient centrifugation. The brush borders were recovered from the light region of the density gradient (20—25% sorbitol) in contrast to intestinal brush borders which are harvested from the heavy end of a sorbitol density gradient (45—55% sorbitol) [12]. This difference in density may be at least partly accounted for by a much higher protein/lipid ratio in the intestinal brush borders related to the important hydrolytic function of the intestine. The yield of brush borders in this analytical study was 20% and they were enriched 10-fold with respect to alkaline phosphatase activity. This is probably a minimum estimate of yield and purification since it is now appreciated that as much as 25% of the alkaline phosphatase may be in the renal basal-lateral membranes [24]. In the intestine,

40—55-fold enrichments of brush borders have been reported using sucrase as a marker enzyme [12]. Trehalase is less than satisfactory as a renal brush border marker owing to inactivation of the enzyme during the isolation procedure (Table I). As judged by the observation that $(Na^+ + K^+)$-ATPase, succinate dehydrogenase, NADPH-cytochrome *c* reductase and DNA are enriched by less than a factor of 0.3, the brush borders prepared by our procedure are essentially free of contamination by basal-lateral membranes, mitochondria, endoplasmic reticulum and nuclear material.

Prior to studies of uric acid and *p*-aminohippuric acid transport, we carried out preliminary experiments to compare the transport properties of these vesicles with those reported in the literature. The stereospecific, sodium-dependent uptake of glucose was essentially identical to that reported previously for rabbit renal brush borders [1]. The ratio of D-/L-glucose uptake at 30 s was 85, and sodium increased the initial rate of D-glucose uptake by a factor of 43. Although the rate of L-glucose uptake was doubled in the presence of a sodium gradient, there was no overshoot and both the D- and L-glucose uptake in the steady state (at 30 min) gave an intravesicular space of 2 $\mu l \cdot mg^{-1}$. The rate of L-glucose uptake and the steady-state glucose spaces were virtually identical to those found previously in rabbit and rat [1,3].

A major difference was observed in the temperature dependence of glucose uptake. Arrhenius plots of the L-glucose uptake in rabbit (Fig. 8) and rat [3] are linear, but in rabbit the activation energy was 9.8 as opposed to 5.8 in the rat. In both rat and rabbit the Arrhenius plots were non-linear for D-glucose, although we obtained a strictly curvilinear plot (Fig. 8) for the rabbit as opposed to the biphasic plot obtained for the rat [3]. In rabbit, the activation energy was 38 kcal/mol below 10°C and approached 11 kcal/mol above 30°C, while in the rat the corresponding activation energies were 18 and 5.5 kcal/mol. The non-linear Arrhenius plots suggest that more than one process controls the rate of D-glucose permeation across the brush border plasma membrane. Similar temperature-dependent activation energies have been observed for facilitated diffusion of D-glucose in renal basal-lateral membranes [3], intestinal basal-lateral membranes [25] and human red blood cells [26]. The molecular interpretation of these effects is unclear.

A final point about the brush border membranes described is that when the membranes were stored at 0°C in mannitol buffer, the initial rate of the sodium-dependent uptake of D-glucose fell by only 5%/day for at least 7 days. Freezing the membranes overnight, on the other hand, reduced transport by 90%.

In the presence of sodium gradients, there was an increase in the initial rate of uptake of D- and L-glucose, *p*-aminohippuric acid and uric acid (Figs. 2 and 4). The effect on D-glucose is undoubtedly due to cotransport of sodium and the sugar across the membrane. However, in the case of L-glucose, *p*-aminohippuric acid and uric acid the increase in uptake is due at least in part to a nonspecific ionic strength effect. This is likely simply to be a general increase in the passive permeability of the membrane. Similar observations have been made with intestinal basal-lateral membranes (Mircheff, A.K. and Wright, E.M., unpublished results).

The initial rate of *p*-aminohippuric acid, uric acid and L-glucose uptakes into the brush border membrane vesicles was measured over the pH range of 8.0—

5.6 (Fig. 7). The pK values of uric acid are 11.3 and 5.4 while the pK for p-aminohippuric acid is 3.8. The uptake of both p-aminohippuric acid and uric acid increased as the pH was decreased from 7.5 to 5.6 but there was no significant change with L-glucose uptake. This suggests that the undissociated form of each of the two weak acids is more permeable than the anionic form. The increase in uptake occurring with the decrease in pH from 7.5 to 5.6 was a factor of 3—4 rather than the factor of 10 expected if $P_{HA} >> P_A$. The fact that the uptake of uric acid was less than that for p-aminohippuric acid at all pH values tested indicates that P_{HA}(uric acid) $< P_{HA}$ p-aminohippuric acid, and this is consistent with our unpublished observation that the p-aminohippuric acid olive oil/water partition coefficient is higher than that for uric acid.

We examined the effects of a number of drugs on the uptake of p-aminohippuric acid, uric acid and D- and L-glucose by brush border membranes (Table II). With the exception of phlorizin, which inhibited D-glucose uptake by 97%, the effects of these compounds on the uptake of sugars were very small and were likely due to nonspecific membrane effects. Probenecid, phenylbutazone, sulfinpyrazone and ethacrynic acid (1 mM) inhibited the uptake of p-aminohippuric acid (50 μM) by about 50%, while the effects of these drugs on uric acid uptake were somewhat smaller. In a previous publication we reported that the effects of these same compounds on the uptake of uric acid by separated renal tubules were substantially greater [22]. For example, $2 \cdot 10^{-5}$ M sulfinpyrazone and $2.6 \cdot 10^{-4}$ M probenecid inhibited the uptake of 1 mM uric acid by 50% [22]. Thus we conclude that the major effect of these drugs on organic acid transport by the proximal tubule must occur at the basal-lateral membrane rather than at the brush border membrane. This agrees with the report of Berner and Kinne [7] who found a much greater effect of probenecid on p-aminohippuric acid transport in basal-lateral membranes as compared with brush border membranes.

The data presented here supports the conclusion of Berner and Kinne [7] that p-aminohippuric acid transport across the renal brush border membrane occurs primarily by a solubility-diffusion mechanism. The same conclusion applies to uric acid transport as well. Although evidence in the literature suggests bidirectional active transport of uric acid in the proximal tubule [27], our data indicate that any 'carrier-mediated' active transport of uric acid must be a property of the renal basal-lateral (peritubular) membrane.

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