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Sugar Transport by Renal Plasma Membrane Vesicles

Characterization of the Systems in the Brush-Border Microvilli and Basal-Lateral Plasma Membranes

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Summary. Uptake studies of D- and L-glucose were performed on vesicles derived from brush-border and basal-lateral membranes. The uptake of the sugars into the vesicles was osmotically sensitive and independent of glucose metabolism. In brush-border vesicles D-glucose but not L-glucose transport was Na^+-dependent, was inhibited by phlorizin, and showed a transitory vesicle/medium ratio > 1, in the presence of an initial Na^+ gradient. Basal-lateral membranes take up D-glucose faster than L-glucose, but the D-glucose uptake is significantly less sensitive to sodium removal and only moderately inhibited by phlorizin as compared to the brush-border fraction.

The asymmetry of an epithelial tissue, where the luminal and basal surfaces of the component cells possess different transport properties, seems to be the mechanism whereby vectorial net transfer of a substance is achieved. In renal proximal tubule, the relative contribution of the brush-border membrane and the basal-lateral membrane to transepithelial transport processes is difficult to assess in the intact organ. More recently, progress has been made by simultaneous microperfusion of both sides of the tubule in the intact kidney *in vivo* (Frömter, Muller & Knauf, 1969), perfusion of the isolated tubule *in vitro* (Tune & Burg, 1971), or experiments on the specialized cyst structure of the flounder tubule (Kleinzeller & McAvoy, 1973).

Simplification of kidney system became feasible with development of methods capable of separating brush-border microvilli and basal-lateral membranes (Heidrich, Kinne, Kinne-Saffran & Hannig, 1972). Studies of

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transport in subcellular vesicles derived from bacterial membranes (Kaback, 1972), sarcoplasmic reticulum (Hasselbach, Fiehn, Makinose & Migala, 1969), neural vesicles (Hazelbauer & Changeux, 1974), red cell ghosts (Benes, Kolínská & Kotyk, 1972), adipocytes (Illiano & Cuatrecasas, 1971), ascites cells (Colombini & Johnstone, 1974), intestinal brush borders (Hopfer, Nelson, Perotto & Isselbacher, 1973), and unfractionated renal membranes (Busse, Elsas & Rosenberg, 1972), for example, suggested further investigation of the transport properties of the separated fractions.

Accordingly, the transport characteristics of isolated brush-border microvilli and basal-lateral membranes for D- and L-glucose have been determined. The data indicate that the isolated membrane fragments differ not only as shown recently in their enzyme content, but also in their transport properties. Some of these data have been presented in a preliminary form (Kinne, Kinne-Saffran & Murer, 1973).

Materials and Methods

Membrane Purification

Partially purified renal membranes were obtained by differential centrifugation from rat kidney following homogenization in isotonic sucrose as previously described (Pockrandt-Hemstedt, Schmitz, Kinne-Saffran & Kinne, 1970). These membranes were then separated into brush-border and basal-lateral membrane fractions using the Desaga FF4 free-flow electrophoresis machine as detailed before (Heidrich *et al.*, 1972).

Criteria of Purity

The fractions were routinely assayed as previously described for enzymes shown to be characteristic of brush-border microvilli, basal-lateral membranes, mitochondria and endoplasmic reticulum, namely alkaline phosphatase, (Na^+K^+)-ATPase, succinic dehydrogenase and glucose-6-phosphatase (Heidrich *et al.*, 1972). Protein was determined after precipitation of the membranes with 5% ice-cold TCA by the Lowry procedure, with bovine serum albumin as a standard (Lowry, Rosebrough, Farr & Randall, 1951).

Transport Studies

The membrane fractions obtained after electrophoretic separation were suspended by homogenization with a teflon glass homogenizer (10 strokes, 1,200 rpm) in 15 ml of a solution containing 100 mM mannitol, 1 mM Tris-HEPES[1] buffer, pH 7.4, and centrifuged for 20 min at $30{,}000 \times g$ at 4 °C. The pellets were resuspended in 1 ml of the same buffer, using a syringe fitted with a fine needle, centrifuged once more, resuspended in the same solution and diluted to a protein concentration of about 10 mg/ml. The incubation medium contained, unless otherwise stated, 100 mM mannitol, 100 mM NaCl, 1 mM Tris-HEPES buffer, pH 7.4, 1 mM D-glucose containing 5 μCi H^3-D-glucose, 1 mM L-glucose containg 2 μCi C^{14}-L-glucose. The stop solution contained 150 mM

1 Tris-N-2-hydroxyethylpiperazine-N′-2-ethane-sulfonic acid.

NaCl, 0.2 mM phlorizin, 10 mM Tris-HEPES buffer, pH 7.4. Osmotic pressure was varied by adding sucrose or raffinose to the incubation medium. In the latter experiments a "stop" solution was used which contained 450 mM NaCl, 10 mM Tris-HEPES, pH 7.4, and 0.2 mM phlorizin.

Uptake experiments were carried out in 1 ml disposable Eppendorf tubes according to the method of Hopfer *et al.* (1973). A portion of 20 μliters of membrane suspension was added at zero to 100 μliters of incubation medium at appropriate temperature and pH (usually 25 °C, pH 7.4). Uptake was stopped by withdrawing 20 μliters of the incubation mixture and adding this to 1 ml of ice-cold "stop" solution. The resultant suspension was rapidly filtered through a millipore filter (HAWP, 0.45 μ) and washed once with 3 ml of ice-cold "stop" solution, the total time being 20 sec. The filter was dried and counted in a Packard liquid scintillation counter in Instagel® (Packard). A medium sample was also counted with each experiment. It was shown by direct efflux measurement that less than 10% of trapped D-glucose counts were lost during the stop and filtration procedure.

Efflux experiments were carried out by preincubating a fivefold greater concentration of membranes in a radioactive D + L sugar medium (100 mM mannitol, 100 mM NaCl, 1 mM Tris-HEPES, 2 mM D- and L-glucose each) for 30 min. At 0 time a 20 μliter sample of the radioactive membrane suspension was added to 80 μliters of medium containing 100 mM mannitol, 1 mM Tris-HEPES buffer, pH 7.4 at 25 °C, and 100 mM NaCl; other additions as noted in the text. Sampling was carried out as before, and the retained counts determined by millipore filtration as described above. In all cases, incubation of membranes denatured by boiling for 150 min, or filtration of membranes added to the stop solution directly before addition of 20 μliters of radioactive incubation medium showed that no significant medium radioactivity was retained on the filters following the washing procedure.

Analysis of Intravesicular Content

Membranes were incubated with a sugar mixture as described above, for 2 and 10 min and filtered on millipore filters. The filters were extracted with 3 ml distilled water for 24 hr at 4 °C, the extract centrifuged and the supernatant concentrated. Separation of potential products of glucose was carried out by thin-layer chromatography on silical gel using H_2O/acetone 1:9 as solvent. Controls were run in which the radioactive glucose was added subsequent to the incubation. The chromatograms were cut into 1-cm sections and the sections counted.

Results

Fractionation of Renal Cortical Plasma Membranes

Fig. 1 shows the relative enrichment of alkaline phosphatase and $(Na^+ + K^+)$-ATPase in the total homogenate, the unfractionated plasma membranes, the brush-border fraction and the basal-lateral membranes isolated in this study. Compared to the rat kidney cortex homogenate, alkaline phosphatase is enriched approximately 10 times in the brush-border fraction, and the specific activity of $(Na^+ + K^+)$-ATPase in the basal-lateral membranes is increased about 12-fold. Less than 10% cross-contamination occurs between brush-border microvilli and basal-lateral

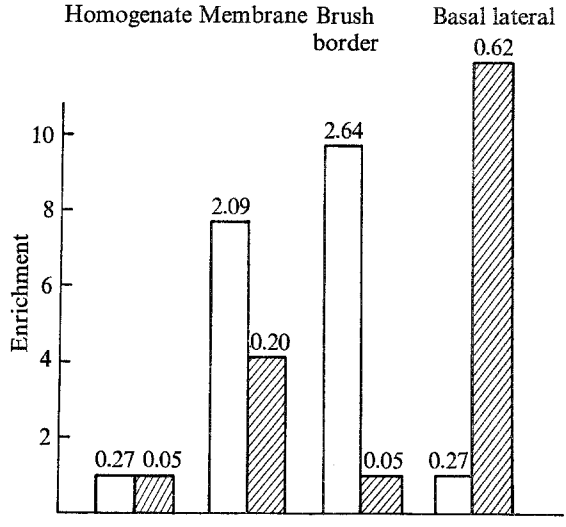

Fig. 1. The activity of alkaline phosphatase (clear) and (Na^+K^+)-ATPase (shaded) in rat kidney cortex homogenates, unfractionated plasma membranes, brush-border microvilli and basal-lateral membranes purified by free-flow electrophoresis. The data (n = 5) are expressed as enrichment compared to the homogenate. Specific activity is expressed as milliunits mg^{-1} protein as stated above the bars

membranes as determined by the specific activities of the marker enzymes in the final preparations. Marker enzymes for other cellular components (soluble protein, mitochondria and endoplasmic reticulum) were always lower in the membrane fraction obtained by electrophoresis than in the starting material (Heidrich *et al.*, 1972).

Uptake, Binding and Metabolism

Uptake of D- and L-glucose by both membrane fractions was found to be time dependent. Crucial to the interpretation of this finding is the distinction between transport, binding and metabolism of the sugars by the membranes. Fig. 2*A* and *B* show that varying the osmotic pressure of incubation medium by the addition of sucrose reduces uptake of D-glucose by either membrane fraction. The data are plotted as uptake against reciprocal of osmolarity of the incubation medium, and indicate an inverse relationship of uptake and osmolarity. At infinite osmolarity there is 0 uptake for both fractions, indicating that the uptake as measured under experimental conditions is a function of an osmotically sensitive intravesicular space and does not represent simple binding to the membranes.

No metabolic conversion of D- or L-glucose was found following uptake into the membrane vesicles. All the counts obtained from the intra-

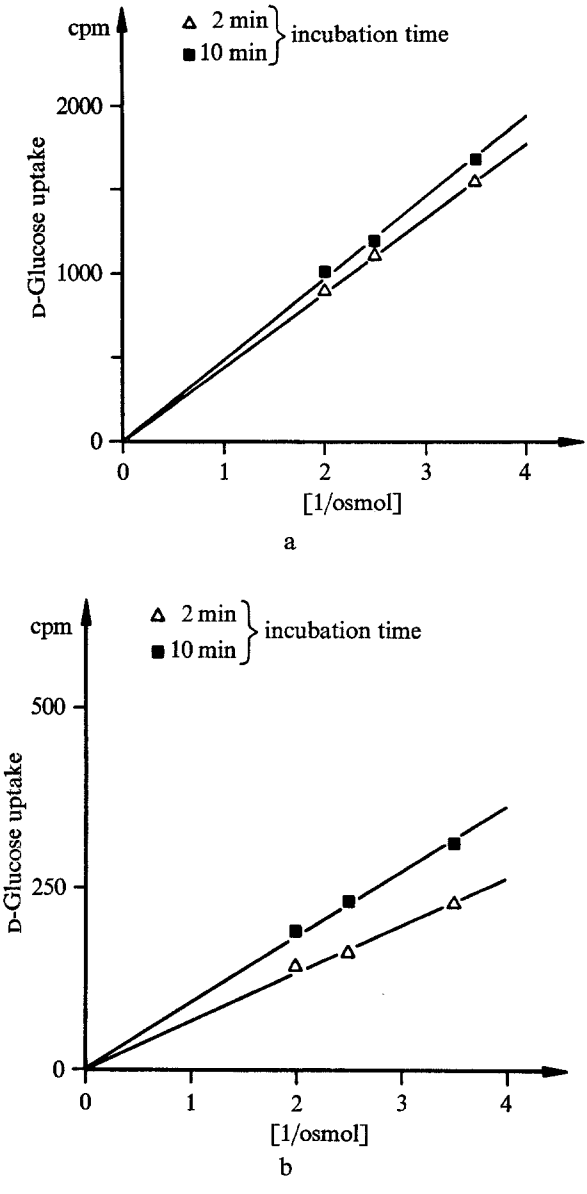

Fig. 2 (a). A plot of D-glucose uptake as a function of medium osmolarity^{-1} showing a straight-line relationship at both 2 and 10 min, and intersection at $y=0$, when osmolarity is ∞, for brush-border membranes. (b) A plot of D-glucose uptake as a function of medium osmolarity^{-1} showing a straight line relationship at both 2 and 10 min, and intersection at $y=0$, when osmolarity is ∞, for basal-lateral membranes

vesicular glucose coincided on the chromatogram with the counts observed with glucose added subsequently to uptake, or with the original radioactive glucose sample.

α-Methyl glucoside, a nonmetabolizable sugar, shows uptake kinetics similar to D-glucose, this result again emphasizing that metabolism does not play a role in our data (*see below*).

Uptake of Sugar by Brush-Border Membrane Vesicles

General Properties. When brush-border membranes are incubated in a medium containing equal concentrations of D- and L-glucose, the initial rate of D-glucose uptake is at least one order of magnitude faster than that of L-glucose (Table 1); therefore the ratio of D-glucose to L-glucose in the membrane vesicles greatly exceeds that of the medium which is set experimentally at 1. After 25 min of incubation the L-glucose level inside the ve-

Table 1. Uptake of D- and L-glucose by brush-border membrane vesicles

Solution (mM)	Time (min)							
	0.25	0.5	1.0	1.5	2	3	5	25
(a) Uptake of D-glucose								
100 NaCl	75.4 ±13.5 (n=5)	89.7 ±3.4 (n=2)	121.2 ±4.1 (n=5)	121 ±7.2 (n=2)	118.6 ±11.2 (n=5) $p<0.02$	118.0 ±12.0 (n=2)	106.2 ±9.9 (n=5)	100%
100 KCl		16.1 ±1.5 (n=4)		26.6 ±4.2 (n=4)		32.0 ±3.3 (n=4)		73.6 ±13.1 (n=4)
100 NaCl +0.1 phlorizin		16.5 ±3.4 (n=4)		24.1 ±5.4 (n=4)		30.7 ±6.1 (n=4)		71.9 ±13.4 (n=4)
(b) Uptake of L-glucose								
100 NaCl		6.3 ±0.8 (n=4)		11.0 ±1.4 (n=4)		15.2 ±0.7 (n=4)		40.6 ±1.9 (n=4)
100 KCl		6.7 ±0.8 (n=4)		12.2 ±3.0 (n=4)		13.9 ±1.6 (n=4)		36.0 ±1.6 (n=4)
100 NaCl +0.1 phlorizin		7.9 ±1.0 (n=4)		11.3 ±0.9 (n=4)		15.9 ±0.8 (n=4)		40.9 ±2.3 (n=4)

Mean values derived from n experiments are given with the standard deviation. The results are expressed as percent of the value obtained for D-glucose after 25 min of incubation in the NaCl-containing medium. This value amounted to 3.2±0.5 nmoles/mg protein (n=4).

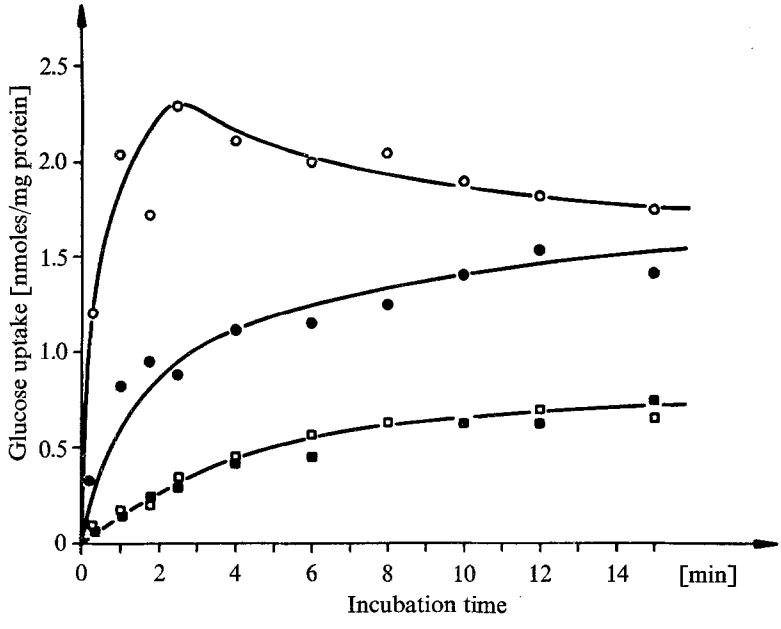

Fig. 3. Uptake of D- and L-glucose by brush-border vesicles in the presence of a Na^+ gradient (o----o D-glucose, □—□ L-glucose) or in the presence of Na^+ with no gradient, obtained by preincubation in NaCl (•----• D-glucose, ▪—▪ L-glucose). The vesicles were preincubated either in a medium containing 100 mM mannitol and 1 mM Tris-HEPES, pH 7.4, or in the same solution containing in addition 100 mM NaCl. Uptake was studied by transferring the membranes to a medium containing 100 mM mannitol, 1 mM Tris-HEPES, and 100 mM NaCl at pH 7.4

sicles is about 40% that of D-glucose, indicating that the rate of L-glucose penetration is quite slow. This rate is identical to that of mannitol as measured in independent experiments. Table 1 also shows that D-glucose but not L-glucose uptake into brush-border membrane vesicles is inhibited by 0.1 mM phlorizin. At 25 min only D-glucose can be considered to have equilibrated with the medium since at 40 min the D-glucose level has not changed whereas L-glucose or D-glucose in the presence of phlorizin have increased but still have not reached the D-glucose level.

Influence of Na^+. When sodium chloride is replaced by potassium chloride the rate of uptake of D-glucose is reduced to the level obtained in the presence of 0.1 mM phlorizin. The sodium dependence of D-glucose uptake by the brush-border vescicles may be due to the Na^+ gradient (medium-vesicle) present in the above-mentioned experiments or due to an interaction of Na^+ with the membrane system facilitating D-glucose movement even in the absence of a gradient. Fig. 3 shows uptake of D- and

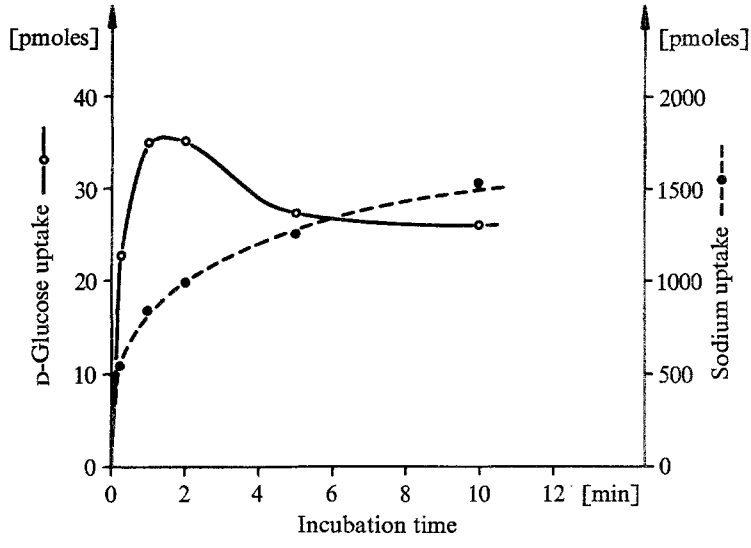

Fig. 4. A plot of D-glucose and Na^+ uptake by brush-border vesicles, using D-glucose-H^3 and Na^{22}, showing approximately a 50-fold greater initial Na^+ uptake, under standard conditions. The medium contained 100 mM mannitol, 1 mM Tris-HEPES and 100 mM sodium chloride labeled with ^{22}Na (specific activity). At the peak of glucose uptake there is still a twofold gradient of Na^+ into the vesicles

L-glucose into brush-border membrane vesicles preincubated in Na^+-free solution or in a solution containing 100 mM NaCl for 20 min at 25 °C. In both cases there was a greater uptake of D-glucose compared to L-glucose. Under the conditions where the intravesicular fluid is initially sodium free (*see* Table 1 and Fig. 3), the time course of D-glucose uptake shows the presence of an overshoot – i.e., the amount of glucose present in the vesicles transiently is higher (about 20–30%) than the amount found after incubation for 25 min. L-glucose uptake is not influenced by sodium or the sodium gradient.

The overshoot probably indicates an accumulation of D-glucose inside the vesicles which is presumably due to the persistence of a Na^+ gradient when the intravesicular glucose has reached the glucose concentration of the outside medium (the starting conditions have a 100:1 Na/glucose ratio in the medium). This implies that Na^+ uptake should be slow enough that equilibration of sodium has not occurred at the time of the glucose overshoot. When D-glucose and Na^{22} uptake were measured simultaneously, the results shown in Fig. 4 were obtained. These show that Na^+ has not reached equilibrium at 2 min at which time D-glucose concentration has reached its peak value.

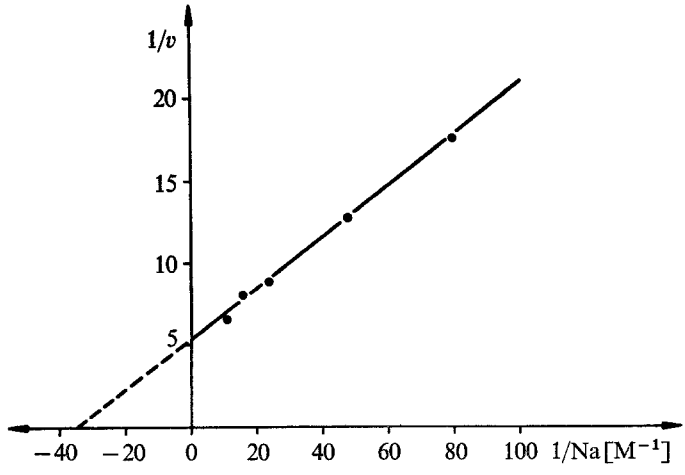

Fig. 5. Effect of $[Na^+]$ on initial rate of D-glucose uptake (15 sec) by brush-border vesicles. The Lineweaver-Burk plot shows the apparent K_m for Na^+ to be 29 mM. In the standard incubation medium sodium was replaced stepwise by potassium

If the $[Na^+]$ dependence of D-glucose transport is studied further by stepwise substitution of Na^+ by K^+, the initial uptake rate (i.e. the uptake in 15 sec) increases as a function of Na^+ concentration. A double-reciprocal plot is shown in Fig. 5, with the apparent K_m for Na^+ being 29 mM (n = 2). Since D-glucose uptake by the brush-border membranes is related to the presence of sodium and a concentration gradient of Na^+, it is possible that Na^+-dependent glucose entry into the brush-border particle is in part anion restricted. Fig. 6 shows uptake data when a highly permeant anion such as SCN^-, and a less permeant anion SO_4^{2-} replaced Cl^-. The presence of SCN^- accentuated the D-glucose overshoot; the presence of SO_4^{2-} abolished the overshoot.

To further investigate the cation site involved in D-glucose uptake, the initial rate of D-glucose uptake was measured in the presence of the other alkali metals, as shown in Table 2. The sequence obtained was Na > K = Cs = Li = Rb: i.e., the acceleration of glucose uptake is Na^+-specific.

Effect of Inhibitors. Table 1 shows that in the first 3 min of incubation 0.1 mM phlorizin reduces the uptake of D-glucose almost to the level of L-glucose without any effect on the latter. At 25 min, however, some D-glucose penetration has occurred in excess of L-glucose, probably due to the competitive nature of the inhibition. Phloretin is a much less effective inhibitor than phlorizin (Table 3); at 5×10^{-5} M, there was only 10% inhibition with phloretin whereas there was 76% inhibition with phlorizin.

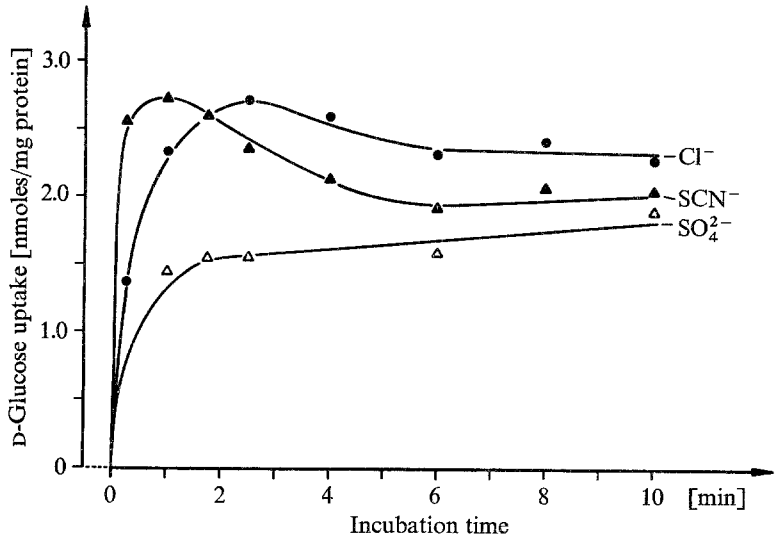

Fig. 6. Uptake of D-glucose by brush-border vesicles in the presence of Na_2SO_4, NaSCN, NaCl: osmolarity was kept constant by increasing the mannitol concentration to 150 mM

Table 2. The effect of cations on D-glucose uptake

Cation	Glucose uptake in % of uptake in the presence of sodium
Na^+	100
K^+	31 ± 5
Cs^+	30 ± 12
Li^+	26 ± 10
Rb^+	21 ± 12

n = 3

Table 3. The effect of phlorizin and phloretin on D-glucose uptake (% inhibition)

Phlorizin (M)	Brush-border	Basal-lateral	Phloretin (M)	Brush-border	Basal-lateral
1×10^{-5}	59.3	26.5	5×10^{-6}	0	0
5×10^{-5}	76.2	29.8	2.5×10^{-5}	0	0
1×10^{-4}	79.2	33.8	5×10^{-5}	9.2	24.3

Mean values of two experiments are given. Results were obtained at a D-glucose concentration of 1 mM after 1 min incubation under standard conditions.

Temperature Dependence. When uptake of D-glucose into brush-border microvilli was studied at various temperatures an Arrhenius plot gave two lines with a different slope intersecting at a temperature of about 15 °C

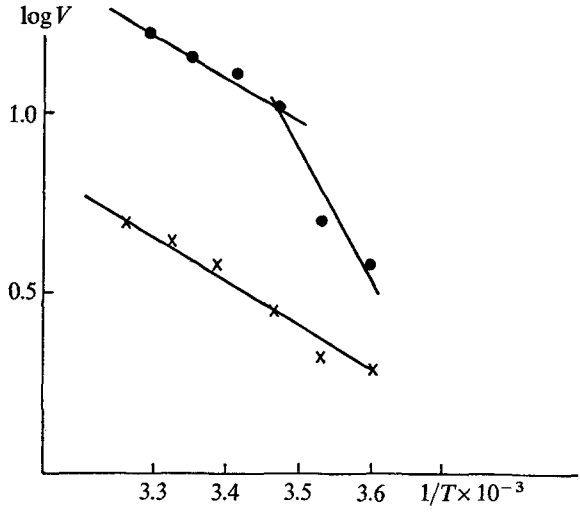

Fig. 7. An Arrhenius plot of D-glucose uptake and L-glucose uptake for brush-border vesicles, at 1 mM sugar concentration where V is amount taken up at 15 sec, •—• D-glucose, × — × L-glucose

Table 4. Apparent activation energies (cal/mole)

	Brush border		Basal-lateral membranes	
	D-glucose	L-glucose	D-glucose	L-glucose
Above transition temp.	5,500	5,800	2,700	6,600
Below transition temp.	17,900		8,400	

(Fig. 7). L-glucose gave a single value for the activation energy. Table 4 shows calculated activation energies under the different conditions.

Efflux Studies. Efflux of sugars from brush-border membrane vesicles is shown in Fig. 8. Efflux of D-glucose expressed as the per cent of sugar initially present was faster than efflux of L-glucose. Phlorizin reduced the efflux of D-glucose from the vesicles, but was less effective as an efflux inhibitor than as an influx inhibitor. It should be noted that since the dilution was 1:5, the 20% level is the equilibrium value. The rate constants are shown in Table 6.

α-Methyl Glucoside Transport. This glucose analog, which is actively transported in a Na^+-dependent fashion with the same rate as D-glucose (Ullrich, Rumrich & Kloss, 1975), is not metabolized by renal tissue. Fig. 9 shows that an overshoot occurs with this compound as it does with

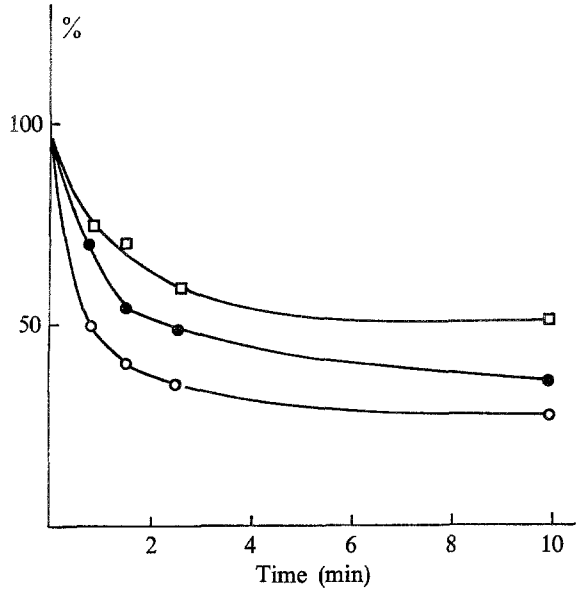

Fig. 8. D- and L-glucose efflux at 25 °C from preloaded brush-border vesicles (*see* Materials and Methods) into a medium containing 100 mM mannitol, 100 mM NaCl and 1 mM Tris-HEPES; D-glucose ∘—∘, D-glucose + phlorizin •—•, and L-glucose ▫—▫). The data are expressed as % counts present in the vesicles at zero time. The expected equilibrium value will be 20% of the counts initially present in the vesicles

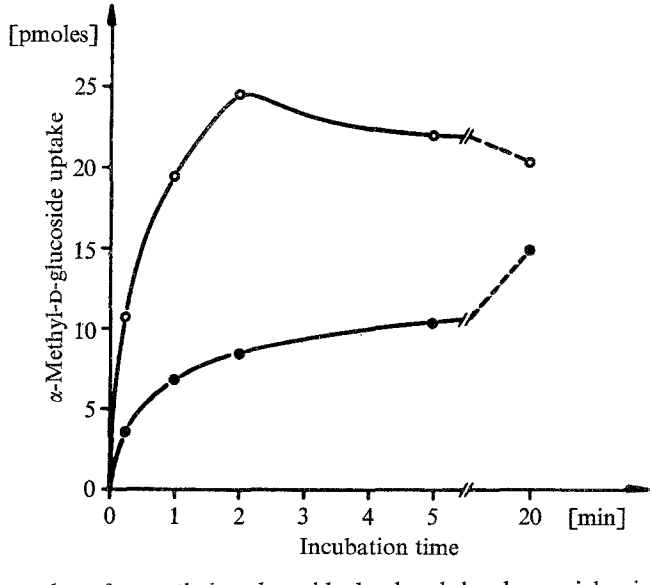

Fig. 9. The uptake of α-methyl-D-glucoside by brush-border vesicles in the absence (∘—∘) and presence (•—•) of 10^{-4} phlorizin at a concentration of 1 mM for the sugar in a medium containing 100 mM mannitol, 100 mM NaCl and 1 mM Tris-HEPES buffer

D-glucose and that phlorizin significantly inhibits the uptake. The data further establish that metabolism of D-glucose plays no role in the data obtained.

Uptake of Sugars by Basal-Lateral Membrane Vesicles

General Properties. Table 5 shows the uptake of D- and L-glucose by basal-lateral membrane vesicles in the presence and absence of phlorizin and Na^+. D-glucose uptake in the first 30 sec was about 3 times faster than that of L-glucose, and was inhibited by phlorizin by 30%. L-glucose uptake was insensitive to the inhibitor. As shown in Table 3, phloretin seems to be more potent in the basal-lateral membranes than phlorizin. Replacement of Na^+ by K^+ reduced uptake of D-glucose by only 40% in contrast to 80% reduction in the brush-border membranes.

Table 5. Uptake of D- and L-glucose by basal-lateral membrane vesicles

Solution (mM)	Time (min)							
	0.25	0.5	1.0	1.5	2	3	5	25
(a) Uptake of D-glucose								
100 NaCl	55.1 ±9.9 (n=5)	56.6 ±12.9 (n=4)	73.6 ±5 (n=5)	76.7 ±22 (n=4)	77.1 ±8.1 (n=5)	76.0 ±16 (n=4)	83.5 ±8.4 (n=5)	100
100 KCl		34.3 ±0.9 (n=4)		50.2 ±8.6 (n=4)		60.2 ±7.9 (n=4)		97.0 ±0.4 (n=4)
100 NaCl +0.1 phlorizin		28.4 ±0.5 (n=4)		50.2 ±5.2 (n=4)		54.8 ±0.14 (n=4)		91.9 ±17.1 (n=4)
(b) Uptake of L-glucose								
100 NaCl		18.7 ±2.5 (n=4)		31.6 ±4.9 (n=4)		38.6 ±9.2 (n=4)		65.8 ±10.3 (n=4)
100 KCl		21.0 ±0.3 (n=4)		32.3 ±4.3 (n=4)		41.9 ±10.9 (n=4)		66.1 ±14.0 (n=4)
100 NaCl +0.1 phlorizin		18.8 ±5.8 (n=4)		30.4 ±5.9 (n=4)		33.1 ±13.9 (n=4)		68.0 ±6.6 (n=4)

Mean values derived from n experiments are given with the standard deviation. The results are expressed as percent of the value obtained for D-glucose after 25 min of incubation in the NaCl-containing medium. This value amounted to 1.79±0.1 nmoles/mg protein (n=3).

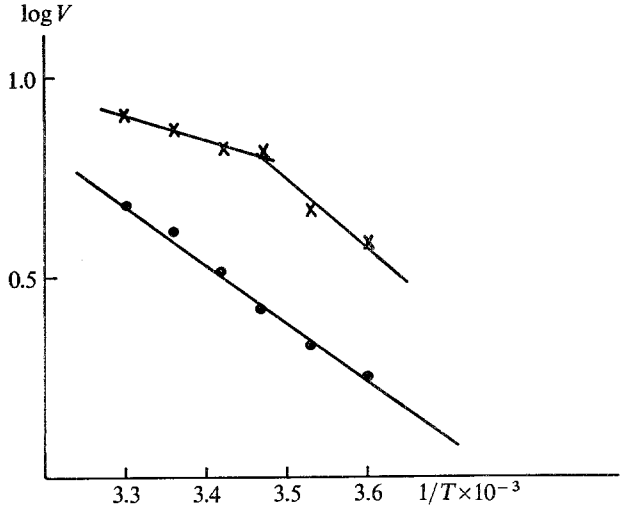

Fig. 10. An Arrhenius plot of D- and L-glucose uptake by basal lateral membrane vesicles, at 1 mM sugar concentration, where V is the amount taken up after 2 min. ×—× D-glucose, •—• L-glucose

Table 6. Efflux rate constants (sec^{-1})

	Brush-border	Basal-lateral
D-glucose	7.9×10^{-3}	5.6×10^{-3}
L-glucose	2.8×10^{-3}	4.2×10^{-3}

Temperature Dependence. Arrhenius plots of the temperature dependence of sugar uptake by basal-lateral membrane vesicles revealed a single activation energy for L-glucose, but again two energies for D-glucose uptake; the transition temperature was about 16 °C (Fig. 10; Table 6).

Efflux Studies. Efflux from preloaded basal-lateral membranes is shown in Fig. 11. D-glucose efflux was found to be faster than that of L-glucose, although the difference was not as pronounced as in the brush-border vesicles. Phlorizin at 0.1 mM did not affect the efflux of D-glucose from basal-lateral membrane vesicles. These findings are consistent with the smaller difference in the uptake of D- and L-glucose by the basal-lateral membranes. Furthermore the initial rate of L-glucose efflux from the basal-lateral membrane vesicles was more than from the brush-border vesicles, which is in agreement with the observed higher initial uptake rate for L-glucose in the basal-lateral membrane component. The rate constants are shown in Table 6.

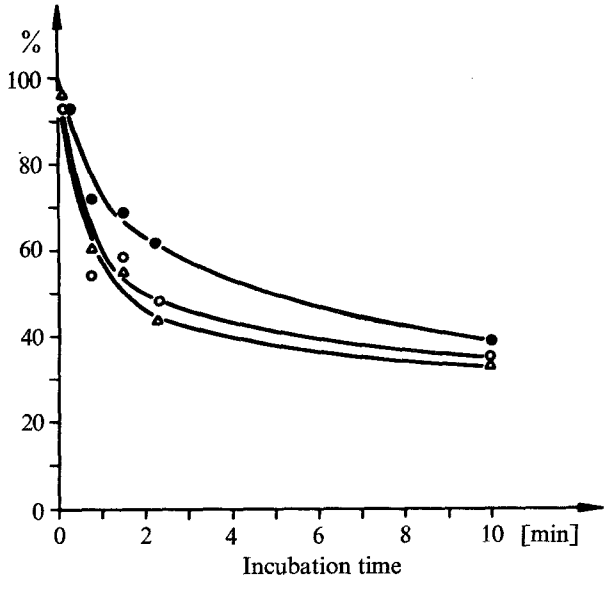

Fig. 11. Efflux curves obtained with basal-lateral membrane vesicles for D-glucose ○—○, D-glucose + 10^{-4} M phlorizin △—△, and L-glucose •—•, expressed as % counts present in the vesicles at zero time (experimental details similar to Fig. 8)

Discussion

The separation methods described in this paper produce adequately purified membrane fractions so that differences between the two surfaces of the proximal tubular cell can be shown in terms of enzyme content, and also transport properties. The separation technique, that of free-flow electrophoresis, seems to be superior to the more common zonal density gradient methods in that the cross-contamination obtained is significantly less (Sachs & Kinne, *unpublished*). It should be pointed out, however, that marker enzyme cross-contamination may be inadequate as a criterion for purity of the vesicle population if not all membrane fragments are vesiculated, or if the vesicular content of the two membrane fractions is different. Brush-border membranes have an apparent intravesicular volume of approximately 3.2 ± 0.5 μliters mg^{-1} protein, whereas basal-lateral membranes have an apparent intravesicular volume of 1.1 ± 0.1 μliters mg^{-1} protein from the D-glucose equilibrium value. Since the vesicular content of the brush-border fractions is about twice that of the basal-lateral membranes, even though marker enzyme shows only 10% cross-contamination, vesicular contamination of the basal-lateral vesicles by brush-border vesicles could run as high as 20%.

Techniques for preparing vesicles are largely empirical. Methods such as sonication, sonication with phospholipid, addition of Ca^{++} or ATP did not improve the yield of vesicles defined by transport activity. Sonication, in fact, abolished the transport properties of the system.

Transport Validation

It is necessary to distinguish between transport, metabolism and binding by subcellular vesicles. Metabolism was excluded by the absence of any detectable metabolic conversion of D-glucose and by the studies on α-methyl glucoside uptake which shows the same characteristics as the uptake of D-glucose. The contribution of binding was assessed by demonstrating zero uptake at infinite osmolarity and by the decrease of efflux from the brush-border membrane by phlorizin. In the case of binding, phlorizin would have completely displaced glucose from its binding site, and therefore the apparent efflux rate would have increased, instead of decreased. Theoretically one would expect that due to the dilution and washing procedure, material bound to membrane fragments is not measured by the methods employed provided the K_m of binding is high, as it is for glucose (Frasch, Frohnert, Bode, Baumann & Kinne, 1970; Glossman & Neville, 1972).

Since glucose is taken up into relatively "tight" vesicles, the degree of tightness of the vesicles membrane may be estimated by the uptake of L-glucose. The initial uptake rate at 25 °C for brush-border vesicles was 0.15 nmoles/mg min and in basal-laterals was 0.41 nmoles/mg min, hence the L-glucose path was more active in the basal-lateral vesicles.

Transport Properties of the Brush-Border Membrane

The isolated brush-border membrane vesicles were shown to contain a stereospecific, phlorizin-sensitive and sodium-dependent glucose uptake system capable of transient intravesicular accumulation of D-glucose.

The movement of D-glucose across the vesicular membrane may be considered to have three components, differing in importance as a function of the conditions of study, namely diffusion, facilitated diffusion and gradient-dependent transport. These processes can be studied separately in the brush-border vesicles.

Simple diffusion may be represented by the uptake of L-glucose, since this pathway is nonselective. It is tempting to equate this process to D-glucose uptake in the presence of phlorizin, or the absence of NaCl. Inspection

of Table 1 shows, however, that this is about as great as that for L-glucose. This may be due to (a) incomplete inhibition by phlorizin, (b) partial effectiveness of K^+, or (c) the presence of an additional D-glucose system, insensitive to Na^+ or phlorizin, perhaps due to contamination with basal-lateral membrane vesicles.

Facilitated diffusion, i.e. movement of hexose in the presence of Na^+ but in the absence of a Na^+ gradient, is shown in Fig. 3. L-glucose does not cross the membrane by this pathway, and initial rate for D-glucose transport by this "carrier-mediated" system is 0.77 nmoles/mg min, i.e. about 4 times greater than for free diffusion, and is fast enough to allow equilibration in about 20 min under our conditions.

Gradient-dependent transport is defined in our system as the additional uptake rate in the presence of a Na^+ gradient. The initial rate is 3.85 nmoles/mg min. In the presence of a sodium gradient the time course of D-glucose uptake shows an overshoot phenomenon; i.e., the amount of D-glucose found in the vesicles transiently exceeds that found after long-term incubation. This might be due to several factors. Firstly, transient swelling of the vesicles could occur which would lead to an increase in intravesicular volume. This would not be expected under our conditions since the vesicles are transferred to an incubation medium which is hypertonic, hence an initial shrinking followed by swelling should occur. Moreover, volume changes should also affect L-glucose uptake, uptake of D-glucose in the absence of a Na^+ gradient (Fig. 3) or uptake of D-glucose in the presence of phlorizin, and no transients were observed in these cases. Whatever volume changes occur, these should be independent of the nature of the alkali cation provided the permeabilities are equal, hence both NaCl and KCl media should produce transients, which also does not occur. Alternatively a transient accumulation of a metabolic product of D-glucose might occur. This should occur in the presence or absence of a Na^+ gradient, but the overshoot occurs only in the presence of a gradient and is a function of the initial gradient present. Also, the observation that an overshoot occurs with α-methyl glucoside, and that no metabolic conversion could be detected in our preparation appears to rule out that possibility. Hence, we are inclined to conclude that the overshoot represents an actual increase in glucose concentration in the vesicles to a value greater than that of the medium. From this it can be concluded that a Na^+ gradient across the membrane seems to be a sufficient condition for uphill transport of D-glucose by brush-border microvilli.

Most studies which have been carried out on tissue preparations (Goldner, Schultz & Curran, 1969; Frömter & Luer, 1973; Ullrich *et al.*, 1975)

involve intact cells where mechanisms are available to maintain the intracellular sodium concentration. However, in the experiments performed in this study, the Na^+ gradient is being dissipated, hence accumulation of glucose against a concentration gradient is only transitory.

There are two pathways for the dissipation of the Na^+ gradient, partly through the glucose carrier pathway, but mostly through a path independent of glucose, since under the conditions of most of our experiments (i.e. with 100 mM and 1 mM glucose) the addition of glucose did not accelerate Na^+ uptake. A potential may also develop across the vesicle membrane due to the differing permeabilities to Na^+ and Cl^- through any pathway. Glucose influx via the carrier pathway is presumably sensitive to this potential since anion substitution altered the initial velocity of uptake.

Substitution of Cl^- with a more permanent ion such as SCN^- increased the glucose influx rate, whereas substitution of Cl^- with a less permeant anion such as SO_4^{2-} delayed influx. Similar data have been obtained for glucose influx into brush-border vesicles from intestine (Murer & Hopfer, 1974). Also, microperfusion experiments have been interpreted as evidence in favor of the electrogenic nature of the glucose-Na^+ carrier system (Frömter & Luer, 1973), and our data tend to substantiate this view.

The Arrhenius plot for the uptake of L-glucose by brush-border vesicles is a straight line giving a calculated activation energy of 5.1 kcal/mole. For D-glucose the Arrhenius plot consists of two lines intersecting at about 15 °C, the slope of the line below this temperature giving an apparent activation energy of 17.4 kcal/mole and above this temperature a lower energy of 5.5 kcal/mole. The effects of temperature on the uptake kinetics of glucose in the presence of a Na^+ gradient may be due to effects on the simple diffusion of glucose, the Na^+ diffusion entry, or on the carrier-linked Na^+ glucose path, but the break in the curve is probably due to phase transitions of the lipid.

Our data give little information on the nature of the D-glucose transport system, for example, whether it is truly a carrier (mobile site) or a channel (fixed site) system. The relative lack of effectiveness of phlorizin in reducing D-glucose efflux is somewhat surprising in that one would expect phlorizin to "freeze" a carrier in the inhibited state on the outside of the vesicle. It should be as effective an efflux as an influx inhibitor.

Properties of Basal-Lateral Membranes

Since this membrane fraction also demonstrated stereospecific uptake of D-glucose and inhibition by phlorizin and phloretin, a carrier system for

sugars seems also to be present on the basal-lateral surface of the tubular cell. The effect of Na^+ replacement by K^+, or the effect of 0.1 mM phlorizin in inhibiting D-glucose uptake by only 30% may well indicate that the basal-lateral "carrier" is significantly less sensitive to Na^+ or phlorizin.

Moreover, since it has not been possible to prepare membrane fractions completely free of contamination by brush-border vesicles, the sensitivity to Na^+ removal or to phlorizin may be due entirely to this contamination.

A study of the temperature dependence of hexose transport also provides evidence for a carrier-mediated D-glucose uptake. Two apparent activation energies exist for D-glucose transport, with a transition temperature of 16 °C, whereas only a single one is present for L-glucose.

Studies on glucose uptake by a kidney membrane fraction have recently been reported by Aronson and Sacktor (1974). In contrast to our data, they show only a 60% stimulation of uptake by a Na^+ gradient, in contrast to the 500% reported here, and there is no Na^+ effect on phlorizin inhibition. In fact their data conform more to our findings on the basal-lateral fraction, than to the brush-border fraction, and this may well be due to the relative impurity of their preparation, as indicated by their high ($Na^+ + K^+$)-ATPase content.

General Comments

Data obtained on the kidney cells demonstrating selective localization of surface receptors, or enzyme markers, to only one surface of the cell can now be extended to transport systems. Accordingly, the lateral fluidity of the bilayer, which allows migration of markers in nonpolar cells such as lymphocytes, is restricted in polar cells, presumably because of the tight junction.

Our results also confirm or provide explanations for many findings on the intact kidney. Thus the differences inferred for the type of D-glucose transport occurring across each membrane of the tubular cell in studies on dog kidney (Silverman, Aganon & Chinard, 1970) or renal cyst of the flounder (Kleinzeller & McAvoy, 1973) are directly demonstrated by our preparations. The electrogenic nature of the transport is also substantiated. In addition, our data demonstrate that in a cell-free system, in the absence of any measurable metabolism of glucose, a Na^+ gradient *per se* is capable of inducing uphill transport of D-glucose.

The asymmetric distribution of the Na^+-sensitive glucose carrier system, and the probably lower transport capacity of the transport process in the basal-lateral membranes account for both net glucose transport when coupled to a ($Na^+ + K^+$)-ATPase capable of maintaining a Na^+

gradient across the brush-border membrane and for the cell's capability of achieving a concentration gradient for glucose.

This work may also be related to Na^+-dependent transport in the intestine. Preparations of brush-border and basal-lateral vesicles give strikingly similar properties to the renal preparation described above (Murer, Hopfer, Kinne-Saffran & Kinne, 1974). A model has been suggested where glucose transport is linked to Na^+-coupled transport via a $(Na^+ + K^+)$-ATPase or a component thereof (Kimmich, 1973), but our data would suggest that such a model is unlikely in the kidney in that the content of ATP in the vesicles is likely to be very low, and certainly not 1 mM as required by the glucose concentration achieved, and $(Na^+ + K^+)$-ATPase activity is low or not detectable in these preparations. The addition of ATP was without effect on D-glucose uptake. The findings of Naftalin and Curran (1974) or those of Bihler and Cybulsky (1973), where the basal surface of the intestine or intestinal cell was shown to have a stereospecific but Na^+-independent hexose uptake mechanism, are quite compatible with our findings in renal tissue. In fact, the mechanism for sugar transport in the proximal tubule appears thus far identical to that of the small intestine.

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