The Na\(^+\) Gradient-dependent Transport of D-Glucose in Renal Brush Border Membranes

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The Na\(^+\)-dependent transport of D-glucose was studied in brush border membrane vesicles isolated from the rabbit renal cortex. The presence of a Na\(^+\) gradient between the external incubation medium and the intravesicular medium induced a marked stimulation of D-glucose uptake. Accumulation of the sugar in the vesicles reached a maximum and then decreased, indicating efflux. The final level of uptake of the sugar in the presence of the Na\(^+\) gradient was identical with that attained in the absence of the gradient, suggesting that equilibrium was established. At the peak of the overshoot the uptake of D-glucose was more than 10-fold the equilibrium value. These results suggest that the imposition of a large extravesicular to intravesicular gradient of Na\(^+\) effects the transient movement of D-glucose into renal brush border membranes against its concentration gradient.

The stimulation of D-glucose uptake into the membranes was specific for Na\(^+\). The rate of uptake was enhanced with increased concentration of Na\(^+\). Increasing Na\(^+\) in the external medium lowered the apparent $K_m$ for D-glucose. The Na\(^+\) gradient effect on D-glucose transport was dissected into a stimulatory effect when Na\(^+\) and sugar were on the same side of the membrane (cis stimulation) and an inhibitory effect when Na\(^+\) and sugar were on opposite sides of the membrane (trans inhibition).

The uptake of D-glucose, at a given concentration of sugar, reflected the sum of the contributions from a Na\(^+\)-dependent transport system and a Na\(^+\)-independent system. The relative stimulation of D-glucose uptake by Na\(^+\) decreased as the sugar concentration increased. It is suggested, however, that at physiological concentrations of D-glucose the asymmetry of Na\(^+\) across the brush border membrane might fully account for uphill D-glucose transport. The physiological significance of the findings is enhanced additionally by observations that the Na\(^+\)-dependent D-glucose transport system in the membranes in vitro possessed the sugar specificities and high phlorizin sensitivity characteristic of more intact preparations. These results provide strong experimental evidence for the role of Na\(^+\) in transporting D-glucose across the renal proximal tubule luminal membrane.

Previous physiological studies with intact animals and renal cortical slices on the reabsorption of D-glucose in the kidney have indicated that D-glucose transport is an uphill transport system, localized in the proximal tubule, saturable, stereospecific, inhibited by specific analogs of D-glucose, highly sensitive to phlorizin, and energy- and Na\(^+\)-dependent (1). Additionally, it has been reported that the steady state concentration of D-glucose in cells of the perfused isolated proximal tubule exceeds the value in the lumen, suggesting that the apical brush border membrane of the cell is the site of the uphill transport step (2). Thus, with the development of techniques for isolating renal brush border membranes, investigations have focused on the mechanisms of transport using this model membrane system (3-11).

In recent studies of the transport of D-glucose with purified renal brush border membranes (10), we have reported accelerated exchange diffusion of D-glucose and the inhibition by phlorizin of sugar efflux. These findings indicate that the uptake of D-glucose by the brush borders represents transport across the membrane into an intravesicular space. The stimulation of D-glucose transport by a Na\(^+\) gradient has also been demonstrated with isolated membranes (10). These studies, which are uncomplicated by significant metabolism of D-glucose or uptake of the sugar by contaminating bacteria (10, 11), clearly distinguish this renal transport system from those reported earlier (5, 6, 9).

In the present paper, experiments are described showing that the Na\(^+\) gradient-dependent transport of D-glucose into the brush border membrane vesicles can be enhanced strikingly, thus enabling this component of the transport process to be characterized kinetically. The high sensitivity of the Na\(^+\) gradient-dependent mechanism to phlorizin and to selective analogs of D-glucose supports the physiological significance of the system. These findings provide direct experimental evidence on the role of Na\(^+\) in transporting D-glucose across the renal proximal tubule luminal membrane.

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EXPERIMENTAL PROCEDURES

Rabbit renal brush border membranes were isolated by a modification of the method described previously (10, 12). In the present procedure, the renal cortex was homogenized in 0.5 M sucrose, with a ratio of 1 g of tissue to 6 ml of medium. The homogenate was centrifuged rapidly and briefly by setting the centrifuge to attain 4,500 x g and when this force was reached stopping the centrifuge immediately. The supernatants were saved. The residues were resuspended in one-half of the original volume of medium, homogenized with three strokes of the Potter-Elvehjem pestle, and recentrifuged in the same manner. The supernatants were combined and layered on sucrose density gradients, as described (12). The pink fluffy brush border layers plus the lower halves of the overlying supernatants were aspirated and resuspended in 0.5 M sucrose to the original volume. The suspension was centrifuged at 4,300 x g for 30 min. The loose pink sediments were sloughed from the pellets and together with the supernatants were recentrifuged at 31,000 x g for 5 min. The resulting supernatants were discarded. The brush border membranes were visible as the loosely packed pink portion of the pellet overlying a dense, tightly packed brownish colored core, containing primarily mitochondria. The brush border membranes were washed off the pellets, resuspended in the sucrose, and centrifuged again for 5 min at 31,000 x g, and for 10 min sequentially at 7,700, 4,300, and 3,000 x g. After each centrifugation, the supernatants were discarded and the loosely packed brownish colored core with membranes were washed off the remaining densely packed containing portion of the pellets. Then, the membranes were suspended in a medium of 300 mM mannitol, buffered with 1 mM Tris-Hepes (1 mM Hepes adjusted with Tris hydroxide) to pH 7.5, and centrifuged at 27,000 x g for 5 min. The supernatants were discarded and the pellets resuspended in the buffered mannitol medium. The washing of the brush border membranes was repeated for a total of three 5-min centrifugations and a final 10-min centrifugation. The brush border membranes, approximately 6 to 10 mg of protein/ rabbit, were finally suspended in 0.3 to 0.5 ml of the buffered mannitol medium so that the protein concentration was approximately 20 mg/ml. The quality of the preparations was randomly evaluated by specific enzyme markers (12) and occasionally by electron microscopy.

Uptake of D-glucose by the renal brush border membranes was measured by the Millipore filtration technique as detailed previously (6, 10), with two modifications. In this study, 10 μl of the membrane suspension was preincubated for 1 min at 20 °C, and incubation at 20 °C was initiated with the addition of 40 μl of buffered mannitol medium containing D-[14C]glucose (2 x 106 cpm) and other constituents, as indicated. The additions replaced mannitol isotonically. The use of the buffered mannitol incubation medium containing predominantly the impermeable saline mannitol contrasts with the completely saline medium used by others (6, 10) and resulted in the marked increase in the Na+ -stimulated transport of D-glucose reported in this paper. The second modification was the use in the present experiments of a solution containing ice-cold 154 mM NaCl and 1 mM Tris-Hepes buffer, pH 7.5, rather than saline-phosphate, to terminate the incubation and wash the filter. Values for the nonspecific retention of radioactivity on the filters (usually 0.010 to 0.015% of the total radioactivity in the incubation mixture) were subtracted from the values of the incubated samples. All incubations were carried out in triplicate with freshly prepared brush border membranes. Each experiment was performed at least three times with different membrane preparations. Although the D-glucose uptake per mg of protein for a given experiment may vary over a 2- to 3-fold range from day to day, relative uptakes normalized to a daily control were consistent and reproducible. The results are expressed in the normalized form as the mean ± the standard error of the mean.

Protein was assayed by the method of Lowry et al. (13).

D-[14C]Glucose (240 Ci/mol) was obtained from Schwarz BioResearch. After thin layer chromatography (14), over 98% of the radioactivity appeared in a single spot having the same Rf as authentic glucose. As reported previously (10, 90% of the radioactive D-glucose accumulated by the brush border membrane vesicles was identified as the brush border membranes. The sugars and other chemicals were of the highest purity available from commercial sources. All water used for preparing media and reagents was triple distilled and deionized, and all solutions were filtered through 0.45-μM Millipore filters (11) prior to use.

RESULTS

Time Course of Uptake of D-Glucose—The uptake of 50 μM D-glucose by renal brush border membrane vesicles during incubations at 20 °C for different lengths of time, either in the standard 300 mM buffered mannitol medium or in a medium in which mannitol was replaced isosmotically by 100 mM NaCl at the initiation of incubation, is illustrated in Fig. 1. In the absence of the Na+ gradient, steady state levels were reached in approximately 80 min. The uptake at this time was 46.7 pmol/mg of membrane protein. The presence of a Na+ gradient between the external incubation medium and the intravesicular medium induced a marked stimulation of D-glucose uptake. The initial (30 s) rate of D-glucose uptake with the Na+ gradient was frequently greater than 40 times the initial rate in the absence of the gradient. Accumulation of the sugar in the renal brush border membranes was maximal at approximately 2 min. Afterward the amount of D-glucose in the vesicles decreased, indicating efflux of the sugar. The final level of uptake of the sugar in the presence of the Na+ gradient was identical with that attained in the absence of the gradient, suggesting that equilibrium was established. Other studies, in which the uptake of D-glucose was measured after 60 min of incubation in different total osmolarities, indicate that at equilibrium the intravesicular space of the membranes incubated in either mannitol or mannitol + NaCl was the same.

As noted, at equilibrium approximately 50 pmol of D-glucose were accumulated/mg of membrane protein and presumably the concentration of D-glucose in the extravesicular space equaled that in the intravesicular space. Since the concentration of D-glucose in the external medium was essentially 50 μM, it follows that the concentration in the membrane vesicles was also 50 μM. Thus, it is estimated that 1 mg of membrane protein in the renal brush borders, as isolated and incubated in this study, represents an intravesicular space of 1 μl. This suggestion is supported by other studies in this laboratory, in which the uptake of amino acid by the same brush border membrane preparations equilibrated with an identical intravesicular space.

At the peak of the "overshoot," at an incubation period of 2 min, the uptake of D-glucose was more than 10-fold the final equilibrium value. These results suggest that the imposition of a large extravesicular to intravesicular gradient of Na+ effects the transient movement of D-glucose into renal brush border membranes against its concentration gradient. A similar observation was reported recently by Murer and Hopfer (15) with intestinal membranes. To substantiate additionally that the Na+ gradient-induced "overshoot" resulted from excess intravesicular uptake rather than binding, i.e., to a Na+ -sensitive surface "carrier," the experiment described in Table I was performed. Renal brush border membranes were incubated with D-glucose in the presence of a Na+ gradient for 2 min. Then, at the peak of the "overshoot," the membranes were diluted 1:10 with sugar-free buffered mannitol media, with and without 1 mM phlorizin, and were reincubated for an additional 30 s. In the absence of phlorizin, approximately two-thirds of the accumulated D-glucose was released. In the presence of phlorizin, release of the previously retained sugar was markedly inhibited, due to inhibition of D-glucose efflux by phlorizin interacting with the membrane external to the site of D-glucose

1 The abbreviation used is: Hepes, N-2-hydroxyethylpiperazine-N'-2-ethanesulfonic acid.

2 Deck and Sacktor, unpublished observations.

3 Hammerman and Sacktor, unpublished observations.
accretion (10). If the excess d-glucose uptake found in the “overshoot” were due to binding, then it would be expected that phlorizin would have displaced the sugar and the release of d-glucose from the membrane would have been accelerated.

**Specificity of Na**<sup>+</sup> **Stimulation**—The specificity of Na**<sup>+</sup> in greatly enhancing the rate of d-glucose uptake by renal brush border membranes is shown in Table II. The membranes were incubated with 50 µM d-glucose for 30 s with 300 mM buffered mannitol or with the mannitol replaced isosmotically with 100 mM Na**<sup>+</sup>, K**<sup>+</sup>, Li**<sup>+</sup> or choline, as chloride salts. The uptake in the mannitol control was 18.4 pmol/min/mg of protein. Neither K**<sup>+</sup> nor choline caused significant stimulation of uptake, but Li**<sup>+</sup> did effect a doubling of the rate. However, Na**<sup>+</sup> increased the rate 45 times.

It is feasible that the isosmotic replacement of mannitol by a solute with a lower reflection coefficient could cause the enlargement of the intravesicular space resulting in the nonspecific stimulation in the rate of d-glucose uptake. The upper limit of this effect would occur when the concentration of mannitol was decreased from 300 mM to 100 mM by water containing no solute. As shown in Table II, a 5-fold enhancement in the rate of d-glucose uptake was observed with 100 mM mannitol relative to that with 300 mM mannitol. This is substantially less than the 45-fold stimulation found with 100 mM mannitol + 100 mM NaCl. Analogously, addition of solute to 300 mM mannitol could effect a shrinkage of the intravesicular space resulting in the inhibition of the rate of d-glucose uptake. Indeed, the rate of d-glucose uptake when the concentration of mannitol was increased from 300 mM to 500 mM was 0.74 relative to that with 300 mM mannitol. Addition of salts while maintaining the mannitol concentration constant at 300 mM should not cause an enlargement of the intravesicular space. As reported in Table II, addition of 100 mM choline or potassium chlorides to 300 mM mannitol resulted in inhibition similar to that found with 500 mM mannitol. Li**<sup>+</sup> neither inhibited nor stimulated. In contrast, 100 mM NaCl + 300 mM mannitol stimulated the rate of d-glucose uptake 42 times despite the hyperosmolarity.

These results demonstrate the specificity of this d-glucose transport system for Na**<sup>+</sup>, in contrast to other monovalent cations. They also eliminate osmotically induced change in intravesicular volume, alteration in ionic strength, or addition

**TABLE II**

<table>
<thead>
<tr>
<th>Incubation medium</th>
<th>Relative rate of d-glucose uptake</th>
</tr>
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<tbody>
<tr>
<td>Mannitol (300 mM)</td>
<td>1.00</td>
</tr>
<tr>
<td>Mannitol (300 mM) + choline Cl (100 mM)</td>
<td>0.90 ± 0.02</td>
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<tr>
<td>Mannitol (100 mM) + KCl (100 mM)</td>
<td>1.19 ± 0.06</td>
</tr>
<tr>
<td>Mannitol (100 mM) + LiCl (100 mM)</td>
<td>2.28 ± 0.16</td>
</tr>
<tr>
<td>Mannitol (100 mM) + NaCl (100 mM)</td>
<td>45.04 ± 5.59</td>
</tr>
<tr>
<td>Mannitol (500 mM)</td>
<td>6.56 ± 0.35</td>
</tr>
</tbody>
</table>

**Fig. 1.** The time course of the uptake of d-glucose by rabbit renal brush border membranes in the standard 300 mM buffered mannitol medium or in a medium in which mannitol was replaced isosmotically by 100 mM NaCl at the initiation of incubation. The concentration of d-glucose was 50 µM and the incubation temperature was 20°. Other details of the procedure are described in the text. The membranes had accumulated 46.7 pmol of d-glucose/mg of protein after 80 min of incubation in the absence of the Na**<sup>+</sup> gradient. This value is designated as having a relative value of 1. Each datum represents the mean ± standard error of the mean for three separate experiments.
of chloride ions as factors that may have accounted for the observed stimulation with NaCl.

**Relationship between Na⁺ Concentration and D-Glucose Uptake**—Fig. 2 describes the effect of different concentrations of NaCl on the rate of uptake of 50 μM d-glucose by renal brush border membranes. In these experiments the rate in the absence of Na⁺ averaged 14.1 pmol/min/mg of protein. There was increased stimulation of uptake with increased concentration of Na⁺ over the entire range of concentrations tested. At 100 mM NaCl, the highest concentration used, the rate of d-glucose uptake was increased 42 times the control rate. Even at this concentration, only partial saturability with respect to Na⁺ was found. This is in agreement with observations on the Na⁺-dependent sugar transport system in renal cortical slices (16).

**Relationship between D-Glucose Concentration and Na⁺-Dependent Uptake of Sugar**—The effect of different concentrations of d-glucose on the rate of sugar uptake, in the absence or presence of 40 mM NaCl, is illustrated in Fig. 3. When examined in the presence of the salt over a wide range of d-glucose concentrations, 10 μM to 20 mM, the relationship between rate of sugar uptake and sugar concentration, plotted according to Eadie-Hofstee, was best described by a nonlinear curve, suggesting more than one transport system. Although not as readily apparent, the curve describing the Na⁺-free uptake of sugar was not linear either. However, if at each d-glucose concentration the Na⁺-free uptake was subtracted from the uptake obtained in the presence of Na⁺, a linear relationship between V and V/S was found. This would be consistent with the hypothesis that the Na⁺-dependent uptake of d-glucose represented a single transport system. It follows that the measured uptake at any glucose concentration might be expressed as the sum of the contributions from a Na⁺-dependent transport system and a Na⁺-independent transport system. The latter system, which shows incomplete saturability, stereospecificity, and partial sensitivity to phlorizin (10), might itself represent the sum of passive diffusion and one or more saturable systems.

It was also evident from this experiment that within the concentration range of 10 to 200 μM d-glucose the Na⁺-independent rate of uptake was essentially negligible compared to the Na⁺-stimulated rate, and Michaelis-Menten kinetic data were apparently obeyed whether or not the Na⁺-free rates of uptake were subtracted from the values determined in the presence of 40 mM NaCl. For the experiments illustrated in Fig. 3 (inset), the Vₘₐₓ value was 0.9 nmol/min/mg of protein and the apparent Kₘ values for d-glucose were 90 and 110 μM when the Na⁺-free rates were subtracted, and the values were 1.0 and 110 when the Na⁺-free rates were not subtracted. Therefore, measurements of the Na⁺-dependent uptake of d-glucose were performed using sugar concentrations within this 20-fold range and results are presented uncorrected for the exceedingly small contribution from the Na⁺-independent system.

Fig. 4 describes the relationship between the relative stimulation of d-glucose uptake by Na⁺ (the ratio of the rate of uptake with 40 mM NaCl to the rate with 0 mM NaCl) and the concentration of d-glucose. Progressively less fractional stimulation was seen as the sugar concentration was increased. It is important to note that even at physiological concentrations of d-glucose, 5 to 10 mM, a fractional stimulation of 2.1- to 1.5-fold was found.

**Effect of Na⁺ on Kinetics of D-Glucose Uptake**—The initial (30 s) rates of uptake of d-glucose were measured using sugar concentrations of 20 to 200 μM in the presence of 10, 20, or 50 mM NaCl, added at the start of the incubation. As shown in Fig. 5, the effect of increasing Na⁺ in the external medium was to lower the apparent Kₘ for transport. The calculated apparent Kₘ values were 500, 220, and 80 μM d-glucose at 10, 20, and 50 mM NaCl, respectively. The Vₘₐₓ was unaffected.

**Fig. 2.** The effect of the concentration of NaCl on the rate of uptake of d-glucose by renal brush border membranes. The membranes were incubated with 50 μM d-glucose for 30 s at 20° as described in the text. NaCl replaced mannitol isosmotically. The rate of uptake of the sugar in a medium of 300 mM buffered mannitol, in the absence of Na⁺, averaged 14.1 pmol/min/mg of protein. This value is designated as having a relative uptake of 1. Each datum represents the mean ± standard error of the mean for three separate experiments.

**Fig. 3.** The relationship between d-glucose concentration and the Na⁺-dependent and Na⁺-independent rates of uptake of the sugar, plotted according to Eadie-Hofstee. The membranes were incubated for 30 s at 20° in the absence or presence of 40 mM NaCl replacing mannitol isosmotically. The d-glucose concentration ranged from 10 μM to 20 mM. The Na⁺-Na⁺-free values were obtained by subtracting the Na⁺-free uptake from the uptake obtained in the presence of Na⁺ at each concentration of d-glucose. The inset shows Lineweaver-Burk plots of the uptakes obtained with concentrations of d-glucose ranging from 10 to 200 μM in the presence of 40 mM NaCl with (●) or without (O) the subtraction of the uptake values obtained in the absence of Na⁺. The Vₘₐₓ values were 0.9 and 1.0 nmol/min/mg of protein and the apparent Kₘ values were 90 and 110 μM d-glucose, with and without, respectively, the subtraction of the Na⁺-free uptakes. The results shown are the means of three experiments.
FIG. 4. The relationship between the relative stimulation of D-glucose by Na\(^+\) and the concentration of D-glucose. The fractional stimulation is defined as the ratio of the rate of uptake with 40 mM NaCl to the rate with no added NaCl. The incubation time was 30 s and the temperature was 20\(^\circ\)C. Other details of the procedure are described in the text.

FIG. 5. The effect of Na\(^+\) on the kinetics of D-glucose uptake. The incubation time was 80 s and the temperature was 20\(^\circ\)C. NaCl replaced mannitol isosmotically. Other details of the procedure are described in the text.

FIG. 6. Effect of preloading the brush border membrane vesicles with Na\(^+\) on the Na\(^+\)-dependent uptake of D-glucose. The membranes were preincubated with 80 mM NaCl at 20\(^\circ\)C for different periods of time prior to initiating sugar uptake measurements. The concentration of D-glucose in the incubation mixture was 20 \(\mu\)M and the final concentration of NaCl was 80 mM in all incubations. The incubation period was 30 s and the temperature was 20\(^\circ\)C. The results are expressed as the uptake relative to the uptake found after 1 min of preincubation in the absence of Na\(^+\). The average uptake of the sugar by these membranes was 175 pmol x min\(^{-1}\) x mg\(^{-1}\) protein, and this value is designated as having a relative uptake of 10. A control experiment in which the brush border membranes were preincubated for 40 min at 20\(^\circ\)C, in the absence of Na\(^+\), is shown (O). Each datum represents the mean \(\pm\) standard error for three separate experiments.

and had an average value of 1.2 nmol/min/mg of protein. These findings for D-glucose with the isolated brush border membranes contrast with those found for galactose (16) and \(\alpha\)-methyl glucoside (17) transport in rabbit renal cortex slices. In the more complex kidney slice it was reported that Na\(^+\) affected \(V_{\text{max}}\) and not \(K_m\). The effect of Na\(^+\) on the kinetics of sugar uptake in intestinal preparations is somewhat conflicting, being either a \(K_m\) (18, 19) or a \(V_{\text{max}}\) (20) alteration, depending on the species and experimental procedure used.

Effect of Preloading Membrane Vesicles with Na\(^+\) on Na\(^+\)-dependent Uptake of D-Glucose—In all of the experiments described above Na\(^+\) was added to the reaction mixture with D-glucose at the start of the incubation. In the experiments illustrated in Fig. 6, the membrane vesicles were preincubated with 50 mM NaCl at 20\(^\circ\)C for varying periods of time prior to initiating sugar uptake measurements. The final concentrations of D-glucose and NaCl were 20 \(\mu\)M and 50 mM, respectively. A rapid decrease in rate of D-glucose uptake was found with preincubation with Na\(^+\). This inhibition increased with longer periods of preincubation and after 40 min of preincubation the rate of uptake was decreased by 90\%. In a control experiment in which the membranes were preincubated for 40 min at 20\(^\circ\)C, but in the absence of Na\(^+\), little loss in the Na\(^+\) stimulation of D-glucose transport was seen. This inhibition presumably reflects the progressive accumulation of Na\(^+\) inside the vesicles during the preincubation period, and this would be in accord with our previous report that preloading brush borders with Na\(^+\) inhibits D-glucose uptake (10). Thus, the Na\(^+\) gradient effect on D-glucose uptake can be dissected into a stimulatory effect of Na\(^+\) on the transport of sugar when D-glucose and Na\(^+\) are on the same side of the membrane (cis stimulation) and an inhibitory effect of Na\(^+\) on the transport when the sugar and Na\(^+\) are on opposite sides of the membrane (trans inhibition).

Effects of Na\(^+\) on Efflux of D-Glucose—Because of the possibility that the inhibition of D-glucose uptake by trans Na\(^+\) could reflect enhancement of backflux rather than true inhibition of influx, the effects of Na\(^+\) on the efflux of D-glucose from the membrane vesicles were determined. That efflux of the sugar was enhanced by a Na\(^+\) gradient of high Na\(^+\) in the inside of the vesicles to low Na\(^+\) in the outside was indicated by the experiments described in Table III. Brush border membranes were incubated for 80 min with 50 \(\mu\)M D-glucose in the presence or absence of 100 mM NaCl. Several incubations were terminated to measure the D-glucose that had accumulated (100\% retained), whereas other incubation mixtures were diluted 1:10 with appropriate sugar-free media so that the final concentration of Na\(^+\) was 100 mM. After 30 s of additional incubation, the D-glucose retained by the membranes was determined. As shown in Table III, only 8\% of the previously accumulated D-glucose was released from the brush borders that originally had been in a Na\(^+\)-free medium. In contrast, 33\% of the D-glucose effluxed from those membranes previously preloaded with Na\(^+\).
Other studies, described in Table IV, demonstrate that efflux of D-glucose was inhibited by trans Na⁺, i.e. relatively high Na⁺ in the outside medium. In these experiments the brush border membranes were incubated with 50 μM D-glucose in the presence of 100 mM NaCl. After 2 min of incubation, at the peak of the "overshoot" (see Fig. 1), the incubation mixtures were diluted 1:10 with sugar-free medium, so that the final concentration of Na⁺ was either 10 mM or 100 mM. The membranes were then reincubated for an additional 30 s. It was found that 67% of the accumulated D-glucose effluxed into the external medium containing 10 mM NaCl, whereas only 34% of the sugar effluxed into the medium containing 100 mM NaCl. As external D-glucose was diluted 1:10 in these experiments, backflux (i.e. reinflux) could not account for the trans inhibition of D-glucose transport by Na⁺.

**Effect of Sugar Analogs on Na⁺-dependent and Na⁺-independent Uptakes of D-Glucose**—Selected analogs of D-glucose were evaluated as inhibitors of the rate of uptake of 50 μM D-glucose, measured in the presence or absence of 100 mM NaCl isosmotically replacing the buffered mannitol medium. As reported in Table V, the Na⁺-dependent uptake of D-glucose was highly sensitive to α-methyl-D-glucoside, D-galactose, and D-glucose tested at an inhibitor concentration of 1 mM. None of the other sugars, including 2-deoxy-D-glucose, D-mannose, D-fructose, D-xylose, or L-glucose, inhibited the Na⁺-dependent D-glucose transport. A slight inhibition (23%) was found with 3-O-methyl-D-glucose. The uptake of D-glucose was almost totally blocked by 1 mM phlorizin. In contrast to these findings, the Na⁺-independent uptake of D-glucose showed a general lack of specificity with respect to the various analogs as well as a relative lack of sensitivity to the inhibitors. For example, except for D-glucose, all of the sugars inhibited D-glucose uptake. However, α-methyl glucoside and D-galactose, at concentrations of 10 mM, were markedly less effective as inhibitors of the Na⁺-independent uptake than as inhibitors of the Na⁺-dependent system even though used at concentrations 10 times higher. This distinction between the Na⁺-independent and Na⁺-dependent D-glucose uptake was also evident by their contrasting sensitivities toward phlorizin. Although these findings suggest that the Na⁺-dependent transport of D-glucose was notably more specific than the Na⁺-independent system, the observations that the Na⁺-independent uptake of D-glucose was inhibited to some extent by phlorizin and sugar analogs, but not by L-glucose, argue that at least part of the Na⁺-independent system represents facilitated transport rather than exclusively passive diffusion.

**TABLE III**

*Effect of preloading brush border membrane vesicles with Na⁺ on efflux of D-glucose*

Brush border membranes were incubated with 50 μM D-glucose for 80 min in the absence or presence of 100 mM NaCl, as described in Fig. 1. The uptakes of D-glucose were measured immediately or the mixtures were diluted 1:10 in an appropriate sugar-free medium so that the final Na⁺ was 10 mM. The membranes had accumulated 49.3 ± 4.2 pmol of D-glucose/mg of protein after the 80-min incubation in the absence or presence of Na⁺, respectively. These values are designated at 100% retained. The diluted membranes were reincubated for an additional 30 s at 20°C. Each datum represents the mean ± standard error for three separate experiments.

<table>
<thead>
<tr>
<th>Experiment</th>
<th>Incubation medium</th>
<th>Na⁺ retained</th>
<th>d-Glucose</th>
<th>Na⁺-independent</th>
<th>Na⁺-dependent</th>
</tr>
</thead>
<tbody>
<tr>
<td>300 mM mannitol</td>
<td>Undiluted control</td>
<td>100</td>
<td>33 ± 3</td>
<td>33 ± 3</td>
<td>66 ± 2</td>
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<tr>
<td>100 mM mannitol</td>
<td>Diluted 1:10, 10 mM Na⁺</td>
<td>93 ± 2</td>
<td>67 ± 6</td>
<td>67 ± 6</td>
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<tr>
<td>NaCl</td>
<td>Undiluted control</td>
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<td>67 ± 6</td>
<td>67 ± 6</td>
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<tr>
<td>NaCl</td>
<td>Diluted 1:10, 10 mM Na⁺</td>
<td>67 ± 6</td>
<td>67 ± 6</td>
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</tr>
</tbody>
</table>

**TABLE IV**

*Effect of external Na⁺ on the efflux of D-glucose from brush border membrane vesicles*

Brush border membranes were incubated with 50 μM D-glucose in the presence of 100 mM NaCl as described in Fig. 1. After 2 min of incubation, at the peak of the "overshoot," the incubation mixtures were diluted 1:10 with an appropriate sugar-free medium, so that the final concentration of Na⁺ was either 10 mM or 100 mM, and reincubated for an additional 30 s at 20°C. The membranes had accumulated 348 pmol of D-glucose/mg of protein prior to dilution, and this value is designated as 100% retained. Each datum represents the mean ± standard error for three separate experiments.

<table>
<thead>
<tr>
<th>Experiment</th>
<th>Incubation medium</th>
<th>Na⁺ retained</th>
<th>d-Glucose</th>
<th>Na⁺-independent</th>
<th>Na⁺-dependent</th>
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<tbody>
<tr>
<td>Control</td>
<td>Undiluted</td>
<td>100</td>
<td>33 ± 3</td>
<td>33 ± 3</td>
<td>66 ± 2</td>
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<tr>
<td>Diluted 1:10 in 10 mM NaCl</td>
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</tbody>
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**TABLE V**

*Effect of sugar analogs on Na⁺-independent and Na⁺-dependent uptake of D-glucose by brush border membranes*

Enol brush border membranes were incubated with 50 μM D-glucose in 300 mM mannitol buffered medium or in 100 mM NaCl and 100 mM mannitol buffered medium for 30 s at 20°C as described in the text. In the absence of Na⁺, the control value (100% relative uptake) was 10.2 pmol of D-glucose x min⁻¹ x mg⁻¹ protein. The concentration of sugar analogs was 10 mM. In the presence of Na⁺, the control value (100% relative uptake) was 5.2 pmol of D-glucose x min⁻¹ x mg⁻¹ protein. The concentration of sugar analogs was 1 mM. The inhibitory effect of phlorizin was measured at 1 mM. Each datum represents the mean ± standard error for three separate experiments.

<table>
<thead>
<tr>
<th>Inhibitor</th>
<th>Relative rate of D-glucose uptake</th>
<th>0 mM Na⁺</th>
<th>100 mM Na⁺</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>100</td>
<td>100</td>
<td>100</td>
</tr>
<tr>
<td>D-Glucose</td>
<td>86 ± 7</td>
<td>97 ± 2</td>
<td>97 ± 2</td>
</tr>
<tr>
<td>D-Fructose</td>
<td>78 ± 5</td>
<td>100 ± 4</td>
<td>100 ± 4</td>
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<tr>
<td>D-Mannose</td>
<td>74 ± 4</td>
<td>96 ± 1</td>
<td>96 ± 1</td>
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<tr>
<td>D-Xylose</td>
<td>74 ± 6</td>
<td>97 ± 3</td>
<td>97 ± 3</td>
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<tr>
<td>D-Galactose</td>
<td>67 ± 3</td>
<td>87 ± 1</td>
<td>87 ± 1</td>
</tr>
<tr>
<td>2-Deoxy D-glucose</td>
<td>63 ± 6</td>
<td>90 ± 2</td>
<td>90 ± 2</td>
</tr>
<tr>
<td>3-O-methyl-D-glucose</td>
<td>59 ± 5</td>
<td>77 ± 1</td>
<td>77 ± 1</td>
</tr>
<tr>
<td>a-Methyl-D-glucoside</td>
<td>76 ± 4</td>
<td>29 ± 0.4</td>
<td>29 ± 0.4</td>
</tr>
<tr>
<td>D-Glucose</td>
<td>59 ± 6</td>
<td>18 ± 0.2</td>
<td>18 ± 0.2</td>
</tr>
<tr>
<td>Phlorizin</td>
<td>41 ± 3</td>
<td>3 ± 0.2</td>
<td>3 ± 0.2</td>
</tr>
</tbody>
</table>
transport systems in the dog, in vivo. The high affinity and specificity of the Na⁺-dependent uptake of D-glucose in renal proximal tubule brush border membranes that reaches a level severalfold higher than that attained at equilibrium. With the membrane preparation the rate of uptake of D-glucose at a given sugar concentration may be considered the sum of contributions from a homogenous Na⁺-dependent transport system and a heterogeneous Na⁺-independent process. The ratio of the rate of uptake in the presence of Na⁺ to the rate of uptake in the absence of Na⁺ is inversely related to the concentration of D-glucose. At physiological concentrations of D-glucose a fractional stimulation of about 2 was found. This value agrees with the steady-state ratio of cellular to luminal D-glucose in isolated rabbit proximal tubule segments perfused with 7 mM D-glucose (2). Thus, it is suggested that asymmetry of Na⁺ across the brush border membrane may fully account for the active transport of D-glucose at physiological concentrations.

The specificity of the Na⁺-dependent sugar transport system in brush border membranes for D-glucose is emphasized by the contrast between the effects of various analogs on the Na⁺-dependent and Na⁺-independent D-glucose uptakes. Of the sugars tested, only D-galactose and α-methyl D-glucoside inhibited the Na⁺-dependent transport of D-glucose. Kleinzeller (23) suggested that in rabbit renal cortical slices the structural requirements for the Na⁺-dependent active sugar transport system are a D-pyranose or furanose ring, a hydrophilic group on C-2, a hydroxy group on C-3 in the same configuration as in D-glucose, a hydroxy group on C-6, but not a hydroxy group on C-1. The results presented in this paper are generally consistent with this view with the additional specifications that the ring must be in the pyranose form and the hydroxyl group on C-2 must be in the D-glucose rather than in the D-mannose configuration. Silverman et al. (24) also stressed the distinction between the β-glucose and β-mannose transport systems in the dog, in vivo. The high affinity Na⁺-dependent binding of phlorizin to rat kidney brush border membranes is likewise inhibited by β-glucose and β-galactose but not by β-mannose (4). However, 2-deoxy-β-glucose interacts with the high affinity phlorizin site in the rat (4) as well as the β-glucose transport system in the dog (24), but this analog does not share the Na⁺-dependent D-glucose transport system in the rabbit renal cortical slice (23) nor in the rabbit membrane preparations used in the present studies. Perhaps there is a species difference in this respect.

The specificity of the D-glucose transport system for Na⁺ is indicated by the failure of K⁺ and choline ion gradients to stimulate uptake and by the relatively small effect found with Li⁺. This does not rule out the influence of these cations on the Na⁺-dependent uptake, such as the competitive inhibition of Na⁺ by K⁺ observed in studies of sugar transport in the hamster intestine (18). These possibilities were not investigated in the present paper. Also, if renal Na⁺-dependent transport proves to be electrogenic, as current investigations in this laboratory suggest or as recently indicated in intestinal membranes (15), other ions may influence the Na⁺-dependent D-glucose transport by altering electrical potential gradients across the luminal membrane.

Previous reports on the influence of Na⁺ on the reabsorption of sugar in the kidney, as studied in vitro in renal cortical slices (17), and in intact animals by microperfusion of proximal tubules (25, 26) and in clearance measurements (27, 28) implicate a role for Na⁺ and fluid reabsorption on the transport of sugar. However, the mechanisms by which Na⁺ enhances D-glucose transport in the kidney remain controversial. The increased movement of the sugar may represent a passive response to bulk water and Na⁺ transport or it may be a specific effect of the Na⁺ electrochemical gradient on the mediated transport of D-glucose across the proximal tubular cell luminal membrane, analogous to the mechanism first proposed by Crane (29) for intestinal transport. In the latter scheme, it is the ouabain-sensitive active extrusion of Na⁺ across the lateral and basal membranes of the cell that maintains the Na⁺ gradient across the luminal membrane. Ouabain is known to inhibit D-glucose reabsorption in the dog (30) and the accumulation of α-methyl glucoside in rabbit renal cortical slices (31). The uptake of D-glucose by brush border preparations is not dependent on ATP nor inhibited by ouabain (10). This, however, is understandable since the uptake by the vesicles deals only with that portion of the transport process which takes place at the luminal membrane, and in the present study the Na⁺ gradients are artificially imposed. The Na⁺ gradient across the brush border membrane affects D-glucose transport in two ways, cis stimulation and trans inhibition, both of which are now described in influx and efflux studies. Thus, the experiments reported in this paper clearly demonstrate that the isolated brush border membrane possesses the necessary properties to enhance D-glucose influx and inhibit D-glucose backflux in the appropriate relationship to imposed Na⁺ gradients that can account for net uphill transport.

The physiological significance of the present findings is enhanced by the observations that the Na⁺-dependent D-glucose transport system in the membranes in vitro has now been shown to possess the sugar specificities and high phlorizin sensitivity characteristic of the more physiologically intact preparations. Thus, these results strongly support the view that the isolated brush border membrane preparation is an effective tool in investigations of the molecular mechanisms of...
transport in the kidney (10). Furthermore, whereas studies of phlorizin binding may prove to be valuable as a marker of the D-glucose “carrier” that may be essential for the isolation and chemical characterization of this membrane component, only studies of D-glucose transport itself can provide insight into such dynamic aspects of the molecular mechanism as the energy transductions involved in coupling sugar flux to electrochemical ion gradients.

REFERENCES
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P S Aronson and B Sacktor


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