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TRANSPORT OF D-GLUCOSE BY BRUSH BORDER MEMBRANES ISOLATED FROM THE RENAL CORTEX

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SUMMARY

The transport of D-glucose by brush border membranes isolated from the rabbit renal cortex was studied. At concentrations less than 2 mM, the rate of D-glucose uptake increased linearly with the concentration of the sugar. No evidence was found for a "high-affinity" (μM) saturable site. Saturation was indicated at concentrations of D-glucose greater than 5 mM. The uptake of D-glucose was stereospecific and selectively inhibited by D-galactose and other sugars. Phlorizin inhibited the uptake of D-glucose in the presence and absence of Na^+. The glycoside was a potent inhibitor of the efflux of D-glucose. Preloading the brush border membrane vesicles with D-glucose, but not with L-glucose, accelerated exchange diffusion of D-glucose. These results demonstrate that the uptake of D-glucose by renal brush borders represents transport into an intravesicular space rather than solely binding. The rate of D-glucose uptake was increased when the Na^+ in the extravesicular medium was high and the membranes were preloaded with a Na^+-free medium. The rate of D-glucose uptake was inhibited by preloading the brush border membranes with Na^+. These results are consistent with the Na^+ gradient hypothesis for D-glucose transport in the kidney. Thus, the presence of a Na^+-dependent facilitated transport of D-glucose in isolated renal brush border membranes is indicated. This finding is consistent with what is known of the transport of the sugar in more physiologically intact preparations and suggests that the membranes serve as an effective model system in examining the mechanism of D-glucose transport in the kidney.

INTRODUCTION

Previous physiological studies have led to the concept that the reabsorption of D-glucose in the kidney is an uphill transport process, energy and Na^+ dependent, sensitive to phlorizin, stereospecific and inhibited by specific analogs of D-glucose [1–9]. The preparations used in these studies have ranged from the intact animal, to

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renal cortical slices to segments of the proximal tubule. Recently, techniques for isolating the apical membranes of proximal tubular cells, the renal brush border, have been developed. These membrane preparations have been used as model systems to examine the biochemical mechanisms of sugar transport. In general, two approaches have been taken. In one, the binding to the membrane of phlorizin, the non-metabolizable and presumably non-transportable inhibitor of D-glucose transport, has been investigated to provide insight into the properties of the proposed D-glucose carrier [10–14]. Discrepancies between the kinetics of phlorizin binding and D-glucose transport, however, have raised questions whether the high-affinity phlorizin-binding site is intimately related to the physiological transport of D-glucose [14, 15]. In the other approach, D-glucose uptake has been measured directly [15–18]. Such studies have been complicated, too, by significant metabolism of the sugar [16], proposals of multiple saturable uptake or binding sites [16–18], and the presence of contaminating bacteria that may mask the kinetics of D-glucose uptake by the brush borders [15]. Moreover, it has been reported that the membranal uptake of D-glucose, at physiological (mM) concentrations of the sugar, is independent of Na^+ [16–18].

The present studies were undertaken to reinvestigate the transport of D-glucose by renal brush border membranes, uncomplicated by some of the aforementioned difficulties. The presence of a Na^+ gradient-dependent mediated transport of D-glucose by the isolated membrane is now demonstrated. Additionally, the system for D-glucose uptake by the brush borders is found to be consistent with the transport of the sugar, as studied in more physiologically intact preparations. Preliminary accounts of part of this investigation have been reported [19, 20].

METHODS

Rabbit renal brush border membranes were isolated by a modification of the method detailed previously [21]. In the present procedure the renal cortices, homogenized in 0.5 M sucrose, were layered on a discontinuous sucrose density gradient comprised of 2.5 ml of 1.7 M sucrose and 6 ml of 1.4 M sucrose and centrifuged at $64\,000 \times g$ for 60 min. The pink fluffy brush border layer plus the overlying supernatant were aspirated and resuspended in 0.5 M sucrose to the original volume. The suspension was centrifuged at $5000 \times g$ for 10 min. The loose pink sediment was sloughed from the pellet and together with the supernatant was recentrifuged at $31\,000 \times g$ for 10 min. The resulting supernatant was discarded. The brush borders were visible as the loosely packed pink portion of the pellet overlying a dense, tightly packed brownish colored core, containing primarily mitochondria. The brush borders were washed off the pellet, resuspended in the sucrose, and centrifuged for 10 min sequentially at 12 000, 8000, 6000, 4000 and $3000 \times g$. After each centrifugation, the supernatant was discarded and the loosely packed brush border pellet was washed off the diminishing densely packed contaminating portion of the pellet. After the last centrifugation contamination of the brush border pellet was no longer visible. Then, the membranes were suspended in saline–phosphate buffer, 130 mM NaCl and 16 mM sodium phosphate, pH 7.4, and centrifuged at $27\,000 \times g$ for 5 min. The pellet was resuspended in the saline buffer and recentrifuged three times at $12\,000 \times g$ for 5 min. The yield ranged from 6 to 10 mg of purified brush border membrane protein per rabbit. The quality of the preparations was randomly

evaluated by specific enzyme markers [21] and occasionally by electron microscopy.

In general, uptake of D-glucose by the brush border membranes was measured as described previously [17]. Except when noted, 0.1 ml of the suspension of brush borders, containing 0.2–0.5 mg of protein, was preincubated for 1 min at 20 °C. Incubation at 20 °C was initiated with the addition of 25 μl of saline–phosphate buffer containing D-[^{14}C]glucose, $2 \cdot 10^5$–$1 \cdot 10^6$ cpm, and other additions, as indicated. The incubation was terminated 0.5 min later by the rapid addition of 4 ml of cold saline buffer, and the mixture was immediately poured on a 5-μm Millipore filter (SMWP 02400), prewetted with 0.5 M sucrose. The incubation tube was washed twice with 4 ml of cold buffer, each wash immediately poured on the filter. The filter and filtering apparatus were rinsed four additional times with 4 ml of the cold medium. The entire washing process was completed in 20–30 s. The filter was dissolved in 10 ml of Aquasol (New England Nuclear Corp.) and radioactivity measured with a Packard Tri-Carb Scintillation counter. Quenching was monitored by automatic external standardization, but random sample to sample variation was negligible. In each experiment, a "blank" or non-specific retention of radioactivity was determined by adding the 25 μl of medium containing D-[^{14}C]glucose to the 0.1 ml of brush border suspension after the 4 ml cold saline–phosphate was added. Then, the mixture was immediately filtered and the tube and filter were washed as described. This non-specific retention was generally 10–30 % of the total radioactivity retained by the normally incubated brush borders and this value was subtracted from the incubated samples to estimate the D-glucose taken up specifically. 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. The absolute magnitude of the D-glucose uptake varied over a 2-fold range from experiment to experiment, but the relative uptakes, expressed as the percentage of a control, were consistent and reproducible. All results are expressed as the mean for the number of experiments indicated $\pm$ the standard error of the mean.

Protein was estimated by the method of Lowry et al. [22].

D-[^{14}C]Glucose (240 Ci/mole) was obtained from Schwarz BioResearch. The sugar was chromatographed to test for purity and over 98 % of the counts appeared as a single spot with the same R_F as authentic glucose. Other chemicals were of the highest purity available from commercial sources.

EXPERIMENTAL RESULTS

Time-course of uptake of D-*glucose*

The uptake of 1 mM D-glucose at 20 °C by the brush border membranes was determined after incubations for different periods of time (Fig. 1). Steady-state levels were attained in 16–24 min and no further increase in uptake was seen in incubations of 1–2 h. About 40 % of the maximal uptake occurred in the first 30 s. The value at 30 s was used to approximate the unidirectional initial rate of D-glucose uptake. In 36 separate determinations the mean value for the uptake of 1 mM D-glucose (2 times the 30-s value) was 0.303 ± 0.015 nmole $\cdot$ min^{-1} $\cdot$ mg^{-1} of protein. For comparison, when the incubations were carried out at 37 °C, maximal steady state levels were achieved in 3–5 min [17].

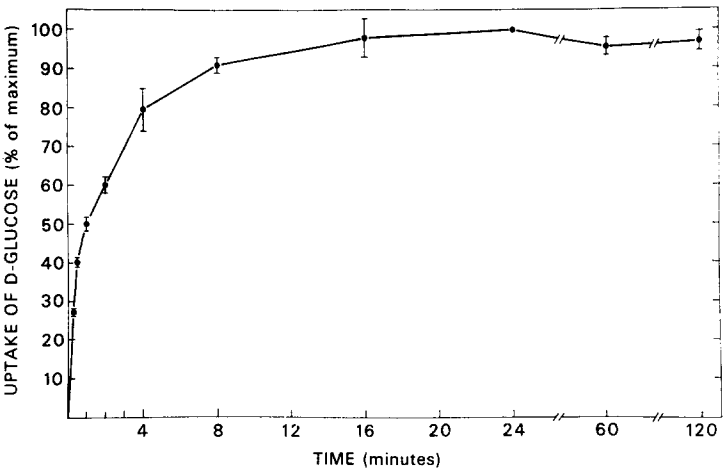

Fig. 1. The time-course of the uptake of D-glucose by renal brush border membranes. Details of the procedure are described in the text. The concentration of D-glucose was 1 mM and the incubation temperature was 20 °C.

Relationship between D-*glucose concentration and uptake*

Fig. 2 describes the effect of D-glucose concentration on the initial rate of uptake. Deviation from a linear relationship, hence suggestion of saturability, was evident with concentrations of D-glucose exceeding 5 mM. Saturation was incomplete, however, in that a plateau was not reached at concentrations as high as 100 mM. At concentrations below 2 mM, D-glucose uptake was dependent in a linear fashion on

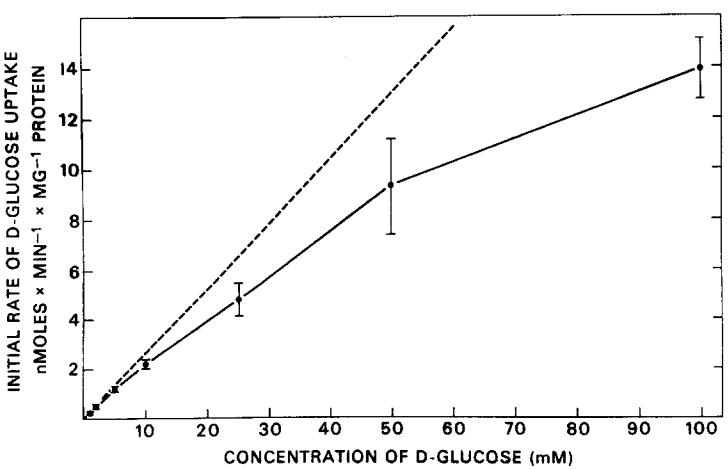

Fig. 2. The dependence of the rate of uptake of D-glucose by renal brush border membranes on the concentration of D-glucose. Details of the procedure are described in the text. Mannitol was added so that the sum of the mannitol and D-glucose concentration was always 100 mM; hence, all incubation mixtures were equiosmotic. The dashed line indicates a hypothetical linear relationship between concentration of sugar and initial rate of uptake.

D-glucose concentration. For example, at 2, 50 and 1000 μM D-glucose the average uptakes were 0.59, 14.7 and 303 pmoles $\cdot$ min^{-1} $\cdot$ mg^{-1} of protein, respectively, increments of 25- and 500-fold. No evidence was found for the presence in the brush borders of a saturable "high-affinity" (μM) site [16–18] even when uptakes were measured at concentrations as low as 0.8 μM. As reported elsewhere [15], this saturable "high-affinity" site was attributable to contamination of the earlier preparations and reagent solutions with *Pseudomonas* bacteria. Thus, the present data refute the hypothesis of multiple saturable systems for the uptake of D-glucose in renal brush border membranes, when measured at incubation periods of 0.5 min and longer.

Identification and recovery of the D-*glucose taken up by the brush borders*

Brush border preparations were incubated with 1 mM D-glucose, containing traces of D-[^{14}C]glucose, at 20 °C for 30 s or 24 min and the membranes were retained on Millipore filters by the standard procedure. The sugar taken up by the membranes was extracted with 2 ml of distilled water by heating the filter in a boiling water bath for 15 min. The filter was reextracted two more times by the same method. The eluates, containing 97 % of the total radioactivity were combined and centrifuged at 30 000 $\times g$ for 15 min. The precipitate containing 1 % of the radioactivity was discarded. The supernatant was concentrated by lyophilization and chromatographed on thin-layer silica gel, as described previously [23], with the modification that the plates were chromatographed three times in one direction rather than once in two directions. The glucose spot and each 2 cm of gel on the plate was scraped and measured for radioactivity. As illustrated in Fig. 3, approximately 90 % of the radioactivity had a R_F identical with that of authentic glucose. No other discrete radioactive spot

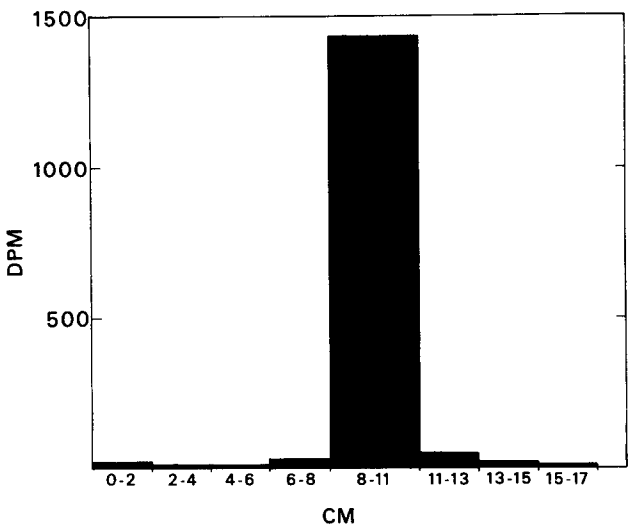

Fig. 3. The recovery of the D-glucose taken up by renal brush border membranes. Brush borders were incubated with 1 mM D-glucose containing traces of D-[^{14}C]glucose for 24 min at 20 °C. The accumulated sugar was extracted from the membranes and chromatographed on silica gel thin-layer plates, as described in the text. Authentic D-glucose migrated to a spot 8–11 cm from the origin.

was detected. There were no significant differences between the recoveries of D-glucose when the incubations were for 30 s or 24 min. The present results contrasted markedly with those of Busse et al. [16] who found that 35 % of the D-glucose accumulated by their preparations of renal brush borders was metabolized to lactate and pyruvate. The present findings did agree, however, with our earlier observations on the recovery of D-glucose after incubation with brush borders [17] and with the absence of glycolytic enzymes from these purified renal brush border preparations [21]. In accord with this, other experiments demonstrated that the hexokinase inhibitor, *N*-acetylglucosamine (10 mM), had no effect on the uptake of 1 mM D-glucose.

Efflux of D-*glucose*

Brush border membranes were incubated with 1 mM D-glucose at 20 °C for 24 min, at which time steady state levels were attained, e.g. Fig. 1. The incubation mixture was then diluted 1 : 10 in D-glucose-free media and reincubated at the same temperature for varying periods of time. As described in Fig. 4, release of the accumulated sugar was rapid, in 30 s approximately 35 % of the D-glucose taken up was released. The 30-s value was used to approximate the unidirectional initial rate of D-glucose efflux. Comparison of the data in Fig. 4 and Fig. 1 showed that the relative initial rates of D-glucose uptake and efflux were similar.

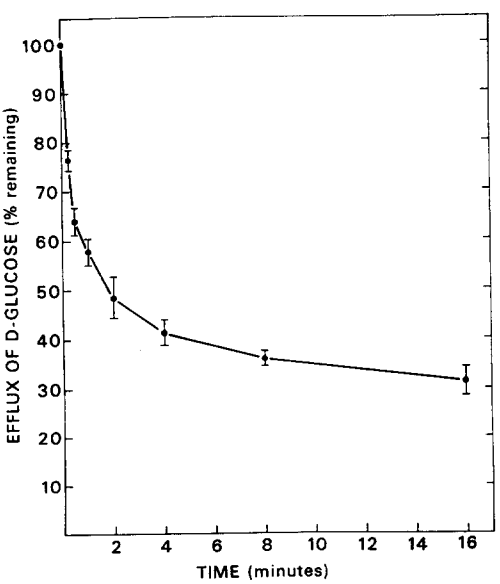

Fig. 4. The time-course of the efflux of D-glucose from renal brush border membrane vesicles, preloaded with the sugar. Brush border membranes were preincubated for 24 min at 20 °C with 1 mM D-glucose. At zero time the incubation mixture was diluted 1:10 with sugar-free media and the D-glucose remaining was measured for various time periods at 20 °C. The zero time (100 %) value was 0.267 nmole/mg of protein.

Effects of temperature on D-*glucose uptake and efflux*

The initial rates of D-glucose uptake and efflux were highly sensitive to temperature. As shown in Table I, at 0 °C the rates of both influx and efflux were decreased to approximately one-third of those at 20 °C. At 37 °C, the rates were 70–80% greater than those at 20 °C.

TABLE I

EFFECTS OF TEMPERATURE ON THE INITIAL RATES OF D-GLUCOSE UPTAKE AND EFFLUX

Initial rates of uptake and efflux of D-glucose were estimated at 30 s as described in the text. Concentration of D-glucose was 1 mM. The values obtained with the standard experimental temperature, 20 °C, were considered as 100%. The 100% values for uptake and efflux averaged 0.27 and 0.23 nmole · min^{-1} · mg^{-1} of protein, respectively. The data are reported as the mean ± S.E. for the number of separate experiments, shown in parenthesis.

	Temperature (°C)	Rate (% of rate at 20 °C)
Uptake	0	37 ± 5 (4)
	20	100 (4)
	37	183 ± 12 (4)
Efflux	0	29 ± 5 (3)
	20	100 (3)
	37	169 ± 3 (3)

Effects of phlorizin on D-*glucose uptake and efflux*

As demonstrated in Table II, phlorizin inhibited the uptake of D-glucose by renal brush border membranes, as had been reported previously [16, 17]. The percent inhibition increased with increasing concentrations of phlorizin. However, concentrations of phlorizin as high as 1 mM inhibited the uptake of 1 mM D-glucose

TABLE II

EFFECT OF PHLORIZIN ON THE INITIAL RATE OF UPTAKE OF D-GLUCOSE

Initial rates of uptake of 1 mM D-glucose were measured as described in the text. In the experiments carried out in the Na^+ media, 130 mM NaCl, 16 mM potassium phosphate, pH 7.4, was used in the last four centrifugations of the preparation of the brush border membranes and in the incubation medium. In the experiments carried out in the choline media, choline chloride replaced NaCl in these steps. The data are reported as the mean ± S.E. for 3 separate experiments. The uptakes in the absence of phlorizin, 0% inhibition, were 0.322 and 0.324 nmole · min^{-1} · mg^{-1} of protein in the sodium and choline media, respectively.

Phlorizin concentration (μM)	Percent inhibition	
	Na^+ media	Choline media
0	0	0
5	6.1 ± 0.7	8.9 ± 6.2
50	17.5 ± 5.4	19.3 ± 4.8
500	35.6 ± 6.0	39.5 ± 6.7
1000	40.9 ± 3.5	–

TABLE III

EFFECT OF PHLORIZIN ON THE INITIAL RATE OF EFFLUX OF D-GLUCOSE

Initial rates of efflux of D-glucose were measured as described in the text. Brush border membranes were preloaded by incubating membranes with 1 mM D-glucose for 24 min at 20 °C. At 0 time, the incubation mixture was diluted 1:10 with sugar-free media, with and without 1 mM phlorizin. Efflux was measured 30-s later. The membranes had accumulated 0.338 nmole of D-glucose per mg of protein prior to dilution. Data are reported as the mean±S.E. for three separate experiments.

Experiment	Efflux (%)
Undiluted	0
Diluted 1:10 with media	34.4±1.8
Diluted 1:10 with media+1 mM phlorizin	0.4±1.6

only 40 %. As also shown in Table II, the inhibitory action of phlorizin was apparently the same when determined in the presence of Na^+ or choline.

Table III demonstrates that 1 mM phlorizin essentially completely inhibited the efflux of D-glucose from the membranes. These findings, therefore, were consistent with the view that the interaction of D-glucose with the renal brush border membranes represented the transport of the sugar across the membrane rather than simply the binding of D-glucose to the surface of the membranes, as had been suggested previously [17, 18]. If the interaction were binding only, then it would be expected that phlorizin would have displaced D-glucose, accelerating the release of the bound D-glucose if phlorizin binding was competitive and the number of sites were limiting, or else would have no effect at all. The fact that phlorizin inhibited the release of the sugar, as well as its uptake, indicated that phlorizin reacted with the membrane at a site external to the site of D-glucose sequestration.

TABLE IV

EFFECT OF PRELOADING WITH UNLABELED SUGARS ON THE UPTAKE OF D-[^{14}C]-GLUCOSE

Renal brush border membranes were preincubated for 24 min at 20 °C with 50 mM mannitol, L-glucose or D-glucose, as indicated. Then, an equal volume of media containing 50 mM D-glucose (if the preincubation had been with mannitol or L-glucose) or 50 mM mannitol (if the preincubation had been with D-glucose), containing tracer amounts of D-[^{14}C]glucose was added. Time of incubation was 30 s at 20 °C. The value obtained when preincubated with mannitol was considered the control value (0 % change). The mean value of D-glucose uptake in the controls was 6.02 nmoles · min^{-1} · mg^{-1} of protein. Data are reported as the mean ±S.E. for 6 separate experiments.

Preincubation sugar (mM)	Incubation sugar (mM)	D-Glucose uptake (% change from control)
Mannitol (50)	Mannitol (25) D-Glucose (25)	0
D-Glucose (50)	Mannitol (25) D-Glucose (25)	+43±11*
L-Glucose (50)	L-Glucose (25) D-Glucose (25)	+ 7±13

* $P<0.02$

Accelerative exchange diffusion

Additional evidence that the uptake of D-glucose by renal brush borders represented transport into an intravesicular space formed by the membrane rather than binding to specific sites was obtained from experiments measuring exchange diffusion. As described in Table IV, brush border membranes were preloaded with 50 mM D-glucose or mannitol. At zero time, D-[^{14}C]glucose was added so that the outside medium was 25 mM D-glucose+25 mM mannitol and the inside medium was either mannitol or D-glucose. There was a marked stimulation (43 %) in the initial rate of D-glucose uptake when the brush borders were preloaded with D-glucose. The effect was stereospecific, as the rate of D-glucose uptake was not augmented by preincubation with L-glucose. Accelerated exchange diffusion could not be explained by the binding hypothesis, because preincubation with unlabeled D-glucose would occupy binding sites and, if anything, would have inhibited the uptake of D-[^{14}C]glucose. On the other hand, models to explain the process of accelerative exchange diffusion all involve the facilitated transport across a membrane [24–26].

Effects of sugar analogs on the rate of D-*glucose uptake*

The effect of several sugars, tested at 100 mM, on the uptake of 1 mM D-glucose is shown in Table V. L-Glucose had no effect on the uptake of the D-isomer, indicating the stereospecificity of the transport system in the brush border membrane. D-Galactose, 3-*O*-methyl-D-glucose and D-glucose, itself, were the most effective inhibitors of D-[^{14}C]glucose uptake.

TABLE V

EFFECTS OF SUGAR ANALOGS ON THE INITIAL RATE OF UPTAKE OF D-GLUCOSE

Initial rates of uptake of labeled 1 mM D-glucose were estimated as described in the text. 100 mM mannitol was added in the control incubations. The mannitol was replaced by 100 mM of each sugar, as indicated. The control value, 0 % inhibition, averaged 0.238 nmole of D-glucose · min^{-1} · mg^{-1} of protein for 15 separate experiments. Data are reported as the mean±S.E. for the number of separate experiments, given in parentheses.

Sugar (100 mM)	Inhibition (%)	
Control	0	(15)
L-Glucose	2.2±3.8	(15)
α-Methyl-D-glucoside	16.5±4.4	(5)
2-Deoxy-D-glucose	21.8±5.7	(5)
D-Mannose	31.9±7.5	(7)
D-Galactose	38.6±3.4	(5)
3-*O*-Methyl-D-glucose	39.3±3.4	(5)
D-Glucose	42.0±3.8	(15)

Effect of sodium on the rate of D-*glucose uptake*

As described in Table VI, brush border membranes were prepared so that the vesicles were preloaded with either Na^+ or choline. When initial rates of 1 mM D-glucose uptake were measured with membranes prepared with 130 mM Na^+ and incubated with 130 mM Na^+ and compared with those of membranes prepared

TABLE VI

EFFECT OF Na^+ ON THE INITIAL RATE OF UPTAKE OF D-GLUCOSE

Initial rates of uptake of 1 mM D-glucose were estimated as described in the text. The medium used for resuspending the brush border pellet during the last four centrifugations and for the final resuspension of the membranes is designated as the preparation medium. At zero time 100 μl of brush border suspension was mixed with 100 μl of solution, containing D-glucose, to yield an incubation medium of the final composition as indicated. The incubation was terminated 30 s later. All media contained 130 mM Cl^-, 29 mM K^+ and 16 mM phosphate, pH 7.4. All data are reported as the percent control ± the standard error from incubations containing 130 mM choline in both the preparation and incubation media. The mean value for the uptake of D-glucose in this control was 0.248 nmole · min^{-1} · mg^{-1} of protein. Values are the means of 5 separate experiments.

Incubation medium (mM)	Preparation medium (mM)	D-Glucose uptake (% change from control)
Choline (130)	choline (130)	100
Na^+ (65) + choline (65)	choline (130)	159.4 ± 6.7*
Na^+ (65) + choline (65)	Na^+ (130)	102.4 ± 6.3**
Na^+ (130)	Na^+ (130)	116.1 ± 9.0
Li^+ (65) + choline (65)	choline (130)	83.9 ± 7.1
Na^+ (65) + Li^+ (65)	Na^+ (130)	97.1 ± 9.0

* The difference between experiments in which the preparation medium was 130 mM choline and the incubation medium was either 130 mM choline or 65 mM Na^+ + 65 mM choline has a significance of $P < 0.001$.

** The difference between experiments in which the incubation medium was 65 mM Na^+ + 65 mM choline and the preparation medium was either 130 mM choline or 130 mM Na^+ has a significance of $P < 0.001$.

and incubated with 130 mM choline, there was only a slight stimulatory effect due to the presence of Na^+. However, when the membranes were preloaded with choline and the Na^+ in the extravesicular medium was relatively high (65 mM Na^+ + 65 mM choline) the rate of D-glucose uptake was increased by about 60 %. The specificity of this effect of Na^+ was evident from the findings that neither Li^+ (Table VI) nor K^+ (not illustrated) could replace Na^+. On the other hand, when the incubation medium was also 65 mM Na^+ + 65 mM choline, but the brush border membranes were preloaded with Na^+, rather than choline, the rate of D-glucose uptake was decreased from 159 to 102 % of the control value. In other experiments, it was found that 1 mM ouabain did not inhibit and 5 mM ATP did not stimulate the uptake of D-glucose in the Na^+ media.

DISCUSSION

In general, previous studies on the accumulation of D-glucose by preparations of renal brush border membranes were equivocal because of significant glucose catabolism, bacterial contamination and claims of multiple saturable uptake or binding sites [15–18]. The present experiments resolve some of these ambiguities. For example, in this study glucose metabolism is negligible. Further, no evidence is found for the presence of "high affinity" saturable sites, even when uptake was measured at D-glucose concentrations as low as 0.8 μM. Mitchell et al. [15] found that earlier prep-

arations of brush borders and reaction media were contaminated with *Pseudomonas* bacteria and that previously proposed sites saturable at μmolar concentrations of D-glucose probably represent uptake of the sugar by bacteria. In other experiments, not reported here, the uptake of D-glucose by sterile brush border preparations [15] was compared directly with those used in this paper. Identical results were obtained. This suggests that when brush borders are incubated with mmolar concentrations of D-glucose, uptake of the sugar by *Pseudomonas* would be insignificant relative to that by the renal membranes. Thus, these findings, in conjunction with those reported elsewhere [15], argue against the proposals for more than one saturable site for D-glucose interaction with the renal brush border. Moreover, the one saturable system which is indicated has apparent saturability for D-glucose in the mmolar range.

The present data strongly indicate that the uptake of D-glucose by the renal brush border membranes represents the transport of the sugar into membrane vesicles rather than simple binding to the membrane. Compelling evidence in support of this view comes from the finding that phlorizin inhibits both the uptake and release of D-glucose. Further support for the transport hypothesis stems from the finding that preloading the membranes with D-glucose augments D-glucose uptake. Also favoring the interpretation that the isolated brush borders behave as membrane vesicles are electron microscopic observations which show that brush borders react to the tonicity of the medium by condensing and swelling in, respectively, hypertonic and hypotonic media [17].

Accepting, then, that the transport of D-glucose into renal brush border membrane vesicles has been examined in this study, the question arises as to how these findings compare with what is known about the physiology of D-glucose transport in the kidney.

The D-glucose transport system in brush border membranes shows a tendency to saturate, but only incompletely. In this respect, the results resemble those found with intestinal brush border membrane vesicles [27]. There are several possible explanations for the imperfect saturability of the membrane system relative to the defined transport maximum (T_m) for D-glucose in the intact kidney. First, in the membrane system studied in this paper initial rates of D-glucose uptake were approximated from the value at 30 s. Despite this short period of incubation, the rate of uptake was not increasing linearly with time and was 40 % of the equilibrium value (Fig. 1). Equilibrium values would tend to increase linearly with concentration. Hence, better saturability with concentration would be expected if truer initial velocities could be measured. Secondly, at least a component of the D-glucose uptake by the isolated brush border membranes may be due to passive diffusion, or leak. L-Glucose was used by Hopfer et al. [27] in intestinal studies to correct for this. However, L-glucose may not be strictly passive in the kidney [28, 29] and the uptake of L-glucose, like that of D-glucose is inhibited by sulfhydryl reagents [17].

The stereospecificity of the transport system in the isolated brush border membranes is indicated from the findings that L-glucose does not inhibit the uptake of D-glucose nor accelerate exchange diffusion. The pattern of selectivity for other sugars, as measured by their relative inhibitions of D-glucose uptake, is in general agreement with that of "System A" reported by Busse et al. [16], though they did not test α-methyl-D-glucoside. It is noted, however, that 3-*O*-methylglucose, an effective in-

hibitor of the uptake of D-glucose by the rabbit brush border membranes, poorly penetrates the luminal membrane of the dog, in vivo [2], and is not taken up by rabbit renal slices, in vitro [30]. On the other hand, 3-*O*-methylglucose was not tested as an inhibitor of D-glucose transport in those systems and it is possible that it may effectively inhibit the interaction of D-glucose with its transport system, yet not itself be transported.

Transport of D-glucose by the brush border membranes is inhibited by phlorizin. However, significant inhibition of the uptake by membranes as well as cortical slices requires phlorizin concentrations of from 10^{-4} to 10^{-3} M [6, 16, 17], whereas concentrations of 10^{-5} to 10^{-7} M inhibit D-glucose reabsorption in vivo [3]. Of additional concern is the observation that the phlorizin inhibition of D-glucose uptake by the brush borders is independent of Na^+. Other studies of the binding of phlorizin to these membranes reveal Na^+-dependent high-affinity (8 μM) and Na^+-independent low-affinity (2.5 mM) binding sites [10–14]. The uptake of phlorizin by renal cortical slices, in contrast, is independent of Na^+, not saturated and not inhibited competitively by sugars [14]. These findings may indicate that phlorizin interacts with a quantitatively large number of binding sites of lesser specificity. It is also feasible that the interaction of phlorizin with the brush border membranes represents the accumulation of the glycoside into vesicles, as suggested from studies with cortical slices [14] and isolated renal tubules [31]. The reasons for these apparent discrepancies are not known at this time, but various explanations are possible, including: only a Na^+-dependent component of D-glucose uptake may be highly sensitive to phlorizin; the high affinity phlorizin binding site may not be related to the physiological transport of D-glucose; and both high- and low-affinity phlorizin sites may play roles.

The present study demonstrates that the uptake of D-glucose into Na^+-free brush border membrane vesicles is stimulated by adding Na^+ to the incubation medium but inhibited by preloading the vesicles with Na^+. These findings are consistent with the reported uphill, Na^+-dependent, ouabain-sensitive uptake of sugar into renal slices [6, 8, 32], and with clearance studies showing glucose transport to be affected by Na^+ transport and inhibited by ouabain [33–35]. It is pointed out that, whereas in more intact systems it is the ouabain-sensitive active extrusion of Na^+ across the lateral and basal membranes of the tubule cell that presumably creates the Na^+ gradient required for uphill sugar transport, in the isolated brush border preparation Na^+ gradients are artificially imposed. Hence, ouabain sensitivity or dependence on ATP is not seen. The recent observation that glucose reabsorption in the presence of Na^+ causes depolarization of the proximal tubule luminal membrane [36] suggests that there may be glucose induced Na^+ cotransport as well. Thus, our studies support the Na^+ gradient hypotheses proposed by Crane [37] and further elaborated upon by Schultz and Curran [38].

The experiments reported in this paper show that the characteristics of D-glucose uptake by isolated renal brush border membranes are in agreement with what is known of the transport of the sugar by more physiologically intact preparations. Thus, the membranes can serve as an effective model system to study the mechanisms of D-glucose transport in the kidney.

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