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THE SPECIFICITY OF THE ACTIVE SUGAR TRANSPORT IN KIDNEY CORTEX CELLS

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SUMMARY

The transport of some monosaccharides into renal tubular cells was investigated using slices of rabbit kidney cortex:

1. An active, Na^+-dependent and phlorrhizin-sensitive cellular accumulation of β-methyl-D-glucoside, D-glucosamine and D-galactosamine was demonstrated. D-Mannitol, L-glucose, *N*-acetyl-D-glucosamine, 3-deoxy-D-glucose, D-allose, D-ribose and 2,6-dideoxy-D-altrose were not actively accumulated against their concentration gradients. 2-Deoxy-D-allose, 2-deoxy-D-ribose and possibly 2-deoxy-D-xylose were transported against a small concentration gradient by a Na^+-independent mechanism.

A comparison of the transport properties of 26 monosaccharides indicates the following structural requirements for active transport: a hemiacetal group, C-3–OH (in a configuration identical with that of D-glucose) and C-6–OH. The Na^+ requirement for active transport is related to the presence of a hydrophilic (–OH or $-NH_2$) group on C-2.

2. The apparent kinetic parameters, K_m and v_{max}, are given for the active transport of α-methyl-D-glucoside, D-galactose, 2-deoxy-D-glucose and 2-deoxy-D-galactose into renal tubular cells. The values of the apparent transport K_m for these sugars varied only slightly between 0.79 ± 0.07 for D-galactose to 3.0 ± 0.11 for 2-deoxy-D-galactose; rather wide variations of v_{max} were found (12 μmoles per g cell water per h for 2-deoxy-D-glucose to 62 ± 4.7 for α-methyl-D-glucoside).

3. The competition for transport between a variety of monosaccharides was investigated: the found K_i was within the range of independently determined K_m for the inhibition by galactose of 2-deoxy-D-galactose and 2-deoxy-D-glucose transport. However, the K_i for the inhibition of D-galactose transport by 2-deoxy-D-galactose was one order of magnitude higher than the K_m of the latter sugar. Similarly, no agreement between the respective K_i and K_m was found for the competitive inhibition between α-methyl-D-glucoside and galactose. D-Glucose, which competitively inhibited the transport of 2-deoxy-D-glucose, had practically no effect on the transport of 2-deoxy-D-galactose even at a molar ratio of 1:50.

4. These kinetic data indicate the existence of several pathways for the active sugar transport into renal tubular cells.

5. A correlation between the reabsorption of sugars in the kidney tubule (*in vivo*) and their active cellular accumulation (*in vitro*) is demonstrated.

INTRODUCTION

Previous studies[1,2] based on a comparison of the transport properties of 13 monosaccharides, showed a marked active, phlorrhizin-sensitive accumulation of the following sugars in cells of kidney cortex: D-glucose, D-fructose, D-galactose, α-methyl-D-glucoside, 2-deoxy-D-glucose and 2-deoxy-D-galactose. This investigation indicated that for the active cellular accumulation of monosaccharides in kidney cortex slices, the following structural requirements of the sugars have to be met: (1) a hydroxyl on C-3, since 3-*O*-methyl-D-glucose was not actively accumulated; (2) a hydroxyl on C-6 (6-deoxyhexoses being accumulated only against small concentration gradients). (3) Furthermore, it was established that the Na^+ dependence of the active sugar accumulation in these cells was related to the presence of a hydroxyl on C-2. 2-Deoxy-D-glucose and 2-deoxy-D-galactose were found to be actively accumulated in renal tubular cells in the absence of external Na^+. This Na^+-independent transport of 2-deoxyhexoses was also insensitive to ouabain, as opposed to the Na^+-dependent entry of sugars containing C-2–OH.

The present communication extends the previous studies with the aim of defining further the structural requirements for the active sugar transport system (or systems) in renal cortex cells as well as for the Na^+ dependence thereof. Kinetic data reported here also indicate that the active sugar transport by renal tubular cells is brought about by more than one pathway (or site).

A preliminary report on some of the results has been presented[3].

METHODS AND MATERIALS

Slices of kidney cortex of adult rabbits were used throughout. The animals were decapitated and the kidneys were immediately placed into ice-cooled dishes and slices. Slices (mean thickness 0.3 mm) were prepared by the method of DEUTSCH[4].

The basic saline of the Krebs–Ringer type, allowing considerable flexibility for modifications of its composition in this and subsequent studies, was prepared as follows (unless otherwise stated, all stock solutions were 0.154 M): NaCl, 95 ml; lithium acetate, 5 ml (acetate serving as metabolic substrate); KCl, 4 ml; $CaCl_2$ (0.11 M), 3 ml; KH_2PO_4, 1 ml; $MgSO_4$, 1 ml; Tris–HCl buffer (pH 7.4), 5 ml. Stock solutions of the saline components were prepared by dissolving analytical-grade reagents in glass-distilled water. Na^+-free salines were prepared by an equivalent replacement of NaCl by Tris–HCl buffer (pH 7.4). It was found previously that Tris–HCl buffer is innocuous for the sugar transport system, identical results having been obtained with salines in which NaCl was replaced by LiCl or choline chloride[1].

The experimental procedure has been described in detail earlier[2] and will be only summarized here: Cut slices were placed into ice-cold Na^+- or $Tris^+$-saline and maintained at 0° for 2.5 h. The tissue was repeatedly washed with the respective salines. In this way, most of the tissue Na^+ was removed from the slices placed in $Tris^+$-saline.

Groups of four to five slices were preincubated aerobically (O_2 as gaseous phase) for 45 min at 25° in a Dubnoff metabolic shaker in 50-ml conical flasks containing 8 ml of the appropriate saline. This procedure ensured a steady-state distribution of tissue water and electrolytes; furthermore, the tissue was also impoverished of endo-

genous glucose which might interfere with the subsequent transport of the tested sugars.

Subsequently, the slices were transferred into 50-ml conical flasks containing 8 ml of identical salines *plus* the tested sugar (usually 1 mM) or sugar and inhibitor, and the tissue was incubated aerobically (O_2) for 60 min, when a steady-state level of sugar was reached.

After the incubation, the slices were removed, blotted between disks of Whatman filter paper No. 541, each slice was weighed on a torsion balance (usually 30–50 mg) and placed into homogenizing tubes containing 3 ml 1.67 % (w/v) $ZnSO_4 \cdot 7H_2O$. The tissue was then homogenized and 1 ml of previously titrated 0.15 M $Ba(OH)_2$ was added. In this way a deproteinized extract of the tissue containing free sugars only[5] was obtained. This extract was then used for sugar determinations. Portions of the incubation media before and after incubation were similarly deproteinized and the supernatant used for the determination of the sugar concentration ($[S]_0$) in the medium.

Analytical procedures

2-Deoxysugars were assayed using the specific procedure of WARAVDEKAR AND SASLAW[6]. The tissue blank was usually so low that it could be safely neglected. In some instances, glucose was determined in the tissue extracts and media using a glucose oxidase reagent (Glucostat, Worthington). All other sugars tested were ^{14}C- or ^{3}H-labeled. Their concentration in the media (0.025 μC/ml for ^{14}C-labeled substrates, 0.1 μC/ml for ^{3}H) and in the tissue extracts was determined by measuring the activity in 1-ml portions of the deproteinized extract. A Packard 3320 liquid-scintillation spectrometer was employed. The scintillation fluid consisted of 10 ml of a toluene–Triton X-100 (2:1, v/v) mixture containing 5 g 2,5-diphenyloxazole and 0.2 g 1,4-bis-(5-phenyloxazol-2-yl)benzene per l (see ref. 7). Since no additional quenching was observed using the external standard technique, no corrections had to be introduced.

The above deproteinizing procedure could not be employed where the accumulation of hexosamines was studied. Preliminary tests showed that a portion of the (^{14}C-labeled) hexosamines was retained in the $Zn(OH)_2$–$BaSO_4$ precipitate. Therefore, the tissue and the media were deproteinized with 5 % (w/v) trichloroacetic acid. The supernatant contained hexosamines as well as considerable amounts of neutral (presumably acetylated) derivatives. This was ascertained as follows: The trichloroacetic acid tissue and medium supernatants (3 ml) were extracted with two 5-ml portions of diethyl ether in order to remove the deproteinizing agent. 0.5-ml portions of the aqueous solution were then used for the determination of the total counts. 2-ml portions of the extract were passed through columns (3.5 cm × 0.5 cm) of Dowex 50-X8 (ref. 8) and the columns washed with 2 ml water. The combined eluate contained the neutral sugar (presumably *N*-acetylhexosamine). The hexosamines were then eluted from the columns with two 1-ml portions of 2 M HCl, and the columns were again washed with 2 ml water. In the combined eluates the activity was again determined after removal of HCl *in vacuo* over solid KOH. The recovery of counts was satisfactory (103.8 % ± 1.3, $n = 18$).

From the analytical data the apparent intracellular concentration of the tested sugars ($[S]_i$) was calculated after correction for the extracellular space. The accumulation ratio, $[S]_i/[S]_0$, was then computed. Each reported value represents the mean

of at least four determinations; where more analytical data were obtained using at least two animals, values ±S.E. are given. From the difference between the amount of sugar originally present in the flask and that after incubation *plus* that accumulated in the tissue, the rate of sugar utilization was computed and expressed in μmoles/g tissue per h.

In experiments where the rate of sugar transport, v, was studied, $[S]_i$ (μmoles/g cell water) equals v if the transport is reasonably linear within the given time period. The tissue was incubated usually for 20–30 min in the presence of the tested sugar. The further procedure for the determination of the sugars in the tissue and the media was as described above. Each experimental point presented was the mean of usually three determinations. The apparent kinetic parameters of transport, K_m and v_{max}, were obtained from experiments in which the relationship between v and $[S]_0$ was investigated, the latter value being determined at the end of incubation. In studies of competition between sugars for transport, K_i was graphically computed[9]. In some experiments K_i was calculated according to the equation

$$\frac{v}{v_i} - 1 = \frac{K_m}{K_m + [S]_0} \cdot \frac{[I]_0}{K_i}$$

which describes competitive inhibition in accordance with the Michaelis–Menten theory (see refs. 9 and 10): v_i is the rate of transport of the substrate in the presence of the inhibiting sugar of concentration $[I]_0$; values of K_m were independently determined.

It ought to be pointed out that the employed technique does not allow the determination of zero-time kinetic parameters. The considerable extracellular space prohibits a desirable shortening of the incubation period necessary to obtain significant values for the intracellular sugar content. Also, if some sugars should employ several pathways of entry, as indicated in RESULTS, the found kinetic parameters represent composite values; this applies particularly to D-galactose.

Materials

The following labeled sugars were obtained from New England Nuclear Corp., Boston and Calbiochem, Los Angeles: D-[1-^{14}C]mannitol, L-[1-^{14}C]glucose, D-[1-^{14}C]galactose, α-methyl-D-[^{14}C]glucoside, β-methyl-D-[^{14}C]glucoside, D-[1-^{14}C]glucosamine, D-[1-^{14}C]galactosamine, *N*-acetyl-D-[1-^{14}C]glucosamine, 2-deoxy-D-[1-^{14}C]glucose, D-[1-^{14}C]ribose, 2-deoxy-D-[1-^{14}C]ribose. D-[^{3}H]Allose and 3-deoxy-D-[^{3}H]glucose were a gift from Dr. R. K. Crane, Rutgers University, New Brunswick, N. J. 2-Deoxy-D-xylose was kindly supplied by Prof. E. Hardegger, Eidg. Techn. Hochschule, Zurich. 2-Deoxy-D-allose and 2,6-dideoxy-D-altrose (digitoxose) were purchased from Gulf South Research Institute, Baton Rouge, La.

RESULTS

Accumulation of monosaccharides in renal tubular cells

Table I summarizes the results of experiments in which the accumulation of various sugars was studied under the following conditions: in standard saline, in Na^+-free ($Tris^+$-) saline, and also the effect of 0.5 mM phlorrhizin and 1 mM D-galactose on the $[S]_i/[S]_0$ in Na^+-saline.

TABLE I

ACCUMULATION OF VARIOUS MONOSACCHARIDES BY KIDNEY CORTEX CELLS

Slices were preincubated aerobically (O_2) for 45 min at 25° in either Na^+-saline or $Tris^+$-saline, then incubated for 60 min under identical conditions in saline media containing the tested sugar (1 mM) without and with 0.5 mM phlorrhizin or 1 mM D-galactose. Mean values ± S.E. are given where more than five analyses were carried out. Values without indicated S.E. are the means of four analyses. Monosaccharide concn. 1 mM.

Monosaccharide	*Rate of utilization (μmoles/g tissue water per h)*	*Na^+-saline*	*$[S]_i/[S]_0$ $Tris^+$-saline*	*Na^+-saline + phlorrhizin*	*Na^+-saline + D-galactose*
D-[^{14}C]Mannitol	<0.1	0.36 ± 0.01	0.39 ± 0.01	0.22 ± 0.004	0.27 ± 0.1
L-[^{14}C]Glucose	<0.1	1.11	0.89	0.63	0.99
β-[^{14}C]Methyl-D-glucoside	0.7	11.7 ± 0.99	0.73	0.97	11.3 ± 1.3
D-[^{14}C]Glucosamine*	1.7	2.32 ± 0.02	1.22 ± 0.01		
N-Acetyl-D-[^{14}C]-glucosamine	0.8	0.33 ± 0.02	0.56 ± 0.02	0.31 ± 0.01	0.31 ± 0.0
D-Digitoxose	<0.1	1.04 ± 0.04	1.00 ± 0.13	0.88 ± 0.02	0.97 ± 0.0
D-[^{14}C]Mannose**	<0.1	1.64	1.18		
3-Deoxy-D-[^{3}H]glucose	<0.1	0.72 ± 0.03	0.52 ± 0.04	0.41 ± 0.03	
D-[^{3}H]Allose	<0.1	1.28	0.94	0.95	0.99
2-Deoxy-D-allose	0.3	1.75	1.83		
D-[^{14}C]Galactosamine*	1.7	2.82	1.38	0.65	1.37
D-[^{14}C]Ribose	1.4	1.01	0.80	0.58	
Deoxy-D-[^{14}C]ribose	0.8	1.77 ± 0.10	1.64 ± 0.14	1.20 ± 0.13	
2-Deoxy-D-xylose	3.2	1.20 ± 0.05	1.40 ± 0.08	1.06 ± 0.08	1.39 ± 0.0

* A considerable portion of the tissue activity and also some activity of the media after incubation passed through the Dowex-1-X8 column and thus represented a non-dissociable sugar, presumably the *N*-acetylhexosamines (see text).

** These values were not corrected for the small amounts of free glucose found in the tissue and media after incubation (see text).

D-Mannitol was found to enter to some extent the cell space and this entry was found to be depressed by both phlorrhizin and galactose. It should be mentioned here that previously[2] sucrose has also been found to enter a cell compartment by a phlorrhizin-sensitive mechanism. The absence of active accumulation of mannitol contrasts with the active transport of various aldohexoses.

L-Glucose did not accumulate against its concentration gradient in renal tubular cells. The entry of this sugar was Na^+ dependent and phlorrhizin sensitive in the same way as previously reported for D-glucose[1].

The transport characteristics of β-methyl-D-glucoside were identical with those previously reported for its α-anomer, *i.e.* the very active accumulation of this sugar was depressed to $[S]_i/[S]_0$ values below 1 by the absence of external Na^+ and by 0.5 mM phlorrhizin. It should be mentioned here that no β-glucosidase activity was detected in the tissue since no free glucose could be found after incubation on both tissue and media.

The epimer of D-glucose on C-2, D-mannose, was found to be sluggishly accumulated against its concentration gradient and this accumulation was Na^+ dependent. In these experiments, glucose was also determined in the tissue and the media after incubation in view of the report[11] that kidney cortex cells convert D-mannose to

D-glucose. It was confirmed that in the presence of D-mannose the tissue concentration of D-glucose was somewhat increased (from 0.2 to 0.7 μmole/g). However, even if the correction for the amounts of glucose in the tissue and the medium was made, the $[S]_i/[S]_o$ was still significantly higher than 1.0. In any case, it is questionable whether such correction is justified since the conversion of mannose to glucose of necessity takes place after mannose has been transported into the cells.

The accumulation ratios for both D-glucosamine and D-galactosamine were found to be considerably higher than 1.0, and this accumulation was markedly decreased by the absence of saline Na^+ and by phlorrhizin. The accumulation of galactosamine was also markedly decreased by galactose. It has been mentioned (see METHODS AND MATERIALS) that a considerable portion of both hexosamines in the tissue and also a fraction of the hexosamines in the media after incubation behaved on the Dowex-50 columns as neutral sugars and presumably represented the *N*-acetylated derivatives. The $[S]_i/[S]_o$ ratios of the free hexosamines were 1.8 and 2.1, respectively. As pointed out above for D-mannose, it is questionable whether such correction is meaningful. By definition, an accumulation ratio higher than 1.0 indicates active transport only if the same molecular species is concerned; however, since the energy-requiring conversion of hexosamines to their acetylated derivatives can take place only after the hexosamines entered the cells, the results reported here indicate both the active transport of these sugars and also the Na^+ dependence and phlorrhizin sensitivity of the transport system. *N*-Acetylglucosamine entered only a small portion of the cell water.

The transport behavior of 3-deoxy-D-glucose and of D-allose was of particular interest in view of the previous observation that D-glucose and D-galactose were actively transported, whereas 3-*O*-methyl-D-glucose was not, indicating a structural requirement of C-3–OH for transport[2]. It will be seen that neither 3-deoxy-D-glucose nor the C-3 epimer of glucose, D-allose, were found to be actively accumulated in renal tubular cells (even if the incubation was carried out for 2 h), thus providing additional evidence that for active transport a hydroxyl on C-3 has to be present, presumably in the position found in D-glucose. The entry of both tested sugars into the cells was significantly inhibited by the absence of Na^+ and by phlorrhizin. 2-Deoxy-allose was to a small degree accumulated in the cells against its concentration gradient, and this accumulation was Na^+-independent.

2,6-Dideoxy-D-altrose (digitoxose) was not actively accumulated by the renal cells and this transport was not affected by the absence of Na^+. The absence of an active transport of digitoxose emphasizes the role of both the configuration of –OH on C-3, this being the same as on D-allose, and that of the hydroxyl on C-6 for the transport system.

As compared with the lack of active accumulation of D-ribose, 2-deoxy-D-ribose was accumulated to a significant degree, and this accumulation was Na^+ independent.

2-Deoxy-D-xylose was of interest from the point of view of the Na^+-independence of 2-deoxysugars, since D-xylose has been previously shown to be sluggishly, but significantly actively transported into renal tubular cells by a Na^+-dependent, phlorrhizin-sensitive mechanism. It will be seen that no marked accumulation of 2-deoxy-D-xylose was found, its entry into the cells being Na^+ independent. However, this sugar was found to be rather rapidly utilized by the tissue as compared with most

other sugars tested so far. A rapid intracellular sugar metabolism would tend to decrease the $[S]_i/[S]_o$.

Kinetic parameters for the transport of some monosaccharides

Previous[2] and present studies (see Table I) showed mutual competition between various monosaccharides for the transport site, indicating the possibility of a common carrier for sugars, in spite of such differences as the Na^+ requirement. On the other hand, reported data indicated marked differences between various sugars as to the phlorrhizin sensitivity of their transport and also as to the molar ratio of competing sugars required for inhibition. Thus, while 0.5 mM phlorrhizin reduced the accumulation ratio of α-methyl-D-glucoside (ref. 2) and β-methyl-D-glucoside (Table I) from values higher than 5 (controls) to below 1.0, the same concentration of inhibitor reduced the $[S]_i/[S]_o$ for 2-deoxyglucose and 2-deoxygalactose only some 40% (ref. 2). Furthermore, while 0.5 mM D-galactose reduced the accumulation of 1 mM 2-deoxy-D-galactose by 65%, a molar ratio of 10 was required to demonstrate a 40% inhibition of the accumulation of galactose by 2-deoxygalactose[2].

In order to elucidate whether the various actively transported sugars employ the same carrier, the kinetic parameters of a group of sugars were first determined. The data, summarized in Table II, show that the apparent K_m for the transport of α-methyl-D-glucoside, D-galactose, 2-deoxyglucose and 2-deoxy-D-galactose vary only slightly. Considerably greater differences were found in the v_{max}, the highest value being found for α-methyl-D-glucoside (65 μmoles/g cell water per h), while that for the other tested sugars was considerably lower. Assuming similar mobilities of the loaded carrier within the membrane, no great differences in v_{max} would be expected if a common pathway were used by these sugars.

TABLE II

APPARENT KINETIC PARAMETERS FOR SUGAR TRANSPORT INTO KIDNEY CORTEX CELLS

Means ± S.E. are given where five or more determinations were carried out.

Monosaccharide	*Number of experiments*	K_m *(mM)*	v_{max} *(μmoles/g cell water per h)*
α-Methyl-D-glucoside	5	1.2 ± 0.1	62 ± 4.7
D-Galactose	10	0.79 ± 0.07	16 ± 2.2
2-Deoxy-D-glucose	3	1.1	12
2-Deoxy-D-galactose	9	3.01 ± 0.11	28.4 ± 3.8

Competition between monosaccharides for the transport site(s)

First experiments showed a clean competitive inhibition of the transport site(s) between some tested sugars. Thus, the transport of 2-deoxy-D-galactose was competitively inhibited by D-galactose (Fig. 1). The found K_i (0.45 mM) was within the range of values of the transport K_m for D-galactose (0.79 ± 0.07 mM, range 0.3–1.05 mM). As a first approximation, a close agreement between K_i of the competing substrate and its transport K_m would be expected if a common carrier were operative (see ref. 12).

Similar results were also obtained for the competitive inhibition of 2-deoxy-D-glucose transport by D-glucose and D-galactose (Fig. 2) (although here the computed K_i for galactose was somewhat high, *i.e.* 3 mM) as well as for the competition between 2-deoxy-D-galactose and 2-deoxy-D-glucose (Fig. 3). Finally, a competitive inhibition of the α-methyl-D-glucoside transport by D-glucose was demonstrated (details are not given here), the found K_i (1.1 mM) being close to that obtained for

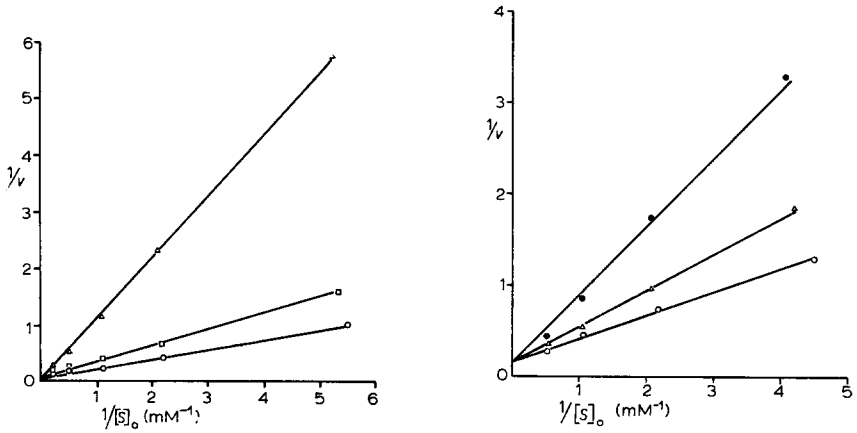

Fig. 1. Lineweaver–Burk plot of the effect of D-galactose on the rate of 2-deoxy-D-galactose transport into kidney cortex cells. Slices were incubated aerobically at 25° for 30 min in standard saline containing 0.2–2 mM 2-deoxy-D-galactose without (○) and with 0.3 (□) and 2 mM (△) D-galactose. v: μmoles/g cell water per 0.5 h. Transport parameters: K_m: 2.5 mM; v_{max}: 25 μmoles per g cell water per h; K_i: 0.45 mM.

Fig. 2. Lineweaver–Burk plot of the effect of D-glucose and D-galactose on the rate of 2-deoxy-D-glucose transport into kidney cortex cells. Slices were incubated aerobically at 25° for 30 min in standard saline containing 0.20–2 mM 2-deoxy-D-glucose without (○) and with 2 mM glucose (●) or 2 mM galactose (△). v: μmoles/g cell water per 0.5 h. Computed transport parameters: K_m: 1.6 mM, v_{max}: 25 μmoles/g cell water per h; K_i for galactose: 3 mM; K_i for glucose: 0.5 mM.

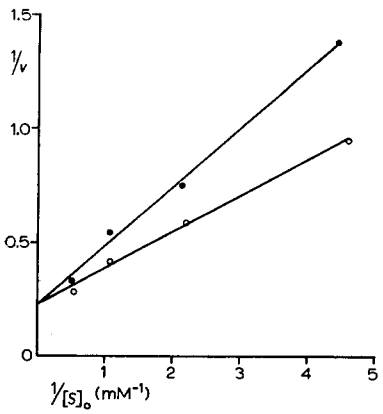

Fig. 3. Lineweaver–Burk plot of the effect of 2-deoxy-D-galactose on the rate of 2-deoxy-D-glucose transport into kidney cortex cells. Slices were incubated aerobically at 25° for 30 min in standard saline containing 0.25–2 mM 2-deoxy-D-glucose without (○) and with 2 mM 2-deoxy-D-galactose (●). v: μmoles/g cell water per 0.5 h. Transport parameters: K_m: 0.7; v_{max}: 9 μmoles/g cell water per h; K_i: 3.5 mM.

TABLE III

COMPETITIVE INHIBITION BETWEEN VARIOUS SUGARS FOR TRANSPORT INTO KIDNEY CORTEX CELLS

Slices were preincubated and incubated aerobically (O_2) at 25°, as described in METHODS AND MATERIALS. v: μmoles per g cell water for the given incubation petiod (20 min for D-galactose and α-methyl-D-glucoside, 30 min for 2-deoxyhexoses); all values are the means of at least four determinations. The mean K_i was calculated according to the equation given in the text, and represents the mean (± S.E.) for the given experiment.

Expt. No.	*Substrate*	*Concn. (mM)*	*Inhibitor*	*Concn. (mM)*	*v*	K_i *(mM)*
1	D-Galactose	0.5	α-Methylglucoside	0	2.67	
		0.5		1	2.40	
		0.5		5	1.98	
		0.5		10	1.96	
		2.0		0	1.50	
		2.0		1	1.35	
		2.0		5	1.16	
		2.0		10	1.15	8.0 ± 2.1
2	α-Methyl-D-glucoside	0.125	D-Galactose	0	3.08	
		0.125		1	2.65	
		0.125		10	2.56	7.0
		0.125	2-Deoxy-D-glucose	0	3.08	
		0.125		1	3.25	
		0.125		5	2.85	
		0.125		10	3.11	
3	D-Galactose	0.2	2-Deoxy-D-galactose	0	1.22	
		0.2		5	1.35	
		0.2		10	1.36	
4		0.2		0	2.70	
		0.2		20	1.59	23
5	2-Deoxy-D-galactose	0.25	D-Glucose	0	1.16	
		0.25		1	1.23	
6		1.0		0	4.71	
		1.0		1	4.80	
		1.0		5	4.32	
		1.0		10	4.45	

the inhibition of 2-deoxy-D-glucose transport by D-glucose, *i.e.* 0.5 mM (see legend to Fig. 2).

However, a further study showed marked differences between the found K_i and K_m for a variety of combinations (Table III). It will be seen that the K_i for the inhibitory effect of α-methyl-D-glucoside on the transport of D-galactose and also the K_i for the inhibition of α-methyl-D-glucoside transport by galactose differ from their respective K_m by nearly one order of magnitude. Moreover, in confirmation of indications given previously[2], a marked inhibition of the D-galactose transport by 2-deoxy-D-galactose could be observed only at high molar ratios (1:100) of these sugars (Table III, Expts. 3 and 4), indicating the K_i for this transport process to be at least one order of magnitude higher than the transport K_m of 2-deoxy-D-galactose. Finally, Expts. 2, 5 and 6 show that even at high molar ratios 2-deoxy-D-glucose

failed to inhibit the transport of α-methyl-D-glucoside, and practically no inhibition of the 2-deoxy-D-galactose transport by D-glucose could be obtained.

The observed data thus indicate the possibility of several pathways (or carriers) for the sugar transport in renal tubular cells.

DISCUSSION

Based on a study of a total of 27 sugars (refs. 1, 2, 13, 14 and Table I of the present communication), the specificity pattern for the active accumulation of monosaccharides in renal tubular cells may now be evaluated.

The presence of a pyranose or furanose ring appears to be one of the structural requirements for the active sugar transport since D-mannitol was found not to be actively transported, as opposed to D-mannose, D-glucose, D-fructose and D-galactose. It may be argued that *myo*-inositol is actively accumulated by renal cortex cells[13,14], thus demonstrating the lack of a requirement for a pyranose or furanose ring. However, it is questionable whether this cyclic polyol is transported by the same pathway as other sugars in view of the observation[13] that even a 10 mM concentration of D-glucose failed to affect the accumulation ratio of *myo*-inositol at an external concentration of 17 μM, *i.e.* at a molar ratio of 600. A hydroxyl on C-1 is not essential for active transport, both α- and β-methyl-D-glucosides being transported against high concentration gradients.

A hydroxyl on C-2 is not required for the active transport of monosaccharides, as evidenced by the fact that all the 2-deoxy-D-hexoses studied so far (2-deoxy-derivatives of D-glucose, D-galactose, D-allose, D-ribose, and possibly also of D-xylose) were found to be accumulated against their concentration gradient. The 2,6-dideoxysugar, digitoxose, was not actively accumulated; however, this sugar is lacking a hydroxyl on C-6 which is of importance for active transport. It appears equally clear that the absence of a hydroxyl on C-2 abolishes the Na^+ requirement for the monosaccharide transport, since the deoxysugars enumerated above were actively transported against their concentration gradient in the absence of saline Na^+ and at an apparent intracellular Na^+ concentration lower than 3 mM. A replacement of the hydroxyl by an amino group also did not prevent an active accumulation of the studied hexosamines; the lack of an accumulation of *N*-acetylglucosamine may be due to steric hindrance. Since the transport of hexosamines was found to be Na^+ dependent, it may be inferred that the Na^+ dependence is related to the presence of a hydrophilic group on C-2. The cation dependence of sugar transport will be considered in more detail in a subsequent communication.

A hydroxyl on C-3 in the same position as that in D-glucose or D-galactose appears to be essential for the active accumulation of monosaccharides. This conclusion is based on the observations that 3-*O*-methyl-D-glucose, 3-deoxy-D-glucose and D-allose were not actively accumulated by the cells. This structural requirement may be responsible for the fact that L-glucose failed to be actively accumulated although some inhibition of its entry by D-galactose was found.

A hydroxyl on C-6 greatly enhances the active accumulation of sugars, as evidenced by the low accumulation ratio of various pentoses as well as by the transport behavior of digitoxose.

The above conclusions as to the structural requirements for active sugar trans-

port into renal tubular cells are in agreement with and represent more detailed specification of those presented earlier[2]; the latter were based on a limited analysis of the transport behavior of 13 sugars. There is also close agreement with the conclusions of SILVERMAN *et al.*[15], based on their findings of the specificity of sugar transport by the kidney tubule under conditions *in vivo*.

The structural requirements for active sugar transport in renal tubular cells thus differ considerably from those described for intestinal transport, where hydrogen bonding between the carrier and C-1, C-2 and C-6 of the sugar appears to be the minimum for active transport to occur[16,17].

The data given here represent evidence against the possibility, envisaged earlier[2,3], of a common carrier for the transport of various monosaccharides into renal tubular cells. For the case of a common carrier, differences in the accumulation levels would essentially be due to varying affinities of the transported sugars for the transport mechanism, v_{max} being identical.

First, significant differences as to the transport v_{max} of some monosaccharides were demonstrated (Table II). Secondly, in some instances the K_i for competitive inhibition between sugars for transport differed markedly from the transport K_m of the inhibiting sugar (Tables I and III). These results indicate the possibility of several, interrelated pathways (or sites) for the transport of the examined sugars. This is particularly apparent when considering the relationship between the transport of both 2-deoxyhexoses tested and D-glucose: 2-Deoxy-D-glucose and 2-deoxy-D-galactose were found to compete for transport, the K_i agreeing reasonably well with the independently determined K_m (Fig. 3); however, glucose, which competitively inhibited the transport of 2-deoxy-D-glucose (Fig. 2), failed to affect the transport of 2-deoxy-D-galactose even at the high molar ratio of 10:1 (Table III, Expts. 5 and 6). No simple assumption of, say, two transport sites with clearly established structural requirements for the sugars appears to fit the obtained data. The possibility of some sugars mutually sharing some pathways (or sites) may have to be considered. Thus, galactose may be sharing a transport pathway with 2-deoxy-D-galactose (the K_i for galactose coinciding with the transport K_m of this sugar), while the reverse does not readily hold (the affinity of 2-deoxygalactose for the galactose carrier appears to be at least one order of magnitude smaller than that for galactose).

Additional evidence for several pathways of sugar transport into renal tubular cells was now obtained: (a) The K_i for the competitive inhibitor of sugar transport, phlorrhizin, was found to vary from 0.01 mM for α-methyl-D-glucoside to 0.06 mM for D-galactose and 0.6 mM for 2-deoxy-D-galactose (KLEINZELLER AND PENN, to be published); (b) considerable differences were observed[18] in the response of monosaccharide transport to variations of pH in the incubating medium. These data also exclude the possibility that the differences of the transport pathways are related solely to the Na^+ requirement for the sugar transport. It ought to be pointed out here that the geometry of the experimental system used may contribute to some anomalous kinetics: using tissue slices, both the luminal and peritubular faces of the cells are accessible to sugars from the external medium. Let us assume that the substrate is transported into the cell only at the luminal face while the competing sugar can enter the cell both at the luminal and peritubular face. This would produce in addition to competitive inhibition of the substrate transport into the cells at the luminal face also some countertransport, yielding kinetic data which would be very difficult to

interpret. Such geometric factors may also be responsible for the failure to demonstrate unequivocally a counterflow of sugars in the slices. It is also obvious that if a sugar uses several transport pathways, the obtained kinetic parameters will represent composite values.

It should be noted that SILVERMAN *et al.*[15] postulated two transport sites for glucose, 2-deoxy-D-glucose, galactose and mannose, localized at the luminal face of the cells proximal to the kidney tubule. In addition, one transport site localized at the antiluminal face of the cells was suggested, mediating the entry of glucose, 3-*O*-methyl-D-glucose, galactose, mannose, 6-deoxy-D-galactose and D-xylose into the cells. Judging from kinetic data presented here, the variety of pathways may be still greater, and it appears premature to offer a detailed model.

TABLE IV

COMPARISON OF CELLULAR SUGAR ACCUMULATION *in vitro* (KIDNEY CORTEX SLICES) AND REABSORPTION OF THESE SUGARS IN THE KIDNEY TUBULE *in vivo*

Symbols: Studies *in vitro*: +, steady-state $[S]_i/[S]_0 > 2$; ±, $[S]_i/[S]_0$ 1–2; −, $[S]_i/[S]_0 < 1$. Studies *in vivo*: +, reabsorption; −, no reabsorption.

Sugar	*Accumulation in vitro*	*Ref.*	*Reabsorption in vivo*	*Ref.*
D-Glucose	+	1	+	20
D-Glucose	−	*	−	21
D-Galactose	+	19, 1	+	22, 15
D-Fructose	+	2	+	22
D-Mannose	±	*	+	15
2-Deoxy-D-glucose	+	2	+	23, 15
2-Deoxy-D-galactose	+	2	+	3
Myo-inositol	+	13, 14	+	24
3-*O*-Methyl-D-glucose	−	2	−	21, 15
6-Deoxy-D-galactose	±	2	−	15
D-Xylose	±	2	±	25, 15

* Present communication.

Finally, a satisfactory correlation can now be established between the active accumulation of sugars in kidney cortex cells, demonstrated *in vitro*, and the reabsorption of sugars in the nephron *in vivo* (Table IV). In addition to the sugars listed by SILVERMAN *et al.*[15] to be reabsorbed by mechanisms localized at the luminal face of the tubular cells (see above), D-fructose[22], and *myo*-inositol[24] should be mentioned on the basis of earlier studies. 2-Deoxy-D-galactose should be added to this list[3]. In the course of reabsorption of this sugar in the rat kidney (sugar/creatinine clearance ratio 0.72 ± 0.017), a marked accumulation of 2-deoxy-D-galactose in the kidney cortex took place with tissue/plasma ratios of up to 9. It will be noted from Table IV that these sugars are also actively accumulated under conditions *in vitro*. On the other hand, sugars which have been shown not to be reabsorbed or which may even be secreted into the tubular lumen, *e.g.* 3-*O*-methyl-D-glucose and L-glucose, also failed to be actively accumulated in the slices. The only discrepancy concerns the transport of D-xylose and possibly 6-deoxy-D-galactose. Whereas earlier data in-

dicated reabsorption of xylose *in vivo*, more recent studies could not show this process to occur; *in vitro*, a slight but significant active transport of D-xylose was demonstrated. As to 6-deoxy-D-galactose, its accumulation *in vitro* was small and a detailed analysis of this process has not yet been carried out.

The good correlation between information obtained under conditions *in vitro* and *in vivo* once again emphasizes the usefulness of the tissue slice technique for the investigation of the mechanism of cellular processes such as transport.

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