Sugar Transport across the Peritubular Face of Renal Cells of the Flounder

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ABSTRACT The transport of some sugars at the antiluminal face of renal cells was studied using teased tubules of flounder (*Pseudopleuronectes americanus*). The analytical procedure allowed the determination of both free and total (free plus phosphorylated) tissue sugars. The inulin space of the preparation was 0.333 ± 0.017 kg/kg wet wt (7 animals, 33 analyses). The nonmetabolizable α-methyl-D-glucoside entered the cells by a carrier-mediated (phloridzin-sensitive), ouabain-insensitive process. The steady-state tissue/medium ratio was systematically below that for diffusion equilibrium. D-Glucose was a poor inhibitor of α-methyl-glucoside transport, D-galactose was ineffective. The phloridzin-sensitive transport processes of 2-deoxy-D-glucose, D-galactose, and 2-deoxy-D-galactose were associated with considerable phosphorylation. Kinetic evidence suggested that these sugars were transported in free form and subsequently were phosphorylated. 2-Deoxy-D-glucose accumulated in the cells against a slight concentration gradient. This transport was greatly inhibited by D-glucose, whereas α-methyl-glucoside and also D-galactose and its 2-deoxy-derivative were ineffective. D-Galactose and 2-deoxy-D-galactose mutually competed for transport; D-glucose, 2-deoxy-D-glucose, and α-methyl-D-glucoside were ineffective. Studies using various sugars as inhibitors suggest the presence of three carrier-mediated pathways of sugar transport at the antiluminal cell face of the flounder renal tubule: the pathway of α-methyl-D-glucoside (not shared by D-glucose); the pathway commonly shared by 2-deoxy-D-glucose and D-glucose; the pathway shared by D-galactose and 2-deoxy-D-galactose.

It is generally assumed that in the course of the reabsorption of sugars in the renal proximal tubule the active step is located at the luminal face of the cells, thus setting up a major concentration gradient between the cellular space and tubular lumen. The accumulated sugar is then assumed to pass across the peritubular face of the renal cells in accordance with its concentration gradient, possibly by a carrier-mediated mechanism (see, e.g. Silverman et al., 1970; Kleinzeller, 1970). Obviously, detailed information as to

the properties of the transport systems for sugars at the peritubular face of the renal tubular cells is essential for an understanding of the reabsorptive process.

Teased tubules of flounder (*Pseudopleuronectes americanus*) kidney are known to close up at their ends and thus form little cysts in which substances secreted into the tubular lumen, e.g. chlorphenol red, can accumulate against concentration gradients of several orders of magnitude (Forster, 1948; Forster and Hong, 1958; Kinter, 1966). It is this property of the preparation which appeared to be attractive for the purpose of studying the mechanism(s) involved in the transport of various sugars across the peritubular cellular face, thus separating this step from the overall transcellular transport. Most studies presented here were carried out using sugars known to be actively transported in slices of mammalian renal cortex, i.e., α-methyl-D-glucoside, 2-deoxy-D-glucose, D-galactose, and 2-deoxy-D-galactose (Kleinzeller, 1970). In view of the observed magnitude of the extracellular (inulin) space, i.e. 0.333 kg/kg tissue wet wt, no attempt has been made at following detailed transport kinetics.

It should be pointed out here that, using the preparation of teased tubules, one may obtain information essentially on the transport properties localized at the peritubular face of renal cells only if the assumption is made that the rate of entry of sugars through the "tight junction" between the tubular cells is minimal, preventing an entry of the studied saccharide into the lumen in amounts sufficient to be actively taken up at the luminal face. Obviously, such event would preclude a simple interpretation of experimental data. In absence of other techniques, such as the use of perfused isolated renal tubules (Tune and Burg, 1971), an experiment was designed to give some limited assurance on this point.

A preliminary report on some of the data reported here has appeared (Kleinzeller and McAvoy, 1972).

METHODS AND MATERIALS

Winter flounders (*Pseudopleuronectes americanus*), usually weighing 150–250 g, were caught in Frenchmans Bay and were kept in tanks with running seawater for several days before experiments.

Teased tubules of flounder kidney were prepared as described by Forster (1948) and Kinter (1966). After removal of the kidney from the decapitated fish, pieces of tissue approximately 3 × 3 × 3 mm were cut with a razor blade and were placed into a dish containing ice-cooled medium. Up to 25 such tissue portions were obtained. Each tissue piece was gently teased with dissecting needles in order to remove adhering hematopoietic cells as much as practicable. The pale mesh of tubules was then stored for not more than 1 h in fresh ice-cold medium until used for incubation. Generally the viability of the preparations was tested by observing the tubular uptake of chlorphenol red at room temperature from a saline containing 0.025 mM of this dye. No in-

dication of a relationship between the visually observed rate of dye accumulation in the tubular lumen and sugar transport was found.

Media

The standard medium was a slightly modified version of that described by Forster (1948), employing a mixture of Tris-TES[1] buffers (13 mM, final concentration) to adjust the pH to the required value (see Kleinzeller et al., 1970): 6.7 mM acetate (final concentration) served as metabolic substrate. Unless otherwise stated, the standard saline had a pH of 7.2. It will be shown below that the accumulation of sugars was affected only slightly by variations of pH between 6.2 and 8.2. Na-free media were prepared by equivalently replacing Na^+ by Li^+: Ca^{2+}-free medium was prepared by omitting $CaCl_2$.

Incubation

Three to six pieces of teased tissue were placed into 25-ml conical flasks with 2.6 ml of the appropriate saline containing labeled sugar and other additions. Unless otherwise stated, 0.02 μCi ^{14}C or 0.2 μCi ^{3}H per ml medium were employed. The flasks were maintained at a constant temperature of 15°C in a bath of running seawater. The low depth of the fluid phase and occasional swirling of the flasks assured a sufficient diffusion of O_2 from the air phase to the slowly respiring tissue. At the end of the incubation the pieces of tissue were removed from the flasks and placed on a disk of filter paper (Whatman No. 541) in a moist chamber, thus allowing a drainage of adhering fluid. Individual pieces of tissue were then weighed on a Cahn microbalance (Cahn Div., Ventron Instruments Corp., Paramount, Calif.) (mostly from 5 to 15 mg, wet wt) and transferred into homogenizing tubes for the assay of tissue sugar.

Determination of Tissue Sugars

Originally, the Somogyi (1945) procedure was employed as previously used in our laboratory for the determination of free tissue sugars (see, e.g., Kleinzeller, 1970). Subsequently, it was observed that the above procedure may lead to erroneous results owing to a breakdown of phosphorylated sugars by a Zn-activated phosphatase, and the technique had to be modified (Kleinzeller and McAvoy, to be published). Tissue was placed into a homogenizing tube containing 2.5 ml H_2O at 100°C and was maintained in a boiling water bath for 10 min; the phosphatase was thus inactivated. After cooling, the tissue was homogenized; after centrifugation, 0.5 ml of the cloudy supernatant was used for the assay of total (free plus metabolized) sugar. The pellet was resuspended in the remaining 2 ml of supernatant, and the acidic (phosphorylated) metabolites were precipitated by the Somogyi procedure (i.e., adding 0.5 ml 5% [wt/vol] $ZnSO_4 \cdot 7\ H_2O$ and 0.5 ml of exactly titrated 0.3 N Ba $[OH]_2$). 1 ml of the supernatant was then used for the determination of the free sugars. A Packard 3320 instrument was used for scintillation spectrometry of the labeled material present in 10 ml of toluene-Triton X-100 coctail (Patterson and Greene, 1965). Experiments to be reported elsewhere (Kleinzeller and McAvoy) demonstrated that thus determined

[1] TES, *N*-tris(hydroxymethyl)methyl-2-aminoethane sulfonic acid.

values of free and phosphorylated (i.e., total minus free) sugars agreed reasonably well with those found when tissue sugars were first extracted by 5% trichloroacetic acid and then determined after separation on columns of ion-exchange resin. 0.1 ml portions of the media before and after incubation were treated as described above for the determination of the specific activity of sugars in the incubating media and their final concentrations. The obtained data allowed the evaluation of the uptake of the sugar by the tissue, and will be expressed either in micromole per gram tissue wet weight, or the tissue/medium (T/M) ratio, using final values of the sugar concentration in the medium. Individual analytical values usually did not differ more than 5–10% from the mean of three determinations.

Determination of the Extracellular (Inulin) Space

Pieces of tissue were incubated for 60 min as described above in media containing 0.1% (wt/vol) inulin labeled with [*methoxy*-^{3}H]inulin (0.5 μCi/ml), blotted, weighed, and subsequently extracted with boiling H_2O. In appropriate portions of the supernatant the activity was determined. Also, the activity in 0.1 ml portions of the medium was measured. From these data the inulin space was calculated and expressed as the T/M ratio, or in the usual fashion, i.e., in kilogram per kilogram tissue wet weight. The former way of expressing data allowed a direct comparison of the respective spaces occupied by the studied sugar and inulin.

Where five or more analytical values were obtained, the data are given as the mean ± standard error. The significance of differences between experimental values was estimated using the Student's *t* test.

Materials

D-[1-^{14}C]galactose, [1-^{14}C]2-deoxy-D-glucose and [*methoxy*-^{3}H]inulin were purchased from New England Nuclear, Boston, Mass. Crude [^{3}H]2-deoxy-D-galactose was prepared by catalytic exchange at The Radiochemical Centre, Amersham, Buckinghamshire, England, and was purified by preparative paper chromatography using the solvent system ethylacetate:acetic acid:H_2O containing 2% (wt/vol) phenylboronic acid (9:2:2, vol/vol), followed by *n*-butanol:ethanol:H_2O (104:66:30, vol/vol). The radiochemically determined R_f value of the pure product was identical with that of authentic 2-deoxy-D-galactose. The authors are indebted to Dr. J. C. Turner, The Radiochemical Centre, for his helpful suggestions concerning the purification of the labeled sugar. α-Methyl-2-deoxy-D-glucoside (mp 90°C, uncorrected) was prepared as described by Bergmann et al. (1922). All other reagents and chemicals were commercial preparations of the highest obtainable purity.

RESULTS

Transport of α-Methyl-D-Glucoside

The mean extracellular (inulin) space determined in the teased tubule preparation of seven animals was 0.333 ± 0.017 kg H_2O/kg tissue wet wt. Fig 1 shows that α-methyl-glucoside entered the cellular space of the teased tubules (i.e., the space from which inulin was excluded) rather slowly. Even after 90

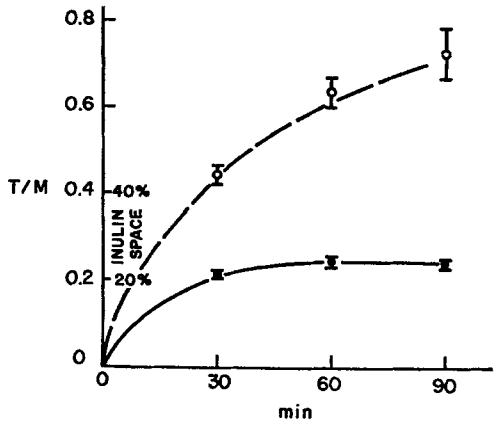


FIGURE 1. Uptake of α-methyl-D-glucoside and inulin by flounder renal tubules. Groups of teased tubules were incubated aerobically at 15°C in standard medium containing 1 mM [^{14}C]α-methyl-D-glucoside and 0.1% (wt/vol) [^{3}H]inulin. Each point is the mean of six determinations ± standard error. Inulin space, ●; T/M for the glycoside, ○.

TABLE I

ENTRY OF α-METHYL-D-GLUCOSIDE INTO TEASED TUBULES OF FLOUNDER KIDNEY

Groups of tissue (five per flask) were incubated aerobically (air) at 15°C for 60 min in standard salines containing 1 mM [^{14}C]α-methyl-glucoside without (control) and with other additions. All values of the T/M are the means ± standard error.

Additions	T/M
mM	
None (control)	0.568 ± 0.030
Phloridzin, 0.5	0.447 ± 0.022
Ouabain, 0.5	0.527 ± 0.015
D-Glucose, 5	0.473 ± 0.016
D-Galactose, 5	0.649 ± 0.020

min incubation the T/M values were well below those expected for a diffusion equilibrium level in tissue water: given the mean-found value of tissue water (see Kleinzeller and Hogben, 1970), i.e., 5.55 ± 0.09 kg H_2O/kg tissue dry wt (12 analyses, 5 animals) the T/M for a diffusion equilibrium in total tissue water would be 0.83. Under all experimental conditions the activity found in the tissue extracts before and after treatment with $ZnSO_4$ + $Ba(OH)_2$ agreed within the limits of analytical error, indicating that this sugar was not phosphorylated in renal cells.

Table I shows that the T/M for α-methyl-D-glucoside was significantly depressed by 0.5 mM phloridzin but not by 0.5 mM ouabain. It may also be noted that 5 mM D-glucose (i.e., at a molar ratio 5 glucose:1 α-methyl-

D-glucoside, though inhibitory, did not abolish the entry of the glucoside into the cellular space, suggesting a relatively small affinity of the sugar for the site involved in the transport of the glucoside. D-Galactose even somewhat increased the cellular uptake of methyl-glucoside; in some other experiments no significant effect of this sugar on the entry of the glucoside was observed. Data not given here in detail showed that the T/M decreased with increasing glycoside concentrations in the medium: at 0.1 mM α-methyl-D-glucoside, a value of 0.845 was found ($n = 4$), whereas at 5 mM glycoside the T/M was 0.604. These data suggest that α-methyl-D-glucoside enters the tubular cells of the flounder kidney tubules by a saturable process mediated by a phloridzin-sensitive carrier.

The possibility was considered that α-methyl-D-glucoside did not enter the

TABLE II

THE EFFECT OF PHLORIDZIN ON THE STEADY-STATE SPACE OF α-METHYL-D-GLUCOSIDE AND INULIN

Groups of tissue (six per flask) were incubated aerobically (air) at 15°C for 60 min in standard saline containing 0.5 mM [^{14}C]α-methyl-D-glucoside (α-MGLU) and 0.1% (wt/vol) [*methoxy*-^{3}H]inulin, without (control) and with 0.5 mM phloridzin. The values of the T/M for the sugar and inulin are the means, ± standard error.

	T/M (α-MGLU)	T/M (inulin)
mM		
Control	0.547 ± 0.028	0.322 ± 0.018
Phloridzin, 0.5	0.399 ± 0.044	0.318 ± 0.029

Significance of differences (*t* test): Phloridzin effect on T/M of α-MGLU: $P < 0.01$; T/M of α-MGLU and inulin in the presence of phloridzin: $0.05 > P > 0.02$.

renal cells at the exposed antiluminal face but rather penetrated through leaky intercellular junctions into the tubular lumen and was there taken up into the cells. Thiscoside is rapidl glyy reabsorbed from the lumen of flounder kidney tubule and this process is associated with a cellular accumulation of the saccharide against a marked concentration gradient (up to tissue/plasma ratio of 2), indicating the presence of a potent active transport system for α-methyl-D-glucoside at the luminal face of the tubular cells (Kleinzeller, et al., 1972). Consequently, even a slow leak of the sugar into the lumen would bring about a marked cellular entry.

The experiment presented in Table II was set up to provide some information on this point. Groups of teased tubules were incubated in salines containing 0.5 mM [^{14}C]α-methyl-D-glucoside plus [^{3}H]inulin, without (control) and with 0.5 mM phloridzin. Assuming the intercellular junction to be tight for both inulin and the saccharide, a complete inhibition of the entry of α-meth-

yl-glucoside into the cells at their exposed antiluminal face would restrict the saccharide to the space occupied by the marker of the extracellular fluid. On the other hand, if the tight junction were leaky for α-methyl-D-glucoside but excluded inulin, the saccharide could also occupy the tubular space, and in case that the relatively large molecule of phloridzin could not pass through the tight junction, the steady-state space of the saccharide would not be affected by the inhibitor. Data in Table II show that phloridzin depressed the cellular entry of α-methyl-D-glucoside from a space of 0.225 to 0.077, i.e. by $\frac{2}{3}$, to a level close to that of the inulin space. It is thus reasonable to assume that the entry of the sugar takes place essentially at the antiluminal face of the tubular cells. The possibility that phloridzin did pass together with α-methyl-glucoside through the tight junction appears to be remote:

TABLE III

UPTAKE OF 2-DEOXY-D-GLUCOSE BY TUBULES OF FLOUNDER KIDNEY: EFFECT OF SUBSTRATE CONCENTRATION

Groups of tissue were incubated for 60 min at 15°C in standard salines containing 2-deoxy-glucose at concentrations varying from 0.05 to 2.5 mM. Data are given in free and total tissue sugar (micromole per gram wet weight, ± standard error (n = 5).

Substrate concentration	Tissue sugar	
	Total	Free
mM	*μmol/g*	*μmol/g*
0.05	0.131 ± 0.0015	0.0191 ± 0.0019
0.10	0.215 ± 0.007	0.043 ± 0.0014
0.50	0.695 ± 0.007	0.233 ± 0.019
1.0	1.161 ± 0.037	0.519 ± 0.037
2.5	2.914 ± 0.058	0.822 ± 0.068

The mol wt of the inhibitor, 436, is close to that of chlorphenol red, 425, which can be accumulated in the tubular lumen against concentration gradients of several orders of magnitude and thus cannot be assumed to leak readily through the tight junction.

Transport of 2-Deoxy-D-Glucose

On incubation of the flounder kidney tubules in the presence of 2-deoxy-D-glucose, both free and phosphorylated sugar (probably 2-deoxy-D-glucose-6-phosphate, see Elsas and Macdonell, 1972) are found in the tissue. Table III shows that variations of the sugar concentration in the medium (S_o) produced an increase of both free and total (free plus phosphorylated) sugar in the tissue; the T/M values for free 2-deoxy-D-glucose varied between 0.41 and 0.61. The observation that the T/M was lower for the free sugar than that corresponding to a diffusion equilibrium (0.83) may be due to a rather fast

phosphorylation of free sugar entering the cells. This possibility was investigated further in an experiment shown in Fig. 2. Groups of tissue were incubated in media at S_o 0.5 and 2.5 mM until levels approaching a steady state were reached. Subsequently, a portion of the tissue was transferred from S_o = 2.5 mM to fresh medium containing 0.5 mM sugar, thus inducing an efflux from the cells. It will be seen that at both external sugar concentrations the levels of free tissue saccharide reached steady states within 30 min, whereas the levels of phosphorylated sugar continued to rise. On transfer of tissue from S_o = 2.5 mM to 0.5 mM, a fast exit of free sugar took place, the new steady-state tissue level being identical with that of the control. The level of

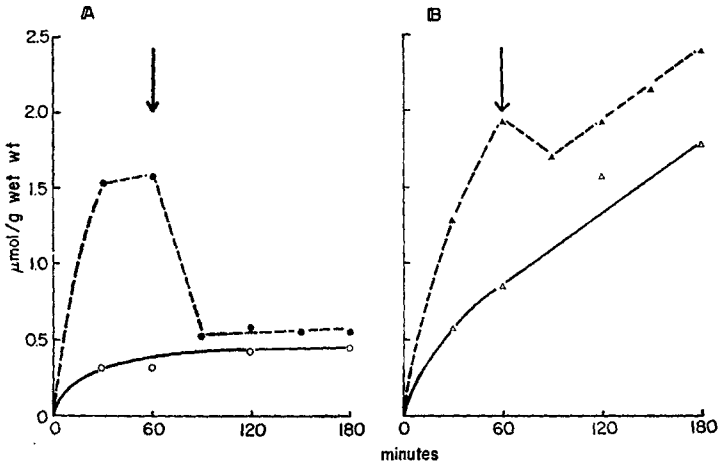


FIGURE 2. Accumulation of 2-deoxy-D-glucose in flounder kidney tubules: reversibility. Teased tubules were first loaded with [^{14}C]2-deoxy-D-glucose at 0.5 mM (○, △) and 2.5 mM(●,▲). At the time indicated by arrows the tissue was transferred from the medium containing 2.5 mM sugar to fresh medium with 0.5 mM saccharide, and the changes in tissue levels of the sugar were followed. Each point is the mean of three analyses. A, free tissue sugar; B, 2-deoxy-D-glucose phosphate (i.e., total minus free tissue sugar).

phosphorylated sugar temporarily somewhat decreased and then resumed its rise. The following interpretation of the data is offered: 2-deoxy-D-glucose entered the cells as free sugar which was subsequently phosphorylated at a rate not greatly differing from the rate of entry. The transient decrease of 2-deoxy-glucose-phosphate during the efflux phase indicates that within the cells an equilibrium between free and phosphorylated sugar exists, the dephosphorylation being brought about by a phosphatase (see Kleinzeller et al., 1973). Since sugar-phosphate obviously acted as a sink for the entered sugar, no unequivocal evidence for an accumulation of 2-deoxy-glucose against its concentration gradient could be obtained. However, the T/M values at the end of the experiment (0.958 in the control; 0.930 after the induced efflux)

are indicative of a concentration gradient compatible with an up-hill transport mechanism.

Fig. 3 shows the result of an experiment where the steady-state uptake of 2-deoxy-glucose was studied as a function of external pH. It will be seen that the total amount of sugar taken up by the tissue increased significantly ($P < 0.02$) when the pH of the medium was raised from 6.2 to 8.2 (see also Kleinzeller and Hogben, 1970). The values of tissue-free sugar were not greatly affected by the pH of the medium.

Data given in Table IV provided further information as to the transport of 2-deoxy-D-glucose. An inspection of the data shows that none of the inhibitors tested affected markedly the level of free tissue sugar, whereas some major inhibition of sugar uptake were observed. The following properties of

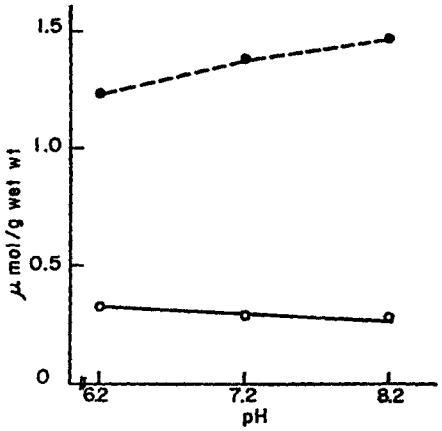


FIGURE 3. Uptake of 2-deoxy-D-glucose by flounder renal tubules: effect of pH. Groups of teased tubules (four per experimental point) were incubated in media containing 0.5 mM [^{14}C]2-deoxy-glucose at external pH varying from 6.2 to 8.2. Free sugar, ○; total tissue sugar, ●.

the transport system for 2-deoxy-glucose are apparent: (*a*) A carrier-mediated transport mechanism is indicated from the marked inhibition of sugar uptake by phloridzin and phloretin (see also Kleinzeller, 1972). An —SH group may be involved here in view of the inhibition by *N*-ethyl-maleimide. (*b*) The transport of 2-deoxy-glucose appears to be independent of Na since ouabain showed no inhibitory action and the uptake of the sugar in the absence of external Na (Li-saline) was even higher than in the control. Unpublished data showed that at the concentration of ouabain employed the Na-pump was inhibited, i.e., a considerable loss of tissue K and uptake of Na took place. Also, it has been shown previously (Kleinzeller, 1970 *a*) that under similar experimental conditions replacement of Na by Li completely abolished the Na-dependent active sugar transport in rabbit renal cells. (*c*) The inhibi-

TABLE IV

UPTAKE OF 2-DEOXY-D-GLUCOSE BY TEASED TUBULES OF FLOUNDER KIDNEY: EFFECT OF INHIBITORS

Groups of tissue were incubated 60 min aerobically (air) at 15°C in standard salines containing 0.5 mM [^{14}C]2-deoxy-D-glucose without (control) and with additions. All values are the means of at least four analyses. Data are presented as micromole per gram wet weight of free and total tissue sugar.

Exp. no.	Additions	Tissue sugar	
		Total	Free
	mM	*μmol/g*	*μmol/g*
1	None (control)	0.92	0.32
	Ouabain, 0.5	0.92	0.28
	2,4-Dinitrophenol, 0.1	0.62	0.33
	3-O-Methyl-D-glucose, 5	0.85	0.27
2	None	1.32	0.28
	D-Glucose, 5	0.50	0.32
	α-Methyl-D-glucoside, 5	1.60	0.24
	α-Methyl-2-deoxy-D-glucoside, 5	1.41	0.23
	D-Galactose, 5	1.09	0.28
	2-Deoxy-D-galactose, 5	1.50	0.27
3	None	1.28	0.32
	L-Glucose, 5	1.07	0.27
	N-Ethyl-maleimide, 1	0.78	0.42
4	None	1.02	0.33
	Phloridzin, 0.5	0.63	0.28
	Phloretin, 0.3	0.70	0.29

tion of total 2-deoxy-glucose uptake by dinitrophenol may have been produced by a decrease of sugar phosphorylation owing to a depletion of cell ATP, but may also reflect a metabolic dependence of an active sugar transport system. (*d*) Of the various sugars tested, D-glucose was the most potent inhibitor, L-glucose being ineffective. This result indicates that 2-deoxy-D-glucose and D-glucose share a transport carrier. D-Galactose was not an effective inhibitor. In another experiment not reported here in detail the difference in total tissue sugar between the control and the experimental value in the presence of a 10-fold molar excess of D-galactose was not significant ($P > 0.10$, $n = 5$). 2-Deoxy-D-galactose also did not inhibit the uptake of 2-deoxy-glucose. These results thus suggest that D-galactose and its 2-deoxy-anomer did not use the glucose-2-deoxy-glucose transport pathway. α-Methyl-D-glucoside did not inhibit the entry of 2-deoxy-D-glucose into the cells; in fact, the uptake of 2-deoxy-glucose was accelerated by the presence of the glucoside by a mechanism not understood so far. However, the fact that α-methyl-D-glucoside did not inhibit the transport of 2-deoxy-D-glucose

suggests that these sugars do not share a transport carrier, thus reinforcing the observation that D-glucose was a poor inhibitor of α-methyl-D-glucoside transport (Table I). It would appear that a free hydroxyl on C_1 is required for a sugar with the glucose configuration to interact with the carrier for glucose or 2-deoxy-glucose since α-methyl-2-deoxy-D-glucoside also did not inhibit the transport of 2-deoxy-glucose.

The Uptake of D-Galactose and 2-Deoxy-D-Galactose

The uptake of these two sugars by teased tubules of flounder kidney has many features in common. Both sugars were found to enter the tissue at a rate similar to that of 2-deoxy-D-glucose (Fig. 4 for D-galactose) and were present in free as well as phosphorylated form. It may be assumed that the products of sugar phosphorylation in the flounder kidney are identical with

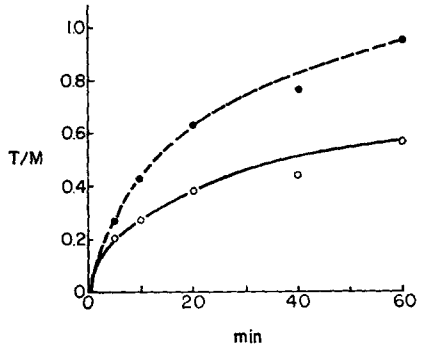


FIGURE 4. Uptake of D-galactose by flounder renal tubules. Groups of teased tubules (four per experimental point) were incubated in standard medium containing 0.5 mM D-[^{14}C]galactose. Free sugar, ○; total tissue sugar, ●.

those identified in rabbit kidney cortex on the basis of chromatographic and chemical behavior, i.e. galactose-1-*P* and the hitherto not described 2-deoxy-D-galactose-1-*P* (Kleinzeller et al., 1973). No marked effects of external pH in the range of pH 6–8 on the sugar uptake were observed, as opposed to the properties of the transport system for these sugars in rabbit kidney cortex. An experiment similar to that shown in Fig. 2 for 2-deoxy-glucose was carried out with D-galactose with identical results, demonstrating (*a*) the reversibility of the transport of free sugar, and (*b*) a temporary breakdown of sugar-*P* associated with the induced exit of free sugar. It may thus be assumed that the free sugar entering the cells is phosphorylated within and both free and phosphorylated sugars are at equilibrium. Under no experimental condition did the T/M values for either sugar exceed 0.83, i.e., the value corresponding to a diffusion equilibrium.

Although no dependence of the T/M on the external sugar concentration

was found, the fact (Tables V and VI) that the uptake of both sugars was inhibited by phloridzin and phloretin indicated a carrier-mediated transport system. Ouabain, or the absence of Na^+, did not affect the sugar uptake, suggesting a lack of Na dependence of the transport mechanism.

TABLE V

UPTAKE OF D-GALACTOSE BY TEASED TUBULES OF FLOUNDER KIDNEY

Groups of tissue were incubated for 60 min aerobically (air) at 15°C in salines containing 0.5 mM D-[^{14}C]galactose without (control) and with various additions. Mean values of at least four analyses of total and free tissue sugar (in micromole per gram tissue wet weight) are presented.

Exp. no.	Additions	Tissue sugar	
		Total	Free
	mM	*μmol/g*	*μmol/g*
1	None (control)	0.94	0.37
	Ouabain, 0.5	1.12	0.32
	D-Glucose, 5	0.71	0.30
	2-Deoxy-D-glucose, 5	0.75	0.30
	2-Deoxy-D-galactose, 5	0.59	0.34
	α-Methyl-D-glucoside, 5	0.99	0.31
	N-Ethyl-maleimide, 1	0.60	0.42
	None (Li-saline)	1.23	0.34
2	None	0.76	0.35
	Phloridzin, 0.5 mM	0.44	0.27
	D-Glucose, 5 mM	0.78	0.33

TABLE VI

UPTAKE OF 2-DEOXY-D-GALACTOSE BY TEASED TUBULES OF FLOUNDER KIDNEY

Groups of tissue were incubated for 60 min aerobically (air) at 15°C in saline containing 0.5 mM [^{3}H]2-deoxy-D-galactose without (control) and with various additions. Mean values of four analyses of total and free tissue sugar (in micromole per gram tissue wet weight) are given.

Additions	Tissue sugar	
	Total	Free
mM	*μmol/g*	*μmol/g*
None (control)	0.82	0.44
D-Glucose, 5	1.00	0.46
2-Deoxy-D-glucose, 5	0.80	0.34
D-Galactose, 5	0.39	0.34
Phloridzin, 0.5	0.35	0.25
Phloretin, 0.3	0.39	0.26

As to the specificity of the carrier-mediated transport, the following points emerge from the data given in Tables V and VI. The sugars markedly inhibited the uptake of each other by the tissue, suggesting a carrier shared by D-galactose and its 2-deoxy-derivative. This carrier appears to differ from that mediating the transport of D-glucose and 2-deoxy-D-glucose, since both these sugars were rather poor inhibitors of the galactose-mediating transport carrier. Finally, the carrier also appears to differ from that mediating the transport of α-methyl-D-glucoside in view of the fact that this glucoside did not inhibit the uptake of either D-galactose or its 2-deoxy-derivative.

DISCUSSION

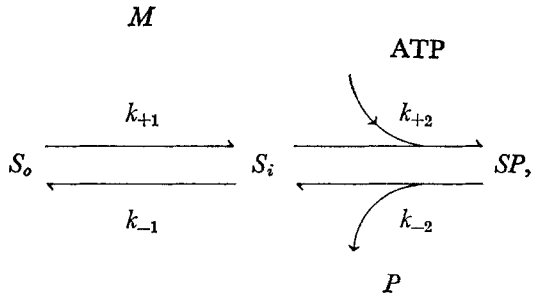
The results presented above showed that all the sugars studied entered a space of the tissue markedly exceeding that of inulin and thus entered the tubular cells. Arguments based on data given in Table II indicated that the observed transport of sugars took place essentially at the outer (antiluminal) face of the renal tubular cells.

The results also indicated that carriers are involved in the transport of the four studied sugars across the cell membrane. This conclusion is based on the marked inhibition of sugar entry by the classical agents blocking sugar transport, i.e. phloridzin and phloretin, and also by observed inhibitory (presumably competitive) effects between sugars for transport (Tables I, IV, V, VI). In the case of α-methyl-glucoside, some indication of a saturation phenomenon was obtained. Experiments not given here in detail showed that the entry of the four sugars into the cells was not affected by the presence of 0.025 mM chlorphenol red; thus, the sugar transport was not related to the simultaneously occurring transcellular transport of the indicator. Also, the transport of sugars was not affected by the absence of external Ca^{2+}; it has been found previously (Kleinzeller, 1970 *a*) that the active sugar transport in slices of rabbit renal cortex was greatly diminished by the absence of Ca^{2+}.

The mechanism of α-methyl-D-glucoside transport appears to be of the facilitated diffusion type since this nonmetabolizable sugar did not enter a tissue space greater than that corresponding to a diffusion equilibrium (Fig. 1, Table I).

The observation that the transport of 2-deoxy-D-glucose, D-galactose, and its 2-deoxy-derivative was associated with considerable phosphorylation (Tables IV, V, and VI) represents a complication in the establishment of the transport mechanism. The results of the experiment shown in Fig. 2 for 2-deoxy-D-glucose (and identical data for D-galactose), i.e. (*a*) reversibility of the sugar transport across the cell membrane, and (*b*) a temporary dephosphorylation of the cellular sugar-phosphate concomitant with the in-

duced efflux of free sugar, are compatible with the following model:



where S is sugar (subscripts o and i denote the outer and intracellular spaces, respectively), SP is sugar-phosphate, and M is membrane. More detailed evidence in favor of such a model will be presented elsewhere for the tubular cell of rabbit kidney. It is obvious that for such a model the steady-state level of S_i will be related to the actual values of the respective rate constants and may be well below that corresponding to a diffusion equilibrium in spite of an uphill transport process. Only in the case of 2-deoxy-D-glucose did the obtained data indicate the possibility of an active transport mechanism: In the experiment shown in Fig. 2 the steady-state T/M values for the free sugar were close to those corresponding to a diffusion equilibrium in spite of a concomitant phosphorylation process; the T/M ratio of 0.95 found at the end of this experiment actually corresponds to a concentration gradient across the cell membrane of 1.2 if the usual assumptions are made as to the calculation of the apparent intracellular concentrations (Kleinzeller, 1970). Some of the properties of the 2-deoxy-D-glucose transport mechanism in the flounder kidney tubule also appear to be similar to those found for the active transport of this sugar in renal tubular cells of the rabbit. Thus the tissue uptake of the sugar was increased by increasing external pH in both flounder (Fig. 2) and rabbit (Kleinzeller et al., 1970) kidney cells, and this uptake was shown to be essentially due to a tissue accumulation of 2-deoxy-glucose phosphate (Fig. 3, and unpublished data for the rabbit tissue).

Particular interest of the data presented above centers on the specificity of the sugar transport systems. The observation that D-glucose was not an impressive inhibitor of the transport of α-methyl-D-glucoside (Table I) was corroborated by the fact that α-methyl-D-glucoside failed to inhibit the transport of 2-deoxy-D-glucose, although here D-glucose was a potent inhibitor (Table IV). These data justify the conclusion that the carrier transport of α-methyl-D-glucoside across the antiluminal face of the flounder tubular cell differs from that which brings about the transport of 2-deoxy-D-glucose (and D-glucose). Applying the same argument, it may also be stated that the carrier for α-methyl-D-glucoside shows no affinity for D-galactose and its 2-deoxy-derivative since the glucose did not inhibit the transport of these sugars

(Tables V and VI). In view of the above data, α-methyl-D-glucoside cannot be used indiscriminately as a nonmetabolizable analogue of D-glucose in transport studies of renal tissue. Further information as to the structural requirements for the α-methyl-glucoside transport pathway, beyond the absence of a free hydroxyl on C_1, is clearly desirable.

No explanation can be offered at present as to the mechanism by which α-methyl-D-glucoside in fact accelerated the uptake of 2-deoxy-D-glucose (Table IV), D-galactose (Table V), and 2-deoxy-D-galactose (Table VI).

From data shown in Table IV on the competitive inhibition of 2-deoxy-D-glucose transport, it appears that the mediating carrier has the following structural requirements: a hydroxyl on C_1 and a hydroxyl on C_4 in a trans-position with regard to C_3-OH. Since mannitol was not markedly transported into renal tubules (the values of T/M were only slightly greater than those for inulin), it may be assumed that a hemiacetal group is also required. No free hydroxyl on C_2 appears to be mandatory. Judging from the lack of inhibition of 2-deoxy-D-glucose transport by 3-O-methyl-D-glucose, C_3-OH also is not required for an interaction with the carrier.

The structural requirements for the carrier mediating the transport of D-galactose and 2-deoxy-D-galactose, based on data shown in Tables V and VI, may be summarized as follows: Free hydroxyls on C_1 and C_4, the latter in a cis-position to that on C_3.

The data given here demonstrate the presence of at least three transport pathways for sugars at the antiluminal face of the flounder renal cells; further details as to the respective structural requirements will have to be supplied, and the possibility of some overlapping of specificities will have to be investigated. The possibility of a variety of transport pathways for sugars in renal tubular cells has been previously recognized on the basis of studies in vivo (Silverman et al., 1970) and in vitro (Kleinzeller, 1970; 1972). The present investigation substantiates such view and specifies more closely the transport processes taking place at the antiluminal face of flounder kidney tubules.

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