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Transport of Monosaccharides in Kidney-Cortex Cells

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1. The aerobic accumulation of various monosaccharides in slices of rabbit kidney cortex at 25° was studied. 2. D-Fructose and α-methyl D-glucoside were readily accumulated against their concentration gradient by a phlorrhizin-sensitive Na^+-dependent active transport. In the absence of external Na^+ the maximal rate of α-methyl glucoside transport was decreased tenfold, the K_m of entry into the cells (8·2mM) not being affected. Phlorrhizin and D-galactose inhibited the entry of α-methyl glucoside also in the absence of external Na^+. 3. D-Xylose, 6-deoxy-D-glucose and 6-deoxy-D-galactose were poorly accumulated ($[S]_i/[S]_o$ ratios slightly above 1·0); this transport was inhibited by phlorrhizin and by the absence of Na^+. 4. 3-*O*-Methyl-D-glucose, D-arabinose and L-arabinose were not actively transported, $[S]_i/[S]_o$ ratios never exceeding 1·0. 5. 2-Deoxy-D-glucose and 2-deoxy-D-galactose were readily accumulated against a high concentration gradient, this transport being Na^+-independent and only slightly sensitive to phlorrhizin. External Na^+ was not required for an inhibitory action of phlorrhizin and D-galactose on the entry of 2-deoxy-D-galactose into the cells. 6. Interference for entry into the cells between the following saccharides was found: D-galactose inhibited α-methyl D-glucoside transport; D-xylose entry was inhibited by D-glucose; D-galactose transport was inhibited by D-xylose; a mutual interference between D-galactose and its 2-deoxy analogue was found. 7. It is concluded that D-glucose, D-galactose, α-methyl D-glucoside, D-xylose and possibly also some other monosaccharides share a common active transport system. 8. The specificity of the Na^+-dependent phlorrhizin-sensitive active transport system for monosaccharides in kidney-cortex cells differs from that in intestinal epithelial cells.

In the preceding paper (Kleinzeller, Kolínská & Beneš, 1967) evidence was presented that glucose is actively accumulated in kidney-cortex cells by a phlorrhizin-sensitive Na^+-dependent mechanism apparently identical with that for galactose. Information on the specificity of the transport mechanism for sugars in renal cells was therefore of interest. In this paper we now report results of experiments on the transport of some hexoses, pentoses and their derivatives in kidney-cortex slices.

METHODS

Experiments were carried out with pooled slices of rabbit kidney cortex; one or two animals were used for each experiment. Experimental conditions and techniques were essentially those given in the preceding paper (Kleinzeller *et al.* 1967) and only departures from these are described below in detail.

Monosaccharide accumulation and oxidation in the tissue. To minimize a possible interference of glucose formed from endogenous substrate (Krebs, Bennett, de Gasquet, Gascoyne & Yoshida, 1963) with the accumulation of the monosaccharides studied, a routine aerobic preincubation of the tissue in a sugar-free saline containing 10mM-sodium acetate as substrate for 30–45min. at 25° was adopted. Only then were groups of slices transferred to 25ml. conical flasks each containing 3ml. of the same saline with the sugar being examined and incubated, usually for 60min.; this time was usually sufficient to obtain a distribution of sugars approaching the steady state. Unless otherwise stated, Na^+-free saline media were prepared by replacing Na^+ in the medium of the Krebs–Ringer type (see Krebs, 1950) by $tris^+$ ion.

Except for the 2-deoxy-D-hexoses, all experiments were carried out with ^{14}C-labelled sugars, usually about 0·3μc/3ml. of incubation saline in the flask. After incubation, sugars were determined in the tissue extract and incubation saline after deproteinization by the $ZnSO_4$–$Ba(OH)_2$ procedure (Somogyi, 1945) either by radioactivity measurements or by chemical analysis; in this way only free sugars in the analysed material were estimated (see also the Results section, Table 6). From the values obtained the apparent $[S]_i/[S]_o$ ratios were computed.

Monosaccharide oxidation was determined on the basis of $^{14}CO_2$ formation only where U-^{14}C-labelled sugars were

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available and the experiments were carried out in a phosphate saline. For the 2-deoxyhexoses, the rate of sugar utilization (μmoles/g. wet wt./hr.) by the cells was determined as the amount of sugar taken up from the medium during incubation after deduction of that accumulated in the tissue.

In some experiments the initial rate of sugar accumulation, v, was determined as the amount of sugar taken up by the cells during 20min. incubation, after correction for the extracellular space, i.e. 250ml./kg. wet wt. of tissue; this procedure is justified by the kinetics of sugar accumulation, the uptake being practically linear during this time-interval. Results are expressed in μmoles/g. wet wt./hr.

Analytical methods. Radioactivity measurements were carried out as described in the preceding paper (Kleinzeller *et al.* 1967) with a Tracerlab scintillation counter. 2-Deoxy-D-hexoses were estimated colorimetrically by the periodate procedure of Waravdekar & Saslaw (1957) with a Hilger Spekker absorptiometer; the values for the tissue had to be corrected for a small blank (usually 0·2–0·4μmole/g.) determined in each experiment. Organic phosphate in the deproteinized tissue extract (after ashing with H_2SO_4–HNO_3) was estimated by the method of Martin & Doty (1949). Results given in the present paper are the means of at least three analyses; where more determinations were carried out, means ± S.E.M. are presented. Variations in results in different experiments did not exceed 20%.

Materials. D-[1-^{14}C]Galactose, D-[U-^{14}C]xylose, D-[U-^{14}C]fructose and D-[1-^{14}C]arabinose were purchased from The Radiochemical Centre, Amersham, Bucks.; L-[1-^{14}C]arabinose was obtained from Calbiochem Inc., Los Angeles, Calif., U.S.A. α-Methyl D-[U-^{14}C]glucoside and 3-*O*[^{14}C]-methyl-D-glucose were prepared by Dr O. Liebster from the Isotope Laboratory, Czechoslovak Academy of Sciences, Prague, Czechoslovakia; 6-deoxy-D-[U-^{14}C]glucose and 6-deoxy-D-[U-^{14}C]galactose were gifts from Professor R. K. Crane, Chicago, Ill., U.S.A. Thiobarbituric acid for the determination of 2-deoxyhexoses was prepared by the method of Michael (1887). All other reagents were commercial preparations of A.R. grade.

RESULTS

Transport of hexoses

Fructose. D-Fructose was readily accumulated against its concentration gradient (see Table 1). This accumulation was markedly inhibited by 0·4mM-phlorrhizin (Table 2) and by the absence of Na^+ from the saline (Table 3).

α-Methyl glucoside. Kidney-cortex slices rapidly accumulated this sugar against a high concentration gradient by a phlorrhizin-sensitive Na^+-dependent mechanism (Tables 1, 2 and 3). As compared with D-galactose (see Kleinzeller *et al.* 1967) the steady-state gradient of α-methyl glucoside established after 60min. incubation was about double at identical $[S]_o$, although the glucoside was relatively rapidly oxidized at a rate of about 1μmole/g./hr. at $[S]_o$ 1mM.

The above-mentioned characteristics of accumulation of this sugar being identical with those for glucose and galactose, further evidence was sought in favour of a common transport mechanism, i.e. competition between these sugars for accumulation in kidney-cortex slices. Results presented in Table 4 provide some evidence for competition for sugar entry into the cells between α-methyl glucoside and D-galactose. It may thus be concluded that α-methyl D-glucoside is transported into renal cells by the same active phlorrhizin-sensitive and Na^+-dependent mechanism as D-glucose and D-galactose. Owing to favourable characteristics of the α-methyl glucoside transport, i.e. fast rate of accumulation and high $[S]_i/[S]_o$ ratios, this sugar may serve as a convenient model for the study of active monosaccharide transport.

Mechanism of the effect of Na^+ on the transport of

Table 1. *Accumulation and oxidation of various monosaccharides in kidney-cortex slices*

The experimental procedure for the determination of the apparent $[S]_i/[S]_o$ ratio and of the rates of oxidation of U-^{14}C-labelled sugars was as described in the Methods section. The saline media used were: bicarbonate saline (B), gas phase O_2+CO_2 (95:5); phosphate saline (P), gas phase O_2. Mean values are given (with numbers of analyses in parentheses), ± S.E.M. if more than three analyses were carried out.

Monosaccharide	Saline	$[S]_i/[S]_o$ ratio found: $[S]_o$ 0·3mM		$[S]_o$ 1·0mM		Rate of oxidation at $[S]_o$ 1·0mM (μmoles/g./hr.)	
D-[U-^{14}C]Fructose	P	2·53 ± 0·08	(5)	2·28 ± 0·09	(5)	0·58	(4)
α-Methyl D-[U-^{14}C]glucoside	P	7·85 ± 0·38	(5)	6·32 ± 0·25	(5)	1·04	(4)
3-*O*[^{14}C]-Methyl-D-glucose	B	0·69 ± 0·06	(5)	0·32 ± 0·03	(5)	—	
D-[U-^{14}C]Xylose	P	1·15	(3)	0·99	(3)	0·02	(4)
D-[1-^{14}C]Arabinose	B	0·15	(5)	0·09	(5)	—	
L-[1-^{14}C]Arabinose	B	0·53	(3)	0·46	(3)	—	
6-Deoxy-D-[U-^{14}C]glucose	P	1·51 ± 0·09	(5)	1·43 ± 0·05	(5)	Not detected	
6-Deoxy-D-[U-^{14}C]galactose	P	1·04 ± 0·04	(5)	1·09 ± 0·05	(5)	Not detected	
2-Deoxy-D-glucose	B	8·64	(3)	5·78	(3)	—	
2-Deoxy-D-galactose	B	9·97	(3)	6·52	(3)	—	

Table 2. *Effect of phlorrhizin on the accumulation of various monosaccharides in kidney-cortex slices*

Slices were incubated aerobically [O_2+CO_2 (95:5)] for 60min. at 25° in bicarbonate saline containing the tested sugar (1mM), without (control) and with 0·4mM-phlorrhizin. Mean values are given (with numbers of analyses in parentheses).

Monosaccharide	$[S]_i/[S]_o$ ratio found: Control		Phlorrhizin	
D-[U-^{14}C]Fructose	1·46	(5)	0·77	(5)
α-Methyl D-[U-^{14}C]glucoside	4·51	(4)	0·60	(4)
D-[U-^{14}C]Xylose	1·46	(3)	0·77	(3)
L-[1-^{14}C]Arabinose	0·46	(3)	0·31	(3)
6-Deoxy-D-[U-^{14}C]glucose	1·27	(4)	1·10	(4)
2-Deoxy-D-galactose	7·32	(5)	4·17	(5)

Table 3. *Effect of* Na^+ *on the accumulation of various monosaccharides in kidney-cortex slices*

Slices were preincubated aerobically (O_2) for 45min. at 25° in either Na^+ saline or $tris^+$ saline containing 10mM-sodium acetate, then incubated for 60min. under identical conditions in saline media containing the tested sugar (1mM). Mean values are given (with numbers of analyses in parentheses).

Monosaccharide	$[S]_i/[S]_o$ ratio found: Na^+ saline ($[Na^+]_o$ 135mM)		$Tris^+$ saline ($[Na^+]_o$ 0)	
D-[U-^{14}C]Fructose	2·28	(5)	0·76	(5)
α-Methyl D-[U-^{14}C]glucoside	4·42	(5)	0·73	(5)
D-[U-^{14}C]Xylose	1·05	(3)	0·69	(3)
L-[1-^{14}C]Arabinose	0·46	(3)	0·37	(3)
6-Deoxy-D-[U-^{14}C]glucose	1·27	(4)	1·09	(4)
2-Deoxy-D-glucose	4·05	(3)	3·98	(3)

α-methyl glucoside. The Na^+ requirement for the active accumulation of glucose, galactose and some other monosaccharides (see Table 3) raises the question of the mechanism of this phenomenon. Information on this point was sought by examining the effect of Na^+ on the kinetic parameters of α-methyl glucoside entry into the cells (Fig. 1): the K_m was identical at both extreme Na^+ concentrations used (K_m 8·2mM) whereas $V_{max.}$ was greatly decreased in the Na^+-free saline (at $[Na^+]_o$ 135mM, $V_{max.}$ was 100μmoles/g./hr.; at $[Na^+]_o$ 0, $V_{max.}$ was 10μmoles/g./hr.).

It was decided to examine whether phlorrhizin and galactose might also affect the entry of α-methyl glucoside taking place in the absence of Na^+, when no accumulation against the concentration gradient occurs. The results in Table 4 show that even in an Na^+-free saline the entry of α-methyl glucoside was markedly decreased by both substances.

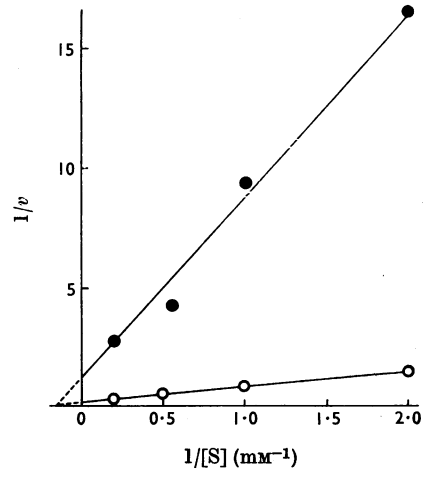

Fig. 1. Lineweaver–Burk plot of the effect of Na^+ on the rate of α-methyl D-glucoside transport into kidney-cortex slices. Slices were incubated aerobically [O_2+CO_2 (95:5)] for 20min. at 25° in bicarbonate saline containing 10mM-lithium acetate and various concentrations of the glucoside. Each point is the mean of three analyses. ○, Control ($[Na^+]_o$ 135mM); ●, $[Na^+]_o$ 0. Initial velocity, v, is expressed in μmoles/g. wet wt./hr.

Table 4. *Effect of various inhibitors on the accumulation of α-methyl D-glucoside in kidney-cortex slices in the presence and absence of* Na^+

Experimental conditions were as given in the legends to Tables 2 and 3, with 0·15mM-α-methyl D-glucoside. The inhibitors used were: 0·4mM-phlorrhizin; 0·03mM-ouabain; 10mM-galactose. Each value is the mean ± S.E.M. of four analyses.

Inhibitor	$[S]_i/[S]_o$ ratio found: Na^+ saline ($[Na^+]_o$ 135mM)	$Tris^+$ saline ($[Na^+]_o$ 0)
None (control)	6·6±0·3	0·78±0·4
Phlorrhizin	1·6±0·3	0·44±0·03
Ouabain	4·5±0·2	0·72±0·05
Galactose	3·8±0·2	0·59±0·02

3-O-*Methylglucose.* This sugar was not transported against its concentration gradient (Table 1). This result is surprising in view of the fact that this sugar is readily transported against the concentration gradient by intestinal epithelial cells (Campbell & Davson, 1948). Thus some differences in the specificity of the sugar transport mechanism in renal and intestinal epithelial cells appear to exist. In view of the lack of active transport of this sugar, the effect of phlorrhizin and Na^+ was not investigated.

Transport of pentoses

D-*Xylose*. No marked accumulation of xylose against its concentration gradient was found at a variety of external concentrations, the $[S]_i/[S]_o$ ratio being usually about 1·0 (Table 1).

The entry of xylose into renal cells and its intracellular concentration after 60min. of incubation were phlorrhizin-sensitive (Table 2) and Na^+-dependent (Fig. 2).

The transport of xylose at $[S]_o$ 1·0mM was decreased by the presence of 0·3mM-glucose in the incubation saline, and to some extent also by 0·1mM-2,4-dinitrophenol (Table 5). Thus, in spite of its poor accumulation, xylose appeared to be transported by a mechanism similar to that for glucose, galactose and some other monosaccharides studied in the present paper.

It ought to be mentioned that a slight $^{14}CO_2$ formation from added [U-^{14}C]xylose (Table 1) was observed, corresponding to a rate of xylose oxidation of 0·02μmole/g./hr. This low value can hardly explain the poor accumulation of xylose in renal tubular cells. Possibly the oxidation of xylose might proceed via intermediate glucose formation (see Krebs & Lund, 1966).

Though the above results indicated a common carrier for the transport of glucose, galactose and xylose into kidney-cortex cells, evidence of a mutual competition between these sugars for the transport site would add considerable weight in favour of such a view. Therefore the effect of glucose on the entry of xylose (Table 5) and the effect of xylose on the accumulation of [^{14}C]galactose was studied. At a D-xylose/D-galactose concentration ratio 100 : 1 in the saline, a significant inhibition of galactose accumulation after 60min. incubation was found [control: $[S]_o$ 0·1mM-galactose, $[S]_i/[S]_o$ ratio 12·0 ± 1·0 ($n = 5$); $[S]_o$ 0·1mM-galactose + 10mM-xylose, $[S]_i/[S]_o$ ratio found 9·9 ± 1·1 ($n = 5$)]. No such inhibition was detected at a 25 : 1 concentration ratio of these sugars.

Thus, in spite of a poor accumulation of D-xylose against its concentration gradient, the transport of this sugar shows the essential features of the mechanism responsible for the uphill transport of glucose and galactose, and the assumption of a common carrier for these three sugars in kidney-cortex cells appears to be justified.

D- *and* L-*arabinose*. D-Arabinose hardly entered renal tubular cells during the 60min. incubation period (Table 1). L-Arabinose was considered to be of greater interest, its configuration corresponding to that of D-galactose. However, L-arabinose also was not markedly accumulated in the cells, although its entry was decreased by phlorrhizin and by the absence of Na^+ from the saline (Tables 1, 2 and 3).

Transport of deoxyhexoses

6-*Deoxy*-D-*glucose and* 6-*deoxy*-D-*galactose*. Unexpectedly, neither of these deoxyhexoses accumulate markedly in kidney-cortex cells (Tables 1, 2 and 3), although in intestinal cells these substances, especially 6-deoxy-D-glucose, are rapidly transported against a high concentration gradient (Crane & Krane, 1956; Wilson & Crane, 1958). The $[S]_i/[S]_o$ ratio for 6-deoxy-D-glucose was somewhat decreased by phlorrhizin and by the absence of Na^+ from the saline.

2-*Deoxy*-D-*glucose and* 2-*deoxy*-D-*galactose*. These

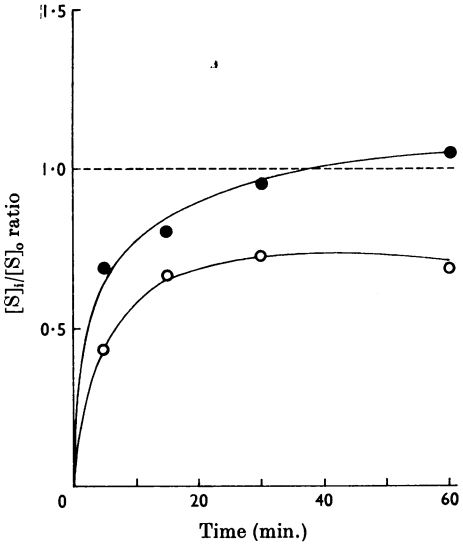

Fig. 2. Effect of external Na^+ on the accumulation of D-xylose in kidney-cortex slices. Conditions of the experiment are given in the legend to Table 3. Each point is the mean of three analyses. ●, Control in Na^+ medium; ○, $tris^+$ medium.

Table 5. *Transport of* D-*xylose in kidney-cortex slices*

Slices were preincubated aerobically (O_2) for 40min. at 25° in phosphate saline, with 10mM-sodium acetate as substrate, then transferred to saline media containing in addition to acetate 1mM-D-[U-^{14}C]xylose and various additions and incubated at 25°. Each value is the mean of three analyses.

Addition	$[S]_i/[S]_o$ ratio	
Incubation time ...	10 min.	30 min.
None (control)	0·74	0·86
Glucose (0·3mM)	0·57	0·71
2,4-Dinitrophenol (0·1mM)	—	0·76

sugars are not actively transported by intestinal epithelial cells (see Crane, 1960). It was therefore rather surprising to find that kidney-cortex cells readily established a high concentration gradient of both these sugars (Table 1); since the transport of 2-deoxyhexoses did not require Na^+ and was considerably less sensitive to phlorrhizin than that of other actively accumulated monosaccharides (Tables 2 and 3), the characteristics of this transport system were investigated in more detail. 2-Deoxy-D-galactose was mostly used as the model substance.

The accumulation of both 2-deoxyhexoses did not reach equilibrium after 60min. incubation, as shown in Fig. 3. Thus $[S]_i/[S]_o$ ratios up to 20 were found after 2hr. of incubation (Fig. 4). The rate of entry and the $[S]_i/[S]_o$ ratios for 2-deoxyglucose were consistently lower than those for the galactose derivative.

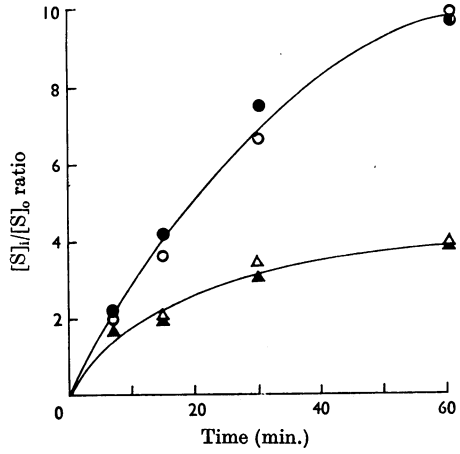

Fig. 3. Transport of 2-deoxyhexoses into kidney-cortex slices. Conditions of the experiment are given in the legend to Table 3. Each point is the mean of two analyses. ○ and ●, 2-Deoxy-D-galactose; ($[S]_o$ 1mM); △ and ▲, 2-deoxy-D-glucose ($[S]_o$ 1mM). ○ and △, Na^+ medium; ● and ▲, $tris^+$ medium.

It might be argued that the high $[S]_i/[S]_o$ ratios for the 2-deoxyhexoses are due to an accumulation in the cells of the phosphorylated derivatives, although the deproteinizing procedure used here (Somogyi, 1945) should yield a practically sugar phosphate-free supernatant. This point was checked by the following experiment. Slices (about 500mg.) were incubated for 2hr. without (control) and with 1mM-2-deoxygalactose or -2-deoxyglucose. After incubation, the tissue and incubation saline media were deproteinized by the zinc sulphate–barium hydroxide procedure, and in the clear supernatant 2-deoxyhexoses, inorganic phosphate and organic phosphate were determined. The values found are shown in Table 6. The increase of organic phosphate on incubation with 2-deoxygalactose would account only for 14% of the accumulated sugar, and practically no increase of organic phosphate on incubation with 2-deoxyglucose was found. Further, a portion of the supernatant was passed through a column (3·5cm. long) of the anion-exchange resin Dowex 1 (X8); within the limits of analytical error the recovery of the 2-deoxyhexoses was quantitative. It is thus concluded that the renal cells accumulate free 2-deoxyhexoses against their concentration gradients and that the transport is an active one.

The concentration gradient of 2-deoxyhexoses established by renal tubular cells was dependent on the external sugar concentration (Fig. 4) in a similar way to that previously shown for D-glucose and D-galactose (Kleinzeller *et al.* 1967) and as also indicated for some other sugars (Table 1). Some utilization of 2-deoxygalactose by the cells occurred: at $[S]_o$ 1mM, about 0·9 μmole/g./hr. (mean of three experiments) was utilized.

Table 6. *Accumulation of 2-deoxyhexoses in kidney-cortex slices*

Slices (500mg.) were incubated aerobically (O_2) for 2hr. at 25° in 15ml. of phosphate saline, with 10mM-sodium acetate as substrate without (control) and with 1mM-2-deoxyhexose. After incubation, 2-deoxyhexose and organic phosphate in the $ZnSO_4$–$Ba(OH)_2$ supernatants were determined.

	2-Deoxyhexose				
		In tissue (μmoles/g.)			
Sugar in incubation saline	In saline (μmole/ml.)	Direct analysis	After Dowex 1 treatment	$[S]_i/[S]_o$ ratio	Organic phosphate in tissue (μmoles/g.)
None (control)	0	0·034	0·022	—	1·18
2-Deoxygalactose	0·69	5·96	6·20	15·4	2·05
2-Deoxyglucose	0·45	2·72	2·81	10·8	1·24

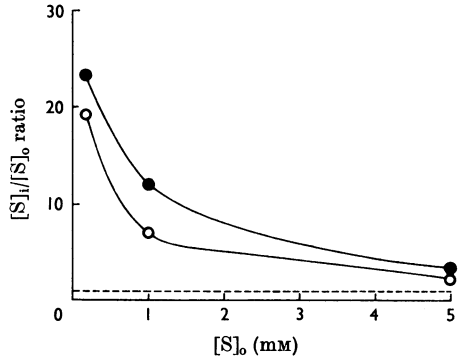

Fig. 4. Effect of sugar concentration on the accumulation of 2-deoxyhexoses in kidney-cortex slices. Slices were incubated aerobically [O_2+CO_2 (95:5)] for 2hr. at 25° in bicarbonate saline with 10mM-sodium acetate and various concentrations of 2-deoxyhexoses. Each point is the mean of three analyses. ○, 2-Deoxy-D-glucose; ●, 2-deoxy-D-galactose.

The accumulation of 2-deoxygalactose was markedly inhibited by 0·1mM-2,4-dinitrophenol (Table 8).

Independence of 2-deoxyhexose transport on external Na^+. The experiment shown in Fig. 3 also supplied evidence that the rate of accumulation of neither 2-deoxyhexoses depended on external Na^+. This aspect was checked further (*a*) by using different Na^+-free saline media, and (*b*) by testing the effect of ouabain.

Results obtained in the experiment given in Table 7 show that, as compared with the control Na^+ saline, the accumulation of 2-deoxygalactose was somewhat decreased in the Na^+-free saline media used, i.e. $tris^+$, Li^+ and $choline^+$ saline media; nevertheless, the $[S]_i/[S]_o$ ratios were considerably higher than 1 in all the media tested.

Ouabain (0·1mM), which inhibits the active accumulation of D-galactose completely (Kleinzeller & Kotyk, 1961), had no effect on the accumulation of 2-deoxy-D-galactose (Table 8).

The conclusion is thus justified that the accumulation of 2-deoxyhexoses does not require external Na^+, in contrast with the transport of other monosaccharides in kidney-cortex cells.

Phlorrhizin inhibition of 2-deoxygalactose accumulation. It was mentioned above that the accumulation of 2-deoxyhexoses was less sensitive to phlorrhizin that that of other monosaccharides tested. Whereas 0·4mM-phlorrhizin decreased the $[S]_i/[S]_o$ ratio of other actively accumulated sugars to below 1, the results in Table 8 show that even at 1mM-phlorrhizin the $[S]_i/[S]_o$ ratio for 2-deoxygalactose was still above 4.

Competition between 2-deoxygalactose and D-galactose for entry into the cells. The above results

Table 7. *Accumulation of 2-deoxy-D-galactose in* Na^+*-free saline media*

Slices were preincubated aerobically (O_2) at 25° for 40min. in the respective saline media, then transferred to saline media containing 1mM-2-deoxy-D-galactose and incubated for 60min. at 25° with 10mM-lithium acetate as substrate. Each value is the mean ± S.E.M. of five analyses.

Saline	$[S]_i/[S]_o$ ratio
Na^+ saline	5·75 ± 0·29
$Tris^+$ saline	4·23 ± 0·10
Li^+ saline	3·26 ± 0·21
$Choline^+$ saline	4·67 ± 0·17

Table 8. *Effect of inhibitors on the accumulation of 2-deoxy-D-galactose in kidney-cortex slices*

Experimental conditions were as given in the legends to Tables 1, 2 and 3, with 1mM-2-deoxy-D-galactose. Each value is the mean of three or four analyses.

Expt. no.	Inhibitor	$[S]_i/[S]_o$ ratio
1	None (control)	5·0
	Ouabain (0·1mM)	6·2
	2,4-Dinitrophenol (0·1mM)	2·1
2	None (control)	8·7
	Phlorrhizin (0·4mM)	5·4
	Phlorrhizin (1mM)	3·9

indicated that 2-deoxyhexoses are actively accumulated in kidney-cortex cells by a mechanism differing from that for other monosaccharides by being independent of external Na^+ and relatively insensitive to phlorrhizin. To test this transport system further, the possibility of competition between 2-deoxygalactose and galactose for the transport site was investigated. The results of such experiments (Table 9) show that mutual competition between the sugars for the transport mechanism takes place, the affinity of the carrier being probably higher for galactose than for 2-deoxygalactose.

Effect of inhibitors on the accumulation of 2-deoxygalactose in the absence of external Na^+. In an attempt to elucidate whether 2-deoxygalactose shares the same active transport system with some other monosaccharides in spite of the observed differences in the Na^+ requirement, the inhibitory effect of D-galactose and phlorrhizin on the entry of 2-deoxy-D-galactose in an Na^+-free saline was studied. The results of such an experiment are shown in Table 10. External Na^+ was not required for an inhibition of 2-deoxygalactose entry into the cells by phlorrhizin and D-galactose; the accumulation of the latter is dependent on external Na^+ (Kleinzeller & Kotyk, 1961).

Table 9. *Competition between* D-*galactose and 2-deoxy-*D-*galactose for entry into kidney-cortex slices*

Slices were preincubated aerobically ($O_2 + CO_2$ (95 : 5)] for 45 min. at 25° in bicarbonate saline with 10 mM-sodium acetate, then transferred to bicarbonate saline containing one or both tested sugars and incubated for 60 min. Mean values are given (with numbers of analyses in parentheses), ± S.E.M. if more than three analyses were carried out.

Expt. no.	Monosaccharide	Addition	$[S]_i/[S]_o$ ratio	
1	D-[1-^{14}C]Galactose (0·1 mM)	None	18·8 ± 1·3	(5)
		2-Deoxy-D-galactose (1 mM)	11·4 ± 0·6	(5)
		2-Deoxy-D-galactose (10 mM)	4·5 ± 0·4	(5)
2	2-Deoxy-D-galactose (1 mM)	None	7·6	(3)
		D-Galactose (0·1 mM)	5·9	(3)
		D-Galactose (0·3 mM)	3·4	(3)
		D-Galactose (1·0 mM)	1·2	(3)
		D-Galactose (3 mM)	0·7	(3)
		D-Galactose (10 mM)	0·6	(3)

Table 10. *Effect of inhibitors on the accumulation of 2-deoxy-*D-*galactose in kidney-cortex slices in the presence and absence of* Na^+

Experimental conditions were as given in the legends to Tables 2 and 3, with 1 mM-2-deoxy-D-galactose. Each value is the mean ± S.E.M. of four analyses.

	Na^+ saline ($[Na^+]_o$ 135 mM)		Tris$^+$ saline ($[Na^+]_o$ 0)	
Inhibitor	$[S]_i/[S]_o$ ratio	%	$[S]_i/[S]_o$ ratio	%
None (control)	7·9 ± 0·2	100	5·6 ± 0·2	100
Phlorrhizin (0·4 mM)	4·2 ± 0·1	53	3·9 ± 0·0	70
Phlorrhizin (1 mM)	3·0 ± 0·1	37	3·2 ± 0·2	57
D-Galactose (0·1 mM)	6·5 ± 0·2	83	4·8 ± 0·3	87
D-Galactose (0·5 mM)	2·2 ± 0·0	28	1·9 ± 0·1	34

DISCUSSION

This paper reports evidence on the accumulation of some monosaccharides in kidney-cortex cells. Having confirmed that in the tissue extract after zinc sulphate–barium hydroxide deproteinization no sugar phosphates are present, $[S]_i/[S]_o$ ratios higher than 1·0 indicate an active transport of sugars by kidney-cortex cells. The results reported are discussed below from the point of view of (*a*) the specificity of the transport mechanism for monosaccharides in renal cortex cells, and (*b*) the Na^+ requirement of the sugar transport.

Specificity of the active transport mechanism for monosaccharides. Results presented in the preceding paper (Kleinzeller *et al.* 1967) and above (Tables 1, 2 and 3) show that the following sugars are readily accumulated in kidney-cortex cells by an active phlorrhizin-sensitive and Na^+-requiring mechanism: D-glucose, D-galactose, α-methyl D-glucoside and D-fructose; the results for fructose may be partly distorted owing to the rapid conversion of fructose into glucose in renal tissue (Krebs & Lund, 1966). From the work of Hauser (1965) it might be inferred that *meso*inositol is also actively accumulated by the same system. Of the poorly accumulated sugars, the transport of D-xylose had the same characteristics. Evidence has also been supplied to show mutual competition of some of these sugars for the transport site. On the basis of these criteria, it appears justified to suggest that the above sugars (and possibly also 6-deoxyhexoses) share a common active transport mechanism.

Available evidence does not allow one to decide whether D-arabinose and L-arabinose employ the same transport carrier for their very slow entry into renal cells; the fact that the $[S]_i/[S]_o$ ratio for L-arabinose, though well below 1·0 after 60 min. of incubation, was decreased by phlorrhizin and absence of Na^+ might point in favour of such suggestion.

The possibility was considered whether for the above monosaccharides two types of transport sites might be present in the membrane, one bringing about an equilibrating transport of the diffusion type, the other one being a metabolically linked (and Na^+-dependent) pump transporting sugars against the concentration gradient. Such a view is unlikely, especially on the basis of the experiment reported in Table 4, showing that even in the

absence of external Na^+ the transport of α-methyl glucoside was inhibited by phlorrhizin and D-galactose although the $[S]_i/[S]_o$ ratio was below diffusion equilibrium. An interaction of monosaccharides with a mobile carrier even in absence of Na^+ has been postulated by Crane (1965) for the active transport system in the brush border of intestinal epithelial cells, and some evidence in favour of such a view has been obtained by Alvarado (1965), i.e. an induced counterflow of sugars in Na^+-free media. On the other hand, Csáky & Rigor (1964) have shown that a considerable portion of the uphill sugar transport into the epithelial cells of the dog choroid plexus did not require external Na^+.

If the suggestion of a common transport mechanism for the monosaccharides enumerated above is correct, the structural requirements for transport ought to be considered:

C-1. The hydroxyl group of the semiacetal group on C-1 does not appear to be essential for the transport, since α-methyl D-glucoside is readily accumulated.

C-2. The accumulation of 2-deoxyhexoses by an active transport system with the described properties raises two possibilities: (*a*) that two different active transport sites for sugars operate in kidney tubular cells, both of them showing similar structural specificities (namely a competition between D-galactose and its 2-deoxy analogue for entry), but only one of them being dependent on external Na^+; (*b*) that all the actively transported sugars, including 2-deoxy derivatives, share one transport mechanism with a rather wide spectrum of specificity; the hydroxyl group on C-2 would then be required for interaction with Na^+ and would also play a role in the phlorrhizin action. This aspect is discussed below.

C-3. A free hydroxyl group on C-3 appears to be required for active accumulation in kidney cells, 3-*O*-methyl-D-glucose not being markedly transported.

C-6. A hydroxyl group of C-6 enhances active accumulation, 6-deoxy analogues of the actively transported D-glucose and D-galactose being but poorly accumulated.

It ought to be pointed out that a correlation appears to exist between the reabsorption in renal tubules of glucose, galactose, fructose, xylose (see Smith, 1951) and inositol (Perlès, Colas & Blayo, 1960) and the active accumulation of these sugars in kidney-cortex slices.

The specificity of the system responsible for active sugar accumulation in kidney-cortex cells differs in some points from the active phlorrhizin-sensitive and Na^+-requiring transport mechanism in intestinal cells: in the former, hydroxyl groups appear to be required on C-3 and C-6 of the pyranose ring for active accumulation, whereas in intestinal epithelial cells C-2 plays the dominant role (Crane, 1960).

Though further quantitative data are clearly required for an assessment of the relative affinities of various monosaccharides for the active transport system in kidney-cortex cells, the data supplied above suggest that considerable differences exist in this respect between the various sugars tested.

Na^+ *requirement for monosaccharide transport.* Crane, Forstner & Eichholz (1965) presented a concept of the Na^+-dependent sugar transport in intestinal cells as a direct interaction of Na^+ with a specific binding site on the sugar carrier; from the results obtained by these authors, i.e. an effect of Na^+ on the K_m for transport without change of $V_{max.}$, the binding sites for both Na^+ and the sugar on the carrier molecule would have to be spatially very close.

In renal cells, Na^+ affected $V_{max.}$ rather than K_m, as observed for the transport of α-methyl glucoside (Fig. 1) and as calculated for the absorption of glucose in frog renal tubules from Fig. 3 of Vogel, Lauterbach & Kröger (1965). This may be compatible with a carrier–sugar–ion complex, as visualized by Crane *et al.* (1965), but may equally well indicate two different, possibly separated, sites for the attachment of Na^+ and the sugar in the transport system.

In this connexion, the peculiar features of the Na^+-independent transport of 2-deoxyhexoses are of note. Two possibilities have been mentioned above for the mechanism of transport of these sugars in kidney-cortex cells. The fact that Na^+ was not required for the inhibitory effect of D-galactose or phlorrhizin does not allow conclusive discrimination between these views. The 2-deoxyhexoses might be actively transported by a specific carrier (not linked to the sodium pump, but competitively inhibited by D-galactose); then one would expect D-galactose to accumulate in the cells also in absence of Na^+, which is not the case. Alternatively, a pumping mechanism of a wide specificity would transport any sugar channelled to it by a carrier, the latter requiring Na^+ for a temporary attachment to C-2 of, e.g., galactose. In this connexion, Thier, Fox, Rosenberg & Segal (1964) observed a non-competitive inhibition of α-amino acid accumulation in kidney-cortex slices by some hexoses, but not by 2-deoxyhexoses, and Alvarado (1966) reported mutual competition for transport between sugars and amino acids in intestinal cells.

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