BBA 75459

ACTIVE SUGAR TRANSPORT IN RENAL CORTEX CELLS: THE ELECTROLYTE REQUIREMENT

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(Received February 3rd, 1970)

SUMMARY

The electrolyte requirement for the active transport of α-methyl-D-glucoside, D-galactose, 2-deoxy-D-glucose and 2-deoxy-D-galactose was investigated using slices of rabbit kidney cortex:

1. As compared with values obtained at $[Na^+]_0 = 0$, the presence of external Na^+ (128 mM) increased the apparent v_{max} of D-galactose transport without affecting the transport K_m.

2. The Na^+ requirement for the transport of α-methyl-D-glucoside and D-galactose was not saturated at 128 mM Na^+. Within the range of $[Na^+]_0$ 25–128 mM, indications of a single Na^+ entering a rate-limiting event in α-methyl-D-glucoside transport were found.

3. A significant fraction of the D-galactose transport was found to be Na^+ independent and insensitive to 2 mM ouabain.

4. As compared with values obtained at $[K^+]_0 = 0$, the presence of 0.5–7 mM K^+ activated the transport of α-methyl-D-glucoside and D-galactose; this K^+ stimulation was saturated at $[K^+]_0$ 1 mM and affected the v_{max}, rather than the K_m, of sugar transport. No such effect of $[K^+]_0$ on the transport of both 2-deoxyhexoses was observed.

5. The absence of external Cl^- had no effect on the transport of the four sugars tested.

6. The absence of saline Ca^{2+} markedly depressed both the influx and the steady-state accumulation level of all four sugars tested; the efflux of D-galactose and 2-deoxy-D-galactose was accelerated. This Ca^{2+} stimulation of the sugar transport was saturated at $[Ca^{2+}]_0$ 0.5 mM and affected the v_{max} rather than the K_m of sugar transport. The Ca^{2+} effect was found to be fairly specific for this ion: whereas Sr^{2+}, Ba^{2+} and, to some extent, Mn^{2+} could replace Ca^{2+} in its effect on the sugar transport, Mg^{2+} and La^{3+} were ineffective.

7. The above results are discussed in relation to hypotheses put forward to explain the mechanism of the Na^+-dependent nonelectrolyte transport in some animal cells. It is concluded that: (a) In kidney cortex cells, the presence of Na^+ (external and intracellular) is not mandatory to bring about an active transport of 2-deoxyhexoses and also a fraction of galactose; (b) the K^+ stimulation of sugar transport is related to the Na^+ requirement for sugar transport; (c) Ca^{2+} stimulates a common step in the transport of all sugars tested.

INTRODUCTION

The present investigation was undertaken with the aim of providing more information as to the nature of the cation requirement of sugar transport in renal tubular cells. When the Na^+ requirement and K^+ stimulation of the active D-galactose transport into renal cells was first observed[1], the view of identical mechanisms of this process in the kidney tubule and in the intestinal mucosa appeared feasible (see also ref. 2). A subsequent study[3] revealed two fundamental differences between the sugar transport in both tissues: (a) marked differences in the mininum structural requirement for active sugar transport, and (b) the observation that as opposed to the intestinal mucosa, 2-deoxyhexoses can be actively transported into kidney cortex cells by a Na^+-independent (and ouabain-insensitive) mechanism. The latter finding could not be readily accomodated within the framework of hypotheses put forward to describe the coupling between electrolyte and nonelectrolyte transport in intestinal mucosa[2,4] and some other animal cells (see ref. 5 for a detailed analysis of the salient views in this field). In addition to the demonstrated Na^+ independence of 2-deoxyhexose transport into renal tubular cells, the puzzling competition for active cellular accumulation between 2-deoxyhexoses and D-galactose (a sugar requiring Na^+ for its transport) had to be taken into account for the description of possible models for sugar transport into kidney cortex cells. Two possibilities were envisaged: (1) Independent pathways for the Na^+-requiring and Na^+-independent sugar transport, the latter also having some affinity for sugars such as D-galactose. (2) A mutual sharing of parts of the transport pathway.

A more detailed model favoring the second alternative has been discussed previously[6]. However, kinetic evidence then indicated the need to postulate several pathways of sugar transport in kidney cortex cells[7].

In this communication quantitative kinetic data will be reported concerning the relationship between electrolyte and monosaccharide transport in renal tubular cells. Four actively transported sugars were chosen as models for this study: D-galactose and α-methyl-D-glucoside (the transport of these sugars being largely Na^+ dependent[3]); 2-deoxy-D-glucose and 2-deoxy-D-galactose, both transported into kidney cortex cells by a Na^+-independent (and ouabain-insensitive) mechanism[3].

Some preliminary data have been presented[6,8].

METHODS

The experiments were carried out using kidney cortex slices of healthy rabbits. Most of the experimental and analytical procedures used were described in detail earlier[7] and only departures from these are given here .

Salines. The standard saline (composition (mM): Na^+, 128.5; K^+, 6.75; Li^+, 6.75; Ca^{2+}, 2.9; Mg^{2+}, 1.35; $Tris^+$, 6.75; Cl^-, 145; $SO^2{}_4{}^-$, 1.35; phosphate, 1.35; pH 7.4) and modifications thereof were used. The saline concentration of individual components will be denoted by the subscript o to the symbol of the respective ionic species.

Na^+-free salines. $Tris^+$ or Li^+-media were prepared by equivalent replacement of Na^+ in the standard saline by the respective cations. Modifications in the contents of K^+ were obtained by equivalent replacements of NaCl by KCl.

Cl^--free salines. The salines were prepared by employing 0.154 M sodium ethane sulfonate, 0.154 M KNO_3 and 0.11 M $Ca(NO_3)_2$ instead of solutions of the respective chlorides.

Ca^{2+}-free media. $CaCl_2$ in the saline was equivalently replaced by NaCl (or LiCl) and usually 0.1 mM EDTA (sodium or lithium salt) was added.

Experimental

The preincubation and incubation of the tissue in the presence of sugars (unless otherwise stated: 25°, O_2 as gaseous phase, 1 mM sugars during incubation, acetate as metabolic substrate) was carried out essentially as described in the previous communication[7]. However, care was taken to impoverish the tissue of those electrolytes the effect of which was investigated. Thus, when studying the transport of sugars in Na^+-, K^+- and Cl^--free media, the slices were first leached in ice-cold salines devoid of the investigated component, then preincubated aerobically for 45 min in the respective salines and only then incubated in the presence of sugars. Using this procedure, the apparent intracellular concentration of Na^+ ($[Na^+]_i$) of tissue incubated in Na^+-free salines was below 3 mM.

In some experiments the distribution of $^{36}Cl^-$ was followed. The experimental procedure was identical with that used for studying the distribution of sugars; however, the incubation saline was labeled with $^{36}Cl^-$ (0.1 μC/ml). After incubation, the blotted slices were placed overnight into 4 ml 0.1 M HNO_3. In 1-ml portions of the tissue extract and of suitably diluted samples of the media, $^{36}Cl^-$ was determined by scintillation spectrometry (Packard Instruments, Model 3320) using 10 ml of the toluene–Triton X-100 scintillation fluid containing fluors (see ref. 9). The wash-out technique[10] was employed to study the efflux of $^{22}Na^+$. Slices were first loaded with the label by aerobic incubation in labeled saline (1 μC/ml) for 45 min. Blotted slices were then placed into tubes with nylon netting and the wash-out of $^{22}Na^+$ into a series of 50-ml tubes each containing 10 ml saline vigorously aerated with O_2 was followed. The activity in the tissue (after loading and after the wash-out) and in the tubes was then determined by scintillation spectrometry using the toluene–Triton scintillation fluid[9]. Similarly, the efflux of sugars was followed (see also ref. 11). From the data obtained the efflux curves were drawn by plotting the log percent of activity remaining in the tissue against time, and the apparent rate constants of efflux and corresponding spaces were calculated using the graphic method for compartmental analysis[12].

Analytical

The methods for the analysis of sugars in the tissue and the media were those described earlier[3,7]. From the data obtained, the following values were calculated: The apparent intracellular sugar concentration, $[S]_i$; the near steady-state accumulation ratio (after 60 min incubation), $[S]_i/[S]_0$; the rate of sugar transport (30 min incubation), v (μmoles per g cell water per 0.5 h); the apparent kinetic transport parameters, K_m and v_{max}.

Details of the methods employed for the analysis of tissue water and electrolytes were given previously[13]. Water was determined gravimetrically from the difference between tissue wet and dry wt. The dry tissue was extracted overnight with 0.1 M HNO_3 (see ref. 14) and in the suitably diluted supernatant tissue cations were analy-

zed by atomic absorption spectrometry (Evans Electroselenium, Halstead, Great Britain, Model 140). Results are expressed in kg water or mequiv/kg dry wt. As indicated earlier[3], the steady-state values of tissue water usually found in standard Na^+-saline (*i.e.* 3 kg water per kg dry wt.) were not affected by the absence of saline Na^+; the same held true for Cl^--free media or other saline modifications employed here. From the data obtained the apparent intracellular cation concentrations (subscript i) were calculated after correction for the extracellular tissue compartment (25 % of tissue wet wt).

From the data of $^{36}Cl^-$ experiments, $[^{36}Cl^-]_i$ and the Donnan ratio, $[^{36}Cl^-]_o/[^{36}Cl^-]_i$ were calculated. It has been shown[15] that within experimental error the steady-state Nernst diffusion potential for $^{36}Cl^-$, *i.e.* $-58\log [^{36}Cl^-]_o/[^{36}Cl^-]_i$, is equal to the directly measured membrane potential of renal tubular cells.

Typical experiments are presented. Each reported value was the mean of at least three analyses (agreeing $\pm$ 5 %); where more determinations were carried out using at least two animals, means $\pm$ S.E. are given.

MATERIALS

D-[1-^{14}C]Galactose, α-methyl-D-[1-^{14}C]glucoside, ^{22}NaCl and H^{36}Cl were purchased from New England Nuclear Corp., Boston and Calbiochem, Los Angeles. 2-Deoxy-D-glucose and 2-deoxy-D-galactose were obtained from Sigma Chemical Co., St. Louis, Mo.: ethane sulfonic acid from K. and K. Laboratories, Plainview, N.Y. All other reagents were commercial preparations of analytical grade.

RESULTS

Na^+ and the monosaccharide transport

It has been shown previously[3] that external Na^+ decreases the v_{max} of the α-methyl-D-glucoside transport without affecting the transport K_m. The same type of response was now obtained for the transport of D-galactose (Fig. 1). The active sugar transport system in renal tubular cells is thus similar in this respect to the rabbit intestinal mucosa[16] and differs from that of the hamster intestine[17].

It was of interest to examine whether a saturation of the D-galactose and α-methyl-D-glucoside transport system by Na^+ could be achieved. Such saturation would be expected if a ternary complex monosaccharide–carrier–Na^+ were formed as postulated for the mechanism of the Na^+ dependence of sugar transport in the intestinal mucosa[2,5]. Fig. 2 shows the results of such an investigation. It will be seen that increasing from 0 to 125 mM failed to produce a saturation of the Na^+ requirement of galactose and α-methylglucoside transport. Essentially the same relationships were obtained when the rates of sugar transport were plotted against the apparent intracellular Na^+ concentrations determined in separate experiments (details not given here). The marked differences in the response of both sugars to $[Na^+]_o$ may reflect differences in their transport pathways[7]. It should be recalled here that a lack of saturation of the Na^+ requirement was also reported for the transport of amino acids in kidney cortex slices[18].

Two additional points arising from these experiments should be noted. First, the dependence of the sugar transport on $[Na^+]_o$ did not differ whether Li^+ or $Tris^+$

was used to replace Na^+. Thus, both cations appear to be innocuous for the transport system. Secondly, at equal external concentrations of both sugars (1 mM) at $[Na^+]_0 = 0$, the rate of α-methyl-D-glucoside transport was considerably lower than that of D-galactose; in fact, even during a 30-min incubation a slight but significant accumulation against the concentration gradient of galactose in the absence of external Na^+ took place. Such an observation was made repeatedly, suggesting that a portion of the D-galactose transport is Na^+ independent. This finding differs from an earlier report and may be the result of improved analytical techniques.

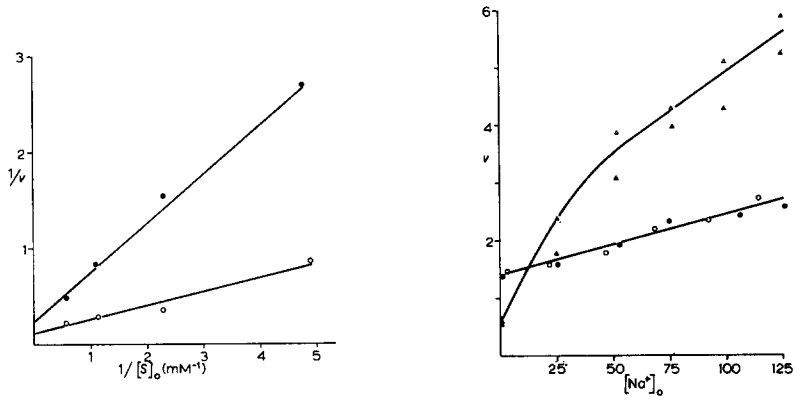


Fig. 1. Lineweaver–Burk plot of the effect of $[Na^+]_0$ on the rate of D-galactose transport into renal tubular cells. Slices were incubated aerobically (O_2) for 30 min at 25° in saline containing various concentrations of D-galactose. Each point is the mean of three analyses. Transport rate, v, is expressed in μmoles per g cell water per 30 min. Apparent kinetic parameters: ○, $[Na^+]_0$ 128 mM: K_m 1.05 mM; v_{max} 14 μmoles per g cell water per h; $[Na^+]_0$ 0 (Tris$^+$-saline). ●, v_{max} 8.7 μmoles per g cell water per h.

Fig. 2. Effect of $[Na^+]_0$ on the rate of sugar transport into renal tubular cells Slices were first leached, then aerobically (O_2) preincubated (45 min) and incubated 30 min in isotonic salines of varying $[Na^+]_0$ containing 1 mM D-galactose (○,●) or α-methyl-D-glucoside (△,▲). Cations replacing Na^+: Li^+ (○,△), Tris$^+$ (●,▲). The transport rate, v, is expressed in μmoles per g cell water per 30 min. Each point is the mean of three analyses.

Independent evidence demonstrating that a portion of the D-galactose transport into renal tubular cells is Na^+ independent was obtained when comparing the effect of ouabain on the rate of transport of α-methyl-D-glucoside and D-galactose (Table I). It will be seen that the transport of α-methyl-D-glucoside was inhibited 50 % by the presence of 0.05 mM ouabain, whereas even 2 mM glycoside did not produce more than a 30 % inhibition of galactose transport; 0.3 mM ouabain was found to be sufficient to bring about a maximal inhibition of Na^+-transport in rabbit kidney cortex slices[19].

A hypothesis for the mechanism of the Na^+ dependence of sugar transport in intestinal cells was advanced[5] suggesting a coupling of fluxes of Na^+ and sugar, thereby producing the observed increase of the potential difference across the intestinal mucosa and of the Na^+ short-circuit current. Similar experiments to test rigorously whether such cotransport of Na^+ and sugar exists also in the kidney would be feasible only using isolated perfused kidney tubules[20]. At first view, an alternative experimental approach might also be envisaged, *i.e.* following the effect of sugar influx on the steady-state efflux of Na^+ from $^{22}Na^+$-loaded tissue. However,

TABLE I

EFFECT OF OUABAIN ON THE RATE OF α-METHYL-D-GLUCOSIDE AND D-GALACTOSE TRANSPORT INTO RENAL CORTEX CELLS

Slices were first preincubated aerobically (O_2) at 25° for 45 min in Na^+-saline, then incubated aerobically for 30 min in the presence of 1 mM α-methyl-D-glucoside or D-galactose and various concentrations of ouabain. Values of v are the means of four analyses.

Ouabain concn. (mM)	*Rate of sugar transport (μmoles/g cell water per 0.5 h)*	
	α-Methyl-D-glucoside	*D-Galactose*
0	4.67	4.35
0.05	2.28	4.10
0.1	2.21	3.93
0.5	1.73	3.51
1.0	1.50	3.13
2.0		2.92

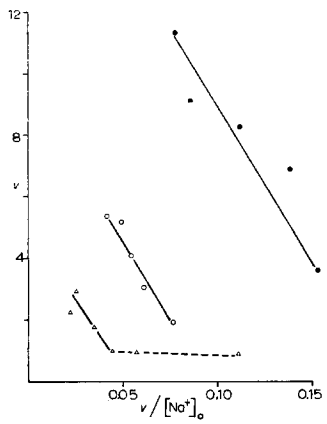


Fig. 3. Effect of $[Na^+]_0$ on the rate of α-methyl-D-glucoside transport into kidney cortex cells. Slices were first leached for 2.5 h in ice-cold Li^+-saline, then aerobically (O_2) preincubated 45 min in salines of varying $[Na^+]_0$ (0–128 mM) and incubated 30 min in these salines containing α-methyl-D-glucoside: △, 0.5 mM; ○, 1 mM; ●, 4 mM. Ordinate: v (μmoles α-methyl-D-glucoside per g cell water per 0.5 h); abscissa: $v/[Na^+]_0$. All values are means of three determinations.

the quantitative relationship between sugar and Na^+ fluxes has to be considered here: The efflux of $^{22}Na^+$ is two orders of magnitude faster than the influx of sugar (*cf.* the rate constants given for the Na^+ efflux (ref. 10) with those for sugars, given below); thus, a significant effect of sugar influx on the rate constants of $^{22}Na^+$ efflux might be demonstrable only if the coupling between Na^+ and sugar transport exceeded a ratio of about 5 Na^+/sugar. No marked effects of α-methyl-D-glucoside or D-galactose influx on the steady-state $^{22}Na^+$ efflux were observed in such experiments.

Evidence for a low molar ratio of Na^+ and sugar coupling was obtained using the approach of VIDAVER[22] and WHEELER *et al.*[21]: Replotting the data given in Fig. 2 using the ratio $v/[Na^+]_0$ on the abscissa, a linear relationship was obtained. Fig. 3 shows such a plot for several α-methyl-D-glucoside concentrations. It will be seen

that for the range of $[Na^+]_0$ between 25 and 128 mM a reasonably linear relationship holds; at lower $[Na^+]_0$, $v/[Na^+]_0$ rapidly increases. It may thus be concluded that in the range of $[Na^+]_0$ 25–128 mM, α-methyl-D-glucoside and Na^+ participate in the rate-limiting transport step at a molar ratio 1:1.

K^+ and the monosaccharide transport

It has been reported briefly[1] that, in addition to Na^+, K^+ also stimulated the accumulation of D-galactose in renal tubular cells. The effect of K^+ on the monosaccharide transport was now investigated in more detail.

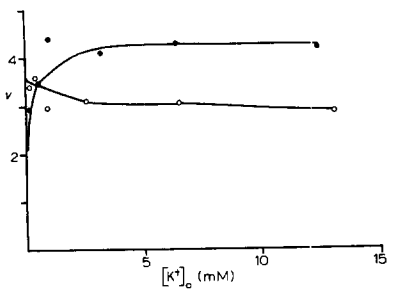
Table II shows that in the absence of external K^+ the transport of α-methyl-D-glucoside and D-galactose was significantly depressed, whereas the Na^+-independent transport of both 2-deoxyhexoses was not influenced. A kinetic study (Fig. 4) showed that in the absence of external K^+ the v_{max} rather than the K_m of D-galactose transport was affected; a further increase of $[K^+]_0$ from 6 to 15 mM did not influence the sugar influx. The stimulating effect of K^+ on the transport of α-methyl-D-glucoside was saturated at about 1 mM, as shown in Fig. 5, whereas the transport of

TABLE II

EFFECT OF K^+ ON THE TRANSPORT OF MONOSACCHARIDES IN KIDNEY CORTEX CELLS

Slices were first leached for 2.5 h at 0° in standard (6.7 mM K^+) and K^+-free salines, then preincubated 45 min at 25° aerobically (O_2) in the respective salines and subsequently incubated 30 min under identical conditions in salines containing 1 mM monosaccharides. Means ± S. E. (eight to twelve analyses, three animals) are given.

Monosaccharide	*Rate of sugar transport (μmoles per g cell water per 0.5 h)*	
	Standard saline	*K^+-free saline*
α-Methyl-D-glucoside	3.92 ± 0.32	2.10 ± 0.06
D-Galactose	2.55 ± 0.16	1.72 ± 0.09
2-Deoxy-D-glucose	2.96 ± 0.11	2.53 ± 0.09
2-Deoxy-D-galactose	4.19 ± 0.22	4.40 ± 0.10



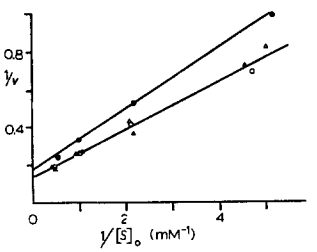


Fig. 4. Effect of $[K^+]_0$ on the rate of sugar transport into kidney cortex cells. Slices were first leached 2.5 h in ice-cold K^+-free saline, then aerobically (O_2) preincubated 45 min at 25° in salines of varying $[K^+]_0$ and subsequently incubated 30 min in identical salines containing 1 mM α-methyl-D-glucoside (●) or 2-deoxy-D-galactose (○). v is expressed in μmoles per g cell water per 0.5 h.

Fig. 5. Lineweaver–Burk plot of the effect of $[K^+]_0$ on the transport of D-galactose into kidney cortex cells. Slices were leached, preincubated and incubated as described in legend to Fig. 4. $[K^+]_0$, (mM): ●, 0; ○, 6.5; △, 14.7; ▲, 29.3. Apparent kinetic parameters: ○, △, ▲: K_m 0.85 mM, v_{max} 11 μmoles per g cell water per h; ●, v_{max} 15 μmoles per g cell water per h.

2-deoxy-D-galactose was not markedly affected by variations of $[K^+]_0$ between 0 and 14 mM. An increase of saline K^+ above 15 mM produced a marked decrease in the rate of transport of both α-methyl-D-glucoside and 2-deoxy-D-galactose, (details are not given here) in agreement with the previously shown depressing effect of higher $[K^+]_0$ on galactose accumulation[1]. An inhibitory effect of higher saline K^+ has been observed for a variety of metabolic processes (see, *e.g.* ref. 23) and may be related to the marked cellular swelling observed under such conditions[14].

The results presented here show that the Na^+-dependent transport of α-methyl-D-glucoside and D-galactose differs from the Na^+-independent by low concentrations transport of 2-deoxyhexoses by the stimulating effect of 1–6 mM $[K^+]_0$.

Variations of saline K^+ greatly affect the membrane potential. In particular, on incubation of kidney cortex slices in K^+-free saline depolarized cells high in Na^+ and low in K^+ are obtained[6]. The above observation that the absence of external K^+ did not affect the active accumulation of 2-deoxyhexoses thus indicates that at least for the transport system of these sugars the membrane potential does not play a role.

TABLE III

EFFECT OF Cl^- ON THE TRANSPORT OF MONOSACCHARIDES IN KIDNEY CORTEX CELLS

Slices were first leached for 2.5 h at 0° in standard (144 mM Cl^-), and Cl^--free salines, then preincubated 45 min at 25° aerobically (O_2) in the respective salines and subsequently incubated 30 min under identical conditions in salines containing 1 mM monosaccharides. Means ± S. E. (five analyses) are given.

Monosaccharide	*Rate of sugar transport (μmoles per g cell water per 0.5 h)*	
	Standard saline	*Cl^--free saline*
α-Methyl-D-glucoside	5.90 ± 0.79	5.91 ± 0.48
D-Galactose	3.07 ± 0.11	3.04 ± 0.12
2-Deoxy-D-glucose	3.82 ± 0.25	4.50 ± 0.11
2-Deoxy-D-galactose	4.49 ± 0.20	4.86 ± 0.12

TABLE IV

EFFECT OF Ca^{2+} ON THE TRANSPORT OF MONOSACCHARIDES IN KIDNEY CORTEX CELLS

Slices were first leached for 2.5 h at 0° in standard 2.7 mM Ca^{2+} and Ca^{2+}-free salines, then preincubated 45 min at 25° aerobically (O_2) in the respective salines and subsequently incubated 30 min under identical conditions in salines containing 1 mM monosaccharides. Means ± S.E. (eight to twelve analyses, three animals) are given.

Monosaccharides	*Rate of sugar transport (μmoles per g cell water per 0.5 h)*	
	Standard saline	*Ca^{2+}-free saline*
α-Methyl-D-glucoside	4.74 ± 0.25	1.90 ± 0.07
D-Galactose	4.07 ± 0.16	1.71 ± 0.18
2-Deoxy-D-glucose	5.15 ± 0.18	3.62 ± 0.19
2-Deoxy-D-galactose	4.05 ± 0.42	3.20 ± 0.22

Cl^- and the monosaccharide transport

The effect of saline Cl^- on the sugar transport was investigated in view of the report[25] that in the turtle intestine the transport of amino acids is linked to the Cl^-, rather than cation, transport. As shown in Table III, the absence of external Cl^- had no significant effect on the transport of all four sugars tested.

Ca^{2+} and the monosaccharide transport

Preliminary experiments showed that, as compared with controls (2.9 mM Ca^{2+}), the absence of saline Ca^{2+} greatly depressed the accumulation of monosaccharides. Fig. 6 shows the result of such an experiment using D-galactose. An identical response was obtained when the effect of saline Ca^{2+} on the transport of 2-deoxy-D-galactose was tested (details not shown here).

It is known[26] that leaching of kidney cortex slices in Ca^{2+}-free salines impoverishes the tissue of a major portion of its Ca^{2+}; this loss can be accelerated by the presence of 0.1 mM EDTA in the leaching saline. On aerobic incubation of the slices in salines containing Ca^{2+} the tissue again takes up this alkaline earth. In the absence of Ca^{2+}, depolarized cells high in Na^+ and low in K^+ are obtained[27]. Thus, an analysis of the effect of Ca^{2+} should be directed to elucidate whether the observed phenomena are directly related to saline Ca^{2+} or, possibly, indirectly reflect effects of Ca^{2+} on the ionic distribution of the cells.

It was first established that the described effect of Ca^{2+} could be demonstrated for all four sugars tested (Table IV).

Fig. 6 indicated that both the rate of sugar transport and also the near steady-state accumulation of sugar was affected by saline Ca^{2+}. Such a result might be produced (a) by an effect of Ca^{2+} on the influx; (b) by Ca^{2+} affecting the sugar efflux from the cells and (c) by a combination of both effects.

Fig. 7 shows that saline Ca^{2+} affects the influx of both D-galactose and 2-deoxy-D-galactose; the stimulation of the sugar transport by Ca^{2+} appeared to be saturated

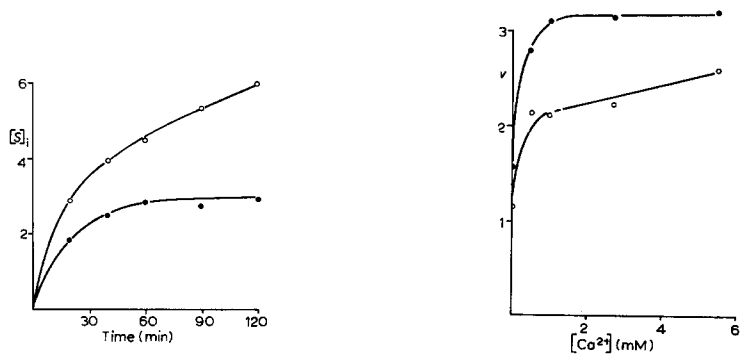


Fig. 6. Effect of $[Ca^{2+}]_0$ on the influx of D-galactose into kidney cortex cells. Slices were first leached in Ca^{2+}-free saline (*plus* 0.1 mM EDTA) for 2.5 h (0°), preincubated aerobically (O_2) 45 min at 25° in salines with 2.9 mM Ca^{2+} (control, ○) and without Ca^{2+} (0.1 mM EDTA present, ●) and then incubated in identical salines containing 1 mM D-galactose.

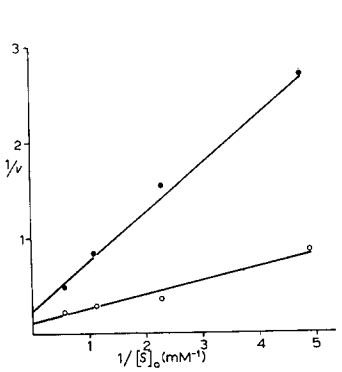
Fig. 7. Effect of $[Ca^{2+}]_0$ on the rate of sugar transport into kidney cortex cells. Slices were leached and preincubated (see legend to Fig. 6), then incubated aerobically (O_2) 30 min in salines of varying $[Ca^{2+}]_0$ containing 1 mM D-galactose (○) or 2-deoxy-D-galactose (●). v: μmoles per g cell water per 0.5 h.

at 0.5 mM Ca^{2+}. The observation that even at $[Ca^{2+}] = 0$ a marked sugar influx takes place may be due to the fact that prolonged leaching and preincubation of the tissue in Ca^{2+}-free salines in the presence of 0.1 mM EDTA fails to remove a sizeable portion of tissue Ca^{2+} (ref. 26). It may thus be inferred that at the described experimental conditions, sufficient Ca^{2+} remained at the site of action to allow some sugar transport to take place.

Fig. 8 shows that, as compared with the control (2.9 mM Ca^{2+}), the absence of saline Ca^{2+} affected the v_{max} of D-galactose transport, rather than the K_m. A similar result was obtained when the effect of Ca^{2+} on the apparent kinetic parameters of 2-deoxy-D-galactose transport was studied and the following values were obtained: control (with 2.9 mM Ca^{2+}): K_m 2.2 mM, v_{max} 40 μmoles per g cell water per h; Ca^{2+}-free saline (containing 0.1 mM EDTA): K_m 2.3 mM, v_{max} 20 μmoles per g cell water per h.

The effect of Ca^{2+} on the sugar efflux was next investigated. Tissue slices were first loaded with the appropriate sugars by incubation in the basic medium (2.9 mM Ca^{2+}) and the wash-out of the sugars was then followed using either the standard saline (control) or Ca^{2+}-free saline containing 0.1 mM EDTA to accelerate the Ca^{2+} loss from the tissue. The result shown in Fig. 9 demonstrates that as compared with the controls, the absence of saline Ca^{2+} increased the efflux of D-galactose. A similar result was obtained with 2-deoxy-D-galactose. Thus, Ca^{2+} affects the efflux of sugars in a similar way as that of a variety of other substances.

Taking the net uptake of D-galactose by the tissue in 60 min, obtained by numerical integration of data shown in Fig. 6, it was found that the presence of Ca^{2+} brought about a 36% increase of sugar transport; this figure should be compared with the integrated efflux data (Fig. 9) where the presence of Ca^{2+} depressed



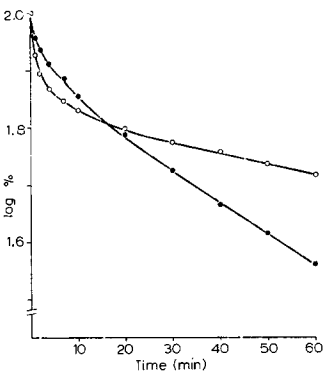


Fig. 8 Lineweaver–Burk plot of the effect of $[Ca^{2+}]_0$ on the transport of D-galactose into kidney cortex cells. Leaching and preincubation of the tissue was carried out as described in the legend to Fig. 6. Slices were then aerobically (O_2) incubated 30 min in salines containing 1 mM D-galactose with 2.9 mM Ca^{2+} (○, control) and without (●) Ca^{2+} (0.1 mM EDTA present). v: μmoles per g cell water per 0.5 h. Apparent kinetic parameters: ○, K_m 0.85 mM, v_{max} 17 μmoles per g cell water per 0.5 h; ●, K_m 0.95 mM, v_{max} 11 μmoles per g cell water per 0.5 h.

Fig. 9. Effect of $[Ca^{2+}]_0$ on the efflux of D-galactose from kidney cortex slices. Slices were first loaded with D-[^{14}C]galactose by aerobic incubation for 90 min at 25° in standard saline containing 1 mM D-galactose. The blotted slices ($[S_1]/[S]_0$ 4.2) were placed into wash-out tubes and the efflux of the sugar into two series of tubes, each containing 10 ml sugar-free saline with 2.9 mM Ca^{2+} (○, control) and without Ca^{2+} (●, 0.1 mM EDTA present) was followed. Ordinate: log % of initial tissue activity.

the loss of D-galactose only by 8.5 %. The above set of experiments thus demonstrates that Ca^{2+} produces its effect on the steady-state level of sugar accumulation by both (a) increasing the influx and (b) decreasing the efflux.

Several aspects of the above efflux experiments are noteworthy. First, a greater number of experimental points in the first min of the experiment allowed a more detailed analysis of the efflux curve then possible hitherto (*cf.* ref. 11): The graphic compartmental analysis yielded three transport components, including a compartment with a fast rate constant (mean: $k'_2 = 0.94$ min^{-1}), suspected previously. This fast component can be ascribed to efflux from the extracellular space, as indicated by the following consideration: From Fig. 10, the computed space for the fastest efflux component was 14.8 %; the tissue content of D-galactose after loading (final $[S]_0$: 0.58 mM) was 1.37 μmoles/g, corresponding to an $[S]_i/[S]_0$ of 4.31. The fastest kinetic component thus corresponded to $0.148 \times 1.37 = 0.202$ μmole galactose per g tissue. This value should be compared with that calculated on the basis of the known extracellular (inulin) space, *i.e.* 25 % tissue wet wt. (assuming that the concentration of the sugar in the extracellular space equals that in the medium): 0.25×0.58 μmole/g $= 0.145$ μmole D-galactose per g. In view of the derived nature of the fast kinetic component, the agreement between both values is satisfactory.

Secondly, it will be noted that the efflux of D-galactose (and also of 2-deoxy-D-galactose) in the controls proceeded somewhat faster in the first 5 min than in the absence of Ca^{2+}. This repeatedly observed phenomenon may be related to net ionic fluxes taking place when slices previously incubated in standard saline are transferred into Ca^{2+}-free media; it has been shown that the associated changes of the membrane potential take about 5 min before a new steady state is reached[27].

The specificity of the described effect of Ca^{2+} on the sugar transport was then investigated. First, it was of interest to see whether the effect of Ca^{2+} could be demonstrated also in the absence of external Na^+. The results of such experiments are

TABLE V

EFFECT OF Ca^{2+} ON THE ACCUMULATION OF SUGARS IN KIDNEY CORTEX CELLS: ROLE OF $[Na^+]_0$, REVERSIBILITY

Slices were first leached 2.5 h in ice-cold salines, then preincubated aerobically at 25° 45 min and incubated 60 min in salines containing 1 mM D-galactose or 2-deoxy-D-galactose. All Ca^{2+}-free salines contained 0.1 mM EDTA. Values of $[S]_i/[S]_0$ are the means of three analyses, or means ± S.E. ($n = 10$, two animals).

Expt. No.	*Sugar*	*Leaching and preincubation saline*		*Incubation saline*		$[S]_i/[S]_0$ *ratio*
		Bulk	$[Ca^{2+}]_0$ *(mM)*	*Bulk*	$[Ca^{2+}]_0$ *(mM)*	
1	D-Galactose	$Tris^+$	0	$Tris^+$	2.9	2.24 ± 0.11
		$Tris^+$	0	$Tris^+$	0	1.57 ± 0.07
2	2-Deoxy-D-galactose	Na^+	2.9	Na^+	2.9	9.1
		Na^+	0	Na^+	0	3.5
		Na^+	0	Na^+	2.9	8.9
		$Tris^+$	2.9	$Tris^+$	2.9	9.2
		$Tris^+$	0	$Tris^+$	0	4.8
		$Tris^+$	0	$Tris^+$	2.9	7.5

presented in Table V. It will be seen that the slight active transport of D-galactose in Na^+-free saline was significantly decreased when Ca^{2+} was omitted from the medium. The same type of response was obtained for 2-deoxy-D-galactose (Expt. 2). The results of this experiment also demonstrate the reversibility of the Ca^{2+} effect: A portion of the slices was first leached and preincubated in Ca^{2+}-free saline (containing 0.1 mM EDTA) and subsequently incubated in standard saline containing sugar *plus* 2.9 mM Ca^{2+}. It will be noted that under these conditions the $[S]_i/[S]_0$ nearly reached values of the controls where the tissue was not improverished of Ca^{2+} before the sugar accumulation was tested. It may thus be concluded that the Ca^{2+} effect on the sugar transport is not related to the presence of external Na^+. Furthermore, the reversibility of the Ca^{2+} effect on the sugar transport indicates that this is not due to some nonspecific tissue damage produced by incubating the slices in Ca^{2+}-free salines.

Sr^{2+} and Ba^{2+} could replace Ca^{2+} on its affect on the accumulation of both D-galactose and 2-deoxy-D-galactose (Table VI, Expt. 1). It may be noted that, as compared with Ca^{2+}, the effect of the mentioned alkaline earths at the lower

TABLE VI

SPECIFICITY OF THE Ca^{2+} EFFECT ON THE ACCUMULATION OF 2-DEOXY-D-GALATOSE AND D-GALACTOSE

Slices were first leached 2.5 h in ice-cold Ca^{2+}-free saline, then preincubated and incubated in the presence of the tested sugars (1 mM) for 60 min in modified media which differed from standard saline only with respect to those constituents shown in Columns 1 and 2. In Expt. 1, a SO_4^{2-}-free saline was used, $MgSO_4$ being replaced by $MgCl_2$. All values of $[S]_i/[S]_0$ are the means of at least four analyses.

Expt. No.	*Saline Ca^{2+} concn. (mM)*	*Additions to, or omissions from saline*	$[S]_i/[S]_0$ *ratio*	
			2-Deoxy-D-galactose	*D-Galactose*
1	0	—	3.4	1.7
	0.5	—	7.7	4.4
	2.7	—	8.0	5.1
	0	0.57 mM Sr^{2+}	5.9	2.7
	0	2.7 mM Sr^{2+}	8.1	5.1
	0	0.57 mM Ba^{2+}	3.6	2.2
	0	2.7 mM Ba^{2+}	8.7	4.6
2	0	—	3.1	3.3
	2.7	—	6.8	5.6
	0	4.8 mM Mg^{2+}	3.2	3.5
3	0	—	3.1	2.1
	2.7	—	7.4	4.1
	2.7	0 mM Mg^{2+}	7.3	3.6
	2.7	0 mM HPO_4^{2-}	5.1	3.4
4	0	—	2.7	2.3
	2.7	—	8.2	3.6
	0	1.35 mM Mn^{2+}	6.6	2.0
	0	2.7 mM Mn^{2+}	7.8	1.8
5	0	—	2.4	
	2.7	—	5.2	
	0	0.01 mM La^{3+}	2.1	
	0	0.05 mM La^{3+}	1.8	
	0	0.1 mM La^{3+}	2.0	

concentration of 0.5 mM was smaller. On the other hand, even increased concentrations of Mg^{2+} could not replace Ca^{2+}. The absence of Mg^{2+} or phosphate from the saline did not markedly affect the accumulation of both sugars. La^{3+}, which has been described as a super-calcium in its effect on the squid axon[28], also could not replace Ca^{2+}. Curiously, Mn^{2+} was able to replace Ca^{2+} in its effect on the accumulation of 2-deoxy-D-galactose but had no effect on the transport of D-galactose.

The results presented in Table VI allow the conclusion that the Ca^{2+} effect on the sugar transport is fairly specific for this alkaline earth and may represent a direct stimulation of one of the steps in the active transport process. Moreover, in view of the observation that both the Na^+-dependent transport of D-galactose and α-methyl-D-glucoside and the Na^+-independent accumulation of 2-deoxyhexoses were equally dependent on saline Ca^{2+}, it may be inferred that both transport processes share such a Ca^{2+}-stimulated step.

DISCUSSION

Based on results reported previously[3,6] and in the present communication, two categories of active sugar transport in cells of rabbit kidney cortex are readily discerned: (a) The active transport of 2-deoxyhexoses which is independent of external (and internal) Na^+ as well as of external K^+. (b) The active, Na^+-requiring transport of hexoses with a hydrophilic (–OH or $–NH_2$) group on C-2, C-3–OH and C-6–OH; using α-methyl-D-glucoside and D-galactose as models, this transport system was shown to be stimulated by K^+.

Both sugar transport systems were shown to be stimulated by Ca^{2+}.

Two major hypotheses have been proposed to explain the observed Na^+ requirement for the active sugar of a variety of nonelectrolytes in several animal cells, particularly for the intestinal mucosa where the Na^+ dependence was found to be nearly absolute (see also ref. 5). (1) According to CSÁKY[4], Na^+ is required to activate a metabolic system directly providing energy for the active transport system for nonelectrolytes. The hypothesis predicts that a decrease of the intracellular Na^+ should depress the active nonelectrolyte transport; at $[Na^+]_i$ near 0, only a transport of the facilitated diffusion type could take place. (2) The Na^+-gradient hypothesis[2] has more recently received greater attention[5,29]. Here the energy for the active nonelectrolyte (sugar) transport is derived from the asymmetric distribution of primarily Na^+ across the cell membrane; this asymmetry is brought about by a coupling of the Na^+ pump with the energy-providing metabolic process. A basic postulate for this hypothesis is the intermediary formation of a ternary complex carrier–sugar–Na^+, the mobility of which favors the ferrying of a sugar molecule across the (luminal) membrane of (intestinal) epithelial cells in a down-hill gradient of Na^+. The kinetic predictions of this hypothesis have been extensively analyzed (see also ref. 30) and various models have accomodated apparently divergent results: while in hamster intestinal epithelium Na^+ affects the affinity of the carrier for the transported sugar, in rabbit ileum the mobility of the ternary complex (*i.e.* v_{max}) is involved.

The possible mechanism(s) involved in the active transport of sugars in kidney cortex cells will now be considered.

The Na^+-independent sugar transport system

From the above summary it is apparent that the Na^+-independent (and ouabain-insensitive) transport of 2-deoxyhexoses cannot be readily accomodated within the framework of either of the hypotheses mentioned above: Both 2-deoxy-D-glucoside and 2-deoxy-D-galactose were found to be accumulated in kidney cortex cells against high concentration gradients in the absence of external Na^+ (and at a $[Na^+]_i < 3$ mM). The data shown here also appear to exclude some other possible ionic fluxes being responsible for the active, metabolically dependent transport of 2-deoxysugars: (a) K^+ flux: Indications of an active K^+ transport system, independent of Na^+, have been obtained for kidney cortex cells[15,31]; however, the absence of external K^+ failed to inhibit the influx of both 2-deoxy-D-glucose and 2-deoxy-D-galactose (Table II, Fig. 4). (b) The small electrochemical gradient of Li^+ across the membrane of kidney cortex cells[31] could hardly be responsible for the 2-deoxyhexose accumulation, particularly since the sugar transport was identical in Li^+-, $Tris^+$- and $choline^+$-salines (Fig. 2, ref. 3). (c) No evidence for an active transport of Cl^- has been obtained in kidney cortex cells[15]; data shown in Table III demonstrate that the 2-deoxyhexose transport was not affected by the absence of Cl^-.

The possibility of a cotransport between Ca^{2+} and sugars cannot be at present excluded in view of the saturable stimulation of sugar influx by Ca^{2+} (Table IV, Fig. 7); kidney cortex cells are capable of actively transporting Ca^{2+} (ref. 26).

The simplest explanation of the Na^+-independent sugar transport would be a direct coupling between a source of metabolic energy and the transport mechanism (carrier) for 2-deoxyhexoses. In view of differences in the responses of 2-deoxy-D-glucose and 2-deoxy-D-galactose transport[31] to changes of pH, two such carrier systems may have to be postulated. The observed[3,6] competive inhibition of 2-deoxy-D-galactose transport by D-galactose might then be readily explained by the finding (Table I) that a significant portion of D-galactose transport is independent of external Na^+.

It should be recalled here that a Na^+-independent active sugar transport has been described[32] in chorioid plexus epithelium and also in bacteria[33]. The kidney cells thus do not represent an exception.

The Na^+-dependent sugar transport system

An inspection of the results reported here reveals the following characteristics of the Na^+-requiring transport system for α-methyl-D-glucoside and D-galactose in rabbit kidney cortex cells: (a) The apparent transport v_{max}, rather than the K_m, is affected by Na^+. (b) No saturation of the Na^+ requirement for transport was observed even at $[Na^+]_0 = 128$ mM, recalling a similar finding for the Na^+ dependence of α-aminoisobutyric acid transport in kidney cortex slices[18]. A saturation of the transport system by Na^+ at relatively low $[Na^+]_0$ would be expected if a ternary complex carrier–sugar–Na^+ were formed. (c) A stoichiometric relationship of 1 Na^+ per 1 sugar molecule describes the rate-limiting event of the transport system, at least for the range of $[Na^+]_0$ 25–128 mM; at lower $[Na^+]_0$, the relationship appeared to be looser as also observed in ascites tumour cells[21]. (d) K^+ stimulates only the Na^+-dependent sugar transport system. Such K^+ stimulation has also been observed for the Na^+-dependent sugar transport in intestinal cells[35]. (e) No Cl^- dependence of this transport system was found. (f) The Na^+-dependent sugar transport system

was stimulated by Ca^{2+} in the same way as the Na^+-independent transport.

The above characteristics might be compatible with a version of the Na^+-gradient hypothesis; some modification of the basic assumptions might also allow an explanation of the observation[7] that sugars not requiring Na^+ for their transport can to some extent competitively inhibit the Na^+-dependent transport system. However, one observation cannot be reconciled with the Na^+-gradient hypothesis: An increase of saline pH produced a marked increase of the Na^+-dependent fraction of D-galactose transport without affecting the α-methyl-D-glucoside accumulation[31]; the hypothesis would predict that changes in the Na^+ gradient (produced by pH) should bring about comparable changes in the sugar transport.

An alternative model might equally well fit the observed data (see ref. 6). The first event in the membrane would be an interaction (particulary at C-2–OH) between sugar and a protein the conformation of which would be poised by the presence of Na^+ held in place by an active transport system for Na^+; K^+ would then stimulate the latter system in a way similar to the stimulation of the membrane ATPase by K^+ (ref. 36). The second step would be the actual transport of the sugar against its concentration gradient, the energy being supplied by a direct coupling with a metabolic process. This second step would be similar to that for the Na^+-independent sugar transport system. This model would offer an explanation for the range of apparent overlapping of the specificities of sugar transport systems in kidney cortex cells, revealed by studies of competitive inhibition[7]; such competition could take place at three levels, *i.e.* at the first and second steps of the Na^+-dependent sugar transport system, as well as for the available source of metabolic energy.

Finally, the stimulating effect of Ca^{2+} (and some alkaline earths) on both sugar transport systems (Table VI) should be commented upon. The depolarizing effect produced by the absence of Ca^{2+} in the saline[27], can hardly be responsible for the observed phenomenon since Ca^{2+} also stimulated the sugar transport in Na^+-free salines. (Table V). The simplest explanation would be an effect of Ca^{2+} on a common step for both Na^+-dependent and Na^+-independent sugar transport, such as the source of metabolic energy. It should be noted that the absence of Ca^{2+} was shown to depress the Na^+-dependent transport of amino acids in kidney cortex slices[37], whereas in pigeon erythrocytes no effect of Ca^{2+} on glycine transport was observed[22].

ACKNOWLEDGMENTS

This investigation was started in the Department of Physiology, University of Rochester, School of Medicine, Rochester (U.S. Public Health Service Grant 1ROl AM 3601), and completed with the support of U.S. Public Health Service Grant No. 1 ROl AM 12619 which is gratefully acknowledged. The invaluable assistance of Miss A. H. Davis with many experiments is greatly appreciated. Thanks are also due to Dr. J. Kutschera, Medical School Computer Facility, University of Pennsylvania (National Institutes of Health Grant No. RR 15), for the computer evaluation of some flux data.

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