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KINETICS OF SUGAR TRANSPORT IN RABBIT KIDNEY CORTEX, *IN VITRO*: MOVEMENT OF D-GALACTOSE, 2-DEOXY-D-GALACTOSE AND α-METHYL-D-GLUCOSIDE*

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SUMMARY

The kinetic parameters of D-galactose, 2-deoxy-D-galactose and α-methyl-D-glucoside transport were studied in rabbit kidney cortex slices. The following observations were made.

1. A portion of the uphill transport of D-galactose does not require extracellular Na^+. In the presence of external 118 mM Na^+ the maximal rate of D-galactose transport was increased 2.5-fold, the apparent K_m of entry being unaffected. With regard to the Na^+ requirement, D-galactose transport appears to be intermediary between the practically Na^+-independent uphill transport of 2-deoxy-D-galactose and the Na^+-requiring uphill transport of α-methyl-D-glucoside.

2. 2-Deoxy-D-galactose is an inhibitor of D-galactose transport (and *vice-versa*) both in the presence and in the absence of Na^+. The respective K_i's are Na^+-independent.

3. The inhibition is of a partially competitive type, indicating that D-galactose and 2-deoxy-D-galactose share a common carrier with separate binding sites for each of the sugars.

4. D-Galactose is a competitive inhibitor of α-methyl-D-glucoside transport.

It may be concluded that the transport of the sugars examined by the kidney cortex cells is affected by a common carrier with several more or less independent binding sites, the Na^+ effect being more quantitative than qualitative in nature.

INTRODUCTION

It was reported from this laboratory[1] that accumulation of 2-deoxyhexoses, namely of 2-deoxy-D-glucose and 2-deoxy-D-galactose in kidney cortex slices does not require external Na^+ in contrast with the transport of some other monosaccharides. The question arose as to whether the feature of Na^+-independent accumulation of 2-deoxyhexoses is due to the existence of a specific carrier for the 2-deoxy sugar transport or a pumping mechanism of a wide specificity for sugars and a graded

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sensitivity to Na^+ which would transport any sugar into kidney cells. On the basis of steady-state sugar levels it could not be decided between these two possibilities. Na^+-independent accumulation of 2-deoxy-D-galactose and lack of effect of ouabain on it might indicate the former possibility, which is supported by the fact that accumulation of D-galactose is highly increased in the presence of Na^+ and completely inhibited by 0.1 mM ouabain[2]. The second alternative was based on experiments showing inhibition of 2-deoxy-D-galactose accumulation by D-galactose, both in the presence and in the absence of Na^+ (ref. 1). Interaction of transport of these two sugars was confirmed *in vivo* with rat renal tubules[3].

The present experiments with kidney slices were intended to examine the relationship between kinetic parameters of transport of D-galactose and its 2-deoxy analogue and the effect of Na^+ thereon. In some experiments α-methyl-D-glucoside whose transport characteristics were previously reported[1] served in this study as a model of sugar transport.

MATERIAL AND METHODS

Tissue incubation

Experiments were performed with free-hand cut slices of rabbit kidney cortex. The media used were based upon the Krebs–Henseleit bicarbonate saline (pH 7.4) with O_2–CO_2 (95:5, v/v) as the gaseous phase and with 10 mM acetate as substrate. Incubations were carried out at 25°. Na^+-free media and those with lower Na^+ concentrations were prepared by replacing NaCl with Tris–HCl, LiCl or KCl. Instead of $NaHCO_3$, Tris–HCO_3 of pH 7.4 was used. In experiments where the effect of external Na^+ on monosaccharide transport was studied a 30-min preincubation of the tissue in corresponding Na^+-deficient media with lithium acetate (10 mM) was used in order to minimize the amount of intracellular Na^+. It ought to be pointed out that in the preincubation procedure applied here, most of the tissue Na^+ is washed out in Na^+-free media within the first 15 min (from 90 to 15 mM); after further 15 min of preincubation in fresh Na^+-free media the residual intracellular Na^+ was about 4 mM. Preincubation for more than 30 min had no further effect, so that only a certain amount of bound Na^+ appears to remain in the tissue and can barely participate substantially in sugar transport.

At the end of the incubation the slices were separated from the incubation media, blotted and homogenized together with balanced solutions of $ZnSO_4$ and $Ba(OH)_2$. The usefulness of this deproteinization procedure has already been discussed before[1,4], the contamination of supernatants by metabolic products of galactose and 2-deoxygalactose being insignificant. The incubation media were processed essentially in the same way and thus the extracellular concentrations of sugars, $[S]_0$, after incubation were obtained.

In deproteinized supernatants ^{14}C-labelled D-galactose and α-methyl-D-glucoside were estimated in a Tracer-lab scintillation counter and 2-deoxy-D-galactose assayed colorimetrically by the periodate procedure[5] and corrected for the tissue blank as previously described[1].

From the results obtained the apparent intracellular concentration, $[S]_1$, was calculated after correction for extracellular space (250 ml/kg wet weight), described in detail in an earlier paper[6]. When a full K^+-medium served for tissue incubation the

extracellular space was decreased due to the tissue swelling in comparison with the control incubated in a Na^+ medium, a proper correction always being made for this.

The initial rate of sugar entry into the cells was determined as the amount entering during 10 or 15 min and it was expressed in μmoles/g intracellular water. The $[S]_i/[S]_0$ ratio indicated the degree of intracellular tissue accumulation.

Materials

D-[1-^{14}C]Galactose and[^{14}C]-α-methyl-D-glucoside (uniformly labeled) were obtained from the Radio-Chemical Centre, Amersham, England. 2-Deoxy-D-galactose was purchased from Koch-Light Laboratories, Colnbrook, England. Thiobarbituric acid for the determination of 2-deoxy-D-galactose was prepared in this laboratory by the method of Michael (1887). All other reagents were commercial preparations of A.R. grade.

RESULTS

Na^+ effect on the steady-state levels of D-galactose, 2-deoxy-D-galactose and α-methyl-D-glucoside

The data in Fig. 1 show that increasing concentrations of Na^+ in the incubation media affect the accumulation of the sugars studied to a widely differing extent. The minimal external concentration of Na^+ necessary for maximum accumulation is also different for different sugars. While at 40 mM Na^+ the process of D-galactose accumulation reaches its optimum, increasing medium concentrations of Na^+ up to 120 mM cause a gradual increase in α-methyl-D-glucoside uptake. Of the three sugars tested only α-methyl-D-glucoside is not accumulated against a concentration difference in Na^+-free media. Na^+-independent accumulation of D-galactose, not previously observed[2] is of interest in view of the fact that this sugar inhibits 2-deoxy-D-galactose

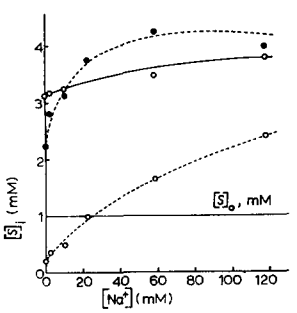

Fig. 1. Effect of external Na^+ on steady-state levels of D-galactose (●---●), 2-deoxy-D-galactose (○—○) and α-methyl-D-glucoside (○---○). Slices were preincubated in the Na^+-free Tris–HCl medium for 30 min with 10 mM lithium acetate and then transferred to the media with different concentrations of Na^+ containing 1 mM sugar and incubated for 60 min.

transport. Recent data of KLEINZELLER *et al.*[7] confirm the absence of an absolute Na^+ requirement for D-galactose transport. At variance with their observation, no transport against the concentration gradient was found for α-methyl-D-glucoside in different Na^+-free media (Table I).

On the basis of the above-mentioned characteristics the question may be asked whether the changes in transport of the three sugars under study reflect only Na^+

concentrations in the media and not the effect of the osmotic replacement of $Tris^+$ which was previously used for this purpose[1,6]. $Tris^+$ does not affect sugar transport in the hamster intestine[8]. It was established explicitly that the capacity of the tissue to transport sugars uphill is not at all affected by the 30-min preincubation generally employed to remove tissue Na^+. It was found to be safe to use $Tris^+$ as a replacement for Na^+ without significant alteration to the final results. Other possible compounds occasionally used for replacing Na^+ (mannitol or choline) were not employed in this study. As preliminary experiments suggested mannitol gave the same results as did $Tris^+$ for 2-deoxy-D-galactose uptake and choline was not used at all because it is transported and oxidized in the kidney, the oxidation being Na^+-dependent[9].

TABLE I

INFLUENCE OF IONIC COMPOSITION OF THE MEDIA ON D-GALACTOSE AND α-METHYL-D-GLUCOSIDE UPTAKE

Slices were incubated in the respective media containing 10 mM lithium acetate as substrate for 30 min and then transferred to the same media containing 1 mM D-galactose or α-methyl-D-glucoside for 60 min.

Medium	$[S]_i/[S]_0$	
	D-*Galactose*	*α-Methyl-D-glucoside*
Na^+	7.80	4.40
$Tris^+$	2.71	0.32
Li^+	3.20	0.19
K^+	2.94	0.46

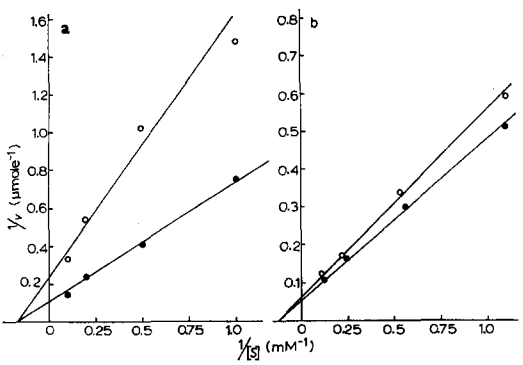

Fig. 2. (a) Lineweaver–Burk plot of the effect of external Na^+ on the rate of entry of D-galactose into kidney cortex cells. Initial velocity measured after 10-min incubation, is expressed in μmoles/g intracellular water. ●, $[Na^+]_0$ = 118 mM; ○, $[Na^+]_0$ = 0. (b) Lineweaver–Burk plot of the effect of external Na^+ on the rate of entry of 2-deoxy-D-galactose into kidney cortex cells. Experimental details were as given in the legend to (a).

Effect of Na^+ on v_{max} of sugar transport

Similarly to the effect of Na^+ on the kinetic parameters of α-methyl-D-glucoside entry into the kidney cells[1] the apparent K_m for both D-galactose and 2-deoxy-D-galactose is not influenced by Na^+ concentration of the medium (Figs. 2a, 2b); whereas the v_{max} of D-galactose transport decreased from 67 μmoles/g per h at

118 mM Na^+ to 27.3 μmoles/g per h at 0 Na^+, only a slight difference of v_{max} at the two extreme Na^+ concentrations having been observed for 2-deoxy-D-galactose entry.

Theoretically, the effect of an ion on the maximum velocity, with the affinity for the substrate remaining constant can be interpreted by assuming a combination of Na^+ with the carrier independently of sugar, and *vice versa*. Under such conditions when Na^+ does not directly interact with the substrate binding site it is less complex to examine, both in the presence and in the absence of Na^+, the competition between structurally related substrates leading usually to a change in the affinity of carrier for the sugar. Information on this point is presented in the next paragraph.

Mutual inhibition between D-*galactose and 2-deoxy-*D-*galactose transport*

Competitive inhibition

When the concentration of 2-deoxy-D-galactose was varied and D-galactose concentration was kept constant, the Lineweaver–Burk plot showed competitive inhibition of 2-deoxy-D-galactose transport (Fig. 3). When D-galactose served as a substrate at different concentrations 2-deoxy-D-galactose inhibited competitively D-galactose entry (Fig. 4). It ought to be noted that mutually competitive inhibition of these two sugars was observed also in the absence of Na^+, the K_i values being not appreciably different from those obtained after incubation in a full Na^+ medium. In all these experiments, the K_m of D-galactose was always higher than its K_i for inhibition of 2-deoxy-D-galactose entry, the K_m of 2-deoxy-D-galactose being lower than its K_i for inhibition of D-galactose (Table II).

Fully or partially competitive inhibition

When applying enzyme kinetics to membrane transport systems a fully competitive inhibitor binds to the same site of an enzyme as the substrate, while a partially competitive inhibitor to a site different from, but closely related to, the substrate binding site. Thus, in hamster intestine phlorizin was designated as a fully competitive inhibitor of sugar transport[10], D-galactose as a partially competitive inhibitor of transport of neutral amino acids[11,12]. One should be able to distinguish whether two

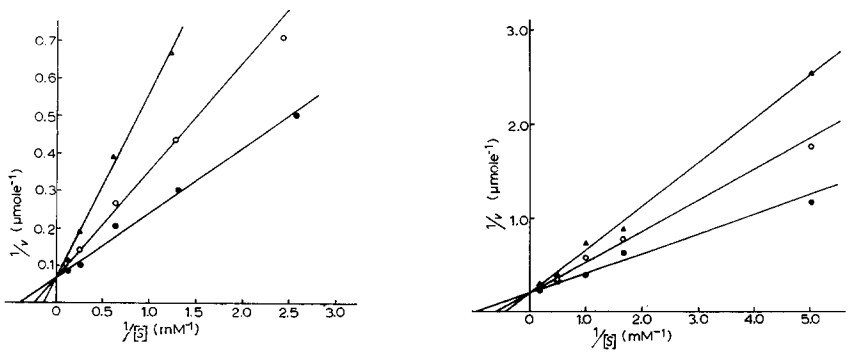

Fig. 3. Lineweaver–Burk plot of D-galactose inhibition of 2-deoxy-D-galactose entry. Kidney cortex slices were incubated for 15 min in Krebs–Henseleit bicarbonate medium. ●, control; ○, 0.2 mM D-galactose; △, 0.5 mM D-galactose.

Fig. 4. Lineweaver–Burk plot of 2-deoxy-D-galactose inhibition of D-galactose entry. Kidney cortex slices were incubated in Krebs–Henseleit bicarbonate medium for 15 min. ●, control; ○, 7.5 mM 2-deoxy-D-galactose; △, 15 mM 2-deoxy-D-galactose.

structurally similar sugars such as D-galactose and its 2-deoxy analogue, using apparently a common carrier (competitive type of inhibition), bind to the same or to different sites on it, by using variable concentrations of inhibitor plotted against v_{max}/v of the substrate according to THORN[13]. With this plot one should get a straight line for fully competitive inhibition. The experimental points, however, do not follow a straight line (Fig. 5). The theoretical curve given in the same figure was calculated

TABLE II

THE AVERAGE K_m AND K_i VALUES (mM) OF UPTAKE OF TESTED SUGARS AT 0 AND 118 mM Na^+

K_i values of D-galactose for inhibition of 2-deoxy-D-galactose entry were obtained with 0.5 mM sugar and those for α-methyl-D-glucoside inhibition with 10 mM sugar. The K_i of 2-deoxy-D-galactose was found with 15 mM sugar; the K_i of α-methyl-D-glucoside was found with 20 mM sugar.

Competing sugar	$[Na^+]$ (*mM*)	*Inhibited sugars*		
		D-*Galactose*	*2-Deoxy-D-galactose*	*α-Methyl-D-glucoside*
D-Galactose	0	1.3	0.32	—
	118	1.3	0.26	6.3
2-Deoxy-D-galactose	0	11.2	3.4	∞
	118	10.9	3.4	
α-Methyl-D-glucoside	0	100.0	∞	4.3
	118	115.0		4.3

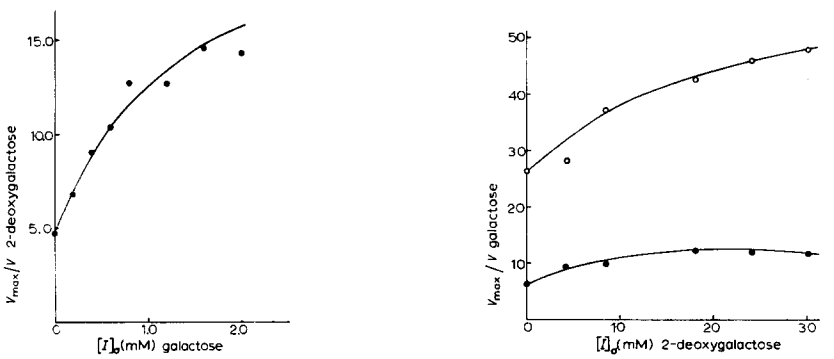

Fig. 5. Thorn plot of D-galactose inhibition of 2-deoxy-D-galactose uptake. Theoretical curve calculated from the equation of partially competitive inhibition

$$\frac{v_{max}}{v} = 1 + \frac{K_m}{[S]} \cdot \frac{1 + \frac{[I]}{K_i}}{1 + \frac{[I]}{K_i}\frac{K_m}{K_m'}}$$

where v_{max} is maximum rate, v initial rate, K_m the dissociation constant of the ES complex and K_m' the dissociation constant of the EIS complex. Initial concentration of 2-deoxy-D-galactose 1 mM. Incubation 10 min. ●, experimental points.

Fig. 6. Thorn plot of 2-deoxy-D-galactose inhibition of D-galactose uptake. Initial concentration of D-galactose 0.3 mM. Incubation 10 min. ●, at $[Na^+]_0 = 118$ mM; ○, at $[Na^+]_0 = 0$.

on the assumption of a partially competitive inhibition using the average K_i value and the average K_m/K_m' ratio calculated from the experimental points. The theoretical curve fits the experimental data satisfactorily.

Fig. 6 shows experimental points obtained for inhibition of D-galactose transport by 2-deoxy-D-galactose in both the presence and the absence of Na^+. On the basis of the results in Figs. 5 and 6, it appears justified to suggest that the above sugars act as partially competitive inhibitors.

Interaction between D-galactose, 2-deoxy-D-galactose and α-methyl-D-glucoside

A Lineweaver–Burk plot (Fig. 7) shows that D-galactose competitively inhibits α-methyl-D-glucoside uptake, the latter sugar being, however, much less an efficient inhibitor of galactose transport (*cf.* Table II, K_i of α-methyl-D-glucoside). As follows from Fig. 7 2-deoxy-D-galactose does not inhibit α-methyl-D-glucoside entry at concentrations 60 times higher than those of α-methyl-D-glucoside. The fact that the K_m values of 2-deoxy-D-galactose and α-methyl-D-glucoside do not substantially differ from each other would allow us to expect two different, possibly separated sites for attachment of these two sugars in the transport system.

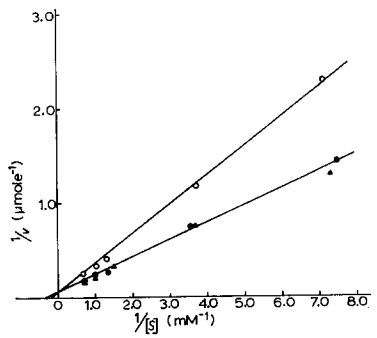

Fig. 7. Lineweaver–Burk plot of D-galactose and 2-deoxy-D-galactose inhibition of α-methyl-D-glucoside transport. Initial velocity measured after 10-min incubation. ●, control; ○, 10 mM D-galactose; △, 10 mM 2-deoxy-D-galactose.

DISCUSSION

Two salient points emerge from the experimental data reported above.

First, galactose displays a partially competitive effect towards 2-deoxygalactose and probably α-methylglucoside during their active transport into the kidney cell.

Second, external Na^+ increases the maximum rate of uptake of the three sugars in different proportions, ranging from a practically negligible effect on 2-deoxygalactose to a virtually essential requirement for α-methylglucoside. The apparent K_m's of the sugars are not appreciably affected by Na^+.

Let us deal now with these points in turn. The luminal membrane of the proximal tubule cell is generally regarded as the site of active sugar transport. However, recent investigations of SILVERMAN *et al.*[14] who used an *in vivo* technique, also show the importance of the antiluminal (basal and lateral) membrane in the overall transport of glucose in dog kidney, whereby the sugar from the peritubular fluid is transported in both directions; into the cell and back again. The mechanism of transport of sugars across the antiluminal membrane has not been yet described in detail but, according

to SILVERMAN *et al.*[15], the chemical specificity of this membrane is substantially different from that of the luminal one. While interaction of the hexose with the antiluminal membrane requires an OH group at C-2, chemical interaction at the luminal membrane involves OH groups at C-2 and C-6.

In view of the sugar interaction with the luminal and the antiluminal membranes it is evident that in our studies with kidney cortex slices the influx measurements represent the sum of transport processes at both cell surfaces. Thus, by analogy with dog kidney, one can predict that galactose will be transported into the kidney cell at both poles, 2-deoxygalactose at the luminal membrane only, and α-methylglucoside possibly at both membranes. The uphill transport at the luminal membrane is a feature shared by all the three sugars.

Dealing now with luminal membrane, it follows from the work of SILVERMAN *et al.*[15] that the sites of attachment of hexose involve four OH groups at C-2, C-3, C-4 and C-6. The authors postulate that the specificity of the binding process resides in the steric configuration of the above-mentioned sites of attachment on the same carrier, a conformational change of these binding sites distinguishing between, say, the glucose- and the mannose-preferring sites. All the three sugars studied here share the critical OH groups at C-3 and C-6. In contrast with galactose, 2-deoxygalactose is not attached by the OH group at C-2 while α-methylglucoside differs in the orientation of the OH group at C-4, the methyl group at C-1 interfering probably with the binding process. The partially competitive inhibition of galactose with the transport of 2-deoxygalactose and α-methylglucoside but a lack of competition between 2-deoxygalactose and α-methylglucoside even at concentrations substantially exceeding the K_m's of the sugars forces one to assume the presence of three binding sites on a single protein, not fully exclusive but rather capable of interacting, with greater or lesser affinity, with the various sugars in question (Fig. 8). D-Galactose, by binding to its receptor sites (or, to a special "effector sites") may allotopically alter the affinity of the respective binding sites for 2-deoxy-D-galactose and α-methyl-D-glucoside. On the other hand, 2-deoxy-D-galactose and α-methyl-D-glucoside are much less

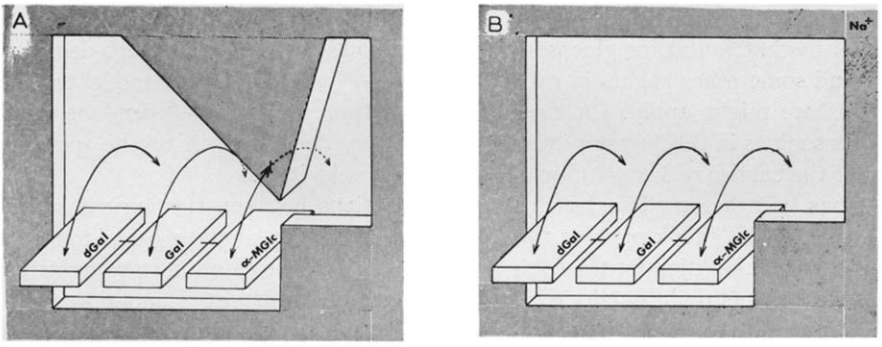

Fig. 8. Schematic illustration of the Na^+-activated sugar carrier in the luminal membrane of proximal tubule. (A) No Na^+. (B) With Na^+. Binding of galactose interferes with that of 2-deoxygalactose and α-methylglucoside but the binding sites for the latter two sugars are too far apart for any interaction to occur. In the absence of Na^+ the back-and-forth movement of 2-deoxygalactose binding site is not sterically hindered, that of α-methylglucoside is completely inhibited, that of galactose binding site being intermediary in this respect.

efficient allotopic effectors of D-galactose binding (*cf.* the K_m's and K_i's in Table II). The K_m of α-methyl-D-glucoside ranged from 3.3 to 8.2 mM while its K_i towards D-galactose uptake was rather erratic but was always above 100 mM.

Obviously, the role of the basal membrane in sugar transport is not amenable to a rigorous study by the slice technique and the above-proposed model might be replaced by a kinetically equivalent, but topographically more complex model, if differences at the two membranes were postulated. The partially competitive inhibition between the sugars studied would then be due to competition between two sugars at the one but not at the other cell pole.

The effect of Na^+ on the uptake of the three different sugars is rather more intriguing. The fact that the v_{max} of the sugars is increased by Na^+ to different degrees practically excludes a simple attachment of Na^+ to the "carrier" molecule, whereby its rate of movement across the membrane would be increased. One is tempted to envisage a steady-state mechanism of transport of the sugars in question, implying that the binding of sugars to carrier is of a comparable order of velocity to the actual movement of the sugar–carrier complex. In such a mechanism, one could account for the different degrees of the Na^+ effect by assuming that Na^+ acts as a co-factor in the binding reaction of substrate to carrier and that in such a function it may exhibit quantitatively different effects. However, since the steady-state mechanism of transport has not been demonstrated unequivocally in any living cell while the equilibrium mechanism has, we prefer to ascribe to Na^+ a rather sterically active role, as depicted in Fig. 8. While the movement of the sugar–carrier complex (whether by rotation or oscillation) in the absence of Na^+ is sterically hindered by a closely located membrane component, in the presence of Na^+ the steric hindrance is removed and hence the maximum rate of uptake is increased.

The effect of Na^+ on the transport of galactose and of α-methylglucoside supports the assumption that these sugars are transported uphill across the luminal membrane since the Na^+ activation of the process resides most probably in this membrane[6]. The fact that 2-deoxy-D-galactose is transported actively across the luminal membrane (see also ref. 15) then places all the three sugar-binding receptors at the luminal membrane and thus vindicates the suggested transport model.

The influence of Na^+ on the maximum rate of sugar uptake observed here is analogous to that found for glucose in frog renal cells[16] and to that for 6-deoxy-D-glucose and some other sugars in rabbit intestine[17,18] where a similar model to that described here might apply. On the other hand, there exist Na^+-dependent sugar transports such as in the hamster intestine, where the effect of Na^+ is on the apparent affinity of the carrier rather than on the maximum velocity[19].

It has been assumed so far that the interactions between the various sugars and the effect of Na^+ took place at the carrier level. It is not simple *a priori* to refute an alternative explanation for the competitive effects, *viz.* one assuming a competition for the source of metabolic energy required for the active transport or, more precisely, for the compound transmitting it to the actual transport system. However, there are at least two types of evidence against such an explanation: (a) total lack of competition between 2-deoxy-D-galactose and α-methyl-D-glucoside and (b) persistence of competition between galactose and 2-deoxy-D-galactose even under anaerobic conditions, which was checked in three experiments not shown here in detail.

Thus, we propose that a model involving three receptors on the same carrier

protein with mutually more or less interlinked binding sites, *plus* a sodium site on a nearby membrane component, might account for the data presented here.

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