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ACTIVE RENAL HEXOSE TRANSPORT

STRUCTURAL REQUIREMENTS

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Summary

The active transport of methyl β-D-galactoside and some other analogs of D-glucose and D-galactose was studied in slices of rabbit renal cortex.

1. The non-metabolizable methyl β-D-galactoside accumulates in renal cortical cells against its concentration gradient. At 1 mM substrate concentration (O_2, 25°C, 60 min incubation) the gradient was 2.36 ± 0.11 S.E. (n = 33). The K_t was 1.50 ± 0.02 mM. The active transport of the substrate was inhibited by dinitrophenol, phlorizin, absence of Na^+ and by ouabain. This inhibition was incomplete, suggesting that the sugar may enter the cells by two separate pathways, only one of which was coupled to the down-hill electrochemical Na gradient.

2. The structural requirements for the interaction between substrate and the carrier were defined:

(a) by testing the transport behavior of some analogs (1,5-anhydro-D-glucitol; methyl β-thio-D-galactoside; 3-deoxy-D-glucose; 4-deoxy-D-glucose; 5-thio-D-glucose; 6-deoxy-D-glucose and methyl-α-6-deoxy-D-glucoside); and

(b) by inhibition analysis of methyl β-D-galactoside transport. The role of each hydroxyl of the sugar molecule was tested by using a total of 41 analogs modified on each C by replacing -OH by -H, -O-CH_3, -F and in some instances also by -SH.

3. The carrier is shared by D-glucose, D-galactose and their methyl glycosides. A pyranose ring is mandatory. The D-*gluco*configuration is preferred for the interaction with the carrier.

Abbreviations: Gal, D-galactose; Glc, D-glucose; Man, D-mannose. Deoxy sugars are abbreviated by indicating the carbon number followed by the prefix d and the appropriate abbreviation of the sugar; the respective methyl glycosides are indicated by the prefix me followed by the symbol of the anomeric configuration (α or β) and the abbreviation of the sugar. DNP, 2,4-dinitrophenol.

4. Replacement of -OH by -H or -S practically abolished (on C_1, C_2, C_3) or greatly reduced (on C_4) the affinity of the analog for the carrier. This was also confirmed by demonstrating that 1-deoxy-, and 3-deoxy-glucose and the thiogalactoside were not actively transported and their entry into the cells was not markedly affected by phlorizin, dinitrophenol, ouabain or absence of Na^+. 4-Deoxy-D-glucose was taken up and its transport was inhibited by agents affecting the transport of methyl β-D-galactoside.

5. Replacement of -OH by -F did not abolish the affinity of the analogs for the carrier, indicating hydrogen bonding between the carrier and the oxygens at C_1, C_2, C_3, and C_4.

6. 5-Thio-D-glucose was not transported against its concentration gradient and also poorly interacted with the carrier as shown by inhibition analysis. Hydrogen bonding between the carrier and the pyranose ring oxygen is suggested.

7. 6-Deoxyglucose is a potent inhibitor of methyl β-D-galactoside transport although it is not actively taken up by the tissue. It is concluded that a hydroxyl at C_6 is required for transport, but is not mandatory for an interaction with the carrier. However, 6-deoxy-D-galactose was ineffective as inhibitor.

8. The specificity of the carrier involved in the renal active transport of D-glucose, D-galactose and their methyl glycosides resembles qualitatively, and mostly also quantitatively that described for intestinal transport of these sugars.

Introduction

The specificity of the renal active, Na^+-dependent, phlorizin-sensitive transport of hexoses has been studied in vivo [1—3] and in vitro [4—6]. The present investigation was undertaken to elucidate the nature of the interaction between the transported hexoses and the involved carrier(s). The results below present data on the uptake of some glucose and galactose analogs, particularly deoxysugars, by renal cells, as well as the inhibition of the cellular uptake of methyl β-D-galactoside by various analogs.

Me-β-Gal is a convenient model for the study of the active, Na^+-dependent sugar transport in renal tubular cells [7,8].

(1) The saccharide is actively reabsorbed from the tubular lumen of the rat kidney in vivo (clearance experiments) and this reabsorption is associated with a cellular accumulation of the free sugar, inferring the localization of the active transport step at the brush border. An active absorption of this substrate was also observed in double-perfused proximal renal tubules of the rate [3].

(2) In vitro, an active, Na^+-dependent accumulation of me-β-Gal is found using cortical slices of rabbit and rat kidney.

(3) Me-β-Gal is not metabolizable; this simplifies the interpretation of transport phenomena.

(4) The carrier involved in the transport of me-β-Gal is shared by several saccharides, including Glc, Gal and the respective methyl glycosides. However, the affinity of me-β-Gal for the carrier is lower than that of Glc and its derivatives. An inhibition analysis of me-β-Gal transport would then reveal the sharing of the carrier more readily than when using a substrate with a high affinity for the transport site(s).

Methods

Slices of renal cortex of healthy adult rabbit and rats were used. Methods for the preparation and handling of the tissue were given previously (see, e.g., Refs. 4 and 9). After incubation of the tissue aerobically (O_2) at 25°C in media containing usually 1 mM substrates (unless otherwise stated, 0.02 μCi ^{14}C or 0.1 μCi ^{3}H per ml) and appropriate additions, the blotted and weighed slices were extracted 10 min with boiling H_2O and total and free sugar were determined in the extract by assaying the label by scintillation spectrometry (Packard Instr., Model 3320). Data for individual experiments (5—6 slices per flask) are given as the mean values of sugar taken up by the tissue, in $\mu mol \cdot g^{-1}$ tissue wet wt. ± S.E. Mean values for several experiments, ± S.E., are also given. The apparent intracellular concentration (S_i) was computed after correction of the tissue values for the extracellular (inulin or poly(ethylene glycol)) space. Where indicated, normalized data (in percent inhibition of the control) and the number of experiments (n) are given. In spite of considerable individual variation in the sugar uptake in controls (see, e.g., Table IV), the % inhibition for a given analog differed not more than 7% from the mean value.

The significance of the difference between controls and experimental data was evaluated by Student's t-test.

Inhibition analysis. The application of the inhibition analysis to the study of the structural requirements for sugar transport in renal slices, as well as some limitations thereof, have been described previously [4]. It would be preferable to define the interference of an analog with the interaction of the substrate, me-β-Gal, and the carrier, in terms of K_i values for competitive inhibition (see, e.g., Ref. 10). Unfortunately, zero-time kinetic parameters cannot be readily assessed in renal slices (see below), and therefore the present inhibition by 5 mM analog of the substrate (1 mM) uptake at 60 min incubation was taken as the relevant criterion. This is justified by the linearity of substrate uptake between 20 and 120 min incubation (Fig. 1). If an analog displays a considerably smaller affinity for the transport site than the substrate, such inhibition may not be easily discernible. In order to minimize this possibility, a 5-fold molar excess of analogs was usually employed. Where required, additional confirmation was sought by varying the concentrations of both analog and substrate. More information may be obtained if it can be shown that the analog itself is transported into the cells and this process is inhibited by me-β-Gal and some other sugars assumed from preliminary tests to share the same transport pathway. Such tests of the competitive nature of the analog effect were carried out with various sugar derivatives, particularly when these were available in labelled form.

The purity of employed sugars is crucial in such experiments. If the affinity of an analog for the transport site exceeds that of the studied substrate, the presence of a minor contamination by such analog in the test system could produce a marked inhibition. D-Glucose has a high affinity for the carrier; at 1 mM me-β-Gal, 0.05 mM Glc had a significant inhibitory effect (Fig. 3 below). In such a system a 1% glucose impurity could simulate inhibition by the analog.

The choice of analogs permits obtaining further information on the nature of the interaction between the sugar and the carrier. At individual hydroxyls of

the sugar molecule interaction may take place by a transient formation of either hydrogen bonds [11] or of a relatively more stable covalent bond (e.g., ether or ester linkage). (1) The role of a free hydroxyl is revealed by testing the effect of the respective O-methyl ethers (at C_1: methyl glycosides) and deoxy-sugars. The possibility of steric hindrance by the rather bulky methyl groups has to be taken into account. Also, as compared to hexoses, the above analogs are markedly less polar and thus lipid solubility may play a role in their entry through the membrane. (2) Fluorine replacing the tested hydroxyl group freely forms hydrogen bonds [12] but is not expected to undergo covalent links. Under the conditions employed, i.e., 13 mM Tris* in the medium hydrolysis of the fluoro-sugars (e.g., glucosyl-fluoride [13] or 3-deoxy-3-fluoro-D-glucose [14]) may be reduced as demonstrated for glucosyl fluoride [13]. (3) Replacement of the hydroxyl by -H (deoxy-sugars) or -SH (thio-sugars) will abolish or greatly diminish hydrogen bonding [15].

The technique employed here does not permit discriminating between genuine effects produced by analogs owing to changes in the configuration on one carbon atom, and possible effects produced by changes in the $C_1 \rightleftharpoons {}^1C$ ratio of conformers between the substrate and the tested analog; hence, two effects might be involved where only one was sought.

Materials

Glucose and galactose analogs. Unless otherwise stated sugars employed in this study were purchased from Sigma, St. Louis, MO, U.S.A.; Pfanstiehl Laboratories, Waukegan, IL, U.S.A.; Koch and Light, Colnbrook, Bucks, U.K., and Sefochem Fine Chemicals, Emek Hayarden, Israel. Methyl α-D-mannoside, me-α-2-dGlc and me-α-2-dGal were prepared in this laboratory [16]. The generous gifts of the following sugars are gratefully acknowledged: 1,5-anhydro-D-glucitol (i.e., 1-dGlc): Dr. A.S. Keston, Mt. Sinai Graduate School, City University of New York, New York; α-D-F-Glc., propyl-α-D-glucoside, 3-dGal, 6-*O*-me-Gal, 6-d-6-F-Gal: Dr. J.E.G. Barnett, Nottingham, U.K.; me-α-6-dGlc: Dr. M. Saier, University of California, La Jolla, CA, U.S.A.; 2-*O*-me-Gal: Dr. J. Chittenden, University of Nijmegen, Nijmegen, The Netherlands; 3-d-Glc: Dr. E. Hardegger, ETH, Zürich, Switzerland; 3-d-3-F-Glc: Dr. N.F. Taylor, University of Windsor, Windsor, Canada; 4-d-Glc: Dr. J. Lehmann, University of Freiburg, Freiburg, F.R.G.; 4-d-4-F-Glc: Dr. A.D. Barford, Chester Beatty Res. Inst., London, U.K.; 5-S-Glc: Dr. R.L. Whistler, Purdue University, Lafayette, IN, U.S.A.; 2-d-2-F-Glc: Dr. E.L. Coe, North-Western University, Chicago, IL, U.S.A.; 6-d-6-F-Glc: Dr. A.P. Wolf, Brookhaven Natl. Lab., Brookhaven, N.Y., U.S.A.; me-α-Gal-furanoside and me-β-Gal-furanoside: Dr. A. Reine, University of Oslo, Oslo, Norway; 4,6-*O*-ethylidene-Glc: Dr. W.F. Widdas, Bedford College, University of London, U.K.

Labelled sugars. [^{14}C]Methyl-β-D-galactopyranoside, methyl-α-D-[U-^{14}C]-glucopyranoside, [^{14}C]methyl-β-D-thiogalactopyranoside, 5-[^{35}S]- and 5-thio-D-[G-^{3}H]glucose were purchased from New England Nuclear Corporation, Boston, MA, U.S.A. The following labelled sugars were generous gifts: [^{3}H]-1-dGlc and [^{3}H]-6-dGlc: Dr. W. Tanner, University of Regensburg, Regensburg, F.R.G.; [^{3}H]-3-dGlc: Dr. G. Semenza, ETH, Zürich, Switzerland.

The purity of all sugars was tested chromatographically and/or radiochromatographically, using Whatman No. 1 paper and propanol/H_2O (4 : 1, v/v) as the solvent mixture. Where required, traces of glucose impurities were removed by treatment of the sugar solution with glucose oxidase [17].

All other reagents were commercial products of analytical grade.

Results

Tissue uptake of some glucose and galactose analogs

The replacement in the sugar molecule of -OH by -H or another group produced the following results (Tables I and II): The non-metabolizable 1,5-anhydro-D-glucitol (i.e., 1-dGlc) was not actively accumulated in the tissue, and the entry of the sugar into the cells was not affected by inhibitors of active hexose transport, i.e., DNP, phlorizin, ouabain, etc. Similar results were obtained with me-β-thio-D-galactoside (details not given). Since me-α-Glc and me-β-Gal are good substrates for the transport system (Refs. 4 and 5, and Table I below), such results suggested that contrary to the prevalent view [1,4,5] a hydroxyl or oxygen atom, at C_1 may be required for transport. C_2: An Na^+-independent active uptake of 2-dGlc has been reported previously [4]. C_3: 3-dGlc was not actively taken up, consistent with the view [1,5] that a hydroxyl on this carbon plays a role in the sugar interaction with the carrier. C_4: 4-Deoxy-D-glucose was taken up to a level close to that of a diffusion equilibrium and some intracellular accumulation of the respective sugar phosphate(s) was found. The results implied a role of C_4-OH. Ring oxygen: 5-Thio-D-Glc was not taken up against a sizeable concentration gradient (3 experiments with different batches of ^{35}S and ^3H-labelled sugars: the highest S_i/S_o was 1.5) but the various inhibitory agents of the Na^+-dependent hexose transport system were effective. Quantitatively, our data contrast with those of Whistler and Lake [18] who reported an Na^+-dependent uptake of this sugar against a considerable concentration gradient. C_6: Finally, 6-dGlc was found

TABLE I

THE UPTAKE OF SOME ANALOGS OF D-GALACTOSE AND D-GLUCOSE IN RABBIT RENAL CORTICAL SLICES

Groups of slices (5—6 per flask) were incubated under standard conditions (60 min, 25°C, O_2) in salines containing 1 mM of the respective labelled substances, without (control) and with further additions: In the blotted and weighed tissue, total and free sugar were determined. Mean values of total sugar ($\mu mol \cdot g^{-1}$) ± S.E. (n = 5—6) are given. Values for free tissue sugar (in parentheses) are presented only for the controls. * Indicates a significance of difference between the control and experimental values at a level of $P < 0.01$.

Additions	Substrate			
	1-Deoxy-D-glucose	3-Deoxy-D-glucose	4-Deoxy-D-glucose	5-Thio-D-glucose
None (control)	0.81 ± 0.02 (0.76 ± 0.02) *	0.92 ± 0.01 (0.88 ± 0.01)	1.44 ± 0.08 (0.88 ± 0.03) *	0.79 ± 0.03 (0.61 ± 0.02) *
Dinitrophenol, 0.1 mM	0.76 ± 0.01	0.79 ± 0.01 *	0.73 ± 0.01 *	0.67 ± 0.01 *
Phlorizin, 0.5 mM	0.77 ± 0.01	0.76 ± 0.01 *	0.97 ± 0.04 *	0.51 ± 0.02 *
Ouabain, 0.5 mM	0.76	0.87 ± 0.02	1.02 ± 0.03 *	0.72 ± 0.03

TABLE II

UPTAKE OF 6-DEOXY-D-[^{3}H]GLUCOSE AND METHYL-α-6-DEOXY-D-[^{3}H]GLUCOSIDE BY RABBIT RENAL CORTICAL SLICES

Conditions of experiments: 1 mM substrates in the media, respective, without (control) and with further additions; 60 min incubation at 25°C, O_2. Values are the means of 5—6 analyses, ± S.E. Significance of difference of experimental value from the control at $P < 0.01$ denoted by *.

Expt. no.	Substrate	Addition	Tissue sugar (μmol · g^{-1})	
			Total	Free
1	6-Deoxy-D-glucose	None (control)	0.82 ± 0.01	0.69 ± 0.006
		Dinitrophenol, 0.1 mM	0.74 ± 0.006 *	0.69 ± 0.009
		Phlorizin, 0.5 mM	0.74 ± 0.002 *	0.66 ± 0.02
		Ouabain, 0.5 mM	0.75 ± 0.003 *	0.66 ± 0.01 *
		None; Na-free medium (Li)	0.63 ± 0.01 *	0.60 ± 0.007 *
2	6-Deoxy-D-glucose	None (control)	0.81 ± 0.01	0.70 ± 0.01
		D-glucose, 5 mM	0.77 ± 0.01	—
		Methyl α-D-glucoside, 5 mM	0.78 ± 0.02	—
		Methyl-β-D-galactoside, 5 mM	0.77 ± 0.01	—
		6-Deoxy-D-glucose, 5 mM	0.75 ± 0.01 *	—

not to be accumulated by renal cells (see also Ref. 5) whereas this sugar potently inhibited hexose transport in perfused renal tubules [2]. A slight formation of an acidic metabolic product of 6-dGlc was apparent from the systematically found difference between total and free tissue sugar. Me-α-6-dGlc also was not taken up by the tissue (details not given).

In the light of the above inconsistencies, particularly concerning the roles of C_1-OH, C_6-OH and the ring oxygen, a further analysis of the structural requirements of the transport system was desirable.

Methyl-β-D-galactoside as a model for the Na-dependent hexose transport

The time curve (Fig. 1) for the uptake of me-β-Gal shows that the apparent intracellular concentration of the saccharide reached that of the medium

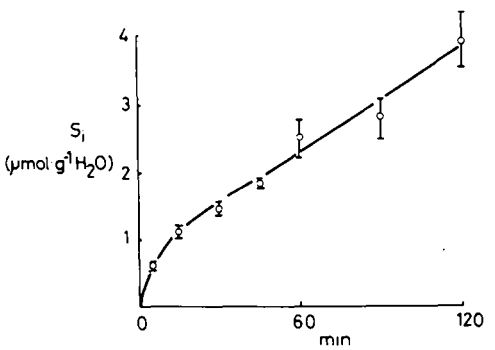

Fig. 1. Time curve of methyl β-D-galactoside utake by slices of rabbit renal cortex. Tissue (5—6 slices per flask) was incubated at 25°C (O_2) in saline containing 1 mM sugar plus 0.1% (w/v) poly(ethylene glycol). Mean values of S_i (μmol · g^{-1} cell water · h^{-1}), ± S.E. are presented.

TABLE III

UPTAKE OF METHYL-β-D-GALACTOSIDE BY SLICES OF RABBIT RENAL CORTEX

Groups of slices (6 per flask) were incubated aerobically (O_2) for 60 min at 25°C in saline containing [^{14}C]me-β-Gal (0.02 μCi · ml^{-1}) without (control) and with further additions. Na-free medium: Na^+ was equivalently replaced by Li^+. Mean values of tissue sugar (μmol · g^{-1}) ± S.E., as well as the computed S_i/S_o data, are given.

Additions	Tissue sugar (μmol · g^{-1})	S_i/S_o
None (control)	1.50 ± 0.07	2.78 ± 0.14
Phlorizin, 0.5 mM	0.53 ± 0.02	0.58 ± 0.05
Phloretin, 0.5 mM	0.77 ± 0.05	1.03 ± 0.07
Dinitrophenol, 0.1 mM	0.63 ± 0.03	0.73 ± 0.04
Ouabain, 0.5 mM	0.90 ± 0.07	1.31 ± 0.09
Na^+-free medium (Li^+)	0.81 ± 0.02	1.01 ± 0.03

(1 mM) within 20 min and then increased linearly against the concn. gradient to attain a S_i/S_o value of 4 after 120 min incubation. The mean value of S_i/S_o after 60 min incubation was 2.36 ± 0.11 S.E. (33 expts.). Data given in Table III established that the basic properties of the transport system for me-β-Gal are identical with those found for the active transport system for sugars located in the brush border; the driving force is the downhill electrogenic flux of Na^+ coupled at the carrier to the uphill flux of the sugar (see, e.g., Ref. 19). This conclusion is based on the observation that the active uptake of me-β-Gal was inhibited by 0.1 mM DNP, by 0.5 mM ouabain and by the absence of external Na^+, as well as by 0.5 mM phlorizin.

No evidence was obtained for the presence of an acidic metabolic product of me-β-Gal, using either the $ZnSO_4$ + $Ba(OH)_2$ method or separation of free sugar from its anionic products on a Dowex 1-X2 ion-exchange resin [9]. Also, chromatography of the tissue extract did not reveal evidence for a possible hydrolysis of the substrate. Thus, me-β-Gal is not appreciably metabolized by the renal tissue.

It should be noted that the S_i/S_o established by the renal cells after 60 min incubation was of the same order as that for free Gal [9], but was less than half that found for its analog, me-α-Glc [4,5]. Differences in the influx of the sugars, reflecting the respective affinities for the transport site, or their efflux, may be responsible for this.

An attempt was made to determine the (zero-time) kinetic transport parameters by measuring the cellular uptake of me-β-D-[^{14}C]Gal within 5—10 min incubation, after correction for the extracellular (poly(ethylene glycol)) space. Such procedure appears to be justified by the observation that D-mannitol fills this space within 2 min of incubation [9]. These experiments did not reveal a saturable component (Fig. 2, insert) as opposed to results obtained when the incubation period was 60 min (Fig. 2). Such data suggest that at short incubation periods a physical event, e.g., diffusion of substrate to the transport site, was rate-limiting. A double reciprocal plot of data for 60 min incubation (Fig. 2) showed a linear saturable component corresponding to an apparent K_t of 1.5 mM ± 0.02 S.E. (computed from the regression coefficient by the least-

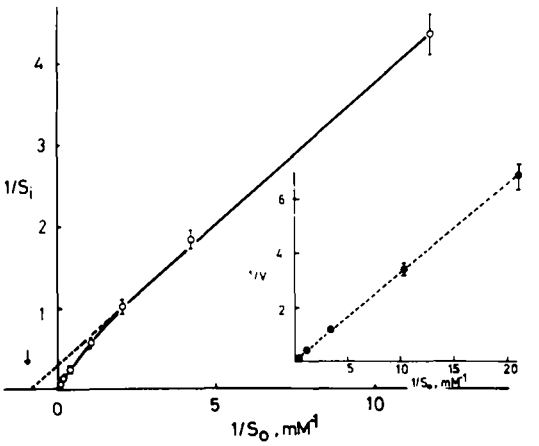

Fig. 2. Double reciprocal plot of concentration dependence of methyl β-D-galactoside uptake. Tissue (5 slices per flask) was incubated under standard conditions (60 min, 25°C, O_2) in salines containing 0.1 to 10 mM sugar plus 0.1% (w/v) poly(ethylene glycol) (PEG). Mean values of S_i, ± S.E. are given. Arrow: Intercept of extrapolated (broken line) indicates value of K_t. Insert: Double reciprocal plot for concentration dependence at 5 min incubation. Ordinate: $1/v$; v, rate of cellular sugar uptake (μmol · g^{-1} cell water · h^{-1}).

squares method), and also a minor component displaying a low affinity between substrate and the carrier.

Varying external pH showed a shallow maximum for the accumulation of me-β-Gal around pH 7.2 (S_i/S_o: pH 6.2 1.04 ± 0.04; pH 7.2 1.61 ± 0.08; pH 8.2 1.31 ± 0.06) as also observed for the glucose analog [20]. The accumulation of me-β-Gal increased upon increasing the incubation temperature from 20° C to 37° C (details not given here).

Inhibition analysis of the structural requirements for methyl-β-D-galactoside transport

The role of C_1-OH. The transport of me-β-Gal was strongly inhibited by its anomer, me-α-Gal, by 5 mM me-β-Gal (self-inhibition), as well as by both anomers of me-Glc (Table IV).

Since Glc and Gal also potently inhibited me-β-Gal uptake (Ref. 1, and below), the above set of results appeared to be compatible with the earlier view [1,4,5] that a free hydroxyl on C_1 is not required for substrate-carrier interaction. The configuration at the anomeric center of the sugar molecule is also of little consequence. Since propyl-α-D-Glc was also inhibitory, it would appear that the molecular arrangement between the carrier and the substrate can accomodate the relatively bulky propyl group.

On the other hand, 1-dGlc did not inhibit the transport of me-β-Gal under standard experimental conditions. Only at a 50-fold molar excess (at 0.1 mM me-β-Gal) did an 24% inhibition ($P < 0.01$) occur. This analog also was not actively taken up by the tissue (Table I). An elucidation of the apparent contradiction between the above sets of data was obtained by the observation that α-D-glucosyl-fluoride was as powerful an inhibitor of me-β-Gal uptake as both methyl glycosides or free Glc (Table IV). This result suggests that it is the

TABLE IV

EFFECT OF STRUCTURAL ANALOGS ON THE TRANSPORT OF METHYL-β-D-GALACTOSIDE BY RABBIT RENAL CORTICAL SLICES

Groups of slices (5—6 per flask) were brought to a steady state of tissue solutes by aerobic (O_2) preincubation for 45 min at 25°C in standard saline. The tissue was then transferred to flasks with saline containing 1 mM me-β-Gal (0.02 μCi/ml) without (control) and with 5 mM analog, and was incubated under identical conditions for 60 min. In the blotted slices the substrate was determined. Means ± S.E. of tissue me-β-Gal (μmol · g^{-1} tissue wet wt.) are given for a representative experiment. The mean percent inhibition (with n = number of experiments), as well as the significance of the difference between control and experimental values are shown: **, $P < 0.01$; *, $P < 0.025$; n.s., not significant.

Ring position tested	Analog	Tissue sugar (μmol · g^{-1})		Mean % of control	n	P
		Control	Inhibitor			
C_1	Methyl α-D-galactopyranoside	2.25 ± 0.06	1.18 ± 0.06	63	4	**
	Methyl β-D-galactopyranoside [a]	1.15 ± 0.03	0.90 ± 0.04	65	3	**
	Methyl α-D-glucopyranoside	1.60 ± 0.07	0.78 ± 0.01	42	3	**
	Methyl β-D-glucopyranoside	1.56 ± 0.09	1.25 ± 0.13	79	2	*
	Propyl αD-glucopyranoside	1.62 ± 0.04	1.00 ± 0.09	62	1	**
	D-Galactose	1.77 ± 0.11	1.29 ± 0.03	68	5	**
	D-Glucose	1.70 ± 0.17	0.94 ± 0.03	59	3	**
	L-Glucose [b]	1.73 ± 0.11	1.64 ± 0.14	91	4	n.s.
	1,5-Anhydro-D-glucitol	1.16 ± 0.06	1.14 ± 0.03	96	4	n.s.
	α-D-Glucosyl fluoride	1.62 ± 0.04	0.59 ± 0.03	41	2	**
	Methyl β-thio-D-galactopyranoside	0.92 ± 0.05	0.98 ± 0.03	103	2	n.s.
	α-Thio-D-glucose	1.31 ± 0.11	1.32 ± 0.05	100	2	n.s.
C_2	2-Deoxy-D-galactose	1.25 ± 0.06	1.30 ± 0.04	83	3	n.s.
	Methyl α-2-deoxy-D-galactoside	1.15 ± 0.09	1.26 ± 0.08	82	3	n.s.
	2-Deoxy-D-glucose	1.16 ± 0.03	1.05 ± 0.02	93	4	n.s.
	Methyl α-2-deoxy-D-glucoside	1.15 ± 0.09	1.31 ± 0.09	105	2	n.s.
	3-*O*-Methyl-D-galactose	1.31 ± 0.11	1.25 ± 0.09	95	2	n.s.
	2-Deoxy-2-fluoro-D-glucose	1.41 ± 0.07	1.00 ± 0.06	71	2	**
	D-Talose	1.31 ± 0.09	1.17 ± 0.11	90	2	n.s.
	D-Mannose	1.02 ± 0.06	1.01 ± 0.03	103	2	n.s.
	Methyl α-D-mannoside	1.15 ± 0.10	1.20 ± 0.08	105	1	n.s.
C_3	3-Deoxy-D-galactose	1.17 ± 0.05	1.17 ± 0.09	99	2	n.s.
	3-Deoxy-D-glucose [c]	0.97 ± 0.01	1.20 ± 0.08	117	2	*
	3-Deoxy-3-fluoro-D-glucose	1.75 ± 0.11	1.05 ± 0.07	58	2	**
	3-*O*-Methyl-D-glucose	1.48 ± 0.05	1.15 ± 0.06	81	4	**
	D-Allose [c]	1.12 ± 0.11	1.32 ± 0.08	132	3	n.s.
C_4	4-Deoxy-D-glucose	1.18 ± 0.08	0.93 ± 0.03	71	2	**
	4-*O*-Methyl D-glucose	0.98 ± 0.04	0.86 ± 0.05	87	1	n.s.
	4-Deoxy-4-fluoro-D-glucose	0.92 ± 0.05	0.60 ± 0.02	69	2	**
	4,6-*O*-Ethylidene-D-glucose	1.62 ± 0.04	1.11 ± 0.03	75	2	**
Ring structure and C_5	D-Galactitol	1.16 ± 0.01	1.11 ± 0.04	108	2	n.s.
	D-Glucitol	1.02 ± 0.06	1.03 ± 0.06	100	2	n.s.
	Methyl α-D-galactofuranoside	1.15 ± 0.03	1.13 ± 0.07	99	2	n.s.
	Methyl β-D-galactofuranoside	1.15 ± 0.03	0.98 ± 0.06	95	3	n.s.
	5-Thio-D-glucose	1.17 ± 0.03	1.04 ± 0.05	84	2	*
C_6	6-Deoxy-D-galactose	1.56 ± 0.09	1.38 ± 0.08	93	4	n.s.
	6-*O*-Methyl D-galactose	1.31 ± 0.11	1.15 ± 0.04	85	2	n.s.
	6-Deoxy-6-fluoro-D-galactose	1.00 ± 0.04	0.96 ± 0.04	99	2	n.s.
	6-Deoxy-D-glucose	1.75 ± 0.11	0.93 ± 0.07	64	3	**
	6-Deoxy-6-fluoro-D-glucose	1.00 ± 0.04	0.96 ± 0.04	101	2	n.s.
	Methyl α-6-deoxy-D-glucopyranoside	1.09 ± 0.02	0.86 ± 0.03	79	2	**
	L-Arabinose	1.77 ± 0.11	1.51 ± 0.14	88	3	n.s.
	D-Xylose	1.02 ± 0.06	1.03 ± 0.06	97	2	n.s.

[a] 'Self-inhibition'.

[b] Test of stereo-isomer specificity.

[c] Actual activation of transport?

oxygen on C_1 which interacts with the carrier via hydrogen bonding, and is also required for the actual active transport process. Such view is further supported by the fact that α-thio-D-glucose and me-β-thio-D-galactoside were not inhibitory. The above data are compatible with the view that me-β-Gal and other substrates transported at the same site interact with the carrier by the formation of a hydrogen bond at C_1-O.

The role of C_2-OH. The mandatory role of C_2-OH in the D-*gluco*-configuration for the interaction of the substrate and the carrier is brought out by the observation that the methyl-α-D-glycopyranosides of 2-dGlc, 2-dGal, and of Man, as well as the sugars 2-dGlc (highly purified), 2-dGal and Man, and the C_2-epimer of D-galactose, i.e., D-talose, were ineffective as inhibitors. 2-Fluoro-2-deoxy-D-glucose was inhibitory. This set of observations indicates the possibility of hydrogen bonding between the carrier and the oxygen at C_2 in the D-*gluco*-configuration. An inhibitory effect of the fluoroderivative of 2-dGlc by a feedback mechanism brought about by its phosphate cannot at present be excluded, but appears to be less likely in view of the considerable chemical inertness of this compound (Coe, E., personal communication). If the suggestion of a mandatory formation of a hydrogen bond between the oxygen at C_2-OH in the D-*gluco*-configuration is acceptable, the observation that 2-*O*-methyl-D-galactose did not affect the transport of me-β-Gal would indicate that the bulk of the methoxy group at C_2-O- cannot be accommodated at the transport site.

The role of C_3-OH. Glucose-free 3-*O*-me-Glc was a rather weak inhibitor of me-β-Gal transport (Table IV). From the concentration dependence of this inhibition (details not given) it was deduced that the methylation of C_3-OH reduced the affinity for the carrier by about two orders of magnitude. These results support previous reports that 3-*O*-me-Glc is a poor substrate for renal sugar transport in vitro [4,5] and in vivo [1,12].

The role of C_3-OH was studied further using available analogs. Table IV shows that the transport of me-β-Gal was not inhibited by 3-dGlc, 3-dGal and by the C_3-epimer of Glc, i.e., D-allose. Moreover, 3-dGlc was not actively taken up by the tissue (Table I). These findings confirm the mandatory role of C_3-O- in the D-*gluco*-configuration for active transport in renal cells. Hydrogen bonding between the oxygen and the carrier is suggested by the fact that 3-fluoro-3-deoxy-D-glucose significantly inhibited the transport of me-β-Gal.

The role of C_4-OH. The transport of me-β-Gal was readily inhibited by Glc (Table IV) and by both anomers of methyl glucoside. These observations support the view that the carrier at the brush border of renal cells is shared by Glc, Gal and their methyl glycosides. However, an interaction between the carrier and C_4-OH is indicated by the following: (a) sugars of the D-glucose series had a markedly higher affinity for the carrier than their D-*galacto*-analogs. Thus, D-glucose is a competitive inhibitor of Gal transport in renal cells [5,21]. From the concentration dependence of the inhibition of me-β-Gal transport by D-glucose (Fig. 3) the affinity of Glc for the carrier could be up to one order of magnitude higher than that of sugars of the Gal-series. Such conclusion is supported by data shown in Fig. 4 demonstrating that while me-α-Glc and me-β-Gal competed for the transport site, the affinity of me-α-Glc for the carrier was markedly higher.

(b) 4-Deoxy-D-glucose entered (actively?) renal cortical cells and this uptake

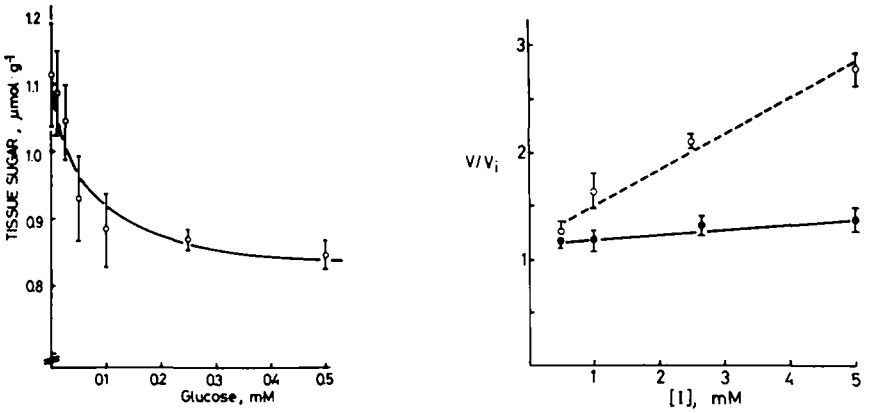

Fig. 3. Effect of varying D-glucose concentrations on tissue uptake of methyl β-D-galactoside. Tissue (5 slices per flask) was incubated (60 min, 25°C, O_2) in saline containing 1 mM substrate plus 0 to 0.5 mM D-glucose. Mean values of tissue me-β-Gal (μmol · g^{-1}), ± S.E.

Fig. 4. Competition of methyl α-D-glucoside and methyl β-D-galactoside for transport sites in renal cells. Tissue was incubated under standard conditions for 60 min. Mean values, ± S.E. are given: (○) 1 mM me-β-Gal, varying me-α-Glc concentration; (●) 1 mM me-α-Glc, varying me-β-Gal concentration. Abscissa: concentration of inhibitor; ordinate: reciprocal of relative inhibition, v, rate of tissue uptake (μmol · g^{-1}).

was inhibited by all inhibitors of the Na^+-dependent sugar transport system (Table I). The absence of an up-hill transport system for 4-dGlc, may be related to the intracellular phosphorylation of this sugar; the sugar phosphate would thus act as a sink for 4-dGlc which entered the cells. However, as compared with the uptake of D-galactose, the uptake of this 4-deoxyderivative appears to be lower. Also, 4-dGlc inhibited me-β-Gal uptake and the affinity of the analog for the carrier was intermediate between that of Glc and Gal (Table IV). Thus, the presence of C_4-OH facilitates the interaction of the sugar with the carrier.

(c) 4-d-4-F-Glc was a potent inhibitor of me-β-Gal uptake suggesting the possibility of hydrogen bonding between C_4-O and the carrier.

The above set of observations indicates that an interaction between the carrier and C_4-O takes place preferably in the D-*gluco*-configuration. Such conclusion is also supported by the observation that 4,6-*O*-ethylidene-D-glucose significantly inhibits the uptake of me-β-Gal (Table IV). The lack of inhibitory action of 4-*O*-me-Glc on the uptake of me-β-Gal may well be due to steric factors.

The role of the ring structure. D-Galactitol and D-glucitol were ineffective as inhibitors. Thus, a ring structure is required for the interaction between the substrate and the carrier. Evidence that the pyranose ring is the preferred structure is shown in Table IV. It will be seen that both α- and β-methyl D-galactofuranoside did not inhibit the uptake of me-β-Gal under conditions where the corresponding pyranose was highly effective. Such result substantiates the widely held view (see, e.g., Refs. 22 and 23) that an aldopyranose ring is required for interaction with the carrier.

As opposed to Glc, 5-thio-D-glucose (5-S-Glc) was a poor inhibitor of me-β-Gal transport ($P < 0.05$) indicating a low affinity of this analog for the carrier. The tissue uptake of this analog was also low compared with Gal, me-β-Gal and

me-α-Glc. The replacement of the ring O by S thus decreases the affinity of the sugar for the carrier, as would also follow from the pooor inhibitory effect of 5-S-Glc on the uptake of me-α-Glc (Ref. 18, Fig. 2). Hydrogen bonding between the ring O of the sugar and the carrier is indicated.

The role of C_6-OH. We reported previously [4] that 6-dGlc, as well as the pentose corresponding to Glc, i.e., D-xylose (Xyl), were taken up by renal cortical cells only against a minor concentration gradient, the S_i/S_o not exceeding 1.5. Such results, and also data obtained in vivo (Ref. 1, see, however, Ref. 24 concerning Xyl) indicated that a free C_6-OH was required for (or enhanced) the transport of these sugars. On the other hand, 6-dGlc, but not 6-dGal, was found to be an effective inhibitor of renal Glc transport [2]. A more detailed analysis of the role of C_6-OH was therefore desirable.

Table IV shows that none of the following C_6-analogs of Glc and Gal inhibited the tissue uptake of me-β-Gal: 6-dGal, D-xylose, L-arabinose, the methyl glycosides of these pentoses as well as 6-*O*-me-Gal. On the other hand, 6-dGlc, as well as its methyl glycoside were potent inhibitors of me-β-Gal transport. Such result is not consistent with a role of C_6-OH in transport. Data on the transport of 6-deoxy-D-[^{3}H]glucose (Table II) are of assistance to resolve the apparent discrepancy. The inhibitory effect of DNP, phlorizin, the absence of Na^+, and of ouabain on the tissue sugar uptake, and also a slight inhibition thereof by other sugars suggests that 6-dGlc does interact with the carrier involved in the transport of Glc, Gal and their glycosides at the brush border of the cells. Essentially similar results were obtained using me-6-dGlc as substrate (details not given). Such results are compatible with the view that while a free C_6-OH is required for a major active transport of the sugar across the membrane, this group is not mandatory for an interaction with the carrier. The presence of C_4-O in the D-*gluco*-configuration greatly facilitates such interaction.

Discussion

Recent evidence [3,25,26] now points in favor of the view [1,5] that several sugar transport pathways with overlapping specificities are present along the nephron. It is reasonable to assume that only one transport system is responsible for the renal active transport of Glc and Gal (and some of their analogs) by the established mechanism, i.e., coupling to the downhill electrogenic flux of Na^+ (see Refs. 19 and 23) at the brush border of the tubular cells [27]. A comparison of the transport properties of some of the analogs (Tables I and II) with data on the inhibition analysis of me-β-Gal transport should therefore provide more detailed information on the structural requirements of the active glucose-galactose (Glc-Gal) transport system.

The use of the me-β-Gal transport as a model system

Results given above (Table III) show that the non-metabolizable me-β-Gal may serve as a useful model for the Glc-Gal transport system. Several points arise from these data:

(1) Renal cells contain large amounts of β-galactosidase [28] and hence hydrolysis of the substrate and subsequent metabolism of galactose might be

expected. A similar discrepancy pertains to the me-α-Glc. It would be of interest to know the distribution of α-glucosidase and β-galactosidase in the cells as well as in different portions of the nephron and to inquire into the possibility of compartmentation of the enzyme and/or substrate within the cells.

(2) Agents known to inhibit the coupled, Na^+-dependent sugar transport i.e., DNP, phlorizin or ouabain, abolished the transport of the substrate against its concentration gradient but did not completely inhibit the (passive) entry of the sugar into the cells. Similarly, 0.5 mM phlorizin did not reduce the S_i/S_o of me-α-Glc markedly below the diffusion equilibrium value of 1.0: in 4 such experiments, the S_i/S_o was 0.74 ± 0.04 (S.E.). Such observations suggest that the glycosides can also enter the cells by one or several pathways relatively insensitive to phlorizin. An equilibrating Na^+-independent relatively phlorizin-insensitive transport system for me-α-Glc (and glucose) has been found at the antiluminal face of renal tubular cells of the flounder [19]; however, no interaction between me-α-Glc and the antiluminal face of the dog nephron has been found by the multiple indicator dilution technique [25]. The existence of several transport pathways for me-β-Gal impedes a meaningful evaluation of kinetic parameters such as K_i in slices.

It has been pointed out that in vitro the tubular lumina of incubated slices are collapsed [30], at least in those from the subcapsular region [31]. These observations, as well as histoautoradiographic data suggesting that the intracellular lumen in renal slices does not permit direct entry (or exit) of solutes from the external medium [31], have been taken to indicate that transport processes observed in slices reflect preponderantly events localized at the antiluminal cell face [32,33]. In order to explain the active transport of solutes by a brush border localized active process, it was assumed [31—33] that the solutes gain access to the transport site by rate-limiting diffusion through the intercellular junctions. Two studies indicate that the above possible interpretation is not applicable to the data presented here: Dennis and Brazy [34] have directly demonstrated that the leak of a sugar through intercellular junction of rabbit renal proximal tubules is very small; less direct evidence on this point has also been obtained for the flounder renal tubule [35]. Second, the time course of the active, Na^+-dependent, phlorizin-sensitive me-α-Glc uptake in renal epithelial cells grown in vitro [36] is identical with that found for this sugar in rat renal cortical slices [37] as well as for me-β-Gal (Fig. 1 above). Since the conditions for measuring sugar transport in epithelial cells grown in vitro precludes diffusion as the rate-limiting step for sugar uptake, this observation indicates that the rather slow active uptake of the methyl glycosides reflects a cellular characteristic seen also in slices, rather than an artifact produced by the geometry of the system. However, the low-affinity transport component for me-β-Gal seen in Fig. 2, as well as the differences seen in the temperature-dependence of the tissue uptake of me-β-Gal and me-α-Glc at 37°C (cf. above and Ref. 37) may be related to problems of geometry in slices.

Specificity and the nature of the interaction between substrate and the carrier involved in the Na^+-dependent active renal sugar transport

An inspection of the data presented above shows that D-glucose and the methyl glycosides are the most powerful inhibitors of me-β-Gal transport. Since

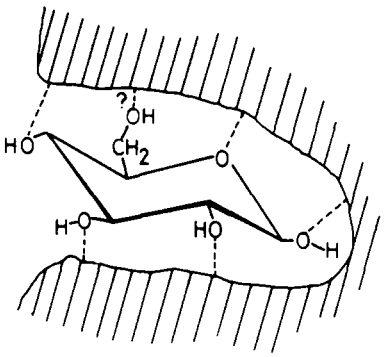

Fig. 5. Possible model of interaction between sugars transported by Glc-Gal system and the carrier. Broken lines: hydrogen bonding.

the practically non-metabolizable methyl glucosides are also readily accumulated by renal cortical cells gainst higher concentration gradients than any other monosaccharide, it follows that the D-*gluco*-configuration is preferred for the substrate-carrier interaction. The affinity of Gal and the methyl galactosides for the transport site are 2-fold lower (see Fig. 4) than that for glucose or methyl glucosides (see also Ref. 21). It is concluded that the above sugars share the carrier of the active Glc-Gal transport system.

The use of analogs revealed the following: Changes in the substrate structure which resulted in a decrease of the capability of hydrogen bond formation between the transport site and the oxygen on the individual carbon atoms (e.g., the use of the respective sugars) decreased or (particularly at C_2) practically abolished the inhibition of me-β-Gal uptake by the tissue as well as the cellular active uptake of the analogs. On the other hand, the affinity of analogs in which the hydroxyl was replaced by -F at C_1, C_2, C_3, and C_4 was close to that of the parent sugar, i.e., D-glucose. Such data are consistent with the view that the interaction between substrate and the carrier takes place at most oxygen atoms of the pyranose ring, C_1, C_2, C_3, probably C_4, and the ring oxygen; the C_4-O- in the D-*gluco*-configuration is the preferred structure. At C_6, a requirement for a hydroxyl for transport of the sugar is suggested, whereas the presence of C_6-OH does not appear to be crucial for interaction of the hexose with the carrier. The above view is supported by experiments where the oxygens of the sugar molecule were replaced by thio groups (i.e., α-thio-D-glucose, methyl-β-thio-D-galactoside and 5-thio-D-glucose; Table I). These analogs either did not inhibit me-β-Gal uptake (and also were not actively transported by the cells) or their affinity for the Glc-Gal carrier was greatly reduced. Fig. 5 represents the appropriate model, similar to that suggested by Barnett et al. [38].

The present study does not permit stating unequivocally whether the reported observations reflect only a marked decrease of the affinity between the deoxy-sugars and the carrier, or a complete loss thereof. Minor inhibitory effects are not readily recognized by the employed technique. However, the fact that the entry into the cells of 1-dGlc, 3-dGlc and 6-dGlc was slightly affected by phlorizin or dinitrophenol argue in favor of the view that none of the

hydrogen bonds (possibly with the exception at C_2-O) between the sugar and the carrier is mandatory; rather, each point of attachment of the sugar at the carrier contributes to increasing the affinity.

The model presented above is in essential points in agreement with hitherto available data on the specificity of sugar transport in renal proximal tubules, derived from studies in vivo [25], in micropuncture experiments [23], as well as in studies using vesicles of brush-border membranes [6,25,27]. Some questions arose previously concerning the renal transport of 2-deoxy-D-glucose which was thought [1,25] to be reabsorbed in the kidney of the dog by the Na^+-dependent transport system shared by Glc and Gal. Our data agree with the conclusions of Ullrich et al. [2,3], of Glossmann and Neville [39] and the recent results of Turner and Silverman [40] that 2-deoxy-D-glucose does not share the carrier involved in the luminal, Na^+-dependent transport of Glc and Gal. This deoxyhexose is reabsorbed from the tubular lumen by a transport system which displays only a minor sensitivity to phlorizin and may be localized in a portion of the nephron different from that of the Glc-Gal transport system investigated in this study [26].

While 3-*O*-me-Glc appears to share the carrier for Glc and Gal transport (Table IV, Refs. 2, 25 and 40), the affinity of this analog for the transport site is considerably reduced by the introduction of the methylgroup on C_3 (see also Ref. 40), possibly for steric reasons. Data from this laboratory [36] indicate that this sugar may enter the cells by two pathways, i.e., one shared with me-β-Gal (Table IV) and me-α-Glc (and this transport system is phlorizin sensitive), and by a phloretin-sensitive, probably equilibrating transport system.

No explanation can be offered here for the discrepancy between data presented here and those of Whistler and Lake [18] concerning the transport behavior of 5-S-Glc. These authors found a marked accumulation of the substrate by renal cortical cells, and a transport K_t of 2.4 mM whereas we observed only a minor accumulation of this analog ($S_i/S_o < 1.5$) (Table I). Since the K_t for me-β-Gal is of the order of 1.5 mM (Fig. 2) a major inhibition of me-β-Gal uptake by 5-S-Glc would be expected when the inhibiting analog was present in a fivefold molar excess. A similar discrepancy between the found inhibitory effect of 5-S-Glc on the uptake of me-α-Glc (K_t 0.7 mM, Ref. 8) is apparent (cf. Fig. 2 of the above authors). Studies using membrane vesicles of microvilli [40] also indicate that the affinity of the analog for the Glc-Gal transport carrier is greatly reduced by the replacement of the ring oxygen by sulfur.

The data reported above on the transport behavior of C_6-analogs of Glc and Gal are helpful in resolving some of the controversial aspects on the role of C_6-OH. The inhibitory effect of 6-dGlc on the transport of Glc in the perfused renal tubule in vivo (2) and on the cellular me-β-Gal uptake in vitro (Table IV) can be reconciled with the lack of active uptake of this sugar and of me-6-dGlc if it is assumed that a free C_6-OH is required for the transport across the membrane but is not mandatory for the interaction of the sugar with the carrier. The observation of Widdas et al. [41,42] is pertinent here in that 4,6-ethylidene-α-D-glucose readily interacts with the sugar carrier of erythrocytes and intestinal cells but is not itself transported across the membrane. The surprising observation that the 6-deoxy-6-fluoro-derivatives of Glc and Gal are not inhibitory of the renal transport of me-β-Gal (Table IV) suggests an important role in

the oxygen atom in C_6. This finding should be contrasted to observations on the sugar transport at the brush border of the intestinal mucosa where both sugars were found to be actively taken up by the cells [10].

Comparison of the specificities of active renal and intestinal sugar transport systems

A comparison of the above data with those on the specificity of intestinal sugar transport [10,11,38,42] revealed that the structural requirements at each carbon appeared to be qualitatively, and mostly also quantitatively, similar. The only relatively minor discrepancies concern the following points:

C_2: A practically absolute requirement for C_2-OH in the D-*gluco*-configuration was seen in renal and intestinal cells (Table IV and Ref. 38). The only difference in the transport behavior of C_2 analogs in both tissues concerns 2-d-2-F-Glc. Whereas in the intestine this analog was completely inactive, a distinct inhibitory effect was found on the renal uptake of me-β-Gal. Such discrepancy impinges on the question whether the interaction between the carrier and the sugar at C_2 is brought about by hydrogen bonding on the oxygen at C_2, as suggested here, or by the formation of a covalent bond. Further information on this point is desirable.

C_3: The differences in the transport behavior of 3-*O*-me-Glc in both tissues [4,5] are only apparent. An inspection of the data of Barnett et al. [11] reveals that as compared with Gal, the introduction of a methyl group on C_3 of glucose decreased the intestinal tissue accumulation by 50% and the affinity for the carrier by more than 80%. From our studies, the affinity of 3-*O*-me-Glc for the carrier is about two orders of magnitude smaller than that of me-β-Gal. Thus, 3-*O*-me-Glc is a rather poor substrate for the Glc-Gal transport system in both epithelial tissues.

C_4: In both tissues the D-*gluco*-configuration is preferred.

C_5: An apparent discrepancy concerns the transport behavior of 5-S-Glc. Whereas, in the intestine, the analog was a powerful inhibitor of galactose uptake and was also taken up by the tissue against a sizeable concentration gradient [38], no such accumulation was found in renal cells by this laboratory (cf., however, Ref. 18). Silverman (personal communication) found that this analog is reabsorbed by the kidney, albeit with a relatively low affinity for the Glc-Gal transport carrier.

C_6: Data in Tables II and IV show that omission of -OH on C_6 in Glc practically abolished the active uptake of the analog but did permit interaction of 6-dGlc with the carrier of the Glc-Gal transport system, as evidenced by its strong inhibition of me-β-Gal accumulation. These results do not differ qualitatively from the transport behavior of 6-dGlc in the intestine where both its active accumulation in the tissue and its affinity for the Glc-Gal carrier were diminished [4]. A role of C_6-OH in the transport process is further supported by the fact that 6-dGal is neither accumulated nor does it interact with the carrier in the kidney (Table I and Ref. 5) and the intestine [10]. The one difference between the tissues which should be commented upon concerns the transport behavior of the 6-d-6-F analogs. In the intestine these analogs shared the Glc-Gal carrier [10] whereas no evidence for an interaction between the analogs and the carrier was seen in the renal cells. Further studies on this point would be informative.

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