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MULTIPLICITY OF CARRIERS FOR FREE GLUCALOGUES IN HAMSTER SMALL INTESTINE

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SUMMARY

The transport of free glucalogues across the mucosal pole of hamster small intestine is carried out by (at least) two transport systems, one of which is common to glucose, galactose, 6-deoxyglucose and 3-methylglucose, whereas the other is essentially specific for glucose and galactose, among the monosaccharides tested. This was shown by:

1. The dependence of the unidirectinal medium–mucosa flux on the substrate concentration which is described by a single horizontal hyperbola in the cases of 6-deoxyglucose and of 3-*O*-methylglucose, but by the sum of two such hyperbolas in the cases of both glucose and galactose.

2. The mutual inhibition among glucalogues and the inhibition by arbutin, which are best described by the existence of two carriers, as given above;

3. The difference in the absorption of glucose and galactose in baby hamsters as compared with adult hamsters.

Both systems are Na^+-dependent and (to a different degree) phlorizin-sensitive. Neither is identical with the fructose transport system.

INTRODUCTION

Although free glucalogues (*i.e.*, monosaccharides of the glucose group) have been generally assumed to be transported in small intestine by a single, Na^+-dependent, phlorizin-sensitive carrier, there have been a number of indications that this picture may be an oversimplification (see Discussion).

This paper presents evidence that the glucalogues are absorbed in the small intestine of the hamster by at least two transport systems. Some results were reported in a preliminary form[1].

MATERIAL AND METHODS

Hamsters approximately 2.5-month-old of either sex were used. Unless stated otherwise, the animals were fed *ad libitum* (for experiments on the effect of fasting, the animals were deprived of food for 3 days, but had access to water). The baby hamsters used (8-day-old) were left with the mother till shortly before sacrifice. The animals were killed by decapitation. The small intestines were rinsed, everted, cut and

mounted in frames as described elsewhere[2], so as to expose only the mucosal side to the medium*. Between the rinsing and the incubation the small intestinal pieces were kept in Krebs–Henseleit buffer[4] under continuous gassing with O_2–CO_2 (95:5, v/v) at room temperature. The time between the death of the animals and the beginning of the incubation never exceeded 15 min**. The intestinal pieces were incubated for 2 min (unless stated otherwise) at 37 °C in small beakers, held in a vigorously shaking Dubnoff water bath. The incubation medium was pregassed Krebs–Henseleit buffer, in which the concentrations of all salts were uniformly reduced (see Table I), the isoosmolarity being made up with monosaccharides, mannitol or Tris–HCl. The incubations were terminated by rinsing the mounted small intestines in ice-cold standard Krebs–Henseleit buffer. The tissues were then punched and weighed[2].

The experiments on glucose uptake were carried out, as suggested by Crane[5], in Krebs–Henseleit buffer, in which 20 mM NaF substituted for $CaCl_2$ and $MgSO_4$.

For experiments in Na^+-free media, the gut was rinsed with either isotonic KCl or choline·HCl, kept in choline-substituted Krebs–Henseleit buffer containing 1 mM ouabain and 2 mM ethacrynic acid[6] and incubated in the choline-substituted Krebs–Henseleit buffer of Table I, containing 1 mM ouabain and 2 mM ethacrynic acid.

Analytical procedures

Various techniques for assay of sugar uptake were used: (a) In double-labeling experiments the tissue was digested in 2 ml Nuclear Chicago Tissue Solubilizer (NCS) and the ^{14}C- and ^{3}H-labeled sugars were determined by liquid scintillation counting. Aliquots of the media were counted in 10 ml Aquasol. (b) Deproteinization was carried out according to Nelson[7], *i.e.* the tissue was homogenized in 1 ml $ZnSO_4$ (0.15 M); 1 ml of equimolar $Ba(OH)_2$ was added, and the precipitate spun down (the media were subjected to the same procedure). Aliquots of the supernatants were taken for sugar assay either by a chemical method as given below or by liquid scintillation counting, or both. (c) In some experiments the tissues were homogenized in water and denatured in a boiling water for 5 min. This partial deproteinization was used for the enzymatic assays only.

Chemical assays. 6-Deoxy-D-glucose was determined according to Dische and Shettles[8], 2-deoxyglucose according to Waravdekar and Saslav[9] and inulin according to Roe *et al.*[10].

Enzymatic assays. Glucose was determined either with glucose oxidase and peroxidase[11,12] or with hexokinase–glucose-6-*P* dehydrogenase[13]; galactose with galactose dehydrogenase[14].

Labeled compounds were determined in a Nuclear Chicago liquid scintillation counter by either of the following methods: (a) Aqueous solutions were either first evaporated to dryness in the counting vial, dissolved in 2 ml methanol with subsequent addition of 10 ml scintillator mixture [2-(4′*tert*-butylphenyl)-5-(4″-biphenyl)-1,3,4-oxadiazol in toluene, 5 g/l] or directly diluted with 10 ml Aquasol. (b) Tissue

* Contrary to the small intestine of baby rats[3], that of baby hamsters can be mounted in our frames.

** At least up to this time the sugar uptake (glucose 0.3 mM) was found to be independent of the time elapsed between death of the hamster and the beginning of the incubation. 6 min elapsed: J_{me}= 1.45 μmole·ml^{-1}·min^{-1}; 16 min, J_{me}= 1.50; 36 min, J_{me}= 1.30.

samples were dissolved in 2 ml Nuclear Chicago Tissue Solubilizer (NCS) and counted after addition of 10 ml scintillator mixture.

Sugar uptake is expressed as μmoles of sugar/min per ml of tissue water (assumed to be 80% of the tissue wet weight)[15] and corrected for the extracellular space.

The extracellular space was determined with either [^{3}H]- or [^{14}C]mannitol (0.25 μM) or 2-deoxyglucose (1.5 mM) for incubations lasting not more than 2 min. Inulin (unlabeled or (*carboxymethyl*-^{14}C) labeled) was also used for longer incubation (in incubations lasting 2 min or less the use of inulin as an extracellular space marker may have led to an underestimation).

Chemicals

D-[U-^{14}C]Fructose, D-[1-^{14}C]galactose, D-[1-^{3}H]mannitol and Aquasol were suppled by New England Nuclear Chemicals, Dreieichenhain, West Germany. 6-Deoxy-D-[1-^{3}H]glucose was prepared by the ^{3}H-labeling service of the Radiochemical Centre Amersham, Bucks, England and purified in our laboratory by paper chromatography as described elsewhere*[16]. D-[U-^{14}C]Glucose, 3-*O*-methyl-(D-[1-^{3}H]-glucose), 3-*O*-methyl-(D-[U-^{14}C]glucose), [1-^{14}C]mannitol and NCS were also supplied by the Radiochemical Centre, Amersham.

6-Deoxy-D-glucose (D-isorhamnose, D-quinovose) was purchased from Koch-Light Laboratories Ltd, Colnbrook, England; 2-deoxy-D-glucose from Sigma, St. Louis, Mo.; 3-*O*-methyl-D-glucopyranose from Calbiochem, Los Angeles, Calif.; α-D-glucose from Serva Entwicklungslabor Heidelberg, West Germany; D-galactose, D-fructose, D-mannitol and inulin from Merck, Darmstadt, West Germany; ethacrynic acid from Merck Sharp and Dohme Research Laboratories, Rahway, N. J.; L-fucose and ouabain from Fluka, Buchs SG, Switzerland. Arbutin (purum, Fluka) was recrystallized from hot water after treatment with charcoal. Phlorizin (purum, Fluka) was recrystallized twice from hot water. D-Fructose (Merck) was purified with the use of glucose oxidase according to Messer and Dahlqvist[17] in order to remove traces of contaminating glucose. All other reagents were of the highest purity grade.

The purity of all tracers used and the identity of the recovered labeled sugars in the intestine were checked by paper chromatography (*n*-butanol–pyridine–water; 30:10:15, by vol.) and by autoradiography. Of all radioactive compounds tested, only in 3-*O*-methyl-(D-[1-^{3}H]glucose) a non negligible radioactive impurity was detected. A number of experiments were, therefore, repeated with ^{14}C-labeled 3-*O*-methylglucose.

Symbols

J_{mc}, unidirectional flux from the mucosal medium into the small intestine (c).

J^{mx}_{mc}, maximum J_{mc}, *i.e.*, J_{mc} at infinitely large substrate concentration (for single hyperbolic functions).

K_t, substrate concentration at $J_{mc}=J^{mx}_{mc}/2$.

Subscripts

1 and 2 refer to Carrier 1 and Carrier 2, respectively, according to the two-carrier model (see Summary and Discussion).

* Specific activity obtained: 0.4 Ci/mmole 6-deoxy-D-glucose.

a, kinetic parameters calculated from J_{mc} values obtained at low substrate concentrations (Table IIA) and assuming a single carrier.

b, kinetic parameters calculated from J_{mc} values obtained at high substrate concentrations (Table IIA) and assuming a single carrier.

R, kinetic parameters calculated from J_{mc} values obtained at both high and low substrate concentrations by forcing them into a single regression line (Table IIB), thereby assuming a single carrier again.

RESULTS

Uptake as a function of time

Fig. 1 shows the uptake of 6-deoxyglucose and D-glucose as a function of incubation time. The uptake is linear up to 6 min (with concentrations up to 30 mM) and up to 5 min (with concentrations up to 60 mM). Therefore, sugar uptake was measured routinely using 2-min incubations, although incubation lasting 5 min yielded comparable results. The correction for the mannitol or inulin space never amounted to more than 5% of tissue water in 2-min incubations. Since the mucosal side only was exposed to the medium, under these conditions the uptake was an accurate measurement of the unidirectional flux from the medium into the small intestine (J_{mc}).

Effect of the "inert" replacement to isoosmolarity

The kinetic experiments to be described in the following paragraphs required incubations at high substrate concentrations and the concentration of NaCl had, therefore, to be reduced to 93 mM. Therefore, rather large concentrations of inert substances had to be added in order to maintain isoosmolarity in those experiments in which the substrate concentrations were low. The effects of Tris–HCl, mannitol and also fructose on the J_{mc} of some monosaccharides were investigated first. The data

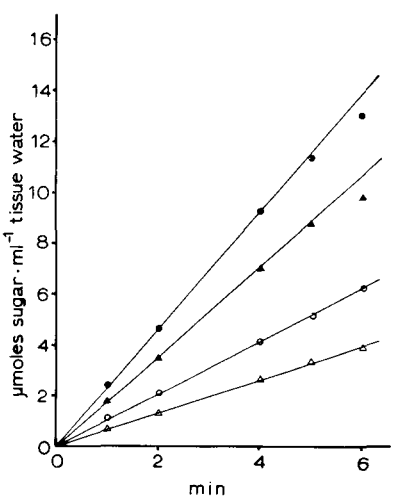

Fig. 1. Uptake of D-glucose, 2 mM (○) and 60 mM (●), and of 6-deoxy-D-glucose, 2 mM (△) and 60 mM (▲), into hamster small intestine as a function of time. Each point is the average of 3 experiments. Conditions described under Material and Methods.

are summarized in Fig. 2. At low substrate concentrations Tris–HCl caused a weak but not significant inhibition of the uptake of glucose (A), galactose (C), 3-*O*-methylglucose (E) and 6-deoxyglucose (G) as compared with mannitol or fructose (K_i for Tris < 200 mM).

At high substrate concentrations no significant difference in J_{mc} values of all

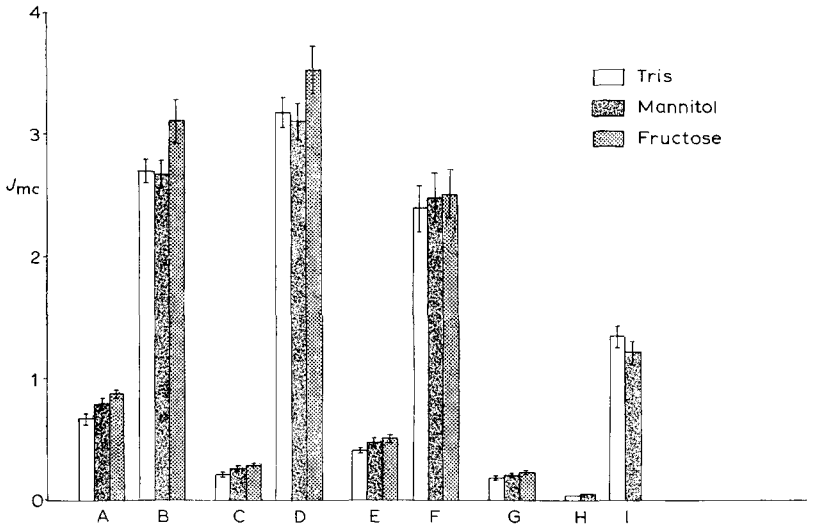

Fig. 2. Uptake of D-glucose, 0.3 mM (A), 30 mM (B); D-galactose, 0.45 mM (C), 30 mM (D); 3-*O*-methylglucose, 2 mM (E), 30 mM (F); 6-deoxyglucose, 1 mM (G); and D-fructose, 0.5 mM (H), 30 mM (I) into hamster small intestine in the presence of Tris–HCl, mannitol or D-fructose at concentrations giving, when added to that of the sugar, a total of 106 mosM (with glucose) or or 100 mosM (with the other monosaccharides).

TABLE I

IONIC COMPOSITION OF THE INCUBATION MEDIA

Uptake of	*Standard incubation mixture (mM)*		*Na^+-free incubation mixture (mM)*	
D-Galactose	Na^+	93	Choline$^+$	93
6-Deoxyglucose	K^+	3.9	K^+	3.9
3-*O*-Methylglucose	Ca^{2+}	2.3	Ca^{2+}	2.3
D-Fructose	Mg^{2+}	0.8	Mg^{2+}	0.8
	Cl^-	85	Cl^-	85
	SO_4^{2-}	0.8	SO_4^{2-}	0.8
	Phosphate	0.8	Phosphate	0.8
	Bicarbonate	16.2	Bicarbonate	16.2
D-Glucose	Na^+	93	Choline$^+$	93
	K^+	3.9	K^+	3.9
	Cl^-	60	Cl^-	80
	Phosphate	0.8	Phosphate	0.8
	HCO_3^-	16.2	HCO_3^-	16.2
	F^-	20		

glucalogues was found in the presence of either Tris or mannitol, whereas in presence of fructose the J_{mc} values of glucose and galactose are slightly increased (B, D). A slight Tris inhibition of D-galactose uptake had already been reported by Barnett *et al.*[18]. For our experiments both Tris–HCl and mannitol were used as inert component. It should be pointed out, however, that the same pattern, *i.e.* the same deviation from linearity in Eadie plots (see next paragraph) were obtained with either inert component. Fructose uptake is not inhibited by Tris (Fig. 2, H and I).

TABLE IIA

APPARENT K_t AND J^{mx}_{mc} VALUES FOR MONOSACCHARIDE UPTAKE IN HAMSTER SMALL INTESTINE, DETERMINED ALTERNATIVELY AT LOW (a) OR AT HIGH (b) SUBSTRATE CONCENTRATION RANGES FROM EADIE PLOTS

The data are expressed as $\bar{x} \pm$S.E. (n).

Substrate	*Concentration range (mM)*	K_{ta} *(mM)*	J^{mc}_{mca}	K_{tb} *(mM)*	J^{mx}_{mcb}
D-Glucose	0.1– 1.0	0.8±0.1 (4)	1.5		
	5.0– 30.0			2.8±0.5 (4)	2.5
D-Galactose	0.1– 3.0	3.5±0.3 (5)	2.1		
	10.0–100			12.3±3 (5)	3.9
3-*O*-Methylglucose	0.3– 10.0	25.4±4 (3)	3.5		
	20.0–100			27.5±4 (3)	3.6
D-Fructose	0.3– 10.0	16.2±2 (3)	2.1		
	20.0–100			19.0±3 (3)	2.3
6-Deoxyglucose	0.1– 3.0	2.7±0.3 (4)	2.0		
	5.0– 30.0			2.9±0.4 (3)	2.2

TABLE IIB

APPARENT K_t AND J^{mx}_{mc} VALUES FOR MONOSACCHARIDE UPTAKE IN HAMSTER SMALL INTESTINE

Values were determined over a wide concentration range from Eadie plots (Figs 3–7) on the assumption of one carrier (by forcing the data into a regression line) as well as on the assumption that glucose and galactose are each transported by two carriers (by a computer program).

Monosaccharide (concn range, mM)	*1 carrier assumed*		*2 carriers assumed*			
	K_{tR} *(mM)*	J^{mx}_{mcR}	K_{t1} *(mM)*	J^{mx}_{mc1}	K_{t2} *(mM)*	J^{mx}_{mc2}
D-Glucose (0.1–60)	1.3	2.2	0.7	1.3	15	1.5
D-Galactose (0.1–100)	5.6	3.1	3.5	1.9	50	2.2
6-Deoxy-D-glucose (0.1–30)	2.8	2.0	2.8	2.0	—	—
3-*O*-Methyl-D-glucose (0.3–100)	28	3.5	28	3.5	—	—
D-Fructose (0.3–100)	17	2.0	17	2.0	—	—

The velocity of uptake as a function of substrate concentration

The medium-small intestine unidirectional flux (J_{mc}) of some monosaccharides was first studied at two different concentration ranges (high and low), and the K_t and $J^{mx}{}_{mc}$ values for each sugar were determined for each range by a regression line in Eadie plots. As Table IIA shows, a sizable difference in the apparent transport constants between high and low substrate concentration ranges was found for both glucose and galactose but not for 6-deoxyglucose, 3-*O*-methylglucose and fructose. In further experiments on monosaccharide uptake over the total concentration range, the results for glucose and galactose again deviated significantly from the linearity expected for a simple carrier. Figs 3–6 report the velocity of uptake (J_{mc}) as a function of substrate concentrations in Eadie plots. A number of possible reasons were considered and ruled out to explain this deviation from the linearity in Eadie plots found for glucose and galactose.

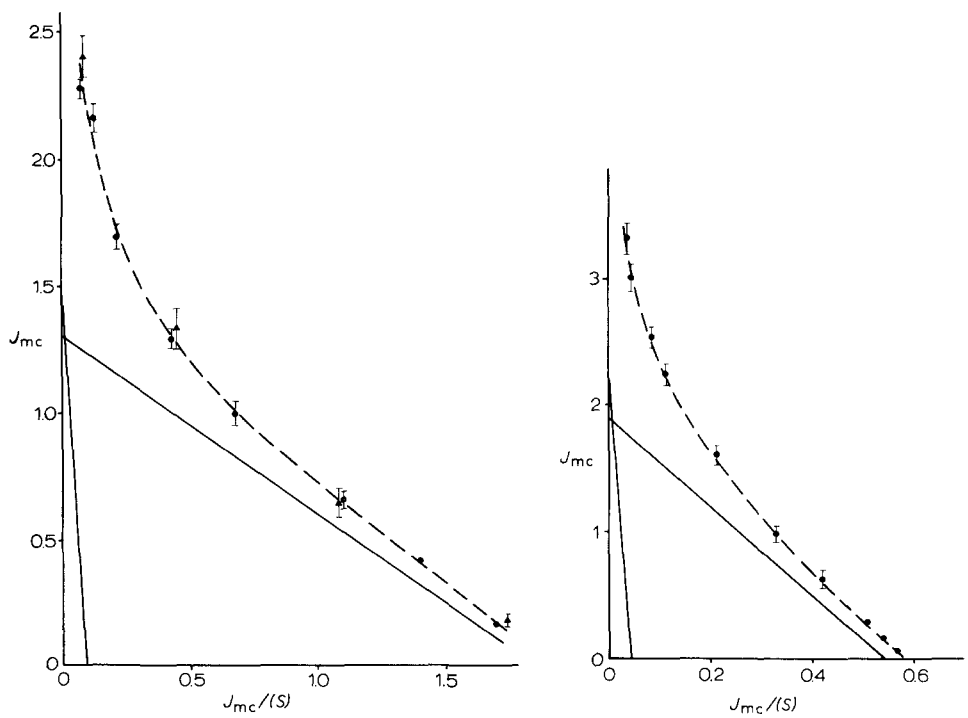

Fig. 3. Unidirectional flux of D-glucose from the medium into hamster small intestine (J_{mc}) as a function of glucose concentration (Eadie plot). Concentration range: 0.1 to 60 mM; 2-min incubations at 37 °C in modified, Ca^{2+}- and Mg^{2+}-free Krebs–Henseleit buffer, pH 7.2, containing 93 mM Na^+ and 20 mM F^-. ●, average of 16 experiments with hamsters fed *ad libitum*; ▲, average of 4 experiments with fasted animals; the bars indicate the S.E. – —, theoretical lines computed by iteration for each of the two carriers[24]; – – –, calculated line for the sum of the two carriers.

Fig. 4. Unidirectional flux of D-galactose from the medium into hamster small intestine (J_{mc}) as a function of glactose concentration (Eadie plot). Concentration range: 0.1 to 100 mM; 2-min incubations at 37 °C in Krebs–Henseleit buffer, pH 7.2, containing 93 mM Na^+. ●, average of 16 experiments; the bars indicate the S.E. ——, theoretical lines computed[24] for each of the two carriers; – – –, calculated line for the sum of the two carriers.

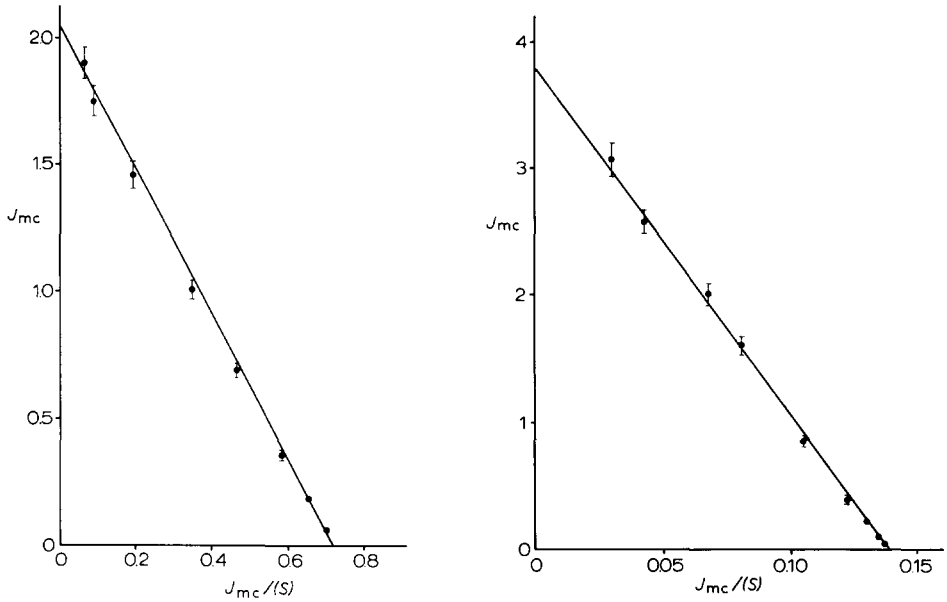

Fig. 5. Unidirectional flux of 6-deoxy-D-glucose from the medium into hamster small intestine (J_{mc}) as a function of 6-deoxyglucose concentration (Eadie plot). Concentration range: 0.1 to 30 mM. ●, average of 6 experiments; the bars indicate the S.E. Conditions as in Fig. 4. The experimental points fall on one single regression line.

Fig. 6. Unidirectional flux of 3-*O*-methyl-D-glucose from the medium into hamster small intestine (J_{mc}) as a function of 3-*O*-methyl-D-glucose concentration (Eadie plot). Concentration range: 0.3 to 100 mM. ●, average of 8 experiments; the bars indicate the S.E. Conditions as described under Fig. 4. The experimental points fall on one single regression line.

(a) Errors in the determination of the substrates in the tissue can be ruled out, because the sugars in question, *i.e.* glucose and galactose, were determined both chemically or enzymatically and by tracer techniques. The extracellular space was accounted for in each incubation mixture; the use of mannitol (which probably slightly overestimates the extracellular space[19]), or of inulin (which probably slightly underestimates it with short incubation times) did not noticeably affect the results.

(b) Glucose, arising from the degradation of intracellular glycogen may enhance the entry of the extracellular sugar by counterflow. However, the same pattern was observed irrespective of whether the animals had fasted prior to sacrifice, or not [animals fasting up to 3 days were investigated (Fig. 3)]. In addition, sugars reported to have the same apparent average K_t (in 140 mM Na^+) such as D-glucose and 6-deoxyglucose[20] yielded different patterns (*cf.* Figs 3 and 5).

(c) Although the incubation times were short, the uptake may have corresponded to the J_{mc} at low substrate concentrations, and to net flux at higher ones. But again, this source of error should have lead to a different kind of deviation from linearity, *i.e.* to an artificially low J_{mc} at high substrate concentrations.

(d) The deviation from linearity might have been due to the inert replacement used to reach isoosmolarity. However, this possibility can be ruled out since: (i) the

inhibition by Tris or mannitol was minimal; (ii) no such deviation was observed for other monosaccharides under similar conditions (Figs 5 and 6).

(e) Unstirred layers effects cannot be made responsible for the deviation from linearity in the plots of Figs 3 and 4, because (i) 6-deoxyglucose and 3 methylglucose under identical conditions did yield linear plots (Figs 5–7) and (ii) the effect of unstirred layers is larger at low than at high substrate concentrations[21]. This would have lead to a deviation from linearity opposite from that which we found (Figs 3 and 4).

(f) Finally, our data are not consistent with an interconversion of two carrier forms having different affinities for the substrate (C–Z interconversion, *e.g.* Wilbrandt and Rosenberg[22])*.

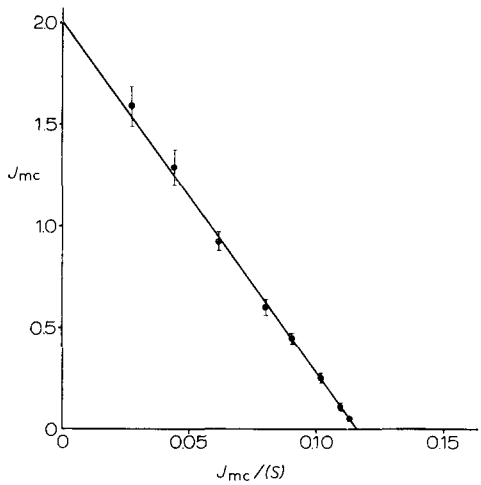

Fig. 7. Unidirectional flux of D-fructose from the medium into hamster small intestine (J_{mc}) as a function of D-fructose concentration (Eadie plot). Concentration range: 0.5 to 60 mM. ●, average of 8 experiments. The bars indicate the S.E. Conditions as described under Fig. 4. The experimental points fall on one single regression line.

Although considerations in the footnote do not entirely rule out possibility f, a likely explanation of the data in Figs 3 and 4 is the existence of at least two transport systems for glucose and galactose. Therefore, the kinetic parameters of Table IIB were calculated.

Mutual inhibition of monosaccharide uptake

In order to check the validity of the different transport parameters for each monosaccharide listed in Table IIB as well as to examine the specificity of the expected

* This argument will be discussed on the basis of the treatment given by Kotyk and Janáček to which the reader is referred (ref. 23, p. 140, eqns 22–24). The data in Figs 3 and 4 could be fitted by the rate equation for this model which contains substrate terms both to the first and to the second power (Eqn 22). From the Michaelian ranges at low and at high substrate concentrations it is possible to calculate their respective maximum velocity terms, the term from the high substrate concentrations $[2c_tm/(1+K_{ZS}/n)]$ always being smaller than the term from the lower substrate concentrations $(2c_tm)$ (Eqns 23, 24). However, in no case (Table IIB) is the "maximum velocity" obtained experimentally from the higher substrate concentrations smaller than the "maximum velocity" obtained from the lower substrate concentrations.

TABLE III

MUTUAL INHIBITION BETWEEN SOME FREE GLUCALOGUES AT VARIOUS CONCENTRATIONS OF SUBSTRATE AND INHIBITOR

The mucosal medium–tissue unidirectional flux (J_{mc}), as percent of that found in the absence of inhibitor, is compared with that calculated under one of the following assumptions: (i) one carrier common to all glucalogues tested (either from the kinetic parameters found at low substrate concentrations, Table II A, see here below, Column *i,a*; or from those obtained by forcing the data into a single regression line, Table II B, see here below, Column *i,R*. (The percent inhibition calculated from the kinetic data found at high concentrations, Table II A fits even less well with the experimental data); (ii) two carriers, of which one is common to all glucalogues tested and the other to glucose and galactose alone (Column *ii*); three carriers, of which one is common to all glucalogues tested, the second is specific for glucose alone, and the third is specific for galactose alone (Column *iii*). Naturally, the three-carrier assumption differs from assumption ii only for interactions between glucose and galactose. Abbreviations: D-glu, D-glucose; D-gal, D-galactose; 6-DG, 6-deoxy-D-glucose; 3-MG, 3-*O*-methyl-D-glucose.

Substrate (*mM*)	*Inhibitor* (*mM*)	*Percent* J_{mc} *found* [$\bar{x} \pm S.E.$ (*n*)]	*Calculated unidirectional flux*			
			(i) One carrier assumed		*(ii) Two carriers assumed*	*(iii) Three carriers assumed*
			(i,a) From K_{ta} *and* J_{mca}^{mx}	*(i,R) From* K_{tR} *and* J_{mcR}^{mx}		
D-glu (0.3)	6-DG (30)	22.9 ± 1.7 (4)	11.0	10.3	**18.0**	
	D-gal (30)	20.2 ± 1.1 (4)	13.8	17.9	**17.7**	20.2
	arbutin (30)	17.5 ± 1.4 (4)	10.3	9.3	**16.9**	
	3-MG (75)	54.3 ± 4.4 (3)	31.4	31.5	**40.0**	
D-gluc (1.0)	D-gal (10)	50.2 ± 1.1 (6)	44.1	48.4	**50.1**	51.8
	(30)	28.4 ± 0.7 (6)	20.8	23.8	**26.7**	30.5
	(60)	18.0 ± 0.3 (6)	11.6	13.5	**16.2**	21.9
	(100)	13.1 ± 0.6 (6)	7.3	8.6	**10.8**	17.8
D-glu (2.0)	D-gal (10)	60.0 ± 1.3 (6)	55.1	57.4	**61.7**	64.0
	(30)	33.5 ± 1.0 (6)	29.0	31.0	**36.4**	41.7
	(60)	22.5 ± 0.8 (6)	17.0	18.3	**23.1**	30.9
	(100)	16.6 ± 0.5 (6)	10.9	11.9	**15.7**	25.4
D-glu (10)	D-gal (60)	52.3 ± 3.2 (4)	44.1	43.4	**50.8**	64.5
	arbutin (60)	54.3 ± 1.9 (4)	36.0	26.6	**58.9**	
	3-MG (75)	84.8 ± 3.2 (3)	74.3	76.4	**89.9**	
D-gal (1.0)	D-glu (10)	12.5 ± 1.6 (4)	9.3	13.4	**13.1**	16.7
	(30)	5.3 ± 0.4 (4)	3.3	4.9	**5.8**	11.8
	(60)	4.0 ± 0.3 (4)	1.7	2.5	**3.2**	10.5
	(100)	2.6 ± 0.3 (4)	1.0	1.5	**2.0**	9.9
D-gal (0.6)	3-MG (10)	75.3 ± 4.2 (3)	74.5	75.6	**78.6**	
	(60)	40.2 ± 2.6 (3)	32.8	34.1	**40.8**	
3-MG (1.0)	D-gal (10)	28.3 ± 2.2 (3)	26.7	35.4	**26.4**	
	(30)	10.9 ± 2.0 (3)	10.8	15.2	**10.7**	
	(60)	5.0 ± 1.5 (3)	5.7	8.1	**5.5**	

TABLE III (*continued*)

Substrate (*mM*)	*Inhibitor* (*mM*)	*Percent* J_{mc} *found* [$\bar{x} \pm$ *S.E.* (*n*)]	*Calculated unidirectional flux*			
			(*i*) *One carrier assumed*		(*ii*) *Two carriers assumed*	(*iii*) *Three carriers assumed*
			(*i,a*) *From* K_{ta} *and* J_{mca}^{mx}	(*i,R*) *From* K_{tR} *and* J_{mcR}^{mx}		
3-MG (7.5)	D-gluc (7.5)	13.5 ± 1.8 (3)	12.2	18.0	**10.6**	
	D-gal (7.5)	40.1 ± 3.4 (3)	37.8	47.2	**37.2**	
	arbutin (7.5)	27.2 ± 2.3 (3)	30.2	29.7	**29.7**	
3-MG (10)	D-gal (10)	31.1 ± 2.8 (3)	32.9	41.8	**32.2**	
	(30)	15.0 ± 1.7 (3)	14.0	19.3	**13.5**	
	(60)	7.6 ± 0.9 (3)	7.6	10.7	**7.3**	
3-MG (10)	D-glu (0.75)	55.0 ± 3.4 (3)	60.0	70.2	**55.9**	
	(10)	9.4 ± 1.8 (3)	10.2	15.0	**8.8**	
	(30)	3.0 ± 1.1 (3)	3.7	5.6	**3.0**	
6-DG (1.0)	3-MG (10)	83.1 ± 3.9 (3)	77.4	79.2	**79.2**	
	(30)	57.2 ± 2.3 (3)	53.2	55.9	**55.9**	
	(100)	28.6 ± 1.7 (3)	25.5	27.5	**27.5**	
6-DG (1.0)	D-gal (10)	30.5 ± 1.9 (6)	32.2	41.8	**32.2**	
	(30)	14.9 ± 1.1 (6)	13.7	19.3	**13.7**	
	(60)	10.0 ± 1.3 (6)	7.3	10.7	**7.3**	
	(100)	8.1 ± 0.4 (6)	4.5	6.7	**4.5**	
6-DG (2.0)	D-gal (10)	43.2 ± 2.4 (11)	37.9	47.6	**37.5**	
	(30)	20.6 ± 0.8 (11)	16.7	23.2	**16.7**	
	(60)	14.2 ± 0.4 (11)	9.1	13.2	**9.1**	
	(100)	11.0 ± 0.3 (11)	5.7	8.3	**5.7**	
6-DG (2.0)	D-glu (10)	13.2 ± 0.4 (3)	12.2	18.2	**10.7**	
	(30)	5.3 ± 0.3 (3)	4.5	6.9	**3.9**	
	(60)	3.0 ± 0.3 (3)	2.2	3.6	**2.0**	
	(100)	2.3 ± 0.4 (3)	1.4	2.2	**1.2**	

different monosaccharide carriers, experiments on mutual inhibition among glucose, galactose, 3-*O*-methylglucose, 6-deoxyglucose and fructose were performed. The experimental data from mutual inhibition experiments were compared with the theoretical data calculated by considering the following two possibilities: (i) a single carrier for all glucalogues; (ii) two monosaccharide carriers for glucose and galactose: one of them common to other glucalogues and the other apparently specific for glucose and galactose. For possibility i the apparent transport constants determined at low substrate concentrations (K_{ta} and $J^{mx}{}_{mca}$), (Table IIA), as well as the apparent transport constants determined over a wide concentration range by regression lines, (K_{tR} and $J^{mx}{}_{mcR}$) (Table IIB), were used. For possibility ii the apparent transport constants determined over a wide concentration range by a computer program[24] were used ($K_{t1,2}$, $J^{mx}{}_{mc1,2}$, see Table IIB).

As Table III shows, the best fit to our results is given by the assumption of two carriers for glucalogues, one common to all of them and another, with larger K_t values and essentially specific for glucose and galactose (among the monosaccharides tested).

Fructose uptake

Fructose is known to be transported by a different carrier system from the supposedly single glucalogue carrier (*e.g.* ref. 25). Fig. 7 reports an Eadie plot which is compatible with a single type of fructose carrier (K_t 17 mM, Table IIB). The K_t values for fructose transport in other species are: in the rat, 0.9 mM (ref. 26), in the rabbit, 18 mM (ref. 27) and in the guinea pig, 4 mM (ref. 28).

Table IV clearly shows that fructose uptake is not inhibited by glucose, 3-*O*-methylglucose, sorbose, *myo*-inositol or arbutin. Thus, the fructose carrier is not identical with any of the glucalogue carriers, or with the inositol carrier[29]. The lack of inhibition of fructose uptake by sorbose has already been reported by others in

TABLE IV

LACK OF EFFECT OF MONOSACCHARIDES, PHLORIZIN OR *myo*-INOSITOL ON D-FRUCTOSE UPTAKE IN HAMSTER SMALL INTESTINE

D-*Fructose* (*mM*)	*Compound added* (*mM*)	*Percent uptake found* [$\bar{x} \pm S.E.$ (*n*)]
1.0	D-glucose (60)	98 ± 3 (3)
85.0	D-glucose (15)	96 ± 2 (3)
7.7	3-*O*-methylglucose (30)	102 ± 2 (3)
60.0	3-*O*-methylglucose (30)	95 ± 4 (3)
0.1	L-sorbose (30)	99 ± 2 (3)
0.5	*myo*-inositol (30)	98 ± 3 (3)
10.0	*myo*-inositol (30)	103 ± 3 (3)
15.0	arbutin (30)	104 ± 3 (3)
15.0	phlorizin (1.2)	96 ± 4 (3)
85.0	phlorizin (0.2)	94 ± 5 (3)

TABLE V

UPTAKE OF MONOSACCHARIDES FROM Na^+-FREE MEDIA IN HAMSTER SMALL INTESTINE

Substrate (*mM*)	*Percent uptake found in Na^+-free media* [$\bar{x} \pm S.E.$ (*n*)]
6-Deoxyglucose (1.0)	5.0 ± 1.1 (4)
D-Glucose (1.0)	5.5 ± 1.3 (4)
6-Deoxyglucose (10)	14.6 ± 1.9 (3)
D-Glucose (10)	13.8 ± 0.6 (3)
D-Fructose (15)	102 ± 2.5 (3)
D-Fructose (90)	96 ± 3.2 (3)

the rat[30]. Phlorizin does not inhibit fructose uptake, as also already reported by a number of other authors.

Conflicting reports have been published on the Na^+-dependence of fructose uptake in the small intestine. Whereas the lack of this cation does not seem to affect fructose uptake in the rabbit[27,30], two contradicting reports are available in the case of rat intestine[26,30]. In the hamster (Table V) fructose uptake is independent of the Na^+ concentration in the medium, both at concentrations close to the K_t value, and well above it.

Arbutin inhibition of monosaccharide uptake

According to Alvarado and Crane[20], arbutin, a β-hydroquinone-glucoside, is transported by the sodium- and phlorizin-sensitive glucose carrier system with a K_t of 2–3 mM (in 140 mM Na^+). Because of this low K_t, arbutin is a suitable compound for inhibition studies on monosaccharide uptake. The question of whether arbutin interacts with both glucose carriers or with one only was approached as follows: the uptake of each glucalogue was measured over a wide concentration range

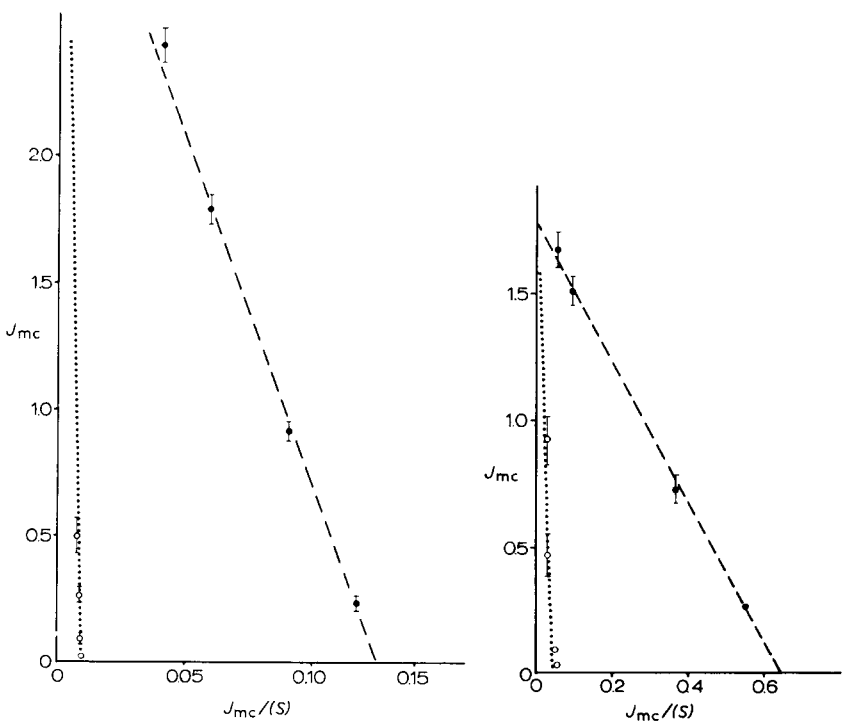

Fig. 8. Unidirectional flux of 6-deoxy-D-glucose from the medium into hamster small intestine (J_{mc}) in the absence (●) or in the presence (○) of arbutin (30 mM). Average of 4 experiments. The bars indicate the S.E. – – –, calculated from the assumption that arbutin inhibits 6-deoxy-D-glucose uptake with K_i 2.5 mM. Conditions as described under Fig. 4.

Fig. 9. Unidirectional flux of 3-*O*-methyl-D-glucose from the medium into hamster small intestine (J_{mc}) in the absence (●) or in the presence (○) of arbutin (30 mM). Average of 3 experiments. The bars indicate the S.E. – – –, calculated from the assumption that arbutin inhibits 3-*O*-methyl-D-glucose uptake with K_i 2.5 mM. Conditions as described under Fig. 4.

in the absence and presence of arbutin (30 mM). The experimental data are plotted according to Eadie (Figs 8–11). From the experiments with 6-deoxyglucose and with 3-*O*-methylglucose (Figs 8 and 9) the K_i value of arbutin for the (single) carrier of these sugars was calculated to be 2.5 ± 0.5 mM.

The data of Table III suggest that the carrier transporting 6-deoxyglucose and 3-*O*-methylglucose is identical with the low K_t carrier (K_{t1}) for glucose and galactose. The inhibition of uptake of 6-deoxyglucose, 3-methylglucose, glucose and galactose by arbutin (Tables III and VI and Figs 10 and 11) is fully accounted for by the interaction of arbutin with the low K_t carrier alone.

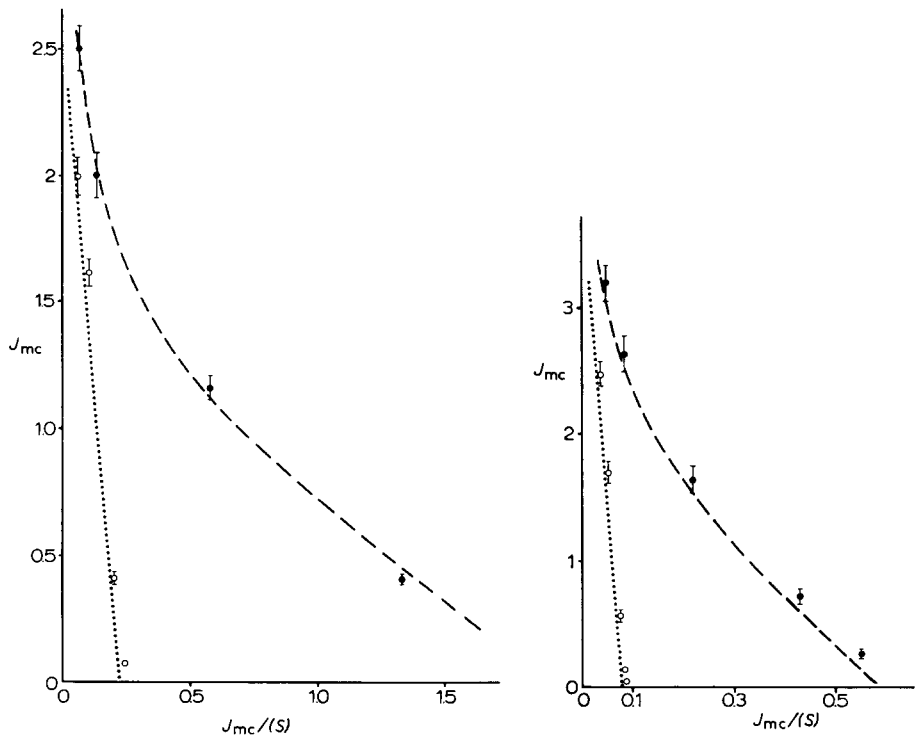

Fig. 10. Unidirectional flux of D-glucose from the medium into hamster small intestine (J_{mc}) in the absence (●) or in the presence (○) of arbutin (30 mM). Average of 5 experiments. The bars indicate the S.E. – – –, is the same as in Fig. 3. · · · ·, calculated from the assumption that arbutin inhibits the less specific low K_t carrier alone, with K_i 2.5 mM. Conditions as described under Fig. 3.

Fig. 11. Unidirectional flux of D-galactose from the medium into hamster small intestine (J_{mc}) in the absence (●) or in the presence (○) of arbutin (30 mM). Average of 4 experiments. The bars indicate the S.E. – – –, is the same as in Fig. 4. · · · ·, calculated from the assumption that arbutin inhibits the less specific low K_t carrier alone, with K_i 2.5 mM. Conditions as described under Fig. 4.

Phlorizin inhibition of monosaccharide uptake

The inhibition of glucose, galactose, 6-deoxyglucose and 3-methylglucose uptake by phlorizin was investigated at various concentrations of both inhibitor and substrate (Fig. 12 and Table VII). Like arbutin inhibition (see previous paragraph), phlorizin inhibition of 6-deoxyglucose and 3-methylglucose uptake is described

TABLE VI

INHIBITION OF GLUCALOGUE UPTAKE BY ARBUTIN IN HAMSTER SMALL INTESTINE

The uptake found (as percent of the uninhibited uptake) is compared with that calculated under any of the following assumptions: one carrier alone, (with which arbutin interacts); two carriers (arbutin interacting with the low K_t carrier alone). The same K_t and $J^{mx}{}_{mc}$ values were used as in the calculations of Table III; K_i for arbutin, 2.5 mM.

Substrate (*mM*)	*Arbutin* (*mM*)	*Percent uptake found* [$\bar{x} \pm S.E.$ (n)]	*Calculated uptake*		
			One carrier assumed		*Two carriers assumed*
			From $K_{t\alpha}$ *and* $J^{mx}{}_{mc\alpha}$ (*Table IIA*)	*From* K_{tR} *and* $J^{mx}{}_{mcR}$ (*Table IIB*)	
Adult hamsters					
D-Glucose (0.3)	30	17.6 ± 1.7 (8)	10.3	9.3	16.9
D-Galactose (0.5)	30	15.5 ± 1.9 (8)	8.7	8.4	16.2
6-Deoxyglucose (0.5)	30	9.5 ± 0.5 (8)	9.0	8.9	8.9
3-*O*-Methylglucose (9.5)	3.0	55.7 ± 4.5 (5)	53.5	52.8	52.7
3-*O*-Methylglucose (9.5)	30	10.6 ± 1.9 (5)	10.3	10.0	10.0
Young hamsters (8 days)					
D-Glucose (0.3)	30	16.9 ± 1.3 (3)	—	—	14.8
D-Galactose (1.5)	30	11.2 ± 1.2 (3)	—	—	16.2
6-Deoxyglucose (1.5)	30	8.3 ± 1.6 (3)	—	—	8.9

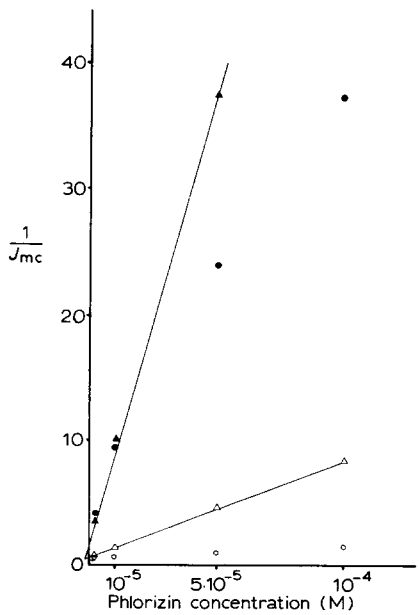

Fig. 12. Inhibition of uptake of D-glucose, 0.3 mM (●) and 10 mM (○), and of 6-deoxy-D-glucose, 1 mM (▲) and 10 mM (△), by phlorizin. (Dixon plot). Data from Table VII.

satisfactorily by the interaction of the glucoside with the single carrier for these sugars. With each of the two substrates a K_i for phlorizin of $(1.8 \pm 0.2) \cdot 10^{-6}$ M was calculated.

TABLE VII

INHIBITION OF GLUCALOGUE UPTAKE BY PHLORIZIN IN HAMSTER SMALL INTESTINE

Substrate (*mM*)	*Phlorizin* (*M*)	*Percent uptake found* [$\bar{x} \pm$ S.E. (*n*)]
6-Deoxyglucose (1.0)	$2 \cdot 10^{-6}$	55.6 ± 1.6 (3)
	$1 \cdot 10^{-5}$	20.5 ± 0.5 (3)
	$5 \cdot 10^{-5}$	6.0 ± 0.9 (3)
6-Deoxyglucose (10)	$2 \cdot 10^{-6}$	84.4 ± 2.8 (3)
	$1 \cdot 10^{-5}$	45.5 ± 1.5 (3)
	$5 \cdot 10^{-5}$	14.0 ± 1.3 (3)
D-Glucose (0.3)	$2 \cdot 10^{-6}$	59.2 ± 3.2 (3)
	$1 \cdot 10^{-5}$	24.7 ± 0.9 (3)
	$5 \cdot 10^{-5}$	10.1 ± 0.7 (3)
	$1 \cdot 10^{-4}$	6.3 ± 0.5 (3)
	$1.2 \cdot 10^{-3}$	1.2 ± 0.2 (3)
D-Glucose (10)	$2 \cdot 10^{-6}$	92.3 ± 5.1 (3)
	$1 \cdot 10^{-5}$	82.0 ± 2.9 (5)
	$5 \cdot 10^{-5}$	52.9 ± 3.5 (3)
	$1 \cdot 10^{-4}$	34.1 ± 2.6 (5)
	$1.2 \cdot 10^{-3}$	9.0 ± 1.5 (5)
D-Galactose (1.9)	$1 \cdot 10^{-5}$	27.6 ± 1.3 (5)
	$5 \cdot 10^{-5}$	7.9 ± 0.7 (5)
	$1 \cdot 10^{-4}$	5.1 ± 0.5 (5)
	$1.2 \cdot 10^{-3}$	0.4 ± 0.4 (5)
D-Galactose (30)	$1 \cdot 10^{-5}$	65.7 ± 1.9 (5)
	$5 \cdot 10^{-5}$	30.9 ± 1.6 (5)
	$1 \cdot 10^{-4}$	20.0 ± 1.1 (5)
	$9.2 \cdot 10^{-4}$	2.4 ± 0.5 (5)
3-Methylglucose (9.5)	$2.5 \cdot 10^{-6}$	49.1 ± 4.6 (5)
	$1 \cdot 10^{-5}$	19.5 ± 2.4 (5)
	$5 \cdot 10^{-5}$	4.6 ± 1.3 (5)

The Dixon plots for glucose (Fig. 12, dots) and for galactose were not linear. With the same assumptions made above for arbutin inhibition, and assuming that phlorizin inhibition of the high-K_t carrier of glucose and galactose is also competitive, phlorizin K_{i2} values of $1.5 \cdot 10^{-4}$ M (with glucose) and $2.0 \cdot 10^{-5}$ M (with galactose) were calculated. The reason for the discrepancy between the K_{i2} values for the same inhibitor is not clear*. The "classical" K_i values of phlorizin inhibition of glucalogue uptake (in 140 mM Na^+) is about 10^{-6} M (refs 20, 31) but a value in the range of 10^{-4} has been reported for the inhibition of glucose uptake from sucrose[32].

* Five experiments only were carried out, and the difference between the K_{i2} values did not reach statistical significance. Since this problem was regarded as a side-issue in the present paper, it was not investigated further.

Na^+-dependence of monosaccharide uptake

The uptake of glucose and of 6-deoxyglucose was tested at 0 and 93 mM Na^+ and at two substrate concentrations (1 and 10 mM) (Table V). Lack of Na^+ inhibited the uptake of both sugars to the same extent, which strongly suggests that both glucalogue carriers are equally Na^+-dependent.

The uptake of glucose, galactose and fructose in baby hamsters

Figs 13 and 14 report the uptake of glucose and galactose by the small intestine of 6–8-day-old hamsters. In these animals also the Eadie plots are described by the sum of two linear functions, the apparent K_{t1} and K_{t2} values of which are identical with those in adult animals. However: (i) both J^{mx}_{mc} are larger than those in the adult animals (possibly due to a larger surface/weight ratio in the baby hamsters), and (ii) the $J^{mx}_{mc1}/J^{mx}_{mc2}$ ratios are 1.47 ± 0.175 (5) [$\bar{x}\pm$S.E. (n)] for glucose and 1.51 ± 0.067 (5) for galactose in baby hamsters, as compared with 0.92 ± 0.085 (10) for glucose and 0.86 ± 0.073 (10) for galactose in adult animals (the difference in the ratios in baby and adult animals is significant with $P<0.01$ for glucose and $P<0.001$ for galactose). This observation supports the conclusion that glucose and galactose are each transported by two carriers. Apparently there is more of Carrier 1 (low K_t), or less of Carrier 2 (high K_t) at birth.

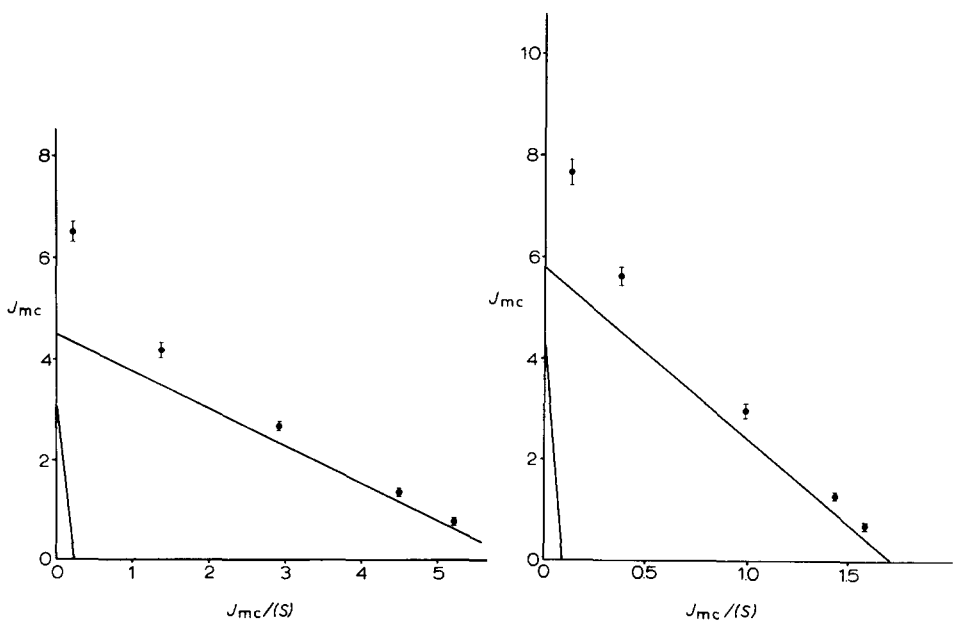

Fig. 13. Unidirectional flux of D-glucose (J_{mc}) from the medium into small intestine of young hamsters (8 days) as a function of glucose concentration (Eadie plot). Concentration range 0.15 to 30 mM. ●, average of 5 experiments. The bars indicate the S.E. For further details see legend to Fig. 3.

Fig. 14. Unidirectional flux of D-galactose (J_{mc}) from the medium into the small intestine of young hamsters (8 days) as a function of galactose concentration (Eadie plot). Concentration range 0.45 to 60 mM. ●, average of 5 experiments. The bars indicate the S.E. For further details see legend to Fig. 4.

Fructose uptake in baby hamsters has the same K_t as in adult animals (16.5 ± 1.6 mM). The J^{mx}_{mc} is, however, not larger than in adults ($1.9\ \mu moles \cdot min^{-1}$), compared with $2.0\ \mu moles \cdot min^{-1} \cdot ml^{-1}$ in adult hamsters.

DISCUSSION

The results reported in the previous sections can be summarized as follows: (1) The dependence of the unidirectional medium–mucosa flux on the substrate concentration is described by a single horizontal hyperbola in the cases of 6-deoxyglucose and of 3-*O*-methylglucose, but by the sum of two such hyperbolas in the cases of both glucose and galactose. (2) The mutual inhibition between glucalogues, as well as the inhibition by arbutin and by phlorizin are not satisfactorily described by the assumption of a single transport system common to all glucalogues, but are compatible with the existence of two transport systems of overlapping specificity; (3) In baby hamsters the K_t values of the two transport systems are identical with those found in adult animals, but the ratio between the two J^{mx}_{mc} values is different from that in the adults.

As we described the individual results, we considered and ruled out a number of alternative explanations. Our data seem to be compatible with one model only, *i.e.* there are in the small intestine of hamster at least two Na^+-dependent and phlorizin-sensitive transport systems for glucalogues. Carrier 1 has lower K_t values (for glucose and galactose) and broad specificity: it interacts with D-glucose, D-galactose, 6-deoxy-D-glucose, 3-*O*-methyl-D-glucose and arbutin. It is the more prominent at birth. The other system, Carrier 2, has larger K_t values for glucose and galactose and seems to interact with these two monosaccharides only among those tested. It is less prominent than Carrier 1 at birth. It is not entirely impossible that Carrier 2 is, perhaps, two carriers; the mucosal to serosal flux of glucose and galactose in adult rats are differently sensitive towards uranyl ions[33]; oral tolerance tests with glucose or galactose do not always yield identical results in human glucose-galactose malabsorption[34]; monosaccharide-induced ΔP values, as well as the apparent K_t values for monosaccharide transport, respond to fasting and hormones in different ways for glucose and galactose in the rat[35,36]. On the other hand, the mutual inhibition of glucose and galactose is fully accounted for by the existence of Carrier 1 + Carrier 2 alone and the $J^{mx}_{mc1}/J^{mx}_{mc2}$ ratio is about the same for glucose and galactose in adult as well as in young hamsters. Thus, the case for more than two carriers for free glucose and galactose is still unsettled.

The small intestine is, of course, composed of a number of cell types and the enterocytes themselves are present at different stages of development. Our data do not indicate whether both transport systems for glucalogues are located in the same cell type(s) or not; although naturally the enterocytes are the most likely cells to carry both systems. With other small intestinal preparations, *e.g.* isolated cells[37] vesicles from brush border membranes[38] which would allow the location of the systems, an easy determination of J_{mc} is not possible at the moment. However, the procedure used by us to determine uptake limits the cell types involved to those located at the mucosal surface, because the mucosa alone was exposed to the medium. Experiments on the uptake of glucose and 6-deoxyglucose along the small intestine show a predominance of Carrier 1 in some segments and of Carrier 2 in others (Honegger, P. and Gershon, E., unpublished).

Is there any relationship between a glucalogue carrier and the disaccharidase-associated transport systems?

Crane *et al.*[32] have reported that the monosaccharides arising from disaccharides can be transported across the brush border membrane by system(s) not accessible to *free* monosaccharides. One of these transport systems has been artificially reconstituted *in vitro* by incorporating the sucrase–isomaltase complex into black lipid membranes[39]. The apparent K_i values of monosaccharides for sucrase have been determined only with glucose (19 mM) and fructose (105 mM) and only for the rabbit enzyme (Semenza, G. and v. Balthasar, A-K., unpublished). However, if the values for hamster sucrase are in the same order of magnitude, one may entertain the possibility that Carrier 2 may be identical with one or more disaccharidases. This may also explain the apparent stimulation of glucose uptake by high concentration of fructose (Fig. 2). However, Tris is a powerful inhibitor of intestinal disaccharidases (reviewed in ref. 40) and competes (at least in rabbit sucrase) for the glucose subsite (Semenza, G. and v. Balthasar, A-K., unpublished). Compared to this, Tris has little effect, if any at all, on glucose uptake at low or at high substrate concentrations (Fig. 2); and the presence of Tris does not alter significantly the Eadie plots of glucose (Honegger, P., unpublished results). In addition, the ratio between the J^{mx}_{mc} of Carrier 1 and the J^{mx}_{mc} of Carrier 2 is the same for glucose and galactose in adult (0.87) as well as in baby hamsters (1.5), although the activity of intestinal α-glucosidases is higher in adult hamsters than in baby hamsters, whereas the converse is true for lactase. If Carrier 2 were identical with one or more of the intestinal disaccharidases, one should have expected that the ratio between the two J^{mx}_{mc} should change with age in a different fashion for glucose (if transported mainly by α-glucosidases) than for galactose (if transported mainly by lactase). This argument, however, is weakened by the present limited knowledge of the properties of the disaccharidases-associated transport systems, and the rather broad "glycone" specificity of small-intestinal phlorizin hydrolase and lactase[3]. At the present moment, a definite conclusion on this specific point cannot be made.

Fructose and myoinositol

The glucalogue carriers identified above do not detectably participate in fructose uptake. In fact, fructose uptake is not inhibited by glucalogues, arbutin, phlorizin (Table IV) or by the absence of Na^+ (Table V). These observations agree with what has been reported by most authors[27,30] for a number of species, but are at variance with one report on fructose uptake in rat small intestine[26]. Methodological differences are not responsible for the discrepancy, because these authors[26] used the same method of measuring J_{mc} as we did[2]. Fructose uptake is apparently due to a single carrier (K_t 17 mM) (Fig. 6) and is not inhibited by sorbose (Table IV). The transport system for *myo*-inositol is independent of both the system for fructose (no inhibition of fructose uptake by *myo*-inositol, Table IV; no inhibition of inositol uptake by fructose, ref. 29) and of those for the glucalogues (no inhibition of glucalogue uptake by *myo*-inositol, Honegger, P., unpublished results; see also ref. 29). Glucalogues, however, do partially inhibit inositol net uptake[29] possibly *via* an effect of Na^+ at the cellular face of the inositol carrier[41].

Previous indications for the existence of more than one system for glucalogue uptake

One may wonder why most previous workers have failed to detect the existence of two, rather than one, transport systems for free glucalogues. There may be a number of reasons for this: (i) Both systems are Na^+-dependent, (Table V). They may be indistinguishable in 0 and 140 mM Na^+, but may have different characteristics at the intermediate Na^+ concentration which we have used (93 mM).

(ii) Tissue preparations in which the serosal side is also exposed to the medium may lose part of the absorbed substrate through it or through the cuts, particularly at longer incubation times. If this loss occurs more at high than at low substrate concentrations, the deviation from the expected hyperbola may be smaller and fall within the experimental error.

(iii) Due to the need of keeping the medium isoosmolar, and to the logical wish of studying sugar transport at optimal, *i.e.* high Na^+ concentrations (140 mM), most workers have confined themselves to relatively narrow substrate concentration ranges.

(iv) Two of the most widely used sugars in studies of this kind (6-deoxyglucose and 3-methylglucose) are absorbed exclusively by Carrier 1.

(v) We have confined ourselves to hamsters. Species differences may exist (as it is known in other respects).

Scattered throughout the years, however, there had been indications and suggestions that the picture of a single transport system common to all glucalogues may be incomplete. After the older observations from Cori[42], Fisher and Parsons[43] and Du Ruisseau and Quastel[44], Newey *et al.*[33] reported in 1966 that the transport of glucose across rat small intestine is more sensitive to uranyl nitrate than that of galactose. Crane[45] pointed out in 1968 some apparent contradictions in the substrate specificity of the assumed single transport system.

The absorption of glucose in human glucose–galactose malabsorption is often less impaired (as judged from oral tolerance tests) than that of galactose; Meeuwisse[34] actually suggested the existence of two transport systems in normal human gut. Our data on the change in relative activities of Carrier 1 and 2 in hamsters with age (see above) and the apparent improvement of glucose tolerance in human glucose–galactose malabsorption on growing[34] make it possible that in normal human baby one glucalogue carrier only is present (or very much prevealing), and that a second carrier will develop on growing; in glucose–galactose malabsorption the first carrier only would be affected.

Finally, Levin's group[35,36] has reported recently that the apparent K_t values for sugar transport and for the monosaccharide-induced ΔP in the rat are affected by hormone and by fasting in different ways for different sugars.

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