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FURTHER EVIDENCE FOR THE MULTIPLICITY OF CARRIERS FOR FREE GLUCALOGUES IN HAMSTER SMALL INTESTINE

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SUMMARY

The ratios between the Na^+-dependent unidirectional fluxes of glucose and 6-deoxyglucose change along the small intestine of hamsters. This observation provides further evidence for the existence of more than a single common carrier for the Na^+-dependent transport of free monosaccharides into the small intestinal mucosa.

INTRODUCTION

Recently, two carrier systems for free glucalogues in adult and baby hamster small intestine have been demonstrated [1]. Carrier 1 is common to glucose, galactose, 6-deoxyglucose, and 3-methylglucose, whereas Carrier 2 is essentially specific for glucose and galactose [1]. This paper reports the different distribution of these two carriers along hamster small intestine, thereby further substantiating the conclusions reached previously.

MATERIALS AND METHODS

Either adult hamsters (approx. 2.5 months old) or baby hamsters (8 days old) of either sex were used.

The adults were fed ad libitum, with free access to water. The babies were left with the mother until shortly before decapitation.

Incubation Method A

The small intestines were rinsed with ice-cold saline, everted over a rod and fixed at both ends. The rod was vertically immersed in a cylinder containing incubation medium [1] (modified Krebs–Henseleit buffer containing 93 mM Na^+). The medium was gassed from the bottom with O_2–CO_2 (95/5, v/v) and maintained at 37 °C with a water jacket. After 2 min incubation, the uptake was stopped by rinsing in ice-cold standard Krebs–Henseleit buffer. The small intestine was removed from the rod, gently blotted, its length measured, and cut into 1.5-cm pieces. The pieces were individually weighed, dissolved in 1 ml Nuclear Chicago Tissue Solubilizer (NCS)

and counted after addition of 10 ml scintillator (0.5% 2-(4′-*tert*-butylphenyl)-5-(4″-biphenyl)-1,3,4-oxadiazol in toluene).

Incubation Method B

The small intestine was rinsed, everted, cut and mounted in frames as described elsewhere [2]. The pieces were incubated individually in a continuous sequence for 2 min at 37 °C in a vigorously shaking water bath. Details on the method and the composition of incubation medium have been described previously [1]. The unidirectional sugar uptake values (J_{mc}) were corrected for extracellular space, as determined with [^{3}H]mannitol (0.25 μM). In the present paper ratios between J_{mc} values obtained under identical conditions were considered throughout.

Although the conclusions as to distribution pattern were independent of the method used to measure uptake, Method A yielded substantially higher values than Method B in adult hamsters. This was possibly due to a better exposure of the mucosal surface. In baby hamsters both methods yielded comparable uptake values.

The chemicals used were the same as in a previous paper [1].

The uptake of D-glucose, 6-deoxy-D-glucose, and D-fructose was measured in separate small segments along the entire length of the small intestine. The individual data were normalized (position in the intestine as percent of total intestinal length and uptake as percent of highest measured uptake per experiment) and plotted. The results from various experiments under identical conditions were averaged, which yielded a characteristic pattern. The experimental data from Method A and Method B were first evaluated separately until they proved to be practically superposable (Fig. 1, A, B). Therefore in later experiments the results from the two methods were pooled (Fig. 1, C, D).

RESULTS AND DISCUSSION

Fig. 1 shows that the uptake of the monosaccharides studied was similarly distributed along the length of the intestine, a maximum being clearly recognizable between the duodenum and jejunum as well as between the jejunum and ileum (Peak 1 and Peak 2, respectively). The apparent splitting of Peak 1 with Method A is due to a large lymph node situated there which was discarded in Method B only. Peak 1 remained relatively constant in position and measured uptake (J_{mc}). Peak 2 was broader and more variable: in some experiments, the height of Peak 2 was about half that of Peak 1, while in other experiments Peak 2 was as high or even higher than Peak 1. The cause of these individual differences is not clear (hormonal [4–6], nervous [7] influences?).

Under the conditions of the experiments in Fig. 1 little difference could be detected between the distribution pattern of monosaccharide uptake mediated by both Carrier 1 and Carrier 2 (glucose), that mediated by Carrier 1 alone (6-deoxyglucose), and that mediated by a further and fundamentally different (Na^+-independent) carrier (fructose). Similar observations were reported by Crane and Mandelstam [3].

In order to reveal more subtle differences in the relative distribution of Carriers 1 and 2 along the intestine, the simultaneous uptake of glucose and 6-deoxyglucose or fructose was studied from the same incubation media with Method A. In order to

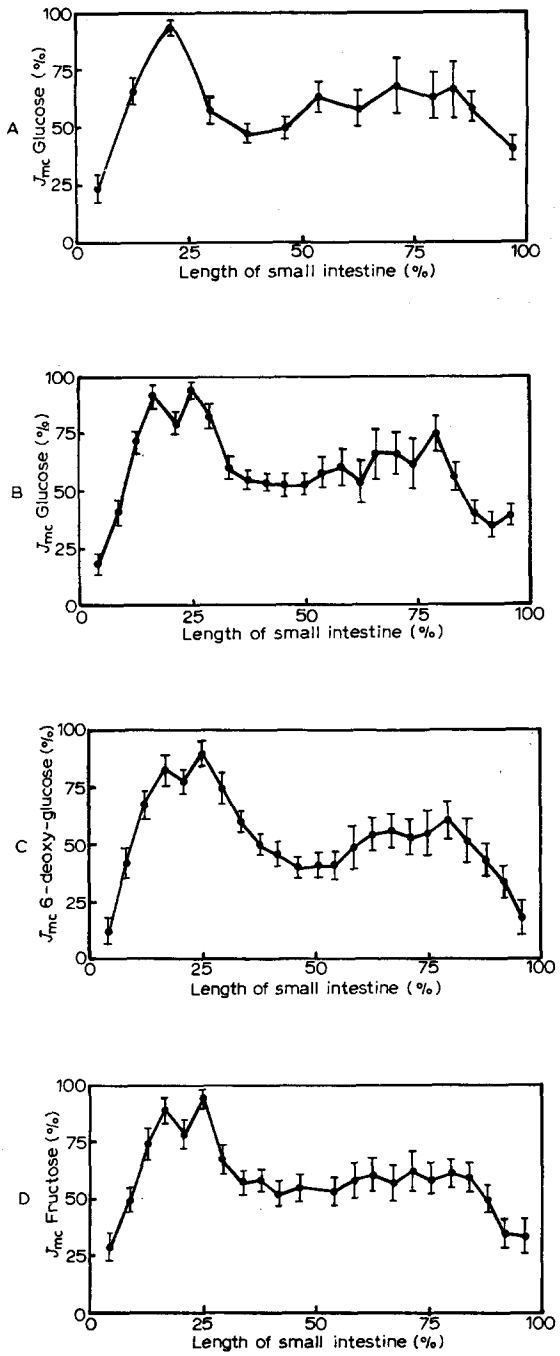

Fig. 1. Monosaccharide uptake along the length of adult hamster small intestine. (A) D-Glucose (1 mM), Method B; $n = 8$ The large lymph node in the region of peak 1 was discarded. (B) D-Glucose (1 mM), Method A; $n = 8$. (C) 6-Deoxyglucose (1 mM), Methods A+B; $n = 10$. (D) D-Fructose (1 mM) Methods A+B; $n = 9$. Each point is the mean ($\pm$S.E.) of n observations.

magnify small differences in some experiments 30 mM arbutin (which inhibits the glucalogue uptake by Carrier 1 only) was added to the media. Both baby and adult hamsters were investigated.

The concentrations of glucose and 6-deoxyglucose which were present simultaneously in the incubation media were approximately equal multiples of their respective apparent K_m values for Carrier 1. (The apparent K_m value of glucose for Carrier 1 is 0.7 mM; that of 6-deoxyglucose is 2.8 mM [1]). In this way the two sugars were expected to inhibit each other's uptake via Carrier 1 to about the same extent.

The extracellular space was assumed to be 2.4%, as it was found consistently in a number of previous experiments with Method A.

The results are reported in Figs 2 (adult) and 3 (baby hamsters). Clearly, the uptake of glucose, (which is transported by both Carrier 1 and 2) relative to that of 6-deoxyglucose (which is transported by Carrier 1 alone) shows a rise in the distal end of ileum. This indicates a higher Carrier 2/Carrier 1 ratio in this area of ileum. The difference compared with other areas of the small intestine is statistically significant (Table I).

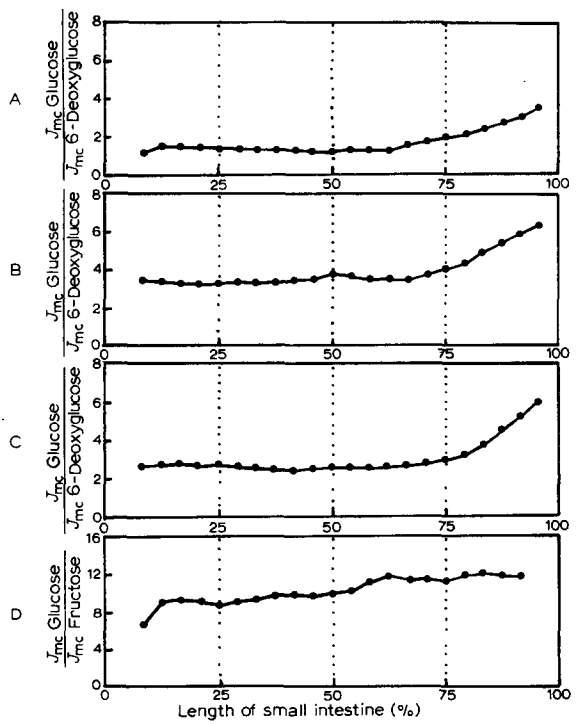

Fig. 2. Simultaneous uptake of D-glucose and 6 deoxyglucose or of D-glucose and D-fructose along the length of adult hamster small intestine (Method A). The data are expressed as ratios between the measured uptake values (J_{mc}), as indicated. Sugar composition in the media: (A) D-Glucose (1 mM), 6-deoxyglucose (3 mM); $n = 6$. (B) D-Glucose (1 mM), 6-deoxyglucose (3 mM), arbutin (30 mM); $n = 6$. (C) D-Glucose (0.3 mM), 6-deoxyglucose (1 mM), arbutin (30 mM); $n = 7$. (D) D-Glucose (1 mM), D-fructose (1 mM); $n = 6$. Each point is the mean of n observations. The average values of the ratios ($\overline{Q} \pm$S.E.) in the individual intestinal sections are shown in Table Ia.

TABLE I

SIMULTANEOUS UPTAKE OF D-GLUCOSE AND 6-DEOXYGLUCOSE OR OF D-GLUCOSE AND D-FRUCTOSE ALONG THE SMALL INTESTINE (METHOD A)

Significance test between the $\bar{Q}$ values of individual intestinal segments (I–IV for adult animals, I–III for baby hamsters). $\bar{Q}$ = average of all Q values in one segment

$$Q = \frac{J_{mc}\text{ (glucose)}}{J_{mc}\text{ (6-deoxyglucose)}} \text{ or } Q = \frac{J_{mc}\text{ (glucose)}}{J_{mc}\text{ (fructose)}}$$

$\bar{Q}_{max}$ = average of the maximum Q values in Peak 1. N.S. = not significant.

Sugar compostion of the media (mM)	n	$\bar{Q}$ (±S.E.)				$\bar{Q}_{max}$ (±S.E.)	P			P		
		I	II	III	IV		I–IV	II–IV	III–IV	I–II	I–III	II–III
(a) Adult hamsters												
D-Glucose (1) 6-deoxyglucose (3)	6	1.6 (±0.1)	1.5 (±0.1)	1.7 (±0.1)	2.1 (±0.2)	1.7 (±0.1)	<0.01	<0.005	<0.01			
D-Glucose (1), 6-deoxyglucose (3), arbutin (30)	6	3.4 (±0.1)	3.5 (±0.1)	3.7 (±0.1)	4.3 (±0.2)	3.4 (±0.2)	<0.0025	<0.0025	<0.0025			
D-Glucose (0.3), 6-deoxyglucose (1), arbutin (30)	7	2.7 (±0.2)	2.7 (±0.1)	2.8 (±0.1)	4.0 (±0.4)	3.0 (±0.3)	<0.005	<0.0025	<0.0025			
D-Glucose (1), D-fructose (1)	6	8.8 (±0.6)	10.0 (±0.7)	11.5 (±1.1)	11.1 (±0.8)	10.2 (±1.5)	<0.01	N.S.	N.S.			
(b) Baby hamsters												
D-Glucose (1), 6-deoxyglucose (3), arbutin (30)	6	1.5 (±0.1)	1.9 (±0.2)	2.7 (±0.2)		1.7 (±0.1)				N.S.	<0.0025	<0.005
D-Glucose (0.3), 6-deoxyglucose (1), arbutin (30)	6	1.9 (±0.1)	2.0 (±0.1)	2.9 (±0.2)		1.9 (±0.1)				N.S.	<0.0025	<0.0025
D-Glucose (1), D-fructose (1)	6	16.2 (±1.1)	19.4 (±2.0)	30.5 (±2.8)		18.1 (±2.2)				N.S.	<0.0025	<0.005

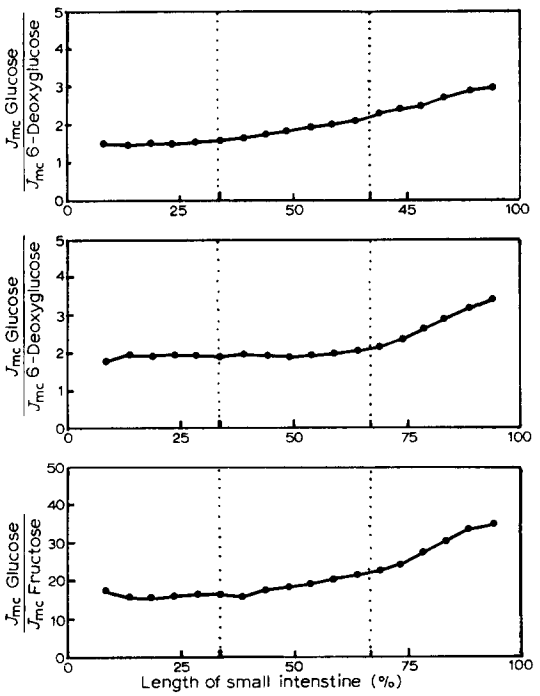

Fig. 3. Simultaneous uptake of D-glucose and 6-deoxyglucose or of D-glucose and D-fructose along the length of baby small intestine (Method A). The data are expressed as in Fig. 2. Sugar composition in the media: (A) D-Glucose (1 mM), 6-deoxyglucose (3 mM), arbutin (30 mM); $n = 6$. (B) D-Glucose (0.3 mM), 6-deoxyglucose (1 mM), arbutin (30 mM); $n = 6$. (C) D-Glucose (1 mM), D-fructose (1 mM); $n = 6$. Each point is the mean of n observations. The average values of the ratios ($\bar{Q} \pm$S.E.) in the individual intestinal sections are shown in Table Ib.

TABLE II

SIMULTANEOUS UPTAKE OF D-GLUCOSE AND 6-DEOXYGLUCOSE OR OF D-GLUCOSE AND D-FRUCTOSE ALONG THE LENGTH OF ADULT HAMSTER SMALL INTESTINE

The uptake in the different thirds of the small intestine was measured with Method B.
$\bar{Q}$ = average of the Q values in one third; N.S. = not significant.

$$Q = \frac{J_{mc}\text{ (glucose)}}{J_{mc}\text{ (6-deoxyglucose)}} \text{ or } Q = \frac{J_{mc}\text{ (glucose)}}{J_{mc}\text{ (fructose)}}$$

Sugar composition in the media (mM)	n	$\bar{Q} \pm$S.E.			P	
		I	II	III	I–III	II–III
D-Glucose (5 mM), 6-deoxy-glucose (5 mM)	3	3.7±0.3	4.2±0.2	6.3±0.2	<0.005	<0.005
D-Glucose (5 mM), 6-deoxyglucose (5 mM) arbutin (30 mM)	3	7.1±0.2	8.0±0.1	10.8±0.5	<0.01	<0.025
D-Glucose (5 mM), D-fructose (5 mM)	3	6.4±0.3	7.0±0.4	7.5±0.6	N.S.	N.S.

The data also indicate similar distribution patterns of Carrier 1 and fructose carrier along the intestine. Similar results were obtained with Method B (Table II).

The conclusion that Carrier 1 and Carrier 2 are not distributed along the length of the small intestine in parallel is in agreement with other findings which indicate that the small intestine is neither anatomically nor physiologically uniform. Rather, the structural and functional properties are characteristically distributed, e.g. villus size [8, 9] and disaccharidase activities [10]. The maximal activity of the Na^+-dependent sugar transport carrier has been localized in jejunum in experiments both in vivo [11–13] and in vitro [3, 14–16]. The transport systems for bile salts [17] and vitamin B_{12} [18], instead, are solely located in ileum, which demonstrates that independent carriers in the small intestine can exhibit different distribution patterns.

The increasing contribution of Carrier 2 toward the distal end of the small intestine raised the question of whether this transport system might extend to the large intestine also. Thus the uptake of glucose was investigated along the proximal third of the large intestine. None was found, in agreement with other observations in hamster [3], rats [19], and humans, cats or guinea pigs [20], and in variance to other observations on dogs and mice [20].

Finally, a comparison between the results on baby hamsters with those on adult animals indicates that the contribution of Carrier 2 to the uptake of glucalogues, relative to that of Carrier 1, increases with the animal's growth. At the same time, the uptake of fructose increases as well (Table I).

In young hamsters approximately the same J_{mc} values were obtained with both methods, while the J_{mc} value for adult hamsters was substantially higher, when using Method A (possibly due to a better exposure of the mucosal surface). Therefore, the uptake observed using Method B was higher in baby than in adult hamsters, while the measurements with Method A led to a small increase in J_{mc} value for adult animals in comparison with young.

The J_{mc} value obtained for fructose with both methods was 4–6 times smaller in young animals, indicating that the age dependence of fructose uptake is much greater than that of glucalogue uptake.

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REFERENCES

1 Honegger, P. and Semenza, G. (1973) Biochim. Biophys. Acta 318, 390–410
2 Semenza, G. (1969) Biochim. Biophys. Acta 173, 104–112
3 Crane, R. K. and Mandelstam, P. (1960) Biochim. Biophys. Acta 45, 460–476
4 Crane, R. K. (1961) Biochem. Biophys. Res. Commun. 4, 436–440
5 Levin, R. J. and Smyth, D. H. (1963) J. Physiol. 169, 755–769
6 Aulsebrook, K. A. (1965) Biochem. Biophys. Res. Commun. 18, 165–169
7 Hardcastle, P. T. and Eggenton, J. (1973) Biochim. Biophys. Acta 298, 95–100
8 Fisher, R. B. and Parsons, D. S. (1950) J. Anat. 84, 272–282
9 Altmann, G. G. and Enesco, M. (1967) Amer. J. Anat. 121, 319–336

10 Semenza, G. (1968) Handbook of Physiology (Code, C. F. et al. eds.) Section 6: Alimentary Canal, Vol. V., pp. 2543–2566, Am. Physiol. Soc., Washington
11 Lium, R. and Florey, H. W. (1939) Q. J. Exp. Physiol. 29, 303–319
12 Goldner, M. G. and Haerem, A. T. (1943) Proc. Soc. Exp. Biol. Med. 52, 186–188
13 Weckesser, E. C., Ankeney, J. L., Portmann, A. F., Price, J. W., Cebul, F. A. (1951) Surgery 30, 465–476
14 Fisher, R. B. and Parsons, D. S. (1953) J. Physiol. 119, 224–232
15 Korelitz, B. I. and Frank, E. D. (1959) Gastroenterology 36, 94–101
16 Barry, B. A., Matthews, J. and Smyth, D. H. (1961) J. Physiol. 157, 279–288
17 Lack, L. and Weiner, I. M. (1961) Am. J. Physiol. 200, 313–317
18 Strauss, E. W. and Wilson, T. H. (1960) Am. J. Physiol. 198, 102–107
19 Parsons, D. S. and Patersons, C. R. (1965) Q. J. Exp. Physiol. 50, 220–231
20 Robinson, J. W. L., Luisier, A. L. and Mirkowitch, V. (1973) Eur. J. Physiol. 345, 317–326