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ACTIVE INTESTINAL TRANSPORT OF D-FRUCTOSE

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SUMMARY

The uptake of D-fructose by the small intestine of the rat was studied *in vitro*.

1. Under the experimental conditions outlined, the small intestine of the rat accumulates D-fructose against a concentration gradient by an energy- and Na^+-dependent process with a K_m of 0.9 mM.

2. As with other actively transported sugars, accumulation against a concentration gradient was not achieved by the large intestine.

3. Phlorizin at a concentration of 0.1 mM did not adversely affect the accumulation of D-fructose but at 0.5 mM and above caused significant inhibition.

4. The actively transported sugars D-glucose and 3-*O*-methylglucose caused only moderate inhibition of the uptake of D-fructose, while L-sorbose had a somewhat more marked adverse effect.

5. The amino acids L-glycine, L-phenylalanine, L-hydroxyproline and L-lysine caused considerable inhibition of D-fructose uptake.

6. We suggest that the intestinal transport of D-fructose occurs *via* an active, carrier-mediated mechanism separate from that involved in the transfer of other actively transported sugars.

INTRODUCTION

Fructose is a common, and increasingly important dietary constituent[1] but the mechanism of its absorption has remained unsolved. In 1925, Cori[2] showed that sugars are absorbed from the gut at widely different rates. This suggests that sugars with intermediate rates of absorption, such as D-xylose and D-fructose, must be absorbed by some mechanism other than passive diffusion. Active transport of D-xylose by the intestine has subsequently been demonstrated[3,4], however, active transport of D-fructose has not been shown before our experiments. The preliminary results of this work have been reported briefly elsewhere[5] and are now given here in detail.

MATERIALS AND METHODS

Incubation technique

Weanling Wistar rats of both sexes and weighing 80–110 g and maintained on a standard laboratory diet were used. They were fasted for 48 h in cages with raised

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wire-mesh floors; water was allowed *ad libitum*. The animals were killed by cervical dislocation and the intestine gently stripped away from the mesentery. The isolated intestine was rinsed with 0.9 % NaCl at 20 °C and the duodenum was discarded. The rest of the small intestine was everted over a glass rod and cut transversely into segments 1.5 cm long; areas containing Peyer's patches were excluded. The segments were randomised in Krebs–Henseleit bicarbonate buffer[6] at 37 °C and were mounted in plexiglass chambers as described by Semenza[7]. The preparation was usually completed within 4 min of sacrifice. The mounted speciments were incubated in a Dubnoff-type metabolic incubator (Gallenkamp) at 37 °C, shaking at 90 oscillations/min.

The standard incubation medium consisted of 1 mM unlabelled D-fructose, 0.067 μCi/ml of D-[^{14}C]-fructose and 0.002 μmole/ml of [^{3}H]mannitol in Krebs–Henseleit bicarbonate buffer. The incubation conditions were altered separately in several ways as follows: (1) by preparing the medium for 1 h and then incubating under an atmosphere of N_2–CO_2 (95:5,v/v); (2) by performing incubations at 4 °C; (3) by adding 2,4-dinitrophenol; (4) by adding phlorizin; (5) by completely replacing Na^+ in the medium by K^+, Li^+ or $Tris^+$; (6) by partial replacement of Na^+ by K^+ in the medium to various intermediate degrees; (7) by adding other sugars and amino acids as indicated subsequently; (8) by altering the pH of the incubation medium. The pH measurements were made with glass electrodes on a Beckman Zeromatic pH meter with a relative accuracy of ± 0.05 pH unit.

The apparent K_m was determined by varying the concentration of unlabelled D-fructose from 0.5 to 5.0 mM while the concentration of D-[^{14}C]-fructose was constant; K_m was determined by the Lineweaver–Burk plot[8].

The uptake of D-fructose by the large intestine was examined using the standard technique given above.

After incubation, the mounted plexiglass chambers were rinsed thoroughly three times in cold Krebs–Henseleit bicarbonate buffer. The central tissue was removed by means of a metal punch, dried carefully on tissue paper and weighed on a torsion balance. The tissue was homogenized in distilled water and deproteinised by boiling.

Compounds

D-fructose, D-glucose, D-galactose, D-mannose, D-xyloxe, L-fructose, L-sorbose, L-glycine, L-lysine, L-phenylalanine and L-hydroxyproline were obtained from Koch-Light Laboratories; 2,4-dinitrophenol and lactic acid (analytical grade) from British Drug Houses; phlorizin from Fluka; 3-*O*-methylglucose from Calbiochem; fructose 1-phosphate, fructose 6-phosphate and fructose 1,6-diphosphate from Sigma Chemical Co. Isotopes were obtained from the Radiochemical Centre, Amerham; the radiochemical purity of D-[^{14}C]-fructose was 99 % and of [^{3}H]mannitol was 98 %. Chromatographic analysis of the unlabelled sugars showed them to be free from appreciable amounts of other carbohydrates.

Analytical methods

Radioactivity in samples of incubation medium and supernatant was determined in a Tracerlab Corumatic CM-200 liquid scintillation spectrometer. Counting vials contained 0.7 ml of the solution to be counted and 10 ml of scintillant as described by Patterson and Greene[9]. Appropriate corrections for background, counting efficiency and quenching were applied to all counts.

The possibility that D-fructose had been metabolised to other compounds during its transport across the intestine was evaluated as follows: Aliquots of supernatant from tissue homogenates and incubation medium at the end of each experimental period were concentrated under vacuum and examined by thin-layer chromatography in a solvent system consisting of *n*-propanol–acetic acid–water (14:1:1, by vol.). D-Fructose, fructose 1-phosphate, fructose 6-phosphate, fructose 1,6-diphosphate, D-glucose and lactic acid were used as reference standards. After separation, 2-cm strips from the origin to the solvent front were scraped into counting vials and their radioactivity determined as described above. The distribution of radioactivity was then related to the position of the reference compounds in guide strips sprayed with aniline–diphenylamine for sugars and fructose esters[10] and aniline–xylose for lactic acid[32].

Calculation of data

Results are expressed as μmoles per ml of tissue water in a stated period, assuming a water content of 80 % of the tissue wet weight[11]. Substrate uptake was calculated assuming the proportion of labelled D-fructose to be the same in the tissue as in the medium; corrections were made for passive diffusion according to final [^{3}H] mannitol concentrations. Results are also expressed as percent filling which was calculated according to the formula:

$$\text{Percent filling} = \frac{\mu\text{moles per ml tissue water}}{\mu\text{moles per ml medium}} \times 100$$

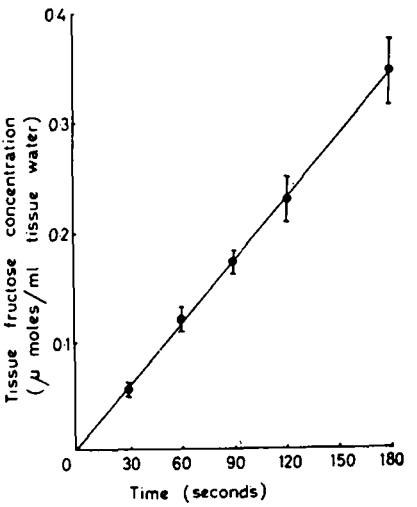
Standard arithmetical methods and Student's *t* test were used in the calculations.

RESULTS

The results of the chromatographic analyses at the conclusion of the experiments indicated that 83 ± 4.7 % of the total radioactivity remained attached to D-fructose. The remainder of the radioactivity was associated with lactic acid. Radioactivity associated with the fructose esters and with D-glucose was not significantly above the background count. All results were appropriately corrected for conversion to lactate and the results given subsequently represent true accumulation of D-fructose.

The early time course of transfer of D-fructose across the brush border is shown in Fig. 1. This illustrates that uptake is a linear function of time for at least 3 min and the line extrapolates to the origin. Under standard conditions, D-fructose accumulated over a period of 50 min against a final concentration gradient of more than 2 to 1 (Fig. 2). Hypoxia and hypothermia markedly inhibited this, so that accumulation against a concentration gradient was not achieved. The final mean tissue concentrations after incubation for 50 min were 0.53 and 0.69 μmoles per ml of tissue water, respectively.

The effect of metabolic inhibitors is shown in Tables I and II. In the presence of 2,4-dinitrophenol at concentrations from 0.25 to 1.0 mM, uptake of D-fructose was significantly less than control values. Substrate uptake was only slightly and insignificantly lowered in the presence of 0.1 mM phlorizin, but with 0.5–5.0 mM phlorizin uptake was considerably less than normal.



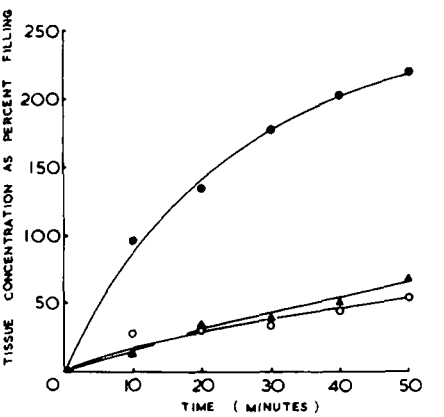


Fig. 1. Transfer of D-fructose across the mucosal border of the small intestine as a function of time. Uptake pattern illustrates the result of exposure of small-intestinal mucosa to a buffered solution containing 1 mM D-fructose, 0.067 μCi/ml of D-[^{14}C]fructose and 0.002 μmole/ml of [^{3}H]mannitol. The data are not corrected for the negligible mannitol space for these short time periods. The five points represent the mean and standard deviation of eight experiments in each instance.

Fig. 2. Active transport of D-fructose by rat small intestine *in vitro* and its inhibition by anoxia and hypothermia. The incubation medium contained 1 mM D-fructose. Under standard conditions, the pH of the medium was 7.4 and it was gassed with O_2–CO_2 (95:5, v/v); n = 5 (●). ○, gaseous phase of N_2–CO_2 (95:5, v/v) in this case n = 6. ▲, experiments performed at 4 °C (n = 2). In the control experiments corrections for mannitol space were made, in the other two categories this was not applied.

TABLE I

EFFECT OF 2,4-DINITROPHENOL ON UPTAKE OF D-FRUCTOSE BY THE INTESTINE

The initial concentration of D-fructose was 1 mM and the concentrations of 2,4-dinitrophenol used are indicated in the table. Duration of incubation was 15 min.

Experimental condition	*Number of experiments*	*Percent filling*	*P*	*Percent of control*
Control	6	102 ± 3	—	100
0.25 mM 2,4-dinitrophenol	4	70 ± 2	<0.001	68.6
0.5 mM 2,4-dinitrophenol	8	57 ± 5	<0.001	55.9
1.0 mM 2,4-dinitrophenol	8	54 ± 7	<0.001	52.9

TABLE II

EFFECT OF PHLORIZIN ON UPTAKE OF D-FRUCTOSE BY THE INTESTINE

Initial concentration of D-fructose was 1 mM. Duration of incubation was 60 min.

Experimental conditions	*Number of experiments*	*Percent filling*	*P*	*Percent of control*
Control	6	230 ± 27	—	100
0.1 mM phlorizin	6	200 ± 18	0.05<*P*<0.1	87.0
0.5 mM phlorizin	6	140 ± 28	<0.001	60.9
1.0 mM phlorizin	6	140 ± 28	<0.001	60.9
5.0 mM phlorizin	6	113 ± 13	<0.001	49.1

Effect of Na^+

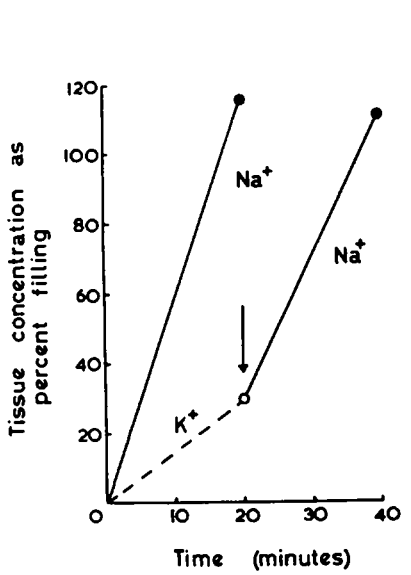
When Na^+ in the incubation medium was completely replaced by the cations K^+, Li^+ or $Tris^+$, the uptake of D-fructose was markedly reduced and accumulation against a concentration gradient was not demonstrated (Table III). To determine whether this effect was due to tissue damage its reversibility was tested by performing the incubation in a medium free from Na^+ (145 mM K^+) for 20 min and then transferring the tissue to the normal 145 mM Na^+-containing medium for a further 20 min. This showed the inhibitory effect to be acutely reversible (see Fig. 3). Graded replace-

TABLE III

EFFECT OF COMPLETE REPLACEMENT OF Na^+ BY K^+, Li^+ OR $Tris^+$ IN THE INCUBATION MEDIUM ON THE UPTAKE OF D-FRUCTOSE BY RAT SMALL INTESTINE

The initial concentration of D-fructose was 1 mM and the incubation time was 30 min.

Experimental condition	*Number of experiments*	*Percent filling*	*P*	*Percent of control*
Control Krebs–Henseleit bicarbonate buffer	5	177 ± 28	—	100
K^+ medium	4	52 ± 5	<0.001	29.4
Li^+ medium	4	44 ± 5	<0.001	24.9
$Tris^+$ medium	4	63 ± 7	<0.001	35.6



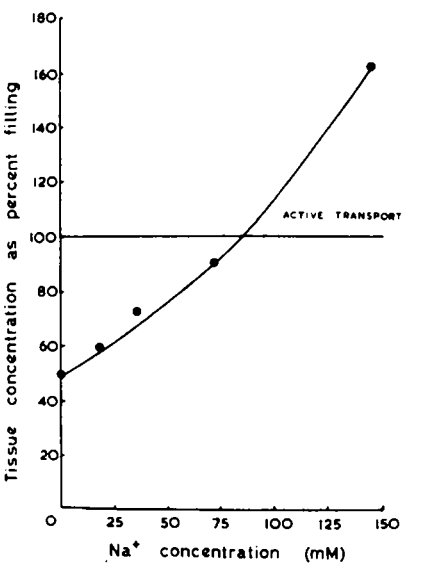


Fig. 3. Reversibility of inhibitory effect of removal of Na^+ from incubation on uptake of D-fructose by rat small intestine. Experiments were begun in a medium free from Na^+ (replaced by 145 mM K^+) for 20 min and tissues were then transferred to the normal 145 mM Na^+ incubation medium for the second part of the experiment. The initial concentration of D-fructose was 1 mM. The data represent a mean of four experiments in each instance.

Fig. 4. Effect of Na^+ on the uptake of D-fructose by rat small intestine. Incubations were performed in a medium containing 1 mM D-fructose at 37 °C and pH 7.4 with Na^+ content of the Krebs–Henseleit bicarbonate buffer replaced, as indicated, by K^+. Results indicate the mean of four experiments in each instance.

ment of Na^+ by K^+ caused different degrees of inhibition of uptake dependent on the extent of ionic replacement. When represented graphically this relationship is shown to be non-linear (Fig. 4).

Effect of other sugars

The influence of the presence of other sugars in the incubation medium on the uptake of D-fructose by the small intestine is detailed in Table IV. In the presence of 2.5 mM D-glucose, 2.5 and 5.0 mM D-galactose and 3 mM 3-*O*-methylglucose, the uptake of D-fructose was moderately reduced. 30 mM D-Mannose, 30 mM D-xylose, 10 mM D-fucose and 30 mM arabinose had little effect. In the presence of 20 mM L-fucose and 20 mM L-sorbose, the uptake of the substrate was significantly less than control values.

Effect of amino acids

In the presence of each of the four L-amino acids tested, the uptake of D-fructose was significantly less than normal ($P>0.001$ in each instance). These results are given in Table V.

TABLE IV

EFFECT OF OTHER SUGARS ON UPTAKE OF D-FRUCTOSE BY RAT SMALL INTESTINE

Sugars were added in the concentrations shown to the standard Krebs–Henseleit bicarbonate buffer containing 1 mM D-fructose. Incubations were performed for 30 min.

Addition to medium	*Number of experiments*	*Percent filling*	*P*	*Percent of control*
Control	5	178 ± 28	—	100
2.5 mM D-glucose	4	135 ± 6	<0.005	75.8
2.5 mM D-galactose	4	124 ± 13	<0.005	69.7
5 mM D-galactose	4	122 ± 17	<0.005	68.5
3 mM 3-*O*-methylglucose	2	148 ± 2	$0.9<P<0.95$	83.2
30 mM D-mannose	4	170 ± 15	$0.8<P<0.9$	95.5
30 mM D-xylose	4	162 ± 15	$0.1<P<0.2$	91.0
10 mM D-fucose	2	165 ± 7	$0.1<P<0.2$	92.7
30 mM arabinose	2	155 ± 7	$0.05<P<0.1$	87.1
20 mM L-fucose	4	138 ± 21	<0.02	77.5
20 mM L-sorbose	4	123 ± 14	<0.025	69.1

TABLE V

EFFECT OF AMINO ACIDS ON UPTAKE OF D-FRUCTOSE BY RAT SMALL INTESTINE

The initial concentration of D-fructose was 1 mM. L-amino acids were added to the incubation medium at a concentration of 10 mM. Incubations were performed for 50 min. In each instance $n = 8$.

Experimental condition	*Percent filling*	*P*	*Percent of control*
Control	235 ± 24	—	100
L-Glycine	128 ± 11	<0.001	54.5
L-Phenylalanine	98 ± 2	<0.001	41.7
L-Hydroxyproline	95 ± 7	<0.001	40.4
L-Lysine	85 ± 8	<0.001	36.2

Effect of pH

The effects of alteration of the pH of the incubation medium are shown in Fig. 5. For pH values below 7.4, a phosphate buffer was used. The pH values presented are those obtained after completion of the incubation. In each experiment a parallel series of media were incubated and used solely for measurement of pH. The relationship between pH and the rate of substrate accumulation is parabolic, with a pH optimum of 6.3.

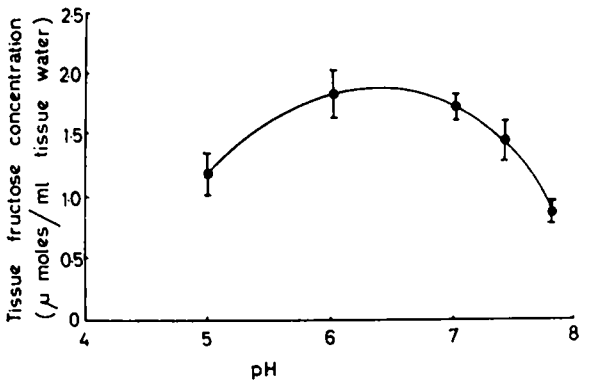


Fig. 5. Effect of pH on uptake of D-fructose by rat small intestine *in vitro*. Incubations were performed for 30 min. The results represent the mean and standard deviation from six experiments in each instance. The estimated pH optimum is 6.3.

Kinetics

The kinetics of entry of D-fructose into rat small intestine are shown diagrammatically in Fig. 6. This shows that D-fructose exhibits typical saturation kinetics with a K_m of 0.9 mM.

Transport of D-fructose by the large intestine

Transport against a concentration gradient did not occur when large intestine was used instead of small intestine under control conditions. The final mean tissue concentration was 0.48 μmole/ml of tissue water after incubation for 50 min.

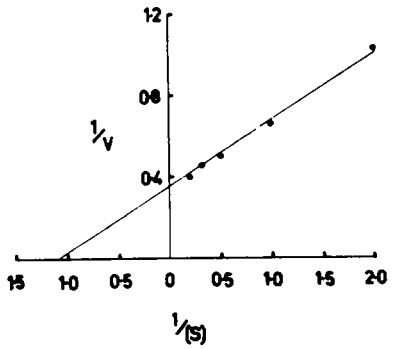


Fig. 6. Kinetics of entry of D-fructose into segments of rat small intestine *in vitro*. Incubations were done for 30 min in O_2–CO_2 (95:5, v/v). Average velocities (ordinate) and substrate concentrations (abscissa) of eight experiments are plotted as reciprocals (Lineweaver–Burk plot).

DISCUSSION

The results of these experiments demonstrate that, in the rat at least, D-fructose is accumulated by the small intestine against a concentration gradient. This process is energy and Na^+ dependent and is interfered with by certain other monosaccharides and the several L-amino acids investigated.

The use of the rat in these studies merits consideration. There are important differences between species in the metabolism of D-fructose by the intestinal mucosa. In the golden hamster[12], the guinea pig[13,14] and the dog[15] large amounts of the sugar may be converted to glucose. Conversion to lactate may also occur, although in man[16,17] and in the rat [13] this is not a major fate of D-fructose. The rat appears to resemble man also in that little conversion of fructose to glucose occurs.

The assay system used allowed an accurate quantitative assessment of the metabolic fate of the substrate. It demonstrated clearly that there was no significant transformation to D-glucose or the fructose esters, fructose 1-phosphate, fructose 6-phosphate or fructose 1,6-diphosphate, and that more than 80% of radioactivity remained attached to D-fructose within the tissue. The remainder of the radioactivity was due to transfer of the ^{14}C label to lactate and appropriate correction for this was made in the calculations. This, therefore, allows the conclusion that the final, estimated tissue levels indicate true concentrations of D-fructose and not some other metabolic by-product. Prolonged fasting causes a sharp decrease in the production of lactate from fructose in the rat[18], and the long period of fasting (48 h) used in the present experiments probably contributed to the demonstration of D-fructose uptake against a concentration gradient.

These experiments show that fructose accumulation in the small intestine of the rat is an energy-dependent process, with an estimated pH optimum of 6.3, and is inhibited by anoxia and hypothermia. As with other actively transported sugars, uptake of D-fructose against a concentration gradient could not be demonstrated in the large intestine[11]. Complete replacement of Na^+ by K^+ in the incubation medium caused significant inhibition of fructose transport, but this effect may to some extent be non-specific and related to tissue swelling[19]. However, marked inhibition also occurred when the replacing ion was Li^+ or $Tris^+$. With other substrates, some degree of activity of intestinal transport remains when Li^+ is used as the replacement ion[20]. Furthermore, the inhibitory effect of complete replacement of Na^+ by K^+ was acutely reversible and therefore not explicable by gross tissue disruption caused by the replacing ion. Graded cation replacement by K^+ demonstrated Na^+ dependence, the extent of which varied with the degree of replacement of Na^+. The non-linear relationship between the extent of inhibition and the degree of replacement of Na^+ may result from competition by the replacing ion for Na^+-binding sites as found for other sugars[21].

The metabolic inhibitor, 2,4-dinitrophenol has been found by others[22,23] to inhibit intestinal transport at concentrations from 0.1 to 1.0 mM. Although the lower concentration was not tested in the present experiments, significant inhibition was found at 0.25 to 1.0 mM; in each case $p<0.001$. Phlorizin at 0.1 mM had an insignificant effect on D-fructose accumulation in our system, in contrast to its effect on D-glucose and similarly transported sugars in many reported studies[24]. However, at concentrations of 0.5 mM and above, phlorizin significantly inhibited D-fructose uptake. This is consistent with the observation of Bihler[25] in a system where active

transport of D-fructose was not demonstrated. Schultz and Strecker[26], in agreement with our results, found that 0.1 mM phlorizin did not inhibit D-fructose uptake. If phlorizin competetively inhibits glucose transport[27] these observations might mean that there is a separate binding site for D-fructose with less affinity than D-glucose for phlorizin. However, current opinions on the effect of phlorizin on intestinal sugar transport are conflicting[25,27] and valid inferences cannot be made on the present information.

The rapidity of absorption of D-fructose by the small intestine has led some investigators[28,29] to suggest that a carrier mechanism must be involved. Recent experimental evidence favours this view[26] and a recent preliminary report[30] supports our earlier claim[5] for active transport of D-fructose in the rat. The early time course for D-fructose uptake demonstrated in our experiments (Fig. 1) illustrates unidirectional influx of the substrate during this time; this was found also by Schultz and Strecker[26]. Furthermore, the Lineweaver–Burk plot (Fig. 6) indicates that the mechanism involved abides by Michaelis–Menten kinetics.

The effect of several other sugars on the uptake of D-fructose suggests that the fructose carrier differs from that for sugars previously reported to be actively transported. The minor degree of inhibition found in the presence of D-glucose, D-galactose and 3-*O*-methylglucose is much less than found by most investigators when studying mutual inhibition between these substances which share the intestinal active transport pathway[24]. In sharp contrast to the findings of Schultz and Strecker[26], we found considerable inhibition of D-fructose uptake in the presence of L-sorbose, a ketohexose with close structural similarities to D-fructose. Considerable inhibition in the presence of the four L-amino acids tested also occurred in our experiments. This is consistent with a binding site for fructose separate from that for glucose and the actively transported sugars. The continuing controversy[31] about the effect of amino acids on small-intestinal sugar transport prevents firm conclusions about this being made from our data at the present time.

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