BBA 76046

SUPPORT FOR THE EXISTENCE OF AN ACTIVE TRANSPORT MECHANISM OF FRUCTOSE IN THE RAT

A. R. MACRAE* AND T. S. NEUDOERFFER
Department of Nutrition, University of Guelph, Guelph, Ontario (Canada)
(Received April 10th, 1972)

SUMMARY

1. The characteristics of the active transport mechanism for fructose entry were studied in the laboratory rat. By the criteria of transport against a concentration gradient, and a dependence of absorption on endogenous energy reserves, active transport of fructose was demonstrated in rat gut segments, measured both as uptake by the whole gut segment and the epithelial tissues.

2. The pattern of fructose absorption at various positions in the small intestine was studied. Fasting was observed to have little effect on fructose uptake. The optimum pH of fructose entry under the conditions used was 6.8.

3. The rate of fructose absorption was intermediate between that for glucose and mannose. High concentrations of sucrose and glucose inhibited fructose uptake, while sorbose and phlorizin did not. Fructose absorption was observed to be possibly Na^+ stimulated, but an absolute dependence on that ion was not observed.

INTRODUCTION

It was recognized by Cori[1] that sugars were not absorbed across the small intestine at similar rates. Subsequently the efficiency of different sugars as nutrients has been an area of concern. Glucose and galactose, for instance, were shown to be very rapidly absorbed by an active transport system[2]. As a result, the nutritive value of these two sugars has always been unquestioned. In contrast, mannose and arabinose are absorbed at very slow rates. Fructose, which has become a major dietary ingredient due to the large consumption of sucrose, was shown to be absorbed at a rate intermediate between the rapid and slow absorbing sugars. Studies by Holdsworth and Dawson[3] have suggested that an efficient transport mechanism for fructose is operating in the small intestine of humans, but they did not define the nature of the mechanism of absorption.

This mechanism of fructose entry appears to vary with species. Whereas in some species such as guinea pig[4,5] and hamster[6], fructose is converted to glucose and lactate to a large extent during absorption, in man[7], chick[8], and the laboratory rat[9], however, fructose is absorbed intact in relatively large quantities. To facilitate the

* Present address: Department of Physiology, Guy's Hospital Medical School, London SE1 9RT, Great Britain.

transport of intact fructose, the existence of an active transport mechanism was recently suggested by Gracey *et al.*[10] in the rat and a specific mechanism for fructose uptake has been demonstrated by Schultz and Strecker[11] in the rabbit. The studies being reported here add support to the observations made by the afore-mentioned authors, and define in greater detail some of the properties of the intestinal transport mechanism of fructose in the rat*.

METHODS

General methods

Rats were killed under ether and their gut removed from the pyloris to the mid-ileal region. Gut content was removed by washing with isotonic saline under moderate pressure. The first sixth of the gut (varying from 10 to 15 cm, depending on the age of the rat) was discarded to minimize any effects due to position in the gut. The remainder was everted over a glass rod and then cut into segments 6–7 cm long. Inbetween operations, the gut was transferred in isotonic saline and the amount of handling was kept to a minimum. Gut segments were then incubated individually in a water bath at 37 °C, in erlenmeyer flasks containing a modified Krebs–Henseleit bicarbonate solution ($CaCl_2$, 1.2 mM) pregassed with 95 % O_2–5 % CO_2 for at least 10 min. The incubation medium had a final pH of 6.8 and contained 1.0 mM fructose, except where otherwise noted. All incubation media contained trace amounts of uniformly labelled [^{14}C]fructose. The time from the death of the rat to incubation of the segments was usually less than 5 min. The incubation was stopped after 15, 30 and 45 min in most cases.

Following the incubation, segments were rinsed in a Krebs–Henseleit bicarbonate solution (20 °C) containing 5.0 mM fructose. Segments were then gently blotted dry on damp paper towels. The damp paper towels were used because they resulted in the least shedding of mucosal cells during drying. The ends of the segments were trimmed off and the center 4 cm of each segment was weighed in a scintillation vial.

Segments were solubilized overnight at 50 °C in 3 ml NCS solubilizer (Nuclear Chicago Solubilizer, supplied by Amersham/Searle, Toronto) and 10 ml scintillation cocktail (6 % Spectrafluor, in toluene) were added and the radioactivity counted by liquid scintillation. Results were corrected for quenching prior to further calculation.

In the individual experiments, the following changes in the general method were used: (a) Optimum pH experiment: In the modified Krebs–Henseleit bicarbonate solution, the amount of bicarbonate present was varied as either the potassium or sodium salt. At the same time, the NaCl and KCl concentrations were varied. The pH of the medium was thus altered over the range of 6.0–8.0 while still maintaining the original Na^+ and K^+ concentrations. (b) The effect of fasting: Young females were fasted for 48 h prior to the absorption experiment. (c) Comparison of glucose, fructose and mannose absorption: Cut segments from young females were incubated normally in Krebs–Henseleit Ringer containing 1.0 mM glucose, fructose or mannose. Calculations were made with no correction for epithelial cell transport (see below) because of possible differences in distribution of label in the tissues. (d) Criteria for active transport: Incubations were carried out at 4 °C and under 95 % N_2–5 % CO_2 and with

* A preliminary report of these findings was presented at the 55th Annual Meeting of the Federation of American Societies for Experimental Biology, April, 1971.

the addition of 20 mM NaF or 0.5 mM 2,4-dinitrophenol. In the NaF studies, the NaCl concentration was lowered by 20 mM. (e) Na^+-free experiments: At the pH of 6.8, all the sodium ions in the Krebs–Henseleit Ringer are added as NaCl. Na^+-free media were obtained by substituting the 140 mM NaCl with 140 mM solutions of KCl, choline chloride, Tris–HCl (pH 6.8) or 280 mM mannitol. In all Na^+-free media, the Na^+ concentration was less than 1 ppm.(f) Inhibition by other monosaccharides: The fructose concentration in these experiments was lowered to 0.1 mM and the concentration of the added glucose, sorbose and sucrose was 20 mM; 20 mM mannitol served as a control. In the case of phlorizin studies, 0.1 mM phlorizin was added to 1.0 mM fructose in Krebs–Henseleit Ringer. (g) Prior to the establishment of the gut segment incubation method described above, preliminary experiments to locate the site of absorption of fructose in the gut were carried out. An unmodified Krebs–Henseleit phosphate ($CaCl_2$, 2.7 mM) solution, pH 7.4 was used. The concentration of fructose was 0.5 g/100 ml (27.7 mM). Segments of various lengths, ranging from 3 cm in the duodenum to 5 cm in the ileum, were incubated for 45 min under 100 % O_2 at 37 °C. Thirteen sites in the gut were tested in 24 animals (mature males) and the results were calculated as μmoles fructose absorbed per cm gut.

The percentage dry weight of small intestine was determined in a pilot study. From this value (26.1 %), and the weight of the blotted dry segments, the volume of cell water containing the fructose was ascertained. The volume of cell water as calculated above includes the cell water present in the underlying muscle tissues. Since these tissues are very likely not involved in the absorption of fructose *in vivo*[14], it seems possible that the radioactive label is concentrated in the tissues involved in the absorption, namely, the epithelial tissues. To confirm this supposition, gut segments were prepared and incubated as described in the general methods above. The method of Levine and Weintraub[12] of low-amplitude shaking was used to permit rapid and reproducible harvesting of sheets of epithelial tissue from each segment. Both the epithelial sheets and the residual muscle tissue were weighed and counted, and a correction factor based on the distribution of radioactivity between these two tissues was obtained for quantitating transport into the epithelial tissue. All calculations of absorption data were carried out on the IBM 370 Computer at the University of Guelph. (h) Before accepting the method for absorption of fructose by the rat gut tissue, the extent of metabolism of fructose in the tissue during incubation was ascertained. Gut segments were prepared and incubated for 45 min as described in the general methods. Following the incubation, segments were homogenized at 8 °C and the proteins were precipitated and separated by centrifugation. The supernatant was ultracentrifuged (100000 $\times$ g) for 5 h and concentrated on a flash evaporator. An aliquot of the concentrated sample was plated on silica gel plates impregnated with boric acid and developed in either isopropanol–water (4:1,v/v) or butanol–formic acid–water (10:2:15, by vol.). Sample spots representing glucose, fructose, fructose esters and lactic acid were scraped off and solubilized in NCS. The silica gel was filtered out before samples were counted as previously described.

RESULTS

The results of the study of fructose absorption at various positions in the gut (Fig. 1) show a rapid decrease of absorption from the duodenal area where absorption

is the highest, followed by an increase to a relatively constant level in the mid-jejunal area. On the basis of these results, it was decided that the jejunal segments should be used in further experiments and that they be randomized before incubation.

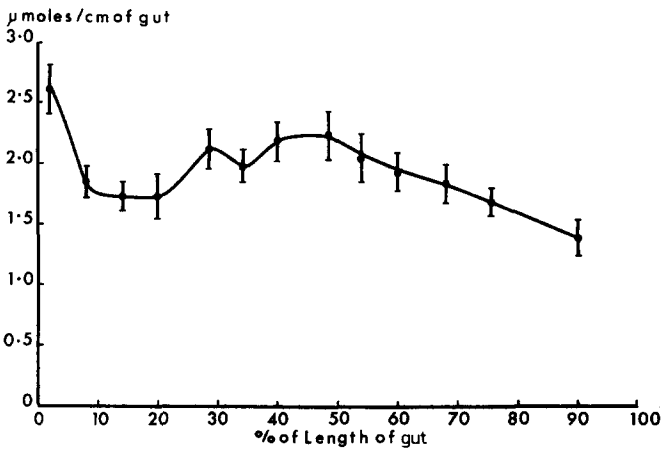

Fig. 1. The pattern of fructose absorption down the length of the small intestine. Gut segments from various positions in the small intestine were incubated for 45 min, as described in Methods (section g). Each point is the mean (± S.E.) of 24 observations. Absorption is expressed as μmoles fructose/cm of gut length.

Significantly more fructose was absorbed at pH 6.8 than at 6.4 or 7.4. Other pH levels (6.0–8.0) were tested and those investigated showed lower fructose absorption than at pH 6.8. The pH of 6.8 was used in further experiments.

Fasting has been shown to have an effect on digestion[13], and studies on the effect of fasting on glucose transport have been carried out by Crane and Mendelstam[14]. The study of the effect of fasting on fructose absorption showed that fasted females absorbed slightly more than did the fed females, but this difference was not significant. However, it is possible that, with further testing and more observations, a significant response may be seen. Fed females were used in further experiments.

Fructose absorption and transport

It was found (Fig. 2) that fructose concentrations in the intestinal wall were higher than 1 μmole/ml in young female rats after 30 min which represents transport against the concentration gradient. This observation led to the suspicion of the existence of an active transport mechanism for fructose in young female rats. At the time of this observation in our laboratory, similar results for young females were published by Gracey *et al.*[10].

These authors based the conclusion of intestinal fructose absorption on 80% fructose recovery from the tissue after incubation. They used a thin-layer chromatography method for the purification of a gut homogenate but they gave no indication that the thin-layer chromatography solvent separated fructose from its phosphate esters. However, fructose and its phosphate esters are usually well separated by the thin-layer chromatographic techniques commonly applied to monosaccharide fractionation.

In our studies on the distribution of label it was found that 78% of the ^{14}C

remained associated with fructose and 19 % as lactate. Glucose and fructose phosphate esters were not labelled. These observations support the suggestion that in some animals, including the rat[5,8,9], fructose metabolism is a minor pathway of translocation and that intact fructose enters the cell where it is resistant to metabolism.

Since intestinal transport is a function of the epithelial cells[14], and sugar appearing in underlying tissue is a result of diffusion from the high concentration provided by the epithelial cells, it was of interest to quantitate the epithelial transport of fructose. With the application of the correction factor for non-specific label accumulation in non-absorbing tissue, absorption against a concentration gradient was even more pronounced than when non-absorbing tissue volume is included (Fig. 2).

A confirmation of Cori's[1] earlier observation was obtained when a comparison of the absorption of fructose was made with mannose and glucose. The results in Fig. 3 show the rate of fructose absorption to be intermediate between the rates observed for mannose and glucose uptake. These results were based on transport into the whole intestinal tissue. All results reported below, however, are based on uptake by the epithelial tissues only.

Both low temperature and anaerobic conditions significantly depressed uptake of fructose (Fig. 4). Similar effects have been noticed by others[10]; however, the magnitude of the anaerobic depression observed by these workers was greater than that seen here. Since the tissue preparation varied from the one used here, the true significance of this discrepancy in anaerobic depression cannot be evaluated at this time.

Both the addition of 20 mM NaF and the addition of 0.5 mM 2,4-dinitrophenol

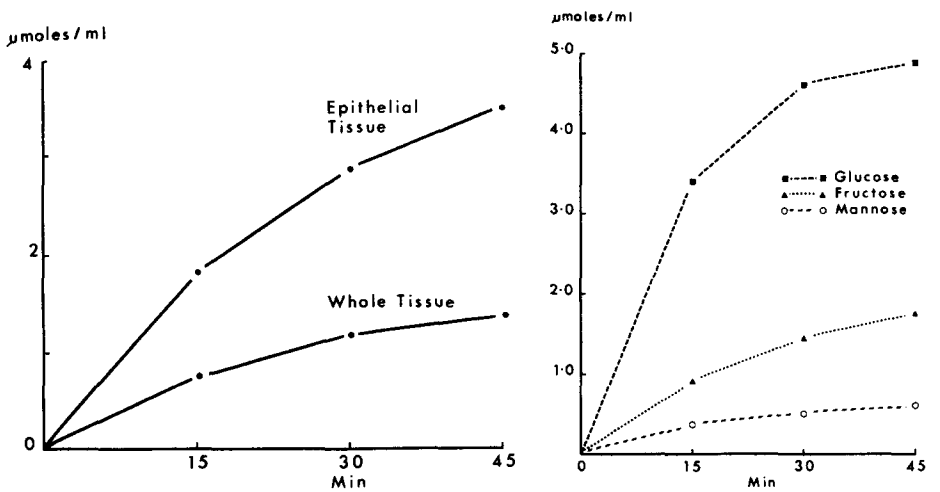

Fig. 2. Fructose absorption in the young female rat based on transport into the whole intestinal wall, and on transport into the epithelial tissues. Jejunal segments were incubated as described in Methods for 15, 30 and 45 min. Each point is the mean of eight observations. Absorption is expressed as μmoles fructose/ml whole tissue water and μmoles fructose/ml epithelial tissue water. Values greater than 1 μmole/ml represent transport against the concentration gradient.

Fig. 3. Comparison of the absorption rates of D-fructose, D-glucose, and D-mannose in the young female rat. Jejunal segments were incubated, as described in Methods (section c), for 15, 30 and 45 min. Each point is the mean of eight determinations. Absorption is expressed as μmoles sugar/ml whole tissue water.

significantly depressed uptake by about 40 % and 60 %, respectively (Fig. 4). The levels of inhibitors used here have been shown to seriously inhibit energy production and reduce the effectiveness of active transport systems[15].

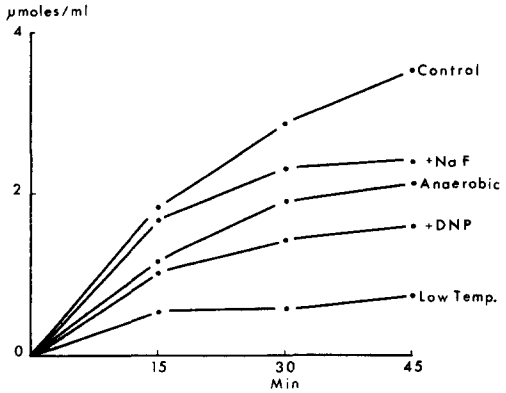

Fig. 4. The effect of general energy inhibitors (low temperature, anaerobic conditions) and more specific energy inhibitors (NaF, 2,4-dinitrophenol (DNP)) on fructose absorption. Jejunal segments were incubated under the conditions described in Methods (section d) for 15, 30 and 45 min. Each point represents the mean of eight observations. Absorption is expressed as μmoles fructose/ml epithelial tissue water.

The dependence of the active transport system of glucose on the presence of Na^+ has been well established[16–19]. The results in Fig. 5 show that in fructose transport, the replacement of Na^+ with K^+ resulted in a moderate but significant decrease in absorption. Conversely, the replacement of NaCl with choline chloride caused a significant increase in fructose absorption. Mannitol substitution also caused a significant reduction in fructose absorption, showing a final absorption similar to that seen with KCl. The greatest reduction (45 %) was seen when Tris–HCl replaced NaCl. The fact that different substitutes for Na^+ gave rise to different levels of absorption and that one replacement (choline chloride), produced a significant increase in absorption, points to a lack of strict dependence on the Na^+ for fructose entry. The results tend to indicate that the varying effects are caused by the replacement ions rather than by the lack of Na^+.

Contrasting results were obtained by Gracey *et al.*[10] who studied fructose absorption from a medium with NaCl replaced by KCl. These workers found a severe inhibition (80 %) of fructose absorption in the absence of Na^+. The work of Schultz and Strecker[11], however, supports the results presented here in that no inhibition of fructose uptake was seen when choline chloride replaced NaCl.

The results being reported here do not rule out the possibility of a moderate Na^+ activation of fructose uptake, but rather, point to a lack of dependence on that ion.

The results in Fig. 6 show the effect of various sugars on fructose absorption in the young female rat. Fructose absorption in the presence of mannitol was similar to that obtained when no sugar was added. In contrast, the substitution of 20 mM glucose for mannitol caused significant decreases in fructose absorption. This observation is in contrast to the results reported earlier by Schultz and Strecker[11] and may in part be due to their use of a shorter incubation period. However, methylated glucose was shown by Bihler[15] to also depress the uptake of fructose.

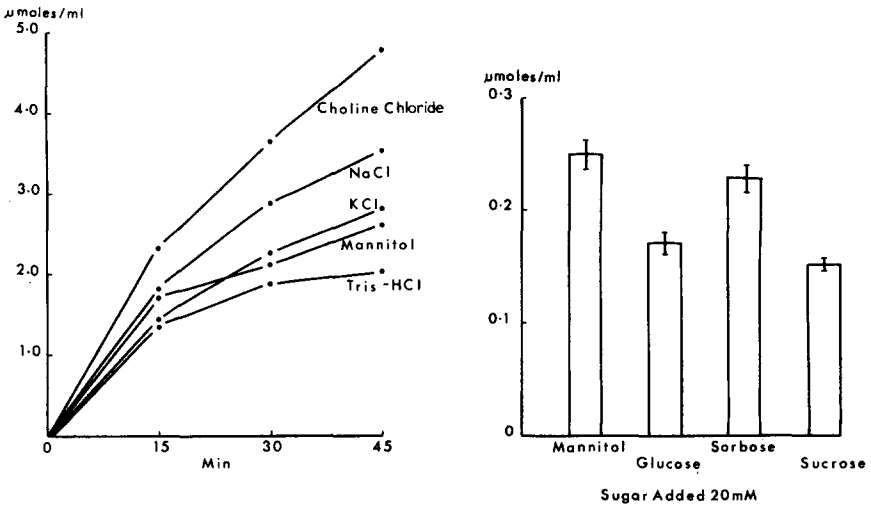

Fig. 5. The effect of Na^+ replacement on fructose absorption. Jejunal segments were incubated, as described in Methods (section e), for 15, 30 and 45 min. Each point represents the mean of four observations. Absorption is expressed as μmoles fructose/ml epithelial tissue water.

Fig. 6. The effect of high concentrations of other sugars on fructose absorption. Jejunal segments were incubated, as described in Methods (section f), for 30 min. Each bar represents the mean (± S.E.) of 24 observations, except for sorbose, which is 36 observations. Absorption is expressed as μmoles fructose/ml epithelial tissue water.

The results of the addition of 20 mM sucrose also show inhibition. This observation may be due to the dilution of the fructose by the fructose released from sucrose hydrolysis. However, there is also the possibility that the unhydrolysed sucrose is interacting with the carrier mechanism for fructose[20]. The 10 % decrease in absorption of fructose in the presence of 20 mM sorbose is similar to that observed by Schultz and Strecker[11]. However, large variations in the effect of this sugar observed in this laboratory makes it difficult to assess the importance of the structurally similar sugar in the interference with the fructose absorption mechanism.

The addition of phlorizin had no effect on the rate of absorption of fructose. This is in spite of the fact that the same concentration of phlorizin severly inhibits glucose and galactose transport[15,21].

DISCUSSION

The transport of glucose and fructose against the concentration gradient points to the existence of active transport for both sugars. However, the difference in the rate of absorption of these two sugars suggests the mechanisms are different. The difference in the two transport mechanisms is further supported by the observation of an optimum pH of 6.8 for fructose absorption compared to 5.9 for glucose uptake[22].

The inhibitory effects on fructose absorption by both the general energy inhibitors (low temperature and anaerobic conditions) and the more specific energy inhibitors (NaF and 2,4-dinitrophenol) indicates a dependence of fructose entry upon energy utilization. The nature of this energy input, however, is still unknown.

The lack of inhibition by phlorizin on fructose entry suggests a mechanism

for fructose transport different from that for glucose. The inhibition of fructose entry by large concentrations of glucose, sorbose and sucrose could suggest the presence of a carrier molecule with a specific binding site for fructose. The point of action of these inhibitors, whether direct (competitive) or indirect (non-competitive) has not yet been determined.

The relatively non-specific effects of Na^+ substitution with other ions could be the result of the interaction of the replacement ion with the carrier rather than the dependence of the carrier on Na^+.

REFERENCES

1 C. F. Cori, *J. Biol. Chem.*, 66 (1925) 691.
2 R. K. Crane, *Fed. Proc.*, 21 (1962) 891.
3 C. D. Holdsworth and A. M. Dawson, *Proc. Soc. Exp. Biol. Med.*, 118 (1965) 142.
4 W. A. Darlington and J. H. Quastel, *Arch. Biochem. Biophys.*, 43 (1953) 194.
5 J. Y. Kiyasu and I. L. Chaikoff, *J. Biol. Chem.*, 224 (1957) 935.
6 T. H. Wilson and T. N. Vincent, *J. Biol. Chem.*, 216 (1955) 851.
7 G. C. Cook, *Clin. Sci.*, 37 (1969) 675.
8 G. A. Leveille, T. K. Akinbami and C. O. Ikediobi, *Proc. Soc. Exp. Biol. Med.*, 135 (1970) 483.
9 V. Ginsberg and H. G. Hers, *Biochim. Biophys. Acta*, 38 (1960) 427.
10 M. Gracey, V. Burke and A. Oshin, *Lancet*, ii (1970) 827.
11 S. G. Schultz and C. K. Strecker, *Biochim. Biophys. Acta*, 211 (1970) 586.
12 P. H. Levine and L. R. Weintraub, *J. Lab. Clin. Med.*, 75 (1970) 1026.
13 L. K. McNeill and J. R. Hamilton, *Pediatrics*, 47 (1971) 65.
14 R. K. Crane and P. Mendelstam, *Biochim. Biophys. Acta*, 45 (1960) 460.
15 I. Bihler, *Biochim. Biophys. Acta*, 183 (1969) 169.
16 E. Riklis and J. H. Quastel, *Can. J. Biochem. Physiol.*, 36 (1958) 363.
17 T. Z. Csaky and L. Zollicoffer, *Am. J. Physiol.*, 198 (1960) 1056.
18 I. Bihler and R. K. Crane, *Biochim. Biophys. Acta*, 59 (1962) 78.
19 R. K. Crane, G. Forstner and A. Eichholz, *Biochim. Biophys. Acta*, 109 (1965) 467.
20 J. A. Carnie and J. W. Porteous, *Biochem. J.*, 89 (1964) 100 P.
21 G. A. Kimmich, *Biochemistry*, 9 (1970) 3669.
22 H. Ida, E. W. Moore, S. A. Broitman and N. Zamcheck, *Proc. Soc. Exp. Biol. Med.*, 127 (1968) 730.