BBA 73127

Fructose influx across the brush border of rabbit ileum

Numerous studies have shown that D-fructose is rapidly absorbed by the small intestine *in vivo* in a variety of species[1-4]. The rapidity with which this sugar disappears from the lumen compared with the slow disappearance rates of nontransported sugars (*e.g.* arabinose) has strongly suggested the participation of a carrier mechanism in fructose absorption. However, because fructose is metabolized after entering the intestinal cell[1-3], studies of net transmural transport or accumulation by segments of intestine *in vitro* are unsuited for the investigation of this transport mechanism. For this reason the characteristics of the brush border mechanisms involved are essentially unknown.

The present investigation is concerned with the characterization of the unidirectional influx of fructose across the brush border of rabbit ileum. It was undertaken in order to compare this process with the previously studied influx mechanism for D-glucose, D-galactose and 3-*O*-methyl-D-glucose[5]. The method employed for the direct determination of the unidirectional influxes of fructose from the mucosal solution across the brush border and into the absorptive epithelium has been described in detail[6]. In essence it involves the brief exposure of a defined area of the mucosal surface to a solution containing D-[^{14}C]fructose and [^{3}H]inulin. The initial rate of fructose uptake is calculated from the ^{14}C content of the tissue after correction for the [^{3}H]inulin space. The results obtained using this method are not influenced by intracellular metabolism, provided the products of these reactions are retained within the tissue. Unless otherwise noted, the buffered electrolyte solution employed for preincubation and influx determination contained: 140 mM NaCl, 10 mM $KHCO_3$, 1.2 mM K_2HPO_4, 0.2 mM KH_2PO_4, 1.2 mM $CaCl_2$ and 1.2 mM $MgCl_2$.

A typical time-course of fructose uptake across the brush border alone is illustrated in Fig. 1. In this experiment, eight mucosal areas of tissue from the same animal were exposed for varying periods of time to a solution containing 10 mM [^{14}C]fructose. Uptake is a linear function of time for at least 1.5 min and the line

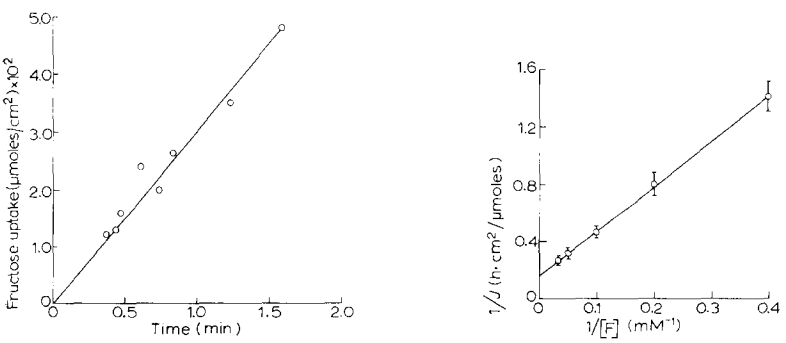

Fig. 1. Uptake of D-fructose across the mucosal border as a function of duration of exposure of the mucosal surface to a solution containing 10 mM [^{14}C]fructose.

Fig. 2. Lineweaver–Burk plot of fructose influx (J) *vs.* fructose concentration ([F]). Points represent the averages of 6 determinations at 2.5 and 30 mM and 12 determinations at 5, 10 and 20 mM. Errors are S.E.

extrapolates to the origin. These results indicate that the 1-min uptake provides a valid measure of the unidirectional influx (*i.e.* initial rate of uptake) of fructose. All of the data to be presented were obtained from experiments in which the duration of exposure was less than 1 min.

Fig. 2 shows a Lineweaver–Burk plot of the unidirectional influx of D-fructose as a function of the fructose concentration in the mucosal solution. In these experiments the fructose concentration varied from 2.5 to 30 mM and the osmolarity of the mucosal solution was maintained constant by the use of appropriate amounts of mannitol. The data clearly describe a straight line, indicating that fructose influx is a saturable function of concentration that conforms to Michaelis–Menten kinetics. The maximal influx is 6.3 μmoles/h·cm^2 and the fructose concentration required to elicit a half-maximal influx (K_t) is 18 mM.

TABLE I

EFFECT OF OTHER MONOSACCHARIDES ON FRUCTOSE INFLUX

All errors are expressed as S.E. Numbers in parentheses designate the number of influx determinations.

Mucosal solution	*Fructose influx* (μmoles/h·cm^2)
0.1 mM D-fructose + 20 mM mannitol	0.029 ± 0.001 (16)
0.1 mM D-fructose + 20 mM D-glucose	0.028 ± 0.002 (6)
0.1 mM D-fructose + 20 mM D-mannoheptulose	0.028 ± 0.001 (6)
0.1 mM D-fructose + 20 mM L-sorbose	0.025 ± 0.002 (16)

The effect of several other sugars on fructose influx is given in Table I. Clearly, the replacement of 20 mM mannitol with either 20 mM D-glucose or 20 mM D-mannoheptulose does not significantly affect fructose influx from a solution containing 0.1 mM fructose. The absence of any inhibition of fructose influx by a 200-fold greater concentration of glucose excludes any significant competitive interaction between glucose and the fructose influx mechanism. The presence of 20 mM L-sorbose results in a small but consistent inhibition of fructose influx that is significant at the 5 % level by unpaired analyses (Student's t test). Paired analyses, in which influx in the presence of sorbose is compared with influx in the absence of sorbose determined on the adjacent segments of tissue from the same animals, indicate a significant inhibition at the 1 % level. The calculated K_i for sorbose, assuming that the effect of sorbose can be attributed to classical competitive inhibition, is approx. 100 mM.

Finally, studies were performed to examine the effects of Na^+ and phlorizin on fructose influx. Fructose influx from a solution rendered Na^+-free by replacement of NaCl with choline chloride, and influx from the normal buffer (140 mM Na^+) containing 0.1 mM phlorizin did not differ significantly from the control.

The present results provide compelling kinetic evidence for the presence of a carrier mechanism that is responsible for the movement of fructose from the lumen of rabbit ileum into the absorptive cells, and these findings thus support the notion derived from earlier *in vivo* studies. This mechanism appears to be relatively specific for fructose in view of the minimal inhibition of influx observed in the presence of a 200-fold concentration of the closely related isomer, L-sorbose. The carrier mecha-

nism responsible for the influx of this hexoketose is distinct from that responsible for the mediated influx of a number of hexoaldoses, such as glucose, galactose and 3-*O*-methylglucose, and, unlike the latter, is neither Na^+-dependent nor phlorizin-sensitive[5]. Further study employing a variety of closely related monosaccharides is necessary to define the minimal structural requirements of this transport process.

This investigation was supported by research grants from the U.S. Public Health Service, National Institute of Arthritis and Metabolic Diseases (AM-13744) and the American Heart Association (67-620). One of us (S.G.S.) is the recipient of a Research Career Development Award (AM-9013) from the National Institute of Arthritis and Metabolic Diseases.

Department of Physiology, STANLEY G. SCHULTZ
University of Pittsburgh School of Medicine, CAROLE K. STRECKER
Pittsburgh, Pa. 15213 (U.S.A.)

1 R. K. CRANE, *Physiol. Rev.*, 40 (1960) 789.
2 G. WISEMAN, *Absorption from the Intestine*, Academic Press, London, 1964, pp. 27–29.
3 T. H. WILSON, *Intestinal Absorption*, W. B. Saunders, Philadelphia, 1962, pp. 91–94.
4 C. D. HOLDSWORTH AND A. M. DAWSON, *Clin. Sci.*, 27 (1964) 371.
5 A. M. GOLDNER, S. G. SCHULTZ AND P. F. CURRAN, *J. Gen. Physiol.*, 53 (1969) 362.
6 S. G. SCHULTZ, P. F. CURRAN, R. A. CHEZ AND R. E. FUISZ, *J. Gen. Physiol.*, 50 (1967) 1241

Received June 5th, 1970