D-Xylose, a Substrate for the Process of Sugar Active Transport by the Small Intestine[1]

With respect to their mode of intestinal absorption (see reviews by CRANE[2] and WILSON[3]), sugars have been classified into two categories: *active* and *non-active*[4]. Active sugars are characterized by transport *uphill* or against a concentration difference and therefore by a dependence of transport velocity on energy-yielding metabolism (see ROSENBERG[5]). In contrast, non-active sugars are negatively defined by an absence of experimental demonstration of any of these features; consequently, the mode of intestinal absorption of this group of sugars is almost completely ignored.

This paper, the first of a series aiming at an understanding of the transport mechanism(s) of the so-called non-active sugars, deals with D-xylose transport. This pentose, hitherto considered as typically non-active, now appears to be transported through a common pathway with the active sugars.

In 1961, SALOMON, ALLUMS and SMITH showed that xylose crosses the intestinal wall through a *downhill*, carrier-mediated process, which they called 'thermal transport' as distinct from active transport[6]. Several considerations, however, pointed to the possibility that xylose transport may be closely related to the sugar active transport process. First, CRANE found that xylose inhibits, although only slightly, active transport of D-glucose and 1,5-anhydro-D-glucitol[7]. Later, SALOMON et al. found that glucose substantially interferes with xylose transport, but were unable to show reciprocal interference of xylose on glucose movement; they concluded that a single carrier must be involved 'with greater affinity for D-glucose than for D-xylose'[6]. It is noteworthy, however, that in both the experiments of CRANE[7] and of SALOMON et al.[6] the same relative affinities hold true, xylose having a much lower affinity than glucose for both the 'xylose thermal transport' and the 'glucose active transport' mechanisms.

Experiments designed to determine the extent of the apparent relationship between these two processes are summarized here. These studies were carried out using the *in vitro* technique of CRANE and MANDELSTAM[8]. Reference will be made only to results obtained with hamster small intestine, but similar results were found using chicken intestine.

Results. Xylose transport is significantly inhibited by 2,4-dinitrophenol (DNP), phlorizin, arbutin and lack of Na^+ from the incubation media (Figure 1); all the above effects would have been expected to take place on the transport of any active sugar. The only difference with these appears to be that xylose is not accumulated against a concentration difference. The lack of similar effects on the epimer at C-2 of xylose, D-lyxose (Figure 1), serves to emphasize the parallelism between xylose and the active sugars. Like the latter, xylose has an –OH group in the same configuration as D-glucose in C-2[2,3].

Similarity between xylose and the active sugars is evinced by the lack of filling above the extracellular space when Na^+ is omitted from the incubation media (Figure 1). As is the case with active sugars[9,10], neither K^+ nor Li^+ can substitute for Na^+.

Xylose transport, like sugar active transport (see ALVARADO and CRANE[11,12]), is inhibited by phlorizin and arbutin (Figure 1), probably due to competition for a common carrier. This is supported by the following facts: (1) phlorizin inhibition is typically competitive (Figure 2), with a Ki on the order of $6 \times 10^{-5} M$; (2) only active compounds such as glucose[6], arbutin, methyl-α- and methyl-β-glucopyranoside significantly inhibit xylose transport; the non-active compounds sorbitol, mannitol, inositol and methyl-α-mannopyranoside, on the contrary, are essentially inert; (3) xylose, but not lyxose, mannitol or mannose, reciprocally interferes with arbutin active transport (see also ALVARADO and CRANE[12]). From these studies a Ki for xylose of the order of 100 mM has been calculated, in good agreement with Km values obtained from saturation experiments by the method of LINEWEAVER and BURK[13]. Km values obtained in this way (Figure 2) have a raw value of 300 mM, but, if corrections are applied for the extracellular space (^{14}C-sorbitol was used for this purpose), the apparent Km decreases to about 100 mM. In contrast, the Ki for phlorizin is not significantly modified by this correction.

Energy requirement for xylose transport. Both DNP (Figure 1) and anaerobiosis strongly depress xylose transport, suggesting that energy is required for at least a

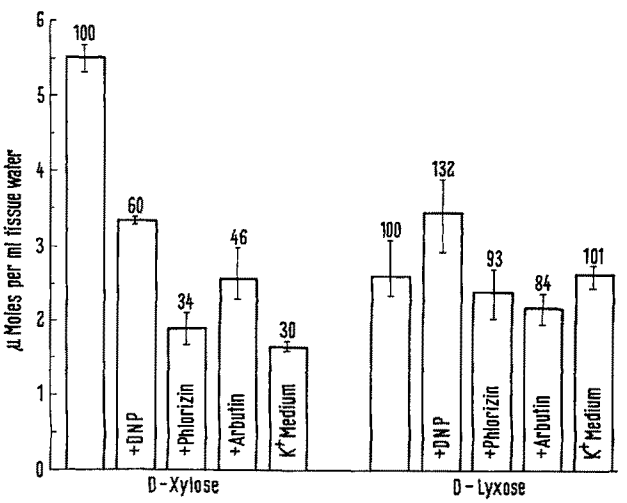

Fig. 1. Rings of everted hamster small intestine were incubated[8] in 4 ml Krebs-Henseleit[17] bicarbonate buffer or in its Na-free, K-substituted modification[9] (K^+ medium), at 37°. Gas phase, 95% O_2:5% CO_2. Initial concentrations were (mM): xylose and lyxose, 10; dinitrophenol (DNP), 0.5; phlorizin, 0.1; arbutin, 5. Incubations were for 10 min (xylose) or for 15 min (lyxose). Pentose was determined with aniline-oxalic acid[18] in deproteinized extracts[18]. Results are expressed as µmoles of substrate per ml tissue water[8,9,12]. Each column is an average of three determinations; figures on top of the columns are mean % values with respect to the controls.

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portion of this process. Since dependence of the transport velocity on energy-yielding metabolism is one of the theoretical requirements for active transport[5], this strongly suggests that xylose is indeed actively transported. Similar evidence was adduced by BIHLER, HAWKINS and CRANE[10] to classify 6-deoxy-1,5-anhydro-D-glucitol as active; although this compound was not found to be accumulated, its transport was inhibited by 4,6-dinitro-*o*-cresol and by anaerobiosis[10].

Evidence for a two-stage mechanism in xylose transport. An appraisal of the above findings suggests that, like the active sugars[10,12,14], xylose transport occurs in two stages: (1) a phlorizin-sensitive, Na^+-dependent, energy-independent *entry* into the epithelial cell and (2) an oxygen-dependent, DNP-sensitive step, probably equivalent to the energy-dependent *accumulation* step, typical of active compounds.

Concluding remarks. It seems clear that xylose, a sugar hitherto considered as non-active, is transported, in the hamster small intestine, through the same pathway as the active sugars. The evidence includes demonstration of Na^+ requirement, competitive inhibition by phlorizin, sugar active transport inhibition by xylose and reciprocal inhibition of active compounds on xylose transport. Although no xylose accumulation was observed, participation of an energy-dependent component in xylose transport in the hamster is evinced by the inhibitory action of anaerobiosis and dinitrophenol. Investigations now in progress[15] seem to indicate that the apparent lack of xylose accumulation against the gradient is due to the small affinity of this pentose for an accumulation process different from entry. This contention is supported by the lack of significant change in apparent Km values for xylose, as determined in aerobiosis or in anaerobiosis. In significant contrast, typical active compounds such as arbutin show a drastic decrease in apparent affinity for the (overall) system when nitrogen instead of oxygen is used while determining these constants. These observations, in agreement with an earlier suggestion by WIDDAS[16], suggest that sugar active transport involves two different processes, the first of which seems to be a typical facilitated diffusion process identical to the carrier-mediated xylose transport mechanism of SALOMON et al.[6]. It seems warranted to conclude that the properties of the overall sugar active transport process are indeed a composite of partial features of at least two distinct stereospecific processes. Evidence in favour of this hypothesis will be presented in a forthcoming series of papers[20].

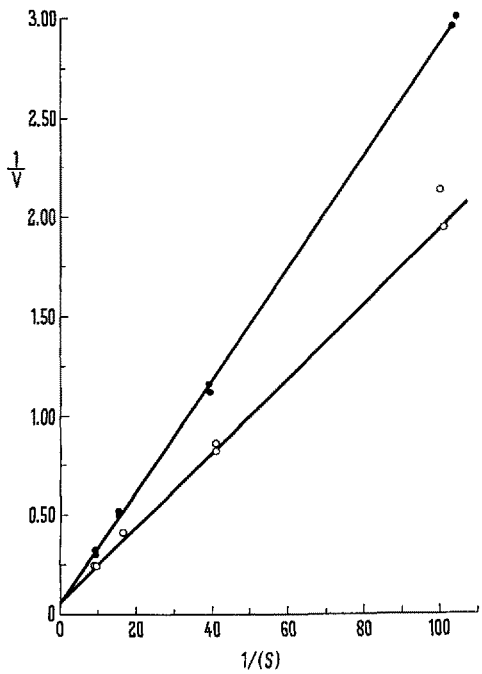

Fig. 2. Phlorizin competitive inhibition on xylose transport. Incubations in oxygen atmosphere were for 10 min in 4 ml Krebs-Henseleit[17] phosphate buffer containing 2 ml of a mixture in varying proportions of isotonic (0.3 *M*) xylose and mannitol. o, xylose; •, xylose plus 3.33×10^{-5} *M* phlorizin. Velocities[8,9,12] and mean substrate concentrations are plotted as reciprocals[13]. Other experimental details as in Figure 1.

Résumé. Dans les cellules épithéliales de l'intestin grêle du Hamster, l'absorption de xylose comprend deux étapes: (1) une *entrée* sensible à la phlorizine, et (2) une étape sensible au dinitrophénol, probablement équivalente à l'étape d'*accumulation* dépendant de l'énergie et typique des sucres actifs.

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Synthetic Peptides Related to Eledoisin[1]

After the structure of eledoisin, a powerful vasodilating and hypotensive peptide isolated from the salivary glands of a mollusc[2], had been elucidated[2] and confirmed by synthesis[3], we prepared a large number of analogues of this substance in order to investigate the influence of structural modifications on its biological properties. In a previous communication[4] we listed in a Table those analogues and partial sequences which have been found to be devoid or almost devoid of biological activity.

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