Hexose transport by hamster intestine in vitro

BERNARD R. LANDAU, LEOYARD BERNSTEIN, AND T. HASTINGS WILSON (With the Technical Assistance of Angela DeCarlo)

Departments of Medicine and Biochemistry, Western Reserve University, Cleveland, Ohio; and Department of Physiology, Harvard Medical School, Boston, Massachusetts

Considerable data bearing upon the specificity and mechanism of hexose transport by the intestine are now available. Structural requirements for active sugar transport by the intestine of the hamster have been partially established (1, 2) and evidence has been presented which appears to exclude phosphorylation from direct participation in the transport mechanism (3, 4).

These observations have now been extended. First, the restrictions which limit active transport of compounds differing from glucose and galactose by the modification of the carbon 1 grouping have been investigated by a study of a series of glycosides. Secondly, the availability of 5-deoxyglucose has permitted a test of the specificity of transport for a hexose differing in structure from glucose at carbon 5. Finally, the use of glucose labeled with tritium has permitted a determination of whether or not reactions involving the rupture of the bonds between the hydrogens and carbon 1 or 6 of glucose participate in the mechanism of active transport. This is especially to be considered with regard to carbon 6 since hexoses, but not pentoses, are transported against a concentration gradient (5).

Experimental Procedures

Methods. The glycosides and 5-deoxyglucose were tested for transport in an everted sac preparation from hamster intestine in the manner previously described (6). For the mucosal solution 3.0 ml of modified Krebs-Henseleit medium containing the sugar to be tested was used and 1.0 ml was introduced into the sac. Only small changes in the volume in the sacs were observed at the completion of incubation. Glycoside concentrations were usually determined by Nelson's copper reduction method after hydrolysis of a Ba(OH)₂ - ZnSO₄ filtrate (7) of the sugar solution in 1 N HCl at 100 C for 1-2 hr, and subsequent neutralization with NaOH. In some experiments an anthrone determination (8) was employed. The absence of reducing properties in the medium prior to treatment with HCl was used to demonstrate that hydrolysis did not occur during incubation. The concentrations of 5-deoxyglucose were determined by the Somogyi-Nelson method (7).

To assess the stability of the carbon-hydrogen bonds of carbon 6 of glucose during transport, glucose-6-C¹⁴ and glucose-6-H³ were added together on the mucosal side of the intestinal sac to make a final concentration of 5 mM and nonradioactive glucose was added to the serosal medium at the same concentration. After incubation for 90 min the ratios of the C¹⁴ to H³ activity in the glucose of the mucosal and serosal solutions were determined and compared with the initial ratio. The determination of the ratios was made by the preparation of glucosazones from aliquots of the solutions after addition of carrier glucose, conversion of the glucosazones to isotriazoles, and assay of the isotriazoles in a scintillation counter using a xylene-ethanol-boric acid solvent with 2,5-diphenyloxazole (DPO) as phosphor (9). Glucose concentrations were determined by the Somogyi-Nelson method on aliquots of the solutions to assure that a concentration gradient had developed during incubation.
To assess the stability of the carbon-hydrogen bond of carbon 1, \( \alpha \)-methylyglucosides from glucose-1-C\(^{14} \) and glucose-1-H\(^{3} \) were prepared (10). These were added to the mucosal side of the sac at a concentration of 5 mM and nonradioactive methylglucoside was added on the serosal side at the same concentration. After incubation the solutions were deproteinized and hydrolyzed and the reducing content was determined on aliquots to make certain that a concentration gradient had been established. The ratio of C\(^{14} \) to H\(^{3} \) in the methylglucoside transported into the serosal contents was then compared to the ratio presented to the sac as follows. Aliquots of the \( \text{Ba(OH)}_{2} - \text{ZnSO}_{4} \) filtrates of the initial mucosal, final mucosal, and final serosal solutions were deionized by passage through columns of Duolite-A-4 packed above Duolite-C-3 ion exchange resins. The eluates were concentrated and applied to Whatman 3 MM paper. It was first demonstrated that methylglucoside was not hydrolyzed during passage through the resins. Descending chromatography was performed with a butanol-pyridine-water system (11). Controls of nonradioactive \( \alpha \)-methylglucoside were chromatographed adjacent to the radioactive concentrates. The spots were visualized by means of a periodate-permanganate spray. The corresponding radioactive segments were not sprayed, but were eluted with water, concentrated in vacuo to 1 ml, and then assayed for H\(^{3} \) and C\(^{14} \) activity in a dioxane solution containing naphthalene and with DPO and \( 1,4 \)-bis-(5-phenyloxazolyl) benzene (POPOP) as phosphors (12).

Calculations. The concentration gradient has been calculated as the ratio of the concentration of sugar in the serosal to that in the mucosal solution at the completion of incubation. The quantity of sugar transported per 100 mg of tissue has been calculated from the difference between the initial and final quantities of sugar present in the serosal solution and the final weight of the intestinal tissue. The per cent recovery of a sugar has been calculated from the total quantity of sugar presented in the mucosal and serosal solutions before and after incubation as measured by reducing content after hydrolysis.

Materials. Generous quantities of many of the glycosides were gifts from Dr. G. N. Bollenback, Corn Products Co., Argo, Ill. Dr. M. L. Wolfrom, Ohio State University, Columbus, Ohio, kindly provided a sample of 5-deoxy-1,2-O-isopropylidene-\( \alpha \)-D-glucose from which the 5-deoxyglucose was prepared (13). Glucose-6-H\(^{3} \) (14) was purchased from the National Bureau of Standards, Washington, D.C., and glucose 1-H\(^{3} \), glucose 1-C\(^{14} \), and glucose-6-C\(^{14} \) from New England Nuclear Corp., Boston, Mass. Arbutin (\( \beta \)-hydroxyphenyl-\( \beta \)-glucoside) was obtained from Aldrich Chemical Co., Milwaukee, Wis., \( \beta \)-nitrophenyl-\( \beta \)-glucoside from California Corp. for Biochemical Research, Los Angeles, Calif., \( \phi \)-nitrophenyl-\( \beta \)-galactoside from Sigma Chemical Co., St. Louis, Mo., methyl-\( \beta \)-thiogalactoside from Mann Research Laboratories, New York, N. Y., and phenyl-\( \beta \)-galactoside from Sigma Chemical Co., St. Louis, Mo.

### Table 1. Effect of \( \beta \)-phenylglucoside (20 mM) on galactose (5 mM) transport

<table>
<thead>
<tr>
<th>Incubation Medium</th>
<th>Galactose Transport, ( \mu )moles/100 mg tissue/hr</th>
</tr>
</thead>
<tbody>
<tr>
<td>Galactose alone</td>
<td>10.0 ± 0.7</td>
</tr>
<tr>
<td>Galactose plus ( \beta )-phenylglucoside</td>
<td>3.1 ± 0.5</td>
</tr>
</tbody>
</table>

Values are means ± SEM. *Sacs of hamster intestine were incubated in pairs, one with galactose alone and the other with galactose plus the glucoside. Incubation was for 1 hr at 37 C. Each value represents the mean of 4 experiments.
TABLE 2. Effect of glucosides on 6-deoxyglucose transport*

<table>
<thead>
<tr>
<th>Inhibitor</th>
<th>Conc. of Inhibitor, mm</th>
<th>Per cent Inhibition</th>
<th>Apparent Kt, μM</th>
</tr>
</thead>
<tbody>
<tr>
<td>α-Methylglucoside</td>
<td>2</td>
<td>58</td>
<td>0.5</td>
</tr>
<tr>
<td>β-Methylglucoside</td>
<td>2</td>
<td>71</td>
<td>0.29</td>
</tr>
<tr>
<td>β-Phenylglucoside</td>
<td>5</td>
<td>65</td>
<td>0.95</td>
</tr>
<tr>
<td>β-N-Hydroxyphenylglucoside</td>
<td>10</td>
<td>76</td>
<td>1.1</td>
</tr>
</tbody>
</table>

* Small rings (ca. 150 mg wet wt.) of hamster intestine were incubated in 5 ml bicarbonate-saline containing 1 mM 6-deoxyglucose-H3 with or without glucoside. Tissue accumulation of 6-deoxyglucose was estimated after a 20-min incubation at 37°C. † Kt, the inhibitor was calculated assuming a common pathway for transport and a K of 0.6 μM for 6-deoxyglucose (15).

Glucoside from Pfanzteh Laboratories, Inc., Waukegan, Ill. In all cases the glucose and galactose derivatives were of the α-configuration.

RESULTS

Figure 1 summarizes the data obtained on the incubation of intestinal sacs with 18 different glucosides. Results obtained with glucose, which was used as the test sugar in one segment from each animal, are shown in the figure for comparison. The ability of the intestine to transport α and β methyl- and ethyl-glucoside and β-isopropyl-glucoside against a concentration gradient is evident (concentration gradients range from 3.2 to 11.9). Also transported, although slowly, were the α- and β-normal-butyl, β-secondary-butyl, β-bromoamyl- and β-ethoxyethyl-glucoside (concentration gradients 1.3 to 1.9). No transport of β-isobutyrylglucoside was detected. It should be noted that even large aglycones such as phenyl, p-chlorophenyl, and p-hydroxy-phenyl were transported (concentration gradients 1.7 to 1.9). No preference by the system for the alpha or beta configuration of a sugar was observed.

Although glycosidases are present in the intestine their activity was low for most of the glucosides used in the present study. There was good recovery of glucosides at the end of incubation (between 90 and 100%), with the exception of α-ethylglucoside with recovery of only 80%. Recoveries for the α- and β-methylgalactosides were 93% and 77%, respectively, while that for β-thiomethylgalactoside was 97%. Glucose was extensively metabolized, only 65% being recovered. β-α-Nitrophenylglucoside and β-α-nitrophenylgalactoside were extensively hydrolyzed, particularly in the jejunum, so that no definite conclusion for or against the occurrence of active transport could be made.

Glycosides were found to inhibit the movement of actively transported sugars in all four cases investigated. Table 1 records data demonstrating the inhibition of galactose transport by β-phenylglucoside. This compound and three other glucosides were also found to inhibit the transport of 6-deoxyglucose (Table 2). The apparent K of (analogous to the K of enzyme kinetics) was calculated from the previously determined K for 6-deoxyglucose (15) and the observed inhibition. It is assumed in the calculation that Michaelis-Menton kinetics exist and that the inhibitor competes with the substrate for a common site in the transport system.

Table 3 records the results of incubations of intestinal sacs with 5-deoxyglucose. This sugar was not transported against a concentration gradient and was recovered unchanged at the end of incubation. It was also found (not recorded in the table) that 5-deoxyglucose (5 mM) did not interfere with the transport of glucose (10 mM).

In Table 4 are recorded the ratios of 14C to 3H activity in the glucose from the mucosal solution before incubation, and in the mucosal and serosal solutions after incubation of sacs with glucose-6-14C, α-14C, and methylglucoside-1-14C. In no experiment did the ratio of 14C to 3H change as a result of absorption across the intestinal wall. The concentrations of glucose and methylglucoside in the serosal solutions at the completion of the incubations were severalfold greater than their initial concentrations so that much, if not all, of the labeled sugar in the serosal solutions at the completion of the incubation was actively transported.

DISCUSSION

Both 6-deoxyglucose and α-methylglucoside have been shown to be actively transported (3, 16) and to compete with glucose for the transport process (15, 17). This would indicate that neither the hydroxyl nor aldehydic
function of the first carbon of glucose is necessary for transport and thus eliminates phosphorylation at carbon 1 as a part of the mechanism (1, 2). The transport of several glycosides, reported here, is in support of the above conclusions, if it is assumed that these glycosides compete for the transport process. In support of this assumption, four of the glycosides have been demonstrated to inhibit the transport of galactose or 6-deoxyglucose, sugars which share a common transport system with glucose (15, 17).

It would appear that steric or configurational factors limit, to some extent, the process of transport. Thus, glycosides with the smallest aglycone, i.e., methyl, ethyl, and isopropyl, were the most extensively concentrated. A somewhat similar situation has been found with 3-O-alkyl ethers, a small group being tolerated better than a large one (16). As the hydroxyl function of carbon 2 must be retained for transport, a large group in the position 1 or 3 may interfere with this function.

On the other hand, a number of rather large substituent groups on the hydroxyl at carbon 1 of the glucose molecule do not completely block transport. Indeed, the apparent affinity for the system of phenyl and p-hydroxyphenyl glycosides is of the same order of magnitude as for α- or β-methylglucoside or glucose itself. It would therefore seem reasonable to conclude that the hydroxyl at carbon 1 is not directly involved in the interactions between the sugar and the transport apparatus.

A few speculations bear on these observations. If the active site of the sugar transport system were in close proximity to small water-filled pores, increasing the size of the sugar molecule would be expected to reduce its affinity. If, on the other hand, the active site was associated with a lipid area of membrane, a large lipophilic aglycone such as the phenyl group might not affect the affinity. The latter possibility is consistent with the experimental observation that phenylglucoside and p-hydroxyphenylglucoside have a high affinity for the sugar transport system. An additional possibility cannot be excluded, namely, that the binding of sugar to the system occurs at a site other than that at which actual transport proceeds, affinity being unaffected by the nature of the transport "channel."

Since 5-deoxyglucose is not transported it might be suggested that the hydroxyl function at carbon 5 is required for active transport. However, since 1-deoxyglucose and the glycosides which are transported are fixed in the pyranose form and therefore lack a hydroxyl function at position 5, the inability to transport the 5-deoxy derivative would rather indicate the requirement for the pyranose ring.

6 Deoxyglucose is transported and competes with glucose for transport (15, 17). This has been interpreted to indicate that the phosphorylation of glucose in the 6 position does not occur and that a function other than hydrogen in the 6 position is not required for transport (1, 2). A mechanism of transport in which an oxidation-reduction reaction involves the hydrogens bonded to carbon 6 has remained a possibility (Crane and Krane (18) considered this as a possible reaction at carbon 2). However, since carbon 6 of glucose has previously been shown to be transported intact (4), and since the C14 to H3 ratio remained constant, the carbon-to-hydrogen bonds of position 6 would appear to remain unbroken during transport. If oxidation did occur during transport, with the resultant removal of the hydrogen bonded to carbon 6 of glucose followed by reduction to reform glucose, it would be expected that the ratio of C14 to H3 would have increased, i.e., the tritium would have been removed during oxidation and replaced by hydrogen derived from the tissue and medium pool.

A similar oxidation-reduction mechanism could conceivably occur at carbon 1 of glucose during transport. Since methylglucoside is not metabolized and appears to be transported by the same system as glucose (15), methylglucoside-1-H3, C14 was tested. The constant ratio of C14 to H3 would indicate that the carbon to hydrogen bond of carbon 1 of glucose is not cleaved.

REFERENCES


4 Only if there was a mechanism by which the tritium removed was the only source of hydrogen available for subsequent addition would this assumption not be valid.