Specificity of sugar transport by
the intestine of the hamster

T. HASTINGS WILSON2 AND BERNARD R. LANDAU3
Departments of Physiology and Biological Chemistry, Harvard Medical
School, Boston, Massachusetts

WILSON, T. HASTINGS AND BERNARD R. LANDAU. Specificity
of sugar transport by intestine of the hamster. Am. J. Physiol.
198(1): 99-102. 1960.—The specificity of the sugar transport
system of the hamster small intestine was tested with 20
sugars and sugar derivatives not previously tested in this
system. The absorption of sugars across the intestinal wall
against a concentration gradient was tested with the everted
sac technique in vitro. 3-Deoxyglucose, 4-o-methylgalactose,
6-deoxy-6-fluoroglucose and α-methylglucoside were trans-
ported while a variety of other sugars were not. From the
data derived from the study of a total of 49 sugars tested in
this system, certain generalizations are made as in the struc-
tural limitations of the sugar-absorbing capacity of the hamster
intestine.

The passage of sugars across many cell membranes
does not obey the laws of simple diffusion; rather there
appear to be more complicated processes involved. The
sugar permeability of yeast cells (1), human erythrocytes
(2) and ascites tumor cells (3) is characterized by sugar
specificity, competitive inhibition between sugars, sub-
strate saturation phenomenon, high temperature co-
efficient and noncompetitive inhibition by a variety of
agents. In these cells there is no effect of insulin nor is
there movement of sugar against a concentration gradient.
Muscle cells (4) and the blood-aqueous humor barrier (5)
possess many of the same characteristics mentioned above but, in addition, show insulin sensi-
tivity. Recently it has been shown that certain bacteria
have the capacity to accumulate sugar intracellularly
against concentration gradients (6).

The small intestine and kidney tubule differ from
other mammalian cells as the passage of sugar probably
occurs across two cell membranes and the transport
occurs against a concentration gradient. Of the two
tissues considerably more information is available
concerning the absorptive process in the small intestine.
The conclusive evidence that expenditure of energy is
required for this process was the demonstration that
certain sugars could be transported across the intestinal
epithelium against a concentration gradient in vivo (7, 8)
and in vitro (9, 10). The stereospecific nature of sugar
absorption from the intestine was first suggested by the
finding that glucose and galactose were absorbed much
more rapidly than other sugars of an equal or smaller
molecular size (11-14). Later, Csák y (15) observed that
3-o-methyl glucose was absorbed from rat intestine at a
rate similar to that of glucose while 2-o-methyl, 5-o-
methyl and 6-o-methyl derivatives were more slowly
absorbed.

The specificity of the sugar transport system has
recently been extended in studies with sacs of everted
hamster intestine in vitro (16-18). The present work is
still a further extension of these studies and utilizes a
variety of derivatives of glucose, fructose and galactose.
From the total of 49 compounds examined to date it is
possible to give a more detailed description of the trans-
port capacity of the small intestine.

METHODS

Golden hamsters weighing 80-150 gm were killed by
a blow on the head and the entire small intestine washed
out in situ with 0.15 M NaCl. The intestine was removed
from the animal and everted with a long stainless steel
probe as previously described (10). Tied sacs of intestine
(about 900 mg wet weight) were prepared containing
about 1 ml of the sugar solution to be tested. The sac
was placed in a 50-ml Erlenmeyer flask with 3-5 ml of
Krebs-Henseleit bicarbonate saline (19) containing
the same sugar. The flask was gassed with 5% CO2 and
95% O2 and incubated at 37° with shaking for 60 or 90
minutes. Following incubation the final volume within
the sac was determined by weighing the sac before and
after emptying. Each sugar was tested in sacs from at
least three hamsters. For each intestine a 'control' sac
with glucose in the medium was incubated to make
certain that the transport system was functioning.
TABLE I. Chromatographic Separation of Sugars With the Butanol:Pyridine:Water Solvent

<table>
<thead>
<tr>
<th>Sugar</th>
<th>Rₚ*</th>
</tr>
</thead>
<tbody>
<tr>
<td>D-glucose and L-glucose</td>
<td>1.0</td>
</tr>
<tr>
<td>D-galactose</td>
<td>0.8</td>
</tr>
<tr>
<td>D-fructose</td>
<td>1.1</td>
</tr>
<tr>
<td>D-mannose</td>
<td>1.1</td>
</tr>
<tr>
<td>D-ribose</td>
<td>1.3</td>
</tr>
<tr>
<td>L-xylene</td>
<td>1.2</td>
</tr>
<tr>
<td>D-lyxose</td>
<td>1.2</td>
</tr>
<tr>
<td>L-arabinose</td>
<td>1.1</td>
</tr>
<tr>
<td>α-methyl glucoside</td>
<td>1.4</td>
</tr>
<tr>
<td>3-o-methylglucose</td>
<td>1.5</td>
</tr>
<tr>
<td>3-o-ethylglucurine</td>
<td>1.9</td>
</tr>
<tr>
<td>3-o-propylglucose</td>
<td>2.4</td>
</tr>
<tr>
<td>3-o-butylglucose</td>
<td>2.6</td>
</tr>
<tr>
<td>3-o-hydroxyethylglucose</td>
<td>1.7</td>
</tr>
<tr>
<td>3-deoxyglucose</td>
<td>1.5</td>
</tr>
<tr>
<td>2-o-methylglucose</td>
<td>1.6</td>
</tr>
<tr>
<td>2-deoxygalactose</td>
<td>1.3</td>
</tr>
<tr>
<td>4-o-methylgalactose</td>
<td>1.2</td>
</tr>
<tr>
<td>2,4-di-o-methylgalactose</td>
<td>1.1</td>
</tr>
<tr>
<td>6-deoxyglucose</td>
<td>1.5</td>
</tr>
<tr>
<td>6-deoxy-6-fluoroglucose</td>
<td>2.2</td>
</tr>
<tr>
<td>D-gulose</td>
<td>1.3</td>
</tr>
<tr>
<td>6-o-methylglucose</td>
<td>1.4</td>
</tr>
<tr>
<td>6-deoxy-6-ido-n-galactose</td>
<td>1.7</td>
</tr>
<tr>
<td>Gold-thioglucose</td>
<td>0</td>
</tr>
</tbody>
</table>

* Cm sugar moved on paper/cm glucose moved on paper.

In each experiment aliquots of the initial and final solutions were chromatographed on paper in the butanol-pyridine-water (6:4:3) system (20). The rates of movement of sugars on the paper relative to glucose (Rₚ) are given in Table 1. The α-methyl glucoside was visualized with a 1 % meta periodate spray followed by 0.5 % KMnO₄ spray. Sugar spots were visualized with a benzidine spray (21). Paper chromatograms of the final solutions showed only a single spot, which corresponded in Rf to the initial test sugar.

Sugar estimations were carried out on the final mucosal and final serosal solutions. The concentrations of 6-o-methyl glucose, 3-deoxyglucose and 4-o-methylgalactose were determined by the Somogyi-Nelson method (22). The methyl glucoside was estimated by the Somogyi-Nelson method following incubation of the sugar in N HCl at 100°C for 60 minutes and neutralization with NaOH. 2-o-Methylglucose and the 3-o-alkyl glucoses were determined by the anthrone method of Roe (23) and 2-deoxygalactose was measured by means of the quinaldine reagent (24). In a few experiments a glucose oxidase method (25) rather than anthrone was employed.

RESULTS

The results obtained with several of the sugar derivatives are given in figures 1–5. In each experiment the initial concentration of sugar was the same on the two sides of the intestinal wall. A fall in concentration on the mucosal side and rise on the serosal side indicated a net movement of sugar across the wall against a concentration gradient. The volume changes on the two sides were either negligible or occurred in the direction of mucosal to serosal sides. The latter movement of fluid tended to reduce the concentration of a nontransported sugar on the serosal side.

The hydroxyl group at carbon 1 of the glucose molecule may be modified by the formation of a glycoside and still retain characteristics favorable for intestinal transport. When α-methylglucoside (10 mM) was placed on both sides of the intestinal sac the sugar was transported across the wall and final concentration gradients of from 5- to 20-fold were observed. Little or no free glucose was found although some sugar loss from the medium occurred. Gold-thioglucose, on the other hand, was not transported. This sugar (at an initial concentration of 4 mM) was tested with five separate experimental sacs including both jejunum and ileum. On chromatograms of the final solutions only one spot was noted (corresponding in position to the initial sugar) and no formation of free glucose was detected. When the hydroxyl group at carbon number 2 of the glucose or galactose molecule was modified by removal (2-deoxygalactose) or by the addition of a methyl group (2-o-methylglucose) transport did not occur (fig. 1). These compounds are not metabolized by the tissue as shown by a 90–100 % recovery of the sugars at the end of the experiment. When the constituent groups at carbon 3 of glucose were modified by removal of the hydroxyl group (3-deoxyglucose) (see fig. 5) or by the
introduction of a methyl group (3-O-methylglucose) transport occurred, but was not as marked as with glucose. With the alkyl group ethyl, propyl or butyl there was no sugar transport (fig. 2). Galactose, which is known to be transported, differs from glucose only in the position of the hydroxyl at carbon 4. When the four position of galactose contained a methyl group, 4-O-methylgalactose, transport also occurred (fig. 3).

The failure of transport of 6-O-methylglucose confirmed the previous work of Csáky with this compound. However, 6-deoxyglucose (17) and 6-deoxy-6-fluoro-glucose were well transported (fig. 4). A summary of the results with other sugars is given in table 2.

Sugar utilization (or, more exactly, disappearance from the incubation medium) occurred with 3-deoxyglucose. The utilization of this sugar compared with glucose is shown in figure 5. Paper chromatography of the final solutions did not reveal any other benzidine-reacting material. In five experiments with an initial concentration of from 5 to 8 mM, 20-60% of the 3-deoxyglucose disappeared during the 1-hour incubation.

**DISCUSSION**

One important criterion for active transport of a neutral molecule across a membrane is its movement against a concentration gradient. The study of such a process in the case of the small intestine has been greatly simplified by in vitro preparations in which the composition of the solutions on both sides of the wall may be controlled. To date, 49 different sugars or related compounds have been tested with everted sacs of hamster intestine.

The intestine shows a considerable degree of selectivity as only 14 of the 49 sugars tested were transported.
against a concentration gradient. The glucose molecule with certain modifications about carbon 1 still retains the capacity to be transported (e.g. 1-deoxyglucose or α-methylglucoside) but the substitution of S-Au fur -OH gives an inactive compound. Two modifications of the hydroxyl at carbon 2 led to inactive compounds confirming the proposal of Crane and Krane (17) that this group is essential for transport. The carbon-bound hydrogen at position 2 is not essential as 3-0-deoxyethyl, 3-o-propyl, 3-o-butyl and 3-o-hydroxyethyl are transported while the 3-o-deoxyethyl derivatives are not active.

In the 6 position the deoxy-iodo and o-methyl are not. There are apparently limitations in size of substituent groups which are required by the transport system. This is clearly shown with a series of compounds modified in the 3 position of the glucose molecule; 3-deoxy and 3-o-methyl derivatives are transported while the 3-o-ethyl, 3-o-propyl, 3-o-butyl and 3-o-hydroxyethyl derivatives are not active. In the 6 position the deoxy and deoxy-fluoro derivatives are active while the deoxy-iodo and o-methyl are not.

Steric orientation is also important as only the D- and deoxy-fluoro derivatives are active while the deoxy-derivatives are not active. In the 6 position the deoxy-iodo and o-methyl are not.

REFERENCES


T. HASTINGS WILSON AND BERNARD R. LANDAU

isomers of glucose, galactose and 6-deoxygalactose are transported. Reversal of the orientation of hydroxyl groups at carbon 3 (allose) or carbon 4 (galactose) does not prevent transport while reversal of both (gulose) does.

A limited amount of information is available concerning the number of sugars transported by a common pathway. Glucose and galactose compete with one another for absorption as shown by in vivo (27) and in vitro (28, 29) experiments. Csáky has shown competition between 3-o-methyl glucose and glucose (30). Keston, on the other hand, has reported that 1-deoxyglucose did not inhibit glucose absorption (31). Further studies are needed to confirm these observations and extend the sugar combinations.

The authors are indebted to E. L. Blakley of the University of Minnesota for 6-deoxy-6-fluoro-n-glucose, to Dr. G. B. Creamer and C. B. Purves of McGill University for 3-o-hydroxyethyl-n-glucose, to Dr. K. Jeanloz of Harvard Medical School for 4-o-methyl-n-galactose and 2,4-di-o-methyl-n-galactose. Gold-thio-glucose was obtained from Merck and Co., 2-deoxy-galactose and 6-deoxy-6-idogalactose from Aldrich Chemical Co., 3-o-methyl, 3-o-ethyl, 3-o-propyl and 3-o-butyl n-glucose and 3-o-methyl fructose from Ayerst, McKenna & Harrison, Ltd. 2-o-methyl-n-glucose was obtained from Dr. E. Tissis and 3-o-deoxyglucose from Dr. J. Pratt of the National Institutes of Health.