The Role of Phosphorylation in Glucose Absorption from the Intestine of the Golden Hamster*

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The exact biochemical mechanism by which glucose is absorbed by the intestine against a concentration gradient is yet to be established. In 1933, Wilbrandt and Laszt (1) proposed the phosphorylation-dephosphorylation hypothesis for sugar absorption by the small intestine. In the same year Lundsgaard (2) suggested the same hypothesis for glucose reabsorption by the kidney tubules. Much of the evidence on which this hypothesis was originally based has since proved inadequate. On the other hand, conclusive evidence against the hypothesis is lacking.

The present study was designed to test the hypothesis of the phosphorylation of glucose during the transport process. Ashmore et al. (3) employed a method for calculating the quantity of glucose which was phosphorylated to glucose 6-phosphate by liver slices and subsequently hydrolyzed to glucose and inorganic phosphate. In a similar manner an attempt has been made in the present study to calculate the quantity of glucose arising from glucose 6-phosphate during the course of intestinal absorption of glucose by sacs of hamster intestine. The results obtained by such experiments indicate that only a small fraction of glucose transported passes through glucose 6-phosphate as an intermediate.

EXPERIMENTAL

Materials and Methods

Preparation of Tissue—Golden hamsters (80 to 150 gm.) fed Purina laboratory chow were used. The animal was killed by a blow on the head, the abdomen opened, and the entire small intestine washed out in situ with 0.9 per cent NaCl. The intestine was stripped from its mesentery and turned inside out with the use of a long probe. After washing the everted intestine carefully in 0.9 per cent NaCl, it was placed in a Petri dish that contained a modified Krebs-Henseleit (4) bicarbonate-sodium chloride medium with the following composition in mmoles: Na, 143; K, 6; Mg, 1.2; Ca, 1.3; Cl, 125; and HCO₃, 25. Tied sacs of intestine (about 200 mg. wet weight of tissue) were prepared in the manner previously described (5). From the gut of a single animal 2 to 8 sacs were prepared.

A measured volume (approximately 1 ml.) of bicarbonate-saline medium was injected into each sac (serosal solution). For the mucosal solution 3 or 5.0 ml. of bicarbonate-sodium chloride medium were used. The filled sacs were placed in 50-ml. flasks containing the mucosal solution. Flasks were gassed with 5 per cent CO₂ and 95 per cent O₂ and incubated with shaking for 1 hour at 37°. The final volume on the serosal side was determined by weighing the sac before and after emptying the contents. The final mucosal volume was calculated from the initial value and the change on the serosal side, assuming no tissue swelling or evaporation.

Experimental Design—Three different studies were performed.

1. To estimate the contribution of the phosphogluconate oxidative pathway to gut metabolism, glucose-1-C¹⁴ or -6-C¹⁴ was present in both the mucosal and serosal solutions of a sac at a concentration of 200 mg. per 100 ml. Sacs from adjacent segments of intestine were used, one labeled with glucose-1-C¹⁴ and the other with -6-C¹⁴. At the completion of the incubation, the sac was cut open and the mucosal and serosal solutions combined. An aliquot of this combined solution was discharged into sulfuric acid, the CO₂ evolved was trapped in sodium hydroxide, and precipitated as barium carbonate for assay. Total CO₂ in the flask, i.e. in the gas phase and the medium, was shown to be about 225 μmoles.

2. To estimate the extent of rearrangement of the glucose carbon skeleton during transport, glucose-6-C¹⁴ was placed at a concentration of 200 mg. per 100 ml. on the mucosal side and the glucose transported during the incubation was isolated as the glucoazone from the serosal solution with the aid of carrier glucose. The glucoazone was assayed and then degraded (7) to the 1,2-bisphenylhydrazone mesoxaldehyde, which was also assayed. This latter compound contained carbons-1, -2, and -3 of the glucose molecule.

3. To estimate the quantity of glucose transported which had glucose 6-phosphate as an intermediate, sacs from adjacent segments were incubated in 5 ml. of bicarbonate-sodium chloride medium containing either galactose-1-C¹⁴ (100 mg. per 100 ml.) and nonradioactive glucose (200 mg. per 100 ml.), or nonradioactive galactose (100 mg. per 100 ml.) and glucose-1-C¹⁴ (200 mg. per 100 ml.). Bicarbonate-saline medium, 1 ml., (containing no sugar) was placed on the serosal side. At the completion of the incubation, the concentration of glucose in the mucosal and sero-
sal solutions was estimated by determining reducing substance (8) before and after incubation with glucose oxidase. Galactose was taken as the reducing sugar unaffected by the enzyme, with a small correction for slow oxidation of the galactose. For the determination of radioactivity in CO$_2$ as described above 1 ml. of mucosal solution was taken.

In order to determine the activity of the glucose and galactose transported, 0.25 ml. of the serosal solution was placed on a 10-cm. line at the origin of a paper chromatogram. The chromatogram was developed for 40 hours in the descending direction with butanol-pyridine-water (6:4:3) (9). This solvent separates glucose from galactose, glucose running in the forward position. After drying, a thin strip of paper was cut from each side of the chromatogram and sprayed with benzidine (10) to develop the sugar spots. By this method it was possible to determine the areas occupied by glucose and galactose in the untreated central portion of the paper. The forward one-half of the glucose band was eluted, the quantity of sugar determined (8), and the glucosazone was prepared with the aid of carrier glucose. A similar procedure was followed with the entire galactose band with carrier galactose added to facilitate the isolation of the galactosazone. An aliquot of the initial incubation medium was treated in the same way, and it was found that the specific activities of the substrates run on paper chromatograms and eluted varied no more than 10 per cent from the specific activity determined by preparation directly of the osazones. There was no contamination of nonradioactive glucose by galactose-1-C$^{14}$ when it was isolated from solution by this chromatographic procedure. Furthermore, the osazones were counted, recrystallized, and counted a second time. Constant specific activity was found in all cases. It was previously found that by such recrystallization galactosazone could be separated from glucosazone (11).

Calculations—The pmoles of C$^{14}$-labeled glucose or galactose oxidized to CO$_2$, the pmoles of glucose-6-C$^{14}$ incorporated into the mesoxaldehyde, and the pmoles of galactose converted to glucose were calculated from (a) the specific activity of the substrates, (b) the specific activity of the metabolic products, and (c) the quantity of metabolic products present. Results were expressed as pmoles per 100 mg. of intestine wet weight per 60 minutes of incubation.

In the derivation of the quantity of glucose on the mucosal side converted to glucose on the serosal side via glucose 6-phosphate, it was assumed that glucose and galactose metabolism in the intestinal epithelial cell was similar to that in other mammalian cells and that most of the metabolism studied in the preparation occurred in the epithelial cells. That is, as shown in Fig. 1, it was assumed that in the metabolism of the intestine glucose and galactose had as their common intermediate glucose 6-phosphate, that carbon dioxide was formed from these substrates only after conversion to glucose 6-phosphate, and that glucose, once phosphorylated to glucose 6-phosphate, could be released as free glucose only directly from glucose 6-phosphate. It was further assumed that glucose and galactose both contributed to the same glucose 6-phosphate pool and that the CO$_2$ collected served as a sampling of the contributions of each to the pool. Therefore, since both CO$_2$ and glucose arose from the same glucose 6-phosphate pool, the relative proportion of glucose and galactose converted to CO$_2$ was the same as the proportion of the two substrates converted to glucose, i.e.:

\[
\frac{\text{Glucose to CO}_2}{\text{Galactose to CO}_2} = \frac{\text{glucose to glucose-6-PO}_4 \text{ to glucose}}{\text{galactose to glucose-6-PO}_4 \text{ to glucose}}
\]

The quantity of glucose converted to glucose via glucose-6-phosphate was then compared with the quantity of glucose that was transported during the experiment. This latter quantity was taken as that quantity of glucose found on the serosal side of the intestinal wall after incubation.

**RESULTS**

Since a knowledge of the extent of the conversion of radioactive glucose to CO$_2$ was required for the determination of glucose phosphorylation, preliminary studies were carried out on the effect of location along the gut on this conversion. Both glucose-1-C$^{14}$ and glucose-6-C$^{14}$ oxidation to CO$_2$ were studied at different locations of the gut to obtain the additional information as to the possible presence of the phosphogluconate oxidative pathway. Table I shows the results obtained in three animals, six sacs of tissue being prepared from each. In all cases but one, the CO$_2$ produced from glucose-1-C$^{14}$ was more radioactive than that from glucose-6-C$^{14}$, suggesting the presence of the oxidative pathway.

One possible mechanism of glucose transport involving glucose 6-phosphate as an intermediate was investigated with glucose-6-C$^{14}$. If the mechanism for transport were the conversion of hexose to triose followed by the resynthesis of hexose, glucose-6-C$^{14}$ entering the cell from the mucosal side would emerge on the opposite side as glucose-1-6-C$^{14}$. An experiment designed to test this possibility is shown in Table II. In each of the four sacs over 95 per cent of the initial glucose-6-C$^{14}$ was absorbed from the mucosal side, an average of 3.3 mg. of the initial 6 mg. being recovered on the serosal side. The average final concentration gradient was 74-fold. The glucose transported to the serosal side was isolated at the end of incubation as the phenylosazone. This was oxidized to the 1,2-bisphenylhydrazone of mesoxaldehyde which contained carbons-1, -2, and -3 of the original glucose molecule. Table II (Column 8) shows that no significant activity was found in the first three carbons (isolated as the mesoxaldehyde). One further point of interest is that glucose emerging on the serosal side had about the same specific activity as that on the mucosal side initially (Columns 3 and 4). This indicates negligible dilution of the glucose in its passage through the tissue.

An attempt was next made to evaluate the role of the conversion of glucose to glucose 6-phosphate in the process of glucose absorption. The experiments were carried out in the presence of both glucose and galactose on the mucosal side of the intestinal wall; in one flask glucose was radioactive, while in the other galactose was radioactive. As there was some variation from one location of the gut to another, three consecutive segments were used; the first and third were incubated in one solution, and the second in the other. The average values for
From each of three hamsters 6 sacs were prepared and alternate sacs were incubated with either glucose-1-Cl\textsuperscript{4} or glucose-6-Cl\textsuperscript{4} at a glucose concentration of 200 mg. per 100 ml. The carbon dioxide formed from the labeled glucose is presented as pmoles of CO\textsubscript{2} per 100 mg. of intestine per 60 minutes of incubation.

### Table I

<table>
<thead>
<tr>
<th>Hamster</th>
<th>Glucose-1-Cl\textsuperscript{4}</th>
<th>Glucose-6-Cl\textsuperscript{4}</th>
<th>Ratio (d/d0)</th>
<th>Glucose-1-Cl\textsuperscript{4}</th>
<th>Glucose-6-Cl\textsuperscript{4}</th>
<th>Ratio (d/d0)</th>
<th>Glucose-1-Cl\textsuperscript{4}</th>
<th>Glucose-6-Cl\textsuperscript{4}</th>
<th>Ratio (d/d0)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>1.55</td>
<td>1.07</td>
<td>1.45</td>
<td>1.09</td>
<td>0.56</td>
<td>1.51</td>
<td>0.80</td>
<td>0.56</td>
<td>1.43</td>
</tr>
<tr>
<td>2</td>
<td>0.84</td>
<td>0.50</td>
<td>1.68</td>
<td>0.59</td>
<td>0.39</td>
<td>1.51</td>
<td>0.76</td>
<td>0.48</td>
<td>1.58</td>
</tr>
<tr>
<td>3</td>
<td>0.84</td>
<td>0.65</td>
<td>1.29</td>
<td>0.81</td>
<td>0.79</td>
<td>1.09</td>
<td>0.47</td>
<td>0.47</td>
<td>1.00</td>
</tr>
<tr>
<td>Mean</td>
<td>1.08</td>
<td>0.74</td>
<td>1.47</td>
<td>0.83</td>
<td>0.55</td>
<td>1.52</td>
<td>0.68</td>
<td>0.50</td>
<td>1.36</td>
</tr>
</tbody>
</table>

### Table II

**Possible conversion of glucose-6-Cl\textsuperscript{4} to glucose-1-6-Cl\textsuperscript{4} during intestinal transport**

Glucose-6-Cl\textsuperscript{4} (200 mg. per 100 ml.) was placed in the mucosal incubation medium of 4 sacs from two hamsters. The specific activity of the glucose employed and of the glucose isolated from the serosal medium at the completion of the incubation (60 minutes) was determined as the glucosazone. The initial and serosal glucosazones were then degraded to their corresponding mesoxaldehydes (which contain carbons 1, 2, and 3 of the glucose) and their activity was determined. Specific activity is given in c.p.m. per μmole of glucosazone or mesoxaldehyde.

### Table III

**Sample calculation* of fraction of transported glucose which was phosphorylated**

Sacs were prepared from three adjacent segments of small intestine of a single hamster. The first and third segments were incubated with galactose-1-Cl\textsuperscript{4} (100 mg. per 100 ml.) in the presence of glucose (200 mg. per 100 ml.) and the second with galactose-1-Cl\textsuperscript{4} (100 mg. per 100 ml.) in the presence of glucose-1-Cl\textsuperscript{4} (200 mg. per 100 ml.). Values are given as μmoles per 100 mg. of tissue per 60 minutes of incubation.

<table>
<thead>
<tr>
<th>Sac No.</th>
<th>Location</th>
<th>Unlabeled sugar</th>
<th>Labeled sugar</th>
<th>Sugar-Cl\textsuperscript{4} to CO\textsubscript{2}</th>
<th>Galactose-1-Cl\textsuperscript{4} to glucose</th>
<th>Glucose transported</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>upper jejunum</td>
<td>glucose</td>
<td>galactose</td>
<td>0.12</td>
<td>0.15</td>
<td>9.68</td>
</tr>
<tr>
<td>2</td>
<td>mid jejunum</td>
<td>galactose</td>
<td>galactose</td>
<td>0.72</td>
<td>0.15</td>
<td>7.20</td>
</tr>
<tr>
<td>3</td>
<td>low jejunum</td>
<td>glucose</td>
<td>galactose</td>
<td>0.06</td>
<td>0.06</td>
<td>7.60</td>
</tr>
</tbody>
</table>

* Glucose 1-Cl\textsuperscript{4} to CO\textsubscript{2}
  
Galactose-1-Cl\textsuperscript{4} to CO\textsubscript{2} =

\[
\frac{\text{Glucose (mucosal) to glucose (serosal) via Glucose-6-P}}{\text{Galactose (mucosal) to glucose (serosal) via Glucose-6-P}}
\]

Substituting we have:

\[
\text{Glucose (mucosal) to glucose (serosal) via glucose-6-P} = \frac{(0.72)(0.5)(0.15 + 0.06)}{0.5(0.12 + 0.06)} = 0.84
\]

Net glucose transported (average of Sacs 1 + 3) = 8.6

Per cent of glucose transported via glucose-6-P = \( \frac{0.84}{8.6} \times 100 \) = 10 per cent
the mucosal side, one sugar or the other being radioactive. No
lactose was still moving down a concentration gradient at the
galactose (100 mg. per 100 ml.). In the presence of glucose, ga-
the utilization rate of galactose. This is illustrated in Table V.

The serosal side
with or without glucose (200 mg. per 100 ml.) on the mucosal side.
The mucosal side contained no sugar. Galactose disappearing from
the medium during incubation is indicated by “galactose util-
ized.” All values are given as millimoles per 100 mg. of tissue per 60
minutes of incubation.

| No. | Glucose to CO₂ | Galactose to glucose | Glucose to glucose via Glucose-6-P | Glucose transported | Sugar phospho-
<table>
<thead>
<tr>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>phosphorylated</td>
</tr>
<tr>
<td>1</td>
<td>8.0</td>
<td>0.105</td>
<td>0.81</td>
<td>8.6</td>
<td>10</td>
</tr>
<tr>
<td>2</td>
<td>12.0</td>
<td>0.050</td>
<td>0.60</td>
<td>4.2</td>
<td>14</td>
</tr>
<tr>
<td>3</td>
<td>5.1</td>
<td>0.069</td>
<td>0.35</td>
<td>8.0</td>
<td>4</td>
</tr>
<tr>
<td>4</td>
<td>9.3</td>
<td>0.030</td>
<td>0.37</td>
<td>7.8</td>
<td>0</td>
</tr>
<tr>
<td>5</td>
<td>22.0</td>
<td>0.037</td>
<td>0.82</td>
<td>5.4</td>
<td>15</td>
</tr>
<tr>
<td>6</td>
<td>9.4</td>
<td>0.039</td>
<td>0.37</td>
<td>9.0</td>
<td>4</td>
</tr>
<tr>
<td></td>
<td>Average</td>
<td></td>
<td></td>
<td></td>
<td>8.7</td>
</tr>
</tbody>
</table>

* Final concentration on serosal side/final concentration on
mucosal.
† Final serosal concentration = 259 mg. per 100 ml.; final
mucosal concentration = 0.

than that on the mucosal side at the termination of the incuba-
tion.

Glucose has a profound effect on both the transport rate and
the utilization rate of galactose. This is illustrated in Table V.
A very striking inhibition of galactose transport was noted with
glucose (200 mg. per 100 ml.) at a concentration twice that of
galactose (100 mg. per 100 ml.). In the presence of glucose, ga-
 lactose was still moving down a concentration gradient at the
time when, in the absence of glucose, a very large concentration
gradient was developing. Also oxidation of galactose to CO₂ and galactose utilization was markedly decreased in the
presence of glucose. Although the inhibition of galactose trans-
port by glucose is well known (12, 13), the inhibition of utili-
zation and CO₂ production has not previously been noted.

**DISCUSSION**

The phosphorylation-dephosphorylation hypothesis of sugar
transport (1, 2) was based primarily on the observations that
iodoacetate and phlorizin (thought at that time to be specific
inhibitors of phosphorylation reactions) inhibited glucose ab-
 sorption by the small intestine (1, 14-17) and kidney (18-20).
Subsequent studies have shown that iodoacetate is not a specific
inhibitor but reacts with the sulfhydryl groups of many enzymes.
Furthermore, the primary site of action of phlorizin is apparently
not on hexokinase or phosphatase but on oxidative pathways (21,
22). Additional evidence for the hypothesis was the finding that
the kinase activity in the intestine for different sugars was pro-
portional to their rate of intestinal absorption (23-25), although
this was subsequently disputed (26). The accumulation of sugar
phosphates within the epithelium which occurs during absorption
(27-32) has also been taken as evidence for the hypothesis.

However, many cells increase the concentration of intermediates
of metabolism on the addition of utilizable sugars and the ac-
cumulation of sugar phosphates cannot be taken as conclusive
evidence for their participation in the transport process itself
(although it is consistent with the hypothesis). Alterations in
the concentration of phosphatases in the kidney (33) and gut
(34) in various states have also been used to support the hypoth-
thesis.

One variation of the general phosphorylation hypothesis in-
volves the conversion of glucose to two triose phosphates fol-
lowed by recondensation to hesose. This possibility was con-
sidered (35) as lactic acid was found during glucose and fructose
absorption both in vitro (5, 36-38) and in vivo (39-41). Con-
siderable evidence against this triose hypothesis, however, has
been presented both for the intestine (35, 42, 43) and the kidney
(44). The present experiments (Table II) provide further evi-
dence that this mechanism does not occur in the small intestine
of the hamster.

Two recent studies have offered what may be considered direct
evidence against the general phosphorylation hypothesis. First,
Sols (26) was unable to detect kinase activity for the sugars
galactose and 3-O-methylglucose, two sugars transported by the
small intestine at concentration gradients (38). However, indi-
eght evidence suggests that galactose is phosphorylated.

The hamster intestine utilizes considerable quantities of galactose
(35), converts some to CO₂ (see Table V) and a small amount to
lactic acid (37). Qualitative tests performed by Kjerulf-Jensen
(30) on the phosphate esters accumulated in the gut epithelium
of the rat during galactose absorption suggested the presence of
a galactose phosphate. Although 3-O-methylglucose is not uti-
lized by the intact rat (45) nor converted to lactic acid by the
intestine (37), its phosphorylation by preparations of rat intestine
has been reported (46). During 3-O-methylglucose absorption
by the hamster intestine small amounts of a sugar phosphate
can be isolated by ion exchange columns, which on elution from
the column and treatment with phosphatase yield a sugar chro-
natographically identical with 3-O-methylglucose.³

The second observation inconsistent with this hypothesis is the
finding of Crane and Krane (47) that both 1-deoxyglucose and
6-deoxyglucose are transported by sacs of hamster intestine.
The assumption was made that these two sugars and glucose are
transported by a common mechanism and that phosphorylation
³ T. H. Wilson, unpublished experiments.
of hydroxyl groups on carbons other than 1 and 6 does not occur in the absorption mechanism. The assumptions appear reasonable, but await experimental verification.

The experiments presented in this paper add further weight of evidence against the participation of glucose 6-phosphate as an intermediate in the transport of glucose across the intestine, at least in the case of the hamster. It should be emphasized that a number of assumptions have been made in the calculations used and the conclusions must be considered tentative until a more direct method for testing the hypothesis is available.

**SUMMARY**

1. The conversion of glucose-1-C\(^14\) and glucose-6-C\(^14\) to CO\(_2\) has been studied with sacs of hamster intestine in vitro. The presence of the phosphogluconate oxidative pathway was indicated by the greater CO\(_2\) production from C-1 than C-6 labeled glucose.

2. The possibility of hexose conversion to triose followed by resynthesis of hexose by the intestine was tested. It was presumed not to occur as glucose-6-C\(^14\) absorbed from the mucosal side of the in vivo preparation did not show randomization on passage across the intestinal wall.

3. By incubating sacs of intestine in paired flasks in the presence of glucose and galactose, the quantity of glucose transported across the intestinal wall.

4. The experiments presented in this paper add further weight of evidence against the participation of glucose 6-phosphate as an intermediate in the transport of glucose across the intestine, side of the in vitro preparation did not show randomization on passage across the intestinal wall.

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