Cerebral Glucose-6-phosphatase and the Movement of 2-Deoxy-d-glucose across Cell Membranes*

(Received for publication, December 16, 1976, and in revised form, June 6, 1977)

J. Michael Anchors, Donald F. Haggerty, and Manfred L. Karnovsky

From the Departments of Biological Chemistry and Psychiatry, Mental Retardation Research Center, University of California School of Medicine, Los Angeles, California 90024 and the Department of Biological Chemistry, Harvard Medical School, Boston, Massachusetts 02115

Glucose-6-phosphatase (glucose-6-phosphohydrolase and its associated phosphotransferase activities) was determined in brain tissue and in several preparations derived from brain tissue. These included purified capillaries and established cell lines of neuronal or glial origin. Since it has been suggested that glucose-6-phosphatase may be involved in sugar transport, the characteristics of that process were examined in these preparations. The pattern of uptake of 2-deoxy-d-glucose in four cell lines was shown to involve transport of the analog across the cell membrane that was more rapid than the subsequent phosphorylation of the sugar in the intracellular compartment. In the remaining cell lines and in purified capillaries, phosphorylation of 2-deoxy-d-glucose was at least as rapid as uptake. No differences could be found between the cells in these two categories with respect to amount or localization of glucose-6-phosphatase, ability to phosphorylate 3-O-methyl-D-glucose, or ability to phosphorylate extracellular and intracellular 2-deoxy-d-glucose. In the course of these experiments, it was found that there was a rapid efflux of 2-deoxy-d-glucose from cells that had taken up this sugar. The efflux involves a dephosphorylation step catalyzed by intracellular phosphatase that releases free sugar in the cytoplasm. Glucose-6-phosphatase thus probably has no major role in the phosphorylation of glucose in brain cells, but acts in the more conventional sense, i.e., as a phosphohydrolase.

We have reported that when radioactive inorganic orthophosphate is infused into the lateral ventricles of rats (1), more radioactivity is incorporated into cerebral glucose-6-phosphatase (EC 3.1.3.9) during sleep than during wakefulness. The phosphate-containing enzyme is presumably the enzymatic intermediate (2). Furthermore, we recently found that the enzymatic activity of cerebral glucose-6-phosphatase increases during sleep relative to wakefulness. We would like to know what role increased activity of cerebral glucose-6-phosphatase might play in sleep, but at present the metabolic function of the enzyme, especially in brain, is incompletely comprehended. Hepatic glucose-6-phosphatase is considered to be involved in the release of glucose into blood (3). Could this be the role of the enzyme in brain, or does the enzyme operate predominantly in the reverse direction, i.e., in the uptake of glucose from blood (3)?

Materials and Methods

Chemicals and Reagents—D-Glucose, 2-deoxy-D-glucose, 3-O-methyl-D-glucose, phlorizin, oligomycin, bovine insulin, glucose-6-phosphate dehydrogenase (type VII, yeast), and the sodium salts of d-glucose 6-phosphate, β-glycerophosphate, inorganic pyrophosphate, and nicotinamide adenine dinucleotide phosphate were purchased from Sigma Chemical Co., St. Louis, Mo. Ferrous sulfate and ammonium molybdate were purchased from Mallinckrodt Inc., St. Louis, Mo. Sodium pentobarbital was obtained from Abbott Laboratories, North Chicago, Ill.

Radiochemicals—2-Deoxy-D-[G-3H]glucose, 3-O-methyl-d-[14C]glucose, 14C-thiourea, and 1H-thiocyanate were purchased from New England Nuclear, Boston, Mass. 2-Deoxy-d-[14C]glucose was obtained from the isotope and nuclear division of ICN Pharmaceuticals, Irvine, Calif.

Cell Culture—The following cell lines were obtained from the sources indicated: B65, B103, and B104 cells, derived from transplacentally induced (ethyl nitrosourea) tumors of the rat central nervous system, from Dr. David Schubert, Salk Institute; NB41-43-2-X1 mouse neuroblastoma cells and 3T3 Swiss mouse embryo fibroblasts from Dr. Harvey Horsman; C6 rat glioma cells from Dr. Jean deVellis, University of California, Los Angeles; and H4-II-E-C3 rat hepatoma cells from Dr. Van R. Potter, University of Wisconsin, to all of whom the authors are most grateful. Each cell line was propagated in 75-cm² Falcon plastic tissue culture flasks (Scientific Products, Santa Ana, Calif.) under conditions optimal for its growth: 2T3 cells were grown in Eagle's minimum essential medium; B65, B103, and B104 cells, derived from transplacentally induced (ethyl nitrosourea) tumors of the rat central nervous system, from Dr. David Schubert, Salk Institute; NB41-43-2-X1 mouse neuroblastoma cells and 3T3 Swiss mouse embryo fibroblasts from Dr. Harvey Horsman; C6 rat glioma cells from Dr. Jean deVellis, University of California, Los Angeles; and H4-II-E-C3 rat hepatoma cells from Dr. Van R. Potter, University of Wisconsin, to all of whom the authors are most grateful. Each cell line was propagated in 75-cm² Falcon plastic tissue culture flasks (Scientific Products, Santa Ana, Calif.) under conditions optimal for its growth: 2T3 cells were grown in Eagle's minimum essential medium; B65, B103, and B104 cells, derived from transplacentally induced (ethyl nitrosourea) tumors of the rat central nervous system, from Dr. David Schubert, Salk Institute; NB41-43-2-X1 mouse neuroblastoma cells and 3T3 Swiss mouse embryo fibroblasts from Dr. Harvey Horsman; C6 rat glioma cells from Dr. Jean deVellis, University of California, Los Angeles; and H4-II-E-C3 rat hepatoma cells from Dr. Van R. Potter, University of Wisconsin, to all of whom the authors are most grateful. Each cell line was propagated in 75-cm² Falcon plastic tissue culture flasks (Scientific Products, Santa Ana, Calif.) under conditions optimal for its growth: 2T3 cells were grown in Eagle's minimum essential medium; B65, B103, and B104 cells, derived from transplacentally induced (ethyl nitrosourea) tumors of the rat central nervous system, from Dr. David Schubert, Salk Institute; NB41-43-2-X1 mouse neuroblastoma cells and 3T3 Swiss mouse embryo fibroblasts from Dr. Harvey Horsman; C6 rat glioma cells from Dr. Jean deVellis, University of California, Los Angeles; and H4-II-E-C3 rat hepatoma cells from Dr. Van R. Potter, University of Wisconsin, to all of whom the authors are most grateful. Each cell line was propagated in 75-cm² Falcon plastic tissue culture flasks (Scientific Products, Santa Ana, Calif.) under conditions optimal for its growth: 2T3 cells were grown in Eagle's minimum essential medium; B65, B103, and B104 cells, derived from transplacentally induced (ethyl nitrosourea) tumors of the rat central nervous system, from Dr. David Schubert, Salk Institute; NB41-43-2-X1 mouse neuroblastoma cells and 3T3 Swiss mouse embryo fibroblasts from Dr. Harvey Horsman; C6 rat glioma cells from Dr. Jean deVellis, University of California, Los Angeles; and H4-II-E-C3 rat hepatoma cells from Dr. Van R. Potter, University of Wisconsin, to all of whom the authors are most grateful. Each cell line was propagated in 75-cm² Falcon plastic tissue culture flasks (Scientific Products, Santa Ana, Calif.) under conditions optimal for its growth: 2T3 cells were grown in Eagle's minimum essential medium; B65, B103, and B104 cells, derived from transplacentally induced (ethyl nitrosourea) tumors of the rat central nervous system, from Dr. David Schubert, Salk Institute; NB41-43-2-X1 mouse neuroblastoma cells and 3T3 Swiss mouse embryo fibroblasts from Dr. Harvey Horsman; C6 rat glioma cells from Dr. Jean deVellis, University of California, Los Angeles; and H4-II-E-C3 rat hepatoma cells from Dr. Van R. Potter, University of Wisconsin, to all of whom the authors are most grateful. Each cell line was propagated in 75-cm² Falcon plastic tissue culture flasks (Scientific Products, Santa Ana, Calif.) under conditions optimal for its growth: 2T3 cells were grown in Eagle's minimum essential medium; B65, B103, and B104 cells, derived from transplacentally induced (ethyl nitrosourea) tumors of the rat central nervous system, from Dr. David Schubert, Salk Institute; NB41-43-2-X1 mouse neuroblastoma cells and 3T3 Swiss mouse embryo fibroblasts from Dr. Harvey Horsman; C6 rat glioma cells from Dr. Jean deVellis, University of California, Los Angeles; and H4-II-E-C3 rat hepatoma cells from Dr. Van R. Potter, University of Wisconsin, to all of whom the authors are most grateful. Each cell line was propagated in 75-cm² Falcon plastic tissue culture flasks (Scientific Products, Santa Ana, Calif.) under conditions optimal for its growth: 2T3 cells were grown in Eagle's minimum essential medium; B65, B103, and B104 cells, derived from transplacentally induced (ethyl nitrosourea) tumors of the rat central nervous system, from Dr. David Schubert, Salk Institute; NB41-43-2-X1 mouse neuroblastoma cells and 3T3 Swiss mouse embryo fibroblasts from Dr. Harvey Horsman; C6 rat glioma cells from Dr. Jean deVellis, University of California, Los Angeles; and H4-II-E-C3 rat hepatoma cells from Dr. Van R. Potter, University of Wisconsin, to all of whom the authors are most grateful.
Enzyme activity of rat liver and brain, and of various rodent cell lines

Each value represents the mean of four determinations. The standard deviations, in all cases, were less than 10% of the mean.

<table>
<thead>
<tr>
<th>Tissues or cell lines</th>
<th>Glucose-6-phosphatase</th>
<th>PP/glucose phosphotransferase</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>nmol min (^{-1}) mg (^{-1}) protein</td>
<td>Hexokinase</td>
</tr>
<tr>
<td>Liver</td>
<td>233</td>
<td>95.7</td>
</tr>
<tr>
<td>Brain</td>
<td>4.8</td>
<td>1.2</td>
</tr>
<tr>
<td>NB41</td>
<td>41.9</td>
<td>13.0</td>
</tr>
<tr>
<td>B60</td>
<td>51.0</td>
<td>18.8</td>
</tr>
<tr>
<td>B103</td>
<td>48.3</td>
<td>16.7</td>
</tr>
<tr>
<td>B104</td>
<td>46.7</td>
<td>14.5</td>
</tr>
<tr>
<td>C6</td>
<td>38.8</td>
<td>13.6</td>
</tr>
<tr>
<td>3T3</td>
<td>40.7</td>
<td>11.5</td>
</tr>
<tr>
<td>H4</td>
<td>54.9</td>
<td>14.6</td>
</tr>
</tbody>
</table>

Enzyme activity of cerebral cortex, liver, and seven selected cell lines (Table I). Both tissues and all seven cell lines contained these enzyme activities. Glucose-6-phosphatase has already been reported in cerebral capillaries (7), and a recent histochemical demonstration of its location in brain has been published (12). The enzymatic activities of the cell lines differed markedly from the corresponding activities of brain tissue. This should not be too surprising since brain tissue contains a miscellany of cell types. Kato and Lowry (13) have reported that the hexokinase activity of a cerebellar homogenate is 32.5 nmol min \(^{-1}\) mg \(^{-1}\) of protein, but the activity of isolated Purkinje cells a component of the cerebellum is only 5.0 nmol min \(^{-1}\) mg \(^{-1}\) of protein.

Enzyme activity in brain has been published (12). The enzymatic activities of these two enzymatic activities in brain may be exaggerated by this simple comparison since normally only a small fraction of cerebral hexokinase is active (15).

Results

Phosphotransferase Activities of Cells and Tissues – Glucose-6-phosphatase, pyrophosphate/glucose phosphotransferase, and hexokinase activities were assayed in cerebral cortex, liver, and seven selected cell lines (Table I). Both tissues and all seven cell lines contained these enzyme activities. Glucose-6-phosphatase has already been reported in cerebral capillaries (7), and a recent histochemical demonstration of its location in brain has been published (12). The enzymatic activities of the cell lines differed markedly from the corresponding activities of brain tissue. This should not be too surprising since brain tissue contains a miscellany of cell types. Kato and Lowry (13) have reported that the hexokinase activity of a cerebellar homogenate is 32.5 nmol min \(^{-1}\) mg \(^{-1}\) of protein, but the activity of isolated Purkinje cells a component of the cerebellum is only 5.0 nmol min \(^{-1}\) mg \(^{-1}\) of protein. The differences in enzymatic activities between the cell lines and brain tissue should not prejudice the use of these cells as a model for glucose transport in brain. That changes do occur in cellular enzymes upon culture is, however, a matter to be borne in mind.

The ratio of glucose-6-phosphatase activities to pyrophosphate/glucose phosphotransferase activities was about 3:1 or 4:1 in all cases. The constancy of this ratio implies that the two activities appertain to the same enzyme (1, 14). If acid phosphatase or alkaline phosphatase had contributed significantly to the hydrolysis of glucose 6-phosphate, the ratios of hydrolytic to phosphotransferase activity would have been higher. Moreover, glycerophosphate, which is an excellent substrate for nonspecific phosphatase, was hydrolyzed very slowly by the membrane fraction of the cells and tissues. Alkaline phosphatase was inactive at pH 5.5, the pH of the assay medium used to assay glucose-6-phosphatase.

From the pH-dependent curves of Norrlie (3), it may be estimated that the activity of pyrophosphate/glucose phosphotransferase at pH 7.4 would be about 20% of the activity at pH 5.5. Thus, at intracellular pH levels (usually considered to be less than 7.4) the activities of pyrophosphate/glucose phosphotransferase would be at least equal to those of hexokinase in eix of the cell lines. H4 cells would probably manifest significantly more hexokinase than pyrophosphate/glucose phosphotransferase at intracellular pH levels, and yet, paradoxically, these cells phosphorylate 2-deoxy-D-glucose more slowly than the other cell lines (see below). The activity of pyrophosphate/glucose phosphotransferase in liver would exceed the activity of hexokinase. By contrast, the activity of hexokinase in brain was manifold greater than the activity of pyrophosphate/glucose phosphotransferase. The dissimilarity of these two enzymatic activities in brain may be exaggerated by this simple comparison since normally only a small fraction of cerebral hexokinase is active (15).

Uptake and Phosphorylation of 2-Deoxy-D-glucose – Cells on coverslips and tissue slices were exposed to 2-deoxy-D-\[^{14}C\]glucose for timed intervals to determine the kinetics of uptake and phosphorylation of this unmetabolizable analog of glucose. The results are shown in Fig. 1. The kinetics described by the curve for ST3 cells, shown in the left panel, are typical of the curves obtained for the tissues and six of the cell lines. The kinetics of the H4 cells, shown in the right panel, were unique in that the rate of net phosphorylation of 2-deoxy-D-glucose was quite low.
A more elaborate experiment was undertaken, based on the design of Colby and Romano (16) to ascertain whether phosphorylation of glucose occurs during or after transport across the cell membrane (Fig. 2). In this experiment, cells, tissue slices, or purified capillaries were incubated with 2-deoxy-o-[14C]glucose for 30 min to label the intracellular pools of phosphorylated and unphosphorylated sugars. The cells, tissues, and capillaries were then exposed to 2-deoxy-o-[3H]glucose for a brief period of 10 or 30 s. Our results with 3T3, NB41, B65, and H4 cells in this experiment agree with the results obtained with 3T3 cells by Colby and Romano (16); in these cell lines, [3H] first appeared in the intracellular pool of unphosphorylated sugars (top left panel in Fig. 2). These cell lines will be collectively referred to as "class A" cell lines. With the remaining cell lines, B103, B104, and C6, and with the purified capillaries, the opposite result was obtained: [3H] appeared immediately (relative to the period of observation; 10 s) in the intracellular pool of phosphorylated sugars (right panels, top and bottom, in Fig. 2). This type of behavior will be referred to as "class B" behavior. When this experiment was performed with brain slices, results of a mixed class were obtained (lower left panel in Fig. 2): at 10 s, [3H] was almost equally divided between the tissue pools of phosphorylated and unphosphorylated sugars.

The following control and supplemental experiments were performed. The effectiveness of the washing procedures was evaluated with cells on coverslips that had been exposed to 5% (w/v) trichloroacetic acid at 0°C for 10 min and with tissue slices that had been boiled in Hank's solution for 5 min. The killed material was incubated at 37°C for 10 min in Hank's solution containing 2 mM 2-deoxy-o-glucose and 2-deoxy-o-[14C]glucose 0.5 Ci/mol. The material was then passed through the appropriate washing procedure and placed in counting vials. The amounts of radioactivity remaining in the killed material after washing were negligible. The extracellular space of brain slices was estimated from the uptake of [14C]inulin to be about 14% of the wet volume of the slices. Radioactive sugars trapped in the extracellular space could have accounted for only about 10% of the radioactivity of the unphosphorylated sugar present in tissue slices in the experiments above. Phlorizin and sodium pentobarbital inhibited both uptake and phosphorylation of 2-deoxy-o-glucose with a $K_i$ on the order of 0.1 mM. KCN and oligomycin at concentrations of 1 mM and 1 $\mu$M, respectively, inhibited phosphorylation of 2-deoxy-o-glucose by about 90%.

One interpretation of the preceding experiment might be that, over a period of 30 min, 2 mM 2-deoxy-o-glucose is toxic to cells, particularly the type A cell lines. Thus, when the cells were subsequently exposed briefly to 2-deoxy-o-[3H]glucose they might not have been able to phosphorylate it due to a lack of ATP. We measured the ATP levels of cells that were incubated in Hank's balanced salt solution supplemented with 2 mM 2-deoxy-o-glucose. In 30 min the ATP levels of the cells declined by about 50%, relative to the ATP levels of cells incubated in their appropriate nutritionally complete media (see "Materials and Methods"). However, the ATP levels do not decline further, even after 24 h of incubation in the presence of 2-deoxy-o-glucose. The level of cellular

![Fig. 1. Uptake of 2-deoxy-o-[14C]glucose. Cells on coverslips or tissue slices were incubated at 37°C for timed intervals in Hank's solution supplemented with 2 mM 2-deoxy-o-glucose and 2-deoxy-o-[14C]glucose, 0.5 Ci/mol. The cells or slices were washed to remove unabsorbed glucose and extracted for 3 min with 1 ml of boiling distilled water. The extracts were centrifuged to remove coagulated protein. The radiolabeled sugars in the extracts were resolved by chromatography on columns of Bio-Gel AG 1-A2, as described by Colby and Romano (16). O, radioactivity of unphosphorylated sugars; @, radioactivity of the phosphorylated sugars. Error bars indicate the ranges of duplicate measurements; where not indicated, ranges were negligible. Two representative types of cell behavior are shown.](http://www.jbc.org/content/284/12/7037/F1.large.jpg)

![Fig. 2. Determination of whether phosphorylation of d-glucose is simultaneous with transport. Cells on coverslips, tissue slices, or purified capillaries were incubated at 37°C for 30 min in Hank's solution supplemented with 2 mM 2-deoxy-o-glucose and 2-deoxy-o-[14C]glucose, 0.5 Ci/mol. The cells, slices, or capillaries were washed to remove unabsorbed glucose and then exposed for 10 or 30 s at 37°C to Hank's solution containing 2 mM 2-deoxy-o-glucose and 2-deoxy-o-[14C]glucose, 25 Ci/mol. The cells, slices, or capillaries were rapidly washed at 0°C to remove adventitious radioactivity and extracted for 3 min with 1 ml of boiling distilled water. The radiolabeled sugars in the extracts were resolved by chromatography as described by Colby and Romano (16), except that Bio-Gel AG 1-A8 was used instead of Bio-Gel AG 1-A2. Unphosphorylated sugars were eluted with distilled water in the first six fractions. Phosphorylated sugars were eluted with 0.5 mM ammonium formate, 0.2 M formic acid buffer in the remaining fractions. O, radioactivity of [3H] (short term exposure, 10 to 30 s); @, radioactivity of [14C] (long term exposure, 30 min). The error bars indicate the ranges of duplicate experiments. For the sake of clarity, only the data from the 10-s incubation with [3H] are shown, and only the data for 3T3 cells (class A) and B103 (class B) are given. The data for the other cell lines resemble those shown.](http://www.jbc.org/content/284/12/7037/F2.large.jpg)
viability (ability to exclude trypan blue) remained greater than 95% up to 48 h of exposure.

Nevertheless, since glucose phosphorylation might be especially sensitive to a decrease in cellular ATP content, we repeated the double-label transport experiment of Fig. 2 using only trace amounts of radiolabeled 2-deoxy-d-glucose (0.52 \textmu M) with 2 mm d-glucose. The results were not significantly different from those of the experiments with 2 mm 2-deoxy-d-glucose above. Most importantly, the distinction between type A and type B cells remained and subnormal concentrations of ATP cannot be implicated as the determining factor that distinguishes the two classes.

**Uptake and Phosphorylation of 3-O-Methyl-d-glucose** - Results of the transport experiment might suggest that hexokinase is responsible for phosphorylation of glucose in class A cell lines and glucose-6-phosphatase is responsible for phosphorylation in class B cell lines and capillaries. If this hypothesis is correct, class B cells should be able to take up and phosphorylate 3-O-methyl-d-glucose since this sugar is a substrate for the phosphotransferase activities of glucose-6-phosphatase (3). Class A cells may be able to take up the sugar, but they should prove unable to phosphorylate it since 3-O-methyl-d-glucose is not a substrate for hexokinase (17). Fig. 3 shows that none of the cell lines were in fact able to phosphorylate 3-O-methyl-d-glucose, although the sugar was taken up.

**Subcellular Distribution of Glucose-6-phosphatase** - If glucose-6-phosphatase is involved in the uptake and phosphorylation of sugars, then the difference between class A and class B cells might lie in different subcellular distributions of the enzyme. Table II shows that in four of the cell lines glucose-6-phosphatase is concentrated in membranous fractions, as it is in the subcellular fractions of tissues (1.3). The data suggest that there may be some differences in the subcellular distribution of the enzyme in the cell lines, but there appears to be little correlation with the classes of sugar uptake; NB41 and B104 cells have similar subcellular distributions of glucose-6-phosphatase, and yet NB41 cells are class A and B104 cells are class B.

**Phosphorylation of Extracellular or Intracellular 2-Deoxy-d-glucose** - Glucose-6-phosphatase may be vectorially arranged in cellular membranes so that glucose bound on one side of the membrane is phosphorylated and released as glucose-6-phosphate on the other side (3, 19, 20). If the phosphorylating activity of glucose-6-phosphatase is responsible for transport of glucose into cells, then one might expect that extracellular 2-deoxy-d-glucose would be phosphorylated more readily than intracellular 2-deoxy-d-glucose. Diamond and Fishman (21) obtained just this result in an experiment in which they preloaded synaptosomes with 2-deoxy-d-[1,14C]glucose at 0°C so that most of the 14C was in the pool of unphosphorylated sugars. The synaptosomes were then exposed to 2-deoxy-d-[1,3H]glucose at 37°C. They observed that only 3H was found in the pool of phosphorylated sugars (21).

We tried this experiment with cells on coverslips. The results are shown in Table III. From the column of the table for 0 min, it is evident that at 0°C, a considerable amount of 2-deoxy-d-[1,14C]glucose had been taken up and that less than 20% of this had been phosphorylated by the cells. After an exposure for 0.5 min at 37°C to medium containing the 14C-sugar, a considerable amount of H had entered the cells, but less than 10% of the 14C remained intracellular, after 5 min, all of the 14C had escaped into the medium. Because of this rapid efflux of 14C-labeled sugar from the cells, phosphorylation of 2-deoxy-d-glucose already intracellular could not be demonstrated, although it may have occurred. It was probable, however, that dephosphorylation of intracellular 2-deoxy-d-glucose 6-phosphate was occurring especially in the case of H4 cells, and this was examined in the next set of experiments.

**Efflux of 2-Deoxy-d-Glucose** - The mechanism of the efflux of 2-deoxy-d-glucose from cells was examined by rearranging the conditions of the previous experiment. Cells on coverslips or tissue slices were incubated at 37°C with 2-deoxy-d-[1,14C]glucose; most of the 14C was incorporated into the intracellular pool of phosphorylated sugars. The cells or slices were then washed and submitted to a second incubation at 0°C with 2-deoxy-d-[1,3H]glucose; most of the 3H remained in the intracellular pool of unphosphorylated sugars. Thus the ratio of 14C to 3H was high in the pool of phosphorylated sugars and low in the pool of unphosphorylated sugars. The difference in these ratios could serve to identify the source of the isotopic sugars released into the medium, as described below.

**Table II**

<table>
<thead>
<tr>
<th>Cell Lines</th>
<th>Whole lysate</th>
<th>Subcellular fractions</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Nuc</td>
<td>M1</td>
</tr>
<tr>
<td>B104</td>
<td>47</td>
<td>57</td>
</tr>
<tr>
<td>NB41</td>
<td>35</td>
<td>50</td>
</tr>
<tr>
<td>3T3</td>
<td>32</td>
<td>51</td>
</tr>
<tr>
<td>H4</td>
<td>34</td>
<td>37</td>
</tr>
</tbody>
</table>
After these incubations, the cells or slices were incubated in a nonradioactive medium at 37°C and the efflux of radioactive sugars was followed (Table IV). During the first 10 min of incubation, the rate of efflux was about half of the corresponding rate of influx determined in the influx experiments (Fig. 1). After 10 min, a steady state was achieved in which the rates of influx and efflux were equal. Virtually all the radioactive sugar released into the medium was unphosphorylated, although most of the radioactive sugar within the cells or slices was phosphorylated. Although tissue slices and cells in culture release phosphatases into the medium, this enzymatic activity was found to be negligible after 10 min of incubation. The most plausible hypothesis to cover the observations, therefore, is that radioactive phosphorylated sugars in the cells were dephosphorylated by an intracellular phosphatase prior to or during efflux.

If glucose-6-phosphatase were the phosphatase involved in efflux, and if glucose-6-phosphatase operated vectorially, binding phosphorylated sugars on the cytoplasmic face of the membrane and releasing dephosphorylated sugars on the external face, then the ratio of \(^{14}C\) to \(^3H\) in the external medium should have been higher than the intracellular ratios at any given time. In fact \(^{14}C/\(^3H\) ratio in the medium in each case in Table IV is actually lower than the ratio in the intracellular pool of unphosphorylated sugars. This can be explained by noting that the \(^{14}C/\(^3H\) ratio in the intracellular pool of unphosphorylated sugar was increasing over the period of the experiment; the extracellular ratio represents the average ratio over the 10 min of the experiment. That the ratio of isotopes in the medium was lower than the intracellular ratios, implies that the phosphatase involved in efflux does not operate vectorially, i.e. it probably releases the dephospho-sugar intracellularly prior to efflux.

**Effect of Insulin on Glucose-6-phosphatase** — Daniel et al. (22) demonstrated that insulin reduces the rate of efflux of \(^{14}C\) glucose from the brain in vivo. We found that insulin did not inhibit the glucose-6-phosphatase of cerebral membranes in vitro, even at concentrations of insulin 50 times higher than those used by Daniel et al. (Table V).

**DISCUSSION**

Glucose-6-phosphatase is a multifunctional enzyme capable of hydrolyzing a number of organic and inorganic phosphates or of transferring the phosphate moiety of these compounds to a variety of sugars (3). The versatility of this enzyme in vitro has perhaps complicated comprehension of its function in cells. Since previous studies had shown that during sleep, the phosphatase of the phosphorylated (intermediate) form of the brain enzyme may turn over more rapidly, and the enzymatic activity itself increases, the focus of our present studies has been the function of the enzyme in cerebral tissue. Since the \(K_m\) of glucose-6-phosphatase is lowest for glucose 6-phosphate among the phosphorylated sugars normally found in tissues, the true function of the enzyme is probably related to glucose metabolism. Since glucose is the only significant source of metabolic energy in the brain under normal conditions (23), glucose-6-phosphatase is likely to be an important enzyme in cerebral metabolism.

A hypothesis frequently encountered is that glucose-6-phosphatase may be involved in the transport of glucose across cellular membranes (3). The evidence for this hypothesis is suggestive, but still inconclusive. The enzyme is vectorially arranged in hepatic microsomes (19) and avian nuclear membranes (19). Glucose-6-phosphatase is inhibited by phlorizin, a potent inhibitor of sugar transport in vivo (24, 25).

In the present study, we have tested the hypothesis that the enzyme is involved in glucose transport in cerebral cells and tissues. The brain is composed of an assortment of cell types whose transport properties differ (26). The ideal situation for our experiments would have been to have pure preparations of functionally normal neurons, glia, and capillaries. We were able to approach this ideal with capillaries, but we reasoned that neurons and glia prepared by the usual methods involving disruption of cerebral tissue and centrifugation on sucrose gradients might be too damaged for meaningful transport experiments. Instead, we used established cell lines derived from neural tumors. Such cells exhibit neuron- or glia-specific proteins, synthesis of neurotransmitters, electrically active membranes, and the formation of neurite-like processes (27). Four of our cell lines were neuronal (NB41, B65, B103, B104) and one glial (C6). We included two cell lines of nonneural origin (3T3 and H4) for the sake of comparison. We recognize that data obtained from established...
of 2-deoxyn-glucose would not appear to apply to these cells. The enzyme that catalyzes both the transport and the phosphorylation of 2-deoxyn-glucose in the cells and in the bathing media were determined. The columns designated U and P represent the unphosphorylated and phosphorylated sugars, respectively. There was no detectable phosphorylated radioactive sugar in the medium from any of the cell lines. In the case of brain and liver slices, some activity was present, presumably due to cell damage in slicing. The relevant numbers are brain: $^{14}C$, 44; $^{3}H$, 34. Liver: $^{14}C$, 49; $^{3}H$, 322. For the situation that exists at the beginning of the final 10-min incubation, consult Table III.

### Table IV

<table>
<thead>
<tr>
<th>Cell line or tissue</th>
<th>Isotope</th>
<th>Intracellular</th>
<th>Medium, U</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>$^{14}C$</td>
<td>U: 686</td>
<td>23,800</td>
</tr>
<tr>
<td></td>
<td></td>
<td>P: 415</td>
<td>5,920</td>
</tr>
<tr>
<td></td>
<td>$^{14}C$/$^{3}H$ ratio</td>
<td>1.65</td>
<td>4.02</td>
</tr>
<tr>
<td>B104</td>
<td>$^{14}C$</td>
<td>811</td>
<td>6,680</td>
</tr>
<tr>
<td></td>
<td>$^{3}H$</td>
<td>377</td>
<td>1,470</td>
</tr>
<tr>
<td></td>
<td>$^{14}C$/$^{3}H$ ratio</td>
<td>2.10</td>
<td>4.50</td>
</tr>
<tr>
<td>NB41</td>
<td>$^{14}C$</td>
<td>434</td>
<td>17,200</td>
</tr>
<tr>
<td></td>
<td>$^{3}H$</td>
<td>217</td>
<td>4,910</td>
</tr>
<tr>
<td></td>
<td>$^{14}C$/$^{3}H$ ratio</td>
<td>2.00</td>
<td>3.50</td>
</tr>
<tr>
<td>3T3</td>
<td>$^{14}C$</td>
<td>502</td>
<td>5,100</td>
</tr>
<tr>
<td></td>
<td>$^{3}H$</td>
<td>247</td>
<td>1,670</td>
</tr>
<tr>
<td></td>
<td>$^{14}C$/$^{3}H$ ratio</td>
<td>2.03</td>
<td>3.00</td>
</tr>
<tr>
<td>H4</td>
<td>$^{14}C$</td>
<td>55.5</td>
<td>671</td>
</tr>
<tr>
<td></td>
<td>$^{3}H$</td>
<td>29.7</td>
<td>186</td>
</tr>
<tr>
<td></td>
<td>$^{14}C$/$^{3}H$ ratio</td>
<td>1.87</td>
<td>3.61</td>
</tr>
<tr>
<td>Brain</td>
<td>$^{14}C$</td>
<td>1170</td>
<td>47.8</td>
</tr>
<tr>
<td></td>
<td>$^{3}H$</td>
<td>959</td>
<td>56.3</td>
</tr>
<tr>
<td></td>
<td>$^{14}C$/$^{3}H$ ratio</td>
<td>1.22</td>
<td>0.85</td>
</tr>
</tbody>
</table>

In class B, phosphorylation was at least as rapid as uptake of 2-deoxyn-glucose. In this instance, the hypothesis is neither confirmed nor refuted. B103, B104, C6 cells, and purified capillaries were class B. Brain tissue itself represents a mixed transport class, probably because it is composed of a number of cell types, some class A, others class B. The differences between classes were not due to toxic effects of 2-deoxyn-glucose per se, under the conditions of our experiments.

Follow-up experiments did not reveal the reason for the differences in the two transport classes. All of these cell lines have glucose-6-phosphatase, and the enzyme is similarly distributed in their membranous subcellular fractions. None of the cell lines or tissues could phosphorylate 3-O-methyl-glucose. Since the similarities among the cell lines with respect to glucose-6-phosphatase are more impressive than the differences, the most economical hypothesis is that transport and phosphorylation of glucose are separate processes in both class A and class B cells. If glucose-6-phosphatase is involved in phosphorylating glucose at all, it seems likely that its action is entirely intracellular. Perhaps, the difference between class A and class B cells lies in their possession of different amounts of a facilitative transport protein in the cell membrane. Where large amounts of this protein exist, glucose might enter more rapidly than it can be phosphorylated (class A); where less of this protein is present, glucose might be phosphorylated as rapidly as it crosses the cellular frontier (class B).

In the course of these experiments, an unexpected observation was made with regard to brain, i.e., that there is a rapid efflux of 2-deoxyn-glucose from cells and tissues that have taken up this sugar. A cellular phosphatase that releases its products into the cytoplasm appears to be involved in the efflux process since the 2-deoxyn-glucose released from cells and tissues originates in the intracellular pool of free 2-deoxyn-glucose which is, in turn, derived from intracellular 2-deoxyn-glucose-6-phosphate. The intracellular phosphatase that catalyzes this reaction is probably glucose-6-phosphatase since it seems unlikely that alkaline phosphatase or acid phosphatase could participate. Alkaline phosphatase is confined to capillaries in brain (7), and is absent entirely from our cell lines. Acid phosphatase is confined in lysosomes (28). 2-Deoxyn-glucose does not disrupt lysosomes significantly since cells remain able to multiply and grow even after a 24-h exposure to 2-deoxyn-glucose.

Efflux of glucose from cells and tissues is probably a normal phenomenon (22), although it is difficult to understand the purpose of the efflux in organs other than liver. If hexokinase and glucose-6-phosphatase are active in the same intracellular compartment, as our results suggest, then these two enzymes would appear to catalyze a futile cycle of phosphorylation and
dephosphorylation. Perhaps the activities of both these enzymes are dependent, but in opposite senses, on the concentration of some intracellular metabolite or circulating hormone so that a small change in the concentration of such molecules would cause an amplified change in the net rate of glucose phosphorylation (29). Such a situation has been described by Newsholme and Start (29) and discussed by Katz and Rognstad (30). This “amplified regulation” could conceivably be an important, rapid, sensitive mechanism in the brain to ensure that energy would be promptly available to support the increased demands that underlie increased electrical activity (31, 32). In particular, one enzyme in the “futile” cycle has already been implicated in the sleep/wakefulness situation, i.e. glucose-6-phosphatase (see introduction to the text).

There is evidence of increased glucose transport and phosphorylation during stimulation of various neural pathways (33, 34). As one example of substances that might be such “reciprocal effectors,” nucleotides have complicated stimulatory and inhibitory effects on both hexokinase (35) and glucose-6-phosphatase (36). The difficulty in this area of research is that the regulatory properties of glucose-6-phosphatase studied in vitro may be altered by detergents or mechanical disruption of membranes (37). Although the efflux of glucose from brain is inhibited in vivo by insulin (22), insulin appeared to exert no effect on the glucose-6-phosphatase activity of membranes disrupted by sonication. Its effect in vivo may also, of course, not have been at the level of dephosphorylation of glucose-6-P, but on the efflux mechanism per se, or on the balance between influx and efflux, i.e. net flux. It is noteworthy, though, that Ashmore et al. (38) found that diabetes in rats was associated with a doubling of hepatic glucose-6-phosphatase activity, while administration of insulin in vivo caused a marked decrease in the enzymatic activity measured in vivo. Further, the incorporation in vivo of pyruvate carbon into free glucose, rather than into glycogen, was favored in the case where the enzyme had been elevated (i.e. in liver slices from diabetic animals).

Our data do not offer support to the concept that glucose-6-phosphatase is of importance in the phosphorylation of glucose. At least in brain, contrary to the proposals of Nordlie (3, 36), but in accordance with the argument of Hers (39), its function appears to be mainly as a phosphohydrolase in the systems studied here.

Acknowledgments — We express our gratitude to Dr. George Popják and Dr. Gary Gibson for their encouragement and counsel and to Barbara Burrows for expert technical assistance. We extend thanks to Dr. Samuel Ward for furnishing high quality photomicrographs of our preparation of purified capillaries.

REFERENCES
Cerebral glucose-6-phosphatase and the movement of 2-deoxy-D-glucose across cell membranes.
J M Anchors, D F Haggerty and M L Karnovsky


Access the most updated version of this article at http://www.jbc.org/content/252/20/7035

Alerts:
- When this article is cited
- When a correction for this article is posted

Click here to choose from all of JBC's e-mail alerts

This article cites 0 references, 0 of which can be accessed free at http://www.jbc.org/content/252/20/7035.full.html#ref-list-1