Permeation of Glucose by Simple and Facilitated Diffusion by Novikoff Rat Hepatoma Cells in Suspension Culture and Its Relationship to Glucose Metabolism*

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SUMMARY

The incorporation of 2-deoxy-D-[14C]glucose by cultured Novikoff rat hepatoma cells was investigated as a function of the deoxyglucose concentration in the medium. The intracellular radioactivity was mainly associated with deoxyglucose 6-phosphate, to a small extent with 6-phosphate-deoxygluconate, and the remainder with free deoxyglucose. Below a concentration of 1 to 2 mM in the medium, the initial rate of deoxyglucose incorporation followed simple Michaelis-Menten kinetics at 22, 27, 32, and 37°C with an apparent $K_m$ of about 2 mM. The $V_{max}$ values increased with a $Q_10$ of about 2.5 with an increase in temperature. Between 2 and 10 mM, on the other hand, the initial rate of incorporation increased linearly with an increase in deoxyglucose concentration and the relative increase in incorporation rate with increase in deoxyglucose concentration was about the same at the temperatures tested. Treatment of the cells with 0.5 mM $p$-chloromercuribenzoate for 15 min or heat shock (48.5°C, 5 min) abolished the saturable uptake component without affecting the nonsaturable component. When combined, the results indicate that at low concentrations, deoxyglucose is taken up mainly by carrier-mediated transport (probably facilitated diffusion), whereas at concentrations above 2 mM, simple diffusion becomes the principal mode of entry of deoxyglucose into the cells. Results from studies on D-glucose and 3-O-methyl-D-glucose incorporation support this conclusion and suggest that all three substrates are transported by a single system.

Persantine competitively inhibited deoxyglucose uptake by whole cells and decreased the metabolism of glucose without affecting the phosphorylation of the substrates. Prior treatment with $p$-chloromercuribenzoate or heat shock also had no effect on the hexokinase activity of the cells. Little if any hexokinase activity was associated with the plasma membrane and the kinetic properties of the in vitro hexokinase reaction differed from that for glucose or deoxyglucose uptake by whole cells. These results indicate that transport of glucose into the cell is a reaction distinct from phosphorylation and is the rate-limiting step in the metabolism of glucose by the cells. Since the cells possess an excess of hexokinase, glucose is phosphorylated as rapidly as it enters the cells and thereby trapped. At low concentrations of glucose in the medium (5 to 50 μM), the glucose 6-phosphate formed was exclusively used by the cells for macromolecular synthesis and oxidative processes without net production of lactate. The rate of CO$_2$ production and incorporation of glucose in macromolecules approached a maximum at about 1 mM glucose in the medium and the excess glucose 6-phosphate formed, particularly at higher concentrations of glucose in the medium, was converted to lactate.

The transformation of mammalian cells by tumor viruses (1-4) or of lymphocytes by phytohemagglutinin (5, 6), as well as the cell density-dependent inhibition of growth of cultured mammalian cells (7-9), is associated with dramatic changes in the capacity of the cells to take up small molecular weight substances such as glucose, nucleosides, or phosphate ions. These changes may be directly related to changes in the metabolism of the cell since the metabolism of certain substrates by mammalian cells may be limited by the rate of their transport into the cell (10-12). Hormones may also affect metabolism by induced changes of the transport systems (12-15).

The fact that the capacity of the cells to take up glucose is affected by tumor virus or phytohemagglutinin-induced cell transformations or by cell density inhibition of growth, and is regulated by hormones is of particular interest in view of the central importance glucose serves as energy source and in the supply of metabolic intermediates for growth. Although the uptake of hexoses by erythrocytes (see Reference 16), adipose tissue (12, 13), and by suspensions of Ehrlich ascites tumor cells harvested from whole animals (17-19) has been investigated extensively, the mechanism of uptake is poorly understood (20, 21). Not even the kinetics of glucose uptake by human erythrocytes has been well defined since different methods yield variant results (22, 23). Further, few studies have concerned themselves with the mode and regulation of uptake of glucose by growing mammalian cells in culture (24) and the relationship between the rate of glucose uptake and the rate of its metabolism. In attempts to gain further understanding of these processes, we

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have first compared the usefulness of various substrates as models for measuring glucose transport by Novikoff rat hepatoma cells growing in suspension culture and then analyzed the mode(s) of uptake of glucose by the cells. Of additional interest was the substrate specificity of the glucose transport system and the question of whether phosphorylation represents an integral part of the transport system (19). We also concerned ourselves with the question of whether the metabolic fate of glucose is influenced by the rate with which it is taken up by the cells, and whether transport is the rate-limiting step in the metabolism of glucose by the cells. A preliminary report of some of our findings has been presented (25).

EXPERIMENTAL PROCEDURE

Materials

Materials were purchased as follows: uniformly labeled D-\(^{[14]C}\)glucose, 2-deoxy-D-\(^{[14]C}\)glucose, 3-O-methyl-D-\(^{[14]C}\)glucose, and L-\(^{[14]C}\)sorbosene from International Chemical and Nuclear Corporation; unlabeled n-glucose, 2-deoxy-D-glucose, D-galactose, D-mannose, D-fructose, L-sorbosene, 3-O-methyl-D-glucose, \(\text{D-glucosamine-HCl} \), D-gluconic acid, D-glucose 6-phosphate, 2-deoxy-D-glucose 6-phosphate, fructose 1,6-bisphosphate, and 6-phospho-D-gluconic acid from Calbiochem; D-glucose 6-phosphate dehydrogenase and NAD* from Fajst Laboratories; and uridine and adenosine from Schwarz BioResearch. Persantine (2,6-bis(dichloroacetonamino)-4,8-dipiperidineimidazole·(5,4,4) pyrimidine) was a gift from Geigy Pharmaceuticals, Yonkers, N. Y. Glucose-free BM42 had the same composition as BM42 (26) except that glucose was omitted. BM42 is a basal medium containing salts, amino acids, vitamins, and antibiotics, but no serum or other body fluids, and is buffered with bicarbonate.

Analysis of \(^{14}CO_2\) and \([^{14}C]Lactate Production—Production of \(^{14}CO_2\) from \([^{14}C]glucose\) was measured by suspending incubations of cells in \(^{14}CO_2\) collection flasks (Wheaton Scientific, Millville, N. J.). The attached scintillation vials contained strips of Whatman No. 3MM paper soaked with 0.3 ml of 10% (w/v) KOH. The vials were replaced at appropriate time intervals of incubation. The cell suspensions were acidified prior to removal of the vials when indicated in the appropriate experiments. The paper was dried in the vials at room temperature, then scintillation fluid was added to the vials, and the radioactivity was determined as described below.

Production of extracellular lactate was measured as follows. Samples of a suspension of \([^{14}C]glucose\)-labeled cells were centrifuged and 25-ml samples of the supernatant fluid (culture fluid) were chromatographed with solvent 36 as described below.

Chromatographic Analyses—All materials were analyzed by ascending chromatography on Whatman No. 3MM paper. Chromatograms of acid extracts of cells and hexokinase reaction mixtures were developed at 30° for 18 hours with solvent 28° (3 volumes of 1 M ammonium acetate, pH 5, and 7 volumes of 95% ethanol). Chromatograms of culture fluid were developed at room temperature for 5 hours with a solvent composed of 3 volumes of ethyl acetate, 1 volume of glacial acetic acid, and 1 volume of H\(_2\)O (solvent 36). Appropriate mixtures of standards were co-chromatographed. Nucleotides were located by examining the developed paper under ultraviolet light, hexoses and hexose derivatives by staining with alkaline silver nitrate reagent (29), and lactate and pyruvate by staining with 0.4% (w/v) methyl orange in 95% ethanol. The developed chromatograms of the experimental samples were cut into 1-cm segments at right angles to the direction of migration. The segments were placed in scintillation vials and agitated with 1 ml of H\(_2\)O at 37° for 30 min. Then scintillation fluid was added and the radioactivity was determined. About 100% of the radioactivity was recovered from the paper.

Methods

Cell Culture—Novikoff rat hepatoma cells (subline N181-67) were propagated in suspension culture in Swim's medium 67 and enumerated by tabulation in a Coulter counter as described previously (26, 27). For experiments, cells were collected by centrifugation at 400 \(\times \) \(g\) for 1 to 2 min from cultures in the exponential phase of growth (2 \(\times\) \(10^6\) to 2.8 \(\times\) \(10^6\) cells per ml) and suspended to 2 \(\times\) \(10^6\) cells per ml in glucose-free BM42 or N-2-hydroxyethylpiperazine-N'-2-ethanesulfonic acid-buffered BM42 as indicated in the appropriate experiments.

Incorporation of Labeled Hexoses into Cell Material and Preparation of Acid-soluble Cell Extracts—Suspensions of cells in glucose-free BM42 were supplemented with \([^{14}C]glucose\) as indicated in the appropriate experiments and incubated on a gyratory shaker at about 200 rpm. At appropriate time intervals, samples of 0.5 or 1.0 ml of suspension were analyzed as follows. (a) For total radioactivity associated with the cells, the cells were collected by centrifugation at 400 \(\times\) \(g\) for 1 to 2 min (0°), rapidly washed in 5 ml of cold BSS, and suspended in 0.2 ml of 0.5 M trichloroacetic acid. (b) For radioactivity in acid-insoluble material, other samples were quickly frozen in a bath of solid CO\(_2\) in ethanol and later thawed and mixed with perchloric acid at 0°, and the precipitated material was washed repeatedly with perchloric acid and trichloroacetic acid as described previously (27). The washed precipitates were mixed with 0.1 ml of 0.5 M trichloroacetic acid and then all samples of (a) and (b) were heated at 70° for 30 min and analyzed for radioactivity. The total radioactivity in the cell suspension was estimated as follows. Samples (0.1-ml) of the suspension were mixed with 0.1 ml of 1 M trichloroacetic acid, heated at 70° for 30 min, and analyzed for radioactivity. The acid-soluble pools were extracted from samples of 1 \(\times\) \(10^7\) to 2 \(\times\) \(10^7\) cells as described previously (11, 28).

Analysis of \(^{14}CO_2\) and \([^{14}C]Lactate Production—Production of \(^{14}CO_2\) from \([^{14}C]glucose\) was measured by suspending incubations of cells in \(^{14}CO_2\) collection flasks (Wheaton Scientific, Millville, N. J.). The attached scintillation vials contained strips of Whatman No. 3MM paper soaked with 0.3 ml of 10% (w/v) KOH. The vials were replaced at appropriate time intervals of incubation. The cell suspensions were acidified prior to removal of the vials when indicated in the appropriate experiments. The paper was dried in the vials at room temperature, then scintillation fluid was added to the vials, and the radioactivity was determined as described below.

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Cell Fractionations—All steps were conducted at 0°-4°. Cell-free extracts were prepared as described previously (28) except for the use of a different buffer solution. In brief, 4 \(\times\) \(10^7\) to 20 \(\times\) \(10^7\) cells were collected by centrifugation from exponential phase cultures, suspended in 2 ml of a solution composed of 0.1 M glycyglycine buffer (pH 7.5) and 1 mM mercaptoethanol, and disrupted by sonication. The suspension was centrifuged at 18,000 \(\times\) \(g\) for 15 min and the supernatant fluid designated cell-free extract. Cells were fractionated into a mitochondrial-microsomal fraction and the cell sap by disrupting the cells by homogenization in B4 followed by differential centrifugation as described in detail elsewhere (30). Cell fractions were prepared in 0.4 M sucrose plus 10 mM Na\(_2\)EDTA in the same manner except for the initial treatment: 1 \(\times\) \(10^6\) cells

* The numbers refer to the solvents described in the 1967 catalog (p. 123) of Schwarz BioResearch, Orangeburg, N.Y.
were suspended in 2.4 ml of H$_2$O and after 10 min at 0° disrupted by five strokes with a Dounce homogenizer. Immediately thereafter, the suspension was mixed with 0.6 ml of 2 M sucrose and 0.06 ml of 0.5 M Na$_2$EDTA and again homogenized with 10 strokes. The supernatant fluid obtained by centrifuging the suspension at 500 x g for 5 min was termed postnuclear fraction.

**Enzyme Assays**—Fractions from sucrose density gradients were assayed for cytochrome oxidase and total ATPase activities as described previously (31). Hexokinase activity was measured either by (a) a spectrophotometric method modified from that of Walker and Parry (32) or (b) a direct method. For method (a), the reaction mixture contained 50 mM glycylglycine buffer (pH 7.5), 10 mM MgSO$_4$, 100 mM KCl, 10 mM ATP, 1 mM NADP, 10 mM glucose, 0.5 unit of glucose 6-phosphate dehydrogenase, and cell fraction in a total volume of 1.0 ml. Incubation was at room temperature and hexokinase activity was estimated from the linear increase in absorbance at 340 nm. No significant reduction of NADP occurred in the absence of glucose.

The composition of the reaction mixture in method (b) was the same as in (a), except that NADP and glucose 6-phosphate dehydrogenase were omitted, the unlabeled glucose was replaced by 0.6 mM [14C]glucose or [14C]deoxyglucose, and the total volume was 0.5 ml. Other substrate concentrations were employed as indicated in the appropriate experiments. The reaction mixtures were incubated at 37° and at appropriate time intervals, 50-µl samples were removed and placed in a bath of boiling water for 1 min. The suspension was clarified by centrifugation and the substrate and the phosphorylated product in the supernatant fluid were separated chromatographically with solvent 28 as already described. Hexokinase activity was estimated from the initial linear portion of a plot of the amount of phosphorylated product formed as a function of time of incubation. One unit of hexokinase was defined as the amount of enzyme that phosphorylated 1 µmole of substrate per min.

**Radioactivity Measurements**—All samples were mixed with 15 ml of modified Bray's solution (26) and the radioactivity was measured with a Beckman or a Packard liquid scintillation spectrometer. The counting efficiencies with the two instruments were approximately 92 and 83%, respectively, for all 14C-labeled samples, except those from 14CO$_2$ collection flasks for which the counting efficiencies were approximately one-third lower.

**RESULTS**

**Modes of Deoxyglucose Uptake by N181-67 Cells**—Fig. 1A illustrates the initial time course of incorporation of [14C]deoxy-
glucose into total cell material by N181-67 cells at various concentrations of deoxyglucose in the medium. At concentrations below 0.5 mM deoxyglucose, incorporation was linear with time for at least 20 min, whereas at higher concentrations, the rate of incorporation decreased rapidly. In agreement with previous results (15), most of the deoxyglucose taken up by the cells was phosphorylated and partially further converted to 6-phosphodeoxygluconate, but not further metabolized. As indicated by the chromatographic analysis of acid extracts from labeled cells (Table I), most of the intracellular label was associated with deoxyglucose 6-phosphate, smaller amounts with 6-phosphodeoxygluconate, and the remainder with free deoxyglucose, regardless of the deoxyglucose concentration in the medium. The relative proportion of free deoxyglucose inside the cell increased from about 10 to 30% with an increase in deoxyglucose concentration in the medium from 0.04 to 4.2 mM. After 20 min of incubation with 4.2 mM deoxyglucose, the cells contained 6.6 nmoles of free deoxyglucose per 10^6 cells. Based on an average over-all volume of 2.5 μl per 10^6 cells (28), this amount represents an over-all intracellular concentration of 2.7 mM free deoxyglucose, which is close to the extracellular concentration.

Lineweaver-Burk plots of the initial rates of uptake up to 1.7 mM deoxyglucose in the medium show that deoxyglucose uptake followed normal Michaelis-Menten kinetics at 22, 27, 32, and 37°C (Fig. 1C). The apparent K_m increased slightly with an increase in temperature, but less than that observed for glucose uptake by human erythrocytes (33). The apparent V_max decreased from 5.0 nmoles of deoxyglucose taken up per 10^6 cells per min at 37°C to 1.25 nmoles per 10^6 cells per min at 22°C with an average Q_10 of 2.5. When the V_max values were analyzed in an Arrhenius plot a straight line was obtained from which an activation energy of 15.6 Cal per mole was calculated (Fig. 1D). The results indicate that at these concentrations deoxyglucose is taken up by the cells by a carrier mediated process.

The data in Fig. 1B, however, show that at concentrations above 2 mM the initial rate of deoxyglucose uptake increased linearly with an increase in the deoxyglucose concentration in the medium and that this increase was relatively unaffected by the changes in temperature of incubation. When analyzed in a Lineweaver-Burk plot the uptake rates above 2 mM deoxyglucose deviated from the straight line in the direction of the origin. These results were similar to those obtained with nucleosides (11, 34) and suggest that at higher concentrations deoxyglucose also entered the cell at a significant rate by simple diffusion. This conclusion is further supported by the effects of p-chloromercuribenzoate treatment and heat shock on deoxyglucose uptake. The results in Fig. 2 demonstrate that preliminary incubation of the cells for 15 min with 0.3 mM or higher concentrations of p-chloromercuribenzoate or heating the cells at 48.5°C for 5 min markedly reduced the capacity of the cells to take up deoxyglucose. These effects seem to be due to an inactivation of the transport system, since the hexokinase of N181-67 cells as measured in cell-free preparations was reduced less than 5% by heating the cells at 48.5°C or by incubation in BM42 containing 0.5 mM p-chloromercuribenzoate for 15 min and the distribution of label among the intracellular components after 90 min of labeling was about the same whether or not the cells had been treated: 65 to 75% of the total label was associated with deoxyglucose 6-phosphate, 10 to 15% with 6-phosphodeoxygluconate, and 17 to 21% with free deoxyglucose. The nucleoside triphosphate levels of the cells were not affected by these treatments (35). As shown by the data in Fig. 3A, uptake of deoxyglucose by simple diffusion was unaffected by treatment of the cells with p-chloromercuribenzoate or heat shock. Only the saturable transport component of the uptake curve (below 2 mM deoxyglucose) seemed to be inactivated by the treatments.

**Table I**

Distribution of label from [14C]deoxyglucose among intracellular components as function of deoxyglucose concentration and time of incubation

The details of the experiment are described in the legend to Fig. 1. At the indicated times of labeling with [14C]deoxyglucose, acid extracts were prepared from 10^6 cells and the acid extracts were analyzed chromatographically as described under "Methods."

<table>
<thead>
<tr>
<th>Deoxyglucose</th>
<th>Time</th>
<th>Incorporated per 10^6 cells</th>
<th>Percentage of total in cell</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>min</td>
<td>nmoles</td>
<td>Deoxyglucose</td>
</tr>
<tr>
<td></td>
<td></td>
<td>6-phosphate</td>
<td>6-Phosphodeoxygluconate</td>
</tr>
<tr>
<td>0.04</td>
<td>10</td>
<td>0.5</td>
<td>81</td>
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<tr>
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<td>20</td>
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<td>78</td>
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<tr>
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<td>6</td>
<td>80</td>
</tr>
<tr>
<td></td>
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<td>10</td>
<td>80</td>
</tr>
<tr>
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</tr>
<tr>
<td></td>
<td>20</td>
<td>21</td>
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</table>

**Fig. 2.** Effects of p-chloromercuribenzoate (PCMB) treatment and heat shock on the uptake of 2-deoxy-o-glucose by N181-67 cells. Samples of 4 × 10^6 cells were collected by centrifugation from an exponential phase culture and further treated as follows. A, the cells were suspended to 4 × 10^6 cells per ml in glucose-free BM42 containing 0.5 mM p-chloromercuribenzoate. The suspensions were incubated on a gyratory shaker at 37°C for 15 min and the cells collected by centrifugation. B, the cell pellets were incubated in water baths at the indicated temperatures for 5 min. Then the cell pellets from A and B were suspended (zero time) to 2 × 10^6 cells per ml in glucose-free BM42 containing 10 μM [14C]deoxyglucose (80 cpm per pmole) and further incubated on a gyratory shaker at 37°C. At the indicated times, duplicate 1-ml samples of each suspension were analyzed for radioactivity in total cell material as described under "Methods." All points are averages of the duplicate samples. At 90 min, about 10% of the cells heated at 50°C or treated with 1 mM p-chloromercuribenzoate were stainable by trypan blue, whereas less than 2% of the cells were stainable in all other suspensions. At 90 min of incubation acid extracts were prepared from 1.0 × 10^6 cells and analyzed chromatographically (see text).
In Fig. 3A we have estimated the rate of deoxyglucose uptake due to simple diffusion as a function of deoxyglucose concentration by drawing a line (---) through the origin and parallel to the straight line portions of the uptake curves. Subtraction of these estimated simple diffusion rates from the over-all rates of uptake yielded an estimate of the rates of uptake due to the transport reaction (O--O). The values give a curve typical of a saturable process. The Lineweaver-Burk plots of the initial rates of total uptake and of the transport rates corrected for simple diffusion show that the V$_{\text{max}}$ and K$_m$ values estimated from the corrected values were only one-half as great as those calculated from the total rates (Fig. 3B).

**Uptake of 3-O-Methylglucose**—3-O-Methylglucose transport has also been frequently used as a model in studying glucose transport, since it is not metabolized by cells (1, 6, 13, 17, 36-38). In agreement with the latter finding, all 3-O-[14C]methylglucose taken up by N181-67 cells was recovered in an unaltered form. However, because 3-O-methylglucose was not phosphorylated by the cells and thereby trapped intracellularly, only very small amounts of the substrate were taken up by the cells and net incorporation ceased when the intracellular concentration approached that in the medium (Fig. 4A). Similar results were obtained with L-[14C]sorbose (not shown). Further, even though the cells were incubated at 20° rather than 37°, it was difficult to obtain accurate initial rates of uptake, because equilibration between extracellular and intracellular concentration was extremely rapid (Fig. 4A).

We did not use temperatures below 20° since the lower the temperature, the greater is the contribution of simple diffusion to the total uptake of substrates by cells (see Fig. 1 and References 30 and 34). Nevertheless, analysis of the 1-min uptake values in a Lineweaver-Burk plot (Fig. 4B) yielded a straight line and estimates for the apparent K$_m$ and V$_{\text{max}}$ of 5 mM and 3 nmoles per 10$^6$ cells per min, respectively. These values are similar to those estimated for the transport of deoxyglucose (Table II).

**Glucose Uptake and Metabolism as Function of Glucose Concentration**—Results from preliminary experiments indicated that the rates of n-glucose uptake by N181-67 cells could not be measured by simply analyzing the amounts of labeled glucose incorporated into total cell material, as has been done in studies with a number of other cell systems (1-3, 13, 24, 37), because almost immediately upon addition of n-[14C]glucose to cell suspensions, significant amounts of glucose began to be converted to extracellular lactate and CO$_2$. Moreover, the relative

![Fig. 3. Initial rates of 2-deoxy-D-glucose uptake by untreated, p-chloromercuribenzoate-treated, and heat-shocked cells as a function of substrate concentration. A, samples of 1.8 x 10$^8$ cells were collected by centrifugation from an exponential phase culture and treated as follows: (a) untreated (---); (b) the cells were suspended to 4 x 10$^8$ cells per ml in glucose-free BM42 containing 0.5 mM p-chloromercuribenzoate, the suspension was incubated on a gyrotary shaker at 37° for 15 min, and the cells were again collected by centrifugation (Δ---Δ); (c) the cell pellet was heated at 48.5° for 5 min (V---V). The cells of all three samples were suspended in glucose-free BM42 to 2 x 10$^8$ cells per ml and samples of each suspension were supplemented with 0.2, 0.33, 0.5, or 1 mM [14C]deoxyglucose (214 cpm per n mole) or 4 mM (53 cpm per n mole) or 10 mM (21.6 cpm per n mole) [14C]-deoxyglucose, and the initial rates of uptake were determined as illustrated in Fig. 1A. For technical reasons the experiment was conducted in parts, one treatment at a time, but the whole experiment, except for the radioactivity analyses, was completed in 45 min. The rate of simple diffusion (---) was estimated by drawing a line parallel to the other straight lines through the origin (see text). The rate of uptake due to facilitated diffusion (O---O) was estimated by subtracting these values from the rates of total uptake by the untreated cells. B, Lineweaver-Burk plot of the initial rates of total uptake by untreated cells (O---O) and the rates due to facilitated diffusion (O---O, total rates minus those estimated for simple diffusion).](http://www.jbc.org/)

![Fig. 4. Uptake of 3-O-methyl-D-glucose by N181-67 cells. A, samples of a suspension of 1 x 10$^6$ cells per ml of glucose-free BM42 equilibrated in a water bath at 20° was supplemented with 0.11 μM 3-O-[14C]methylglucose (1,500 cpm per n mole) or 0.11 μM 3-O-[14C]methylglucose plus unlabeled 3-O-methylglucose to final concentrations of 0.21, 0.31, 0.53, 1.53, 4, or 10 mM. The samples were incubated at 20° and, at the indicated times, duplicate 1-ml samples were analyzed for radioactivity in total cell material as described under “Methods.” All points are averages of the duplicate samples. For technical reasons the experiment was conducted in two parts, the four higher and the four lower concentrations together. B, Lineweaver-Burk plot of the 1-min uptake values from A.](http://www.jbc.org/)
amounts of glucose converted to lactate and CO₂ and assimilated into cell material varied markedly with the glucose concentration in the medium. Further, the rates of incorporation of a substrate into intracellular intermediates can only be equated with the rate of uptake of the substrate if it can be demonstrated that uptake is the rate-limiting step in the conversion of the substrate to intracellular intermediates (10). The time courses of conversion of [¹⁴C]glucose by N181-67 cells into total cell material, acid-insoluble material (macromolecular), and extracellular lactate and CO₂ at four concentrations of glucose in the medium are illustrated in Fig. 5. At all glucose concentrations, label began to appear rapidly in extracellular lactate as determined by chromatographic analysis of the culture fluid (see Fig. 6A). However, at 10 and 55 µM glucose in the medium, the rate of lactate production decreased rapidly and net production ceased altogether at 30 to 45 min, while the incorporation of glucose into total cell material and acid-soluble material and its conversion to CO₂ continued at undiminished rates (see also Fig. 7). Subsequently, the labeled lactate began to disappear from the medium (Fig. 5, A and B). As shown in Fig. 8, prior incubation of the cells in glucose-free BM42 for 30 min before addition of the labeled glucose further diminished the net production of lactate from glucose when present at low concentrations in the medium, without affecting the assimilation of glucose into total and acid-insoluble cell material (compare Fig. 5, A and B, and Fig. 8). At concentrations of 0.5 or 5 mM glucose in the medium, the other hand, lactate production continued at a rapid rate and the cells converted 3 and 10 times more glucose to lactate at 0.5 and 5 mM, respectively, than they assimilated into cell material during 65 min of incubation (Fig. 5, C and D). The incorporation of [¹⁴C]glucose into acid insoluble cell material and its conversion to CO₂ occurred at close to maximum rates at 0.5 mM glucose in the medium, since about the same amounts of glucose were incorporated or converted to CO₂ at 5 mM as at 0.5 mM (compare Fig. 5, C and D, and Fig. 7C). In contrast a 3-fold greater amount of lactate was produced at 5 mM than at 0.5 mM glucose.

The values for CO₂ production included in Fig. 5 were determined in an independent experiment by the use of [¹⁴C]CO₂ collection flasks (Fig. 7). From one suspension of cells the accumulated [¹⁴C]CO₂ was analyzed at the indicated time intervals and the accumulative values are presented (solid symbols). To a duplicate suspension of cells trichloroacetic acid was added after peaks (see “Methods” and Fig. 6A). [¹⁴C]CO₂ production (— — — — —) was measured in a separate experiment by incubating the cell suspensions in [¹⁴C]CO₂ collection flasks (see Fig. 7). All values are expressed in glucose equivalents. The curves designated TOTAL (○—○) represent the sum of the amounts of glucose converted to total cell material, lactate, and CO₂. At 5, 20, and 65 min of incubation, acid extracts were prepared from 1 × 10⁷ cells and analyzed chromatographically (see Fig. 6B). In C and D about 100% of the radioactivity lost from glucose was recovered in total cell material, lactate, and CO₂ in A and B the recovery was about 90%.

### Table II

<table>
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<tr>
<th>Substrate</th>
<th>Preparation</th>
<th>V&lt;sub&gt;max&lt;/sub&gt; (nmol/10&lt;sup&gt;6&lt;/sup&gt; cells/min)</th>
<th>K&lt;sub&gt;m&lt;/sub&gt; (mM)</th>
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Fig. 6. Chromatographic analyses of culture fluid (A) and acid-soluble cell extracts (B) from cultures incubated with d-[14C]glucose. The details of the experiment are described in the legend to Fig. 5. Samples of 25 μl of culture fluid from cell suspensions after 65 min of incubation with 10 or 500 μM [14C]glucose (A) or acid extracts from 20-min labeled cells (B) were chromatographed as described under "Methods" with solvents 36 and 28, respectively.

Fig. 7. 14CO2 production by N1SI-67 cells at various concentrations of d-[14C]glucose in the medium. Samples of a suspension of 2 × 10^5 cells per ml of N-2-hydroxyethylpiperazine-N'-2-ethanesulfonic acid-buffered BM42 were supplemented with [14C]glucose and unlabeled glucose as described in the legend to Fig. 5. Two 10-ml portions of each suspension were incubated in 14CO2 collection flasks (see "Methods"). On one flask, the counting vials were replaced at the indicated times and analyzed for radioactivity, and the accumulative amounts of CO2 produced are presented ( ) and ( △). The duplicate flasks were incubated until 210 min and then 1 ml of 1 N trichloroacetic acid was added. The flasks were kept at 0°C for 30 min before the radioactivity in COT was determined ( ).

210 min of incubation. The mixture was incubated for 30 min, and the total amount of labeled-CO2 produced was determined ( ). The results indicate that the rate of CO2 production at the four glucose concentrations was relatively constant throughout the incubation period and that the lag period observed in the time course of CO2 production probably represented the time required for the CO2 to be released from the suspension. In this type of experiment the cells were incubated in N-2-hydroxyethylpiperazine-N'-2-ethanesulfonic acid-buffered BM42. The results from other experiments have shown that the relationship between the time courses of the incorporation of glucose into cell material and extracellular lactate was about the same whether the cells were incubated in N-2-hydroxyethylpiperazine-N'-2-ethanesulfonic acid-buffered BM42 or normal BM42 which contains bicarbonate buffer.

It is clear from the data in Fig. 5 that the amounts of glucose converted to CO2 and assimilated into cell material changed similarly with an increase in glucose concentration in the medium, whereas the amounts of lactate produced changed in an entirely different manner. At all glucose concentrations tested, the amount of glucose converted to CO2 represented between one-half to two-thirds of the amount incorporated into acid-insoluble cell material ( ), acid-insoluble material ( ), and extracellular lactate ( ) as described in the legend to Fig. 5.

2 P. G. W. Plagemann and F. D. Renner, unpublished data.
press entirely de novo synthesis of pyrimidine and purine nu-
cides. In contrast the production of lactate and pyruvate was
affected significantly by the presence of the nucleosides (Fig.
s). At appropriate times of incubation, duplicate 0.5-ml samples of suspension were analyzed for radioactivity in total cell material or acid-insoluble material (A) as described under "Methods." All points represent averages of the duplicate samples. The amounts of lactate and pyruvate produced (B) were estimated by chromatographic analysis of 25-µl samples of culture fluid (see Fig. 6A). At 90 min of incubation, acid extracts were prepared from 1 × 10^6 cells and 50-µl samples of the acid extract from the control cells (O—O) and from cells labeled in the presence of 1 mM uridine and adenosine (▲▲▲). Chroma-
tographed with solvent 98 (C) as described under "Methods." acid-insoluble material was recovered in nucleic acids. In other experiments the labeled nucleotides were further purified and hydrolyzed by boiling in 10 N perchloric acid. Chromato-
graphic analysis of the hydrolysates showed that at least 60% of the label in the nucleotide fraction was associated with the ribose moiety. The results indicate that about 50% of the glu-
cose assimilated by the cells is used for nucleic acid synthesis and enters the nucleic acids mainly via ribose of the nucleotides. In agreement with this conclusion is the finding that most of the CO₂ produced by N181-67 cells from glucose is liberated in the pentose pathway. This was indicated by the fact that 14CO₂ is liberated from 1-C but not 6-C-labeled glucose. The data in Fig. 9 show that the presence of 1 mM uridine and adenosine in the medium markedly reduced the incorporation of glucose into total and acid-insoluble cell material. As indicated in Fig. 9C, the labeling of the major intracellular acid-soluble fraction was specifically inhibited by the presence of the nucleo-
tides. In contrast the production of lactate and pyruvate was not affected significantly by the presence of the nucleosides (Fig. 9D). It has been demonstrated elsewhere (11, 39) that these concentrations of uridine and adenosine are sufficient to sup-
press entirely de novo synthesis of pyrimidine and purine nu-
cleotides without affecting macromolecular synthesis by the cells. Thus in the presence of nucleosides the cells had a diminished requirement for the synthesis of ribose.

Fig. 10 illustrates the initial rates of glucose incorporation into total cell material, acid-insoluble material, extracellular lactate, and CO₂ as a function of the glucose concentration in the medium. We have taken the sum of the initial rates of glucose incorporation into total cell material, extracellular lactate, and CO₂ to represent an estimate of the initial rate of glucose uptake by the cells (Fig. 10, TOTAL). The kinetic curve estimated from these combined values resembled that for deoxy-

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1. E. D. Renner, P. G. W. Plagemann, and R. W. Bernlohr, un-
published data.
The Kᵢ and Vₘₐₓ values for the incorporation of glucose into total cell material (Fig. 12A) do not, however, represent the real values for glucose transport, since they do not take into consideration the production of extracellular lactate and CO₂. The apparent Kᵢ values were about one order of magnitude lower than the values estimated for glucose transport from the combined rates of glucose conversion to cell material, lactate, and CO₂ (Fig. 13). The results indicate that the sole effect of Persantine was an inhibition of the transport reaction. The slightly greater effect of Persantine on lactate production (Fig. 13B) than on the incorporation into cell material (Fig. 13A) or CO₂ (Fig. 13C) was expected, since Persantine lowered the amount of glucose taken up by the cells and thereby mimicked the effect of lowering the glucose concentration in the medium (Fig. 5). The effect of Persantine was quite different from that of the nucleosides which inhibit glucose incorporation into nucleotides and thus reduce its incorporation into total cell material without affecting glucose transport per se.

Glucose and mannose, on the other hand, competitively inhibited both the uptake of deoxyglucose by whole cells and the cell-free phosphorylation of glucose or deoxyglucose, but the apparent Kᵢ values for the inhibition of these two processes differed significantly. The Kᵢ values for the inhibition of deoxyglucose uptake by mannose and glucose were 2.8 and 1.3 mM, respectively (Fig. 11), whereas the Kᵢ for the inhibition of the hexokinase reaction by both glucose and mannose was about 0.25 mM (Fig. 14). The latter Kᵢ was about the same as the Kₛ for the phosphorylation of glucose (Fig. 14). The Vₘₐₓ for the phosphorylation of glucose by cell-free extracts from N1S1-67 cells was approximately 3 times higher than that for deoxyglucose, and the apparent Kₛ for glucose was about one-third lower than that for deoxyglucose. The Kₛ values for the phosphorylation of glucose and deoxyglucose by cell-free preparations differed significantly from the corresponding values for the uptake of the substrates by whole cells (Table II). When

**Fig. 12.** Lineweaver-Burk plot of the initial rates of glucose incorporation into total cell material at various temperatures (A) and Arrhenius plot of the apparent Vₘₐₓ values (B). Cells were suspended to 2 x 10⁶ cells per ml in glucose-free BM42 equilibrated at the indicated temperatures. Samples of each suspension were supplemented with 20, 30, 40, 60, 100, 200, or 400 µM [¹⁴C]glucose (1.25 cpm per pmole) and incubated at the appropriate temperature. Duplicate 1-ml samples were analysed for radioactivity in total cell material at 5, 10, and 20 min of incubation and the initial rates of incorporation were estimated from these values.

Persantine has been previously shown to inhibit the uptake of monosaccharides by human erythrocytes (40) and the transport of nucleosides by various types of cells (10, 35, 41, 42). The results in Fig. 11 show that the uptake of deoxyglucose by N1S1-67 cells was competitively inhibited by Persantine and 3-O-methylglucose. In contrast, neither substance had any effect on the phosphorylation of glucose or deoxyglucose by cell-free preparations, even when added at relatively high concentrations (5 to 20 mM, not shown), and 3-O-methylglucose was not a substrate for the hexokinase (Table II). Also Persantine reduced to a similar extent the incorporation of 100 µM [¹⁴C]glucose into total cell material, acid-insoluble material, extracellular lactate, and CO₂ (Fig. 13). The results indicate that the sole effect of Persantine was an inhibition of the transport reaction. The slightly greater effect of Persantine on lactate production (Fig. 13B) than on the incorporation into cell material (Fig. 13A) or CO₂ (Fig. 13C) was expected, since Persantine lowered the amount of glucose taken up by the cells and thereby mimicked the effect of lowering the glucose concentration in the medium (Fig. 5). The effect of Persantine was quite different from that of the nucleosides which inhibit glucose incorporation into nucleotides and thus reduce its incorporation into total cell material without affecting glucose transport per se.

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membrane fragments as reported for other hepatoma cells (46), this must have represented only a very small proportion of the total activity of the cells (Fig. 15). Incubation of the cells for 2 hours in the absence or presence of 20 mM glucose had no effect on the relative proportion of hexokinase activity in the various cell fractions or its sucrose density gradient profile (not shown).

**DISCUSSION**

The over-all results demonstrate that two different processes are involved in the uptake of glucose and deoxyglucose by NISI-67 cells. Similarly, Sala and Coe (19) recognized two modes of glucose uptake by Ehrlich ascites cells. These investigators suggested that the uptake at high glucose concentrations reflects the glucose transport system, whereas the system with an apparent Km of 0.2 mM represents coordinated transport and phosphorylation. Their data and the present results, however, are more consistent with the conclusion that the main mode of uptake below 1 to 2 mM glucose or deoxyglucose represents carrier-mediated transport whereas at concentrations above 2 mM simple diffusion becomes the main mode of entry of the hexoses into the cell. At 5 to 10 mM deoxyglucose in the medium, which is the range of glucose concentrations present in many cell culture media or blood plasma, 60 to 70% of the initial rate of uptake is due to simple diffusion and only 30 to 40% to the transport reaction (Fig. 3). That the catalyzed uptake reaction detectable at low substrate concentrations represents facilitated diffusion and not active transport is suggested by the finding that O-methylglucose does not accumulate intracellularly against a concentration gradient.

**Fig. 13.** Effect of Persantine on the incorporation of D-[14C]glucose into cell material (A), lactate and pyruvate (B), and CO2 (C). Cells were suspended to 2 x 10^6 per ml in glucose-free BM42 (A and B) or N-hydroxyethylpyperazine-N2-ethanesulfonic acid-BM12 (C). The suspensions were supplemented with 0.1 mM [14C]glucose (2.5 μCi per mmole) and where indicated with 0.2 mM Persantine. At appropriate times of incubation, duplicate 0.5-ml samples of each suspension were analyzed for radioactivity in total cell material or acid-insoluble material (A) and 25-μl samples of culture fluid were chromatographed with solvent B (B). CO2 production (C) was measured by incubating the suspensions in 14CO2 collection flasks. The scintillation vials were replaced at 30-min intervals and the cumulative radioactivity values are presented.

**Fig. 14.** Phosphorylation of D-glucose and 2-deoxy-D-glucose by a cell-free preparation from NISI-67 cells. A cell-free extract was prepared from 4 x 10^6 cells in 2 ml of glycollyglycine buffer (pH 7.5) and assayed for hexokinase activity as described under "Methods." The final reaction mixture of 0.5 ml contained 50 mM glycollyglycine buffer (pH 7.5), 10 mM MgSO4, 100 mM KCl, 20 mM ATP, and 0.5 μCi of [14C]glucose plus unlabeled glucose to final concentrations of 0.1, 0.2, 0.3, 0.5, or 1 mM, or 0.35 μCi of [14C]deoxyglucose plus unlabeled deoxyglucose to 0.15, 0.2, 0.3, 0.5, or 1 mM. Where indicated, [14C]deoxyglucose reaction mixtures were supplemented with 0.2 mM glucose or mannose. At appropriate time intervals of incubation at 37°C, 50-μ1 samples were removed, placed in a boiling water bath for 1 min, and clarified by centrifugation, and the supernatant fluid was chromatographed as described under "Methods." Phosphorylation was linear with time until 10 to 30% of the total substrate was converted. The initial velocities were estimated from the linear portions of the reaction curves.

combined, the foregoing results indicate that the transport or diffusion of glucose into the cell are reactions distinct from the phosphorylation step and that permeation is the rate-limiting step in its further metabolism by the cells.

We also determined the distribution of hexokinase activity among various centrifugal fractions of NISI-67 cells. Upon fractionation of the cells in B4 or a 0.4 m sucrose solution containing 10 mM Na2EDTA, 60 to 70% of total cellular hexokinase activity was recovered in the cell sap, 5 to 10% in the nuclear fraction, and 20 to 30% in a combined mitochondrial-microsomal fraction. The complete cytoplasmic fraction prepared by fractionating cells in sucrose-EDTA was also analyzed by isopycnic centrifugation in a sucrose density gradient (Fig. 15). In this type of gradient the mitochondria of exponential phase NISI-67 cells equilibrate at a density of about 1.15 g per cm^3 as indicated by the distribution of cytochrome oxidase activity, and the fragments of the plasma membrane equilibrate at about 1.15 g per cm^3 as indicated by a peak of ATPase activity (31; see Fig. 15). As indicated by the profiles in Fig. 15, most of the hexokinase activity was recovered in the upper 8 ml of the gradient and thus was not associated with particulate membrane components of the cell. As in other cell systems (43-45), an appreciable proportion of the activity was located in the mitochondrial band. If any activity was associated with the plasma membrane fragments as reported for other hepatoma cells (46), this must have represented only a very small proportion of the total activity of the cells (Fig. 15). Incubation of the cells for 2 hours in the absence or presence of 20 mM glucose had no effect on the relative proportion of hexokinase activity in the various cell fractions or its sucrose density gradient profile (not shown).
uptake at the maximum rate. However, high concentrations of glucose are difficult to obtain with N1S1-67 cells because of the characteristics similar to the transport systems for nucleosides (35). The fact that p-chloromercuribenzoate inactivates the various transport systems suggests the involvement of sulphydryl groups in these transport reactions, and this conclusion is supported by the finding that the p-chloromercuribenzoate effect is readily reversed by incubating the cells in BM42 containing 1 mM dithiothreitol. Further, with all these substrates, transport seems to be a reaction distinct from phosphorylation and is the rate-limiting step in the phosphorylation of the substrates by whole cells and their subsequent metabolism. The kinases are present in excess in exponentially growing N1S1-67 and the natural substrates are phosphorylated as rapidly as they enter the cells and are thereby trapped. That the rate of glucose metabolism by various tissues is limited by the rate at which glucose is taken up by the cells has been suggested previously by a number of investigators (12-15, 48). It has also been shown that the failure of red blood cells from certain species to metabolize glucose is solely due to the lack of a glucose transport system (49). That glucose metabolism by N1S1-67 cells is limited by the rate of uptake is indicated by the fact that no free glucose is present intracellularly, even when the cells are incubated with high concentrations of glucose (Fig. 6B) and that the inhibition of glucose transport by Persantin (Fig. 13), 3-O-methylglucose, or hydrocortisone (15), or its inactivation by p-chloromercuribenzoate treatment or heat shock (Fig. 2), results in a proportional inhibition of the metabolism of glucose. This does not rule out that in some slow growing tumors the rate of glucose metabolism and of growth may be limited by a low level of hexokinase or some other enzyme (50, 51).

The rate limitation of the transport reaction may also be responsible for the concentration-dependent difference in the metabolic fate of the glucose taken up by N1S1-67 cells. At low concentrations in the medium, the small amount of glucose available to the cells is apparently used for the synthesis of macromolecules and for energy production by oxidative processes. As the glucose concentration in the medium is increased, the amount of glucose taken up and phosphorylated by the cells increases and the synthetic and oxidative pathways seem to become saturated. This is indicated by the fact that the rates of incorporation of glucose into macromolecules and of CO$_2$ production approach a maximum at about 1 mM glucose in the medium (Fig. 10). All excess glucose 6-phosphate formed from the glucose taken up by the cells will then be fermented to lactate. It has been shown that various lines of cultured mammalian cells grow at close to maximum rates in the absence of any net production of lactate if the glucose concentration in the medium is maintained at a very low level (52-54). Also, some lines of cultured cells grow as well with galactose as with glucose (52). Thus under aerobic conditions, the increased energy production from the glycolysis of the excess glucose taken up by the cells is not required for the growth of these cells (52-56). Under these conditions, therefore, glycolysis may largely serve to remove the excess of glucose 6-phosphate formed by the cells from the glucose taken up. The influx of glucose either by facilitated or simple diffusion is clearly a function of its concentration in the medium. It follows that the high concentrations of glucose present in most cell culture media are far in excess of what is required for cell replication and may even be detrimental to cell growth (52-54). Excess glucose 6-phosphate could also be converted to glycogen, but we have been unable to detect any glycogen formation in N1S1-67 cells so far, although certain strains of the Novikoff hepatoma propagated in rats (57) and other cells in culture (48) accumulate glycogen in the presence of high concentrations of glucose.
At low concentrations of glucose in the medium, cell growth may be limited by the rate at which glucose is transported into the cells. In this respect, mammalian cells may behave in the same manner as yeast (58). An increased capacity to take up glucose may be essential for the growth potential of tumor cells (1-4) or phytohemagglutinin-stimulated lymphocytes (6).

It has been suggested (46) that the ability of rapidly growing rat tumors to utilize glucose efficiently and their high rate of glycolysis is related to the presence of hexokinase in their plasma membrane and its involvement in glucose transport since hexokinase activity is absent from rat liver plasma membranes. The present results do not support this hypothesis. Glucose transport is clearly a process distinct from phosphorylation and if some hexokinase of NLSl-67 cells is associated with the plasma membrane, it represents only a very small proportion of the total activity of the cells (Fig. 15). Our results are more consistent with the view previously expressed by Eagle et al. (52) that the high rate of glycolysis exhibited by many tumor cells at physiological concentrations of glucose may be simply a consequence of an increased capacity of the tumor cells to transport the substrate into the cell.

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REFERENCES

35. Plagemann, P. G. W. (1971) Biochim Biophys Acta 233, 688
41. Kühner, W., and Breschneider, H. J. (1964) Arch Gesamte Physiol Menschen Tiere (Pfluegers) 280, 141
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