Selectivity of the 2-Deoxyglucose Transport System in Human and Guinea Pig Polymorphonuclear Leukocytes

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To determine whether the deleterious action of p-galactose upon phagocyte function could be related to inhibition of glucose uptake, the properties of glucose transport were investigated by following the incorporation of [G-3H]2-deoxyglucose into human and guinea pig polymorphonuclear leukocytes (PMN). Uptake of [G-3H]2-deoxyglucose by guinea pig PMN proceeded in vitro with a $K_m$ of 1.8 mM and $V_{max}$ of 0.67 nmol/min per $10^6$ cells. This system was competitively inhibited by glucose and mannose but was not significantly affected by galactose, fructose, or 3-O-methylglucose. Maximal uptake of 2-deoxyglucose occurred at 41°C, and phosphorylation was necessary for its intracellular concentration. Transport of 2-deoxyglucose, although not altered by uncouplers of oxidative phosphorylation, was sensitive to inhibitors of glycolysis. Preincubation of cells with 2 mM iodoacetate for 30 min significantly reduced the uptake of 2-deoxyglucose and the intracellular levels of adenosine-5'-triphosphate without decreasing cell viability. These results indicated that uptake of 2-deoxyglucose in guinea pig PMN occurred by facilitated diffusion with subsequent phosphorylation. Similar results were obtained with PMN isolated from human peripheral blood.

Phagocytosis and intracellular killing of microorganisms are two primary functions of neutrophilic polymorphonuclear leukocytes (PMN). These activities are of particular importance to the protection of a host against infection. However, in certain disorders, such as diabetes mellitus (4, 24) and galactosemia (18), the capacity of PMN to ingest and destroy bacteria is impaired. Previous studies indicate that this impairment directly results from elevated levels of plasma carbohydrate (4, 18, 24) and is not an effect of insulin or opsonin deficiencies (4).

Since phagocytosis is sensitive to inhibitors of glycolysis (25) and glucose transport (9), the deleterious effect of galactose on PMN function (18) could largely result from a competitive inhibition of glucose transport. To test this suggestion, we undertook a detailed study of hexose transport in human and guinea pig PMN, using the non-metabolizable glucose analogue, 2-deoxyglucose. The kinetics and selectivity of this transport system are herein described.

MATERIALS AND METHODS

Materials. All radioisotopes were purchased from New England Nuclear Corp. with the exception of [1.4C]fructose which was obtained from Amersham Searle Corp. All carbohydrates were of the $n$ configuration and were purchased from Sigma Chemical Co., Mallinkrodt Chemical Works, and Nutritional Biochemicals Corp. Other reagents were primarily from Sigma with the following exceptions: iodoacetic acid, Matheson Coleman and Bell Manufacturing Chemists; potassium cyanide, Baker Chemical Co.; p-chloromercuribenzoate and cytochalasin B, Calbiochem; phlorizin, Nutritional Biochemicals Corp.; N-ethylmaleimide, Aldrich Chemical Co.; U-80 regular Iletin insulin, Eli Lilly and Co.; dextan 250, Pharmacia Fine Chemicals, Inc.; guinea pig serum and Hanks balanced salt solution, Grand Island Biological Co.; and polystyrene latex particles (1.1-μm diameter), Dow Diagnostics. All glassware was silanized with a 1% solution of Silicol (Clay Adams).

Cell preparations. PMN were isolated from adult male guinea pigs (Connaught Laboratories, Ltd.) fed a commercial diet and water with 0.04% L-ascorbic acid ad libitum. Guinea pigs were injected intraperitoneally with 5.0 ml of sterile 1% casein in saline, and exudates were removed 15 to 20 h later by flushing peritoneal cavities with Hanks balanced salt solution. Exudate cells composed of greater than 95% PMN were pooled and centrifuged at 250 x g at room temperature for 5 min. Cells were washed and suspended in Krebs-Ringer phosphate solution without calcium (pH 7.4) (KRPS).

Human PMN were prepared from 10-ml samples of venous blood taken from 15 healthy adult volun-
teers. Samples were pooled, supplemented with 1.2% dextran 250, and allowed to sediment for 1 h at room temperature. Leukocyte-rich supernatants
were removed and centrifuged as described above. To lyse remaining erythrocytes, pellets of leukocytes were suspended twice and incubated for 1 h at 37°C in 200 ml of 0.015 M tris(hydroxymethyl)aminomethane (pH 7.2), containing 0.75% NH4Cl. After the second incubation, cells were again centrifuged and suspended in 5.0 ml of fresh autologous plasma. Cells in plasma were then applied to 20 ml of silicized glass beads (0.3-mm diameter), and PMN were isolated as described by Rabinowitz (23). Human PMN of at least 90% purity as judged by visual count were washed and suspended in KRPS. Cell viability was determined by exclusion of 0.04% trypan blue (22).

**Oxidation of carbohydrate.** Conversion of 14C-labeled carbohydrate to 14CO2 by guinea pig PMN was performed in sealed 25-ml Erlenmeyer flasks as previously described (25). Cell suspensions (7 × 106 PMN) were incubated for 1 h at 37°C in 1.0-ml aliquots of KRPS containing 10% guinea pig serum and 1.0 mM labeled carbohydrate (0.25 μCi). Reactions were stopped by adding 0.2 ml of 7.3 N H2SO4 to the cells, and evolved CO2 was collected on folded strips (1 by 3 cm) of Whatman 3MM paper as previously described (22). This paper was wetted with whatman silica gel and then dried. Radioactivity in the paper was determined by counting cells in a hemocytometer. Cell counts were performed at least in quadruplicate with a Universal model Zeiss microscope using phase optics.

Intracellular levels of phosphorylated 2-deoxyglucose were measured after the uptake experiments were performed. Scintillation fluid from each vial was dried, and carbohydrate was removed from each naphthalene residue by extracting with 20 ml of distilled water. Extracts were concentrated under a stream of nitrogen and spotted on Whatman 3MM paper for ascending chromatography. After development for 15 h with 7:3 (vol/vol) ethanol-1 M ammonium acetate, chromatograms were cut into segments corresponding to fast- and slow-migrating bands of radioactivity. Phosphorylated 2-deoxyglucose associated with the heterogenecous fraction. Intracellular 2-deoxyglucose was determined by subtracting extracellular tritium counts from total tritium counts in each pellet. Levels of ATP. PMN (15.2 × 106 cells per assay) were incubated either with or without inhibitors at 37°C in 1.0-ml aliquots of KRPS. After 30 min of incubation, cells were centrifuged for 1 min at 3,700 × g. Pellets were immediately frozen by immersing tubes in liquid nitrogen, and cells were stored at −80°C until homogenizing with 0.21 ml of 3 N perchloric acid. Homogenates were centrifuged at 5,000 × g for 10 min to remove precipitated protein, and supernatants were neutralized with 0.18 ml of 2 N KOH-0.4 M imidazole-0.4 M KCl. Levels of adenine 5′-triphosphate (ATP) were determined in each supernatant by monitoring the reduction of nicotinamide adenine dinucleotide phosphate at 340 nm in the presence of excess glucose-6-phosphate dehydrogenase (EC 1.1.1.49) and hexokinase (EC 2.7.1.1) (19).

**RESULTS**

Oxidation of carbohydrates to 14CO2. Table 1 shows the levels of 14CO2 produced by guinea pig PMN during a 1-h incubation at 37°C with various 14C-labeled carbohydrates. Values obtained with [1-14C]glucose were higher than those obtained with other carbohydrates, and participation of the hexose monophosphate shunt in this process is indicated by comparing values for the oxidation of [1-14C]glucose to those of [6-14C]glucose. Addition of latex particles, which stimulates hexose monophosphate shunt activity (1), enhanced 14CO2 production by the following extents: glucose, 2.6-fold; mannose, 2.6-fold; galactose, 2.8-fold; fructose, 1.3-fold. Increases in 14CO2 evolution during phagocytosis were not observed when 14C-labeled 2-deoxyglucose, 3-O-methylglucose, or sucrose was employed. The latter carbohydrates were not readily converted to 14CO2 by PMN. That [U-14C]sucrose was not taken up by PMN was further demonstrated by monitoring the radioactivity associated with pellets of cells. This radioactivity (approximately 900 cpm/106
cells) did not change significantly over the course of a 2-h incubation. In contrast to \(^3\)H counts, all \(^14\)C counts could be removed from pellets of cells by washing with cold KRPS (data not shown).

**Time course for 2-deoxyglucose uptake.** Data in Fig. 1 indicate the time course for uptake of 5.0 mM \([G-\text{\textsuperscript{3}H}]2\text{-deoxyglucose}\) into guinea pig PMN. Uptake was essentially linear with time for the first 10 min, after which a maximal value of 4.8 nmol/10\(^6\) cells was reached. Intracellular levels of 2-deoxyglucose were calculated upon dividing uptake by the intracellular water space of 0.42 \(\mu l/10^6\) PMN (7). An approximate twofold concentration of sugar then became apparent (Fig. 1, right ordinate). Intracellular 2-deoxyglucose was 69.1 to 84.9% phosphorylated. Uptake of 2-deoxyglucose was linear with cell concentration up to 8 to 10\(^6\) PMN per ml (data not shown).

**Kinetics of uptake.** Values indicated in Fig. 2 were determined by stopping cell incubations after 5 min and measuring the penetration of labeled 2-deoxyglucose under conditions of varying substrate concentrations. Initial velocities for uptake were therefore not directly measured but were calculated by dividing the average nanomoles of 2-deoxyglucose per 10\(^6\) cells by the 5-min time interval. Penetration of 2-deoxyglucose clearly followed saturation-type kinetics and yielded a \(K_m\) and \(V_{max}\) of 1.8 mM and 0.67 nmol/min per 10\(^6\) cells, respectively, when analyzed by the method of Lineweaver and Burk (17).

**Effects of heterologous carbohydrates on uptake.** When PMN suspensions were incubated with either 4.0 mM glucose or mannose together with 0.2 mM \([G-\text{\textsuperscript{3}H}]2\text{-deoxyglucose}\) (Table 2), the uptake of labeled sugar was significantly impaired. However, inhibition by similar levels of galactose and fructose was not observed. Addition of 3-O-methylglucose or insulin had no significant effects on 2-deoxyglucose uptake, and the presence of insulin did not increase the inhibition by glucose.

**Experiments were then conducted using various 2-deoxyglucose levels at fixed concentrations of glucose or mannose (Fig. 3A and B, respectively). Both glucose and mannose were competitive inhibitors of 2-deoxyglucose uptake; the former giving a \(K_i\) of 2.67 ± 0.32 mM and the latter giving a \(K_i\) of 2.28 ± 0.33 mM. All lines in Fig. 3 were fit to the data by the method of least squares using a weighted computer program as previously described (5).

**Effects of temperature and metabolic inhibitors.** The velocity of 0.2 mM \([G-\text{\textsuperscript{3}H}]2\text{-deoxyglucose}\) penetration rose significantly with increasing temperature until 41°C (Fig. 4). Beyond this point velocity decreased and was particularly sensitive to temperatures between 48 and 53°C.

The dependence of 2-deoxyglucose uptake upon temperature suggested that this process relied, in part, upon metabolic activity. To test this suggestion, PMN were preincubated for 30 min at 37°C with various metabolic inhibitors, and the rates of \([G-\text{\textsuperscript{3}H}]2\text{-deoxyglucose}\) uptake as well as phosphorylation were measured (Table 3). In the presence of 2.0 mM iodoacetate, rates of both uptake and phosphorylation were significantly reduced to 33 and 11% of the control values, respectively. Preincubation of cells with either 40 mM sodium fluoride or 0.2 mM iodoacetate resulted in similar degrees of inhibition in both uptake and phosphorylation. However, mitochondrial effectors, such as antimycin A, potassium cyanide, and dinitrophenol, did not show significant effects. Phlorizin, \(N\)-ethylmaleimide, and \(p\)-chloromercuribenzen-
oate produced slight reductions of 2-deoxyglucose uptake, but these were only significant when the former two agents were employed. In subsequent experiments, cytochalasin B at 0.5 and 1.0 µg/ml also inhibited uptake by 30.3 and 35.7%, respectively. These differences were significant at the $P < 0.01$ level.

A number of other agents were preincubated with cells for 30 min but were without significant effects on [G-3H]2-deoxyglucose uptake. These included 10 mM sodium barbital, 3 mM theophylline, 3 mM caffeine, 4 mM myo-inositol, 5 mM colchicine, 1 mM 3',5'-cyclic adenosine monophosphate (cAMP), 1 mM dibutryl cAMP, 1 mM 3',5'-cyclic guanosine monophosphate (cGMP), and 1 mM dibutryl cGMP. Preincubation of cells with polystyrene latex particles (200:1, particles:cell) or 0.5 µU of insulin did not significantly affect uptake rates. Effects of insulin were not altered by substituting Krebs-Henseleit bicarbonate solution for KRPS. However, rates for [G-3H]2-deoxyglucose uptake were 30% lower when the former medium was employed.

ATP levels. Intracellular levels of ATP in guinea pig PMN were significantly lowered by the presence of 2.0 mM iodoacetate (Table 4). Conditions during the incubation of cells were identical with those employed during the inhibitor experiments (Table 3).

**Effects of heterologous carbohydrates on uptake by human PMN.** The uptake of 0.2 mM [G-3H]2-deoxyglucose by human PMN was approximately twofold greater than the uptake by guinea pig PMN 0.027 versus 0.100 nmol/min per 10⁶ cells). Despite this difference, inhibitors of uptake for human PMN (Table 5) were very similar to those for guinea pig PMN (Table 2). Galactose, fructose, and 3-O-methyglucose did not significantly impair 2-deoxyglucose uptake, whereas glucose and mannose resulted in significant inhibition. Mannose in this case was a slightly better inhibitor than glucose, and some inhibition did occur in the presence of 30 mM galactose.

![Graph](image)

**Fig. 1.** Time-course for uptake at 5.0 mM 2-deoxyglucose. Assays were performed in quadruplicate using $7 \times 10^6$ PMN per experiment at 37°C. Line bars indicate standard deviations from the mean. Intracellular concentrations (right ordinate) are derived from the uptake values (left ordinate).

![Graph](image)

**Fig. 2.** Dependence of 2-deoxyglucose uptake on levels of external homologous carbohydrate. Velocity is plotted (A) against external substrate concentrations, and these values are replotted (B) according to Lineweaver and Burk (17). Each point is a mean of values from three experiments that were performed in triplicate. Line bars indicate standard deviation from the mean.
DISCUSSION

Although the stimulation of [1-\(^{14}\)C]glucose oxidation by PMN during phagocytosis has been frequently reported (11, 12), studies on the oxidation of other carbohydrates during phagocytosis have not been previously made. Our observations (Table 1) demonstrate that [1-\(^{14}\)C]mannose, [1-\(^{14}\)C]galactose, and [1-\(^{14}\)C]fructose are also metabolized to \(^{14}\)CO\(_2\), and that this oxidative metabolism is stimulated by phagocytosis. The degree of stimulation of [1-\(^{14}\)C]glucose oxidation is comparable to values previously reported (12), and the rate of oxidation by phagocytosing cells agrees with the 20 nmol/h per 10\(^6\) cells observed by Stjernholm et al. (26). That a constant degree of stimulation (2.6- to 2.8-fold) occurred, when either labeled glucose, mannose, or galactose (but not fructose) was employed, strongly suggests that the former three hexoses are converted into a common intermediate, i.e., glucose-6-phosphate, prior to oxidation by the hexose monophosphate shunt. Support for this suggestion is threefold: (i) PMN contain a complete Leloir pathway and are capable of converting galactose to glucose-6-phosphate (16, 18); (ii) \(^{14}\)C-labeled mannose is converted to lactate, via mannose-6-phosphate and fructose-6-phosphate, with the same labeling pattern as lactate derived from \(^{14}\)C-labeled glucose (8); and (iii) PMN are not glucogenic and are not able to convert fructose-1-phosphate or fructose-1,6-bisphosphate to glucose-6-phosphate (21).

The inability of guinea pig PMN to transport or oxidize \(^{14}\)C]sucrose is in agreement with results on human PMN described by Englhardt and Metz (6) and with observations on rabbit alveolar macrophages described by Gee et al. (9). Both of these studies similarly employed the exclusion of sucrose to correct for extracellular space during hexose transport experiments. The use of this technique also corrects for a nonspecific type of uptake encountered during phagocytosis. Esman refers to this as "piggy-back" phagocytosis or the "concomitant engulfment of extracellular medium with the phagocytosis of particles" (7). Such nonspecific uptake increases the insulin space in pellets of leukocytes by 4 to 6% (21) and, thus, could result in elevated values for 2-deoxyglucose uptake if \(^{14}\)C]sucrose was not also employed.

When 5 mM [G-\(^{3}\)H]2-deoxyglucose is presented to guinea pig PMN, maximal intracellular levels of label approach 11.4 mM after 40 min of incubation (Fig. 1). Since 69 to 85% of this label is phosphorylated, the maximal intracellular levels of free 2-deoxyglucose can be calculated to range from 1.7 to 3.5 mM. These values suggest that entry of 2-deoxyglucose occurs in the free form and that phosphorylation is necessary for 2-deoxyglucose concentration. These results are in accord with those of Esman (7), who found free intracellular glucose in PMN at low external glucose concentrations.

Whereas the data in Fig. 1 eliminate an active transport system for 2-deoxyglucose uptake, those from Fig. 2 and 3 rule out simple diffusion and indicate a facilitated transport mechanism. Uptake of 2-deoxyglucose displayed saturation-type kinetics and was competitively inhibited by glucose and mannose, which is suggestive of a common carrier-mediated transport system. Similar inhibition of 2-deoxyglucose uptake by glucose occurs in rabbit alveolar macrophages (9), and inhibition by glucose and mannose, but not galactose and fructose, is reported for rat diaphragm muscle (14). Studies on guinea pig lymph node cells by Helmreich and Eisen (10) also imply a common carrier mechanism for hexose transport; however, this mechanism appears to be more selective in PMN, since our results did not show competitive inhibition by fructose or 3-O-methylglucose.

Further evidence for facilitated diffusion of 2-deoxyglucose arises from the temperature dependence of [G-\(^{3}\)H]2-deoxyglucose uptake (Fig. 4) and from the effects of metabolic inhibitors upon this process (Table 3). Since hexose phosphorylation is required for sequestering of label, inhibition of phosphorylation by depleting

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**Table 2. Effect of carbohydrate and insulin on uptake of [G-\(^{3}\)H]2-deoxyglucose by guinea pig PMN**

<table>
<thead>
<tr>
<th>Additions</th>
<th>Uptake (% ± SD)</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>None</td>
<td>100 ± 13.1</td>
<td></td>
</tr>
<tr>
<td>Insulin</td>
<td>95.7 ± 4.9</td>
<td>NS</td>
</tr>
<tr>
<td>Glucose</td>
<td>42.5 ± 10.1</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Glucose + insulin</td>
<td>44.5 ± 3.6</td>
<td>&lt;0.001c</td>
</tr>
<tr>
<td>Mannose</td>
<td>46.9 ± 4.2</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Galactose</td>
<td>95.8 ± 7.5</td>
<td>NS</td>
</tr>
<tr>
<td>Fructose</td>
<td>95.8 ± 6.8</td>
<td>NS</td>
</tr>
<tr>
<td>3-O-methylglucose</td>
<td>87.0 ± 7.1</td>
<td>NS</td>
</tr>
</tbody>
</table>

* PMN were incubated at 37°C in 1.0 ml of buffer. All additions were made at zero time. 2-Deoxyglucose was 0.2 mM, whereas levels of carbohydrate and insulin were 4.0 mM and 0.5 mU/ml, respectively. P indicates levels of significance between values with additions and values without additions. Abbreviations: SD, standard deviation; NS, no significance.

* Uptake rate without additions was 0.142 ± 0.019 nmol/min per 10\(^6\) cells, N = 6.

* No significance between values obtained with glucose and values obtained with both glucose and insulin.
intracellular ATP would be expected to lower the diffusion gradient for free 2-deoxyglucose and, thus, impair the rate of 2-deoxyglucose entry. We observed significantly lower rates of entry when guinea pig PMN were incubated at temperatures below 37°C, when an isotonic bicarbonate solution was substituted for KRPS, and when cells were preincubated with 40 mM sodium fluoride or 2.0 mM iodoacetate. Preincubation with the latter inhibitor not only impaired uptake and phosphorylation to the greatest extents but also significantly decreased the intracellular levels of ATP (Table 4). These results, together with the lack of significant inhibition by potassium cyanide, antimycin A, and dinitrophenol, further demonstrate that active glycolysis, and not oxidative phosphorylation, is the primary source of ATP in PMN. This agrees with previous observations that PMN contain few mitochondria and that inhibitors of glycolysis can depress phagocytic function (25).

The lack of an in vitro insulin effect on either 2-deoxyglucose uptake or the inhibition of 2-deoxyglucose uptake by glucose (Table 2) is consistent with our findings that the rate of
Carbohydrate entry was greater than the rate of phosphorylation. However, this also implies that phosphorylation of 2-deoxyglucose is not affected by the presence of insulin. Similar results were obtained by Beck (2), who could not find insulin sensitivity in leukocyte hexokinase, by Englhardt and Metz (6), who did not observe an insulin effect upon glucose transport.

**Fig. 4.** Temperature dependence of 0.2 mM 2-deoxyglucose uptake. Line bars indicate standard deviations from the mean at values from five experiments.

**TABLE 3.** Effect of metabolic inhibitors on rates of \([G-3H]2\text{-deoxyglucose uptake and phosphorylation by guinea pig PMN}^a\)

<table>
<thead>
<tr>
<th>Additions</th>
<th>Uptake (nmol/10^6 PMN)</th>
<th>Phosphorylation (nmol/10^6 PMN)</th>
</tr>
</thead>
<tbody>
<tr>
<td>None</td>
<td>0.100</td>
<td>0.064</td>
</tr>
<tr>
<td>Iodoacetate (0.2 mM)</td>
<td>0.057 (A)</td>
<td>0.018</td>
</tr>
<tr>
<td>Iodoacetate (2.0 mM)</td>
<td>0.033 (B)</td>
<td>0.007</td>
</tr>
<tr>
<td>Fluoride (40 mM)</td>
<td>0.064 (A)</td>
<td>0.017</td>
</tr>
<tr>
<td>Antimycin A (1.0 µg/ml)</td>
<td>0.086 (C)</td>
<td>0.042</td>
</tr>
<tr>
<td>Cyanide (6.0 mM)</td>
<td>0.086 (C)</td>
<td>0.042</td>
</tr>
<tr>
<td>Dinitrophenol (10.0 mM)</td>
<td>0.096 (C)</td>
<td>0.058</td>
</tr>
<tr>
<td>Phlorizin (10.0 mM)</td>
<td>0.069 (D)</td>
<td></td>
</tr>
<tr>
<td>N-ethylmaleimide (0.2 mM)</td>
<td>0.070 (E)</td>
<td></td>
</tr>
<tr>
<td>Chloromercuribenzoate (0.2 mM)</td>
<td>0.081 (C)</td>
<td></td>
</tr>
</tbody>
</table>

*PMN (2.81 x 10^6 cells per assay) were preincubated with or without additions for 30 min at 37°C. After this period [G-3H]2-deoxyglucose was added to a final concentration of 0.2 mM, and uptake rates were measured. Each assay was performed five times, and levels of significance between values with additions and values without additions are indicated by (A) 0.01, (B) 0.005, (C) no significance, (D) 0.05 and (E) 0.02.

**TABLE 4.** Effect of iodoacetate and galactose on ATP levels in guinea pig PMN*

<table>
<thead>
<tr>
<th>Additions</th>
<th>ATP (nmol/10^6 cells ± SD)</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>None</td>
<td>1.32 ± 0.46</td>
<td></td>
</tr>
<tr>
<td>Iodoacetate (2.0 mM)</td>
<td>0.41 ± 0.33</td>
<td>&lt;0.05</td>
</tr>
<tr>
<td>Galactose (30.0 mM)</td>
<td>0.78 ± 0.27</td>
<td>NS</td>
</tr>
</tbody>
</table>

*PMN (15.2 x 10^6 cells) were incubated with or without additions for 30 min at 37°C. After incubation cells were immediately centrifuged at 37°C for 1 min at 3,700 x g. Pellets were quickly frozen by immersing tubes in liquid nitrogen. Cells were stored at -80°C until assay; see Materials and Methods for assay conditions. P refers to levels of significance between values with additions and values without additions. Abbreviations: SD, standard deviation; NS, no significance.

**TABLE 5.** Effect of carbohydrate on uptake of \([G-3H]2\text{-deoxyglucose by human PMN}^a\)

<table>
<thead>
<tr>
<th>Additions</th>
<th>Uptake (% ± SD)</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>None</td>
<td>100 ± 20.1</td>
<td></td>
</tr>
<tr>
<td>Mannose (4.0 mM)</td>
<td>39.1 ± 7.8</td>
<td>&lt;0.02</td>
</tr>
<tr>
<td>Glucose (4.0 mM)</td>
<td>50.8 ± 4.6</td>
<td>&lt;0.02</td>
</tr>
<tr>
<td>Fructose (4.0 mM)</td>
<td>78.2 ± 9.9</td>
<td>NS</td>
</tr>
<tr>
<td>Galactose (4.0 mM)</td>
<td>78.2 ± 6.6</td>
<td>NS</td>
</tr>
<tr>
<td>Galactose (30.0 mM)</td>
<td>64.3 ± 11.9</td>
<td>&lt;0.05</td>
</tr>
<tr>
<td>3-O-methylglucose (4.0 mM)</td>
<td>98.8 ± 8.8</td>
<td>NS</td>
</tr>
</tbody>
</table>

*PMN (0.848 x 10^6 cells per ml) were incubated in 1.0 ml of buffer at 37°C. All additions were made at zero time. [G-3H]2-deoxyglucose was 0.2 mM, and uptake rate without additions was 0.227 ± 0.046 nmol/min per 10^6 cells; N = 5. P refers to levels of significance between values with additions and values without additions. Abbreviations: SD, standard deviation; NS, no significance.

Into human PMN, and by Helmreich and Eisen (10), who were unable to demonstrate an insulin effect upon glucose uptake into guinea pig lymph node cells. On the other hand, Luzzatzo (20) observed an increase in xylose penetration into leukocytes at a non-physiological level of insulin (1 U/ml), and Kalant and Schucher (15) found an increased disappearance of glucose when leukocytes were incubated with 0.3 U of insulin per ml. Levels of insulin used in our studies (0.5 mU/ml) were only slightly higher than physiological.

Although decreases in cell metabolism, as well as glucose uptake, have been noted in concentrated PMN suspensions, we did not encounter this during our experiments. This so-called "crowding effect" has been attributed to changes in extracellular pH as a result of lactate accumulation and to the subsequent inhibition of phosphofructokinase activity (7). Al-
though Englard and Metz (6) observed this in their experiments with 7.6 × 10⁶ PMN/ml during a 1-h incubation, our experiments avoided this effect by utilizing fewer cells and shorter (5 min) incubation times. Therefore, lactic acid was not allowed to build up, and linearity between 2-deoxyglucose uptake and cell concentration was established. This method also circumvented any problems associated with excessive 2-deoxyglucose-6-phosphate accumulation which could non-competitively inhibit the hexokinase reaction (14).

Similarities between the uptake of 2-deoxyglucose by guinea pig PMN and the uptake by human PMN isolated from peripheral blood are apparent from comparing values in Table 2 to those in Table 5. Although the rate of uptake with human cells was approximately twofold greater than the rate with guinea pig cells at 37°C, uptake in both cases was substantially inhibited by the presence of glucose or mannose. Galactose, fructose, and 3-O-methylglucose at 4 mM did not significantly impair 0.2 mM 2-deoxyglucose entry into either cell type. However, uptake was slightly decreased when human PMN were incubated with 30 mM galactose. This latter effect was not observed with guinea pig PMN under similar conditions but was found, together with lower ATP levels (Table 4), after a 30-min preincubation with elevated galactose. It therefore appears that galactose may impair 2-deoxyglucose uptake by blocking its phosphorylation rather than by competing with its uptake. Although competition in this case with human PMN cannot be eliminated per se, it seems unlikely that elevated levels of galactose, as encountered during galactosemia, could impair phagocyte function by competing with glucose uptake. Thus, impaired phagocytic activity in the presence of galactose (18) may be primarily attributed to other factors, such as (i) the presence of a futile adenosine triphosphatase cycle or (ii) the intracellular accumulation of galactose or one of its metabolites with subsequent inhibition of glycolysis (18).

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LITERATURE CITED


