The Role of Glucose 6-Phosphate in the Regulation of Glucose Metabolism in Human Erythrocytes*

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At concentrations of glucose ranging from the physiological (1) to very high values (2), the rate of equilibration of external glucose with human red cells is much greater than the rate of glucose utilization by cellular reactions, the first of which is thought to be hexokinase. In mammalian tissues glucose 6-phosphate may undergo the following fates: return to glucose via phosphatase action, passage into glycogen, or entry into the energy-yielding Embden-Meyerhof path or into the 6-phosphogluconate path. Of these only the last two are reported to be of importance in the human red cell, since glycogen is apparently not present (3) nor are other important storage materials. The possibility that a phosphatase acting on glucose-6-P may function in situ has not been resolved. In mature erythrocytes, the amounts of glucose-6-P entering the Embden-Meyerhof path or the oxidative pathway are readily assessed since there exists no pathway for the oxidation of pyruvate to CO₂ (4).

Under conditions of steady state, the rate of metabolism of glucose will be equal to the rate of net flow from glucose to glucose-6-P, which is determined by the relative rates of two irreversible steps, the kinase and phosphatase. In the mature red cell, with glycolysis the major source of adenosine triphosphate, it would be forbidden to have either of these steps much faster than the net flux as this would result in a proportionally large hydrolysis of ATP. Thus control of the steady state flux must be effected through the control of one or both of these two steps in cells in which glucose transport is rapid.

In 1928, Barron and Harrop (5) reported that in the presence of methylene blue, the disappearance of glucose from mammalian blood was accelerated. This observation has been repeated and extended with purified human red cells (4, 6). One possible explanation for this effect is that methylene blue promotes the removal of glucose-6-P through the oxidative path (4) so that its return to glucose by phosphatase reaction is diminished. Another hypothesis would be that the lowered glucose-6-P would cause diminished inhibition of hexokinase, an action consistent with the inhibitory effect of glucose-6-P on mammalian hexokinases (7-9). On the other hand, one could consider either direct activation of hexokinase or inactivation of phosphatase by methylene blue, a compound known to interact with a number of enzymes.

The present experiments show that the extent of increase in utilization seen in the presence of methylene blue is correlated with decreased glucose-6-P content and that there is no functional glucose 6-phosphatase in the erythrocyte, but that red cell hexokinase is inhibited by glucose-6-P to a degree consistent with the conclusion that it is the concentration of glucose-6-P which controls glucose utilization through its effect on hexokinase. Additional experiments seeking to explain the inhibitory effect of inosine upon glucose uptake in red cells indicate that in this case also the controlling factor is the cellular concentration of glucose-6-P.

EXPERIMENTAL PROCEDURE

Human blood was drawn by venipuncture into ethylenediaminetetraacetic and was used without storage for the preparation of red cells for most experiments. Blood stored by the local blood bank in acid-citrate-dextrose medium for periods of 1 to 2 weeks could be used interchangeably with freshly drawn blood, and data obtained with cells from this source are seen in Table IV. After centrifugation, the plasma and the upper one-fifth of the cells were removed by suction; the cells were washed three times with isotonic NaCl and suspended in an equal volume of an isotonic phosphate-Locke solution (10) modified to contain 0.033 M phosphate buffer. The pH of all whole cell experiments was 7.2 and was unchanged during the course of incubation.

The amount and routes of glucose utilization in mature erythrocytes are readily determined by use of glucose-1-¹⁴C. Experiments were conducted in conventional Warburg flasks with NaOH in the center well. The completed vessels were stoppered and shaken at 37° for the required period, after which 0.3 ml of 3 M HClO₄ was tipped in from the side arm. Shaking was continued for at least 30 minutes to allow complete collection of CO₂. An acid extract of the residue was made by centrifugation and by three washes of the precipitate with 0.3 M HClO₄. The combined supernatant solutions were carefully neutralized with dilute KOH in the cold and left on ice overnight, and the resulting supernatant was used for the determinations of radioactive anions. A portion of the solution was diluted 10-fold and passed through a column of Dowex 1, acetate form. The column was washed well with water, and 20 ml of 1 N HCl were passed through to elute all radioactivity. The eluate was concentrated to near dryness. The radioactivity in this fraction was taken as a measure of compounds derived from glucose and which retain the radioactivity of the C-1 position. It would thus represent lactate and pyruvate as well as all of the phosphorylated intermediates derived from glucose-1-¹⁴C, including glucose-6-P and 6-P-gluconate. Since the latter compound was found to be present in undetectable amount, the counts in this fraction represent the Embden-Meyerhof path including glucose-6-P, which is common to the phosphogluconate pathway. The values reported for anion counts, anion micromoles, or analyzed amount of glucose-6-P have been corrected for incomplete recovery of radioactivity in the acid extract fraction.

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when proper account has been taken of the CO\textsubscript{2} fraction. In general, the recovery of radioactivity was 85 to 95%.

The specific activity of glucose was determined on a portion of the original effluent of the column described above. The sample was converted in a spectrophotometric assay to glucose-6-P dehydrogenase. The entire assay was placed on Dowex 1-acetate, and the column was washed and eluted as above. The retained counts and the absorbance change in the assay provided a measure of the specific activity of glucose. The amount of glucose-6-P in the acid extract fraction of the cells was determined by fluorometric assay with glucose-6-P dehydrogenase. The amount of ATP in the acid extract was measured spectrophotometrically with the hexokinase-glucose-6-P dehydrogenase system. The specific activity of glucose-6-P in the acid extract from cells incubated with either glucose-1-1\textsuperscript{4}C or glucose-2-\textsuperscript{3}H was determined by the specific conversion of the radioactive atom to a volatile form. In the case of -1\textsuperscript{4}C labeling, the acid extract was treated in turn with glucose-6-P dehydrogenase in a spectrophotometer and 6-P-gluconate dehydrogenase, the latter step being done in a closed system so that capture of CO\textsubscript{2} in an alkali center well was possible. To determine the degree of labeling of glucose-6-P in the 2-position with tritium, a sample of extract was repeatedly taken to dryness to remove volatile radioactivity. Samples were taken to determine the further formation of volatile counts resulting from incubation with phosphoglucose isomerase before and after treatment with glucose-6-P dehydrogenase. In both determinations, small corrections were made for nonenzymatic liberation of radioactivity and incubations were performed with internal controls to be certain that completeness of reaction had been achieved. The values reported represent means of three determinations, and additional experiments gave similar results.

A preparation of hexokinase was made by following the procedure of Hennessey et al. (11) for the separation of erythrocyte enzymes from hemoglobin. A stable preparation resulted from the ammonium sulfate precipitate of the DEAE-cellulose eluate. Kinetic properties were determined spectrophotometrically in the presence of excess glucose-6-P dehydrogenase plus TPN. When the effect of glucose-6-P was determined, an isotopic method was used in which the extent of conversion of glucose-\textsuperscript{14}C to a form held by Dowex 1-acetate was measured. In this latter method, the radioactivity of the substrate was the highest available, and the reaction was terminated at a time when an amount of product had formed which was very small compared to the K\textsubscript{d} determined for glucose-6-P.

Glucose-2-\textsuperscript{3}H was prepared by the incubation of glucose-6-P with phosphoglucose isomerase in tritiated water. The crystalline barium salt was isolated and recrystallized until free of material which reacts in the Roe test for fructose (12). Ester phosphate was hydrolyzed by incubation with potato phosphatase, and the resulting glucose solution was deionized by ion exchange. This preparation is completely detritiated by combined treatment with hexokinase and isomerase, and should correspond to material previously shown to be labeled only at C-2 (13).

The enzymes used for the present studies were either obtained commercially (glucose-6-P dehydrogenase, P-glucose isomerase, pyruvate kinase, and lactic dehydrogenase, from Boehringer, and potato phosphatase, California Corporation for Biochemical Research) or prepared in this laboratory (6-P-gluconate dehydrogenase (14), prepared by Mr. Gustav Lienhard, and hexokinase (15) of yeast).

All counting was done by liquid scintillation detection in a solution of phosphors in ethanol-toluene (1:2) as previously reported (16).

### EXPERIMENTS AND RESULTS

**Effect of Methylene Blue on Rate of Glucose Utilization and on Glucose-6-P Content of Red Cells**—Cells were suspended in 3 volumes of buffered salts medium with glucose-1-1\textsuperscript{4}C, 4.5 mm, and varying amounts of methylene blue as described in Table I. The effect of methylene blue in stimulating the utilization of glucose is entirely attributable to its effect on the phosphogluco-transport pathway measured by the formation of radioactive CO\textsubscript{2}. Part of this is at the expense of the Embden-Meyerhof path, which is seen to decrease to about half the control value under conditions of a much greater fall in the level of glucose-6-P.

To examine the possibility that the relation between velocity and glucose-6-P concentration is that to be expected for product inhibition of hexokinase, the reciprocal of the velocity was plotted against glucose-6-P (Fig. 1). If this were the mechanism by

### Table I

<table>
<thead>
<tr>
<th>Methylene blue</th>
<th>Glucose-1-\textsuperscript{14}C utilization</th>
<th>Glucose-6-P</th>
</tr>
</thead>
<tbody>
<tr>
<td>(\mu\text{M})</td>
<td>(\mu\text{mole/hr/ml packed cells})</td>
<td>CO\textsubscript{2}</td>
</tr>
<tr>
<td>0</td>
<td>0.142</td>
<td>0.99</td>
</tr>
<tr>
<td>0.54</td>
<td>1.27</td>
<td>0.64</td>
</tr>
<tr>
<td>2.7</td>
<td>1.44</td>
<td>0.635</td>
</tr>
<tr>
<td>6.7</td>
<td>2.39</td>
<td>0.482</td>
</tr>
<tr>
<td>134</td>
<td>2.79</td>
<td>0.415</td>
</tr>
</tbody>
</table>

![Fig. 1](http://www.jbc.org/) Plot of the reciprocal of the total glucose utilized at the observed intracellular concentrations of glucose-6-P. Data from Table I.
which glucose-6-P is related to velocity, such a linear relationship could be expected only if the other parameters controlling hexokinase, such as glucose and ATP concentration, were constant or at noncontrolling levels over the range of conditions in the five incubations. Analysis for ATP proved it to be almost constant at 1.0 μmole per ml of packed cells. The glucose concentration of the cell should correspond to that of the medium and should have been only slightly affected by the degree of utilization which occurred, 5 mm reduced to 4.5 or 3.7 mm in tubes 1 or 5, respectively, by the 90-minute incubation. The $K_m$ of glucose for red cell hexokinase is reported to be 0.18 mM (17).

**Question of Effect of Methylene Blue per se**—Although the results of Fig. 1 support the concept of an inverse relation between rate of utilization and the amount of glucose-6-P found in the acid extract, it might be argued that this is a spurious relation and that the increased glucose uptake is rather a response to methylene blue itself or to an effect of methylene blue other than on glucose-6-P levels. Thus methylene blue might either stimulate hexokinase or inhibit a glucose 6-phosphatase. In order to exclude these possibilities, a comparison was made of the effect of methylene blue on normal and glucose-6-P dehydrogenase-deficient red blood cells. From the data given in Table I it seems that methylene blue causes a stimulation of glucose utilization only when the dehydrogenase is active enough to cause a significant lowering in the glucose-6-P concentration of the cell.

**Test of Functional Glucose 6-Phosphatase in Glycolyzing Red Cell**—As stated in the introductory section, one mechanism by which the glucose-6-P concentration could control utilization is the regulation of glucose 6-phosphatase. At concentrations of glucose-6-P less than the $K_m$ for the phosphatase there would result a relation between glucose-6-P and the reciprocal of velocity of utilization similar to that observed in Fig. 1. The technique used to assay the functional phosphatase in the glycolyzing condition is based on the following rationale: when glucose-6-P is related to velocity, such a linear relation could be expected only if the other parameters controlling the reaction were constant or at noncontrolling levels over the range of conditions in the five incubations. Analysis for ATP proved it to be almost constant at 1.0 μmole per ml of packed cells. The glucose concentration of the cell should correspond to that of the medium and should have been only slightly affected by the degree of utilization which occurred, 5 mm reduced to 4.5 or 3.7 mm in tubes 1 or 5, respectively, by the 90-minute incubation. The $K_m$ of glucose for red cell hexokinase is reported to be 0.18 mM (17).

**TABLE II**  
**Effect of methylene blue on normal and glucose-6-P dehydrogenase-deficient cells**

<table>
<thead>
<tr>
<th>Cells</th>
<th>Methylene blue</th>
<th>Glucose-1-14C utilized</th>
<th>Glucose-6-P</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>CO₂</td>
<td>Anions</td>
</tr>
<tr>
<td>Normal</td>
<td>0</td>
<td>0.24</td>
<td>1.0</td>
</tr>
<tr>
<td></td>
<td>130</td>
<td>2.8</td>
<td>0.42</td>
</tr>
<tr>
<td>Deficient</td>
<td>0</td>
<td>0.06</td>
<td>1.14</td>
</tr>
<tr>
<td></td>
<td>130</td>
<td>0.80</td>
<td>0.88</td>
</tr>
</tbody>
</table>

**TABLE III**  
**Test for functioning glucose 6-phosphatase in glycolyzing cells**

<table>
<thead>
<tr>
<th>Time (min)</th>
<th>Glucose used</th>
<th>Specific activity</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Glycose-1-14C added</td>
<td>Glucose-2-3H added</td>
</tr>
<tr>
<td></td>
<td>c.p.m./μmole</td>
<td>c.p.m./μmole</td>
</tr>
<tr>
<td>0</td>
<td>0</td>
<td>70,000</td>
</tr>
<tr>
<td>40</td>
<td>30</td>
<td>74,000</td>
</tr>
<tr>
<td>150</td>
<td>70</td>
<td>105,000</td>
</tr>
</tbody>
</table>

every cycling of glucose-6-P to fructose-6-P and back, the specific activity of glucose-6-P-2-3H would be reduced to 25%. If isomerase were rapid in the red cell it would act to reduce the specific activity of the glucose-6-P pool to a value well below that of the starting glucose-2-3H. Under these conditions, the action of glucose 6-phosphatase would serve to decrease the specific activity of the glucose remaining in the medium. The experiment shown in Table III is a critical test of this. After an incubation of 40 minutes, the glucose-6-P of the acid extract was found to have the same specific activity as the starting glucose-1-14C, whereas it had only about 12% of the starting glucose-2-3H. This result suggests that the fructose-6-P of the cell was recycled in the phosphoglucose isomerase reaction about 10 times as readily as it was removed in catabolism. That there is no glucose 6-phosphatase activity in the cell is shown by the fact that at a time when 86% of the glucose had been utilized, the specific activity of the total glucose recovered had not changed significantly from its starting value.

**Inhibition of Red Cell Hexokinase by Glucose-6-P**—The results of the preceding experiments support the hypothesis that glucose utilization is controlled by the effect of glucose-6-P upon hexokinase. From the plot of Fig. 1, an inhibition constant of 0.013 μmole per ml of packed cells is obtained. The effect of glucose-6-P upon red cell hexokinase was studied through a range of ATP concentrations and at pH 8.0 and 7.2. Fig. 2A contains typical data in the form of a plot of $v$ versus glucose-6-P concentration in the concentration range of ATP from 1.5 to 6.0 mm, pH 8.0, 37°C. The values of $K_i$ fall in the range 0.035 to 0.073 μmole per ml depending on ATP concentration. The inhibition is only partly competitive with ATP (Fig. 2B).

**Effect of Inosine upon Glucose Utilization**—Inosine has been reported to curtail the utilization of glucose by red cells (18). That inosine is utilized preferentially is shown by the large production of lactic acid under conditions of almost no glucose uptake. Red cells stored in the presence of inosine have been found to contain increased amounts of various sugar phosphates, including glucose-6-P (19). To test the possibility that accumulated glucose-6-P might be related to the depressed rate of glu-
FIG. 2. Effect of glucose-6-P and ATP on red cell hexokinase.

Each incubation contained, in a final volume of 0.20 ml, triethanolamine chloride (0.12 M, pH 8.0); glucose-1-¹⁴C (0.3 mM, 910,000 c.p.m. per pmole); ATP and MgCl₂ in a ratio of 1:2 with ATP at 1.5, 2.25, 3.0, or 6.0 mM; glucose-6-P at 0, 0.06, or 0.12 mM; and red cell hexokinase. The reaction was started by the addition of enzyme to the 37° incubation mixture and terminated after intervals ranging from 5 to 20 minutes, so that the amount of glucose-6-P formed was in the range 0.006 to 0.01 mM for all incubations. The reaction was terminated by the addition of 1,000 µmoles of unlabeled glucose, and the radioactivity retained by Dowex 1-acetate was determined. A control tube contained the carrier glucose added prior to the enzyme. A, The reciprocal of radioactivity retained by the anion exchange resin plotted against glucose-6-P concentration with ATP at 1.5 (○), 2.25 (■), 3.0 (△), and 6.0 mM (X). The values of glucose-6-P estimated to correspond to half-maximal velocity were 0.035, 0.046, 0.051, and 0.073, respectively. B, The reciprocal of radioactive anions plotted against reciprocal ATP concentration with glucose-6-P at 0 (○), 0.06 (●), or 0.12 mM (△). The calculated Kₘ values for ATP were 1.48, 5.40, and 6.24 mM, respectively.

TABLE IV

Effects of inosine and methylene blue on glucose utilization and glucose-6-P levels

Blood obtained from the blood bank after 1 week of storage was used. Red cells were initially incubated in a PO₄-Locke solution containing glucose, 0.3 mM, and the additions noted in the table. After 30 minutes at 37° the cells were centrifuged, resuspended in the same medium, and again incubated for 30 minutes. The cells were recovered by centrifugation and washed with 5 volumes of buffer. Finally 0.50 ml of packed cells was transferred to a Warburg flask, providing an incubation mixture which contained glucose-1-¹⁴C (1.2 mM and 100,000 c.p.m. per pmole) and the same concentration of inosine used in the initial incubations. No addition of methylene blue was made to any of the final incubations, since that which was present in the initial incubations was retained by the cells through the washing. The cells were incubated at 37° for 30 minutes before acid was tipped in.

<table>
<thead>
<tr>
<th>Additions</th>
<th>Concentration</th>
<th>Glucose-1-¹⁴C utilization</th>
<th>Glucose-6-P</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>µmoles/hr/ml packed cells</td>
<td>µmol/ml cells</td>
</tr>
<tr>
<td></td>
<td></td>
<td>CO₂</td>
<td>Anions</td>
</tr>
<tr>
<td>None</td>
<td></td>
<td>0.06</td>
<td>0.84</td>
</tr>
<tr>
<td>Methylene blue</td>
<td>0.002</td>
<td>1.17</td>
<td>0.52</td>
</tr>
<tr>
<td></td>
<td>0.020</td>
<td>2.20</td>
<td>0.31</td>
</tr>
<tr>
<td></td>
<td>0.090</td>
<td>2.47</td>
<td>0.34</td>
</tr>
<tr>
<td>Inosine</td>
<td>0.18</td>
<td>0.021</td>
<td>0.767</td>
</tr>
<tr>
<td></td>
<td>0.36</td>
<td>0.014</td>
<td>0.575</td>
</tr>
<tr>
<td></td>
<td>0.72</td>
<td>0.008</td>
<td>0.334</td>
</tr>
<tr>
<td></td>
<td>1.44</td>
<td>0.006</td>
<td>0.21</td>
</tr>
</tbody>
</table>

FIG. 3. The rate of total glucose utilization as the reciprocal is plotted against the amount of glucose-6-P per ml of packed cells to show the effects of methylene blue (○) or inosine (●) relative to the control (△). Data taken from Table IV.
from this plot is 0.036 μmole per ml of packed cells. In order to be certain that the effect of inosine was not on glucose transport, a study of the partition of glucose between cells and medium was made with cells initially incubated with inosine. According to the method of Murphy (1), no difference could be detected either in the size of the intracellular compartment or in the rapidity with which full equilibration was achieved as a result of inosine treatment. The individual acid extracts did not differ appreciably in content of ATP or ADP, and hemolysates were found to contain a constant activity of hexokinase and phosphofructokinase activity, independent of pretreatment with methylene blue or inosine.

**DISCUSSION**

The results of the whole cell experiments with varying amounts of methylene blue and with inosine support the conclusion that the glucose-6-P level in the cell exercises control over glucose utilization. The concept that glucose-6-P inhibition of hexokinase might be an important regulatory factor has been frequently invoked (20-22) since its original proposal by Crane and Sols (8). There seem to exist no reports of the desired correlation of rate of glucose utilization with glucose-6-P level over a substantial range of concentration. It is perhaps the simplicity of the mature red cell in terms of reactions involving glucose 6-P that has lent itself to these studies. It should be remarked, however, that any compound or effect which varied in exact proportionality with glucose-6-P, such as fructose-6-P, glucose-1-P, and perhaps glucose-1,6-di-P, might also contribute to the linear relation of inverse velocity to amount of glucose-6-P. Such other compounds would have the effect of lowering the apparent Kᵢ below that which could be ascribed to glucose-6-P alone. The interpretation of the size of the Kᵢ determined has an additional arbitrary quality in that the volume in which the glucose-6-P is distributed is certainly less than the packed cell volume, but it may also be less than the intracellular space, about 0.65 the packed cell volume. When calculated on the basis of intracellular water space, the values for apparent Kᵢ of glucose-6-P become 0.02 and 0.055 mm in the two experiments reported in Figs. 1 and 3, values which are comparable to the Kᵢ determined for hexokinase (Fig. 2).

A point worthy of comment is the tendency of many authors to denote a single enzyme or compound as the rate-limiting factor in glycolysis. It would seem correct to assume that the rate equation for glycolysis in the red cell would have hexokinase concentration in the numerator and glucose-6-P concentration in the denominator. However, the control of glucose-6-P concentration by enzymes that utilize it either directly or indirectly, and the control of these enzymes, may all be of importance for a complete description of the kinetics of glycolysis in the steady state as long as glucose-6-P is at a concentration similar to or greater than its Kᵢ for hexokinase. In examining the question of what other factors are important, we can clearly eliminate phosphoglucone isomerase activity, since several lines of evidence indicate that the interconversion of glucose-6-P and fructose-6-P is much more rapid than the net flux: (a) the dilution in the tritium specific activity of the glucose-6-P found in Table III is evidence of rapid recycling in this step; (b) the two sugar phosphates are found in a ratio approximating the equilibrium ratio in cells metabolizing glucose at the normal rate; and (c) no isotope effect is found with glucose-2-2H either on glucose utilization rate or on the concentration of glucose-6-P. The rate of glucose-6-P reaction in the oxidative shunt pathway may be of importance in determining the rate of glucose utilization at low pH (18) or in the presence of strongly oxidizing conditions. Normally, however, it accounts for less than 20% of the rate of glucose-6-P. If phosphofructokinase is not significantly “reversed” by phosphatase action, then this step must be uniquely important in determining the glucose-6-P level in the red cell. In this case, the factors that contribute to the steady state velocity of the phosphofructokinase reaction would enter into the rate equation for glucose utilization. Ideas similar to these have been previously expressed (8, 21-23).

The glucose-6-P level usually found in the red cell is near the Kᵢ value for the hexokinase acting at the observed level of ATP. Thus the glycolytic rate will be highly responsive to effects which alter the net removal of glucose-6-P. Although the ATP level of the cell is such that it should exert maximal control over the hexokinase rate, being near its Kᵢ concentration, it is not likely that regulation of ATP concentration is important for the rapid adjustment of the hexokinase rate in the intact red cell. The amount of ATP, about 1 μmole per ml of packed cells, is large compared to the rate of ATP generation, about 0.05 μmole per minute per ml of packed cells, and hence large compared to the rate of hydrolysis to ADP in the steady state. Thus fluctuations in ATP level due to changes in these rates will be small compared to the absolute ATP level. This argument assumes that hexokinase is responsive to the whole ATP of the cell. On the other hand, the concentration of glucose-6-P in the cell, 0.05 to 0.1 μmole per ml of packed cells, is in a range that can easily be affected by changes in the rate of phosphofructokinase, normally at least 0.025 μmole per minute per ml of packed cells. The correlation of the rate of glucose utilization with a very wide range of glucose 6-P levels in the cell supports the conclusion that the hexokinase reaction rate is responsive to a single uniform pool of glucose-6-P.

**SUMMARY**

The rate of glucose utilization by intact human red blood cells is inversely proportional to the amount of glucose 6-phosphate present in the cell over a very wide range of variation (40-fold in glucose-6-P). The rate of glucose utilization is half-maximal at a glucose-6-P level in the neighborhood of the Kᵢ value of glucose-6-P for red cell hexokinase. Phosphatase action on glucose-6-P in the glycolyzing cell is negligible. The data support the conclusion that the normal rate of glucose utilization is strongly controlled by factors that determine the rate of removal of glucose-6-P.

**Acknowledgments**—We wish to thank the blood bank of the Grace-New Haven Hospital for making blood available, and Dr. Paul Marks, Columbia University College of Physicians and Surgeons, for providing us with samples of blood from glucose-6-phosphate dehydrogenase-deficient subjects.

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