Clinical Studies on Glucose Metabolism via the Pentose Phosphate Pathway in Human Erythrocytes

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Glucose metabolism via the pentose phosphate pathway was studied by measuring accelerated $O_2$ uptake of human erythrocytes in the presence of methylene blue. Erythrocyte $O_2$ uptake was independent of age in 67 normal patients. Erythrocyte $O_2$ uptake was increased in pernicious anemia in relapse and in 5 of 6 jaundiced patients but was normal in pernicious anemia in remission and in 20 cases of malignancies of various types. In the latter cases there was no correlation between erythrocyte $O_2$ uptake and hemoglobin content or reticulocyte count.

Mature mammalian erythrocytes metabolize glucose by the anaerobic Embden-Meyerhof pathway and the aerobic pentose phosphate pathway. Erythrocytes in the presence of methylene blue metabolize glucose largely via the pentose phosphate pathway (1, 2) and about 85% of the $O_2$ uptake by erythrocytes in the presence of methylene blue can be accounted for by this mechanism.

Methods

Whole blood (10 ml.) was drawn by venipuncture into tubes containing heparin from patients who had fasted 16–20 hr. The specimen was centrifuged at 1400 $g$ for 0.5 hr. and the supernatant and buffy coat removed. The cells were washed once in Krebs-Ringer phosphate (K-R-P) solution and again centrifuged for 0.5 hr. at 1400 $g$. The supernatant was again removed and the top of the cell column blotted gently with a cotton applicator.

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K-R-P solution (2 ml.) containing 100 mg./100 ml. glucose was pipetted into the main compartments of Warburg flasks. K-R-P solution (1 ml.) containing $10^{-4} M$ methylene blue was pipetted into side arms and fluted filter paper saturated with 20% (w/v) KOH was placed in the center wells. One milliliter of packed erythrocytes was suspended in the fluid of the main compartments. The flasks were gassed for 5 min. with $O_2$ and equilibrated in the water bath at 38° before stopcocks were closed.

Flasks oscillated through an arc of 180° at a rate of 65 oscillations per minute. The system was run for 30 min., after which the apparatus was stopped and the manometers were removed and tilted to mix the contents of the side arms with the main compartments. Oxygen uptake was then measured during the next 30 min. and was recorded as microliters $O_2$ consumed per milliliter of erythrocytes per 30 min. Determinations were performed in duplicate on each blood sample.

**Results**

Sixty-seven normal male patients ranging in age from 21 to 87 years were studied by the above method. The number of patients in each age group was as follows: 21–30 years, 10; 31–40 years, 10; 41–50 years, 11; 61–70 years, 12; 71–80 years, 4. Mean $O_2$ uptake for the whole group calculated from the mean of duplicate samples was 46.68 $\mu$L/ml RBC/30 min. (standard error = 1.98). There was no significant correlation between erythrocyte $O_2$ uptake and patient age ($r = 0.240$).

A sequential analysis of data was calculated according to the method of Wald (3, 4) to determine whether erythrocyte $O_2$ uptake was significantly different from normal in specific disease states using the hypothesis in each case that the erythrocyte $O_2$ uptake in a given disease state was not significantly different from normal.

Patients with pernicious anemia in relapse showed a significantly higher erythrocyte $O_2$ uptake than normals (Fig. 1). This elevated $O_2$ uptake returned to normal with treatment, and studies of 6 other patients with known pernicious anemia in remission showed normal $O_2$ uptake.

Twenty patients with varying types of malignancy were studied, including carcinoma of the prostate 6, carcinoma of the colon 4, multiple myeloma 1, lymphosarcoma 2, reticulum cell sarcoma 1, carcinoma of the lip 1, Hodgkins disease, metastatic carcinoma, primary undetermined 3. Hemoglobin in these patients ranged from 8.2 to 16.5 gm. and reticulocyte count from 1.1 to 3.8%. When considered as a
group, there was no significant difference in erythrocyte O₂ uptake when compared with normal patients (Fig. 1). Further, there was no correlation between O₂ uptake and hemoglobin content (r = 0.23) nor between O₂ uptake and reticulocyte count (r = 0.07).

An interesting finding in the present study was the fact that 5 of 6 jaundiced patients had significantly increased erythrocyte O₂ uptake (greater than 2 standard deviations from the normal mean). Three patients had portal cirrhosis and 3 had carcinoma of the head of the pancreas. In one of the cases of carcinoma of the head of the pancreas the O₂ uptake reached 124.3 μL/30 min., or nearly 3 times the normal mean (Table 1).

**Discussion**

Methylene blue acts as an electron acceptor through which the oxidation of TPNH by molecular O₂ can take place. Since in the metabolism of glucose by the erythrocyte TPNH is formed only via the pen-

<table>
<thead>
<tr>
<th>Patient</th>
<th>Age</th>
<th>Serum bilirubin (mg/100 ml.)</th>
<th>Qmax (80 min./ml. RBC)</th>
<th>Diagnosis</th>
</tr>
</thead>
<tbody>
<tr>
<td>C. G.</td>
<td>51</td>
<td>2.0</td>
<td>67.8</td>
<td>Portal cirrhosis</td>
</tr>
<tr>
<td>R. F.</td>
<td>39</td>
<td>34.1</td>
<td>79.6</td>
<td>Portal cirrhosis</td>
</tr>
<tr>
<td>D. S.</td>
<td>48</td>
<td>3.9</td>
<td>108.9</td>
<td>Portal cirrhosis</td>
</tr>
<tr>
<td>O. B.</td>
<td>82</td>
<td>2.2</td>
<td>83.5</td>
<td>Carcinoma head of pancreas</td>
</tr>
<tr>
<td>J. X.</td>
<td>68</td>
<td>15.3</td>
<td>90.8</td>
<td>Carcinoma head of pancreas</td>
</tr>
<tr>
<td>G. O.</td>
<td>74</td>
<td>11.6</td>
<td>124.3</td>
<td>Carcinoma head of pancreas</td>
</tr>
</tbody>
</table>
Pentose pathway, the erythrocyte O₂ uptake in the presence of methylene blue is a direct monitor of the amount of TPNH formed. Glucose-6-phosphate dehydrogenase (G-6-PD) is the priming enzyme for the initiation of glucose metabolism via the pentose phosphate pathway. Decrease in this enzyme occurs in primaquine-sensitive individuals (5) and in some cases of nonspherocytic hemolytic anemia (6–8), resulting in a decreased production of TPNH and a consequent decrease in the erythrocyte O₂ uptake in the presence of methylene blue (9).

In the present studies, erythrocyte O₂ uptake in the presence of methylene blue was increased in patients with pernicious anemia in relapse and jaundice, indicating increased amounts of TPNH available for oxidation. An increased level of G-6-PD in these cells, with a resultant increase in TPNH, could account for this result. That this explanation may hold in cases of jaundice is suggested by the work of Pitcher and Williams (10), who demonstrated by biochemical studies of erythrocytes in jaundiced patients that the levels of G-6-PD were elevated in some cases. Examination of this paper indicated that in 4 cases of portal cirrhosis the G-6-PD level was normal in 1 and elevated in 3.

The data reported herein are in agreement with those of Oka and Puranen (11), who found an increased O₂ uptake in erythrocytes in the presence of methylene blue in patients with pernicious anemia in relapse and also found no correlation between erythrocyte O₂ uptake and reticulocyte count. However, while these authors found a correlation between O₂ uptake and hemoglobin content (r = 0.64), we found no such correlation in patients with malignancy and anemia (r = 0.24). This difference in results may be due to the fact that the former authors reported O₂ uptake per gram of hemoglobin and the present study reports O₂ uptake per milliliter of erythrocytes.

References