Diagnosis of Erythrocyte Glucose-6-Phosphate Dehydrogenase Deficiency in the Negro Male Despite Hemolytic Crisis

By FRITZ HERZ, EUGENE KAPLAN AND ELSIE S. SCHEYE

ERYTHROCYTE GLUCOSE-6-PHOSPHATE DEHYDROGENASE (G-6-PD) DEFICIENCY is a frequent clinically-significant genetically-determined abnormality of man. On the basis of gene frequency of the enzyme deficiency, about 3,000,000 Negroes in the United States carry genes for this disorder. When exposed to certain drugs and chemical agents, clinically normal persons with G-6-PD deficiency experience hemolysis.

The diagnosis of G-6-PD deficiency in the Negro is often made difficult by falsely-normal estimates of red cell enzyme activity during acute hemolytic crisis. The distribution of enzyme activity in the red cell population is age dependent, with increased activity in the younger red cells and decreased activity in the older red cells. Since susceptibility of red cells to chemical agents in this disorder is inversely proportional to enzyme activity, the older cells with the least G-6-PD activity are preferentially eliminated in hemolytic crisis, leaving a disproportionate number of young red cells with high enzyme levels. Measurement of red cell G-6-PD activity in whole blood samples during hemolytic crisis may result in values in the normal range. Proper recognition of G-6-PD deficiency is therefore usually postponed until the hemolytic episode and its reticulocytic response have subsided.

To make possible the recognition of G-6-PD deficiency despite hemolytic crisis, we have employed a simple technique based on density changes associated with red cell aging, for detecting the persisting, aged, enzyme-deficient erythrocytes in Negro patients with chemically induced hemolytic anemia who have not received blood transfusions within the past five months.

MATERIALS AND METHODS

Capillary blood from newborn infants and venous blood from older children and adult individuals was collected with sequestrene or heparin. A 1.5-ml aliquot of the blood sample was transferred to a 15- x 125-mm. test tube and the red cells were washed twice with 15 volumes of ice-cold 0.15 M NaCl solution by centrifugation at 800 x g for five minutes. To facilitate their subsequent handling, the cells were transferred to a
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10- × 75-mm. test tube after the second spinning and washed once again with saline solution. The supernatant liquid and the huffy coat were carefully suctioned off after each centrifugation. Uniform erythrocyte suspensions were prepared by mixing on a Vortex Jr. mixer, and a 0.1 ml. aliquot was adjusted to a hematocrit value of approximately 50 per cent with saline solution and set aside for the determination of enzyme activity in unseparated red cells.

Four microhematocrit capillary tubes were filled with the thrice-washed, homogenous cell suspension. One end of the tubes was sealed with vinyl plastic putty and then centrifuged for five minutes at 15,500 × g. in a microhematocrit centrifuge. After measuring the total length of the spun red cell columns, the lower 10 per cent of the columns was gently broken off at incision points made with a glass scorer and placed in a 10- × 75-mm. test tube containing 0.12 ml. of 0.15 M NaCl solution. The red cells were expelled from the glass segments by shaking the tube with a Vortex Jr. mixer. This additional mechanical stress did not cause hemolysis. An aliquot of this separated, heavy red cell suspension, and of the unseparated sample were diluted 1:20 in cold distilled water and the resultant lysates were assayed in duplicate for G-6-PD activity according to the method of Zinkham. Enzyme activity was related to hemoglobin concentration, the latter measured at 540 μm as cyanmethemoglobin. Specific activity was expressed as μmoles of TPN reduced/min./g. hemoglobin.

RESULTS AND DISCUSSION

Representative examples of the usefulness of the procedure described above for the diagnosis of G-6-PD deficiency during hemolytic states are summarized in Table 1. As can be seen, the enzyme activity of the unseparated red cell specimens in three out of the four patients was within the limits of normal individuals. By measuring the activity of the heaviest (oldest) erythrocytes, the sex-linked enzyme defect was unequivocally established without having to wait until after hematological recovery had taken place and without having to perform enzyme studies on the immediate family of the patients. In no instance were we unable to recognize the enzyme defect during the time of a hemolytic crisis, and the diagnosis of G-6-PD deficiency was confirmed when blood was re-examined after the hemolytic episode had subsided. The finding of G-6-PD deficiency during hemolytic episodes permits the physician to take appropriate remedial action immediately. Our inability to detect any G-6-PD activity in the heaviest erythrocytes could be due to in vitro enzyme inactivation, since the cells were hemolysed in the absence of TPN, mercaptoethanol, and EDTA. This explanation, however, is not supported by the failure of hemolysing solutions containing TPN and mercaptoethanol to increase the G-6-PD activity in red cells of subjects with the Mediterranean variant of this enzyme abnormality. Our preference would be to believe that the absence of detectable activity in the oldest cells represents a valid observation, and that any activity present in these red cell preparations was of too low an order to be detected by the method employed. We have also used the microgravimetric technique as an aid in the diagnosis of G-6-PD deficiency in homozygous and certain heterozygous Negro females during hemolytic states, although in these cases the precipitating hemolytic agent has not been as clearly defined as in the patients indicated in Table 1.

Although we are using quantitative measurements of G-6-PD activity, it should be possible to apply the microgravimetric procedure to the several qualitative screening tests suggested by others. The method employed in
Table 1.—G-6-PD Activity of Negro Males in Hemolytic Crisis

<table>
<thead>
<tr>
<th></th>
<th>Precipitating Agent</th>
<th>Age</th>
<th>Hematocrit</th>
<th>Per Cent Reticulocytes</th>
<th>G-6-PD Activity Unseparated Red Cells</th>
<th>Heaviest Cells</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ra</td>
<td>Naphthalene</td>
<td>2</td>
<td>24</td>
<td>12.8</td>
<td>6.2</td>
<td>0</td>
</tr>
<tr>
<td>Ha</td>
<td>Naphthalene</td>
<td>2</td>
<td>19</td>
<td>16.4</td>
<td>5.3</td>
<td>0</td>
</tr>
<tr>
<td>Fi</td>
<td>Aspirin</td>
<td>7</td>
<td>26</td>
<td>5.9</td>
<td>9.6</td>
<td>0</td>
</tr>
<tr>
<td>Qu</td>
<td>Aspirin</td>
<td>42</td>
<td>25</td>
<td>5.4</td>
<td>6.3</td>
<td>0</td>
</tr>
<tr>
<td>Normals (31)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>5.5–9.3, 3.2–5.1</td>
</tr>
</tbody>
</table>

* G-6-PD activity expressed as micromoles of TPN reduced/min./g. hemoglobin.

isolating the oldest red cells is simple, does not require special laboratory equipment, and provides results within one hour. Diagnosis in individuals with other genetic variants of erythrocyte G-6-PD deficiency does not require this special laboratory procedure. In these individuals reduced enzyme activity is distributed throughout the red cell population, and the measurement of G-6-PD activity in whole blood readily reveals the necessary information.

The ever-growing list of drugs capable of inducing hemolysis in a large segment of the population and the increasing incidence of drug ingestion, both prescribed and self-administered, as well as the accidental ingestion by sensitive children of noxious chemical agents, would indicate that a simple procedure which permits the diagnosis of G-6-PD deficiency at the time of a hemolytic episode can be of significance in patient management.

**SUMMARY**

A simple procedure for the diagnosis of erythrocyte glucose-6-phosphate dehydrogenase deficiency in Negro males experiencing hemolytic crisis is described. By measuring enzyme activity in the persisting older erythrocytes, the deficiency can be recognized without having to wait until the hemolytic episode and its reticulocyte response have subsided.

**REFERENCES**


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