Correlation of S Hemoglobin with Glucose-6-Phosphate Dehydrogenase Deficiency and Its Significance

By ROGER A. LEWIS AND MICHAEL HATHORN

FROM A RELATIVELY small series of cases, Lewis and Hathorn concluded that there is a correlation between the incidence of glucose-6-phosphate dehydrogenase deficiency (G-6-PD) and S hemoglobin. It was suggested that G-6-PD defect protects against the hazards of sickle cell disease, and that this might account for the high incidence of the enzyme deficiency in individuals with an abnormal hemoglobin pattern. Now that more cases have been studied, the original findings have been confirmed, and it is possible to compare the incidence of the defect in sickle cell-hemoglobin C disease, sickle cell anemia and sickle cell thalassemia. Meanwhile, Allison has drawn our attention to conflicting observations made by Naylor et al. Their data are compared with ours and the conflicting conclusions are explained by differences in methodology and interpretation.

MATERIAL AND METHODS

The figures for normal hemoglobin pattern, sickle cell trait and hemoglobin C trait were drawn from previous studies of 100 normal soldiers picked at random and 159 consecutive pregnant women supplemented by a few cases of AS hemoglobin pattern picked from the hospital population. The figures for sickle cell disease were obtained from patients admitted to or attending the various clinics and hospitals of Accra, chiefly the Korle Bu Hospital.

Relatives of patients with sickle cell disease were studied but not included in the series. As far as was known, there are no relatives in the series except for 1 pair of twins with G-6-PD defect and SC hemoglobin and a pair of half-siblings with normal G-6-PD and SC hemoglobin.

Hemoglobin patterns were determined by paper electrophoresis of washed, laked red blood cells, even when the sickling test was negative. This method does not differentiate between hemoglobin S and D, so that among the cases described as having SS hemoglobin pattern, there may be an occasional case of SD hemoglobin. Starch block electrophoresis was used to examine the blood of nonsickling parents of cases with sickle cell disease.

G-6-PD was estimated by the technic described by Brewer et al. using glucose, sodium nitrite and methylene blue incubated with blood treated with heparin or ethylenediamine-tetraacetic acid. The test is interpreted in terms of G-6-PD although it is a measure of the methylene blue accelerated reduction of methemoglobin and may be influenced by other factors. When the per cent of methemoglobin remaining after incubation for 3 hours was 80-100, the defect was considered to be complete, and when the per cent of methemoglobin was 10-70, the defect was considered to be partial. It is well-known that a considerable fraction of those cases with the genotype of partial defect are not detected by this test, although it is one of the most sensitive of the methods available.

In several instances the parents of females with complete defect and sickle cell anemia were investigated and found to have sickle cell trait and G-6-PD defect.

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Table 1.—Partial and Complete Methemoglobin Reduction in Sickle Cell-Hemoglobin C Disease and Sickle Cell Anemia

<table>
<thead>
<tr>
<th></th>
<th>Sickle Cell-Hemoglobin C Disease</th>
<th>Sickle Cell Anemia</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Normal</td>
<td>Partial</td>
</tr>
<tr>
<td>Male</td>
<td>25</td>
<td>9</td>
</tr>
<tr>
<td>Female</td>
<td>10</td>
<td>8</td>
</tr>
<tr>
<td>Total</td>
<td>35</td>
<td>17</td>
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</tbody>
</table>

Table 2.—Incidence of Glucose-6-Phosphate Dehydrogenase Defect According to the Hemoglobin Pattern

<table>
<thead>
<tr>
<th>Hemoglobin Pattern</th>
<th>AA</th>
<th>AC</th>
<th>AS</th>
<th>Sth</th>
<th>SC</th>
<th>SS</th>
</tr>
</thead>
<tbody>
<tr>
<td>Male</td>
<td>16</td>
<td>2</td>
<td>8</td>
<td>0</td>
<td>12</td>
<td>25</td>
</tr>
<tr>
<td></td>
<td>109</td>
<td>11</td>
<td>21</td>
<td>4</td>
<td>37</td>
<td>57</td>
</tr>
<tr>
<td>Female</td>
<td>12</td>
<td>2</td>
<td>8</td>
<td>1</td>
<td>9</td>
<td>23</td>
</tr>
<tr>
<td></td>
<td>88</td>
<td>15</td>
<td>30</td>
<td>3</td>
<td>19</td>
<td>43</td>
</tr>
<tr>
<td>Total</td>
<td>28</td>
<td>4</td>
<td>14</td>
<td>1</td>
<td>21</td>
<td>48</td>
</tr>
</tbody>
</table>

RESULTS

The incidence of G-6-PD defect in cases of sickle cell disease can be calculated from the data in table 1. It is interesting to note the high proportion of males with partial methemoglobin reduction in sickle cell hemoglobin C disease, and an even higher proportion in sickle cell anemia. Males with partial methemoglobin reduction were not encountered among individuals with normal hemoglobin pattern or sickle cell trait.

The incidence of G-6-PD defect in the most frequently encountered hemoglobin patterns is given in table 2, which separates males from females and gives the number of individuals tested. The differences can be seen more easily in table 3 which gives the same data in per cent. The defect seems to occur more frequently in individuals with sickle cell trait than in persons with normal hemoglobin pattern, even more frequently in persons with sickle cell disease, and the rate is higher in sickle cell anemia than in sickle cell hemoglobin C disease.

Table 3 also shows that there is good agreement between our figures and those of Naylor et al. in each hemoglobin pattern except sickle cell anemia where our figures are much higher.

The number of cases of sickle cell thalassemia and hemoglobin C trait are too small for statistical analysis. However, using the chi-square test for the differences between normal hemoglobin pattern and sickle cell trait, sickle cell hemoglobin C disease and sickle cell anemia, the figures for probability of the results being due to chance are 0.05, 0.001 and 0.0001. Obviously, the difference between our results and those of Naylor et al. is not due to the size of the series and is probably the result of differences in the technics used.
Table 3.—Per Cent of Cases with Glucose-6-Phosphate Dehydrogenase Defect

<table>
<thead>
<tr>
<th></th>
<th>AA</th>
<th>AS</th>
<th>Sth</th>
<th>SC</th>
<th>SS</th>
</tr>
</thead>
<tbody>
<tr>
<td>Our series</td>
<td>14</td>
<td>28</td>
<td>14</td>
<td>37</td>
<td>48f</td>
</tr>
<tr>
<td>Naylor et al.</td>
<td>11</td>
<td>29</td>
<td>17</td>
<td>36</td>
<td>111</td>
</tr>
</tbody>
</table>

*Our series compared with results of Naylor et al.

G-6-PD activity estimated by per cent methemoglobin remaining after incubation with glucose, sodium nitrite and methylene blue.

G-6-PD activity measured by change in optical density reflecting production of NADP and expressed in units of enzyme activity per unit of red blood cells.

**DISCUSSION**

Many research workers have investigated the incidence of G-6-PD defect in the hemoglobinopathies.1,3,7,12 Discrepancies in results with the sickle cell trait may be due to sampling errors or population stratification.2 But the discrepancies with respect to sickle cell anemia are more probably due to differences in the techniques used for the estimation and calculation of enzyme activity.

Naylor et al. discarded their figures for AS hemoglobin pattern because, in their opinion, the selection of cases was biased. They disregarded their figures for SC hemoglobin pattern, presumably because the number of cases was small. They concluded that "the incidence of the enzyme defect in the erythrocytes of those with sickle cell anemia . . . does not differ from that of a similar Negro population having normal hemoglobin." Perhaps, their figures for AS and SC pattern are correct, whereas their figures for SS may underestimate the true incidence of the genotype for G-6-PD defect because of the high level of G-6-PD in hemolytic anemias.7,8 which can be explained by the high level of the enzyme in very young red blood cells.9,10

We have occasionally encountered a normal G-6-PD test in a patient with sickle cell crisis, which reverted to complete defect after an interval of several weeks, or on treatment with promazine.11 Allison et al.12 made no effort to determine the G-6-PD status of "cases of sickle cell disease and its genetic variants and thalassemia major because the high proportion of young cells in the peripheral blood increases the G-6-PD levels and makes classification difficult."

Naylor et al. used the method described by Zinkham13 to measure G-6-PD and this estimates the enzyme activity per unit of red blood cells. The method we have used makes no correction for the hematocrit and gives a much lower estimate of G-6-PD activity in cases of severe anemia. However the Brewer method is quite sound as there is a built in correction factor for samples with reduced hemoglobin levels. In such cases, less methemoglobin is formed, and it is the reduction of this methemoglobin that is measured. When the hematocrit level falls below 20 per cent it is customary to remove some plasma before performing the test as there would be an excess of the reagents. The correction factor used in the Zinkham method overcorrects for the anemia, and although it may give a true picture of the G-6-PD activity, it does not reflect the genotype of the individual.
Our observation that there is an increased incidence of G-6-PD defect in sickle cell trait may be the result of population stratification and further study of different population groups should be performed. The magnitude of the difference in incidence of G-6-PD defect between individuals with normal hemoglobin pattern and sickle cell disease cannot be explained by population stratification.

The high incidence of G-6-PD defect in sickle cell disease may be the result of a protective influence of the defect on the course of the disease. This explanation of the findings has important implications on the specific therapy of sickle cell disease and on the currently accepted theories of genetic polymorphism.

**SUMMARY**

Hemoglobin electrophoresis and methemoglobin reduction tests have been carried out on 438 individuals with various hemoglobin patterns. The incidence of the enzyme defect rises stepwise from AA pattern through AS, Sth and SC to SS being greatest in the latter. From the data presented it is not possible to decide what factors have produced this selection.

**SUMMARIO IN INTERLINGUA**

Electrophorése de hemoglobina e essayage de dehydrogenase de glucosa-6-phosphato esseva effectuate in 438 subjectos con varie configurationes de hemoglobina. Le incidentia de defectivitate de dehydrogenase de glucosa-6-phosphato monta passo a passo ab le configuration AA ad le configuration AS, Sth, SC, e SS, con cifras maxime in SS. Isto suggere que le defecto del enzyma protege contra le hasardos de morbo a cellulas falciforme.

**REFERENCES**

10. Marks, P. A.: Red cell glucose-6-phosphate and 6-phosphogluconate de-


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