Glucose-6-Phosphate Dehydrogenase Deficiency in Egypt:
With a Note on the Methemoglobin Reduction Test

By Ronald P. McCaffrey and Ammhed Y. Awny

Six hundred and fifty Egyptian males were screened for glucose-6-phosphate dehydrogenase (G6PD) deficiency. The ascorbate-cyanide test and methemoglobin reduction test were used. An incidence of 4.9 per cent G6PD deficiency was found, in contrast to the previously reported incidence of 26.4 per cent. Good agreement was found between the ascorbate-cyanide test and methemoglobin reduction test. Unreliable results were obtained when previously prepared air-dried reagent tubes (modified methemoglobin reduction test) were used.

Despite its location in a part of the world where many extensive and detailed surveys for red cell glucose-6-phosphate dehydrogenase (G6PD) deficiency have already been undertaken, information on G6PD deficiency in Egypt has, until recently, been unavailable. The first report on the incidence of this red cell defect among the Egyptians appeared in early 1966, following an earlier W.H.O. Report which listed, as "nonexistent," information on G6PD deficiency in Egypt.1 In this first study,2 500 male Egyptian Army recruits, representing a geographic cross section of Egypt, were screened for this enzyme deficiency, using the methemoglobin reduction test of Brewer.3 A group of 132 subjects, 26.4 per cent, were reported to be G6PD-deficient.

Because of the far-reaching implications of such a high incidence, and also because it was at variance with the clinical impression of medical practitioners working in Egypt with large numbers of patients who were given potentially hemolytic drugs, we began a second survey in an attempt to further document these findings.

Materials and Methods

Six hundred and fifty Egyptian males were screened. The subjects were randomly selected from prospective blood donors at the Ein Shams University Hospital, Cairo; and from surgical in-patients and out-patients with nonhematologic disease at the same hos-
Table 1.—Geographic Distribution of the Subjects Tested and Incidence of Deficiency from Each Area

<table>
<thead>
<tr>
<th>Area</th>
<th>Number of Subjects</th>
<th>Total Number Deficient</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cities</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Cairo</td>
<td>320</td>
<td></td>
</tr>
<tr>
<td>Alexandria</td>
<td>30</td>
<td>18 (5%)</td>
</tr>
<tr>
<td>Port Said</td>
<td>10</td>
<td></td>
</tr>
<tr>
<td>Lower Egypt</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Beheira</td>
<td>20</td>
<td></td>
</tr>
<tr>
<td>Minufiya</td>
<td>28</td>
<td>4 (5.7%)</td>
</tr>
<tr>
<td>Qalyubia</td>
<td>21</td>
<td></td>
</tr>
<tr>
<td>Upper Egypt</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Giza</td>
<td>10</td>
<td></td>
</tr>
<tr>
<td>Faiyum</td>
<td>20</td>
<td></td>
</tr>
<tr>
<td>Beni Suef</td>
<td>22</td>
<td>7 (4.7%)</td>
</tr>
<tr>
<td>Assiut</td>
<td>30</td>
<td></td>
</tr>
<tr>
<td>Shohag</td>
<td>8</td>
<td></td>
</tr>
<tr>
<td>Asswan</td>
<td>55</td>
<td></td>
</tr>
<tr>
<td>Unclassified</td>
<td>76</td>
<td>3 (3.9%)</td>
</tr>
<tr>
<td>Total</td>
<td>650</td>
<td>32 (4.9%)</td>
</tr>
</tbody>
</table>

Venous blood was collected in ACD solution with inosine added. All samples were stored at 4°C until tested, which was always within 5 days of collection.

Two screening tests were used: the ascorbate-cyanide test of Jacob and Jandl; and the methemoglobin reduction test of Brewer, both by the original and "modified" techniques. The modified technique involves the use of previously prepared air-dried reagent tubes containing methylene blue, sodium nitrite and dextrose; in place of "wet" reagent tubes prepared at the time of testing.

In addition to the screening tests, all bloods abnormal on the methemoglobin reduction test, and 50 of those normal on this screen, were assayed directly for red cell G6PD activity.

RESULTS

The geographic distribution of the subjects tested, and the incidence of deficient subjects from each area is illustrated in Table 1. The overall incidence of deficient subjects was 32 of the 650 tested, or 4.9 per cent. There was little variation on a regional basis.

Of the 650 people tested, 467 were screened simultaneously with both the ascorbate-cyanide test and the methemoglobin reduction test. In 108 subjects, only the ascorbate-cyanide test was done, and 75 subjects had the methemoglobin reduction test alone. There was excellent agreement between the

* The Nubian bloods were supplied through the courtesy of Dr. Badr, Genetics Section, National Research Center, Cairo, Egypt.
Table 2.—Comparison of Original Versus Modified Methemoglobin Reduction Tests

<table>
<thead>
<tr>
<th></th>
<th>Original “Wet” Method</th>
<th>Modified “Dry” Method</th>
</tr>
</thead>
<tbody>
<tr>
<td>Number of subjects</td>
<td>463</td>
<td>79</td>
</tr>
<tr>
<td>Number deficient (%)</td>
<td>20 (4.3%)</td>
<td>5 (6.3%)</td>
</tr>
<tr>
<td>Normal residual methemoglobin</td>
<td>4.2% ± 1.2%</td>
<td>5.5% ± 1%</td>
</tr>
<tr>
<td>Range</td>
<td>0–8%</td>
<td>0–25%</td>
</tr>
</tbody>
</table>

ascorbate-cyanide test and the methemoglobin reduction test. Of the 467 subjects who were screened by both methods, 21 were positive (G6PD-deficient) in both, with a 22nd subject positive in the Jacob-Jandl test alone.

Comparison was made between the original (wet) methemoglobin reduction test, and the later modified method involving the use of previously prepared air-dried reagents. The results are tabulated in Table 2. Of the 542 subjects screened by both wet and dry methods, 463 had residual methemoglobin levels quantitated according to the original wet procedure. Of these, 20 subjects were considered enzyme-deficient, with residual methemoglobin levels in excess of 70 per cent. These were confirmed by direct assay for G6PD. In the 443 other subjects in this group, the residual methemoglobin levels averaged 4.2 per cent ± 1.2 per cent, with a range of 0–8 per cent. Fifty of these had normal G6PD activity on direct assay. Using the evaporated dry reagent modification, a total of 79 people were screened. Five were positive, with residual methemoglobin levels in excess of 70 per cent, and low enzyme activity on direct assay. Of the remaining 74 subjects, the average residual methemoglobin level was 5.5 per cent ± 1 per cent, with a range of 0–25 per cent. Seven subjects from this group had values in excess of 8 per cent: direct assays of G6PD activity in these gave normal results. Five of the seven were retested using the original wet reagent technique. At this time, residual methemoglobin values were all below 8 per cent.

DISCUSSION

While we appear to be studying a group of Egyptian males comparable to that screened in the first Egyptian survey, and by the same techniques, our results are in marked conflict with those reported. Our overall incidence of approximately 5 per cent is in distinct contrast to the report of 26 per cent among the Army recruits, but is more consistent with the relative infrequency of clinical hemolytic reactions seen in Egypt. We are unable to explain the differences reported.

We found the ascorbate-cyanide procedure to be simple, convenient, easy to interpret and suitable for use in a population survey. There was excellent agreement between the results obtained with this screen and the methemoglobin reduction technique. The interpretation of the single blood which tested abnormal in the ascorbate-cyanide test but not in the methemoglobin reduction test remains open. Fairbanks and Fernandez have reported “false” positive results with the ascorbate-cyanide in a variety of disorders, including glutathione reductase deficiency, glutathione peroxidase deficiency, pyruvate kinase deficiency and several unstable hemoglobins.
Our unexpected findings on the variation between residual methemoglobin values obtained with the original wet reagent technique, compared to the modified air-dried reagent method, need to be emphasized and explored further. Here, using the modification of the previously prepared air-dried methylene blue-sodium nitrite-dextrose reagent tubes in place of wet reagents prepared at the time of use, seven subjects were identified with residual methemoglobin levels, in the range seen with intermediate Mediterranean deficiency, or with female heterozygotes.8 On retesting with freshly prepared wet reagents, and on direct enzyme assay, these subjects were found to be normal. The basis for this variability is uncertain. It may be related to inactivation of the methylene blue during drying or storage. Because of the experience described here, one must be cautious about assigning subjects to the intermediate deficient group, or to the heterozygous deficient group, unless freshly prepared reagents are used, and/or further studies are performed.

REFERENCES
Glucose-6-Phosphate Dehydrogenase Deficiency in Egypt: With a Note on the Methemoglobin Reduction Test

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