Hereditary Hemolytic Anemia Associated with Glucosephosphate Isomerase (GPI) Deficiency—a New Enzyme Defect of Human Erythrocytes

By MARJORIE A. BAUGHAN, WILLIAM N. VALENTINE, DONALD E. PAGLIA, PETER O. WAYS, ERNEST R. SIMONS AND QUIN B. DEMARSH

IN RECENT YEARS, specific inborn erythrocyte enzyme deficiencies have been implicated in the etiology of certain hereditary hemolytic anemias not characterized by spherocytosis. This report defines still another instance in which hereditary hemolytic anemia has been found to be associated with a deficiency in a specific glycolytic enzyme. The propositus has had a hemolytic syndrome present since birth. Erythrocytes and leukocytes both exhibit subnormal activity of the enzyme glucosephosphate isomerase (D-glucose-6-phosphate ketol-isomerase, EC 5.3.1.9). The hematologic, biochemical, and genetic studies of the patient and his family constitute the basis of this report.

MATERIALS AND METHODS

Routine hematologic studies were performed by standard methods. Erythrocyte and leukocyte glucosephosphate isomerase (GPI) were assayed by a modification of the method described by Chapman et al. The enzyme catalyzes the interconversion of glucose-6-phosphate (G-6-P) and fructose-6-phosphate (F-6-P). In the assay employed, F-6-P serves as substrate. G-6-P generated is converted to 6-phosphogluconate (6-P-G) by added crystalline glucose-6-phosphate dehydrogenase (G-6-PD) in the presence of nicotinamide-adenine dinucleotide phosphate (NADP). The conversion of the latter to its reduced form (NADPH) is followed spectrophotometrically at 340 μm and 37 C. for 10 min. following equilibration, employing a Beckman DU Spectrophotometer linked to a Gilford Multiple Sample Absorbance (A) Recording System. Correction for the...

From the Department of Medicine, University of California School of Medicine, Los Angeles, California; the Department of Medicine, University of Washington School of Medicine, Seattle, Washington; and the Department of Medicine, University of New Mexico School of Medicine, Albuquerque, New Mexico.

The study was supported by the U.S. Public Health Service Grant Nos. HE-01069 and HE-07326, AM-08131, and the Louis B. Mayer Foundation Fund for Medical Education and Research.

First submitted October 12, 1967; accepted for publication January 25, 1968.

MARJORIE A. BAUGHAN, M.D.: Assistant Professor of Medicine, Department of Medicine, University of California School of Medicine, Los Angeles, California. WILLIAM N. VALENTINE, M.D.: Professor and Chairman, Department of Medicine, University of California School of Medicine, Los Angeles, California. DONALD E. PAGLIA, M.D.: Postgraduate Research Hematologist, Department of Medicine, University of California School of Medicine, Los Angeles, California. PETER O. WAYS, M.D.: Assistant Professor of Medicine, Department of Medicine, University of Washington School of Medicine, Seattle, Washington. ERNEST R. SIMONS, M.D.: Associate Professor of Medicine, Department of Medicine, University of New Mexico School of Medicine, Albuquerque, New Mexico. QUIN B. DEMARSH, M.D.: Clinical Professor of Medicine, Department of Medicine, University of Washington School of Medicine, Seattle, Washington.

236 BLOOD, VOL. 32, NO. 2 (AUGUST), 1968
presence of known numbers of leukocytes in the erythrocyte suspensions and for the presence of known numbers of erythrocytes in the leukocyte suspensions is made in each assay on the basis of simultaneously performed erythrocyte and leukocyte assays. The system contains in a final volume of 3.02 ml. the following: glycylglycine buffer, 0.05 M, pH 8.1, 25 μmoles; F-6-P, 0.01 M, 5 μmoles; NADP 1.25 × 10⁻⁴ M, Sigma, 1 μmole; G-6-1PD, 1 enzyme unit, Sigma; and an hemolysate equivalent to approximately 3.0 × 10⁶ erythrocytes or to approximately 4.0 × 10⁴ leukocytes. Results are expressed as micromoles of NADPH generated per minute per 10¹⁰ erythrocytes or leukocytes (Fig. 1). The activities of erythrocyte hexokinase (HK), phosphofructokinase (PFK), fructose-phosphate aldolase, (FPA), glyceraldehydephosphate dehydrogenase (G-3-PD), triosephosphate isomerase (TPI), phosphoglycerate kinase (PGK), phosphoglyceromutase (PGM), phosphopyruvate hydratase, pyruvate kinase (PK), lactate dehydrogenase (LDH), G-6-PD, 6-P-GD, and glutathione reductase (GSSCR) were assayed as previously described. The activity of glutathione peroxidase (GSH-Px) was determined by a method developed in this laboratory. All the spectrophotometric assays were performed at 37 C., except for GSH-Px which was assayed at 25 C. Except for the enzyme activities found for glucosephosphate isomerase, summary tables showing the results of these erythrocyte enzyme assays have not been included since they have been published elsewhere.

A screening procedure for determining the combined activity of the terminal enzymes in the pentose phosphate pathway, i.e., ribosephosphate isomerase (RPI), ribulosephosphate 3-epimerase (RP-3-E), transketolase (TK), and transaldolase (TA), employed the quantitation of the amount of F-6-P produced following the incubation of a 1.0 ml mixture containing hemolysate (approximately 9.0 × 10⁸ RBC) and 10 μmoles of ribose-5-phosphate (R-5-P). After incubation for 150 min. at 37 C., the reaction was halted by placing the tubes in ice water. A 0.100 ml aliquot of the incubation mixture was then assayed enzymatically for F-6-P by converting this intermediate quantitatively to 6-P-G in the presence of a system containing in a final volume of 3.0 ml. the following: glycylglycine buffer, 0.05 M, pH 7.6, 124 μmoles; NADP, 0.524 μmoles; PCI, 0.010 ml., Sigma; and C-6-PD, 0.010 ml., Sigma. This multistep screening procedure depends upon the assumption that a deficiency of significant degree in any of the four enzymes mentioned above would be reflected in diminished generation of F-6-P. The absence of more than very small amounts of adenosine-5'-triphosphate (ATP), nicotinamide-adenine dinucleotide (NAD), and inorganic phosphorus (Pi) during the initial incubation employing hemolysates of washed blood cells essentially prevents further metabolism of either F-6-P or triose formed prior to the final assay of F-6-P. Although, admittedly, only a semiquantitative test, F-6-P production shows good linearity to concentration for at least 180 min. incubation under the conditions stated.

Glutathione (GSH) was determined as mg. per 100 ml. of packed cells collected in acid-citrate-dextrose (ACD) as described by Flanagan et al., and glutathione stability was measured as described by Beutler modified to employ the 5.5' dithiobis (2-nitrobenzoic
Table 1.—Hematologic Data Obtained at the Time of Enzyme Studies On the Patient and His Immediate Family

<table>
<thead>
<tr>
<th>Subject</th>
<th>Hemoglobin Gm./100 ml.</th>
<th>Red Cell Count $\times 10^6$ per mm.$^3$</th>
<th>Volume of Packed Cells %</th>
<th>Reticulocytes %</th>
<th>Mean Corpuscular Volume $\mu$m</th>
<th>Mean Corpuscular Hemoglobin g/m$^3$</th>
<th>Mean Corpuscular Hemoglobin Concentration %</th>
<th>White Cell Count $\times 10^9$ per mm.$^3$</th>
</tr>
</thead>
<tbody>
<tr>
<td>Patient</td>
<td>9.8</td>
<td>2.47</td>
<td>33.0</td>
<td>28.3</td>
<td>133.6</td>
<td>39.7</td>
<td>29.7</td>
<td>8.77</td>
</tr>
<tr>
<td>Father</td>
<td>14.3</td>
<td>4.71</td>
<td>44.0</td>
<td>1.9</td>
<td>93.4</td>
<td>30.4</td>
<td>32.5</td>
<td>5.21</td>
</tr>
<tr>
<td>Mother</td>
<td>12.9</td>
<td>4.26</td>
<td>41.5</td>
<td>0.6</td>
<td>97.4</td>
<td>30.3</td>
<td>30.2</td>
<td>4.54</td>
</tr>
<tr>
<td>Brother</td>
<td>13.6</td>
<td>4.83</td>
<td>42.0</td>
<td>1.8</td>
<td>86.9</td>
<td>28.2</td>
<td>32.4</td>
<td>4.92</td>
</tr>
</tbody>
</table>

acid) quantitation of glutathione according to Beutler et al.\textsuperscript{11} Heinz-body induction was quantitated as described by Beutler et al.\textsuperscript{12}

The methods for determining erythrocyte osmotic fragility of fresh and incubated cells, autohemolysis, glucose disappearance, lactate formation, recovery of $^{14}$CO$_2$ from isotopically labeled glucose with and without methylene blue stimulation, methemoglobin reduction, ATP, and adenosine-5'-diphosphate (ADP) have been previously described by Simon and Ways.\textsuperscript{13}

Red cell lipid analyses were done by previously described methods.\textsuperscript{14} The method employed in measuring red cell survival with radioactive sodium chromate ($\text{Na}_2\text{CrO}_4$) is described by Donohue et al.\textsuperscript{15}

Bovine serum albumin discontinuous-gradient fractionation of erythrocytes was done according to a modification of the method described by Bishop and Prentice,\textsuperscript{16} and by Leif and Vinograd.\textsuperscript{17}

CASE REPORT

J. M., a caucasian male of French and Irish descent, was born June 29, 1952, following an uneventful pregnancy. Birth weight was 7 lb., 3 oz. The patient, his father, and his mother were all Rh positive. There was no consanguinity in the family. Throughout the child's life there has been evidence of a chronic hemolytic process documented by numerous moderately to markedly elevated reticulocyte counts, increased total and indirect serum bilirubin, elevated stool urobilinogen, and absence of haptoglobin. In 1957, the defect was defined as intrinsic following studies in which normal $^{51}$chromium-labeled O negative cells were found to have a half-life of 39–44 days after injection into the patient, whereas the patient's cells labeled with $^{51}$chromium and injected into a normal subject had a half-life of only 4.5 days.

In the first seven months of life, hemoglobin values ranged between 4.7 and 6.8 Gm./100 ml. of whole blood, but increased to 9 Gm. in one instance following folic acid therapy given because of the development of a megaloblastic marrow. In February 1953, during an episode of gastroenteritis, the patient developed an anergic crisis with the hemoglobin decreasing to 2.1 Gm./100 ml. of whole blood. He was maintained with transfusions of whole blood until March 1953, age 9 months, when he underwent an uneventful splenectomy because of the persistent hemolysis, splenomegaly, and the suspected diagnosis of hereditary spherocytosis. A 90 Gm. spleen and a small accessory spleen were removed. Histologic examination showed no increase in reticuloendothelial or fibrous tissue, no deposition of hemosiderin in the pulp space, no increase in prominence of the sinusoids, and no extramedullary hematopoiesis or other criteria of hereditary spherocytosis.

Following splenectomy, the patient's hemoglobin values ranged between 8 and 10 Gm./100 ml. of whole blood, except during respiratory infections, tonsillitis, otitis media, and “flu.” During these episodes, the hemoglobin would decrease, sometimes to levels requiring transfusions.
In the last three to four years, the patient has participated actively in sports, including football, his only complaint being that he fatigues slightly more rapidly than friends his age. He has grown and developed normally, and the only abnormality which has been detected on physical examination is an apical systolic murmur which has been described variously as "soft" to "grade II/VI."

RESULTS

Hematologic Data

Routine hematologic data on the patient and his immediate family members are summarized in Table 1. Although the patient was splenectomized at 9 months of age, he has continued to show evidence of hemolysis. At the time of study, he displayed moderate macrocytic anemia with a hemoglobin of 9.8 Gm./100 ml. of whole blood, RBC of 2.47 million/cu.mm., a packed cell volume of 33.0 ml./100 ml. of whole blood, and a reticulocytosis ranging from 24 to 37 per cent. Peripheral blood smears have shown a predominance of macrocytes, moderate polychromatophilia and anisopoikilocytosis, numerous Pappenheimer bodies, Howell-Jolly bodies and cells with basophilic stippling, and a few burr-shaped cells, target cells, and nucleated erythrocytes. Spherocytes were noted rarely (Fig. 2). The antiglobulin test was negative, and no hemoglobin abnormality was evident on electrophoresis. Fetal hemoglobin was present in essentially normal amount.

The immediate family members had normal routine hematologic studies.

Osmotic Fragility Test

No deviation from normal was observed when erythrocyte osmotic fragility
Table 2.—Autohemolysis Studies on Red Cells of the Patient

<table>
<thead>
<tr>
<th>Compound</th>
<th>Final Concentration M</th>
<th>Normal %</th>
<th>2 S.D.* %</th>
<th>Patient %</th>
</tr>
</thead>
<tbody>
<tr>
<td>37 C.</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>None</td>
<td>—</td>
<td>3.50</td>
<td>2.70</td>
<td>7.9</td>
</tr>
<tr>
<td>Glucose</td>
<td>0.028</td>
<td>0.66</td>
<td>0.36</td>
<td>3.7</td>
</tr>
<tr>
<td>Inosine</td>
<td>0.009</td>
<td>0.66</td>
<td>0.38</td>
<td>2.4</td>
</tr>
<tr>
<td>22 C.</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>None</td>
<td>—</td>
<td>0.31</td>
<td>0.24</td>
<td>0.46</td>
</tr>
<tr>
<td>4 C.</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>None</td>
<td>—</td>
<td>0.24</td>
<td>0.20</td>
<td>0.46</td>
</tr>
</tbody>
</table>

*Standard deviation.

was determined on the patient’s fresh, defibrinated blood. After 24 hour incubation at 37 C., however, some increase in osmotic fragility was noted. The osmotic fragility was entirely normal in the heterozygous subjects studied.

Autohemolysis Test

The results are shown in Table 2. A moderately greater than normal degree of autohemolysis, only partially corrected by the addition of glucose and inosine, is demonstrated at 37 C. The pattern is most consistent with Type 1 autohemolysis as designated by Selwyn and Dacie.18 Autohemolysis of defibrinated blood was found to be within the normal range at 22 C. and 4 C.

Enzyme and Metabolic Studies

General. With the exception of GPI, which is discussed below, all erythrocyte enzymes assayed as described under Materials and Methods exhibited normal or elevated values in the blood cells of the propositus. Many had markedly increased activity in a pattern consistent with that characteristically found in assays of young cell populations in hemolytic anemias. In addition, F-6-P production from R-5-P, measured as described in Materials and Methods, was substantially above that found repeatedly in our laboratory when cells from normal blood were assayed. No enzyme deficiencies other than that of GPI were noted in either the Embden-Meyerhof or pentose phosphate pathway. In addition, GSSG-R activity was increased and GSH-Px activity was normal. The enzyme assays done on the blood from family members were essentially within normal limits.

Glucosephosphate Isomerase. A summary of both the erythrocyte and leukocyte assays for this enzyme is shown in Table 3. Determinations on four separate occasions and four separate samples of the patient’s blood showed a marked decrease in activity with a mean erythrocyte enzyme value of approximately 19 percent of normal, and a mean leukocyte enzyme value of approximately 23 per cent of normal, even in the presence of a mean reticulocyte count of 28.3 per cent. The father, mother, and brother had erythrocyte assay values of 6.6 units, 6.0 units, and 6.3 units, respectively. These approxi-
Table 3.—Glucosephosphate Isomerase Activity of Erythrocytes and Leukocytes of the Patient and Selected Family Members

<table>
<thead>
<tr>
<th>Subject</th>
<th>Enzyme Activity (μmoles of NADPH generated/min./10^6 cells)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Erythrocytes</td>
</tr>
<tr>
<td>Normal mean and 2 standard deviations</td>
<td>13.6 ± 1.9</td>
</tr>
<tr>
<td>Patient, III-3 (mean of 4 determinations)</td>
<td>2.61</td>
</tr>
<tr>
<td>Father, II-4</td>
<td>6.55</td>
</tr>
<tr>
<td>Mother, II-5</td>
<td>6.03</td>
</tr>
<tr>
<td>Brother, III-4</td>
<td>6.31</td>
</tr>
<tr>
<td>Paternal grandmother, I-11</td>
<td>12.57</td>
</tr>
<tr>
<td>Paternal aunt, II-2</td>
<td>7.30</td>
</tr>
<tr>
<td>Paternal uncle, II-3</td>
<td>15.38</td>
</tr>
<tr>
<td>Maternal aunt, II-7</td>
<td>15.04</td>
</tr>
<tr>
<td>Maternal aunt, II-9</td>
<td>14.42</td>
</tr>
<tr>
<td>Maternal uncle, II-10</td>
<td>16.58</td>
</tr>
<tr>
<td>Maternal uncle, II-12</td>
<td>5.27</td>
</tr>
<tr>
<td>Maternal uncle, II-14</td>
<td>13.63</td>
</tr>
<tr>
<td>Maternal aunt, II-17</td>
<td>13.36</td>
</tr>
<tr>
<td>Maternal 1st cousin, III-20</td>
<td>6.39</td>
</tr>
<tr>
<td>Maternal 1st cousin, III-21</td>
<td>6.11</td>
</tr>
</tbody>
</table>

mate 50 per cent of the normal adult mean value of 13.6 ± 1.9* units in our laboratory. All samples were assayed in duplicate or quadruplicate and showed similar activity. The data suggest that all the immediate family members are heterozygous for the abnormality. Leukocyte assays in the same subjects are all within the low normal range. It should be noted that two standard deviations from mean activity is much greater in the case of leukocytes than is true for erythrocytes. Thirty-five persons related directly to the patient and five persons related by marriage were also studied. An intermediate decrease in erythrocyte activity and low normal leukocyte activity similar to that observed in parents and sibling were noted in 4 family members; a paternal aunt, a maternal uncle, and 2 maternal first cousins. The remainder were all within normal limits. No measurable enzyme activity was demonstrated in the plasma of the patient, whereas plasma from normal subjects consistently exhibited measurable activity of this enzyme.

**Enzyme Ratios.** It has generally been found that certain enzymes show increased activities in young cell populations. HK, in particular, shows a marked increase in activity, whereas more moderate increases have been noted particularly in PK, G-6-PD, and fructosephosphate aldolase. Density gradient fractionation studies in our laboratory have also shown that GPI activity is increased very slightly in younger, less dense cells.

Ratios of GPI to HK, GPI to PK, and GPI to G-6-PD in terms of the enzyme units employed in our laboratory showed the following: 56.7, 4.9, and 4.6, respectively, for normal control blood; 18.5, 4.3, and 3.5, respectively, for reticulocyte-rich blood (10 cases of miscellaneous hemolytic anemias with a mean reticulocyte count of 26 per cent); and 2.7, 0.4, and 0.3, respectively, for the patient's blood (average of 4 determinations). The much lower GPI to HK

*Two standard deviations.
Table 4.—Carbon Labeled Glucose Radioisotope Studies of Red Cell Metabolism

<table>
<thead>
<tr>
<th>Glucose Catabolism</th>
<th>Normal Mean ± 2 S.D.</th>
<th>Patient</th>
<th>% of Normal</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>(% 14)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Glucose disappearance</td>
<td>2.20 ± 0.28</td>
<td>3.54</td>
<td>161</td>
</tr>
<tr>
<td>Lactate formation</td>
<td>3.60 ± 0.29</td>
<td>6.89</td>
<td>191</td>
</tr>
<tr>
<td>14C-1-O2 formation</td>
<td>0.092 ± 0.02</td>
<td>0.186</td>
<td>202</td>
</tr>
<tr>
<td>With methylene blue (MB)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Glucose disappearance</td>
<td>2.69 ± 0.35</td>
<td>4.45</td>
<td>165</td>
</tr>
<tr>
<td>Lactate formation</td>
<td>3.96 ± 0.30</td>
<td>7.49</td>
<td>189</td>
</tr>
<tr>
<td>14C-1-O2 formation</td>
<td>1.46 ± 0.23</td>
<td>1.26</td>
<td>86</td>
</tr>
<tr>
<td>14C-1-O2 formation</td>
<td>0.43 ± 0.12</td>
<td>0.043</td>
<td>10</td>
</tr>
</tbody>
</table>

*umoles/10^10 red cells/hour.
†Parentheses indicate number of normal specimens tested.
‡Standard deviation.
§Normal = 100 per cent.

ratios in reticulocyte-rich blood compared with that of normal controls indicates a disproportionately greater increase in HK activity in these cells compared with the increased activity of the other enzymes studied. The marked decrease in each ratio for the propositus, on the other hand, indicates extremely low GPI activity compared with the activities of the other enzymes measured, whether comparison is made to either normal or reticulocyte-rich blood. The decreased ratios clearly reflect the GPI deficiency in the cells of the propositus.

Erythrocyte Glycolysis. Glucose catabolism studies are shown in Table 4. In the cells of the propositus, total glucose consumption was 161 per cent and lactate production 191 per cent higher than the mean value for normal blood (expressed here as 100 per cent). Percentage changes similarly expressed are recorded for studies with methylene blue stimulation.

Carbon dioxide production resulting from the oxidation of carbon 1 of glucose in studies employing glucose-1-14C was found to be increased to twice that of cells from normal subjects. Under the same experimental conditions, stimulation of the pentose shunt by methylene blue, however, showed only an increased carbon dioxide production approximately equal or perhaps somewhat less than the usual normal increase under these conditions. In striking contrast to the increased oxidation of carbon 1 glucose, when glucose-2-14C was employed as substrate methylene, blue stimulation resulted in only 10 per cent of the amount of 14CO2 obtained from normal cells under the same circumstances.

Methemoglobin Reduction. The capacity of the cells of the propositus to reduce methemoglobin is compared with that of normal cells in the presence of glucose and inosine in Table 5. Normally, in the presence of methylene blue, methemoglobin was found to be reduced slightly more rapidly with inosine than with glucose as substrate under the conditions of the test. Glucose provides the NADPH necessary for methemoglobin-reductase activity when phosphorylated to G-6-P and oxidatively metabolized; inosine performs similarly but enters the oxidative pathway after conversion to F-6-P and isomerization to G-6-P. The presence of methylene blue enhances the recycling of NADPH to its oxidized form which is then again available for shunt-pathway
metabolism. In the case of the patient's cells, 23.7 μmoles of methemoglobin were reduced per hour in the presence of glucose plus methylene blue, compared with a mean normal value of 13.2 μmoles and a value of 13.2 μmoles in simultaneously assayed control cells. The order of activity was reversed with inosine as substrate. The patient's erythrocytes reduced only 14.7 μmoles of methemoglobin while the mean normal value was 15.3 μmoles, and simultaneously assayed control blood reduced 20.6 μmoles. In contrast to normal erythrocytes, in the patient's cells methemoglobin was more actively reduced in the presence of glucose than when inosine was substrate.

Inhibition Studies. Enzyme activities were determined in hemolysates prepared from varying amounts of erythrocytes and leukocytes, respectively, and were found to increase in proportion to the amount of hemolysate assayed. Known amounts of lysates of the patient's erythrocytes or leukocytes were also mixed with similar lysates prepared from normal erythrocytes or leukocytes and incubated for 2 hours at 37 C. The activities on assay in each case closely approximated the arithmatic sum of the individual activities. No inhibition was observed nor could any be demonstrated with mixtures of hemolysates of the patient's cells and commercially obtained GPI.

Glutathione Stability

Erythrocyte GSH was normal and was stable on incubation with acetyl-phenylhydrazine in a modification of the test devised by Beutler. In addition, Heinz-body formation when the patient's cells were incubated with acetyl-phenylhydrazine was not abnormal.

ATP and ADP Content

Levels of both ATP and ADP were increased in the reticulocyte-rich erythrocytes of the propositus. In micromoles per gram hemoglobin, the value for ATP was 6.14; for ADP, 1.02. Normal values for ATP are 4.04 ± 1.26 and for ADP, 0.57 ± 0.32.

Enzyme Kinetics

Erythrocyte and leukocyte GPI assays were performed using varying concentrations of F-6-P as substrate with the Michaelis-Menton constant determined from the resultant activity. The $K_m$ value for the leukocytes appeared nearly identical with that of 5 normal controls. The $K_m$ values for the erythrocytes,
however, appeared slightly higher than that of the 5 controls. Because of the precision limitations for this determination, no conclusions regarding kinetic abnormalities of the deficient enzyme were possible.

**Erythrocyte Stroma Lipid Studies**

In total lipid per cell, cholesterol per cell, lipid phosphorous per cell, phospholipid distribution, and total phospholipid fatty acid distribution, no significant differences from normal erythrocytes of comparable cell age were observed.

**Electrophoretic Studies**

Preliminary electrophoretic studies of the patient’s erythrocyte glucosephosphate isomerase indicate that the deficient enzyme had different electrophoretic properties than the wild type, and that each parent was of a different phenotype. These experiments, as well as a family study, will be published separately as part of a study on glucosephosphate-isomerase variants in red blood cells and white blood cells.

**Genetics**

Genetic studies are summarized in Figure 3. The only subject with clinical evidence of a hemolytic anemia is the propositus (III-3). Biochemically, there is also a marked deficiency in the erythrocyte and leukocyte glucosephosphate-isomerase activity. Evidence for an inherited defect is shown by the finding of half-normal or intermediate levels of erythrocyte glucosephosphate-isomerase activity in the brother of the propositus (III-4), as well as in his mother (II-5) and his father (II-4). In addition, similar levels were found in the paternal aunt (II-2), a maternal uncle (II-12), and in two maternal first cousins (III-20 and III-21), the offspring of the maternal uncle (II-12). A deceased brother of the patient died of a congenital cranial malformation in very early infancy without known evidence of a hemolytic anemia. The presence of a defective gene in the father and brother without clinical evidence of disease would tend to rule out an X-chromosome–linked transmission of the deficiency.

It is clear that about half-normal glucosephosphate-isomerase activity is present in each parent. It cannot be stated whether this reduction is due to the
same or variant genetic defects in the two parents. However, it appears that patient has inherited an abnormal gene from each parent resulting in a markedly decreased activity in glucosephosphate isomerase.

**DISCUSSION**

The propositus represents still another and previously undescribed instance in which hereditary hemolytic anemia is associated with an inborn error in red cell metabolism. The deficient enzyme, glucosephosphate isomerase, reversibly catalyses the second step in the Embden-Meyerhof glycolytic pathway, namely, the interconversion of glucose-6-phosphate and fructose-6-phosphate. The inborn error is substantiated by direct measurement of the markedly reduced activity of the enzyme involved in the propositus; the demonstration of partial deficiency in both parents, a sibling, and certain family members; the kinetic data employing isotope-labeled glucose; and in studies involving methemoglobin reduction in the presence of glucose and inosine. Morphologically, the anemia demonstrates a now familiar pattern: reticulocytosis, modest anisocytosis and poikilocytosis, and small numbers of acanthocyte-like forms. Genetically the pattern is also familiar. The homozygous propositus has clinically apparent disease; the heterozygous parents and relatives are phenotypically normal but biochemically detectable. The defective gene appears to reside on an autosome.

On sterile incubation of blood for 48 hours, an autohemolysis pattern like that categorized by Selwyn and Dacie at Type 1 is observed. Autohemolysis is modestly increased beyond normal and partly corrected by additives of glucose, inosine, and adenosine. In enzyme deficiency hemolytic anemias, the autohemolysis pattern varies with the point of metabolic impairment, but the precise explanation for the disparate patterns is not clear. In hemolytic disease associated with HK deficiency, and usually with G-6-PD deficiency, the pattern closely resembles that of the propositus. In PK deficiency the most prevalent pattern is that of severe autohemolysis corrected poorly or not at all by glucose or adenosine additives, but corrected by neutralized ATP. In TPI deficiency, autohemolysis is like that in hereditary spherocytosis and correctable by glucose and adenosine. However, it has been observed that even in the same enzymatic defect pattern, differences occur and appear to depend on the severity of the associated hemolytic anemia.

In the present case, leukocytes are also enzyme-deficient, a finding analogous to that in TPI deficiency, but unlike HK or PK deficiency. Likewise, plasma GPI, whose tissue origins are obscure, appears distinctly low. There is good evidence that in erythrocyte-TPI-deficiency, other tissues, such as muscle and those of the nervous system, are also deficient. A progressive neurologic disorder appears to be part of the characteristic syndrome, and a general body deficiency in the enzyme is distinctly possible. In the present case, a generalized deficiency, though unproved, may conceivably be present, but, if so, at the clinical level this has no obvious import on cells other than the erythrocyte. In the propositus, no clinical evidence of neurologic involvement, mental re-
tardation, muscle or leukocyte dysfunction is apparent. Tissues other than the erythrocytes, leukocytes, and plasma have thus far not been studied.

The data employing glucose isotopically labeled at either carbon 1 or carbon 2 conform to expectation for a young erythrocyte population with substantially impaired enzymatic activity at the GPI step in glycolysis. Despite any inhibition of HK by increased levels of G-6-P which presumably could exist in cells with significant GPI deficiency, net production of G-6-P must be greater than normal since over-all glucose consumption and lactate production are increased in the propositus. This is possible because the increased level of enzymatic activity in young cells, particularly reticulocytes, and particularly in rate-limiting hexokinase, assures a substantial supply of glycolytic intermediates even in the face of deficient, but not totally absent, glucosephosphate isomerase. It is evident from Table 4 that the reticulocyte-rich blood of the propositus can in the over-all metabolize substantially more glucose to lactate than do erythrocytes of normal blood. In the absence of methylene-blue stimulation, the percentage of total glucose shunted through the pentose phosphate pathway (i.e., as measured by $^{14}$CO$_2$ production of C-1 labeled glucose) was about the same as that measured in normal blood, 5 per cent and 4 per cent, respectively.

Thus, because of the more active metabolic machinery in very young erythrocytes, the remaining 95 per cent of the total glucose metabolized was able to traverse the point of partial blockade in the anaerobic pathway. With methylene-blue stimulation, the enzyme-deficient cells shunted about the same absolute amount of glucose through the pentose phosphate pathway, but the per cent of shunted glucose was only 30 per cent, as compared with 50 per cent in normal cells.

Production of $^{14}$CO$_2$ from carbon-2 labeled glucose in methylene-blue-stimulated cells reflects that proportion of glucose which traverses the oxidative shunt, re-enters the Embden-Meyerhof pathway via F-6-P, and is then recycled via G-6-P through the oxidative pathway. In the GPI-deficient cells, recycling was far below normal (Table 4), presumably because the competition for F-6-P between substantially deficient GPI and phosphofructokinase, the next enzymatic step in the anaerobic pathway, is greatly weighted in favor of the latter at the F-6-P intracellular concentrations pertaining in these experiments.

Further evidence for a block in recycling was provided by tests for methemoglobin reduction under the stimulation of methylene blue. When glucose was the substrate (Table 5), the GPI-deficient cells reduced methemoglobin by the shunt pathway at about twice the normal rate, reflecting more active glucose metabolism by the young cells of the propositus. With inosine as substrate, shunt activity was slightly greater in normal blood and considerably more active in the normal control subject than when glucose was substrate. In contrast, propositus cells exhibited markedly reduced shunt activity with inosine as substrate when comparison was made to the activity with glucose as substrate. Since inosine enters the shunt pathway as pentose phosphate, it must then enter the Embden-Meyerhof pathway as F-6-P and be isomerized to G-6-P before being cycled preferentially through the shunt pathway under
the stimulating effect of methylene blue. Since recycling is substantially blocked at the GPI step in the cells of the propositus, inosine is appreciably less effective than glucose in methemoglobin reduction.

Why then do not the metabolically impaired cells of the propositus survive normally? In the young cell population analyzed, there is substantial glycolysis, and ATP levels appear reasonably maintained. The answer is undoubtedly complex and at present unknown. First of all, it is imperative to recognize that the metabolic status of the cell population assayed applies only to the young cells and reticulocytes present at the moment of assessment. From such studies, the status of a cell 30 or 60 or more days in the circulation cannot be inferred. It is not possible to project the metabolic capacities of these young assayed cells to some hypothetically more mature state in their existence. Yet hemolytic anemia, by definition, exists when metabolic efficiency is not preserved to a degree compatible with normal erythrocyte survival. Certain facts suggest that a persisting efficient metabolism would not be expected in the case of the impaired cells of the propositus. As the cell matures, HK activity falls to a marked degree and, hence, is less capable of priming glycolysis with G-6-P. Perhaps also crucial, the reticulocyte possesses metabolic machinery not available to the mature erythrocyte which may render its use of the alternative oxidative shunt pathway more effective. It still possesses the mechanisms of oxidative phosphorylation whereby not only a means of generating ATP but also additional means of oxidizing NADPH are operative. The capacities of the shunt pathway are dependent on the concomitant mechanisms for recycling NADP and NADPH, and these pyridine nucleotide cofactors are present in very small amounts. The young cell, then, may survive for a time utilizing metabolic processes which will soon be largely lost, and deficiencies still compatible with survival and even effective function in youth may become crucial and fatal as the cell matures. The actual events preceding destruction may also relate to changing patterns of intermediate accumulation and their concomitant role in feedback inhibition. In any event, cell viability is clearly impaired in the GPI deficient erythrocytes and there seems little doubt that the enzyme defect is causally related to the chronic hemolytic anemia present since birth in the propositus.

Summary

A new congenital hemolytic anemia not characterized by spherocytosis has been defined as due to a deficiency in another glycolytic enzyme, glucosephosphate isomerase, the catalyst specific for the second step of the Embden-Meyerhof glycolytic pathway. The leukocytes and the plasma are involved as well as the erythrocytes, but there is no clinical evidence of dysfunction other than the hemolytic anemia. Family studies are consistent with an autosomal-recessive mode of inheritance with the asymptomatic heterozygotes demonstrating an intermediate enzyme deficiency, and the symptomatic homozygote, the propositus, demonstrating a marked enzyme deficiency.

SUMMARIO IN INTERLINGUA

Un nove congenite anemia hemolytic que non es caracterisate per spherocytosis es definite como efecto de un carentia in un altere enzyme glycolytic, i.e., in isomerase
glucoso-phosphatic, le catalysta specific pro le secunde passo in le circuito de Embden-Meyerhof. Le leucocytos e le plasma es afficite e etiam le erythrocytos, sed nulle evidentia clinic de dysfunctionamento es presente altere que le anemia hemolytic. Studios familial supporta le postulato de un modo autosomal-recessive de hereditamento, con asymptomatic heterozygotos demonstrante un carencia de enzyme intermediari e le symptomatic homozygotos, le probando, demonstrante un carencia de enzyme marcate.

ADDENDUM

Since the preparation of this manuscript, a second kindred has been studied in collaboration with Dr. Phillip Holland of the University of Kentucky Medical School. Three of seven children exhibited severe hemolytic anemia and were found to be homozygous for the same glucosephosphate-isomerase deficiency. Again, the deficiency was apparent in both the erythrocytes and leukocytes. The results of this family study will be included in a subsequent report.

ACKNOWLEDGMENTS

We are indebted to Mrs. J. Wittenberg, Miss M. L. Wilson, Mrs. T. Gabor, Mr. K. Kürschner, Miss M. Mattson, and Miss L. Miller for their technologic assistance.

REFERENCES


Hereditary Hemolytic Anemia Associated with Glucosephosphate Isomerase (GPI) Deficiency— a New Enzyme Defect of Human Erythrocytes

MARJORIE A. BAUGHAN, WILLIAM N. VALENTINE, DONALD E. PAGLIA, PETER O. WAYS, ERNEST R. SIMONS and QUIN B. DEMARSH

Updated information and services can be found at:
http://www.bloodjournal.org/content/32/2/236.full.html

Articles on similar topics can be found in the following Blood collections

Information about reproducing this article in parts or in its entirety may be found online at:
http://www.bloodjournal.org/site/misc/rights.xhtml#repub_requests

Information about ordering reprints may be found online at:
http://www.bloodjournal.org/site/misc/rights.xhtml#reprints

Information about subscriptions and ASH membership may be found online at:
http://www.bloodjournal.org/site/subscriptions/index.xhtml