CLINICAL PROFILE

Hereditary Hemolytic Disorders and Enzymatic Deficiencies of Human Erythrocytes

By Ernst R. Jaffe

The internist or pediatrician occasionally, and the hematologist not infrequently, is confronted with a patient with a long-standing hemolytic disorder. Since rational therapy depends upon defining the underlying abnormality, making an etiologic diagnosis is more than just an academic exercise. After excluding such diverse etiologies as iso- and autoimmune disorders, hemolysis associated with hematologic and nonhematologic diseases, hemolysis due to infectious, physical, chemical or toxic agents, hereditary hemoglobinopathies and unstable hemoglobins, thalassemic syndromes, hereditary spherocytosis and elliptocytosis, paroxysmal nocturnal hemoglobinuria, and erythropoietic porphyria, a significant number of patients remain without a diagnosis. These patients are characterized by having a nonspherocytic hemolytic disorder in which large numbers of spherocytes are not detected in the peripheral blood, either upon examination of a stained smear or upon determination of the osmotic fragility of the erythrocytes both before and after incubation of whole blood for 24 hr. Initially, this heterogeneous group of patients had to be classified on the basis of the results obtained in the autohemolysis test of Selwyn and Dacie. More recently, many of these patients have been found to have erythrocytes with deficiencies of specific enzymes whose activities are involved in the essential metabolic processes of these cells. A few appear to have abnormalities in the membrane of the erythrocyte, the component which, until recently, has been investigated the least.

This review will attempt to summarize the clinical and biochemical abnormalities which have been demonstrated in the 14 enzyme deficiencies which have been reported to be associated with hemolytic disorders. Although a few general principles have become apparent, it must be recognized that much of the diagnostic evaluation still involves the exclusion of other etiologies.

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The investigations in the author’s laboratory have been supported by USPHS Grants HE-10041 and AM-13698.
First submitted September 17, 1969; accepted for publication October 15, 1969.

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Fig. 1.—Pathways by which glucose is metabolized in human erythrocytes. Reactions enclosed in shaded area comprise the hexosemonophosphate shunt pathway. Interrupted lines indicate omission of several reactions. Bold bars indicate enzymatic deficiencies whose association with hereditary hemolytic disorders is well established, lighter bars indicate enzymatic deficiencies which have been reported to be associated with hemolytic disorders.

RELATIONSHIP OF METABOLIC ACTIVITY TO FUNCTION IN THE HUMAN ERYTHROCYTE*

The mature human erythrocyte is limited to glycolysis as the only significant source of the energy required for its functions (Fig. 1). For an expenditure of two molecules of adenosine triphosphate (ATP) per molecule of glucose utilized, the erythrocyte can generate four, a potential net gain of two. The full potential is rarely if ever attained because of the alternative metabolic pathways which are discussed below. The ATP generated can be used for cation transport by the ATPase system which appears to be required to

maintain the high intracellular potassium and low sodium contents characteristic of human erythrocytes against concentration gradients and passive leaks. ATP can also be utilized for the resynthesis of purine nucleotides from adenine by the salvage pathway, and for the synthesis of pyridine nucleotides from small molecule precursors. Human erythrocytes have a striking and perhaps unique capacity among the erythrocytes of mammals to synthesize large amounts of nicotinamide adenine dinucleotide (NAD or DPN) and, less certainly, nicotinamide adenine dinucleotide phosphate (NADP or TPN). Purine and pyridine nucleotides, of course, are essential cofactors for glycolysis. Although synthesis de novo of the lipid components of erythrocyte membranes probably does not occur in the mature cell, there is evidence for the exchange of cholesterol, phospholipids, and the fatty acids of the phospholipids between the plasma and the membrane. These processes also appear to require metabolic activity, and the exchange of fatty acids has been shown to require ATP. Recent studies have emphasized the relationship between oxygen transport and the concentration of the organic phosphate ester, 2,3-diphosphoglycerate (2,3-DPG). The binding of 2,3-DPG to deoxyhemoglobin changes the configuration of the molecule and decreases its affinity for oxygen. Increased concentrations of 2,3-DPG may, therefore, make oxygen more readily available for release to tissues at low oxygen tensions. The 2,3-DPG or Rapoport-Luebering cycle provides the mechanism for the generation of 2,3-DPG, the organic phosphate compound present in the highest concentration in human erythrocytes. This cycle also permits a bypass of an ATP-generating step and may, thereby, regulate the production of ATP. Metabolic activity is also involved in maintaining hemoglobin in a state suitable for the reversible binding of oxygen, a process which requires that the iron of the heme moiety be in the ferrous state. Reduced nicotinamide adenine dinucleotide (NADH or DPNH) is required for the reduction of any methemoglobin (ferrihemoglobin), which is formed to (ferro)hemoglobin. The shunting of the metabolites of glucose into 2,3-DPG may also serve to conserve DPNH required for this process. Finally, it has been presumed that metabolic activity is required to maintain the organization, shape, and structure of the erythrocyte. How these physical properties are dependent upon metabolic activity, however, remains unknown.

Since the mature erythrocyte does not possess an intact tricarboxylic acid cycle, lactate is the normal end product of the Embden-Meyerhof (E–M) pathway of glycolysis, and pyruvate cannot be shunted off. In addition to the direct pathway from glucose to lactate, the erythrocyte possesses an active hexosemonophosphate (HMP) or pentose phosphate shunt through which normally about five to ten per cent of the glucose utilized passes before re-entering the mainstream of glycolysis. This shunt is the only source for the generation of reduced nicotinamide adenine dinucleotide phosphate (NADPH or TPNH), the preferred cofactor for the reduction of oxidized glutathione (GSSG) to reduced glutathione (GSH). There may be other requirements for TPNH in mature erythrocytes, but they are unknown at present. Although its precise function also is still unknown, the human erythrocyte contains about half as many molecules of GSH as of hemoglobin. HMP shunt activity
is required to maintain the intracellular concentration of this sulfhydryl-containing tripeptide which may be involved in protecting hemoglobin against irreversible oxidative denaturation, guarding membrane lipids against peroxidation, and shielding essential enzymes against inactivation.

In contrast to the mature erythrocyte, the immature cell or reticulocyte is capable of much more metabolic activity. Reticulocytes can synthesize ribonucleic acids, proteins, lipids, heme and purine nucleotides, can perform oxidative phosphorylation with an intact cytochrome system and tricarboxylic acid cycle, and may be able to utilize amino acids and fatty acids as energy sources. The concentrations of ATP and, probably, other organic phosphate esters such as 2,3-DPG, are higher and the activities of several, but not all, enzymes are considerably greater in these young cells. These fundamental differences between mature and immature erythrocytes contribute to the difficulty often encountered in evaluating deficiencies in enzymatic activity in erythrocytes obtained from patients with hemolytic disorders where the number of reticulocytes is characteristically elevated.

With a relatively limited metabolic apparatus, the normal mature human erythrocyte can support all of its essential functions for about 120 days. This limited capacity, however, makes it not too surprising that the survival of erythrocytes deficient in the activity of any one of the enzymes involved is jeopardized. The greater metabolic potential of the reticulocytes probably makes it possible for the young cells to survive at least long enough to prevent the patient’s death.

**Abnormalities Associated with the Embden-Meyerhof Pathway of Glycolysis**

The significant hematologic characteristics and biochemical abnormalities which have been described in hemolytic disorders associated with deficiencies in the activities of enzymes, in or related to the E-M pathway, are summarized in Table 1.

*Hexokinase (HK)*

This enzyme catalyzes the initial reaction in the utilization of glucose, has the lowest activity of all of the glycolytic enzymes of human erythrocytes, and its activity is extremely age dependent with a pronounced decline as the cell ages. An 11-year-old girl of English and Polish extraction with a congenital hemolytic anemia was found to have erythrocytes with markedly reduced HK activity, especially when the activity was related to that in red cells from blood samples with comparable numbers of reticulocytes. Somewhat decreased HK activity was noted in the erythrocytes of her asymptomatic parents and two siblings, but one brother without evidence of a hemolytic disorder had erythrocytes whose HK activity was about 50 per cent of that of the patient’s cells. Despite this puzzling observation, an autosomal recessive mode of inheritance was postulated and the patient was considered to be a homozygote.

The decreased utilization of glucose and the decreased concentration of ATP, when related to the level of reticulocytes, are consistent with the metabolic block. An attempt to compensate for this handicap may be reflected
Table 1.—Abnormalities Associated with Deficiencies in Enzymes Related to Embden-Meyerhof Pathway

<table>
<thead>
<tr>
<th>Enzyme</th>
<th>No. of Cases</th>
<th>Severity of Anemia</th>
<th>Retic. Per Cent</th>
<th>Autohemolysis</th>
<th>Enzyme Per Cent of Normal RBC</th>
<th>Enzyme Activity</th>
<th>Enzyme Kinetics</th>
<th>Glucose Utilization</th>
<th>ATP of RBC</th>
<th>Electrophoresis</th>
<th>Mode of Inheritance</th>
<th>References</th>
</tr>
</thead>
<tbody>
<tr>
<td>HK</td>
<td>1*</td>
<td>Moderate</td>
<td>7-24</td>
<td>I</td>
<td>14R,62M</td>
<td>N</td>
<td>N</td>
<td>↓</td>
<td>↓R,NM</td>
<td>N</td>
<td>AR</td>
<td>5</td>
</tr>
<tr>
<td></td>
<td>1</td>
<td>Mild</td>
<td>4-13</td>
<td>N</td>
<td>50</td>
<td>N</td>
<td>A</td>
<td>↓</td>
<td>↓M</td>
<td>N</td>
<td>AR</td>
<td>6</td>
</tr>
<tr>
<td></td>
<td>1</td>
<td>Severe</td>
<td>5-7</td>
<td>I</td>
<td>←N</td>
<td>←N</td>
<td>A</td>
<td>N</td>
<td>↑</td>
<td>A</td>
<td></td>
<td>7</td>
</tr>
<tr>
<td>PHI</td>
<td>1*</td>
<td>Moderate</td>
<td>24-37</td>
<td>I</td>
<td>19</td>
<td>23</td>
<td>N</td>
<td>↑</td>
<td>↑M</td>
<td>A</td>
<td>AR</td>
<td>8</td>
</tr>
<tr>
<td></td>
<td>3</td>
<td>Moderate</td>
<td>12-82</td>
<td>I</td>
<td>14-30</td>
<td>5</td>
<td>N</td>
<td>←</td>
<td>←</td>
<td>A</td>
<td>AR</td>
<td>9</td>
</tr>
<tr>
<td></td>
<td>3</td>
<td>Very mild</td>
<td>4-6</td>
<td></td>
<td>30-48</td>
<td>←</td>
<td>←</td>
<td>←</td>
<td>←</td>
<td>←</td>
<td></td>
<td>10</td>
</tr>
<tr>
<td>PFK</td>
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<td>Very mild</td>
<td>3-4</td>
<td></td>
<td></td>
<td>50</td>
<td>N</td>
<td>←</td>
<td>←</td>
<td>←</td>
<td>AR?</td>
<td>11</td>
</tr>
<tr>
<td></td>
<td>1</td>
<td>Very mild</td>
<td>10</td>
<td>N</td>
<td>60</td>
<td>N</td>
<td>A</td>
<td>NR</td>
<td>↓R,M</td>
<td>X</td>
<td></td>
<td>12</td>
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<tr>
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<td>5-20</td>
<td>HS</td>
<td>38-82</td>
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<td>↑,?↓</td>
<td>↑</td>
<td>N</td>
<td>AD</td>
<td></td>
<td>13</td>
</tr>
<tr>
<td>TPI</td>
<td>9</td>
<td>Severe</td>
<td>10-33</td>
<td>HS</td>
<td>6-14</td>
<td>9-14</td>
<td>N</td>
<td>↑</td>
<td>N,↓</td>
<td>A</td>
<td>AR</td>
<td>14,15</td>
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<tr>
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<td>2*</td>
<td>Moderate</td>
<td>3-7</td>
<td>I</td>
<td>←</td>
<td>←</td>
<td>←</td>
<td>↓</td>
<td>↓</td>
<td>←</td>
<td>AD</td>
<td>17</td>
</tr>
<tr>
<td></td>
<td>6</td>
<td>Moderate</td>
<td>2-7</td>
<td>←</td>
<td>←</td>
<td>←</td>
<td>←</td>
<td>←</td>
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<td>←</td>
<td>AD</td>
<td>18</td>
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<tr>
<td>PGK</td>
<td>1*</td>
<td>Moderate</td>
<td>6-11</td>
<td>II</td>
<td>80</td>
<td>N</td>
<td>←</td>
<td>←</td>
<td>↓R</td>
<td>↓N,↑</td>
<td>?</td>
<td>20</td>
</tr>
<tr>
<td></td>
<td>7</td>
<td>Severe</td>
<td>10-25</td>
<td>I</td>
<td>&lt; 5</td>
<td>5-6</td>
<td>↓R</td>
<td>↓R</td>
<td>↓</td>
<td>X</td>
<td></td>
<td>21</td>
</tr>
<tr>
<td>PK</td>
<td>&gt;100</td>
<td>Mild to severe</td>
<td>5-80</td>
<td>II,I</td>
<td>5-20</td>
<td>N</td>
<td>N,A</td>
<td>↓R</td>
<td>↓R</td>
<td>↓N,↑</td>
<td>N,A?</td>
<td>AR 22-24</td>
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<tr>
<td>ATPase</td>
<td>3</td>
<td>Moderate</td>
<td>2-4</td>
<td></td>
<td>44-63</td>
<td>←</td>
<td>←</td>
<td>←</td>
<td>←</td>
<td>←</td>
<td>AD?</td>
<td>26</td>
</tr>
<tr>
<td></td>
<td>1</td>
<td>Moderate</td>
<td>1</td>
<td></td>
<td>50</td>
<td>N</td>
<td>←</td>
<td>←</td>
<td>←</td>
<td>←</td>
<td>?</td>
<td>27</td>
</tr>
</tbody>
</table>

*All subjects postplenectomy when studies performed.

Abbreviations: For enzyme names, see text. RBC = erythrocytes; WBC = leukocytes; Autohemolysis: I = increased, partly corrected by glucose, II = increased, corrected by ATP and/or not by glucose, HS = pattern seen in hereditary spheroerythrocytosis; N = normal; R = when related to reticulocyte-rich samples; M = when related to mature erythrocytes; A = abnormal; AR = autosomal recessive; AD = autosomal dominant; X = sex linked.
in the increased percentage of glucose which is metabolized via the HMP shunt.

Recently, hexokinases with decreased abilities to utilize glucose at extremely low concentrations have been described in the erythrocytes of two unrelated patients. A 50 per cent decrease in the concentration of glucose-6-phosphate (G-6-P) and 2,3-DPG was noted in one patient's erythrocytes, but a similar decrease in the level of G-6-P was also demonstrated in erythrocytes of asymptomatic relatives. The activity of HK from both erythrocytes and leukocytes of the other patient declined rapidly with decreasing glucose concentrations, although it was nearly normal at conventional levels. The band corresponding to type-III HK was absent on electrophoresis. The mechanism for hemolysis in these patients has not been defined. Presumably, the loss of HK activity normally associated with aging of the erythrocyte may impose a major handicap on the abnormal cells which are then rapidly removed from the circulation, leaving only those with demonstrable activity available for biopsy by the syringe. Erythrocytes possessing a HK with aberrant kinetics also may be more susceptible to premature destruction in organs such as the spleen where the blood glucose concentration may be extremely low.

Phosphohexose Isomerase (PHI)

One infant male of French and Irish descent with moderately severe hemolytic anemia, and two boys and one girl from an unrelated Anglo-Saxon family with more severe hemolysis were found to have erythrocytes and leukocytes markedly deficient in PHI activity. The deficiency was particularly striking when PHI activity of erythrocytes was related to that of other enzymes whose activities would be expected to be increased because of the marked reticulocytosis. Despite the low leukocyte and plasma PHI activities, these children did not have an increased susceptibility to infection, and neurologic or developmental abnormalities were not noted. The electrophoretic migration of the PHI of the erythrocytes from both kindreds was abnormal, but the alterations were different in the two families.

Erythrocytes deficient in PHI activity may be able to bypass the metabolic block by utilizing the HMP shunt. Their ability to recycle the metabolites from this shunt through fructose-6-phosphate (F-6-P) back to G-6-P, however, is impaired. The relatively normal concentration of ATP and the increased glucose utilization may reflect the compensatory mechanisms available to the immature erythrocyte.

Phosphofructokinase (PFK)

Initially, PFK deficiency was detected in Japanese and Jewish kindreds with Type-VII glycogen storage disease and probable myoglobinuria. Although the deficiency in muscle was extremely severe, PFK activity in the erythrocytes was reduced by only 50 per cent with evidence of a mild, compensated hemolytic disorder. From the results of immunological studies, the conclusion was drawn that the decrease in PFK activity in the erythrocytes was due to a deficiency in the muscle type of PFK and that there was genetic heterogeneity of the PFK protein in red cells.
Recently, a young adult male with a compensated hemolytic process, but without evidence of a muscle disorder, and a 40 per cent decrease in PFK activity in the erythrocytes, was described. A similar decrease in PFK activity was also noted in the red cells of his mother and maternal grandmother, and it is conceivable that this deficiency may be linked to the X chromosome. Although enzyme kinetics of the PFK were similar to those of the normal, the pH optimum curve was altered, the enzyme was extremely sensitive to ATP inhibition and it was unusually unstable upon storage at 4°C. An abnormal muscle type of PFK in the erythrocytes was postulated.\textsuperscript{12}

\textit{Aldolase (Ald)}\textsuperscript{13}

Despite many decades and numerous investigations, the basic defect in hereditary spherocytosis has defied definition. Disagreement persists between the advocates of the thesis that the disorder is the consequence of an excessive cation leak, and those who consider that losses of pieces of the erythrocyte membrane are responsible. Recently, the activity of Ald has been reported to be 38–82 per cent of the predicted value in erythrocytes of patients with typical hereditary spherocytosis. Thus, it is postulated that the disease occurs in subjects heterozygous for a deficiency in Ald activity. Since the description of Ald deficiency involves several mathematical and biological assumptions, confirmation of this observation is needed.

\textit{Triosephosphate Isomerase (TPI)}\textsuperscript{14,16}

This severely incapacitating disorder, first described in children of French Louisiana and Negro origins, has subsequently been observed in individuals of Anglo-Saxon extraction. At least nine patients are now known, and all but one has died before the age of six with a progressive neuromuscular disorder. A 21-year-old woman with TPI deficiency, discovered recently, had been labeled as an atypical case of cerebral palsy since the age of seven months. All patients appeared to develop normally, except for evidence of hemolysis, during the first six to seven months of life, and then experienced progressive neuromuscular impairment with initial generalized spasticity, and subsequent weakness and muscle atrophy. The sudden deaths, often while recovering from one of the frequent infections, may have reflected cardiac involvement.

Deficient TPI activity has been documented in erythrocytes, leukocytes, skeletal muscle, serum, and spinal fluid of affected subjects. The occurrence of such a widespread deficiency has led to speculation about the effect of the metabolic abnormality on the functions of all tissues. TPI activity is required to convert half of each molecule of glucose metabolized from dihydroxyacetone phosphate (DHAP) to glyceraldehyde-3-phosphate (G-3-P). The latter compound is metabolized further to lactate, but DHAP ordinarily has no other place to go in the mature erythrocyte which lacks significant \(\alpha\)-glycerophosphate dehydrogenase activity to convert DHAP to \(\alpha\)-glycerophosphate, an essential substrate for the synthesis of lipids. Striking accumulations of DHAP and, to a slight degree, of fructose-1,6-diphosphate (F-1,6-diP) have been observed in erythrocytes deficient in TPI activity. DHAP, directly or indirectly, may be toxic to erythrocytes and, conceivably, to other cells as well.
Utilization of glucose by erythrocytes deficient in TPI activity is increased even more than expected for the level of reticulocytosis. While HMP shunt activity may be normal for the number of reticulocytes, the ability of the shunt to increase its activity upon stimulation is limited. Whether or not this failure to respond to stimulation is related to the increased tendency for erythrocytes with decreased TPI activity to accumulate Heinz bodies upon prolonged incubation with oxidative stress is unknown. Increased HMP shunt activity, however, may help to maintain a normal or near-normal ATP concentration.

2,3-Diphosphoglycerate Mutase (2,3-DPG mutase)\textsuperscript{17-19}

Hemolytic disorders attributed to deficient 2,3-DPG mutase activity were described in German, French, and English subjects. Decreased concentrations of 2,3-DPG, inability to regenerate 2,3-DPG after depletion in vitro, increased content of F-1,6-diP, and slightly reduced concentrations of ATP in the erythrocytes were indirect evidences for this defect.

A German male infant with severe hemolytic anemia without marked reticulocytosis expired at age three months, having required repeated blood transfusions with evidence of accelerated destruction of donor cells. The activity of 2,3-DPG mutase in the erythrocytes of his unaffected parents who were first cousins, one sister, and a paternal grandmother, was 55-60 per cent of normal. The propositus was assumed to have had severe 2,3-DPG mutase deficiency. The atypical features of the hemolysis in this patient may invalidate the conclusion about cause and effect. The disorder was considered to have been inherited as an autosomal recessive characteristic.\textsuperscript{19}

Phosphoglycerate Kinase (PGK)\textsuperscript{20,21}

A moderate deficiency in PGK activity, especially in the older erythrocytes, was considered to be the cause of a life-long hemolytic disorder in a Caucasian woman. Unfortunately, family studies and additional investigations have not been possible.\textsuperscript{29}

A more severe hemolytic disorder associated with a deficiency in the activity of PGK in both erythrocytes and leukocytes was demonstrated in a large Chinese kindred. Two young males were most affected and had, in addition to hemolysis, a neurological disorder with abnormal behavior, emotional lability, and impaired speech. Five male relatives in the maternal portion of the family tree who died in early childhood may have had the same disorder. The family history and the results of biochemical investigations were interpreted as indicating that mosaicism for PGK activity may be present in the erythrocytes of the affected women with mild or no hemolysis, and that the affected males were hemizygotes.\textsuperscript{21}

Deficiency in PGK activity would be expected to impose a severe metabolic handicap because this enzyme catalyzes one of the two ATP-generating steps. Although the 2,3-DPG cycle provides a bypass to this metabolic block, metabolism of glucose via this cycle will result in no net gain in ATP. An increased concentration of 2,3-DPG is present in the erythrocytes of the one male patient studied most extensively. Reticulocytes may be able to survive because
of their additional metabolic pathways. Generalized manifestations of PGK deficiency may be reflected in the neurologic syndrome since, as in TPI deficiency, the abnormality is shared by the leukocytes.

*Pyruvate Kinase (PK)*\(^{22-25}\)

Hereditary hemolytic anemia associated with a deficiency in the activity of PK probably is second in frequency to G-6-P dehydrogenase deficiency, and has been the subject of extensive investigations and numerous publications. Considerable heterogeneity in both clinical severity and, more recently, in the biochemical abnormality, has been described. The severity of the anemia appears to have improved in most patients after splenectomy and the degree of reticulocytosis also has been higher after removal of the spleen. Although the disease is most common in people of Northern European origin, it has been found in Italians and Japanese, a Mexican child, an American Negro, and a Syrian girl.

Characteristically, the concentrations of 2,3-DPG, phosphoenol pyruvate (PEP), 3-phosphoglycerate (3-PG), and even G-6-P have been elevated in erythrocytes deficient in PK activity, while the ATP, pyruvate and lactate contents, and the utilization of glucose have been decreased. Anomalous results, however, have been obtained in studies where the kinetics of the PK have been so abnormal that deficient activity could be demonstrated only at very low concentrations of PEP in the assay system.\(^{24}\) Decreased HMP shunt activity has been noted and may reflect the generalized decrease in utilization of glucose. The normal or even elevated concentrations of ATP which have been reported in erythrocytes deficient in PK activity may reflect the utilization of alternative metabolic pathways by the immature erythrocytes.\(^{25}\)

*Adenosine Triphosphatase (ATPase)*\(^{26,27}\)

Three Danish and one French patient with mild to moderate hemolytic disorders, but without marked reticulocytosis, have been reported in which the hemolysis was attributed to a decrease of about 50 per cent in the activity of ATPase. Insufficient data are available to permit conclusions about the mode of inheritance, and no other results of biochemical studies have been described. Recently, decreased activities of ATPase and elevated sodium concentrations have been demonstrated in the erythrocytes of many members of a large family in which the abnormality appears to have been inherited as a recessive characteristic. Hemolysis, however, is not apparent in the affected individuals (Welt, L., personal communication). The relationship between decreased ATPase activity and hemolysis, therefore, must be reexamined.

If ATPase activity is involved in maintaining the osmotic equilibrium of erythrocytes, increased osmotic fragility of the erythrocytes might be expected to resemble the pattern observed in hereditary spherocytosis. Decreased osmotic fragility, however, was noted in the erythrocytes of the patient reported from France.\(^{27}\)

**General Comment**

Considerable variability in the severity of the hemolysis associated with
deficiencies of enzymes related to the E–M pathway is obvious. Most patients, however, have been noted to have jaundice, splenomegaly, and mild to marked reticulocytosis, especially after splenectomy. Increased need for blood transfusions in association with infections has been noted, especially in patients with HK, PHI, TPI, and PGK deficiencies. While it has not been as curative of the anemia as in hereditary spherocytosis, splenectomy appears to have ameliorated the anemia in many patients, especially in those with PK deficiency and, perhaps, in those with HK, PHI, 2,3-DPG mutase, and PGK deficiencies. On the other hand, it is sometimes difficult to be certain if this improvement has resulted from the splenectomy or if it is coincident with the subsidence of the rapid growth phase of early childhood.

Mild to moderate anisocytosis and poikilocytosis with macrocytosis, polychromatophilia, basophilic stippling, target cells, burr cells, fragmented erythrocytes, and rare spherocytes are often described in the stained peripheral blood smear. The changes in morphology induced by splenectomy are often seen. Markedly distorted and fragmented erythrocytes have been described in severe PK deficiency hemolytic anemia, and densely staining, spiculated microspherocytes are common in PHI deficiency. The morphologic findings, however, are nonspecific. The initial osmotic fragility of the erythrocytes is characteristically normal or even decreased, but a small population of cells with increased fragility or a flattening of the curve has been noted after incubation of the blood at 37°C for 24 hr. As noted in Table 1, the findings in the autohemolysis test are variable and nonspecific.

Of particular interest are those enzyme deficiencies which are present in erythrocytes, leukocytes, and other tissues. A severe neuromuscular disorder accompanies TPI deficiency, a neurologic abnormality may be associated with PGK deficiency, and a glycogen storage disease is seen in some patients with PFK deficiency. A systemic disorder, however, has not been apparent in PHI deficiency where the leukocytes are as deficient as the erythrocytes. In all of the other disorders discussed, the leukocytes, where examined, have had normal enzyme activity and there has been no associated neurological disorder or increased susceptibility to infections.

The hemolytic process is often apparent from birth, but not infrequently and especially in the milder forms, the illness is not detected until adult life. In keeping with the autosomal recessive mode of inheritance of most of these enzyme deficiencies, parent to child transmission has been rare and, as in the case of PK deficiency, has usually been explicable on the basis of a mating between heterozygous and homozygous subjects. Hereditary spherocytosis, which may well not belong in this category of diseases, appears to be transmitted as a dominant characteristic and the original reports of 2,3-DPG mutase deficiency implied a dominant mode of inheritance. PGK deficiency appears to be sex linked with severe manifestations in the hemizygous male and it is possible, but certainly not proved, that PFK deficiency without muscle involvement may also be sex linked.

Significant impairment of an enzymatic activity would be expected to result in the accumulation of metabolites proximal to the block and a decrease in those distal to the defect. Such confirmatory evidence has been obtained in at
Table 2.—Abnormalities Associated with Deficiencies in Enzymes Related to Hexosemonophosphate Shunt Pathway

<table>
<thead>
<tr>
<th>Enzyme</th>
<th>No. of Cases</th>
<th>Severity of Anemia</th>
<th>Retic. Per Cent</th>
<th>Auto-hemolysis</th>
<th>Glutathione Stabil-ity*</th>
<th>Enzyme Per Cent of Normal</th>
<th>Enzyme Activity Percent of Normal WBC</th>
<th>Glucose Utilization</th>
<th>ATP of RBC</th>
<th>Electro- phoresis</th>
<th>Mode of Inheritance</th>
<th>References</th>
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<td>Mild to severe</td>
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<td>60–75 ↓</td>
<td>+</td>
<td>8–20</td>
<td>N, A</td>
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<td>Severe</td>
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<td>30–64</td>
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<td>–</td>
<td>N</td>
<td>AR</td>
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*Stability upon incubation with acetylphenylhydrazine.
†All subjects post-splenectomy when studies performed.
Abbreviations otherwise as in Table 1. HB test = Heinz Body test.
least one type of HK deficiency, presumed 2,3-DPG mutase deficiency, TPI, PGK, and PK deficiency. Because of the presence of alternative pathways of metabolism, such as the HMP and 2,3-DPG shunts and, in reticulocytes, possible tricarboxylic acid cycle activity, the expected alterations in the concentrations of intermediates may not always occur. Accurate determinations of the intermediates of glycolysis in blood deproteinized immediately after collection have only recently become practical and additional data are expected.

The molecular defects in the abnormal enzymes which characterize these disorders have not been determined. Deficient enzymatic activity may be due to decreased synthesis or increased destruction of a normal enzyme protein, or may be due to the formation of a structurally abnormal enzyme with altered catalytic capacity, kinetics, or stability. Abnormalities in electrophoretic migration have been described in HK, PHI, PFK, and TPI deficiency, but the basis for the alterations has not been defined. So far, none of the enzymatic deficiencies have been attributable to the presence of an inhibitor of a normal enzymatic activity.

Abnormalities Associated with the Hexosemonophosphate Shunt Pathway

The important features of the hemolytic disorders associated with deficiencies in the activities of enzymes in the HMP shunt, or in closely related reactions, are summarized in Table 2.

Glucose-6-Phosphate Dehydrogenase (G-6-PD)

The world-wide incidence of G-6-PD deficiency, the prototype of the enzymatic deficiencies of human erythrocytes, has led to investigations of import for clinical medicine, pediatrics, pharmacology, biochemistry, genetics, and anthropology. Over 50 different variants of G-6-PD have been defined on the basis of altered electrophoretic mobilities, kinetics, or physicochemical properties of the enzyme. Many of these variants are associated with no clinical effect and no significant decrease in activity, while others are associated with mild to moderately severe nonspherocytic hemolytic anemia even in the absence of any known precipitating agent. The three most common variants with decreased activities, G-6-PD A−, Mediterranean and Canton, are present in erythrocytes with normal or nearly normal lifespans, but are associated with drug-induced hemolytic anemia and, in the case of the latter two, with favism. G-6-PD deficiency has been held responsible for an increased incidence of neonatal hemolytic jaundice in some population groups. Viral and bacterial infections, such as hepatitis and pneumonia, acidosis and other intercurrent diseases have been incriminated in the precipitation of hemolysis in G-6-PD deficient subjects. Significantly decreased activity in tissues other than erythrocytes has not been observed in Negro subjects with G-6-PD A−, but leukocytes and other tissues share the deficiency in other types. Although G-6-PD deficiency in tissues other than erythrocytes may have physiological effects, no specific clinical syndromes attributable to this abnormality have been defined.

The data from all investigations so far reported are consistent with the
thesis that a gene on the X chromosome determines G-6-PD activity in man, as well as in horses, donkeys, and rabbits. The locus for G-6-PD appears to be closely linked to those for color blindness and hemophilia A and B, but not for blood group Xg\(^b\). In addition, the study of G-6-PD deficiency in man has provided substantial evidence for genetic mosaicism in human females. Thus, the ill effects of G-6-PD deficiency are characteristically manifested in hemizygous men, while heterozygous women may or may not suffer the consequences.

The biochemical abnormalities in G-6-PD deficient erythrocytes reflect the decreased ability of the HMP shunt to respond to stimulation. The concentrations of GSH in deficient erythrocytes are slightly reduced, but the GSH falls dramatically upon incubation of the cells with agents such as acetylphenylhydrazine (APH). The decline in the concentration of GSH under these conditions, as well as during a hemolytic episode, is accompanied by the formation of Heinz bodies which probably represent denatured hemoglobin precipitated within the cell. The hemolytic property of certain drugs in G-6-PD deficiency has been attributed to their ability to generate hydrogen peroxide (H\(_2\)O\(_2\)), peroxynemoglobin, or free radicals within the erythrocytes. Normally, any H\(_2\)O\(_2\) formed can be destroyed by GSH-peroxidase activity (Fig. 1). The GSSG formed is reduced to GSH through the activity of GSSG-reductase, a reaction which utilizes the TPNH generated by G-6-PD activity. This detoxification mechanism is defective in cells unable to regenerate GSH. This hypothesis, however, does not adequately explain the chronic hemolytic process in Caucasian G-6-PD deficient individuals with hemolysis in the absence of any known precipitating factor. The hemolytic consequences of G-6-PD deficiency may involve a series of simultaneous, related, or unrelated events: oxidation of GSH, oxidative denaturation of hemoglobin (Heinz bodies), lipid peroxidation and membrane destruction with removal of the damaged erythrocytes by the reticuloendothelial system.

Sufficient purification of G-6-PD from large volumes of blood has permitted the definition of a single amino acid difference, asparagine instead of aspartic acid, between the two most common variants, B and A\(^+\), with normal activity.\(^{30}\) In G-6-PD Hektoen, a variant associated with markedly increased enzyme activity in erythrocytes and leukocytes and not associated with hemolytic anemia, a tyrosine residue has replaced a histidine of the normal B enzyme (Yoshida, A., personal communication). G-6-PD A\(^-\) appears to have normal catalytic activity, but to be unusually susceptible to deterioration during the normal aging of the erythrocyte. Information about the other variants has not yet reached this stage of molecular refinement.

6-Phosphogluconate Dehydrogenase (6-PGD)\(^{31-33}\)

Deficiency in the activity of the second enzyme in the HMP shunt which can generate TPNH might be expected to produce effects similar to those resulting from G-6-PD deficiency. Only two patients, of French and German origin, have been reported with hemolytic anemia attributed to 6-PGD deficiency. Instability of GSH upon incubation with APH and a positive Heinz body test were observed in one,\(^{32}\) but not the other.\(^{33}\) The rate of hemolysis in the
latter patient was not increased upon administration of primaquine. Several subjects with partial deficiency in 6-PGD activity in both erythrocytes and leukocytes have been described, but without apparent clinical effects and with questionable minimal acceleration of destruction of erythrocytes in one subject given primaquine. Thus, the cause-and-effect relationship between deficient 6-PGD activity and hemolysis must be questioned. Instability of the enzyme upon incubation, especially the enzyme from leukocytes, has been noted, but the nature of the molecular defect has not been defined. While family studies in the patients with hemolysis were inconclusive, 6-PGD appears to be controlled by a gene on an autosome.

**Oxidized Glutathione Reductase (GSSG-R)**

An extremely varied assortment of illnesses ranging from drug-induced hemolysis, nonspherocytic hemolytic processes with or without thrombocytopenia or leukopenia, pancytopenia, leukemia, hemophilia B, and nonhematologic diseases has been described in subjects with GSSG-R deficiency. Effects similar to those observed in G-6-PD deficiency would be expected in the presence of a significant deficiency in the activity of the enzyme required to reduce GSSG to GSH, especially after oxidative stress.

In many subjects whose erythrocytes were deficient in GSSG-R activity, the activity was increased markedly by the administration of riboflavin in vivo, or the addition of flavin adenine dinucleotide (the coenzyme of GSSG-R) to the hemolysate in vitro. The severity of hemolysis in a woman homozygous for hemoglobin C with approximately 50 per cent of normal GSSG-R activity in her erythrocytes was not influenced appreciably by the administration of riboflavin, although normal GSSG-R activity could be demonstrated after therapy for a few days. Thus, the relationship between GSSG-R deficiency and the associated diseases must be reevaluated.

**Glutathione Peroxidase (GSH-Px)**

Persistent mild reticulocytosis, but acute hemolysis after autotransfusion, were described in a Puerto Rican male whose erythrocytes were markedly deficient in the activity of GSH-Px, the enzyme presumed to be of primary importance in destroying $H_2O_2$ in human erythrocytes. Transient neonatal hemolytic jaundice and hemolysis after nitrofurantoin and sulfonamide therapy have been attributed to partial, presumably heterozygous, GSH-Px deficiency. Whether or not catalase, which can also destroy $H_2O_2$ and whose activity is transiently decreased in the newborn period, may serve to protect the erythrocytes of heterozygous subjects has not been evaluated. The only known presumably homozygous subject has not been challenged with drugs. Thus, GSH-Px deficiency may be associated with drug-induced hemolytic episodes analogous to those observed in subjects with G-6-PD A-.

Although GSH-Px deficient erythrocytes demonstrate increased Heinz body formation after incubation with APH, the stability of the GSH is normal. If the concept of GSH-Px activity is correct, increased Heinz body formation with normal or elevated and stable concentrations of GSH are to be expected.
**GSH Deficiency (GSH Synthetase)**

Five members of a large Dutch family, all offspring of consanguineous marriages, with a well-compensated hemolytic disorder were found to have less than 10 per cent of the normal content of GSH in their erythrocytes. Hemolysis increased after ingestion of fava beans or primaquine. The Heinz body test was positive, but GSH stability could not be evaluated because of the low initial concentrations. As a consequence of the low GSH content, glyoxylase and GSH-Px activities were decreased, but those of all other enzymes measured were normal or elevated. No abnormalities were observed in studies of obligatory carriers. Two unrelated French patients with similar clinical and biochemical findings, one of whom appeared to have less severe hemolysis after splenectomy, have been described. An acute hemolytic episode after irradiation was attributed to a 50 per cent reduction in GSH in the erythrocytes of a German woman.

Although the erythrocytes of the French patients were able to incorporate labeled glutamine into glutamylcysteine, cells from neither the Dutch nor the French patients were able to incorporate glycine into GSH. No abnormalities could be demonstrated with the erythrocytes of obligatory heterozygotes. The deficiency, however, was considered to be the consequence of decreased GSH synthetase activity.

The well-compensated hemolytic process in GSH-deficient subjects is in marked contrast to the more severe hemolysis in Caucasian subjects with G-6-PD deficiency and nearly normal concentrations of GSH in their erythrocytes. Extremely low concentrations of GSH might be expected to produce all of the deleterious consequences of an inability to regenerate GSH from GSSG.

**General Comment**

So far, abnormalities in enzymes in or closely related to the HMP shunt all appear to have produced their hemolytic effects as a consequence of alterations in the metabolic regulation of the GSH content of erythrocytes. Without stress, glycolysis in the enzyme-deficient cells is not impaired. In the absence of oxidant drug administration, these defects are associated with somewhat less severe hemolysis than deficiencies in the E-M pathway, and none has been incriminated in generalized, systemic disorders. The spontaneous hemolysis in some patients with HMP shunt deficiencies may have improved slightly after splenectomy, but the indications for this operation are even less clear in these cases than they are in patients with deficiencies of enzymes in the E-M pathway.

None of the routine hematologic tests is of diagnostic help in detecting deficiencies in the HMP shunt enzymes. The results of the autohemolysis test are not helpful. The morphology of the erythrocytes may be entirely normal, as in G-6-PD deficiency without hemolysis, or the alterations are nonspecific. The demonstration of a low or unstable erythrocyte GSH may provide a clue to such a deficiency, as may the development of increased numbers of Heinz bodies after incubation of blood with APH. The development of Heinz bodies, however, is also nonspecific in that they may be seen in erythrocytes with TPI deficiency and in cells containing unstable hemoglobins.
Speculations About the Future

The problem of identifying known deficiencies of enzymes in erythrocytes has been eased considerably by the development of a number of simple screening tests. These tests have been evaluated carefully recently and, in conjunction with appropriate examinations to exclude the numerous other causes of hemolysis, they can lead to a presumptive diagnosis. A definitive diagnosis, however, requires the demonstration of a significant enzyme deficiency, preferably by direct assay, in a laboratory equipped with more than routine methodology and interest.

Enzymes are usually present in great excess over the minimum required for normal metabolic activity and the activities are rarely if ever totally absent in deficient cells. Enzyme activities are also determined under optimal assay conditions. Activities under such ideal settings may not reflect the true state of the enzyme within the intact erythrocyte in the circulation, especially when only a selected population of young cells is available for study. For these reasons, it is not surprising that such parameters of metabolic activity as total glucose consumption, lactate production, and the reduction of methemoglobin often do not mirror the enzymatic deficiency. Because of these considerations, attention has recently been directed to the determination of the concentrations of intermediates of glycolysis to provide clues as to the site of the "metabolic hangup." Caution is required in interpreting the results of such studies because of the alternative metabolic pathways available, even to a cell as disadvantaged as the erythrocyte. In the final analysis, the definition of an enzymatic deficiency probably should include: (1) significantly decreased enzyme activity in the hemolysate relative to the activity in a hemolysate of erythrocytes of comparable age, (2) increased concentrations of substrates preceding the metabolic block, (3) decreased concentrations of compounds dependent upon the deficient enzyme activity, and, ideally, (4) similar but less pronounced abnormalities in erythrocytes of blood relatives with minimal or no evidence of hemolysis.

What of other enzyme deficiencies as causes for hemolysis? Initially, it was thought that a significant deficiency in the activity of HK in the erythrocyte would be incompatible with life. Since this premise has been refuted, any enzymatic deficiency could be possible. There remain only a few enzymes in the pathways of glycolysis for which deficiencies have not been described. Their detection may require careful investigations in line with the concepts outlined above, and their presence may not even be associated with hemolysis. A tentative presumption of deficiency in the activity of 2,3-DPG phosphatase in erythrocytes has been described, but in association with retarded development, cerebral dysgenesis, muscle hypotonia, and 3 per cent reticulocytes without anemia. In the future, a deficiency of lactate dehydrogenase, an enzyme with an extremely high activity in human erythrocytes, may be discovered. The pyruvate which should accumulate with such a deficiency might diffuse out of the cell in vivo to be metabolized in other tissues without producing a deleterious effect on the erythrocyte. On the other hand, the accumulation of DPNH and the relative deficiency in DPN might result in indirect inhibition...
of glycolysis. The complex reactions of the HMP shunt which have not been considered here (phosphoketopentopimerase, transketolase, transaldolase) might be impaired in some hemolytic disorders. The decreased transketolase activity observed in thiamine deficiency (beriberi), however, is not associated with hemolysis. Hereditary defects in enzyme systems other than those related to glycolysis might also be associated with hemolytic disorders. While deficiencies in the activities of catalase and galactose-1-phosphate uridylic transferase can be demonstrated in the erythrocytes of subjects with acatalasemia and galactosemia, respectively, these disorders are not associated with significant hemolysis.

While much has been learned, additional information is required to explain the mechanisms for hemolysis in hereditary enzymatic deficiencies of the erythrocytes. Future studies should look at any and all enzyme systems which can be measured, and should examine more closely the detailed structure of the erythrocyte membrane and the relationship of structure to metabolic activity.

REFERENCES

The references cited have been limited to review articles, definitive discussions and, when essential, preliminary reports to provide a source for additional information. An exhaustive bibliography, therefore, has not been included.

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Clinical Profile: Hereditary Hemolytic Disorders and Enzymatic Deficiencies of Human Erythrocytes

ERNST R. JAFFÉ