REVIEW

Red Cell Enzyme Defects as Nondiseases and as Diseases

By Ernest Beutler

FOR A MODEST SUM, Sigma Chemical Company will supply a diagnostic kit that will enable one to determine the glutathione reductase activity of the erythrocytes in a patient with hemolytic anemia. This kit or one or another assay procedure for glutathione reductase is used by conscientious physicians in the work-up of patients with chronic hemolysis. Unfortunately, the money and effort are wasted. Glutathione reductase deficiency does not produce chronic hemolytic anemia.

Within the past two decades, recognition of clear-cut relationships between hemolytic disease and deficiencies of glucose-6-phosphate dehydrogenase, pyruvate kinase, glucose-phosphate isomerase, pyrimidine 5'-nucleotidase, and a number of other enzymes has greatly advanced our understanding of the origin of hemolytic disease and of certain basic aspects of red cell metabolism. Although the cause of hereditary nonspherocytic hemolytic anemia has thus been correctly identified in some patients with this disorder, the etiology of hemolysis in other patients has eluded the investigator of red cell biochemistry. Quite naturally, such patients are being investigated in research laboratories with ever-enlarging panels of red cell enzyme assays. When deficiencies have been found, it has often been assumed that a cause-and-effect relationship exists between enzyme deficiency on the one hand and chronic hemolysis on the other.

Glutathione reductase is a case in point. Many articles and reviews, even quite recent ones, have listed a deficiency of this enzyme as one of the causes of chronic hemolytic anemia.\(^1-3\) However, we now recognize that in the vast majority of cases, partial glutathione reductase deficiency is due to suboptimal riboflavin nutrition.\(^4-8\) Glutathione reductase is an enzyme that contains flavine-adenine dinucleotide (FAD). In red cells its activity is markedly influenced by riboflavin intake.\(^7\) Whether nutritional or hereditary in origin, one thing is quite clear: partial glutathione reductase deficiency has no known hematologic effect. Virtually
complete glutathione reductase deficiency has been documented in only one consanguineous family, and the deficient individuals in this family were quite well; only a single acute hemolytic episode was observed in a deficient member after the ingestion of fava beans. Thus, although glutathione reductase deficiency cannot, in a strict sense, be classified as a nondisease, a search for this defect in patients with chronic hemolysis is almost certain to be unrewarding.

Another enzyme deficiency often erroneously regarded as a cause of hemolytic anemia is glutathione peroxidase deficiency. A hemolytic episode following autotransfusion in a Puerto Rican patient with severe cardiac disease was attributed to lowered glutathione peroxidase levels, and subsequently, glutathione peroxidase levels of approximately one-half normal were described in a number of patients with hemolytic syndromes. It was generally assumed that the low glutathione peroxidase activity was responsible for hemolysis. Some years ago, the author's laboratory was attempting to devise an improved technique for glutathione peroxidase assay by substituting t-butylhydroperoxide as substrate for hydrogen peroxide. In the course of defining normal values in standardizing the new assay, a blood sample provided by the author's son proved to have "one-half normal" red cell glutathione peroxidase activity. Similar levels were found in the red cells of the author himself, his three other children, and to a lesser extent, his wife. Enlarging the survey disclosed that a gene for low glutathione peroxidase activity, designated GSHPx, was particularly prevalent in the Jewish population, reaching a gene frequency of 0.56. Thus, approximately 30% of individuals of Jewish ancestry were homozygous for this gene and had glutathione peroxidase activity approximately one-half of normal. Other Mediterranean peoples were found to share the high gene frequency for the GSHPx gene, and it was also found in people who were not known to be of Mediterranean ancestry. Yet no hematologic disorder is evident in these people with genetically lowered GSHPx activity. (From an ethnocentric point of view, one might even suggest that the GSHPx/GSHPx genotype produces the normal glutathione peroxidase activity and that many people, particularly those whose ancestry is not Mediterranean, have twice normal GSHPx activity.) Dietary factors, too, apparently can influence GSHPx activity. Purification of GSHPx from red cells has revealed that it is a selenium-containing enzyme. In New Zealand, where the selenium content of the soil is very low, decreased red cell GSHPx activities have been observed in normal subjects. Since glutathione peroxidase "deficiency" is the consequence of a common benign genetic polymorphism, and since it can readily be produced by change in diet without any overt hematologic findings, why should its occurrence in a few persons with chronic hemolytic disease by anything but a coincidence? It may be that someday severely deficient variants of glutathione peroxidase or those with markedly abnormal kinetic properties will be discovered and be shown by pedigree analysis to bear a cause-and-effect relationship to hemolytic anemia. However, no such variant has been discovered to date and, for the present, GSHPx deficiency must be regarded as a nondisease.

The suggestion has also been made that 6-phosphogluconate dehydrogenase (6-PGD) deficiency may produce hemolytic anemia. Perhaps because of a readiness to believe that a deficiency of any enzyme involved in the hexose monophosphate shunt will lead to drug sensitivity, 6-PGD deficiency is sometimes included among those that can produce hemolytic anemia. Possibly the fact that
glucose-6-phosphate dehydrogenase (G-6-PD) deficiency was coincidentally present in some patients with 6-PGD deficiency played a role in this misconception. However, there is good reason to believe that 6-PGD deficiency is a nondisease. The most severely deficient subjects ever described were detected by Parr and Fitch in the course of a population survey. These subjects had only 2.4% and 4.5% of mean normal 6-PGD activity, but they showed no obvious clinical manifestations that could be attributed to red cell 6-PGD deficiency. Other red cell enzyme deficiencies presumed at times to be associated with hemolytic anemia, but in which the data do not stand scrutiny, include those of ATPase, enolase, and glyceraldehyde phosphate dehydrogenase.

Nonetheless, certain red cell enzyme deficiencies do cause hemolytic anemia, and it is precisely this fact that is responsible for the confusion surrounding the cause of hemolytic anemia coincident with enzyme deficiency. Table 1 indicates those enzymes for which a cause-and-effect relationship between the deficiency and chronic hemolysis is well documented. Detailed accounts of the clinical manifestations of these enzyme deficiencies are readily available to the reader. However, there are no data concerning the relative frequency of the occurrence of hemolytic anemia due to red cell enzyme defects.

In order to gain some insight into this problem, we have reviewed the results of enzyme assays carried out on blood samples from our patients or samples referred to us from 350 unrelated patients with hematologic disorders, most with hemolytic anemia, during the past 10 years. Unless a deficiency of pyruvate kinase or G-6-PD was detected, most samples were assayed for the following enzymes: hexokinase, glucose phosphate isomerase, phosphofructokinase, aldolase, triose phosphate isomerase, glyceraldehyde phosphate dehydrogenase, diphosphoglycerate mutase, Table 1. Occurrence of Red Cell Enzyme Deficiencies in Patients With Hemolytic Anemia

<table>
<thead>
<tr>
<th>Enzyme</th>
<th>Literature Documentation of Chronic Hemolysis</th>
<th>Previously Undiagnosed</th>
<th>Age Known</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>All Cases (350)</td>
<td>All Cases (304)</td>
<td>0-20 yr (124)</td>
</tr>
<tr>
<td>Hexokinase</td>
<td>+ 1 (0.3%)</td>
<td>1 (0.3%)</td>
<td>1 (0.8%)</td>
</tr>
<tr>
<td>Glucose phosphate isomerase</td>
<td>+ 6 (1.7%)</td>
<td>6 (2.0%)</td>
<td>4 (3.3%)</td>
</tr>
<tr>
<td>Phosphofructokinase</td>
<td>+ 0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Aldolase</td>
<td>+ 1 (0.3%)</td>
<td>1 (0.3%)</td>
<td>1 (0.8%)</td>
</tr>
<tr>
<td>Triose phosphate isomerase</td>
<td>+ 3 (0.9%)</td>
<td>3 (1.0%)</td>
<td>3</td>
</tr>
<tr>
<td>Glyceraldehyde phosphate dehydrogenase</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Phosphoglycerate kinase</td>
<td>+ 0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Diphosphoglycerate mutase phosphatase</td>
<td>+ 1 (0.3%)</td>
<td>1 (0.3%)</td>
<td>0</td>
</tr>
<tr>
<td>Monophosphoglycerate mutase</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Enolase</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Pyruvate kinase</td>
<td>+ 37 (9.9%)</td>
<td>31 (10.1%)</td>
<td>16 (12.8%)</td>
</tr>
<tr>
<td>Lactate dehydrogenase</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>G-6-PD</td>
<td>+ 49 (13.9%)</td>
<td>11 (3.6%)</td>
<td>3 (2.4%)</td>
</tr>
<tr>
<td>Glutathione reductase</td>
<td>0</td>
<td>0</td>
<td>0</td>
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<tr>
<td>Glutathione peroxidase</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Glutathione synthetase</td>
<td>+ 0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>y-Glutamyl cysteine synthetase</td>
<td>+ 0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>6-PGD</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Pyrimidine 5'-nucleotidase</td>
<td>+ 5 (1.4%)</td>
<td>5 (1.7%)</td>
<td>3 (2.4%)</td>
</tr>
<tr>
<td>Unstable Hb</td>
<td>+ 6 (1.7%)</td>
<td>4 (1.3%)</td>
<td>2 (1.6%)</td>
</tr>
</tbody>
</table>
monophosphoglyceromutase, enolase, pyruvate kinase, lactic dehydrogenase, G-6-PD, phosphogluconic dehydrogenase, glutathione reductase (with and without added FAD), glutathione peroxidase, glutamic oxaloacetic transaminase (with and without added pyridoxal-5-phosphate), and adenylate kinase. These determinations were carried out by methods previously described, using more than one substrate level and testing allosteric reactions where indicated. The isopropanol test for unstable hemoglobin was also performed. In the last 80 samples examined, the pyrimidine 5'-nucleotidase activity was also measured. Glutathione levels were studied in many samples, and levels of ATP and 2,3-DPG were measured in the 41 samples for which adequately prepared perchloric acid extracts were available to us.

Of the 350 samples submitted, 38 were already known or suspected to be G-6-PD-deficient and were sent for confirmation of diagnosis or characterization of the mutant enzyme. Since the diagnosis of G-6-PD deficiency is relatively simple to make, it is not surprising that we were able to confirm the diagnosis in all of these cases. A diagnosis of G-6-PD deficiency was established for the first time in 11 additional cases. In one case, the G-6-PD activity had been regarded as normal, but the patient was actually a heterozygote for G-6-PD deficiency. Seventeen of the patients studied had nonspherocytic hemolytic anemia due to unusual G-6-PD variants; the remainder, if characterized, were G-6-PD A- and G-6-PD Mediterranean.

Pyruvate kinase deficiency is particularly difficult to diagnose because kinetic abnormalities that markedly influence enzyme activity intracellularly may not be correspondingly reflected in in vitro activity measurements. Sometimes it is necessary to carry out heat stability tests and kinetic measurements in order to detect a pyruvate-kinase-deficient variant. Moreover, incomplete removal of leukocytes from a red cell preparation being assayed for this enzyme may obscure a deficiency. Not surprisingly, then, among the 37 patients with unequivocal pyruvate-kinase deficiency that we detected, 7 patients had been considered to be normal with respect to pyruvate kinase activity after examination in another laboratory, and only 6 patients had been previously diagnosed as pyruvate-kinase-deficient. The oldest patient in whom we detected a previously unknown enzyme deficiency was a 67-yr-old pyruvate-kinase-deficient American Indian male whose red cell pyruvate kinase activity had previously been regarded as being normal. Our own record in the diagnosis of pyruvate kinase deficiency was not perfect. Among the 242 samples in which we were unable to detect an enzyme deficiency or unstable hemoglobin, pyruvate kinase deficiency was reliably documented in another laboratory in 1 case several years later. Our error in this case was probably due to the fact that the patient carried a considerable proportion of transfused cells at the time of our examination and that these studies were carried out at a time when our red cell preparations were freed of leukocytes by centrifuging, removing the buffy coat, and washing. The filtration of red cells through small cellulose columns, freeing them almost entirely of leukocytes, will render such an occurrence less likely. Three individuals who appeared to be heterozygous for deficiency were encountered in the series. It seems unlikely that the heterozygous state was in any way responsible for their hemolytic anemia. Indeed, approximately 1% of persons in the general population are believed to be
heterozygous for pyruvate kinase deficiency, and thus the incidence of heterozygotes in this population is no larger than might be expected.

Next in frequency to G-6-PD deficiency and pyruvate kinase deficiency were glucose phosphate isomerase deficiency and the unstable hemoglobins. We found 6 cases of each of these disorders among the patients referred to us for the diagnosis of possible enzyme deficiencies. In 2 of the patients with unstable hemoglobins, the diagnosis had been established previously, but in 1 patient with hemoglobin H disease, the hemoglobin had been examined previously and thought to be normal. The diagnosis of glucose phosphate isomerase deficiency had not been previously established in any of the 6 unrelated cases that we detected. One of the 6 patients with glucose phosphate isomerase deficiency had previously been incorrectly diagnosed as having pyruvate kinase deficiency. The incidence of pyrimidine 5'-nucleotidase deficiency, a disorder only relatively recently recognized by Valentine et al., was surprisingly high. Although assays for this enzyme were carried out on only the last 80 cases investigated, 5 unrelated patients with this disorder were found. All had the characteristic stippled red cells and abnormal nucleotide pattern. At least 3 of the patients were mentally retarded, a finding that has not previously been associated with pyrimidine 5'-nucleotidase deficiency.

Three unrelated patients with triose phosphate isomerase deficiency were detected. All manifested the characteristic picture of severe neurologic deficit, rapid downhill course, and early death. One case of hexokinase deficiency, 1 case of diphosphoglyceromutase deficiency, and 1 case of aldolase deficiency were also recorded in the series.

Some of the samples assayed were from patients for whom a hematologic diagnosis had already been considered to be established or could readily be established on the basis of the data submitted with the sample. We examined blood from a patient with Rh null disease, from 2 patients with S hemoglobinopathies, from 4 patients with diagnoses of congenital dyserythropoietic anemia (CDA), and from 14 patients with diagnoses of hereditary spherocytosis (HS). One of the patients with a diagnosis of HS was in reality pyrimidine-5'-nucleotidase-deficient, whereas another was pyruvate-kinase-deficient. One patient diagnosed as having congenital dyserythropoietic anemia proved to be deficient in pyruvate kinase.

The results of our studies are summarized in Table 1 and Fig. 1. Certain serious biases in case selection must be acknowledged. There is a tendency, first of all, for only problem cases to be referred. Since assays for certain enzymes, such as G-6-PD and pyruvate kinase, are relatively readily available, the likelihood that such patients would be referred is decreased. On the other hand, because our laboratory has been interested in G-6-PD deficiency for many years, there would be a tendency to receive more samples from patients already known to be G-6-PD-deficient. In spite of such limitations, certain important conclusions may be drawn:

1. It is quite clear that many patients with chronic hemolytic anemia of unknown origin do not have a detectable red cell enzyme defect; this includes those who are quite young and in whom there is a strong likelihood that the anemia is hereditary in origin.

2. There seems to be little question that G-6-PD deficiency and pyruvate kinase deficiency are the most common of the enzyme deficiencies that are detected.
Fig. 1. Incidences of various defects in the blood samples submitted to our laboratory in the past 10 yr. All patients were anemic, usually with evidence of hemolysis. The 21 patients previously diagnosed as having disorders such as hereditary spherocytosis or congenital dyserythropoietic anemia are included in the overall analysis, since some of these proved, in reality, to have enzyme defects. Abbreviations: G-6-PD - glucose-6-phosphate dehydrogenase; PK - pyruvate kinase; GPI - glucose phosphate isomerase; PSN - pyrimidine 5'-nucleotidase; TPI - triose phosphate isomerase; Hx - hexokinase; DPGM - diphosphoglycerate mutase.

Much less common than these two defects, but clearly more prevalent than any of the other remaining defects, are deficiencies of glucose phosphate isomerase and pyrimidine 5'-nucleotidase activity. It is particularly notable that although assays for pyrimidine 5'-nucleotidase were performed on only 80 samples, 5 cases of this relatively rarely reported enzyme deficiency were encountered.

3. Applicable numbers of blood samples from patients in whom the diagnosis of pyruvate kinase deficiency had been excluded on the basis of tests carried out in commercial or other laboratories were, in reality, clearly pyruvate-kinase-deficient.

4. Study of this relatively large group of patients with hemolytic anemia, believed possibly to be due to enzyme deficiencies, did not yield a single patient with glutathione reductase deficiency or with 6-phosphogluconic dehydrogenase deficiency.

REFERENCES


8. Waller HD, Benöhr HC: Hematological manifestations in enzymatic deficiencies of glutathione reduction, in Flohe L, Benöhr HC, Sies H,


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