REVIEW ARTICLE

G6PD Deficiency

By Ernest Beutler

THIRTY-FIVE YEARS ago Dr William Dameshek, the first editor of the emerging journal Blood, invited me to write a review on “The Hemolytic Effect of Primaquine.” At the time, primaquine sensitivity, which had just recently been shown to be caused by a deficiency of the enzyme glucose-6-phosphate dehydrogenase (G6PD), represented a unique example of an inherited deficiency of an enzyme that caused hemolytic anemia.

Although many other red blood cell (RBC) enzyme deficiencies are now known, G6PD deficiency still reigns as the most common of all clinically significant enzyme defects, not only in hematology, but in human biology as a whole. A variety of drugs and infections cause hemolytic anemia in persons with the deficiency, and nonhematologic sequelae have been claimed as well. Using classical biochemical techniques, enormous apparent diversity of mutations causing G6PD deficiency was documented in hundreds of publications. The distribution of the deficiency in different populations has been investigated exhaustively, and gene frequencies of over 0.5 have been observed in some ethnic groups. With the advances made possible by the cloning of G6PD cDNA and gene has come a better understanding of the diversity that exists. In this review, I will attempt to put what we have learned in the past 35 years into perspective and to touch upon what still needs to be learned.

CLINICAL MANIFESTATIONS

Hemolytic Anemia

Drug-induced hemolysis. G6PD deficiency was discovered as a result of a series of investigations performed to understand why some persons were uniquely sensitive to the development of hemolytic anemia when they ingested the 8-aminoquinoline antimalarial drug primaquine. Thus, the first and best known morbid effect of G6PD deficiency was drug-induced hemolysis. Primaquine is but one of many drugs that shortens RBC life span in G6PD-deficient persons (see below). The administration of such drugs is followed, after a 1- or 2-day delay, by a fall in the hemoglobin (Hb) concentration. Heinz bodies, particles of denatured protein adherent to the RBC membrane, appear in the early stages of drug administration and disappear as hemolysis progresses. Another morphologic feature observed on the blood film is the appearance of RBCs that have variously been designated “irregularly contracted RBCs,” “eccentrocytes,” “hemi-ghosts,” “double-colored RBC,” and “cross-bonded cells.” The Hb of these cells is confined to one side of the erythrocyte, leaving the other part as a flat, Hb-free ghost. In this portion of the cell, the inside surface of the membrane is tightly bonded. Often Heinz bodies are included in the flattened region, where they may bulge visibly out of the leaflet. When hemolysis is severe, the urine turns dark and the patient may complain of back pain. When G6PD deficiency is relatively mild, as in the class 3 G6PD A-, the hemolytic anemia is self-limited because only the older RBCs are destroyed and young RBCs have normal or near-normal enzyme activity. In patients with more severe forms of enzyme deficiency such as G6PD Mediterranean, young cells are severely deficient in G6PD, and as a consequence, hemolysis continues until well after the administration of drug is stopped.

The fact that primaquine was only one of many drugs that precipitated hemolysis in G6PD-deficient individuals was recognized early in our studies by in vivo challenge of Cr-labeled erythrocyte. Therefore, in the 1950s, when a person with G6PD deficiency developed hemolytic anemia, it was generally assumed that hemolysis had been precipitated by a drug, and whatever drug had been ingested was considered to be culpable. As a result, a long list of drugs thought to cause hemolysis evolved. On more careful study many of them have been proven to be quite innocent with respect to the cause of hemolytic anemia in G6PD deficiency.

As a matter of fact, it is difficult to be certain in some cases, whether a cause-and-effect relationship exists between ingestion of a drug and hemolysis. The most robust data regarding the potential hemolytic effect of drugs and chemicals comes from clinical investigations with Cr-labeled erythrocytes. However, even results obtained using RBC survival studies can be misleading. Individual inherited differences in drug metabolism such as acetylator status play a significant role in determining whether a drug will be hemolytic. Thus, if a recipient who efficiently catabolizes the active hemolytic metabolite of a drug is challenged, hemolysis will not be apparent, but the drug may be hemolytic in a subset of individuals who metabolize the drug less efficiently. Moreover, even when a drug does shorten RBC life span, as shown by performing sensitive studies with Cr-labeled erythrocytes, the degree of hemolysis may be so modest as to be of no clinical significance. Sulfaamethoxazole, a component of the commonly used combination Septra® and Bactrim®, has been shown to produce shortening of the

* G6PD variants have been classified as follows: class 1, hereditary nonspherocytic hemolytic anemia; class 2, severe deficiency; class 3, mild deficiency; class 4, not deficient variant.

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RBC life span in Asian subjects with G6PD deficiency, but no significant hemolysis could be shown when this combination was used clinically in patients with G6PD A−.24 RBCs from subjects with severe class 2 variants such as G6PD Mediterranean may be sensitive to drugs when those with milder defects such as G6PD A− are not. The data obtained from 51Cr survival must be supplemented with less reliable information gained from clinical observations. Clinical studies are confounded by the effect of intercurrent infections which may be responsible for hemolysis rather than the drug that has been administered. For example, the clinical observations that hemolytic anemia is caused by acetaminophen have been made during the concurrent presence of infection; investigations of the putative hemolytic effect of this drug with 51Cr-labeled erythrocytes fail to show shortening of RBC life span.25,26 Reports of single cases implicating agents such as melphalan,27 dimercaprol,28 doxorubicin,29 and sodium metaarsenophan noramidipyrine30 are difficult to interpret. When more than a decade has passed without any confirming report, one is inclined to regard the originally reported episode as being coincidental rather than etiologic.

Detailed analysis of the evidence regarding the hemolytic potential of a large number of drugs and chemicals has been published previously.19 Table 1 lists drugs and chemicals that appear, on the basis of the available evidence, to cause clinically significant hemolytic anemia. Drugs that can be given safely to G6PD-deficient persons are listed in Table 2.

Favism. A clinical manifestation of G6PD deficiency closely related to drug-induced hemolysis is the hemolytic anemia induced by ingestion of the fava bean, Vicia faba. Favism, this hemolytic anemia, has been known since antiquity. Indeed, the demise of Pythagoras has been attributed to unwillingness to enter a bean field, possibly because of favism,31 although the evidence supporting this interpretation is feeble. Patients with favism are always G6PD deficient, but not all G6PD-deficient individuals develop hemolysis when they ingest fava beans. Thus, G6PD deficiency is a necessary but not sufficient cause of favism. Presumably some other factor, probably also genetic32 and very likely related to metabolism of the active ingredients in the beans, is involved. The vast majority of cases of favism occurs in individuals with severely deficient (class 2) variants of G6PD, but occasionally favism has been observed in a patient with G6PD A−.34,35 Although at times the onset of hemolysis in favism may be more explosive than occurs as a result of drug administration,36 in general the course of hemolysis in favism is very similar to that occurring after drug ingestion. Hemolysis does not usually begin for 24 hours after ingestion of the beans and hemoglobinuria may continue for several days.11

Mechanism of hemolysis. The mechanism by which drugs and fava beans produce hemolytic anemia is not well understood. Such drugs do not lyse RBCs in vitro.33 Instead, they appear to inflict oxidative injury on the erythrocytes and, therefore, are often designated as oxidative drugs. Because of its relatively high frequency in some areas in the Mediterranean region, the mechanism by which fava beans produce hemolysis has received special attention, with the suggestion that the pathogenesis of favism and drug-induced hemolytic anemia may be essentially the same.35 Vicine, convicine, ascorbate, and L-DOPA are abundant in fava beans and have been considered candidate toxins. The most likely offenders are vicine and convicine, β-glucosides of pyrimi-

Table 1. Drugs and Chemicals That Should Be Avoided by Persons With G6PD Deficiency

<table>
<thead>
<tr>
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Table 2. Some Common Drugs That Can Safely Be Administered in Therapeutic Doses to G6PD-Deficient Subjects Without Nonspherocytic Hemolytic Anemia

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* Very high "therapeutic" doses (~80 g administered intravenously) have precipitated severe, even fatal, hemolysis.
dine compounds that are converted by β-glucosidases to their aglycones, vicine and isouramil, respectively. These compounds form reactive semiquinone-free radicals and can generate active oxygen species. This results in the formation of ferrylhemoglobin, methemoglobin, and inactivation of various enzymes. The reactions that occur are complex and varied and, therefore, largely unpredictable. 

New drugs continue to be introduced into medical practice, and it would be extremely useful to be able to predict which of these cannot safely be given to patients with G6PD deficiency. Unfortunately those drugs that do produce hemolysis have no clearly understood common denominator either in structure or in chemical properties. Moreover, in some (perhaps in most) instances the injury to the enzyme-deficient erythrocyte is not mediated by the chemical compound that is administered, but rather by a metabolic product. In vitro systems have been devised in an attempt to mimic what occurs in the body. The RBCs of some animal species, notably sheep, regularly have low RBC G6PD levels. Moreover, hereditary deficiencies occurring within species are documented, but these have limited appeal with respect to attempts to predict the hemolytic potential of drugs because of species differences in drug metabolism and of RBC metabolism.

Infection-induced hemolysis. Although, for historical reasons, drug-induced hemolysis has attracted the most attention, it is likely that hemolysis induced by infection may be a more common cause of clinically significant hemolysis. Numerous reports attest to the importance of infection in causing hemolytic anemia. It is clear that many different types of infections may trigger hemolysis in the G6PD-deficient patient. The mechanism by which this occurs is not clear, but an imaginative suggestion has been that during phagocytosis, leukocytes damage erythrocytes in their environment by discharging active oxygen species during phagocytosis. Perhaps nitric oxide might also play such a role. It is unlikely that such a mechanism is operative in the case of viral infections such as hepatitis, but it may play a role in some infections.

Diabetes mellitus-induced hemolysis. It has been suggested that episodes of diabetic acidosis may precipitate hemolytic episodes in persons with G6PD deficiency, but in one study, no evidence was found that such an effect existed. It has also been reported that hypoglycemia may precipitate hemolysis in G6PD deficiency.

Hereditary nonspherocytic hemolytic anemia. It was in 1958, not long after G6PD deficiency was identified as the cause of primaquine sensitivity, that it was recognized that the enzyme deficiency could cause chronic hemolysis as well. The syndrome of hereditary nonspherocytic hemolytic anemia did not occur in persons who inherited the common, polymorphic variants of G6PD such as G6PD A– or G6PD Mediterranean, but rather in patients who had inherited rare mutations, designated class 1 because of their association with chronic hemolysis. (Exceptional instances in which class 2 variants have seemed to be associated with chronic hemolytic anemia are discussed below.) The severity of hemolysis varies greatly. Although it is usually mild, the patient with "G6PD Campinas" has transfusion-dependent hemolysis resembling thalassemia major.

Presumably class 1 variants produce chronic hemolysis because the functional severity of the defect is so great that the erythrocyte cannot even withstand the normal stresses that it encounters in the circulation. The functional severity in these patients is not usually reflected by the level of the enzyme as it is measured in the laboratory. The RBCs of patients with class 1 variants may have residual G6PD activity as high as 35% of normal when measured under standard conditions. The functional impairment that leads to the shortening of the RBC life span in these patients may include such factors as susceptibility to inhibition by NADPH and in vivo lability. Possibly the most consistent common feature of class 1 variants is the location of the mutation. In the great majority of cases, it is in the region of the putative NADP-binding or glucose-6-phosphate binding site of the molecule. (see below and Fig 1)

Neonatal Jaundice

Neonatal jaundice is one of the most life- and health-threatening consequences of G6PD deficiency, and kernicterus may occur in these infants. It is often erroneously assumed that the jaundice is the result of hemolysis. However, this is apparently not usually the case. Anemia is not present in G6PD-deficient infants that develop neonatal icterus. Instead of the icterus being a manifestation of accelerated RBC destruction, it now seems likely that it is largely the result of the impairment of liver function, presumably because of a deficiency of the enzyme in the liver. It is entirely possible that some shortening of RBC life span also plays a role. Neonatal jaundice has occurred primarily in Asian and Mediterranean infants. In one study, G6PD Aures has been associated with a particu-
larly high incidence of jaundice. Early reports from the United States suggested that African-American infants did not have a significantly increased incidence of neonatal jaundice.\textsuperscript{112,114} However, anecdotal observations from the United States\textsuperscript{315} and surveys in Jamaica\textsuperscript{100} and in Africa\textsuperscript{116-118} all suggest that an increased incidence of neonatal icterus may occur also in infants with G6PD A−. 

**Transfusion With G6PD-Deficient Blood**

There is evidence that G6PD-deficient RBCs maintain viability less well than do normal cells even without being subjected to oxidative stress.\textsuperscript{119} However, the consequences of transfusing a single unit of G6PD-deficient RBCs into an adult are probably minor. It has been pointed out that in the case of G6PD A−, the number of cells that would be destroyed if a hemolytic stress occurred would be no greater than the number of nonviable cells in a unit of blood nearing its expiration date.\textsuperscript{120} Transfusion of G6PD-deficient blood may be an issue of greater potential importance in parts of the world in which the incidence of the defect is very high and where more severely deficient class 2 variants such as G6PD Mediterranean are prevalent. In such areas, it is possible for a patient to receive, by chance, several units of deficient blood. In one instance, it has been suggested that fatal hemolysis occurred in a young woman as a result of receiving G6PD-deficient blood,\textsuperscript{111} but the reports of such severe consequences are not themselves convincing of a cause-and-effect relationship. In a controlled study, only minor increases in bilirubin levels were found in individuals receiving a unit of severely G6PD-deficient blood,\textsuperscript{122} but the changes might be greater if a hemolytic stress were present.

In general, it has not been the practice to screen blood bank blood for G6PD deficiency, even in areas in which the gene frequency is very high.\textsuperscript{123} However, caution is justified in the exchange transfusion of newborn infants. Here, in contrast to adults, the proportion of deficient cells could be very high, and the products of Hb catabolism disposed of inefficiently by the immature liver.\textsuperscript{124-126}

**Other Manifestations of G6PD Deficiency**

It is reasonable to assume that a genetic trait that has reached such high frequencies in many populations that it is carried by some 200,000,000 persons would not have a readily apparent effect on fitness. For this reason, if no other, it has been generally assumed that those who carry polymorphic genes for G6PD deficiency would not suffer from any morbidity. Nonetheless, a number of studies have suggested that G6PD-deficient individuals might, even in the absence of any stress, have some clinical abnormalities.

**Tissue distribution of the deficiency.** Early studies indicated that the deficiency of G6PD activity was limited to the RBCs; liver and leukocyte activity was reported to be normal, and it was even suggested that the defect might not be in the G6PD gene itself, but rather some other gene that influenced the stability of the enzyme in the erythrocyte.\textsuperscript{127} Platelet activity was found to average about 40% of normal.\textsuperscript{128}

These studies were probably performed on patients with G6PD A−, and subsequent investigations in patients with more severely deficient variants indicated that other tissues were indeed involved in G6PD deficiency. Mediterranean subjects were found to have leukocyte enzyme activity that was only 22%\textsuperscript{129} and 39%\textsuperscript{130} of normal, platelet activity that was 19% of normal,\textsuperscript{120} saliva activity that was 7% of normal,\textsuperscript{111} and liver activity that was 60% of normal.\textsuperscript{121} In one study of liver biopsy samples, marked variability in the level of enzyme was found, but the activity was consistently lower in G6PD A− subjects than in most subjects who did not have RBC G6PD deficiency.\textsuperscript{133} G6PD activity could not be detected in the breast milk of severely deficient mothers.\textsuperscript{114} Cultured fibroblasts from a deficient male from Ferrara, Italy had only 10% of normal G6PD activity.\textsuperscript{113} In Chinese patients with severe RBC enzyme deficiency, the leukocyte G6PD activity was 25% of normal, platelets 28% of normal, liver 49% of normal, adrenal 13% of normal and kidneys 13% of normal.\textsuperscript{136} Lenses from patients with G6PD A− were found to contain about 40% of normal activity\textsuperscript{127} and cataractous lenses of Mediterranean subjects were devoid of detectable enzyme when cataracts of patients with normal erythrocyte G6PD levels averaged 5 mU/mg protein.\textsuperscript{138}

**Hematologic effects.** In our original studies of G6PD deficiency, \textsuperscript{14}Cr erythrocyte survival was determined on many primaquine sensitive subjects, who now would be designated as being hemizygotes for G6PD A−. The baseline RBC survivals of these subjects was normal.\textsuperscript{10,18,110} Subsequently, studies of \textsuperscript{32}DFP and \textsuperscript{51}Cr RBC survival in such G6PD A− subjects were claimed to show marked shortening of the erythrocyte life span, with a mean \textsuperscript{51}Cr half-life of only 20.2 days in three subjects with a control mean of 28.7 days.\textsuperscript{140} The average \textsuperscript{32}DFP-labeled RBC half-life of G6PD-deficient subjects was reported to be 48 days with a mean normal of 66.1 days.\textsuperscript{148} Such shortening of RBC life-span has not been observed either before or after this one investigation, even with much more severe forms of G6PD deficiency, and the finding of marked shortening of RBC life span in G6PD A− subjects in the absence of a stress cannot be regarded as valid. However, some minimal shortening of RBC life span has been observed in some studies of Mediterranean subjects with G6PD deficiency: mean \textsuperscript{51}Cr half-lives of 22.9 days,\textsuperscript{141} 26 days,\textsuperscript{142} and 28.9 days.\textsuperscript{143} Interestingly, even though the RBC life span of these subjects is nearly normal, three reports document a slight decrease in the Hb concentration of the blood of normal subjects with the Mediterranean form of G6PD deficiency.\textsuperscript{144,146} In one of these studies,\textsuperscript{145} the mean difference between the Hb levels of deficient and normal subjects was nearly 2 g/dL and was accompanied by an increase in the average reticulocyte count of 0.28%, and of the mean corpuscular volume of 3.9 fl., all of these differences being statistically significant. There have also been occasional cases of apparent G6PD Mediterranean with low-grade hemolysis,\textsuperscript{143,145} although these studies were performed before verification of the genotype by DNA analysis was possible. Thus, it appears that under certain circumstances, either genetic or environmental, low-grade hemolysis can occur in persons with G6PD Mediterranean. It is doubtful whether this actually occurs in the milder, class 3 variants such as G6PD A−.

**Life expectancy.** Large-scale studies have assessed the effect of G6PD A− on the overall health of Afro-American
veterans. In an investigation of 1,413 black males Petrakis et al. found that the incidence of G6PD deficiency was 12.1% in the 5- to 20-year age group, 5.6% in the 21- to 49-year age group, and only 3.8% of those above the age of 49. While acknowledging that there might be a number of explanations for this, they concluded that G6PD-deficient subjects had a reduced life span. However, this seems unlikely in view of the fact that it would require a very high excess mortality rate among persons with G6PD deficiency. Indeed, a study of 65,154 black male patients admitted to US Veteran's Administration hospitals showed no increased mortality among patients who were G6PD deficient and no significant difference in the mean ages of G6PD-deficient and -nondeficient patients.

Cancer. Epidemiologic studies suggested to some that the incidence of cancer may be lower in G6PD-deficient persons. However, these investigations were generally based on screening methods that do not efficiently ascertain G6PD deficiency in heterozygotes, and even in hemizygotes who have a disorder that might decrease RBC life span. Indeed, in one study it was shown that the RBC G6PD activity of cancer patients is higher than that of controls. More recent studies tend not to show any differences between the incidence of cancer in G6PD-deficient and normal subjects.

Other putative clinical and laboratory abnormalities. The many other possible clinical and laboratory consequences of G6PD deficiency that have been proposed, either on the basis of anecdotal observations or more detailed studies are summarized in Table 3. In many instances, contradictory data have also been presented and it is difficult to judge the validity of the many claims that have been made.

### Table 3. Putative Abnormalities Suggested to Occur in Persons With G6PD Deficiency

<table>
<thead>
<tr>
<th>Proposed Effect</th>
<th>Evidence</th>
<th>Class*</th>
<th>Reference</th>
<th>Contradictory Reference</th>
</tr>
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<tbody>
<tr>
<td>Platelet abnormalities</td>
<td>A</td>
<td>2</td>
<td>366, 367</td>
<td>368</td>
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<tr>
<td>Skin pedicle flap loss</td>
<td>A</td>
<td>3</td>
<td>369</td>
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<tr>
<td>Athletic performance, impairment</td>
<td>C</td>
<td>3</td>
<td>370</td>
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<tr>
<td>Seizure disorders</td>
<td>A</td>
<td>1, 2</td>
<td>371, 372</td>
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<tr>
<td>Cataracts, increased incidence</td>
<td>C</td>
<td>2</td>
<td>371, 373-375</td>
<td>377</td>
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<tr>
<td>Manifestations of schizophrenia or depression</td>
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<td>3</td>
<td>376</td>
<td>378, 379</td>
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<td>Renin release, impairment</td>
<td>C</td>
<td>2</td>
<td>382</td>
<td></td>
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<td>Glucose tolerance tests, abnormalities or diabetes</td>
<td>C</td>
<td>3</td>
<td>383, 384</td>
<td>385-387</td>
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<tr>
<td>Insulin release, abnormalities</td>
<td>C</td>
<td></td>
<td>388</td>
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<tr>
<td>Cortisol production, decrease</td>
<td>C</td>
<td>2, 3</td>
<td>388</td>
<td>389</td>
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<tr>
<td>Survival, decrease</td>
<td>C</td>
<td>3</td>
<td>147</td>
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<td>Cardiovascular disease, risk factor</td>
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<td>Cholelithiasis</td>
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<td>Myoglobinuria</td>
<td>A</td>
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<td>393, 394</td>
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<tr>
<td>Susceptibility to infection, increased</td>
<td>C</td>
<td>21</td>
<td>395</td>
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<tr>
<td>Coronary artery disease, decreased incidence</td>
<td>C</td>
<td>3, 4</td>
<td>396</td>
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<tr>
<td>Leukocyte function, abnormality</td>
<td>A</td>
<td>12</td>
<td>396, 399</td>
<td>400</td>
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<td>Increased jaundice in hepatitis</td>
<td>A</td>
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<tr>
<td>Mental retardation</td>
<td>C</td>
<td>2</td>
<td>402</td>
<td></td>
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<tr>
<td>Dehydroepiandrosterone sulfate, increased serum levels</td>
<td>C</td>
<td>2</td>
<td>403</td>
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</tbody>
</table>

Abbreviations: A, anecodal; C, controlled series.

* Class 1, hereditary nonspherocytic hemolytic anemia; class 2, severe deficiency; class 3, mild deficiency; class 4, not deficient (eg, G6PD A+). Classification based on racial origin if data not given.

### The Enzyme

#### Structure

The G6PD monomer consists of 515 amino acid subunits with a calculated molecular weight of 59,256 daltons. The active enzyme exists as a dimer and contains tightly bound NADP. Aggregation of the inactive monomers into catalytically active dimers and higher forms requires the presence of NADP. Thus, NADP appears to be bound to the enzyme both as a structural component and as one of the substrates of the reaction. The binding sites for this coenzyme have not been identified at the structural level, but examination of mutants has suggested that amino acids 386 and 387, the basic amino acids lysine and arginine, respectively, seem to bind one of the phosphates of NADP. The evidence that this site is involved in the binding of NADP is as follows: (1) all mutants that rapidly lose activity at a 10 μmol/L NADP concentration, but are reactivated at high concentrations of NADP have been shown to have mutations in this region; (2) mutations in this region result in paradoxical electrophoretic migration of the enzyme as if it had become more positively charged, even when the amino acid change adds a negative charge, suggesting failure of binding of negatively charged NADP. It has also been suggested, on the basis of the deduced conformation of the peptide chain of the yeast enzyme, that the NADP binding site may be elsewhere, but the data on the human enzyme seems much more compelling to me. The glucose-6-phosphate binding site has been identified at amino acid 205 by locating a lysine at this position that is reactive with pyridoxal phosphate in competition with glucose-6-phosphate.
<table>
<thead>
<tr>
<th>Variant</th>
<th>Nucleotide Substitution</th>
<th>WHO Class</th>
<th>Amino Acid Substitution</th>
<th>References</th>
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<td>32 His → Arg</td>
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<td>&quot;Sunderland&quot;</td>
<td>105-107 del</td>
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<td>35 Ile → del</td>
<td>405</td>
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<tr>
<td>&quot;Aures&quot;</td>
<td>143 T → C</td>
<td>2</td>
<td>48 Ile → Thr</td>
<td>406</td>
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<tr>
<td>Metaponto</td>
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<td>58 Asp → Asn</td>
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<td>Distrito Federal</td>
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<td>&quot;Matera&quot;</td>
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<td>Castilla</td>
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<td>Ferrara</td>
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<td>Ube</td>
<td>241 C → T</td>
<td>3</td>
<td>81 Arg → Cys</td>
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<td>Konan</td>
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<td>[317 C → G]</td>
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<td>[544 C → T]</td>
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<td>[592 C → T]</td>
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<td>São Borna</td>
<td>337 G → A</td>
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<td>113 Asp → Asn</td>
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<td>A</td>
<td>376 A → G</td>
<td>4</td>
<td>126 Asn → Asp</td>
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<td>163 Gly → Asp</td>
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<td>165 Asn → Asp</td>
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<td>527 A → G</td>
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<td>Santamaria</td>
<td>[542 A → T]</td>
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<td>[181 Asp → Val]</td>
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<td>[126 Asn → Asp]</td>
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<td>Dallas</td>
<td>563 C → T</td>
<td>2</td>
<td>188 Ser → Phe</td>
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<td>Birmingham</td>
<td></td>
<td></td>
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<td>&quot;Coimbra&quot;</td>
<td>592 C → T</td>
<td>2</td>
<td>198 Arg → Cys</td>
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<td>&quot;Santiago&quot;</td>
<td>593 G → C</td>
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<td>198 Arg → Pro</td>
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<td>Sibari</td>
<td>634 A → G</td>
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<td>Minnesota</td>
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<td>213 Val → Leu</td>
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<td>&quot;Harilaou&quot;</td>
<td>648 T → G</td>
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<td>216 Phe → Leu</td>
<td>217, 420</td>
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<tr>
<td>&quot;Mexico City&quot;</td>
<td>688 G → A</td>
<td>3</td>
<td>227 Arg → Gln</td>
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(Continued on following page)
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<tr>
<th>Variant</th>
<th>Nucleotide Substitution</th>
<th>WHO Class</th>
<th>Amino Acid Substitution</th>
<th>References</th>
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</table>
| A-      | 680 G → T  
376 A → G | 3         | 227 Arg → Leu  
126 Asn → Asp | 204 |
| "Stonybrook" | 724-729  
GCC del | 1         | 242-243 | 219 |
| Wayne   | 769 G → C | 1         | 257 Arg → Gly  
Gly & Thr | 424 |
| "Cleveland" | 820 G → A | 1         | 274 Glu → Lys | 219 |
| "Chinese-1" | 835 A → T | 2         | 279 Thr → Ser | 250 |
| Seattle | 844 G → C | 2         | 282 Asp → His  
| Lodi   | "Modena" | 2         | 285 Arg → His | 426 |
| "Montalbano" | 854 G → A | 3         | 285 Arg → His | 426 |
| Viangchan | 871 G → A | 2         | 291 Val → Met | 424 |
| Jammu   | "West Virginia" | 1         | 303 Val → Phe | 219 |
| Kalyam  | 949 G → A | 3         | 317 Glu → Lys | 427 |
| Kerala  | "Nara" | 968 T → C  
376 A → G | 3         | 323 Leu → Pro  
126 Asn → Asp | 204 |
| Betica  | 953-976 del | 1         | 319-326 del | 428 |
| Selma   | 1003 G → A | 3         | 335 Ala → Thr | 407 |
| Chatham | 1004 C → A | 2         | 335 Ala → Asp | 219 |
| "Fushan" | 1024 C → T | 1         | 342 Leu → Phe | 416 |
| "Chinese-5" | 1057 C → T | 2         | 353 Pro → Ser | 423 |
| "Urepeta" | 1069 C → A | 1         | 363 Asn → Lys | 216 |
| Loma Linda | 1114 T → C | 1         | 381 Phe → Leu | 219 |
| Tomah   | 1153 T → C | 1         | 385 Cys → Arg | 407 |
| Iowa    | 1156 A → G | 1         | 386 Lys → Glu | 163 |
| Walter Reed Iowa City Springfield |         |       |       | |
| Guadalajara | 1159 C → T | 1         | 387 Arg → Gys | 423 |
| "Mt. Sinai" | 1159 C → T  
376 A → G | 1         | 387 Arg → Cys  
126 Asn → Asp | 248 |
| Beverly Hills Genova Worcester |         | 1         | 387 Arg → His | 163 |
| "Praba" | 1160 G → A | 1         | 387 Arg → His | 429 |
| Genova Worcester |         | 1         | 387 Arg → His | 409 |
| Nashville Anaheim |         | 1         | 393 Arg → His | 216 |
| "Calgary" "Portici" |         | 1         | 393 Arg → His | 430 |
| Alhambra | 1180 G → C | 1         | 394 Val → Leu | 423 |
| "Puerto Limon" | 1192 G → A | 1         | 398 Glu → Lys | 420 |

(Continued on following page)
Table 4. G6PD Variants That Have Been Characterized at the DNA Level (Cont’ed)

<table>
<thead>
<tr>
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<th>Amino Acid Substitution</th>
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<tr>
<td>Taiwan-Hakka Gifu-like</td>
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<td>459 Arg → Leu</td>
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</table>

Class 1, nonspherocytic hemolytic anemia; class 2, severe deficiency; class 3, moderate deficiency; class 4, not deficient.

Enzymology

G6PD catalyzes the first step in the hexose monophosphate pathway (HMP). It oxidizes glucose-6-phosphate to 6-phosphogluconolactone, reducing NADP to NADPH. The HMP is the only source of NADPH in the erythrocytes and it also serves to produce the ribose needed for synthesis of nucleotides in the salvage pathways. The main function of the pathway seems to be to protect the RBC against oxidative damage. Glutathione peroxidase (GSHPx) removes peroxide from the erythrocyte. Reduced glutathione (GSH) serves as a substrate for this enzyme, and because NADPH is required for the reduction of oxidized glutathione and protein sulfhydryl groups, it is an essential factor in the chain of reactions that defends the RBC against peroxide. RBCs are a particularly rich source of catalase, but this enzyme is relatively inefficient at removing low levels of peroxide levels. Moreover, catalase has the ability to bind NADPH tightly and the inactive form, compound II, is reactivated by NADPH. Thus, the activity of the HMP serves to remove peroxide not only through the action of GSHPx, but also by activating catalase. The long-standing controversy about which is the more important, catalase or GSHPx, seems to me to be largely irrelevant and futile. Clearly, both enzymes may play a role and serve as backup mechanisms for each other. Which is more active at any one time may depend on the particular conditions under which the measurements are being made and very likely the particular peroxide substrate that is being catabolized.

The Km of G6PD for NADP is very low, roughly 2 to 4 μmol/L, and the enzyme is strongly inhibited competitively by NADPH. Thus, the NADP/NADPH ratio within the RBC controls the rate of the reaction in an autoregulatory manner. In the quiescent state, the NADP/NADPH ratio is very high, and G6PD is nearly completely inhibited. When NADPH is oxidized, as when oxidized glutathione is reduced in the glutathione reductase reaction, NADPH is converted to NADP and G6PD becomes active, reducing NADP to NADPH. G6PD-deficient cells are unable to respond adequately to such an oxidative stress. When the susceptibility of a mutant enzyme to inhibition by NADPH is greater than normal, this compounds the metabolic difficulty of the cell.

GENETICS

X-Linkage and X-Inactivation

Even before the basic defect was known, X-linkage of primaquine sensitivity was established by application of the glutathione stability test, the earliest reliable means for
the detection of the defect of primaquine sensitivity. Pedigrees of Afri-
co-American families showed that glutathione instability was most
frequently transmitted from mother to son, although there were
instances in which the defect could not be detected in the mother
and even where apparent father-to-son transmission occurred. It was
correctly presumed that these anomalies were caused by inadequ-
date ascertainment of heterozygous females with the relatively crude
technology then available. With the recognition that the basic
defect was a deficiency of G6PD, X-linkage was confirmed by
estimation of enzyme activity, studies of electrophoretic
mobility, and study of linkage with color blindness. Still, there
were families in which genetic transmission aberrant for
ference was observed. These aberrations led us to suggest
that one of the two X-chromosomes might be
inactive in human females, at the same time and quite
independently of the proposal made by Lyon on the basis
of X-linked traits in mice.

More recently, it has been appreciated that G6PD is one
of a cluster of genes on the distal long arm of the X chromo-
some (q28). Included in this group of genes are those for
the fragile X, hemophilia A, color vision, a putative gene
for bipolar affective illness, the ABP-280
filamin gene, Bornholm eye disease, clasped-thumb
mental retardation (MASA) syndrome, and dyskeratosis
congenita.

The G6PD Gene

G6PD was cloned and sequenced by Persico et al. and then independently by Takizawa and
Yoshida. The gene contains 13 exons and is over 20 Kb in length. The first exon
contains no coding sequence and the intron between
exons 2 and 3 is extraordinarily long, extending for 9,857
bp. The sequence of the entire gene is known. At the 5'
end of the gene is a cytidine-guanine dinucleotide (CpG)-
rich island. Differential demethylation of some of the CpG’s
is associated with expression of the gene on the active X
chromosome and this island appears to be preserved be-
tween man and mouse. A 2,850-bp segment of the 5’ end
has been fused to a reporter and deletional analysis showed
that a 436-bp domain was sufficient for full expression.

Some heterogeneity of G6PD mRNA has been found, but
its functional significance is doubtful. The existence of an
altematively spliced form has been documented, but the amount of this mRNA, which contains 138 nucleotides
of what is usually the 3’ end of intron 7 without losing
frame, is always very small. Production of the enzyme has
been accomplished in vitro in Cos cells and in Echerichia
coli. A suggestion that G6PD was, in reality, a trans-
lation product made from two separate mRNAs has proved to be
based on an artifact.

Mutations

Biochemical characterization has led to the description of
no less than 442 variants of G6PD believed to be distinct.
Two hundred ninety nine of these were characterized by
methods agreed upon by a World Health Organization (WHO) expert group and were considered, at least by those
who described them, as being different from the others that
had been published. Because most mutants of G6PD had
abnormal properties (either electrophoretic, kinetic, or both),
it was to be expected that the mutations affecting this enzyme
would be found in the coding region. This has indeed proven to be the case. Facile polymerase chain reaction (PCR)-based
methods for the detection of mutations have been de-
veloped, and these have made it possible to define the
mutations in many individuals (Table 4).

Distribution and nature of mutations. As of this writing, 60 mutations or combination of mutations have been
documented in G6PD (Table 4); all but one of these are associated
with enzyme deficiency. The types of mutations found are
more restricted than is the case with many other genes. It
appears that total G6PD deficiency is not compatible with
life. Thus, most mutations are missense point mutations and
deletions (of which three are known) and are found in multi-
plies of three nucleotides so that a framesshift does not occur.
Only one splicing mutation has been found and no promoter
mutations have been identified. There is only one exception
to the rule that mutations found in patients do not preclude
the synthesis of enzyme; a mutation that we have designated
“Georgia” changes Tyr28 to a stop codon. Eighty-three percent
of the peptide chain would have been synthesized by the
time the stop codon were encountered. Perhaps the
truncated protein made is partially functional. It is also note-
worthy that the mutation was found in a female heterozygote,
and it is conceivable that unbalanced X-inactivation helped
to prevent the dire consequences that might otherwise have
been expected from a null mutation.

The distribution of mutations along the length of the
cDNA is also not random, as shown in Fig 1. Point mutations
that cause the formation of class 1 variants, which are those
associated with nonspherocytic hemolytic anemia, are
largely confined to two areas, one of which approximates
the NADP or NADPH binding site of the enzyme and the
other of which is in the region of the glucose-6-P binding
site. As shown in Table 4, of the 23 point mutations that are
associated with class 1 variants, 5 cause substitutions in the
amino acid range 198 to 257 and 15 in the range of 363 to
447. Thus, 87% of these mutations are found in two areas
that comprise only 28% of the polypeptide. There are, of
course, exceptions and cases in which changing a single
codon produces different clinical syndromes. Thus, changing
Met to Val produces a class 1 variant, G6PD Santiago,
whereas changing the same amino acid to Cys produces the
class 2 variant Coimbra. Similarly, the common class 2 vari-
ant G6PD Union is the result of a mutation of Arg to Cys,
whereas a change of the same amino acid to His produces a
variant, G6PD Andalus, associated with mild hemolytic
anemia.

Frequency of mutations in various populations. The
frequency of G6PD deficiency differs markedly among differ-
ent populations. Among black Americans, the gene fre-
quency of enzyme deficiency is 0.10 to 0.11. The
frequency of G6PD Mediterranean is 0.70 among Kurdish
Jews, the highest incidence of G6PD deficiency in any
population. In a Greek survey of over 1,200,000 in-
fants, a gene frequency of 0.045 was documented. In Asia
too, high frequencies are encountered. Detailed population
frequency data may be found in a number of comprehen-
sive reviews,\textsuperscript{19,224,227} but these data were compiled before mutation analysis was possible.

In the era before mutation identification was possible at the DNA level, it was generally recognized that certain types of mutations were characteristic of certain populations. The electrophoretic mobility of deficient samples from Africans was almost always rapid, and the variant characteristic of this ethnic group was designated G6PD $A^{-}$.\textsuperscript{185} In contrast to the electrophoretically rapid, normally active G6PD $A^{+}$ enzyme found in the same population. The more severe deficiency, found in Mediterranean populations, in which the residual enzyme had a normal electrophoretic mobility was designated G6PD Mediterranean.\textsuperscript{228} However, on the basis of biochemical characterization it was believed that there was great heterogeneity among the different variants found in this region of the world.\textsuperscript{229-232} In Asia too, many different variants were characterized.\textsuperscript{233-241}

With development of the ability to define the mutations in the G6PD gene, some aspects of the situation became more complicated, but others were simplified. G6PD $A^{-}$, which had generally been regarded as a distinct, homogeneous mutation, proved to be the result of the superimposition of several point substitutions on the background of G6PD $A^{+}$.\textsuperscript{376G} The mutation always found in G6PD $A^{-}$ is characteristic of G6PD $A^{+}$. In most cases, the second mutation is 202A, but it can also be 968C or 680T (Table 4). The fact that African deficiency mutations of the G6PD $A^{-}$ type appear to occur only in the context of the 376G mutation of G6PD $A^{+}$ suggested to us at one time that the primordial human G6PD may have been G6PD $A^{+}$.\textsuperscript{242,243} It is now clear on the basis of haplotype analysis that the $A^{-}$ mutation is more recent in origin than the prototypal G6PD B.\textsuperscript{244,245} A more attractive explanation for the association of the 376G mutation with other African deficiency mutations is provided by the finding of Town et al\textsuperscript{246} that the 202A mutation produced by site-directed mutagenesis alone is not enough to produce enzyme deficiency; the 376G mutation, which ordinarily does not produce enzyme deficiency, is required. Thus, it is possible that the mutations at nt 202, 680, and 968 would have had no selective advantage against malaria when they occurred in a G6PD gene that did not have the 376G mutation. However, two mutations that have been found to produce hemolytic anemia when present together with the 376G mutation also result in deficiency when found alone. These are 542TZ4' and 159T.\textsuperscript{248}

The data regarding the incidence of G6PD deficiency in various populations\textsuperscript{233-235} can now begin to be viewed from the perspective of which mutations are actually found. The emerging data regarding the distribution of different mutations in various populations is summarized in Table 5. In general, mutations are limited to contiguous geographical areas or to areas where population migrations are well documented. Thus, G6PD $A^{-}$ has a broad distribution that includes all of Africa, Southern Europe, and wherever the slave trade brought Africans to the New World. G6PD Mediterranean is found in Southern Europe, throughout the middle East and in India, and G6PD Canton is found in Asia. An interesting exception to this rule is provided by G6PD Union\textsuperscript{1507T}. This mutation was described originally in the Philippines and has indeed been documented at the DNA level of Jews, Kurdish

| Population Distribution of Common G6PD Mutations |
|---------------------|---------------------|---------------------|
| Mutation            | Population          | Reference           |
| Gaohe\textsuperscript{HGO}; Gaozhou\textsuperscript{HGO} | China               | 219, 249, 404       |
| Aures\textsuperscript{A3C} | Algeria             | 406                 |
|                    | Saudi Arabia        | 103                 |
|                    | Spain               | 247                 |
| Ube\textsuperscript{241T}; Konan\textsuperscript{241T} | Japan               | 411                 |
| A\textsuperscript{252AAT76G+} | Africa             | 246, 266, 436       |
|                    | Italy               | 410, 412            |
|                    | Spain               | 242, 247            |
|                    | Canary Islands      | 219                 |
|                    | Mexico              | 408                 |
| "Chinese-4"\textsuperscript{380T} | China             | 336, 416            |
| "Chinese-3"\textsuperscript{490G} | Philippines       | 249                 |
| Mahidol\textsuperscript{B0A} | Southeast Asia      | 249, 417            |
|                    | China/Taiwan        | 218, 336            |
| Santenmarie\textsuperscript{376G642T} | Costa Rica        | 420                 |
|                    | Canary Islands      | 219                 |
|                    | Italy               | 412                 |
| Mediterranean\textsuperscript{507T} | Italy              | 407, 412            |
|                    | Sardinia            | 219, 421            |
|                    | Greece              | 219                 |
|                    | Saudi Arabia        | 103, 335            |
|                    | Iran                | 335                 |
|                    | Iraq                | 335                 |
|                    | Egypt               | 335                 |
|                    | Jews, Kurdish       | 221                 |
|                    | Jews, Ashkenazi     | 409                 |
| Seattle\textsuperscript{504C} | Italy              | 250                 |
|                    | Spain               | 247                 |
|                    | Sardinia            | 219                 |
|                    | Canary Islands      | 219                 |
| Viangchan\textsuperscript{513A} | India              | 424                 |
|                    | China               | 219                 |
|                    | Laos                | 249, 250, 424       |
|                    | Philippines         | 249, 250            |
| A\textsuperscript{376G640C} | Africa              | 204                 |
|                    | Spain               | 242, 247            |
|                    | Canary Islands      | 219                 |
| Kalyan\textsuperscript{B4AA1} | India              | 427                 |
| Chatham\textsuperscript{1003A} | Philippines        | 249                 |
| "Chinese-6"\textsuperscript{3024T} | China/Taiwan     | 336                 |
| Union\textsuperscript{1507T} | Philippines/Laos    | 249, 250            |
|                    | China               | 219, 336            |
|                    | Japan               | 249                 |
|                    | Spain               | 247                 |
|                    | Italy               | 251                 |
| Canton\textsuperscript{157H7T} | China              | 219, 336, 434, 435  |
| Kaiping\textsuperscript{3890} | China              | 219, 336, 434       |
|                    | Laos                | 250                 |

* See Table 4 for additional designations for the same variant.
G6PD Deficiency

The geographic distribution of G6PD deficiency led Motulsky,272 Siniscalco et al,273 Allison,274 and Allison and Clyde275 to suggest nearly 35 years ago that G6PD deficiency is also one of the polymorphisms that confers resistance to infection with falciparum malaria. The evidence for this, recently reviewed in detail by Greene,276 comes from several types of studies:

1. Epidemiologic investigations indicate that the highest gene frequencies are present among populations living in low-lying areas in which the incidence of malaria is high.277,278 These relationships have been questioned and it has been suggested that an additional factor, perhaps oxidative stress,279,280 may be required for G6PD deficiency to confer immunity to malaria. The malaria parasite appears to be sensitive to oxidative stress,279,280 and it has been suggested that the eating of fava beans protects synergistically with G6PD deficiency against malaria,281,282,283 but it is difficult to explain protection against malaria on this basis in sub-Saharan Africa where fava beans are not cultivated.

2. Decreased parasitemia among patients with G6PD deficiency when compared with normal individuals was originally reported by Allison274 and has been confirmed in a number of studies.277,278,284 A number of negative studies have also been reported,285 but are considered to be
flawed. 275 Although one study indicated that protection extended only to heterozygous females, 298 this conclusion has not been borne out in other investigations, and it seems likely that hemizygous males are also protected.275 However, based on the now-disputed finding that it is heterozygotes that are resistant to malaria, an interesting explanation was devised. It was suggested that when deficient cells are parasitized that resistant to malaria, an interesting explanation was devised. fer an advantage on patients with sickle cell disease, pro-
parasites present in the cells with normal enzyme activity that for G6PD deficiency in the African population has led to many investigations regarding the possible relationships for G6PD deficiency, Luzzatto et al.274 showed that more individuals in whom the proportion of African genes was higher probability of carrying both of these defects than individuals in whom the proportion of African genes was lower. 279 Indeed, it has been shown that G6PD deficiency does not affect the clinical course of sickle disease,294 neither increasing its severity as had been suggested 292 or decreasing it as had also been proposed.296 Moreover, most studies of fairly homogeneous populations show the incidence of hemoglobin S and G6PD deficiency are quite independent.293,297

DIAGNOSIS

Detection of G6PD Deficiency

Before the underlying defect, G6PD deficiency, had been uncovered, two methods for detecting individuals sensitive to the hemolytic effect of primaquine had been developed, the Heinz body test288 and the GSH stability test.296 Although still occasionally used, these surrogate tests are obsolete and no longer have a role in the diagnosis of G6PD deficiency. Instead, quantitative assays or screening tests that detect severe deficiency should be used to diagnose the disorder. Quantitation of G6PD activity in erythrocytes. The simplest type of quantitative assay measures the reduction of NADP to NADPH in the presence of glucose-6-P and hemolysate. In reality, this type of assay measures both G6PD and 6-phosphogluconate dehydrogenase (6-PGD) activity. In the reaction mixture, as in the cell, the immediate product of the G6PD reaction, 6-phosphogluconolactone is converted to 6-phosphogluconate which serves as substrate for the 6-PGD reaction. Thus, 2 moles of NADP are reduced for each mole of glucose-6-P consumed in the mixture. Although methods that measure G6PD activity independently of 6-PGD deficiency have been available for many years,308,312 such methods have little additional utility in diagnosing the deficiency state, because 6-PGD does not usually limit the rate of the reaction, particularly in G6PD-deficient individuals.

Screening for G6PD deficiency. In hemizygous males who are not undergoing hemolysis, as will be found in population surveys, semi-quantitative or nonquantitative screening methods are entirely adequate. Dye reduction tests, first introduced by Motulsky and Campbell-Kraut 213 as the brillant cresyl blue decolorization test, have been widely used. Other receptors for the electrons from NADPH generated in the G6PD and 6-PGD reactions include methylene blue, 314 MTT tetrazolium, 313 and methemoglobin.316 A test in which protection against denaturation of Hb under oxidative stress serves as an endpoint has also been developed. 309,317 Although all of these tests are still sometimes used, particularly in population surveys, they have largely been replaced by the fluorescent spot test, in which the generation of NADPH is detected directly visually under ultraviolet light.309,317,321

Detection of G6PD deficiency in patients undergoing hemolysis. While the diagnosis of deficient males ordinarily poses no special difficulties, the same cannot be written about the detection of G6PD deficiency in patients with some of the milder G6PD-deficient variants (class 3) undergoing a hemolytic episode. Because the older members of the RBC population are selectively removed in patients with variants such as G6PD A+−,14 leaving the younger cells with near-normal activity in the circulation,15 a screening test may give quite normal results, at least for a week or two after the hemolytic episode. The same problem in diagnosis does not exist in the case of severe (class 2) variants because in these variants, even the very young cells are severely enzyme deficient.15,16

Several different approaches may be used to diagnose patients who have just undergone hemolysis. The simplest is merely to wait for a week or two or to perform family studies. Alternatively, one may deplete the sample being studied of reticulocytes by centrifugation. The denser cells, although not truly old as has sometimes been believed, are depleted of very young RBCs.322 Accordingly, it has been found that even during hemolysis, the dense fraction of cells is G6PD deficient.323,324 Another approach is to compare the activity of G6PD with that of another age-dependent RBC enzyme such as hexokinase or glutamic oxaloacetic transaminase.325 This approach has been used also to detect the G6PD A− genotype in patients with sickle cell disease, in which the mean RBC age is greatly decreased.299

The most powerful approach for establishing the diagnosis
in the context of hemolysis is analysis of genomic DNA obtained from circulating leukocytes (see below). Neither the presence of young erythrocytes nor, for that matter, of transfused cells confounds the results obtained from such an analysis.

**Heterozygote detection.** Detection of heterozygotes for G6PD deficiency poses special problems. Because of X-inactivation, heterozygotes have two RBC populations,\(^ {110,336}\) One of these populations consists of normal RBCs and the other of RBCs that are as deficient as those of a hemizygous male with the same deficient variant. On the average, half of the cells are normal and half are deficient. However, in some heterozygous women most of the cells are deficient; in others most are normal. The result of assaying the activity of enzyme per gram Hb reflects the proportion of normal and abnormal cells in the individual being studied, and some heterozygous women will have normal RBC enzyme activity whereas others will be grossly deficient in enzyme activity. Thus, the usual RBC enzyme activity measurements cannot be relied upon for the detection of heterozygotes.

A more acceptable approach is to use techniques in which each RBC acts as an independent metabolic unit. Methemoglobin reduction can be used for this purpose, but only if the dye that links methemoglobin reduction to NADP reduction does not result in cell-to-cell interaction. Nile blue sulfate can be used for this purpose, but not methylene blue.\(^ {327,328}\) Reduction of a tetrazolium dye can also serve as an endpoint.\(^ {330-332}\) Although such methods may be able to identify heterozygotes with as few as 5% to 10% normal or abnormal cells, some heterozygotes will escape detection because virtually no normal or no abnormal cells are present in the circulation.

The most accurate method for heterozygote detection is to detect the mutation in genomic DNA. Although X-inactivation may alter the methylation pattern on the inactive X-chromosome\(^ {331,333}\) and prevent transcription of the inactive gene,\(^ {334}\) it does not prevent the detection of the difference in the nucleotide sequence of the gene. Thus, heterozygote detection by DNA analysis is entirely reliable, provided that the mutation to be detected is known.

**Identification of G6PD variants.** It became apparent early in the study of G6PD deficiency that there were differences in the characteristics of the residual enzyme in different deficient individuals. Fortunately, a WHO expert committee standardized the methods for the purification and characterization of G6PD variants in 1967,\(^ {13}\) and most investigators subsequently used the same techniques for the examination of different variants. The technology that was agreed upon consists of partially purifying the enzyme by absorption on and elution from diethylaminoethyl cellulose, followed by ammonium sulfate fractionation. The partially purified enzyme is then examined kinetically, electrophoretically, and by measuring its thermal stability. This technology proved to be useful in obtaining a general impression of the degree of diversity of G6PD in various populations. However, the volumes of blood required were large, and it was often difficult to be certain whether relatively minor differences in properties were caused by the existence of new variants or whether the observed variation was methodologic. As pointed out above, 442 variants have been claimed to be distinct. Variants that were believed to be likely to be different, specifically, G6PD Cornell and Chicago, were shown to be from members of the same extended family.\(^ {334}\)

The development of a number of PCR-based methods for the detection of known mutations in G6PD has made it possible to detect G6PD deficiency and to identify the specific mutation responsible with relative ease. The advantage of the use of this type of technology is that DNA samples are much more stable than the enzyme in blood samples, and that very small volumes suffice for diagnosis. Methods of detection include the use of restriction endonucleases to cleave naturally occurring restriction sites\(^ {335,336}\) or restriction sites produced by making mismatched oligonucleotides\(^ {335,336}\) and allele-specific oligonucleotide hybridization.\(^ {349}\) These methods are sufficiently facile for population screening and require so small a sample that they can be used for prenatal diagnosis.\(^ {337}\)

**TREATMENT**

When hemolytic episodes occur in G6PD-deficient individuals, the inciting agent, drug or infection, should be removed whenever possible. However, in patients who have class 3 variants such as G6PD A+, \(^ {130,333}\) it may be possible to continue essential drug therapy with careful monitoring of the blood count. Blood transfusion is only occasionally required to support patients who have undergone severe hemolytic episodes, usually in patients with favism.

It has been suggested\(^ {338}\) that attacks of favism may be ameliorated by the administration of desferrioxamine. In one study,\(^ {339}\) patients with favism who received a single 500-mg dose of desferrioxamine and packed RBC transfusions had a shorter duration of hemoglobinuria, greater rise in Hb level and more rapid drop in reticulocyte count than control patients who received packed cells alone. However, it was not clear that both groups received the same volume of transfusion.

To permit NADPH to be produced by a different route, xylitol administration has also been proposed as a way to prevent or treat hemolysis of G6PD deficiency.\(^ {340}\) Clinical studies in which two severely G6PD-deficient volunteers were pretreated with 10 g xylitol per day and then given primamaque and 20 g xylitol per day showed no protection against hemolysis.\(^ {341}\)

It has been suggested that vitamin E, by virtue of its antioxidant effect, might protect against chronic hemolysis in G6PD deficiency causing chronic hemolytic anemia. Some studies have shown a favorable response to this vitamin,\(^ {141,342,344}\) others have not.\(^ {345,346}\)

The most dangerous consequence of G6PD deficiency is neonatal icterus. Kernicterus has been documented repeatedly in populations in which class 2 variants are common,\(^ {106,109,107,347-352}\) and it has been pointed out this is an important preventable form of mental retardation.\(^ {308}\) Phototherapy\(^ {111,353,354}\) has been used to reduce bilirubin levels, and phenobarbital has been used prophylactically with some success.\(^ {104}\) Agar, given to reduce bilirubin reabsorption, was found to be ineffective.\(^ {355}\) Exchange transfusion is required if the bilirubin exceeds 20 mg/dL,\(^ {111}\) but G6PD-deficient blood should not be used for this purpose.
Future Prospects

It has now been almost 40 years since G6PD deficiency was identified as the cause of primaquine sensitivity, and many thousands of papers documenting clinical events, population distribution, biochemical characteristics, and molecular biology have been published. Are there any questions that remain to be answered? The natural occurrence of many mutations and documentation of their biochemical effects is a rich resource for furthering our understanding of structure-function relationships of enzymes. For this reason, both our group and Luzzatto's, working together with crystallographers, have invested considerable effort in attempting to solve the three-dimensional structure of the enzyme. Although we have succeeded in crystallizing the enzyme (Fig 2), it is apparently too inhomogeneous to allow useful information to be obtained. Thus, our understanding of the functional sites has been limited to inferences drawn from the location of mutations that have well-defined biochemical defects. Answers to other questions are needed as well. We would like to understand the difference between individuals who develop favism and those who do not. Are divicine and isouramil really the active principles of the beans? What are the actual mechanisms of drug-induced and infection-induced hemolysis? What is the mechanism of neonatal icterus? Why is it the RBCs that are primarily affected in the deficiency state?

It is of the nature of science that as we solve problems new problems arise. G6PD deficiency is no exception to this rule.

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G6PD deficiency

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