Glucose-6-Phosphate Dehydrogenase Deficiency

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Most hemolytic anemias can be categorized, at least at first approximation, as being either inherited or acquired, secondary to either intracorpuscular or extracorpuscular causes. Hemolytic anemia associated with deficiency of glucose-6-phosphate dehydrogenase (G6PD) glaringly defies this categorization. Indeed, the majority of persons with inherited G6PD deficiency have no anemia and almost no hemolysis. Both develop only as a result of challenge by exogenous agents. Because the metabolic role of G6PD in red blood cells is primarily related to its reductive potential, the threat to G6PD-deficient red cells is oxidative damage. Therefore, to understand hemolytic anemia associated with G6PD deficiency, we first need to define the physiologic role of G6PD and pinpoint why the red cells are deficient in G6PD.

**G6PD IN RED CELL METABOLISM**

In metabolic maps, G6PD is commonly referred to as the first enzyme of the hexose monophosphate shunt, or the pentose phosphate pathway (Fig. 17-1). Although these time-honored phrases persist in textbooks, it is now clear that the main role of G6PD is not glucose utilization (G6PD accounts for less than 10% of that); rather, it is production of the reduced form of nicotinamide adenine dinucleotide phosphate (NADPH). NADPH has a crucial role in preventing oxidative damage to proteins and to other molecules in all cells (Fig. 17-2). This role is particularly crucial in red cells because being oxygen carriers par excellence, they have a literally built-in danger of damage by oxygen radicals generated continuously in the course of methemoglobin formation. The highly reactive oxygen radicals either decay spontaneously or are converted by superoxide dismutase to hydrogen peroxide (H$_2$O$_2$), which is still highly toxic. Detoxification of H$_2$O$_2$ to H$_2$O is effected by catalase and by glutathione peroxidase (GSHPX). GSHPX is crucial for the function of both these enzymes; it is a structural component of catalase, and it is required as a substrate by glutathione reductase, which regenerates GSH when it has been oxidized to GSSG by GSHPX (Fig. 17-2). G6PD-deficient red cells are highly vulnerable to oxidative damage (see later), even though G6PD deficiency is never complete in humans. When complete G6PD deficiency was produced in mouse embryonic stem cells (ESCs) by targeted inactivation of the G6PD gene, the G6PD-null cells thus obtained were viable, but they formed colonies only in a low-oxygen environment; even so, they had an impaired capacity to form erythroid colonies. When G6PD-null ESCs were injected into mouse blastocysts, chimeric embryos were obtained, and germ-line transmission was achieved; however, only female heterozygous mice were obtained because hemizygous male embryos died. Thus, a G6PD-null mutation is an embryonically lethal condition.

**Structure and Biochemistry of G6PD**

G6PD is a ubiquitous enzyme that must be quite ancient in evolution because it has been found in all organisms, from prokaryotes to yeasts, to protozoa, to plants and animals. In mammals, G6PD is a good example of...
a housekeeping enzyme produced by a housekeeping gene; indeed, the gene is expressed in all cells of the body. G6PD is a typical cytoplasmic enzyme, although some G6PD activity is associated with peroxisomes in liver and kidney cells. This is consistent with the view that these organelles have evolved as part of the need of early eukaryotes to defend against oxygen, which is germane to the role of G6PD today.

The enzymatically active form of G6PD is either a dimer or a tetramer of a single polypeptide subunit of about 59 kd. The complete primary structure of the human enzyme has been deduced from the sequence of a full-length complementary DNA clone. The amino acid sequence of rat liver G6PD shows 94% homology to the human sequence and provides evidence that the N-terminal amino acid is N-acetylalanine, which must result from post-translational cleavage of the N-terminal methionine. The same is probably true of the human enzyme.

Extensive data are available on the kinetics of G6PD. Its coenzyme specificity is exquisite; human G6PD has practically no activity with nicotinamide adenine dinucleotide (NAD). Its substrate specificity is also very high because activity on other hexose phosphates (e.g., mannose 6-phosphate or galactose 6-phosphate) is negligible. The affinity for nicotinamide adenine dinucleotide phosphate (NADP) is about 1 order of magnitude higher than the affinity for glucose 6-phosphate (G6P). The tertiary structure of the molecule has been determined (Fig. 17-3). The G6P binding site is near K205, and the critical role of this amino acid in electron transfer has been confirmed by showing that its replacement with threonine nearly abolishes catalytic activity. The NADP binding site is located near a fan of β-sheet structures, with a critical G-X-X-G-X-X peptide motif corresponding to amino acids 38 to 43 encoded in exon 3.

Although many natural and non-natural substances can affect the activity of G6PD, it is not certain which ones may be important physiologically. NADPH, one product of the G6PD reaction, is a potent quasi-competitive inhibitor, and since most of the coenzyme in cells is in the reduced form, it can be assumed that G6PD is normally under strong inhibition. Because the Kₘ values for both G6P and NADP are higher than their normal respective intracellular concentrations, it is likely that these two substrates themselves are the main regulators of intracellular G6PD activity, together with NADPH. Any oxidative event affecting the cell will alter the NADPH/NADP ratio in favor of NADP. The simultaneous increase in NADP and decrease in NADPH act additively to increase G6PD activity by increasing the substrate drive on the reaction rate and decreasing...
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product inhibition. Under most conditions this may be the most important short-term regulatory signal, although it is, of course, possible that other regulatory effects play a role as well.

**Notes on Terminology**

G6PD is the accepted abbreviation for the enzyme glucose-6-phosphate dehydrogenase (E.C. 1.1.1.49); the G6PD gene is designated *Gd*.

In this chapter the terms G6PD normal and G6PD deficient are used to designate the phenotypes of persons; G6PD(+) and G6PD(−) are used to designate the phenotypes of individual cells. Because *Gd* is X-linked (see later), males can be only normal hemizygotes (*Gd*+/−) or deficient hemizygotes (*Gd*−/−); females can be normal homozygotes (*Gd*/+Gd*), deficient homozygotes (*Gd*/Gd*−), or heterozygotes (*Gd*/Gd*+). The phenotype of the last group is often referred to as “intermediate” because their overall red cell G6PD level usually lies in between the normal and the deficient range; however, exceptions do occur (see later).

Consequently, G6PD deficiency should not be regarded as a recessive but rather as a codominant trait. According to a classification introduced in 1966 (Table 17-1), G6PD-deficient variants that result in congenital nonspherocytic hemolytic anemia (CNSHA) are designated class I; G6PD-deficient variants that do not result in CNSHA are designated class II or class III, depending on the severity of the reduction in enzyme activity in red cells. The separation between class II and class III is blurred and probably no longer useful. Class IV variants are those with normal activity. Class V was reserved for variants with increased activity, but after an initial report (G6PD Hektoen31), none has been found. In practice, because the majority of G6PD-deficient persons are mostly asymptomatic, their G6PD deficiency is referred to as mild, simple, or common (corresponding to class II or III); the minority of persons who have CNSHA are referred to as having rare, sporadic, or severe G6PD deficiency (corresponding to class I).

When a diagnostic test for G6PD is carried out, the phrase “positive result” is sometimes used to indicate that the test has shown G6PD deficiency; this has caused confusion and should be avoided by saying that the test has shown either a normal result or a deficient result.

**Genetics of G6PD**

*Gd*, the G6PD gene, is located near the telomeric region of the long arm of the X chromosome (band Xq28); historically, *Gd* has been a valuable X-linked genetic marker and a precious tool for studying the X-chromosome inactivation phenomenon and clonal populations of somatic cells.

The *Gd* region was also one of the first regions of the human genome to be fully sequenced.

X-linkage of *Gd* has three major consequences: (1) *Gd* mutations display the typical pattern of mendelian X-linked inheritance; (2) severe G6PD deficiency is much more common in males than in females; and (3) as a result of X-chromosome inactivation, females heterozygous for two different *Gd* alleles exhibit somatic cell mosaicism.

This means that if one of the alleles entails enzyme deficiency, about half the cells will be G6PD(+) and the other half will be G6PD(−), although there is a large range of variation around that average for various reasons, not all of them fully known. At first approximation it can be assumed that X-inactivation takes place at random, and therefore a binomial distribution would be expected; the width of this distribution depends on the number of cells in the embryo or in individual embryonic tissue at the time of X-inactivation. If this number is 32 to 64, a fraction of about 2% of women with an “extreme phenotype” is predicted, that is, with less than 5% of one of the two cell types; this is in good agreement with observation in an unselected sample of *Gd*/*Gd* heterozygotes, although some studies have suggested an even larger proportion.

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**TABLE 17-1**

<table>
<thead>
<tr>
<th>Class*</th>
<th>Clinical Manifestations1</th>
<th>G6PD Activity (% of Normal)</th>
<th>Number of Known Mutant Alleles2</th>
<th>Examples</th>
<th>Comments</th>
</tr>
</thead>
<tbody>
<tr>
<td>IV</td>
<td>None</td>
<td>&gt;85</td>
<td>2</td>
<td>A, B</td>
<td>G6PD B is the normal “wild type”</td>
</tr>
<tr>
<td>II + III</td>
<td>Asymptomatic in the steady state, but risk for NNJ, AHA, favism</td>
<td>&lt;30</td>
<td>75</td>
<td>Med, A−, Orissa, Mahidol, Canton, Vanua Lava, Seattle</td>
<td>Most of these variants are known to be polymorphic</td>
</tr>
<tr>
<td>II</td>
<td>NNJ (severe), CNSHA, acute exacerbations</td>
<td>&lt;10 in most cases</td>
<td>61</td>
<td>Sunderland, Nara, Guadalajara</td>
<td>Never polymorphic; but same mutation can recur</td>
</tr>
</tbody>
</table>

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1The separation between class II and class III is blurred and, in the authors’ opinion, no longer useful.
2In brief, variants in class II and III can be regarded as having a mild phenotype confined to acute episodes; variants in class I have a severe phenotype with chronic illness.
3We have counted only variants for which the mutation is known and the clinical expression clearly reported.
4AHA, acute hemolytic anemia; CNSHA, congenital nonspherocytic hemolytic anemia. NNJ, neonatal jaundice.
Thus, in many cases unbalanced phenotypes may arise simply by chance, according to the laws of statistics. However, in certain cases there is evidence of selection at the somatic cell level after X-chromosome inactivation. This was first well established in women who are heterozygous for hypoxanthine phosphoribosyltransferase (HPRT) deficiency, and it has also been observed in several women heterozygous for severely deficient G6PD variants. In these cases one has to infer that the G6PD(-) state is a selective disadvantage for hematopoietic stem cells; interestingly, this disadvantage is cell lineage specific because it does not affect, for instance, fibroblasts. Finally, because selection might take place at other X-linked loci, heterozygotes exhibiting extreme phenotypes by analysis of G6PD may arise from selection acting on an allele at another locus (a “hitchhiking effect”), as has been suggested in a family with G6PD Ilesha.

At the genomic level, the Gd gene (Fig. 17-4A) consists of 13 exons, the first of which is noncoding. The total length of the gene is about 18.5 kilobases (kb), much of which (about 12 kb) consists of intron 2. The significance of this large intron is unknown; it may be important for efficient transcription or for processing because it is still the largest intron, even in the compressed version of the G6PD gene found in the puffer fish Fugu rubripes. The promoter region is highly enriched in guanine and cytosine residues (i.e., GC rich), as found characteristically in other housekeeping genes. Deletion analysis has revealed that the “essential” portion of the promoter is only about 150 bp long. Within this region, two Sp1 binding sites have been identified, either of which is essential for promoter activity. This and other regions are highly conserved between human and mouse, thus supporting the notion that they are important for gene regulation.

Features of G6PD in Red Cells

There is only one structural gene for G6PD, although a related autosomal hexose dehydrogenase also exists. Biochemical evidence is in keeping with the notion that the G6PD protein in red cells is the same as that in other somatic cells; thus, when red cells are severely deficient in G6PD, this deficiency is also found to a greater or lesser degree in other somatic cells. However, a significant difference in the metabolism of G6PD arises from the characteristic inability of mature red cells to synthesize protein. As a result, whereas in most somatic cells G6PD is subject to turnover, in red cells any G6PD molecule undergoing denaturation or proteolytic breakdown cannot be replaced (this is true, of course, not only for G6PD but also for most other red cell enzymes). In normal red cells, the decay of G6PD approximates an exponential with a half-life of about 60 days, although it has been claimed that it may more closely approximate a two-slope curve with very fast breakdown when reticulocytes mature to erythrocytes and much slower breakdown subsequently. The age dependence of red cell G6PD activity is so characteristic that it can almost be regarded as a marker of red cell age. In normal blood, reticulocytes have about five times more activity than the 10% oldest red cells.

![Figure 17-4](image-url)
Molecular Basis of G6PD Deficiency

In principle, genetically determined deficiency of G6PD, like that of any other protein, might be due either to quantitative changes, such as mutations that affect the amount of the enzyme but not its structure, or to qualitative changes, such as mutations that affect the structure of the enzyme and hence its stability or catalytic efficiency. Extensive investigations of G6PD from G6PD-deficient cells, mostly carried out before the sequence of G6PD was known, revealed that (1) enzyme activity, even when severely reduced (sometimes to less than 1% of normal), is never completely absent and (2) enzymic properties ($K_m$, $V$ max, activity on substrate analogues, thermostability, etc.) are often different from those of the normal enzyme (i.e., G6PD deficiency is associated with qualitative abnormalities). These data are consistent with point mutations in the coding region. In fact, from a database of nearly 150 mutant alleles now known a reasonably clear pattern has emerged.

1. In nearly all the G6PD variants there is a single amino acid replacement caused by a single missense point mutation.

2. In a few cases (namely, G6PD Santamaria, G6PD Mount Sinai, G6PD Akrotirinths, and the three types of G6PD A), two amino acid replacements are found; in all these cases one of the replacements is G6PD A (N126D). Because this nondeficient G6PD variant is polymorphic in Africa, the most likely explanation is that a second point mutation has taken place in a Gd gene. In another case, the two mutations G6PD Cassano (Q449H) and G6PD Union (R454C) are found in tandem in G6PD Hermoupolis; because both of the former are polymorphic, the latter might have arisen through intragenic recombination.

3. Three small in-frame deletions have been discovered: one removes a single amino acid (G6PD Sunderland), one removes two adjacent amino acids (G6PD Stonybrook), and one removes eight adjacent amino acids (G6PD Nara).

4. Only one mutation affecting splicing has been discovered thus far.

5. The majority of the mutations in the database are sporadic, and most of them have been detected because they result in G6PD deficiency and CNSHA by causing sufficient loss of activity in red cells to become limiting for their in vivo survival. Sporadic variants associated with CNSHA are not likely to spread by genetic drift. However, in several instances the same variant has been encountered recurrently (for instance, we have found G6PD Tokyo in Scotland, G6PD Guadalajara has turned up in Japan and in Belfast, and G6PD Nara has been observed at least three times). That same variation may have been found recurrently in people who are almost certainly not ancestrally related is not trivial; these observations corroborate the notion that there must be specific subtle constraints whereby a particular G6PD variant has a distinctly severe clinical expression but remains compatible with life.

6. Polymorphic mutations (Fig. 17-5). The majority of known mutations in this category again are associated with G6PD deficiency, and there is overwhelming evidence that they have become polymorphic as a result of malaria selection (see later). For these mutations we can visualize more stringent constraints; indeed, though still causing deficiency in red cells, they must not affect them so severely that the advantage with respect to malaria is outweighed. Thus, it is not surprising that nearly all the polymorphic variants fall into classes II or III and none of them cause CNSHA (class I).

7. The pattern of G6PD mutations, whereby null mutations (such as nonsense mutations, frameshifts, or large deletions) are conspicuously absent, is in striking contrast to that seen in other inherited disorders, such as the thalassemias, hemophilia, and muscular dystrophy. An important functional difference between these conditions and G6PD deficiency is that the former result from mutations in tissue-specific genes whereas Gd is a housekeeping gene. When a tissue-specific gene is totally inactivated by a mutation, it may cause severe disease but will not necessarily have interfered with embryonic development. By contrast, we know that G6PD-null mutations are lethal to the mouse embryo; they probably occur in humans, but we never see them because they must be lethal in the human embryo as well. Some clue regarding what mutation can cause G6PD deficiency has come from alignment of the amino acid sequence of G6PD from 42 different organisms; there was a striking correlation between the amino acid replacements that cause G6PD deficiency in humans and the sequence conservation of G6PD. Interestingly two thirds of such replacements are in highly and moderately conserved (50% to 99%) amino acids; relatively few are in fully conserved amino acids (where they might be lethal) or in poorly conserved amino acids, where presumably they simply would not cause G6PD deficiency.

8. The biochemical mechanism of G6PD deficiency is strongly contingent on the fact that in normal red cells, G6PD decreases exponentially as red cells age, with a half-life of about 50 days (see earlier). Because G6PD is an oligomeric globular protein, it is not surprising that many point mutations producing replacement of individual amino acids can further decrease the stability of G6PD; in fact, for most mutants, enzyme instability is the main mechanism of enzyme deficiency (Fig. 17-6). The instability is most extreme when the amino acid involved is at the interface between the two subunits of G6PD; in such cases the dimer does not form or falls apart. This situation applies to G6PD mutants that cause the most severe clinical phenotypes (that of CNSHA; see later). In a few cases (e.g., G6PD Orissa), altered catalytic func-
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Frequency of G6PD deficient males

- <0.5%
- 0.5–2.9%
- 3.0–6.9%
- 7.0–9.9%
- 10.0–14.9%
- 15.0–126.0%

Polymorphic G6PD variants

- A-(202A)
- A-(968C)
- Aures
- Canton
- Kaiping
- Chatham
- Coimbra
- Mahidol
- Cosenza
- Santamaria
- Seattle
- Mediterranean
- Union
- Taipei
- Viangchan
- Local variant


Epidemiology of G6PD Deficiency

The geographic distribution of G6PD deficiency is rather extraordinary in two ways. First, it has a very high prevalence overall, with more than 500 million people estimated to be involved (most of them mostly asymptomatic); second, it has a high prevalence in many populations in most tropical and subtropical parts of the world because of malaria selection (see Fig. 17-5 and the last section in this chapter). Thus, G6PD is prevalent in all five continents; it has never been reported in Amerindians, and one might speculate that this is related to the fact that they have been exposed to malaria only over the past 3 centuries.

Clinical Manifestations of G6PD Deficiency

The most classic manifestation of G6PD deficiency is acute hemolytic anemia (AHA); in children, however, another syndrome of great clinical and public health importance is neonatal jaundice (NNJ). CNSHA is a much rarer manifestation of G6PD deficiency and a lifelong hemolytic process. These different clinical manifestations are discussed in turn.

Acute Hemolytic Anemia

Clinical Picture. A child with G6PD deficiency is clinically and hematologically normal most of the time, and this can be designated as a steady-state condition. However, a rather dramatic clinical picture can develop upon ingestion of fava beans (favism), during the course of infection, or after exposure to certain oxidative agents (see Table 17-3). With infection or drugs the clinical picture may be more complicated than with favism; therefore, here we will describe favism as a paradigm. After a lag of hours the child may become fractious and irritable or subdued and even lethargic. Within 24 to 48 hours the child’s temperature is often moderately elevated. Nausea, abdominal pain, diarrhea, and rarely vomiting may be present. In striking contrast to these relatively nonspecific symptoms, the patient or a parent will observe, within 6 to 24 hours, the telltale and rather frightening
event that the urine is discolored (Fig. 17-7). It will be reported as dark, as red, brown, or black, or as “passing blood instead of water;” it will be said, depending on experience, culture, and socioeconomic background, to resemble Coke or strong tea or port wine. At about the same time jaundice will become obvious. Physical examination may reveal little more than the signs corresponding to these symptoms. The child will be pale and tachycardic; in severe cases there may be evidence of hypovolemic shock or, less likely, heart failure. The spleen is usually moderately enlarged, and the liver may also be enlarged; either or both may be tender.

**Laboratory Findings.** Anemia may range from moderate to extremely severe (hemoglobin values of 2.5 g/dL have been recorded). In the absence of other preexisting hematologic abnormalities the anemia is normocytic and normochromic. The morphology of the red cells may be striking (Fig. 17-8). There is often marked anisocytosis (reflected as a wide red cell size distribution on the electronic counter) because of the coexistence of large polychromatic cells and “contracted” cells, some of which can be frankly classified as spherocytes. There is also marked poikilocytosis with the presence of distorted red cells, “irregularly contracted” red cells, and red cells with apparently uneven distribution of the hemoglobin inside them (hemighosts). Although some of these appearances are probably smearing artifacts, electron microscopic evidence suggests that in some of the cells, opposing surfaces of the membrane have become “cross-linked.” Probably the most characteristic poikilocytes are those in which the cell margin literally appears dented, as though a portion has been plucked out or bitten away (“bite cells”; see Fig. 17-8). The reticulocyte count is increased and may reach peaks of 30% or greater (blood counters using immunofluorescent technology will show an elevated percentage of the immature reticulocyte fraction); this reflects a prompt and effective bone marrow response, which will take place provided that there is no preexisting or concomitant bone marrow pathology. Careful inspection of reticulocyte preparations may reveal inclusion bodies different from those normally seen in reticulocytes; these bodies are discrete, round, and 1 to 3 μm in diameter, and they usually appear to be leaning, from the interior, against the cell membrane. These inclusions are more clearly displayed by supravital staining with methyl violet and are referred to as *Heinz bodies*. They consist of precipitates of denatured hemoglobin,
and they are the vivid manifestation of the oxidative insult that this protein and the cell itself has suffered. However, Heinz bodies are a very transient finding because they tend to be promptly “pinched off” by the spleen82 (thus giving rise to bite cells) and the red cells containing them are very rapidly removed from the circulation. Haptoglobin is reduced to the point of being undetectable. In severe cases it is possible to demonstrate free hemoglobin in plasma (hemoglobinemia). The white blood cell count is usually moderately elevated, with a predominance of granulocytes. The platelet count may be normal, increased, or moderately decreased. The unconjugated bilirubin level is elevated, but “liver enzyme” levels are generally normal. The dark urine tests strongly positive for blood because of the presence of free hemoglobin (see the differential diagnosis of this sign in Table 17-2).

Clinical Course. In the majority of cases the hemolytic attack, even if severe, is self-limited and tends to resolve spontaneously. Depending on the proportion of red cells that have been destroyed (as reflected by the severity of the anemia), the hemoglobin level may be back to normal in 3 to 6 weeks. Although the blood urea level may be transiently elevated, the development of renal failure in children is exceedingly rare, even in the presence of massive hemoglobinuria (see Fig. 17-7A).

Diagnosis. With a history of fava bean ingestion followed by hemoglobinuria (see Table 17-2), the diagnosis is almost always straightforward, and it can be made quite confidently even before obtaining the final proof that the patient is G6PD deficient (see later). If the hemoglobinuria has already subsided and the history is uncertain, one is faced instead with the much wider differential diagnosis of AHA. A negative direct antiglobulin test will militate against autoimmune hemolytic anemia. In endemic areas it will be important to exclude malaria or the much rarer babesiosis. In hemolytic-uremic syndrome, the red cell morphology is different and there will be evidence of impaired renal function. In all cases, demonstration of G6PD deficiency will be conclusive, and in uncertain cases it will be crucial.

Pathophysiology. The clinical picture of AHA in a G6PD-deficient child is a vivid expression of the fact that as we know already, hemolysis results from the action of an exogenous factor on intrinsically abnormal red cells. Hemoglobinemia and hemoglobinuria unambiguously indicate that the hemolysis is at least in part intravascular. At first approximation we can visualize the following sequence of events: (1) an oxidative agent causes conversion of GSH to GSSG; (2) because of the limited capacity of G6PD-deficient red cells to regenerate GSH, their GSH reserve is rapidly depleted; (3) once GSH is exhausted, the sulphydryl groups of hemoglobin and

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### TABLE 17-2 Hemoglobinuria in Children

<table>
<thead>
<tr>
<th>Condition</th>
<th>Circumstances</th>
<th>Diagnostic Approach</th>
</tr>
</thead>
<tbody>
<tr>
<td>G6PD deficiency</td>
<td>Exposure to a trigger of hemolysis</td>
<td>Test for G6PD activity</td>
</tr>
<tr>
<td>Blackwater fever</td>
<td>Relatively rare complication of malaria</td>
<td>Blood slide for malaria parasites</td>
</tr>
<tr>
<td>Paroxysmal cold hemoglobinuria</td>
<td>Usually associated with viral infection</td>
<td>Test for Donath-Landsteiner antibody</td>
</tr>
<tr>
<td>Mismatched blood transfusion</td>
<td>Usually ABO incompatibility</td>
<td>Repeat crossmatch</td>
</tr>
<tr>
<td>Paroxysmal nocturnal hemoglobinuria</td>
<td>Tends to recur</td>
<td>Flow cytometry for CD59</td>
</tr>
<tr>
<td><em>Clostridium welchii</em> septicemia</td>
<td>Burns, severe open trauma, transfusion of contaminated blood</td>
<td>Culture of blood or appropriate patient material</td>
</tr>
</tbody>
</table>

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**FIGURE 17-8.** Blood smear in G6PD deficiency. A, Acute hemolytic anemia (favism) characterized by marked morphologic abnormalities of red blood cells with anisocytosis, polychromasia, bizarre poikilocytes, “bite cells,” and “hemighosts.” Note the nucleated red blood cell and polymorphonuclear leukocytosis with a marked shift to the left. B, Chronic nonspherocytic hemolytic anemia. The morphologic abnormalities are much less pronounced, but several poikilocytes and occasional bite cells are seen.
probably other proteins are oxidized to disulfides or sulf-
oxides; and (4) coarse precipitates of denatured hemoglo-
in cause irreversible damage to the membrane, and
the red cells lyse.

Not all of these steps from oxidative attack to final
hemolysis have been fully documented in vivo, in part
because of one major difficulty: in the course of AHA the
red cells sampled from the patient are obviously, at any
given stage, those that have not yet hemolyzed. However,
GSH depletion is a most characteristic finding when red
cells are subjected to oxidative challenge, and in one
careful study it has been demonstrated that during that
attack of favism, the first measurable biochemical change
is a fall in NADPH, followed by a fall in GSH, in
keeping with stages 1 and 2 described earlier. Heinz
bodies are the visible expression of stage 3. Stage 4 is less
clearly defined, although studies suggest that the abnor-
mal proteolytic activity is associated with increased intra-
cellular calcium, as well as binding of hemichromes
(arising from denaturation of hemoglobin) to band 3
molecules.

Even though intravascular hemolysis in AHA associ-
ated with G6PD deficiency is important in pathophys-
ology and diagnosis, a substantial proportion of the
hemolysis is extravascular (as shown by the enlarged
spleen). Probably the most severely damaged red cells
hemolyze in the bloodstream on their own, whereas less
severely damaged red cells will be recognized as abnor-
mal by macrophages and will undergo extravascular
hemolysis in the reticuloendothelial system. This process
has been referred to as an example of an “innocent
bystander” phenomenon (although the red cells, by
virtue of being G6PD deficient, are not that innocent),
in which complement and immunoglobulins may be
involved. Finally, it is important to remember that the
red cell destruction in AHA associated with G6PD
deficiency is an orderly function of red cell age. The oldest
red cells with the least G6PD are the first to hemolyze,
and the hemolytic process progresses upstream toward
cells with more and more G6PD. As a result, there is a
selective enrichment in red cells that despite being genet-
ically G6PD deficient, have relatively higher levels of
G6PD. This phenomenon can be so marked with certain
G6PD variants that patients in the post-hemolytic state
are found to be relatively resistant to further challenge;
thus, the patient may be in a state of compensated
hemolysis.

**Triggers and Mechanism of Hemolysis.** G6PD defi-
ciency was first discovered by investigating a possible
(genetic basis for sensitivity to primaquine. Since that
time, numerous other drugs have been reported as being
potentially dangerous in G6PD-deficient individuals (Table 17-3). There is no obvious relationship in chemi-
structure among all of these substances, but they have
in common the ability to stimulate the pentose phosphate
pathway in red cells, which must mean that they are
able to oxidize NADPH, directly or indirectly. Extensive

<table>
<thead>
<tr>
<th>Category of Drug</th>
<th>Definite Risk</th>
<th>Possible Risk</th>
</tr>
</thead>
<tbody>
<tr>
<td>Antimalarials</td>
<td>Primaquine</td>
<td>Chloroquine</td>
</tr>
<tr>
<td></td>
<td>Dapsone†</td>
<td>Quinine</td>
</tr>
<tr>
<td></td>
<td>containing combinations†</td>
<td></td>
</tr>
<tr>
<td>Analgesics</td>
<td>Acetanilid</td>
<td>Aspirin</td>
</tr>
<tr>
<td>Sulfonamides/</td>
<td>Sulfamethoxazole/</td>
<td></td>
</tr>
<tr>
<td>sulfones</td>
<td>co-trimoxazole</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Dapsone†</td>
<td>Sulfasalazine</td>
</tr>
<tr>
<td>Quinolones</td>
<td>Nalidixic acid</td>
<td>Sulfadiazone</td>
</tr>
<tr>
<td></td>
<td>Ciprofloxacin</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Norfloxacin</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Moxifloxacin</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Ofloxacin</td>
<td></td>
</tr>
<tr>
<td>Other antimicrobials</td>
<td>Nitrofurantoin</td>
<td>Chloramphenicol</td>
</tr>
<tr>
<td>Other antimicrobials</td>
<td>Methylene blue</td>
<td></td>
</tr>
<tr>
<td>Other antimicrobials</td>
<td>Niriadazole</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Vitamin K</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Rasburicase</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Ascorbic acid</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Glibenclamide</td>
<td></td>
</tr>
</tbody>
</table>

*For all drugs the risk of hemolysis is dose related, as well as the severity of hemolysis. For instance, aspirin up to 20 mg/kg is probably safe; three times that dose will almost certainly cause some hemolysis.
†Dapsone can cause hemolysis even in non–G6PD-deficient children.
but viral infections of the upper respiratory or gastrointestinal tract may also trigger hemolysis.\textsuperscript{101} Recently it has been reported\textsuperscript{102} that in a group of patients who suffered major trauma in road traffic accidents, the frequency and severity of infection were significantly higher in patients who were G6PD deficient; in addition, more severe anemia occurred in this group.

Release of peroxides during phagocytosis of bacteria by granulocytes might explain the hemolytic process in bacterial infections.\textsuperscript{103} In this respect it is possible that hemolysis has sometimes been attributed to drugs used for treating infection when it should have been blamed on the infection itself.

**Treatment.** A child with AHA may be a diagnostic problem that once solved, does not require any specific treatment at all, or the child may be a medical emergency requiring immediate action. The most urgent question is whether a blood transfusion is needed. It is difficult to give absolute directives, but the following guidelines may be useful\textsuperscript{104}:

1. If the hemoglobin level is below 7 g/dL, the child should be transfused forthwith.
2. If the hemoglobin level is below 9 g/dL and there is evidence of persistent brisk hemolysis (hemoglobinuria), immediate blood transfusion is also indicated.
3. If the hemoglobin level is above 9 g/dL but hemoglobinuria persists or if the hemoglobin level is between 7 and 9 g/dL but there is no hemoglobinuria, the child is kept under close observation for at least 48 hours and transfused if either condition 1 or 2 develops.

The most important complication that may require treatment is acute renal failure, which is exceedingly rare in children.

**Neonatal Jaundice**

From an epidemiologic point of view, it is noteworthy that the frequency of NNJ varies widely in different populations; in many populations in which G6PD deficiency is prevalent, the rate of pregnancies at risk for Rhesus incompatibility happens to be low.\textsuperscript{105,106} In addition, as rhesus-related hemolytic disease of the newborn (HDN) is disappearing thanks to the implementation of appropriate prophylaxis, one can expect G6PD-related NNJ to be generally on the increase, at least in relative terms.

**Clinical Features.** NNJ related to G6PD deficiency is very rarely present at birth; it has a peak incidence between day 2 and day 3.\textsuperscript{107} There is more jaundice than anemia, and the anemia is very rarely severe.\textsuperscript{108} For this reason the terms HDN and NNJ cannot be regarded as interchangeable, at least in the context of G6PD deficiency. The severity of G6PD-related NNJ varies from being subclinical to imposing the threat of kernicterus if not treated. Thus, prompt recognition of the problem is extremely important to avoid crippling neurologic sequelae.\textsuperscript{109-112}

**Nature of the Association between G6PD Deficiency and Neonatal Jaundice.** It is not fully explained why NNJ develops in some but not all G6PD-deficient newborns. Several clinical studies have established beyond any possible doubt that the association is statistically much higher than could be expected by chance (Table 17-4).\textsuperscript{113} However, because not all G6PD-deficient newborns have NNJ (Table 17-5), it is likely that genetic or environmental factors (or both) are involved in addition to G6PD deficiency and that the same factors can also, if more extreme, make the NNJ more severe. NNJ is not the prerogative of some G6PD variant because it is prevalent in widely remote parts of the world (e.g., Nigeria).\textsuperscript{113}

### TABLE 17-4 Association between G6PD Deficiency and Jaundice in Newborns

<table>
<thead>
<tr>
<th>Number</th>
<th>% G6PD Deficient</th>
</tr>
</thead>
<tbody>
<tr>
<td>Normal</td>
<td>500</td>
</tr>
<tr>
<td>Mild jaundice (bilirubin 150-200 μmol/L)</td>
<td>38</td>
</tr>
<tr>
<td>Severe jaundice (bilirubin &gt;230 μmol/L)</td>
<td>70</td>
</tr>
<tr>
<td>Admitted with kernicterus</td>
<td>20</td>
</tr>
</tbody>
</table>

Data collected in Ibadan, Nigeria, on consecutive babies born in or admitted to a teaching hospital; see Biemde A, Effiong CE, Luzzatto L. Erythrocyte glucose 6-phosphate dehydrogenase deficiency (G6PD type A) and neonatal jaundice. Acta Paediatr Scand. 1976;65:701-704.

### TABLE 17-5 Features of Neonatal Jaundice in 500 African American Male Babies

<table>
<thead>
<tr>
<th></th>
<th>G6PD Normal (n = 436)</th>
<th>G6PD Deficient (n = 64)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Plasma total bilirubin (PTB), mmol/L</td>
<td>139 ± 48</td>
<td>157 ± 43</td>
</tr>
<tr>
<td>Median end-tidal CO\textsubscript{2}, ppm</td>
<td>2.1 (1.7-2.5)</td>
<td>2.4 (2.0-2.9)</td>
</tr>
<tr>
<td>Babies with PTB &gt;75th percentile (%)</td>
<td>23.4</td>
<td>48</td>
</tr>
<tr>
<td>Babies with PTB &gt;95th percentile (%)</td>
<td>6.7</td>
<td>22</td>
</tr>
<tr>
<td>Mean age at which the highest PTB occurs (hr)</td>
<td>64</td>
<td>55</td>
</tr>
<tr>
<td>Babies requiring phototherapy (%)</td>
<td>5.7</td>
<td>20.3</td>
</tr>
</tbody>
</table>

HEMOLYTIC ANEMIAS

Sardinia, Singapore, China in which the Gd alleles underlying G6PD deficiency are different; moreover, both within Sardinia and within Taiwan, NNJ has been found in babies with any of several different G6PD variants. The possibility that NNJ correlates with the quantitative level of residual G6PD activity is controversial. Specific features of erythrocytes in newborns, such as elevated levels of ascorbic acid, depressed activity of glutathione reductase, and low levels of vitamin E, GSHPX, and other enzymes, could contribute to the degree of jaundice. It must be noted that there is a remarkable dissociation between hyperbilirubinemia and anemia in G6PD-deficient neonates. Indeed, in one series there was no difference in the distribution of hematocrit values in cord blood and on day 3 between jaundiced and nonjaundiced G6PD-deficient newborns, thus suggesting that to a large extent this jaundice may be of hepatic origin rather than hemolytic. In keeping with this notion, measurements of the bilirubin production-conjugation index have demonstrated that the NNJ in otherwise healthy G6PD-deficient neonates depends more on inefficient bilirubin conjugation than on hemolysis. The UDPGT1 mutation characteristic of Gilbert’s disease is associated with a much higher risk for NNJ and kernicterus in G6PD-deficient babies. In addition, environmental factors can certainly exacerbate NNJ, in some cases by causing hemolysis in individual G6PD-deficient newborns; such factors include prematurity, breast-feeding, naphthalene (camphor balls) inhalation, acidosi, hypoxia, infection such as viral hepatitis, oxidant drugs, and ingestion of drugs (favin in uterus) by the mother before delivery.

In summary, there are probably two different types of NNJ associated with G6PD deficiency: (1) a more common type can best be visualized as a marked exaggeration of “physiologic jaundice”; this type is usually clinical benign and not greatly influenced by the environment; and (2) a rarer, frankly hemolytic type, more severe, can be visualized as AHA occurring in a newborn baby because the baby happened to be exposed to one of the same agents that could cause AHA even in an adult. Severe NNJ can occur in girls heterozygous for G6PD deficiency. In addition, in geographic areas where G6PD deficiency is very common, female newborns might be homozygous for the trait, thus behaving like a hemizygous G6PD-deficient male newborn.

Treatment. Management of NNJ associated with G6PD deficiency does not differ from that recommended for other causes. Thus, mild cases do not require treatment; intermediate cases require phototherapy; and severe cases require exchange transfusion, just as in NNJ caused by “classic” HDN. Kernicterus is still an impending threat, especially when severe NNJ is associated with anemia, hypoxia, or infection. Clinical practice guidelines for the management of hyperbilirubinemia in newborns quote: “measurement of the glucose-6-phosphate dehydrogenase (G6PD) is recommended for a jaundiced infant who is receiving phototherapy and whose family history or ethnic or geographic origin suggest the likelihood of G6PD deficiency or for an infant in whom the response to the phototherapy is poor.” G6PD-deficient newborns must be considered as being “at high risk” and therefore requiring greater surveillance and more intensive treatment of hyperbilirubinemia. Specifically, it is recommended that in full-term newborns, exchange transfusion be carried out if the serum bilirubin level exceeds 15 mg/dL in the first 2 days of life or 19 mg/dL at any time in the first week of life.

**Congenital Nonspherocytic Hemolytic Anemia**

A small minority of children with G6PD deficiency have hemolytic anemia not only when it is triggered by an exogenous factor but even in the steady state (Fig. 17-9). These children have special G6PD mutations (class I); all of them are rare, but they are scattered worldwide, regardless of whether G6PD deficiency is endemic in the region. The patient is invariably male and in general is evaluated because of unexplained jaundice, frequently at birth with NNJ. (The only known exception is a woman heterozygous for G6PD Volendam, who had an extremely skewed X-inactivation pattern favoring, paradoxically, the X chromosome with the abnormal G6PD allele.) Unfortunately, anemia recurs and the jaundice fails to clear completely, thus requiring further investigation. In other cases, NNJ may have been overlooked or forgotten and the patient is investigated only much later in life (e.g., because of gallstones in a boy or in a young adult). The severity of the anemia ranges from being borderline to being transfusion dependent in different patients (Table 17-6). The anemia is usually normochromic but somewhat macrocytic because a large proportion of reticulocytes (up to 20% or more) will cause an increased mean corpuscular volume and a shifted, wider than normal red cell size distribution curve. The red cell morphology is mostly not characteristic, and for this reason it is referred to in the negative as being “nonspherocytic.”

**TABLE 17-6 Clinical Spectrum of Chronic Nonspherocytic Hemolytic Anemia Caused by Severe G6PD Deficiency**

<table>
<thead>
<tr>
<th>Patients studied</th>
<th>25 (all male)</th>
</tr>
</thead>
<tbody>
<tr>
<td>% With neonatal jaundice</td>
<td>100</td>
</tr>
<tr>
<td>% Requiring exchange transfusion</td>
<td>24</td>
</tr>
<tr>
<td>% With splenomegaly</td>
<td>68</td>
</tr>
<tr>
<td>Steady-state hemoglobin, range (g/dL)</td>
<td>6-14</td>
</tr>
<tr>
<td>Lowest hemoglobin known, range (g/dL)</td>
<td>3.8-9.3</td>
</tr>
<tr>
<td>Reticulocytes, range (%)</td>
<td>3-51</td>
</tr>
<tr>
<td>% Requiring multiple blood transfusion</td>
<td>72</td>
</tr>
</tbody>
</table>
The bone marrow shows normoblastic hyperplasia, unless the increased requirement for folic acid associated with the high red cell turnover has caused it to become megaloblastic. There is chronic hyperbilirubinemia, decreased haptoglobin, and increased lactate dehydrogenase. Hemo- globinuria is rare, but hemosiderinuria may be detected sometimes. The spleen is usually moderately enlarged in small children, and subsequently it may increase in size sufficiently to cause mechanical discomfort, hypersplenism, or both.

**Pathophysiology.** The pattern of hemolysis in these patients is different from that described earlier for AHA associated with G6PD deficiency and instead more reminiscent of the chronic hemolysis seen in hereditary spherocytosis. However, oxidative stress caused by exposure to the same agents that cause AHA will cause acute exacerbation of hemolysis in CNSHA. Unlike the situation in thalassemia major, even in severe cases there is no evidence of ineffective erythropoiesis. The fact that there is no hemoglobinuria, at least in the steady state, suggests that the hemolysis is mainly extravascular and therefore that its mechanism is different from that of AHA. Studies of red cell membrane proteins have revealed the presence of high-molecular-weight aggregates consisting largely of spectrin, which seem to make the membrane abnormally susceptible to shear-induced fragmentation. These abnormalities in the red cells of G6PD-deficient patients with CNSHA are not found in asymptomatic G6PD-deficient subjects, thus suggesting that whereas in the latter the reductive potential of residual G6PD is adequate in the steady state, in the former, continuous oxidation of sulfhydryl groups takes place, followed by irreversible changes in the configuration of membrane proteins. The reason why the severity of CNSHA associated with G6PD deficiency is so variable is that almost every case is due to a different mutation (see later) and each mutation will have a different effect on stability of the enzyme, on its kinetic properties, or on both.

**Diagnosis.** Laboratory diagnosis of G6PD deficiency is discussed next, but a special problem in relation to CNSHA is to firmly establish the causal link between the former and the latter. If the patient is, for example, a Swede of Swedish ancestry or a Japanese of Japanese ancestry, the link will be taken for granted, and this is generally justified, given the rarity of G6PD deficiency in these populations. On the other hand, if the patient is from a population in which G6PD deficiency is common, its presence in a patient with CNSHA might be a mere coincidence, and the cause of the CNSHA might be something else altogether. In such cases, while other causes of CNSHA are being ruled out, it becomes essential to characterize the G6PD of the patient. If it is a common variant known to be asymptomatic in other subjects, it can certainly be exonerated, whereas if it is a new unique variant, it is likely to be the culprit.

**Treatment.** In general terms, management of CNSHA associated with G6PD deficiency does not differ from that of CNSHA related to other causes (e.g., pyruvate kinase deficiency). If the anemia is not severe, regular folic acid supplements and regular hematologic surveillance suffice. It is important to avoid exposure to potentially hemolytic drugs, and blood transfusion may be indicated when exacerbations occur, mostly in concomitance with intercurrent infection. In rare patients, the anemia is so severe that it must be regarded as transfusion dependent. In these cases blood transfusion will

![Figure 17-9](image-url)
probably be needed at approximately 2-month intervals to keep the hemoglobin in the 8- to 10-g/dL range. A hypertransfusion regimen aiming to maintain a normal hemoglobin level is not indicated (because there is no ineffective erythropoiesis in the bone marrow). However, depending on the extent of the blood transfusion requirement, appropriate iron chelation should be instituted from the age of 2 years onward and must be continued as long as transfusion treatment is necessary; sometimes the transfusion requirement may decrease after puberty.

A special problem is that of splenectomy. There is no evidence of selective red cell destruction in the spleen, as in hereditary spherocytosis. However, the fact that the spleen is usually enlarged suggests that its role in hemolysis is not negligible. In practice, there are three indications for splenectomy: (1) if splenomegaly becomes a physical encumbrance; (2) if there is evidence of hypersplenism; and (3) if the anemia is severe, even in the absence of the first two indications. Splenectomy may reduce the overall rate of hemolysis (see Fig. 17-9) just enough to make a transfusion-dependent child become transfusion independent. This is doubly important because it will make it possible to dispense with iron chelation. Active immunizations before splenectomy and penicillin prophylaxis after splenectomy must be started according to national guidelines such as that produced by the British Committee for the Standards in Haematology (http://www.bcsghguidelines.com/pdf/SPLEEN21.pdf).

When a diagnosis of CNSHA is made, the family must be given genetic counseling, and it is advisable to establish whether the mother is a heterozygote. If she is, the chance of recurrence is 1:2 for every subsequent male pregnancy. Prenatal diagnosis should be offered; although it could be carried out on amniotic fluid cells, it would be much preferable to determine the mutation involved and test for it on DNA from chorionic villi. G6PD-deficient patients with severe CNSHA (see Fig. 17-9), because its clinical manifestations are limited to blood cells, would in principle be good candidates for gene therapy by gene transfer into hematopoietic stem cells. This approach has not yet reached the stage of clinical application, but preclinical experiments have been carried out successfully in mice and Rhesus monkeys.

LABORATORY DIAGNOSIS OF G6PD DEFICIENCY

Although the clinical picture of favism and other forms of AHA associated with G6PD deficiency is characteristic, the final diagnosis must rely on direct demonstration of decreased activity of this enzyme in red cells. In NNJ and CNSHA, the differential diagnosis is much wider, and therefore this test is even more important. Fortunately, the enzyme assay is very easy, and numerous “screening tests” can be used as substitutes if a spectrophotometer is not available. However, a number of potential pitfalls and sources of error must be understood, and the use of commercial kits is not a substitute for such understanding. Here the value and limitations of the regular quantitative assay are discussed first, and then the use of alternatives is mentioned.

Tests for G6PD Deficiency

G6PD can be assayed by the classic spectrophotometric method, which directly measures the rate of formation of NADPH through its characteristic absorption peak in the near ultraviolet spectrum at 340 nm. Red cell activity is expressed in international units (micromoles of NADPH produced per minute) per gram of hemoglobin; therefore, it is best to assay the enzyme activity and the hemoglobin concentration in the same hemolysate and work out the ratio. Because G6PD activity is much higher in leukocytes (particularly in granulocytes) than in erythrocytes, for accurate measurements it is essential to remove all leukocytes by the Ficoll-Hypaque method or by filtration through cellulose powder rather than by the crude approach of sucking off the buffy coat; however, for the purpose of clinical diagnosis of G6PD deficiency, this is not strictly necessary. In normal red cells the range of G6PD activity, measured at 30°C, is 7 to 10 IU/g of hemoglobin.

Several “screening tests” for G6PD deficiency are useful and reliable provided that they are run properly and their limitations are understood. The most popular are the dye decolorization tests, the methemoglobin reduction test, and the fluorescence spot test. Recently, a formazan-based screening test has been developed and field-tested. All these methods are semiquantitative, and they are meant to classify a sample simply as “normal” or “deficient.” The cutoff point can be set by following the appropriate instructions and by trial and error in the individual diagnostic laboratory; one should aim to classify as deficient any sample having less than 30% of normal activity because above this level one is unlikely to encounter clinical manifestations. Screening tests are of course especially useful for testing large numbers of samples. They are also perfectly adequate for diagnostic purposes in patients who are in the steady state but not for patients in the post-hemolytic period or with other complications; in addition, they cannot be expected to identify all heterozygotes. Finally, an ideal screening test ought not to give “false-negative” results (i.e., it should not misclassify a G6PD-deficient subject as normal), but it can be allowed to give a few “false-positive” results (i.e., a G6PD-normal subject might be misclassified as being G6PD deficient). Ideally, every patient found to be G6PD deficient by screening should be confirmed by the spectrophotometric assay. For special purposes, formazan-based cytochemical methods are also available.

The Effect of Red Cell Age and Selective Hemolysis. Because G6PD decreases gradually as red cells age, any condition associated with reticulocytosis will entail an
increase in G6PD activity (Table 17-7). This means that if a subject is genetically G6PD normal, in the course of hemolysis, red cell G6PD activity will now be above the normal range. This does not affect the diagnosis because G6PD deficiency will be correctly ruled out. However, if the subject is genetically G6PD deficient, red cell G6PD may now be raised to the extent of being near or even within the normal range, and the patient might therefore be misclassified as being G6PD normal (even though within the normal range, and the patient might therefore be overcorrected; this finding suggests that the patient is either G6PD deficient or normal). This does not affect the diagnosis because G6PD deficiency will be correctly ruled out. However, if the subject is genetically G6PD deficient, red cell G6PD may now be raised to the extent of being near or even within the normal range, and the patient might therefore be misclassified as being G6PD normal (even though within the normal range, and the patient might therefore be overcorrected; this finding suggests that the patient is either G6PD deficient or normal).

Thus, after a hemolytic attack two circumstances concur to cause a risk of misdiagnosis: first, the older cells have been destroyed selectively; second, the marrow response has caused a sudden upsurge of young cells into the peripheral blood. (A third confusing factor may be admixture of G6PD-normal red cells if the patient has been transfused.) Although the reticulocyte count is a good warning to avoid this mistake, it must be realized that because reticulocytes turn into morphologically “mature” erythrocytes within 1 to 2 days, their count is not a sensitive index of mean red cell age; in other words, mean red cell age may be significantly younger than normal even when the reticulocyte count is normal. There are several ways to circumvent these problems. First, a G6PD level in the low-normal range (as opposed to higher than normal) in the presence of reticulocytosis is always suspicious; indeed, this finding suggests that the patient is actually G6PD deficient. Second, if the patient is suffering or is recovering from AHA, the suspicion generated from the finding just mentioned can be simply kept in store for a few weeks, when the situation will be evolving toward the steady state, and a repeat test will prove whether the patient is indeed G6PD deficient. Third, if either the urgency of some clinical decision or academic curiosity demands a more prompt solution of the problem, the presence of severely G6PD-deficient red cells can be demonstrated either by enzyme assay of the oldest cells (fractionated by sedimentation) or by a cytochemical method (see earlier).

G6PD Deficiency in Heterozygotes. For hemolysis to be clinically significant in heterozygous girls, at least 50% of the red cells must be deficient, and therefore the G6PD level will be about 50% of normal or less. This level of deficiency can be diagnosed by a quantitative test; however, the problems associated with current or recent hemolysis outlined for male patients will be compounded in the case of heterozygous females, and they can usually be overcome by a similar approach, particularly with the use of a cytochemical test. A different matter is the biologic diagnosis of heterozygosity, regardless of immediate clinical implications, as may sometimes be required for appropriate genetic counseling. In cases of “extreme phenotypes” (sometimes referred to as arising from “imbalanced lyonization”), the G6PD-deficient red cells may be so few that the only way to identify heterozygous G6PD deficiency will be by DNA analysis, for which the underlying mutation must be known or identified for the purpose. A special situation involves heterozygotes for G6PD variants associated with CNSHA. In the authors’ experience, the mothers of (male) patients with this condition are often G6PD normal, either because the variant in the offspring is due to a de novo mutation or because the mother is a heterozygote but is phenotypically normal, presumably because somatic selection has favored the hematopoietic progenitor cells with the normal G6PD allele.48

Molecular Analysis of the G6PD Gene. In the vast majority of cases, the family history, the clinical course, and a G6PD assay are sufficient to establish the diagnosis of conditions associated with G6PD deficiency. In special cases (see the previous section for heterozygote diagnosis) and particularly in the case of CNSHA, identification of mutations in the G6PD gene can be carried out, with reference to the large numbers of mutations already known (see Human Gene Mutation Database, http://www.hgmd.cf.ac.uk/ac/index.php?gene=G6PD). Furthermore, in this way new mutations are still likely to be discovered.

GENOTYPE-PHENOTYPE CORRELATIONS

A rigorous definition of a polymorphic allele is one with a frequency higher than can be accounted for by recur-
rent mutation; however, for convenience, a conservative practical criterion is any allele with a frequency of at least 1% in at least one population. By this criterion there are 26 well-mapped Gd polymorphic alleles already known.\textsuperscript{56} The ratio of subjects having G6PD deficiency associated with CNSHA to those having “simple” G6PD deficiency varies in different populations. For instance, in Japan, where G6PD deficiency is, on the whole, very rare, the majority of patients with G6PD deficiency have been reported to have CNSHA.\textsuperscript{152} This suggests that CNSHA, caused by rare sporadic variants, many of which may result from recent mutations, reflects the intrinsic mutation rate of the human Gd gene, which is likely to be uniform throughout the world; in contrast, “simple” G6PD deficiency almost always results from common variants that have arisen many generations ago and spread through biologic selection.

The three variants mentioned previously, G6PD A\textsuperscript{−}, G6PD Mediterranean, and G6PD Mahidol, are probably those for which clinical expression has best been characterized.\textsuperscript{157} Although the former is often quoted as having fewer clinical manifestations than the others, the differences are marginal, and they are unlikely to be relevant with respect to patient management. The severity of hemolysis and whether it is “self-limited” depend on the offending agent, on its dose, and on the time course of exposure probably more than it depends on the G6PD variant involved. Notably, favism has been unambiguously documented with G6PD A\textsuperscript{−}.\textsuperscript{154-156}

In terms of clinical expression, the demarcation between class I variants and all others is, by definition, much more clear-cut. Indeed, one of the outstanding questions in the biochemical genetics of G6PD is why a particular variant can cause CNSHA rather than just AHA. In certain cases the level of residual enzyme activity is not the whole answer—qualitative differences are also important, as first suggested by Kirkman and Riley.\textsuperscript{157} The most likely way in which a structural change can significantly alter the function of G6PD, given the same level of deficiency, is by affecting the binding of one of the main ligands (i.e., G6P, NADP, NADPH). For instance, G6PD Mediterranean and G6PD Coimbra both have mutations near the G6P binding site, and both have increased affinity for G6P. Perhaps because of this, although they are both severely deficient, they belong to class II and not to class I. G6PD Orissa, the main polymorphic variant in tribal Indian populations\textsuperscript{158} also belongs to class II despite having decreased affinity for NADP. Thus, the affinity for G6P appears to be of greater importance. Indeed, comparison of the distribution of $K_m^{G6P}$ values of class I variants with that of class II and III variants shows that they are significantly lower in the latter group.\textsuperscript{8}

However, in most cases the main factor responsible for causing CNSHA is a very low level of residual activity in red cells, which in turn is due to marked in vivo instability. It is quite remarkable that although G6PD mutations as a whole are evenly spread throughout the gene’s coding sequence, the majority of class I mutations are clustered in exons 10 and 11 (see Fig. 17-4B). The three-dimensional model of the human G6PD dimer has, at long last, provided a reasonable explanation for this finding\textsuperscript{66,159} Indeed, these two exons encode the protein region that constitutes the interface between the two identical subunits of the enzyme. As a result, any mutation in this region causes the respective amino acid replacements in the two subunits to be quite near each other in the dimer structure, thus potentially increasing their deleterious effects. Even more important, because there is no covalent link between the subunits, it stands to reason that any interference in the shape of the interface surfaces may affect their mutual fit and therefore dramatically destabilize the active form of the enzyme. Defects in protein folding have been studied in detail for certain G6PD variants.\textsuperscript{150,161}

### G6PD Deficiency and Preventive Medicine

Because NNJ and AHA are the most common manifestations of G6PD deficiency, it is most important to consider how they can be prevented. The first step is to identify G6PD-deficient individuals, which is where screening is most pertinent. Of course, whether population-wide screening is both desirable and feasible depends primarily on the prevalence of G6PD deficiency in any particular community; this will determine the cost-benefit ratio. If screening is done at all, it is best done on cord blood. Once a subject is known to be G6PD deficient, the two main implications are the risk for NNJ and the importance of avoiding exposure to agents that can cause AHA. NNJ cannot be prevented as yet, but awareness of G6PD deficiency must entail surveillance for NNJ until at least day 4 and special recommendations with respect to factors, such as naphthalene, that can cause it or make it worse. By contrast, at least one type of AHA, namely, favism, is completely preventable (Fig. 17-10). Prevention of infection-induced hemolysis is obviously more difficult. Prevention of drug-induced hemolysis is possible in most cases by choosing alternative drugs, but it may be difficult when none are available. The most common problem is the need to administer primaquine for the eradication of malaria caused by Plasmodium vivax or Plasmodium malariae. In these cases, administration of a lower dosage for a longer time is the recommended approach. Hemolysis will still occur, but under appropriate surveillance it will be of an acceptably mild degree.

A special problem in prevention is what to do about new drugs, the hemolytic potential of which is unknown. Although in vitro methods to test drugs in this respect do exist,\textsuperscript{162,163} such tests are unfortunately not routinely carried out before drugs are released on the market, so their hemolytic potential will become apparent only from clinical observation. Recently, a combination of dapsone and chlorproguanil, used as an antimalarial in
several African countries has been withdrawn from the market because of life-threatening hemolytic complications.

**G6PD DEFICIENCY IN NONERYTHROID CELLS**

As mentioned earlier, because nucleated somatic cells have the capability to synthesize G6PD constitutively, they are affected less than red cells by G6PD deficiency. For instance, subjects with G6PD Mediterranean have less than 5% G6PD activity in red cells but about 30% of normal in granulocytes; subjects with G6PD A have about 12% G6PD activity in red cells but nearly normal activity in granulocytes. On the other hand, if deficiency results from a drastic change in catalytic efficiency or in substrate affinity, deficiency may be more universal. In practice, the only well-documented pathologic effect is expressed in granulocytes. Very few of the class I G6PD variants cause not only CNSHA but also granulocyte dysfunction, mainly in the way of impaired killing of phagocytosed bacteria (an example is G6PD Barcelona). Patients with these variants have increased susceptibility to bacterial infection, particularly with *Staphylococcus aureus.* Recently, in a group of patients who suffered major trauma in road traffic accidents, Spolarics and colleagues made the interesting observation that the frequency and severity of infection were significantly higher in patients who were G6PD deficient; thus, neutrophil function may be affected in extreme situations, even in the common type of G6PD deficiency (the patients in the clinical study all had G6PD A). The mechanism whereby G6PD deficiency impairs phagocytosis is a defect of the oxidative burst caused by a shortage in NADPH supply, similar to that observed in chronic granulomatous disease, in which one of the components of the cytochrome- system is defective. Functional abnormalities have also been demonstrated in macrophages from G6PD-deficient mice.

Erythrocytes are not the only non-nucleated cells in the body. Another example is in the eye lens, and juvenile cataracts have been reported occasionally in subjects with G6PD deficiency. Whether G6PD deficiency is more generally associated with a higher frequency or earlier onset of cataracts appears to still be controversial.

**G6PD DEFICIENCY COEXISTING WITH OTHER DISORDERS**

In areas where G6PD deficiency is common, it is not infrequent that it may be encountered in the same patient together with another condition, regardless of whether the patient is hemolytic.

**Association with Other Hematologic Diseases.** The combination of G6PD deficiency with the sickle cell trait has been no more frequent than could be expected by chance. Several studies have shown that there are no significant differences in a variety of clinical and hematologic parameters between two otherwise comparable groups of patients with sickle cell anemia, those...
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with and without G6PD deficiency, but it must be borne in mind that acute intravascular hemolysis superimposed on chronic severe extravascular hemolysis is an added risk with this association. The combination of G6PD deficiency with the β-thalassemia trait has been found to cause a significant increase in mean corpuscular volume, which remains, however, below the normal range. Association of G6PD deficiency with thalassemia major is unlikely to be a problem because patients with the latter condition are treated with regular blood transfusions or with bone marrow transplantation. However, the association may be significant in patients with various forms of thalassemia intermedia syndromes (e.g., hemoglobin E-β-thalassemia).182

Occasionally, G6PD deficiency has been observed in association with much rarer red cell abnormalities, such as pyruvate kinase deficiency,183,184 congenital dyserythropoietic anemia type II,185-187 and hereditary elliptocytosis.188 In these cases the two abnormalities seem to produce only additive clinical effects, but in at least one family G6PD deficiency was synergistic with hereditary spherocytosis in causing moderately severe chronic hemolytic anemia.189

Association with Nonhematologic Diseases. Several studies have reported variable degrees of association between diabetes and G6PD deficiency. It now appears clear that diabetic ketoacidosis does not trigger hemolysis; on the other hand, downregulation of G6PD has been reported in some West African patients with type 1 diabetes predisposed to ketoacidosis.190

Trauma and G6PD Deficiency. Although G6PD deficiency protects against malaria, there are reports indicating that it worsens the clinical course of traumatic patients.191 Altered cytokine response would be the underlying pathogenetic mechanism predisposing to infections in G6PD-deficient individuals.192 Apart from the fact that hepatitis can precipitate hemolysis in G6PD-deficient children, ribavirin, now much used in the treatment of hepatitis C, can itself cause hemolytic anemia (regardless of whether the patient is G6PD deficient). On the other hand, it has recently been shown that G6PD-deficient patients with hepatitis C can be treated safely with ribavirin and pegylated interferon.200

G6PD POLYMORPHISM AND MALARIA

The striking correlation between the worldwide distribution of G6PD deficiency (see Fig. 17-5) and that of Plasmodium falciparum prompted formulation of the "malaria hypothesis" nearly half a century ago.201,202 Since that time, numerous more detailed epidemiologic studies, which can be referred to as “micromapping,” as well as clinical studies, have supported the notion that G6PD deficiency confers some degree of resistance to the potentially lethal malaria parasite P. falciparum and that in malaria-endemic areas, G6PD alleles associated with enzyme deficiency have therefore been subjected to positive darwinian selection.203-207 A geographic correlation certainly does not amount to proof, but three large controlled clinical studies, all carried out in Africa, have shown concordantly that children with G6PD deficiency tend to have less severe malaria and, therefore, presumably a decreased risk of dying of malaria. It is puzzling that in one of these studies (from Nigeria), increased resistance to the parasite (relative to that in appropriate controls) was significant only in heterozygous females; in another study (from Gambia and Kenya), a protective effect was seen in both males and females; and in the third study (from Mali), a protective effect was noted only in males. These differences might result from genetic variations (other than G6PD) in different populations, from the use of different criteria to measure the severity of malaria, from different levels of statistical power, or from any combination of these factors. However, all three studies concur in supporting the notion of malaria selection for G6PD deficiency. A more difficult issue is understanding the mechanism of this phenomenon. In vitro studies have shown that invasion of G6PD-deficient red cells by P. falciparum takes place normally and the parasite can multiply efficiently in G6PD-deficient red cells, at least after several cycles. On the other hand, in vitro experiments have shown that parasitized red cells are recognized more promptly by macrophages when they are G6PD deficient; thus, accelerated removal of parasitized red cells (suicidal infection) may be the most important mechanism of protection, as it is for subjects with the sickle cell trait. An additional strong argument in favor of malaria selection is the genetic heterogeneity of polymorphic G6 alleles in itself. Indeed, each one of these alleles, having arisen through an independent mutational event, must have increased in frequency on its own in a particular geographic area where malaria was or still is endemic—a good example of convergent evolution driven by the same selective force.

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