Red Cell Enzymopathies in the Newborn
II. Inherited Deficiencies of
Red Cell Enzymes*

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ABSTRACT

The mature, anucleate human red cell is incapable of synthesizing enzymes and is dependent upon anaerobic metabolism to meet its energy requirements. Thus, inherited red cell enzyme deficiencies of the glycolytic pathway often result in chronic nonspherocytic hemolytic anemia (CNSHA). The hexosemonophosphate (HMP) shunt is normally relatively inactive and enzyme deficiencies, such as G-6-PD deficiency, usually result in episodic hemolysis secondary to oxidative stress. Deficiency of G-6-PD is the most common red cell enzymopathy. Pyruvate kinase (PK) deficiency is the most common enzyme deficiency of the glycolytic pathway, followed in frequency by glucose phosphate isomerase (GPI) deficiency. Other glycolytic enzyme deficiencies are rare. Red cell phosphofructokinase deficiency can be associated with muscle weakness; triosephosphate isomerase (TPI) and phosphoglycerate kinase deficiencies with neurologic abnormalities. Since its original description, pyrimidine-5'-nucleotidase deficiency has been recognized with greater frequency as a cause of CNSHA. Enolase, glyceraldehyde phosphate dehydrogenase, and lactate dehydrogenase deficiencies are not associated with hemolysis.

The newborn with CNSHA can develop anemia and significant jaundice in the first 24 hrs of life. Precise diagnosis may be difficult owing to the volume of blood required and the laboratory facilities available. Screening tests for G-6-PD, GPI, TPI, and PK deficiencies and the red cell concentration of 2,3-diphosphoglycerate can all be done with small amounts of blood obtained with a heel puncture.

Data obtained for red cell glycolytic intermediates and quantitative enzyme assays must be compared to normal values for neonates since red cells at birth and during the first two months of life have unique metabolic characteristics.

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Introduction

The mature, anucleate, human red cell is devoid of mitochondria and other intracellular organelles and is incapable of synthesizing hemoglobin, membrane protein or metabolic enzymes. It lacks an intact citric acid cycle and is essentially dependent upon anaerobic metabolism to meet its energy requirements. Under normal circumstances, both the metabolic capacity and energy demands are delicately balanced so that the red cell survives 120 days and can fulfill its primary role, which is to deliver oxygen from the lungs to the tissues.

An inherited enzyme deficiency that results in disruption of this delicate balance could result in premature destruction of the red cell and, by definition, hemolytic anemia. The purpose of this paper is to review the metabolic and clinical aspects of these enzyme deficiencies, with particular reference to the newborn period. More comprehensive reviews of red cell enzymopathies have been published recently.47,48,64,88

Red Cell Metabolism

The mature human red cell is basically an anaerobic cell. Glucose is the normal substrate which enters the red cell by a process of facilitated diffusion93; the intracellular concentration approximates, on a water basis, that in the extracellular environment. Within the red cell, approximately 97 percent is phosphorylated to glucose-6-phosphate (G-6-P) via the hexokinase (HK) reaction. Metabolism of G-6-P then proceeds through the hexose monophosphate (HMP) shunt or the Embden-Meyerhof (glycolytic) pathway.

Under normal conditions, 90 percent or more of the G-6-P is metabolized via the glycolytic pathway, with the conversion of one mole of G-6-P to two moles of lactic acid. Metabolism of one mole of glucose via this pathway results in the net production of two moles of adenosine triphosphate (ATP). The ATP serves as an energy source for maintenance of the normal biconcave disc shape of the red cell and plasticity of the red cell membrane, membrane lipid, active cationic pumping of potassium into the cell and sodium out of the cell, prevention of calcium accumulation in the red cell membrane, and glutathione synthesis.

The glycolytic pathway also provides reduced pyridine nucleotide (NADH) at the glyceraldehyde-3-phosphate dehydrogenase step, which can be utilized by the NADH-dependent methemoglobin reductase to reduce methemoglobin formed within the red cell. The Rapoport-Luebering or 2,3-diphosphoglycerate (2,3-DPG) shunt is an important alternate metabolic pathway of glycolysis, whereby 1,3-diphosphoglycerate is converted to 2,3-DPG by the enzyme, 2,3-diphosphoglycerate mutase (DPGM). The 2,3-DPG combines with deoxyhemoglobin and reduces the affinity of hemoglobin for oxygen,6,18 thereby shifting the oxygen-hemoglobin equilibrium curve to the right, facilitating the unloading of oxygen from the red cell to the tissue.

Metabolism of glucose via the HMP shunt results in the net production of two moles of reduced nicotinamide adenine dinucleotide phosphate (NADPH) and one mole of CO₂ for every mole of G-6-P metabolized. The NADPH generated via this pathway is vital to the red cell since it acts as a co-factor to maintain glutathione in the reduced state (GSH) via the glutathione reductase reaction. Thus, GSH, in turn, protects sulphydryl groups of hemoglobin against oxidative denaturation, as well as stabilizing certain intracellular enzymes. GSH detoxifies the primary denaturant, hydrogen peroxide, by serving as a substrate for the enzyme glutathione peroxidase.

Thus, glucose metabolism via the glycolytic pathway: (1) fulfills the energy requirements of the red cell by providing ATP which maintains the integrity and deformability of the red cell membrane, (2) provides 2,3-DPG via the Rapoport-Luebering shunt which enables the red cell to modulate its hemoglobin-oxygen
affinity, and (3) provides NADH that can be utilized by the NADH-dependent methemoglobin reductase.

Metabolism via the HMP shunt provides a means of protecting the red cell against oxidative damage. Perturbations of metabolism caused by inherited defects of the enzymes of either of these pathways can disrupt the harmonious balance of structure and function usually present in the mature red cell and lead to premature cell death.

General Clinical and Laboratory Features

The congenital non-spherocytic hemolytic anemias (CNSHA) share many clinical characteristics. These include: (1) lifelong hemolysis; (2) hyperbilirubinemia, usually with a history of neonatal jaundice, which may have required exchange transfusion; (3) a variable degree of splenomegaly, which tends to increase with age; (4) an increased incidence of cholelithiasis; (5) increased severity of anemia with infection; (6) variable severity of anemia, ranging from a mild compensated state to a severe hemolytic process which is transfusion dependent; (7) mild to moderate improvement after splenectomy, but not cure of the hemolytic process as observed in hereditary spherocytosis; and (8) familial incidence.

Deficiencies involving enzymes of the glycolytic pathway result in CNSHA in contrast to defects in the HMP shunt which usually result in acute episodic hemolysis secondary to oxidative stress, infection, or acidosis, although there are variants of G-6-PD with CNSHA.

The laboratory findings in general are variable, and precise diagnosis cannot be made without specific tests. The red cell morphology reflects the hemolytic state and young red cell population and includes a variable degree of macro- and anisocytosis and polychromasia. Spiculated microspherocytes are present in glucosephosphate isomerase deficiency and irregularly contracted cells are observed in pyruvate kinase deficiency and other enzyme deficiencies of the glycolytic pathway. In G-6-PD deficiency, during a hemolytic episode, occasional spherocytes, red cell fragmentation, and Heinz bodies may be observed. Basophilic stippling is prominent in pyrimidine-5'-nucleotidase deficiency.

Other non-specific laboratory features include hyperbilirubinemia of the indirect type, low plasma haptoglobin and hemopexin, and shortened autologous 51Cr red cell survival. The osmotic fragility is usually normal and may be increased or decreased at 24 hours, unlike hereditary spherocytosis which results in a uniform increase in osmotic fragility after incubation. The autohemolysis test serves as a non-specific screening test and is rarely used.

A useful adjunct in the diagnosis of glycolytic enzyme deficiencies is analysis of the pattern of glycolytic intermediates and adenosine triphosphate (ATP). A block in glycolysis at a specific enzyme step should result in an increase in the concentration of substrates prior to that step and a reciprocal decrease in those distal to the step, and the crossover site may then offer a rational approach as to the specific enzyme to be tested. Similarly, a decreased concentration of GSH may reflect an enzyme deficiency in the HMP shunt or glutathione metabolism.

The most common enzymopathy is G-6-PD deficiency. Deficiency of PK is the most common enzyme defect in the glycolytic pathway, followed by GPI deficiency; the other deficiencies, such as HK, PFK and DPGM, are rare. Deficiency of PD-5'-N has been diagnosed with greater frequency since its description.

Hereditary Enzyme Deficiencies of the Glycolytic Pathway

HEXOKINASE DEFICIENCY

Hexokinase (HK) catalyzes the phosphorylation of glucose to glucose-6-phos-
Glucose + ATP $\xrightarrow{\text{Mg}^{++}}$ Glucose-6-P + ADP

This is the initial enzymatic step that allows glucose to enter the glycolytic or HMP pathways. The activity of HK in the human red cell is normally very low, and it is one of the rate limiting steps that controls glycolysis. Deficiency of HK generally results in a decreased rate of red cell glucose consumption, decreased concentrations of G-6-P, and other intermediates distal to this step and reduced red cell ATP. The glutathione content and glutathione stability are normal.

Deficiency of HK results in a chronic hemolytic state and has been associated with hyperbilirubinemia in the newborn. It has also been described in three cases of Fanconi’s aplastic anemia, but it is not characteristic of this disorder. Splenectomy usually results in improvement regarding transfusion requirements, but an uncompensated hemolytic state persists.

Although this disorder is rare, genetic polymorphism is apparent. An autosomal recessive mode of inheritance has been proposed, but Necheles et al have reported a family where both the father and son were affected. When red cell HK is studied electrophoretically, four bands have been described, a predominant triple HK, Type I, and a more anodal faint HK, Type III. Deficient kindreds of HK have been reported that have: (1) a normal electrophoretic pattern but the enzyme is unstable in hemolysates, and has an altered substrate affinity; (2) absence of all bands except band 1 with normal enzyme kinetics; (3) absence of Type III with altered substrate affinity; and (4) others.

Activity of HK is markedly increased in reticulocytes and its activity declines markedly as the red cell ages. Thus, it should be emphasized that the HK activity in the red cells of a suspected deficient patient must be compared to that in cells of a similar mean age. By employing reticulocyte rich blood as a control, it becomes readily apparent that the “normal” activity that may be obtained in some HK deficient subjects with marked reticulocytosis is, in reality, greatly diminished for red cell age. This is particularly pertinent in evaluating the newborn with hemolysis, since HK in cord blood is normally very elevated.

The concentration of G-6-P is also increased in young red cells, so that “normal” G-6-P may be obtained in some HK deficient subjects rather than the expected decrease in this glycolytic intermediate. This is particularly relevant to the newborn since the concentration of G-6-P in normal infants is elevated, out of proportion to the age of the red cell population until one to two months of age and then declines to levels that are appropriately increased for mean cell age.

The concentration of 2,3-DPG is decreased in HK deficiency, and the subject cannot compensate for his anemia with an increase in 2,3-DPG resulting in decreased exercise tolerance for the degree of anemia. This decrease in 2,3-DPG has little clinical application in the newborn owing to the presence of fetal hemoglobin.

**GLUCOSEPHOSPHATE ISOMERASE DEFICIENCY**

Glucosephosphate isomerase (GPI) catalyzes the reaction:

Glucose-6-P $\xrightarrow{}$ Fructose-6-P

Deficiency of red cell GPI results in a moderate chronic hemolytic process. Isoenzymes of GPI from different tissues have the same electrophoretic properties, and decreased activity has been demonstrated in leukocytes, skin fibroblasts, and platelets in GPI deficient subjects. There is no associated leukocyte dysfunction or systemic disease except one report of a subject with mental retardation and increased hepatic glycogen. Deficiency of GPI is clearly inherited as an autosomal recessive trait; heterozygotes have approximately one-half normal activity and
no evidence of hemolysis. Electrophoretic studies have revealed the genetic polymorphism of this disorder.

Splenectomy results in moderate improvement, but an uncompensated hemolytic state persists. Attempts to bypass the block in glycolysis by stimulating metabolism of G-6-P through the HMP shunt with such agents as ascorbic acid or methylene blue have been unsuccessful in improving the anemia in GPI deficiency.

Neonatal jaundice is not uncommon and may require exchange transfusion. Although specific morphologic findings are uncommon in glycolytic enzyme deficiencies, spherocytes have been reported in this disorder.

Deficiency of GPI results in an increased concentration of red cell G-6-P and decreased concentration of fructose-6-phosphate (F-6-P). Since the concentrations of G-6-P and F-6-P are normally elevated in red cells with a young mean cell age and are markedly increased in the first month of postnatal life, a low F-6-P in a newborn with hemolysis is of particular significance and highly suggestive of GPI deficiency. A reliable screening test for detecting GPI deficiency is available and is particularly helpful in the neonate since it can be done by obtaining blood from a heel puncture.

**Phosphofructokinase Deficiency**

Phosphofructokinase (PFK) catalyzes the reaction:

\[
\text{Fructose-6-P + ATP} \rightarrow \text{Fructose-1,6-diphosphate + ATP} \quad \text{Mg}^{++}
\]

PFK is one of the major rate limiting enzymes in the control of red cell glycolysis. Deficiency of this enzyme has been associated with a severe myopathy referred to as Tarui’s disease or Type VII glycogen storage disease. This disorder is characterized by virtual absence of skeletal muscle PFK and approximately 50 percent of normal red cell PFK activity. These patients have a mild non-spherocytic hemolytic anemia. Autosomal recessive inheritance has been postulated.

Deficiency of PFK without myopathy has also been described. In one of these individuals, hemolytic anemia and splanchnomagly was associated with a 50 percent reduction in red cell enzyme activity, and an unstable enzyme with altered kinetic properties. In another family without muscle involvement, red cell PFK activity was barely detectable. Vora et al have defined the molecular basis of PFK deficiency by demonstrating that PFK is composed of two separate subunits, M (muscle-type) and L (liver-type) and red cell PFK is a mixture of five tetrameric isozymes: M₄, M₃L, M₂L₂, ML₃L, and L₄. In type VII glycogenolysis, the M subunit was absent, so that red cell PFK consisted exclusively of the L₄ isozyme.

Hemolysis is generally mild in this disorder. Deficiency of PFK results in an increased concentration of red cell G-6-P and F-6-P and a decreased concentration of 2,3-DPG. Subjects with PFK deficiency may not be anemic owing to compensatory erythrocytosis secondary to this decrease in 2,3-DPG.

Red cell G-6-P and F-6-P are increased and PFK activity is decreased in normal infants during the first two months of life. In neonates with a hemolytic process, these transiently abnormal metabolic characteristics should not be mistaken for congenital PFK deficiency.

**Aldolase Deficiency**

Aldolase (ALD) catalyzes the reaction:

\[
\text{Fructose-1,6-diphosphate} \rightarrow \text{glyceraldehyde-3-phosphate + dihydroxyacetone phosphate}
\]

A single patient with aldolase deficiency has been reported. The parents were first cousins. Deficiency of ALD was associated with moderate mental retardation, increased hepatic glycogen, and mild CNSHA.
TRIOSEPHOSPHATE ISOMERASE DEFICIENCY

Triosephosphate isomerase (TPI) catalyzes the reaction:

\[
\text{Dihydroxyacetone phosphate} \quad \longrightarrow \quad \text{Glyceraldehyde-3-phosphate}
\]

Red cell TPI deficiency is associated with a moderate to marked hemolytic anemia. In all cases reported to date, a severe and usually progressive neurologic disorder is present that begins after 6 months of age. The enzyme deficiency is transmitted as an autosomal recessive trait and is widespread, involving leukocytes, spinal fluid, muscle, and skin fibroblasts. An increased susceptibility to infections has been reported. Three affected individuals have died suddenly from presumed cardiac arrest, which is suggestive of myocardial involvement in this disorder. It is postulated that the generalized nature of the enzyme deficiency is responsible for the spectrum of clinical symptoms present in this disorder. Heterozygotes have approximately one-half normal red cell TPI activity and are clinically asymptomatic.

Two instances of cri-du-chat syndrome in association with decreased red cell TPI activity have been reported. Other cases, however, have not been associated with decreased TPI activity.

Deficiency of TPI results in marked accumulation of dihydroxyacetone phosphate.

Although the systemic manifestations of this disorder develop early, they are generally not present in the newborn period. Hemolytic anemia with jaundice, however, does present in the neonate. A screening test is available that can be performed with blood obtained from a heel stick.

2,3-DIPHOSPHOGLYCERATE MUTASE DEFICIENCY

2,3-Diphosphoglycerate mutase (2,3-DPGM) catalyzes the reaction:

\[
\text{1,3-Diphosphoglycerate} \quad \longrightarrow \quad \text{2,3-diphosphoglycerate}
\]

This reaction represents the proximal limb of the Rapoport-Luebering shunt. 3-Phosphoglycerate (3-PG) is a necessary co-factor. This shunt represents the only point in the red cell where net synthesis of 2,3-DPG occurs. 2,3-DPG is converted to 3-PG by the enzyme, diphosphoglycerate phosphatase (DPGP). It appears that DPGM, DGP, and monophosphoglyceromutase represent active sites on the same molecule.

Red cell DPGM deficiency with chronic hemolytic anemia associated with mild jaundice has been reported in several patients. In two of these case reports, much of the evidence was indirect, based on a decreased content of red cell 2,3-DPG. In another family, the parents of a child with a severe hemolytic process had 50 percent of normal red cell DPGM activity, but the enzyme could not be measured in the child owing to the presence of large amounts of transfused blood. It was presumed that the parents, who were clinically unaffected were heterozygotes and the infant was homozygous, suggesting an autosomal recessive mode of inheritance. Autosomal dominant inheritance was demonstrated in three generations of a kindred with DPGM deficiency and well compensated hemolysis. Activity of DPGM was reduced to 52 to 61 percent of normal in affected members, DPGP was reduced to 40 percent of normal, and MPGM was normal. The hemoglobin was normal but hemolysis was documented with elevated reticulocyte counts, decreased haptoglobins, and decreased 51Cr red cell survival. No other cause for hemolysis was identified. In contrast, a patient with virtually complete absence of DPGM and 2,3-DPG was healthy and had no evidence of hemolysis. His only manifestation was polycythemia. There is obviously marked variation in the clinical expression of this disorder.
Deficiency of DPGM is not known to be associated with problems in the newborn period.

**Phosphoglycerate Kinase Deficiency**

Phosphoglycerate kinase (PGK) catalyzes the reaction:

\[
\text{1,3-Diphosphoglycerate + ADP } \rightarrow \text{3-Phosphoglycerate + ATP} \\
\text{Mg}^{2+} \text{ and } \text{K}^+ 
\]

Red cell PGK deficiency has been studied in a large Chinese kindred. The inheritance of this disorder is X-linked. Affected males had a moderately severe hemolytic anemia, with marked deficiency of erythrocyte and leukocyte PGK and mild mental retardation with behavioral disturbances. Increased susceptibility to infection has not been described.

Other cases of PGK deficiency have been reported and are similar. However, one PGK variant, PGK München, had moderately decreased enzyme activity, thermal instability, and no clinical symptoms. Recently, this variant was shown to be secondary to one amino acid substitution, an asparagine for an aspartic acid.

In PGK deficiency, glycolysis is "blocked" at an ATP generating step, but 1,3-diphosphoglycerate can be metabolized to lactate via the Rapoport-Luebering shunt. This "bypass" results in no net gain of ATP. There is marked elevation of the red cell 2,3-DPG concentration. Hemolytic anemia and jaundice can appear in the newborn period, but the neurologic signs generally do not appear until after the first year of life.

**Pyruvate Kinase Deficiency**

Pyruvate kinase (PK) catalyzes the reaction:

\[
\text{Phosphoenolpyruvate + ADP } \rightarrow \text{pyruvate + ATP} \\
\text{Mg}^{2+}, \text{K}^+ 
\]

Deficiency of PK is the most common and best described enzyme deficiency of the glycolytic pathway, and recent comprehensive reviews are available. Its distribution is world wide but is most prevalent in persons of Northern European extraction. In the United States, a particularly high incidence has been noted among the Amish in Mifflin County, Pennsylvania.

Deficiency of PK is inherited as an autosomal recessive trait. The heterozygotes have approximately 50 percent of normal PK activity in their red cells and are unaffected clinically. The homozygous deficient individuals demonstrate variability in the clinical expression of their disease, ranging from a mild compensated hemolytic process to severe, transfusion-dependent hemolytic state that may be life-threatening. This broad clinical spectrum may be present in the same family. The PK activity of leukocytes is normal. Acquired PK deficiency has been described in hematologic disorders, such as malignant and dyserythropoietic states.

The majority of patients exhibit moderate to severe anemia, hyperbilirubinemia, and splenomegaly. Cholelithiasis at an early age is not uncommon. Most instances of PK deficiency are noted in infancy or childhood, but rare cases escape detection until adult life. Anemia and hyperbilirubinemia, requiring exchange transfusion, have been observed in newborns, often in the first 24 hours of life, and kernicterus has been reported. In severely affected children, widened diploic spaces and a "hair on end" appearance of the skull can be seen radiographically, similar to the roentgenographic changes observed in thalassemia major. Infection is usually associated with an accelerated rate of hemolysis and/or transient hypoplastic crisis.

Splenectomy usually affords some degree of improvement with a decrease in or elimination of transfusion requirements and variable rise in hemoglobin level, but an uncompensated hemolytic state persists. The reticulocyte count increases
markedly post-splenectomy, sometimes to levels as high as 80 percent. It has been demonstrated that PK-deficient reticuloocytes are sequestered in the spleen in patients with this disorder. It has been proposed that these metabolically affected young cells are doomed to early extinction, but splenectomy allows them to survive longer in the circulation and accounts for the marked increase in reticuloocyte counts observed after removal of the spleen.

The peripheral smear in PK deficiency is not specific and is characterized by macrocytosis, polychromasia, and a variable number of irregularly contracted red cells. These abnormal appearing red cells increase in number after splenectomy. A variable number of normoblasts may be present. The autohemolysis pattern that had previously been considered characteristic of PK deficiency is Dacie type II (marked hemolysis in saline and glucose, with correction with ATP). This pattern is not only variable but is non-specific.

Genetic polymorphism is common in PK deficiency. Variants with altered substrate affinity for phosphoenolpyruvate (PEP) and abnormalities in fructose diphosphate activation, ATP inhibition and thermal stability are frequently noted. "Homozygous" PK deficient subjects that are the product of non-consanguineous matings are usually doubly heterozygous for two different red cell PK variants. Those with increased substrate requirements (high K_m PEP) usually have normal or slightly decreased enzyme activity, since optimal concentrations of PEP are employed in the PK assay system and this concentration is far in excess of that found in red cells. Since the discovery of these mutants, PK activity is routinely measured at high and low PEP concentrations. A screening test for PK deficiency is available and useful, but a negative screen does not rule out PK deficiency owing to these kinetic mutants with normal activity.

Like HK, PK is an age dependent enzyme. Leukocyte PK activity is many times greater than red cell activity and is a different isozyme. Thus, the white cells of PK deficient subjects have normal activity, and it is important to remove the white cells prior to PK assay.

Red cells deficient in PK are characterized by impaired glycolysis and increased concentrations of 2,3-DPG and PEP. Reduced concentrations of red cell ATP would be anticipated owing to a block in glycolysis at an ATP generating step. Measurement of red cell ATP has yielded variable results, however, since the patients who have extreme reticulocytosis are capable of maintaining red cell ATP via oxidative phosphorylation.

**Other Glycolytic Enzyme Deficiencies**

Red cell enolase deficiency has been reported in two sisters who exhibited a mild CNSHA and an accelerated rate of hemolysis after ingestion of nitrofurantoin. No other case of enolase deficiency has been described. Red cell glycerolaldehyde phosphate dehydrogenase and lactic dehydrogenase deficiencies have been reported but were unassociated with hemolytic anemia.

**Deficiencies of the Hexose Monophosphate Shunt**

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

Deficiency of G-6-PD is the most common inborn error of metabolism, and extensive investigations have made this abnormality the best understood and most thoroughly studied red cell enzymopathy. It is widely distributed geographically and occurs with greatest frequency among Blacks, Chinese, and Caucasians of Mediterranean ancestry, such as Italians, Greeks, and Sephardic Jews. The geographic distribution of G-6-PD deficiency is strikingly similar to that of the epidemicity of falciparum malaria. Luzzatto et al demonstrated that when the red cells of heterozygous G-6-PD deficient fe-
males were simultaneously stained for both G-6-PD activity and the malarial parasite, the normal red cells were more heavily parasitized than the deficient cells and it is likely that the high frequency of G-6-PD deficiency in malarial zones was maintained by the advantage conferred upon the heterozygous female.

The incidence of G-6-PD deficiency varies widely in different parts of the world. For example, in the United States approximately 11 percent of Black males and 1 percent of Black females are G-6-PD deficient, whereas in certain African tribes the incidence is as high as 28 percent among males. Italian Americans have an incidence of G-6-PD deficiency of approximately 2 percent; in Sardinia, it is as high as 30 percent.

The X-linked nature of this disorder is well established. Owing to the random inactivation of one of the two X chromosomes, the heterozygous female (X⁺X⁻) can have low, intermediate, or normal G-6-PD activity.

Over 160 variants of G-6-PD have been described. These enzymes are classified into five major groups based on enzyme activity:

I. Severe enzyme deficiency associated with chronic nonspherocytic hemolytic anemia; II. Severe enzyme deficiency; III. Moderate to mild enzyme deficiency; IV. Very mild or no enzyme deficiency; and V. Increased enzyme activity. They are further classified according to electrophoretic mobility (fast, normal, or slow). Further characterization is made within each group utilizing different biochemical techniques, such as the Michaelis constant (Kₘ), pH activity curves; heat denaturation; and capacity of the enzyme to utilize substrate analogues.

The normal enzyme (or wild type) is designated as B and is the most prevalent type. An electrophoretically fast variant with slightly reduced activity that is unassociated with hemolysis is found in approximately 20 percent of American Blacks and is designated as type A. The most common deficient variants are G-6-PD A-, found in Blacks, G-6-PD Mediterranean, prevalent in Italians, Greeks, and Sephardic Jews; and possibly G-6-PD Canton and Hong Kong-Pokfulam common in Orientals. Fingerprinting techniques have demonstrated that the difference between the mutant type A and the wild type B resides in one amino acid substitution, an aspartic acid for an asparagine, respectively.

Current observations suggest that the deficiency in the common variants, G-6-PD A- and G-6-PD Mediterranean is due to an unstable enzyme. The normal enzyme has a half-life (t½) of 62 days. In comparison, G-6-PD A- has a t½ of 13 days. The instability of G-6-PD Mediterranean is so great that activity can only be detected in reticulocytes. Since G-6-PD is an age-dependent enzyme, the differences in the life-span of the enzyme have clinical relevance. In the G-6-PD A-, ingestion of an "oxidant" drug results in destruction of old G-6-PD deficient red cells and compensatory hyperactive erythropoiesis producing a young red cell population with near-normal G-6-PD activity, which is resistant to further "oxidant" stress. Thus, the hemolytic process is self-limited, despite further ingestion of the drug. The G-6-PD Mediterranean enzyme, however, is inactivated so rapidly that reticulocytes emerging from the bone marrow already have decreased G-6-PD activity; the increase in G-6-PD activity during a hemolytic episode is barely detectable, and hemolysis may not be self-limited.

G-6-PD is the first enzyme of the HMP shunt and catalyzes the conversion of G-6-P to 6-phosphogluconate. 6-Phosphogluconate is further metabolized to ribulose-5-P and CO₂. The ribulose-5-P is then converted to F-6-P or G-3-P through the action of transketolase or transaldolase. These substrates then re-enter the glycolytic pathway. The F-6-P can be metabolized to lactic acid or to glucose-6-P and then be recycled through the HMP shunt.

For every mole of G-6-P that enters the HMP shunt, two moles of NADPH are
produced. In turn, NADPH serves to maintain glutathione in the reduced state (GSH) by serving as a co-factor for the enzyme glutathione reductase (GR). The GSH reduces hydrogen peroxide (H₂O₂) to H₂O in the glutathione peroxidase (GSH-Px) reaction. NADPH also participates as a coenzyme in the minor pathway of methemoglobin reduction.

Under normal circumstances, the HMP shunt is relatively inactive, and metabolism proceeds via the glycolytic pathway. Activity of G-6-PD is stimulated by increased concentrations of erythrocyte NADP.76 "Oxidant" compounds appear to decrease the concentration of NADPH and, thereby, increase the concentration of NADP. Through these mechanisms, G-6-PD can counterbalance "oxidant stress" by accelerating flow through the HMP shunt. Subjects deficient in G-6-PD are incapable of compensating in this manner and little NADPH regeneration can occur. In the absence of an intact NADPH generating system, mixed disulfides accumulate in the cell, which leads to instability and heme loss with irreversible denaturation and precipitation of globin as Heinz bodies.

Persons deficient in G-6-PD are clinically asymptomatic unless challenged by "oxidant stress," which may be secondary to a drug or its active metabolite, infection, especially hepatitis, and acidosis.

A certain number of G-6-PD variants manifest a moderate to severe chronic hemolytic state (CNSHA)43,65 in addition to acute exacerbations secondary to drug administration and/or infection. The exact mechanism for chronic hemolysis in these patients is not completely understood.

Deficiency of G-6-PD is often implicated as a cause of neonatal jaundice and kernicterus in otherwise healthy newborns. Term American Black infants94 do not have a significantly increased incidence of jaundice, but term Jamaican,27 African14 and premature24 Black neonates and both term and premature Caucasian23,25 and Oriental42 infants are at increased risk. The appearance of jaundice in otherwise healthy neonates with G-6-PD deficiency tends to be familial.25

The peak bilirubin is usually attained at three to four days and may remain elevated for one to two weeks. The hyperbilirubinemia may be severe and require exchange transfusion to prevent kernicterus. Variants with CNSHA tend to develop jaundice in the first 24 hours and may require early exchange transfusion.46

In an infant with G-6-PD deficiency and hemolysis, a search for an initiating mechanism should be sought, such as maternal drug ingestion, infection, hypoxia, or acidosis. Other causes of hemolysis should be considered, since G-6-PD deficiency may be an "innocent bystander" in certain unresolved cases of neonatal jaundice.

Parents of G-6-PD deficient infants should be advised concerning avoidance of certain drugs and naphthalene containing mothballs. Mothers who are nursing G-6-PD deficient infants should take similar precautions, since these agents may be excreted into breast milk. Heterozygous females should also be advised against the use of any of these offending agents in future pregnancies, since they may cross the placenta.

The diagnosis of G-6-PD deficiency can be made with relative ease employing a screening test10 or the quantitative spectrophotometric enzyme assay.10 It should be reemphasized that the Black deficient with reticulocytosis secondary to drug induced hemolysis or other causes, such as sickle cell anemia, may have a normal G-6-PD screening test. When reticulocytosis is present in G-6-PD Mediterranean, the screening test can be used with confidence. Heterozygous females are more difficult to diagnose. Comparison of the enzyme activity in subjects with G-6-PD A- and reticulocytosis with non-deficient red cells of a similar mean age will reveal that the "low-normal" activity is, in reality, greatly diminished for red cell age.
6-PHOSPHOGLUCONATE DEHYDROGENASE (6-PGD) DEFICIENCY

The conversion of 6-phosphogluconate to ribulose-5-P and CO₂ is catalyzed by 6-PGD. Deficiency of this enzyme²¹ is extremely rare, and there is usually no evidence of hemolysis.

GLUTATHIONE REDUCTASE (GR) DEFICIENCY

Glutathione reductase, as discussed previously, catalyzes the reduction of oxidized glutathione (GSSG) to reduced glutathione (GSH) with NADPH serving as the hydrogen donor. GR exists in two forms, the active flavin-adenine dinucleotide (FAD)-bound form and an inactive form, lacking FAD. Reduction of red cell GR to half normal activity does not impair the rate of reduction of GSSG to GSH. Partial deficiency of GR is quite common and has been reported as an inherited deficiency and found in many diverse disorders, including pancytopenia and hemoglobin C disease. It has recently been recognized that the majority of cases with GR deficiency were dietary in origin and secondary to riboflavin deficiency; administration of riboflavin to these individuals restored GR activity to normal.³ The relationship of GR deficiency to hematologic disease is, at present, ill-defined and of questionable significance.¹¹

GLUTATHIONE PEROXIDASE DEFICIENCY

Glutathione peroxidase (GSH-Px) catalyzes the conversion of H₂O₂ to H₂O. Deficiency of this enzyme³² is transmitted as an autosomal recessive trait and has been reported to be associated with CNSHA, Heinz body formation, drug-induced acceleration of hemolysis, and neonatal jaundice.

However, transient GSH-Px deficiency²⁸ is a normal finding in neonates and has not been associated with neonatal hyperbilirubinemia. GSH-Px is a selenium containing enzyme, and acquired GSH-Px deficiency⁷⁰ is associated with selenium deficiency; there is no evidence of hemolysis in these subjects. Also, 30 percent of individuals of Jewish ancestry were found to have GSH-Px activity that was half normal and had no evidence of hemolysis.¹² Thus, it is probable that GSH-Px deficiency is not a cause of CNSHA¹¹ or neonatal jaundice.

GLUTATHIONE DEFICIENCY

Synthesis of GSH occurs in two steps. In the first step, glutamic acid and cysteine form glutamyl-cysteine; in the second step, glycine combines with glutamyl-cysteine to form the tripeptide, GSH. In affected individuals, either the first step (γ-glutamyl-cysteine synthetase deficiency)⁵¹ or the second step (glutathione synthetase deficiency)³⁷ can be involved.

Deficiency of GSH is transmitted as an autosomal recessive trait. Affected individuals manifest a mild CNSHA and drug-induced exacerbation of hemolysis. Generalized glutathione synthetase deficiency has been associated with oxoprolinuria, chronic metabolic acidosis and, in some instances, neurologic findings.²⁹

Disorders of Nucleotide Metabolism

PYRIMIDINE-5'-NUCLEOTIDASE DEFICIENCY

Pyrimidine-5'-nucleotidase (PD-5'-N) catalyzes the reaction:

Uridine  + Cytidine Monophosphate + H₂O →  Thymidine

Deficiency of PD-5'-N is associated with mild to moderate CNSHA. Neonatal hyperbilirubinemia has been reported. Markedly increased levels of red cell "ATP" and total "adenine nucleotides," elevated glutathione levels, and promi-
nent basophilic stippling of the red cells are characteristic. The increased concentration of ATP reported originally is spurious since they are pyrimidine nucleotides. Deficiency of PD-5'-N also occurs as an acquired disease as a result of lead poisoning.86

**ADENYLATE KINASE DEFICIENCY**

Adenylate kinase (AK) catalyzes the reaction:

\[
2 \text{ADP} \rightarrow \text{ATP} + \text{AMP}
\]

Deficiency of AK77 is associated with a moderate CNSHA and is probably inherited as an autosomal recessive trait. The parents of the affected subjects have intermediate enzyme activity and no hematologic abnormality. The mechanism of the hemolysis has not been defined.

**INCREASED ADENOSINE DEAMINASE ACTIVITY**

Adenosine deaminase (ADA) catalyzes the reaction:

\[
\text{Adenosine} + \text{H}_2\text{O} \rightarrow \text{Inosine} + \text{NH}_3
\]

Decreased ADA activity is associated with combined immunodeficiency and no evidence of hemolysis. Increased ADA activity,87 however, is associated with mild CNSHA. Red cell ATP concentration is characteristically reduced to about 50 to 60 percent of normal.

**Conclusion**

The previously discussed enzymopathies can cause chronic non-spherocytic hemolytic anemia (CNSHA) which may lead to anemia and jaundice in the newborn period. The use of enzyme screening tests for G-6-PD, PK, and GPI deficiencies require small amounts of blood that may be obtained by heel puncture and are helpful in the diagnosis of the three most common enzymopathies of the HMP shunt and glycolytic pathway. However, a negative screen does not definitely rule out PK or G-6-PD deficiency, as previously discussed. Another more common enzymopathy, PD-5'-N deficiency, is characterized by basophilic stippling of the red cells. Analysis of the pattern of glycolytic intermediates may reveal a crossover site and suggest a specific enzymopathy. The 2,3-DPG concentration can be measured with blood obtained from a heel puncture and may be decreased (suggesting an enzyme deficiency at or above the DPGM step) or increased (suggestive of an enzymopathy distal to DPGM).

In many instances, however, after exhaustive investigation of known enzyme deficiencies in specialized laboratories, a specific enzyme deficiency may not be identified.

**References**


