HEMOLYTIC ANEMIAS: RED BLOOD CELL MEMBRANE AND METABOLIC DEFECTS

PATRICK G. GALLAGHER

The mature erythrocyte differs from all other cells in the body. Lacking a nucleus, DNA, RNA, and ribosomes, it cannot synthesize RNA, DNA, or protein. It does not divide, it has no mitochondria, it cannot perform the Krebs cycle, and it lacks an electron transport system for oxidative phosphorylation. After enucleation, the reticulocyte, the precursor of the mature erythrocyte, leaves the marrow and enters the circulation equipped with a full complement of enzymes, transporters, signaling molecules, and all other proteins necessary to perform the essential functions of the red blood cell (RBC) during its lifespan.

The erythrocyte membrane accounts for only about 1% of the total weight of an RBC, yet it plays a critical role in the maintenance of normal RBC homeostasis through a number of mechanisms. These include retention of vital compounds and removal of metabolic waste, regulation of erythrocyte metabolism and pH, and import of iron required for hemoglobin (Hb) synthesis during erythropoiesis. The membrane maintains a slippery exterior so that erythrocytes do not aggregate or adhere to endothelial cells. The membrane skeleton, a network of proteins on the inner surface of the RBC, provides the strength and flexibility needed to maintain the normal shape and deformability of the erythrocyte.

The principal functions of erythrocyte metabolism in the mature erythrocyte include maintenance of adequate supplies of adenosine triphosphate (ATP), production of reducing substances to act as antioxidants, and control of oxygen affinity of Hb by production of adequate amounts of 2,3-diphosphoglycerate (2,3-DPG). Because the mature erythrocyte has lost its ability to perform oxidative phosphorylation, its energy is supplied by anaerobic glycolysis through the Embden-Meyerhof pathway, by oxidative glycolysis through the hexose monophosphate (HMP) shunt, and through nucleotide salvage pathways.

**THE ERYTHROCYTE MEMBRANE**

Composed of a lipid bilayer and an underlying cortical membrane skeleton (Fig. 161-1), the membrane provides the erythrocyte the deformability and stability required to withstand its travels through the circulation. In one circulatory cycle throughout the body, an erythrocyte is subjected to high shear stress in the arterial system, dramatic size and shape changes in the microcirculation with capillary diameters as small as 7.5 μm and marked fluctuations in tonicity, pH, and Po₂.

**Membrane Lipids**

Red blood cell membrane lipids are asymmetrically distributed across the bilayer membrane, reflecting a steady state involving a constant exchange of phospholipids between the two bilayer hemileaflets. Glycolipids and cholesterol are intercalated between the phospholipids in the bilayer with their long axes perpendicular to the bilayer plane. Glycolipids, located in the external half of the bilayer with their carbohydrate moieties extending into the aqueous phase, carry several important RBC antigens and serve other important functions. Phospholipids are asymmetrically organized, with the choline phospholipids, phosphatidylcholine and sphingomyelin, primarily in the outer half of the bilayer, and the amino phospholipids, phosphatidylethanolamine and phosphatidylserine (PS), in the inner half of the bilayer. In pathologic states, such as thalassemia, sickle cell disease, and diabetes, loss of phospholipid asymmetry with externalization of PS leads to activation of blood clotting through conversion of prothrombin to thrombin and facilitates macrophage attachment to erythrocytes, marking them for destruction. Mature erythrocytes are unable to synthesize fatty acids, phospholipids, or cholesterol de novo and depend on lipid exchange and fatty acid acylation as mechanisms for phospholipid repair and renewal.

**FIGURE 161-1** The erythrocyte membrane. A model of the major proteins of the erythrocyte membrane is shown: α and β spectrin, ankyrin, band 3 (the anion exchanger), 4.1 (protein 4.1) and 4.2 (protein 4.2), actin, and glycophorin. (From Perrotta S, Gallagher PG, Mohandas N. Hereditary spherocytosis. Lancet. 2008;372:1411-1426.)
Hereditary spherocytosis affects approximately one in 2000 to 3000 individuals of northern European ancestry. Found worldwide, it is much more common in whites than individuals of African ancestry.

Pathobiology

The primary defect in HS is the loss of erythrocyte membrane surface area caused by defects in erythrocyte membrane proteins, including α spectrin, β spectrin, ankyrin, band 3, and protein 4.2. Qualitative or quantitative defects of one or more of these membrane proteins lead to membrane instability, which, in turn, leads to membrane loss. In approximately two thirds of HS patients, inheritance is autosomal dominant. In the remaining patients, inheritance is nondominant owing to a de novo mutation or autosomal recessive inheritance. Cases with autosomal recessive inheritance are caused by defects in either α spectrin or protein 4.2. Rare cases of homozygous HS have been reported, resulting in fetal death or severe hemolytic anemia. In most cases, HS mutations are "private," that is, each individual has a unique mutation, implying that there is no selective advantage to HS.

The spleen plays a critical, albeit secondary, role in the pathophysiology of HS. Splenic destruction of poorly deformable spherocytes is the primary cause of hemolysis experienced by HS patients. Abnormal erythrocytes are trapped in the splenic microcirculation and ingested by phagocytes. Moreover, the splenic environment is hostile to erythrocytes, with low pH, low glucose, and low ATP concentrations and high local concentrations of toxic free radicals produced by adjacent phagocytes, all contributing to membrane damage.

Clinical Manifestations

The clinical manifestations of the spherocytosis syndromes vary widely. The classic triad of HS is anemia, jaundice, and splenomegaly. Rarely, patients may have severe hemolytic anemia presenting in utero or shortly after birth and continuing through the first year of life. These patients may require multiple blood transfusions, and in some cases, splenectomy in the first year of life. Many patients with HS escape detection throughout childhood. In these patients, the diagnosis of HS may not be made until they are being evaluated for unrelated disorders later in life or when complications related to anemia or chronic hemolysis occur. Although the lifespan of an erythrocyte in these patients may be shortened to only 20 to 30 days, they adequately compensate for their hemolysis with increased bone marrow erythropoiesis.

Chronic hemolysis leads to the formation of bilirubinate gallstones, which are the most frequently reported complication in patients with HS. Although gallstones have been observed in early childhood, most appear in adolescents and young adults. Routine interval ultrasonography to detect gallstones should be performed even if patients are asymptomatic.

Other complications of HS include aplastic, hemolytic, and megaloblastic crises. Aplastic crises occur after virally induced bone marrow suppression and present with anemia, jaundice, fever, and vomiting. The most common etiologic agent in these cases is parvovirus B19 (Chapter 371). Hemolytic crises, usually associated with viral illnesses and occurring before 6 years of age, are generally mild and present with jaundice, increased spleen size, and a decrease in hematocrit. Megaloblastic crises occur in HS patients with increased folate demands, such as the pregnant patient, growing children, or patients recovering from an aplastic crisis.

Uncommon manifestations of HS include skin ulceration, gout, chronic leg dermatitis, cardiomyopathy, spinal cord dysfunction, movement disorders, and extramedullary erythropoiesis. In patients with untreated severe HS, poor growth and findings attributable to extramedullary hematopoiesis, such as hand and skull deformities, may be found.

Diagnosis

Patients with HS may present at any age, usually with anemia, hyperbilirubinemia, or an abnormal blood smear. In evaluating a patient with suspected HS, particular attention should be paid to the family history, including questions about anemia, jaundice, gallstones, and splenectomy. The initial laboratory investigation should include a complete blood count with a peripheral smear, reticulocyte count, direct antiglobulin test (Coombs test), and serum bilirubin. When the peripheral smear or family history is suggestive of HS, an incubated osmotic fragility test or flow cytometric analysis of eosin-5-maleimide–labeled erythrocytes (EMA binding) (discussed later) should be obtained. Rarely, additional, specialized testing is required to confirm the diagnosis.
Overall, laboratory findings in HS are heterogeneous. Erythrocyte morphology is distinctive but not diagnostic (Fig. 161-2, A). Typical HS patients have blood smears with easily identifiable spherocytes lacking central pallor. Some patients present with only a few spherocytes on peripheral smear, but others present with numerous small, dense spherocytes and bizarre erythrocyte morphology. Specific morphologic findings have been identified in patients with certain membrane protein defects such as punctured erythrocytes (band 3) or spherocytic acanthocytes (β spectrin). When examining a smear in a case of suspected spherocytosis, it is important to have a high-quality smear with the erythrocytes well separated and some cells with central pallor in the field of examination because spherocytes are a common artifact on peripheral blood smears. The presence of spherocytosis on peripheral blood smear is not diagnostic of HS. Other disorders with spherocytes on peripheral blood smear are listed in Table 161-1.

The mean corpuscular hemoglobin concentration (MCHC) is increased (between 34.5 and 38) owing to relative cellular dehydration. The mean corpuscular volume (MCV) is usually normal or slightly decreased. Many cell counters provide a histogram of MCHCs claimed to be accurate enough to identify nearly all patients with HS.

In a normal erythrocyte, a redundancy of cell membrane gives the cell its characteristic discoid shape and provides it abundant surface area. In spherocytes, there is a decrease in surface area relative to cell volume, resulting in their abnormal shape. This change is reflected in the increased osmotic fragility found in these cells. Osmotic fragility is tested by adding increasingly hypotonic concentrations of saline to RBCs. Normal erythrocytes are able to increase their volume by swelling, but spherocytes, which are already at maximal volume for surface area, burst at higher saline concentrations than normal. Approximately one fourth of HS individuals will have a normal osmotic fragility on freshly drawn RBCs, with the osmotic fragility curve approximating the number of spherocytes seen on peripheral smear. However, after incubation at 37°C for 24 hours, HS RBCs lose membrane surface area more readily than normal because their membranes have become leaky and unstable. Thus, incubation accentuates the defect in HS erythrocytes and brings out the defect on osmotic fragility, making incubated osmotic fragility the standard test in diagnosing HS (Fig. 161-3, bottom panel). When the spleen is present, a subpopulation of very fragile erythrocytes that have been conditioned by the spleen form the tail of the osmotic fragility curve. This tail disappears after splenectomy. The osmotic fragility test suffers from poor sensitivity, with as many as 20% of mild cases of HS missed after incubation. It is unreliable in patients who have small numbers of spherocytes and in patients who have been recently transfused. It is abnormal in other conditions in which spherocytes are present.

Eosin-5-maleimide binding is a flow cytometry-based test used in the diagnosis of HS. EMA is a fluorescent dye that binds to band 3 and Rh-related proteins in the erythrocyte membrane. In HS, the mean fluorescence of EMA-stained erythrocytes is lower compared with control because of the reduction of band 3 and related proteins, typically decreased to approximately 65% of normal (Fig. 161-3, top panel). Although primary defects of band 3 protein are seen in only about 25% of HS patients, decreased fluorescence intensity is also observed in the erythrocyte membranes of HS patients with defects in other membrane proteins such as ankyrin and spectrin. This is thought to be attributable to transmission of long-range effects of mutant protein defects across the membrane lattice, ultimately influencing the amount of EMA binding to band 3. EMA binding has good sensitivity and specificity and is simple and rapidly performed.

Specialized testing is available for studying difficult cases or cases in which additional information is desired. Useful tests for these purposes include structural and functional studies of erythrocyte membrane proteins, such as protein quantitation, limited tryptic digestion of spectrin, spectrin, and ion transport. Membrane rigidity and fragility may be examined using an ektacytometer. Complementary DNA and genomic DNA analyses are available when a molecular diagnosis is desired.

Other laboratory manifestations in HS are manifestations of ongoing hemolysis. Increased serum bilirubin, increased lactate dehydrogenase,

### Table 161-1

**DISORDERS WITH SPHEROCYTES ON PERIPHERAL BLOOD FILM**

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increased urinary and fecal urobilinogen, and decreased serum haptoglobin reflect increased erythrocyte destruction.

After diagnosing a patient with HS, family members should be examined for the presence of HS. This can be of great epidemiologic importance, particularly for very old and very young patients. Prenatal diagnosis of HS has been made in a few cases, but this is rarely necessary.

Hereditary Elliptocytosis and Related Disorders

**DEFINITION**

Hereditary elliptocytosis is characterized by the presence of elliptical or oval cigar-shaped erythrocytes on peripheral blood smears of affected individuals (see Fig. 161-2, B).

**EPIDEMIOLOGY**

Hereditary elliptocytosis has been estimated to occur in approximately one in 2000 to 4000 individuals. The true incidence of HE is unknown because its clinical severity is heterogeneous, and many patients are asymptomatic. It is common in African Americans and people of Mediterranean ancestry, presumably because elliptocytes confer some resistance to malaria. In parts of Africa, the incidence of HE approaches one in 100.

**PATHOBIOLOGY**

The principal defect in HE is mechanical weakness or fragility of the erythrocyte membrane skeleton. Qualitative and quantitative defects in a number of RBC membrane proteins have been described in HE, including α-spectrin, β-spectrin, protein 4.1, and glycoporphin C. Most defects occur in spectrin, which is the principal structural protein of the erythrocyte membrane skeleton. Several mutations have been described in the α-spectrin, β-spectrin, and glycoporphin C genes, including point mutations, gene deletions and insertions, and messenger RNA processing defects. Several mutations have been identified in a number of individuals of the same genetic background, suggesting a "founder effect" for these mutants, which supports the

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**FIGURE 161-2.** Testing in hereditary spherocytosis. Top panel, Eosin-5-maleimide (EMA) binding. Histogram of fluorescence of EMA-labeled erythrocytes from a normal control and a patient with typical hereditary spherocytosis. Decreased fluorescence in observed from HS erythrocytes. Bottom panel, Osmotic fragility curves in hereditary spherocytosis. The shaded region is the normal range. Results representative of both typical, and severe spherocytosis are shown. A tail, representing fragile erythrocytes conditioned by the spleen, is common in spherocytosis patients prior to splenectomy. (From Gallagher PG. Abnormalities of the erythrocyte membrane. *Pediatr Clin North Am* 2013;60:1349-1352.)

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**TREATMENT AND PROGNOSIS**

Splenectomy is indicated, laparoscopic splenectomy has become the method of choice. This technique results in less postoperative discomfort, a quicker return to preoperative diet and activities, shorter hospitalization, decreased costs, and smaller scars. Even massive spleens can be removed laparoscopically because the spleen is placed in a large bag, diced intraoperatively, and eliminated through suction catheters. Partial splenectomy, initially advocated for infants and young children with significant anemia associated with erythrocyte membrane disorders to allow for palliation of hemolysis and anemia while maintaining some residual splenic immune function, is now being suggested by some for most HS patients. Updated UK guidelines, which reflect changes in current opinion about surgical management, include (1) preference for a laparoscopic approach, (2) performance of splenectomy ideally after the age of 6 years, (3) no indication for extended thrombosis prophylaxis after splenectomy for HS, and (4) avoidance of splenectomy in patients with some forms of hereditary stomatocytosis because of an increased risk of venous thromboembolism.

Before splenectomy, patients should be immunized with vaccines against *Haemophilus influenzae* type B, and meningococcus. Postsplenectomy care includes counseling of patients or parents to seek prompt medical care in case of febrile illness. Use of routine antibiotics after splenectomy for prevention of pneumococcal sepsis is controversial. Data are lacking to indicate or refute their prescription. Before splenectomy and, in severe cases, after splenectomy, HS patients should take folic acid (1 mg/day orally) to prevent folate deficiency.

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**CHAPTER 161 HEMOLYTIC ANEMIAS**
the hypothesis that there has been genetic selection for elliptocytosis because these RBCs confer some resistance to malaria. Most cases of HE are inherited in an autosomal dominant pattern, with rare cases of de novo mutations.

**CLINICAL MANIFESTATIONS**

The clinical presentation of HE is heterogeneous, ranging from asymptomatic carriers to patients with severe, life-threatening anemia. Most patients with HE are asymptomatic and are diagnosed incidentally during testing for unrelated conditions. Asymptomatic carriers have been identified who possess the same molecular defect as an affected HE relative but who have normal peripheral blood smears. The erythrocyte lifespan, normal in most patients, is decreased in only about 10% of patients. This subset of HE patients with decreased erythrocyte lifespan experience hemolysis, anemia, splenomegaly, and intermittent jaundice. Many of these patients have parents with typical HE and thus are homozygotes or compound heterozygotes for defects inherited from each of the parents. Symptoms may vary among members of the same family, indeed, they may vary in the same individual at different times.

**Hereditary Pyropoikilocytosis**

Hereditary pyropoikilocytosis is a rare cause of anemia with distinctive erythrocyte morphology on peripheral blood smear (see Fig. 161-2, C) and has a picture similar to that seen in patients with severe burns. Patients typically present in infancy with severe anemia and peripheral blood smear findings of elliptocytosis, poikilocytosis, pyknocytosis, and fragmentation. Microspherocytosis is common, and the MCV is usually very low (40-70 fl). Most patients are of African ancestry, and at least one third of HPP patients have a parent or sibling with typical HE. Patients with HPP tend to experience severe hemolysis and anemia in infancy that gradually improves, evolving toward typical HE later in life.

**DIAGNOSIS**

Cigar-shaped elliptocytes on peripheral blood smear are the hallmark of HE (see Fig. 161-2, B). These normochromic, normocytic elliptocytes vary in number from a few to 100%, with the likelihood of hemolysis not correlating with the number of elliptocytes present. Ovalocytes, spherocytes, stomatocytes, and fragmented cells may also be seen. In some cases, poikilocytes may be prominent. Elliptocytes may be seen in association with other disorders, including megaloblastic anemias, hypochromic microcytic anemias (iron deficiency anemia and thalassemia), myelodysplastic syndromes, and myelofibrosis; however, elliptocytes generally make up less than one third of RBCs in these conditions. History and additional laboratory testing usually clarify the diagnosis of these disorders. In typical cases, the incubated osmotic fragility is normal, but in severe HE and HPP, incubated osmotic fragility is increased, and EMA binding is decreased.

Other laboratory findings in HE are similar to those found in other hemolytic anemias and are nonspecific markers of increased erythrocyte production and destruction. The reticulocyte count generally is less than 5% but may be higher when hemolysis is severe. Similar to HS, specialized laboratory procedures are available to study the erythrocyte membranes of HE and HPP patients. These studies are not routinely required to make the diagnosis of HE or HPP, but they may be helpful in studying problematic cases and in elucidating the underlying molecular defects.

**TREATMENT**

Therapy is rarely needed in patients with HE. In rare cases, occasional RBCs transfusions may be required. In cases of severe HE and HPP, splenectomy has been palliative because the spleen is the site of erythrocyte sequestration and destruction. Many practitioners think that the same indications for splenectomy in HS should be applied to patients with symptomatic HE or HPP. Post-splenectomy patients with HE or HPP experience increased hemolysis, decreased reticulocyte counts, and improvement in clinical symptoms. Patients should be followed for signs of decompensation during acute illnesses, interval ultrasonography to detect gallstones should be performed. In patients with significant hemolysis, folate should be administered daily.

**Hereditary Stomatocytosis Syndromes**

Red blood cell hydration is primarily determined by the intracellular concentration of monovalent cations. A net increase in sodium and potassium ions causes water to enter, forming stomatocytes (see Fig. 161-2, D) or hydrocytes, but a net loss of sodium and potassium produces dehydrated RBCs, or xeroocytes. Numerous descriptions of congenital or familial hemolytic anemias associated with abnormal cation permeability and, in some cases, disturbed RBC hydration have been reported. These span the range from severe hydropsysis to severe xerocytosis. In many cases, the molecular bases of this group of disorders are unknown. An unusual characteristic of the stomatocytosis syndromes is a predisposition to thrombosis after splenectomy. Acquired stomatocytosis has been associated with acute alcoholism and hepatobiliary disease, vinca alkaloid administration, neoplasms, and cardiovascular disease. Stomatocytosis is also sometimes observed as a processing artifact.

**OVERHYDRATED HEREDITARY STOMATOCYTOSIS (HYDROCYTOSIS)**

This group of disorders is characterized by stomatocytes, erythrocytes with a mouth-shaped (stoma) area of central pallor on peripheral blood smear (see Fig. 161-2, D), severe hemolysis, macrocytosis (110-150 fl), elevated erythrocyte sodium concentration, reduced potassium concentration, and increased total Na⁺ and K⁺ content. The excess cations expand cell water, producing large, osmotically fragile cells with low MCHCs (24%-30%). The clinical severity of overhydrated hereditary stomatocytosis is variable; some patients experience hemolysis and anemia, but others are asymptomatic. Missense mutations in the Rh-associated glycophorin (RhAG) have been identified in a subset of hydrocytosis patients.

**DEHYDRATED HEREDITARY STOMATOCYTOSIS (XEROCYTOSIS)**

Blood smears from patients with dehydrated hereditary stomatocytosis exhibit contracted and spiculated RBCs, dessicocytes, a variable number of stomatocytes, and target cells. Most patients have nearly normal erythrocyte morphology, with only a few target cells and an occasional echinocyte or stomatocyte. The MCV (95-115 fl) and MCHC are increased, and the osmotic fragility is reduced (i.e., resistance to osmotic lysis). The characteristic biochemical abnormality is a decreased potassium concentration and total monovalent cation content. Dominantly inherited mutations in PIEZO1, encoded by the FAM38A gene, have been identified in xerocytosis patients. PIEZO proteins are the recently identified pore-forming subunits of channels that mediate mechanotransduction in mammalian cells. Association of PIEZO variants with changes in erythrocyte hydration suggest that these proteins play an important role in erythrocyte volume homeostasis.

**INTERMEDIATE SYNDROMES AND HEREDITARY STOMATOCYTOSIS VARIANTS**

Hydrocytosis and xerocytosis represent the extremes of a spectrum of RBC permeability defects. A number of families with features of both conditions have been reported. Some patients with severe permeability defects have little or no hemolysis. The proportion of stomatocytes and the degree of sodium influx do not correlate with each other, and neither correlates with the amount of hemolysis or anemia.

**ERYTHROCYTE METABOLISM**

The primary functions of the erythrocyte, gas transport and exchange, are maintained without a net change in energy state. However, several critical functions of the erythrocyte depend on the production and expenditure of energy. As erythrocytes age, glucose utilization and ATP levels fall, leading to decreased membrane deformability and, ultimately, a shortened lifespan. Lower potassium levels, higher sodium levels, and decreased membrane lipids are also seen in ATP-deficient, aging erythrocytes.

Erythrocytes do not undergo oxidative phosphorylation and do not store glycogen; thus, they must constantly catabolize glucose from the blood stream through the Embden-Meyerhof pathway and the HMP shunt as a source of energy (Fig. 161-4). Erythrocytes incorporate glucose from the plasma through facilitated transfer, with erythrocyte glucose levels rapidly equilibrating with changes in blood glucose levels. Glucose is the preferred carbohydrate of the RBC, but fructose and mannose are metabolized almost as readily. Inside the erythrocyte, glucose is converted to glucose-6-phosphate or to fructose by sorbitol. Glucose-6-phosphate follows one of three pathways: (1) most (~90%) enters the Embden-Meyerhof pathway, where it is converted into lactate, pyruvate, and ATP; (2) some (~5%-10%) enters the HMP shunt to produce reduced intermediates and ribulose 5-phosphate, the latter of which eventually enters the Embden-Meyerhof pathway; and (3) a tiny fraction (~1%) is converted to glucose-1-phosphate and then to glycogen.

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**Embden-Meyerhof Pathway**

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Embden-Meyerhof Pathway

The Embden-Meyerhof pathway of glycolysis is the primary source of ATP, 2,3-DPG and nicotinamide adenine dinucleotide, reduced form (NADH) in erythrocytes (see Fig. 161-4). Most of the energy generated by erythrocytes is through the Embden-Meyerhof pathway followed by storage as high-energy phosphates such as ATP or as reducing energy in the form of glutathione or pyridine nucleotides (NADH and nicotinamide adenine dinucleotide phosphate, reduced form [NADPH]). This pathway metabolizes about 90% of erythrocyte glucose with the catabolism of 1 mole of glucose yielding 2 moles of ATP and 2 moles of lactate. Two moles of ATP per mole of metabolized glucose seems insignificant compared with the Krebs cycle of intermediary metabolism, in which 1 mole of glucose metabolized produces 38 moles of ATP. However, this ATP production is adequate to renew 150% to 200% of the total RBC ATP every hour.

The Embden-Meyerhof pathway is also the primary source of NADH, a necessary cofactor for NADH methemoglobin reductase, which maintains heme iron in the reduced state. Without this reaction, heme iron would be oxidized to methemoglobin, which is not a functional oxygen transporter.

Finally, the Rapoport-Luebering shunt of the Embden-Meyerhof pathway (see Fig. 161-4) produces 2,3-DPG, a compound found in high concentrations in erythrocytes but in low concentrations in other cells. After it is formed, under physiologic conditions of pH and solute concentrations, 2,3-DPG binds reversibly to tetramers of deoxyhemoglobin with greater affinity than it does to oxyhemoglobin. By binding to deoxyhemoglobin, it allosterically upregulates the release of the remaining oxygen bound to the Hb, enhancing the ability of erythrocytes to release oxygen near tissues that need it most.

Hexose Monophosphate Shunt (Pentose Phosphate Pathway)

In the HMP shunt (see Fig. 161-4), glucose-6-phosphate undergoes oxidation followed by a series of reactions to yield fructose-6-phosphate and glyceraldehyde-3-phosphate, intermediates in the glycolytic pathway. The HMP shunt is the primary source of erythrocyte NADPH, with 2 moles of NADPH produced for each mole of glucose metabolized. NADPH is required for the reduction of oxidized glutathione and some protein sulphydryl groups.

Mature erythrocytes synthesize large amounts of reduced glutathione (GSH). GSH protects erythrocytes from oxidants, including hydrogen peroxide (H₂O₂), superoxide anions (O₂⁻), and hydroxyl radicals (OH), which are produced as byproducts of the oxidation of heme by oxygen. Oxidants are also produced by activated phagocytes (e.g., during infection) and by erythrocytes after exposure to certain agents. When oxidants accumulate, they damage cellular proteins and lipids. Detoxification of H₂O₂ is significantly enhanced by glutathione peroxidase. GSH is converted to oxidized glutathione (GSSG) and to mixed disulfides with protein thiols. GSH levels are restored by glutathione reductase. In this process, NADPH is oxidized to nicotinamide adenine dinucleotide phosphate (NADP⁺), which stimulates the HMP shunt to regenerate NADPH. After oxidant stress, hypoxia, or acidosis, erythrocytes can increase the amount of glucose metabolized through the HMP shunt up to 10- to 20-fold to generate...
increased amounts of reduced glutathione. The tight coupling of glutathione metabolism with the HMP shunt protects the mature erythrocyte from oxidative stress.

**DISORDERS OF ERYTHROCYTE METABOLISM**

**Congenital nonspherocytic hemolytic anemia (CNSHA)** traditionally includes erythrocyte disorders not due to defects of the RBC membrane or Hb, immune-mediated disease, or other diseases such as paroxysmal nocturnal hemoglobinuria. CNSHA is a heterogeneous group of disorders associated with various metabolic abnormalities of the erythrocytes, including enzymopathies of glucose, glutathione, and nucleotide metabolism. Similar to the membrane disorders, clinical, biochemical, and genetic heterogeneity are typical within the enzymopathies. Hemolysis may develop as a result of either enzyme or antioxidant deficiency or dysfunction (e.g., abnormal substrate or cofactor binding), altered activation or inhibition characteristics, or decreased stability or specific activity.

Peripheral blood smears in CNSHA, with the exception of pyrimidine S'-nucleotidase (PSN) deficiency, are unremarkable. Osmotic fragility of fresh erythrocytes is normal. Response to splenectomy is variable. Inheritance is heterogeneous. A thorough family history is important and may be of assistance in determining the diagnosis. Manifestations of the metabolic defect are usually confined to the erythrocyte but may occasionally involve nonerythroid cells.

Definitive diagnosis of metabolic abnormalities of the RBCs depends on qualitative or quantitative assays of specific enzyme activity or identification of the specific genetic mutation by DNA analysis. Results of enzyme assays should be interpreted with caution because (1) they only sample surviving RBCs in the peripheral blood, and the metabolic milieu of these cells is not necessarily comparable to cells already hemolyzed; (2) in vitro enzyme assay conditions may not accurately reflect the in vivo environment; (3) transfusions before the assay may obscure the underlying metabolic defect; and (4) leukocyte contamination may lead to spurious results. Finally, average enzyme activity may not accurately reflect activity in subpopulations of erythrocytes. This is particularly true when there is reticulocytosis, which may yield artificially elevated mean enzyme activity owing to higher enzyme levels found in reticulocytes.

**Disorders of the Embden-Meyerhof Pathway**

Defects of the Embden-Meyerhof pathway are inherited in an autosomal recessive fashion, and usually hemolysis is seen only in homozygotes or compound heterozygotes. Heterozygotes, whose erythrocytes contain less than normal amounts of mutant enzyme, are clinically normal.

An exception is phosphoglycerate kinase deficiency, an X-linked disorder with hemolysis found only in males. In this group of disorders, hemolysis is chronic, is not typically influenced by drugs or other inciting agents, and is attributed to insufficient levels of erythrocyte ATP. Splenomegaly from trapping of mutant erythrocytes is common. The hostile splenic environment contributes to the shortened erythrocyte lifespan. When performing specific diagnostic enzyme assays, measurement of glycolytic intermediates may assist in diagnosis because concentrations of intermediates are increased upstream of a defect and decreased downstream of a defect.

**PYRUVATE KINASE DEFICIENCY**

Pyruvate kinase (PK) deficiency accounts for approximately 90% of inherited defects of the Embden-Meyerhof pathway and is the second most common inherited erythrocyte enzymopathy associated with anemia after glucose-6-phosphate dehydrogenase (G6PD) deficiency (see later). PK deficiency is found worldwide, but it is most common in individuals of northern European descent.

**PATHOBIOLOGY**

Pyruvate kinase catalyzes the conversion of phosphoenolpyruvate (PEP) to pyruvate, generating ATP. Deficient or defective PK leads to decreased levels of erythrocyte ATP, disturbing many cellular processes such as signaling and maintenance of water and ion content, leading to energy failure and dehydroxylation. Upstream catabolites accumulate in the erythrocyte, including 2,3-DPG, which shifts the oxygen dissociation curve to the right, enhancing tissue oxygenation and ameliorating some of the physiologic effects of anemia. Early PK-deficient reticulocytes retain the ability to use oxidative phosphorylation to produce ATP, bypassing their defect. This ability is lost as reticulocytes mature and is markedly dampened in the hypoxic environment of the spleen.

Pyruvate kinase deficiency is inherited in an autosomal recessive manner. Affected individuals are homozygous or compound heterozygotes for PK defects. Heterozygotes are clinically normal or exhibit very minimal hemolysis.

**CLINICAL MANIFESTATIONS**

Clinical manifestations in PK deficiency are heterogeneous, ranging from asymptomatic to transfusion-dependent hemolytic anemia. More severely affected patients present in infancy or early childhood with anemia, jaundice, and splenomegaly. Occasionally, patients may escape detection until later in life when complications related to anemia and chronic hemolysis occur such as cholelithiasis or aplastic crisis or when the diagnosis is made during evaluation of the patient for another condition.

**DIAGNOSIS**

The peripheral blood smear demonstrates normocytic, normochromic erythrocytes, sometimes with spiculations (Fig. 161-S, A). Poikilocytes and acanthocytes may also be seen. Reticulocytosis is common. Osmotic fragility of fresh erythrocytes is usually normal. Occasional patients exhibit a population of osmotically fragile cells after incubation.

NADH fluorescence under ultraviolet light is a commonly used screening test for PK deficiency. PEP and NADH are mixed with the patient’s blood, incubated, and spotted on filter paper, and fluorescence is measured. Direct enzyme assay, which uses PEP as substrate for PK, can be performed on leukocyte-free hemolysate to confirm abnormal fluorescence tests. Leukocytes must be carefully depleted from the samples because they contain more than 300 times the PK activity of erythrocytes.

**TREATMENT**

Most patients require only expectant management, with only rare transfusions, such as during an aplastic episode. In severe cases, patients may be transfusion dependent. In these cases, splenectomy typically lessens hemolysis and ameliorates the anemia. After splenectomy, some patients develop marked reticulocytosis, up to 50% to 70%. This paradoxical reticulocytosis is attributed to increased reticulocyte survival after removal of the hostile splenic environment.

**OTHER DISORDERS OF THE EMBDEN-MEYERHOF PATHWAY**

Other abnormalities of the Embden-Meyerhof pathway have been described. Hexokinase deficiency is quite uncommon, with great phenotypic variability in reported cases. Severely affected patients have had anemia beginning in infancy and may require blood transfusions. Glucose phosphate isomerase (GPI) deficiency is the third most common hemolytic enzymopathy. GPI deficiency usually presents in infancy or early childhood with moderate to severe hemolytic anemia. Rare cases of GPI deficiency may also be complicated by neurologic symptomatology. Phosphofructokinase deficiency may involve erythrocytes, muscle, or both. The presentation is usually in adolescence with exertional myopathy (Chapter 207). Hemolytic anemia has been described in isolated cases of 2,3-bisphosphoglycerate mutase deficiency and phosphoglycerate kinase deficiency.

**Disorders of Nucleotide Metabolism**

Mature erythrocytes lack the ability to synthesize purine and pyrimidine nucleotides de novo. However, they are able to form some nucleotides through salvage pathways.

**PYRIMIDINE 5'-NUCLEOTIDASE DEFICIENCY**

Pyrimidine 5'-nucleotidase degrades the pyrimidine nucleotides of RNA to cytidine and uridine, which can diffuse out of the cell. When PSN is deficient, nondiffusible, partially degraded RNAs accumulate, leading to the marked basophilic stippling characteristic of PSN-deficient erythrocytes (see Fig. 161-S, B). These accumulated pyrimidine nucleotides inhibit the transport of GSSG (oxidized glutathione) out of RBCs, leading to high levels of erythrocyte glutathione. Clinically, the patient has mild to moderate hemolytic anemia and splenomegaly. The cause of the hemolysis remains cryptic. Typically, splenectomy does not ameliorate the hemolysis and anemia.
Disorders of the Hexose Monophosphate Shunt (Phosphate Pathway) and Associated Pathways

Disorders of the HMP shunt or of the glutathione metabolic pathways (see Fig. 161-4) compromise the ability of the RBC to respond adequately to oxidative stress. In normal erythrocytes, GSH detoxifies oxidants produced by various agents and infection. In G6PD-deficient erythrocytes, because of the inability to generate NADPH, GSH levels are inadequate, leaving the cell susceptible to oxidant stress. Oxidation of Hb sulfhydryl groups leads to the production of methemoglobin and intracellular Hb precipitates called Heinz bodies. Heinz bodies (see Fig. 161-5, D), usually visualized on peripheral blood smears with supravital stains such as methyl violet, attach to and damage the erythrocyte membrane. They induce clustering of immunoglobulins and band 3 protein, marking the erythrocyte for opsonization by phagocytes and eventual removal from the circulation. Heinz bodies are “pitted” from circulating cells by the spleen and are commonly seen on smears of patients after splenectomy. “Bite cells,” erythrocytes with localized invagination, possibly at the site of Heinz body injury or removal, are seen during acute hemolytic episodes. In addition to damage from Heinz body formation, GSH-deficient erythrocytes undergo peroxidation of membrane phospholipids and oxidative cross-linking of spectrin, decreasing membrane deformability and further promoting splenic trapping.

GLUCOSE-6-PHOSPHATE DEHYDROGENASE DEFICIENCY

G6PD deficiency is the most common inherited disorder of erythrocyte metabolism, affecting more than 400 million people worldwide. The high prevalence of G6PD deficiency is thought to be attributable to genetic selection because G6PD-deficient erythrocytes have a selective advantage against invasion by the malaria parasite Plasmodium falciparum.

Epidemiology and Pathobiology

G6PD is the initial and rate-limiting step in the HMP shunt (see Fig. 161-4), which converts NADP into NADPH. NADPH is required for the generation of glutathione, a critical constituent in the prevention of oxidative damage to the cell. G6PD-deficient patients may develop acute hemolytic anemia after exposure to oxidative stress. Although G6PD is a ubiquitous enzyme, erythroid cells are particularly susceptible to oxidative stress because the HMP shunt is their only source of NADPH.

Hundreds of G6PD variants have been described, but only a few are common. Variants are classified on the basis of biochemical characteristics; electrophoretic mobility; ability to use substrate analogue, Km for NADP and G6PD; pH activity profile; and thermal stability. The normal enzyme, Gd^a^, is present in 99% of white Americans and 70% of African Americans. A normal variant, Gd^bb^, found in 20% of African Americans, has a faster electrophoretic mobility than Gd^a^, Gd^bb^, the most common variant associated with hemolysis, is found in about 10% of African Americans and in many Africans. Gd^c^ has decreased catalytic activity compared with Gd^a^, Gd^bb^, the second most common variant associated with hemolysis, is common in the Mediterranean area, in India, and in Southeast Asia, with a prevalence of up to 5% to 50%. Gd^bb^ exhibits markedly decreased catalytic activity. Gd^a^, a variant common in Asian populations, produces a clinical syndrome similar to Gd^bb^.

Gd^a^ activity decreases as normal cells age, with a half-life of approximately 60 days. Despite very low levels of or no active G6PD, older erythrocytes maintain the ability to produce NADPH and maintain a GSH response to oxidative stress. The Gd^a^ variant has a half-life of only 13 days, so young cells have a normal amount of enzyme activity, but older RBCs are grossly deficient. Because of this heterogeneity in G6PD levels, individuals with the Gd^a^ variant experience only limited hemolysis after oxidant exposure.

More than 100 mutations in the G6PD gene, localized to Xq28, have been described. Most mutations are amino acid substitutions that influence enzyme kinetics, stability, or both, with a few rare deletions and splicing mutations described. Because it is X-linked, G6PD deficiency primarily affects males. Males have only one G6PD allele and express only one G6PD type. Females can express one or two G6PD types. The Lyon hypothesis specifies that only one X chromosome is active in any given cell; thus, any given cell in a heterozygous female is either normal or deficient. In females who are heterozygous for G6PD deficiency, average G6PD activity may be normal or mildly, moderately, or severely reduced, depending on the degree of lyonization. G6PD-deficient erythrocytes in heterozygous females are susceptible to the same oxidant stress as G6PD-deficient cells in males, but, typically, the overall degree of hemolysis is less because there is a smaller population of vulnerable cells.

Clinical Manifestations

G6PD deficiency is divided into five classes based on clinical severity and degree of enzyme deficiency. Class I is characterized by CNSHA without precipitating cause and severe G6PD deficiency. Class II is characterized by intermittent hemolysis and severe G6PD deficiency. Class III is characterized by hemolysis after oxidant stress and mild G6PD deficiency. Class II and III...
Acute hemolytic anemia is the most dramatic clinical presentation of G6PD deficiency with acute intravascular hemolysis after exposure to an oxidative stress. Oxidative stresses include ingestion of certain drugs such as primaquine or sulfa-containing compounds, exposure to naphthalene (mothballs), ingestion of fava beans, or infection, the latter being the most common cause of hemolysis. Table 161-2 lists drugs that should be avoided in G6PD-deficient patients. Presenting symptoms include irritability, fever, jaundice, and anemia ensue. The spleen and liver may be enlarged and tender. Cases with severe anemia may precipitate congestive heart failure. Laboratory findings include a normochromic, normocytic anemia with anisocytosis and reticulocytosis. Poikilocytes and bite cells may be seen. Heinz bodies, a classic finding in G6PD deficiency, may be seen but are an inconsistent finding because these damaged cells are rapidly cleared from the circulation in the spleen. Additional laboratory findings may include hemoglobinuria and the presence of free Hb in the blood.

Another clinically significant syndrome of G6PD deficiency is NNJ. Jaundice is seldom present at birth, with the peak incidence of onset between days 2 and 3 of life. The severity of hyperbilirubinemia is variable. It may be severe, resulting in kernicterus or even death. In most cases, however, hyperbilirubinemia is adequately treated with phototherapy. In NNJ, it is important to note that the anemia is very rarely severe. The etiology of NNJ remains controversial. NNJ is increased in G6PD-deficient infants who also carry a polymorphism of the uridine diphosphoglucuronyl transferase (UDPGT1) gene associated with Gilbert syndrome.

Chronic nonspherocytic hemolytic anemia is associated with uncommon variants of G6PD deficiency, usually mutant enzymes unable to maintain basal NADPH production. Presentation may be in the neonatal period when NNJ is accompanied by anemia in a male. The degree of chronic anemia in CNSHA caused by G6PD deficiency has been variable. Some patients have compensated hemolysis, but others require intermittent transfusions. Transfusion dependence occurs in the most severe cases.

The G6PD reaction (glucose-6-phosphate + NADP+ → 6-phosphoglucono-δ-lactone + NADPH + H+) reduces NADP+ to NADPH. Formation of NADPH and NADH can be observed directly because they fluorescence in the visible spectrum when illuminated with long-wave ultraviolet light. Based on this observation, several simple screening tests performed using inexpensive long-wave ultraviolet light have been devised. These tests are semiquantitative, categorizing a sample as normal or deficient. They are unreliable after an acute hemolytic episode and do not typically detect female heterozygotes. Positive screening test results should be confirmed by spectrophotometric assay or DNA studies.

Definitive assay of the enzyme depends on direct spectrophotometric measurement of NADPH production. Although more sensitive than screening tests, this still requires 20% to 30% G6PD-deficient cells to obtain an abnormal result. Sensitivity can be increased by comparing the level of G6PD deficiency with levels of other age-dependent erythrocyte enzymes, especially when testing is temporally in close proximity to an acute hemolytic episode. The cyanide-ascorbate test measures the ability of erythrocytes to prevent the oxidation of Hb by ascorbate. Using intact erythrocytes, as few as 10% to 15% deficient cells can be detected, making this test useful for detecting female heterozygotes and males after a hemolytic episode. This test also detects other perturbations of the HMP shunt or glutathione metabolism.

**DIAGNOSIS**

The best treatment for an individual with AHA is careful prescription of medications and avoidance of inciting agents (see Table 161-2). Outside of acute hemolytic episodes, these patients do not require any special therapy. AHA episodes are managed with particular attention to hematologic, cardiovascular, renal, and pulmonary complications of hemolysis. Management of NNJ does not differ from that recommended for other causes of neonatal hyperbilirubinemia. In CNSHA, management is expectant. Exposure to oxidant stresses should be avoided. Blood transfusions may be necessary during acute hemolytic episodes. In severe cases of CNSHA, splenectomy may ameliorate the anemia.

**TREATMENT**

Disorders of Glutathione Metabolism

Deficits of glutathione metabolism may be associated with hemolysis. Erythrocytes from patients lacking glutathione synthetase or γ-glutamylcysteine synthetase, enzymes involved in glutathione synthesis, have very low levels of GSH. Clinically, these disorders resemble G6PD deficiency. There is mild to moderate chronic hemolytic anemia with increased susceptibility to oxidant stress.

**GENERAL REFERENCES**

For the General References and other additional features, please visit Expert Consult at https://expertconsult.inkling.com.
1. A man recently diagnosed with glucose-6-phosphate dehydrogenase (G6PD) deficiency after an episode of hemolysis after taking chloroquine malaria prophylaxis before visiting Benin. He has many questions, including about starting a family. At the end of the visit, you provide several suggestions. These include all of the following except

A. always inform health care providers that he is G6PD deficient, particularly before they prescribe any medication.
B. seek medical attention if he is feeling tired, short of breath, and experiencing palpitations and if dark-colored urine is observed.
C. he should avoid eating fava beans and fava plant–derived food.
D. if his wife does not have G6PD deficiency, all sons will be unaffected, and all daughters will be carriers.
E. if his wife is a G6PD carrier, one of two daughters will be G6PD deficient, one out of two daughters will be G6PD carriers, and his sons will not be affected.

**Answer:** E. Glucose-6-phosphate dehydrogenase (G6PD) deficiency is inherited in an X-linked manner. Thus, if his wife does not have G6PD deficiency, all sons will be unaffected, and all daughters will be carriers (answer D). However, if his wife is a G6PD carrier, one of two daughters will be G6PD deficient, one of two daughters will be G6PD carriers, one son of two will be G6PD deficient, and one son of two will be unaffected. With the diagnosis of G6PD deficiency, he should always tell his health care providers, especially when they are prescribing medications, and he should avoid fava beans and all fava-derived products. Finally, he should seek medical attention when experiencing the symptoms of a hemolytic reaction, tiredness, shortness of breath, palpitations, and dark-colored urine.

2. You have just diagnosed a 23-year-old patient with hereditary spherocytosis. Which of the following are appropriate actions?  

A. Obtain ultrasonography of the spleen and gallbladder.
B. Counsel the patient to avoid oxidant-inducing foods and medications.
C. Determine if other family members could be affected and should be evaluated for the presence of hereditary spherocytosis.
D. Counsel regarding possible occurrence of hemolytic and aplastic crises.
E. A, B, and C
F. A, C, and D
G. All of the above

**Answer:** F. Assessing spleen size and the biliary tract for the presence of cholelithiasis (answer A), determining if other family members could be affected after diagnosing a patient with hereditary spherocytosis (answer C), and counseling regarding hemolytic and aplastic crises (answer D) are all appropriate actions after diagnosing a patient with hereditary spherocytosis. Avoidance of oxidant-inducing food and medications (answer B), which is appropriate in patients with metabolic disease of the erythrocyte, especially glucose-6-phosphate dehydrogenase deficiency, is not necessary in hereditary spherocytosis.

3. You are asked to see a jaundiced 62-year-old man with cirrhotic liver disease complicated by portal hypertension and hepatosplenomegaly because of stomatocytes on peripheral smear. He is being evaluated for splenectomy. His total leukocyte count is 4000/mm³ and his platelet count is 105,000/mm³. Hemoglobin and hematocrit are 8.4 g/dL and 26%, respectively. Peripheral blood smear shows decreased numbers of leukocytes and platelets. Erythrocyte morphology reveals nearly 100% stomatocytes, erythrocytes with a central bar, and a few target cells. Your most appropriate response would be

A. the presence of stomatocytes makes splenectomy an absolute contraindication.
B. obtain erythrocyte electrolyte determinations.
C. prominent stomatocytosis is common in patients with severe liver disease and no additional specific therapy is indicated.
D. obtain cholesterol and triglyceride levels to correlate with severity of stomatocytes and target cells and consider lipid lowering therapy.
E. obtain osmotic fragility or EMA binding test.

**Answer:** C. Prominent stomatocytosis is common in patients with severe liver disease, and no additional specific therapy is indicated. Marked stomatocytosis is very common in advanced liver disease. Target cells may also be seen in advanced liver disease. Splenectomy is contraindicated in cases of hereditary stomatocytosis, not in cases of acquired stomatocytosis (answer A). Evaluation for hereditary stomatocytosis or hereditary spherocytosis via erythrocyte electrolytes (answer B), osmotic fragility or EMA binding (answer E) without additional historical data is not necessary. Although erythrocyte membrane lipids and cholesterol are perturbed in stomatocytosis, specific therapy is not warranted.

4. A 31-year old woman presents with a history of lifelong hemolytic anemia. Her parents are normal, but a sister also has anemia. She tells you she had a splenectomy as a teenager, but “it didn’t work.” Laboratory evaluation reveals hemoglobin of 10 g/dL, hematocrit of 31%, mean corpuscular volume of 96 fl, and reticulocyte count of 15%. The lactate dehydrogenase level is 750 IU/L. Peripheral blood smear shows evidence of hemolysis; many erythrocytes show prominent basophilic stippling. Her likely diagnosis is

A. pyruvate kinase deficiency.
B. glucose-6-phosphate dehydrogenase deficiency.
C. hereditary spherocytosis.
D. hereditary stomatocytosis.
E. pyrimidine 5′-nucleotidase deficiency.

**Answer:** E. All of the disorders listed may be associated with a history of lifelong anemia. Pyruvate kinase and pyrimidine 5′-nucleotidase (PSN) deficiency are both recessively inherited (normal parents, affected sister). Spleenectomy failure is typical in PSN deficiency and some cases of hereditary stomatocytosis, in which splenectomy is contraindicated. Basophilic stippling of erythrocytes is a characteristic finding in PSN deficiency because of the accumulation of partially degraded, nondiffusible RNAs in the cell.

5. Evaluation of a patient with hemolytic anemia reveals the presence of stomatocytes on peripheral blood smear. Which disorder is NOT in the differential diagnosis?

A. Heinz body hemolytic anemia
B. Autoimmune hemolytic anemia
C. Hereditary stomatocytosis
D. Hereditary spherocytosis
E. Liver disease

**Answer:** C. Although the results of osmotic fragility testing may be similar in patients with hereditary stomatocytosis and hereditary spherocytosis, stomatocytes are rarely seen on the peripheral blood smear in those with hereditary stomatocytosis. In addition to hereditary spherocytosis, stomatocytes may be seen on the peripheral blood smear of patients with Heinz body hemolytic anemia, autoimmune hemolytic anemia, and liver disease.