**Congenital Hypoplastic Anemia**  
(Diamond-Blackfan Syndrome)

This rare condition usually becomes symptomatic in early infancy, frequently with pallor in the neonatal period, but may first be noted later in childhood. About 75% of cases are diagnosed by 3mo of age. The most characteristic features are macrocytic anemia, reticulocytopenia, and a deficiency or absence of red blood cell (RBC) precursors in an otherwise normally cellular bone marrow.

**ETIOLOGY.**

Dominant or recessive patterns of inheritance in about 15% of patients. Erythroid progenitors in this disorder have an unusual sensitivity to withdrawal of EPO, with resultant increased apoptosis (programmed cell death).

Although hematopoiesis is generally adequate in fetal life, some affected infants appear pale in the first days after birth; rarely, hydrops fetalis occurs. Profound anemia usually becomes evident by 2–6mo of age. The liver and spleen are not enlarged initially. About one third of affected children have congenital anomalies, most commonly craniofacial deformities or defects of the upper extremities, including triphalangeal thumbs.

RBCs are usually macrocytic, with elevated levels of folic acid and vitamin B₁₂. Elevated fetal hemoglobin (Hb F) and increased expression of “i” antigen. Adenosine deaminase activity is increased in RBCs of patients with this disorder. These findings may help distinguish congenital RBC aplasia from acquired transient erythroblastopenia of childhood. Reticulocytes are diminished,

... RBC precursors are markedly reduced in the marrow in most patients, but other marrow elements are usually normal. Serum iron levels are elevated.

**DIFFERENTIAL DIAGNOSIS.**

Congenital hypoplastic anemia must be differentiated from other anemias with low reticulocyte counts.

The anemia of the convalescent phase of hemolytic disease of the newborn may, on occasion, be associated with markedly reduced erythropoiesis. This terminates spontaneously at 5–8wk of age.

Aplastic crises characterized by reticulocytopenia and by decreased numbers of RBC precursors, frequently caused by parvovirus B19 infections, may complicate various types of hemolytic disease, but usually after the first several months of life.

The syndrome of transient erythroblastopenia of childhood.

**PROGNOSIS.**
Median survival is more than 40yr of age. The outlook is best in those who respond to corticosteroid therapy. About half of the patients are long-term responders. In the others, survival depends on transfusions. Some spontaneous remissions (about 14%)

Hemosiderosis may result unless chelation therapy for excess iron is carried out appropriately.

The liver and spleen enlarge, and secondary hypersplenism with leukopenia and thrombocytopenia may occur in children who do not receive adequate chelation.

TREATMENT.

Corticosteroid therapy is frequently beneficial if begun early; three fourths of patients respond initially. The mechanism of its effect is unknown.

Prednisone in three or four divided doses totaling 2mg/kg/24hr is used as an initial trial. RBC precursors appear in bone marrow 1–3wk after therapy is begun, and then normoblastosis and a brisk peripheral reticulocytosis occur. The hemoglobin may reach normal levels in 4–6wk. The dose of corticosteroid may then be reduced gradually by tapering divided doses and then by eliminating all except a single, lowest effective daily dose.

In patients who do not respond to corticosteroid therapy, transfusions at intervals of 4–8wk are necessary to sustain life. Chelation therapy for iron overload with deferoxamine.

Other therapies, including androgens, cyclosporine, cyclophosphamide, antithymocyte globulin (ATG), high-dose intravenous immunoglobulin, EPO, and interleukin-3 have not had a consistent beneficial effect and may have a high incidence of side effects.

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ETIOLOGY.

Dominant or recessive patterns of inheritance are indicated by familial occurrence in about 15% of patients. In about 25% of patients, there are mutations in the gene for ribosomal protein S19 (one of 79 ribosomal proteins), mapped to chromosome 19q13. The patients were heterozygous for deficiency of the protein and were sporadic or familial cases. Most evidence indicates that the primary defects are in the erythroid precursor and are not due to immunologic damage to normal stem cells. High levels of
EPO are present in serum and urine. A search for mutations in the EPO receptor gene has been negative. In patients, no defects have been found in the genes for mast/stem cell growth factor (MGF) or its receptor, c-kit, nor does prednisone correct the anemias in mice with deficiencies of MGF or c-kit. Erythroid progenitors in this disorder have an unusual sensitivity to withdrawal of EPO, with resultant increased apoptosis (programmed cell death).

Although hematopoiesis is generally adequate in fetal life, some affected infants appear pale in the first days after birth; rarely, hydrops fetalis occurs. Profound anemia usually becomes evident by 2–6mo of age, occasionally somewhat later. The liver and spleen are not enlarged initially. About one third of affected children have congenital anomalies, most commonly craniofacial deformities or defects of the upper extremities, including tripalangeal thumbs. The abnormalities are diverse, with no specific pattern emerging in the majority of those affected.

RBCs are usually macrocytic, with elevated levels of folic acid and vitamin B_{12}. Assay of RBCs reveals a pattern characteristic of a “young” RBC population, including elevated fetal hemoglobin (Hb F) and increased expression of “i” antigen. Adenosine deaminase activity is increased in RBCs of patients with this disorder. These findings may help distinguish congenital RBC aplasia from acquired transient erythroblastopenia of childhood (Chapter 456). Thrombocytosis or thrombocytopenia and occasionally neutropenia may also be present initially. Reticulocytes are diminished, even when the anemia is severe. RBC precursors are markedly reduced in the marrow in most patients, but other marrow elements are usually normal. Serum iron levels are elevated.

DIFFERENTIAL DIAGNOSIS.

Congenital hypoplastic anemia must be differentiated from other anemias with low reticulocyte counts. The anemia of the convalescent phase of hemolytic disease of the newborn may, on occasion, be associated with markedly reduced erythropoiesis. This terminates spontaneously at 5–8wk of age. Aplastic crises characterized by reticulocytopenia and by decreased numbers of RBC precursors, frequently caused by parvovirus B19 infections, may complicate various types of hemolytic disease, but usually after the first several months of life. Infection with this virus in utero may also cause pure RBC aplasia in infancy, even with hydrops fetalis at birth. The syndrome of transient erythroblastopenia of childhood (Chapter 456) may be differentiated from Diamond-Blackfan syndrome by its relatively late onset (although it may occasionally develop in infants younger than 6mo) and by biochemical differences in RBCs.

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PROGNOSIS.

Median survival is more than 40yr of age. The outlook is best in those who respond to corticosteroid therapy. About half of the patients are long-term responders. In the others, survival depends on transfusions. Some children in each group may eventually have spontaneous remissions (about 14%). By late childhood, children who do not respond to corticosteroids may have had 100 or more transfusions, and hemosiderosis may result unless chelation therapy for excess iron is carried out appropriately. The liver and spleen enlarge, and secondary hypersplenism with leukopenia and thrombocytopenia may occur in children who do not receive adequate chelation or in those with chronic hepatitis acquired from transfusions. The complications of chronic transfusions are similar to those in β-thalassemia major, and prevention and treatment of iron overload should be equally aggressive in both groups of transfused patients (see Chapter 468.9).

TREATMENT.

Corticosteroid therapy is frequently beneficial if begun early; three fourths of patients respond initially. The mechanism of its effect is unknown.

Prednisone in three or four divided doses totaling 2mg/kg/24hr is used as an initial trial. RBC precursors appear in bone marrow 1–3wk after therapy is begun, and then normoblastosis and a brisk peripheral reticulocytosis occur. The hemoglobin may reach normal levels in 4–6wk. The dose of corticosteroid may then be reduced gradually by
tapering divided doses and then by eliminating all except a single, lowest effective daily
dose. This dose should then be doubled, used on alternate days, and tapered still further
while maintaining the hemoglobin level at 10g/dL or above. In some patients, very small
amounts of prednisone, as low as 2.5mg, may be sufficient to sustain adequate
erthropoiesis.

In patients who do not respond to corticosteroid therapy, transfusions at intervals of 4–
8wk are necessary to sustain life. Chelation therapy for iron overload with deferoxamine
administered subcutaneously via a battery-powered portable pump should be begun when
excess iron accumulation is reflected by serum ferritin levels exceeding 1,000mg/dL, but
preferably after 5yr of age, because the medication may interfere with normal growth. An
oral iron chelator, deferasirox (L1), is in clinical trials and may offer an alternative if it is
shown to be effective and to have acceptable toxicity. Other therapies, including
androgens, cyclosporine, cyclophosphamide, antithymocyte globulin (ATG), high-dose
intravenous immunoglobulin, EPO, and interleukin-3 have not had a consistent beneficial
effect and may have a high incidence of side effects. High-dose methylprednisolone (30–
100mg/kg/24hr; tapered after 3 days) has been beneficial in some patients. Splenectomy
may decrease the need for transfusion if hypersplenism or isoimmunization has
developed. Bone marrow transplantation has a role in children who do not respond to
corticosteroids and who have a histocompatible donor. The rate of engraftment is high,
providing further evidence that immunosuppression is not the primary cause of this
disorder. Transplantation of umbilical cord cells from an HLA-compatible newborn
sibling has resulted in successful hematopoietic reconstitution.

Chapter 461 Iron Deficiency Anemia

Anemia resulting from lack of sufficient iron for synthesis of hemoglobin is the most
common hematologic disease of infancy and childhood. Its frequency is related to certain
basic aspects of iron metabolism and nutrition. The body of a newborn infant contains
about 0.5g of iron, whereas the adult content is estimated at 5g. To make up for this
discrepancy, an average of 0.8mg of iron must be absorbed each day during the first 15yr
of life. In addition to this growth requirement, a small amount is necessary to balance
normal losses of iron by shedding of cells. Accordingly, to maintain positive iron balance
in childhood, about 1mg of iron must be absorbed each day.

Iron is absorbed in the proximal small intestine, mediated in part by duodenal proteins
(HFE, hephaestin, Nramp², and mobilferrin). Because absorption of dietary iron is
assumed to be about 10%, a diet containing 8–10mg of iron daily is necessary for optimal
nutrition. Iron is absorbed two to three times more efficiently from human milk than from
cow's milk, perhaps partly because of differences in calcium content. Breast-fed infants
may, therefore, require less iron from other foods. During the first years of life, because
relatively small quantities of iron-rich foods are eaten, it is often difficult to attain
sufficient iron. For this reason, the diet should include such foods as infant cereals or
formulas that have been fortified with iron; both of these are very effective in preventing
iron deficiency. Formulas with 7–12mg Fe/L for full-term infants and premature infant
formulas with 15mg/L for infants less than 1,800g at birth are effective. Infants breast-fed
exclusively should receive iron supplementation from 4mo of age. At best, an infant is in a precarious situation with respect to iron. Should the diet become inadequate or external blood loss occur, anemia ensues rapidly.

Adolescents are also susceptible to iron deficiency because of high requirements due to the growth spurt, dietary deficiencies, and menstrual blood loss. In the United States, about 9% of 1–2yr-olds are iron deficient; 3% have anemia. Of adolescent girls, 9% are iron deficient and 2% have anemia. In boys, a 50% decrease in stored iron occurs as puberty progresses.

Select an item below

- **ETIOLOGY.**
- **CLINICAL MANIFESTATIONS.**
- **LABORATORY FINDINGS.**
- **DIFFERENTIAL DIAGNOSIS (see Table 453–2).**
- **TREATMENT.**
- **TABLES**

ETIOLOGY.

Low birthweight and unusual perinatal hemorrhage are associated with decreases in neonatal hemoglobin mass and stores of iron. As the high hemoglobin concentration of the newborn falls during the first 2–3mo of life, considerable iron is reclaimed and stored (Chapter 99). These reclaimed stores are usually sufficient for blood formation in the first 6–9mo of life in term infants. In low birthweight infants or those with perinatal blood loss, stored iron may be depleted earlier, and dietary sources become of paramount importance. Anemia caused solely by inadequate dietary iron is unusual before 4–6mo but becomes common at 9–24mo of age. Thereafter, it is relatively infrequent. The usual dietary pattern observed in infants with iron deficiency anemia is consumption of large amounts of cow's milk and of foods not supplemented with iron.

Blood loss must be considered a possible cause in every case of iron deficiency anemia, particularly in older children. Chronic iron deficiency anemia from occult bleeding may be caused by a lesion of the gastrointestinal (GI) tract, such as a peptic ulcer, Meckel's diverticulum, a polyp, or hemangioma, or by inflammatory bowel disease. In some geographic areas, hookworm infestation is an important cause of iron deficiency. Pulmonary hemosiderosis may be associated with unrecognized bleeding in the lungs and recurrent iron deficiency after treatment with iron. Chronic diarrhea in early childhood may be associated with considerable unrecognized blood loss. Some infants with severe iron deficiency in the United States have chronic intestinal blood loss induced by exposure to a heat-labile protein in whole cow's milk. Loss of blood in the stools each day can be prevented either by reducing the quantity of whole cow's milk to 1pint/24hr or less, by using heated or evaporated milk, or by feeding a milk substitute. This GI reaction is not related to enzymatic abnormalities in the mucosa, such as lactase deficiency, or to
typical “milk allergy.” Involved infants characteristically develop anemia that is more severe and occurs earlier than would be expected simply from an inadequate intake of iron.

Histologic abnormalities of the mucosa of the GI tract, such as blunting of the villi, are present in advanced iron deficiency anemia and may cause leakage of blood and decreased absorption of iron, further compounding the problem.

Intense exercise conditioning, as occurs in competitive athletics in high school, may result in iron depletion in girls; this occurs less commonly in boys.

CLINICAL MANIFESTATIONS.

Pallor is the most important clue to iron deficiency. Blue scleras are also common, although also found in normal infants. In mild to moderate iron deficiency (hemoglobin levels of 6–10g/dL), compensatory mechanisms, including increased levels of 2,3-diphosphoglycerate (2,3-DPG) and a shift of the oxygen dissociation curve, may be so effective that few symptoms of anemia are noted, although affected children may be irritable. Pagophagia, the desire to ingest unusual substances such as ice or dirt, may be present. In some children, ingestion of lead-containing substances may lead to concomitant plumbism. When the hemoglobin level falls below 5g/dL, irritability and anorexia are prominent. Tachycardia and cardiac dilation occur, and systolic murmurs are often present.

The spleen is enlarged to palpation in 10–15% of patients. In long-standing cases, widening of the diploë of the skull similar to that in congenital hemolytic anemias may occur. These changes resolve slowly with adequate replacement therapy. Children with iron deficiency anemia may be obese or may be underweight, with other evidence of poor nutrition. The irritability and anorexia characteristic of advanced cases may reflect deficiency in tissue iron, because with iron therapy striking improvement in behavior frequently occurs before significant hematologic improvement.

Iron deficiency may have effects on neurologic and intellectual function. A number of reports suggest that iron deficiency anemia, and even iron deficiency without significant anemia, affects attention span, alertness, and learning of both infants and adolescents. In a controlled trial, adolescent girls with serum ferritin levels of 12 ng/L or less but without anemia improved verbal learning and memory after taking iron for 8wk.

Monoamine oxidase (MAO), an iron-dependent enzyme, has a crucial role in neurochemical reactions in the central nervous system. Iron deficiency produces decreases in the activities of enzymes such as catalase and cytochromes. Catalase and peroxidase contain iron, but their biologic essentiality is not well established. Iron deficiency causes rigidity of red blood cells (RBCs) and may be associated with stroke in young children. Administration of iron may decrease the frequency of breath-holding spells, suggesting a role for iron deficiency.
LABORATORY FINDINGS.

In progressive iron deficiency, a sequence of biochemical and hematologic events occurs. First, the tissue iron stores represented by bone marrow hemosiderin disappear. The level of serum ferritin, an iron-storage protein, provides a relatively accurate estimate of body iron stores in the absence of inflammatory disease. Normal ranges are age dependent, and decreased levels accompany iron deficiency. Next, serum iron level decreases (also age dependent), the iron-binding capacity of the serum increases, and the percent saturation falls below normal (also varies with age). When the availability of iron becomes rate limiting for hemoglobin synthesis, a moderate accumulation of heme precursors, free erythrocyte protoporphyrins (FEP), results.

As the deficiency progresses, the RBCs become smaller than normal and their hemoglobin content decreases. The morphologic characteristics of RBCs are best quantified by the determination of mean corpuscular hemoglobin (MCH) and mean corpuscular volume (MCV). Developmental changes in MCV require the use of age-related standards for diagnosis of microcytosis (see Table 453–1). With increasing deficiency, the RBCs become deformed and misshapen and present characteristic microcytosis, hypochromia, poikilocytosis, and increased RBC distribution width (RDW); see Fig. 453–1 C). The reticulocyte percentage may be normal or moderately elevated, but absolute reticulocyte counts indicate an insufficient response to anemia. Nucleated RBCs may occasionally be seen in the peripheral blood. White blood cell counts are normal. Thrombocytosis, sometimes of a striking degree (600,000–1,000,000/mm$^3$), may occur or, in a few cases, thrombocytopenia. The mechanisms of these platelet abnormalities are not clear. They appear to be a direct consequence of iron deficiency, perhaps with associated GI blood loss or associated folate deficiency, and they return to normal with iron therapy and dietary change. The bone marrow is hypercellular, with erythroid hyperplasia. The normoblasts may have scanty, fragmented cytoplasm with poor hemoglobinization. Leukocytes and megakaryocytes are normal. Hemosiderin cannot be demonstrated in marrow specimens by Prussian blue staining. In about a third of cases, occult blood can be detected in the stools.

**TABLE 453–1. Hematologic Values During Infancy and Childhood**

**FIGURE 453–1** Morphologic abnormalities of the red blood cell. A, Normal. B, Macrocytes (folic acid or vitamin B$_{12}$ deficiency). C, Hypochromic microcytes (iron deficiency). D, Target cells (Hb CC disease). E, Schizocytes (hemolytic-uremic syndrome). (Provided by Dr. E. Schwartz.)
DIFFERENTIAL DIAGNOSIS (see Table 453–2).

TABLE 453–2. Classification of Anemia

Iron deficiency must be differentiated from other hypochromic microcytic anemias. In lead poisoning associated with iron deficiency, the RBCs are morphologically similar, but coarse basophilic stippling of the RBCs, an artifact of drying the slide, is frequently prominent. Elevations of blood lead, FEP, and urinary coproporphyrin levels are seen (Chapter 721). The blood changes of β-thalassemia trait resemble those of iron deficiency (Chapter 468.9), but RDW is usually normal or only slightly elevated. α-Thalassemia trait occurs in about 3% of blacks in the United States and in many Southeast Asian peoples. The diagnosis requires direct identification of DNA defects or difficult globin synthesis studies after the newborn period. The diagnosis can be assumed when a patient having familial hypochromic microcytic anemia with normal iron studies, including ferritin, has normal levels of Hb A2 and Hb F and normal hemoglobin electrophoresis. In the newborn period, infants with α-thalassemia trait have 3–10% Bart hemoglobin and the MCV is decreased (Chapter 468.9). Thalassemia major, with its pronounced erythroblastosis and hemolytic component, should present no diagnostic confusion. Hb H disease, a form of α-thalassemia with hypochromia and microcytosis, also has a hemolytic component due to instability of the β-chain tetramers resulting from a deficiency of α globin. The RBC morphology of chronic inflammation and infection, though usually normocytic, may be microcytic, but in these conditions both the serum iron level and iron-binding ability are reduced and serum ferritin levels are normal or elevated. The serum transferrin receptor (TfR) level is useful in the distinction between iron deficiency anemia and anemia of chronic disease, because it is not affected by inflammation. The concentration is elevated in iron deficiency and within the normal range in anemia of chronic disease. An elevation of the TfR/log ferritin ratio is especially sensitive in detecting iron deficiency anemia. Elevations of FEP are not specific to iron deficiency and are observed in patients with lead poisoning, chronic hemolytic anemia, anemia associated with chronic disorders, and some of the porphyrias.

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**Part XX:** Diseases of the Blood  
**Section 2:** Anemias of Inadequate Production  
**Chapter 461:** Iron Deficiency Anemia

**TREATMENT.**

The regular response of iron deficiency anemia to adequate amounts of iron is an important diagnostic and therapeutic feature. Oral administration of simple ferrous salts (sulfate, gluconate, fumarate) provides inexpensive and satisfactory therapy. No evidence shows that addition of any trace metal, vitamin, or other hematin substance significantly increases the response to simple ferrous salts. For routine clinical use, physicians should be familiar with an inexpensive preparation of one of the simple ferrous compounds. The therapeutic dose should be calculated in terms of elemental iron; ferrous sulfate is 20% elemental iron by weight. A daily total of 6mg/kg of elemental iron in three divided doses provides an optimal amount of iron for the stimulated bone marrow to use. Intolerance to oral iron is uncommon in children. A parenteral iron preparation (iron dextran) is an effective form of iron and is usually safe when given in a properly calculated dose, but the response to parenteral iron is no more rapid or complete than that obtained with proper oral administration of iron, unless malabsorption is a factor.

While adequate iron medication is given, the family must be educated about the patient's diet, and the consumption of milk should be limited to a reasonable quantity, preferably 500mL (1 pint)/24hr or less. This reduction has a dual effect: The amount of iron-rich foods is increased, and blood loss from intolerance to cow's milk proteins is reduced. When the re-education of child and parent is not successful, parenteral iron medication
may be indicated. Iron deficiency can be prevented in high-risk populations by providing iron-fortified formula or cereals during infancy.

The expected clinical and hematologic responses to iron therapy are described in Table 461–1.

**TABLE 461–1. Responses to Iron Therapy in Iron Deficiency Anemia**

Within 72–96 hr after administration of iron to an anemic child, peripheral reticulocytosis is noted. The height of this response is inversely proportional to the severity of the anemia. Reticulocytosis is followed by a rise in the hemoglobin level, which may increase as much as 0.5 g/dL/24 hr. Iron medication should be continued for 8 wk after blood values are normal. Failures of iron therapy occur when a child does not receive the prescribed medication, when iron is given in a form that is poorly absorbed, or when there is continuing unrecognized blood loss, such as intestinal or pulmonary loss, or with menstrual periods. An incorrect original diagnosis of nutritional iron deficiency may be revealed by therapeutic failure of iron medication.

Because a rapid hematologic response can be confidently predicted in typical iron deficiency, blood transfusion is indicated only when the anemia is very severe or when superimposed infection may interfere with the response. It is not necessary to attempt rapid correction of severe anemia by transfusion; the procedure may be dangerous because of associated hypervolemia and cardiac dilatation. Packed or sedimented RBCs should be administered slowly in an amount sufficient to raise the hemoglobin to a safe level at which the response to iron therapy can be awaited. In general, severely anemic children with hemoglobin values less than 4 g/dL should be given only 2–3 mL/kg of packed cells at any one time (furosemide may also be administered as a diuretic). If there is evidence of frank congestive heart failure, a modified exchange transfusion using fresh-packed RBCs should be considered, although diuretics followed by slow infusion of packed RBCs may suffice.

**Anemia of Chronic Disorders and Renal Disease**

Anemia complicates a number of chronic systemic diseases associated with infection, inflammation, or tissue breakdown. Examples of such conditions include chronic pyogenic infections, such as bronchiectasis and osteomyelitis; chronic inflammatory processes, such as rheumatoid arthritis, systemic lupus erythematosus, and ulcerative colitis; malignancies; and advanced renal disease. In the latter, an additional major component is decreased production of erythropoietin (EPO) due to damage of the cells producing this cytokine. Despite diverse underlying causes, the erythroid abnormalities are similar. Red blood cell (RBC) life span is moderately decreased, reflecting increased RBC destruction by a hyperactive reticuloendothelial system. The increased hemolysis is less important, however, than a relative failure of bone marrow response, reflecting both hypoactivity of marrow and an EPO production inadequate for the degree of anemia. Another finding is abnormalities of iron metabolism, including defective iron release from tissues into the plasma. Suppression of the erythroid response in the marrow appears
to result primarily from an increase in tumor necrosis factor (TNF), which acts on bone marrow stromal cells to produce interferon (IFN)-β as a primary mediator, and an increase in interleukin-1 (IL-1), which acts on T cells to produce IFN-γ as a primary mediator. IL-6 levels may also be elevated. Recombinant human EPO can overcome this effect if the EPO level in a patient is less than 500mU/mL. TNF and IL-1 decrease EPO production in perfused kidneys and hepatoma cells, corresponding to the two sites of EPO production, accounting for the inadequate EPO response in this type of anemia. The specific stimulant of increased TNF and IL-1 production in these patients has not been identified.

Select an item below

- CLINICAL MANIFESTATIONS.
- LABORATORY FINDINGS.
- TREATMENT AND PROGNOSIS.

LABORATORY FINDINGS.

Hemoglobin concentrations usually range from 6 to 9g/dL. The anemia is usually normochromic and normocytic; about one third of patients may have modest hypochromia and microcytosis. Absolute reticulocyte counts are normal or low, and leukocytosis is common. Free erythrocyte protoporphyrin (FEP) levels are frequently elevated and provide a sensitive reflection of derangements of iron metabolism. They return to normal after successful treatment of the primary disease. The serum iron level is low, without the increase in total iron-binding capacity that occurs in iron deficiency. This pattern of low serum iron and low to normal iron-binding protein is a regular and valuable diagnostic feature. Serum ferritin level may be elevated. Serum transferrin receptor (TfR) level is normal, unless iron deficiency is present. The bone marrow has normal cellularity; the RBC precursors are low to adequate, marrow hemosiderin may be increased, and granulocytic hyperplasia may be present. A frequent clinical challenge is to identify concomitant iron deficiency in patients with an inflammatory disease. Measurement of TfR/ferritin ratio may be useful, because it is elevated when iron deficiency is present. A trial of iron therapy may resolve the issue, although there may be no response when inflammation due to the primary disease persists. Intravenous iron saccharate is effective in iron deficiency associated with juvenile rheumatoid arthritis.

TREATMENT AND PROGNOSIS.

Because these anemias are secondary to other disease processes, they do not respond to iron or hematinsics unless there is concomitant deficiency. Transfusions raise the hemoglobin concentration only temporarily and are rarely indicated. If the underlying systemic disease can be controlled, the anemia is corrected spontaneously. Recombinant human EPO can increase the hemoglobin level and improve activity and the sense of well-being in patients with cancer and end-stage renal failure and in those with anemia of
chronic inflammation. Treatment with iron is usually necessary for an optimal EPO effect.

Chapter 460 Megaloblastic Anemias

The megaloblastic anemias have in common certain abnormalities of red blood cell (RBC) morphology and maturation. The RBCs at every stage of development are larger than normal and have an open, finely dispersed nuclear chromatin and an asynchrony between maturation of nucleus and cytoplasm, with the delay in nuclear progression being more evident with further cell divisions. Megaloblastic morphology may be seen in a number of conditions; almost all cases in children result from a deficiency of folic acid, vitamin B$_{12}$, or both. Both substances are cofactors required in the synthesis of nucleoproteins, and deficiencies result in defective synthesis of DNA and, to a lesser extent, RNA and protein. Ineffective erythropoiesis results from arrest in development or premature death of cells in the marrow. In the peripheral blood, RBCs are large (increased mean corpuscular volume [MCV]) and frequently oval, hypersegmented neutrophils appear, and giant platelets may also be found. In the marrow, the late nucleated megaloblastic RBC may appear well hemoglobinized but still retains an immature nucleus rather than the usual clumped chromatin. Giant metamyelocytes and bands are also present in the marrow. Megaloblastic anemias due to malnutrition are relatively uncommon in the United States.

Select an item below

- 460.1 Folic Acid Deficiencies
- 460.2 Vitamin B12 (Cobalamin) Deficiency
- 460.3 Rare Megaloblastic Anemias

Chapter 460 Megaloblastic Anemias

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- 460.2 Vitamin B12 (Cobalamin) Deficiency
- 460.3 Rare Megaloblastic Anemias

MEGALOBLASTIC ANEMIA OF INFANCY

This disease is caused by a deficient intake or absorption of folic acid (see also Chapters 40 and 44.8). Folates are abundant in many foods, including green vegetables, fruits, and animal organs (liver, kidney). Folic acid is absorbed throughout the small intestine, after pteroylglutamate reacts with membrane-associated folate-binding proteins. Pteroylpolyglutamates, found in cabbage, lettuce, and other foods, are absorbed less efficiently than pteroylmonoglutamate (folic acid). Pteroly polyglutamate hydrolase activity in the brush border aids the conversion to the monoglutamate. Surgical removal or disorders of the small intestine may lead to folate deficiency. There is an active enterohepatic circulation. Much of the folate in the plasma is loosely bound to albumin. Pteroylglutamate is not biologically active. It is reduced by dihydrofolate reductase to tetrahydropteroylglutamate (tetrahydrofolate), which is transported into tissue cells and polyglutamated. Dietary deficiency is usually compounded by rapid growth or infection, which may increase folic acid requirements. The normal adult daily requirement is about 100μg/24hr, which rises to 350μg/24hr in pregnancy. The requirements on a weight basis are higher in the pediatric age range in comparison with adults owing to the increased needs of growth. The needs are also increased with accelerated tissue turnover, as in hemolytic anemia. Human and cow's milk provide adequate amounts of folic acid. Goat's milk is clearly deficient; folic acid supplementation must be given when it is the main food. Unless supplemented, powered milk may also be a poor source of folic acid.

CLINICAL MANIFESTATIONS.

Mild megaloblastic anemia has been reported in very low birthweight infants, and routine folic acid supplementation is advised. Megaloblastic anemia has its peak incidence at 4–7mo of age, somewhat earlier than iron deficiency anemia, although the two may be present concomitantly in infants with poor nutrition. Besides having the usual clinical features of anemia, affected infants with folate deficiency are irritable, fail to gain weight adequately, and have chronic diarrhea. Hemorrhages due to thrombocytopenia occur in advanced cases. Folic acid deficiency may accompany kwashiorkor, marasmus, or sprue.

LABORATORY FINDINGS.
The anemia is macrocytic (MCV >100 fl). Variations in RBC shape and size are common (see Fig. 453-1B). The reticulocyte count is low, and nucleated RBCs demonstrating megaloblastic morphology are often seen in the blood. Neutropenia and thrombocytopenia may be present, particularly in long-standing deficiencies. The neutrophils are large, some with hypersegmented nuclei; more than 5% of neutrophils have five or more nuclear segments. Normal serum folic acid levels are 5–20ng/mL; deficiency is accompanied by levels less than 3ng/mL. Levels of RBC folate are a better indicator of chronic deficiency. The normal RBC folate level is 150–600ng/mL of packed cells. Levels of iron and vitamin B₁₂ in serum are usually normal or elevated. Serum activity of lactic acid dehydrogenase (LDH) is markedly elevated. The bone marrow is hypercellular because of erythroid hyperplasia. Megaloblastic changes are prominent, although some normal RBC precursors may also be found. Large, abnormal neutrophilic forms (giant metamyelocytes) with cytoplasmic vacuolation are seen, as well as hypersegmentation of the nuclei of megakaryocytes.

TREATMENT.

When the diagnosis is established or in severely ill children, folic acid may be administered orally or parenterally in a dose of 1–5mg/24hr. If the specific diagnosis is in doubt, 50–100μg/24hr of folate may be used for a week as a diagnostic test, or 1μg/24hr of cyanocobalamin parenterally for suspected vitamin B₁₂ deficiency. Because a hematologic response can be expected within 72hr, transfusions are indicated only when the anemia is severe or the child is very ill. Folic acid therapy should be continued for 3–4wk. If juvenile pernicious anemia is present or if the anemia recurs after therapy, the prolonged use of folic acid should be avoided, because in pernicious anemia folic acid may produce a partial response to the anemia without decreasing the neurologic abnormalities.

FOLIC ACID DEFICIENCY IN MALABSORPTION SYNDROMES

Diffuse inflammatory or degenerative disease of the intestine may reduce intestinal pteroylpolyglutamate hydrolase activity as well as markedly impair absorption of folate. Celiac disease, chronic infectious enteritis, and enteroenteric fistulas may lead to folic acid deficiency and megaloblastic anemia. Measurement of serum folate is used to assess small intestinal absorptive functions in malabsorptive disorders. Oral folic acid supplements of 1mg/24hr may be indicated in these states (see Chapter 340).

FOLIC ACID DEFICIENCY ASSOCIATED WITH ANTICONVULSANTS AND OTHER DRUGS

Many patients have low serum levels of folic acid during therapy with certain anticonvulsant drugs (e.g., phenytoin, primidone, phenobarbital), but they usually do not develop anemia. Frank megaloblastic anemia is rare and responds to folic acid therapy, even if administration of the offending drug is continued. Absorption of folic acid is impaired by anticonvulsant drugs, but increased use of folate also occurs. Megaloblastic anemia has occurred in users of oral contraceptives, but the cause is not clear.
A number of drugs have antifolic acid activity as their primary pharmacologic effect and regularly produce megaloblastic anemia. Methotrexate binds to dihydrofolate reductase and prevents formation of tetrahydrofolate, the active form. Pyrimethamine, used in the therapy of toxoplasmosis, and trimethoprim, used for treatment of various infections, may induce folic acid deficiency and, occasionally, megaloblastic anemia. Therapy with folinic acid (5-formyltetrahydrofolate) is usually beneficial.

460.2 Vitamin B<sub>12</sub> (Cobalamin) Deficiency

Vitamin B<sub>12</sub> is derived from cobalamin in food, mainly animal sources, secondary to production by microorganisms. Humans cannot synthesize vitamin B<sub>12</sub>. The cobalamins are released in the acidity of the stomach and combine there with R proteins and intrinsic factor (IF), traverse the duodenum, where pancreatic proteases break down the R proteins, and are absorbed in the distal ileum via specific receptors for IF-cobalamin. In addition, some vitamin B<sub>12</sub> from large doses may diffuse through mucosa in the intestine and mouth. In plasma, vitamin B<sub>12</sub> is bound to transcobalamin (TC) II, the physiologically important transporter, as well as to TCI and TCIII. TCII-cobalamin enters cells by receptor-mediated endocytosis, and cobalamin is converted to active forms (methylcobalamin and adenosylcobalamin) important in the transfer of methyl groups and DNA synthesis.

Vitamin B<sub>12</sub> deficiency may therefore result from inadequate intake, surgery involving the stomach or terminal ileum, lack of secretion of IF by the stomach, consumption or inhibition of the B<sub>12</sub>-IF complex, abnormalities involving the receptor sites in the terminal ileum, or abnormalities of TCII. Although TCI binds 80% of serum cobalamin, a deficiency of this protein results in low serum B<sub>12</sub> levels but not in megaloblastic anemia (see Chapter 44.2).

Because vitamin B<sub>12</sub> is present in many foods, dietary deficiency is rare. It may occur in cases of extreme dietary restriction (strict vegetarians: “vegans”) in which no animal products are consumed. Vitamin B<sub>12</sub> deficiency is not common in kwashiorkor or infantile marasms. Cases occur in breast-fed infants whose mothers have deficient diets or pernicious anemia.

Select an item below

- **JUVENILE PERNICIOUS ANEMIA**
- **TRANSCOBALAMIN DEFICIENCY**
- **VITAMIN B12 MALABSORPTION DUE TO INTESTINAL CAUSES**
- **VITAMIN B12 DEFICIENCY IN OLDER CHILDREN**

460.2 Vitamin B<sub>12</sub> (Cobalamin) Deficiency

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Select an item below

- JUVENILE PERNICIOUS ANEMIA
- TRANSCOBALAMIN DEFICIENCY
- VITAMIN B₁₂ MALABSORPTION DUE TO INTESTINAL CAUSES
- VITAMIN B₁₂ DEFICIENCY IN OLDER CHILDREN

VITAMIN B₁₂ MALABSORPTION DUE TO INTESTINAL CAUSES

Cases have been reported of familial occurrence of absence or defect of the receptor for IF-B₁₂ in the terminal ileum, in some instances associated with proteinuria (Imerslund-Grasbeck syndrome). Decreased receptor activity may be detected in the urine of affected patients by a radioisotope binding assay. Histology of the stomach is normal, and IF and acid are present in gastric secretions. This autosomal recessive disorder is due to defects in the CUBN gene on chromosome 10p12.1, resulting in decreased expression of the IF-B₁₂ receptor, cubilin. Parenteral treatment with vitamin B₁₂ monthly corrects the deficiency.

Surgical resection of the terminal ileum, inflammatory diseases such as regional enteritis, neonatal necrotizing enterocolitis, and tuberculosis may also impair absorption of vitamin B₁₂. When the terminal ileum has been removed, lifelong parenteral administration should be used if the Schilling test indicates that vitamin B₁₂ is not absorbed.
An overgrowth of intestinal bacteria within diverticula or duplications of the small intestine may cause vitamin B\textsubscript{12} deficiency by consumption of or competition for the vitamin or by splitting of its complex with IF. In these cases, hematologic response may follow appropriate antibiotic therapy. Similar mechanisms may operate when the fish tapeworm *Diphyllobothrium latum* infests the upper small intestine. When megaloblastic anemia occurs in these situations, the serum vitamin B\textsubscript{12} level is low, the gastric juice contains intrinsic factor, and the abnormal Schilling test result is not corrected by addition of exogenous IF.

**VITAMIN B\textsubscript{12} DEFICIENCY IN OLDER CHILDREN**

In some cases of vitamin B\textsubscript{12} malabsorption in adolescence, atrophy of the gastric mucosa and achlorhydria have been noted. These cases may be related to the syndrome of malabsorption of vitamin B\textsubscript{12} occurring in combination with cutaneous candidiasis, hypoparathyroidism, and other endocrine deficiencies. The serum contains antibodies against IF and parietal cells. An abnormal Schilling result is corrected by addition of exogenous IF. Parenteral vitamin B\textsubscript{12} should be administered regularly to these patients.

Chapter 463 Definitions and Classification of Hemolytic Anemias

George B. Segel

Hemolysis is defined as the premature destruction of red blood cells (RBCs). If the rate of destruction exceeds the capacity of the marrow to produce RBCs, anemia results. Normal RBC survival time is 110–120 days, and approximately 1% of RBCs (the senescent ones) are removed each day and replaced by the marrow to maintain the RBC count. During hemolysis, RBC survival is shortened, and increased marrow activity results in a heightened reticulocyte percentage and number. Hemolysis should be suspected as a cause of anemia if an elevated reticulocyte count is present in the absence of bleeding or administration of hematinic therapy. The marrow can increase its output two- to threefold acutely, with a maximum of six- to eightfold if hemolysis is long standing. The reticulocyte percentage can be corrected to measure the magnitude of the marrow production in response to hemolysis as follows:

\[
\text{Reticulocyte index} = \text{reticulocyte } \% \times \frac{\text{observed hematocrit}}{\text{normal hematocrit}} \times \frac{1}{\mu}
\]

where \(\mu\) is a maturation factor related to the severity of the anemia (Fig. 463–1). In the absence of hemolysis, the reticulocyte index is 1.0, representing normal marrow activity.
As anemia becomes more severe, there is more erythropoietin stimulation of erythropoiesis, and reticulocytes are released from the marrow earlier, spending more than 1 day as reticulocytes in the blood. In terms of measuring the marrow response, it is inappropriate to count reticulocytes produced yesterday in today’s calculation of the reticulocyte index. The maturation factor, μ, provides this correction (Fig. 463–1). The usual marrow response in a chronic hemolytic anemia is reflected by a reticulocyte index of 3–4, with a maximum of 6–8 corresponding to maximal marrow output.

The erythroid hyperplasia resulting from chronic hemolytic anemia in children, especially thalassemia, may be so extensive that the medullary spaces expand at the expense of the cortical bone. These changes may be evident on physical examination or on x-rays of the skull and long bones (see Fig. 468–3). A propensity to fracture long bones can occur also.

Direct assessment of the severity of hemolysis requires measurement of the RBC survival time using RBCs tagged with the radioisotope Na$_2^{51}$CrO$_4$. The normal value for the $^{51}$Cr half-life is 25–35 days. This value is less than the expected half-life of 50–60 days because of the elution of $^{51}$Cr from the labeled RBCs at the rate of about 1% per day.
Several other plasma, urinary, or fecal chemical alterations reflect the presence of hemolysis. The degradation of hemoglobin results in the biliary excretion of heme pigments and increased fecal urobilinogen (Fig. 463–2). Elevations of serum unconjugated bilirubin also may accompany hemolysis.

FIGURE 463–2 Red cell destruction and the catabolism of hemoglobin (Hb) based on the description by Hillman and Finch. (From Hillman RS, Finch CA: Red Cell Manual. Philadelphia, FA Davis, 1983.)

Gallstones composed of calcium bilirubinate may be formed in children as young as 4 yr of age. Three heme-binding proteins in the plasma are altered during hemolysis (Fig. 463–2). Hemoglobin binds to haptoglobin and hemopexin, both of which are reduced. Oxidized heme binds to albumin to form methemalbumin, which is increased. When the capacity of these binding molecules is exceeded, free hemoglobin appears in the plasma and can be seen easily if the RBCs are sedimented in a capillary hematocrit tube. If present, free hemoglobin in the plasma is prima facie evidence of intravascular hemolysis. When the tubular reabsorptive capacity of the kidneys for hemoglobin is exceeded, free hemoglobin appears in the urine. Even in the absence of hemoglobinuria, iron loss may result from reabsorbed hemoglobin and the shedding of renal epithelial cells containing hemosiderin. This may lead to secondary iron deficiency during chronic intravascular hemolysis. When hemoglobin is degraded, an α-methene bridge is broken in the cyclic tetrapyrrole of the heme moiety, with release of carbon monoxide (CO) (Fig. 463–2). The amount of CO in the blood or expired air provides a dynamic measure of the hemolytic rate. End-tidal CO is being evaluated in several research laboratories but is not used in clinical laboratories to measure hemolysis.

The hematocrit during hemolysis is dependent on the severity of the hemolysis and on the marrow response in producing RBCs. The shortened RBC life span and heightened RBC production result in a marked susceptibility to aplastic or hypoplastic crises, characterized by erythroid marrow failure and reticulocytopenia, accompanied by a rapid reduction in hemoglobin and hematocrit. The most common cause of aplastic crises is parvovirus B19, which is erythrocytotropic in marrow culture in vitro (see Chapters 244 and 468). Aplastic crises may produce a precipitous and life-threatening decline in the hematocrit, which usually lasts 10–14 days. Such transient erythroid marrow failure has little effect on persons with a normal RBC life span but has a proportionately greater effect as the RBC life span is shortened by hemolysis. A second infection with parvovirus is uncommon, but other infections may compromise the erythroid marrow output, resulting in various degrees of hypoplasia or hypoplastic crises.

The hemolytic anemias may be classified as either (1) cellular, resulting from intrinsic abnormalities of the membrane, enzymes, or hemoglobin; or (2) extracellular, resulting
from antibodies, mechanical factors, or plasma factors. Most of the cellular defects are inherited (paroxysmal nocturnal hemoglobinuria is acquired), and most of the extracellular defects are acquired (abetalipoproteinemia with acanthocytosis is inherited). Table 463–1 shows the most common hemolytic anemias, their underlying defects, the diagnostic laboratory tests, and the current recommendations for treatment.

TABLE 463–1. Hemolytic Anemias and Their Treatment

Select an item below

- FIGURES
- TABLES

Chapter 464 Hereditary Spherocytosis

George B. Segel

Hereditary spherocytosis is a common cause of hemolysis and hemolytic anemia, with a prevalence of approximately 1/5,000 in people of Northern European extraction. It is the most common familial and congenital abnormality of the red blood cell (RBC) membrane. Affected individuals may be asymptomatic without anemia and with minimal hemolysis or may have a severe hemolytic anemia. Hereditary spherocytosis has been described in most ethnic groups but is most common among persons of Northern European origin.

Select an item below

- ETIOLOGY
- CLINICAL MANIFESTATIONS
- LABORATORY FINDINGS
- DIFFERENTIAL DIAGNOSIS
- TREATMENT
- FIGURES

ETIOLOGY.

Hereditary spherocytosis usually is transmitted as an autosomal dominant and, less frequently, as an autosomal recessive disorder. As many as 25% of patients have no previous family history. Of these patients, most represent new mutations, and a few result from recessive inheritance. The most common molecular defects are abnormalities of spectrin or ankyrin, which are major components of the cytoskeleton responsible for RBC shape. A recessive defect has been described in \( \alpha \)-spectrin; dominant defects, in \( \beta \)-spectrin and in protein 3; and dominant and recessive defects, in ankyrin. A deficiency in
spectrin, protein 3, or ankyrin results in uncoupling in the “vertical” interactions of the lipid bilayer skeleton and the loss of membrane microvesicles (Fig. 464–1). The loss of membrane without a proportional loss of volume causes spheroiding of the RBCs and an associated increase in cation permeability, cation transport, adenosine triphosphate utilization, and glycolytic metabolism. The decreased deformability of the spherocytic RBCs impairs cell passage from the splenic cords to the splenic sinuses, and the spherocytic RBCs are destroyed prematurely in the spleen. Splenectomy markedly improves the RBC life span and cures the anemia.

**FIGURE 464–1** Vertical and horizontal interactions of membrane proteins and the pathobiology of the red cell lesion in hereditary spherocytosis (HS) and hereditary elliptocytosis/hereditary pyropoikilocytosis (HE/HPP). *Left:* A defect of vertical or transverse interactions as exemplified by the red cell membrane lesion in HS. Partial deficiencies of spectrin, ankyrin (band 2.1), or band 3 protein lead to uncoupling of the membrane lipid bilayer from the underlying skeleton (*arrow*) followed by a formation of spectrin-free microvesicles of approximately 0.2–0.5 µm in diameter (*arrowheads*). These vesicles can be visualized by transmission electron microscopy, but they are not seen during examination of blood films. The subsequent loss of cell surface and a decrease in the surface/volume ratio leads to spherocytosis. *Right:* Defect of horizontal or parallel interactions of skeletal proteins as exemplified by the membrane lesion in hemolytic forms of HE associated with a defect of spectrin heterodimer self-association. The molecular lesion involving a weakened self-association of spectrin heterodimers to tetramers represents a horizontal defect of the stress-supporting protein interactions. It leads to a disruption of the membrane skeletal lattice and, consequently, whole cell destabilization followed by red cell fragmentation and poikilocytosis. Such fragments are readily seen on stained blood films. (Modified from Palek J, Jarolim P: Clinical expression and laboratory detection of red blood cell membrane protein mutations. Semin Hematol 30:249, 1993.)

**CLINICAL MANIFESTATIONS.**

Hereditary spherocytosis may be a cause of hemolytic disease in the newborn and may present with anemia and hyperbilirubinemia sufficiently severe to require phototherapy or exchange transfusions. The severity in infants and children is variable. Some children remain asymptomatic into adulthood, but others may have severe anemia with pallor,
jaundice, fatigue, and exercise intolerance. Severe cases may be marked by expansion of
the diploë of the skull and the medullary region of other bones, but to a lesser extent than
in thalassemia major. After infancy, the spleen is usually enlarged, and pigmentary
(bilirubin) gallstones may form as early as age 4–5yr. At least 50% of unsplenectomized
patients ultimately form gallstones, although for the most part, they remain
asymptomatic. Because of the high RBC turnover and heightened erythroid marrow
activity, children with hereditary spherocytosis are susceptible to aplastic crisis, primarily
as a result of parvovirus, and to hypoplastic crises associated with various other
infections. Such erythroid marrow failure may result rapidly in profound anemia
(hematocrit <10%), high-output heart failure, hypoxia, cardiovascular collapse, and
death. LABORATORY FINDINGS.

Evidence for hemolysis includes reticulocytosis and hyperbilirubinemia. The hemoglobin
level usually is 6–10g/dL, but it can be in the normal range. The reticulocyte count often
is heightened to 6–20%, with a mean of approximately 10%. The mean corpuscular
volume is normal, whereas the mean corpuscular hemoglobin concentration often is
increased (36–38g/dL RBCs). The RBCs on the blood film vary in size and include
polychromatophilic reticulocytes and spherocytes (Fig. 464–2 A). The spherocytes are
smaller in diameter and on the blood film are hyperchromic as a result of the high
hemoglobin concentration. The central pallor is less conspicuous than in normal cells.
Spherocytes may be the predominant cell or may be relatively sparse, depending on
severity of the disease, but they usually account for more than 15–20% of the cells when
hemolytic anemia is present. Erythroid hyperplasia is evident in the marrow aspirate or
biopsy. The marrow expansion may be evident on routine roentgenographic examination.
Evidence of hemolysis may include elevated indirect bilirubin, decreased haptoglobin,
and the presence of gallstones by ultrasonography.

![Fig. 464–2 Morphology of abnormal red cells. A, Hereditary spherocytosis; B, hereditary elliptocytosis; C, hereditary pyropoikilocytosis; D, hereditary stomatocytosis; E, acanthocytosis; F, fragmentation hemolysis.](image)

The diagnosis of hereditary spherocytosis usually is established clinically from the blood
film, showing many spherocytes and reticulocytes, from the family history, and from
splenomegaly. The presence of spherocytes in the blood can be confirmed with an osmotic fragility test. The RBCs are incubated in progressive dilutions of an iso-osmotic buffered salt solution. Exposure to hypotonic saline causes RBCs to swell, and the spherocytes lyse more readily than biconcave cells in hypotonic solutions. This feature is accentuated by depriving the cells of glucose for 24hr at 37°C, a so-called incubated osmotic fragility test.

As a research tool, the specific protein abnormality can be established in 80% of these patients by RBC membrane protein analysis using gel electrophoresis and densitometric quantitation. The protein abnormalities are more evident in patients who have had a splenectomy. Studies to define the underlying defects in the cytoskeleton may require assessment of protein synthesis, stability, assembly, and binding to the other membrane proteins.

TREATMENT.

Because the spherocytes in hereditary spherocytosis are destroyed almost exclusively in the spleen, splenectomy eliminates most of the hemolysis associated with this disorder. After splenectomy, osmotic fragility often improves with loss of the abnormal “tail” because of diminished splenic conditioning and less RBC membrane loss, and the anemia, reticulocytosis, and hyperbilirubinemia resolve. Whether all patients with hereditary spherocytosis should undergo splenectomy is controversial. Some hematologists do not recommend splenectomy for those patients whose hemoglobin values exceed 10g/dL and whose reticulocyte counts are less than 10%. Folic acid, 1mg/24hr, should be administered to prevent secondary folic acid deficiency. For patients with more severe anemia and reticulocytosis or those with hypoplastic or aplastic crises, poor growth, or cardiomegaly, splenectomy is recommended after age 5–6yr to avoid the heightened risk of postsplenectomy sepsis in younger children. The introduction of laparoscopic splenectomy decreases the length of hospital stay and may replace open splenectomy. Vaccines for encapsulated organisms such as pneumococcus, meningococcus, and Haemophilus influenzae type b should be administered before splenectomy, and oral prophylactic penicillin V (age <5yr: 125mg/12hr; age ≥5yr through adulthood: 250mg/12hr) administered thereafter. Postsplenectomy thrombocytosis is commonly observed but needs no treatment and usually resolves spontaneously. In one report, partial splenectomy provided substantial increases in hemoglobin and reductions in the reticulocyte count, with potential maintenance of splenic phagocytic and immune function. This technique, if substantiated, would be particularly useful for those children younger than 5yr with severe disease and could be used in older patients with mild disease.

FIGURES
Vertical and horizontal interactions of membrane proteins and the pathobiology of the red cell lesion in hereditary spherocytosis (HS) and hereditary elliptocytosis/hereditary pyropoikilocytosis (HE/HPP). 

**Left:** A defect of vertical or transverse interactions as exemplified by the red cell membrane lesion in HS. Partial deficiencies of spectrin, ankyrin (band 2.1), or band 3 protein lead to uncoupling of the membrane lipid bilayer from the underlying skeleton (arrow) followed by a formation of spectrin-free microvesicles of approximately 0.2–0.5 μm in diameter (arrowheads). These vesicles can be visualized by transmission electron microscopy, but they are not seen during examination of blood films. The subsequent loss of cell surface and a decrease in the surface/volume ratio leads to spherocytosis. 

**Right:** Defect of horizontal or parallel interactions of skeletal proteins as exemplified by the membrane lesion in hemolytic forms of HE associated with a defect of spectrin heterodimer self-association. The molecular lesion involving a weakened self-association of spectrin heterodimers to tetramers represents a horizontal defect of the stress-supporting protein interactions. It leads to a disruption of the membrane skeletal lattice and, consequently, whole cell destabilization followed by red cell fragmentation and poikilocytosis. Such fragments are readily seen on stained blood films. (Modified from Palek J, Jarolim P: Clinical expression and laboratory detection of red blood cell membrane protein mutations. Semin Hematol 30:249, 1993.)

**FIGURE 464–2** Morphology of abnormal red cells. 

- A, Hereditary spherocytosis; 
- B, hereditary elliptocytosis; 
- C, hereditary pyropoikilocytosis; 
- D, hereditary stomatocytosis; 
- E, acanthocytosis; 
- F, fragmentation hemolysis.
Chapter 468 Hemoglobin Disorders

George R. Honig

The clinical disorders that result from abnormalities of the globin genes comprise a diverse group of hematologic diseases. Normal hemoglobins are tetrameric molecules containing pairs of α or α-like and β or β-like globin-heme subunits. The normal postnatal hemoglobins include hemoglobin (Hb) A (α₂ β₂), Hb F (α₂ γ₂), and Hb A₂ (α₂ δ₂). The embryonic hemoglobins, which usually disappear before birth, include Hb Gower-1 (ζ₂ ε₂), Hb Gower-2 (α₂ ε₂), and Hb Portland (ζ₂ γ₂). The genes for the α and ζ chains are encoded on chromosome 16; those for the β group have been localized to chromosome 11. The nucleotide sequences of all these genes have been determined, and many globin-gene abnormalities have been characterized at the molecular level.

The hemoglobin disorders are subdivided into three major groups. The structural abnormalities, including the hemoglobinopathies, result from changes in the amino acid sequences of the globin chains. Most have a single amino acid substitution; in others, however, amino acids may be deleted or inserted, or other, more complex structural changes may be present. The thalassemias are expressed as quantitative defects, in which the synthesis of one or more of the globin chains is decreased or, in the most severe forms, is totally suppressed. The hereditary persistence of fetal hemoglobin (HPFH) syndromes are characterized by elevated levels of Hb F continuing throughout adult life. Almost all these abnormalities result from the same types of molecular defects: Nucleotides may be substituted, deleted, or inserted into globin-gene DNA.

Select an item below

- HEMOGLOBIN STRUCTURAL ABNORMALITIES (Hemoglobinopathies)
- 468.1 Sickle Cell Hemoglobinopathies
- 468.2 Sickle Cell Trait (Heterozygous Hb S; Hb AS)
- 468.3 Other Hemoglobinopathies
- 468.4 Unstable Hemoglobin Disorders (Congenital Heinz Body Anemia)
- 468.5 Abnormal Hemoglobins with Increased Oxygen Affinity
- 468.6 Abnormal Hemoglobins Causing Cyanosis
- 468.7 Hereditary Methemoglobinemia
- 468.8 Syndromes of Hereditary Persistence of Fetal Hemoglobin
- 468.9 Thalassemia Syndromes
- 468.10 Hemochromatosis
- BIBLIOGRAPHY Hemoglobin Disorders
- FIGURES
- TABLES
HEMOGLOBIN STRUCTURAL ABNORMALITIES
(Hemoglobinopathies)

More than 700 structural variants of hemoglobin have been identified. Most are rare, but a few, including some severely pathologic forms, occur with high frequency in certain populations. Many abnormal hemoglobins are readily identified by electrophoresis, but some are electrophoretically “silent” and require other laboratory studies for identification. Numerous hemoglobin variants that have abnormal electrophoretic mobility, including both benign and pathologic forms, exhibit very similar electrophoresis findings and cannot be specifically identified by this means alone.

468.1 Sickle Cell Hemoglobinopathies

Sickle hemoglobin (Hb S) differs from normal adult hemoglobin by a substitution of glutamic acid at the 6th position of its β chains by valine. In the oxygenated state, Hb S functions normally. When this hemoglobin is deoxygenated, an interaction between the β6 valine and complementary regions on the β chains of an adjacent molecule results in formation of highly ordered molecular polymers; these elongate to form filamentous structures, which aggregate into rigid, crystal-like rods. This process of molecular polymerization is responsible for the spiny, brittle character of sickle erythrocytes (RBCs) under conditions of decreased oxygenation. Certain other abnormal hemoglobins, notably Hb C, Hb D Los Angeles, and Hb O Arab, participate in the molecular polymerization of deoxy-Hb S. Hb A does so to a smaller degree, but Hb F is totally excluded from the deoxy-Hb S polymer.

RBCs of heterozygous (sickle cell trait) individuals have been shown to resist invasion by malarial parasites, and this resistance appears to have provided protection against the frequently lethal falciparum form of the disease. The β^S gene is found in high frequency in those living in regions in which Plasmodium falciparum malaria has been endemic, including many parts of Africa, the Mediterranean area, and parts of Turkey, the Middle East, and India. In individuals from several geographic areas, the sickle mutation has been shown to exist in genetic linkage with discrete sets of closely associated markers. Some of these Hb S “haplotypes” appear to be predictive of the degree of severity of the sickle cell disease. Those associated with particularly mild disease are accompanied by significantly higher levels of Hb F. Patients who have sickle cell disease and who co-inherit genes for α-thalassemia may also have disease of modified severity.

Hb S is readily identified by electrophoresis. A confirmatory solubility test excludes other abnormal hemoglobins with similar electrophoretic mobility. Although affected newborns express only small quantities of Hb S, because of the predominance of Hb F at birth, the sickle cell syndromes can nevertheless be identified reliably in neonates by various laboratory methods. Neonatal screening programs for detecting infants with sickle cell disease are widely established in the United States. These disorders can also be determined antenatally using amniocyte or chorionic villus DNA by methods that identify the specific β^S nucleotide substitution.
SICKLE CELL ANEMIA
(Homozygous Hb S)

This disorder is characterized by severe chronic hemolytic disease resulting from premature destruction of the brittle, poorly deformable RBCs. Other manifestations of sickle cell anemia are attributable to ischemic changes resulting from vascular occlusion by masses of sickled cells. The clinical course of affected children is typically associated with intermittent episodic events, often referred to as “crises.”

CLINICAL MANIFESTATIONS.

Affected newborns seldom exhibit clinical features of sickle cell disease; hemolytic anemia gradually develops over the 1st 2–4mo, parallelling the replacement of much of the fetal hemoglobin by Hb S. Other clinical manifestations are uncommon before 5–6mo of age. Acute sickle dactylitis, presenting as the hand-foot syndrome, is frequently the 1st overt evidence that sickle cell disease is present in an infant. Its associated findings include painful, usually symmetric swelling of the hands and feet. The underlying abnormality is ischemic necrosis of the small bones, believed to be caused by a choking off of the blood supply as a result of the rapidly expanding bone marrow. Roentgenograms are not informative in the acute phase but later show evidence of extensive bony destruction and repair (Fig. 468–1).

![Figure 468-1](image)

**FIGURE 468–1** Roentgenograms of an infant with sickle cell anemia and acute dactylitis. *A*, The bones appear normal at the onset of the episode. *B*, Destructive changes and periosteal reaction are evident 2wk later.

Acute painful episodes represent the most frequent and prominent manifestation of sickle cell disease. Most patients experience some pain on a nearly daily basis. Episodes of severe pain that require hospitalization and parenteral analgesic administration average about 1/yr in children with Hb SS, but this interval varies considerably. Some patients
never experience severe pain, and others require hospital admission with such frequency that they become seriously disabled. In young children, pain often involves the extremities; in older patients, head, chest, abdominal, and back pain occur more commonly. In an individual patient, pain tends to recur in a limited number of sites. Intercurrent illnesses accompanied by fever, hypoxia, and acidosis, all of which promote the deoxygenation of Hb S, may precipitate sickle pain episodes, but acute pain also develops frequently without an apparent antecedent event. Sickle-related abdominal pain may mimic that of an acute surgical condition.

More extensive vaso-occlusive events in these patients can produce gross ischemic damage. Acute pain episodes may progress to infarction of bone marrow or bone. Splenic infarcts are common in children, causing pain and contributing to the process of “autosplenectomy.” Pulmonary infarction, often occurring in association with pneumonitis or microscopic fat emboli (from bone marrow infarction) may produce the severe clinical picture of acute chest syndrome. Strokes caused by cerebrovascular occlusion are among the most catastrophic acute events and are a frequent cause of hemiplegia. As many as 10% of children with sickle cell anemia, mainly preadolescent and older patients, exhibit sequelae of cerebrovascular occlusion. Findings of increased blood flow velocity by transcranial Doppler studies have been shown to be predictive of increased risk of stroke in these patients, and this may help to identify children who will benefit from preventive therapy. Ischemic damage may also affect the myocardium, liver, and kidneys. Renal function is progressively impaired by diffuse glomerular and tubular fibrosis, and hyposthenuria accompanied by polyuria is a characteristic finding in patients older than 5yr. Renal papillary necrosis and nephrotic syndrome also develop occasionally. Priapism is a relatively frequent complication that results from the pooling of blood in the corpora cavernosa, causing obstruction of the venous outflow.

Young children with Hb SS may have splenic enlargement associated with their hemolytic disease, with progression to the syndrome of hypersplenism accompanied by worsening anemia and sometimes thrombocytopenia. Acute splenic sequestration is a distinct and episodic event that occurs in infants and young children with sickle cell anemia, often following an acute febrile illness. For unknown reasons, large amounts of blood become acutely pooled in the spleen, which becomes massively enlarged, and signs of circulatory collapse rapidly develop. Blood transfusions in the acute phase may be lifesaving.

Altered splenic function in young children with sickle cell disease is a significant factor leading to their increased susceptibility to meningitis, sepsis, and other serious infections, mainly caused by pneumococci and Haemophilus influenzae. In the absence of specific antibody to the polysaccharide capsular antigens of these organisms, splenic activity is essential for removing these bacteria when they invade the blood. Despite frequent enlargement of the spleen in young patients with Hb SS, its phagocytic and reticuloendothelial functions have been shown to be markedly reduced. As an additional risk factor, children with sickle cell disease have also been shown to have deficient levels of serum opsonins, of the alternate complement pathway, against pneumococci. Children
with sickle cell disease also have increased susceptibility to *Salmonella* osteomyelitis (partly because of bone necrosis).

In common with patients having other forms of chronic hemolytic anemia, children with Hb SS are at risk of developing a rapid, potentially life-threatening decrease in their hemoglobin level (aplastic episodes) in association with parvovirus B19 infection (see Chapter 244).

An additional group of sickle cell sequelae is attributable primarily to the hemolytic anemia that accompanies this disorder. Cardiomegaly is invariably present in older children, often caused partly by sickle-related cardiomyopathy. Increased iron absorption contributes to parenchymal damage of the liver, pancreas, and heart. Symptomatic gallstone formation is common in adolescent and older patients, occasionally occurring in children as young as 5yr.

By midchildhood, most patients are underweight, and puberty is frequently delayed. Zinc deficiency, which is prevalent in children with sickle cell disease, may contribute to their poor growth and delayed maturation. Chronic leg ulcers are relatively uncommon in children, seldom occurring before late adolescence.

LABORATORY FINDINGS.

Hemoglobin concentrations usually range from 5–9g/dL. The peripheral blood smear typically contains target cells, poikilocytes, and irreversibly sickled cells (Fig. 468–2A). These findings allow Hb SS and most of the other forms of sickle cell disease to be readily distinguished from sickle cell trait and other clinically benign conditions. Reticulocyte counts usually range from 5–15%, and nucleated RBCs and Howell-Jolly bodies may be present. The total white blood cell count is elevated to 12,000–20,000/mm$^3$, with a predominance of neutrophils. The platelet count is usually increased; the sedimentation rate is slow. Other changes include abnormal liver function test results, hyperbilirubinemia, and diffuse hypergammaglobulinemia. The bone marrow is markedly hyperplastic and shows erythroid predominance. Roentgenograms show expanded marrow spaces and osteoporosis.

RBCs stained with supravital stain (brilliant cresyl blue) reveal intracellular inclusions. E, Homozygous $\beta^0$-thalassemia: severe hypochromia with deformed RBCs and normoblasts. F, Hemoglobin H disease ($\alpha$-thalassemia): anisopoikilocytosis with target cells. (Courtesy of Dr. John Bolles, The ASH Collection, University of Washington, Seattle, WA.)

**DIAGNOSIS.**

The diagnosis is normally established by hemoglobin studies. Electrophoresis at an alkaline pH demonstrates a characteristic mobility, intermediate between those of Hb A and Hb $A_2$. To distinguish Hb S from other hemoglobins with similar electrophoretic properties, another (confirmatory) test is required, such as electrophoresis at an acidic pH, a sickle cell preparation in which sickling is observed when the cells are deoxygenated, or, most commonly, a hemoglobin solubility test. In the Hb S solubility test, a measured amount of hemoglobin is added to a concentrated buffer that contains a reducing agent; a turbid precipitate forms when more than about 15% Hb S is present. Beyond infancy, RBCs from patients with Hb SS contain between 2% and 20% Hb F with normal level of Hb $A_2$. Hb A is notably absent. The identification of Hb S in each parent provides supportive evidence for the diagnosis of sickle cell anemia.

**DIFFERENTIAL DIAGNOSIS.**

The various clinical manifestations of sickle cell disease, including limb pain, heart murmurs, hepatosplenomegaly, and anemia, may suggest a number of other diagnoses, including rheumatic fever or rheumatoid arthritis, osteomyelitis, and leukemia. In patients who have a Hb SS electrophoresis pattern and concomitant microcytosis (mean corpuscular volume [MCV] <78fL), possibilities that require consideration include iron deficiency or a combination of Hb S with $\alpha$- or $\beta^0$-thalassemia (Table 468–1).

**TABLE 468–1. Clinically Important Sickle Cell Syndromes**

**TREATMENT.**

Measures directed toward preventing serious complications of sickle cell disease are among the most important elements of treatment. Maintaining full immunization status is particularly important. Administration of a polyvalent pneumococcal vaccine may be beneficial, but unfortunately, the forms of these vaccines currently available appear to be poorly immunogenic in children who have Hb SS and who are younger than 5yr. *H. influenzae* immunization has been shown to be efficacious in infants with sickle cell disease, and this as well as hepatitis B immunizations are indicated. Prophylactic penicillin is highly effective in preventing serious pneumococcal infections and should be administered to all young children with sickle cell disease. Oral penicillin V (age <5 yr: 125 mg/12 hr; age $\geq$5 yr: 250 mg/12 hr) is given starting by 4 mo of age. By 5 yr of age,
except in children who have had a severe pneumococcal infection or splenectomy, penicillin prophylaxis usually can be discontinued. Parents of children with Hb SS also need to be aware of the need to bring the child promptly to medical attention for acute illness, especially with a temperature exceeding 38.5°C regardless of the use of prophylaxis. Because of the substantial risk of life-threatening bacterial infections, prompt parenteral antibiotic therapy is indicated for infants and young children with an acute onset of high fever. Febrile patients with temperatures greater than 40°C, those who appear toxic or with findings suggestive of meningitis or other serious infection, and those who have previously had pneumococcal sepsis represent acute medical emergencies. Blood culture, intravenous ceftriaxone, and hospital admission for further antibiotic treatment are indicated. Other febrile patients older than 6mo generally can be treated effectively on an outpatient basis. In low-risk, well-appearing children, after blood cultures are obtained, intravenous ceftriaxone is given, and the dose is repeated the next day.

Parents and caretakers of these children should also be informed about the manifestations of acute splenic sequestration and the need for immediate medical attention for a child with rapid splenic enlargement and pallor.

Painful episodes can frequently be managed with oral acetaminophen, alone or with codeine. More severe episodes may require hospitalization and parenteral administration of narcotics. Anti-inflammatory agents, especially ketorolac, may decrease or eliminate the need for narcotic analgesics. Dehydration or acidosis should be rapidly corrected by the intravenous route, but overhydration should be avoided. Blood transfusions are seldom indicated for painful episodes, and it is doubtful whether transfusion can ameliorate the course of a pain crisis. For patients with disabling chronic pain, for those with ischemic organ damage (acute chest, priapism) or stroke, or in preparation for major surgery, however, transfusions of normal RBCs can provide symptomatic relief and prevent further ischemic complications. A first stroke may be prevented by transfusion of children with sickle cell disease and abnormal transcranial Doppler ultrasonography. For children with stroke, cardiomyopathy, and other severe complications, chronic long-term transfusion regimens are a mainstay of therapy. These patients also may require iron chelation treatment to prevent the development of hemosiderosis. Packed RBC transfusions are specifically indicated for acute splenic sequestration and aplastic episodes. Repeated episodes of splenic sequestration are an indication for splenectomy.

Bone marrow transplantation from a normal donor can be curative in patients with sickle cell disease, but the risks and morbidity associated with this procedure limit its application to highly selected patients. European experience, mainly from young children without chronic organ damage, has shown a high success rate following transplantation. In the United States, allogenic bone marrow transplantation has been used primarily in patients with severe complications of sickle cell disease. A majority of these children achieved successful engraftment with stabilization of their disease sequelae.

Chemotherapy regimens that stimulate Hb F synthesis have been used with beneficial effect, on an experimental basis, in a number of children with sickle cell disease. These
agents, which include hydroxyurea and butyrate, offer considerable promise of more effective means for treating these patients.

OTHER SICKLE CELL SYNDROMES

Sickling disorders of various degrees of severity result from Hb S existing in combination with other abnormal hemoglobins or thalassemias (see Table 468–1). Several of these syndromes, including Hb SD Los Angeles, Hb SO Arab, and Hb S–β0-thalassemia, present a clinical picture virtually indistinguishable from that of sickle cell anemia. Most of the others produce less severe manifestations.

**TABLE 468–1. Clinically Important Sickle Cell Syndromes**

*Hb SC disease* results from the concurrence of genes for Hb S and Hb C. Painful episodes and other vaso-occlusive manifestations are usually less severe in this condition than those associated with Hb SS. Most affected children have persistent splenomegaly, and bone infarcts occur more frequently than in those with Hb SS. Septicemia may also occur. Retinal vascular changes, predominantly in adolescents and adults, may lead to hemorrhage with retinal detachment. The hemoglobin concentration averages 9–10g/dL, with the blood smear showing target cells and characteristic spindle-shaped RBCs.

468.2 Sickle Cell Trait
(Heterozygous Hb S; Hb AS)

Heterozygous expression of the gene for Hb S is usually associated with a totally benign clinical course. About 8% of African-Americans have sickle cell trait; 35–45% of their hemoglobin is Hb S. This low level of Hb S is insufficient to produce sickling manifestations under usual circumstances, but under conditions of severe hypoxia, vaso-occlusive complications may occur. Splenic infarcts and other ischemic sequelae may occur in individuals with Hb AS as a result of hypoxia associated with general anesthesia. Hyposthenuria is usually present in older children and adults. Gross hematuria occasionally develops in otherwise well individuals. The hematologic findings in sickle cell trait are indistinguishable from normal ([Fig. 468–2](#)). The diagnosis is established by hemoglobin electrophoresis, with confirmatory sickle testing.

468.9 Thalassemia Syndromes

The thalassemias are a heterogeneous group of heritable hypochromic anemias of various degrees of severity. Underlying genetic defects include total or partial deletions of globin chain genes and nucleotide substitutions, deletions, or insertions. The consequences of these various changes are a decrease or absence of mRNA for one or more of the globin chains or the formation of functionally defective mRNA. The result is a decrease or total suppression of hemoglobin polypeptide chain synthesis. More than 200 distinct mutations are known to produce thalassemia phenotypes; many of these mutations are unique to localized geographic regions. In general, the globin chains synthesized in thalassemic RBCs are structurally normal. In severe forms of α-thalassemia, abnormal homotetramer hemoglobins (β₄ or γ₄) are formed, but their component globin polypeptides have normal structure. Conversely, a number of structurally abnormal hemoglobins also produce thalassemia-like hematologic changes. In characterizing the expression of the various thalassemia genes, superscript designations are used to distinguish those that produce a demonstrable globin chain product, although at decreased levels (e.g., β⁺-thalassemia) from those in which the synthesis of the affected globin chain is totally suppressed (e.g., β₀-thalassemia).

Thalassemia genes are remarkably widespread, and these abnormalities are believed to be the most prevalent of all human genetic diseases. Their main distribution includes areas bordering the Mediterranean Sea, much of Africa, the Middle East, the Indian subcontinent, and Southeast Asia. From 3–8% of Americans of Italian or Greek ancestry and 0.5% of black Americans carry a gene for β-thalassemia. In some regions of Southeast Asia, as many as 40% of the population have one or more thalassemia genes. The geographic areas in which thalassemia is prevalent closely parallel the regions in which *P. falciparum* malaria was formerly endemic. Resistance to lethal malarial infections by carriers of thalassemia genes apparently represented a strong selective force that favored their survival in these areas of endemic disease.

Select an item below

- **HOMOZYGOUS b₀-THALASSEMAIA (Cooley Anemia; Thalassemia Major)**
- **OTHER b-THALASSEMAIA SYNDROMES**
- **a-THALASSEMAIA**
HOMOZYGOUS $\beta^0$-THALASSEMIA
(Cooley Anemia; Thalassemia Major)

CLINICAL MANIFESTATIONS.

Homozygous $\beta^0$-thalassemia usually becomes symptomatic as a severe, progressive hemolytic anemia during the 2nd 6mo of life. Regular blood transfusions are necessary in these patients to prevent the profound weakness and cardiac decompensation caused by the anemia. Without transfusion, life expectancy is no more than a few years. In untreated cases or in those receiving infrequent transfusions at times of severe anemia, hypertrophy of erythropoietic tissue occurs in medullary and extramedullary locations. The bones become thin, and pathologic fractures may occur. Massive expansion of the marrow of the face and skull (Fig. 468–3) produces characteristic facies. Pallor, hemosiderosis, and jaundice combine to produce a greenish-brown complexion. The spleen and liver are enlarged by extramedullary hematopoiesis and hemosiderosis. In older patients, the spleen may become so enlarged that it causes mechanical discomfort and secondary hypersplenism. Growth is impaired in older children; puberty is delayed or absent because of secondary endocrine abnormalities. Diabetes mellitus resulting from pancreatic siderosis may also occur. Cardiac complications, including intractable arrhythmias and chronic congestive failure caused by myocardial siderosis, have been common terminal events. With modern regimens of comprehensive care for these patients, many of these complications can be prevented and others ameliorated and delayed in their onset.

FIGURE 468–3 A, Facial deformities in an inadequately transfused patient with thalassemia major (Cooley anemia). Severe maxillary hyperplasia and malocclusion are present. B, Roentgenogram of the skull demonstrates the maxillary overgrowth and shows prominent widening of the diploic spaces, with the “hair-on-end” appearance caused by vertical trabeculae. These changes can usually be prevented by an appropriate transfusion regimen.

LABORATORY FINDINGS.
The RBC morphologic abnormalities in untransfused patients with homozygous $\beta^0$-thalassemia are extreme. In addition to severe hypochromia and microcytosis (see Fig. 468–2E), many bizarre, fragmented poikilocytes and target cells are present. Large numbers of nucleated RBCs circulate, especially after splenectomy. Intraerythrocytic inclusions, which represent precipitated excess $\alpha$ chains, are also seen after splenectomy. The hemoglobin level falls progressively to lower than 5g/dL unless transfusions are given. The unconjugated serum bilirubin level is elevated. The serum iron level is high, with saturation of the transferrin. A striking biochemical feature is the presence of very high levels of Hb F in the RBCs (Table 468–2). Dipyrrolic compounds render the urine dark brown, especially after splenectomy.

TABLE 468–2. Clinical and Hematologic Features of the Principal Forms of Thalassemias


TREATMENT.

Transfusions are given on a regular basis to maintain the hemoglobin level above 10g/dL. This “hypertransfusion” regimen has striking clinical benefits; it permits normal activity with comfort, prevents progressive marrow expansion and cosmetic problems associated with facial bone changes, and minimizes cardiac dilatation and osteoporosis. Transfusions of 15–20mL/kg of packed cells are usually necessary every 4–5wk.

Hemosiderosis is an inevitable consequence of prolonged transfusion therapy, because each 500mL of blood delivers to the tissues about 200mg of iron that cannot be excreted by physiologic means. Myocardial siderosis has been a significant contributing factor in the early death of these patients. Hemosiderosis can be decreased or even prevented with
parenteral administration of the iron-chelating drug deferoxamine, which forms an iron complex that can be excreted in the urine. A sustained high blood level of deferoxamine is needed for adequate iron excretion. The drug is usually administered subcutaneously over an 8- to 12-hr period using a small portable pump (during sleep), 5 or 6 nights/wk. Patients who adhere to this regimen are able to control the accumulation of excessive body iron. Lethal complications of hepatic and myocardial siderosis can thus be prevented or significantly delayed. An orally active iron chelating agent, deferiprone, has been studied in a number of clinical trials and has been shown to promote iron excretion from patients with transfusion-related siderosis. A significant percentage of patients treated with deferiprone, however, have been shown to have progressive increases in their hepatic iron stores. This agent alone does not therefore appear to be sufficient for preventing long-term iron toxicity in these patients.

Hypertransfusion in these patients effectively prevents massive splenomegaly resulting from extramedullary erythropoiesis. Splenectomy eventually becomes necessary, however, because of the size of the organ or because of secondary hypersplenism. Splenectomy increases the risk of severe, overwhelming sepsis; therefore, the operation should be performed only for significant indications (see Chapter 493) and should be deferred as long as possible. The most frequent indication for splenectomy is an increased need for transfusion. A transfusion requirement exceeding 240mL/kg of packed RBCs/yr is usually evidence of hypersplenism and is an indication for considering splenectomy. Immunization of these patients with hepatitis B, H. influenzae type b, pneumococcal, and meningococcal vaccines is desirable, and prophylactic penicillin therapy is also recommended.

Bone marrow transplantation is curative in these patients and has been performed with increasing success, even in patients who have been transfused extensively. This procedure, however, carries considerable risks of morbidity and mortality and generally can be used only for patients who have nonaffected histocompatible siblings.

α-THALASSEmia

Microcytic anemias resulting from deficient synthesis of α-globin chains are prevalent in Africa, Mediterranean area countries, and much of Asia. Deletions of α-globin genes account for most of these abnormalities. Four α-globin genes are present in normal individuals, and four distinct forms of α-thalassemia have been identified as corresponding to deletions of one, two, three, or all four of these genes (see Table 468–2).

**Table 468–2. Clinical and Hematologic Features of the Principal Forms of Thalassemias**

*Deletion of a single α-globin gene* produces the silent carrier α-thalassemia phenotype. No hematologic abnormality is usually evident, except for mild microcytosis. Approximately 25% of black Americans have this form of α-thalassemia.
Individuals lacking two α-globin genes exhibit the feature of α-thalassemia trait, with mild microcytic anemia. In affected newborns, small quantities of Hb Bart's (γ4) can be identified by hemoglobin electrophoresis. Beyond about 1mo of age, Hb Bart's is no longer detectable, and the levels of Hb A2 and F are characteristically normal. Inclusions of precipitated hemoglobin may be visualized in RBC smears, however, after supravital staining.

**Deletion of three of the four α-globin genes** is associated with a thalassemia intermedia–like syndrome, Hb H disease. Microcytic anemia in this condition is accompanied by abnormal RBC morphology (see Fig. 468–2), with prominent intracellular inclusions present in the RBCs after supravital staining. Hb H (β4) is highly unstable; it can be readily identified by electrophoresis, but unless special measures are taken to prevent its precipitation during sample preparation, it may escape detection.


The most severe form of α-thalassemia, resulting from deletion of all of the α-globin genes, is accompanied by a total absence of α-chain synthesis. Because Hb F, A, and A2 all contain α chains, none of these hemoglobins is produced. Hb Bart's (γ4) accounts for most of the hemoglobin in affected infants, and because γ4 has a high oxygen affinity and therefore cannot transport oxygen to the tissues, these infants are severely hypoxic. Their RBCs also contain small quantities of the normal embryonic Hb Portland (ζ2 γ2), which functions as an effective oxygen transporter. Most of these infants are stillborn, and most who are born alive die within a few hours. These infants are severely hydropic, with congestive heart failure and massive generalized edema. Those that survive with aggressive neonatal management are also transfusion dependent.
The types of α-thalassemia genes vary among affected populations, and these differences account for the α-thalassemia syndromes that predominate in specific population groups. In black Americans, α-thalassemia genes are prevalent, with almost all affected individuals having the deletion arrangement (–, α) that produces a single α-locus chromosome. In this population, therefore, α-thalassemia occurs mainly as the silent carrier phenotype (–, α/α, α) or as α-thalassemia trait (–, α/–, α). Chromosomes with deletions of both of the α loci (–, –) are prevalent in both Mediterranean and Asian populations, and Hb H disease (–, α/–, –) therefore occurs with significant frequency in both groups. The two α-locus deletion defects in Asians are often accompanied by retention of the ζ-globin genes (i.e., ζ–, –), whereas those from Mediterranean countries usually are not (–, –). The latter type of defect, therefore, cannot support the synthesis of Hb Portland (ζ2 γ2), which appears to be essential for intrauterine survival of fetuses with the hydrops fetalis form of α-thalassemia. Accordingly, the hydrops fetalis form almost exclusively affects infants of Asian ancestry. An acquired α-thalassemia syndrome, which may be associated with a large deletion involving the α-globin genes, includes Hb H disease accompanied by mental retardation, microcephaly, and hypogonadism.

A number of abnormal hemoglobins also produce α-thalassemia–like changes. The α-chain variant Hb Constant Spring occurs commonly in Far Eastern populations and is frequently observed in patients with Hb H disease who have the genotype (αA, αCo Sp /–, –). The gene for Hb G Philadelphia, which is the most prevalent α-chain abnormality of black Americans, usually occurs on a single-locus chromosome (–, αG). Individuals who express this abnormal hemoglobin therefore may also exhibit α-thalassemia–like hematologic changes.

OTHER β-THALASSEMA SYNDROMES

The homozygous expression of milder (β+) thalassemia genes produces a Cooley's anemia-like syndrome of lesser severity ("thalassemia intermedia"; see Table 468–2). Skeletal deformities and hepatosplenomegaly develop in these patients, but their hemoglobin levels are usually maintained at 6–8 g/dL without transfusion. Nevertheless, they may develop severe hemosiderosis, attributable to their greatly increased gastrointestinal iron absorption. For such patients, who do not receive deferoxamine chelation therapy, a low-iron diet is indicated.

TABLE 468–2. Clinical and Hematologic Features of the Principal Forms of Thalassemias

Several structurally abnormal hemoglobins produce β-thalassemia–like hematologic changes and, when present in combination with a gene for β-thalassemia, also result in a thalassemia intermedia syndrome. Among these are the Hb Lepore variants, which are composed of α chains in combination with hybrid δβ fusion globin chains. The Lepore hemoglobins are identified by electrophoresis, in which they exhibit Hb S–like mobility.
Most forms of heterozygous \( \beta \)-thalassemia are associated with mild anemia. The hemoglobin concentration typically averages 2–3g/dL lower than age-related normal values. The RBCs are hypochromic and microcytic, with poikilocytosis, ovalocytosis, and often basophilic stippling. Target cells may be present but usually are not prominent and are not specific for thalassemia. The MCV is low, averaging 65fL, and the mean corpuscular hemoglobin (MCH) values are also low (<26pg). A mild decrease in RBC survival can be shown, but overt signs of hemolysis are usually absent. The serum iron level is normal or elevated.

Individuals with thalassemia trait are often misdiagnosed as having iron deficiency anemia and may be inappropriately treated with iron for extended periods. More than 90% of persons with \( \beta \)-thalassemia trait have diagnostic elevations of Hb A2 of 3.4–7%. About 50% of these individuals also have slight elevations of Hb F, about 2–6%. In a small number of otherwise typical cases, normal levels of Hb A2 with Hb F levels ranging from 5–15% are found, representing the \( \delta \beta \) type of thalassemia (see Table 468–2). The silent carrier form of \( \beta \)-thalassemia produces no demonstrable abnormality in heterozygous individuals (see Table 468–2), but the gene for this condition, when inherited together with a gene for \( \beta^0 \)-thalassemia, results in a thalassemia intermedia syndrome.

A rare type of deletion defect, which involves the \( \gamma \)-, \( \delta \)-, and \( \beta \)-globin genes, produces a clinical picture similar to that of \( \delta \beta \)-thalassemia trait in heterozygous individuals. In the newborn period, however, this defect is accompanied by significant hemolytic disease with microcytosis, normoblastemia, and splenomegaly (see Table 468–2). The hemolytic process is self-limited, but supportive transfusions

**Congenital Hypoplastic Anemia**
(Diamond-Blackfan Syndrome)

This rare condition usually becomes symptomatic in early infancy, frequently with pallor in the neonatal period, but may first be noted later in childhood. About 75% of cases are diagnosed by 3mo of age. The most characteristic features are macrocytic anemia, reticulocytopenia, and a deficiency or absence of red blood cell (RBC) precursors in an otherwise normally cellular bone marrow.

**ETIOLOGY.**

Dominant or recessive patterns of inheritance are indicated by familial occurrence in about 15% of patients. In about 25% of patients, there are mutations in the gene for ribosomal protein S19 (one of 79 ribosomal proteins), mapped to chromosome 19q13. The patients were heterozygous for deficiency of the protein and were sporadic or familial cases. Most evidence indicates that the primary defects are in the erythroid precursor and are not due to immunologic damage to normal stem cells. High levels of EPO are present in serum and urine. A search for mutations in the EPO receptor gene has
been negative. In patients, no defects have been found in the genes for mast/stem cell growth factor (MGF) or its receptor, c-kit, nor does prednisone correct the anemias in mice with deficiencies of MGF or c-kit. Erythroid progenitors in this disorder have an unusual sensitivity to withdrawal of EPO, with resultant increased apoptosis (programmed cell death).

Although hematopoiesis is generally adequate in fetal life, some affected infants appear pale in the first days after birth; rarely, hydrops fetalis occurs. Profound anemia usually becomes evident by 2–6 mo of age, occasionally somewhat later. The liver and spleen are not enlarged initially. About one third of affected children have congenital anomalies, most commonly craniofacial deformities or defects of the upper extremities, including triphalangeal thumbs. The abnormalities are diverse, with no specific pattern emerging in the majority of those affected.

RBCs are usually macrocytic, with elevated levels of folic acid and vitamin B₁₂. Assay of RBCs reveals a pattern characteristic of a “young” RBC population, including elevated fetal hemoglobin (Hb F) and increased expression of “i” antigen. Adenosine deaminase activity is increased in RBCs of patients with this disorder. These findings may help distinguish congenital RBC aplasia from acquired transient erythroblastopenia of childhood (Chapter 456). Thrombocytosis or thrombocytopenia and occasionally neutropenia may also be present initially. Reticulocytes are diminished, even when the anemia is severe. RBC precursors are markedly reduced in the marrow in most patients, but other marrow elements are usually normal. Serum iron levels are elevated.

DIFFERENTIAL DIAGNOSIS.

Congenital hypoplastic anemia must be differentiated from other anemias with low reticulocyte counts. The anemia of the convalescent phase of hemolytic disease of the newborn may, on occasion, be associated with markedly reduced erythropoiesis. This terminates spontaneously at 5–8 wk of age. Aplastic crises characterized by reticulocytopenia and by decreased numbers of RBC precursors, frequently caused by parvovirus B19 infections, may complicate various types of hemolytic disease, but usually after the first several months of life. Infection with this virus in utero may also cause pure RBC aplasia in infancy, even with hydrops fetalis at birth. The syndrome of transient erythroblastopenia of childhood (Chapter 456) may be differentiated from Diamond-Blackfan syndrome by its relatively late onset (although it may occasionally develop in infants younger than 6 mo) and by biochemical differences in RBCs.

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**PROGNOSIS.**

Median survival is more than 40yr of age. The outlook is best in those who respond to corticosteroid therapy. About half of the patients are long-term responders. In the others, survival depends on transfusions. Some children in each group may eventually have spontaneous remissions (about 14%). By late childhood, children who do not respond to corticosteroids may have had 100 or more transfusions, and hemosiderosis may result unless chelation therapy for excess iron is carried out appropriately. The liver and spleen enlarge, and secondary hypersplenism with leukopenia and thrombocytopenia may occur in children who do not receive adequate chelation or in those with chronic hepatitis acquired from transfusions. The complications of chronic transfusions are similar to those in β-thalassemia major, and prevention and treatment of iron overload should be equally aggressive in both groups of transfused patients (see Chapter 468, 9).

**TREATMENT.**

Corticosteroid therapy is frequently beneficial if begun early; three fourths of patients respond initially. The mechanism of its effect is unknown.

**Prednisone** in three or four divided doses totaling 2mg/kg/24hr is used as an initial trial. RBC precursors appear in bone marrow 1–3wk after therapy is begun, and then normoblastosis and a brisk peripheral reticulocytosis occur. The hemoglobin may reach normal levels in 4–6wk. The dose of corticosteroid may then be reduced gradually by tapering divided doses and then by eliminating all except a single, lowest effective daily.
dose. This dose should then be doubled, used on alternate days, and tapered still further while maintaining the hemoglobin level at 10g/dL or above. In some patients, very small amounts of prednisone, as low as 2.5mg, may be sufficient to sustain adequate erythropoiesis.

In patients who do not respond to corticosteroid therapy, transfusions at intervals of 4–8wk are necessary to sustain life. Chelation therapy for iron overload with deferoxamine administered subcutaneously via a battery-powered portable pump should be begun when excess iron accumulation is reflected by serum ferritin levels exceeding 1,000mg/dL, but preferably after 5yr of age, because the medication may interfere with normal growth. An oral iron chelator, deferiprone (L1), is in clinical trials and may offer an alternative if it is shown to be effective and to have acceptable toxicity. Other therapies, including androgens, cyclosporine, cyclophosphamide, antithymocyte globulin (ATG), high-dose intravenous immunoglobulin, EPO, and interleukin-3 have not had a consistent beneficial effect and may have a high incidence of side effects. High-dose methylprednisolone (30–100mg/kg/24hr; tapered after 3 days) has been beneficial in some patients. Splenectomy may decrease the need for transfusion if hypersplenism or isoimmunization has developed. Bone marrow transplantation has a role in children who do not respond to corticosteroids and who have a histocompatible donor. The rate of engraftment is high, providing further evidence that immunosuppression is not the primary cause of this disorder. Transplantation of umbilical cord cells from an HLA-compatible newborn sibling has resulted in successful hematopoietic reconstitution.

Chapter 461 Iron Deficiency Anemia

Anemia resulting from lack of sufficient iron for synthesis of hemoglobin is the most common hematologic disease of infancy and childhood. Its frequency is related to certain basic aspects of iron metabolism and nutrition. The body of a newborn infant contains about 0.5g of iron, whereas the adult content is estimated at 5g. To make up for this discrepancy, an average of 0.8mg of iron must be absorbed each day during the first 15yr of life. In addition to this growth requirement, a small amount is necessary to balance normal losses of iron by shedding of cells. Accordingly, to maintain positive iron balance in childhood, about 1mg of iron must be absorbed each day.

Iron is absorbed in the proximal small intestine, mediated in part by duodenal proteins (HFE, hephaestin, Nramp², and mobilferrin). Because absorption of dietary iron is assumed to be about 10%, a diet containing 8–10mg of iron daily is necessary for optimal nutrition. Iron is absorbed two to three times more efficiently from human milk than from cow's milk, perhaps partly because of differences in calcium content. Breast-fed infants may, therefore, require less iron from other foods. During the first years of life, because relatively small quantities of iron-rich foods are eaten, it is often difficult to attain sufficient iron. For this reason, the diet should include such foods as infant cereals or formulas that have been fortified with iron; both of these are very effective in preventing iron deficiency. Formulas with 7–12mg Fe/L for full-term infants and premature infant formulas with 15mg/L for infants less than 1,800g at birth are effective. Infants breast-fed exclusively should receive iron supplementation from 4mo of age. At best, an infant is in
a precarious situation with respect to iron. Should the diet become inadequate or external blood loss occur, anemia ensues rapidly.

Adolescents are also susceptible to iron deficiency because of high requirements due to the growth spurt, dietary deficiencies, and menstrual blood loss. In the United States, about 9% of 1–2yr-olds are iron deficient; 3% have anemia. Of adolescent girls, 9% are iron deficient and 2% have anemia. In boys, a 50% decrease in stored iron occurs as puberty progresses.

Select an item below

- ETIOLOGY
- CLINICAL MANIFESTATIONS
- LABORATORY FINDINGS
- DIFFERENTIAL DIAGNOSIS (see Table 453–2)
- TREATMENT
- TABLES

ETIOLOGY.

Low birthweight and unusual perinatal hemorrhage are associated with decreases in neonatal hemoglobin mass and stores of iron. As the high hemoglobin concentration of the newborn falls during the first 2–3mo of life, considerable iron is reclaimed and stored (Chapter 99). These reclaimed stores are usually sufficient for blood formation in the first 6–9mo of life in term infants. In low birthweight infants or those with perinatal blood loss, stored iron may be depleted earlier, and dietary sources become of paramount importance. Anemia caused solely by inadequate dietary iron is unusual before 4–6mo but becomes common at 9–24mo of age. Thereafter, it is relatively infrequent. The usual dietary pattern observed in infants with iron deficiency anemia is consumption of large amounts of cow's milk and of foods not supplemented with iron.

Blood loss must be considered a possible cause in every case of iron deficiency anemia, particularly in older children. Chronic iron deficiency anemia from occult bleeding may be caused by a lesion of the gastrointestinal (GI) tract, such as a peptic ulcer, Meckel's diverticulum, a polyp, or hemangioma, or by inflammatory bowel disease. In some geographic areas, hookworm infestation is an important cause of iron deficiency. Pulmonary hemosiderosis may be associated with unrecognized bleeding in the lungs and recurrent iron deficiency after treatment with iron. Chronic diarrhea in early childhood may be associated with considerable unrecognized blood loss. Some infants with severe iron deficiency in the United States have chronic intestinal blood loss induced by exposure to a heat-labile protein in whole cow's milk. Loss of blood in the stools each day can be prevented either by reducing the quantity of whole cow's milk to 1pint/24hr or less, by using heated or evaporated milk, or by feeding a milk substitute. This GI reaction is not related to enzymatic abnormalities in the mucosa, such as lactase deficiency, or to typical “milk allergy.” Involved infants characteristically develop anemia that is more
severe and occurs earlier than would be expected simply from an inadequate intake of iron.

Histologic abnormalities of the mucosa of the GI tract, such as blunting of the villi, are present in advanced iron deficiency anemia and may cause leakage of blood and decreased absorption of iron, further compounding the problem.

Intense exercise conditioning, as occurs in competitive athletics in high school, may result in iron depletion in girls; this occurs less commonly in boys.

CLINICAL MANIFESTATIONS.

Pallor is the most important clue to iron deficiency. Blue scleras are also common, although also found in normal infants. In mild to moderate iron deficiency (hemoglobin levels of 6–10g/dL), compensatory mechanisms, including increased levels of 2,3-diphosphoglycerate (2,3-DPG) and a shift of the oxygen dissociation curve, may be so effective that few symptoms of anemia are noted, although affected children may be irritable. Pagophagia, the desire to ingest unusual substances such as ice or dirt, may be present. In some children, ingestion of lead-containing substances may lead to concomitant plumbism. When the hemoglobin level falls below 5g/dL, irritability and anorexia are prominent. Tachycardia and cardiac dilation occur, and systolic murmurs are often present.

The spleen is enlarged to palpation in 10–15% of patients. In long-standing cases, widening of the diploë of the skull similar to that in congenital hemolytic anemias may occur. These changes resolve slowly with adequate replacement therapy. Children with iron deficiency anemia may be obese or may be underweight, with other evidence of poor nutrition. The irritability and anorexia characteristic of advanced cases may reflect deficiency in tissue iron, because with iron therapy striking improvement in behavior frequently occurs before significant hematologic improvement.

Iron deficiency may have effects on neurologic and intellectual function. A number of reports suggest that iron deficiency anemia, and even iron deficiency without significant anemia, affects attention span, alertness, and learning of both infants and adolescents. In a controlled trial, adolescent girls with serum ferritin levels of 12 ng/L or less but without anemia improved verbal learning and memory after taking iron for 8wk.

Monoamine oxidase (MAO), an iron-dependent enzyme, has a crucial role in neurochemical reactions in the central nervous system. Iron deficiency produces decreases in the activities of enzymes such as catalase and cytochromes. Catalase and peroxidase contain iron, but their biologic essentiality is not well established. Iron deficiency causes rigidity of red blood cells (RBCs) and may be associated with stroke in young children. Administration of iron may decrease the frequency of breath-holding spells, suggesting a role for iron deficiency

LABORATORY FINDINGS.
In progressive iron deficiency, a sequence of biochemical and hematologic events occurs. First, the tissue iron stores represented by bone marrow hemosiderin disappear. The level of serum ferritin, an iron-storage protein, provides a relatively accurate estimate of body iron stores in the absence of inflammatory disease. Normal ranges are age dependent, and decreased levels accompany iron deficiency. Next, serum iron level decreases (also age dependent), the iron-binding capacity of the serum increases, and the percent saturation falls below normal (also varies with age). When the availability of iron becomes rate limiting for hemoglobin synthesis, a moderate accumulation of heme precursors, free erythrocyte protoporphyrins (FEP), results.

As the deficiency progresses, the RBCs become smaller than normal and their hemoglobin content decreases. The morphologic characteristics of RBCs are best quantified by the determination of mean corpuscular hemoglobin (MCH) and mean corpuscular volume (MCV). Developmental changes in MCV require the use of age-related standards for diagnosis of microcytosis (see Table 453–1). With increasing deficiency, the RBCs become deformed and misshapen and present characteristic microcytosis, hypochromia, poikilocytosis, and increased RBC distribution width (RDW); see Fig. 453–1 C). The reticulocyte percentage may be normal or moderately elevated, but absolute reticulocyte counts indicate an insufficient response to anemia. Nucleated RBCs may occasionally be seen in the peripheral blood. White blood cell counts are normal. Thrombocytosis, sometimes of a striking degree (600,000–1,000,000/mm³), may occur or, in a few cases, thrombocytopenia. The mechanisms of these platelet abnormalities are not clear. They appear to be a direct consequence of iron deficiency, perhaps with associated GI blood loss or associated folate deficiency, and they return to normal with iron therapy and dietary change. The bone marrow is hypercellular, with erythroid hyperplasia. The normoblasts may have scanty, fragmented cytoplasm with poor hemoglobinization. Leukocytes and megakaryocytes are normal. Hemosiderin cannot be demonstrated in marrow specimens by Prussian blue staining. In about a third of cases, occult blood can be detected in the stools.

**TABLE 453–1. Hematologic Values During Infancy and Childhood**

**FIGURE 453–1** Morphologic abnormalities of the red blood cell. A, Normal. B, Macrocytes (folic acid or vitamin B₁₂ deficiency). C, Hypochromic microcytes (iron deficiency). D, Target cells (Hb CC disease). E, Schizocytes (hemolytic-uremic syndrome). (Provided by Dr. E. Schwartz.)

**DIFFERENTIAL DIAGNOSIS** (see Table 453–2).
Iron deficiency must be differentiated from other hypochromic microcytic anemias. In lead poisoning associated with iron deficiency, the RBCs are morphologically similar, but coarse basophilic stippling of the RBCs, an artifact of drying the slide, is frequently prominent. Elevations of blood lead, FEP, and urinary coproporphyrin levels are seen (Chapter 721). The blood changes of β-thalassemia trait resemble those of iron deficiency (Chapter 468.9), but RDW is usually normal or only slightly elevated. α-Thalassemia trait occurs in about 3% of blacks in the United States and in many Southeast Asian peoples. The diagnosis requires direct identification of DNA defects or difficult globin synthesis studies after the newborn period. The diagnosis can be assumed when a patient having familial hypochromic microcytic anemia with normal iron studies, including ferritin, has normal levels of Hb A2 and Hb F and normal hemoglobin electrophoresis. In the newborn period, infants with α-thalassemia trait have 3–10% Bart hemoglobin and the MCV is decreased (Chapter 468.9). Thalassemia major, with its pronounced erythroblastosis and hemolytic component, should present no diagnostic confusion. Hb H disease, a form of α-thalassemia with hypochromia and microcytosis, also has a hemolytic component due to instability of the β-chain tetramers resulting from a deficiency of α globin. The RBC morphology of chronic inflammation and infection, though usually normocytic, may be microcytic, but in these conditions both the serum iron level and iron-binding ability are reduced and serum ferritin levels are normal or elevated. The serum transferrin receptor (TfR) level is useful in the distinction between iron deficiency anemia and anemia of chronic disease, because it is not affected by inflammation. The concentration is elevated in iron deficiency and within the normal range in anemia of chronic disease. An elevation of the TfR/log ferritin ratio is especially sensitive in detecting iron deficiency anemia. Elevations of FEP are not specific to iron deficiency and are observed in patients with lead poisoning, chronic hemolytic anemia, anemia associated with chronic disorders, and some of the porphyrias.

DIFFERENTIAL DIAGNOSIS (see Table 453–2).

**TABLE 453–2. Classification of Anemia**

Iron deficiency must be differentiated from other hypochromic microcytic anemias. In lead poisoning associated with iron deficiency, the RBCs are morphologically similar, but coarse basophilic stippling of the RBCs, an artifact of drying the slide, is frequently prominent. Elevations of blood lead, FEP, and urinary coproporphyrin levels are seen (Chapter 721). The blood changes of β-thalassemia trait resemble those of iron deficiency (Chapter 468.9), but RDW is usually normal or only slightly elevated. α-Thalassemia trait occurs in about 3% of blacks in the United States and in many Southeast Asian peoples. The diagnosis requires direct identification of DNA defects or difficult globin synthesis studies after the newborn period. The diagnosis can be assumed when a patient having familial hypochromic microcytic anemia with normal iron studies, including ferritin, has normal levels of Hb A2 and Hb F and normal hemoglobin electrophoresis. In the newborn period, infants with α-thalassemia trait have 3–10% Bart hemoglobin and the MCV is decreased (Chapter 468.9). Thalassemia major, with its pronounced
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**Part XX:** Diseases of the Blood  
**Section 2:** Anemias of Inadequate Production  
**Chapter 461:** Iron Deficiency Anemia

**TREATMENT.**

The regular response of iron deficiency anemia to adequate amounts of iron is an important diagnostic and therapeutic feature. Oral administration of simple ferrous salts (sulfate, gluconate, fumarate) provides inexpensive and satisfactory therapy. No evidence shows that addition of any trace metal, vitamin, or other hematinic substance significantly increases the response to simple ferrous salts. For routine clinical use, physicians should be familiar with an inexpensive preparation of one of the simple ferrous compounds. The therapeutic dose should be calculated in terms of elemental iron; ferrous sulfate is 20% elemental iron by weight. A daily total of 6mg/kg of elemental iron in three divided doses provides an optimal amount of iron for the stimulated bone marrow to use. Intolerance to oral iron is uncommon in children. A parenteral iron preparation (iron dextran) is an effective form of iron and is usually safe when given in a properly calculated dose, but the response to parenteral iron is no more rapid or complete than that obtained with proper oral administration of iron, unless malabsorption is a factor.

While adequate iron medication is given, the family must be educated about the patient's diet, and the consumption of milk should be limited to a reasonable quantity, preferably 500mL (1 pint)/24hr or less. This reduction has a dual effect: The amount of iron-rich foods is increased, and blood loss from intolerance to cow's milk proteins is reduced. When the re-education of child and parent is not successful, parenteral iron medication may be indicated. Iron deficiency can be prevented in high-risk populations by providing iron-fortified formula or cereals during infancy.
The expected clinical and hematologic responses to iron therapy are described in Table 461–1.

**TABLE 461–1. Responses to Iron Therapy in Iron Deficiency Anemia**

Within 72–96 hr after administration of iron to an anemic child, peripheral reticulocytosis is noted. The height of this response is inversely proportional to the severity of the anemia. Reticulocytosis is followed by a rise in the hemoglobin level, which may increase as much as 0.5 g/dL/24 hr. Iron medication should be continued for 8 wk after blood values are normal. Failures of iron therapy occur when a child does not receive the prescribed medication, when iron is given in a form that is poorly absorbed, or when there is continuing unrecognized blood loss, such as intestinal or pulmonary loss, or with menstrual periods. An incorrect original diagnosis of nutritional iron deficiency may be revealed by therapeutic failure of iron medication.

Because a rapid hematologic response can be confidently predicted in typical iron deficiency, blood transfusion is indicated only when the anemia is very severe or when superimposed infection may interfere with the response. It is not necessary to attempt rapid correction of severe anemia by transfusion; the procedure may be dangerous because of associated hypervolemia and cardiac dilatation. Packed or sedimented RBCs should be administered slowly in an amount sufficient to raise the hemoglobin to a safe level at which the response to iron therapy can be awaited. In general, severely anemic children with hemoglobin values less than 4 g/dL should be given only 2–3 mL/kg of packed cells at any one time (*furosemide* may also be administered as a diuretic). If there is evidence of frank congestive heart failure, a modified exchange transfusion using fresh-packed RBCs should be considered, although diuretics followed by slow infusion of packed RBCs may suffice.

**Anemia of Chronic Disorders and Renal Disease**

Anemia complicates a number of chronic systemic diseases associated with infection, inflammation, or tissue breakdown. Examples of such conditions include chronic pyogenic infections, such as bronchiectasis and osteomyelitis; chronic inflammatory processes, such as rheumatoid arthritis, systemic lupus erythematosus, and ulcerative colitis; malignancies; and advanced renal disease. In the latter, an additional major component is decreased production of erythropoietin (EPO) due to damage of the cells producing this cytokine. Despite diverse underlying causes, the erythroid abnormalities are similar. Red blood cell (RBC) life span is moderately decreased, reflecting increased RBC destruction by a hyperactive reticuloendothelial system. The increased hemolysis is less important, however, than a relative failure of bone marrow response, reflecting both hypoactivity of marrow and an EPO production inadequate for the degree of anemia. Another finding is abnormalities of iron metabolism, including defective iron release from tissues into the plasma. Suppression of the erythroid response in the marrow appears to result primarily from an increase in tumor necrosis factor (TNF), which acts on bone marrow stromal cells to produce interferon (IFN)-β as a primary mediator, and an increase in interleukin-1 (IL-1), which acts on T cells to produce IFN-γ as a primary
mediator. IL-6 levels may also be elevated. Recombinant human EPO can overcome this effect if the EPO level in a patient is less than 500mU/mL. TNF and IL-1 decrease EPO production in perfused kidneys and hepatoma cells, corresponding to the two sites of EPO production, accounting for the inadequate EPO response in this type of anemia. The specific stimulant of increased TNF and IL-1 production in these patients has not been identified.

Select an item below

- CLINICAL MANIFESTATIONS.
- LABORATORY FINDINGS.
- TREATMENT AND PROGNOSIS.

LABORATORY FINDINGS.

Hemoglobin concentrations usually range from 6 to 9g/dL. The anemia is usually normochromic and normocytic; about one third of patients may have modest hypochromia and microcytosis. Absolute reticulocyte counts are normal or low, and leukocytosis is common. Free erythrocyte protoporphyrin (FEP) levels are frequently elevated and provide a sensitive reflection of derangements of iron metabolism. They return to normal after successful treatment of the primary disease. The serum iron level is low, without the increase in total iron-binding capacity that occurs in iron deficiency. This pattern of low serum iron and low to normal iron-binding protein is a regular and valuable diagnostic feature. Serum ferritin level may be elevated. Serum transferrin receptor (TfR) level is normal, unless iron deficiency is present. The bone marrow has normal cellularity; the RBC precursors are low to adequate, marrow hemosiderin may be increased, and granulocytic hyperplasia may be present. A frequent clinical challenge is to identify concomitant iron deficiency in patients with an inflammatory disease. Measurement of TfR/ferritin ratio may be useful, because it is elevated when iron deficiency is present. A trial of iron therapy may resolve the issue, although there may be no response when inflammation due to the primary disease persists. Intravenous iron saccharate is effective in iron deficiency associated with juvenile rheumatoid arthritis.

TREATMENT AND PROGNOSIS.

Because these anemias are secondary to other disease processes, they do not respond to iron or hematins unless there is concomitant deficiency. Transfusions raise the hemoglobin concentration only temporarily and are rarely indicated. If the underlying systemic disease can be controlled, the anemia is corrected spontaneously. Recombinant human EPO can increase the hemoglobin level and improve activity and the sense of well-being in patients with cancer and end-stage renal failure and in those with anemia of chronic inflammation. Treatment with iron is usually necessary for an optimal EPO effect.

Chapter 460 Megaloblastic Anemias
The megaloblastic anemias have in common certain abnormalities of red blood cell (RBC) morphology and maturation. The RBCs at every stage of development are larger than normal and have an open, finely dispersed nuclear chromatin and an asynchrony between maturation of nucleus and cytoplasm, with the delay in nuclear progression being more evident with further cell divisions. Megaloblastic morphology may be seen in a number of conditions; almost all cases in children result from a deficiency of folic acid, vitamin B<sub>12</sub>, or both. Both substances are cofactors required in the synthesis of nucleoproteins, and deficiencies result in defective synthesis of DNA and, to a lesser extent, RNA and protein. Ineffective erythropoiesis results from arrest in development or premature death of cells in the marrow. In the peripheral blood, RBCs are large (increased mean corpuscular volume [MCV]) and frequently oval, hypersegmented neutrophils appear, and giant platelets may also be found. In the marrow, the late nucleated megaloblastic RBC may appear well hemoglobinized but still retains an immature nucleus rather than the usual clumped chromatin. Giant metamyelocytes and bands are also present in the marrow. Megaloblastic anemias due to malnutrition are relatively uncommon in the United States.

Select an item below

- 460.1 Folic Acid Deficiencies
- 460.2 Vitamin B12 (Cobalamin) Deficiency
- 460.3 Rare Megaloblastic Anemias

Chapter 460 Megaloblastic Anemias

The megaloblastic anemias have in common certain abnormalities of red blood cell (RBC) morphology and maturation. The RBCs at every stage of development are larger than normal and have an open, finely dispersed nuclear chromatin and an asynchrony between maturation of nucleus and cytoplasm, with the delay in nuclear progression being more evident with further cell divisions. Megaloblastic morphology may be seen in a number of conditions; almost all cases in children result from a deficiency of folic acid, vitamin B<sub>12</sub>, or both. Both substances are cofactors required in the synthesis of nucleoproteins, and deficiencies result in defective synthesis of DNA and, to a lesser extent, RNA and protein. Ineffective erythropoiesis results from arrest in development or premature death of cells in the marrow. In the peripheral blood, RBCs are large (increased mean corpuscular volume [MCV]) and frequently oval, hypersegmented neutrophils appear, and giant platelets may also be found. In the marrow, the late nucleated megaloblastic RBC may appear well hemoglobinized but still retains an immature nucleus rather than the usual clumped chromatin. Giant metamyelocytes and bands are also present in the marrow. Megaloblastic anemias due to malnutrition are relatively uncommon in the United States.
MEGALOBLASTIC ANEMIA OF INFANCY

This disease is caused by a deficient intake or absorption of folic acid (see also Chapters 40 and 44.8). Folates are abundant in many foods, including green vegetables, fruits, and animal organs (liver, kidney). Folic acid is absorbed throughout the small intestine, after pteroylglutamate reacts with membrane-associated folate-binding proteins. Pteroylpolyglutamates, found in cabbage, lettuce, and other foods, are absorbed less efficiently than pteroylmonoglutamate (folic acid). Pteroyl polyglutamate hydrolase activity in the brush border aids the conversion to the monoglutamate. Surgical removal or disorders of the small intestine may lead to folate deficiency. There is an active enterohepatic circulation. Much of the folate in the plasma is loosely bound to albumin. Pteroylglutamate is not biologically active. It is reduced by dihydrofolate reductase to tetrahydropteroylglutamate (tetrahydrofolate), which is transported into tissue cells and polyglutamated. Dietary deficiency is usually compounded by rapid growth or infection, which may increase folic acid requirements. The normal adult daily requirement is about 100μg/24hr, which rises to 350μg/24hr in pregnancy. The requirements on a weight basis are higher in the pediatric age range in comparison with adults owing to the increased needs of growth. The needs are also increased with accelerated tissue turnover, as in hemolytic anemia. Human and cow's milk provide adequate amounts of folic acid. Goat's milk is clearly deficient; folic acid supplementation must be given when it is the main food. Unless supplemented, powered milk may also be a poor source of folic acid.

CLINICAL MANIFESTATIONS.

Mild megaloblastic anemia has been reported in very low birthweight infants, and routine folic acid supplementation is advised. Megaloblastic anemia has its peak incidence at 4–7mo of age, somewhat earlier than iron deficiency anemia, although the two may be present concomitantly in infants with poor nutrition. Besides having the usual clinical features of anemia, affected infants with folate deficiency are irritable, fail to gain weight adequately, and have chronic diarrhea. Hemorrhages due to thrombocytopenia occur in advanced cases. Folic acid deficiency may accompany kwashiorkor, marasmus, or sprue.

LABORATORY FINDINGS.

The anemia is macrocytic (MCV >100 fl). Variations in RBC shape and size are common (see Fig. 453-1B). The reticulocyte count is low, and nucleated RBCs demonstrating megaloblastic morphology are often seen in the blood. Neutropenia and thrombocytopenia may be present, particularly in long-standing deficiencies. The neutrophils are large, some with hypersegmented nuclei; more than 5% of neutrophils have five or more nuclear segments. Normal serum folic acid levels are 5–20ng/mL;
deficiency is accompanied by levels less than 3ng/mL. Levels of RBC folate are a better indicator of chronic deficiency. The normal RBC folate level is 150–600ng/mL of packed cells. Levels of iron and vitamin B₁₂ in serum are usually normal or elevated. Serum activity of lactic acid dehydrogenase (LDH) is markedly elevated. The bone marrow is hypercellular because of erythroid hyperplasia. Megaloblastic changes are prominent, although some normal RBC precursors may also be found. Large, abnormal neutrophilic forms (giant metamyelocytes) with cytoplasmic vacuolation are seen, as well as hypersegmentation of the nuclei of megakaryocytes.

TREATMENT.

When the diagnosis is established or in severely ill children, folic acid may be administered orally or parenterally in a dose of 1–5mg/24hr. If the specific diagnosis is in doubt, 50–100μg/24hr of folate may be used for a week as a diagnostic test, or 1μg/24hr of cyanocobalamin parenterally for suspected vitamin B₁₂ deficiency. Because a hematologic response can be expected within 72hr, transfusions are indicated only when the anemia is severe or the child is very ill. Folic acid therapy should be continued for 3–4wk. If juvenile pernicious anemia is present or if the anemia recurs after therapy, the prolonged use of folic acid should be avoided, because in pernicious anemia folic acid may produce a partial response to the anemia without decreasing the neurologic abnormalities.

FOLIC ACID DEFICIENCY IN MALABSORPTION SYNDROMES

Diffuse inflammatory or degenerative disease of the intestine may reduce intestinal pteroylpolyglutamate hydrolase activity as well as markedly impair absorption of folate. Celiac disease, chronic infectious enteritis, and enteroenteric fistulas may lead to folic acid deficiency and megaloblastic anemia. Measurement of serum folate is used to assess small intestinal absorptive functions in malabsorptive disorders. Oral folic acid supplements of 1mg/24hr may be indicated in these states (see Chapter 340).

FOLIC ACID DEFICIENCY ASSOCIATED WITH ANTICONVULSANTS AND OTHER DRUGS

Many patients have low serum levels of folic acid during therapy with certain anticonvulsant drugs (e.g., phenytoin, primidone, phenobarbital), but they usually do not develop anemia. Frank megaloblastic anemia is rare and responds to folic acid therapy, even if administration of the offending drug is continued. Absorption of folic acid is impaired by anticonvulsant drugs, but increased use of folate also occurs. Megaloblastic anemia has occurred in users of oral contraceptives, but the cause is not clear.

A number of drugs have antifolic acid activity as their primary pharmacologic effect and regularly produce megaloblastic anemia. Methotrexate binds to dihydrofolate reductase and prevents formation of tetrahydrofolate, the active form. Pyrimethamine, used in the therapy of toxoplasmosis, and trimethoprim, used for treatment of various infections, may
induce folic acid deficiency and, occasionally, megaloblastic anemia. Therapy with folic acid (5-formyltetrahydrofolate) is usually beneficial.

460.2 Vitamin B\textsubscript{12} (Cobalamin) Deficiency

Vitamin B\textsubscript{12} is derived from cobalamin in food, mainly animal sources, secondary to production by microorganisms. Humans cannot synthesize vitamin B\textsubscript{12}. The cobalamins are released in the acidity of the stomach and combine there with R proteins and intrinsic factor (IF), traverse the duodenum, where pancreatic proteases break down the R proteins, and are absorbed in the distal ileum via specific receptors for IF-cobalamin. In addition, some vitamin B\textsubscript{12} from large doses may diffuse through mucosa in the intestine and mouth. In plasma, vitamin B\textsubscript{12} is bound to transcobalamin (TC) II, the physiologically important transporter, as well as to TCI and TCIII. TCII-cobalamin enters cells by receptor-mediated endocytosis, and cobalamin is converted to active forms (methylcobalamin and adenosylcobalamin) important in the transfer of methyl groups and DNA synthesis.

Vitamin B\textsubscript{12} deficiency may therefore result from inadequate intake, surgery involving the stomach or terminal ileum, lack of secretion of IF by the stomach, consumption or inhibition of the B\textsubscript{12}-IF complex, abnormalities involving the receptor sites in the terminal ileum, or abnormalities of TCII. Although TCI binds 80% of serum cobalamin, a deficiency of this protein results in low serum B\textsubscript{12} levels but not in megaloblastic anemia (see Chapter 44.2).

Because vitamin B\textsubscript{12} is present in many foods, dietary deficiency is rare. It may occur in cases of extreme dietary restriction (strict vegetarians: “vegans”) in which no animal products are consumed. Vitamin B\textsubscript{12} deficiency is not common in kwashiorkor or infantile marasmus. Cases occur in breast-fed infants whose mothers have deficient diets or pernicious anemia.

Select an item below

- JUVENILE PERNICIOUS ANEMIA
- TRANSCOBALAMIN DEFICIENCY
- VITAMIN B12 MALABSORPTION DUE TO INTESTINAL CAUSES
- VITAMIN B12 DEFICIENCY IN OLDER CHILDREN

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Select an item below

- JUVENILE PERNICIOUS ANEMIA
- TRANSCOBALAMIN DEFICIENCY
- VITAMIN B₁₂ MALABSORPTION DUE TO INTESTINAL CAUSES
- VITAMIN B₁₂ DEFICIENCY IN OLDER CHILDREN

VITAMIN B₁₂ MALABSORPTION DUE TO INTESTINAL CAUSES

Cases have been reported of familial occurrence of absence or defect of the receptor for IF-B₁₂ in the terminal ileum, in some instances associated with proteinuria (Imerslund-Grasbeck syndrome). Decreased receptor activity may be detected in the urine of affected patients by a radioisotope binding assay. Histology of the stomach is normal, and IF and acid are present in gastric secretions. This autosomal recessive disorder is due to defects in the CUBN gene on chromosome 10p12.1, resulting in decreased expression of the IF-B₁₂ receptor, cubilin. Parenteral treatment with vitamin B₁₂ monthly corrects the deficiency.

Surgical resection of the terminal ileum, inflammatory diseases such as regional enteritis, neonatal necrotizing enterocolitis, and tuberculosis may also impair absorption of vitamin B₁₂. When the terminal ileum has been removed, lifelong parenteral administration should be used if the Schilling test indicates that vitamin B₁₂ is not absorbed.

An overgrowth of intestinal bacteria within diverticula or duplications of the small intestine may cause vitamin B₁₂ deficiency by consumption of or competition for the vitamin or by splitting of its complex with IF. In these cases, hematologic response may
follow appropriate antibiotic therapy. Similar mechanisms may operate when the fish tapeworm *Diphyllobothrium latum* infests the upper small intestine. When megaloblastic anemia occurs in these situations, the serum vitamin B₁₂ level is low, the gastric juice contains intrinsic factor, and the abnormal Schilling test result is not corrected by addition of exogenous IF.

**VITAMIN B₁₂ DEFICIENCY IN OLDER CHILDREN**

In some cases of vitamin B₁₂ malabsorption in adolescence, atrophy of the gastric mucosa and achlorhydria have been noted. These cases may be related to the syndrome of malabsorption of vitamin B₁₂ occurring in combination with cutaneous candidiasis, hypoparathyroidism, and other endocrine deficiencies. The serum contains antibodies against IF and parietal cells. An abnormal Schilling result is corrected by addition of exogenous IF. Parenteral vitamin B₁₂ should be administered regularly to these patients.

Chapter 463 Definitions and Classification of Hemolytic Anemias

*George B. Segel*

Hemolysis is defined as the premature destruction of red blood cells (RBCs). If the rate of destruction exceeds the capacity of the marrow to produce RBCs, anemia results. Normal RBC survival time is 110–120 days, and approximately 1% of RBCs (the senescent ones) are removed each day and replaced by the marrow to maintain the RBC count. During hemolysis, RBC survival is shortened, and increased marrow activity results in a heightened reticulocyte percentage and number. Hemolysis should be suspected as a cause of anemia if an elevated reticulocyte count is present in the absence of bleeding or administration of hematinic therapy. The marrow can increase its output two- to threefold acutely, with a maximum of six- to eightfold if hemolysis is long standing. The reticulocyte percentage can be corrected to measure the magnitude of the marrow production in response to hemolysis as follows:

\[
\text{Reticulocyte index} = \text{reticulocyte %} \times \frac{\text{observed hematocrit}}{\text{normal hematocrit}} \times \frac{1}{\mu}
\]

where \(\mu\) is a maturation factor related to the severity of the anemia ([Fig. 463–1](#)). In the absence of hemolysis, the reticulocyte index is 1.0, representing normal marrow activity.
FIGURE 463–1 Number of days for maturation of reticulocytes in the marrow and blood. (Modified from Hillman RS, Finch CA: Red Cell Manual. Philadelphia, FA Davis, 1983.) The duration of maturation as blood reticulocytes is taken as \( \mu \).

As anemia becomes more severe, there is more erythropoietin stimulation of erythropoiesis, and reticulocytes are released from the marrow earlier, spending more than 1 day as reticulocytes in the blood. In terms of measuring the marrow response, it is inappropriate to count reticulocytes produced yesterday in today's calculation of the reticulocyte index. The maturation factor, \( \mu \), provides this correction (Fig. 463–1). The usual marrow response in a chronic hemolytic anemia is reflected by a reticulocyte index of 3–4, with a maximum of 6–8 corresponding to maximal marrow output.

The erythroid hyperplasia resulting from chronic hemolytic anemia in children, especially thalassemia, may be so extensive that the medullary spaces expand at the expense of the cortical bone. These changes may be evident on physical examination or on x-rays of the skull and long bones (see Fig. 468–3). A propensity to fracture long bones can occur also.

FIGURE 468–3 A, Facial deformities in an inadequately transfused patient with thalassemia major (Cooley anemia). Severe maxillary hyperplasia and malocclusion are present. B, Roentgenogram of the skull demonstrates the maxillary overgrowth and shows prominent widening of the diploic spaces, with the “hair-on-end” appearance caused by vertical trabeculae. These changes can usually be prevented by an appropriate transfusion regimen.

Direct assessment of the severity of hemolysis requires measurement of the RBC survival time using RBCs tagged with the radioisotope Na\(^{51}\) CrO\(_4\). The normal value for the \(^{51}\)Cr half-life is 25–35 days. This value is less than the expected half-life of 50–60 days because of the elution of \(^{51}\)Cr from the labeled RBCs at the rate of about 1% per day.
Several other plasma, urinary, or fecal chemical alterations reflect the presence of hemolysis. The degradation of hemoglobin results in the biliary excretion of heme pigments and increased fecal urobilinogen (Fig. 463–2). Elevations of serum unconjugated bilirubin also may accompany hemolysis.

Gallstones composed of calcium bilirubinate may be formed in children as young as 4 yr of age. Three heme-binding proteins in the plasma are altered during hemolysis (Fig. 463–2). Hemoglobin binds to haptoglobin and hemopexin, both of which are reduced. Oxidized heme binds to albumin to form methemalbumin, which is increased. When the capacity of these binding molecules is exceeded, free hemoglobin appears in the plasma and can be seen easily if the RBCs are sedimented in a capillary hematocrit tube. If present, free hemoglobin in the plasma is prima facie evidence of intravascular hemolysis. When the tubular reabsorptive capacity of the kidneys for hemoglobin is exceeded, free hemoglobin appears in the urine. Even in the absence of hemoglobinuria, iron loss may result from reabsorbed hemoglobin and the shedding of renal epithelial cells containing hemosiderin. This may lead to secondary iron deficiency during chronic intravascular hemolysis. When hemoglobin is degraded, an α-methene bridge is broken in the cyclic tetrapyrole of the heme moiety, with release of carbon monoxide (CO) (Fig. 463–2). The amount of CO in the blood or expired air provides a dynamic measure of the hemolytic rate. End-tidal CO is being evaluated in several research laboratories but is not used in clinical laboratories to measure hemolysis.

The hematocrit during hemolysis is dependent on the severity of the hemolysis and on the marrow response in producing RBCs. The shortened RBC life span and heightened RBC production result in a marked susceptibility to aplastic or hypoplastic crises, characterized by erythroid marrow failure and reticulocytopenia, accompanied by a rapid reduction in hemoglobin and hematocrit. The most common cause of aplastic crises is parvovirus B19, which is erythrocytotropic in marrow culture in vitro (see Chapters 244 and 468). Aplastic crises may produce a precipitous and life-threatening decline in the hematocrit, which usually lasts 10–14 days. Such transient erythroid marrow failure has little effect on persons with a normal RBC life span but has a proportionately greater effect as the RBC life span is shortened by hemolysis. A second infection with parvovirus is uncommon, but other infections may compromise the erythroid marrow output, resulting in various degrees of hypoplasia or hypoplastic crises.

The hemolytic anemias may be classified as either (1) cellular, resulting from intrinsic abnormalities of the membrane, enzymes, or hemoglobin; or (2) extracellular, resulting
from antibodies, mechanical factors, or plasma factors. Most of the cellular defects are inherited (paroxysmal nocturnal hemoglobinuria is acquired), and most of the extracellular defects are acquired (abetalipoproteinemia with acanthocytosis is inherited). Table 463–1 shows the most common hemolytic anemias, their underlying defects, the diagnostic laboratory tests, and the current recommendations for treatment.

**TABLE 463–1. Hemolytic Anemias and Their Treatment**

Select an item below

- FIGURES
- TABLES

Chapter 464 Hereditary Spherocytosis

George B. Segel

Hereditary spherocytosis is a common cause of hemolysis and hemolytic anemia, with a prevalence of approximately 1/5,000 in people of Northern European extraction. It is the most common familial and congenital abnormality of the red blood cell (RBC) membrane. Affected individuals may be asymptomatic without anemia and with minimal hemolysis or may have a severe hemolytic anemia. Hereditary spherocytosis has been described in most ethnic groups but is most common among persons of Northern European origin.

Select an item below

- Etiology.
- Clinical Manifestations.
- Laboratory Findings.
- Differential Diagnosis.
- Treatment.
- Figures

Etiology.

Hereditary spherocytosis usually is transmitted as an autosomal dominant and, less frequently, as an autosomal recessive disorder. As many as 25% of patients have no previous family history. Of these patients, most represent new mutations, and a few result from recessive inheritance. The most common molecular defects are abnormalities of spectrin or ankyrin, which are major components of the cytoskeleton responsible for RBC shape. A recessive defect has been described in α-spectrin; dominant defects, in β-spectrin and in protein 3; and dominant and recessive defects, in ankyrin. A deficiency in
spectrin, protein 3, or ankyrin results in uncoupling in the “vertical” interactions of the lipid bilayer skeleton and the loss of membrane microvesicles (Fig. 464–1). The loss of membrane without a proportional loss of volume causes sphering of the RBCs and an associated increase in cation permeability, cation transport, adenosine triphosphate utilization, and glycolytic metabolism. The decreased deformability of the spherocytic RBCs impairs cell passage from the splenic cords to the splenic sinuses, and the spherocytic RBCs are destroyed prematurely in the spleen. Splenectomy markedly improves the RBC life span and cures the anemia.

**FIGURE 464–1** Vertical and horizontal interactions of membrane proteins and the pathobiology of the red cell lesion in hereditary spherocytosis (HS) and hereditary elliptocytosis/hereditary pyropoikilocytosis (HE/HPP). Left: A defect of vertical or transverse interactions as exemplified by the red cell membrane lesion in HS. Partial deficiencies of spectrin, ankyrin (band 2.1), or band 3 protein lead to uncoupling of the membrane lipid bilayer from the underlying skeleton (arrow) followed by a formation of spectrin-free microvesicles of approximately 0.2–0.5μm in diameter (arrowheads). These vesicles can be visualized by transmission electron microscopy, but they are not seen during examination of blood films. The subsequent loss of cell surface and a decrease in the surface/volume ratio leads to spherocytosis. Right: Defect of horizontal or parallel interactions of skeletal proteins as exemplified by the membrane lesion in hemolytic forms of HE associated with a defect of spectrin heterodimer self-association. The molecular lesion involving a weakened self-association of spectrin heterodimers to tetramers represents a horizontal defect of the stress-supporting protein interactions. It leads to a disruption of the membrane skeletal lattice and, consequently, whole cell destabilization followed by red cell fragmentation and poikilocytosis. Such fragments are readily seen on stained blood films. (Modified from Palek J, Jarolim P: Clinical expression and laboratory detection of red blood cell membrane protein mutations. Semin Hematol 30:249, 1993.)

**CLINICAL MANIFESTATIONS.**

Hereditary spherocytosis may be a cause of hemolytic disease in the newborn and may present with anemia and hyperbilirubinemia sufficiently severe to require phototherapy or exchange transfusions. The severity in infants and children is variable. Some children remain asymptomatic into adulthood, but others may have severe anemia with pallor,
jaundice, fatigue, and exercise intolerance. Severe cases may be marked by expansion of the diploë of the skull and the medullary region of other bones, but to a lesser extent than in thalassemia major. After infancy, the spleen is usually enlarged, and pigmented (bilirubin) gallstones may form as early as age 4–5 yr. At least 50% of unsplenectomized patients ultimately form gallstones, although for the most part, they remain asymptomatic. Because of the high RBC turnover and heightened erythroid marrow activity, children with hereditary spherocytosis are susceptible to aplastic crisis, primarily as a result of parvovirus, and to hypoplastic crises associated with various other infections. Such erythroid marrow failure may result rapidly in profound anemia (hematocrit <10%), high-output heart failure, hypoxia, cardiovascular collapse, and death. LABORATORY FINDINGS.

Evidence for hemolysis includes reticulocytosis and hyperbilirubinemia. The hemoglobin level usually is 6–10 g/dL, but it can be in the normal range. The reticulocyte count often is heightened to 6–20%, with a mean of approximately 10%. The mean corpuscular volume is normal, whereas the mean corpuscular hemoglobin concentration often is increased (36–38 g/dL RBCs). The RBCs on the blood film vary in size and include polychromatophilic reticulocytes and spherocytes (Fig. 464–2 A). The spherocytes are smaller in diameter and on the blood film are hyperchromic as a result of the high hemoglobin concentration. The central pallor is less conspicuous than in normal cells. Spherocytes may be the predominant cell or may be relatively sparse, depending on severity of the disease, but they usually account for more than 15–20% of the cells when hemolytic anemia is present. Erythroid hyperplasia is evident in the marrow aspirate or biopsy. The marrow expansion may be evident on routine roentgenographic examination. Evidence of hemolysis may include elevated indirect bilirubin, decreased haptoglobin, and the presence of gallstones by ultrasonography.

The diagnosis of hereditary spherocytosis usually is established clinically from the blood film, showing many spherocytes and reticulocytes, from the family history, and from
splenomegaly. The presence of spherocytes in the blood can be confirmed with an osmotic fragility test. The RBCs are incubated in progressive dilutions of an iso-osmotic buffered salt solution. Exposure to hypotonic saline causes RBCs to swell, and the spherocytes lyse more readily than biconcave cells in hypotonic solutions. This feature is accentuated by depriving the cells of glucose for 24hr at 37°C, a so-called incubated osmotic fragility test.

As a research tool, the specific protein abnormality can be established in 80% of these patients by RBC membrane protein analysis using gel electrophoresis and densitometric quantitation. The protein abnormalities are more evident in patients who have had a splenectomy. Studies to define the underlying defects in the cytoskeleton may require assessment of protein synthesis, stability, assembly, and binding to the other membrane proteins.

TREATMENT.

Because the spherocytes in hereditary spherocytosis are destroyed almost exclusively in the spleen, splenectomy eliminates most of the hemolysis associated with this disorder. After splenectomy, osmotic fragility often improves with loss of the abnormal “tail” because of diminished splenic conditioning and less RBC membrane loss, and the anemia, reticulocytosis, and hyperbilirubinemia resolve. Whether all patients with hereditary spherocytosis should undergo splenectomy is controversial. Some hematologists do not recommend splenectomy for those patients whose hemoglobin values exceed 10g/dL and whose reticulocyte counts are less than 10%. Folic acid, 1mg/24hr, should be administered to prevent secondary folic acid deficiency. For patients with more severe anemia and reticulocytosis or those with hypoplastic or aplastic crises, poor growth, or cardiomegaly, splenectomy is recommended after age 5–6yr to avoid the heightened risk of postsplenectomy sepsis in younger children. The introduction of laparoscopic splenectomy decreases the length of hospital stay and may replace open splenectomy. Vaccines for encapsulated organisms such as pneumococcus, meningococcus, and Haemophilus influenzae type b should be administered before splenectomy, and oral prophylactic penicillin V (age <5yr: 125mg/12hr; age ≥5yr through adulthood: 250mg/12hr) administered thereafter. Postsplenectomy thrombocytosis is commonly observed but needs no treatment and usually resolves spontaneously. In one report, partial splenectomy provided substantial increases in hemoglobin and reductions in the reticulocyte count, with potential maintenance of splenic phagocytic and immune function. This technique, if substantiated, would be particularly useful for those children younger than 5yr with severe disease and could be used in older patients with mild disease.

FIGURES
Vertical and horizontal interactions of membrane proteins and the pathobiology of the red cell lesion in hereditary spherocytosis (HS) and hereditary elliptocytosis/hereditary pyropoikilocytosis (HE/HPP). *Left:* A defect of vertical or transverse interactions as exemplified by the red cell membrane lesion in HS. Partial deficiencies of spectrin, ankyrin (band 2.1), or band 3 protein lead to uncoupling of the membrane lipid bilayer from the underlying skeleton (arrow) followed by a formation of spectrin-free microvesicles of approximately 0.2–0.5µm in diameter (arrowheads). These vesicles can be visualized by transmission electron microscopy, but they are not seen during examination of blood films. The subsequent loss of cell surface and a decrease in the surface/volume ratio leads to spherocytosis. *Right:* Defect of horizontal or parallel interactions of skeletal proteins as exemplified by the membrane lesion in hemolytic forms of HE associated with a defect of spectrin heterodimer self-association. The molecular lesion involving a weakened self-association of spectrin heterodimers to tetramers represents a horizontal defect of the stress-supporting protein interactions. It leads to a disruption of the membrane skeletal lattice and, consequently, whole cell destabilization followed by red cell fragmentation and poikilocytosis. Such fragments are readily seen on stained blood films. (Modified from Palek J, Jarolim P: Clinical expression and laboratory detection of red blood cell membrane protein mutations. Semin Hematol 30:249, 1993.)

**FIGURE 464–2** Morphology of abnormal red cells. *A,* Hereditary spherocytosis; *B,* hereditary elliptocytosis; *C,* hereditary pyropoikilocytosis; *D,* hereditary stomatocytosis; *E,* acanthocytosis; *F,* fragmentation hemolysis.
Chapter 468 Hemoglobin Disorders

George R. Honig

The clinical disorders that result from abnormalities of the globin genes comprise a diverse group of hematologic diseases. Normal hemoglobins are tetrameric molecules containing pairs of α or α-like and β or β-like globin-heme subunits. The normal postnatal hemoglobins include hemoglobin (Hb) A (α₂ β₂), Hb F (α₂ γ₂), and Hb A₂ (α₂ δ₂). The embryonic hemoglobins, which usually disappear before birth, include Hb Gower-1 (ζ₂ ε₂), Hb Gower-2 (α₂ ε₂), and Hb Portland (ζ₂ γ₂). The genes for the α and ζ chains are encoded on chromosome 16; those for the β group have been localized to chromosome 11. The nucleotide sequences of all these genes have been determined, and many globin-gene abnormalities have been characterized at the molecular level.

The hemoglobin disorders are subdivided into three major groups. The structural abnormalities, including the hemoglobinopathies, result from changes in the amino acid sequences of the globin chains. Most have a single amino acid substitution; in others, however, amino acids may be deleted or inserted, or other, more complex structural changes may be present. The thalassemias are expressed as quantitative defects, in which the synthesis of one or more of the globin chains is decreased or, in the most severe forms, is totally suppressed. The hereditary persistence of fetal hemoglobin (HPFH) syndromes are characterized by elevated levels of Hb F continuing throughout adult life. Almost all these abnormalities result from the same types of molecular defects: Nucleotides may be substituted, deleted, or inserted into globin-gene DNA.

Select an item below

- HEMOGLOBIN STRUCTURAL ABNORMALITIES (Hemoglobinopathies)
- 468.1 Sickle Cell Hemoglobinopathies
- 468.2 Sickle Cell Trait (Heterozygous Hb S; Hb AS)
- 468.3 Other Hemoglobinopathies
- 468.4 Unstable Hemoglobin Disorders (Congenital Heinz Body Anemia)
- 468.5 Abnormal Hemoglobins with Increased Oxygen Affinity
- 468.6 Abnormal Hemoglobins Causing Cyanosis
- 468.7 Hereditary Methemoglobinemia
- 468.8 Syndromes of Hereditary Persistence of Fetal Hemoglobin
- 468.9 Thalassemia Syndromes
- 468.10 Hemochromatosis
- BIBLIOGRAPHYHemoglobin Disorders
- FIGURES
- TABLES
HEMOGLOBIN STRUCTURAL ABNORMALITIES
(Hemoglobinopathies)

More than 700 structural variants of hemoglobin have been identified. Most are rare, but a few, including some severely pathologic forms, occur with high frequency in certain populations. Many abnormal hemoglobins are readily identified by electrophoresis, but some are electrophoretically “silent” and require other laboratory studies for identification. Numerous hemoglobin variants that have abnormal electrophoretic mobility, including both benign and pathologic forms, exhibit very similar electrophoresis findings and cannot be specifically identified by this means alone.

468.1 Sickle Cell Hemoglobinopathies

Sickle hemoglobin (Hb S) differs from normal adult hemoglobin by a substitution of glutamic acid at the 6th position of its β chains by valine. In the oxygenated state, Hb S functions normally. When this hemoglobin is deoxygenated, an interaction between the β6 valine and complementary regions on the β chains of an adjacent molecule results in formation of highly ordered molecular polymers; these elongate to form filamentous structures, which aggregate into rigid, crystal-like rods. This process of molecular polymerization is responsible for the spiny, brittle character of sickle erythrocytes (RBCs) under conditions of decreased oxygenation. Certain other abnormal hemoglobins, notably Hb C, Hb D Los Angeles, and Hb O Arab, participate in the molecular polymerization of deoxy-Hb S. Hb A does so to a smaller degree, but Hb F is totally excluded from the deoxy-Hb S polymer.

RBCs of heterozygous (sickle cell trait) individuals have been shown to resist invasion by malarial parasites, and this resistance appears to have provided protection against the frequently lethal falciparum form of the disease. The βS gene is found in high frequency in those living in regions in which Plasmodium falciparum malaria has been endemic, including many parts of Africa, the Mediterranean area, and parts of Turkey, the Middle East, and India. In individuals from several geographic areas, the sickle mutation has been shown to exist in genetic linkage with discrete sets of closely associated markers. Some of these Hb S “haplotypes” appear to be predictive of the degree of severity of the sickle cell disease. Those associated with particularly mild disease are accompanied by significantly higher levels of Hb F. Patients who have sickle cell disease and who co-inherit genes for α-thalassemia may also have disease of modified severity.

Hb S is readily identified by electrophoresis. A confirmatory solubility test excludes other abnormal hemoglobins with similar electrophoretic mobility. Although affected newborns express only small quantities of Hb S, because of the predominance of Hb F at birth, the sickle cell syndromes can nevertheless be identified reliably in neonates by various laboratory methods. Neonatal screening programs for detecting infants with sickle cell disease are widely established in the United States. These disorders can also be determined antenatally using amniocyte or chorionic villus DNA by methods that identify the specific βS nucleotide substitution.
SICKLE CELL ANEMIA (Homozygous Hb S)

This disorder is characterized by severe chronic hemolytic disease resulting from premature destruction of the brittle, poorly deformable RBCs. Other manifestations of sickle cell anemia are attributable to ischemic changes resulting from vascular occlusion by masses of sickled cells. The clinical course of affected children is typically associated with intermittent episodic events, often referred to as “crises.”

CLINICAL MANIFESTATIONS.

Affected newborns seldom exhibit clinical features of sickle cell disease; hemolytic anemia gradually develops over the 1st 2–4mo, paralleling the replacement of much of the fetal hemoglobin by Hb S. Other clinical manifestations are uncommon before 5–6mo of age. Acute sickle dactylitis, presenting as the hand-foot syndrome, is frequently the 1st overt evidence that sickle cell disease is present in an infant. Its associated findings include painful, usually symmetric swelling of the hands and feet. The underlying abnormality is ischemic necrosis of the small bones, believed to be caused by a choking off of the blood supply as a result of the rapidly expanding bone marrow. Roentgenograms are not informative in the acute phase but later show evidence of extensive bony destruction and repair (Fig. 468–1).

Acute painful episodes represent the most frequent and prominent manifestation of sickle cell disease. Most patients experience some pain on a nearly daily basis. Episodes of severe pain that require hospitalization and parenteral analgesic administration average about 1/yr in children with Hb SS, but this interval varies considerably. Some patients
never experience severe pain, and others require hospital admission with such frequency that they become seriously disabled. In young children, pain often involves the extremities; in older patients, head, chest, abdominal, and back pain occur more commonly. In an individual patient, pain tends to recur in a limited number of sites. Intercurrent illnesses accompanied by fever, hypoxia, and acidosis, all of which promote the deoxygenation of Hb S, may precipitate sickle pain episodes, but acute pain also develops frequently without an apparent antecedent event. Sickle-related abdominal pain may mimic that of an acute surgical condition.

More extensive vaso-occlusive events in these patients can produce gross ischemic damage. Acute pain episodes may progress to infarction of bone marrow or bone. Splenic infarcts are common in children, causing pain and contributing to the process of “autosplenectomy.” Pulmonary infarction, often occurring in association with pneumonitis or microscopic fat emboli (from bone marrow infarction) may produce the severe clinical picture of acute chest syndrome. Strokes caused by cerebrovascular occlusion are among the most catastrophic acute events and are a frequent cause of hemiplegia. As many as 10% of children with sickle cell anemia, mainly preadolescent and older patients, exhibit sequelae of cerebrovascular occlusion. Findings of increased blood flow velocity by transcranial Doppler studies have been shown to be predictive of increased risk of stroke in these patients, and this may help to identify children who will benefit from preventive therapy. Ischemic damage may also affect the myocardium, liver, and kidneys. Renal function is progressively impaired by diffuse glomerular and tubular fibrosis, and hyposthenuria accompanied by polyuria is a characteristic finding in patients older than 5yr. Renal papillary necrosis and nephrotic syndrome also develop occasionally. Priapism is a relatively frequent complication that results from the pooling of blood in the corpora cavernosa, causing obstruction of the venous outflow.

Young children with Hb SS may have splenic enlargement associated with their hemolytic disease, with progression to the syndrome of hypersplenism accompanied by worsening anemia and sometimes thrombocytopenia. Acute splenic sequestration is a distinct and episodic event that occurs in infants and young children with sickle cell anemia, often following an acute febrile illness. For unknown reasons, large amounts of blood become acutely pooled in the spleen, which becomes massively enlarged, and signs of circulatory collapse rapidly develop. Blood transfusions in the acute phase may be lifesaving.

Altered splenic function in young children with sickle cell disease is a significant factor leading to their increased susceptibility to meningitis, sepsis, and other serious infections, mainly caused by pneumococci and Haemophilus influenzae. In the absence of specific antibody to the polysaccharide capsular antigens of these organisms, splenic activity is essential for removing these bacteria when they invade the blood. Despite frequent enlargement of the spleen in young patients with Hb SS, its phagocytic and reticuloendothelial functions have been shown to be markedly reduced. As an additional risk factor, children with sickle cell disease have also been shown to have deficient levels of serum opsonins, of the alternate complement pathway, against pneumococci. Children
with sickle cell disease also have increased susceptibility to *Salmonella* osteomyelitis (partly because of bone necrosis).

In common with patients having other forms of chronic hemolytic anemia, children with Hb SS are at risk of developing a rapid, potentially life-threatening decrease in their hemoglobin level (aplastic episodes) in association with parvovirus B19 infection (see Chapter 244).

An additional group of sickle cell sequelae is attributable primarily to the hemolytic anemia that accompanies this disorder. Cardiomegaly is invariably present in older children, often caused partly by sickle-related cardiomyopathy. Increased iron absorption contributes to parenchymal damage of the liver, pancreas, and heart. Symptomatic gallstone formation is common in adolescent and older patients, occasionally occurring in children as young as 5yr.

By midchildhood, most patients are underweight, and puberty is frequently delayed. Zinc deficiency, which is prevalent in children with sickle cell disease, may contribute to their poor growth and delayed maturation. Chronic leg ulcers are relatively uncommon in children, seldom occurring before late adolescence.

**LABORATORY FINDINGS.**

Hemoglobin concentrations usually range from 5–9g/dL. The peripheral blood smear typically contains target cells, poikilocytes, and irreversibly sickled cells (Fig. 468–2A). These findings allow Hb SS and most of the other forms of sickle cell disease to be readily distinguished from sickle cell trait and other clinically benign conditions. Reticulocyte counts usually range from 5–15%, and nucleated RBCs and Howell-Jolly bodies may be present. The total white blood cell count is elevated to 12,000–20,000/mm³, with a predominance of neutrophils. The platelet count is usually increased; the sedimentation rate is slow. Other changes include abnormal liver function test results, hyperbilirubinemia, and diffuse hypergammaglobulinemia. The bone marrow is markedly hyperplastic and shows erythroid predominance. Roentgenograms show expanded marrow spaces and osteoporosis.

RBCs stained with supravital stain (brilliant cresyl blue) reveal intracellular inclusions. 

E, Homozygous $\beta^{0}$-thalassemia: severe hypochromia with deformed RBCs and normoblasts.

F, Hemoglobin H disease ($\alpha$-thalassemia): anisopoikilocytosis with target cells.
(Courtesy of Dr. John Bolles, The ASH Collection, University of Washington, Seattle, WA.)

DIAGNOSIS.

The diagnosis is normally established by hemoglobin studies. Electrophoresis at an alkaline pH demonstrates a characteristic mobility, intermediate between those of Hb A and Hb $\bar{A}_2$. To distinguish Hb S from other hemoglobins with similar electrophoretic properties, another (confirmatory) test is required, such as electrophoresis at an acidic pH, a sickle cell preparation in which sickling is observed when the cells are deoxygenated, or, most commonly, a hemoglobin solubility test. In the Hb S solubility test, a measured amount of hemoglobin is added to a concentrated buffer that contains a reducing agent; a turbid precipitate forms when more than about 15% Hb S is present. Beyond infancy, RBCs from patients with Hb SS contain between 2% and 20% Hb F with normal level of Hb $A_2$. Hb A is notably absent. The identification of Hb S in each parent provides supportive evidence for the diagnosis of sickle cell anemia.

DIFFERENTIAL DIAGNOSIS.

The various clinical manifestations of sickle cell disease, including limb pain, heart murmurs, hepatosplenomegaly, and anemia, may suggest a number of other diagnoses, including rheumatic fever or rheumatoid arthritis, osteomyelitis, and leukemia. In patients who have a Hb SS electrophoresis pattern and concomitant microcytosis (mean corpuscular volume [MCV] <78fL), possibilities that require consideration include iron deficiency or a combination of Hb S with $\alpha$- or $\beta^{0}$-thalassemia (Table 468–1).

**TABLE 468–1. Clinically Important Sickle Cell Syndromes**

TREATMENT.

Measures directed toward preventing serious complications of sickle cell disease are among the most important elements of treatment. Maintaining full immunization status is particularly important. Administration of a polyvalent pneumococcal vaccine may be beneficial, but unfortunately, the forms of these vaccines currently available appear to be poorly immunogenic in children who have Hb SS and who are younger than 5yr. *H. influenzae* immunization has been shown to be efficacious in infants with sickle cell disease, and this as well as hepatitis B immunizations are indicated. Prophylactic penicillin is highly effective in preventing serious pneumococcal infections and should be administered to all young children with sickle cell disease. Oral penicillin V (age <5 yr: 125 mg/12 hr; age ≥5 yr: 250 mg/12 hr) is given starting by 4 mo of age. By 5 yr of age,
except in children who have had a severe pneumococcal infection or splenectomy, penicillin prophylaxis usually can be discontinued. Parents of children with Hb SS also need to be aware of the need to bring the child promptly to medical attention for acute illness, especially with a temperature exceeding 38.5°C regardless of the use of prophylaxis. Because of the substantial risk of life-threatening bacterial infections, prompt parenteral antibiotic therapy is indicated for infants and young children with an acute onset of high fever. Febrile patients with temperatures greater than 40°C, those who appear toxic or with findings suggestive of meningitis or other serious infection, and those who have previously had pneumococcal sepsis represent acute medical emergencies. Blood culture, intravenous ceftriaxone, and hospital admission for further antibiotic treatment are indicated. Other febrile patients older than 6mo generally can be treated effectively on an outpatient basis. In low-risk, well-appearing children, after blood cultures are obtained, intravenous ceftriaxone is given, and the dose is repeated the next day.

Parents and caretakers of these children should also be informed about the manifestations of acute splenic sequestration and the need for immediate medical attention for a child with rapid splenic enlargement and pallor.

Painful episodes can frequently be managed with oral acetaminophen, alone or with codeine. More severe episodes may require hospitalization and parenteral administration of narcotics. Anti-inflammatory agents, especially ketorolac, may decrease or eliminate the need for narcotic analgesics. Dehydration or acidosis should be rapidly corrected by the intravenous route, but overhydration should be avoided. Blood transfusions are seldom indicated for painful episodes, and it is doubtful whether transfusion can ameliorate the course of a pain crisis. For patients with disabling chronic pain, for those with ischemic organ damage (acute chest, priapism) or stroke, or in preparation for major surgery, however, transfusions of normal RBCs can provide symptomatic relief and prevent further ischemic complications. A first stroke may be prevented by transfusion of children with sickle cell disease and abnormal transcranial Doppler ultrasonography. For children with stroke, cardiomyopathy, and other severe complications, chronic long-term transfusion regimens are a mainstay of therapy. These patients also may require iron chelation treatment to prevent the development of hemosiderosis. Packed RBC transfusions are specifically indicated for acute splenic sequestration and aplastic episodes. Repeated episodes of splenic sequestration are an indication for splenectomy.

Bone marrow transplantation from a normal donor can be curative in patients with sickle cell disease, but the risks and morbidity associated with this procedure limit its application to highly selected patients. European experience, mainly from young children without chronic organ damage, has shown a high success rate following transplantation. In the United States, allogenic bone marrow transplantation has been used primarily in patients with severe complications of sickle cell disease. A majority of these children achieved successful engraftment with stabilization of their disease sequelae.

Chemotherapy regimens that stimulate Hb F synthesis have been used with beneficial effect, on an experimental basis, in a number of children with sickle cell disease. These
agents, which include hydroxyurea and butyrate, offer considerable promise of more effective means for treating these patients.

OTHER SICKLE CELL SYNDROMES

Sickling disorders of various degrees of severity result from Hb S existing in combination with other abnormal hemoglobins or thalassemias (see Table 468–1). Several of these syndromes, including Hb SD Los Angeles, Hb SO Arab, and Hb S–β⁰-thalassemia, present a clinical picture virtually indistinguishable from that of sickle cell anemia. Most of the others produce less severe manifestations.

**TABLE 468–1. Clinically Important Sickle Cell Syndromes**

*Hb SC disease* results from the concurrence of genes for Hb S and Hb C. Painful episodes and other vaso-occlusive manifestations are usually less severe in this condition than those associated with Hb SS. Most affected children have persistent splenomegaly, and bone infarcts occur more frequently than in those with Hb SS. Septicemia may also occur. Retinal vascular changes, predominantly in adolescents and adults, may lead to hemorrhage with retinal detachment. The hemoglobin concentration averages 9–10 g/dL, with the blood smear showing target cells and characteristic spindle-shaped RBCs.

468.2 Sickle Cell Trait
(Heterozygous Hb S; Hb AS)

Heterozygous expression of the gene for Hb S is usually associated with a totally benign clinical course. About 8% of African-Americans have sickle cell trait; 35–45% of their hemoglobin is Hb S. This low level of Hb S is insufficient to produce sickling manifestations under usual circumstances, but under conditions of severe hypoxia, vaso-occlusive complications may occur. Splenic infarcts and other ischemic sequelae may occur in individuals with Hb AS as a result of hypoxia associated with general anesthesia. Hyposthenuria is usually present in older children and adults. Gross hematuria occasionally develops in otherwise well individuals. The hematologic findings in sickle cell trait are indistinguishable from normal ([Fig. 468–2B](#)). The diagnosis is established by hemoglobin electrophoresis, with confirmatory sickle testing.

and occasional spherocytes. D, Congenital Heinz body anemia (unstable hemoglobin): RBCs stained with supravital stain (brilliant cresyl blue) reveal intracellular inclusions. E, Homozygous \( \beta^0 \)-thalassemia: severe hypochromia with deformed RBCs and normoblasts. F, Hemoglobin H disease (\( \alpha \)-thalassemia): anisopoikilocytosis with target cells. (Courtesy of Dr. John Bolles, The ASH Collection, University of Washington, Seattle, WA.)

468.9 Thalassemia Syndromes

The thalassemias are a heterogeneous group of heritable hypochromic anemias of various degrees of severity. Underlying genetic defects include total or partial deletions of globin chain genes and nucleotide substitutions, deletions, or insertions. The consequences of these various changes are a decrease or absence of mRNA for one or more of the globin chains or the formation of functionally defective mRNA. The result is a decrease or total suppression of hemoglobin polypeptide chain synthesis. More than 200 distinct mutations are known to produce thalassemia phenotypes; many of these mutations are unique to localized geographic regions. In general, the globin chains synthesized in thalassemic RBCs are structurally normal. In severe forms of \( \alpha \)-thalassemia, abnormal homotetramer hemoglobins (\( \beta_4 \) or \( \gamma_4 \)) are formed, but their component globin polypeptides have normal structure. Conversely, a number of structurally abnormal hemoglobins also produce thalassemia-like hematologic changes. In characterizing the expression of the various thalassemia genes, superscript designations are used to distinguish those that produce a demonstrable globin chain product, although at decreased levels (e.g., \( \beta^+ \)-thalassemia) from those in which the synthesis of the affected globin chain is totally suppressed (e.g., \( \beta^0 \)-thalassemia).

Thalassemia genes are remarkably widespread, and these abnormalities are believed to be the most prevalent of all human genetic diseases. Their main distribution includes areas bordering the Mediterranean Sea, much of Africa, the Middle East, the Indian subcontinent, and Southeast Asia. From 3–8% of Americans of Italian or Greek ancestry and 0.5% of black Americans carry a gene for \( \beta \)-thalassemia. In some regions of Southeast Asia, as many as 40% of the population have one or more thalassemia genes. The geographic areas in which thalassemia is prevalent closely parallel the regions in which \( P. falciparum \) malaria was formerly endemic. Resistance to lethal malarial infections by carriers of thalassemia genes apparently represented a strong selective force that favored their survival in these areas of endemic disease.

Select an item below

- HOMOZYGOUS \( \beta^0 \)-THALASSEMIA (Cooley Anemia; Thalassemia Major)
- OTHER \( \beta \)-THALASSEMIA SYNDROMES
- \( \alpha \)-THALASSEMIA
HOMOZYGOUS β⁰-THALASSEMIA
(Cooley Anemia; Thalassemia Major)

CLINICAL MANIFESTATIONS.

Homozygous β⁰-thalassemia usually becomes symptomatic as a severe, progressive hemolytic anemia during the 2nd 6mo of life. Regular blood transfusions are necessary in these patients to prevent the profound weakness and cardiac decompensation caused by the anemia. Without transfusion, life expectancy is no more than a few years. In untreated cases or in those receiving infrequent transfusions at times of severe anemia, hypertrophy of erythropoietic tissue occurs in medullary and extramedullary locations. The bones become thin, and pathologic fractures may occur. Massive expansion of the marrow of the face and skull (Fig. 468–3) produces characteristic facies. Pallor, hemosiderosis, and jaundice combine to produce a greenish-brown complexion. The spleen and liver are enlarged by extramedullary hematopoiesis and hemosiderosis. In older patients, the spleen may become so enlarged that it causes mechanical discomfort and secondary hypersplenism. Growth is impaired in older children; puberty is delayed or absent because of secondary endocrine abnormalities. Diabetes mellitus resulting from pancreatic siderosis may also occur. Cardiac complications, including intractable arrhythmias and chronic congestive failure caused by myocardial siderosis, have been common terminal events. With modern regimens of comprehensive care for these patients, many of these complications can be prevented and others ameliorated and delayed in their onset.

FIGURE 468–3  A, Facial deformities in an inadequately transfused patient with thalassemia major (Cooley anemia). Severe maxillary hyperplasia and malocclusion are present. B, Roentgenogram of the skull demonstrates the maxillary overgrowth and shows prominent widening of the diploic spaces, with the “hair-on-end” appearance caused by vertical trabeculae. These changes can usually be prevented by an appropriate transfusion regimen.

LABORATORY FINDINGS.
The RBC morphologic abnormalities in untransfused patients with homozygous \( \beta^0 - \) thalassemia are extreme. In addition to severe hypochromia and microcytosis (see Fig. 468–2E), many bizarre, fragmented poikilocytes and target cells are present. Large numbers of nucleated RBCs circulate, especially after splenectomy. Intraerythrocytic inclusions, which represent precipitated excess \( \alpha \) chains, are also seen after splenectomy. The hemoglobin level falls progressively to lower than 5g/dL unless transfusions are given. The unconjugated serum bilirubin level is elevated. The serum iron level is high, with saturation of the transferrin. A striking biochemical feature is the presence of very high levels of Hb F in the RBCs (Table 468–2). Dipyrrolic compounds render the urine dark brown, especially after splenectomy.

**TABLE 468–2. Clinical and Hematologic Features of the Principal Forms of Thalassemias**


**TREATMENT.**

Transfusions are given on a regular basis to maintain the hemoglobin level above 10g/dL. This “hypertransfusion” regimen has striking clinical benefits; it permits normal activity with comfort, prevents progressive marrow expansion and cosmetic problems associated with facial bone changes, and minimizes cardiac dilatation and osteoporosis. Transfusions of 15–20mL/kg of packed cells are usually necessary every 4–5wk.

*Hemosiderosis* is an inevitable consequence of prolonged transfusion therapy, because each 500mL of blood delivers to the tissues about 200mg of iron that cannot be excreted by physiologic means. Myocardial siderosis has been a significant contributing factor in the early death of these patients. Hemosiderosis can be decreased or even prevented with
Parenteral administration of the iron-chelating drug deferoxamine, which forms an iron complex that can be excreted in the urine. A sustained high blood level of deferoxamine is needed for adequate iron excretion. The drug is usually administered subcutaneously over an 8- to 12-hr period using a small portable pump (during sleep), 5 or 6 nights/wk. Patients who adhere to this regimen are able to control the accumulation of excessive body iron. Lethal complications of hepatic and myocardial siderosis can thus be prevented or significantly delayed. An orally active iron chelating agent, deferiprone, has been studied in a number of clinical trials and has been shown to promote iron excretion from patients with transfusion-related siderosis. A significant percentage of patients treated with deferiprone, however, have been shown to have progressive increases in their hepatic iron stores. This agent alone does not therefore appear to be sufficient for preventing long-term iron toxicity in these patients.

Hypertransfusion in these patients effectively prevents massive splenomegaly resulting from extramedullary erythropoiesis. Splenectomy eventually becomes necessary, however, because of the size of the organ or because of secondary hypersplenism. Splenectomy increases the risk of severe, overwhelming sepsis; therefore, the operation should be performed only for significant indications (see Chapter 493) and should be deferred as long as possible. The most frequent indication for splenectomy is an increased need for transfusion. A transfusion requirement exceeding 240mL/kg of packed RBCs/yr is usually evidence of hypersplenism and is an indication for considering splenectomy. Immunization of these patients with hepatitis B, H. influenzae type b, pneumococcal, and meningococcal vaccines is desirable, and prophylactic penicillin therapy is also recommended.

Bone marrow transplantation is curative in these patients and has been performed with increasing success, even in patients who have been transfused extensively. This procedure, however, carries considerable risks of morbidity and mortality and generally can be used only for patients who have nonaffected histocompatible siblings.

α-THALASSEMIA

Microcytic anemias resulting from deficient synthesis of α-globin chains are prevalent in Africa, Mediterranean area countries, and much of Asia. Deletions of α-globin genes account for most of these abnormalities. Four α-globin genes are present in normal individuals, and four distinct forms of α-thalassemia have been identified as corresponding to deletions of one, two, three, or all four of these genes (see Table 468–2).

**TABLE 468–2. Clinical and Hematologic Features of the Principal Forms of Thalassemias**

*Deletion of a single α-globin gene* produces the silent carrier α-thalassemia phenotype. No hematologic abnormality is usually evident, except for mild microcytosis. Approximately 25% of black Americans have this form of α-thalassemia.
Individuals *lacking two α-globin genes* exhibit the feature of α-thalassemia trait, with mild microcytic anemia. In affected newborns, small quantities of Hb Bart's (γ₄) can be identified by hemoglobin electrophoresis. Beyond about 1 mo of age, Hb Bart's is no longer detectable, and the levels of Hb A₂ and F are characteristically normal. Inclusions of precipitated hemoglobin may be visualized in RBC smears, however, after supravital staining.

*Deletion of three of the four α-globin genes* is associated with a thalassemia intermedia–like syndrome, Hb H disease. Microcytic anemia in this condition is accompanied by abnormal RBC morphology (see Fig. 468–2F), with prominent intracellular inclusions present in the RBCs after supravital staining. Hb H (β₄) is highly unstable; it can be readily identified by electrophoresis, but unless special measures are taken to prevent its precipitation during sample preparation, it may escape detection.

![FIGURE 468–2](image)


The most severe form of α-thalassemia, resulting from *deletion of all of the α-globin genes*, is accompanied by a total absence of α-chain synthesis. Because Hb F, A, and A₂ all contain α chains, none of these hemoglobins is produced. Hb Bart's (γ₄) accounts for most of the hemoglobin in affected infants, and because γ₄ has a high oxygen affinity and therefore cannot transport oxygen to the tissues, these infants are severely hypoxic. Their RBCs also contain small quantities of the normal embryonic Hb Portland (ζ₂ γ₂), which functions as an effective oxygen transporter. Most of these infants are stillborn, and most who are born alive die within a few hours. These infants are severely hydropic, with congestive heart failure and massive generalized edema. Those that survive with aggressive neonatal management are also transfusion dependent.
The types of α-thalassemia genes vary among affected populations, and these differences account for the α-thalassemia syndromes that predominate in specific population groups. In black Americans, α-thalassemia genes are prevalent, with almost all affected individuals having the deletion arrangement (−, α) that produces a single α-locus chromosome. In this population, therefore, α-thalassemia occurs mainly as the silent carrier phenotype (−, α/α, α) or as α-thalassemia trait (−, α/−, α). Chromosomes with deletions of both of the α loci (−, −) are prevalent in both Mediterranean and Asian populations, and Hb H disease (−, α/−, −) therefore occurs with significant frequency in both groups. The two α-locus deletion defects in Asians are often accompanied by retention of the ζ-globin genes (i.e., ζ−, −), whereas those from Mediterranean countries usually are not (−, −). The latter type of defect, therefore, cannot support the synthesis of Hb Portland (ζ2 γ2), which appears to be essential for intrauterine survival of fetuses with the hydrops fetalis form of α-thalassemia. Accordingly, the hydrops fetalis form almost exclusively affects infants of Asian ancestry. An acquired α-thalassemia syndrome, which may be associated with a large deletion involving the α-globin genes, includes Hb H disease accompanied by mental retardation, microcephaly, and hypogonadism.

A number of abnormal hemoglobins also produce α-thalassemia–like changes. The α-chain variant Hb Constant Spring occurs commonly in Far Eastern populations and is frequently observed in patients with Hb H disease who have the genotype (αA, αCo Sp/−, −). The gene for Hb G Philadelphia, which is the most prevalent α-chain abnormality of black Americans, usually occurs on a single-locus chromosome (−, αG). Individuals who express this abnormal hemoglobin therefore may also exhibit α-thalassemia–like hematologic changes.

OTHER β-THALASSEMIA SYNDROMES

The homozygous expression of milder (β+) thalassemia genes produces a Cooley's anemia-like syndrome of lesser severity (“thalassemia intermedia”; see Table 468–2). Skeletal deformities and hepatosplenomegaly develop in these patients, but their hemoglobin levels are usually maintained at 6–8 g/dL without transfusion. Nevertheless, they may develop severe hemosiderosis, attributable to their greatly increased gastrointestinal iron absorption. For such patients, who do not receive deferoxamine chelation therapy, a low-iron diet is indicated.

**TABLE 468–2. Clinical and Hematologic Features of the Principal Forms of Thalassemias**

Several structurally abnormal hemoglobins produce β-thalassemia–like hematologic changes and, when present in combination with a gene for β-thalassemia, also result in a thalassemia intermedia syndrome. Among these are the Hb Lepore variants, which are composed of α chains in combination with hybrid δβ fusion globin chains. The Lepore hemoglobins are identified by electrophoresis, in which they exhibit Hb S–like mobility.
Most forms of *heterozygous* β-thalassemia are associated with mild anemia. The hemoglobin concentration typically averages 2–3g/dL lower than age-related normal values. The RBCs are hypochromic and microcytic, with poikilocytosis, ovalocytosis, and often basophilic stippling. Target cells may be present but usually are not prominent and are not specific for thalassemia. The MCV is low, averaging 65fL, and the mean corpuscular hemoglobin (MCH) values are also low (<26pg). A mild decrease in RBC survival can be shown, but overt signs of hemolysis are usually absent. The serum iron level is normal or elevated.

Individuals with thalassemia trait are often misdiagnosed as having iron deficiency anemia and may be inappropriately treated with iron for extended periods. More than 90% of persons with β-thalassemia trait have diagnostic elevations of Hb A₂ of 3.4–7%. About 50% of these individuals also have slight elevations of Hb F, about 2–6%. In a small number of otherwise typical cases, normal levels of Hb A₂ with Hb F levels ranging from 5–15% are found, representing the δβ type of thalassemia (see Table 468–2). The silent carrier form of β-thalassemia produces no demonstrable abnormality in heterozygous individuals (see Table 468–2), but the gene for this condition, when inherited together with a gene for β⁰-thalassemia, results in a thalassemia intermedia syndrome.

A rare type of deletion defect, which involves the γ-, δ-, and β-globin genes, produces a clinical picture similar to that of δβ-thalassemia trait in heterozygous individuals. In the newborn period, however, this defect is accompanied by significant hemolytic disease with microcytosis, normoblastemia, and splenomegaly (see Table 468–2). The hemolytic process is self-limited, but supportive transfusions