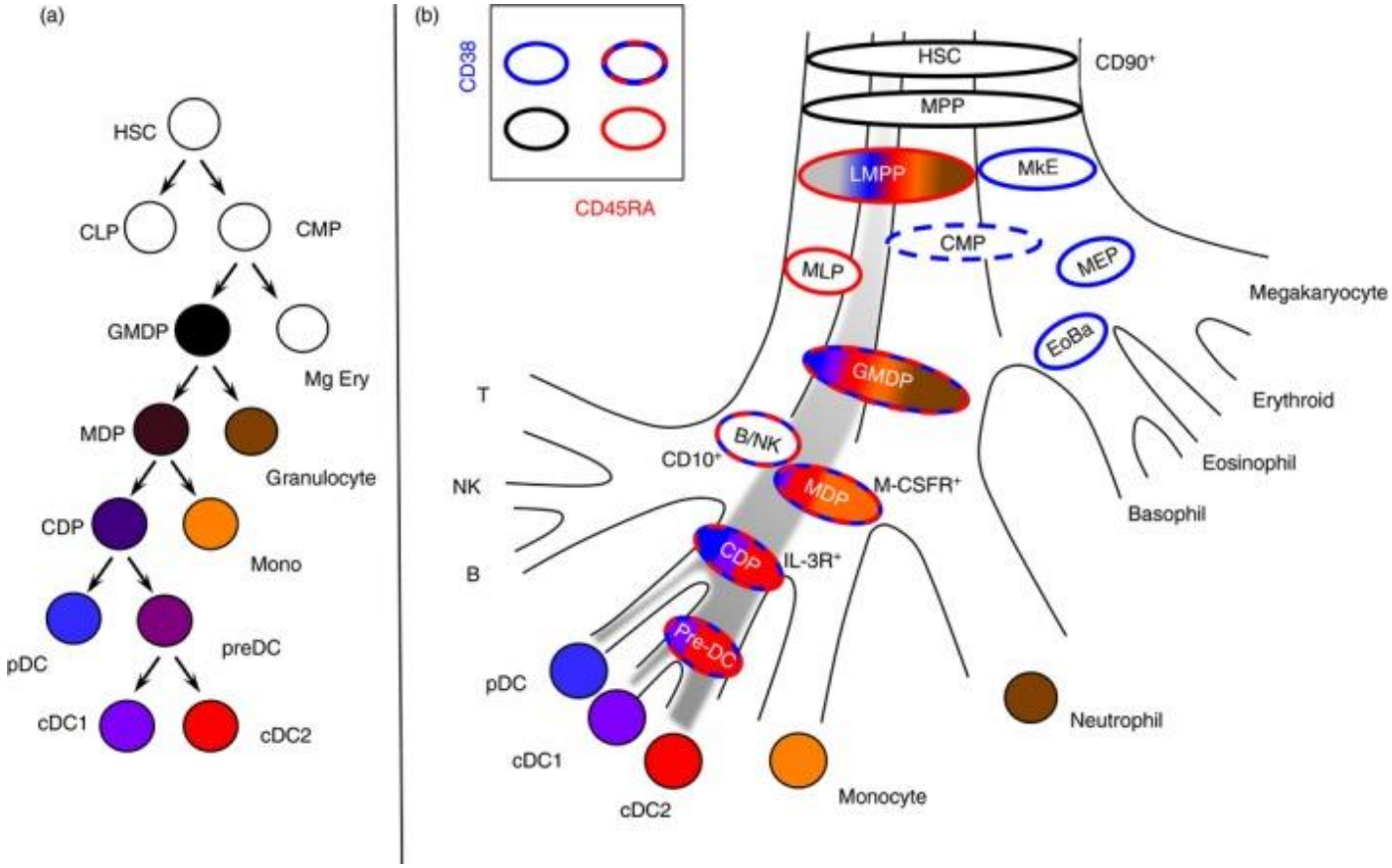
HEMATOLOGY
ERYTHROID SERIES DISORDERS

Kenneth Alonso, MD, FACP

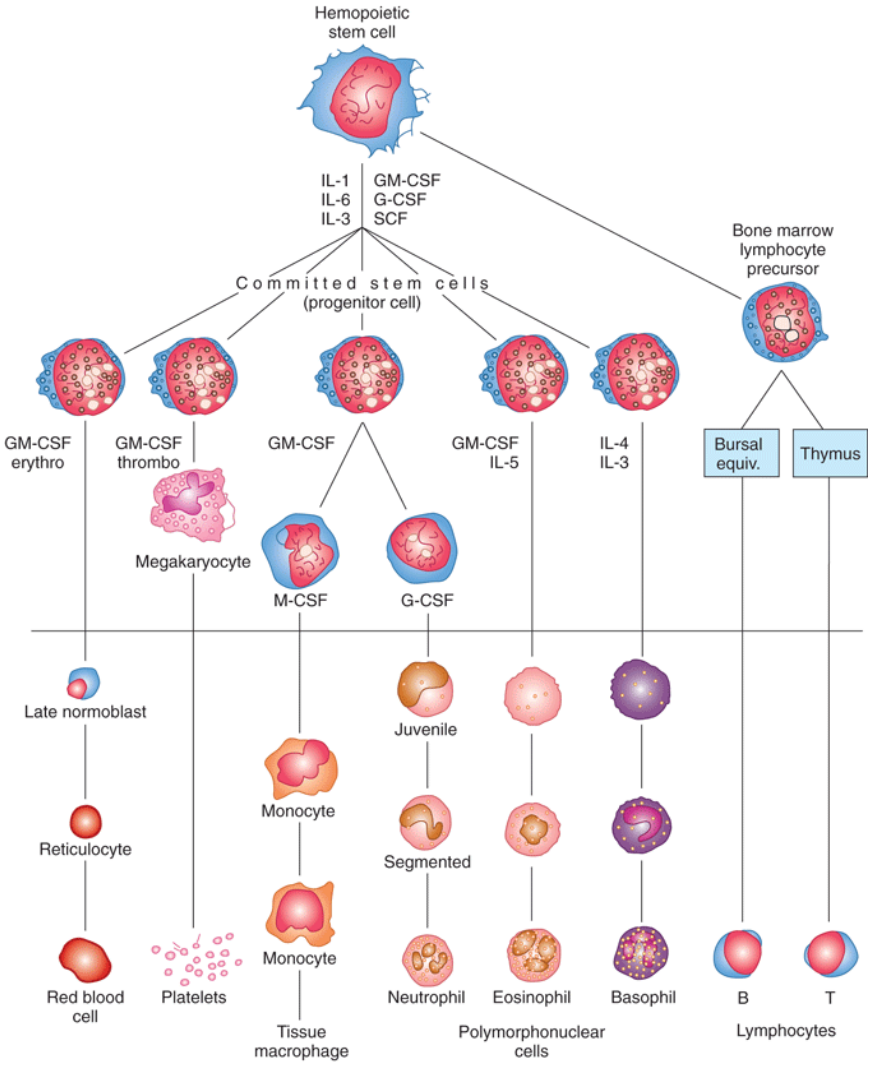
Classic and revised models of hematopoiesis



Common myeloid progenitors are mixtures of mega-erythroid and myeloid precursors and the most significant early partitioning of cell fate occurs when megakaryocyte and erythroid potentials separate from lympho-myeloid potentials.

Doi: 10.1111/imm.12888

Maturation of blood cells



Source: Barrett KE, Barman SM, Boitano S, Brooks H: *Ganong's Review of Medical Physiology, 23rd Edition*: http://www.accessmedicine.com

Fig. 32-3
Accessed 03/01/2010

Sites of red cell formation

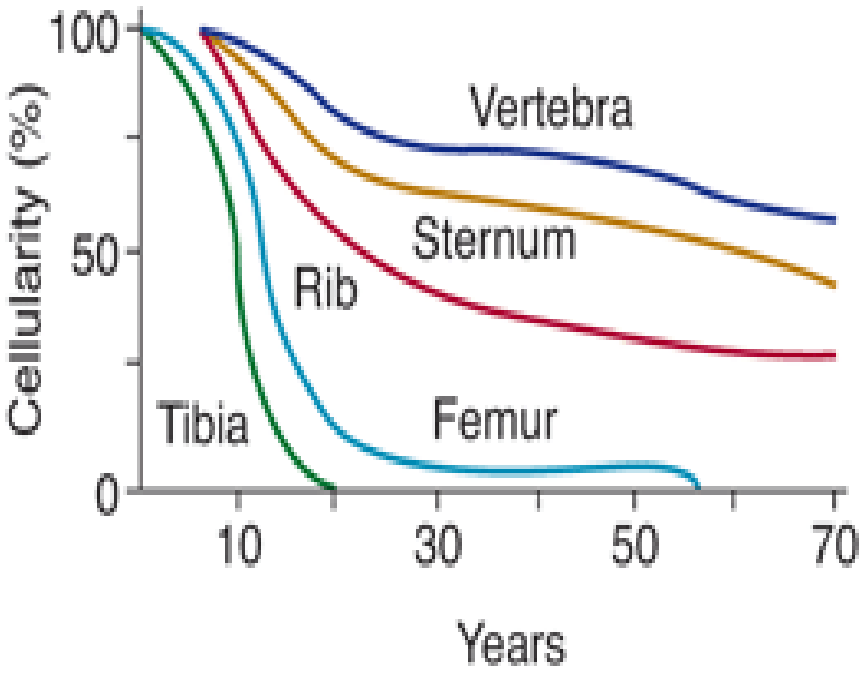


Fig. 32-2 Accessed 03/01/2010

Erythroid series

- Pro-erythroblasts are the earliest, least mature cells in the erythrocyte forming series (erythropoiesis).
- Pro-erythroblasts are characterized by their size (about 20 μm), and by having a very dense nuclear structure with a narrow layer of cytoplasm, homogeneous in appearance, with a lighter zone at the center; they stain deep blue after Romanowsky staining.
- The results of mitosis of erythroblasts are called normoblasts.

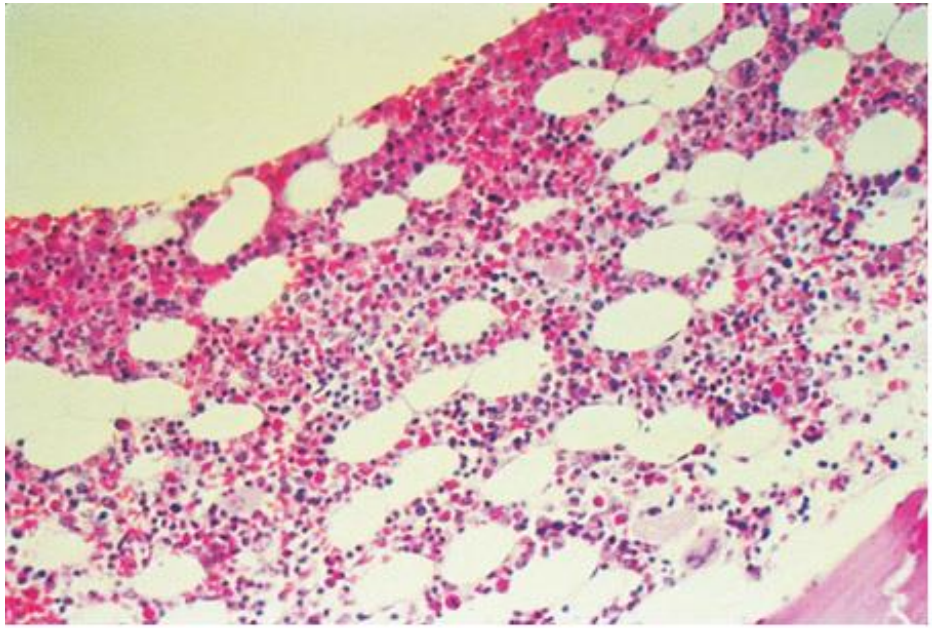
Erythroid series

- There are two cell types with relatively dense round nuclei and grayish stained cytoplasm.
- The polychromatic erythroblasts are immature cells in which the cytoplasm displays a grayish blue hue. They are still able to divide.
- The orthochromatic erythroblasts are cells in which the cytoplasm is already taking on a pink hue contain hemoglobin and are no longer able to divide. The nuclei of these gradually condense into small black spheres without structural definition and are expelled from the cells.

Erythroid series

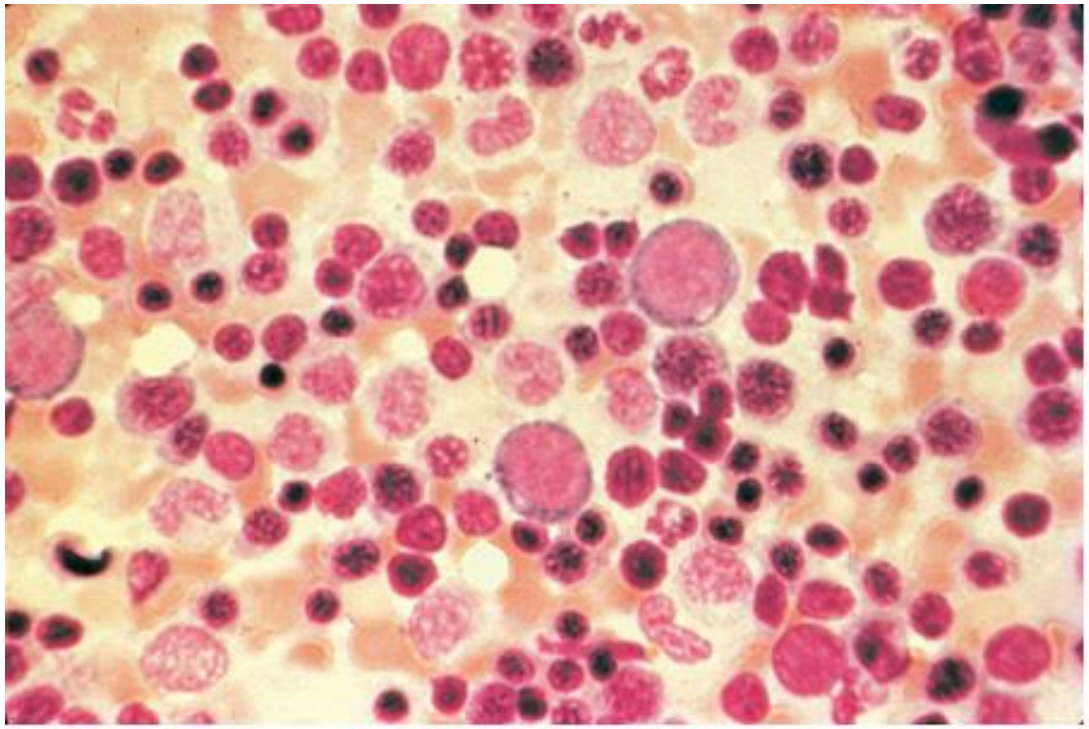
- The now enucleated young erythrocytes (reticulocytes) contain copious ribosomes that precipitate into reticular (net-like) structures identified with supravital stains.
- The maturation of cells in the erythrocyte series is closely linked to the activity of macrophages which phagocytise nuclei expelled from normoblasts and iron from senescent erythrocytes, and pass these cell components on to developing erythrocytes.

Normal bone marrow



Low-power view of normal adult marrow (H&E stain), showing a mix of fat cells (clear areas) and hematopoietic cells. The percentage of the space that is hematopoietic cells is referred to as marrow cellularity. In adults, normal marrow cellularity is 35–40%. If demands for increased marrow production occur, cellularity may increase to meet the demand. As we age, the marrow cellularity decreases and the marrow fat increases. Patients >70 years may have a 20–30% marrow cellularity.

Erythroid maturation



Marrow aspirate specimen with a myeloid/erythroid ratio (M/E ratio) of 1:1–2, typical for a patient with a hemolytic anemia or recovering from blood loss. The cells with large nuclei and a small rim of blue cytoplasm are pro-erythroblasts. Those with dark, small nuclei and red cytoplasm are orthochromatic normoblasts.

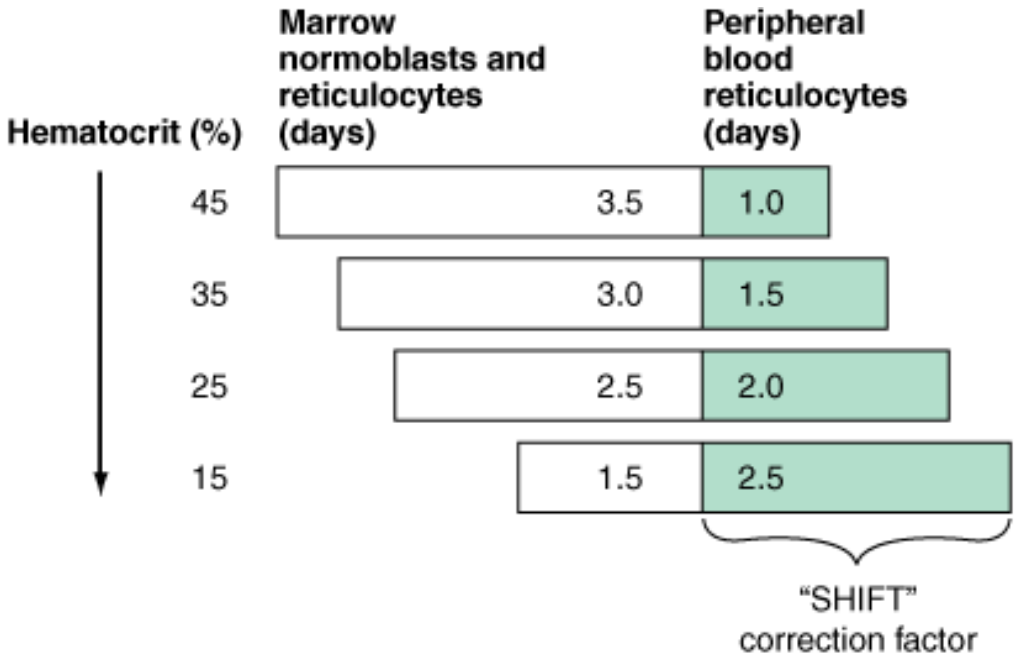
Erythropoiesis

- RBC progenitors express receptors for inflammatory cytokines which are necessary for normal differentiation to basophilic normoblast (Fas/TRAILR/TNFR, IFN-γ).
- Fas/FasL/TRAIL/TNF-α are expressed during maturation from polychromatic normoblast.
- In the absence of erythopoietin, these cytokines promote erythroid cell death.
- (HIF-1α, the transcription factor responsible for EPO gene transcription, is stimulated by iron deficiency and hypoxemia.)

Erythropoiesis

- From the pluripotent stem cell to the mature red cell, approximately 21 days have transpired.
- From pro-erythroblasts to orthochromatic normoblasts, approximately 10-13 days have transpired. Erythropoietin stimulates pro-erythroblasts. The colony forming unit is erythropoietin dependent.
- Iron dependence begins with the orthochromatic normoblasts and terminates with the mature red cell. Its duration is 3-4 days.
- Reticulocytes circulate 1-2 days.

Reticulocytes



Source: Fauci AS, Kasper DL, Braunwald E, Hauser SL, Longo DL, Jameson JL, Loscalzo J: *Harrison's Principles of Internal Medicine*, 17th Edition: http://www.accessmedicine.com

Iron metabolism

- Most important trace element in the human body (4-5mg).
- 75% in heme proteins; 1% in Iron-Sulfur clusters (respiratory chain); the remainder as transferrin and ferritin.
- Intestine only absorbs ferrous form (maximum, 70mg/day). The reducing agent, ascorbate, promotes absorption. The majority of Iron in the body originates in red cell breakdown (150-200mg/day).
- Equal amounts of Iron are found in muscle and bone marrow (300mg). The liver holds 1000mg in storage; the reticuloendothelial system, 600mg.

Iron metabolism

- Hepcidin is a peptide that inhibits ferroprotein found in enterocytes (iron absorption) and macrophages (iron release). It regulates iron absorption and release.
- Iron is transported in the blood as transferrin (in other fluids as lactoferrins); stored, as ferritin (the excess, as hemosiderin). Reticulocyte hemoglobin reflects best iron stores and transport.
- Transport proteins maintain free Iron at very low levels, inhibiting bacterial growth.
- Iron is incorporated into heme by a ferrochelatase as the final step in biosynthesis.

Vitamin B_{12} absorption

- Bound B_{12} released from proteins during digestion and free B_{12} bind to the haptocorrin protein, transcobalamin I, released by salivary glands and gastric mucosa.
- In intestine, digestion of transalbumin I releases B_{12} which then binds to “intrinsic factor” (a glycoprotein secreted by parietal cells of the stomach).
- Intrinsic factor binds to receptors in the ileum and is internalized. (Pernicious Anemia is caused by the lack of intrinsic factor.)
- B_{12} then binds to transcobalamin II and is released to circulation. Transcobalamin II receptors on cells take up the B_{12}.

Vitamin B12 absorption

- Liver takes up about 50% of B_{12} and can store 3-6 years worth of B_{12}.
- In cobalamin deficiency, the methylation of homocysteine to methionine is impaired.
- Homocysteine accumulates.
- Methylation requires the methyl group of the folate intermediate, 5-methyl tetrahydrofolate.
- 5-MTHF enters the cell from the plasma and accumulates. It is the first step in the pathway that generates all other intracellular folate coenzymes.
- THF, the substrate on which all other intracellular folate intermediates depend, falls, impairing DNA synthesis, leading to megaloblastic change.

Vitamin B_{12} absorption

- Accumulation of methyl-malonyl-CoA blocks myelin formation by competitive inhibition of malonyl-CoA carboxylase (key enzyme in folic acid synthesis)
- Accumulation of methyl-malonyl-CoA disrupts membrane structure through incorporation of methyl-malonyl-CoA producing branched fatty acids.
- Leads to “megaloblastic madness”.

Heme

- Protoporphyrin IX rings are arranged about a chelated ferrous ion (bound to the four Nitrogens) to form heme. The step of ferrochelation is also blocked in lead poisoning.
- Heme is held in a hydrophobic pocket in the aporpotein. Iron is also bound to the histidine of the apoprotein and to Oxygen.
- Heme also stimulates globin synthesis.
- Heme is scavenged from red cells and catabolized (opening of the ring) to biliverdin and to bilirubin. Bilirubin is then conjugated in the liver and excreted.

Heme synthesis

- Glycine and succinyl-CoA condense in the presence of ALA synthase and produce δ-aminolevolinic acid (δ-ALA). This step is regulated by heme. This step is blocked in Lead poisoning. This occurs in the mitochondrion.
- Two δ-ALA molecules condense to form one pyrrole ring in the cytoplasm, pophobilinogen. Four pyrrole rings condense to form the porphorinogen series: uroporphoringoen, coproporphyrinogen (again, in the mitochondrion), and, finally, protopoprhyrin IX.
- The porphyrias reflect abnormal heme synthesis.

Porphyrias

Enzyme involved	Type	Presentation
ALA synthase	X-linked sideroblastic anemia	Anemia
ALA dehydratase	Deficiency	Abdominal pain, neuropsychiatric problems
Uroporphinogen I synthase	Acute intermittent porphyria	Abdominal pain, neuropsychiatric problems
Uroporphinogen III synthase	Congenital erythropoietic porphyria	No photosensitivity
Uroporphinogen decarboxylase	Porphyria cutanea tarda	With photosensitivity
Coproporphyrinogen oxidase	Hereditary coproporphyria	Photosensitivity, abdominal pain, neuropsychiatric problems
Protoporphyrinogen oxidase	Variegate porphyria	Photosensitivity, abdominal pain, neuropsychiatric problems
Ferrochetalase	Protoporphyria	Photosensitivity

Erythrocyte metabolism

- Maintenance metabolism involves anerobic glycolysis and the pentose phosphate pathway.
- ATP formed through glycolysis serves to supply Sodium/Potassium ATPase and maintain the membrane potential of the cell.
- NADPH is supplied through the pentose phosphate pathway and is used to regenerate glutathione with the assistance of glutathione reductase.

Erythrocyte metabolism

- Glutathione serves as a coenzyme for the Selenium containing glutathione reductase that detoxifies peroxides.
- The reduction of Iron in methemoglobin (ferric) to hemoglobin (ferrous) is accomplished by glutathione or ascorbate by non- enzymatic pathways.
- This reduction occurs in the mitochondrion and involves cytochrome b5 and NADH.

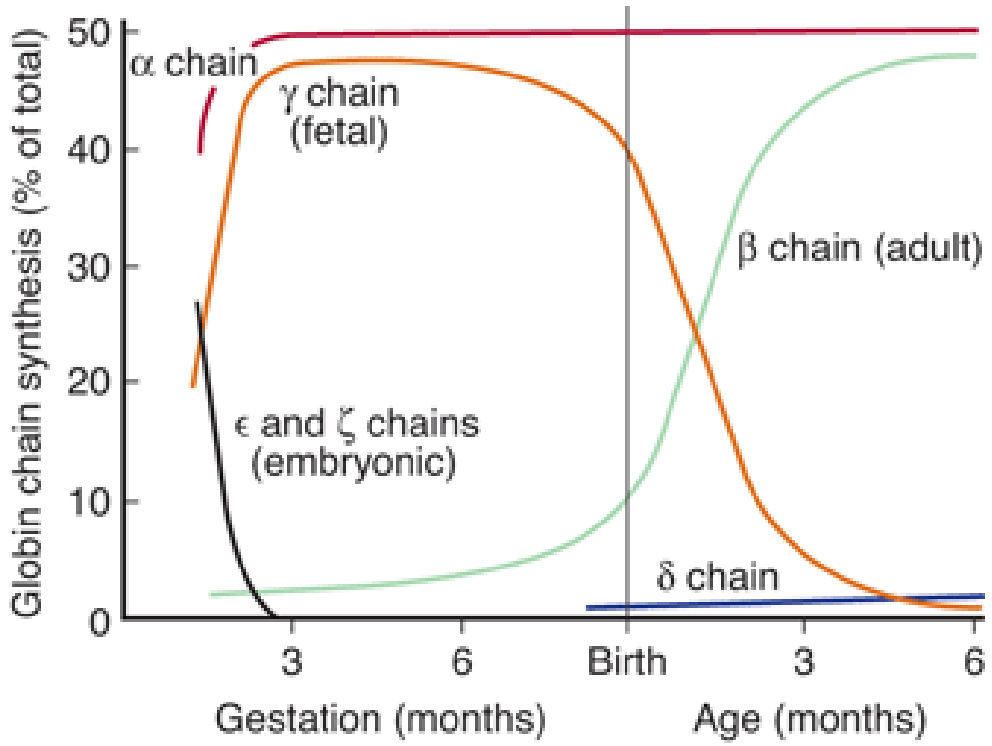
Oxygen transport

- Hemoglobin can transport 70 times the amount of Oxygen as that which is physically soluble in blood.
- The concentration of hemoglobin in erythrocytes is nearly double the concentration of plasma proteins in blood. It is responsible for the majority of the acid buffering capacity of plasma proteins.
- Hemoglobin A1 is a heterotetramer consisting of two α-chains and two β-chains, similarly folded.
- Each subunit carries a heme group with a central ferrous ion. Its oxidation state does not change with Oxygen binding.

Hemoglobins

- HbA1 consists of two α and two β chains.
- HbA2 consists of two α and δ chains.
- Hemoglobin F consists of two α and γ chains. Low affinity for 2,3-BPG (high affinity for Oxygen).
- Embryonic hemoglobins include ζ2ε2 (Gower) and ζ2γ2 (Portland).

Hemoglobin production



Source: Barrett KE, Barman SM, Boitano S, Brooks H: *Ganong's Review of Medical Physiology, 23rd Edition*: http://www.accessmedicine.com

Fig. 32-8 Accessed 03/01/2010

Structure of hemoglobin A_1

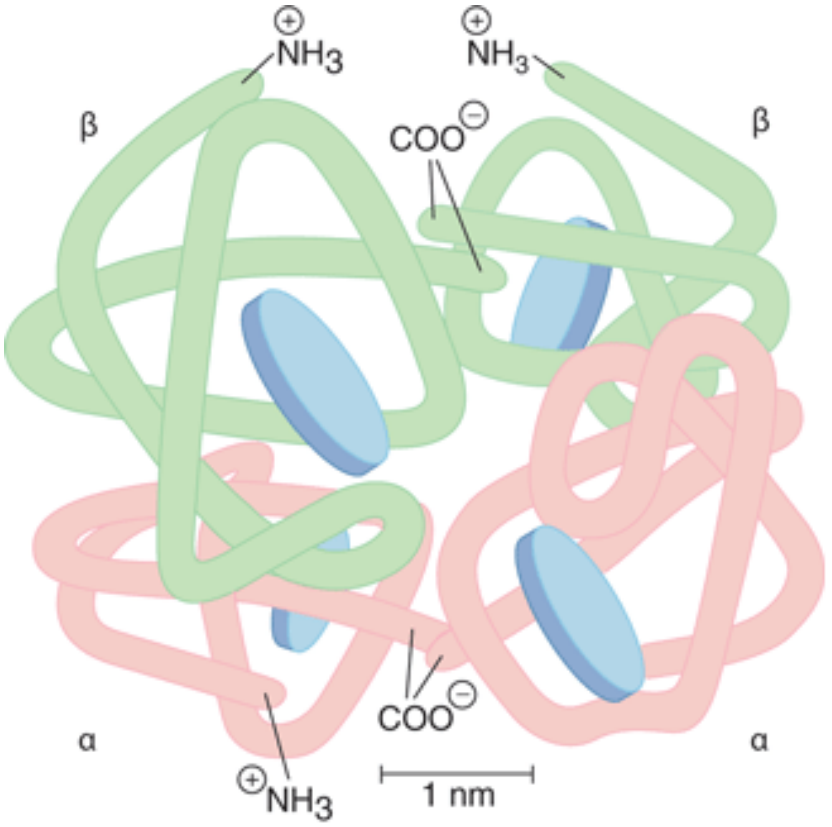


Fig. 32-6
Accessed 03/01/2010

Oxygen transport

- Four of the six Iron coordination sites in hemoglobin are occuppied by the Nitrogen atoms of the pyrrol rings and a fifth is occuppied by the (proximal) histidine of the globin molecule. The sixth is coordinated with Oxygen (or water if the hemoglobin is deoxygenated).
- Hemoglobin can exist in allosteric forms; the tense form has low Oxygen affinity. As Oxygen is bound, more molecules change to the high affinity relaxed form. The Oxygen saturation curve is sigmoidal.
- CO_2 and H^+ are heterotropic effectors of hemoglobin.

Hemoglobin states

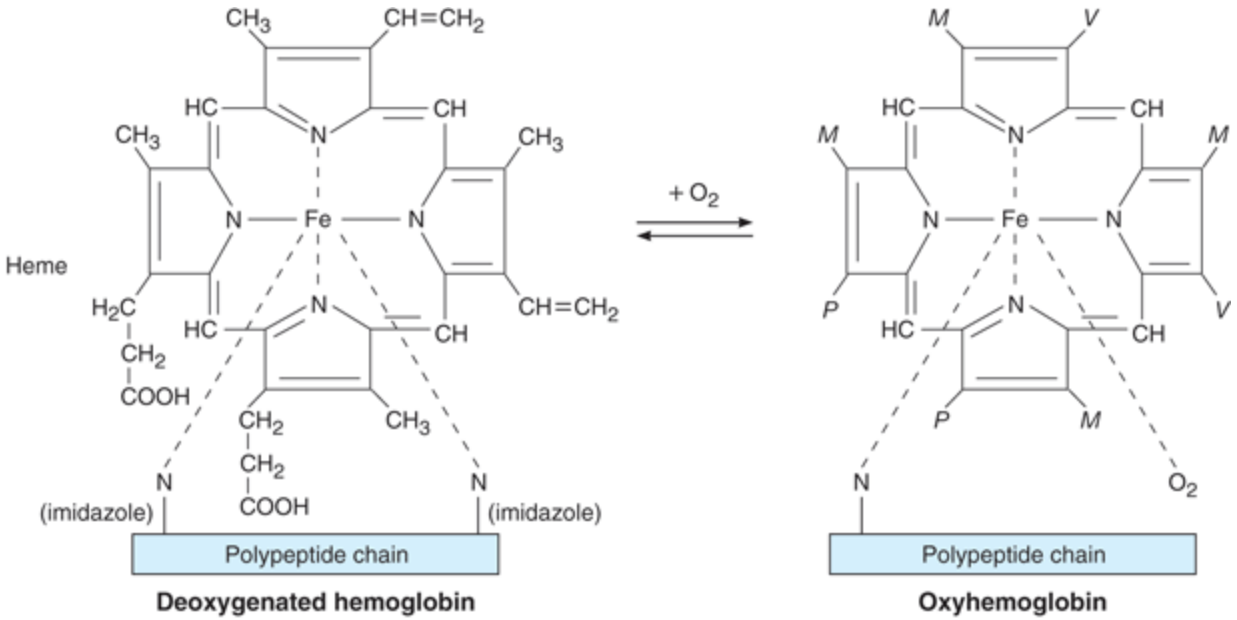


Fig. 32-7
Accessed 03/01/2010

Oxygen transport

- 2,3 biphosphoglycerate (2,3-BPG or DPG), synthesized from an intermediate of glycolysis, also acts as a heterotropic effector of hemoglobin. It can be returned to glycolysis as 2-phospho-glycerol acetate (2-PGA), but without making ATP.
- BPG binds selectively to dexoy-hemoglobin, effectively increasing the release of Oxygen at a constant pO_2. (right shift)
- CO_2 and H^+ act in the same direction as 2-3, BPG. Their influence is called the Bohr effect.
- The effects of CO_2 and BPG are additive.

Carbon dioxide transport

- 5% of CO_2 arising in tissues is soluble in blood.
- 5% of CO_2 arising in tissues is covalently bound to the N-terminus of hemoglobin (carbaminohemoglobin).
- 90% of CO_2 arising in tissues is first converted into bicarbonate. In the lungs, CO_2 is regenerated.
- Deoxy-hemoglobin is a stronger base than is oxyhemoglobin. It therefore binds additional protons and promotes the formation of bicarbonate from CO_2 in the peripheral tissues.
- (Myoglobin does not release O_2 until $pO_2<20$. Myogoblon does not have a quartenary structure).

Carbon dioxide transport

- The resulting bicarbonate is released into the plasma via an antiport in the red cell membrane in exchange for chloride ion, and passes from the plasma to the lungs.
- In the lung, deoxyhemoglobin is oxygenated, causing a release of protons. This shifts the HCO_3^-/ CO_2 equilibrium to the left, promoting CO_2 release. In the erythrocyte, the equilibrium is catalyzed by carbonic anydrase.
- O_2 binding, then, is regulated by the pH.
- High CO_2 concentrations lead to elevated numbers of H^+, reducing the affinity of hemoglobin for O_2, promoting O_2 release.

Bicarbonate buffering system

Responsible for maintaining pH on a real-time basis.

1. CO_2 generated through normal metabolism (TCA cycle) is released to the blood.
2. Erythrocytes take up the CO_2 and convert it to carbonic acid via carbonic anhydrase.
3. Carbonic acid ($H_2CO_{3)}$ dissociates into bicarbonate (HCO_3^-) and a proton (H^+).

Bicarbonate buffering system

4. Hemoglobin can absorb H^+ and buffer changes in pH because of histidine present within the hemoglobin molecule.
5. HCO_3^- can be transported out of the erythrocyte in exchange for Cl^- .
6. Excess H^+ inside the erythrocyte or other cells can be combined with PO_4^{2-} for additional bufferng.
7. Intracellular proteins that contain histidine can also act as buffers against changes in pH.

Erythrocyte deformability

- Ankyrin-3 bridges spectrin (inside the cell) with the outer plasma membrane.
- Actin 4.1 bridges spectrin with glycophorins that extend through the plasma membrane.
- Within the cell, α and β spectrin chains self-associate.
- Maintains cell shape.
- Hereditary spherocytosis or elliptocytosis noted as result of mutation.

Red cell morphology

- Agglutination refers to aggregation or clumping of red cells.
- This is seen in paraproteinemia as well as in autoimmune hemolytic anemia.
- Rouleaux formation refers to red cells lying in single rows as if they were stacked.
- This reflects abnormal serum protein levels.
- Anisocytosis refers to variation in cell size.
- Poikilocytosis refers to variation in cell shape.

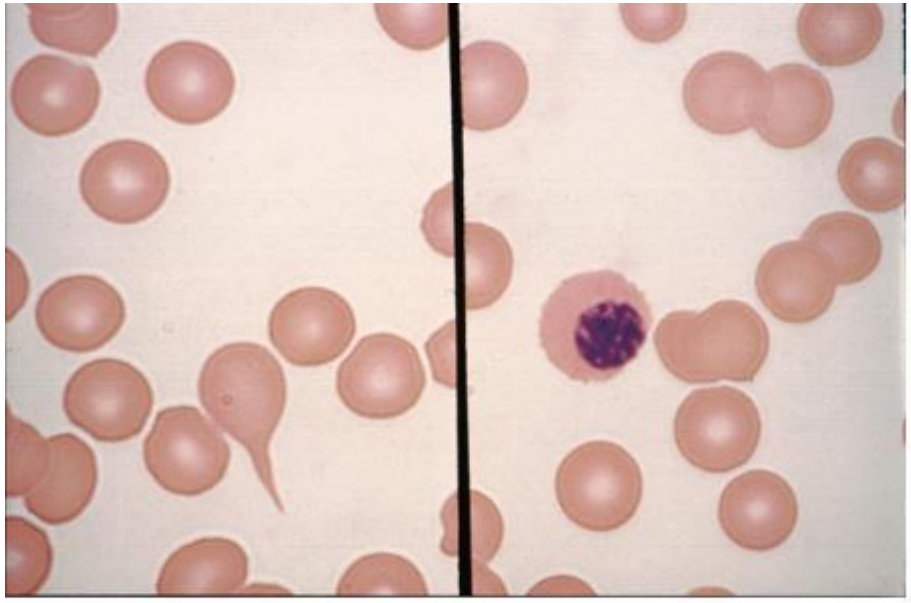
Red cell morphology

- Cells are either normal in color (normochromic) or pale (hypochromic).
- If too much hemoglobin is produced, the cells become larger, not darker.
- Polychromatophilia is the persistence of a bluish hue to the cytoplasm, reflecting the presence of ribosomes actively producing hemoglobin in a young cell.
- Basophilic stippling refers to the diffuse fine or coarse blue dots in the cytoplasm representing nucleic acid residue.
- It is a common finding in lead poisoning.

Red cell morphology

- Erythroid cells mature in 4.5 days.
- They are released into the circulation on the final day of maturation with RNA still present in the cytoplasm (reticulocytes).
- In anemia, erythroid precursors are released early.
- At hematocrits of 35%, reticulocytes may circulate for 1.5 days; at 15%, 2.5 days.
- A correction for this shift reflecting early release is incorporated into the reported reticulocyte count.
- Nucleated red cells are those released from the marrow prior to maturation and may reflect myelofibrosis.

Myelofibrosis

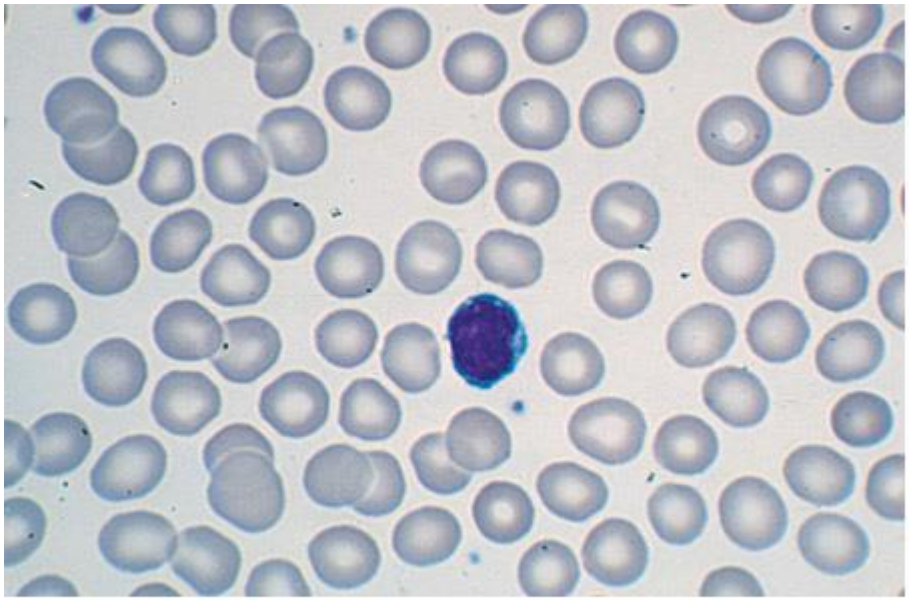


A teardrop-shaped red blood cell (left panel) and a nucleated red blood cell (right panel) as typically seen with myelofibrosis and extramedullary hematopoiesis.

Red cell morphology

- Marked anisocytosis and poikilocytosis with polychromatophilia and basal stippling suggest a maturation disorder.
- The reticulocyte count is low.
- Howell-Jolly bodies are dense blue circular inclusions that represent DNA remnants.
- Their presence points to functional hyposplenia
- Conspicuous erythrocyte inclusions suggest malaria.
- Supravital stains are necessary to see precipitated hemoglobin called Heinz bodies.
- These may be seen in α-thalassemia (hemoglobin H disease), G6PD deficiency, or liver disease.

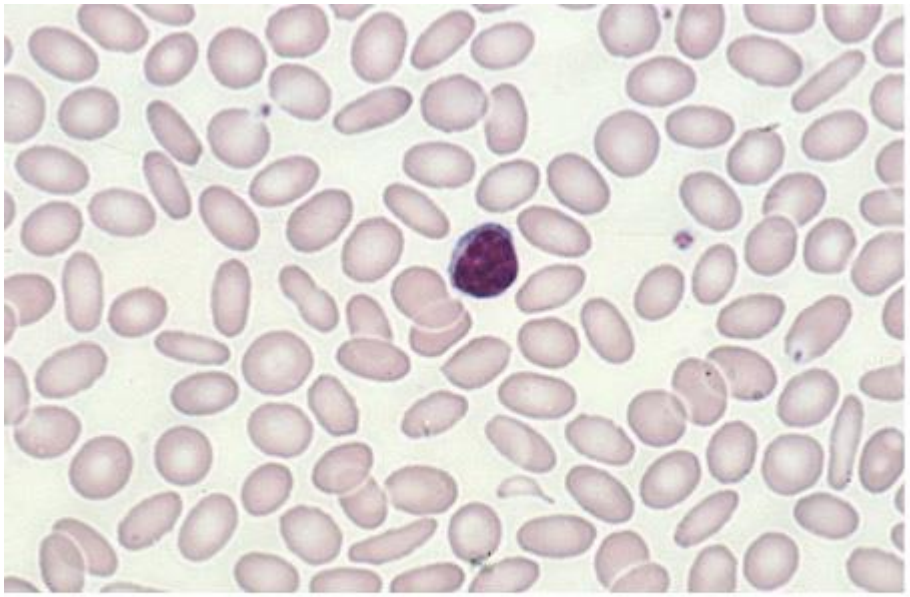
Normal



The normal red cell is slightly smaller than the small lymphocyte.

Fig. e11-1
Accessed 03/01/2010

Abnormal red cell morphology

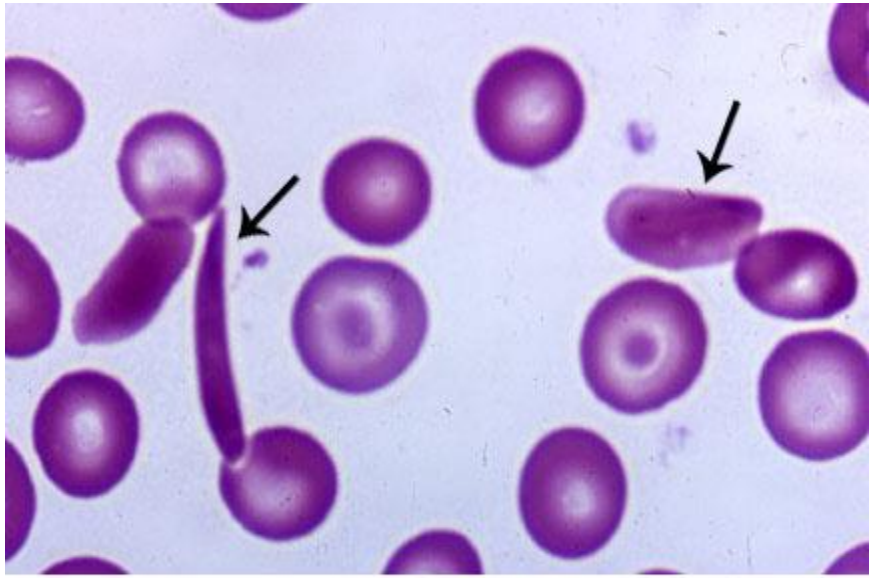


In elliptocytosis the elliptical shape of red cells is related to weakened membrane structure, usually due to mutations in spectrin.

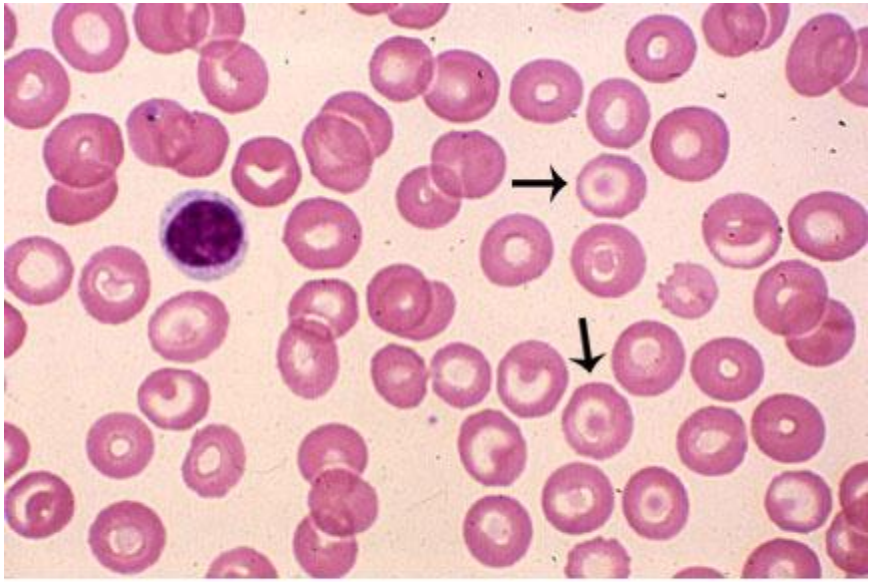
Fig. e11-18
Accessed 03/01/2010

Abnormal red cell morphology

- Sickle Cells (HbS)

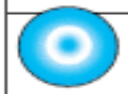


Target Cells (Hb C)



Figs. I.A.26 and I.A.30
Accessed 03/01/2010

Abnormal red cell morphology

	Name	Characteristic	Also seen in
	Spherocyte	Hereditary spherocytosis Immune hemolytic anemia	Clostridial sepsis, hemolytic anemia of Wilson disease, hemoglobin CC disease
	Elliptocyte	Hereditary elliptocytosis (HE)	Iron deficiency, MDS megaloblastic anemia, thalassemias,
	Dacrocyte	Hemolytic hereditary elliptocytosis, hereditary pyropoikilocytosis	Severe iron deficiency, megaloblastic anemia, thalassemias, myelofibrosis, MDS
	Schistocyte	Microangiopathic and fragmentation hemolytic anemias	
	Echinocyte	Renal failure, malnutrition	Common in vitro artifact after blood storage
	Acanthocyte	Spur cell anemia, abetalipoproteinemia	Splenectomy
	Target cell	Cholestasis, hemoglobin C trait and CC disease	Iron deficiency, thalassemias
	Stomatocyte	Hereditary stomatocytosis	Alcoholism

Source: Lichtman MA, Beutler E, Kipps TJ, Seligsohn U, Kaushansky K, Prchal JT: *Williams Hematology*, 7th Edition: http://www.accessmedicine.com

Fig. 2-5
Accessed 03/01/2010

RED CELL FORMS

FORM	ASSOCIATED ABNORMALITY
Biconcave	Normal
Spherocyte	Hereditary spherocytosis; autoimmune hemolysis
Macrocyte	Megaloblastic anemia
Helmet cell (schistocyte)	Diffuse intravascular coaqulopathy; traumatic hemolysis
Sickle cell	HbS
Tear drop cell	Myelofibrosis
Acanthocyte	Abetalipoproteinemia
Target cell	Thalassemia, HbC, asplenia
Poikilocytes	Microangiopathy; diffuse intravascular coagulopathy; thrombotic thrombocytopenic purpura (hemolytic uremic syndrome
Burr cell	Thrombotic thrombocytopenic purpura (hemolytic uremic syndrome

Hematology instruments

- Hematology instruments measure the numbers of red and white cells, platelets, hemoglobin, and the size of red cells (MCV) and platelets (MPV).
- Hematocrit, MCH, MCHC are calculated.
- White cells are differentiated by size and electrical activity.
- Stains are infrequently applied.
- Reticulocytes are measured separately.
- A Giemsa-Wright stained peripheral smear must be examined microscopically to evaluate cellular morphology.

Hemoglobin as screening tool

- The incidence of previously undetected hemoglobin abnormalities in women ranges from 6-13%.
- The incidence of previously undetected hemoglobin abnormalities in men rises to 6% after age 60.
- The WHO defines anemia as a hemoglobin <12.0g/dl

Table 14-1 Classification of Anemia According to Underlying Mechanism

Mechanism	Specific Examples
Blood Loss	
Acute blood loss	Trauma
Chronic blood loss	Gastrointestinal tract lesions, gynecologic disturbances*
Increased Red Cell Destruction (Hemolysis)	
Inherited genetic defects	
Red cell membrane disorders	Hereditary spherocytosis, hereditary elliptocytosis
Enzyme deficiencies	
Hexose monophosphate shunt enzyme deficiencies	G6PD deficiency, glutathione synthetase deficiency
Glycolytic enzyme deficiencies	Pyruvate kinase deficiency, hexokinase deficiency
Hemoglobin abnormalities	
Deficient globin synthesis	Thalassemia syndromes
Structurally abnormal globins (hemoglobinopathies)	Sickle cell disease, unstable hemoglobins
Acquired genetic defects	
Deficiency of phosphatidylinositol-linked glycoproteins	Paroxysmal nocturnal hemoglobinuria
Antibody-mediated destruction	Hemolytic disease of the newborn (Rh disease), transfusion reactions, drug-induced, autoimmune disorders
Mechanical trauma	
Microangiopathic hemolytic anemias	Hemolytic uremic syndrome, disseminated intravascular coagulation, thrombotic thrombocytopenia purpura
Cardiac traumatic hemolysis	Defective cardiac valves
Repetitive physical trauma	Bongo drumming, marathon running, karate chopping
Infections of red cells	Malaria, babesiosis
Toxic or chemical injury	Clostridial sepsis, snake venom, lead poisoning
Membrane lipid abnormalities	Abetalipoproteinemia, severe hepatocellular liver disease
Sequestration	Hypersplenism
Decreased Red Cell Production	
Inherited genetic defects	
Defects leading to stem cell depletion	Fanconi anemia, telomerase defects
Defects affecting erythroblast maturation	Thalassemia syndromes
Nutritional deficiencies	
Deficiencies affecting DNA synthesis	B_{12} and folate deficiencies
Deficiencies affecting hemoglobin synthesis	Iron deficiency anemia
Erythropoietin deficiency	Renal failure, anemia of chronic disease
Immune-mediated injury of progenitors	Aplastic anemia, pure red cell aplasia
Inflammation-mediated iron sequestration	Anemia of chronic disease
Primary hematopoietic neoplasms	Acute leukemia, myelodysplasia, myeloproliferative disorders (Chapter 13)
Space-occupying marrow lesions	Metastatic neoplasms, granulomatous disease
Infections of red cell progenitors	Parvovirus B19 infection
Unknown mechanisms	Endocrine disorders, hepatocellular liver disease

G6PD, Glucose-6-phosphate dehydrogenase.
*Most often cause of anemia is iron deficiency, not bleeding per se.

Acute anemia

- Acute anemia usually results from blood loss or hemolysis.
- In extravascular hemolysis, red cells are cleared by the reticuloendothelial system
- Generally caused by changes that make the red cell less deformable
- Anemia, splenomegaly, and elevated unconjugated bilirubin
- Haptoglobin diminished
- Intravascular hemolysis due to trauma
- Cardiac valves, repetitive mechanical injury, microangiopathy

Acute anemia

- Intravascular hemolysis due to complement fixation
- Transfusion reaction
- Intravascular hemolysis due to toxic injury
- Clostridial sepsis
- Intravascular hemolysis due to intracellular parasites
- Malaria
- Anemia, free hemoglobin in serum, elevated unconjugated bilirubin
- Haptoglobin diminished
- When haptoglobin overwhelmed, free hemoglobin is oxidized to methemoglobin
- Reabsorbed in renal tubules
- Excess excreted in urine (brown color)

Acute anemia

- Hypovolemia dominates the clinical picture in acute blood loss.
- Hematocrit and hemoglobin levels do not reflect the volume of blood lost.
- With acute loss of 10–15% of the total blood volume, hypotension and diminished organ perfusion are present.
- Peripheral vasoconstriction and redistribution of organ blood flow are present.

Acute anemia

- With acute loss of >30% of the blood volume is lost suddenly (>2.0-3.0 g/dL hemoglobin lost), usual physiologic responses are impaired.
- Postural hypotension and tachycardia are evident.
- Shock with confusion, dyspnea, diaphoresis, hypotension, and tachycardia predominate if the volume of blood lost acutely is >40%.
- Immediate volume replacement is indicated.

Table 14-4 Classification of Immunohemolytic Anemias

Warm Antibody Type (IgG Antibodies Active at 37°C)
Primary (idiopathic)
Secondary
Autoimmune disorders (particularly systemic lupus erythematosus)
Drugs
Lymphoid neoplasms
Cold Agglutinin Type (IgM Antibodies Active Below 37°C)
Acute (mycoplasmal infection, infectious mononucleosis)
Chronic
Idiopathic
Lymphoid neoplasms
Cold Hemolysin Type (IgG Antibodies Active Below 37°C)
Rare; occurs mainly in children following viral infections

Autoimmune hemolytic anemia

- 50% have complement fixing IgG antibodies reactive at 37C.
- IgG antibodies bind to Fc receptors on phagocytes
- Membrane defects removed, converting cells to spherocytes that are later removed in spleen
- Extravascular hemolysis
- Often due to drugs such as penicillins or cephalosporins or quinidine that bind to cell surface.
- α-methyldopa induces production of antibodies to red cell antigens, principally Rh type.
- 10% of patients
- Occasionally antibodies are of IgA type.

Autoimmune hemolytic anemia

- Cold agglutinins
- Up to 30% associated with IgM antibodies reactive below 37C (usually 0-4C).
- May follow infection
- Mycoplasma, EBV, CMV, influenza, HIV.
- As cells recirculate from cooler extremities to core, IgM lost but sufficient C3b present to lead to phagocytosis
- Extravascular hemolysis

Autoimmune hemolytic anemia

- Cold hemolysins
- May follow infection.
- IgG antibodies to the P blood group are reactive below 37C as well.
- Extravascular hemolysis

Transfusion reaction

- Intravascular hemolysis generally follows transfusion with (usually ABO) incompatible blood.
- There is release of free hemoglobin.
- There may be a sense of doom, acute back pain, free hemoglobin in the plasma and urine, and renal failure.
- The transfusion must be stopped Saline diuresis is begun to limit precipitation of hemoglobin in renal tubules.
- Pressors may be required
- Heparinization is begun if DIC is present.

Diagnostic screen

- Direct Coombs positive
- Red cells coated by antibody and/or complement.
- Indirect Coombs positive
- Patient serum contains antibodies
- Define antigen target and optimal temperatures

Histopathology

- Erythropoietin increased.
- Increased numbers of erythroid precursors (normoblasts) in the marrow.
- Compensatory increases in erythropoiesis result in a prominent reticulocytosis in the peripheral blood.
- Hemosiderin in spleen, liver, and marrow
- Extramedullary hematopoiesis in severe cases
- If longstanding hemolysis, may see pigmented gallstones

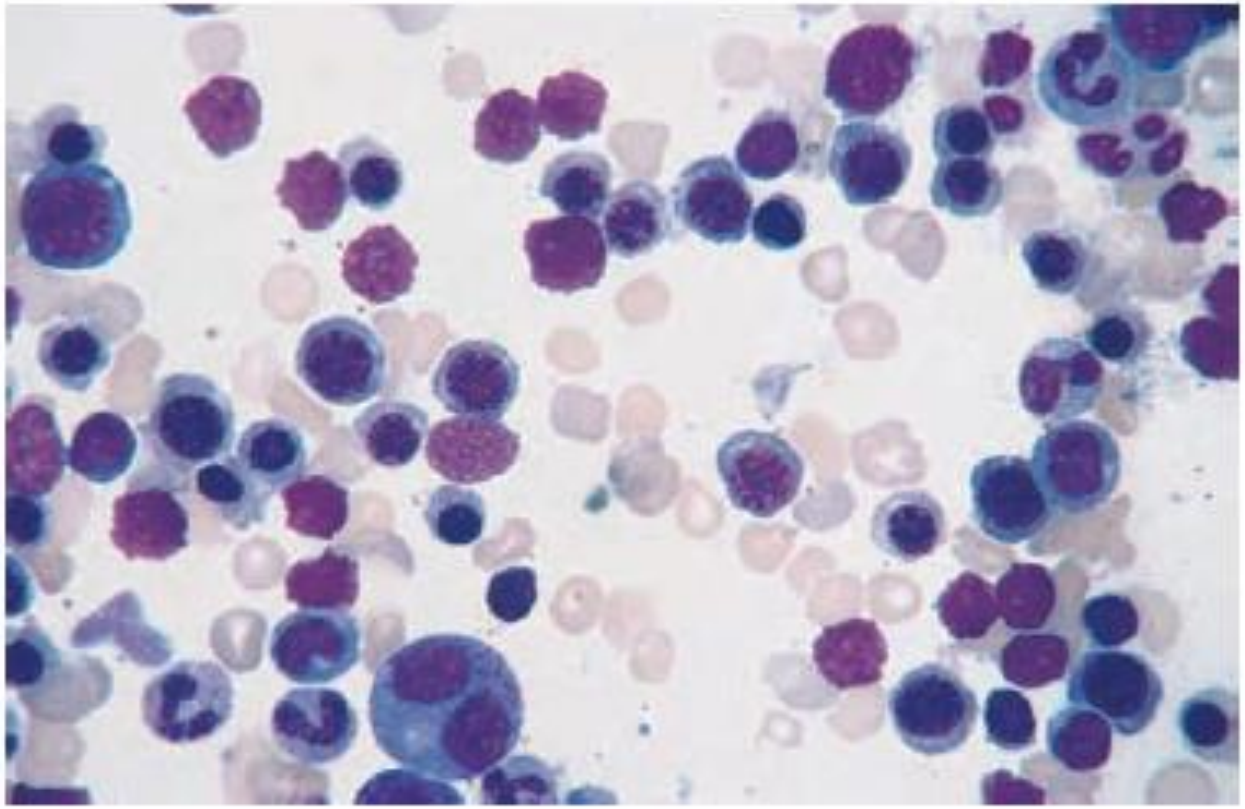


Figure 14-1 Marrow smear from a patient with hemolytic anemia. The marrow reveals increased numbers of maturing erythroid progenitors (normoblasts). (Courtesy Dr. Steven Kroft, Department of Pathology, University of Texas Southwestern Medical School, Dallas, Texas.)

Chronic anemia

- RDW is the mean of distribution of red cell sizes and is an indicator of the chronic nature of the anemia.
- A wide RDW suggests the presence of two red cell populations.
- The usual cause of chronic anemia is uncompensated blood loss.
- Menstruation, gastrointestinal bleeding, or bleeding in the urinary tract must also be explored.
- In elderly patients this may represent myelodysplastic syndrome.
- Bone marrow examination may be required for diagnosis.

Chronic anemia

- Fatigue and loss of stamina are prominent.
- Total blood volume is normal or increased in chronic anemia.
- Cardiac output is maintained.
- Redistribution of organ blood flow away from the skin, gastrointestinal tract, and kidney compensates for loss of Oxygen carrying capacity.

Chronic anemia

- The skin and mucous membranes may be pale if the hemoglobin is <8.0–10.0 g/dL (positive likelihood ratio of pallor of conjunctival rim, LR+, 16.7).
- If the palmar creases are lighter in color than the surrounding skin when the hand is hyperextended, the hemoglobin level is usually <8 g/dL (LR+, 8).
- In the younger patient, symptoms may not be present until the anemia is severe (hemoglobin of <7.0 g/dL).
- The bone marrow normally responds by increasing erythroid cell production.

Normochromic, normocytic anemia

- An MCV between 80-100 fl is seen in anemia related to acute blood loss.
- Generally there is an adequate reticulocyte response (>2.5%).
- An elevated LDH and a depressed haptoglobin level are diagnostic of hemolysis.
- A direct Coombs test to detect red cells coated with immunoglobulin as well as an indirect Coombs test to detect complement sensitivity may be required to evaluate hemolysis.

Normocytic, normochromic anemia

- Non-immune causes of hemolysis include:
- Pyruvate kinase deficiency
- Hereditary elliptocytosis
- Hereditary spherocytosis.
- G6PD deficiency is commonly associated with hemoglobinopathy.

Normocytic, normochromic anemia

- If a chronic condition, consider:
- Anemia of chronic disease
- IL-6 increases hepcidin expression
- Degrades ferroportin
- Inhibits iron release from macophages
- Elevated ferritin but low serum iron
- Chronic renal disease
- Relative erythropoietin deficiency
- Liver disease
- Androgen deficiency.
- Fanconi anemia
- Autoimmune disease

Microcytic, hypochromic anemia

- 60 days are required for changes in hemoglobin levels to reflect uncompensated blood loss.
- Cell size, reflected in the MCV, is the first parameter to change.
- Iron deficiency is the most common cause of an MCV <80 fl.
- Low serum ferritin levels are diagnostic (positive likelihood ratio, LR+, 25-50).
- 20% of women are iron deficient.
- Search for source of bleeding loss.

Iron metabolism

- 80% of the functional iron (Fe^{2+}, ferrous) is found in hemoglobin
- Myoglobin and iron-containing enzymes such as catalase and the cytochromes contain the rest.
- The storage pool represented by hemosiderin and ferritin contains about 15% to 20% of total body iron.
- The major sites of iron storage are the liver and mononuclear phagocytes.
- Stored as Fe^{3+} (ferric)
- Iron in the body is recycled between the functional and storage pools.

Iron metabolism

- Iron absorption is regulated by hepcidin
- Peptide is synthesized and released from the liver in response to increases in intrahepatic iron levels
- Elevated with chronic inflammation (IL-6)
- Diminished in hemochromatosis
- Luminal non-heme iron is mostly in the Fe^{3+} (ferric) state
- Newly absorbed Fe^{3+} iron binds rapidly to transferrin
- Glycoprotein is synthesized in the liver
- Delivers iron to cells, where it is phagocytized
- Erythroid precursors contain high-affinity receptors for transferrin

Iron metabolism

- Fe^{3+} reduced to Fe^{2+} (ferrous) by duodenal cytochrome Bb and STEAP3.
- Upregulated by HIF2α
- Upregulated in low iron states
- Fe^{2+} iron destined for the circulation is transported from the cytoplasm across the basolateral enterocyte membrane by ferroportin.
- Ferroportin also governs iron release from macrophages
- Ferroportin inhibited by hepcidin

Iron metabolism

- Fe^{2+} iron is then transported across the apical membrane by divalent metal transporter 1 (DMT1).
- Mediates iron derived from phagocytized transferrin across lysosomal membranes into cytosol of red cell precursors in the marrow
- Upregulated by low iron states
- This process is coupled to the oxidation of Fe^{2+} to Fe^{3+} by the copper containing enzymes hephaestin and ceruloplasmin
- Transferred to transferrin

Iron metabolism

- Free Fe^{3+} is toxic
- Forms hydroxyl radicals via Fenton reaction.
- No regulation of excretion
- Ascorbic acid enhances absorption at low pH
- Whatever damages duodenal mucosa diminishes iron absorption
- Diminished stainable iron in bone marrow macrophages

Iron metabolism

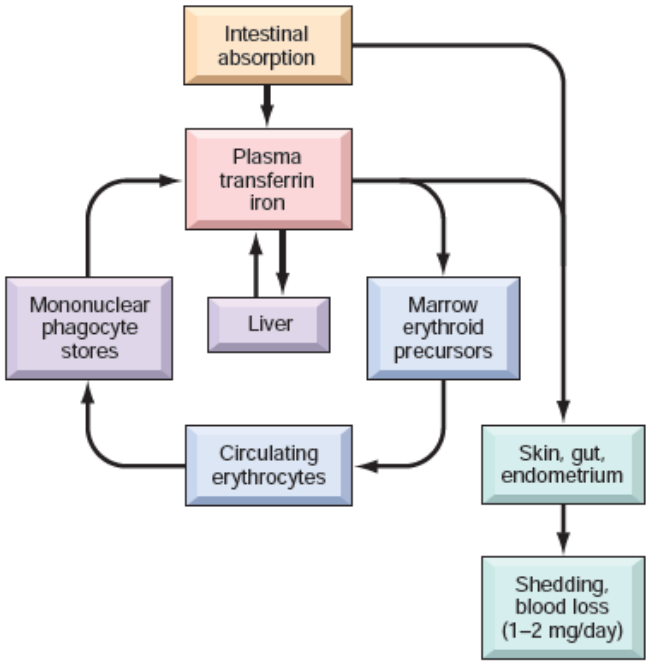


Figure 14-21 Iron metabolism. Iron absorbed from the gut is bound to plasma transferrin and transported to the marrow, where it is delivered to developing red cells and incorporated into hemoglobin. Mature red cells are released into the circulation and, after 120 days, are ingested by macrophages, primarily in the spleen, liver, and bone marrow. Here iron is extracted from hemoglobin and recycled to plasma transferrin. At equilibrium, iron absorbed from the gut is balanced by losses in shed keratinocytes, enterocytes, and (in women) endometrium.

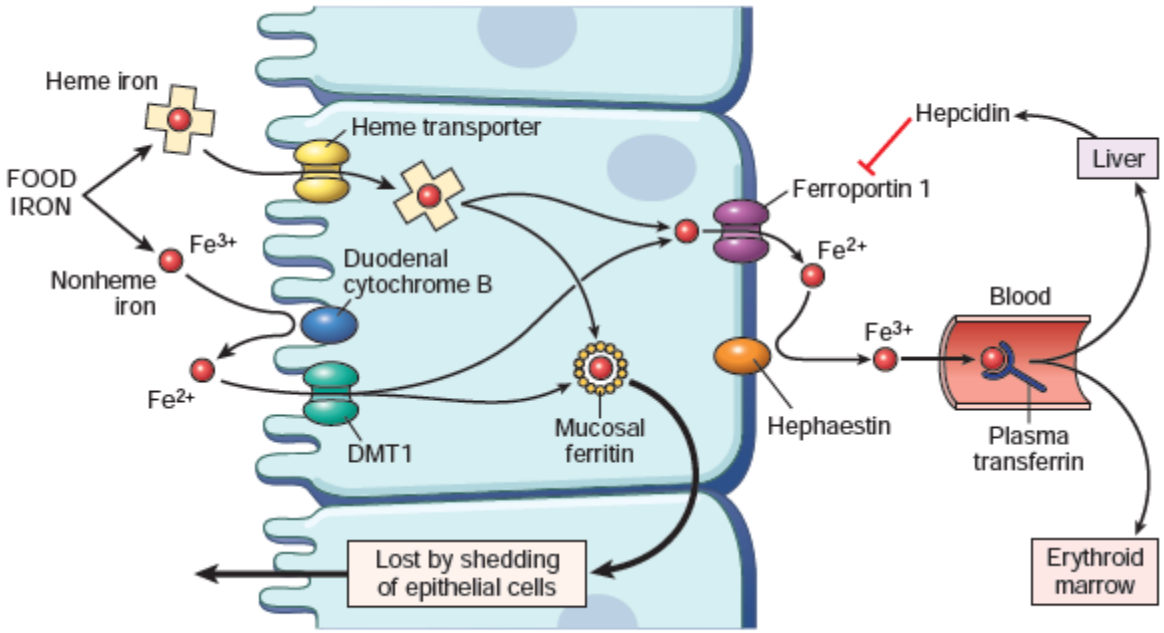


Figure 14-22 Regulation of iron absorption. Duodenal epithelial cell uptake of heme and nonheme iron is depicted. When the storage sites of the body are replete with iron and erythropoietic activity is normal, plasma hepcidin levels are high. This leads to down-regulation of ferroportin and trapping of most of the absorbed iron, which is lost when duodenal epithelial cells are shed into the gut. Conversely, when body iron stores decrease or when erythropoiesis is stimulated, hepcidin levels fall and ferroportin activity increases, allowing a greater fraction of the absorbed iron to be transferred to plasma transferrin. *DMT1,* Divalent metal transporter 1.

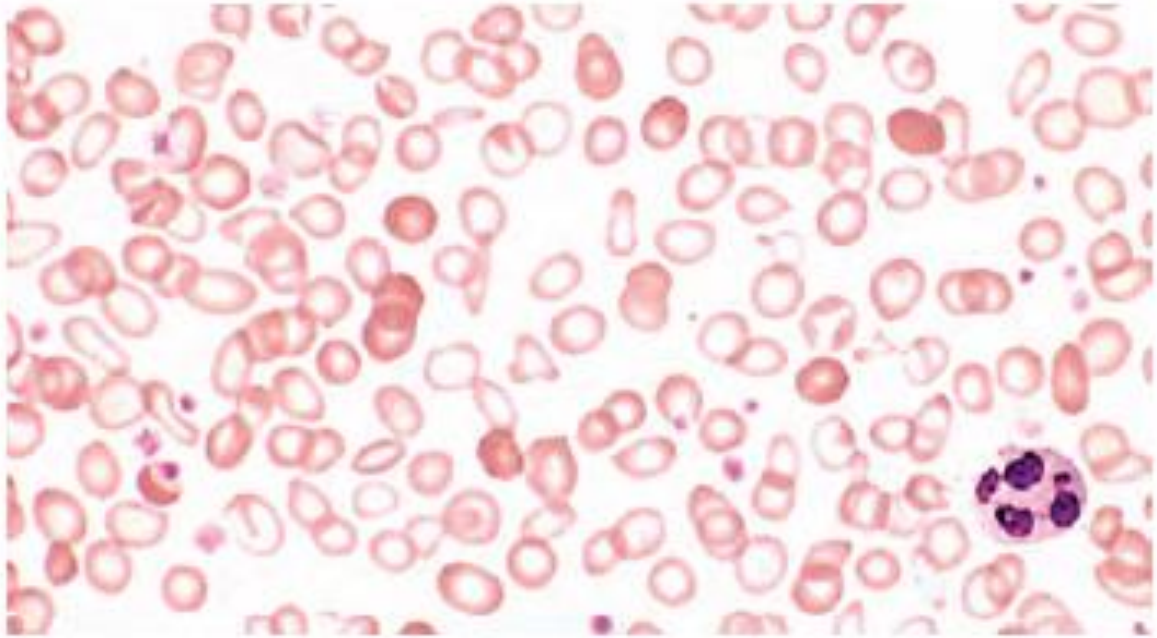


Figure 14-23 Hypochromic microcytic anemia of iron deficiency (peripheral blood smear). Note the small red cells containing a narrow rim of peripheral hemoglobin. Scattered fully hemoglobinized cells, present due to recent blood transfusion, stand in contrast. (Courtesy Dr. Robert W. McKenna, Department of Pathology, University of Texas Southwestern Medical School, Dallas, Texas.)

Microcytic, hypochromic anemia

- Hb <8 g/dL suggests a cause for anemia that is not that of chronic disease.
- If ferritin levels are normal but RDW >15%, anemia of chronic disease is likely.
- If ferritin levels are normal as is the RDW, the thalassemias must be considered.
- Target cells may be present.
- Lead intoxication (>10 µg/dL) and siderobalstic anemia are also causes of microcytic anemia.

Table 14-5 Causes of Megaloblastic Anemia

Vitamin B_{12} Deficiency
Decreased Intake
Inadequate diet, vegetarianism
Impaired Absorption
Intrinsic factor deficiency
Pernicious anemia
Gastrectomy
Malabsorption states
Diffuse intestinal disease (e.g., lymphoma, systemic sclerosis)
Ileal resection, ileitis
Competitive parasitic uptake
Fish tapeworm infestation
Bacterial overgrowth in blind loops and diverticula of bowel
Folic Acid Deficiency
Decreased Intake
Inadequate diet, alcoholism, infancy
Impaired Absorption
Malabsorption states
Intrinsic intestinal disease
Anticonvulsants, oral contraceptives
Increased Loss
Hemodialysis
Increased Requirement
Pregnancy, infancy, disseminated cancer, markedly increased hematopoiesis
Impaired Utilization
Folic acid antagonists
Unresponsive to Vitamin B_{12} or Folic Acid Therapy
Metabolic Inhibitors of DNA Synthesis and/or Folate Metabolism (e.g., Methotrexate)

Modified from Beck WS: Megaloblastic anemias. In Wyngaarden JB, Smith LH (eds): Cecil Textbook of Medicine, 18th ed. Philadelphia, WB Saunders, 1988, p. 900.

Macrocytic anemia

- Macro-ovalocytes
- Hyperchromic
- MCHC is not elevated
- Neutrophils are larger than normal and hypersegmented
- Few reticulocytes
- Hypercellular mrow
- Megaloblastic changes at all stages of erythropoiesis

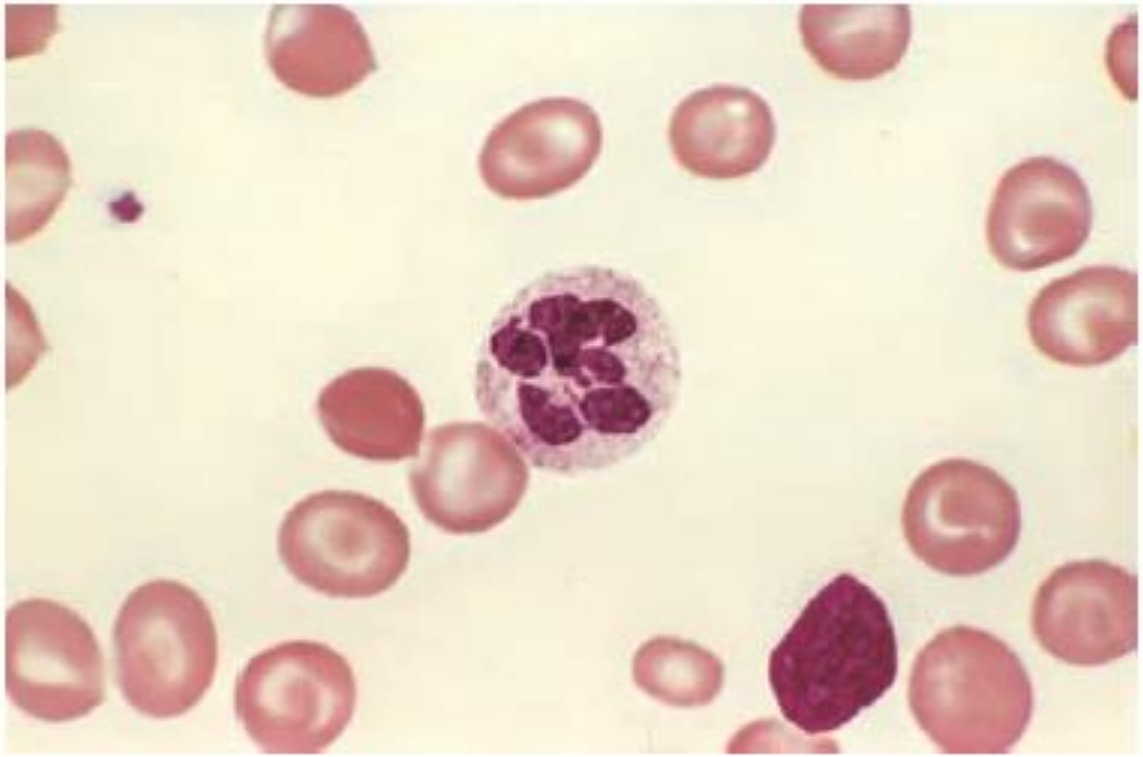


Figure 14-16 Megaloblastic anemia. A peripheral blood smear shows a hypersegmented neutrophil with a six-lobed nucleus. (Courtesy Dr. Robert W. McKenna, Department of Pathology, University of Texas Southwestern Medical School, Dallas, Texas.)

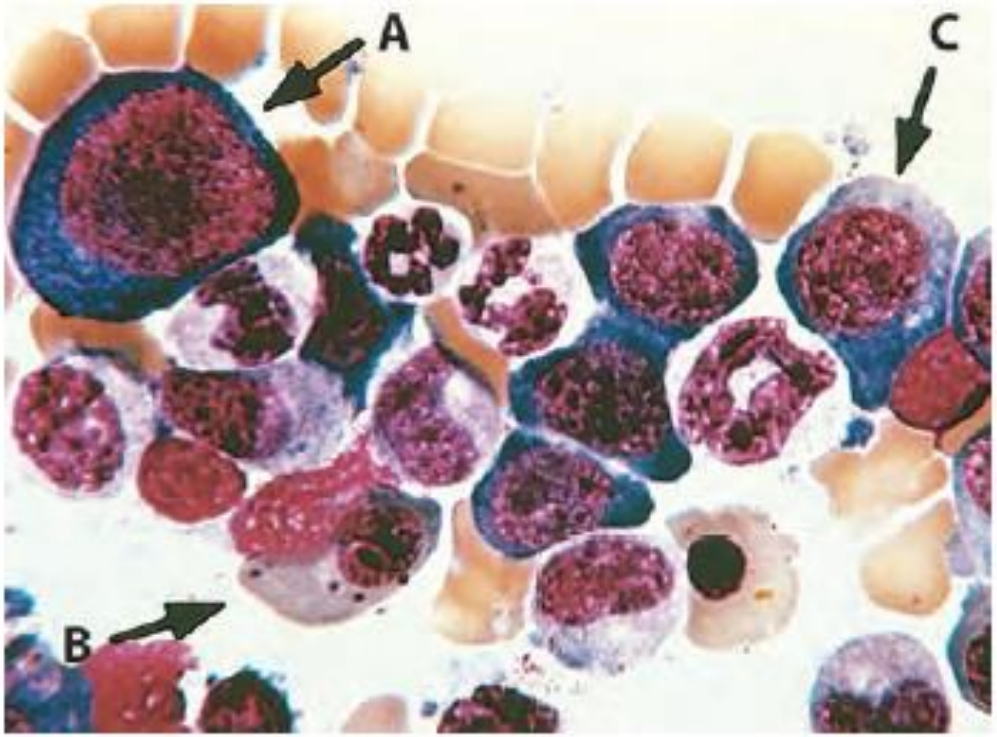


Figure 14-17 Megaloblastic anemia (bone marrow aspirate). **A** to **C**, Megaloblasts in various stages of differentiation. Note that the orthochromatic megaloblast (**B**) is hemoglobinized (as revealed by cytoplasmic color), but in contrast to normal orthochromatic normoblasts, the nucleus is not pyknotic. The early erythroid precursors (**A** and **C**) and the granulocytic precursors are also large and have abnormally immature chromatin. (Courtesy Dr. Jose Hernandez, Department of Pathology, University of Texas Southwestern Medical School, Dallas, Texas.)

Macrocytic anemia

- MCV >100 fl is associated with inadequate levels of either Folic Acid or Vitamin B_{12} or both.
- Common in older persons
- May be nutritional in origin:
- Vegetarian diet, alcohol abuse.
- May reflect malabsorption.
- May reflect liver disease.
- May be tapeworm.
- Relative deficiency noted in pregnancy
- Associated with neural tube defects

Macrocytic anemia

- Methotrexate, zidovudine as other causes.
- May reflect autoimmune hypothyroidism.
- Anti-parietal cell antibody may be present.
- With elevated LDH, intravascular hemolysis.
- Any disorder associated with reticulocytosis will elevate measured MCV.

Macrocytic anemia

- Pernicious anemia
- >60 years of age
- Chronic atrophic gastritis
- Fundic gland atrophy
- Intestinal metaplasia
- 75%, antibody blocking vitamin B12 binding to intrinsic factor (Type I)
- Type II antibodies prevent binding of the complex to ileal cells
- Type III antibodies attack the gastric proton pump
- Megaloblastic change precedes dorsal and lateral spinal tract damage

Vitamin B12 absorption

- Vitamin B12 is freed from binding proteins in food through the action of pepsin in the stomach.
- Binds to a salivary protein (haptocorrin).
- In the duodenum, bound vitamin B12 is released from haptocorrin by the action of pancreatic proteases
- Associates with intrinsic factor produced by parietal cells in the stomach.
- This complex is transported to the ileum, where it is endocytozed by ileal enterocytes that express a receptor for intrinsic factor (cubilin) on their surfaces.

Vitamin B12 absorption

- Within ileal cells, vitamin B12 associates with transcobalmin II and is secreted into plasma.
- Transcobalmin II carries vitamin B12 to the liver and other cells.

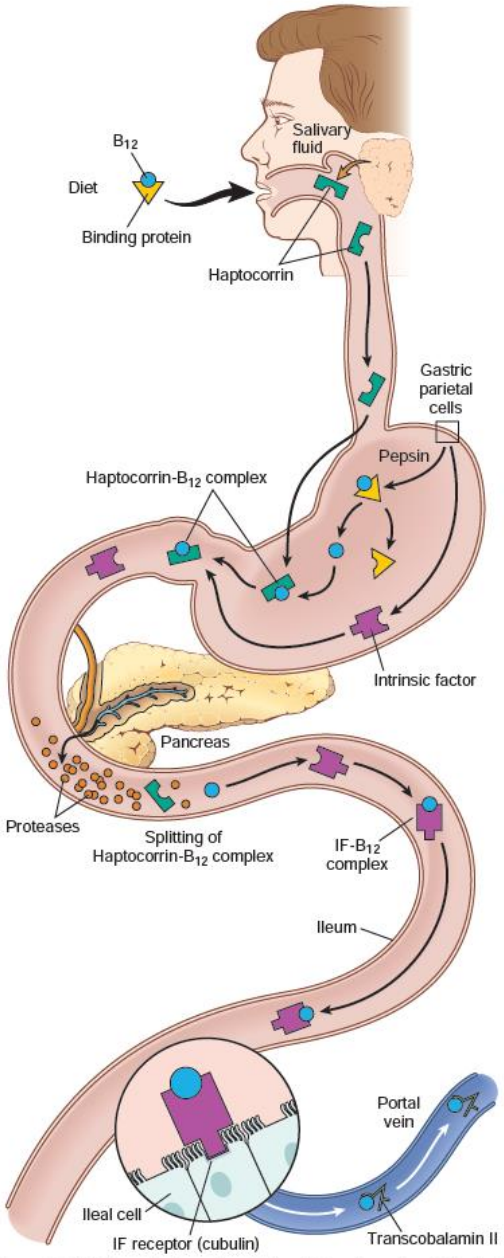


Figure 14-18 Schematic illustration of vitamin B_{12} absorption. IF, Intrinsic factor; haptocorrin, cubilin, see text.

Macrocytic anemia

- In cobalamin deficiency, the methylation of homocysteine to methionine by methionine synthase is impaired.
- Methylation requires the methyl group of the folate intermediate, 5-methyl tetrahydrofolate (5-MTHF).
- Enters the cell from the plasma and accumulates.
- It is the first step in the pathway that generates all other intracellular folate coenzymes.
- THF, the substrate on which all other intracellular folate intermediates depend, falls, impairing DNA synthesis, leading to megaloblastic change.

Macrocytic anemia

- Isomerization of methylmalonyl CoA to succinyl CoA impaired
- Proprionate and methylmalonate accumulate and are incorporated into abnormal neuronal lipids

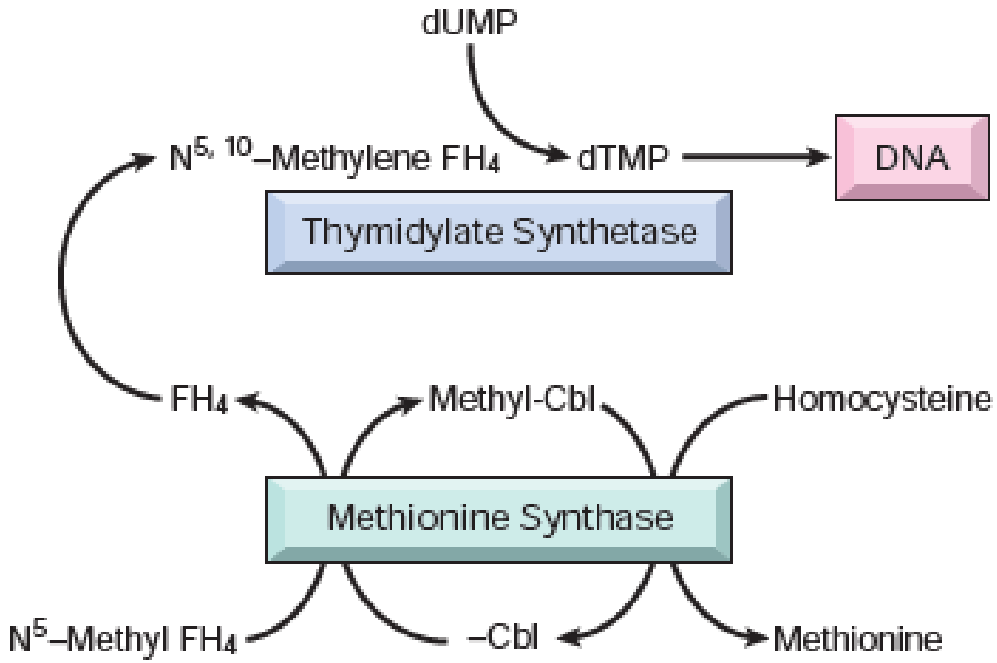


Figure 14-19 Relationship of N^5-methyl FH_4, methionine synthase, and thymidylate synthetase. In cobalamin (Cbl) deficiency, folate is sequestered as N^5-methyl FH_4. This ultimately deprives thymidylate synthetase of its folate coenzyme ($N^{5,10}$-methylene FH_4), thereby impairing DNA synthesis. FH_4, Tetrahydrofolic acid.

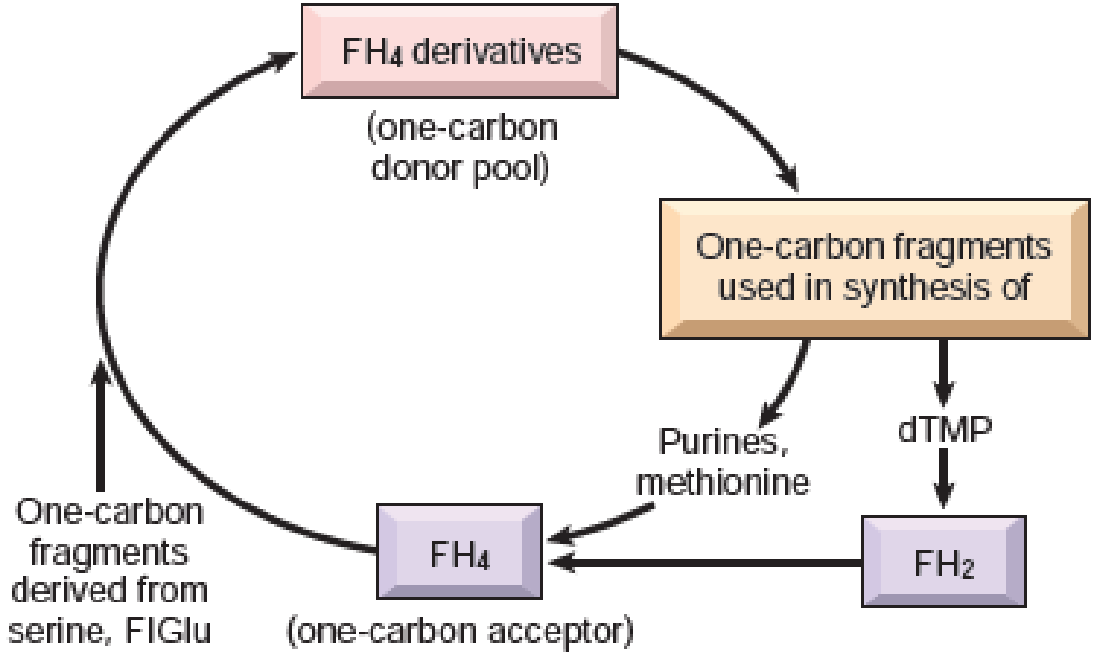


Figure 14-20 Role of folate derivatives in the transfer of one-carbon fragments for synthesis of biologic macromolecules. FH_4, Tetrahydrofolic acid; FH_2, dihydrofolic acid; FIGlu, formiminoglutamate; dTMP, deoxythymidine monophosphate.

Macrocytic anemia

- Both folic acid and vitamin B_{12} levels should be determined in evaluating patients with elevated MCV.
- The assays are interdependent.
- RBC folate determinations correlate with long term folate levels but offer little additional information.
- Serum folate is elevated in B_{12} deficiency while intracellular folate is low.
- Methylmalonic acid elevation and homocysteine elevation are compatible with B_{12} deficiency.

Macrocytic anemia

- Little need for Schilling test in view of low cost of vitamin B_{12} therapy.
- MCV >100 fl with normal folic acid and vitamin B_{12} levels may indicate pyridoxine deficiency.
- Bone marrow examination will be necessary to diagnose myelodysplastic syndrome.
- In the older patient, myelodysplastic syndrome should be considered.

Hypoproliferative anemia

- Pure red cell aplasia is rare.
- Acquired acute transient hypoproliferative anemia in adults as well as children is usually caused by a viral infection
- Often parvovirus B19.
- Non-A, B, or G hepatitis
- Chronic acquired hypoproliferative anemia involving red blood cells is generally associated with thymoma and has an autoimmune basis.
- Reticulocytes are rare.
- Activated T_{H1} cells produce IFN-γ and TNF
- Suppress and kill hematopoietic progenitors

Hypoproliferative anemia

- Fanconi anemia
- Most common inherited aplastic anemia
- Fanconi complex mutation inhibits repair of inter-strand cross-links in DNA
- 60-70% have mutations in FANCA gene at 16q24.3
- 10-15% FANCC gene at 9q22.32
- Ashkenazi
- 10%, FANCG gene at 9p13.3
- TERT mutation
- Adults
- 5-10% of inherited aplastic anemias

Hypoproliferative anemia

- Diamond–Blackfan anemia (congenital hypoplastic anemia) is the congenital form of red cell aplasia
- Chronic acquired erythroblastophthisis is frequently associated with thymoma and has an autoimmune etiology.
- Normochromic anemia.
- Reticulocytes rare.

Table 14-7 Major Causes of Aplastic Anemia

Acquired
Idiopathic
Acquired stem cell defects
Immune mediated
Chemical Agents
Dose related
Alkylating agents
Antimetabolites
Benzene
Chloramphenicol
Inorganic arsenicals
Idiosyncratic
Chloramphenicol
Phenylbutazone
Organic arsenicals
Methylphenylethylhydantoin
Carbamazepine
Penicillamine
Gold salts
Physical Agents
Whole-body irradiation
Viral Infections
Hepatitis (unknown virus)
Cytomegalovirus infections
Epstein-Barr virus infections
Herpes zoster (varicella zoster)
Inherited
Fanconi anemia
Telomerase defects

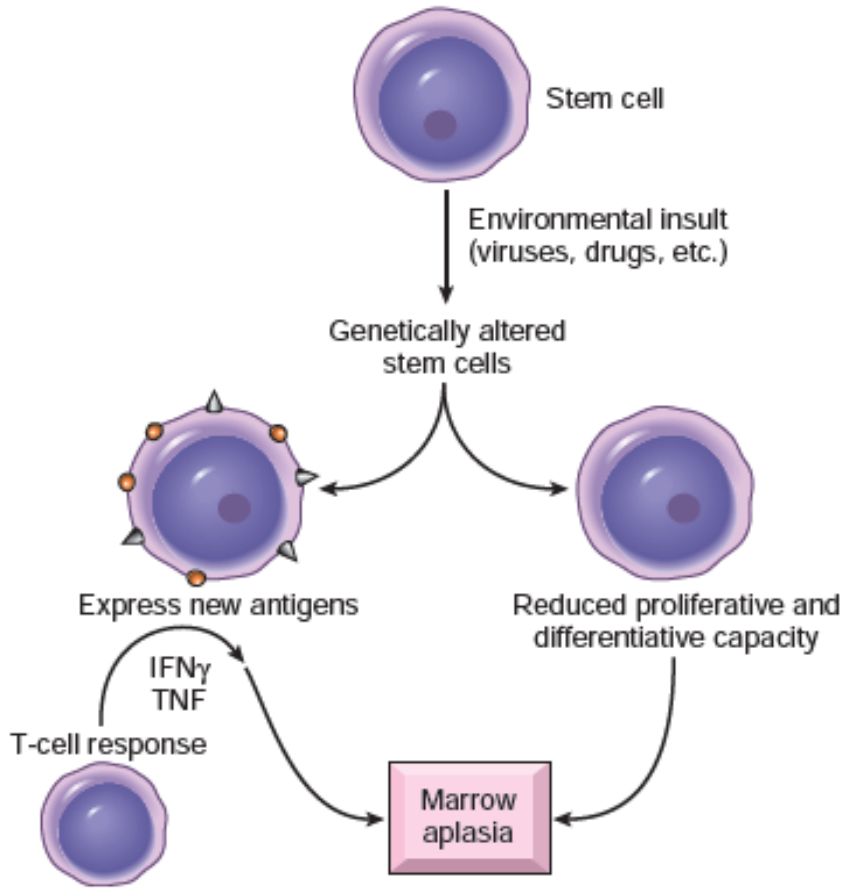


Figure 14-24 Pathophysiology of aplastic anemia. Damaged stem cells can produce progeny expressing neoantigens that evoke an autoimmune reaction, or give rise to a clonal population with reduced proliferative capacity. Either pathway could lead to marrow aplasia. See text for abbreviations.

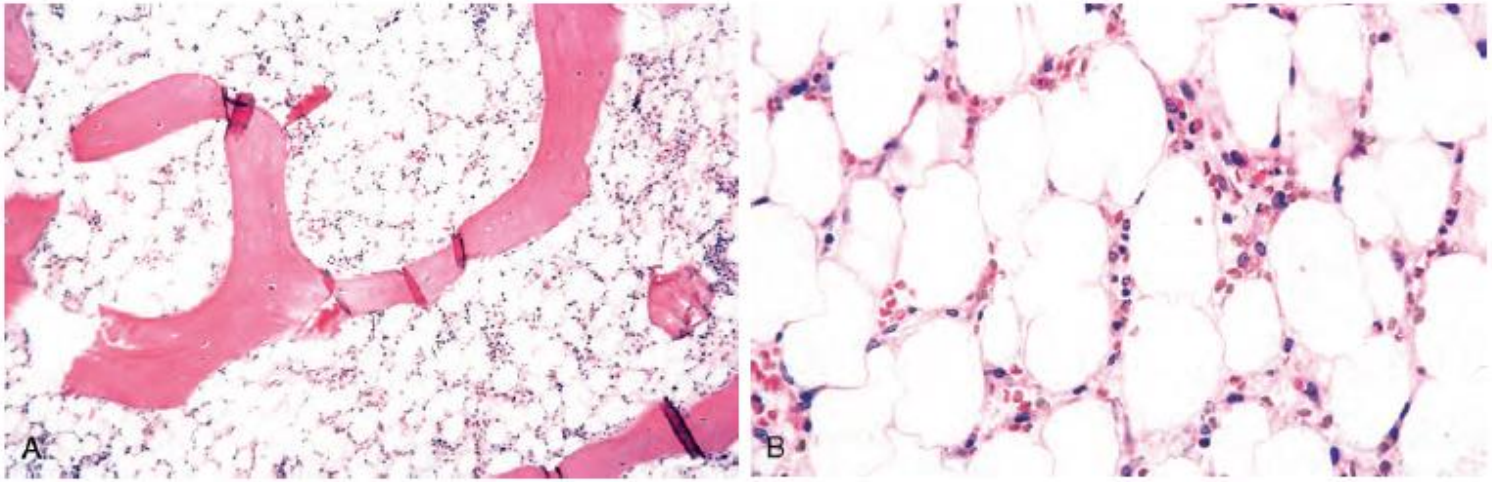


Figure 14-25 Aplastic anemia (bone marrow biopsy). Markedly hypocellular marrow contains mainly fat cells. **A,** Low power. **B,** High power. (Courtesy Dr. Steven Kroft, Department of Pathology, University of Texas Southwestern Medical School, Dallas, Texas.)

Hypoproliferative anemia

- Myelophthysic anemia
- Marrow infiltration with fibrosis
- Carcinoma
- Granulomatous disease
- Leukoerythroblastosis

Red cell membrane exoskeleton

- Spectrin consists of two polypeptide chains (α, β) which form helical flexible heterodimers.
- The “head” region of spectrin dimers self-associate to form tetramers.
- The “tail” region associates with actin oligomers.
- Each actin oligomer binds multiple spectrin tetramers.
- Ankyrin (band 4.2) binds spectrin to the transmembrane ion transporter (band 3).
- Protein 4.1 binds the “tail”region of spectrin to the transmembrane protein, glycophorin A.

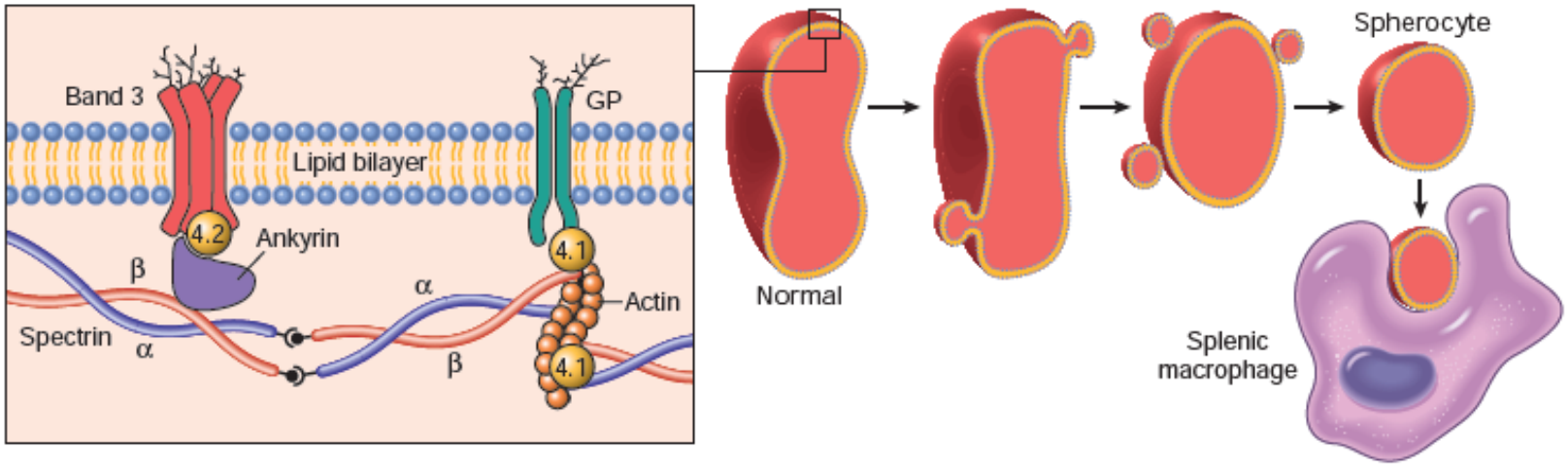


Figure 14-2 Role of the red cell membrane skeleton in hereditary spherocytosis. The left panel shows the normal organization of the major red cell membrane skeletal proteins. Various mutations involving α-spectrin, β-spectrin, ankyrin, band 4.2, or band 3 that weaken the interactions between these proteins cause red cells to lose membrane fragments. To accommodate the resultant change in the ratio of surface area to volume these cells adopt a spherical shape. Spherocytic cells are less deformable than normal ones and therefore become trapped in the splenic cords, where they are phagocytosed by macrophages. GP, Glycophorin.

Hereditary spherocytosis

- Anemia, splenomegaly, jaundice are characteristic features.
- May see gallstones as well.
- Highest prevalence in northern Europe
- Red cells are spherical (lack central zone of pallor).
- Plasma membrane unstable
- Fragments lost as red cell ages.
- Osmotically fragile.
- Parvovirus may produce aplastic crisis.

Hereditary spherocytosis

- 75% autosomal dominant
- Deficiency of cytoskeletal protein ankyrin (or, in fewer patients, spectrin).
- Loss of membrane fragments leads to spherical shape in plasma (tonicity). Lyse readily in hypotonic solution.
- Remainder result from inheritance of two different defects (compound heterozygosity) and present as more severe form.
- Slow transit times because of shape (Erythrostasis).
- Cells accumulate lactic acid, do not generate ATP readily (do not extrude Sodium).

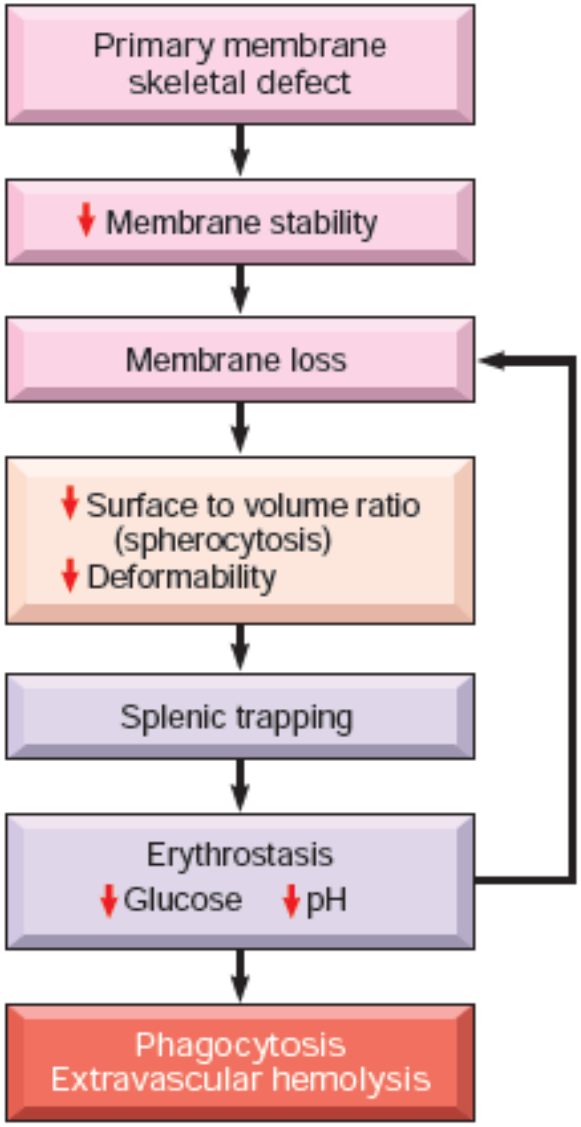


Figure 14-3 Pathophysiology of hereditary spherocytosis.

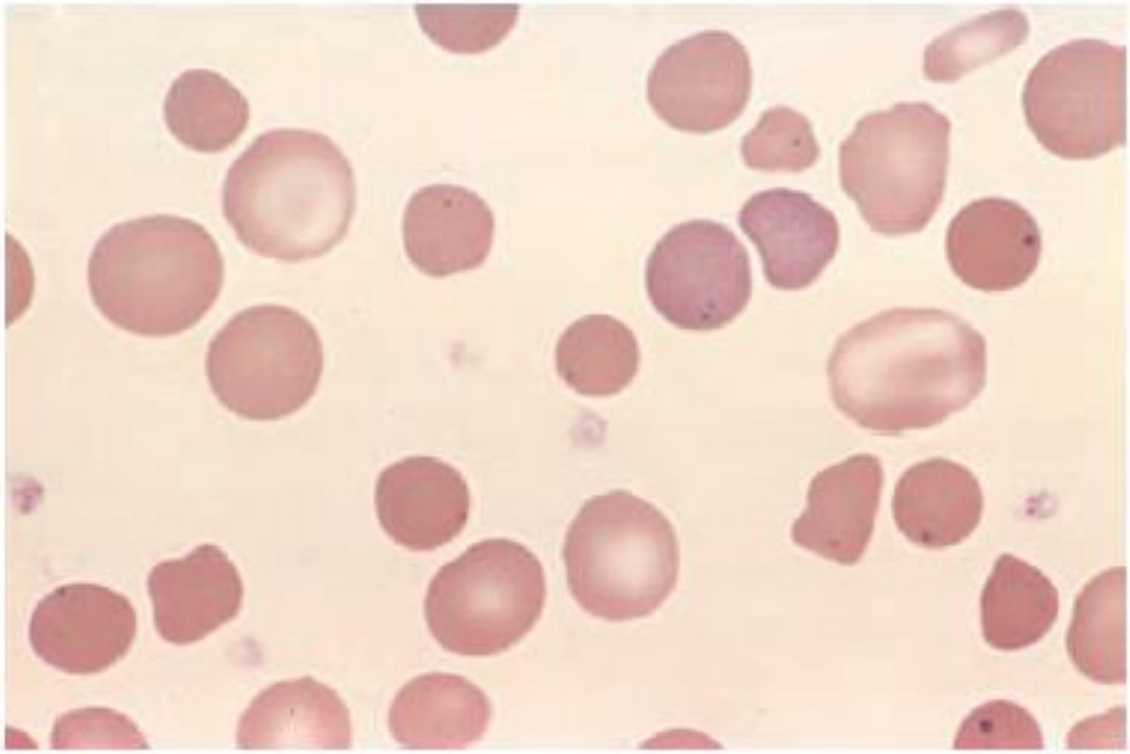


Figure 14-4 Hereditary spherocytosis (peripheral smear). Note the anisocytosis and several dark-appearing spherocytes with no central pallor. Howell-Jolly bodies (small dark nuclear remnants) are also present in red cells of this asplenic patient. (Courtesy Dr. Robert W. McKenna, Department of Pathology, University of Texas Southwestern Medical School, Dallas, Texas.)

G6PD deficiency

- Glucose-6-Phosphate Dehydrogenase (G6PD) is the only source of NADPH that protects the red cell against oxidative stresses.
- G6PD reduces NADP to NADH while oxidizing G6P.
- NADH provides protons required for conversion of oxidized glutathione to reduced glutathione.
- Reduced glutathione protects the cell against oxidant injury.
- Protein abnormally folded.
- Reduces ability of erythrocyte to withstand oxidative stress.

G6PD deficiency

- Leads to oxidation of sulfhydryl groups on hemoglobin, cross-link, then precipitate as Heinz bodies.
- Intravascular and extravascular hemolysis noted.
- X-linked recessive.
- Common in Eastern Mediterranean basin and Arabian peninsula (G6PD Mediterranean), northeastern corner of Africa (G6PDA).
- African form is less severe
- G6PD deficiency is X-linked recessive trait. (Xq28)
- As enzyme levels fall (aging), hemolysis results.

G6PD deficiency

- Fava beans and primaquine are known oxidant stresses.
- Oxidant exposure precipitates intravascular hemolysis.
- Self-limited when only enzyme rich young red cells remain.
- Viral hepatitis, pneumonia, typhoid fever as precipitants
- Vitamin E and Folic acid (anti-oxidants) may be protective.
- Anemia, hemoglobinemia, hemoglobinuria noted.
- Recovery phase heralded by reticulocytosis

Pyruvate kinase deficiency

- Autosomal recessive.
- PKLR gene at 1q22
- PEP does not transfer phosphate to ADP
- Chronic anemia.
- May present in utero (hydrops fetalis)
- Stabilizes by adulthood

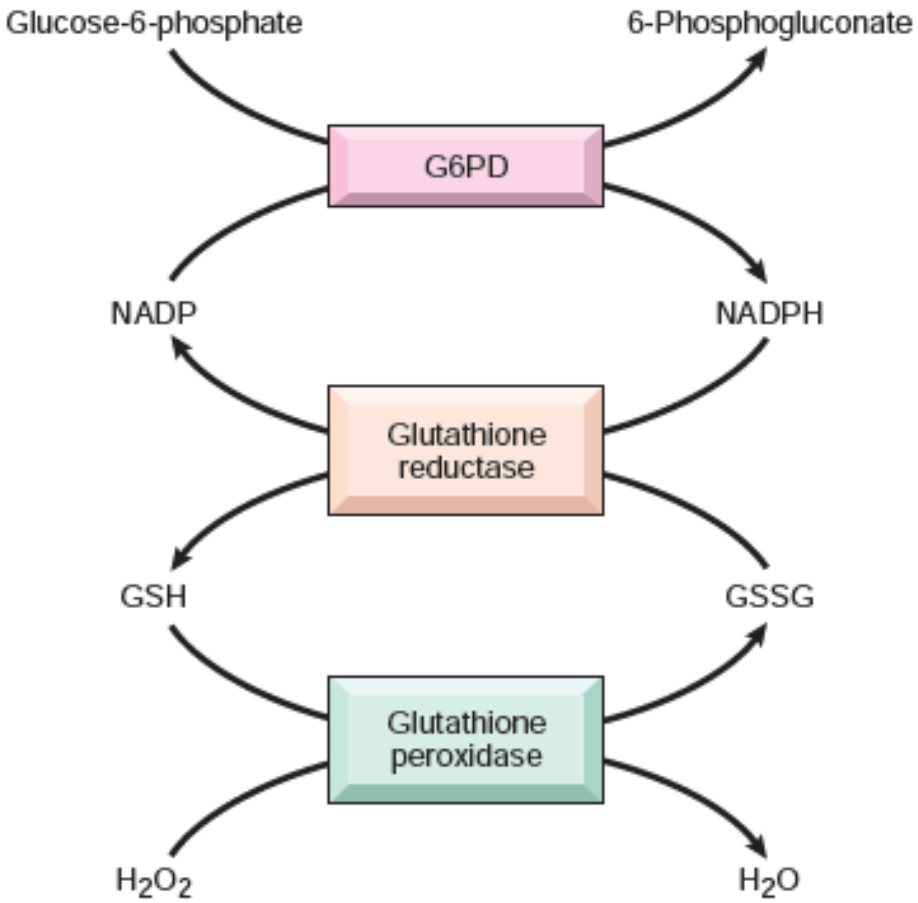


Figure 14-5 Role of glucose-6-phosphate dehydrogenase (G6PD) in defense against oxidant injury. The disposal of H_2O_2, a potential oxidant, is dependent on the adequacy of reduced glutathione (GSH), which is generated by the action of the reduced form of nicotinamide adenine dinucleotide (NADPH). The synthesis of NADPH is dependent on the activity of G6PD. GSSG, Oxidized glutathione.

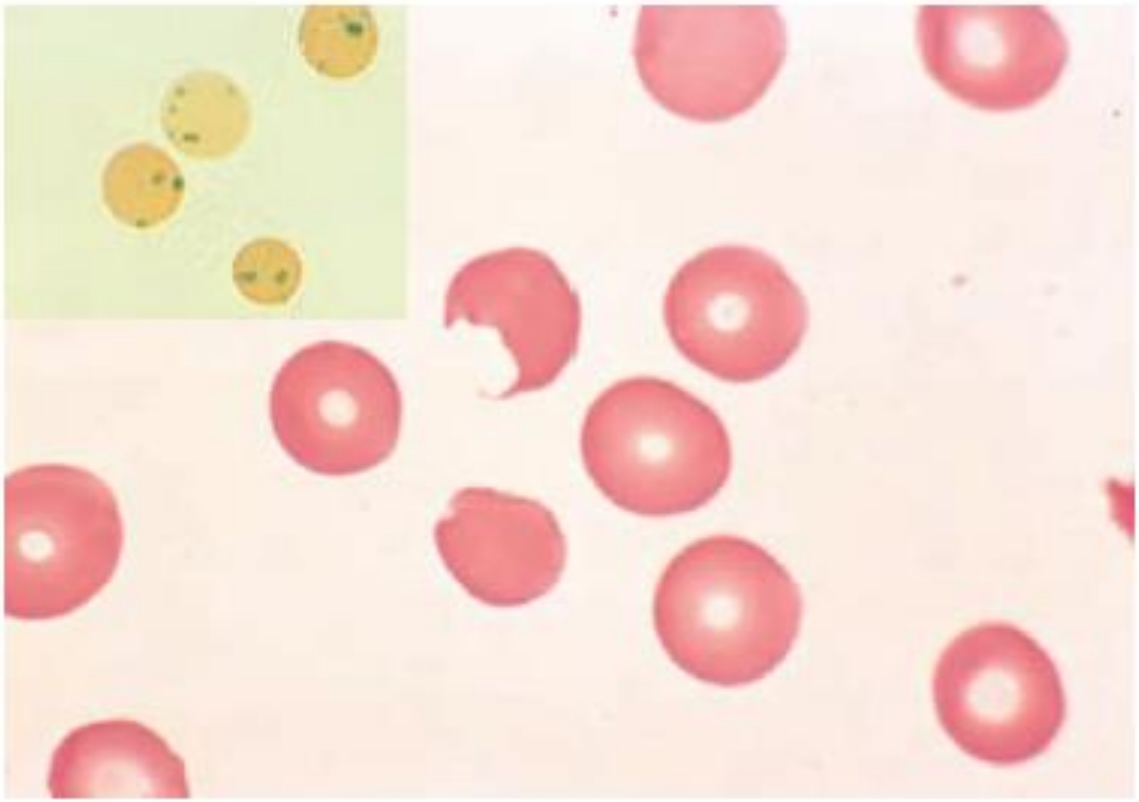


Figure 14-6 Glucose-6-phosphate dehydrogenase deficiency: effects of oxidant drug exposure (peripheral blood smear). *Inset,* Red cells with precipitates of denatured globin (Heinz bodies) revealed by supravital staining. As the splenic macrophages pluck out these inclusions, "bite cells" like the one in this smear are produced. (Courtesy Dr. Robert W. McKenna, Department of Pathology, University of Texas Southwestern Medical School, Dallas, Texas.)

PNH

- Paroxysmal nocturnal hemoglobinuria
- Chronic anemia (intravascular hemolysis)
- Increases complement activity
- 25%, paroxysmal and when asleep
- pH fall activates complement
- Venous thrombosis in 40% (hepatic, renal, cerebral veins frequently).
- Up to 10% will develop myelodysplastic syndrome.
- Diagnosed by flow cytometry.
- Marrow transplantation curative.

PNH

- Mutation in phosphatidylinositol glycan complementation group A (PIGA) gene at Xp22.2.
- All clonal progeny affected
- Glycosylphosphatidyl inositol protein produced by the PIGA gene serves to covalently link transmembrane proteins in plasma membrane.
- Affected cells lack complement decay accelerating factor (CD55)
- Deficiency of membrane inhibitor of reactive lysis (CD59), a potent inhibitor of C3 convertase
- Deficiency of C8 binding protein.
- Cells sensitive to C5b-C9 membrane attack complex.

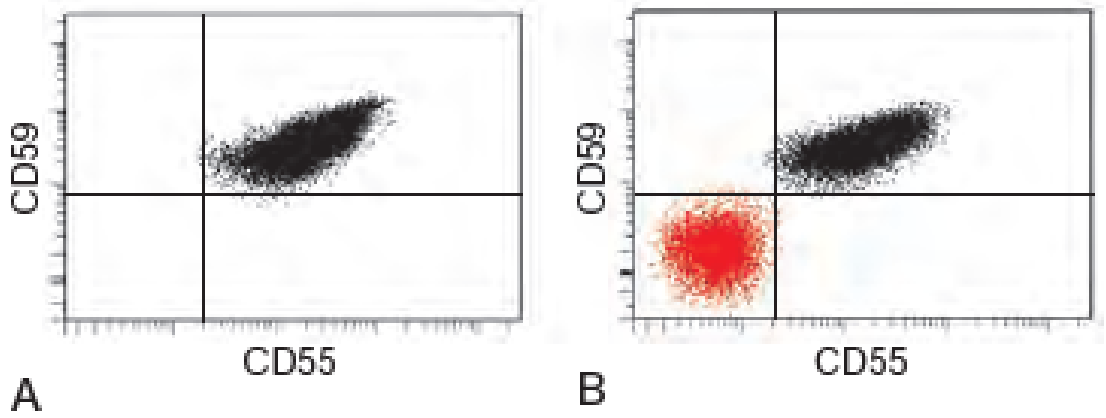


Figure 14-14 Paroxysmal nocturnal hemoglobinuria (PNH). **A,** Flow cytogram of blood from a normal individual shows that the red cells express two phosphatidylinositol glycan (PIG)-linked membrane proteins, CD55 and CD59, on their surfaces. **B,** Flow cytogram of blood from a patient with PNH shows a population of red cells that is deficient in both CD55 and CD59. As is typical of PNH, a second population of CD55+/CD59+ red cells that is derived from residual normal hematopoietic stem cells is also present. (Courtesy Dr. Scott Rodig, Department of Pathology, Brigham and Women's Hospital, Boston, Mass.)

Hemoglobinopathy

- Hemoglobinopathy may be associated with anemia.
- G6PD deficiency may be present with hemoglobinopathy.
- Hemoglobin S or C is seen in those with tropical African or South Mediterranean or coastal Indian Ocean ancestry.
- Hemoglobin electrophoresis only identifies Hemoglobins A, C, F, and S.
- MCV elevated in sickle cell disease while depressed in thalassemia.

Hemoglobinopathy

- Thalassemia is seen in those with tropical or coastal African, South Mediterranean, coastal Indian Ocean, or South Asian ancestry.
- Hemoglobin E is principally found in Indochina.
- Single point mutations are noted in β-globin.
- Elevated Hemoglobin F is suggestive of β-thalassemia.
- Hemoglobin A_2 is often elevated as a percentage of A.
- Measure Hemoglobin A_2 by column chromatography.

Sickle cell disease

- Hemolytic anemia, reticulocytosis common.
- Splenomegaly early in disease course
- Sequestration crisis results from splenic trapping of red cells with marked hypovolemia being noted.
- Late in the disease course, little splenic function noted. (Autosplenectomy)
- Sickling in renal medulla due to hypertonicity may lead to inability to concentrate urine (hyposthenuria)

Sickle cell disease

- Fetal hemoglobin (HbF) inhibits the polymerization of HbS
- Newborns do not manifest the disease until they are 5 to 6 months of age, when the amount of HbF in the cells falls close to adult levels.

Sickle cell disease crises

- Sequestration crises occur in children with intact spleens.
- Massive sequestration of sickled red cells leads to rapid splenic enlargement, hypovolemia, and sometimes shock.

Sickle cell disease crises

- Vascular occlusive (pain) crises represent episodes of hypoxic injury and infarction
- Commonly involve bones, lungs, liver, brain, spleen, and penis.
- May precipitate hypercoagulable state

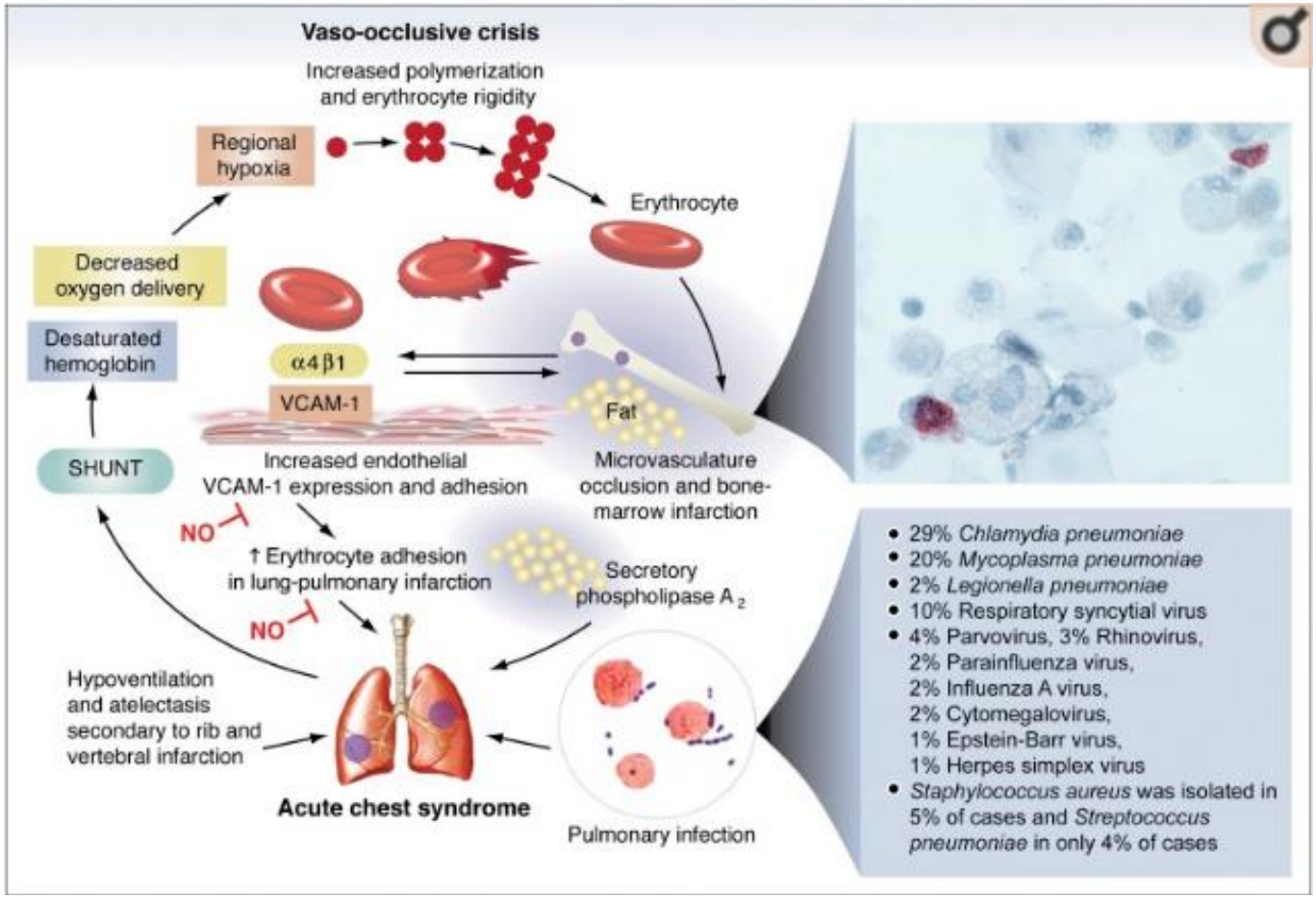
Sickle cell disease crises

- In children, painful bone crises are extremely common and often difficult to distinguish from acute osteomyelitis.
- May lead to fat embolism.
- Acute chest syndrome presents with fever, cough, chest pain, and pulmonary infiltrates.
- Reactive airways common
- Atelectasis common
- Systemic hypoxemia may result.
- At risk for secondary infection

Sickle cell disease crises

- Aplastic crises precipitated by Parvovirus B19 infection.
- there is a transient cessation of bone marrow erythropoiesis due to an acute infection of erythroid progenitor cells by parvovirus B19.
- Reticulocytes disappear from the peripheral blood, causing a sudden and rapid worsening of anemia.

Sickle cell disease crises



Stroke complications

- Peak incidence in cerebral infarction between the ages of 2-5 years and again between the ages of 35-45 years.
- Transcranial Doppler (TCD) screening should be done annually beginning at 2 years of age.
- TCD velocities >200 cm/sec associated with high stroke risk.

Renal disease complications

- There is an inability to concentrate urine
- Urine osmolality 400-450 mOsm/kg
- Renal tubular acidosis, type IV, characterized by low renin and aldosterone
- Hyperkalemia, acidosis, impaired ammonia production
- Hematuria if papillary necrosis
- Proteinuria common
- Priapism frequently occurs during sleep (mean duration, 125 minutes).

HbS

- Hemoglobin S involves a valine for glutamate substitution in the sixth codon of β-globin permits hemoglobin molecules to polymerize when deoxygenated
- Deoxygenated hemoglobin S forms needle-like fibers in red cells, leading to sickle deformation.
- Hemoglobin C involves a lysine for glutamate substitution in the sixth codon.
- HbSC cells are 50% HbS
- HbAS cells are 40% HbS

HbS

- Herniation of HbS polymers through the red cell membrane leads to Ca^{2+} entry and activation of a K^{+}-efflux ion channel with resultant intracellular dehydration.
- O_2 binding lost.
- Sickle cells express elevated levels of adhesion molecules as well as induce endothelial activation.
- Free hemoglobin released from lysed cell binds nitric oxide
- Leads to further vascular constriction and platelet activation.

HbS

- Higher HbS concentrations increase the probability that aggregation and polymerization will occur during any given period of deoxygenation.
- A decrease in pH reduces the O_2 affinity of hemoglobin.
- Intracellular dehydration, which increases the MCHC, facilitates sickling and vascular occlusion.
- Hemolysis and microvascular occlusion are facilitated by dehydration, acidosis, and long transit time in microvascular beds

HbS

- The nonpolar amino acid valine has replaced the polar surface residue Glu6 of the β subunit, generating a hydrophobic “sticky patch” on the surface of the β subunit of both oxy-HbS and deoxy-HbS.
- Both HbA and HbS contain a complementary sticky patch on their surfaces that is exposed only in the deoxygenated state.
- Thus, at low pO_2, deoxy-HbS can polymerize to form long, insoluble fibers.
- Binding of deoxy-HbA terminates fiber polymerization, as HbA lacks the second sticky patch necessary to bind another Hb molecule.

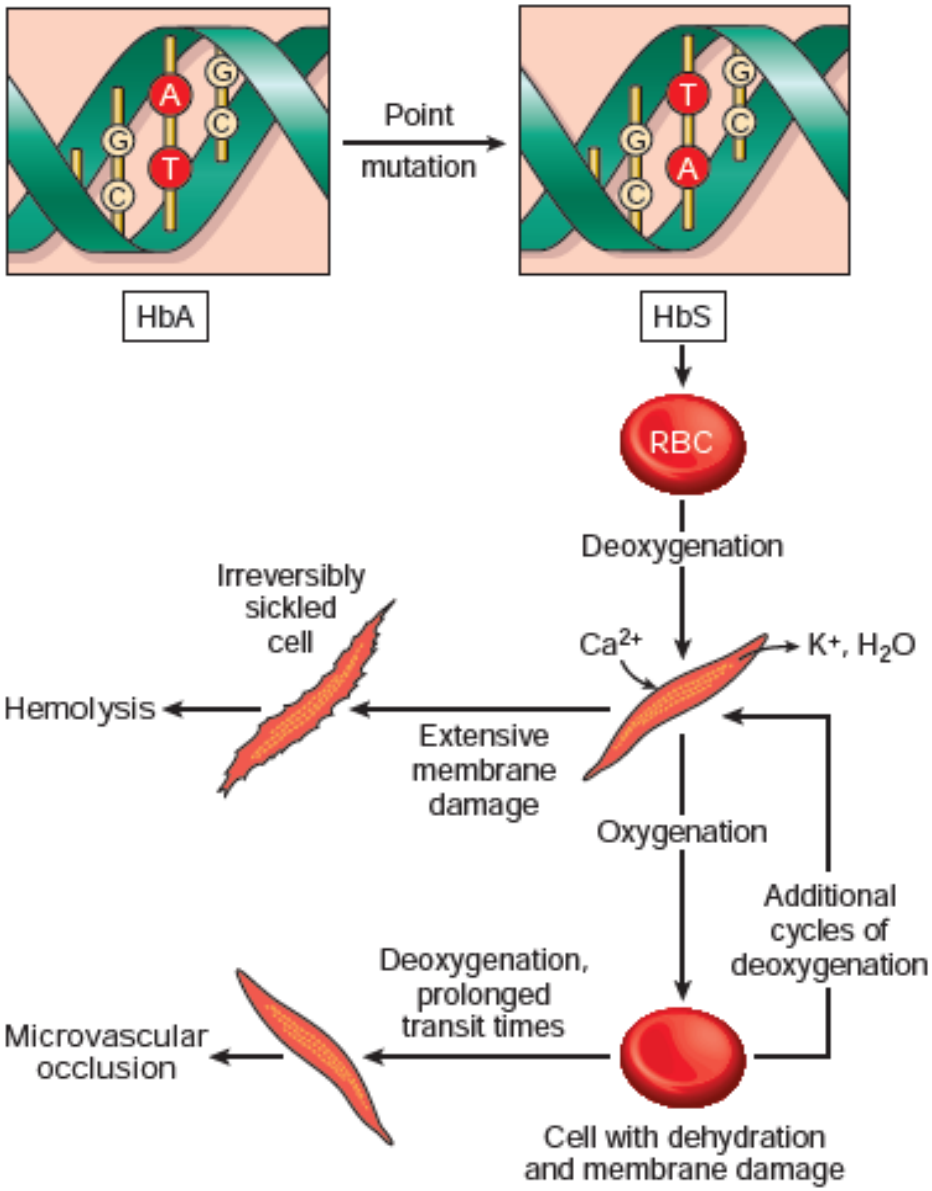


Figure 14-7 Pathophysiology of sickle cell disease.

Sickle cell trait

- Heterozygotes
- Approximately 40% of the hemoglobin is HbS, the rest being HbA, which interacts only weakly with HbS when deoxygenated.
- Both the relatively low concentration of HbS and the presence of interfering HbA act to prevent efficient HbS aggregation and polymerization
- Red cells in heterozygous individuals do not sickle except under conditions of severe hypoxia.

HbSC disease

- HbC (Lys6 substitution) has a greater tendency to form aggregates with deoxygenated HbS than does HbA.
- As a result, individuals with HbS and HbC have a symptomatic sickling disorder (designated HbSC disease) that is generally milder than sickle cell anemia.
- However, retinopathy more common with HbSC disease

Sickle-thalassemia

- Thalassemia reduces globin synthesis and limits the total hemoglobin concentration per cell.
- HbS-α- thalassemia is a milder disease.
- A decrease in pH reduces the O_2 affinity of hemoglobin.
- Normal transit times for red cells passing through capillaries are not sufficient for significant aggregation of deoxygenated HbS to occur.

Histopathology

- The peripheral blood demonstrates variable numbers of irreversibly sickled cells, reticulocytosis, and target cells, which result from red cell dehydration.
- Howell-Jolly bodies (small nuclear remnants) are also present in some red cells due to the asplenia.
- The bone marrow is hyperplastic as a result of a compensatory erythroid hyperplasia.
- Expansion of the marrow leads to bone resorption and secondary new bone formation, resulting in prominent cheekbones and changes in the skull that resemble a “crewcut” on x-ray studies.
- Extramedullary hematopoiesis can also appear.

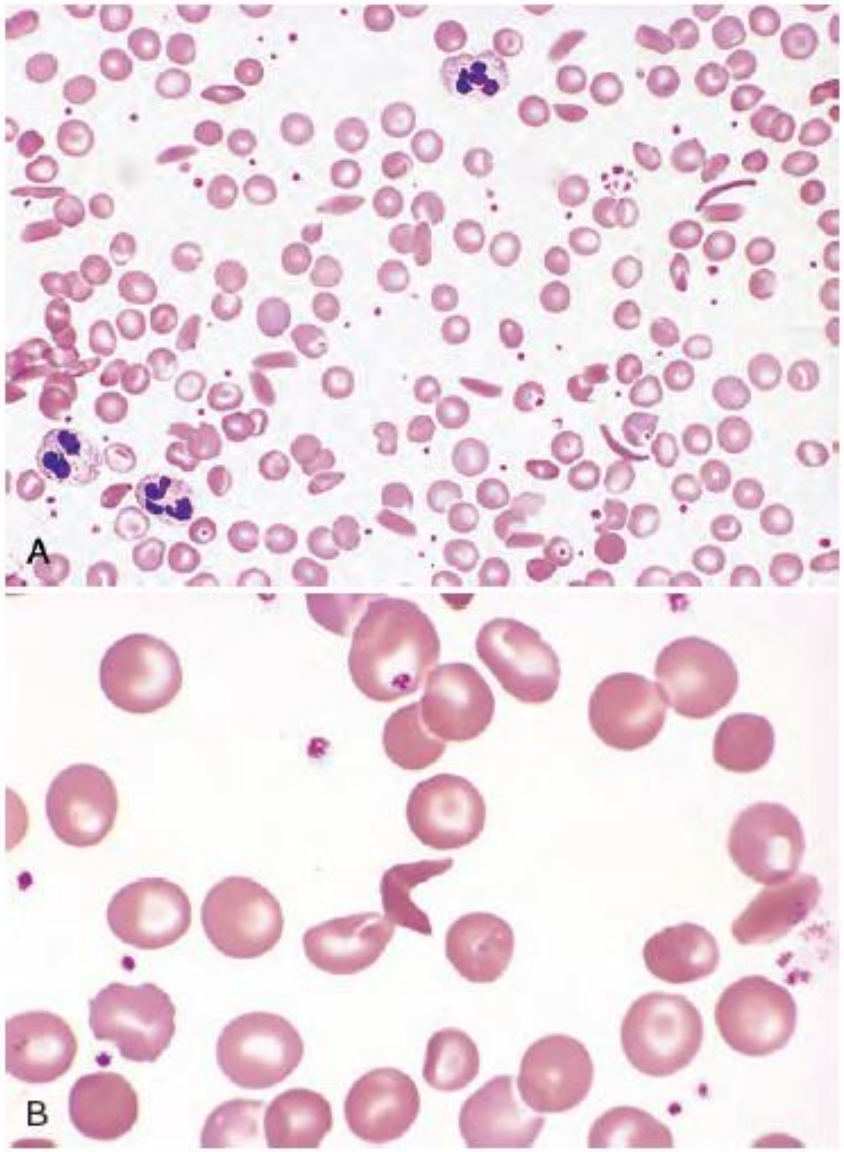


Figure 14-8 Sickle cell disease (peripheral blood smear). **A,** Low magnification shows sickle cells, anisocytosis, and poikilocytosis. **B,** Higher magnification shows an irreversibly sickled cell in the center. (Courtesy Dr. Robert W. McKenna, Department of Pathology, University of Texas Southwestern Medical School, Dallas, Texas.)

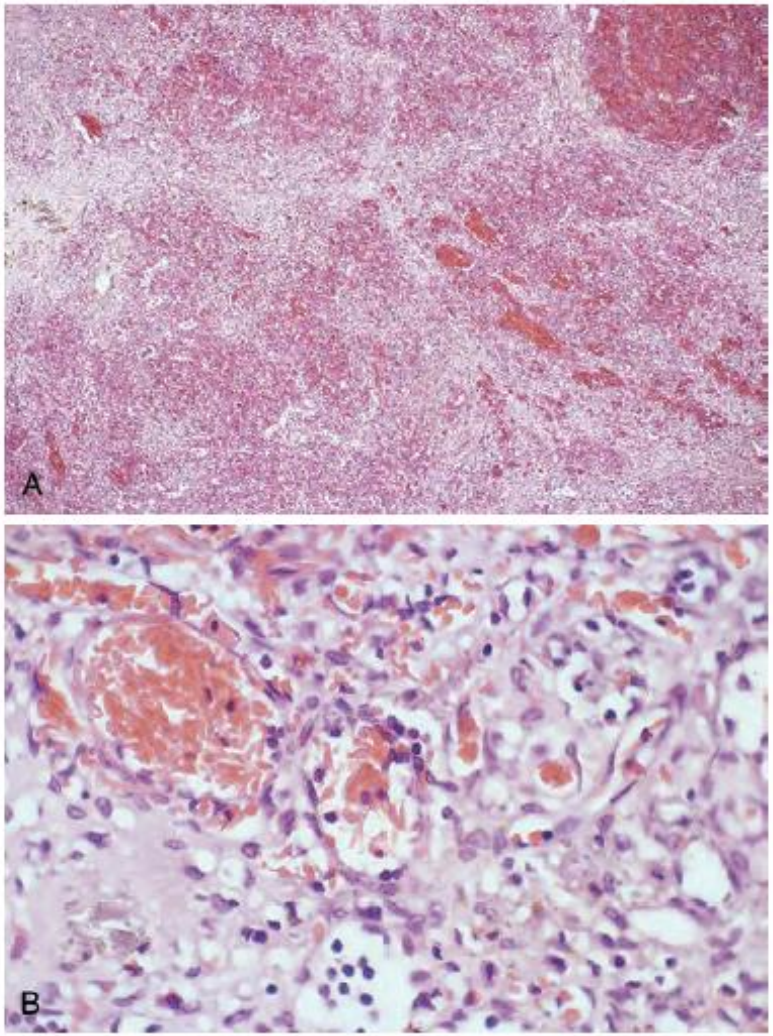


Figure 14-9 **A,** Spleen in sickle cell disease (low power). Red pulp cords and sinusoids are markedly congested; between the congested areas, pale areas of fibrosis resulting from ischemic damage are evident. **B,** Under high power, splenic sinusoids are dilated and filled with sickled red cells. (Courtesy Dr. Darren Wirthwein, Department of Pathology, University of Texas Southwestern Medical School, Dallas, Texas.)

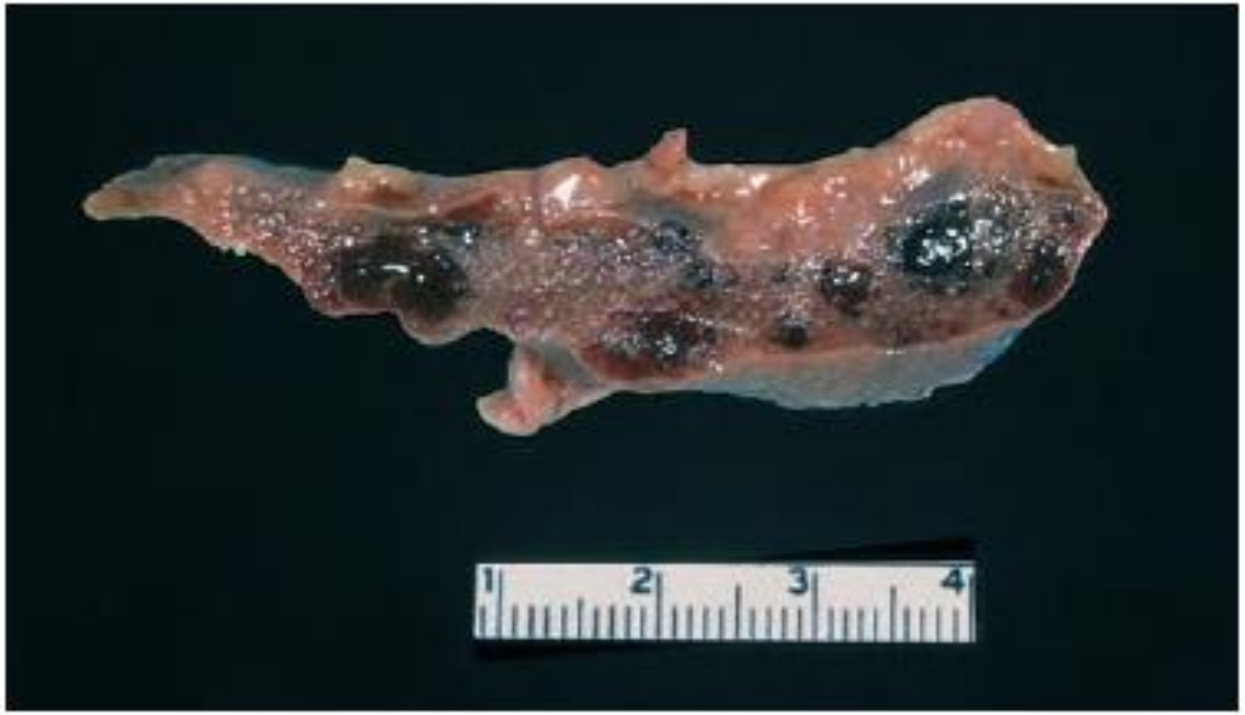


Figure 14-10 "Autoinfarcted" splenic remnant in sickle cell disease. (Courtesy Drs. Dennis Burns and Darren Wirthwein, Department of Pathology, University of Texas Southwestern Medical School, Dallas, Texas.)

α-thalassemia

- Production of the globin chain is affected.
- A decrease in α-globins allows for greater tetrameric hemoglobin formation by β-chains (and vice versa).
- Two loci encode the β-chain on chromosome 11; four loci encode the α-chain on chromosome 16.
- The severity of α-thalassemia is correlated with the number of affected loci.

α-thalassemia

- Four α genes deleted.
- HbH (δ4) and Hb Barts (γ4) associated with death in utero.
- Three α genes deleted
- HbH disease
- -/- -/α genotype
- HbH, Hb Barts, and Hb A_2 found.
- Hemoglobin A_2 levels are low
- Higher O_2 binding occurs, resulting in poor O_2 delivery to tissue.
- Childhood death.

α-thalassemia

- Two α genes deleted
- Thalassemia trait.
- -/- α/α genotype noted in Asia
- -/α -/α genotype noted in Africa.
- HbA_2 and Hb Barts found.
- Occurs early in midlife.
- Anemia and splenomegaly.
- One α gene deleted
- The carrier state
- Normal HbA_1.

β-thalassemia

- Thalassemia is seen in those with tropical or coastal African, South Mediterranean, coastal Indian Ocean, or South Asian ancestry.
- Anemia, anisocytosis and poikilocytosis, hypochromia, basophilic stippling noted.
- Expansion of hematopoietic marrow (with new bone formation in a sunburst appearance)
- Extramedullary hematopoiesis noted.

β-thalassemia

- Excess α-globins bind red cell membranes, producing membrane damage, and, at high concentrations, form toxic aggregates.
- Apoptosis follows
- May see extravascular hemolysis
- Ineffective erythropoiesis
- Hepcidin suppressed
- Intestinal iron absorption increased

β-thalassemia

- Thalassemia major
- Both β genes are affected.
- HbF and HbA_2 are the main isotypes.
- Poor O_2 carrying capacity.

β-thalassemia

- Thalassemia intermedia
- Both genes are affected
- Severity related to β^+ or β^0 mutation
- β^+, some β-globin formation
- β^0, no β-globin formation
- Tetramers do not form
- HbA_1 is present in greater quantity than in thalassemia major.

β-thalassemia

- Thalassemia minor
- Only one gene is affected.
- HbA_1 is normal
- HbA_2 is increased.

β-thalassemia genes

- Mutations are characterized as to whether they prevent (β^0) or permit (β^+) formation of β-chains.
- Splicing, promoter region mutations common in β^+ disease.
- May also see promoter gene mutations
- Both lead to some β-globin formation
- Chain termination mutations common in β^0 disease.
- Stop codon introduced or frame shift
- No β-globin formation
- No tetramers

β-thalassemia

- It is possible to have β-thalassemia and Hb S together.
- Elevated Hemoglobin F is suggestive of β-thalassemia.
- Hemoglobin A_2 is often elevated as a percentage of A.
- Measure Hemoglobin A_2 by column chromatography.

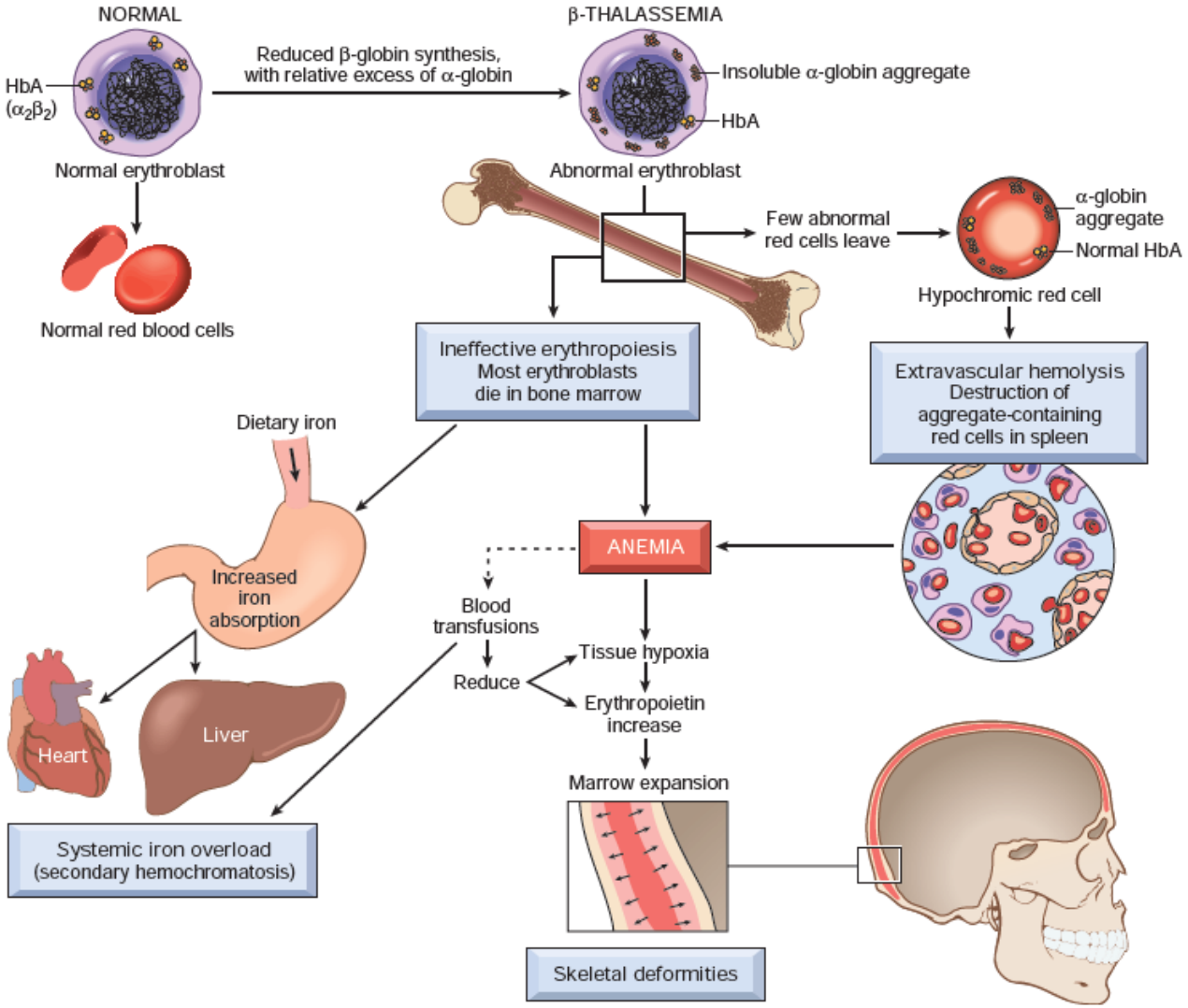


Figure 14-12 Pathogenesis of β-thalassemia major. Note that the aggregates of unpaired α-globin chains, a hallmark of the disease, are not visible in routinely stained blood smears. Blood transfusions are a double-edged sword, diminishing the anemia and its attendant complications, but also adding to the systemic iron overload.

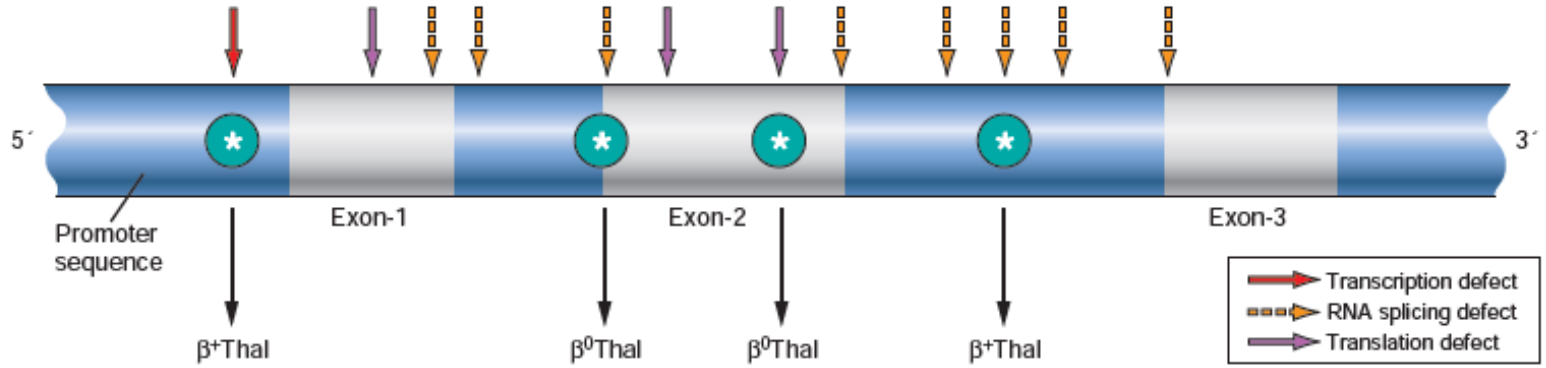


Figure 14-11 Distribution of β-globin gene mutations associated with β-thalassemia. Arrows denote sites where point mutations giving rise to β^0 or β^+ thalassemia have been identified.

11q.

Elevated red cell counts

- Chronic tobacco use, chronic lung disease, residence at high altitude are associated with low arterial oxygen tension and may lead to elevated red cell counts.
- Elevated erythropoietin levels require a search for tumor source
- Renal carcinoma or cyst, cerebellar hemangioblastoma, uterine leiomyoma, hepatoma.

Polycythemia vera

- Polycythemia vera patients generally present following a thrombotic or hemorrhagic event.
- Headache, weakness, epigastric distress, and pruritis may be present.
- Polycythemia vera is part of the spectrum of myelodysplastic syndrome
- Diagnosed with determination of red cell mass and bone marrow examination.
- Erythropoietin levels are low.

Therapy of Iron deficiency anemia

- The intestine cannot absorb more than 50-60 mg/d of elemental Iron.
- An individual with a normally functioning marrow and appropriate erythropoietin stimulus should absorb 50 mg/d if receiving a daily oral dose of 200–300 mg of ferrous sulfate.
- This should double red cell production.
- 1 mg/d (men), 1.4 mg/d (women) normal iron need.

Therapy of Iron deficiency anemia

- Sustained treatment for a period of 6–12 months after correction of the anemia will be necessary to provide stores of at least 0.5–1.0 g of iron.
- The reticulocyte count should begin to increase within 4–7 days after initiation of therapy and peak at 1½ weeks.
- It may reach 15%.

Treatment of macrocytic anemias

- Replenishment of body stores of vitamin B12 should be complete with six 1000-mg IM injections of hydroxocobalamin given at 3- to 7-day intervals.
- There is no evidence that more frequent injections produce a better response in patients with neuropathy.
- For maintenance therapy, 1000 mg hydroxocobalamin sublingually weekly is satisfactory.
- Cyanocobalmin is not as well absorbed.
- Oral doses of 5–15 mg folic acid daily are satisfactory to treat folic acid deficiency..

Treatment of hypoproliferative anemias

- Endogenous erythropoietin levels are inappropriately low in the hypoproliferative anemias.
- Iron stores must be restored to obtain optimal effects from erythropoietin.
- Hemoglobin levels of 10–12 g/dL are usually reached within 4–6 weeks if iron levels are adequate.
- Once a target hemoglobin level is achieved, the erythropoietin dose can be decreased.

Treatment of hypoproliferative anemias

- >90% of those with chronic renal disease respond to erythropoietin therapy.
- However, only 60% of cancer patients respond to erythropoietin therapy.
- There is an increased risk of vascular thrombosis with erythropoietin therapy.
- A fall in hemoglobin level occurring in the face of erythropoietin therapy usually signifies the development of an infection or iron depletion.

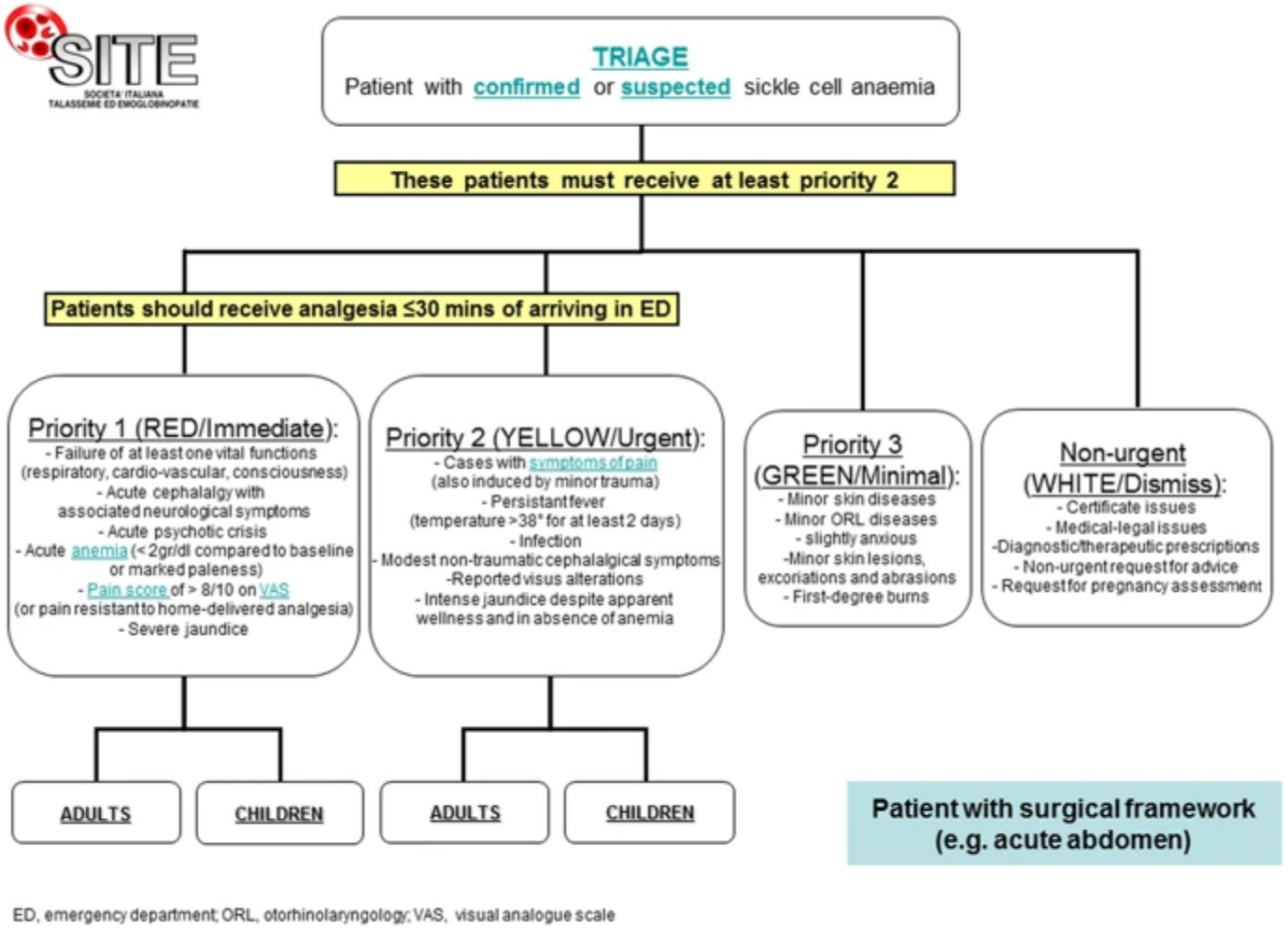
Treatment of hypoproliferative anemias

- Aluminum toxicity and hyperparathyroidism can also compromise the erythropoietin response in dialysis patients.
- When an infection intervenes, it is best to interrupt the erythropoietin therapy and rely on transfusion to correct the anemia until the infection is adequately treated.

Treatment of sickle cell disease

- Hydroxyurea inhibits DNA synthesis and leads to increased HbF levels, limiting hemoglobin polymerization.
- Stroke risk reduced by frequent transfusions
- Proteinuria may respond to ACE inhibitors
- Priapism responds to injection with α-adrenergic drugs.
- Both sequestration crises and the acute chest syndrome may require treatment with exchange transfusions if the patient is to survive.
- Allogenic marrow transplant often curative in severe hemoglobinopathy

Fig. 1



ED, emergency department; ORL, otorhinolaryngology; VAS, visual analogue scale

Algorithm for the management of patients with sickle cell disease in the emergency department. Figure reproduced with permission from Forni et al. 2014 [35]

Forni GL, Finco G, Graziadei G, Balocco M, Rigano P, Perrotta S, et al. Development of interactive algorithm for clinical management of acute events related to sickle cell disease in emergency department. Orphanet J Rare Dis. 2014;9:91. doi: 10.1186/1750-1172-9-91