

# Anemia

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# Definition

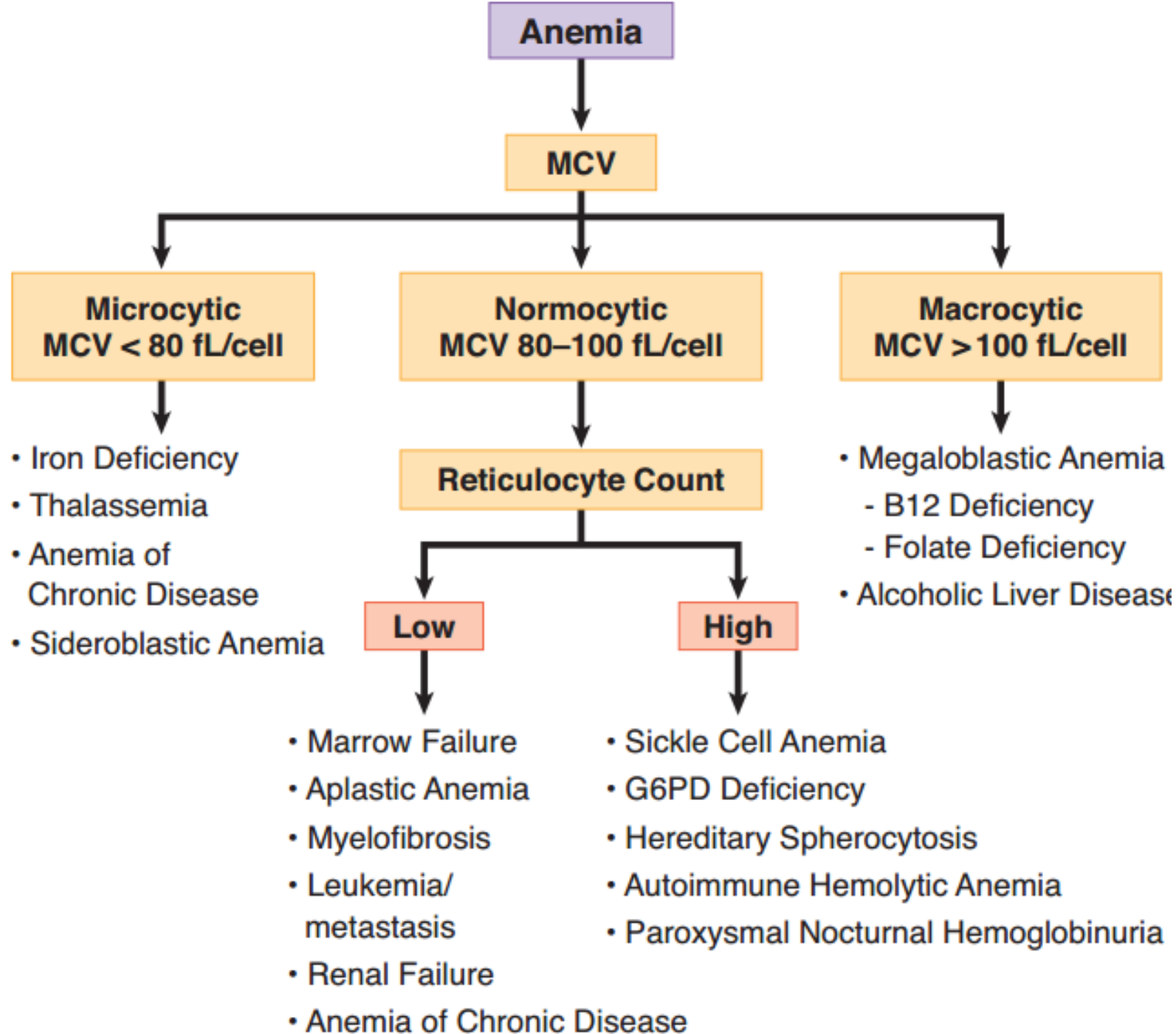
Anemia is defined as a reduction of the total circulating red cell mass below normal limits

In practice, anemia is usually diagnosed based on a reduction in the **hematocrit** (the ratio of packed red cells to total blood volume) and the **hemoglobin concentration** of the blood to levels that are below the normal range.

# Classification of Anemia

Size and morphology and degree of hemoglobinization

- Microcytic hypochromic
- Normocytic normochromic
- Macrocytic



## Morphologic Classification of Anemia

### Macrocytic

- Megaloblastic

- Alcohol use

- Liver disease

- Hypothyroidism

- Reticulocytosis

- Primary bone marrow disease

### Microcytic

- Iron deficiency

- Anemia of chronic disease  
inflammation

- Thalassemias

- Sideroblastic anemias

### Normocytic

- Anemia of chronic disease/in-  
flammation

- Anemia of renal disease

- Acute blood loss

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# Classification of Anemia

Based on underlying pathophysiological mechanism

- Reduced production
- Ineffective production
- Increased destruction
- Blood loss

# Classification of Anemia

## Reduced production

- Aplastic anemia
- Pure red cell aplasia
- Iron deficiency anemia
- Anemia of renal disease
- Anemia of chronic disease
- Myelophthisic anemia
- Anemia of lead poisoning

# Classification of Anemia

## Ineffective production

Megaloblastic anemia

### $\beta$ Thalassemias

- Homozygous thalassemia (thalassemia major)
- Heterozygous thalassemia (thalassemia minor)

### $\alpha$ Thalassemias

- Silent carrier  $\alpha$  thalassemia  $\alpha$ Thalassemia trait
- Hemoglobin H disease
- Homozygous  $\alpha$  thalassemia (hydrops fetalis)

# Classification of Anemia

## Increased destruction

- Extravascular hemolysis

  - Membranopathy: spherocytosis and elliptocytosis, acanthocytosis

  - Hemoglobinopathy: Sickle cell disease, Hemoglobin C and Hemoglobin E disease

  - Warm antibody induced immune hemolytic anemia

- Extravascular or intravascular hemolysis

  - Cold antibody induced immune hemolytic anemia

  - G6PD deficiency

- Intravascular hemolysis

  - Cold hemolysin disease

  - Mechanical red cell fragmentation syndrome

  - Paroxysmal nocturnal hemoglobinuria

# Clinical manifestations of anemia

Pallor, Weakness, malaise, easy fatigability and dyspnea on mild exertion.

Hypoxia can cause fatty change in the liver, myocardium, and kidney.

Progression of fatty changes in the myocardium can lead to heart failure further aggravating tissue hypoxia

Central nervous system hypoxia can cause headache, dimness of vision, and faintness.

# Characteristics of human red cells

# Morphologic Features of Various Anemias

| Red cell abnormality                                                                            | Causes                                                                | Red cell abnormality                                                                                     | Causes                                                              |
|-------------------------------------------------------------------------------------------------|-----------------------------------------------------------------------|----------------------------------------------------------------------------------------------------------|---------------------------------------------------------------------|
|  Normal        |                                                                       |  Microspherocyte       | Hereditary spherocytosis, autoimmune haemolytic anaemia, septicæmia |
|  Macrocyte     | Liver disease, alcoholism. Oval in megaloblastic anaemia              |  Fragments             | DIC, microangiopathy, HUS, TTP, burns, cardiac valves               |
|  Target cell   | Iron deficiency, liver disease, haemoglobinopathies, post-splenectomy |  Elliptocyte           | Hereditary elliptocytosis                                           |
|  Stomatocyte   | Liver disease, alcoholism                                             |  Tear drop poikilocyte | Myelofibrosis, extramedullary haemopoiesis                          |
|  Pencil cell  | Iron deficiency                                                       |  Basket cell          | Oxidant damage—e.g. G6PD deficiency, unstable haemoglobin           |
|  Echinocyte  | Liver disease, post-splenectomy                                       |  Sickle cell        | Sickle cell anaemia                                                 |
|  Acanthocyte | Liver disease, abetalipoproteinaemia, renal failure                   |  Microcyte          | Iron deficiency, haemoglobinopathy                                  |

# Anemia Caused by Decreased Red Blood Cell Production

Iron deficiency anemia is the most common and will be discussed in a separate lecture

# Aplastic Anemia

A disorder of pluripotential stem cells that leads to bone marrow failure, with a reduction of all precursor elements within the bone marrow

Bone marrow demonstrates variably reduced cellularity, increase in the amount of fat

## **Etiology of Aplastic Anemia**

Idiopathic (two thirds of cases)

Ionizing radiation

Drugs

Chemotherapeutic agents

Chloramphenicol

Anticonvulsants

Nonsteroidal antiinflammatory  
agents

Gold

Chemicals

Benzene

Viruses

Hepatitis C virus (HCV)

Epstein-Barr virus (EBV)

HIV

Parvovirus B19

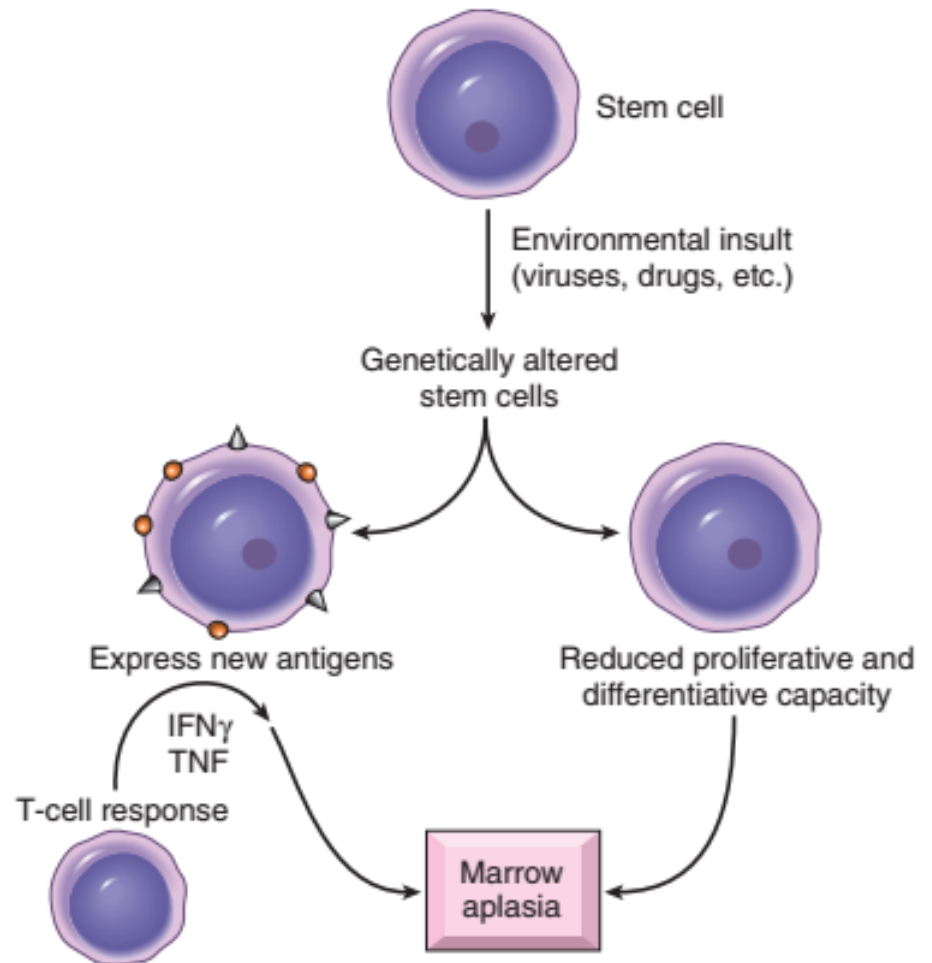
Hereditary

Fanconi anemia (germline *FAC*  
mutation leading to chromosomal  
instability)

# Aplastic Anemia

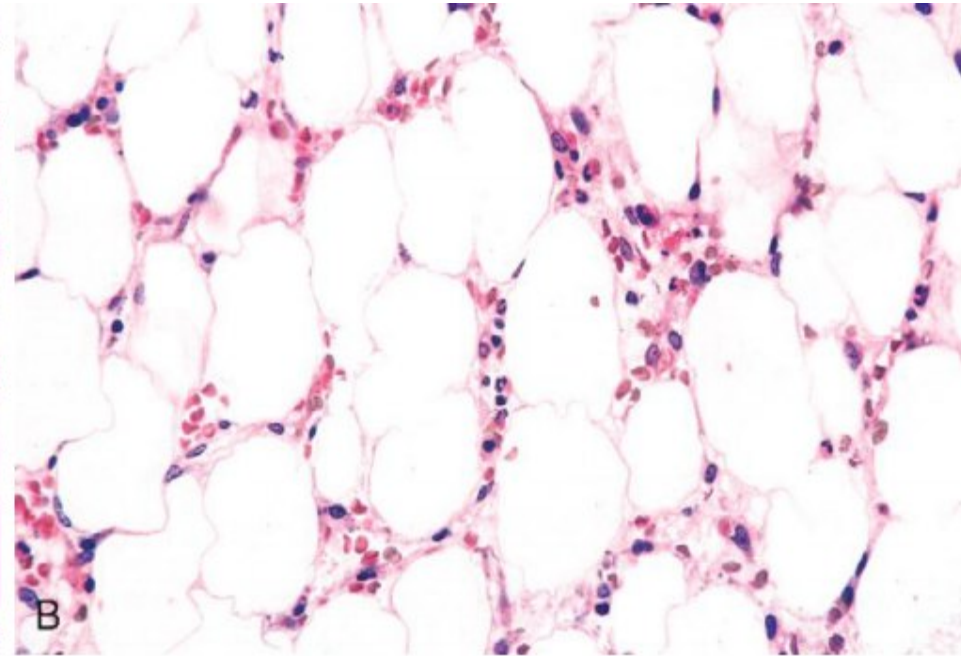
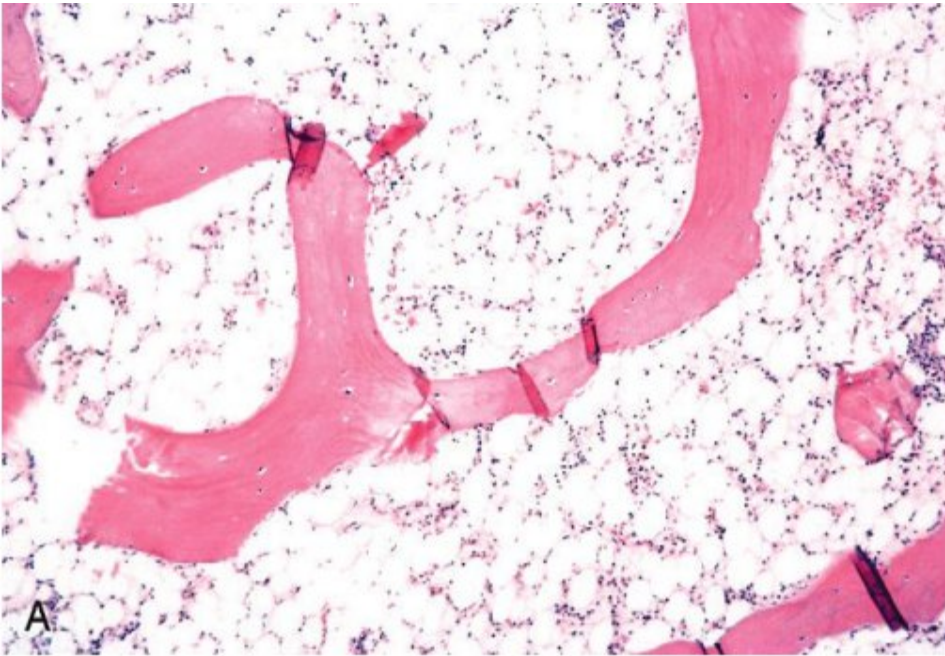
## Pathogenesis

- Extrinsic, immune-mediated suppression of marrow progenitors
  - Upregulated genes involved in apoptosis
  - Recovery in counts after T cell targeting therapy
- Intrinsic abnormality of stem cells
  - Karyotypic aberrations
  - Progression to cancerous and precancerous



# Aplastic Anemia

## biopsy under light microscopy



# Aplastic Anemia

## Clinically:

- weakness, fatigue, infections, and bleeding
- Fanconi anemia: first decade of life presentation, hypoplastic thumbs, absent radii, skin pigmentation, and renal anomalies

## Peripheral blood

- Anemia, leukopenia (primarily granulocytopenia) and thrombocytopenia
- Red blood cells are often mildly macrocytic
- Reticulocytes very low or not identified

## The treatment of aplastic anemia:

- Immunosuppressive therapy
- Bone marrow or stem cell transplantation.

# Pure Red Cell Aplasia (PRCA)

Selective lack of erythroid precursor production in the bone marrow

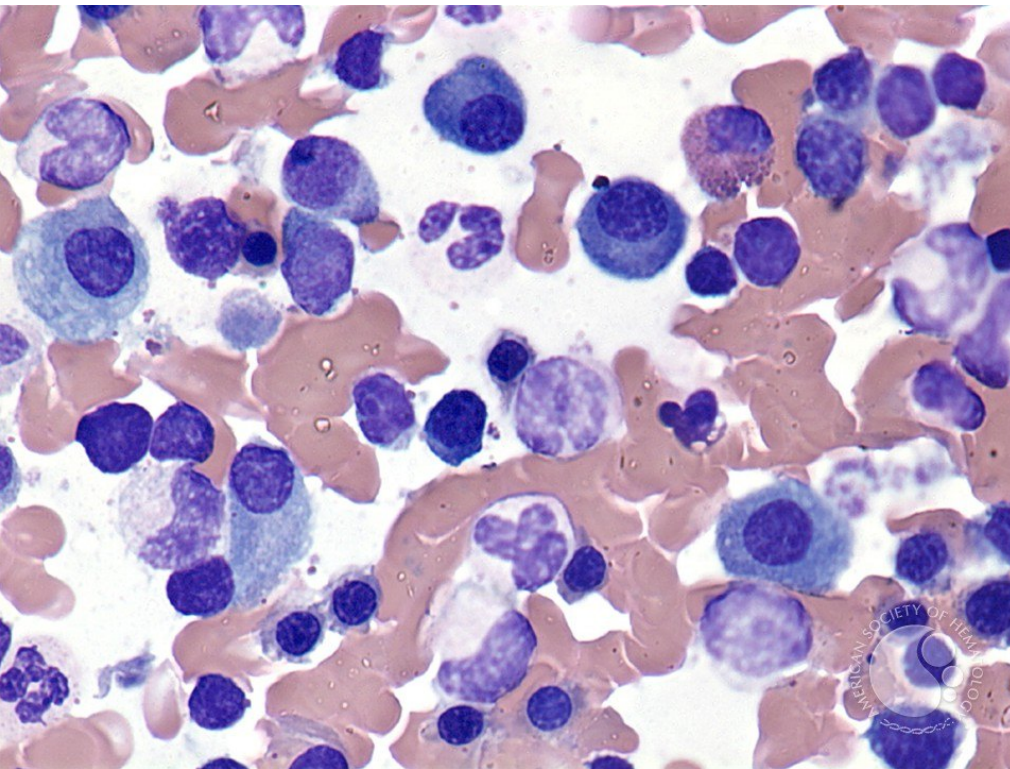
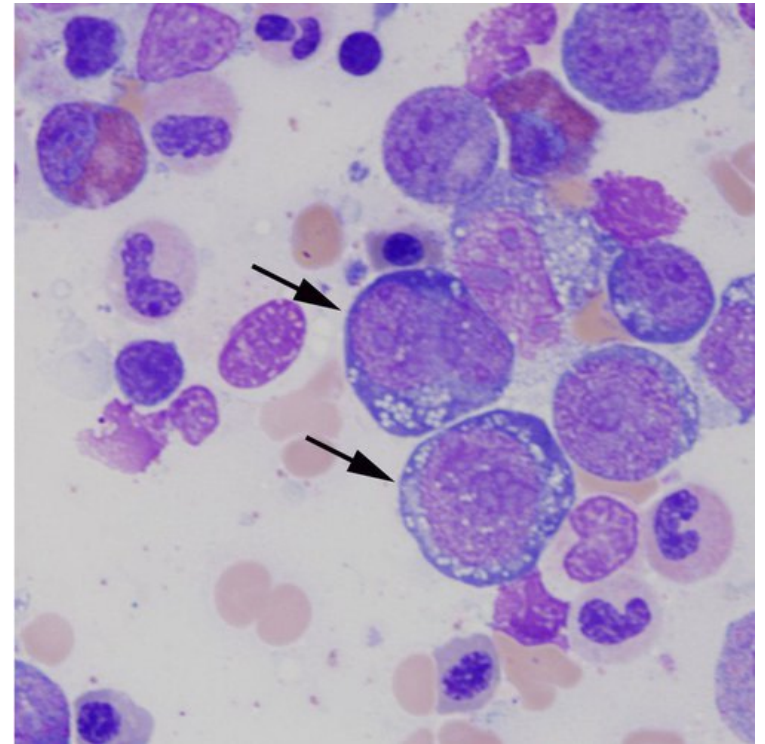
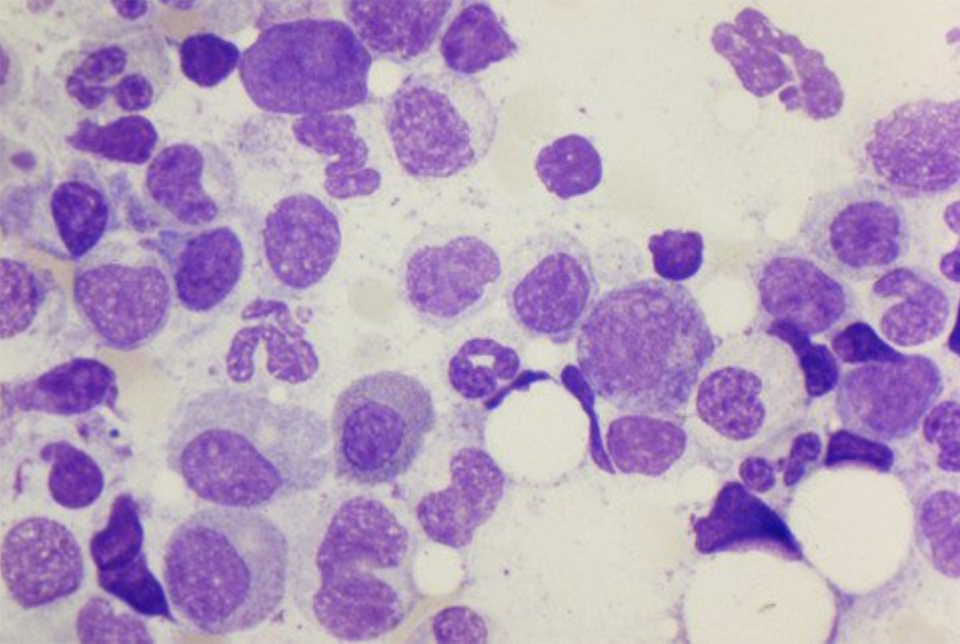
## Etiology

- Idiopathic
- Viral infections especially Parvovirus 19
- Thymic lesions i.e. thymoma
- Autoimmune/collagen vascular disorders
- Hereditary, Diamond-Blackfan anemia, infants less than 1 year

## Microscopically

- Peripheral blood: moderate to severe macrocytic anemia, increased erythropoietin, reticulocyte count very low
- Bone marrow is normocellular, selective absence of erythroid precursors
- In cases of parvovirus B19 infection, nuclear inclusions may be found

Treatment involves transfusion, in cases of thymic lesion, thymectomy, Parvovirus B19 associated are often self limiting



# Anemia of Renal Disease

Anemia of chronic renal insufficiency is multifactorial

- Decreased erythropoietin production
- A uremic toxin suppression of erythropoiesis
- Malnutrition
- Blood loss e.g. dialysis, bleeding from uremic PLT dysfunction
- Reduced RBCs survival

Examination of the blood reveals

- Normocytic, normochromic anemia
- Cells containing scalloped membranes (echinocytes, burr cells)
- If hypertension red cell fragments and schistocytes

Treatment involves administration of recombinant erythropoietin

# Anemia of Chronic Disease

Chronic inflammatory conditions, malignancies

Pathophysiology

- Ineffective use of iron stored within bone marrow macrophages (↑ hepcidin).
- Effects from inflammatory cytokines.

The bone marrow: increased amounts of stored iron by Prussian blue staining.

Examination of the blood: mild to moderate micro/normocytic anemia, decreased serum iron levels.

The total iron binding capacity is decreased

Treatment: control of underlying disease.

# Anemias of Blood Loss

## Acute blood loss

Massive hemorrhage can lead to cardiovascular collapse, shock, and death

After bleeding the blood volume is rapidly restored by the intravascular shift of water from the interstitial fluid compartment resulting in hemodilution

Increased secretion of erythropoietin from the kidney to stimulate more erythropoiesis

Bleeding into tissues: the iron in hemoglobin will be recaptured

Bleeding external: iron loss leads to iron deficiency, which can hamper the restoration of normal red cell counts.

# Anemias of Blood Loss

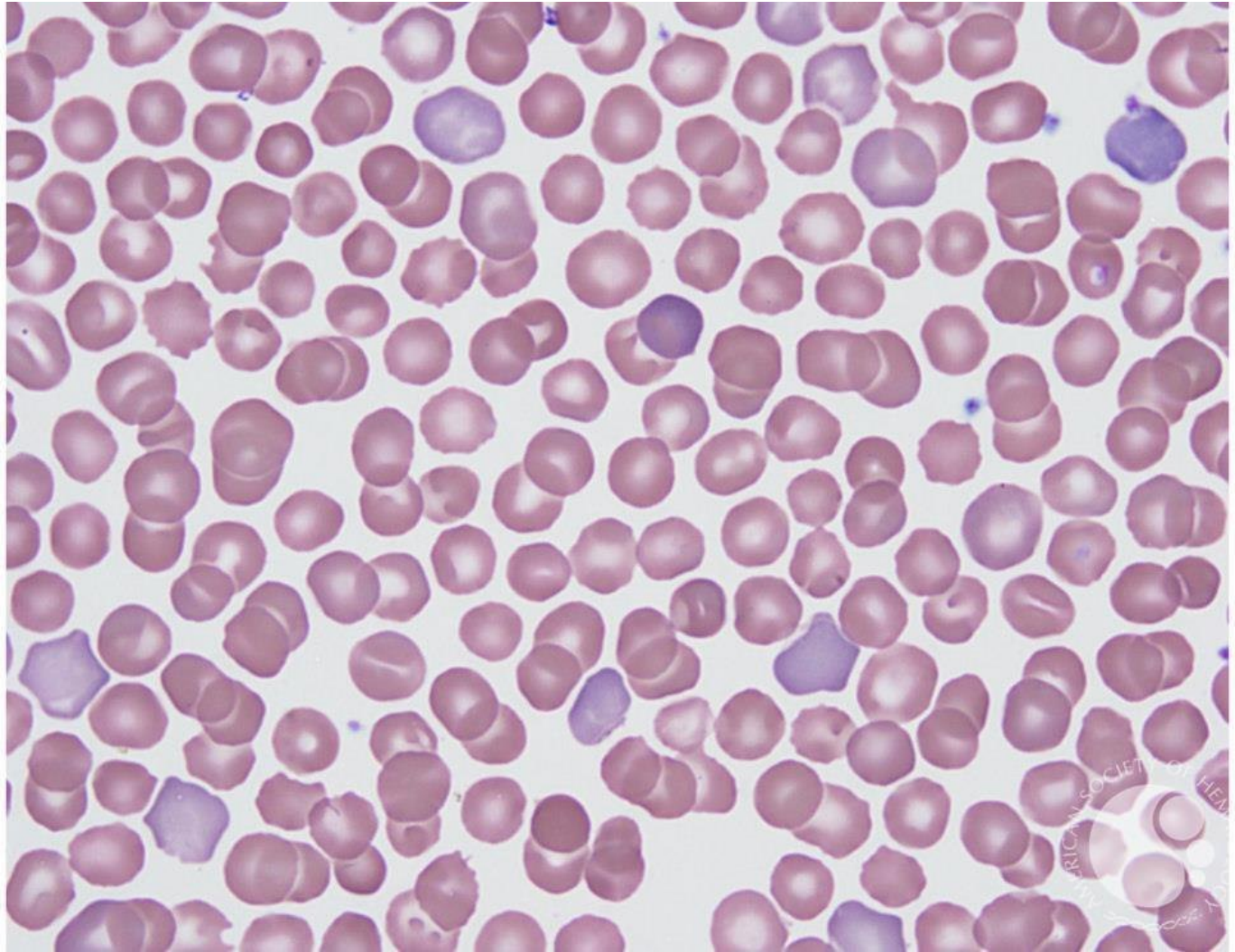
## Acute blood loss

Significant bleeding results in predictable changes in other blood cells

- Leukocytosis,
- Thrombocytosis
- Reticulocytosis

10% to 15% after 7 days,

Reticulocytes are larger in size than normal red cells (macrocytes) and have a blue-red polychromatophilic cytoplasm, on the blood smear, this is called polychromasia



# Anemias of Blood Loss

## chronic blood loss

When the rate of loss exceeds the regenerative capacity of the marrow

Depletion of iron reserves leads to iron deficiency anemia

# Hemolytic Anemias

Shortened RBCs life span, increase in erythropoiesis and accumulation of hemoglobin degradation products

If uncomplicated, the excess serum bilirubin is **unconjugated**. When the liver is normal, jaundice is rarely severe.

Excessive bilirubin excreted by the liver

- Gastrointestinal tract leads to increased formation and fecal excretion of urobilin,
- Gallstones derived from heme pigments.

In the great majority of hemolytic anemias, hemolysis occur in phagocytes, referred to as extravascular, less commonly intravascular hemolysis predominate

# Extravascular (EV) hemolysis

Reduced deformability of RBCs make it difficult to navigate splenic sinusoids leading to their sequestration and phagocytosis by macrophages mainly in the spleen and to lesser extent in the liver leading to either **splenomegaly** or hepatomegaly respectively

There is **increased bilirubin** and decreased haptoglobin, but not to the degree seen with intravascular hemolysis.

Bilirubin-rich gallstones (pigment stones) on the long term

No hemoglobinemia, hemoglobinuria, and methemoglobin formation.

EV hemolysis may occur in membranopathies as hereditary spherocytosis, hemoglobinopathies as sickle cell disease and in warm antibody induced hemolysis

# Intravascular (IV) hemolysis

Release of hemoglobin into the blood causes **hemoglobinemia** and **hemoglobinuria**

Markedly decreased hemoglobin-binding proteins in the blood, such as haptoglobin and hemosiderinuria (**characteristic feature**) results

The hemoglobin may be oxidized to methemoglobin, which causes methemoglobinemia and methemoglobinuria.

Increased bilirubin causes jaundice and an increased risk of pigment (bilirubin) gallstones.

**No splenomegaly** is seen.

IV hemolysis may be caused by mechanical injury like prosthetic cardiac valves, thrombotic narrowing of the microcirculation, glucose 6 phosphate dehydrogenate deficiency, Complement fixation in cold antibody induced autoimmune hemolytic anemia.

# Changes in Hemolytic Anemia

**Increased numbers of erythroid precursors (normoblasts)**

**Prominent reticulocytosis and polychromasia** in the peripheral blood.

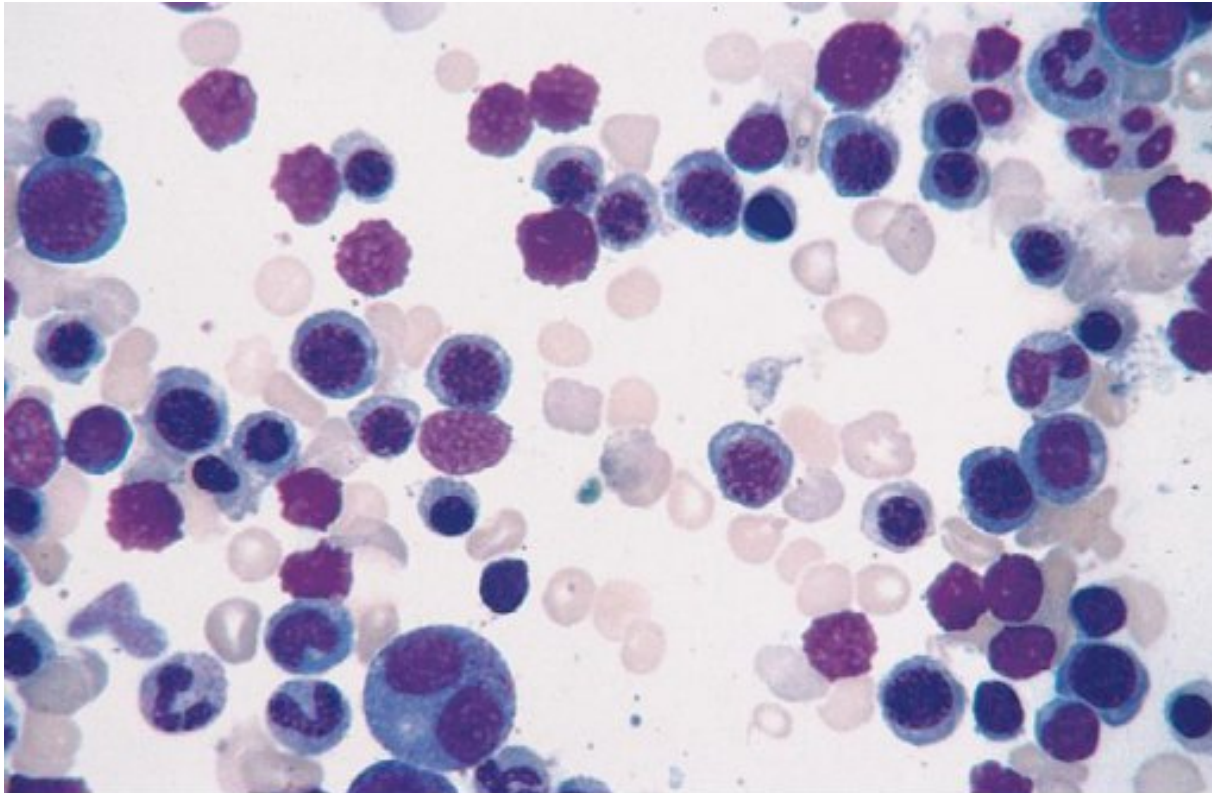
Accumulation of **hemosiderin**, a condition referred to as **hemosiderosis**

**Renal hemosiderosis is characteristic of IV hemolysis**

**Pigment gallstones**

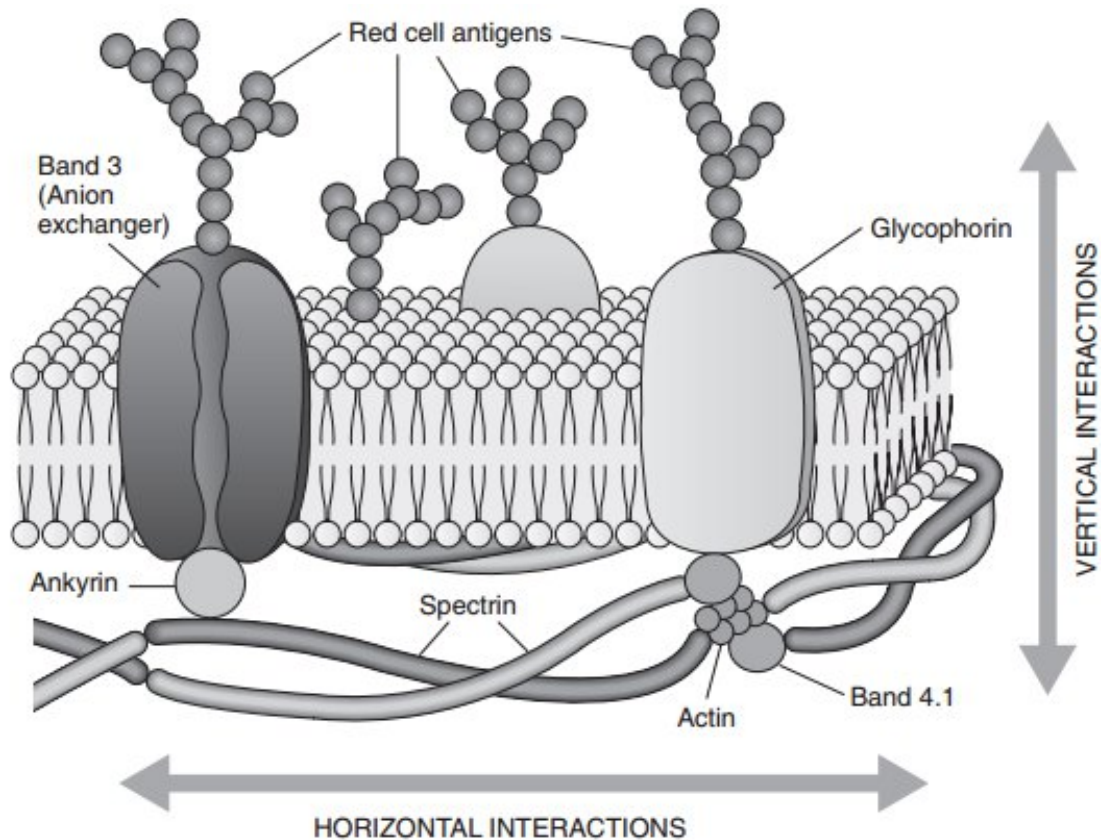
**Extramedullary hematopoiesis** can appear in the liver, spleen, and lymph nodes.

# Marrow changes in hemolytic anemia



# Hereditary Spherocytosis (HS)

## Structure of the erythrocyte plasma membrane



# Hereditary Spherocytosis (HS)

Inherited disorder caused by intrinsic defects in the red cell membrane skeleton

An autosomal dominant inheritance pattern is seen in about 75% of cases

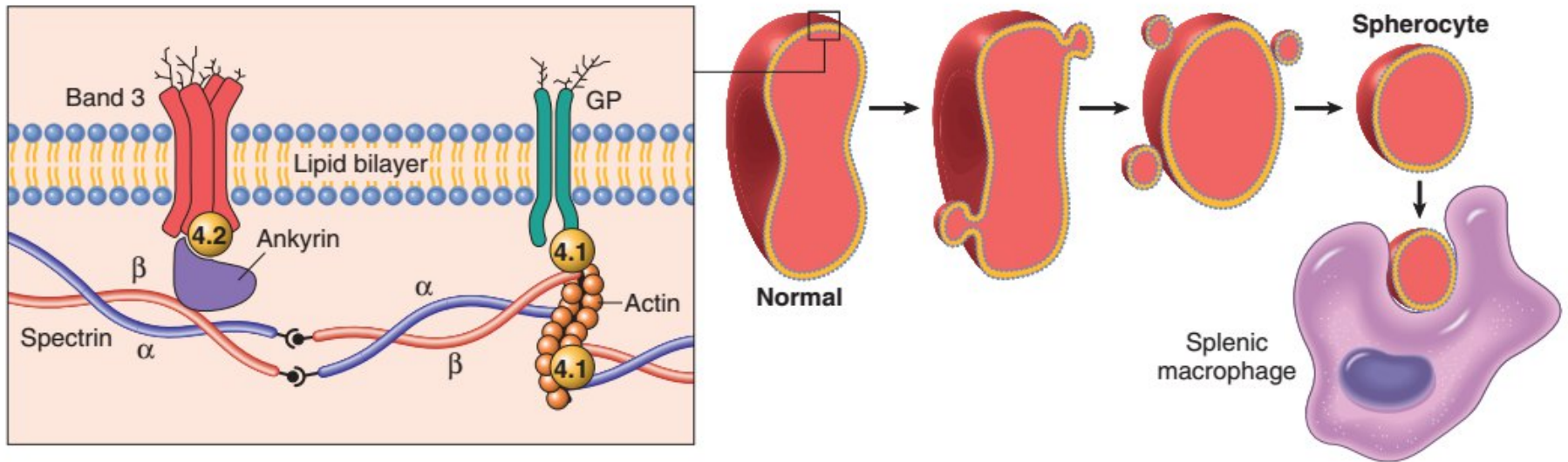
The pathogenic mutations most commonly affect ankyrin, band 3, spectrin, or band 4.2, the mutated allele fails to produce any protein.

Disturbed assembly of the skeleton as a whole, destabilizing the overlying plasma membrane

Red cells become spheroid, less deformable, and vulnerable to splenic sequestration and destruction.

# Hereditary Spherocytosis (HS)

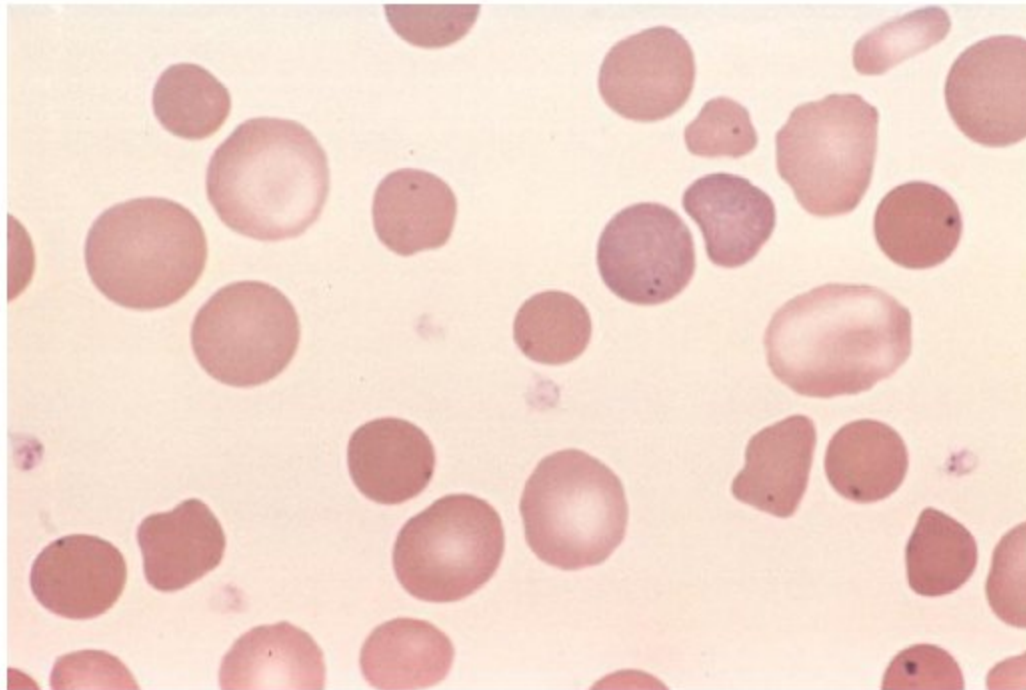
Young HS red cells are normal in shape, but the destabilized lipid bilayer sheds membrane fragments as red cells age in the circulation. The loss of membrane relative to cytoplasm “forces” the cells to assume the smallest possible diameter for a given volume, namely, a sphere



Spherocytes are less deformable, trapped in the splenic cords, where they are engulfed macrophages

The most specific morphologic finding is **spherocytosis**, apparent on smears as small, dark-staining (hyperchromic) red cells lacking the central zone of pallor

Spherocytes are also seen in other disorders associated with membrane loss, such as in autoimmune hemolytic anemia



# Hereditary Spherocytosis (HS)

## Clinical Features

Asymptomatic In 20% to 30% of patients mild disease, here the decreased red cell survival is readily compensated for by increased erythropoiesis.

More likely chronic hemolytic anemia of mild to moderate severity.

- **Anemia, splenomegaly, and jaundice** with greatly variable severity
- Jaundice at birth In minority (mainly compound heterozygotes).

The diagnosis is based on family history, hematologic findings, and laboratory evidence.

HS red cells have an increased mean cell hemoglobin concentration

In two thirds of the patients the red cells are abnormally sensitive to osmotic lysis when incubated in hypotonic salt solutions, which causes the influx of water into spherocytes with little margin for expansion.

# Incubated Osmotic Fragility

# **Hereditary Spherocytosis (HS) complications**

## **Aplastic crises**

- Usually triggered by an acute parvovirus infection which kills red cell progenitors causing red cell production to cease until immune response commences in 1-2 weeks

## **Hemolytic crises**

- Produced by intercurrent events leading to increased splenic destruction of red cells (e.g., infectious mononucleosis); these are clinically less significant than aplastic crises.

## **Cholelithiasis with bilirubin gallstones**

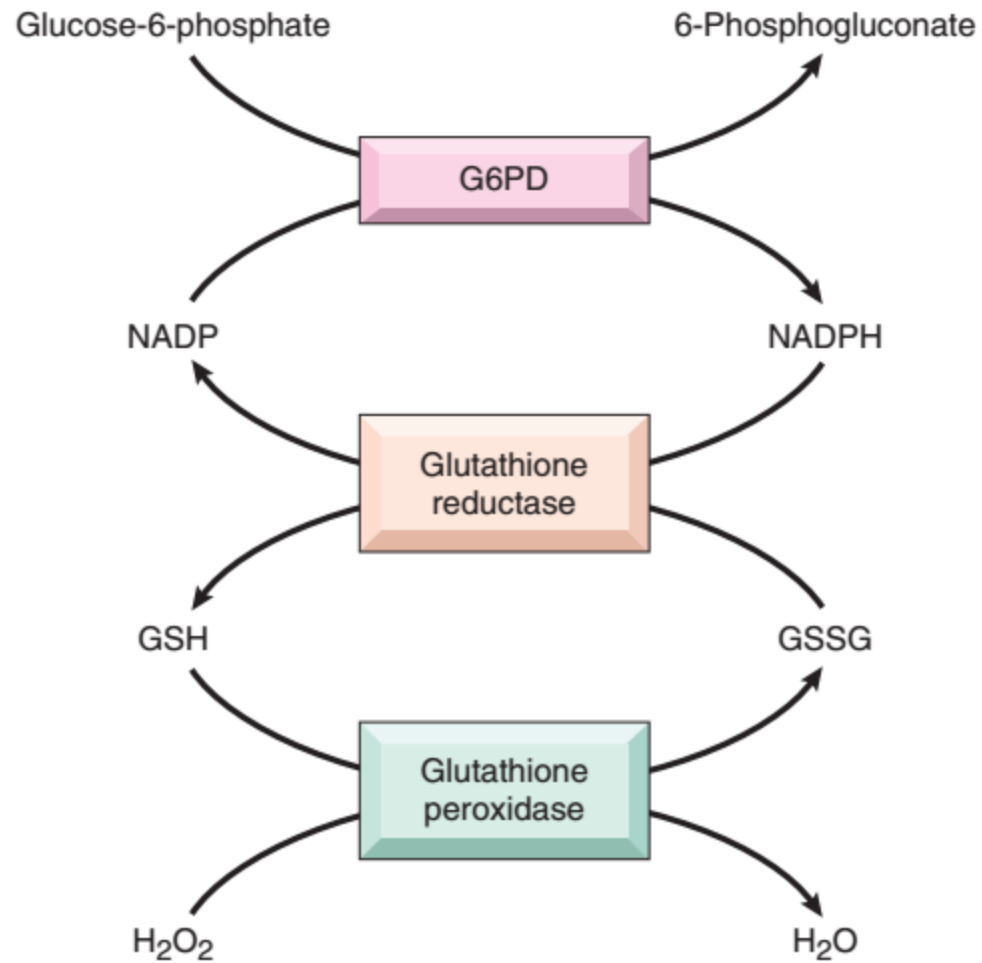
# Glucose-6-phosphate dehydrogenase (G6PD) deficiency

G6PD deficiency is a genetic disorder affecting the hexose monophosphate shunt pathway leading to decreased levels of the antioxidant glutathione (GSH)

G6PD reduces nicotinamide adenine dinucleotide phosphate (NADP) to NADPH while oxidizing glucose-6-phosphate

NADPH then provides reducing equivalents needed for conversion of oxidized glutathione to reduced glutathione, which protects against oxidant injury by participating as a cofactor in reactions that neutralize compounds such as  $H_2O_2$

G6PD deficiency is a recessive X-linked trait, placing males at higher risk for symptomatic disease.



# G6PD deficiency

G6PD variants associated with hemolysis result in misfolding of the protein, making it more susceptible to proteolytic degradation. Two variants cause most of the clinically significant hemolytic anemias.

- G6PD (**A- type**): the half-life of G6PD – is moderately reduced, The hemolysis is intermittent because only older erythrocytes have decreased levels of glucose-6 phosphate dehydrogenase.
- G6PD **Mediterranean**: the half-life of G6PD is more markedly abnormal, the hemolysis is greater in individuals with the highly unstable G6PD Mediterranean variant, in addition to decreased stability there is decreased synthesis

# G6PD deficiency

The episodic hemolysis that is characteristic of G6PD deficiency is caused by exposures that generate oxidant stress

- **Infections**; most common, viral hepatitis, pneumonia, and typhoid fever are most likely.
- The oxidant drugs including antimalarials (e.g., primaquine and chloroquine), sulfonamides, nitrofurantoin, and others.
- **Fava bean**, which generates oxidants when metabolized. “**Favism**” is prevalent in the Mediterranean, Middle East, and parts of Africa where consumption is prevalent

# G6PD deficiency

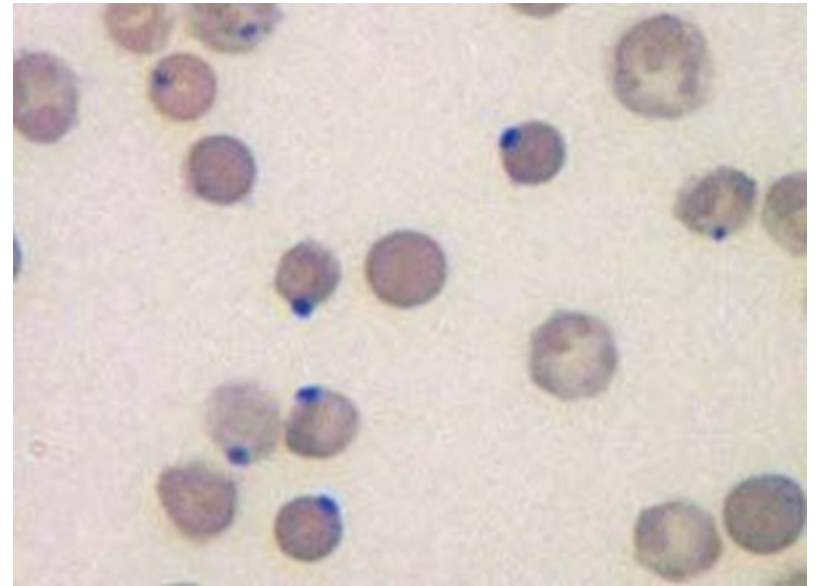
Uncommonly, G6PD deficiency presents as neonatal jaundice or a chronic low-grade hemolytic anemia in the absence of infection or known environmental triggers.

Acute intravascular hemolysis, marked by anemia, hemoglobinemia, and hemoglobinuria, usually begins 2 to 3 days following exposure of G6PD-deficient individuals to oxidants and is greater in individuals with the highly unstable G6PD Mediterranean variant

# G6PD deficiency

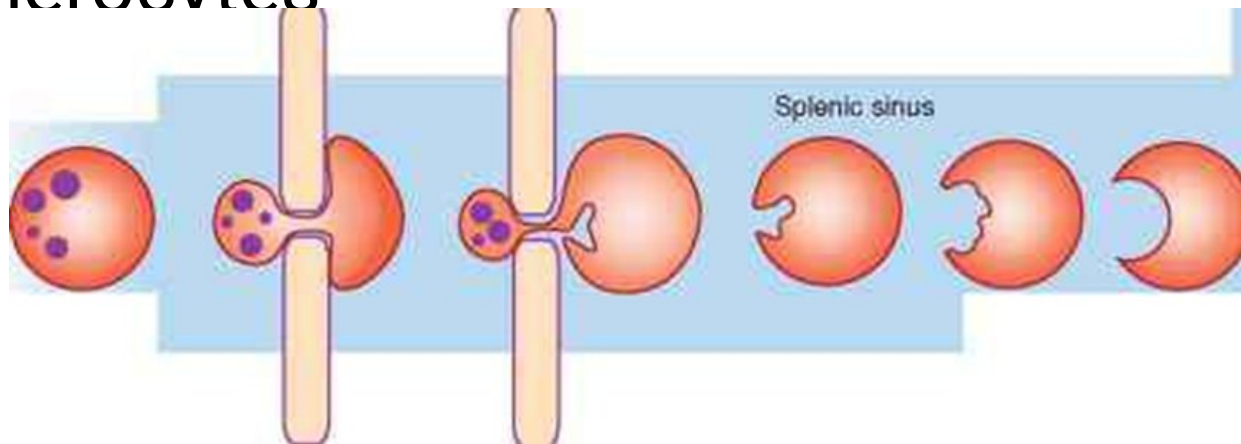
Oxidants cause both intravascular and extravascular hemolysis in G6PD-deficient individuals.

High levels of oxidants causes denaturation of hemoglobin and form membrane bound precipitates known as **Heinz bodies** which can damage the membrane sufficiently to cause intravascular hemolysis

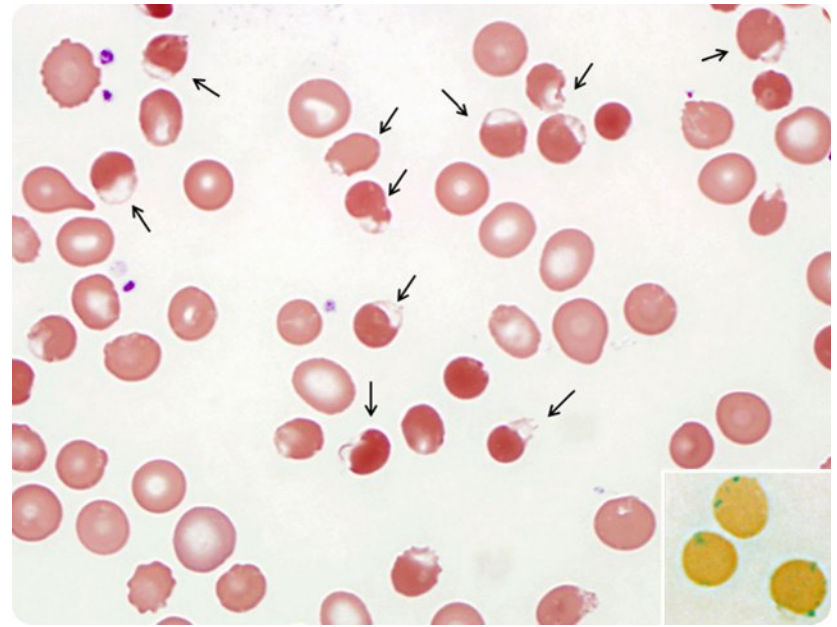
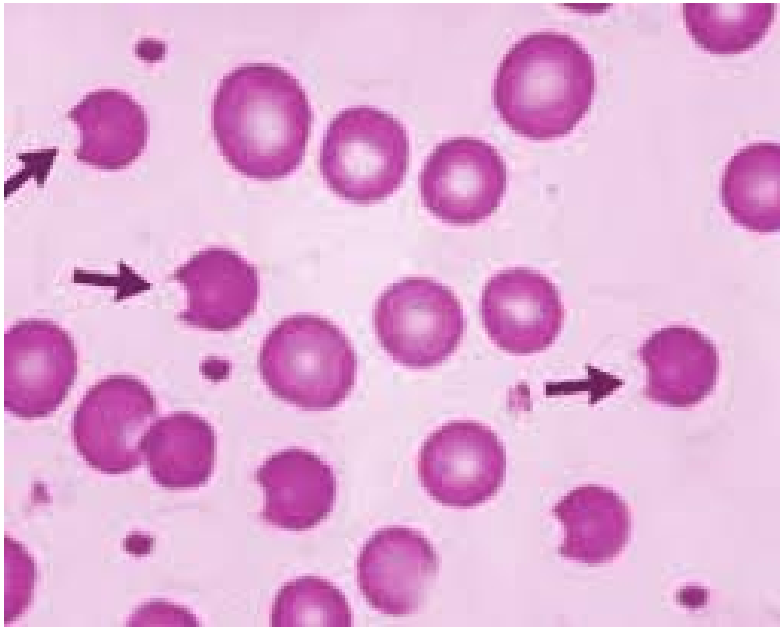


As inclusion-bearing red cells pass through the splenic cords, macrophages pluck out the Heinz bodies giving the RBCs the characteristic appearance of bite cells

Less severe membrane damage results in decreased red cell deformability i.e spherocytes



# G6PD deficiency



# G6PD deficiency

Both bite cells and spherocytes are trapped in splenic cords and removed rapidly by phagocytes

Because hemolytic episodes related to G6PD deficiency occur intermittently, features related to chronic hemolysis (e.g., splenomegaly, cholelithiasis) are absent.

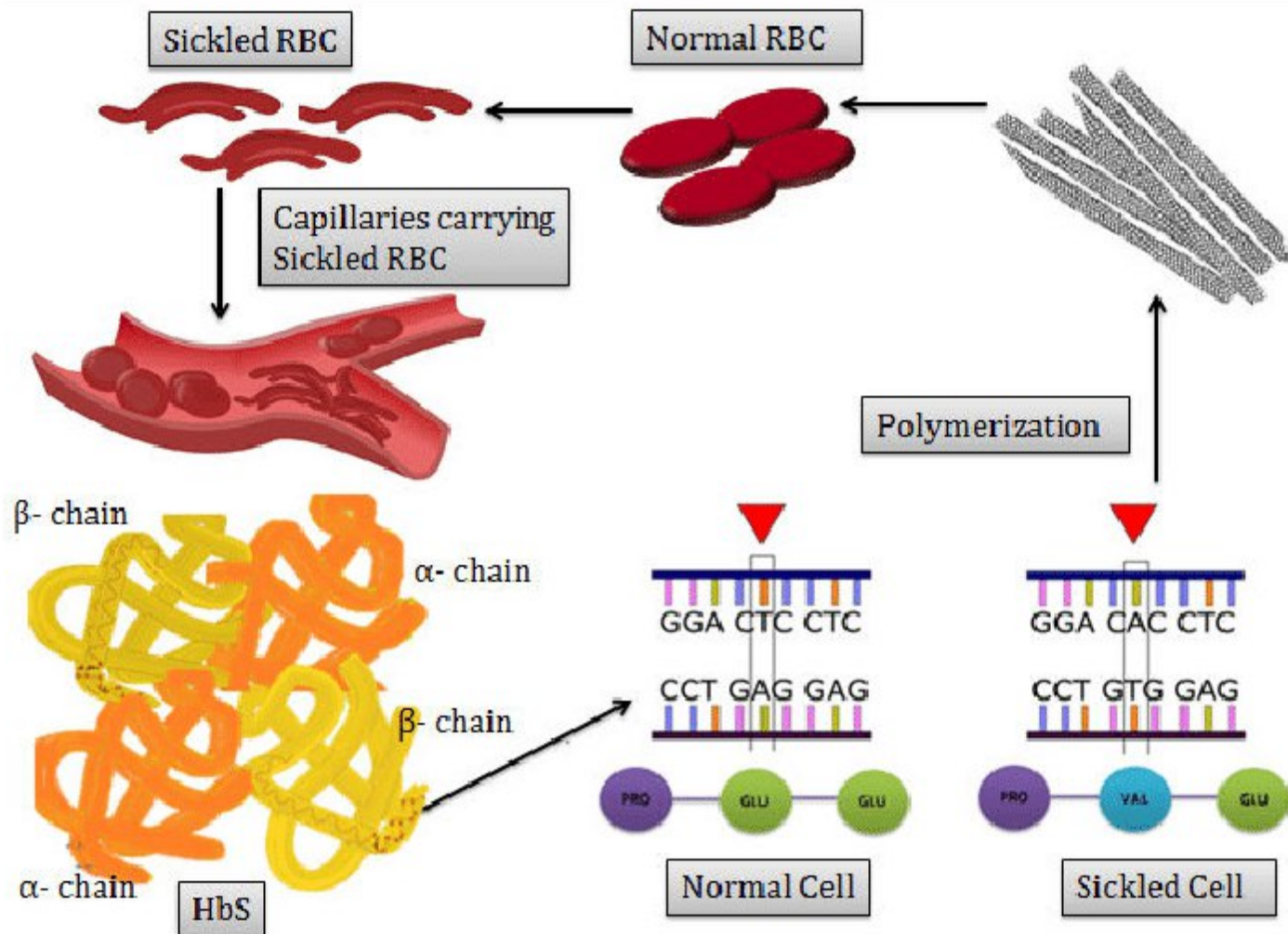
Only older red cells are at risk for lysis, as younger RBCs has near normal levels of G6PD which is unstable and has shorter half life, the RBCs become more G6PD deficient as they mature, hemolysis ceases when only younger G6PD-replete red cells remain

# Sickle Cell Disease

Sickle cell disease is caused by a point mutation in the sixth codon of  $\beta$ -globin gene that leads to the replacement of a glutamate amino acid with a valine amino acid.

Happens in patients who are homozygous ( has 2 mutated allele) for HbS, a condition called **HbSS** disease, one mutated allele leads to a carrier state called **HbAS**

Resultant hemoglobin undergoes polymerization, leading to red cell distortion, hemolytic anemia, microvascular obstruction, and ischemic tissue damage.



# Sickle Cell Disease

## Pathogenesis

The RBCs is sickle or holly-leaf in shape, non deformable.

The major pathologic manifestations are **chronic hemolysis, microvascular occlusions, and tissue damage**

Sickled red cells are also mechanically fragile, leading to **some** intravascular hemolysis as well.

# Variables affecting the degree of sickling

Interaction of HbS with the other types of hemoglobin in the cell:

- Greatest sickling in HbSS
- In Sick cell trait (HbAS), HbA interferes with HbS polymerization,
- HbF inhibits the polymerization of HbS, infants do not become symptomatic until they reach 5 or 6 months

Mean cell hemoglobin concentration (MCHC).

- Higher HbS concentrations increase the probability of sickling
- Lower MCHC e.g.  $\alpha$  thalassemia decrease sickling

# variables affecting the degree of sickling

A decrease in Intracellular pH. augments the tendency for sickling.

High Transit time of red cells through microvascular beds e.g. inflamed tissues

# Sickle Cell Disease

## Pathogenesis

**Microvascular occlusions**, are responsible for the most serious clinical features, these are dependent on

- Subtle red cell membrane damage
- Local factors, such as inflammation or vasoconstriction
- Increased adhesion molecules on sickle red cells

Stagnation of RBCs lead to prolonged exposure to low oxygen that augments sickling that in turn worsens occlusion and generate a vicious circle of occlusion and further sickling

# Sickle Cell Disease

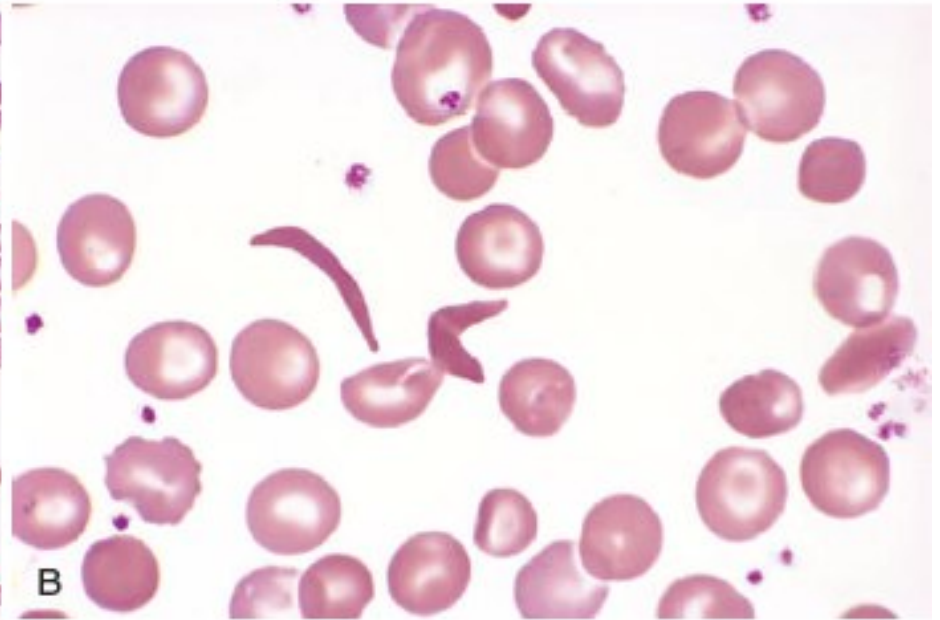
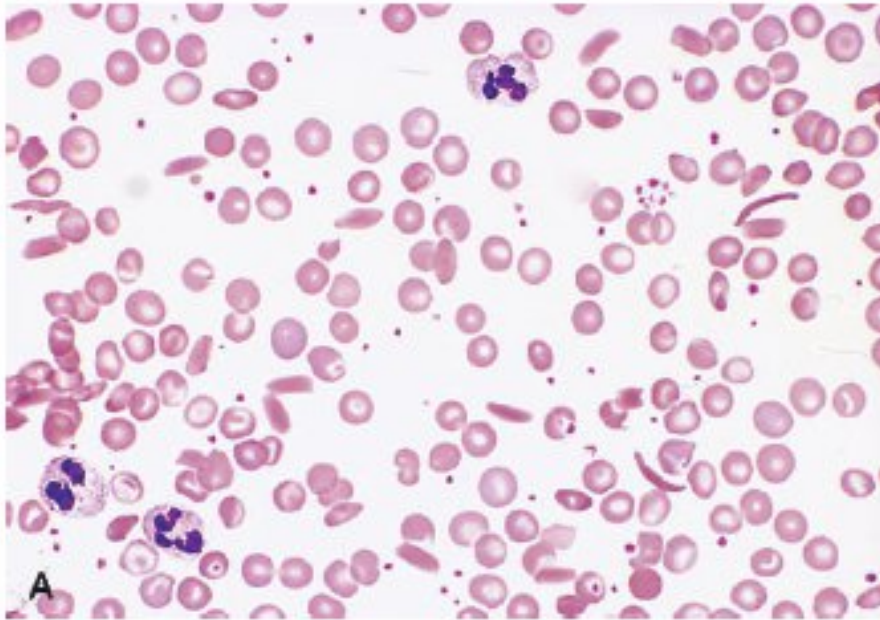
## Pathogenesis

In early childhood, trapping of sickled red cells occurs in the spleen leading to its enlargement

With time, splenic infarction, fibrosis, and progressive shrinkage, so that by adolescence or early adulthood only a fibrous splenic tissue is left; this process is called **autosplenectomy**, leading to significant risk of infection by encapsulated organisms in these patients

# Sickle Cell Disease

## Morphology



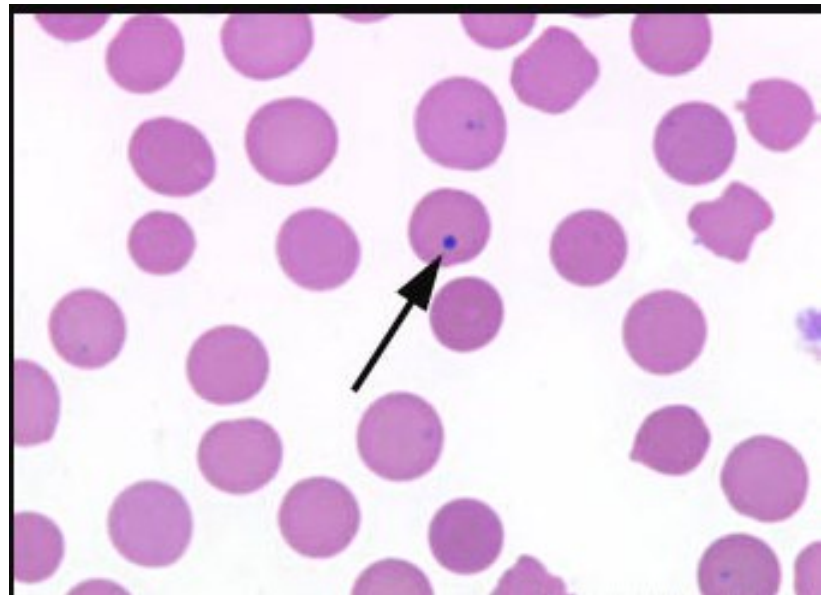
# Sickle Cell Disease

## Morphology

The peripheral blood demonstrates variable numbers of **sickled cells and** target cells and polychromasia from reticulocytosis

**Howell-Jolly bodies** (small nuclear remnants) are present in some red cells due to the asplenia

The bone marrow is hyperplastic as a result of a compensatory erythroid hyperplasia.



# Sickle Cell Disease

## Clinical Features

Manifestations of anemia and jaundice from hyperbilirubinemia

Course is characterized by a variety of acute complications called “crises”

### **Vaso-occlusive crises or pain crises**

- hypoxic injury and infarction that cause severe pain
- Most commonly involved sites are the bones, lungs, and splanchnic vessels
- Infection, dehydration, acidosis

### **Sequestration crises**

- Entrapment of sickle red cells leads to rapid splenic enlargement, hypovolemia, and sometimes shock
- In children

# **Sickle Cell Disease**

## **Clinical Features**

**Aplastic crises**, infection of red cell progenitors by parvovirus B19

### **Acute chest syndrome**

- Vaso-occlusive crisis involving the lungs
- Fever, cough, chest pain, and pulmonary infiltrates

**Priapism, stroke and retinopathy**

# Sickle Cell Disease

## Clinical Features

### Chronic complications

- Impairment of growth and development,
- organ damage affecting the spleen, heart, kidneys, and lungs and loss of concentrating ability of the kidney

Increased susceptibility to **infection** with encapsulated organisms, **Pneumococcus pneumoniae** and **Haemophilus influenza** **septicemia and meningitis**

Diagnosis based on Hb electrophoresis and DNA analysis

Mainstay of treatment is the disease modifying agent hydroxyurea

- Increase red cell Hb F levels, which occurs by unknown mechanisms
- Antiinflammatory effect, stems from an inhibition of leukocyte production.

Thank you and good Luck