Lecture 2A:
O_2 and CO_2 Transport
RBC Life Cycle
Anemias

Oxygen and Carbon Dioxide Transport

Erythrocytes (RBCs) are dedicated to respiratory gas transport.

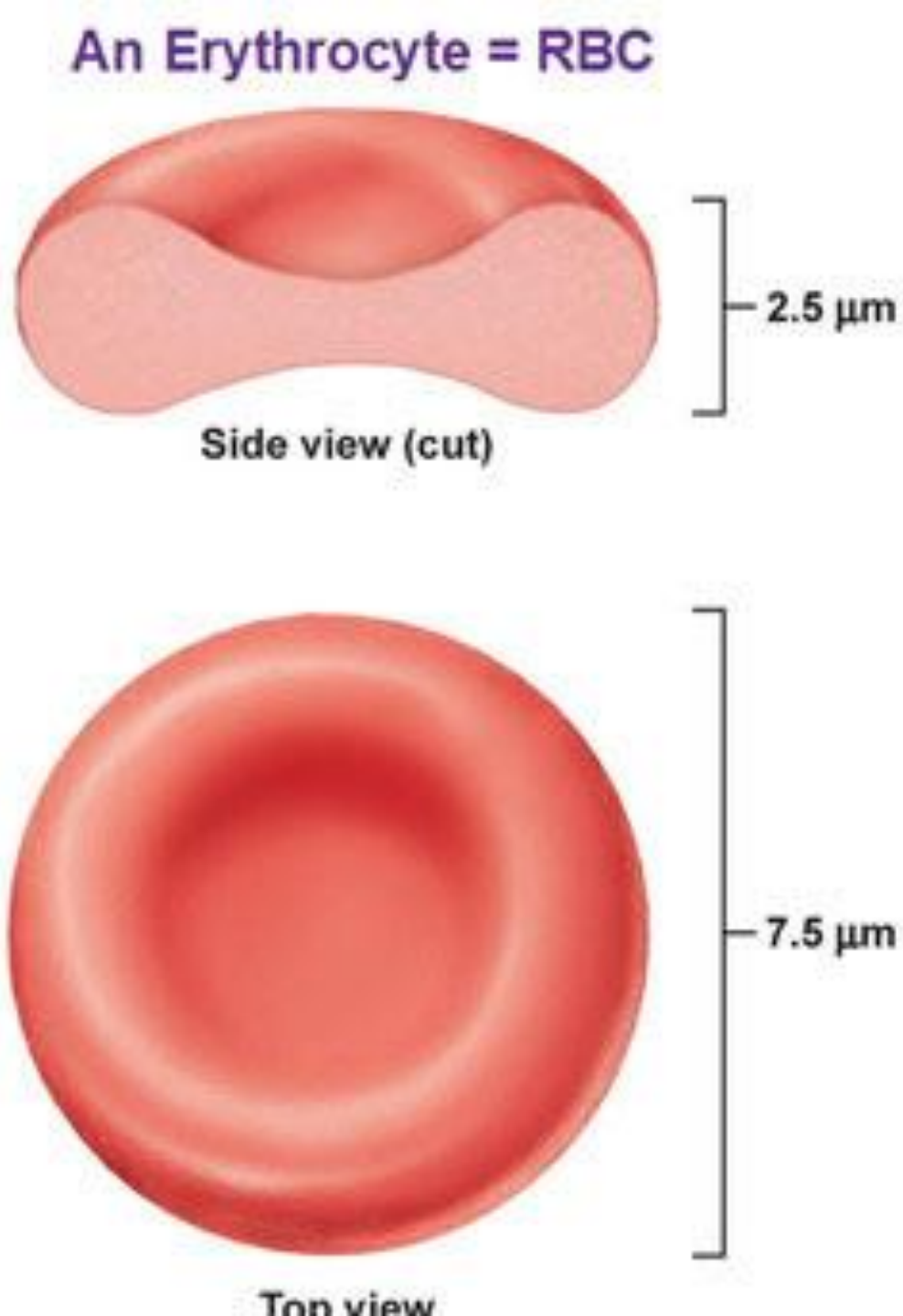
- About 97% of blood oxygen is transported to tissue cells inside RBCs bound to hemoglobin molecules. The rest is transported in the plasma as a dissolved gas.
- 20% of blood carbon dioxide is transported away from tissues cells inside RBCs bound to hemoglobin. The rest is transported in the plasma as a dissolved gas or as bicarbonate ions.
- Hemoglobin binds **reversibly** with both oxygen and carbon dioxide.
- Each erythrocyte contains about **250 million** hemoglobin molecules! Each hemoglobin molecule binds up to four oxygen molecules. That's about a **billion O2 molecules** per cell for arterial red blood cells.

Oxygen and Carbon Dioxide Transport 1

Erythrocyte structure supports function:

- **Biconcave shape** (like a donut without a hole) provides a huge surface area relative to volume. This facilitates gas diffusion between the erythrocyte and blood plasma during capillary gas exchanges.
- **>97% of the RBC volume** is occupied by hemoglobin protein. The absence of nuclei and organelles allows more space for hemoglobin.
- Mature RBCs have **no mitochondria**, so no O2 is used up in generation of ATP by RBCs. All of the O2 is transported to other cells.
- RBC plasma membranes have proteins that provide flexibility**. Cells must often flex when travelling** through tiny capillaries. The absence of organelles also allows flexing.

Biconcave Disc Shape of an Erythrocyte

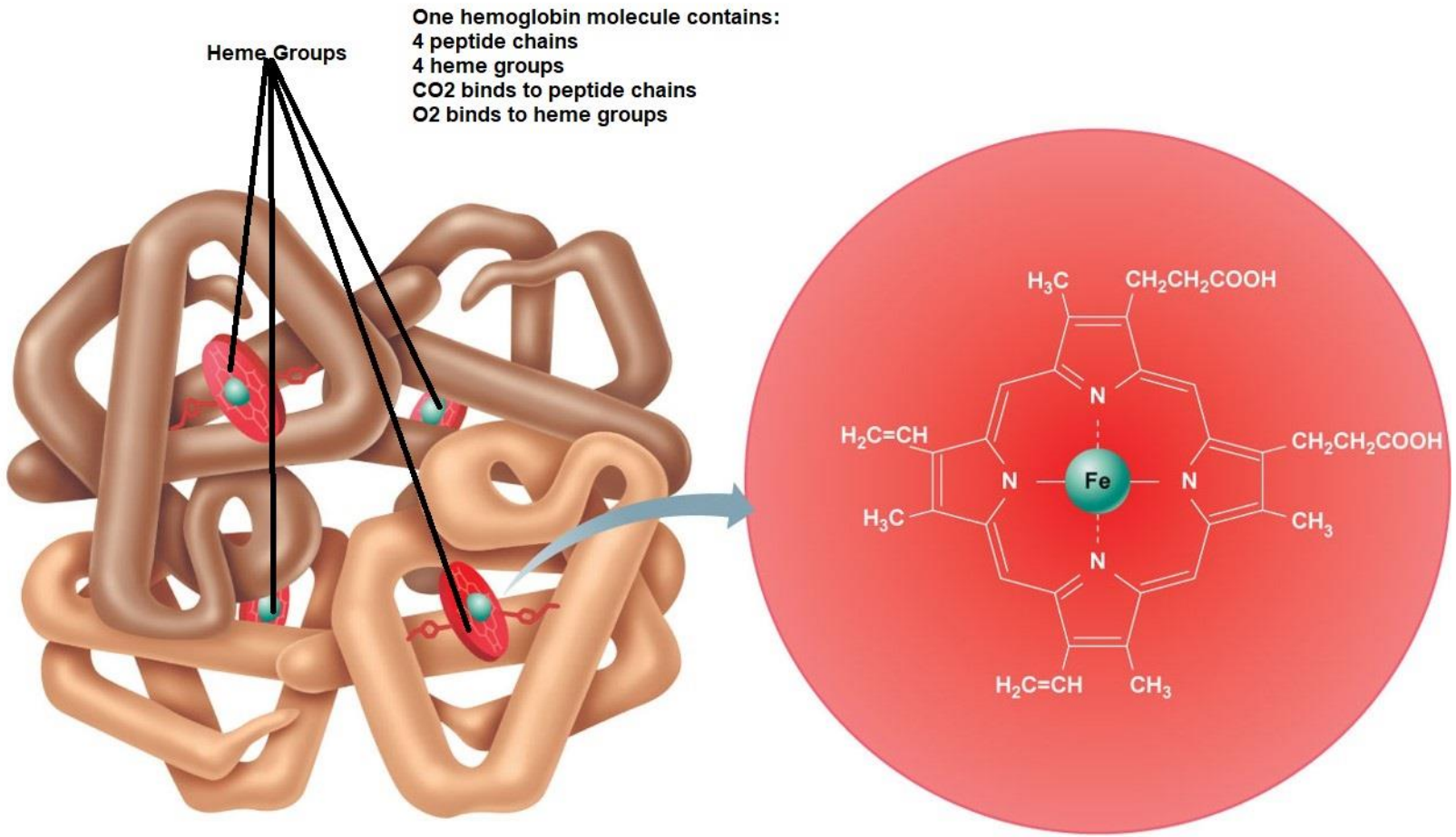


Oxygen and Carbon Dioxide Transport 2

Hemoglobin structure:

- Each hemoglobin molecule contains **four globin protein molecules**: two alpha globin molecules and two beta globin molecules.
- One **heme group** is bonded to each of the four globin chains. Each heme group has an iron atom in its center. The iron atom in a heme group can bind one **oxygen** molecule. Heme is a pigment. It gives blood its red color.
- **Carbon dioxide** molecules bind to the globin chains rather than to the heme groups of hemoglobin molecules.
- Each of the four globin chains can bind one CO2 molecule.
- Hemoglobin molecules have fewer O2 molecules bound in venous blood RBCs than in arterial blood RBCs. And hemoglobin molecules have fewer CO2 molecules bound in arterial blood RBCs than in venous blood RBCs.

A Hemoglobin Molecule

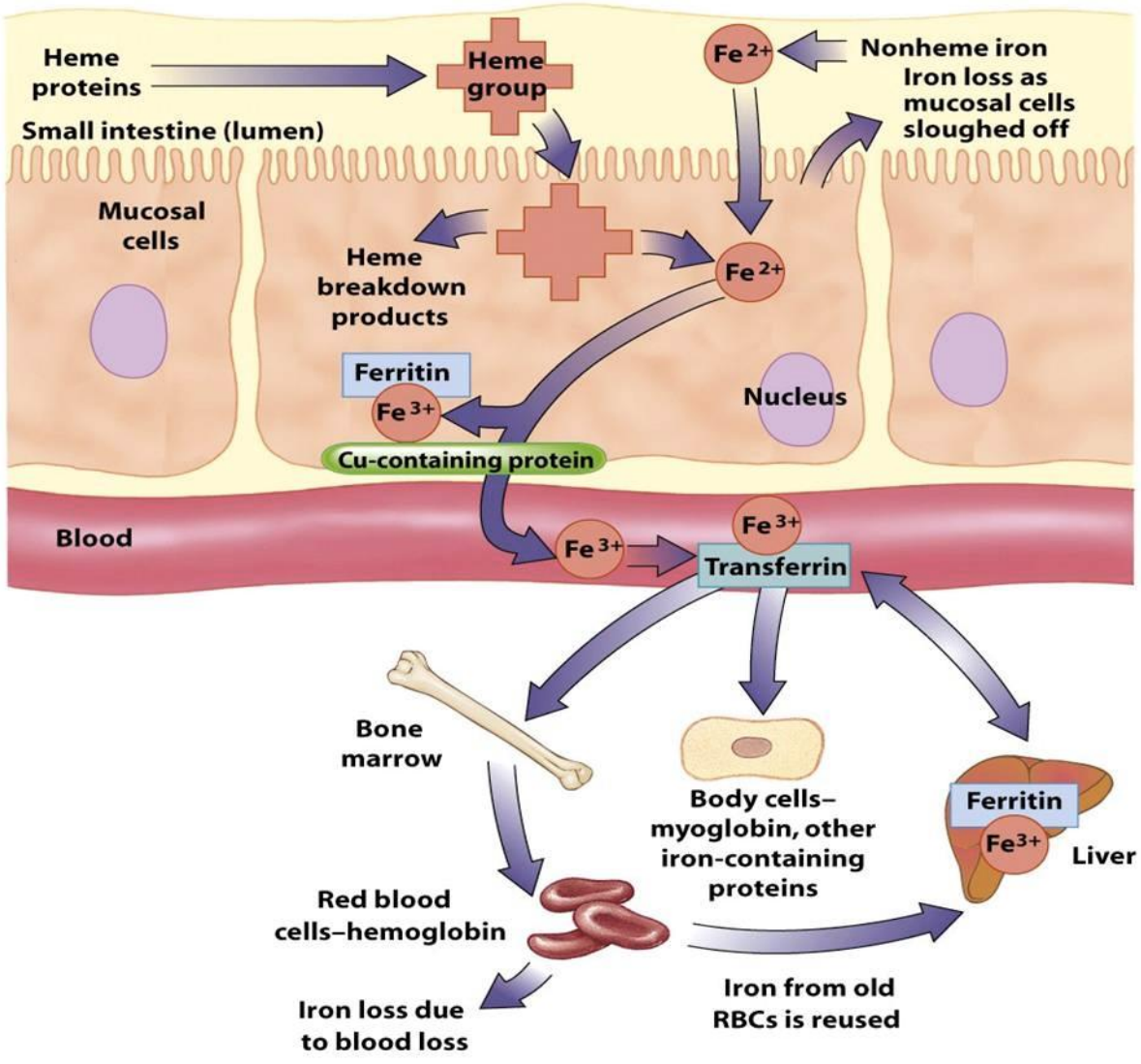


Oxygen and Carbon Dioxide Transport 3

Iron and Heme:

- In order for heme groups to be synthesized by developing erythroblasts, iron must be transported to the bone marrow from the digestive tract or from the liver where it may be stored after being absorbed from foods by the digestive system.
- Iron is transported in the blood plasma by a protein, **transferrin**. Bone marrow erythroblasts (and liver and spleen cells) have membrane receptors for transferrin-iron complexes. The complexes are endocytosed, the iron is freed from the transferrin, and in erythroblasts, the iron is then either used to form heme or bound to **ferritin**, an iron storage protein. The **apotransferrin** (transferrin with no iron bound) is exocytosed for reuse.

Iron Absorption and Transport



Important Normal Blood Values

- RBC count
  - Females 4.2-5.4 million/mm^3
  - Males 4.7-6.1 million/mm^3
- Platelet count
  - 150,000-400,000/mm^3
- Hemoglobin
  - Adult Females 12-16 g/dl
  - Adult Males 14-18 g/dl
  - Infants 4-20 g/dl
- Hematocrit (The %volume of whole blood occupied by RBCs)
  - Females 37-47%
  - Males 42-52%
- NOTE: A cubic millimeter (mm^3) is the same as a microliter (µl). A deciliter (dl) is the same as 100 ml.

STUDENTS: KNOW THESE VALUES FOR QUIZZES AND EXAMS.

Oxygen and Carbon Dioxide Transport 4

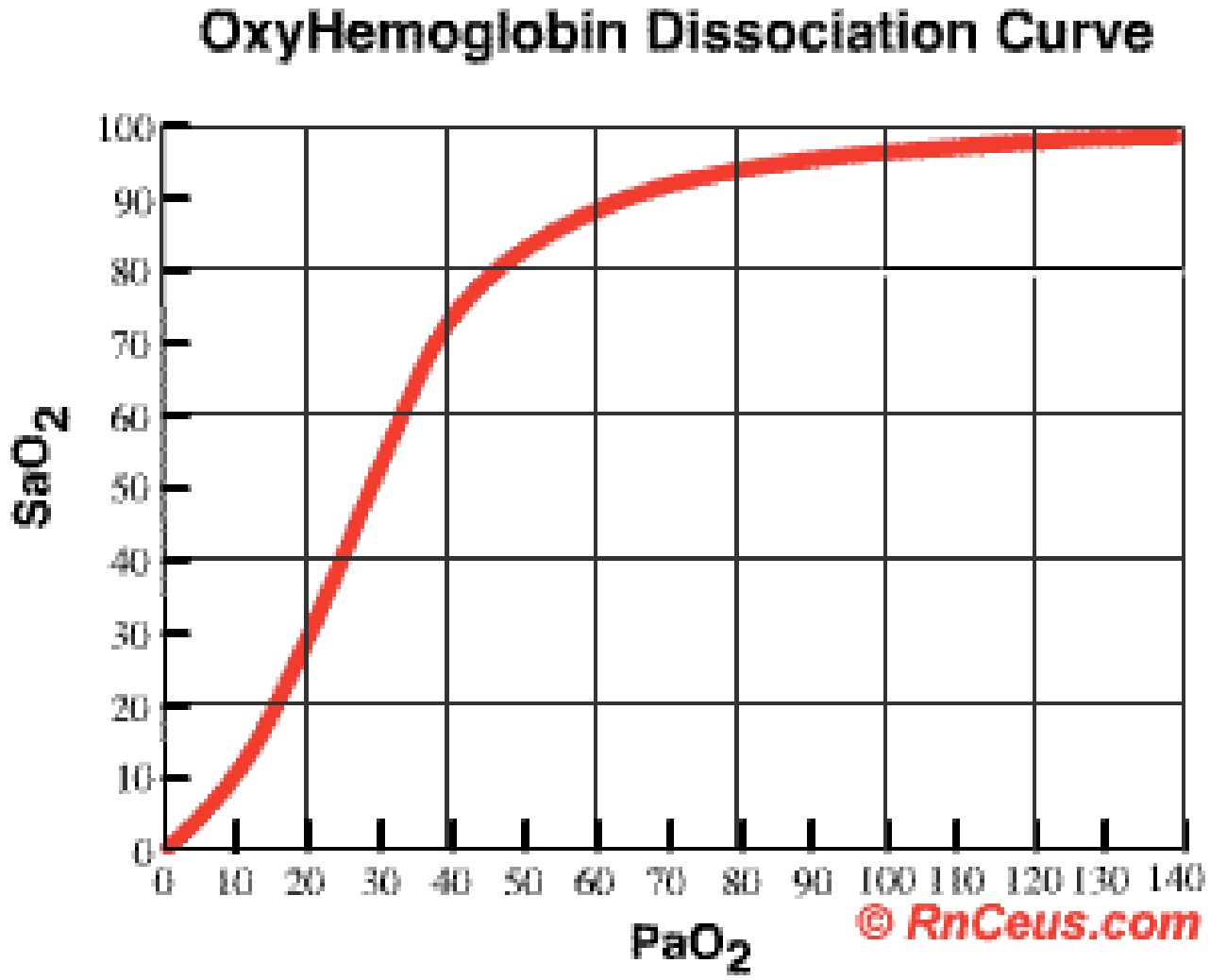
Adult Hemoglobin vs Fetal Hemoglobin

- Infants require more hemoglobin per unit blood volume than adults because their hemoglobin has a different structure.
- **Adult hemoglobin (HbA)** contains 2 alpha chains and 2 beta chains.
- **Fetal hemoglobin (HbF)** contains 2 alpha chains and 2 gamma chains.
- Gamma chains bind O2 more tightly (download less of it to tissues) than beta chains, so a higher volume of blood per unit of tissue mass is required to keep fetal/infant tissues oxygenated than is required for the same mass of adult tissue.
- **HbF is gradually replaced by HbA** during the first few months after birth. This is due to a change in gene expression in the red bone marrow stem cells that produce RBCs.

Oxygen and Carbon Dioxide Transport 5

- The amount of oxygen bound to hemoglobin is determine by the partial pressure of oxygen (P_{O2}) of the fluid surrounding the hemoglobin. The P_{O2} is the pressure created by the 1.5% of blood oxygen gas that is dissolved in the blood plasma.
- The higher the P_{O2} the greater the level of saturation of hemoglobin with oxygen. But the relationship is **NOT linear**. See the diagram on the next slide. The slope of the curve gets much steeper as P_{O2} drops below **60 mm Hg**.
- The P_{O2} of **arteria**l blood plasma is close to **100 mm Hg.** Nearly all the heme binding sites for oxygen are occupied by oxygen at that partial pressure. Hemoglobin is about **98% saturated.**
- The P_{O2} of **venous** blood is about **40 mm Hg**. At that level only about 3 of every 4 heme binding sites for oxygen are occupied by oxygen molecules. Hemoglobin is about **75% saturated**.

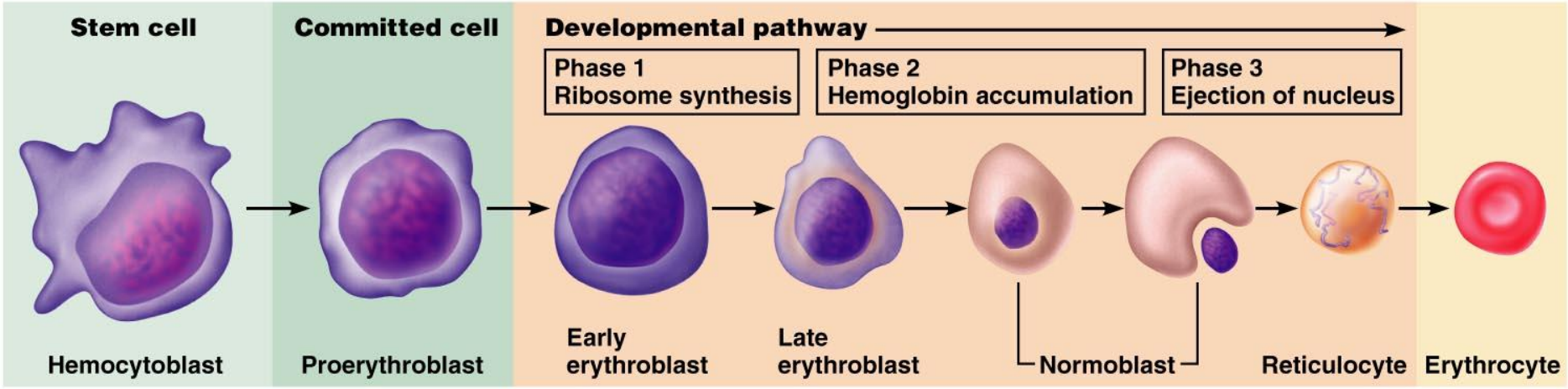
Graph of Oxygen Dissociation from Hemoglobin



Oxygen and Carbon Dioxide Transport 6

- **Venous blood becomes oxygenated by passing through the pulmonary capillary beds of the lungs.** Lung tissue fluid has a relatively high P_{O2}. O2 molecules from the tissue will quickly diffuse into the blood as blood moves through the capillaries due to the pressure gradient. The P_{CO2} of lung tissue is low, so CO2 molecules will rapidly diffuse out of the blood into lung tissue to be exhaled.
- **Arterial blood becomes deoxygenated by passing through systemic capillary beds.** Body tissue fluid (interstitial fluid) has a relatively low P_{O2}. O2 molecules in the blood are released from hemoglobin, enter the blood plasma and will quickly diffuse into the tissue fluid (and then into cells) as blood moves through the capillaries. (Cellular respiration consumes O2 and emits CO2.) The P_{CO2} of interstitial fluid is relatively high, so CO2 molecules will rapidly diffuse out of the interstitial fluid into the blood to be transported back to the lungs.

The RBC Life Cycle

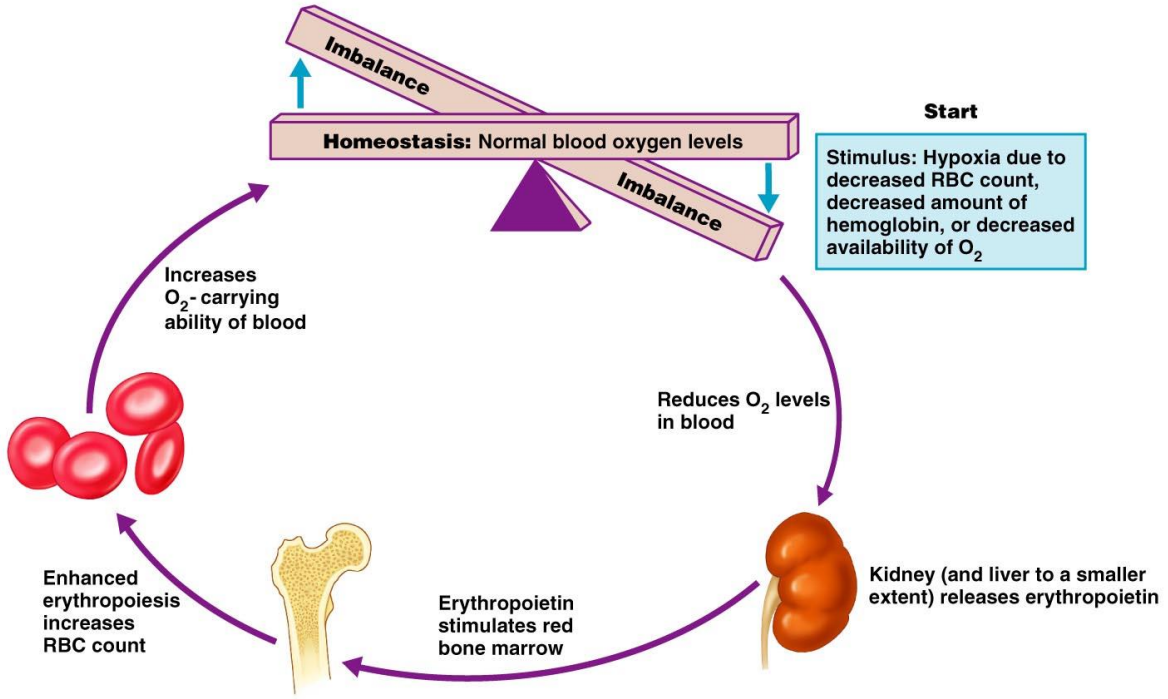


- This diagram depicts **erythropoiesis**, the development of a red blood cell in the red bone marrow. NOTE the three phases.
- Normoblasts eject their nuclei. Reticulocytes eject cell organelles including mitochondria.
- Mature RBCs have no nuclei or organelles. They are full of hemoglobin molecules!
- Red blood cell synthesis requires **three dietary components:**
  - Iron for the production of heme groups
  - Vitamin B12 for DNA synthesis
  - Folic Acid (Vitamin B9) for DNA synthesis

The RBC Life Cycle 2

Regulation of Erythropoiesis

- The red bone marrow produces RBCs at the rate of **2 million cells per second!**
- **Erythropoiesis**, is stimulated by **erythropoietin (EPO)**, a hormone released by **kidney** cells in response to hypoxia.



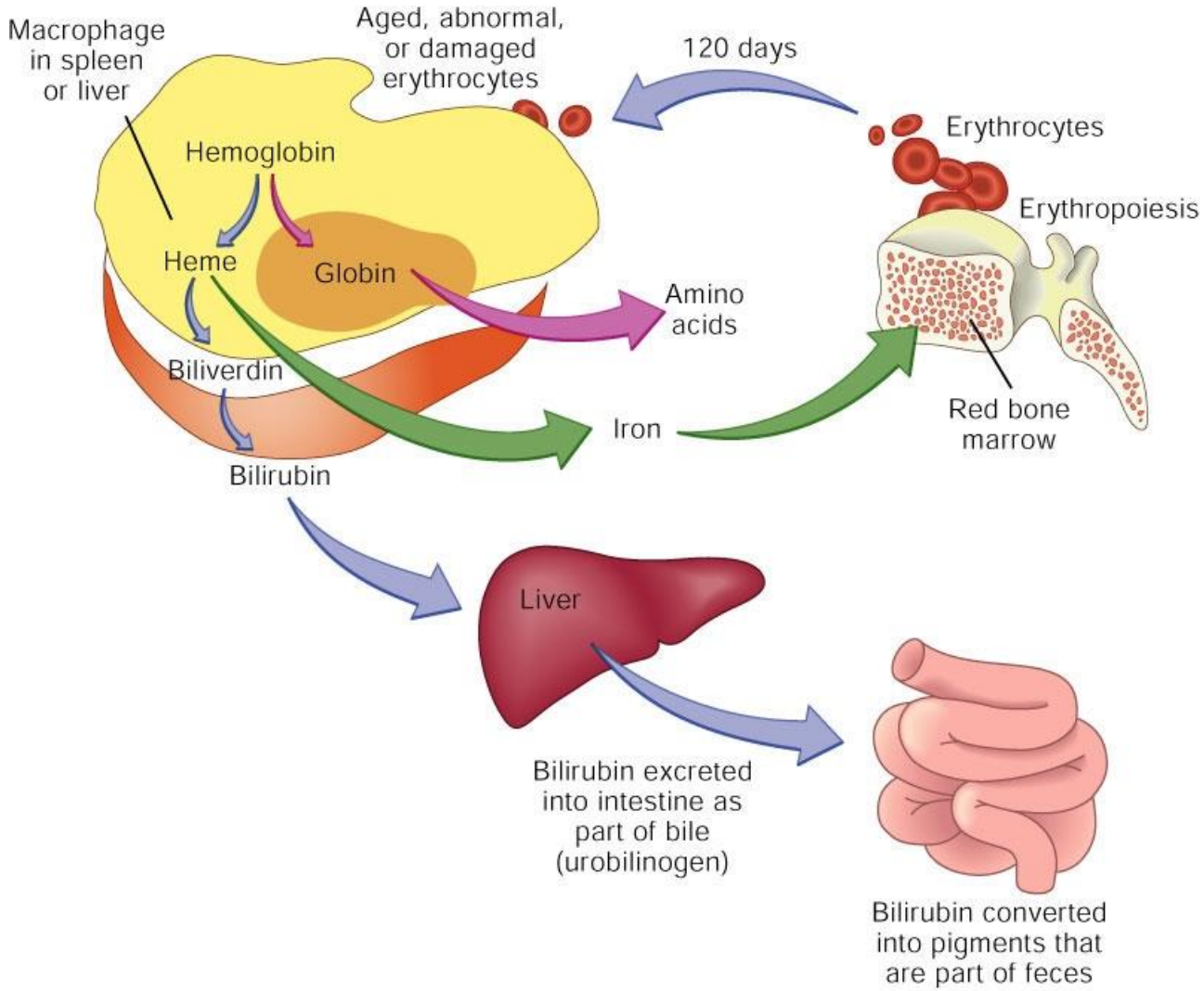
The RBC Life Cycle 3

- Erythrocytes have a life span of about **120 days**. To survive for 120 days they need a means of ATP production mostly for the operation of ion pumps. The functions of respiratory gas transport don't require ATP.
- Since RBCs have no mitochondria their mode of ATP production is quite different from the cellular respiration pathways of other body cell types.
- The two prominent **anaerobic** pathways in RBCs:
  - **The Embden-Meyerhof Pathway**-accounts for about 90% of the glucose-use of RBCs. It is similar to glycolysis. It converts glucose to lactic acid and produces ATP.
  - **The Hexose Monophosphate Shunt**-accounts for about 10% of the glucose-use of RBCs; produces NADPH to perform reduction of reactive oxygen species (oxygen free radicals). No ATP is made.

The RBC Life Cycle 4

- Old worn-out RBCs are destroyed by macrophages in the **spleen** and, to a lesser extent, in the liver.
- The **globin chains** of hemoglobin are broken down into amino acids and the amino acids are reused.
- The **heme groups** free their iron for reuse and become **bilirubin**, the pigment (yellow green) component of bile.
- Bilirubin is **conjugated** (bound to **glucaronic acid**, making it water-soluble) by liver cells and is excreted in feces and to some extent in urine.
- **Liver disease or excessive RBC degradation** may cause unconjugated bilirubin to accumulate in the blood. **Unconjugated bilirubin (lipid-soluble) cannot be excreted**. This causes **jaundice**, a yellowing of the skin and the sclera. Severe serum bilirubin accumulation in the brain can cause brain damage.

The RBC Life Cycle 5



From Thibodeau GA, Patton KT: Anatomy and physiology, ed 5, St Louis, 2003, Mosby, p 537.

Alterations in Oxygen Transport: Anemias

- **Definition of Anemia**
  - Any condition that decreases the transport of oxygen by red blood cells. There are several forms of anemia.
  - It is important to distinguish among the various types of anemia to determine the proper treatment.
- **Three Categories of Anemia:**
  - Decreased Production of RBCs
  - Genetically Abnormal RBCs
  - Destruction/Loss of Normal RBCs
- **General Symptoms of Anemia:**
  - vasoconstriction, pallor, tachypnea (elevated breathing rate), dyspnea (difficultly breathing), tachycardia (elevated heart rate), transient murmurs, angina (chest pain), heart failure, intermittent claudication (leg cramps), headache, postural hypotension, light-headedness, tinnitus (ringing in the ears), syncope (fainting).

Alterations in Oxygen Transport: Anemias 2

- **Lab Tests Important in Anemia**
  - **CBC With Platelets**-measures numbers of all formed elements per mm^3
  - **Blood Smear**-allows microscopic inspection of formed element size, shape, color and relative number
  - **Hemoglobin**-measures the grams of hemoglobin per 100 ml of blood.
  - **Hematocrit-**measures the volume of whole blood that consists of RBCs
  - **Reticulocyte Count**-measures the % of circulating cells that are reticulocytes (immature RBCs)
    - **High Retic Count**-The bone marrow is trying to make up for excessive RBC destruction.
    - **Low Retic Count-**The bone marrow is making red blood cells at a reduced rate.
  - **MCV (mean corpuscular volume)-**measures the average size of RBCs
    - **Low MCV** indicates a problem synthesizing hemoglobin=**microcytic anemia.**
    - **High MCV** indicates a problem with DNA synthesis=**macrocytic anemia (Cells grow larger because they are less able to divide.).**

Alterations in Oxygen Transport: Anemias 3

- **Anemias Related to Decreased RBC Production**
  - Aplastic Anemia
  - Anemia of Chronic Renal Failure
  - Anemia of Vitamin B12 or Folate Deficiency
  - Iron Deficiency Anemia

Alterations in Oxygen Transport: Decreased RBC Production

Aplastic Anemia:

- **Etiology and Pathogenesis**
  - Decrease in functional bone marrow activity caused by injury to marrow stem cells
  - May be due to **leukemia** and bone marrow crowding.
  - Commonly associated with **cancer chemotherapies** that interfere with mitosis in rapidly-dividing cells, both cancerous and non-cancerous. Bone marrow cells divide very rapidly.
  - Decreased hematopoiesis, fatty (yellow) marrow replaces red marrow, and **pancytopenia** (ALL blood cells and platelets are low in number.) occurs.
- **Laboratory Features**
  - Low RBCs, WBCs, and platelets
  - Elevated bleeding time (due to low platelets)

Alterations in Oxygen Transport: Decreased RBC Production 2

Aplastic Anemia:

- **Clinical Manifestations**
  - Due to low WBC count (neutropenia): infection, fever and chills
  - Due to low platelet count (thrombocytopenia): petechiae (pin point flat red marks on skin), bruising, nosebleeds, retinal hemorrhage, and increased menstrual flow.
- **Treatment and Prognosis**
  - Bone marrow transplant
  - Prevent infection
  - Maintain hemoglobin and platelets
  - Stimulate hematopoiesis in patients not suited to bone marrow transplant
  - There is a 90% survival rate if the bone marrow transplant is from an HLA-matched sibling donor.

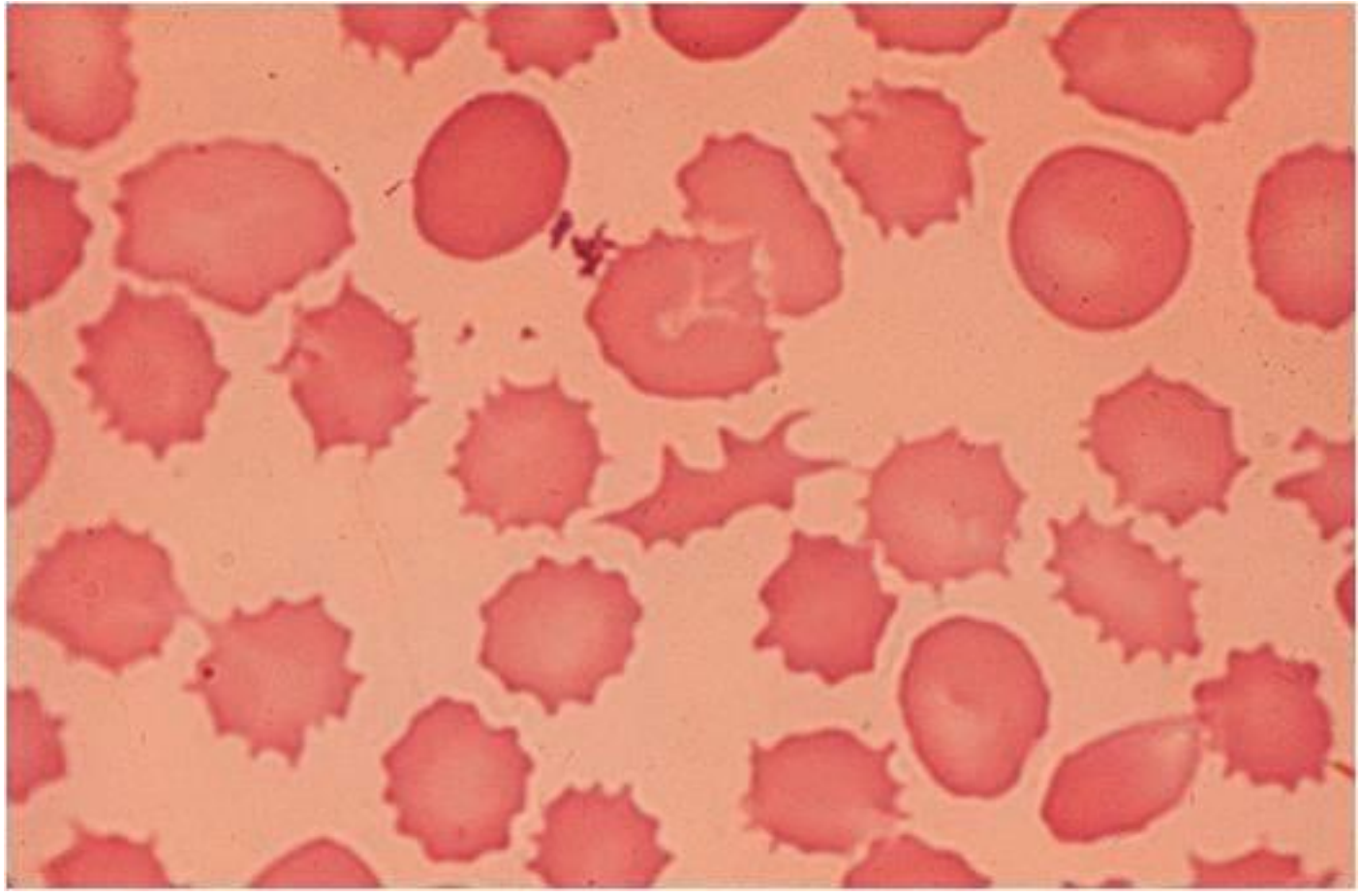
Alterations in Oxygen Transport: Decreased RBC Production 3

Anemia of Chronic Renal Failure:

- Etiology and Pathogenesis
  - There is impaired erythropoietin (EPO) synthesis and secretion secondary to kidney disease.
- Laboratory Features
  - Low RBCs, low hemoglobin, low hematocrit
  - Deformed RBCs **(Burr cells**); RBCs have spikey membranes.
  - WBCs and platelets are normal in number.
  - Mean corpuscular values are normal.
    - **MCV-mean corpuscular volume**=average RBC volume
    - **MCH-mean corpuscular hemoglobin**=average amount of hemoglobin per RBC
    - **MCHC-mean corpuscular hemoglobin concentration**=average amount (grams) of hemoglobin per dl (100 ml) of RBCs

Alterations in Oxygen Transport: Decreased RBC Production 4

blood smear from patient with chronic renal failure
Note: Burr Cells



Alterations in Oxygen Transport: Decreased RBC Production 5

Anemia of Chronic Renal Failure

- **Clinical Manifestations**
  - Hematocrit falls to 20% or less; normal is ~45%
  - Uremia (urea in the blood) occurs if the GFR (glomerular filtration rate) falls below 40 mL/min (125mL/min is normal.). GFR is the rate at which the kidneys form the renal filtrate. The renal filtrate becomes urine.
- **Treatment and Prognosis**
  - Erythropoietin supplementation
  - Dietary iron, vitamin B12, and folic acid
  - 95% respond to EPO.

Alterations in Oxygen Transport: Decreased RBC Production 6

Anemia of Vitamin B12 or Folate Deficiency

- **Etiology and Pathogenesis**
  - Without sufficient uptake of dietary Vitamin B12 or folate (aka Vitamin B9 or folic acid) important enzyme cofactors become deficient. This affects many enzymatic activities.
  - Three of the most important functions that suffer as a result are:
    - DNA synthesis and therefore cell division.
    - Hemoglobin synthesis.
    - Myelin sheath formation (due mostly to Vitamin B12 deficiency)

Alterations in Oxygen Transport: Decreased RBC Production 7

Anemia of Vitamin B12 or Folate Deficiency

- **Pernicious anemia** is due decreased absorption of Vitamin B12 from the digestive tract. It is caused by the lack of **intrinsic factor**.
  - Intrinsic factor is made by cells of the **stomach** mucosa.
  - Intrinsic factor must bind to Vitamin B12 to allow its absorption across the wall of the **small intestine** and into the blood.
  - Intrinsic factor production decreases in elderly individuals.
  - Its production also decreases in cases of gastric bypass surgery and partial or complete removal of the stomach due to gastric cancer.

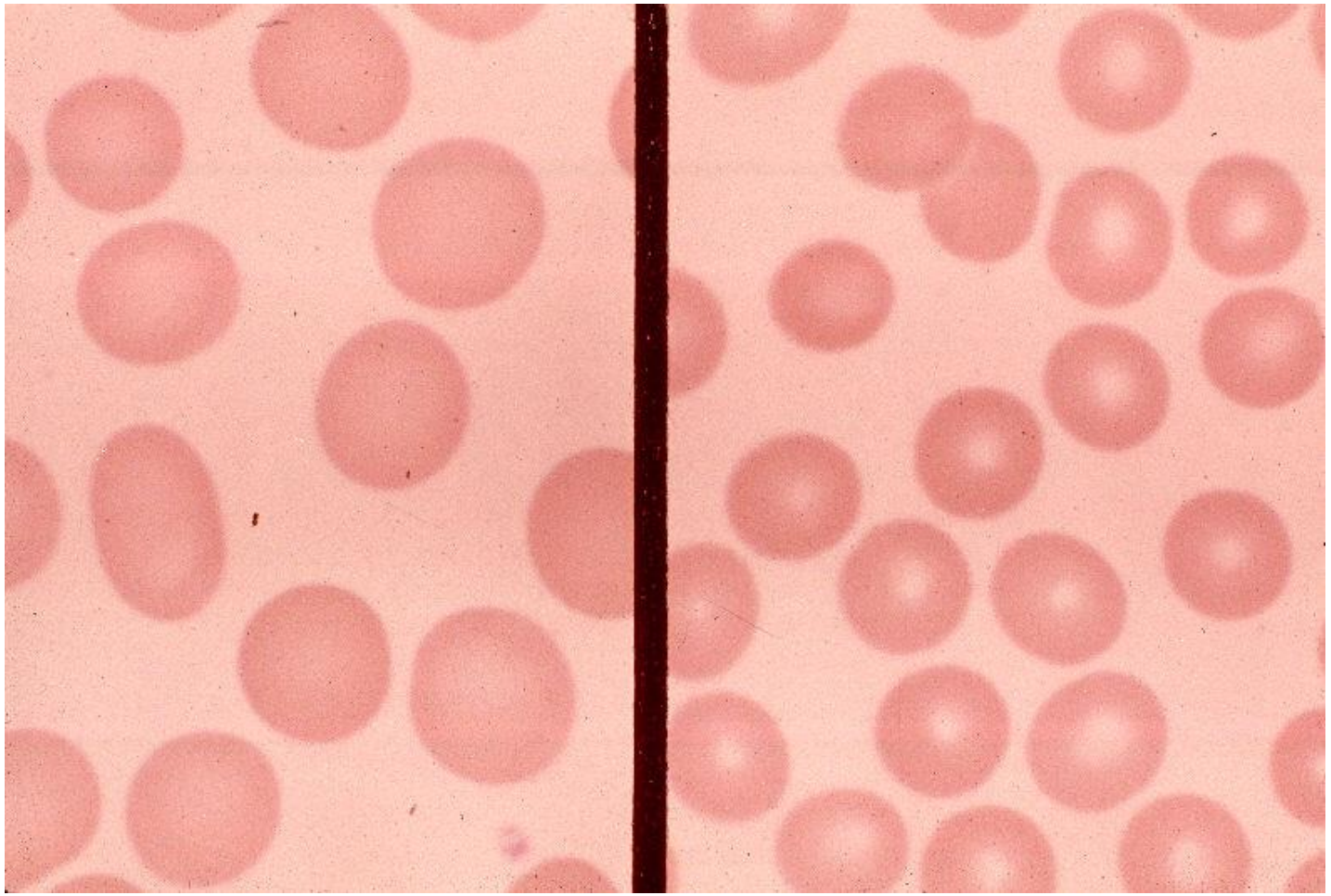
Alterations in Oxygen Transport: Decreased RBC Production 8

Anemia of Vitamin B12 or Folate Deficiency

- **Laboratory Features**
  - RBC, WBC, and platelet values are low, but not as low as in aplastic anemia.
  - Erythropoiesis produces abnormally large cells. The cells **spend more time in G1** of the cell cycle because the S phase is delayed due to problems with DNA synthesis.
  - Bone marrow contains **megaloblasts** (unusually large RBC precursor cell type).
  - Blood contains **macrocytic RBCs** (unusually large).
  - **Hypersegmented neutrophils** in peripheral blood (nuclei contain 6 or more lobes, normally only 3 or 4 occur)
  - MCV and MCH are increased, but MCHC is normal.
  - **Schilling test** (excretion of vitamin B12 in the urine) is low.
  - **Serum vitamin B12** is low.

Alterations in Oxygen Transport: Decreased RBC Production 9

Macrocytic RBCs Blood Smear	Normal RBCs Blood Smear



Alterations in Oxygen Transport: Decreased RBC Production 10

- **Clinical Manifestations Specific to Vitamin B12 Deficiency:**
  - “Megaloblastic madness” (paranoia, dementia, cognitive dysfunction, delusions, hallucinations)
  - Symmetric paresthesias of feet and hands
  - Proprioception disturbances (Proprioceptors sense body position.)
  - Paresthesias advance to spastic ataxia with spinal cord involvement.
  - Cerebral signs include memory loss, somnolescence, irritability, and perversion of taste, smell, and vision.
- **Clinical Manifestations Specific to Folate Deficiency:**
  - Less severe neurologic symptoms than in Vitamin B12 deficiency
  - Arthralgia, arthritis
  - Blotchy brown skin pigmentation in nail beds and skin creases

Alterations in Oxygen Transport: Decreased RBC Production 11

Anemia Related to Vitamin B12 or Folate Deficiency

- **Treatment**
  - Vitamin B12 replacement
  - For pernicious anemia Vitamin B12 must be administered by intramuscular injection, sublingual pill or patch. If it was administered orally it wouldn’t be absorbed due to lack of intrinsic factor.
  - Folic acid (oral) replacement
  - Transfusion in critically ill or elderly patients

Alterations in Oxygen Transport: Decreased RBC Production 12

Iron Deficiency Anemia

- **Etiology and Pathogenesis**
  - Most common cause of anemia in the world
  - Due to low intake, low absorption, increased requirement, or loss of iron
  - A healthy diet contains adequate amounts of iron for men, children, and postmenopausal women.
  - Menstruating and pregnant women require iron supplements.
  - Chronic inflammation causes macrophages to sequester iron. This situation presents as iron deficiency anemia.

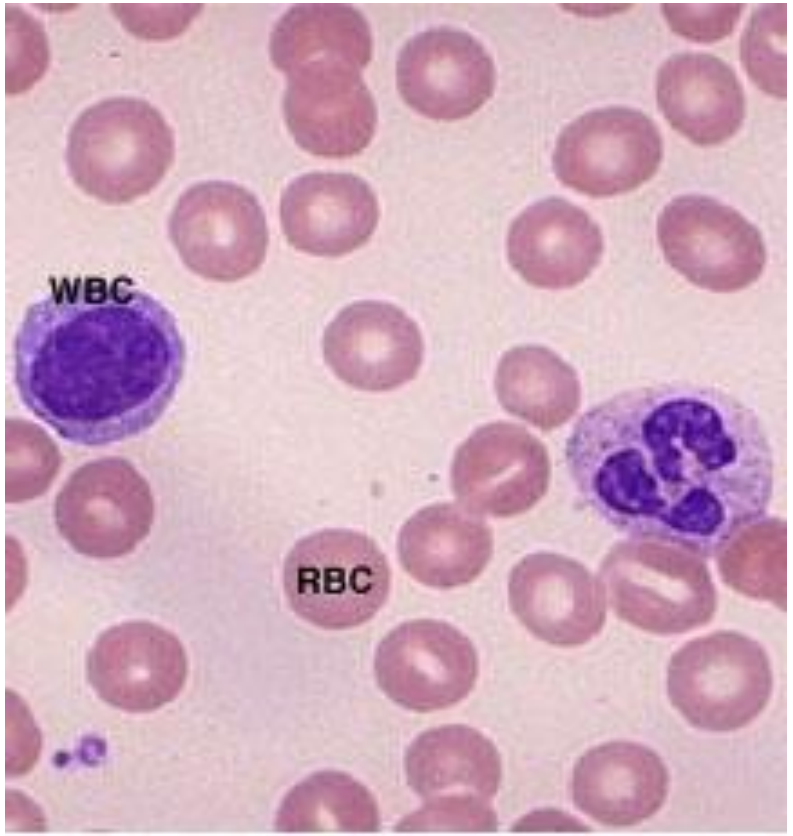
Alterations in Oxygen Transport: Decreased RBC Production 13

Iron Deficiency Anemia

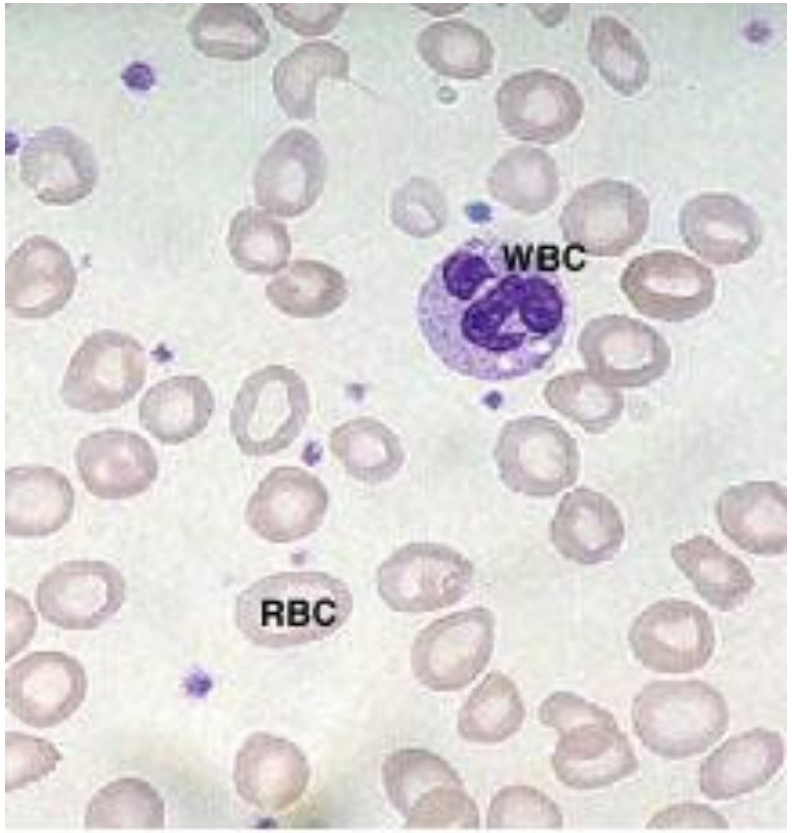
- Laboratory Features
  - RBC number usually low
  - RBCs are smaller than normal (**microcytic**) and pale in color (**hypochromic)**.
  - Mean corpuscular values (MCV and MCH) are low due to reduced cell size.
  - WBCs and platelets are normal.
  - **Serum ferritin** (iron storage protein) and serum iron are decreased
  - Transferrin saturation is reduced.
  - **Total iron binding capacity (TIBC)** is increased
    - TIBC-=a measure of free iron binding sites on iron-binding plasma proteins.

Alterations in Oxygen Transport: Decreased RBC Production 14

Microcytic, Hypochromic RBCs in Iron Deficiency Anemia



Normal Blood Smear



Iron Deficiency Anemia

Alterations in Oxygen Transport: Decreased RBC Production 15

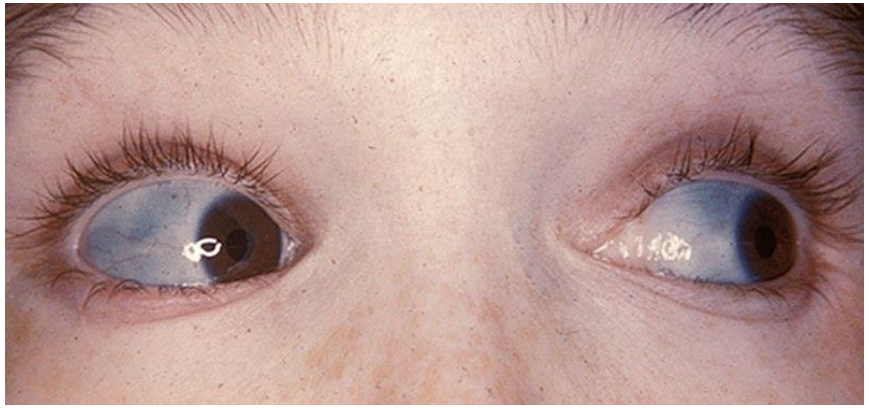
Iron Deficiency Anemia

- **Clinical Manifestations**
  - May be asymptomatic or exhibit general manifestations of anemia
  - Symptoms specific to iron deficiency anemia:
    - **Pica** (cravings for non-food substances such as dirt, clay, ice, hair, cardboard, laundry starch etc.)
    - **Koilonychia**-spoon-shaped nails
    - **Blue sclera**
    - **Restless leg syndrome (RLS)**, especially in the elderly
    - In severe cases, GI symptoms and splenomegaly
- **Treatment**
  - Oral iron supplements (ferrous sulfate) usually alleviates symptoms in a few days.
  - Supplements are continued for 4-6 months to rebuild stores.
  - The hemoglobin level usually normalizes after about 2 months.

Alterations in Oxygen Transport: Decreased RBC Production 16

Manifestations of iron deficiency anemia

Blue sclera



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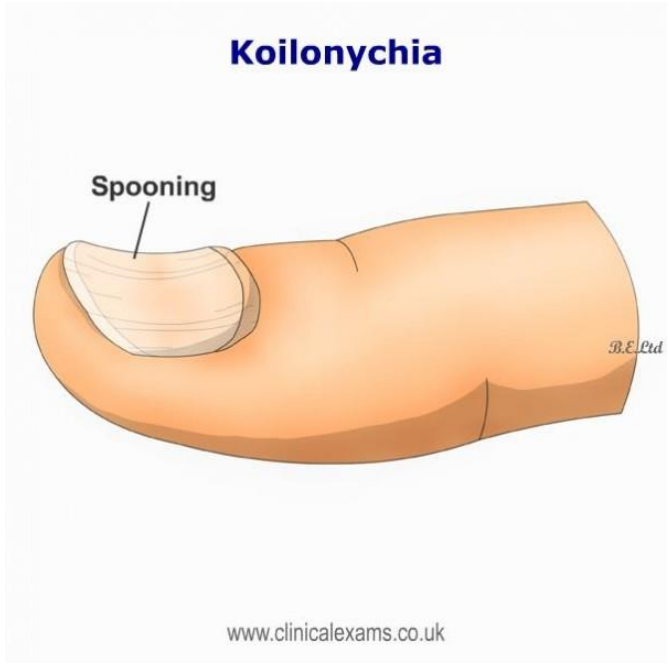


Alterations in Oxygen Transport: Genetically Abnormal RBCs

- **Anemias Related to Inherited Erythrocyte Disorders (Hemolytic Anemias)**
  - Thalassemia
  - Sickle Cell Anemia
  - Hereditary Spherocytosis
  - Glucose-6-phosphate Dehydrogenase Deficiency

  - These disorders produce abnormal red blood cells that experience an **increased rate of destruction by macrophages in the spleen.** The resulting anemia is termed **hemolytic anemia.**

Alterations in Oxygen Transport: Genetically Abnormal RBCs 1

Thalassemia

•Etiology and Pathogenesis

•Thalassemias are a group autosomal genetic diseases. There are two forms, alpha and beta.

•Suppression of the synthesis of one type of globin (alpha or beta) chain occurs due to a genetic mutation, while the "other", globin type builds up and precipitates inside the RBCs.

•The precipitation of normal chains damages RBC cell membranes, leading to **premature RBC destruction** by macrophages in the spleen.

•Hypoxia results and stimulates secretion of EPO. EPO causes increased intestinal iron absorption and profound **erythroblastic (immature RBCs) hyperplasia** in the bone marrow.

•Iron accumulates in the liver and spleen (sites of RBC destruction)**:** **"iron overload"** can be toxic.

Alterations in Oxygen Transport: Genetically Abnormal RBCs 2

Thalassemia

- **Laboratory Features**
  - RBC count is reduced
  - RBCs are microcytic and hypochromic with abnormalities in structure
  - Mean corpuscular values are decreased.
  - Reticulocyte count is elevated. The bone marrow tries to mediate the loss of RBCs by releasing immature RBCs to the circulation.
  - Platelets are normal
  - WBCs are often increased due to stimulation of the immune system.
  - Serum haptoglobin is elevated. Haptoglobin is a protein that binds to the hemoglobin released from RBCs that are destroyed.
  - Serum bilirubin is increased
  - Urine urobilinogen (the pigment in the urine which is synthesized from bilirubin) is increased
  - Hemoglobin electrophoresis is used to determine the type of thalassemia (alpha or beta).
- **Evidence of hemolysis**
  - Splenomegaly (enlarged spleen), the high level of activity causes the organ to enlarge.
  - Splenomegaly may lead to platelet sequestration and bleeding issues.
  - Jaundice, yellowing of skin and sclera, is due to elevated bilirubin

Alterations in Oxygen Transport: Genetically Abnormal RBCs 3

α-Thalassemia

- Most common among those of **Asian** descent.
- Humans have two genes (HBA1 and HBA2) for alpha globin chains on each chromosome 16.
- If a heterozygous mutation occurs in either gene, **α-thalassemia minor** occurs, anemia is mild.
- If a homozygous mutation occurs in one gene and a heterozygous mutation in the other, **α-thalassemia intermedia** occurs.
  - **Hemoglobin H (HbH)**, a tetramer of β chains, accumulates.
  - There are not enough α chains being made to form normal hemoglobin.
  - Hemoglobin H does not carry oxygen properly.
  - Hemoglobin H accumulation damages RBC membranes.
  - Blood transfusions may be needed.

Alterations in Oxygen Transport: Genetically Abnormal RBCs 4

α-Thalassemia

- If homozygous mutations occur in both genes, **α-thalassemia major (Bart's hemoglobin hydrops fetalis)** occurs.
  - Fetuses do not survive to be born.
  - Bart's hemoglobin (HbB) accumulates in the fetal blood.
  - Bart's hemoglobin is a tetramer of gamma (γ) chains. There are not enough alpha chains to produce fetal hemoglobin.
  - Recall that a molecule of fetal hemoglobin (HbF) contains 2 alpha globin chains and two gamma globin chains.

Alterations in Oxygen Transport: Genetically Abnormal RBCs 5

β-Thalassemia

- Humans have one gene for beta globin chains (HBB) on each chromosome 11.
- β-thalassemia occurs most often in those of **Mediterranean** descent.
- Three forms have been identified. The disorder is not a problem in fetuses and infants because their hemoglobin (HbF) doesn't contain beta chains yet.
- If just one beta gene is abnormal, heterozygous, **β-thalassemia minor/trait** occurs.
  - Beta chains are still made but are reduced in number by about 50%.
  - Anemia is mild, if it occurs.
  - RBCs may be microcytic, so the disorder may be misdiagnosed as iron-deficiency anemia.

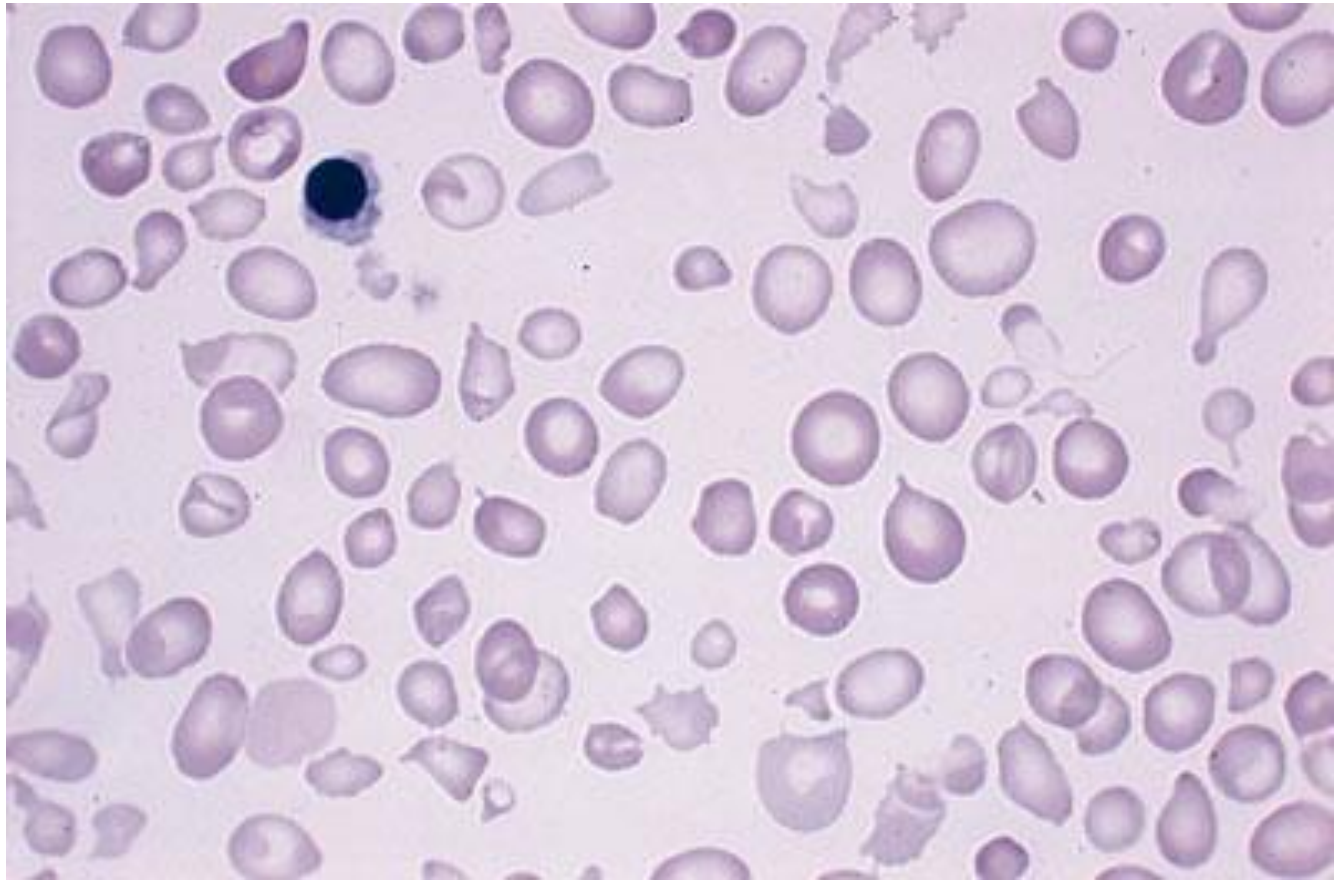
Alterations in Oxygen Transport: Genetically Abnormal RBCs 6

β-Thalassemia

- If both beta globin genes are missing or very defective, **β-thalassemia major** occurs. Fetuses and infants are not affected.
  - As HbF converts to HbA hemolytic anemia becomes severe and life threatening.
  - Red bone marrow expansion leads to bone/joint deformation. Hepatomegaly, splenomegaly and jaundice occur.
  - Survival requires frequent blood transfusions adding to iron overload. Iron chelation therapy is crucial.
    - A chelating agent, **deferoxamine**, binds to iron and is then excreted in the urine. A chelating agent is a chemical that binds to a metal.
    - Treatment is 8-12 hours daily 5 days a week!
  - Bone marrow transplantation may be curative.
  - **Hydroxyurea** is also a useful treatment. It delays conversion of HbF to HbA (delays the production of beta chains.)
- **β-thalassemia intermedia** refers to cases with symptoms intermediate between those of **β-thalassemia minor and β-thalassemia major.**

Alterations in Oxygen Transport: Genetically Abnormal RBCs 7

β-Thalassemia Major, Untreated



Lecture 2B:
Anemias, cont.
Acute Blood Loss
Polycythemia
The Process of Hemostasis

Alterations in Oxygen Transport: Genetically Abnormal RBCs 8

Sickle Cell Anemia

- **Etiology and Pathogenesis**
  - Single base substitution in the beta chain gene on chromosome #11 causes a single amino acid change.
  - Occurs almost entirely among those of African descent.
  - Abnormal hemoglobin S (HbS) is produced.
  - At reduced oxygen tension HbS polymerizes and precipitates, causing RBCs to assume a sickle shape.
  - Sickled cells have a decreased survival time leading to their premature destruction by macrophages in the spleen.
  - Sickled cells cause vascular occlusion leading to: capillary stasis (slow flow), venous thrombosis, arterial emboli. (A thrombus is a stationary blood clot. An embolus is a blood clot traveling in the blood.)
  - The sickling of cells infected with the malarial parasite reduces the severity of malaria. This selective advantage prevents the defective gene from being eliminated by natural selection in regions where malaria is prevalent.

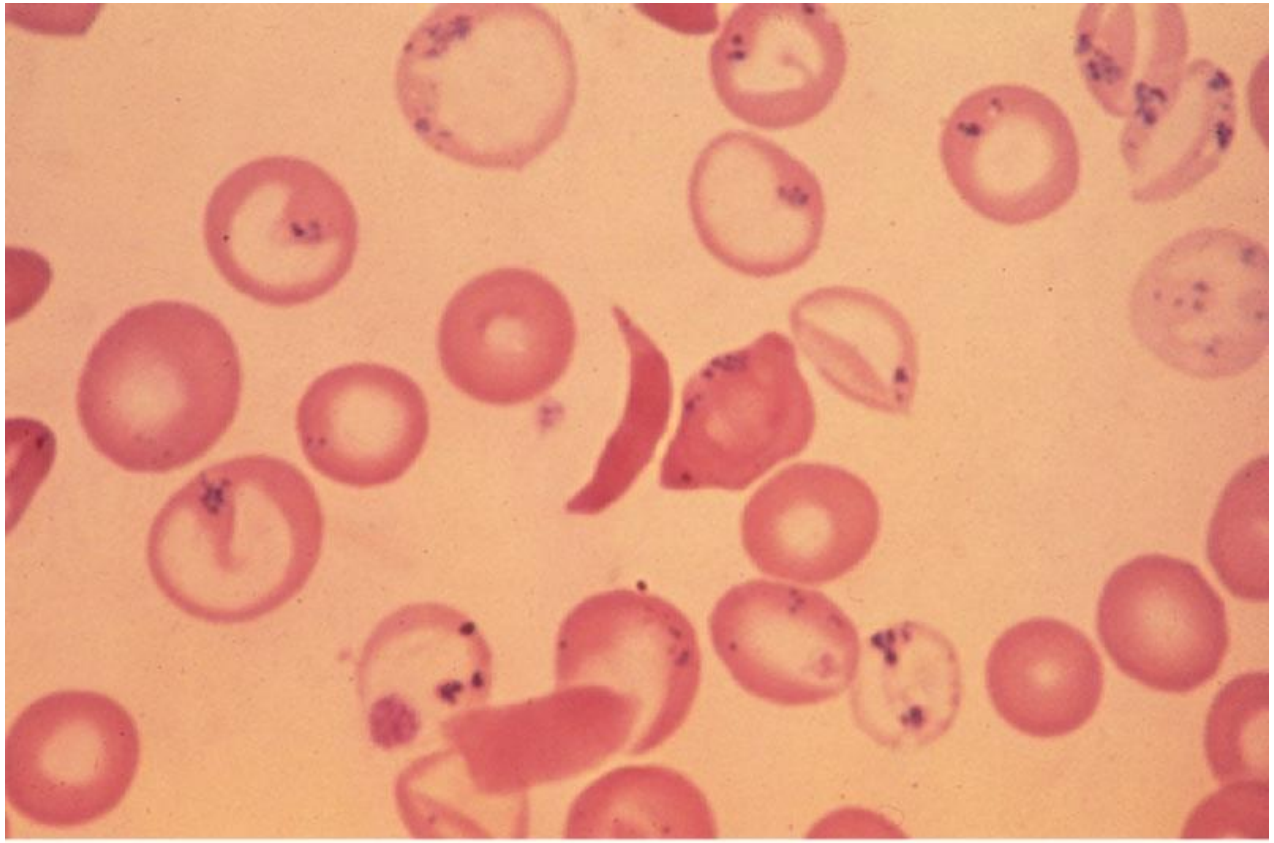
Alterations in Oxygen Transport: Genetically Abnormal RBCs 9

Sickle Cell Anemia

- **Laboratory Features**
  - Anemia is severe if both beta chain genes are abnormal (homozygous form).
    - Lab features are similar to thalassemia.
    - RBCs of different shapes and sizes are seen on smears.
  - The heterozygous form, called **sickle cell trait**, is much less severe. Sickle cell trait still produces sickled cells under conditions of low oxygen tension. Thus it confers resistance to severe infection by the malarial parasite, *Plasmodium falciparum.*

Alterations in Oxygen Transport: Genetically Abnormal RBCs 10

Sickle Cell Anemia

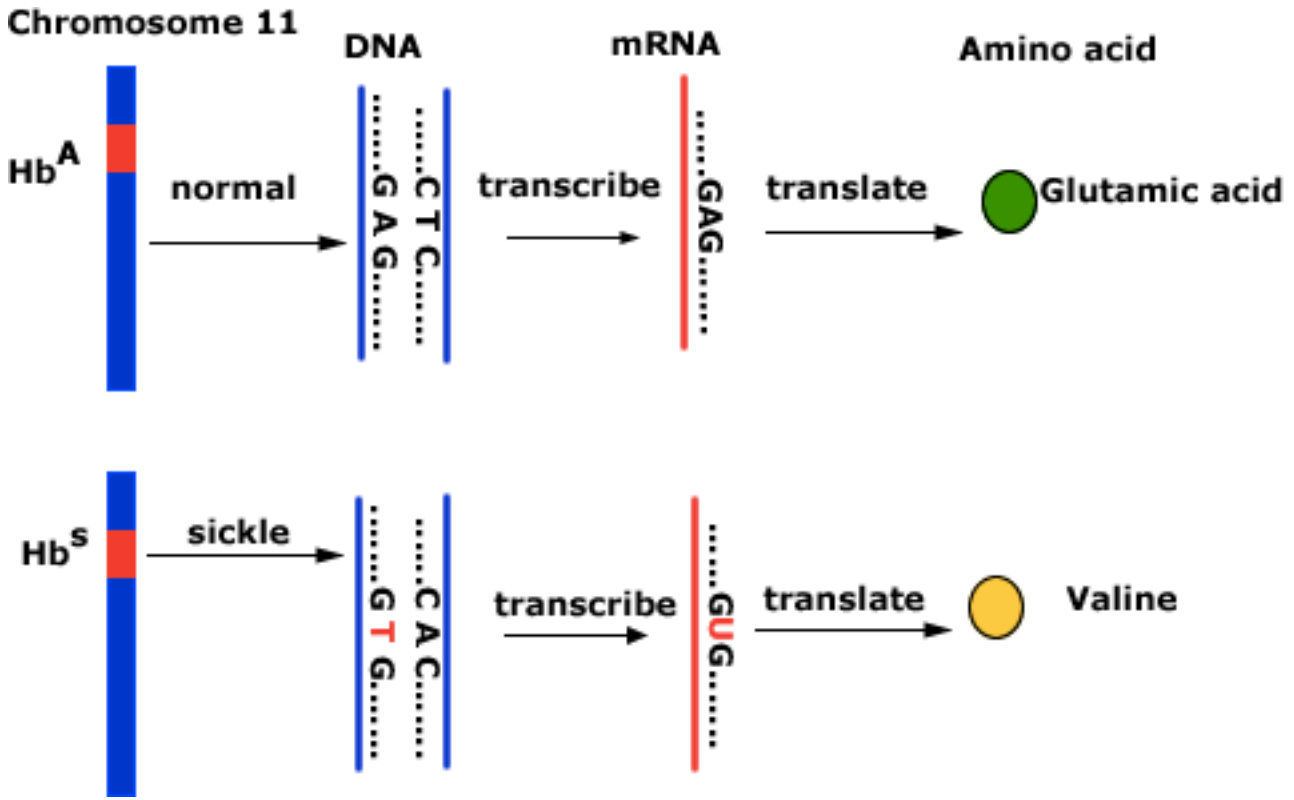


Courtesy Beth Payne, Sacred Heart Medical Center, Spokane, Wash.

Note sickled cells and granules of precipitated hemoglobin S.

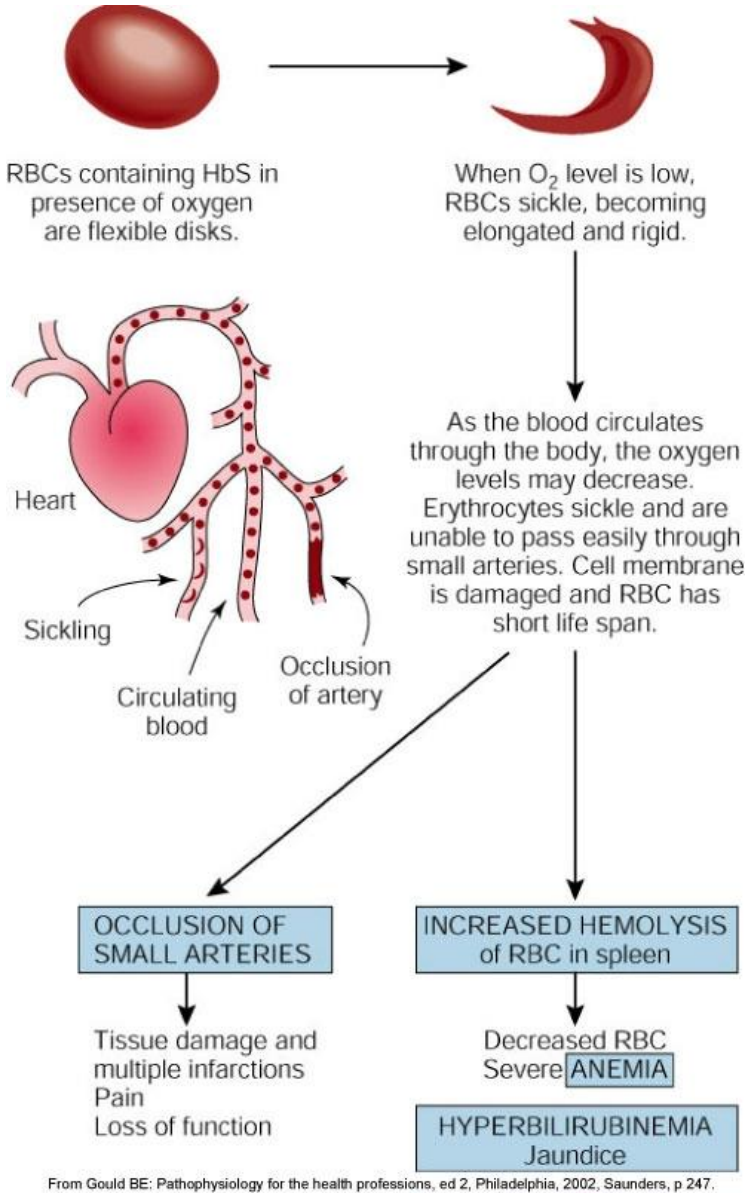
Alterations in Oxygen Transport: Genetically Abnormal RBCs 11

The mutation that causes sickle cell anemia:



Alterations in Oxygen Transport: Genetically Abnormal RBCs 12

Sickle Cell Anemia



From Gould BE: Pathophysiology for the health professions, ed 2, Philadelphia, 2002, Saunders, p 247.

Alterations in Oxygen Transport: Genetically Abnormal RBCs 13

Sickle Cell Anemia

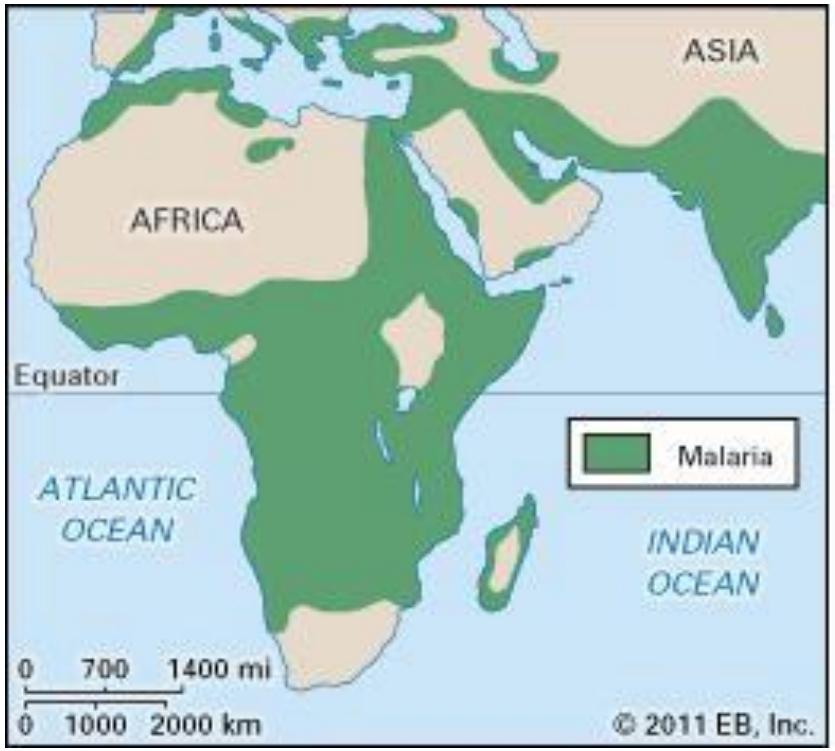
- **Clinical Manifestations**
  - Pooling of RBCs in the spleen can cause **splenic sequestration crisis**. There are too few circulating RBCs! This is a **common cause of deaths** that occur in the first years of life.
  - Delayed growth and delayed puberty
  - Bony abnormalities, **"hand-foot" syndrome**: inflammation in wrist, ankle, and digits
  - **Priapism** (prolonged, painful erection) with impotence
  - **Acute chest syndrome** is more serious in adults: hypoventilation, pulmonary infarct, sternal pain, fat emboli
  - Retinal vessel obstruction can cause blindness
  - Stroke, leg ulcers, infections
  - Pregnant women may exhibit pyelonephritis, pulmonary infarction, pneumonia, antepartum hemorrhage, premature delivery, and fetal death.

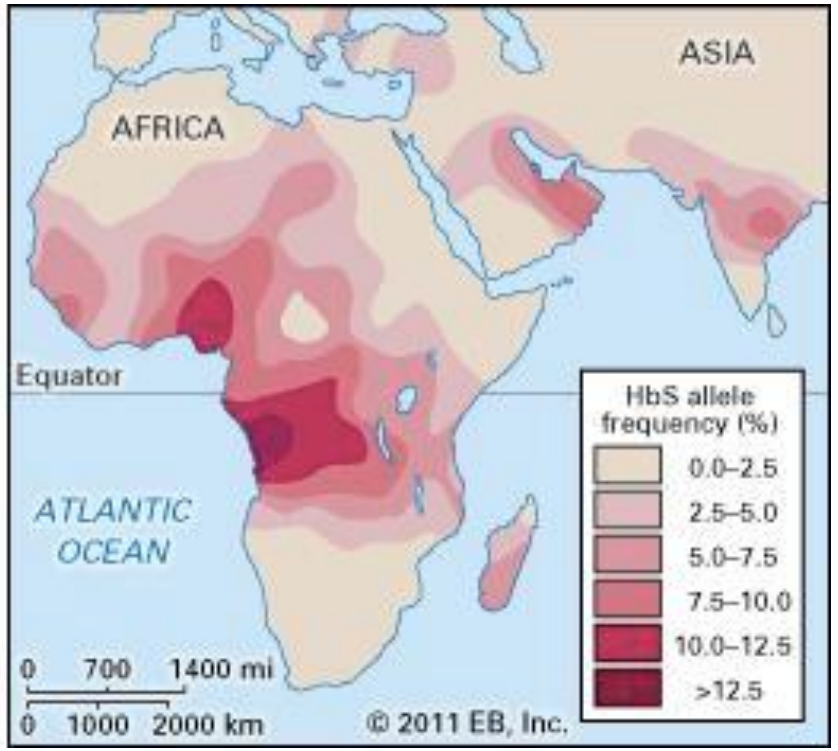
Alterations in Oxygen Transport: Genetically Abnormal RBCs 14

Sickle Cell Anemia

- **Treatment and Prognosis**
  - Bone marrow stem cell transplant is curative.
  - To avoid hemoglobin precipitation: prevent dehydration, infection, fever, acidosis, hypoxemia, cold temps
  - **Transfusion** to restore normal hematocrit
  - **Hydroxyurea** to prevent Hb F conversion to Hb A
  - Damage to the spleen **predisposes patient to infections**. Childhood vaccinations are especially important.
  - Without bone marrow transplant patients live into the third and fourth decade in developed countries. Survival past childhood is unlikely in underdeveloped countries.
  - In heterozygous sickle cell trait a few sickled cells are present and provide resistance to malaria. The kidneys may be affected, but otherwise heterozygotes are usually asymptomatic.
  - Genetic testing is available to prospective parents and also prenatally.

Maps: Incidence Malaria vs Incidence of Sickle Cell Anemia





The allele for sickle cell anemia persists in regions where malaria is common.

Alterations in Oxygen Transport: Genetically Abnormal RBCs 15

Hereditary Spherocytosis

- **Etiology and Pathogenesis**
  - The most common hereditary hemolytic anemia
  - Most common in those of northern European descent.
  - Genetic defect in red blood cell membrane proteins.
    - Mutations in at least 5 different genes produce this disorder.
    - Most often an autosomal dominant mutation in the **ANK-1 gene on chromosome 8**. ANK-1 codes for ankyrin-1, a membrane protein.
    - An autosomal recessive form exists
  - Red cells are very small and have lost their biconcave shape. They are spherical. These fragile **microspheroctyes** subject to destruction by the spleen.
- **Laboratory Features**
  - Lab features are similar to thalassemia.
  - Blood smear shows microspherocytes and reticulocytosis.

Alterations in Oxygen Transport: Genetically Abnormal RBCs 16

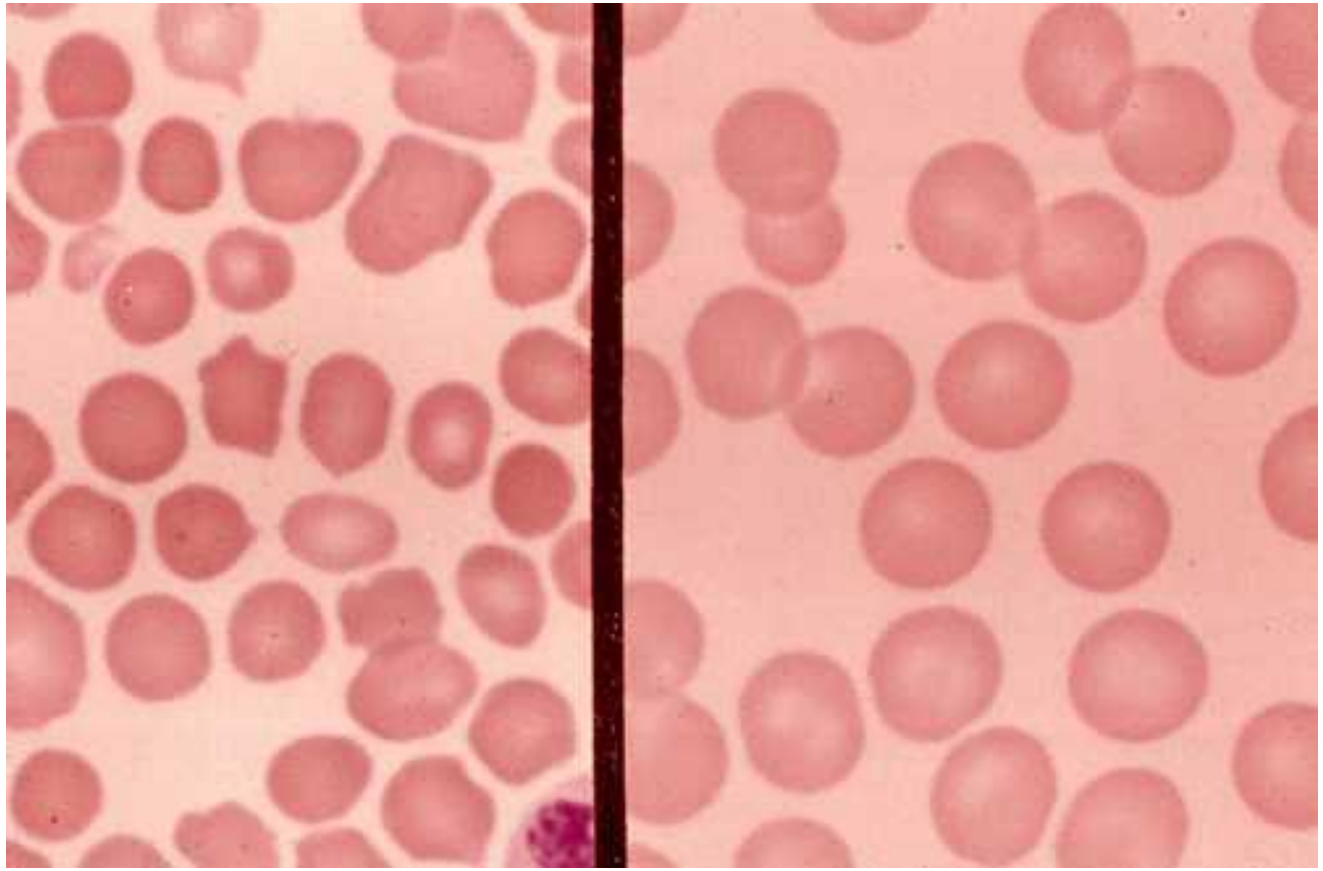
Hereditary Spherocytosis

- **Clinical Manifestations**
  - Most patients have mild to moderate chronic anemia.
  - Jaundice, splenomegaly and bile pigment gallstones occur.
  - **Aplastic crisis** (bone marrow suppression) may be precipitated by infection, usually by **parvovirus.** If so, flu-like symptoms, pallor and tachycardia occur. Shock is a danger.
- **Treatment and Prognosis**
  - **Splenectomy** (removal of the spleen) is usually performed in childhood.
  - Splenectomy is curative with subsequent risk of infections. The spleen contains many immune system cells.
  - Children should be vaccinated prior to splenectomy.
  - Oral penicillin and folic acid therapy should follow for several years after splenectomy.
  - Transfusion is performed in the case of aplastic crisis.

Blood Smears: Hereditary Spherocytosis vs Normal

Blood Smear in Hereditary Spherocytosis

Normal Blood Smear



Alterations in Oxygen Transport: Genetically Abnormal RBCs 17

Glucose-6-phosphate Dehydrogenase (G6PD) Deficiency

- **Etiology and Pathogenesis**
  - Due to an X-linked recessive gene for G6PD
  - Occurs most often in **males** of **African** descent and in **male Sephardic Jews**.
  - G6PD is an enzyme of the **hexose monophosphate shunt**, the anaerobic pathway used by RBCs to produce NADPH.
    - NADPH is required to produce **glutathione (GSH)**, a substances that protects RBCs against **oxygen free radical** damage.
    - G6PD deficiency provides a selective advantage against **malaria**.
- No anemia occurs unless the individual is challenged by infection or chemicals that deplete glutathione stores.
- Without glutathione, RBCs may accumulate defects that cause them to be phagocytized in the spleen.

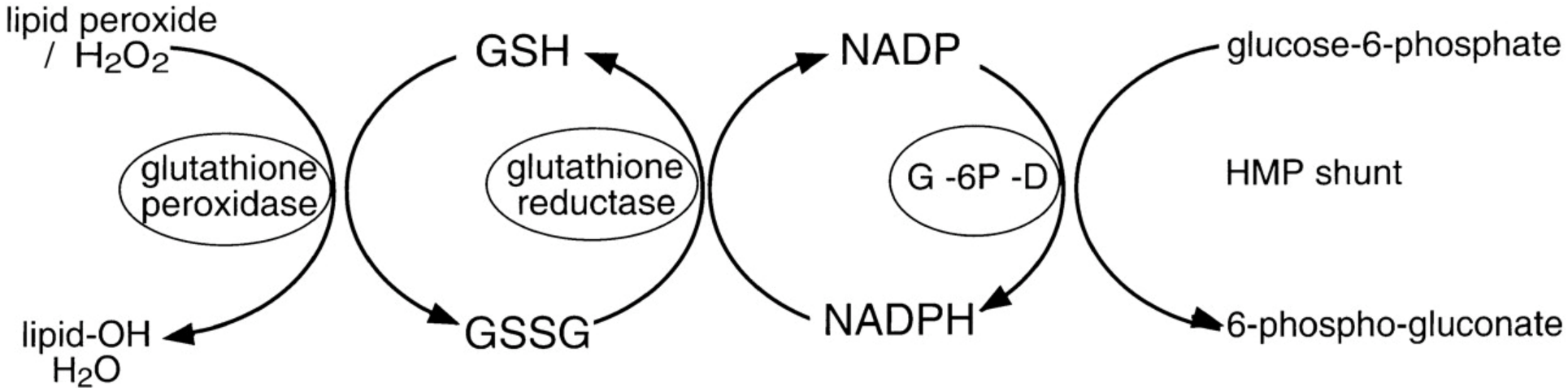
Alterations in Oxygen Transport: Genetically Abnormal RBCs 18

Glucose-6-phosphate Dehydrogenase Deficiency

- **Laboratory Features**
  - Diagnosis is based on specific testing for G6PD enzymatic activity.
- **Clinical Manifestations**
  - As for hemolytic anemia
- **Treatment**
  - Avoid infections
  - Avoid drugs known to deplete glutathione: sulfa drugs, anti-malaria drugs, aspirin, vitamin C
  - Transfusion, if severe

Production and Action of Glutathione

NADP is reduced to form NADPH as G6PD oxidizes glucose-6-phosphate to 6-phospho-gluconate. Glutathione disulfide (GSSG) is reduced to glutathione (GSH) as NADPH is oxidized to NADP. **GSH is oxidized back to GSSG as peroxide (an oxygen free radical) is reduced to water.** Thus GSH protects RBCs from oxygen free radicals.



Alterations in Oxygen Transport: Destruction/Loss of Normal RBCs

- **Anemia Related to Extrinsic Red Cell Destruction or Loss**
  - Hemolytic Disease of the Newborn
  - Antibody-Mediated Drug Reaction
  - Acute Blood Loss

Alterations in Oxygen Transport: Anemias Destruction/Loss of Normal RBCs 1

Hemolytic Disease of the Newborn (HDNB)

- **Etiology and Pathogenesis**
  - Most serious form occurs when an Rh+ fetus is carried by an Rh- mother. This means the father is Rh+.
  - If Rh+ fetal red cells enter the mother's blood, her immune system becomes sensitized against Rh antigens on the fetal cells and begins to mount a primary immune response against it. (If fetal red cells enter the mother's system, it usually happens at birth.)
  - Since it takes a couple weeks for anti-Rh antibodies to be synthesized by the mother's immune system, **her first Rh+ baby is usually unaffected.**
  - In a subsequent pregnancy with an Rh+ fetus, the mother's sensitized immune system will react quickly against Rh antigens. Maternal anti-Rh antibodies (IgG), will cross the placenta, bind to fetal RBCs and cause the hemolysis of fetal RBCs. In severe cases **hydrops fetalis (edema and fluid accumulation in serous cavities)** may occur.

Alterations in Oxygen Transport: Destruction/Loss of Normal RBCs 2

Hemolytic Disease of the Newborn

- **Laboratory Features**
  - Anemia with **erythroblastosis** (excessive immature RBCs, blast cells in the bone marrow and peripheral blood)
  - **Leukocytosis** (elevated WBCs due to the inflammatory response) is present, but platelets are usually normal.
  - At birth the baby's serum bilirubin level reflects **both** the severity of the hemolytic disease process and the inability of the immature liver to conjugate and excrete bilirubin.
  - Cord red blood cells show a positive anti-globulin test (**Coomb's test**), indicating that maternal antibodies are bound to the baby's red blood cells.
  - During pregnancy testing the amniotic fluid for bilirubin and antibodies will indicate the likelihood that the fetus will be affected by HDNB.

Alterations in Oxygen Transport: Anemias Destruction/Loss of Normal RBCs 3

Hemolytic Disease of the Newborn (HDNB)

- **Clinical Manifestations**
  - Hemolytic anemia
  - Hyperbilirubinemia (increased bilirubin in the blood)
  - Jaundice
  - Petechiae
  - Splenomegaly, hepatomegaly
  - Heart failure
  - Fetal hydrops=fluid overload (pulmonary edema, pleural effusion, pericardial effusion, ascites, edema)
  - **Kernicterus** (bilirubin in brain tissue) and associated neural issues
  - Diffuse intravascular coagulation (DIC)
  - Before prenatal monitoring and treatment were available, many HDNB infants died *in utero*.

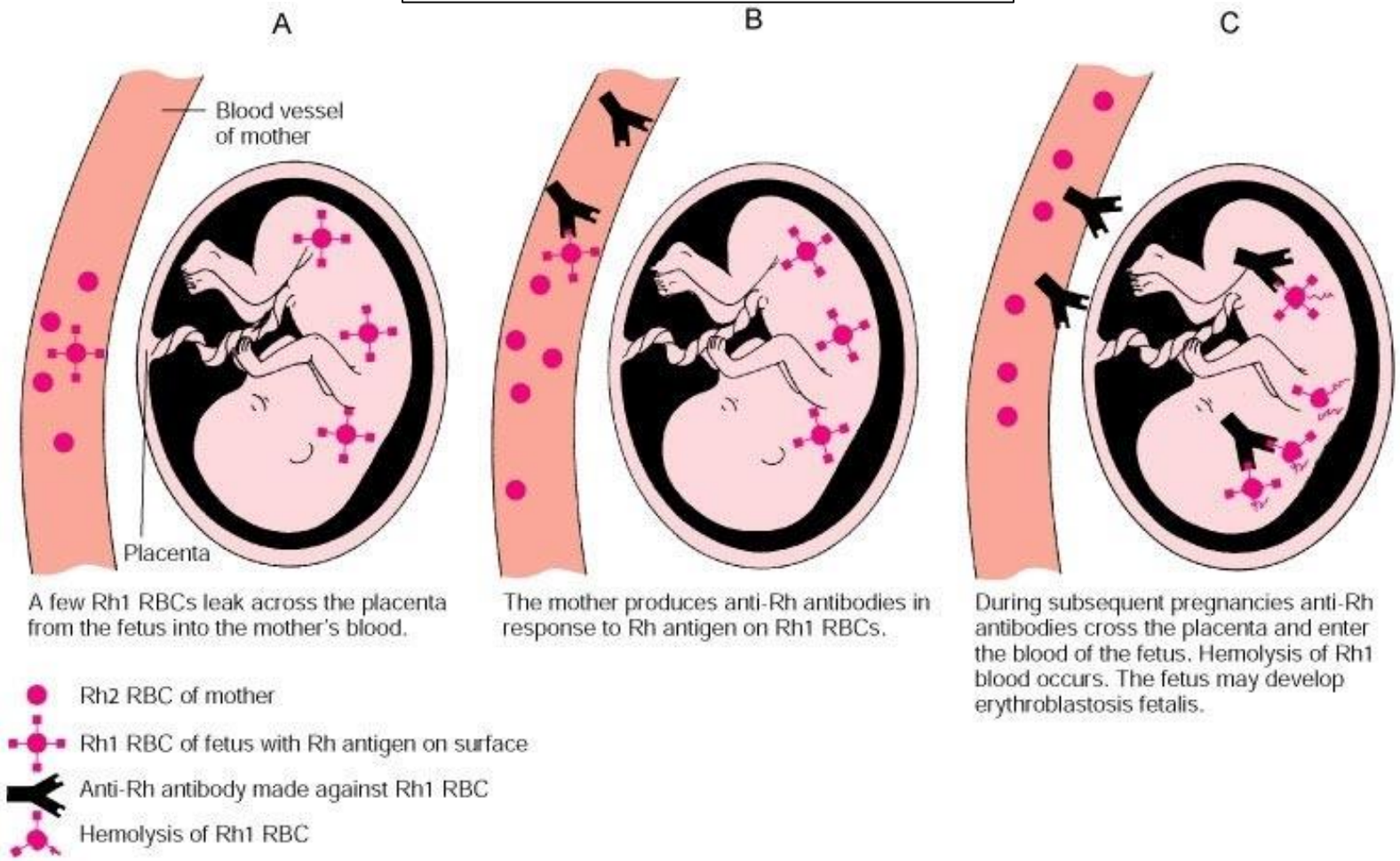
Alterations in Oxygen Transport: Destruction/Loss of Normal RBCs 4

Hemolytic Disease of the Newborn

- **Treatment and Prognosis**
  - **RhoGAM (solution of anti-Rh antibodies)** is given prophylactically to the Rh- mother before and after delivery to destroy any fetal red cells that may have entered the mother's circulation. So RhoGAM prevents the mother's immune system from becoming sensitized against Rh antigen.
  - RhoGam will not negatively affect the Rh+ fetus because the antibodies in RhoGAM (IgA), unlike the antibodies made by a sensitized mother (IgG), are **too large to cross the placenta**.
  - Successful RhoGAM programs have reduced perinatal mortality to 1-2%.
  - *In utero* transfusion and early delivery may be performed in cases of sensitized mothers. Transfusion lowers the level of bilirubin and anti-Rh antibodies in the blood of the fetus.
  - After birth, serum bilirubin is reduced by **phototherapy** (UV light breaks down bilirubin.) and **phenobarbita**l (stimulates liver enzymes that breakdown bilirubin).

Alterations in Oxygen Transport: Destruction/Loss of Normal RBCs 5

Rh Incompatibility



From Solomon EP: Introduction to human anatomy and physiology, ed 2, St Louis, 2003, Mosby, p 172.

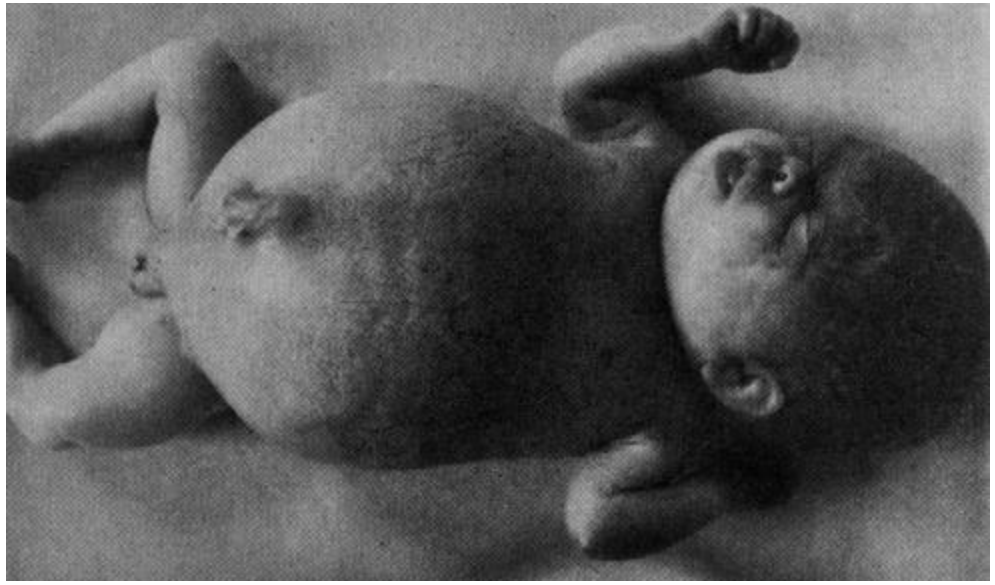
Alterations in Oxygen Transport: Destruction/Loss of Normal RBCs 6

Prevention of HDNB

Alterations in Oxygen Transport: Destruction/Loss of Normal RBCs 7

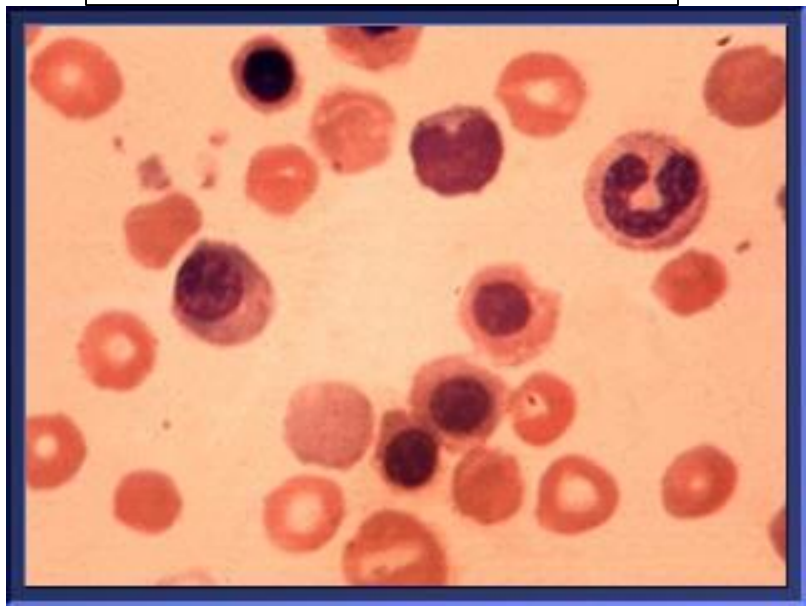
Hemolytic Disease of the Newborn

Hydrops Fetalis



Erythroblastosis

(Several RBCs have nuclei. Abnormal!)



Alterations in Oxygen Transport: Destruction/Loss of Normal RBCs 8

- **Antibody-Mediated Drug Reaction**
  - Drugs, particularly antibiotics, can lead to RBC destruction via an **autoimmune reaction**. The person's own immune system synthesizes antibodies that bind to molecules on the surfaces of their own RBCs. This is known as a **type II hypersensitivity reaction,** a topic in Module 3**.**
  - The drugs alter the RBC membrane so that the immune system reacts to the RBCs as if they are foreign antigens.
  - Some of the drugs associated with this type of reaction:
    - Penicillin-antibiotic
    - Quinidine-used to treat heart rhythm abnormalities
    - α-Methyldopa-used to treat high blood pressure
    - Cephalosporins-antibiotics

Alterations in Oxygen Transport: Destruction/Loss of Normal RBCs 9

- **Etiology and Clinical Manifestations of Acute Blood Loss**
  - Acute blood loss is often associated with gastric ulcers and GI disease in men.
  - It is most often associated with abnormal menstrual bleeding in women.
  - In a 70-kg (154 lb) person with a 5000 mL total blood volume:
    - 30% (1500mL) blood loss leads to flat neck veins when supine, postural hypotension, exercise tachycardia.
    - 40% (2000mL) blood loss leads to a fall in venous pressure, cardiac output and arterial pressure both supine and at rest, with associated air hunger, tachycardia, and cold, clammy skin.
    - 50% (2500mL) blood loss causes shock and death.

Alterations in Oxygen Transport: Destruction/Loss of Normal RBCs 9

- **Treatment of Acute Blood Loss**
  - Blood **volume replacement** with blood plasma, albumin, or dextran occurs first to **prevent shock**. (Transfusion of cells can occur later, if necessary.)
  - Natural replacement of the red cell mass occurs over 2 to 5 days as marrow stem cells proliferate and mature.
  - Maximal RBC production is usually seen by the 10th day after hemorrhage.
- **Transfusion Therapy**
- Donor blood is tested before transfusion:
  - ABO and Rh typing
  - Screening for syphilis, HIV, hepatitis B and C, and T-cell lymphotropic virus.
- Blood may be treated to remove donor WBCs and antibodies before transfusing the recipient. This reduces the risk of a GVH (graft versus host) immune reaction and also the sensitization of the recipient against the donor WBCs and antibodies.

Excessive RBC Production

- **Polycythemia**-RBCs are present in excess causing an increase in blood viscosity and blood pressure. There are three types:
  - Polycythemia Vera
  - Secondary Polycythemia
  - Relative (Spurious) Polycythemia

Excessive RBC Production

- **Polycythemia Vera**
  - **Etiology:** a rare blood cancer; the red bone marrow produces excessive numbers of red blood cells
  - **Symptoms:** itchiness, neuropathies (tingling and numbness in hands and feet), nose bleeds
  - **Complications**: blood clots, enlarged spleen

- **Secondary Polycythemia**
  - **Etiology:** chronic hypoxemia as occurs in chronic obstructive pulmonary disease (COPD), sleep apnea, obesity

- **Relative Polycythemia**
  - **Etiology:** hypovolemia (decreased blood plasma volume) as occurs in dehydration or burn injury**.**

The Process of Hemostasis

Hemostasis, the body's mechanism to stop bleeding, has multiple steps:

Primary Hemostasis:

- **Vascular Spasm**
  - **Contraction of vascular smooth muscle in the wall of the injured blood vessel**
- **Platelet Plug Formation**
  - **Platelet adherence, activation, aggregation and recruitment**

Secondary Hemostasis:

- **Coagulation**
  - **Formation of fibrin**
- **Clot Retraction**
  - **Contraction of clot proteins**
- **Fibrinolysis**
  - **Removal of blood clots**

The Process of Hemostasis: Platelet Plug Formation

Platelets

- Normal platelet count **150,000-400,000/mm^3** in peripheral blood
  - **70%** of platelets are in the circulation.
  - **30%** of platelets are stored in the spleen.
- Platelet life span is about **10 days**. They are removed by phagocytes (mostly in the spleen).
- They are kept **inactive** by the NO (**nitric oxide**) and **prostaglandin-12** (AKA prostacyclin or PG-12) both chemicals are released by endothelial cells.
- Platelets are activated by binding to **von Willebrand factor** (VWF) a protein that binds to subendothelial **collagen fibers** that have been exposed by vessel injury. This process is called **platelet adherence**. VWF is synthesized by endothelial cells and by megakaryoctyes.

The Process of Hemostasis: Platelet Plug Formation 1

Platelet Activation

- Platelet activation causes **platelet degranulation** or the "platelet release reaction"**.** There are two types of granules in platelets.
  - **Alpha granules release proteins:** fibrinogen, von Willebrand factor, clotting factors V and VIII and growth factors.
  - **Dense granules release:** ADP, ATP, Ca^{2+} and serotonin.
- ADP and exposed collagen activate the enzyme, **phospholipase,** in platelets. As a result, **arachidonic acid,** a membrane phospholipid, is released**,** and the **arachidonic acid pathway** is stimulated. One of the products of the pathway is **thromboxane A2.**
  - Thromboxane A2 stimulates increased expression of **fibrinogen receptors** on platelets.

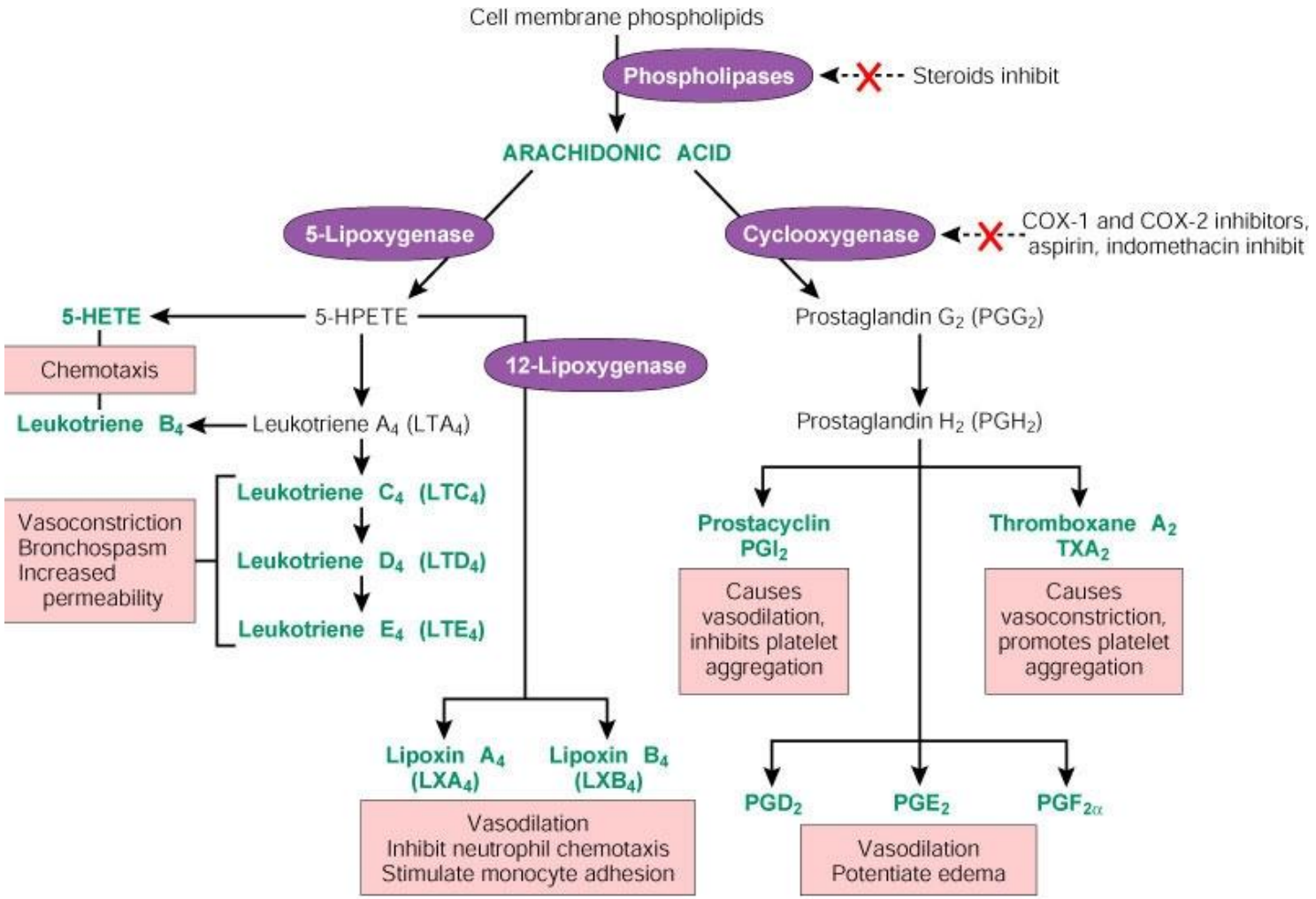
The Process of Hemostasis: Platelet Plug Formation 2

- Fibrinogen links fibrinogen receptors on separate platelets together to cause **platelet aggregation.**
- **COX (cyclooxygenase)** is an enzyme that catalyzes an early reaction in the arachidonic acid pathway.
- **COX inhibitors**, including aspirin, block the production of thromboxane A2 to prevent platelet aggregation.

- The arachidonic acid pathway produces a number of powerful inflammatory chemicals. Phospholipase is an enzyme that is commonly activated in injured cells. More on this later in the course.
- **ADP and thromboxane A2** act to recruit additional platelets to the site of vessel injury by binding to receptors in the platelet plasma membrane.

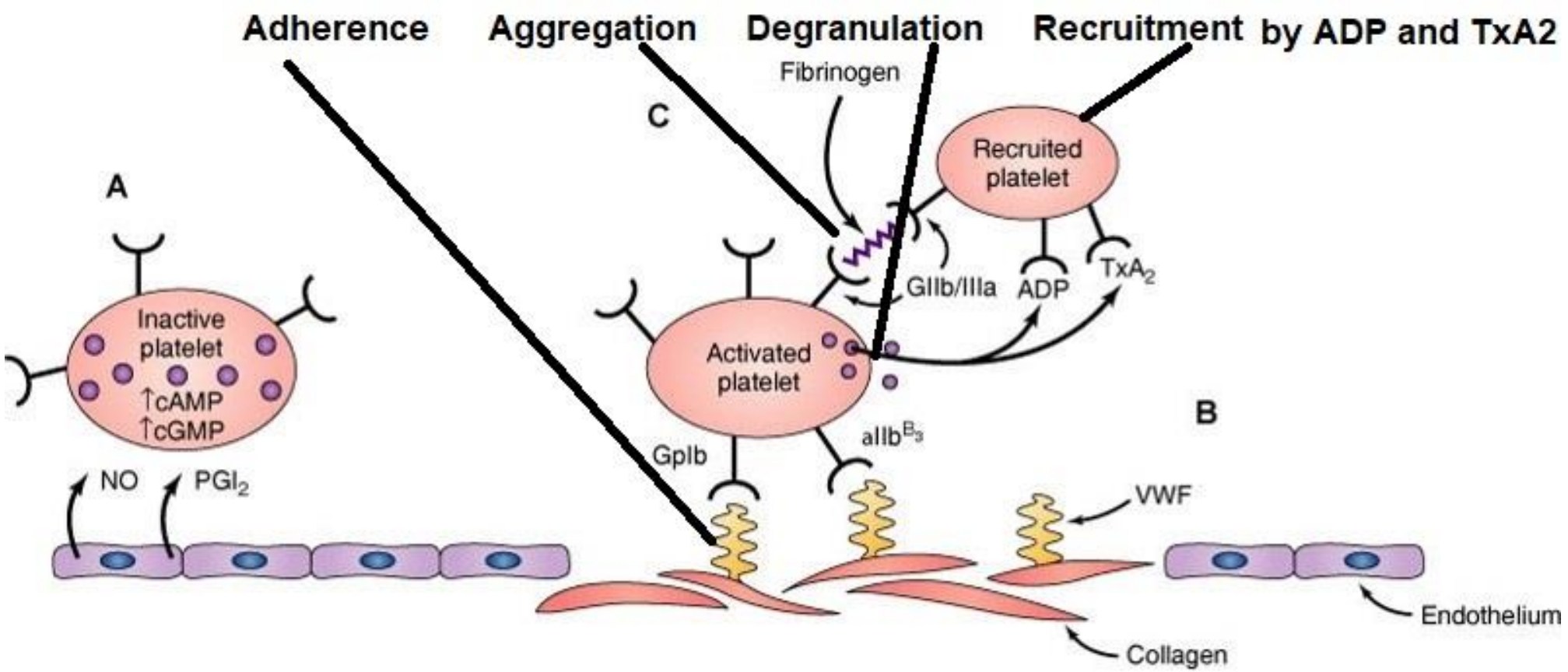
The Process of Hemostasis: Platelet Plug Formation 3

Thromboxane A2: The Arachidonic Acid Pathway:



From Kumar V, Cotran RS, Robbins SL: Robbins basic pathology, ed 7, Philadelphia, 2003, Saunders, p 281.

4 Processes in Platelet Plug Formation



The Process of Hemostasis: Formation of Fibrin

Coagulation Pathways (Fibrin Clot Formation)

- **Extrinsic pathway (occurs outside the blood vessel)**
  - Initiated when **tissue thromboplastin** (aka tissue factor= TF=**Factor III**) is released by injured tissue. It activates Factor VII in the presence of Ca2+ (**Factor IV**).
  - **Factor VIIa/TF complexes** form and **activate Factor X**.
  - Factor X is activated **faster by the extrinsic** pathway.
- **Intrinsic pathway (occurs inside the blood vessel)**
  - Initiated when blood contacts altered endothelium (or, in the lab, a negatively charged surface like glass) and causes activation of Factor XII.
  - Factor XIIa activates Factor XI. Factor XIa activates Factor IX. **Factor IXa activates Factor X** in the presence of **Factor VIIIa,** platelet membrane phospholipid (PL) and Ca2+ **(Factor IV)**.

The Process of Hemostasis: Formation of Fibrin 1

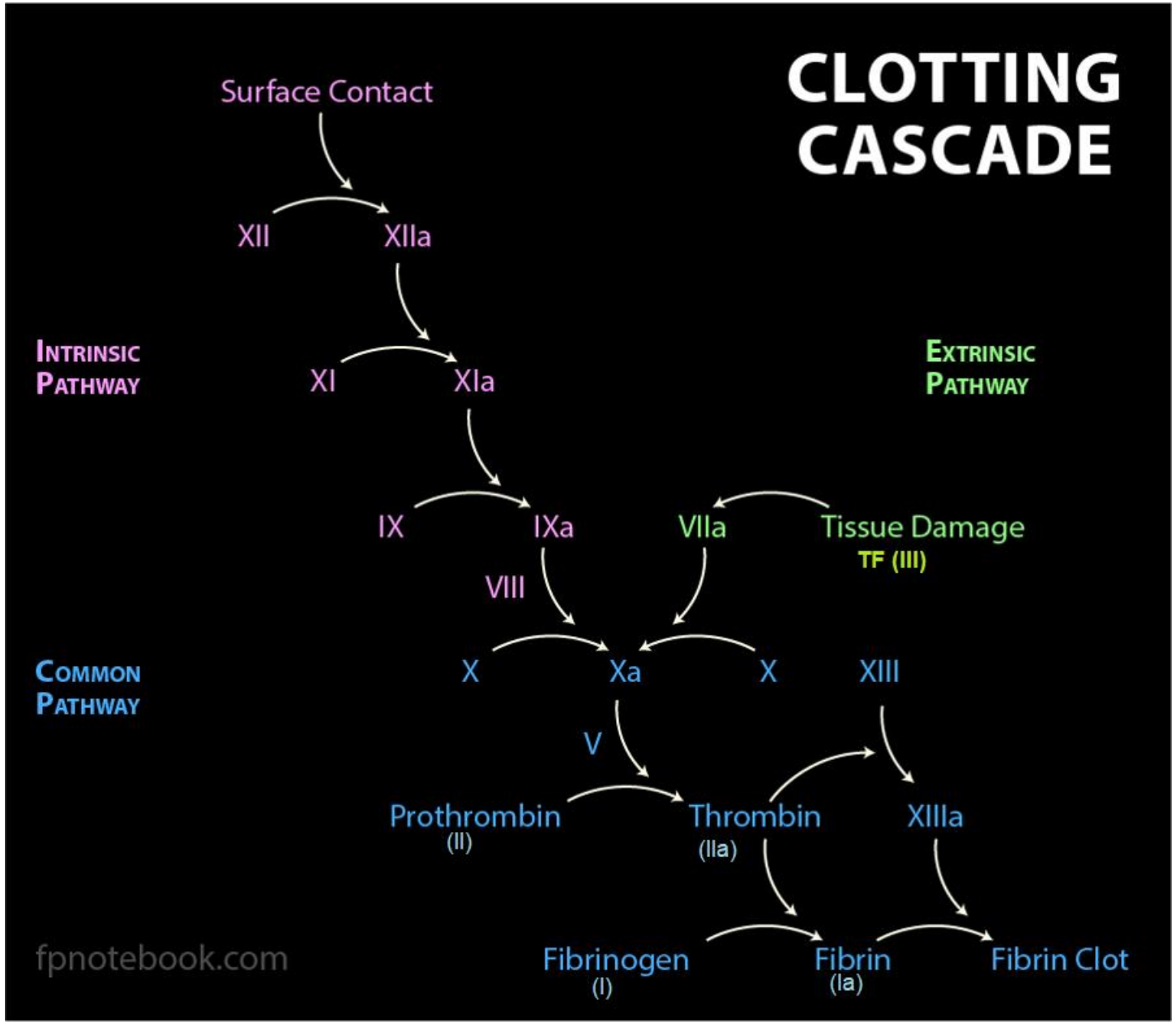
Coagulation Pathways (Fibrin Clot Formation)

- **Common pathway**
  - Factor Xa, in the presence of platelet membrane phospholipid (PL), **Factor Va**, and calcium ions (**Factor IV**), convert **prothrombin (Factor II) into thrombin (Factor IIa)**.
  - Thrombin activates both **Factor V and Factor VIII** as required by the intrinsic pathway.
  - Thrombin is the enzyme that converts **fibrinogen (Factor I) into fibrin (Factor Ia).**
  - Factor XIII is also activated by thrombin. Factor XIIIa **crosslinks fibrin** to finish the clot.
  - Continued activity of the Factor XIII, released by platelets, causes **clot retraction.**

The Process of Hemostasis: Formation of Fibrin 2

- Factor I - fibrinogen
- Factor II – prothrombin (**zymogen** for thrombin)
- Factor III - tissue thromboplastin (tissue factor)
- Factor IV - ionized calcium (Ca++)
- Factor V
- Factor VII - **zymogen**
- Factor VIII
- Factor IX - **zymogen**
- Factor X - **zymogen**
- Factor XI
- Factor XII - **zymogen**
- Factor XIII
- The zymogen factors, when activated, are **proteases**. The proteases clip peptides out of other zymogens to activate them. The lower case “a” after the factor # indicates an activated factor.

The Coagulation Cascade



The Process of Hemostasis
Formation of Fibrin 3

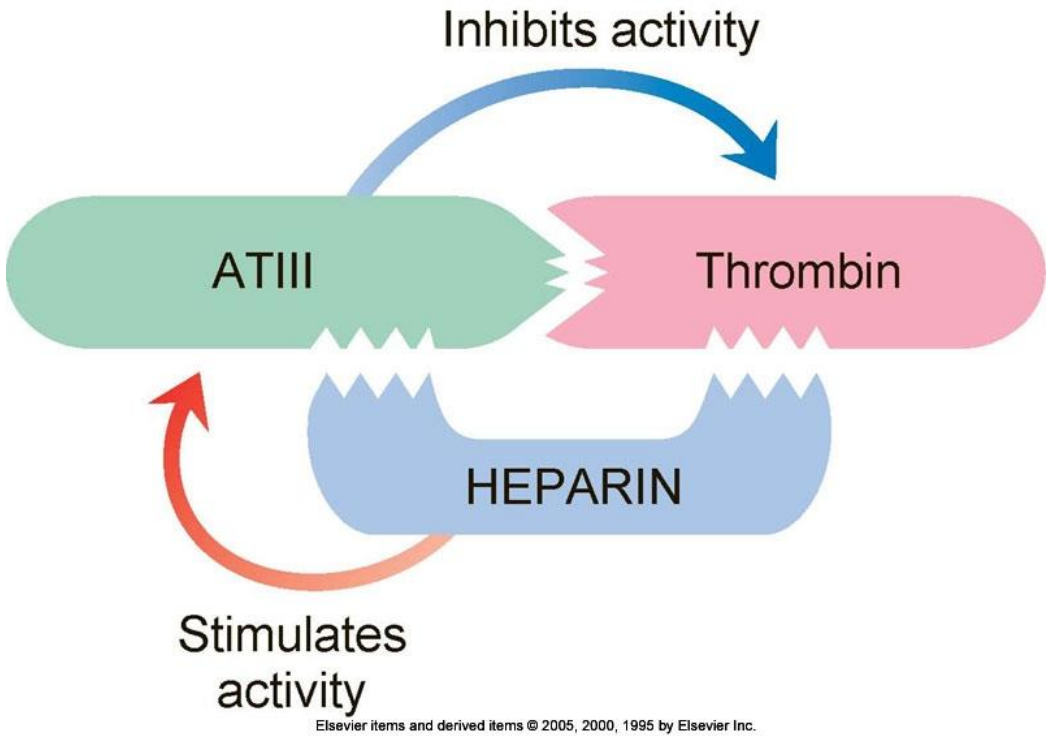
Blood Coagulation Factors

- Factors are numbered by Roman numerals in order of their discovery.
- The **liver** is the primary site of synthesis of the protein clotting factors. Some protein clotting factors are synthesized both by the liver and by cells such as megakaryocytes and endothelial cells.
  - **Factors II, VII, IX, and X** require **Vitamin K** for synthesis by hepatocytes. (Thus all 3 coagulation pathways are affected by Vitamin K.)
  - The anticoagulant, **Coumadin (Warfarin)**, inhibits vitamin K activity.
- The liver synthesizes **antithrombin III,** an inhibitor of **thrombin** activity. Thrombin is the enzyme that forms fibrin from fibrinogen. **Fibrin** is the insoluble protein in blood clots.
- The natural anticoagulant, **heparin**, enhances the activity of antithrombin III.

The Process of Hemostasis: Formation of Fibrin 4

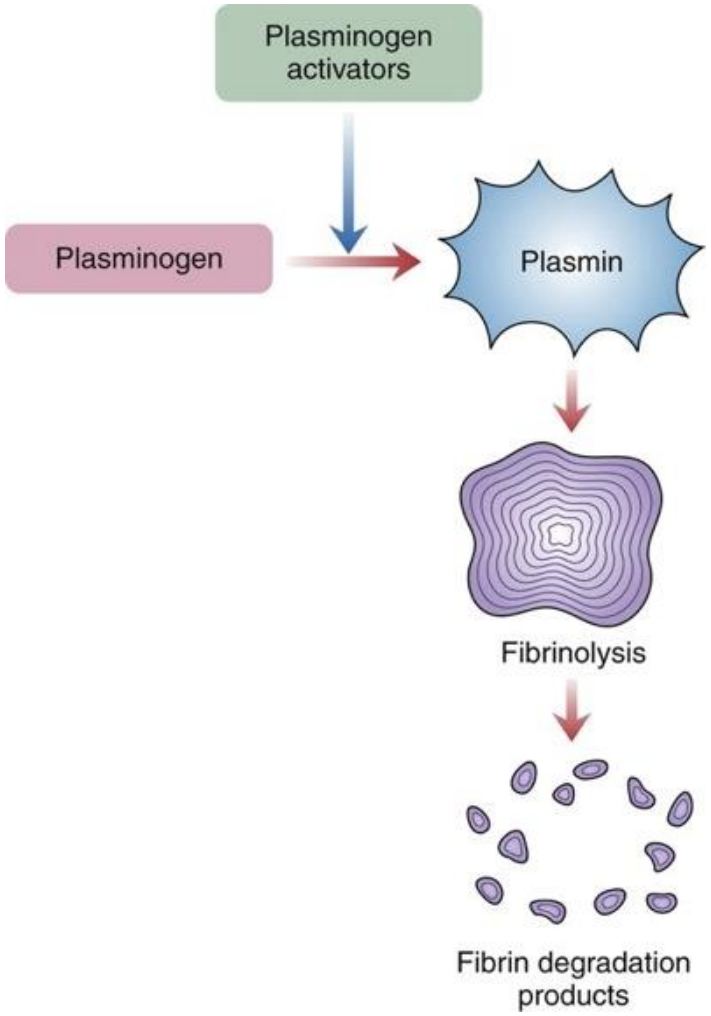
Heparin Stimulates Antithrombin III.

Antithrombin III (ATIII) inhibits thrombin activity.



Fibrinolysis: Degradation of Fibrin in a Blood Clot

- Blood clots are removed by the activity of the enzyme, **plasmin**.
- Plasmin breaks down fibrin.
- Plasmin is activated by **tPA=tissue plasminogen activator,** an enzyme synthesized by endothelial cells. (Plasminogen is the inactive precursor of plasmin.)
- tPA, aka the **"clot buster"** is administered clinically to avert strokes.



Antiplatelets, Anticoagulants, Thrombolytics

- Clinically there are various agents that can interfere with the formation of blood clots.
- Aspirin is an **antiplatelet** agent. It interferes with the production of thromboxane A2 by the arachidonic acid pathway.
- Warfarin, heparin and antithrombin III are **anticoagulants**. They interfere with the production or activity of clotting factors.
- tPA is a **thrombolytic**. It destroys blood clots that have already formed.

QUIZ 2AB

- COMPLETE QUIZ 2AB.
- THEN GO ON TO Module 2CD PPT.