Pathology

USMLE STEP 1

Volume 1: Basic Pathology

Chapters 1–9

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USMLE Step 1

Pathology

Volume 1: Basic Pathology
Chapters 1–9

Volume 2: Systemic Pathology
Chapters 10–28

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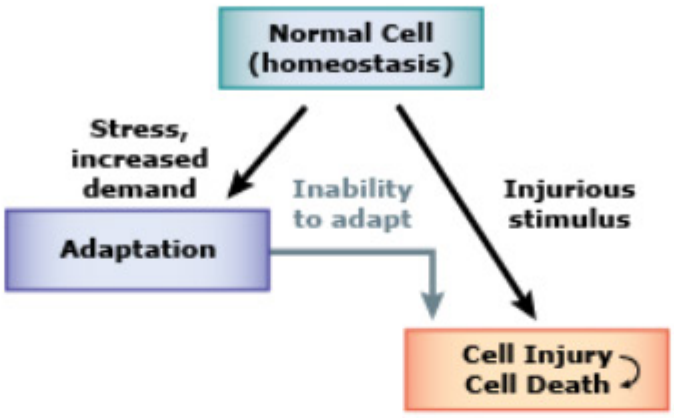
Unit 1

General Principles of Pathology

1 Overview of Cellular Pathophysiology

1.1 Homeostasis

The normal cell is capable of making responses to physiologic demands and maintaining a steady state of function referred to as *homeostasis*. When a new steady state is achieved in response to altered physiologic stress, the process is referred to as *adaptation*. Adaptations are reversible changes that allow cells to survive and function. If the ability of cells to adapt is exceeded, *cell injury* results, and this is also reversible to a point. If the injurious stimulus persists, and adaptation is no longer possible, *irreversible cell injury* and *cell death* ensue.



▲ **Figure 1–1.1** Cellular Response to Stress and Injury

1.2 Causes of Cell Injury

Physiologic adaptation to stress can progress to significant cell injury if the stimulus is not removed. A variety of causes of injury exist, including:

- Hypoxia: A deficiency of oxygen that reduces aerobic oxidative respiration
- Chemical agents/drugs
- Infectious agents
- Immunologic reactions
- Congenital/genetic derangements
- Mechanical trauma
- Nutritional deficiency or excess

1.3 Variables in Cell Injury

The response of cells to injurious stimuli depends on a number of variables:

- The nature of the injurious stimulus
- The duration of action or injury
- The adaptability of specific cells to injury; for example, anoxia causes cell death in:
  - Neurons: 3 to 5 minutes
  - Myocardium: 1 to 2 hours
  - Fibroblasts: hours

USMLE® Key Concepts

For Step 1, you must be able to:

- Describe the limitations of cellular adaptation and the causes of cell injury.
- Define the distinctions between reversible and irreversible cell injury.
- Explain the causes and pathophysiologic results of tissue hypoxia.
- Identify the microscopic changes involved in cell injury from free radicals and aging.
- Describe hyperplasia, hypertrophy, atrophy, metaplasia, and dysplasia as cellular responses to stress.
- List the major types of tissue necrosis.

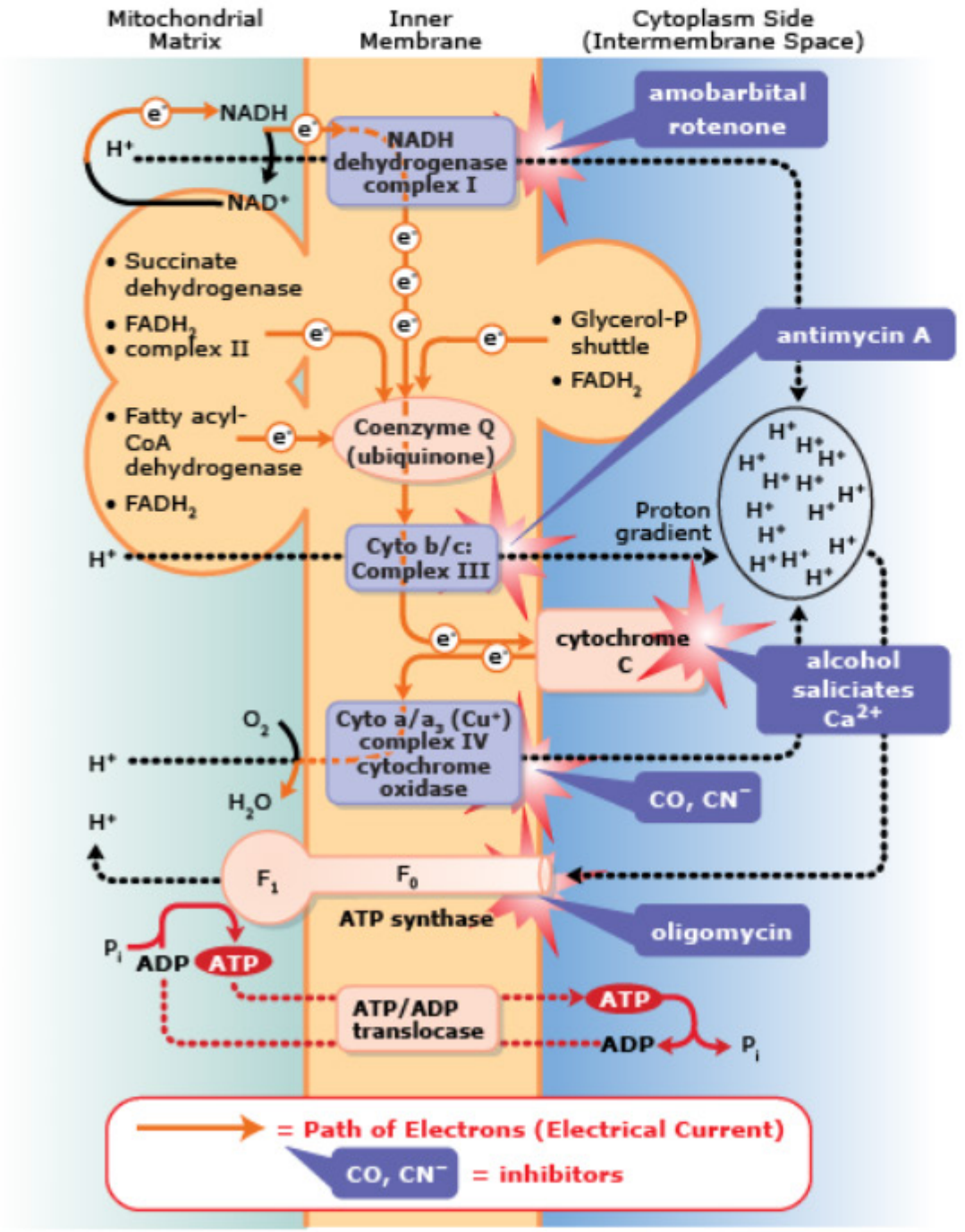
2 Tissue Hypoxia

Hypoxia, or inadequate oxygenation of tissue, is the most common cause of cell injury. The necessity for a cell to use anaerobic metabolism results in the following *reversible* changes:

- The production of fewer molecules of ATP.
- Impairment of the Na^+ pump causes cellular swelling.
- Increased lactic acid.
- Decrease in pH.

Irreversible changes may follow:

- Continued pH decrease causes chromatin clumping.
- Lactic acid denatures lysosomal enzymes and causes coagulation necrosis.
- Increased lactic acidosis causes an increase in metabolic acidosis.



▲ **Figure 1–2.0A** Oxidative Phosphorylation

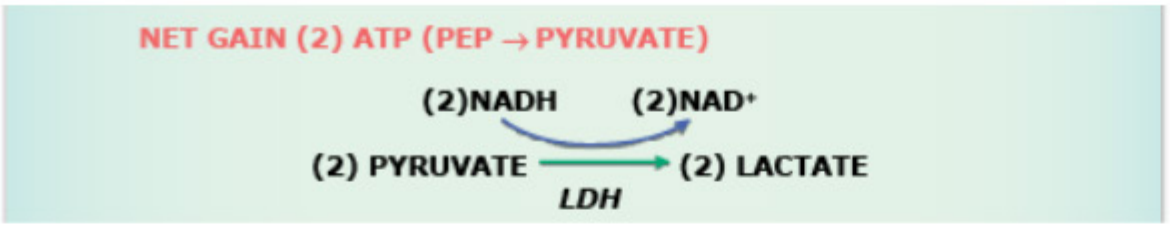
Connection to Biochemistry

Oxidative Phosphorylation and the Electron Transport Chain

Inadequate oxygenation decreases synthesis of adenosine triphosphate (ATP):

1. ATP synthesis occurs in the inner mitochondrial membrane by the process of oxidative phosphorylation.
2. O_2 is an electron acceptor located at the end of the electron transport chain (ETC) in the oxidative pathway.
3. A lack of O_2 or a defect in oxidative phosphorylation culminates in a decrease in ATP synthesis.

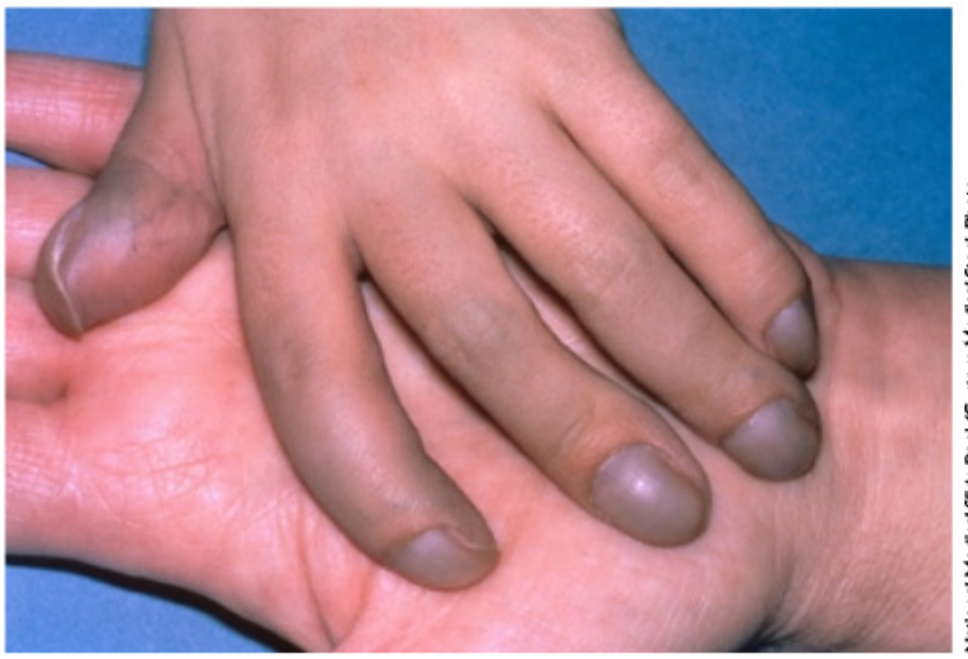
The only way to produce ATP when oxidative phosphorylation is compromised by hypoxia is through anaerobic glycolysis. Cancer cells predominantly use anaerobic glycolysis for energy.



Clinical Application

▼ **Table 1–2.0** Oxygen (O_2) Content

Term	Definition	Contributing Factor	Significance
Hemoglobin	Most important O_2 carrier	Marrow or extramedullary synthesis	Anemia vs. polycythemia
PaO_2	• Pressure keeping O_2 dissolved in the plasma of arterial blood ($0.003 \times PaO_2$) • Driving force for diffusion of O_2 into tissue	Percentage of O_2 in inspired air, atmospheric pressure, normal O_2 exchange in lungs	Reduced in hypoxemia
SaO_2	Average percentage of four heme groups binding O_2	• PaO_2 and valence of heme iron in each of the four heme groups • O_2 binds to Fe^{2+} and does not bind to Fe^{3+}	SaO_2 <80% produces cyanosis of skin and mucous membranes
O_2 content = $(Hb \times 1.34) \times SaO_2 + (PaO_2 \times 0.003)$	Total amount of O_2 carried in blood	• Hemoglobin concentration in RBCs, PaO_2, SaO_2 • Hemoglobin concentration determines total amount of O_2 delivered to tissue	Hemoglobin is the most important carrier of O_2



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▲ **Figure 1–2.0B** Cyanosis in Patient With Tetralogy of Fallot

Connection to Pharmacology

The down-regulation of O_2 results in the up-regulation of EPO. Conversely, the up-regulation of O_2 results in the down-regulation of EPO.

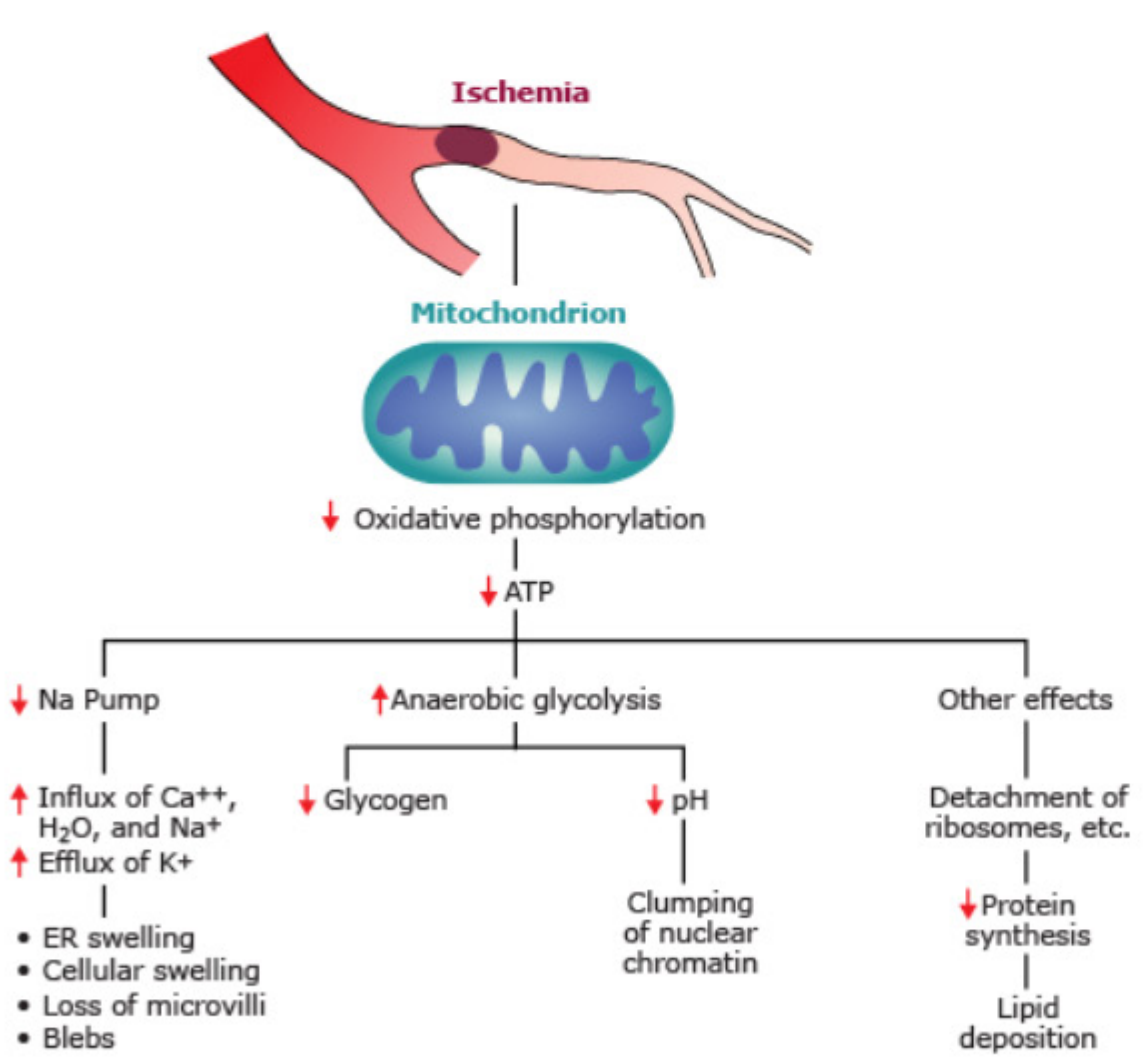
Clinical Application

Pulse oximetry is a noninvasive test for measuring SaO_2 using a probe that is clipped over the patient's finger. This test detects oxy- and deoxyhemoglobins, but does not identify dyshemoglobins such as methemoglobin and carboxyhemoglobin. In the presence of such dyshemoglobins, the oximeter calculates a falsely high SaO_2. The co-oximeter is able to calculate an accurate SaO_2 in the presence of dyshemoglobins.

2.1 Causes of Tissue Hypoxia

2.1.1 Ischemia

Ischemia is the most common cause of hypoxia, and is defined as a decrease in arterial blood flow or venous outflow of blood. The most common cause of ischemia is atherosclerosis. Other causes include decreased cardiac output (e.g., heart failure) and thrombosis of the blood vessels. The results of tissue hypoxia include infarction, organ dysfunction, and tissue atrophy.

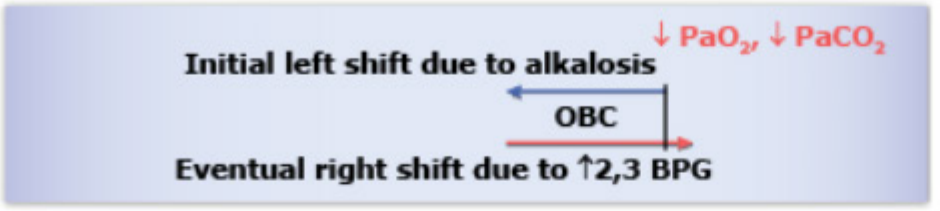


▲ **Figure 1–2.1A** Ischemia

2.1.2 Hypoxemia

Hypoxemia is defined as ↓ PaO_2. It may be caused by any of the following:

Decreased inspired PO_2 (PiO_2) High altitude is the most common cause.



▲ **Figure 1–2.1B** Hypoxemia

Hypoventilation and Respiratory Acidosis

- ↑$PaCO_2$ → ↓PAO_2 → ↓PaO_2 → ↓SaO_2
- A change of any kind (increase or decrease) to alveolar CO_2 which is represented by P_ACO_2, will cause a directly proportionate change in PaO_2:
  ↑P_ACO_2 → ↓P_aO_2 → ↓SaO_2
- A ↓SaO_2 may occur without a ↓PAO_2 and ↓PaO_2 (e.g., methemoglobinemia, *CO poisoning*)

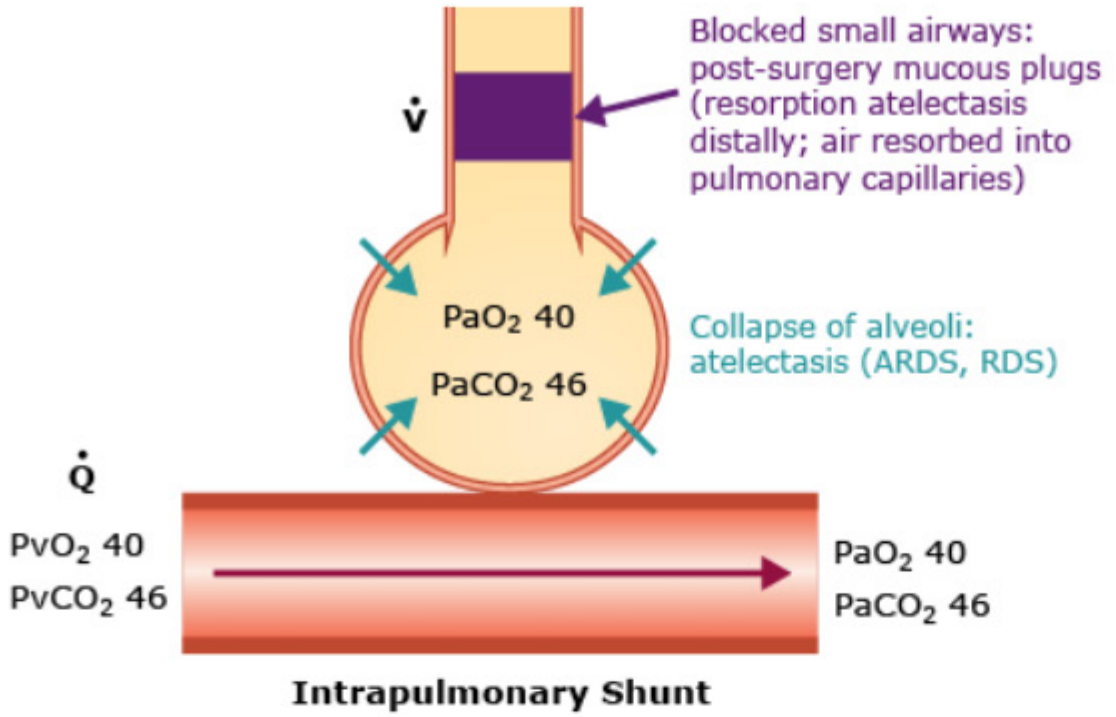
Ventilation Defect A common example is respiratory distress syndrome with impaired O_2 delivery to the alveoli (e.g., respiratory distress syndrome, acute respiratory distress syndrome, resorption atelectasis following surgery).

- No O_2 exchange in the pulmonary vasculature due to lack of ventilation, even though the alveoli are being perfused.
- Diffuse alveolar disease produces intrapulmonary *shunting* of alveoli.
- Consequence is that the pulmonary capillary blood has the same PO_2 and PCO_2 as venous blood returning from tissue.
- Inspired increase in percentage of O_2 from 0.24% to 0.28% or greater does not significantly increase the PaO_2.
- This only applies to diffuse ventilation defects involving lungs being injured bilaterally.
- Smaller pulmonary injuries are compensated by normally ventilated lungs.
- Respiratory distress syndrome: Giving O_2 does *not* significantly increase the PaO_2 (all alveoli in both lungs are collapsed).

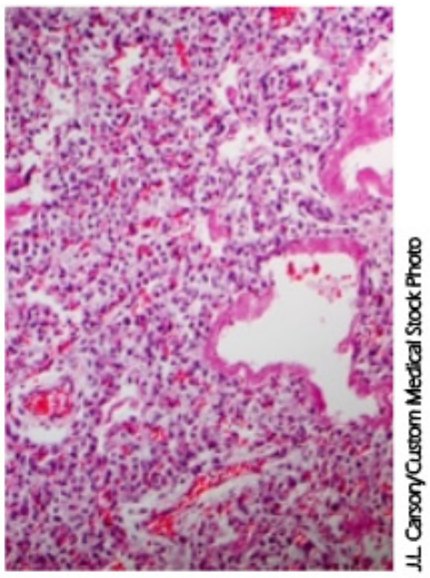
Clinical Application

Left-Shift of OBC

At *high altitudes*, the atmospheric pressure is decreased; however, the percentage of O_2 in the atmosphere remains the same (i.e., 21%). Hypoxemia stimulates peripheral chemoreceptors such as the carotid body causing respiratory alkalosis, which shifts the oxygen binding curve (OBC) to the left. Alkalosis activates phosphofructokinase, the rate-limiting enzyme in glycolysis, causing increased production of 1,3-BPG, which is converted to 2,3-BPG. This eventually shifts the OBC to the right, leading to increased release of O_2 to tissue.



▲ **Figure 1–2.1C** Airway Obstruction



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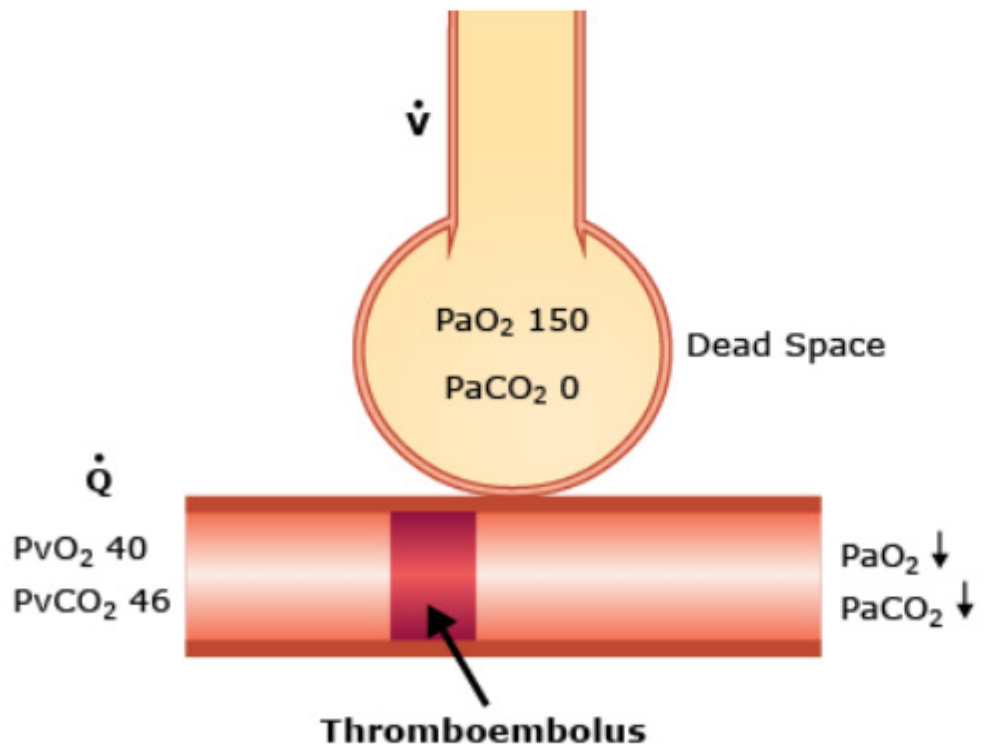
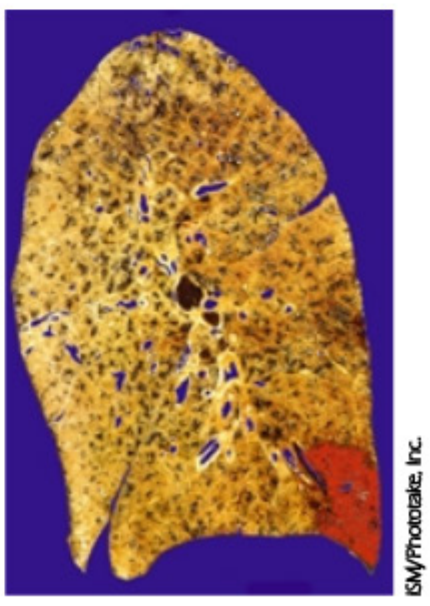
▲ **Figure 1–2.1D** Respiratory Distress Syndrome

Perfusion Defect

- Absence of blood flow to alveoli (e.g., pulmonary embolus or fat embolism).
- No O_2 exchange in the pulmonary vasculature due to lack of perfusion, even though the alveoli are being ventilated.
- Produces an increase in *dead space.*
- Inspired percentage of O_2 from 0.24% to 0.28% or greater *does* increase the PaO_2.
- Giving O_2 ↑PaO_2 (areas of lung not infarcted have normal gas exchange).



▲ **Figure 1–2.1E** Pulmonary Embolus



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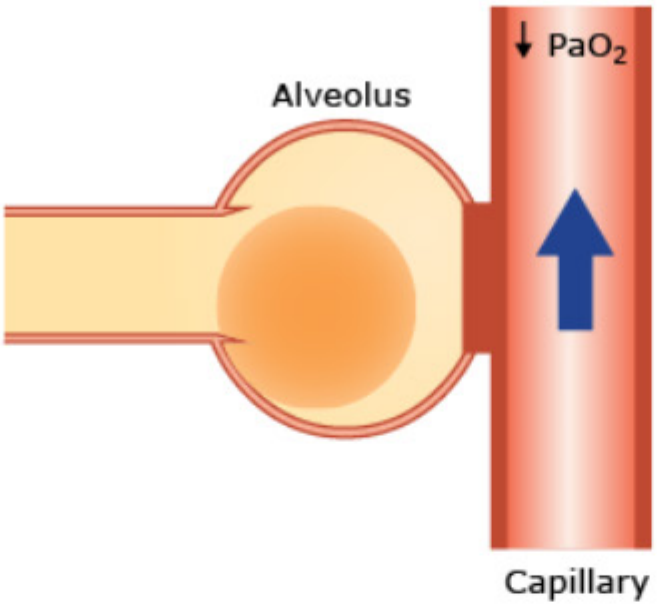
▲ **Figure 1–2.1F** Pulmonary Infarction

Diffusion Defects Cause decreased O_2 diffusion through the alveolar-capillary interface.

- Interstitial fibrosis (black lung or pneumoconiosis; e.g., sarcoidosis)
- Pulmonary edema



▲ **Figure 1–2.1G** Diffusion Defect

Alveolar-arterial (A-a) Gradient

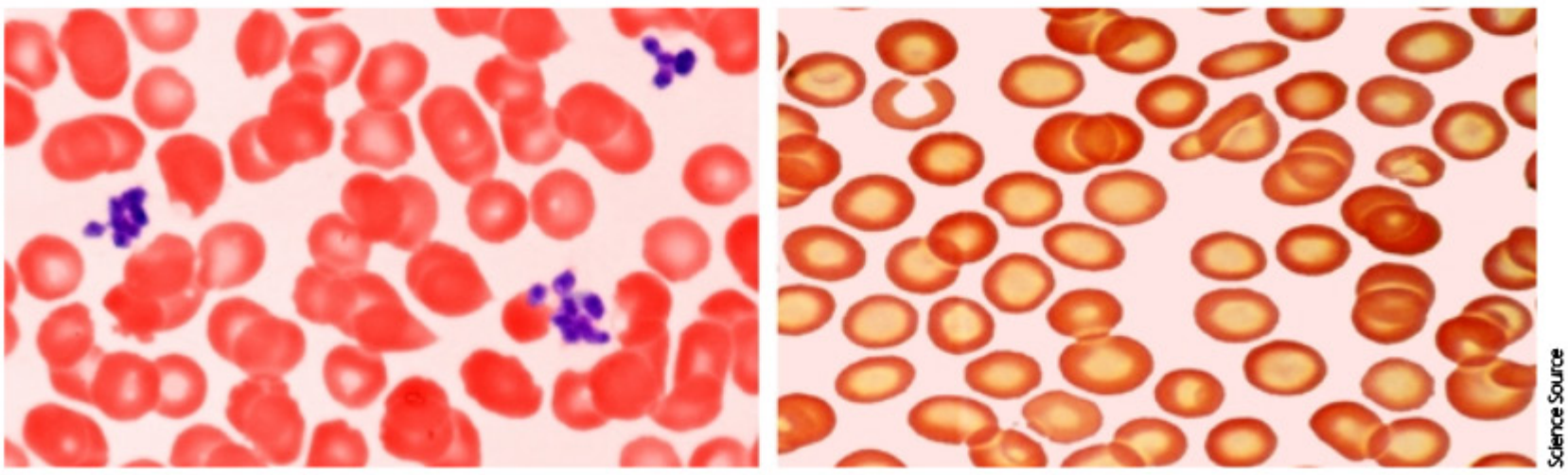
- A = P_AO_2 = [P_{atm}-Humidified air in the trachea (47 mmHg)] × %O_2 ambient (0.21) − $PaCO_2$/0.8
- a = P_aCO_2; provided by arterial blood gas (ABG)
- Normal A-a gradient: Up to 30 mmHg
- Ventilation, perfusion, and diffusion defects create an increase in A-a gradient and refer to intrapulmonary manifestations

2.1.3 Hemoglobin-Related Abnormalities

Anemia

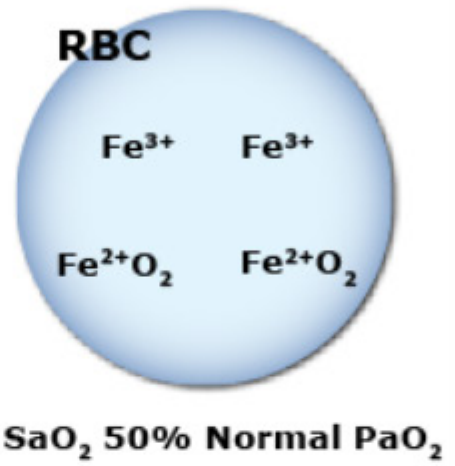
- Decreased hemoglobin concentration of <7 g/dL.
- Causes are vast and numerous (see chapter 8 on Hematology). Iron deficiency is the most common overall anemia due to long-term bleeding.
  - Microcytic anemias
  - Macrocytic anemias
  - Normochromic anemias
- Normal PaO_2 and SaO_2 with decreased O_2 content.

▼ **Figure 1–2.1H** Anemia



Methemoglobinemia

- Methemoglobin is the oxidized (ferric, or Fe^{3+}) form of the heme group which cannot bind O_2.
- Produced by oxidant stresses:
  - Nitrite and sulfur-containing drugs
  - Sepsis
  - Local anesthetics (e.g., benzocaine)
- Congenital deficiency of cytochrome b_5 reductase.
- Pathogenesis:
  - Ferric heme groups impair unloading of O_2 by oxygenated ferrous (Fe^{2+}) heme, causing a left shift of the OBC.
  - Clinically evident cyanosis occurs at metHb levels greater than 1.5 g/dL.
  - *Methemoglobin* is converted to the ferrous state (Fe^{2+}) by the reduced nicotinamide adenine dinucleotide (NA***D***H) reductase system located off the glycolytic pathway in RBCs.
  - Electrons from NADH are transferred to cytochrome b_5 and then to metHb by cytochrome b_5 reductase to produce *ferrous Hb.*
  - *Newborns* are particularly at risk for developing methemoglobinemia after oxidant stresses owing to decreased levels of cytochrome b_5 reductase until at least 4 months of age.

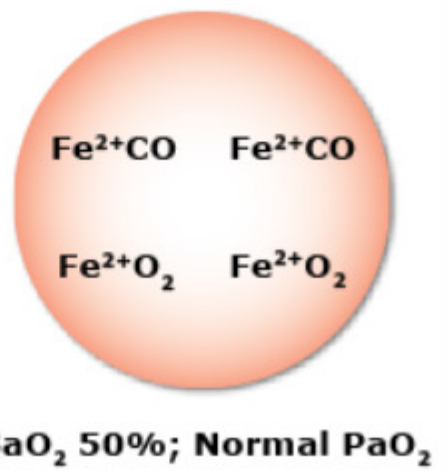


▲ **Figure 1–2.1l** Methemoglobinemia

  - Patients with *methemoglobinemia* have increased concentration of deoxyhemoglobin and cyanosis resulting in *"chocolate"* coloration of blood.
  - Skin color does *not* return to normal after administration of O_2.
- Treatment: Methylene blue acts as an artificial electron carrier in the reduced NAD***P***H metHb reductase system of the pentose phosphate shunt.

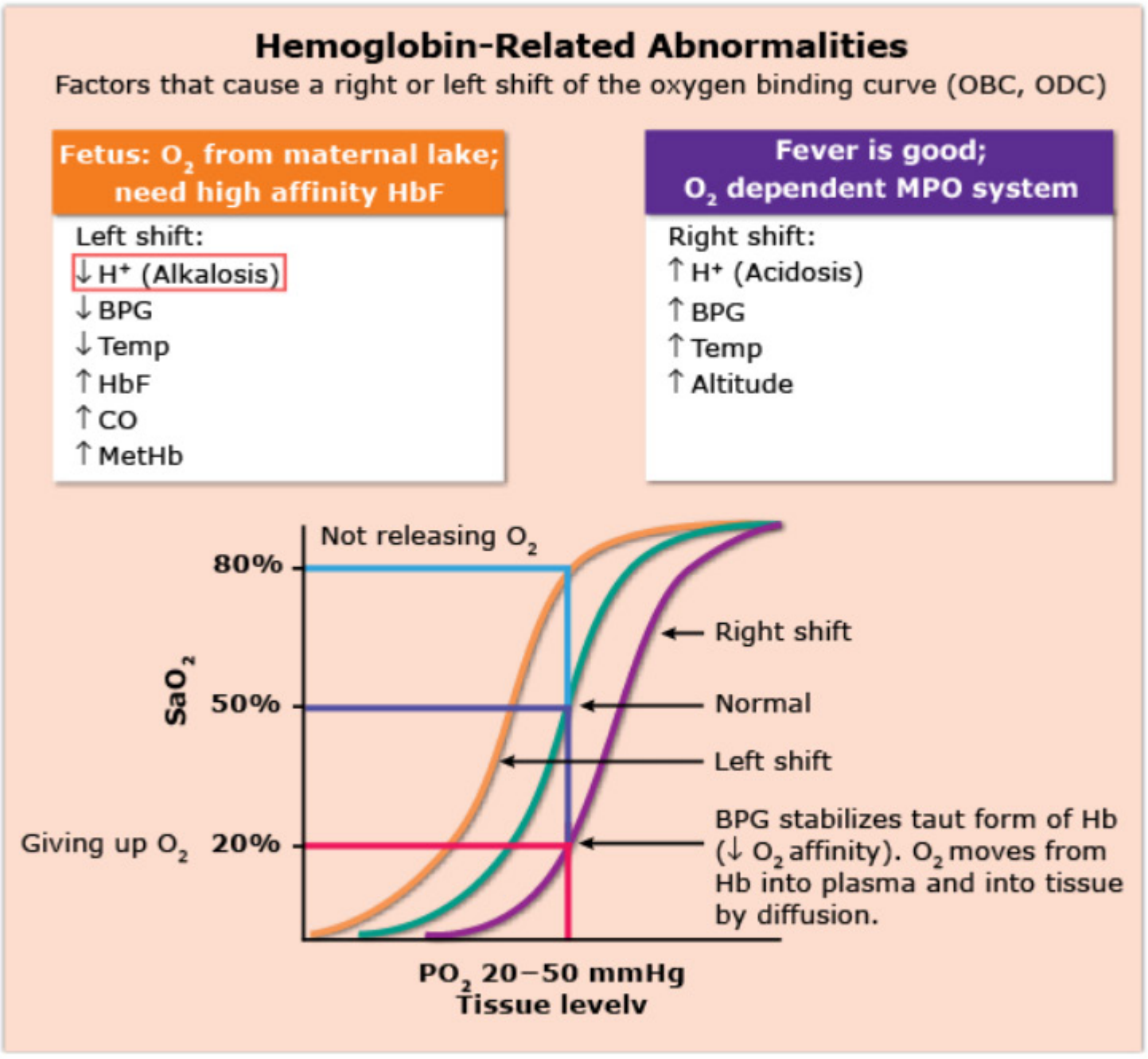
Carbon Monoxide (CO)

- Leading cause of death due to poisoning.
- Produced by incomplete combustion of carbon containing compounds (automobile exhaust, wood stoves, paint thinner).
- Pathogenesis:
  - Competes with O_2 for binding sites on Hb.
  - Causes left shift of the OBC.
  - Inhibits cytochrome oxidase in the electron transport chain, which is part of complex IV.



▲ **Figure 1–2.1J** Carbon Monoxide Poisoning

- Clinical Findings:
  - Cherry-red discoloration of the skin and blood.
  - CO intoxication levels and their associated set of symptoms:
    —CO levels of 10% to 20% → Headache
    —CO levels of 20% to 30% → Dyspnea and dizziness
    —CO levels of 50%to 60% → Seizures and coma
- Treatment: O_2 via nonbreather mask or endotracheal tube (100% O_2



▲ **Figure 1–2.1K** O_2-Binding Curve (OBC)

2.2 Tissue Susceptible to Hypoxia

2.2.1 Watershed Areas

- Lie between the terminal branches of major arterial blood supplies.
- The blood supply from the two vessels does not overlap.
- Examples:
  - In the brain between the anterior and middle cerebral arteries.
  - In the splenic flexure between the superior and inferior mesenteric arteries.

2.2.2 Subendocardial Tissue

This tissue receives the least oxygenation and hence is most susceptible to hypoxia.

Clinical Application

Coronary Artery Blood Flow

Factors decreasing coronary artery blood flow (e.g., coronary artery atherosclerosis) produce subendocardial ischemia, which is manifested by chest pain (i.e., angina) and ST-segment depression in an electrocardiogram (ECG). Increased thickness of the left ventricle (i.e., hypertrophy) in the presence of increased myocardial demand for O_2 (e.g., exercise) also can produce subendocardial ischemia.

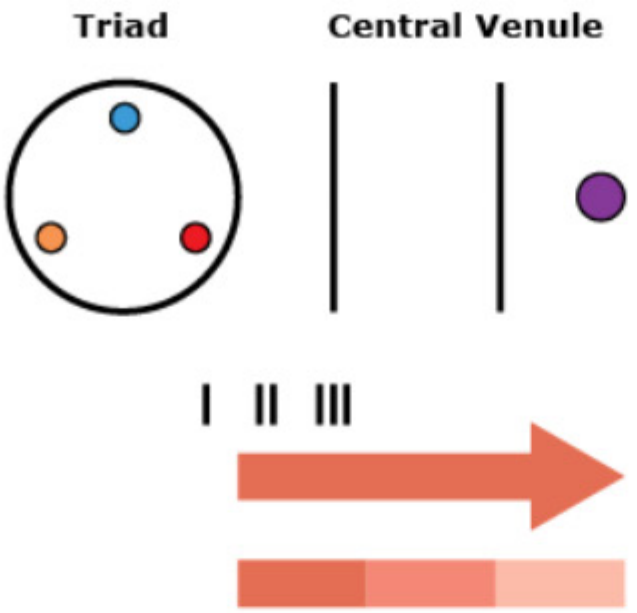
2.2.3 Renal Cortex and Medulla

- In the cortex, the straight portion of the proximal tubule is most susceptible to hypoxia.
- In the medulla, the Na^+-K^+-$2Cl^-$ cotransport channel in the thick ascending limb is most susceptible to hypoxia.

2.2.4 Neurons in the Central Nervous System

- Examples: Purkinje cells in cerebellum; neurons in layers 3, 5, and 6 of the cerebral cortex.
- Irreversible injury occurs in ~5 minutes after global hypoxia.

2.2.5 Hepatocytes Located Around the Central Vein



▲ **Figure 1–2.2** Hepatic Portal System

Clinical Application

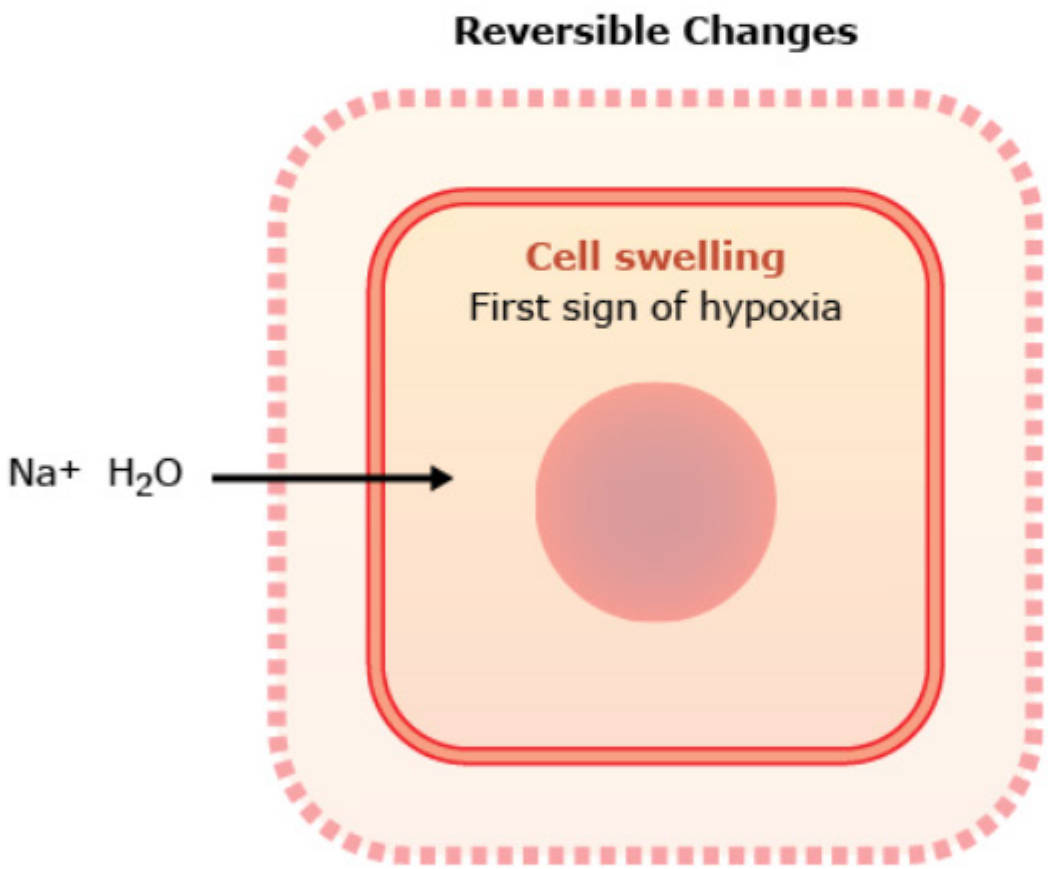
In the *portal triads*, tributaries of the hepatic artery carrying oxygenated blood and portal vein tributaries carrying unoxygenated blood empty into the liver sinusoids, which drain blood into the central veins (terminal hepatic venules). The central veins become the hepatic vein, which empties into the inferior vena cava. Therefore, hepatocytes closest to the portal triads (zone I) receive the most oxygen and nutrients, and those farthest from the portal triads (zone III around the central vein) receive the least amount of oxygen and nutrients. For this reason, production of free radicals from drugs (e.g., acetaminophen), tissue hypoxia (e.g., shock, CO poisoning), and alcohol-related fatty change cause most initial damage to zone III hepatocytes.

2.3 Consequences of Hypoxic Cell Injury

2.3.1 Reversible Cellular Injury

Anaerobic glycolysis is used for ATP synthesis and is accompanied by several changes:

- Activation of phosphofructokinase
  - Caused by low citrate levels and increased adenosine monophosphate
  - Net gain of 2 ATP instead of aerobic results of 36 ATP
- Decrease in intracellular pH caused by an excess of lactate
  - Also accumulates in blood, producing lactic acidosis
  - Denatures structural and enzyme proteins
- Impaired Na^+/K^+-ATPase pump which actively keeps $2K^+$ intracellularly for $3Na^+$ extracellularly
  - Diffusion of Na^+ and H_2O into cells causes cellular swelling
  - Potentially reversible with restoration of O_2
- Decreased protein synthesis
- Due to detachment of ribosomes (potentially reversible)

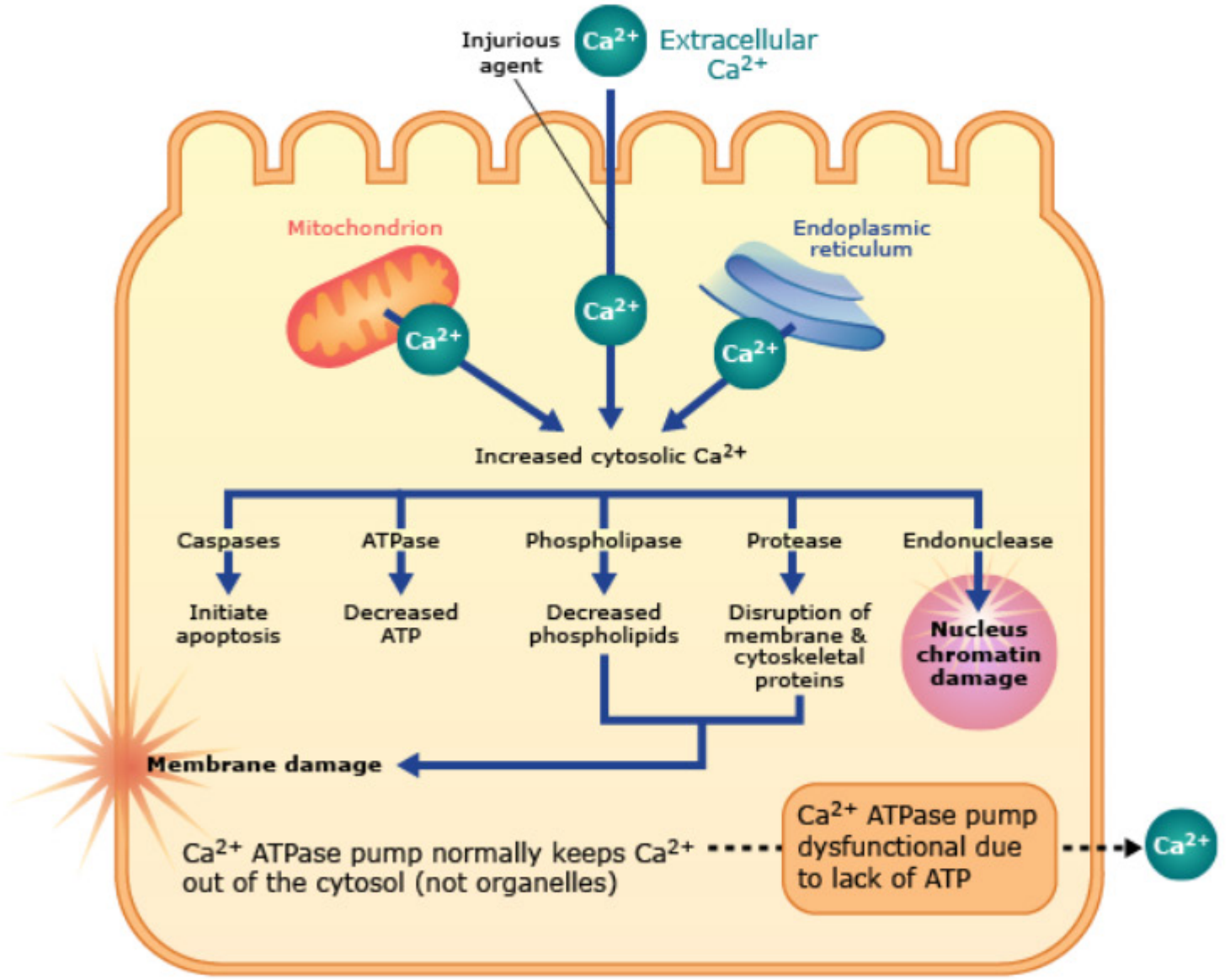


▲ **Figure 1–2.3A** Anaerobic Glycolysis: Diffusion of Na+ and H_2O Into Cell

2.3.2 Irreversible Cellular Changes

Calcium (Ca^{2+}) entry into the cell is an indication of irreversible cellular injury because the Ca^{2+}-ATPase pump normally functions to keep Ca^{2+} out of the cytosol. Impairment of the Ca^{2+}-pump resulting in increased cytosolic Ca^{2+} has two lethal effects:

- **Enzyme Activation**
  - Phospholipase increases cell and organelle membrane permeability
  - Proteases damage the cytoskeleton
  - Endonucleases cause fading of nuclear chromatin (karyolysis)
  - ATPases destroy ATP
- **Re-entry of Ca^{2+} Into Mitochondria:** Increases mitochondrial membrane permeability resulting in release of cytochrome c and subsequent apoptosis through activation of caspases



▲ **Figure 1–2.3B** Re-entry of Ca^{2+} Into Mitochondria

2.3.3 Damage to Cellular Organelles

Hypoxia can damage cellular organelles, affecting virtually all aspects of cellular physiology.

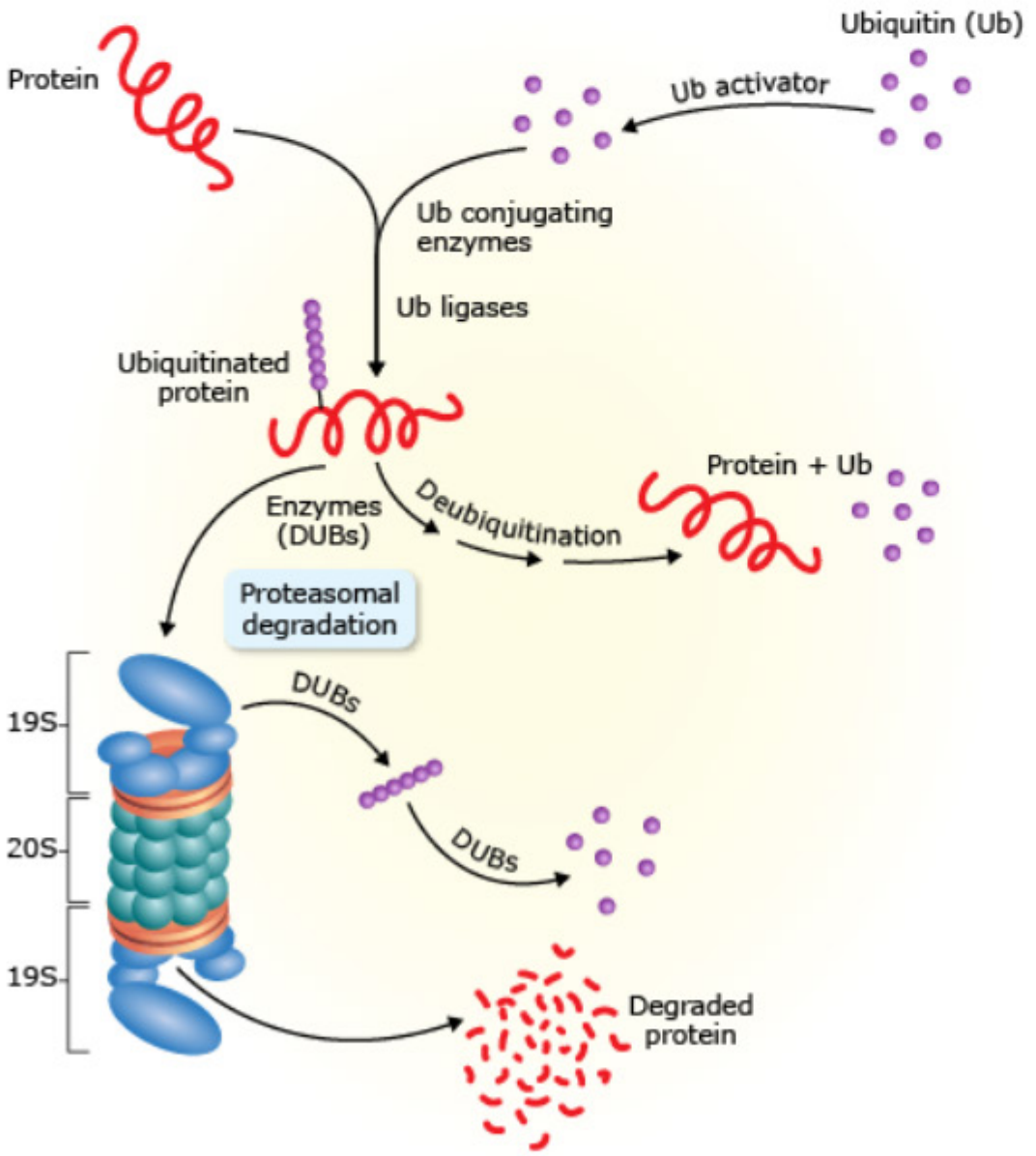
Cytoskeleton

Normally, the cytoskeleton is a network of microtubules, actin filaments, and intermediate filaments in the cytosol that control the shape and motility of cells.

- Microtubules are polymers composed of the protein tubulin.
- Thick (myosin) and thin (actin) filaments are involved in the contractile process.
- Intermediate filaments are important in the anchoring of cell organelles.

Ubiquitination

- *Ubiquitin*, a stress protein, binds to damaged intermediate filaments, and marks them for degradation in proteasomes and lysosomes of the cytosol.
- Hallmark of atrophy: *autophagic vacuoles*.



▲ **Figure 1–2.3C** Ubiquitination

Connection to Pharmacology

Several anticancer drugs target the formation of the mitotic spindle:

- Tubulin depolymerization inhibitors: paclitaxel
- Tubulin polymerization inhibitor: colchicine, vinblastine

Intermediate Filament Defects

- **Mallory Bodies:** Ubiquitinated cytokeratin intermediate filaments in hepatocytes in alcoholic liver disease.
- **Lewy Bodies:**
  - Damaged neurofilaments in idiopathic Parkinson disease.
  - Eosinophilic cytoplasmic inclusion in degenerating substantia nigra neurons.

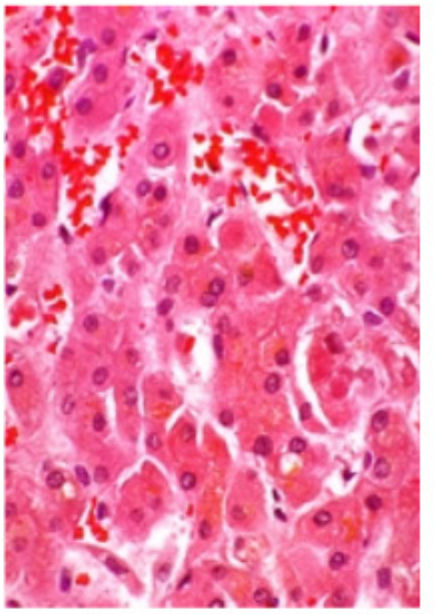
Mitochondria

Mitochondrial Injury

- Enzyme inhibition of oxidative phosphorylation results in decreased ATP synthesis.
- CO and cyanide (CN) inhibit cytochrome oxidase in Complex IV of ETC.
- Damaging agents such as alcohol, salicylates, calcium → release cytochrome c → activation of caspases → apoptosis.
- Uncouplers carry protons in the intermembranous space through the inner mitochondrial membrane into the matrix without damaging the mitochondrial membrane.
  - This wasted oxidative energy is channeled to produce heat rather than intended ATP.
  - Dinitrophenol, found in TNT and nitroprusside, synthesizes heat, increasing the risk for hyperthermia in adults.

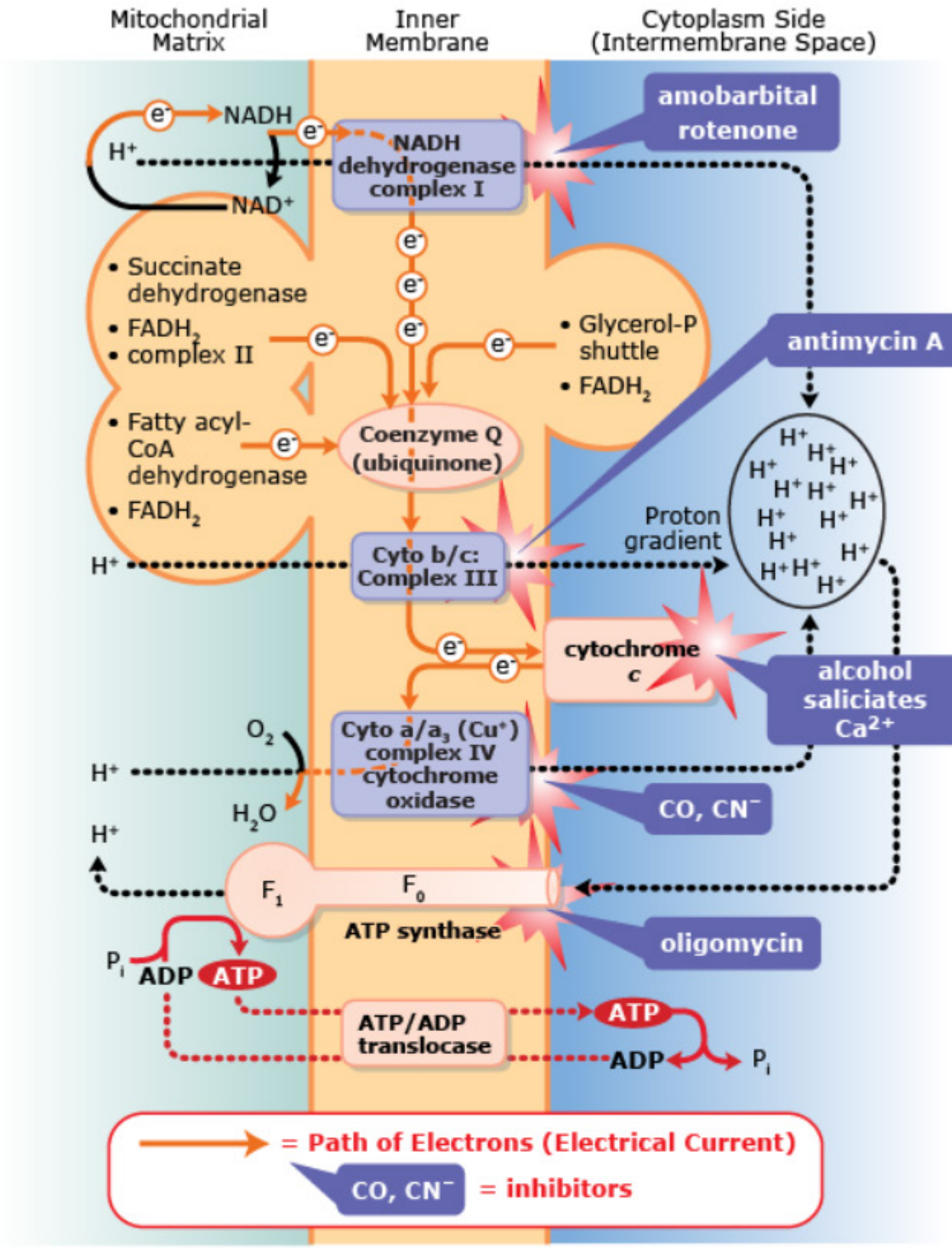
Cyanide

- Causes:
  - Drugs such as nitroprusside.
  - Combustion of polyurethane products in house fires.
- Pathogenesis:
  - It produces initial central nervous system and cardiovascular stimulation followed by depression and death.
  - It produces increased venous P_{mvO2} and saturation.
  - Tissue cannot extract O_2.
  - Drugs (e.g., nitroprusside); house fires (combustion from synthetic fiber is the most common cause).
  - Inhibits cytochrome oxidase (normally converts O_2 to water); shift to anaerobic glycolysis for ATP (lactic acidosis).
  - Bitter almond smell to breath.
  - SaO_2 is *not* decreased in venous blood and is similar to the SaO_2 in arterial blood.
  - When the ETC is blocked, O_2 does *not* diffuse into tissue (gradient is lost), because it no longer is being utilized to generate ATP.
- Treatment:
  - Option 1: Two-step protocol
    1. Amyl nitrite forms cyanmethemoglobin; CN can only bind to oxidized (Fe^{3+}), not reduced, iron (Fe^{2+}).
    2. Thiosulfate binds with CN and becomes thiocyanate, which is excreted.
  - Option 2: Hydroxycobalamin, a precursor to vitamin B12, is converted to cyanocobalamin.

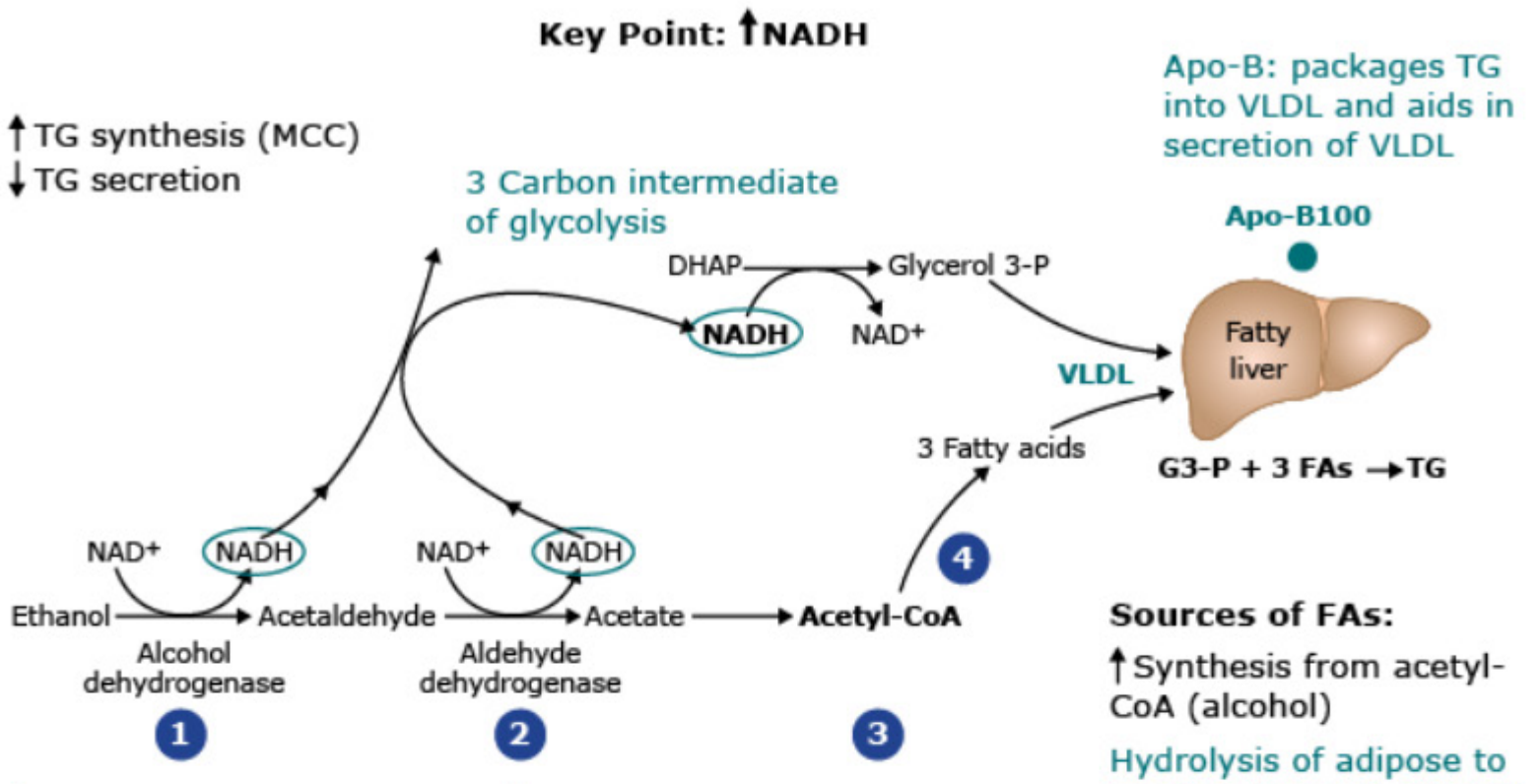


Courtesy of Dr. Edwin P. Ewing, Jr./CDC

▲ **Figure 1–2.3D**
Mallory Body



▲ **Figure 1–2.3E** Electron Transport Chain: BCL_2 Gene and Cytochrome *c*



▲ **Figure 1–2.3F** NADH

▼ **Table 1–2.3** Causes of Hypoxia

Injury	PaO_2	SaO_2	O_2-Binding Curve (OBC)	Cytochrome Oxidase
Anemia	Normal	Normal	Normal	Normal
Carbon monoxide	Normal	Decreased	Left-shifted	Inhibited
Methemoglobin	Normal	Decreased	Left-shifted	Normal
Cyanide	Normal	Normal (O_2 not removed from blood)	Normal	Inhibited

2.3.4 Smooth Endoplasmic Reticulum (SER)

Smooth endoplasmic reticulum responsible for detoxification through cytochrome P450 system (CYP450).

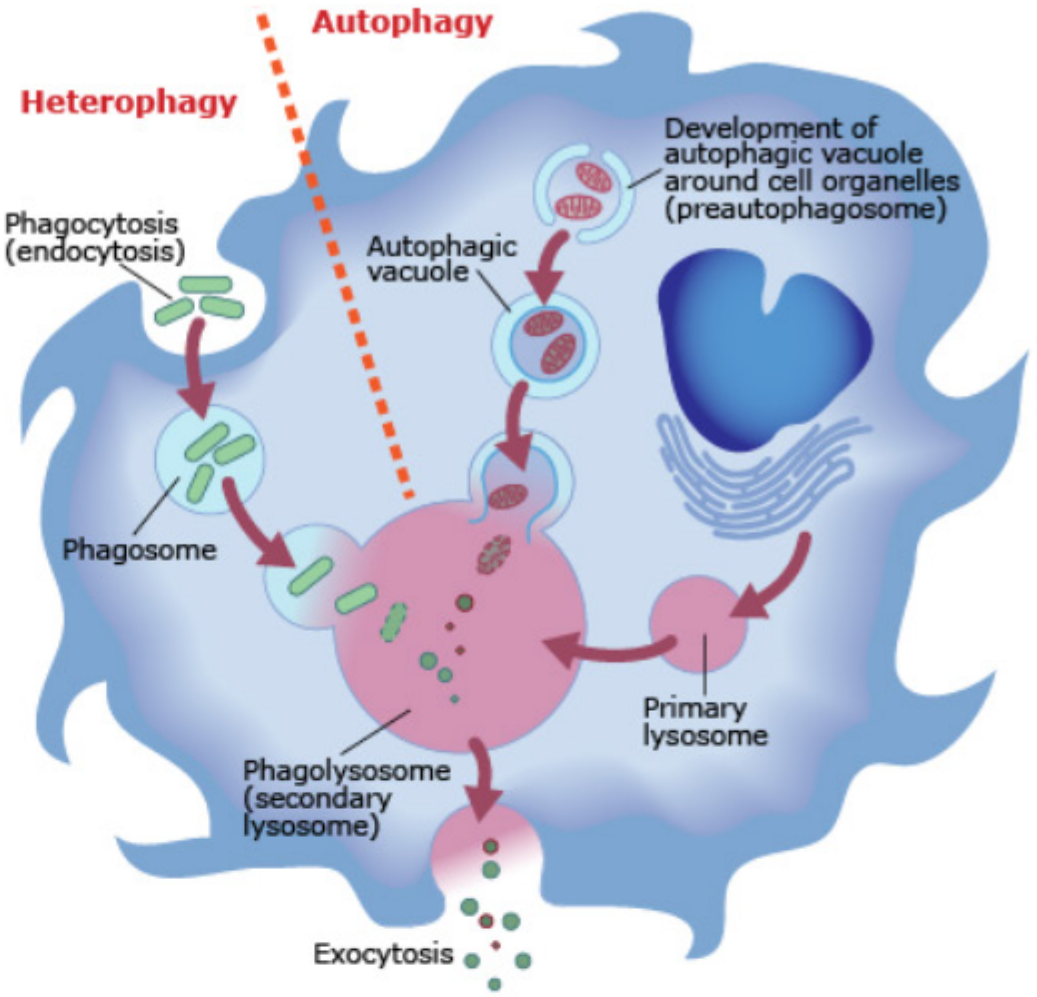
- Drugs (phenytoin, barbiturates) may speed the CYP450 system and cause faster metabolism of drugs or decrease their efficacy (for example, patient taking phenytoin to prevent seizures may become pregnant even when she is taking oral contraceptives).
- Drugs may inhibit the CYP450 system and increase drug toxicity.

2.3.5 Lysosomes

Formation

Hydrolytic enzymes synthesized by the rough endoplasmic reticulum (RER) are transported to the Golgi apparatus for posttranslational modification.

- Modification involves attaching phosphate (via phosphotransferase) to mannose residues on hydrolytic enzymes to produce mannose 6-phosphate.
- The marked lysosomal enzymes attach to specific *mannose 6-phosphate receptors on the Golgi membrane*.
- Vesicles containing the receptor-bound lysosomal enzymes pinch off the Golgi membrane to form primary lysosomes in the cytosol.
- Fusion of additional vesicles to the primary lysosomes further increases their content of hydrolytic enzymes.
- Small vesicles containing only the receptors pinch off the primary lysosomes and return to the Golgi apparatus to bind more marked lysosomal enzymes so the cycle can repeat itself.



▲ **Figure 1–2.3G** Heterophagy and Autophagy

Functions

- **Heterophagy:** Phagosome (phagocytic leukocyte) containing ingested microbes becomes phagolysosome (secondary lysosome) after lysosomal fusion.
- **Autophagy:** Destruction of cell organelles.
- Degradation of complex substrates (e.g., sphingolipids, glycosaminoglycans).

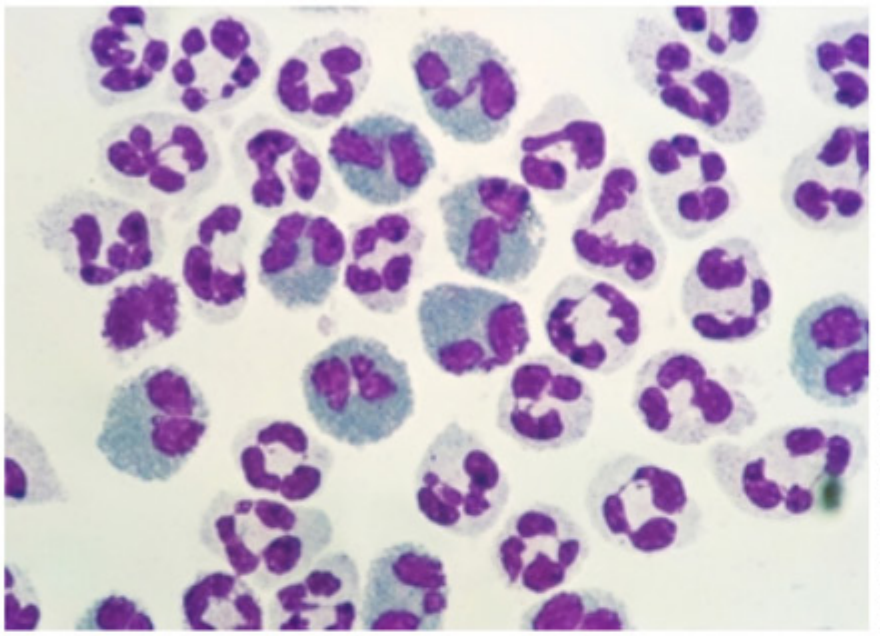
Lysosomal Disorders

Inclusion (I)-Cell Disease

- Inherited deficiency of phosphotransferase involved in marking newly formed lysosomal enzymes.
- Without mannose 6-phosphate to direct the enzymes to lysosomes, the enzymes are emptied into the extracellular spaces to be degraded in the bloodstream.
- Undigested substrates, such as carbohydrates, lipids, and proteins, accumulate in the cytosol as inclusions.
- Individuals will display psychomotor retardation and early death.

Chédiak-Higashi Syndrome

- Autosomal recessive defect in lysosomal transport protein which affects formation of:
  - Lysosomes in leukocytes
  - Azurophilic granules in neutrophils
  - Dense bodies in platelets
  - Melanosomes in melanocytes
- Granules fuse together to become megagranules
- Defect of microtubule function prevents phagolysosome formation



Dr. Cecil H. Fox/Science Source

▲ **Figure 1–2.3H** Megagranule Formation in CHS

Lysosomal Storage Diseases

These diseases result from deficiencies of important lysosomal enzymes involved in the degradation of complex substrates. As a result, incompletely digested substrates accumulate in lysosomes.

- **Gaucher Disease:** Glucocerebrosidase deficiency causes accumulation of *glucocerebrosides* in the lysosome.
- **Pompe Disease:** Deficiency of α-1,4-glucosidase causes an accumulation of *glycogen* in the lysosome.

3 Free Radical Cell Injury

Free radicals (FRs) are unstable chemical compounds with an unpaired electron in their outermost orbital. They cause the loss of electrons from other molecules, resulting in a chain reaction which culminates in cell death.

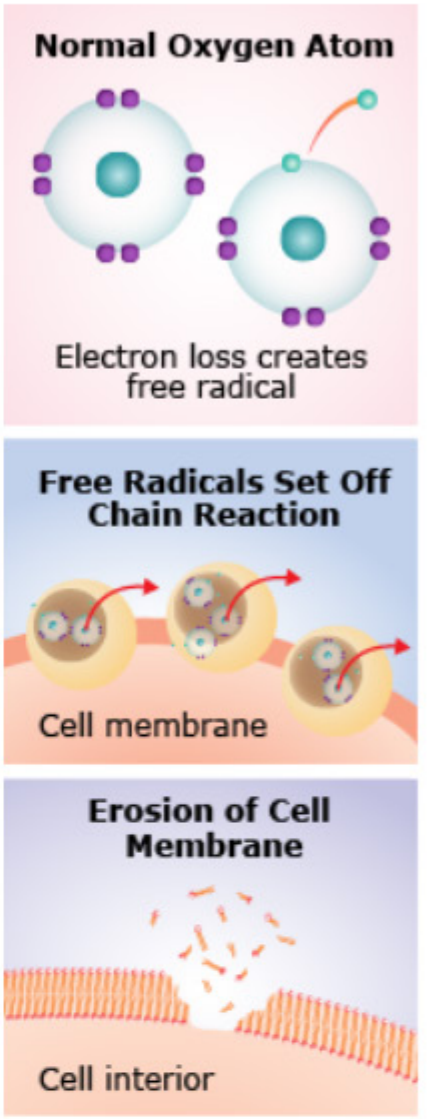
3.1 Mechanisms of Action

- FRs produce DNA fragmentation and dissolution.
- FRs initiate lipid peroxidation of polyunsaturated lipids in cell and mitochondrial membrane.
- FRs combine with O_2 increasing membrane permeability to Ca^{2+}, leading to irreversible cellular injury.

3.2 Injuries Causing Free Radical Formation

▼ **Table 1–3.2** Free Radical Formation

Injury	Form of Free Radical
Ionizing radiation	Hydroxyl FRs
Damage to mitochondria	Superoxide FRs
High oxygen concentration	Superoxide and hydroxyl FRs: Hydrogen peroxide → Hydroxyl and peroxide FRs
NADPH oxidase reactions	H_2O_2 + MPO → HOCl FRs
Xanthine oxidase	Superoxide FRs
Acetaminophen	Acetaminophen FRs in liver
Carbon tetrachloride	CCl_3 FRs in liver
Low-density lipoproteins (LDLs)	Oxidized to LDL FR by macrophages, smooth muscle cells, and endothelial cells
Cigarette smoke	Quinone/hydroquinone FRs from tar, nitric oxide (FR gas)
Nitrogen dioxide, ozone (pollution)	Nitrate FRs
Iron, copper	Hydroxyl FRs (the Fenton reaction)

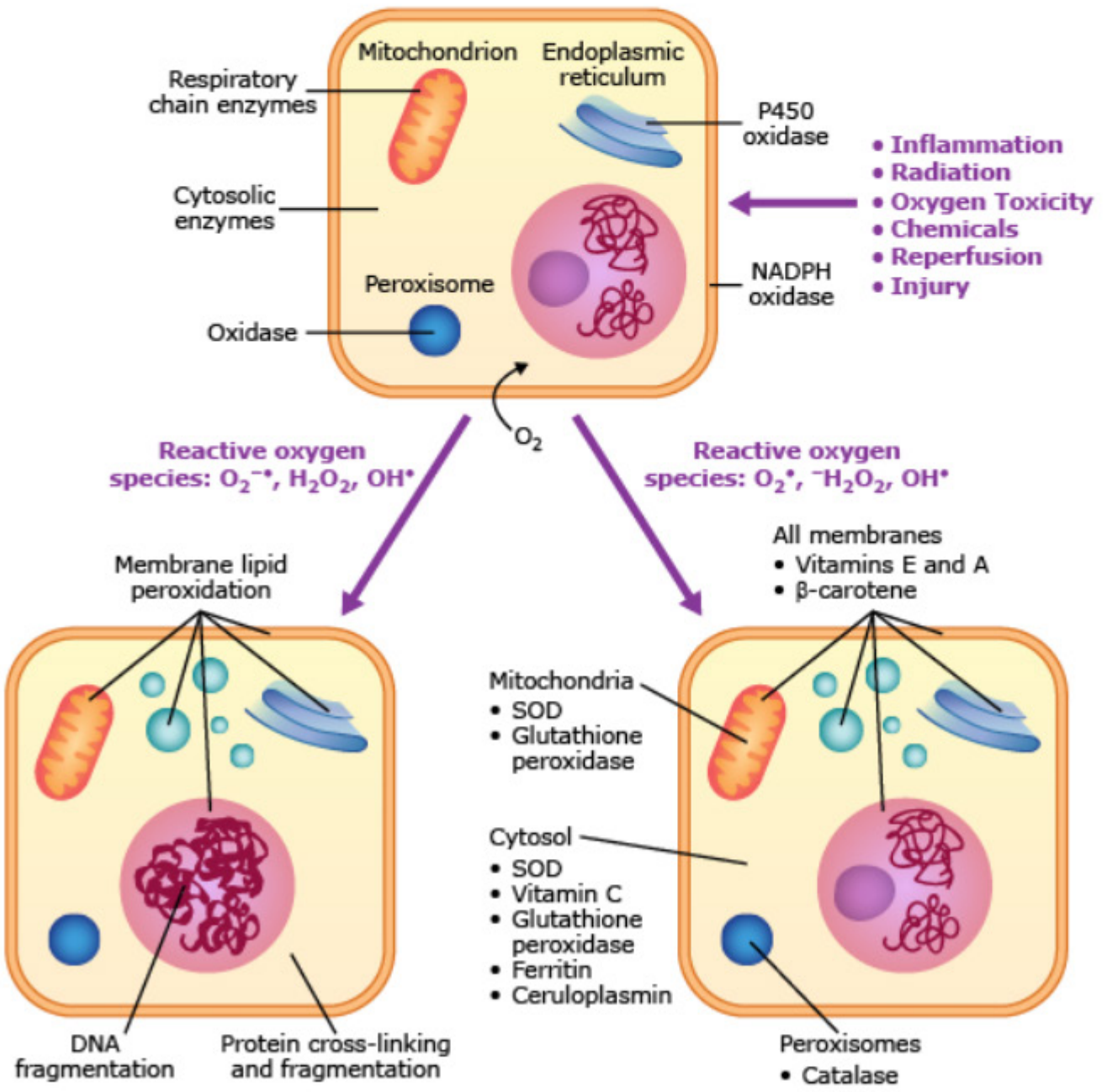


▲ **Figure 1–3.0** Role of Free Radicals in Cell Death

3.3 Free Radical Neutralization

▼ Table 1–3.3 Free Radical Neutralization

Agent	Action
Superoxide dismutase (SOD)	Converts superoxide FRs to peroxide and O_2
Glutathione peroxidase	Located in the hexose monophosphate (HMP) shunt, this agent neutralizes H_2O_2, hydroxyl, and acetaminophen FRs
Catalase	Present in peroxisomes, this agent degrades peroxide into O_2 and water
Vitamin E	• Prevents lipid peroxidation in cell membranes • Neutralizes oxidized LDL
Vitamin C	• Neutralizes FRs produced by pollutants and cigarette smoke • Best neutralizer of hydroxyl FRs
Selenium	Neutralizes FRs in the cytosol



▲ Figure 1–3.3 Neutralization of Free Radicals

3.4 Examples of Free Radical Injury

Clinical Application

Free Radical Injury by Acetaminophen

Acetaminophen is normally metabolized by sulfation or glucoronidation. In cases of overdose, however, cytochrome P450 will cause the formation of N-acetyl-p-benzoquinonimine (NAPQI), which then causes diffuse chemical hepatitis. Liver cell necrosis initially occurs around zone III and may occur at otherwise nontoxic levels of acetaminophen usage in alcoholics. This produces a transient decrease in functional factor VII, which prolongs the prothrombin time. When used with nonsteroidal anti-inflammatories (NSAIDs), acetaminophen may result in renal papillary necrosis. Free radical injury of this sort is treated with N-acetylcysteine.

3.4.1 Reperfusion Injury

The combined effects of superoxide and Ca^{2+} cause damage.

- Irreversibly damaged myocytes are destroyed.
- Undamaged myocytes remain intact.

3.4.2 Retinopathy of Prematurity

Treatment of respiratory distress syndrome with $O^2 > 50\%$ may result in blindness due to free radical synthesis.

3.4.3 Fenton Reaction Disorders

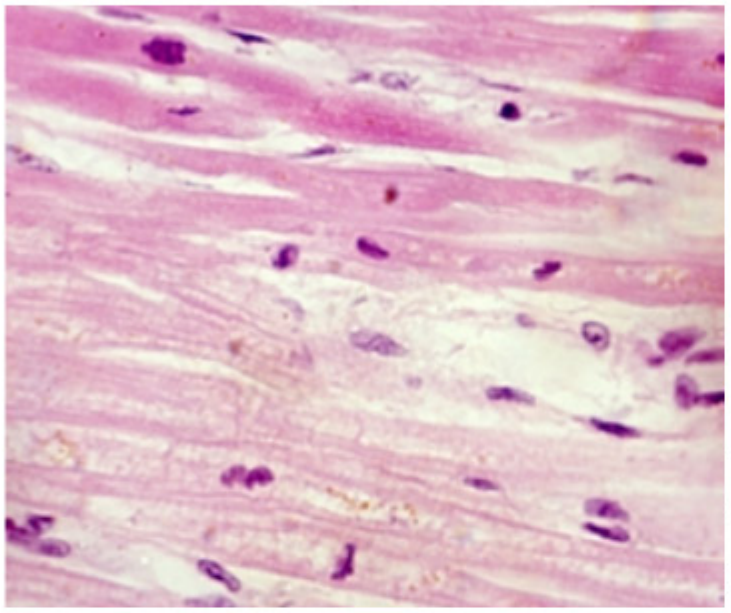
- Iron overload disorders: Hemochromatosis and hemosiderosis.
- Copper overload disorders: Wilson disease.
- Intracellular iron produces hydroxyl FR, which damages parenchymal cells.
- Examples: Cirrhosis, exocrine/endocrine pancreatic dysfunction.

4 Cellular Accumulations and Reversible Changes

A variety of materials caused by cellular injury can accumulate intracellularly.

4.1 Lipofuscin

Lipofuscin is a "wear and tear" pigment, an indigestible lipid derived from lipid peroxidation of cell membranes that results from free radical damage to tissue. When it occurs along with atrophy of an organ, lipofuscin accumulation is referred to as *brown atrophy*.



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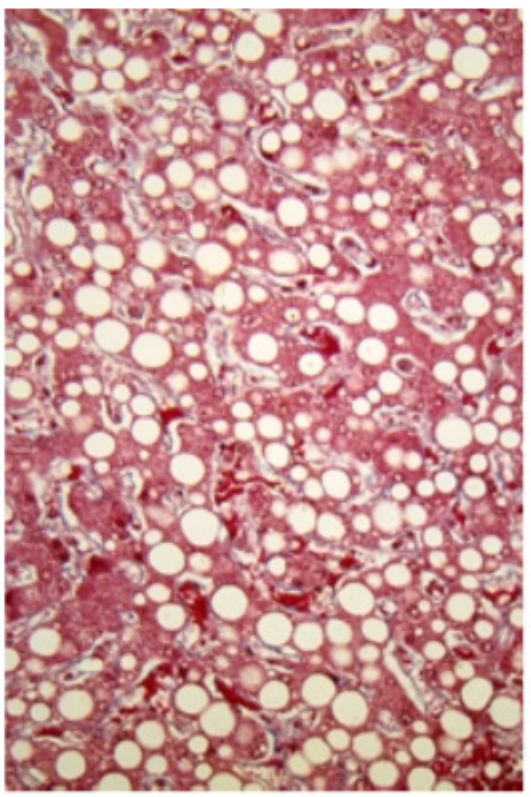
▲ **Figure 1–4.1** Lipofuscin

4.2 Triglycerides

Fatty changes occur in the liver, heart, and kidney due to cytosolic accumulation of triglycerides. The main causes of fatty changes include alcohol, malnutrition, and carbon tetrachloride.

- Alcohol:
  - Impairs mitochondrial function.
  - Increased production of NADH from alcohol metabolism accelerates conversion of DHAP to G3-P.
- Protein malnutrition (kwashiorkor): Increased mobilization of fatty acids from triglyceride stores in adipose tissue by hormone sensitive lipase.
- Carbon tetrachloride (CCl_4): Decreased synthesis of apoproteins.



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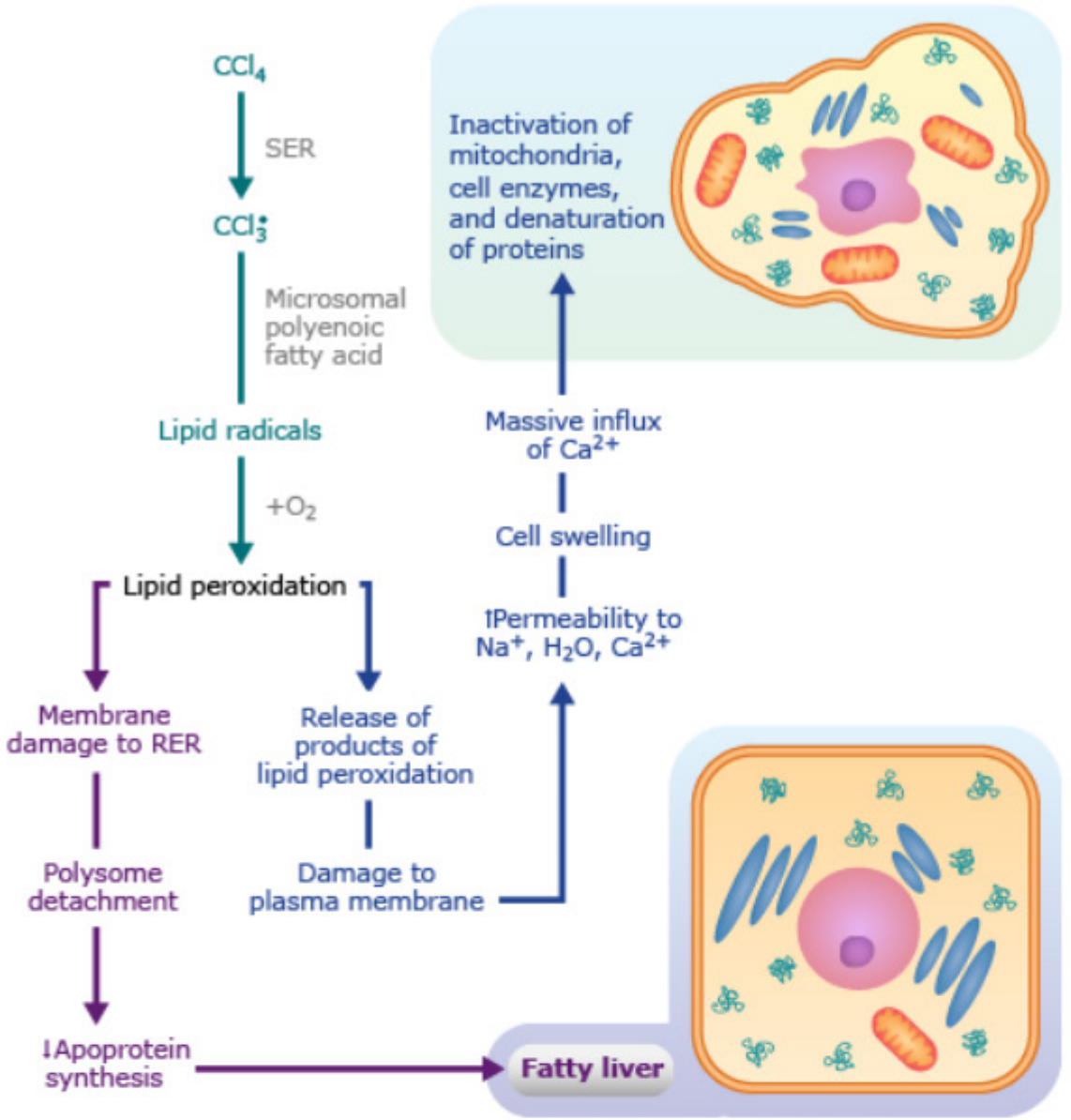
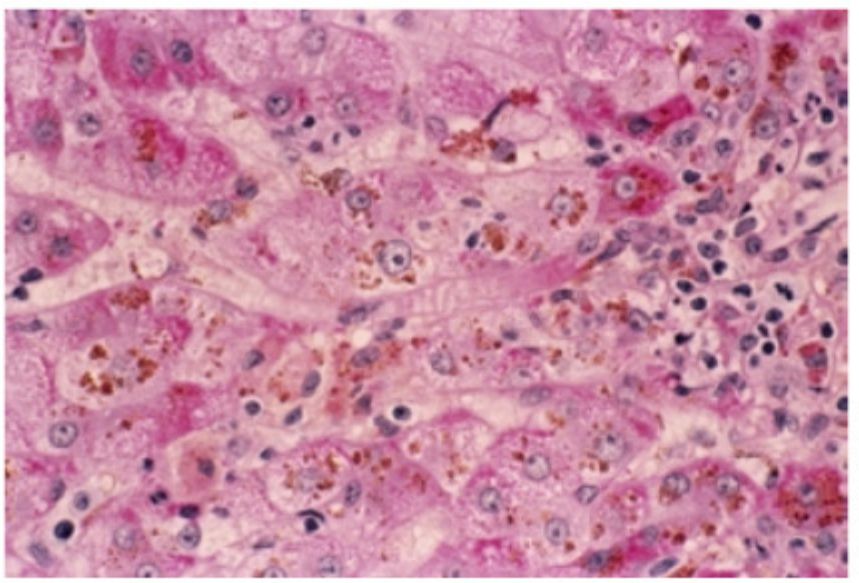
▲ **Figure 1–4.2A** Fat Accumulation in Liver Cells



▲ **Figure 1–4.2B** Role of CCl_4 in Fat Accumulation

4.3 Bilirubin

- **Hemolytic Jaundice:** Excessive destruction of red blood cells; *pre-hepatic*.
- **Hepatocellular Jaundice:** Associated with liver damage; *hepatic*.
- **Obstructive Jaundice:** Stone, cancer in biliary tract; *post-hepatic*.



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▲ **Figure 1–4.3** Bilirubin Accumulation in Liver Tissue

Looking Ahead

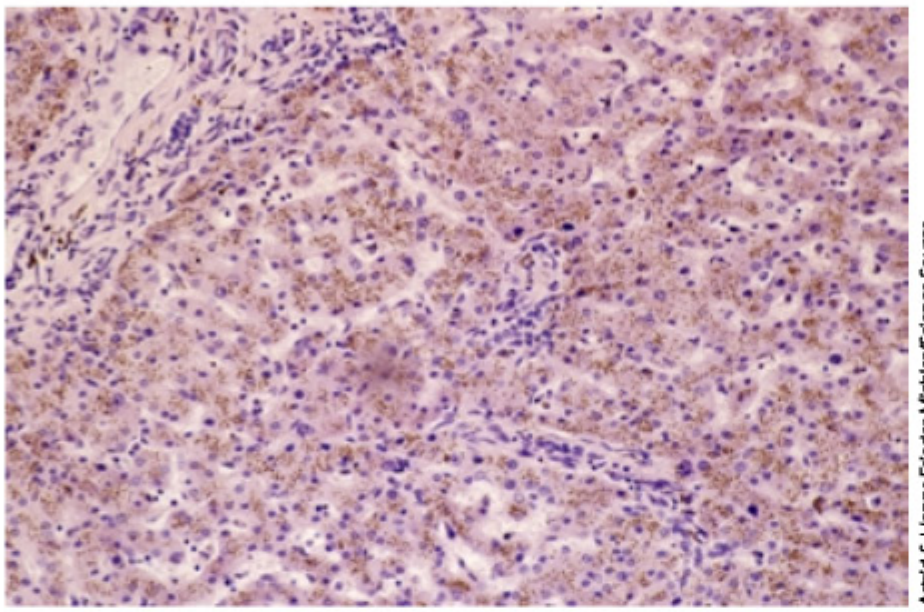
For more on the three types of jaundice, see chapter 15, on "Hepatobiliary Pathology."

4.4 Hemosiderin

Iron is normally stored as a soluble product, ferritin, in bone marrow macrophages and hepatocytes. A small amount circulates in the serum.

4.4.1 Hemosiderosis

This is a system overload of hemosiderin deposits in macrophages with no tissue damage.



Astrid & Hanns-Frieder Michler/Science Source

▲ **Figure 1–4.4** Hemosiderin Deposits: Prussian Blue Stain

4.4.2 Hemochromatosis

In extensive iron overload, hemosiderin deposits form intracellularly and in the interstitium, causing tissue dysfunction and scarring.

4.5 Calcium

4.5.1 Dystrophic Calcification

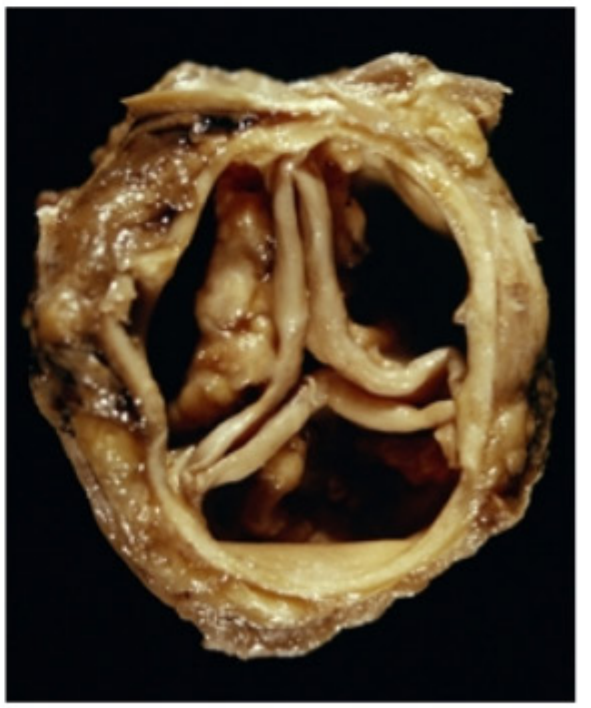
Dystrophic calcification is the deposition of calcium phosphate in necrotic tissue. It occurs in alcohol-induced chronic pancreatitis, senile aortic stenotic valves, atheromatous plaques, calcified aortic valves, and periventricular calcification of congenital CMV.

4.5.2 Metastatic Calcification

Metastatic calcification is the deposition of calcium phosphate in *normal tissue* due to increased calcium levels for *any* reason.

Manifestation Nephrocalcinosis of collecting duct interfering with the normal functioning of ADH resulting in nephrogenic diabetes insipidus.

- Primary hyperparathyroidism resulting in ↑serum Ca^{2+}.
- Chronic renal failure resulting in hyperphosphatemia (↑PO_4):
  - ↑PO_4 drives calcium into normal tissue.
  - Normal function is to drive calcium into osteoid in bone.



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▲ **Figure 1–4.5** Dystrophic Calcification

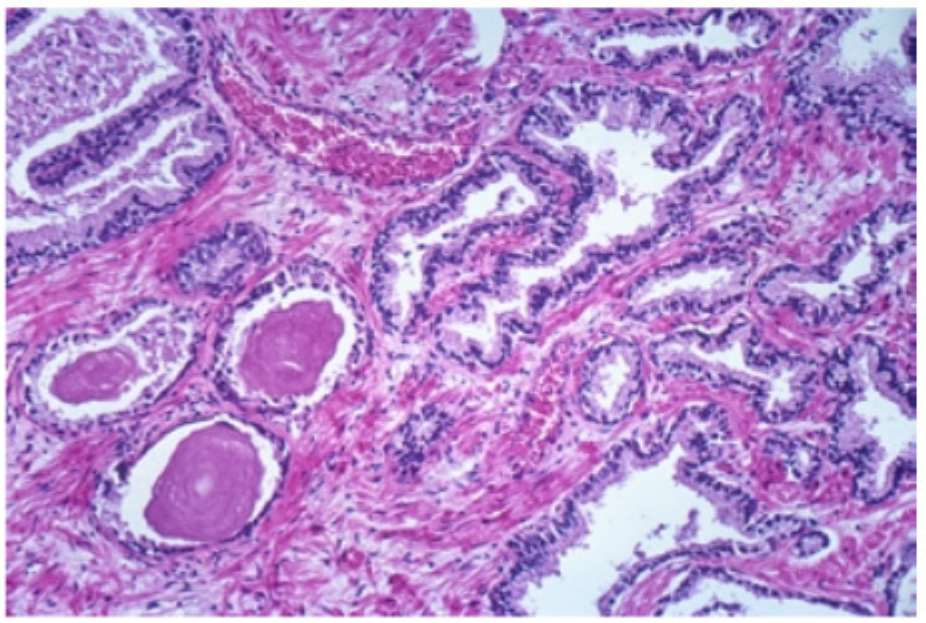
5 Cellular Responses to Stress

Cellular adaptations to stress can include hyperplasia (increase in cell number), hypertrophy (increase in cell size), atrophy (decrease in cell size), metaplasia (replacement of one cell type with another), or dysplasia (disordered cell growth).

5.1 Hyperplasia

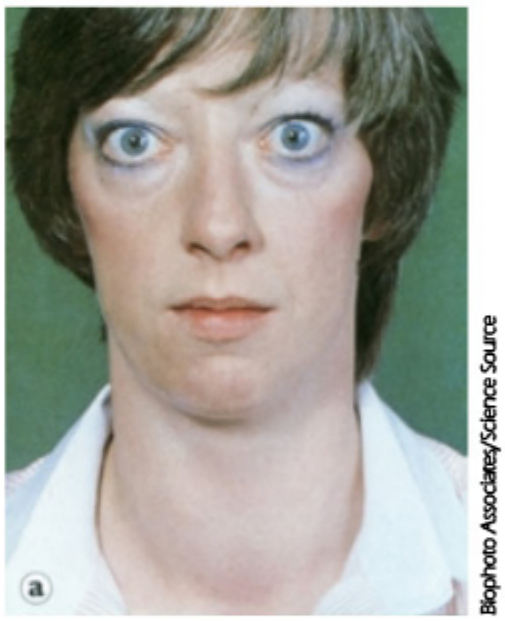
Hyperplasia is an increase in the *number* of cells, resulting in increased organ size.

- Can be physiologic (e.g., breast tissue during pregnancy) or pathologic (e.g., endometrial hyperplasia)
- Pathologic hyperplasia is a *soil* for cancerous proliferation
- Mechanisms:
  - *Hormonal Stimulation:*
    —Benign prostatic hyperplasia: Dihydrotestosterone
    —Acromegaly: Growth hormone and insulin-like growth factor 1
  - *Antibody Stimulation:* Graves disease
  - *Mechanical Stimulation:* Epidermis from friction (Callus)
  - *Viral Stimulation:* Epidermal hyperplasia (Humanpapilloma virus)



Michael Abbey/Visuals Unlimited, Inc.

▲ **Figure 1–5.1A** Prostate Hyperplasia



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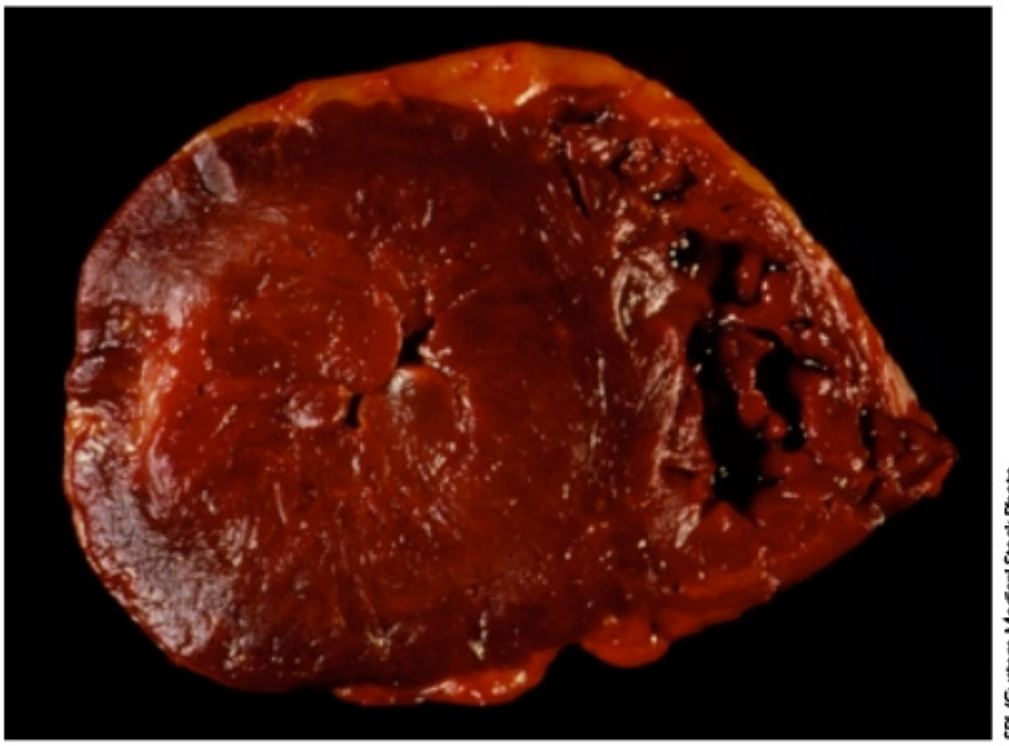
▲ **Figure 1–5.1B** Hyperplasia: Graves Disease Goiter

5.2 Hypertrophy

Hypertrophy is an increase in the *size* of cells.

- Increased number of intracellular organelles and cytosol
- Examples:
  - Cardiac myocyte hypertrophy in hypertension
  - Skeletal muscle in exercise
- No cellular proliferation, but induction of select genes and proteins
- Hyperplasia and hypertrophy can occur together (pregnant uterus) but, if cell cannot divide, then hypertrophy occurs alone
- Mechanisms:
  - Growth factor stimulation: Insulin-like growth factor-1
  - Neuroendocrine stimulation: Sympathetic stimulation
  - Ion channels: Calcium channel activity may induce calcineurin
  - Other chemical mediators: Nitric oxide (NO), angiotensin II, bradykinin
  - Oxygen supply: Increased functional demand on cells induces angiogenesis
  - Hypertrophy antagonist: Atrial and B-type natriuretic factors, high concentration of NO



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▲ **Figure 1–5.2** Hypertrophy

Connection to

Pharmacology

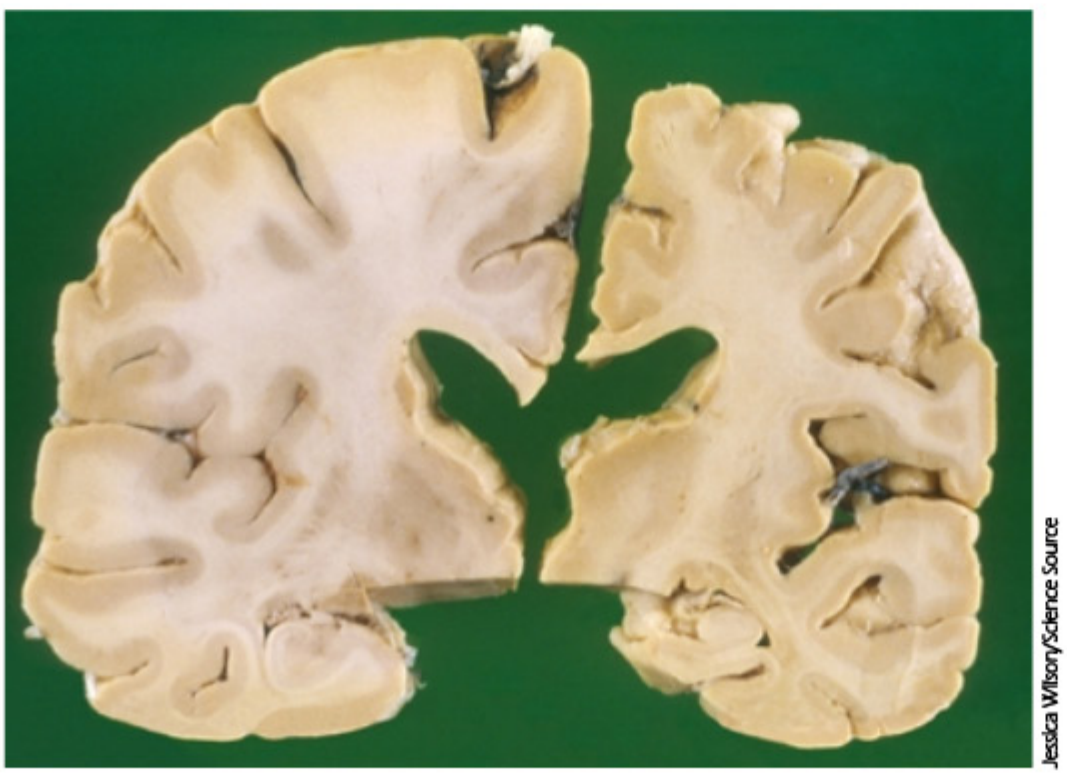
Tacrolimus, a widely-prescribed immunosuppressant drug, inhibits calcineurin.

5.3 Atrophy

Atrophy is the decrease in the size and weight of an organ due to disuse, aging, or loss of nervous or hormonal input.

▼ **Table 1–5.3** Causes of Atrophy

Cause	Result
↓ **Innervation/activity**	Muscle atrophy in ALS, cast, skeletal muscle atrophy with lower motor neuron denervation
↓ **Hormone stimulation**	Hypopituitarism (target organs undergo atrophy), ovarian atrophy following loss of estrogen with menopause
↓ **Nutrients**	Marasmus: Total calorie deprivation
↓ **Blood flow (atherosclerosis is the most common cause)**	Cerebral atrophy: Loss of neurons in layers 3, 5, and 6
Occlusion secretory ducts in pancreas	Thick ductal secretions in cystic fibrosis: Dilation ducts, atrophy exocrine glands, possible compression atrophy
Ureteral obstruction	Compression atrophy of the cortex/ medulla causing hydronephrosis



Jessica Wilson/Science Source

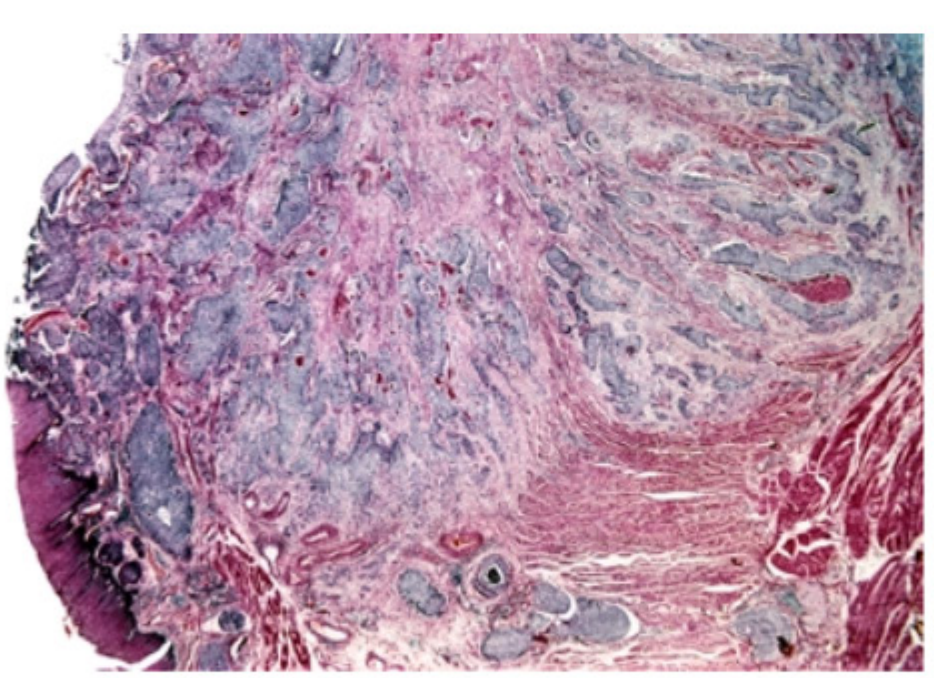
▲ **Figure 1–5.3** Cerebral Atrophy

5.4 Metaplasia

Metaplasia is the replacement of one adult cell type with another adult cell type. It arises due to re-programming of stem cells and has the possibility to transform to cancer.

▼ **Table 1–5.4** Causes of Metaplasia

Cause	Result
Acid reflux	Barrett esophagus; distal esophagus epithelium has increase of goblet cells and mucus-secreting cells
Helicobacter pylori	Increase in goblet and paneth cells in antrum and pylorus
Smoking	Columnar ciliated epithelium in bronchus replaced with squamous epithelium
Schistosoma haematobium	Squamous metaplasia of bladder



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▲ **Figure 1–5.4** Glandular Metaplasia in Barrett Esophagus

5.5 Dysplasia

Dysplasia is disordered cell growth with loss of cellular uniformity and architectural orientation. It is a precursor to malignant transformation and may result from persistence of the stress that caused metaplasia or pathologic hyperplasia—a clinically noteworthy example would be dysplasia of cervical epithelium in HPV infection.

6 Cell Death (Necrosis)

If cells are incapable of adaptation to injury, cell death occurs. The patterns of necrosis are coagulative, liquefactive, caseous, fibrinoid, and fat.

6.1 Coagulative Necrosis

Coagulative necrosis is the result of infarction or ischemia of all tissues except the brain. This type of necrosis involves denaturation of proteins with cellular outlines preserved. Two forms of infarction may result in coagulative necrosis: *white (pale) infarcts* and *red infarcts*.

6.1.1 White (Pale) Infarct

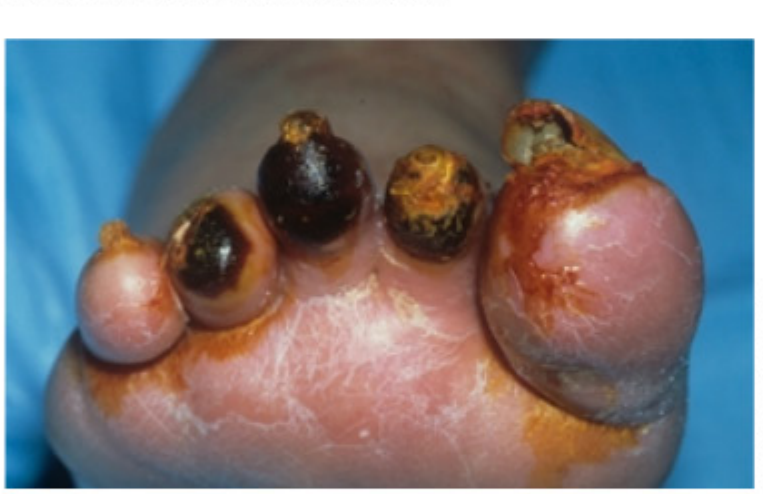
- Solid organs (heart, kidney)
- Single blood supply

6.1.2 Red Infarct

- Hemorrhagic
- "Soft" organ (lung, intestines)
- Dual blood supply
- Also seen in reperfusion of infarct in solid organs

6.1.3 Dry Gangrene

Coagulation necrosis due to infarction.



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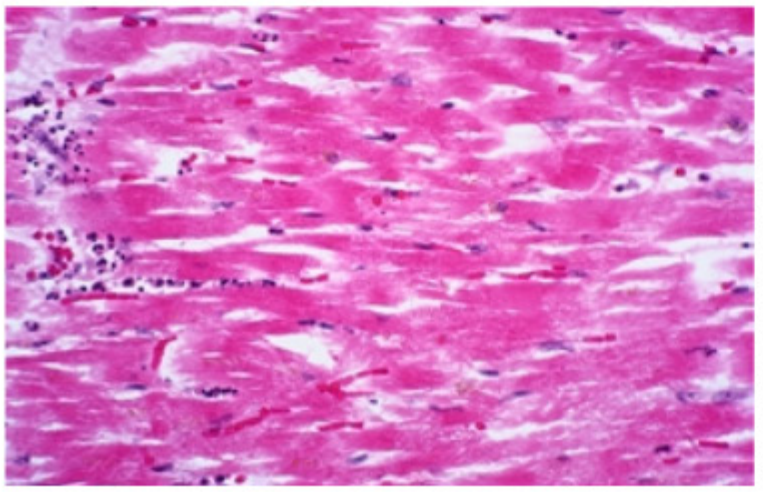
▲ **Figure 1–6.1A** Coagulative Necrosis: Dry Gangrene Caused by Diabetes

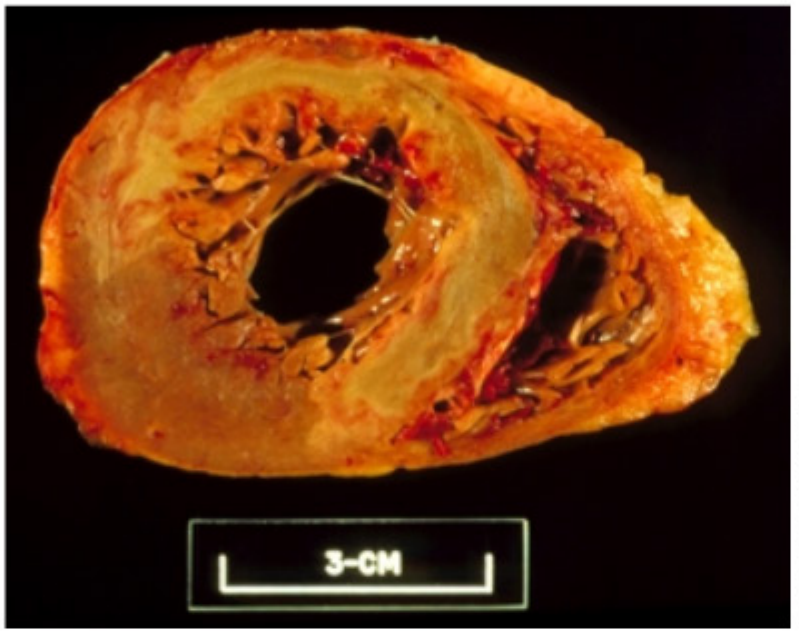


Dr. Frederick Silvera/Visuals Unlimited, Inc.

▲ **Figure 1–6.1B** Coagulative Necrosis

6.1.4 Microscopic Appearance

- Intact architecture of cells
- Ghostly outline preserved
- Nuclei disintegrating/absent:
  - *Pyknosis*: Chromatin clumping and shrinking with increased basophilia
  - *Karyorrhexis*: Fragmentation of chromatin
  - *Karyolysis*: Fading of chromatin material

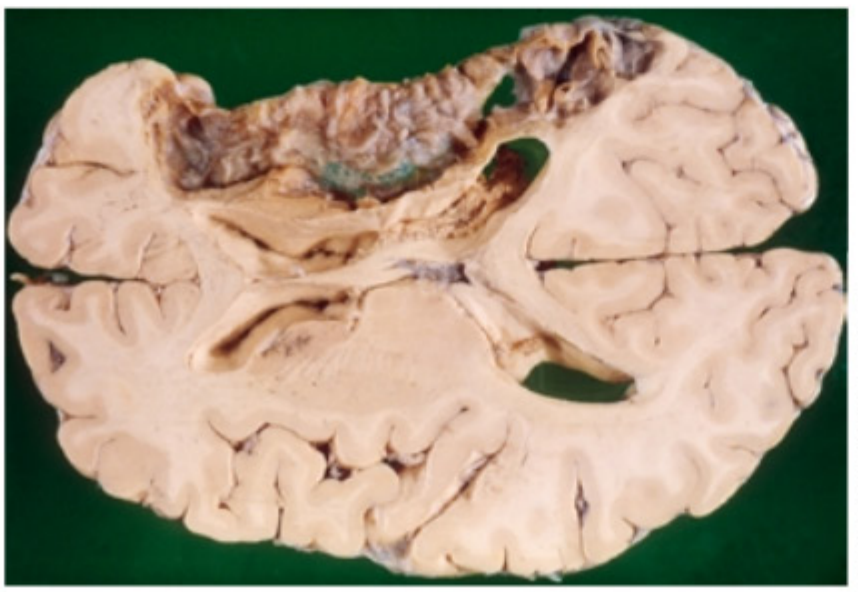


Dr. E. Walker/Science Source

▲ **Figure 1–6.1C** Myocardial Infarction

6.2 Liquefactive Necrosis

- Ischemic brain infarction or bacterial infection of any organ.
- Digestion of tissue by hydrolytic enzymes.
- Enzyme source:
  - *Neutrophils* in bacterial infections; e.g., abscess.
  - *Neuroglial cells* in brain infarction.
- Macroscopic morphology: Formation of a cystic cavity.
- *Wet gangrene* occurs when a bacterial infection is superimposed on dry gangrene and liquefactive necrosis results.



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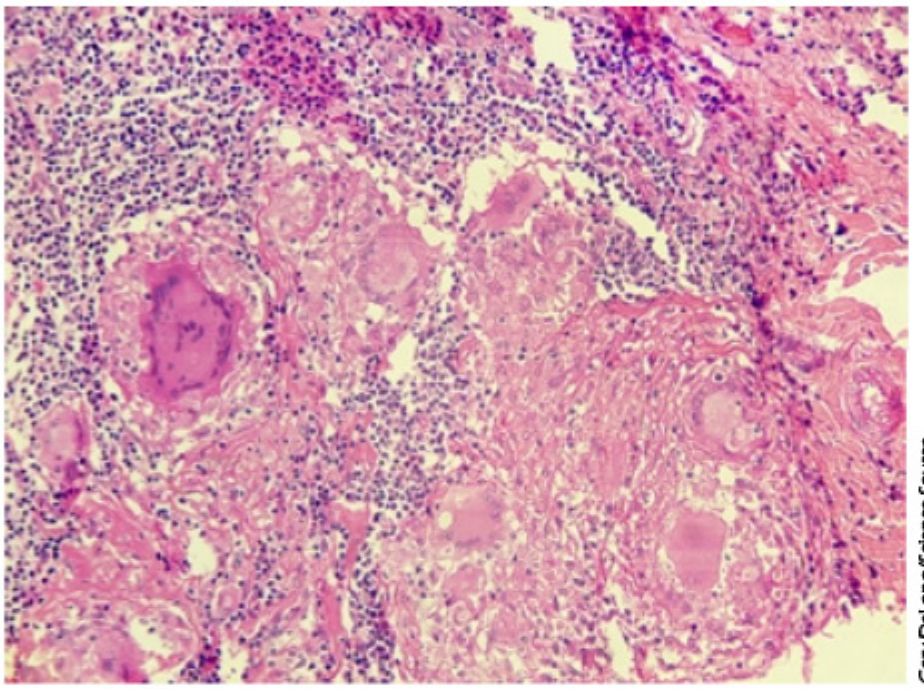
▲ **Figure 1–6.2** Liquefactive Necrosis: Cystic Cavity in the Brain

6.3 Caseous Necrosis

Caseous necrosis is a distinct form of coagulative necrosis that occurs when lipid is released following the granulomatous inflammation caused by tuberculosis and fungal infections.

- Macroscopic morphology: Granular, *"cheese-like"* material.
- Microscopic morphology:
  - Granuloma: Multinucleated giant cells are fused macrophages. Other nucleated cells are CD4 Th1 cells.
  - Central caseation occurs due to the action of macrophage enzymes on lipids from cell walls of *Mycobacteria* or systemic fungi.



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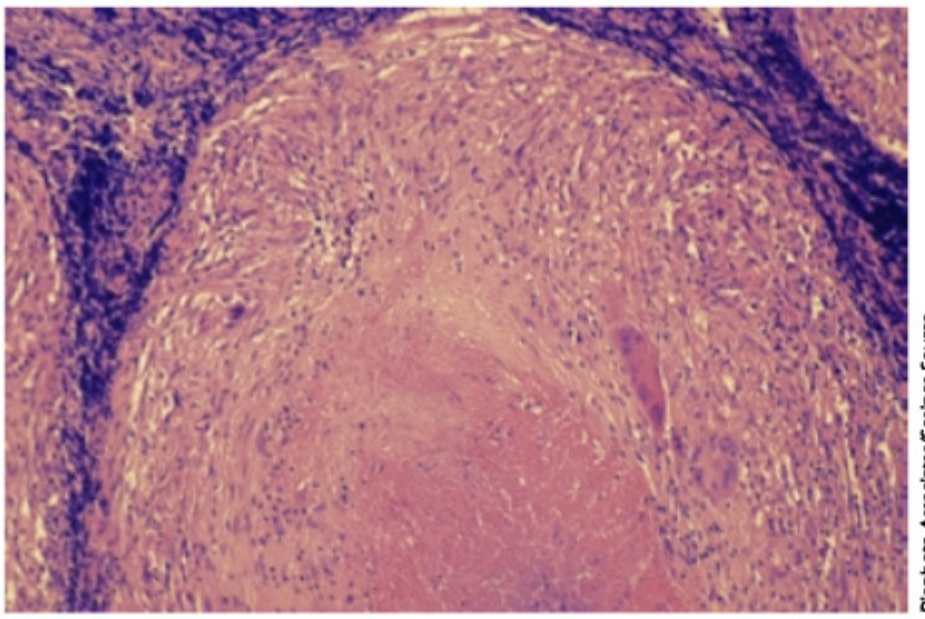
▲ **Figure 1–6.3A** Caseous Necrosis: Macroscopic Morphology



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▲ **Figure 1–6.3B** Caseous Necrosis: Microscopic Morphology

6.4 Fibrinoid Necrosis

Fibrinoid necrosis is limited to small muscular arteries, arterioles, venules, and glomerular capillaries. It results from the deposition of proteinaceous material in damaged vessel walls and is associated with immune vasculitis and malignant hypertension.

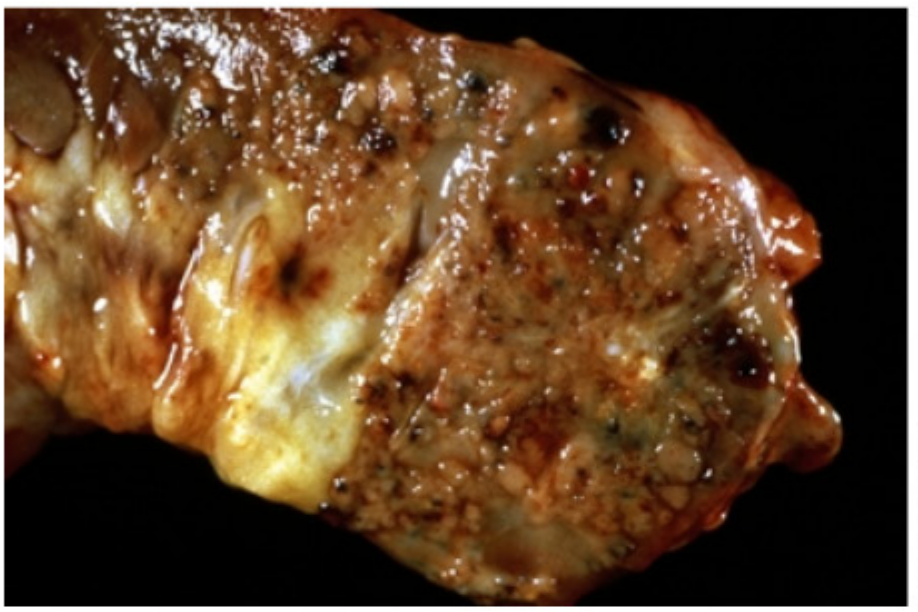
6.5 Fat Necrosis

6.5.1 Enzymatic

- Adipose tissue located around acute pancreatic inflammation can undergo enzymatic autodigestion by lipases.
- Released free fatty acids are saponified with calcium.

6.5.2 Traumatic

Injury to fatty tissue (e.g., breast) can result in fat necrosis that is not enzymatically mediated.



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▲ **Figure 1–6.5** Enzymatic Fat Necrosis of Pancreas With Saponification

6.5.3 Morphology of Fat Necrosis

- Macroscopic: Chalky, whitish deposits surrounding the pancreas.
- Microscopic: Dense, inflammatory infiltrate surrounding digested tissues.
  - Residual fat cells
  - Basophilic calcified areas

6.6 Summary of Types of Necrosis

▼ **Table 1–6.6** Types of Necrosis

Type	Mechanism of Cellular Change	Pathologic Changes
Coagulative necrosis	Ischemia leading to denaturation of cellular proteins and cytoplamsic RNA—*dry gangrene* may result.	Cellular architecture preserved, except for nuclear changes.
Liquefactive necrosis	Enzymatic destruction of necrotic tissue, generally in CNS, due to ischemia or infection—*wet gangrene* may result.	Necrotic tissue soft and liquefied.
Caseous necrosis	Coagulation and liquefactive necrosis; tuberculous granulomas.	Structure lost, but tissue not liquefied; appearance resembles cheese.
Fibrinoid necrosis	Deposits of fibrin materials in walls of arteries.	Thick, pink rings in vascular walls; may not have true necrosis.
Fat necrosis	Digestion of pancreatic tissue by pancreatic enzymes.	Necrosis of fat cells, forming calcium soaps. Inflammation, hemorrhage.

7 Apoptosis

Apoptosis is programmed, enzyme-mediated cell death. Both physiologic and pathologic processes are associated with apoptosis, which is activated by two possible mechanisms: an intrinsic pathway and an extrinsic pathway.

7.1 Characteristics of Apoptosis

- Cell degrades its own nuclear and cytoplasmic proteins
- Cell membrane *does not* break down
- Cell particles are phagocytosed
- No inflammation

7.2 Functions of Apoptosis

7.2.1 Physiologic

- Embryogenesis (loss of webs between digits)
- Hormone withdrawal (endometrium)
- Elimination of self-reactive T cells in thymus

7.2.2 Pathologic

- Viral infections (T cell induced)
- DNA damaged cells (p53 mechanism)

7.3 Pathways of Apoptotic Activation

The signal to initiate cellular apoptosis may originate inside the cell (*intrinsic*, mitochondrial) or outside the cell (*extrinsic*, death receptor initiated).

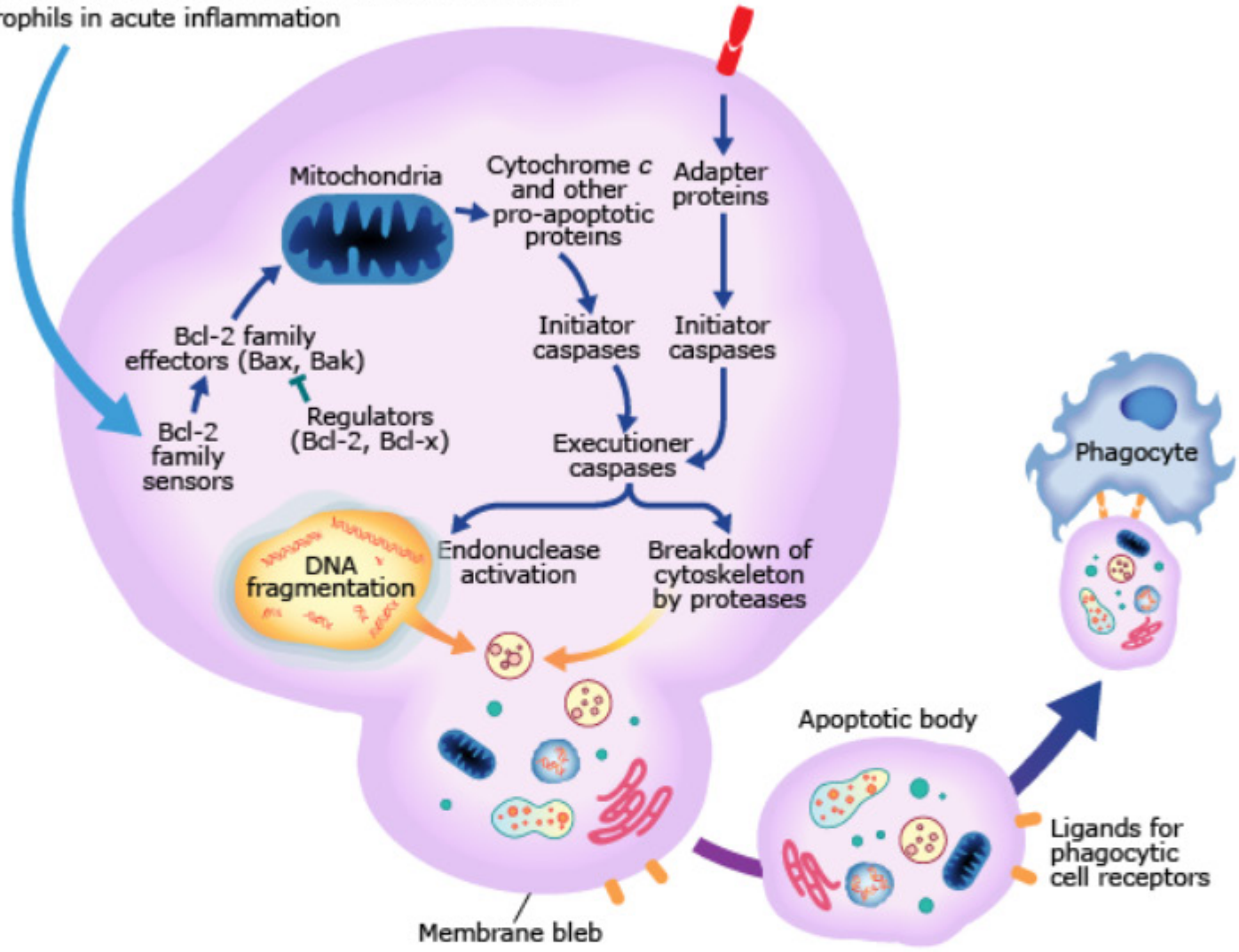
Mitochondrial (Intrinsic) Pathway

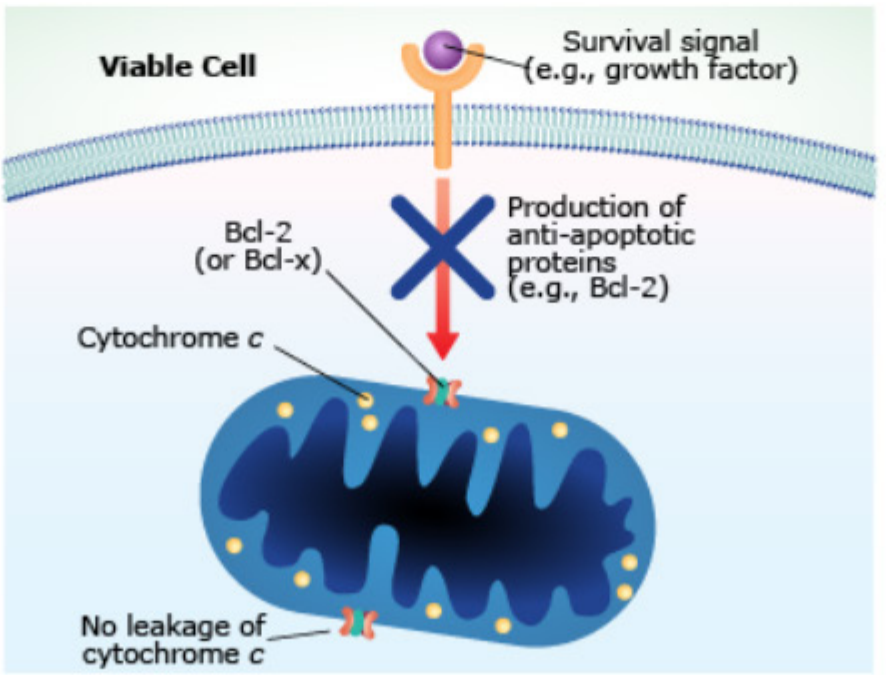
Cell injury, DNA damage, misfolded proteins (e.g., prions), embryogenesis (MIS [müllerian inhibiting substance] apoptosis of müllerian structures, tissue between fingers, cardiac morphogenesis), menses (abrupt withdrawal of estrogen/progesterone), thymus involution, CD8 T cell destruction virus-infected cell/tumor cell, removal of neutrophils in acute inflammation

Death Receptor (Extrinsic) Pathway

Receptor-ligand interactions

- Fas
- TNF-α / TNFR1 receptor





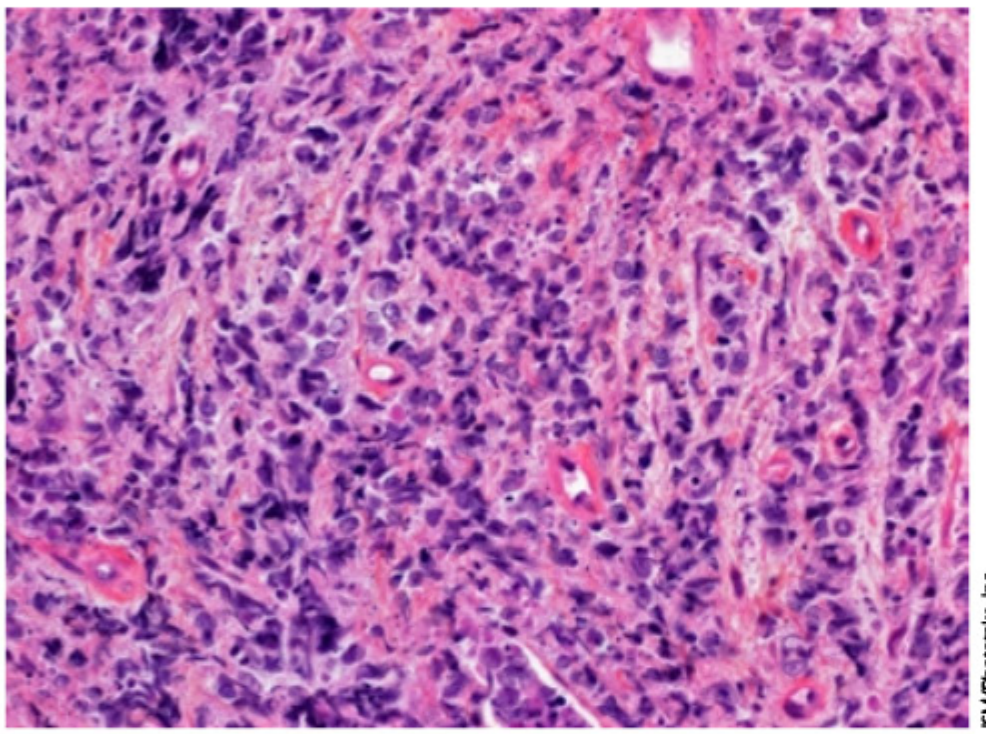
▲ **Figure 1–7.3A** Pathways of Apoptosis

7.3.1 Intrinsic Pathway

- Cellular injury may result in growth factor withdrawal
  - Anti-apoptotic proteins not produced
  - Cytochrome c released
- Cytochrome c interacts with *Apaf-1*
  - → Activation of *caspase-9*
  - → Activation of downstream caspases
  - → Enzymatic digestion of cell content

7.3.2 Extrinisic Pathway

- Mediated by receptors including FAS and TNF
- FAS-TNF receptor ligands signal a series of events, activating caspases
- *Cytotoxic T cell activation: Granzyme B*, a cytotoxic T cell protease, directly activates executioner caspases



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▲ **Figure 1–7.3B** Apoptotic Body

CHAPTER 2 The Inflammatory Reaction

1 Overview of Inflammation

Inflammation is the term used to describe how blood vessels and immune cells react to injury, resulting in fluid and leukocytes accumulating in extravascular tissue. There are many steps in inflammation, including the release of fluid from vessels, the attraction of leukocytes, activation of chemical mediators (such as cytokines), the removal of debris, and the repair of damaged tissue. The *two main players* in the inflammatory system are *antibodies* and *leukocytes*.

1.1 Types of Inflammation

Inflammation can be divided into two types, based on cells involved, not the time-course of the reaction, although sometimes the two correlate. *Acute inflammation* primarily involves neutrophils. *Chronic inflammation*, on the other hand, tends to occur with more persistent insults and mostly involves lymphocytes and macrophages.

1.1.1 Overview of Acute Inflammation

The acute inflammatory response is an immediate response to any injury. It is considered a component of the innate immune response. It has the following characteristics:

- It onsets within minutes.
- It usually resolves within a few days.
- It may progress to chronic inflammation.

1.1.2 Overview of Chronic Inflammation

The chronic inflammatory response results from inflammation of long duration, often due to the persistence of the injurious stimulus. It has the following characteristics:

- It has slower onset (weeks to years).
- It has a longer time-course.
- It results in loss of functional tissue with repair by fibrosis.

USMLE® Key Concepts

For Step 1, you must be able to:

- Identify the histology of acute and chronic inflammation.
- Describe the chemical, cellular, and hemodynamic changes that result from acute and chronic inflammation.
- Explain the molecular origins of leukocyte function diseases such as leukocyte adhesion deficiency, Chédiak-Higashi syndrome, and chronic granulomatous disease.

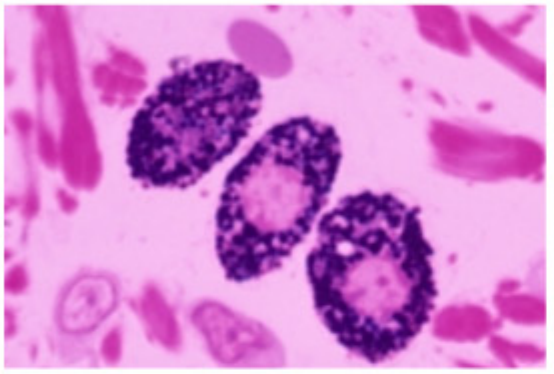
1.2 Inflammatory Cells

1.2.1 Mast Cells and Basophils

Mast cells and basophils look and act similar and differentiate from a common CD34+ bone marrow precursor. Basophils, however, emerge fully mature from the bone marrow, whereas mast cells circulate in immature form before homing to a particular tissue. Both cells contain granules that are filled with histamine and heparin, and play a role in allergy, anaphylaxis, and as a defense against helminthic parasites. Basophils can be distinguished by their bilobed nuclei.

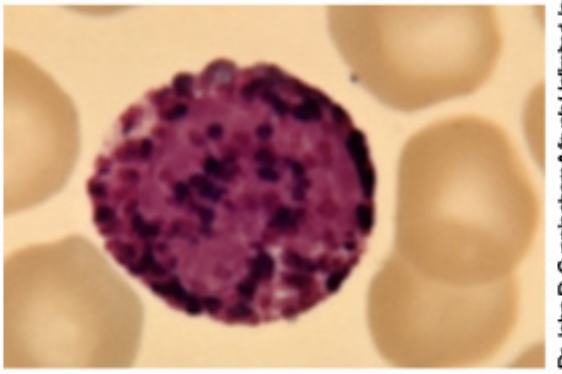
Memory Aid

The letter "B" represents a fully mature **B**asophil from the **B**one marrow.



Dr. Robert Calentine/Visuals Unlimited, Inc.

▲ **Figure 2–1.2A** Mast Cell

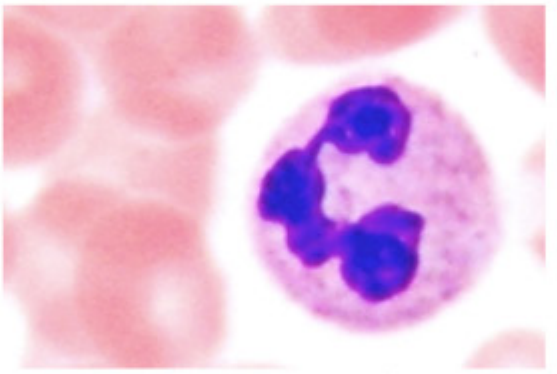


Dr. John D. Cunningham/Visuals Unlimited, Inc.

▲ **Figure 2–1.2B** Basophil

1.2.2 Neutrophils

Neutrophils are the first leukocytes to respond to injury and their influx will peak within five to six hours. Neutrophilia is particularly associated with bacterial infection and infarction. The major function of neutrophils is in phagocytosis and the release of microbicidal chemicals that contribute to the formation of abscesses.



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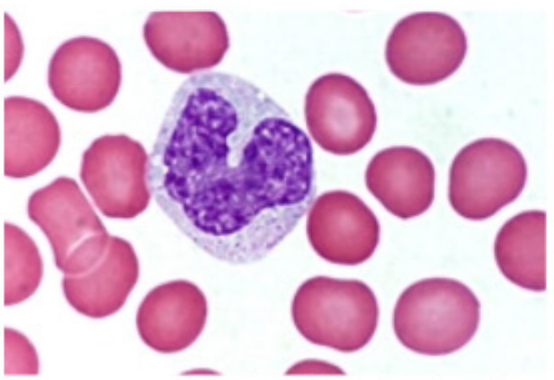
▲ **Figure 2–1.2C** Neutrophil

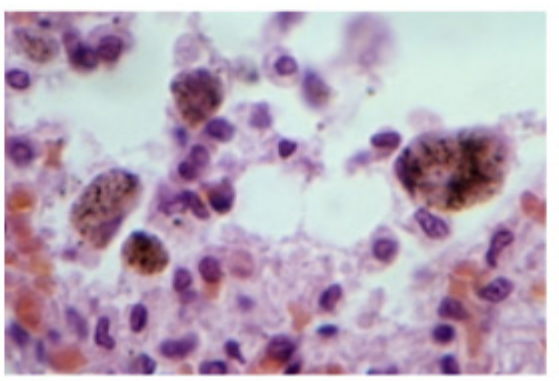
1.2.3 Monocytes and Macrophages

Monocytes and macrophages typically appear in an inflammatory response two to three days after the injury. Both cells are highly phagocytic and play important roles in cleaning debris and infection from the area, but additionally, macrophages are important cytokine-secreting cells and antigen-presenting cells for the development of acquired immunity.



Michael Ross/Science Source

▲ **Figure 2–1.2D** Monocyte

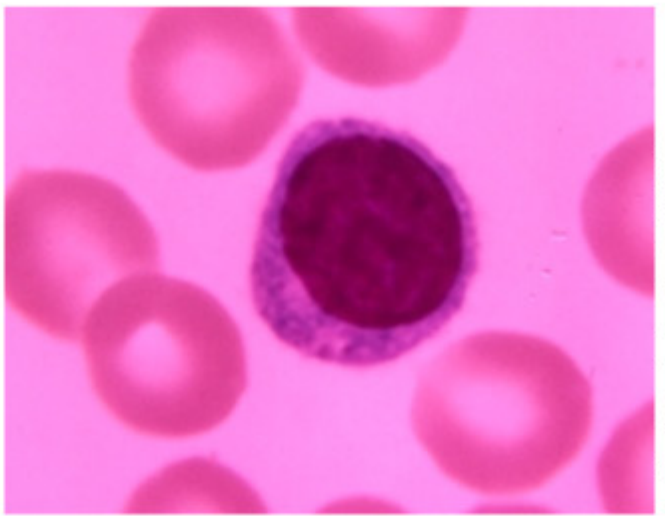


Ralph Hutchings/Visuals Unlimited, Inc.

▲ **Figure 2–1.2E** Macrophage

1.2.4 Lymphocytes

Lymphocytes come in two forms. Large lymphocytes include cells such as the natural killer cells, whereas the small lymphocytes consist of T and B cells. Natural killer cells belong to the innate immune response and protect against some viruses and malignancies. B and T lymphocytes are cells of the acquired immune response, and protect against virtually all other forms of pathogen.

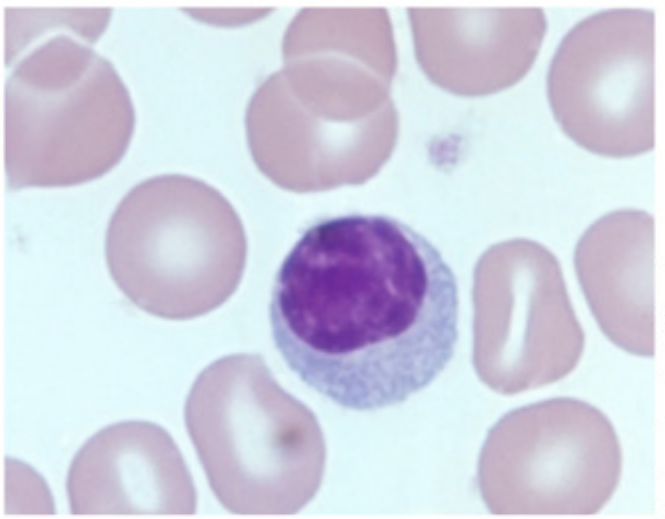


Dr. John D. Cunningham/Visuals Unlimited, Inc.

▲ **Figure 2–1.2F** Lymphocyte

1.2.5 Plasma Cells

Plasma cells are the end-cell of B lymphocyte differentiation. They are cellular factories of antibody synthesis and are found most frequently in the lymphoid follicles of the lymph nodes and spleen.

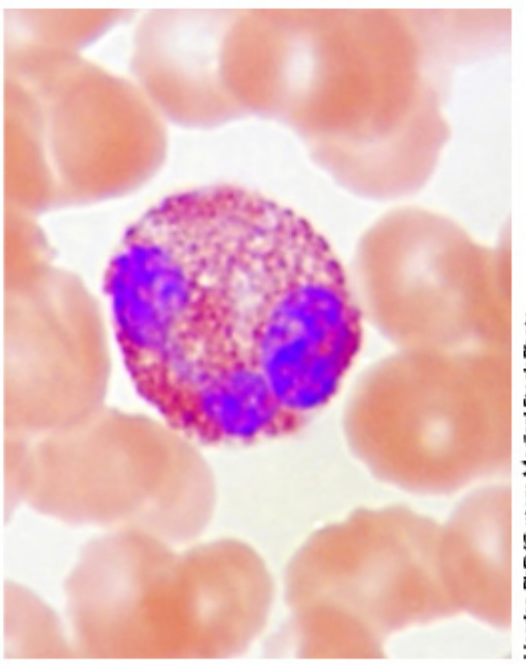


Biophoto Associates/Science Source

▲ **Figure 2–1.2G** Plasma Cell

1.2.6 Eosinophils

Eosinophils are associated with allergic and parasitic infections. They are attracted to inflammatory foci by the release of the granular contents of mast cells and basophils. They release a major basic protein that is toxic to the cuticle of helminthic parasites.



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▲ **Figure 2–1.2H** Eosinophil

Memory Aid

Mnemonic for conditions in which eosinophils will be seen:

NAACP

N = **N**eoplasia

A = **A**llergies

A = **A**sthma

C = **C**ollagen vascular disease

P = **P**arasites

2 The Process of Acute Inflammation

Acute inflammation is really just an early response to injury of any kind. The end goal of acute inflammation is to get white blood cells into the extravascular space to help limit the damage and allow the tissue to repair. There are three main components of acute inflammation:

- **Vasodilation:** Increases blood flow to the affected tissue.
- **Endothelial Cell Disruption:** Allows the contents of the blood to exit blood vessels.
- **Leukocyte Margination and Diapedesis:** Allows white blood cells to exit the blood vessel.

The result of these vascular and chemical responses is the production of the five cardinal signs of acute inflammation:

- **Rubor:** Redness
- **Tumor:** Swelling
- **Calor:** Heat
- **Dolor:** Pain
- **Functio Laesa:** Loss of function

Clinical Application

The Buildup of Interstitial Fluids

Edema: An increase in interstitial fluid.

Anasarca: An extreme form of generalized edema that is characterized by widespread swelling of the skin. It usually only occurs in response to liver or renal failure/ disease, though some medications can also induce anasarca.

Exudate: A form of interstitial fluid with a very high protein concentration; in essence, exudates form when cells and proteins are able to migrate out of the blood vessels and the fluid then follows these cells and proteins via osmosis. As the proteins and cells break down, they generate a lot of cellular debris. One of the hallmarks of an exudate is a *high specific gravity and high protein content.*

Pus: An exudate that is filled with neutrophils and other debris.

Transudate: A form of interstitial fluid marked by *low specific gravity and low protein content*. Transudate forms when fluid is squeezed out of the blood vessels due to some hydrostatic imbalance—thus, it is essentially a filtered form of plasma.

2.1 The Components of Acute Inflammation

2.1.1 Vasoactive Changes

- Transient initial vasoconstriction of arterioles is neurogenic.
- Massive vasodilation follows, due to histamine and nitric oxide which relax vascular smooth muscle.
- Increased blood flow increases hydrostatic pressure.

2.1.2 Increased Capillary Permeability

- Increased capillary permeability releases a transudate into the interstitium:
  - Chemical mediators of permeability include:
    —Vasoactive amines (histamine, serotonin)
    —Bradykinin from the kinin cascade
    —Leukotrienes (LTC4, LTD4, LTE4)
  - Cellular contributors to permeability include:
    —Endothelial cell and pericyte contraction
    —Direct endothelial cell injury
- The outflow of fluid surpasses lymphatic drainage ability.
- Blood stasis due to increased viscosity allows leukocyte margination.

2.1.3 Leukocyte Emigration

The process by which leukocytes leave the blood occurs in four steps:

1. **Margination:** Leukocytes move toward the walls of the blood vessels, a process known as margination.
2. **Rolling:** In this stage, leukocytes actually attach loosely to newly expressed selectin molecules on the endothelial cells and gradually decelerate.
   - **Selectins:** A group of adhesive proteins on the surface of the endothelium that play a key role in leukocyte rolling. The two main selectins are E and P selectins, which bind to oligosaccharide motifs (such as sialyl-Lewis-X) on the leukocytes. Selectins are redistributed on the endothelium by chemicals such as histamine and thrombin, and inflammatory mediators such as IL-1 and TNF.
   - **Activation by Chemokines:** The binding of leukocytes to endothelial selectins allows them to become activated by chemokines diffusing from the focus of injury. This activation causes a change in configuration of a second set of adhesion molecules on the surface of the leukocyte known as integrins. An example of this activation would be an important interaction of chemokines such as C5a and LTB4 activating neutrophilic integrins. Important clinical associations of integrins include CD11a:CD18 and β2 integrin.
3. **Adhesion:** Tight adhesion of leukocytes is mediated by the endothelial molecules ICAM-1 and VCAM-1 binding to complementary activated integrins on the surface of the leukocytes. This stops the leukocytes in the circulation and allows them time to extend their pseudopodia and move through the endothelium into the area of injury.

Clinical Application

Leukocyte Adhesion Deficiency is a set of autosomal recessive disorders in which leukocyte diapedesis is defective. LAD type 1 is a deficiency of CD11a:CD18, and LAD type 2 is a deficiency of a selectin which binds neutrophils. Clinical findings include delayed separation of the umbilical cord, extreme gingivitis, poor wound healing, and peripheral leukocytosis.

4. **Transmigration:** This is the last step in delivering leukocytes to the areas of infection. There are two phases: Crossing over the vessel walls and then traveling to area of infection or injury.
   - **Diapedesis:** The technical term for the leukocyte transmigration across the endothelium. CD31 (PECAM-1; platelet endothelial cell adhesion molecule) plays a major role in this movement. Neutrophils dissolve the basement membrane and enter the interstitium.
   - **Chemotaxis:** The movement of leukocytes along a chemical gradient toward the area of infection/injury. This movement can be mediated by both exogenous agents (such as bacterial products) or by endogenously produced chemicals (C5a, LTB4, and cytokines such as IL-8). Binding causes the release of calcium, which increases neutrophil motility. Chemotaxis can be measured by the Boyden chamber technique.

Connection to Microbiology

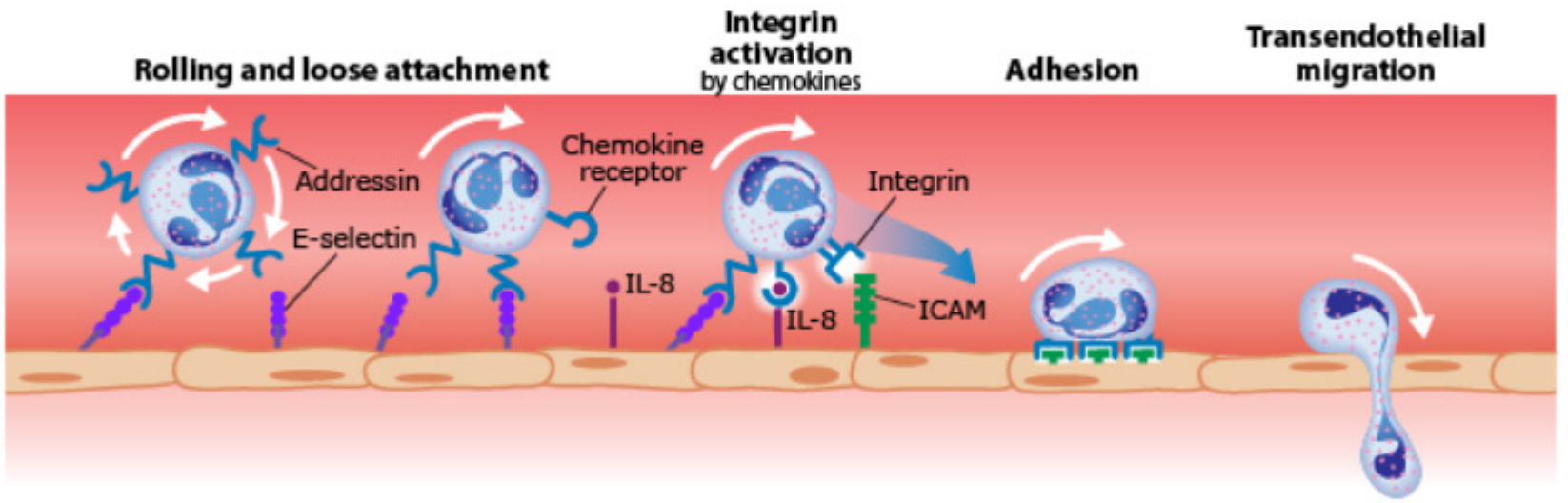
Endotoxin enhances activation of adhesion molecules and, therefore, removes leukocytes from the circulating pool.

Connection to Pharmacology

Catecholamines, corticosteroids, and lithium inhibit activation of adhesion molecules.

▼ **Table 2–2.1** Chemotaxins

Chemotaxin	Source
Leukotriene B4	Phospholipid membrane damage activates arachidonic acid cascade and lipoxygenase pathway
Interleukin-8	Resident and entering leukocytes
C5a	Classical, alternative, or lectin complement cascades
Formyl methionyl peptides	Bacteria introduced into the injury
Fibrinopeptides	Activation of the clotting cascade



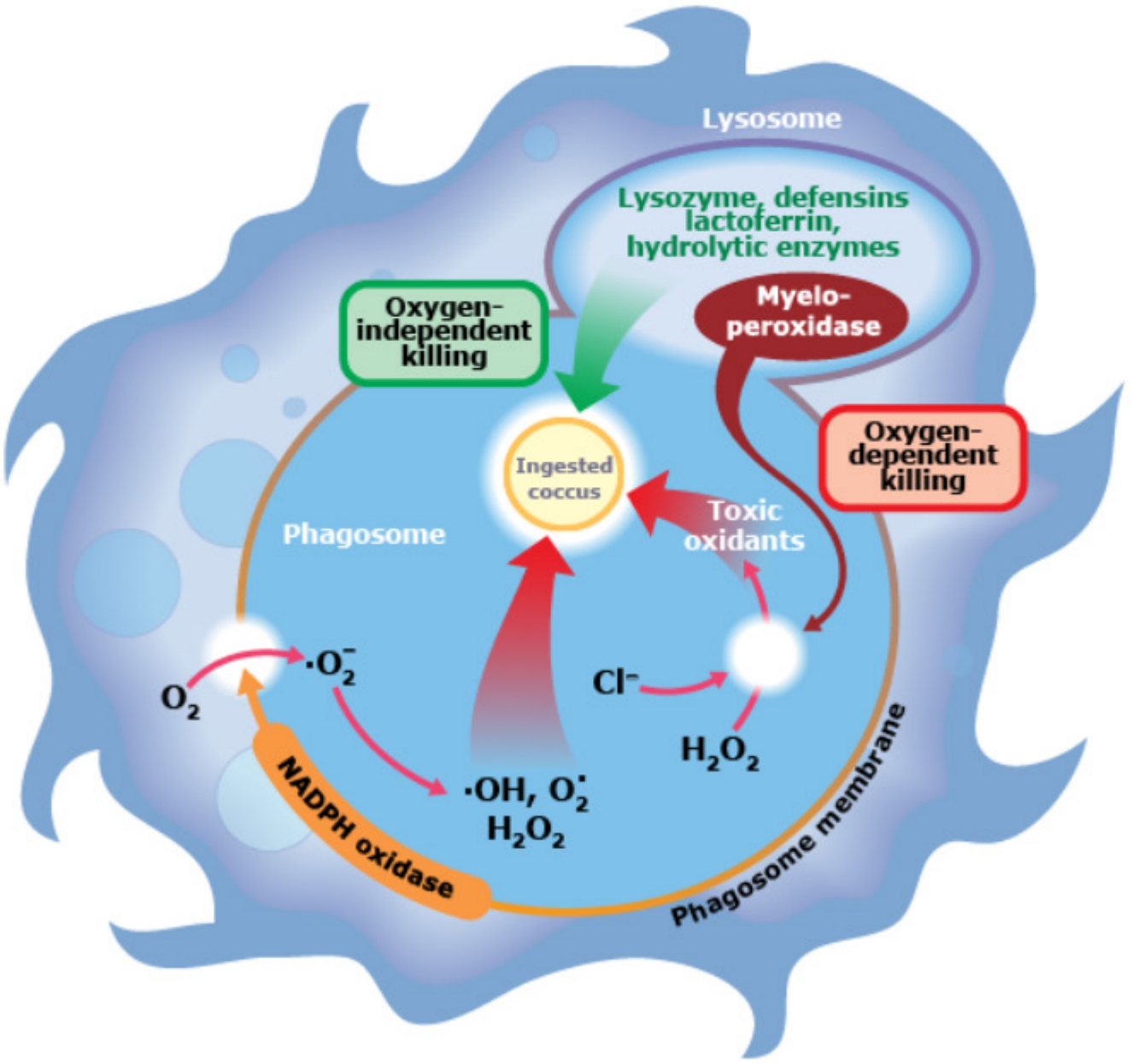
▲ **Figure 2–2.1** Steps of Leukocyte Extravasation

2.2 Phagocytosis

Phagocytosis is the process by which phagocytes remove the cellular debris that remains after injury.

2.2.1 Steps of Phagocytosis

The first step of phagocytosis is recognition and attachment, which may occur via TLR receptors and be enhanced by opsonization with IgG and C3b. The debris is engulfed into vesicles called phagosomes, which are then fused with lysosomes to produce *phagolysosomes*. Inside the phagolysosome there are two different mechanisms by which the digestion can occur. The most efficient is the oxygen-dependent system, which begins with the formation of hydrogen peroxide by NADPH oxidase. Next, myeloperoxidase (along with a halide such as chloride) converts hydrogen peroxide to hypochlorite. Oxygen-independent killing is much less effective and mediated by enzymes such as lysozyme.



▲ **Figure 2–2.2** Mechanisms of Intracellular Killing

2.3 Chemical Mediators

The chemical mediators of inflammation originate from plasma, leukocytes, or other cells.

2.3.1 Vasoactive Amines

Two of the key chemical mediators of inflammation are known as vasoactive amines—this is because they are either derived from amino acids or are amino acids themselves.

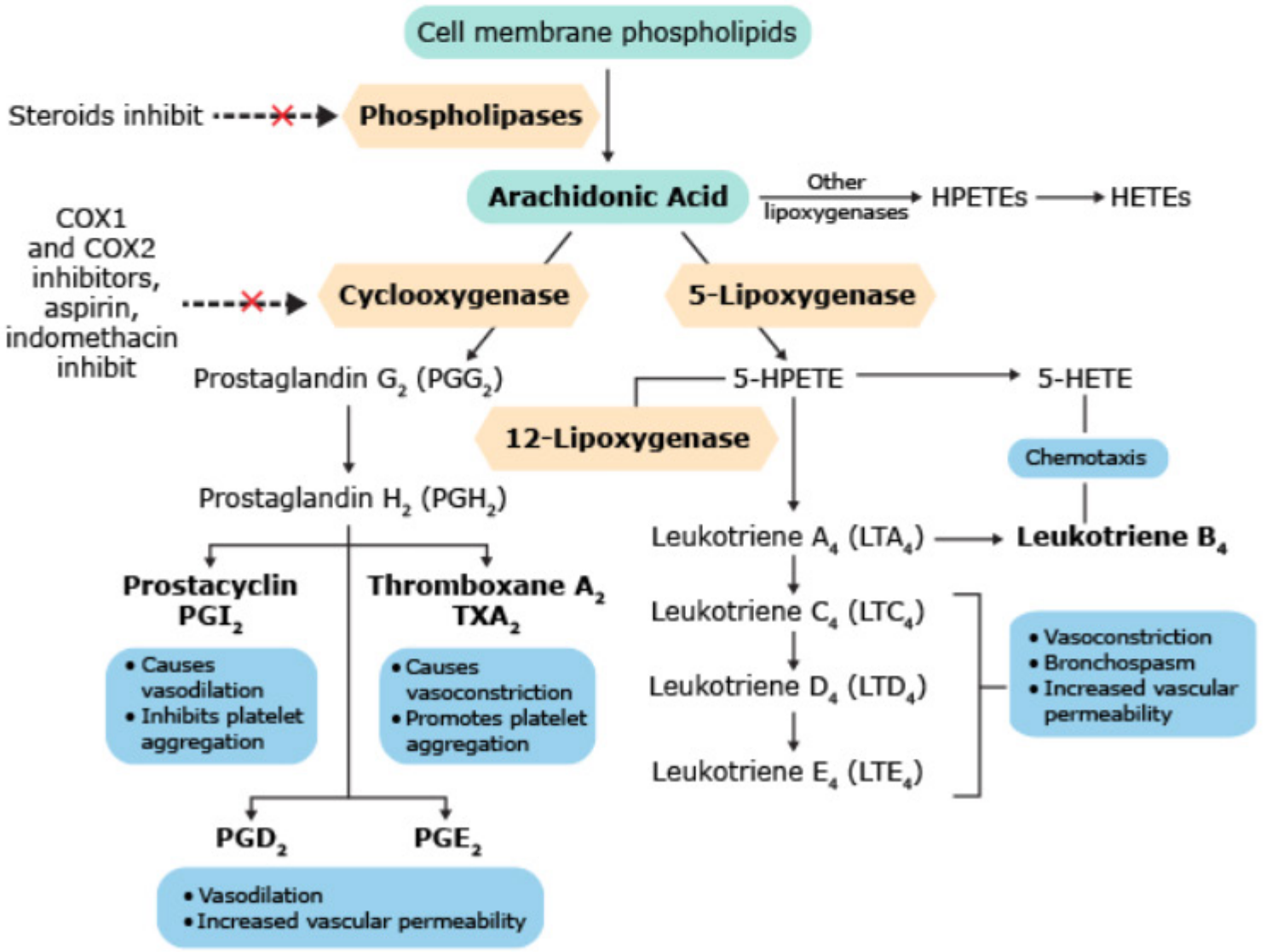
- **Histamine:** Increases capillary permeability.
  - Released by mast cells and basophils when IgE molecules on their membranes are cross-linked by antigen.
  - Released by platelets in a *platelet aggregation and release* response that can be *triggered* by endothelial injury, thrombosis, or the presence of platelet-activating factor.
- **Serotonin:** Increases capillary permeability, but is much *more potent*. It is found in *platelets* and is released with *platelet aggregation*.

2.3.2 Arachidonic Acid Cascade

Arachidonic acid is another chemical mediator that is active in inflammation by itself and via its metabolites. It is released from membrane phospholipids by phospholipase A2. Arachidonic acid is *metabolized via the cyclooxygenase or the lipoxygenase pathways*.

Important Concept

It's key to distinguish phospholipase A2 from phospholipase C, part of the G_q–calcium second messenger cascade. Link the "c" in calcium with phospholipase C. The complex "A2" represents the two branches seen with the arachidonic acid pathway.



▲ **Figure 2–2.3A** Arachidonic Acid Cascade

- **Cyclooxygenase Pathway**
  - Uses the proteins COX1 and COX2, breaks arachidonic acid into *thromboxanes and prostaglandins*.
  - Thromboxane A2 causes *vasoconstriction and platelet aggregation*.
  - Prostaglandins cause *vasodilation and inhibition of platelet aggregation*.
  - The cyclooxygenase pathway is inhibited by NSAIDs, aspirin, and COX-inhibitors.
- **Lipoxygenase:** This pathway metabolizes arachidonic acid into leukotrienes.
  - A4 and B4 cause *vasodilation* and inflammation.
  - C4, D4, and E4 cause *bronchoconstriction and vasoconstriction*.
  - One treatment used for asthma is leukotriene inhibitors.
- **Lipoxins**
  - Formed from platelets and neutrophils.
  - Promote resolution of inflammation.

2.3.3 Nitric Oxide

- Produced by macrophages and endothelial cells.
- Produced during conversion of arginine to citrulline by NO synthase.
- Causes vasodilation, decreased leukocyte adhesion, and reduced platelet adhesion. Has microbicidal activities.

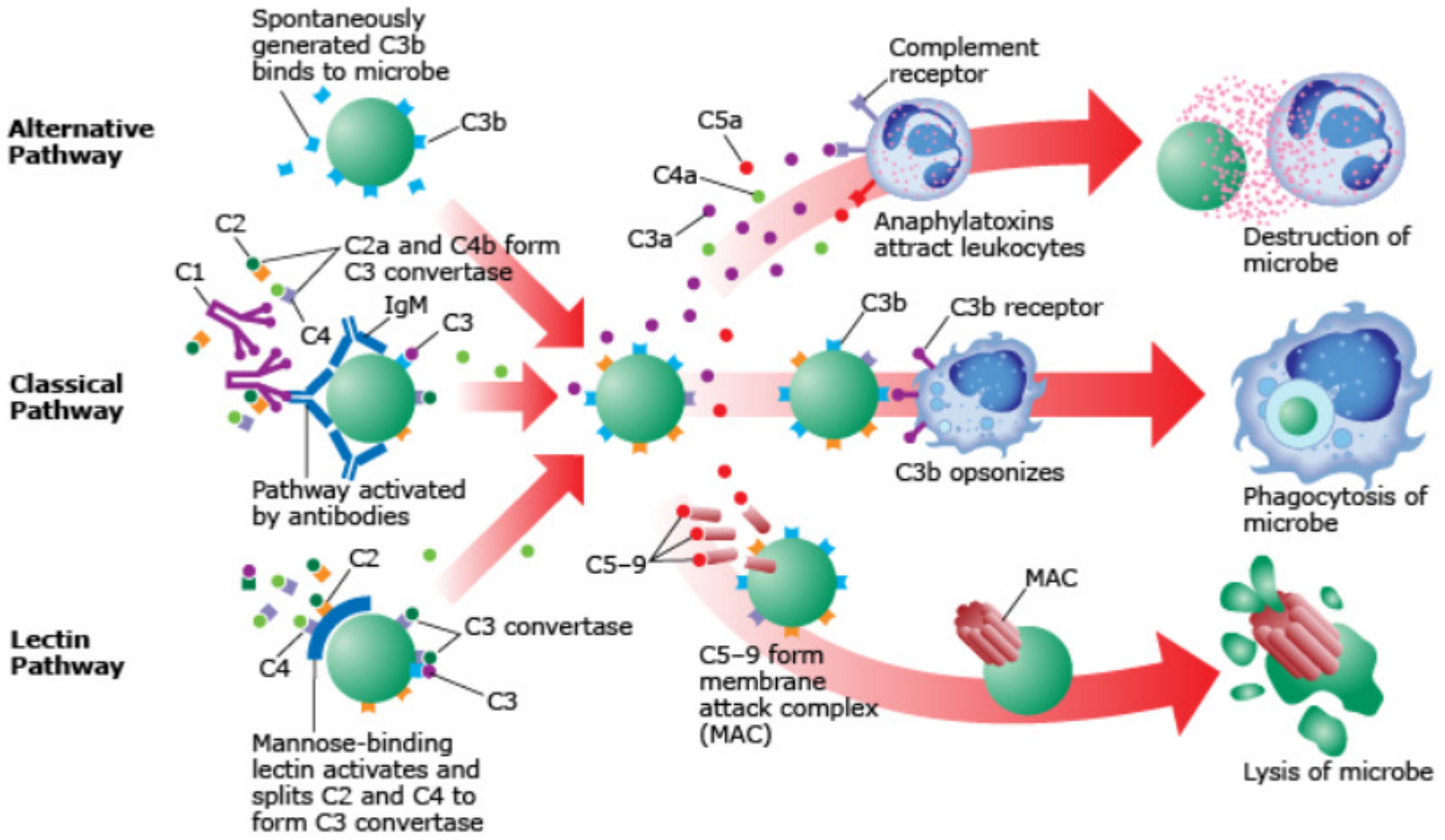
2.3.4 Cytokines

Soluble proteins secreted by inflammatory cells—IL-1 and TNF are secreted by monocytes and macrophages and produce the *acute-phase response*, a response to inflammation that involves fever, malaise, increased sleep, neutrophilia, and decreased appetite.

2.3.5 Complement

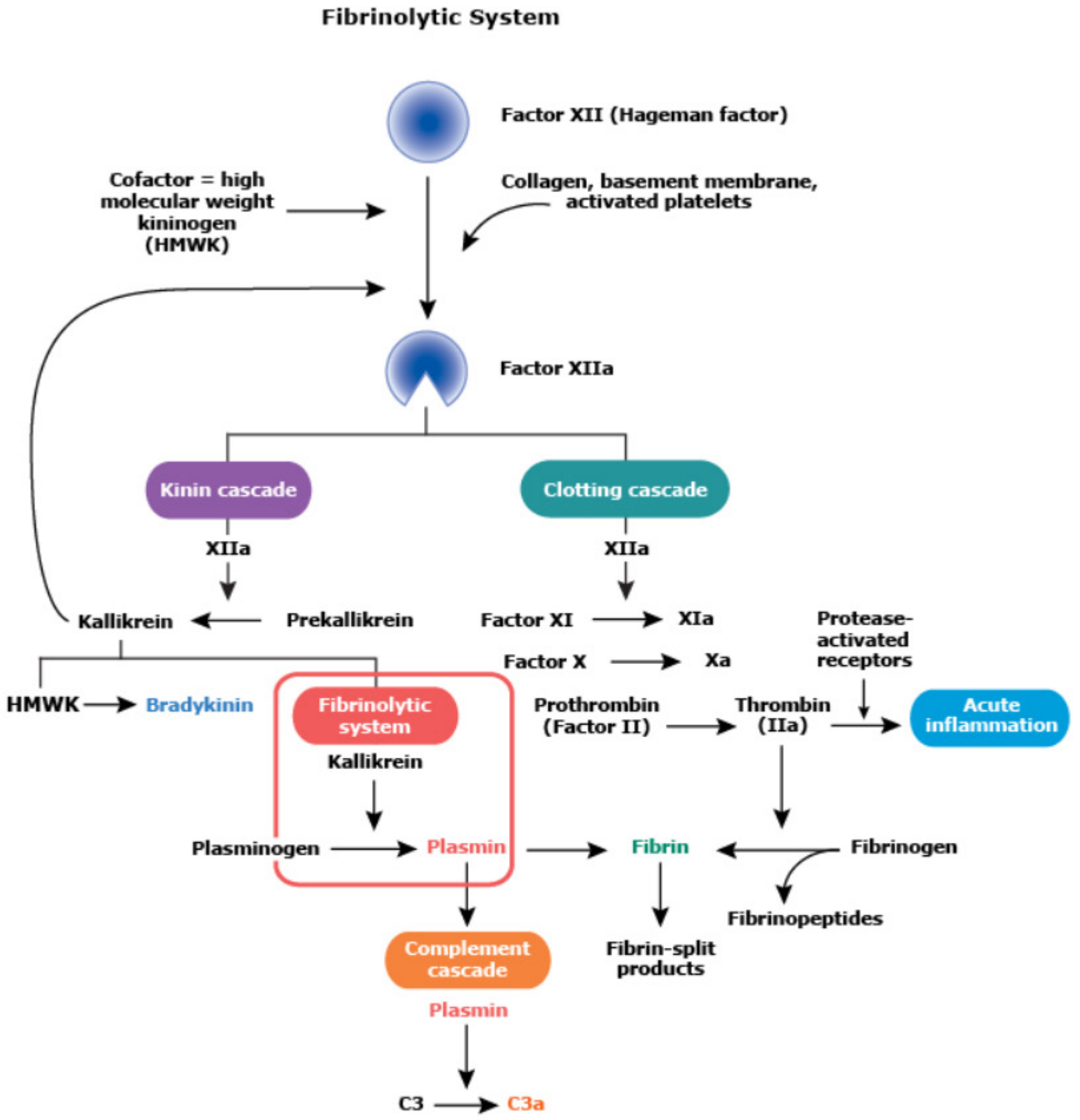
A set of plasma proteins that assist in the lysis of cells in addition to mediating inflammation, phagocytosis, and lysis of microbes.

- There are three pathways to generate the complement cascade from a set of zymogenic serum precursors: classical, alternative, and lectin.
- All pathways generate split products which attract inflammatory cells (C3a, C4a, C5a), encourage opsonization (C3b), and generate membrane lysis (C5-9; membrane attack complex).



▲ **Figure 2–2.3B** Complement Split Products

2.3.6 Coagulation Cascade



▲ **Figure 2–2.3C** Clotting Cascade

1. **Kinin Cascade:** The kinin cascade is activated by factor XIIa (also known as Hageman factor) of the coagulation cascade. This causes high molecular weight kininogen to be converted into *bradykinin*. Bradykinin is a 9 amino-acid peptide that mediates vascular permeability, dilation of the arterioles, and pain.
2. **Complement Activation**
3. **Thrombin:** (Factor IIa) formation
4. **Fibrinolytic System**

2.4 Defects in Acute Inflammatory Response

2.4.1 Defects of the Complement Cascade

- Defects of complement activation
- Defects of complement regulation

Clinical Application

Complement Cascade Pathologies

Deficiencies of Complement Activation Complement deficiencies result in a decreased concentration or functioning of one or more of the components of the complement system. These can cause increased susceptibility to infections, which tend to manifest on the milder side.

- **Defective Formation of MAC:** Patients unable to form an effective membrane attack complex have an increased risk of *Neisseria* bacteremias.

Deficiencies in Complement Regulation

- **Hereditary Angioedema—**C1 inhibitor (C1NH) deficiency: There are inhibitors of complement activation at the level of C1, C3, and C5. Deficiency of the inhibitor of C1 binding causes hereditary angioedema. A deficiency of C1INH causes overactivation of the kinin system, resulting in massive edema due to increased vascular permeability
- **Paroxysmal Nocturnal Hemoglobinuria—**Decay-accelerating factor (DAF) deficiency: DAF is a protein found on the surface of cells that destroys the C3 convertase protein, preventing the activation of C3. A lack of DAF as well as another inhibitor, called *membrane inhibitor of reactive lysis* (MIRL), which inhibits the formation of the membrane attack complex, causes a disease known as *paroxysmal nocturnal hemoglobinuria*.

2.4.2 Defects in Neutrophils

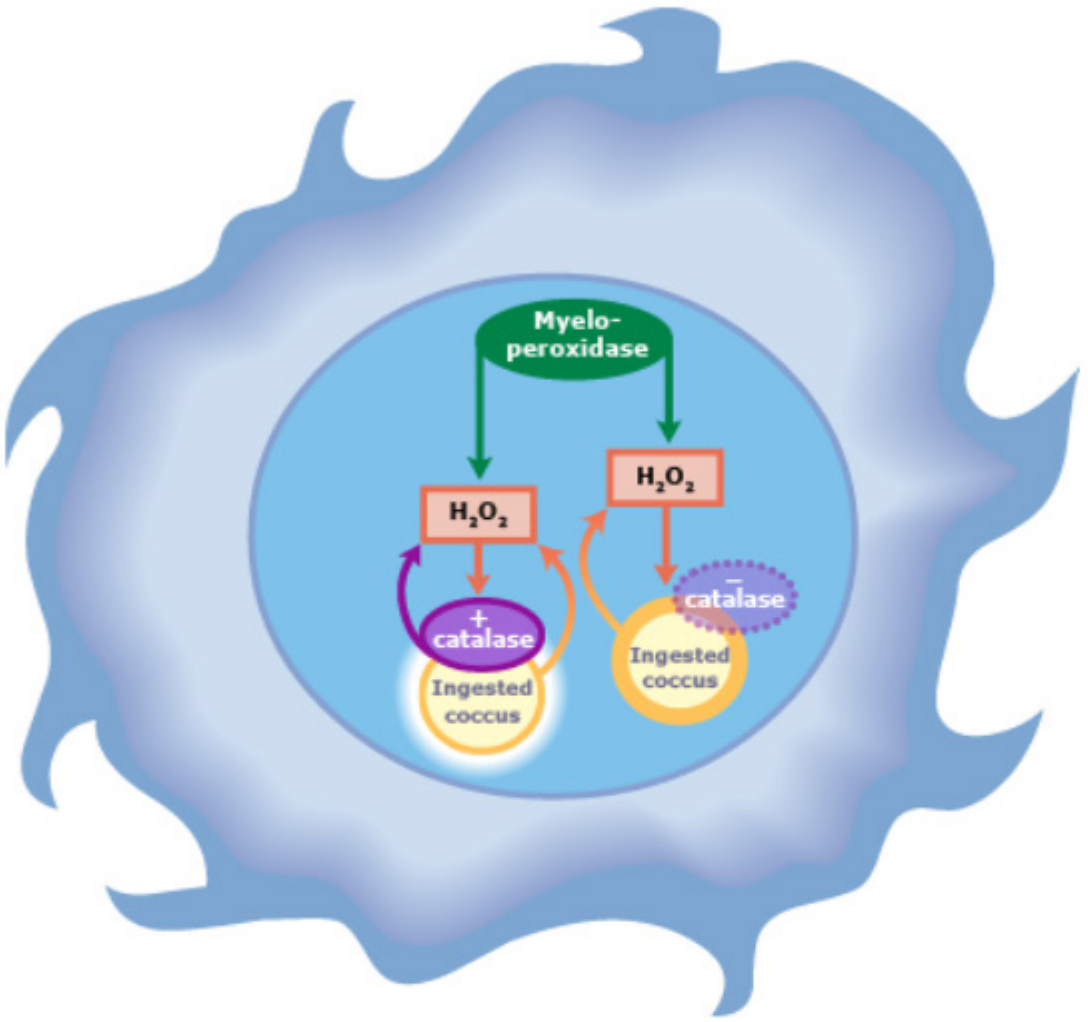
Neutrophilic defects can be due to low neutrophil levels, neutropenia, or impaired neutrophil function, as can be seen with leukocyte adhesion deficiencies and other genetic disorders. Because of the central role of neutrophils in fighting disease, these problems can cause significant susceptibility to infection from extracellular pathogens.

Looking Ahead

Paroxysmal Nocturnal Hemoglobinuria is covered more extensively in chapter 6.

Chronic Granulomatous Disease

- Defect in NADPH oxidase, which prevents the formation of oxygen radicals and hydrogen peroxide (the substrate for myeloperoxidase).
- Catalase positive microbes destroy their own metabolic catalase and therefore cannot be killed by the myeloperoxidase; both oxygen-dependent mechanisms become nonfunctional.



▲ **Figure 2–2.4A** Intracellular Killing in CGD

Memory Aid

Staph '**N** **E**nterobacteriaceae **A**re **L**isted **C**atalase **P**ositive:

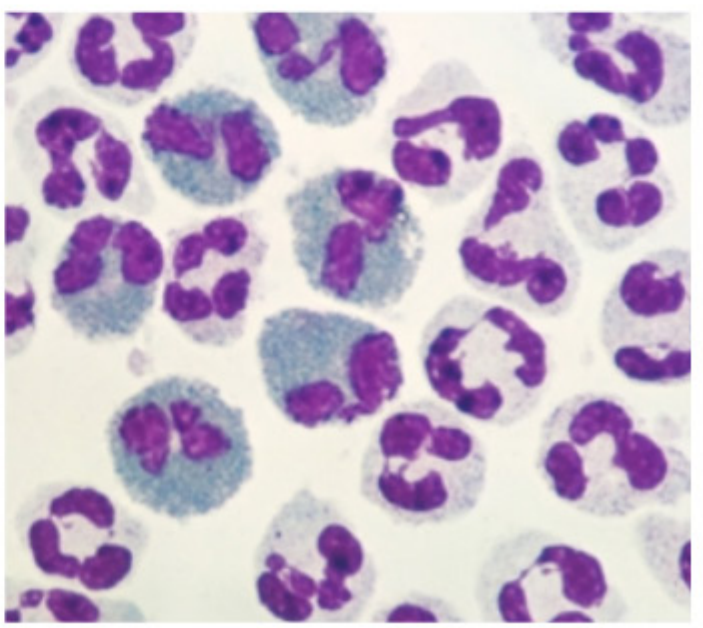
- ***S**taphylococcus*
- ***N**ocardia*
- ***E**nterobacteriaceae*
- ***A**spergillus*
- ***L**isteria*
- ***C**andida*
- ***P**seudomonas*

Myeloperoxidase Deficiency

- Generally asymptomatic or, at most, an increased susceptibility to *Candida* infections.
- As long as one of the two oxygen-dependent mechanisms is functional, the case will be subclinical.

Chédiak-Higashi Syndrome (CHS)

- Autosomal recessive disorder that affects microtubule formation so lysosomal enzymes cannot be transferred into phagocytic vesicles.
- Affects melanocytes (causing albinism) and the cells of the nervous system (resulting in peripheral neuropathy).
- Results in neutropenia, defective granulation, and delayed killing of microbes. Large cytoplasmic vacuoles appear in phagocytes and natural killer cells as defective granules accumulate.



Dr. Cecil H. Fox/Science Source

▲ **Figure 2–2.4B** Phagocyte in CHS

3 Chronic Inflammation

When the acute inflammatory response is insufficient to remove or heal an injury, progression to chronic inflammation may occur.

3.1 Causes of Chronic Inflammation

In general, chronic inflammation results from the persistence of injury or infection:

- **Infections:** Tuberculosis, leprosy, hepatitis C
- **Autoimmune Disease:** Rheumatoid arthritis, Crohn disease
- **Foreign Sterile Agents:** Breast implants, silica, uric acid

3.2 Morphology of Chronic Inflammation

Chronic inflammation occurs when there is simultaneous tissue damage due to active inflammation coupled with attempts at tissue repair. Chronic inflammation has a somewhat different inflammatory cell profile than acute inflammation.

3.2.1 Mononuclear Cell Infiltration

The main inflammatory cells involved in chronic inflammation are mononuclear cells. These include lymphocytes, macrophages, and plasma cells.

3.2.2 Tissue Destruction and Fibrosis

Ultimately, chronic inflammation can cause permanent tissue damage and resulting fibrosis.

3.2.3 Granulomatous Inflammation

A possible consequence of chronic inflammation, a granuloma, which possesses a unique cellular profile.

- **Macrophages:** These are the predominant cell found in granulomatous inflammation. They are transformed into epithelioid cells and are also called *epithelioid* histiocytes because of their epithelial appearance and highly secretory activity. This transformation is induced by the cytokines of Th1 lymphocytes, which serve as the organizer of the lesion through secretion of IFN-γ.
- **Caseating vs. Non-caseating:** The two main forms of granulomas are *caseating* and *non-caseating*. Caseating granulomas have areas of central, "cheesy" necrosis, which results from the growth of the lesion beyond the capacity of the blood supply.
- **Granuloma Triggers:** Chronic infection or the presence of foreign bodies triggers granuloma formation.

CHAPTER 3

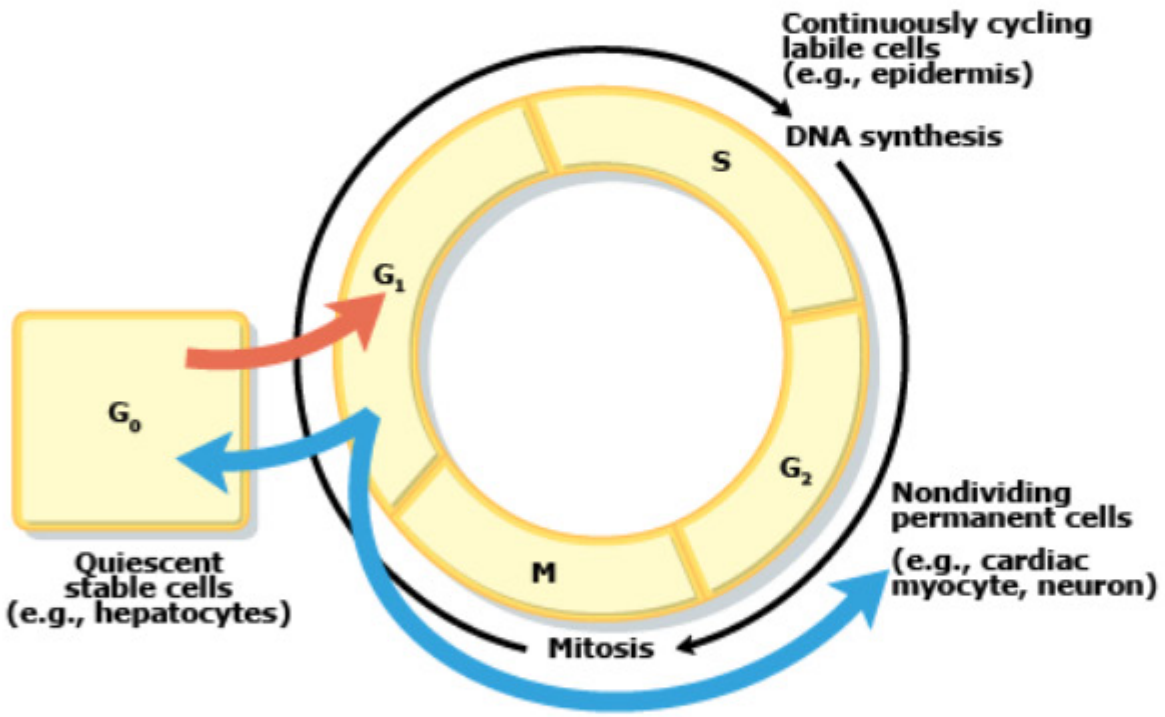
Tissue Repair and Wound Healing

1 Overview of Regeneration and Repair

When cells and tissues are injured, recovery is accomplished via regeneration or repair. Regeneration requires replicative cells to replace dead cells, and repair involves the deposition of collagen, new connective tissue, and scar formation.

2 Repair by Regeneration

2.1 The Cell Cycle and Cell Types



▲ **Figure 3–2.1** Cell Cycle

- **Labile Cells:** *Regenerate perpetually*
  - Stem cells: Embryonic and adult
  - Bone marrow cells
  - Surface epithelial cells
  - Intestinal crypts
- **Stable (Quiescent) Cells:** *Divide if stimulated* (TNF-α and IL-6 important for G_0/G_1 transition)
  - Hepatocytes
  - Endothelium
  - Renal tubular cells
- **Permanent Cells:** *Unable to divide*
  - Neurons
  - Cardiac myocytes

USMLE® Key Concepts

For Step 1, you must be able to:

- Identify the stages of wound healing and predict their outcome in specific tissues.
- Explain the mechanisms of healing by first and second intention.
- Diagnose the cellular and molecular basis of defects of wound healing.

2.2 Growth and Regeneration Signals

2.2.1 Platelet-Derived Growth Factor (PDGF)

- Produced by many cells, especially platelets.
- Attracts and causes *fibroblasts and smooth muscle cells* to proliferate.

2.2.2 Epidermal Growth Factor (EGF)

This is a progression factor for endothelial cells and fibroblasts.

2.2.3 Fibroblast Growth Factor (FGF)

- Promotes synthesis of extracellular matrix proteins.
- Attracts *fibroblasts and endothelial cell*.

2.2.4 Transforming Growth Factor (TGF)

- TGF-α is like EGF.
- TGF-β is a *growth inhibitor*.

2.2.5 Vascular Endothelial Growth Factor (VEGF)

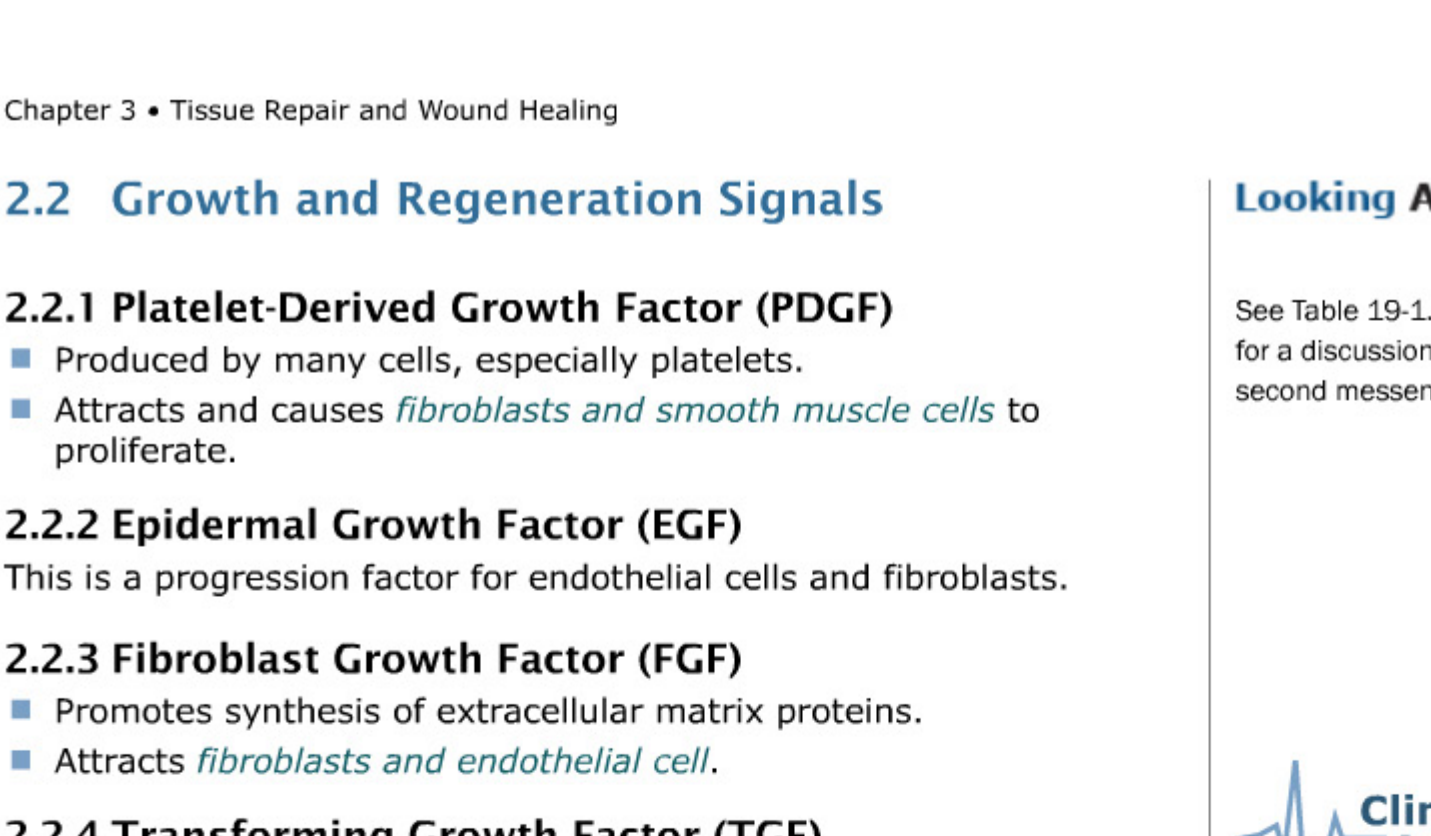
Stimulates angiogenesis, the formation of new blood vessels from existing vessels.

Looking Ahead

See Table 19-1.0A in chapter 19 for a discussion of where all the second messenger goes.

Clinical Application

In diabetic retinopathy neovascularization caused VEGF results in retinal detachment.



▲ **Figure 3–2.2** Growth and Regeneration Signals

2.3 Fibrosis and Remodeling

When injury is severe or persistent, the damaged area must be replaced with a connective tissue scar. If the damage is such that there is loss of the basement membrane and its accompanying connective tissue infrastructure, then full regeneration of function is not possible.

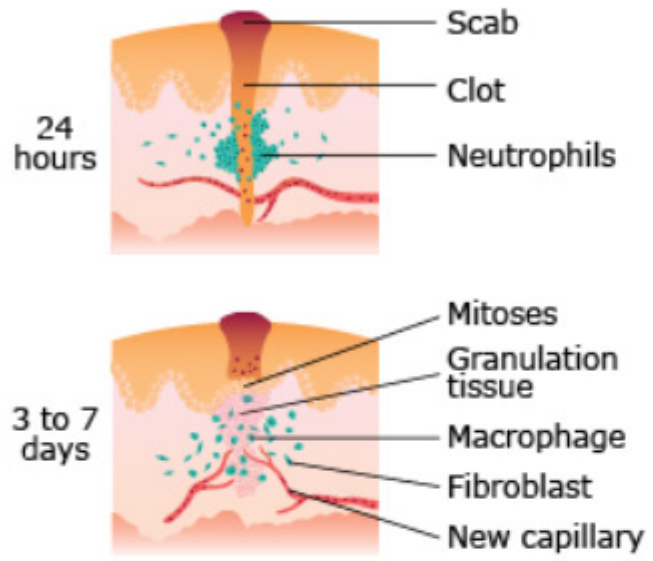
Steps in repair include:

- Neutrophils transmigrate to liquefy injured tissue, and macrophages transmigrate to remove debris.
- *Granulation tissue* composed of fibroblasts and angiogenesis accumulates in the extracellular matrix.
- Fibroblasts produce type III collagen initially.
- Remodeling by metalloproteinases (collagenases) replaces type III collagen with type I collagen, increases tensile strength of tissue to approximately 80% of the original tissue.

3 Types of Wound Healing

Wound healing is defined as occurring via *primary intention (primary union)* when wound edges are closely approximated and the process results in a minimal scar. Wound healing by *second intention (second union)* occurs when wounds are larger or the ends of the wound are not in contact.

3.1 Healing by Primary Intention



Day 1:
- Fibrin clot (hematoma)
- Neutrophils infiltrate

Day 2:
- Squamous cells seal off wound
- Macrophages emigrate into wound

Day 3:
- Granulation tissue begins to form
- Initial deposition of type III collagen
- Macrophages replace neutrophils

Days 4–6:
- Peak granulation tissue formation
- Fibronectin key glycoprotein

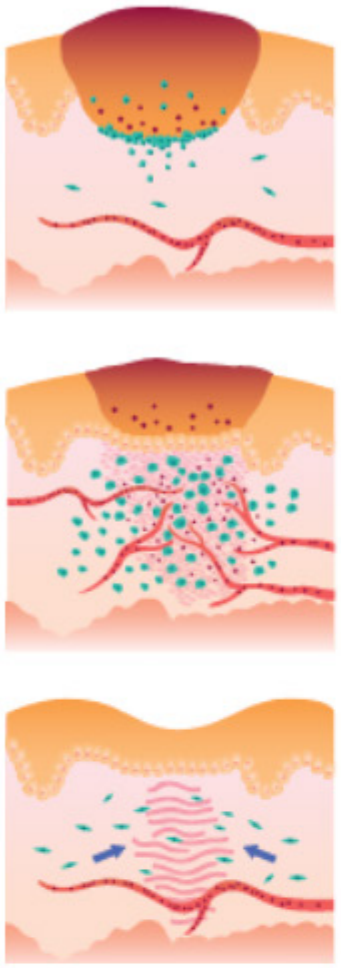
Week 2:
- Tensile strength ~10%

Month 1:
- Remodeling of wound (collagenase/lysyl oxidase)
- Tensile strength ~80% in 3 months

▲ **Figure 3–3.1** Healing by Primary Intention

3.2 Healing by Second Intention

Wounds that are more extensive result in more intense inflammatory reactions and more granulation tissue production. Myofibroblasts will cause wound contraction and more significant residual scarring.



- Increased deposition of granulation tissue along with myofibroblasts arriving
- Angiogenic factors such as VEGF arriving to increase blood supply to the injured area
- Increased deposition of collagen leads to the formation of scar tissue and thus wound contraction

▲ **Figure 3–3.2** Healing by Secondary Intention

Connection to Pharmacology

- Glucocorticoids interfere with collagen formation and decrease tensile strength, so they are used clinically to prevent excessive scar formation.
- Dexamethasone and antibiotics are used to prevent granulation tissue and scar formation in bacterial meningitis.

Clinical Application

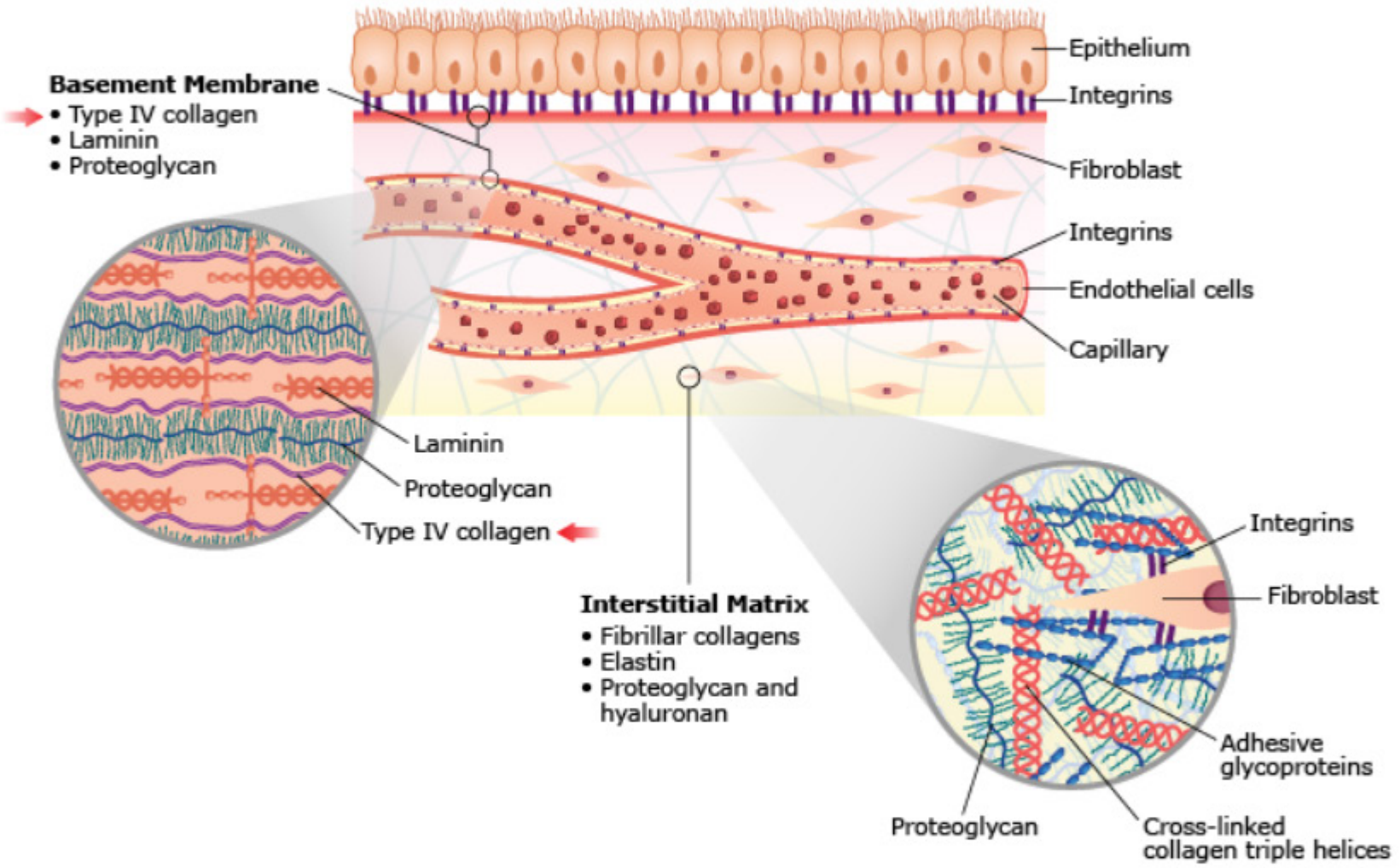
Conditions Causing Delayed Wound Healing

- Persistent infection (e.g., *Staphylococcus aureus*)
- Metabolic disorders (e.g., diabetes mellitus may cause ischemia and raise tissue levels of glucose, creating an ideal environment for infections)
- Nutritional factors:
  - Kwashiorkor resulting from severe protein malnutrition
  - Vitamin C deficiency resulting in decreased cross-linking of tropocollagen and reduction of tensile strength
  - *Trace metal deficiencies*
    - Copper deficiency leads to decreased cross-linking in collagen
    - Zinc deficiency results in decreased metalloproteinase activity and remodeling

4 Extracellular Matrix

An extracellular matrix is a locally secreted network of structural molecules that consists of:

- **Interstitial Matrix**
  - Fibrillar collagens
  - Proteoglycans
  - Hyaluronic acids
- **Basement Membrane**
  - Collagen IV
  - Laminin



▲ **Figure 3–4.0** Extracellular Matrix: Collagen

▼ Table 3–4.0 Collagen Types

Group	Type	Structures
Fibrillar	I	Hard (*bone* and cartilage) and soft tissues (skin)
	II	*Cartilage*
	III and V	Vessels and skin
Basement membrane	IV	*Basement membranes*

Clinical Application

Vitamin C Deficiency (Scurvy)

- Children: Hemorrhage (purpura and ecchymoses), joint space bleeding, and bone disease
- Adults: Hemorrhage and healing defects

Connection to Biochemistry

Collagen

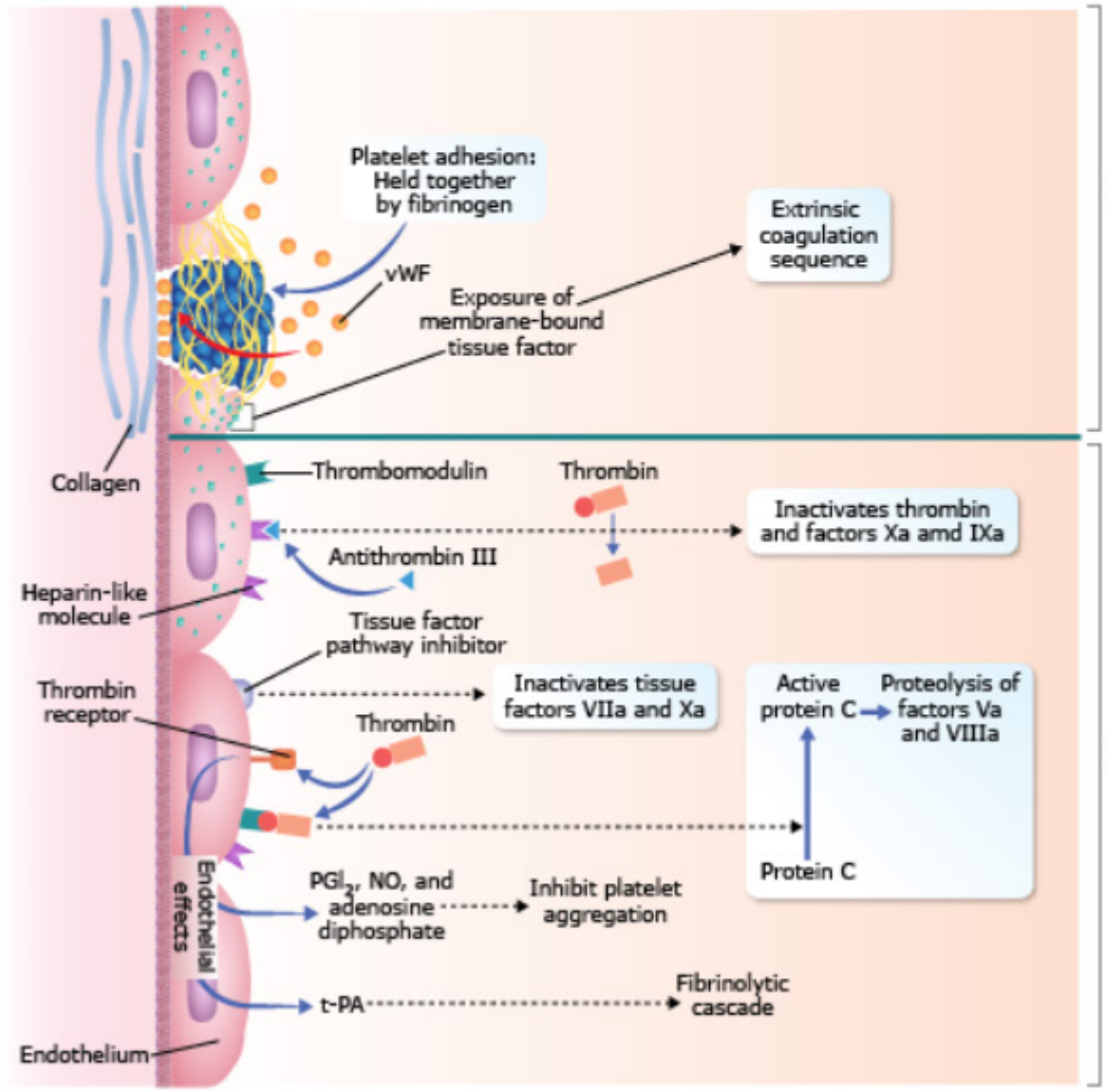
- Triple helix of three polypeptide alpha chains with gly-x-y repeats.
- Collagen chains produced in the cell form triple helix called procollagen.
- Procollagen secreted from the cell and cross-linked to form collagen.
- Vitamin C activates *prolyl* and *lysyl hydroxylases* from inactive precursors, which then hydroxylate procollagen, providing strength.

1 Normal Vascular Hemostasis

Normal vascular hemostasis results from the action of carefully regulated processes that maintain the blood as a clot-free fluid in normal vessels and yet allow the formation of a rapid hemostatic plug at a site of injury. The pathologic consequence of hemostasis is referred to as thrombosis.

1.1 Contributors to Hemostasis

- Endothelium
- Clotting cascade
- Platelets



▲ **Figure 4–1.1** Thrombotic Hemostasis

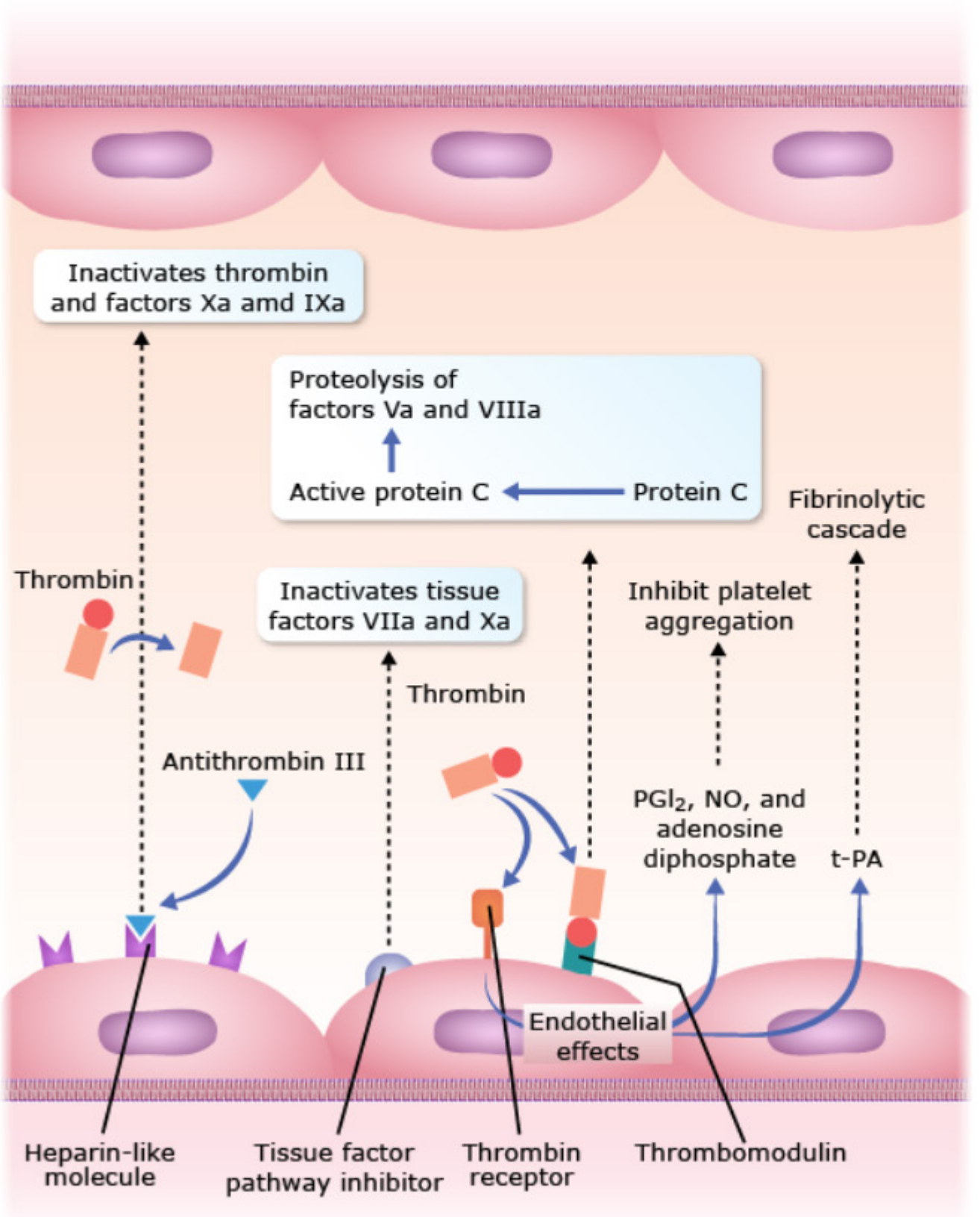
USMLE® Key Concepts

For Step 1, you must be able to:

- Describe the physiologic mechanisms controlling hemostasis.
- Explain the causes of thrombosis (Virchow triad) and its sequela (embolism and infarction).
- Diagnose and describe the molecular basis of the diseases of hypercoagulability.
- Identify the types of emboli and their pathologic sequelae.
- Diagnose cases of hyperemia, congestion, and edema.

1.2 Antithrombotic Activities of Endothelial Cells

- Produce heparin-like molecules that bind and activate antithrombin III, which neutralizes thrombin (factor IIa) and coagulation factors IXa and Xa.
- Produce tissue plasminogen activator (t-PA).
- Inactivate and remove thrombin.
- Synthesize thrombomodulin, which activates protein C.
- Synthesize protein S, PGI_2, and nitric oxide.



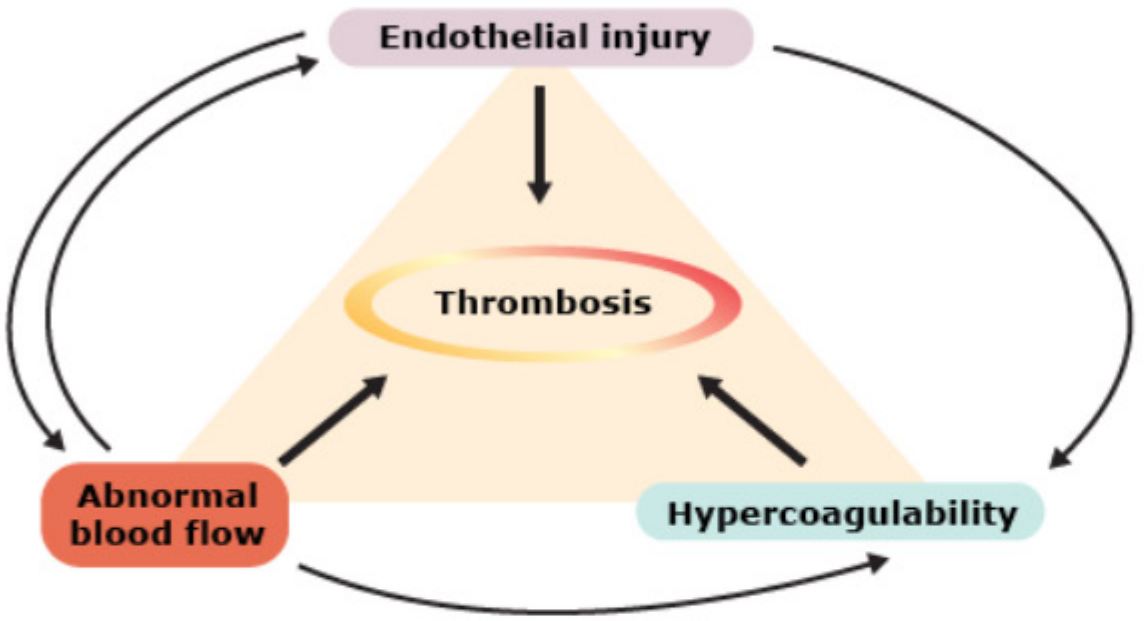
▲ **Figure 4–1.2** Antithrombotic Activities of Endothelial Cells

2 Hemodynamic Dysfunction

Disturbances of the delicate balance of vascular hemostasis can result in thrombosis, embolism, infarction, hyperemia, congestion, edema, and shock.

2.1 Thrombosis

Trauma and inflammation of endothelial cells often begin the process of thrombosis, or intravascular coagulation of blood. Thrombogenesis can result from the activity of three possible stimuli (Virchow triad): endothelial injury, hypercoagulability, and abnormal blood flow.



▲ **Figure 4–2.1A** Thrombogenesis

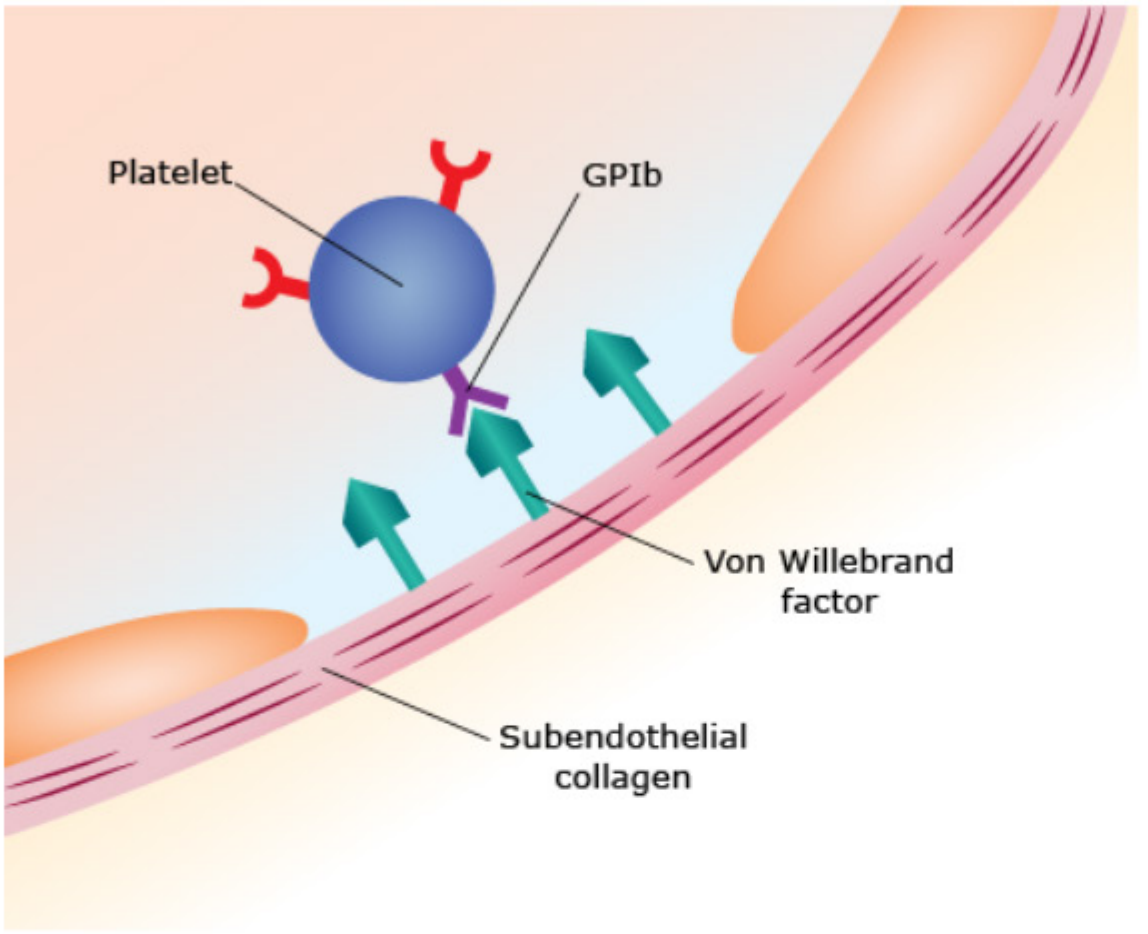
2.1.1 Endothelial Injury

Causes of endothelial injury include:

- Hypertension
- Turbulent flow over scarred valves
- *Bacterial endotoxins*
- Homocystinuria
- Hypercholesterolemia
- Radiation
- Cigarette smoke chemicals

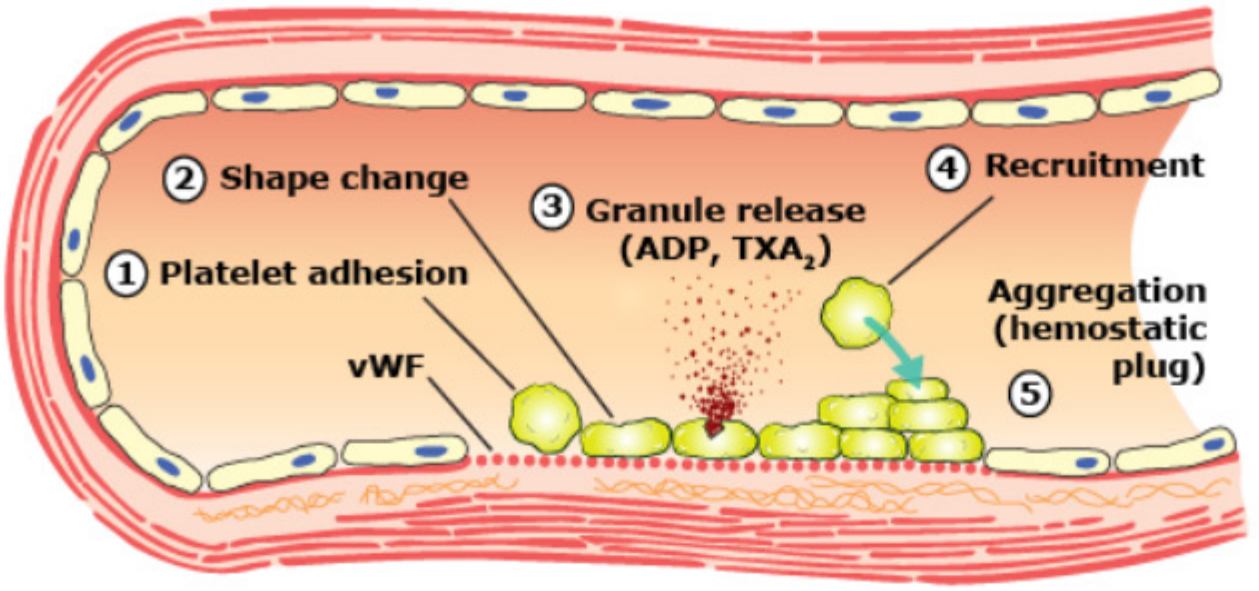
The Function of Platelets Platelets contribute to thrombogenesis through a three-step process involving adhesion, the release reaction, and aggregation.

- **Platelet Adhesion**
  - Vascular endothelial damage exposes subendothelial collagen causing platelet *adhesion* to the surface.
  - *Von Willebrand factor* mediates the interaction between platelet surface glycoprotein receptors (*GPIb*) and subendothelial collagen.



▲ **Figure 4–2.1B** Platelet Adhesion

- **The Release Reaction:** Once platelets are fixed to the subendothelial collagen, platelets release.
  - **Dense Granules**
    - —Adenosine diphosphate (ADP)
    - —Serotonin
    - —Calcium and magnesium
    - —Cyclooxygenase
  - **Alpha Granules**
    - —Hemostatic factors (Factor V, plasminogen activator inhibitor 1, von Willebrand Factor, fibrinogen)
    - —Angiogenic factors (vascular endothelial growth factor)
    - —Anti-angiogenic factors (platelet factor 4)
    - —High molecular weight kininogen (HMWK)
    - —Platelet-derived growth factor
    - —Fibroblast growth factor



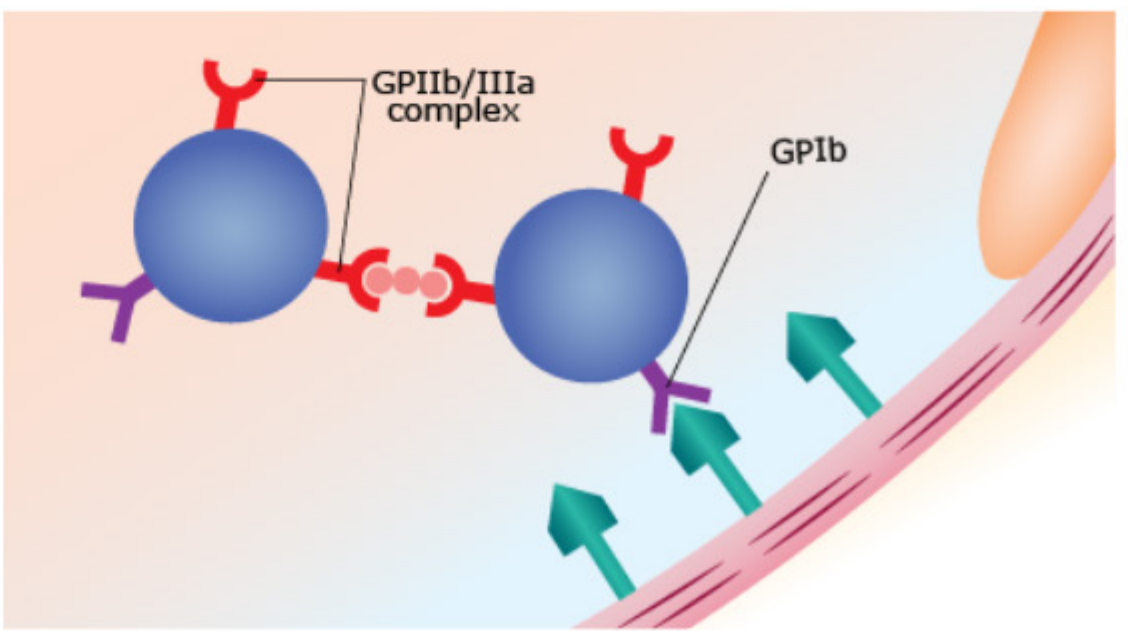
▲ **Figure 4–2.1C** Primary Hemostasis

- **Aggregation:** Substances secreted during the release reaction cause platelets to stick together, forming the primary platelet plug. Aggregation is facilitated by the glycoprotein IIb-IIIa complex on the surface of platelets.
  - Binds fibrinogen and links platelets
  - Fibrinogen bridges stabilize the platelet plug
  - Controlled by the *arachidonic acid cascade:*
    - —Platelet phospholipase produces arachidonic acid through the cyclooxygenase pathway, which produces thromboxane A_2 (TxA_2).
    - —Platelet TxA_2 constricts blood vessels and causes platelet aggregation.
    - —Endothelial prostacyclin (PGI_2) blocks platelet TxA_2 and limits further platelet aggregation.
  - Other stimuli for platelet aggregation:
    - —Thrombin
    - —Platelet-activating factor (PAF)
    - —ADP
    - —Collagen
    - —Epinephrine

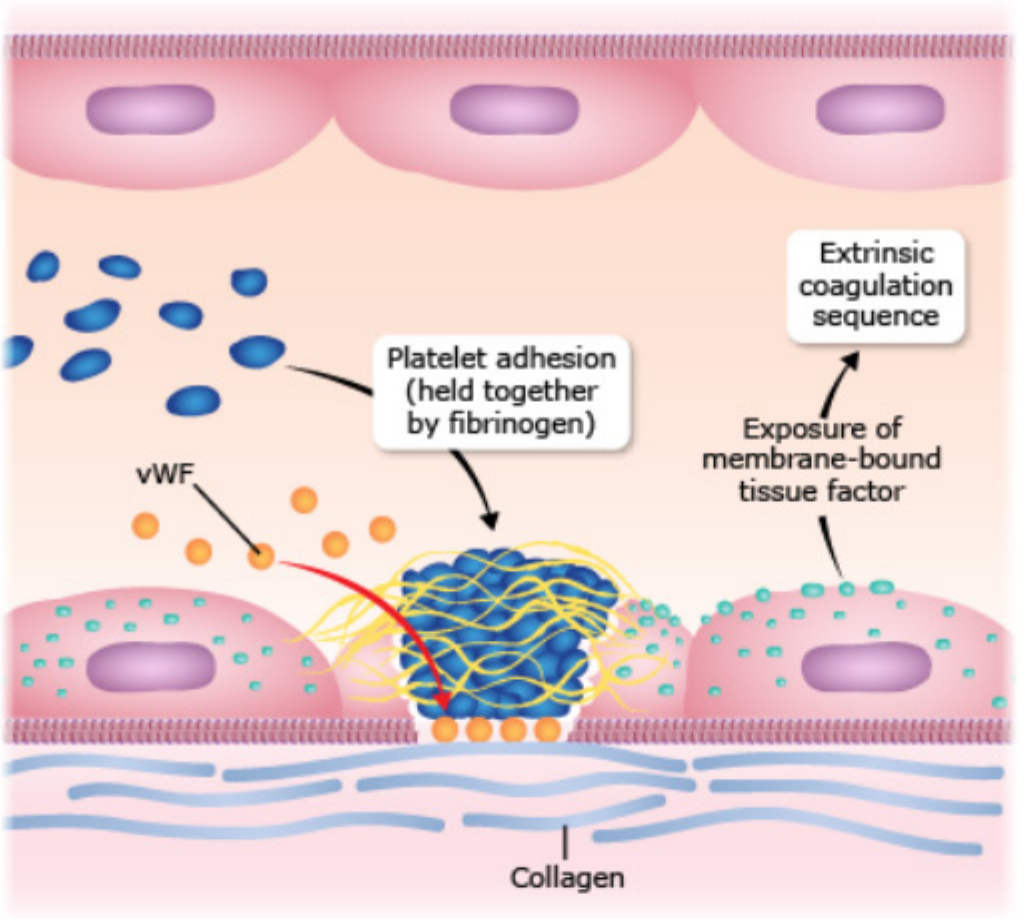
Clinical Application

Aspirin

The production of thromboxane A_2 via the conversion of arachidonic acid by the enzyme cyclooxygenase-1 is inhibited by low-dose aspirin for the entire life span of the platelet, which is about 7 to 10 days.



▶ **Figure 4–2.1D** Aggregation

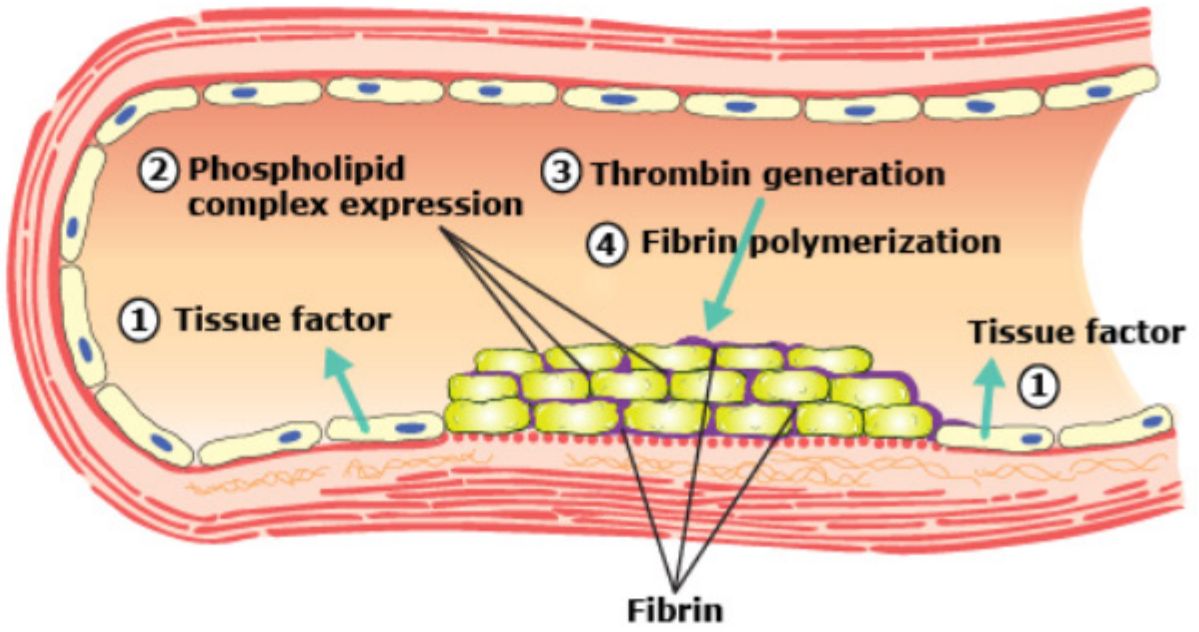


▲ **Figure 4–2.1E** Primary Hemostasis

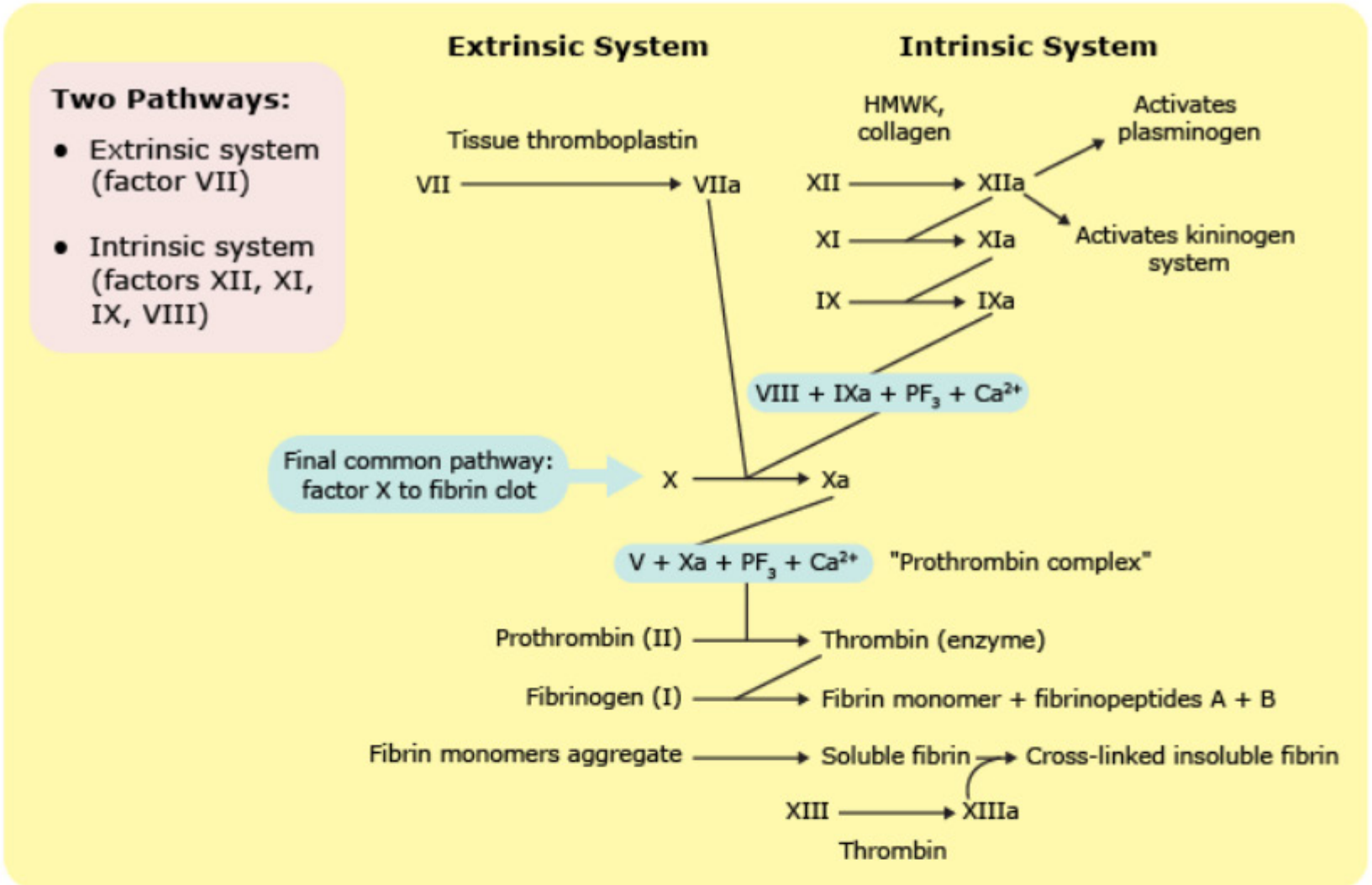
Other Functions of Platelets

- Activation of *arachidonic acid metabolism*.
- Activation of the *coagulation cascade*, which enhances the primary hemostatic plug (platelets).
- Platelets expose a *phospholipid complex*, which activates the intrinsic coagulation cascade, producing thrombin.
- Thrombin converts fibrinogen to fibrin.
- *Fibrin polymerization forms the secondary platelet plug.*

Pathways of the Coagulation Cascade There are two major pathways of activation of the coagulation cascade: The extrinsic system, which begins with tissue thromboplastin activating factor VII; and the intrinsic system, which begins with the activation of factors IX, XI, or XII by HMWK or collagen. Both pathways feed together at the level of factor X to finally produce the fibrin clot.



▶ **Figure 4–2.1F** Coagulation Cascade



▲ **Figure 4–2.1G** Extrinsic and Intrinsic Pathways

- **Extrinsic Pathway**
  - The extrinsic pathway of coagulation begins when factor VII is converted to factor VIIa by *tissue thromboplastin (tissue factor)*.
  - Factor VIIa converts factor X to Xa and converts factor IX to factor IXa.
  - Factor Xa then converts prothrombin (factor II) to thrombin (factor IIa).
  - Factor Va is a cofactor required in the conversion of prothrombin to thrombin.
  - Thrombin converts fibrinogen to fibrin.
  - Cleavage of fibrinogen creates a fibrin monomer, which is stabilized by factor XIII, and forms a fibrin clot.
  - The laboratory test of the extrinsic pathway is *prothrombin time (PT)*.
  - This measures factors II, V, VII, X, and fibrinogen.

- **Intrinsic Pathway**
  - The intrinsic pathway of coagulation involves the activation of all clotting factors, except factors VII (extrinsic pathway) and XIII (common pathway).
  - Factor XII (Hageman factor) is activated by:
    —Exposed subendothelial collagen
    —High molecular weight kininogen (HMWK)
  - It may also be initiated by factor VIIa (from the extrinsic pathway), activating *factor IX* to *IXa*.
  - Factor IXa leads to the conversion of factor X to factor Xa, catalyzed by *factor VIIIa*.
  - The intrinsic pathway also is activated by the *platelet phospholipid complex*.
  - The laboratory test of the intrinsic pathway is *partial thromboplastin time (PTT)*.
  - This measures factors II, V, VIII, IX, X, XI, XII, and fibrinogen.

Fibrinolysis Lysis of the vascular thrombus, which restores blood flow in occluded vessels.

- *Plasminogen* (a proenzyme) is converted to *plasmin*, a fibrinolytic protease. Plasmin then splits fibrin.
- The conversion of factor XII to XIIa initiates the fibrinolytic, coagulation, complement, and kinin systems.

Lab Tests for Bleeding Dyscrasias

- **Platelets**
  - Bleeding time
  - Platelet count
- **Coagulation Proteins**
  - PTT (intrinsic pathway; used to monitor heparin therapy)
  - PT (extrinsic pathway; used to monitor warfarin therapy)
- **Fibrinolytic System:** Fibrin split products (D-dimers)

Connection to Microbiology

Hageman factor (factor XII, intrinsic pathway) is also activated by lipid A, the toxic moiety in gram-negative bacterial endotoxin. In endotoxin shock, the paradoxical combination of disseminated intravascular coagulation in conjunction with hemorrhage usually precipitates death.

Clinical Application

▼Table 4-2.1A Use of Bleeding Parameters in Diagnosis

Circumstance	Platelet Count	Bleeding Time	PT	PTT
Aspirin	Normal	↑	Normal	Normal
Thrombocytopenia	↓	↑	Normal	Normal
VWD	Normal	↑	Normal	↑
Hemophilia A	Normal	Normal	Normal	↑
DIC	↓	↑	↑	↑
Warfarin/heparin	Normal	Normal	↑	↑

▼Table 4-2.1B Diagnostic Parameters of Bleeding

Circumstance	Platelet Dysfunction	Coagulation Factor Deficiency
Bleeding superficial scratches	Yes	No
Petechia	Yes *(only thrombocytopenia, not aspirin)*	No
Late rebleeding	No	Yes
Hemarthroses	No	Yes *(only very severe facator deficiencies)*
Epistaxis, menorrhagia, GI/GU bleeding, hematuria, easy bruising	Yes	Yes
Ecchymoses/purpura	Yes	Yes

3 Diseases of Hypercoagulability

3.1 Primary Thrombophilia

Primary hypercoagulable states generally result from mutations affecting the factor V, prothrombin genes.

3.1.1 Hereditary Thrombophilia

- Prothrombotic familial syndrome in young women.
- Lack of antithrombotic proteins (antithrombin III, protein C, and protein S).

3.1.2 Factor V Leiden

- Mutation resulting in substitution of glutamine for normal *arginine* at position 506.
- Renders activated factor V protein *resistant to cleavage by protein C*.
- *Most common cause* of hereditary thrombophilia.

3.1.3 Prothrombin 20210A

- G-to-A mutation in the 3'-untranslated region of prothrombin gene.
- *Second most common cause* of hereditary thrombophilia.

3.2 Secondary Hypercoagulability

Many diseases that affect blood flow or stasis will result in secondary increases in the coagulatory cascades.

3.2.1 Oral Contraceptive Use and Pregnancy

- Increased synthesis of coagulation factors.
- Reduced synthesis of antithrombin III.

3.2.2 Cancer

Cancer creates tumor products that can lead to thrombosis.

3.2.3 Advancing Age

- Increased platelet aggregation.
- Reduced endothelial PGI_2 release.

3.2.4 Smoking and Obesity

The association of hypercoagulability with smoking and obesity is suspected but not yet clinically confirmed.

3.2.5 Antiphospholipid Antibody Syndrome

- Prothrombotic disorder with autoantibodies against protein antigens complexed to phospholipids.
- Clinical Features:
  - Recurrent venous and arterial *thromboembolism, fetal loss,* thrombocytopenia, and neurologic manifestations.
  - Paradoxical prolonged PTT; still treat with coumadin.
  - *False positive test for syphilis* (antigens in test are bound to cardiolipin).
  - Sometimes *associated with SLE* (hence, also referred to as lupus anticoagulant).

3.3 Abnormal Blood Flow

The final contributor to the Virchow triad is disturbance of blood flow. Stasis and turbulence are alterations in normal blood flow that contribute to thrombus formation. Causes include:

- Ulcerated atherosclerotic plaques
- Aneurysms (cause local stasis)
- Myocardial infarctions
- Mitral valve stenosis
- Atrial fibrillation
- Polycythemia
- Sickle cell anemia

4 Thrombosis

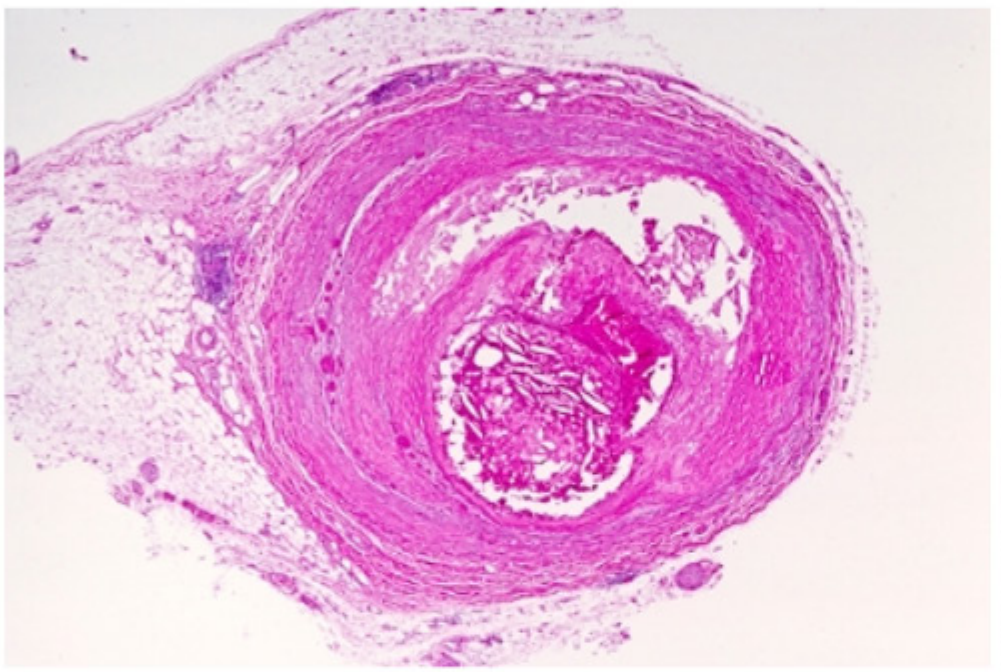
A thrombus is an intravascular clot composed of coagulation factors, erythrocytes, and platelets that is attached to the vessel wall. Thrombi may develop in arteries or veins. Thrombi are formed in flowing blood (in vivo) and will develop with alternating layers of light and dark (lines of Zahn). The pale layers consist of platelets and fibrin, and the darker layers consist of RBC and fibrin.

4.1 Arterial or Cardiac Thrombi

Thrombi of this sort develop due to endothelial injury or turbulence and tend to grow "backward."

4.2 Venous Thrombi

These thrombi tend to develop at sites of stasis and grow "forward."



Carolina Biological Supply Company/Phototake, Inc.

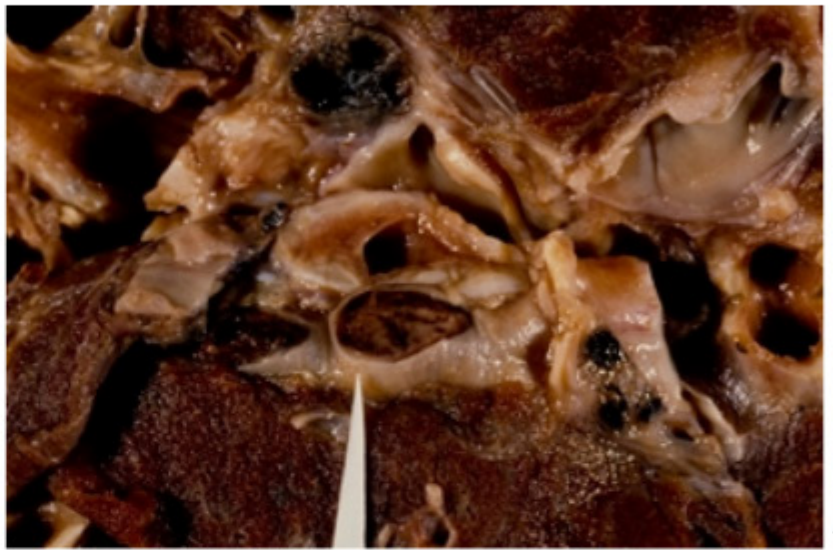
▲ **Figure 4–4.2** Arterial Thrombus

5 Embolism

An embolus is a detached mass that travels through the vasculature to a distant site where it eventually becomes trapped. Most emboli are formed when thrombi on vascular walls detach and are washed downstream, where they lodge and block blood flow. Emboli may be arterial (from mural thrombi), pulmonary (from venous thrombosis), or due to bubbles of gas, fat, or amniotic fluid.

5.1 Arterial Emboli

- Arterial emboli generally develop from detached mural thrombi from the heart or a large vessel. Common causes include:
  - Atrial fibrillation
  - Status post infarction
- Common arterial emboli scenarios:
  - Carotid artery and infarct of middle cerebral artery
  - Mesenteric artery and hemorrhagic infarct of the intestinal tract
  - Renal artery and pale wedge infarct of renal cortex



Martin Rotker/Phototake, Inc.

▲ **Figure 4–5.1** Saddle Embolus

5.2 Pulmonary Emboli

- Immobilization and venous stasis lead to *venous thrombosis* in the lower extremities.
- Portions of the thrombus break away, travel through the venous circulation, and lodge in branches of the pulmonary artery.
- These frequently occur in immobilized hospitalized patients.
- *Saddle emboli* can obstruct the pulmonary artery and cause sudden death.

5.3 Gas Emboli

These emboli, the cause of *decompression sickness*, are the result of nitrogen gas dissolving in tissue under pressure. Rapid ascent causes nitrogen bubbles to form in vessels and tissue, producing ischemic damage.

5.4 Fat Emboli

Trauma releases fat from bone marrow and globules of fat block the microvasculature of brain, lungs, and kidneys.

5.5 Amniotic Fluid Emboli

Tears in placental membranes or uterine veins can infuse amniotic fluid into the maternal circulation. Fetal squames/vernix may be found in the maternal pulmonary circulation. Amniotic fluid emboli carry 60% to 80% maternal mortality rates.

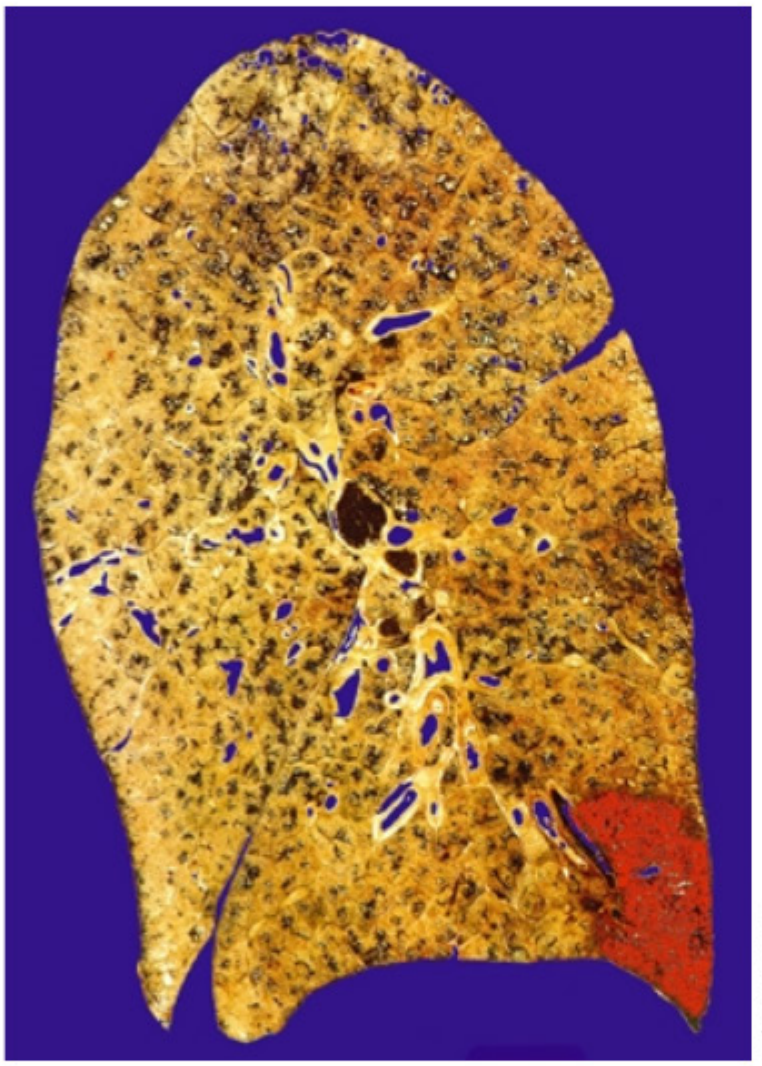
6 Infarction

An infarction is an area of ischemic necrosis caused by occlusion of the arterial or venous system, usually due to thrombotic or ischemic events.

6.1 Red Infarct (Hemorrhagic Infarct)

Tissues with overlapping circulation:

- Typically in lung, liver, and GI tract—redundant blood supplies.
- Arterial occlusion with cell death, followed by perfusion from overlapping circulation.
- Venous occlusions.
- Loose tissues (blood collects).
- Often wedge-shaped.



ISM/Phototake, Inc.

▲ **Figure 4–6.1** Red Infarction

6.2 White Infarct

- Anemic infarct.
- Solid tissue with occlusion of single blood supply.
- Classic example of ischemic coagulative necrosis.

7 Hyperemia, Congestion, and Edema

7.1 Hyperemia

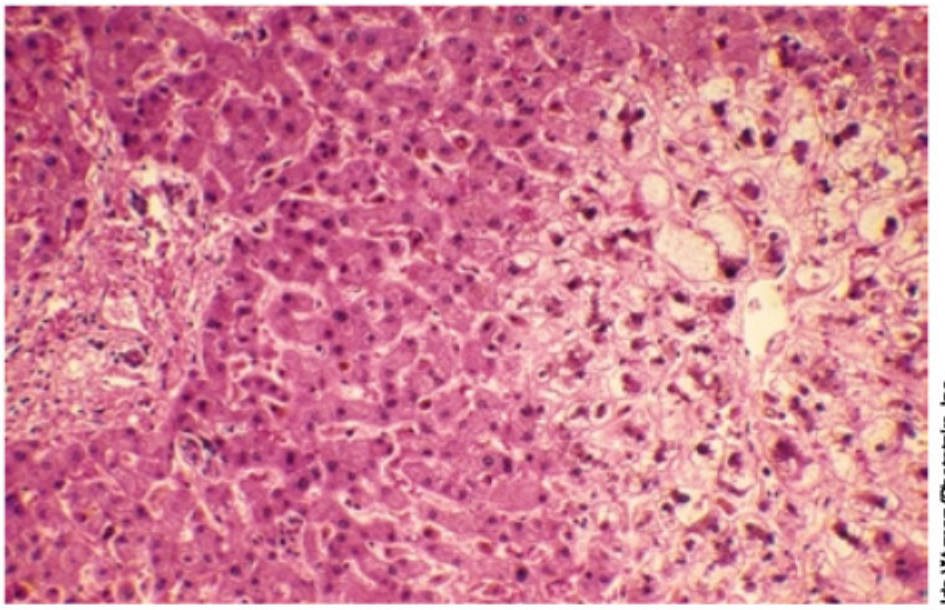
Hyperemia is an active process whereby increased blood flow into an area results from arteriolar dilation. It occurs during inflammation and exercise. The affected tissue will be red with oxygenated blood.

▲ **Figure 4–7.1** Hyperemia

7.2 Congestion

Congestion is a passive process whereby impaired venous return from the tissues prevents normal perfusion of the tissue. It happens systemically in cardiac failure or locally when there is isolated venous obstruction. The tissue will be blue (cyanotic).

▲ **Figure 4–7.2A** Congestion



Ida Wymary/Phototake, Inc.

▲ **Figure 4–7.2B** Centrilobular Necrosis Resulting From Congestive Heart Failure

7.3 Edema

Edema is defined as the presence of increased fluid in the interstitial spaces. Anasarca refers to generalized edema, and ascites refers to edema of the peritoneal cavity.

7.3.1 Types of Edema Fluid

- Transudate
- Exudate
- Lymphedema
- Myxedema

7.3.2 Causes of Edema

- **Increased hydrostatic pressure:** Exemplified by CHF
  - Right heart failure: *Peripheral edema*
  - Left heart failure: *Pulmonary edema*
- **Decreased oncotic pressure:** Secondary to hypoalbuminemia
  - Oncotic protein loss in *nephrotic syndrome*
  - Decreased albumin (oncotic protein) production with *cirrhosis of the liver and liver failure*
- **Increased capillary permeability:** Secondary to inflammation or injury to capillary endothelium

8 Shock

Shock results when reduced perfusion of tissue results in impaired oxygenation of tissue. The types of shock are defined by their cause: hypovolemic, cardiogenic, and septic.

8.1 Hypovolemic Shock

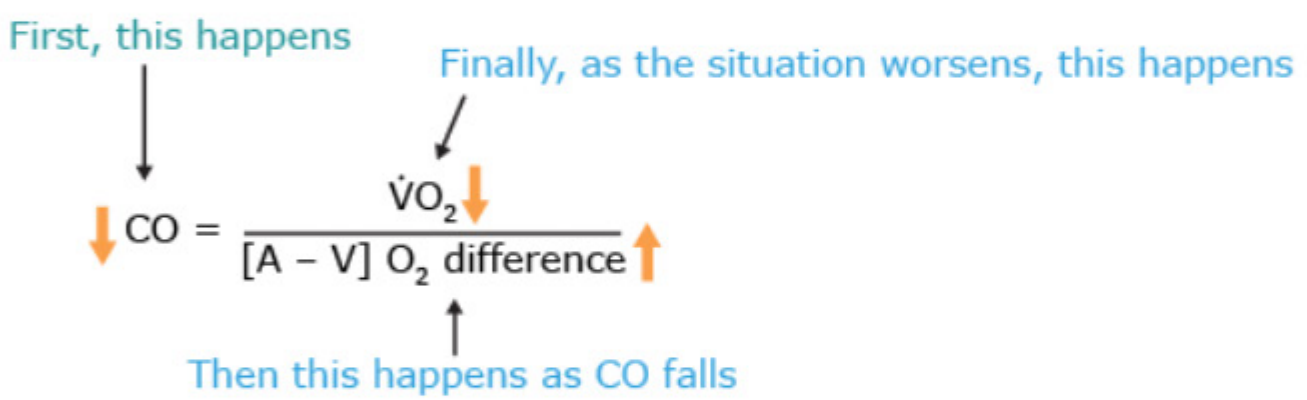
Hypovolemic shock results from excessive loss of fluid (dehydration) or blood (hemorrhage).

- Cold, clammy skin
- Hypotension, weak pulse

8.2 Cardiogenic Shock

- Pump failure with circulatory collapse
- Hypotension, weak pulse, cool skin
- *Changes in Fick equation parameters*

Changes in Fick equation parameters:



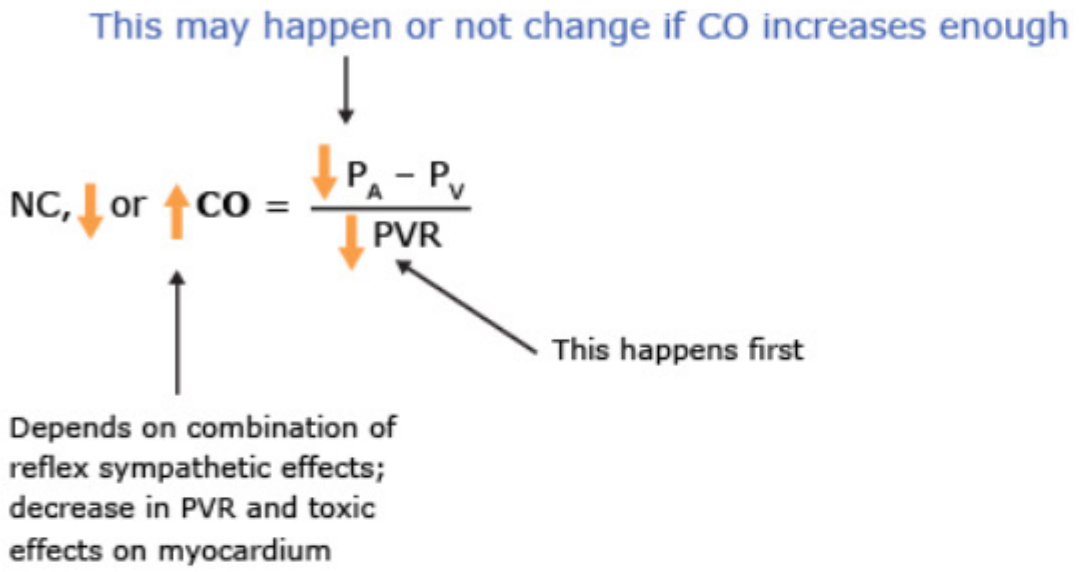
- $\dot{V}O_2$ falls because tissues begin switching to anaerobic metabolism
- Changes in Fick equation parameters are used to guide therapy

▲ **Figure 4–8.1** Fick Equation

8.3 Septic Shock

Septic shock results from the multiplication of microbes (usually gram-negative bacteria) in the circulation.

- *Endotoxin* from gram-negative bacilli activates macrophages by binding to CD14
- This activates a cytokine cascade, the complement and kinin systems, and causes direct toxic injury to vessels
- Hypotension, weak pulse, and warm skin due to peripheral vasodilation



▲ **Figure 4–8.3** Septic Shock

8.4 Irreversible Shock

Irreversible shock results in multiple-organ damage:

- Kidney: Acute tubular necrosis, causing renal insufficiency
- Brain: Focal necrosis
- Liver: Centrilobular necrosis of liver, causing hepatic insufficiency
- Colon: Mucosal hemorrhages
- Lungs: Edema, causing respiratory compromise

▼ **Table 4–8.4** Shock Differentials

	CO	PVR	LVEDP
Hypovolemic	↓	↑	↓
Cardiogenic	↓	↑	↑
Endotoxic	↑	↓	↓

CHAPTER 5 Neoplasia

1 Types of Neoplasms

Neoplasia refers to new growth that is not reversible. It may arise from dysplasia, the reversible condition of disordered growth. Neoplasms may be benign or malignant.

1.1 Characteristics of Benign and Malignant Neoplasms

▼ **Table 5–1.1** Benign vs. Malignant Neoplasms

Benign	Malignant
Monoclonal origin	Monoclonal origin
Small size	Variable size
Slow-growing	Variable growth rate
Well-demarcated borders	Invasive into adjacent tissues
Well-differentiated cells	Variable differentiation of cells, pleomorphism
Nuclear/cytoplasmic ratio near normal; normal mitotic spindles	Increased nuclear/cytoplasmic ratio, often atypical mitoses, prominent nucleoli, up-regulated telomerase activity

1.2 Naming Tumors

▼ **Table 5–1.2** Names for Benign and Malignant Neoplasms

Tissue	Benign	Malignant
Epithelium	Adenoma, papilloma	Adenocarcinoma, papillary carcinoma
Mesenchyme	Fibroma, lipoma	Sarcoma
Melanocytes	Nevus	Melanoma
Lymphocytes		Lymphoma

USMLE® Key Concepts

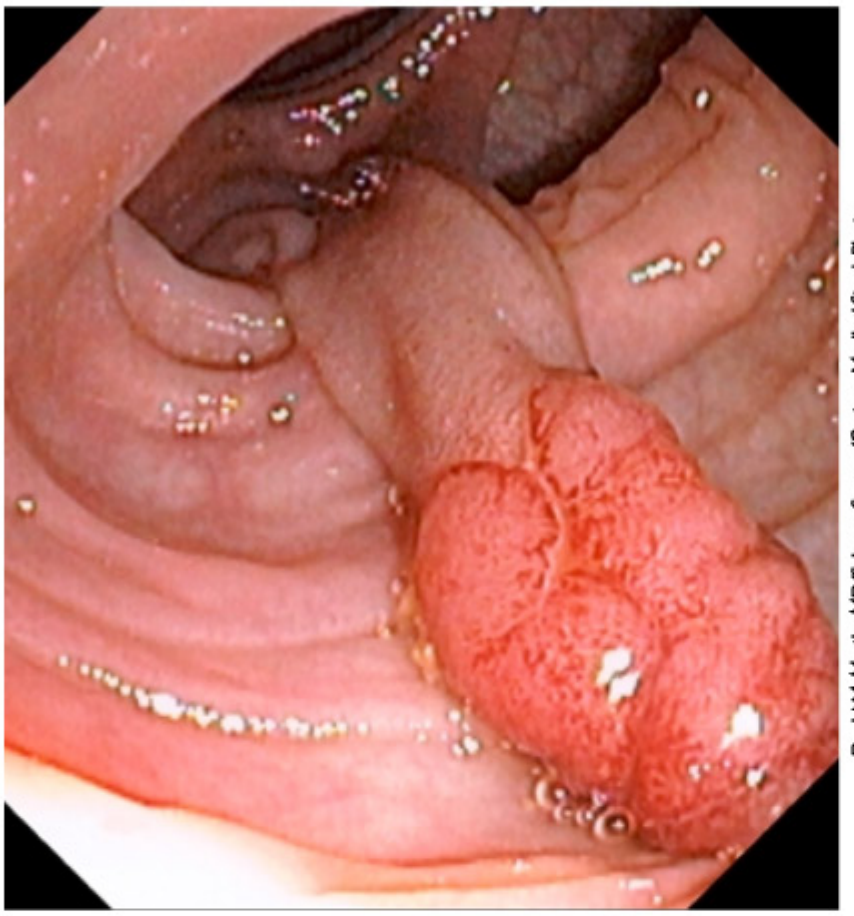
For Step 1, you must be able to:

- Explain the steps of neoplasia and differentiate between benign and malignant neoplasms.
- Describe the molecular control of oncogenes, tumor suppressor genes, and apoptotic signals in the development of cancer.
- Identify the histological and cytochemical techniques used for diagnosis of cancers.
- Explain the epidemiology of the most common cancers in the U.S.
- Identify the paraneoplastic syndromes that accompany cancer.
- Describe strategies for cancer prevention.

1.3 Benign Tumors

1.3.1 Benign Tumors of Epithelial Origin

- Arise from ectoderm or endoderm
- Example: Tubular adenoma arising from glands in the colon (adenomatous polyp)



David M. Martin, MD/Science Source/Custom Medical Stock Photo

▲ **Figure 5–1.3A** Tubular Adenoma

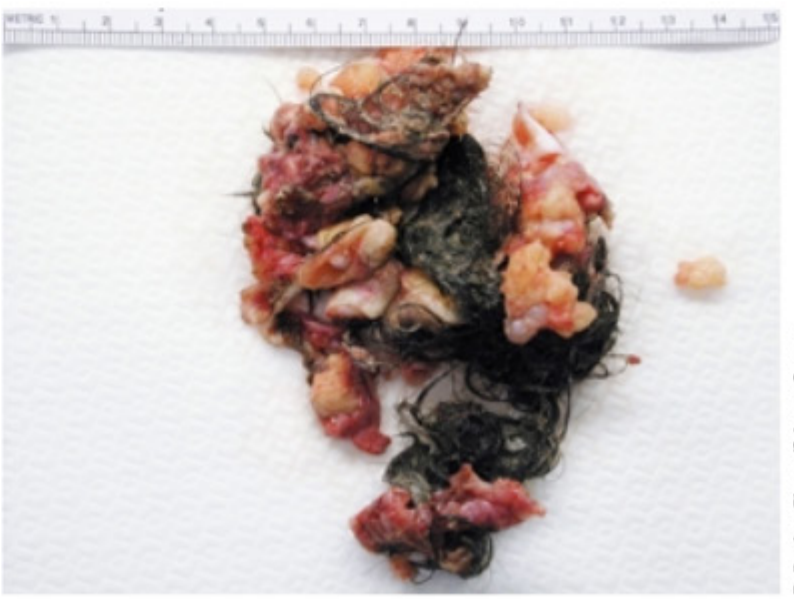
1.3.2 Benign Tumors of Connective Tissue

- Arise from mesoderm
- Example: Lipoma from adipose tissue

1.3.3 Other Benign Tumor-Like Conditions

Teratoma

- Tumors that derive from more than one germ-cell layer
- Derived from ectoderm, endoderm, or mesoderm
- Calcifications on x-ray
- Tend to be located toward the midline: Ovary/testis, anterior mediastinum, pineal gland, sacrococcygeal in children



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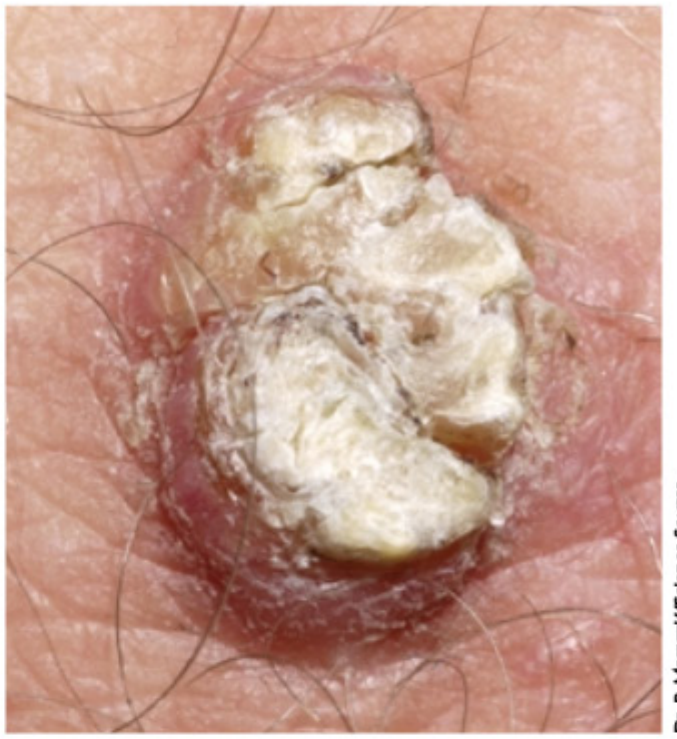
▶ **Figure 5–1.3B** Teratoma

Hamartoma

- A disorganized mixture of cells and tissue indigenous to a particular site
- *Not neoplastic*
- Common sites: Bronchial (detected on x-ray), Peutz-Jeghers polyp

Choristoma (Heterotopic Rest)

- Non-neoplastic tissue in an ectopic site
- Examples:
  - Pancreatic tissue in the stomach wall
  - Parietal cells in Meckel diverticulum



Dr. P. Marazzi/Science Source

▲ **Figure 5–1.3C** Hamartoma

1.4 Malignant Neoplasms

1.4.1 Carcinomas

These forms of cancer are ectodermal or endodermal in origin.

- **Squamous Cell Carcinoma**
  - Stratified squamous epithelial carcinoma. Commonly found on the skin, mouth, esophagus, or vagina.
  - Also found in areas of squamous metaplasia, including the bronchi and squamocolumnar junction of the uterine cervix.
- **Adenocarcinoma**
  - *Glandular epithelial carcinoma*. Commonly occur in mucosa of GI track, pancreas, breast, prostate, and uterine endometrium.
  - Adenocarcinomas may induce reactive proliferation of non-neoplastic fibrous connective tissue, a process called *desmoplasia*.
  - Transitional cell carcinoma.
- **Transitional Cell Epithelium of the Urinary Tract**

1.4.2 Sarcomas

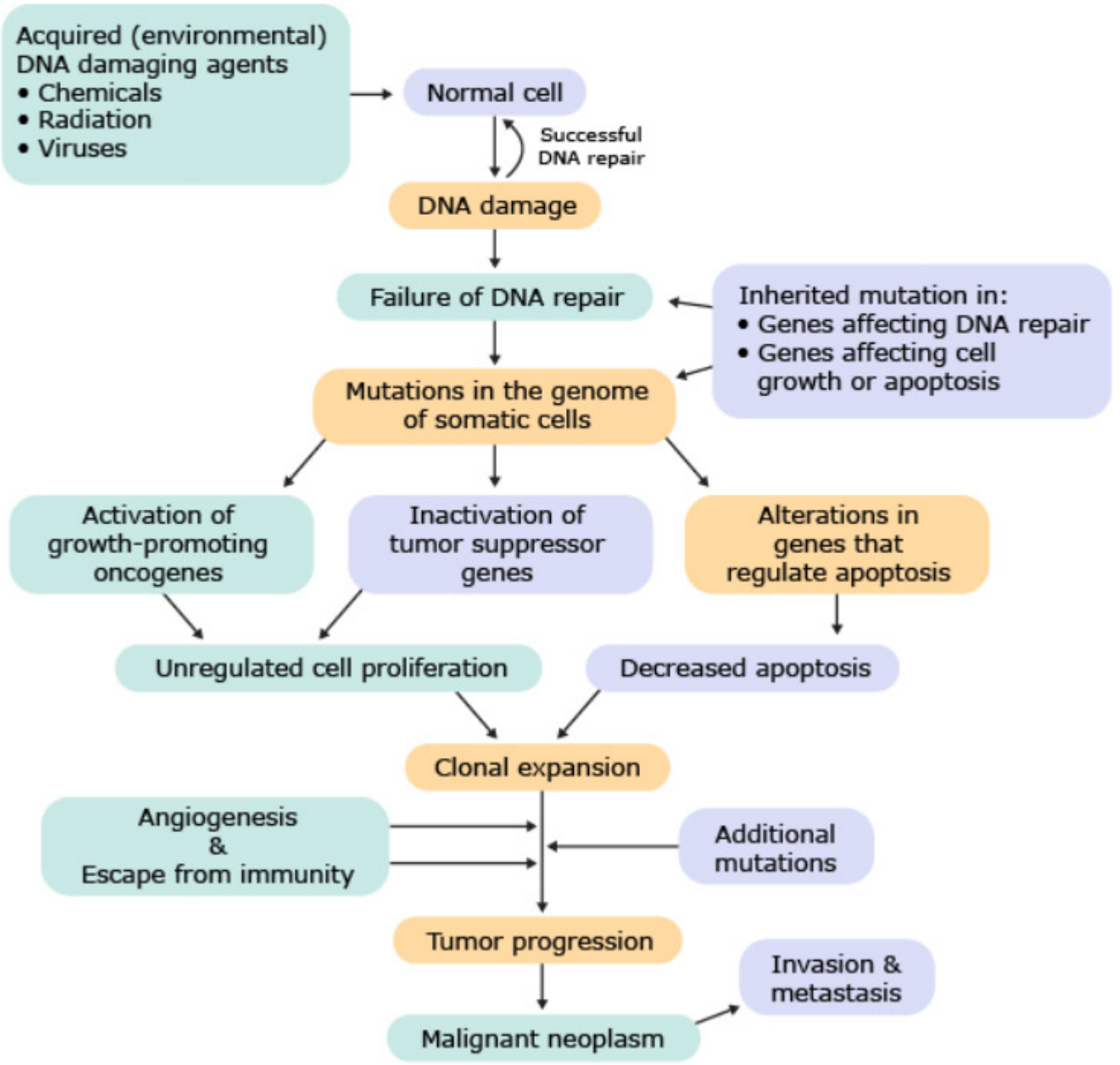
▼ **Table 5–1.4** Sarcomas

Cancer	Tissue of origin
Osteosarcoma	Bone
Leiomyosarcoma	Smooth muscle
Chondrosarcoma	Cartilage
Rhabdomysarcoma	Skeletal muscle
Liposarcoma	Fat
Lymphosarcoma	Lymphoid tissue
Hemangiosarcoma	Blood vessels

2 Mechanisms of Carcinogenesis

For tumorigenesis to occur, there is the necessity for nonlethal genetic damage. Tumors arise by clonal expansion of a single genetically damaged precursor cell (they are monoclonal). Mistakes made in DNA replication are normally corrected by DNA repair genes. Tumorigenesis occurs as a multistep process. Four classes of normal regulatory genes are often damaged:

- DNA repair genes
- Growth-promoting oncogenes
- Growth-inhibiting tumor suppressor genes
- Apoptosis-regulating genes



▲ **Figure 5–2.0** Carcinogenesis

2.1 Acquired DNA Damaging Agents

2.1.1 Chemicals

▼ **Table 5–2.1A** DNA Damaging Chemicals

Chemical Exposure	Associated Cancer
Nitrosamines (smoked meat products)	Gastric adenocarcinoma
Asbestos	Bronchogenic carcinoma, mesotheliomas
Nickel, uranium, chromium	Lung carcinoma
Arsenic	Squamous cell carcinoma of skin and lung, angiosarcoma of liver
Aflatoxin B1 (*Aspergillus* infection)	Hepatocellular carcinoma
Vinyl chloride and polyvinyl chloride (plastics)	Angiosarcoma of liver
Alkylating agents (cancer chemotherapy)	Leukemia, lymphoma
Aniline dyes, aromatic amines, β-naphthylamine	Transitional cell bladder cancer
Thorotrast (contrast medium in radiology in 1940s)	Hepatic hemangiosarcoma (angiosarcoma)

2.1.2 Physical Injury

- Third-Degree Burns: Squamous cell carcinoma
- Chronically Draining Sinuses: Squamous cell carcinoma

2.1.3 Ultraviolet Radiation (UVB)

- Produces *pyrimidine dimers* in DNA, leading to transcriptional errors and mutations of oncogenes and tumor suppressor genes.
- Nucleotide excision repair pathway is "knocked out."

2.1.4 Ionizing Radiation

▼ **Table 5–2.1B** Radiation and Associated Cancers

Source	Associated Cancer
Atomic blast and nuclear radiation	Leukemias, thyroid cancer, etc.
Uranium mining	Lung cancer
Radium exposure	Osteosarcoma
Radiology	Skin cancer, myeloid leukemias

2.1.5 Viruses

DNA Viruses

- **Human Papillomavirus**
  - Cervical carcinoma
  - *HPV 16 and 18* responsible for 70% of cervical cancers
  - *HPV 6 and 11* are "low risk" viruses
- **EBV:** Infects B cells and epithelial cells of nasopharynx via CD21
  - Burkitt lymphoma
  - B cell lymphomas in immunocompromised patients
  - Hodgkin lymphoma
  - Nasopharyngeal carcinomas
- **Hepatitis B:** Hepatocellular carcinoma

RNA Viruses

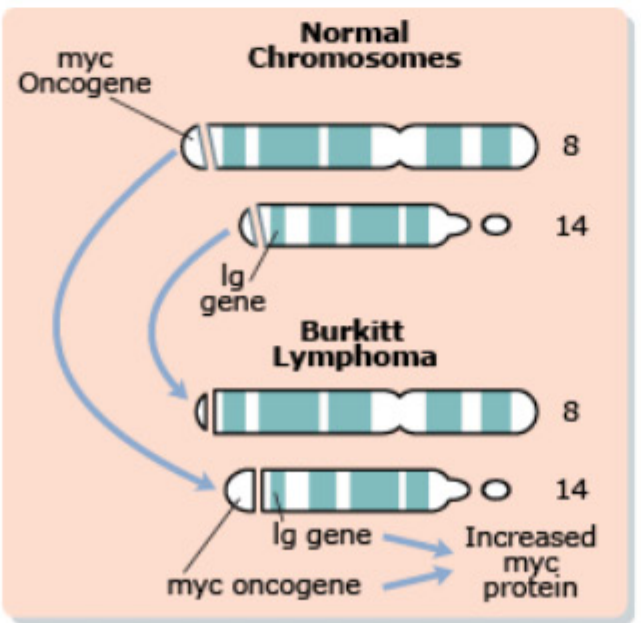
- **Human T-Cell Leukemia Virus Type-1:** Infects CD4+ T-cells to produce T-cell leukemia/lymphoma
- **Hepatitis C Virus:** Hepatocellular carcinoma

2.2 Activation of Oncogenes

- Growth-promoting oncogenes *(protooncogenes)* are normal cellular genes that promote growth and cellular differentiation.
- Oncogenes are derived from protooncogenes, but are not regulated. They result in unregulated cell proliferation.

2.2.1 Mechanisms of Oncogene Activation

- **Point Mutations** (e.g., ras)
- **Chromosomal Translocations**
  - **t(8;14) Burkitt lymphoma:** c-myc on 8 translocated to IgH on 14
  - **t(9;22) Chronic myeloid leukemia:** c-abl on 9 translocated to bcr on 22 forming bcr-abl *(Philadelphia chromosome)*
- **Gene Amplification:** *N-myc* linked to neuroblastoma



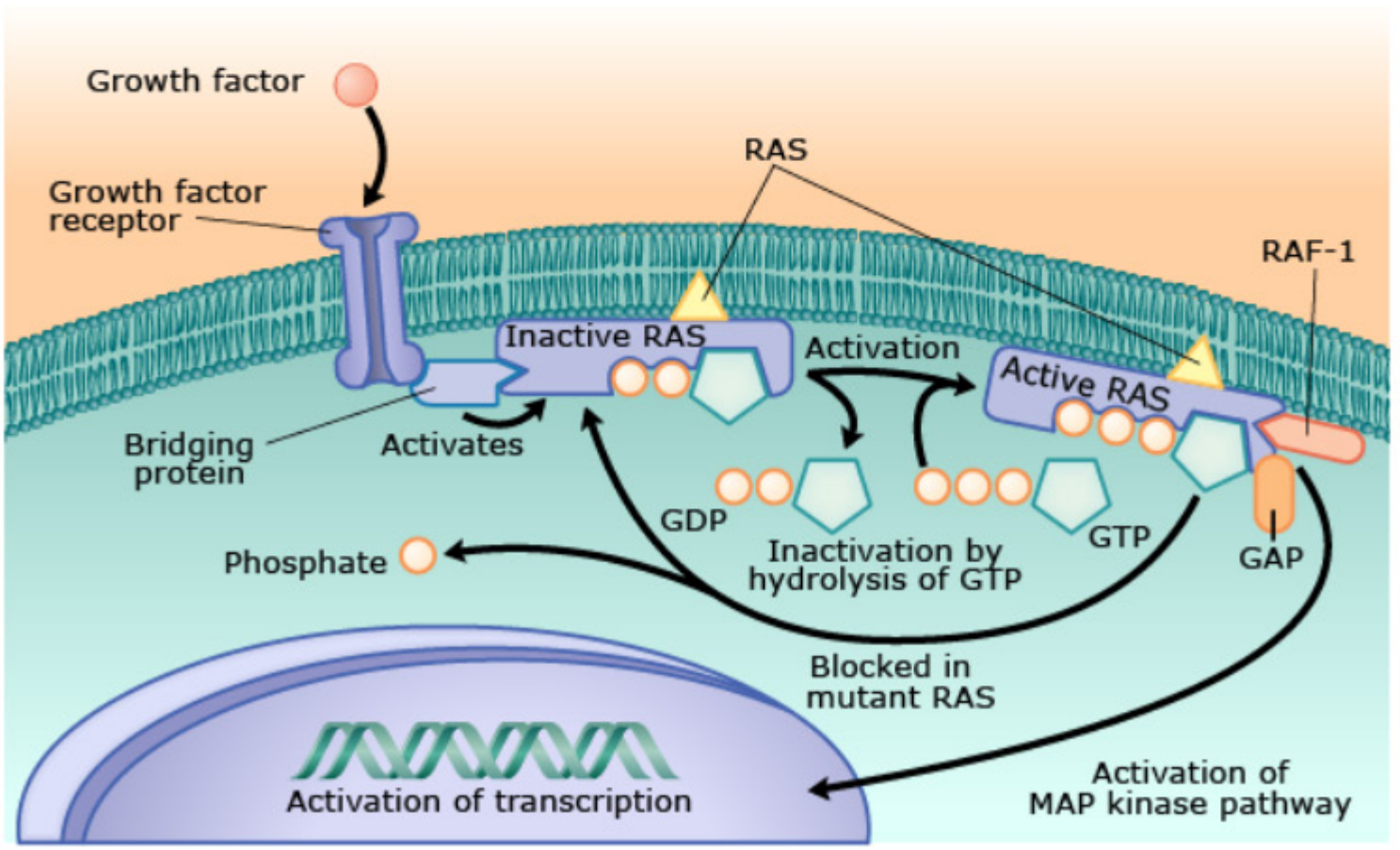
▲ **Figure 5–2.2A** Chromosomal Translocation

2.2.2 Oncogenes Affecting Growth Factors and Associated Receptors

Normal cells require growth factors produced by neighboring cells to induce proliferation (paracrine stimulation). Many cancer cells acquire the ability to synthesize the growth factors to which they will ultimately respond (autocrine stimulation). Some oncogenes encode mutated growth factor receptors that remain constitutively dimerized and activated without binding to the growth factor. This delivers a constant mitogenic signal to the cell in the absence of growth factor stimulation.

▼ **Table 5–2.2A** Oncogenes Affecting Growth Factors

Protooncogene	Growth Factor	Associated Tumor
Growth Factors		
SIS	PDGF-β	Astrocytoma, osteosarcoma
TGFA	TGF-α	Astrocytomas, hepatocellular carcinomas
Growth Factor Receptors		
ERBB1, ERBB2	EGF-receptor family	Squamous cell carcinoma of lung, gliomas
RET	Receptor for neurotropic factors	Leukemia, multiple endocrine neoplasia 2A and 2B, familial medullary thyroid carcinomas
PDGFRB	PDGF receptor	Gliomas, leukemia



▲ **Figure 5–2.2B** Mechanism of RAS Carcinogenesis

2.2.3 Oncogenes Affecting Signal Transduction or Nuclear Regulatory Proteins

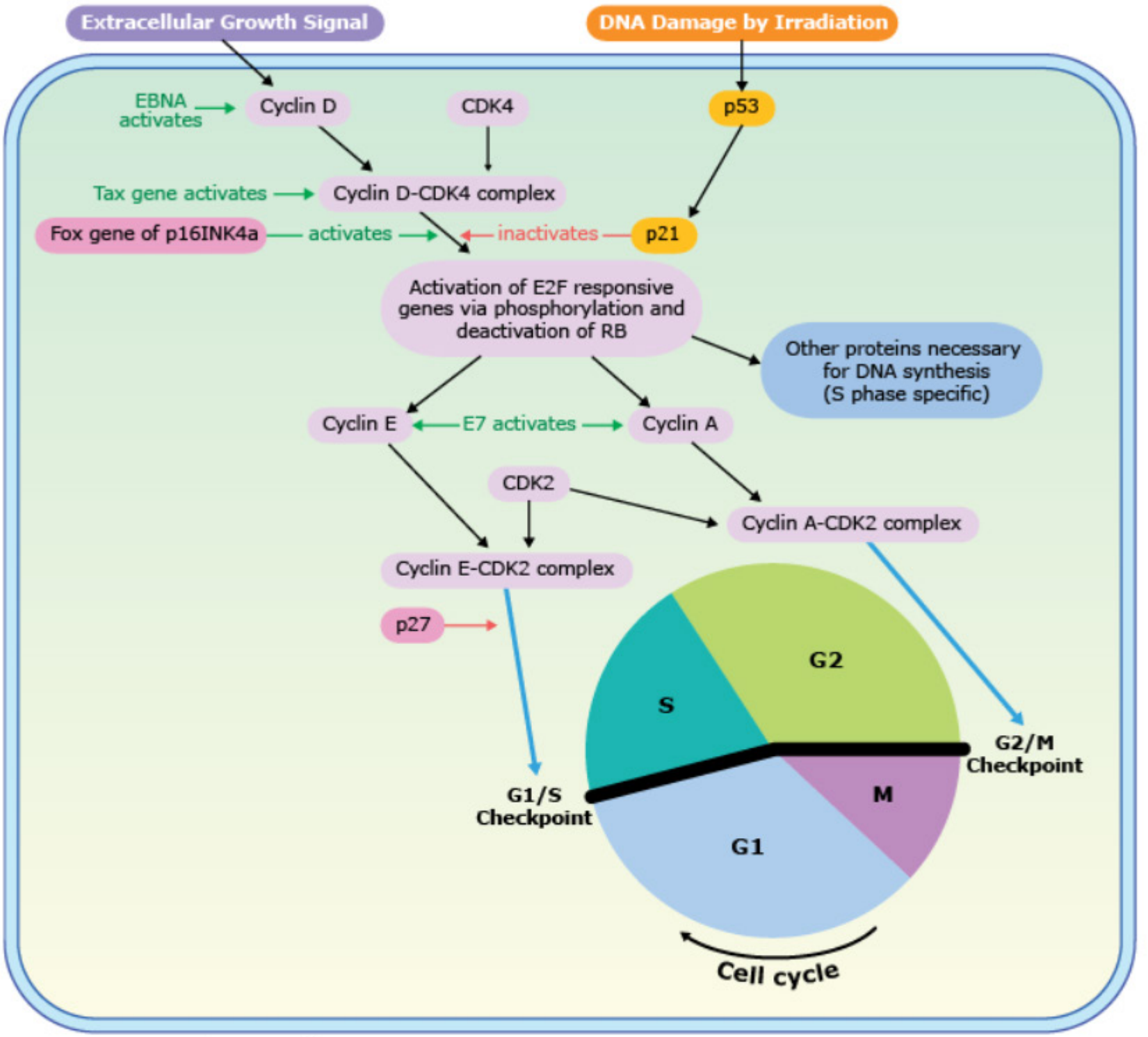
▼ **Table 5–2.2B** Oncogenes Affecting Signal Transduction

Protooncogene	Protein	Associated Tumors
Signal Transduction Proteins		
KRAS	GTP-binding	Colon, lung, pancreatic tumors
HRAS	GTP-binding	Bladder and kidney tumors
NRAS	GTP-binding	Melanomas, leukemias
ABL	Non-receptor tyrosine kinase	Chronic myelogenous leukemia, acute lymphoblastic leukemia
BRAF	RAS signal transduction	Melanomas
β-catenin	WNT signal transduction	Hepatoblastomas, hepatocellular carcinoma
Nuclear-Regulatory Proteins		
C-MYC	Transcriptional activators	Burkitt lymphoma
N-MYC	Transcriptional activators	Neuroblastoma, small cell carcinoma of lung
L-MYC	Transcriptional activators	Small cell carcinoma of lung

2.2.4 Oncogenes Affecting Cell Cycle Regulation

▼ **Table 5–2.2C** Oncogenes Affecting Cell Cycle Regulation

Protooncogene	Protein	Associated Tumors
Cyclin D	Cyclins	Mantle cell lymphoma, breast and esophageal cancers
Cyclin E		Breast cancer
CDK4	Cyclin-dependent kinase	Glioblastoma, melanoma, sarcoma



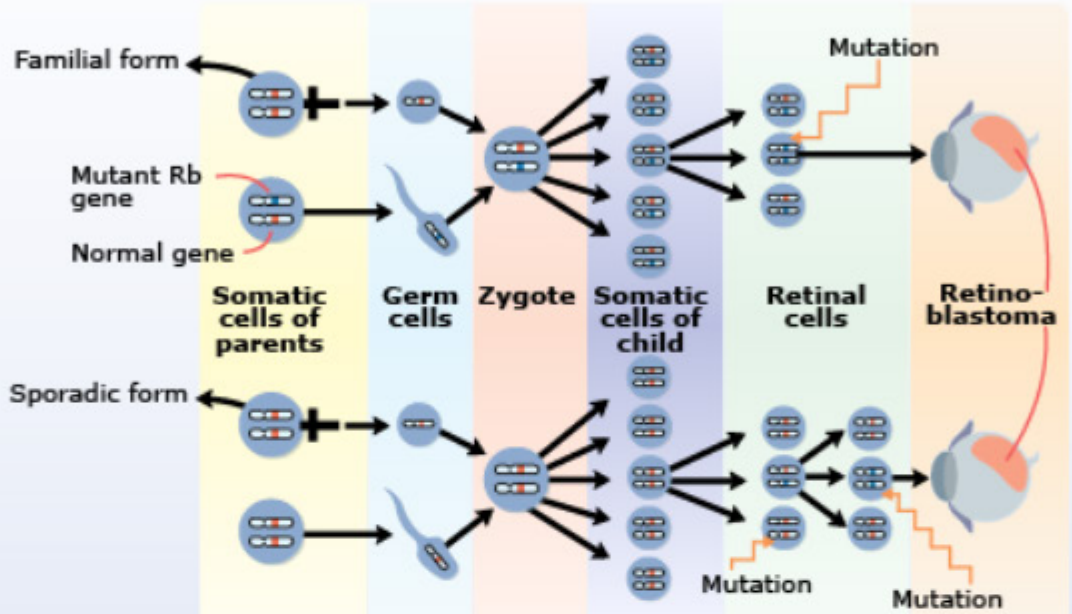
CDK = Cyclin-Dependent Kinase

▲ **Figure 5–2.2C** Oncogenesis

Clinical Application

Retinoblastoma

Retinoblastoma occurs in both inherited and sporadic forms. In the inherited form, the individual inherits one defective copy of the RB gene from a carrier parent and the "second hit" occurs via mutation in the retina. In the sporadic form, the individual inherits two normal copies of the RB gene and both become inactivated by somatic cell mutation. The RB protein is a nuclear phosphoprotein that plays a crucial role in the enforcement of G1, the gap between mitosis (M) and DNA replication (S) phases of the cell cycle.



▲ **Figure 5–2.3A** Pathogenesis of Retinoblastoma

2.3 Inactivation of Tumor Suppressor Genes

While oncogenes promote the proliferation of cells, tumor suppressor genes apply brakes to cellular proliferation. With tumor suppressor genes, both copies of the gene must be inactivated for the result of oncogenesis.

- First hit: Inherited germline or inherited somatic mutation.
- Second hit: Acquired somatic mutation.

▼ **Table 5–2.3** Inactivation of Tumor Suppressor Genes

Gene	Function	Tumors of Somatic Mutations	Tumors of Inherited Mutations
RB1	Cell cycle regulation	Retinoblastoma, osteosarcoma, carcinomas of breast, colon, lung	Retinoblastoma, osteosarcoma
P53	Cell cycle arrest for DNA repair	Most human cancers	Li-Fraumeni syndrome, multiple carcinomas and sarcomas
WT1	Nuclear transcription	Wilms tumor	Wilms tumor
P16/INK4a	Inhibition of CDKs	Pancreatic, breast, esophageal cancers	Malignant melanoma
BRCA1 and 2	DNA repair	Unknown	Carcinomas of breast and ovary

Clinical Application

Wilms Tumor

This childhood renal disease is commonly found with the "constellation" of signs and symptoms at the time of presentation. The inactivation of the WT1 tumor-suppressor gene initiates these signs and symptoms, which are covered by the acronym *WAGR:*

- **W** = *W*ilms Tumor which is where *WT1* (*not* WT2), specifically, plays role
- **A** = *A*niridia
- **G** = *G*enitourinary anomalies
- **R** = Mental *R*etardation/intellectual disability

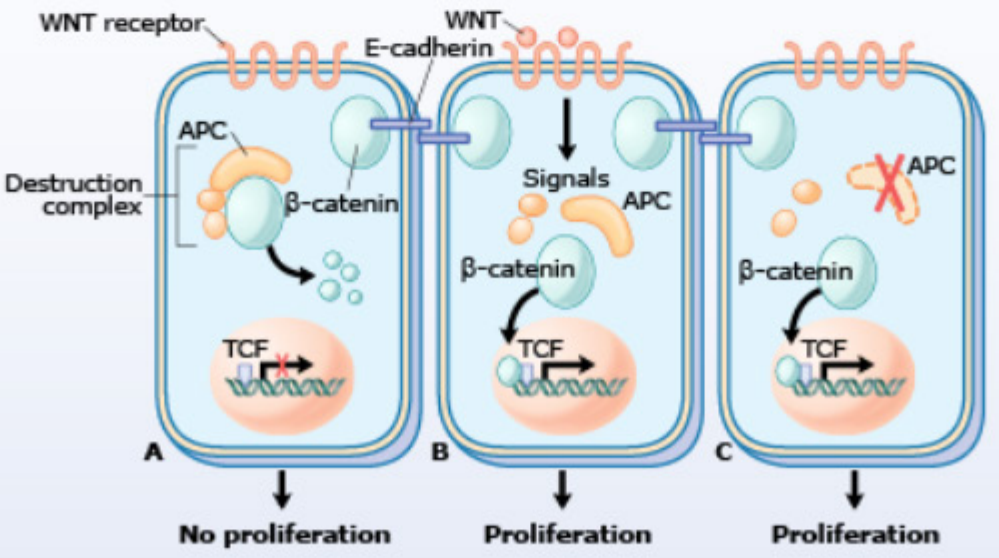
Do not confuse the WAGR acronym with another childhood constellation of presenting symptoms, *VATER* (or more precisely *VACTERL*)

- **V** = *V*ertebral abnormalities
- **A** = *A*nal atresia
- **C** = *C*ardiovascular anomalies
- **TE** = *T*racheo-*E*sophageal fistula
- **R** = *R*enal abnormalities (not to be confused with Wilms tumor)
- **L** = *L*imb defects (most commonly the radial bone of the arm)

Clinical Application

Familial Adenomatous Polyposis

This condition has an approximately 100% transformation rate into colorectal cancer. It is caused by a mutation in the adenomatous polyposis coli (APC) gene, which normally controls the activity rate of β-catenin associated transcription. When the transmembrane growth receptor WNT is stimulated by certain carcinogenic growth factors, the regulation of APC is lost and β-catenin is allowed to run amuck. The resulting overstimulation of DNA may cause unregulated cellular proliferation and thus widespread polyp formation, which in turn increases the risk of malignant transformation.



▲ **Figure 5–2.3B** Pathogenesis of Familial Adenomatous Polyposis

2.4 Dysregulation of Apoptosis

In addition to the action of oncogenes and inactivation of tumor suppressor genes, accumulation of neoplastic cells may result from mutations in the genes that regulate apoptosis. In the intrinsic pathway of apoptosis, which is stimulated by stress and injury, mitochondrial membrane permeability is controlled by a balance of pro-apoptotic and anti-apoptotic members of the BCL2 family of proteins.

- BAX and BAK proteins: pro-apoptotic
- BCL2 and BCL-XL: anti-apoptotic
- BH3-only proteins (BAD, BID, PUMA) regulate the balance between pro- and anti-apoptotic signals
- BH3-only proteins sense death-inducing stimuli and promote apoptosis by neutralizing the actions of BCL2 and BCL-XL

2.5 Limitless Clonal Expansion

2.5.1 Telomerase

Normal cells are capable of 60 to 70 doublings, after which the progressive shortening of telomeres at the ends of chromosomes seem to be recognized by DNA-repair machinery as breaks that necessitate cell cycle arrest. Telomerase maintenance is observed in virtually all malignant cells, so this normal cellular aging is not observed, and cancer cells are, therefore, immortal.

2.5.2 Angiogenesis

Tumors are unable to grow beyond 1–2 mm in diameter unless they are supplied with oxygen and nutrients by neoangiogenesis. This provides the necessary perfusion of supplies and nutrients, provides the secretion of growth factors, and provides access to the vasculature necessary for metastasis.

2.6 Escape From Immune Destruction

- Immunosurveillence should destroy neoplastic cells via recognition of "nonself" antigens.
- Both humoral and cell-mediated immune responses should play a role.
- Tumors mount "countermeasures" to evade the immune response.
- Patients with immune system dysfunction have an increased number of neoplasms.

2.7 Tumor Progression

Each cancer must result from the accumulation of multiple (on average 90) mutations. These mutations to oncogenes, tumor suppressor genes, and anti-apoptotic signals must accumulate incrementally over time. This is demonstrated in the progression of the development of colon carcinoma, through easily identifiable states of epithelial hyperplasia to formation of adenomas which ultimately undergo malignant transformation.

Looking Back

For details on the intrinsic and extrinsic pathways of apoptosis, see topic 7 in chapter 1, "Cellular Pathophysiology."

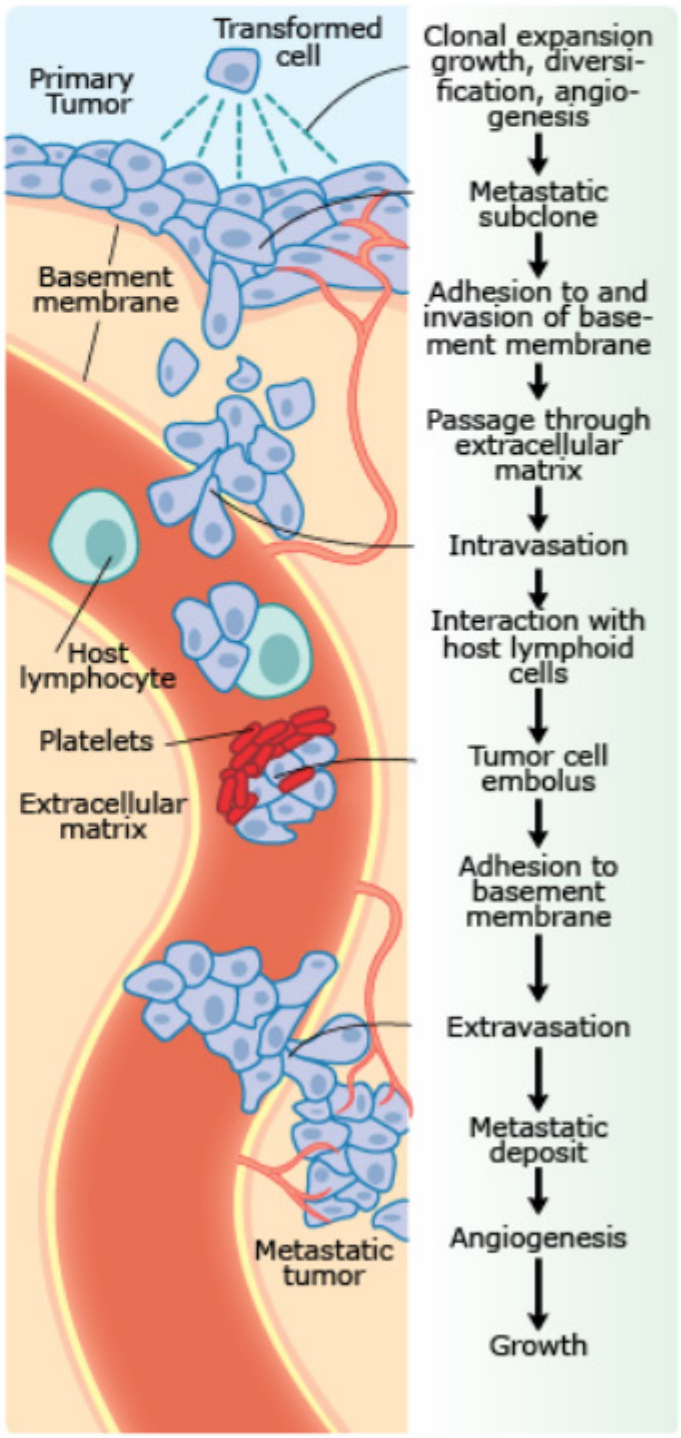
2.8 Invasion and Metastasis

2.8.1 Invasion

- Loss of intercellular adherence.
- Cell invasion takes place:
  - Cell receptors attach to laminin in basement membrane.
  - Cells release type IV collagenase, which dissolves the basement membrane.
  - Cell receptors attach to fibronectin in the extracellular matrix.
  - Cells produce cytokines to stimulate locomotion and proteases to dissolve connective tissue.
  - Cells produce factors that stimulate angiogenesis (vascular endothelial growth factor and basic fibroblast growth factor).

2.8.2 Metastasis

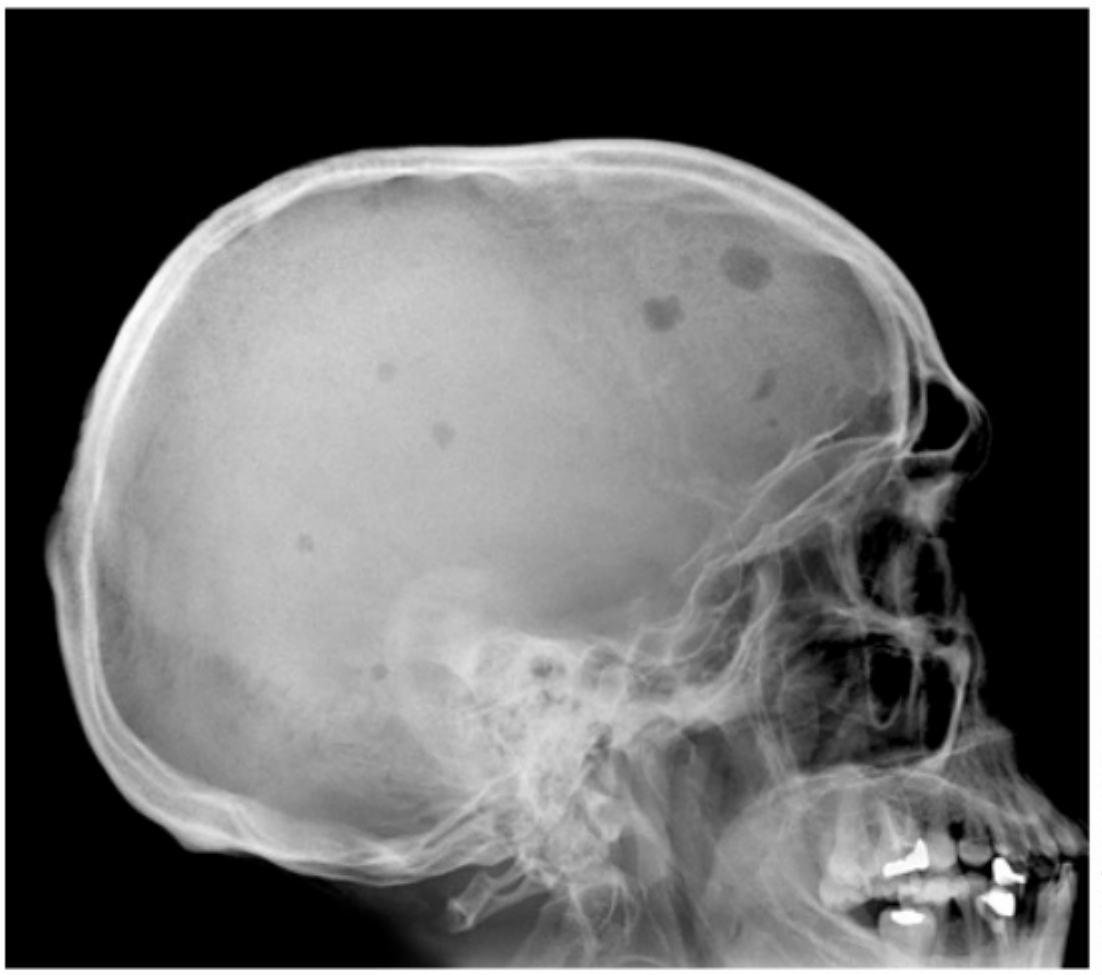
- Requires malignant cells to detach from tumor, be transported in blood or lymphatics to distant sites, and survive (multiply) in new location.
  - *Sarcomas* tend to spread *hematogenously*.
  - *Carcinomas* spread initially via lymphatics.
- **Seeding:** Malignant cells exfoliate from a surface and implant and invade tissue in a body cavity.
  - Primary surface-derived ovarian cancers (serous cystadenocarcinoma) commonly seeds the omentum.
  - Peripherally located lung cancers commonly seed the parietal and visceral pleura.
  - Glioblastoma multiforme commonly seeds the cerebrospinal fluid, causing spread to the brain and spinal cord.



▲ **Figure 5–2.8A** Metastasis

Bone Metastasis

- The most common site is the vertebra site through the Batson paravertebral plexus.
- Osteoblastic activity will result in bone-building activity and raising levels of alkaline phosphatase (ALP).
- Osteolytic activity will result in lytic bone lesions with increased serum levels of calcium.



▲ **Figure 5–2.8B** Radiograph of Metastasis

3 Cancer Epidemiology

3.1 Adults

▼ **Table 5–3.1** Cancer Incidence and Mortality in Adults

Gender	Type	Incidence	Mortality
Male	Prostate	29%	11%
	Lung	14%	31%
	Colorectal	10%	10%
Female	Breast	30%	15%
	Lung	14%	26%
	Colorectal	11%	9%
	Uterine corpus	6%	3%
	Ovarian	3%	6%
	Cervical	0.7%	2%

3.2 Children

In the U.S., from one to two children develop cancer each year for each 10,000 in the population. Of these, more than 50% of the new cases are leukemias (acute lymphoblastic leukemia is most common) and cancers of the brain and central nervous system (gliomas and medulloblastomas are most common).

4 Diagnosis of Cancer

4.1 Types of Monoclonal Isoenzymes

- **Human Androgen Receptor Gene (HUMARA):** This is the most common marker used to determine monoclonality. It examines methylation patterns.
- **Glucose-6-Phosphate Dehydrogenase (G6PD):** The use of this monoclonal isoenzyme for cancer detection is limited to females of African heritage.

4.2 Serum Tumor Markers

▼ **Table 5–4.2** Serum Tumor Markers

Molecular Marker	Associated Cancer
α-fetoprotein	Hepatocellular carcinoma, yolk sac
Bence Jones protein	Multiple myeloma, Waldenström
Ca-125	Surface-derived ovarian
Carcinoembryonic antigen	Colorectal, pancreatic
Prostate-specific antigen	Prostate

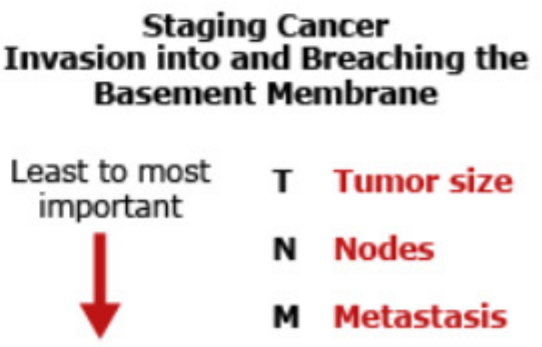
4.3 Immunohistochemistry

▼ **Table 5–4.3** Immunohistochemistry

Identifying Molecule	Cancer Source
Cytokeratin	Epithelial cells
Desmin	Muscle cells
Vimentin	Mesenchymal cells
Actin	Smooth and skeletal muscle
CD markers	Hematopoietic cells
Estrogen receptors	Breast
S100	Melanoma and neural tumors
Thyroglobulin	Thyroid

4.4 Staging Cancer

Staging is more significant than grading when it comes to determining prognosis of a cancer. The reason falls back on the understanding that when unrestricted cellular proliferation starts invading deeper into the tissue and encroaches the basement membrane, it is then rendered susceptible to rupture. If such an event takes place, malignant cells gain access to the preferred route of metastasis (e.g., sarcoma = hematogenous; carcinoma = lymphatics). Once a cancer has reached the level of metastasis, the prognosis of the condition drops significantly (e.g., breast cancer metastasizing to the axillary lymph nodes; prostate cancer metastasizing to the vertebrae).



▲ **Figure 5–4.4** Clinical Basis for Staging Cancer

5 Paraneoplastic Syndromes

5.1 Paraneoplastic Syndromes

▼ **Table 5–5.1** Paraneoplastic Syndromes

Syndrome	Associated Cancer
Acanthosis nigricans	Gastric adenocarcinoma
Lambert-Eaton syndrome	Small cell carcinoma of lung
Hypertrophic osteoarthropathy (HOA)	Bronchogenic carcinoma
NBTE	Mucus-secreting pancreatic/colorectal carcinomas
Seborrheic keratosis (Leser-Trélat sign)	Gastric adenocarcinoma
Superficial migratory thrombophlebitis	Pancreatic carcinoma

5.2 Paraneoplastic Endocrinopathies

▼ **Table 5–5.2** Paraneoplastic Endocrinopathies

Disease	Cell Type	Hormone
Cushing	Small cell carcinoma of the lung	ACTH
Gynecomastia	Choriocarcinoma	hCG
Hypercalcemia	Renal cell, squamous cell carcinoma of the lung	PTH-related peptide
Hypocalcemia	Medullary carcinoma	Calcitonin
Hyponatremia	Small cell carcinoma of the lung	ADH
Polycythemia	Renal cell, hepatocellular carcinoma	Epo

6 Cancer Prevention

6.1 Lifestyle Modification

- Cessation of smoking and alcohol
- Increased intake of fiber
- Weight loss
- Avoidance of environmental carcinogens

6.2 Vaccination

- Hepatitis B virus (HBV) vaccine decreases the risk of hepatocellular carcinoma.
- Human Papillomavirus (HPV) vaccine decreases the risk of cervical cancer.

6.3 Screening

- Pap smear
- Colonoscopy
- Mammography
- Prostate-specific antigen

6.4 Preemptive Treatment for Precursor Conditions

- Treatment for regimen for *Helicobacter pylori* may prevent malignancies such as mucosa-associated lymphoid tissue cancers.
- Treatment for gastroesophageal reflux disease (GERD) may prevent the development of adenocarcinoma of the distal esophagus.

Unit 2

Hematology

CHAPTER 6 Red Blood Cell Pathology

1 Red Blood Cells

1.1 Mature Red Blood Cells

Mature red blood cells do not contain mitochondria or HLA. The biochemical pathways that mature red blood cells contain include:

- The only source of energy derived from anaerobic glycolysis
- Pentose phosphate pathway
- Methemoglobin reductase pathway
- Synthesis of 2,3-Bisphosphoglycerate (BPG)

1.2 Red Blood Cell Labs

1.2.1 Blood Counts

Hemoglobin (Hgb) Concentration of hemoglobin in blood.

- Normal Adult Female Range: 12–16 g/dL
- Normal Adult Male Range: 14–18 g/dL

Hematocrit (Hct) Percentage of RBCs by volume.

- Normal Adult Female Range: 37%–47%
- Normal Adult Male Range 40%–54%

RBC Count Number of RBCs per unit volume.

- Normal Adult Female Range: 3.9–5.2 mill/mcL
- Normal Adult Male Range: 4.2–5.6 mill/mcL

1.2.2 Red Cell Indices

Mean Corpuscular Volume (*MCV*) Average volume of individual RBCs.

- <80 mm^3 = Microcytic
- 80–100 mm^3 = Normocytic
- >100 mm^3 = Macrocytic

Red Blood Cell Distribution Width (*RDW*) Reflects variability in red cell size (anisocytosis).

Mean Corpuscular Hemoglobin Concentration (*MCHC*) Increased when RBCs are misshapen (spherocytosis—lack of a pale center microscopically).

- Average hemoglobin concentration per RBC
- Normal Adult Range: 27–33 pg
- Decreased: Hypochromic
- Normal: Normochromic
- Increased: Hyperchromic

USMLE® Key Concepts

For Step 1, you must be able to:

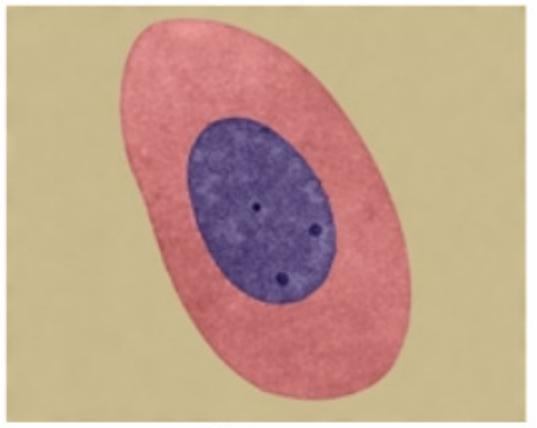
- List the various types of red blood cell labs and explain their diagnostic functions.
- Classify anemias based on their presentation and characteristics.
- Identify the types of polycythemia.

1.3 Red Blood Cell Life Cycle

RBC conception begins in the bone marrow with the help of erythropoietin being supplied from the renals. This is a six- to seven-day process that leads to the development of the reticulocyte, which then enters the circulation.

- **Reticulocyte:** Immature RBC that requires 24 hours to mature upon entry into circulation. The quantity of immature RBCs in the bone marrow reflects the degree of production.
- **Reticulocyte Count:** Percentage of RBCs that are reticulocytes
  - Corrected reticulocyte count corrects for degree of anemia
  - Corrected count = count × (Hct/45)
  - *>3% = effective erythropoiesis*
  - *<3% = ineffective erythropoiesis*

The lifespan of an RBC is 120 days, after which it is referred to as senescent RBC and is discarded through the reticuloendothelial system (RES), which mainly includes the spleen, but also the liver and lymph nodes.



Dennis Kunkel Microscopy, Inc./Visuals Unlimited, Inc.

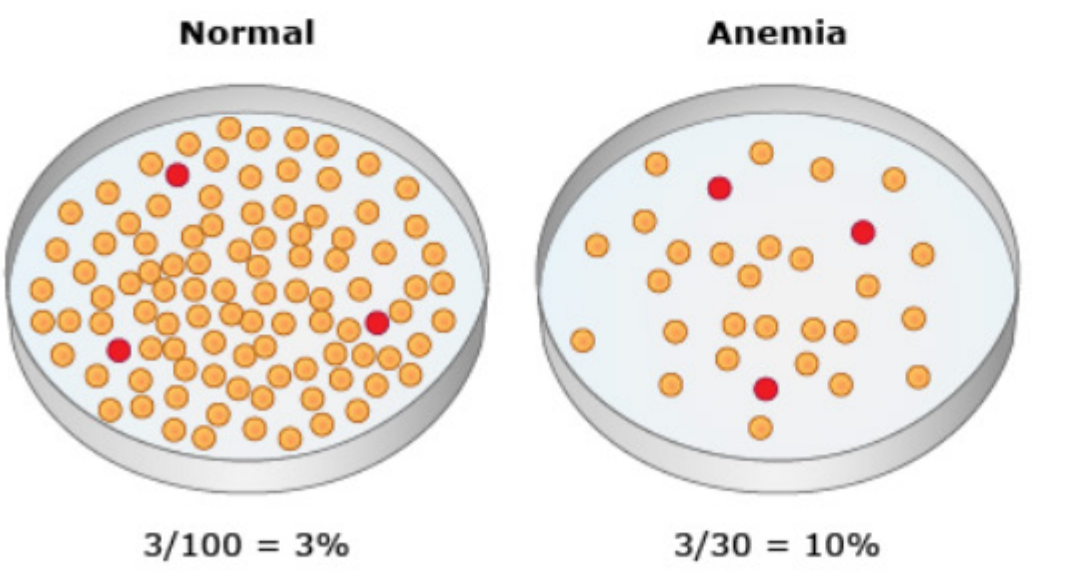
▲ **Figure 6–1.3A** Reticulocyte

Connection to

Immunology

Hematopoietic Stem Cell Development

In the bone marrow, a pluripotent stem cell differentiates down one of two lineages in the presence of specific *cytokines* or soluble mediators. The common myeloid progenitor is created in the presence of granulocyte-monocyte colony stimulating factor (GM-CSF) or *interleukin-3 (IL-3)*. This cell is the originator of erythrocytes, platelets, granulocytes (neutrophils, basophils, and eosinophils), monocytes, macrophages, and dendritic cells. The common lymphoid progenitor is created in the presence of *interleukin-7 (IL-7)*. This cell ultimately differentiates into B lymphocytes, T lymphocytes, or natural killer (NK) cells. The T lymphocyte precursor leaves the bone marrow and migrates to the second primary lymphoid organ, the *thymus*, to undergo further differentiation into *helper T cells (Th)* or *cytotoxic T cells (Tc)*.



▲ **Figure 6–1.3B** Inaccurate Estimate of Reticulocytosis

Clinical Application

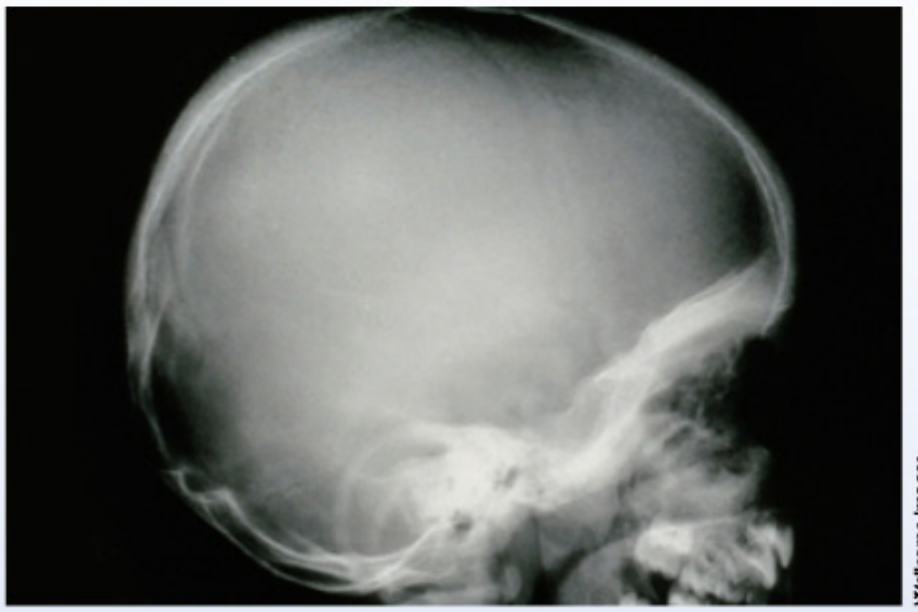
Reticulocytosis

- Reticulocytosis refers to 6% of the peripheral blood smear being populated by reticulocytes, which seems to be effective on the surface, but because it is anemia, one must take the patient's hematocrit into consideration and adjust the erythropoiesis accordingly.
- HCT/45 × COUNT
- e.g. HCT 15%, COUNT 6%
- 15/45 × 6 = 2%

Clinical Application

Accelerated Erythropoiesis

- Sickle cell disease or Cooley anemia (β-thalassemia major).
- The body must compensate and thus normally unresponsive sources of erythropoiesis are recruited.
- Radiographs of skull show "hair-on-end" appearance.

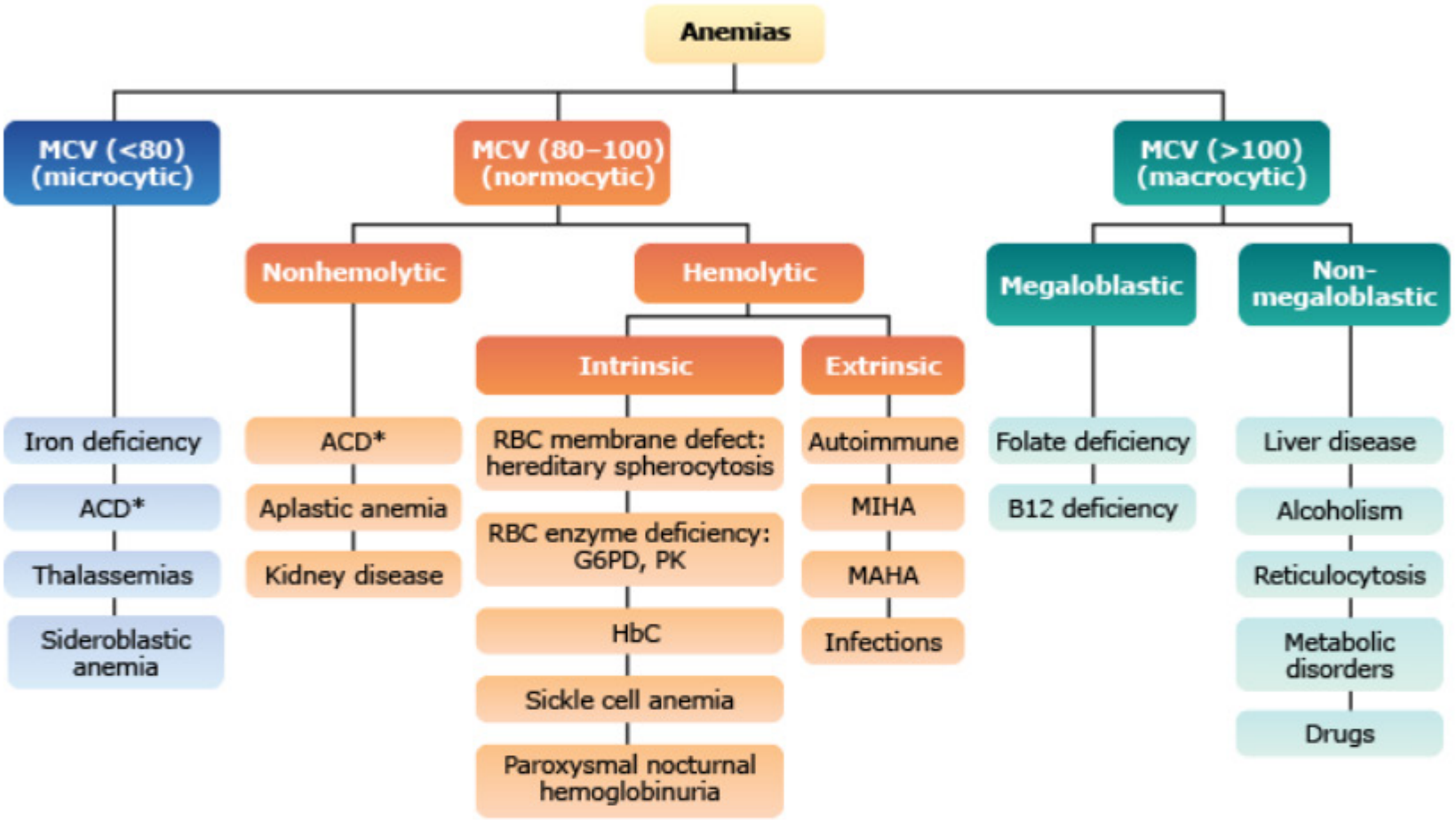


▲ **Figure 6–1.3C** Erythropoiesis: Hair-on-End Appearance

2 Anemia

Anemia is the reduction in oxygen-carrying capacity of the blood. It represents the decrease in total number of RBCs, hemoglobin, or circulating RBC mass. Anemia is usually reflected in decreased hemoglobin and hematocrit, in which case the RBC count may be increased, decreased, or normal.

2.1 Classification of Anemia



*ACD may present first as a normocytic anemia and then progress to a microcytic anemia.

▲ **Figure 6–2.1A** Types of Anemia

Memory Aid

To remember anemias, use the mnemonic **MAD**:

Membrane-defect anemias:
- PNH
- Hereditary spherocytosis

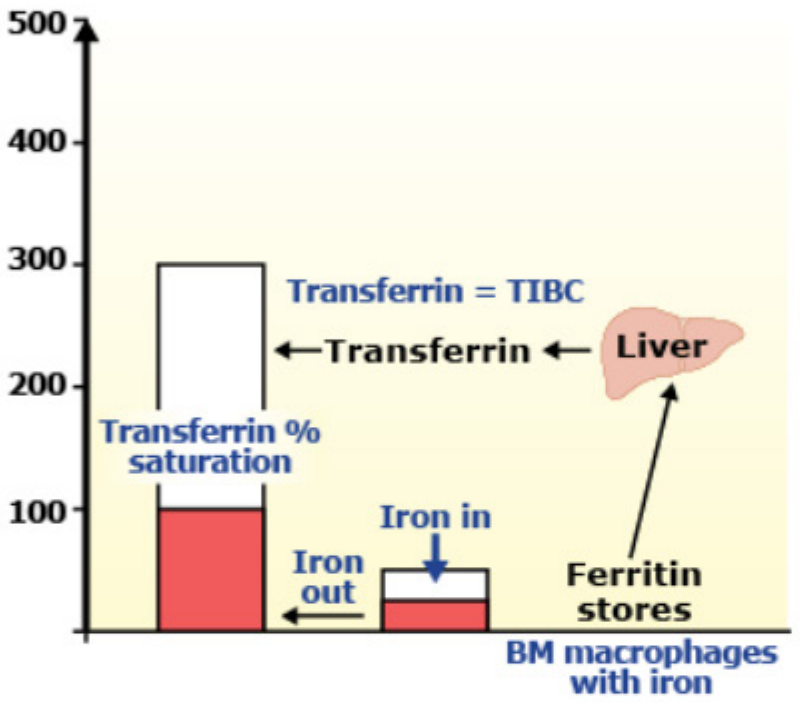
Abnormal hemoglobin anemias:
- Sickle cell disease
- HbC disease

Deficiency of enzyme anemias:
- G6PD deficiency
- Pyruvate kinase deficiency

2.1.1 Normal Red Blood Count Labs

Normal RBC labs required to diagnosis various forms of anemia include:

- **Serum Iron:** The iron levels found upon a blood draw.
- **Ferritin:** The serum iron is then stored in ferritin that is made by macrophages and located in bone marrow.
- **Transferrin:** When bodily demands for iron are increased, it is transported to the target tissue in the bound form only. This transport mechanism is known as transferrin, and is synthesized by the liver. Clinically, this is known as total iron binding capacity S(TIBC).
- **Transferrin Saturation:** The percentage of iron that is occupying transferrin.



▲ **Figure 6–2.1B** Normal Iron Labs

3 Microcytic Anemias

Microcytic anemias result from problems with hemoglobin synthesis. The two types are:

1. **Defective Heme Synthesis:** Iron deficiency anemia, anemia of chronic disease, and sideroblastic anemia.
2. **Defective Globin Synthesis:** α-thalassemia and β-thalassemia.

3.1 Defective Heme Synthesis

3.1.1 Iron Deficiency Anemia

Etiologies

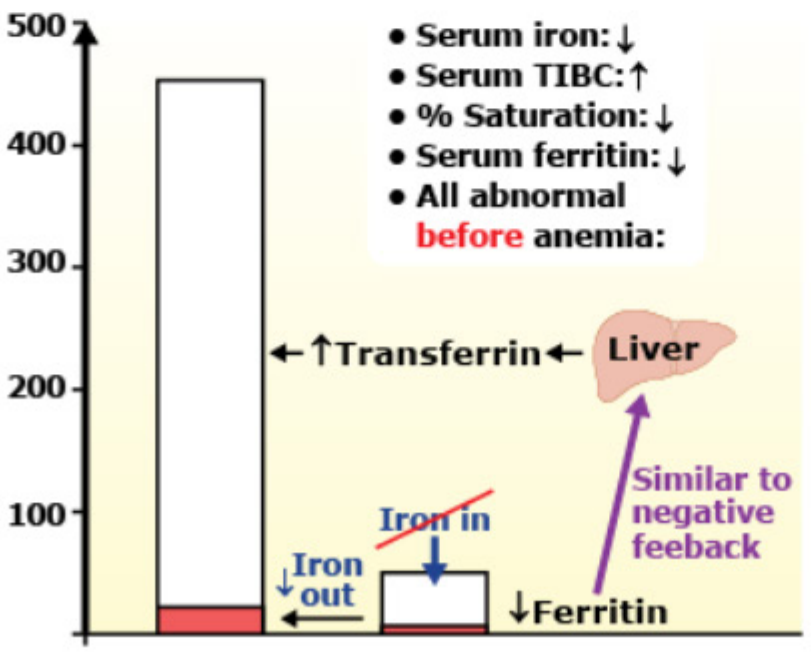
- **Chronic blood loss, depleting iron stores**
  - GI bleeding—ulcer, diverticulosis, colon cancer
  - GYN bleeding—menorrhagia
- **Dietary deficiency**
  - Very young and very old
  - Associated with ascorbic acid deficiency (vitamin C)
- **Malabsorption**
  - Decreased gastric acid secretion (achlorhydria)
  - Small bowel resection

Decreased Functional Iron Sequence of events leading to decreased hemoglobin:

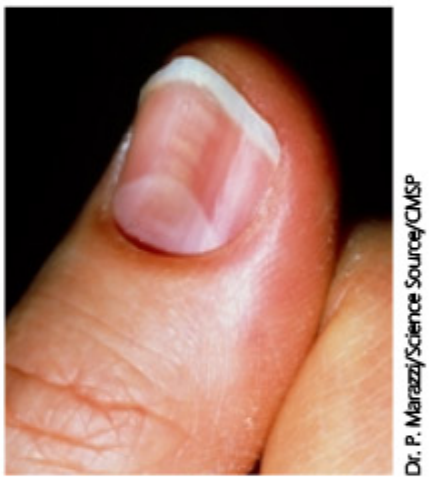
- Decreased serum iron
- Decreased ferritin
- Increased TIBC
- Decreased saturation

Signs and Symptoms

- Most commonly weakness, pallor, tachycardia
- Glossitis, koilonychia (spooning of nails)
- Chronic iron deficiency may cause pica (an appetite for non-nutritive substances such as dirt)

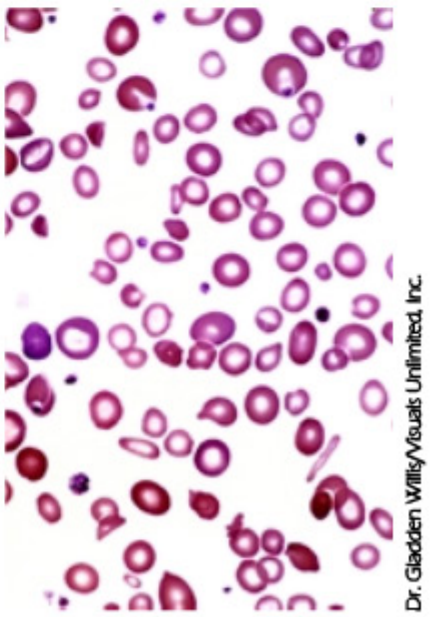


▲ **Figure 6–3.1A** Iron Deficiency



Dr. P. Marazzi/Science Source/CMSP

▲ **Figure 6–3.1B** Koilonychia



Dr. Gladden Willis/Visuals Unlimited, Inc.

▲ **Figure 6–3.1C** Red Blood Cell Distribution Width (RDW)

Plummer-Vinson Syndrome

- Caused by chronic iron deficiency
- Esophageal web form causing dysphagia for solid foods (not liquids)
- Associated with achlorhydria, glossitis, koilonychia

3.1.2 Anemia of Chronic Disease

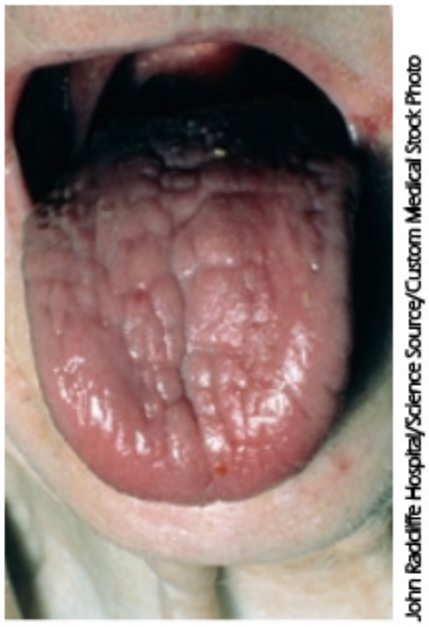
This form of anemia is most common in hospitalized patients. Its causes range from infection, autoimmune conditions (rheumatoid arthritis), cancer, renal disease, and alcohol abuse. In such cases, the most important diagnostic tool in establishing the cause of such anemia will be a good clinical history.

Pathogenesis

- Chronic inflammation causes sequestration of iron in bone marrow macrophages.
- This effect seems to be mediated by IL-1.
- Hepcidin is a hepatic peptide released secondary to inflammation that blocks the transfer of iron from macrophages within the bone marrow to transferrin.

Clinical Features

- Laboratory findings:
  - Mildly decreased Hgb (usually not <9 mg/dL) and Hct
  - RBCs usually normocytic to mildly microcytic
  - RDW is normal to slightly increased
- Main distinguishing features between iron deficiency anemia and anemia of chronic disease include:
  - Increased iron storage in bone marrow (ferritin)
  - Reduced total iron-binding capacity



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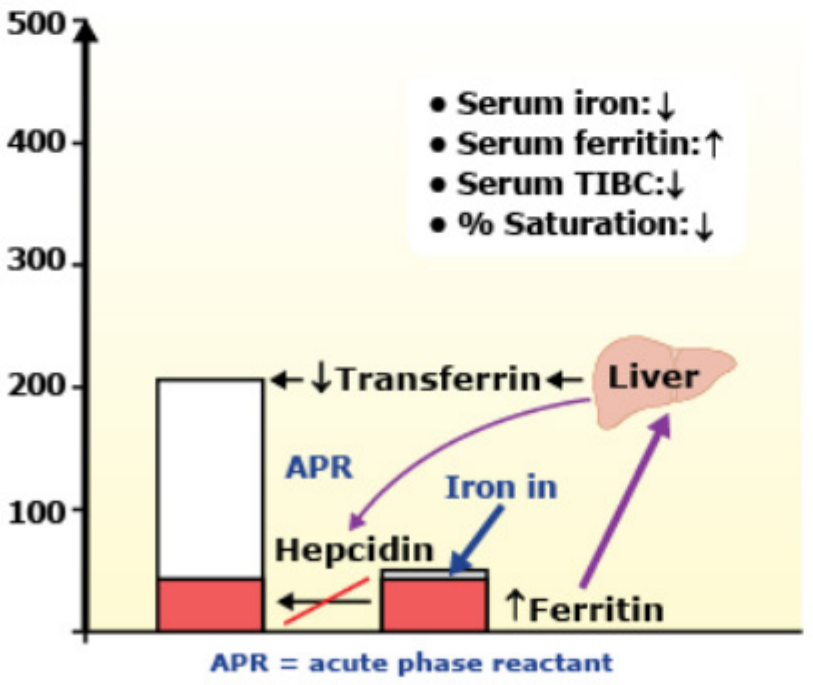
▲ Figure 6–3.1D Glossitis Seen With Iron-Deficiency Anemia



▲ Figure 6–3.1E Anemia of Chronic Disease: Laboratory Findings

3.1.3 Sideroblastic Anemia

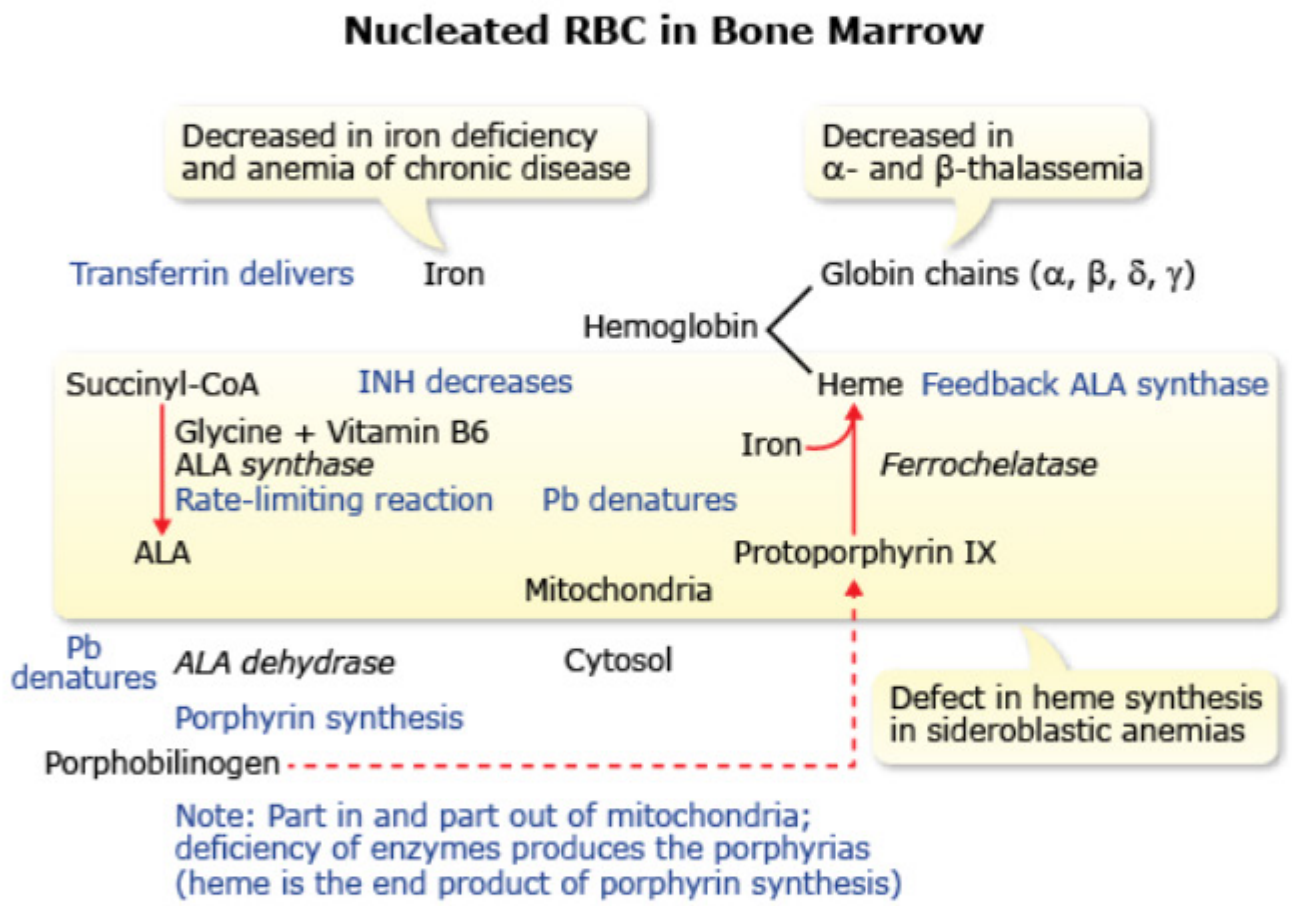
Decreased heme synthesis, which may be caused by any number of reasons, results in increased levels of iron.

- **Sideroblast:** Erythroblast containing granules of iron.
- **Ringed Sideroblast:** Nucleated erythroblast containing perinuclear iron granules in its cytoplasm.

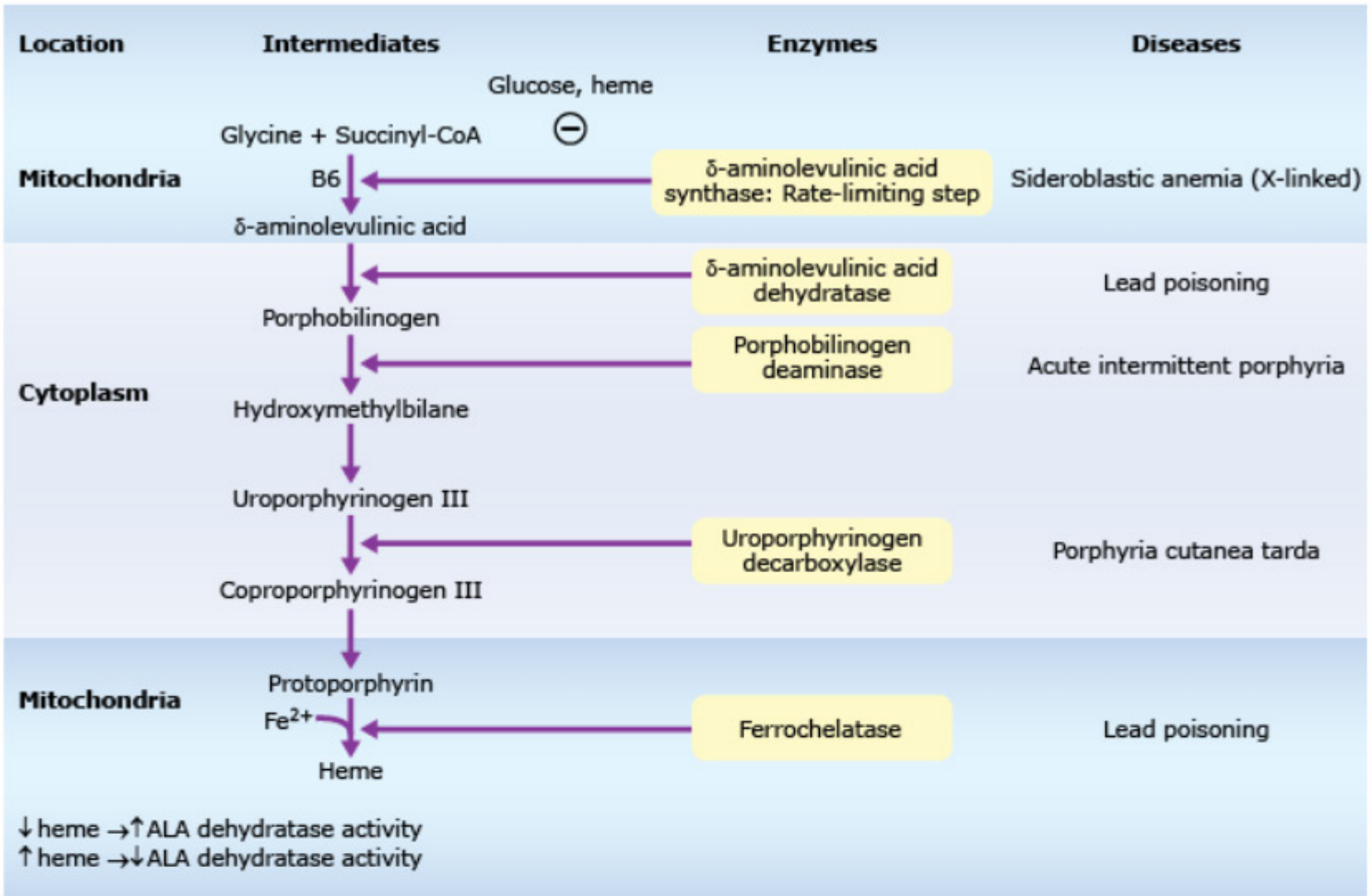
Etiology

- Alcohol abuse (most common cause)
- Lead poisoning
- Pyridoxine (vitamin B6) deficiency
- Myelodysplastic syndrome

Porphyrias These are conditions of defective heme synthesis that lead to the accumulation of heme precursors. Lead inhibits specific enzymes needed in heme synthesis, leading to a similar condition.



▲ **Figure 6–3.1F** Porphyria Pathway

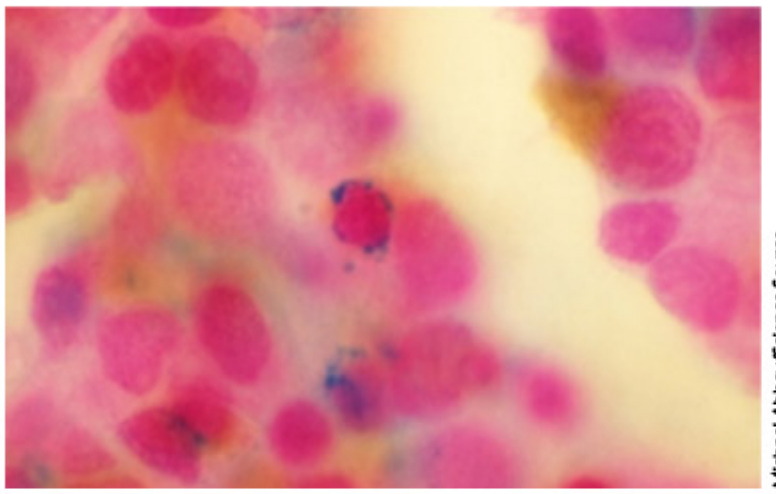


▲ **Figure 6–3.1G** Heme Synthesis, Porphyrias, and Lead Poisoning

Pathogenesis Alcohol abuse, lead poisoning, and pyridoxine (vitamin B6) deficiency all inhibit mitochondrial heme synthesis via different mechanisms:

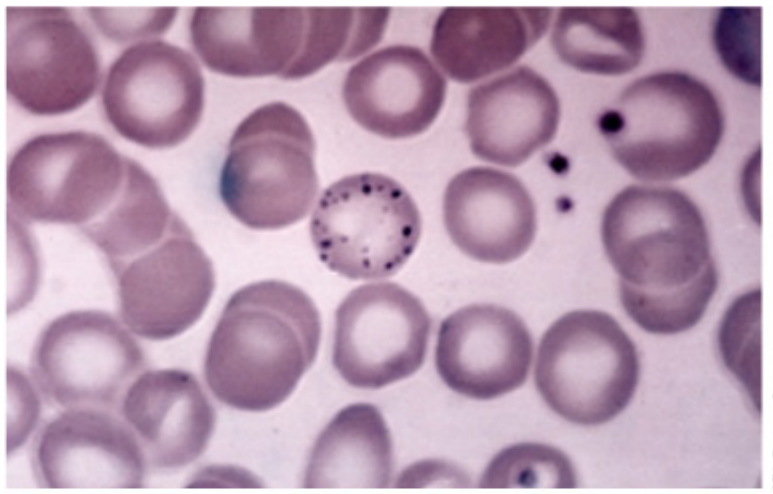
- **Alcohol:** The mitochondrial membrane plays a pivotal role in the proper functioning of the porphyria pathway, necessary for heme synthesis. Chronic ethanol exposure acts as mitochondrial membrane poison and thus interrupts heme synthesis, resulting in the most common cause of sideroblastic anemia.
- **Lead Poisoning**
  - Most common in children 1 to 5 years old.
  - Lead may be found in car radiators, battery factories, paint used in homes before 1978 (exfoliated as paint chips), and pottery.
  - Causes basophilic stippling of RBC.
  - Associated with learning disabilities and growth retardation in children.
  - In addition to disrupting heme synthesis, it also causes:
    —Abdominal pain
    —Encephalopathy
    —Peripheral neuropathy in adults

- **Pyridoxine (Vitamin B6) Deficiency:**
  - **Clinical Pathology**
    - — B6 is a cofactor for δ-aminolevulinic acid synthase, a rate-limiting step in heme synthesis.
    - — Isoniazid (INH) in TB treatment competes with pyridoxine and can lead to B6 deficiency.
    - — Vitamin B6 deficiency also causes peripheral neuropathy.
  - **Lab Findings**
    - — Increased serum iron
    - — Increased ferritin
    - — Decreased TIBC
    - — Increased transferrin saturation
  - **Bone Marrow Smear:** Show ringed sideroblasts



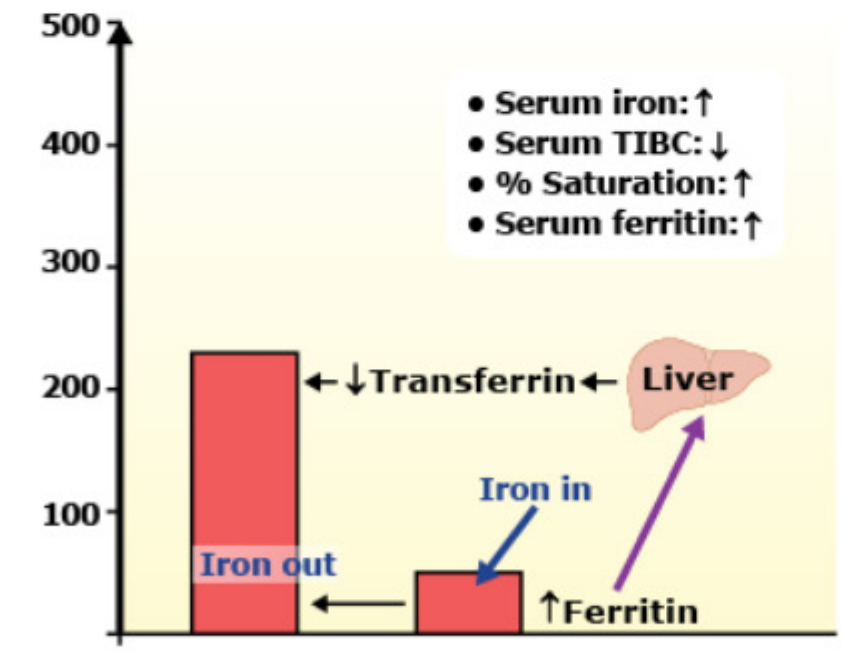
Michael Abbey/Science Source

▲ **Figure 6–3.1H** Ringed Sideroblasts



Wellcome Images

▲ **Figure 6–3.1I** Basophilic Stippling



▲ **Figure 6–3.1J** Iron Overload: Sideroblastic Anemia

3.2 Defective Globin Synthesis

Thalassemias are caused by gene mutations leading to decreased production of globin protein.

3.2.1 α-Thalassemia

- Deletions of α-globin genes cause α-thalassemia.
- There are two α-globin genes closely linked on chromosome 16.
- Total of four alleles.

Etiology Characteristics and severity of the disease depend on the number of genes deleted.

▼ Table 6–3.2A α-Thalassemia Genotype Expression

Syndrome	Genotype	Clinical Features
Silent carrier	-α / αα	Asymptomatic
α-Thalassemia trait	-- / αα -α / -α	Asymptomatic, with mild anemia
HbH disease	-- / -α	Intermediate to severe chronic anemia
Hydrops fetalis	-- /--	Lethal in utero or neonatal

Pathogenesis

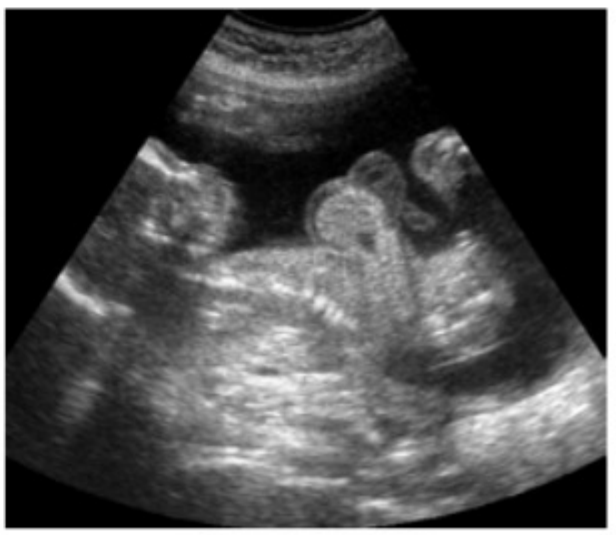
- α-Thalassemia trait:
  - Most common thalassemia in patients of Southeast Asia.
  - Two inheritance patterns observed based on ethnic descent:
    - —Asian (-- allele) descent
    - —African (-α allele) descent
- There are *two consequences* of 3-deletion α-globin deficiency:
  - Quantitative imbalance between α- and β-globin proteins resulting in the formation of insoluble 4β-globin-*HbH* or 4γ-globin-*Hb Barts* aggregates in the RBC.
  - These RBCs are often cleared in the spleen and liver, worsening the anemia.

Memory Aid

Mnemonic for α-Thalassemia (Hemoglobin Barts) disease: "4-gamma rays are affecting Bart."

Clinical Pathology

- **HbH Disease**
  - Signs and symptoms of anemia.
  - Chronic hemolysis with variable jaundice and cholelithiasis (bilirubin stones).
  - Extramedullary hematopoiesis with frontal bossing and hepatosplenomegaly.
- **Hydrops Fetalis**
  - Anasarca (generalized edema) from high-output heart failure.
  - Hepatosplenomegaly.
  - Causes death in the prenatal period.

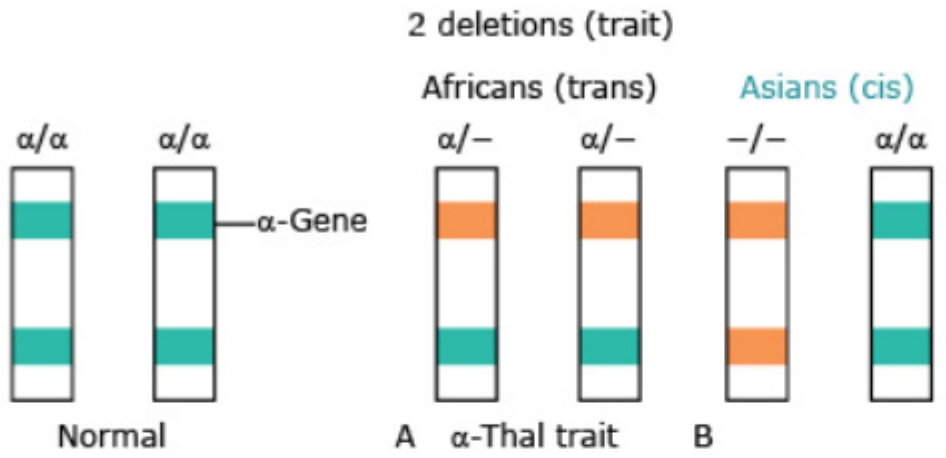


Dr. Najeeb Layyous/Science Source

▲ Figure 6–3.2A Hydrops Fetalis

Laboratory Studies

- Decreased Hgb, Hct, MCV.
- Often an increased RBC count, RDW.
- HbH on hemoglobin electrophoresis.
- Iron studies normal.

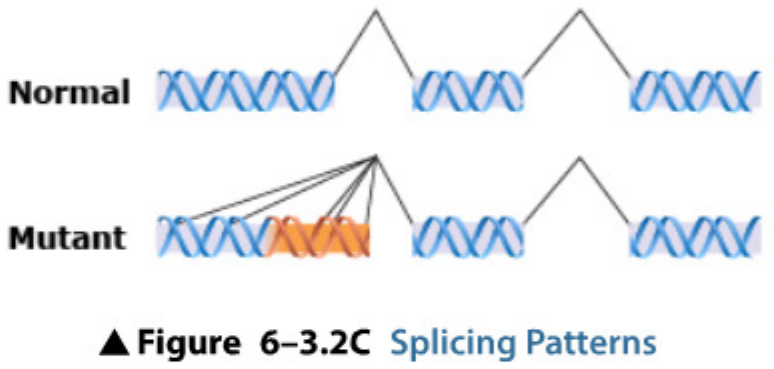


▲ **Figure 6–3.2B** α-Thalassemia Trait

3.2.2 β-Thalassemia

Etiology and Epidemiology Found in African, Italian, and Greek populations:

- There is only one β-globin gene on chromosome 11.
- Total of *two alleles* (α-thalassemia has issues with four alleles).
- β-thalassemia is primarily caused by splicing mutation for minor and nonsense mutation (stop codon) for major.
- There are two types of mutations in the β-globin gene:
  - β^+ mutations: Variable decreased expression.
  - β^0 mutations: Absent expression.



▲ **Figure 6–3.2C** Splicing Patterns

▼ **Table 6–3.2B** β-Thalassemia Genotype Expression

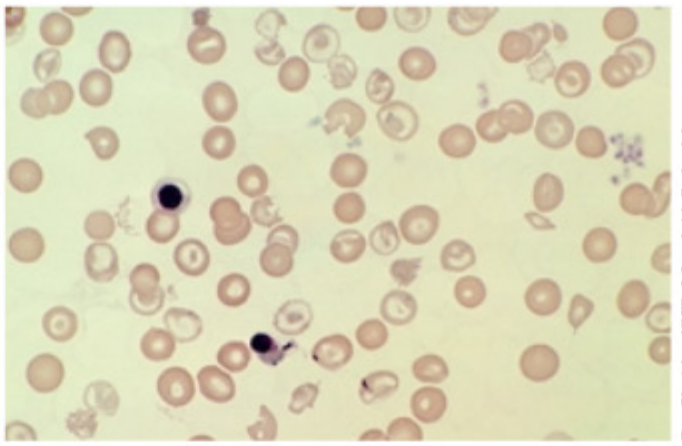
Syndrome	Genotype	Clinical Features
Thalassemia minor	β^0/β or β^+/β	Asymptomatic; mild anemia
Thalassemia intermedia	β^0/β or β^+/β^+	Variable moderate anemia, requiring occasional transfusion
Thalassemia major aka Cooley anemia	β^0/β^0 or β^+/β^+	Severe, transfusion-dependent anemia

Pathogenesis Similar to α-thalassemia.

Morphology Similar to α-thalassemia with erythroblastosis, anisopoikilocytosis, and target cells.

Clinical Pathology β-thalassemia major (Cooley anemia):

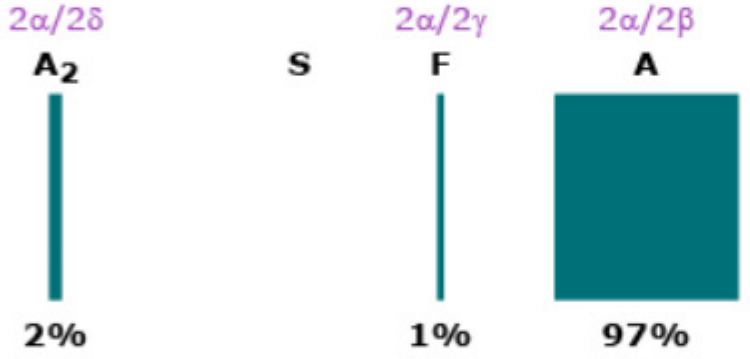
- Causes severe transfusion-dependent anemia that develops at a few months of age (as HbF declines).
- If adequately transfused, children will develop normally, but will develop secondary hemochromatosis and die of heart disease in their 20s.
- If not transfused, there is stunted growth, bony changes, and high-output heart failure with death in infancy.



▲ **Figure 6–3.2D** Peripheral Blood Smear

Laboratory Findings

- Decreased Hgb, Hct, and MCV
- Often an increased RBC count
- Hemoglobin electrophoresis varies depending on the severity of the disease:
  - Decreased HbA
  - Increased HbF (α_2Y_2)
  - increased HbA2 (α2δ2)



Newborns: Splenic macrophages phagocytose and destroy RBCs with HbF and replace with HbA and HbA_2; takes a few months.

▲ **Figure 6–3.2E** Normal HbE

Anemia, but no change in percentage of Hb A, A_2, or F (all of them need α-globin chains for their synthesis).

▲ **Figure 6–3.2F** α-Thalassemia Trait

A_2 S F A 5% 2% 93%	Microcytic β/β^+	β-Thal minor. Note that HbA is decreased, because β-globin chain synthesis is decreased. There is a corresponding increase in HbA_2 and HbF.
A_2 S F A 10% 90%	Microcytic β^0/β^0	β-Thal major. Note that there is no synthesis of HbA.

β = normal
β^+ = some
β^0 = none

▲ **Figure 6–3.2G** HbE of β-Thalassemia Minor

3.3 Summary of Microcytic Anemias

▼ **Table 6–3.3** Microcytic Anemias

Test	Iron Deficiency Anemia	Anemia of Chronic Disease	Thalassemia	Sideroblastic Anemia
RBC Count	↓	↓	↑	↓
MCV	↓	N/↓	↓	↓
RDW	↑	N	N/↑	N
Ferritin	↓	N/↑	N	↑
Serum Iron	↓	↓	N	↑
TIBC	↑	↓	N	↓
Tfn Saturation	↓	↓	N	↑

4 Macrocytic Anemias

The most common causes of macrocytic anemia are vitamin B12 deficiency and folate deficiency.

- Vitamin B12 and folate are required for multiple steps in DNA synthesis.
- Vitamin B12 and folate deficiencies cause defects in DNA synthesis and cell division.

4.1 Vitamin B12

Steps of vitamin B12 metabolism:

1. Consumption of vitamin B12 in meat products.
2. Vitamin B12 binds to R factor in saliva, which protects it from acid destruction.
3. Gastric acid converts pepsinogen to pepsin, which frees vitamin B12 from ingested proteins.
4. Parietal cells (body/fundus) synthesize intrinsic factor (IF).
5. Pancreatic enzymes cleave off R factor in duodenum, allowing vitamin B12 to form complex with IF.
6. Vitamin B12: IF complex reabsorbed in terminal ileum.
7. Vitamin B12 binds to transcobalamin II and is delivered to liver, marrow, and actively dividing cells.

▼ **Table 6–4.1** Vitamin B12 Deficiency

Type	Cause	Discussion
Decreased intake	Vegan diet	Breast fed infants of vegans
	Malnutrition	Elderly
Malabsorption	Decreased intrinsic factor	Autoimmune destruction of parietal cells
	Decreased gastric acid	Cannot activate pepsinogen
	Decreased intestinal reabsorption	Crohn disease, celiac disease, bacterial overgrowth, fish tapeworm, chronic pancreatitis
Increased use	Pregnancy/lactation	Deficiency in vegan

4.2 Folate

Steps of folic acid metabolism:

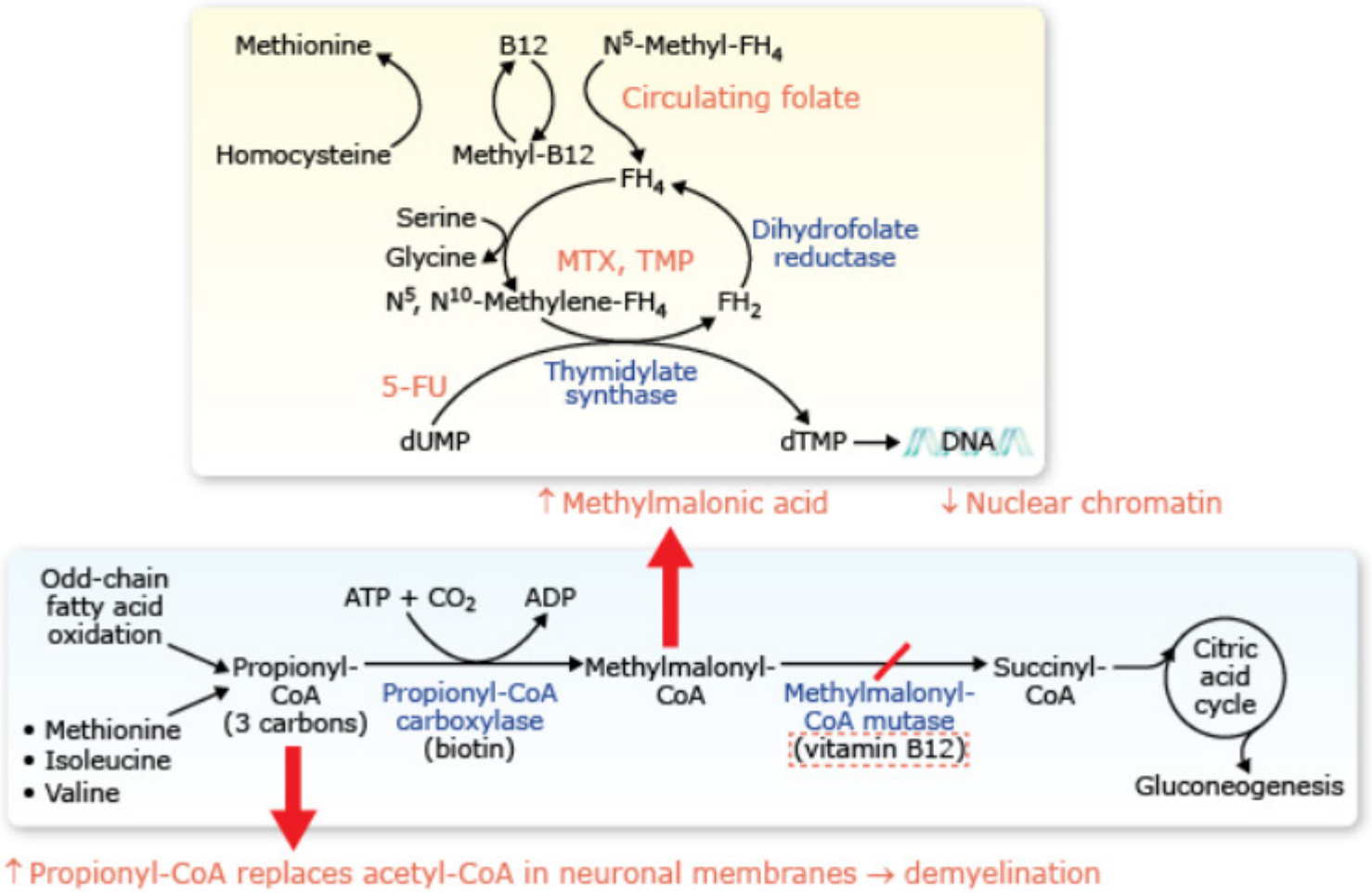
1. Consumption of folic acid in green vegetables and meat products.
2. Polyglutamate converted to monoglutamate by intestinal conjugase in jejunum.
3. Monoglutamate reabsorbed in jejunum.

Important Concept

There is only a three- to four-month supply of folate in liver.

▼ **Table 6–4.2** Folic Acid Deficiency

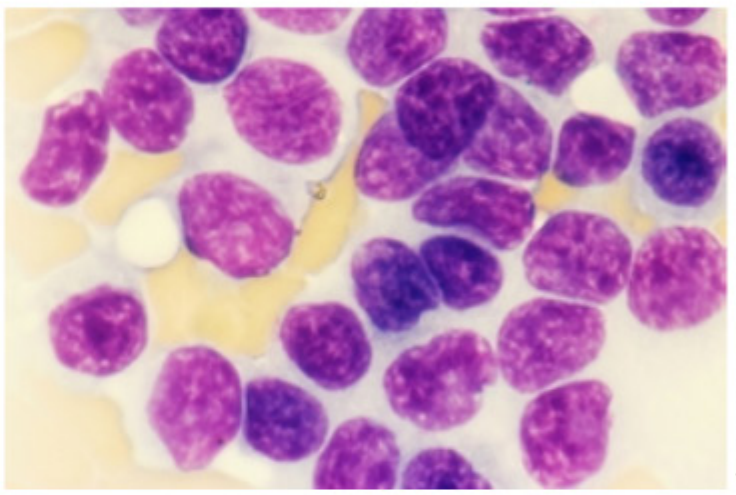
Type	Cause
Decreased intake	Infants and elderly
	Malnutrition
	Chronic alcoholism
	Consumption of goat's milk
Malabsorption	Celiac disease
Drug inhibition	5-Fluorouracil
	Methotrexate, TMP
	Phenytoin
	OCP, alcohol
Increased use	Pregnancy/lactation
	Disseminated malignancy
	Severe hemolytic anemia



▲ **Figure 6–4.2** Biochemical Pathways of Folate and Vitamin B12

4.3 Clinical Features

- **Combined Folate and Vitamin B12 Deficiency:**
  - Signs and symptoms of anemia
  - May present with glossitis
- **Folate Deficiency:** May present as neural tube defects in neonate
- **Vitamin B12 Deficiency:**
  - Causes peripheral neuropathy with sensorimotor dysfunction
  - Subacute combined degeneration (demyelination) of spinal cord
    - —Demyelination of the posterior columns (loss of vibratory sensation and proprioception)
    - —Demyelination of dorsal spinocerebellar tract (ataxia and wide-based gait)
    - —Lateral corticospinal tract of the spinal cord (spasticity)
- **Laboratory Findings:**
  - *Decreased* Hgb and Hct
  - *Increased* MCV and RDW
  - Hypersegmented neutrophils
  - Folate deficiency
    - —Decreased serum and RBC folate
    - —Increased homocysteine
  - B12 deficiency
    - —Decreased serum B12
    - —Increased serum homocysteine and methylmalonic acid



Michael Abbey/Science Source

▲ **Figure 6–4.3** Megaloblast

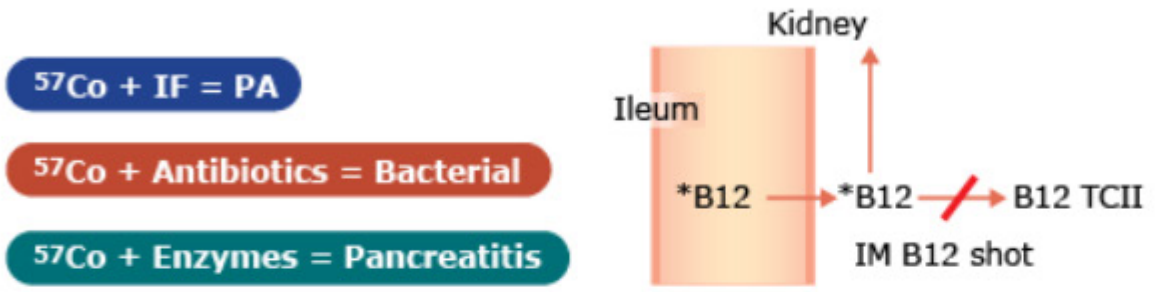
▼ **Table 6–4.3** Clinical Features and Laboratory Findings

Finding	Pernicious Anemia	Other Vitamin B12 Deficiencies	Folate Deficiency
Achlorhydria	*Present*	Absent	Absent
Autoantibodies	*Present*	Absent	Absent
Chronic atrophic gastritis	*Present*	Absent	Absent
Gastric carcinoma risk	Increased	None	None
Hypersegmented neutrophils	Present	Present	Present
Mean corpuscular volume	Increased	Increased	Increased
Neurologic disease	Present	Present	*None*
Pancytopenia	Present	Present	Present
Plasma homocysteine	Increased	Increased	Increased
Serum gastrin level	*Increased*	Normal	Normal
Urine methylmalonic acid	Increased	Increased	*Normal*

4.4 Schilling Test

Now rarely used, the Schilling test is sometimes performed because of its historical significance in demonstrating the impairment of vitamin B12 reabsorption.

- Administration of radioactive vitamin B12 combined with intrinsic factor (IF) followed by a 24-hour urine collection to measure excreted B12.
- Pernicious anemia diagnosed if increased levels of radioactive vitamin B12 are found in urine.



* Giving radioactive B12 in large doses blocks all the transcobalamin II binding sites, so any radioactive B12 must be excreted in urine.

▲ **Figure 6–4.4** Schilling Test

4.5 Treatment

- **Folate Deficiency:** Treated with oral folic acid supplements and suggestions for improved diet. Symptoms resolve with treatment.
- **Vitamin B12 Deficiency:** Treated with supplemental intramuscular injections. However, the neurologic manifestation (subacute combined degeneration) is irreversible if left untreated for too long.

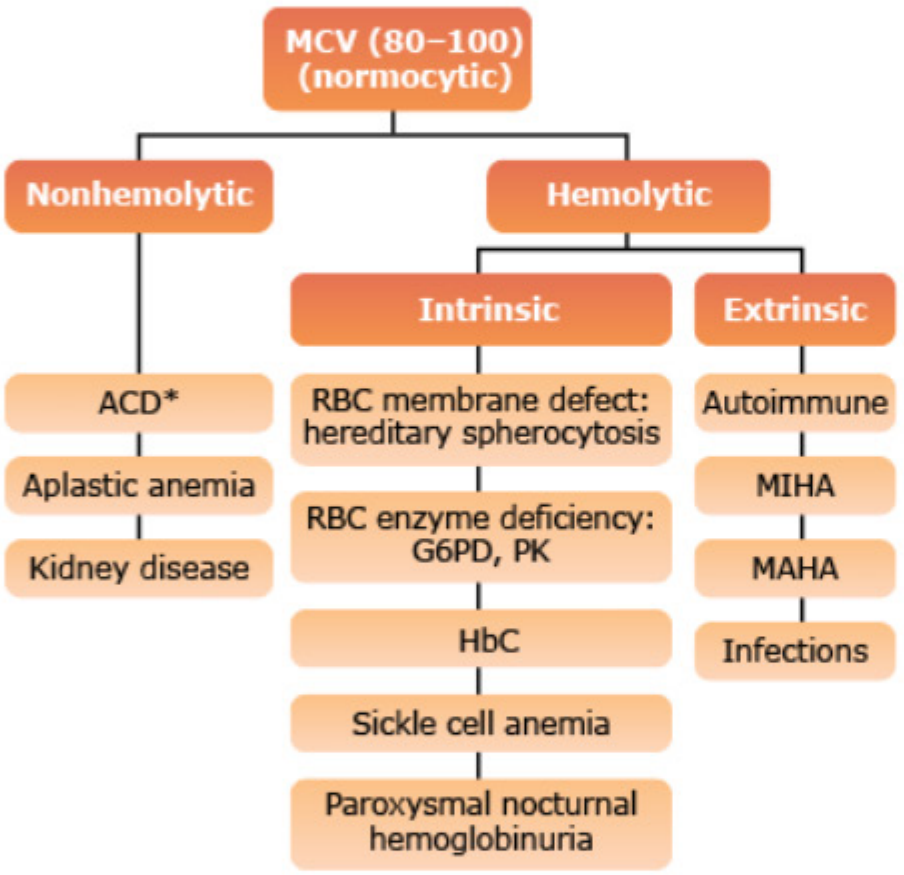
Important Concept

If a negligent doctor only prescribes folate supplements for megaloblastic anemia caused by vitamin B12 deficiency, neurological deterioration will persist to the point of irreversibility.

5 Normocytic Anemia

5.1 Overview of Normocytic Anemia

Normocytic anemias are the largest classification of anemias. This type of anemia refers to a red blood cell appearing normochromic (normal MCHC) and normocytic (normal MCV). Globally, the hemoglobin levels are decreased, and the reticulocyte count is altered. This alteration of the reticulocyte count is the true determinant of the various categories of normocytic anemia. The category of normocytic anemia with corrected reticulocyte count is less than 3% and primarily due to bone marrow failure; these are considered nonhemolytic anemias. If the corrected reticulocyte count is found to be greater than 3%, these anemias are considered to be hemolytic, and fall under either intravascular or extravascular damage, as will be discussed below.

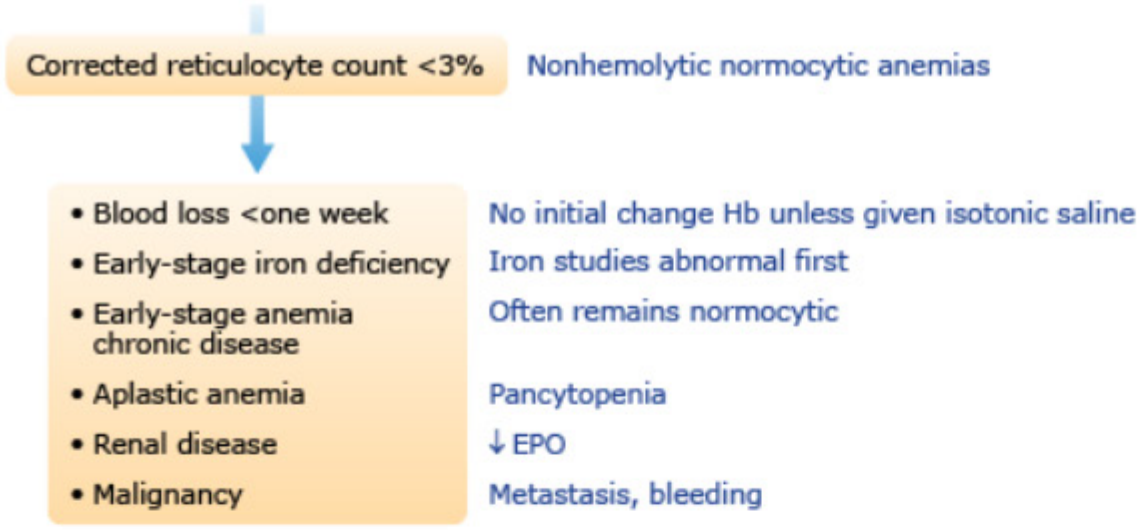


▲ **Figure 6–5.1** Normocytic Anemias

5.2 Nonhemolytic Anemia

5.2.1 Classification

- Corrected reticulocyte count <3% (acute blood loss of less than a week)
- Decreased RBC production (no reticulocytosis):
  - Aplastic anemia (bone marrow failure)
  - Anemia of renal disease (insufficient erythropoietin)
  - Myelophthisic anemia (bone marrow destruction due to metastasis into bone marrow from a primary cancer)



▲ **Figure 6–5.2A** Corrected Reticulocyte Count

5.2.2 Acute Blood Loss

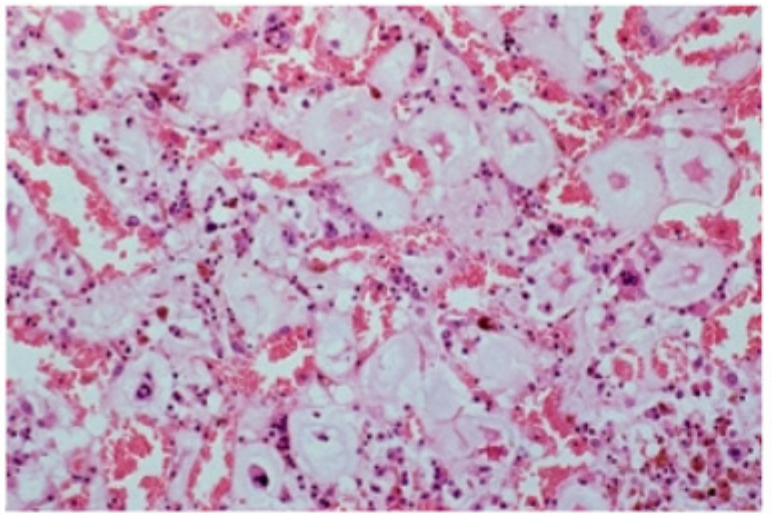
- Major causes:
  - Trauma
  - GI bleeding: Ulcer, varices, diverticuli
- No anemia initially as plasma is lost with RBCs; when a whole blood compartment gets smaller, it loses its ability to dilute and thus does not initially show signs of anemia.
- Plasma is replaced first by retaining volume in the kidneys; this reveals the anemia by diluting the RBCs.
- Bone marrow takes five to seven days to respond with reticulocytosis.

5.2.3 Bone Marrow Failure

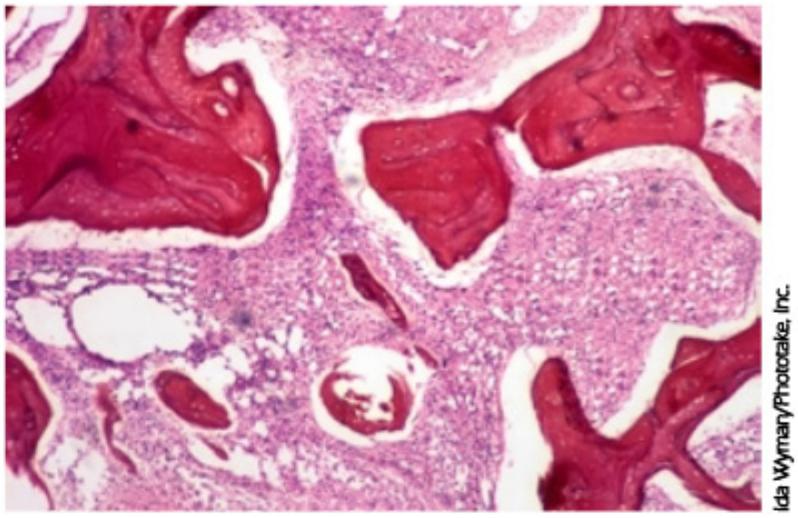
- **Aplastic Anemia**
  - Pancytopenia
  - Normocytic anemia without reticulocytosis
    —Leukopenia: Susceptibility to infection
    —Thrombocytopenia: Bleeding diathesis
  - Etiology of aplastic anemia:
    —Idiopathic/autoimmune most common
    —Drugs (chloramphenicol, chemotherapy)
    —Infection (EBV, CMV, parvovirus B19)
    —Congenital (Diamond-Blackfan syndrome, Fanconi anemia)
- **Anemia of Renal Failure:** Caused by decreased production of erythropoietin by the kidneys.
- **Myelophthisic Anemia:** Replacement of bone marrow by space-occupying lesions:
  - Leukemia or metastatic cancer
  - Fibrosis: Chronic idiopathic myelofibrosis

Important Concept

Don't confuse Fanconi anemia and Fanconi syndrome—both are frequently tested in Step 1.



▲ **Figure 6–5.2B** Normal Bone Marrow



▲ **Figure 6–5.2C** Fibrosed Bone Marrow Secondary to Breast Cancer Metastasis

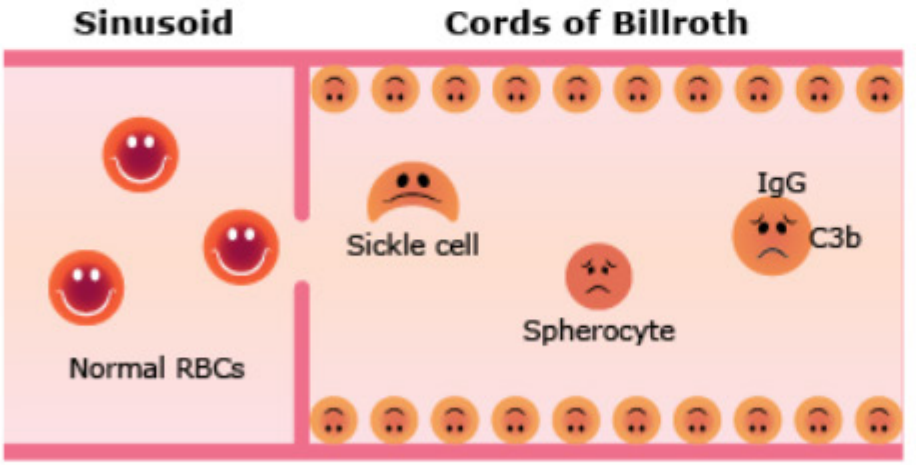
5.3 Hemolytic Normocytic Anemia

These anemias are caused by intrinsic or extrinsic defects:

- **Intrinsic Defects:** These are part of the RBC itself—structural proteins, enzymes, membrane defects, etc.
- **Extrinsic Defects:** These are outside of the RBC—mechanical destruction, autoimmune destruction, etc.

Hemolytic anemias can also be classified by site of RBC destruction:

- **Intravascular Hemolysis:** Hemolysis occurs in the circulation. Hemoglobinuria results in decrease in haptoglobin and increase in LDH. Haptoglobin complexes with Hb and is removed by macrophages (the amount of UCB is usually not high enough to produce jaundice).
- **Extravascular Hemolysis:** RBCs are removed from circulation by the reticuloendothelial system (macrophages in the liver and spleen) and destroyed.



- Unconjugated bilirubin = Jaundice
- Lactate dehydrogenase (nonspecific)

▲ **Figure 6–5.3A** Extravascular Hemolysis

5.3.1 Clinical Features

- Hemoglobinemia/hemoglobinuria
- Jaundice
- Decreased serum haptoglobin
- Increased serum LDH
- Cholelithiasis with bilirubin stones
- Hepatosplenomegaly
- Corrected reticulocyte count ≥3%

5.3.2 Hereditary Spherocytosis

- Intrinsic
- Extravascular

Etiology Autosomal dominant disorder of RBC membrane proteins that maintain the integrity of the cell membranes. These include spectrin and ankyrin.

Pathogenesis

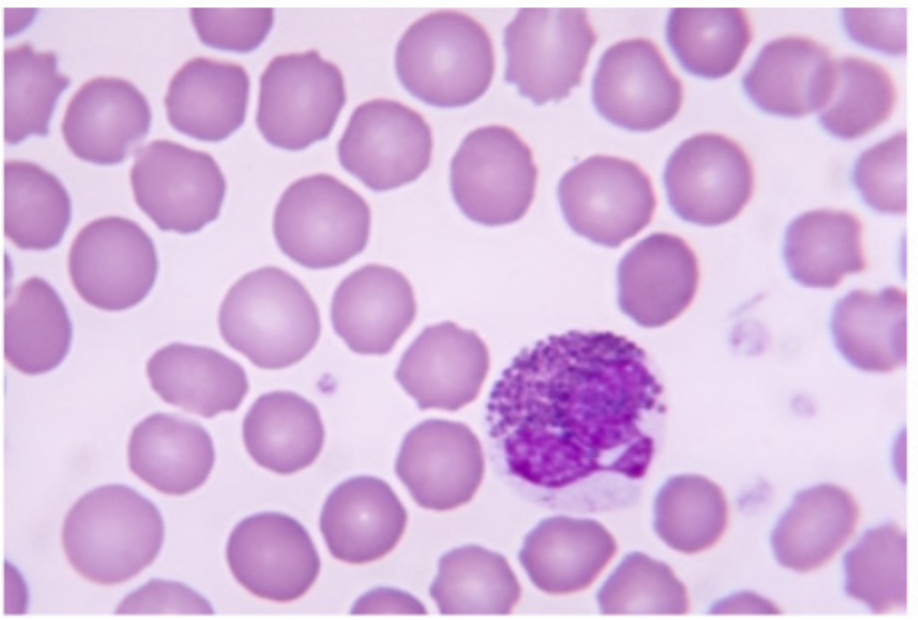
- Loss of membrane fragments causes the RBCs to assume a spherical, rather than biconcave, shape.
- The spherocytes are small, round, and thus MCHC is increased.
- Damage to the Na^+/K^+ ATPase pump from the mutation results in increased osmotic pressure within the cell.

Clinical Pathology

- Because spherocytes are destined for extravascular hemolysis, splenomegaly is observed.
- *Aplastic crisis* with *parvovirus B19* infection is due to decreased RBC life span.

Diagnosis

- Diagnosed with *osmotic fragility test* (increased hemolysis in hypotonic solution).
- Coombs negative (i.e., no antibodies on RBCs).



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▲ **Figure 6–5.3C** Spherocytes

Corrected reticulocyte count ≥3%

Intrinsic RBC defect

- Membrane defects
  - Hereditary spherocytosis
  - Hereditary elliptocytosis
  - Paroxysmal nocturnal hemoglobinuria
- Abnormal hemoglobins
  - Sickle cell disease
- Deficient enzymes
  - G6PD deficiency
  - Pyruvate kinase deficiency

▲ **Figure 6–5.3B** Reticulocyte Count of >3%

Memory Aid

To remember the intrinsic RBC defects that cause hemolytic anemia, use the mnemonic **MAD:**

- **M**embrane defects
- **A**bnormal hemoglobin
- **D**eficiency of enzymes

5.3.3 Paroxysmal Nocturnal Hemoglobinuria

- Intrinsic
- Intravascular

Etiology Clonal deficiency in glycosyl phosphatidyl inositol (GPI)-linked proteins on RBCs, neutrophils, and platelets.

Pathophysiology One of the GPI-linked proteins is decay-accelerating factor (DAF), which neutralizes complement attached to RBCs, neutrophils, and platelets. In the absence of this protein, RBCs are susceptible to complement-mediated intravascular hemolysis.

Clinical Pathology

- Causes episodic *(paroxysmal)* hemolysis when complement is activated by mild acidosis.
- Respiratory acidosis during sleep *(nocturnal)*.
- Lactic acidosis during exercise.
- Over time, can lead to iron deficiency from chronic blood loss.

Diagnosis

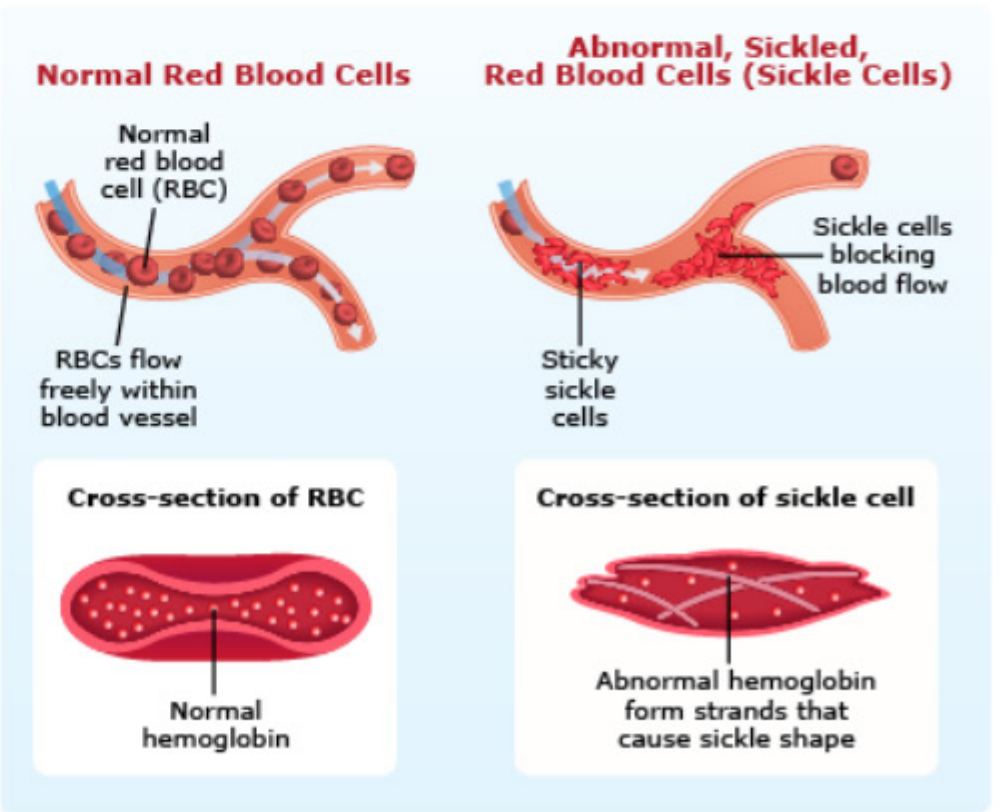
- Lysis of RBCs in sucrose (sucrose lysis test) or acid (Ham test).
- Flow cytometry to detect GPI-linked proteins.

5.3.4 Sickle Cell Disease

- Intrinsic
- Extravascular

Etiology

- Sickle cell disease is an autosomal recessive genetic disorder caused by a missense mutation (GAG → GTG) at the second nucleotide of the sixth codon in the HBB gene on chromosome 11, which results in a glutamic acid → valine substitution (E6V) in the β-globin subunit of hemoglobin.
- The significance of *Hemoglobin C* along with Hemoglobin S → HbSC is that sickle cell disease may also be caused by coinheritance of the E6V HBB gene mutation with another HBB gene mutation, as follows:
  - E6V HBB gene mutation + is a missense mutation, which results in a glutamic acid → lysine substitution (E6K) in the HBB gene forming HbC.
  - The result is the coexistence of both E6V HbS and E6K HbC within red blood cells (HbSC).



▲ **Figure 6–5.3D** Sickling of Red Blood Cell

Pathogenesis

- *Deoxyhemoglobin S* has a tendency to polymerize.
- Polymerization causes sickling of RBCs under conditions of low oxygen tension:
  - Infection
  - Dehydration
  - Hypoxia
- Sickled cells:
  - Are cleared in the spleen causing hemolytic anemia.
  - Block the microvasculature causing vaso-occlusion.

Clinical Pathology

- Vaso-occlusion can lead to pain crises (microvascular ischemia):
  - 100% of SS patients are eventually addicted to opiates.
  - Dactylitis—inflammation and pain of the digits due to ischemia.
- Acute chest syndrome: Hypoxemia caused by microvascular disease of the lung.
- Autosplenectomy: Involution of the spleen causing susceptibility to infection by encapsulated bacteria (100% of SS patients autosplenectomized by adulthood).
- Stroke.
- Painful priapism.
- Also susceptible to:
  - Aplastic crisis with parvovirus B19 infection.
  - *Salmonella* osteomyelitis.
  - Other sequelae of chronic hemolytic anemia.

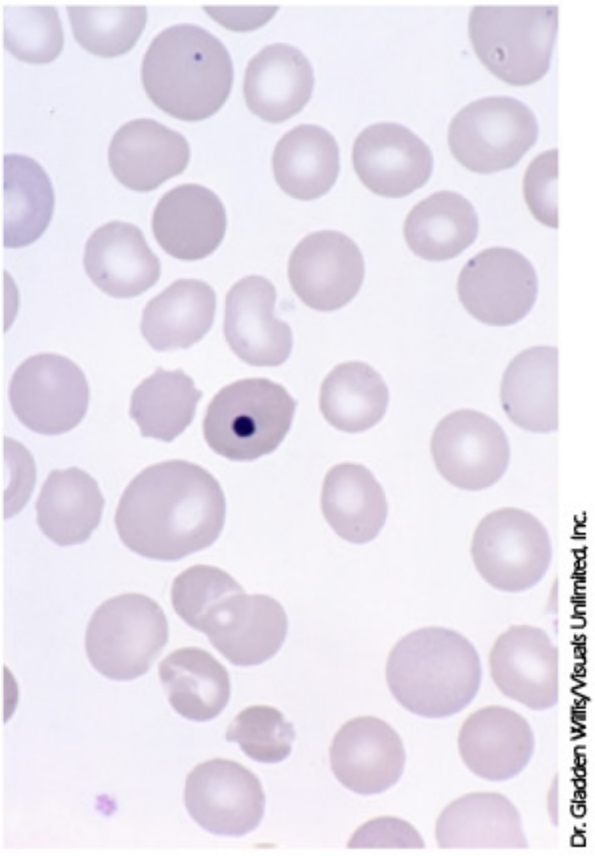
Diagnosis

- Peripheral blood smear:
  - Target cells are a nonspecific finding (i.e., found in thalassemias) and represent any sign of hemoglobinopathy.
  - Sickled cells: RBCs take on a sickled configuration, and when these accumulate in smaller tributaries, they cause ischemia to the distal target tissue.
  - Howell-Jolly bodies are inclusions found in RBCs that represent the first sign of splenic dysfunction. Their presence leaves the patient with increased risk of infection from encapsulated organisms:
    —*Streptococcus pneumoniae*
    —*Neisseria meningitides*
    —*Klebsiella pneumoniae*
    —*Haemophilus influenzae*
    —*Salmonella typhi*
    —*Cryptococcus neoformans*
    —*Pseudomonas aeruginosa*
- Newborn screening: Genetic test for E6V mutation.
- Sickle prep: Peripheral blood sickles when exposed to sodium metabisulfite (reduces oxygen tension).
- Hemoglobin electrophoresis.

Memory Aid

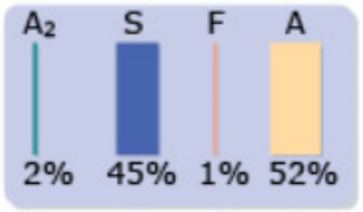
Some **N**asty **K**illers **H**ave **S**lime **C**apsules for **P**rotection:

- ***S****treptococcus pneumoniae*
- ***N****eisseria meningitides*
- ***K****lebsiella pneumoniae*
- ***H****aemophilus influenzae*
- ***S****almonella typhi*
- ***C****ryptococcus neoformans*
- ***P****seudomonas aeruginosa*



Dr. Gladden Willis/Visuals Unlimited, Inc.

▲ **Figure 6–5.3E** Howell-Jolly Bodies

	No anemia	Sickle cell trait. Note that there is not enough HbS to cause spontaneous sickling in the peripheral blood.
	Normocytic	Sickle cell disease. Note that there is no HbA. There is enough HbS to cause spontaneous sickling.

▲ **Figure 6–5.3F** Sickle Cell Trait vs. Sickle Cell Disease

5.3.5 Glucose-6-Phosphate Dehydrogenase (G6PD) Deficiency

- Intrinsic
- Intravascular is the most common manifestation; however, extravascular hemolysis may also take place.

Epidemiology

- Mostly seen in African population where the severity is mild.
- Severe forms are seen in Greeks and Italians.

Etiology

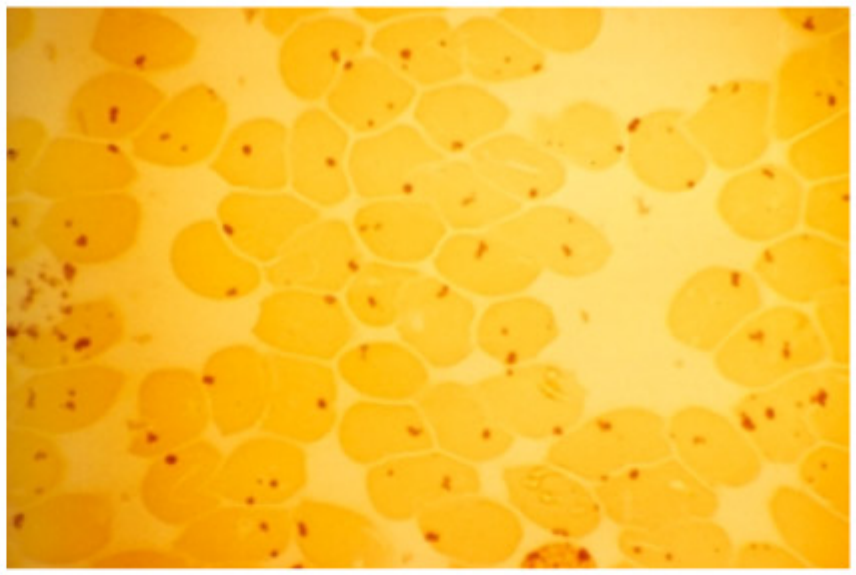
- X-linked recessive inheritance pattern is the most common presentation.
- Mutation in glucose-6-phosphate dehydrogenase (G6PD), the rate-limiting step in the pentose phosphate shunt; the mutation decreases the half-life of the enzyme ($t^1/_2$=13 days, normal 62).
- Agents that provide oxidant stress:
  - Infections
  - Oxidizing drugs:
    - —Primaquine
    - —Dapsone
    - —Chloroquine
    - —Nitrofurantoin
    - —Sulfonamides
  - Fava beans

Pathophysiology

- The pentose phosphate shunt is the only source of NADPH in RBCs.
- NADPH is required for recycling of glutathione.
- Reduced glutathione, the plenished form of glutathione with NADPH, acts as an antioxidant. NADPH is synthesized in the pentose phosphate shunt with the help of G6PD.
- G6PD deficiency leads to oxidative damage, including hemoglobin precipitation *(Heinz bodies)* and membrane damage *(bite cells)*.
- Heinz bodies = denatured hemoglobin.
- Bite cells = RBCs with membrane damage, partially consumed by macrophages.
- The damaged RBCs are destined to be cleared by the spleen if the damage is incomplete (bite cells). If the macrophages aggressively attack RBCs due to presence of Heinz bodies, and phagocytosis is complete, then this form of hemolysis is intravascular.

Clinical Features

- Clinical phases of G6PD deficiencies:
  - With acute hemolysis, a Heinz body prep is performed, *not* enzyme assay.
  - Post-hemolysis will show *decreased* levels of enzyme.
- Causes episodic hemolytic anemia (pallor, fatigue, jaundice) with oxidative stressors.
- May present with hemolytic anemia concomitantly with recurring Staphylococci infections due to decreased supplies of NADPH.

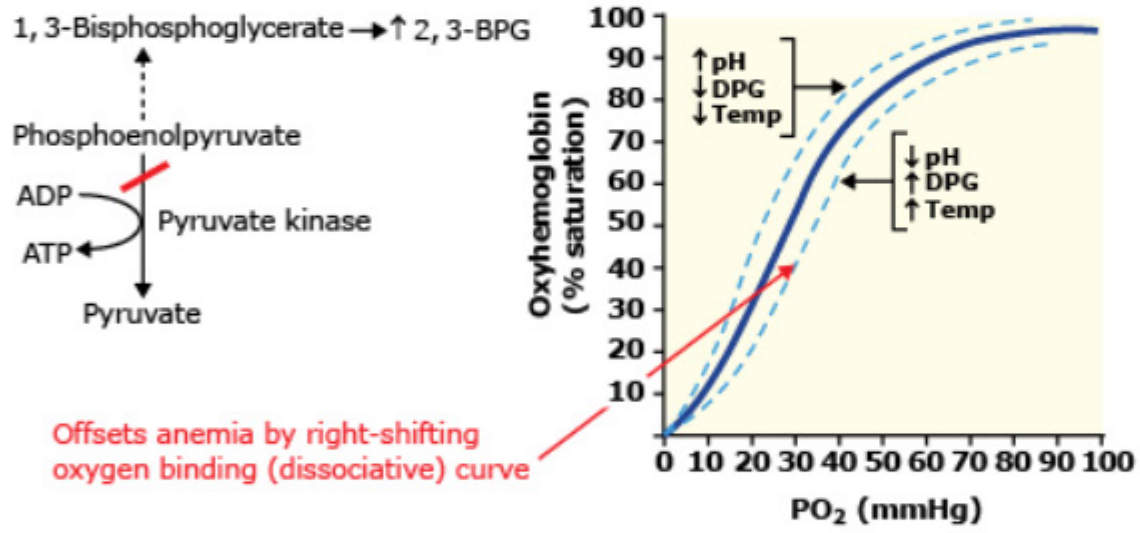


▲ **Figure 6–5.3G** Heinz Bodies

5.3.6 Pyruvate Kinase Deficiency

- Intrinsic
- Extravascular

Etiology Caused by genetic mutations that decrease the activity of pyruvate kinase, the last enzyme in glycolysis.



▲ **Figure 6–5.3H** Role of Pyruvate Kinase in Glycolysis

Pathogenesis

- The RBC has only glycolysis to generate energy (no mitochondria).
- In pyruvate kinase deficiency, therefore, there is little ATP or NADH production.
- ATP deficiency affects the Na^+/K^+ ATPase, leading to osmotic instability and misshapen RBCs that are cleared in the spleen.
- NADH deficiency decreases the reducing power required to convert methemoglobin (Hbg-Fe^{3+}) to hemoglobin (Fe^{2+}).
- Because of the distal block, 2,3-BPG is increased.
- 2,3-BPG binds to hemoglobin and alters its conformation *(allosteric inhibition)*, shifting the oxygen saturation curve to the right.

Morphology Damage to red blood cell membrane causes spiculated RBCs.

Clinical Pathology

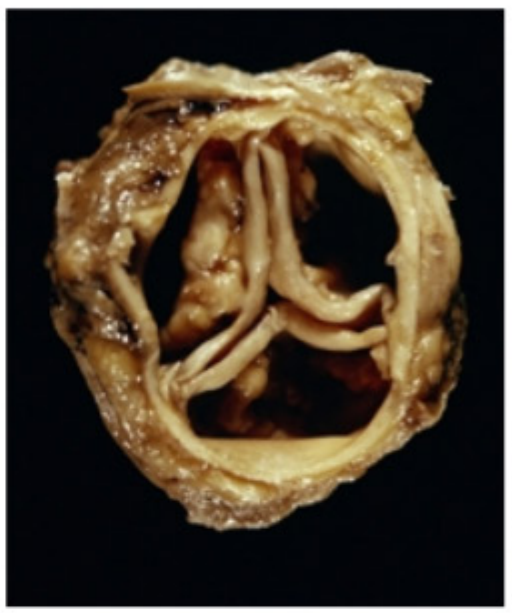
- Usually diagnosed in childhood as a chronic hemolytic anemia.
- Serum methemoglobin is elevated.
- Although pyruvate kinase deficiency (PKD) is the most common of the glycolysis errors, it is still a very rare disease.

5.3.7 Microangiopathic Hemolytic Anemia

- Extrinsic
- Intravascular

Etiology Mechanical damage to RBCs:

- Mechanical or stenotic heart valves (Waring blender effect).
- Concomitant iron deficiency anemia may be present, indicating replacement of the valve.
- Malignant hypertension.
- Diffuse microthrombus formation (fibrin and/or platelets):
  - Disseminated intravascular coagulation (DIC).
  - Thrombotic thrombocytopenic purpura (TTP).
  - Hemolytic uremic syndrome (HUS).



▲ **Figure 6–5.3I** Aortic Stenosis

Pathogenesis RBCs are "sheared" by encountering vascular defects in circulation.

Morphology Schistocytes on peripheral smear.

5.3.8 Autoimmune Hemolytic Anemia

There are two types of this anemia:

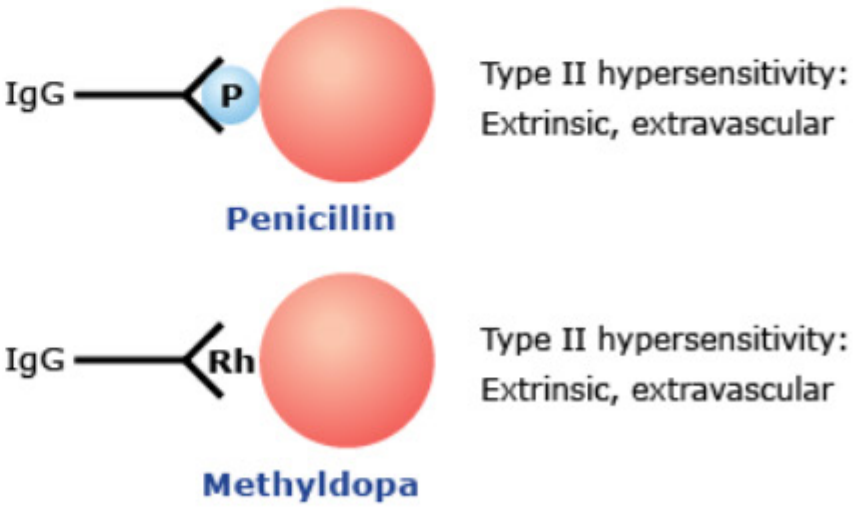
- Warm autoimmune hemolytic anemia (WAIHA)
- Cold autoimmune hemolytic anemia (CAIHA)

Warm Autoimmune Hemolytic Anemia (WAIHA)

- Extrinsic
- Extravascular

Etiology Most cases are idiopathic. Warm (IgG) antibodies are associated with:

- Autoimmune diseases (especially SLE).
- Chronic lymphocytic leukemia (CLL).
- Drugs causing IgG coating:
  - Through the process of adsorption of penicillin on the membrane.
  - α-Methyldopa disrupts the red blood cell membrane so drastically that it renders it foreign to the host's immune system.



▲ **Figure 6–5.3J** WAIHA: Role of Penicillin and α-Methyldopa

Pathogenesis

- Warm-reacting (37°C) IgG autoantibodies formed against RBC surface antigens.
- Coated antibodies are cleared in the spleen.
- Extravascular type of hemolysis.
- Microspherocytes generated when splenic macrophages remove part of antibody-coated membrane.
- Nearly impossible to differentiate from hereditary spherocytosis on a smear alone.

Clinical Features

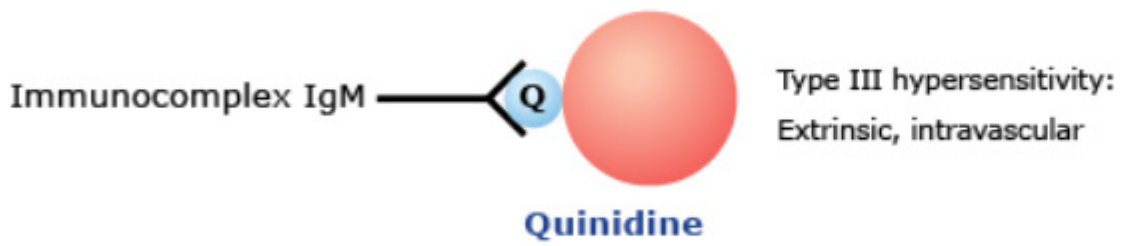
- Signs and symptoms of hemolytic anemia.
- Diagnosed by direct Coombs test.
- Anti-IgG immune globulin is mixed with patient RBCs and incubated at 37°C.
- If the cells agglutinate, this indicates the presence of IgG coating the RBC membrane.
- For transfusions, all blood will have a positive cross-match, so the transfusions are all labeled "incompatible."

Cold Autoimmune Hemolytic Anemia (CAIHA)

- Extrinsic
- Intravascular

Etiology

- CAIHA often follows a viral infection, especially *Mycoplasma pneumonia*.
- Class Ia antiarrhythmic agent, such as quinidine, forms an immune complex with IgM.



The line linking quinidine (Q) to IgM demonstrates its role within the immune globulin complex and thus in the activation of the classical complement cascade and intravascular hemolysis.

▲ **Figure 6–5.3K** CAIHA: Role of Quinidine

Pathogenesis

- Mediated by IgM autoantibodies.
- These antibodies bind RBCs in cold extremities.
- Antibody binding causes complement fixation.
- As the complex travels to warmer parts of the body complement is activated and RBCs hemolyze (intravascular hemolysis).

Clinical Pathology

- Diagnosis is made by identifying cold-reacting (1°–4°C) antibodies by direct Coombs test.
- Cold autoantibodies are fairly common and transient.
- It is uncommon for them to cause serious clinical consequences.

Clinical Application

Using Coombs Test to Diagnose Autoimmune Hemolytic Anemia:

- **Direct Coombs test**
  - Incubate patient RBCs with anti-IgG or IgM (Coombs reagent)
  - If RBCs agglutinate → positive
  - IgG = "warm"
  - IgM = "cold"
- **Indirect Coombs test**
  - Maternal prenatal testing
  - Prior to a blood transfusion
  - Detects unbound anti-RBC antibodies that are in the patient's serum
  - Patient serum is incubated with RBCs of known antigenicity
  - Agglutination → positive

5.3.9 Malaria

- Extrinsic
- Intravascular

Etiology

- Female *Anopheles* mosquito transmits *Plasmodia* to humans
- Intraerythrocytic parasite causes intravascular hemolysis
- Occurrence of intravascular hemolysis correlates with fever spikes

Pathogenesis

- *Plasmodium vivax*
  - Most common
  - Tertian fever every 48 hours
  - Duffy (Fy) antigen on RBCs is the binding site
- *Plasmodium falciparum*
  - Most lethal
  - Quotidian daily fever spikes with no pattern
- *Plasmodium malariae*
  - Association with nephrotic syndrome
  - Quartan fever pattern every 72 hours

6 Myelofibrosis

Excess fibrosis in the bone marrow damages red blood cells by squeezing them through "narrowed" passages.

- A mutation of JAK2 myeloid stem cell results in extramedullary hematopoiesis with neoplastic hematopoietic cells present in the spleen.
- The development of massive splenomegaly commonly causes splenic infarcts.
- Peripheral blood smear present with the following:
  - Tear drop cells due to squeezing of the remaining red blood cells through the fibrosed bone marrow.
  - Immature white blood cells and nucleated red blood cells (NRBCs).

7 Polycythemia

Polycythemia is an excess of red blood cells in the blood.

7.1 Etiology

- Primary: Neoplastic, independent of erythropoietin (polycythemia vera)
- Secondary: Due to increased erythropoietin
  - Chronic hypoxemia (lung disease, congenital heart disease, high altitude)
  - Paraneoplastic syndrome (RCC/HCC)
  - Blood "doping"
- One of the *myeloproliferative disorders*:
  - Essential thrombocythemia (ET)
  - Chronic myelogenous leukemia (CML)

7.2 Pathogenesis

7.2.1 Primary Polycythemia

- Mutation of JAK2 gene on short arm of chromosome 9 of myeloid stem cell results in proliferation in red blood cells, platelets, and white blood cells (except for those of the lymphoid lineage).
- There is a *normalization of SaO_2* with a normal negative feedback effect on *EPO*, which is *decreased*.

7.2.2 Secondary Polycythemia

- Hypoxia for any reason, for example high altitude, results in *decreased SaO_2* and thus an appropriate *increase in EPO*.
- Ectopic production of EPO most commonly from renal cell carcinoma resulting in an *increase in EPO* and *normalization of SaO_2*.

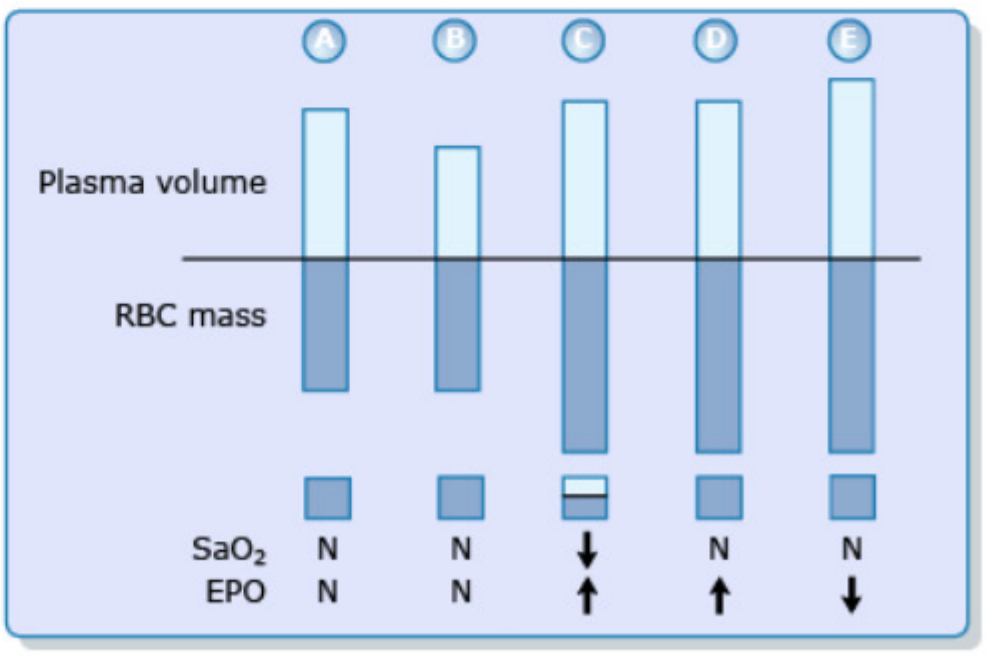
7.3 Laboratory Findings

- Markedly increased RBCs on peripheral smear.
- Increased RBC and myeloid precursors in bone marrow.

7.4 Diagnosis

In figure 6-7.4A, the parameters to take into consideration for proper designation of condition include:

- Plasma volume
- Red Blood Cell mass which is measured as:
  - RBCs mL/kg body wt.
  - RBC count cells/µL
- Percent saturation of oxygen (SaO_2)
- Plasma erythropoietin (EPO) levels



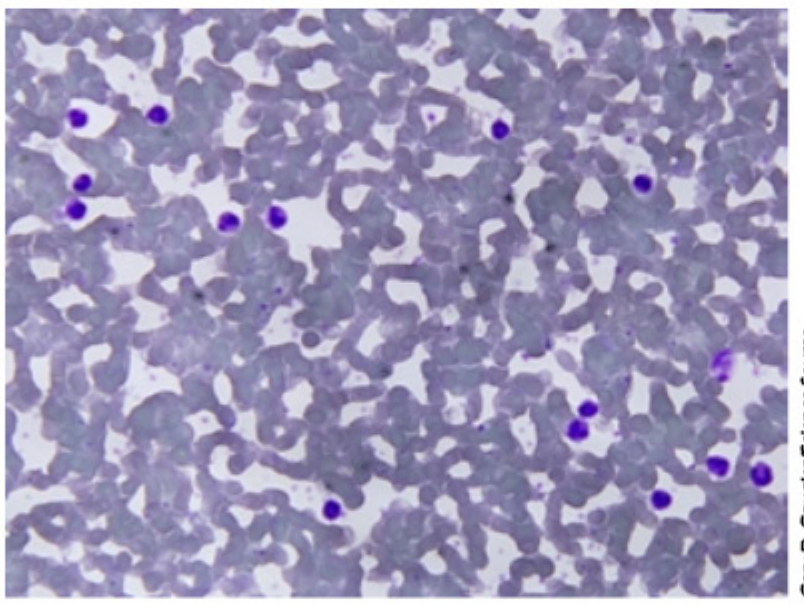
▲ **Figure 6–7.4** Polycythemia

▼Table 6–7.4 Differentials of Polycythemia

Differentials of Polycythemia	Plasma Volume	RBC Mass	SaO_2	EPO
Volume depletion (e.g., excessive sweating, secretory diarrhea)	Decreased	Normal	Normal	Normal
Appropriate absolute (e.g., cyanotic CHD, COPD, high altitude)	Normal	Increased	Decreased	Increased
Inappropriate absolute: Ectopic EPO (renal cell cancer)	Normal	Increased	Normal	Increased
Polycythemia vera	Increased	Increased	Normal	Decreased

7.5 Clinical Features

- Increased RBC mass is more viscous and puts patients at risk for thrombosis (stroke, bowel infarction, hepatic vein thrombosis, etc.). This is the most common cause of death in these patients.
- Pruritus after bathing, due to histamine release from mast cells in the skin.



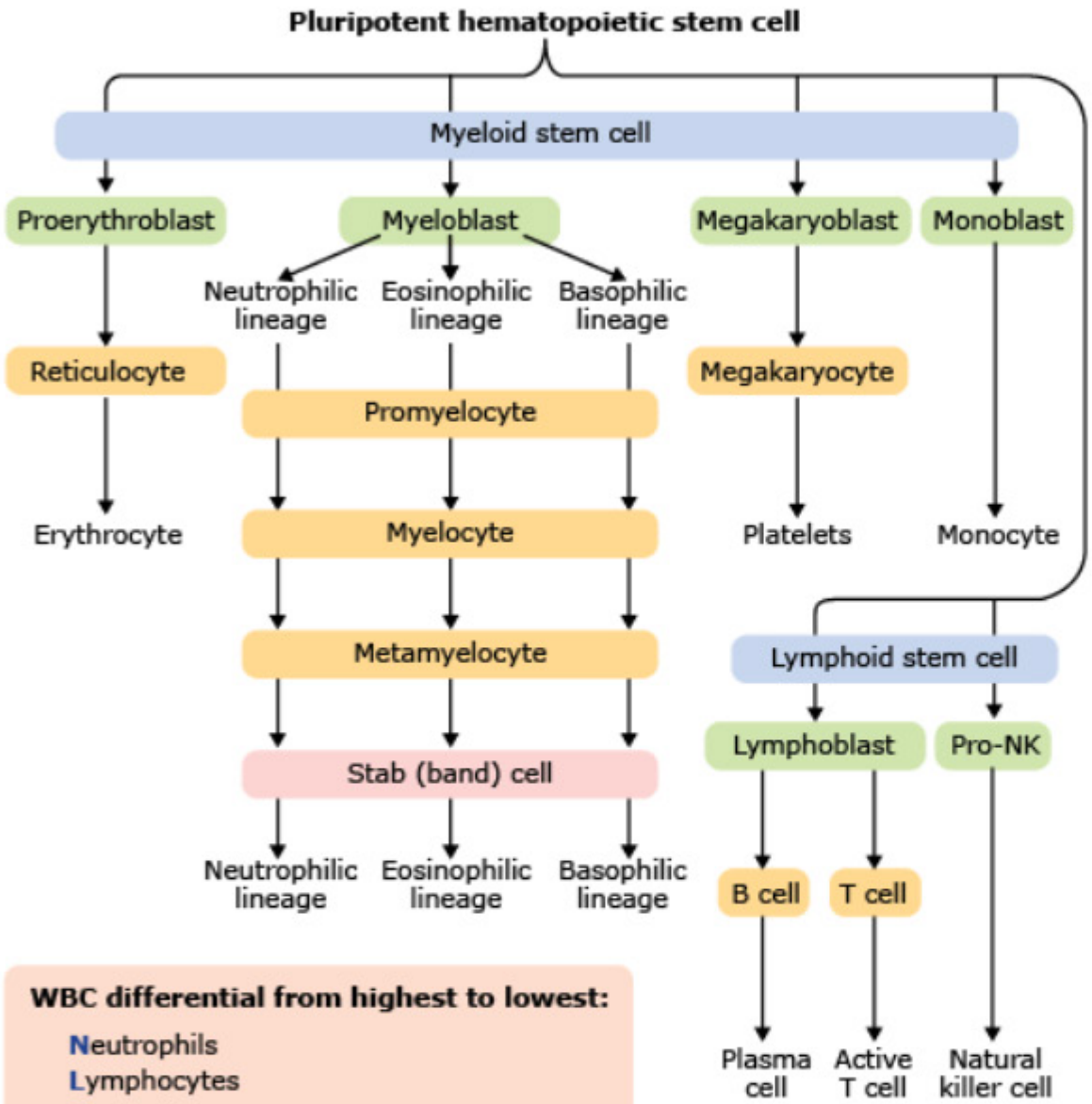
Gary D. Gaugler/Science Source

▲Figure 6–7.5 Polycythemia Vera Bone Marrow

▼Table 6–7.5 Types of Polycythemia

Type	RBCs	WBC	Platelets	Philadelphia Chromosome	JAK2 Mutations
Polycythemia vera	Increased	Increased	Increased	Negative	Positive
Essential thrombocytosis	Unaffected	Unaffected	Increased	Negative	Positive (30%–50%)
Chronic myelogenous leukemia	Decreased	Increased	Increased	Positive	Negative
Myelofibrosis	Decreased	Variable	Variable	Negative	Positive (30%–50%)

1 Blood Cell Differentiation



WBC differential from highest to lowest:

Neutrophils
Lymphocytes
Monocytes
Eosinophils
Basophils

Neutrophils **L**ike **M**aking **E**verything **B**etter

▲ **Figure 7–1.0** Blood Cell Differentiation

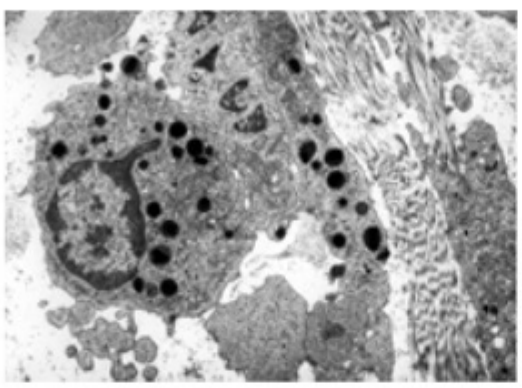
USMLE® Key Concepts

For Step 1, you must be able to:

- Describe the morphology of white blood cells.
- Explain the quantitative disorders of white blood cells, leukocytosis and leukopenia.
- Differentiate acute and chronic leukemias.

2 Review of Morphology

2.1 Types of White Blood Cells



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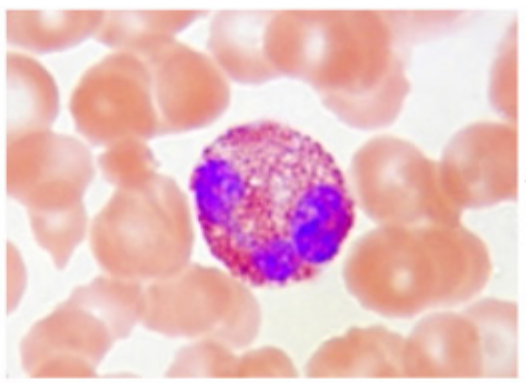
Eosinophil



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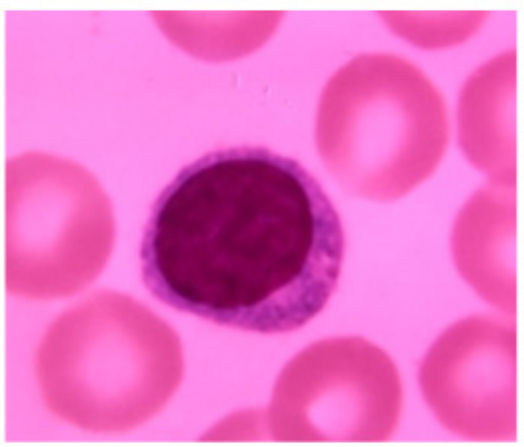
Eosinophil



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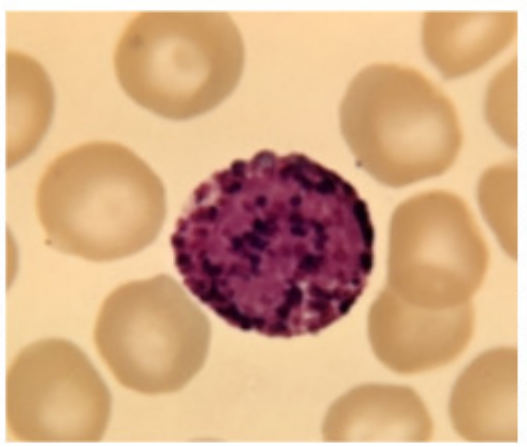
Lymphocyte



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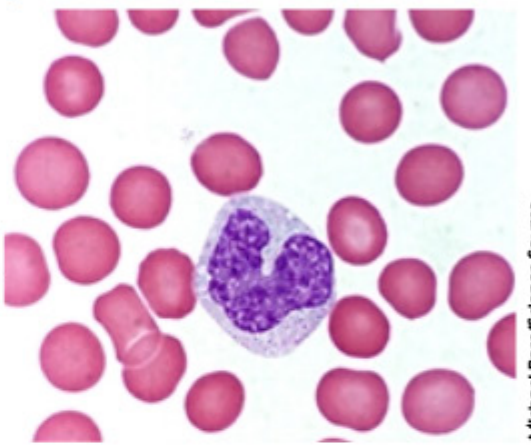
Basophil



Michael Ross/Science Source

Monocyte

▲ **Figure 7–2.1** White Blood Cells

2.2 Peripheral Smear

2.3 Relative Proportions of Leukocytes

- Total WBC count: 4,000–10,000/mm^3
- Segmented neutrophils: 56%
- Lymphocytes: 34%
- Monocytes: 4%
- Bands: 3%
- Eosinophils: 3%
- Basophils: 0.3%

3 Quantitative Disorders

3.1 Leukocytosis

3.1.1 Neutrophilic Leukocytosis

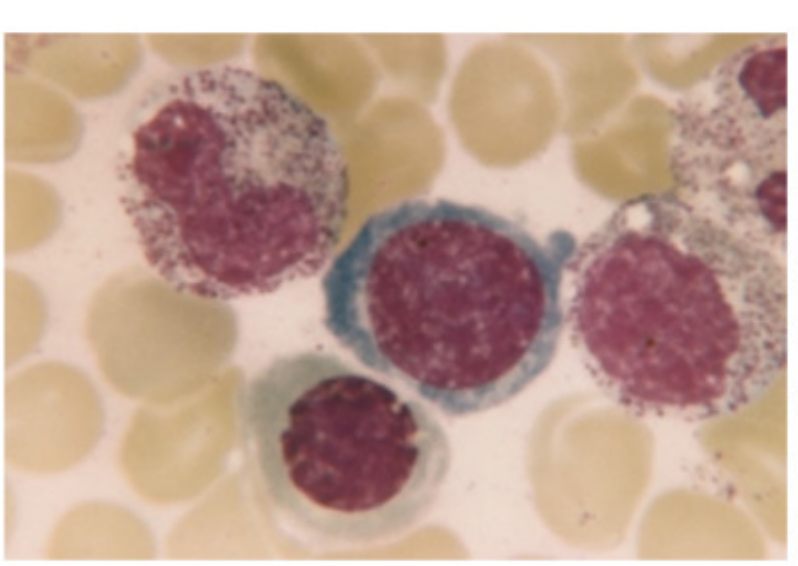
Definition: Elevated WBC count with absolute neutrophil count >7,000 (NL 2,200–5,600/mm^3)

Etiology

- Infection (especially bacterial infection)
- Sterile inflammation with necrosis (for example, myocardial infarction)
- Drugs (most commonly corticosteroids)

Pathogenesis

- Cytokines stimulate:
  - Increased production from bone marrow.
  - Early release—this causes "left shift," such as an increase in immature forms (*for example,* bands).
- Corticosteroids cause apparent neutrophilia by decreasing adhesion of neutrophils to endothelium (*demargination*).



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▲ **Figure 7–3.1A** Leukoerythroblastic

- Two extreme cases:
  - Leukemoid Reaction
    - —Exaggerated response to serious infection (perforating appendicitis, sepsis, etc.).
    - —The presentation appears like a leukemia, but it is not. There is no anemia, thrombocytopenia, or blast population.
    - —WBC count >50,000.
    - —May involve all cell types.
  - Leukoerythroblastic Reaction
    - —It is like having bone marrow cells in the peripheral blood.
    - —Increase in immature WBCs, including myeloblasts.
    - —Caused by infiltrative bone marrow diseases or multiple fractures.

3.1.2 Lymphocytosis

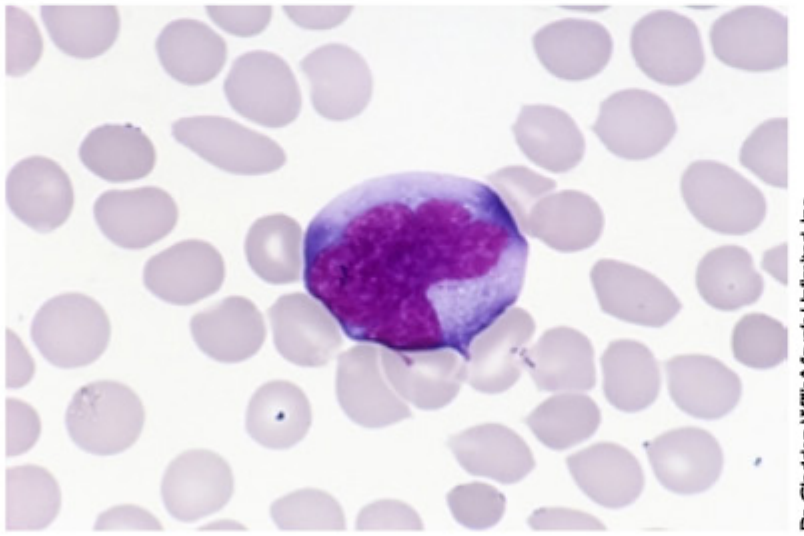
Definition: Elevated WBC count with absolute lymphocyte count >4,000 (adults) or >8,000 (children).

Etiology

- Infection—especially viral infection, but also bacterial infection
- Chronic inflammation
- Drugs (for example, phenytoin)

Pathogenesis

- Increased production
- Decreased entry into lymph nodes (seen particularly in pertussis)
- Antigenic stimulation and activation of T cells causes them to take on an atypical morphology especially seen in:
  - Infectious mononucleosis (EBV)
    —Virus is found in the saliva and is responsible for exudative and painful lymphadenopathy; tests such as heterophile antibodies in the acute phase (Monospot test) are very sensitive, especially in adolescents and young adults; IgM antibodies against horse, bovine, and sheep RBCs; antiviral capsid antigen (VCA) IgM/IgG is the best screening test; EBNA only positive after a few months.
  - Viral hepatitis
  - Cytomegalovirus (CMV)
  - Toxoplasmosis



▲ **Figure 7–3.1B** Atypical Lymphocytes

3.1.3 Eosinophilia

- Allergies/asthma (Type I hypersensitivity reaction)
- Parasitic infections

3.1.4 Basophilia

Myeloproliferative disorder (CML) is an example of where we might see basophilia.

3.1.5 Monocytosis

Infections (EBV, TB, *Salmonella*, *Listeria*, syphilis)

3.2 Leukopenia

3.2.1 Neutropenia

- Absolute neutrophil count <1,500
- Caused by:
  - Ineffective granulopoiesis (MDS, megaloblastic anemia)
  - Bone marrow infiltration (leukemia, metastatic carcinoma, storage diseases, myelofibrosis)
- Splenic sequestration (blood cells trapped by spleen)
- Drugs that cause neutropenia:
  - Chemotherapeutic drugs
  - Alkylating agents
  - Antimetabolites
  - Chlorpromazine
  - Clozapine
  - Sulfonamides
  - Chloramphenicol

3.2.2 Lymphopenia

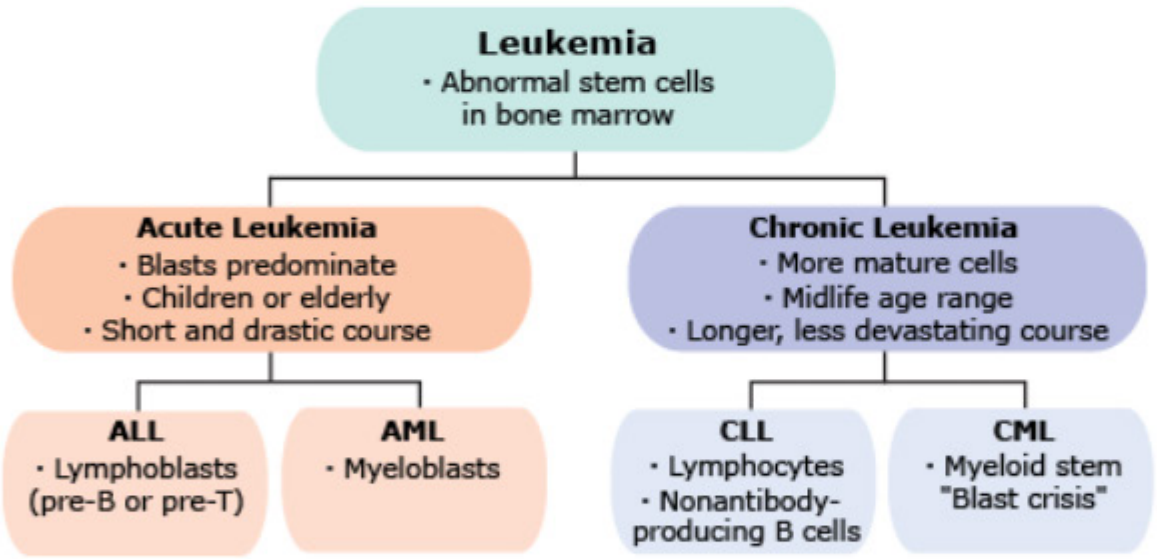
- Caused by:
  - HIV infection (CD4)
  - Congenital immunodeficiency (DiGeorge, SCID)
  - Autoimmune disease
  - Cytotoxic drugs (for example, chemotherapy)
  - Glucocorticoids (long term)
  - Malnutrition

4 Leukemias

- Malignancy of stem cells in bone marrow with widespread metastasis. It is important to understand that malignant lymphomas are malignancies that arise from lymph nodes, with the most common extranodal site being the stomach.
- Leukemias are broken down further by using *age brackets* and percentage of blasts of the condition.

▼Table 7–4.0 Leukemias

Age Bracket	Blasts Percentage
Acute lymphoblastic leukemia (ALL) • Newborn–14 years of age	>20% blasts
Acute myeloblastic leukemia (AML) • 15–39 years of age	>20% blasts
AML and chronic myelogenous leukemia (CML) • 40–60 years of age	<10% blasts
Chronic lymphocytic leukemia (CLL) • Greater than 60 years of age • Most common overall	<10% blasts



▲Figure 7–4.0 Classification of Leukemias

4.1 Acute Leukemia

- Disease of neoplastic leukocytes.
- Predominance of immature forms, especially blasts (myeloblasts or lymphoblasts).
- Disease defined by >20% blasts in the bone marrow.
- Symptoms due to marrow failure secondary to leukemia infiltration pancytopenia—anemia, leukopenia, and thrombocytopenia.
- Can be associated with Philadelphia chromosome t(9;22).
  - This translocation is not only associated with chronic myelogenous leukemia (CML).

Etiology

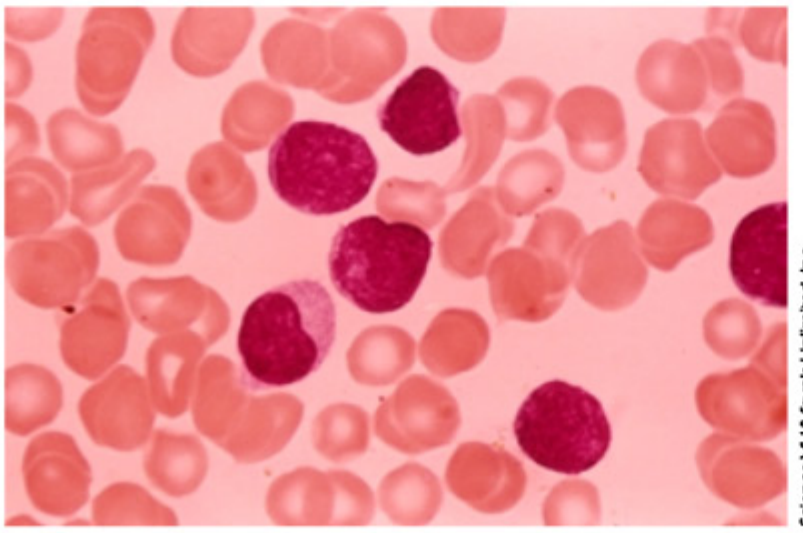
- Chromosomal abnormalities (for example, Down syndrome)
- Ionizing radiation
- Chemical exposure
- Alkylating agents (chemotherapy)
- Age

4.1.1 Acute Lymphocytic (Lymphoblastic) Leukemia (ALL)

- Disease of immature lymphocyte—pre-B and pre-T ALLs
- Typically seen in children up to age 15
  - No. 1 leukemia in this age group

Pathogenesis

- *Most common malignancy in children.*
- Defective maturation of lymphocyte precursors.
- Common ALL Antigen (CALLA) + (CD10).
- Terminal deoxynucleotidyl transferase (TdT)+ is the most common type.
- Translocation t(12;21) has good prognosis.
- Can be pre-B or pre-T ALL.
- Can be extramedullary (lymphoblastic lymphoma).
- Metastasizes to CNS, testicles.



▲ **Figure 7–4.1A** Acute Lymphocytic Leukemia (ALL)

▼ **Table 7–4.1** Onset of Leukemias

	Pre-B ALL	Pre-T ALL
Frequency	80%	20%
Age of Onset	Childhood	Adolescence
Site	Blood/BM	Mediastinal mass
WBC Count	Low-normal	High
Prognosis	Good	Poor
Symptoms	Pancytopenia, neurologic symptoms, bone pain	

4.1.2 Acute Myeloid Leukemia (AML)

- Disease of imiaure granulocytes
- Seen in young to middle-aged adults (15–60)

Morphology: Classification (FAB)

M0—undifferentiated
M1—AML without maturation
M2—AML with maturation
M3—Acute promyelocytic leukemia (APL)
M4—Myelomonocytic
M5—Monocytic/monoblastic
M6—Erythroleukemia
M7—Megakaryoblastic leukemia

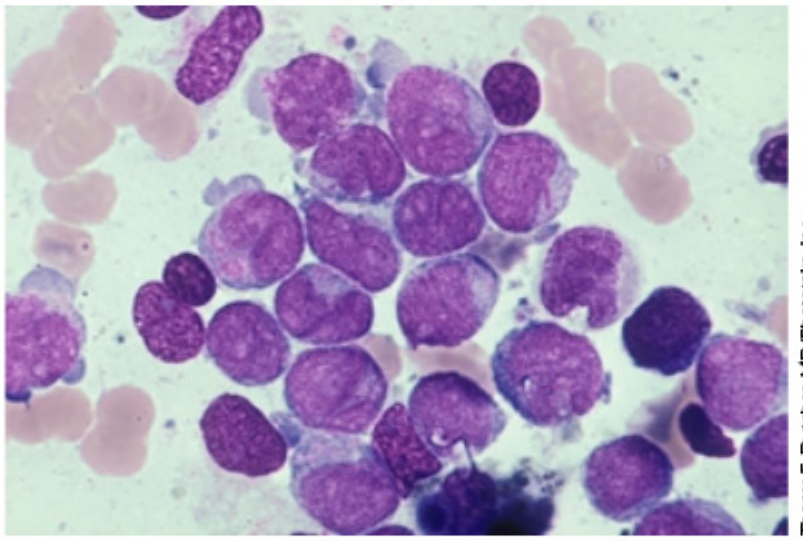
Pathogenesis

- *Myelodysplastic syndrome (MDS):*
  - May precede AML—"*pre*leukemic" condition
  - Pancytopenia
  - Shift to immaturity in granulocytes, but <20% blasts
  - Dysplasia in one or more lineages
  - Tends to occur in *older* individuals
  - Especially common in those treated with *prior chemotherapy*
- Associated with recurrent cytogenetic abnormalities:
  - t(15;17)—*acute promyelocytic leukemia (APL)*
  - t(8;21)
  - 11q23 abnormalities
- Genetic abnormalities lead to defects in stem cell maturation and clonal expansion of leukemic blasts.

4.1.3 M3—Acute Promyelocytic Leukemia (APL)

Morphology Characterized by abnormal myeloid blasts

- Large nuclei
- Prominent nucleoli
- Cytoplasmic granules with occasional *Auer rods.*
  - Auer rods are needle-shaped azurophilic intracytoplasmic inclusion bodies.
  - These contain peroxidase and lysosomal enzymes, which can stain with antibody against myeloperoxidase (MPO).
  - Upon peripheral blood smear, if you see blasts with Auer rods you know it is some type of acute leukemia.
  - These acute leukemias include myeloblastic, promyelocytic, myelomonocytic, and monocytic.
  - Other conditions in which Auer rods are seen are myelodysplastic syndromes (MDS) (RAEB-2) and chronic monomyeloid leukemia.
  - Keep in mind that the converse is not true: Just because you *do not* see Auer rods does not mean that the blast is *not* a myeloblast.



▲ **Figure 7–4.1B** Acute Myeloid Leukemia (AML)

Clinical Findings

- Symptoms:
  - Weakness, pallor, fatigue (normocytic anemia)
  - Infection susceptibility (leukopenia)
  - Easy bleeding/bruising (thrombocytopenia)
  - Bone pain (marrow infiltration)
- M3 (APL) associated with DIC—can be effectively treated with *all-trans retinoic acid (ATRA)*

4.2 Chronic Leukemia

- Chronic leukemias are derived from more mature leukocytes
- Chronic (mature) leukemias
  - Chronic lymphoctyic leukemia
  - Chronic myelogenous leukemia
  - Hairy cell leukemia
  - Adult T cell leukemia/lymphoma

Pathogenesis

- Neoplasm of maturing peripheral lymphocytes
- May present as concurrent or isolated lymphoma (small lymphocytic lymphoma—SLL)
- Bone marrow is always involved
- Spleen and liver also can be involved

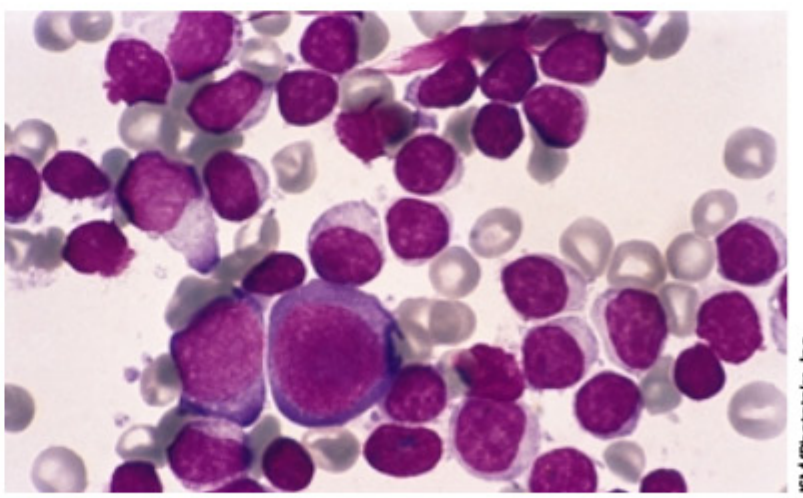
4.2.1 Chronic Lymphocytic Leukemia (CLL)

Pathogenesis

- CLL disrupts normal immune function:
  - Hypogammaglobulinemia and increased infections
  - Autoimmune hemolytic anemia (AIHA)
    —Most commonly associated with "warm" type AIHA
  - Idiopathic thrombocytopenic purpura (ITP)

Clinical Findings

- Usually occurs in older patients, median age of 60.
  - CLL is the most common cause of generalized *painless* lymphadenopathy.
  - WBC count varies from normal to very high (>100,000).
  - Hypogammaglobulinemia is a common finding.
  - Insidious onset with nonspecific symptoms—fatigue, weight loss, anorexia.
  - Lymphadenopathy and hepatosplenomegaly are often present.
  - Progression is slow—median survival is four to six years, less than one year if disease transforms.
    —Some CLLs transform to more aggressive forms: Prolymphocytic transformation and *Richter syndrome*—transformation to diffuse large B-cell lymphoma
  - Peripheral blood smear:
    —"Smudge" cells
    —Small, monomorphic lymphocytes with "cracked" chromatin



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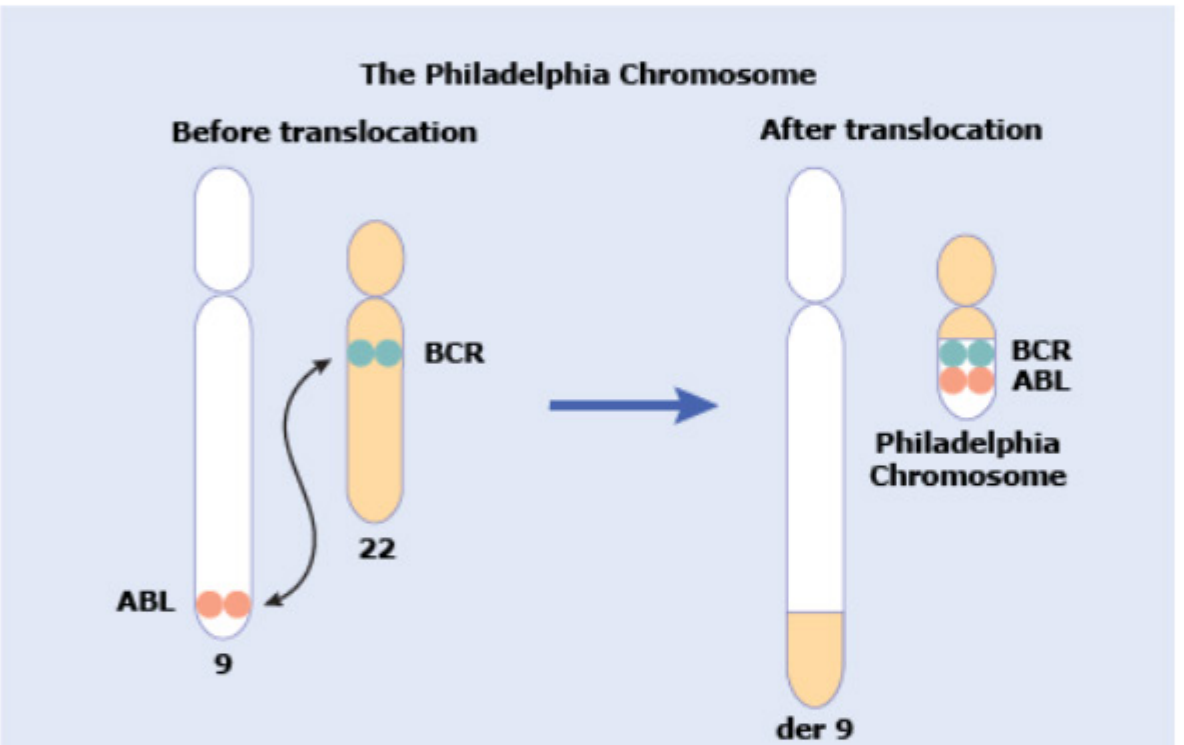
▲ **Figure 7–4.2A** Chronic Lymphoid Leukemia

4.2.2 Chronic Myelogenous Leukemia (CML)

Etiology/Pathogenesis

- CML is a neoplastic transformation of pluripotent stem cells for both myeloid and lymphoid series.
- It is the only leukemia that may present with thrombocytosis (40% to 50%).
- CML is defined by the presence of *the Philadelphia chromosome t(9;22)*; this translocation also may be found in other acute leukemias.
- This results *in fusion of the BCR and ABL genes.*
- The *ABL* gene product is a tyrosine kinase that controls cell growth.
- The BCR/ABL fusion protein results in increased, unregulated activity of ABL leading to uncontrolled growth of maturing myeloid cells.

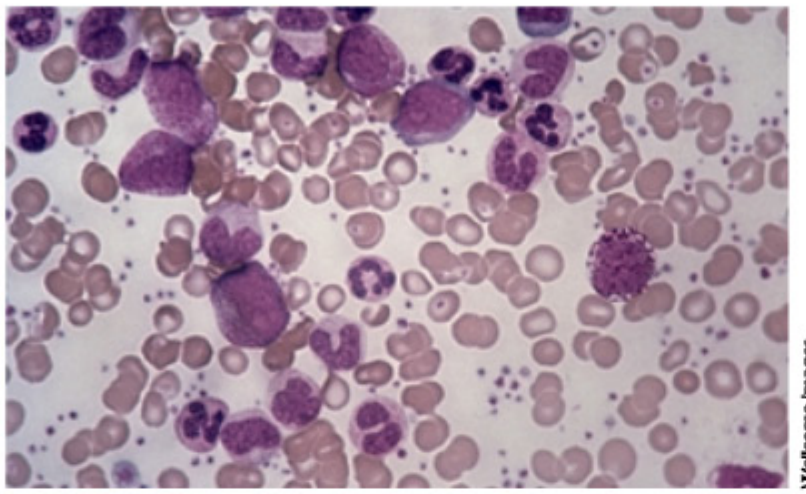


The Philadelphia chromosome results when a piece of chromosome 9 switches places with a piece of chromosome 22. The translocation forms an extra-long chromosome 9 (called der 9) and an extra-short chromosome 22, which is the Philadelphia chromosome that contains the abnormal, fused BCR-ABL gene.

▲ **Figure 7–4.2B** The Philadelphia Chromosome

Morphology

- Peripheral blood shows leukocytosis with increased immature granulocytes—myelocytes, metamyelocytes, bands, etc.
- There is an absolute basophilia.
  - Notice the increased number of granules and intense basophilia in the image.



▲ **Figure 7–4.2C** Chronic Myelogenous Leukemia

Clinical Findings

- Over 40 years old
- Onset is slow and insidious with nonspecific symptoms
- Symptoms come from:
  - Anemia—weakness, fatigue, pallor, etc.
  - Splenomegaly—abdominal fullness, pain
- Disease slowly progresses over years to:
  - Accelerated phase—failure of treatment and increasing cytopenias
  - *Blast crisis*—rapid and marked increase in bone marrow or peripheral blood blast count

Treatment

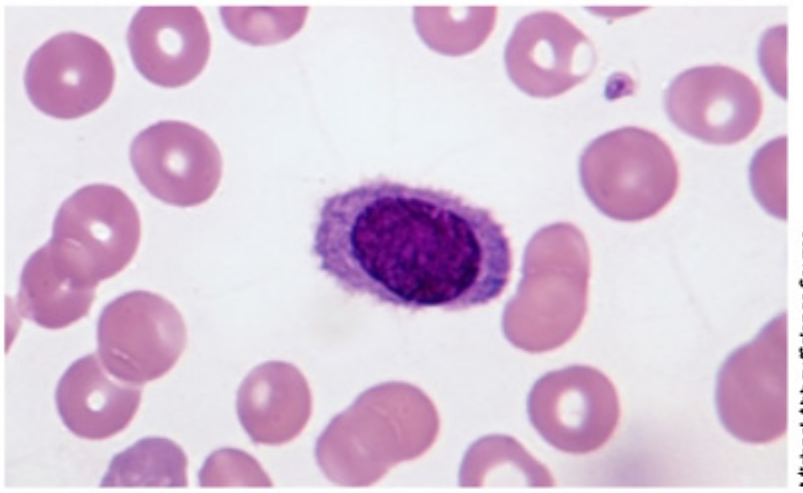
- Chronic phase treated successfully with *Gleevec* (ABL tyrosine kinase inhibitor)
- Gleevec-resistant disease treated with bone marrow transplant

▼ **Table 7–4.2** Indications of Leukemias

	ALL	AML	CML	CLL
Age	Children	Young adults	Middle-aged	Elderly
Onset	Abrupt/acute		Chronic/insidious	
Symptoms	Cytopenias, bone pain		Nonspecific, fatigue, weakness, etc.	
Prognosis	Excellent (two thirds cured)	Moderate (30% cured)	OK with Gleevec; otherwise poor	Poor, but slowly progressing

4.2.3 Hairy Cell Leukemia

- Rare mature B-cell leukemia
  - Hair-like projections due to irregular cytoplasmic membrane on malignant B lymphocytes.
  - Tartrate-resistant acid phosphatase (TRAP) stain used to identify.
- Occurs predominantly in older males.
- Patients present with splenomegaly due to source of blast proliferation.
- Pancytopenia, especially monocytopenia.
- Complications include opportunistic infections and vasculitis.
- Prognosis is good with treatment with purine nucleoside analogs and/or splenectomy.



Michael Abbey/Science Source

▲ **Figure 7–4.2D** Hairy Cell Leukemia

4.2.4 Adult T-Cell Leukemia/Lymphoma (ATLL)

- ATLL is caused by a retrovirus—human T-cell leukemia virus, type 1 (HTLV-1).
- It is primarily found in *HTLV-1 endemic areas*—Japan, the Caribbean, and central Africa.

Pathogenesis

- Activation of TAX gene, which inhibits the TP53 suppressor gene.
- There is monoclonal proliferation of neoplastic CD4Th cells.

Clinical Findings

- The disease has a long latency, causing leukemia/lymphoma decades after infection.
- Patients may present with generalized lymphadenopathy and hepatosplenomegaly.
- Skin infiltration is commonly involved.
- Lymphoblasts release osteoclastic activating factor, which causes widespread lytic bone lesions, resulting in hypercalcemia.
- Prognosis varies, but is generally poor, with death usually from opportunistic infections.

CHAPTER 8 Lymphoid Pathology

1 Lymphoid Pathology Overview

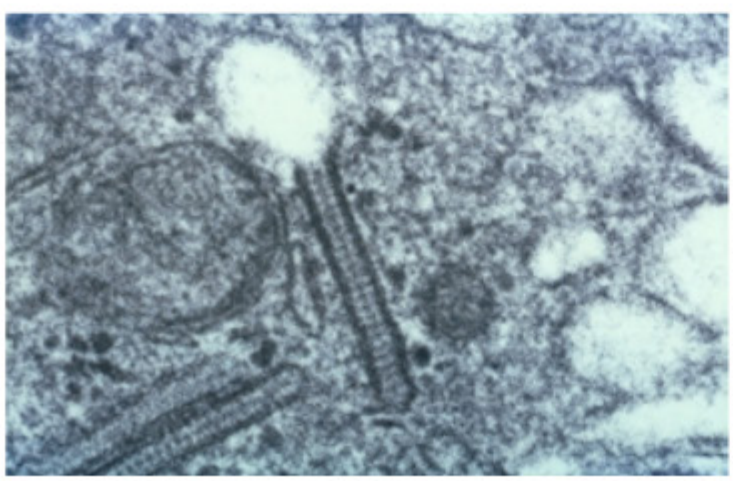
- Lymph nodes
  - Normal lymph node
  - Benign lymphadenopathy
  - Lymphoid neoplasms
- Spleen
- Thymus
- Plasma cell neoplasms

2 Lymph Nodes

2.1 Anatomy: Normal Lymph Node

2.1.1 Sinus: Location of First Site of Metastasis

- Clinical correlate
- Histiocytoses
  - Group of clinical syndromes characterized by an abnormal proliferation of histiocytes capable of migrating from skin to lymph nodes.
  - Clinically, its manifestations range from isolated bone lesions to multisystem disease.
  - *Langerhans cell histiocytosis (LCH)* is a rare clonal proliferative disorder of dendritic (Langerhans) cells from bone marrow of monocyte lineage.
    - —Cells are functionally immature and do not efficiently stimulate T lymphocytes via antigen presentation.
    - —Cells express S-100 and CD1a.
    - —Electron microscopy: Birbeck granules ("tennis rackets")



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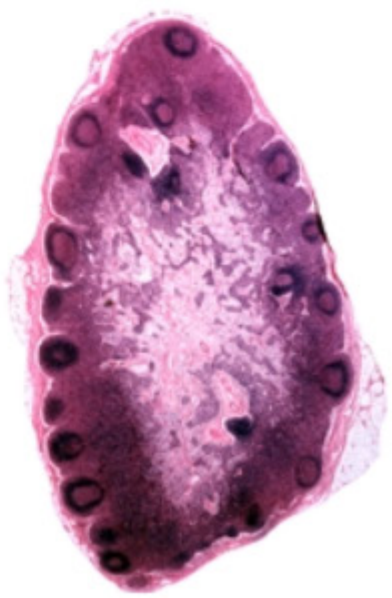
▲ **Figure 8–2.1B** Birbeck Granules

USMLE® Key Concepts

For Step 1, you must be able to:

- Differentiate among the various types of histiocytoses.
- Differentiate between benign vs. chronic lymphadenopathy.
- Differentiate between Hodgkin vs. non-Hodgkin lymphomas.
- Differentiate among the various types of plasma cell neoplasms.

▼ **Figure 8–2.1A** Normal Lymph Node



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—LCH Subtypes:
- *Eosinophilic granuloma* (unifocal LCH): Benign lytic bone lesions with no extraskeletal involvement.
- *Hand-Schüller-Christian* (multifocal unisystem): Mostly found in children with a triad of lytic skull lesions, central diabetes insipidus, infiltration of the orbit resulting in exophthalmos.
- *Letterer-Siwe disease* (multifocal multisystem): Seen in children younger than 2 with Langerhans cells in various tissues: Rash, lytic lesions, lymphadenopathy.

2.1.2 Germinal Follicles (B Cells)

Site for B cell proliferation

- Clinical correlate
  - Follicular B cell lymphoma and Reed-Sternberg cells from Hodgkin lymphoma originate here.
  - Germinal follicle are absent in Bruton agammaglobulinemia resulting in B cell and, therefore, plasma cell deficiency.

2.1.3 Paracortex (T Cells)

Site of T cell maturation resulting in decreased production of B cells and thus plasma cell deficiency.

- Clinical correlate
  - T cell lymphomas originate here.
  - The paracortex is absent in DiGeorge syndrome.

2.1.4 Severe Combined Immunodeficiency

- SCID is the absence of the enzyme adenine deaminase, which, along with the absence of both the germinal centers and paracortex, impairs proper manufacturing of B and T cells.

2.2 Benign Lymphadenopathy

2.2.1 Acute Nonspecific Lymphadenitis

- Inflammation of lymph nodes characterized by cortical and/or paracortical hyperplasia.
- Usually caused by *acute infections*.
  - Strep/staph most common
  - Cat-scratch disease (*Bartonella*)
  - Tularemia (rabbits)
- Most frequent presentation is *tender enlarged* lymph nodes.
  - Site depends on nature of disease.

2.2.2 Chronic Nonspecific Lymphadenitis

- Usually causes long-standing, *non-tender* lymphadenopathy.
- Etiologies include:
  - Neoplasms
  - Autoimmune disease
  - HIV
  - Chronic infections
  - Drugs

Memory Aid

Clinical pearls of lymphadenopathy:

- If the involved lymph nodes are painful, think inflammatory, the most common etiology.
- If the involved lymph nodes are painless, think of either malignancies. Common lymphadenopathy associations:
  - —Seminoma spreading to para-aortic nodes.
  - —Stomach cancer to the left supraclavicular lymph nodes.

2.3 Lymphoid Neoplasms

- *Leukemias* of either acute or chronic types may invade lymph nodes and give rise to a lymphoma type of picture.
  - Leukemias are covered in chapter 7, "White Blood Cell Pathology."
- Hodgkin lymphoma
  - Nodular sclerosis
  - Mixed cellularity
  - Lymphocyte rich
  - Lymphocyte depleted
  - Nodular lymphocyte predominant
- Non-Hodgkin lymphoma
  - Follicular lymphoma
  - Diffuse large B cell lymphoma
  - Burkitt lymphoma
  - Mantle cell (see topic 2.5.2)
  - T cell lymphoma
- Plasma cell neoplasms may invade lymph nodes and give rise to a lymphoma type of picture.

2.4 Hodgkin Lymphoma

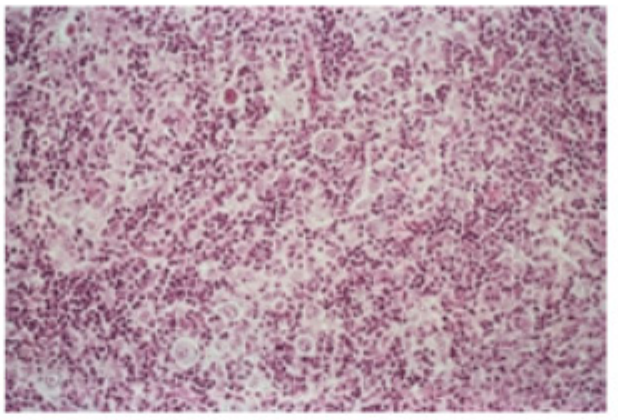
- Presents in a single lymph node or several adjacent lymph nodes.
- Is confined to lymph nodes with little extranodal component.
- Spreads in a contiguous fashion (for example, from one node to an adjacent node).
- Consists of a few neoplastic cells (Reed-Sternberg or RS cells) surrounded by many benign reactive cells.
- Reed-Sternberg cells arise from B cell germinal center origin. They are mirror-image nuclei, each with eosinophilic nucleolus surrounded by a clear halo.

▼ Table 8–2.4A Ann Arbor Classification of Tumor Staging

Stage	Description
I	One lymph node or group
II	Two or more lymph nodes or groups on one side of the diaphragm
III	Two or more lymph nodes or groups on both sides of the diaphragm
IV	Extralymphatic spread

- There are four types of High-Yield morphologies of RS cell to know:

1. **Classic:** Two mirror image nuclei with eosinophilic nucleolus surrounded by a clear halo.
2. **Lacunar:** Pale cell with multilobed nucleus containing many small nucleoli found in sclerosing type Hodgkin disease.
3. **Mononuclear variant:** Single nucleus with prominent nucleolus found in mixed cellularity type Hodgkin disease.
4. **Lymphocytic and Histiocytic (L&H) variant:** Large, pale staining, multilobed cell found in lymphocyte predominant type Hodgkin disease.



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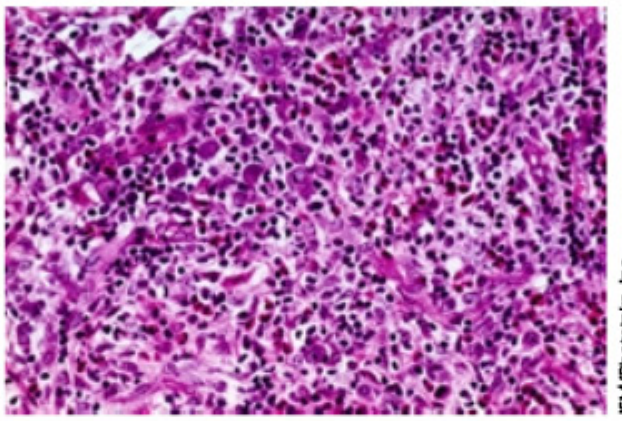
Classic RS Cell



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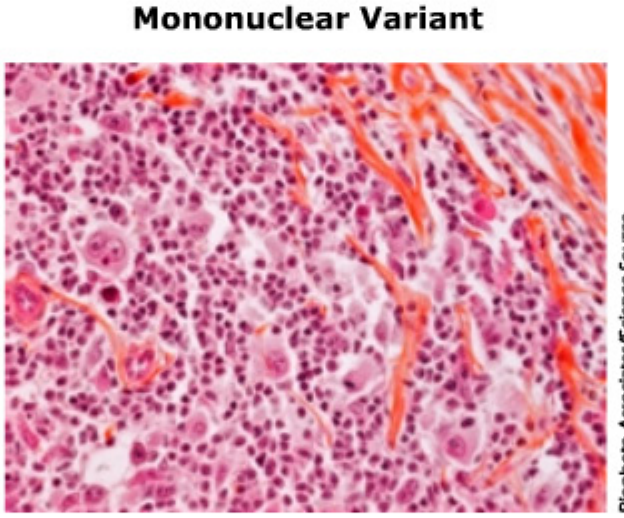
Mononuclear Variant



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L&H ("Popcorn") Variant

▲ **Figure 8–2.4** Hodgkin Lymphoma

Clinical Pathology

- Presents as enlarging lymphadenopathy (usually cervical/ supraclavicular) with or without "B" symptoms—fever, weight loss, night sweats.
- Prognosis depends on stage, but is generally good.
- Even stage IV disease has a 60% to 70% five-year survival.
- CD (clusters of differentiation) markers.
- Generally, **CD 15+, 30+, 45**—*except* lymphocyte predominant form (CD 15**-**, 30**-**, 45+).

▼ **Table 8–2.4B** Characteristics of Subtypes

Subtype	Neoplastic Cells	Background Cells	Clinical Features
Nodular sclerosis	Lacunar Classic RS	Mixed Bands of fibrosis	60%–80% of cases, M=F, anterior mediastinal + either cervical or supra-clavicular lymph nodes
Mixed cellularity	Mononuclear Classic RS	Mixed	30% of cases, M(>55 years)>F, HIV common, EBV association
Lymphocyte rich	Mononuclear Classic RS	T lymphocytes	Older, M>F, EBV association
Lymphocyte depleted	Classic RS and variants	Few, some diffuse fibrosis	Rare, older, HIV
Nodular lymphocyte predominant (NLPHL)	L&H (popcorn) cells	B cells Dendritic cells	Uncommon, young, M>F Cervical, axillary

2.5 Non-Hodgkin Lymphomas (NHLs)

2.5.1 Characteristics of Non-Hodgkin Lymphomas

Non-Hodgkin lymphomas are most commonly of *B cell origin.* Usually present with:

- Widespread lymphadenopathy.
- "B symptoms"—fever, weight loss, night sweats.
- Spread *noncontiguously.*
- Often involve extranodal sites.
- Risk factors
  - Viral
    —Epstein-Barr virus (EBV)
    —Human T cell leukemia virus type I—T cell lymphoma or leukemia.
    —Hepatitis C virus
  - *Helicobacter pylori*
  - Autoimmune disease
    —Sjögren syndrome; Hashimoto
    - Immunodeficiency syndromes and immunosuppressive therapy
    - High-dose radiation as in treatment for Hodgkin leukemia
    - NHL CD markers—"clusters of differentiation."
  - Common markers to remember:
    —CD 19, 20—B cell markers
    —CD 3, 5—T cell markers
    —CD 138, kappa, lambda—plasma cell markers

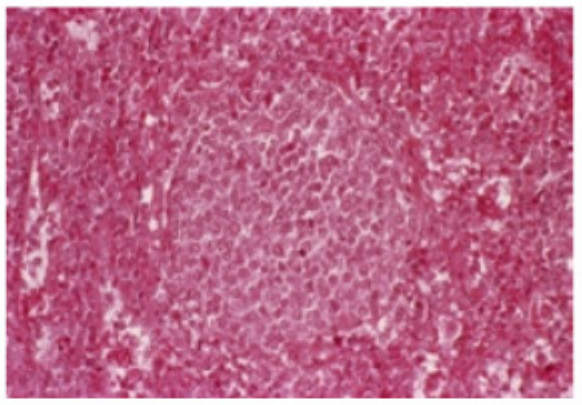
2.5.2 Most Common Types of Non-Hodgkin Lymphoma (NHL)

- Follicular lymphoma
- Diffuse large B cell lymphoma
- Burkitt lymphoma
- Mantle cell lymphoma
- T cell lymphomas

Follicular Lymphoma

Etiology/Pathogenesis

- Most follicular lymphomas carry a t(14;18) translocation.
- This juxtaposes the *BCL2 gene with the immunoglobulin heavy chain gene*.
- This causes unregulated expression of BCL2, an inhibitor of apoptosis.
- This causes increased survival of germinal center cells, leading to lymphoma.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▲ **Figure 8–2.5A** Non-Hodgkin Lymphoma/Follicular Lymphoma

Morphology

- Replacement of lymph nodes with enlarged follicles, consisting of small, cleaved lymphocytes.

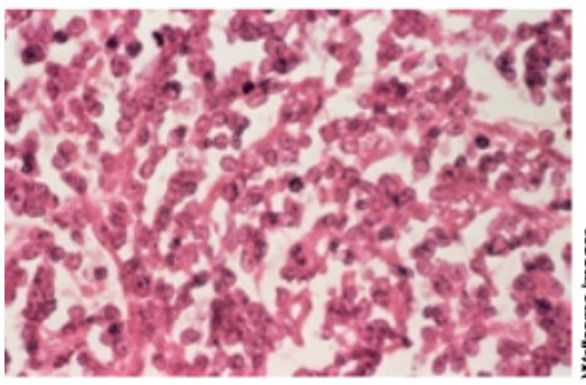
Clinical Pathology

- Follicular lymphoma is the most common of the NHLs.
- It usually presents in middle age.
- It is fairly indolent with average survival: ~8 years, but not amenable to chemotherapy.
- ~30–50% of follicular lymphomas transform to more aggressive large B cell lymphomas.
- *Not* equivalent to Richter syndrome.

Diffuse Large B Cell Lymphoma (DLBCL)

Etiology/Pathogenesis

- Most common type of NHL.
- 30% carry t(14;18), indicating transformed follicular lymphoma.
- 20% to 30% carry translocations involving *BCL6*, a gene involved in regulation of B cell differentiation.
- Diffuse large B cell lymphoma (DLBCL) is common in immunodeficiency states, a result of transformation of lymphocytes due to EBV or HHV-8 infection.
- May be mature T cell in origin of approximately 20% of the time.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▲ **Figure 8–2.5B** Non-Hodgkin Lymphoma/Diffuse Large B Cell Lymphoma

Morphology

- Diffuse replacement of lymph node parenchyma with large, pleomorphic cells.

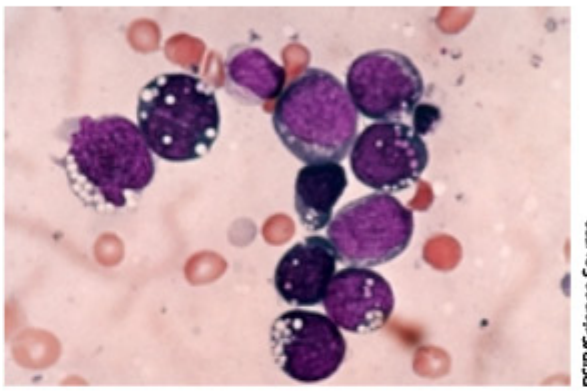
Clinical Pathology

- Most occur in older patients, but has a wide age range, including children.
- Patients usually present with a rapidly enlarging mass at either a nodal or extranodal site.
- Symptoms, if present, are usually due to mass effect or tissue destruction.
- DLBCL is quite aggressive, but usually responds to chemotherapy, with lasting remission seen in ~50% of patients.

Burkitt Lymphoma

Etiology/Pathogenesis

- *Endemic and sporadic forms are associated with latent EBV infection.*
- Most carry translocations involving the *c-myc gene* on chromosome 8.
- The most common of these is **t(8;14),** which juxtaposes the *c-myc* gene with the immunoglobulin heavy chain gene.
- These translocations lead to unregulated overexpression of *c-myc*, an oncogene that promotes cell growth and division.



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▲ **Figure 8–2.5C** Non-Hodgkin Lymphoma/Burkitt Lymphoma

Morphology

- Diffuse infiltrate of medium-sized cells.
- Large numbers of mitotic and apoptotic cells.
- Scattered benign macrophages—"*starry sky*."

Clinical Pathology

- All forms occur at extranodal sites.
- Burkitt lymphomas tend to appear more frequently in children and young adults, except for the HIV form.
- Three types of Burkitt lymphoma:
  1. **Endemic (African):** Most typically occurs in the mandible.
  2. **Sporadic:** Presents as an abdominal mass.
  3. **HIV-associated:** Very aggressive form.

Scott Camazine/Phototake, Inc.

▲ **Figure 8–2.5D** Non-Hodgkin Lymphoma/Burkitt Lymphoma

▼Table 8–2.5 Hodgkin vs. Non-Hodgkin

Hodgkin	Non-Hodgkin
Normally localized to a single group of nodes (cervical, mediastinal, para-aortic)	More frequent involvement of multiple peripheral nodes
Contiguous spread	Noncontiguous spread
Rarely involve mesenteric nodes or Waldeyer ring	Mesenteric nodes and Waldeyer ring commonly involved
Extranodal involvement uncommon	Extranodal involvement common

Mantle Cell Lymphoma

- Tends to occur in older males.
- Associated with translocation t(11;14).
- Pathogenesis is the inactivation of cyclin D regulatory gene.
- Poor prognosis with association of CD5+.

T Cell Lymphomas

Adult T Cell Lymphoma/Leukemia See chapter 7, "White Blood Cell Pathology," topic 4.

Mycosis Fungoides/Sézary Syndrome

- CD4 T cell lymphoma in adults.
- Begins in the skin as a rash and progresses to plaques and nodular masses.
- Groups of neoplastic cells in skin called Pautrier microabscesses.
- Sézary syndrome: mycosis fungoides with a leukemic phase; circulating cells called Sézary cells.

Important Concept

Skin involvement is common in T cell lymphomas/leukemias).

3 Spleen

3.1 Splenomegaly

Etiology

- Passive congestion:
  - Cirrhosis
  - Portal vein thrombosis
  - Heart failure
  - Infiltration
- Lymphoproliferative diseases
- *Marginal zone lymphoma*
- Storage diseases
- Amyloidosis
- Infection (for example, EBV):
  - In an infectious mononucleosis patient, worry about splenic rupture when there is trauma to the abdomen.
  - Worry about trauma to the abdomen in someone with mono → splenic rupture.

3.2 Hypersplenism

- Congestion of blood in the spleen causes:
  - Sequestration of blood elements.
  - Activation of reticuloendothelial cells.
  - Both processes lead to *anemia* and *thrombocytopenia.*
  - In some cases, there is increased risk of traumatic splenic rupture, causing potentially fatal hemoperitoneum.

3.3 Splenic Dysfunction/Autosplenectomy

- Predisposition to infections by encapsulated pathogens.
  - *Streptococcus pneumoniae*
  - *Haemophilus influenzae*
  - *Salmonella typhi*
  - *Neisseria meningitidis*
- Must immunize patients with splenic dysfunction or splenectomy.

Looking Back

For information on autosplenectomy seen with patients suffering from sickle cell disease, see chapter 6, "Red Blood Cell Pathology," topic 5.3.4.

4 Thymus

4.1 Congenital Disorders

4.1.1 DiGeorge Syndrome

- Thymic hypoplasia and parathyroid hypoplasia; *22q11 deletion.*
  - Abnormal development of the *third and fourth pharyngeal pouches.*
  - Varying degrees of T cell immunodeficiency

4.1.2 Thymic Hyperplasia

- Clinical features of acquired hypogammaglobulinemia are the same in DiGeorge syndrome and thymic hyperplasia.

4.2 Autoimmune Disorders

- *Myasthenia gravis*
- Good syndrome (thymoma + combined immunodeficiency and hypogammaglobulinemia)
- Pure red cell aplasia
- Graves disease
- Pernicious anemia
- Dermatomyositis-polymyositis
- Cushing syndrome

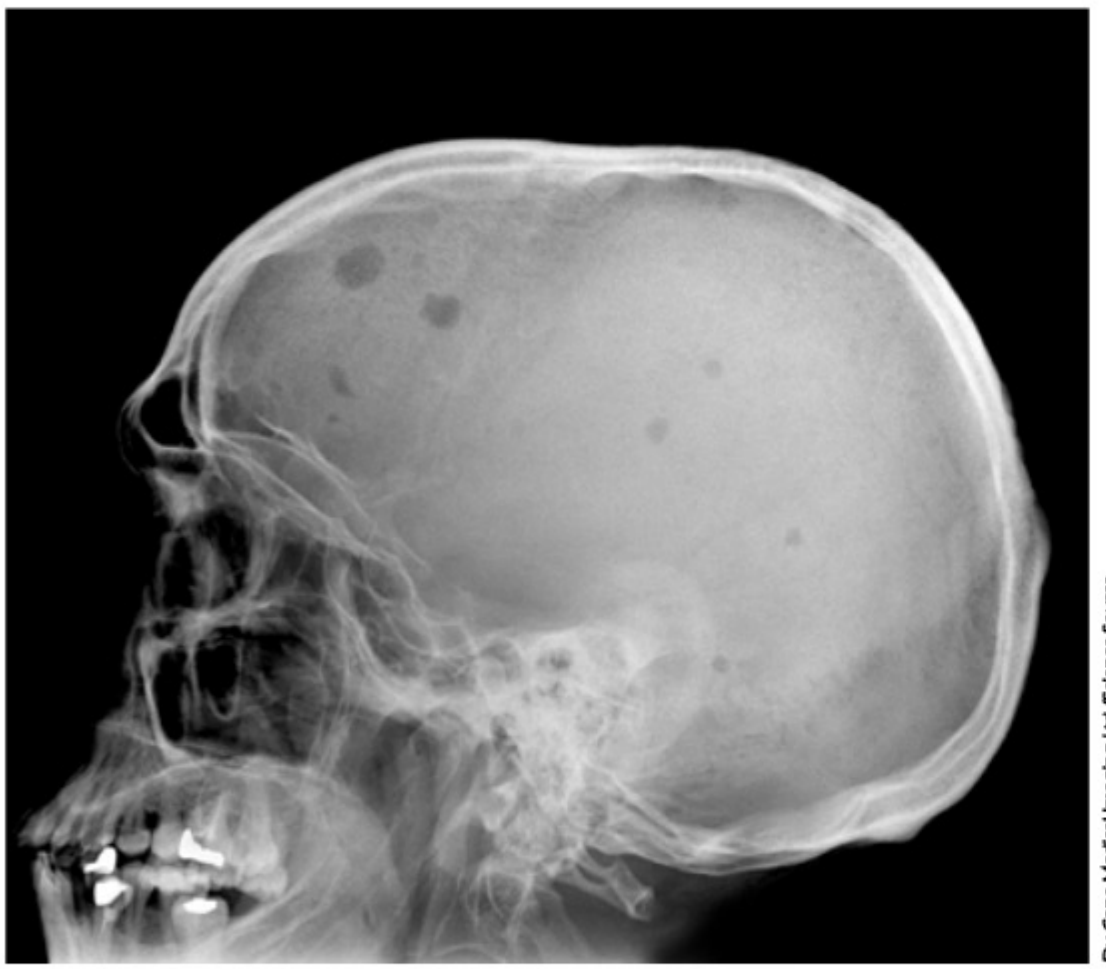
5 Plasma Cell Neoplasms

- White cell neoplasms that secrete monoclonal immunoglobulin
- Includes:
  - Plasma cell myelomas
  - Plasmacytoma
  - Monoclonal Gammopathy of Undetermined Significance (MGUS)
  - Lymphoplasmacytic lymphoma (Waldenström macroglobulinemia)

5.1 Plasma Cell Myelomas

5.1.1 Plasma Cell Multiple Myeloma

- Characterized by large neoplastic plasma cells derived from B lymphocytes.
- Neoplastic plasma cells produce an *osteoclast-activating factor* secreted by the neoplastic cells.
- Cause *lytic lesions in bone* (punched-out lesions), especially in the skull and axial skeleton.



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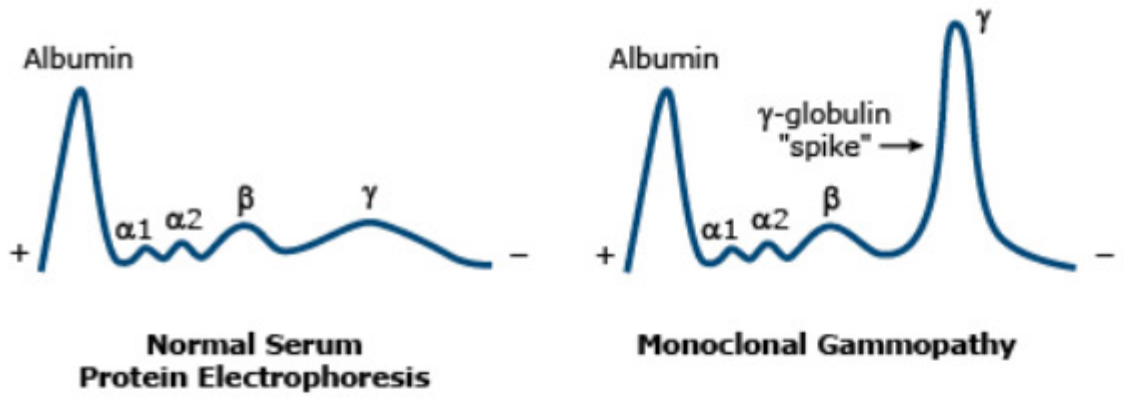
▲ **Figure 8–5.1A** Multiple Myeloma

- Products secreted:
  - Intact monoclonal immunoglobulin ("M protein") *not* to be confused with IgM
  - Heavy (mostly *IgG* and *IgA*) and light chains (*kappa* and *lambda*)
  - Free light chains (Bence Jones protein) spilled into urine



▲ **Figure 8–5.1B** Special Staining of CD138

Clinical Pathology

- Seen almost exclusively in patients >50 years old.
- Incidence increases with age.
- Prognosis is poor—median survival is ~3 years with treatment.
- Signs and symptoms from:
  - *Bony infiltration*
  - Chronic pain
  - Pathologic fractures
  - Hypercalcemia
  - *Increased Ig production*
  - Renal failure
  - Amyloid deposition
  - *Impaired immunity*—recurrent infections
  - *Haemophilus influenzae*, strep pneumonia
  - Normocytic anemia with *rouleaux formation*

5.1.2 Plasmacytoma

- Solitary skeletal plasmacytoma
  - Bony plasmacytomas usually eventually progress to plasma cell myeloma within 10 to 20 years.
- Extramedullary (most often in upper respiratory tract)

5.1.3 Monoclonal Gammopathy of Undetermined Significance (MGUS)

- Most common monoclonal gammopathy.
- Serum monoclonal M protein, but no other signs or symptoms of myeloma.
- Found in 1% of people >50 years old, and incidence increases with age.
- Only a small percentage (~1% per year) ever progress to overt myeloma.

5.1.4 Lymphoplasmacytic Lymphoma (Waldenström Macroglobulinemia)

- B cell neoplasm of cells that are in a developmental stage between B lymphocytes and plasma cells.
  - Referred to as *plasmacytoid lymphocytes.*
- *MGUS is a major risk factor*.
- Neoplastic B cells secrete *monoclonal IgM.*
- Elevated serum IgM causes *hyperviscosity syndrome*:
  - Visual impairment
  - Neurologic problems
  - Bleeding
- Differences compared with multiple myeloma: No lytic lesions; does *not* arise in the bone marrow, but does metastasize to the marrow.

1 ABO Blood Group Antigens

1.1 Blood Group O Characteristics

- Most common blood group
- No blood group antigens are present on the RBC membrane
- Natural antibodies (isohemagglutinins) in serum
  - Anti-A IgM, IgG and anti-B IgM, IgG may be present

1.2 Blood Group A Characteristics

- Anti-B IgM antibodies
- Increased incidence of gastric carcinoma

1.3 Blood Group B Characteristics

- Anti-A IgM antibodies

1.4 Blood Group AB Characteristics

- Least common blood group
- No natural antibodies
- Contains A and B antigens

1.5 Paternity Issues in Newborns

- Blood group AB parents cannot have an O child.
- Blood group O parents cannot have an AB, A, or B child.
- AO + BO parents *may* have an O child.

Mother / Father	A	O
B	AB	BO
O	AO	OO

▲ **Figure 9–1.5** Parents' Blood Types

▼ **Table 9–1.5** Parental-Fetal Blood Group Combinations With Viability Options

AO + BO Parents	with	O child	Newborn has greatest chance of viability
O Parents	with	AB, A, B, child	Newborn has the least chance of viability
A + B Parents	with	O child	Newborn is at moderate risk for non-viability

USMLE® Key Concepts

For Step 1, you must be able to:

- Determine ABO grouping properties including forward, backward typing.
- Interpret clinical consequences of Rh and non-Rh antigen systems.
- Interpret the clinical significance of patient cross-matching.
- Identify various types of transfusion reactions including allergic, febrile and hemolytic transfusion reactions.
- Interpret the development of hemolytic disease of the newborn (HDN).

2 Determining the ABO Group

2.1 Forward Type

- Identifies the blood group antigen.
  - Patient's red blood cells are added to test tubes that contain either anti-A or anti-B test serum.
  - For example, a patient with blood group B red blood cells is added to a test tube containing serum antibodies (anti-B IgM), resulting in agglutination.

2.2 Back Type

- Identifies natural antibodies.
  - Patient's serum is added to test tubes containing either group A or B test red blood cells.
  - For example, a patient with blood group B serum (anti-A IgM) is added to a test tube containing blood group A test red blood cells, resulting in agglutination.

	Forward Type		Back Type	
Blood group	**Anti-A**	**Anti-B**	**A RBCs**	**B RBCs**
O	–	–	+	+
A	+	–	–	+
B	–	+	+	–
AB	+	+	–	–

▲ **Figure 9–2.2** Determining ABO Group

3 Rh Antigen and Non-Rh Antigen Systems

3.1 Rh Antigen System

- It has three adjoining gene loci.
  - Locus coding for D antigen; d antigen does not exist.
  - Locus coding for C and c antigen
  - Locus coding for E and e antigen
- Autosomal codominant inheritance
  - One of the sets of three Rh antigens from each parent is transmitted to each child.
  - For example, a child with cDe from the mother and cde from the father results in the child lacking E antigen.

Mother / Father	cDe	CDE
cde	cde/cDe	cde/CDE
CDE	CDE/cDe	CDE/CDE

▲ **Figure 9–3.1** C, D, E Antigens

- An individual who is Rh positive is D antigen positive.
  - Approximately 85% of the population has a D antigen.
  - Those lacking a D antigen are considered Rh negative.
- An example of an Rh phenotype individual would be a patient with C, c, D, E (Rh-positive) antigen who would be negative for e antigen.

3.2 Alloimmunization

Antibodies develop against foreign antigens.

- Production of an antibody against a foreign antigen not present on patient's red blood cells.
- Examples:
  - If a patient develops antibodies after exposure to Rh antigens, then this patient would be considered Rh-negative.
  - If a patient develops antibodies to non-Rh antigens, the patient is considered to be lacking Kell antigen, for example.
  - These antibodies are called *atypical antibodies:* The patient is considered to be sensitized if atypical antibodies are present.

- *What is the significance of atypical antibodies?*
  - They may lead to *hemolytic transfusion reaction (HTR)*.
  - An example of this would be a patient with anti-Kell antibodies who is exposed to Kell antigen positive red blood cells.
  - Of the antibodies synthesized, IgG antibodies are more likely to produce HTR than IgM antibodies.
    - —IgG antibodies react best in warm temperatures, whereas IgM antibodies react best in cold.
    - —Clinical significance of transfusion requirements calls for a protocol to check for atypical antibodies.
    - —Transfusion recipients must receive blood that is negative for foreign antigens.
    - —For example, a patient with anti-Kell antibodies must receive Kell antigen negative blood.

3.3 Clinically Important Non-Rh Antigens

- Duffy (Fy) antigens
  - Fy antigens are the binding sites for invading organisms such as *Plasmodium vivax* into red blood cells.
  - The African populace lacks the Fy antigen and therefore may be rendered resistant to *P. vivax* infestation.
- I and i antigen systems
  - IgM antibodies, considered to be cold agglutinins, may develop I or i antigens.
  - Examples include anti-i hemolytic anemia in infectious mononucleosis in patients and anti-I hemolytic anemia in patients with *Mycoplasma pneumoniae* infections.

4 Patient Crossmatch

4.1 Components of a Standard Crossmatch

- ABO group and Rh type
- Antibody screen for atypical antibodies
- Direct Coombs test to identify atypical IgG antibodies bound to red blood cells
- Major crossmatch

4.2 Major Crossmatch

- *What is the purpose of a major crossmatch?*
  - To detect atypical antibodies directed against foreign antigens on donor red blood cells.
- Sample of red blood cells from a donor unit is mixed with the patient's serum.
- Lack of red blood cell agglutination or hemolysis indicates a compatible crossmatch.
- *Clinical Pearl*: Even though there might be a compatible major crosssmatch, this does not guarantee that there will not be a transfusion reaction.

5 Transfusion Reactions

5.1 Allergic Reaction

- The most common transfusion reaction.
- Type I IgE-mediated hypersensitivity reaction against some protein in the donor blood.

5.2 Febrile

- Recipient has anti-human leukocyte antigen (HLA) antibodies directed against HLA antigens on donor leukocytes.
  - Remember: Red blood cells do not have HLA antigens.
- This is an example of a type II hypersensitivity reaction.

5.3 Hemolytic Transfusion Reaction (HTR)

5.3.1 Intravascular Hemolysis: ABO Blood Group Incompatibility

For example, a blood group B recipient received blood group A donor blood, which then elicits a type II hypersensitivity reaction in which the recipient's IgM antibodies will react with the donor's blood group A antigens, resulting in complement activation and rapid intravascular hemolysis.

5.3.2 Extravascular Hemolysis

- For example, atypical antibodies react with foreign antigens on donor's red blood cells
- These atypical antibody-coated red blood cells are then taken to the recipient's spleen for extravascular hemolysis. Jaundice might be noticed in this patient as a typical finding.

5.4 Clinical Presentation and at Risk

- Fever, back pain, hypotension
- Disseminated intravascular coagulation, acute renal failure

5.5 Lab Findings

- Positive Direct (coated RBC)
- Indirect (atypical antibodies)

Memory Aid

The pattern of intra- and extra-hemolysis behavior exhibited by both IgG and IgM antibodies in Hemolytic Transfusion Reaction (HTR) is similar to that found with Autoimmune Hemolytic Anemia (AIHA).

6 Hemolytic Disease of the Newborn (HDN)

HDN results from the transplacental passage of maternal IgG antibodies as seen with anti-D antibodies, anti-A and anti-B antibodies in group O mothers resulting in extravascular hemolytic anemia in the fetus.

6.1 ABO HDN

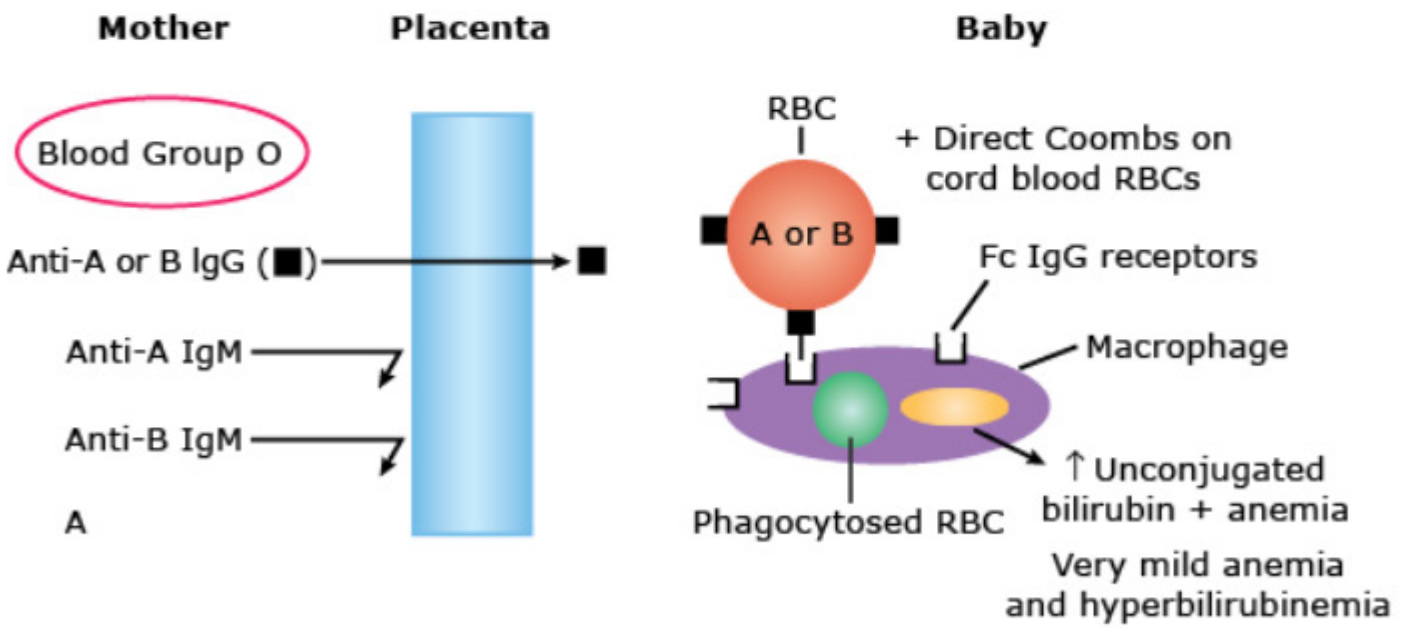
The most common occurrence, presenting in 20% to 25% of pregnancies.

Pathogenesis

- Blood group O mothers have anti-A and anti-B IgG antibodies.
- IgG antibodies will cross transplacentally and coat the fetal red blood cells.
- The fetal spleen will respond by causing extravascular hemolysis, resulting in the release of unconjugated bilirubin.
- This unconjugated bilirubin is eliminated by the mother's liver, thus diminishing the effects of the unconjugated bilirubin damage, mainly to the fetal nervous system (i.e. kernicterus).

Clinical and Lab Findings

- ABO HDN is the most common cause of jaundice in the first 24 hours due to the fact that the fetal liver has not developed a fully operating conjugating mechanism.
- ABO HDN gives a positive direct Coombs test on fetal cord red blood cells.



▲ **Figure 9–6.1** ABO HDN

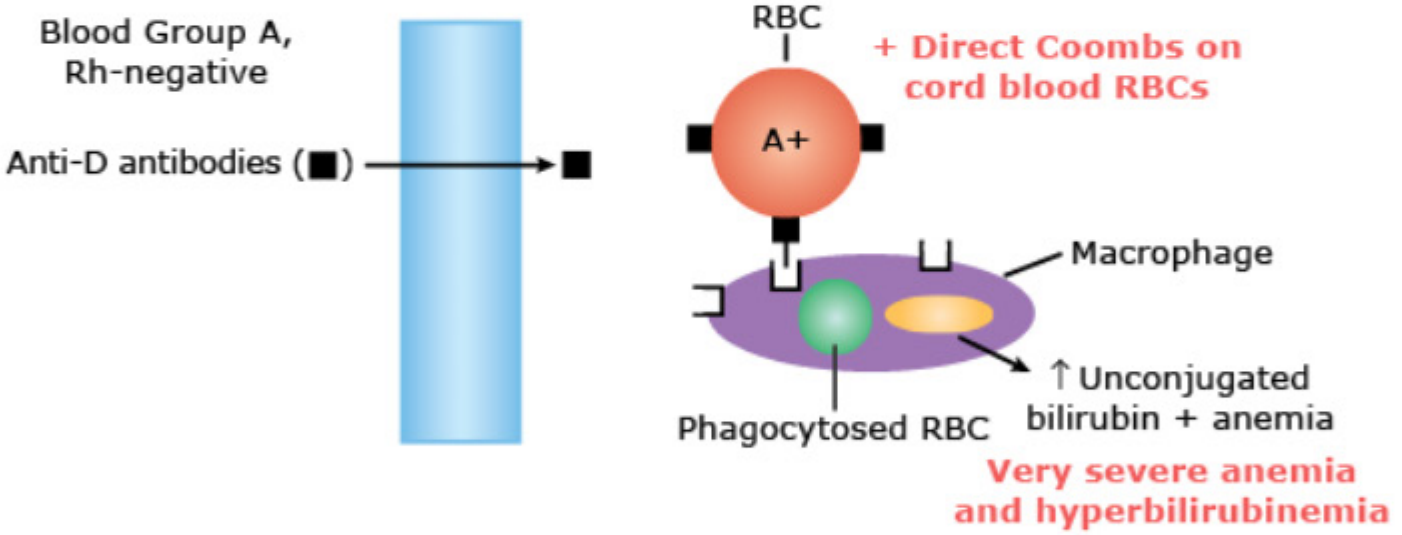
6.2 Rh HDN

Pathogenesis

- Mother is Rh (D antigen) negative and the fetus is Rh positive.
- Fetomaternal exposure, in which the mother will develop anti-D IgG antibodies that will not cause significant harm to the first newborn.
- Subsequent pregnancies under the same conditions will cause demise to the fetus due to maternal pre-formed IgG antibodies.
- The extravascular hemolysis and anemia that ensues results in high-output cardiac failure leading to a condition called hydrops fetalis.
- The degree of jaundice is more pronounced than it is with ABO HDN, and an increased risk for kernicterus also exists.

Prevention

- Administration of anti-D IgG globulin to D-negative mothers with D-positive child at 28th week of pregnancy.
- Given at the time of delivery or termination of pregnancy.
- Causes antibody-mediated removal of fetal red cells from the maternal circulation, preventing maternal alloimmunization.
- *Clinical Pearl*: ABO HDN protects against Rh HDN.



▲ **Figure 9–6.2** Kernicterus

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