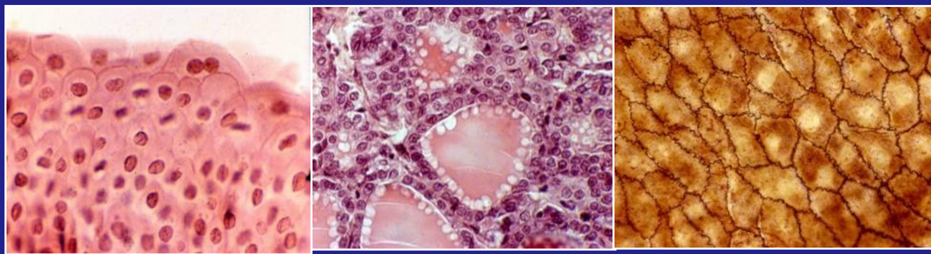
University of Teramo

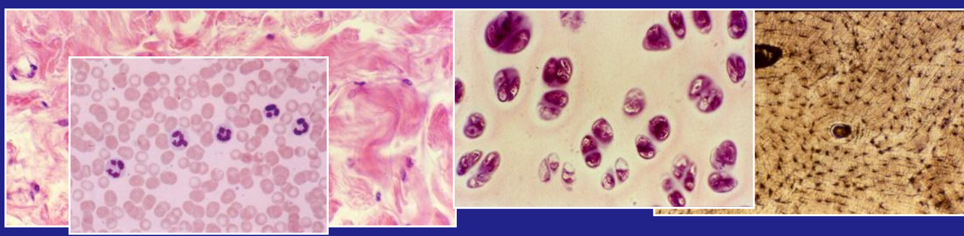
Degree Program in Biotechnology

COURSE OF CYTOLOGY AND HISTOLOGY
Prof. Mauro

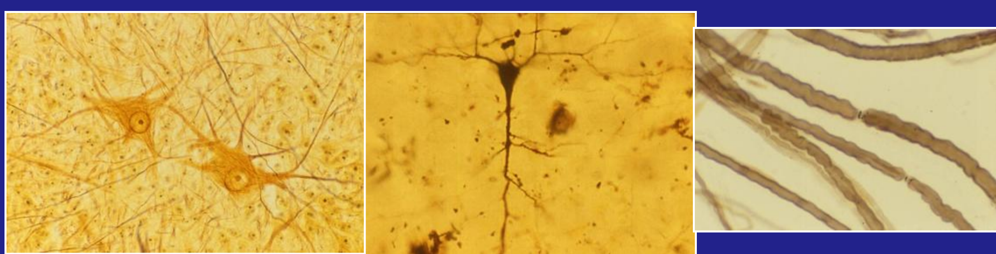
TESSUES



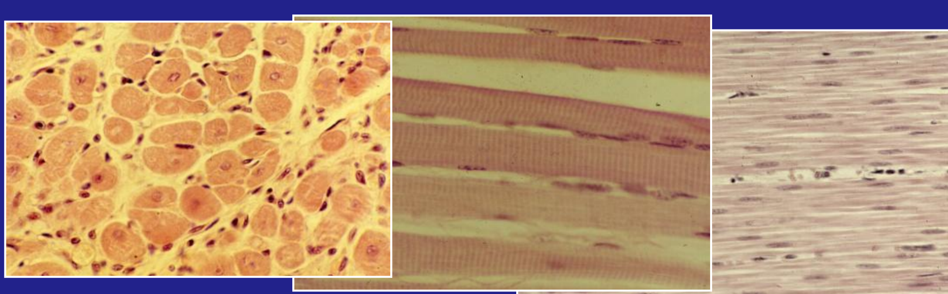
EPITHELIAL TISSUE



CONNECTIVE TISSUE



NERVOUS TISSUE

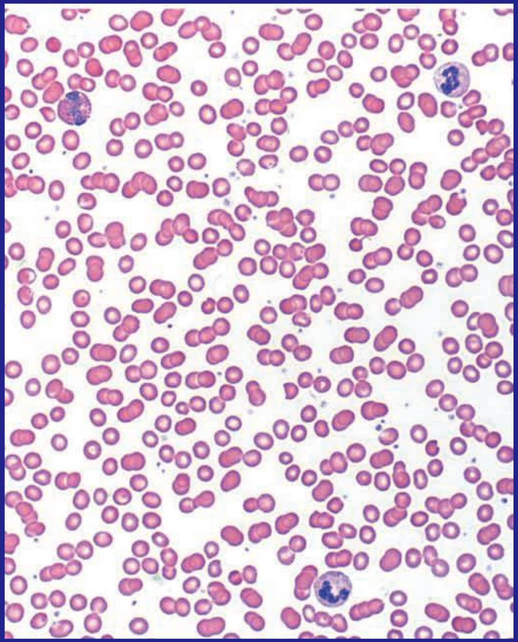


MUSCLE TISSUE

FLUID CONNECTIVE TISSUE: BLOOD

Blood is a fluid connective tissue that circulates through the cardiovascular system.

It consists of CELLS and their derivatives and a protein rich fluid called PLASMA.

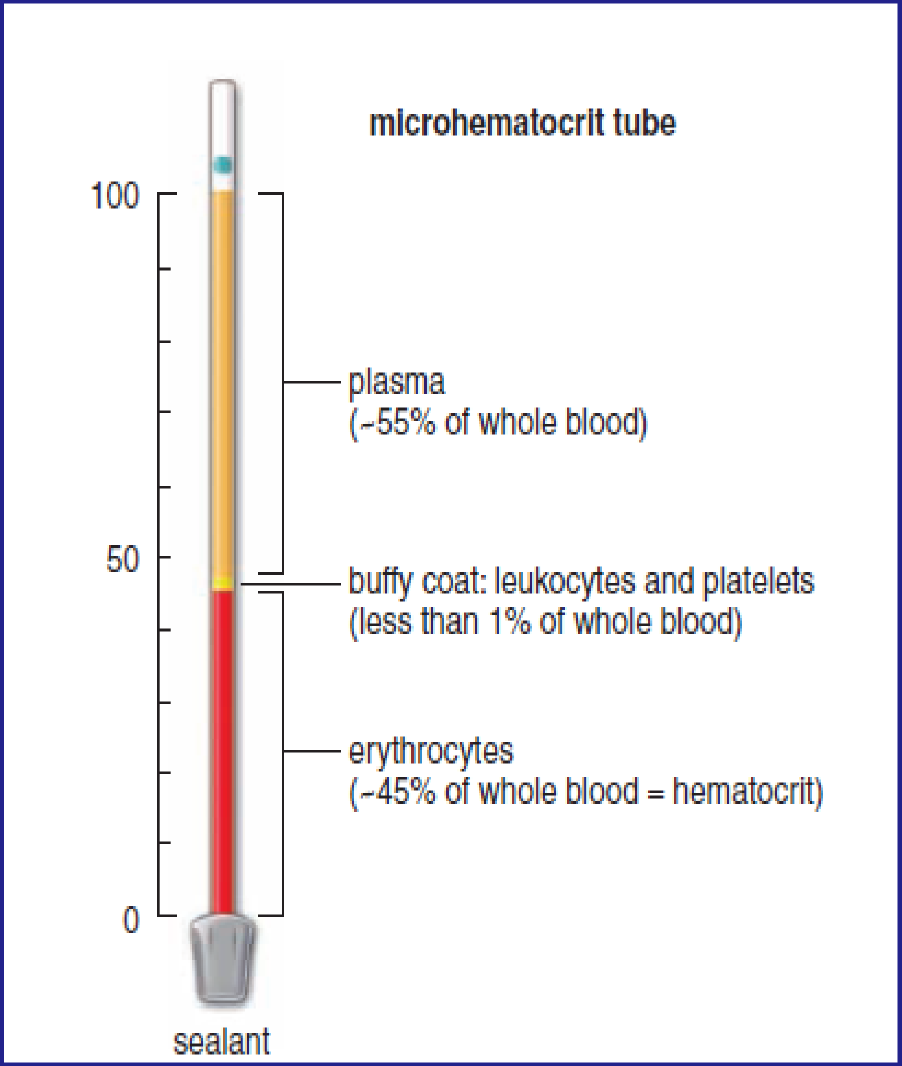


BLOOD CELLS and their derivatives include:

- **erythrocytes**, also called red blood cells (RBCs);
- **leukocytes**, also known as white blood cells (WBCs); and
- **thrombocytes**, also termed platelets.

PLASMA is the liquid extracellular material that imparts fluid properties to blood

FLUID CONNECTIVE TISSUE: BLOOD



The **hematocrit** is measured by centrifuging a blood sample to which anticoagulants have been added, and then calculating the percentage of the centrifuge tube volume occupied by the erythrocytes compared with that of the whole blood.

Erythrocytes = 45%
Buffy coat (leukocytes and platelets) = 1%
Plasma = 55%

Blood's many functions include:

• **delivery of nutrients and oxygen** directly or indirectly to cells,

• **transport of wastes and carbon dioxide** away from cells,

• **delivery of hormones and other regulatory substances** to and from cells and tissues,

• **maintenance of homeostasis** by acting as a buff er and participating in **coagulation and thermoregulation**, and

• **transport of humoral agents** and cells of the immune system that protect the body from pathogenic agents, foreign proteins, and transformed cells (i.e., cancer cells)

Blood's PLASMA

TABLE 10.2 Composition of Blood Plasma

Component	%
Water	91–92
Protein (albumin, globulins, fibrinogen)	7–8
Other solutes:	1–2
Electrolytes (Na^+, K^+, Ca^{2+}, Mg^{2+}, Cl^-, HCO^{3-}, PO_4^{3-}, SO_4^{2-})	
Nonprotein nitrogen substances (urea, uric acid, creatine, creatinine, ammonium salts)	
Nutrients (glucose, lipids, amino acids)	
Blood gases (oxygen, carbon dioxide, nitrogen)	
Regulatory substances (hormones, enzymes)	

90% of plasma by weight is water, which serves as the solvent for a variety of **solutes**, including proteins, dissolved gases, electrolytes, nutrients, regulatory substances, and waste materials.

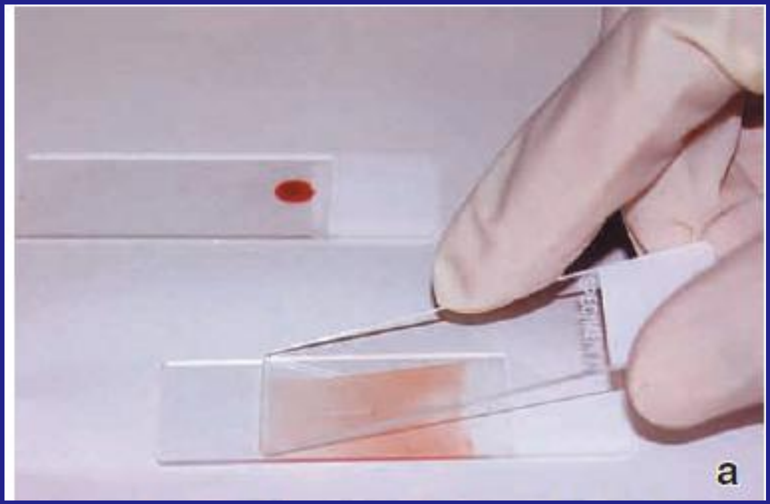
Solutes in the plasma help maintain **homeostasis.**

Plasma that lacks coagulation factors is called **serum**.

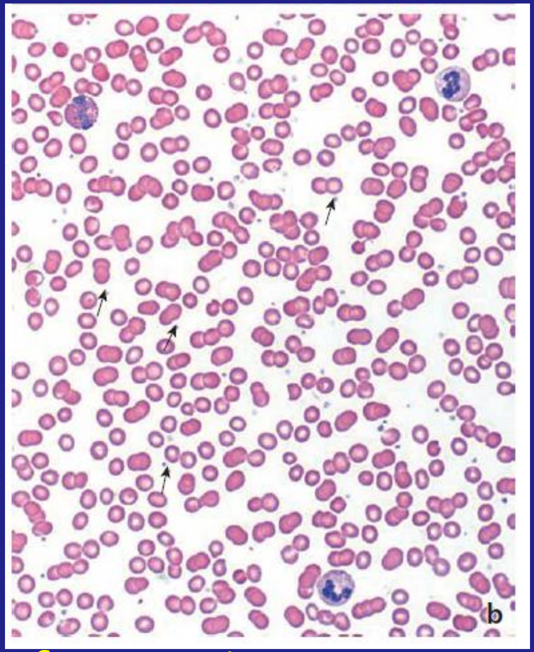
Plasma proteins: albumin, globulins, and fibrinogen.

With the exception of these large plasma proteins and regulatory substances, most plasma constituents are small enough to pass through the blood vessel wall into the extracellular spaces of the adjacent connective tissue.

Examination of blood cells requires special preparation and staining



May Grunwald-Giemsa stain (MGG)



May Grunwald-Giemsa stain is a combination of two stains:
May Grunwald stain and **Giemsa stain.**

- May Grunwald stain is alcohol-based stain composed of methylene blue and eosin.
- Giemsa stain is alcohol-based stain composed of methylene blue, eosin and azure B.

- **Erythrocytes**: Light pink to light purple
- **Platelets**: Granules – Reddish purple
- **Lymphocytes/monocytes**: Nuclei – Dark purple, Cytoplasm – Sky blue
- **Neutrophils**: Nuclei – Dark blue, Granules – Reddish purple, Cytoplasm – Pale pink
- **Eosinophils**: Nuclei – Blue, Granules – Red/orange red, Cytoplasm – Blue
- **Basophils**: Nuclei – Dark blue, Granules – Purple

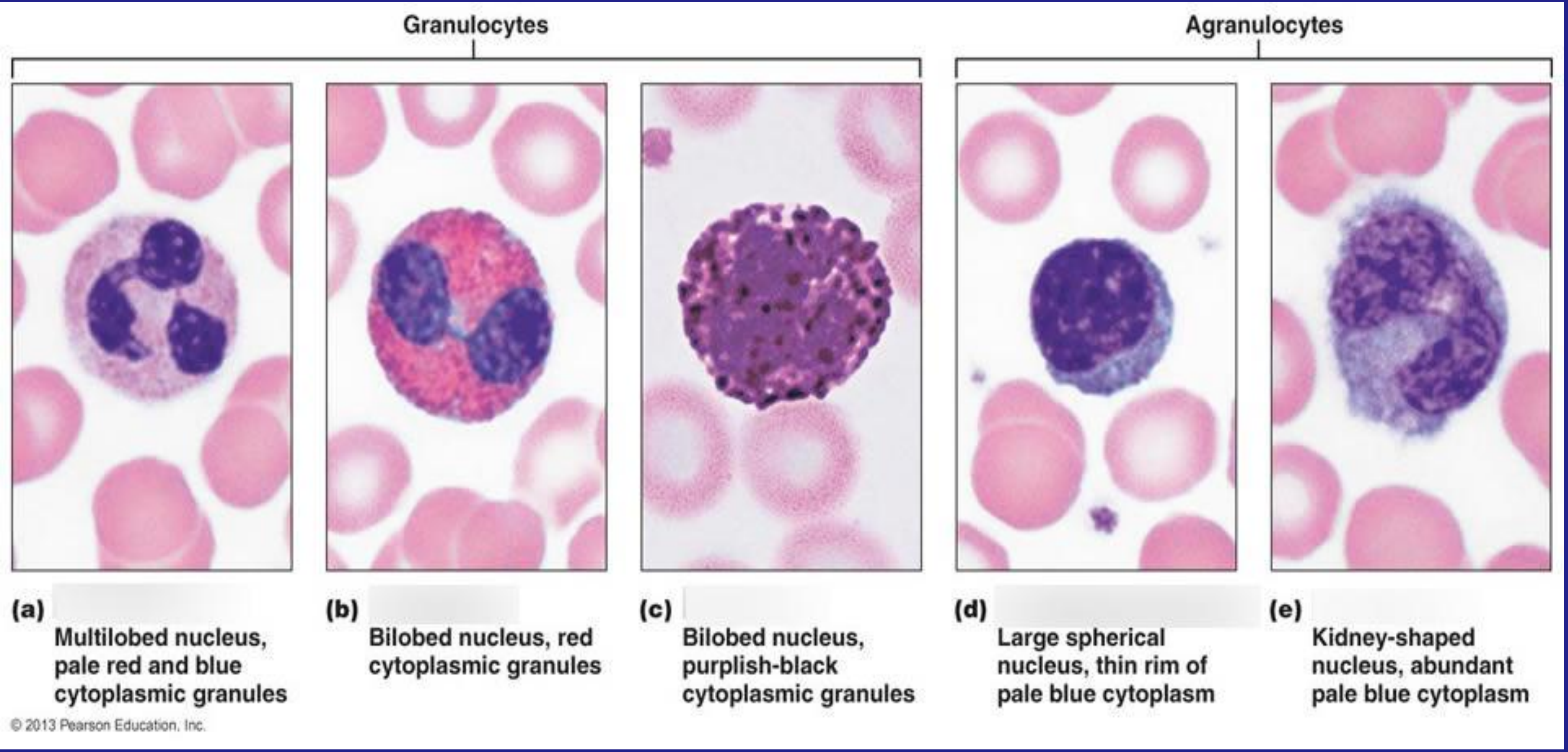
https://www.jove.com/v/20264/may-grunwald-giemsa-staining-a-method-to-stain-bone-marrow-cells

Blood's CELLS: LEUKOCYTES

LEUKOCYTES (or **White Blood Cells**) account 1% of blood volume and are divided into:

-Granulocytes (neutrophils, eosinophils, and basophils) and

-Agranulocytes (lymphocytes and monocytes).



Blood's CELLS: LEUKOCYTES

Neutrophils (polymorphonuclear neutrophils)

the most numerous WBCs as well as the most common granulocytes.

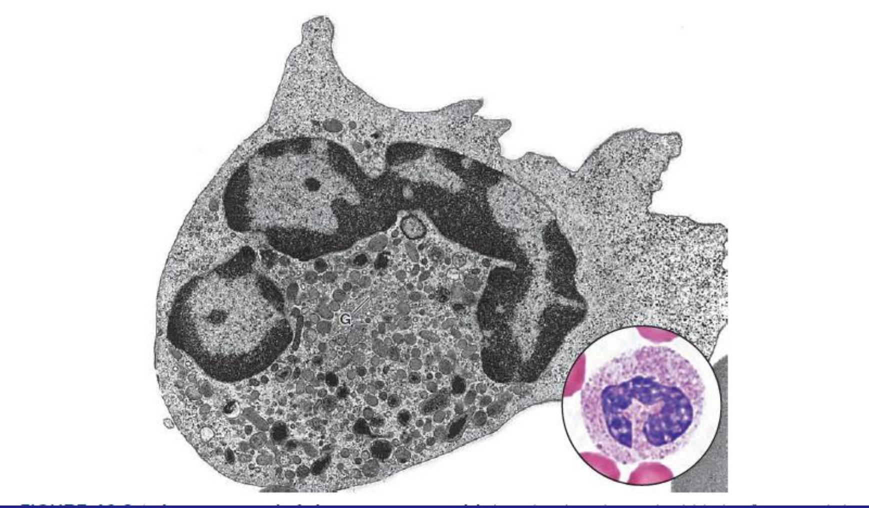
10 to 12 um in diameter;
multilobal nucleus (2-4 lobes)

Neutrophils contain three types of granules that reflect the various phagocytotic functions of the cell:

-Azurophilic granules (primary granules)

-Specific granules (secondary granules)

-Tertiary granules



Neutrophils are motile cells

Neutrophils are active phagocytes that utilize a variety of surface receptors to recognize bacteria and other infectious agents at the site of inflammation.

Neutrophils are motile cells: they leave the circulation and migrate to their site of action in the connective tissue (diapedesis)

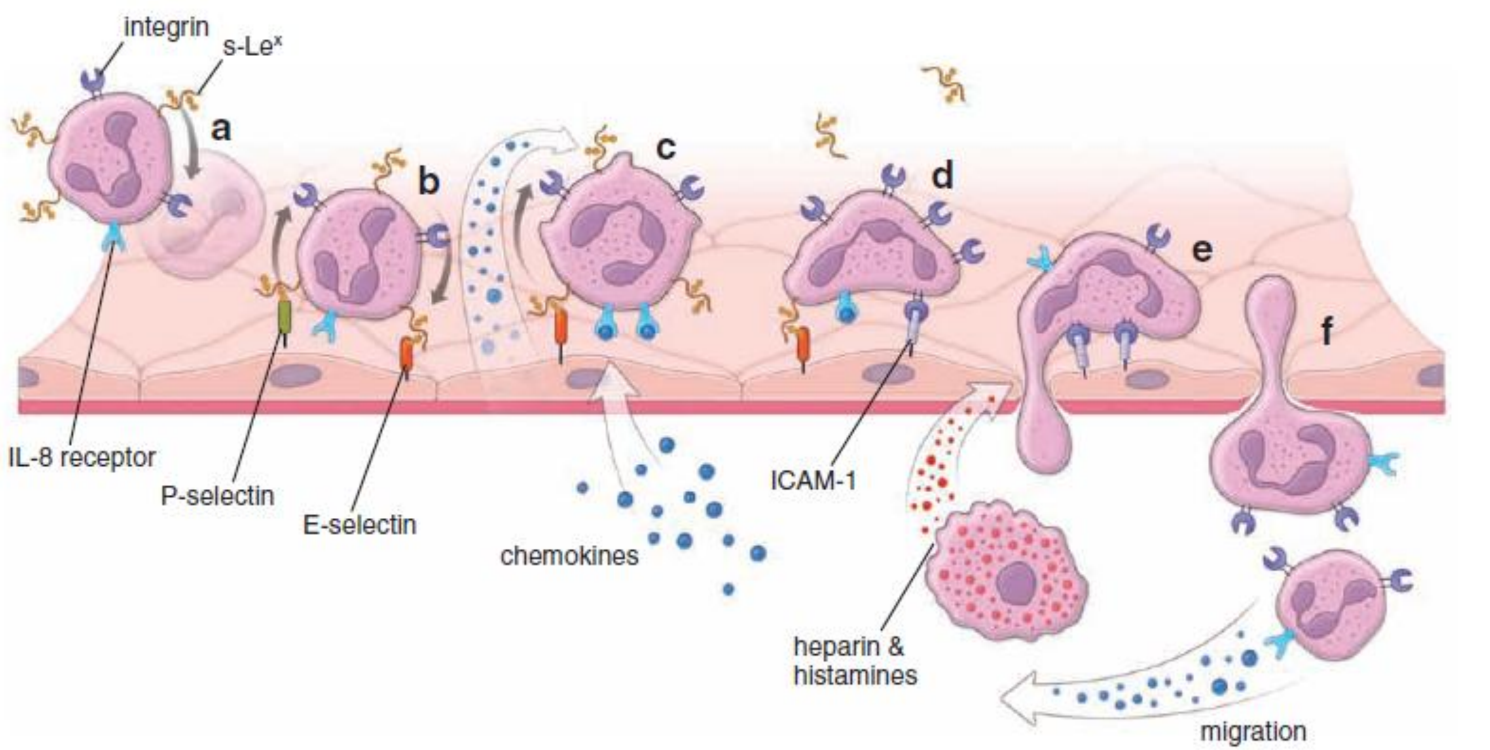


FIGURE 10.9 ▲ Diagram of events in the migration of a neutrophil from a postcapillary venule into the connective tissue. **a.** A neutrophil traveling in the blood vessel expresses a high number of cell-to-cell recognition molecules, such as Sialyl Lewis^x (s-Le^x) carbohydrates, integrin, and interleukin receptors. **b.** Circulating neutrophils are slowed down by the interaction of their surface s-Le^x molecules with E- and P-selectins expressed on the endothelium of the postcapillary venule. **c.** As a result of this interaction, the cell rolls on the surface of the endothelium. The neutrophil then adheres to the endothelium and responds to chemokines (e.g., interleukin-8) secreted by the endothelial cells. **d.** Their secretion induces the expression of other adhesion molecules on the surface of the neutrophil, such as integrins (e.g., VLA-5), which provide tight bonds with the immunoglobulin superfamily of adhesion molecules (e.g., intercellular adhesion molecule-1 [ICAM-1]) expressed on the surface of the endothelium. These interactions provide firm adhesion of the neutrophil to the endothelial surface. **e.** The neutrophil then extends a pseudopod to an intercellular junction previously opened by histamine and heparin released from the mast cells in the connective tissue, allowing the neutrophil to migrate through the vessel wall. **f.** Once the neutrophil leaves the circulation and enters the connective tissue, its further migration is directed by chemoattractant molecules that interact with specific receptors on its surface.

Neutrophils are active phagocytes that utilize a variety of surface receptors to recognize bacteria and other infectious agents at the site of inflammation.

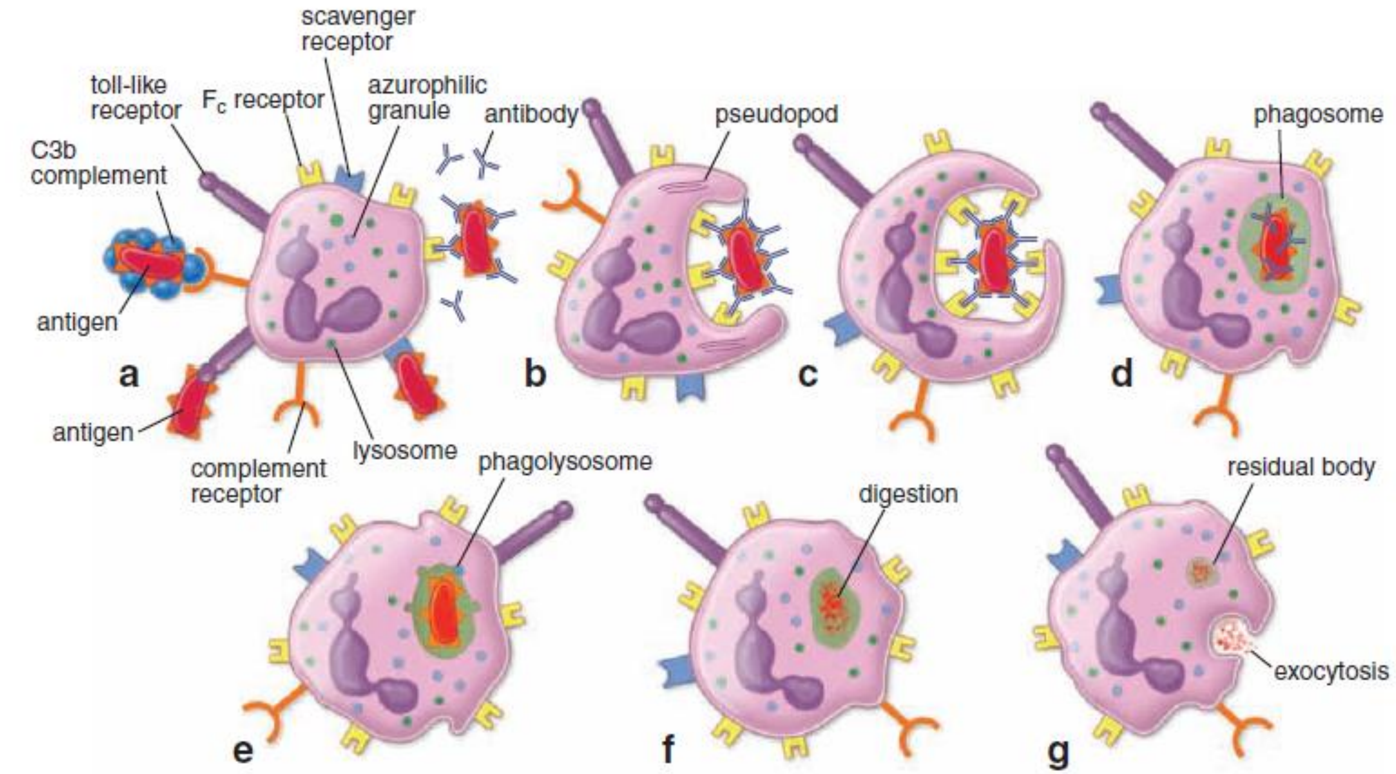


FIGURE 10.10 ▲ **Neutrophil phagocytosis. a.** Phagocytosis begins with recognition and attachment of foreign material (antigen), mainly by F_c receptors that interact with the F_c region of antibodies bound to the antigen. **b.** The antigen is then engulfed by pseudopods of the neutrophil. **c.** As the pseudopods come together and fuse, the antigen is internalized. **d.** Once the phagosome is formed, digestion is initiated by activation of membrane-bounded oxidases of the phagosome. **e.** Next, both specific and azurophilic granules fuse with the phagosome and release their contents, forming a phagolysosome. This fusion and release of granules is called *degranulation*. **f.** The enzymatic contents of the granules are responsible for killing and digesting the microorganism. The entire digestive process occurs within the phagolysosome, which protects the cell from self-injury. **g.** The digested material is either exocytosed into the extracellular space or stored as residual bodies within the neutrophil.

Blood's CELLS: LEUKOCYTES

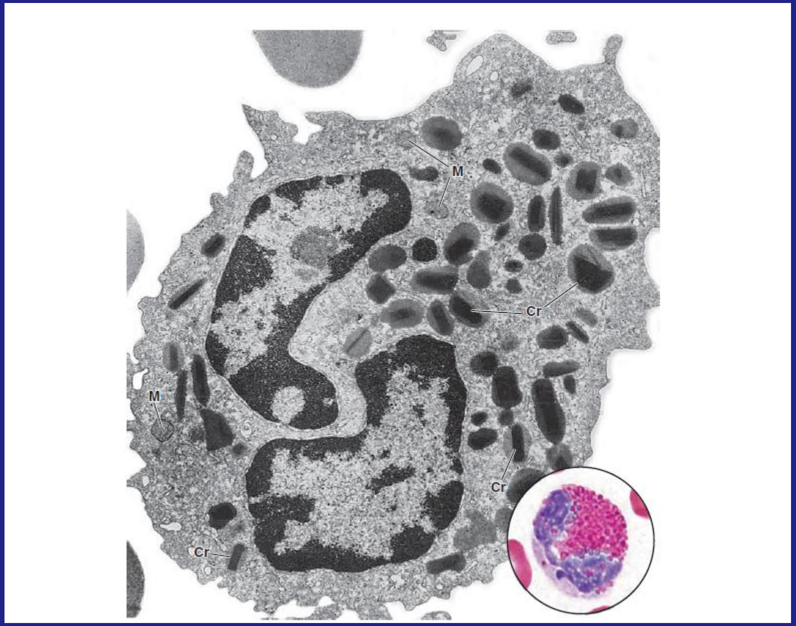
Eosinophils are named for the large, eosinophilic, refractile granules in their cytoplasm.

10 to 12 um in diameter;
bilobed nucleus (2 lobes)

Eosinophils contain two types of granules:

-Azurophilic granules (primary granules)

-Specific granules (secondary granules)



Eosinophils are associated with allergic reactions, parasitic infections, and chronic inflammation.

Blood's CELLS: LEUKOCYTES

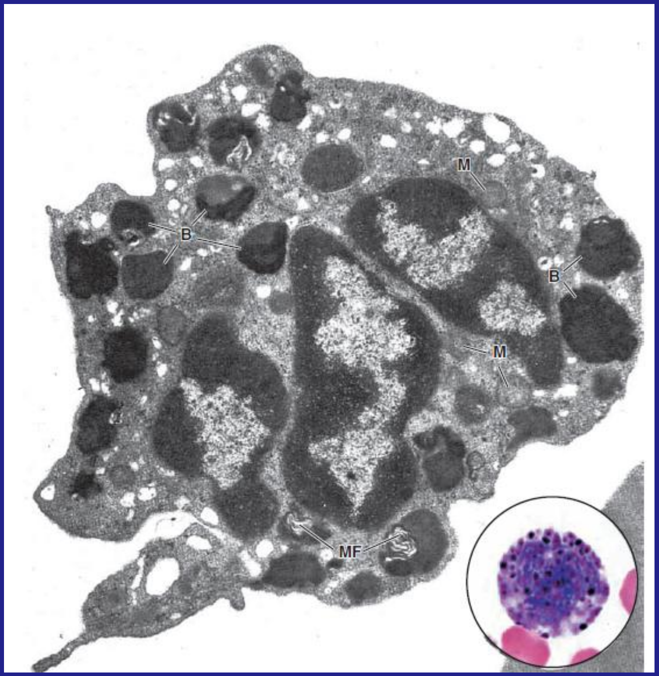
Basophils are the least numerous of the white blood cells, accounting for less than 0.5% of total leukocytes

10 to 12 um in diameter;
bilobed nucleus (2 lobes)

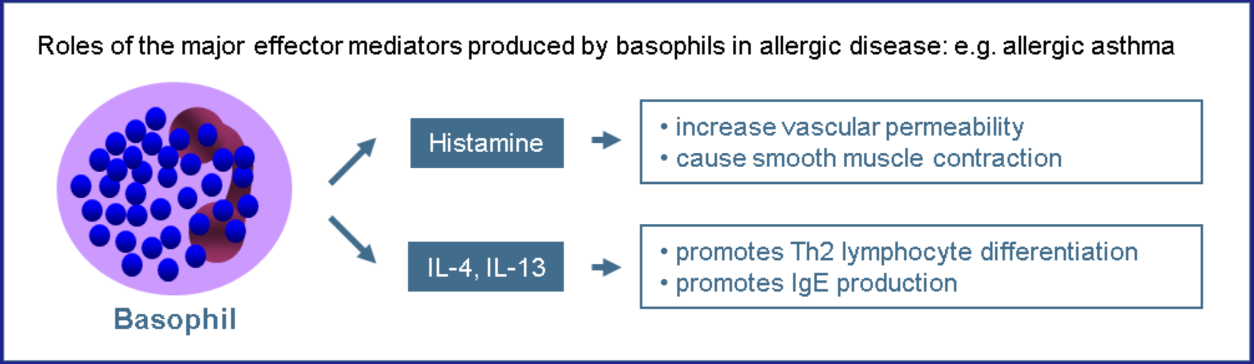
Basophils contain two types of granules:

-Azurophilic granules (primary granules)

-Specific granules (secondary granules)



The function of basophils is closely related to that of mast cells of connective tissue



Blood's CELLS: LEUKOCYTES

Lymphocytes are the main functional cells of the lymphatic or immune system. They are the most common agranulocytes and account for about 30% of the total blood leukocytes

lymphocytes are different in several aspects from other leukocytes:

- Lymphocytes are not terminally differentiated cell

- Lymphocytes can exit from the lumen of blood vessels into tissues and subsequently can recirculate back into blood vessels

- Despite originate in the bone marrow, lymphocytes are capable of developing outside the bone marrow in tissues associated with the immune system

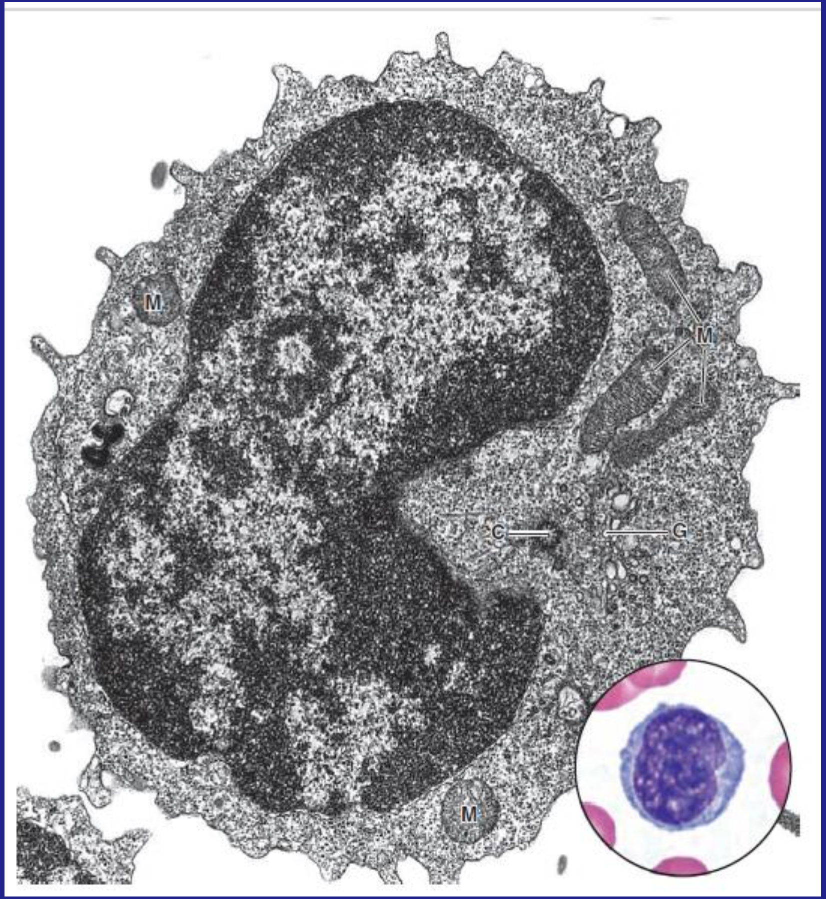
Blood's CELLS: LEUKOCYTES

Lymphocytes , with spherical nucleus, can be identified in **three groups** according to size ranging in diameter from 6 to 30 um:

-SMALL LYMPHOCYTES (6 um in diameter)

-MEDIUM LYMPHOCYTES (15 um in diameter)

-LARGE LYMPHOCYTES (30 um in diameter), also called **activated lymphocytes**



Blood's CELLS: LEUKOCYTES

The characterization of **lymphocyte** types is based on their function, not on their size or morphology

Three functionally distinct types of lymphocytes are present in the body:

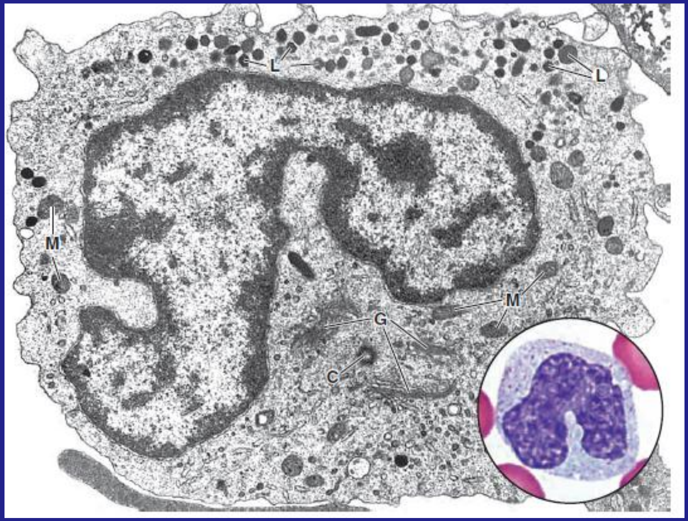
- **T LYMPHOCYTES** involved in cell mediated immunity, are characterized by the presence of cell-surface recognition proteins called **T-cell receptors (TCRs)**
- **B LYMPHOCYTES** involved in the production of circulating antibodies. Mature B cells in blood express **IgM** and **IgD** and **MHC II** molecules on their surface.
- **NATURAL KILLER (NK) CELLS** are programmed during their development to kill certain virus-infected cells and some types of tumor cells. They secrete an antiviral agent, **interferon (IFN-γ)**

T and B cells are indistinguishable in blood smears and tissue sections; immunocytochemical staining for diff erent types of markers and receptors on their cell surface must be used to identify them.
NK lymphocytes can be identified in the light microscope by size, nuclear shape, and presence of cytoplasmic granules; however, immunocytochemical staining for their specifi c markers is used to confi rm microscopic identifi cation.

Blood's CELLS: LEUKOCYTES

Monocytes are the precursors of the cells of the mononuclear phagocytotic system. Even if are classified as agranular, they contain small, dense, azurophilic granules

18 um in diameter;
One nucleus, kidney-shaped



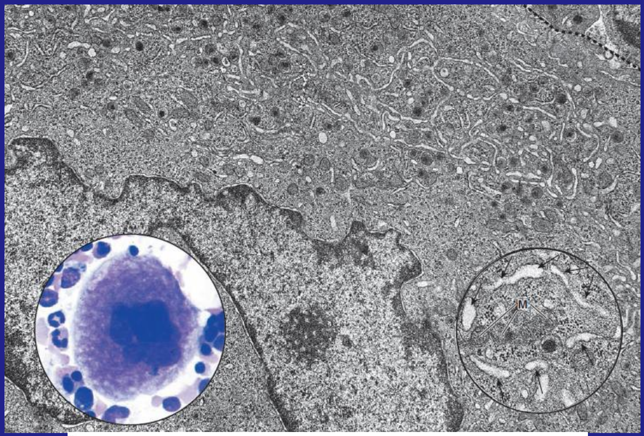
Monocytes transform into macrophages, which function as antigen-presenting cells in the immune system.

Phagocytoses bacteria, other cells, and tissue debris

Blood’s CELLS: LEUKOCYTES

Thrombocytes (platelets) are small, membrane-bounded, anucleate cytoplasmic fragments derived from megakaryocytes fragmentation.

Platelets circulate as discoid structures about 2 to 3 um in diameter.



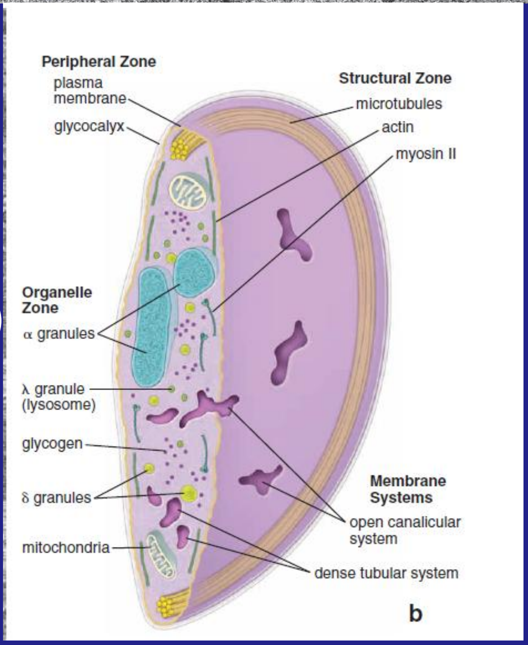
Thrombocyte cytoplasm can be categorized into the four zones:

peripheral zone, cell membrane covered by a thick surface coat of glycocalyx.

structural zone, near the periphery, comprises microtubules, actin filaments, myosin, and actin-binding proteins that form a network supporting the plasma membrane.

organelle zone with mitochondria, peroxisomes, glycogen particles, and at least three types of granules dispersed within the cytoplasm.

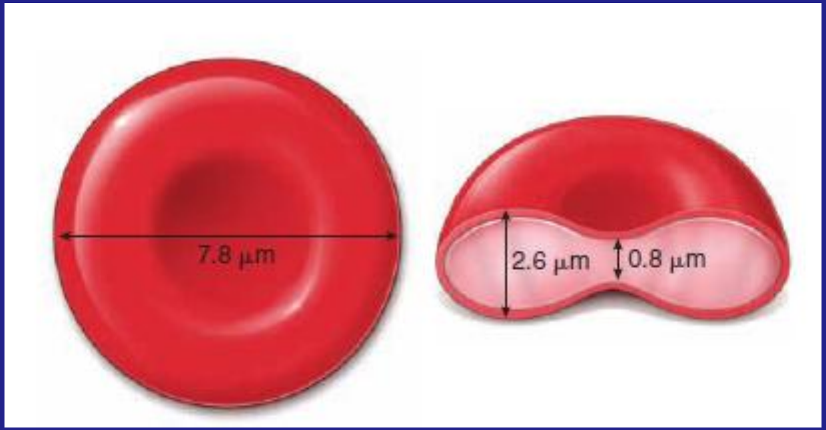
membrane zone, consists of two types of membrane channels, the **open canalicular system (OCS)** and **dense tubular system (DTS)**

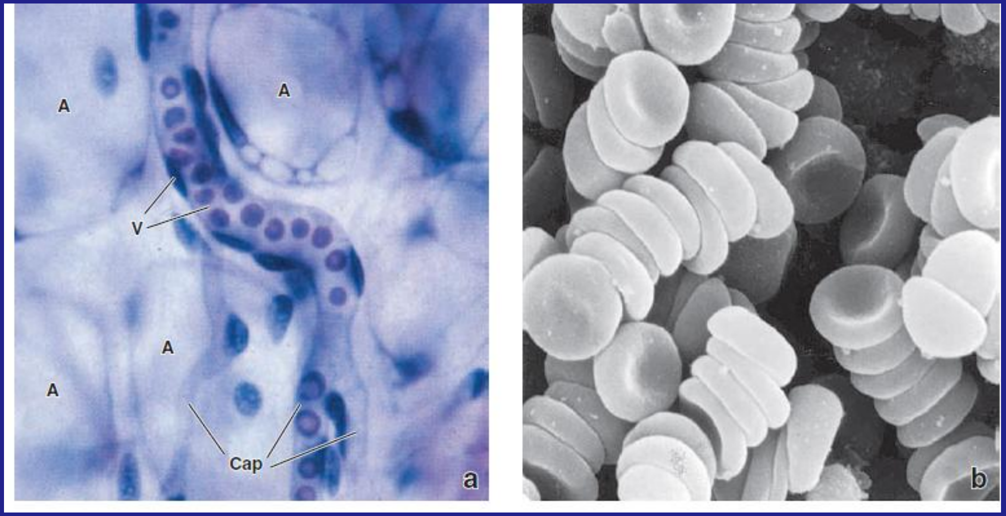


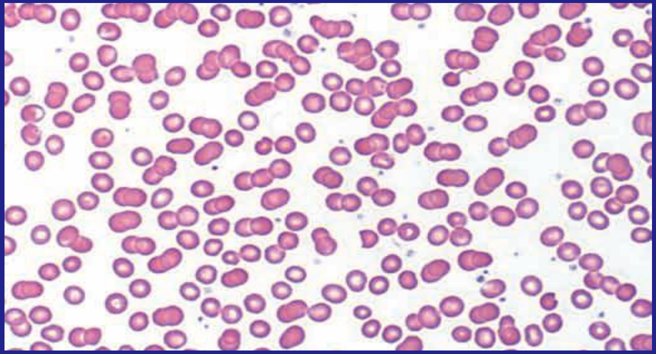
Platelets function in continuous surveillance of blood vessels, blood clot formation, and repair of injured tissue.

Blood's CELLS: ERYTHROCYTES

Erythrocytes or red blood cells (RBCs) are anucleate cells devoid of typical organelles



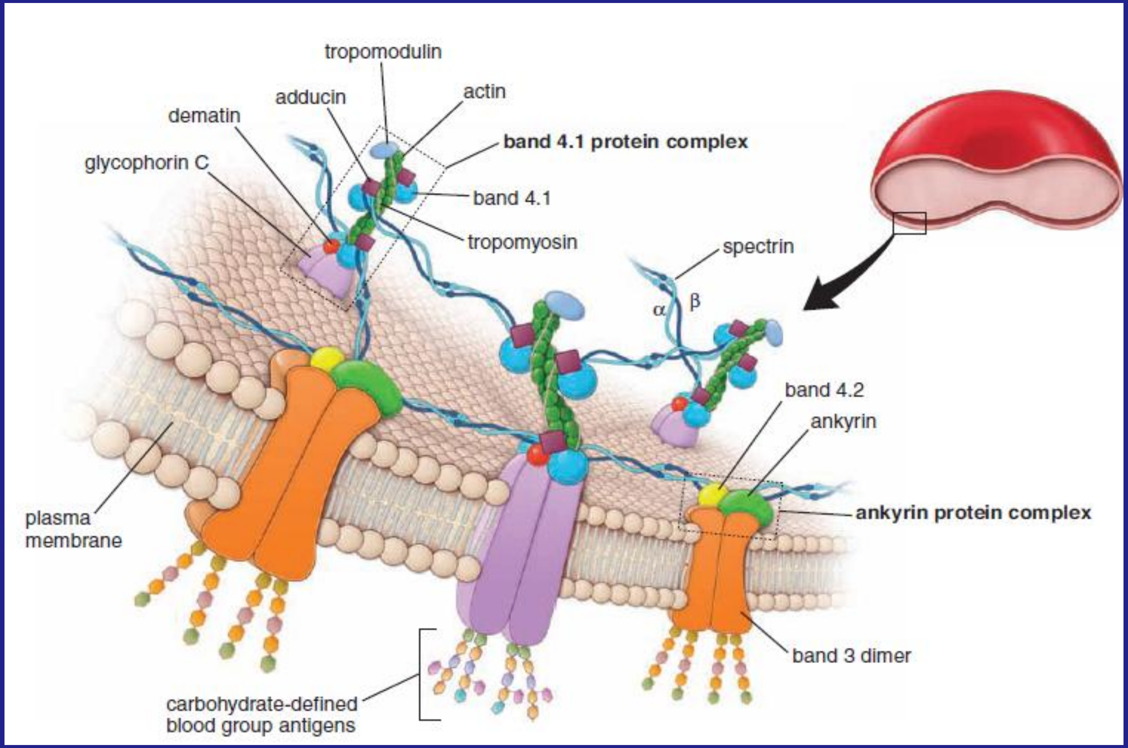




They function only within the bloodstream to bind oxygen for delivery to the tissues and, in exchange, bind carbon dioxide for removal from the tissues

Blood's CELLS: ERYTHROCYTES

The shape of the erythrocyte is maintained by a specialized cytoskeleton that provides the mechanical stability and flexibility necessary to withstand forces experienced during circulation.



Th is unique cytoskeletal arrangement contributes to the shape of the erythrocyte and imparts elastic properties and stability to the membrane.

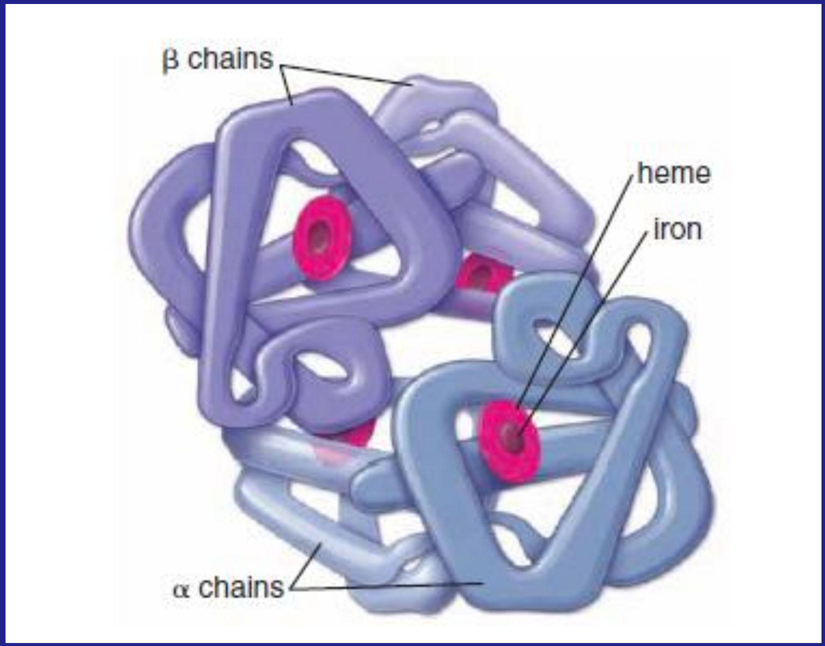
Blood's CELLS: ERYTHROCYTES

Erythrocytes contain HEMOGLOBIN, a protein specialized for the transport of oxygen and carbon dioxide.

Hemoglobin consists of four polypeptide chains of globin α, β, δ, and γ, each complexed to an iron-containing heme group

- Hemoglobin HbA is most prevalent in adults, accounting for about 96% of total hemoglobin. It is a tetramer with two α and two β chains ($\alpha_2\beta_2$).
- Hemoglobin HbA$_2$ accounts for 1.5% to 3% of total hemoglobin in adults. It consists of two α and two δ chains ($\alpha_2\delta_2$).
- Hemoglobin HbF accounts for less than 1% of total hemoglobin in adults. It contains two α and two γ chains ($\alpha_2\gamma_2$) and is the principal form of hemoglobin in the fetus. HbF production falls dramatically after birth;

Hemoglobin HbA



During gestational and postnatal periods, the synthesis of hemoglobin polypeptide chains varies, resulting in different hemoglobin types

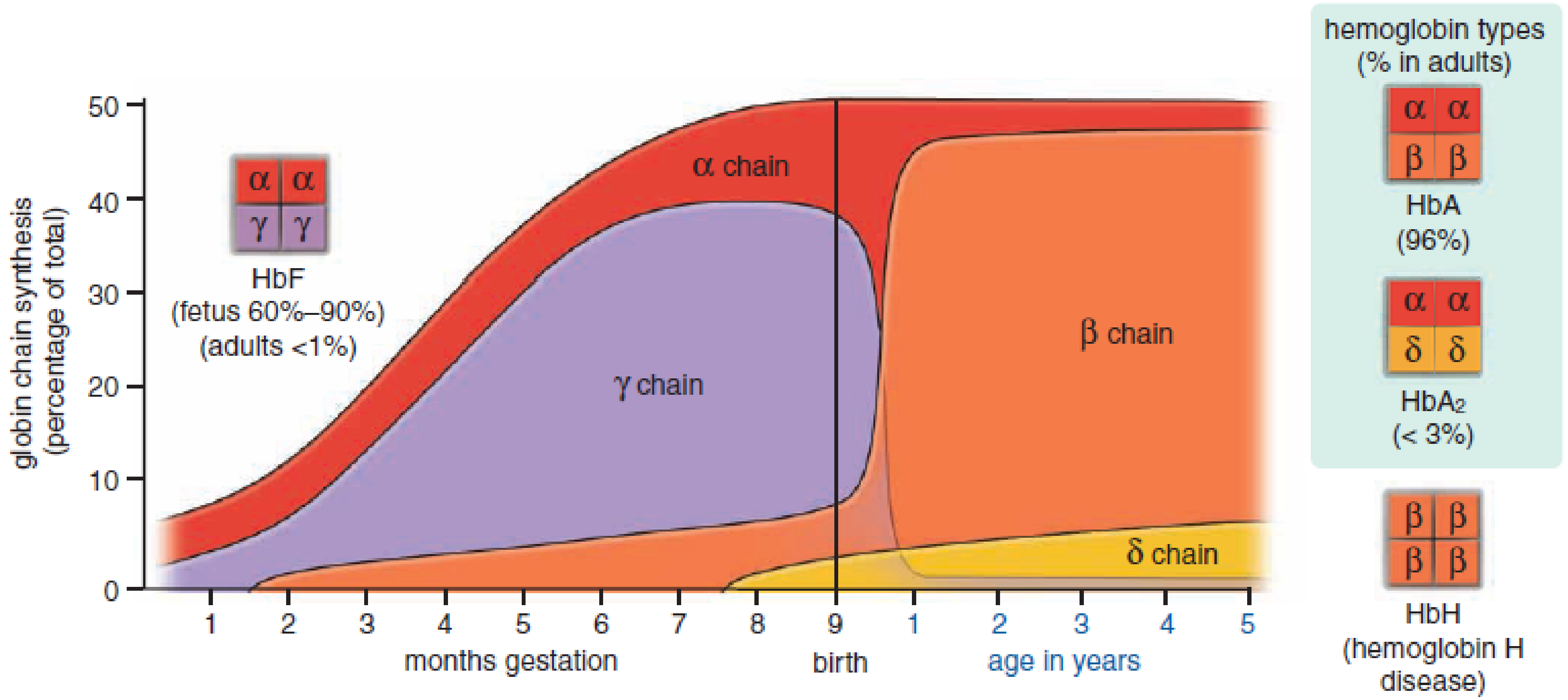


FIGURE 10.7 ▲ Major globin chain synthesis and hemoglobin composition in prenatal and postnatal periods. The type of hemoglobin differs in the gestational and postnatal periods. This diagram represents a timeline for the synthesis of the four major globin chains (α, β, δ, and γ) and for hemoglobin composition. In early stages of development, α and γ chains form fetal hemoglobin (HbF), which is predominate at birth. In the second month of gestation, synthesis of β chains gradually increases. After birth, it drastically escalates to form with α chains, predominately adult hemoglobin (HbA). During this time, γ chain synthesis declines. Later in prenatal age, δ chain production is initiated to form hemoglobin containing two δ and two α chains (HbA_2). Adult hemoglobin HbA (96%) and HbA_2 (<3%) within the *blue box* are regarded as normal hemoglobin types. Traces of hemoglobin HbF is considered normal in levels below 1%. An example of the pathological hemoglobin shown in this diagram is hemoglobin HbH, which is formed as a tetramer of β chains.

Sickle Cell Disease

Sickle cell disease is caused by a single-point mutation in the gene that encodes the **Beta -globin chain of hemoglobin A (HbA)**.

The result of this mutation is an abnormal Beta -globin chain in which the amino acid valine is substituted for glutamic acid in position 6. Hemoglobin containing this abnormal Beta-globin chain is designated **sickle hemoglobin (HbS)**.

The substitution of the hydrophobic valine for the hydrophilic glutamic acid causes HbS molecules in a condition of low oxygen saturation to aggregate and grow in length beyond the diameter of the erythrocyte.

Instead of the normal biconcave disc, many of the erythrocytes become sickle-shaped at low oxygen tension.

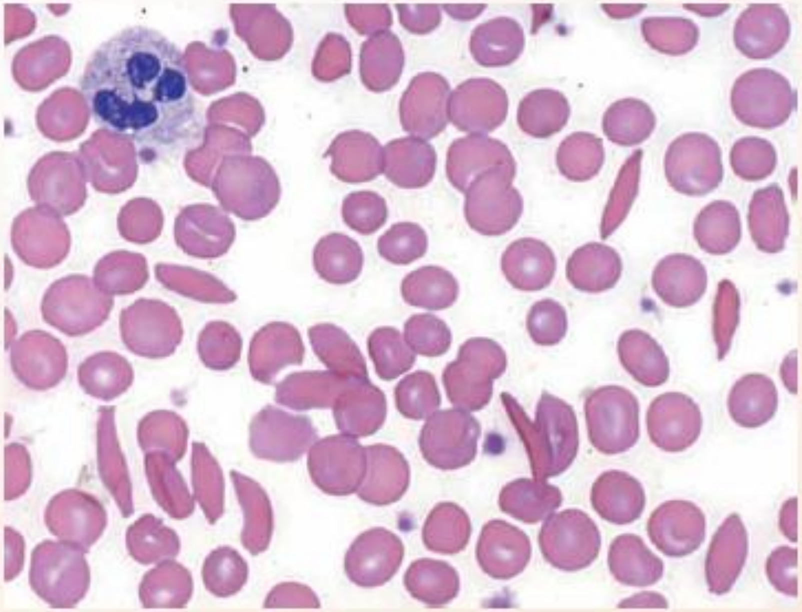


FIGURE F10.3.1 ▲ Photomicrograph of a sickle cell anemia blood smear. Blood smear stained with Wright's stain shows abnormal "boat"- and "sickle"-shaped cells from an individual with sickle cell anemia. ×400.

Sickled erythrocytes are more rigid than normal cells and adhere more readily to the endothelial surface. Thus, the blood becomes more viscous and sickled erythrocytes may pile up in the smallest capillaries, depriving portions of tissues and organs of oxygen and nutrients

FORMATION OF BLOOD CELLS (HEMOPOIESIS)

Hemopoiesis (hematopoiesis) includes

erythropoiesis (development of red blood cells)
leukopoiesis (development of white blood cells)
thrombopoiesis (development of platelets)

Hemopoiesis is initiated in early embryonic development.

The first or **yolk-sac phase** of hemopoiesis begins in the third week of gestation

The second, or **hepatic phase**, early in fetal developmentduring the second trimester

The third or **bone marrow phase** of fetal hemopoiesis and leukopoiesis involves the bone marrow (and other lymphatic tissues) and begins during the second trimester of pregnancy.
After birth, hemopoiesis takes place only in the red bone marrow and some lymphatic tissues, as in the adult.

Blood cells are derived from a common hemopoietic stem cell (HSC)

HSC in the bone marrow gives rise to multiple colonies of progenitor stem cells.

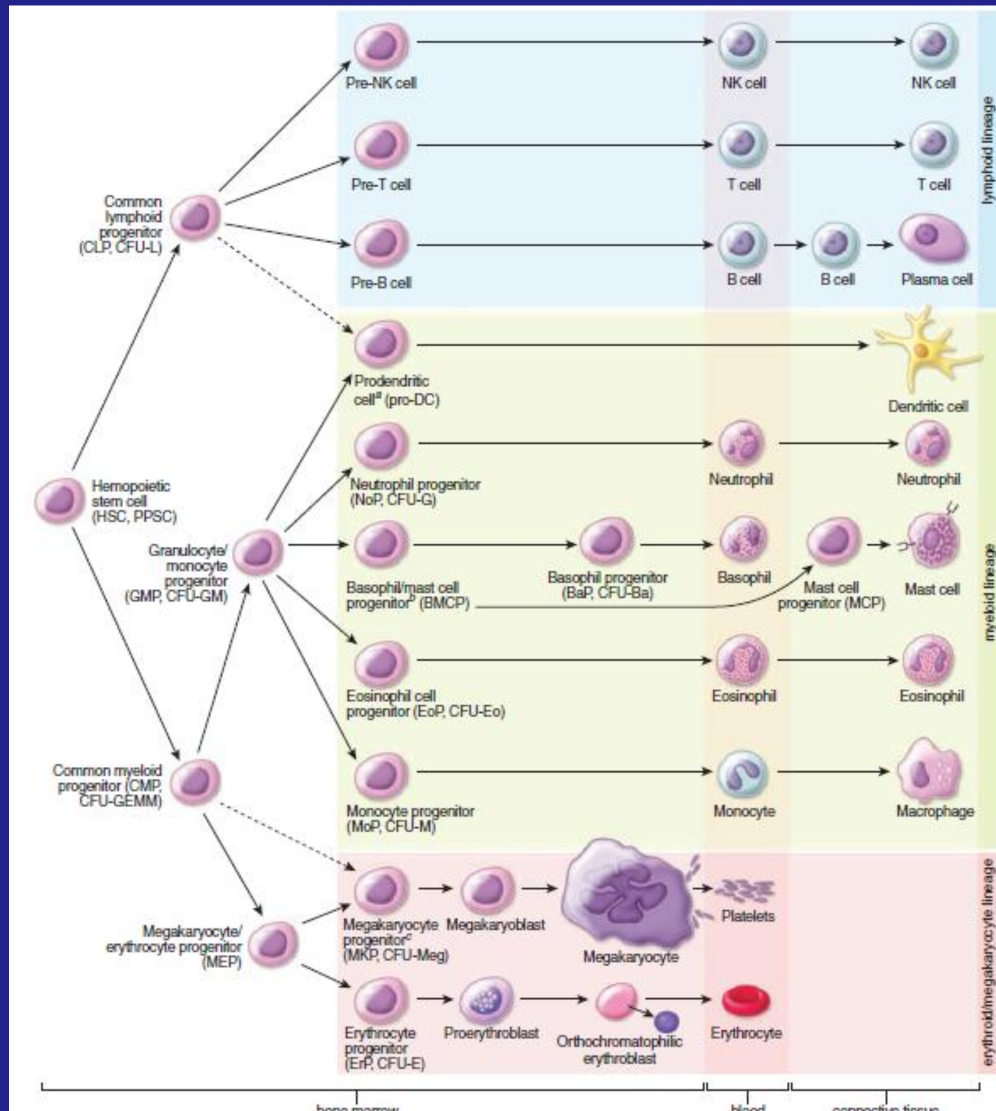


FIGURE 10.19 ▲ **Hemopoiesis.** This chart is based on the most recent concepts in hemopoiesis. It shows blood cells' development from

FORMATION OF BLOOD CELLS (HEMOPOIESIS)

- **Hemopoiesis** (hematopoiesis) is initiated in early embryonic development and includes **erythropoiesis** (development of red blood cells), **leukopoiesis** (development of white blood cells), and **thrombopoiesis** (development of platelets).
- In adults, **hemopoietic stem cells (HSCs)** reside in the bone marrow. Under the influence of cytokines and growth factors, they differentiate into **common myeloid progenitor (CMP) cells** (give rise to megakaryocytes, erythrocytes, neutrophils, eosinophils, basophils and/or mast cells, and monocytes) and **common lymphoid progenitor (CLP) cells** (give rise to T cells, B cells, and NK cells).
- During **erythropoiesis**, erythrocytes evolve from **proerythroblasts** and **basophilic**, **polychromatophilic**, and **orthochromatophilic erythroblasts** to **polychromatophilic** and mature **erythrocytes**.
- Developing red blood cells become smaller, change their cytoplasm appearance (from blue to red) due to intense accumulation of hemoglobin, and extrude their nuclei.
- In **thrombopoiesis**, thrombocytes (platelets) are produced in the bone marrow by **megakaryocytes** (large polyploid cells of the red bone marrow) that developed from the same CMP stem cells as the erythroblasts.
- In **granulopoiesis**, granulocytes originate from the CMP stem cell, which differentiates into **granulocyte/monocyte progenitors (GMPs; also give rise to monocytes)**. CMP stem cells also give rise to monocytes.
- **Neutrophil progenitor (NoP)** cells undergo six morphologically identifiable stages in development: **myeloblast**, **promyelocyte**, **myelocyte** (first to exhibit specific granules), **metamyelocyte**, **band (immature) cell**, and **mature neutrophil**. The development of other granulocytes follows a similar path.
- In **lymphopoiesis**, lymphocytes develop from the CLP stem cell and depend on the expression of specific transcription factors. They differentiate in the bone marrow and other lymphatic tissues.

BONE MARROW

- **Red bone marrow** contains cords of active hemopoietic cells that lie within the medullary cavity in children and within the spaces of spongy bone in adults.
- Bone marrow contains specialized blood vessels (**sinusoids**) into which newly developed blood cells and platelets are released.
- Bone marrow not active in hemopoiesis contains predominately adipose cells and is called **yellow bone marrow**.