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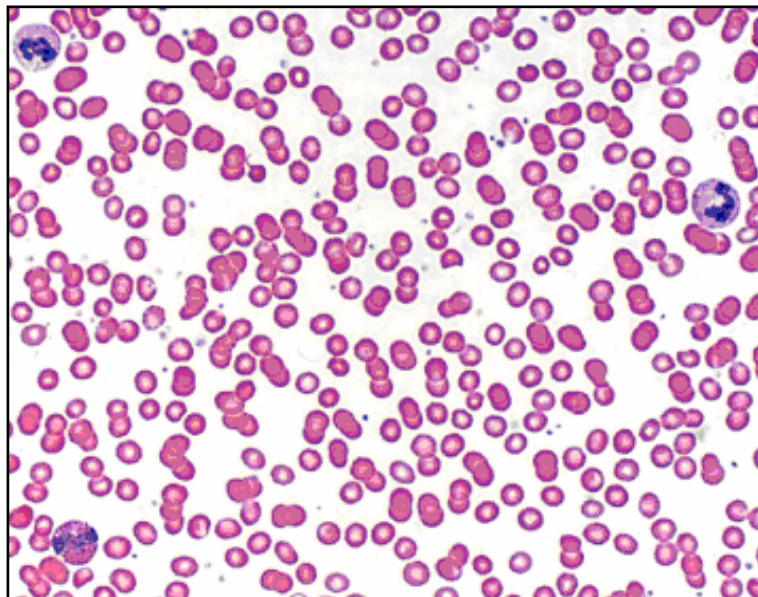
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Blood

- **Blood** is a viscous fluid formed of cellular element suspended in plasma.
- The **cellular element** composed of: Erythrocytes (red blood cells), Leucocytes (white blood cells), and Platelets.
- **Plasma** is a viscous, translucent, yellowish fluid composed of water (90%), proteins (7%), organic salts (1%), and organic compound (2%) such as amino acids, lipids, and vitamins.
- The total blood volume in human is about 5 L (depending on body size).
- Outside the blood vessels, blood undergoes a complex reaction called coagulation or clot formation, which plays an important role in repairing damaged blood vessels and preventing blood loss.
- Erythrocytes and blood platelets perform their functions inside the blood vessels, whereas leukocytes reside temporarily in the blood vessels and then leave the blood stream through the capillary walls and venules to enter either the connective tissues or lymphoid tissues.
- The ratio of erythrocytes to the total blood volume is about 43% and is known as haematocrit.



Blood film showing red blood cells and different types of white blood cells

COMPOSITION OF PLASMA

A. Water: constitutes 90% of plasma volume.

B. Solutes: constitutes 10% of plasma. and include plasma proteins and other organic compounds as well as inorganic salts.

1. Plasma proteins. Plasma contains a rich variety of soluble proteins, 7% by volume. Important examples include:

a. Albumin. This is the most abundant plasma protein (3.5-5 g/dL of blood) and is mainly responsible for maintaining the osmotic pressure of blood.

b. Globulins (Alpha, beta, and gamma globulins) are globular proteins dissolved in the plasma. The gamma globulins include the antibodies, or immunoglobulins, synthesized by plasma cells.

c. Blood coagulation proteins: such as prothrombin, fibrinogen which is converted into fibrin during clot formation. Fibrinogen is synthesized and secreted by the liver.

2. Organic compounds. They include nutrients such as amino acids and glucose, vitamins, and a variety of regulatory peptides, steroid hormones, and lipids.

3. Inorganic salts. They constitute 0.9% of plasma volume, include blood electrolytes such as sodium, potassium, and calcium salts.

Serum: portion of plasma that separates from coagulum after clotting.

When anticoagulants (heparin, citrate, etc) are added, blood samples can be separated by centrifugation into 3 major fractions:-

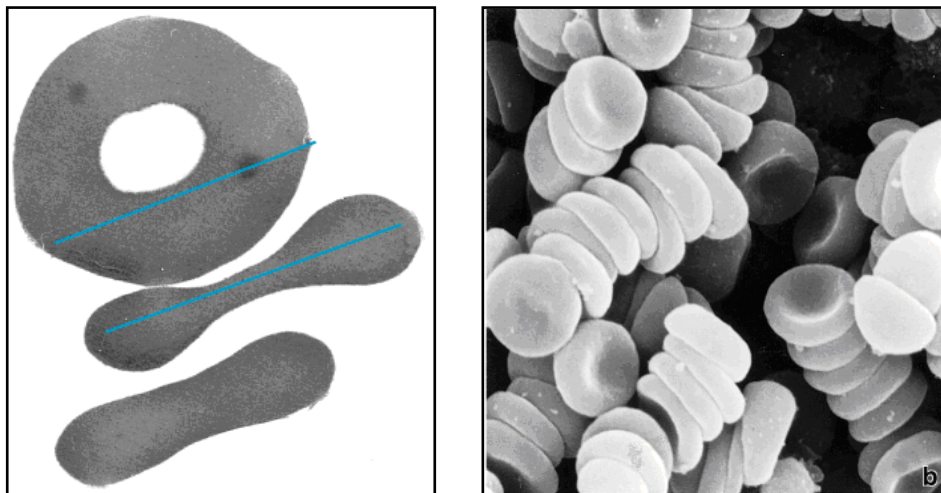
- **The erythrocytes** constitute the densest fraction and end up at the bottom of the tube.
 - The percentage of packed erythrocytes per unit volume of blood is termed **hematocrit**.
 - In adults, normal hematocrit values vary from 35 to 50% and are sex dependent.
- **Leukocytes** are less dense and less numerous (about 1 % of blood volume) and form a thin white or grayish layer over the erythrocytes (buffy coat).

- On top of the buffy coat is a thin layer of platelets.
- The least dense is the **clear layer of plasma**, which constitutes 42 - 47% of the blood and overlies the buffy coat.

The cellular or formed elements of blood

A- Erythrocytes (RBC)

RBCs are structurally and functionally specialized to transport oxygen from the lungs to other tissues. Their cytoplasm contains the oxygen binding protein hemoglobin. Mature RBCs lack nuclei and cytoplasmic organelles, which they lose during differentiation. Mature erythrocytes therefore have a limited lifespan (120 days) in the circulation before they are removed by macrophages in the spleen and bone marrow.



- Erythrocytes (RBC) are anucleated corpuscles (nucleated in embryonic and fetal mammals and in other vertebrates).
- They are biconcave disks about $\sim 7\mu\text{m}$ in diameter, $2\mu\text{m}$ thick at its rim and less than $1\mu\text{m}$ at its center.
- They contain hemoglobin, which fills almost the entire cytoplasm.
- Erythrocytes are elastic and can withstand deformation.
- Their number is about 4.5-5 million/ mm^3 , the number is more in males than in females.

- The lifespan of an erythrocyte in the bloodstream is 100-120 days i.e. about 5×10^{11} erythrocytes are formed/ destroyed each day.
- Erythron (census): whole mass of RBCs & their precursors in bone marrow.
- RBCs are considered as 'yardstick' for estimating dimensions of other cells in sections, (the diameters of relative cells are about $7\mu\text{m}$).

Plasmalemma and stroma

The stroma is composed of proteins such as *spectrin* that are associated with the inner surface of the plasmalemma; it maintains the biconcave shape of the RBC. The external surface of the plasmalemma is covered by a carbohydrate-rich glycocalyx, which contains genetically determined antigens that allow blood types (including A, B, O, AB groups) to be distinguished.

Hemoglobin

Each hemoglobin molecule consists of polypeptide subunits, each includes an iron-containing heme group. Hemoglobin (Hb) exists in a different forms, distinguishable on the basis of the amino acid sequence of their subunits. In humans, only 3 forms are considered normal in postnatal life: HbA1 constitutes 97%, HbA2 2%, and HbF 1% of the hemoglobin of healthy adults. HbF makes up around 80% of the hemoglobin of newborns, however; this proportion gradually decreases until normal adult levels are reached at about 8 months of age.

HbS is an abnormal form of HbA that is found in patients with sickle cell anemia. Unlike HbA, HbS becomes insoluble at low oxygen tensions and crystallizes into inflexible rods that deform the RBCs, giving them the characteristic sickle shape. When the rigid sickled cells pass through narrow capillaries, they cannot bend as normal RBCs do. They may become trapped, obstructing blood flow through the capillary, or rupture, decreasing the number of RBCs available for oxygen transport (anemia).

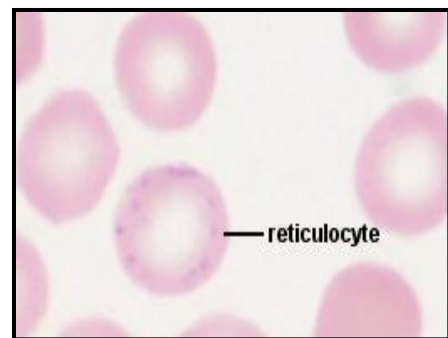
Abnormalities of RBCs: usually named as anemia that are due to changes in shape, number, or hemoglobin content.

Anisocytosis: refers to the presence of a high percentage of RBCs with great variations in size. Those larger than $9\mu\text{m}$ in diameter are termed **macrocytes**, and those smaller than $6\mu\text{m}$ are termed **microcytes**.

Nuclear fragments: in some diseases nuclear fragments or *Howell-Jolly bodies* remain in some mature RBCs. When these form circular filaments they are termed *Cabot rings*.

Reticulocytes are immature RBCs released from bone marrow; normally estimated $\sim 1\%$ of circulating RBCs. Reticulocytes contain a small amount of residual RER and ribosomes, and are stained with supravital staining (brilliant cresyl blue) in fresh blood film.

Reticulocytosis means increase reticulocyte count which indicate an increased demand for oxygen carrying capacity (eg, from loss of RBCs due to hemorrhage or anemia).



B- White blood cells (WBC) or leucocytes

- Leukocytes can be subdivided into granular leukocytes (neutrophils, basophils and eosinophils) and non-granular leukocytes (monocytes and lymphocytes).
- In healthy individuals the total number of circulating leukocyte is about $4000-10.000/\text{mm}^3$.
- Increase the leucocytes count above the upper range is called *leucocytosis*; which occurs in infection, inflammatory conditions, and in leukemia.
- Whereas, decrease the count below the lower range is called *leucopenia*; which occurs in excessive exposure to X-ray and after prolonged treatment with steroids.

Differential Cell Count: Blood is also studied by spreading a drop on a slide to produce a single layer of cells (blood smear). The cells are stained, differentiated by type, and counted to reveal disease-related changes in their relative numbers. The smears are usually stained with dye mixtures (eg, Wright's or Giemsa) containing eosin and methylene blue. Blood cells and their components exhibit 4 major staining properties that allow the cell types to be distinguished:

- **Basophilia:** is affinity for methylene blue. Basophilic structures stain purple to black.
- **Azurophilia:** is affinity for the oxidation products of methylene blue called azures. Azurophilic structures stain reddish-purple.
- **Eosinophilia**, or acidophilia: is affinity for eosin. Eosinophilic structures stain pink to orange.
- **Neutrophilia:** is affinity for a complex of dyes (originally thought to be neutral) in the mixture. Neutrophilic structures stain pink.

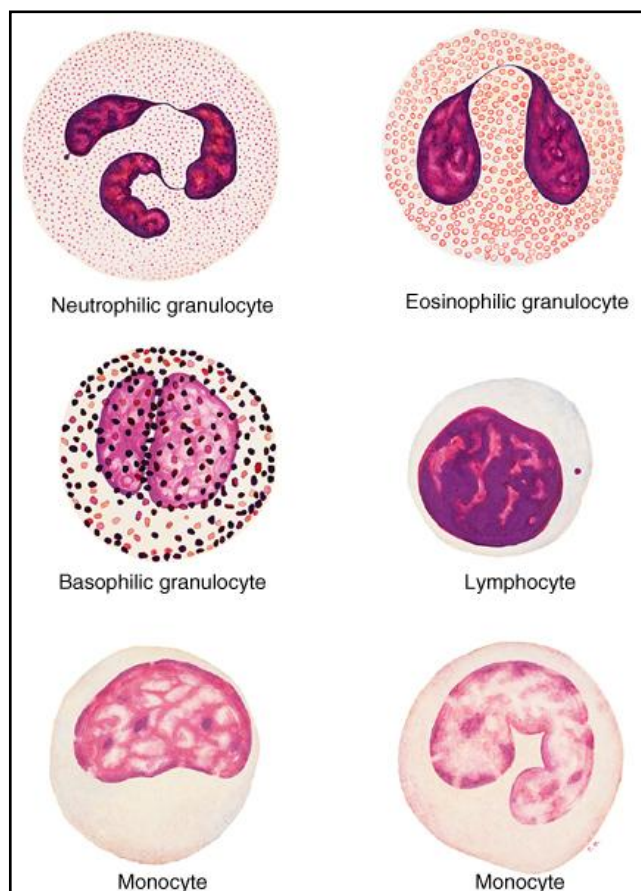
The differential leukocytic count is the percentage of each type of WBCs in blood, and would typically produce the following cell frequencies:

- ~ 60% neutrophils (50% - 70%)
- ~ 4% eosinophils (>0% - 5%)
- ~ 0.5-1% basophils (>0% - 2%)
- ~ 5% monocytes (1% - 9%)
- ~ 40% lymphocytes (20% - 40%)

Changes in their relative numbers indicate that something abnormal is happening in individual.

A larger than usual number of neutrophils (*neutrophilia*) would indicate e.g. an acute or chronic infection.

The number of basophils and eosinophils may increase (*eosinophilia* or *basophilia*) as a consequence of e.g. allergic disorders.



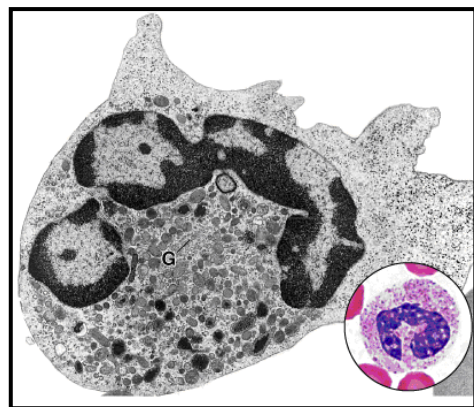
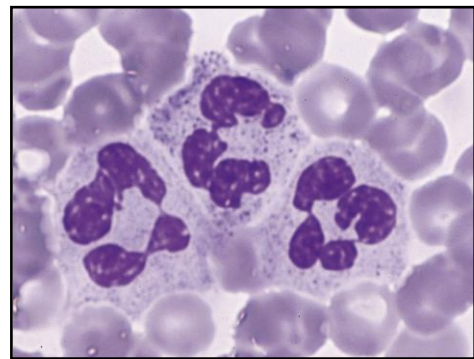
Granular Leukocytes

- Granular leukocytes are all approximately the same size (12-15 μm in diameter).
- Their nuclei are lobulated and nucleoli cannot be seen.

- The number of nuclear lobes varies according to cell type.
- All granulocytes are motile.
- The term granulocyte refers to the presence of granules in the cytoplasm of these cells.
- The granules correspond to secretory vesicles and lysosomes.
- Specific granules are the granules which are only found in one particular type of granulocytes.

1- Neutrophils

- Neutrophils (polymorphonuclear granulocytes): one of granular leucocytes that have a very characteristic nucleus.
- It is divided into 3-5 lobes, which are connected together by thin strands of chromatin.
- The number of lobes increases with cell age. Up to 7 lobes can be found in very old neutrophils (hypersegmented cells).
- Barr body is a drumstick chromosome or condensed chromatin visible in about 3% peripheral blood of females.
- Neutrophils contain all the organelles that make up a typical cell.
- In addition to the usual complement of organelles, they also contain three types of granules that stain "neutral", hence the term neutrophil



Types of neutrophilic granules:

- Primary (azurophilic granules): large Lysosomes contain hydrolytic enzymes such as myeloperoxidase and acid phosphatase that have microbicidal effect.
- Secondary (specific) granules: smaller, twice as numerous; alkaline phosphatase = phosphasome (light fraction of membranes)

- Tertiary: contain gelatinase; enhance phagocytosis

Neutrophilia: Means increased number of neutrophils in circulation as in acute bacterial infection, tissue injury and malignancy.

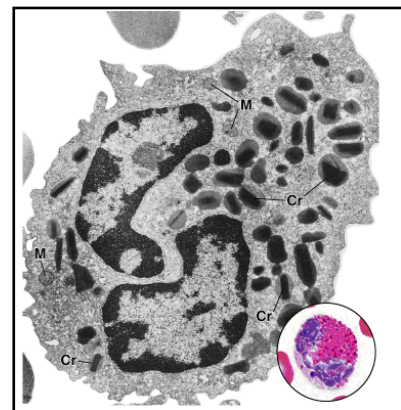
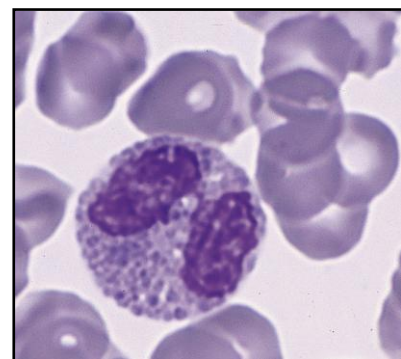
Neutropenia: Means decreased number of neutrophils in circulation as in viral infection, chronic bacterial infection such as typhoid fever and tuberculosis.

Functions:

- Their lifespan is only about one week inside blood vessels, and then pass to the connective tissue where they last for another 1-4 days.
- Neutrophils play a central role in inflammatory processes.
- Neutrophils are the first wave of cells invading infection sites.
- Receptors in their plasma membrane allow them to recognize foreign bodies, e.g. bacteria, and tissue debris, which begin to phagocytose and destroy.
- The phagocytotic activity of neutrophils is further stimulated if invading microorganisms are "tagged" with antibodies (or *opsonised*).
- Dead neutrophils and tissue debris are the major components of pus.

2- Eosinophils

- Eosinophils are small 12-18 μ in diameter, slightly larger than neutrophils. Eosinophils represent 1-6% of the total white cell count, and show diurnal variation (greatest in morning; least in afternoon).
- Their nuclei usually have two lobes that hidden by the numerous granules, which cover almost all of the cytoplasm.
- As the term "eosinophil" indicates, these granules are stained red or pink with eosin or other similar dyes.



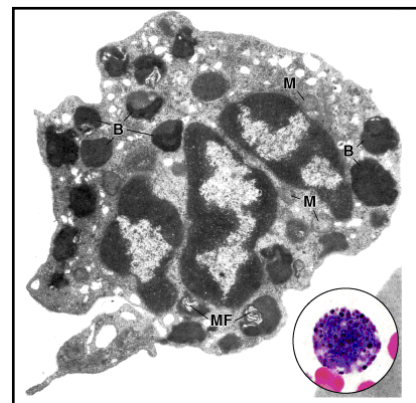
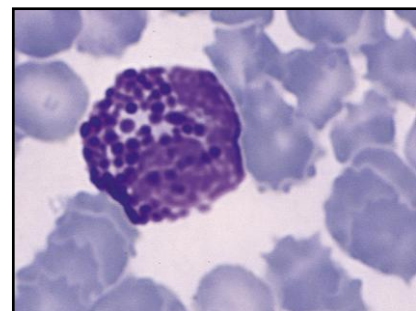
- Eosinophils contain some large rounded vesicles (~1 μm) in their cytoplasm.
- The specific granules contain lysosomal enzymes, and electron-dense, proteinaceous crystal composed of major basic protein (MBP).
- Smaller granules: contain aryl sulfatase and acid phosphatase.
- Life span: Eosinophils circulate in blood for 3 to 8 hours before migrating to connective tissue where they last for 10-12 days.
- Eosinophilia: Increase the number of eosinophils above the normal as in parasitic disease; and in allergic disorders.

Functions

- Contain receptors for IgE which stimulates the immune system.
- Their granules contain histaminase and arylsulfatase enzymes that break down histamine and leukotrienes.
- Major Basic Protein, which can function as a cytotoxin, and involved in the response of the body against parasitic infections.
- Produce eosinophil-derived-inhibitor, which inhibits mast cell degranulation.

3- Basophils

- Small granulocytes with a diameter of 8 to 10 μm .
- Their nucleus is bilobed which hidden by the large cytoplasmic granules.
- Their number is ~1% of differential white cell count.
- The granules are not as numerous as those in eosinophils and have metachromatic stainability with toluidine blue.



- Their specific granules (about 0.5 μm) appear quite dark in EM pictures. They contain heparin, histamine lysosomal enzymes and leukotrienes.
- Their cell membrane contains receptors for IgE (produced in response to allergens); that triggers rapid exocytosis of granular contents (degranulation).

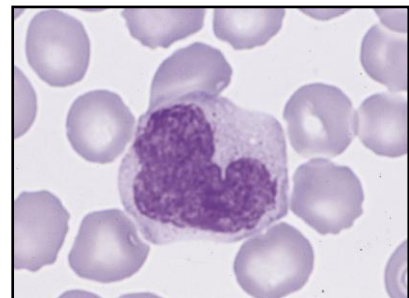
Functions

- Heparin and histamine are vasoactive substances, dilate the blood vessels, make vessel walls more permeable and prevent blood coagulation.
- They facilitate the access of lymphocytes and other antibodies to the site of infection.

Non-granular leukocytes

1- Monocytes

- Monocytes are large cells, 12-18 μm in diameter; represent 2-10 % of the differential cell count.
- Monocytes are highly motile and phagocytic cells; i.e. they are the precursor of tissue phagocytes that migrate into tissues.
- Their nucleus less dense than lymphocytes; deeply indented, kidney or C-shaped.
- Their cytoplasm is pale grayish blue with small pink to purple stained lysosomal granules, and contain cytoplasmic vacuoles (frosted glass).
- Monocytes contain granules (visible in the EM) which are similar to the primary granules of neutrophils, i.e. Lysosomes containing acid phosphatase, aryl granules.
- They contain also secondary granules of unknown function.

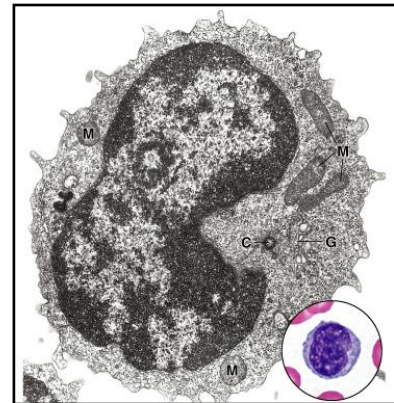
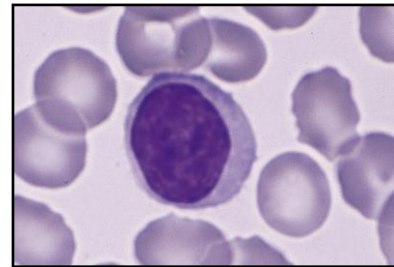


Functions

- Once monocytes enter the connective tissue they differentiate into macrophages that phagocytose microorganisms, tissue debris and the dead neutrophils.
- Monocytes also give rise to mononuclear phagocytic system: which include histiocytes; multinucleate giant cells; hepatic macrophages (Kupffer) cells; microglia of CNS; macrophages (Langerhans cells) of skin; antigen-presenting cells (APCs) of lymphoid organs; and osteoclasts of bone.
- Monocytosis: increase numbers of monocytes more than 8% as in lymphoma and monocytic leukemia; subacute bacterial endocarditis; some chronic infection and malaria.

2- Lymphocytes

- Lymphocytes represent 20 to 40% of the differential white cell count.
- There are two structural types:
 - Small lymphocytes: $\sim 5\mu\text{m}$ in diameter, and represent 3% of lymphocytes in peripheral blood. Most small lymphocytes in the blood stream belong to either the group of B-lymphocytes ($\sim 5\%$) or the group of T-lymphocytes ($\sim 90\%$).
 - Large lymphocytes: 9 to $15\mu\text{m}$ in diameter, possibly natural killer cells; possibly dividing lymphocytes.



- The cells are rounded with densely stained nuclei, small amount of pale basophilic cytoplasm with free ribosomes; short microvilli (seen in EM) more numerous on B-lymphocytes than T lymphocytes.
- Unless they become activated, the two groups cannot easily be distinguished using routine light or electron microscopy.

- Only blood lymphocyte capable of division outside the bone marrow.

Functional types of lymphocytes:

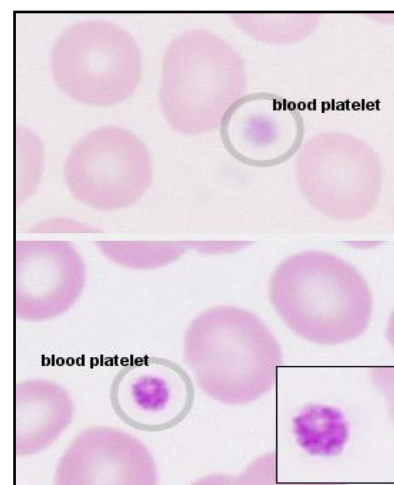
- B-lymphocytes: responsible for humoral immune response and produce antibodies.
- T- lymphocytes: responsible for cell mediated immune response.
- T- helper lymphocytes
- T- suppressor lymphocytes
- T- memory lymphocytes
- Cytotoxic T- lymphocytes (Killer cells)
- Natural killer lymphocytes

Functions

- Upon exposure to antigens B-lymphocytes differentiate into antibody producing plasma cells that produce antibodies which directed against foreign antigen.
- T-lymphocytes represent the "cellular arm" of the immune response (cytotoxic T cells) and may attack foreign cells, cancer cells and cells infected by e.g. a virus.
- Lymphocytosis: increased numbers above the normal as in viral infection, chronic bacterial infection such as typhoid fever and tuberculosis; lymphoma and lymphocytic leukemia.
- Lymphopenia: decrease the number of lymphocytes less than 20% as in AIDS, and in aplastic anemia.

C- Blood Platelets (Thrombocytes)

- Blood platelets or thrombocytes, are the smallest formed elements in the blood.
- They are cytoplasmic fragments of very large thrombocyte (megakaryocytes) that are found in the bone marrow.
- Their number is 150,000 - 400,000/mm³, with a lifespan of about 8 days, and appear in clumps in blood smears.

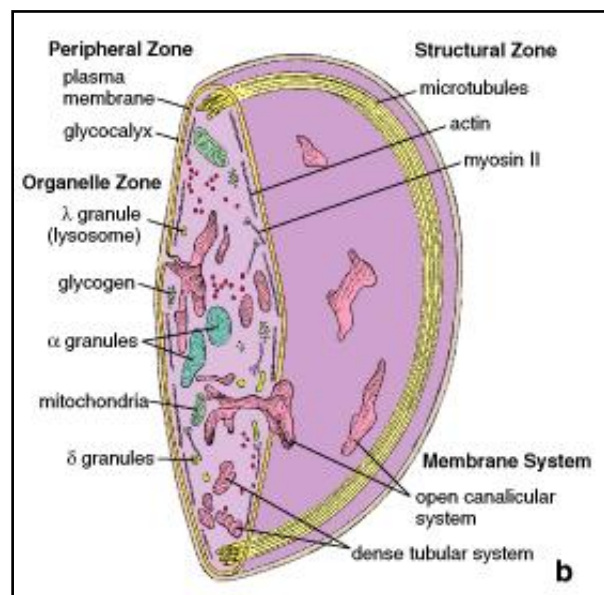


- They are rounded or oval, biconvex discs, 1.5 to 3.5 μ m in diameter.
- The cytoplasm is divided into two zones: an outer hyalomere, and an inner granulomere, which contains a few mitochondria, glycogen granules and a variety of purple granules.
- The hyalomere contains cytoskeletal fibers, which include actin and myosin filaments.
- Different types of vesicles contain either serotonin (electron-dense delta granules) or compounds important for blood coagulation (alpha granules), they also contain platelet-derived growth factor (PDGF) which may play a role in the repair of damaged tissue.

- Their cytoplasm is purple-staining, granular; organelles concentrated toward the center; granules constitute about 20% of the volume.

- The glycocalyx is rich in glycosaminoglycans and is associated with adhesion, the major functional characteristic of platelets.

- Platelets have an important physical role in plugging wounds, and they contribute to the cascade of molecular interactions among the various clotting factors dissolved in the plasma.



The Clot and Serum: Clotted blood consists of 2 parts:

- the clot, or thrombus, which includes the formed elements and some of the proteins dissolved in the plasma,
- the serum, a clear yellow liquid that is similar to plasma except that it lacks fibrinogen and contains more serotonin.

Clotting Factors:

Clotting involves a cascade of molecular interactions among several plasma proteins and ions (clotting factors I - XIII). The cascade can be initiated by 2 converging pathways, each of which results in the conversion of fibrinogen into fibrin by the enzyme thrombin.

Other factors act as promoters and accelerators of the clotting process or help stabilize the fibrin once it has formed. An inherited abnormality in factor VIII results in the clotting disorder known as hemophilia.

The Role of Platelets:

- **Primary aggregation.** Platelets in the damaged region attach to collagen revealed by the discontinuity in the vessel wall, forming a platelet plug.
- **Secondary aggregation.** Platelets in the plug release the contents of their alpha and delta granules. This release of serotonin explains the higher concentration of serotonin in serum than in plasma. Serotonin, a vasoconstrictor, restricts blood flow to the damaged area by causing contraction of vascular smooth muscle.
- **Blood coagulation.** Platelets release fibrinogen in addition to that normally found in the plasma. The fibrinogen is converted by the clotting factor cascade into fibrin, which forms a dense fibrous mat to which more platelets and other blood cells attach, forming a clot and plugging the opening in the blood vessel wall.

Clot Retraction: The clot (thrombus) initially bulges into the vessel lumen, but later it contracts and condenses through the interactions of thrombosthenin (a contractile protein) and platelet actin, myosin, and ATP.

Clot Removal: As the vessel wall heals and the protection afforded by the clot is no longer needed, the clot is removed by the enzyme plasmin. Plasmin is formed by the action of plasminogen activators (from endothelial cells) on the plasma proenzyme plasminogen (from the liver). Enzymes released by the lambda granules (lysosomes) of the platelets also aid in clot digestion.

The following table summarizes the types and function of blood cells.

Cell Type	Main Products	Main Functions
Erythrocyte	Hemoglobin	CO ₂ and O ₂ transport
Leukocytes	Specific granules and modified lysosomes (azurophilic granules)	Phagocytosis of bacteria
Neutrophil		
Eosinophil	Specific granules, pharmacologically active substances	Defense against parasitic helminths; modulation of inflammatory processes
Basophil	Specific granules containing histamine and heparin	Release of histamine and other inflammation mediators
Monocyte	Granules with lysosomal enzymes	Generation of mononuclear-phagocyte system cells in tissues; phagocytosis and digestion of protozoa and virus and senescent cells
B lymphocyte	Immunoglobulins	Generation of antibody-producing terminal cells (plasma cells)
T lymphocyte	Substances that kill cells. Substances that control the activity of other leukocytes (interleukins)	Killing of virus-infected cells
Natural killer cell (lacks T and B cell markers)	Attacks virus-infected cells and cancer cells without previous stimulation	Killing of some tumor and virus-infected cells
Platelet	Blood-clotting factors	Clotting of blood

Haemopoiesis:

During foetal development, the formation of blood cells (*haemopoiesis*) commences in wall of the yolk sac. After the second month of foetal development, the liver, and the spleen become the dominant sites of haemopoiesis. From the 6th month, and dominating from the 7th month onwards, the formation of blood cells occurs in bone marrow, which is the major site of formation blood cells in normal adult man.

Haemopoietic Cells

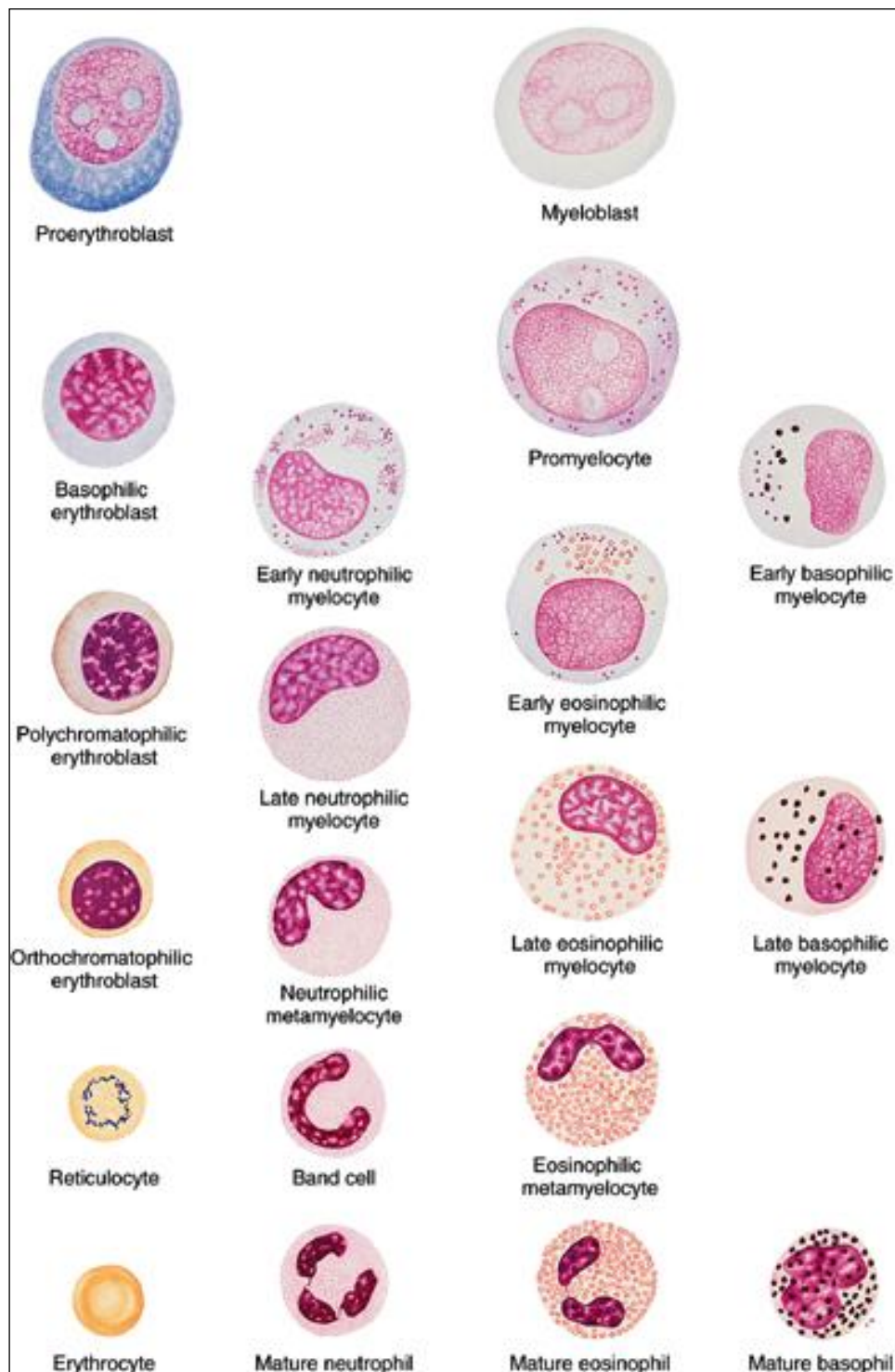
The basis of haemopoiesis is a small population of self-replicating stem cells, which ultimately can generate all types of blood cells. Their progeny may develop into either **lymphocytic stem cells** or **pluripotent haemal stem cells** (colony-forming unit - stem cell -

CFU-S). The latter type gives rise to stem cells which can form the major groups of blood cells other than lymphocytes. Depending on their progeny it is possible to differentiate

- **burst-forming unit** of the **erythroid line** (BFU-E),
- **colony-forming unit** - **granulocytes** and **macrophages** (CFU-G/M), and
- **colony-forming unit** - **megakaryocytes** (CFU-Mk).

The following table shows the stages of blood cells development

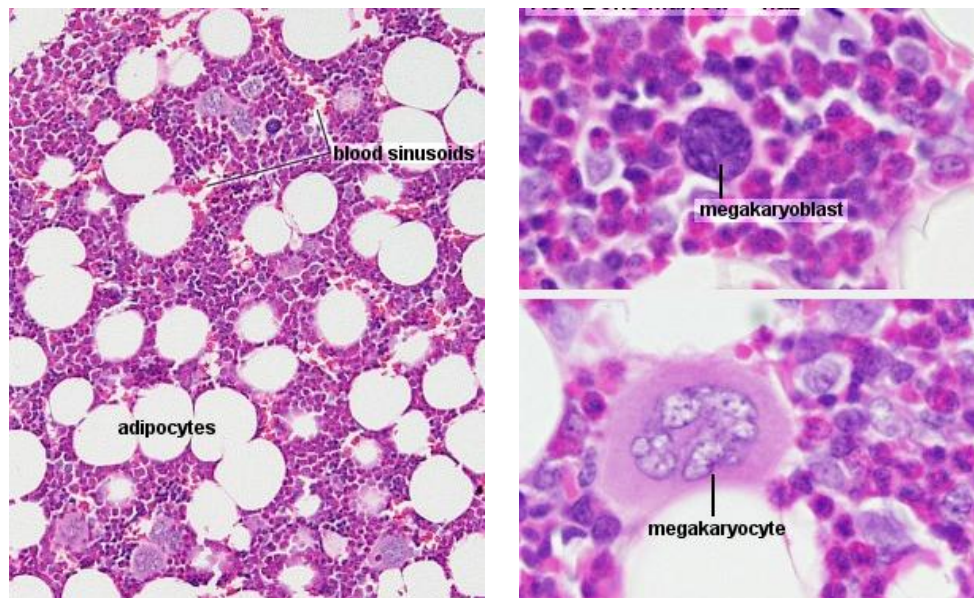
Phase	Stem Cells	Progenitor Cells	Precursor Cells (Blasts)	Mature Cells
Early morphologic	Not morphologically distinguishable; have the general aspect of lymphocytes		Beginning of morphologic differentiation	Clear morphologic differentiation
Mitotic activity	Low mitotic activity; self-renewing; scarce in bone marrow	High mitotic activity; self-renewing; common in marrow and lymphoid organs; mono- or bipotential	High mitotic activity; not self-renewing; common in marrow and lymphoid organs; monopotential	No mitotic activity; abundant in blood and hematopoietic organs
	Lymphoid multipotential cells Pluripotent cell Myeloid multipotential cells remain in bone marrow	Migrate to lymphoid organs Lymphocyte-colony-forming cell (LCFC) Erythrocyte-colony-forming cell (ECFC) Megakaryocyte-forming cell Monocyte-colony-forming cell (MCFC) MGCFC Granulocyte-colony-forming cell (GCFC) Eosinophil-colony-forming cell (EoCFC) Basophil-colony-forming cell (BCFC)	Lymphoblast Erythroblast Megakaryoblast Promonocyte Neutrophilic myelocyte Eosinophilic myelocyte Basophilic myelocyte	B and T lymphocytes Erythrocyte Megakaryocyte Monocyte Neutrophilic granulocyte Eosinophilic granulocyte Basophilic granulocyte



A diagram shows the different types of developed blood cells.

Bone marrow:

Functionally and histologically, there are two types of bone marrow; yellow and red bone marrow. Yellow bone marrow, which harbours mainly adipocytes, dominates in the hollow of the diaphysis of adult long bones. Haemopoiesis occurs in red bone marrow, which is typically found between the trabeculae of spongy bone in the epiphysis of adult long bones. Both age and demands on haemopoiesis may affect the relative amounts of red and yellow bone marrow. Haemopoietic cells surround the vascular sinusoids and are supported by reticular connective tissue. In addition to the endothelial cells of the sinusoids and the reticulocytes of the connective tissue, macrophages are frequent in red bone marrow.



Red bone marrow showing adipocytes, megakaryocyte, and other developing cells.