PIO 201: INTRODUCTION TO PHYSIOLOGY AND BLOOD

Haemopoiesis
WBC and Differential Count

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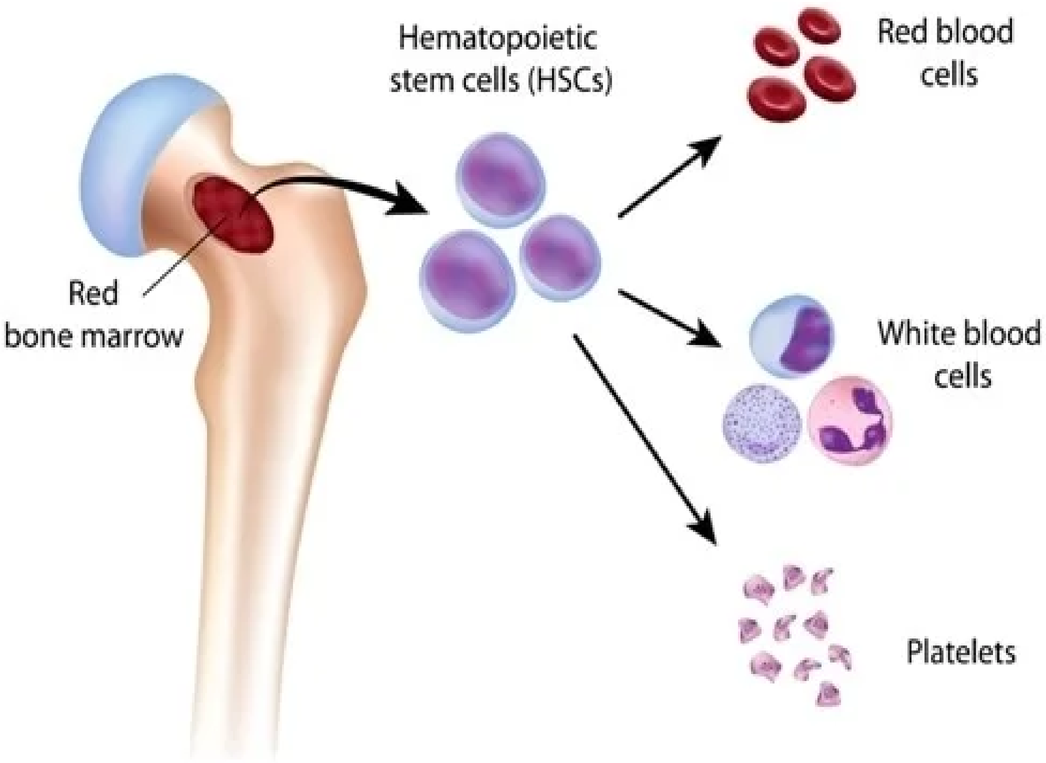
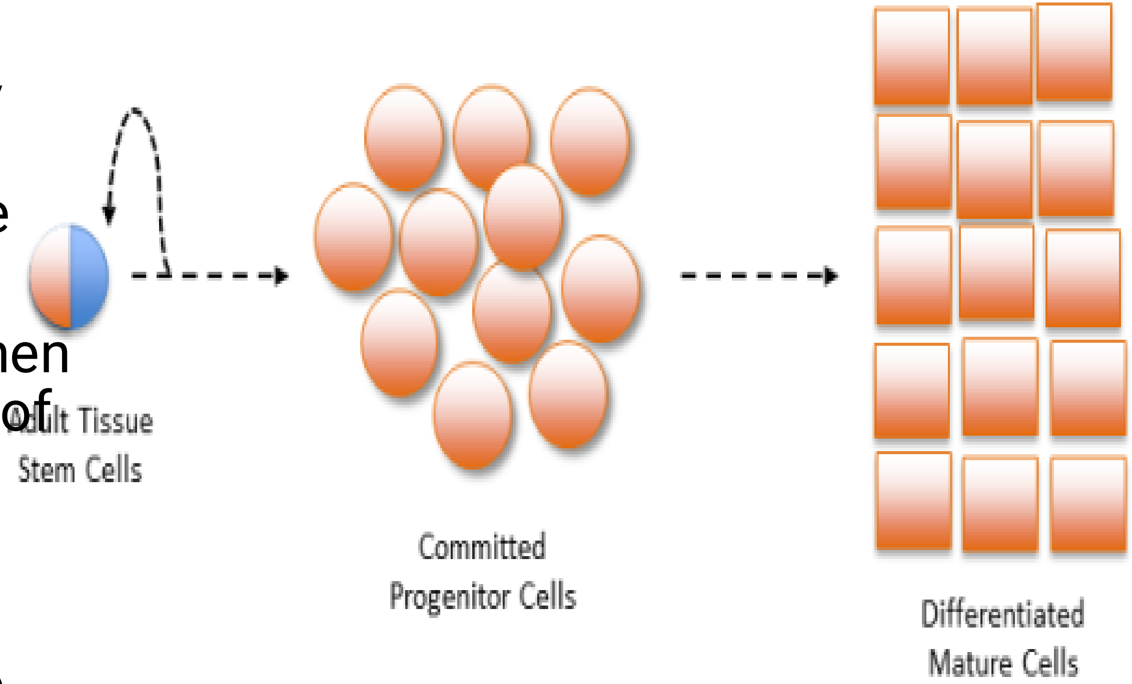
Introduction

- **Haemopoiesis or haematopoiesis** is the process of origin, development and maturation of all the blood cells.
- The blood cells begin their lives in the bone marrow from a single type of cell called the ***uncommitted pluripotential hematopoietic stem cell (PHSC)***.
- As these cells reproduce, a small portion of them remains exactly like the original pluripotential cells and is retained in the bone marrow to maintain a supply of these, although their numbers diminish with age.



- Most of the reproduced cells, however, differentiate to form the other cell types.
- The intermediate stage cells are very much like the PHSCs, even though they have already become committed to a particular line of cells and are therefore called *committed PHSCs*.
- The different committed stem cells, when grown in culture, will produce colonies of specific types of blood cells.
- Committed PHSCs are of two types:

1. Lymphoid stem cells (LSC) which give rise to lymphocytes and natural killer (NK) cells.



2. Colony forming blastocytes, which give rise to myeloid cells.

Different units of colony forming cells are:

- i. Colony forming unit-erythrocytes (CFU-E) – Cells of this unit develop into erythrocytes
- ii. Colony forming unit-granulocytes/ monocytes (CFU-GM) – These cells give rise to granulocytes (neutrophils, basophils and eosinophils) and monocytes
- iii. Colony forming unit-megakaryocytes (CFU-M) – Platelets are developed from these cells.

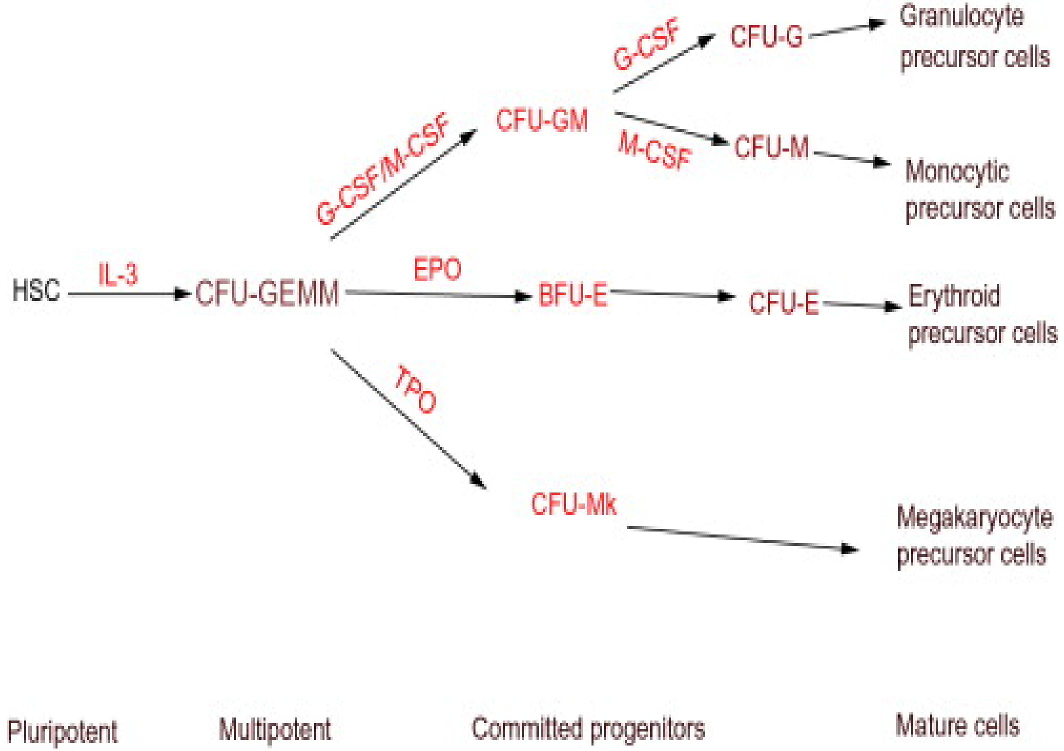
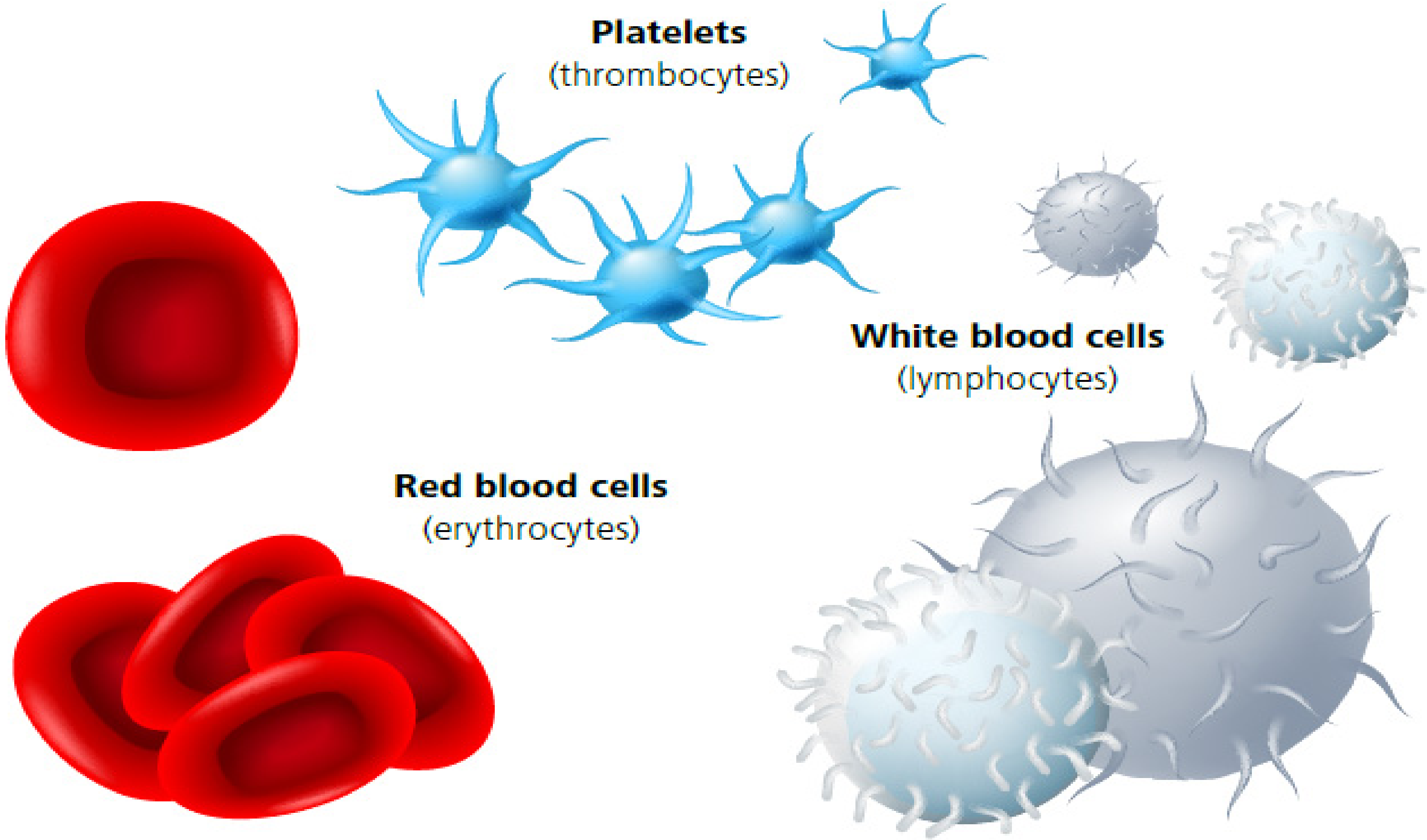


Fig: Blood cells

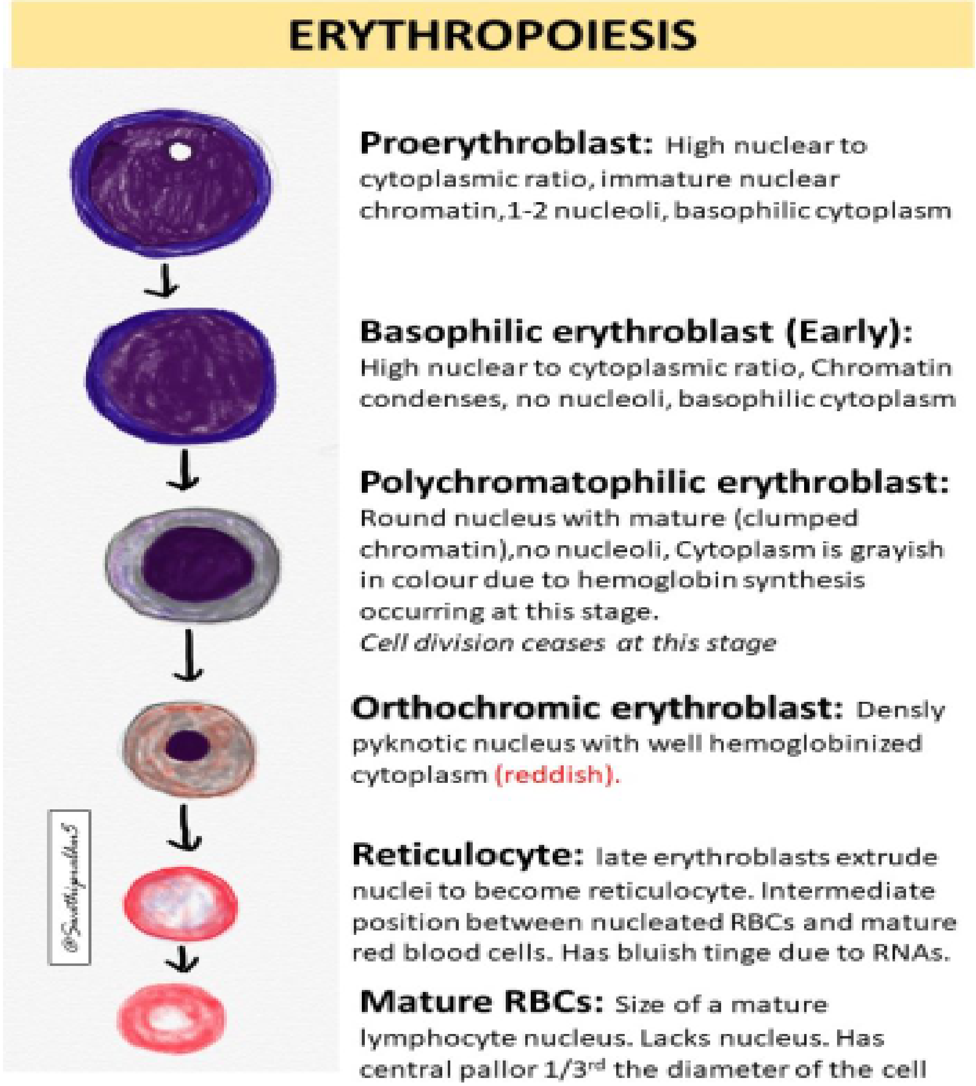


- Growth and reproduction of the different stem cells are controlled by multiple proteins called *growth inducers*.
- Four major growth inducers have been identified, each having different characteristics.
- One of these, *interleukin-3*, promotes growth and reproduction of virtually all the different types of committed stem cells, whereas the others induce growth of only specific types of cells.
- Differentiation of the cells is the function of another set of proteins called *differentiation inducers*. Each of these causes one type of committed stem cell to differentiate one or more steps toward a final adult blood cell.

Stages of Differentiation of Red Blood Cells

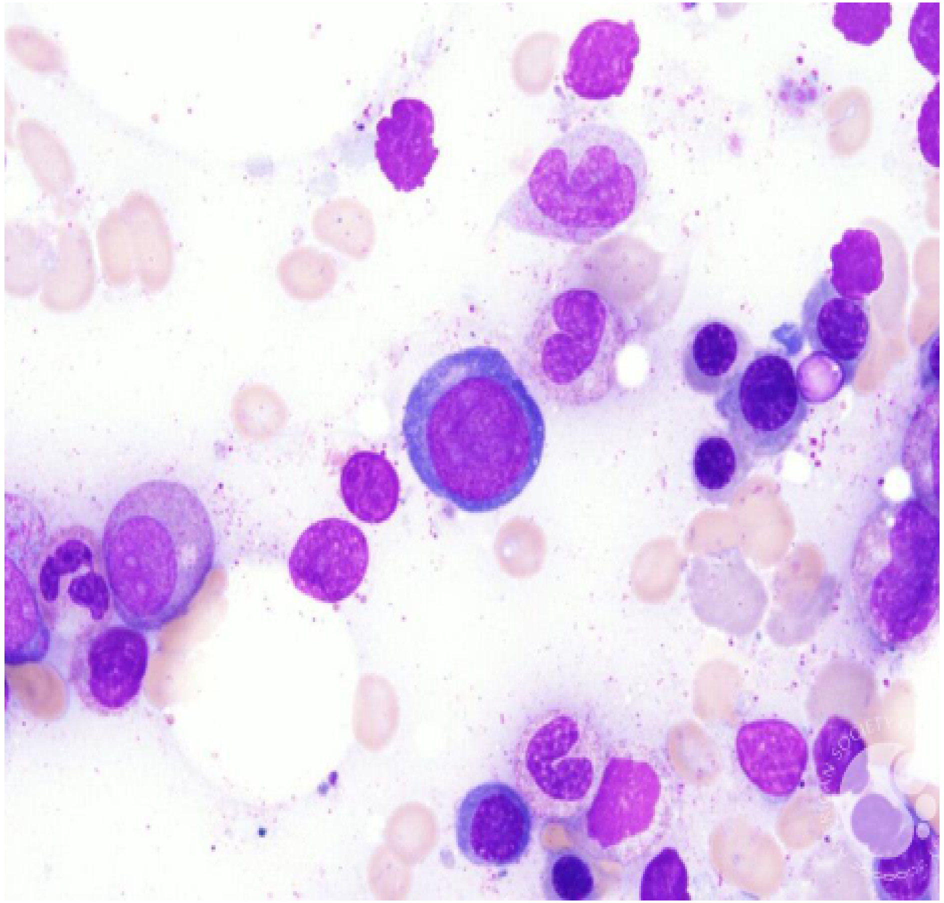
- Various stages between CFU-E cells and matured RBCs are:

1. Proerythroblast
2. Early normoblast
3. Intermediate normoblast
4. Late normoblast
5. Reticulocyte
6. Matured erythrocyte



Proerythroblast

- Very large in size with a diameter of about 20µ.
- Its nucleus is large and occupies the cell almost completely.
- The nucleus has two or more nucleoli and a reticular network.
- Proerythroblast does not contain hemoglobin.
- The cytoplasm is basophilic in nature.
- Proerythroblast multiplies several times and finally forms the cell of next stage called early normoblast.
- Synthesis of hemoglobin starts in this stage. However, appearance of hemoglobin occurs only in intermediate normoblast.

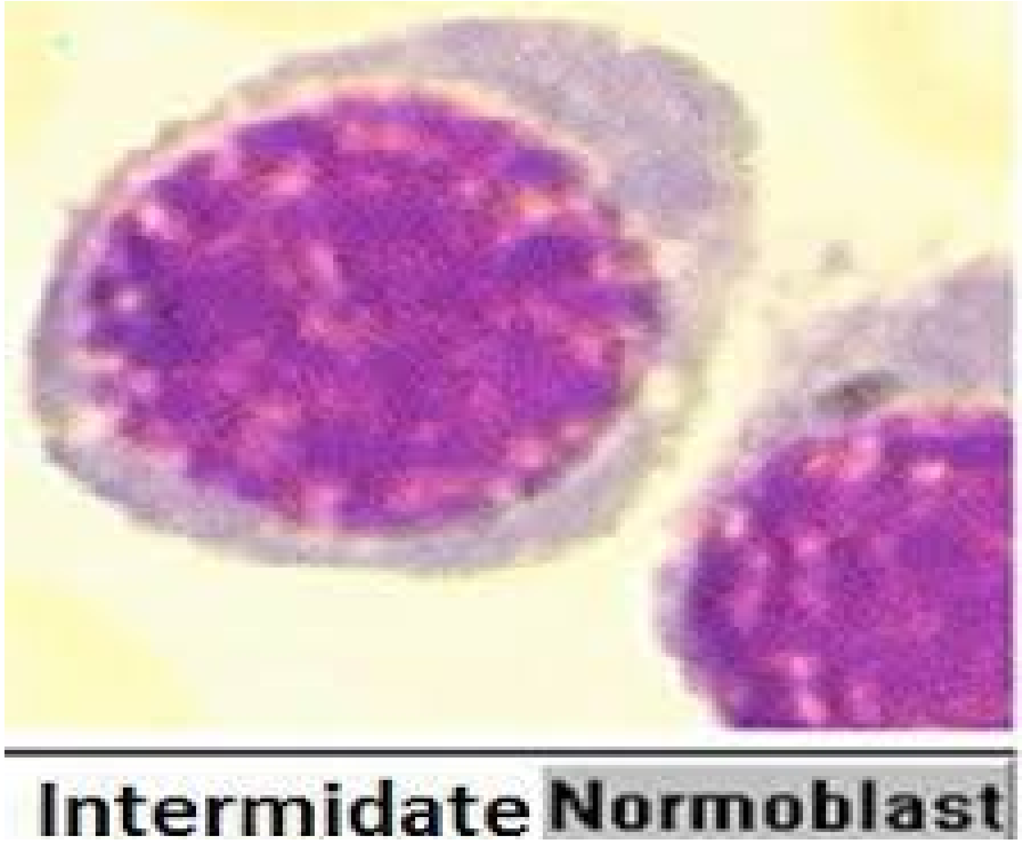


Early Normoblast

- The early normoblast is little smaller than proerythroblast with a diameter of about 15μ.
- In the nucleus, the nucleoli disappear.
- Condensation of chromatin network occur and becomes dense.
- The cytoplasm is basophilic in nature. So, this cell is also called **basophilic erythroblast.**
- This cell develops into next stage called intermediate normoblast.
- The cell at this time has accumulated very little hemoglobin.

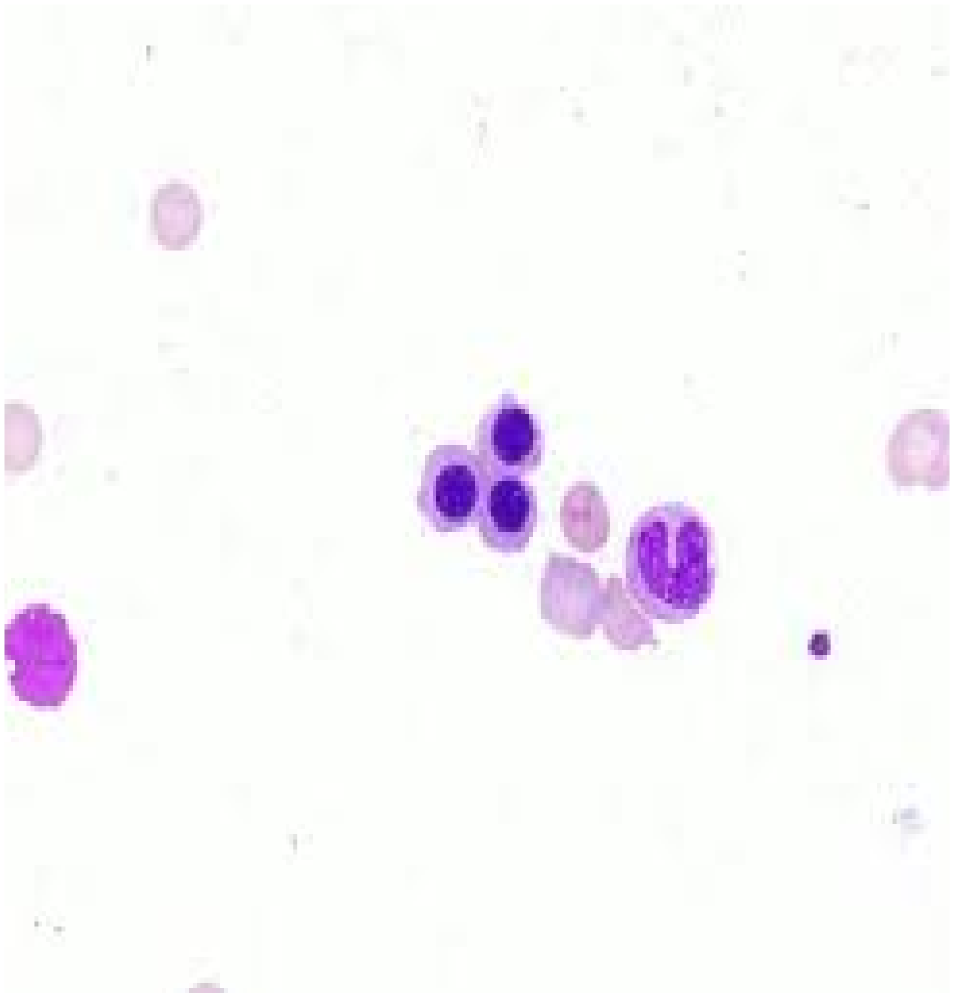
Intermediate Normoblast

- Cell is smaller than the early normoblast with a diameter of 10 to 12 μ.
- The nucleus is still present but, the chromatin network shows further condensation.
- The hemoglobin starts appearing.
- Cytoplasm is already basophilic. Now, because of the presence of hemoglobin, it stains with both acidic as well as basic stains.
- So, this cell is called polychromophilic or polychromatic erythroblast.
- This cell develops into next stage called late normoblast.



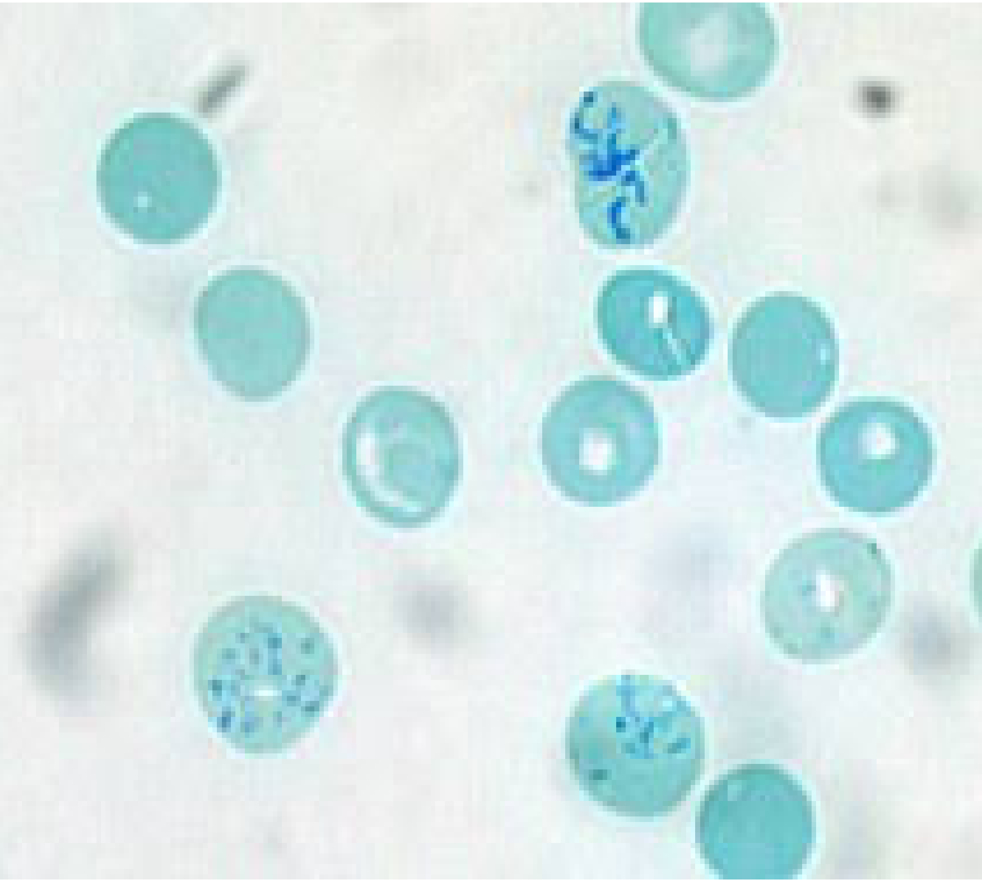
Late Normoblast

- Diameter of the cell decreases further to about 8 to 10µ.
- Nucleus becomes very small with very much condensed chromatin network and it is known as ink-spot nucleus.
- Quantity of hemoglobin increases. And the cytoplasm becomes almost acidophilic. So, the cell is now called orthochromic erythroblast.
- In the final stage of late normoblast just before it passes to next stage, the nucleus disintegrates and disappears.
- The process by which nucleus disappears is called pyknosis.
- The final remnant is extruded from the cell.
- Late normoblast develops into the next stage called reticulocyte



Reticulocyte

- Reticulocyte is otherwise known as **immature RBC**.
- The cytoplasm contains the reticular network or reticulum, which is formed by remnants of disintegrated organelles.
- Due to the reticular network, the cell is called reticulocyte. The reticulum of reticulocyte stains with **supravital stain**.
- Reticulocyte is basophilic due to the presence of remnants of disintegrated Golgi apparatus, mitochondria and other organelles of cytoplasm.
- During this stage, the cells enter the blood capillaries through capillary membrane from site of production by diapedesis.



Matured Erythrocyte

- Reticular network disappears and the cell becomes the matured RBC and attains the biconcave shape.
- The cell decreases in size to 7.2 μ diameter.
- The matured RBC is with **hemoglobin but without nucleus**.
- It requires 7 days for the development and maturation of RBC from proerythroblast.
- It requires 5 days up to the stage of reticulocyte (Reticulocyte takes 2 more days to become the matured RBC).

Red Blood Cells

Fig: Important events during erythropoiesis

Stages of erythropoiesis	Important event
Proerythroblast	Synthesis of hemoglobin starts
Early normoblast	Nucleoli disappear
Intermediate normoblast	Hemoglobin starts appearing
Late normoblast	Nucleus disappears
Reticulocyte	Reticulum is formed Cell enters capillary from site of production
Matured RBC	Reticulum disappears Cell attains biconcavity

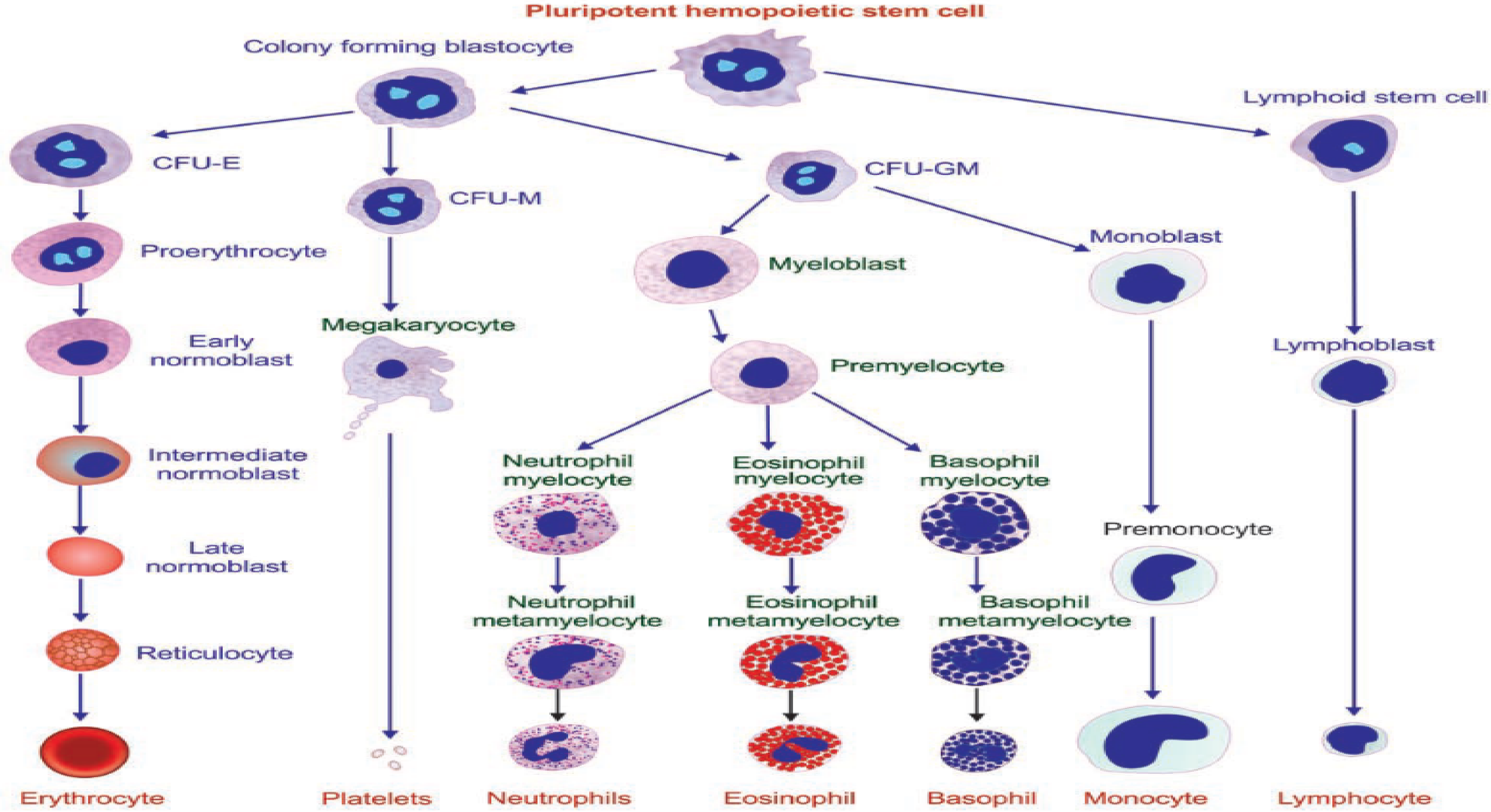
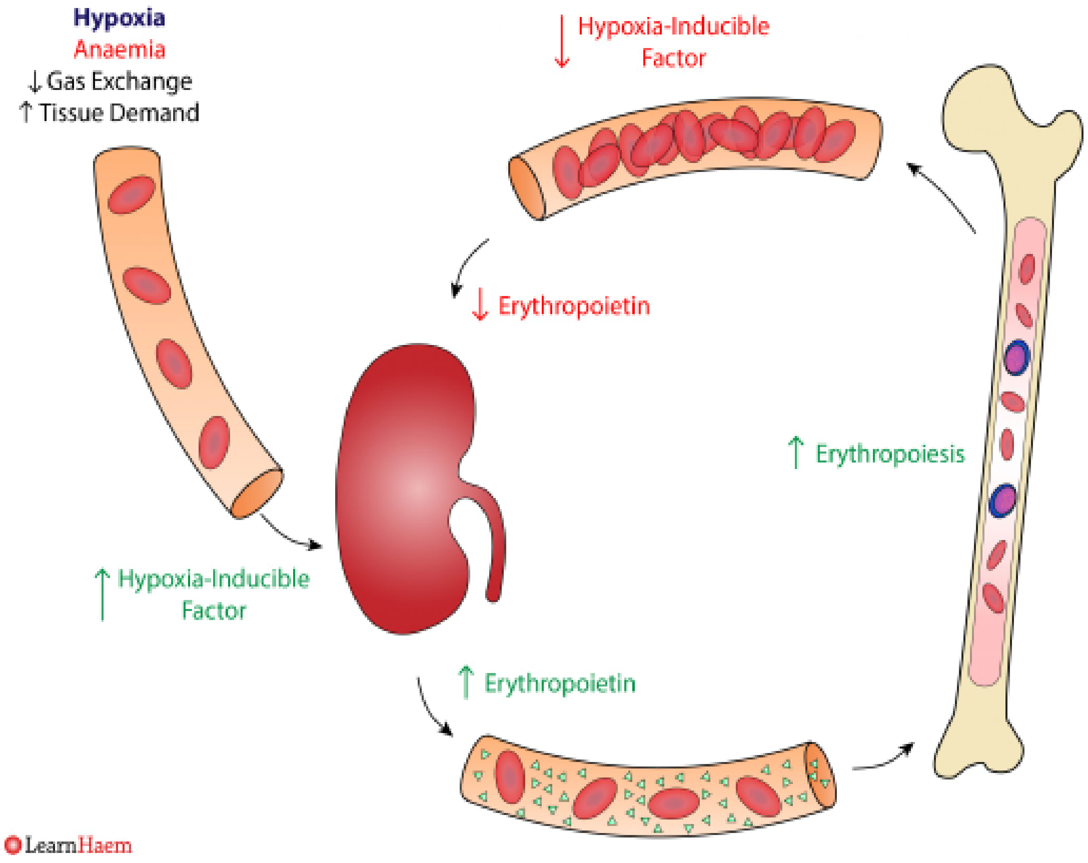


Fig: Stages of haemopoiesis

Regulation of RBC Production—Role of Erythropoietin

- The most important general factor for erythropoiesis is the hormone called erythropoietin.
- Major quantity of erythropoietin is secreted by peritubular capillaries of kidney. A small quantity is also secreted from liver and brain.
- Hypoxia and anemia are stimulants for the secretion of erythropoietin.
- Erythropoietin causes formation and release of new RBCs into circulation.



- Erythropoietin promotes the following processes:

a. Production of proerythroblasts from CFU-E of the bone marrow

b. Development of proerythroblasts into matured RBCs through the several stages – early normoblast, intermediate normoblast, late normoblast and reticulocyte.

c. Release of matured erythrocytes into blood.

Site of erythropoiesis

IN FETAL LIFE

- In fetal life, the erythropoiesis occurs in three stages:

1. **Mesoblastic Stage** – During the first two months of intrauterine life, the RBCs are produced from **mesenchyme** of yolk sac.
2. **Hepatic Stage** – From third month of intrauterine life, **liver** is the main organ that produces RBCs. **Spleen** and **lymphoid organs** are also involved in erythropoiesis.
3. **Myeloid Stage** – During the last three months of intrauterine life, the RBCs are produced from red **bone marrow** and **liver**.

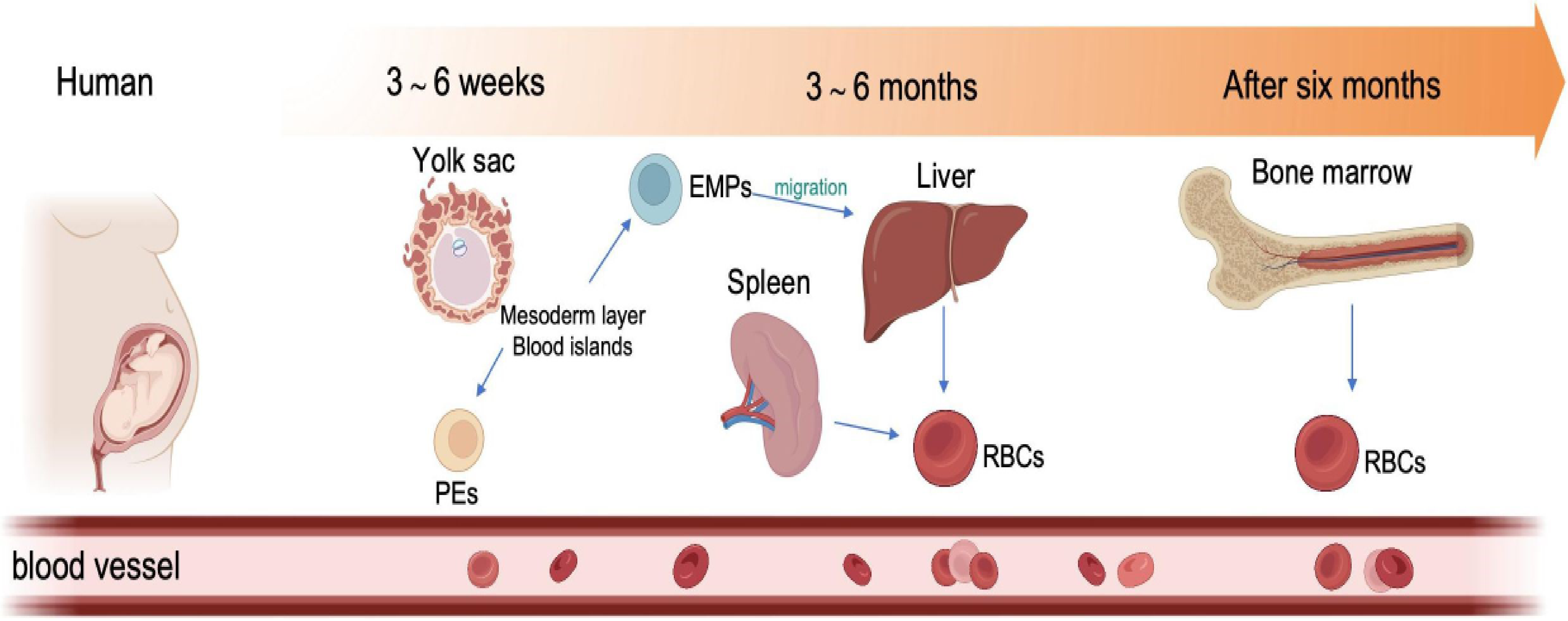
IN NEWBORN BABIES, CHILDREN AND ADULTS

- In newborn babies, growing children and adults, RBCs are produced only from the red bone marrow.

1. *Up to the age of 20 years:* RBCs are produced from red bone marrow of all bones (long bones and all the flat bones).

2. *After the age of 20 years:* RBCs are produced from membranous bones like vertebra, sternum, ribs, scapula, iliac bones and skull bones.

Fig: Site of erythropoiesis



Red blood Cells

- Red blood cells (RBCs) are the **non-nucleated** formed elements in the blood.
- RBCs are also known as erythrocytes (erythros = red).
- Red color of the RBC is due to the presence of the coloring pigment called hemoglobin.
- RBCs play a vital role in transport of respiratory gases.
- RBCs are larger in number compared to the other two blood cells.
- RBC count ranges between 4 and 5.5 million/cu mm of blood.
- In adult males, it is 5 million/cu mm and in adult females, it is 4.5 million/cu mm.

Morphology of RBCs

SHAPE

- Normally, the RBCs are disk shaped and biconcave (dumbbell shaped).
- Central portion is thinner and periphery is thicker.

Advantages of Biconcave Shape of RBCs

- Helps in equal and rapid diffusion of oxygen and other substances into the interior of the cell.
- Large surface area is provided for absorption or removal of different substances.
- Minimal tension is offered on the membrane when the volume of cell alters.
- RBCs squeeze through the capillaries very easily without getting damaged.

SIZE

- Diameter: 7.2μ (6.9 to 7.4 μ).
- Thickness: At the periphery it is thicker with 2.2μ and at the center it is thinner with 1μ.
- Surface area: 120 sq μ.
- Volume: 85 to 90 cu μ.

STRUCTURE

- Red blood cells are nonnucleated.

Properties

- ROULEAUX FORMATION – When blood is taken out of the blood vessel, the RBCs pile up one above another like the pile of coins.
- SPECIFIC GRAVITY – Specific gravity of RBC is 1.092 to 1.101.
- PACKED CELL VOLUME – Packed cell volume (PCV) is the proportion of blood occupied by RBCs expressed in percentage. It is also called hematocrit value. It is 45% of the blood and the plasma volume is 55%.
- SUSPENSION STABILITY – During circulation, the RBCs remain suspended uniformly in the blood.

Lifespan of Red blood cells

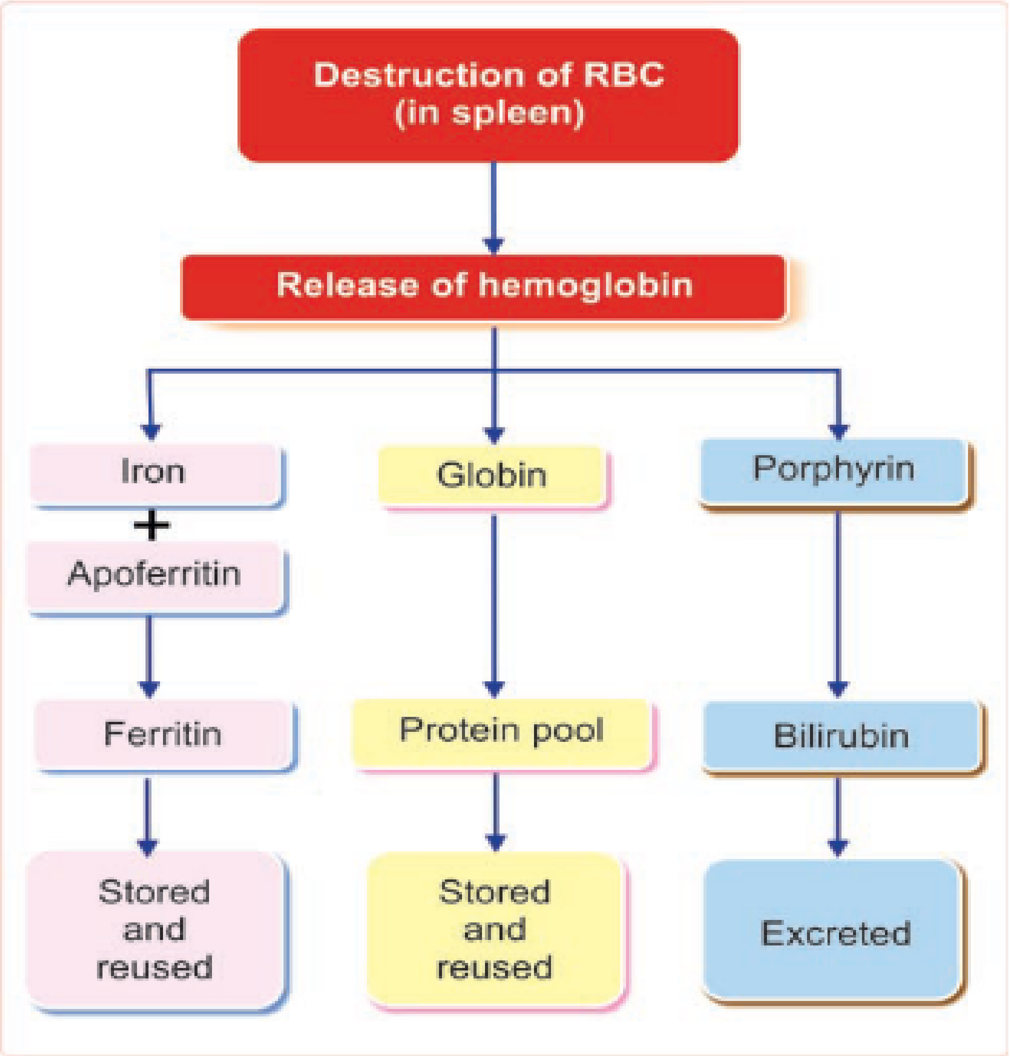
- The average lifespan of RBC is about **120 days**.
- After the lifetime the senile (old) RBCs are destroyed in **reticuloendothelial system**.

Fate of red blood cells

- When the cells become older (120 days), the cell membrane becomes more fragile.
- Because of the fragile nature, older cells are destroyed while trying to squeeze through the capillaries.
- The destruction occurs mainly in the capillaries of red pulp of spleen because the diameter of splenic capillaries is very small. So, the spleen is called **'graveyard of RBCs'**.
- Destroyed RBCs are fragmented and hemoglobin is released from the fragmented parts.

- Hemoglobin is immediately phagocytized by macrophages of the body, particularly the macrophages present in liver (Kupffer cells), spleen and bone marrow.
- Hemoglobin is degraded into iron, globin and porphyrin.
- Iron combines with the protein called apoferritin to form ferritin, which is stored in the body and reused later.
- Globin enters the protein depot for later use. Porphyrin is degraded into bilirubin, which is excreted by liver through bile.
- Daily, 10% RBCs which are senile, are destroyed in normal young healthy adults.
- This causes release of about 0.6 g/dL of hemoglobin into the plasma. From

Fig: Fate of RBC



Functions of Red blood cells

- Major function of RBCs is the transport of respiratory gases.

1. Transport of Oxygen from the Lungs to the Tissues

Hemoglobin in RBC combines with oxygen to form oxyhemoglobin. About 97% of oxygen is transported in blood in the form of oxyhemoglobin.

2. Transport of Carbon Dioxide from the Tissues to the Lungs

Hemoglobin combines with carbon dioxide and form carbhemoglobin. About 30% of carbon dioxide is transported in this form.

RBCs contain a large amount of the carbonic anhydrase. This enzyme is necessary for the formation of bicarbonate from water and carbon dioxide. Thus, it helps to transport carbon dioxide in the form of bicarbonate from tissues to lungs. About 63% of carbon dioxide is transported in this form.

- Other functions of RBCs are:

3. Buffering Action in Blood

Hemoglobin functions as a good buffer. By this action, it regulates the hydrogen ion concentration and thereby plays a role in the maintenance of acid-base balance.

4. In Blood Group Determination

- RBCs carry the blood group antigens like A antigen, B antigen and Rh factor. This helps in determination of blood group and enables to prevent reactions due to incompatible blood transfusion.

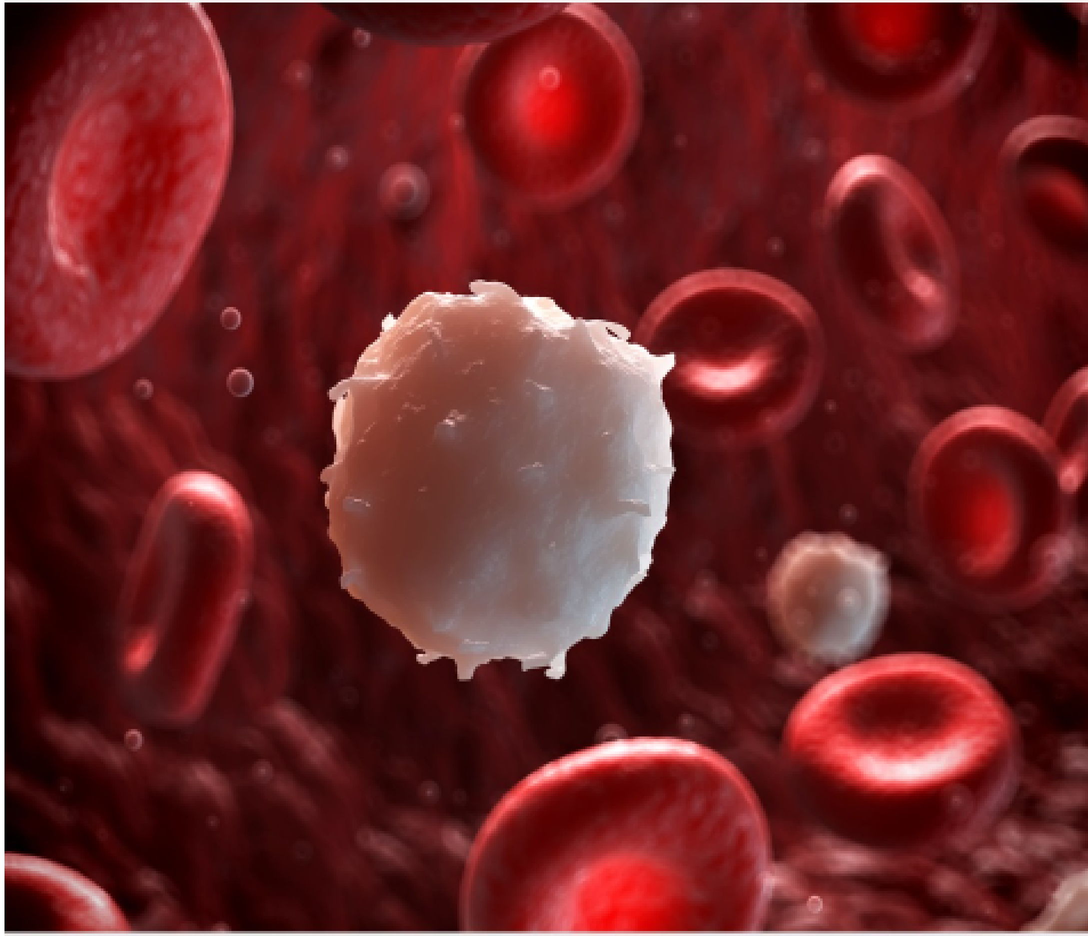
Genesis of the White Blood Cells (WBCs)

- Leukopoiesis is the development and maturation of leukocytes.
- Committed pluripotent stem cell gives rise to leukocytes through various stages.
- The proliferation and self renewal of hematopoietic stem cells (HSCs) depends on hemopoietic growth factors and colony stimulating factors
- Aside from those cells committed to form red blood cells, two major lineages of *white blood cells* are formed, the **myelocytic** (beginning with the *myeloblast);* and the **lymphocytic** (beginning with the *lymphoblast)* lineages.
- The granulocytes and monocytes are formed only in the bone marrow.
- Lymphocytes and plasma cells are produced mainly in the various lymphogenous tissues—especially the lymph glands, spleen, thymus, tonsils, and various pockets of lymphoid tissue elsewhere in the body, such as in the bone marrow and in so-called Peyer's patches underneath the epithelium in the gut wall.

- The white blood cells formed in the bone marrow are stored within the marrow until they are needed in the circulatory system. Then, when the need arises, various factors cause them to be released.
- Normally, about three times as many white blood cells are stored in the marrow as circulate in the entire blood. This represents about a 6-day supply of these cells.
- The lymphocytes are mostly stored in the various lymphoid tissues, except for a small number that are temporarily being transported in the blood.
- Megakaryocytes are also formed in the bone marrow.
- These megakaryocytes fragment in the bone marrow; the small fragments, known as *platelets* (or *thrombocytes*), then pass into the blood.
- They are very important in the initiation of blood clotting.

White blood cells

- White blood cells (WBCs) or leukocytes (leuko is derived from Greek word leukos = white) are the colourless and nucleated formed elements of blood.
- Functionally, these cells play important role in defense mechanism of body and protect the body from invading organisms by acting like soldiers.
- Leukocytes contain nuclei and mitochondria and can move in an amoeboid fashion.
- Because of their amoeboid ability, leukocytes can squeeze through pores in capillary walls and move to a site of infection, in a process known as diapedesis.



- WBCs are attracted to inflamed tissue areas by **Chemotaxis**
- Total WBC count ranges between 4,000 to 11,000/cu mm of blood
- Variations in WBC count occur due to several physiological and pathological conditions

  - **Physiological:**

1. Age: Higher in infants and children, lower in adults
2. Sex: Slightly more in males than females
3. Diurnal: Minimum in early morning and maximum in the afternoon
4. Exercise: Slightly increases
5. Sleep: Decreases

6. Emotional conditions like anxiety: Increases
7. Pregnancy and Parturition: Increases
8. Menstruation: Increases

- **Pathological**:

1. Leukocytosis: Increase in total WBC count. Occurs due to infections, allergy, common cold, tuberculosis.
2. Leukemia: Cancer of the blood
3. Leukopenia: Decrease in total WBC count. Occurs due to liver cirrhosis, pernicious anemia, typhoid, viral infections.

Characteristics of WBCs

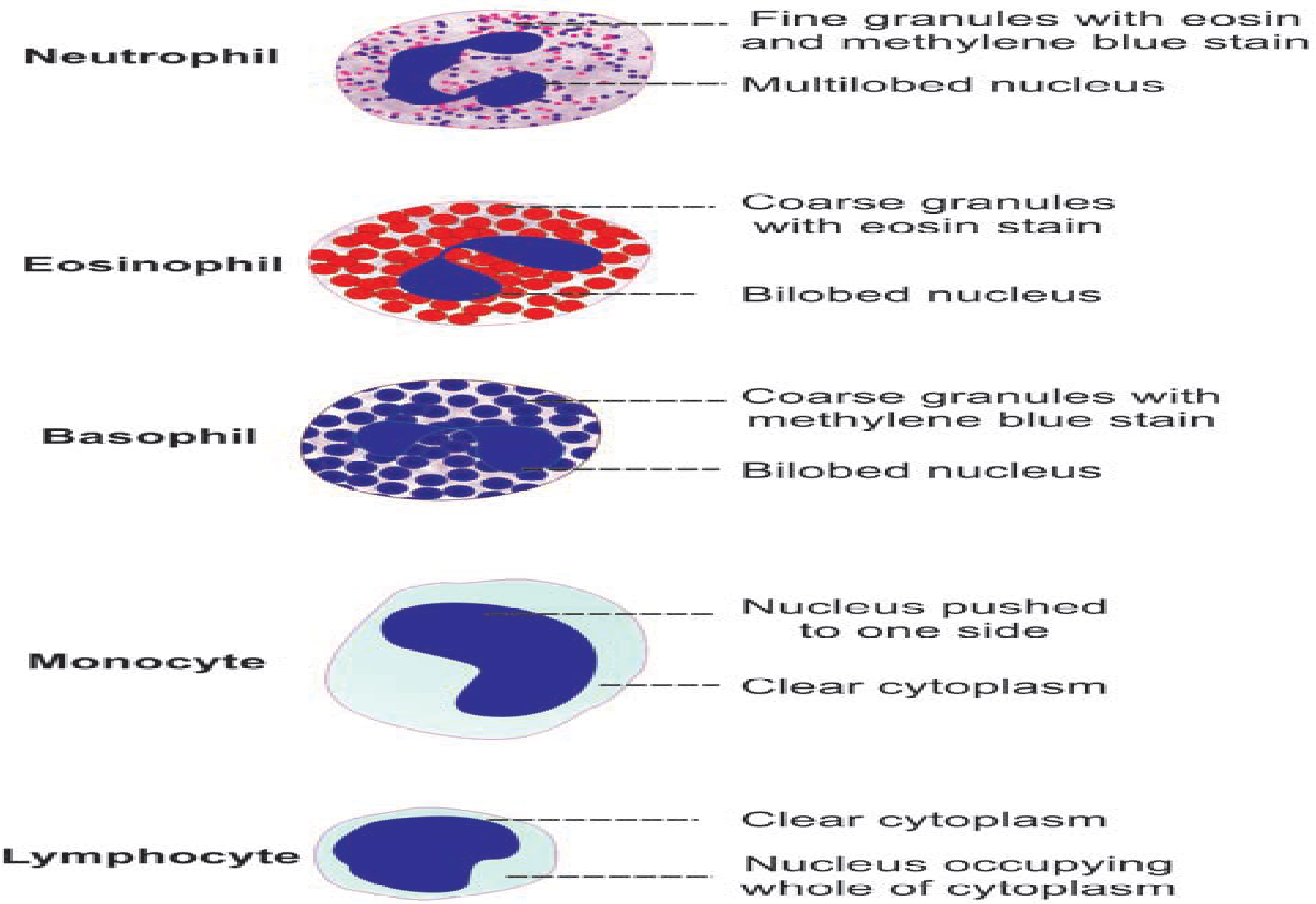
- Larger in size
- Irregular in shape (Amoeboid)
- Nucleated
- Many types
- Presence of granules in some types of WBCs
- Shorter lifespan (Lifespan may be as short as half a day or it may be as long as 3 to 6 months)

Types/Classification

- Broadly, WBCs are classified into two:
  - Granulocytes: These WBCs (polymorphonuclear cells, PMNs) possess granules in their cytoplasm. They are grouped into three depending on their staining property.
    i. Eosinophils (with granules taking acidic stain)
    ii. Basophils (with granules taking basic stain)
    iii. Neutrophils (with granules taking both acidic and basic stains)

  - Agranulocytes: These WBCs possess clear cytoplasm without visible granules. They are:
    i. Monocytes
    ii. Lymphocytes

Fig: Different types of White Blood Cells



Features of the Types of WBCs

- **Eosinophils** have coarse (larger) granules in the cytoplasm, which stain pink or red with eosin. Nucleus is bilobed and spectacle-shaped. Diameter of the cell varies between 10 and 14μ.

- **Basophils** also have coarse granules in the cytoplasm. The granules stain purple blue with methylene blue. Nucleus is bilobed. Diameter of the cell is 8 to 10μ.

- **Neutrophils** are the most abundant type of leukocyte, accounting for 50% to 70% of the leukocytes in the blood. Nucleus of neutrophils is multilobed which depends upon the age of cell. In younger cells, the nucleus is not lobed. And in older neutrophils, the nucleus has 2 to 5 lobes. The diameter of cell is 10 to 12μ

Features of the Types of WBCs

- Monocytes are the largest leukocytes with diameter of 14 to 18μ. The cytoplasm is clear without granules.
- Nucleus is round, oval and horseshoe shaped, bean shaped or kidney shaped. Nucleus is placed either in the center of the cell or pushed to one side and a large amount of cytoplasm is seen.

- Lymphocytes also do not have granules in the cytoplasm. Nucleus is oval, bean-shaped or kidney-shaped. Nucleus occupies the whole of the cytoplasm. A rim of cytoplasm may or may not be seen
  - Depending upon the size, lymphocytes are divided into two groups:

1. Large lymphocytes: Younger cells with a diameter of 10 to 12μ.
2. Small lymphocytes: Older cells with a diameter of 7 to 10 μ.

Functions of WBCs

- WBCs are majorly involved in the defense mechanism of the body. However, each type of WBCs acts through different ways.

Neutrophils

- Neutrophils provide the first line of defense against invading microorganisms, along with monocytes. Neutrophils are the free cells in the body and wander freely through the tissue.
- Each neutrophil can hold about 15 to 20 microorganisms at a time. These cells engulf the bacteria and then destroy them by means of phagocytosis.
- Granules of neutrophils contain enzymes like proteases, myeloperoxidases, elastases and metalloproteinases.
- The granules also contain antibody like peptides called cathelicidins and defensins, which are antimicrobial peptides and are active against bacteria and fungi.

- Membrane of neutrophils contains an enzyme called NADPH oxidase (**dihydronicotinamide adenine dinucleotide phosphate oxidase**). It is activated by the toxic metabolites released from infected tissues.
- The activated NADPH oxidase is responsible for bactericidal action of neutrophils.
- Neutrophils also secrete platelet-activating factor (PAF)**, which accelerates the aggregation** of platelets during injury to the blood vessel, resulting in prevention of excess loss of blood.

Eosinophils

- Eosinophils play an important role in the defense mechanism of the body against parasites.
- They are especially abundant in the mucosa of the gastrointestinal tract, respiratory and urinary tracts.
- Circulating eosinophils are increased also during allergic diseases like asthma.
- Eosinophils are responsible for detoxification, disintegration and removal of foreign proteins.
- Eosinophils attack them by some special type of cytotoxic substances present in their granules. When released over the invading parasites from the granules, these substances become lethal and destroy the parasites.

Basophils

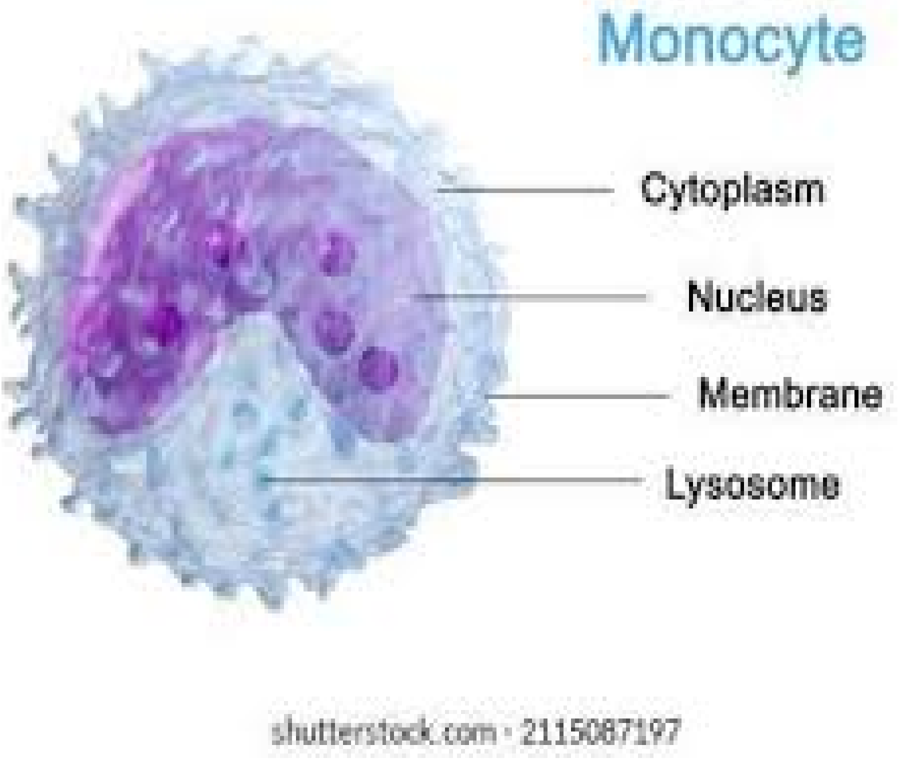
- Basophils also enter tissues and release proteins and cytokines.
- They play an important role in healing processes.
- They are characterized by the presence of receptors for IgE in basophil membrane which are essential for immediate-type hypersensitivity reactions (allergy).
- Functions of basophils are executed by the release of some important substances from their granules such as: Heparin; Histamine, slow-reacting substances of anaphylaxis, bradykinin and serotonin; Proteases and myeloperoxidase; and Cytokines.

Mast Cells

- Mast cells are large tissue cells resembling basophil. Generally, they are heavily granulated cells of the connective tissue and prominently seen in the areas such as skin, mucosa of the lungs and digestive tract, mouth, conjunctiva and nose.
- Mast cells usually do not enter the bloodstream but play an important role in producing the hypersensitivity reactions like allergy and anaphylaxis.
- When activated, the mast cell immediately releases various chemical mediators from its granules into the interstitium.

Monocytes

- Monocytes are the largest cells among the leukocytes. Like neutrophils, monocytes also are motile and phagocytic in nature.
- Along with neutrophils, these leukocytes provide the first line of defense. Monocytes secrete:
  - 1. Interleukin-1 (IL-1)
  - 2. Colony stimulating factor (M-CSF)
  - 3. Platelet-activating factor (PAF)
- Monocytes are the precursors of the tissue macrophages. Matured monocytes stay in the blood only for few hours. Afterwards, these cells enter the tissues from the blood and become tissue macrophages.
- Examples of tissue macrophages are Kupffer cells in liver, alveolar macrophages in lungs, microglia and macrophages in spleen.



Formation of Pus

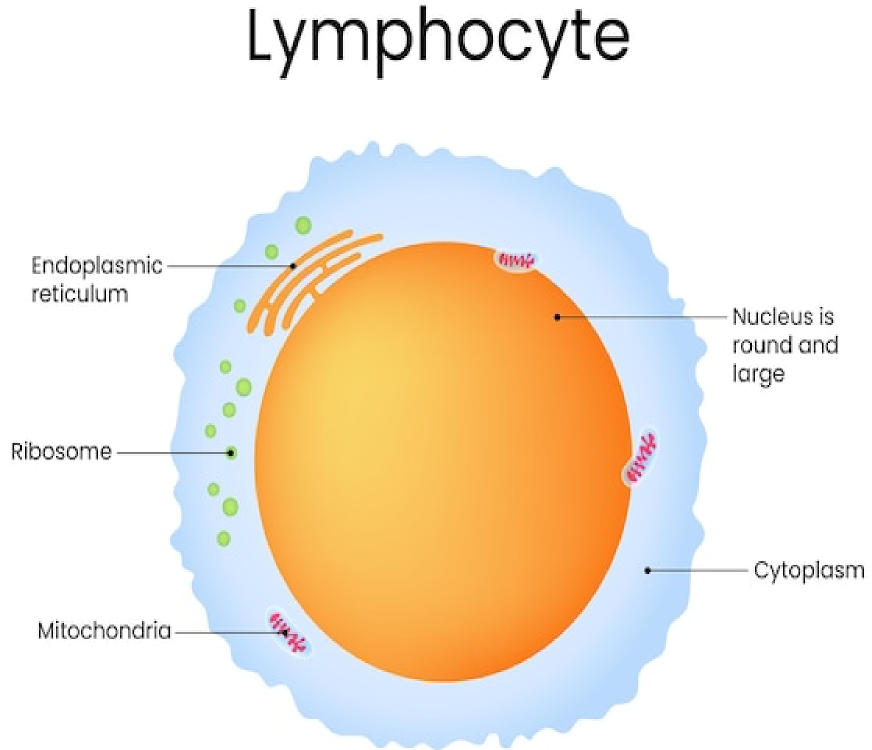
- When neutrophils and macrophages engulf large numbers of bacteria and necrotic tissue, essentially all the neutrophils and many, if not most, of the macrophages eventually die.
- After several days, a cavity is often excavated in the inflamed tissues that contains varying portions of necrotic tissue, dead neutrophils, dead macrophages, and tissue fluid. This mixture is commonly known as **pus**.
- After the infection has been suppressed, the dead cells and necrotic tissue in the pus gradually autolyze over a period of days, and the end products are eventually absorbed into the surrounding tissues and lymph until most of the evidence of tissue damage is gone.

Lymphocytes

- Lymphocytes play an important role in immunity.
- After birth, some lymphocytes are formed in the bone marrow. However, most are formed in the lymph nodes, thymus and spleen, from precursor cells that originally came from the bone marrow and were processed in the thymus.
- Functionally, the lymphocytes are classified into two categories, namely T lymphocytes and B lymphocytes.

- T lymphocytes are responsible for the development of cellular immunity

- B lymphocytes are responsible for the development of humoral immunity.

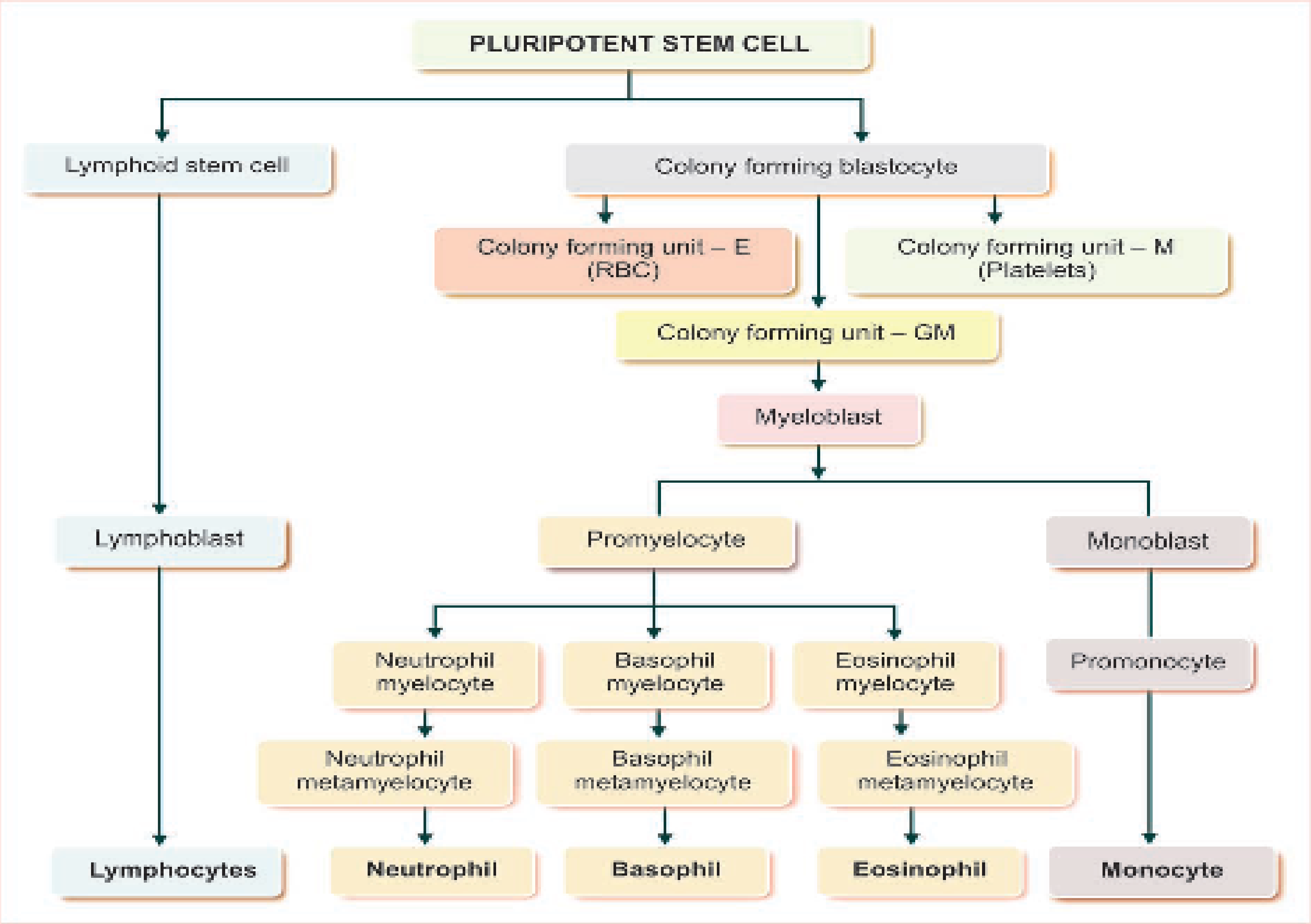


Colony stimulating Factors

- Colony stimulating factors (CSF) are proteins which cause the formation of colony forming blastocytes. CSF are of three types:

1. Granulocyte-CSF (G-CSF) secreted by monocytes and endothelial cells
2. Granulocyte-monocyte-CSF (GM-CSF) secreted by monocytes, endothelial cells and T lymphocytes
3. Monocyte-CSF (M-CSF) secreted by monocytes and endothelial cells.

Fig: Leukopoiesis



Differential Count

WBC	Percentage	Absolute value per cu mm
Neutrophils	50 to 70	3,000 to 6,000
Eosinophils	2 to 4	150 to 450
Basophils	0 to 1	0 to 100
Monocytes	2 to 6	200 to 600
Lymphocytes	20 to 30	1,500 to 2,700

Table: Normal values of different WBCs

THANK YOU

FOR

LISTENING