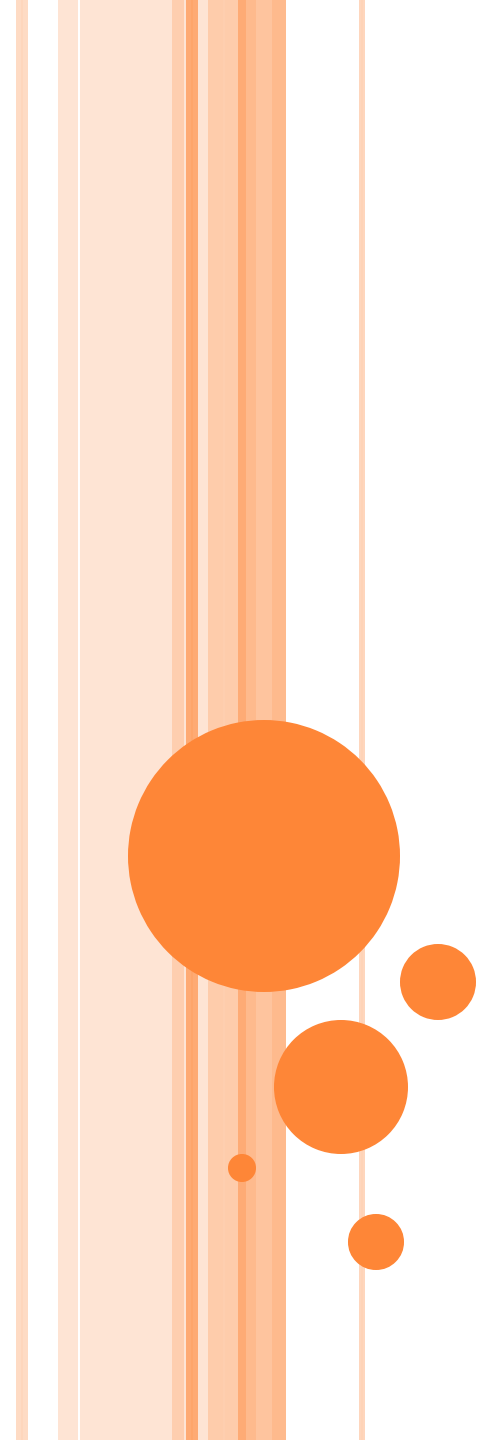




Histology lab- Hematopoiesis

Blood

It is a specialized type of connective tissue that composed of:

- 1- The plasma: represents the matrix.
- 2- The formed blood elements: represent the cells.

Blood has no fibers.



The formed blood elements

1- Red blood corpuscles (RBCs).

2- White blood cells (WBCs) or leukocytes including:

-Granular leukocytes: neutrophils, eosinophils and basophils.

-Agranular leukocytes: lymphocytes and monocytes.

3- Blood platelets.



Preparation of a blood smear

1-Giemsa's stain (methylene blue+ eosin) is used which differentiate blood cells by their nuclei and cytoplasmic granules:

- basophilic (violet)
- eosinophilic (pink)
- azurophilic (red purple)



2-Blood count:

It is the average number of a formed blood element per cubic millimeter blood.

RBCs count.

Total leukocytic count.

Platelets count.

Differential leukocytic count: it is the percentage of each type of leukocytes relative to the total number of WBCs.



Red blood corpuscles

Average RBC count :

In males: 5-5.5 millions/mm³.

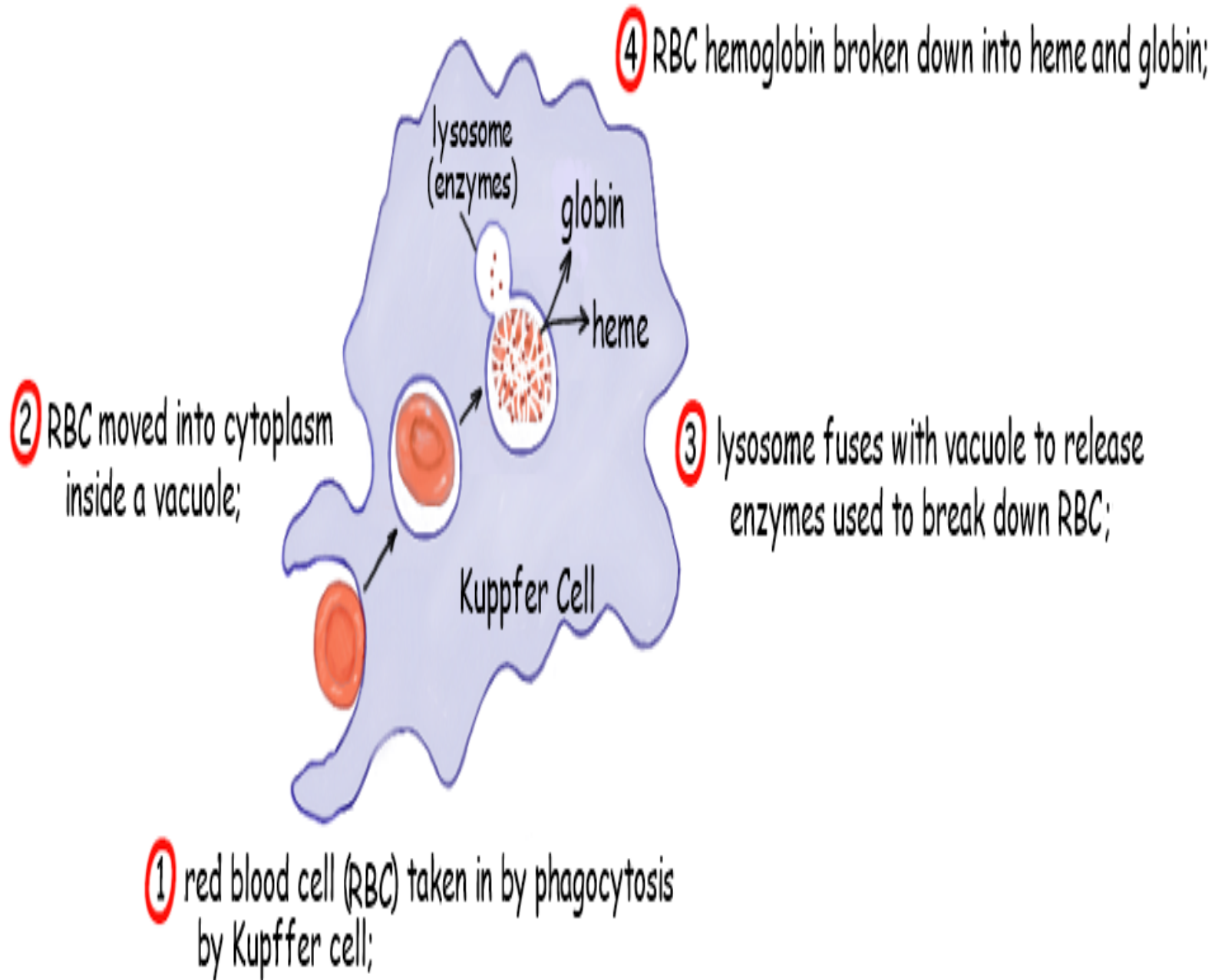
In females: 4.5-5 millions/mm³.

Life span of RBCs:

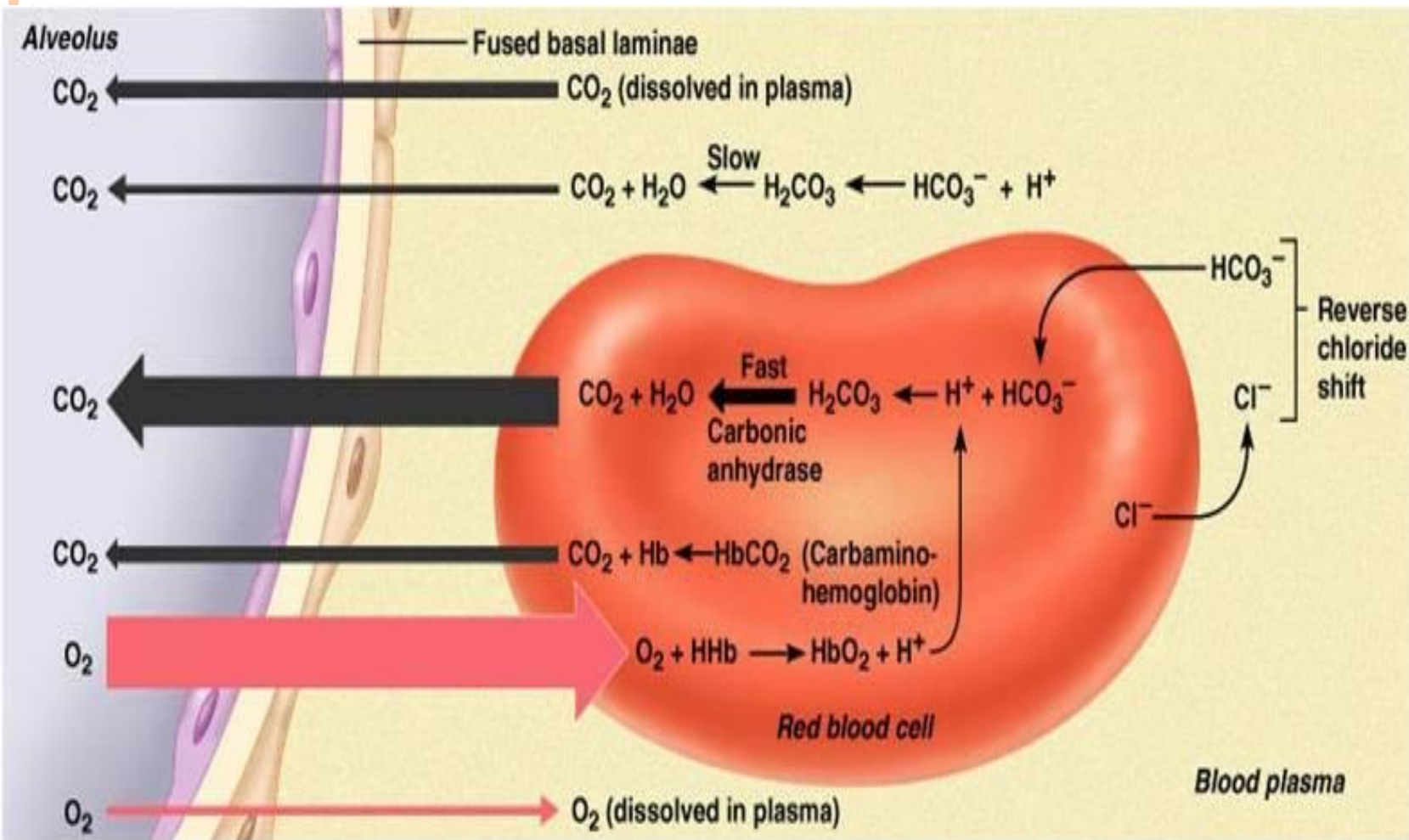
It is 100-120 days.

Old & deformed RBCs are phagocytosed by the macrophages in the spleen and liver.





Function of Red blood corpuscles



(b) Oxygen pickup and carbon dioxide release in the lungs

LM picture of RBCs

Shape: eosinophilic biconcave discs.

Diameter: 7.2-7.8 μ m (=the size of the smallest blood capillaries).

Nucleus: absent.

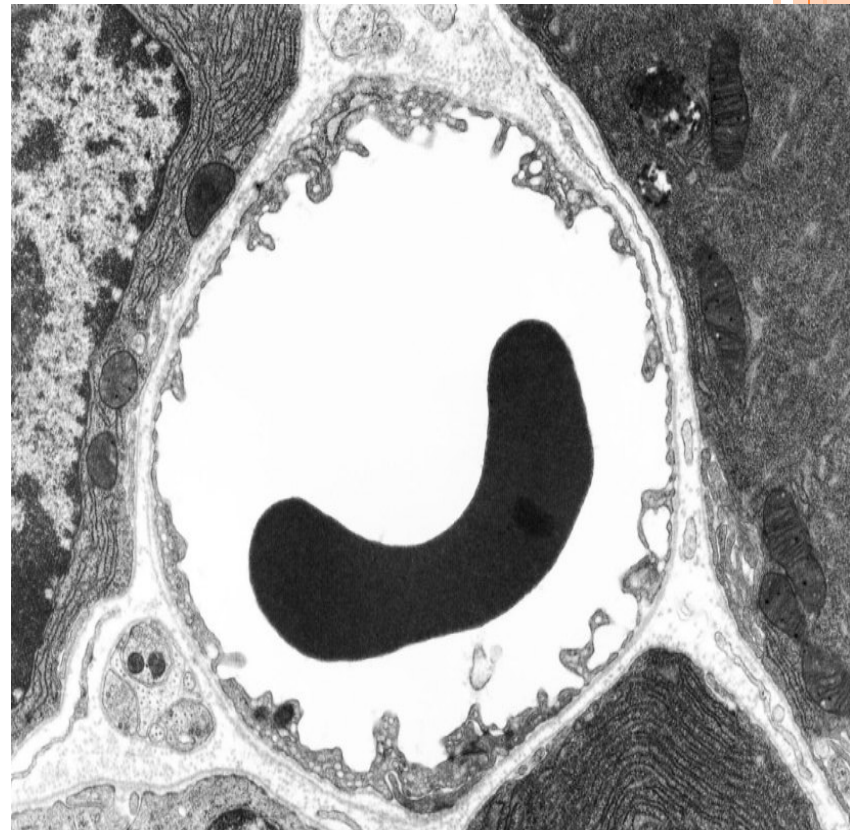
Cytoplasm: hemoglobin occupies about 33% of the corpuscular volume & is more concentrated at the periphery.



EM picture of RBCs

Shape: membranous electron dense biconcave discs.

Cell organelles: no nucleus& no organelles.

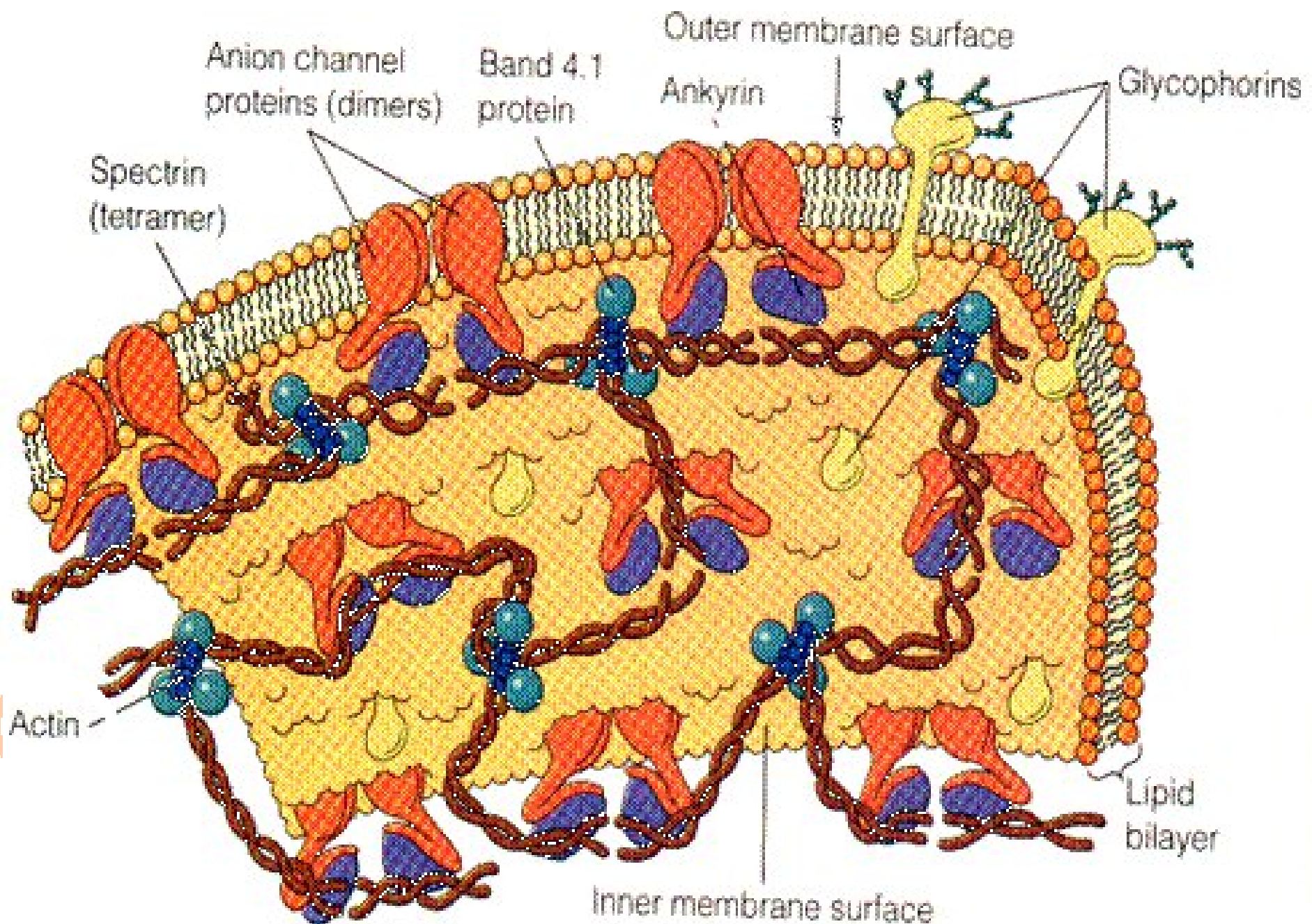


RBC membrane

- 1- **From outside:** It is covered by a well developed **glycocalyx** which is responsible for **the blood grouping**.
- 2- **From inside:** It is supported by a well developed **cytoskeleton**, which is responsible for the **flexibility** of RBCs.

It is formed of microfilaments (actin & spectrin) that are attached to a peripheral membrane protein (ankyrin).





How do the RBCs adapt to its function?

1- **The biconcave discoid shape** increases the surface area of the RBCs by 20-30% more than if they had a spherical shape.

2- **The lack of a nucleus and organelles** allows more space in the cytoplasm for carrying the maximal amount of hemoglobin. →

3- **The concentration of hemoglobin at the periphery of the cytoplasm** facilitates its binding to oxygen and carbon dioxide.

4- **The structure of the cytoskeleton** helps the RBCs to be squeezed through the narrowest blood capillaries without being damaged.

5- **The selective permeability of the cell membrane to oxygen and carbon dioxide** to enhance their diffusion rates during gas exchange. →



Myeloid tissue

- **Sites:**

- 1- The central bone marrow cavity in young long bones.
- 2- The marrow cavities of cancellous bone.

- **Types of bone marrow:**

- 1- **Red bone marrow:** active in hemopoiesis.
- 2- **Yellow bone marrow:**

inactive bone marrow in which the blood formation has stopped.

Its yellow color is due to the presence of fat cells.

It can revert to the red type in hemorrhage and anemia.



Structure of myeloid tissue

I- The stroma:

A-Reticular fibers: support the myeloid cells and blood sinusoids.

B-Stromal cells: the **fixed** cell population of the bone marrow. They include:

1-Reticular cells: have 3 functions:

- Produce the reticular fibers.

- Secrete growth factors that stimulate the hemopoietic stem cells.

- In yellow bone marrow, these cells are changed to fat cells.

2- Macrophages:

- Phagocytosis of aged RBCs & the malformed blood elements.

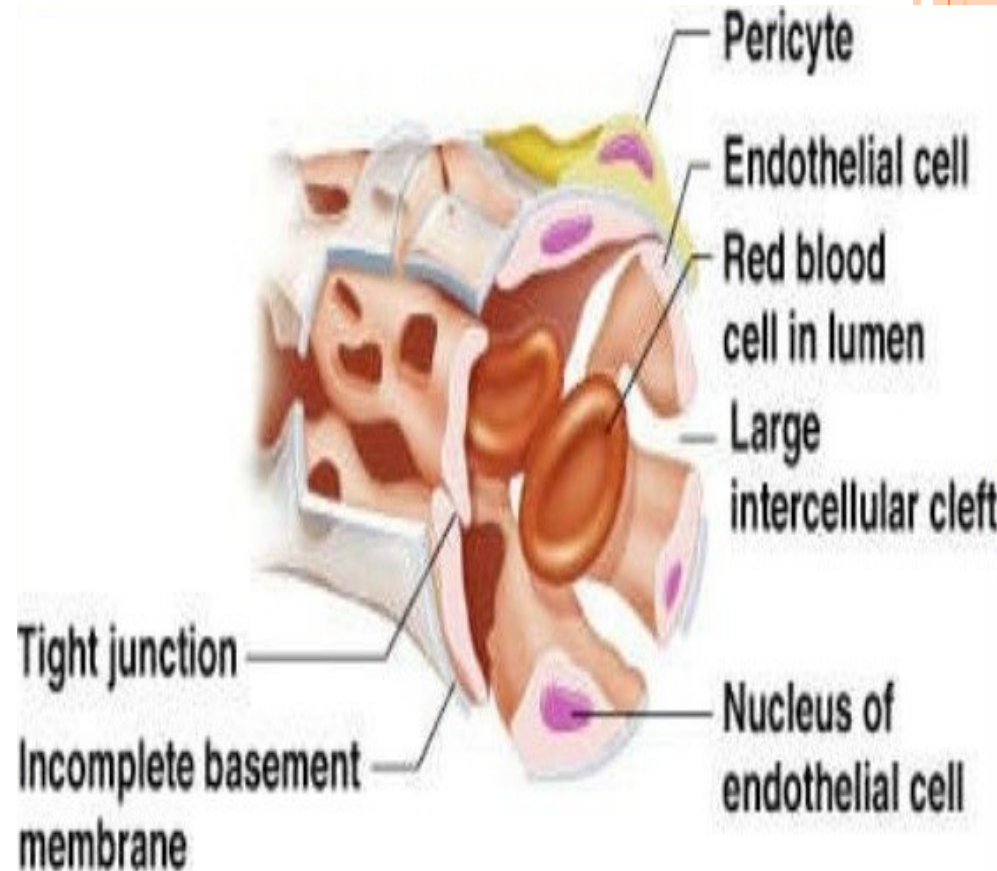
- Storage of iron for reuse.



II- The blood sinusoids:

Wide & irregular blood vessel.

Lined by fenestrated endothelial cells.



III- The myeloid cells:

- The **free** cell population of the bone marrow.
- They include:

1- **Pluripotent hemopoietic stem cells (PHSCs):**

- **Shape:** small cells with pale basophilic cytoplasm and pale nuclei.
- **Function:** they divide, give rise to daughter cells (half remains as a reserve of the HPSCs while the other half develops into multipotent hemopoietic stem cells).



2- **Multipotent hemopoietic stem cells (MHSCs)**: They give rise to cell colonies for each of the formed blood elements.

3- **Colony forming units (CFUs)**: 2 types:

A-Colony forming unit- lymphocyte (CFU- Ly): give rise to lymphocytes.

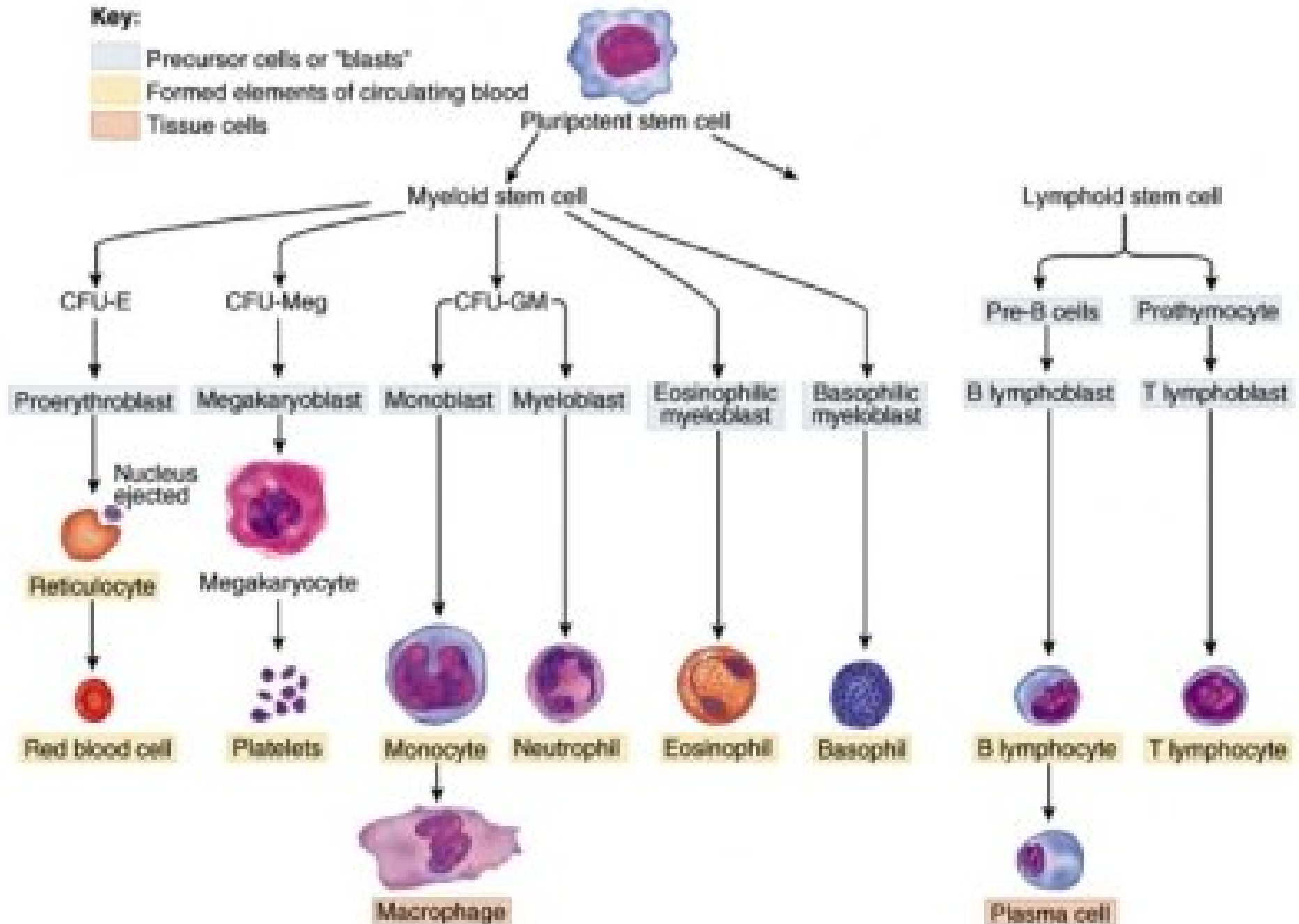
B- Colony forming unit- granulocyte, erythrocyte, monocyte, megakaryocyte (CFU- GEMM): give rise to unipotent colony:

- CFU-Erythrocyte (CFU-E).
 - CFU-Megakaryocyte (CFU-Meg).
 - CFU-monocyte (CFU-M)
 - CFU-Granulocyte: give rise to CFU-Neutrophil.
 - CFU-Eosinophil (CFU-Eo).
 - CFU-Basophil (CFU-Ba).
- These cells are larger with more basophilic cytoplasm (abundant ribosomes for synthesis of the specific products of each blood element).



Key:

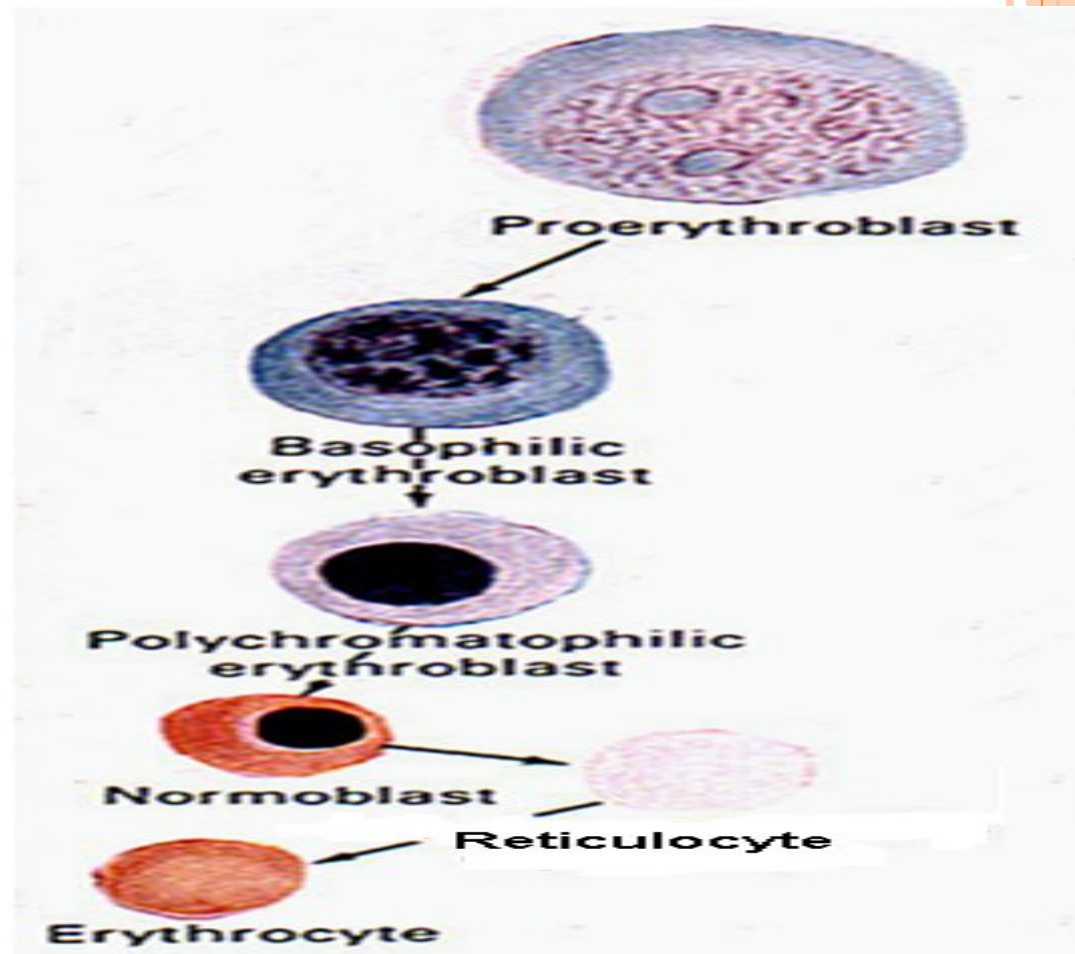
- Precursor cells or "blasts"
- Formed elements of circulating blood
- Tissue cells



Erythropoiesis

Definition: It is the process of formation of RBCs.

Duration: 7 days.



- 1- PHSCs.
- 2- MHSCs.
- 3- Colony forming unit- erythrocytes (CFU-E).
- 4- Proerythroblasts:
 - ✓ They are the first recognizable erythrocyte precursor.
 - ✓ They are large cells with basophilic cytoplasm.



5- Basophilic erythroblasts:

- ✓ Hemoglobin synthesis is most active.
- ✓ The cytoplasm is strongly basophilic due to abundant polysomes.

6- Polychromatophilic erythroblasts:

- ✓ Hemoglobin is accumulating in large amount.
- ✓ Ribosomes are still abundant.
- ✓ The cytoplasm shows eosinophilic areas alternating with basophilic spots.
- ✓ They are the last stage in which the cells undergo cell divisions. The next phases involve morphological changes of the erythroblasts.



7- Orthochromatophilic erythroblasts (normoblasts):

- ✓ The synthesis of hemoglobin is completed.
- ✓ Ribosomes are much reduced in number.
- ✓ The nucleus becomes small and condensed, pushed towards the periphery, extruded from the cell and phagocytosed by macrophages.



8- Reticulocytes:

- ✓ Non-nucleated.
- ✓ They differ from mature RBCs in that:
 - Slightly larger.
 - Their cytoplasm contains remnants of ribosomes and other organelles which on staining with cresyl blue stain gives a reticulate pattern.
- ✓ They represent about 1% of all RBCs in a normal blood film(an increase in their percentage indicates an increase in the rate of erythropoiesis as in case of anemia or hemorrhage) .

9- Mature RBCs.



White blood cells=leukocytes

- **Normal count:** 4000-10,000 / mm³ blood.

- **Types:**

Granular leukocytes (granulocytes) which have specific granules in their cytoplasm:

1- Neutrophils.

2- Eosinophils.

3- Basophils.

Agranular leukocytes (agranulocytes) which lack specific granules in their cytoplasm:

1-Monocytes.

2-Lymphocytes.



Function of white blood cells

They do not function within the bloodstream but, they circulate in the blood stream and when they receive a stimulus, they leave the bloodstream to enter the connective tissue and perform an immune function.



WBCs

Never	Let	Monkey	Eat	Banana
60-70	20-30	3-8	1-4	0.5-1

Neutrophils

60-70 %

Lymphocytes

20-30 %

Monocytes

3-8 %

Eosinophils

1-4 %

Basophils

0.5-1 %

Never

Let

Monkey

Eat

Banana

Granular leukocytes

I-Neutrophils (polymorphonuclear leukocytes

Why it is named neutrophils? because their granules have a neutral staining affinity to the stains in blood film.

Why it is named PNLs?

Because the cells may contains from 2-5 lobes of nuclei according to its age.



Function of the neutrophils

The first line of defense against any invading micro-organism

Killing strategy

- 1- **Go to battlefield:** they leave the circulation and migrate to the site of action in connective tissue.
- 2- **Identify the enemy.**
- 3- **Phagocytosis** (so they are called microphage).



During the inflammatory reaction, neutrophils themselves are damaged by the bacterial toxins causing their death and they are called **pus cells**.



Neutrophils

Differential count: 60-70% of the total leukocytic count.

LM:

Cytoplasm: lightly stained.

Nucleus: multilobed formed of 2-5 segments that are connected with a thin chromatin thread.

In females with a normal karyotyping (44+XX chromosomes), one of the X-chromosomes is condensed and attached to the nucleus forming a drumstick appearance (**Barr body**).



How do the neutrophils adapt to its function?

EM:

Shape: have an irregular outline due to extension of pseudopodia motility & phagocytosis

Cytoplasm: contains two types of granules phagocytosis:

1- **Small specific granules:** contain protease enzymes e.g. collagenase.

2- **Larger azurophilic granules(non specific):** containing lysosomal hydrolytic enzymes and the antibacterial defensins



II-Eosinophils

Why it is called eosinophils? because their granules have a strong affinity to the eosin component of the stains.

Differential count: 1-4% of the total leukocytic count.



Functions of the eosinophils

- 1-They are responsible for **regulation of allergic reactions** (secrete histaminase).
- 2- In parasitic infections: **killing of parasites.**



Killing Strategy:

- 1- **Go to battlefield:** They circulate in the blood and then migrate to the connective tissue.
- 2- **Identify the enemy.**
- 3- **The proteins in their specific granules** act on the surface of the parasitic worm _____forming pores facilitating entry of lethal substances in the granules to the parasite death of parasite.



LM:

Nucleus: dense, bilobed, C-shaped.

Cytoplasm: contains large, shiny reddish granules.



How do the eosinophils adapt to its function?

EM:

The cytoplasm contains two types of granules:

- 1- **Large specific granules:** containing major basic protein and histaminase enzyme.
- 2- **Small non specific azurophilic granules:** containing lysosomal hydrolytic enzymes.



III-Basophils = mast cell of the blood

Why it is called basophils?

Because their granules have strong affinity to the basic component of the stains.

Why it is called mast cell of the blood?

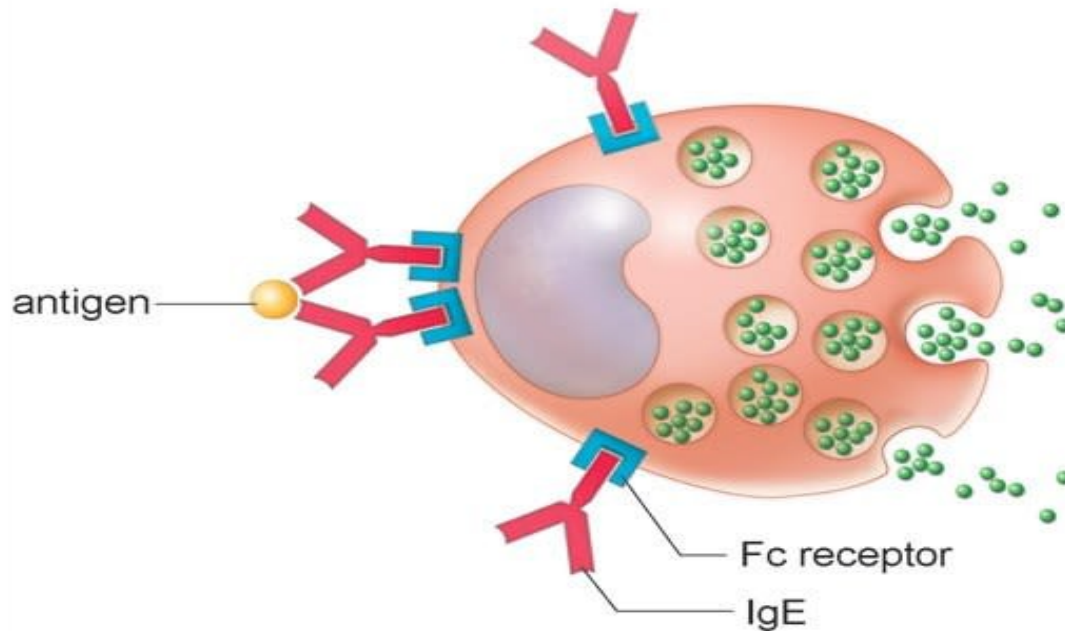
Because they secrete heparin and histamine like the connective tissue mast cells.

Differential count: 0.5- 1 % of the total leukocytic count.



Function of basophils

Basophils are responsible for the **release of histamine and heparin** in systemic hypersensitivity allergic reaction.



LM:

Nucleus: bilobed, S-shaped, obscured by the abundant basophilic granules in the cytoplasm.

Cytoplasm: contains abundant basophilic granules

Basophils show a **metachromasia** when stained with toluidine blue (the granules appear red instead of blue because of their content of heparin).



How do the basophils adapt to its function?

EM:

The cytoplasm contains two types of granules:

- 1- **Large specific granules:** containing heparin and histamine.
- 2- **Small non specific azurophilic granules:** containing lysosomal enzymes



Cell type	Neutrophil	Eosinophil	Basophil
Differential leukocytic count	60-70%	1-4%	0.5-1%
LM Nucleus Cytoplasm	Multilobed, Barr body in females. Lightly stained.	Bilobed C shaped. Shiny reddish granules.	Bilobed S shaped. Basophilic granules obscuring the nucleus. Metachromatic staining.
EM Specific granules Azurophilic granules	Small=collagenase Large	Large=major basic protein, histaminase. Small	Large= histamin, heparin. Small
Functions	First line of defense against any invading microorganism; microphage.	1-Activated in parasitic infection. 2- down regulate allergic reaction.	Involved in hypersensetivity reaction.

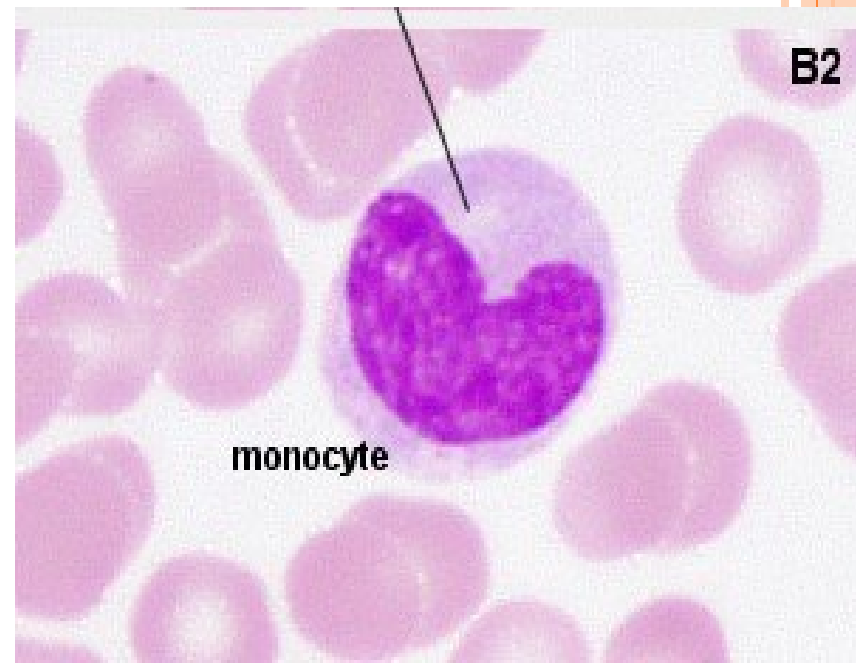
Agranular leukocytes

I-Monocyte

Why it is called monocyte?

Because they have a single, non-segmented nucleus.

Differential count: 3-8 % of the total leukocytic count.



Function of monocytes

Monocytes circulate in the blood and then migrate to the different body tissues where they differentiate to specific tissue phagocytic cells that constitute **the mononuclear phagocytic system:**

CT: Macrophages.

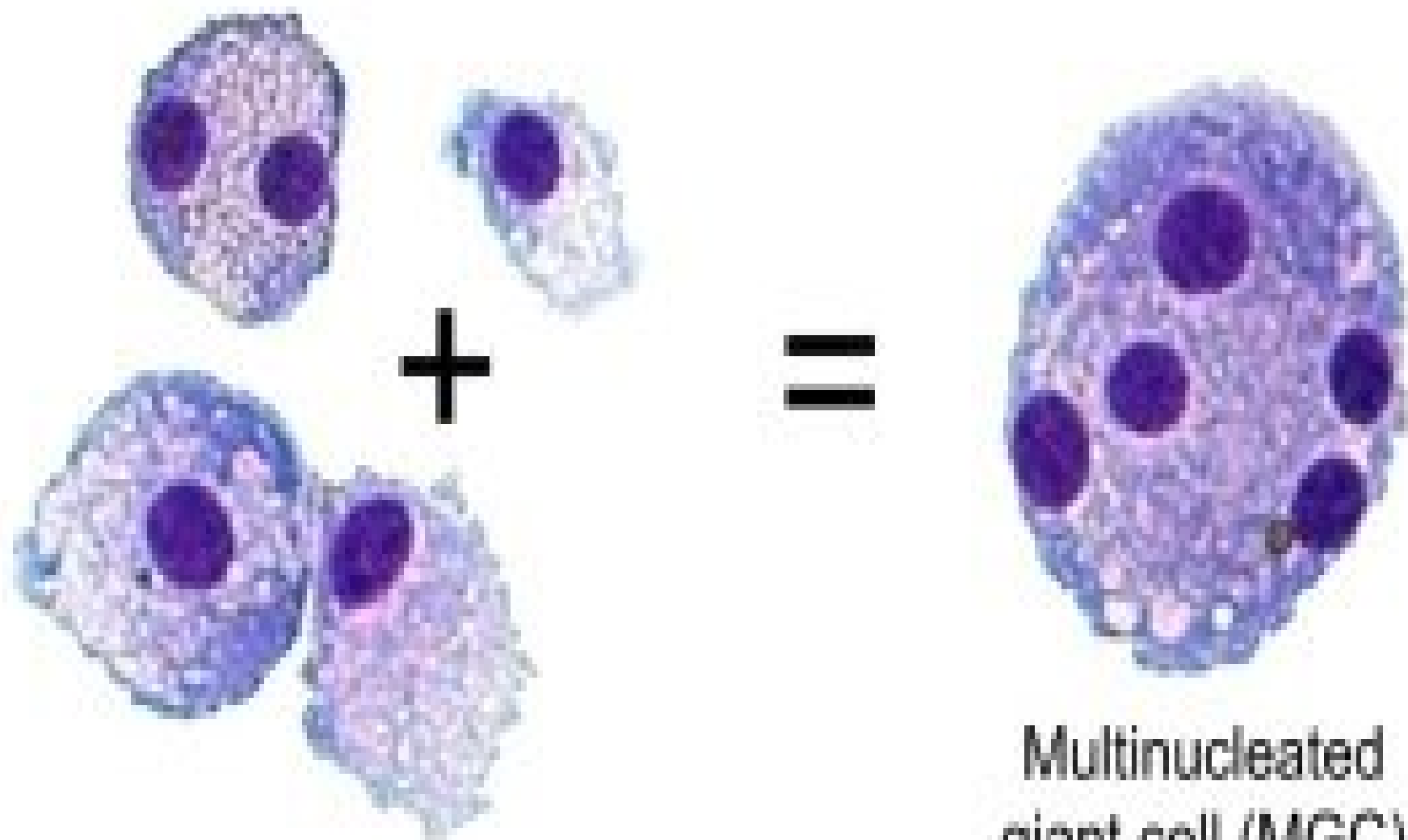
Bone : Osteoclasts.

CNS: Microglia.

Liver: Von Kupffer cells.

In chronic infections : macrophages fuse with one another forming **multinucleated giant cells.**





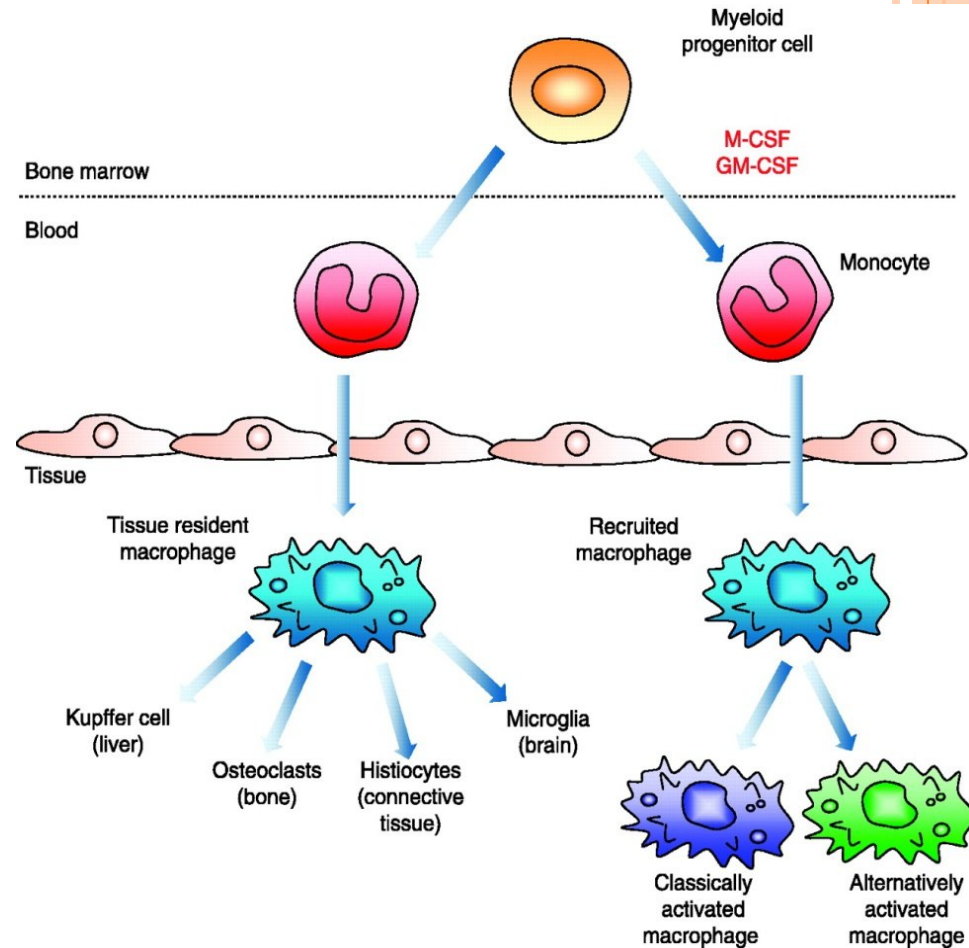
Macrophage fusion
induced by interleukin-4

Multinucleated
giant cell (MGC)

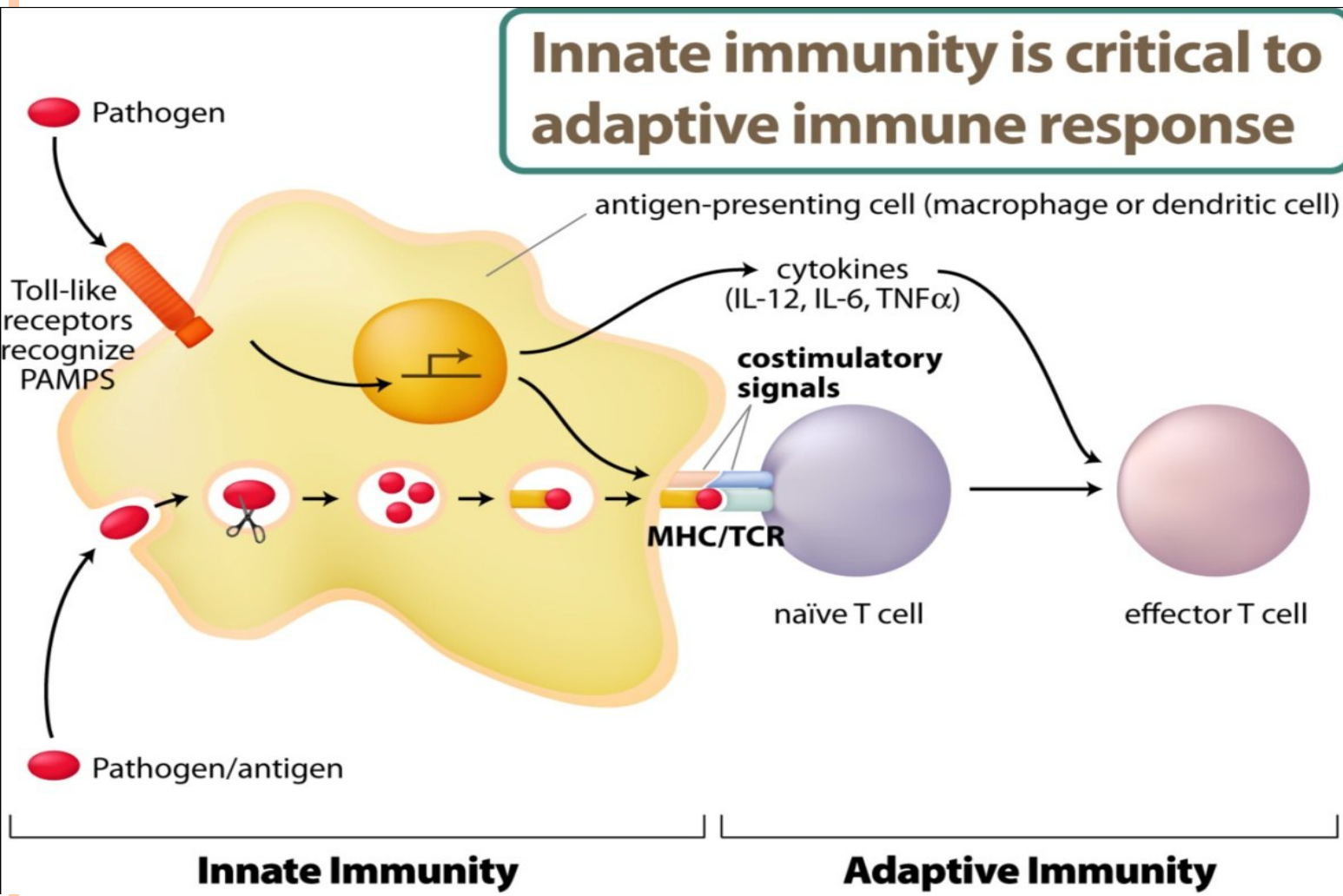
Phagocytosis of foreign antigens.

Killing Strategy:

- 1- Go to battlefield .
- 2-Differentiate into specific phagocytic cell.
- 3- Identify the enemy.
- 4- Phagocytosis.



Antigen presenting cells: they process antigens and present them to T lymphocytes

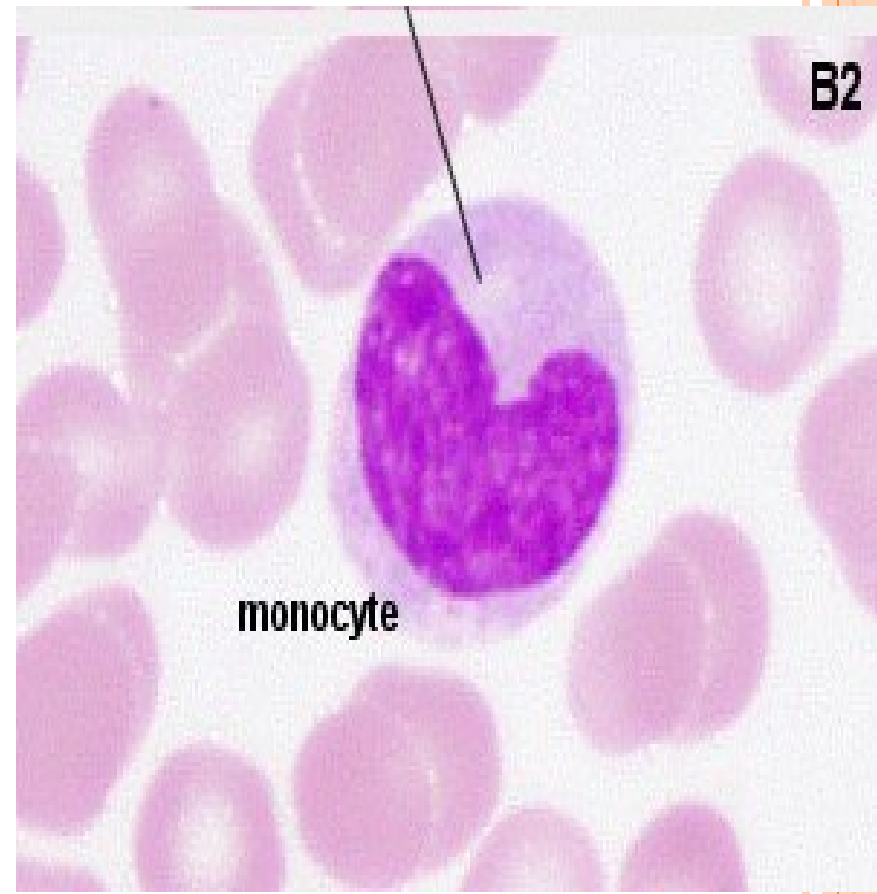


LM:

Size: the largest leukocytes.

Nucleus: large, eccentric and kidney shaped or horse-shoe.

Cytoplasm: abundant, pale basophilic and finely granular.

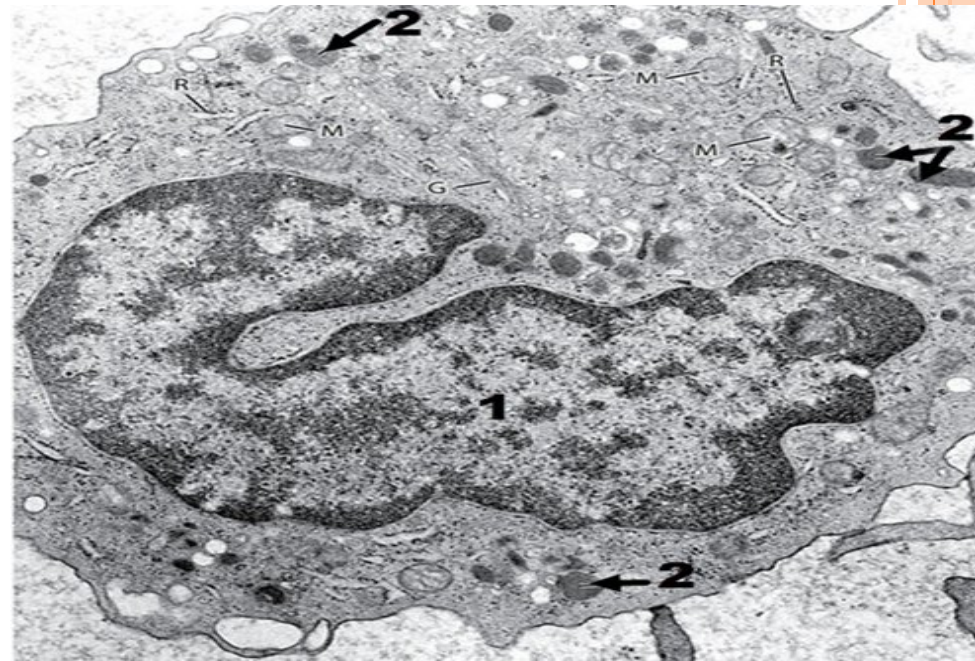


How do the monocyte adapt to its function?

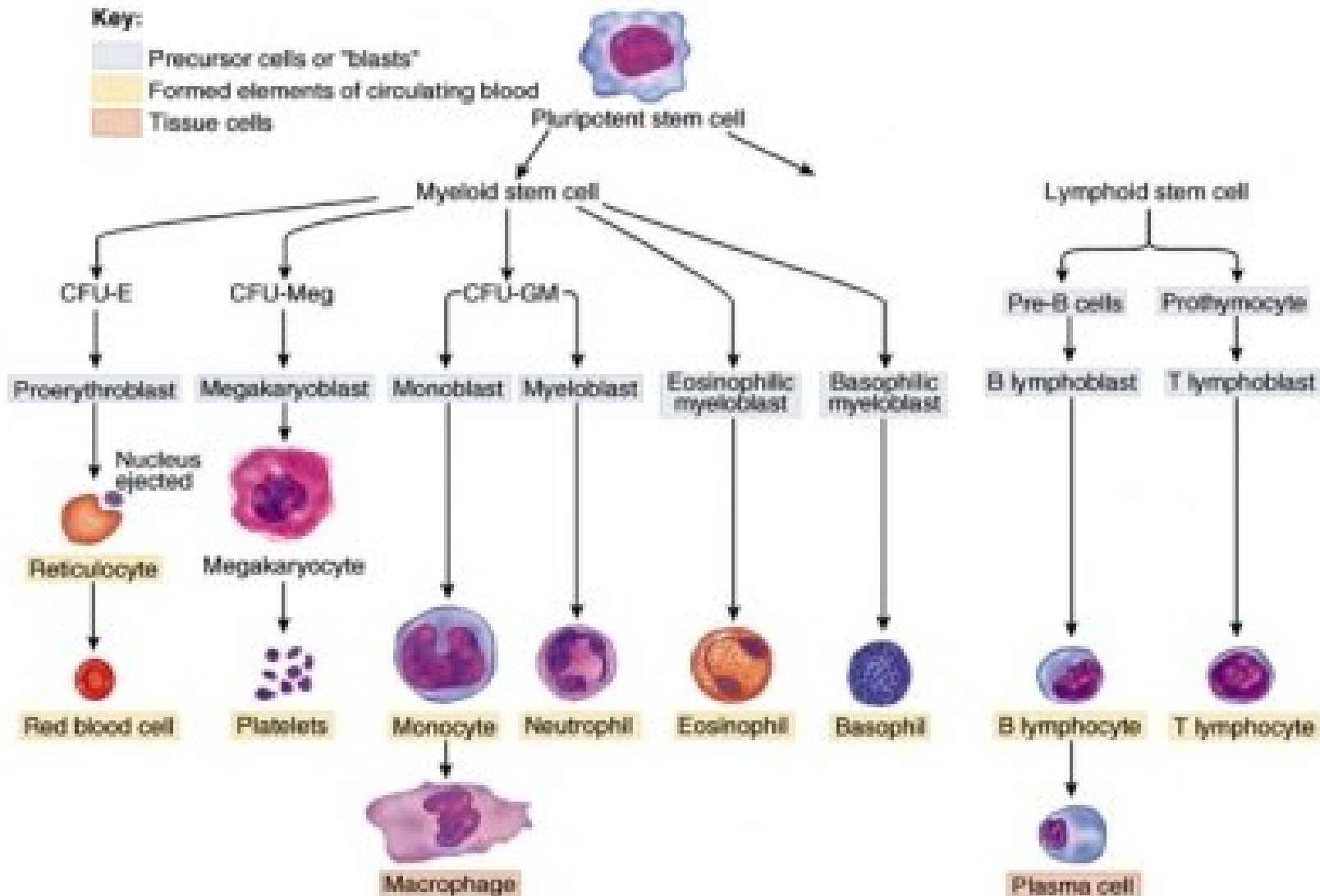
EM:

Shape: has an irregular outline due to extension of pseudopodia → motility & phagocytosis

Cytoplasm: contains small dense azurophilic granules containing lysosomal hydrolytic enzymes.



Granulopoiesis



- **Promyelocytes:**

Cytoplasm is more basophilic: as active protein synthesis occurs and the production of azurophilic granules begins.

- **Myelocytes:**

The specific granules for each type of the granulocytes start to appear in addition to the azurophilic granules.

The cytoplasmic affinity changes: it shows decreased basophilia and increased eosinophilia in neutrophils and eosinophils; whereas basophilia dominates in basophils.

This stage represents the last stage in which the cell can undergoes cell division, the next stages represent only morphological changes.



- **Metamyelocytes:**

The specific granules are more abundant.

The cytoplasm shows the staining affinity characteristic for each of the granular leukocytes.

Specifically, the neutrophils present by the end of these stage with an additional phase known as the band cells:

1- immature neutrophils

2-with a band-shaped nucleus.

3- They are 0-3% in the blood. This percentage increases if there is an acute infection.

- **Mature granulocytes:**

The nucleus becomes segmented in the pattern characteristic for each type of the granulocytes.



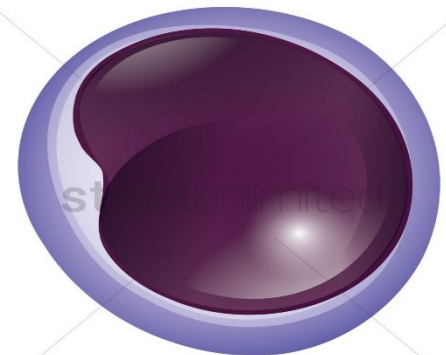
Lymphocytes& immune system

Why it is called lymphocyte?

Because they are the main cell population of the lymphoid organs.

Differential count: 20-30 %of the total leukocytic count.

Life span: for several months. The memory cells can live for several years.



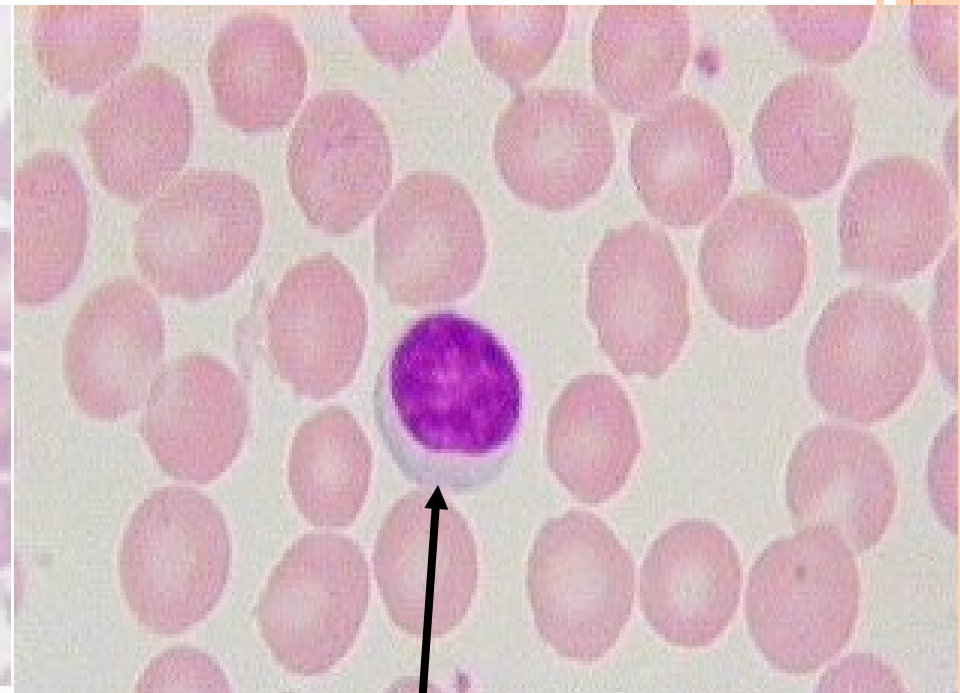
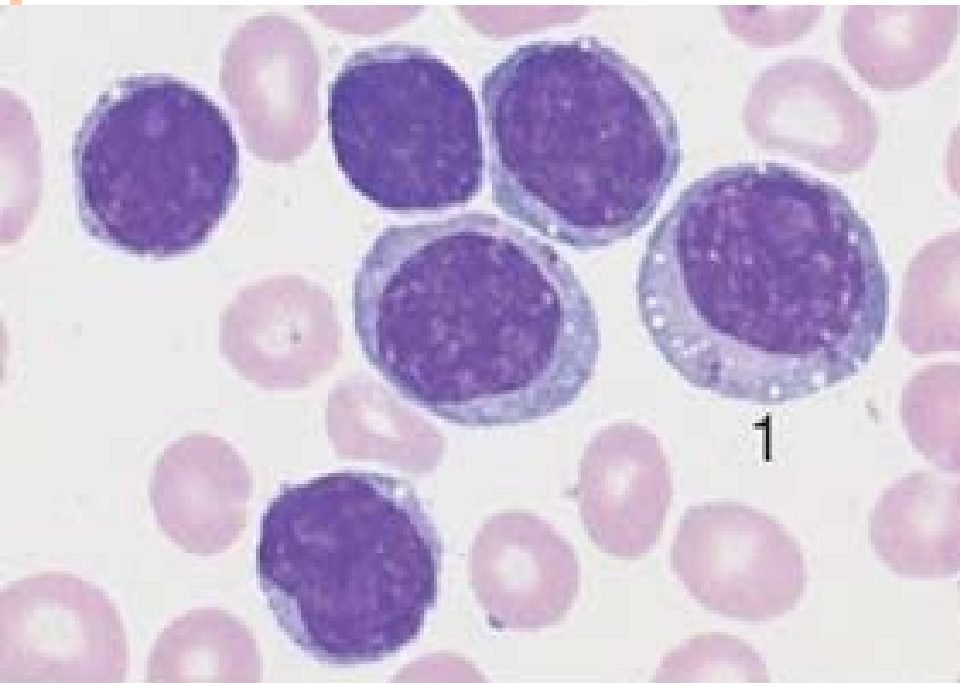
Lymphocyte

LM:

Shape& size: rounded cells with variable diameter.

Nucleus: large, dense and slightly indented.

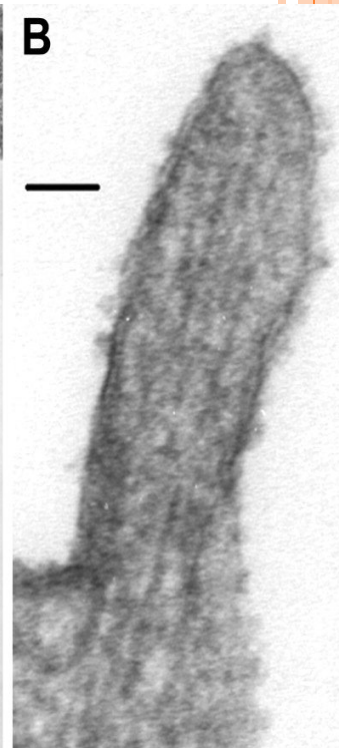
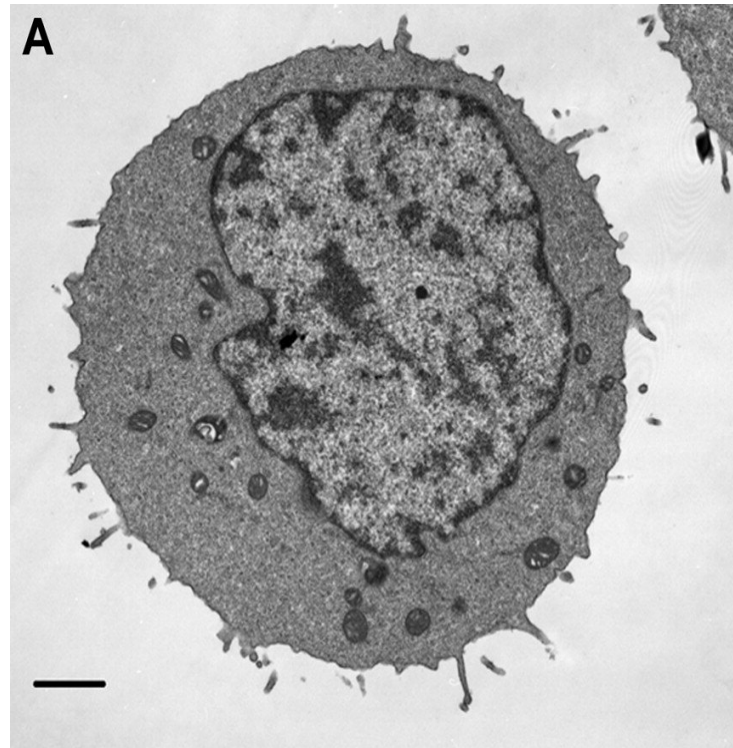
Cytoplasm: narrow rim of basophilic cytoplasm.



EM:

Cytoplasm: small dense azurophilic granules containing lysosomal enzymes.

The cell coat: contains receptors acting as antigenic markers e.g. the cluster of differentiation → classifying the lymphocytes into subtypes according to their functions.



Types and functions of lymphocytes

- According to their size:

Large lymphocytes.

Medium sized lymphocytes.

Both the large and medium-sized lymphocytes are functionally less differentiated lymphocytes.

Small lymphocytes: represents most of the circulating lymphocytes in blood. They represent the mature form of lymphocytes.

- According to the function:

1- T lymphocytes: 60-80% of small lymphocytes.

2- B lymphocytes: 20-30% of small lymphocytes.

3- Natural Killer cells 5-10%



Lymphopoiesis

1-PHSCs.

2- MHSCs.

3- Colony forming unit- lymphocyte (CFU- Ly):

Some daughters of these cells: migrate during fetal and early postnatal life to the cortex of the thymus where they differentiate into colony forming unit -T lymphocyte.

The rest: remains in the bone marrow and differentiate to colony forming unit- B lymphocyte.



4- Colony forming unit- T lymphocyte:

They divide in the thymus giving rise to T-lymphoblasts.

T lymphoblasts divide to give immunocompetent T-lymphocytes (expressing specific surface receptors).

Colony forming unit- B lymphocyte:

They divide in the bone marrow giving rise to B-lymphoblasts.

They divide to give immunocompetent B-lymphocytes (expressing specific surface receptors).



Blood platelets

Normal count: 150,000-400,000/ mm³.

Structure:

LM:

Shape: biconvex discs.

Diameter: 2-3 μm .

Nucleus: absent.

The granulomere: granular central core contains azurophilic granules.

The hyalomere: pale basophilic area at the periphery.



Platelets



Function of Blood Platelets

Blood platelets are concerned with the process of **thrombus formation** (blood clotting) in response to any vascular endothelial injury to prevent excessive blood loss.

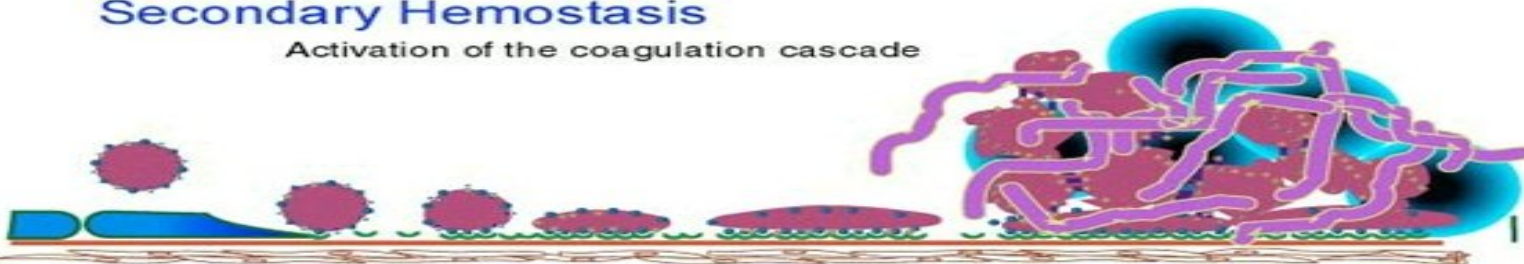
Formation of a Hemostatic Plug

Primary Hemostasis



Secondary Hemostasis

Activation of the coagulation cascade



How do the blood platelets adapt to its function?

EM:

Shape: irregular outline(when activated) due to presence of pseudopodia motility.

The platelet membrane: is covered by a well developed glycocalyx helps in platelets adhesion & aggregation.



How do the blood platelets adapt to its function?

The **hyalomere** contains:

- 1- **A circumferential bundle of microtubules** — maintains the discoid shape of platelets.
- 2- **Actin and myosin filaments** — contraction of the platelet for motility and clot retraction.
- 3- **Two systems of membranes** — regulation of the intraplatelet calcium concentration and thus the control of platelet degranulation. These are:

The open canalicular system: small canaliculi that open on the platelet surface.

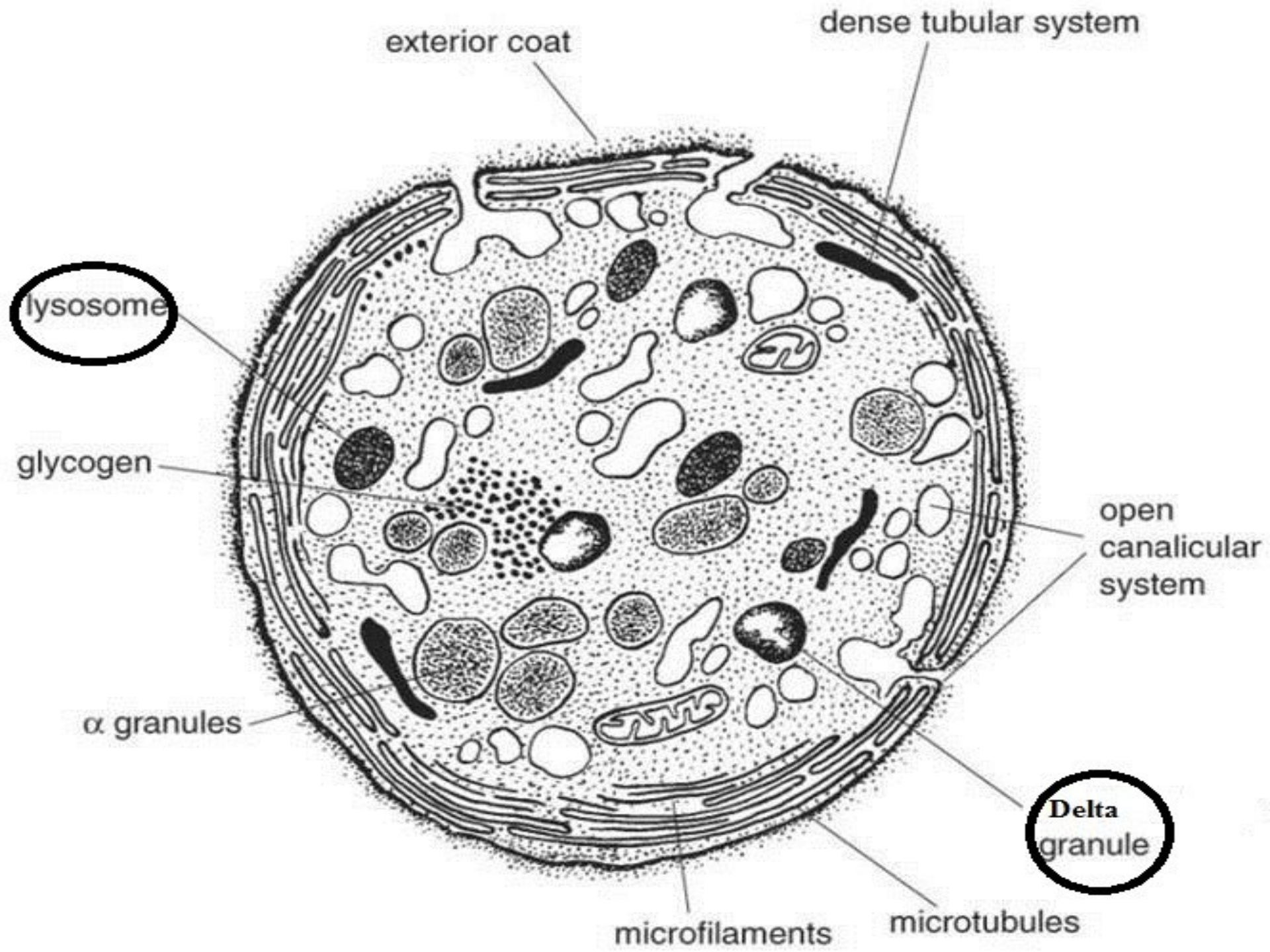
The dense tubular system: remnants of the endoplasmic reticulum of the platelets mother cell (the megakaryocyte).



The granulomere contains:

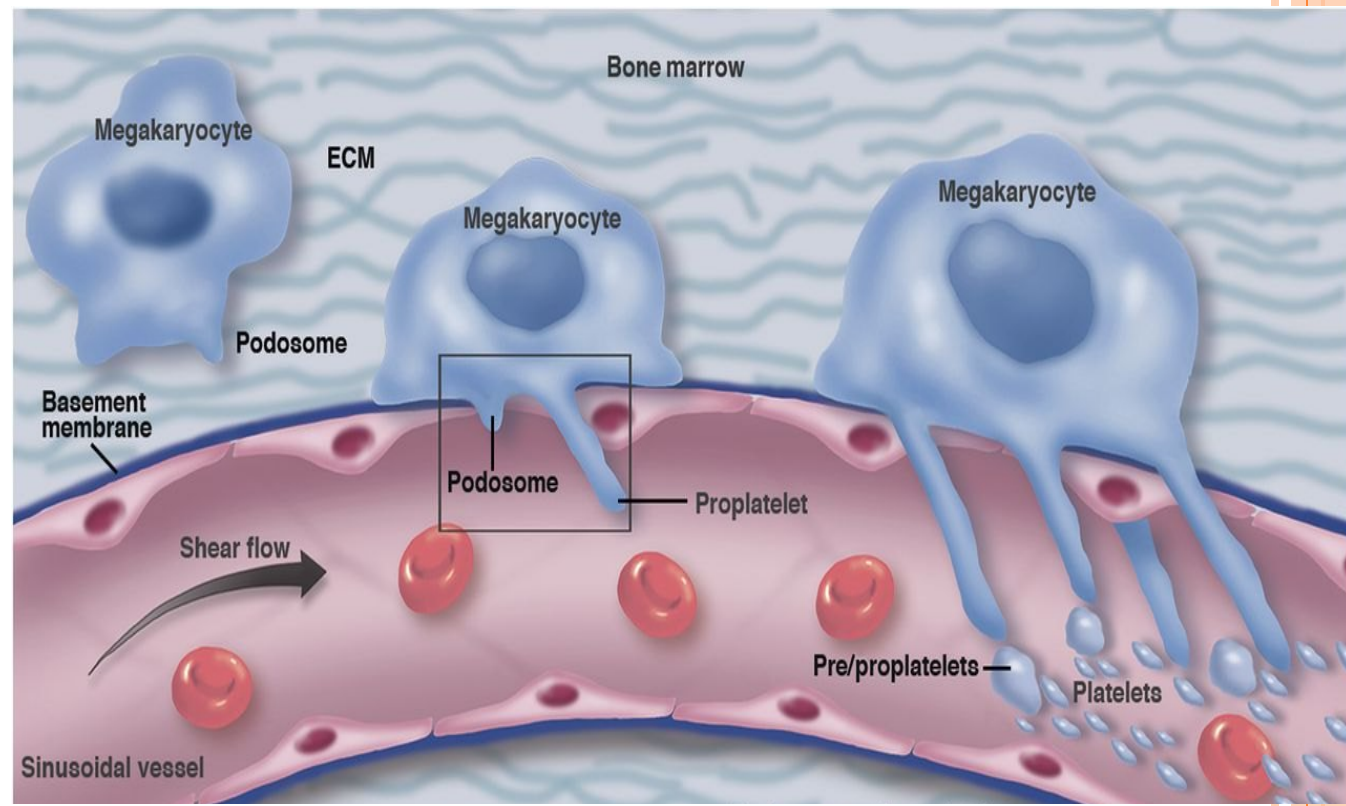
- 1- Few ribosomes, mitochondria and glycogen particles.
- 2- Three types of **membrane bounded granules** which are released by exocytosis during the process of thrombus formation. These are:
 - **Alpha granules** : abundant, large granules which contain fibrinogen and other coagulation factors.
 - **Delta granules**: less abundant, medium- sized granules which contain histamine, serotonin, ATP, ADP.
 - **Lambda granules (lysosomes)**: few small dense granules that contain lysosomal hydrolytic enzymes.





Thrombopoiesis

Definition: It is the formation of blood platelets. The release of mature platelets from the bone marrow into the bloodstream takes about 10 days



Thrombopoiesis

- 1- PHSCs.
- 2- MHSCs.
- 3- Colony forming unit-GEMM.
- 4- Colony forming unit- megakaryocyte (CFU- Meg).
- 5- Megakaryoblasts.
- 6- Megakaryocytes



Megakaryocyte

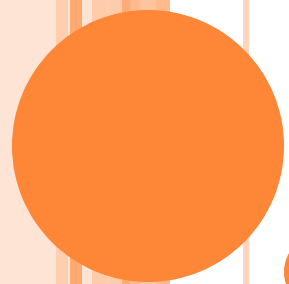
LM:

- **Shape:** Giant cells (50-150µm).
- **Nucleus:** large, lobulated containing the polyploid number of the ordinary DNA sets.
- **Cytoplasm:** basophilic contains many organelles together with the alpha, delta and lambda granules.

EM: the cytoplasm shows **membranous demarcation lines** surrounding groups of granules (lines of cleavage of the megakaryocytes into about 1200 platelets per megakaryocyte).

- Megakaryocytes are located near the bone marrow sinusoids, they extend their processes to release the platelets in bloodstream.





Thank You