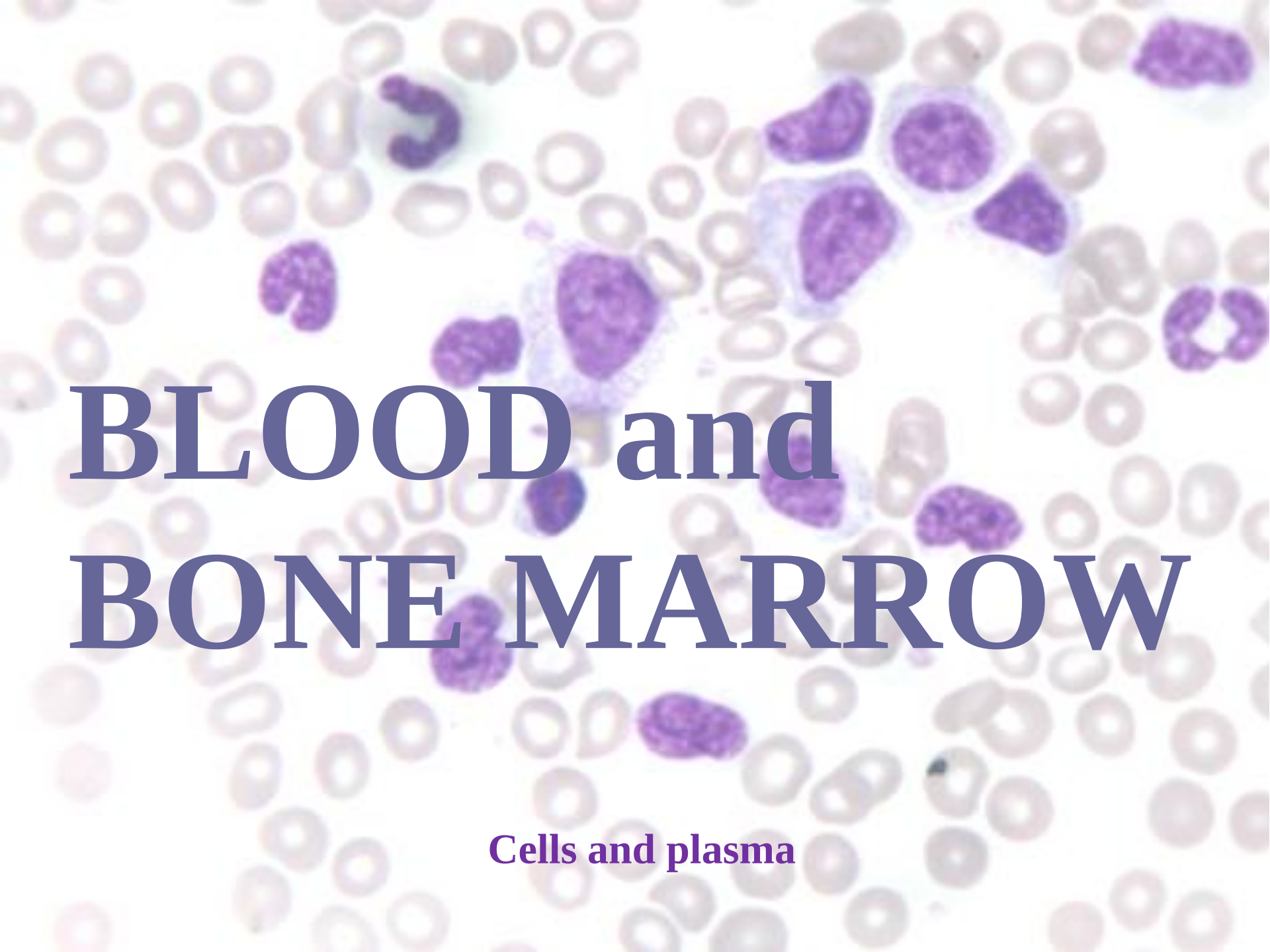


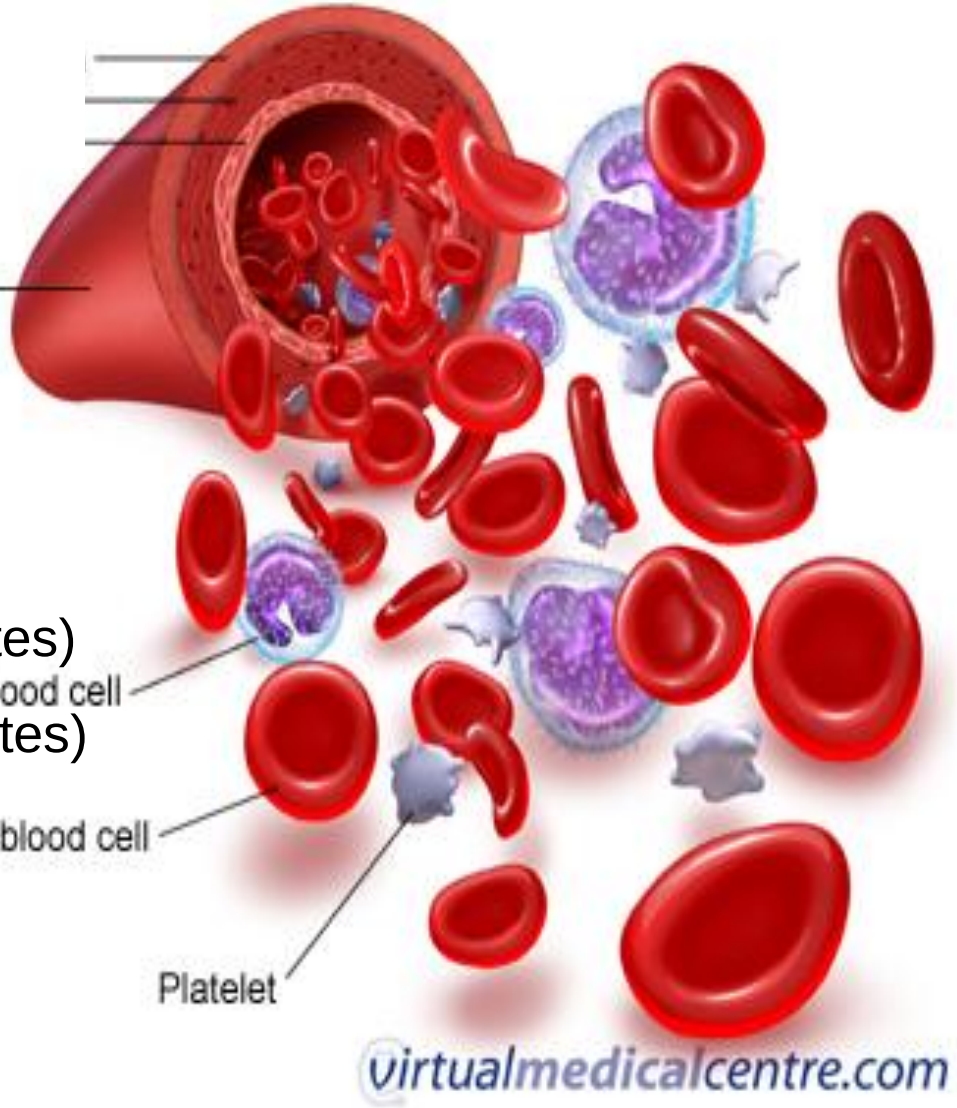
BLOOD and BONE MARROW

Cells and plasma

BLOOD

- is a specialized connective tissue (total volume of blood is about 5L)
- is composed of:
  - **formed elements**
  - fluid element

(**extracellular matrix – plasma**)



Formed elements of blood:

- Red blood cells (RBCs, erythrocytes)
- White blood cells (WBCs, leukocytes)
- Platelets

Function

- **Transports of materials:**

Dissolved gases namely oxygen, and carbon dioxide, between the lungs and rest of the body

Waste products of metabolism (e.g. water, urea)

Nutrients from the digestive tract and storage sites of the body (glucose, amino acids, vitamins, minerals, fatty acids, glycerol)

Hormones from the glands in which they are produced to their target cells

- **Protection**

Blood has several roles in inflammation:

- White blood cells, destroy invading microorganisms and cancer cells
- Antibodies and other proteins destroy pathogenic substances
- Platelet factors initiate blood clotting and help minimise blood loss

- **Regulation**

Blood helps regulate:

- pH by interacting with acids and bases

The pH of blood must remain in the range 6.8 to 7.4, otherwise it begins to damage cells

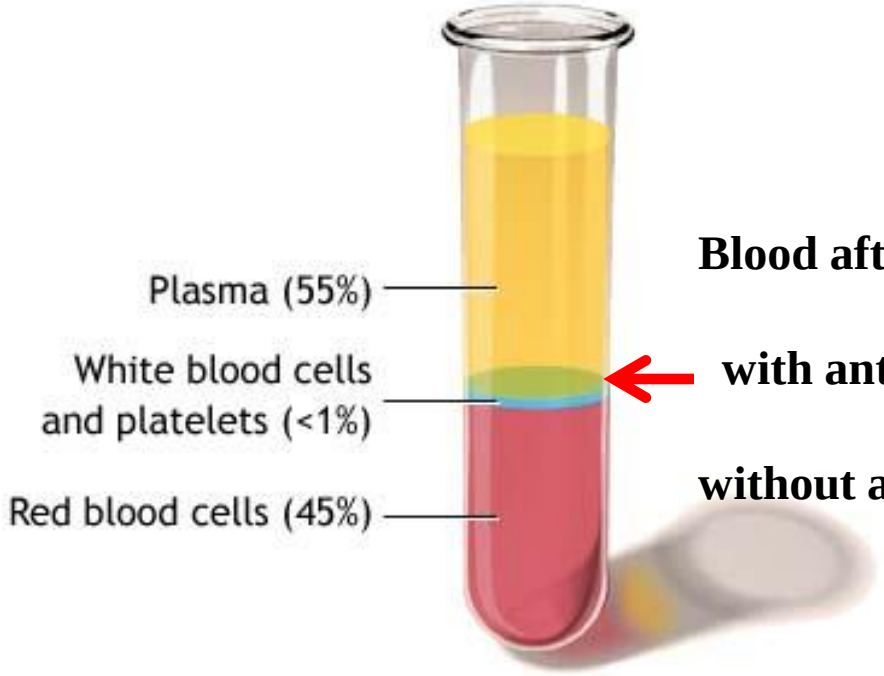
- Water balance by transferring water to and from tissues

Maintains Body Temperature

Blood plasma is the pale-yellow liquid component of blood in which formed elements are suspended.

- makes up about 55% of total blood volume
- is mostly water (92% by volume), and contains dissolved proteins (i.e.—albumins, globulins, and fibrinogen), glucose, clotting factors, electrolytes (Na^+, Ca^{2+}, Mg^{2+}, HCO_3^- Cl^- etc.), hormones and carbon dioxide

Blood serum is blood plasma without fibrinogen and clotting factors



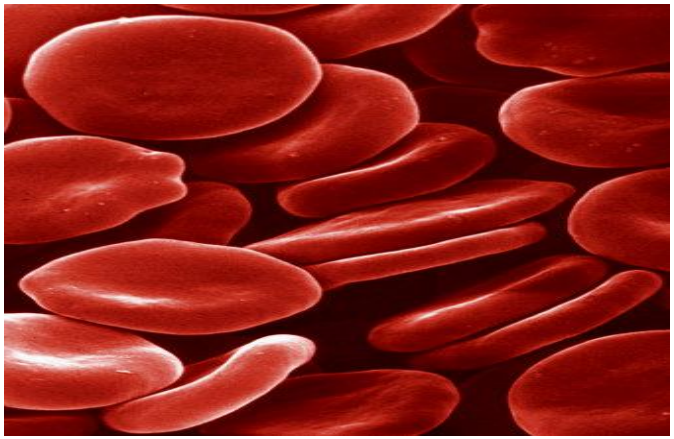
Blood after centrifugation

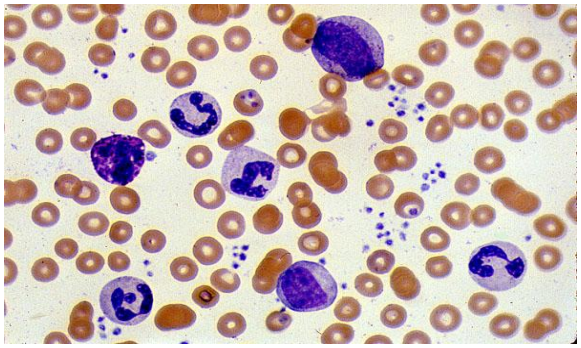
with anticoagulant

without anticoagulant

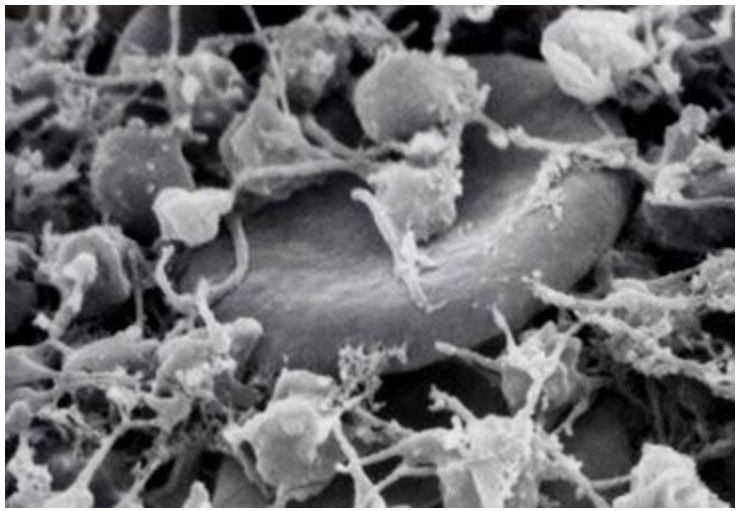
One microliter (µl, mm^3) of blood contains:

Red blood cells (RBCs) - 4.7 to 6.1 millions (male), 4.2 to 5.4 millions (female)





White blood cells (WBCs) - 4,000–11,000



Platelets - 200,000–450,000

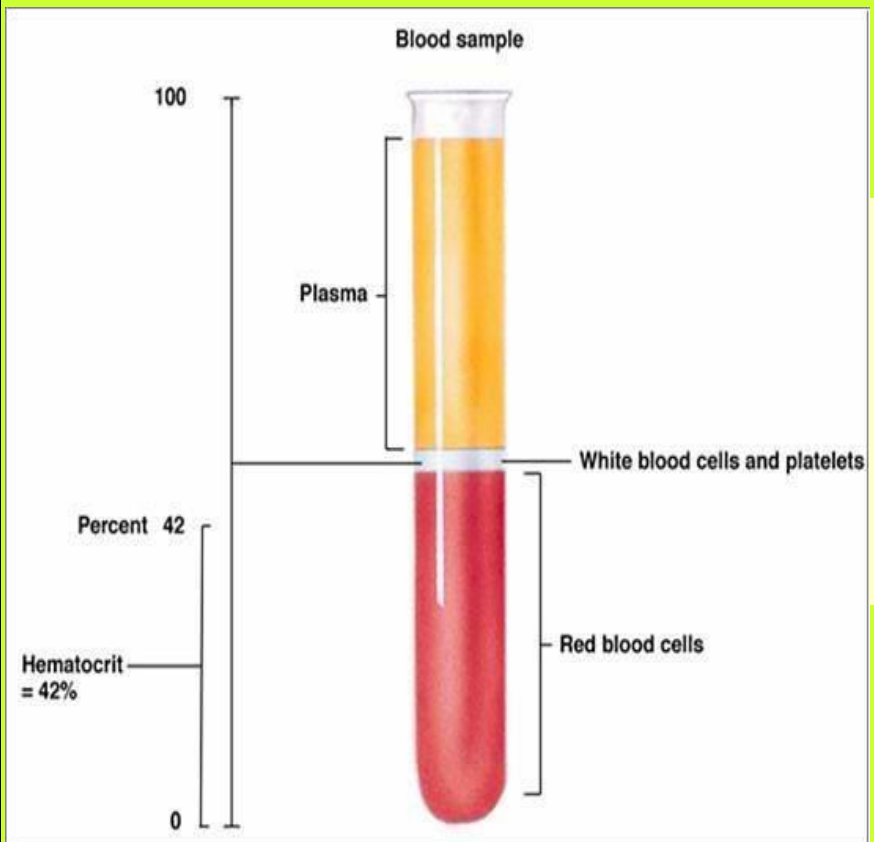
Complete blood count

Hemoglobin 13.5-18.0 g/dl (male); 12.0-16.0 (female)

Hematocrit 45 ± 7 (38–52%) for males

42 ± 5 (37–47%) for females

dl – deciliter = 10^{-1} L



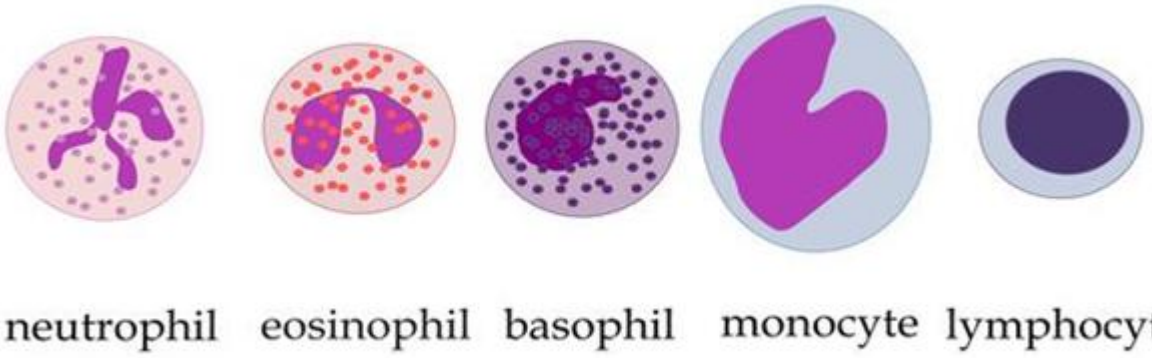
Hematocrit –
The relative volume of cells (erythrocytes) and plasma measured after centrifugation of blood sample with anticoagulant added

White blood cells (leukocytes)

Granulocytes
- Neutrophils
- Eosinophils
- Basophils

Agranulocytes
- Lymphocytes
- Monocytes

Both granulocytes and agranulocytes possess nonspecific (azurophilic) granules



The previous and the next slide must be memorized !

You must remember it even if you

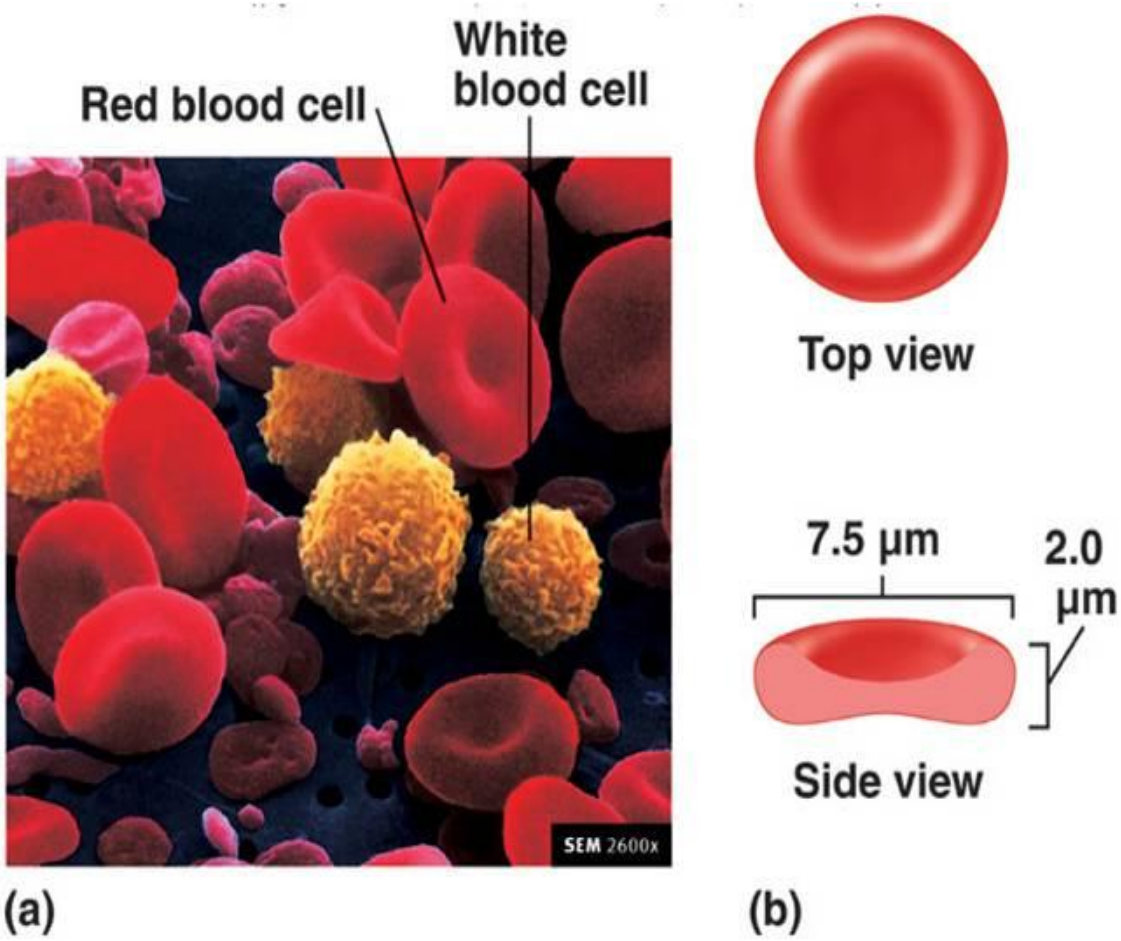
are awaked from deep sleep!

The percentage of granulocytes and agranulocytes in white blood cells

Cell type	Range	Percent
Total WBC	4500-11000/mm^3	
Neutrophils	4100-6500	**45-70**
Eosinophils	0-700	**1-4**
Basophils	0-150	**0,5-1**
Lymphocytes	1500-4000	**20-40**
Monocytes	200-950	**2-8**

Red blood cells (erythrocytes)

- the smallest and most numerous cells of blood
- have no nuclei
- are responsible for the transport of oxygen and carbon dioxide to and from the tissues
- have average life span of 120 days

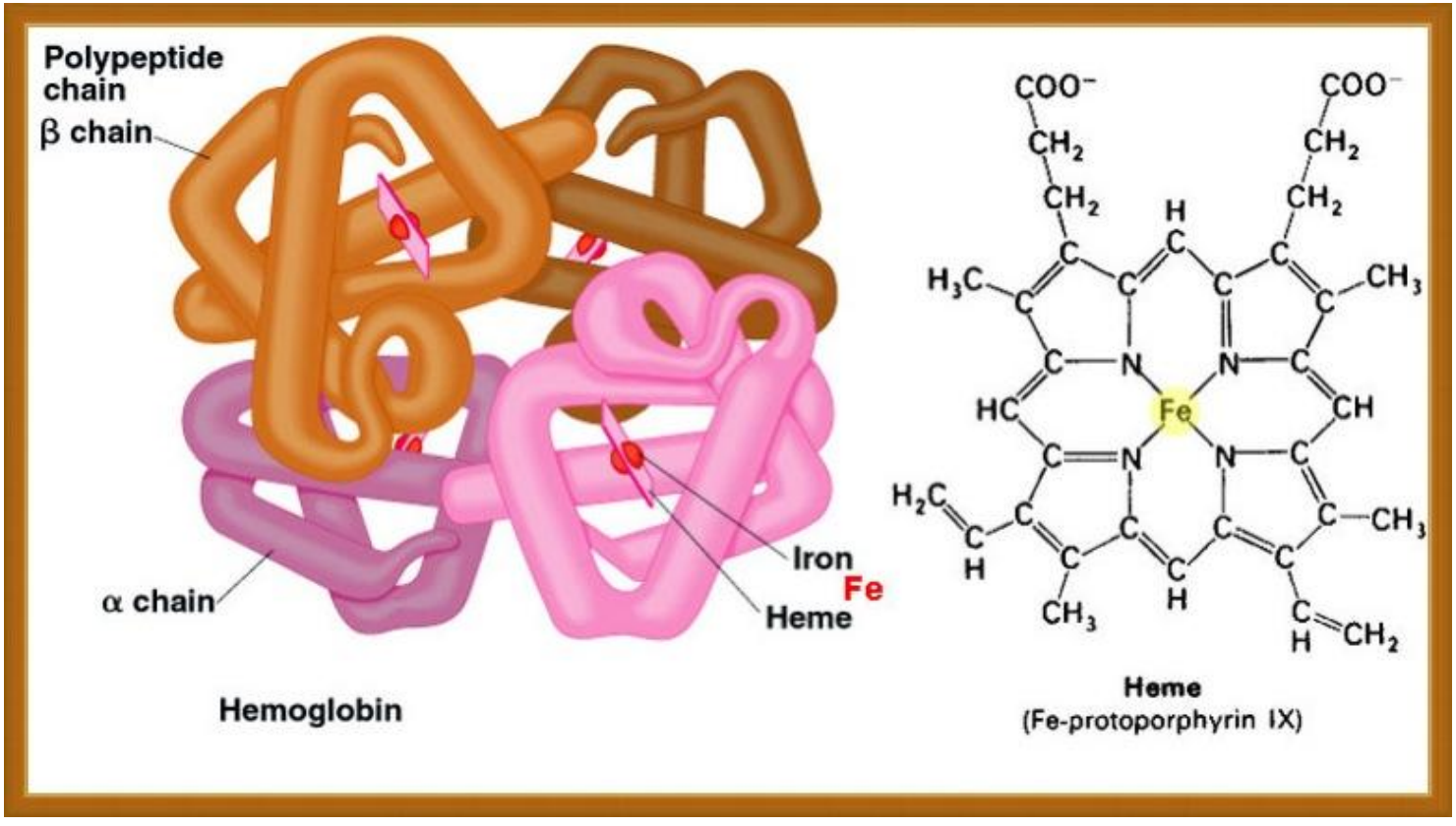


- Males: 5x 10^6 per mm^3
- Females: 4,5x 10^6 per mm^3

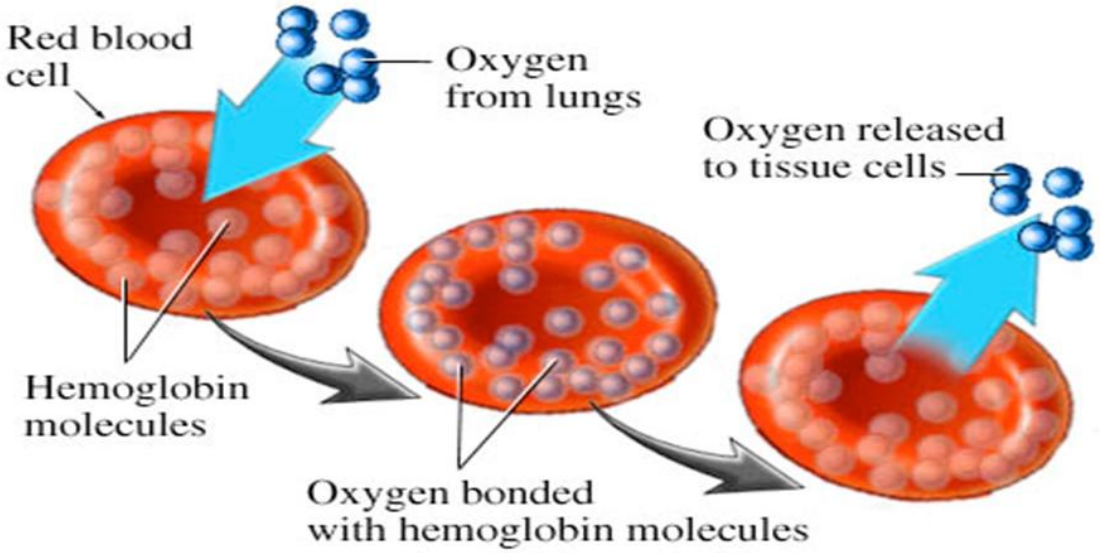
Biconcave-shaped disk - this shape provides the cell with a large surface area relative to its volume, thus enhancing its capability for gaseous exchange

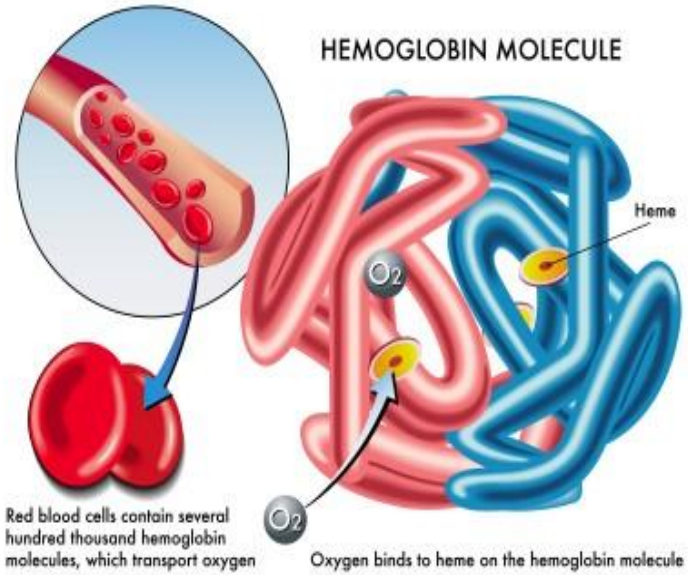
Hemoglobin (HB)

- molecule is an assembly of four globular protein subunits
- each subunit is composed of a protein chain tightly associated with a non-protein heme group
- a heme group consists of an iron (Fe) ion



Hemoglobin in the blood carries oxygen from the respiratory organs (lungs) to the rest of the body (i.e. the tissues) where it releases the oxygen to burn nutrients to provide energy to power the functions of the organism

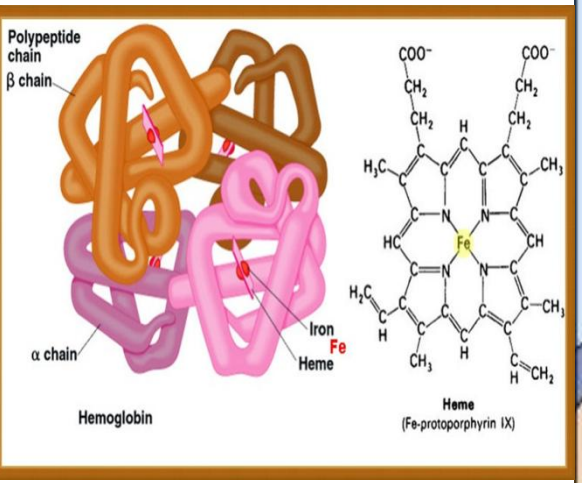


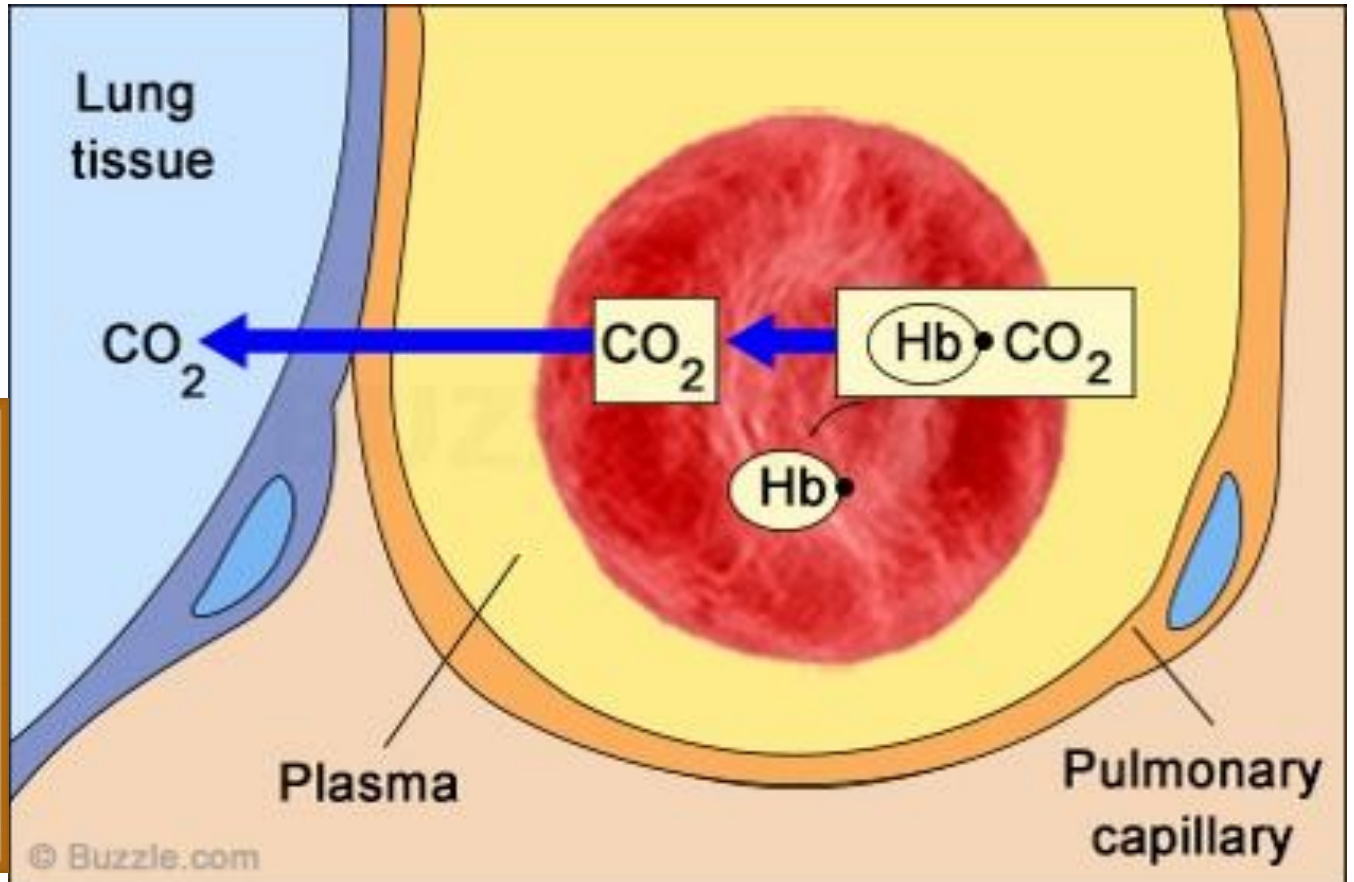


Oxygen binds to the heme in the hemoglobin molecule - oxyhemoglobin

Hemoglobin is involved in the transport of other gases: it carries some of the body's respiratory carbon dioxide (about 10% of the total, 85% carried in blood as bicarbonate 5% carried as free CO_2, in solution) as **carbaminohemoglobin,** in which CO_2 is bound to the globin protein.

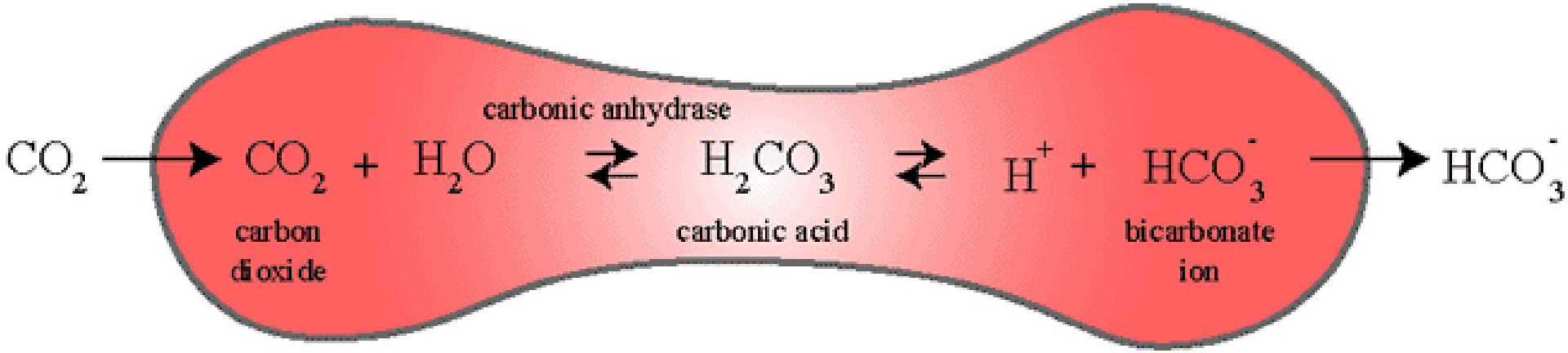
CO_2 is bound to the globin protein - carbaminohemoglobin





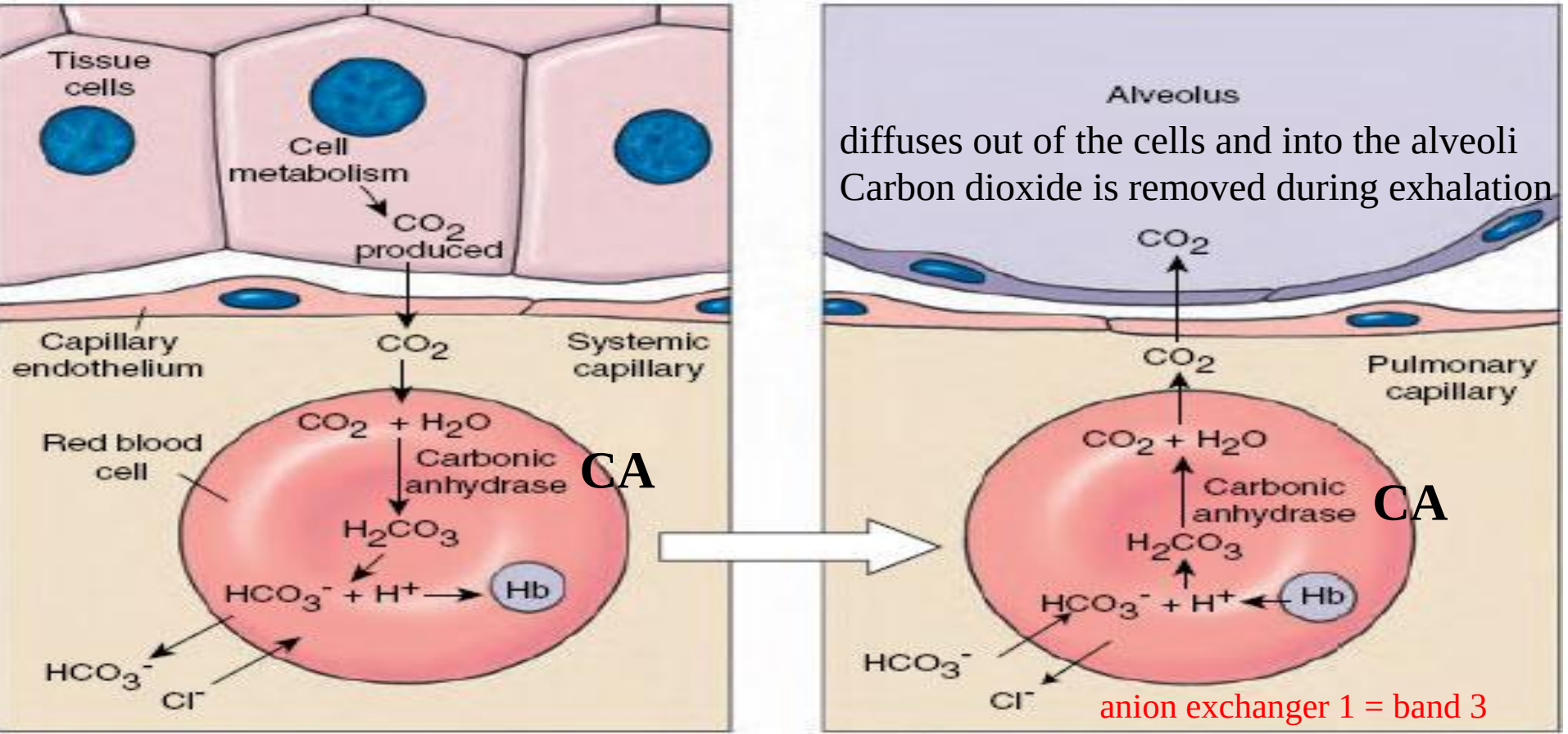
Carbonic anhydrase – facilitates the formation of carbonic acid from CO_2 and water (and the reverse reaction). This acid dissociates to form bicarbonate and hydrogen.

- maintaining acid-base balance in blood and other tissues
- transport carbon dioxide out of tissues.

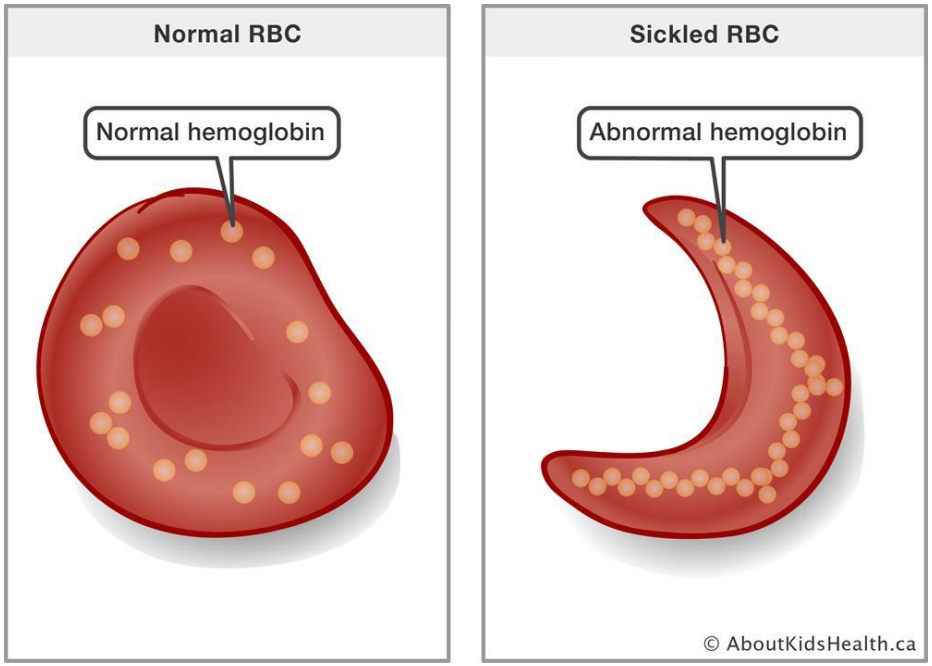


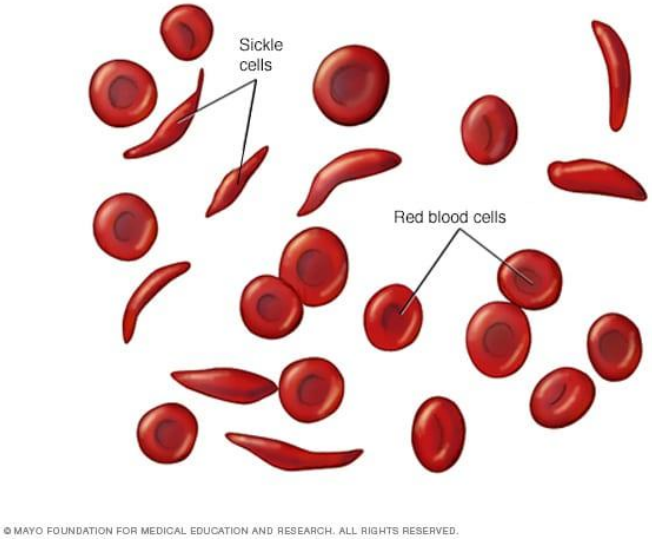
Carbon dioxide is transported mainly in the form of bicarbonate (HCO3–)

- carbon dioxide enters red blood cells in the tissue capillaries
- carbon dioxide and water form carbonic acid (CA)
- carbonic acid dissociates to bicarbonate and hydrogen ions
- bicarbonate ions diffuse out of the cell into the plasma
- in lungs bicarbonate ions enter the red blood cells and form carbonic acid with hydrogen ions
- carbonic acid is decomposed into carbon dioxide and water



sickle cell anemia

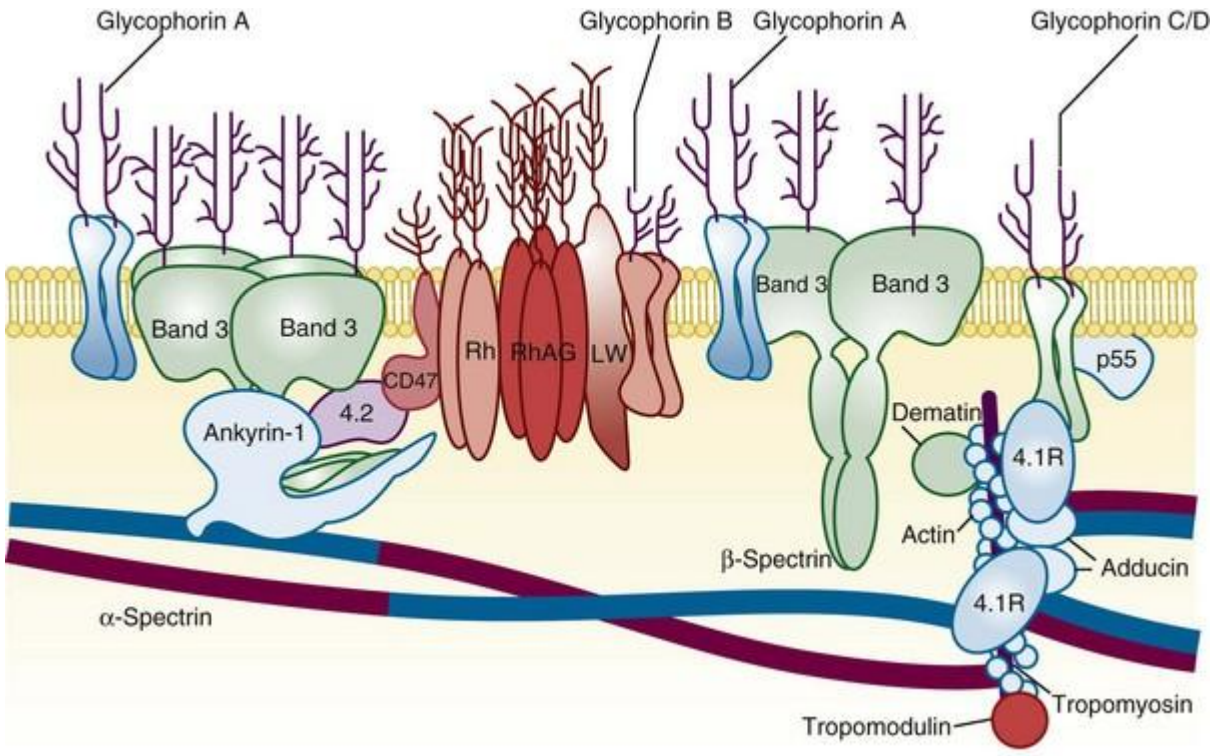




Healthy red blood cells are flexible so that they can move through the smallest blood vessels. In sickle cell disease, the hemoglobin is abnormal, causing the red blood cells to be rigid and shaped like a "C" or sickle, the shape from which the disease takes its name. Sickle cells can get stuck and block blood flow, causing pain and infections. Complications of sickle cell disease occur because the sickled cells block blood flow to specific organs. The worst complications include stroke, acute chest syndrome (a condition that lowers the level of oxygen in the blood), organ damage, other disabilities, and in some cases premature death.

ERYTROCYTE CELL MEMBRANE

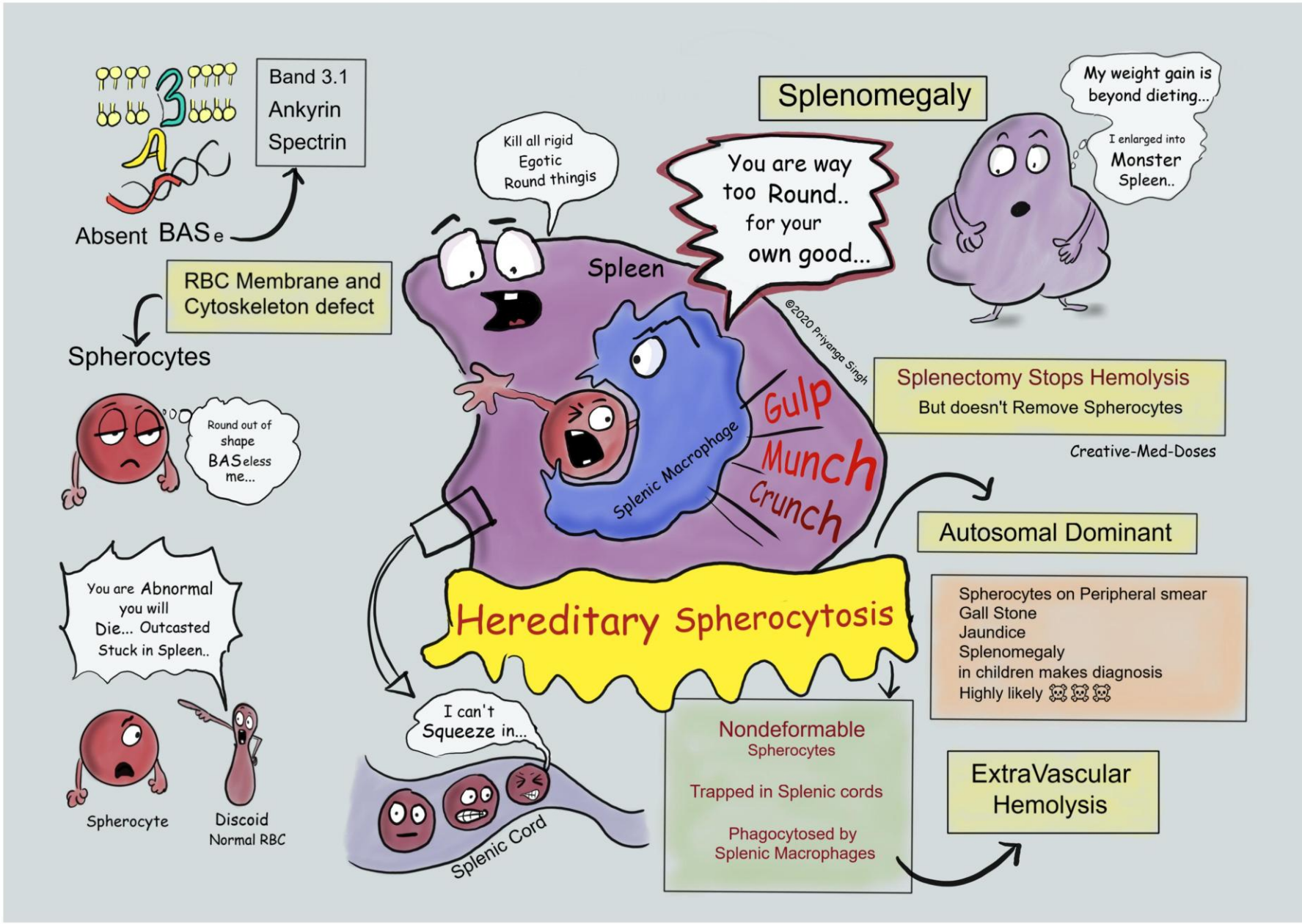
anion transporter (bicarbonate ion crosses cell membrane through Band 3)



A peripheral proteins: **spectrin** and **ankyrin** are anchoraged to the cell membrane by **band 3 protein**, and **glycophorin A**.

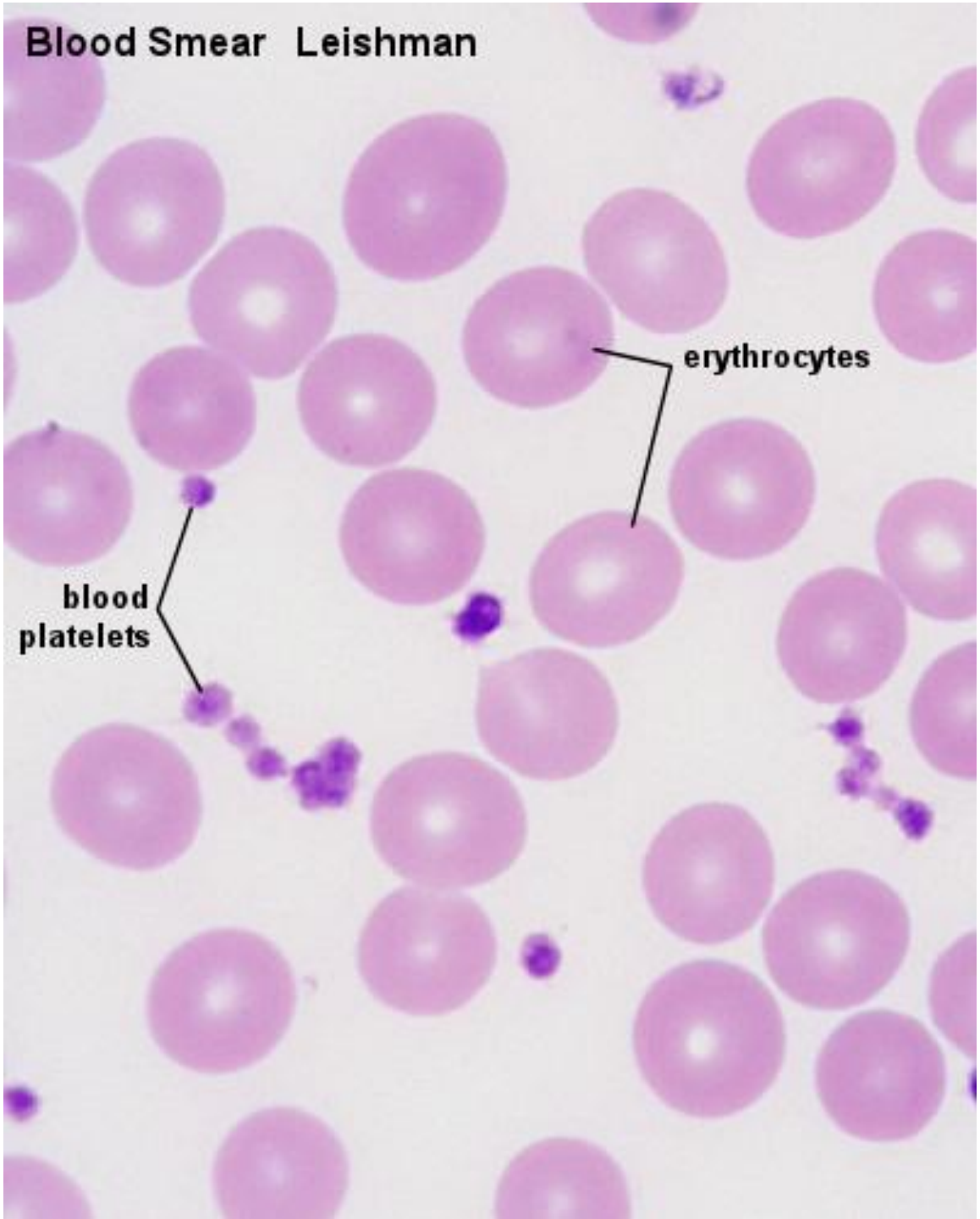
Subplasmalemmal cytoskeleton helps to maintain the biconcave disk shape

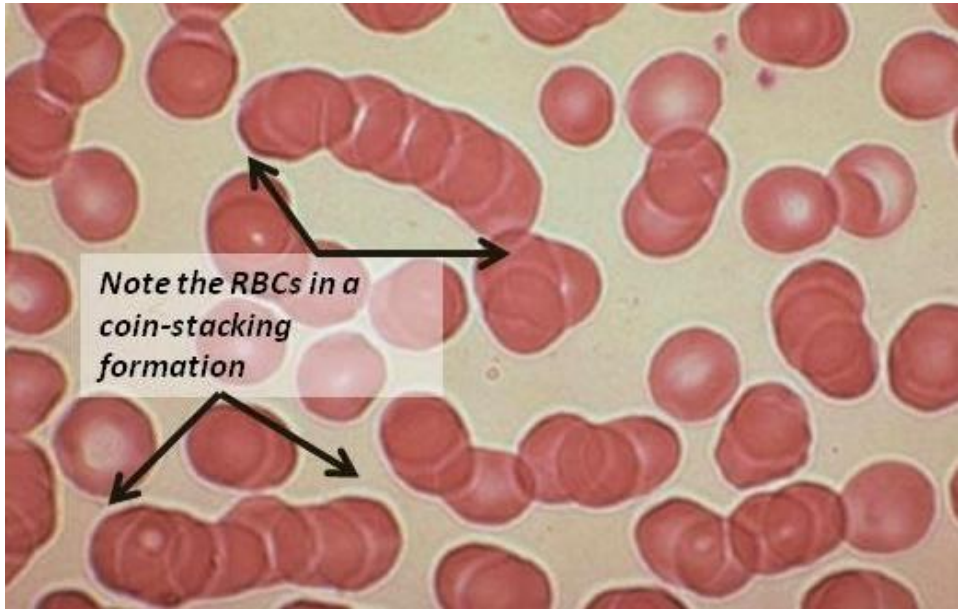
Spectrin forms the meshwork that provides red blood cells their shape

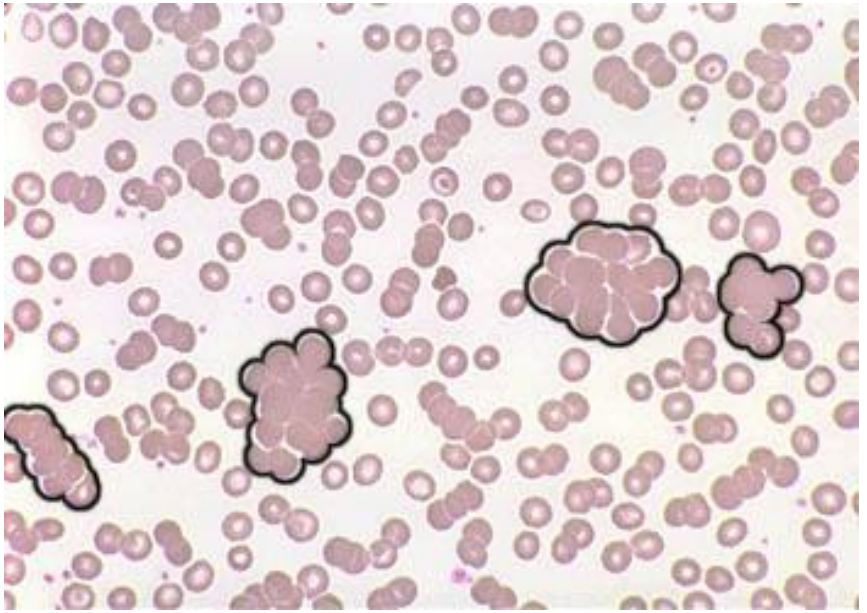

Band 3.1
Ankyrin
Spectrin
Absent BASe
RBC Membrane and Cytoskeleton defect
Spherocytes
Round out of shape BASeless me...
You are Abnormal you will Die... Outcasted Stuck in Spleen..
Spherocyte
Discoid Normal RBC
Splenomegaly
My weight gain is beyond dieting...
I enlarged into Monster Spleen..
Kill all rigid Egotic Round thingis
You are way too Round.. for your own good...
Spleen
Splenic Macrophage
Gulp
Munch
Crunch
©2020 Priyanga Singh
Splenectomy Stops Hemolysis
But doesn't Remove Spherocytes
Creative-Med-Doses
Autosomal Dominant
Hereditary Spherocytosis
Spherocytes on Peripheral smear
Gall Stone
Jaundice
Splenomegaly
in children makes diagnosis
Highly likely
I can't Squeeze in...
Splenic Cord
Nondeformable
Spherocytes
Trapped in Splenic cords
Phagocytosed by Splenic Macrophages
ExtraVascular Hemolysis

Erythrocytes in the blood smear

their centers look lighter than their periphery (due to the bicocave shapes)



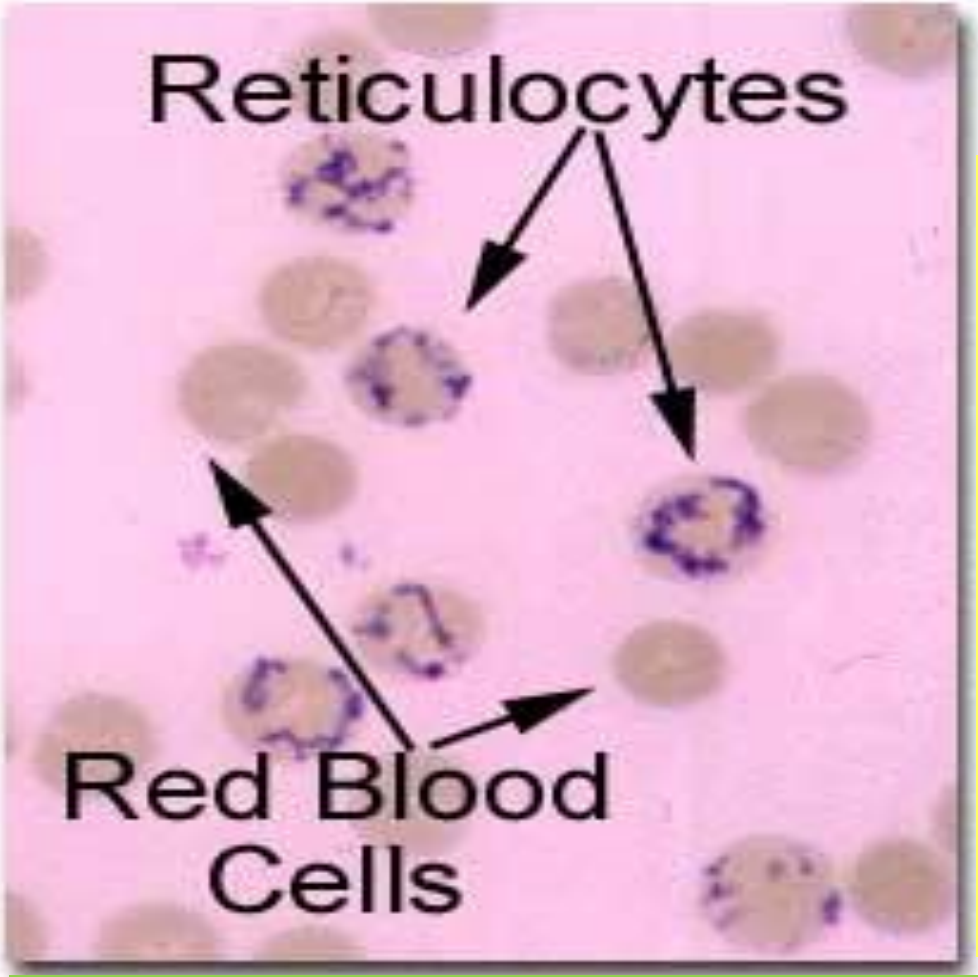




Rouleaux are clumps of red blood cells that look like stacked plates. They usually form as a result of abnormal quantities of certain proteins (immunoglobulin, fibrinogen) in the blood. Rouleaux are a non-specific indication of the presence of a pathology.

Red cell autoagglutination is the process whereby red cells clump together forming aggregates. This is seen in Waldenström's macroglobulinemia, cold agglutinin disease and Infection with Mycoplasma Pneumonia or Infectious mononucleosis.

Reticulocytes



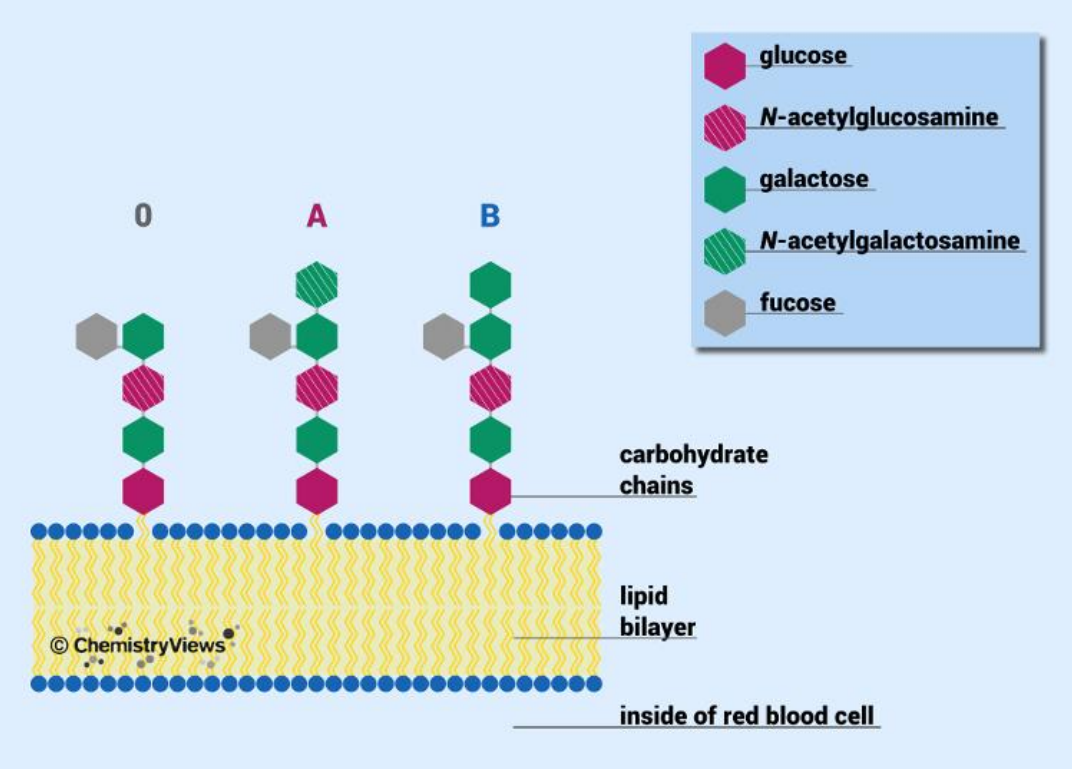
Reticulocytes –

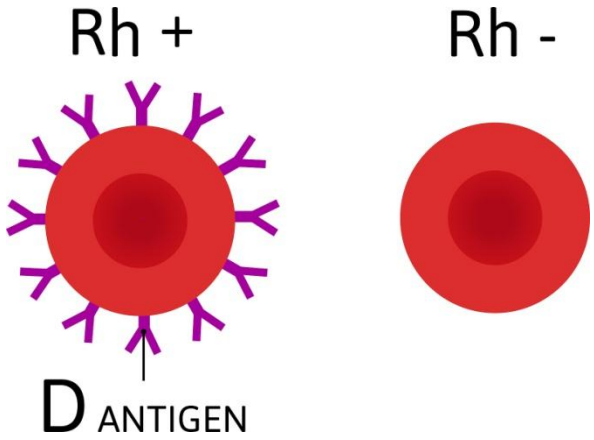
immature red blood cells

- develop and mature in the red bone Marrow and then circulate for about a day in the blood stream before developing into mature red blood cells.
- like mature red blood cells, reticulocytes do not have a cell nucleus. They are called reticulocytes because of a remnants of RER, ribosomes and mitochondria.

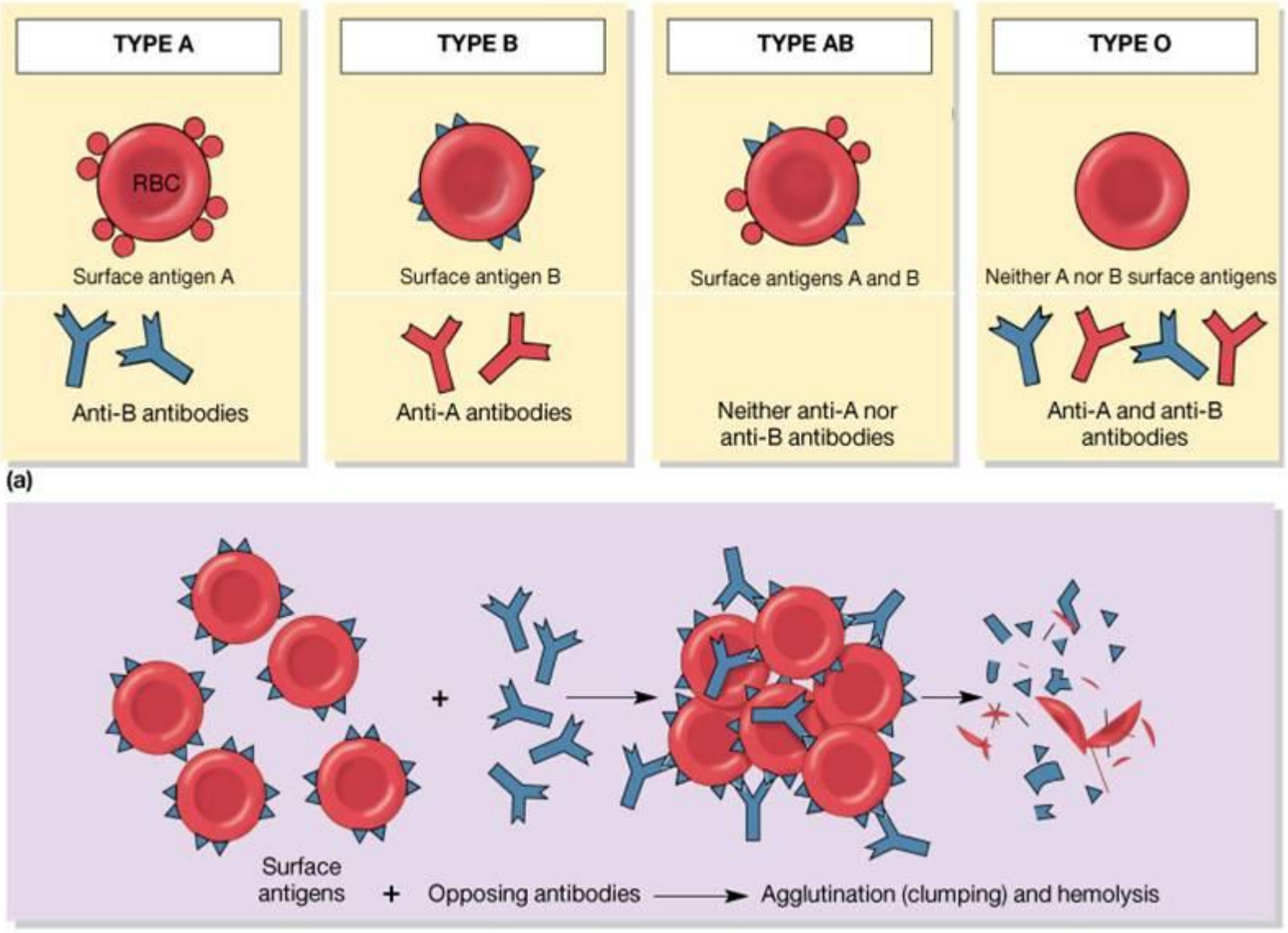
Reticulocytes constitute 1-2% of red blood cells.Their number in peripheral blood increases after bleeding or at high altitude.

Specific inherited carbohydrate chains act as antigens and determine the **blood group** of an individual.
- Antigens **A** and **B** determine four primary blood groups **A, B, AB** and **0**



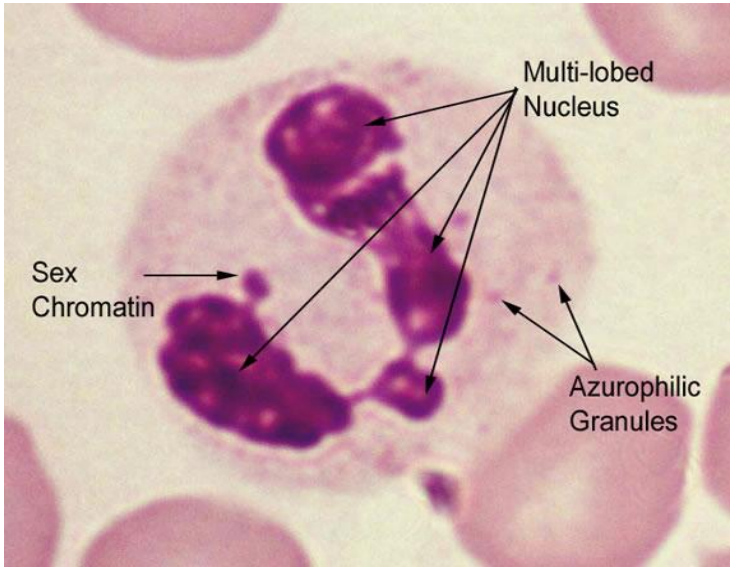


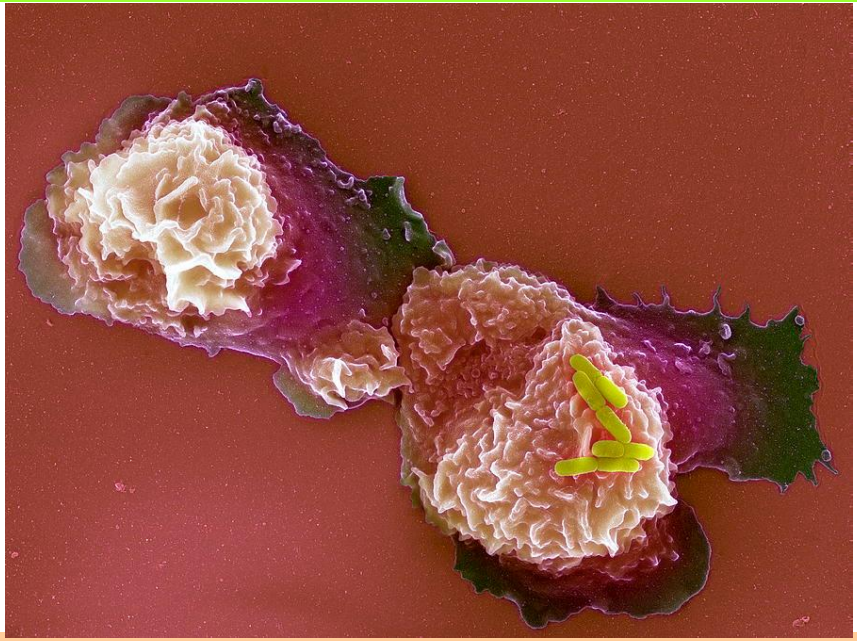
Rh antigens (first identified in rhesus monkeys) determine the Rh-positive (Rh^+) group. Individuals lacking these antigens are Rh-negative (Rh^-)


TYPE A
RBC
Surface antigen A
Anti-B antibodies
TYPE B
Surface antigen B
Anti-A antibodies
TYPE AB
Surface antigens A and B
Neither anti-A nor anti-B antibodies
TYPE O
Neither A nor B surface antigens
Anti-A and anti-B antibodies
(a)
Surface antigens
+
Opposing antibodies
Agglutination (clumping) and hemolysis
(b)

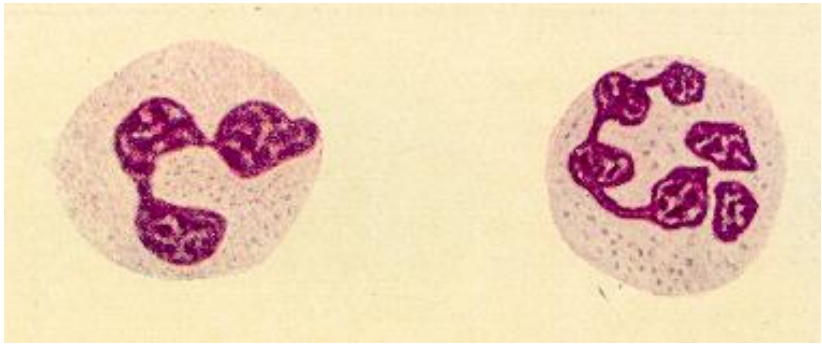
Neutrophils (polymorphonuclear leukocytes)

- the most numerous of the white blood cells
- have a multilobed nuclei (lobes are connected to each other by slender chromatin threads)
- in females the nucleus presents a small appendage, the „drumstick” which contains inactive X chromosome



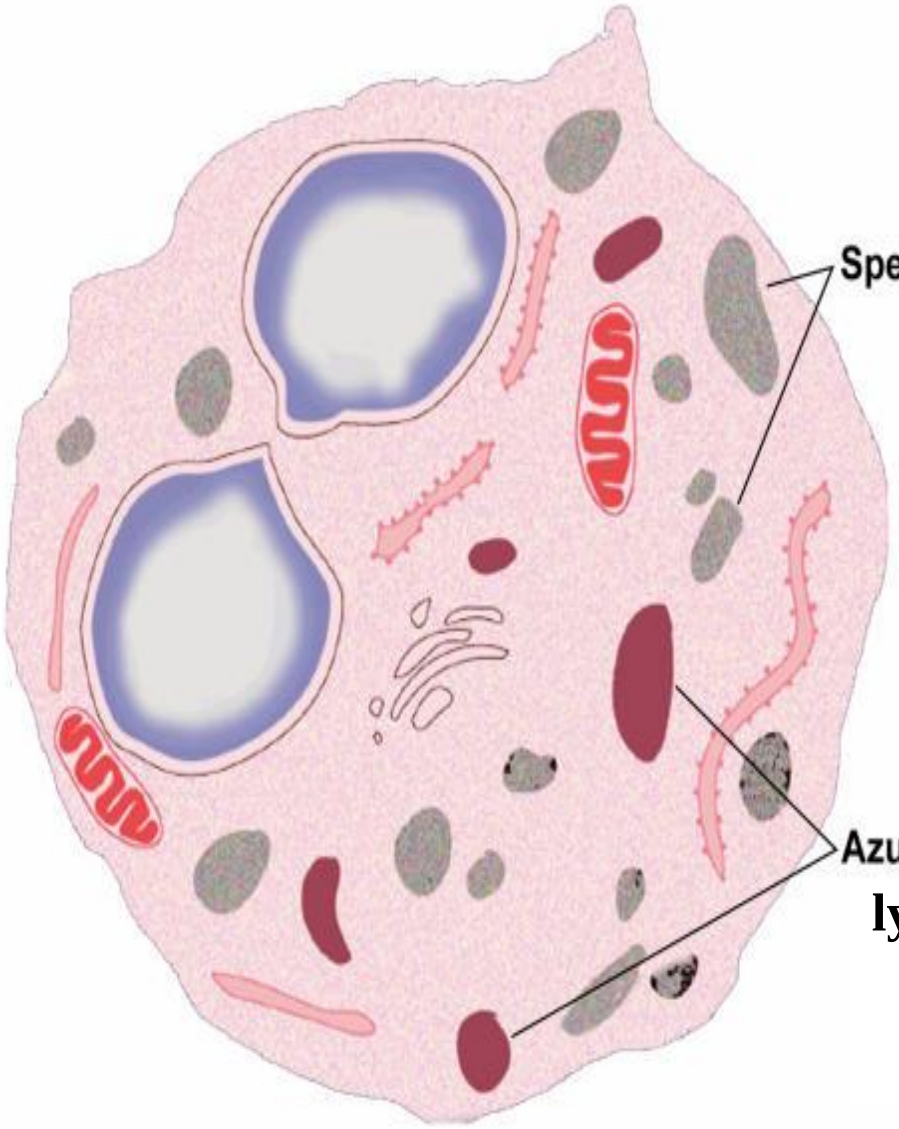


Phagocytose and destroy invading bacteria



45-70% of leukocytes

Neutrophils (polymorphonuclear leukocytes)



contain enzymes: type IV collagenase alkaline phosphatase and pharmacological agents: lysozyme, lactoferrin

Tertiary granules
contain gelatinase and cathepsins

lysosomes, contain hydrolytic enzymes, myeloperoxidase, defensins

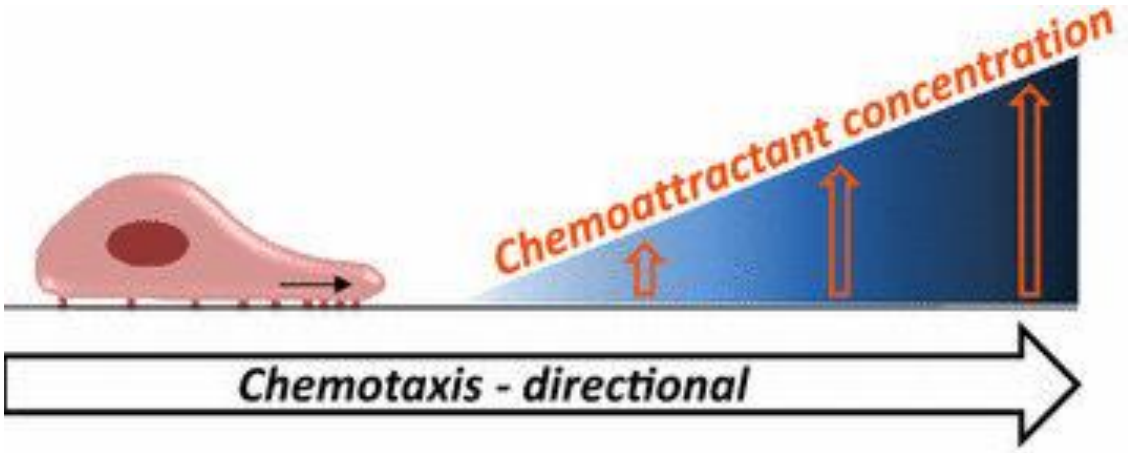
Components of granules are necessary for destroying of microorganisms

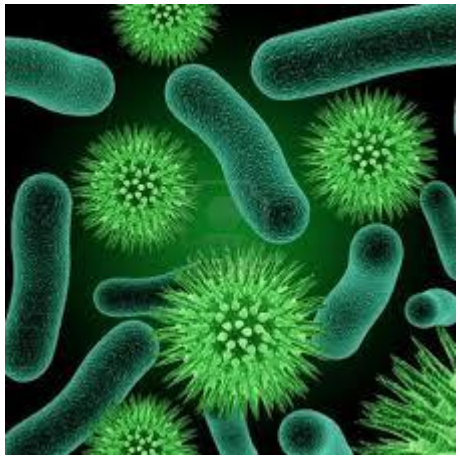
Function of neutrophils (microphages)

- are responsible for the phagocytosis and destroying of bacteria

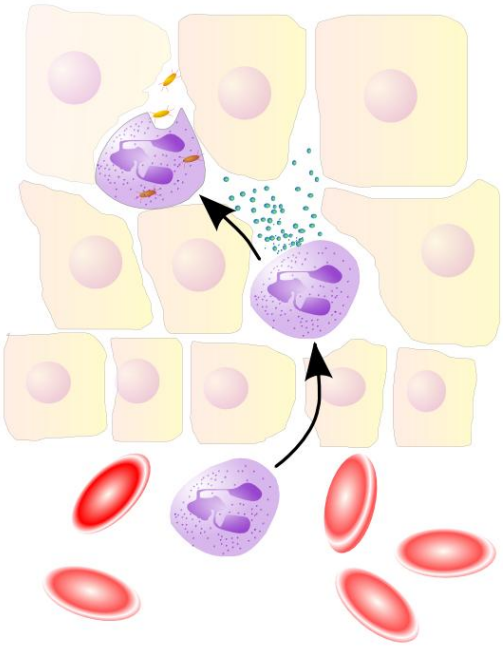
1. **Neutrophils undergo a process called chemotaxis, which allows them to migrate toward sites of infection or inflammation.**
Cell surface receptors allow neutrophils to detect chemical gradients of molecules which these cells use to direct the path of their migration

2. **Chemotactic agents facilitate the release of the contents of tertiary granules into the extracellular matrix (gelatinase degrades the basal lamina, facilitating neutrophil migration)**





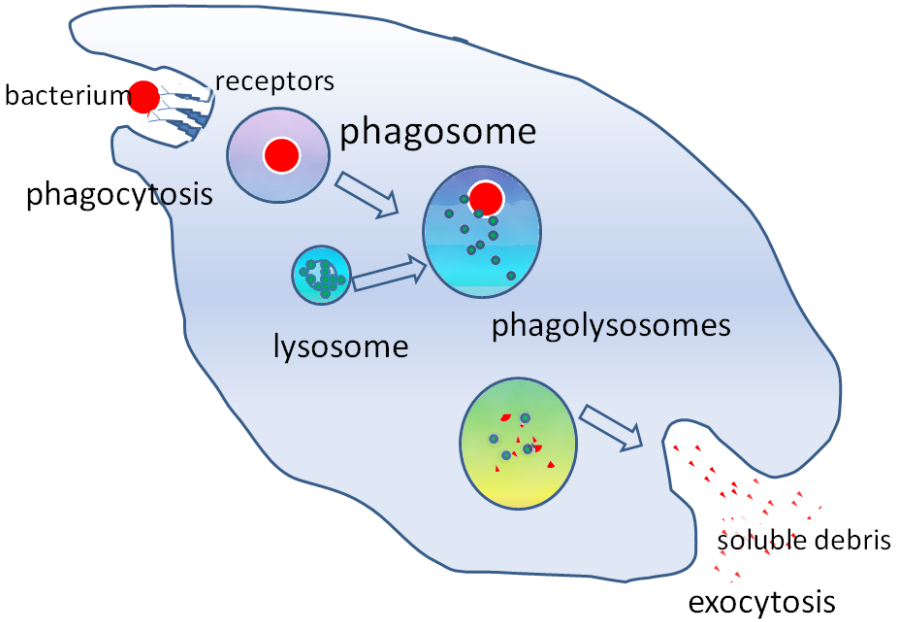
Phagocytosis



3. **The content of specific granules is released into the extracellular matrix (they attack the microorganisms and aid neutrophil migration)**

4. **Microorganisms, phagocytosed by neutrophils, become enclosed in phagosomes (which fused with lysosomes)**

5. **Enzymes and pharmacological agents of the azurophilic granules (lysosomes) destroy the ingested microorganisms**

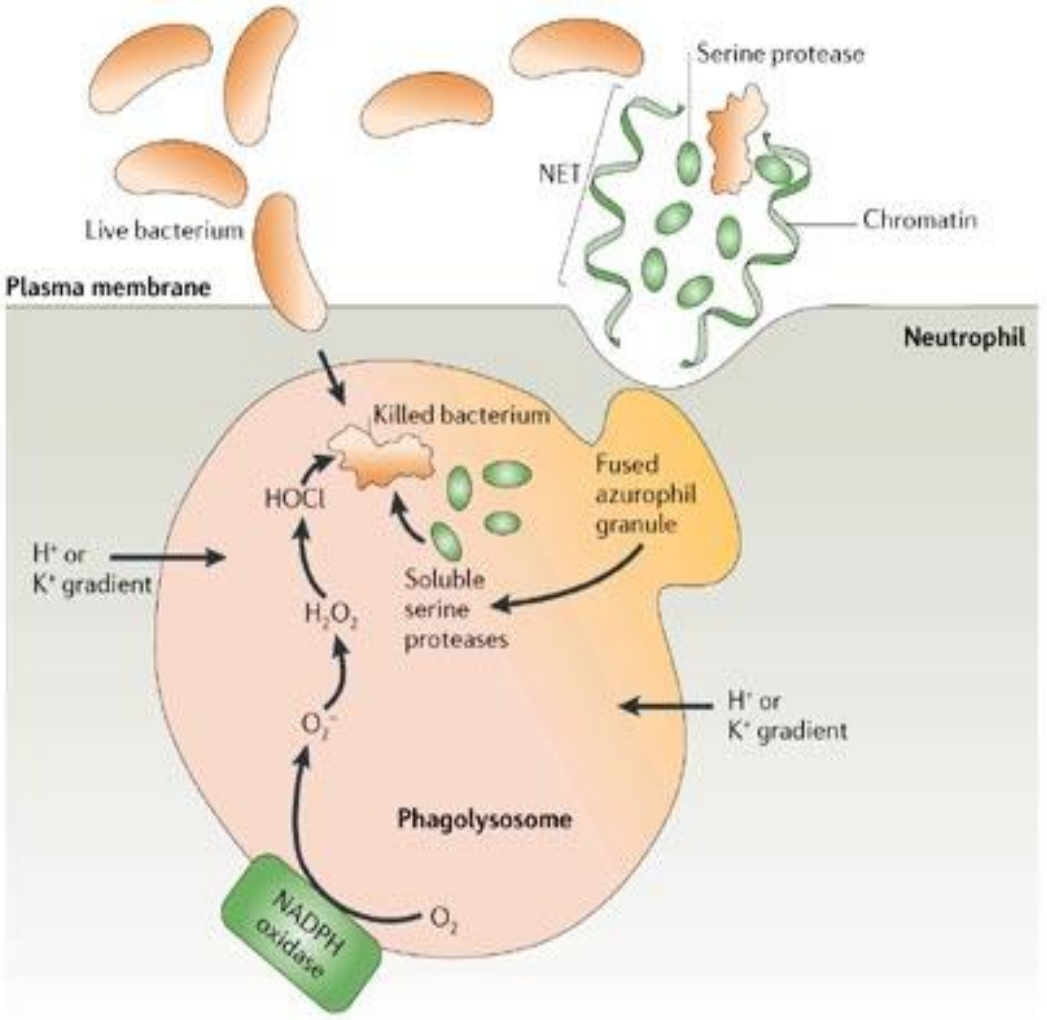


In neutrophils microorganisms are killed also by the formation of reactive oxygen compounds within the phagosomes:

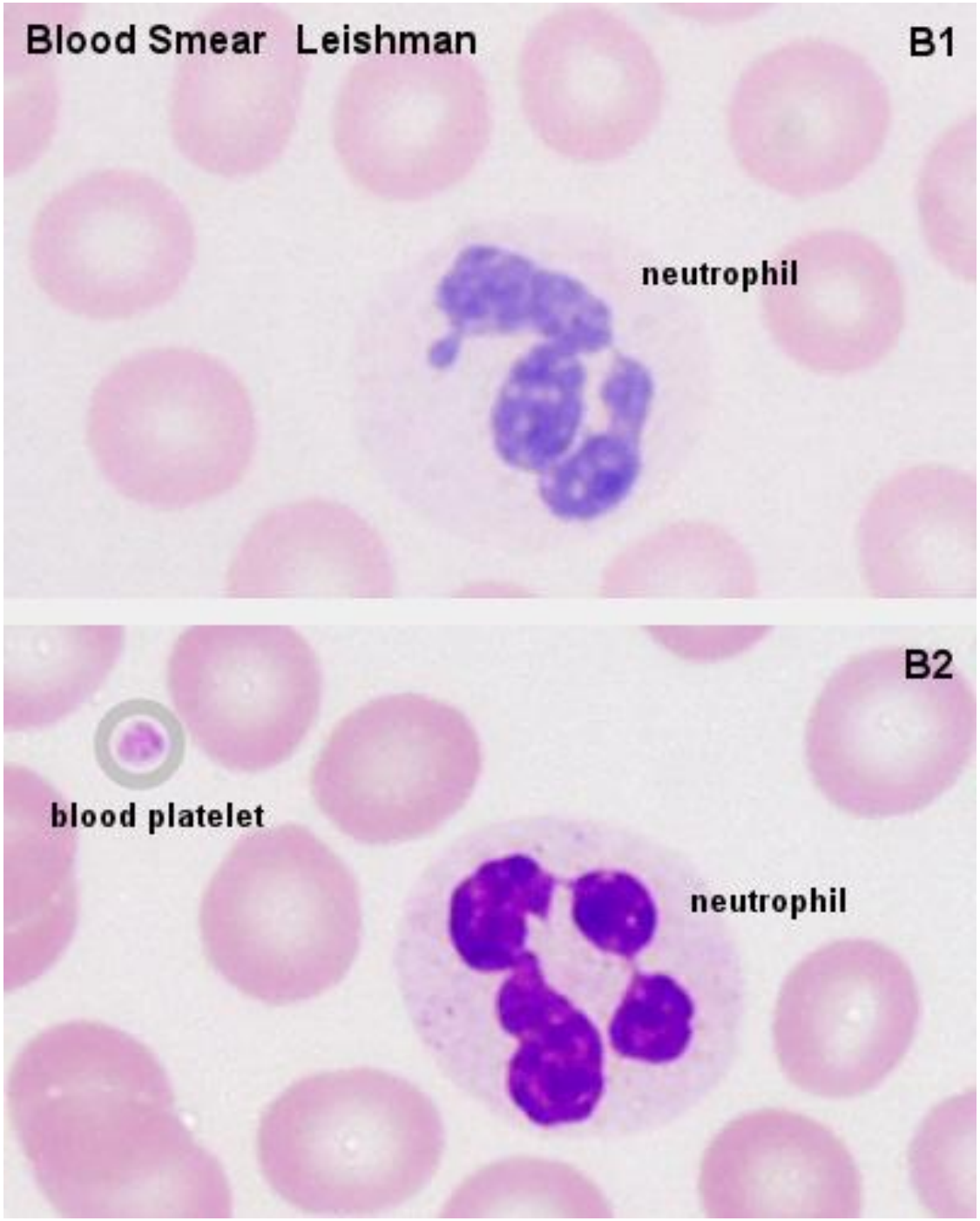
Superoxide (O_2^-)

Hydrogen peroxide (H_2O_2)

Hypochlorous acid (HOCl)



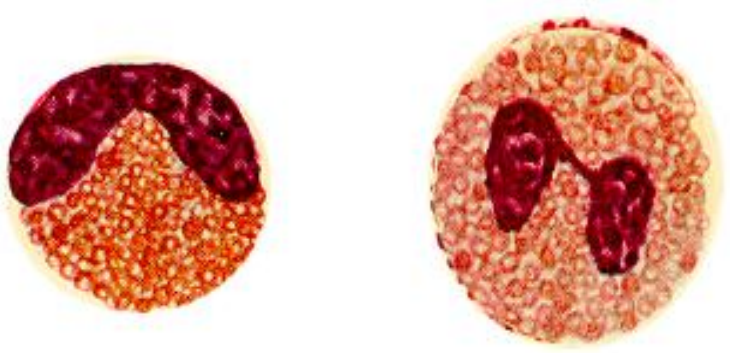
Nucleus of
The neutrophil is divided into 3-5 lobes which are connected by thin strands of chromatin



Eosinophils

- constitute less then 4% of the total white blood cell
- have bilobed nucleus (lobes are connected by thin chromatin strand)
- contain pink specific granules and nonspecific granules (lysosomes)

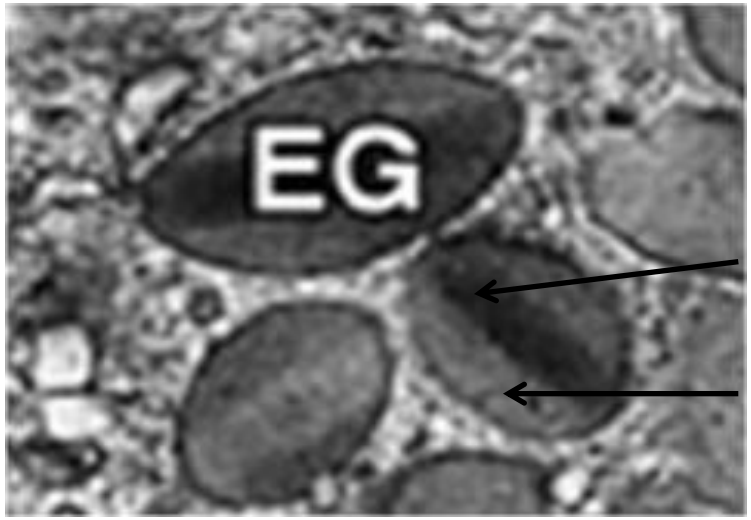
Destroy larger parasites and modulate allergic inflammatory responses



1-3% of leukocytes

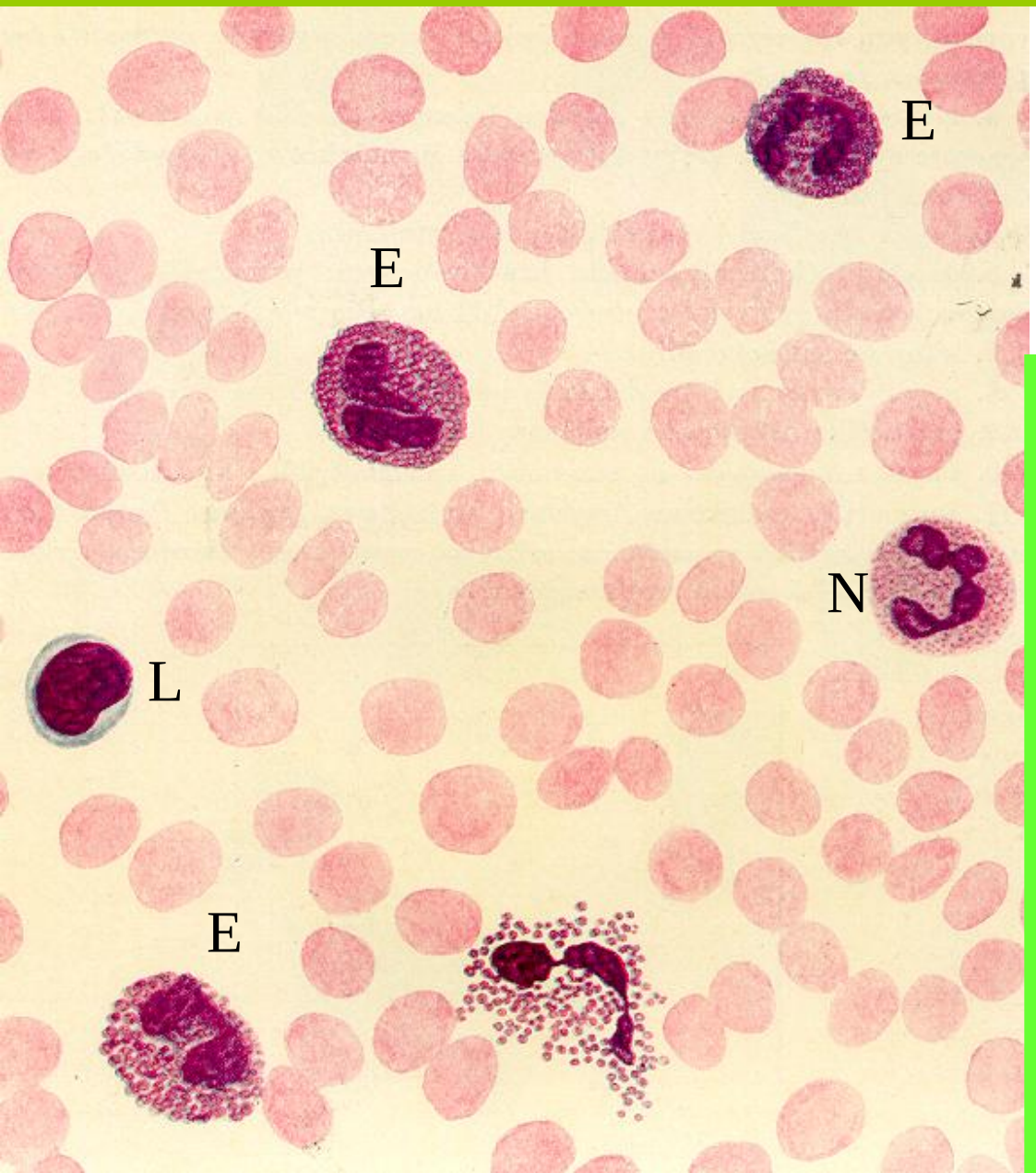
specific granules

- **have the internum** (a **crystal-like**, electron-dense center) surrounded by a less electron-dense externum



Internum – contains **major basic protein**, eosinophilic cationic protein and eosinophil-derived neurotoxin (agents responsible for the combating parasites)

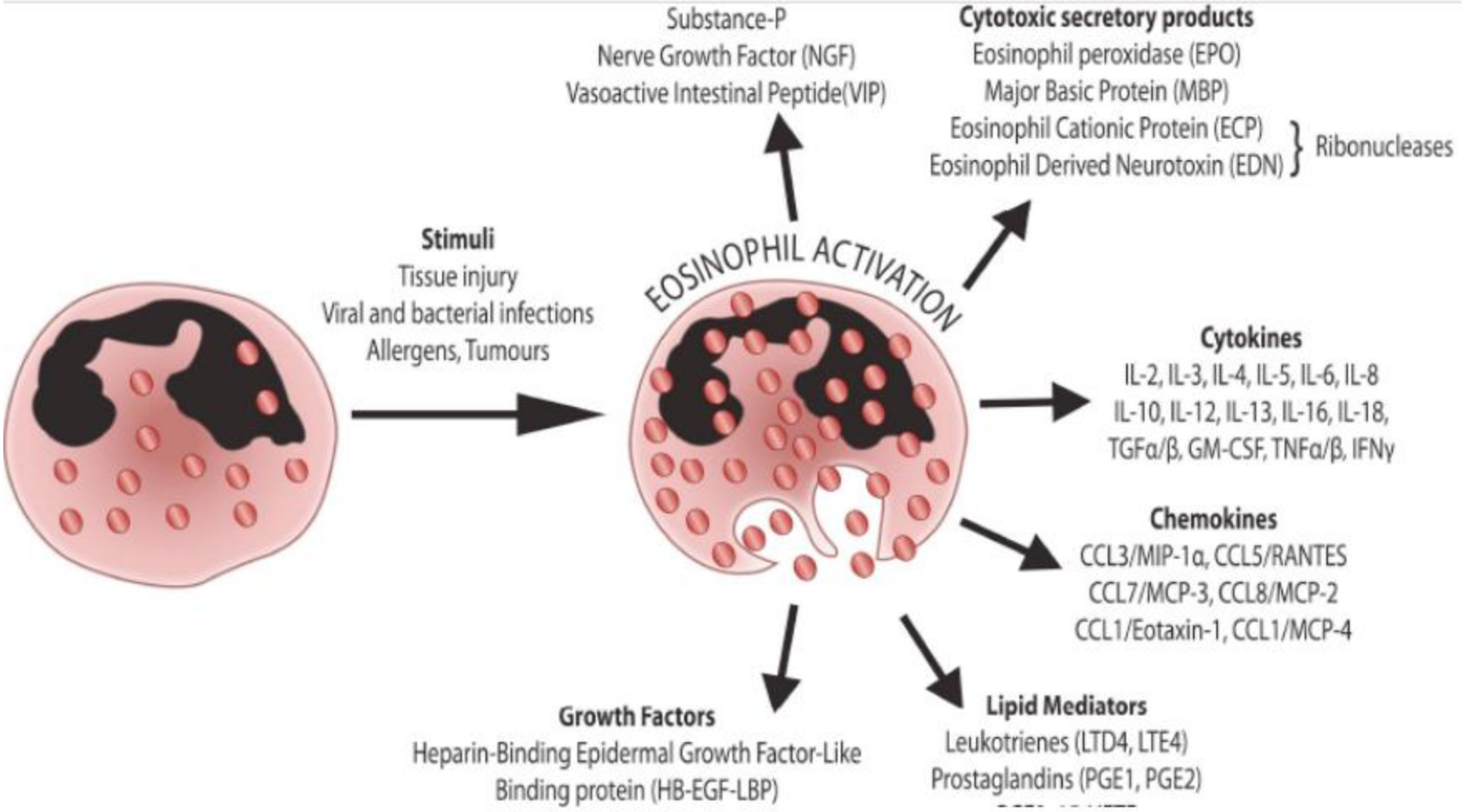
Eosinophilia in blood



Eosinophilia - eosinophil count in the peripheral blood exceeds the norm.

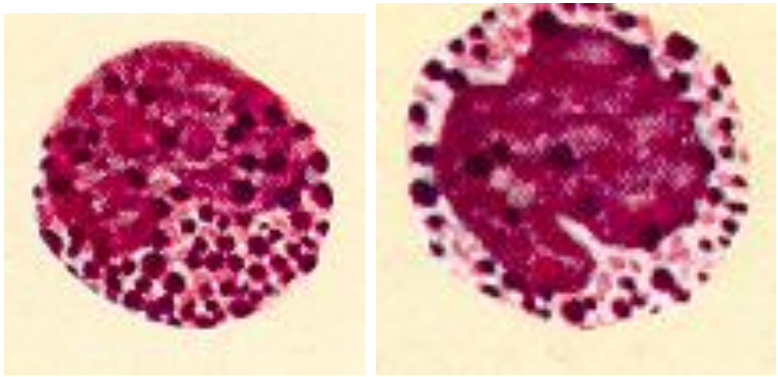
It is associated with allergic reactions and parasitic infections.

Degranulation of granules – formation of pores in parasites, killing by superoxides and hydrogen peroxide.

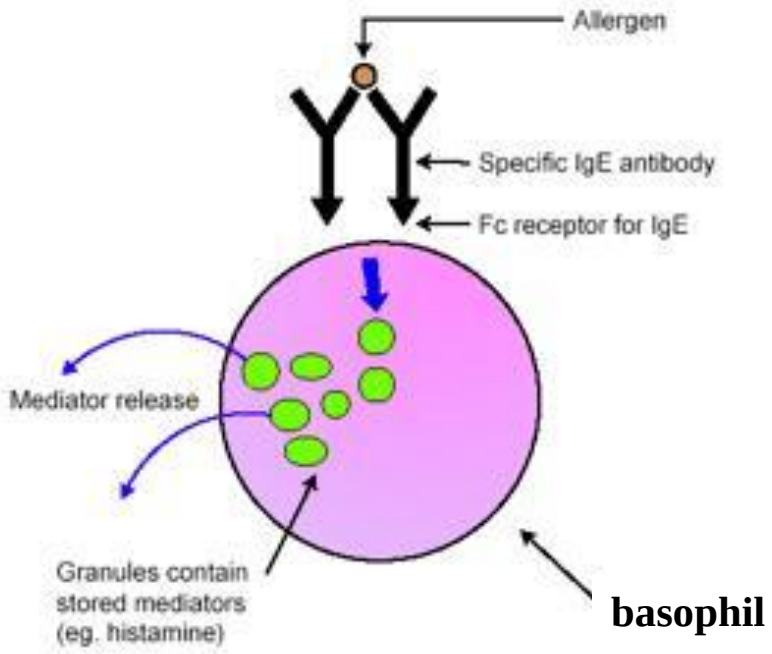

Substance-P
Nerve Growth Factor (NGF)
Vasoactive Intestinal Peptide(VIP)
Cytotoxic secretory products
Eosinophil peroxidase (EPO)
Major Basic Protein (MBP)
Eosinophil Cationic Protein (ECP)
Eosinophil Derived Neurotoxin (EDN)
Ribonucleases
Stimuli
Tissue injury
Viral and bacterial infections
Allergens, Tumours
EOSINOPHIL ACTIVATION
Cytokines
IL-2, IL-3, IL-4, IL-5, IL-6, IL-8
IL-10, IL-12, IL-13, IL-16, IL-18,
TGFα/β, GM-CSF, TNFα/β, IFNγ
Chemokines
CCL3/MIP-1α, CCL5/RANTES
CCL7/MCP-3, CCL8/MCP-2
CCL1/Eotaxin-1, CCL1/MCP-4
Growth Factors
Heparin-Binding Epidermal Growth Factor-Like
Binding protein (HB-EGF-LBP)
Lipid Mediators
Leukotrienes (LTD4, LTE4)
Prostaglandins (PGE1, PGE2)

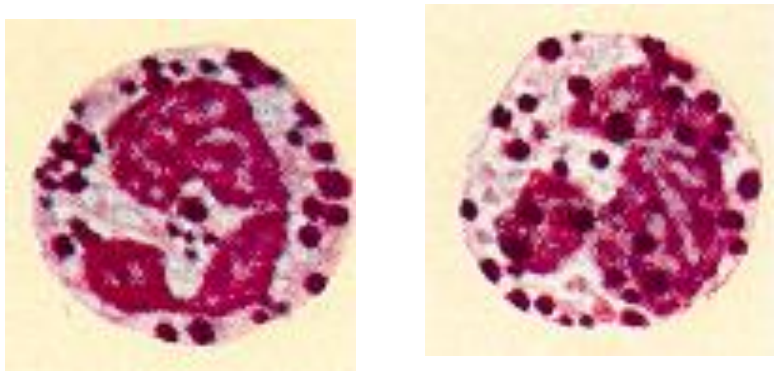
Basophils

- constitute less then 1% of total leukocyte population
- have S-shaped nucleus, masked by dark-blue specific granules



Similar to mast cells, have receptors for **IgE**. Release histamine (and in some species serotonine) produce leukotrienes and prostaglandins





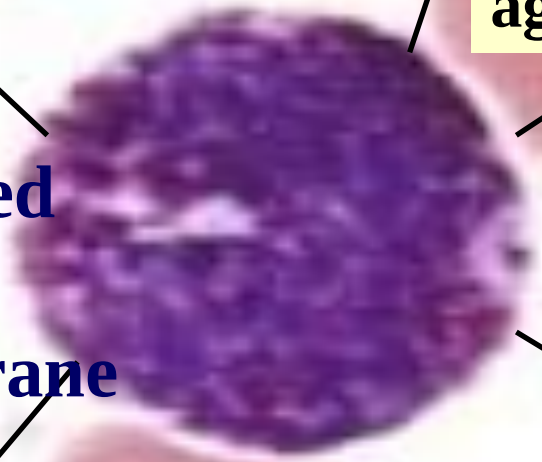
0 –1 % of leukocytes

After stimulation, cells release the contents of its specific granule

Heparin – anticoagulant – prevents the formation of clots

Histamine – couses vasodilation, smooth muscle contraction in the bronchial tree and leakiness of blood vessels

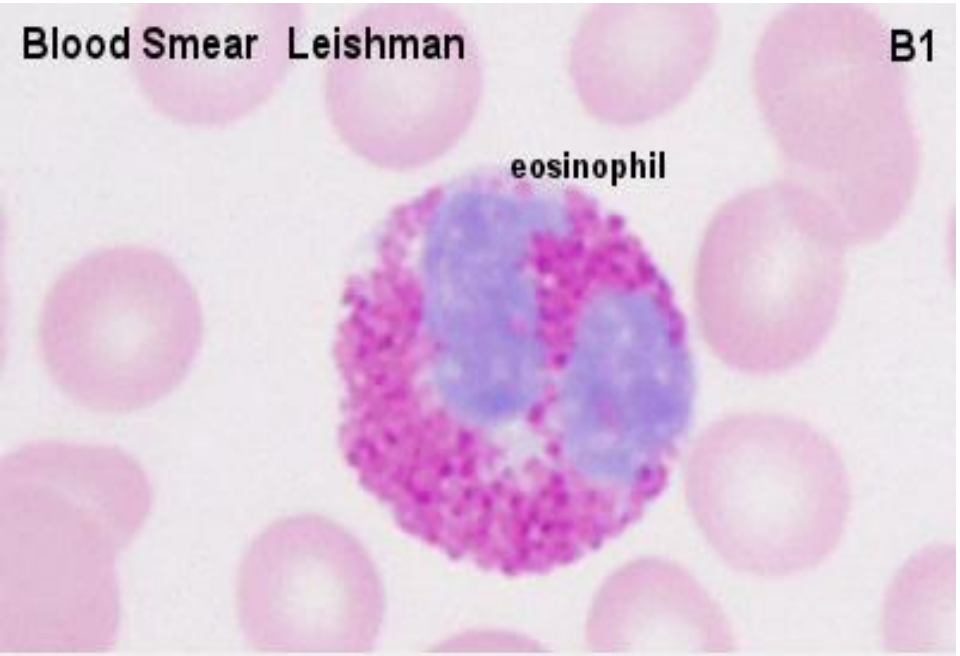
Tromboxane – causes platelet aggregation

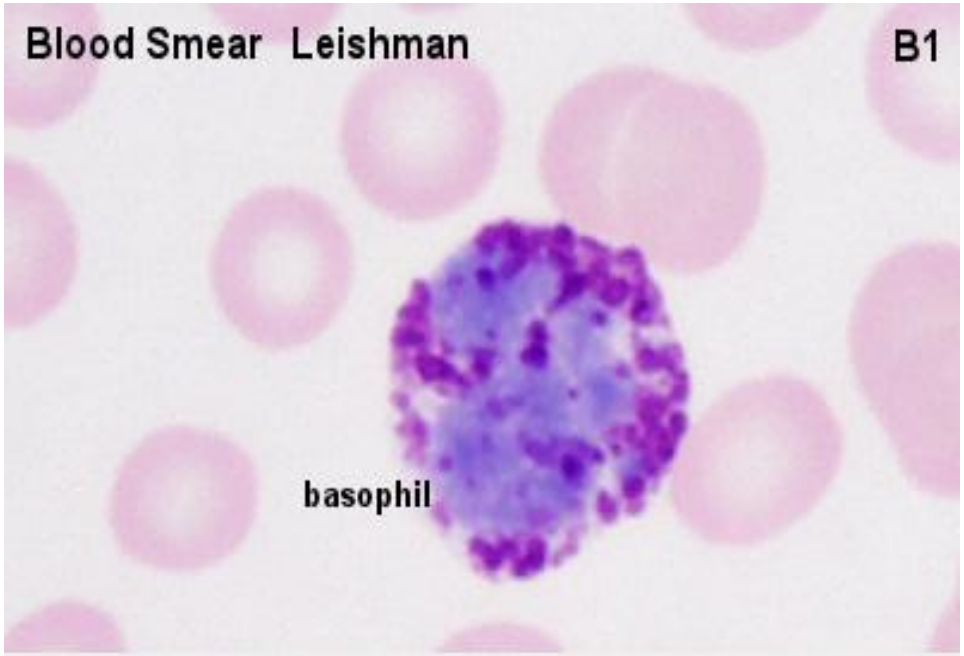


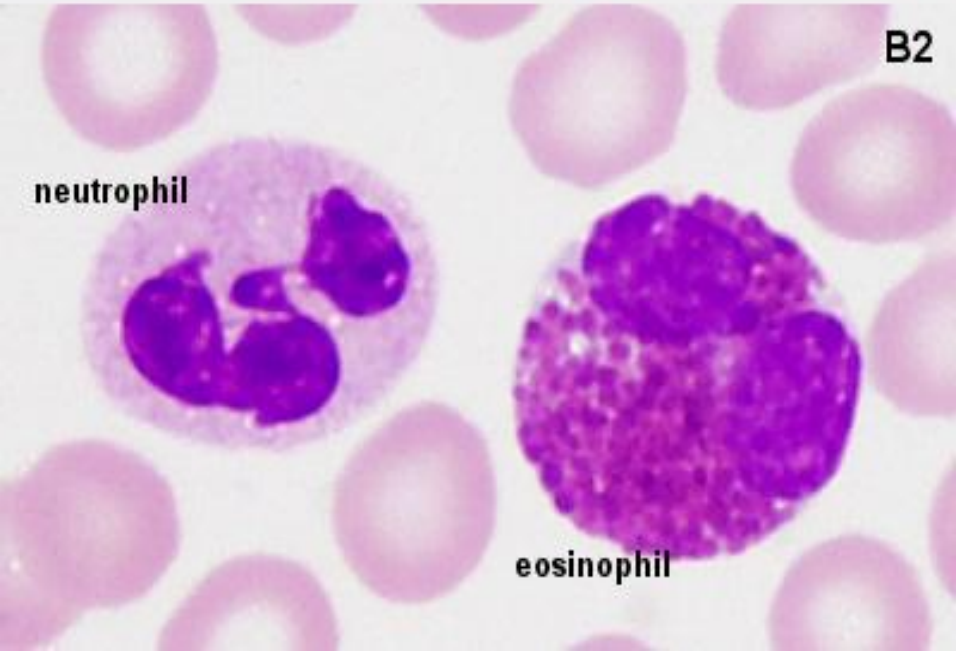
mediators produced from arachidonic acid of cell membrane

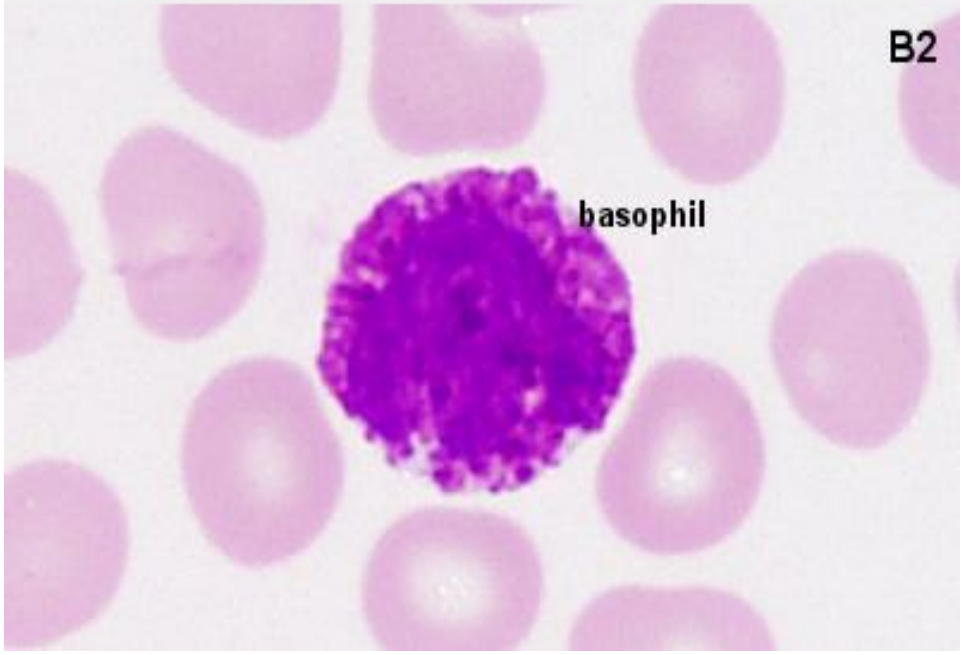
Leukotrienes – effects similar to histamine (action is slower)

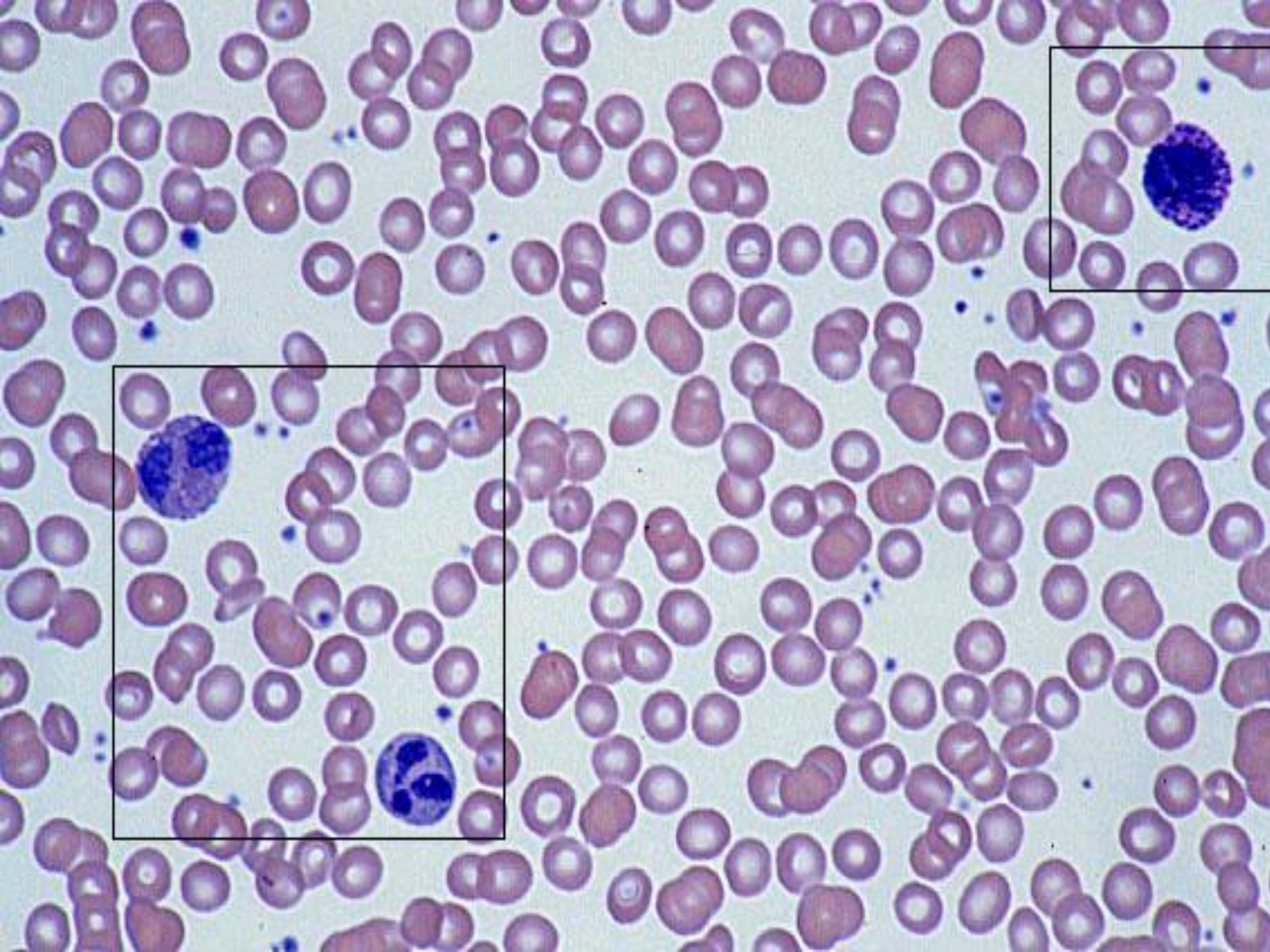
Prostaglandin D_2 – contraction of bronchial smooth muscle, vasoconstriction


Blood Smear Leishman
B1
eosinophil


Blood Smear Leishman
B1
basophil


B2
neutrophil
eosinophil

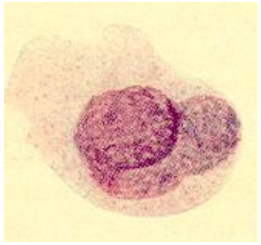

B2
basophil

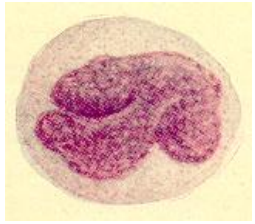


Monocytes

- the largest of the circulating blood cells
- constitute 2 – 8% of the leukocyte
- have a large kidney-shaped nucleus, lobe-like extensions of the nucleus seem to overlap one another
- have numerous azurophilic granules (lysosomes) but no specific granules

Monocytes become tissue macrophages, which phagocytose and digest invading microorganisms and foreign bodies as well as damaged senescent cells. Members of mononuclear phagosyte system.



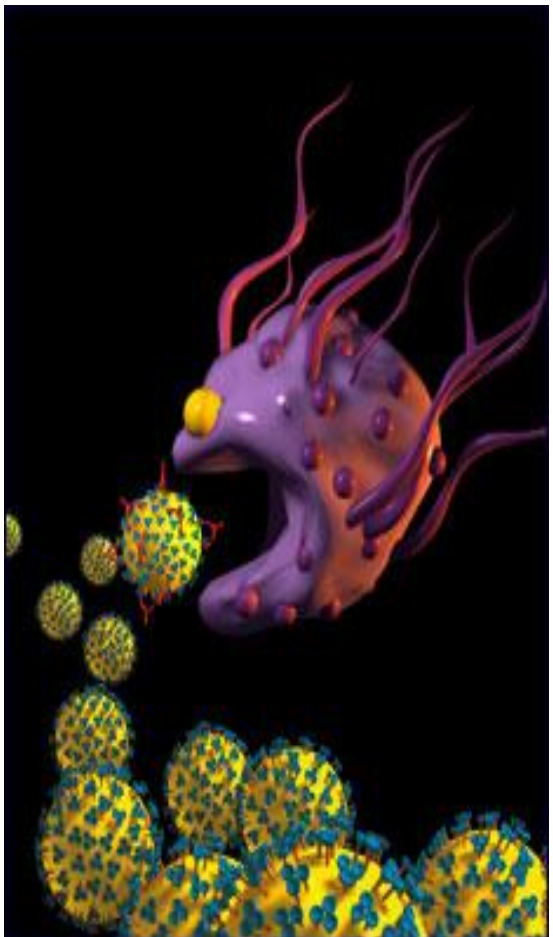


2 –8 % of white blood cells

MONOCYTES

- stay in blood vessels for only a few days
- migrate through the endothelium of venules and capillaries

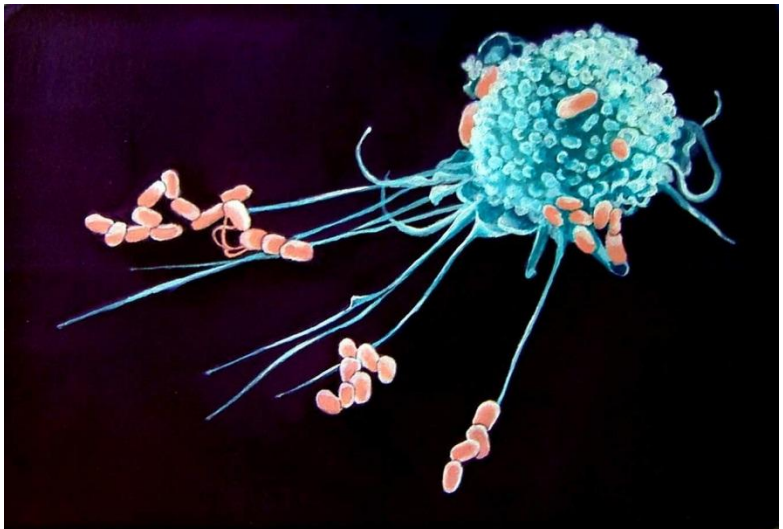
into the connective tissue – differeniate into **macrophages**

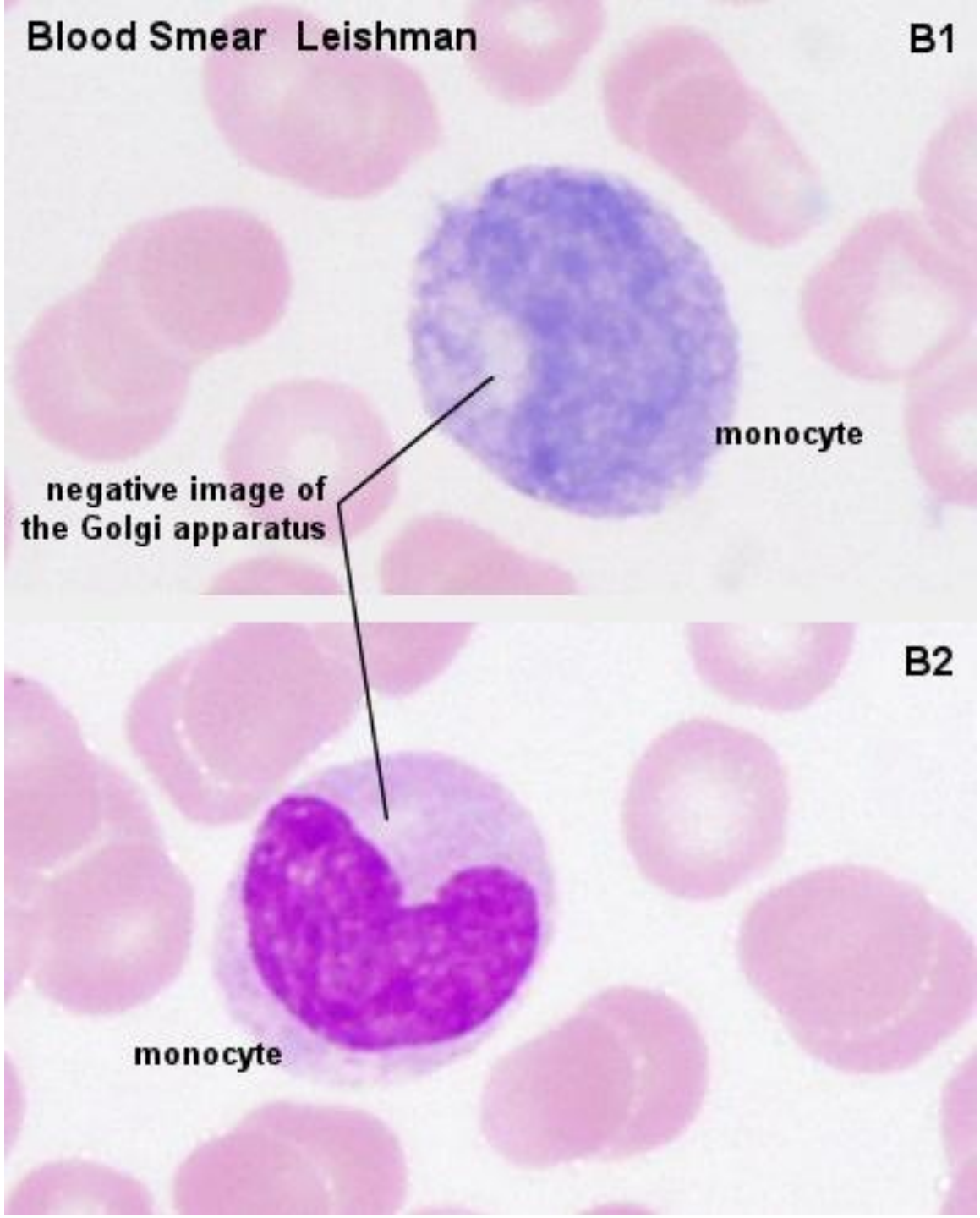


Macrophages – are phagocytes (members of the mononuclear phagocyte system), phagocytose:

- antigens and foreign particles (bacteria)
- dead and defunct cells (senescent erythrocytes)

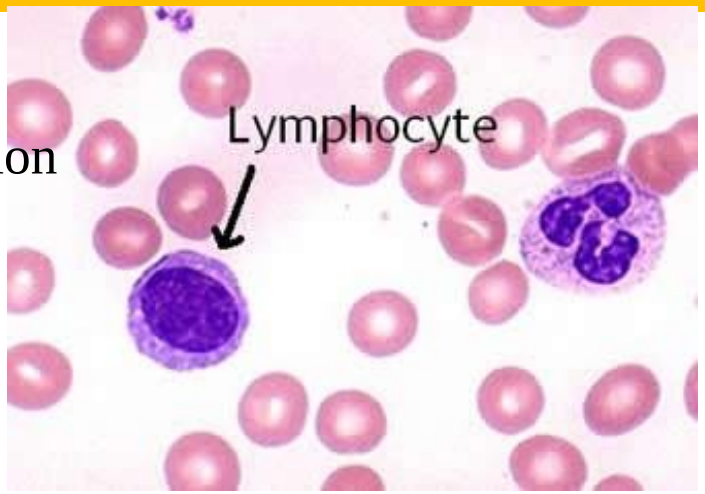
Macrophages are involved in the immune response




Blood Smear Leishman
B1
monocyte
negative image of
the Golgi apparatus
B2
monocyte

Lymphocytes

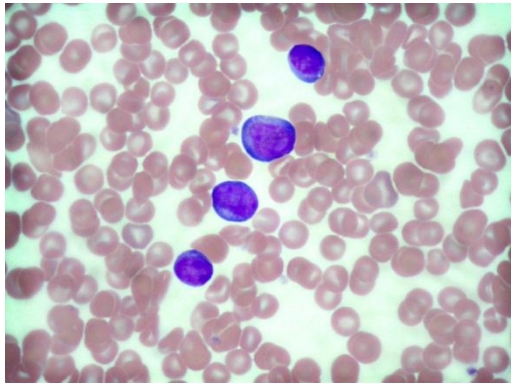
- constitute 22% to 44% of the total leukocyte population
- have round nucleus that occupies most of the cell
- contain a few azurophilic but no specific granules



Lymphocytes are subdivided into three functional groups:

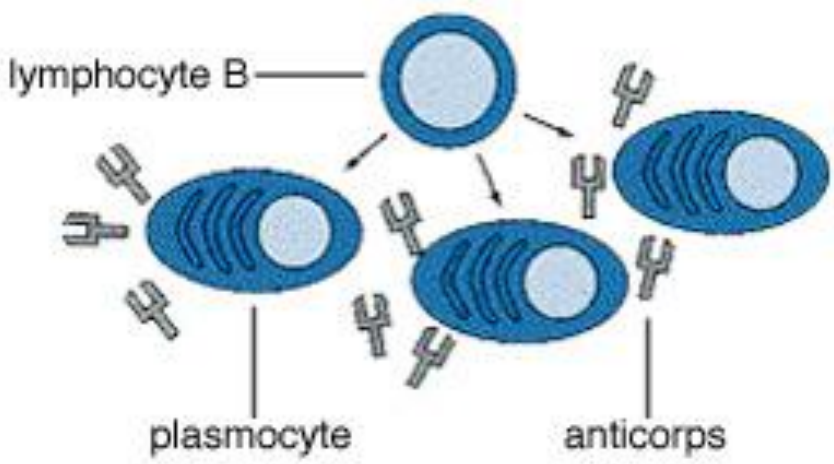
- B lymphocytes (15% of the circulating lymphocytes)
- T lymphocytes (80% of the circulating lymphocytes)
- Null cells (NK cells) (5% of the circulating lymphocytes)

Morphologically are indistinguishable



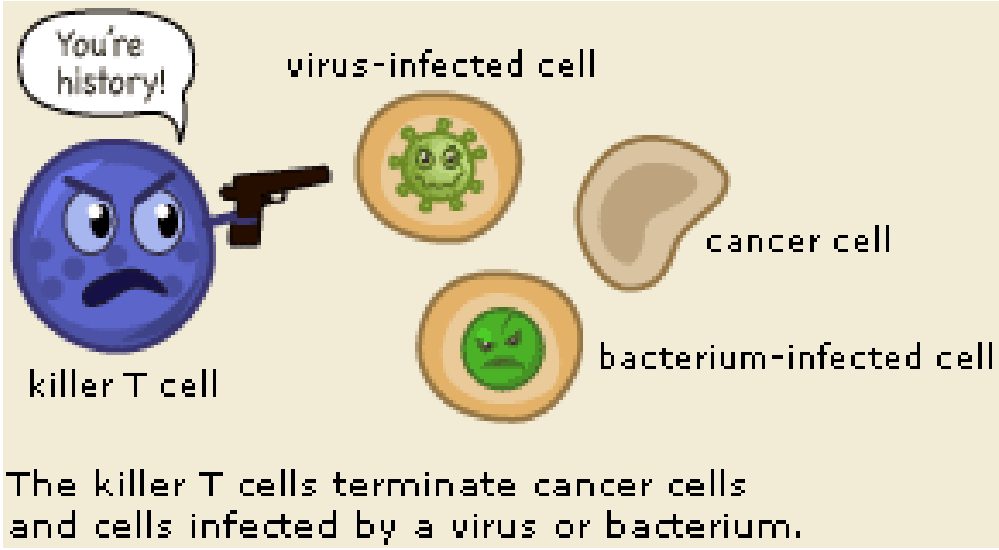
LYMPHOCYTES - function

B cells – responsible for the humoral immune response

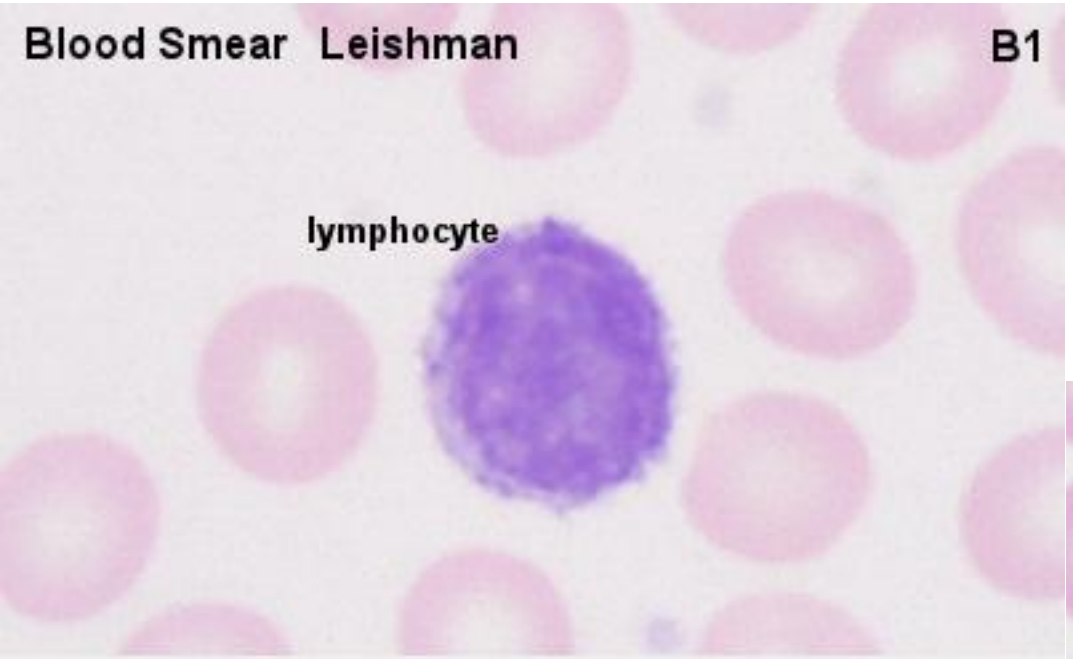


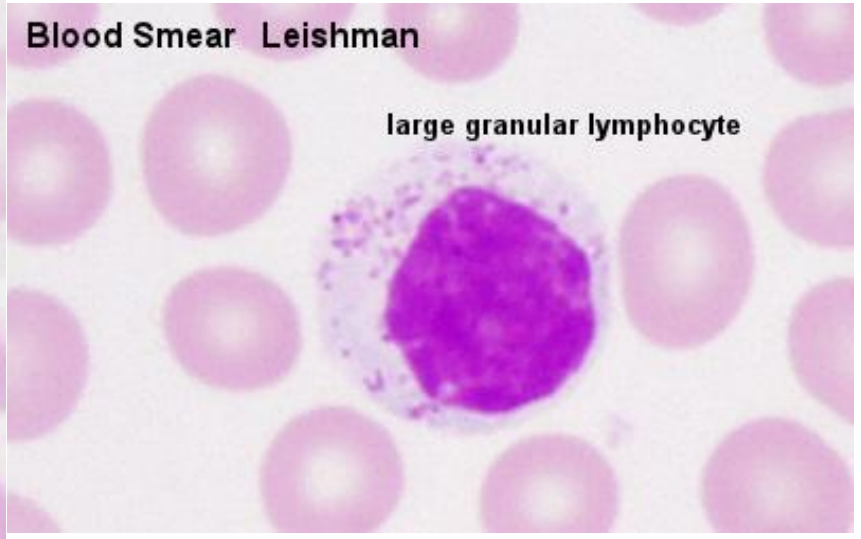
1. Contact with antigens
2. Differentiate into plasmocytes
3. Produce antibodies against antigens

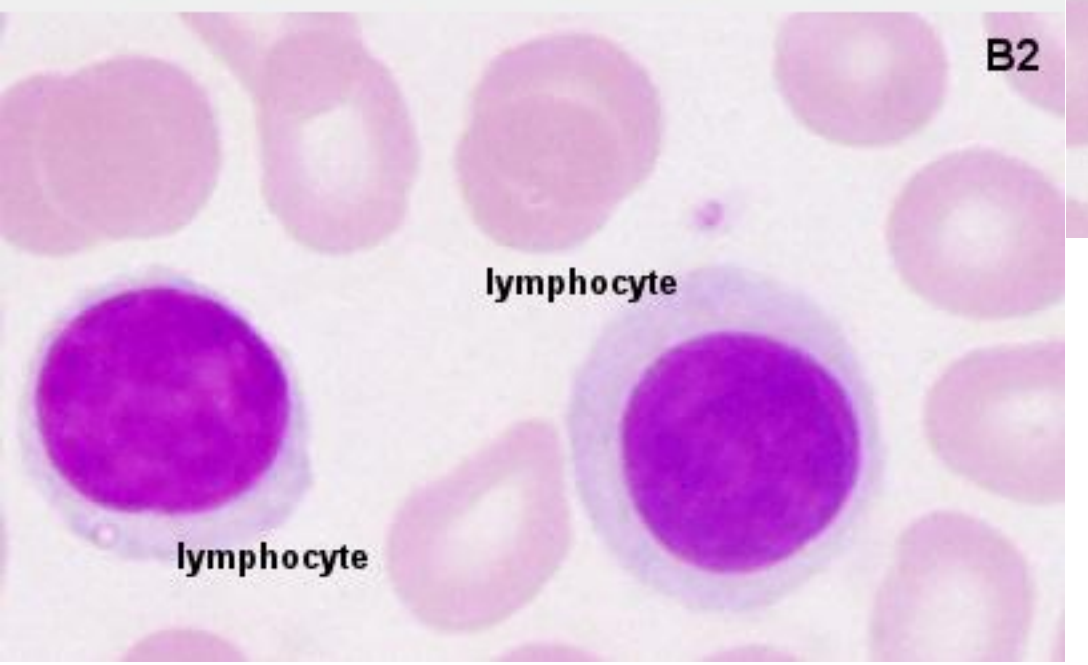
T cells – responsible for the cellular immune response



1. Some T cells differentiate into cytotoxic T cells (T killer cells)
2. Some of them differentiate into T helper cells and produce cytokines (signaling molecules)

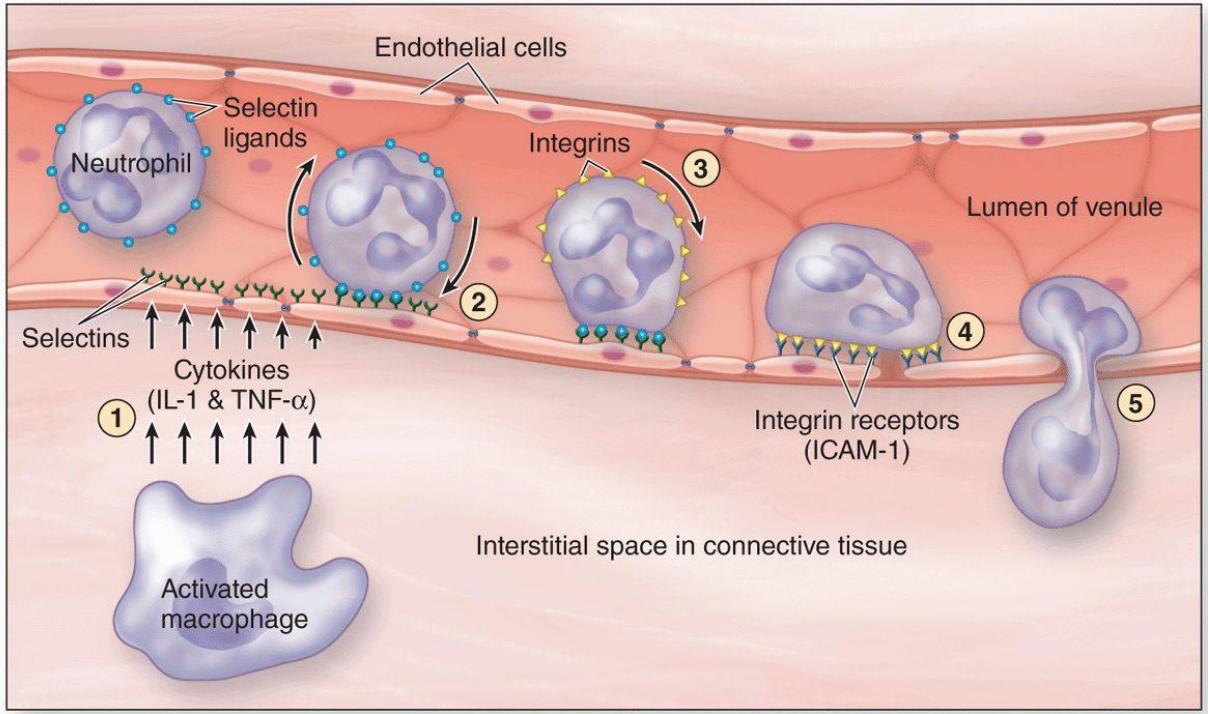

Blood Smear Leishman
B1
lymphocyte


Blood Smear Leishman
large granular lymphocyte


B2
lymphocyte
lymphocyte

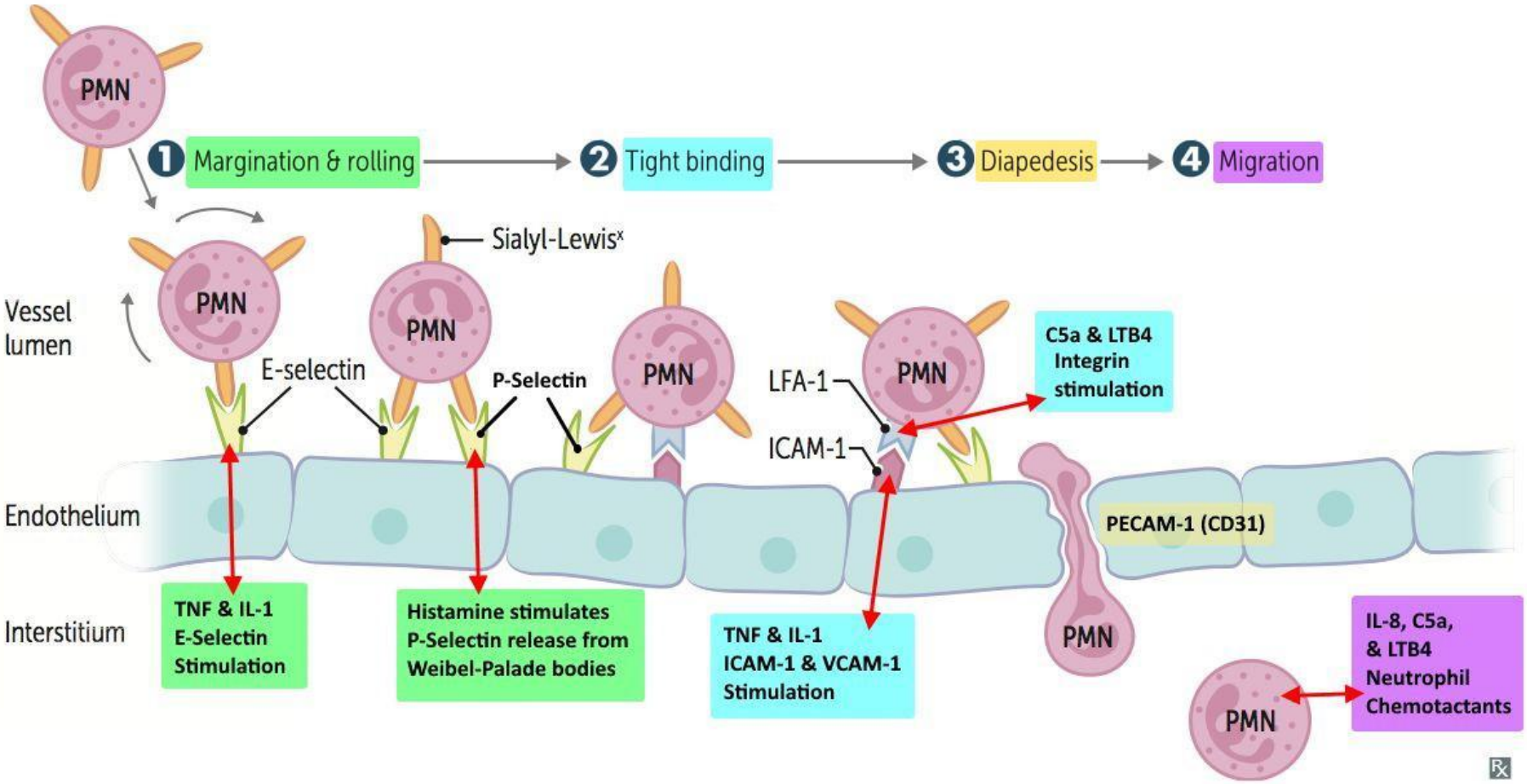
The migration of white blood cells out of the bloodstream during an inflammatory response

Leukocytes do not function within the bloodstream but they migrate
between the endothelial cells of blood vessels into the connection tissue
- DIAPEDESIS



1. Leukocytes roll slowly on the surface of endothelial cells.
2. Damaged tissue release chemokines (small signaling proteins which have ability to induce directed chemotaxis)
3. Endothelial cells bind leukocyte
4. Passage through the endothelium of the venule to enter the connective tissue compartment

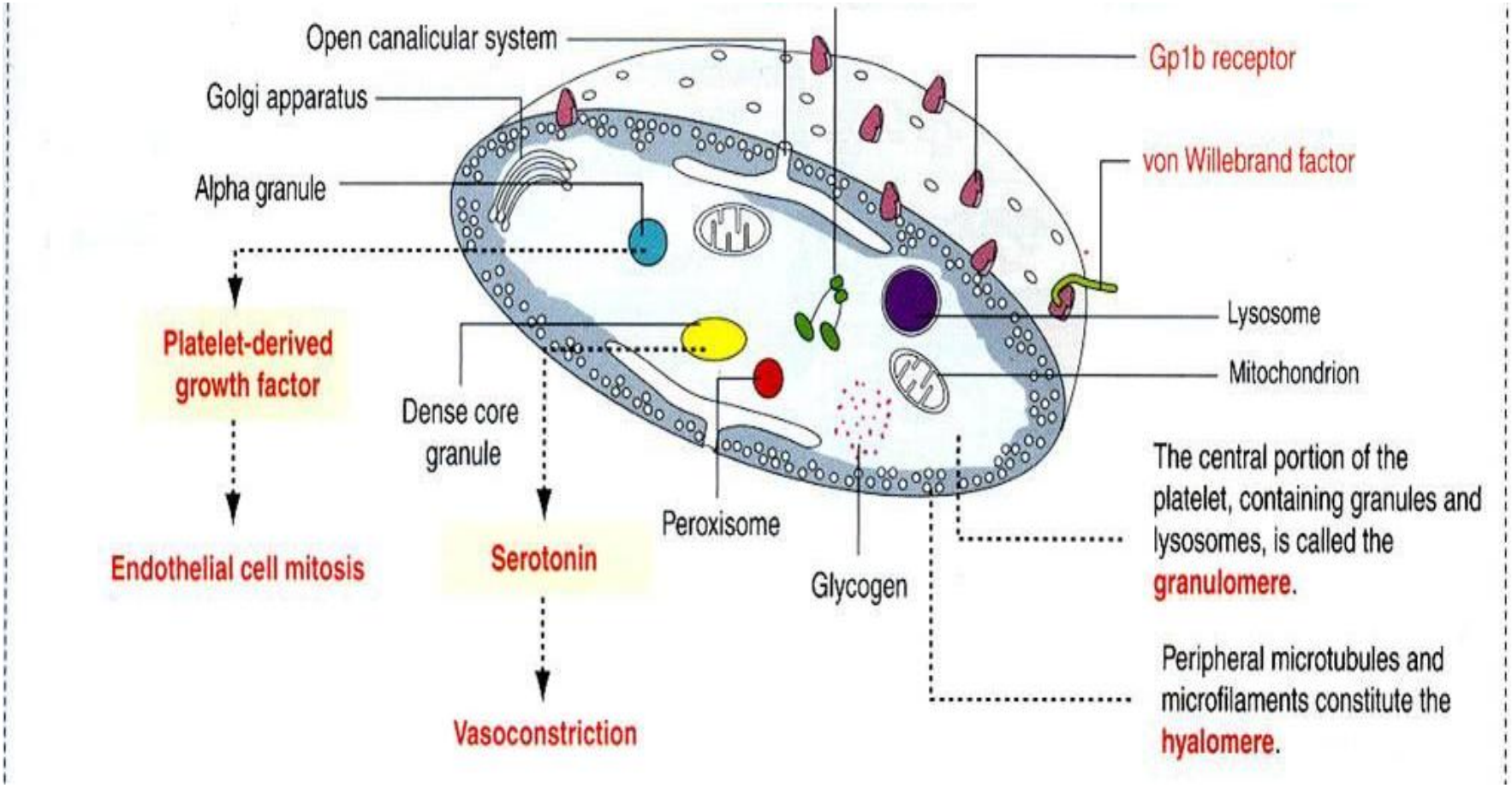
Margination diapedesis migration chemotaxis



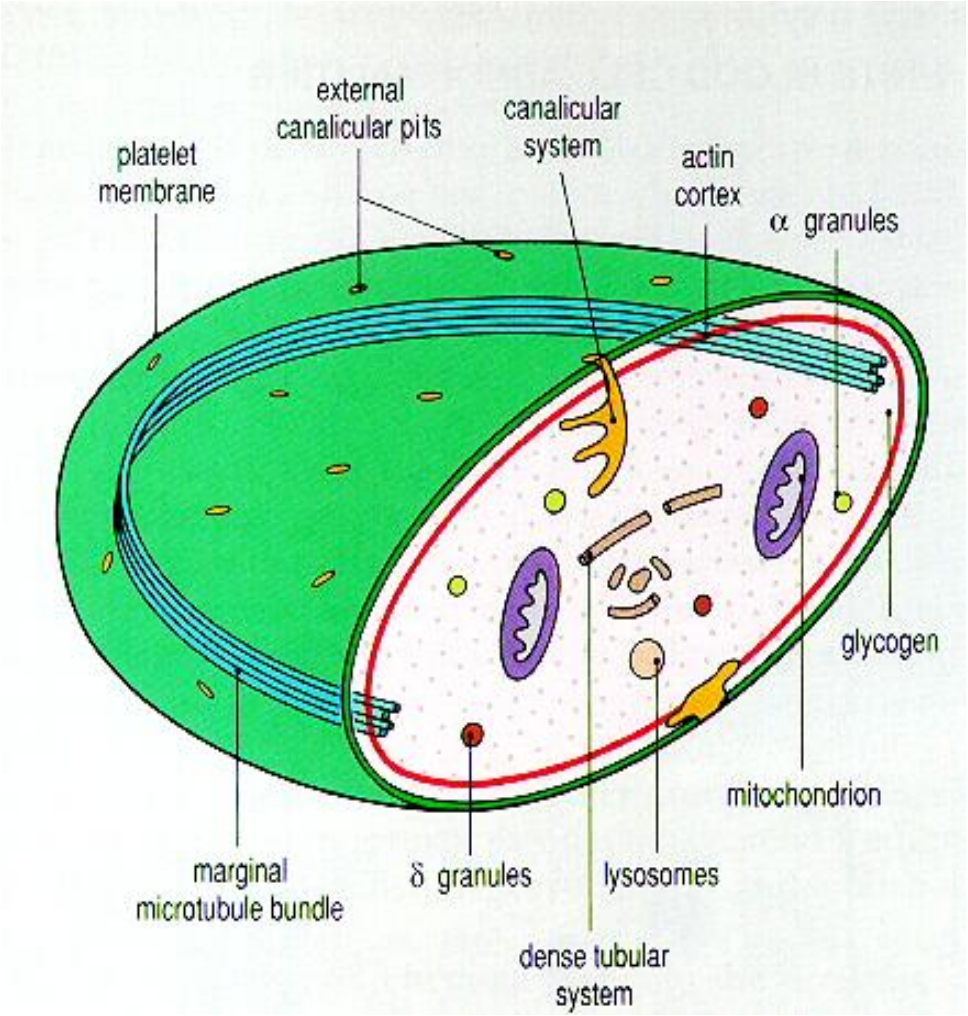
Platelets (Thrombocytes)

Non-nucleated fragments of megakaryocyte cytoplasm, 2-4 mm in diameter, with a life-span about 10 days.
200.000 – 400.000 per microliter of blood

Platelet
- has two regions: hyalomere and granulomere



Platelets (Thrombocytes)



Two tubular systems of platelets

(in hyalomere)

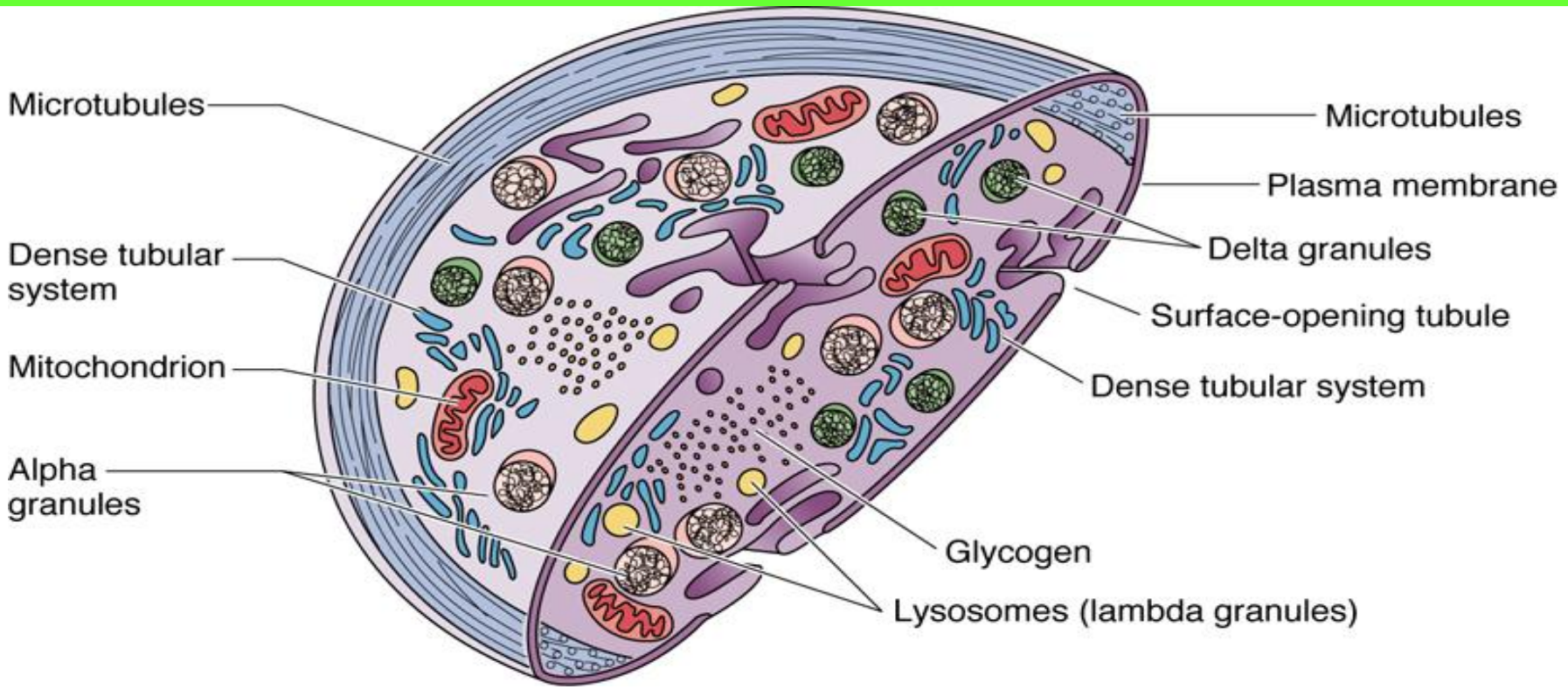
Dense tubular system – site of prostaglandin synthesis?

The surface opening canalicular system – coiled system within the platelet which communicates with the outside, increases surface of platelet (7-8 times)

Microtubules

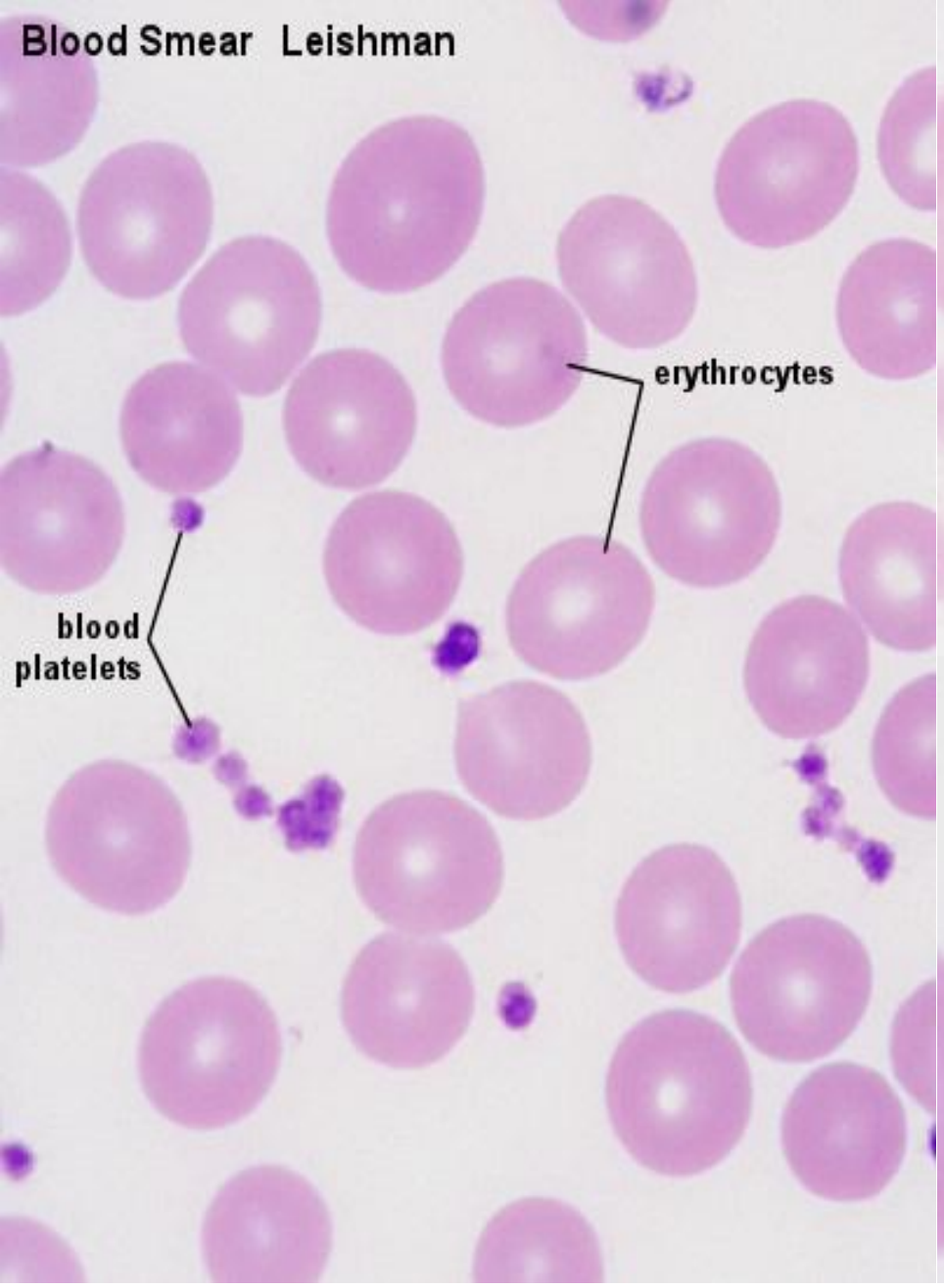
- are arranged parallel to each other and form a ring within the hyalomere
- assist platelets in maintaining their diskoid morphology

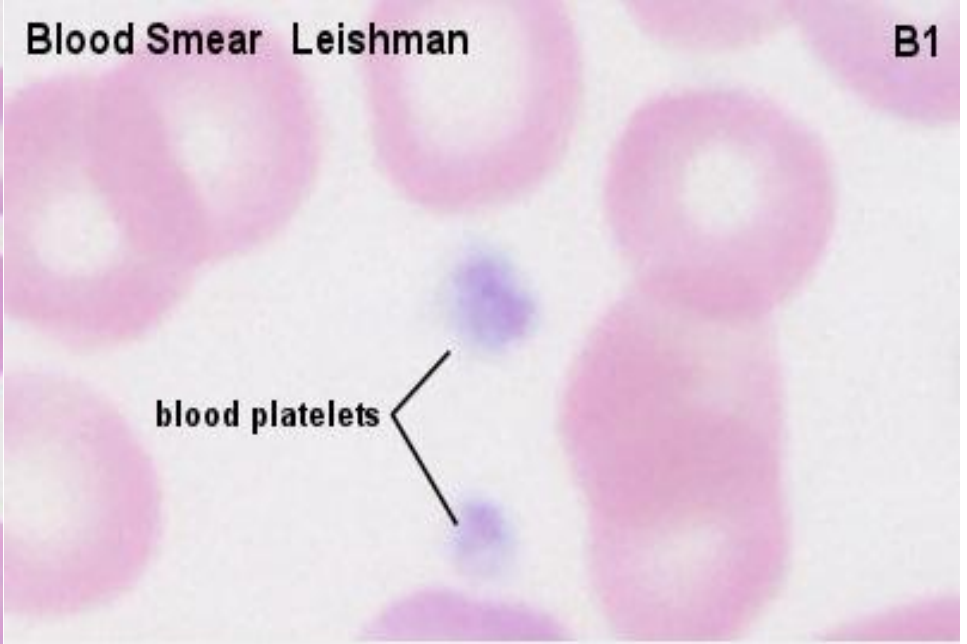
Platelets (Thrombocytes)

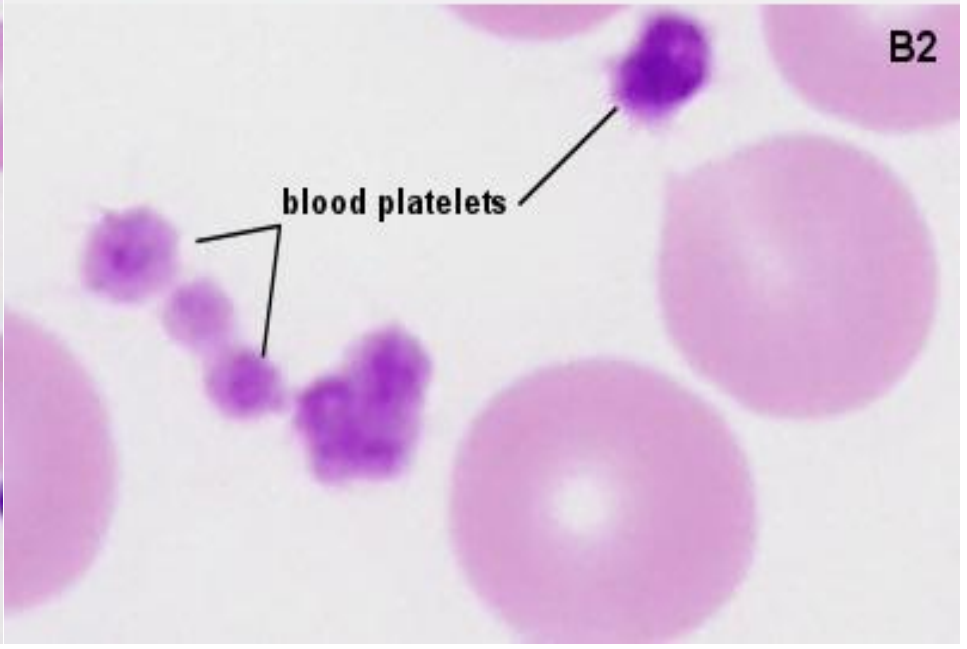


Three types of granules:

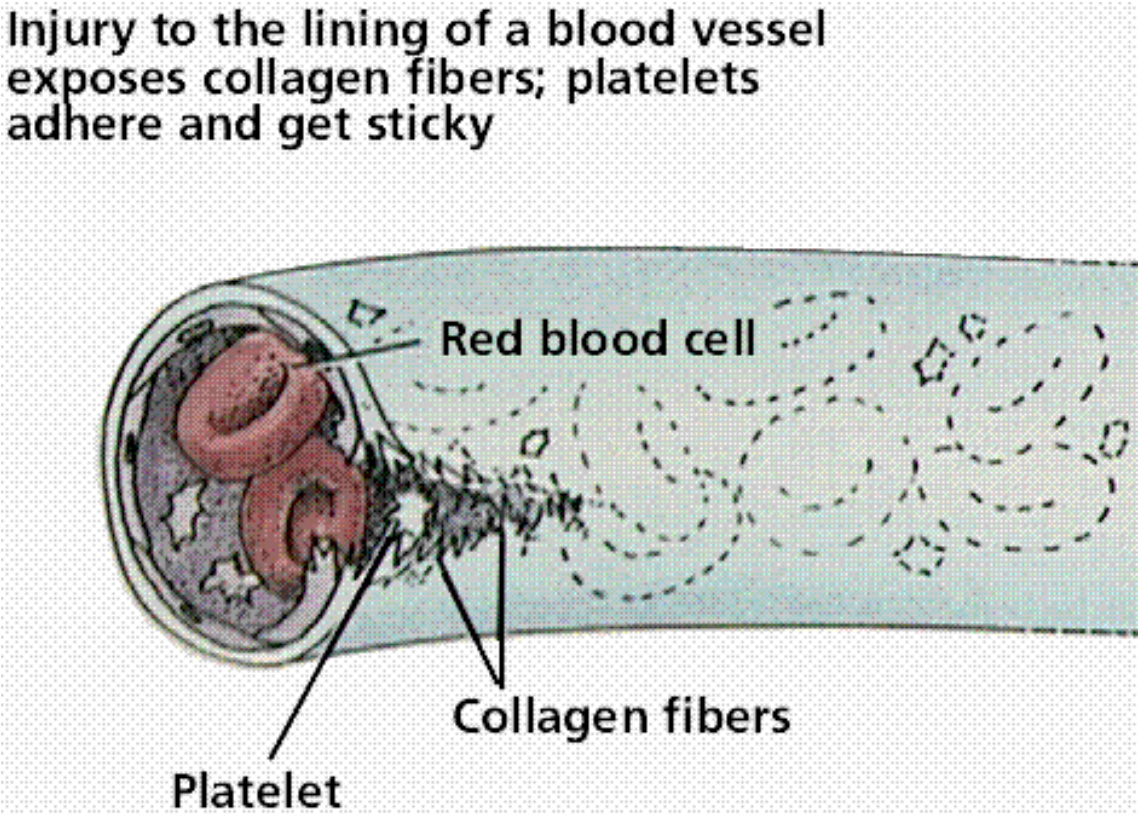
- alpha granules - fibrinogen, PDGF, TGF, von Willebrand factor, thromboplastin.
- delta granules - calcium ions, ADP, ATP, serotonin (enhances platelet procoagulant properties)
- lambda granules (lysosomes) – hydrolytic enzymes


Blood Smear Leishman
erythrocytes
blood platelets


Blood Smear Leishman
B1
blood platelets

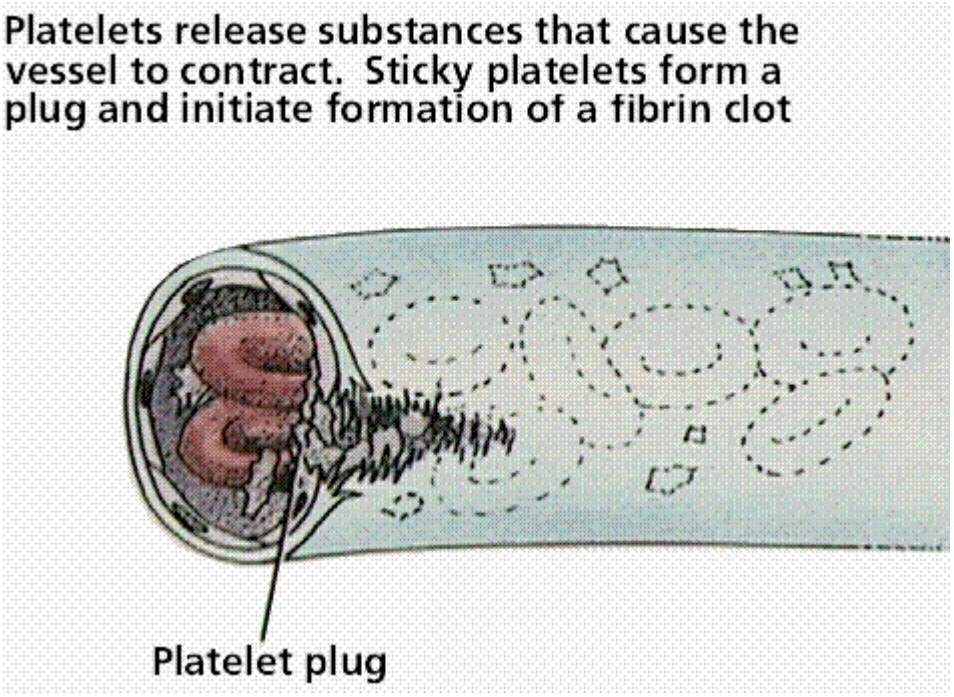

B2
blood platelets

Platelets circulating in blood are inactive



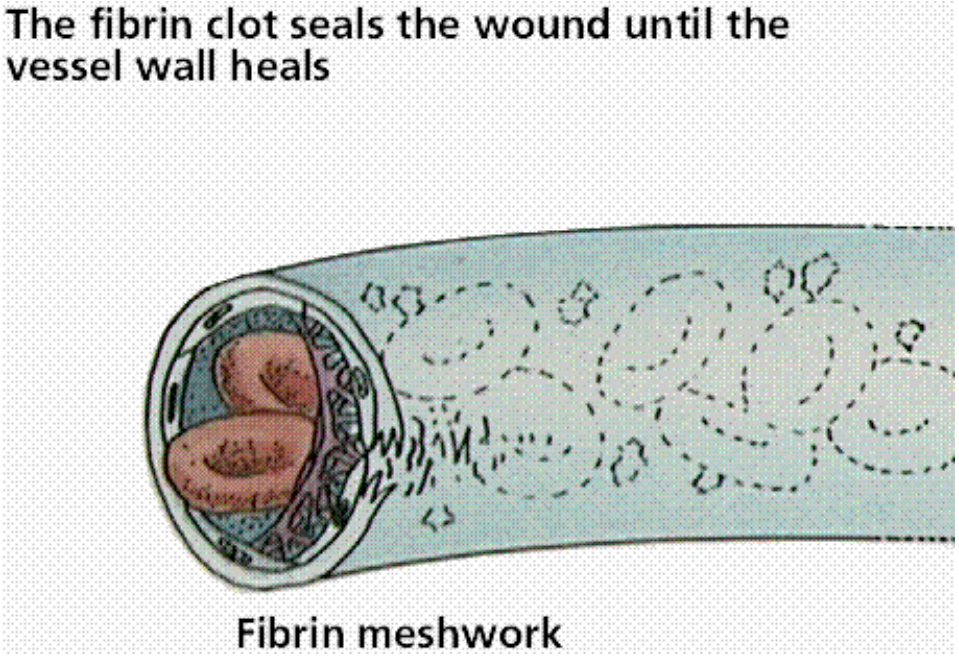
Contact with collagen from damaged vessel activates them. They attach to collagen directly by membrane receptors (integrins)

or with the help of von Willebrand factor (from alpha granules).



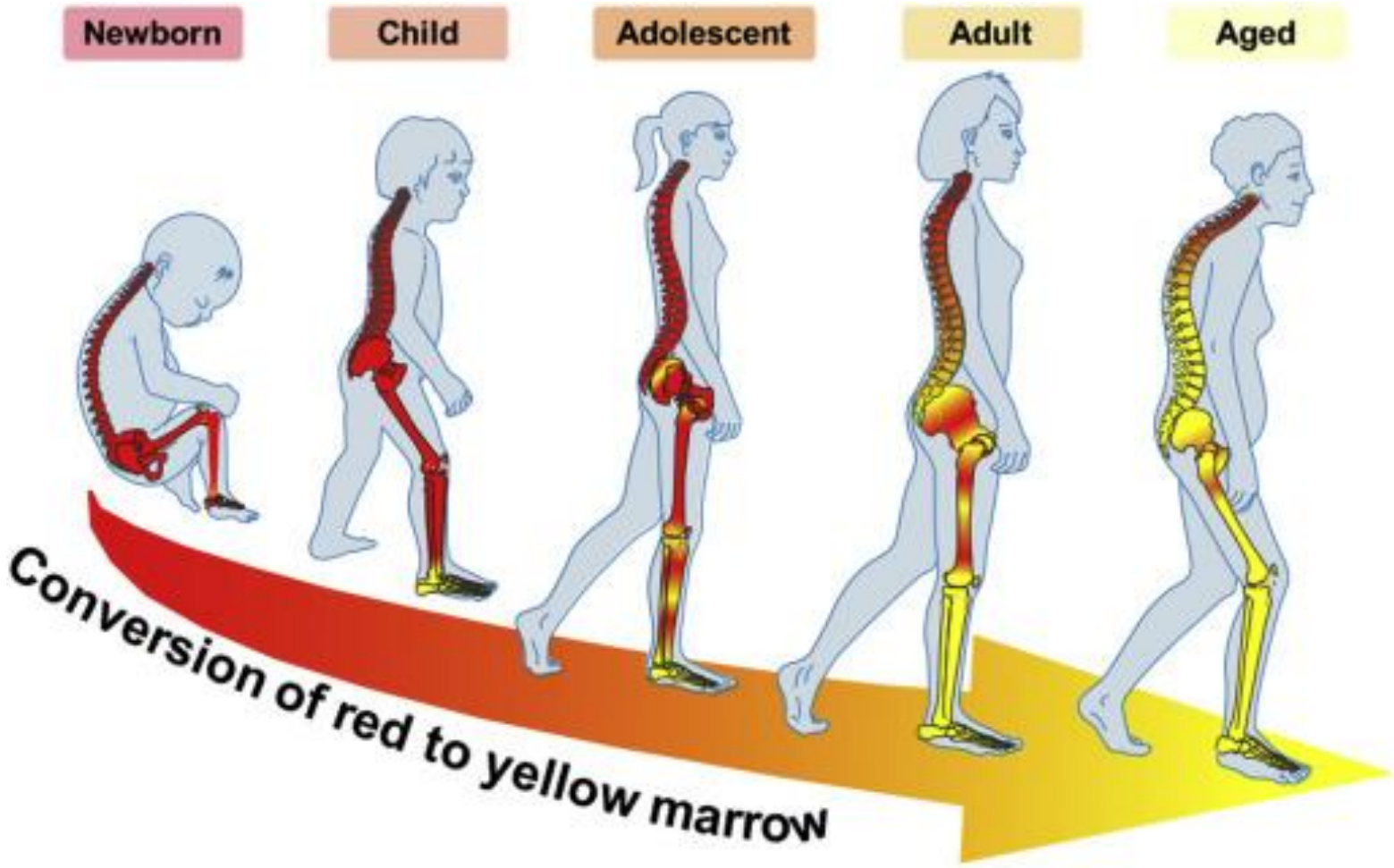
Platelets form tromboxane A2 (TXA2) which contracts blood vessels.

Platelets aggregate and form a platelet plug, inhibiting bleeding and initiate blood clotting.



Thromboplastin converts prothrombin into thrombin. Thrombin converts fibrinogen into fibrin – clot is formed in the presence of calcium ions

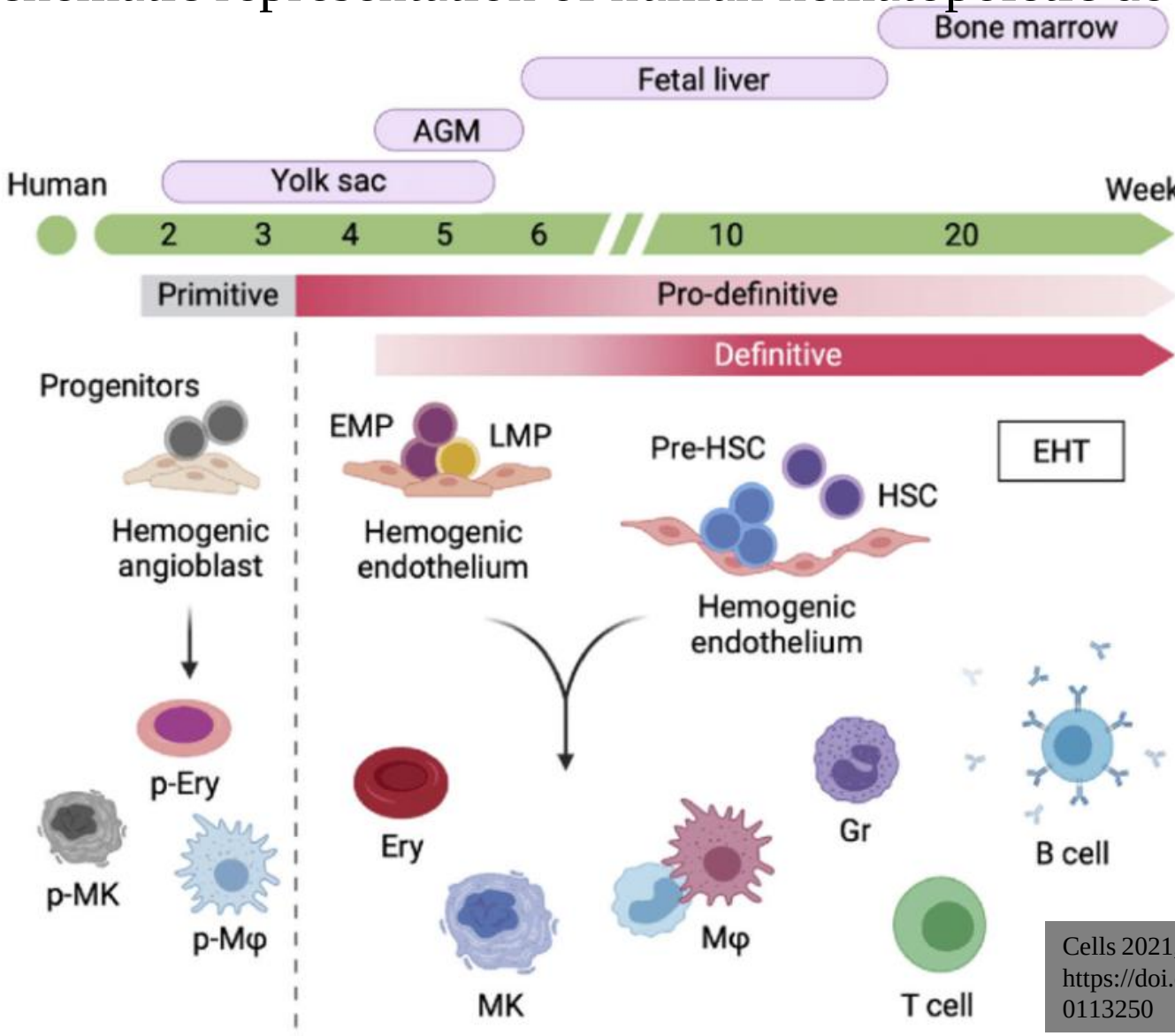
Hemopoiesis - formation of blood cellular components



In a healthy adult person, approximately 10^{11}–10^{12} new blood cells are produced daily in order to maintain steady state levels in the peripheral circulation.

In adults, it occurs mainly in the pelvis, cranium, vertebrae, and sternum.

Schematic representation of human hematopoietic development.



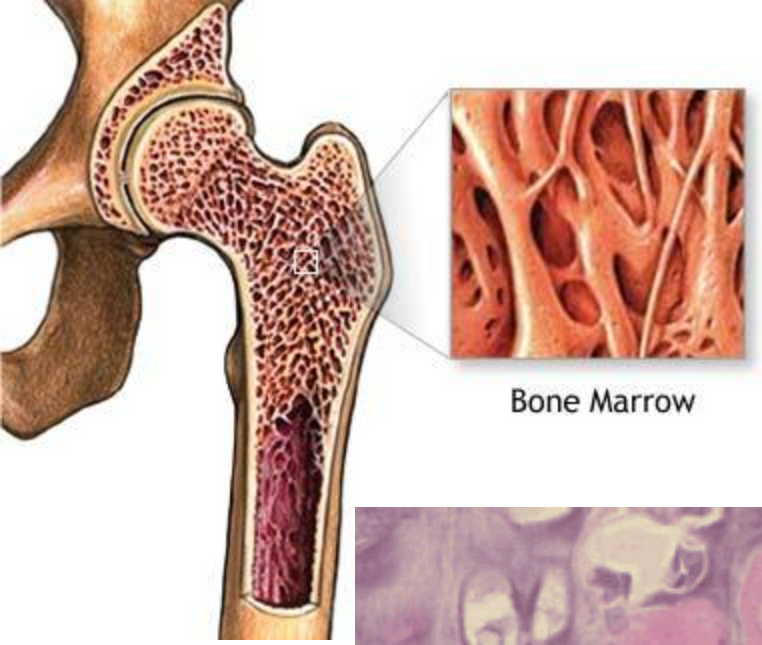
Cells 2021, 10, 3250. https://doi.org/10.3390/cells10113250

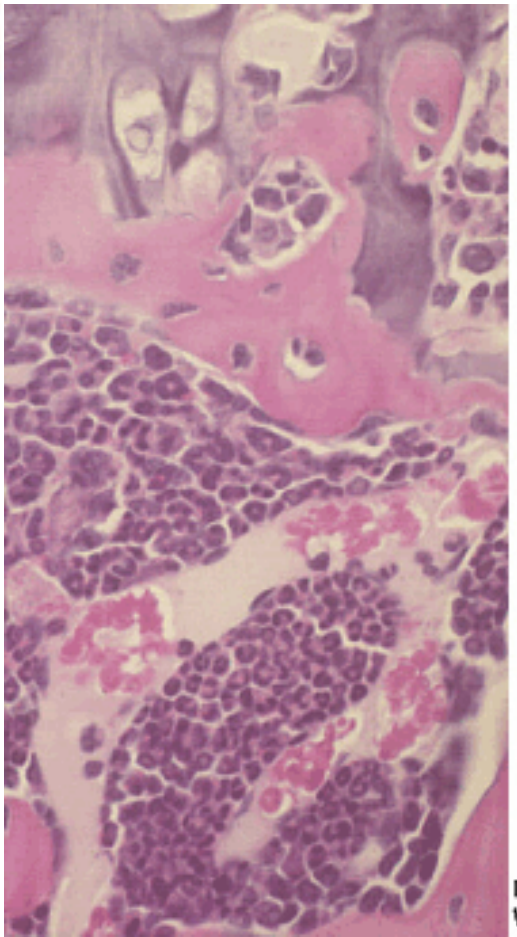
AGM, aorta-gonad-mesonephros; EHT, endothelial-to-hematopoietic transition; EMP, erythro-myeloid progenitor; LMP, lympho-myeloid progenitor; HSC, hematopoietic stem cell; Ery, erythrocyte; MK, megakaryocyte; Mφ, macrophage; Gr, granulocyte.

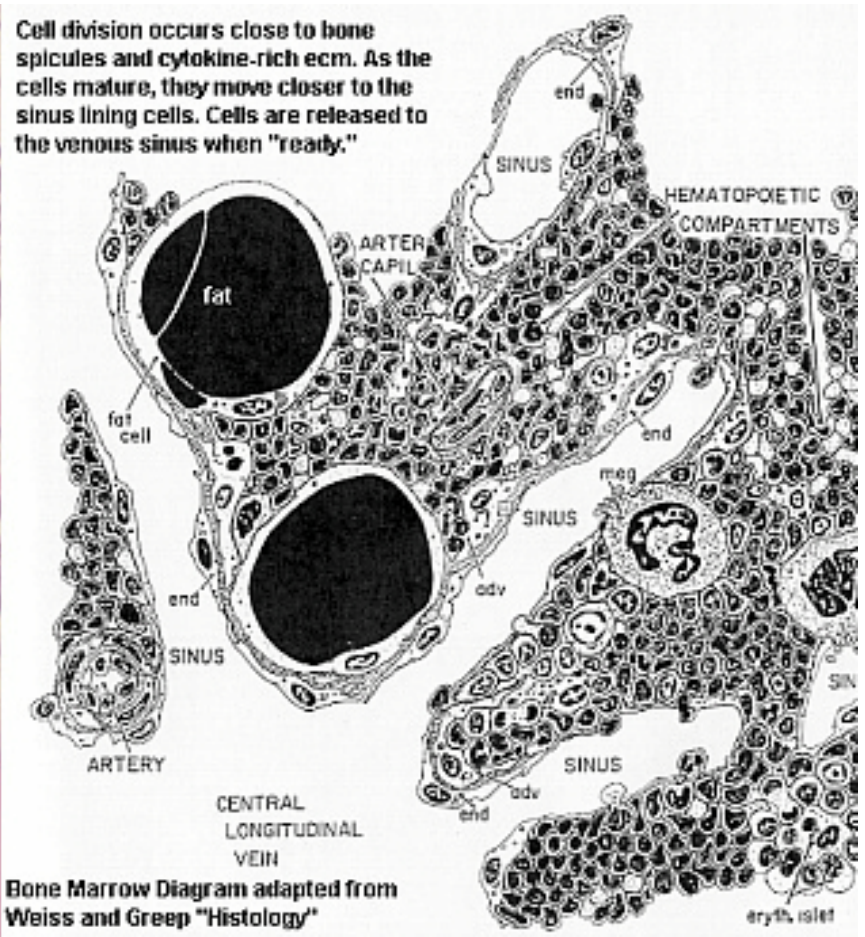
Bone marrow

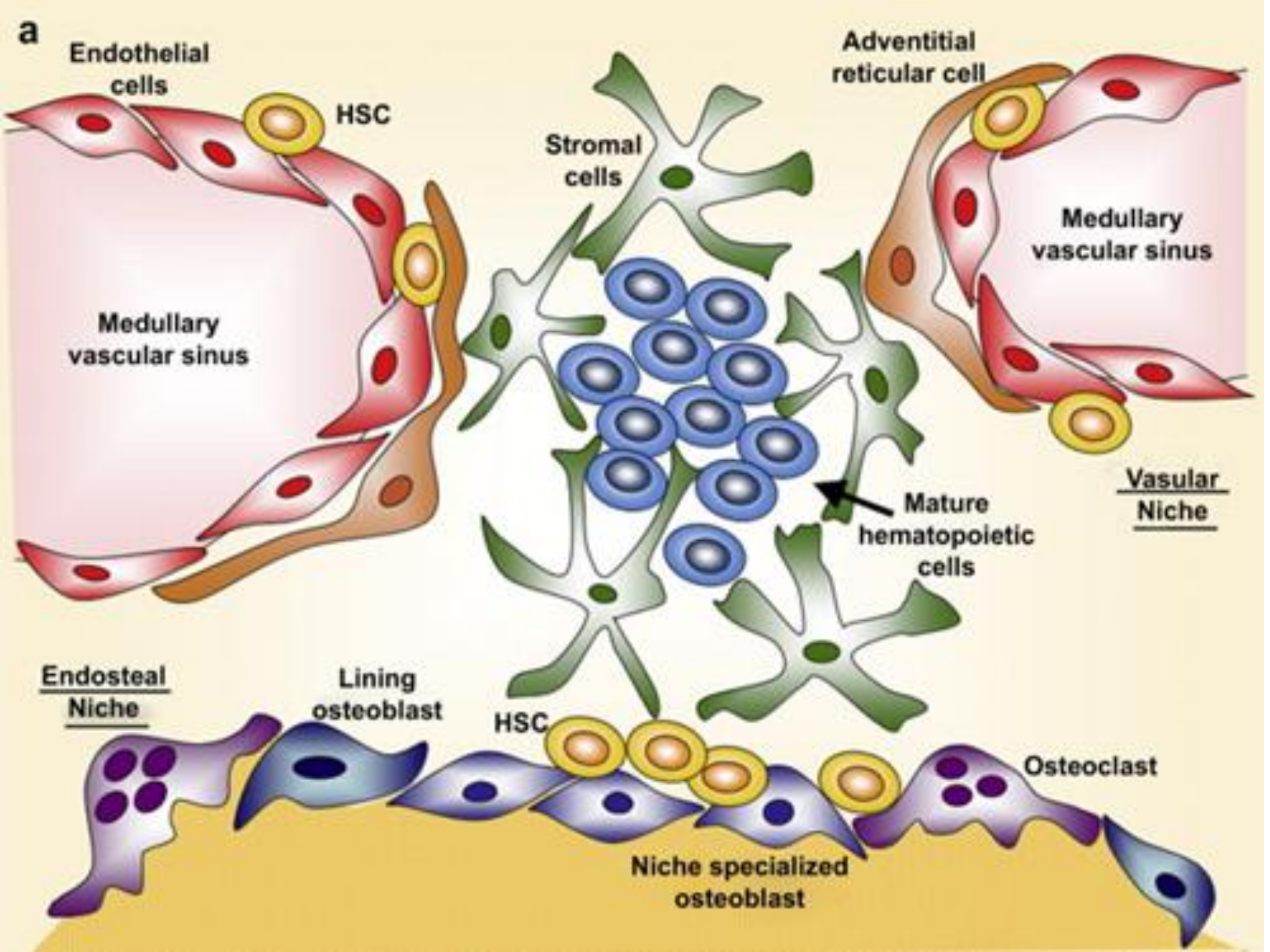
- vascular connective tissue
- located in marrow cavity
- contains cells responsible

for hemopoiesis - hematopoietic stem cells








a
Endothelial cells
HSC
Stromal cells
Adventitial reticular cell
Medullary vascular sinus
Medullary vascular sinus
Vasular Niche
Mature hematopoietic cells
Endosteal Niche
Lining osteoblast
HSC
Osteoclast
Niche specialized osteoblast

Multipotent hemopoietic stem cells (PHSCs)

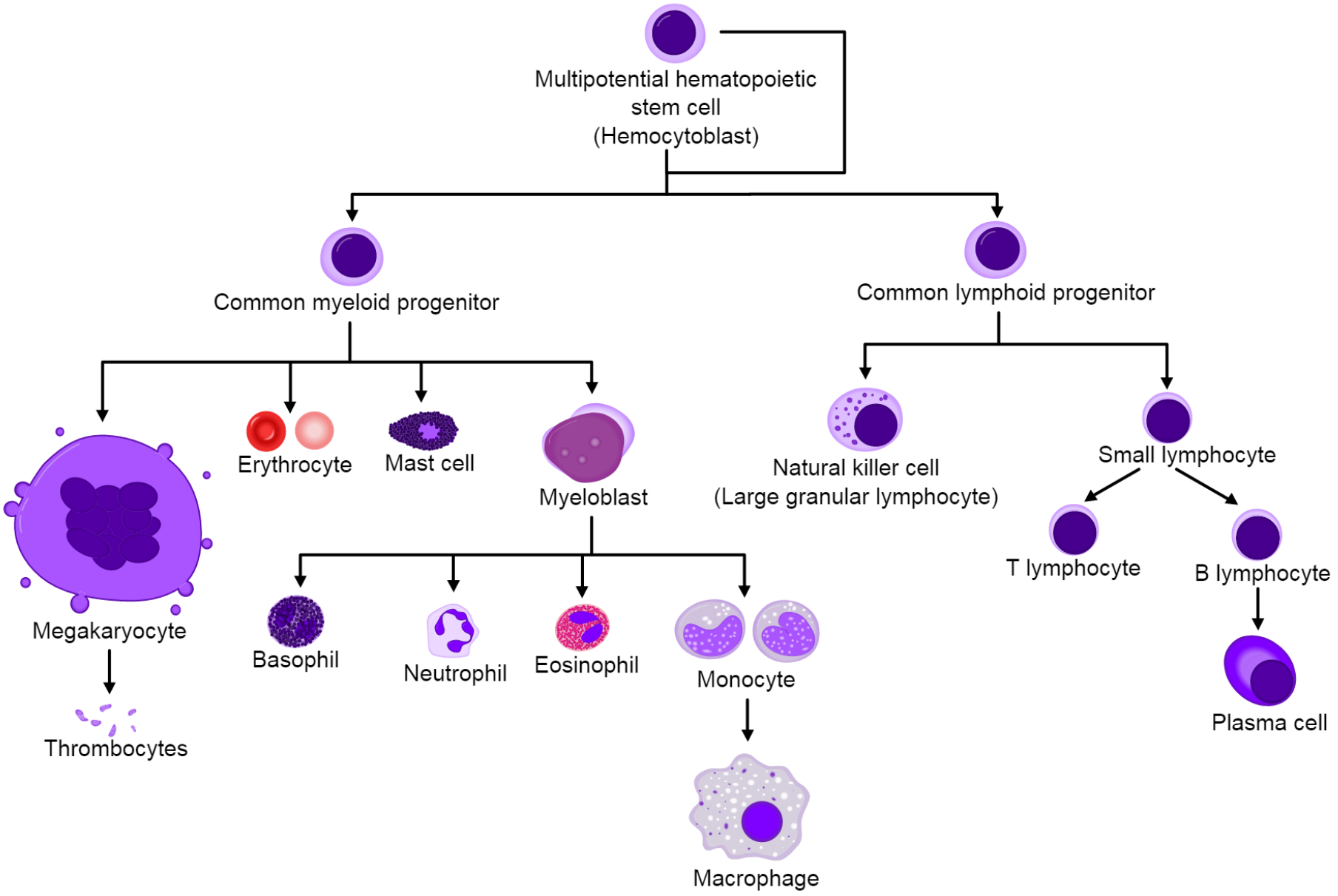
- account for about 0.1% of cell population of bone marrow undergo bursts of cell division giving rise to more **PHSCs** as well as two types:

- **common myeloid progenitor**

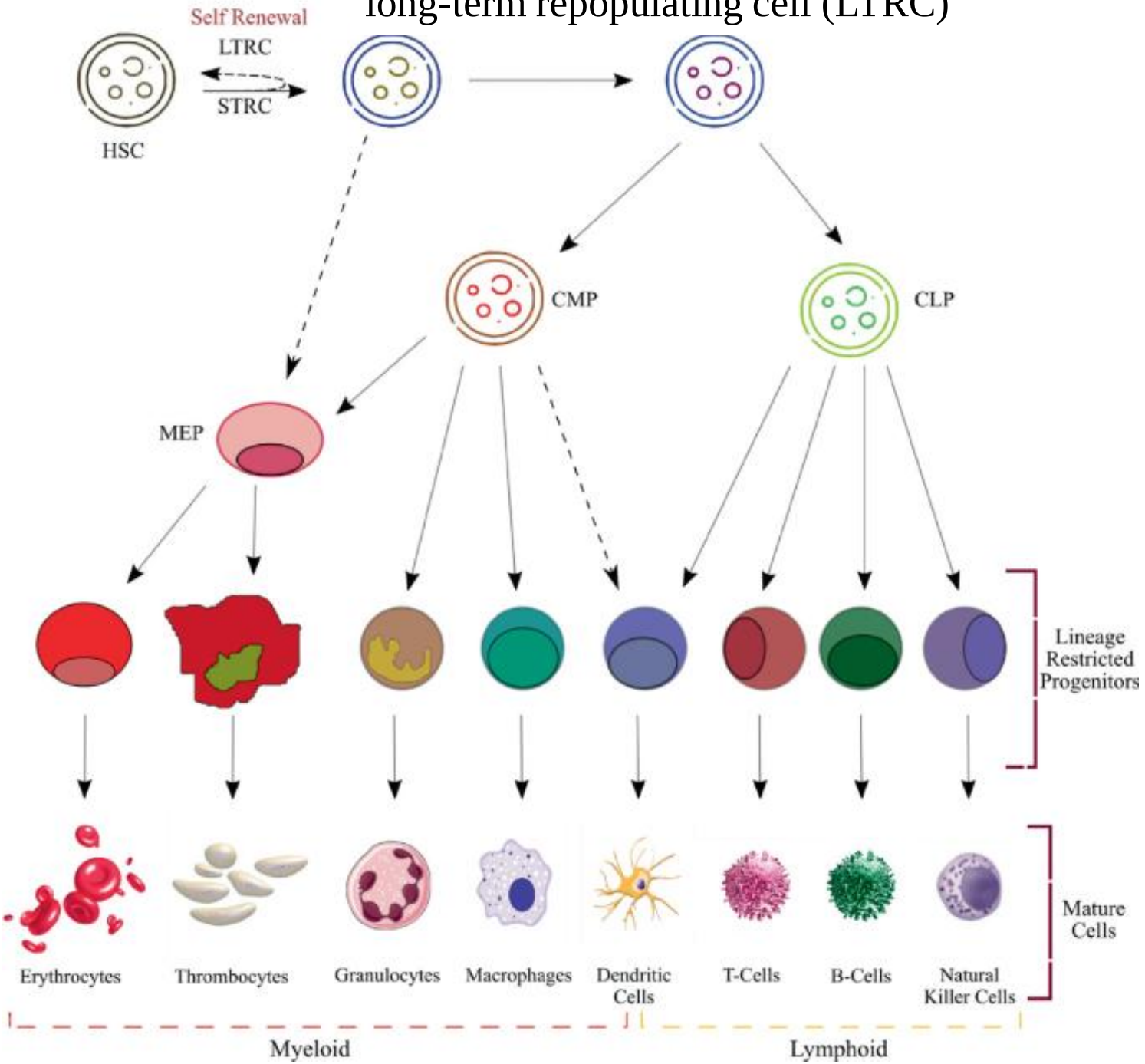
colony forming unit-granulocyte, erythrocyte, monocyte, megakaryocyte (CFU-GEMM) – responsible for the formation of myeloid cell lines (erythrocytes, granulocytes, monocytes, and platelets)

- **common lymphoid progenitor**

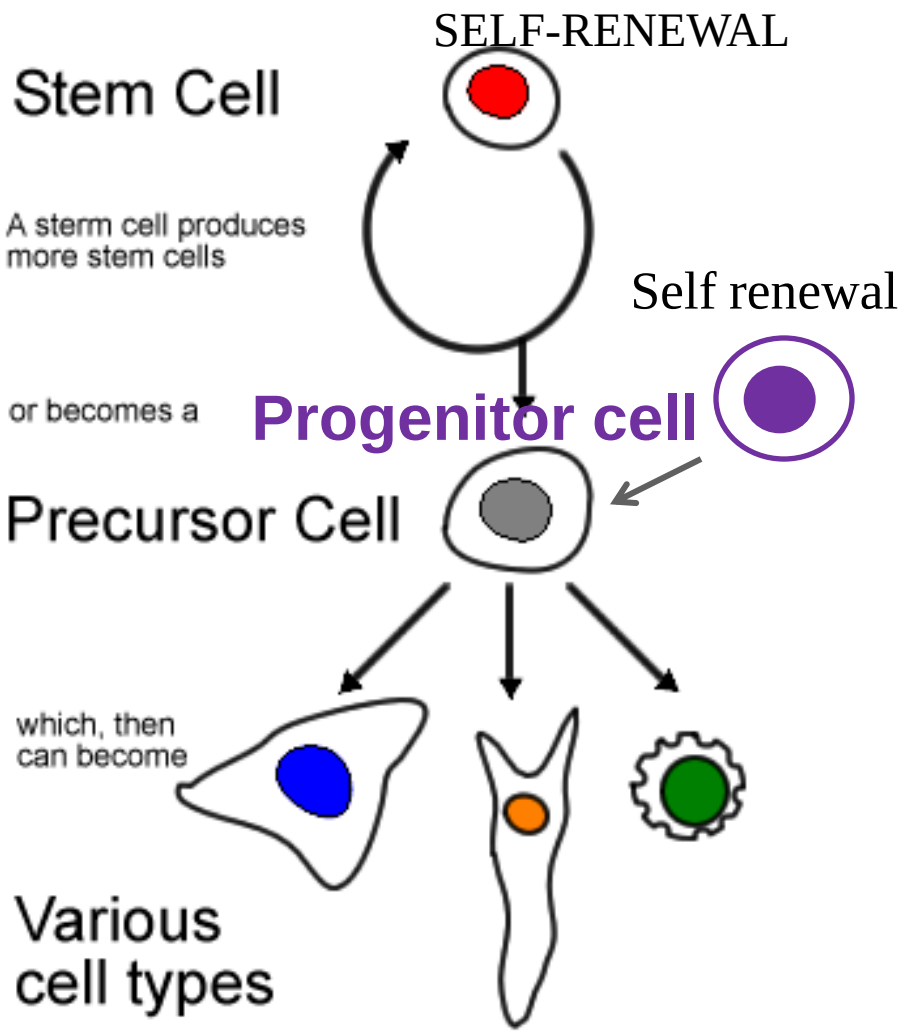
colony forming unit–lymphocyte (CFU-Ly) – responsible for the formation of lymphoid cell lines (T cells and B lymphocytes)

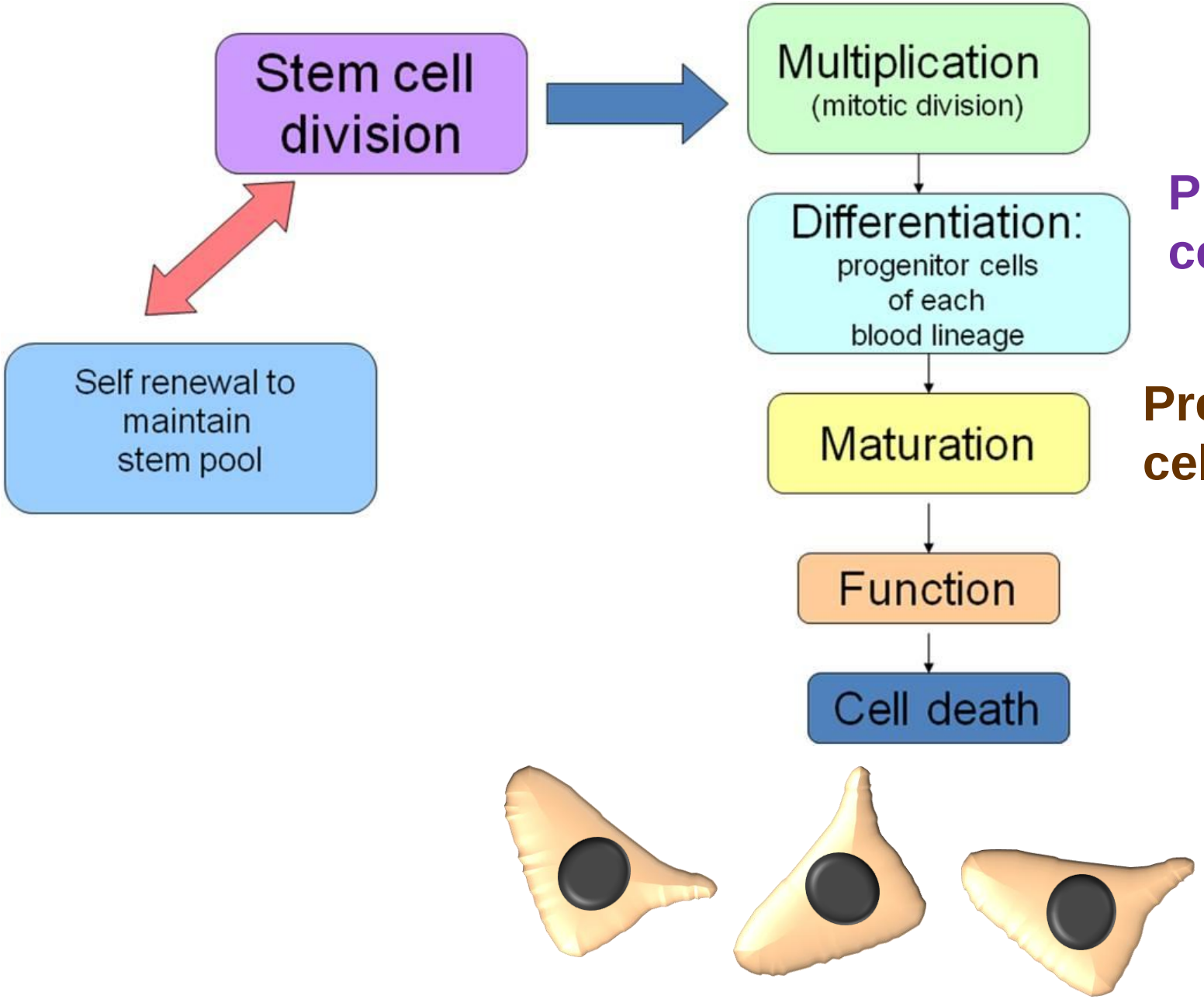

Multipotential hematopoietic stem cell (Hemocytoblast)
Common myeloid progenitor
Common lymphoid progenitor
Megakaryocyte
Thrombocytes
Erythrocyte
Mast cell
Myeloblast
Basophil
Neutrophil
Eosinophil
Monocyte
Macrophage
Natural killer cell (Large granular lymphocyte)
Small lymphocyte
T lymphocyte
B lymphocyte
Plasma cell

long-term repopulating cell (LTRC)



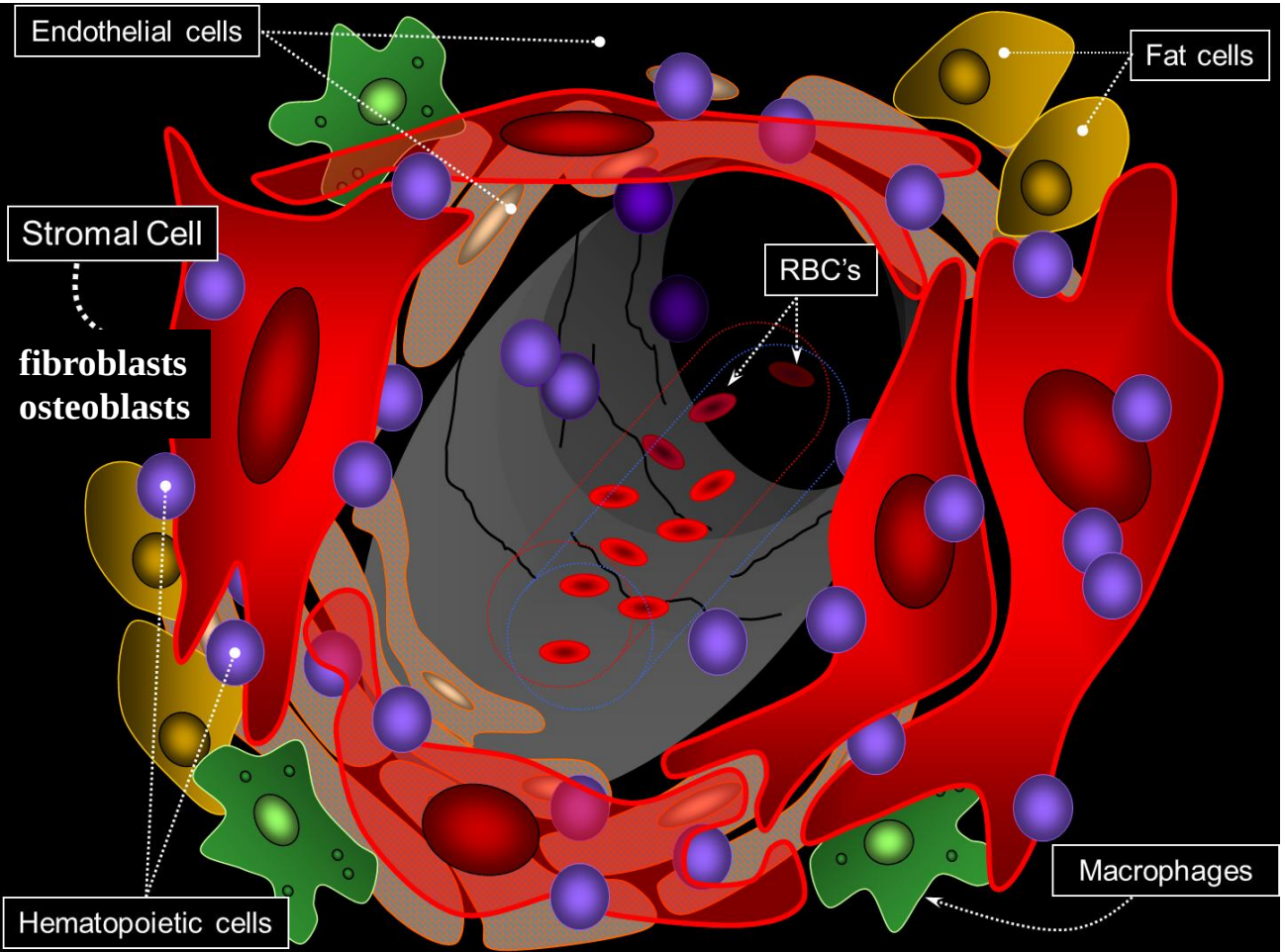
The main difference between progenitor and precursor cells is that **progenitor cells** are mainly multipotent cells that can differentiate into **many types** of cells, whereas **precursor cells** are unipotent cells that can only differentiate into a **particular type** of cells. Furthermore, progenitor cells are less differentiated cells, while precursor cells are more differentiated than progenitor cells.




Stem cell division
Multiplication
(mitotic division)
Differentiation:
progenitor cells
of each
blood lineage
Maturation
Function
Cell death
Self renewal to
maintain
stem pool
Progenitor cell
Precursor cell

Hemopoietic Growth Factors

1. Stem cell factor – promotes hemopoiesis – produced by stromal cells of bone marrow (is inserted into their cell membranes). Acts on multipotential stem cells



Stem cells must come in contact with stromal cells before division

2. Colony-stimulating factors (CSFs)

- are secreted glycoproteins that bind to receptor proteins on the surfaces of hemopoietic stem cells or progenitor cells, thereby activating intracellular signaling pathways that can cause the cells to proliferate and differentiate into a specific kind of blood cell (usually white blood cells)
- produced by T cells, macrophages and endothelial cells

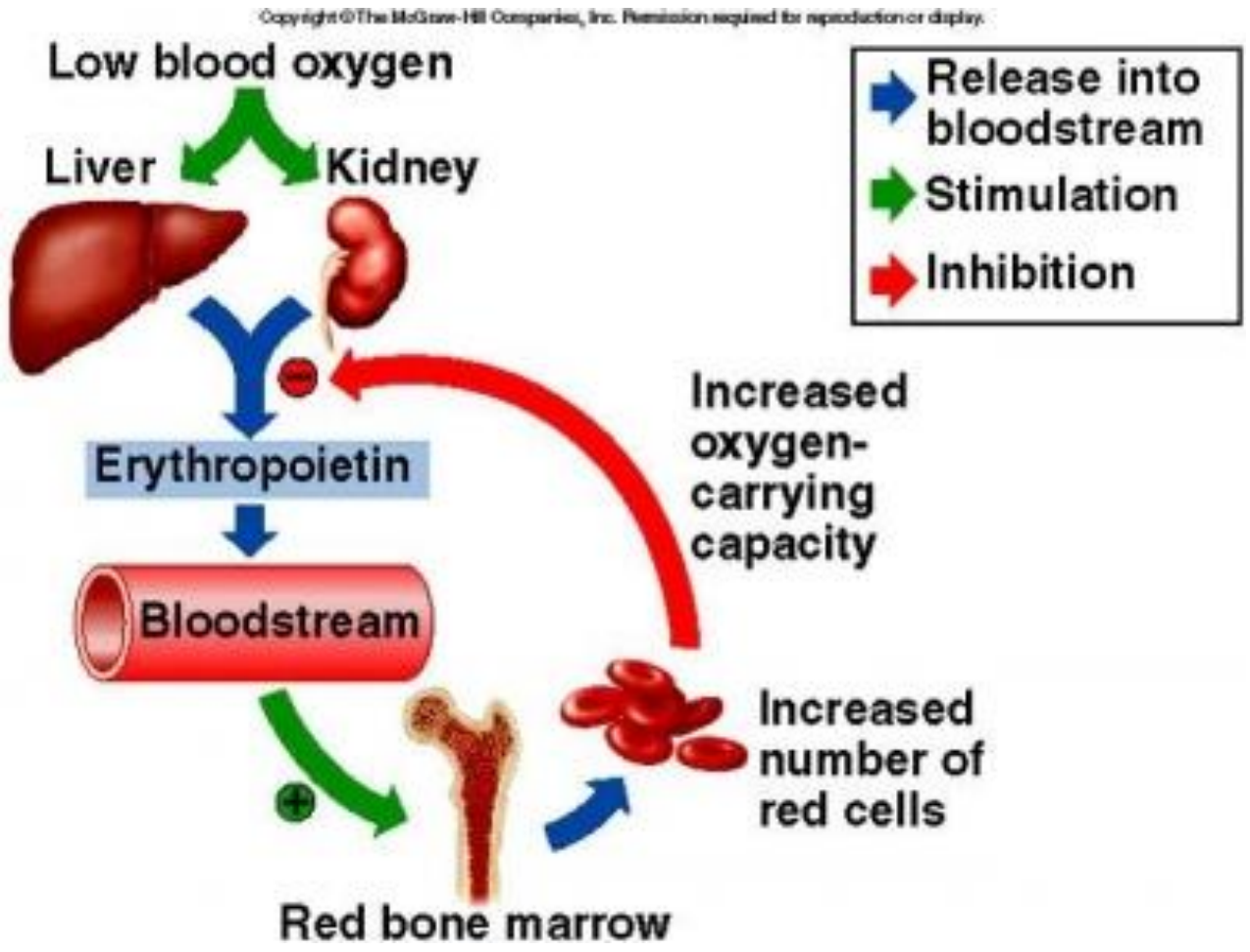
Granulocyte-macrophage colony-stimulating factor (**GM-CSF**) – is a cytokine that functions as a white blood cell growth factor. GM-CSF stimulates stem cells to produce granulocytes (neutrophils, eosinophils, and basophils) and monocytes.

Granulocyte colony-stimulating factor (**G-CSF) -** is a glycoprotein that stimulates the bone marrow to produce granulocytes

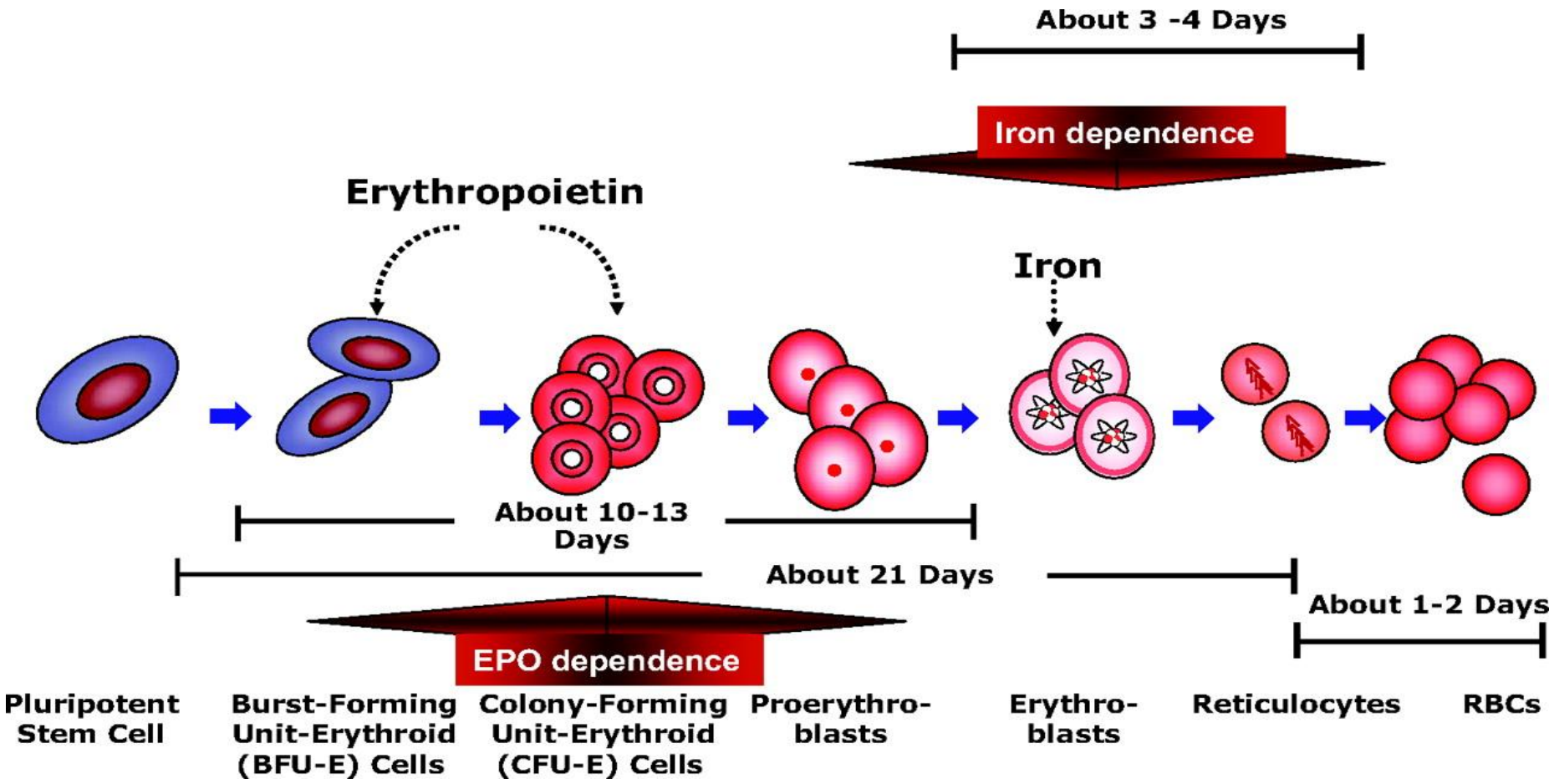
Macrophage colony-stimulating factor (M-CSF) - is a secreted cytokine which influences hematopoietic stem cells to differentiate into macrophages

Erythropoietin (EPO) - is a glycoprotein hormone that controls red blood cell production.

- produced by endothelial cells of kidney and hepatocytes of the liver

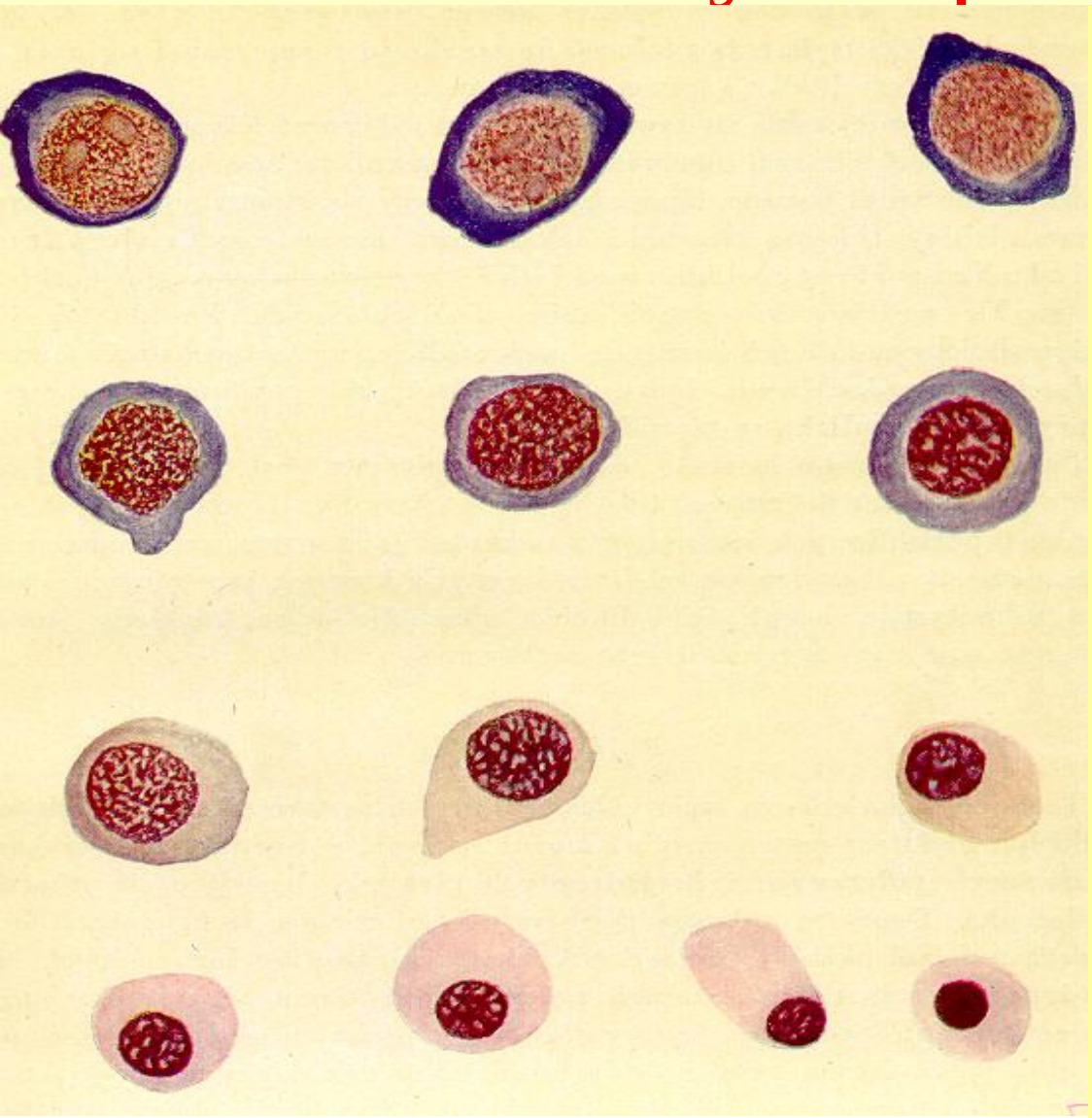


Erythropoiesis



- Generates 2.5 x 10^{11} erythrocytes every day

Erythropoiesis



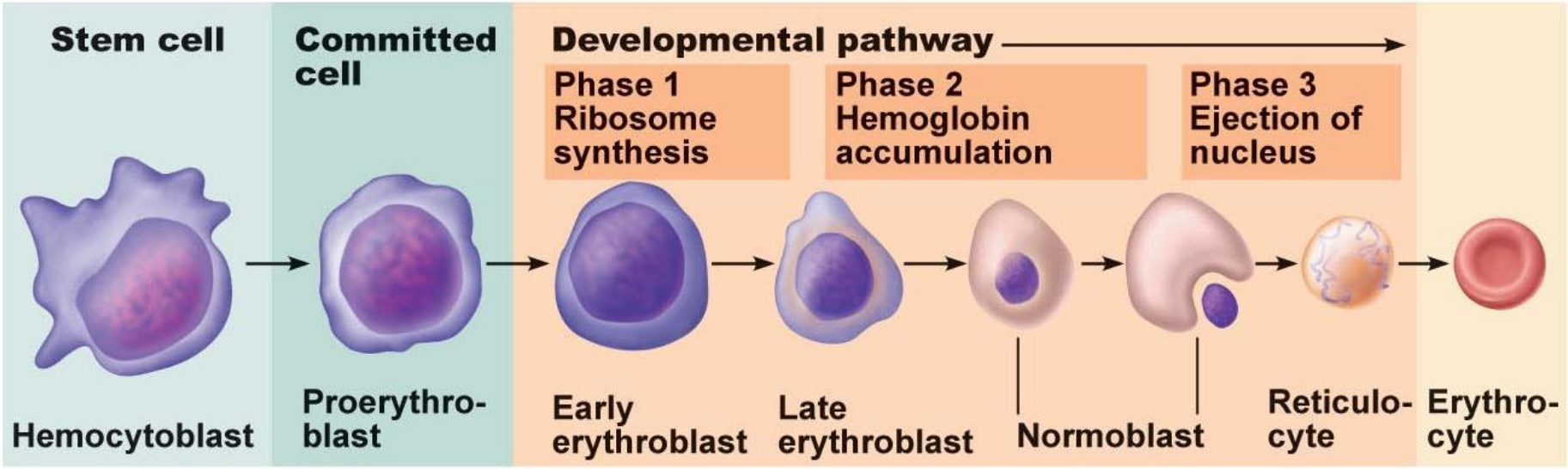
PROERYTHROBLASTS - the first identifiable stage of erythropoiesis. Proerythroblasts proliferate to generate:

Basophilic erythroblasts

Polychromatophilic erythroblasts

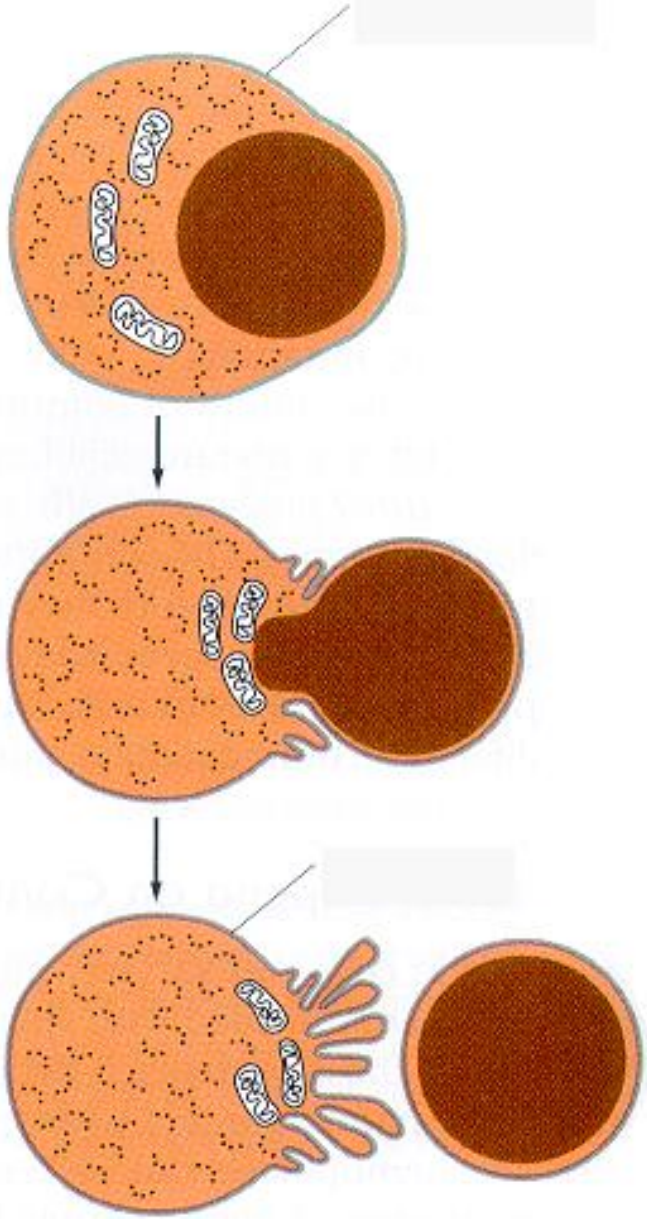
Ortochromatophilic erythroblasts (Normoblasts)

Cells are named after changes in the staining characteristic of their cytoplasm



Erythroblast – extrusion of nucleus

The nucleus is extruded from the normoblast



Ortochromatophilic erythroblast (normoblast)

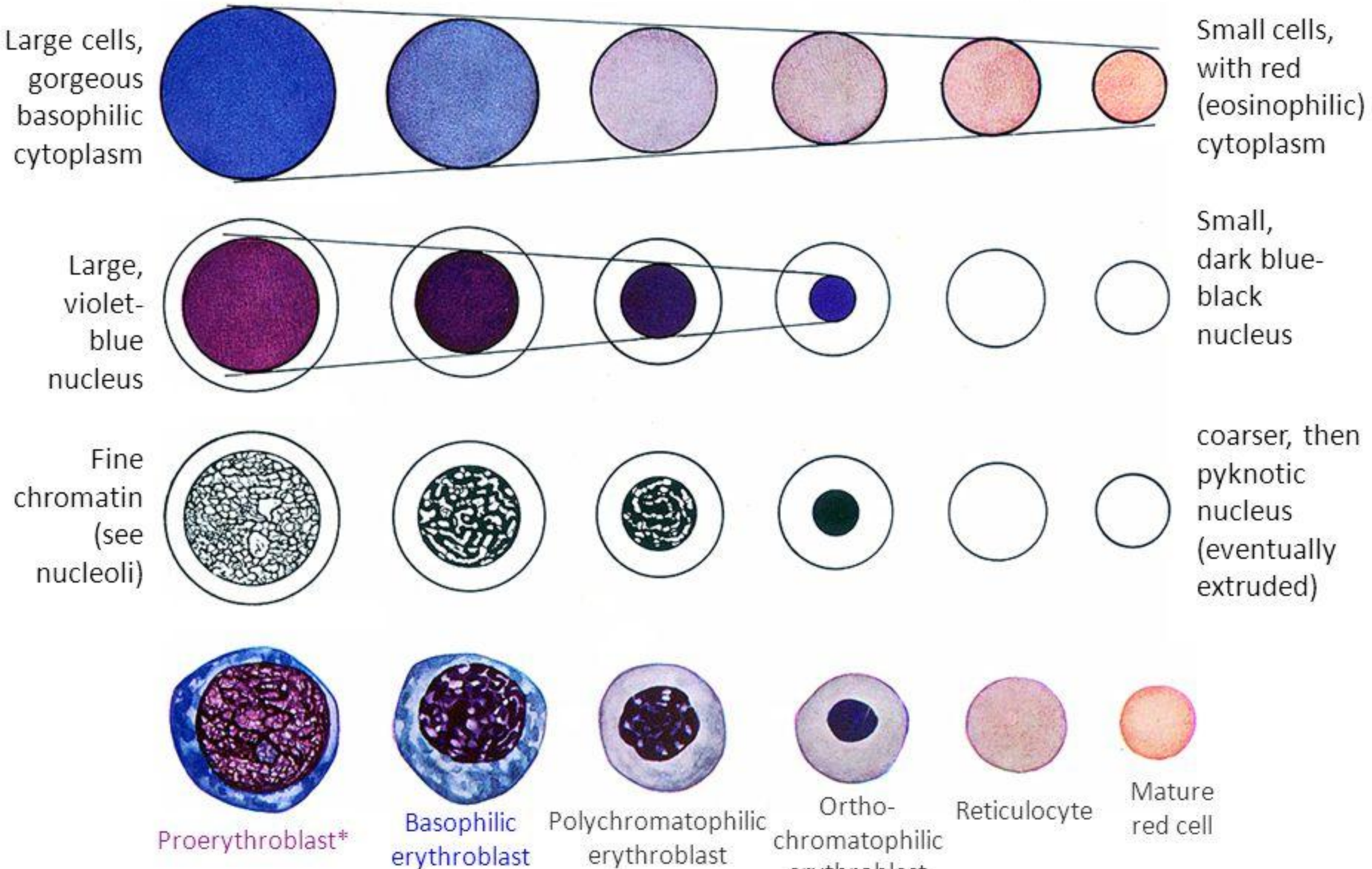
Extrusion of nucleus

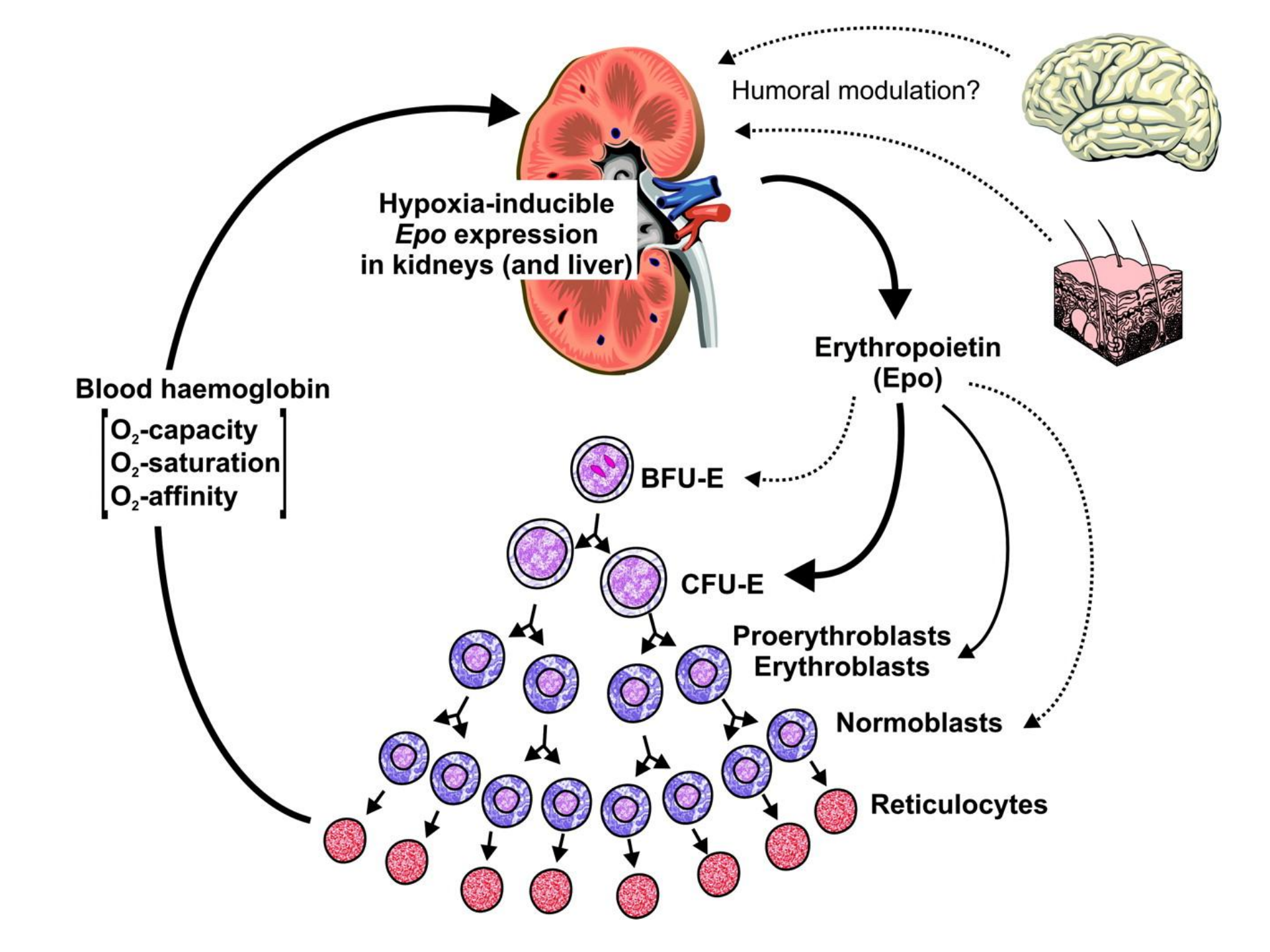
The cell enters circulation as a reticulocyte, which still contains some organelles

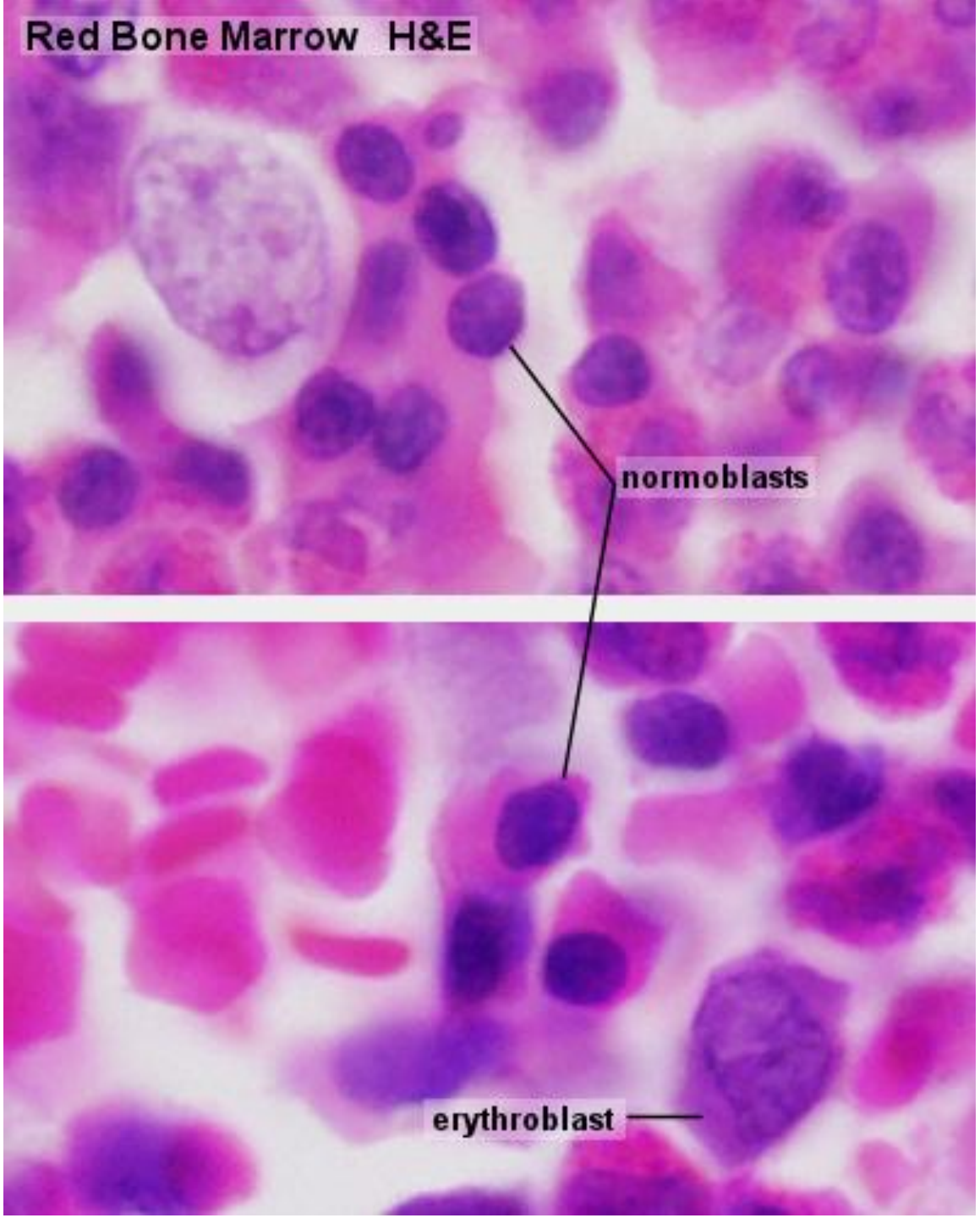
Reticulocyte

Morphologic Changes in Erythropoiesis

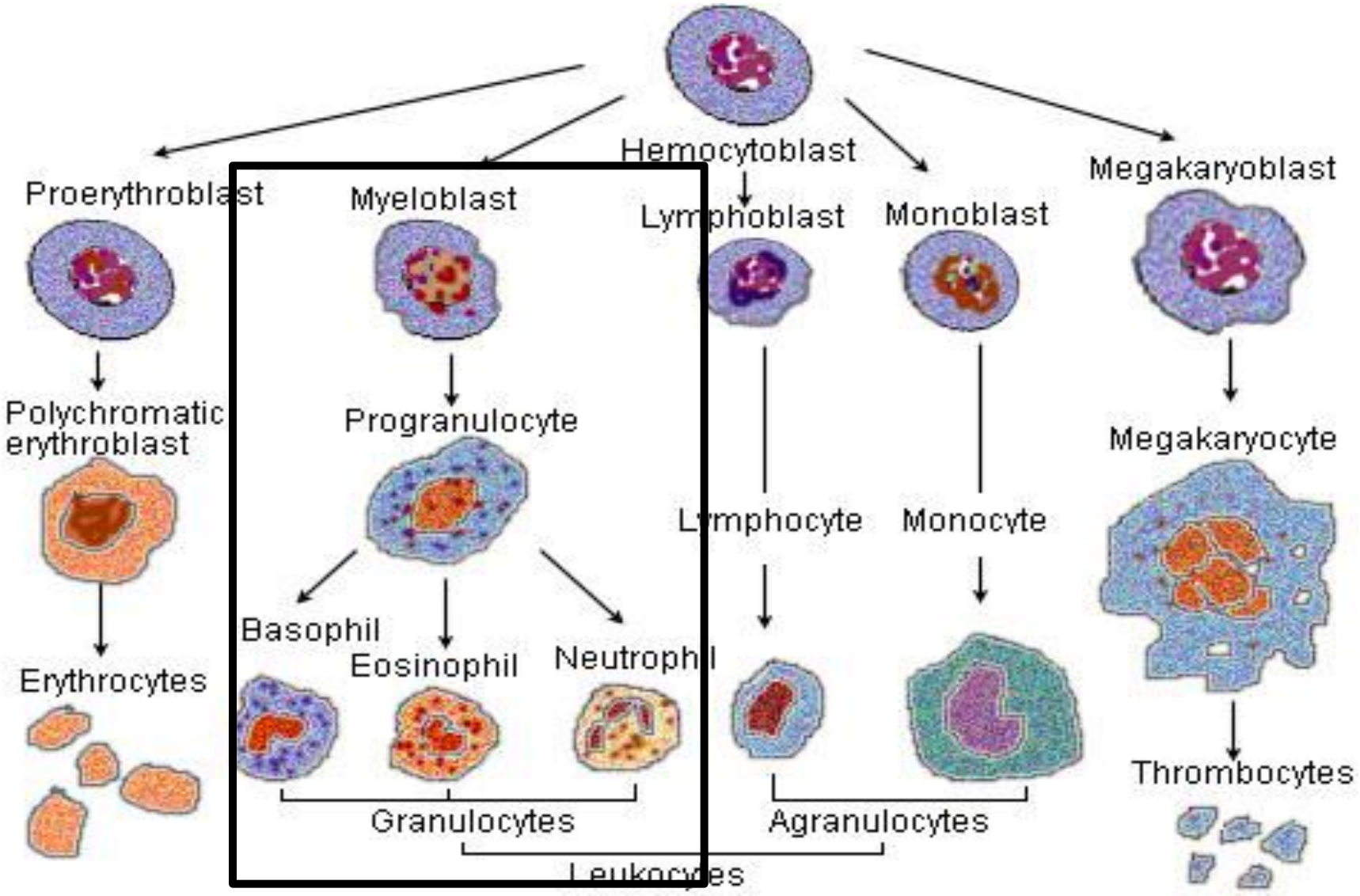
Immature ----------> Mature



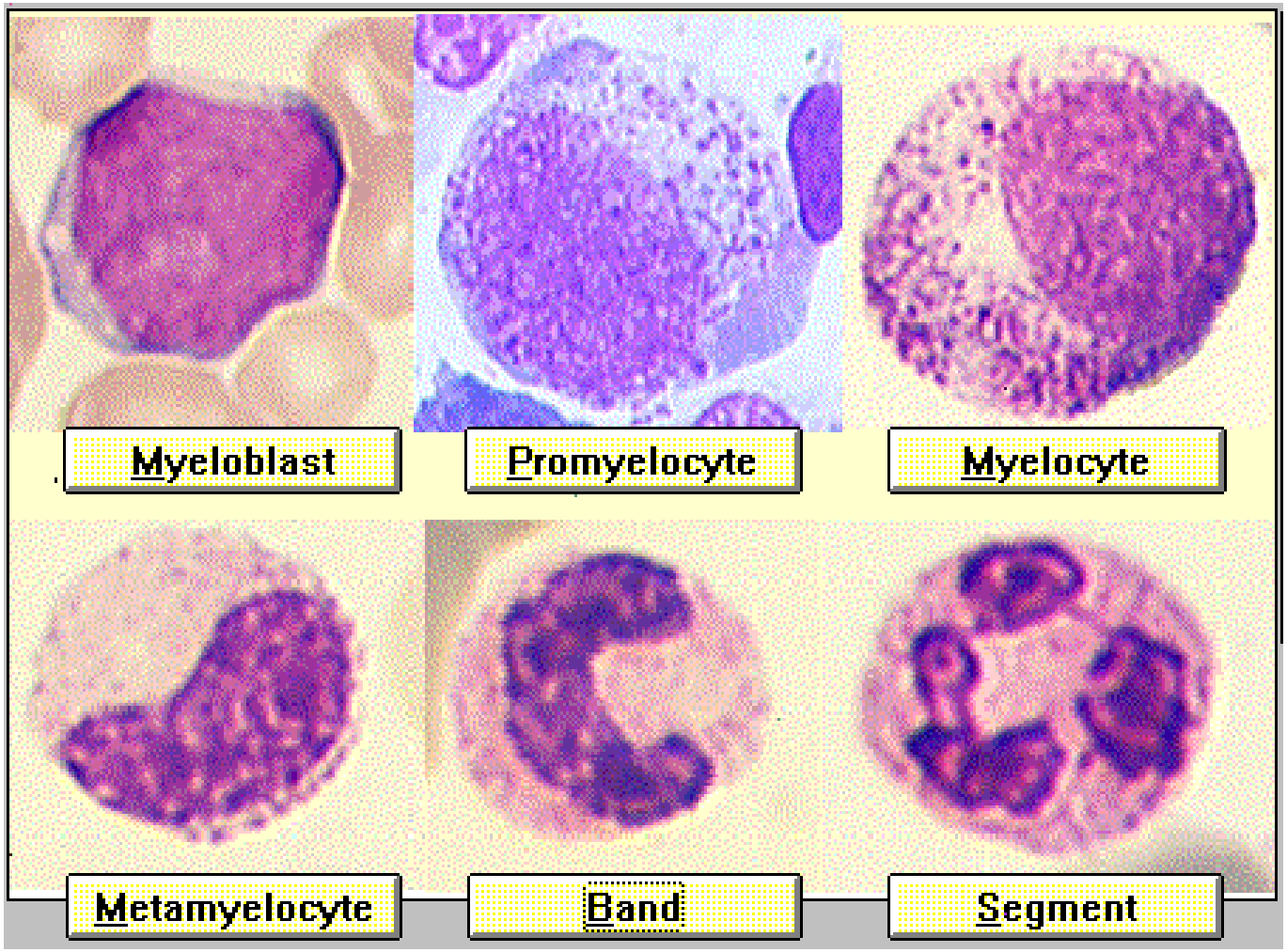

Humoral modulation?
Hypoxia-inducible
Epo expression
in kidneys (and liver)
Erythropoietin
(Epo)
Blood haemoglobin
O_2-capacity
O_2-saturation
O_2-affinity
BFU-E
CFU-E
Proerythroblasts
Erythroblasts
Normoblasts
Reticulocytes


Red Bone Marrow H&E
normoblasts
erythroblast

Granulocytopoiesis - formation of granulocytse (basophols, eosinophils and neutrophils) from precursor cell - myeloblast. Myeloblast derived from multipotent stem cell



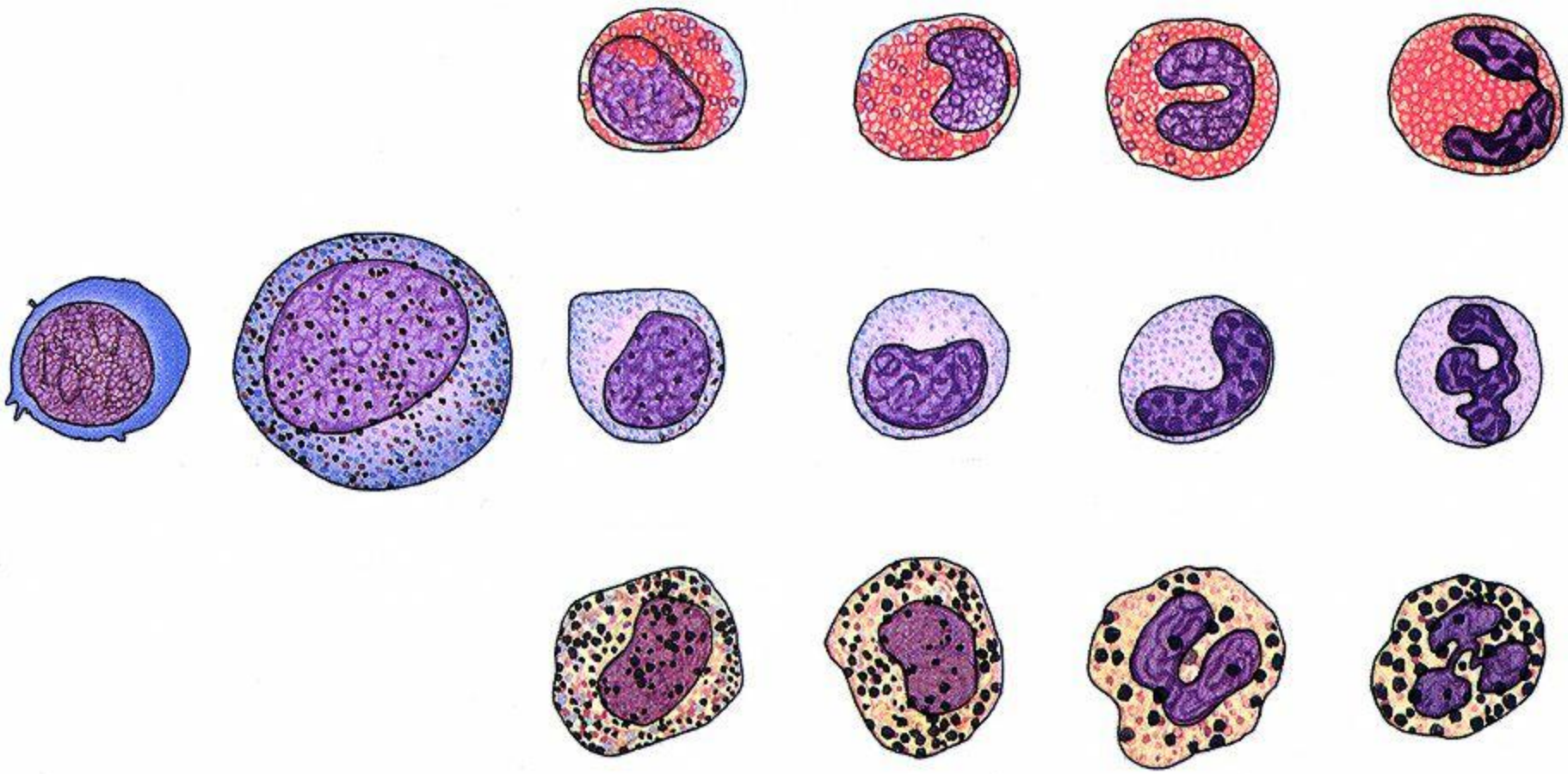
Myeloblasts - **proliferate to generate promyelocytes**



Promyelocytes contain nonspecific azurophilic granules (modified lysosomes). Myelocytes, metamyelocytes and mature cells contain azurophilic granules and specific granules

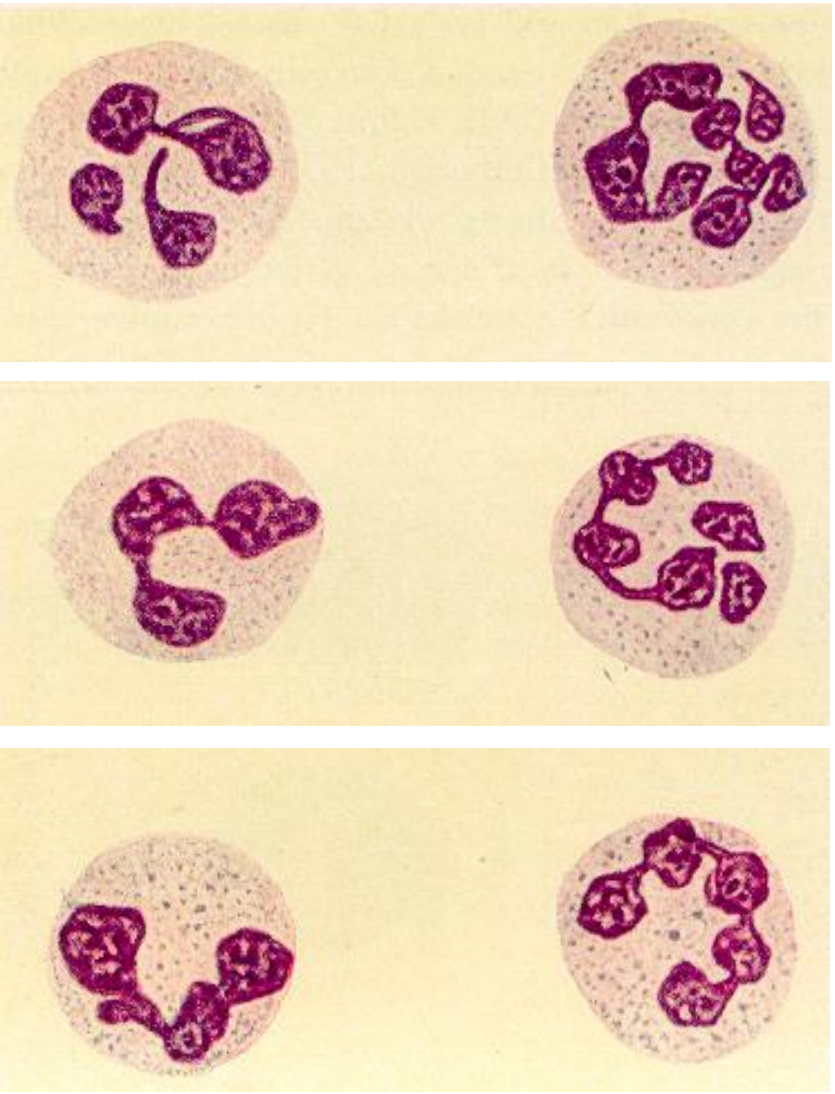
Morphologic Changes in Granulopoiesis

Immature ----------> Mature



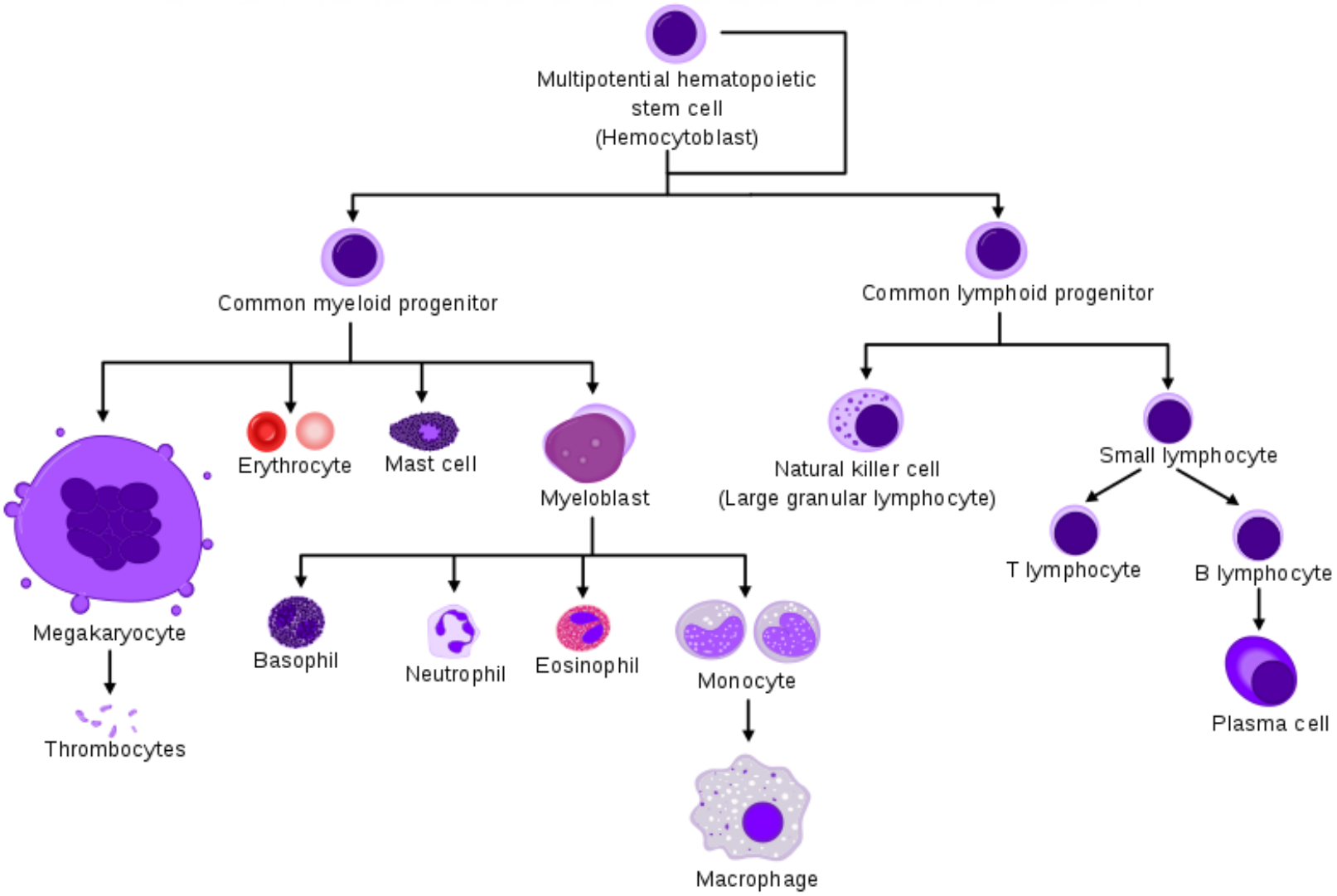
Cytoplasm loses its basophilic color. Chromatin becomes more condensed. Specific granules increase in number.

Old neutrophils



The lobulation of nuclei increases in ageing neutrophils. Neutrophils in peripheral blood stay for 8 – 12h and then pass into tissues. They remain active as microphages for 1-2 days and either degenerate or are eliminated with saliva, faeces or urine.

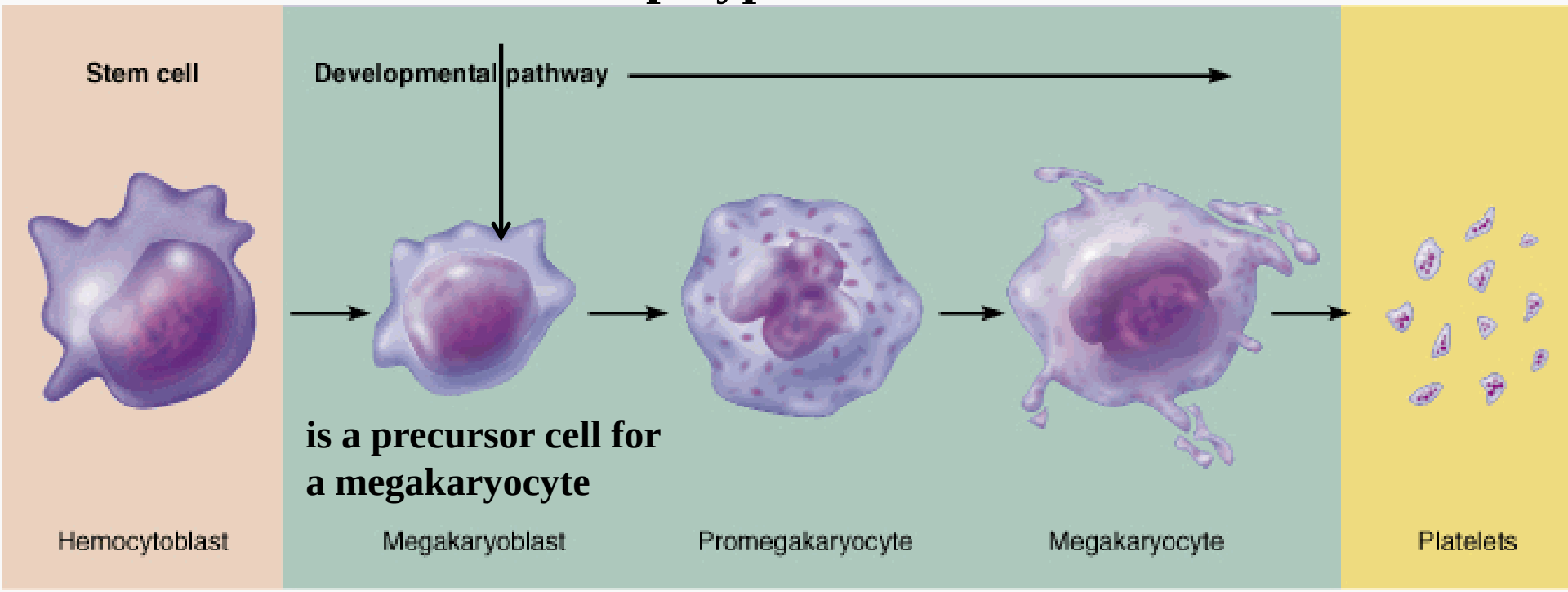
LYMPHOPOIESIS



undergo endomitosis nuclei become polyploid

Platelets formation

derived from fragmentation of megakaryocytes

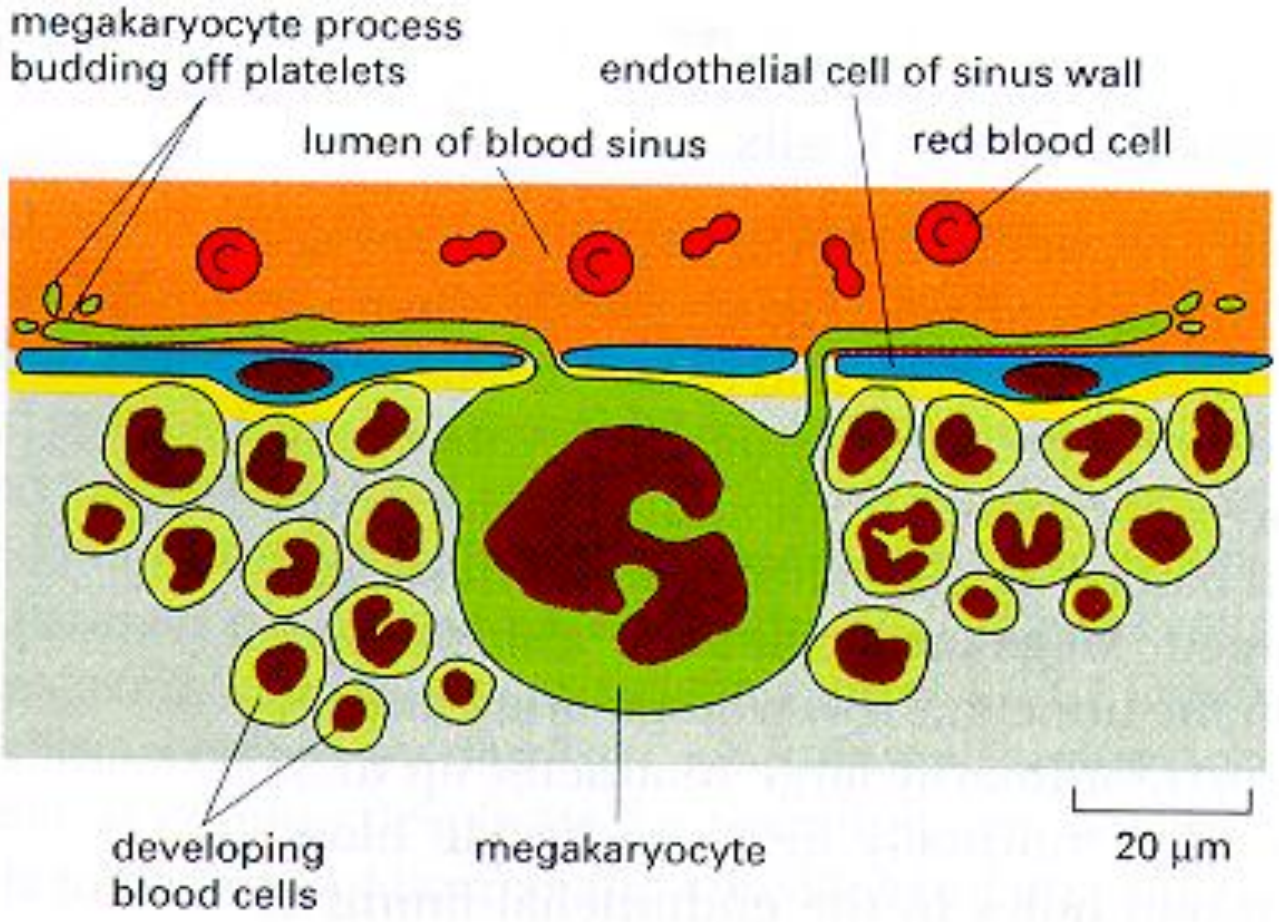


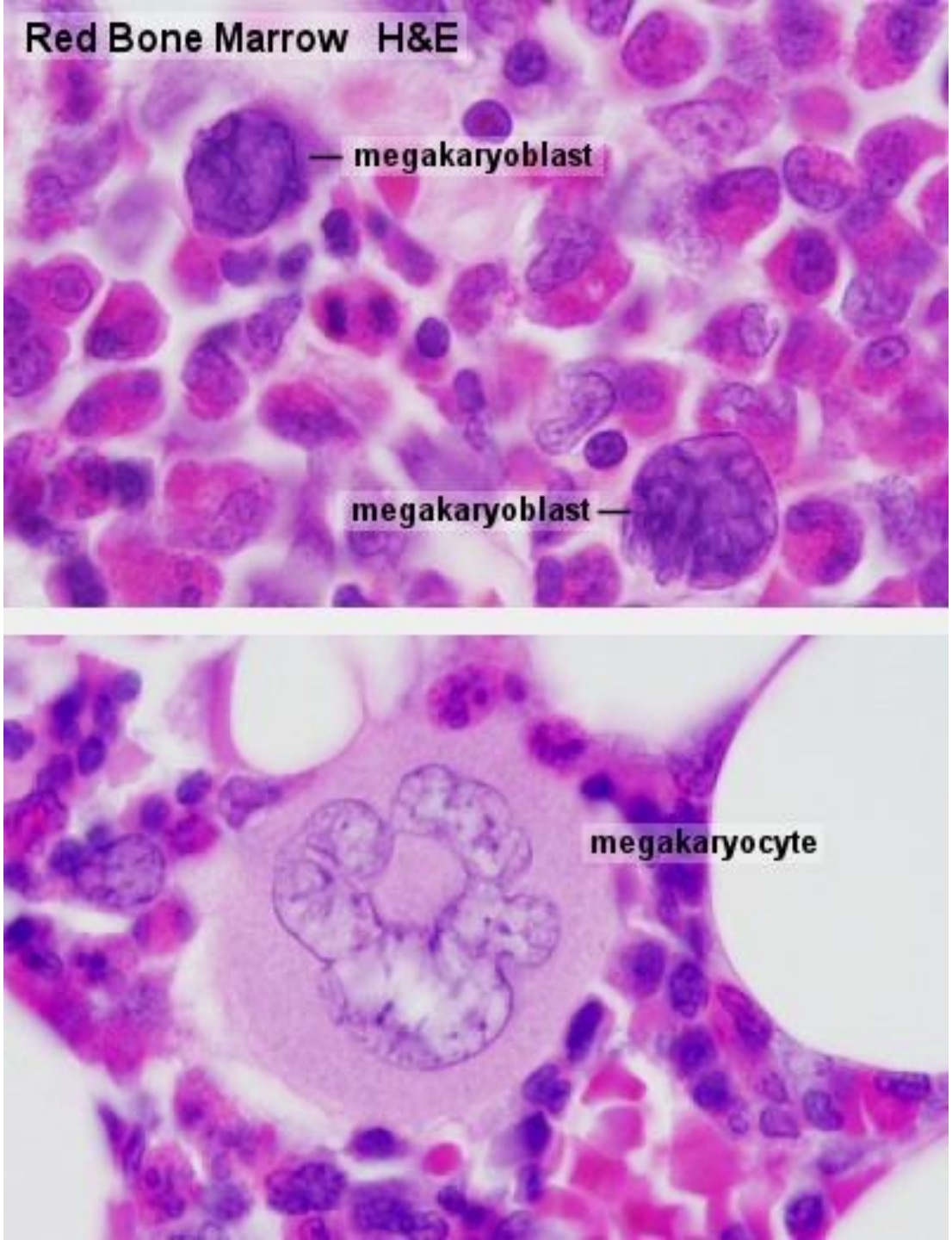
Endomitosis is a variant of mitosis without nuclear or cellular division, resulting in cells with many copies of the same chromosome occupying a single nucleus

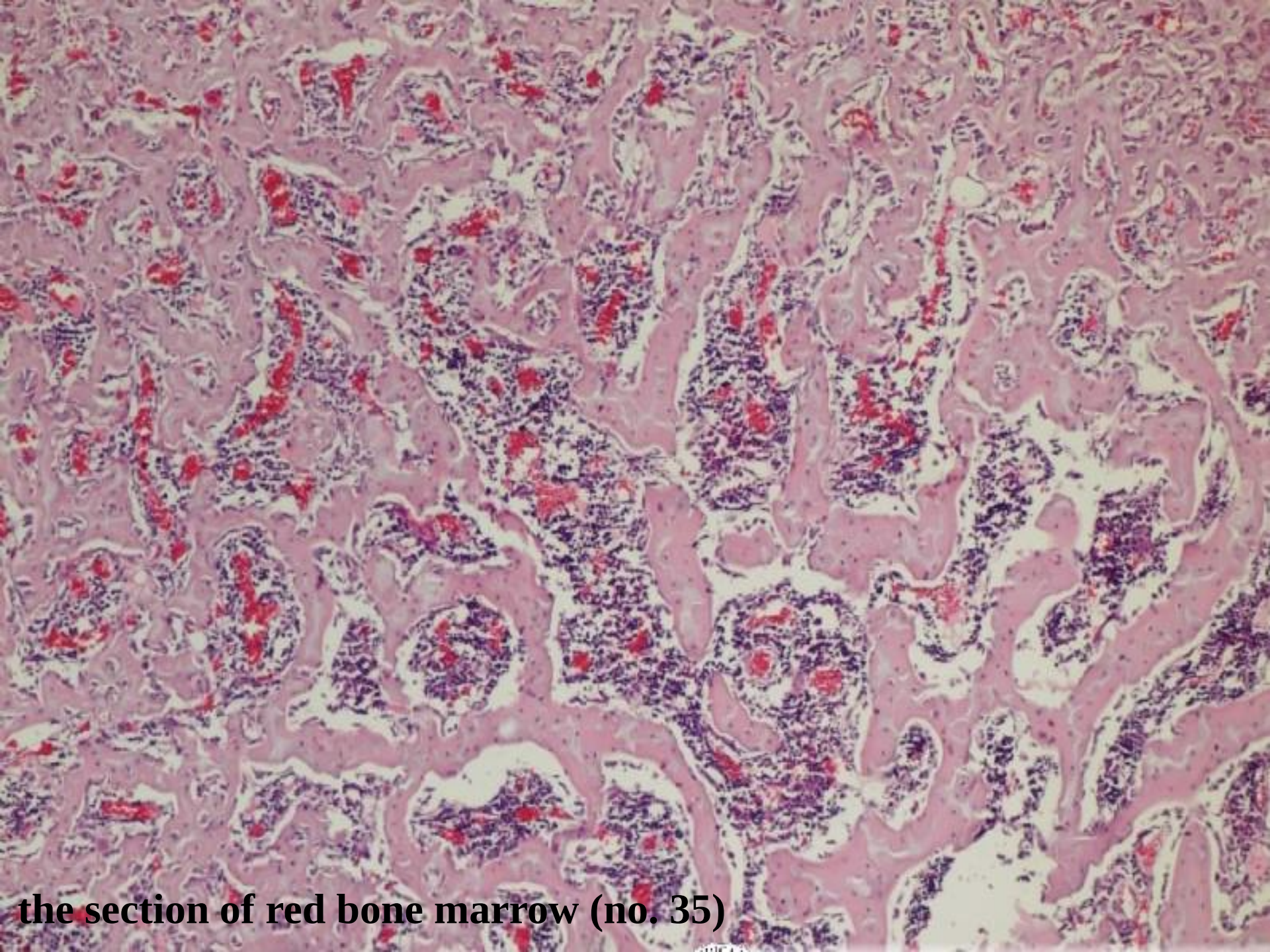
Thrombopoietin (THPO) also known as **megakaryocyte growth and development factor** (MGDF)

Platelets - Origin

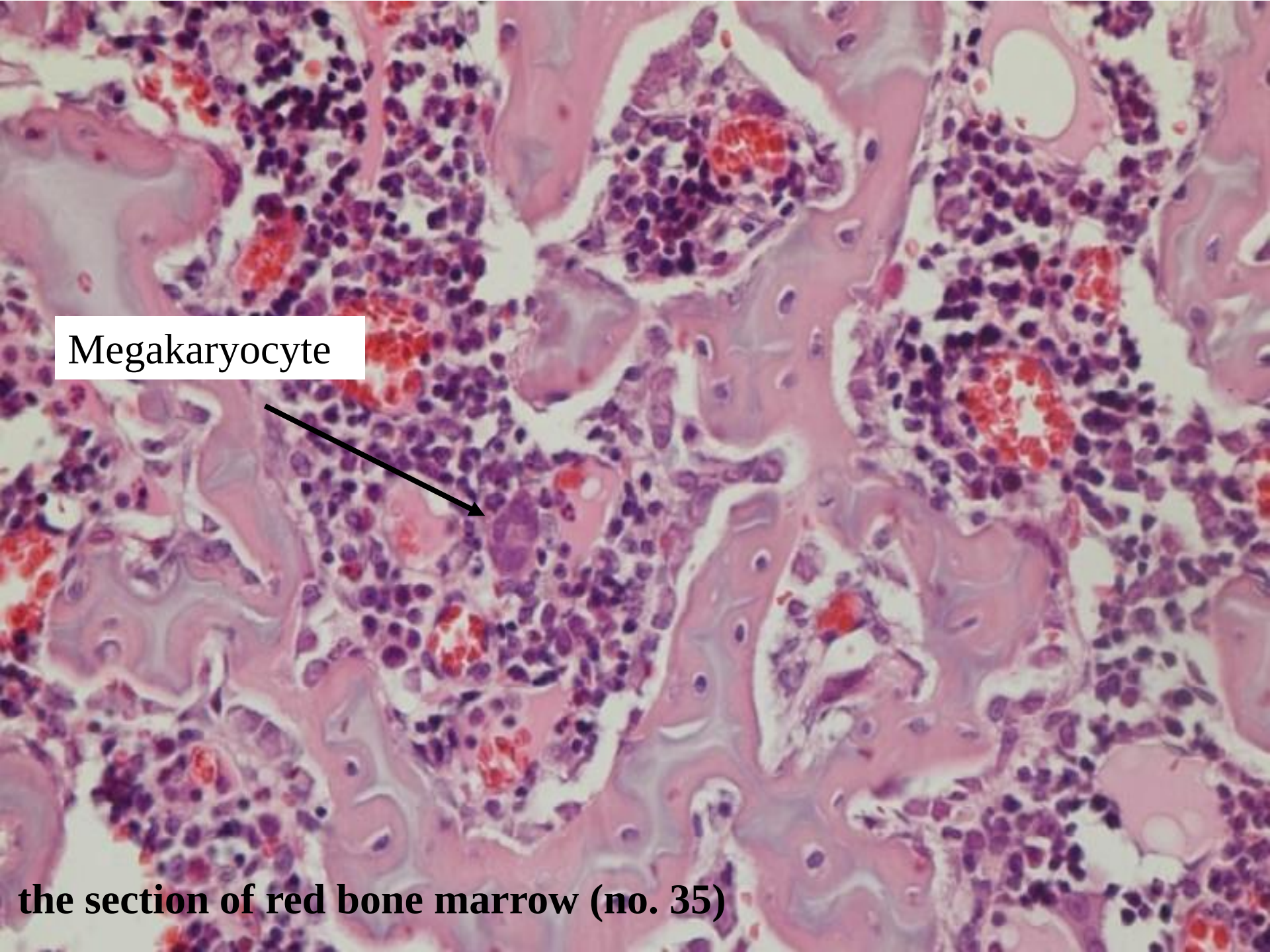
- megakaryocytes are located next to sinusoids, into which they protrude their cytoplasmic processes



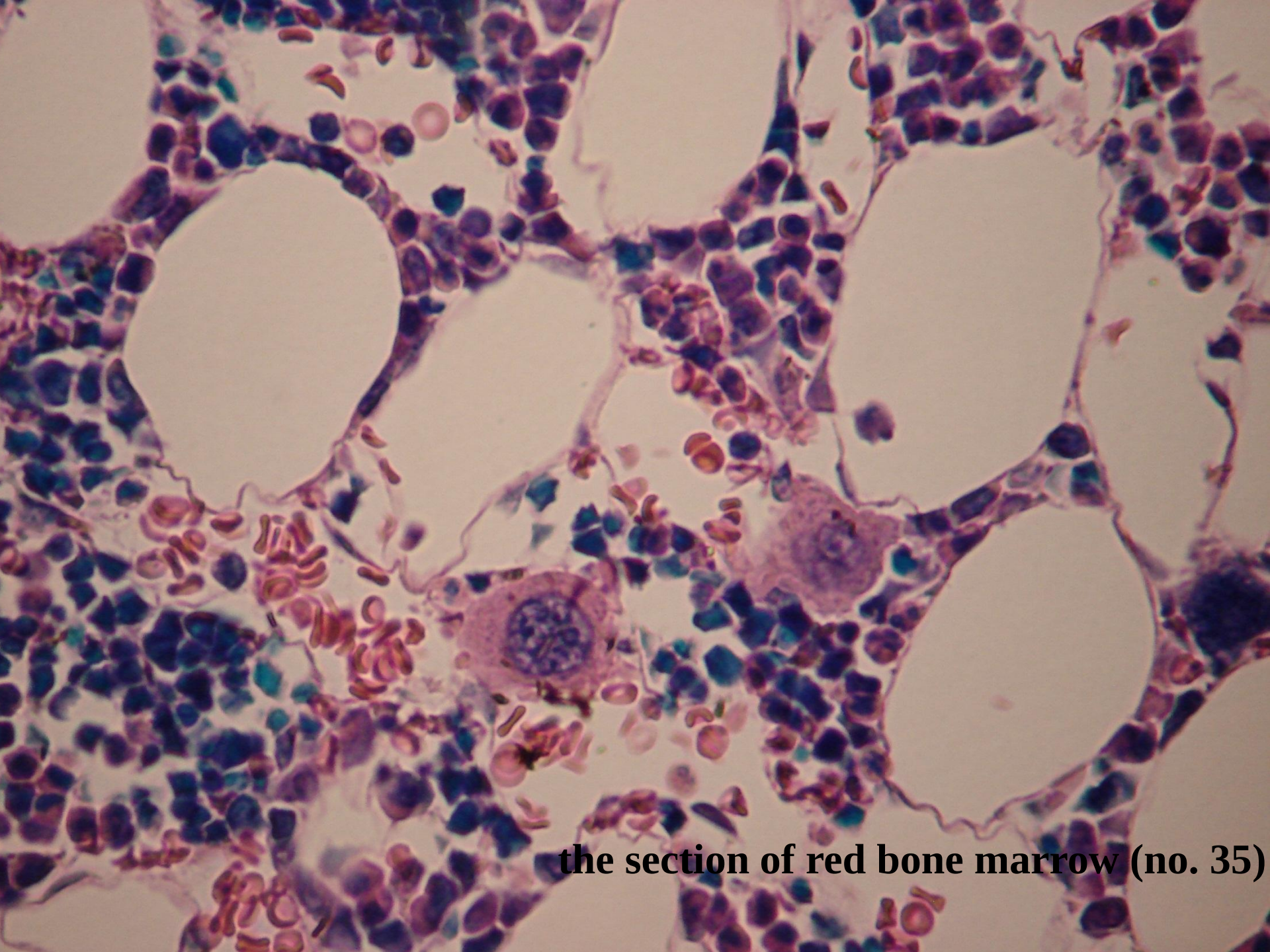

Red Bone Marrow H&E
megakaryoblast
megakaryoblast
megakaryocyte



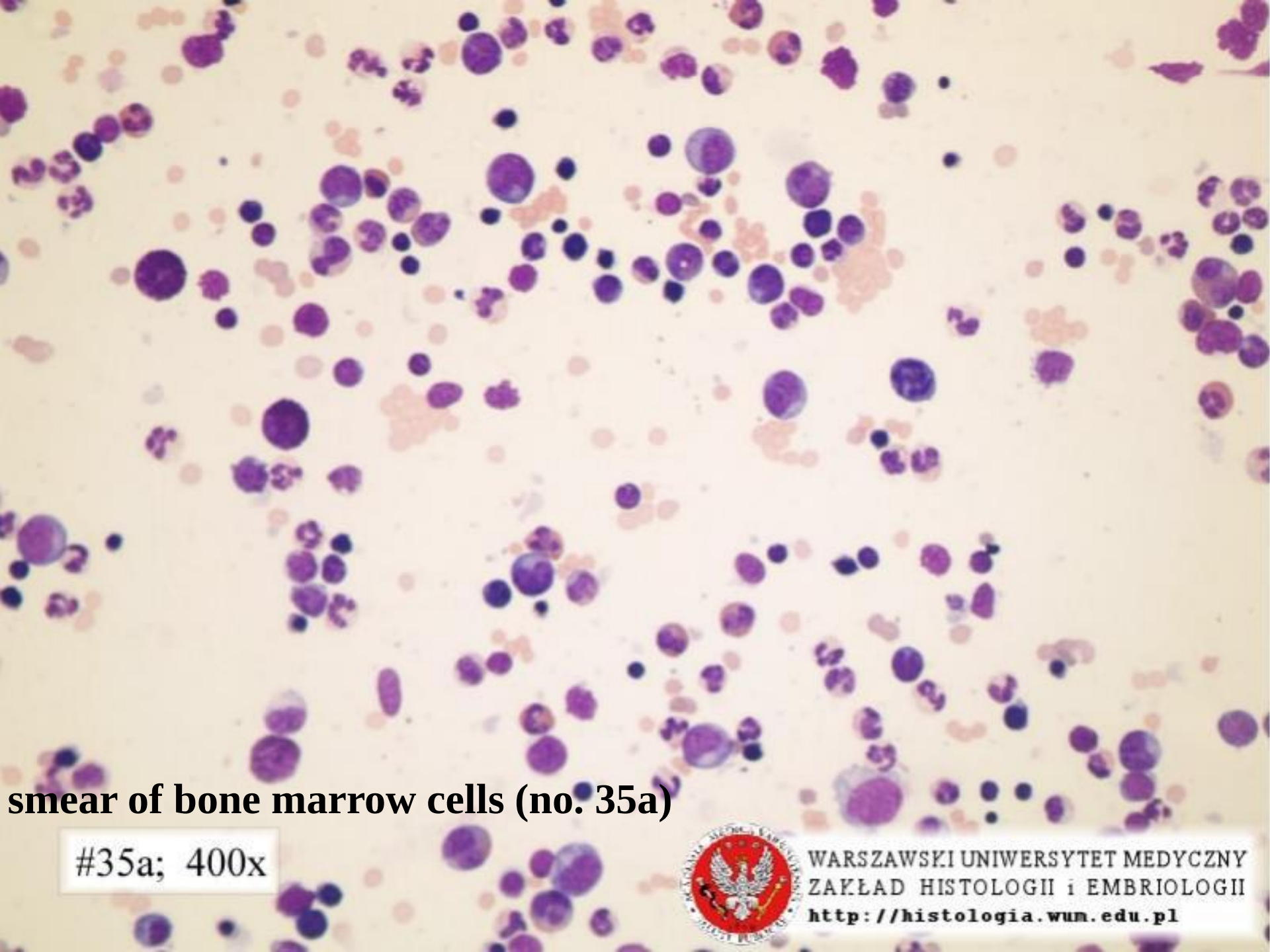
the section of red bone marrow (no. 35)



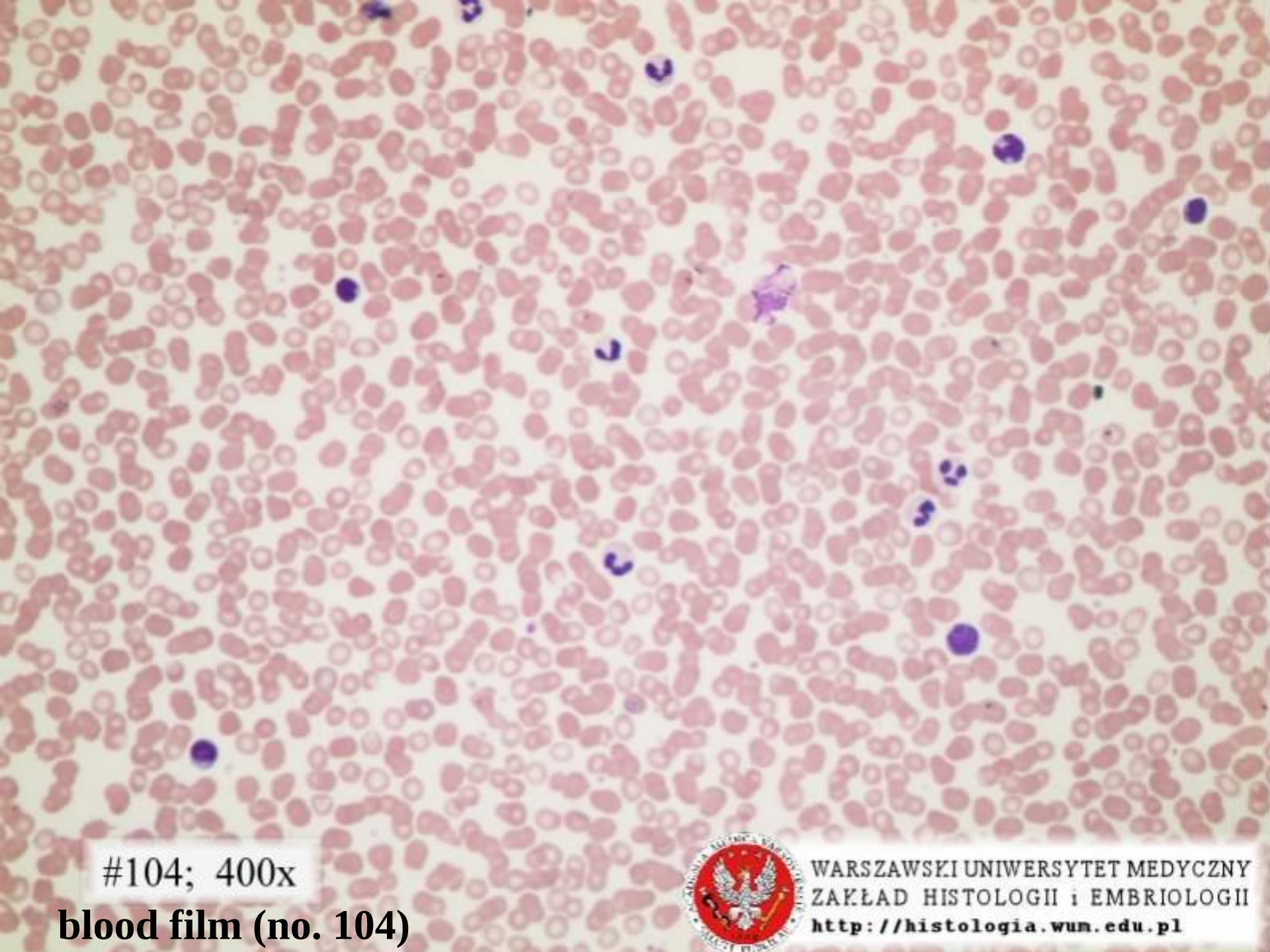
the section of red bone marrow (no. 35)



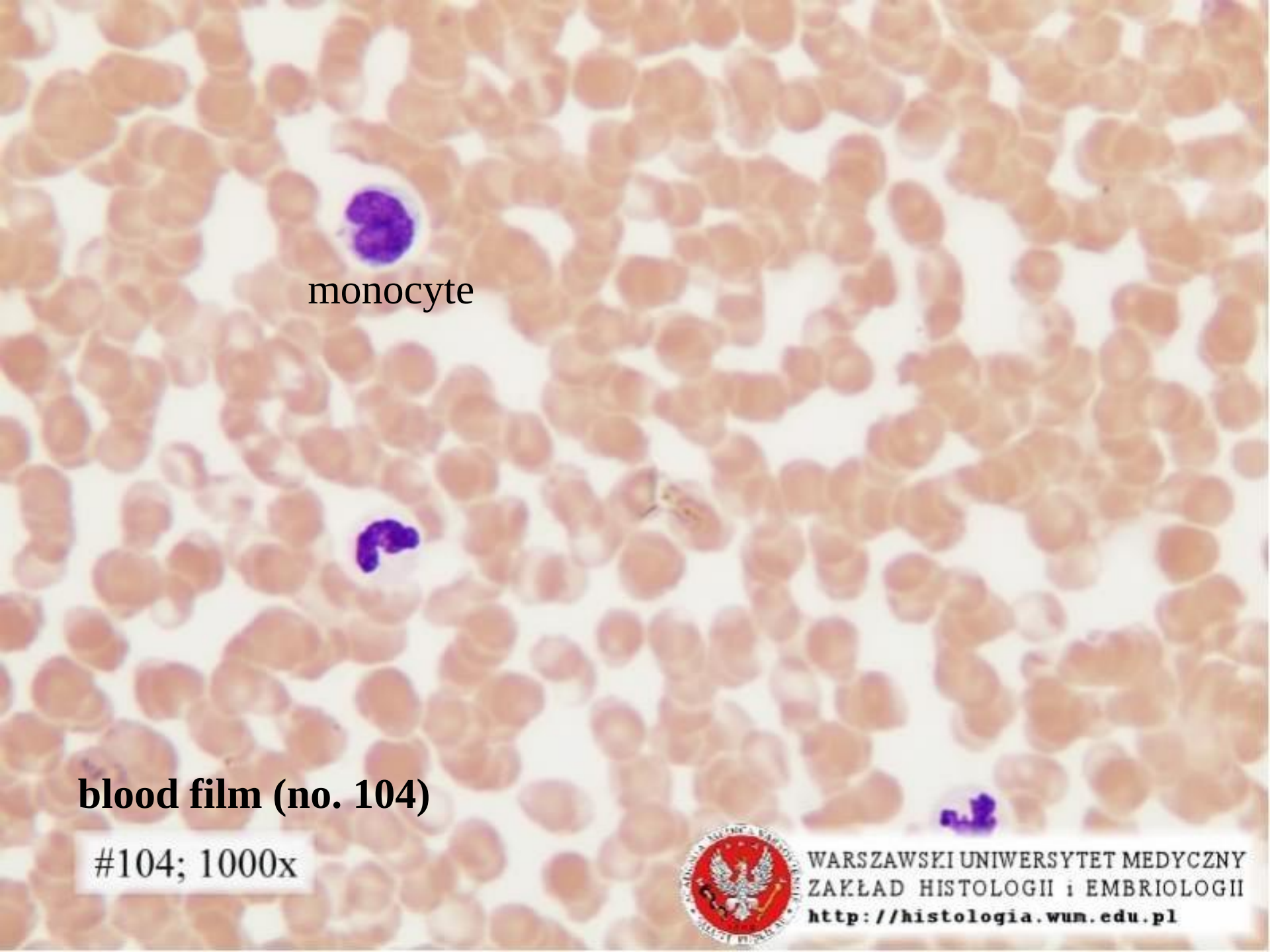
the section of red bone marrow (no. 35)

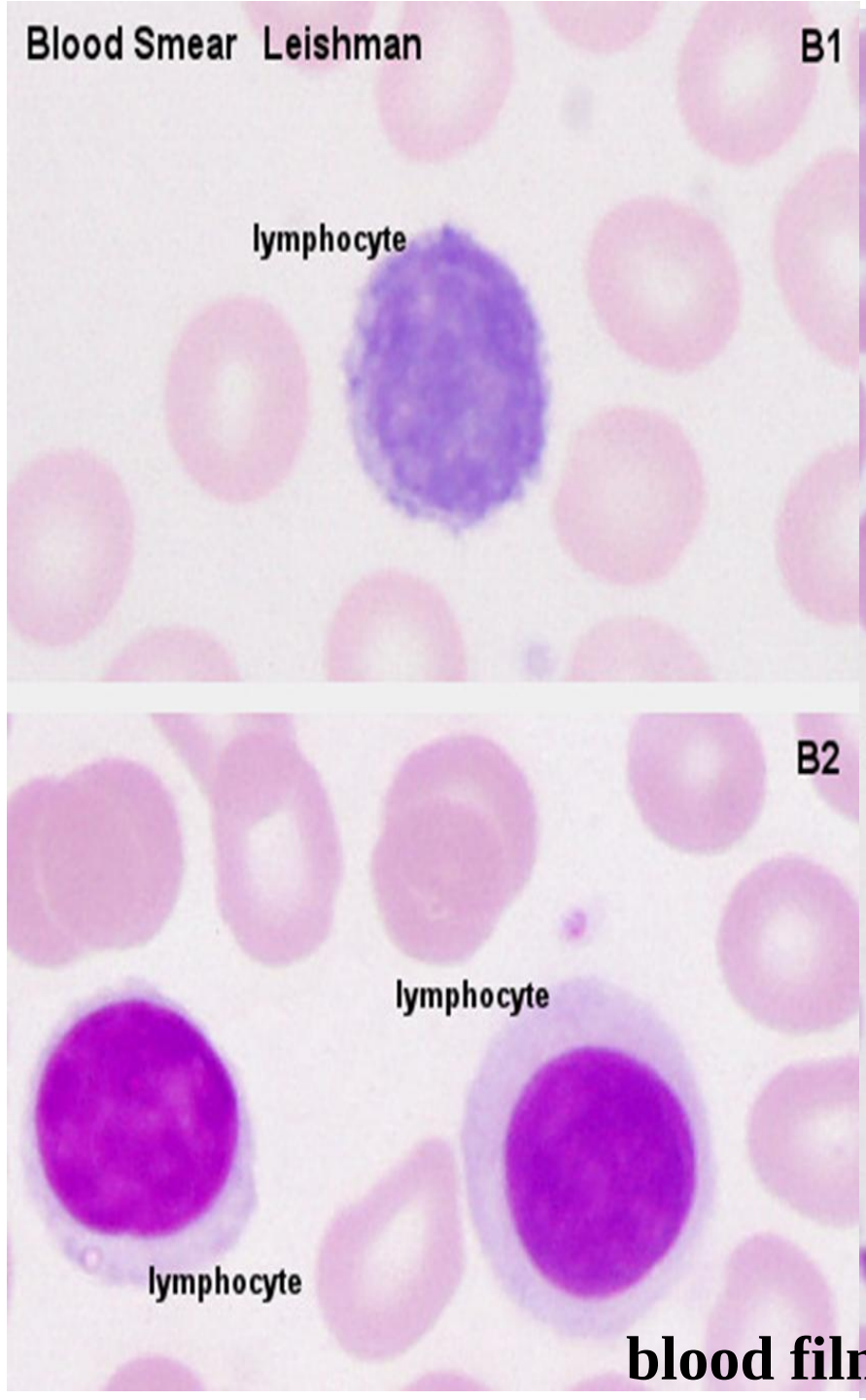


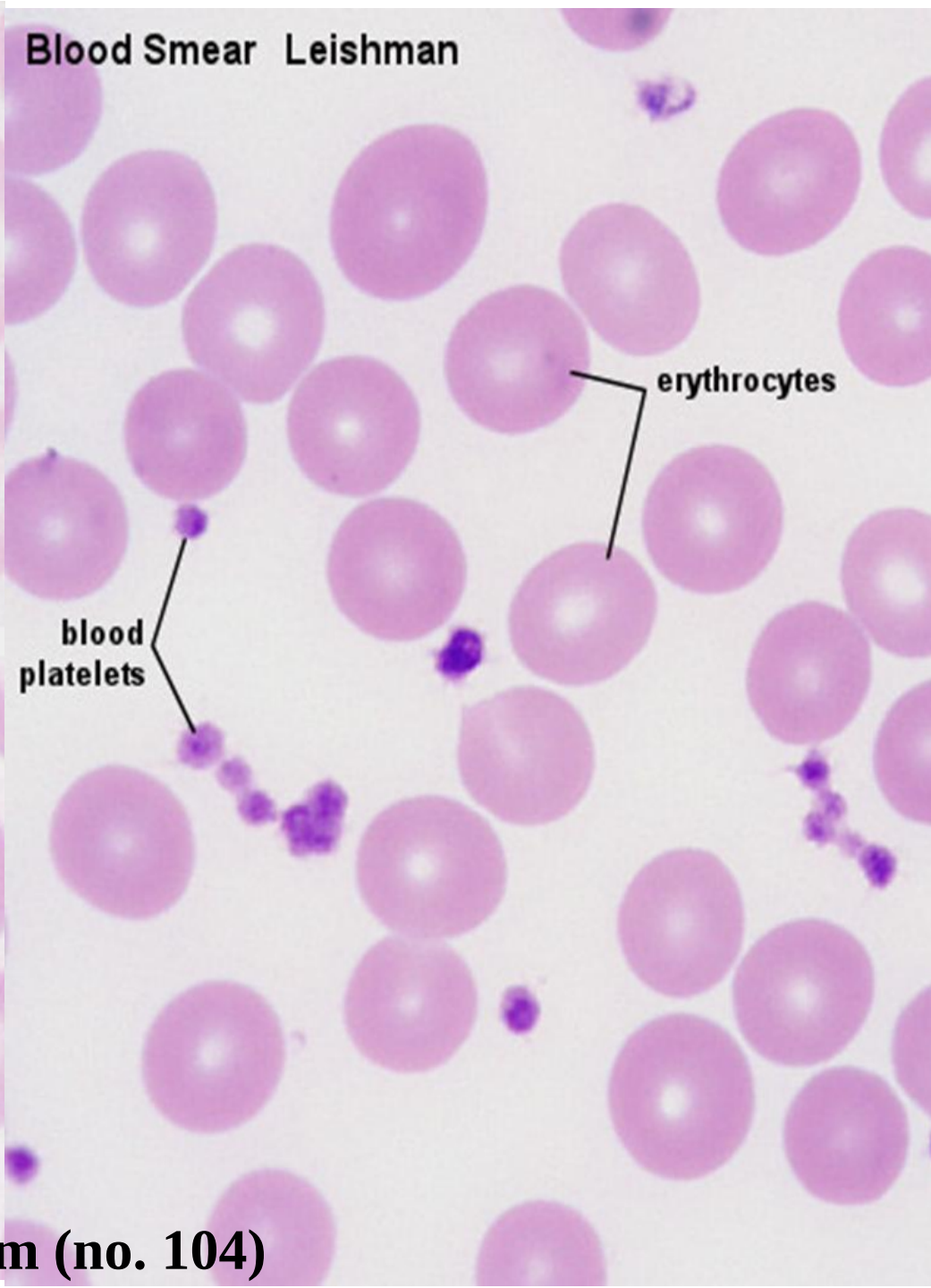
smear of bone marrow cells (no. 35a)



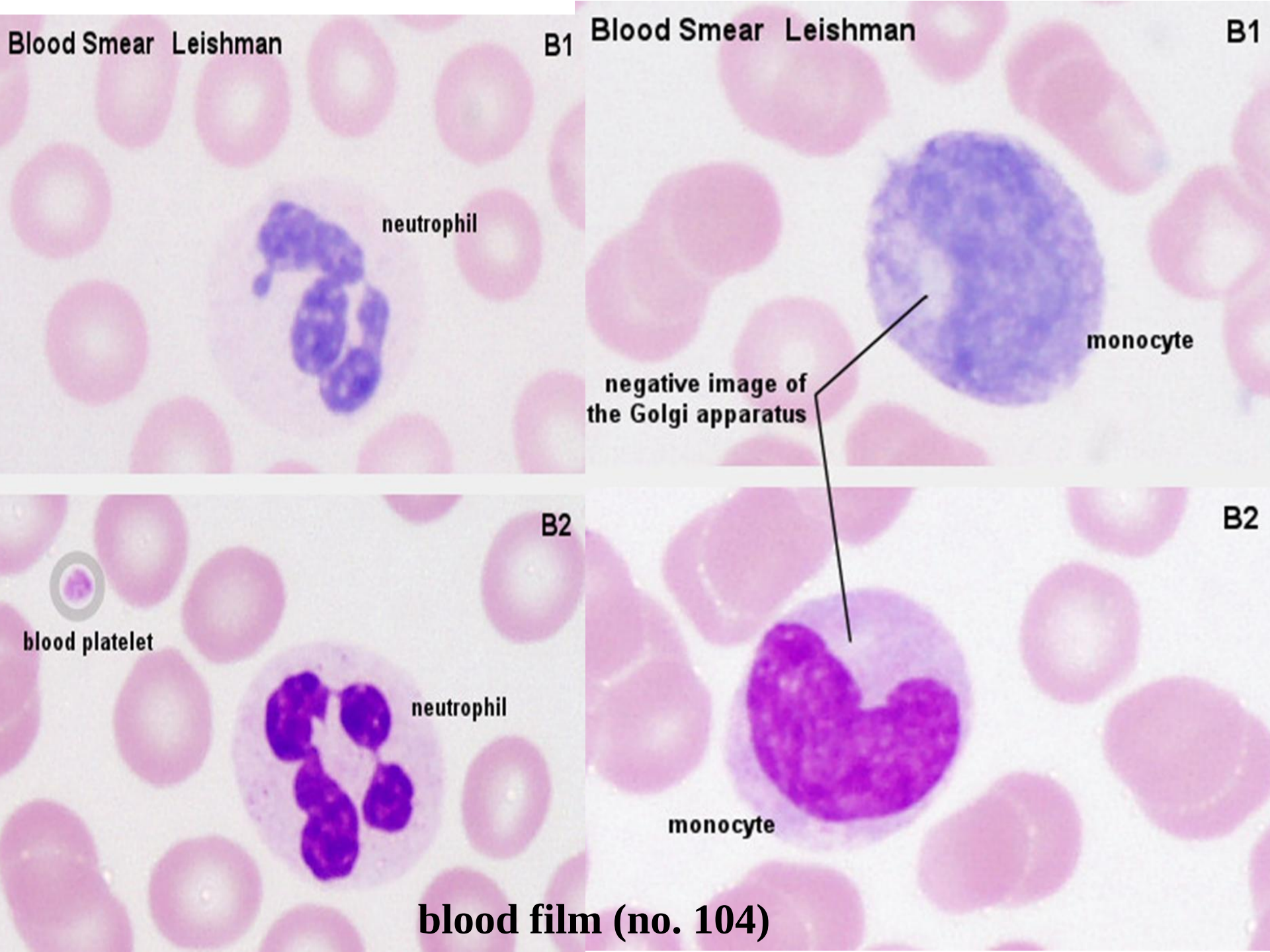
blood film (no. 104)


monocyte
blood film (no. 104)
#104; 1000x
WARSZAWSKI UNIWERSYTET MEDYCZNY
ZAKŁAD HISTOLOGII i EMBRIOLOGII
http://histologia.wum.edu.pl

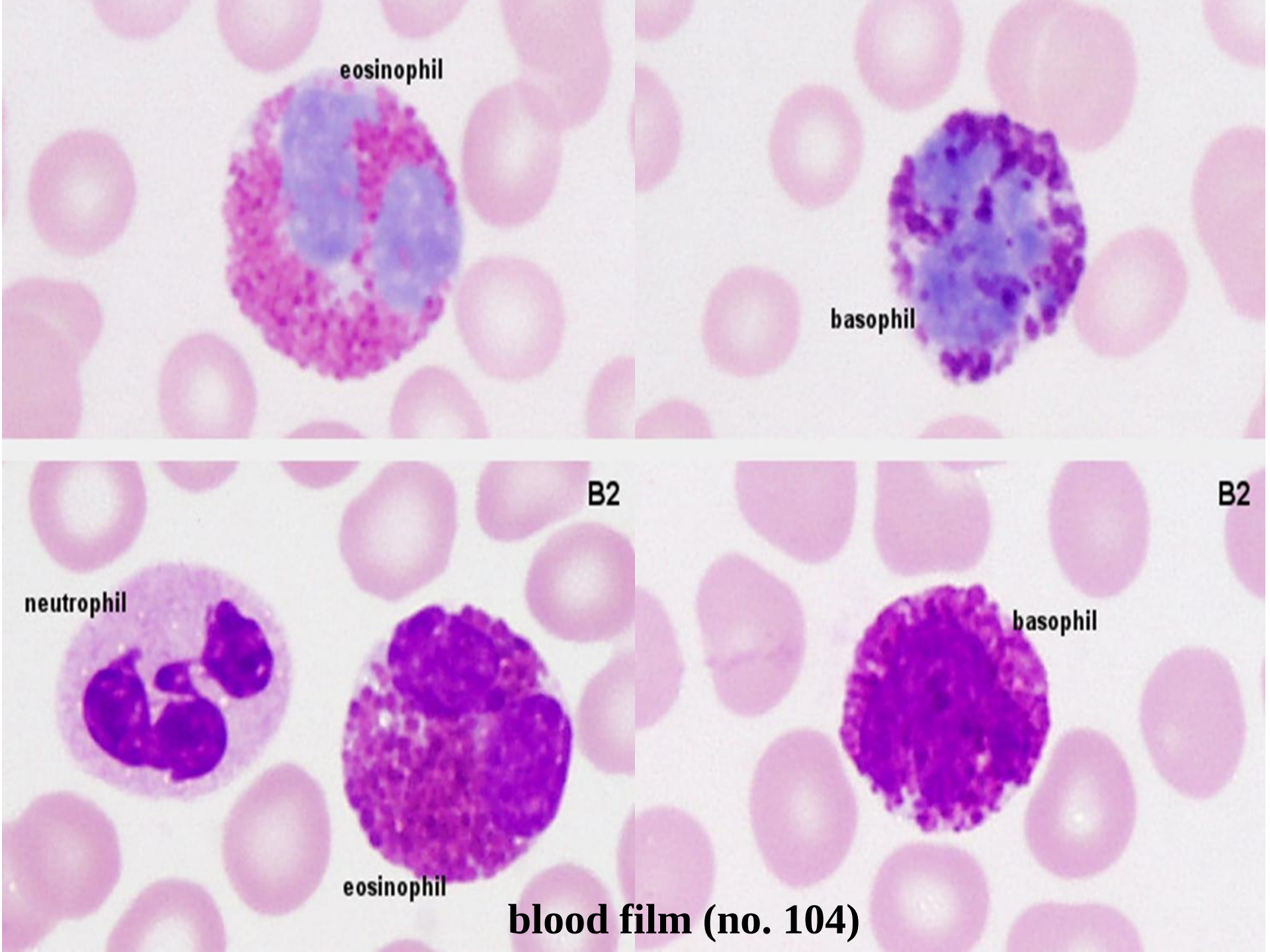




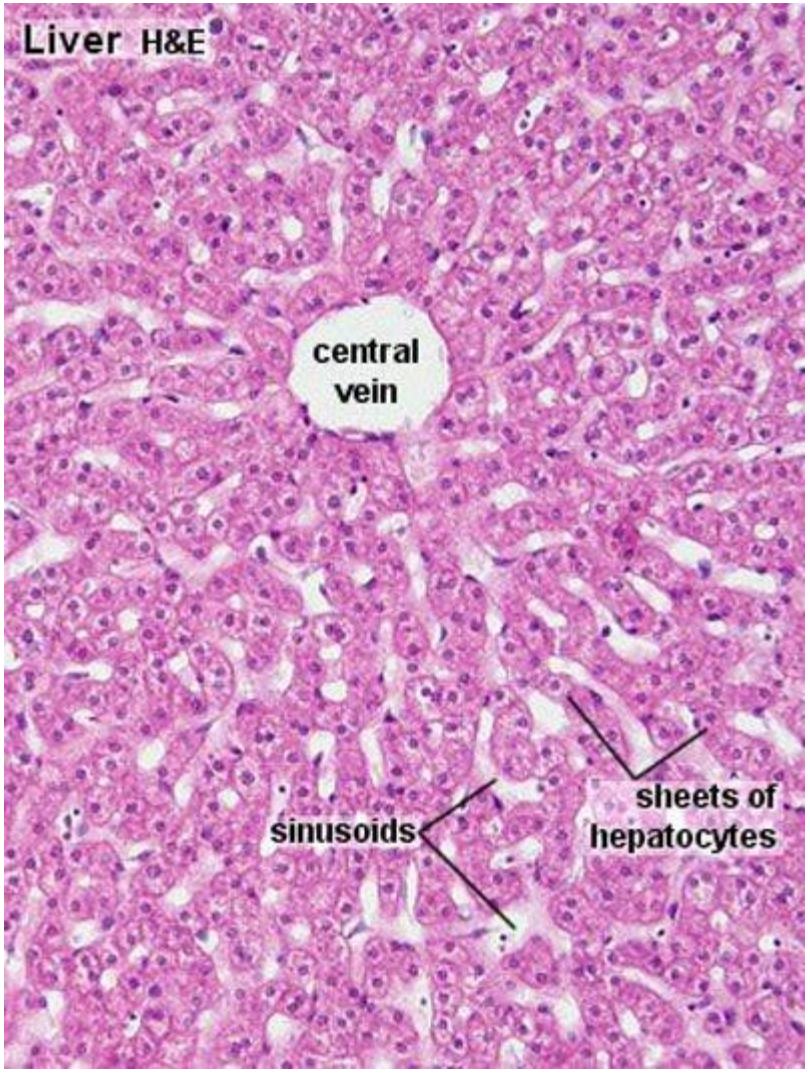
blood film (no. 104)



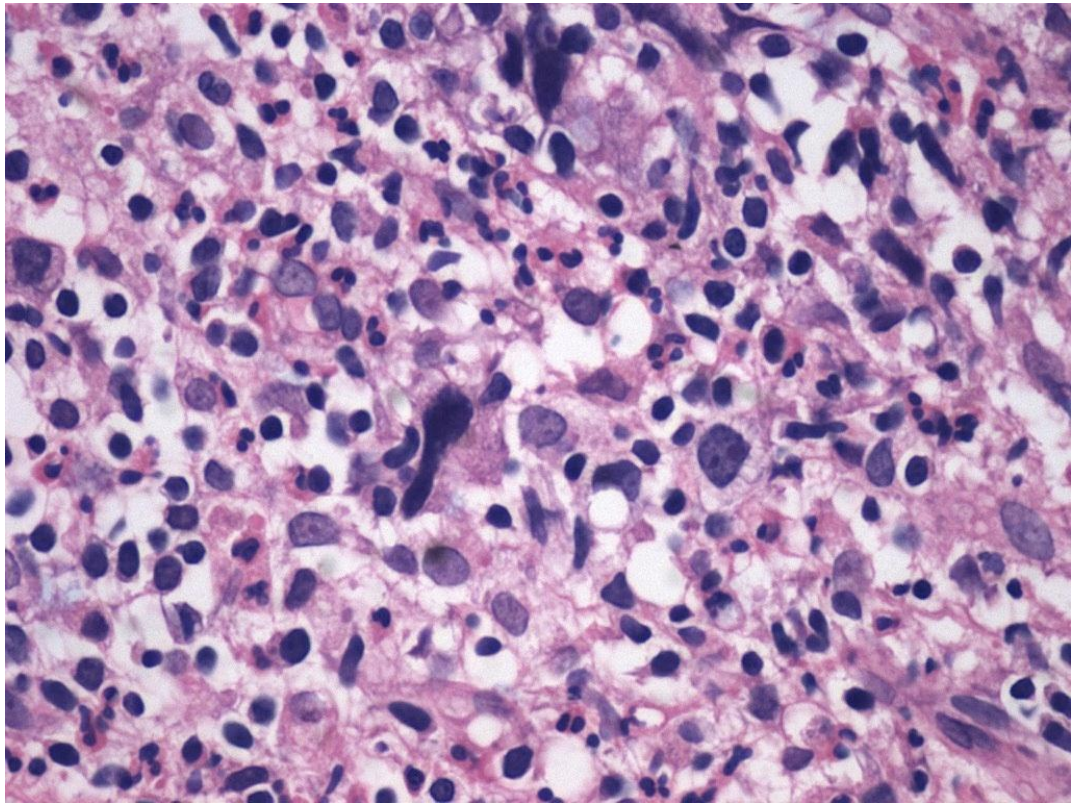
blood film (no. 104)



blood film (no. 104)



hemopoiesis



Circulatory system