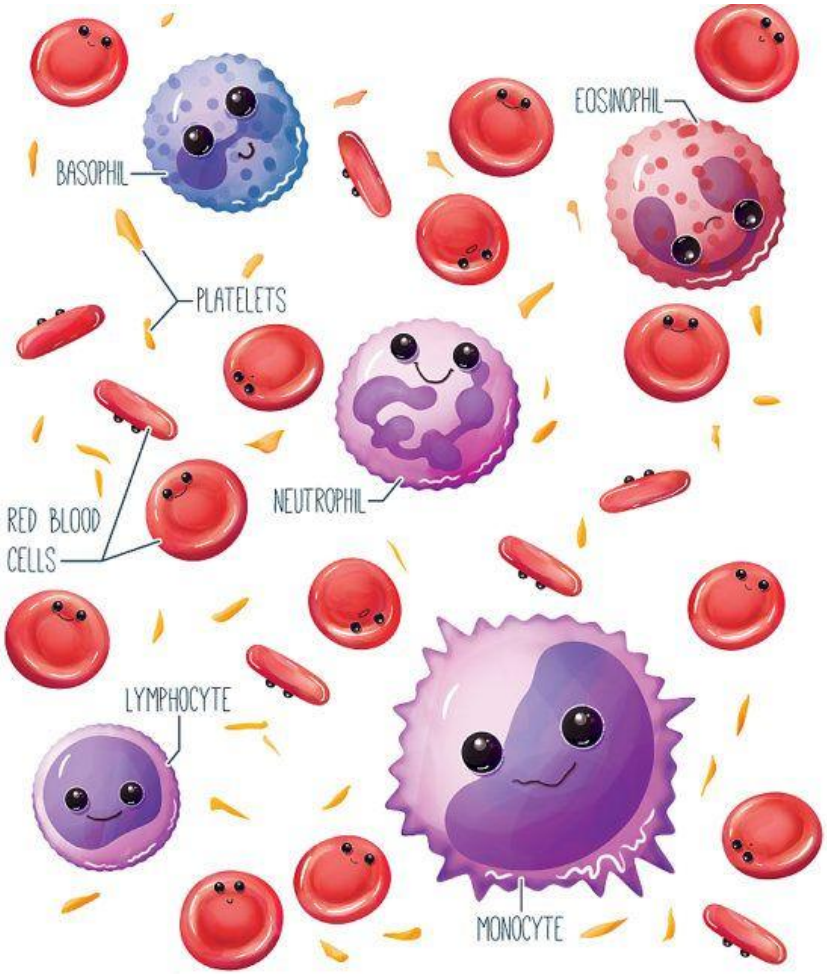
BLOOD
BONE MARROW

Seminars

FUNCTIONS OF BLOOD

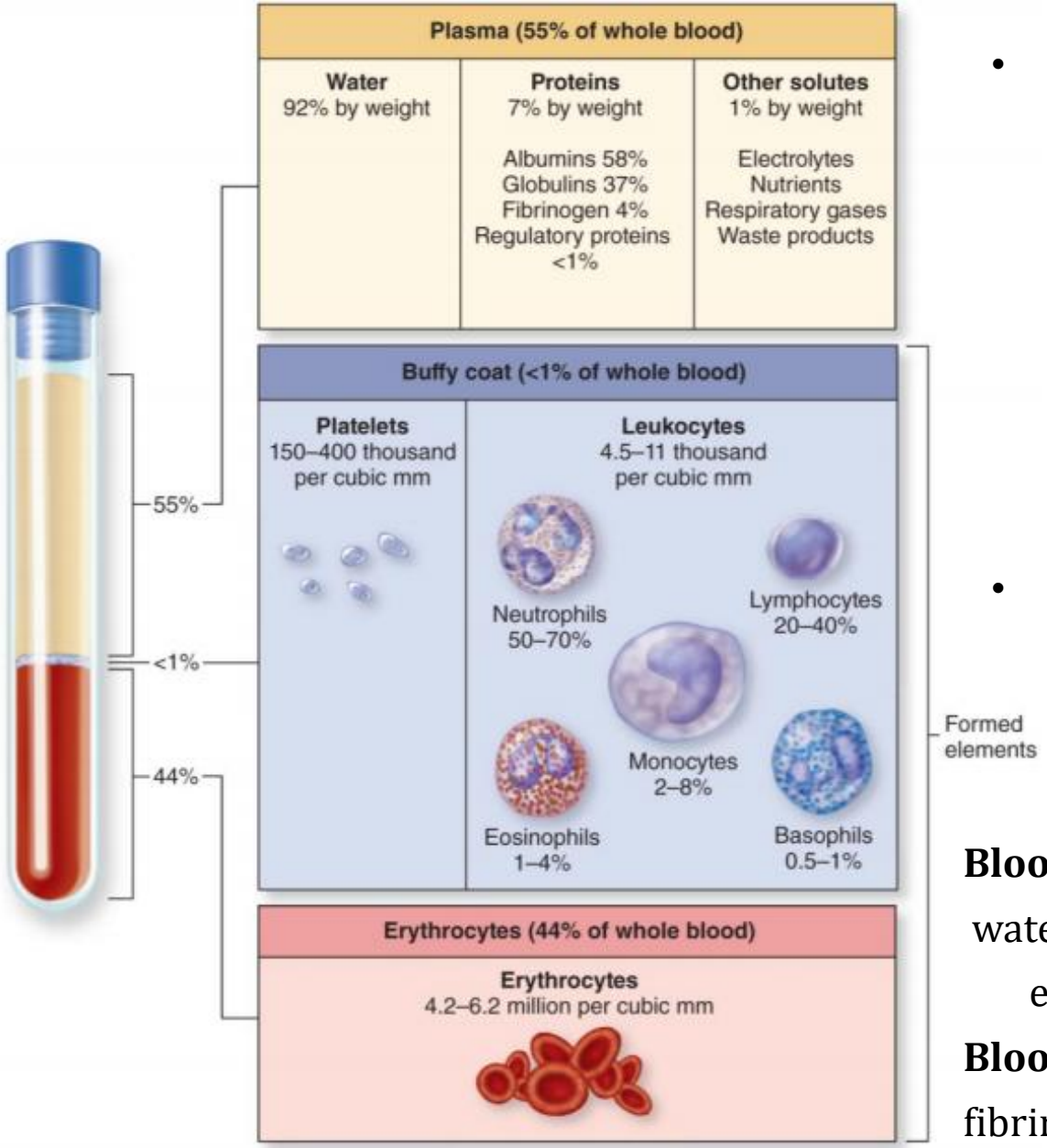


1. **Transports of materials:**
   - Dissolved gases (oxygen, and carbon dioxide)
   - Nutrients (glucose, amino acids, vitamins, minerals, fatty acids, glycerol)
   - Hormones (glands - target cells)
   - Waste products of metabolism (e.g. water, urea)

2. **Protection**
   - Phagocytosis
   - Production of antibodies
   - Blood clotting

3. **Regulation**
   - pH (6,8 -7,4) - interaction with acids and bases
   - Water balance
   - Body Temperature

BLOOD COMPONENTS



- **Formed elements**:
  - Red blood cells (RBCs, erythrocytes)
  - White blood cells (WBCs, leukocytes)
  - Platelets (cells fragments)

- **Fluid element** (extracellular matrix – plasma)

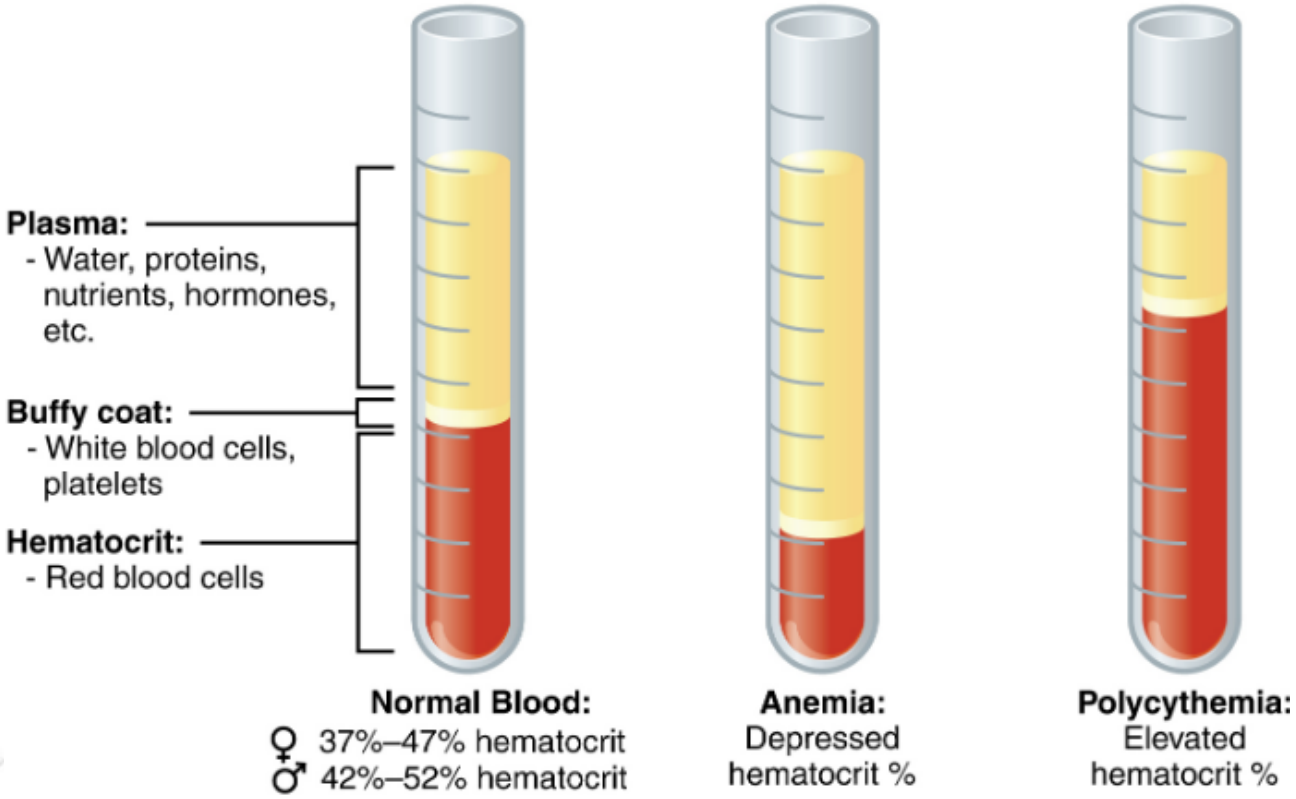
Blood plasma:
water, proteins, glucose, clotting factors, electrolytes, carbon dioxide

Blood serum = blood plasma without fibrinogen and clotting factors

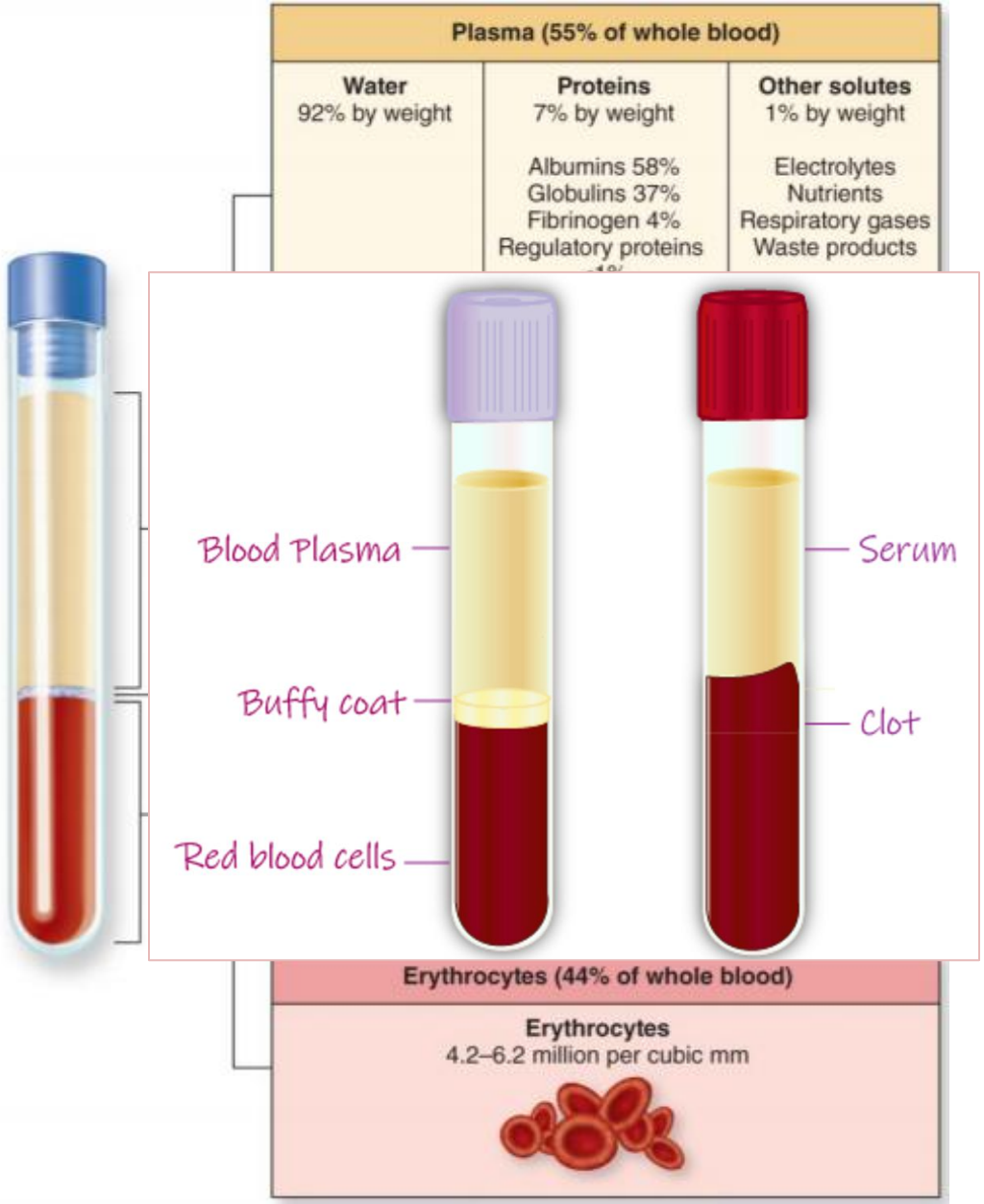
HEMATOCRIT

The relative volume of cells and plasma measured after centrifugation of blood sample with anticoagulant added

- 45 ± 7 (38–52%) for males
- 42 ± 5 (37–47%) for females



BLOOD PLASMA

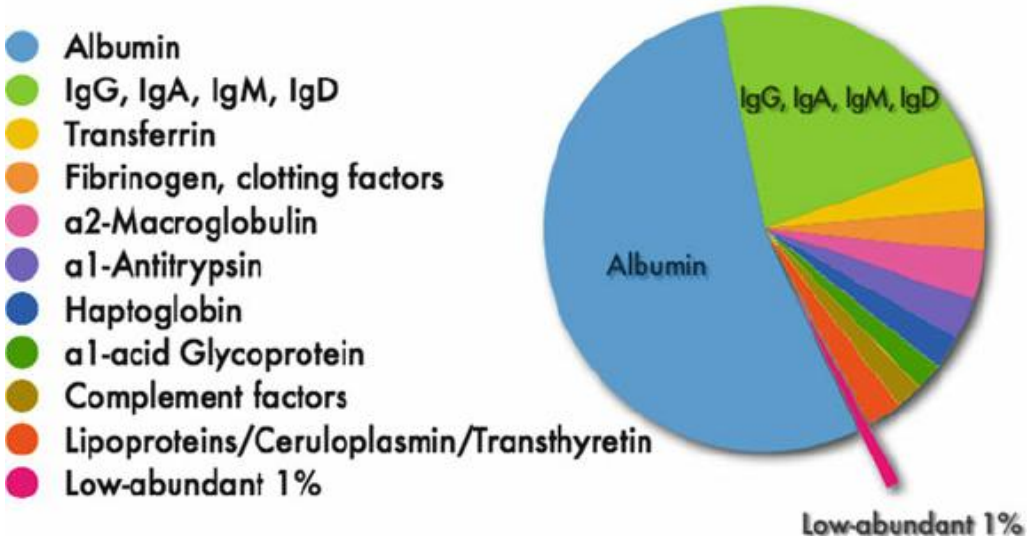


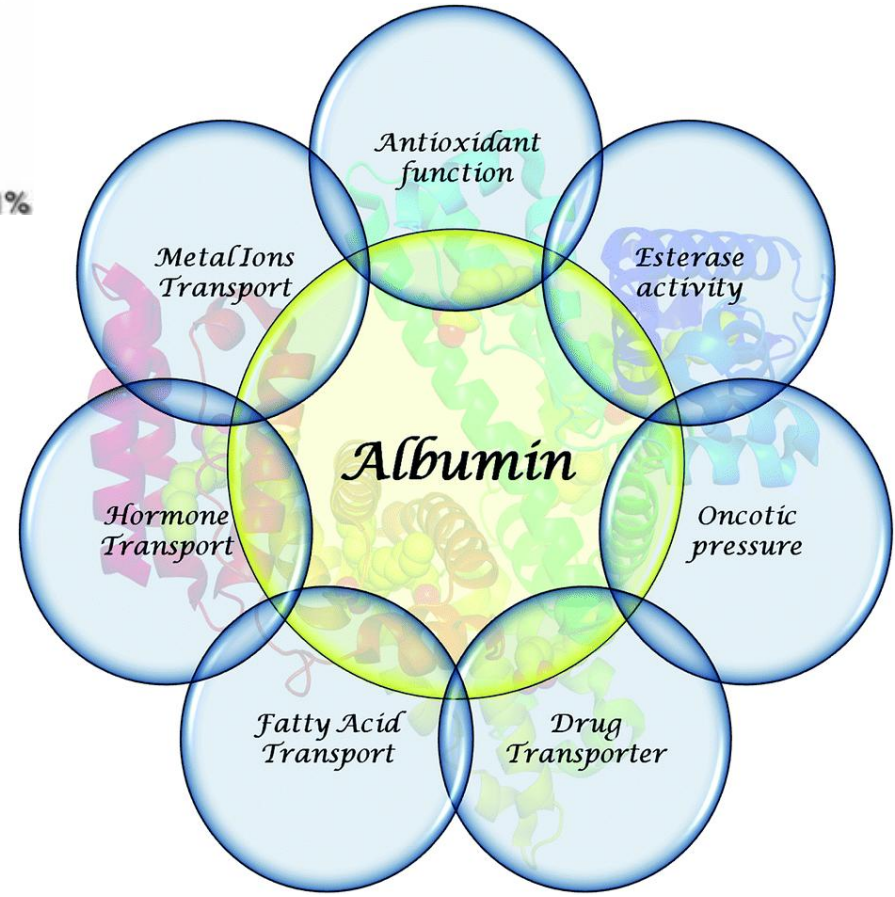
Blood plasma: pale-yellow liquid component of blood in which formed elements are suspended

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

Blood serum = blood plasma without fibrinogen and clotting factors

PLASMA PROTEINS



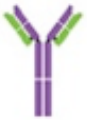


- Albumin and the immunoglobulins account for approximately 75 % of the total protein weight.
- Albumin, which constitutes about half of the blood serum protein, transports hormones and fatty acids, buffers pH, and maintains osmotic pressures.

Sources:

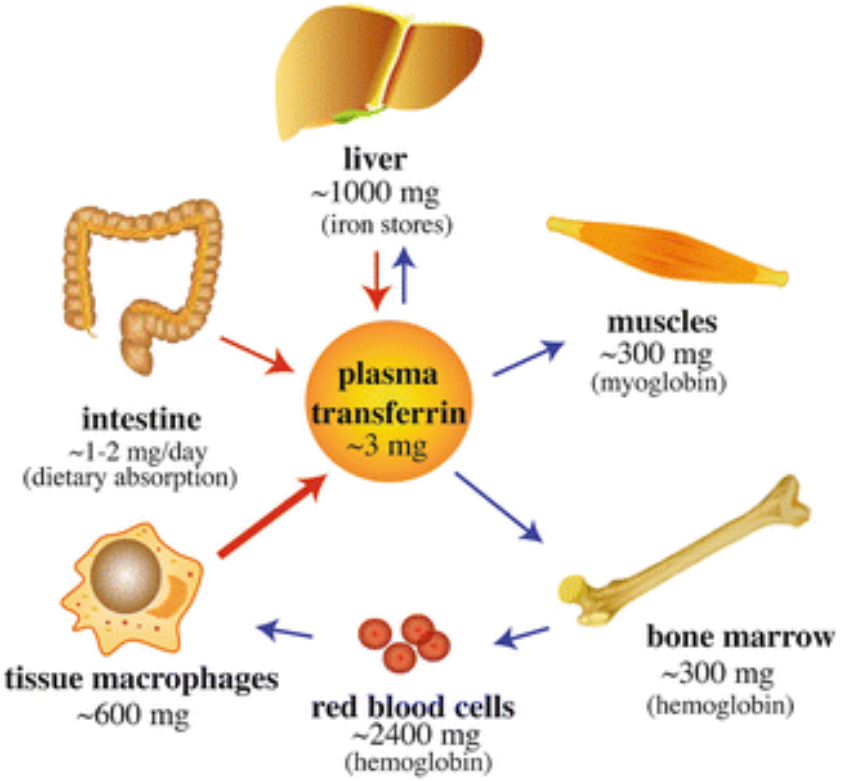
- https://doi.org/10.1007/978-1-62703-360-2_1
- https://doi.org/10.1039/C6RA08283A

PLASMA PROTEINS

					
	IgM	IgG	IgA	IgE	IgD
Heavy Chain	μ (mu)	γ (gamma)	α (alpha)	ε (epsilon)	δ (delta)
MW (Da)	900k	150k	385k	200k	180k
% of total antibody in serum	6%	80%	13%	0.002%	1%
Fixes complement	Yes	Yes	No	No	No
Function	Primary response, fixes complement. Monomer serves as B-cell receptor	Main blood antibody, neutralizes toxins, opsonization	Secreted into mucus, tears, saliva	Antibody of allergy and anti-parasitic activity	B cell Receptor

Immunoglobulins (antibodies) - glycoprotein molecules produced by plasma cells (white blood cells). They act as a critical part of the immune response.

Transferrin - secreted glycoprotein that transports ferric iron (Fe^{3+}) from extracellular fluids to tissues.



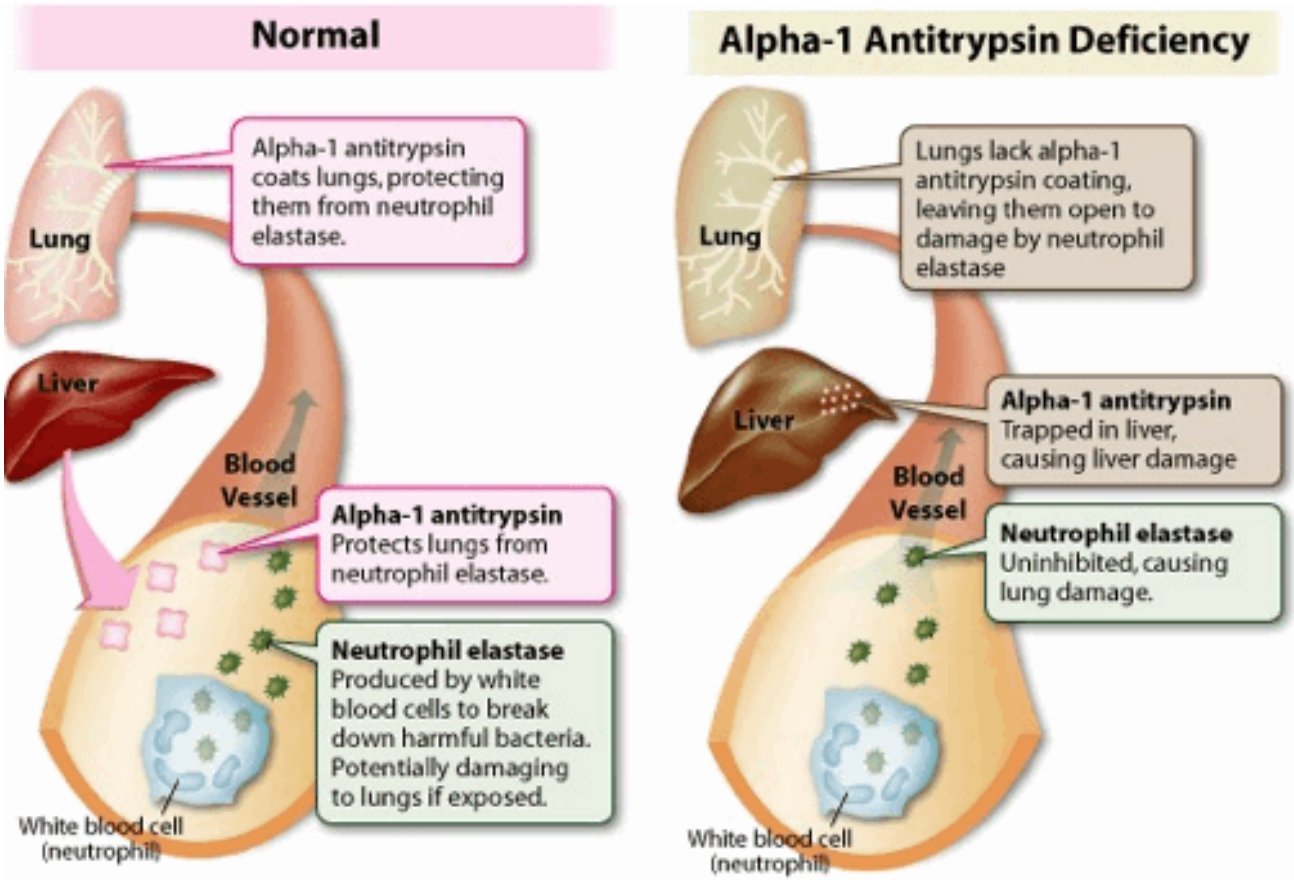
PLASMA PROTEINS

Alpha 2 macroglobulin acts as an antiprotease (inactivation of variety of proteinases).

- Inhibitor of fibrinolysis (inhibition of plasmin and kallikrein).
- Inhibitor of coagulation (inhibition of thrombin).

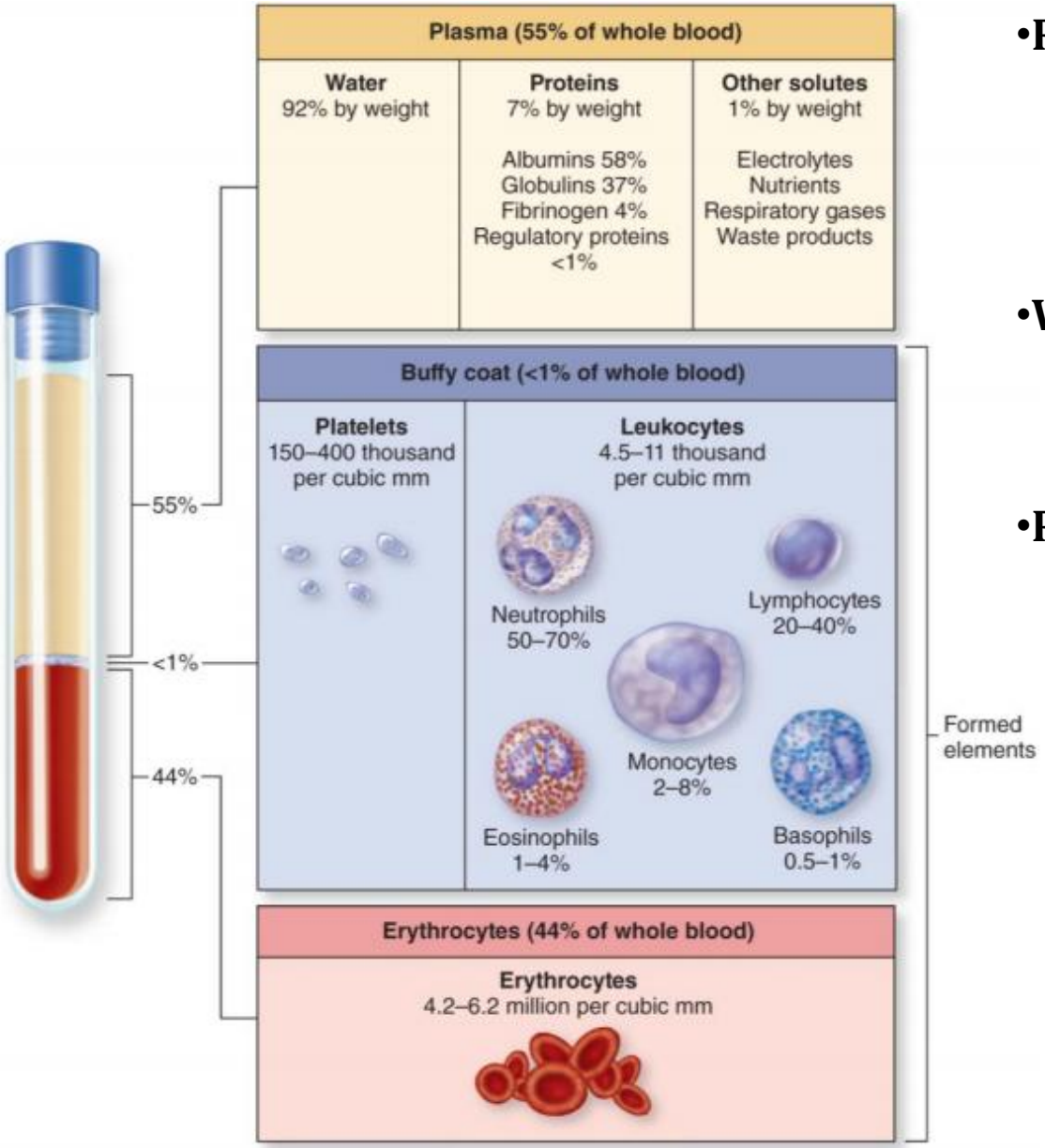
α1-antitrypsin - inhibition of neutrophil elastase

Haptoglobin – binds free hemoglobin (Hb) with very high affinity (prevention of the loss of iron after intravascular hemolysis and prevention Hb-mediated renal injury).



Sources: https://doi.org/10.1002/jcp.24266

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



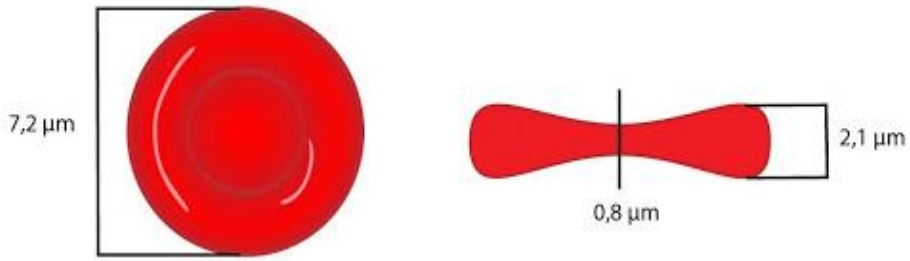
- **Red blood cells (RBCs):**
  - 4.2 to 5.4 millions (female)
  - 4.7 to 6.1 millions (male)
- **White blood cells (WBCs):**
  - 4,000–11,000
- **Platelets**:
  - 200,000–450,000

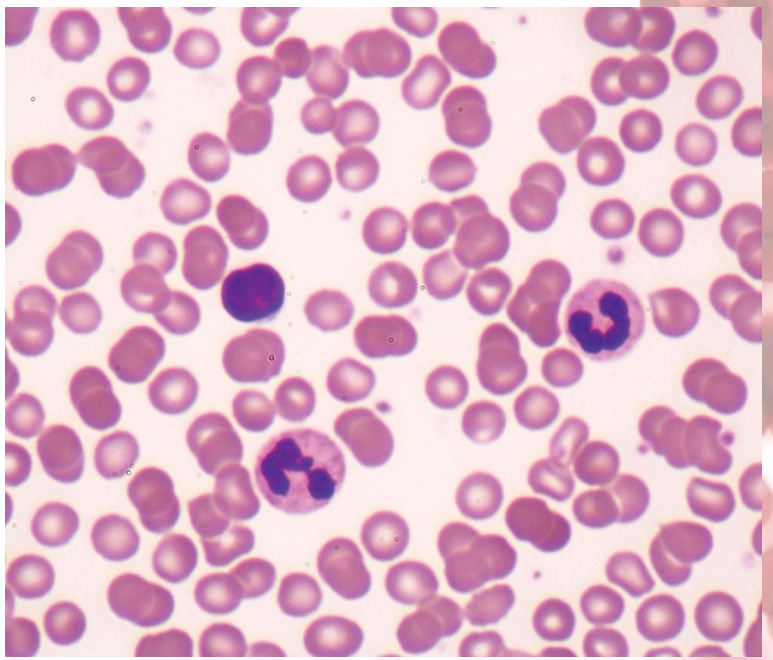
Red blood cells (erythrocytes)

- The most numerous cells of blood
- Have no nuclei
- Average life span of 120 days

Female: 4.2 to 5.4 millions /mm^3

Male: 4.7 to 6.1 millions /mm^3

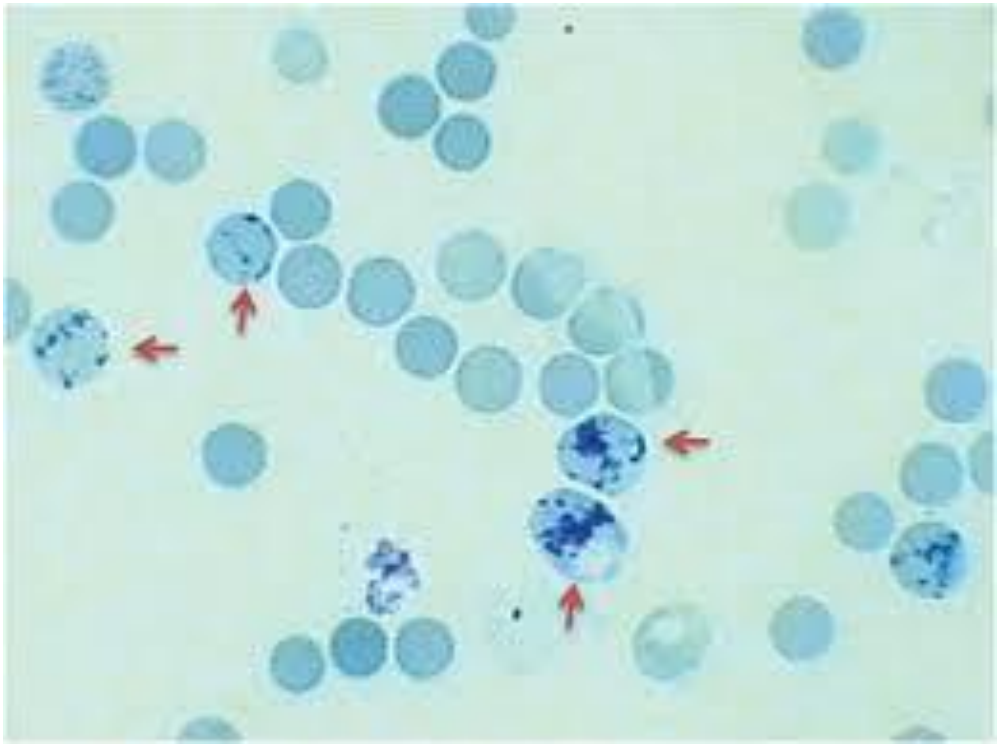




Transport of oxygen and carbon dioxide to and from the tissues

RETICULOCYTES

Reticulocytes are young RBCs that lack a **nucleus** but still contain residual ribonucleic acid (RNA) to complete the production of hemoglobin. This residual RNA generally is lost progressively during the 24 hours after the cell enters the circulation.

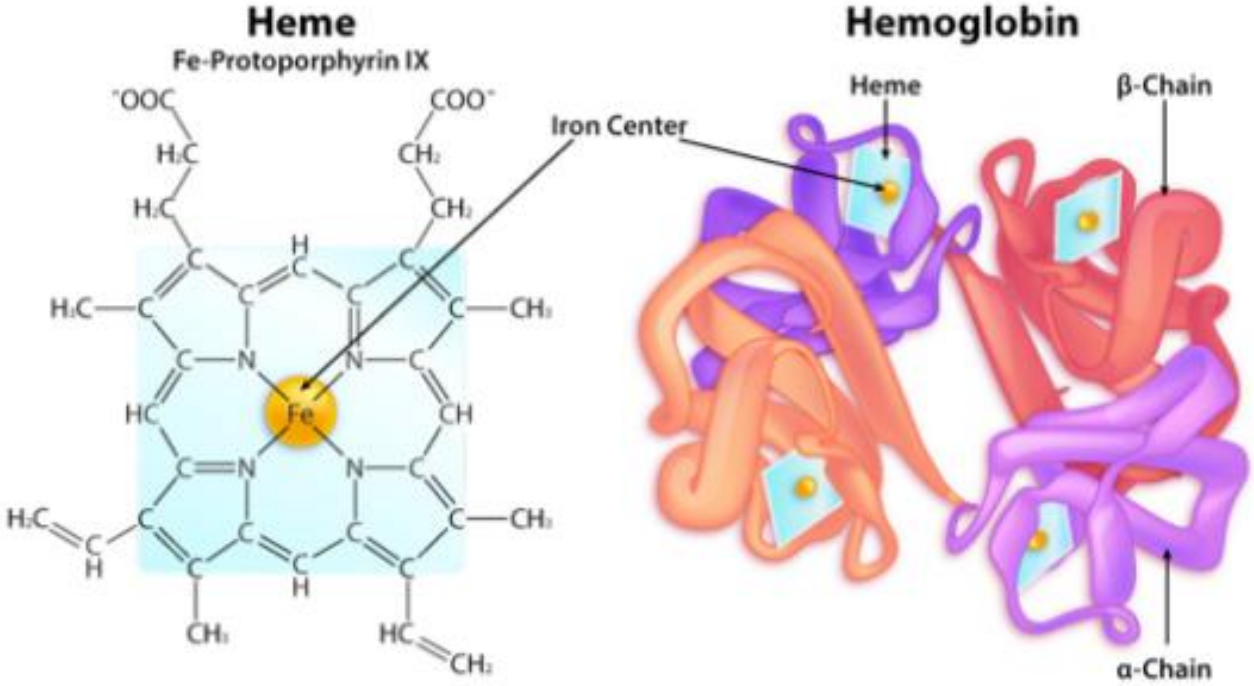


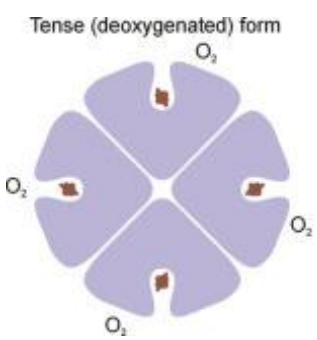
Reticulocytes constitute 1-2% of red blood cells.
Their number in peripheral blood increases after bleeding or at high altitude.

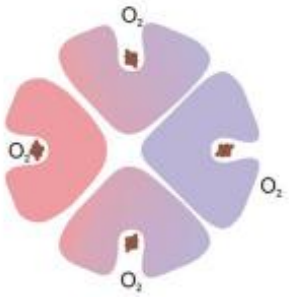
Hemoglobin (HB)

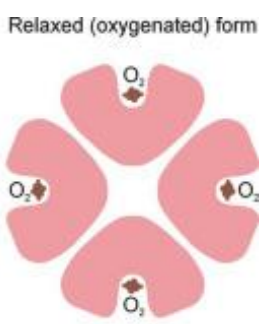
- Four globular protein subunits
- Each subunit is composed of a protein chain tightly associated with a non-protein heme group
- Heme group consists of an iron (Fe) ion in the center of the heterocyclic porphyrin ring

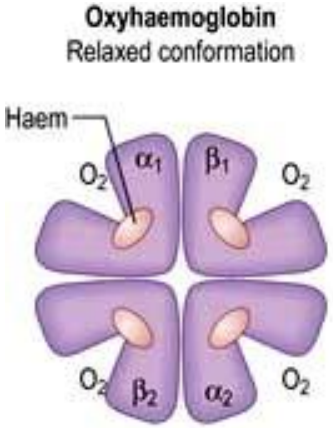
13.5-18.0 g/dl (male)
12.0-16.0 g/dl (female)

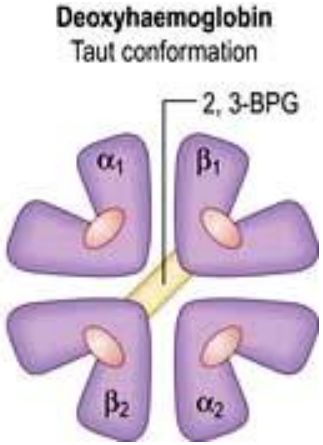








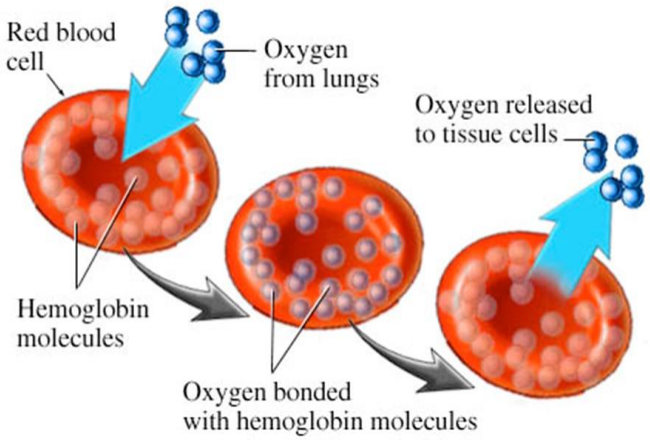


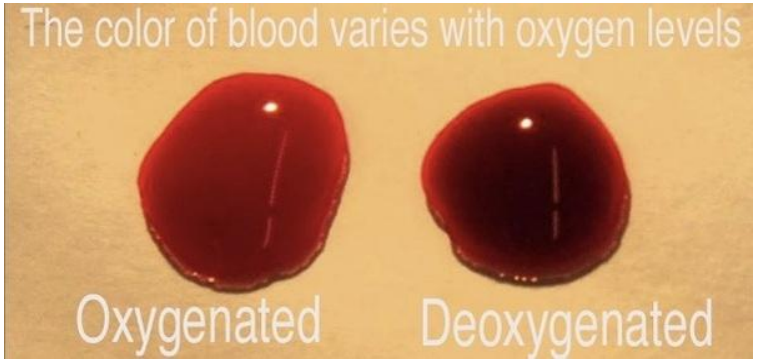


• In the blood stream hemoglobin exists in two forms distinguished by the presence [oxyhemoglobin] or absence of attached molecular oxygen [deoxyhemoglobin]

• Oxygen binds to hemoglobin through positive cooperation.

• 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.

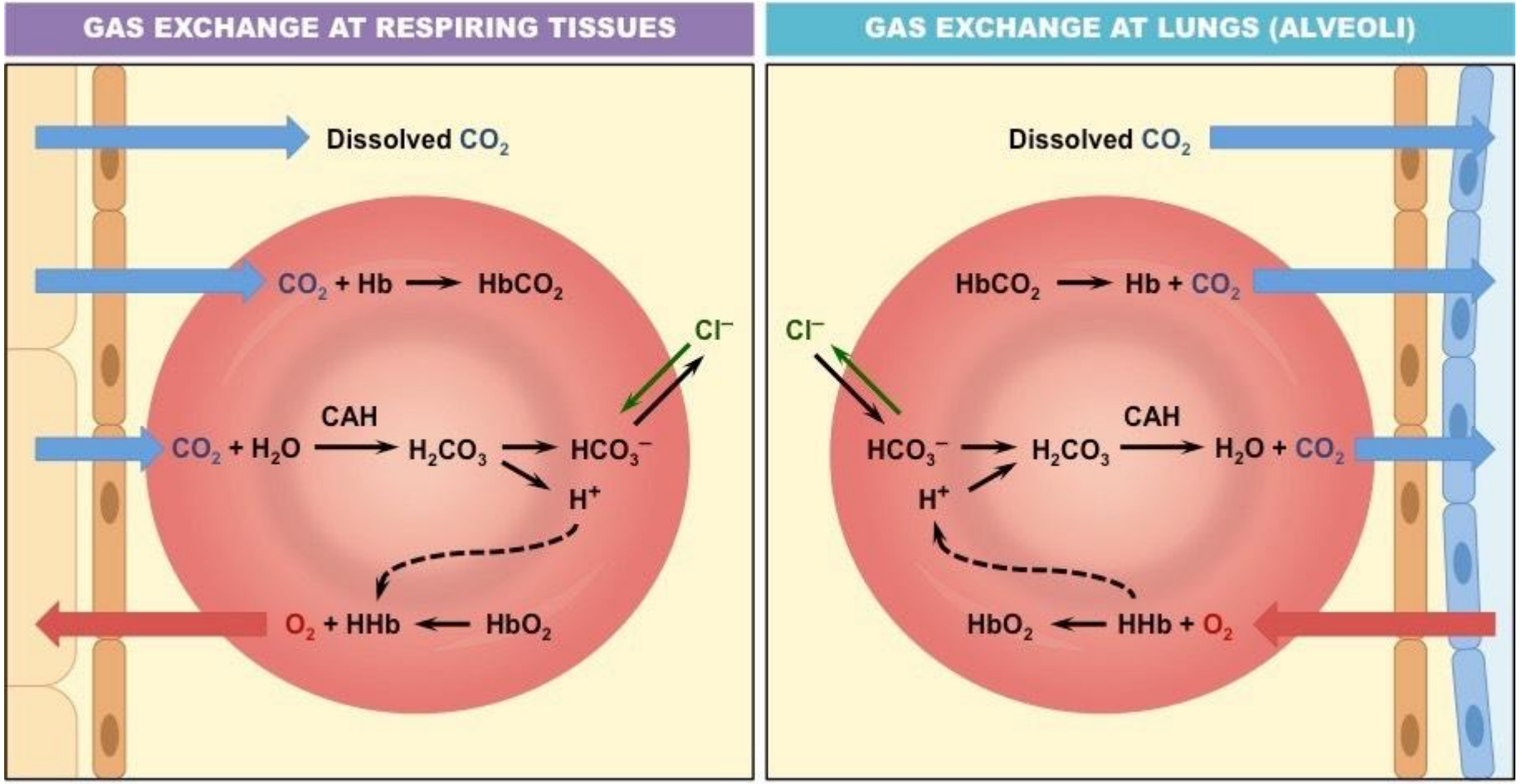


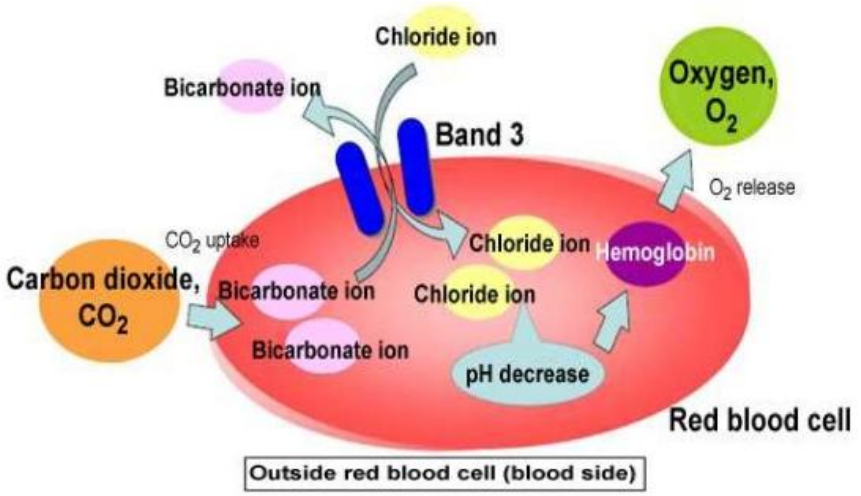


CARBON DIOXIDE - transported mainly in the form of bicarbonate (HCO_3^-)

85% - in blood as bicarbonate ; about 10% - erythrocyte; 5% - free CO2

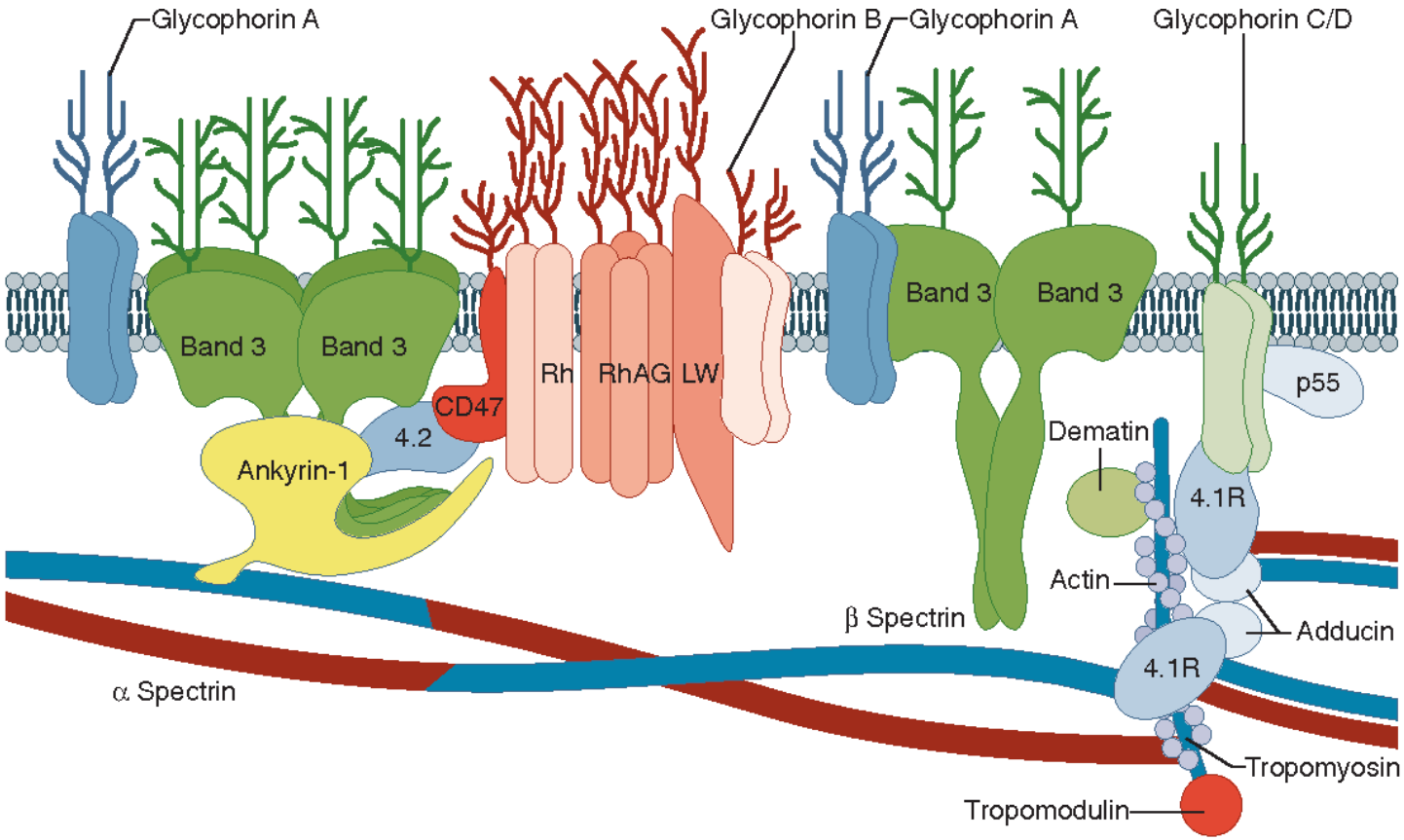
CARBONIC ANHYDRASE – facilitates the formation of carbonic acid from CO_2 and water (and the reverse reaction). This acid dissociates to form bicarbonate and hydrogen



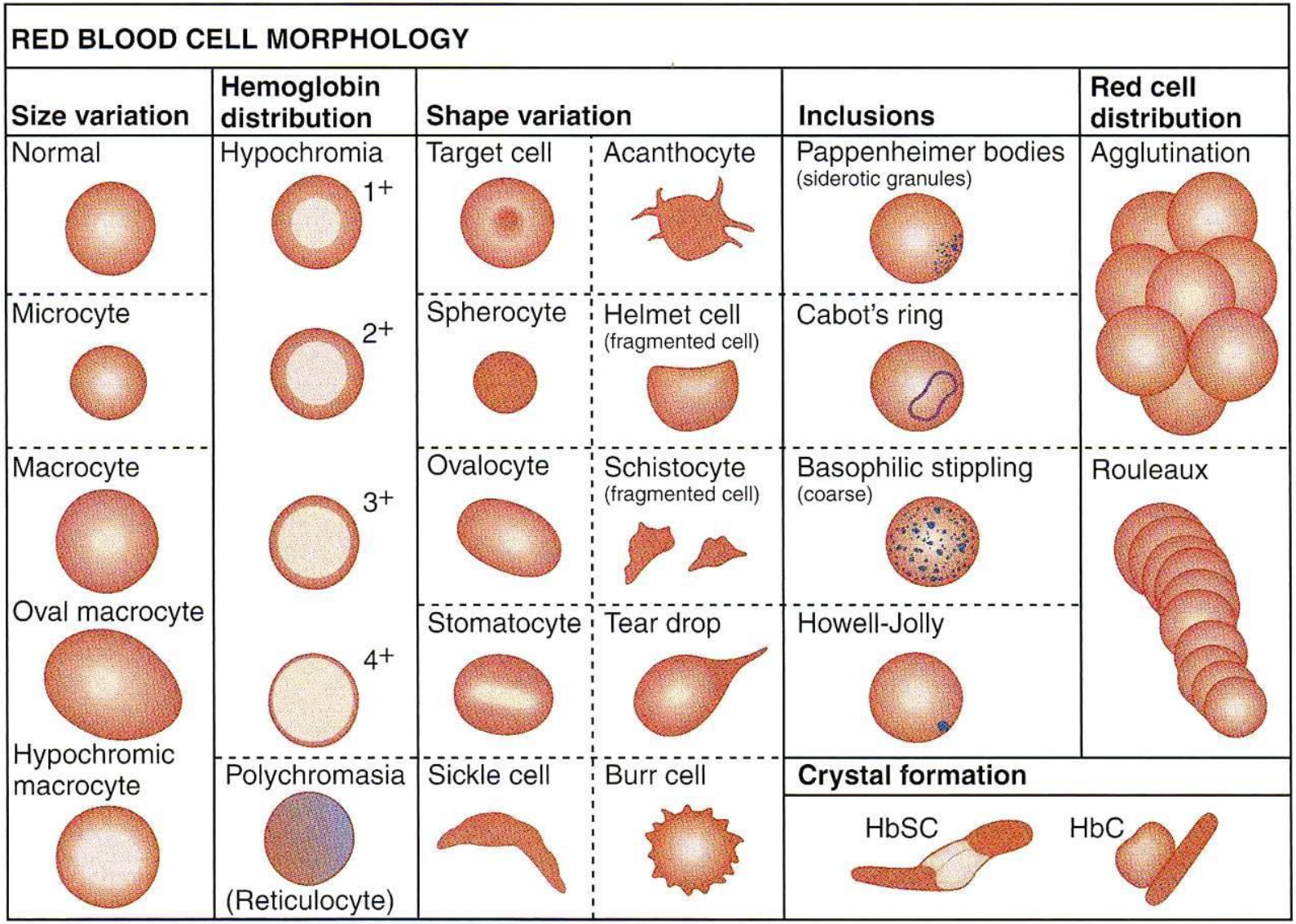


CHLORIDE SHIFT

Band 3 - coupled anion transporter. Exchange of bicarbonate for chloride.

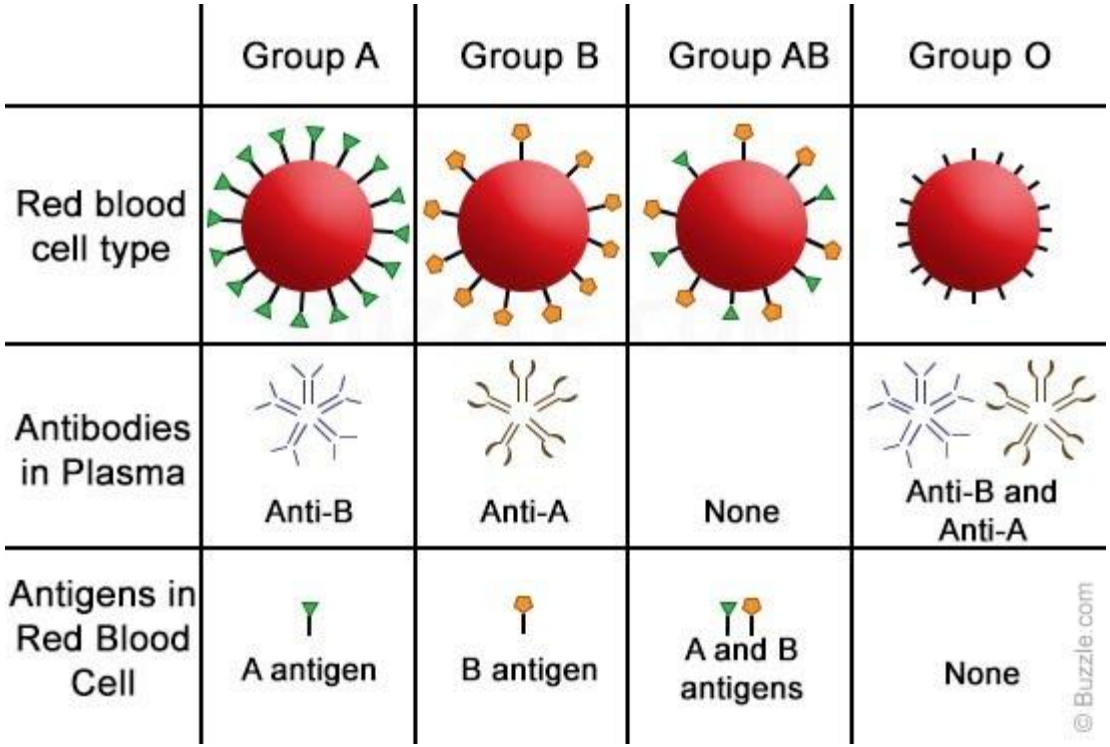


Erythrocyte cell membrane



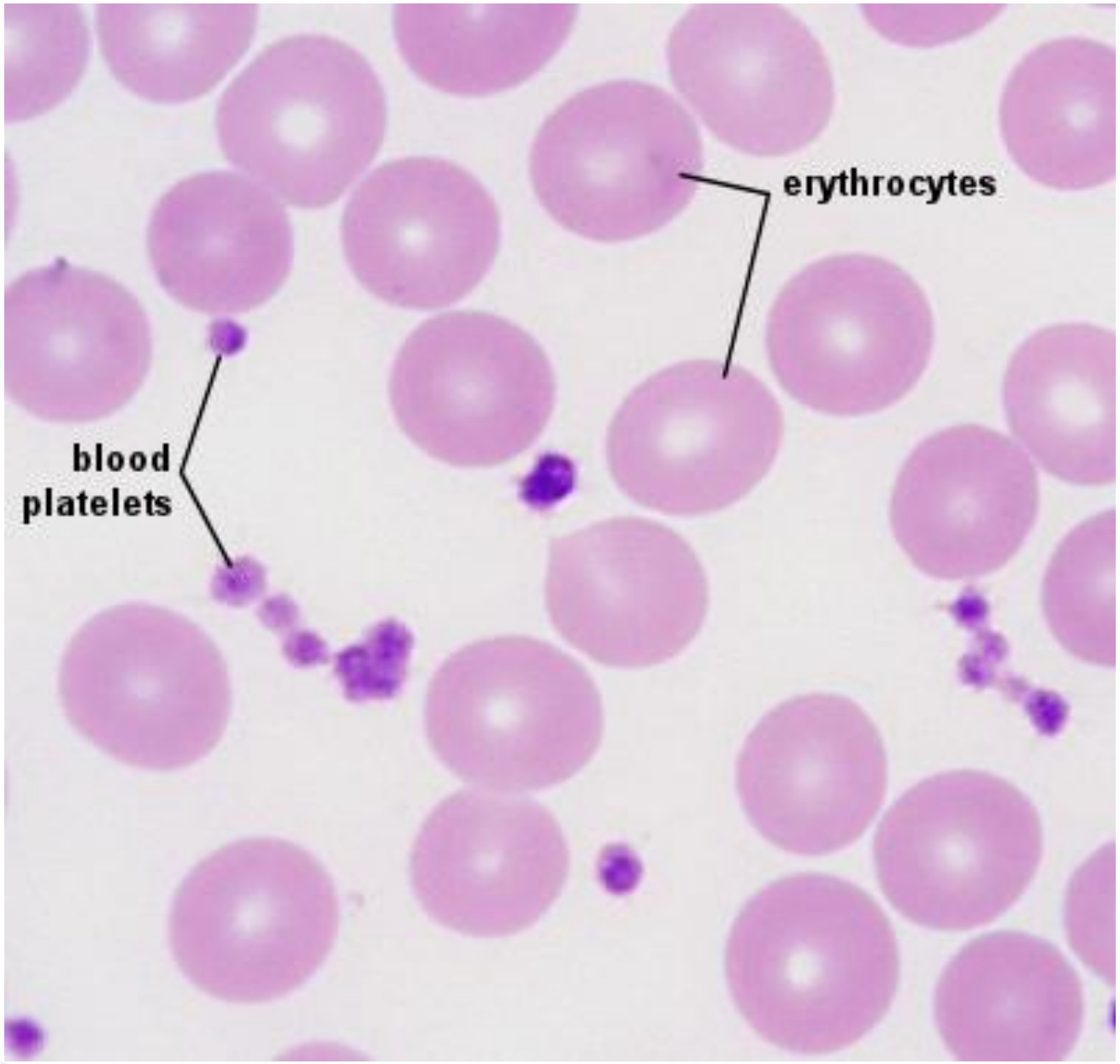
Specific inherited carbohydrate chains act as antigens and determine the **blood group** of an individual.

Antigens **A** and **B** determine four primary blood groups **A, B, AB** and **0**

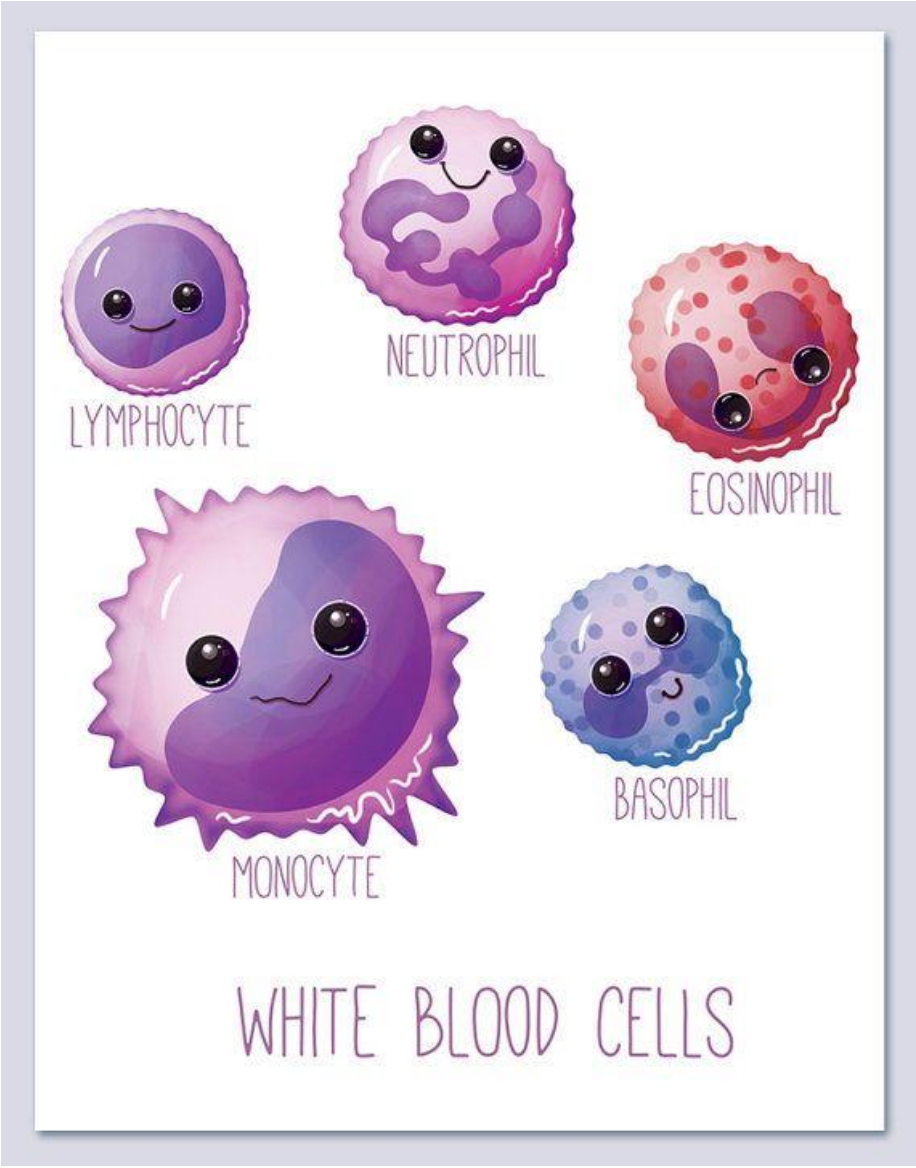


Rh antigens (first identified in rhesus monkeys) determine the Rh-positive (Rh^+) group. Individuals lacking these antigens are Rh-negative (Rh^-)

Erythrocytes in the blood smear



White blood cells (leukocytes)



Granulocytes (have specyfic granules)

- Neutrophils
- Eosinophils
- Basophils

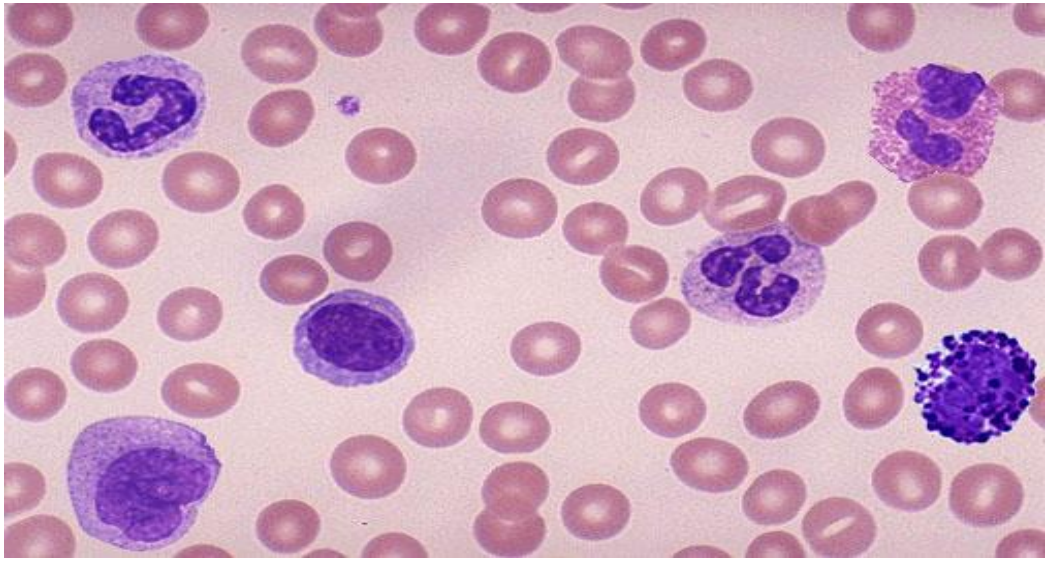
Agranulocytes (lack specific granules)

- Lymphocytes
- Monocytes

Both granulocytes and agranulocytes possess nonspecific (azurophilic) granules

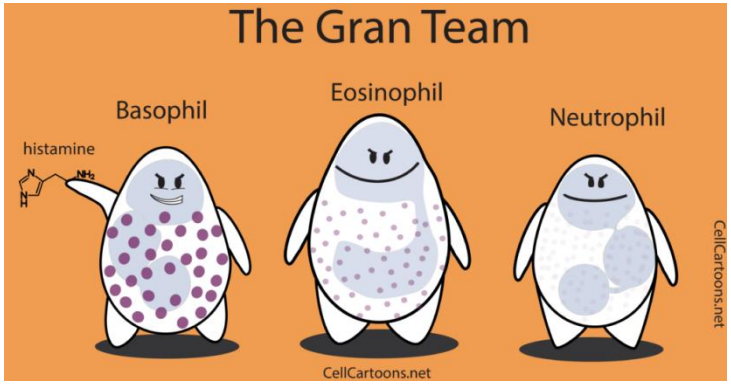
The percentage of granulocytes and agranulocytes in white blood cells

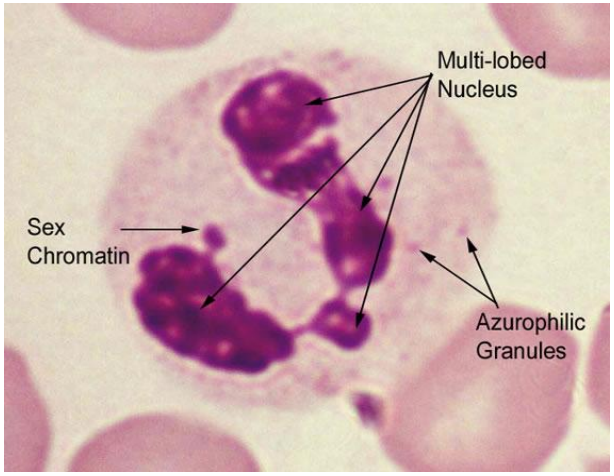
Cell type	Range	Percent
Total WBC	**6 500 - 10 000/mm^3**	
Neutrophils	4 100-6 500	60-70
Lymphocytes	1 500- 2 500	20-25
Monocytes	200- 800	3-8
Eosinophils	150-400	2-4
Basophils	50-100	0-1

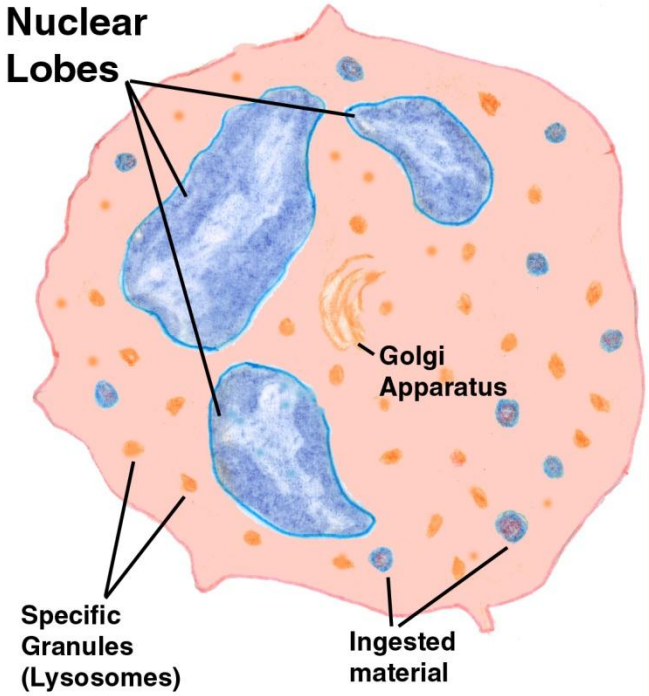


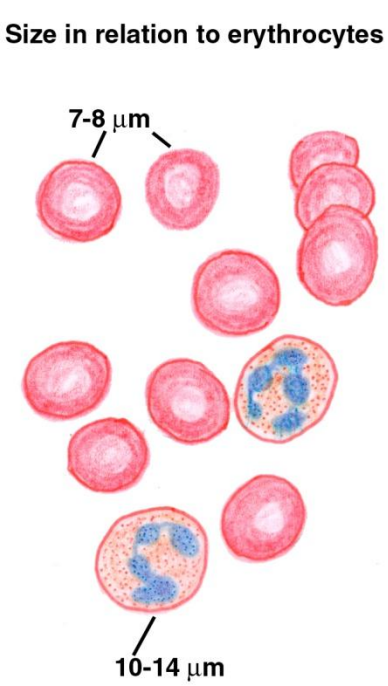
NEUTROPHILS (polymorphonuclear leukocytes)

- The most numerous of the white blood cells
- Phagocytose and destroy invading bacteria
- In females - the „drumstick” which contains inactive X chromosome







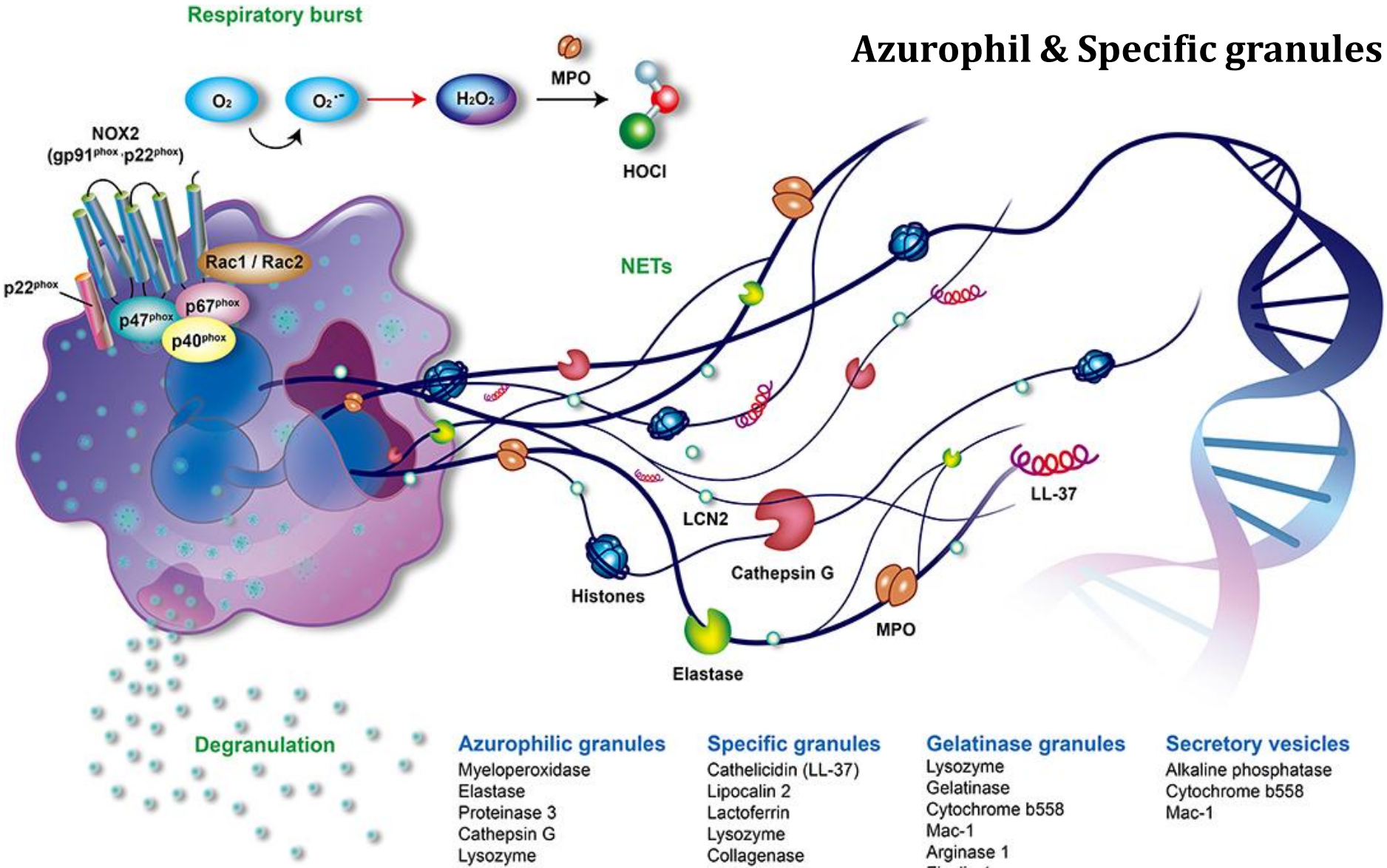


Sources:

• DOI:10.1126/sciimmunol.aat4579
• https://www.doctorc.net/Labs/Lab6/lab6.htm

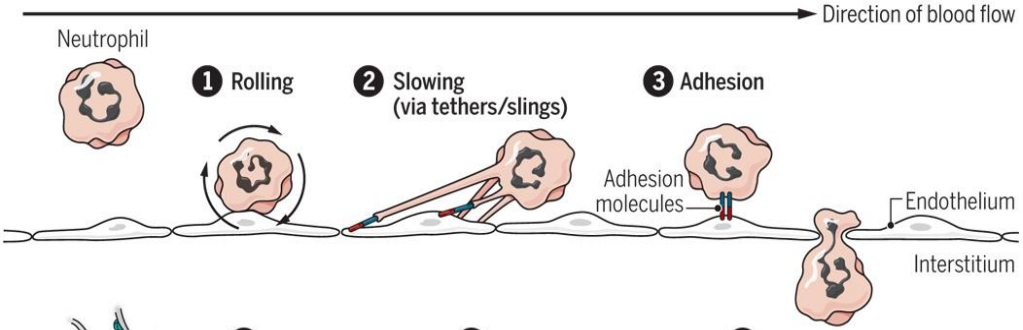
NEUTROPHILS (polymorphonuclear leukocytes)

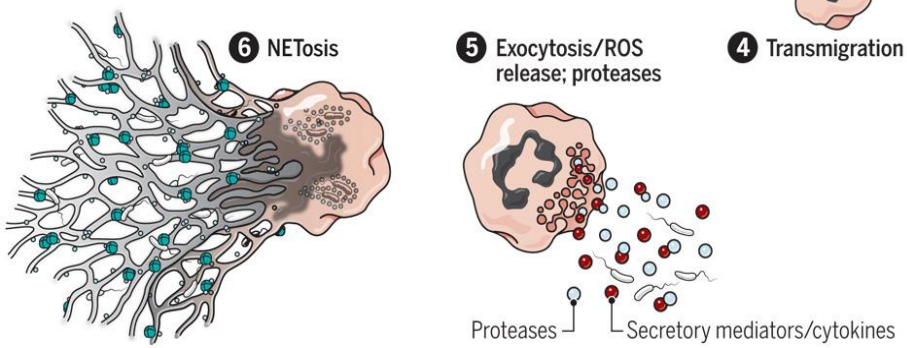
Azurophil & Specific granules

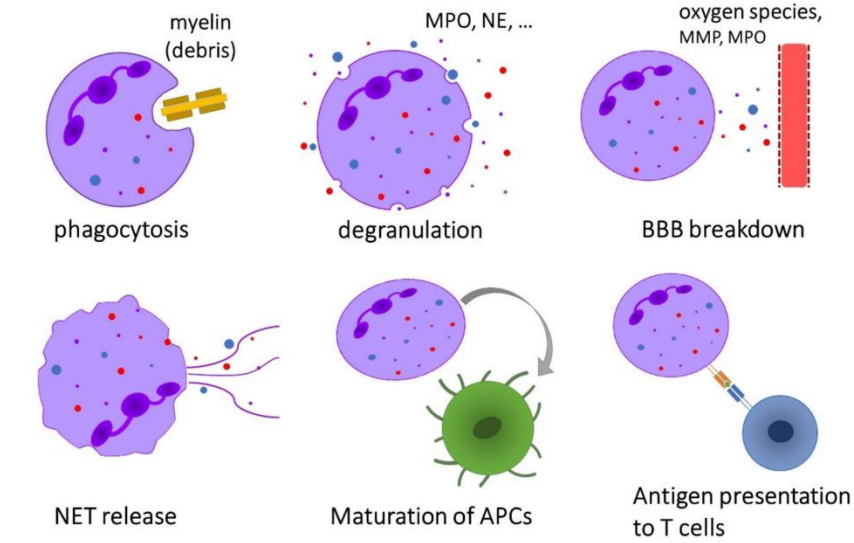


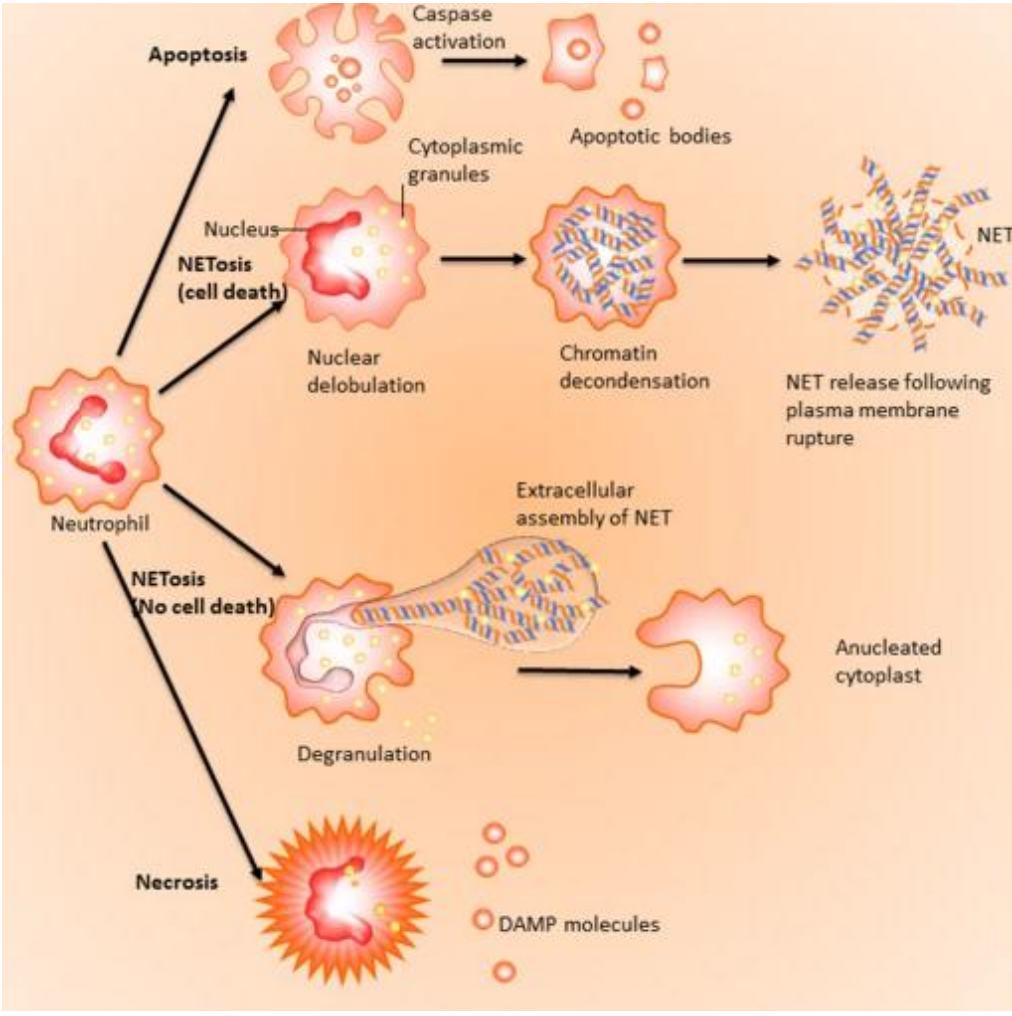
Source:
https://doi.org/10.3389/fimmu.2019.02376

NEUTROPHILS









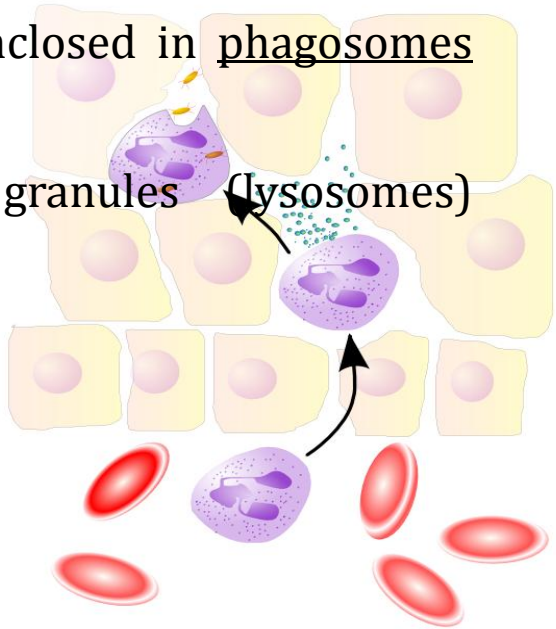
Source:

- DOI:10.1126/sciimmunol.aat4579
- DOI: 10.1007/s10753-020-01294-x
- DOI: 10.3390/ijms21124558

FUNCTIONS OF NEUTROPHILS (MICROPHAGES)

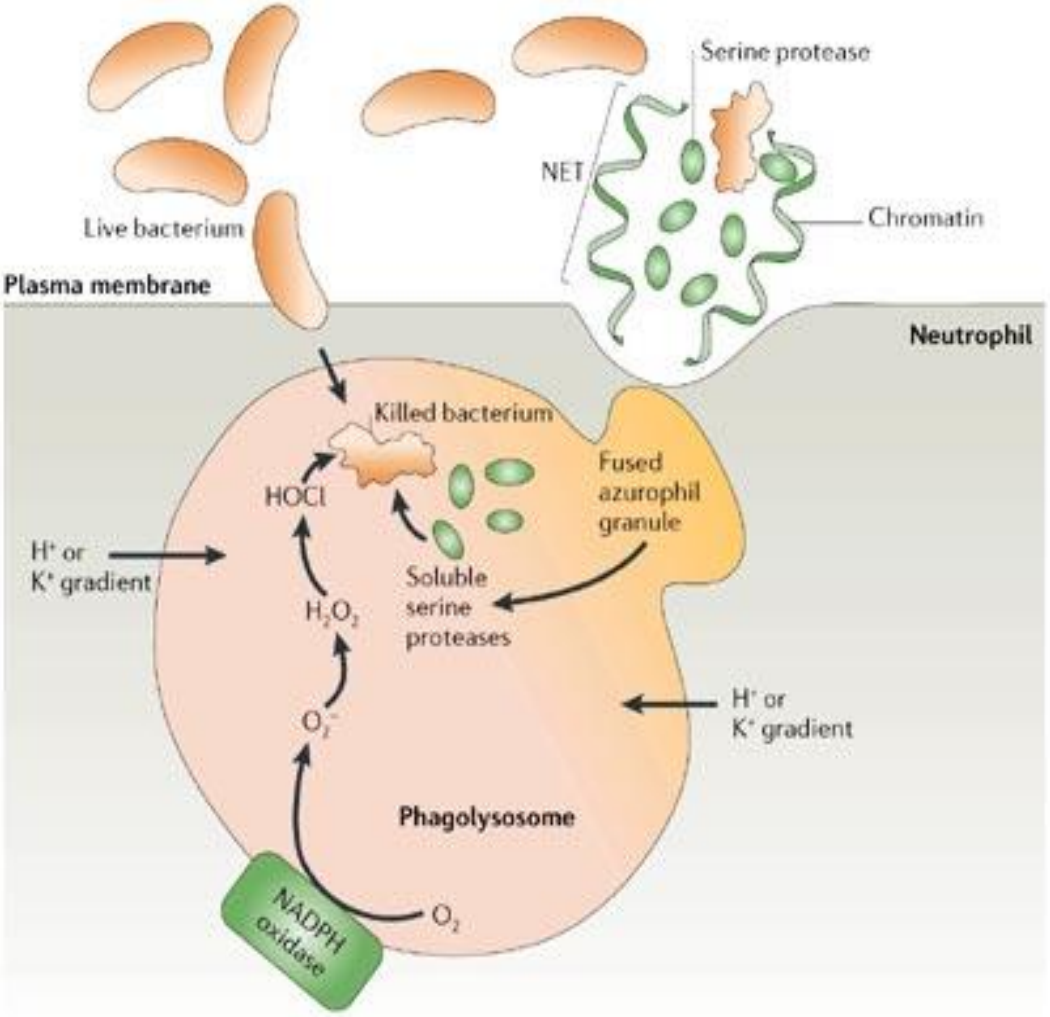
Responsible for the phagocytosis and destroying of bacteria

1. Neutrophils undergo a process called chemotaxis – migration toward sites of infection or inflammation. Cell surface receptors– detection of chematractants
2. Chemotactic agents facilitate the release of the contents of tertiary granules into the extracellular matrix (gelatinase degrades the basal lamina, facilitating neutrophil migration)
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)



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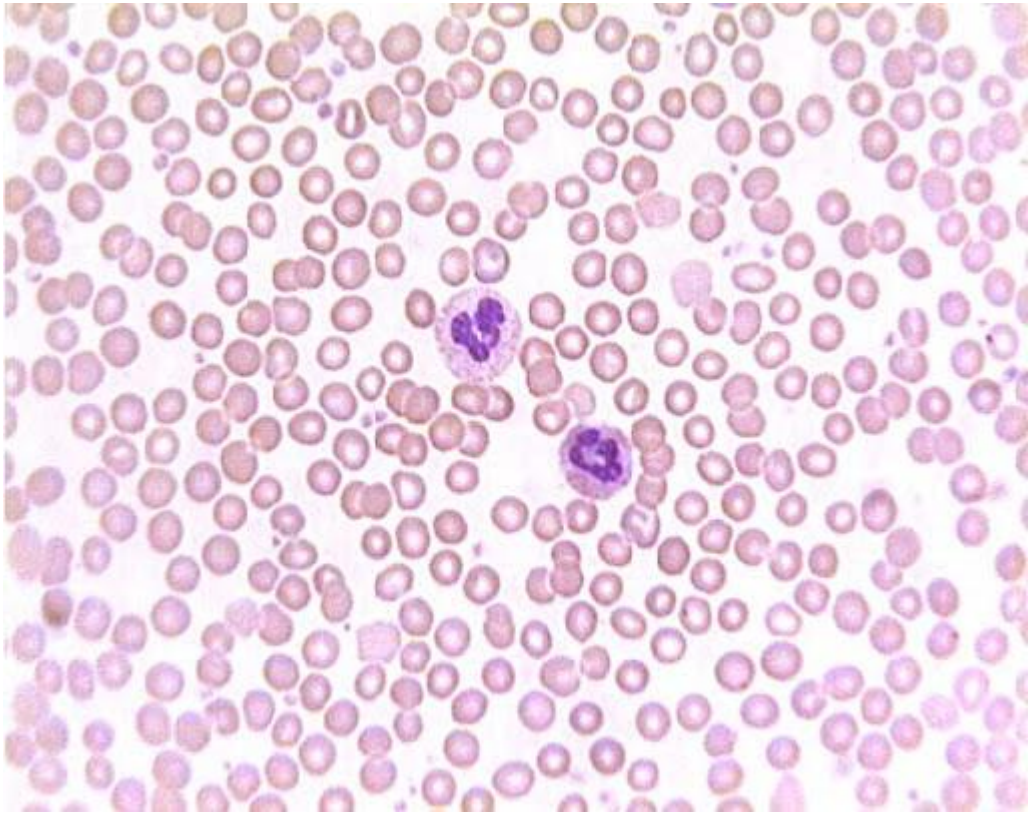
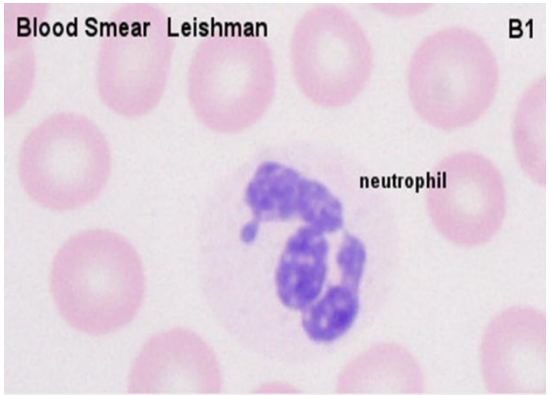
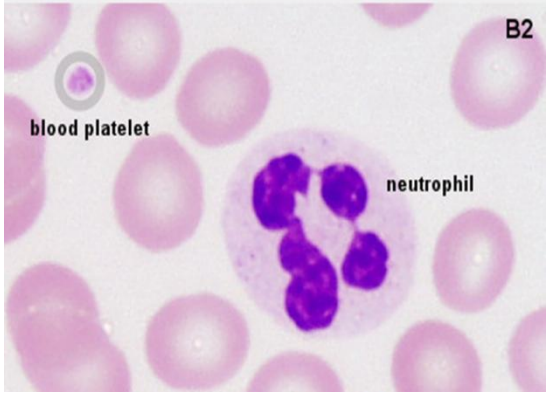
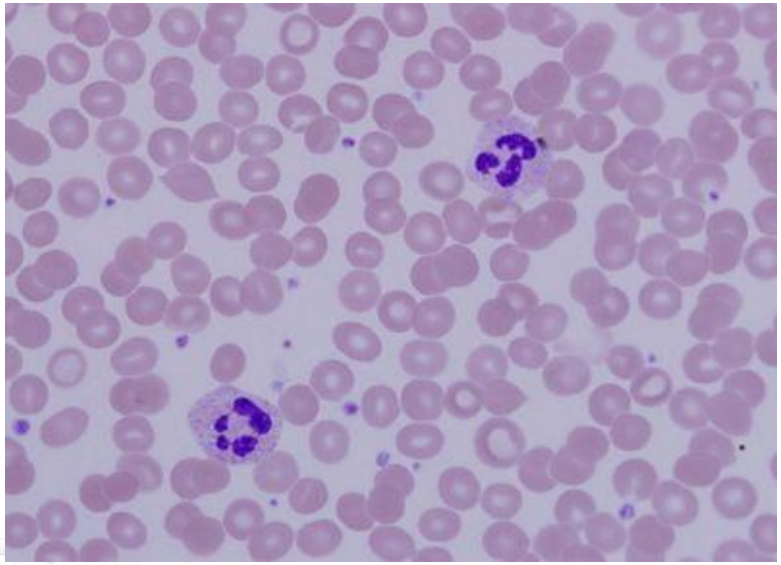
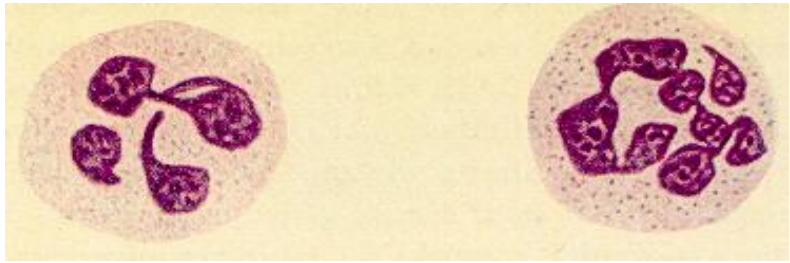
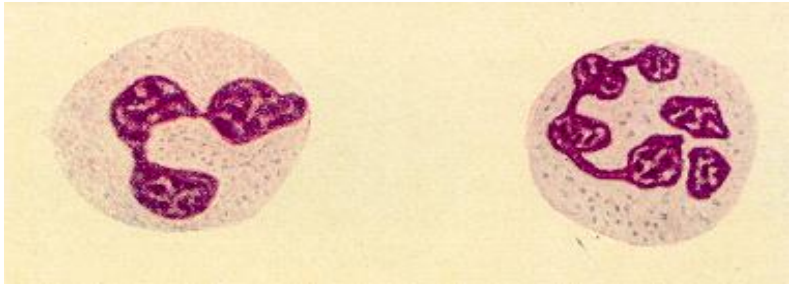



Blood Smear Leishman
B1
neutrophil

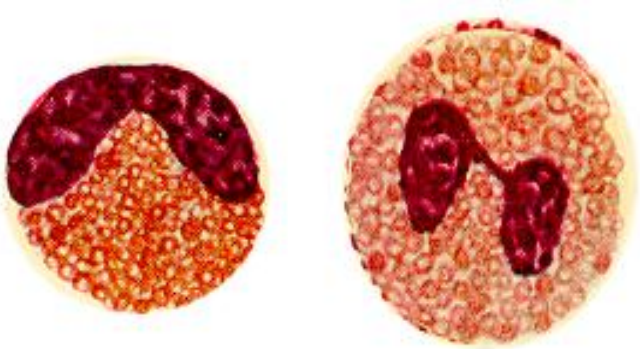

B2
blood platelet
neutrophil



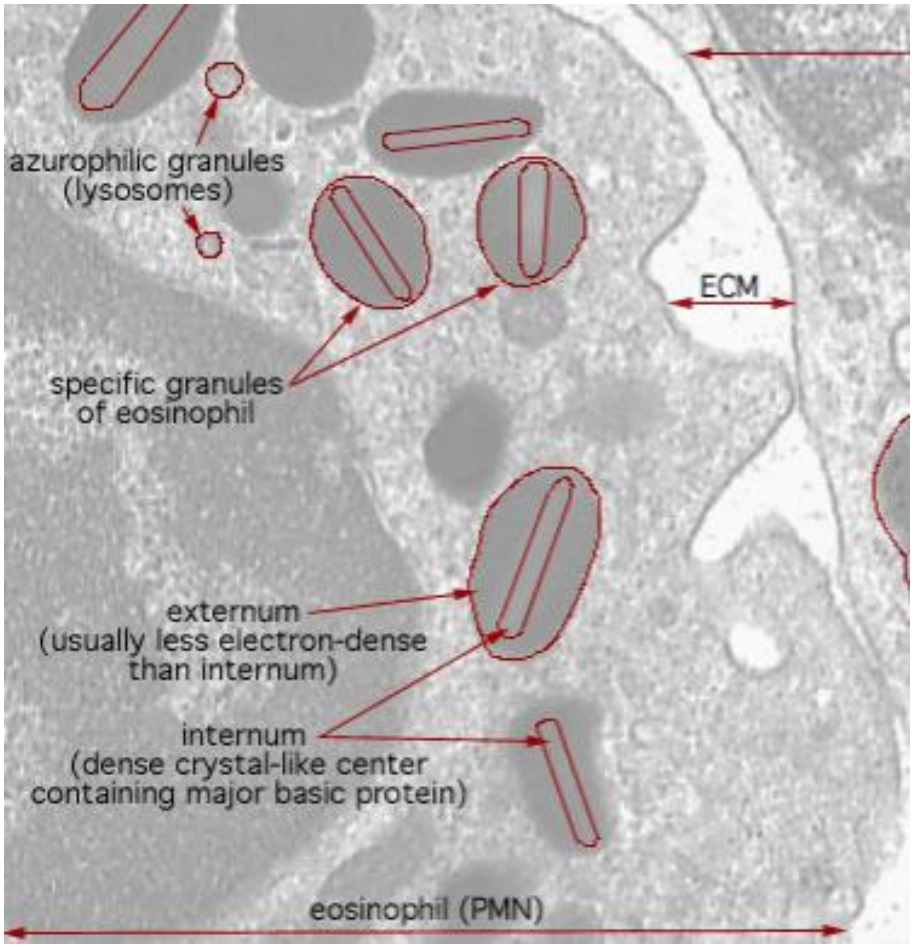




EOSINOPHILS



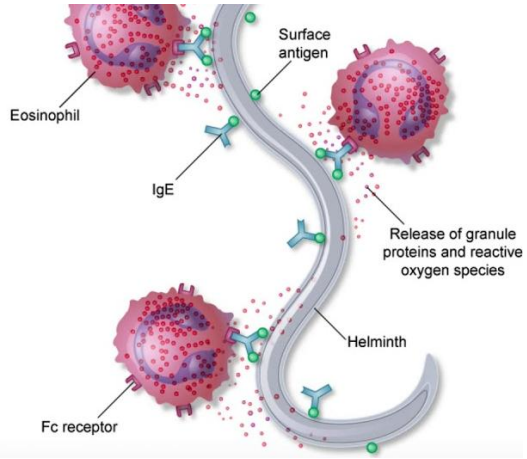
- Constitute less then 4% of the total white blood cell
- Have bilobed nucleus (lobes are connected by thin chromatin strand)
- Contain nonspecific (azurophilic) granules (lysosomes with hydrolitic enzymes) and pink specific granules

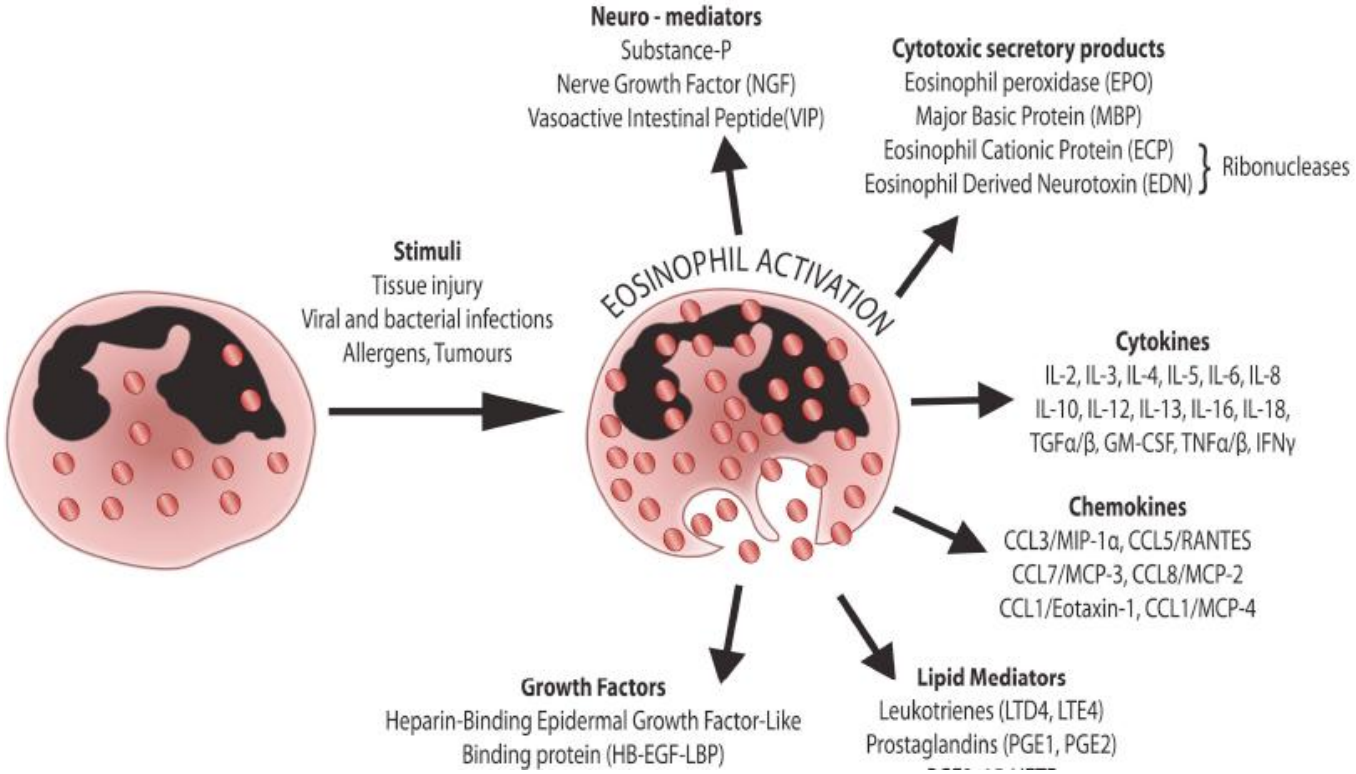


Specific granules contain (within internum):

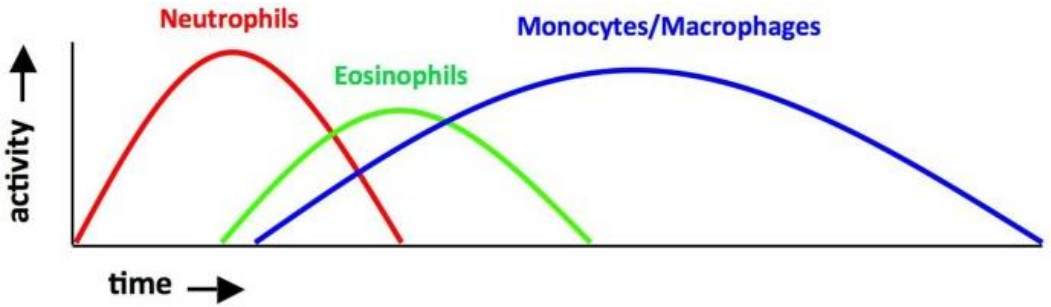
- Major basic protein
- Eosinophilic cationic protein
- Eosinophil-derived neurotoxin

EOSINOPHILS



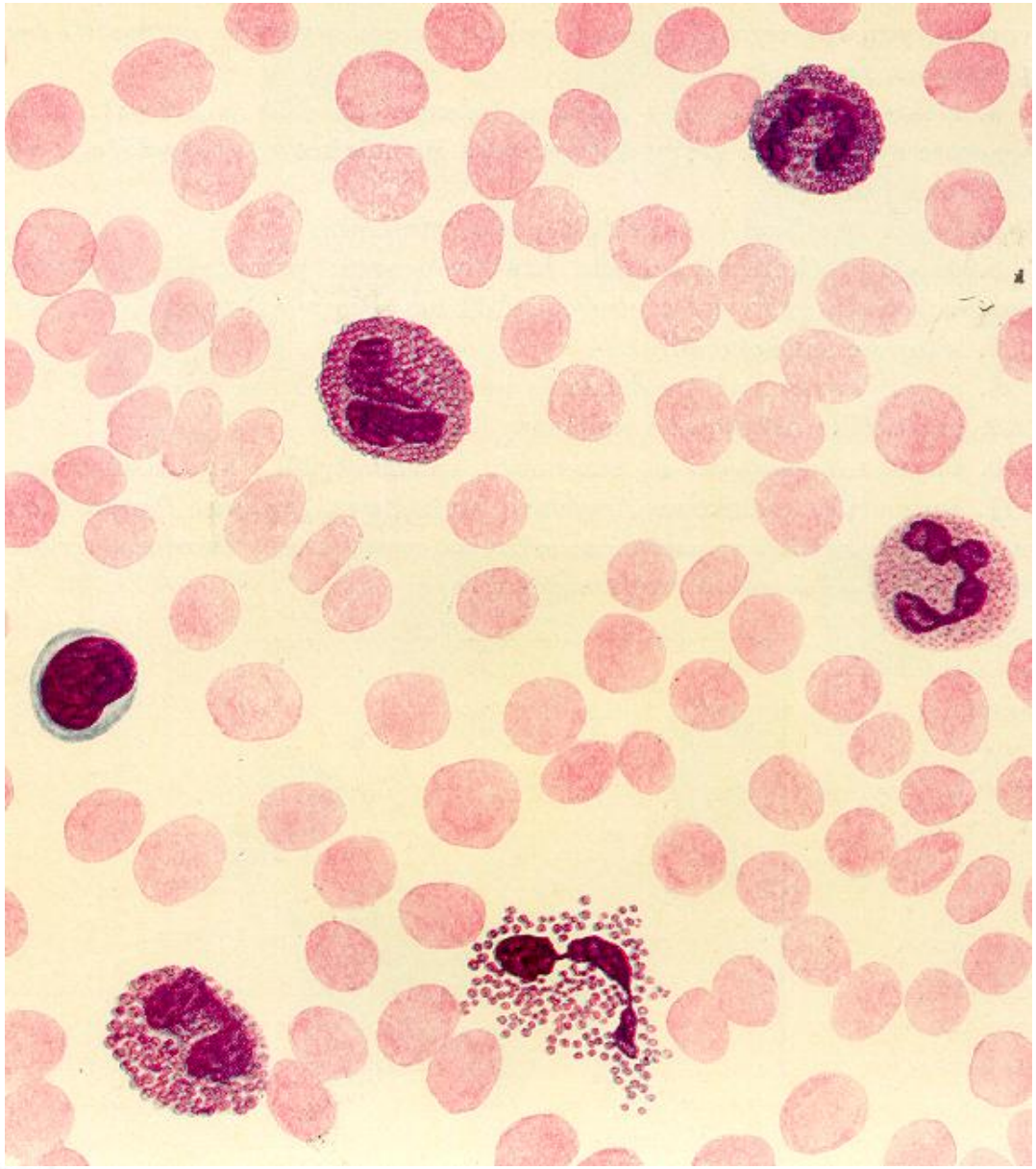


Eosinophils can be activated by a variety of stimuli that include non-specific tissue injury, infections, allergens and tumors.



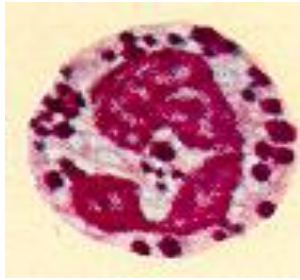
Eosinophils release histaminase during allergic reactions and are associated with inflammatory responses and helminthic responses to parasitic and protozoan infections.

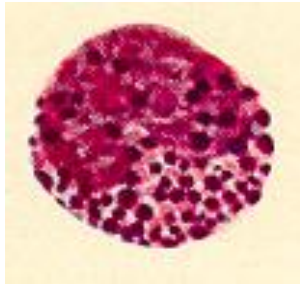
Eosinophilia in blood

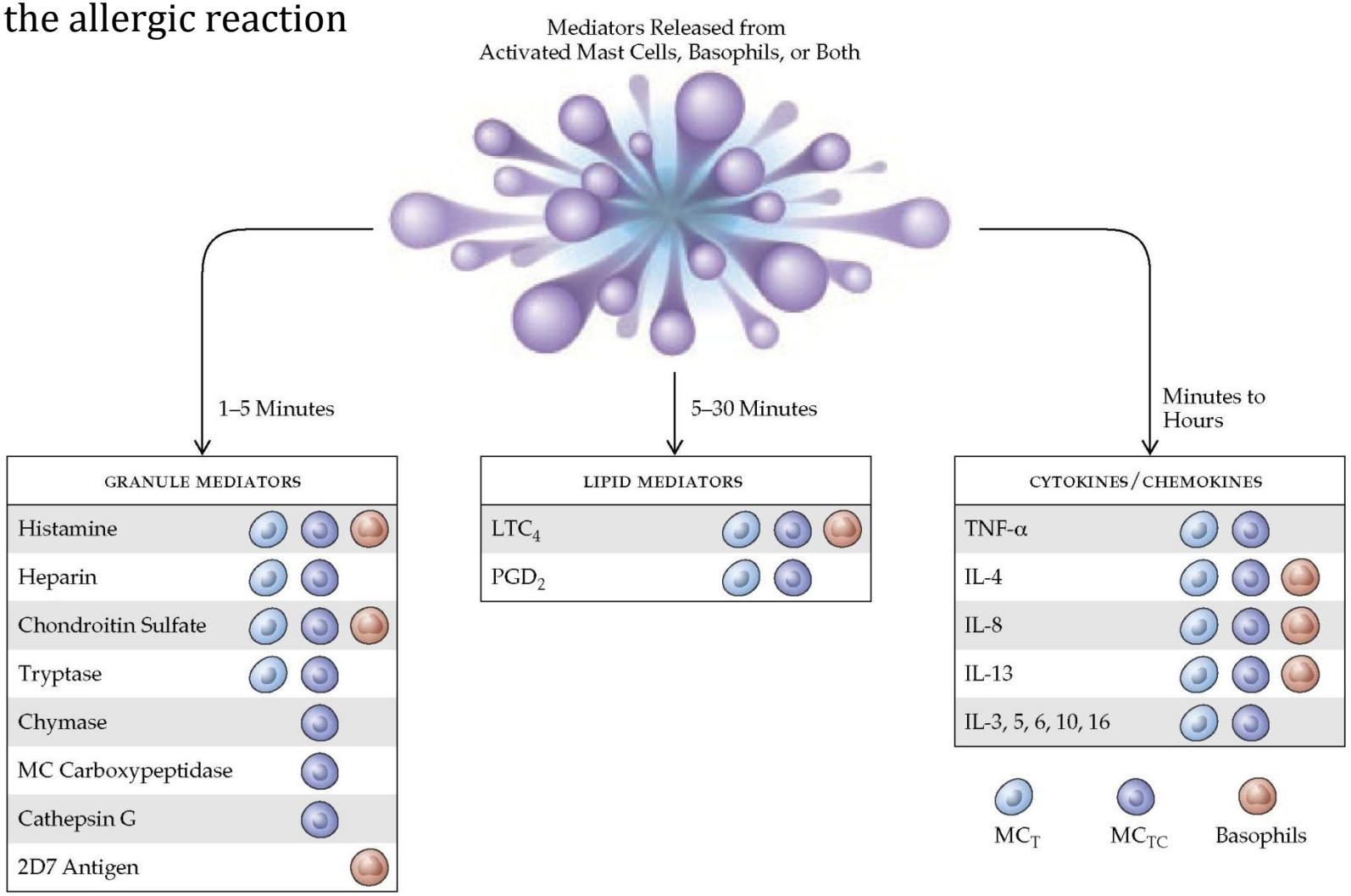


BASOPHILS

- Constitute less then 1% of total leukocyte population
- S-shaped nucleus, masked by dark-blue specific granules
- Basophils are associated with allergic responses as well.
- Release of histamine and vasoactive agents (blood vessels dilatation) – intensification of the allergic reaction

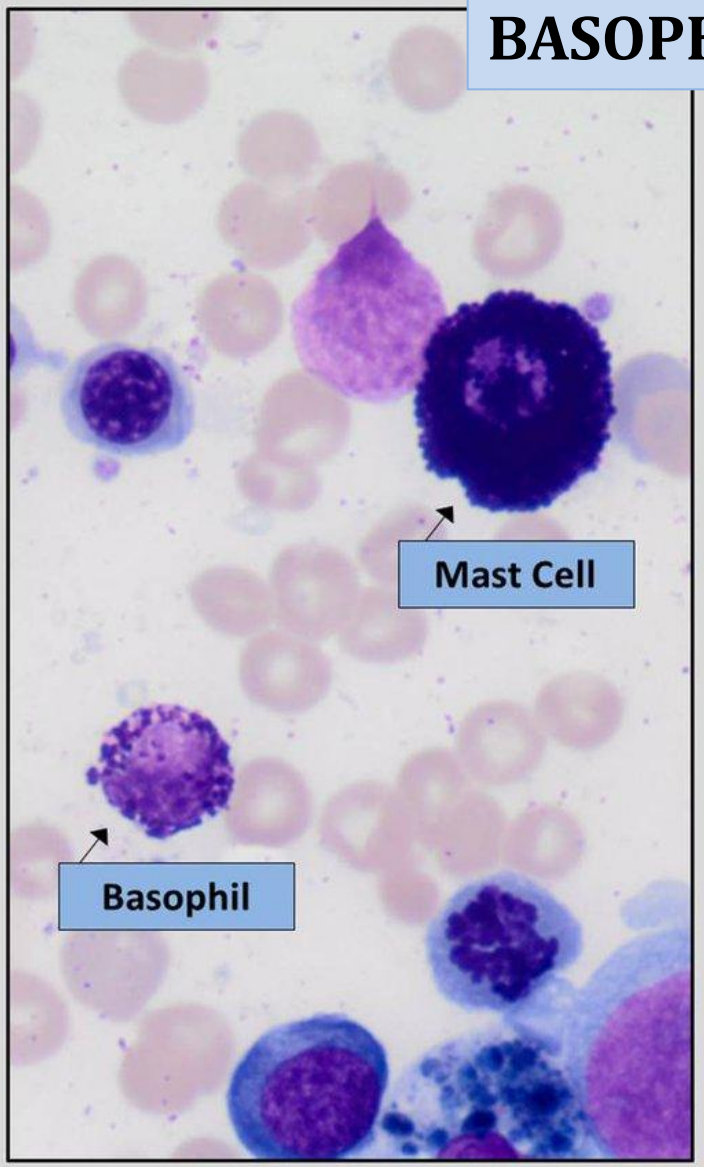






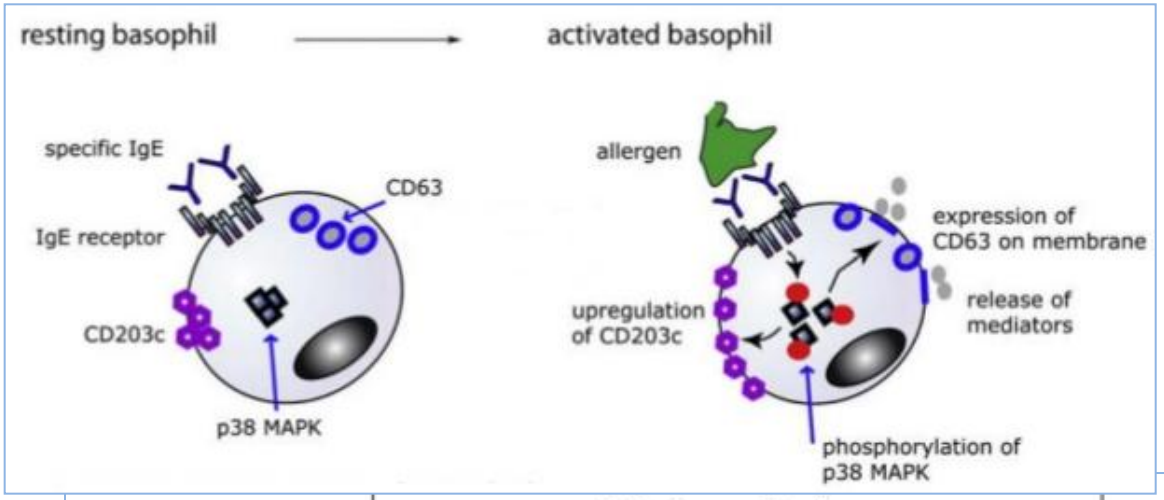
BASOPHILS

	Basophils	Mast Cells
Cell Size	Smaller (10-15 μm)	Larger (15-30 μm)
Cell Shape	Round	Oval
Nuclear Shape	Lobated	Round
Cytoplasmic Granules	Many fewer than in mast cells Granules often partially obscure the nucleus Cell border appears smooth	Densely pack the cytoplasm Granules often partially obscure the nucleus and obscure the cytoplasmic border Cell border appears bumpy
Life Span	Days	Months



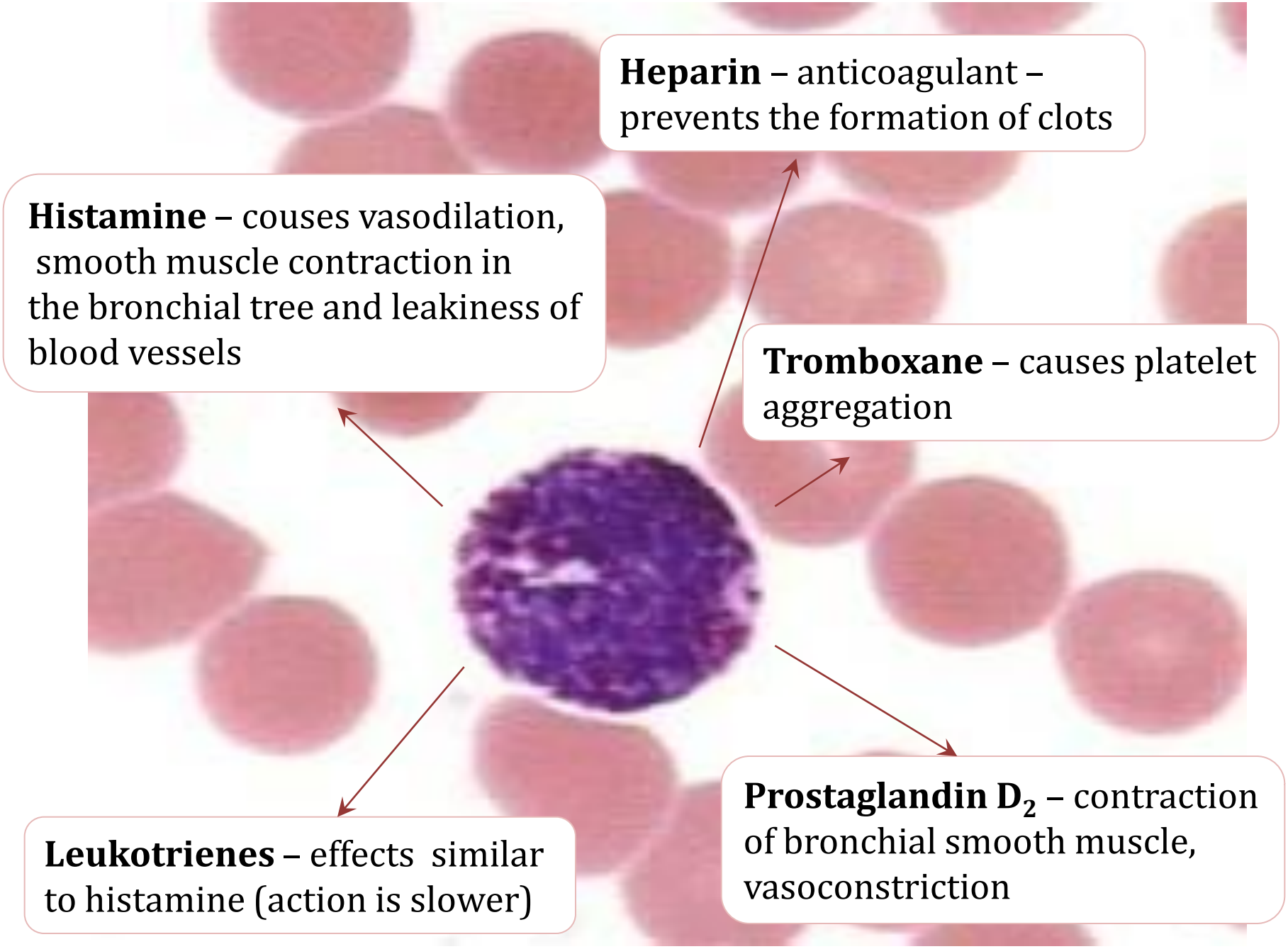
Basophil differentiation from myeloid progenitors is driven by **IL-3** and their expression of **IL-3R α chain** (distinction from mast cells).

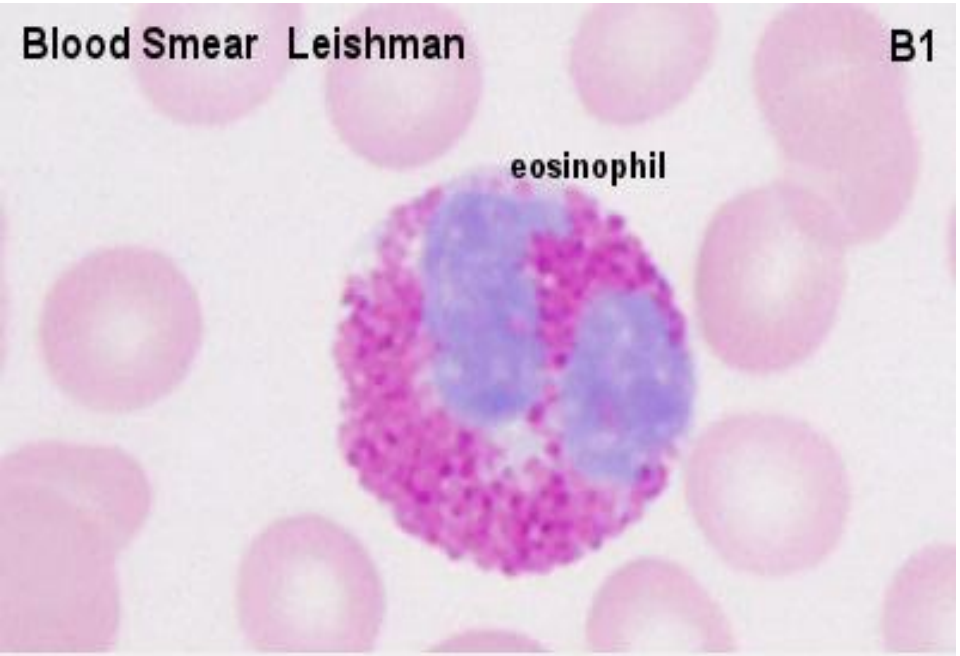
BASOPHILS

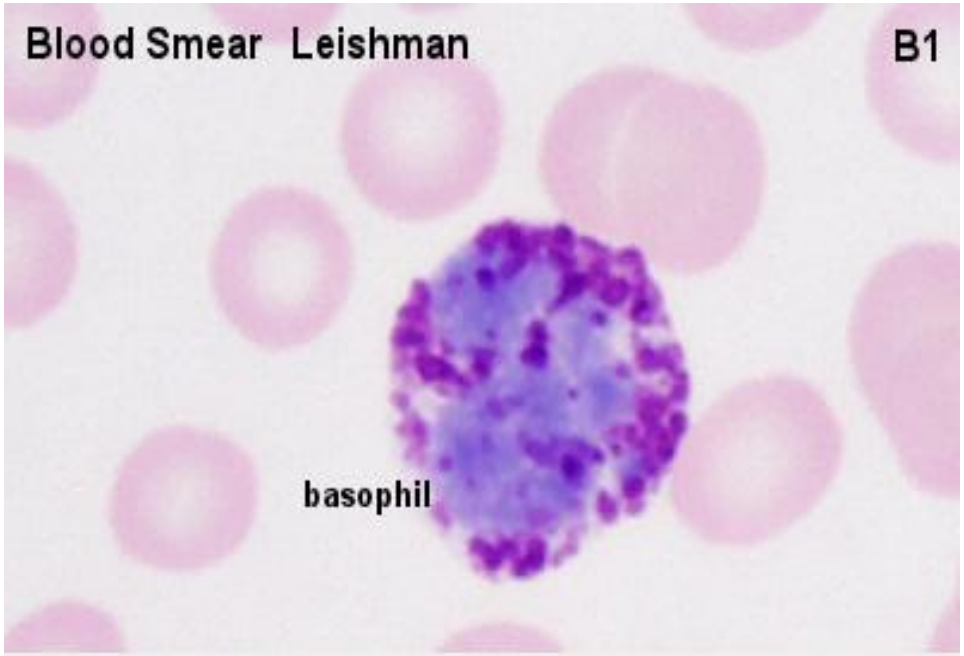


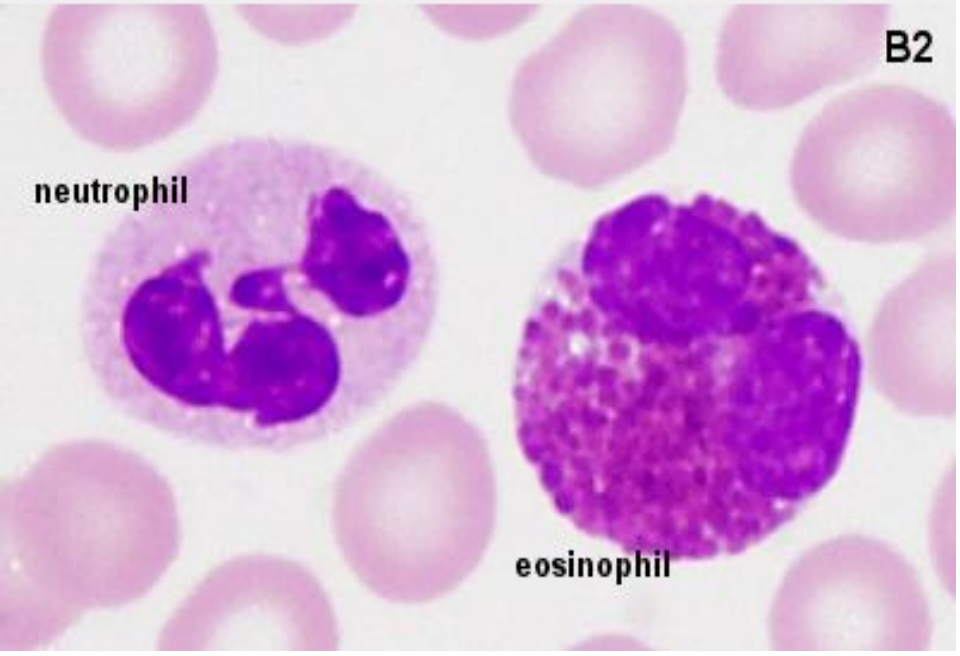
		IgE-dependent		IgE-independent
		IL-3-elicited basophil		TSLP-elicited basophil
Activation		IgE, Antigen, IgE, FcεR1	IL-3, FcεR1, IL-3R	IL-3R, TSLPR, IL-33R, IL-18R
Function		Degranulation Cytokine secretion		Cytokine secretion
Effector molecules	Mouse	IL-4, Histamine	IL-4, 13	IL-4, 6, 13, Histamine CCL3, CCL4, CCL9, CCL12, CXCL2
	Human	IL-4, 13, Histamine, leukotriene Platelet-activating factor	IL-13, Histamine, leukotriene amphiregulin	IL-4, 5, 6, 13
Related human disease		Chronic idiopathic urticaria (CIU) Food allergy Allergic rhinitis	Asthma	Atopic dermatitis (AD) Eosinophilic esophagitis (EoE)

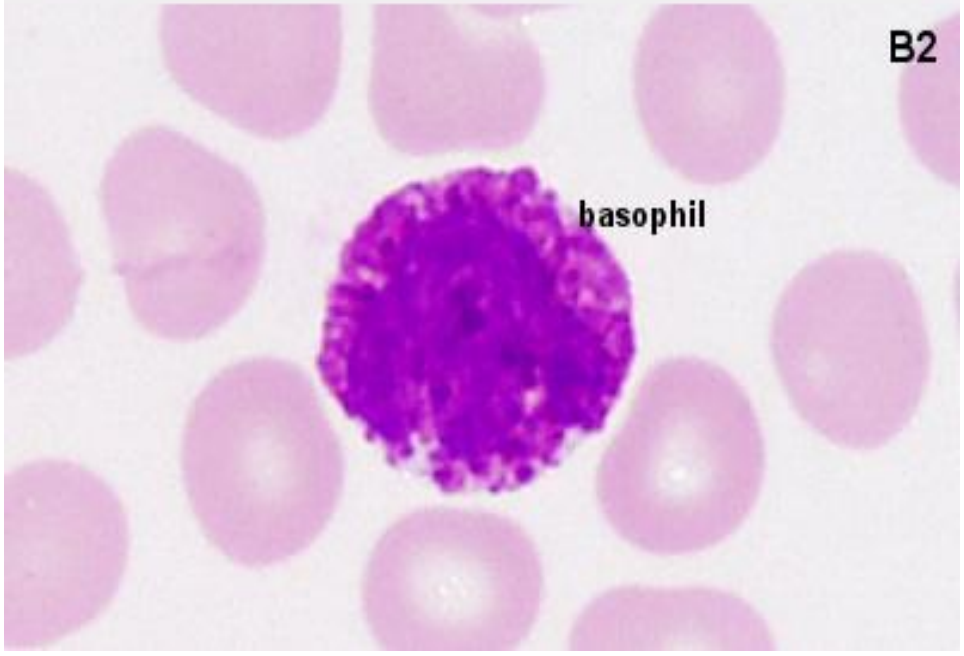
doi.org/10.1016/j.jdermsci.2018.03.007

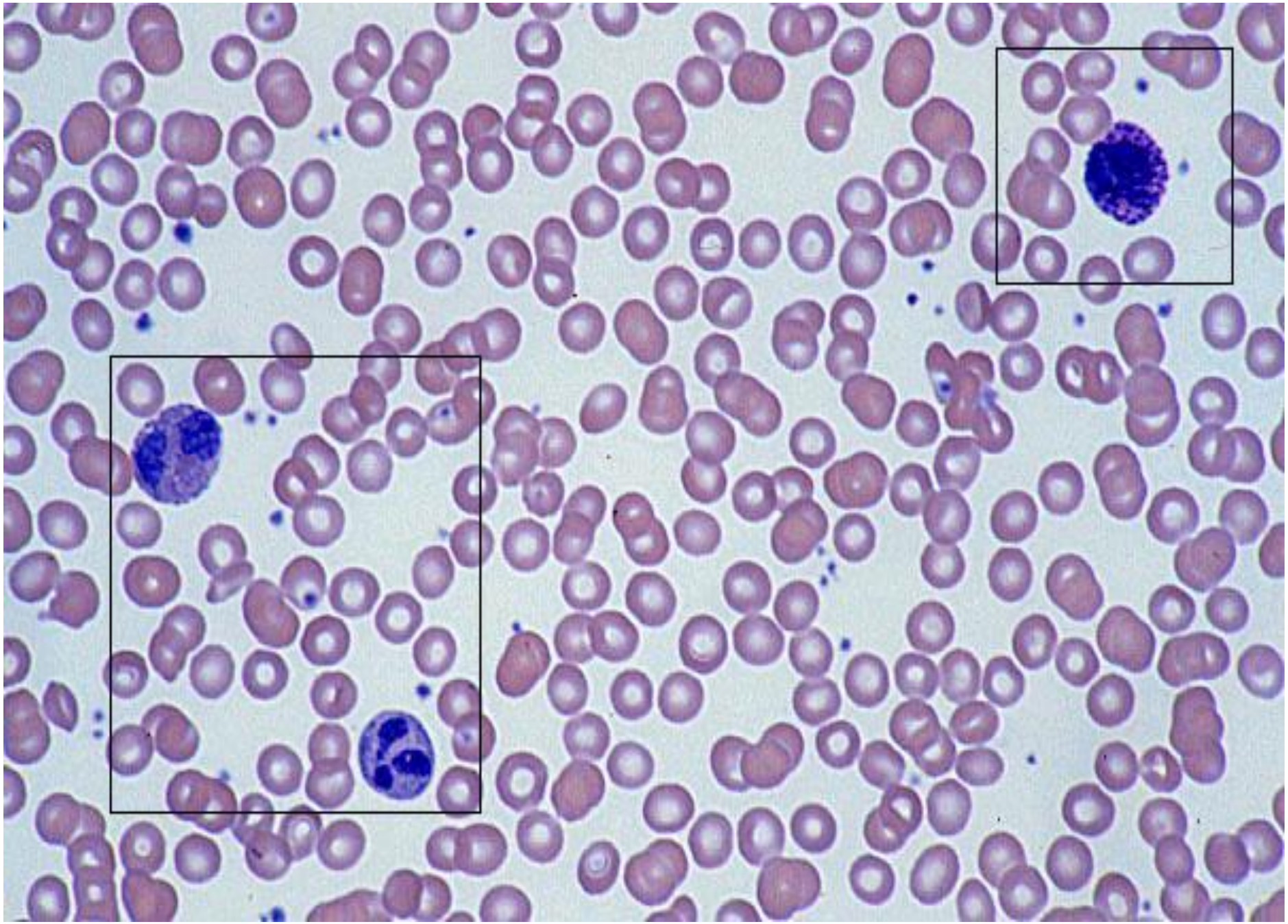

Heparin – anticoagulant – prevents the formation of clots
Histamine – couses vasodilation, smooth muscle contraction in the bronchial tree and leakiness of blood vessels
Tromboxane – causes platelet aggregation
Leukotrienes – effects similar to histamine (action is slower)
Prostaglandin D_2 – contraction of bronchial smooth muscle, vasoconstriction


Blood Smear Leishman
B1
eosinophil


Blood Smear Leishman
B1
basophil

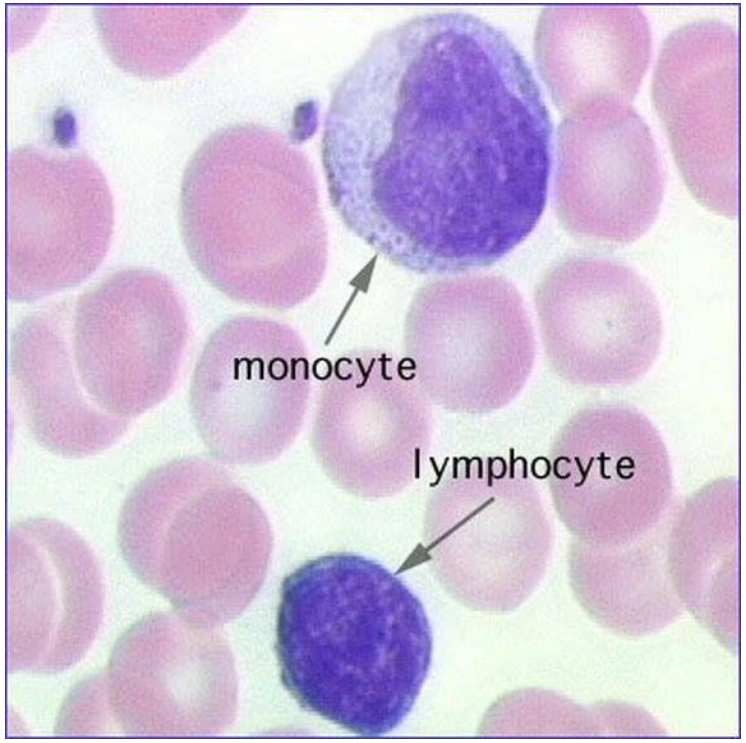

B2
neutrophil
eosinophil


B2
basophil

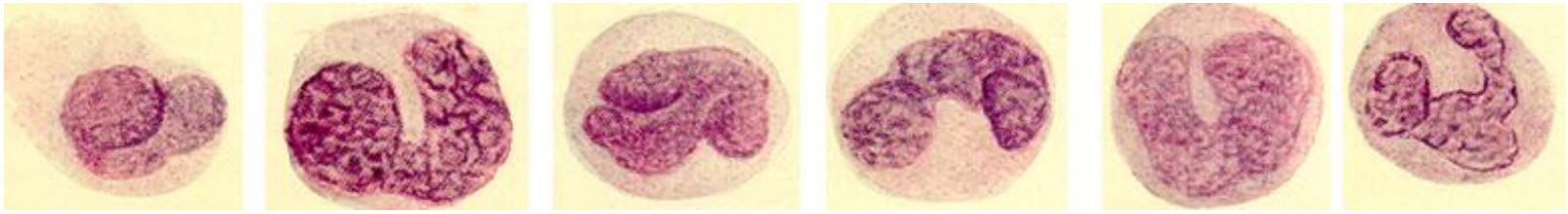


MONOCYTES

- The largest of the circulating blood cells constitute 3 – 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**

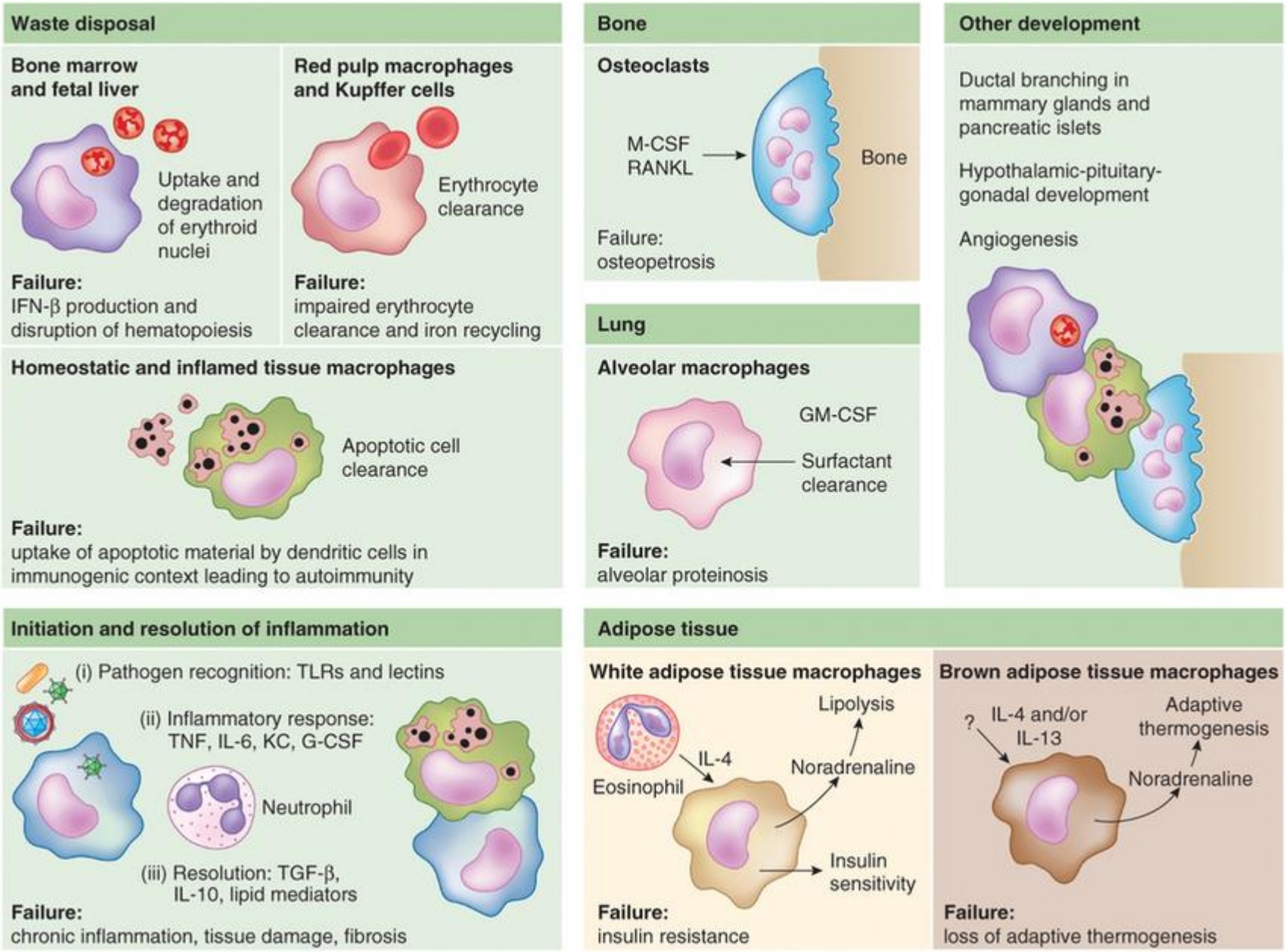


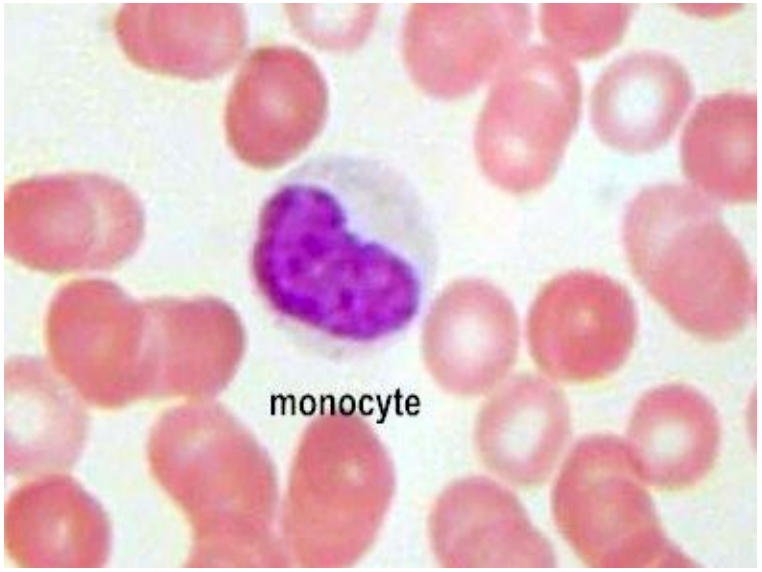
Monocytes become tissue macrophages, which phagocytose and digest invading microorganisms and foreign bodies as well as damaged senescent cells. Members of mononuclear phagosyte system.

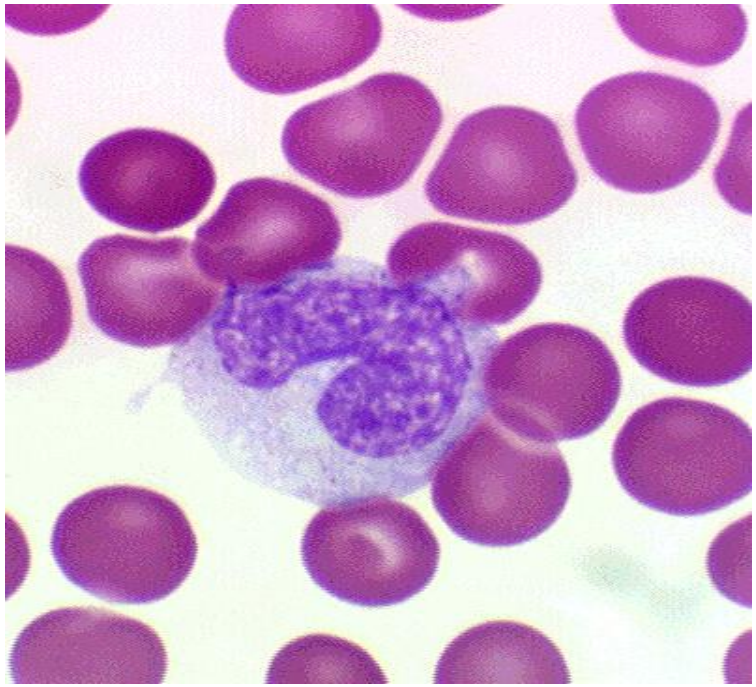


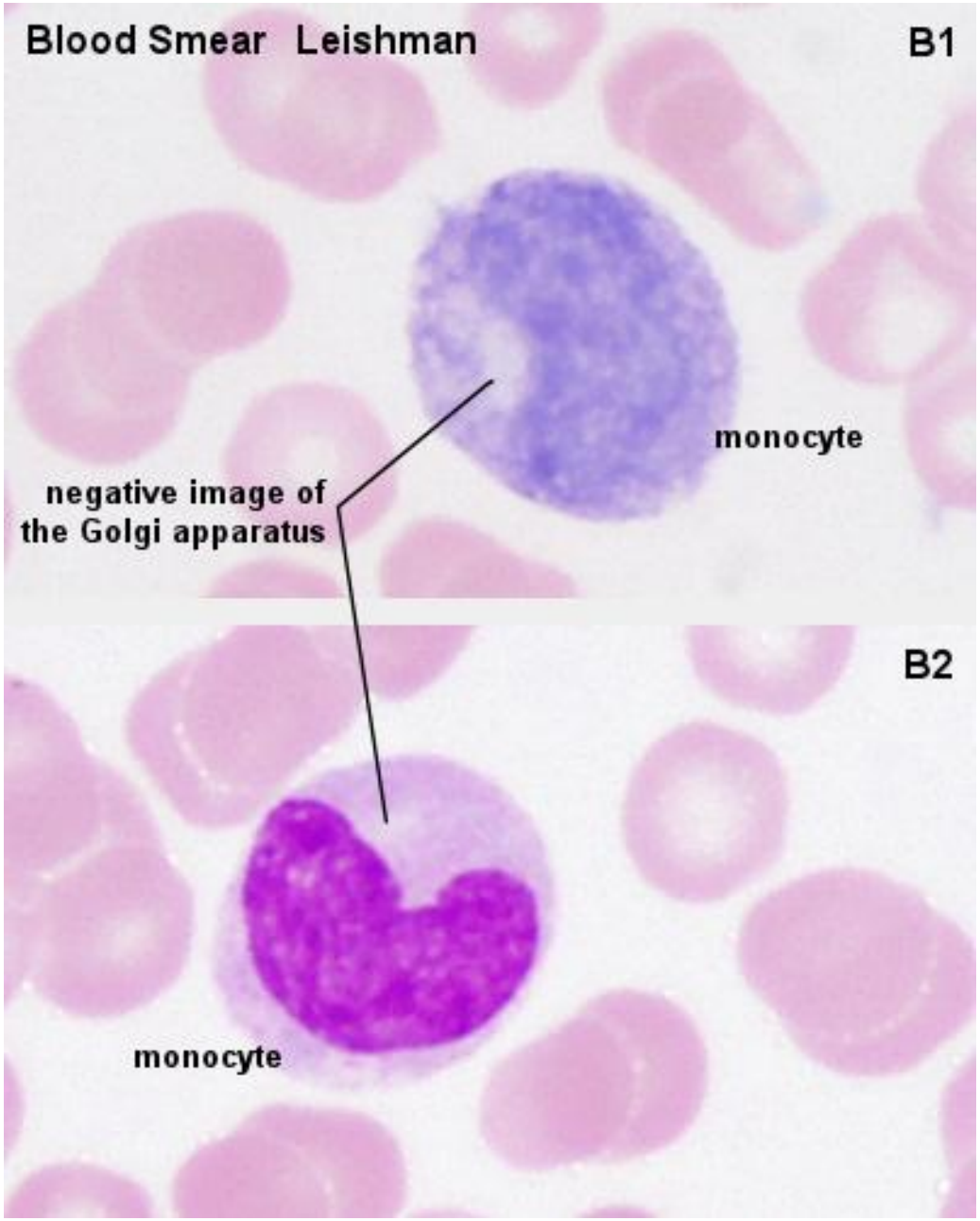
MONOCYTES

Source: doi:10.1038/ni.2705.




monocyte



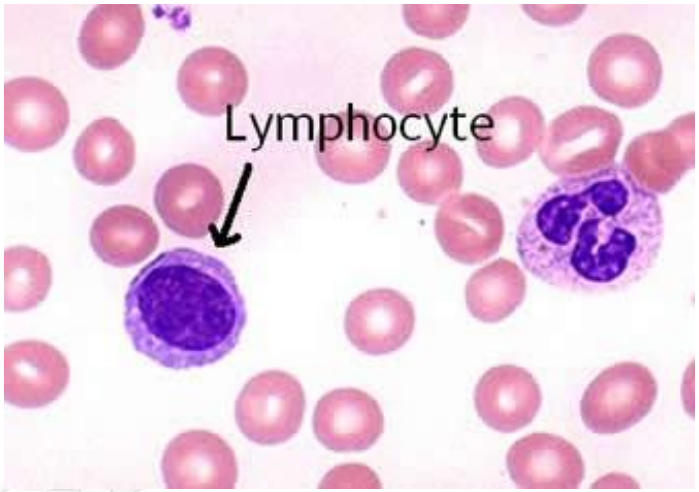

Blood Smear Leishman
B1
monocyte
negative image of
the Golgi apparatus
B2
monocyte

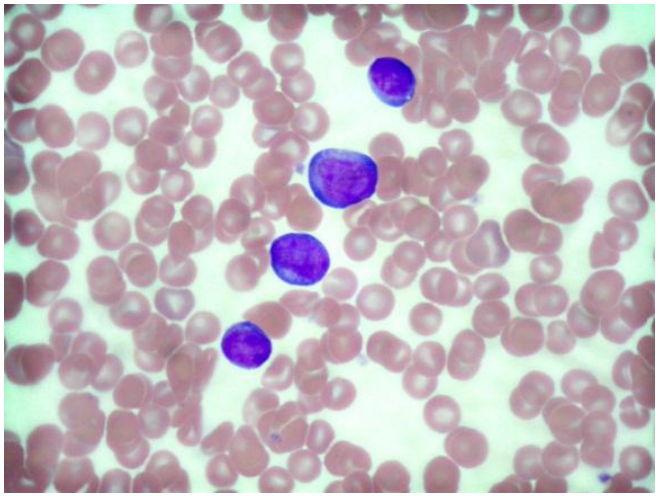
LYMPHOCYTES

- Constitute 20% to 25% 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 (5% of the circulating lymphocytes)





LYMPHOCYTES -FUNCTIONS

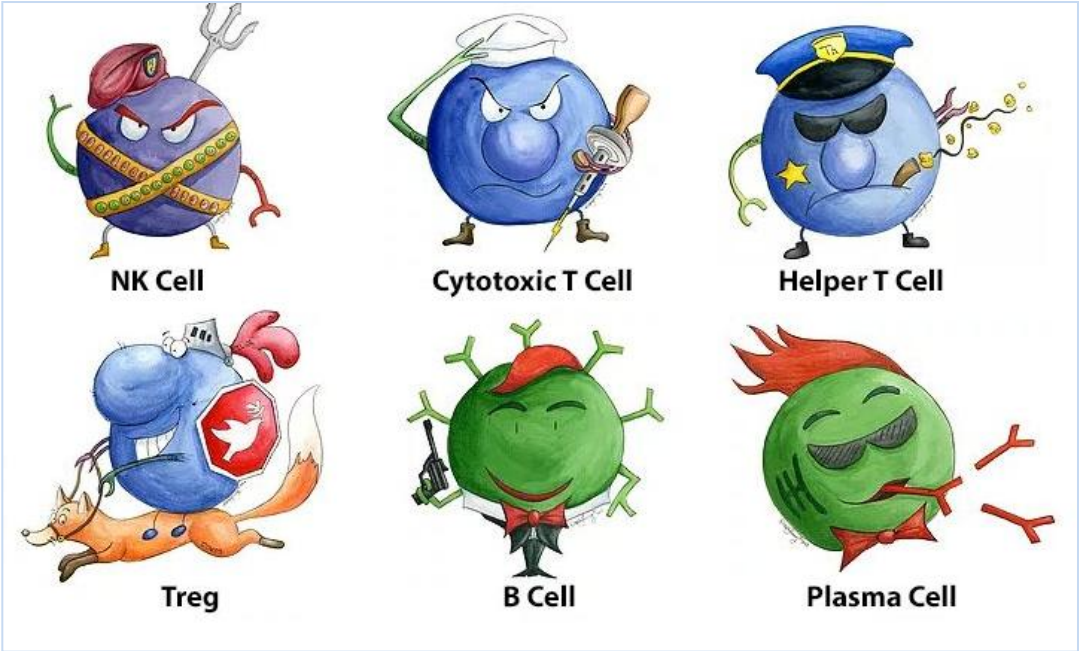
B cells – responsible for the humoral immune response:

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

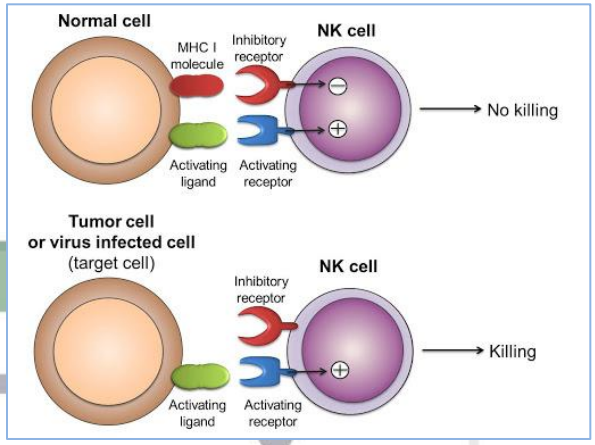
T cells – responsible for the cellular immune response:

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)

Morphologically are indistinguishable



LYMPHOCYTES -FUNCTIONS



Classes of Lymphocytes

T Cells

Account for approximately 80 percent of circulating lymphocytes; are of three major types

Cytotoxic T Cells

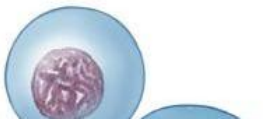
Attack foreign cells or body cells infected by viruses, commonly by direct contact; are the primary cells involved in the production of cell-mediated immunity (cellular immunity)

Helper T Cells

Stimulate the activation and function of both T cells and B cells

Suppressor T Cells

Inhibit the activation and function of both T cells and B cells; the interplay between suppressor T cells and helper T cells helps establish and control the sensitivity of the immune response



B Cells

Account for 10–15 percent of circulating lymphocytes

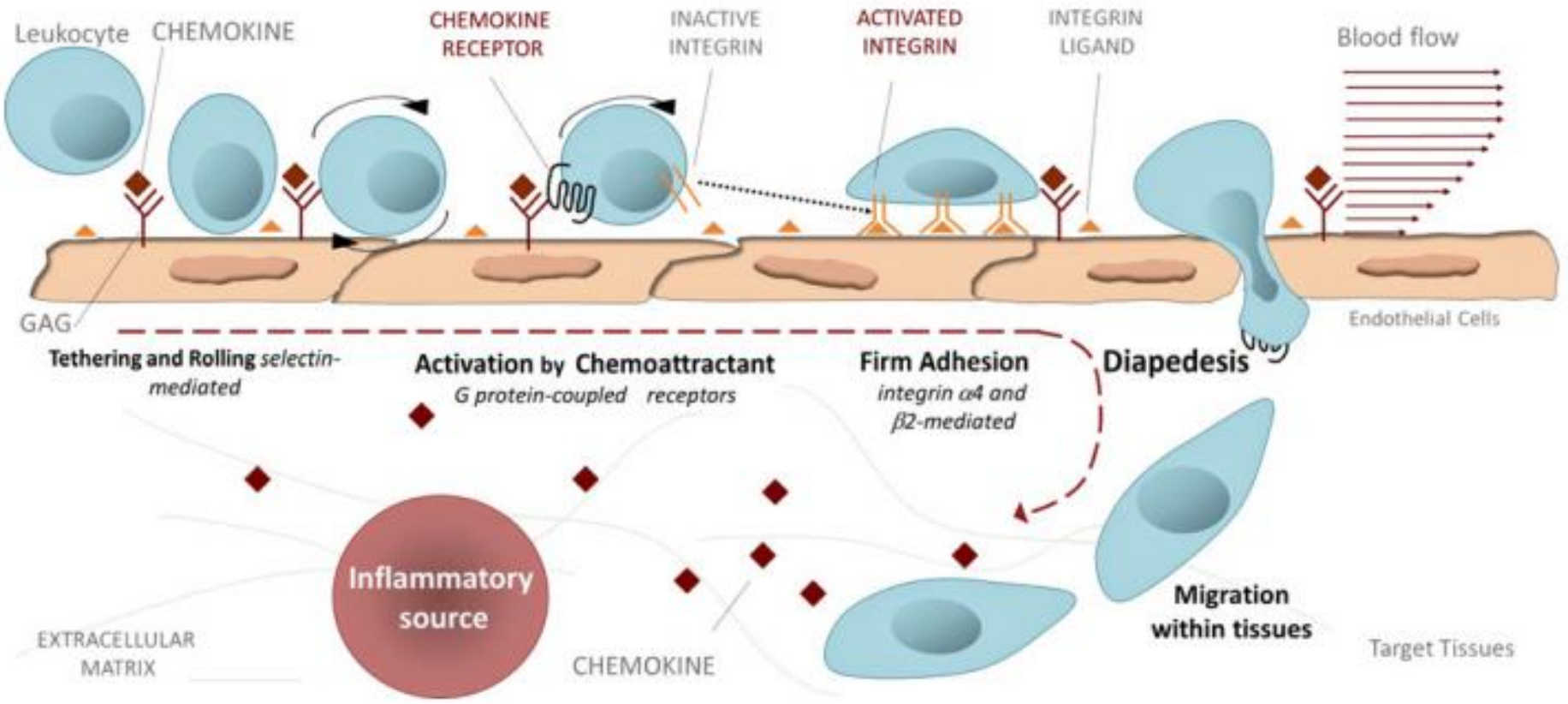
Plasma Cells

When stimulated can differentiate into plasma cells, which produce and secrete antibodies; are said to be responsible for antibody-mediated immunity (humoral immunity) because antibodies circulate widely in body fluids

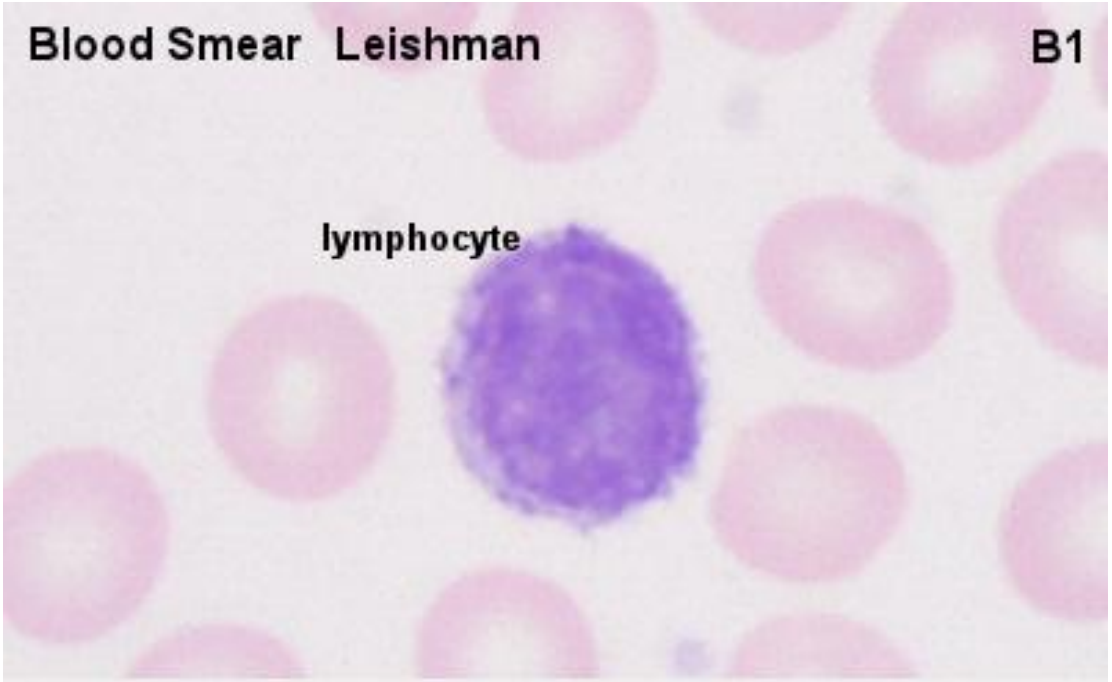
NK Cells

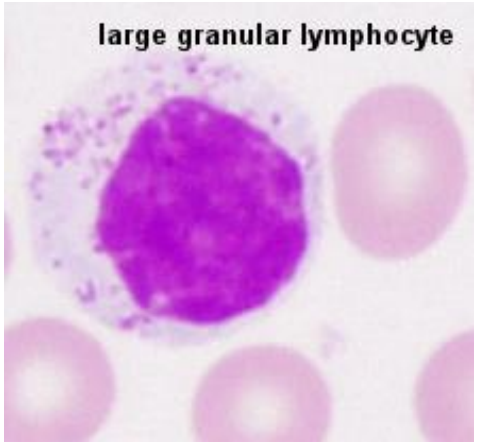
Account for 5–10 percent of circulating lymphocytes; perform immune surveillance, attacking foreign cells, body cells infected with viruses, and cancer cells that appear in normal tissues

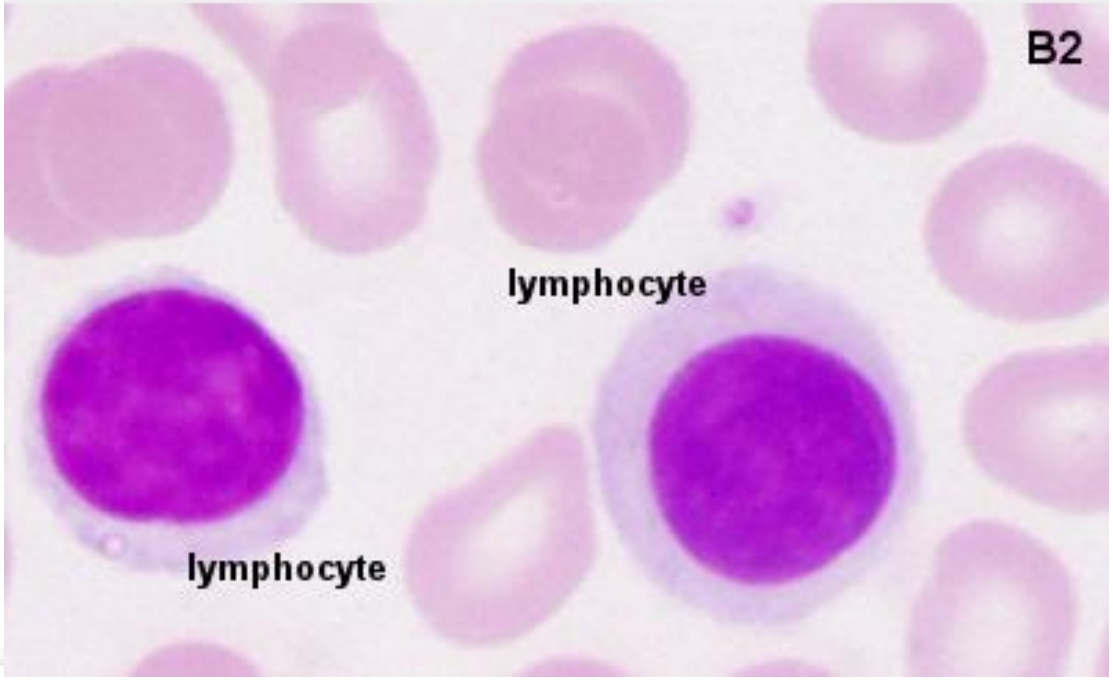
Leukocytes do not function only within the bloodstream but they migrate between the endothelial cells of blood vessels into the connective tissue during an inflammatory response - DIAPEDESIS

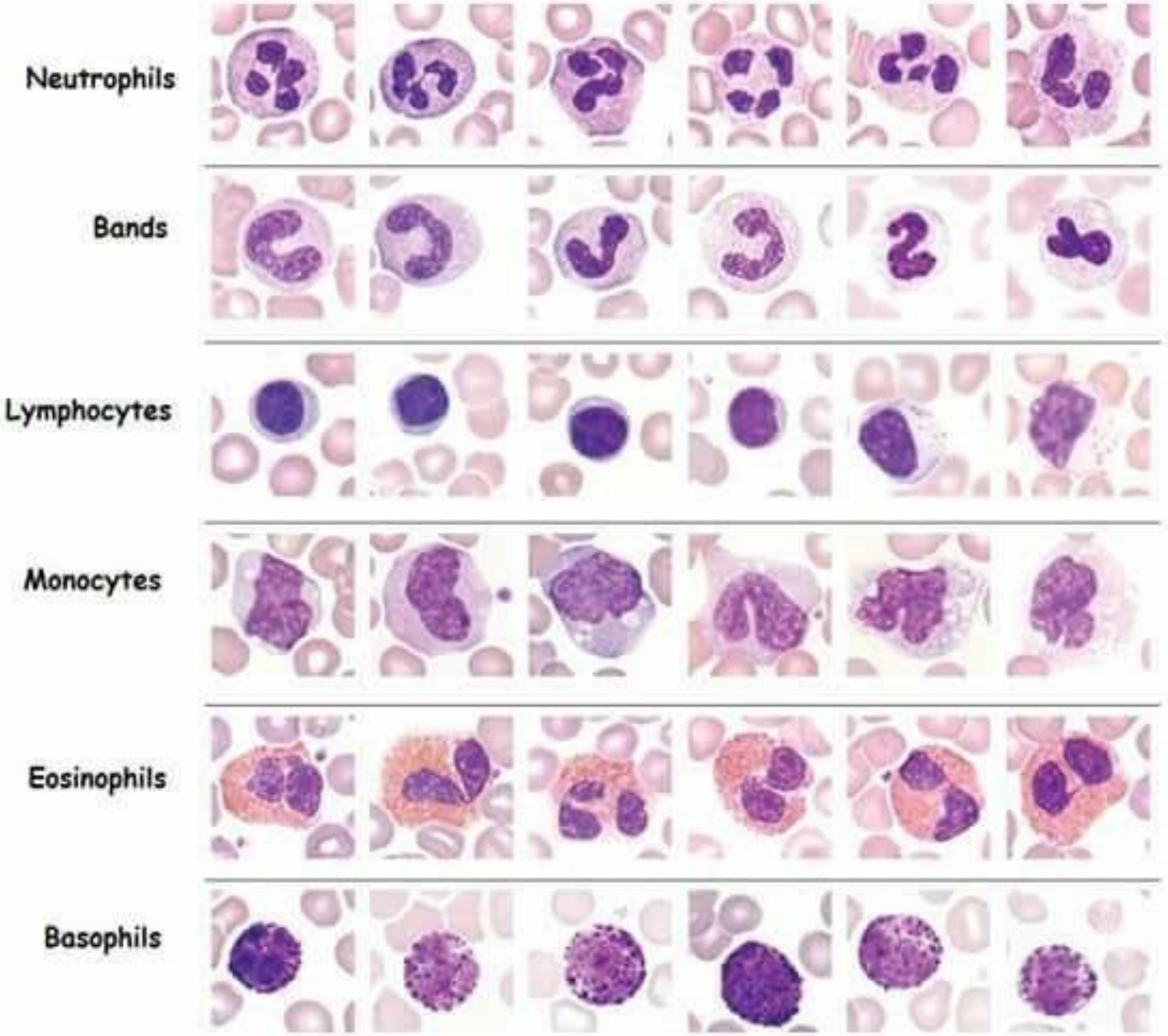


Source:
http://dx.doi.org/10.1016/j.jbiomech.2012.10.024


Blood Smear Leishman
B1
lymphocyte

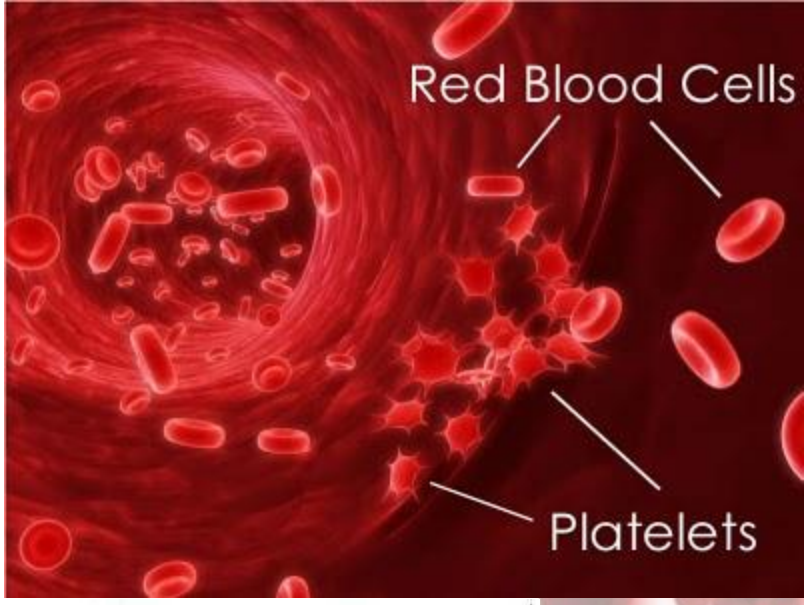

large granular lymphocyte


B2
lymphocyte
lymphocyte

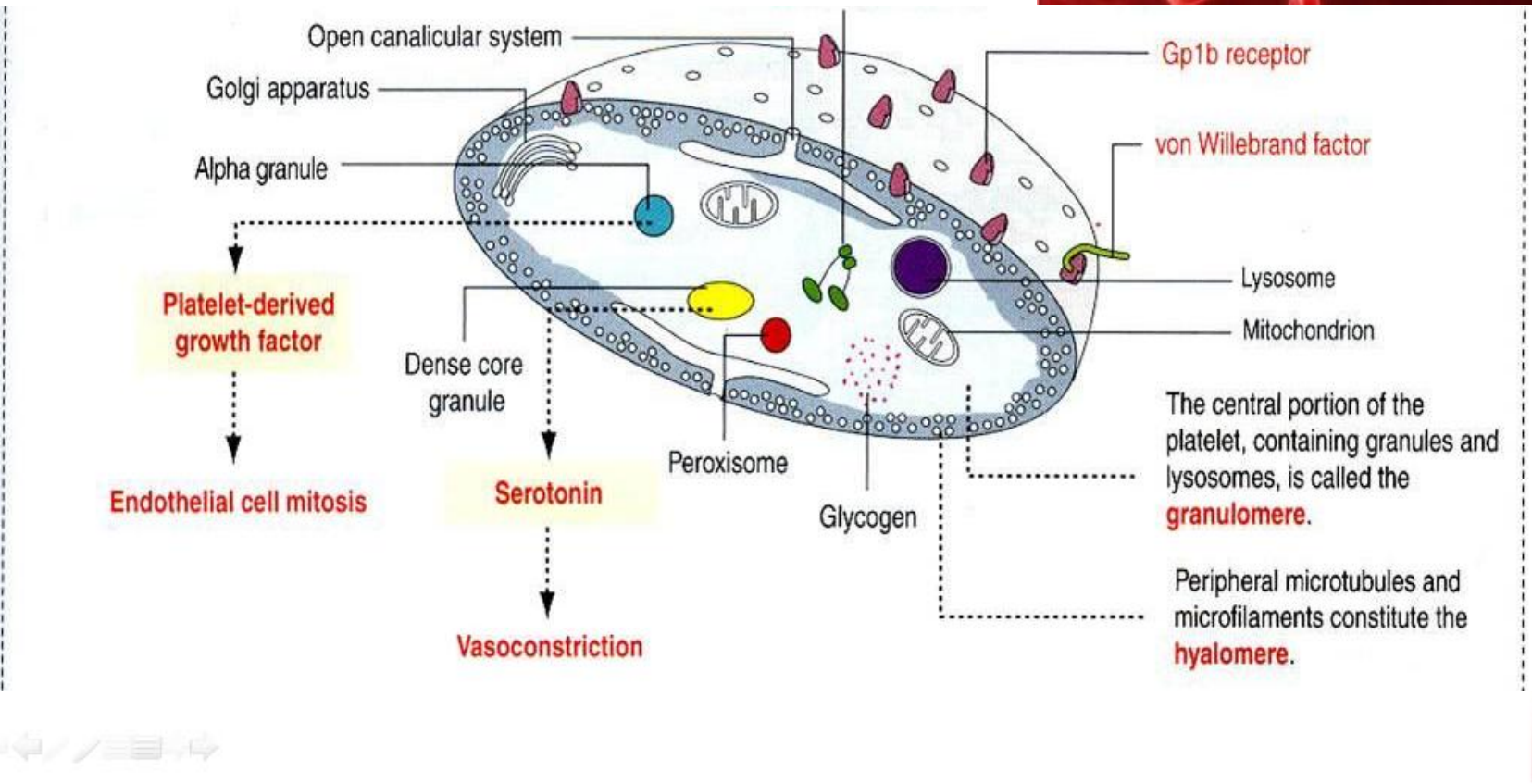


>> laboratoryinfo.com

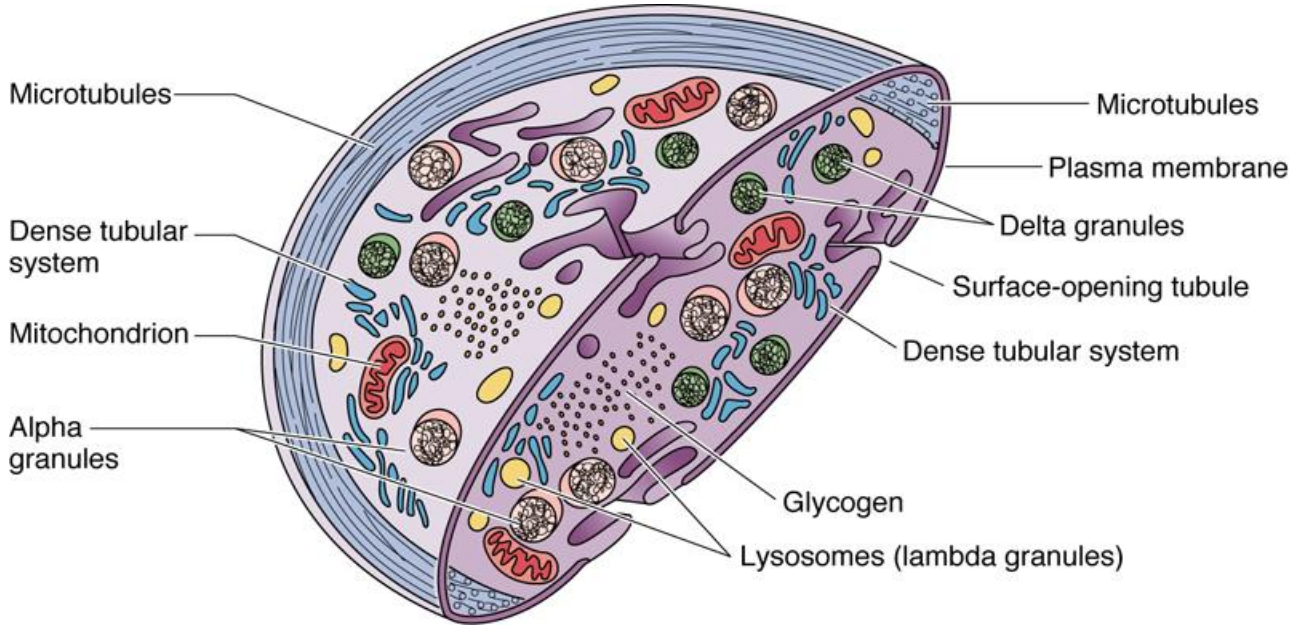
PLATELETS (THROMBOCYTES)



- Non-nucleated fragments of megakaryocyte cytoplasm, 2-4 μm in diameter, with a life-span less than 14 days.
- 250.000 – 400.000 per microliter of blood



PLATELETS (THROMBOCYTES)



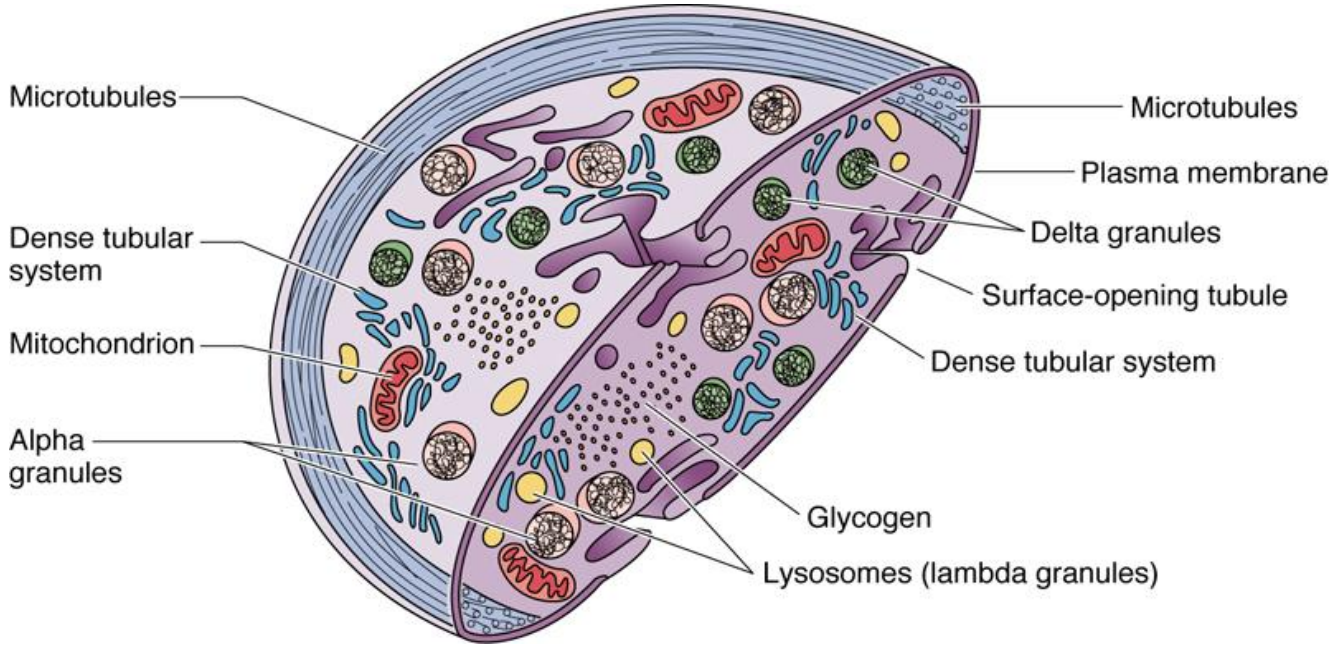
Two tubular systems of platelets

• **Dense tubular system (in hyalomere)** – calcium ions sequestration to prevent platelet stickiness?

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

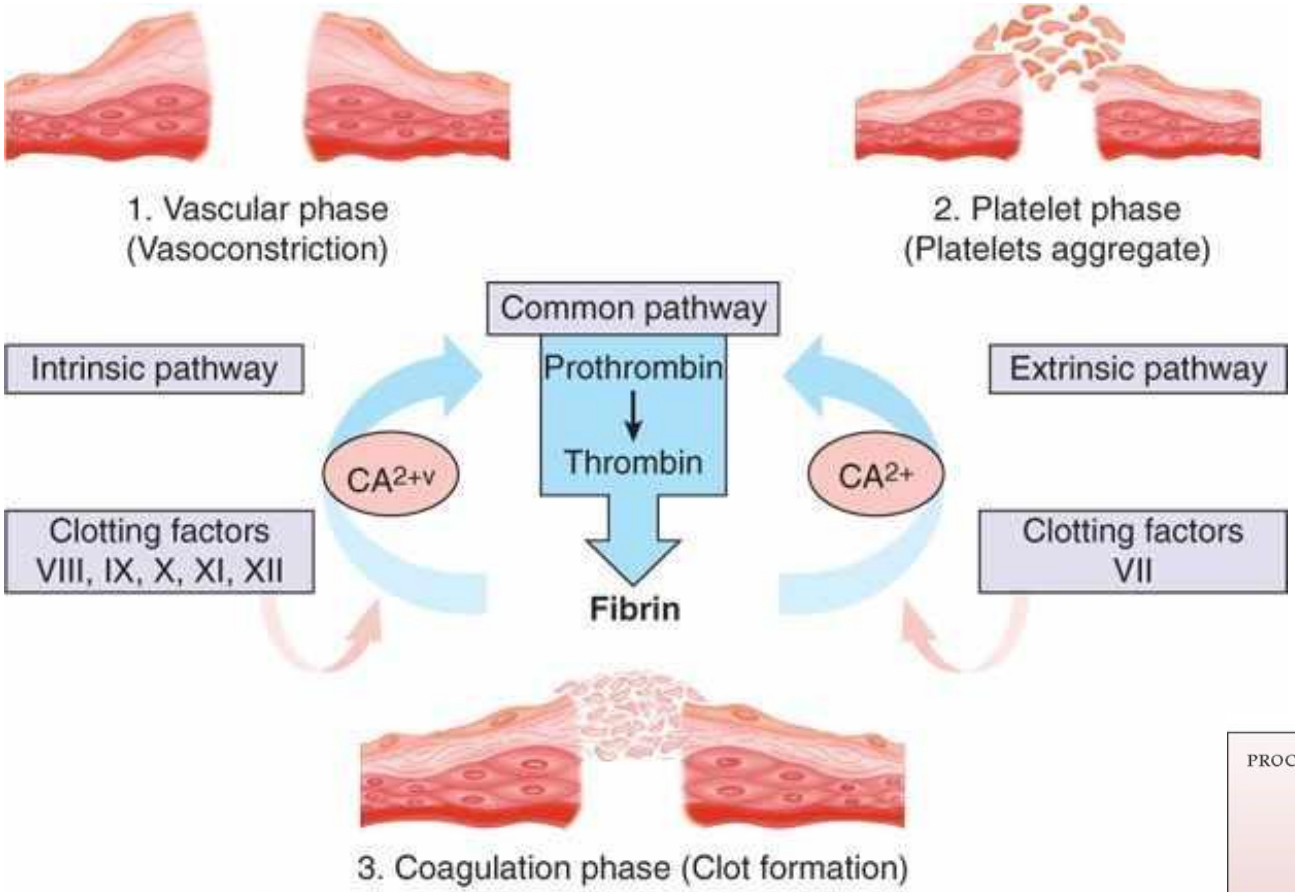
Microtubules (in hyalomere): are arranged parallel to each other and form a ring within the hyalomere, assist platelets in maintaining their diskoid morphology

PLATELETS (THROMBOCYTES)



Three types of granules:

- **alpha granules** (most numerous)- fibrinogen, PDGF, TGF (growth factors), von Willebrand factor, thromboplastin (formation of blood clots).
- **delta granules** - calcium ions, ADP, ATP, serotonin, histamine (faciliate platelet adhesion + vasoconstriction in the area of injured vessel)
- **lambda granules** (lysosomes) – hydrolytic enzymes (clot resorption in later stages of vessel repair)



Source: F.C. Brunicardi, D.K. Andersen, T.R. Billiar, D.L. Dunn, L.S. Kao, J.G. Hunter, J.B. Matthews, R.E. Pollock: Schwartz's Principles of Surgery, 11e
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Damaged endothelium:

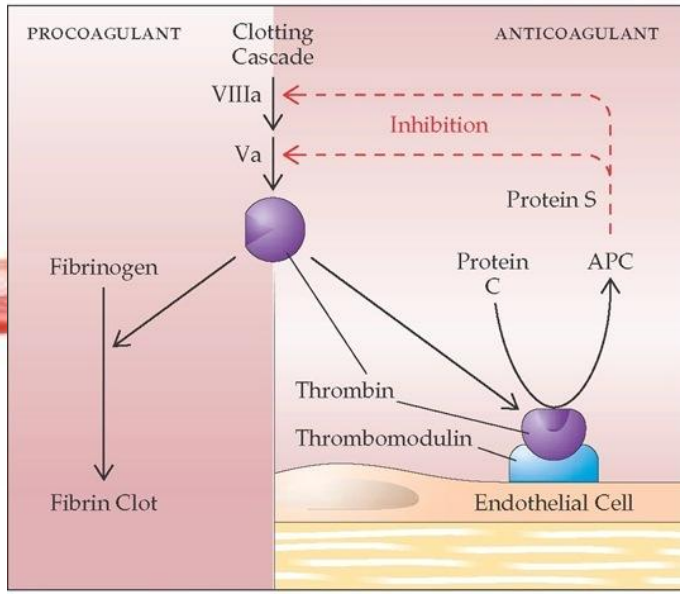
1.Von Willebrandt factor
2.Tissue thromboplastin
3.Endothelin

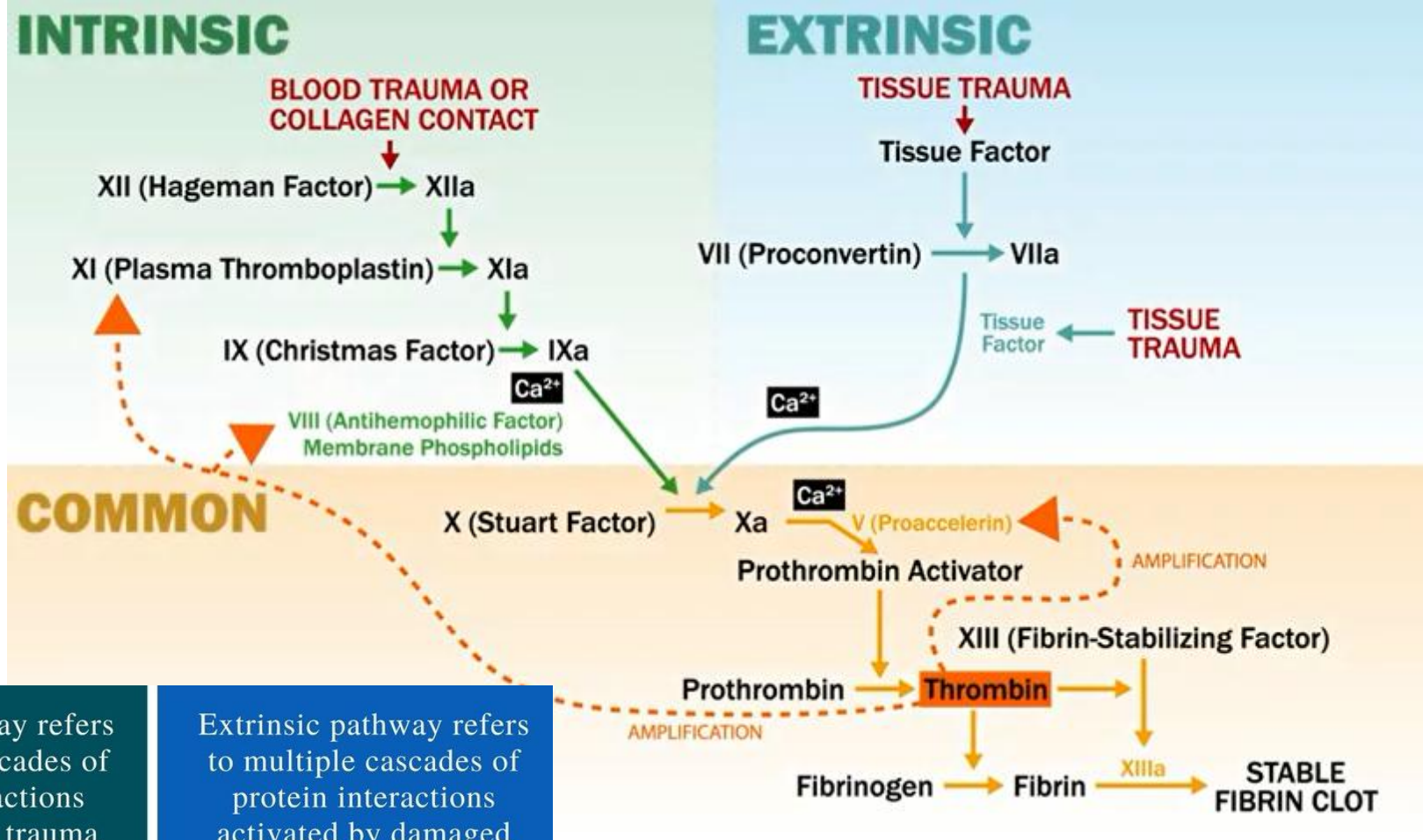
Activated platelets:

1.ADP
2.Thrombospondin
3.Thromboxane A2
4.Calcium
5.Coagulation factors
6.Fibrinogen
7.thromboplastin

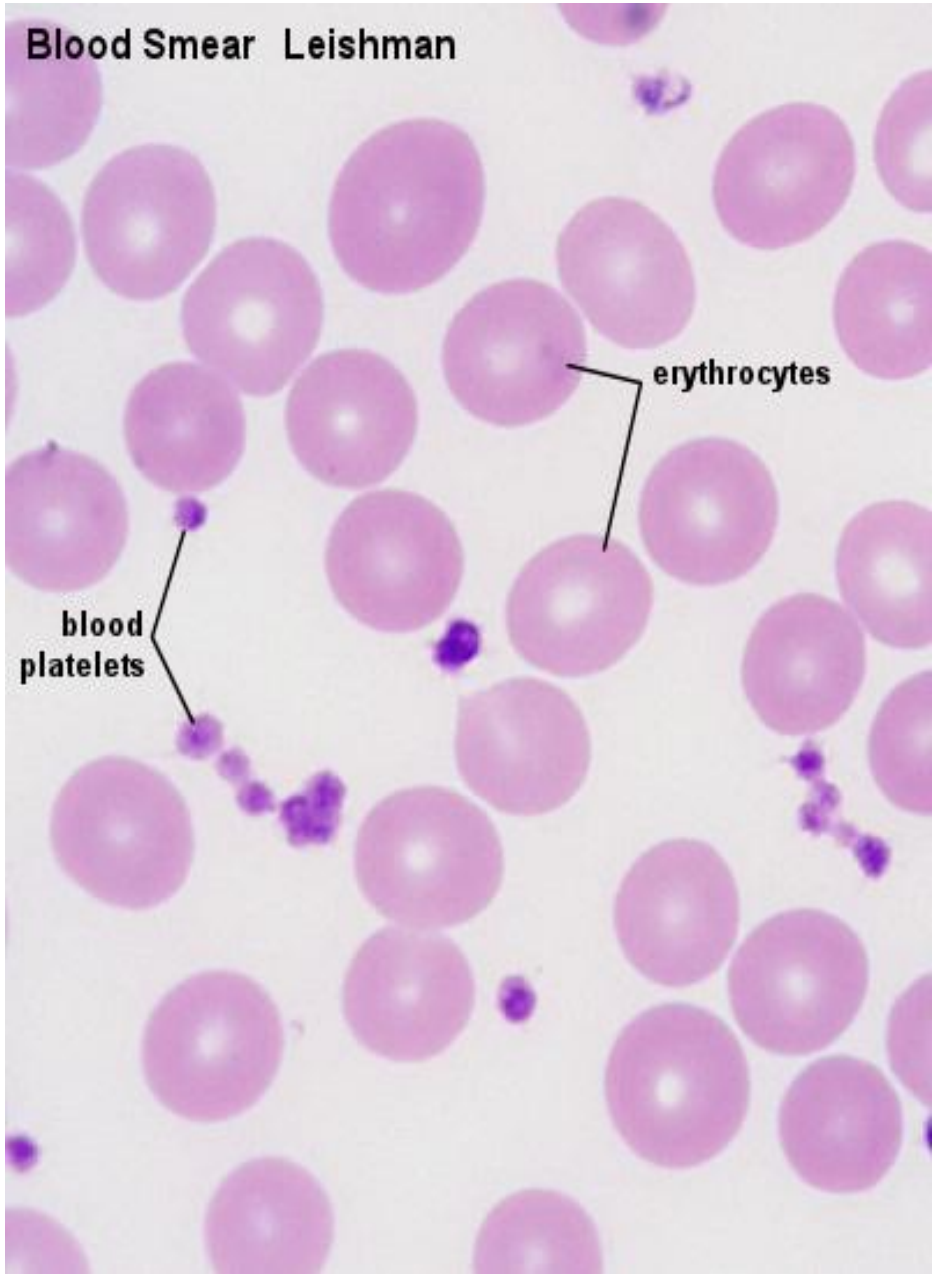
Liver cells:

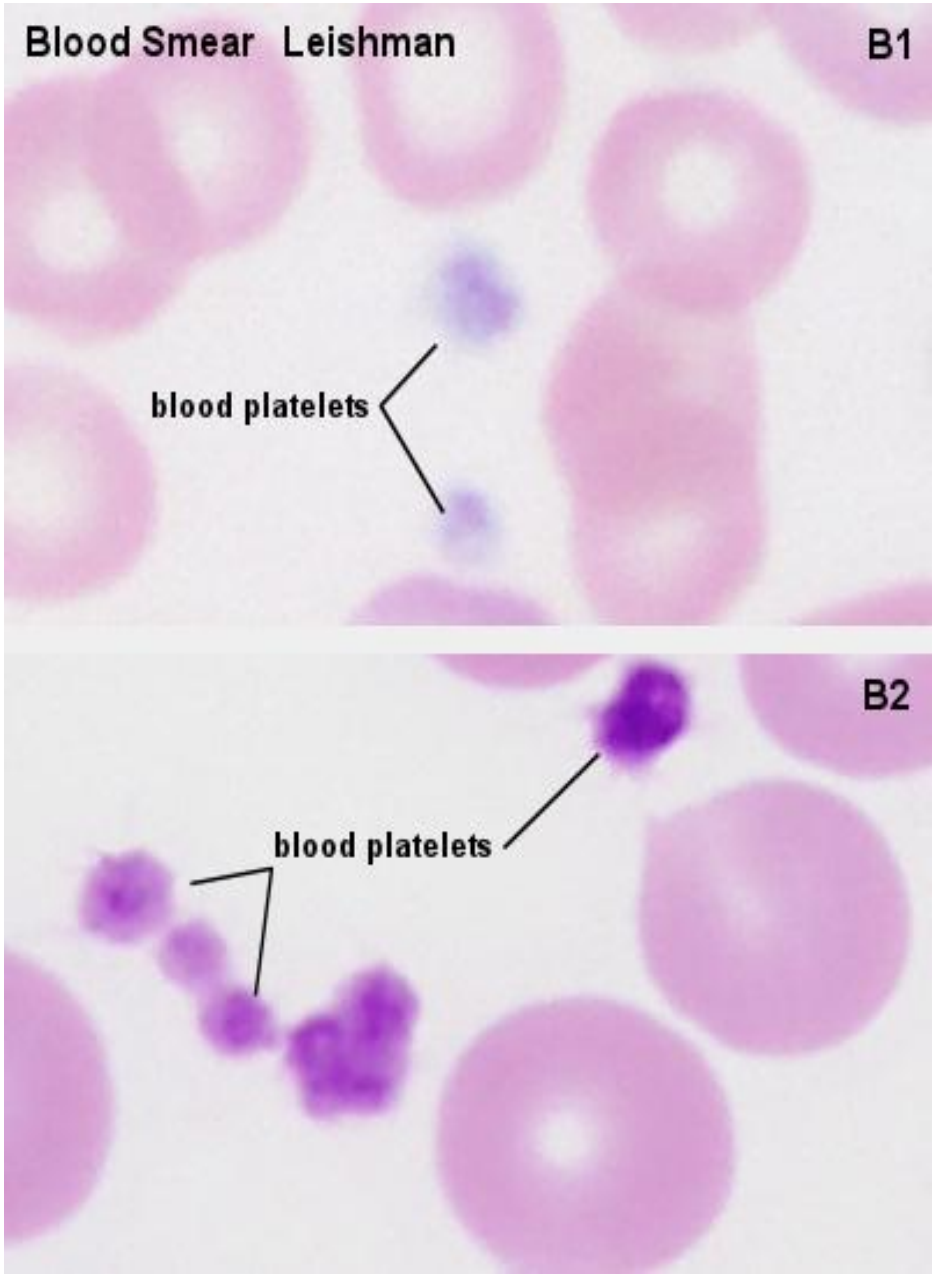
1. Prothrombin





Intrinsic pathway refers to multiple cascades of protein interactions activated by a trauma inside blood vessels	Extrinsic pathway refers to multiple cascades of protein interactions activated by damaged external surfaces
Activated by internal trauma	Activated by external trauma
Factors VIII, IX, XI, and XII are involved	Factor VII is involved
Slow	Fast

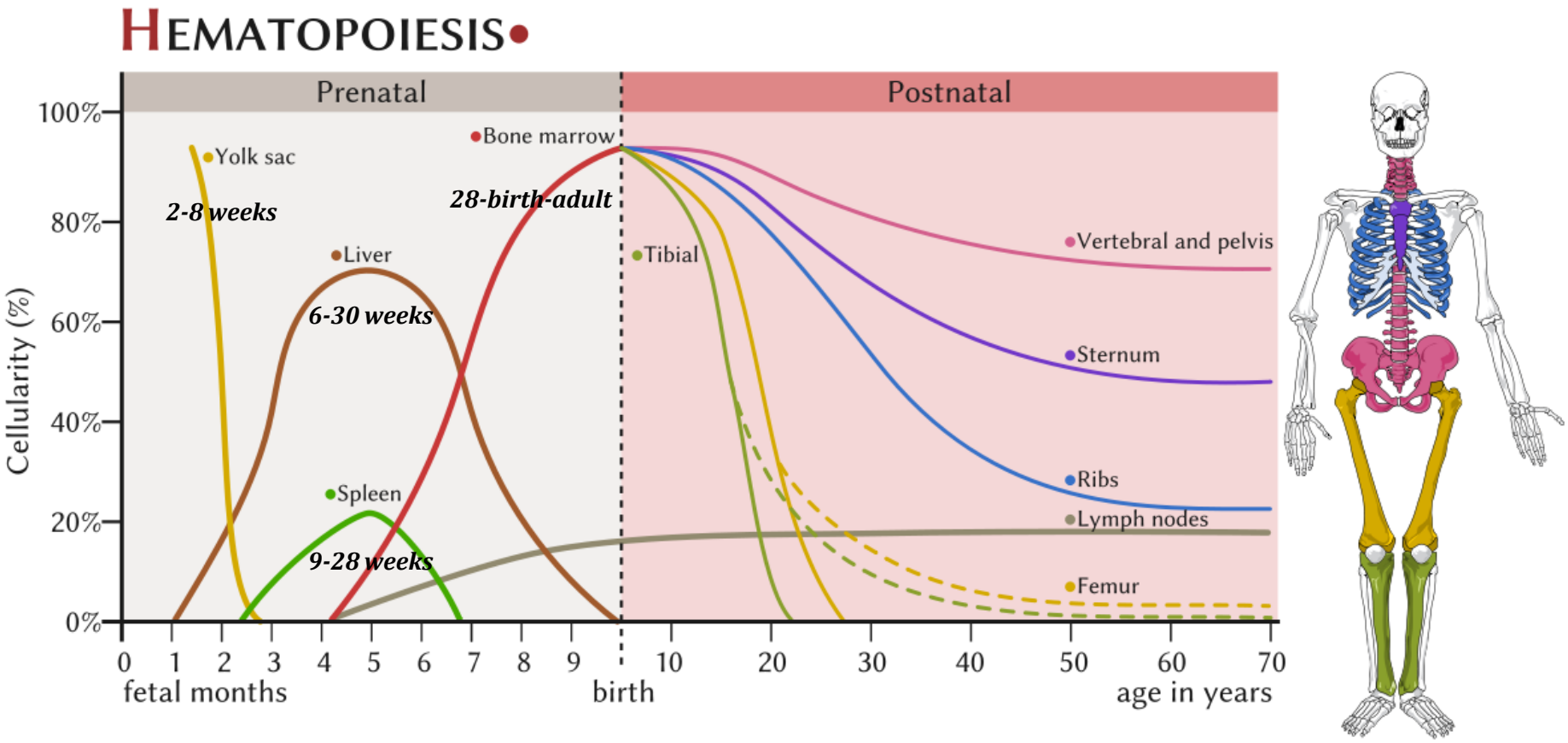

Blood Smear Leishman
erythrocytes
blood platelets


Blood Smear Leishman
B1
blood platelets
B2
blood platelets

Hemopoiesis - is the formation of blood cellular components.

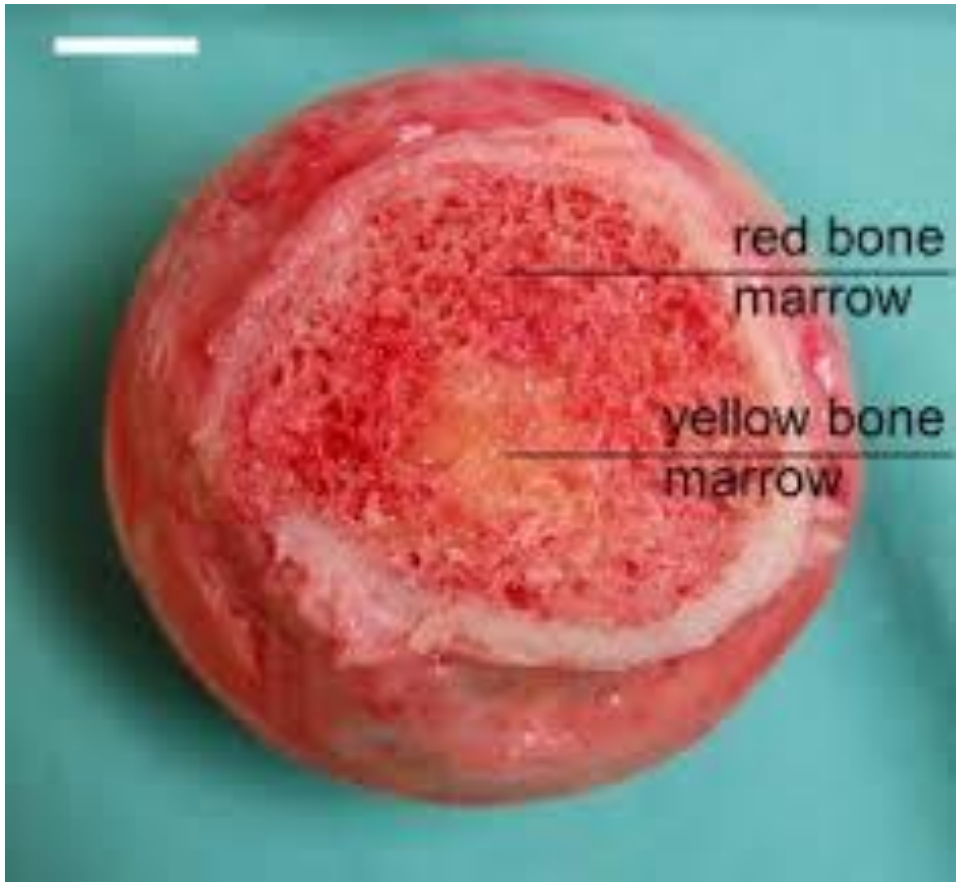
All cellular blood components are derived from **haematopoietic stem cells.**

In children, haematopoiesis occurs in the **bone marrow** of the long bones such as the femur and tibia. In adults, it occurs mainly in the pelvis, cranium, vertebrae, and sternum.



Red bone marrow – consists mainly of hematopoietic tissue
Yellow bone marrow - is mainly made up of fat cells.

At birth, all bone marrow is red.
With age, more of it is converted to the yellow type; only around half of adult bone marrow is red.

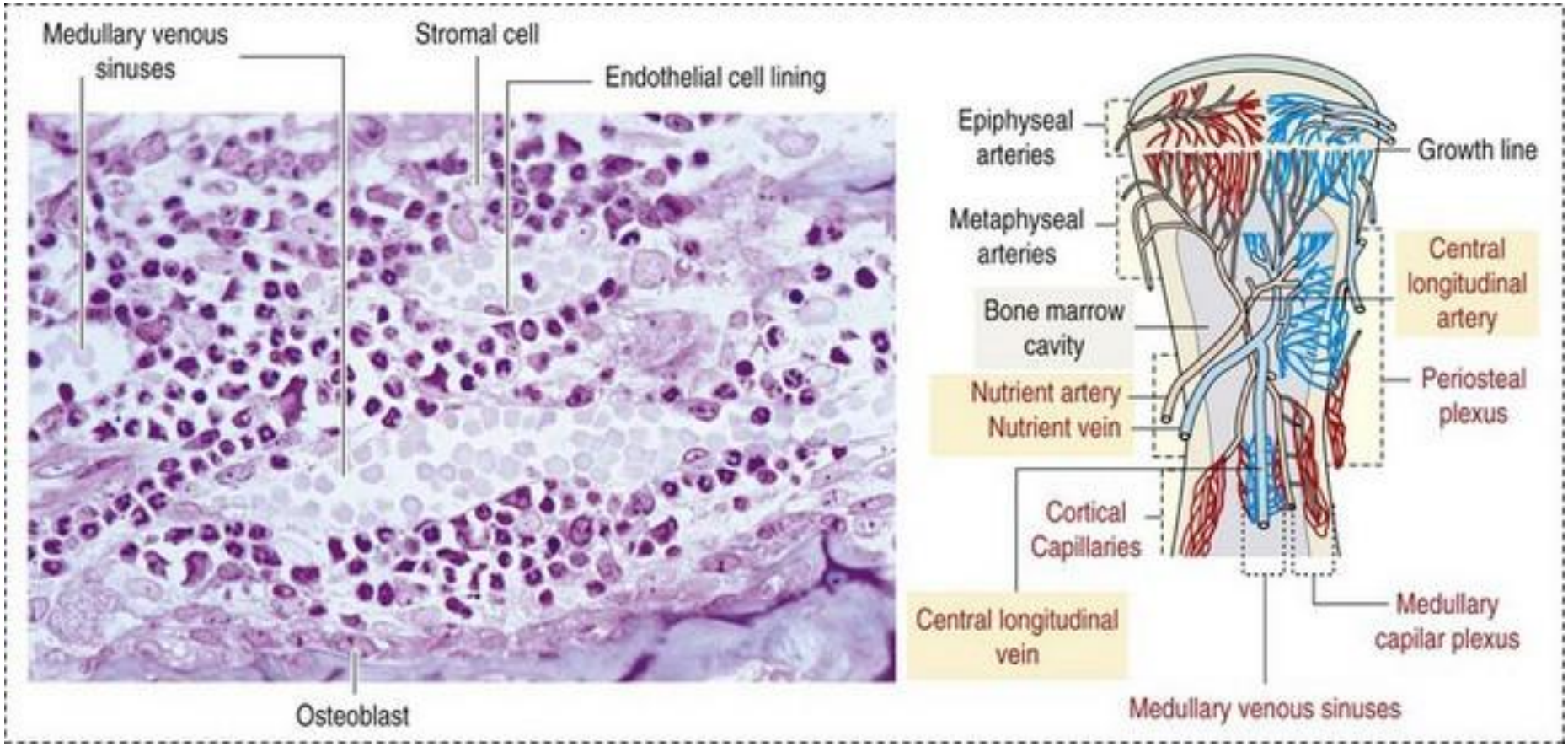


In adults red marrow is found mainly in the flat bones and in epiphyseal ends of long bones. Yellow marrow is found in the medullary cavity of long bones.
In cases of severe blood loss, the body can convert yellow marrow back to red marrow to increase blood cell production.

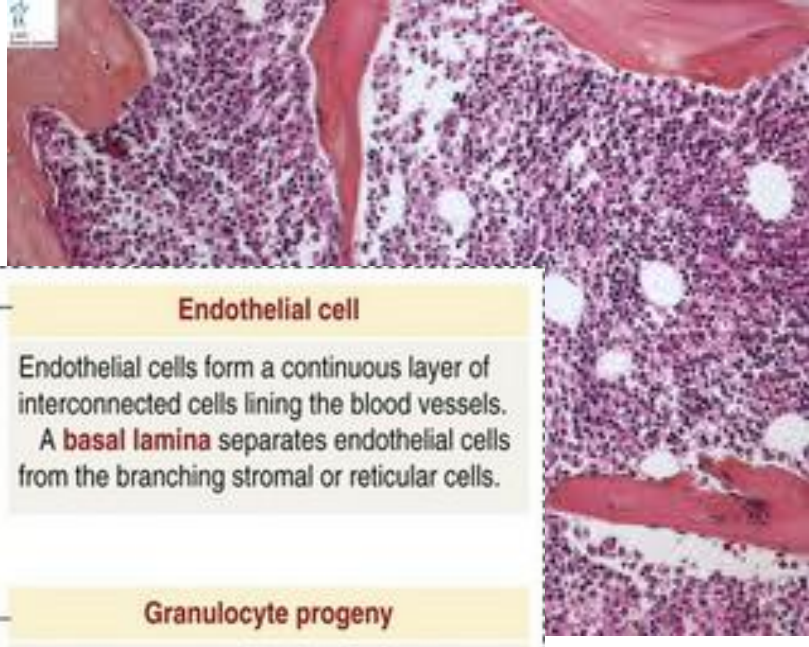
BONE MARROW

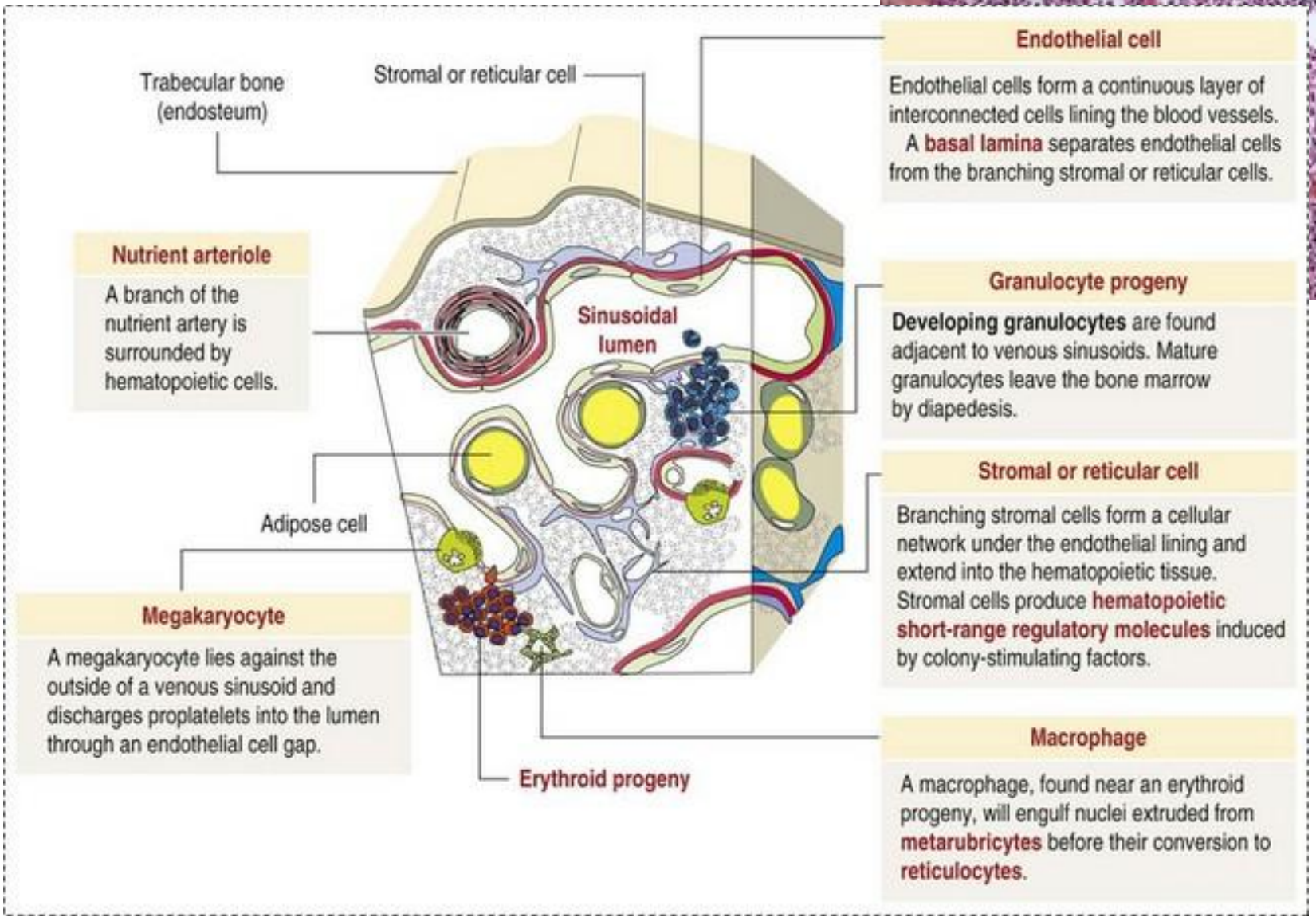
All „formed elements„ of blood are formed in the bone marrow

- Vascular connective tissue
- Located in marrow cavity
- Contains cells responsible for hemopoiesis

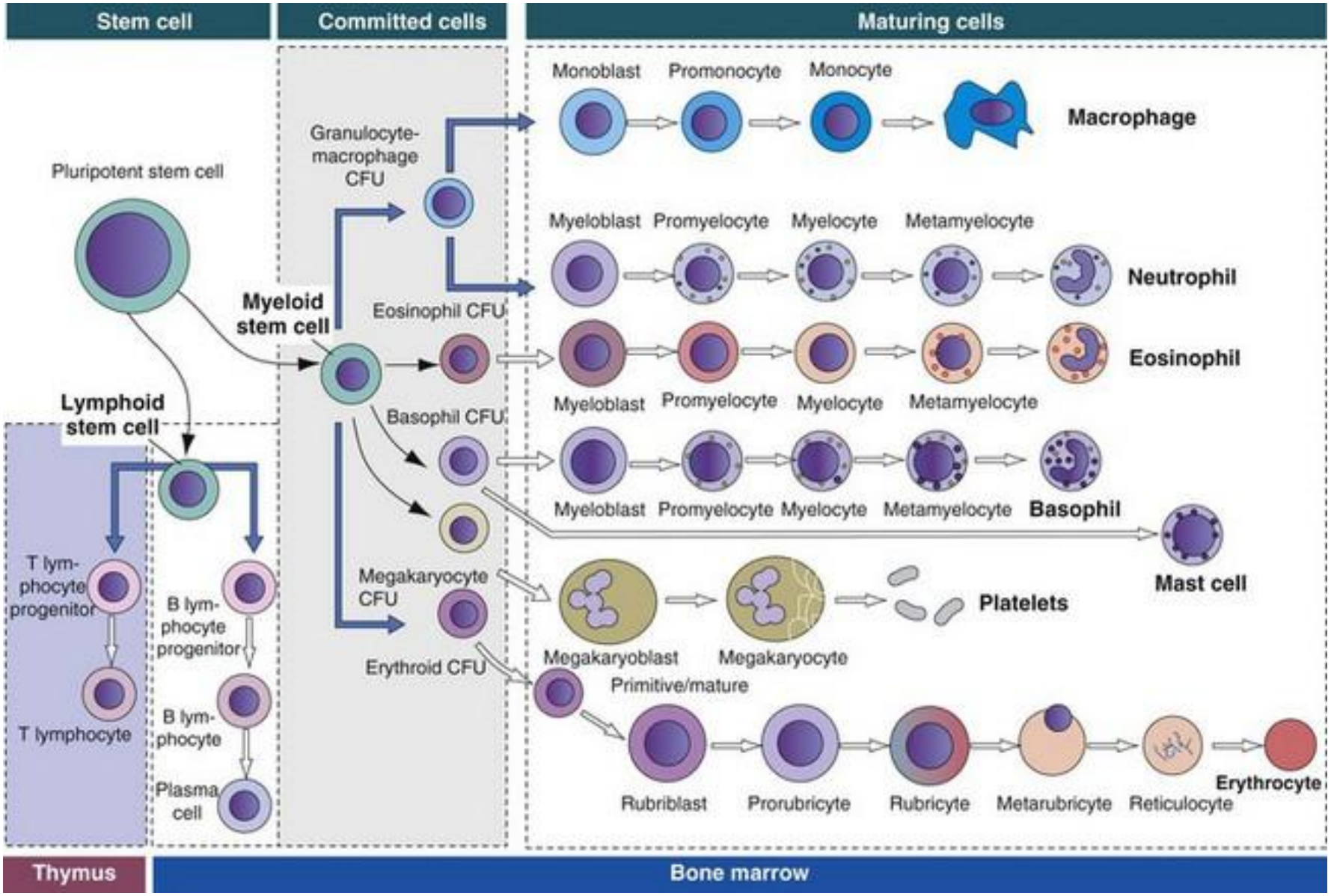


BONE MARROW




Trabecular bone (endosteum)
Stromal or reticular cell
Nutrient arteriole
A branch of the nutrient artery is surrounded by hematopoietic cells.
Sinusoidal lumen
Adipose cell
Megakaryocyte
A megakaryocyte lies against the outside of a venous sinusoid and discharges proplatelets into the lumen through an endothelial cell gap.
Erythroid progeny
Endothelial cell
Endothelial cells form a continuous layer of interconnected cells lining the blood vessels. A basal lamina separates endothelial cells from the branching stromal or reticular cells.
Granulocyte progeny
Developing granulocytes are found adjacent to venous sinusoids. Mature granulocytes leave the bone marrow by diapedesis.
Stromal or reticular cell
Branching stromal cells form a cellular network under the endothelial lining and extend into the hematopoietic tissue. Stromal cells produce hematopoietic short-range regulatory molecules induced by colony-stimulating factors.
Macrophage
A macrophage, found near an erythroid progeny, will engulf nuclei extruded from metarubricytes before their conversion to reticulocytes.

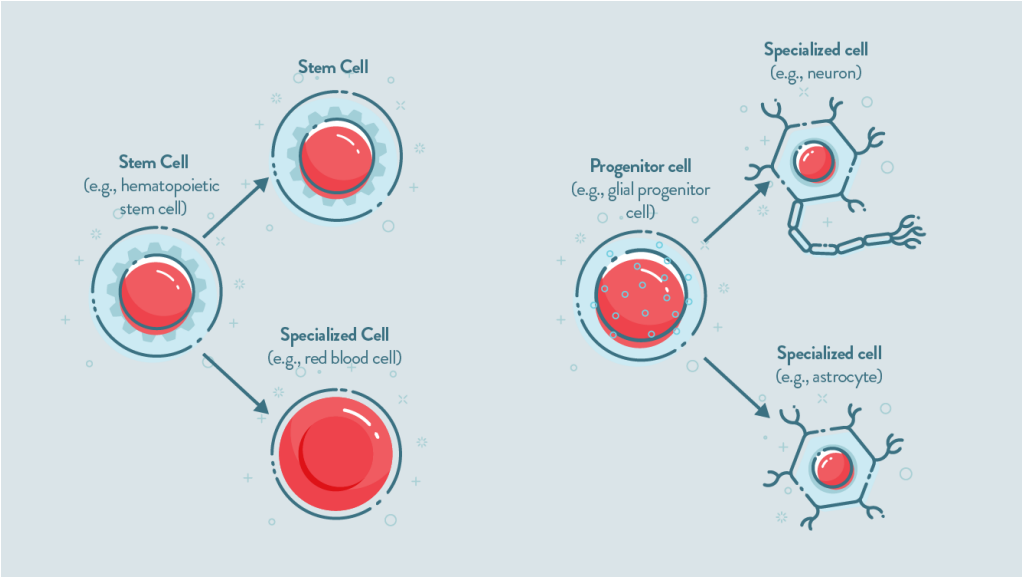
HEMATOPOIETIC CELLS IN THE BONE MARROW



Pluripotential hemopoietic stem cells (PHSCs)

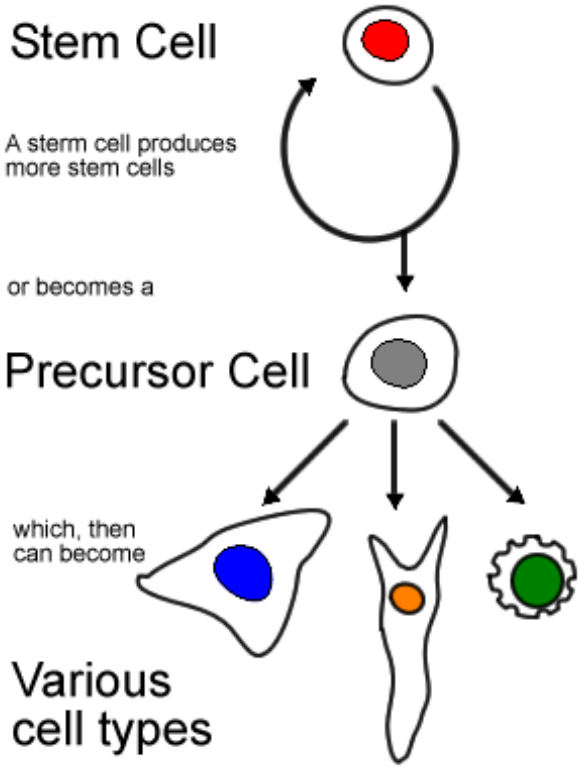
- Account for about 0.1% of cell population of bone marrow
- Undergo bursts of cell division giving rise to more PHSCs as well as two types **multipotential hemopoietic stem cells (MHSCs)** – multipotent progenitors:
  - CFU-GEMM- (colony forming unit-granulocyte, erythrocyte, monocyte, megakaryocyte) formation of myeloid cell lines (erythrocytes, granulocytes, monocytes, and platelets)
  - CFU-Ly - (colony forming unit–lymphocyte) – formation of lymphoid cell lines T cells and B lymphocytes

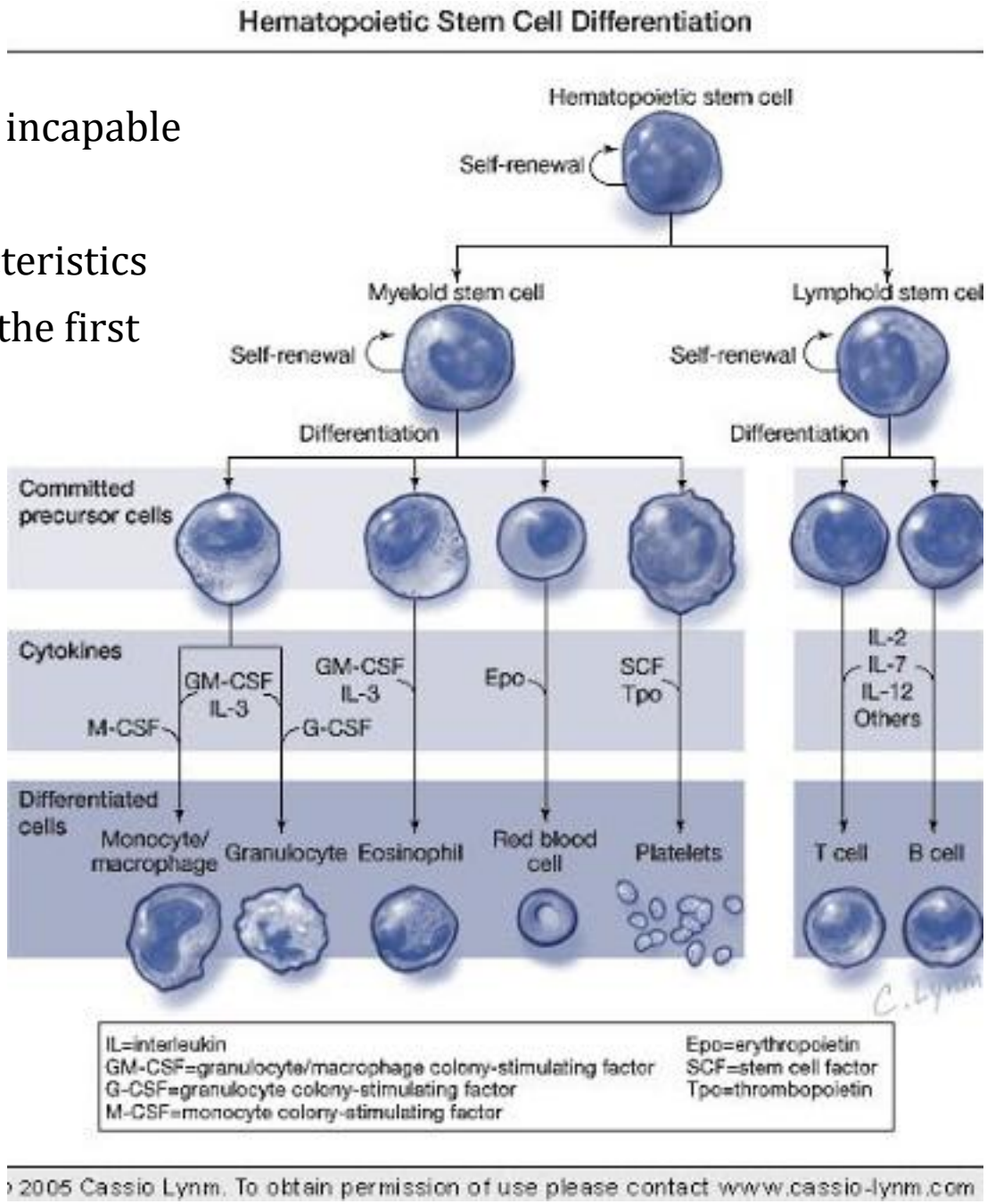
Stem cells can replicate indefinitely, whereas progenitor cells can divide only a limited number of times

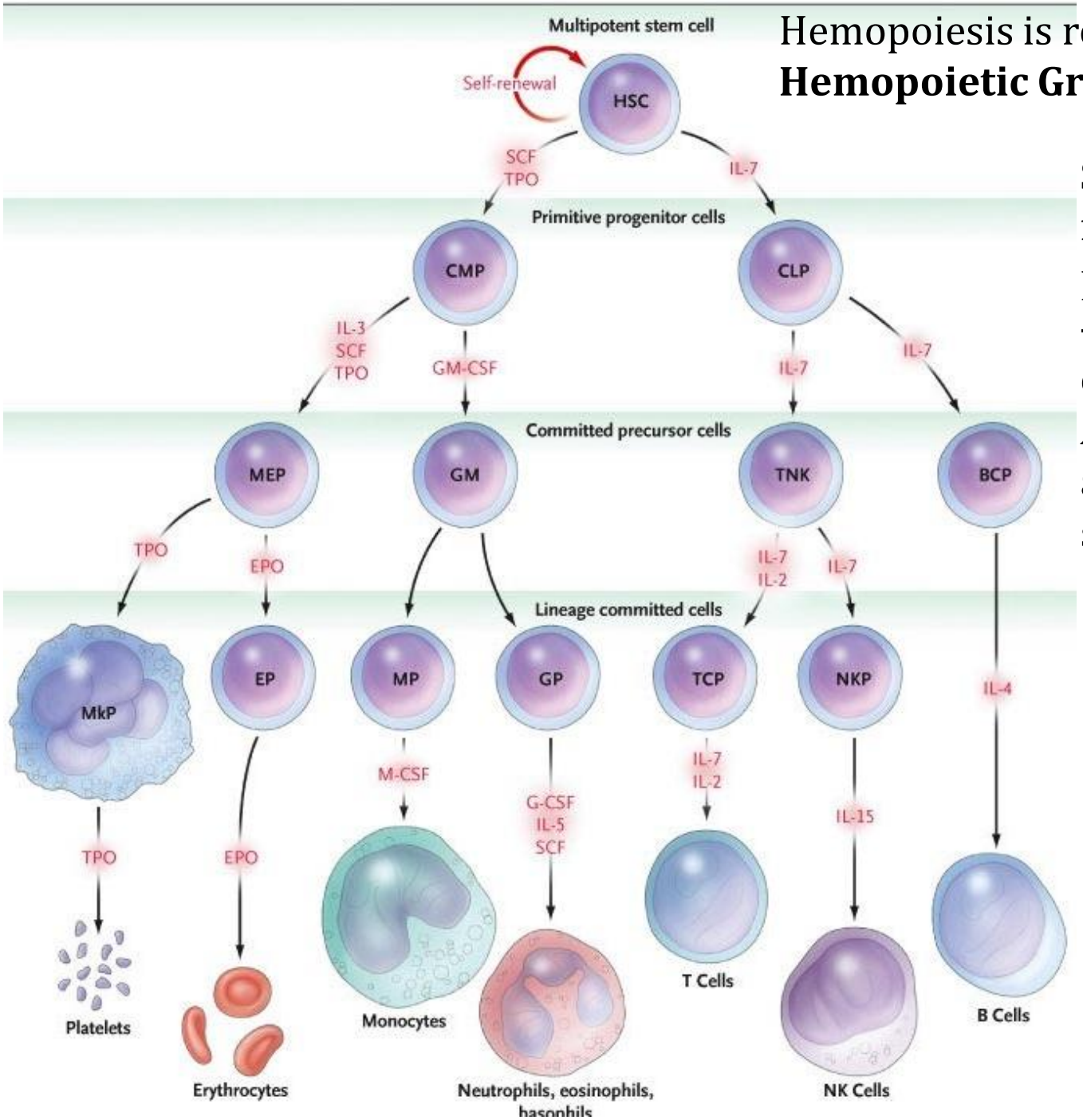


Precursor cells

- Arise from progenitor cells and are incapable of self-renewal:
- Have specific morphological characteristics that permit them to be recognized as the first cell of a particular cell line



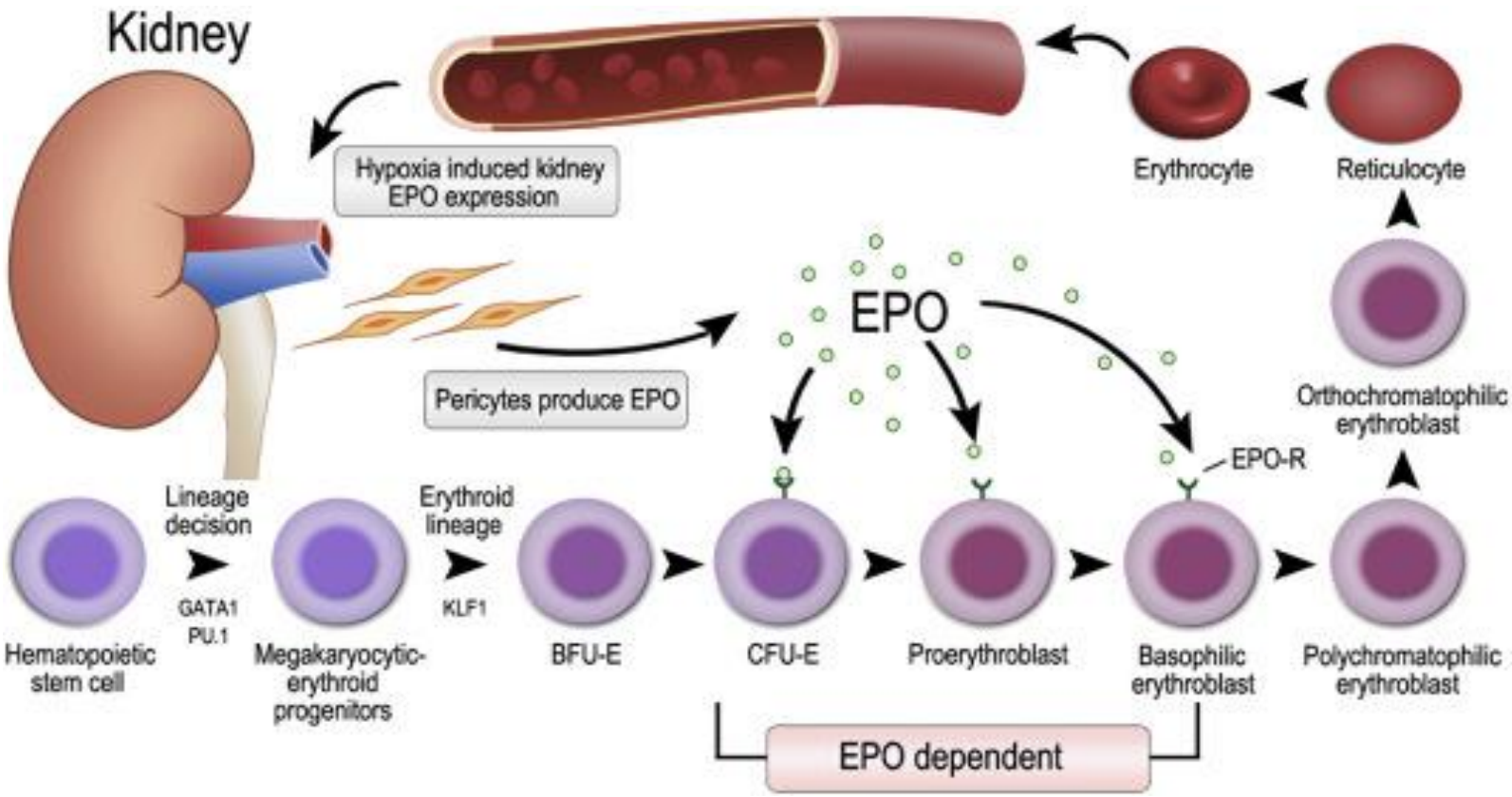




Hemopoiesis is regulated by numerous **Hemopoietic Growth Factors**

Stem cell factor (steel factor, c-kit ligand) – promotes hemopoiesis – produced by **stromal cells of bone marrow.** Acts on pluripotential and multipotential stem cells

ERYTHROPOIESIS

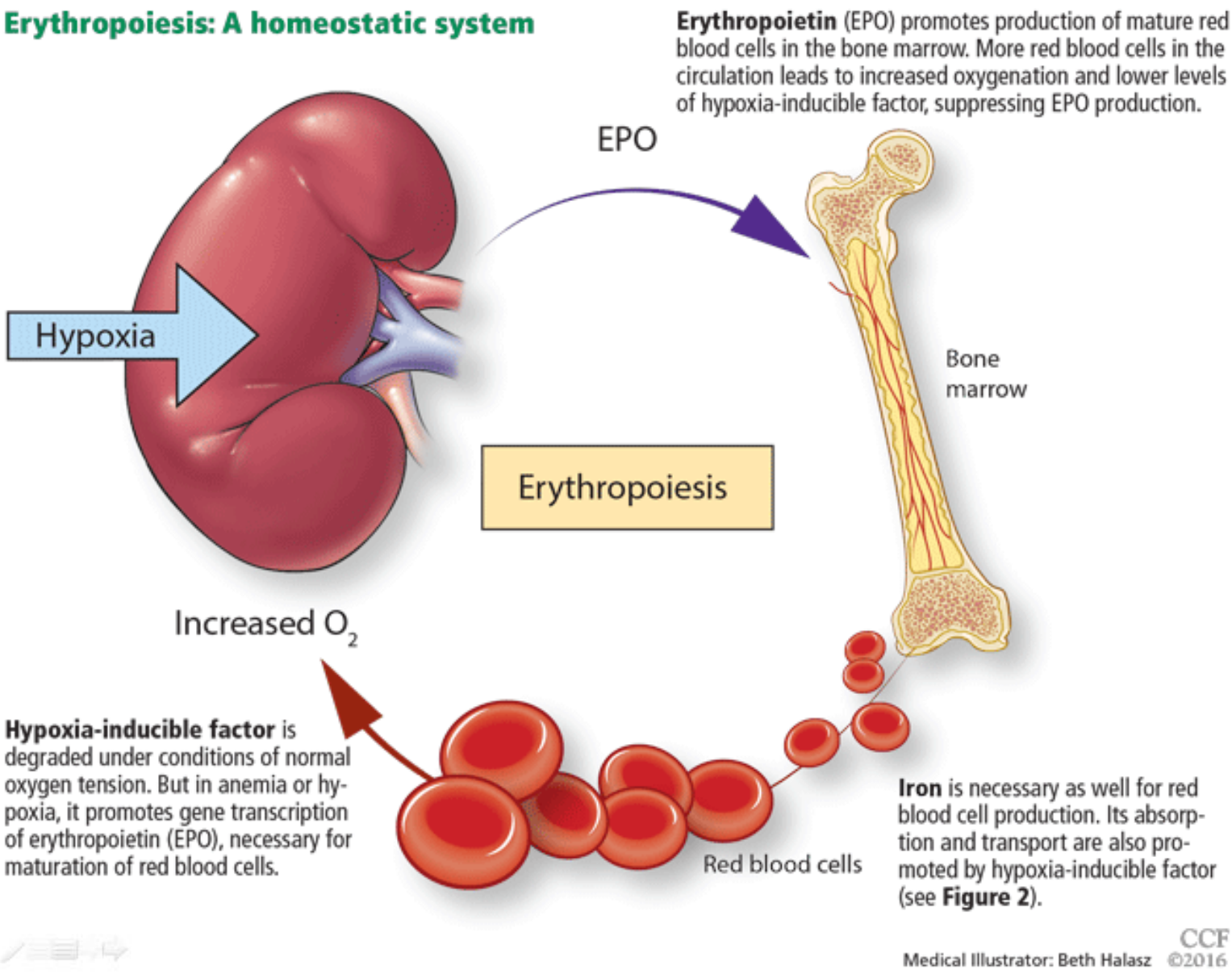


Once hemoglobin concentration decreases, pericytes sense hypoxia and produce erythropoietin (EPO), which binds to EPO receptors (expressed on CFU-E, proerythroblasts and early basophilic erythroblasts) – prevents from apoptosis.

https://doi.org/10.1016/j.jfma.2018.03.017

Erythropoietin (EPO) (hormone that controls red blood cell production)

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



Erythroblast – extrusion of nucleus

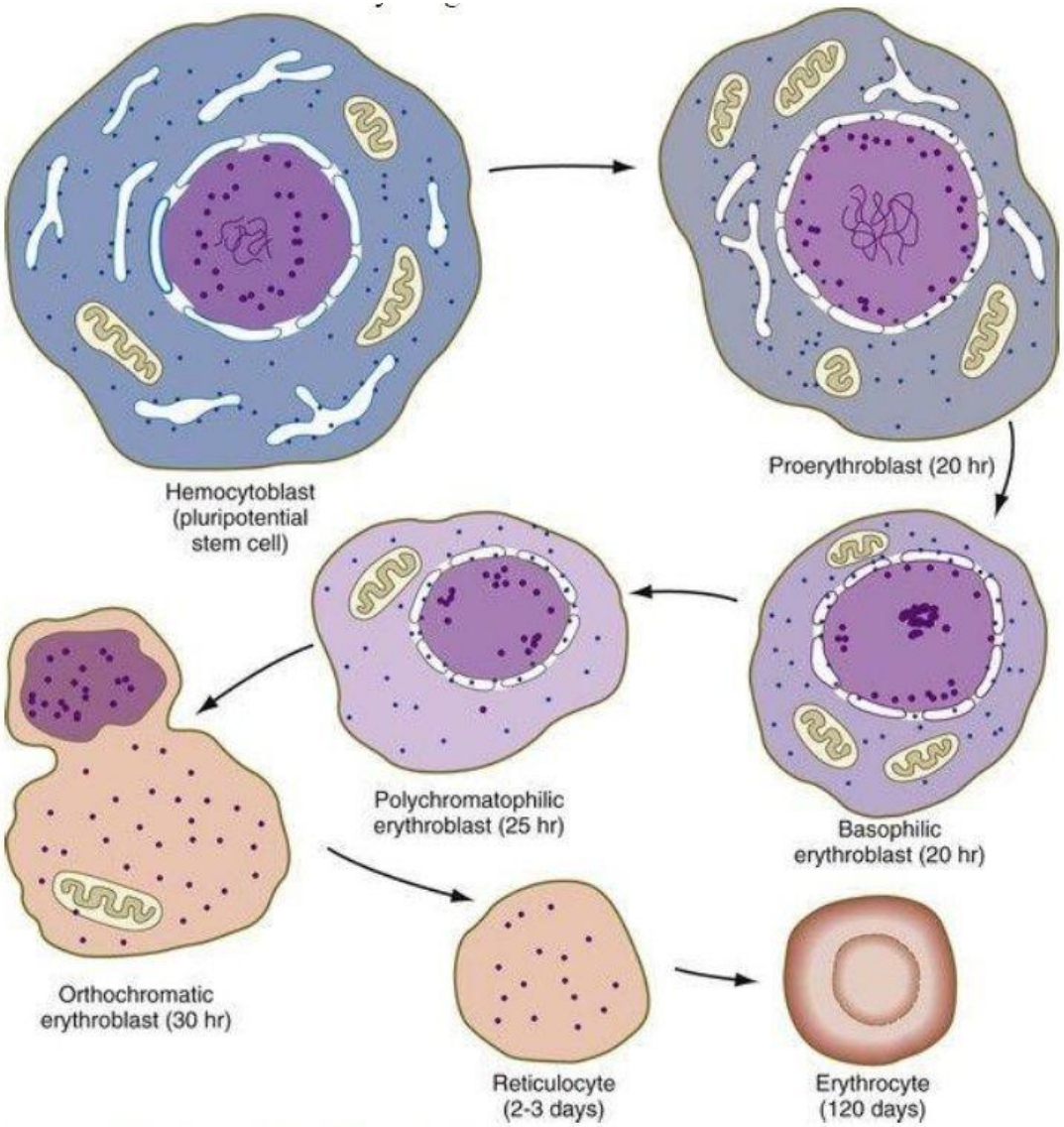
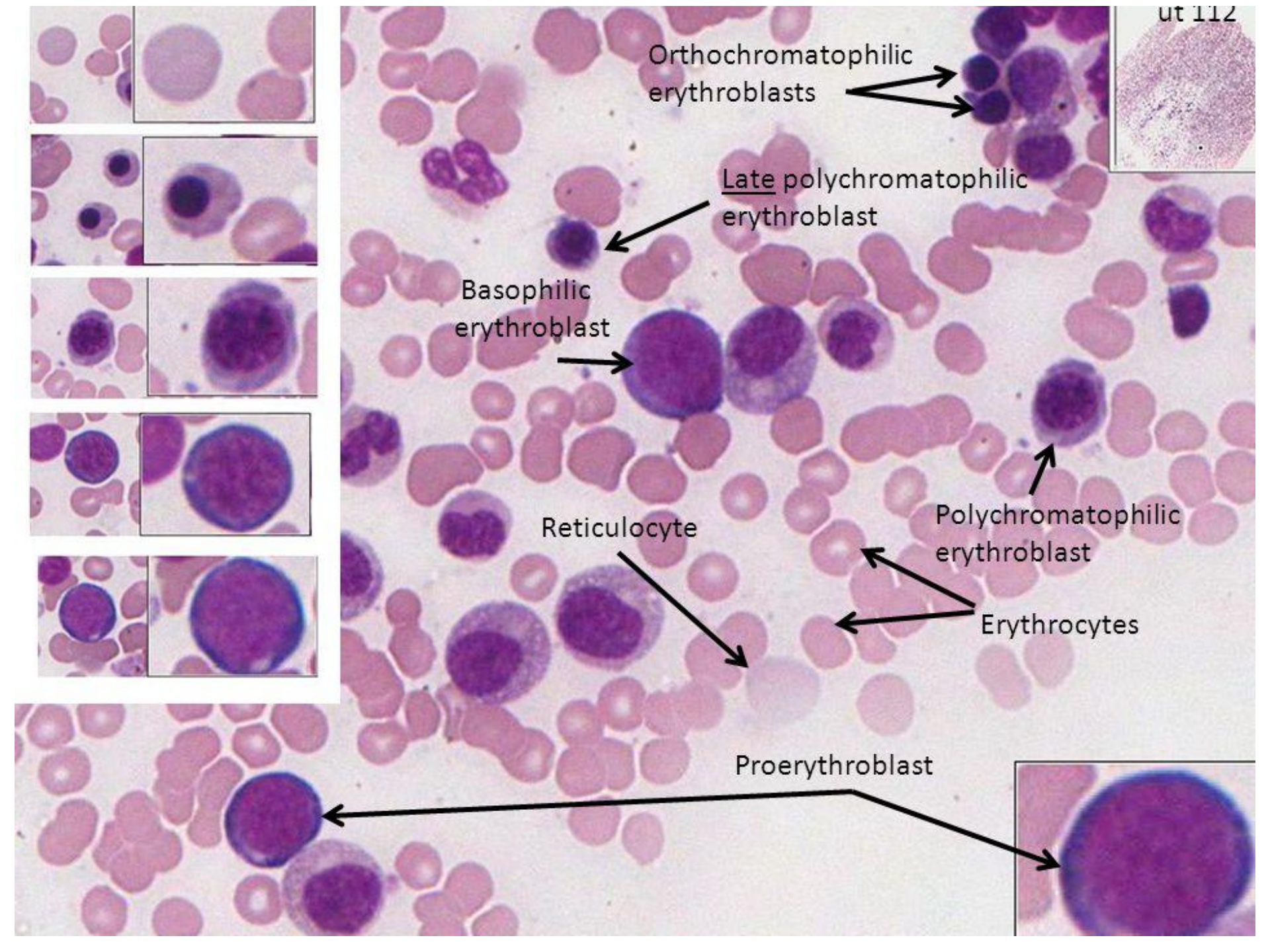


Fig. 17.5

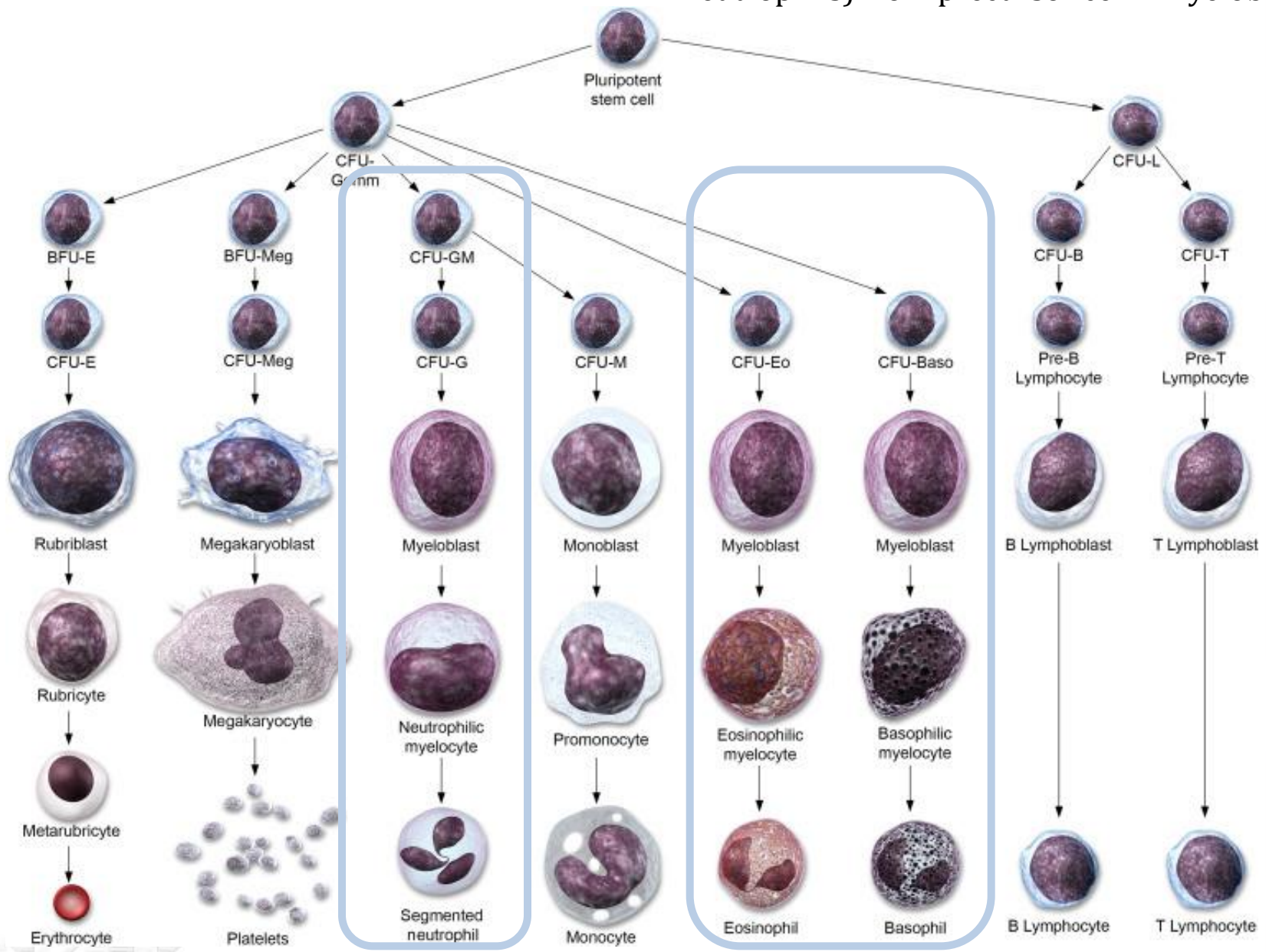
Structural features of erythropoiesis.
In successive stages, cytoplasmic basophilia decreases and the concentration of hemoglobin increases in the cells.

- The nucleus is extruded from the orthochromatic erytroblast
- The cell enters circulation as a reticulocyte, which still contains some organelles

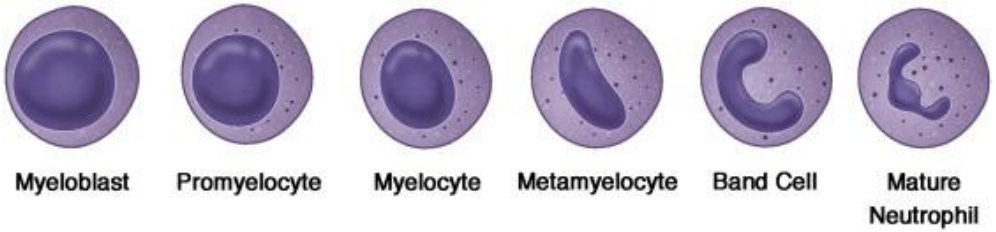

Orthochromatophilic erythroblasts
Late polychromatophilic erythroblast
Basophilic erythroblast
Polychromatophilic erythroblast
Reticulocyte
Erythrocytes
Proerythroblast

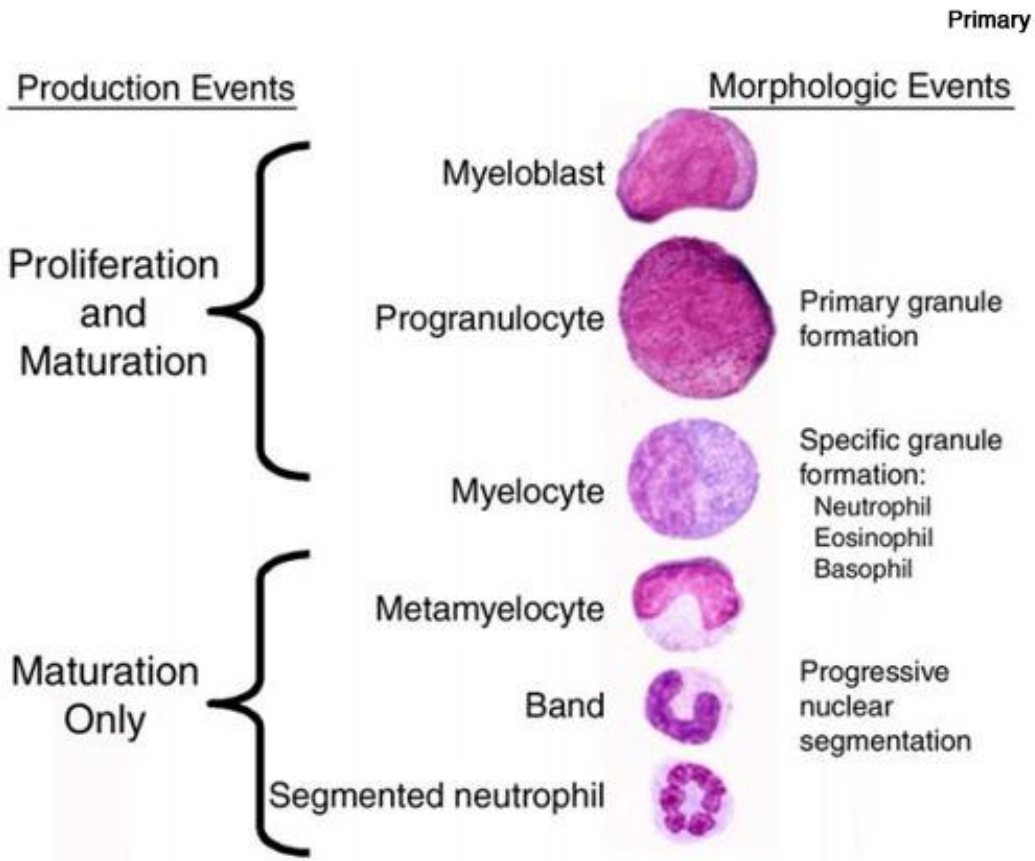
GRANULOCYTOPOIESIS

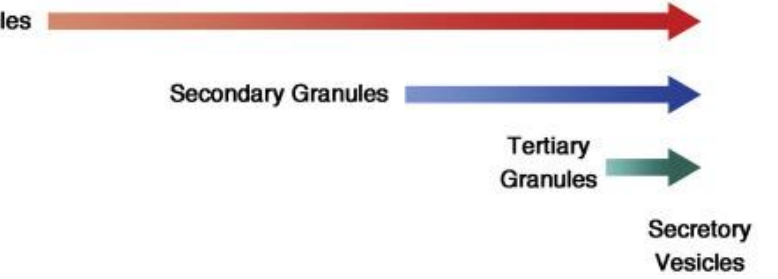
Formation of granulocytes (basophils, eosinophils and neutrophils) from precursor cell – myeloblasts.

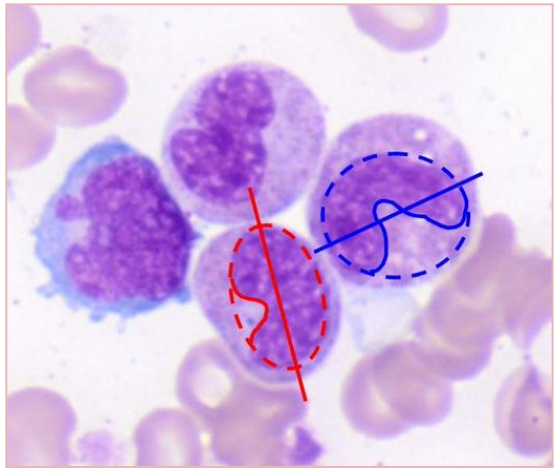


Myeloblasts - proliferate to generate promyelocytes







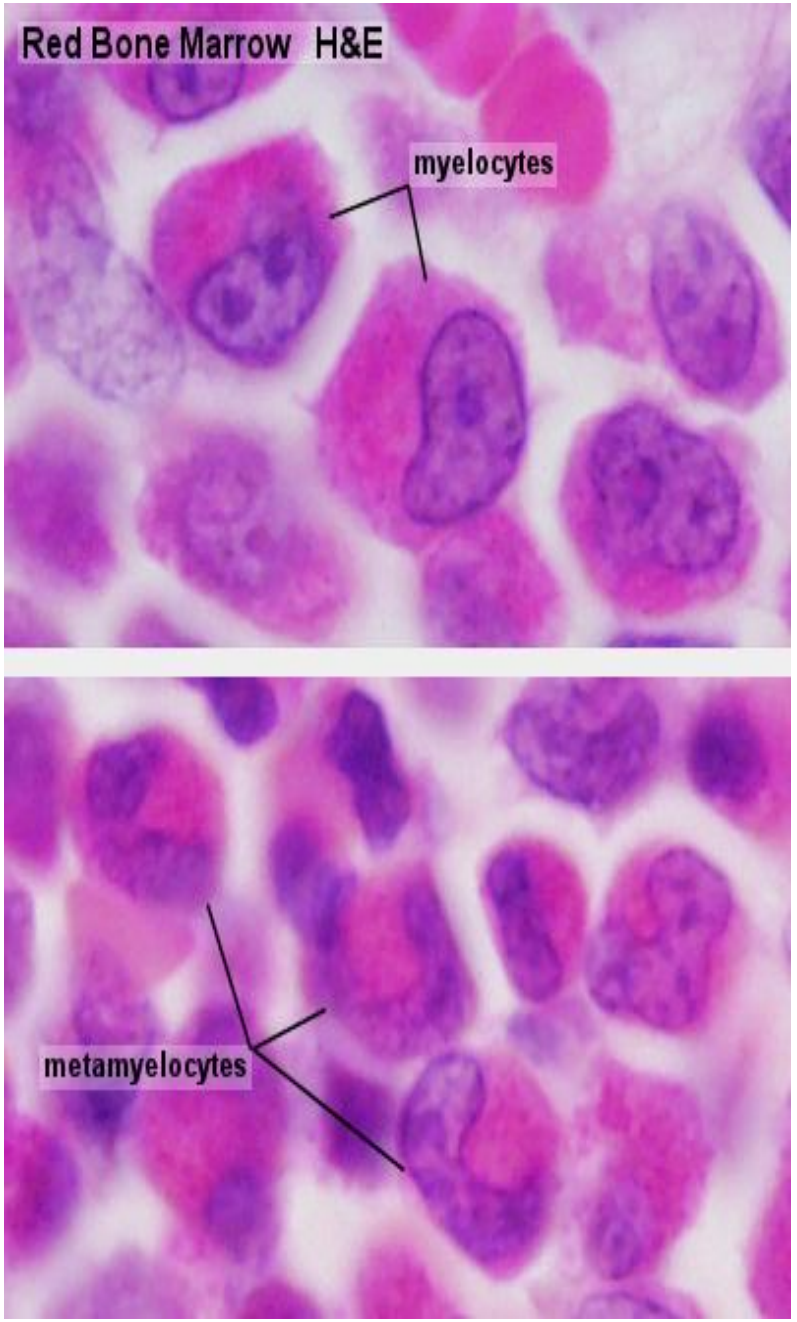


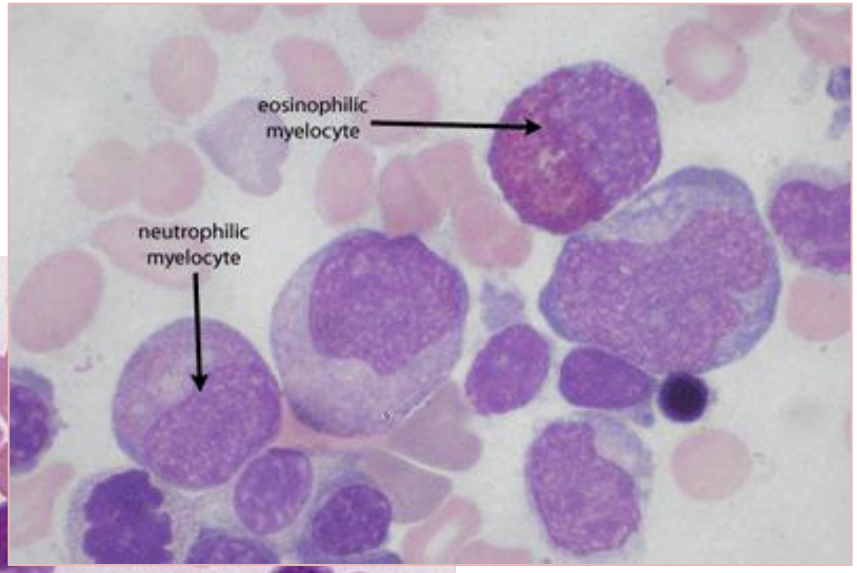
- Promyelocytes contain nonspecific azurophilic granules (modified lysosomes).
- Myelocytes, metamyelocytes and mature cells contain azurophilic granules and specific granules

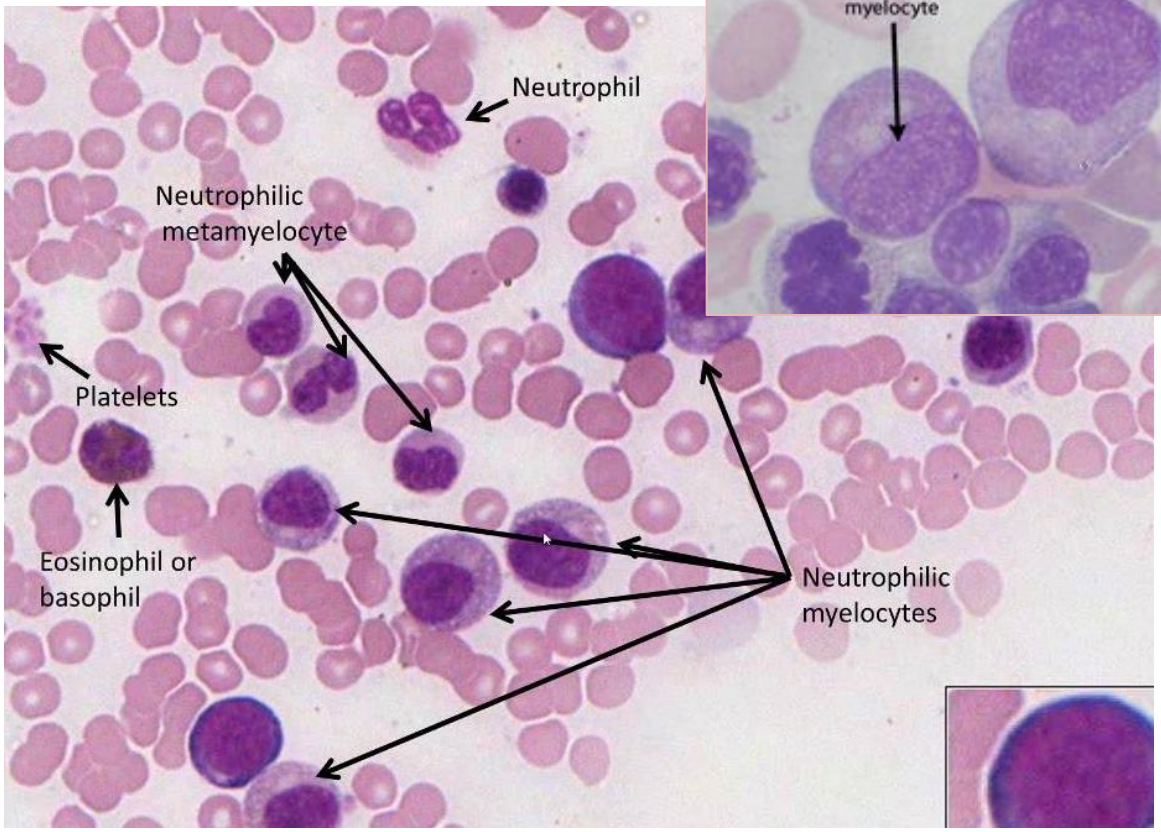
Granulocytopoiesis is under the influence of:

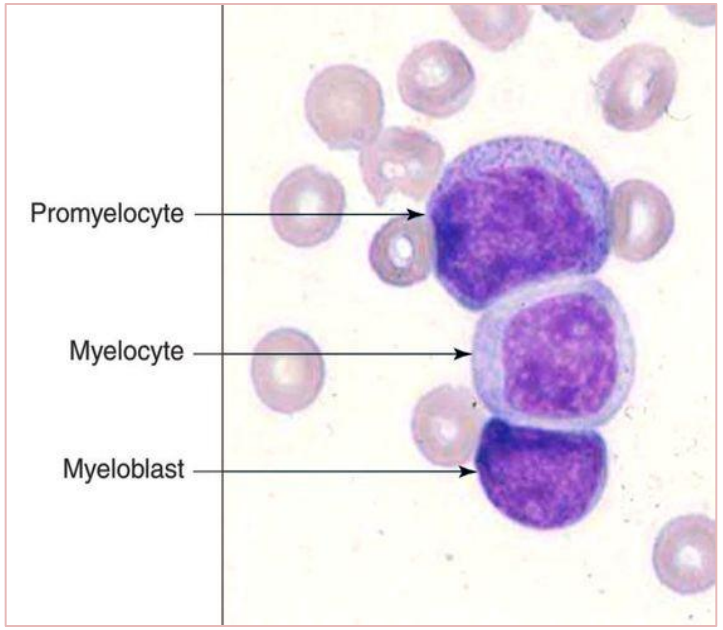
- G-CSF, GM-CSF and cytokines: IL-3, IL-5

- G-CSF: Granulocyte colony-stimulating factor
- GM-CSF: Granulocyte-macrophage colony-stimulating factor
- IL-5: also known as eosinophil differentiation factor




eosinophilic
myelocyte
neutrophilic
myelocyte

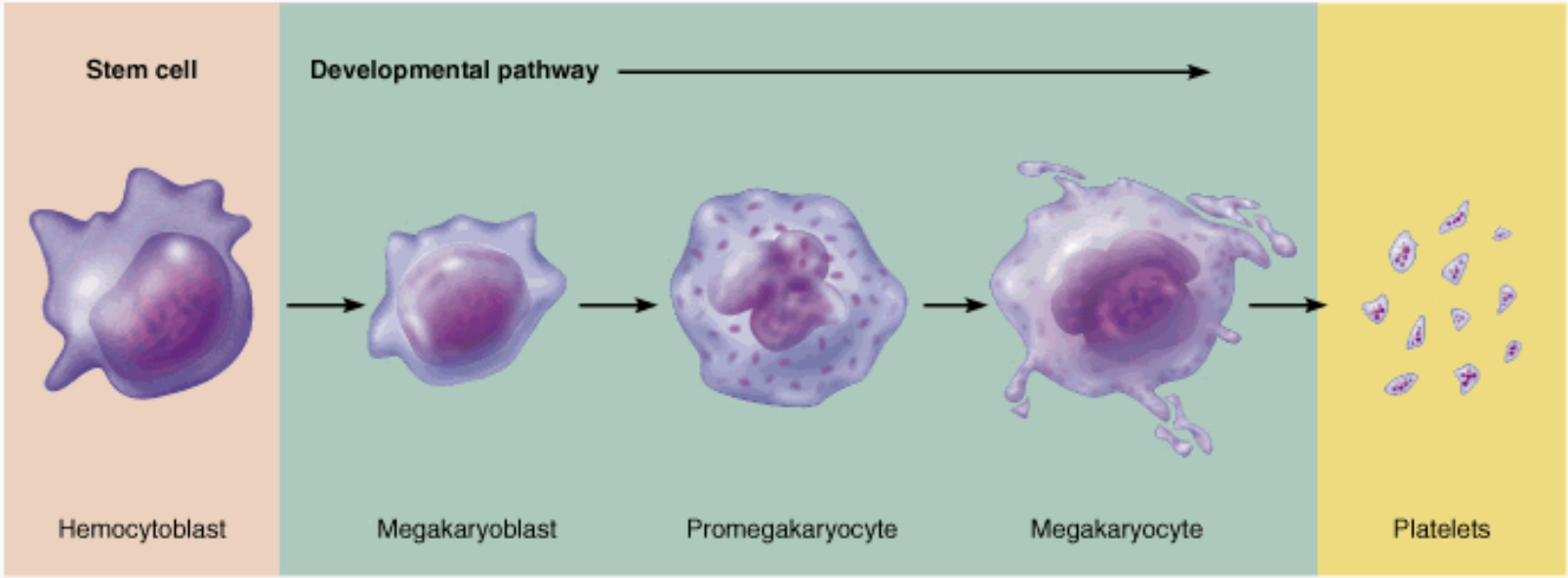

Neutrophil
Neutrophilic
metamyelocyte
Platelets
Eosinophil or
basophil
Neutrophilic
myelocytes


Promyelocyte
Myelocyte
Myeloblast

PLATELETS FORMATION

Megakaryocytes break into pieces, each one making 2000-3000 platelets.

Thrombopoietin (THPO) - **megakaryocyte growth and development factor** (MGDF)



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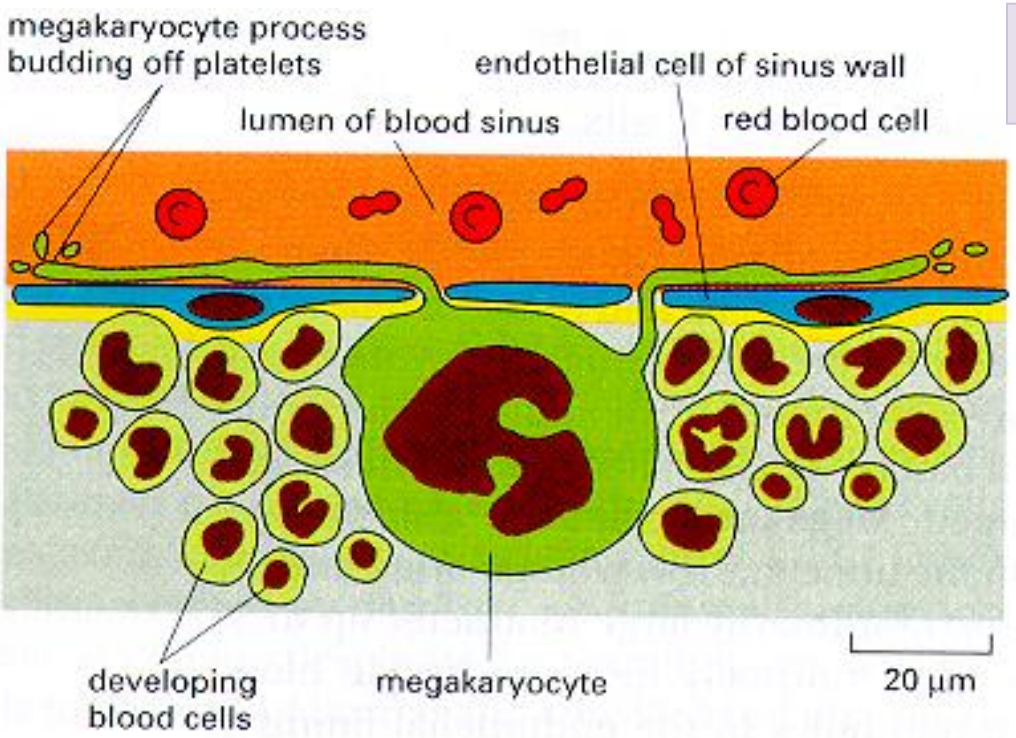
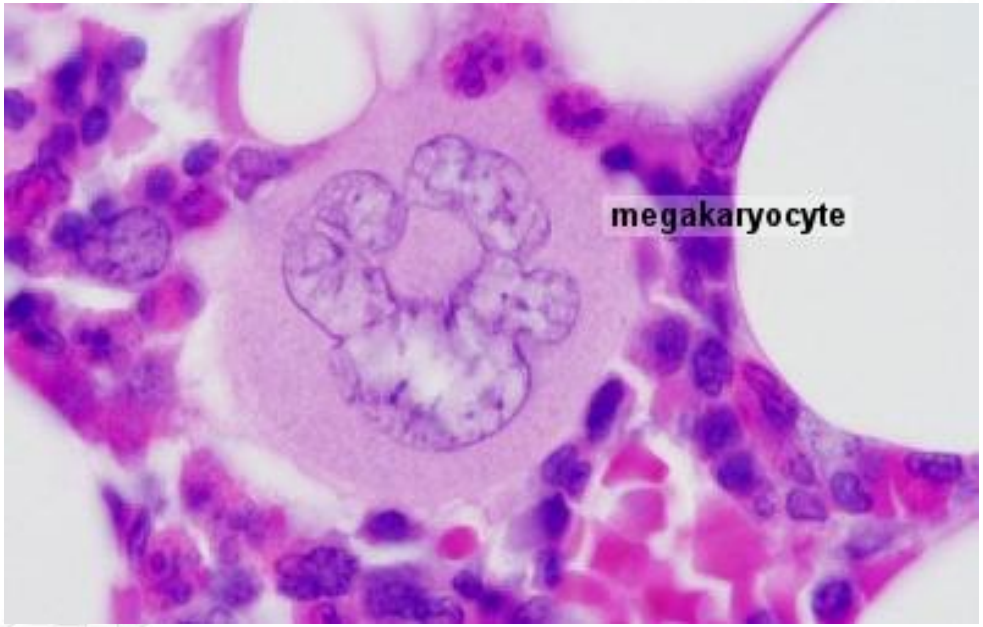
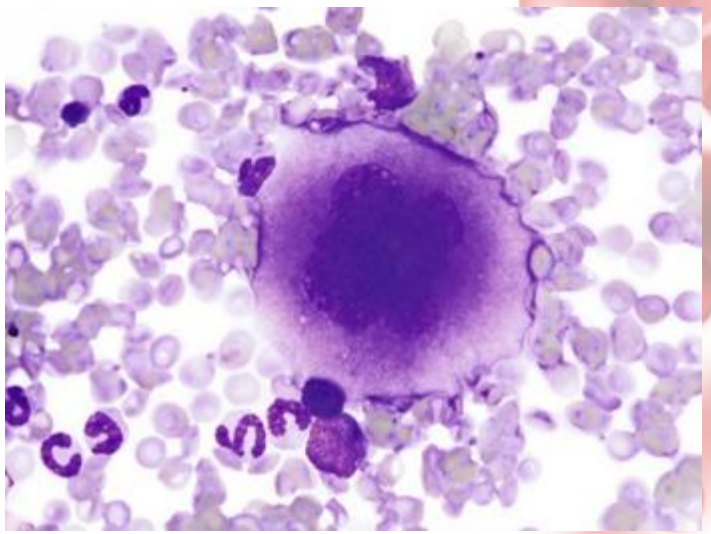
PLATELETS FORMATION

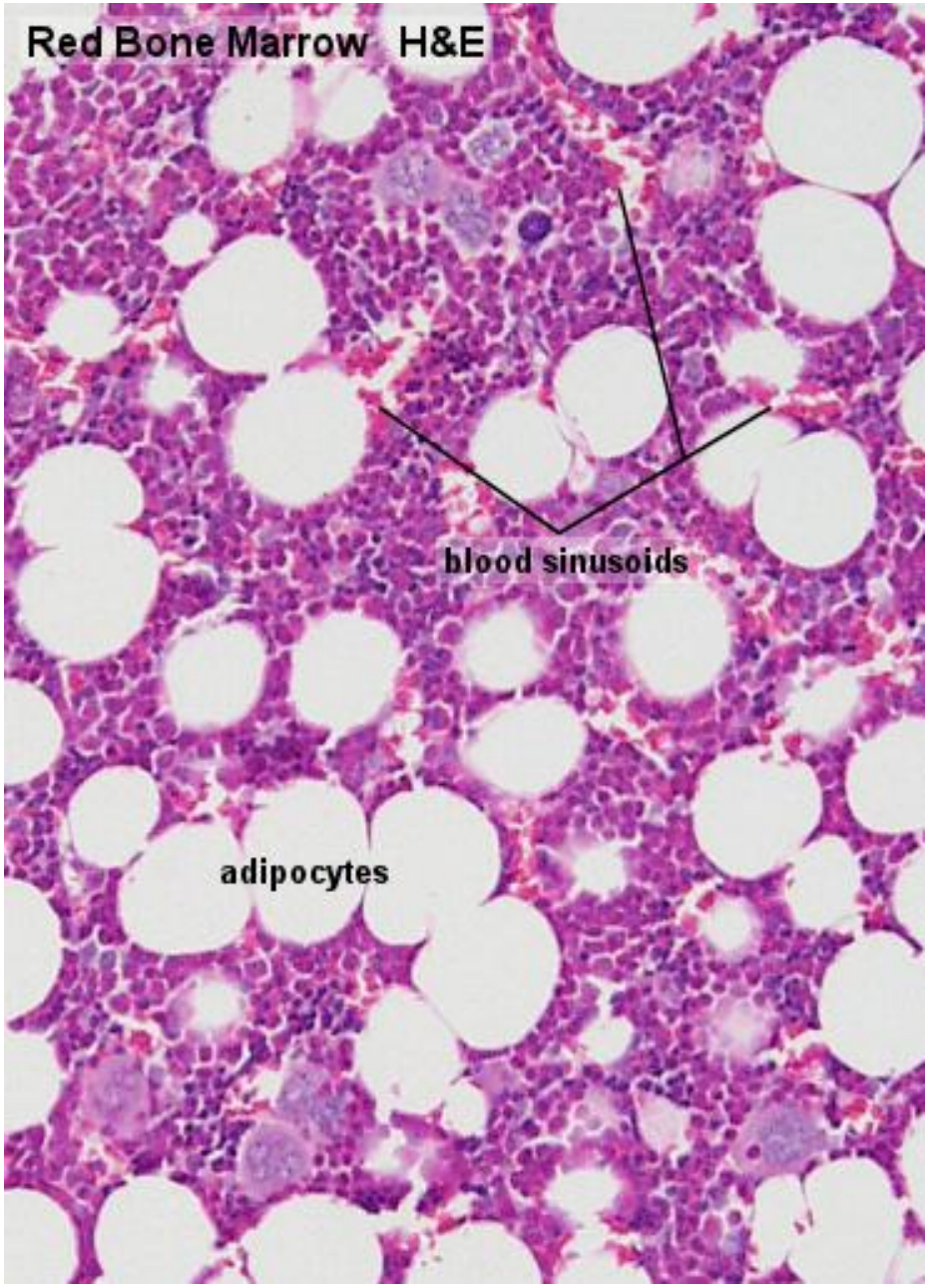


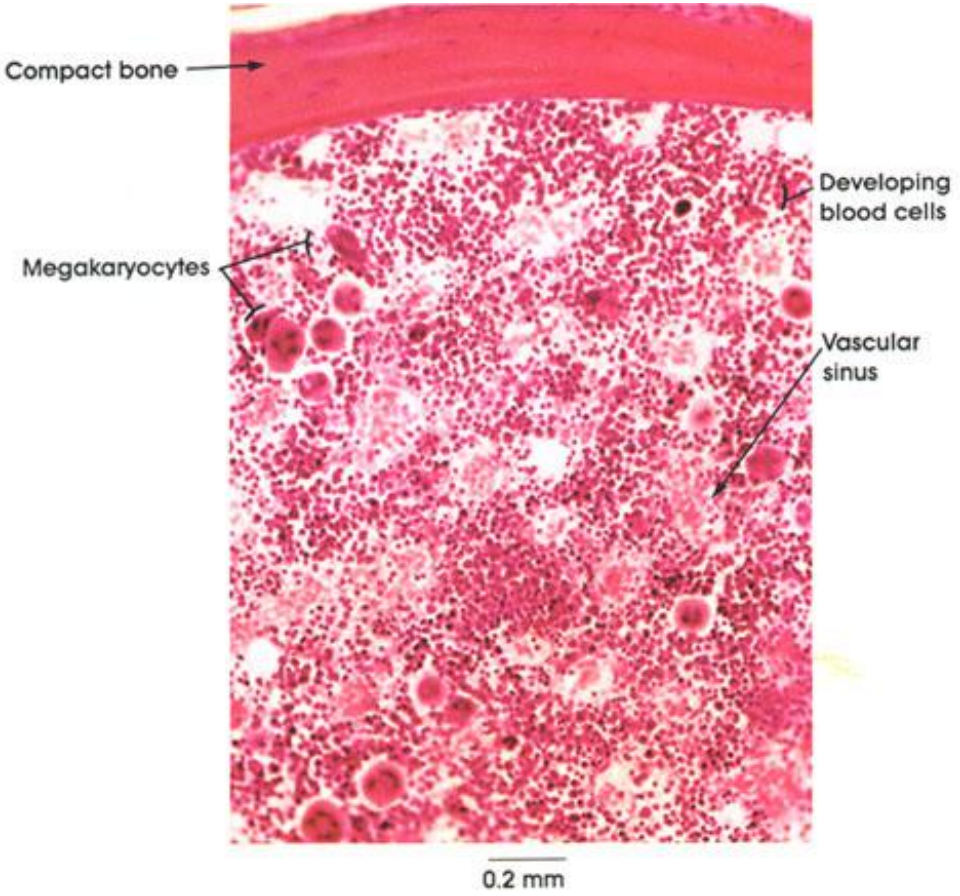
Megakaryocytes are located next to sinusoids, into which they protrude their cytoplasmic processes

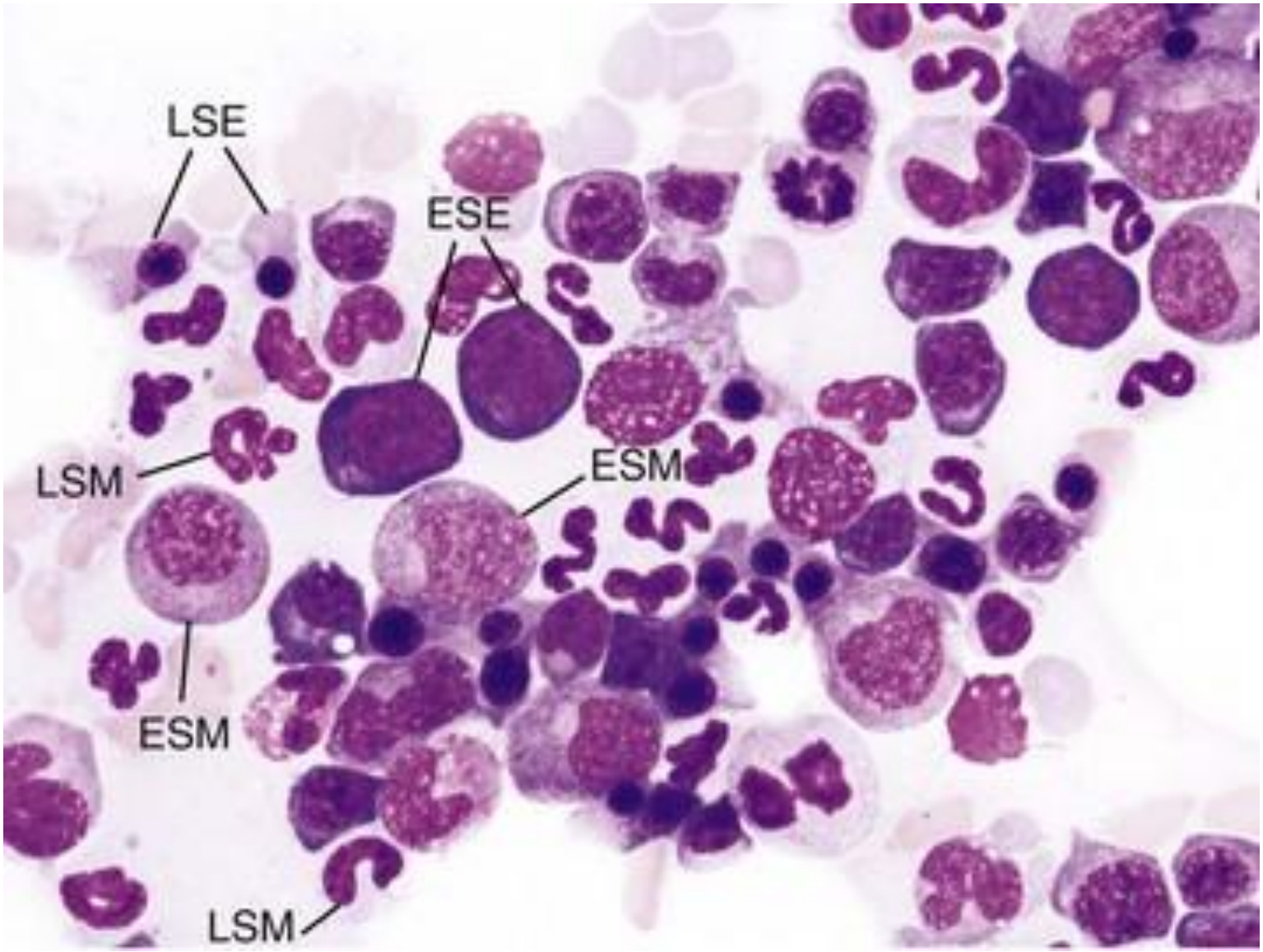




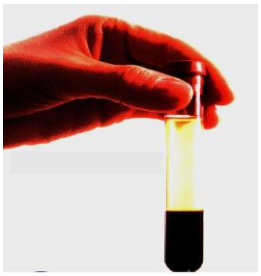
PLATELETS FORMATION






LSE
ESE
LSM
ESM
ESM
LSM

Seminar: Formation of particular types of blood cells.
Practical class: Blood and bone marrow.

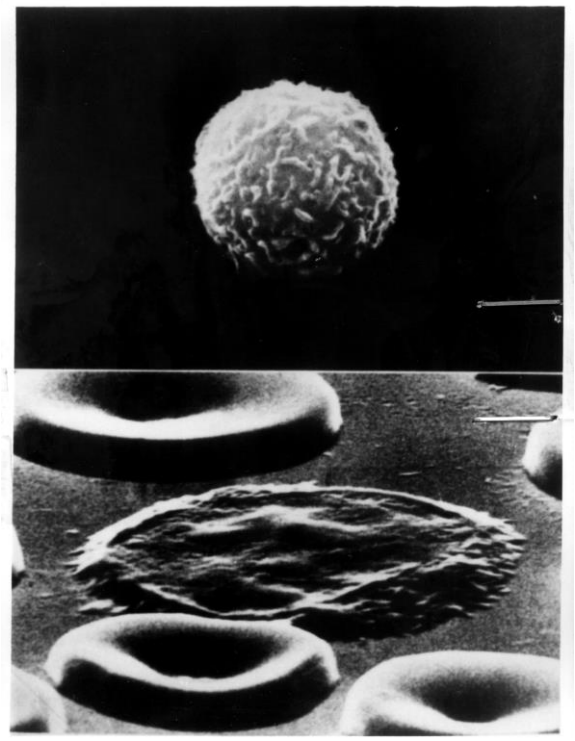


blood in the tube

- blood film (slide # 104),
- smear of bone marrow cells (slide # 35a),
- the section of red bone marrow (slide # 35),
- foetal liver (no. 54a),
- lymphocytes fixed as a suspension and in the smear (EM # 59),
- scheme of platelet function (fig. # 68).
- blood morphology analysis by flow cytometry (text & fig. # 67).

EM # 59

Lymphocytes as seen when fixed when in suspension (apper) and on the smear.



Text & fig. # 68

Thrombocyti (blood platelets)

Morphology

Blood platelet (BP) is a fragment of megakariocyte cytoplasm. BP is composed of:

Dense granules (containing ADP, serotonin and high concentration of Ca2+)
Alpha granules (containing platelet-derived growth factor - PDGF;
von Willebrand protein - present also in endothelial cells;
factor 4 - ounteracting anticoagulation activity of heparin;
thromboplastin and fibrinogen - proteins involved in blood coagulation).

1. **Lyzosomes**
2. **Mitochondriae**
3. **Glycogen**
4. **Microtubules - distributed in the periphery of BP**
5. **Thrombostein - a complex of actin and myosin**

Function

BP are involved in a blood coagulation:

6. **Their aggregates fill defects in blood vessels**
7. **They release factors which activate blood coagulation**

In case of detachment of endothelial cell from a basal membrane, BP attach via von Willebrand protein to the basal membrane collagen. Throm-bostein constricts BP aggregates, thus stabilising them. On contact with collagen BP release the granules contents and the arachidonic acid - pre-cursor of prostaglandines and thromboxan A_2. Thromboxan A_2 enhances the release of BP granules contents.

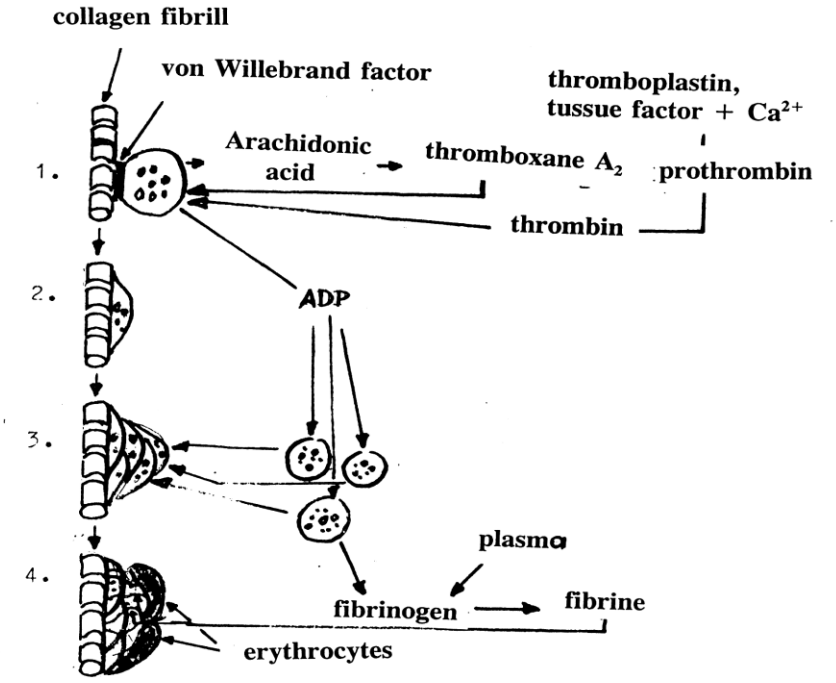
In the presence of Ca^{2+} thromboplastin and other blood coagulation factors, which are present in blood plasma and in tissues, convert pro-thrombin in to the thrombin. Thrombin, in turn, enhances the release of granules contents in the BP: ADP, serotonin and Ca^{2+}. ADP is responsible for a rapid aggregation of BP, while serotonin constricts blood vessels.

Thrombin transforms fibrinogen into fibrin. Fibrin fibrilles attach erythrocytes to the BP aggregates, thus the aggregates enlarge.

PDGF, released from the alpha granules, is responsible for mitotic activity of myocytes. Proliferating myocytes can be a nucleation site for atherosclerosis foci.

The participation of blood platelets in the blood coagulation process is presented on Fig.1

Fig. # 1



1. **Stages of coagulum (clot) formation.**
2. **Blood platelet attaches, via von Willebrand protein, to the collagen of endothelium basal membrane.**
3. **Blood platelet spreading.**
4. **Blood platelets aggregation.**
5. **Aggregate enlargement thanks to the attachment of erythrocytes and fibrin fibres.**

TEXT & FIG. # 67
Blood morphology analysis by flow cytometry

The increasing number of urgent blood analyses resulted in automatization of employed methods. Flow cytometry technique is a fast (the analysis takes approx. 30-40 sec.) and objective (independent of technician) method, that is commonly used by numerous analytical laboratories.
The principle of this method is to pump patient's blood sample through a system of capillaries, which is lighted by a laser beam. The flow of blood cells induces changes of laser light parameters detected by several sensors located around the capillaries. The data obtained are collected and transformed by a computer to numeric results or a graph display.
The analysis includes measurement of several morphologic and biochemical blood parameters. It consists of estimation of a size and some internal structure parameters of various blood cells, such as granularity and complexity, or lobularity of nuclei. The obtained parameters are compared to those of reference pattern. Thus, it is possible to automatically identify cell types and estimate their number (K, kilo=10^3 or M, mega=10^6) per volume (µl).

In case of leukocytes (WBC, white blood cells) WBC percentage pattern is also calculated:
WBC – leukocytes (total); normal range: 4-11 K/µl
NEU – neutrophiles (neutrophilic granulocytes); normal range: 2-6,9 K/µl; 50-66% WBC
LYM – lymphocytes; normal range: 0,6-3,4 K/µl; 20-40% WBC
MONO – monocytes; normal range: < 0,9 K/µl; 3-10% WBC
EOS – eosinophiles; (eosinophilic granulocytes); normal range: < 0,7 K/µl; < 7% WBC
BASO – basophiles; (basophilic granulocytes); normal range: < 0,2 K/µl; < 2% WBC

Most often causes of the changes in WBC number and percentage

WBC type	Increase	Decrease
WBC (total)	Infection, leukemia, lymphoma	Immunodeficiency (also – AIDS), reaction to some drugs
NEU	Bacterial infection, myeloma	See above, also advanced neoplasm
LYM/MONO	Viral infection, leukemia, lymphoma	See above, also advanced neoplasm
EOS	Allergy, parasite invasion	-
BASO	Allergy, parasite invasion	-

In addition to the red blood cell (RBC) number estimation (normal range: 3,8 – 6,3 M/µl), their analysis also includes:

RBC – number of red blood cells; normal range: 3,8 – 6,3 M/µl
HGB – total hemoglobin concentration; normal range: 12 – 18 g/dl
HCT – hematocrite (RBC volume/whole blood volume ratio); normal range: 37 – 54%
MCV – mean cell volume (mean RBC volume); normal range: 80 – 97 fl
MCH – mean cell hemoglobin (mean hemoglobin content in RBC); normal range: 27 – 31,2 pg
MCHC - mean cell hemoglobin concentration (mean hemoglobin concentration in RBC); normal range: 30 – 36 g/dl

Most often causes of RBC parameter changes

Parameter	Increase	Decrease
RBC/HGB/HCT	dehydratation, stress, adoptive response to cardiac and pulmonary diseases or high altitude	Bleeding, various anemia (also as a result of neoplasm)
MCV/HCT	B12 vitamin deficiency	Fe deficiency

In addition to a platelet (PLT) number estimation, the analysis also includes measurement of mean platelet volume (MPV; normal range: <99fl).
The two distinct populations (peeks) of blood cells are visible on a RBC graph, showing relation of RBC number to their volume. The right one (higher blood cell volume values) is a real RBC, the left one (with lower volume values) corresponds to PLT population. It is worth noticing that PLT graph originates from magnification of the left population of RBC graph.

Example no 1. Healthy control blood analysis

SAMODZIELNY PUBLICZNY CENTRALNY SZPITAL KLINICZNY

PRACOWNIA HEMATOLOGII

Wynik morfologii krwi

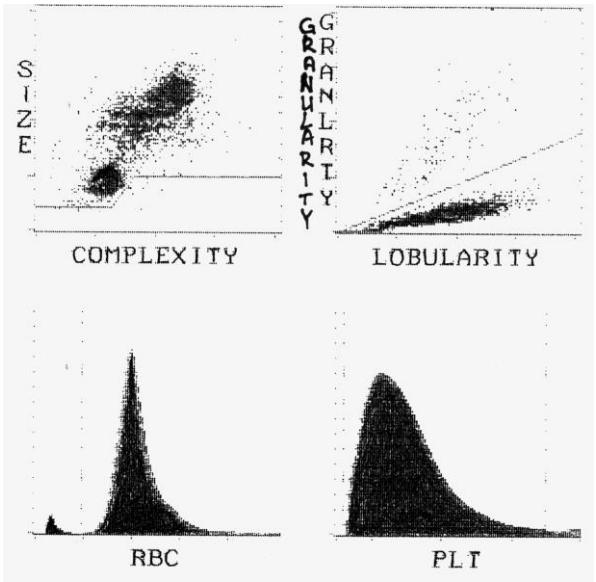
Analizator CELL-DYN 3700

Specimen ID — 31 Aug 2000 09:14
Patient — Operator ID 1
Sex — DOB — sequence 130
Dr LAB/LON — Open Sampler
Param: 1 — Limits: 1

WBC	6. 03 k/:L	
NEU	3 . 25	53.9 %N
LYM	1. 95	32.3 %L
MONO	.553	9.16 %M
EOS	.218	3.62 %E
BASO	.061	1.01%B

RBC	4.41 M/:L
HGB	13.0 g/dL
HTC	40.7 %
MCV	92.3 fL
MCH	29.5 pg
MCHC	32.0 g/dL

PLT	224 K/:L
MPV	10.8 fl



PATIENT LIMITS SET 1

WBC	6.00-11.0		RBC 3.80-6.30
NEU	2. 00- 6.90	50.0-66.0 %N	HGB 12.0-18.0
LYM	. 600-3.40	20.0-40.0%L	HCT 37.0-54.0
MONO	0.00-900	3.00-10.0%M	MCV 80.0-97.0
EOS	0.00-700	0.00-7.00 %E	MCH 27.0-31.2
BASO	0.00-200	0.00-2.00 %B	MCHC 30.0-36.0
			ROW 11.6-14.8

PLT 150.-400
MPV 0.00-99.9

Example no 2. Blood analysis of myeloid leukemia-suffering patient

SAMODZIELNY PUBLICZNY CENTRALNY SZPITAL KLINICZNY

PRACOWNIA HEMATOLOGII

Wynik morfologii krwi

Analizator CELL-DYN 3700

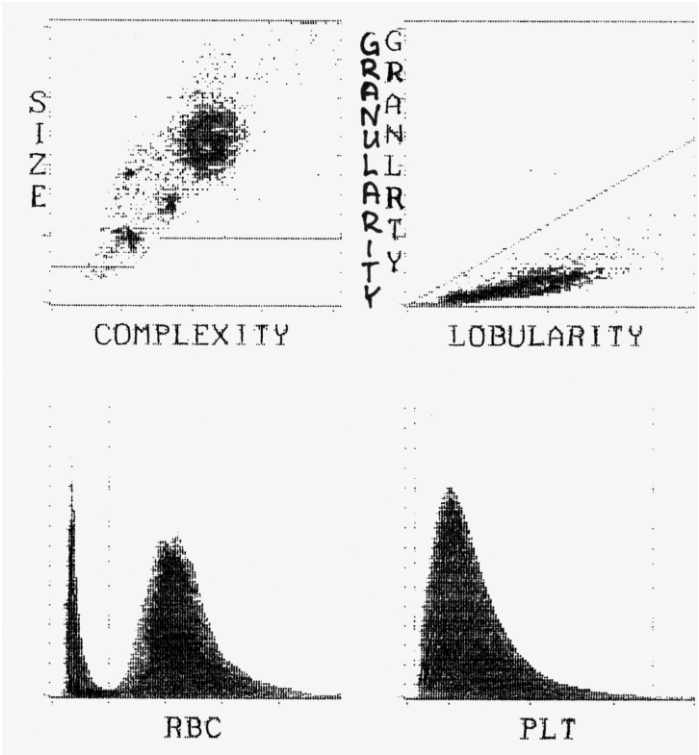
Specimen ID — 30 Aug 2000 13:10
Patient — Operator ID 1
Sex DOB — sequence 62
Dr 40 — Open Sampler
Param: 1 Limits: 1

SUSPECT

WBC	29.4	K/:L	
NEU	23.9	81.3 %N	IG
LYM	2.27	7.73 %L	
MONO	1.37	4.66 %M	
EOS	.022	.073 %E	
BASO	1.83	6.23%B	

RBC	2.63 M/:L	
HGB	9.02 g/Dl	
HTC	26.2 %	
MCV	99.6 fL	RBC MORPH
MCH	34.3 pg	
MCHC	34.4 g/dL	

PLT 1978 K/:L
MPV 7.79 fL



The underlined values exceed the normal limits. You can see high leukocytosis (WBC number) with dominant immature neutrophiles, decrease of RBC number, low HGB and HCT, and very high PLT number.