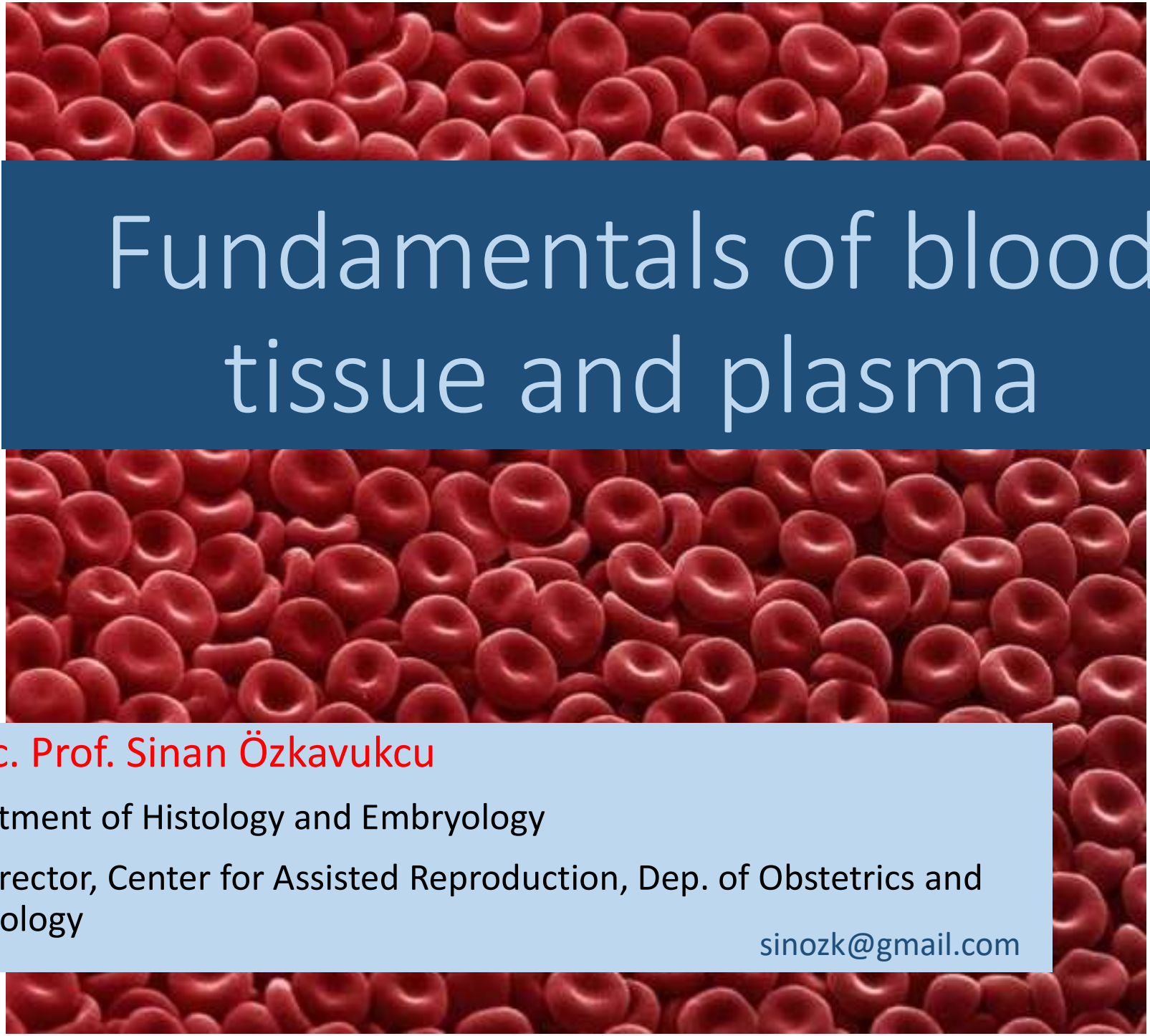

Fundamentals of blood tissue and plasma

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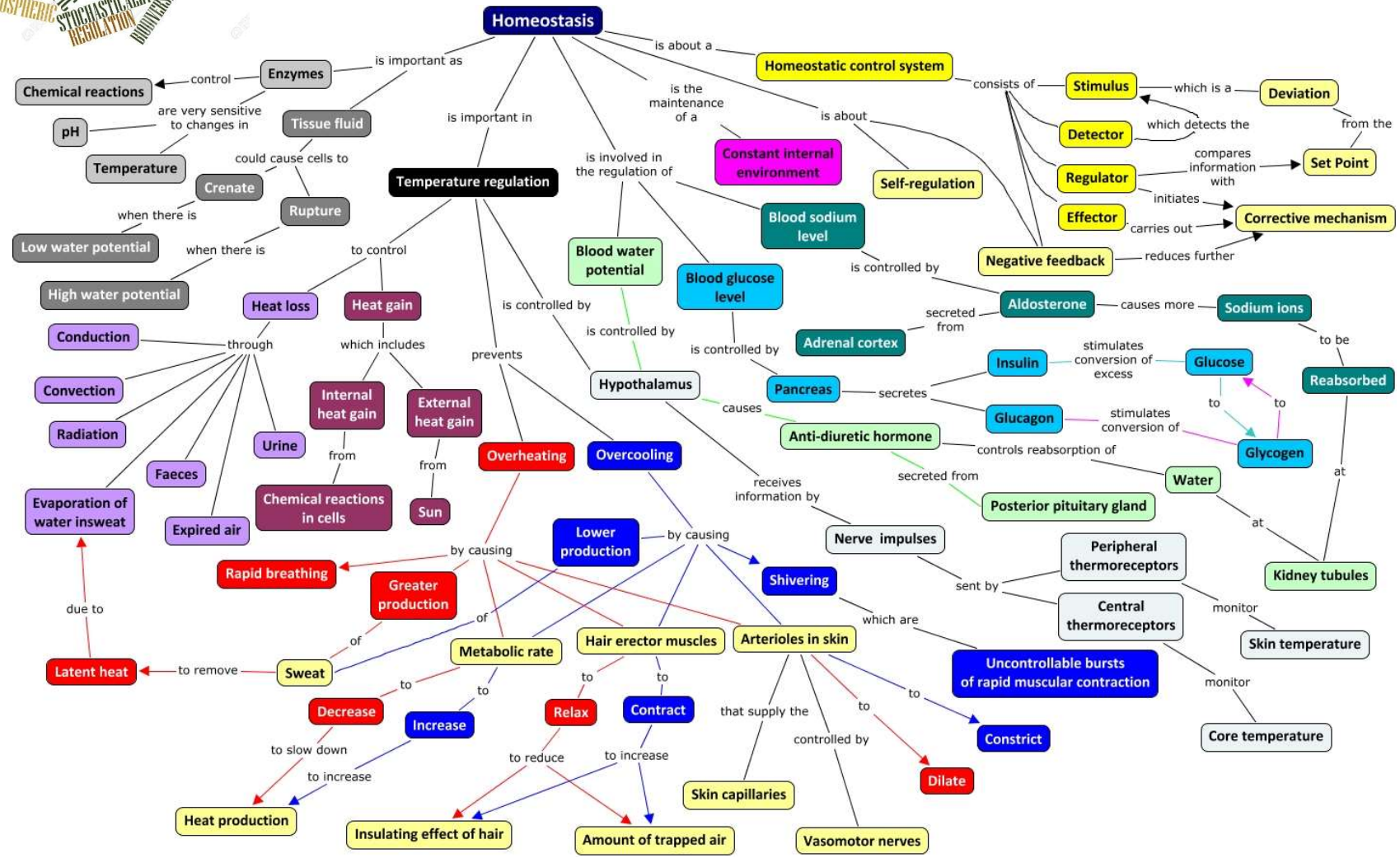
BLOOD

- Blood; is a liquid connective tissue, contains cells and extracellular compounds.
- There are about 5-6 liters of blood in adults and it constitutes 7-8% of body weight.

Duties:

- Delivering oxygen and nutrients directly or indirectly to the cells
- Removal of carbon dioxide and waste materials from cells
- Transmitting hormones and regulatory substances to tissues and cells
- To maintain homeostasis with tasks such as; acid-base balance (protection of pH), coagulation, thermoregulation
- Defensing body against pathogenic agents, proteins and cancer cells by transporting cells and molecules of the immune system

Homeostasis is the state of steady internal physical and chemical conditions maintained by living systems



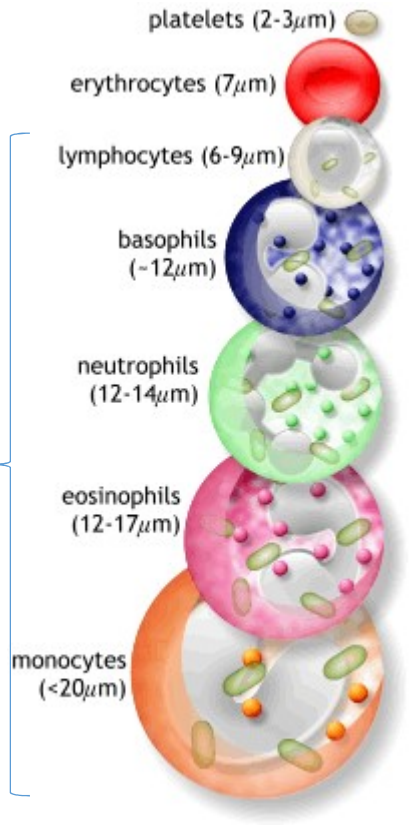
Not to be confused with hemostasis.

BLOOD TISSUE

CELLS

- Erythrocytes → Red Blood Cells (RBC)
- Leukocytes → White Blood Cells (WBC)
- Thrombocytes → Platelets

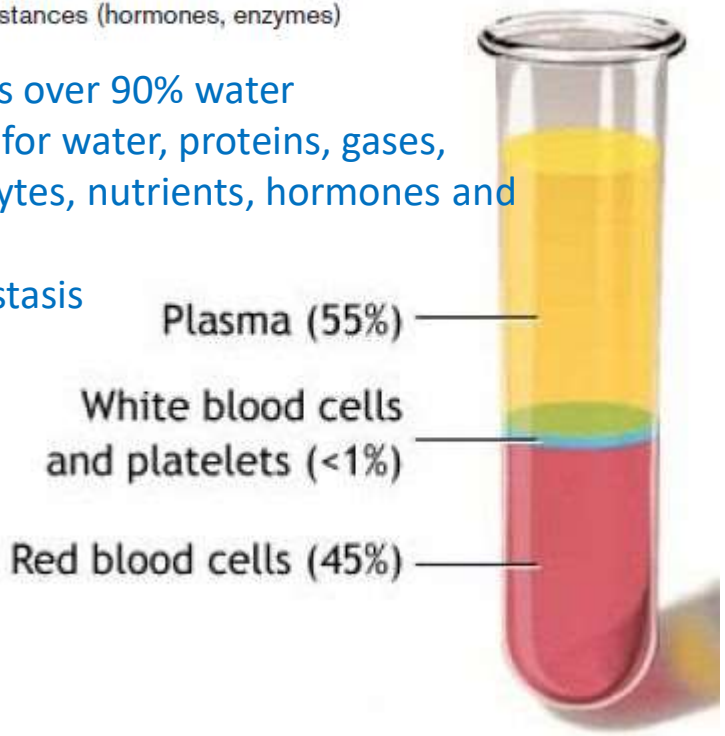
- Leukocytes:
  - Agranulocytes
    - Lymphocytes
    - Monocytes
  - Granulocytes
    - Neutrophils
    - Basophils
    - Eosinophils



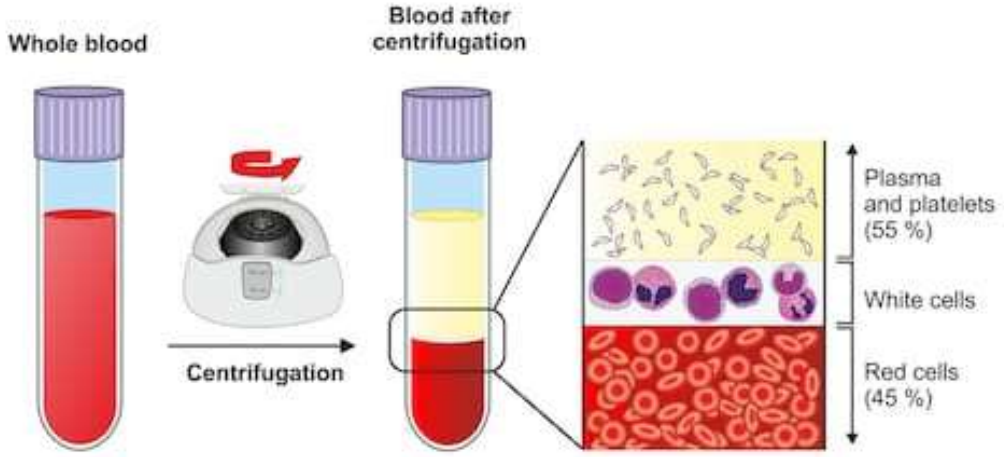
PLASMA

Component	%
Water	91–92
Protein (albumin, globulins, fibrinogen)	7–8
Other solutes: • Electrolytes (Na^+, K^+, Ca^{2+}, Mg^{2+}, Cl^-, HCO_3^-, PO_4^{3-}, SO_4^{2-}) • Nonprotein nitrogen substances (urea, uric acid, creatine, creatinine, ammonium salts) • Nutrients (glucose, lipids, amino acids) • Blood gases (oxygen, carbon dioxide, nitrogen) • Regulatory substances (hormones, enzymes)	1–2

- Contains over 90% water
- Solvent for water, proteins, gases, electrolytes, nutrients, hormones and waste
- Homeostasis



Buffy-coat



- The buffy coat is the fraction of an anticoagulated blood sample that contains most of the white blood cells and platelets following centrifugation of the blood.

- Leukocytes are less dense and small in number (1% of the blood volume).
- They form a thin white or grayish layer on the erythrocytes in the tube, which is called "buffy coat".
- The upper surface of the buffy coat contains platelets as a thin layer.

Plasma
the extracellular matrix of the blood tissue

- Water
- Proteins
- Solutes
  - electrolytes
  - non protein nitrogen
  - nutrients
  - gas
  - hormones, enzymes

Plasma Albumin

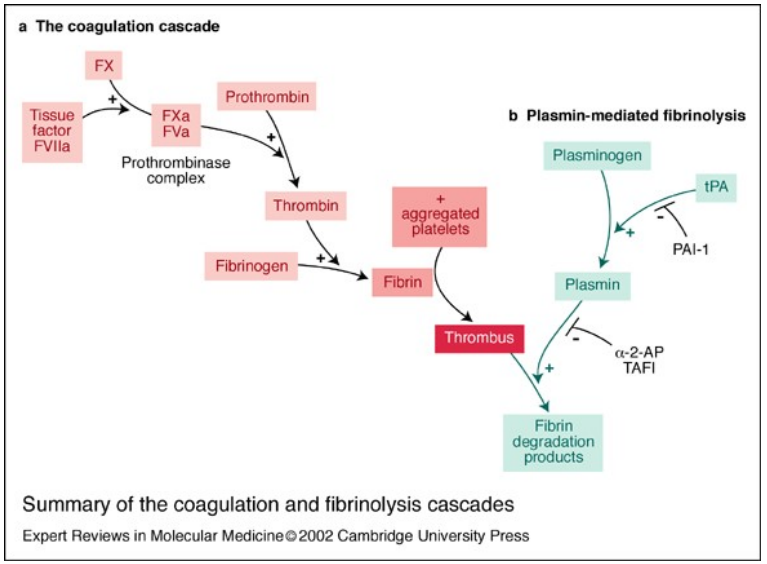
- The main protein component of plasma is ALBUMIN (50%).
- Smallest plasma protein (70kd), produced in the liver
- It is responsible for colloid osmotic pressure (oncotic pressure) and maintains fluid balance between tissue ECM and vessel lumen.
- Albumin also acts as carrier protein in the blood. Many hormones (thyroxine), metabolites (bilirubin) and drugs (barbiturates) are transported by binding to albumin.
- Colloid osmotic pressure decreases in the diseases with albumin loss (impaired production in the liver, excessive excretion from the kidneys), fluid accumulates in the tissues and in the body cavities (edema, pleural effusion, ascites).

Plasma Globulins

- γ (gamma) Globulins = immunoglobulins
  - They are functional immune system molecules called antibodies and produced by plasma cells
- Nonimmune globulins (α and β globulins)
  - Produced by the liver
  - Provide intravenous oncotic pressure
  - Bind and transport some substances
    - Ceruloplasmin (copper)
    - Transferrin (iron)
    - Haptoglobin (hemoglobin)
    - Fibronectin, lipoproteins, coagulation factors

Plasma Fibrinogen

- It is the largest plasma protein (340 kd) and is produced in the liver
- Normally present as dissolved in plasma, it is transformed into insoluble fibrin structure as a result of stepwise reactions of coagulation factors and enables clotting



Summary of the coagulation and fibrinolysis cascades

PROTEIN	PLACE SYNTHESIZED	FUNCTION
Albumin (Mostly found)	Liver	1. Adjusts colloidal osmotic pressure 2. Plays a role in substance transport
α, β Globulins (Nonimmune globulins)	Liver	Carries metals and fat-soluble vitamins
δ Globulins (immune globulins)	Plasma Cells	Immune system
Coagulation proteins (fibrinogen)	Liver	Important in the formation of fibrin during coagulation
Plasma lipoproteins:		
chylomicrons	Intestinal epithelium	Carries triglyceride to the liver
VLDL	Liver	Carries triglyceride between liver and cells
LDL	Liver	Carries cholesterol between liver and cells

Serum

= has the same content as plasma, obtained by removing coagulation factors

- Plasma = Serum + Coagulum
- Serum = Plasma – Coagulation factors

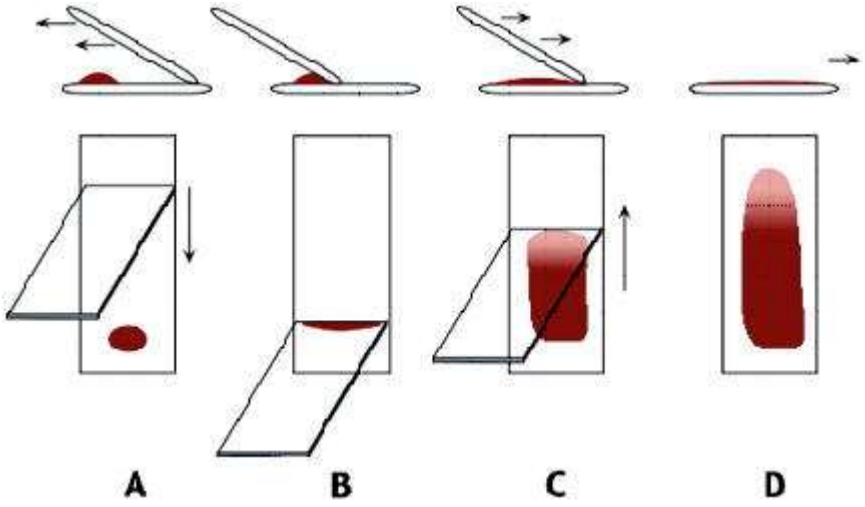
- Clotting occurs as soon as blood comes out of the vein
- Blood is drawn into tubes with citrate or heparin (anticoagulant) to get accurate measurements for laboratory studies
- High volume of serum can be obtained after centrifugation

PHYSICAL PROPERTIES OF BLOOD

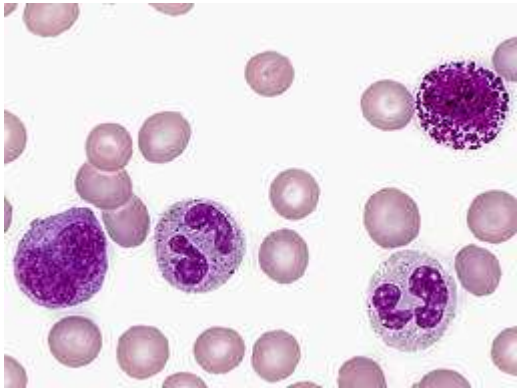
- COLOR: Red color originates from hemoglobin; As hemoglobin is saturated with oxygen, the color gets brighter.
- DENSITY: A mean density value of plasma and specific cells (1050-1060).
- REACTION: pH is around 7.35-7.45; higher in arterial blood and lower in venous blood
- VISCOSITY: Around 3.5-5.4; it is higher in venous blood due to the increased carbon dioxide.
- *** Blood plasma is isotonic at 0.9% NaCl

Blood smear

- The easiest method used in the examination of blood cells
- A drop of blood is placed directly on a glass slide and spread over its surface with the edge of another slide.
- After air drying, it is stained with modified Romanovsky type staining mixtures (Wright or Giemsa).
- Methylene blue (basic dye)
- Azure (basic dye)
- Eosin (acidic dye)



The granules of leukocytes can be distinguished as they have different staining properties (nuclei and basophil granules receive basic dyes)

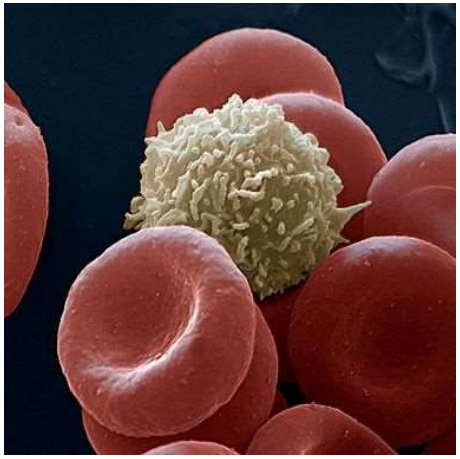


Cellular elements of blood

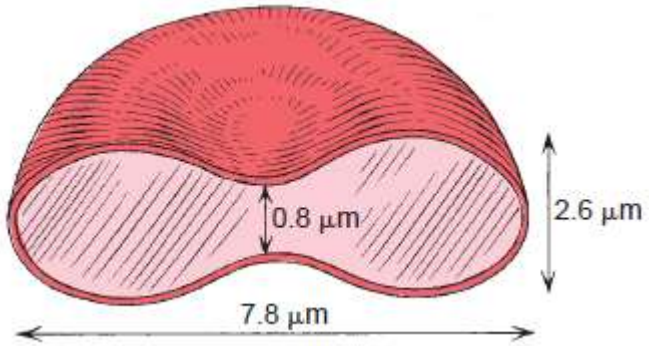
Formed Elements	Cells/L		%	
	Male	Female		
Erythrocytes	$4.3–5.7 \times 10^{12}$	$3.9–5.0 \times 10^{12}$		4,5-5,5 million/mm³
Leukocytes	$3.5–10.5 \times 10^{9}$	$3.5–10.5 \times 10^{9}$	100	5-9 thousand/mm³
Agranulocytes				
Lymphocytes	$0.9–2.9 \times 10^{9}$	$0.9–2.9 \times 10^{9}$	25.7–27.6[a]	
Monocytes	$0.3–0.9 \times 10^{9}$	$0.3–0.9 \times 10^{9}$	8.6[a]	
Granulocytes				
Neutrophils	$1.7–7.0 \times 10^{9}$	$1.7–7.0 \times 10^{9}$	48.6–66.7[a]	
Eosinophils	$0.05–0.5 \times 10^{9}$	$0.05–0.5 \times 10^{9}$	1.4–4.8[a]	
Basophils	$0–0.03 \times 10^{9}$	$0–0.03 \times 10^{9}$	0–0.3[a]	
Thrombocytes (platelets)	$150–450 \times 10^{9}$	$150–450 \times 10^{9}$		250 thousand/mm³

[a] Percentage of leukocytes

Erythrocytes



- Red blood cells
- No typical organelles and nuclei (anuclear)
- Transports oxygen to tissues and removes released carbon dioxide from tissues
- It is in the form of a biconcave disc, thus increasing surface area (advantage in gas exchange)
- Total surface area of erythrocytes is 2000 times higher than the whole body surface area

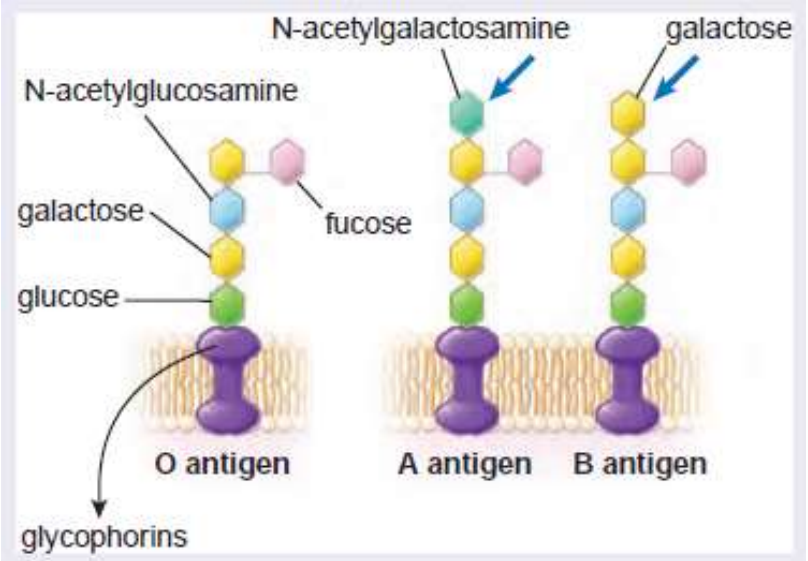


microscopic ruler

- Their lifetime is up to 120 days
- They are eliminated especially in the spleen and also in bone marrow and liver, with phagocytosis.
- A small part of it is eliminated in the circulation and releases hemoglobin
- Extremely flexible and capable folding in the capillaries

ABO Blood Group Systems

- ABO group compliance is important in blood transfusions..
  - It is determined by the surface antigens on erythrocytes
  - These antibodies are glycoprotein and glycolipid structures that extend outside the cell on the internal membrane protein glycophorins.
  - O antigen can be synthesized in all individuals
  - Individuals with group A add N-acetylgalactosamine on the O group with N-acetylgalactosamine transferase (A-glycosyltransferase)
  - Individuals with group B add galactose on group O with galactose transferase (B-glycosyltransferase)



- People with AB group have both enzymes, whereas those with O group do not have any of these enzymes.
- Therefore, there are antibodies against:
  - B antigen in A group,
  - A antigen in B group, and
  - both antigens in O group.
- AB group individuals can be a general recipient as they produce no antibody.

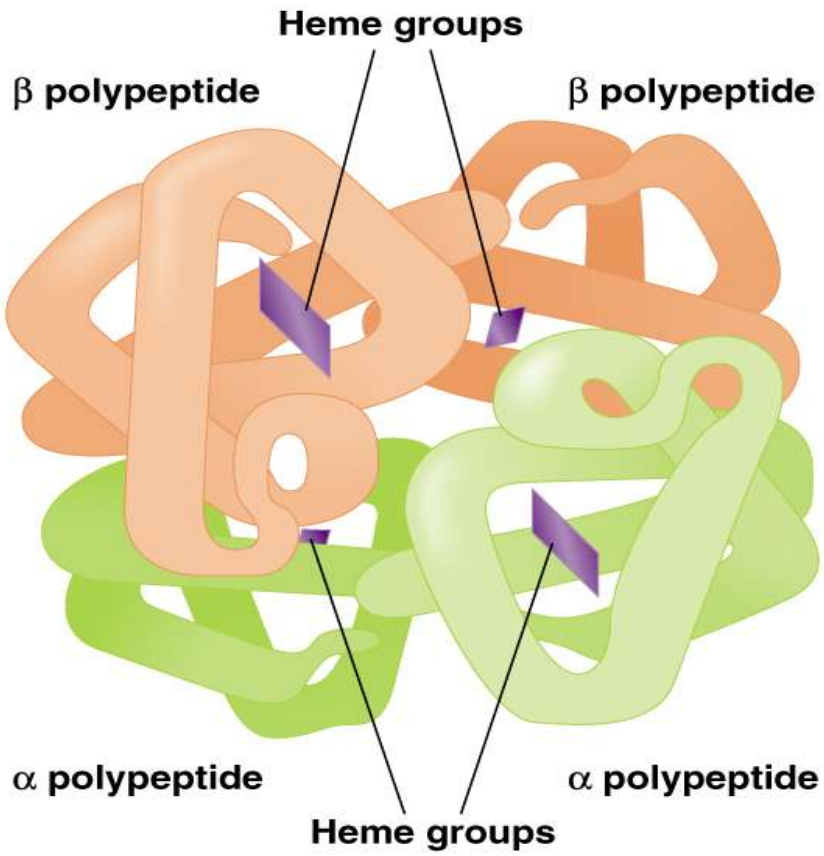
Rh Blood Group Systems

- Rhesus (Rh) antigen is also important for transplantation and in newborn infants
- Expressed with D, C, E antigens identified by Rh30 polypeptide and Rh50 glycoprotein
- Immunoglobulins develop against erythrocytes of Rh (D +) babies carried by Rh (D-) mothers.
- These antibodies cause erythroblastosis fetalis by hemolizing the erythrocytes of the baby in the second pregnancy.

- Administration of anti-D antibodies (RhoGAM) to the mother during pregnancy and after parturition destroys any circulating Rh(D+) fetal erythrocytes that persist in the mother's blood, thus preventing Rh-incompatibility reactions in future pregnancies.

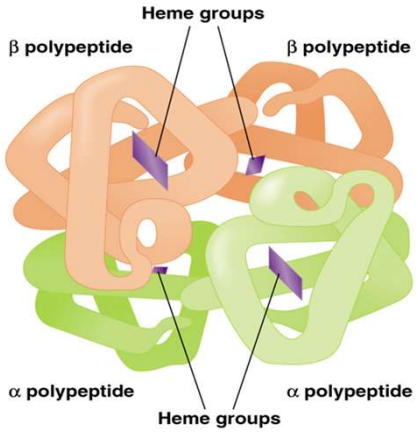
Hemoglobin

- A specialized protein for transport, by binding oxygen and carbon dioxide
- The erythrocytes contain 33% dissolved hemoglobin surrounded by cell membranes.
- It is found more intense close to the cell membrane for gas exchange
- It makes erythrocytes stain acidophilic with eosin.
- Hemoglobin is a natural colored compound protein.
- It is made of globulin, a colorless protein, and 4 *Heme* molecules attached to it.



- Hemoglobin molecule has 4 globulin complexes
- The globulin complexes are formed by the binding of α, β, δ or γ globulin to a group of iron-containing heme groups in the form of a polypeptide subunit pair.
- Hemoglobin can bound reversibly to oxygen and forms oxyhemoglobin, and forms carbominohemoglobin when bound to carbon dioxide.

- Hemoglobin forms an **irreversible** bond with carbon monoxide (carboxyhemoglobin). In this case, the oxygen carrying capacity of the blood is reduced. It is seen in cases of asphyxia during fire, suffocation from the chimney and stove.



In humans, only 3 forms are considered normal in postnatal life.

Types of hemoglobin

HbA is found in 97% of normal adults. It carries two beta chains.

- HbA → 2α + **2 beta chains**, 2α,2β

HbA_2 is found 2%. Carries two delta chains.

- HbA_2 → 2α + **2 delta chains**, 2α,2δ

- HbF → 2α + **2 gama chains**, 2α,2γ

HbF is present in 1% of healthy adults.
HbF is 80% in the newborn. However, this goes down to the normal adult level at about 8 years old.
It carries two gamma chains.

Although 550 types of abnormal hemoglobin molecule types have been identified, the majority of these do not cause any clinical conditions.

Leukocytes

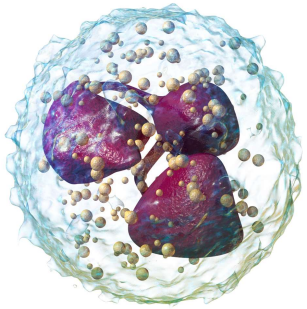
White Blood Cells-WBC

They are subdivided according to the presence or absence of specific granules.

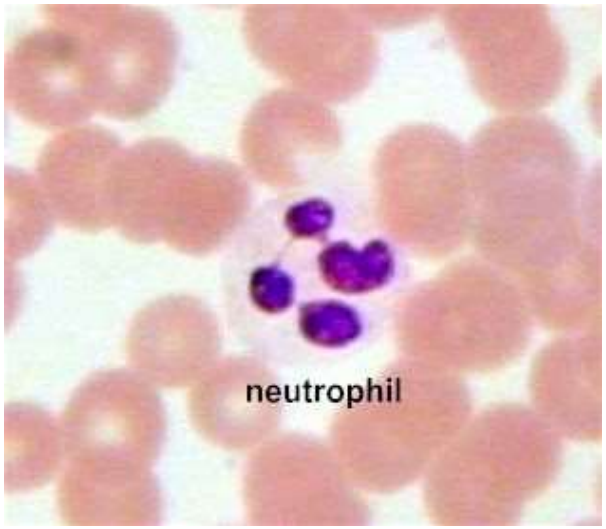
However, all leukocytes have non-specific azurophilic granules of lysosome structure.

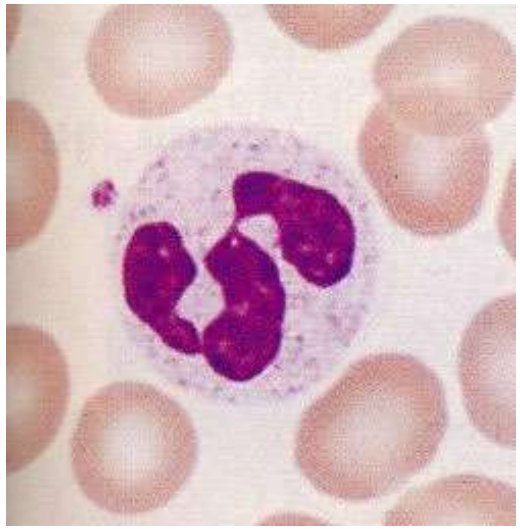
- Granulocytes: Neutrophils, Eosinophils, Basophils
- Agranulocytes: Lymphocytes, Monocytes

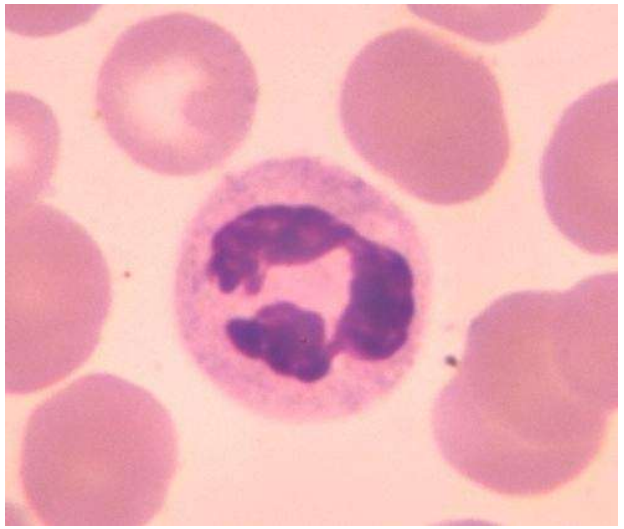
Neutrophils

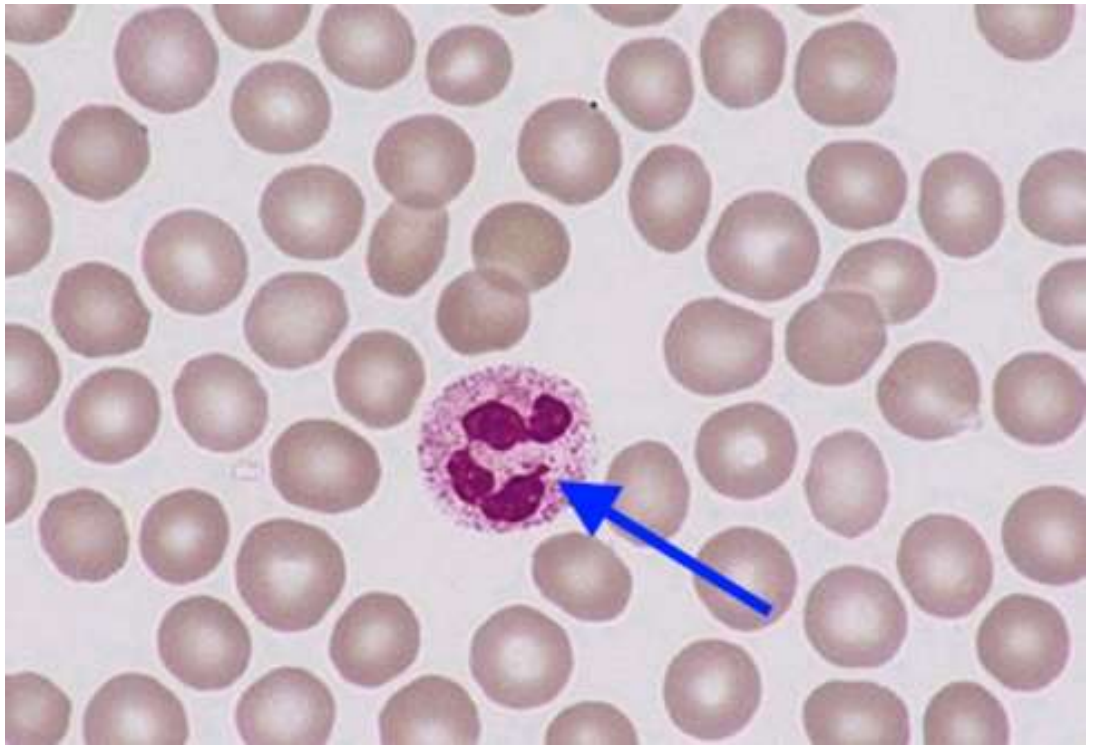


- They are the largest number of leukocytes, they are 10-12 µm in diameter.
- Since they have no characteristic staining in their cytoplasm, they are named neutral.
- Because their nuclei are multi-lobed, they are also called polymorphonuclear neutrophils (PMN / PNL).
- The Barr body (named after discoverer Murray Barr) in which the inactive X chromosome in females, is concentrated and stored can be detected in the PMN leukocyte nucleus (small **drumstick**-like projection).


neutrophil





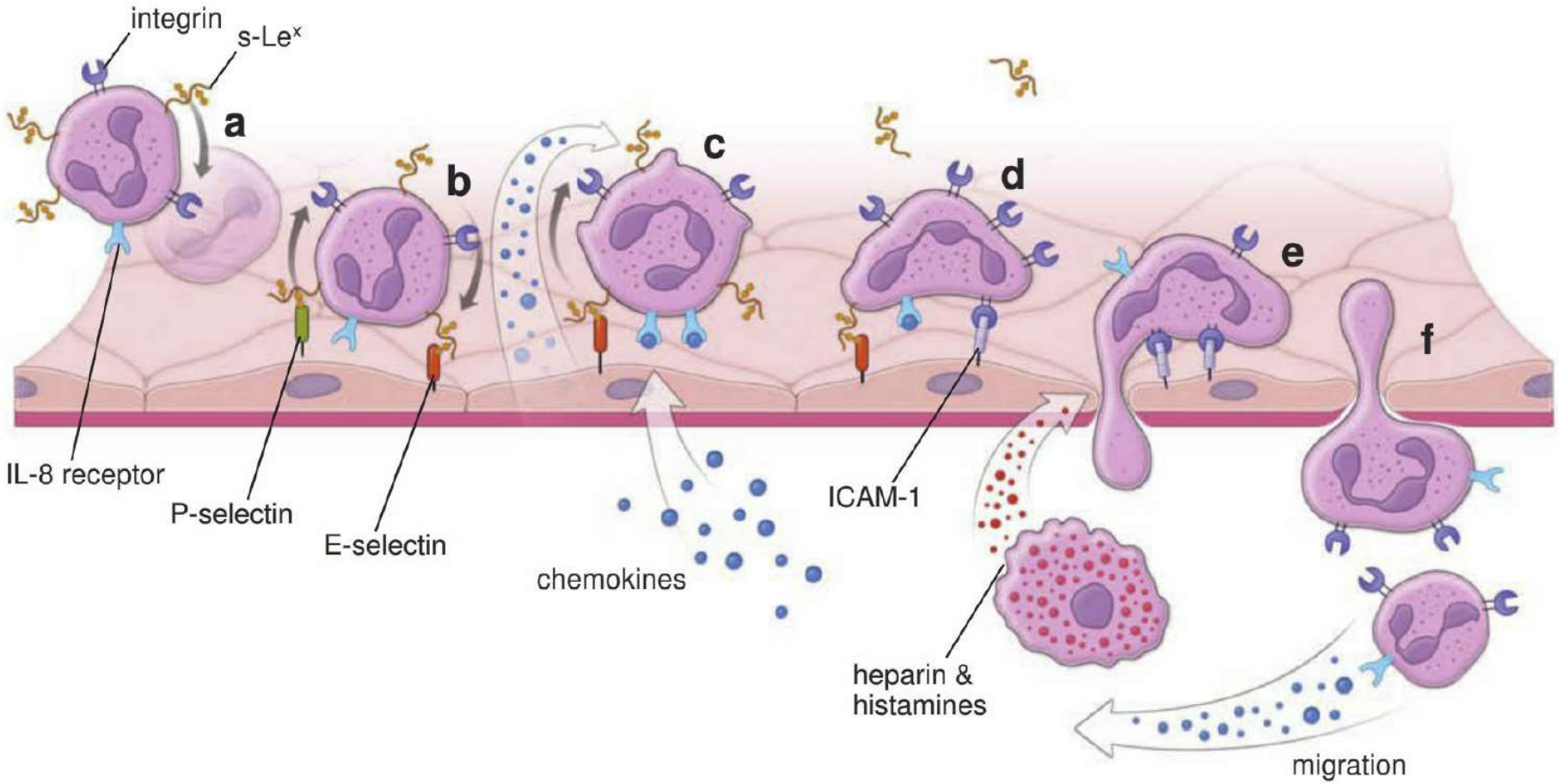


Granules of neutrophils

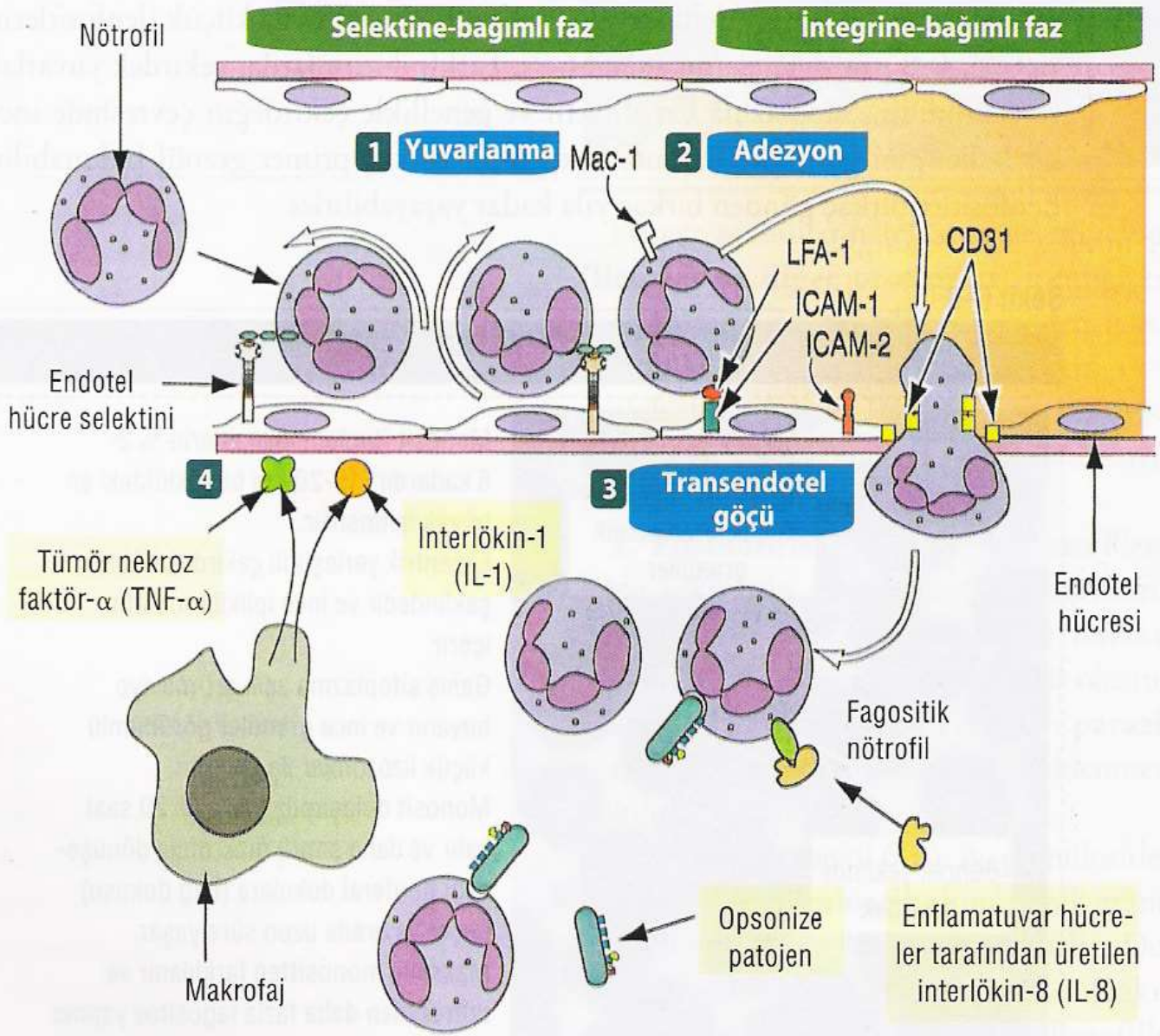
- Specific (*secondary*) granules
  - They are the smallest granules
  - Their number is more than double the azurophilic granules
  - They are difficult to observe under the light microscope, they are monitored in the form of an ellipse with EM
  - They contain enzymes (type IV collagenase, phospholipase) and complement activators.
- Azurophilic (*primary*) granules
  - They are larger but fewer
  - They are bactericidal lysosomes containing myeloperoxidase
  - They also contain acid hydrolase, defensin (helps antibodies), cathelicidin (antimicrobial)
- Tertiary granules
  - There are two types: those containing phosphatases that remove a phosphate group from a molecule and metalloproteinases such as gelatinase, collagenase, which wash away connective tissue and helps migration into the tissue.

Motile

- Neutrophils have migration - movement abilities and are the first cells to reach the area after any tissue damage.
- Circulating neutrophils express adhesion molecules on their surface under the action of cytokines, and these molecules match their counterparts on the endothelium and slow them down, allowing them to enter into the connective tissue.


integrin
s-Le^x
a
b
c
d
e
f
IL-8 receptor
P-selectin
E-selectin
chemokines
ICAM-1
heparin &
histamines
migration

Homing ve inflamasyon



1 Yuvarlanma ve bağlanma
Lökositler (şekilde nötrofil) endotel hücre yüzeyinde bulunan selektinler ve nötrofil yüzeyinde bulunan karbonhidrat ligandları ile geri dönüşümlü bir bağlanma meydana getirirler. Bu bağlanma güçlü değildir ve hücre yuvarlanmasına devam eder.

2 Adezyon (yapışma)
Nötrofil ve endotel (yapışma) hücre arasında güçlü bir etkileşim meydana gelir. Bu etkileşim endotel üzerindeki intersellüler adezyon molekülleri **ICAM-1 ve ICAM-2** ile **Mac-1** ve **LFA-1 integrinleri** (lenfosit fonksiyonuna-bağlı antijen) aracılığı ile olur. ICAM-1 inflamasyon varlığında salınır.

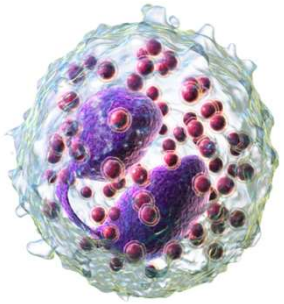
3 Transendotel göçü
T hücresi enflamatuar hücreler tarafından üretilen **IL-8** konsantrasyonunun artışı ile endotelden göç eder. **CD31** diapedezi kolaylaştırır.

4 Aktive olmuş makrofajlar endotel hücrelerden selektinlerin salgılanmalarını uyaran **TNF-α ve IL-1**'i salgılarlar.

Pus

the accumulation
of dead bacteria and dead neutrophils
constitutes the thick exudate called **pus.**
The yellow—green color of the
pus comes from the heme pigment of
myeloperoxidase enzyme in azurophilic
granules of neutrophils.

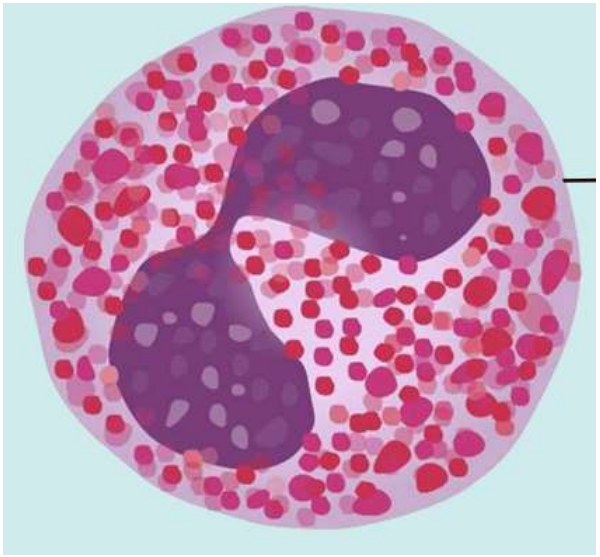
Eosinophils

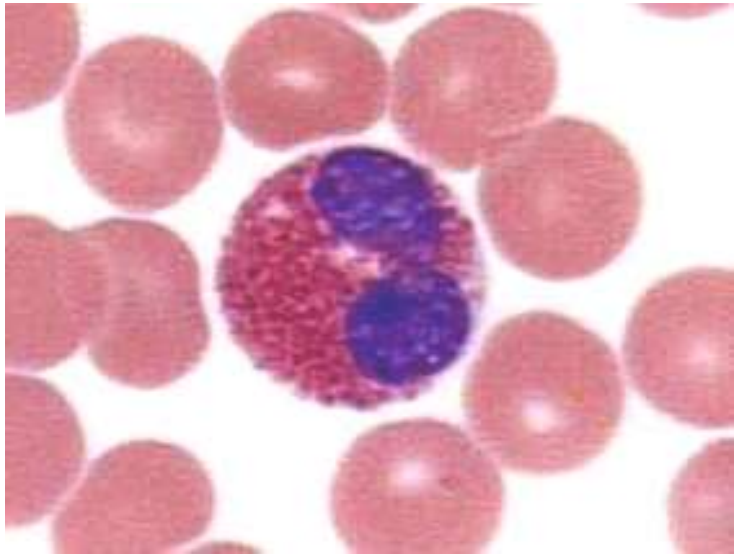


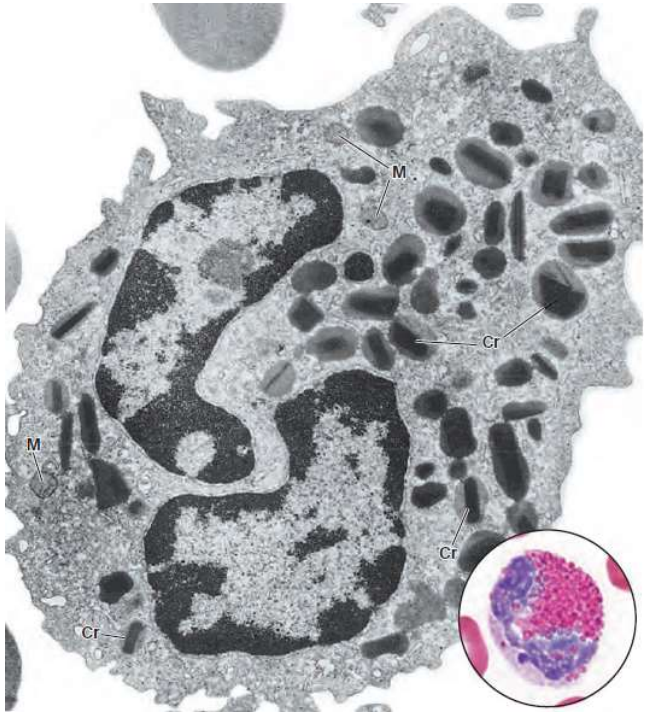
- Its dimensions are neutrophil-sized, and its nuclei have typically two lobes (*bi-lobed*).
- They contain large, eosinophilic, refractile granules in their cytoplasm
- Large, elongated specific granules
  - They contain crystalloid body that can be seen with TEM (refractivity in the light microscope)
    - Major basic protein (inside crystalloid body), eosinophil cationic protein, eosinophil peroxidase, eosinophil-derived neurotoxin
    - Toxic activity against parasites (protozoa and helminths)
    - Neurotoxin against parasites
    - Histaminase, arylsulphatase, collagenase, cathepsin
- Azurophilic granules (lysosomes)
  - Lysosomal acid hydrolases

Functions of eosinophils

- They take part in the allergic reactions and defense of parasitic infections (protozoans and helminthic parasites)
- After production in bone marrow, they move into the connective tissue from blood and get activated with IgG, IgA and secretory IgA.
- The count of eosinophils in blood samples of individuals with allergies and parasitic infections is usually high (eosinophilia).
- Abundant in the lamina propria of lung and intestines and in patients with asthma
- Participates in other immunologic responses and phagocytoses antigen-antibody complexes



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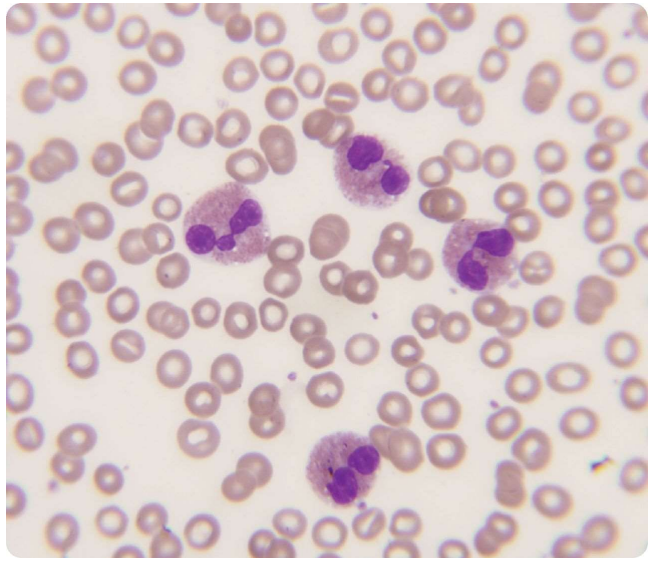
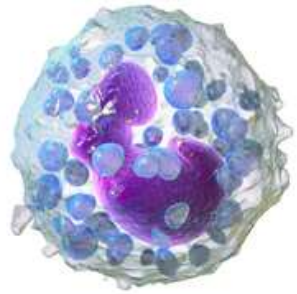


Basophils



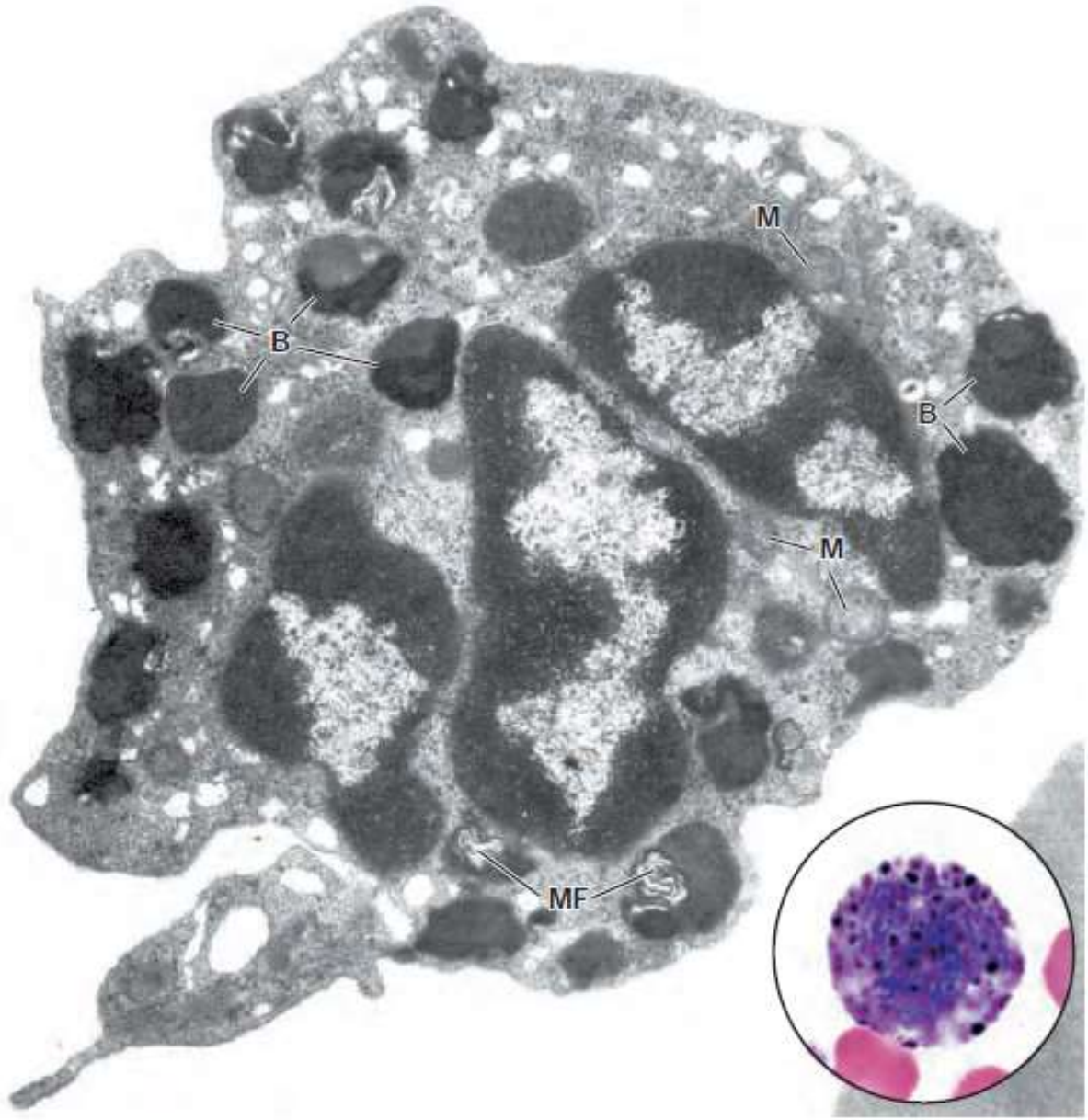
- They are cells of the same size as neutrophils, with a large number of basic stained granules.
- They are the least numerous white blood cells, up to 0.5% of the total leukocytes.
- It has a lobed, hardly selected nucleus due to dense granules in stained blood smears
- The basophil plasma membrane possesses numerous high-affinity **Fc receptors** for IgE antibodies.

Granules of basophils

- Specific granules
  - Granules are larger than neutrophil's
  - They contain heparin, histamine, heparan sulfate, leukotrienes, IL-4, and IL-13.
  - Anticoagulant, vasodilation, smooth muscle contraction, stimulation of IgE synthesis
- Azurophilic granules
  - Lysosomal acid hydrolases

Functions of basophils

- functionally related to, but not identical with, mast cells of the connective tissue
- Both mast cells and basophils bind an antibody secreted by plasma cells, **IgE,** through high-affinity Fc receptors expressed on their cell surface. Exposure to a specific antigen (allergen) instantly or later in life triggers the activation of the basophil and release of vasoactive substances from granules
- Responsible for hypersensitivity reactions and anaphylaxis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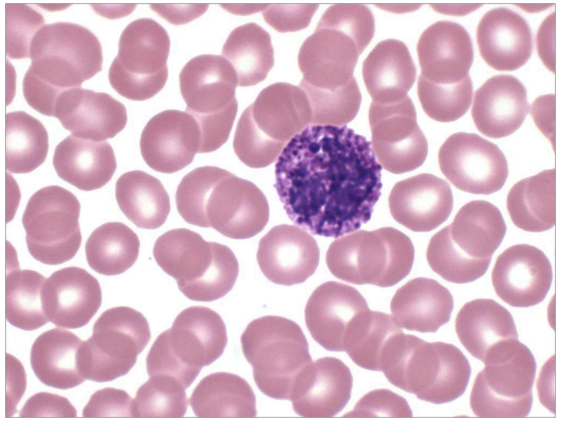
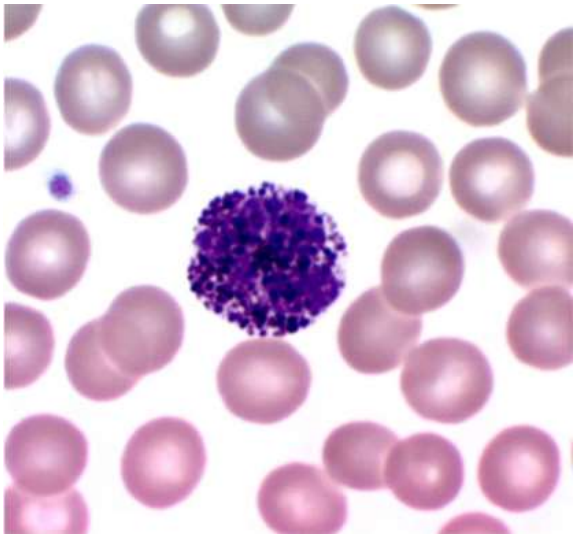


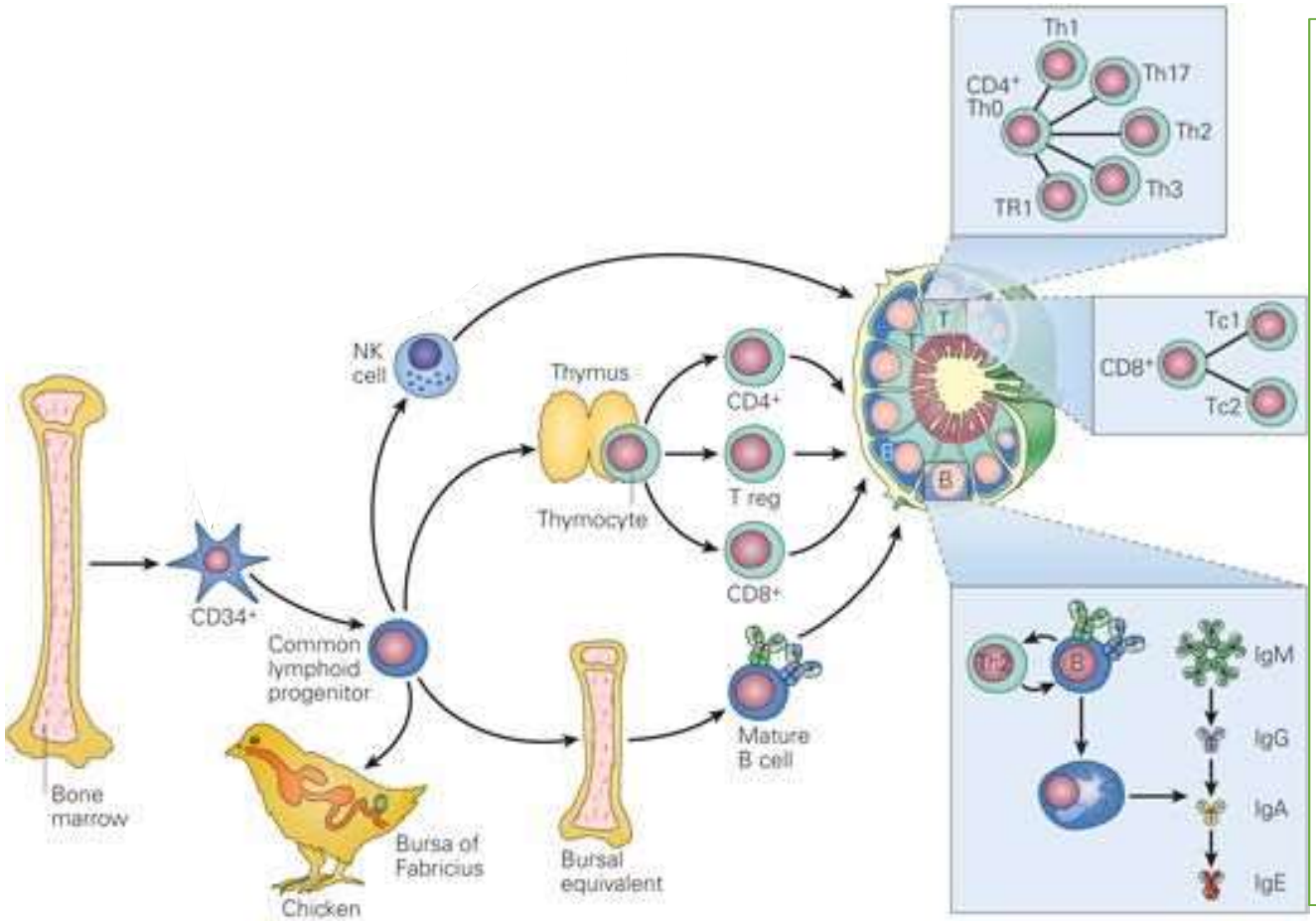


Agranulocytes

Lymphocytes

- They are the basic cells of the lymphatic and immune system
- Constitutes 30% of all blood leukocytes
- Three types of lymphocytes are defined according to their size in the immune system: small, medium and large (activated lymphocytes or natural killer (NK) cells).
- 6-30 µm

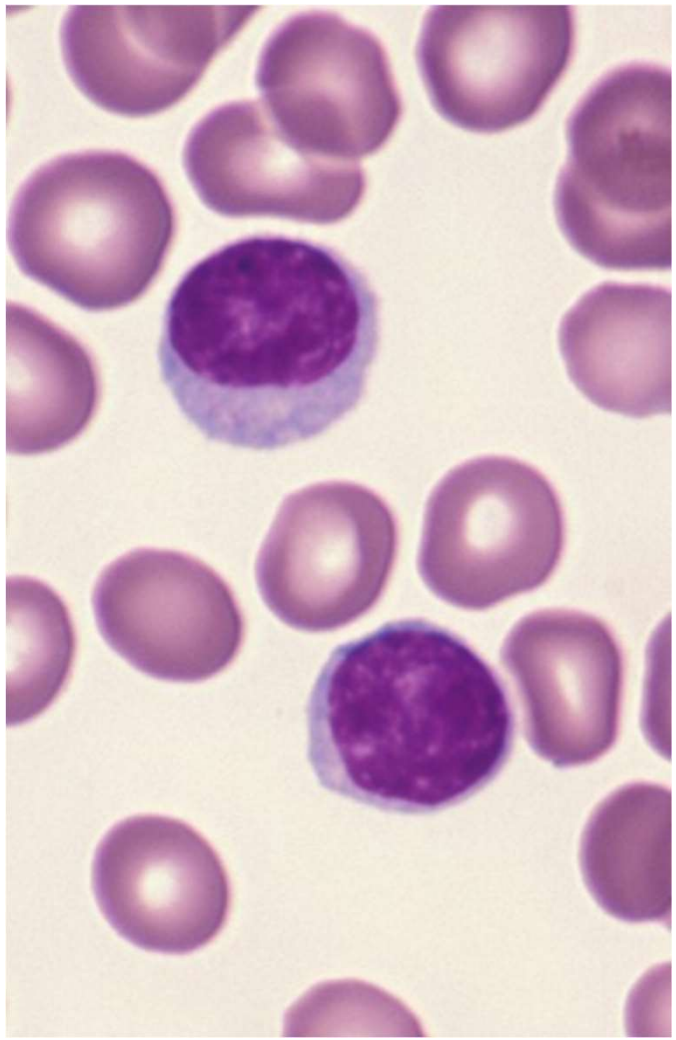
Origins of Lymphocytes

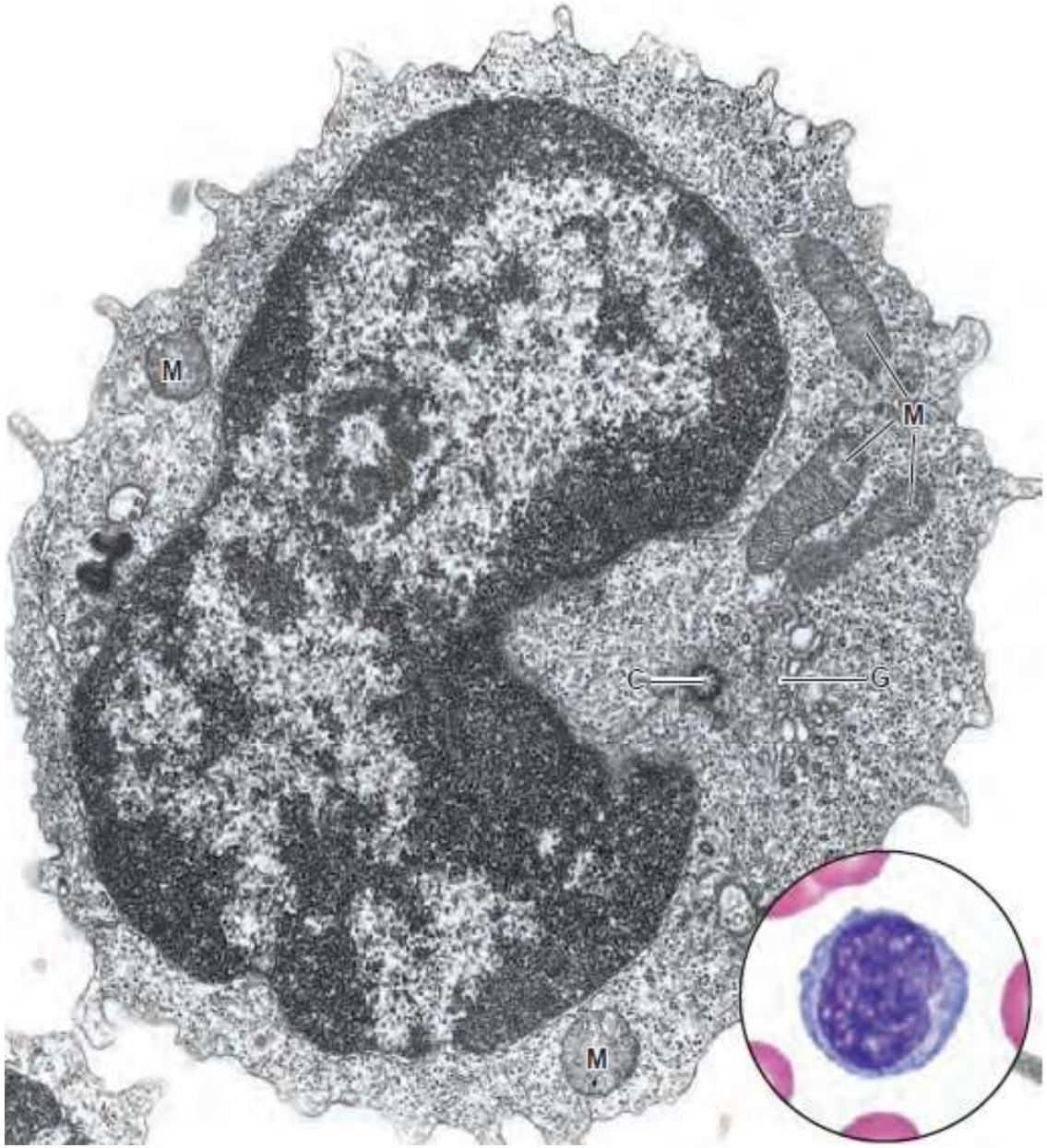


- not terminally differentiated cells
- capable of undergoing divisions and differentiations into other types of effector cells
- originate in the bone marrow, lymphocytes are capable of developing outside the bone marrow in tissues associated with the immune system

Morphology

- Small lymphocytes are the 90% of the circulating lymphocytes and they are around the size of an erythrocyte.
- They have an intensely staining, slightly indented, spherical nucleus almost filling the cytoplasm
- The cytoplasm appears as a very thin, pale blue rim surrounding the nucleus

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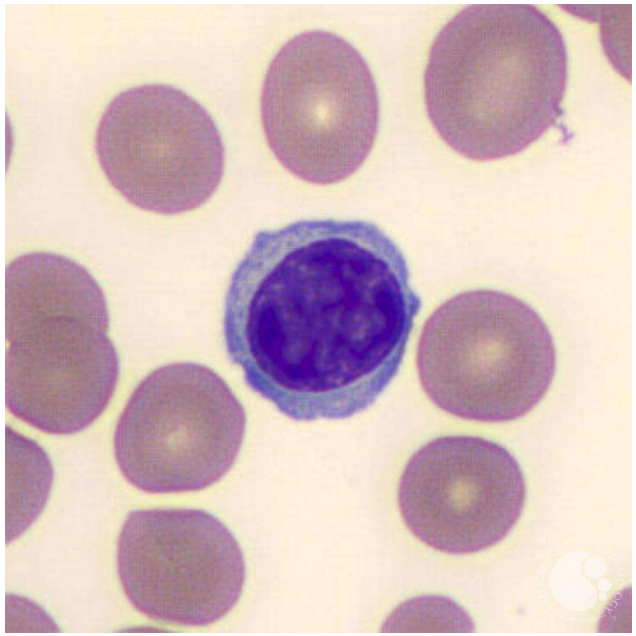


Three functionally distinct types of lymphocytes are present in the body

1. T lymphocytes (matured in Thymus)
2. B lymphocytes (matured in Bursa fabricius in birds and Bone marrow in mammals)
3. NK cells

CD: Cluster of Differentiation
a protocol used for the identification and investigation of cell surface molecules providing targets for immunophenotyping of cells

Type of cell	CD markers
stem cells	CD34+, CD31-, CD117
all leukocyte groups	CD45+
Granulocyte	CD45+, CD11b, CD15+, CD24+, CD114+, CD182+[16]
Monocyte	CD4, CD45+, CD14+, CD114+, CD11a, CD11b, CD91+,[16] CD16+[17]
T lymphocyte	CD45+, CD3+
T helper cell	CD45+, CD3+, CD4+
T regulatory cell	CD4, CD25, FOXP3 (a transcription factor)
Cytotoxic T cell	CD45+, CD3+, CD8+
B lymphocyte	CD45+, CD19+, CD20+, CD24+, CD38, CD22
Thrombocyte	CD45+, CD61+
Natural killer cell	CD16+, CD56+, CD3-, CD31, CD30, CD38

T-Lymphocytes

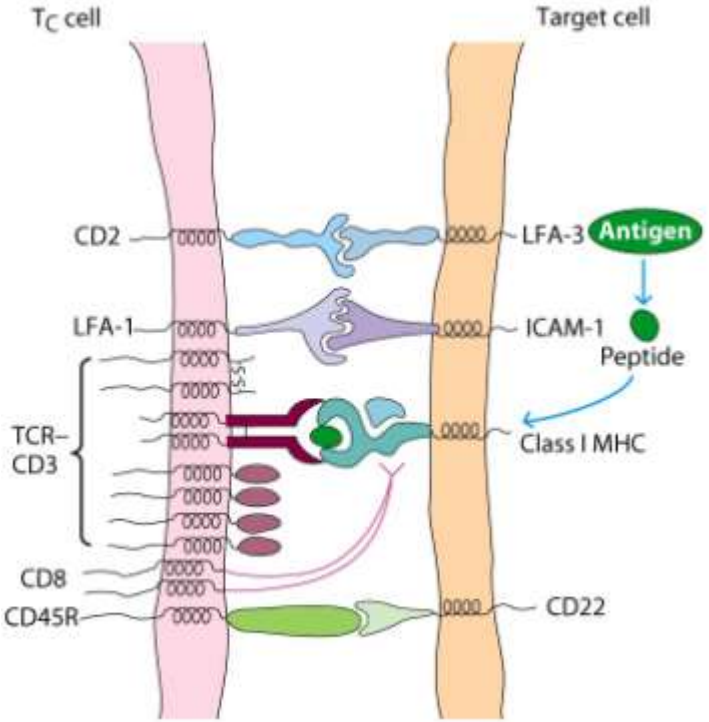
- They are responsible for cell-mediated immunity
- There are cytotoxic, helper, suppressor and gamma/delta types.
- However, the main functional distinction depends on CD4 and CD8 expressions.
  - CD4+ T_{helper} lymphocytes: recognize antigens presented with MHC-II
  - CD8+ $T_{cytotoxic}$ lymphocytes : recognize antigens bound to MHC-I

MHC: major histocompatability complex

MHC-I molecules are expressed by all nucleated cells

$CD8^+$ T-lymphocytes

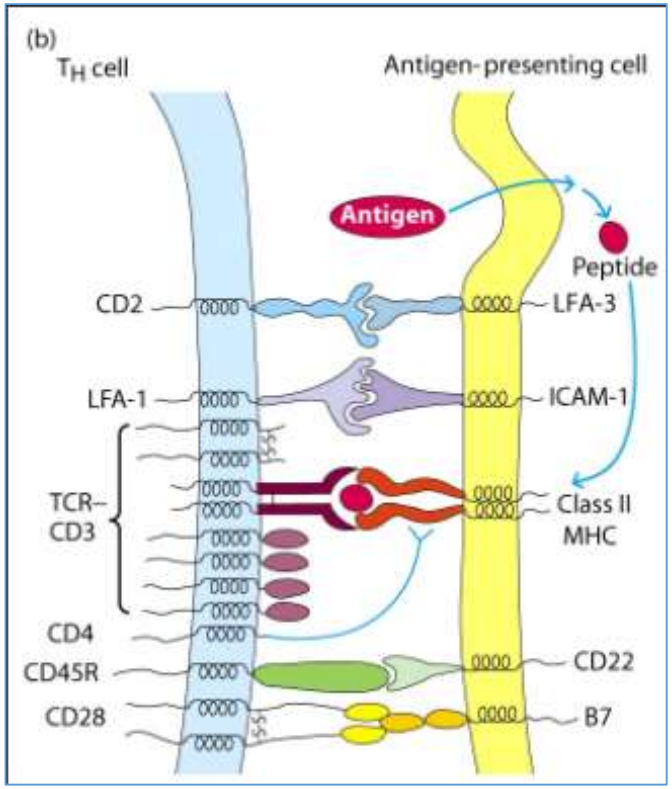
- It is the main effector cell of cell-mediated immunity
- Sensitive to foreign, virus-infected and neoplastic cell surface antigens



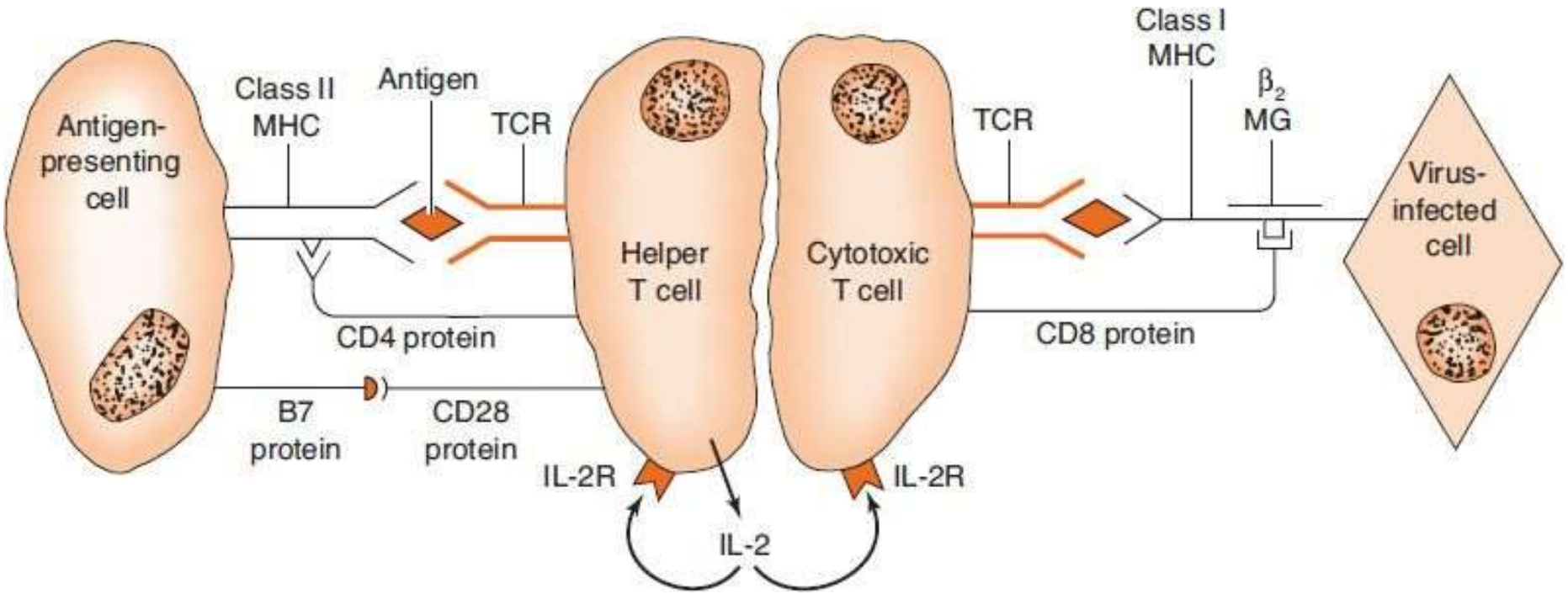
- When TCR binds to the MHC-I-antigen complex, Tc cells secrete substances called lymphokines and perforin, causing lysis in enemy cells, killed by membrane damage.
- Cytotoxic T lymphocytes play a major role in organ and tissue rejection and tumor immunity.

$CD4^+$ T-lymphocytes

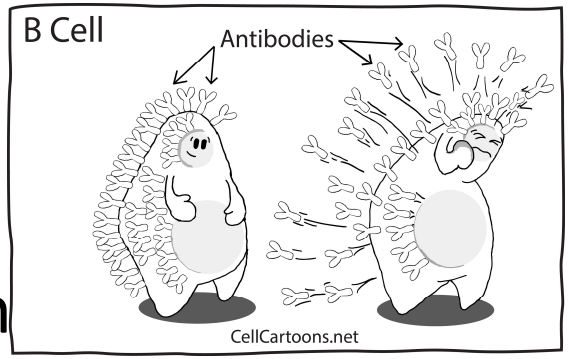
- Important in initiating an immune reaction response to a foreign antigen



- Antigen bound to MHC II molecules is presented by «antigen-presenting cells» such as macrophages
- Activated helper CD4 T-lymphocytes produce interleukins (IL-2) to stimulate more T-lymphocytes and to differantiate B-lymphocytes into plasma cells for antibody production


Antigen-presenting cell
Class II MHC
Antigen
TCR
Helper T cell
Cytotoxic T cell
TCR
Class I MHC
β_2 MG
Virus-infected cell
CD4 protein
CD8 protein
B7 protein
CD28 protein
IL-2R
IL-2R
IL-2

B cells

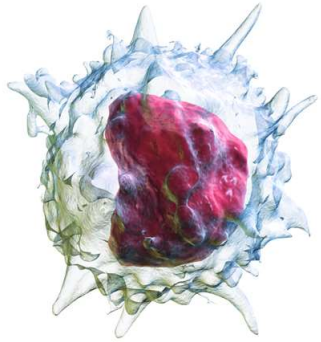


- Responsible for antibody production
- Mature B cells in the blood express IgM, IgD and MHC-II molecules on their surface

NK cells

- Programmed to kill virus-infected cells and malign cells
- They secrete an antiviral substance: interferon γ
- Also referred to as large granular lymphocyte (LGL)
- (~ 15 µm)

Monocyte

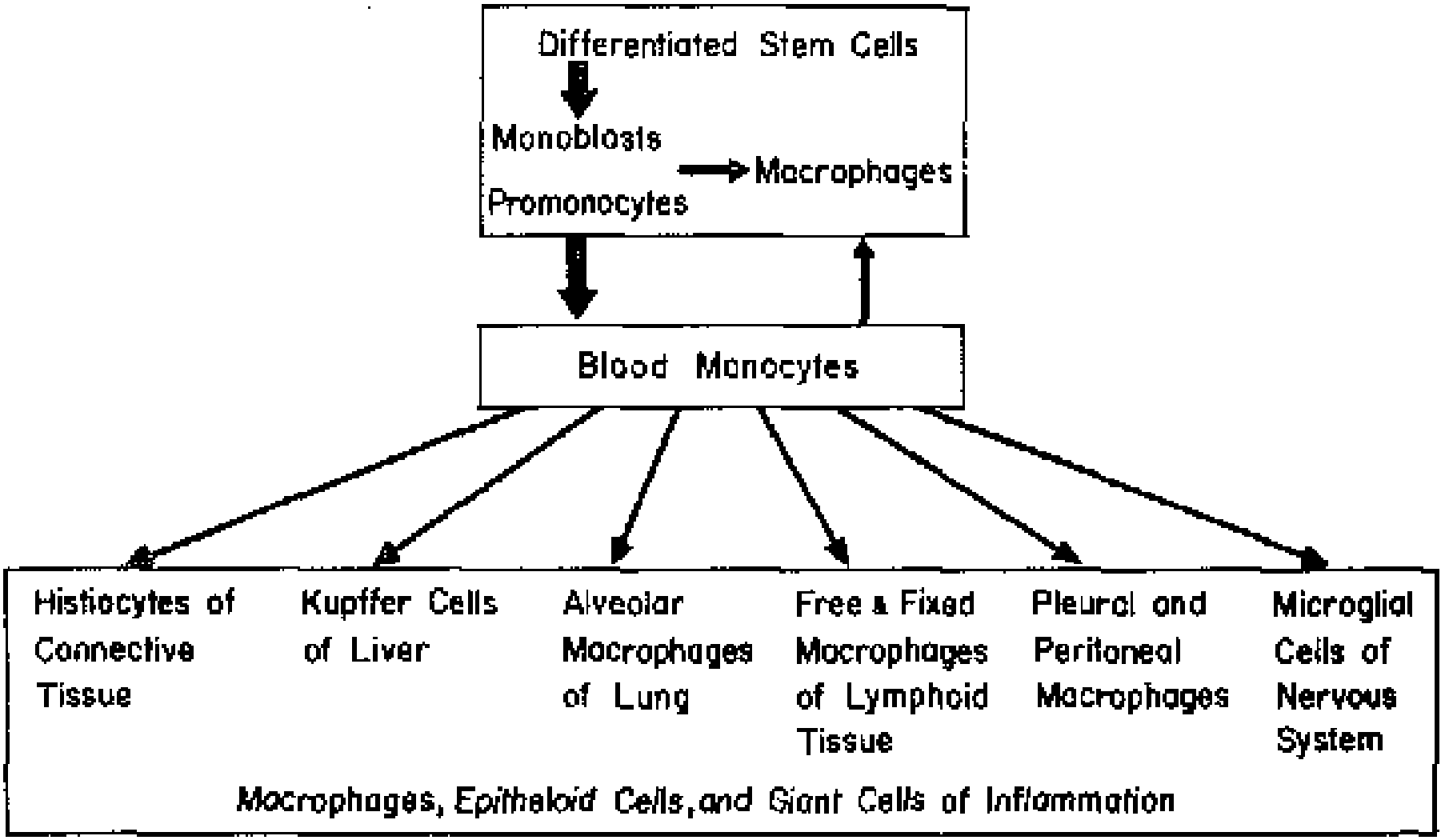


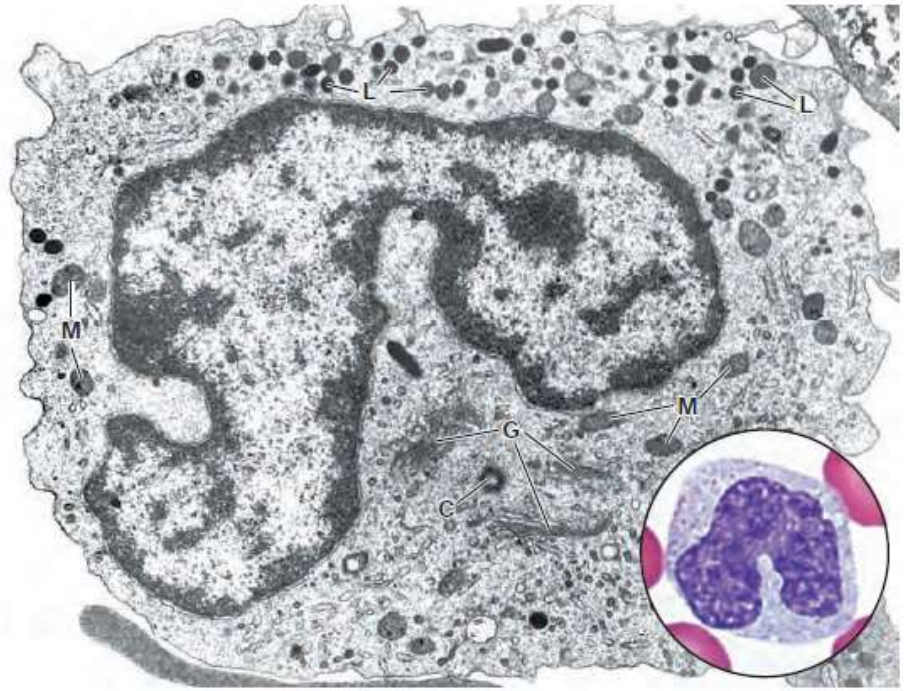
- They are the precursors cells of the **mononuclear phagocytotic system**
- They are the largest sized leukocytes in blood smear
- 18 µm
- After it is produced in the bone marrow, it remains in the circulation for 1-3 days and transits into the tissues, where it turns into cells responsible for phagocytosis: Mononuclear phagocytotic system:
  - Macrophages
  - Osteoclasts
  - Alveolar macrophage
  - Kupffer cells

Macrophages function as antigen-presenting cells in the immune system

professional antigen-presenting cells are **dendritic cells, macrophages and B cells**

mononuclear phagocytotic system

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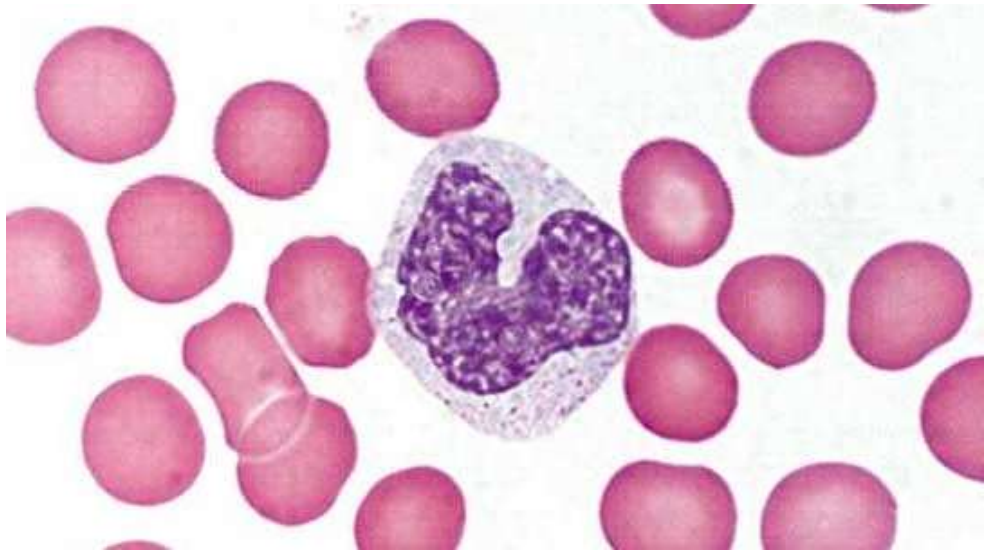
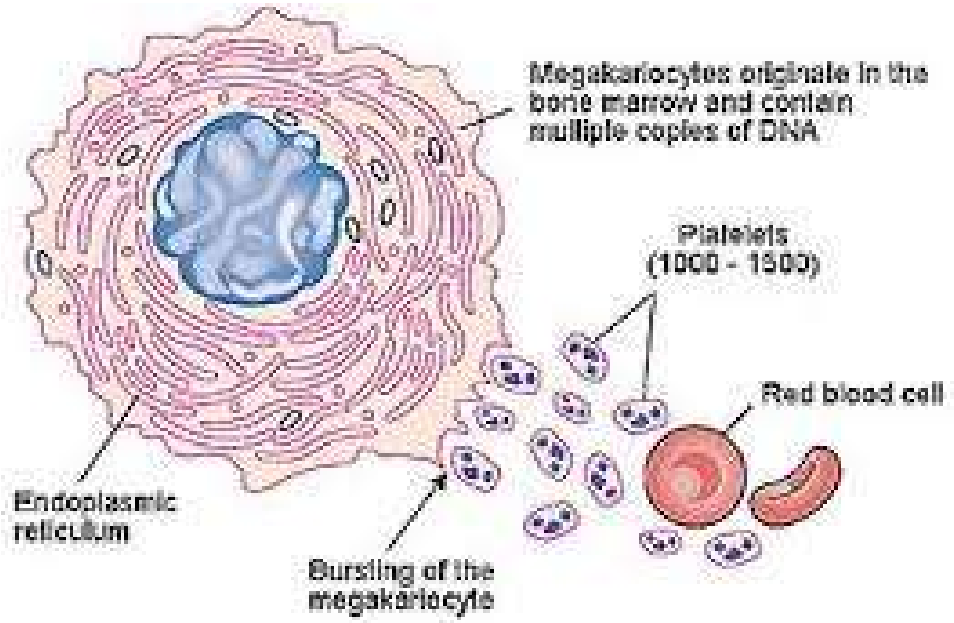
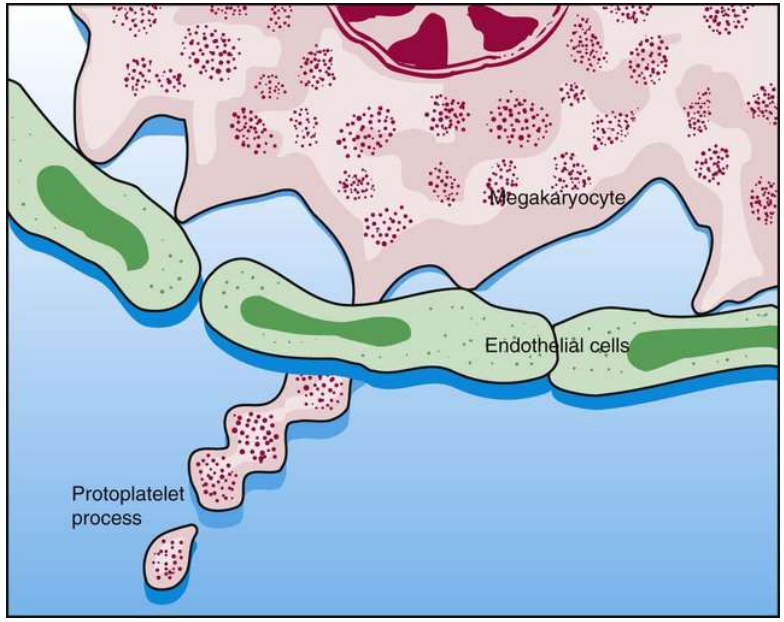


Thrombocytes = Platelets

- **Thrombocytes are small, membrane-bounded, anucleate cytoplasmic fragments derived from megakaryocytes**
- 150.000-400.000/mm^3 (2-4 µm sized)
- They are colorless, oval or biconvex disc shaped structures. Life expectancy in circulation is 8-10 days
- Platelets are formed under the control of thrombopoietin produced in liver and kidneys

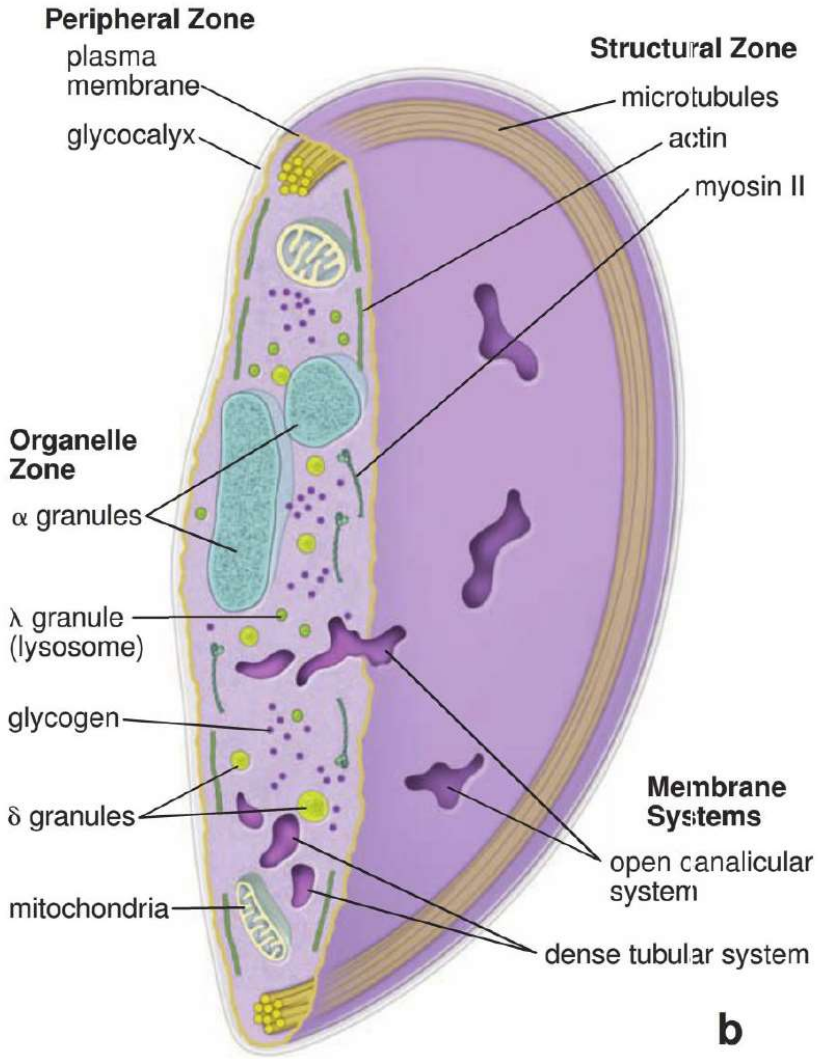


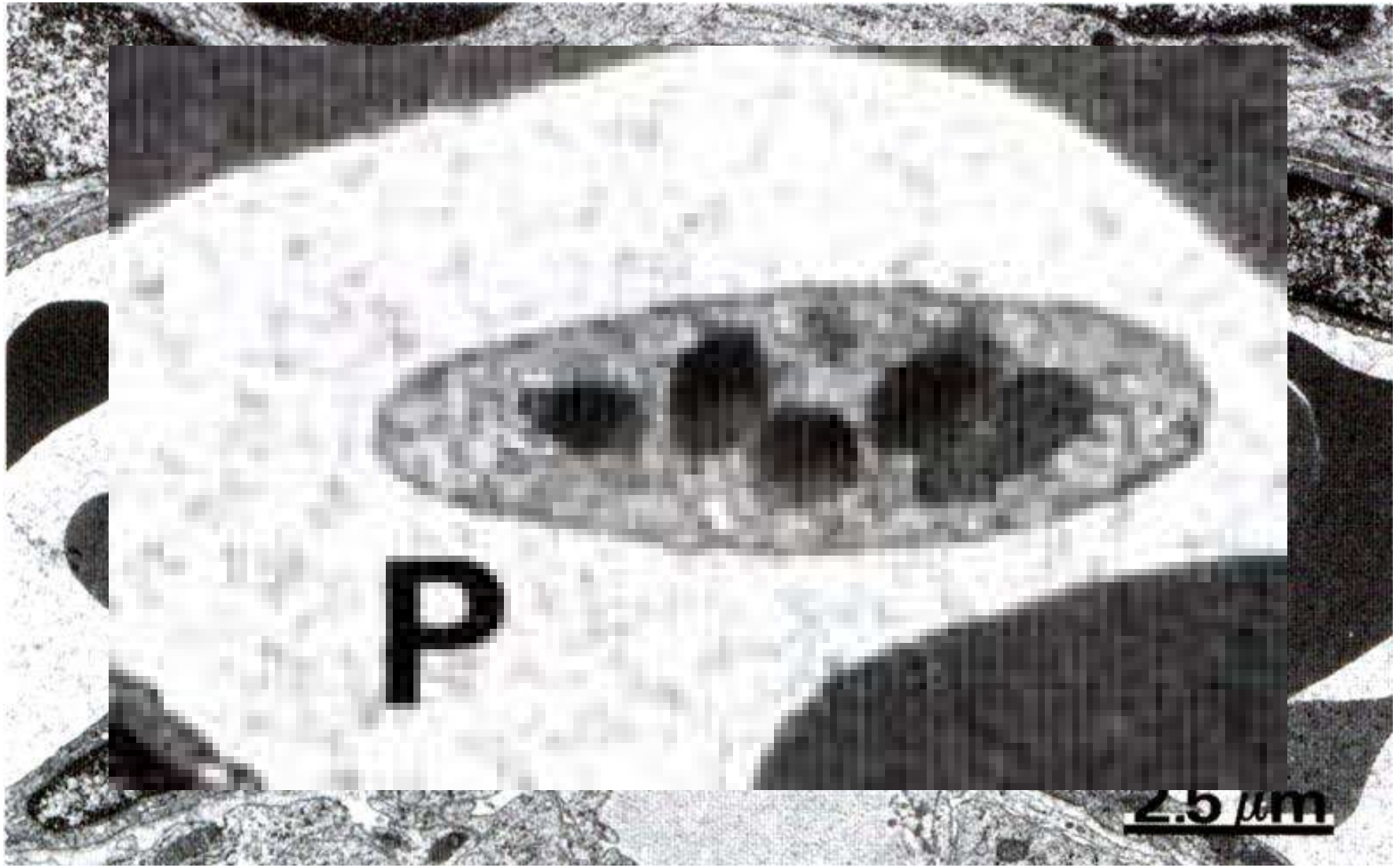


Formation: small bits of cytoplasm are separated from the peripheral regions of the megakaryocyte by extensive **platelet demarcation channels.** The breakdown of the megakaryocyte cytoplasm in the bone marrow and the surrounding of these parts by the membrane, cytoplasmic fragments form individual platelets

Platelet Morphology in 4 Zones

1. **Peripheral zone**
   - cell membrane covered by a thick surface coat of glycocalyx (=glycoproteins, GAGs, coagulation factors)
2. **Structural zone**
   - microtubules, actin filaments, myosin, and actin-binding proteins that form a network supporting the plasma membrane
3. **Organelle zone**
   - mitochondria, peroxisomes, glycogen particles, and at least three types of granules
4. **Membrane zone**
   - two types of membrane channels:
     - open canalicular system (OCS)
     - dense tubular system (DTS)
       - sER –Ca storage

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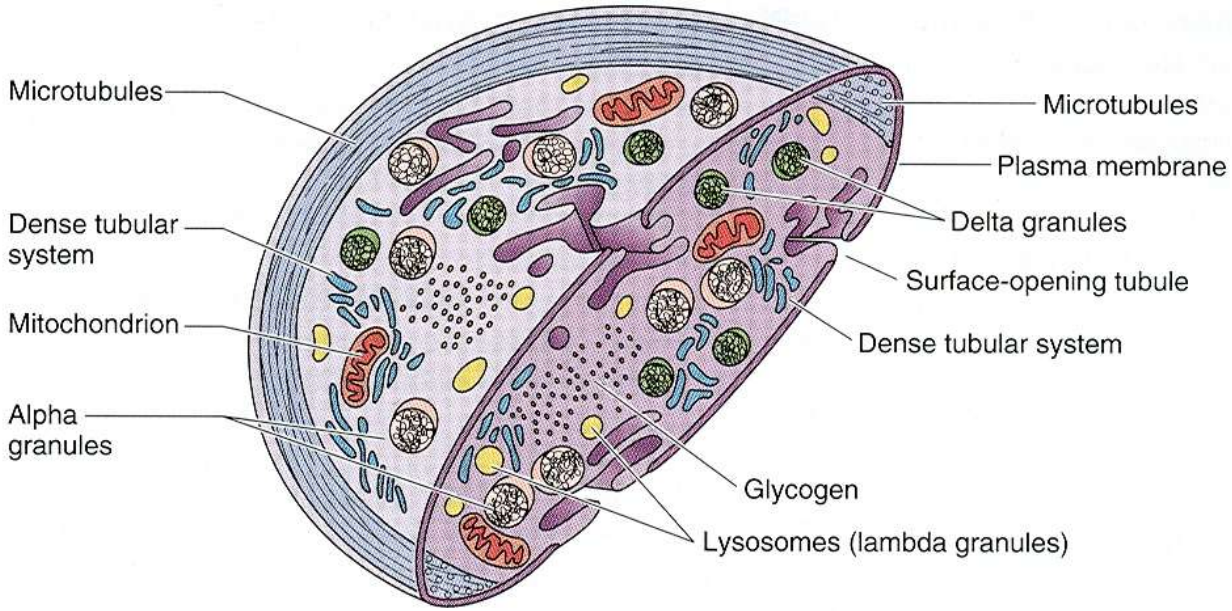
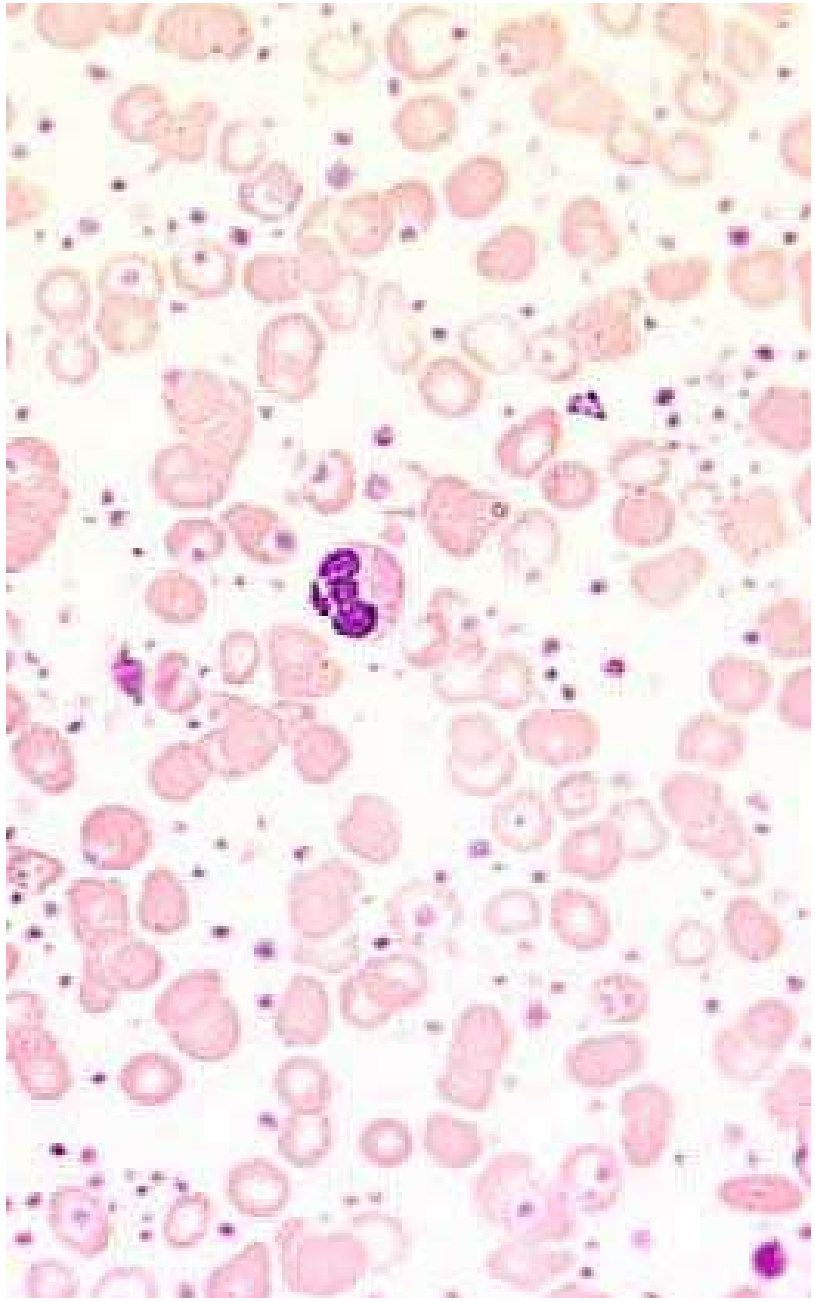
2.5 μm

Organelle Zone

- The purple colored central part where the granules settle. There are few mitochondria, glycogen particles and granules of different properties (alpha, delta and lambda granules).



Thrombocytosis



Thrombocytopenia