

A 3D illustration of a blood vessel cross-section. The vessel is shown in a reddish-brown color, with a textured interior. Inside the vessel, there are numerous red blood cells (erythrocytes) depicted as biconcave discs, and several white blood cells (leukocytes) shown as larger, spherical cells with distinct nuclei. The overall scene is set against a dark red background, suggesting the interior of a blood vessel.

BLOOD AND HEMATOPOIESIS

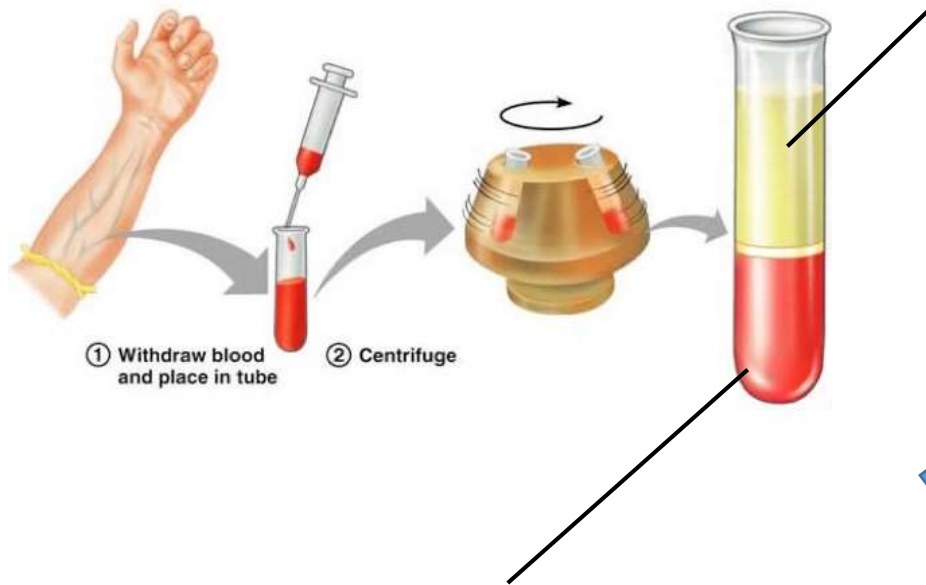
Petr Vaňhara

Department of Histology and Embryology
LF MU

BLOOD

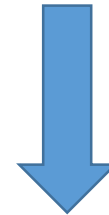
Blood is body fluid

- transport medium (O₂, CO₂, metabolites, hormones, nutrients...)
- homeostasis of inner body environment (thermoregulation, acidobasic equilibrium, oncotic pressure)
- integrity of cardiovascular system (clotting cascade)
- immune reactions



plasma

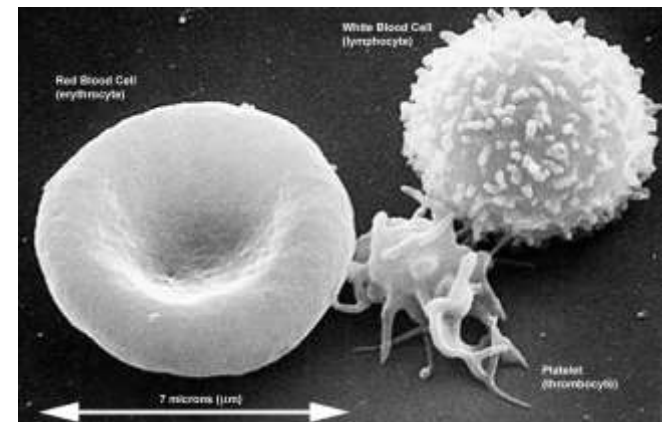
- ions, proteins, low mass organic compounds
- fluid ECM



Blood can be considered as a specialized connective tissue

formed blood elements

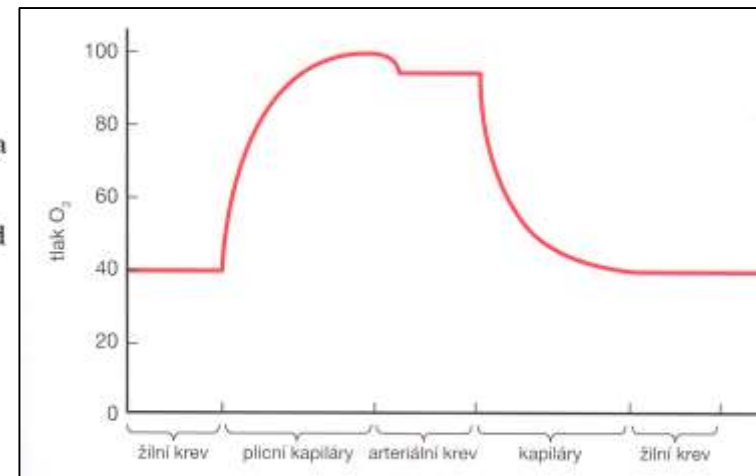
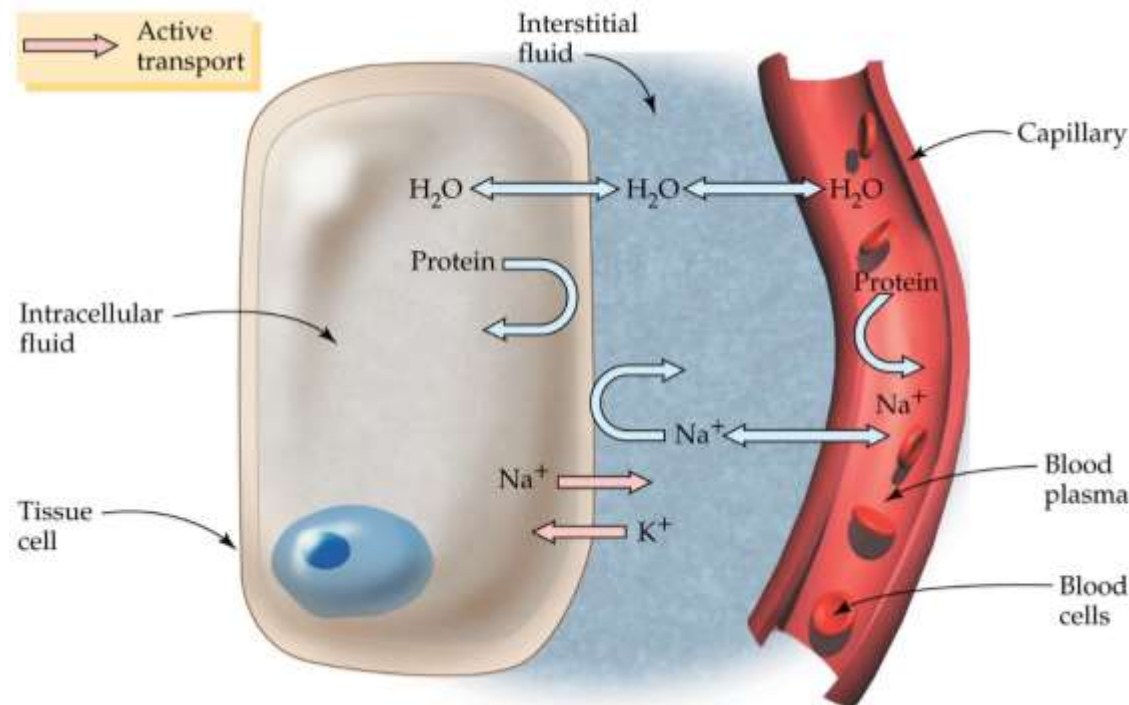
- erythrocytes
- leukocytes
- thrombocytes



BLOOD PLASMA AND TISSUE FLUID

plasma

- 2,8-3,5 l
- pH 7.4 (\pm 0.05)
- ~ 92% **water**
- ~ 1% **ions** (Na^+ , K^+ , Ca^{2+} , Mg^{2+} , Cl^- , HCO_3^-), **low mass organic compounds** (glucose, aminoacids, cholesterol, lipids, waste products of metabolism), **respiration gases**
- ~ 7% **proteins** (albumins, globulins, fibrinogen)



IONS AND LOW MASS MOLECULES OF BLOOD PLASMA (~1%)

- ~ 1% **ions** (Na^+ , K^+ , Ca^{2+} , Mg^{2+} , Cl^- , HCO_3^-), **low mass organic compounds** (glucose, aminoacids, cholesterol, lipids, waste products of metabolism), **respiration gases**

	Sodium	136–148 mmol/l	Osmotic pressure, volume, pH
	Potassium	3,7–5,0 mmol/l	Membrane potential of cells (nerve, muscle)
Cations	Calcium	2,15–2,61 mmol/l	Permeability of membranes, blood clotting, neuromuscular junctions
	Magnesium	0,66–0,94 mmol/l	Cofactor of enzymes, neuronal conduction
	Iron ♂	12–27 $\mu\text{mol/l}$	Cofactor of enzymes, in hem of hemoglobin
	Iron ♀	10–24 $\mu\text{mol/l}$	
	Copper	12–22 $\mu\text{mol/l}$	Cofactor of enzymes
	Chlorides	95–110 mmol/l	Osmotic pressure, volume, pH
Anions	Bicarbonates $[\text{HCO}_3]^-$	22–26 mmol/l	Transport of CO_2 , buffer - pH
	P_i	0,6–1,4 mmol/l	Buffer - pH
	Iodide	276–630 $\mu\text{mol/l}$	Hormones of thyroid gland

IONS AND LOW MASS MOLECULES OF BLOOD PLASMA (~1%)

- ~ 1% **ions** (Na^+ , K^+ , Ca^+ , Mg^+ , Cl^- , HCO_3^-), **low mass organic compounds** (glucose, aminoacids, cholesterol, lipids, waste products of metabolism), **respiration gases**

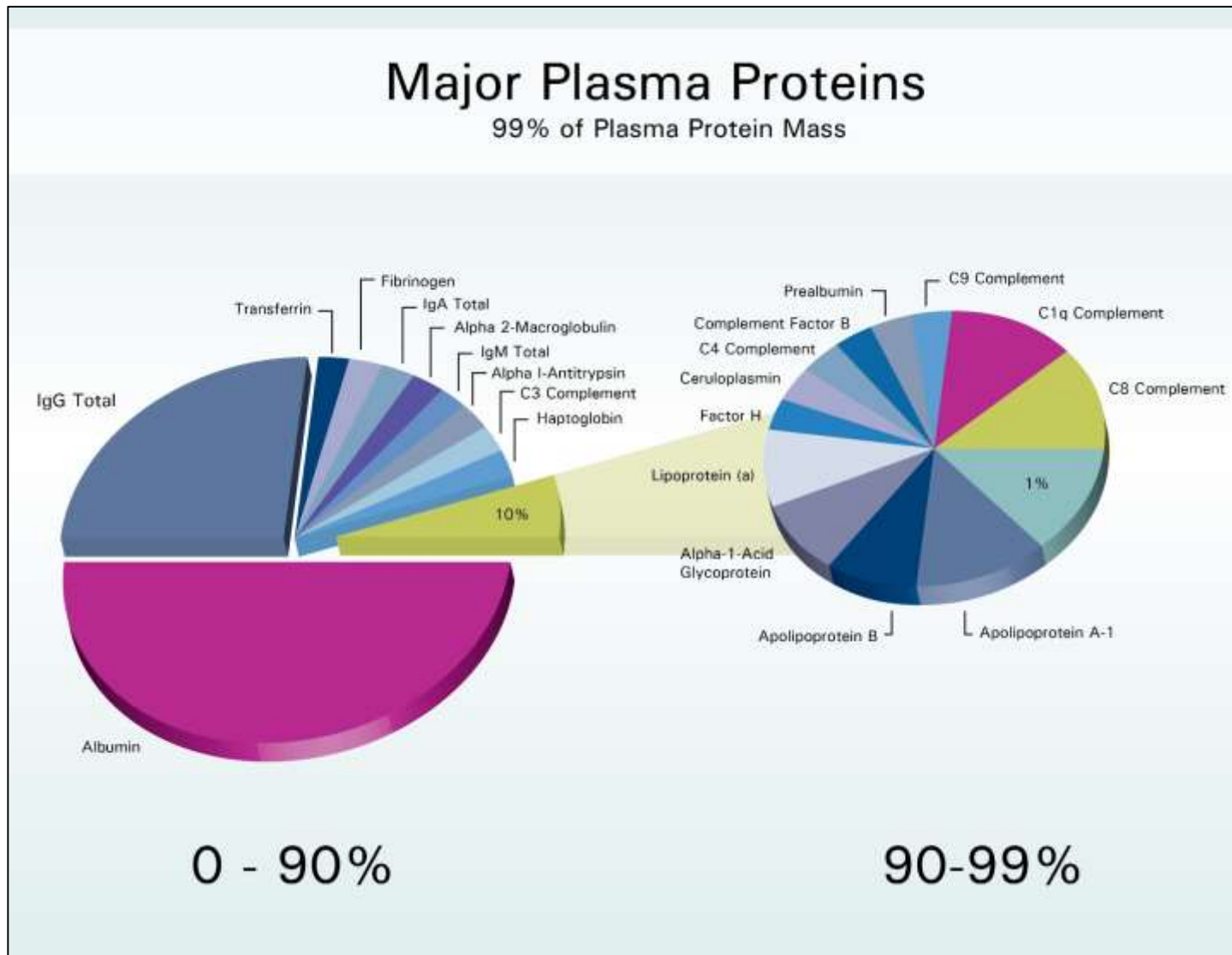
Glucose	3,3–6,1 mmol/l
Aminoacids	2,3–3,9 mmol/l
Urea	3,0–7,6 mmol/l
Lipids	4–9 g/l
Triacylglycerols	0,5–1,8 mmol/l
Phospholipids	1,8–2,5 g/l
Creatinine	55–110 $\mu\text{mol/l}$
Cholesterol (total)	3,5–5,2 mmol/l
Bilirubin	3,3–18,0 $\mu\text{mol/l}$
Lactate	0,55–2,22 mmol/l



COMPOSITION OF BLOOD PLASMA IS CONSTANT
regulated in narrow range → essential for clinical medicine

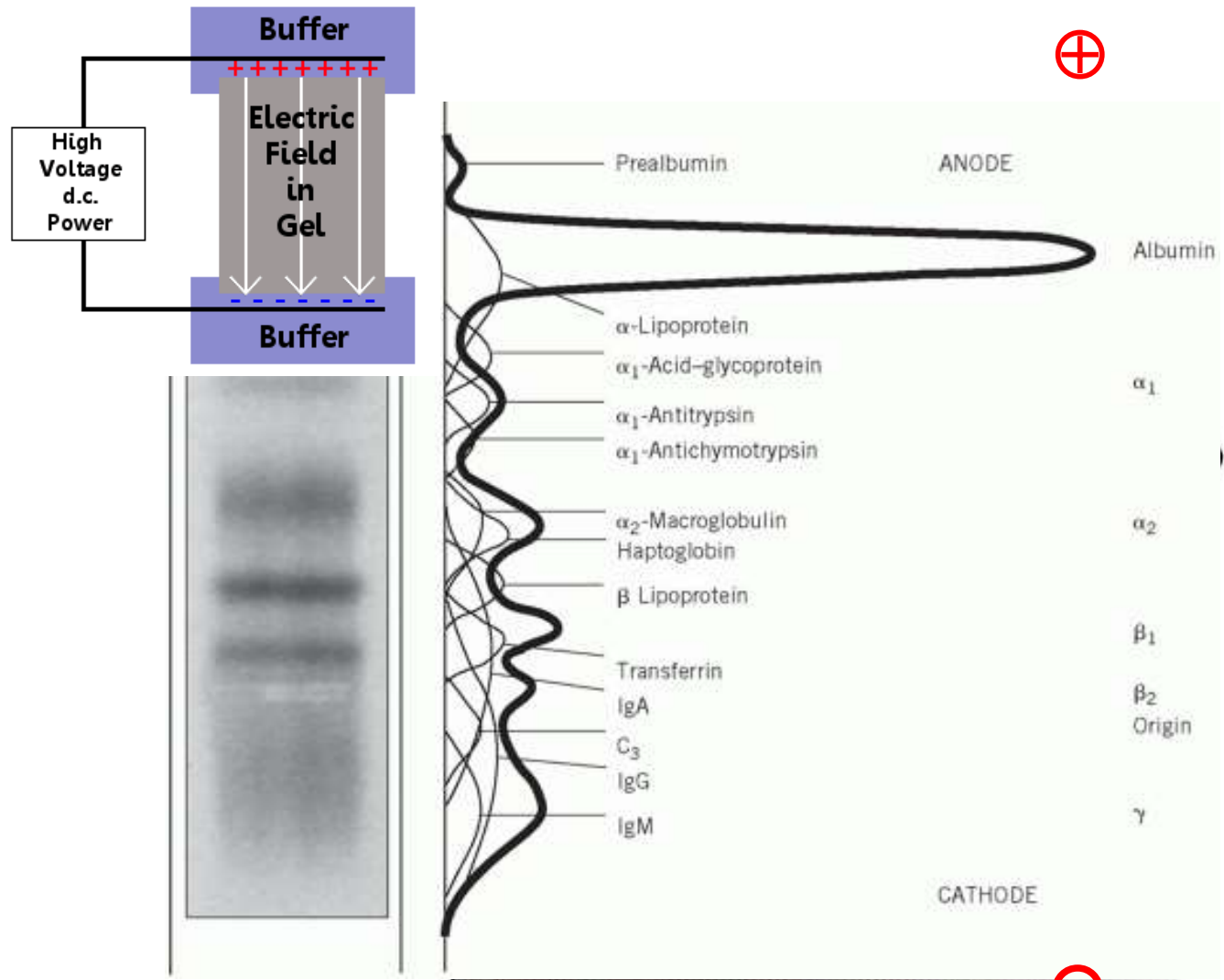
PROTEINS OF BLOOD PLASMA (7%)

- oncotic blood pressure
- transport
- coagulation
- immune response
- regulatory proteins



PROTEINY KREVŇÍ PLAZMY (7%)

- **prealbumin**
 - transport
- **albumin**
 - 68kDa
 - transport
 - osmotic pressure
- **α1 region**
 - α1 lipoprotein (HDL)
 - α1 acid glycoprotein
 - α1 antitrypsin
 - (α1 fetoprotein)
- **α2 region**
 - α2 macroglobulin
 - haptoglobin
- **β1 region**
 - transferrin
 - hemopexin
- **β2 region**
 - β lipoprotein (LDL)
 - C4 (complement)
- **β2 region**
 - CRP
 - fibrinogen
 - β2 microglobulin
 - C3 (complement)
- **γ region**
 - IgA, IgG, IgM

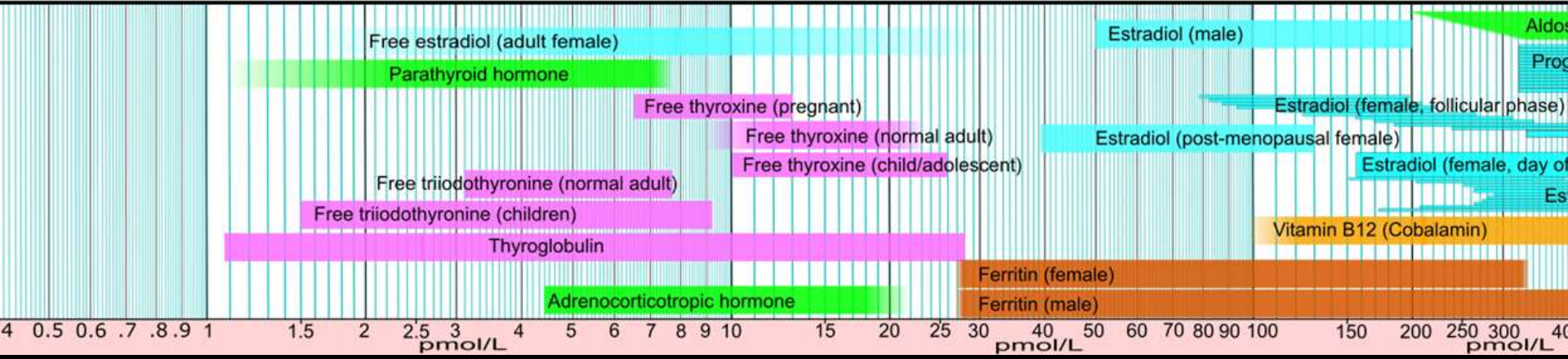
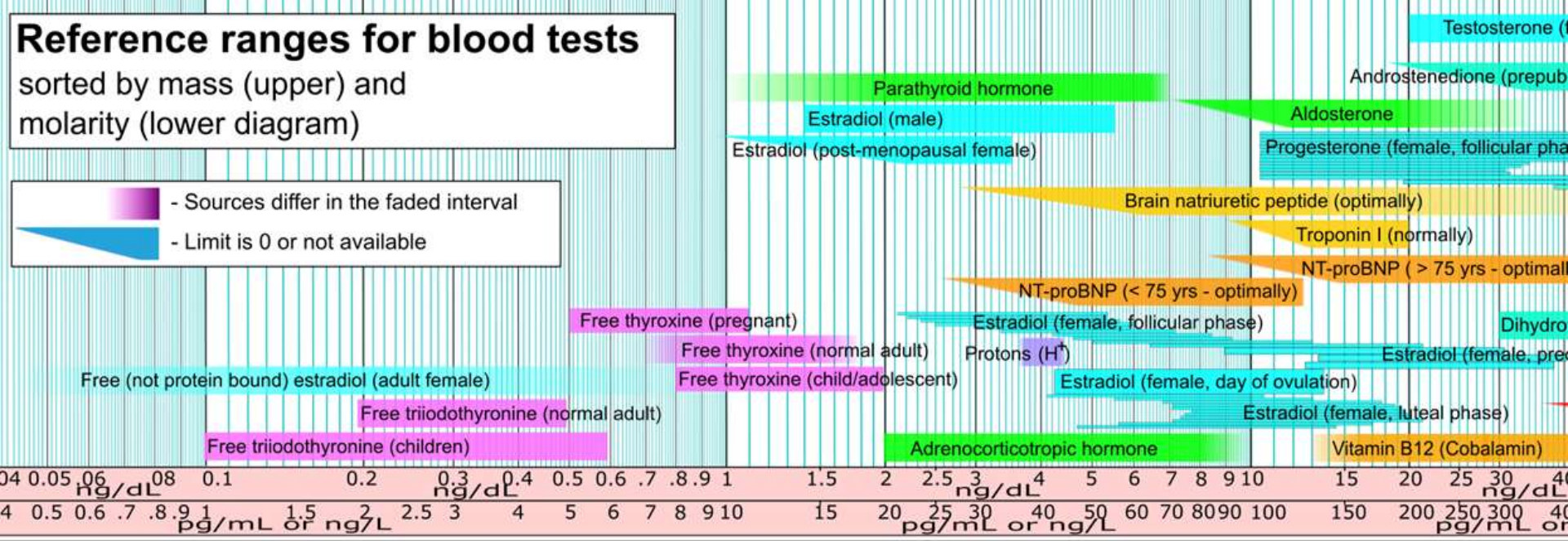


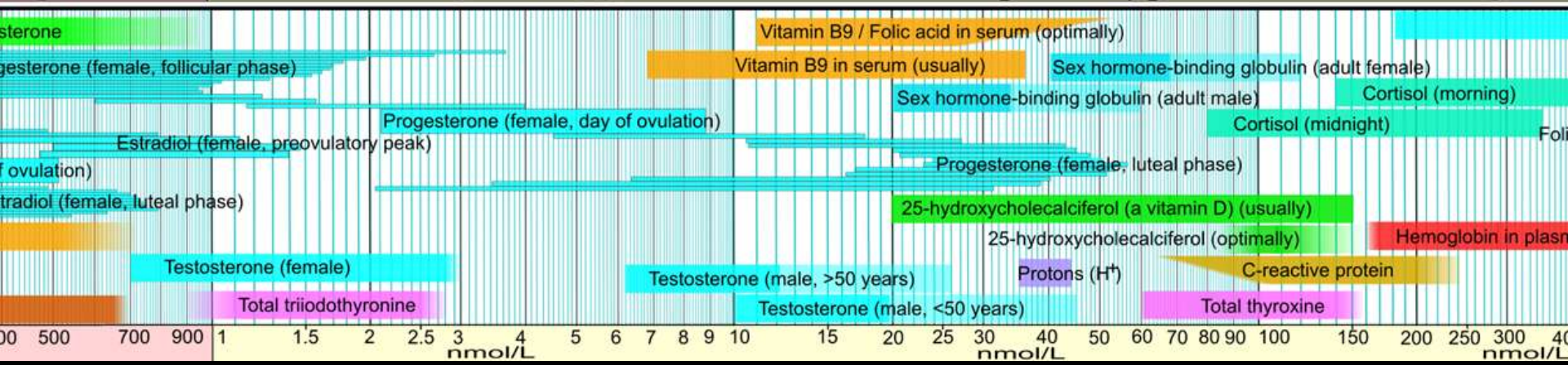
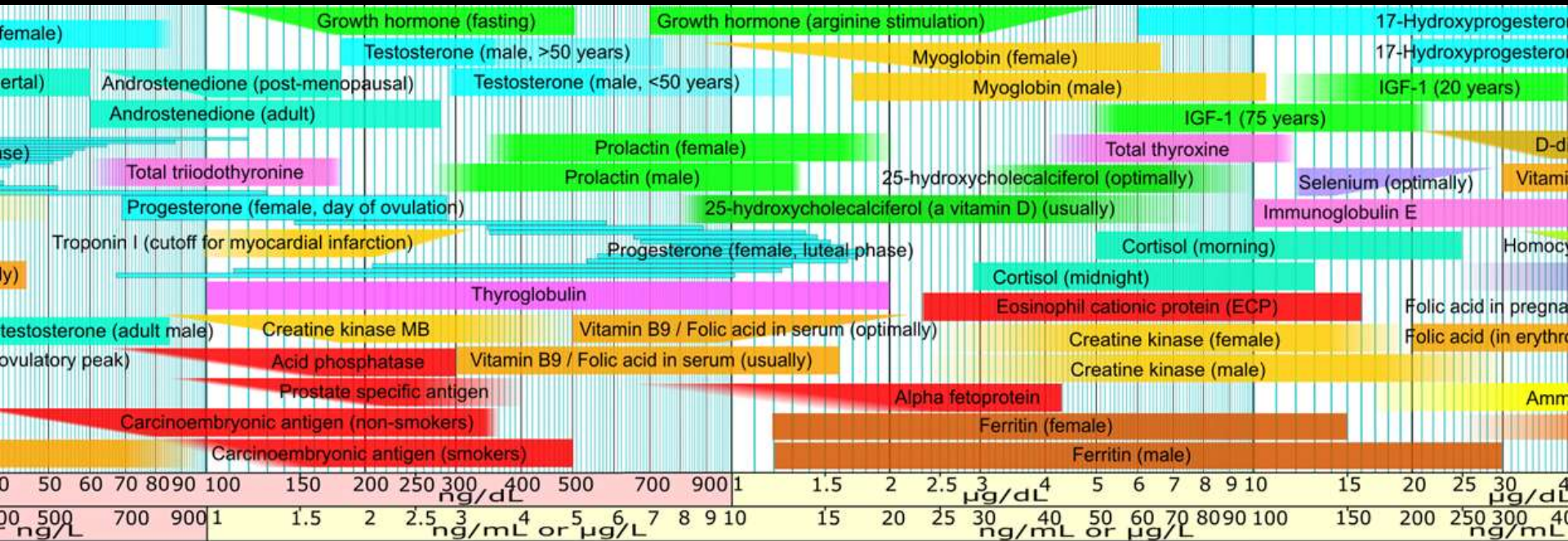
Electrophoretic separation of serum proteins
A. Electrophoretogram of normal serum on cellulose acetate strip
B. Densitometric scanning from cellulose acetate strip converts bands to characteristic peaks of albumin, α₁-globulin, α₂-globulin, β-globulin and γ-globulin

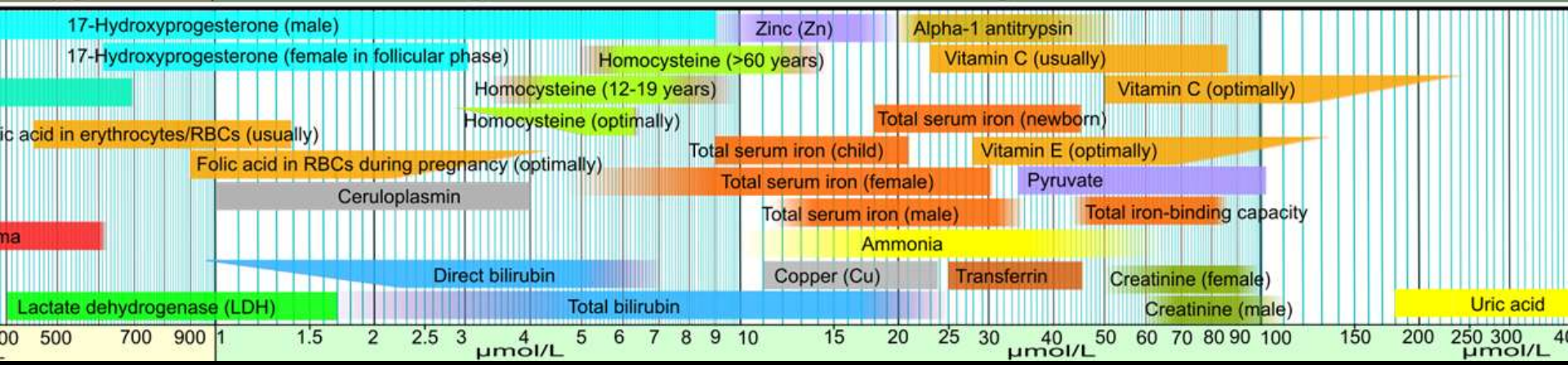
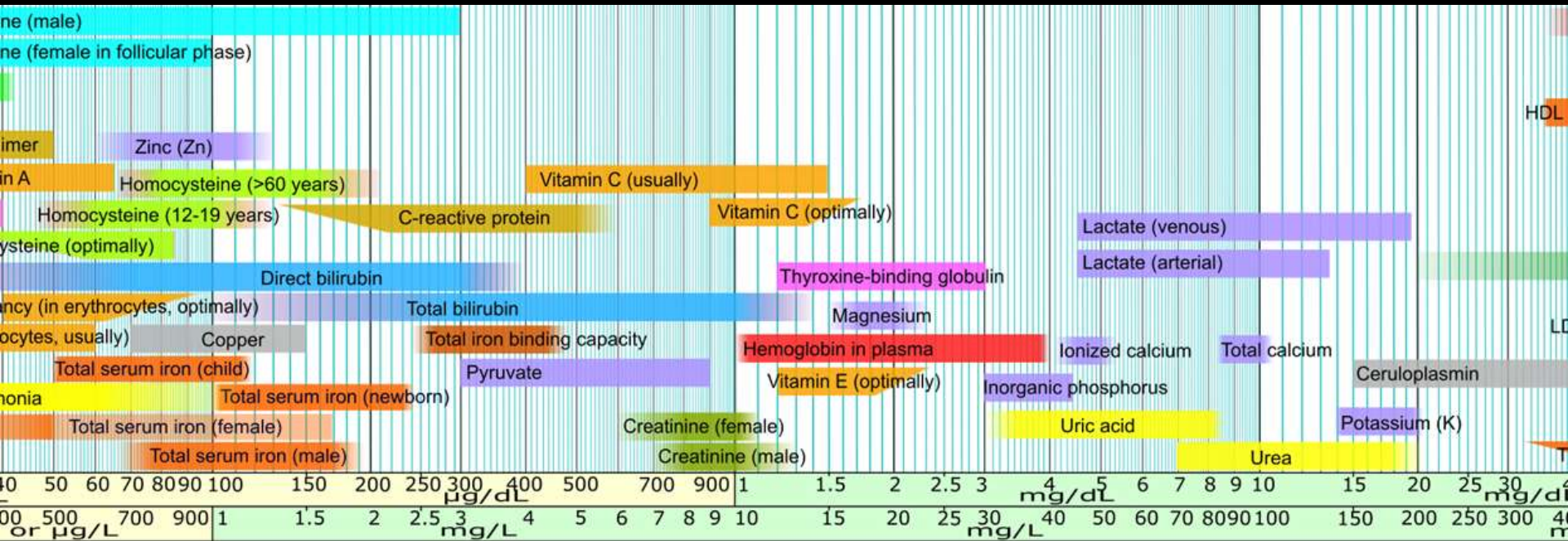
Reference ranges for blood tests

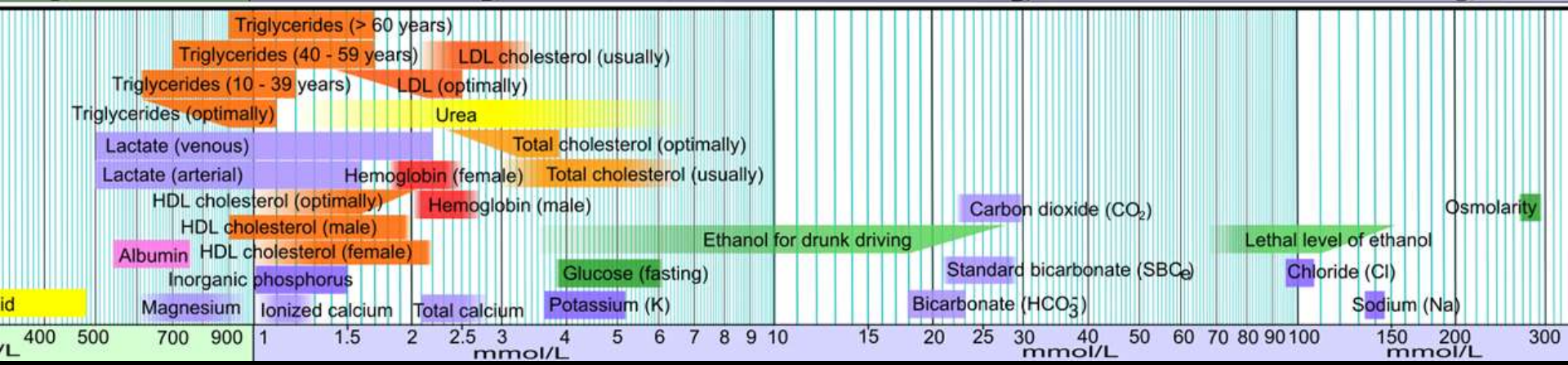
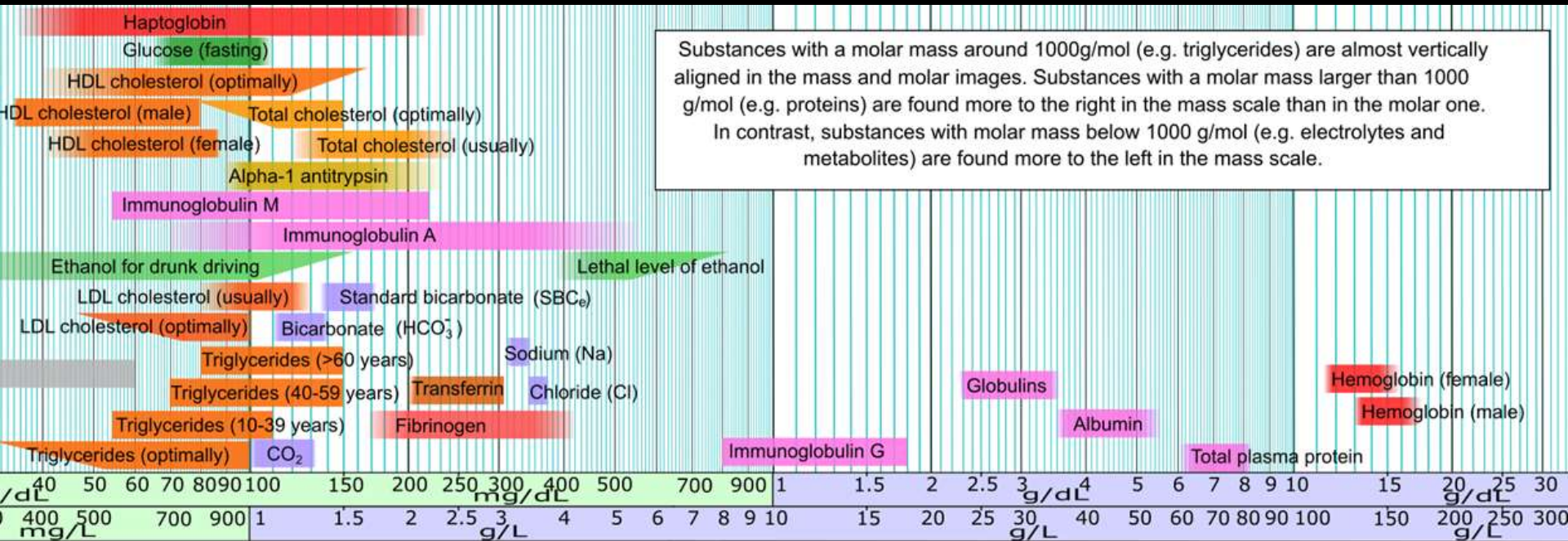
sorted by mass (upper) and molarity (lower diagram)

- Sources differ in the faded interval
- Limit is 0 or not available



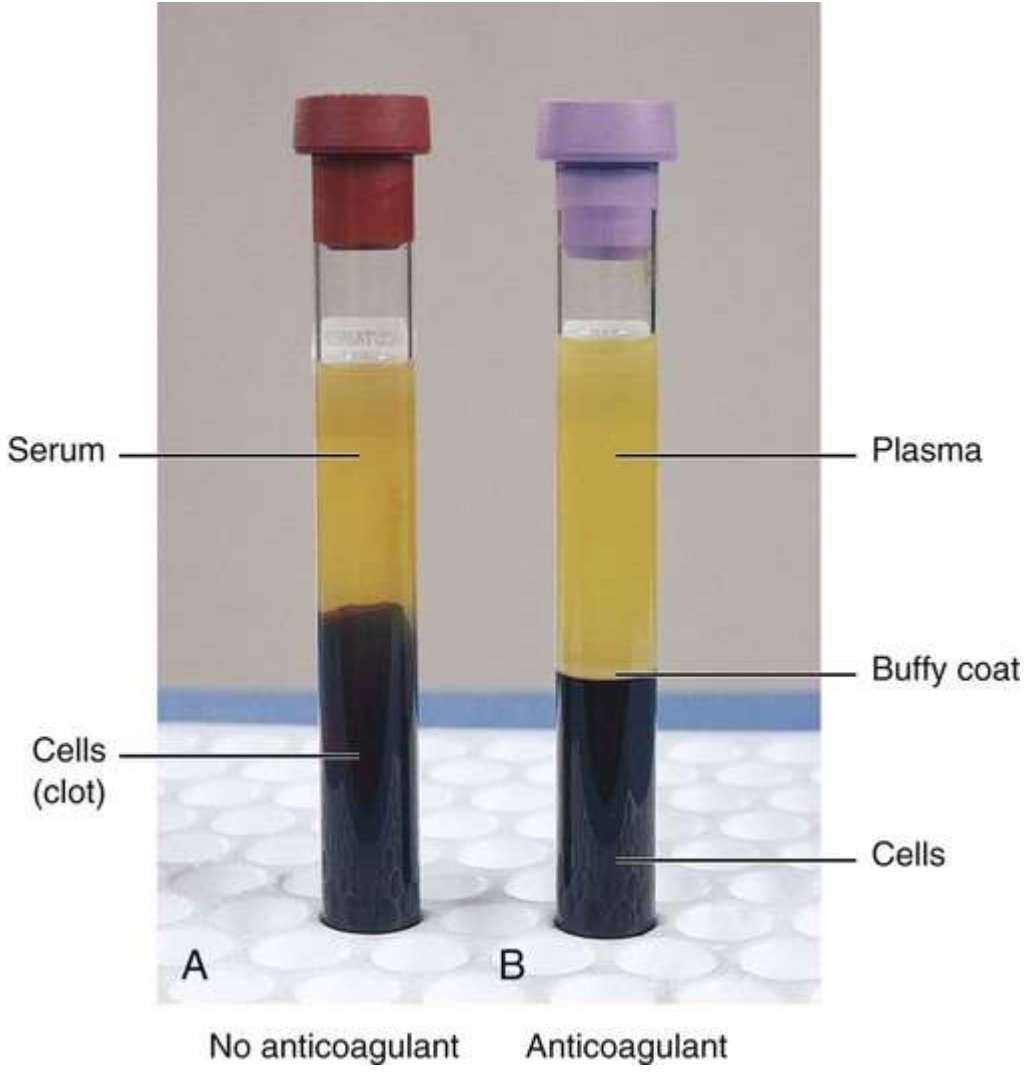




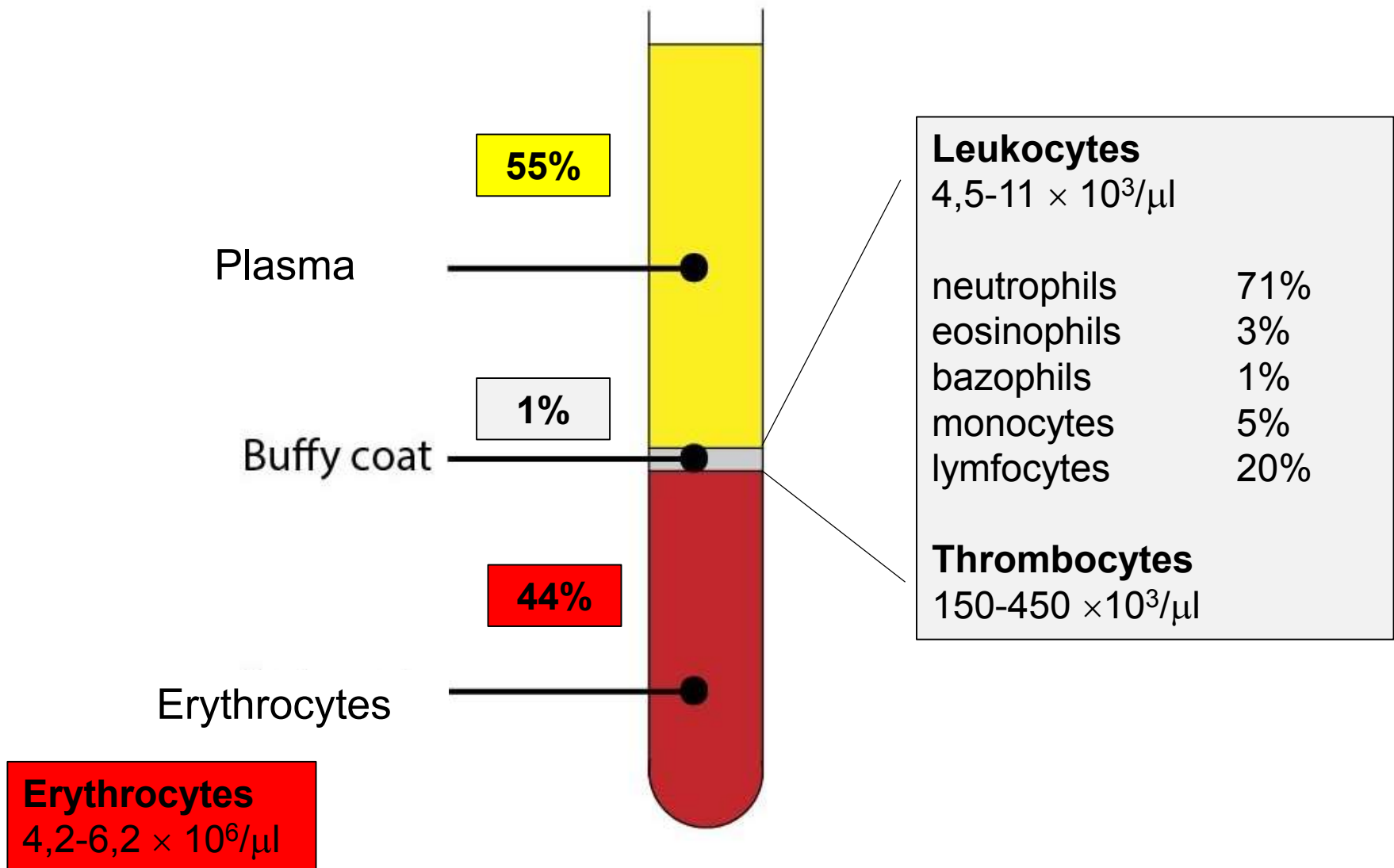


BLOOD PLASMA AND SERUM

- serum ≠ plasma



FORMED BLOOD ELEMENTS



HEMATOCRIT

Ratio of erythrocyte mass volume to volume of full blood

Erythrocytes
 $4,2-6,2 \times 10^6/\mu\text{l}$

HEMATOCRIT



47±5%

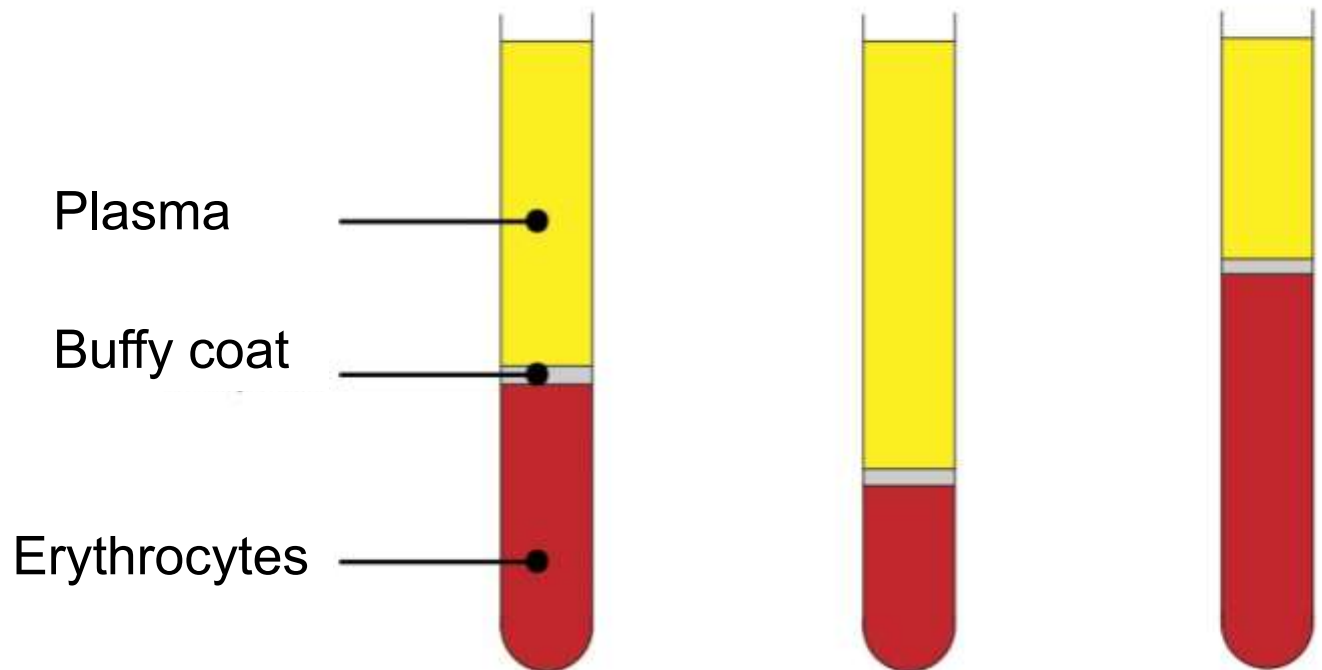


42±4%

Norm

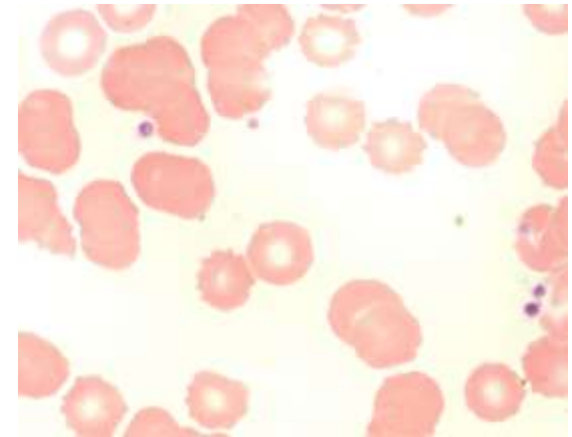
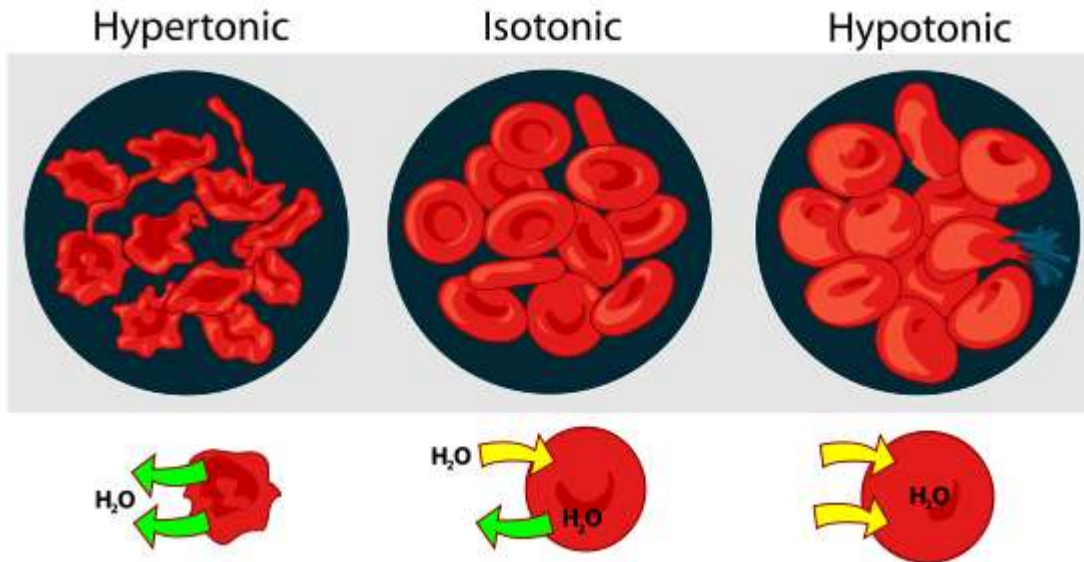
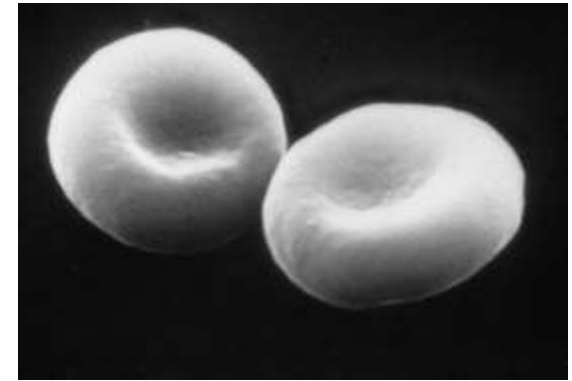
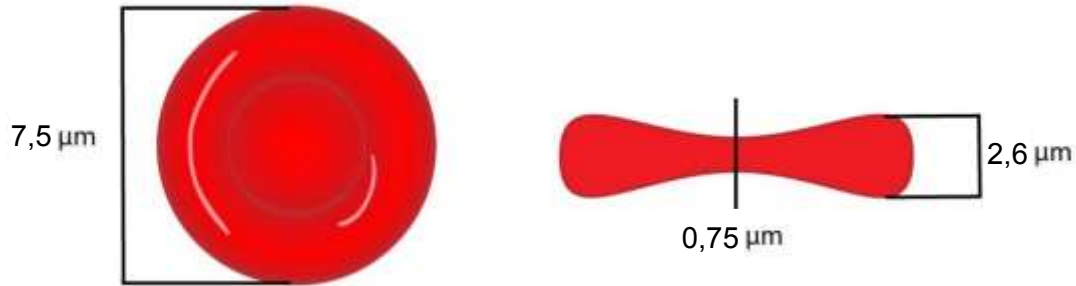
Anemia

Polycythemia



ERYTHROCYTES

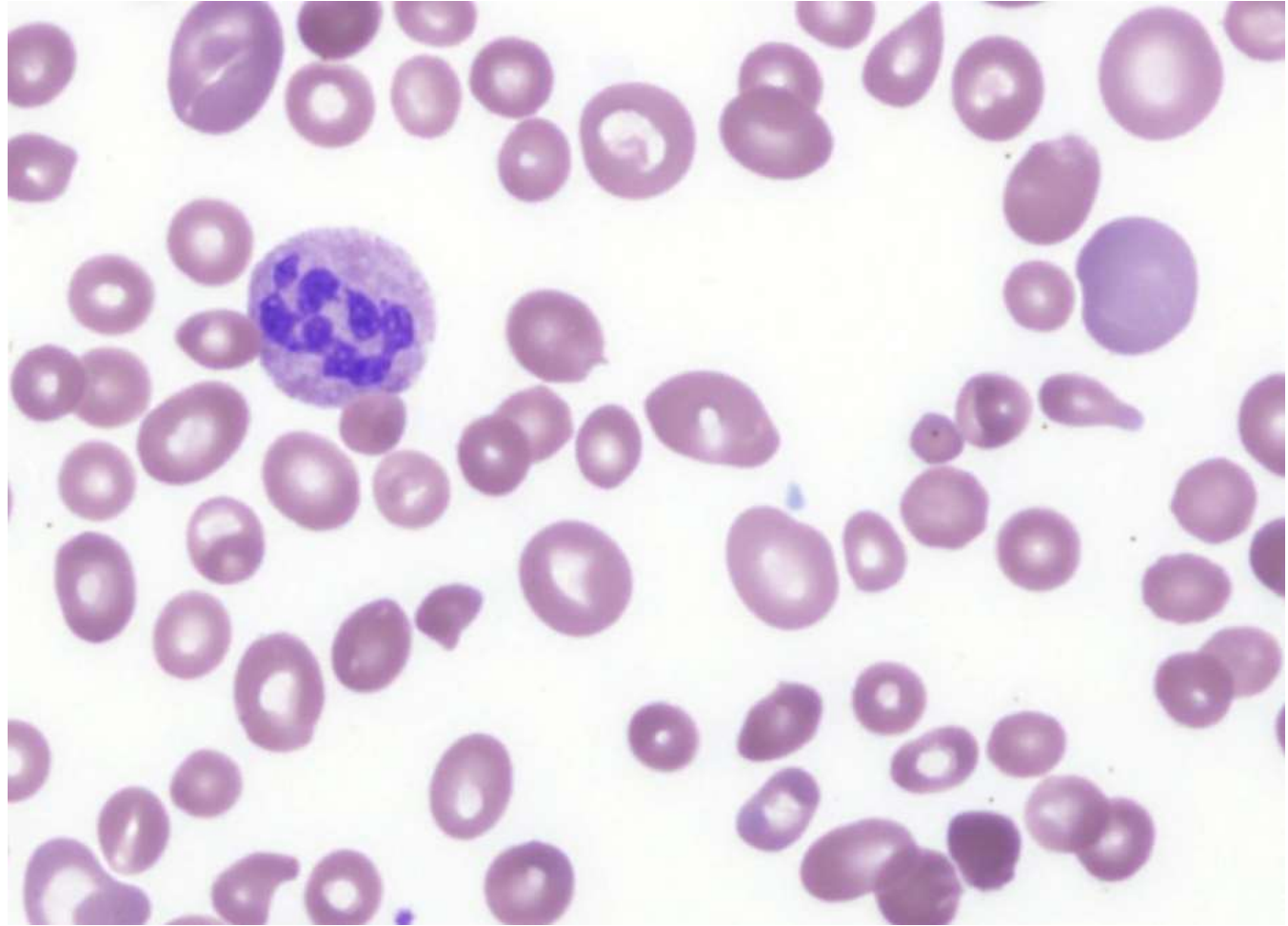
Size depends on osmotic pressure of environment



ERYTHROCYTES

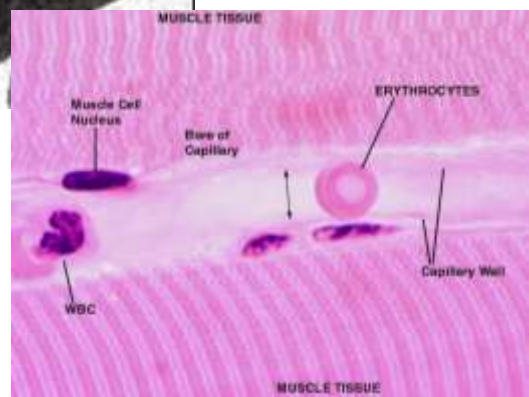
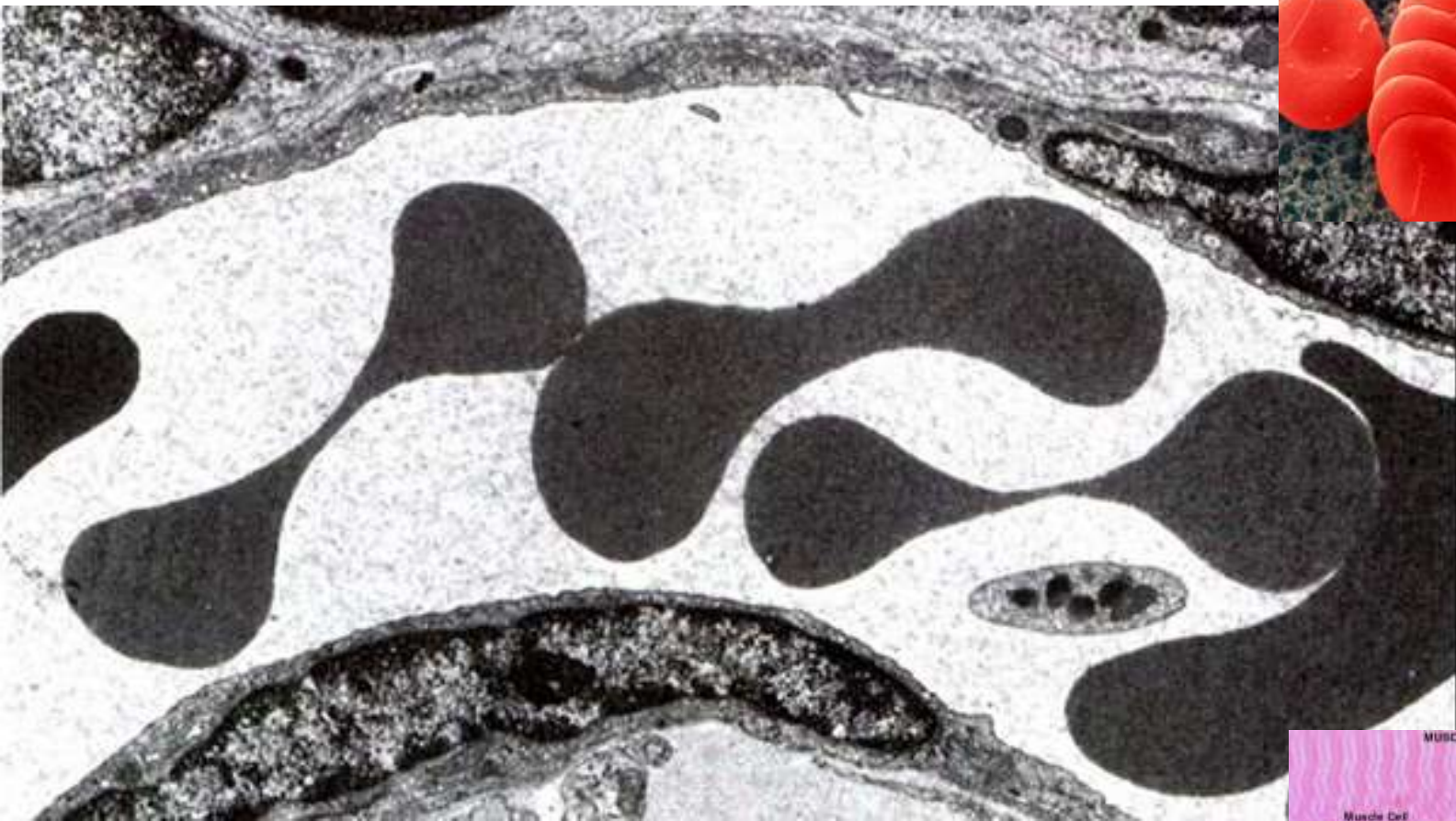
Deviations from normal size

- **anisocytosis**
 - macrocytes ($>9\ \mu\text{m}$)
 - microcytes ($<6\ \mu\text{m}$)



ERYTHROCYTES

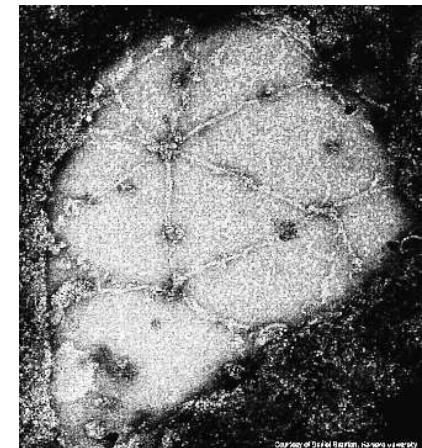
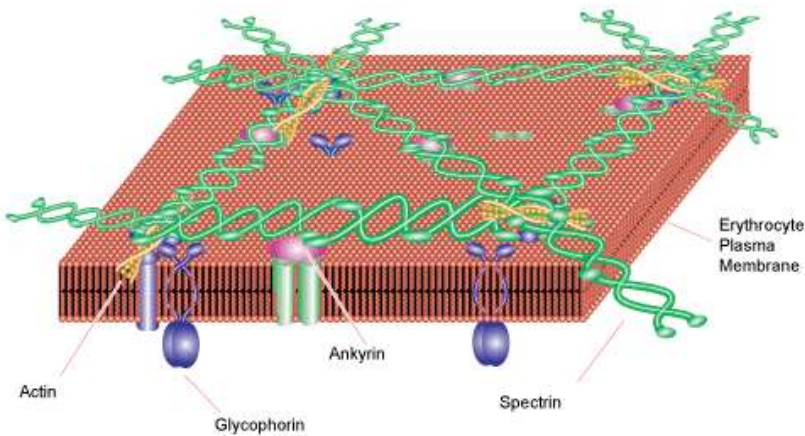
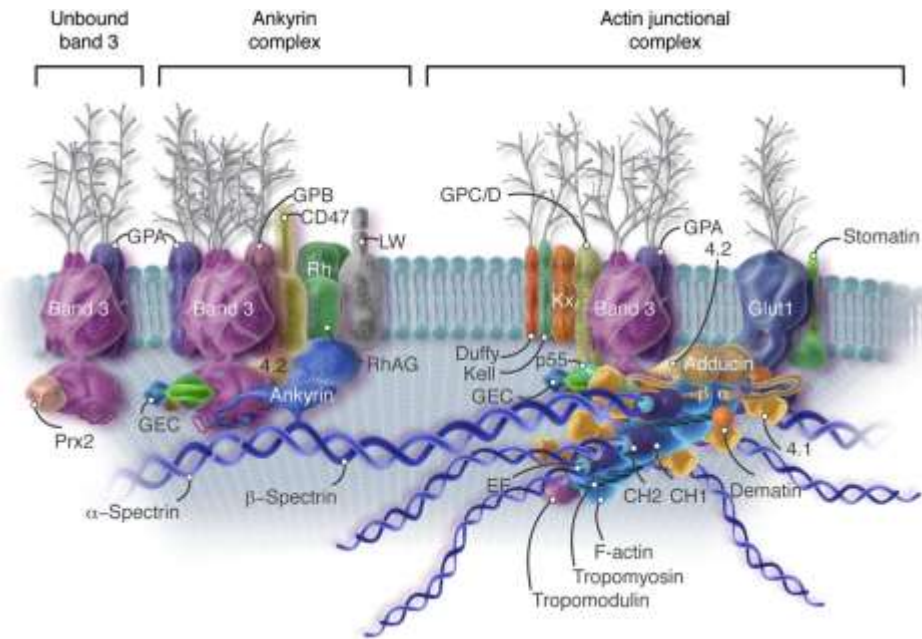
Erythrocyte is amazingly flexible cell



ERYTHROCYTES

Shape of erythrocytes

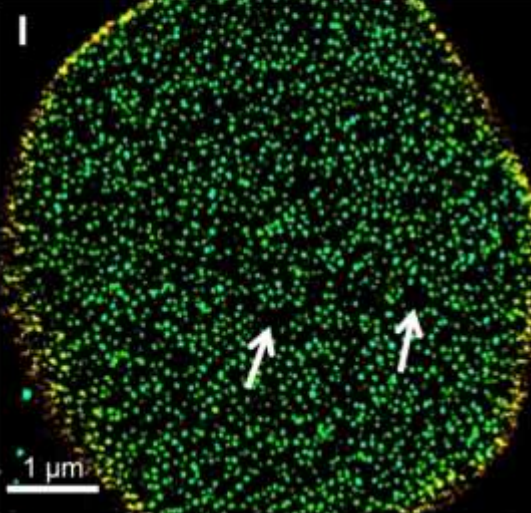
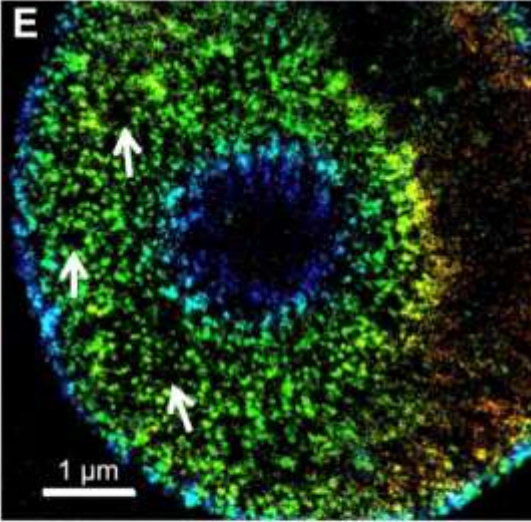
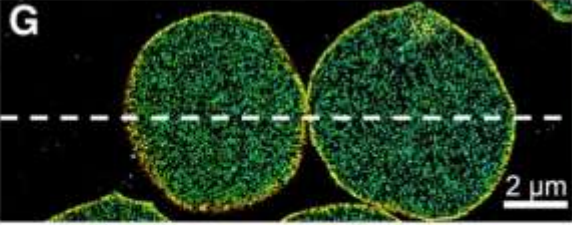
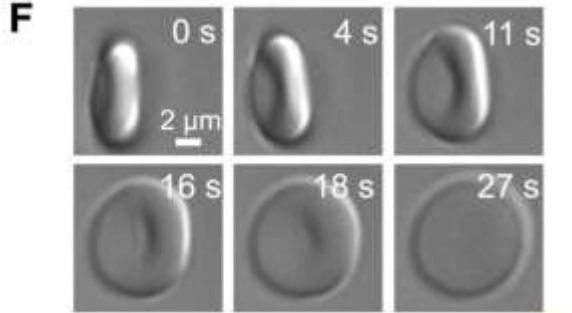
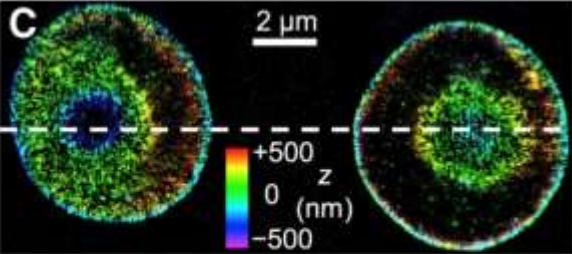
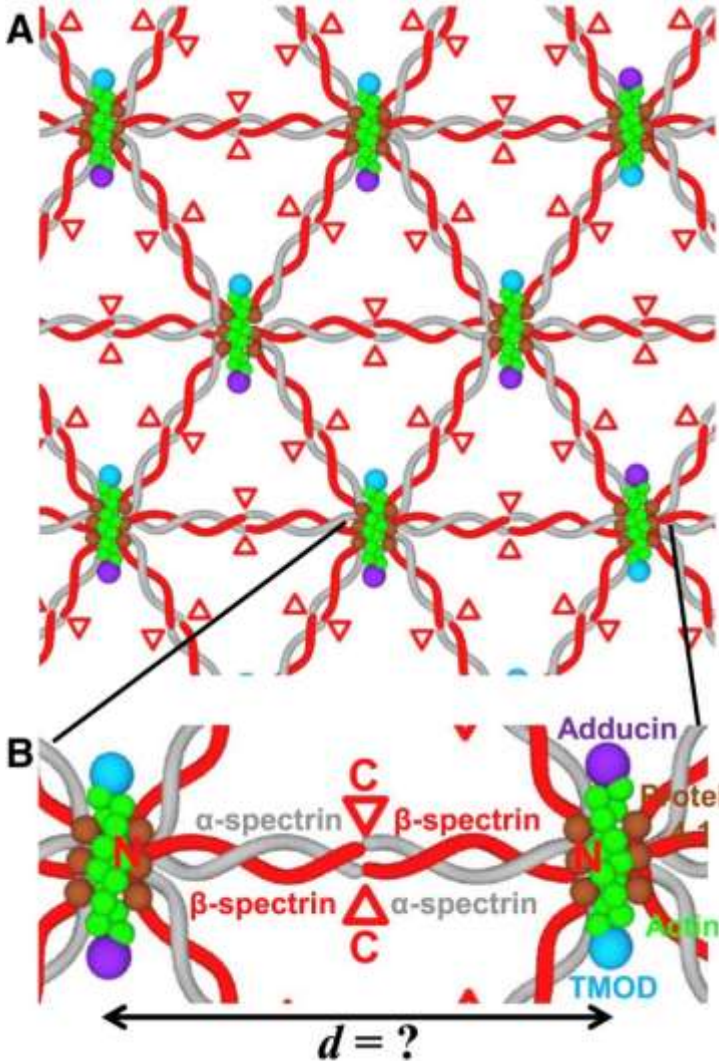
- **integral proteins**
 - band 3, glycoprotein A (ion transporters)
- **spectrin**
- **ankyrin**
- **aktin a s aktinem asociované proteiny**
 - tropomodulin, tropomyosin
- **hemoglobin**



ERYTHROCYTES

Super-Resolution Microscopy for the Cytoskeleton of Membrane-Preserved Erythrocytes

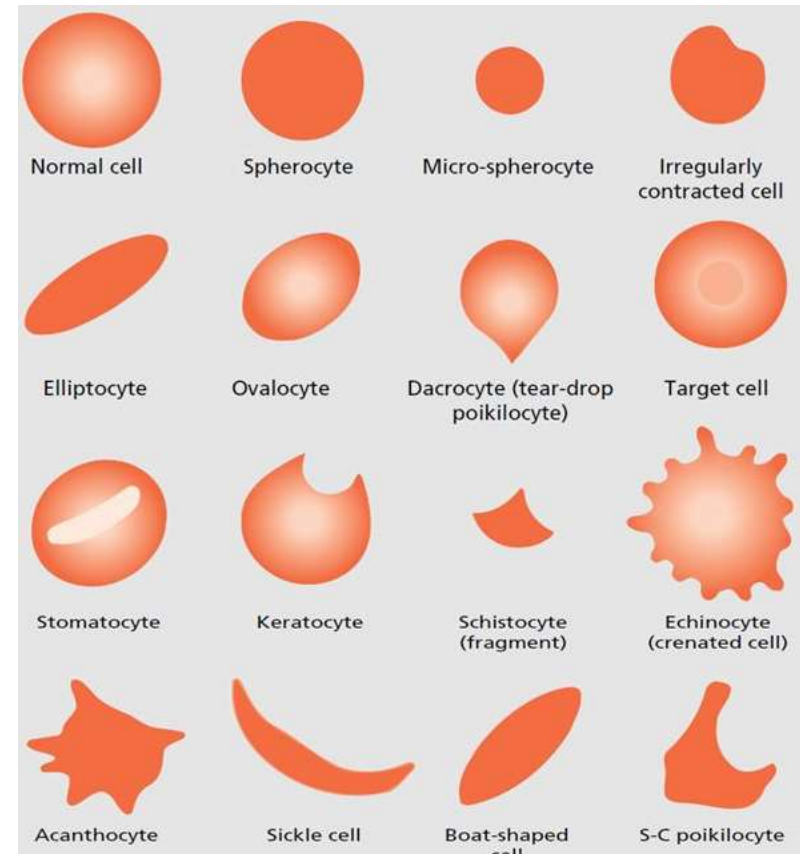
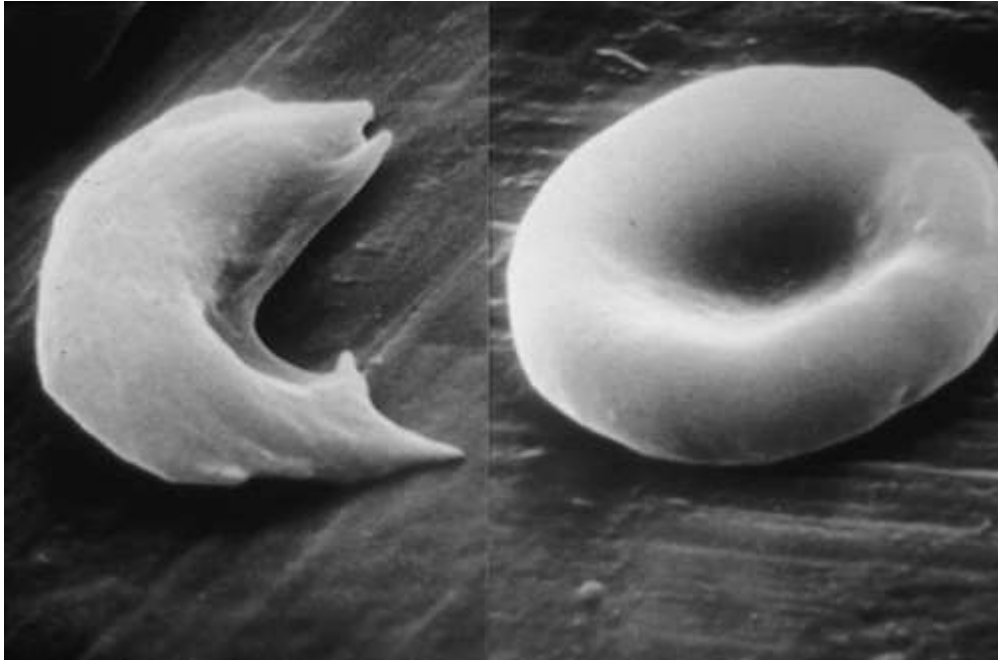
doi: 10.1016/j.celrep.2017.12.107.



ERYTHROCYTES

Deviations from biconcave shape

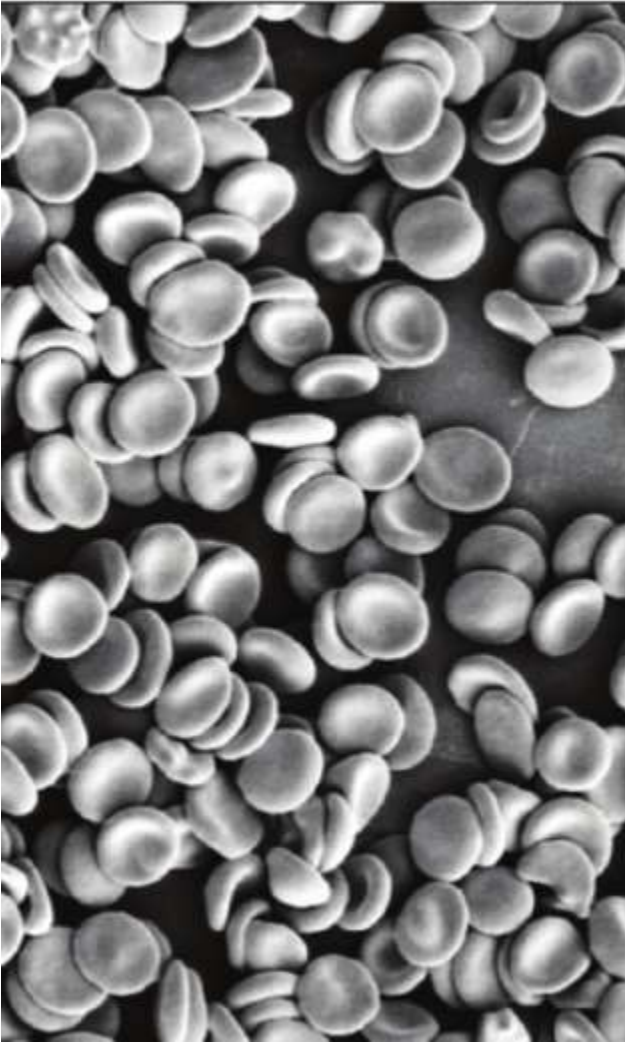
- **poikilocytosis**
 - **acanthocytes** (irregular spikes)
 - **codocytes** („tyre “)
 - **echinocytes** (spiked membrane)
 - **eliptocytes** (elliptic)
 - **spherocytes** (spheroidal)
 - **stomatocytes** (some parts missing or other irregularities)
 - **drepanocytes** (sickle)
 - **dacrocytes** (tear drop)



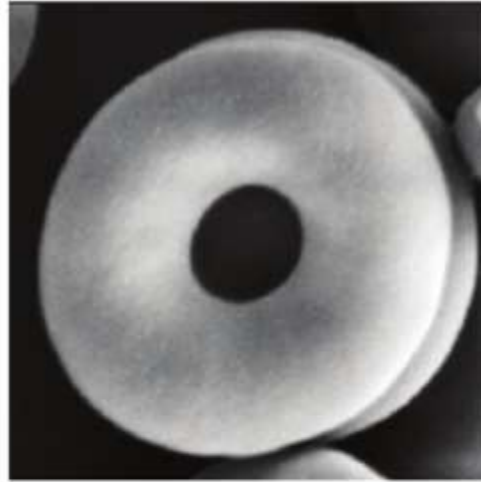
ERYTHROCYTES

Deviations from biconcave shape

Normal

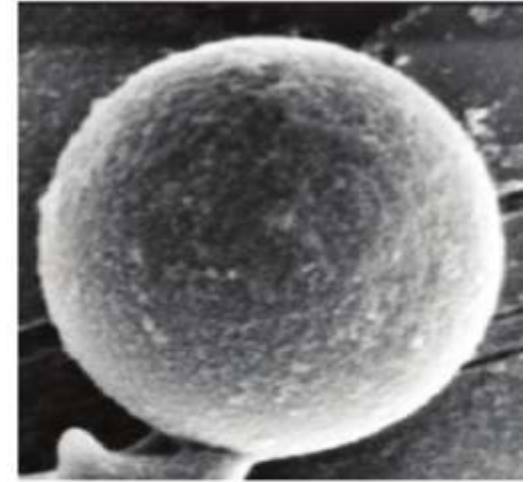


Codocyte

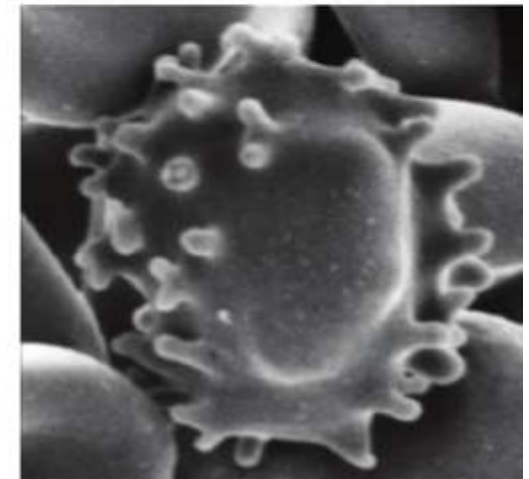
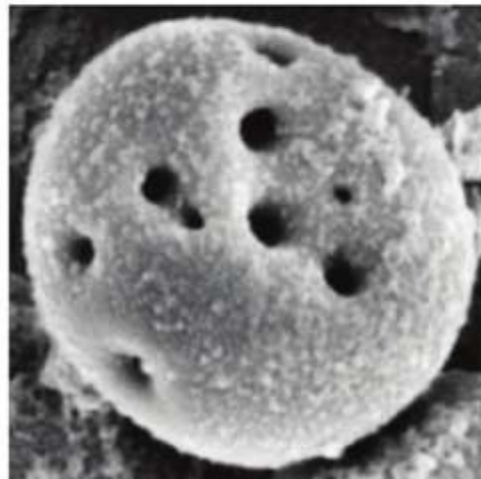


(b)

Spherocyte



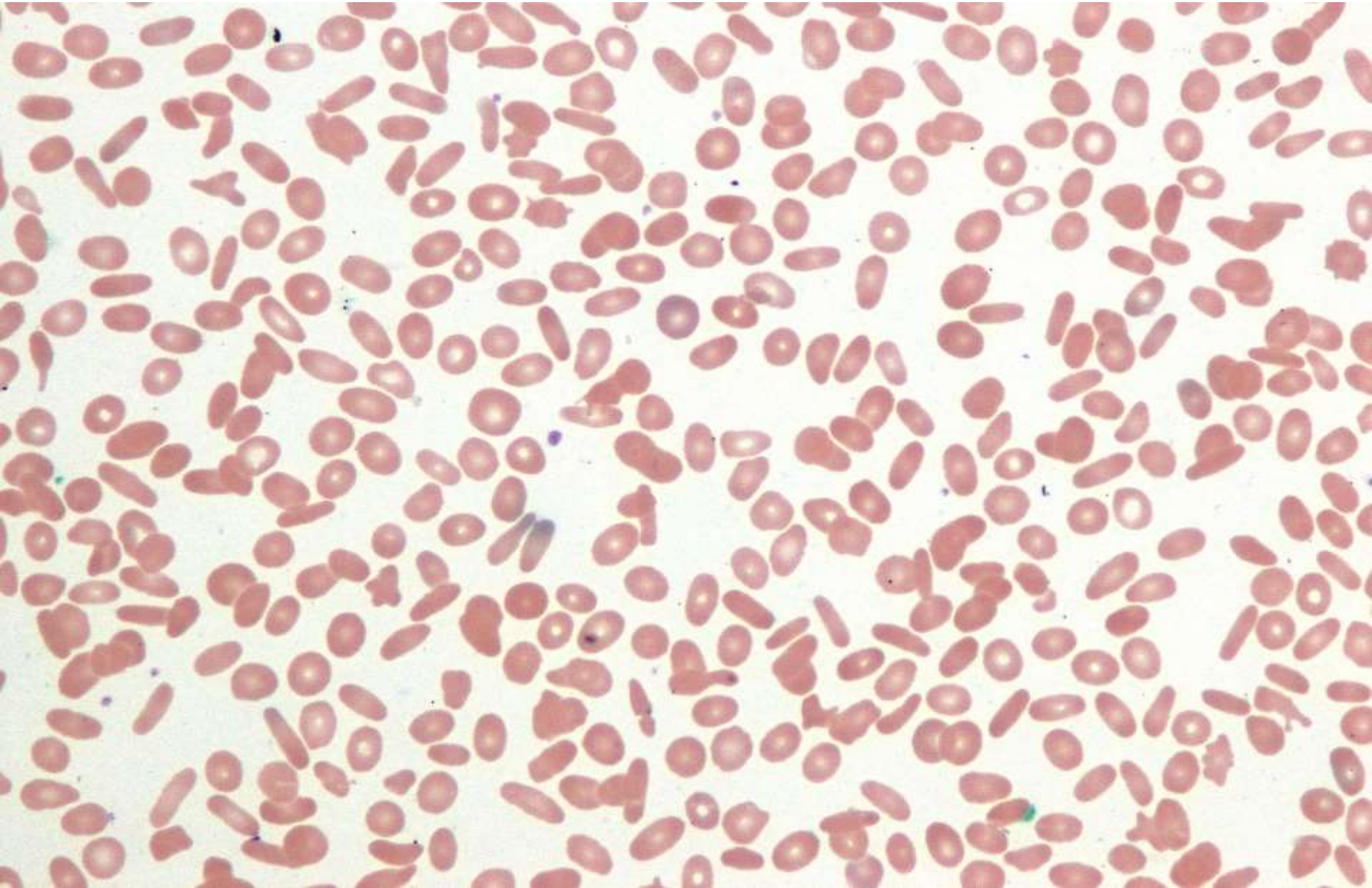
(d)



Echinocyte

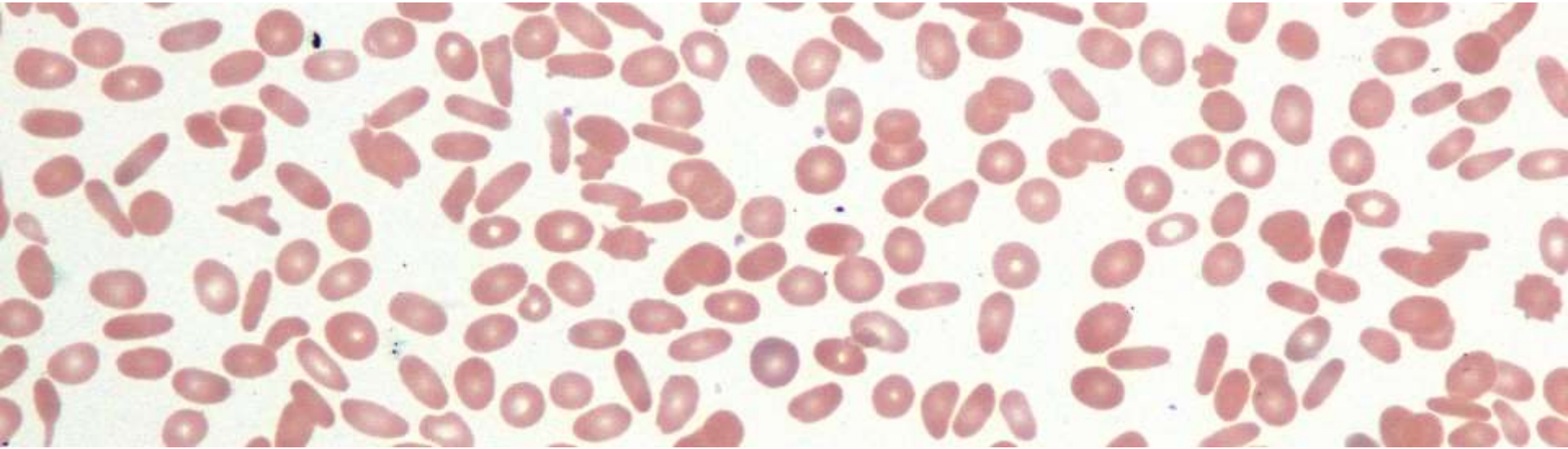
ERYTHROCYTY

Hereditary elliptocytosis

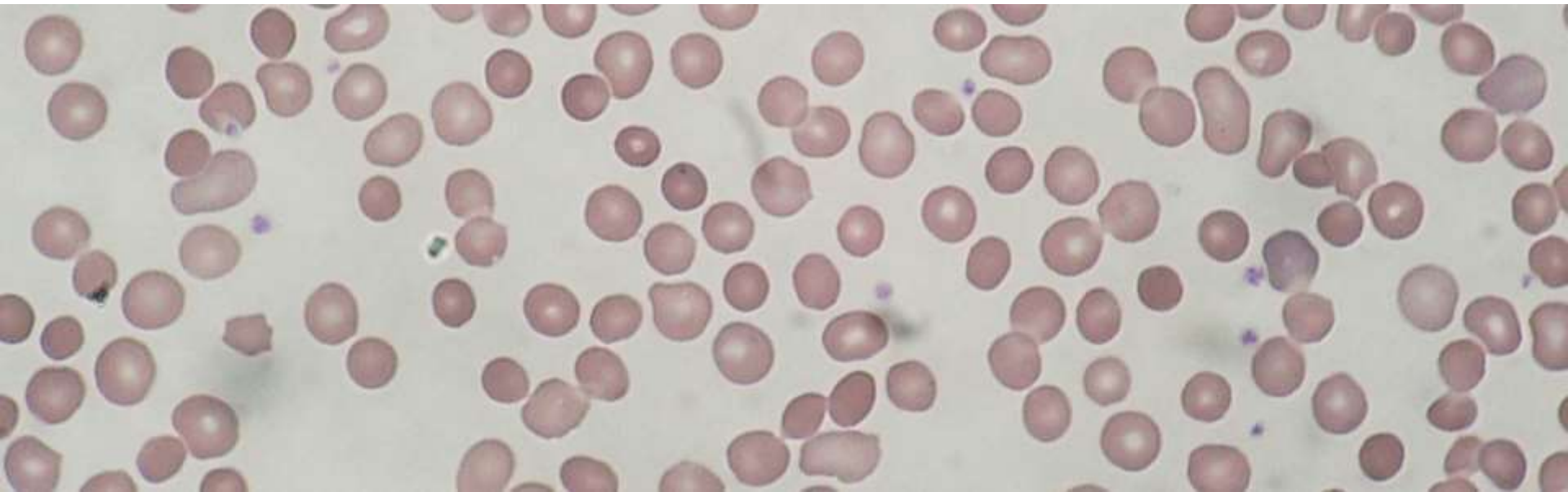


ERYTHROCYTES

Hereditární eliptocytóza



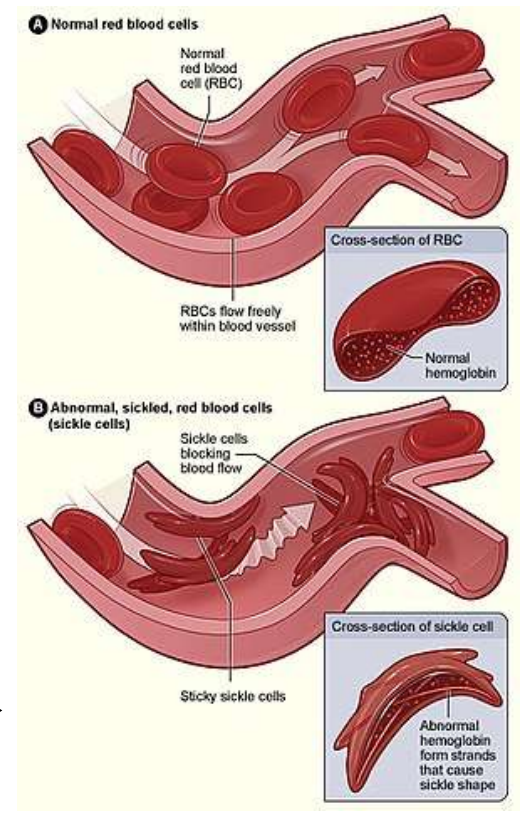
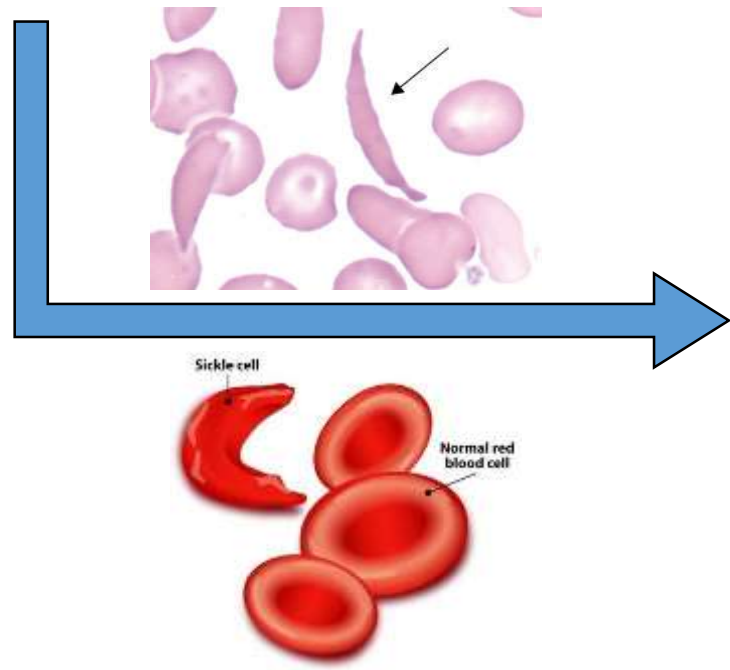
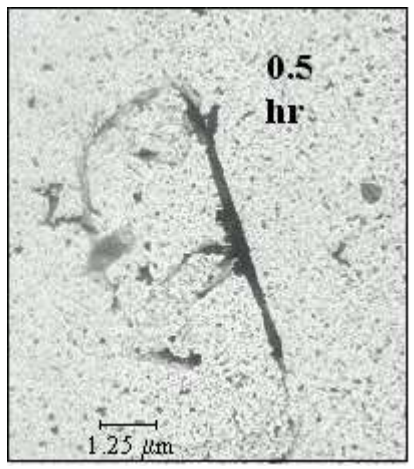
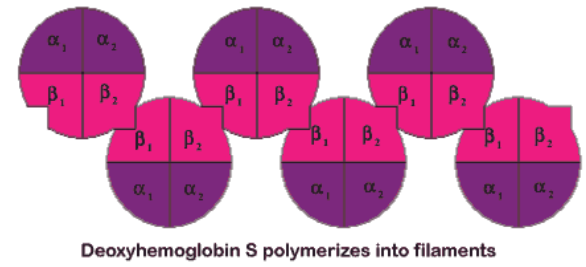
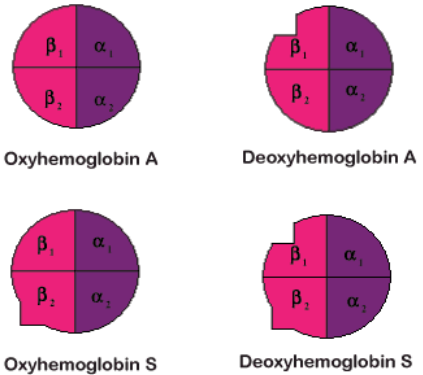
Hereditary spherocytosis



ERYTHROCYTES

Sickle cell anemia

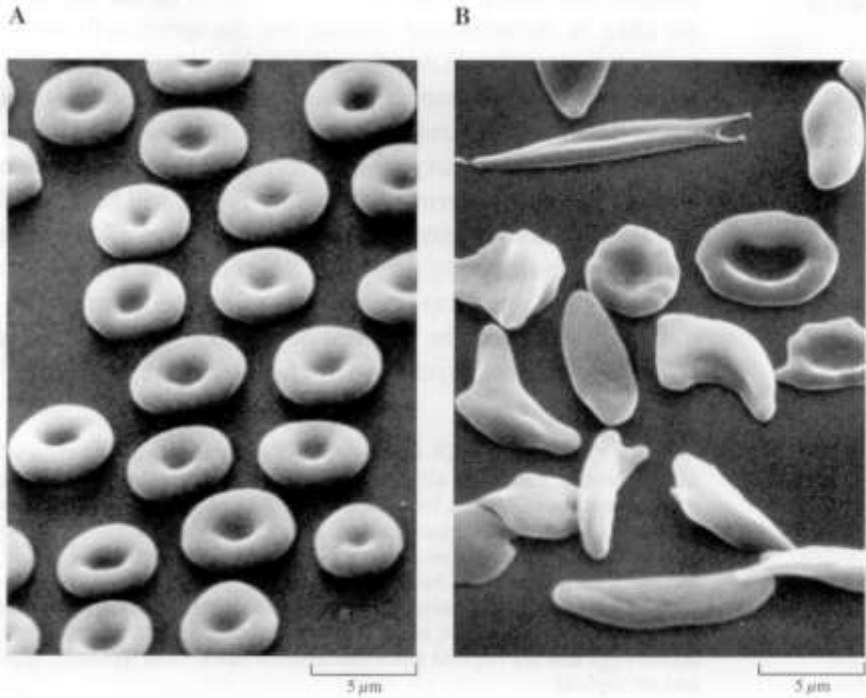
- Abnormal hemoglobin (hemoglobin S)



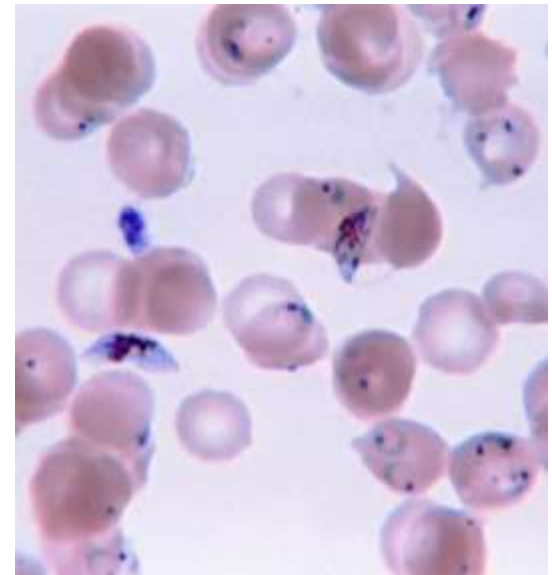
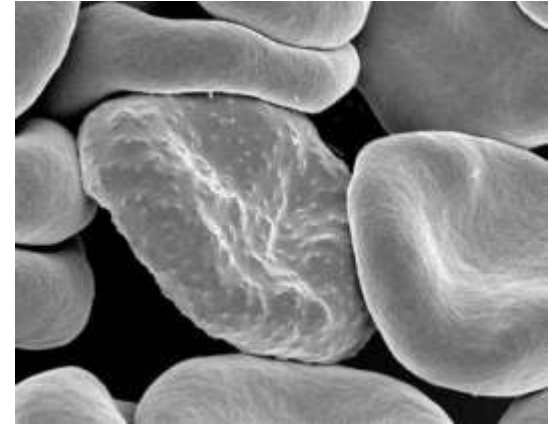
ERYTHROCYTES

Sickle cell anemia

- pathological genotype (heterozygote HbS/HbA) is beneficial

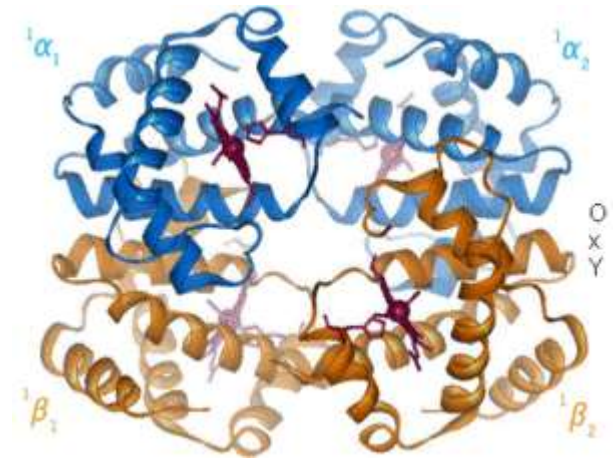
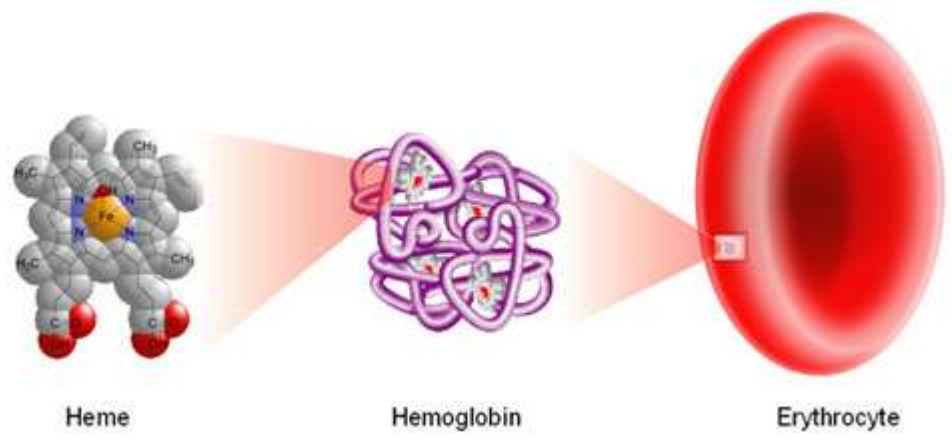
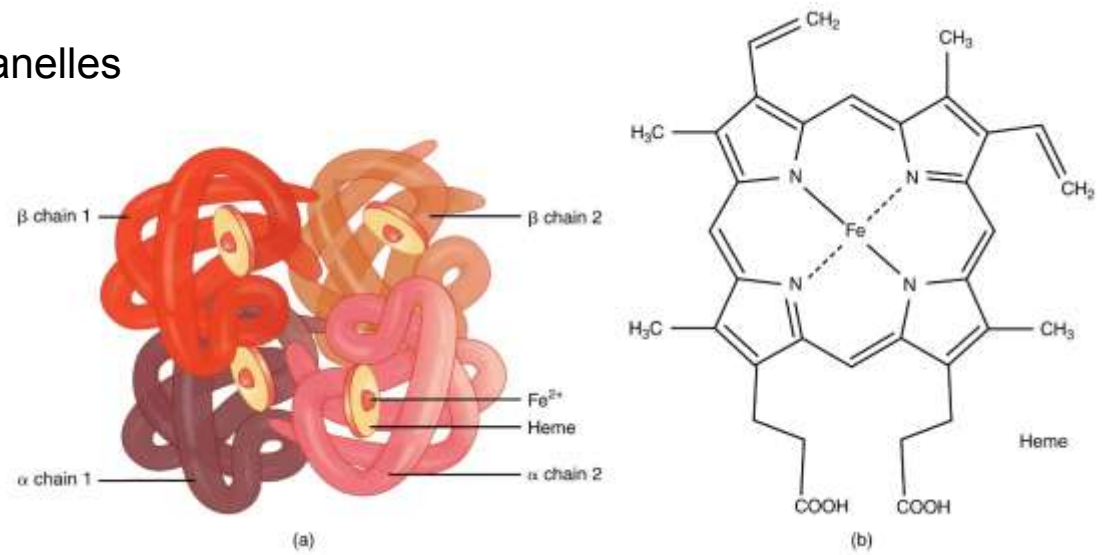


Malaria

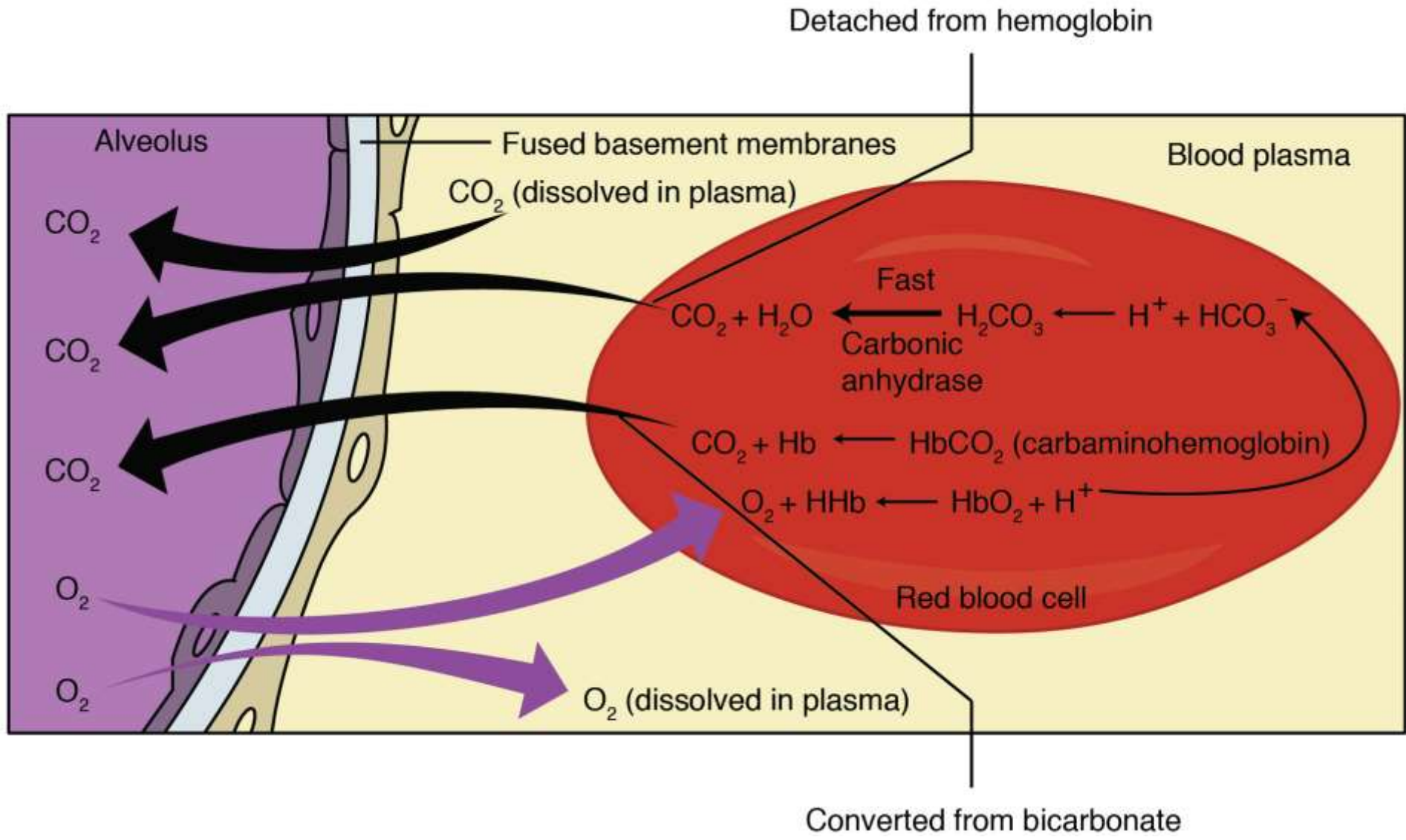


ERYTHROCYTES

- Erythrocytes lack nucleus and organelles
- Anaerobic glykolysis
- Hemoglobin

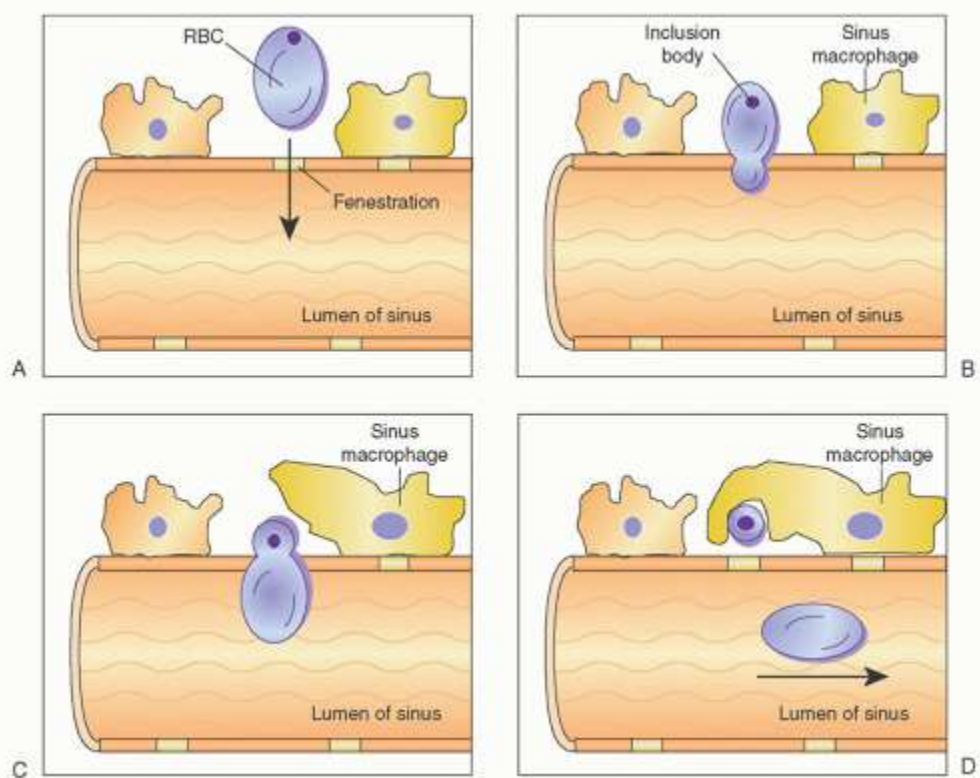
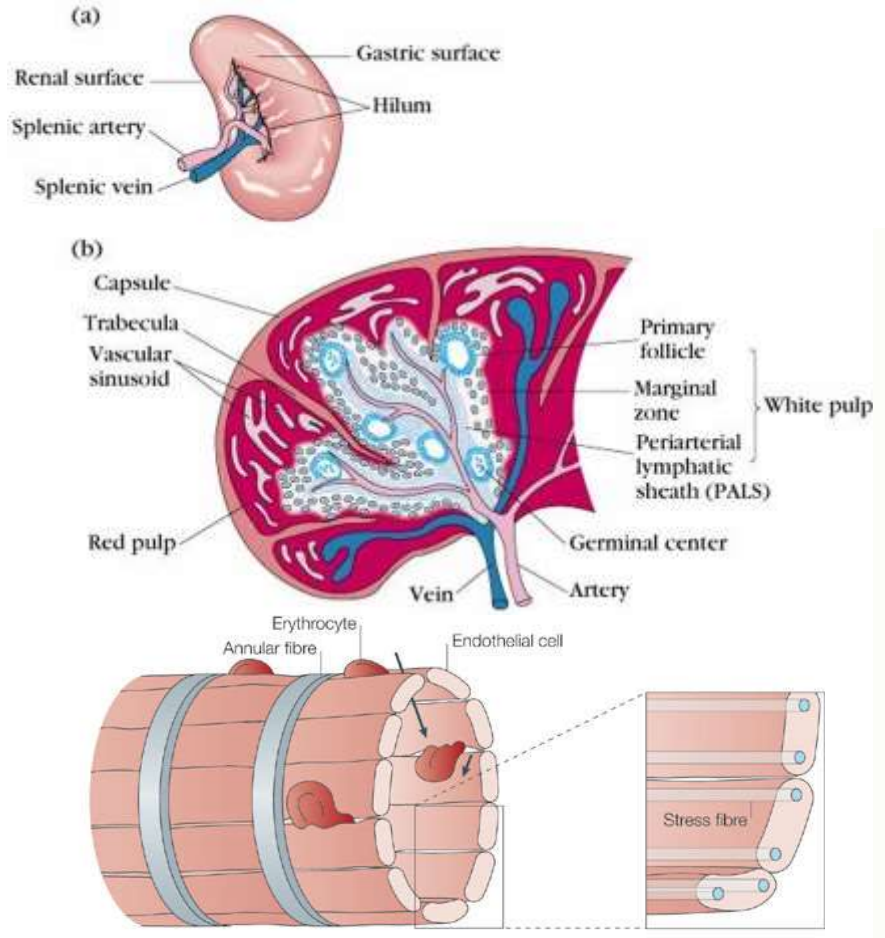
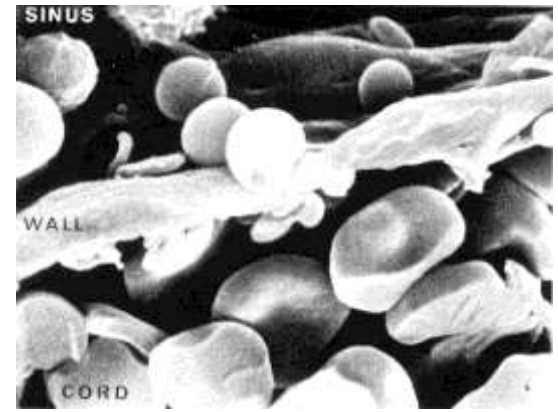


ERYTHROCYTES



ERYTHROCYTES

- Life span 120 days
- Constant abrasion
- No regeneration
- Removal of aged or damaged erythrocytes in spleen



LEUCOCYTES

- immune response
- morphological classification – **cytoplasmic granules**
(does not follow hematopoiesis)

Granulocytes

Agranulocytes

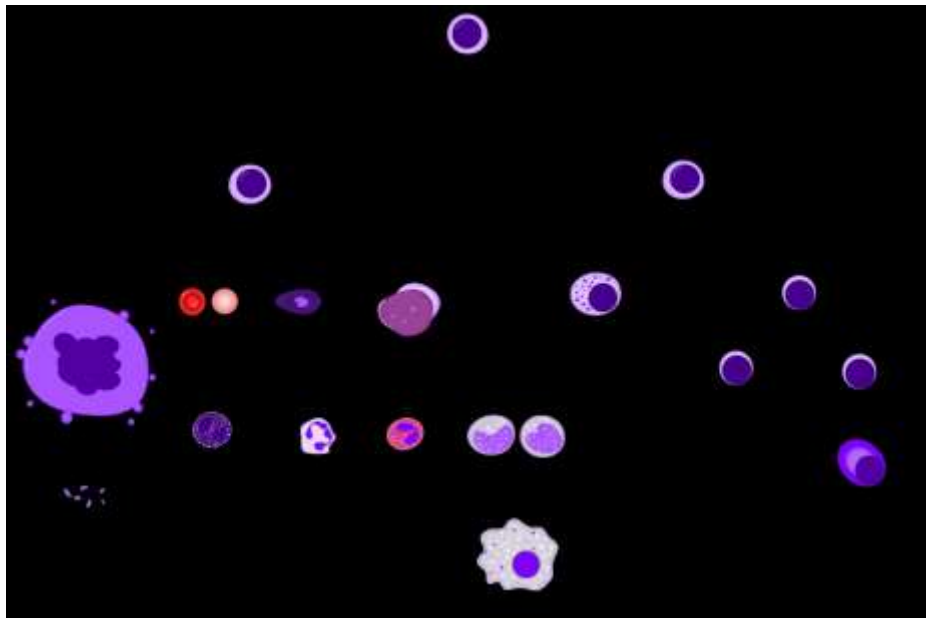
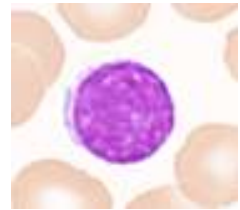
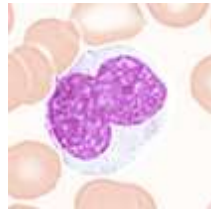
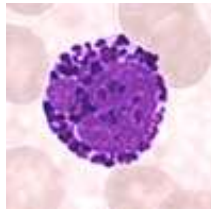
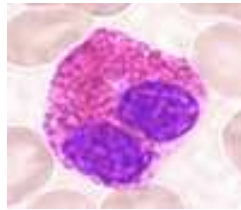
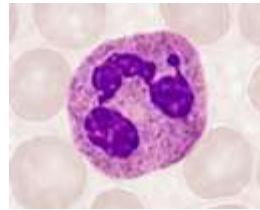
Neutrophils

Eosinophils

Basophils

Monocytes

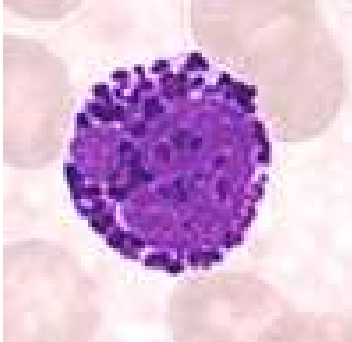
Lymphocytes



GRANULOCYTES

- Lysosomes (primary, azurophilic, nonspecific granules)
- Specific (secondary) granules
- Polymorphic nucleus
- Terminally differentiated
- Short lifespan (hours)
- Reduced ER, GA, mitochondria (anaerobic glycolysis)
- Apoptosis

Basophil



Eosinophil

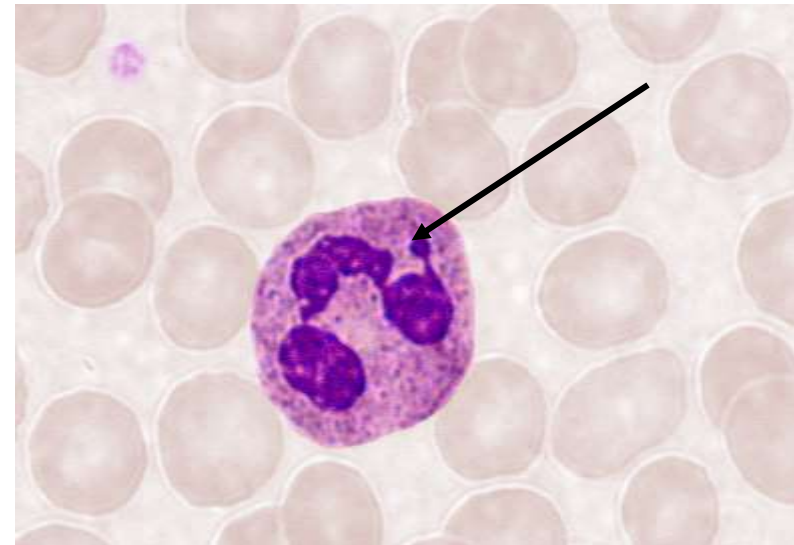
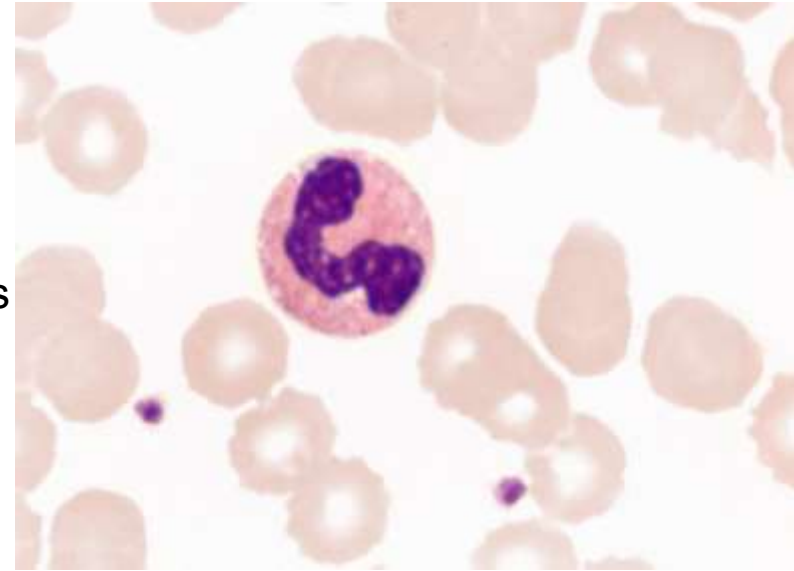
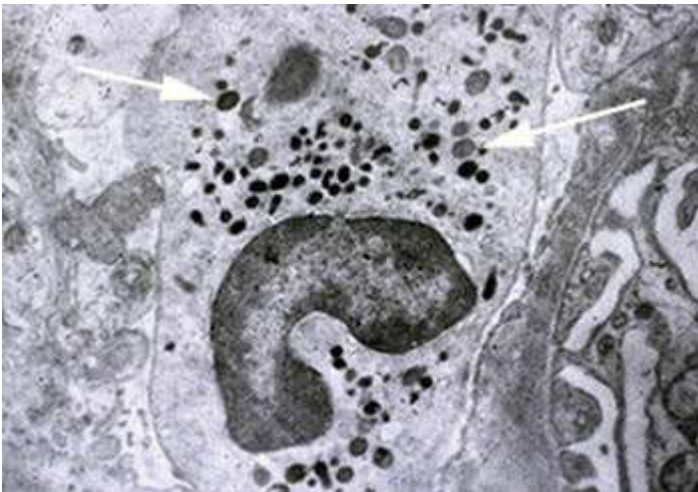


Neutrophil



NEUTROPHILIC GRANULOCYTES

- **Neutrophils**
 - 50-70% of leukocytes in circulation
 - $\varnothing > 12 \mu\text{m}$
 - Segmented nucleus
 - Barr's body in females
 - **Azurophilic (primary) granules**
 - myeloperoxidase, lysozyme, proteases, defensins
 - **Neutrophilic (secondary) granules**
 - collagenase, bactericidal enzymes
 - Chemotaxis of other leukocytes
 - Microphages
- **Neutrophilic band**
- **Neutrophilic segment**

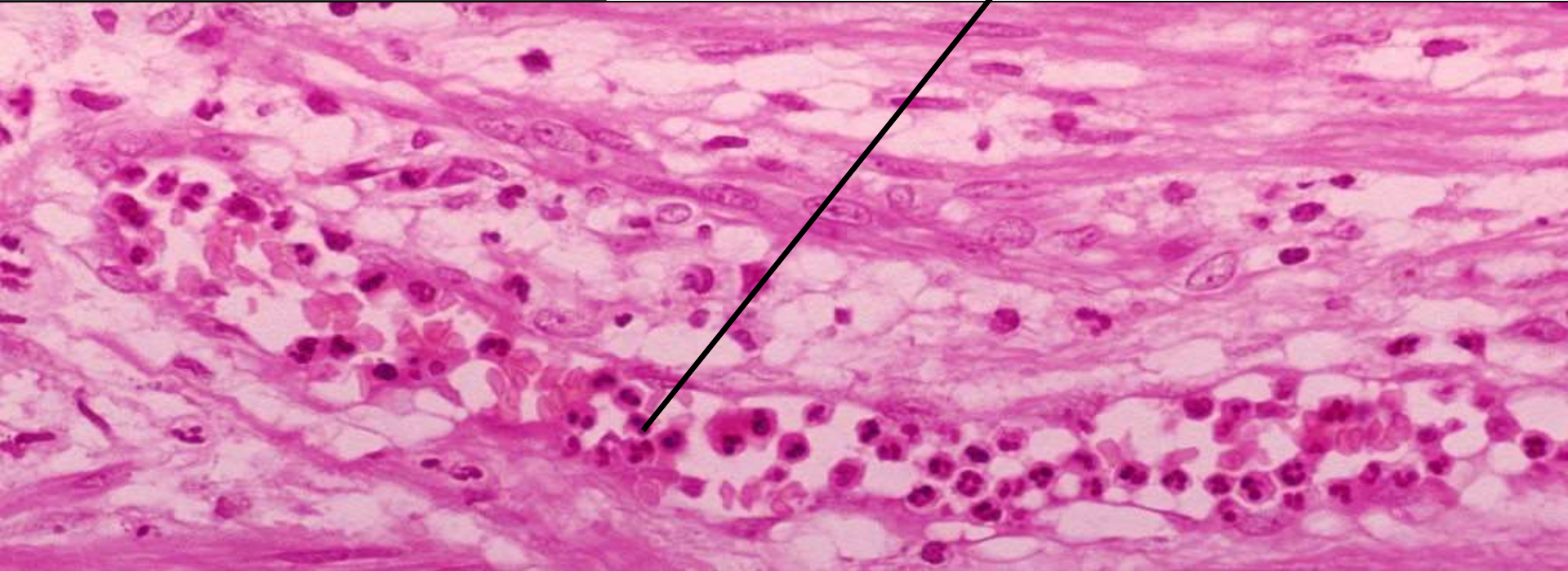
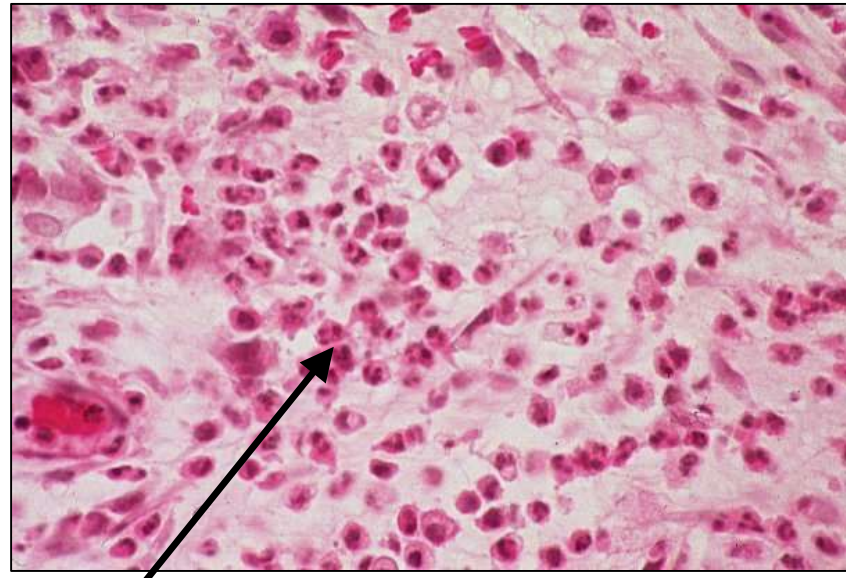
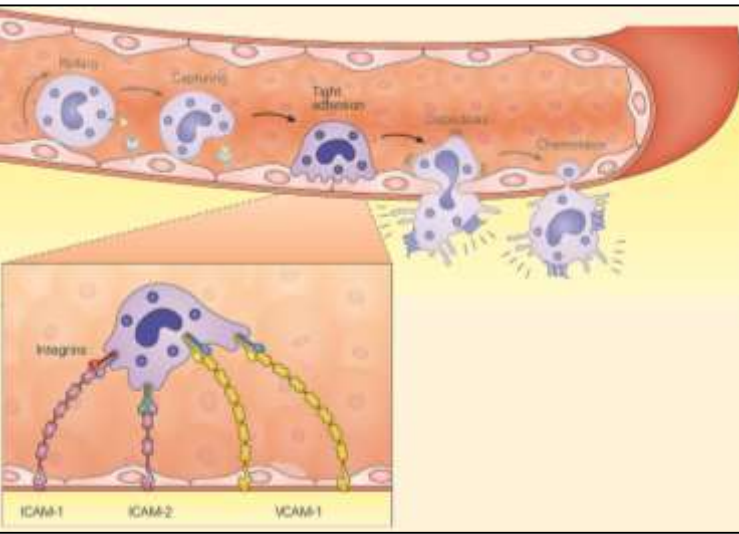


NEUTROPHILIC GRANULOCYTES



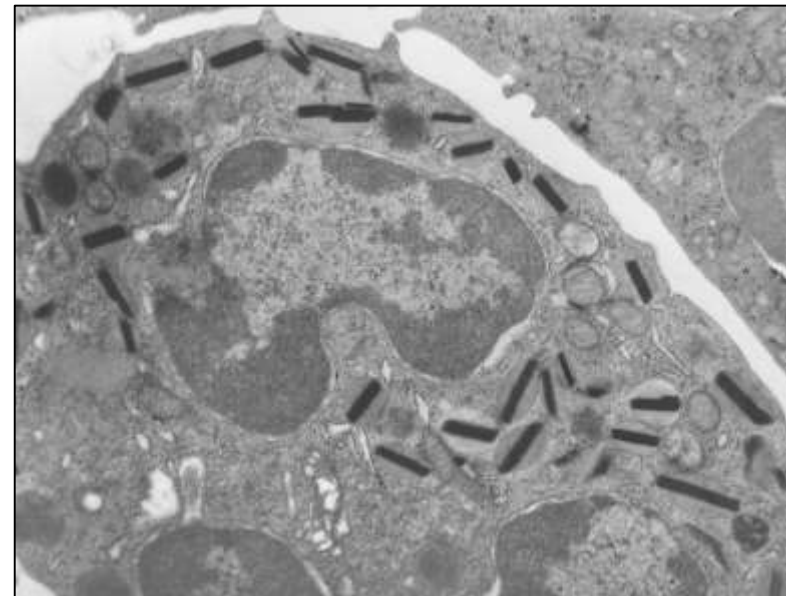
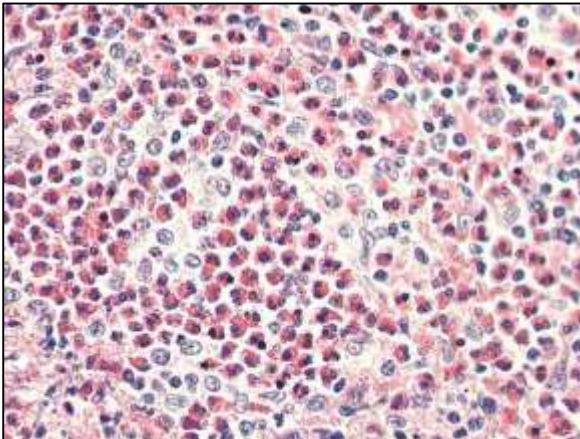
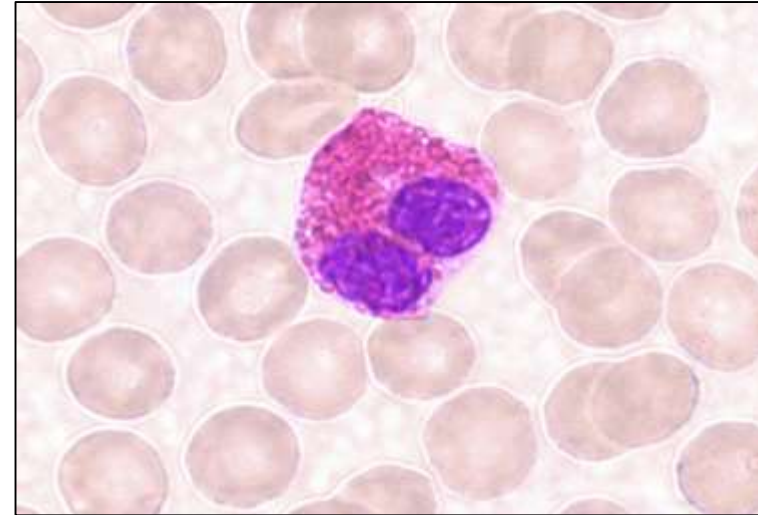
NEUTROPHILIC GRANULOCYTES

- Extravasation (diapedesis)



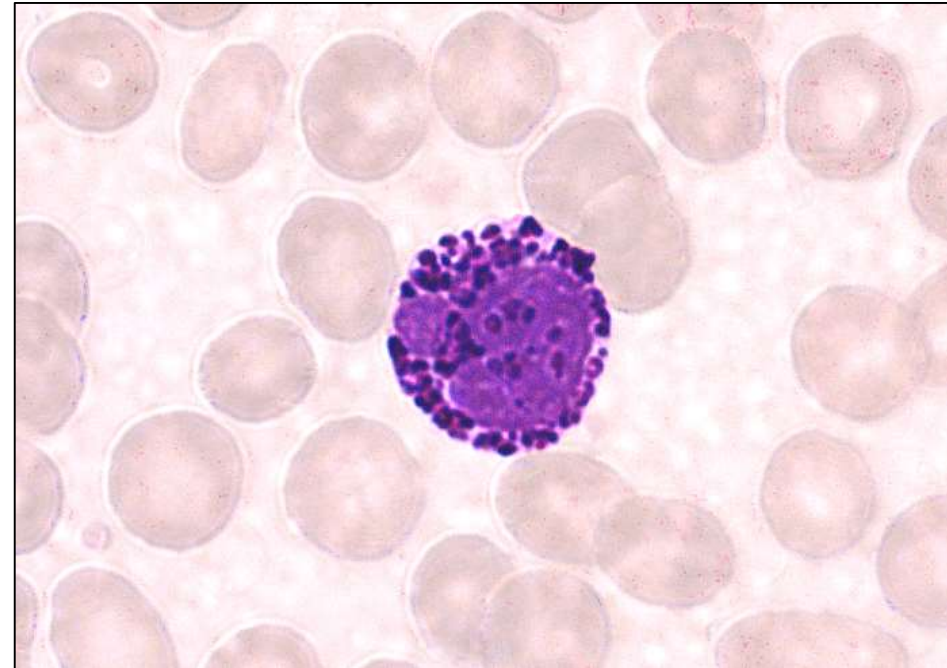
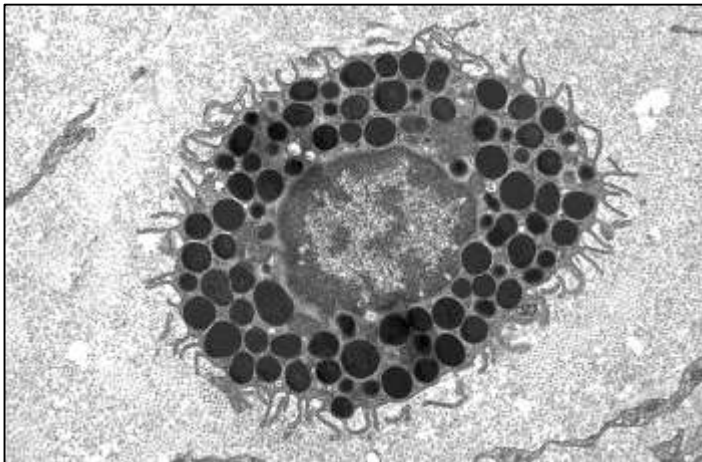
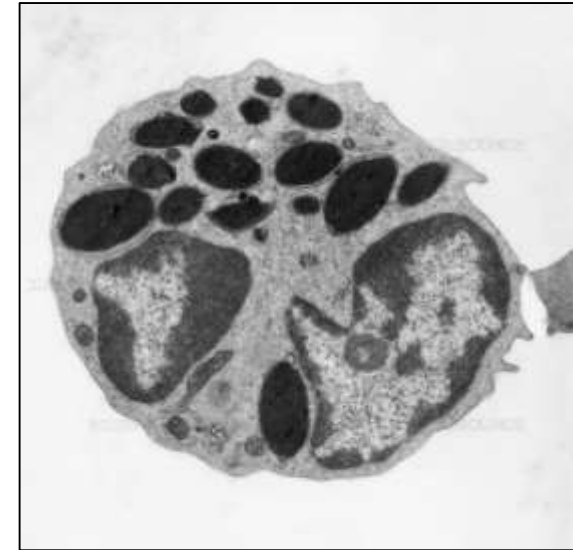
EOSINOPHILIC GRANULOCYTES

- **Eosinophils**
 - 1-4% of leukocytes in circulation
 - \varnothing 12-15 μm
 - Irregular, characteristic bi-segmented nucleus
 - **Azurophilic (primary) granules**
 - myeloperoxidase, lysozyme, proteases, defensins
 - **Eosinophilic (secondary) granules**
 - bright red (eosinophilic)
 - major acidic protein
 - peroxidase
 - cytokines, chemokines
- Chemotaxis of other leukocytes
- Phagocytosis of antibody-antigen complexes
- Parasitic infections, allergic reaction
- Chronic inflammation



Basophilic Granulocytes

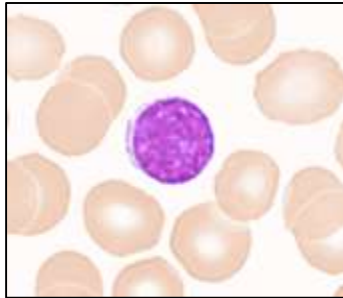
- **Bazophils**
 - <1% of leukocytes in circulation
 - \varnothing 12 μm
 - Irregular, bisegmented nucleus, masked by granules
- **Azurophilic (primary) granules**
 - myeloperoxidase, lysozyme, proteases, defensins
- **Basophilic (secondary) granules**
 - 0.5 μm
 - large, dark (basophilic)
 - heparin, histamin - vasodilatation
 - phospholipase A
- Analogs of mast cells
- Receptors for IgE
- Allergy, anaphylaxis, inflammation



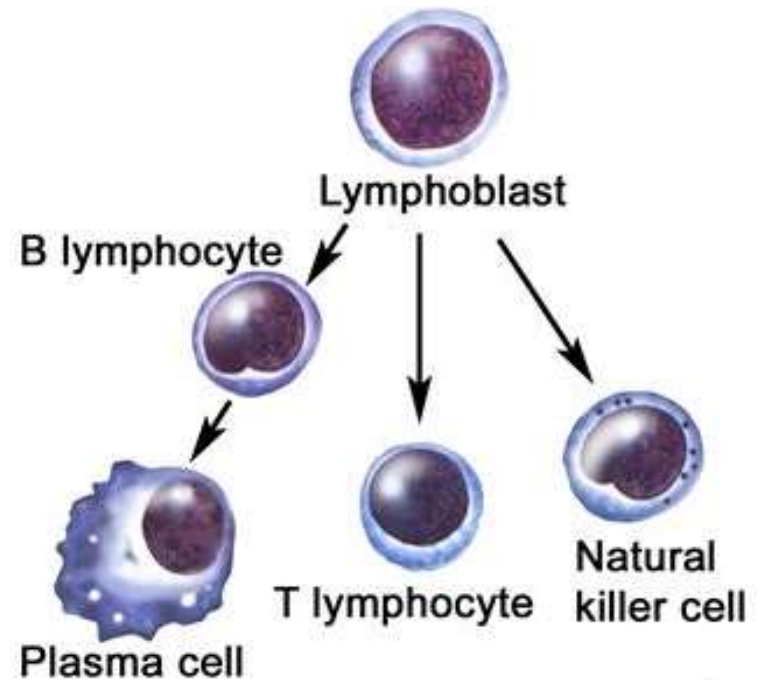
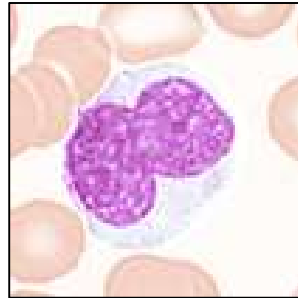
AGRANULOCYTES

- Lysosomes only (azurophilic, nonspecific granules)
- Specific granules absent
- Nonsegmented nucleus

Lymphocyty

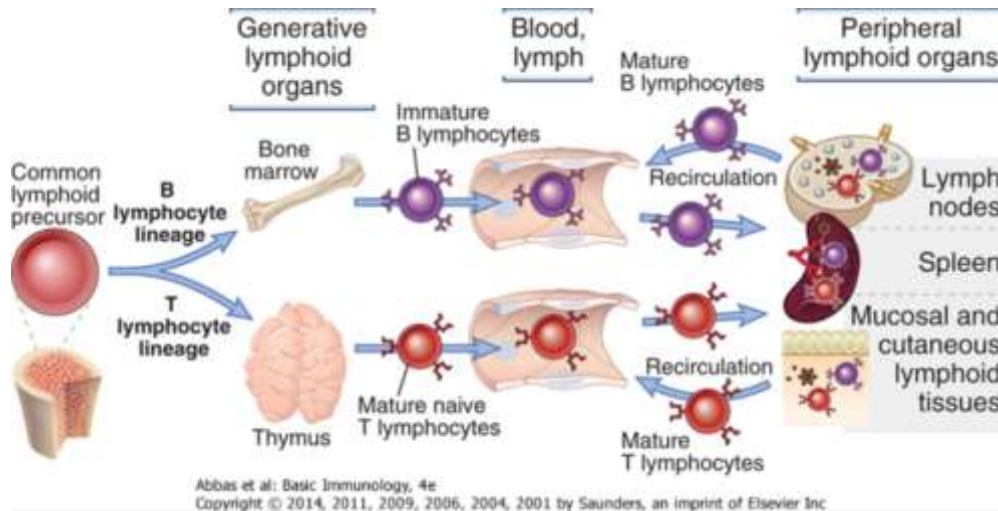
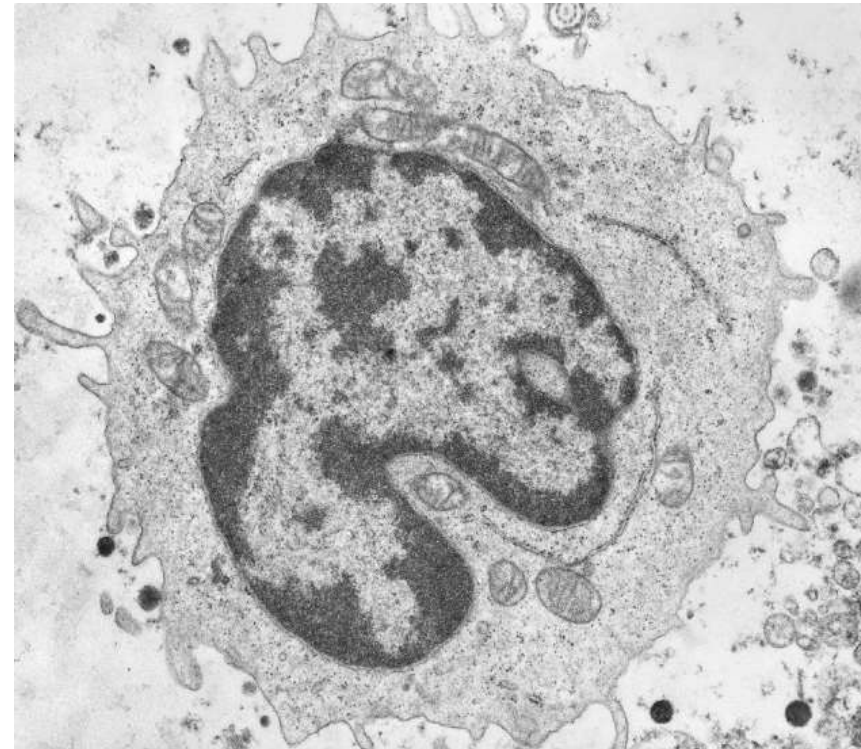
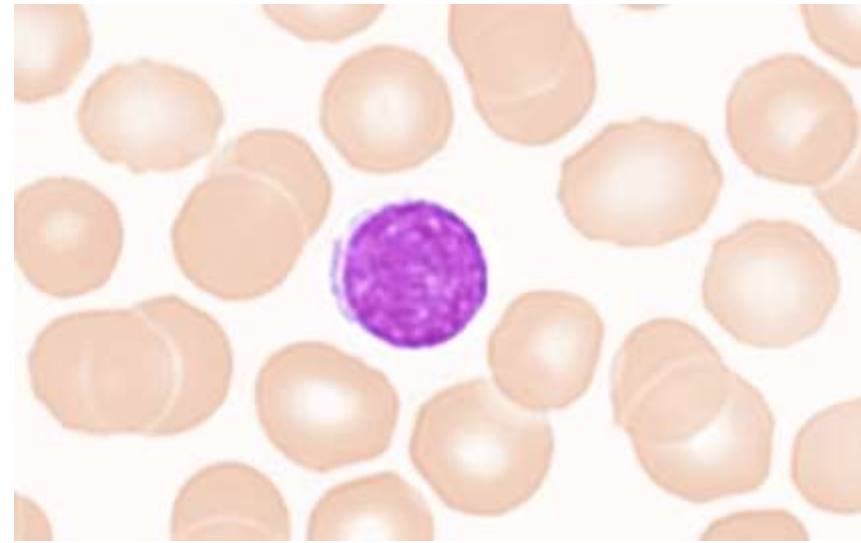


Monocyty



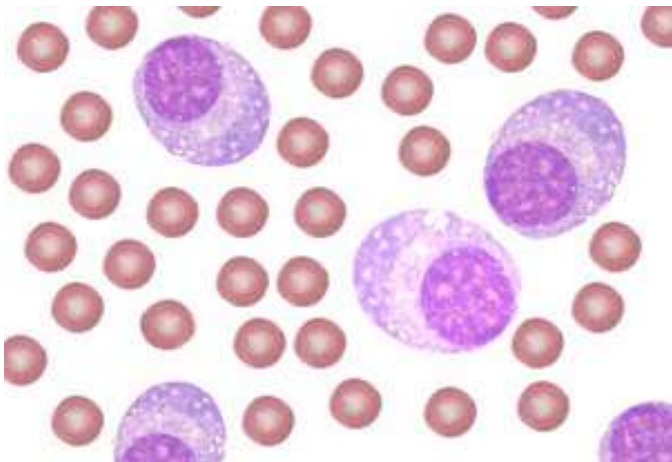
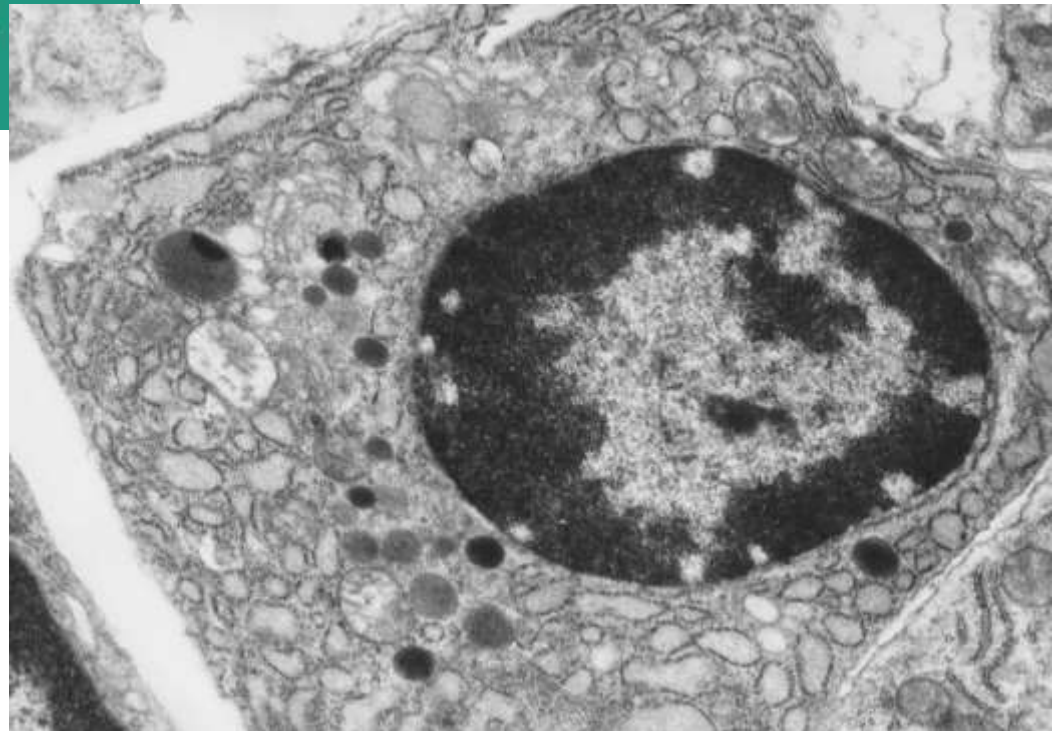
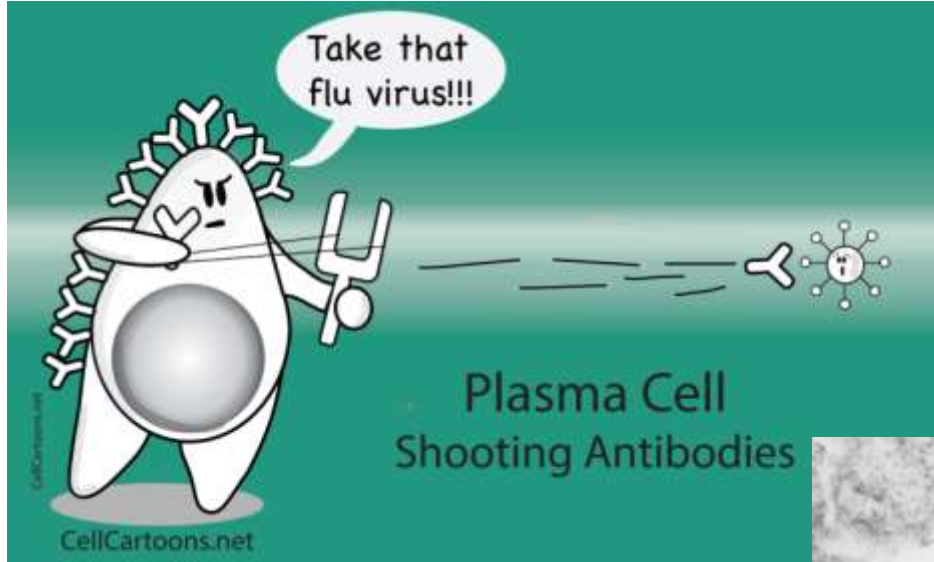
LYMPHOCYTES

- Round dominant nucleus
- Basophilic cytoplasm
- Variable lifespan
- Biologically different lymphocytes (B, T, NK) are morphologically indistinguishable
- Small, medium, large
- Bone marrow, thymus
- BCR/TCR/Antibodies with amazing variability



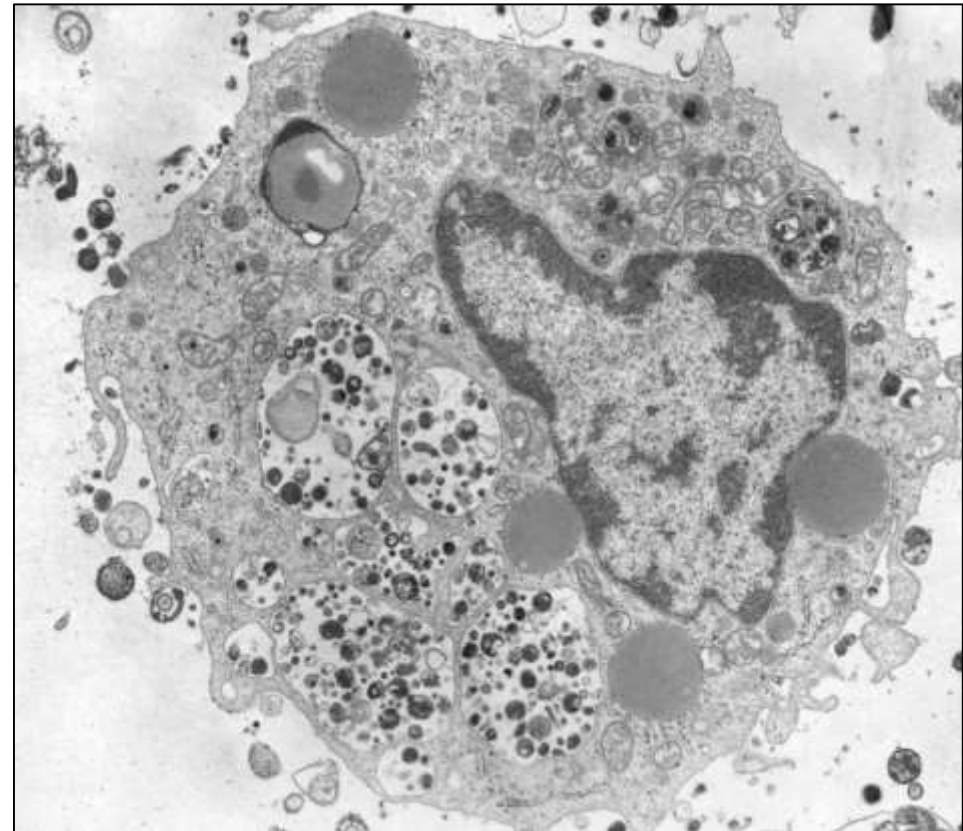
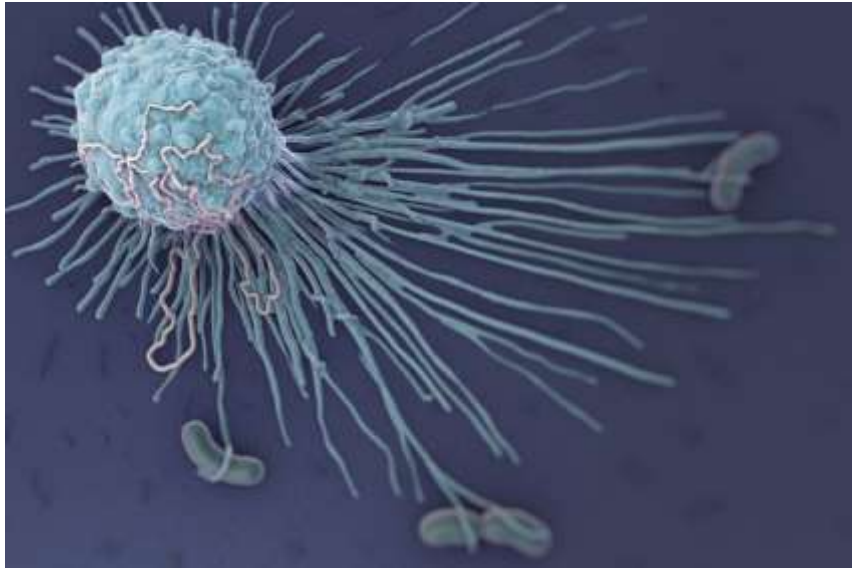
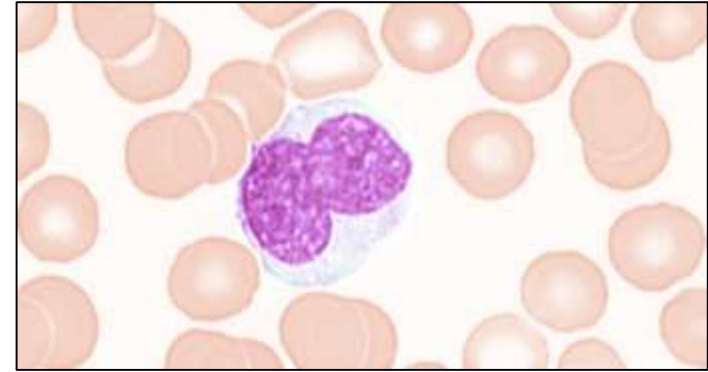
LYMPHOCYTES

- Plasma cells



MONOCYTES

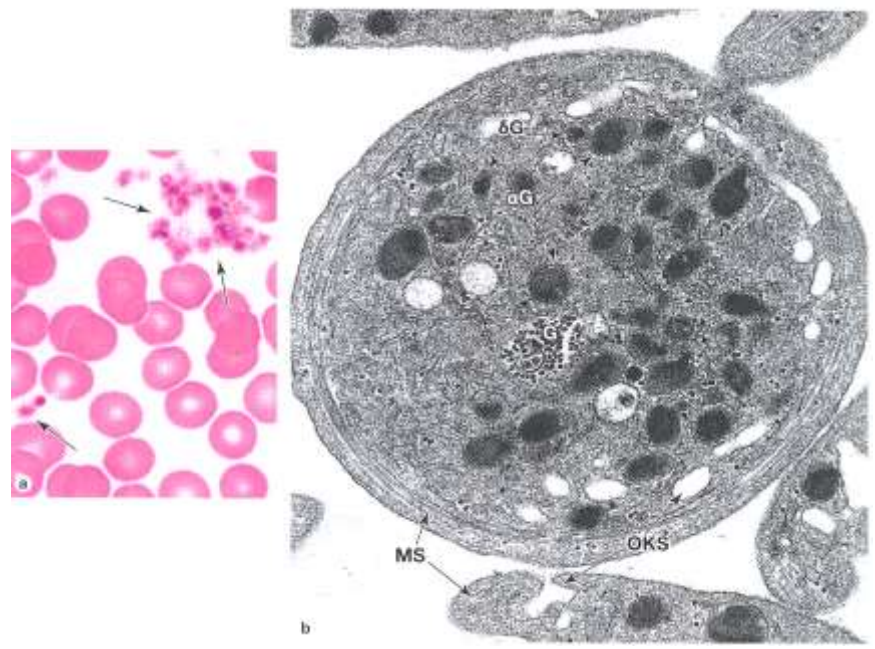
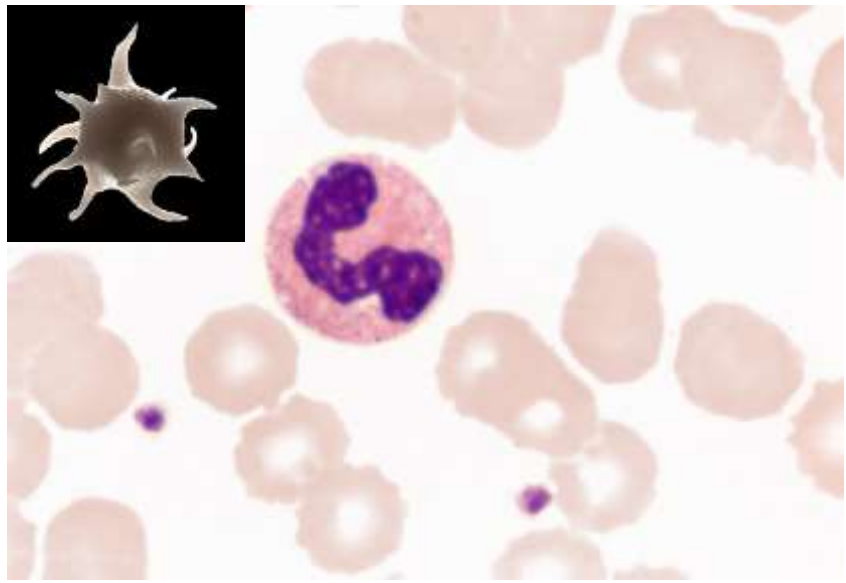
- \varnothing 12-15 μm
- Circulating precursors of macrophages, osteoclasts, microglia, Kupfer cells and dendritic cells
- Mononuclear phagocytic system
- Large, oval (bean, kidney) nucleus with less condensed chromatin and 2-3 nucleoli
- Basophilic cytoplasm
- Azurophilic granules



THROMBOCYTES

- Cell fragments without nucleus
- \varnothing 2-3 μm , discoid shape
- hyalomere, granulomere
- $150-400 \times 10^3/\mu\text{l}$
- blood clotting, repair of vessel wall

α-granules 300-500 nm	fibrinogen, PDGF
δ-granules 250-300 nm	serotonin, Ca^{++} pyrophosphate ADP, ATP
λ-granules 175-200 nm	lysosomal enzymes



THROMBOCYTES

1. Primary aggregation of platelets

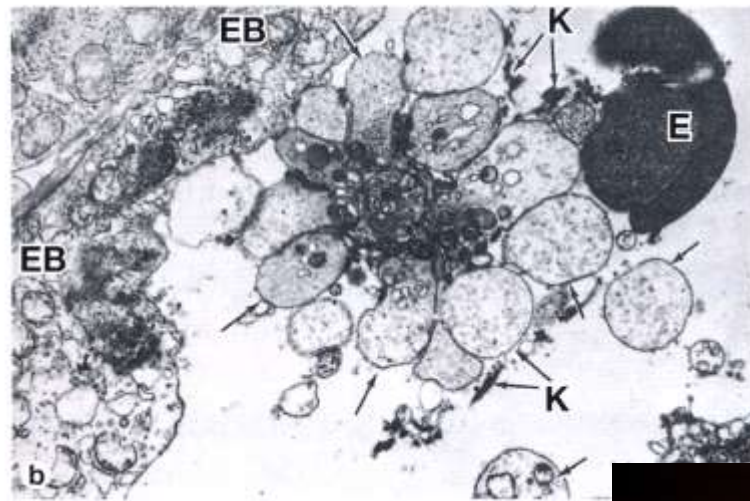
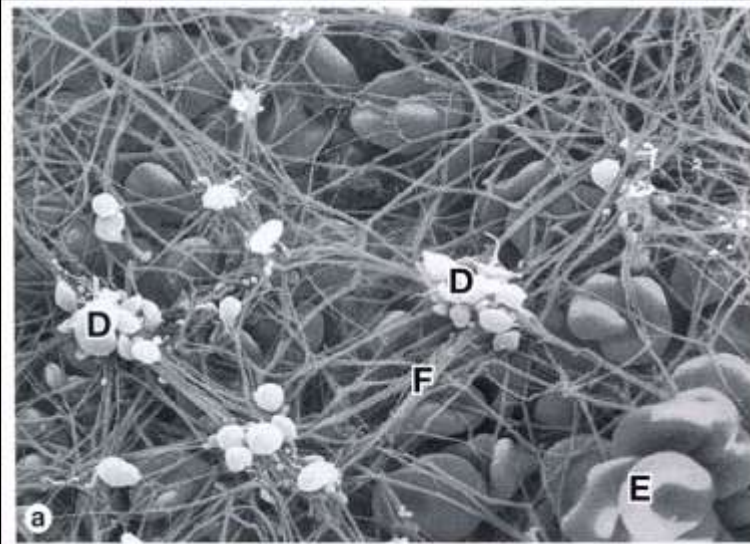
- collagen fibers exposed by endothelial rupture
- platelet clot

2. Secondary aggregation of platelets

- clotting factors, ADP from thrombocytes attracts other platelets – *white thrombus*

3. Coagulation – blood clotting

- fibrin mesh capturing erythrocytes – *red thrombus*



4. Thrombus retraction

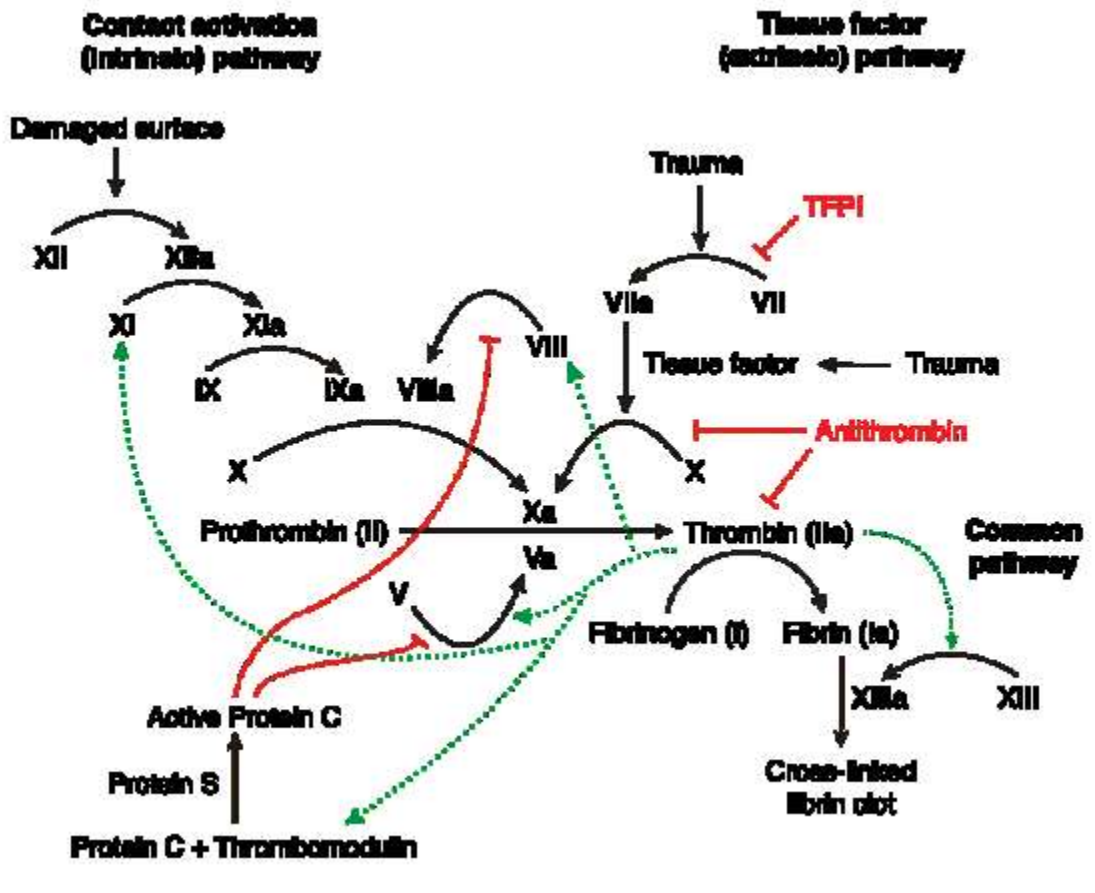
- contraction of thrombus (platelet actin and myosin)

5. Thrombolysis

- dissolving of thrombus (plasmin) and tissue regeneration



TROMBOCYTY



DIFFERENTIAL WHITE BLOOD CELL COUNT

Norm

Neutrophil	band	4 %
------------	------	-----

	segment	67 %
--	---------	------

1:17

shift to the left

shift to the right

more bands

more segments

Eosinophils		3 %
-------------	--	-----

Basophils		1 %
-----------	--	-----

Lymphocytes		20 %
-------------	--	------

Monocytes		5 %
-----------	--	-----

$\Sigma = 100 \%$

DIFFERENTIAL WHITE BLOOD CELL COUNT

Deviations from norm

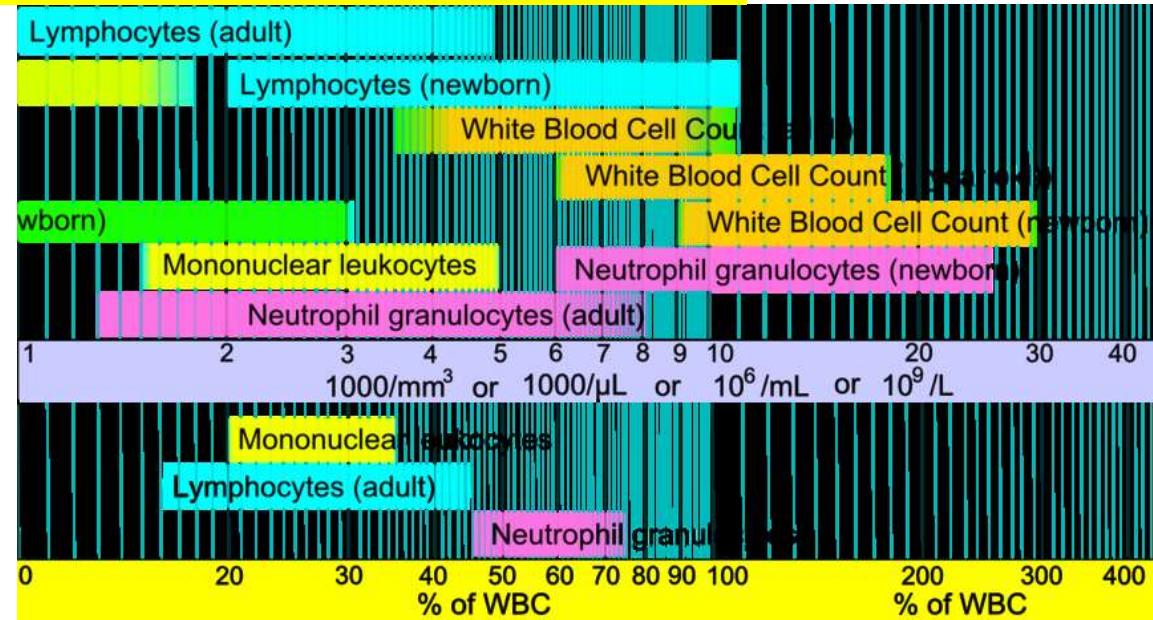
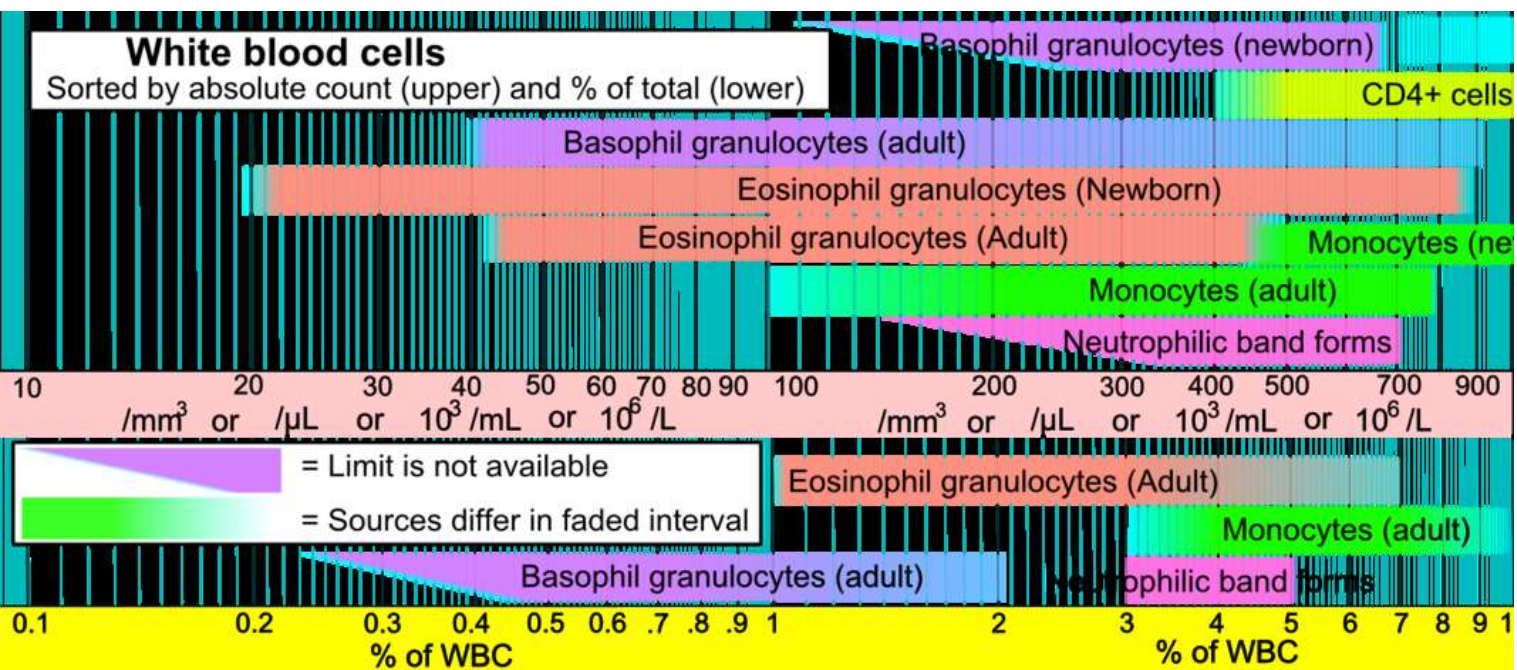
	↑ Increased	↓ Decreased
Neutrophils	neutrophil granulocytosis	neutrophil granulocytopenia
Eosinophils	eosinophil granulocytosis	eosinophil granulocytopenia
Basophils	basophil granulocytosis	basophil granulocytopenia
Lymphocytes	lymphocytosis	lymphocytopenia
Monocytes	monocytosis	monocytopenia

DIFFERENTIAL WHITE BLOOD CELL COUNT

Example of population variability

Neutrophils	bands	0-5 %
	segments	35-85 %
Eosinopils		0-4 %
Basophils		0-1 %
Lymphocytes		20-50 %
Monocytes		2-6 %

DIFFERENTIAL WHITE BLOOD CELL COUNT



Wikipedia

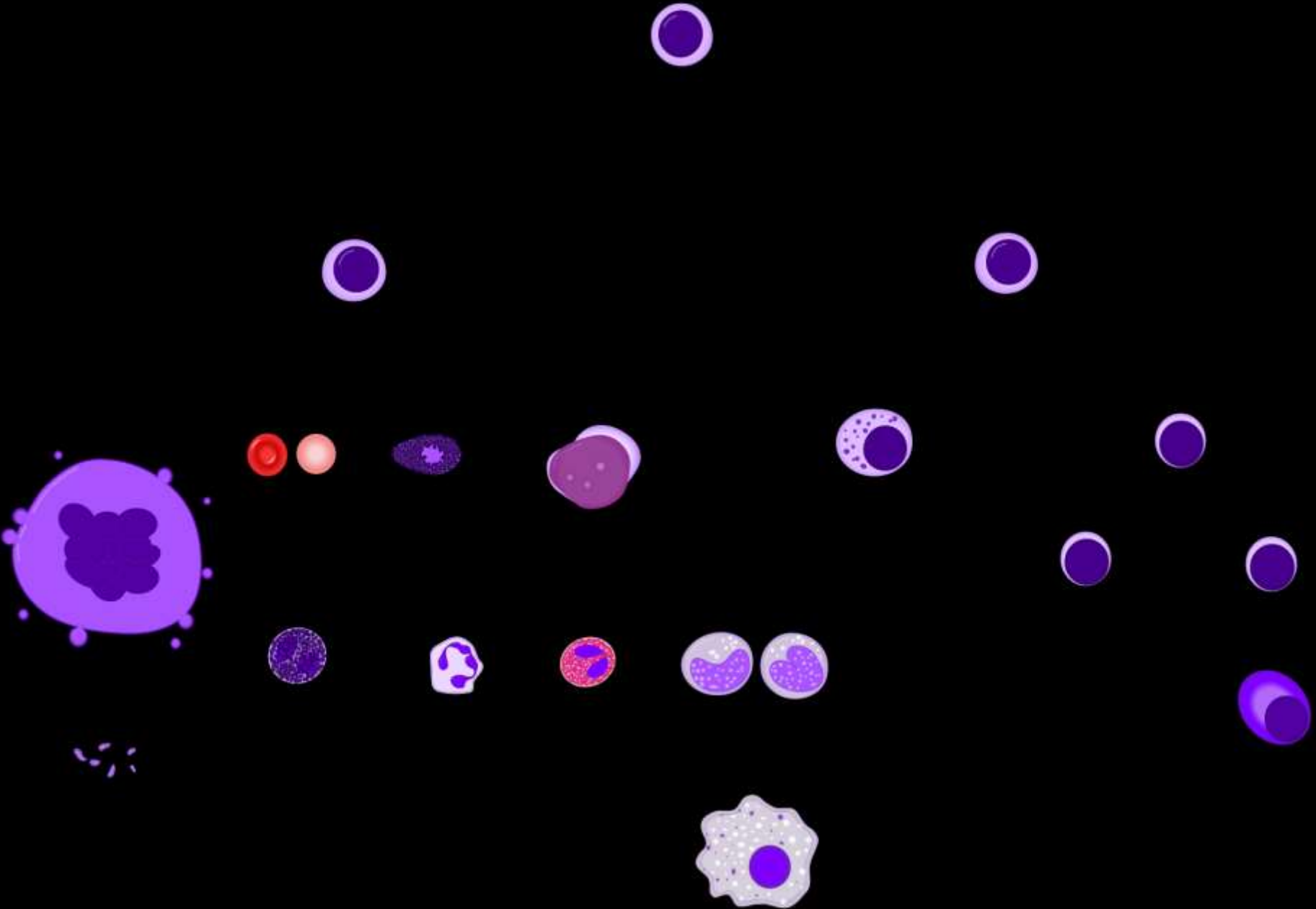
DIFFERENTIAL WHITE BLOOD CELL COUNT

Age dependence

Age	Leukocytes ($\times 10^3$)	Neutrophils (%)	Lymphocytes (%)	Monocytes (%)	Eosinophils (%)
Birth	18	61	31	6	2
1 week	12.2	45	41	9	4
1 mo	10.8	35	56	7	3
6 mo	11.9	32	61	5	3
1 yr	11.4	31	61	5	3
4 yr	9.1	42	50	5	3
10 yr	8.1	54	38	4	2
16 yr	7.8	57	35	4	3

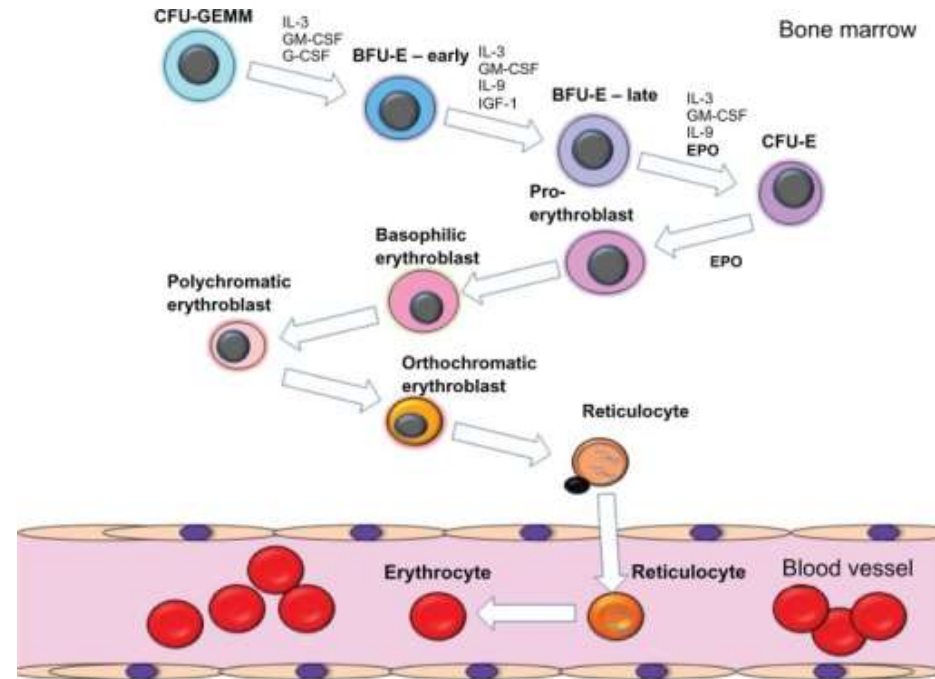
WBC, White blood cell.

HEMATOPOIESIS

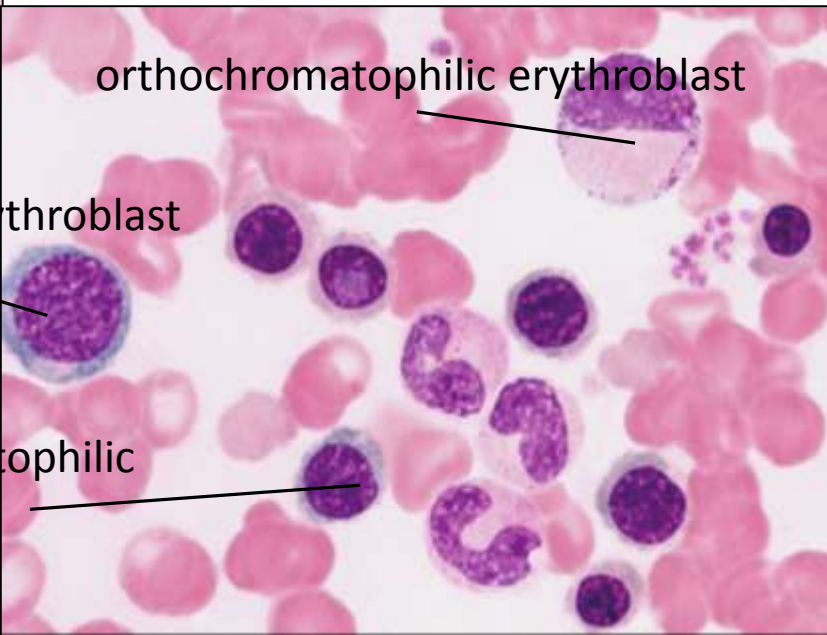
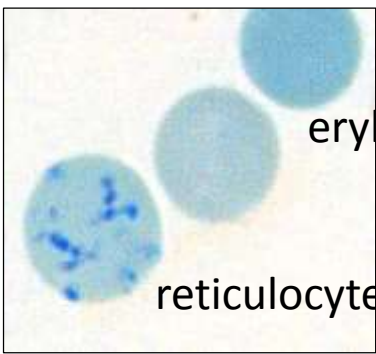
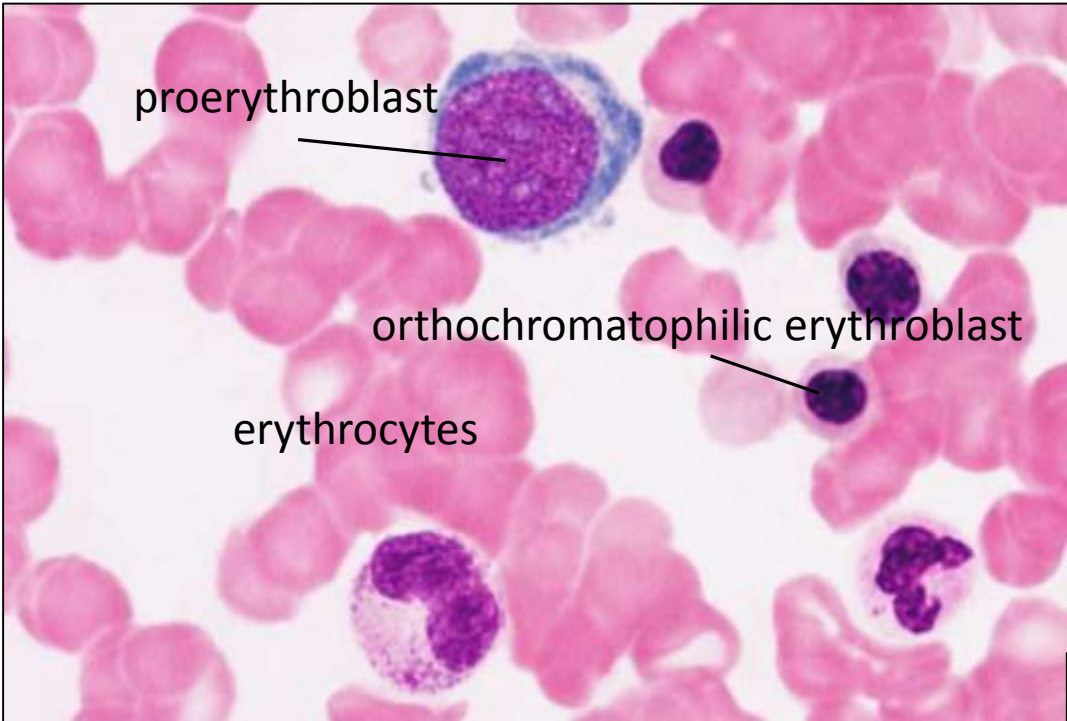


ERYTHROPOIESIS

- **2×10^{11} of new erythrocytes daily**
- **proerythroblast** (~14-19 μm)
 - mitotically active
 - dominant, round nucleus with 1-2 nucleoli
 - mildly basophilic cytoplasm
- **basophilic erythroblast** (~13-16 μm)
 - mitotically active
 - heterochromatic nucleus with inconspicuous nucleoli
 - basophilic cytoplasm (sometimes more than in proerythroblast)
- **polychromatophilic erythroblast** (~13-16 μm)
 - mitotically active
 - **production of hemoglobin**
 - blue-gray cytoplasm due to combined basophilic (polyribosomes) and acidophilic aspects (hemoglobin)
 - heterochromatic nucleus (checkerboard appearance)
- **orthochromatophilic erythroblast** (~8-10 μm)
 - mitotically inactive
 - small, compact, eccentric, pyknotic nucleus \rightarrow **extrusion**
 - mildly acidophilic cytoplasm with basophilic residues
- **reticulocyte** (polychromatophilic erythrocyte, ~ 7-8 μm)
 - **lacks nucleus, still spheroid shape**
 - acidophilic cytoplasm
 - *substantia reticulofilamentosa* visible by supravital staining (brilliant cresyl blue)
- **erythrocyte** (~7-8 μm)
 - anucleate, biconcave disc
 - acidophilic cytoplasm



ERYTHROPOIESIS



proerythroblast

orthochromatophilic erythroblast

erythrocytes

erythrocytes

reticulocyte

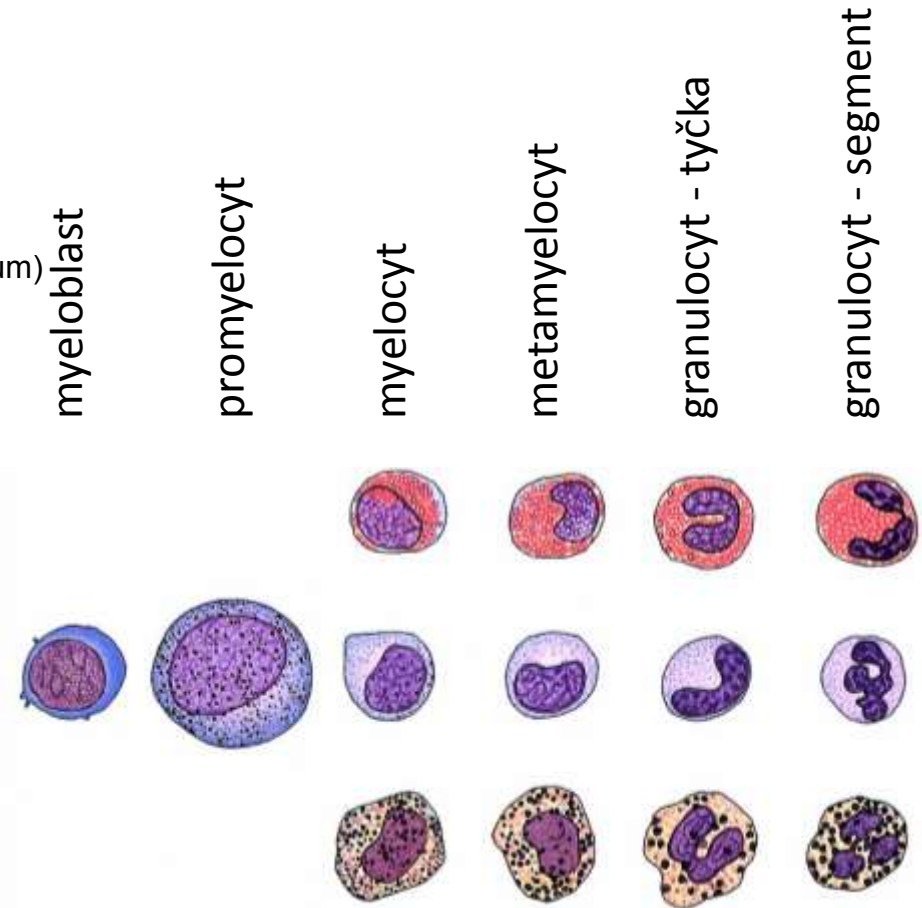
orthochromatophilic erythroblast

basophilic erythroblast

polychromatophilic erythroblast

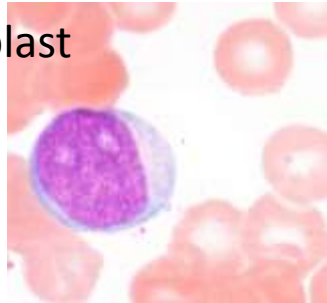
GRANULOPOIESIS

- **myeloblast** (~15 µm)
 - mitotically active
 - round-oval, euchromatic nucleus
 - 2-6 apparent nucleoli
 - weakly basophilic cytoplasm without granules
- **promyelocyte** (~15-24 µm)
 - mitotically active
 - round-oval nucleus with partly condensed chromatin
 - basophilic cytoplasm with azurophilic^③ granules
- neutrophilic, eosinophilic or basophilic **myelocyte** (~10-16 µm)
 - mitotically active
 - oval or bean-shaped nucleus with condensed chromatin
 - increasing number of specific granules in cytoplasm
- neutrophilic, eosinophilic or basophilic **metamyelocyte** (~10-12 µm)
 - mitotically inactive
 - horseshoe-like nucleus with condensed chromatin
- neutrophilic, eosinophilic or basophilic **granulocyte** (~10-12 µm)
 - segmentation of nucleus
 - cytoplasm rich in specific and azurophilic granules

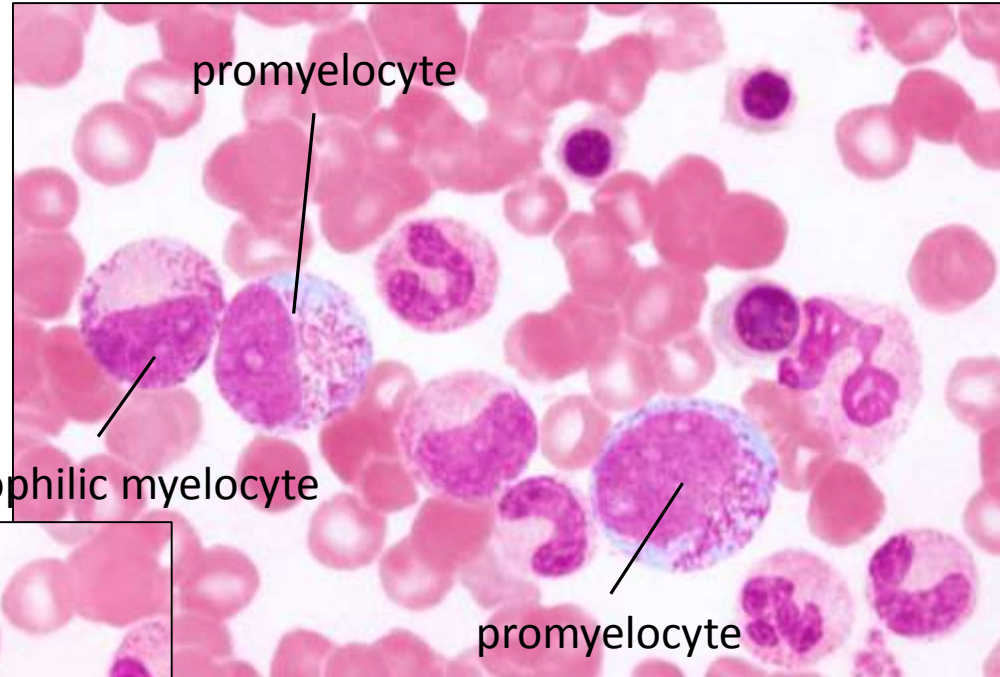


GRANULOPOIESIS

myeloblast



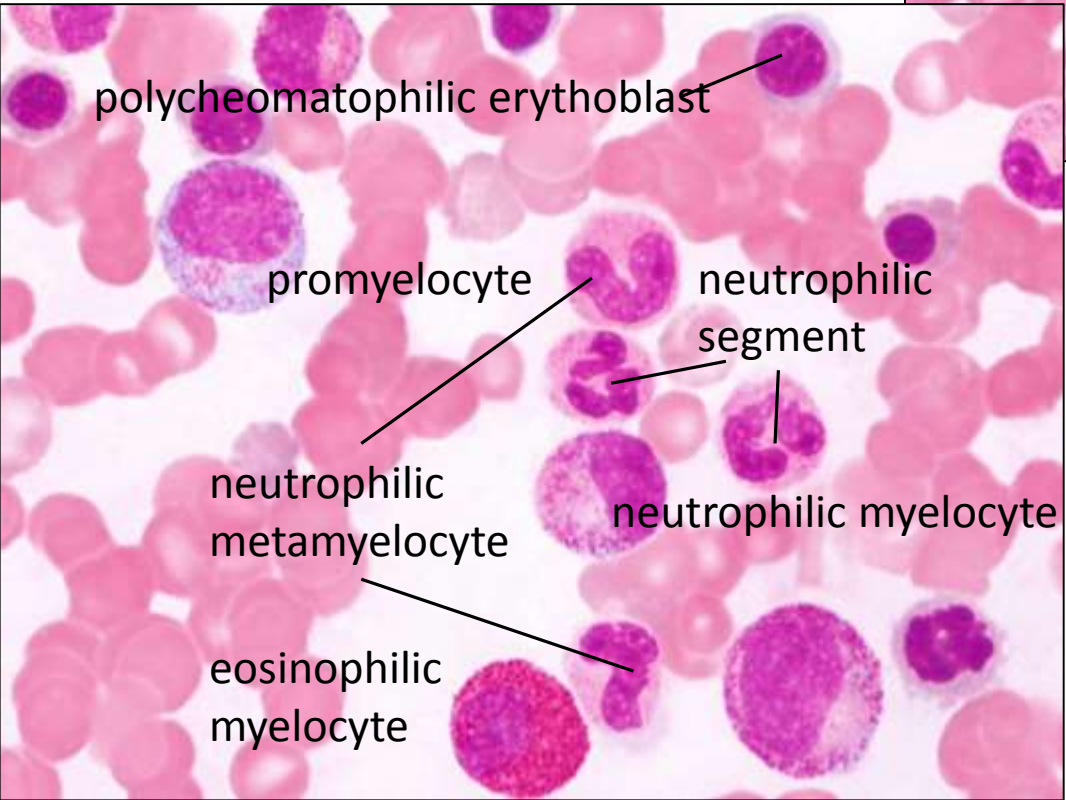
promyelocyte



neutrophilic myelocyte

promyelocyte

polychromatophilic erythroblast



promyelocyte

neutrophilic segment

neutrophilic metamyelocyte

neutrophilic myelocyte

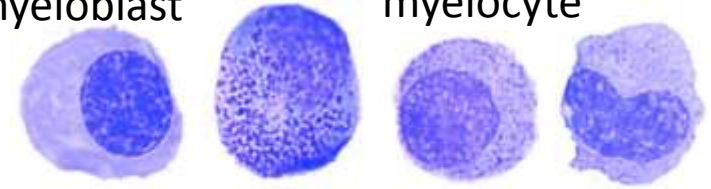
eosinophilic myelocyte

promyelocyte

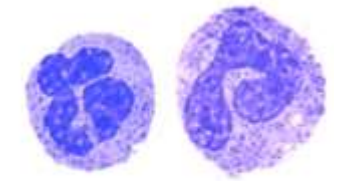
metamyelocyte

myeloblast

myelocyte

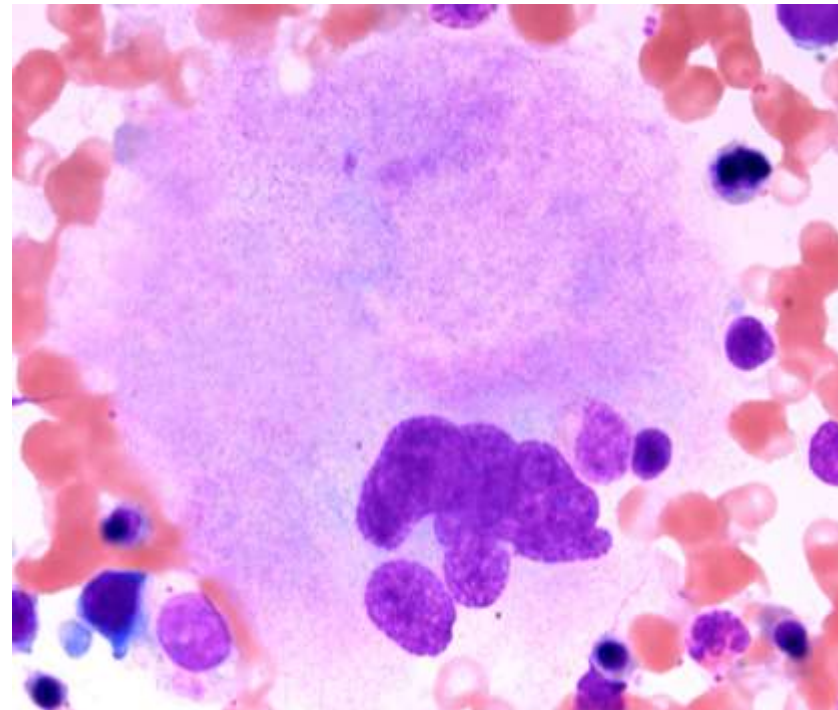
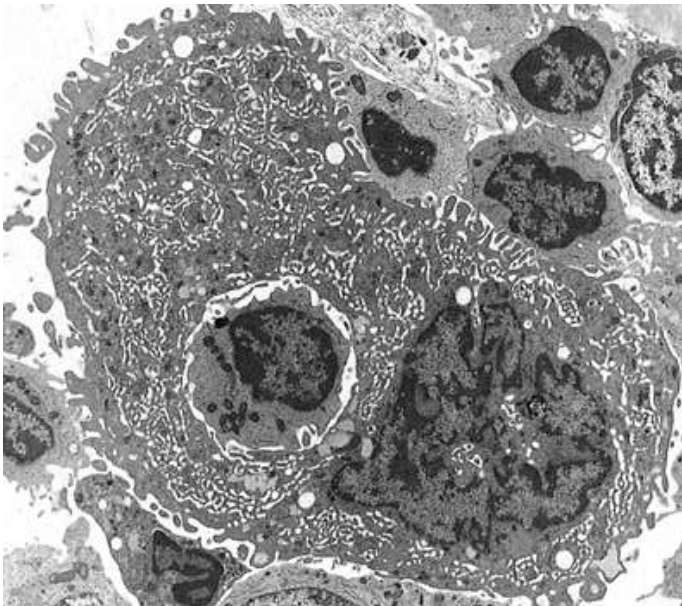
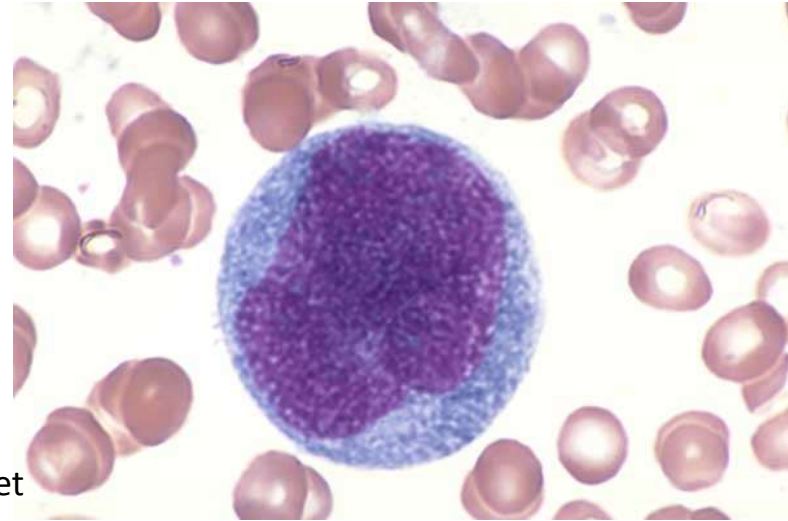


granulocyte



THROMBOPOIESIS

- **megakaryoblast** (up to 30 μm)
 - large oval, nonlobed nucleus with prominent nucleoli
 - basophilic cytoplasm
 - successive endomitoses without karyokinesis and cytokinesis
- **promegakaryocyte** (up to 100 μm)
 - large cell with polyploid nucleus (8n-64n)
- **megakaryocyte** (80-150 μm)
 - polyploid, multilobed nucleus (8n-64n)
 - azurophilic and platelet granules
 - multiple centrioles, ER and Golgi apparatus
 - numerous peripheral invaginations of plasma membrane – platelet demarcation channels defining individual thrombocytes
 - release of **thrombocytes** into bone marrow sinusoids



MONOCYTOPOIESIS AND LYMPHOPOIESIS

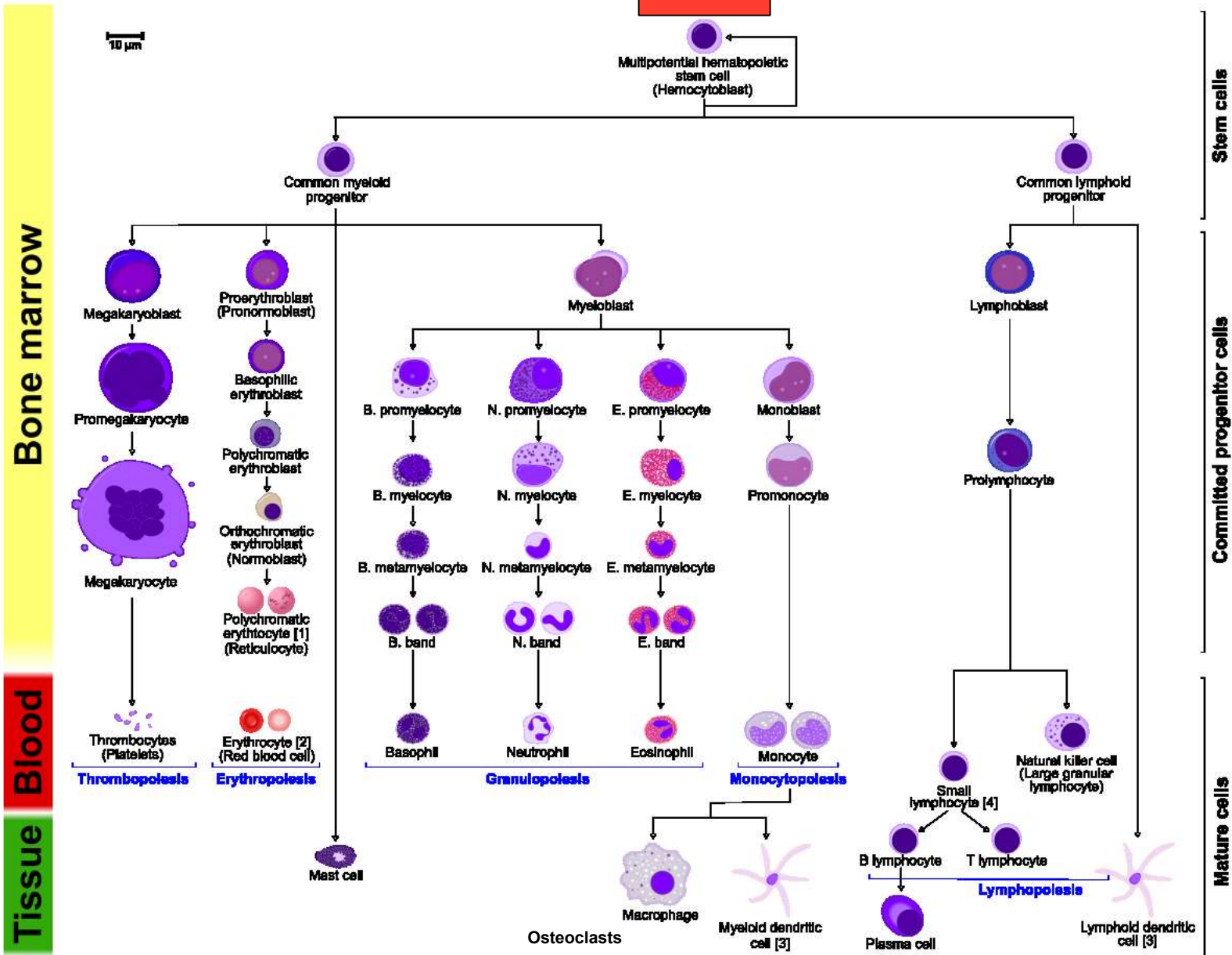
MONOCYTOPOIESIS

- **monoblast** (~16 μm)
 - round, bean shaped nucleus with 2-6 nucleoli
 - mildly basophilic cytoplasm
- **promonocyte** (~16-20 μm)
 - mitotically active (1-2 divisions)
 - large nucleus with mild indentation, unapparent nucleoli
 - basophilic cytoplasm
 - azurophilic granules
- **monocyte**
 - short-time in circulation, then extravasation and differentiation to tissue macrophages

LYMPHOPOIESIS

- **lymphoblast** (~18-20 μm)
 - round-oval nucleus with several nucleoli
 - mildly-basophilic cytoplasm without azurophilic granules
- **prolymphocyte** (~12-15 μm)
 - morphological transition and maturation to lymphocytes
- **lymphocyte**
 - further maturation and differentiation outside bone marrow

OVERVIEW OF ADULT HEMATOPOIESIS



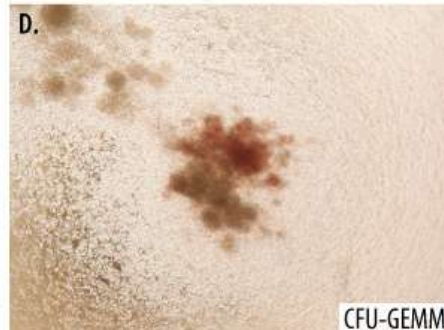
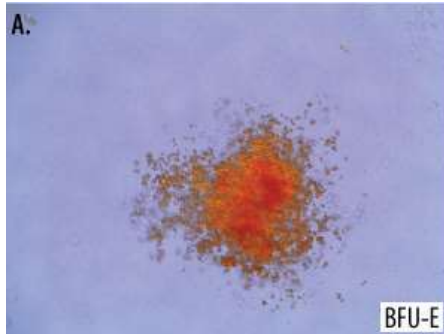
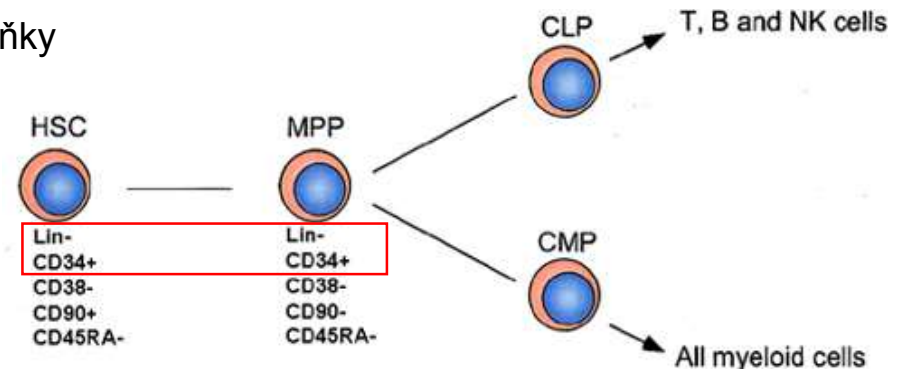
HEMATOPOIETIC STEM CELLS AND PROGENITORS

- **Hematopoietic stem cell**

- Quiescent, slow cell cycle
- Transmembrane phosphoglycoprotein CD34⁺ - adhesion within niche
- No expression of lineage surface markers (Lineage negative or Lin⁻)
- Transplantations

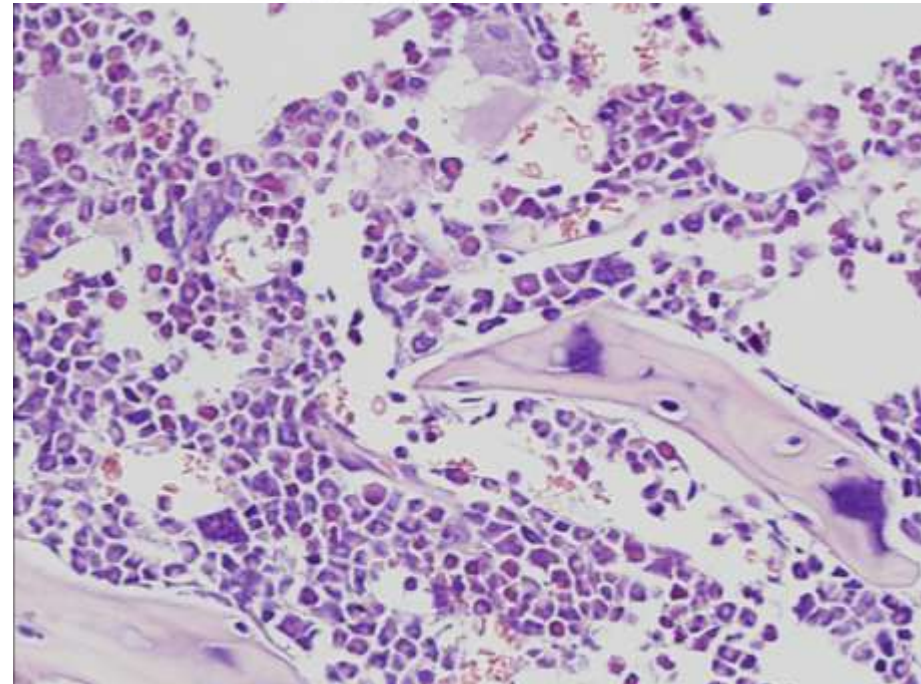
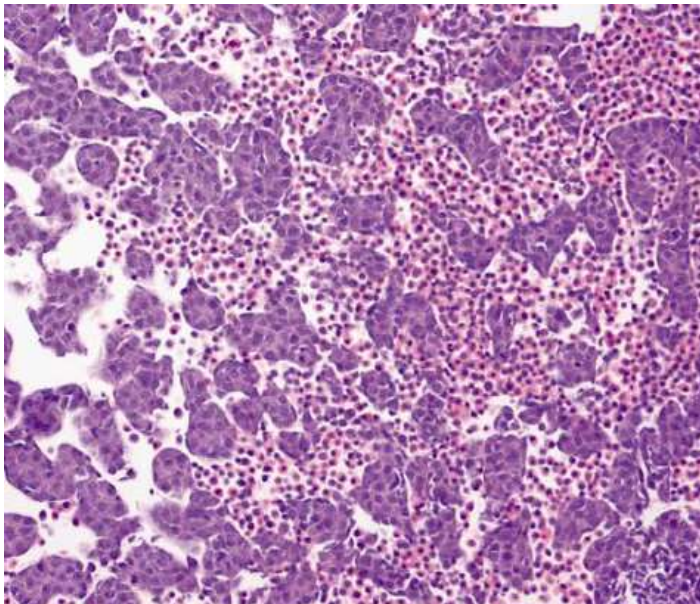
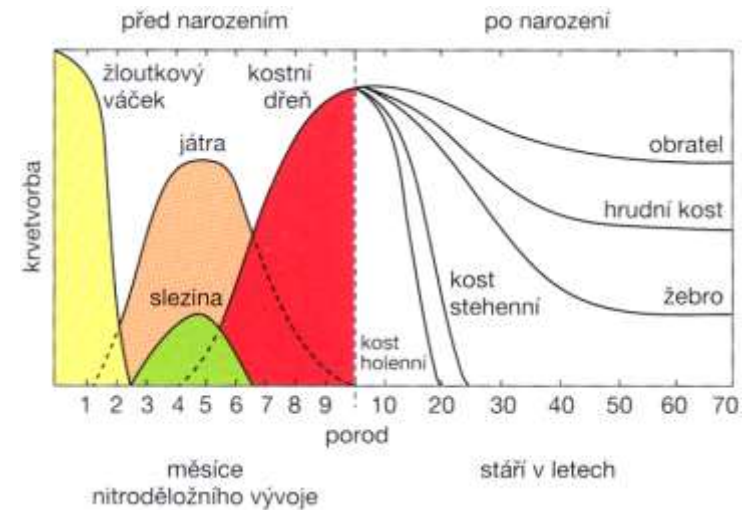
- **Colony/Burst – Forming Unit – CFU/BFU**

- Progenitory jednotlivých linií – unipotentní kmenové buňky
- Tvoří kolonie in vitro



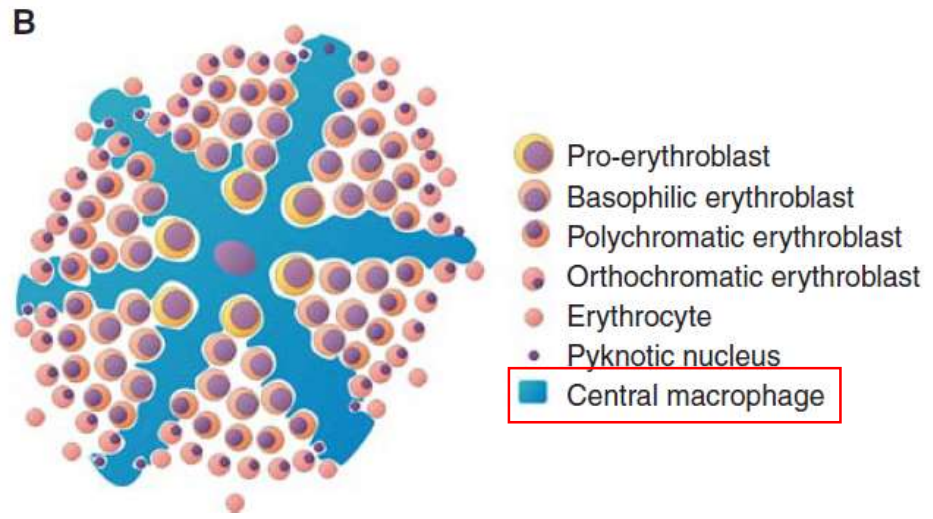
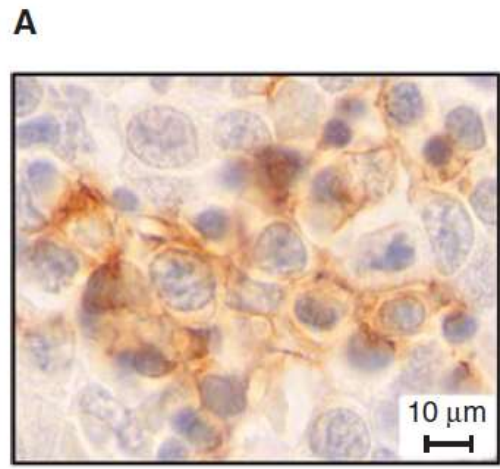
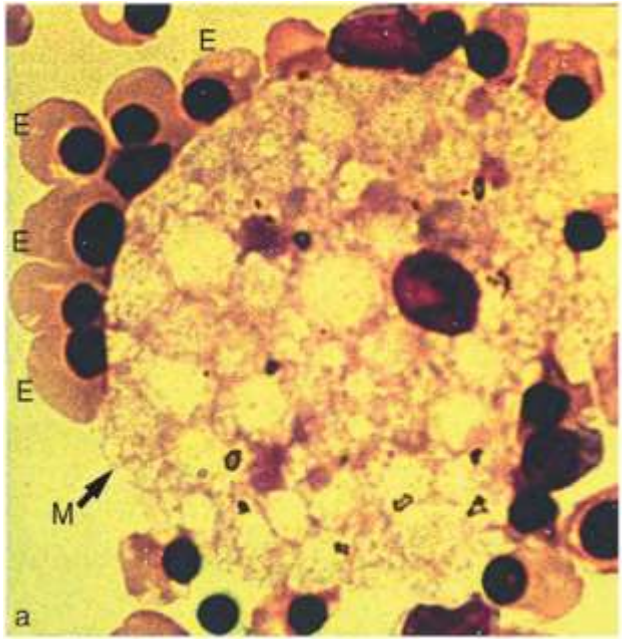
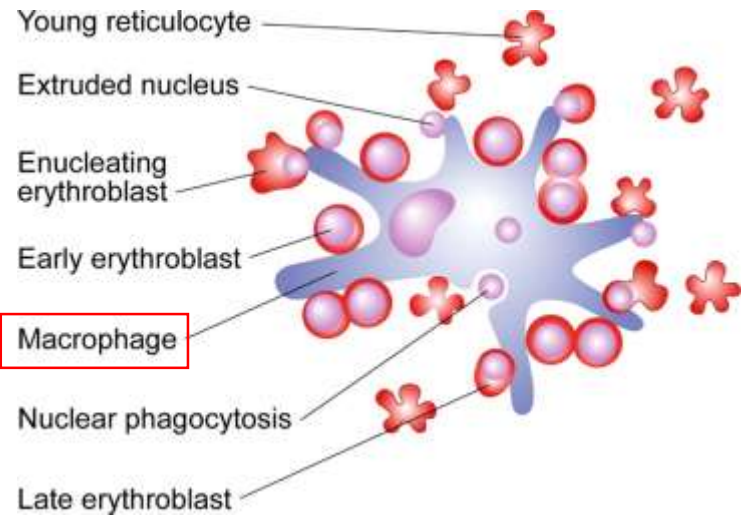
EMBRYONIC HEMATOPOIESIS

- **Extraembryonic mesoblastic period (day 16-20 – week 8)**
 - yolk sac
 - classical model – hemangioblasts (bipotent cells)
 - large, nucleated erythroid cells
- **aorta-gonad-mesonephros (day 28 – week 4)**
- **hepatolienal period (month 1 – birth)**
 - colonization of fetal liver and spleen
- **medullary period (month 4-6. – life)**
 - bone marrow



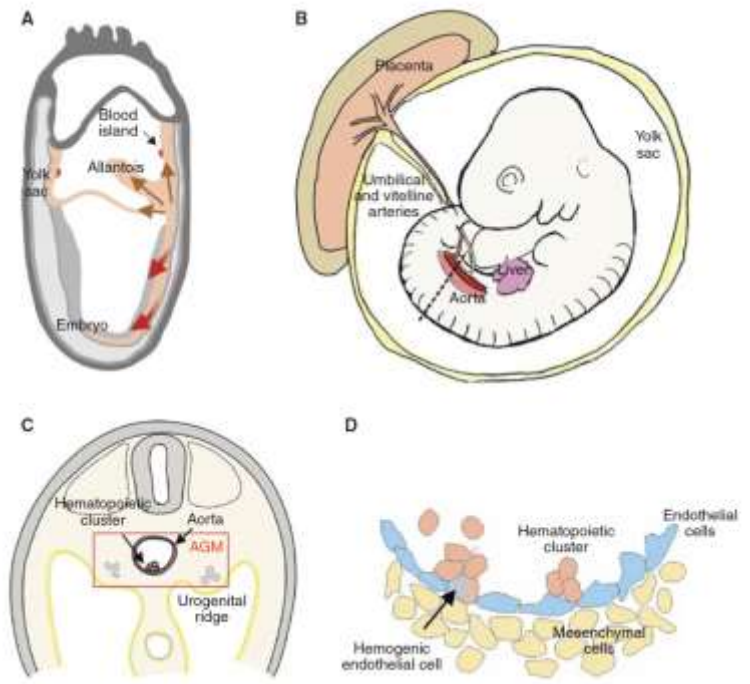
HEMATOPOIETIC ISLANDS

- hepatolienal and bone marrow hematopoiesis
- Erythroblast islands

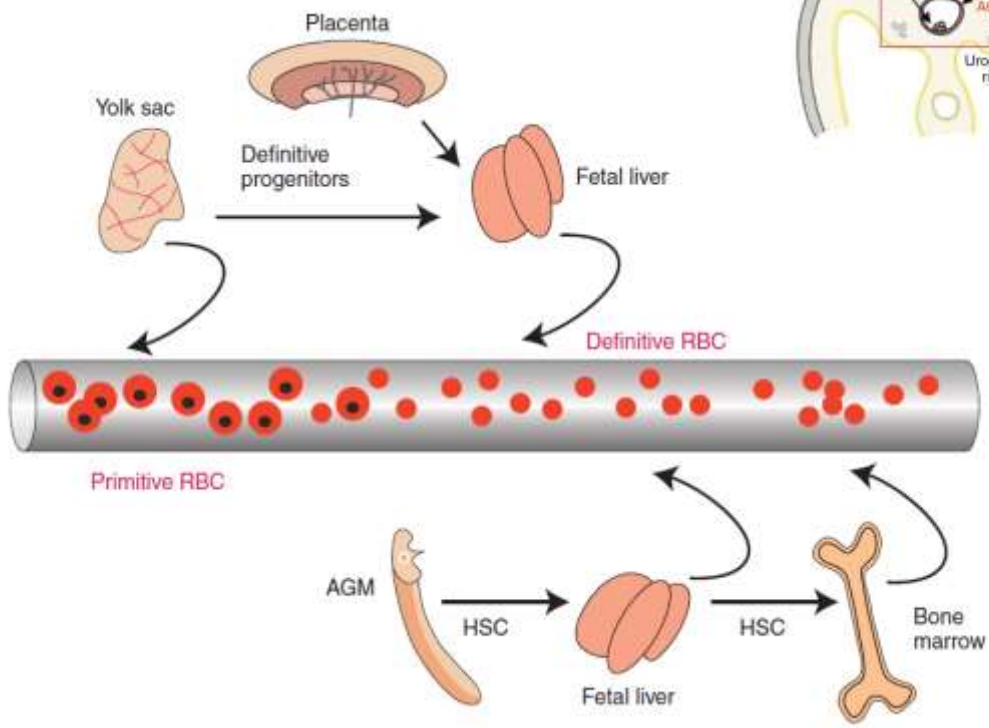


INTRAEMBRYONIC HEMATOPOIESIS

- **Aorta-gonad-mesonephros (day 28 – week 4)**
- para-aortic clusters in mesoderm of splanchnopleura
- source of embryonic HSCs



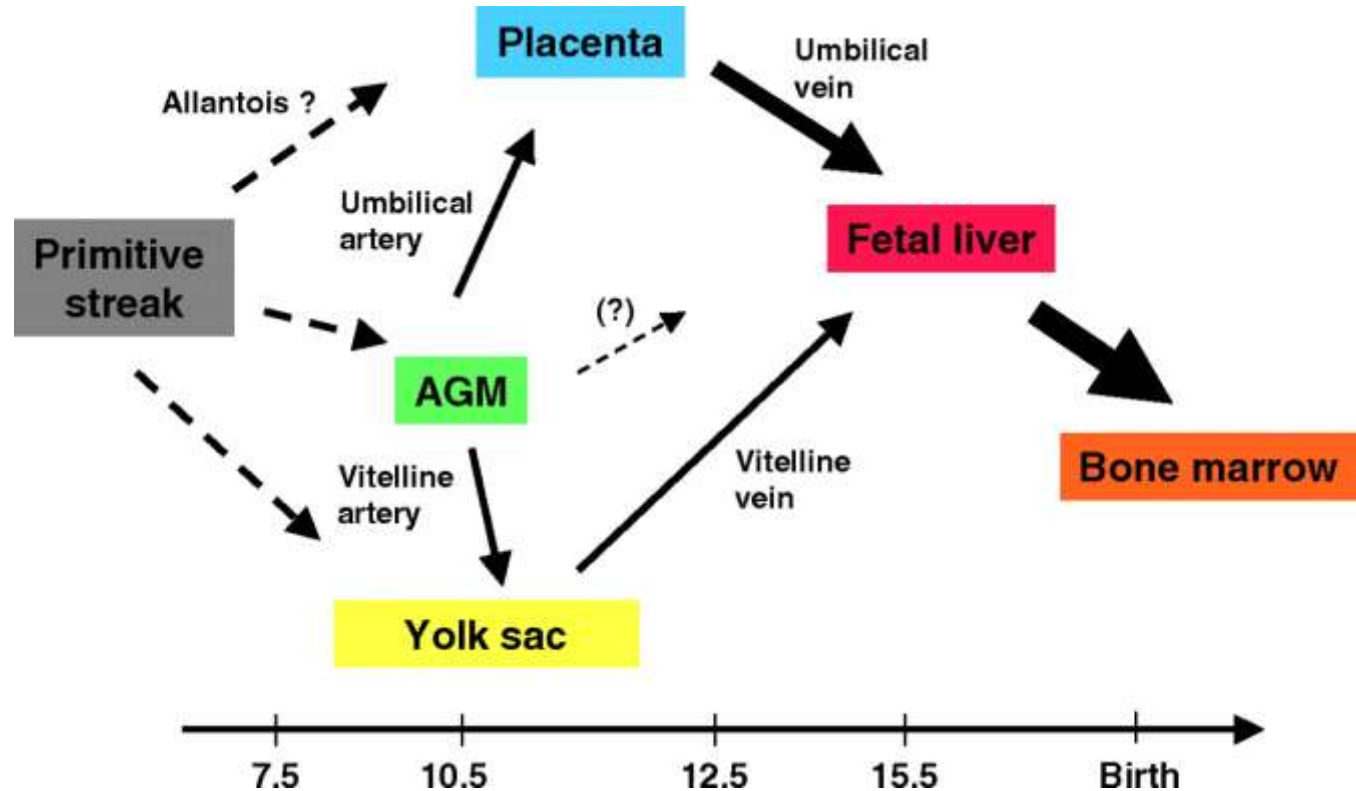
- **Placenta**



SUMMARY OF HEMATOPOIESIS

Embryonic

- yolk sac
- AGM
- liver and spleen
- bone marrow



Adult

- bone marrow (yellow, red)
- extramedullar hematopoiesis rare (pathology)

DĚKUJI ZA POZORNOST

pvanhara@med.muni.cz
www.med.muni.cz/histology

