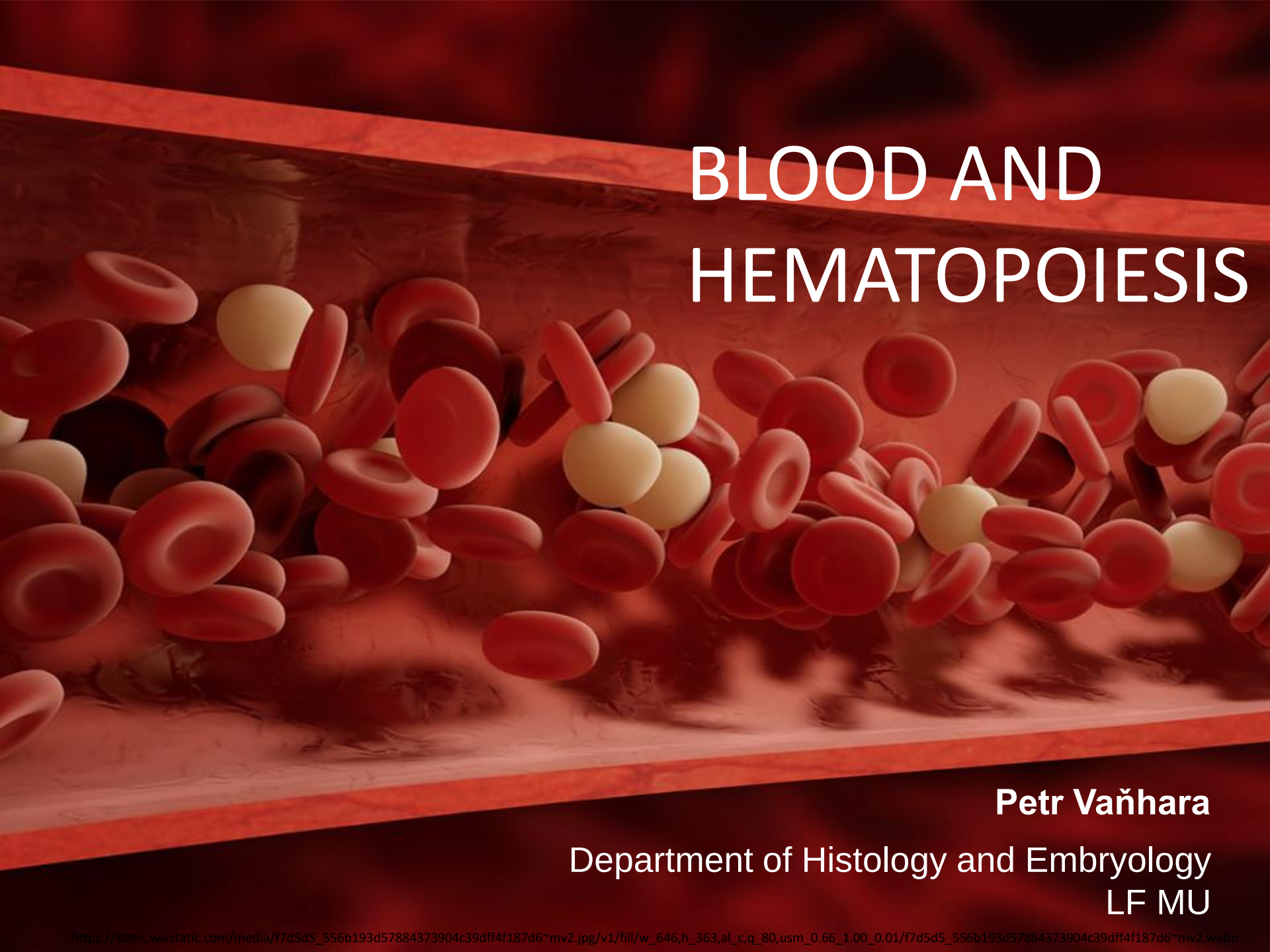

BLOOD AND HEMATOPOIESIS

Petr Vaňhara

Department of Histology and Embryology
LF MU

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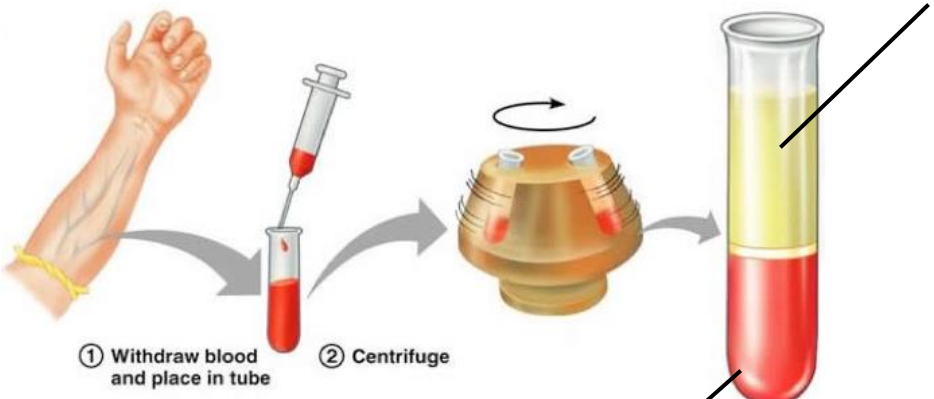
BLOOD

Blood is body fluid

- transport medium (O_2, CO_2, metabolites, hormones, nutrients...)
- homeostasis of inner body environment (thermoregulation, acidobasic equilibrium, oncotic pressure)
- integrity of cardiovascular system (clotting cascade)
- immune reactions



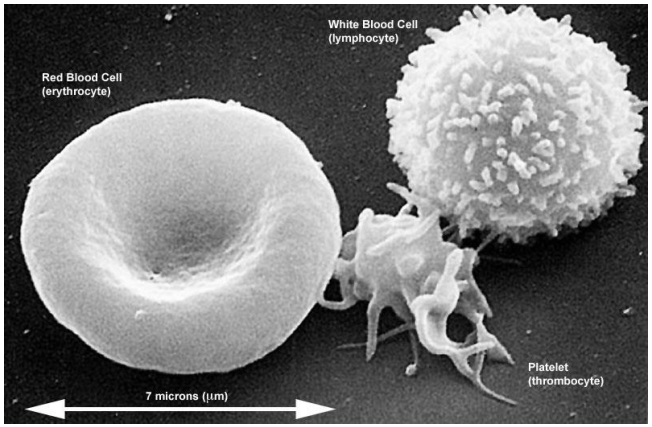
plasma

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

Blood can be considered as a specialized connective tissue

formed blood elements

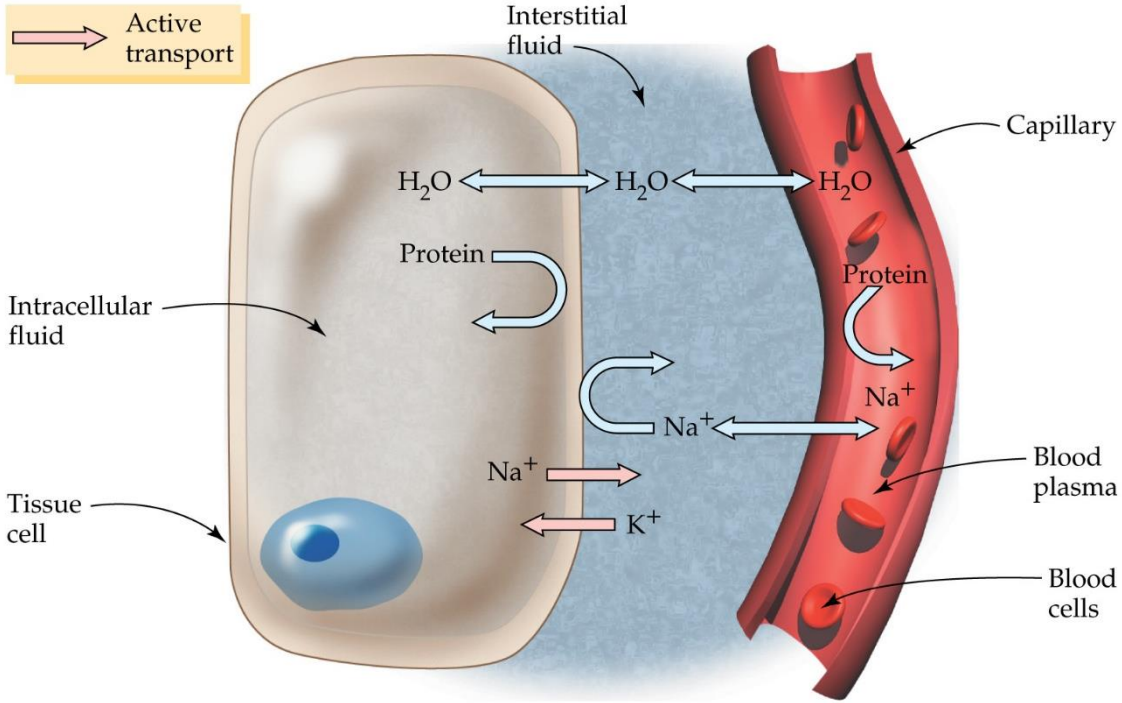
- erythrocytes
- leukocytes
- thrombocytes

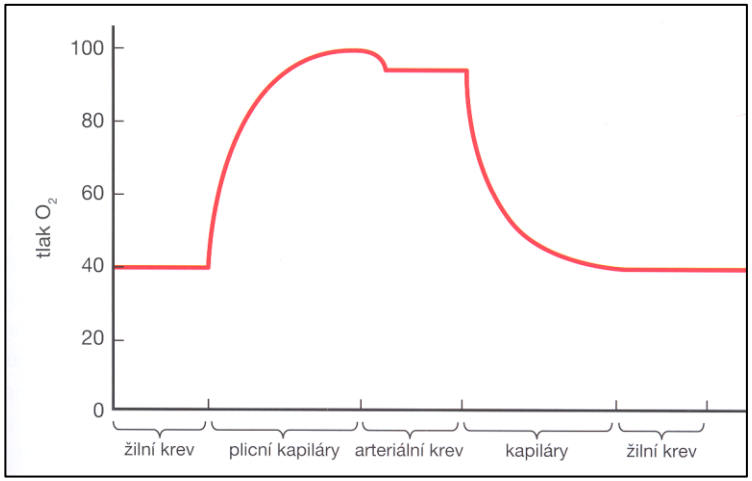


BLOOD PLAMA AND TISSUE FLUID

plasma

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





Venous blood

Lung capillaries

Arterial blood

Capillaries

Venous blood

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

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

Cations	Sodium	136–148 mmol/l	Osmotic pressure, volume, pH
	Potassium	3,7–5,0 mmol/l	Membrane potential of cells (nerve, muscle)
	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 ♂ Iron ♀	12–27 µmol/l 10–24 µmol/l	Cofactor of enzymes, in hem of hemoglobin
	Copper	12–22 µmol/l	Cofactor of enzymes
Anions	Chlorides	95–110 mmol/l	Osmotic pressure, volume, pH
	Bicarbonates $[HCO_3]^-$	22–26 mmol/l	Transport of CO_2, buffer - pH
	P_i	0,6–1,4 mmol/l	Buffer - pH
	Iodide	276–630 µmol/l	Hormones of thyroid gland

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

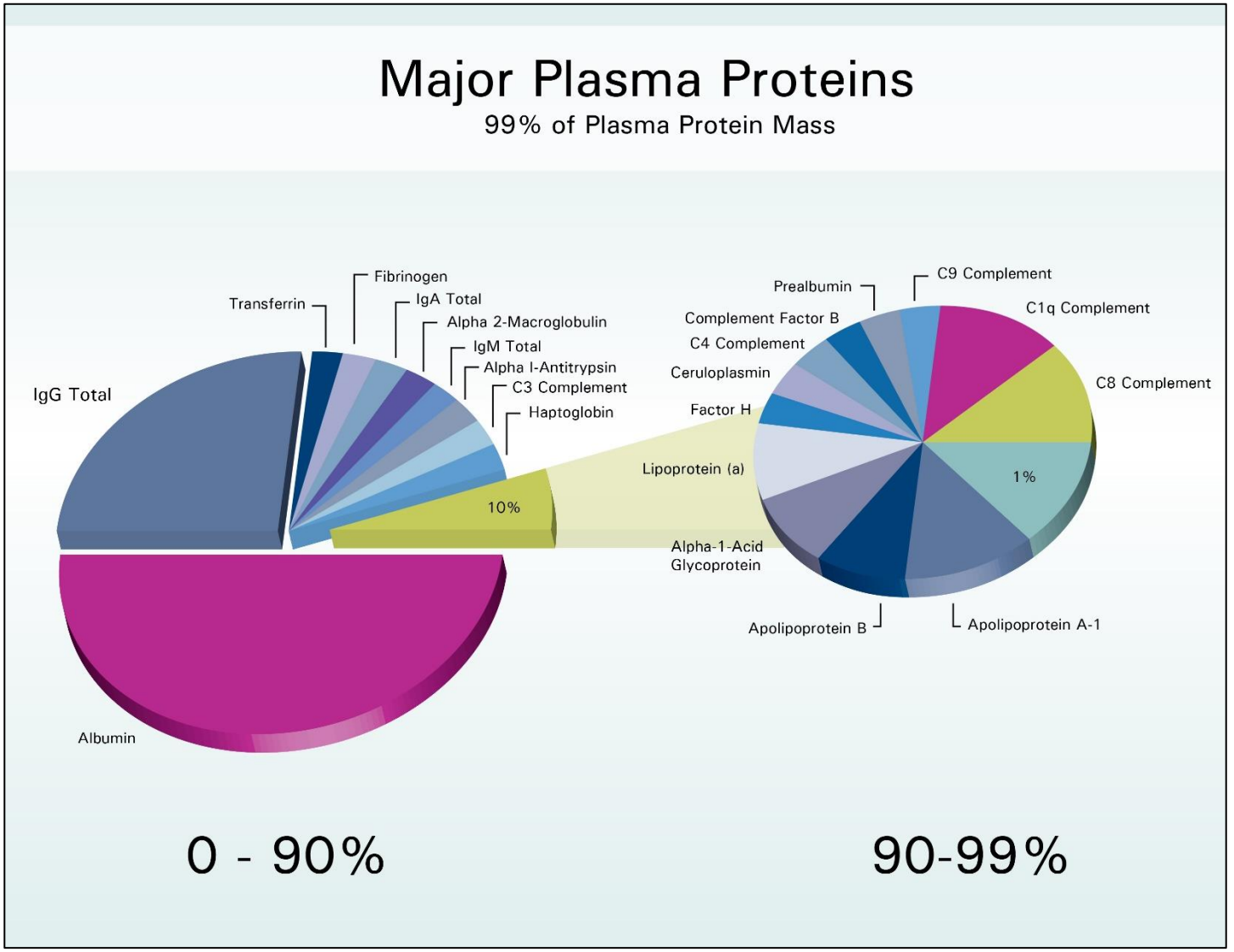
- ~ 1% **ions** (Na^+, K^+, Ca^+, Mg^+, Cl^-, HCO_3^-), **low mass organic compounds** (glucose, aminoacids, cholesterol, lipids, waste products of metabolism), **respiration gases**

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 µmol/l
Cholesterol (total)	3,5–5,2 mmol/l
Bilirubin	3,3–18,0 µmol/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



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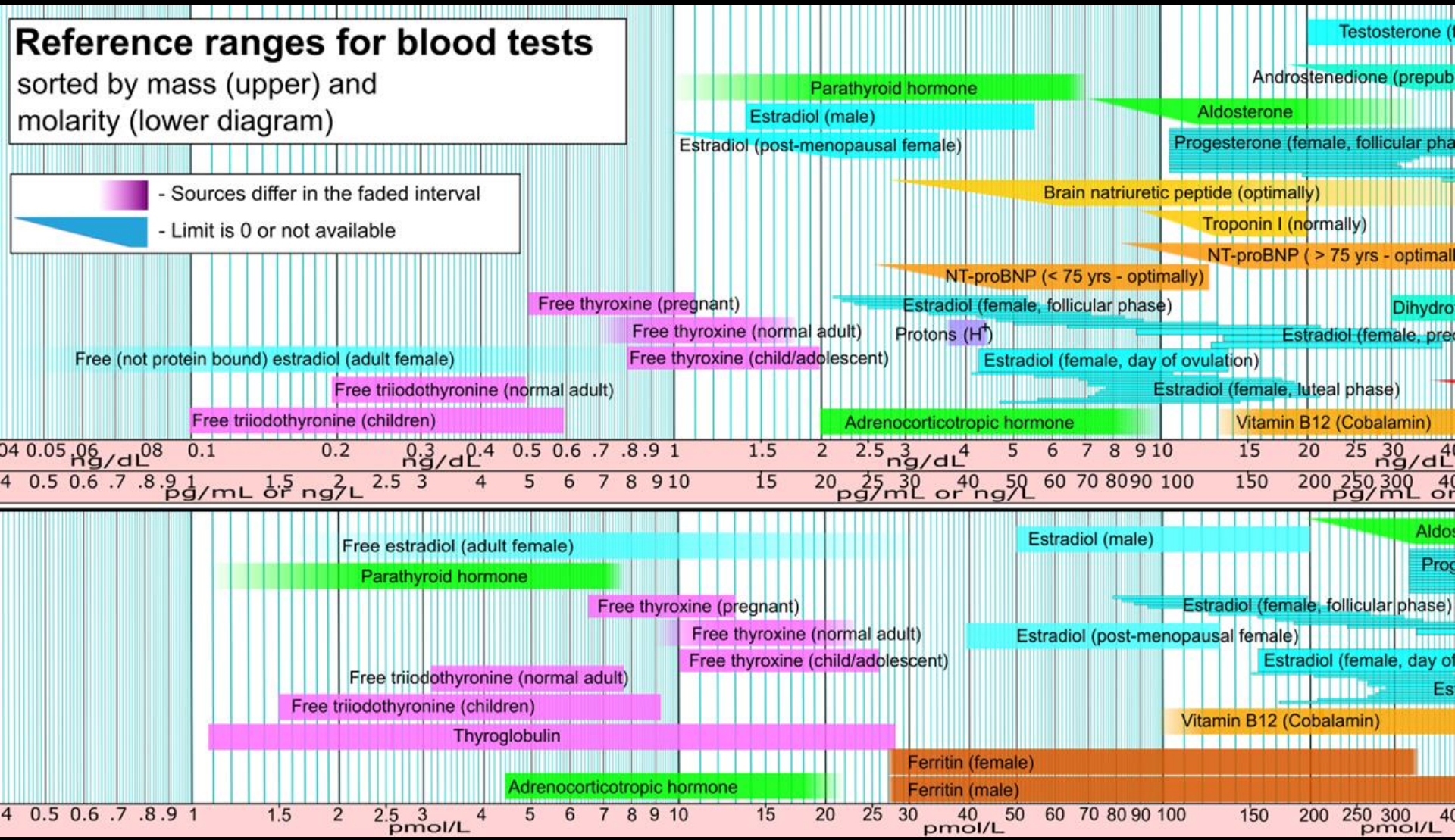
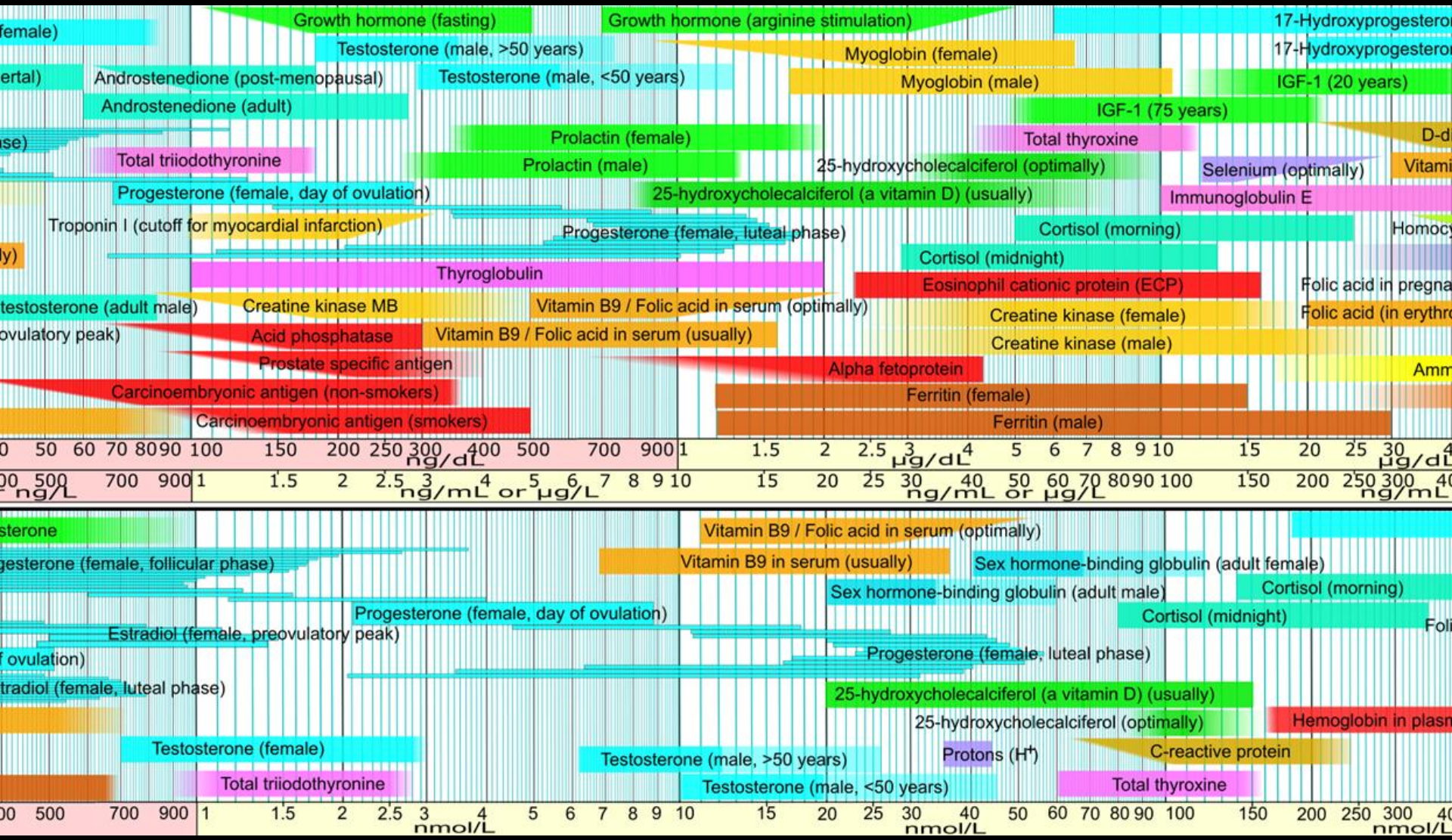


https://en.wikipedia.org/wiki/Reference_ranges_for_blood_tests



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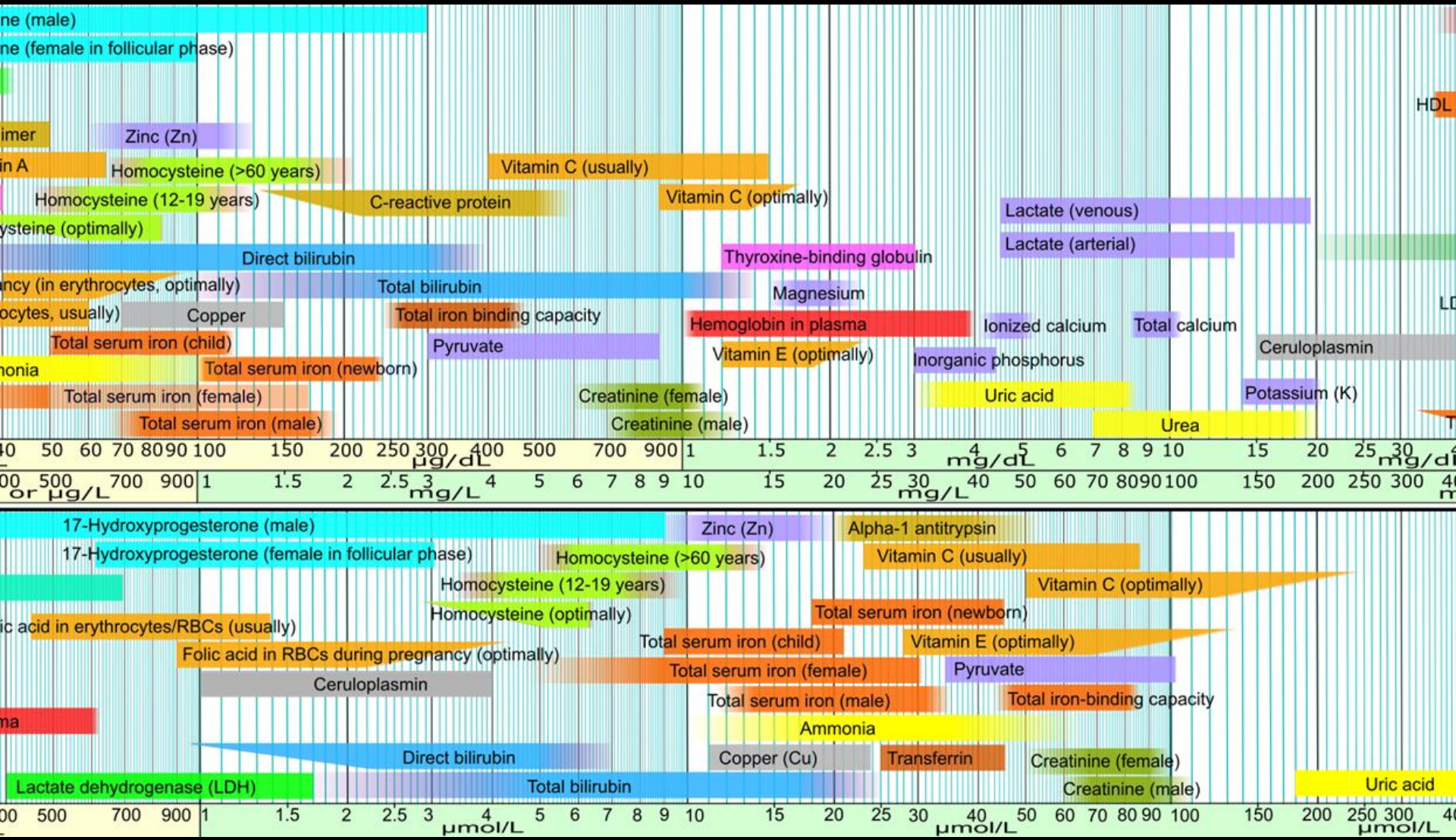

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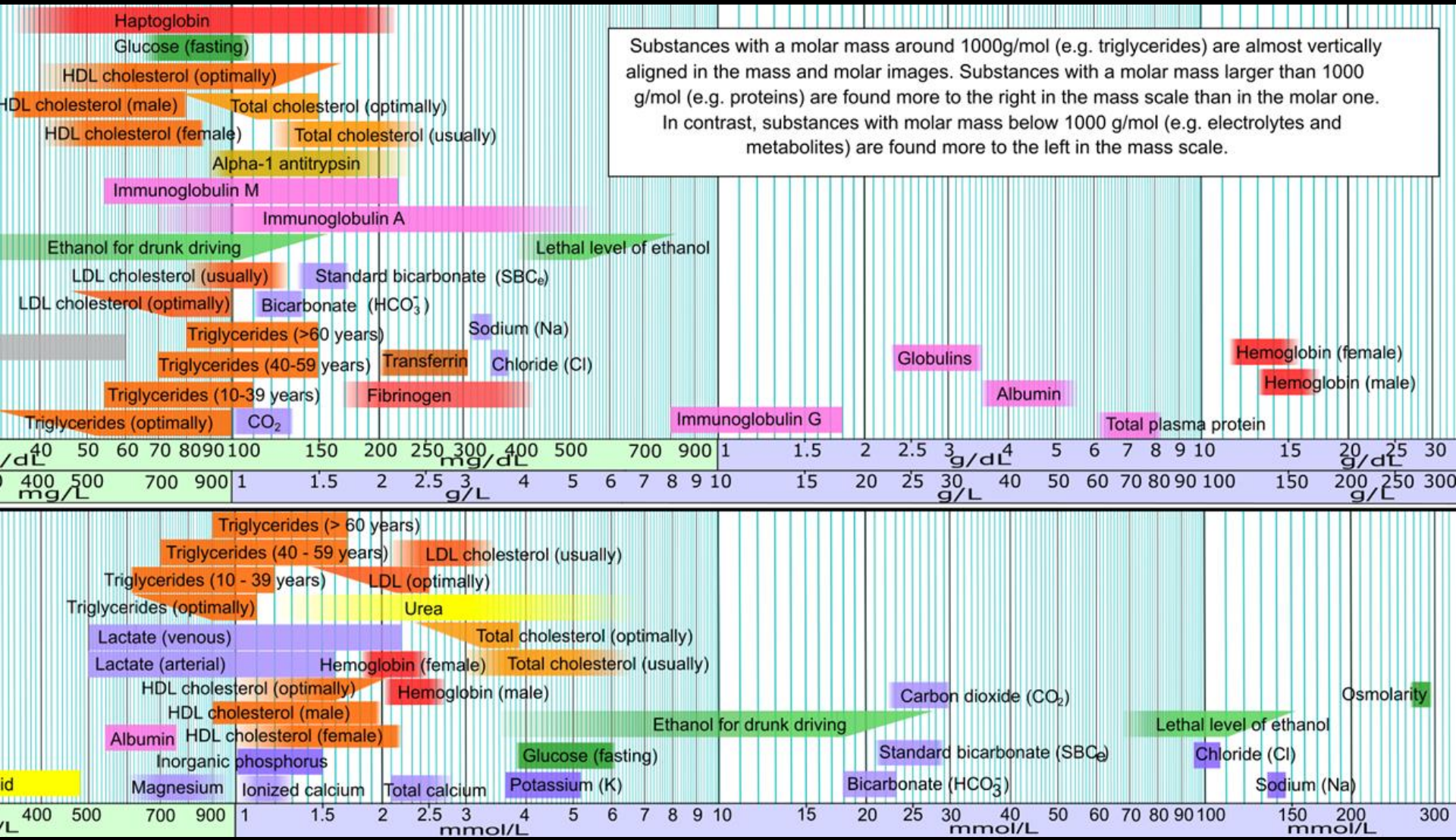
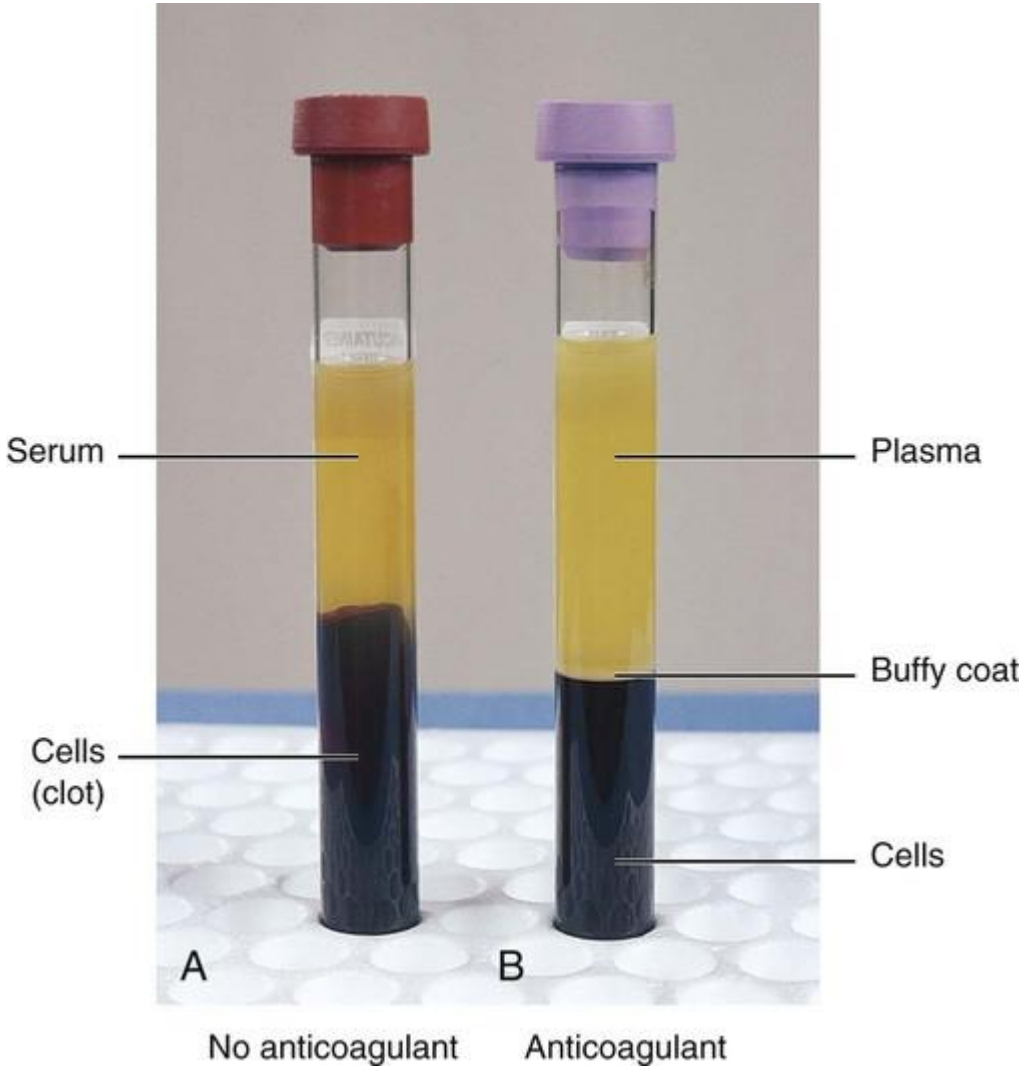


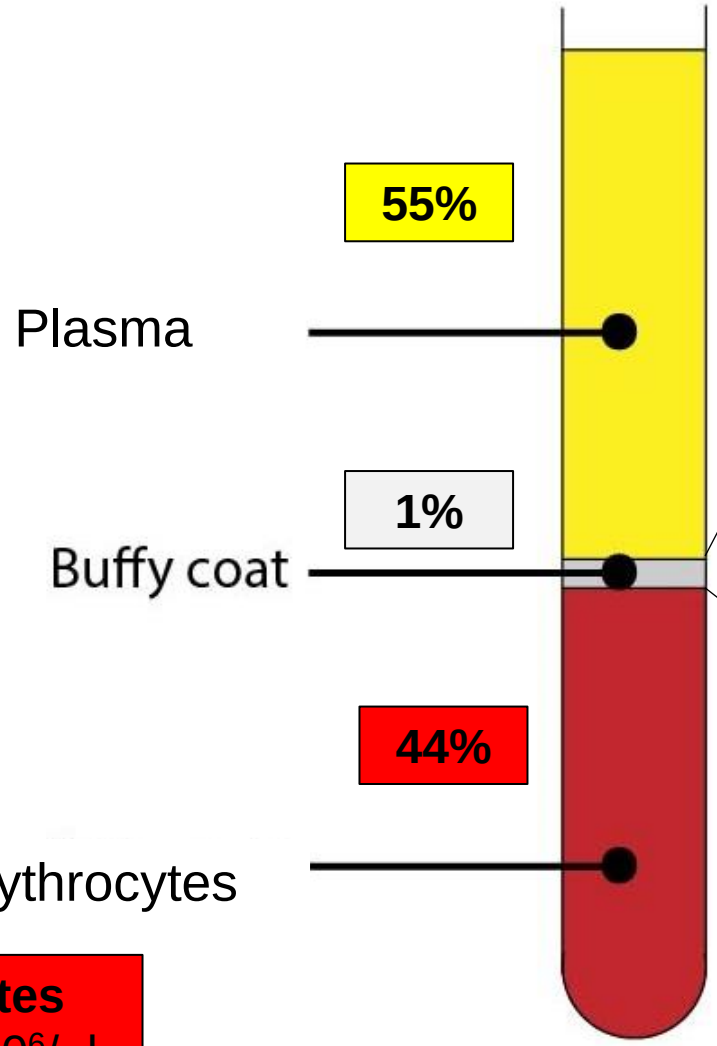
https://en.wikipedia.org/wiki/Reference_ranges_for_blood_tests

BLOOD PLASMA AND SERUM

- **serum ≠ plasma**



FORMED BLOOD ELEMENTS



Leukocytes
4.5-11 × 10^3/μl

neutrophils	71%
eosinophils	3%
bazophils	1%
monocytes	5%
lymfocytes	20%

Thrombocytes
150-450 ×10^3/μl

Erythrocytes
4.2-6.2 × 10^6/μl

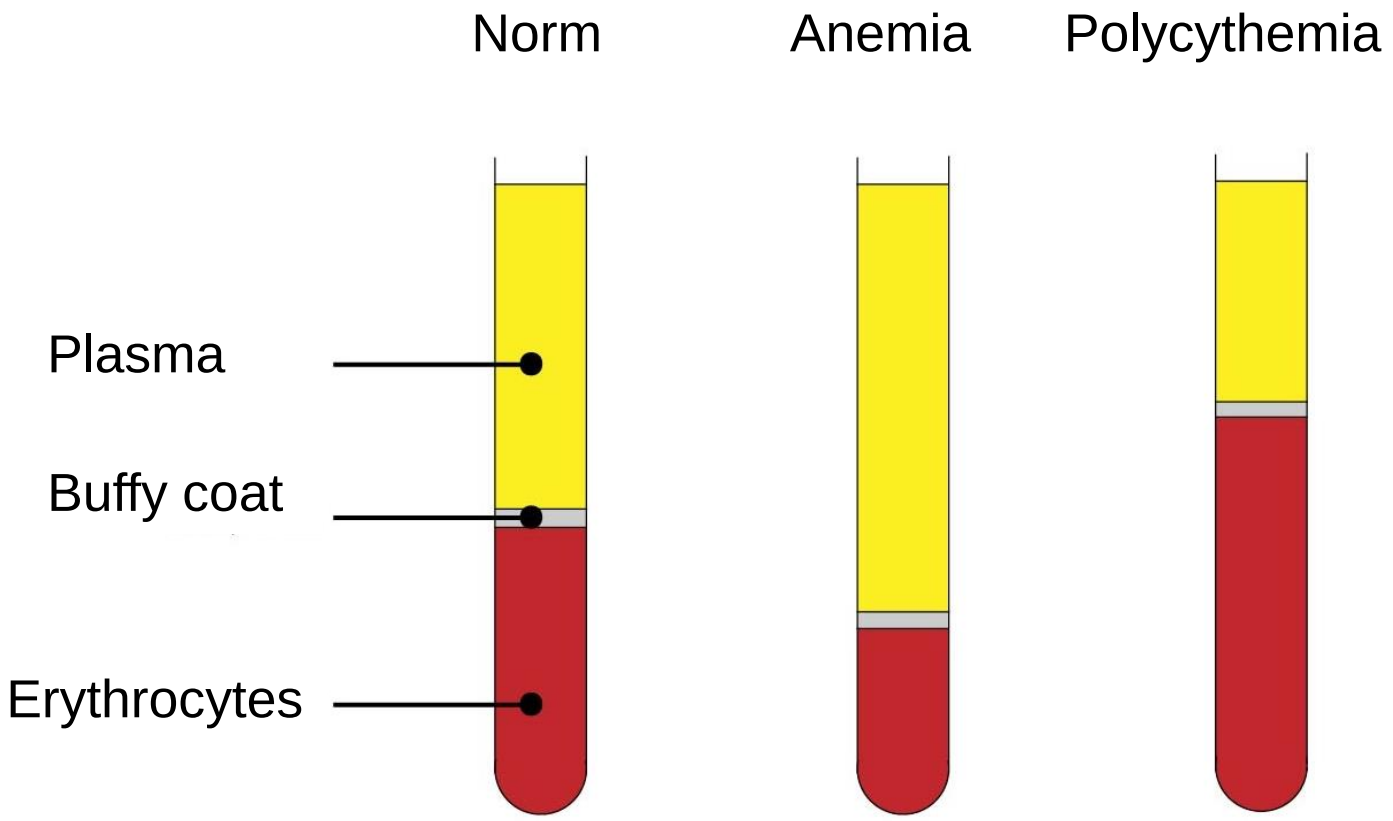
Ratio of erythrocyte mass volume to volume of full blood

Erythrocytes
4.2-6.2 × 10^6/μl

HEMATOCRIT

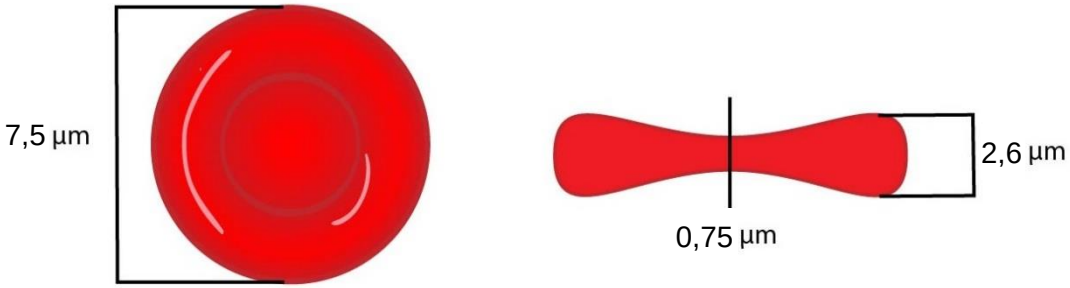
♂ **47±5%**

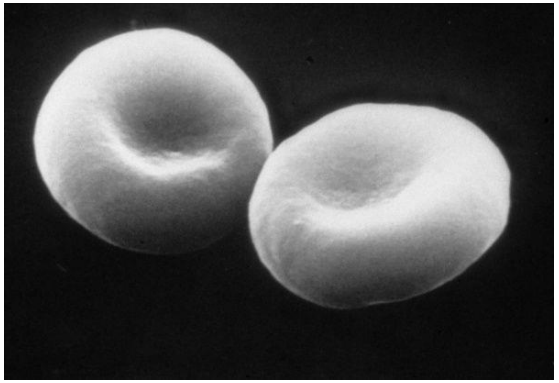
♀ **42±4%**

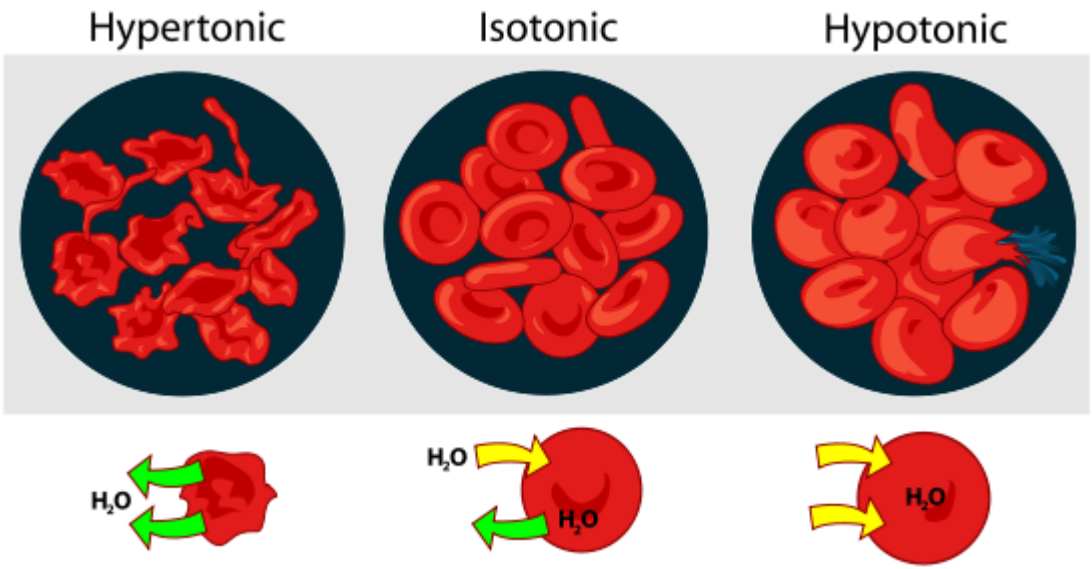


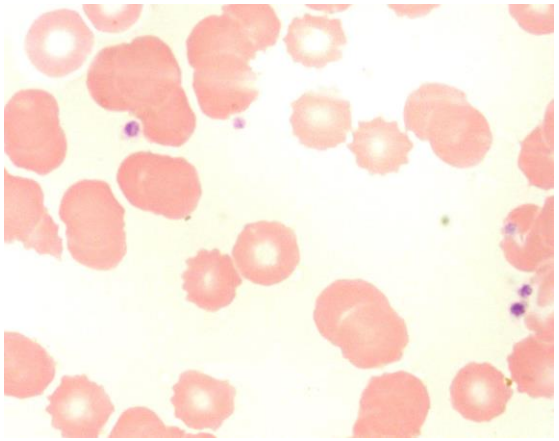
ERYTHROCYTES

Size depends on osmotic pressure of environment





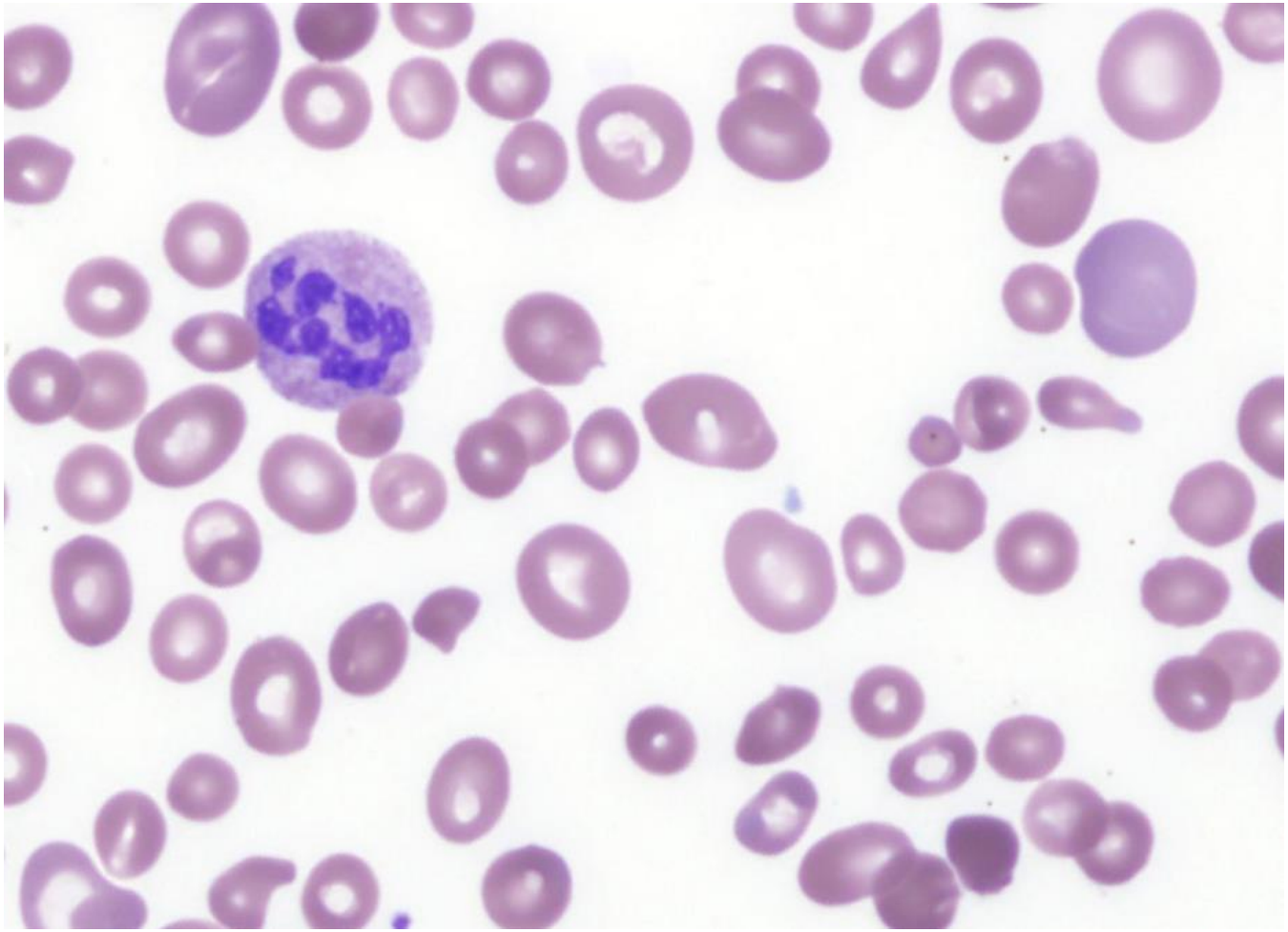




ERYTHROCYTES

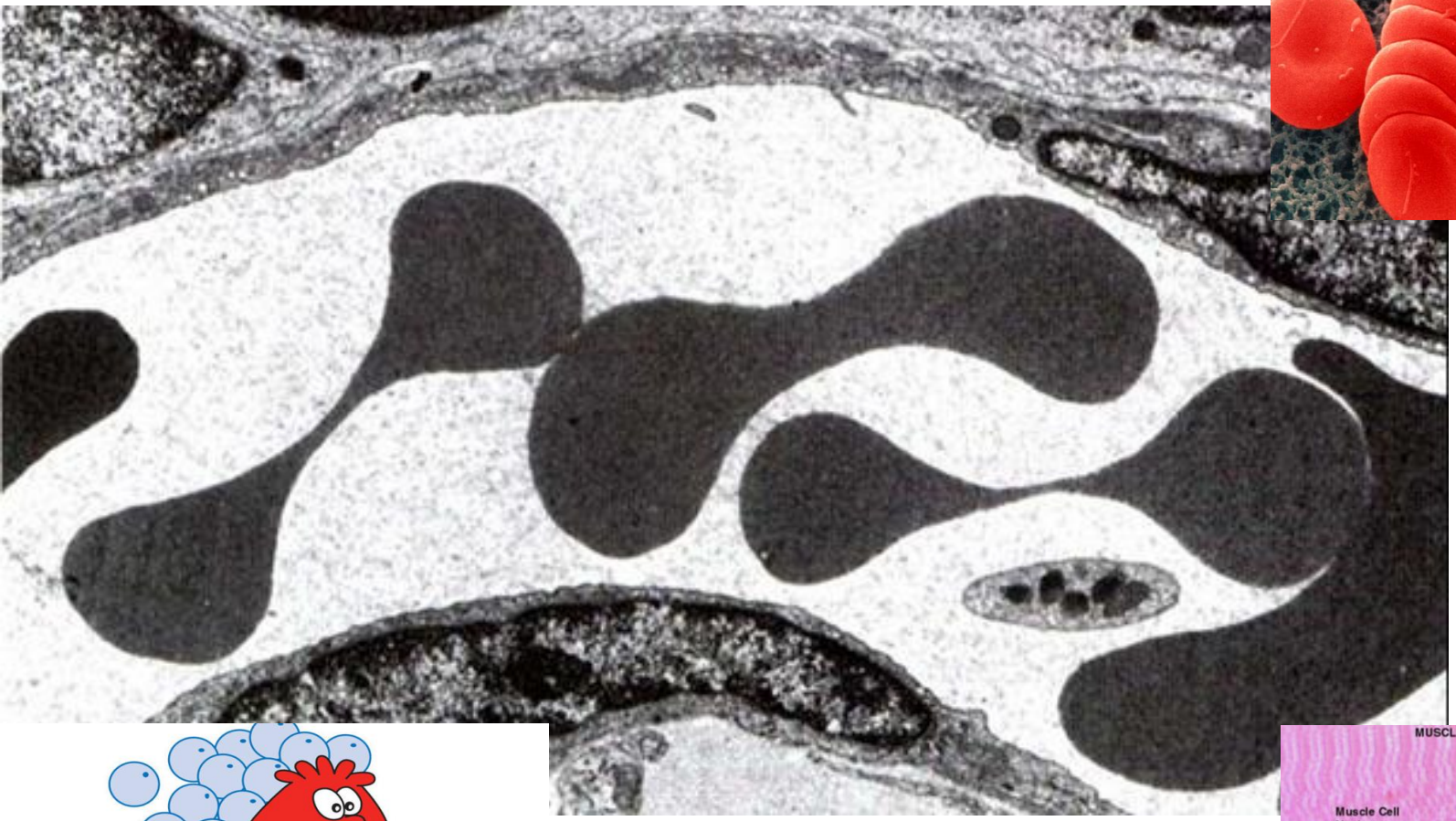
Deviations from normal size

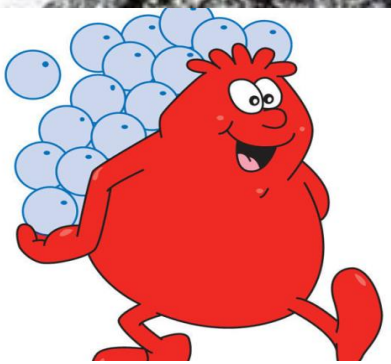
- **anisocytosis**
- macrocytes (>9 μm)
- microcytes (<6 μm)

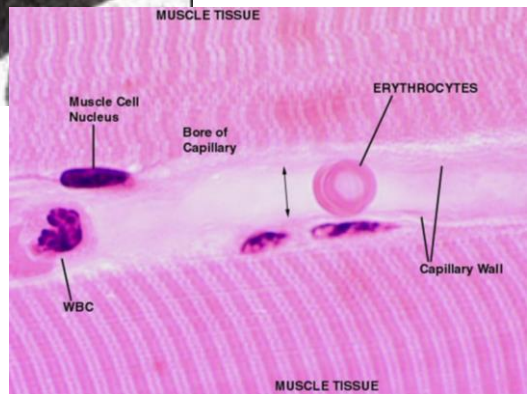


ERYTHROCYTES

Erythrocyte is an amazingly flexible cell







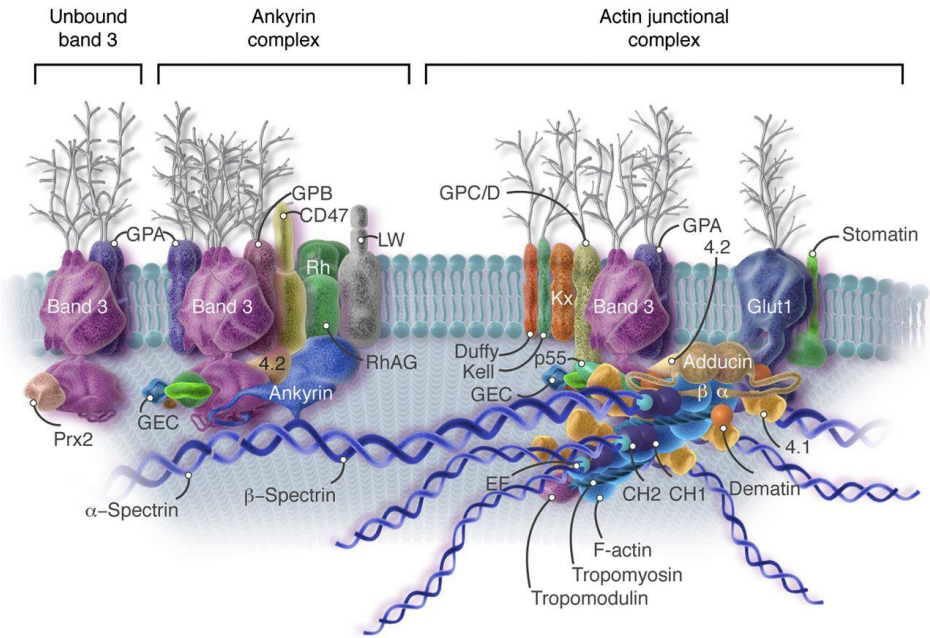
ERYTHROCYTES

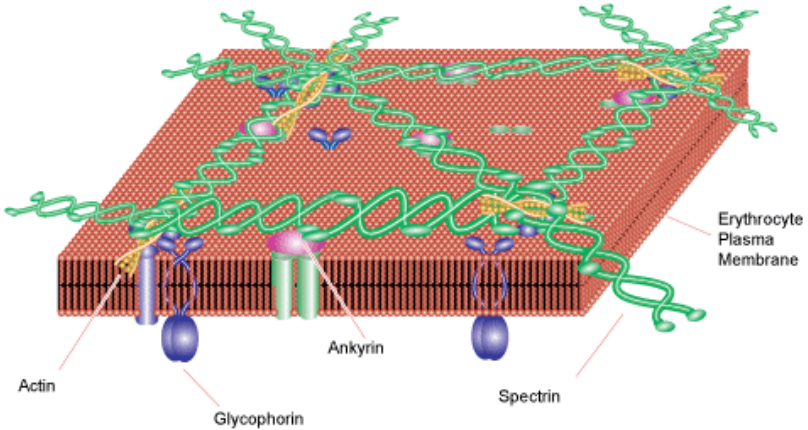
Shape of erythrocytes

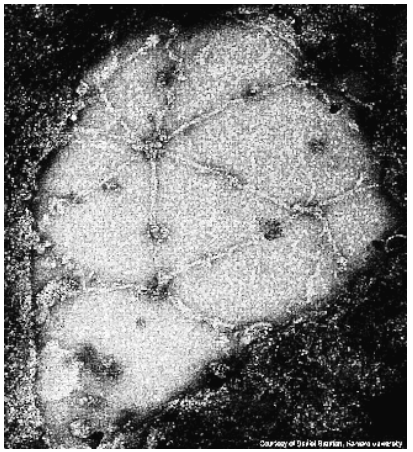
- **integral proteins**
- band 3, glycoprotein A (ion transporters)
- **spectrin**
- **ankyrin**

- **aktin a s aktinem asociované proteiny**
- tropomodulin, tropomyosin

- **hemoglobin**





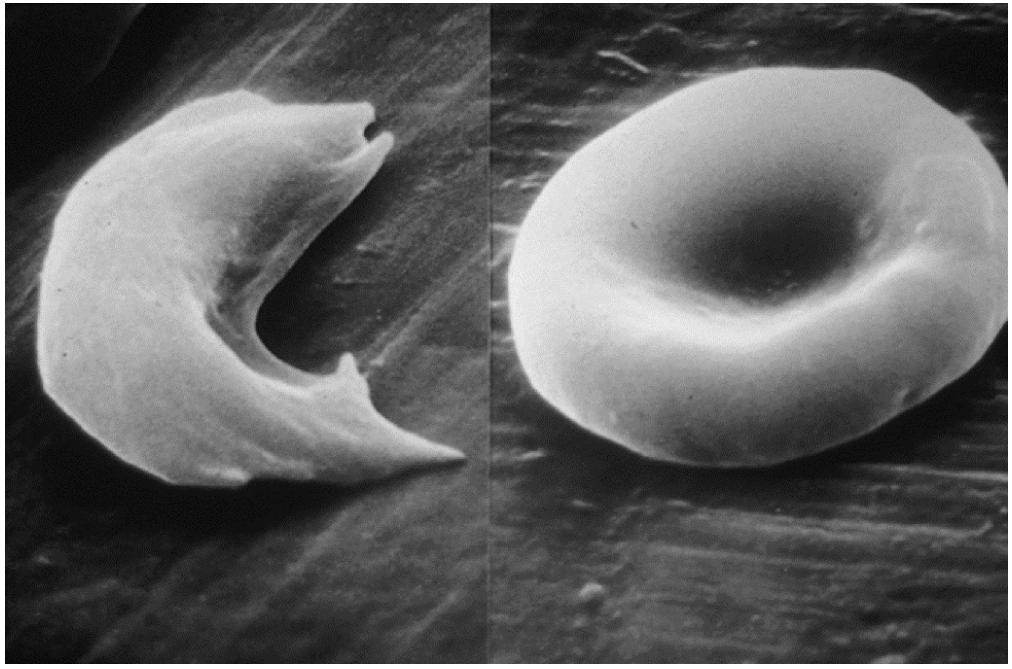


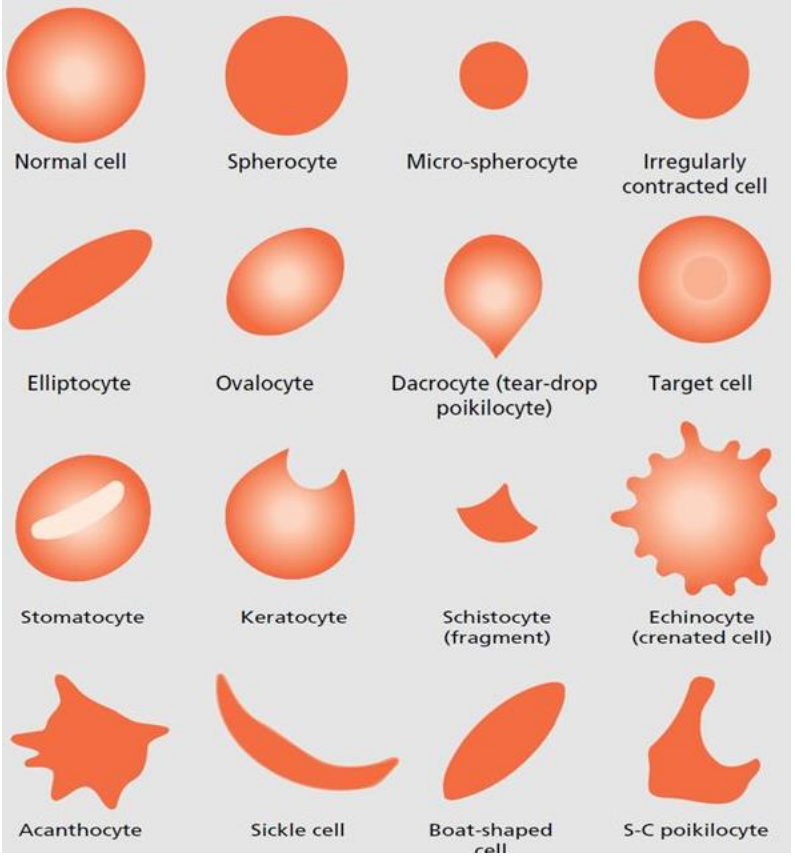
ERYTHROCYTES

Deviations from biconcave shape

- **poikilocytosis**

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

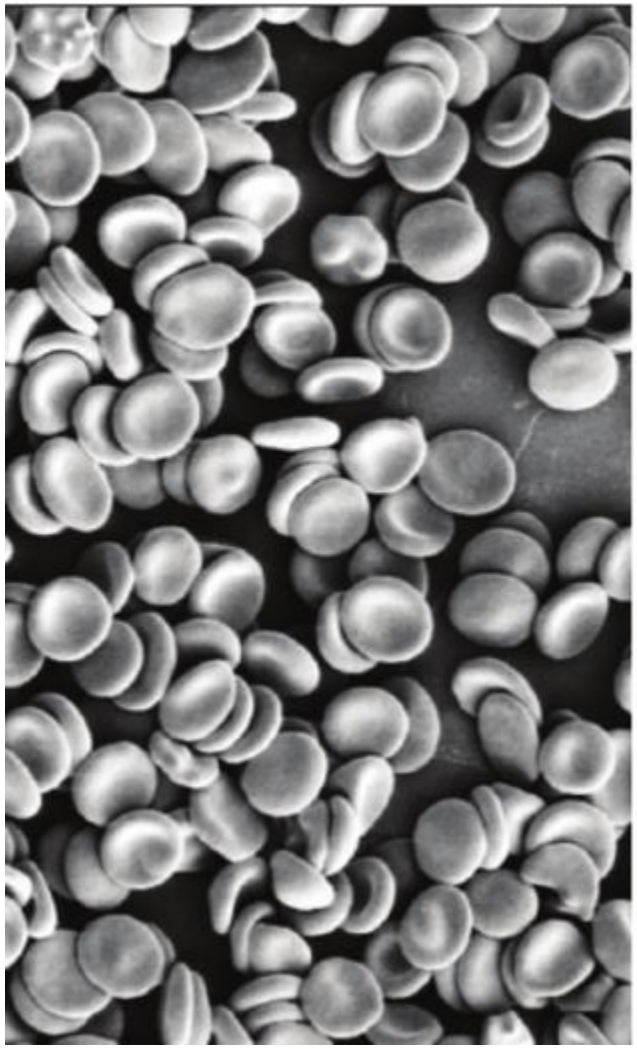




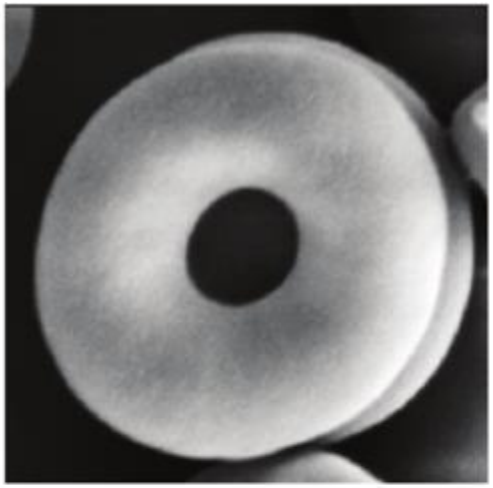
ERYTHROCYTES

Deviations from biconcave shape

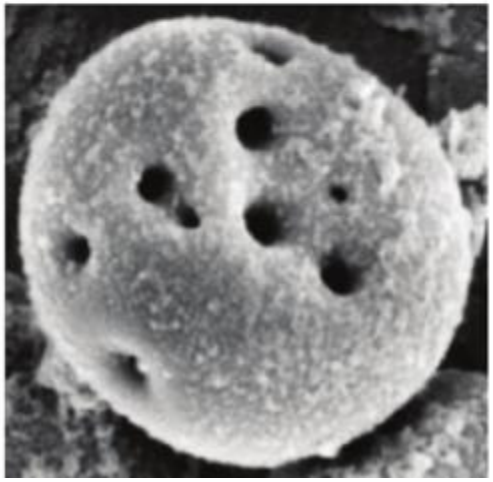
Normal



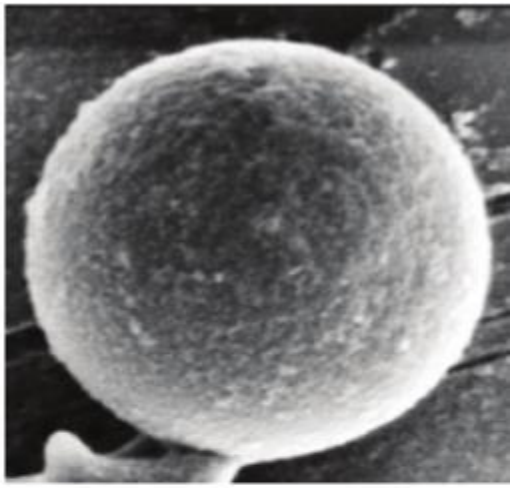
Codocyte



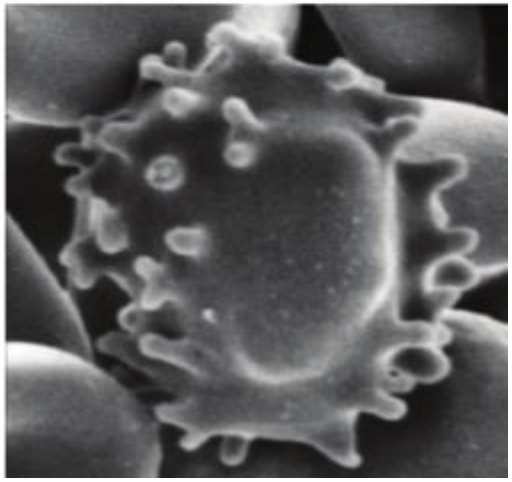
(b)



Spherocyte



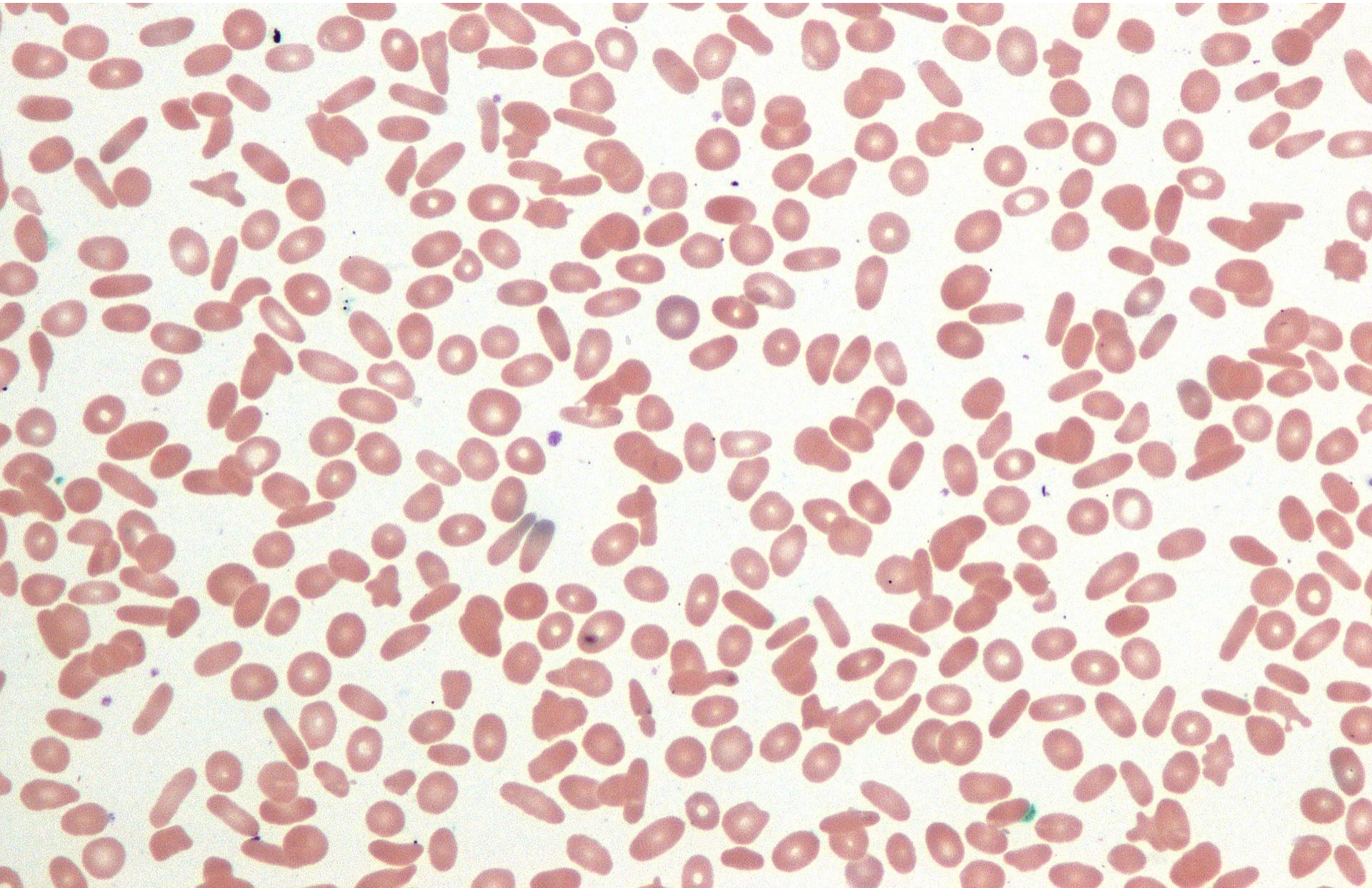
(d)



Echinocyte

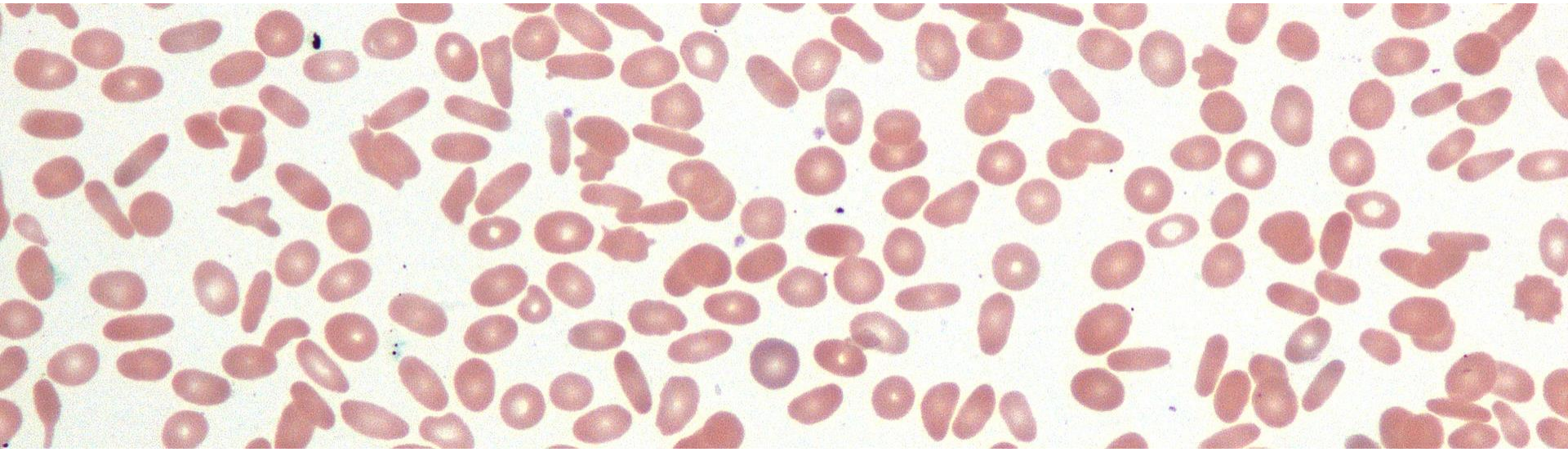
ERYTHROCYTY

Hereditary eliptocytosis

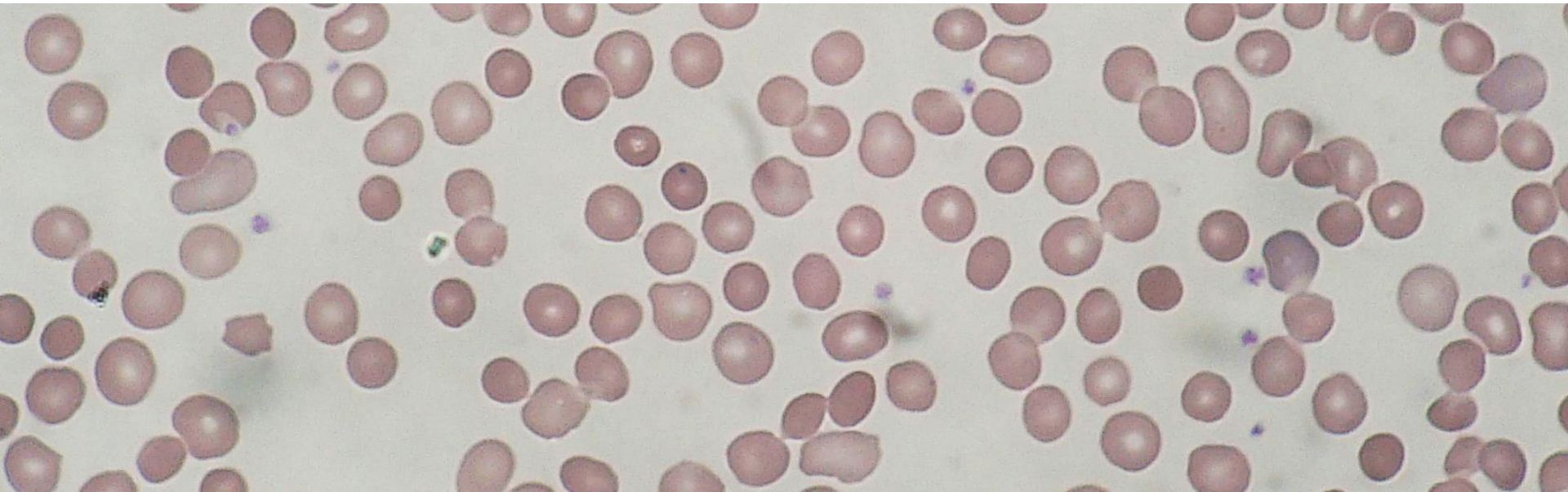


ERYTHROCYTES

Hereditary eliptocytosis

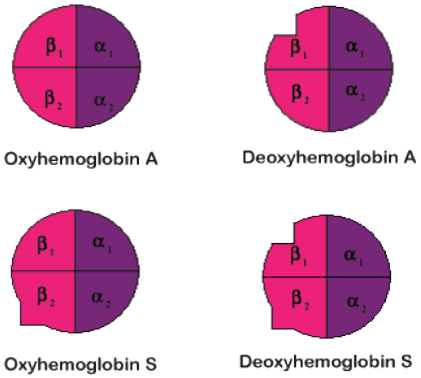


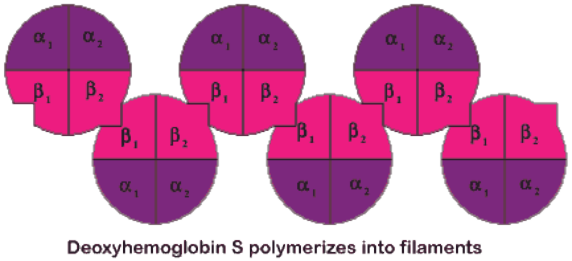
Hereditary spherocytosis

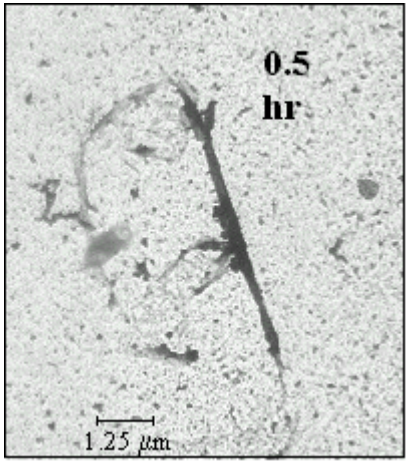


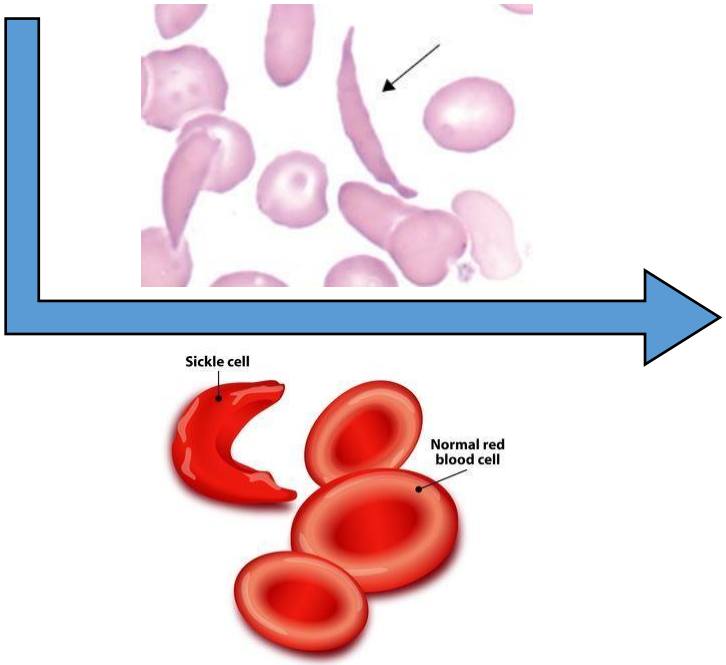
Sickle cell anemia

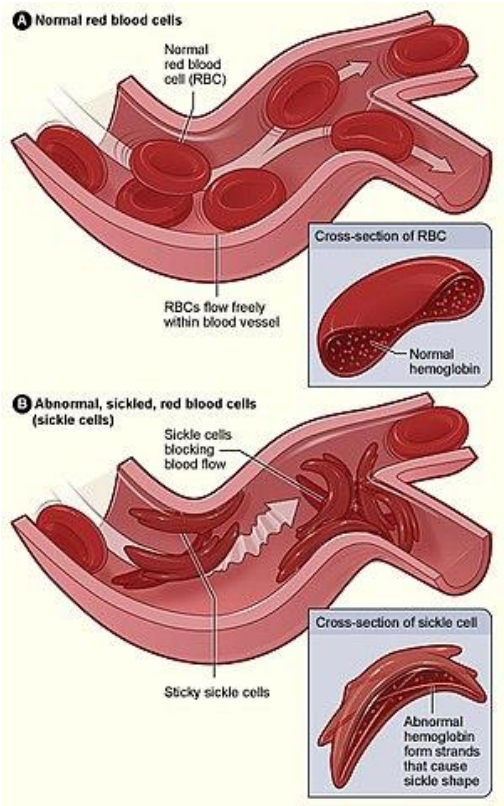
- Abnormal hemoglobin (hemoglobin S)







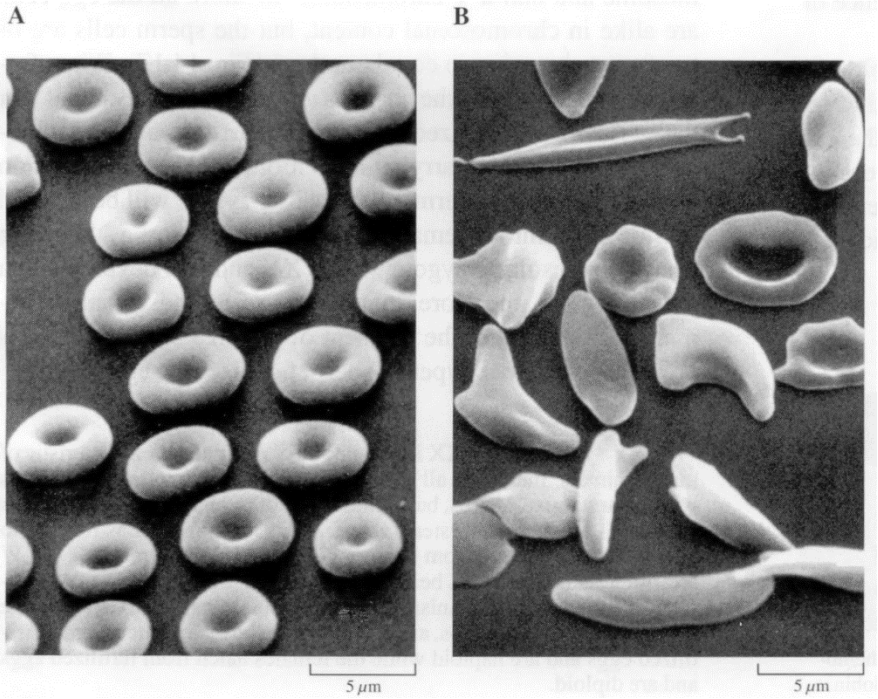




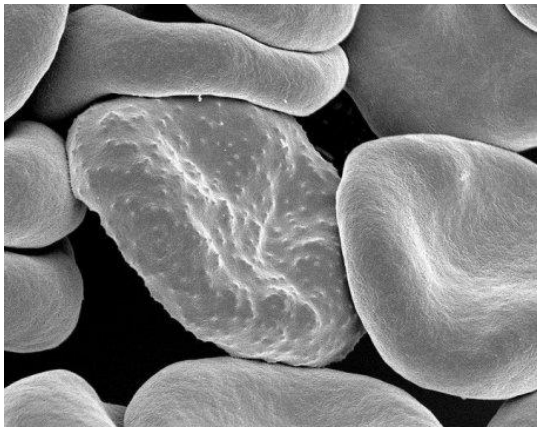
ERYTHROCYTES

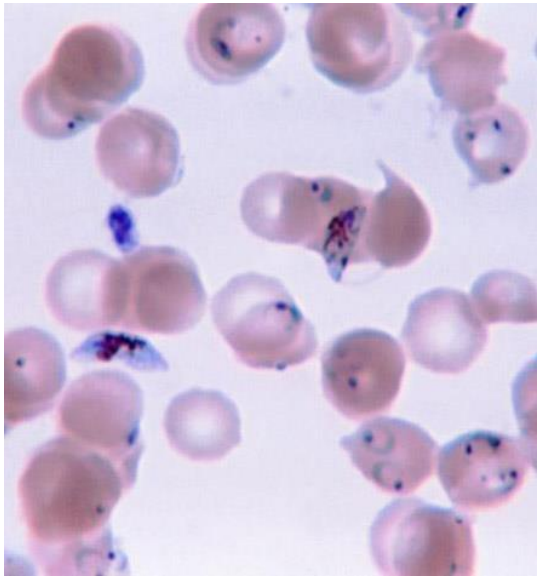
Sickle cell anemia

- pathological genotype (heterozygote HbS/HbA) is beneficial



Malaria

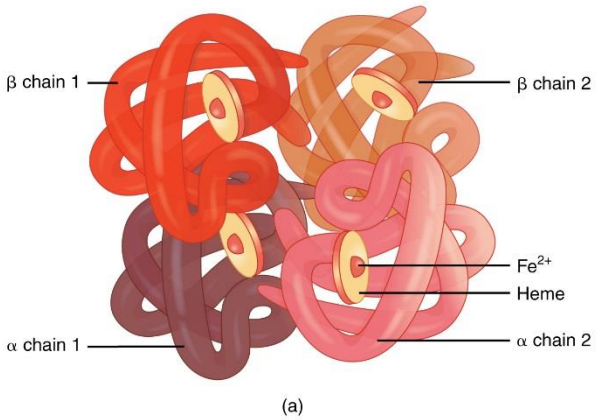


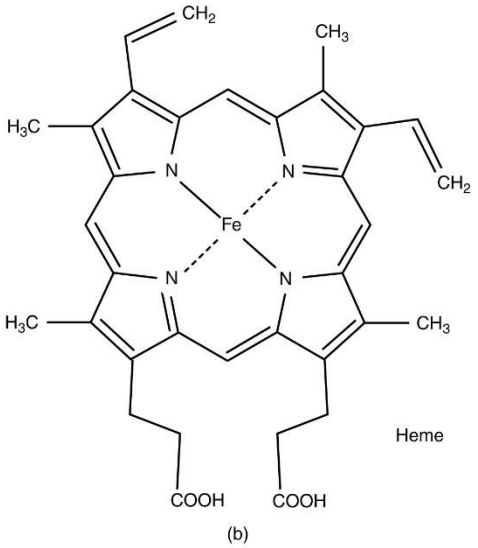


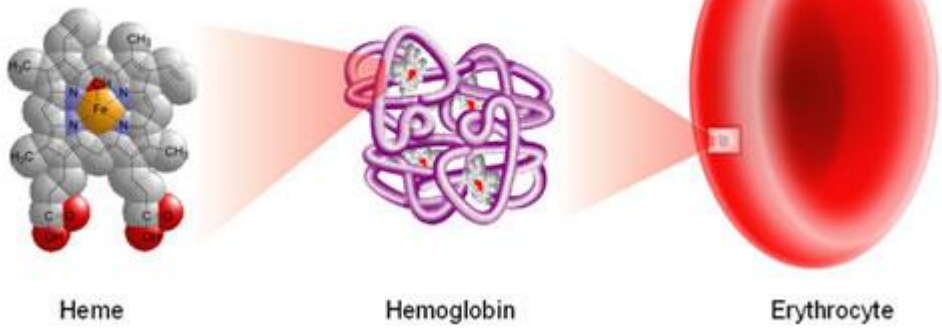
ERYTHROCYTES

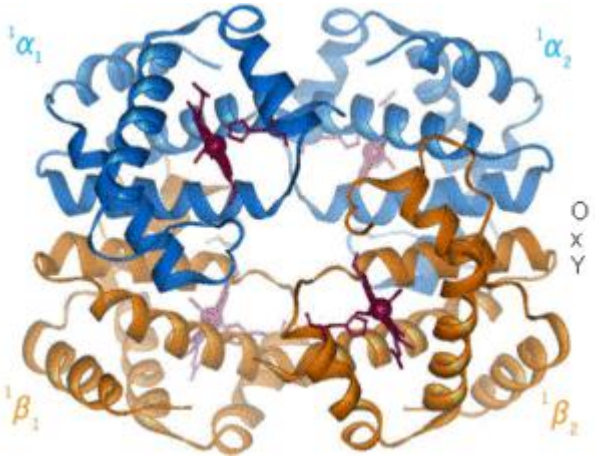
- Erythrocytes lack nucleus and organelles
- Anaerobic glykolysis

- Hemoglobin



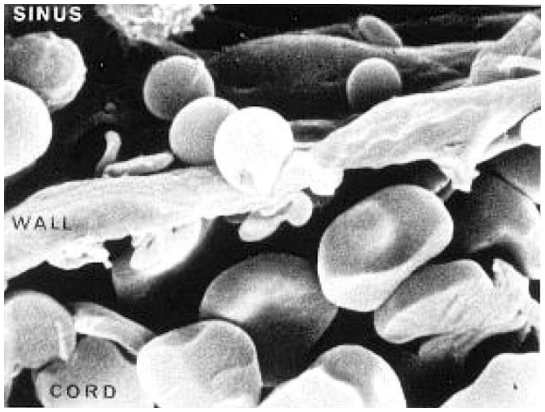


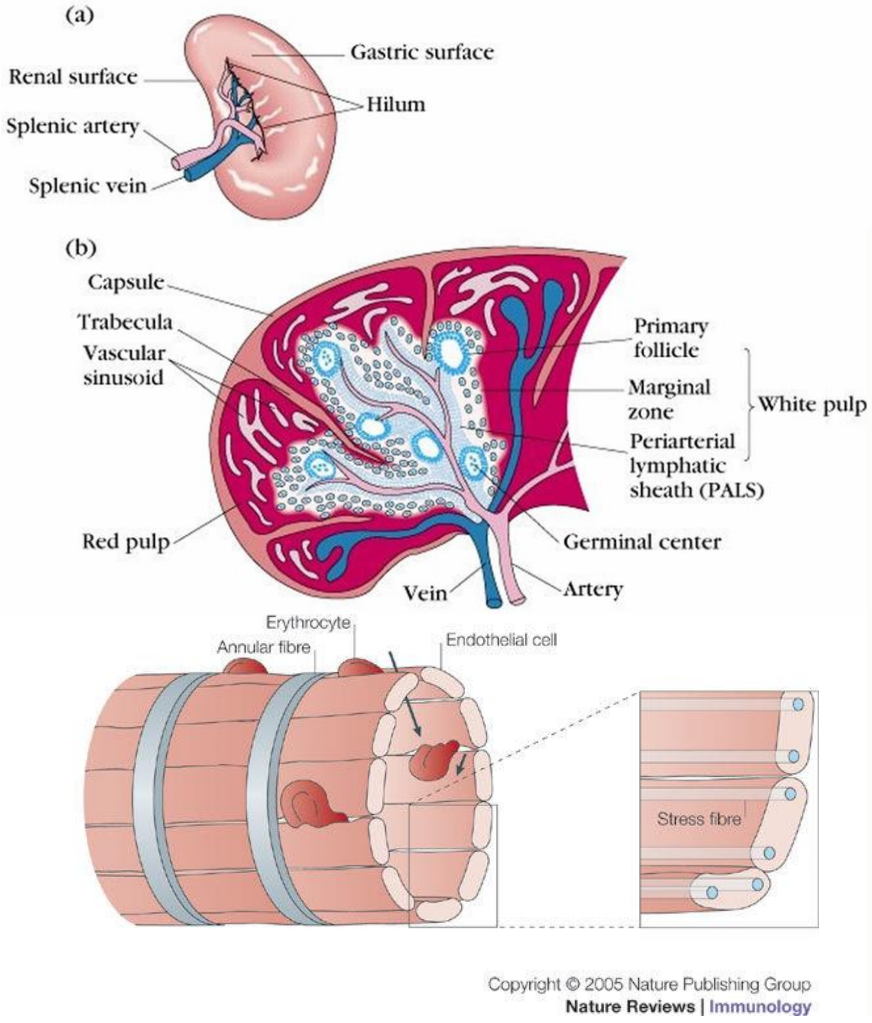


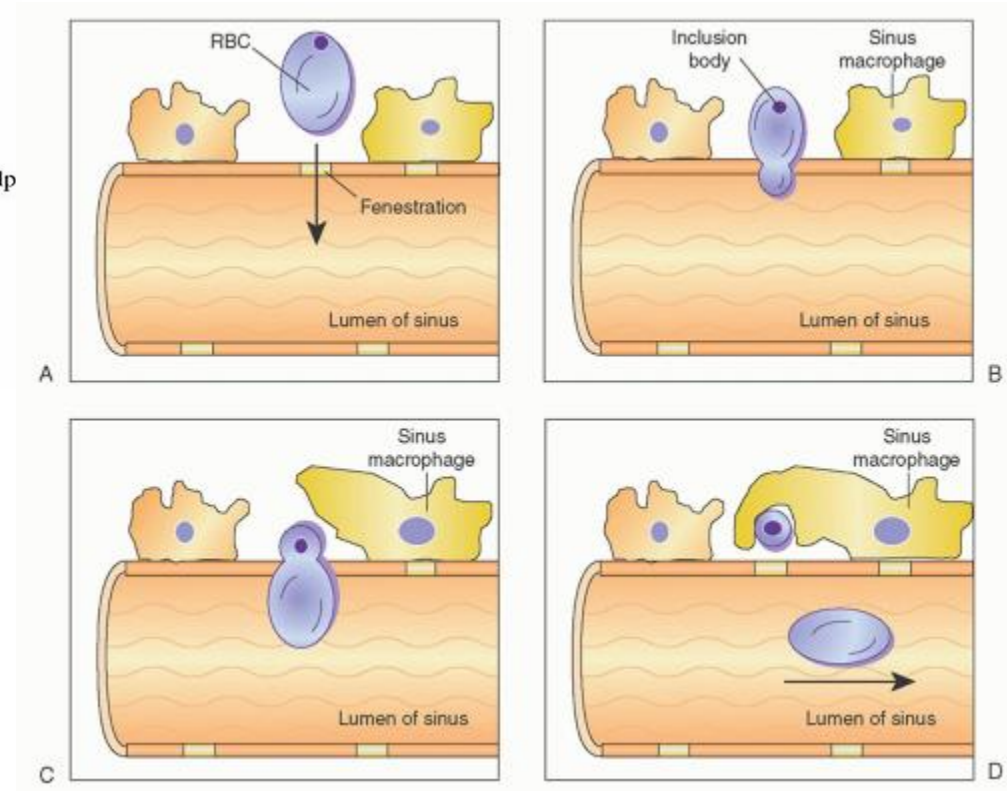


ERYTHROCYTES

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







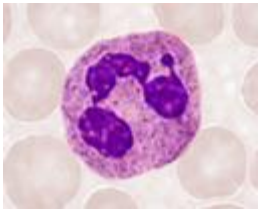
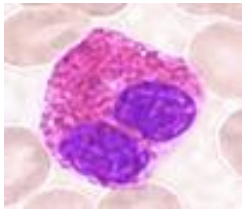
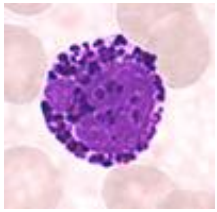
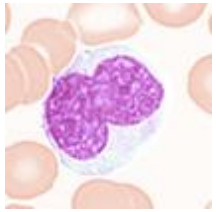
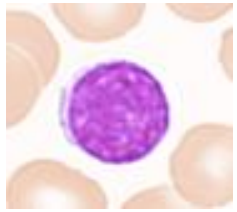
LEUCOCYTES

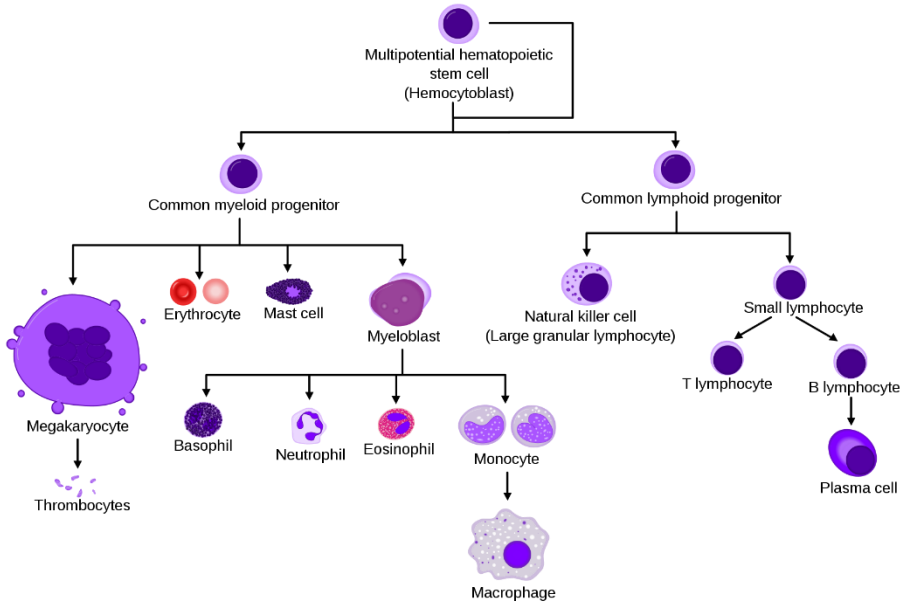
- immune response
- morphological classification – **cytoplasmic granules**

(does not follow hematopoesis)

Granulocytes **Agranulocytes**

Neutrophils Eosinophils Basophils Monocytes Lymphocytes

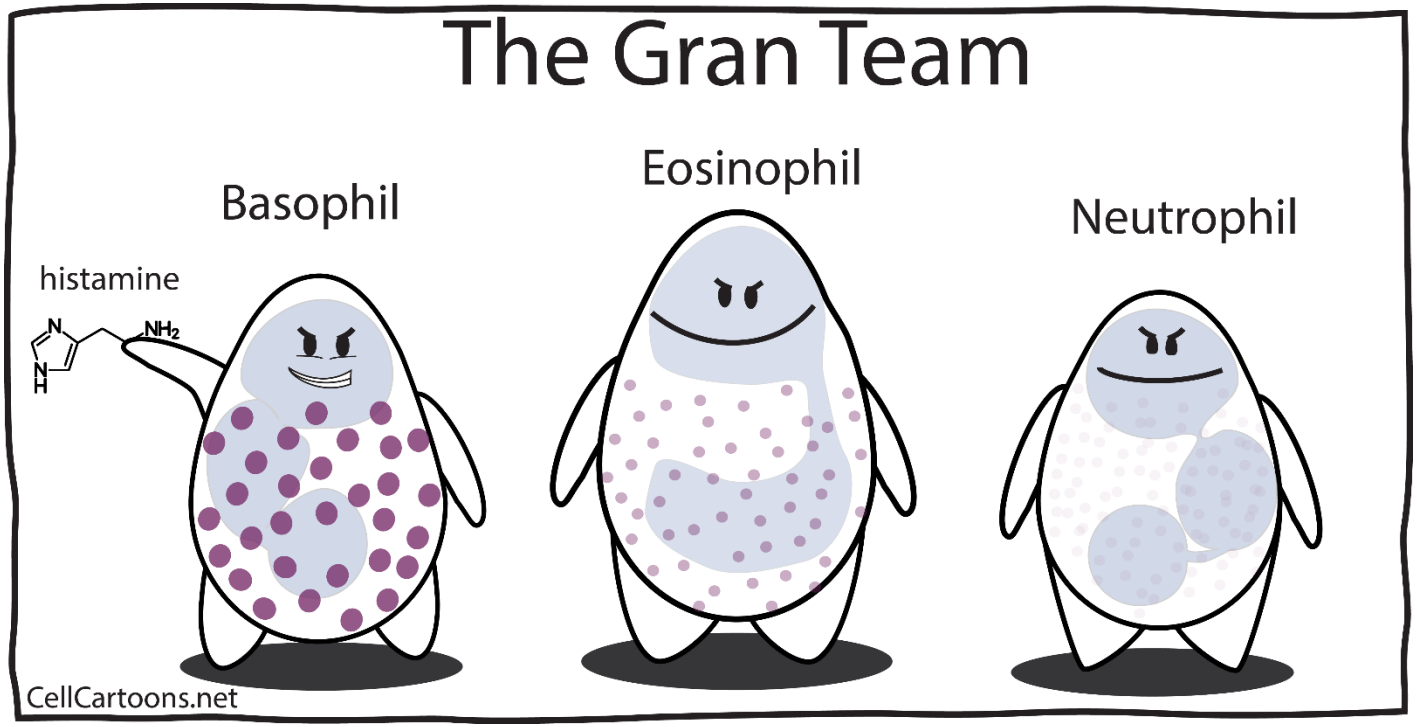








GRANULOCYTES

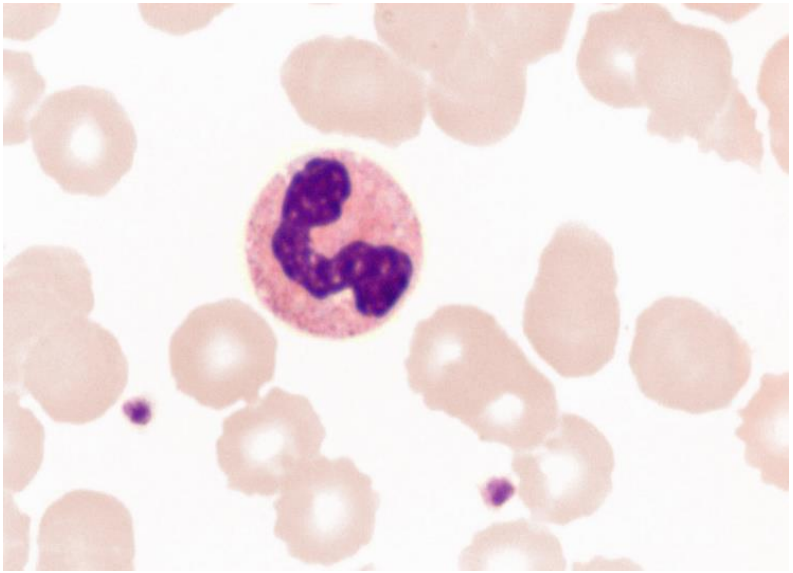
- Lysosomes (primary, azurophilic, nonspecific granules)
- Specific (secondary) granules
- Polymorphic nucleus

- Terminally differentiated
- Short lifespan (hours)
- Reduced ER, GA, mitochondria (anaerobic glycolysis)
- Apoptosis

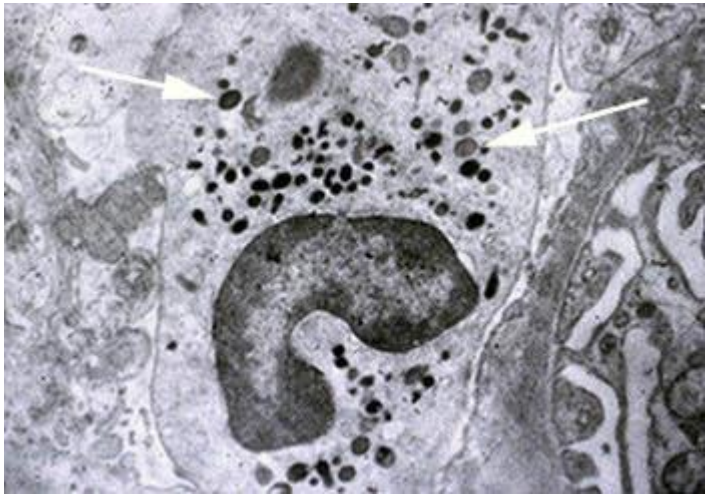


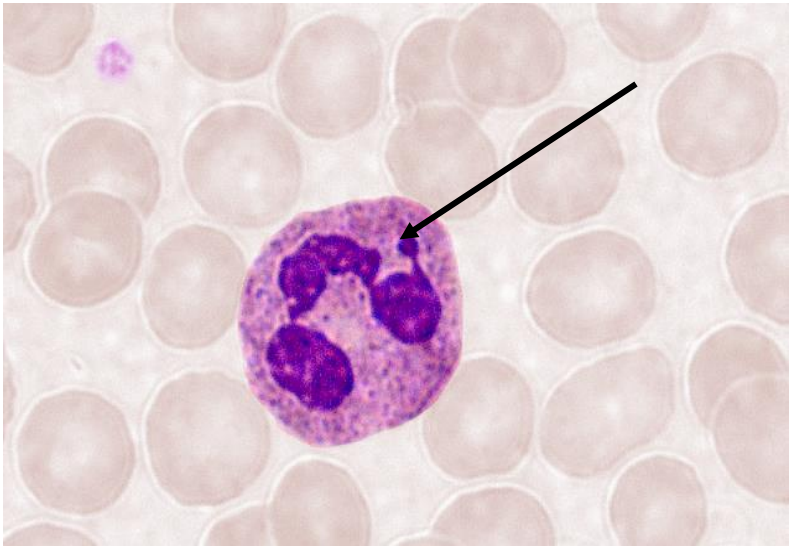
NEUTROPHILIC GRANULOCYTES

- **Neutrophils**
- 50-70% of leukocytes in circulation
- ∅ >12 μm
- 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

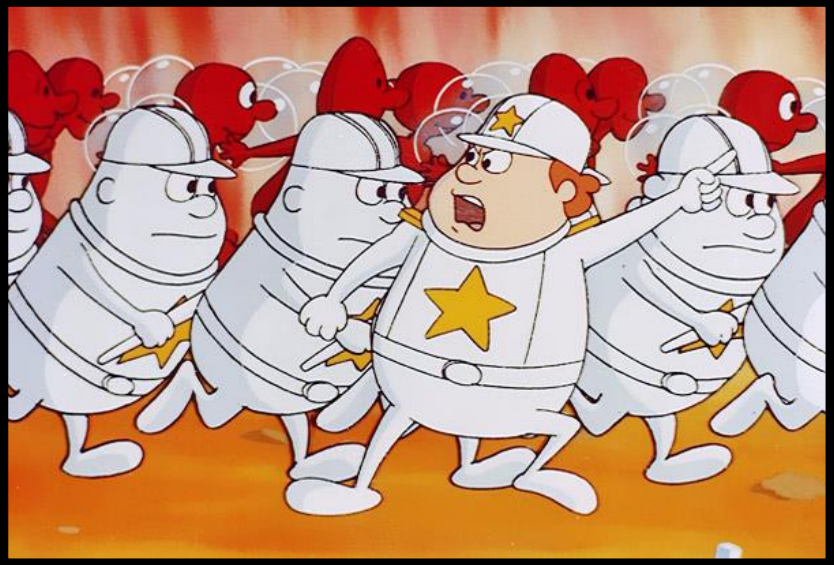


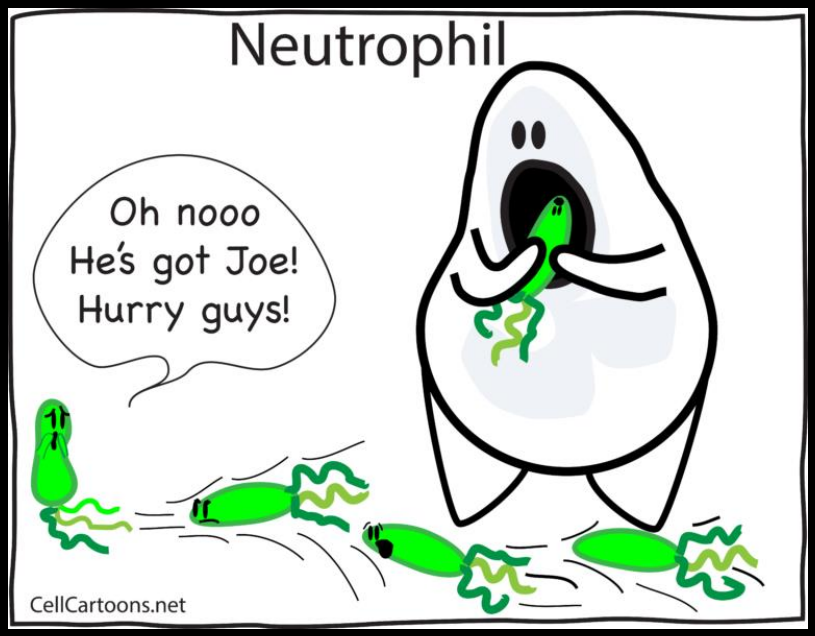
- **Neutrophilic band**
- **Neutrophilic segment**





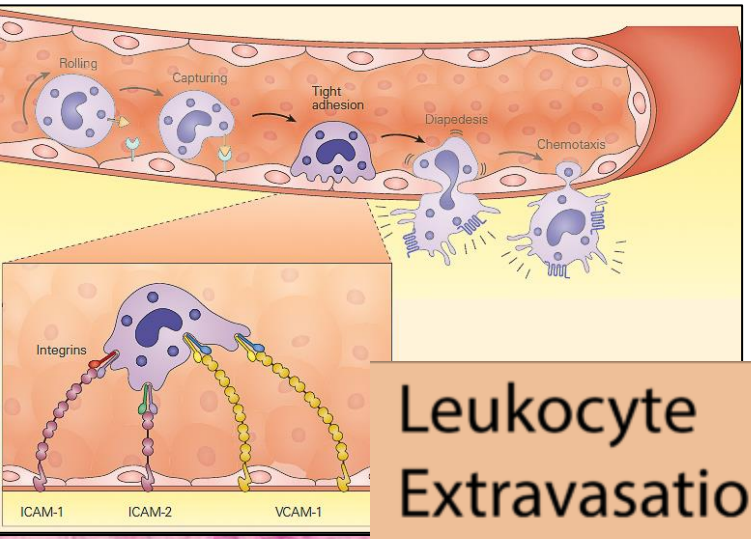
NEUTROPHILIC GRANULOCYTES

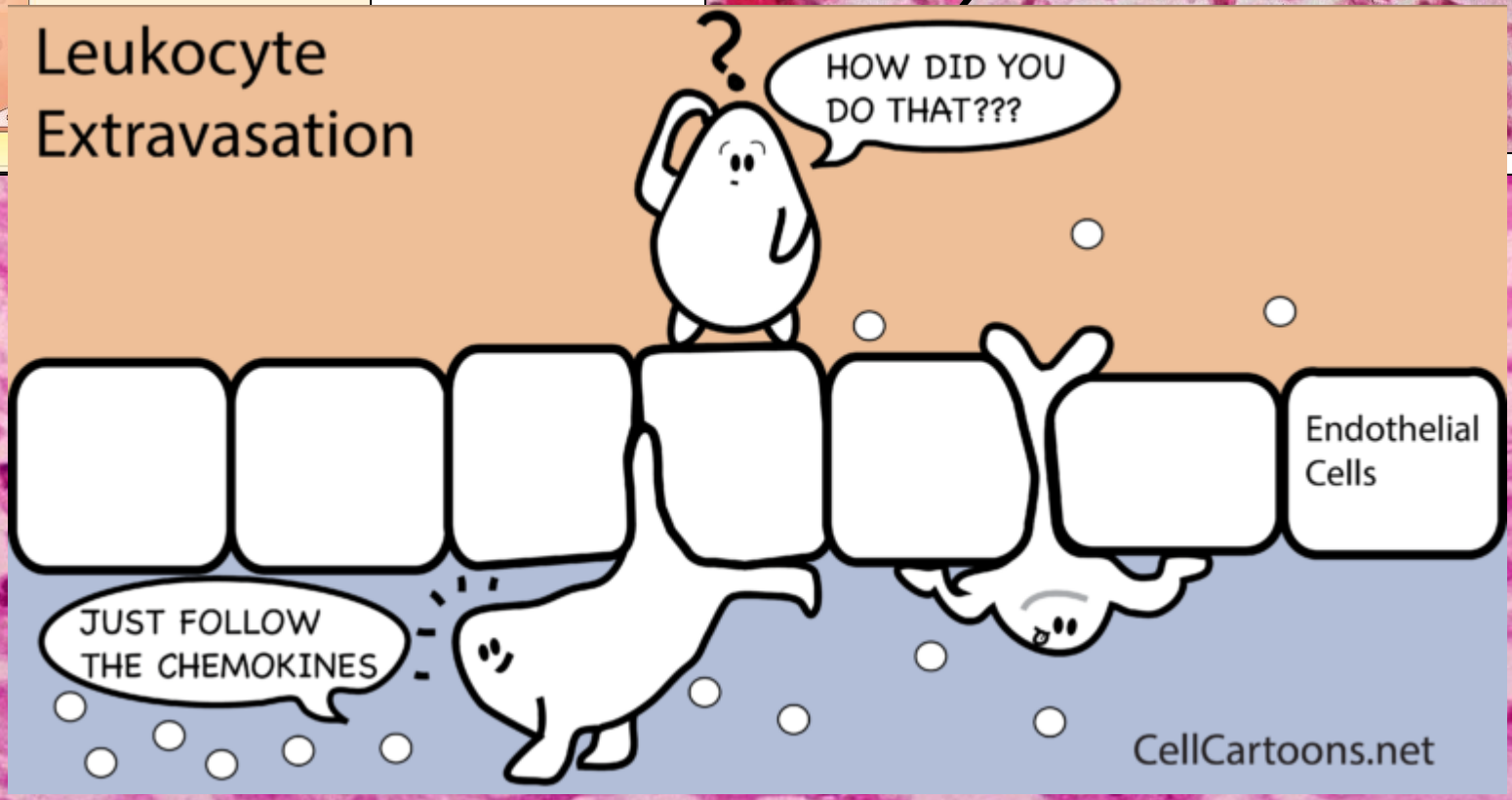




NEUTROPHILIC GRANULOCYTES

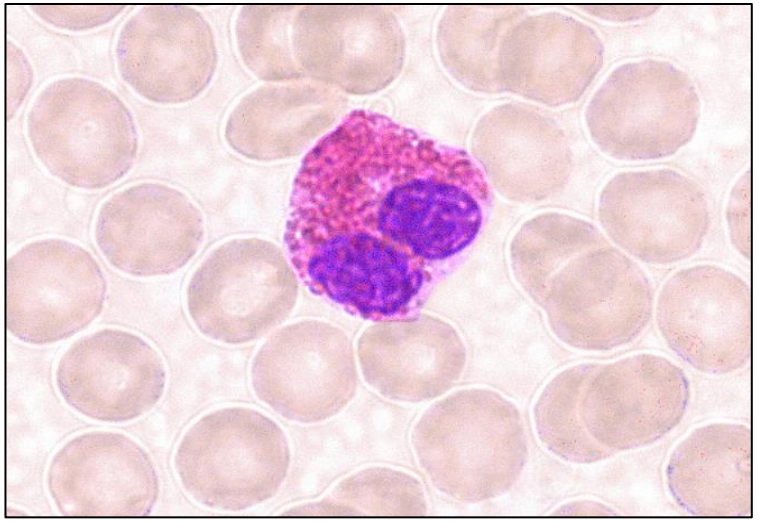
- **Extravasation** (diapedesis)



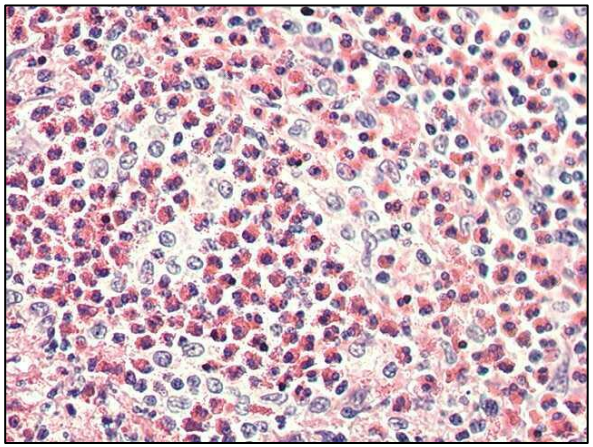


EOSINOPHILIC GRANULOCYTES

- **Eosinophils**
- 1-4% of leukocytes in circulation
- ∅ 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

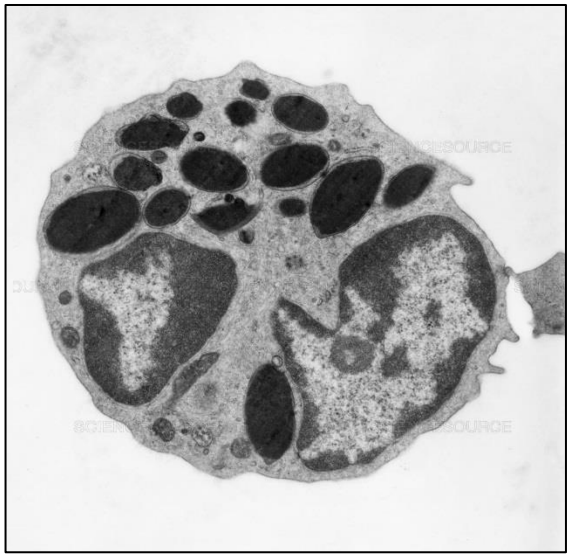


- Chemotaxis of other leukocytes
- Phagocytosis of antibody-antigen complexes
- Parasitic infections, allergic reaction
- Chronic inflammation

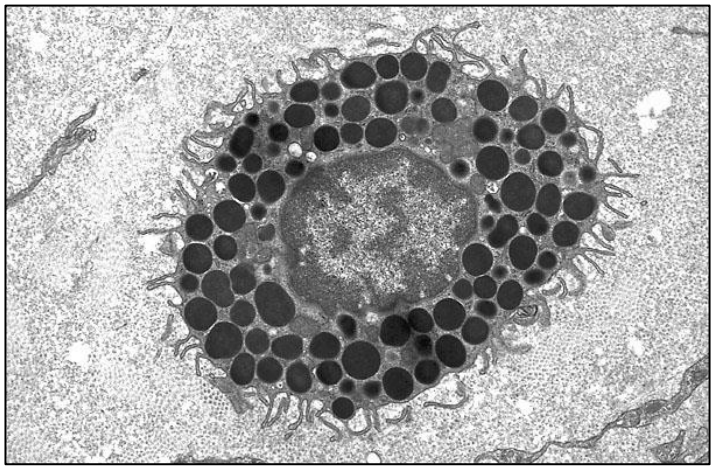


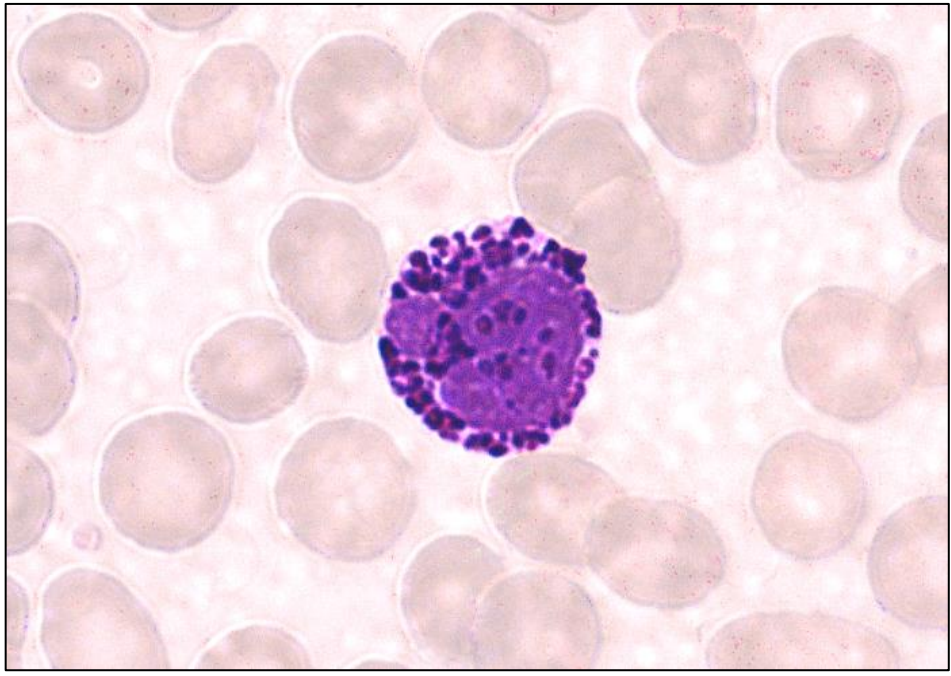
BASOPHILIC GRANULOCYTES

- **Basophils**
- <1% of leukocytes in circulation
- ∅ 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

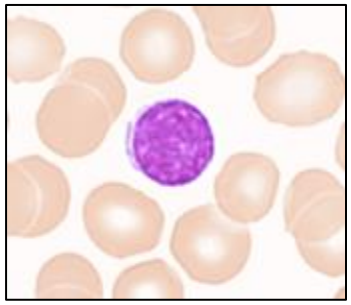




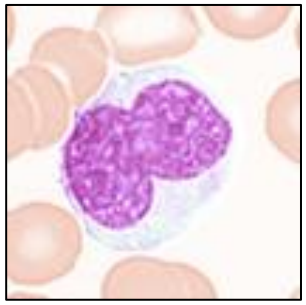
AGRANULOCYTES

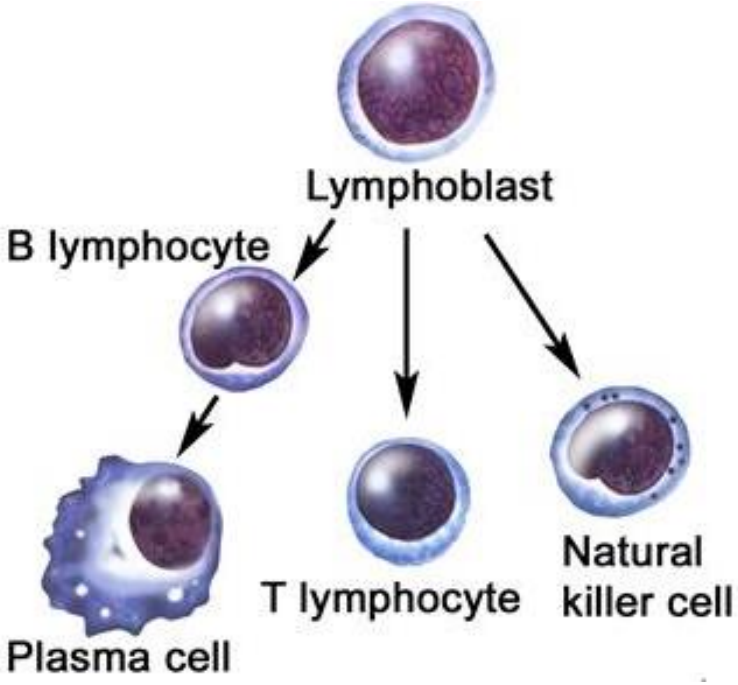
- Lysosomes only (azurophilic, nonspecific granules)
- Specific granules absent
- Nonsegmented nucleus

Lymfocytes

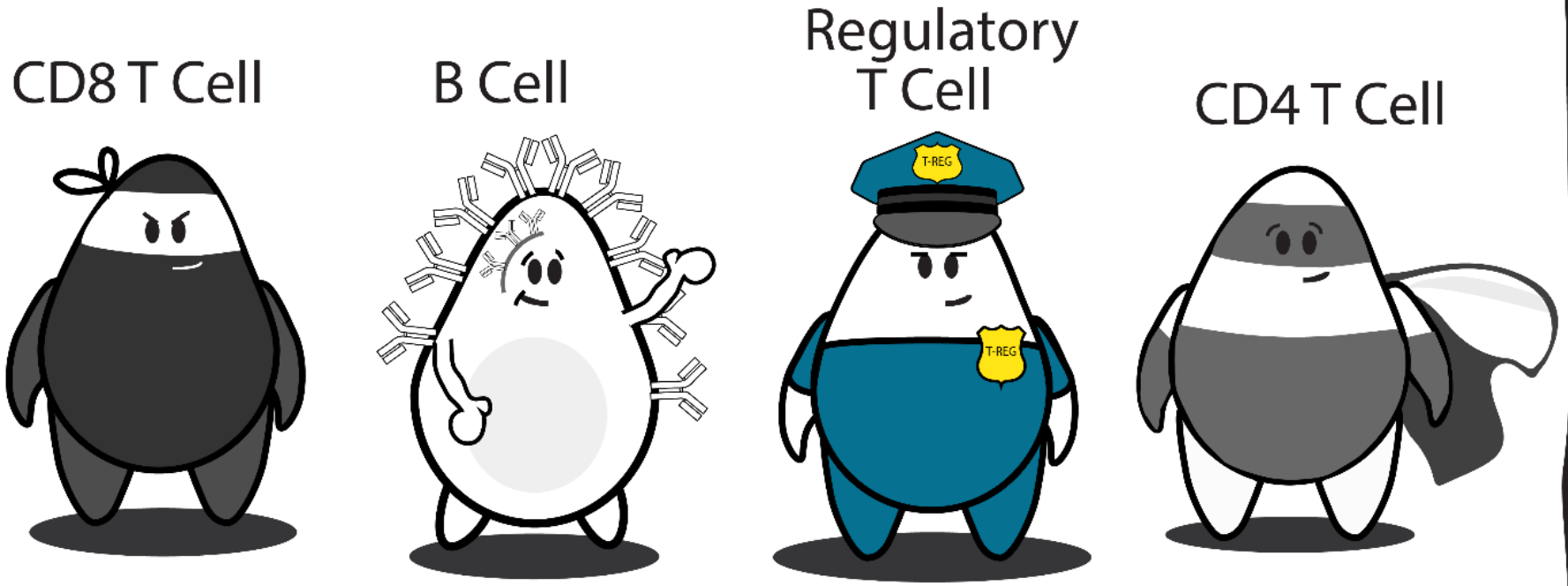


Monocytes



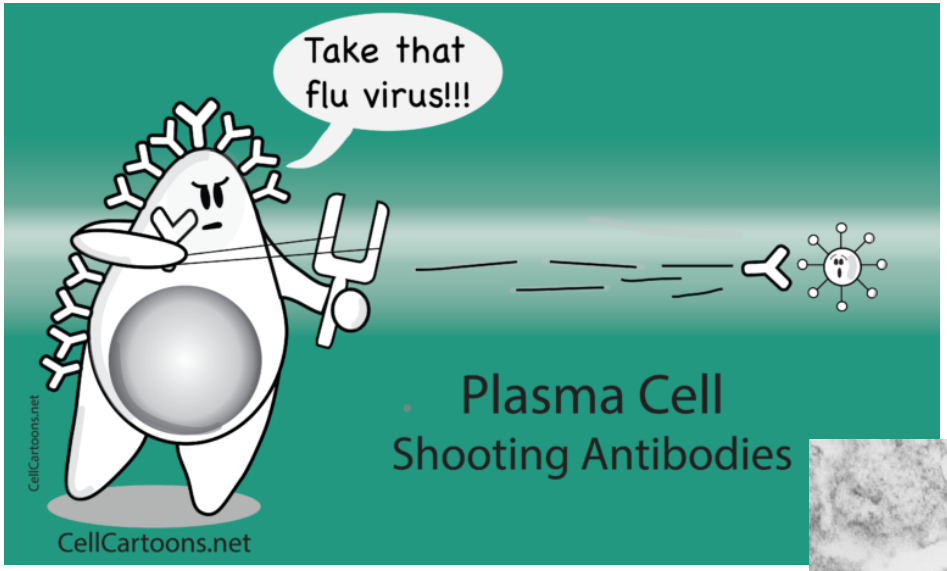


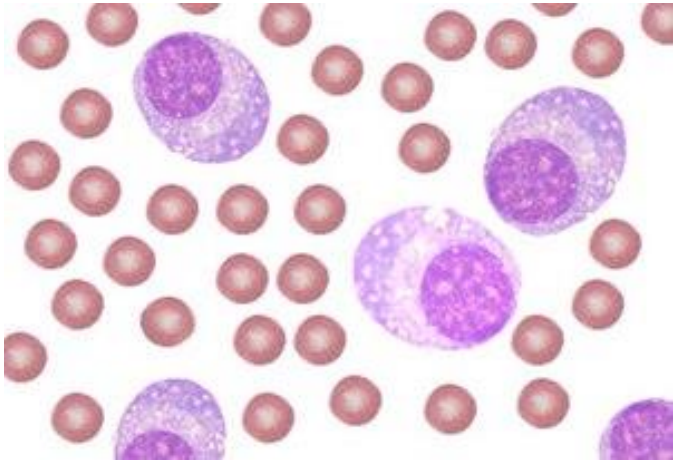
Lymphocytes

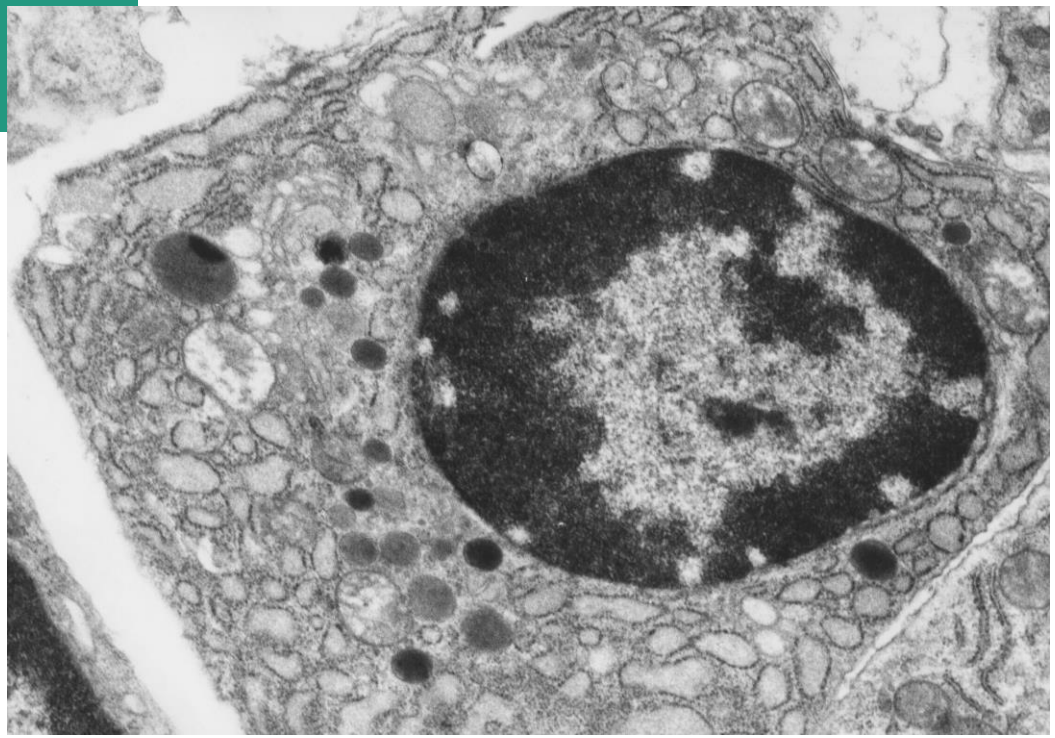


LYMPHOCYTES

- Plasma cells

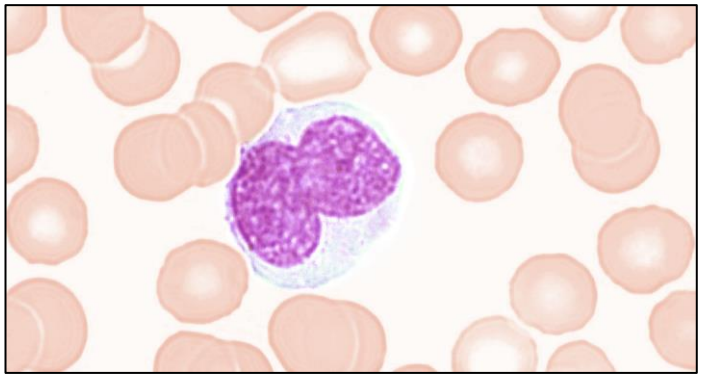


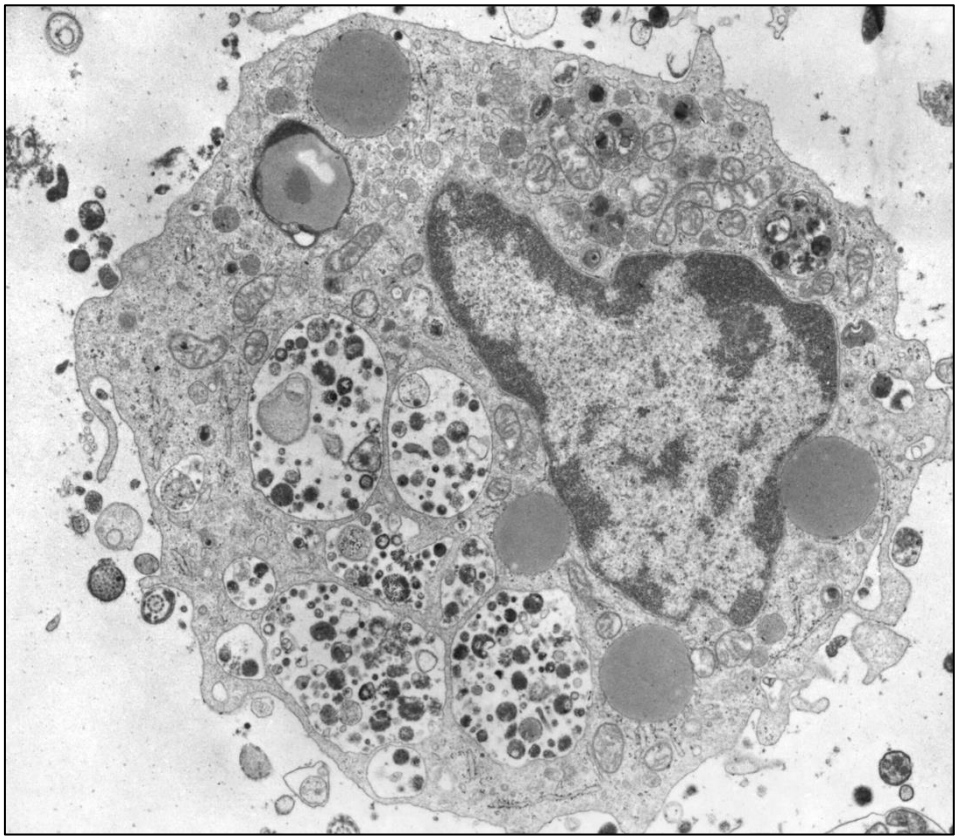


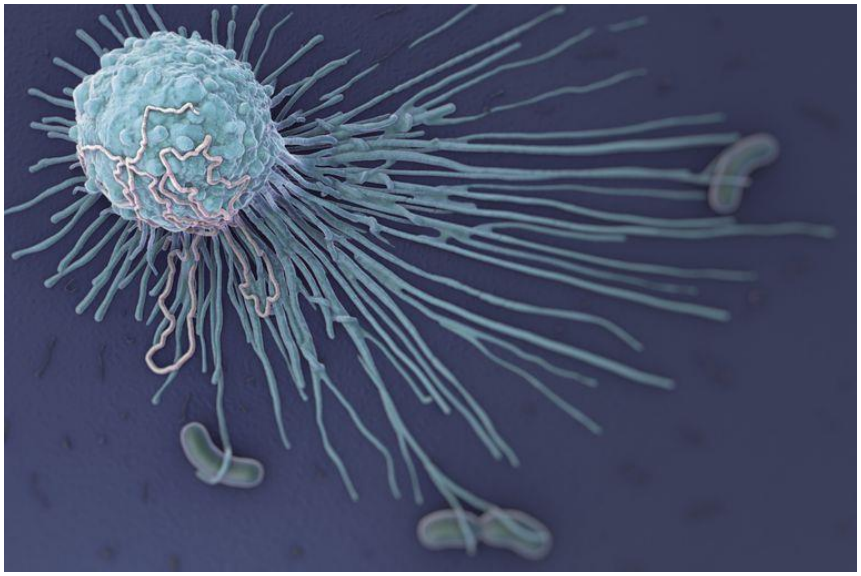


MONOCYTES

- ∅ 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



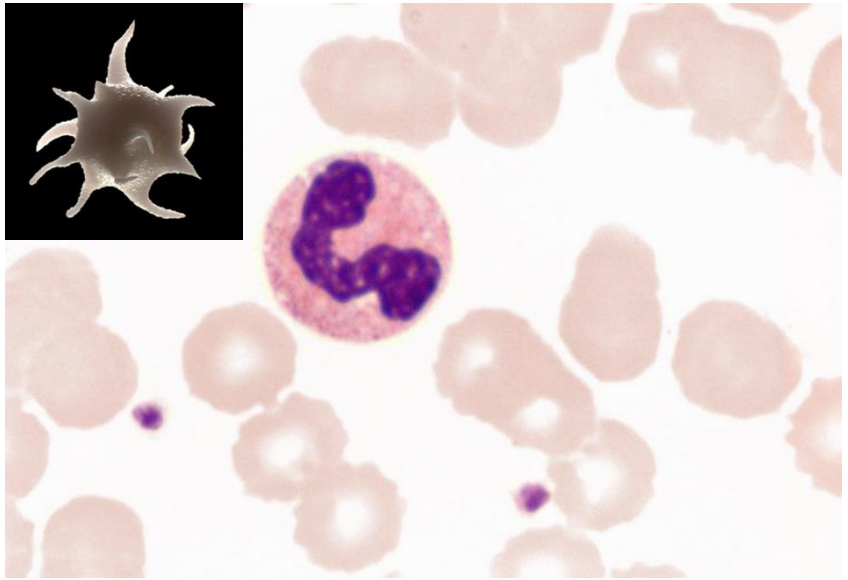


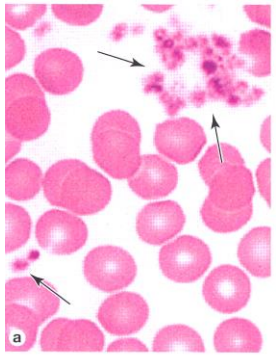


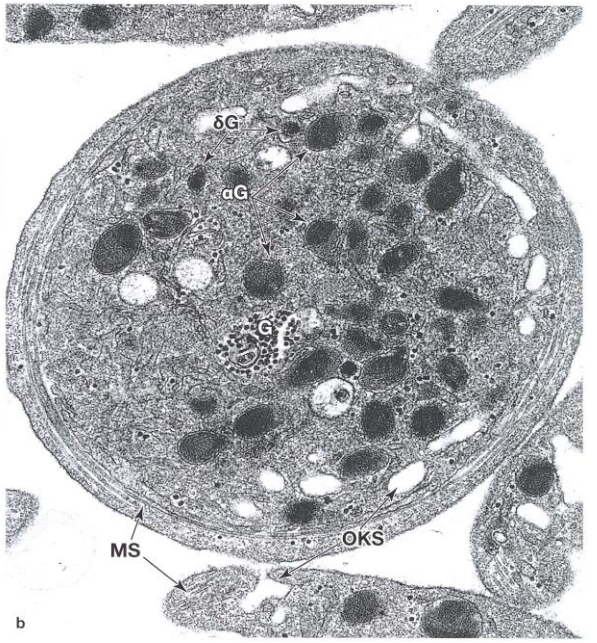
THROMBOCYTES

- Cell fragments without nucleus
- ∅ 2-3 μm, discoid shape
- hyalomere, granulomere
- 150-400 × 10^3/μl
- blood clotting, repair of vessel wall

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

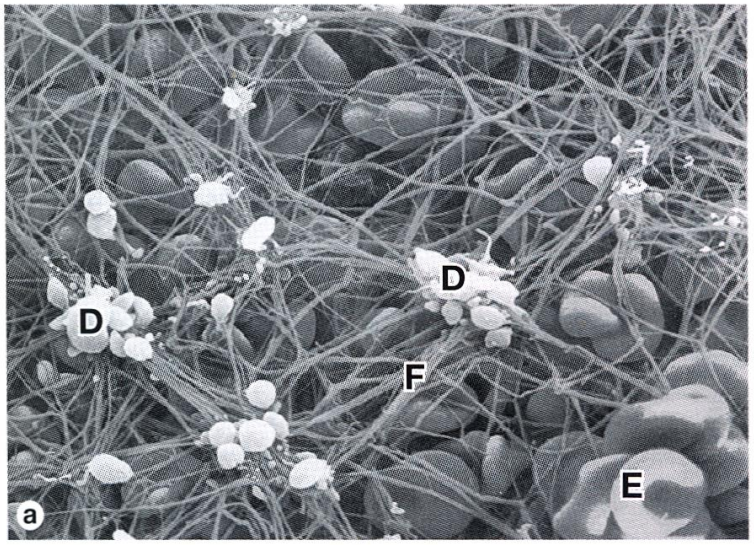


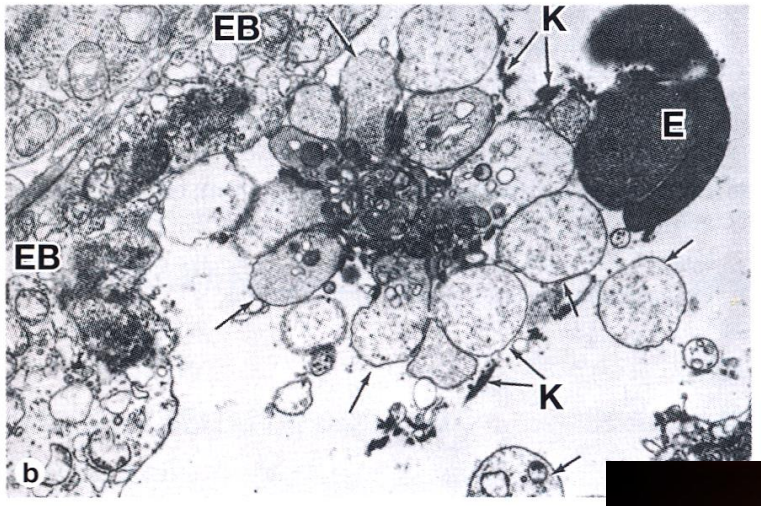




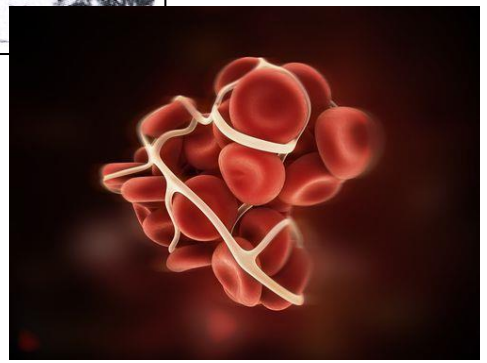
THROMBOCYTES

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***

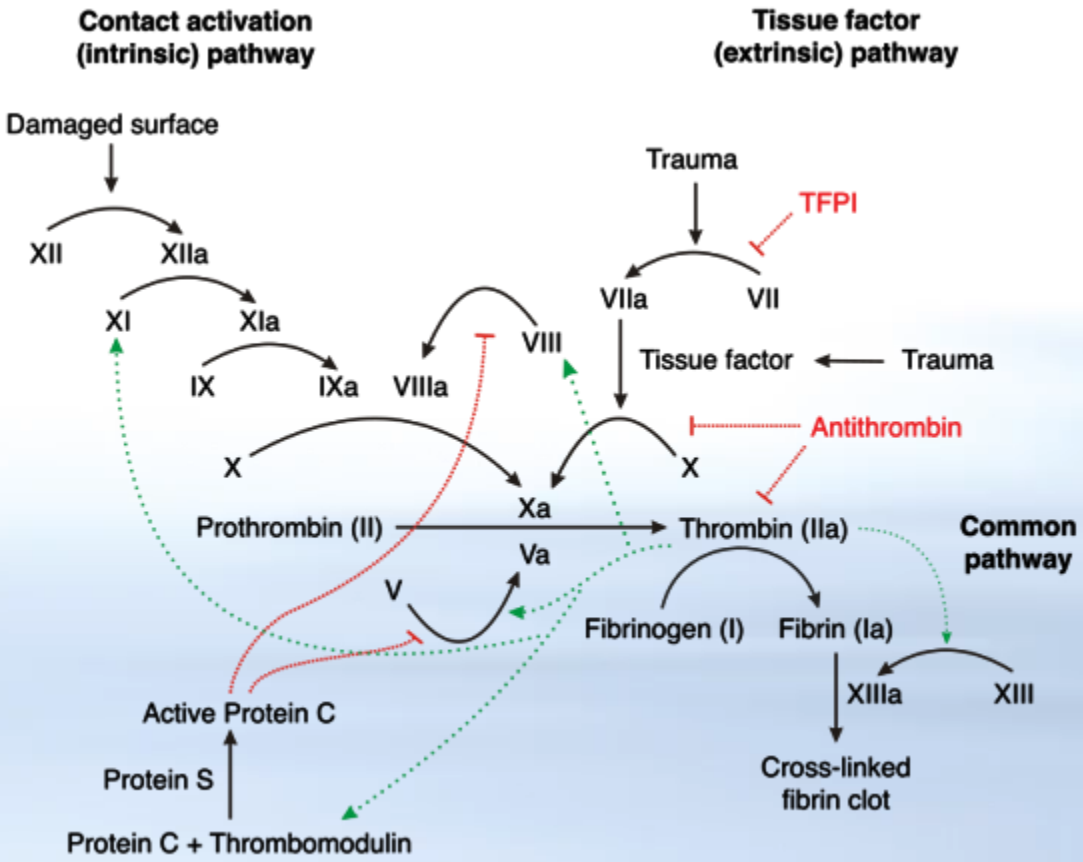




4. **Thrombus retraction**
   - contraction of thrombus (platelet actin and myosin)
5. **Thrombolysis**
   - dissolving of thrombus (plasmin) and tissue regeneration



THROMBOCYTES



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 %**

∑ = 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

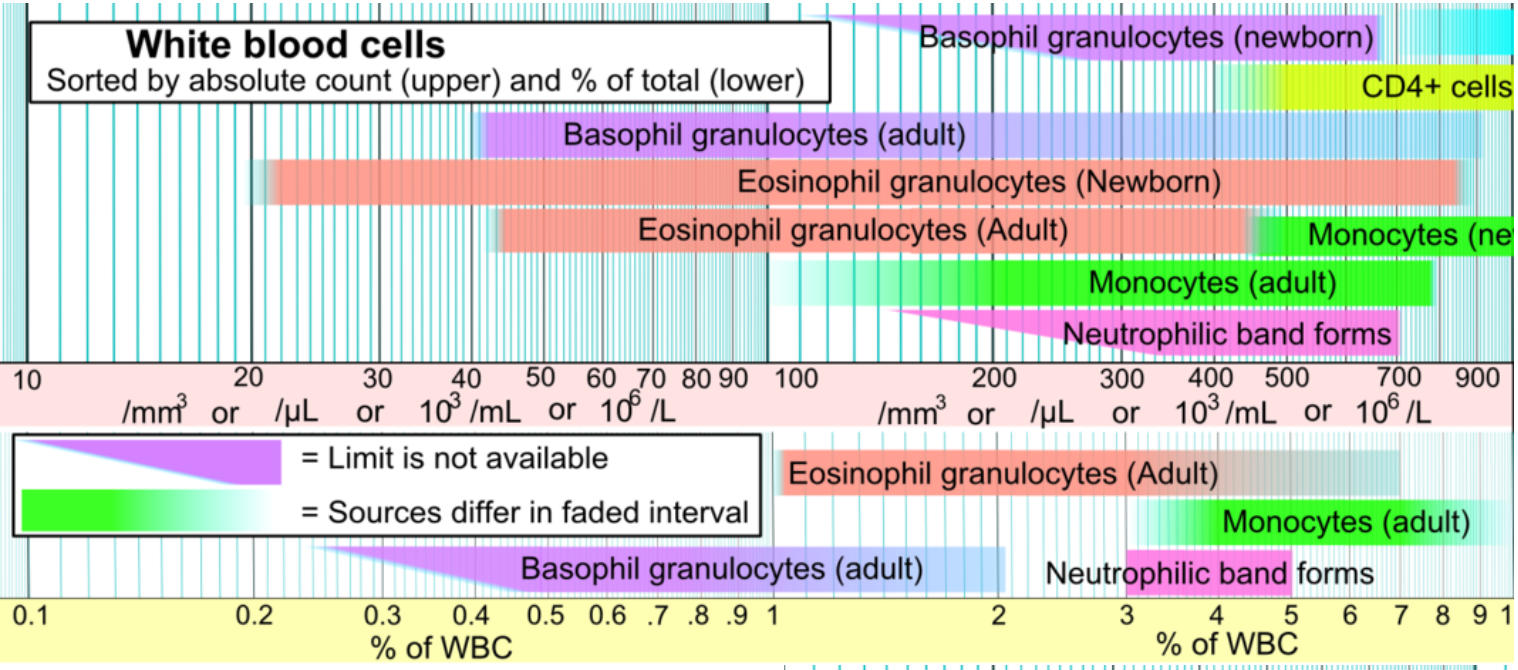
DIFFERENTIAL WHITE BLOOD CELL COUNT

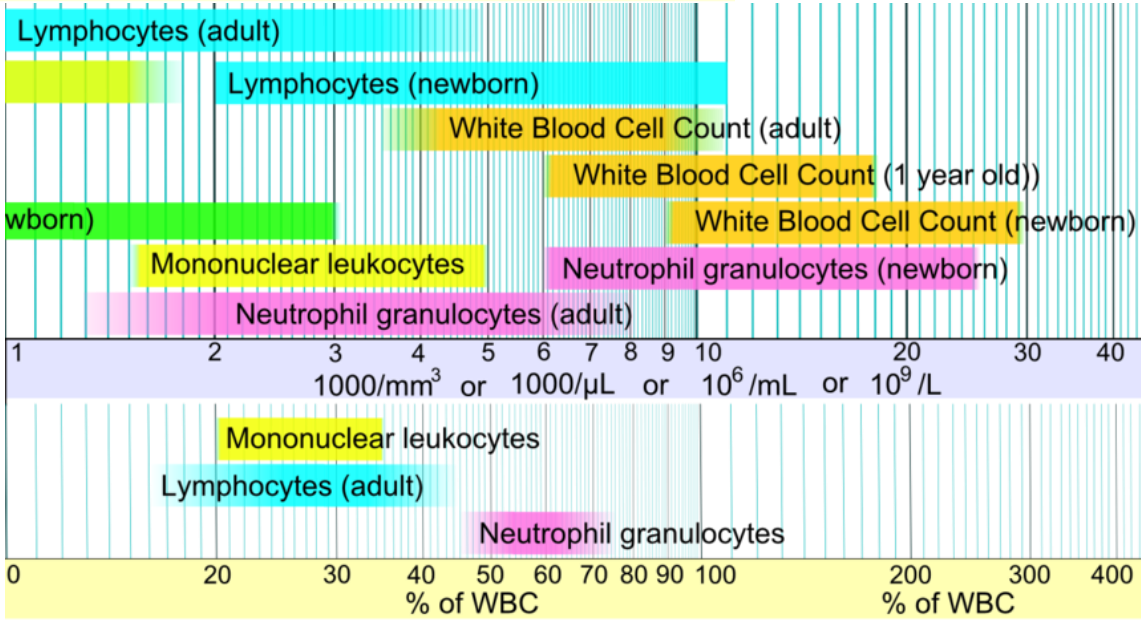
Example of population variability

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

Podle Haferlach et al. Kapesní atlas hematologie. Grada 2014

DIFFERENTIAL WHITE BLOOD CELL COUNT





Wikipedia

DIFFERENTIAL WHITE BLOOD CELL COUNT

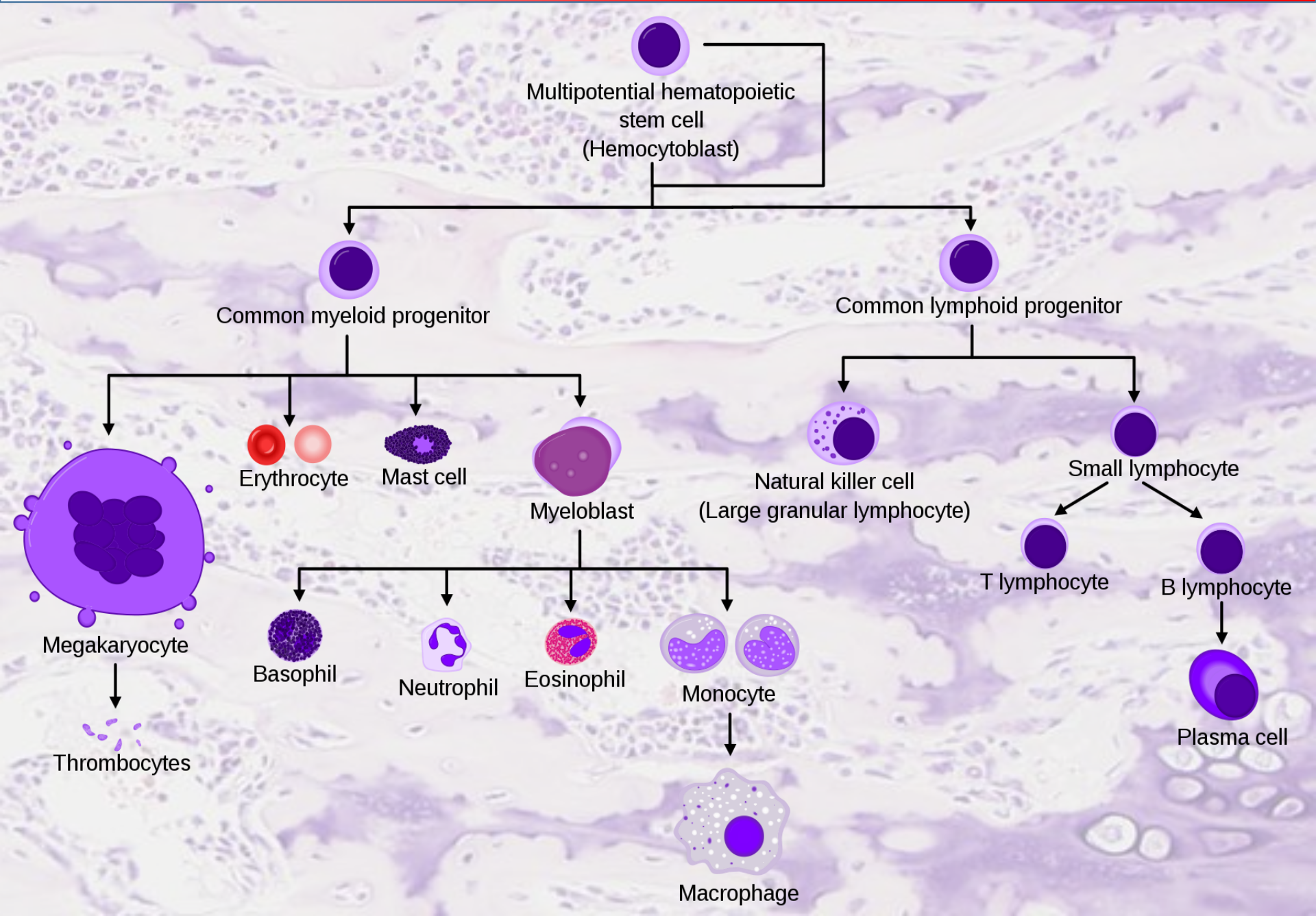
Age dependence

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.

https://obgynkey.com/elevated-or-depressed-white-blood-cell-count/

HEMATOPOIESIS



ERYTHROPOIESIS

- **2×10^{11} of new erythroyctes daily**

- **proerythroblast** (~14-19 μm)
- mitotically active
- dominant, round nucleus with 1-2 nucleoli
- mildly basophilic cytoplasm

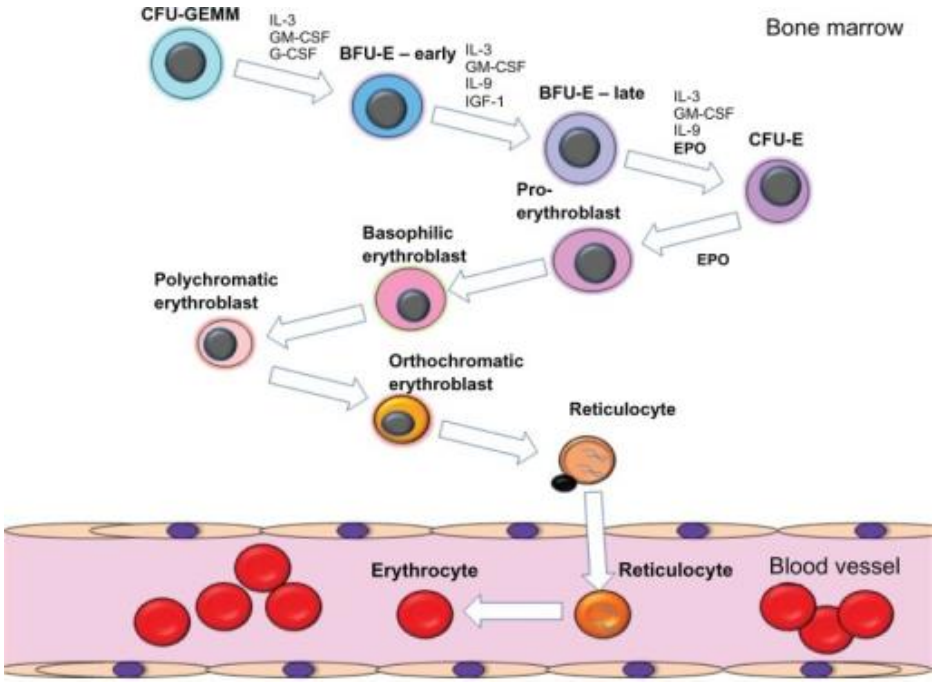
- **basophilic erythroblast** (~13-16 μm)
- mitotically active
- heterochromatic nucleus with inconspicuous nucleoli
- basophilic cytoplasm (sometimes more than in proerythroblast)

- **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)

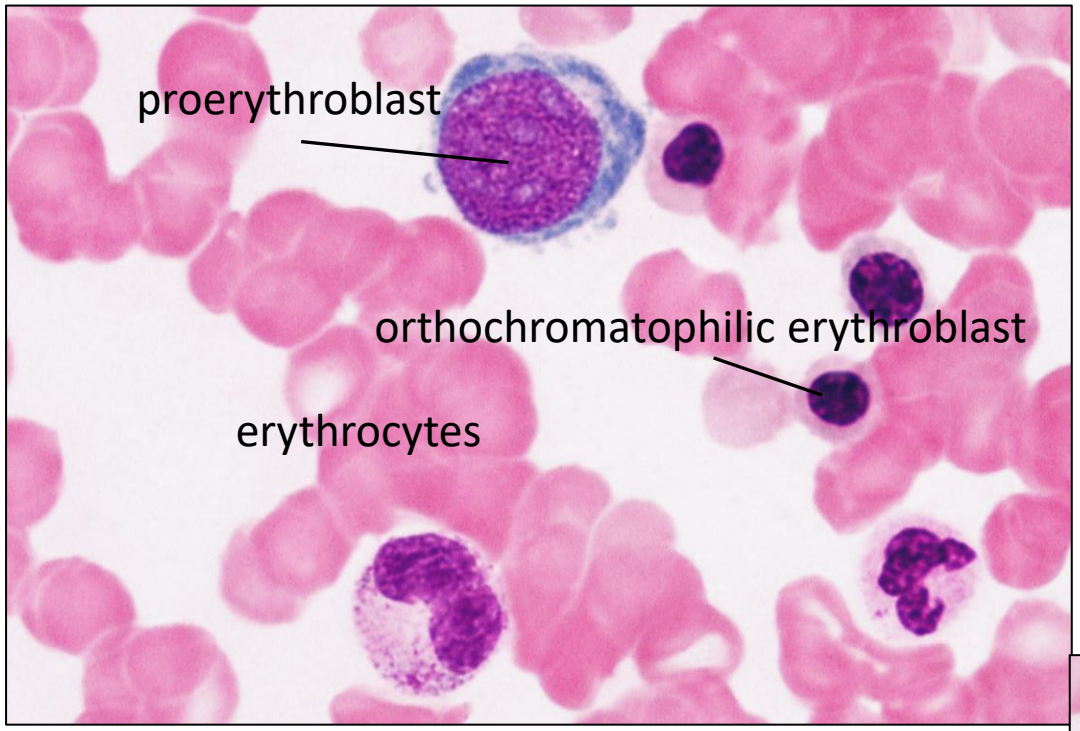
- **orthochromatophilic erythroblast** (~8-10 μm)
- mitotically inactive
- small, compact, eccentric, pycnotic nucleus → **extrusion**
- mildly acidophilic cytoplasm with basophilic residues

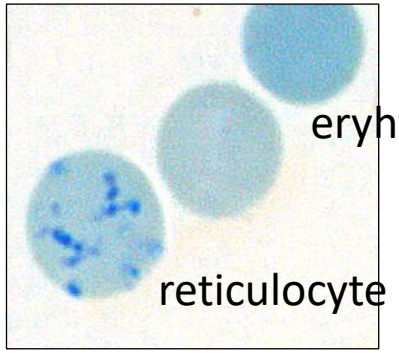
- **reticulocyte** (polychromatophilic erythrocyte, ~ 7-8 μm)
- **lacks nucleus, still spheroid shape**
- acidophilic cytoplasm
- *substantia reticulofilamentosa* visible by supravital staining (brilliant cresyl blue)

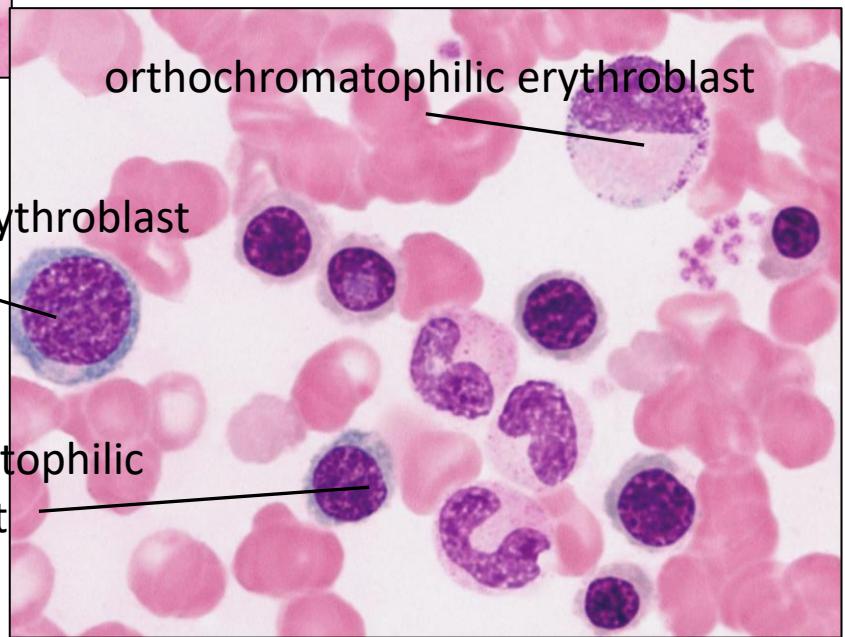
- **erythrocyte** (~7-8 μm)
- anucleate, biconcave disc
- acidophilic cytoplasm



ERYTHROPOIESIS







GRANULOPOIESIS

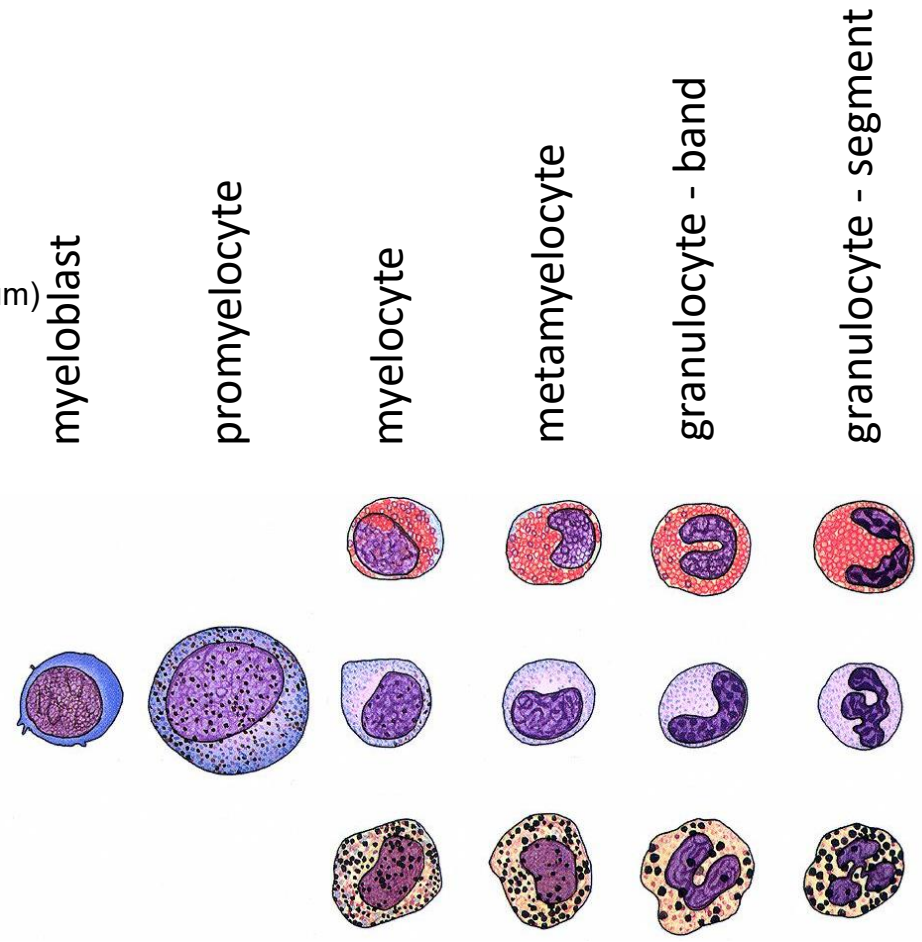
- **myeloblast** (~15 μm)
- mitotically active
- round-oval, euchromatic nucleus
- 2-6 apparent nucleoli
- weakly basophilic cytoplasm without granules

- **promyelocyte** (~15-24 μm)
- mitotically active
- round-oval nucleus with partly condensed chromatin
- basophilic cytoplasm with azurophilic 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

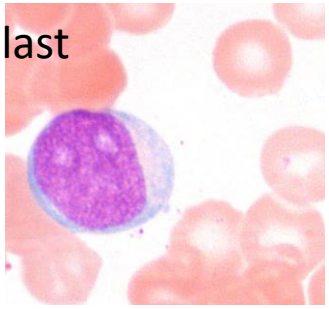
- neutrophilic, eosinophilic or basophilic **metamyleocyte** (~10-12 μm)
- mitotically inactive
- horseshoe-like nucleus with condensed chromatin

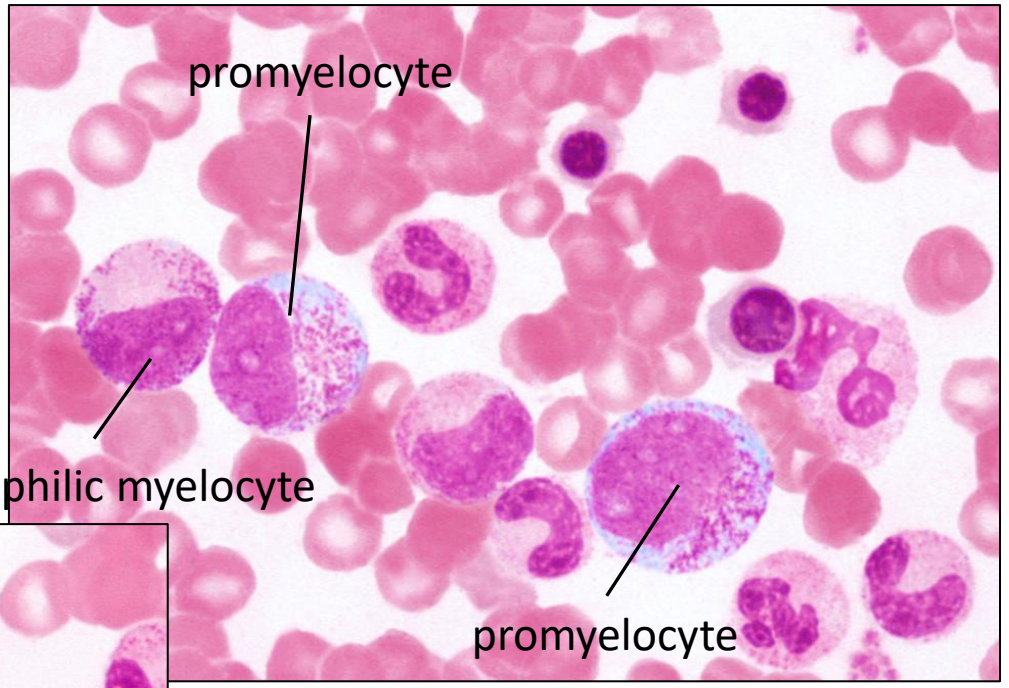
- neutrophilic, eosinophilic or basophilic **granulocyte** (~10-12 μm)
- segmentation of nucleus
- cytoplasm rich in specific and azurophilic granules

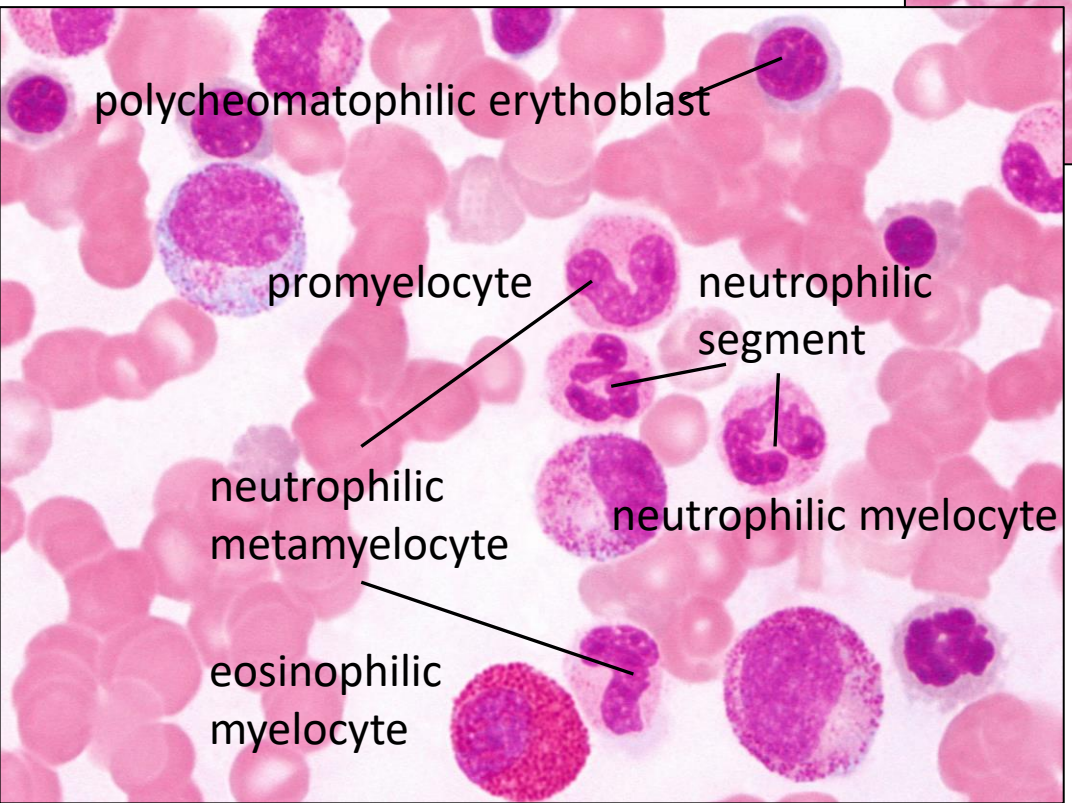


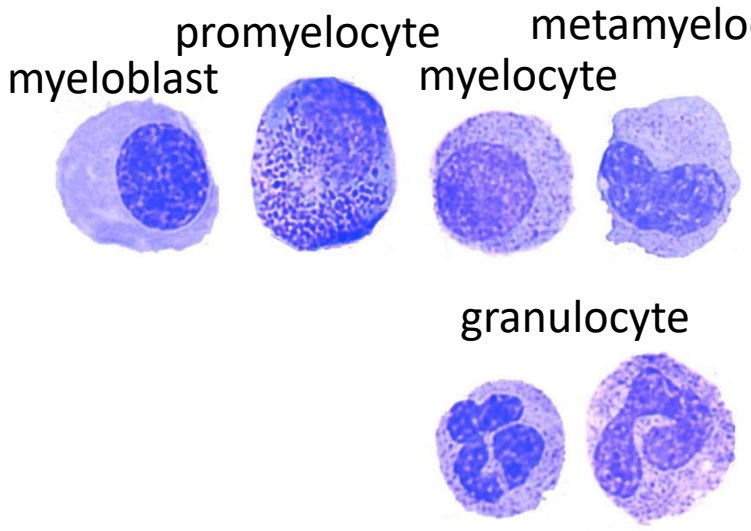
GRANULOPOIESIS

myeloblast



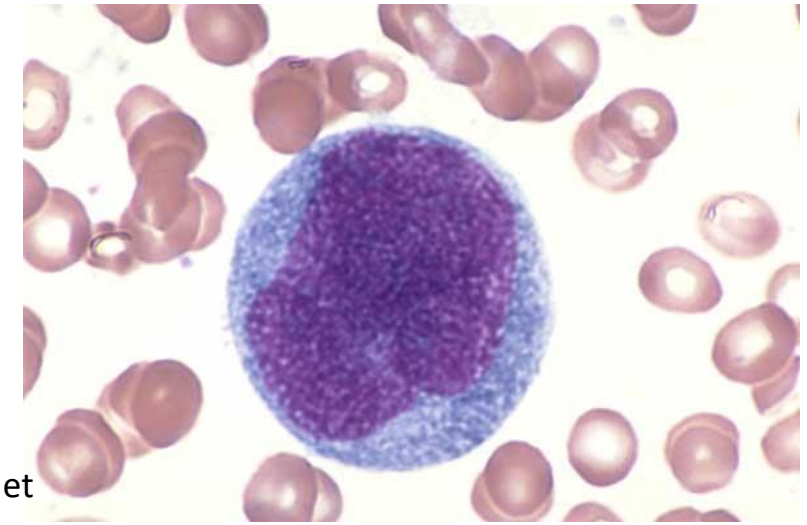


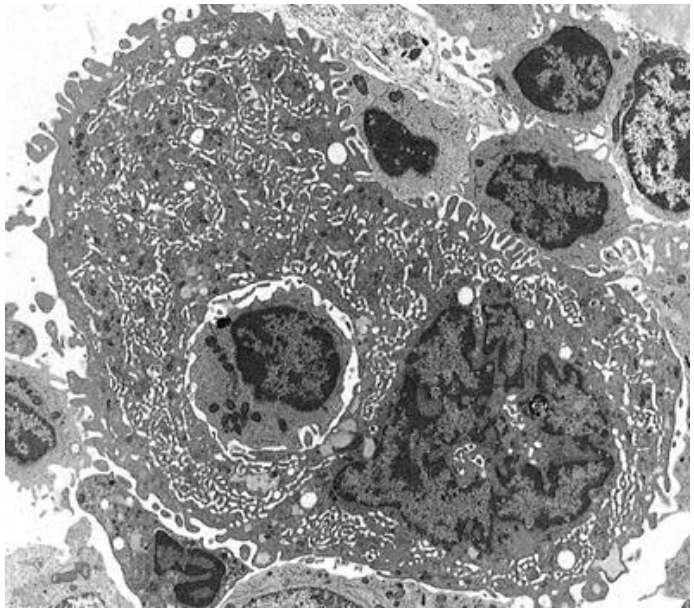


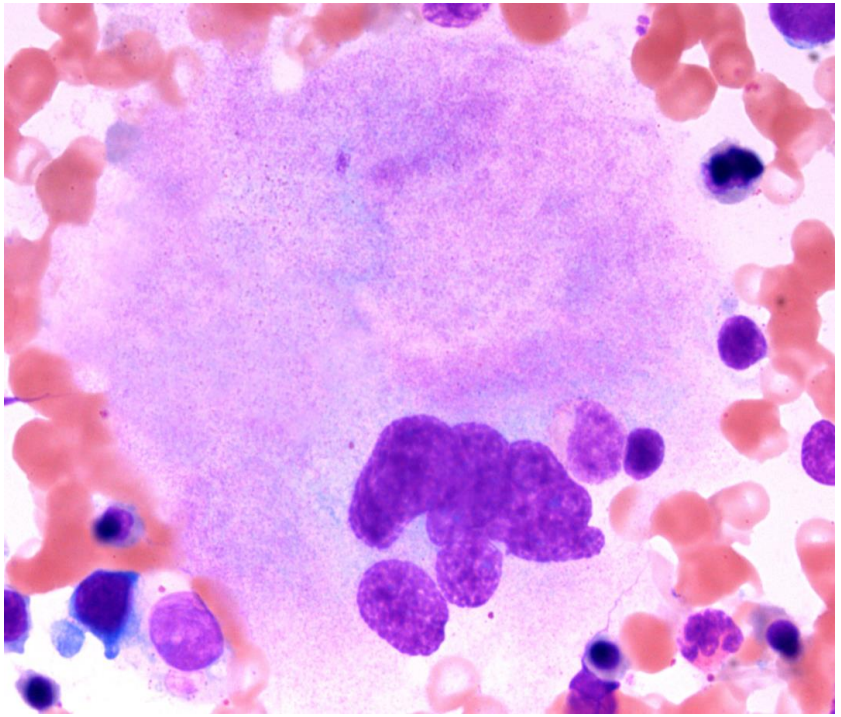


THROMBOPOIESIS

- **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

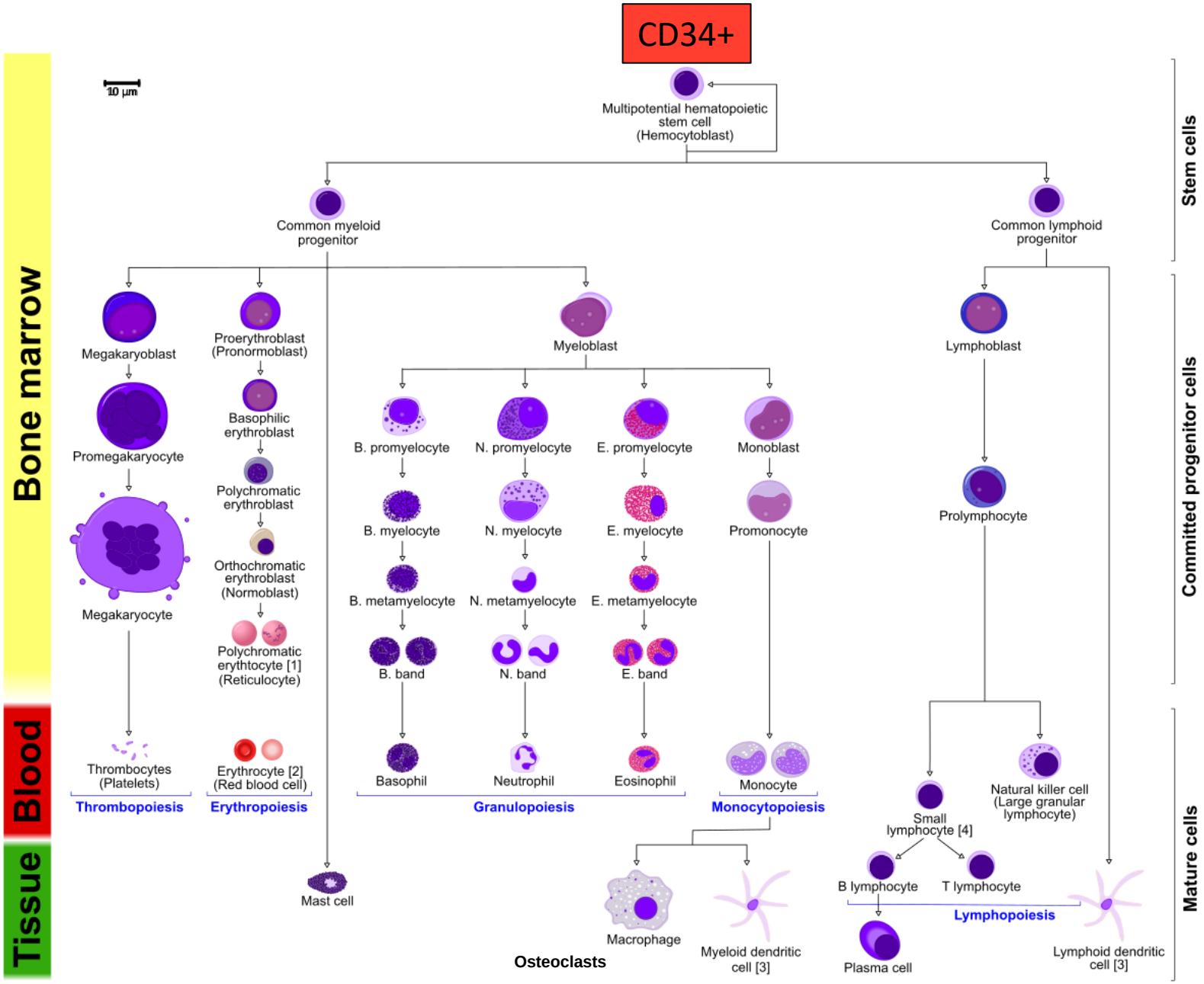
MONOCYTOPOIESIS

- **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



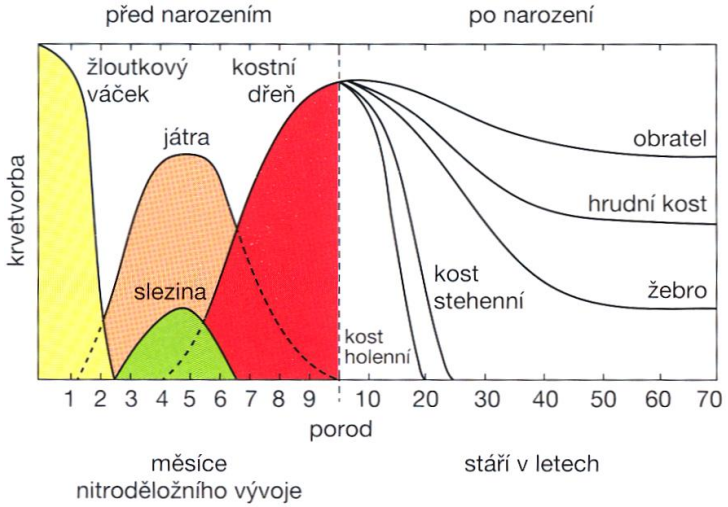
EMBRYONIC HEMATOPOIESIS

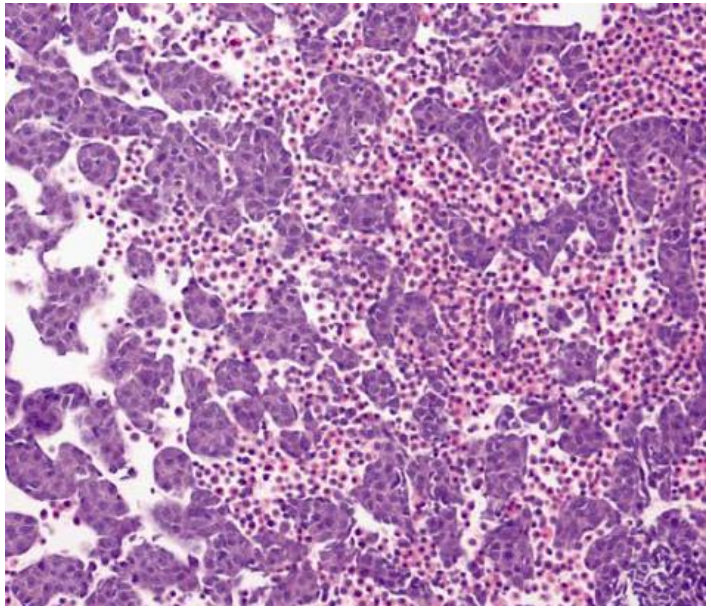
- **Extraembryonic mesoblastic period (day 16-20 – week 8)**
- yolk sac
- classical model – hemangioblasts (bipotent cells)
- large, nucleated erythroid cells

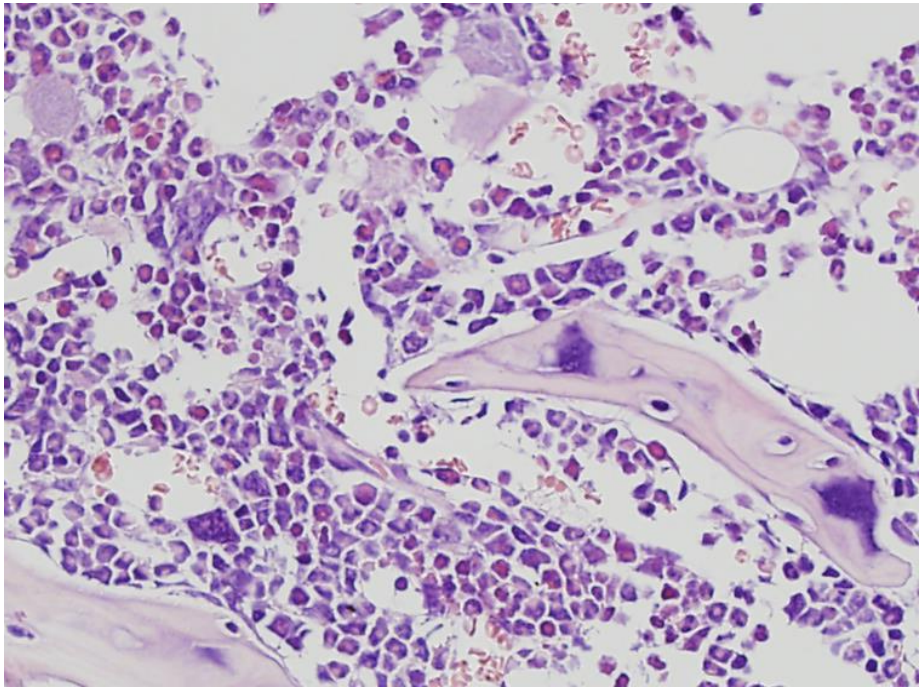
- **aorta-gonad-mesonephros (day 28 – week 4)**

- **hepatolienal period (month 1 – birth)**
- colonization of fetal liver and spleen

- **medullary period (month 4-6. – rest of life)**
- bone marrow

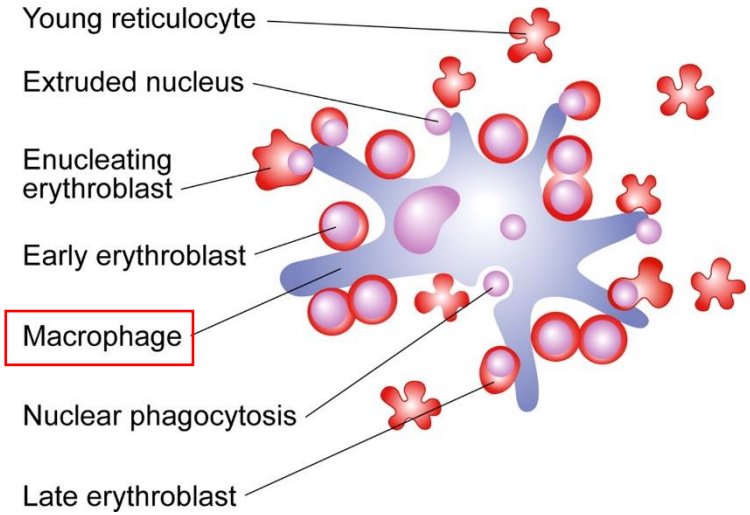


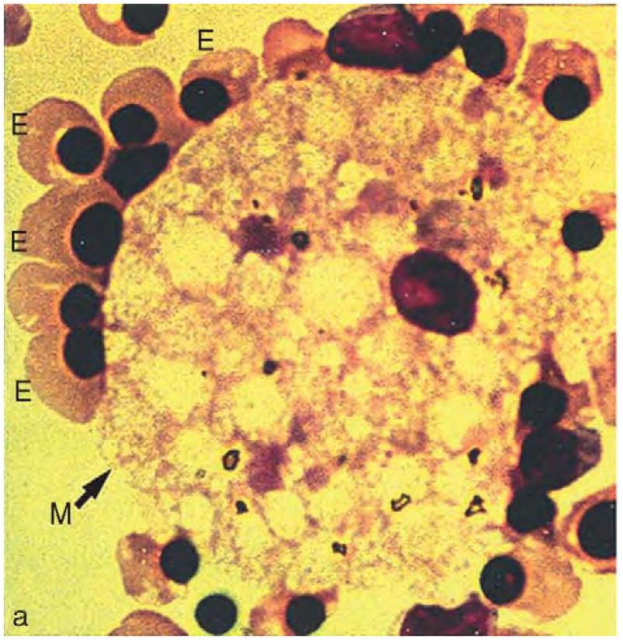




HEMATOPOIETIC ISLANDS

- **hepatolienal and bone marrow hematopoiesis**
- erythroblast islands





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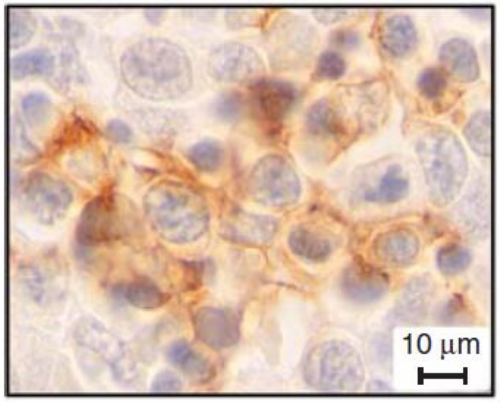

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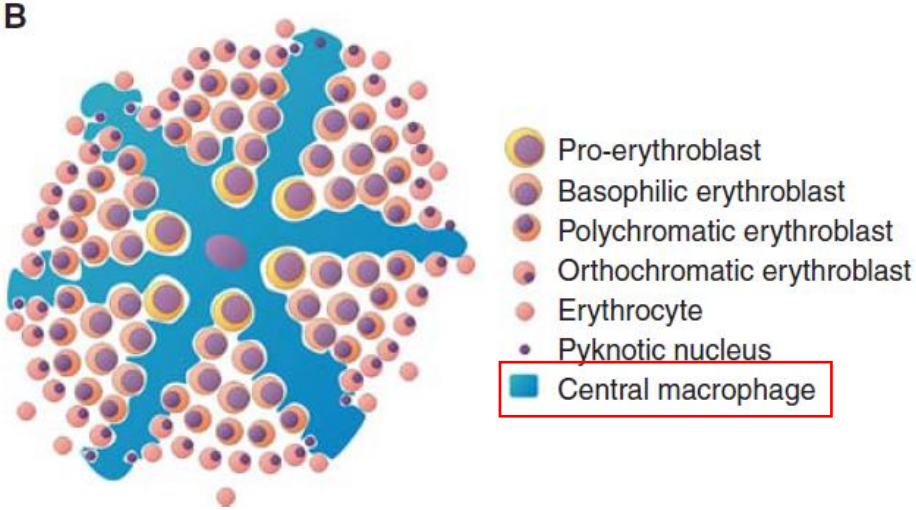

Thank you for attention

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