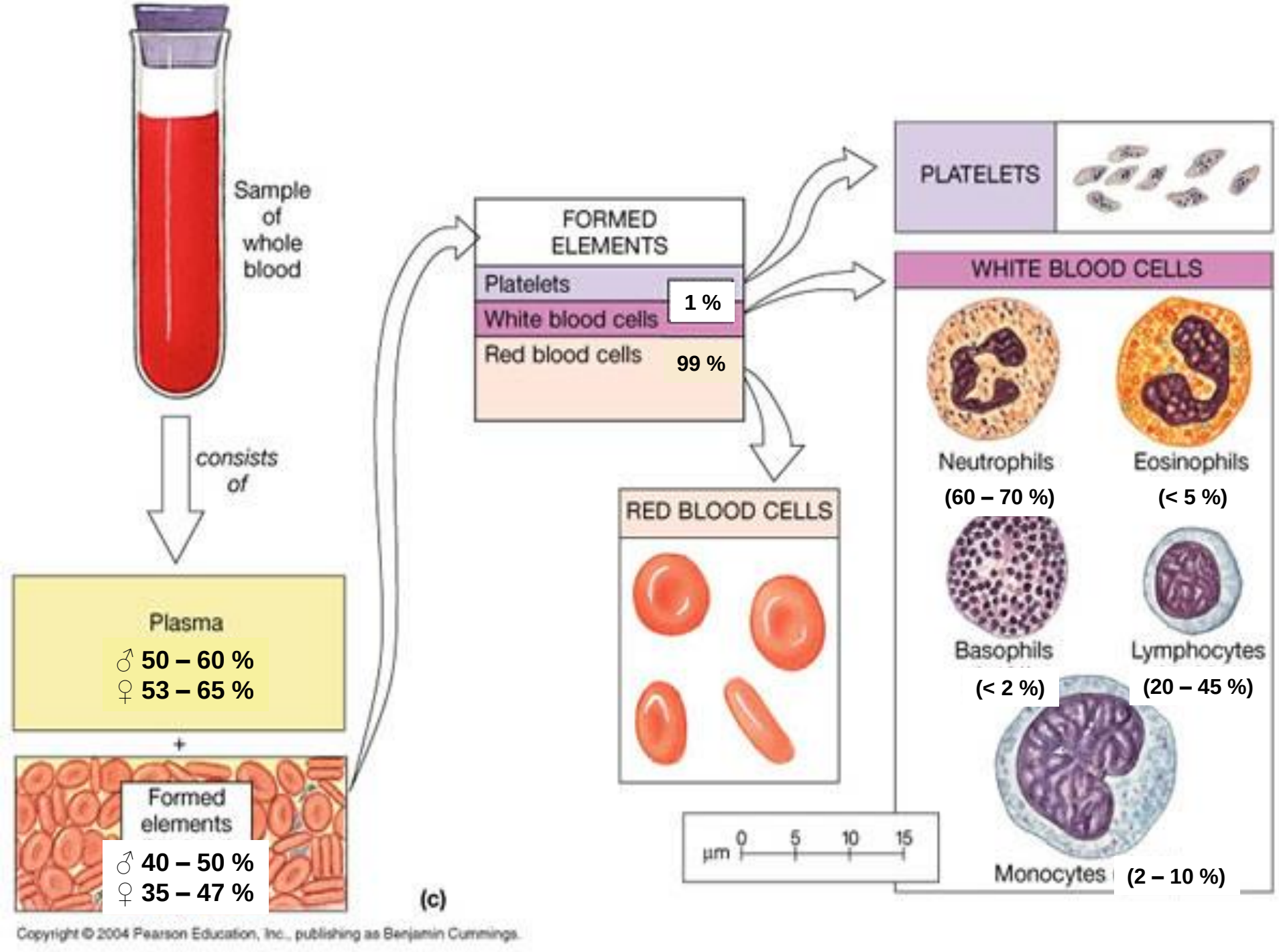
Blood

Body fluids - blood, lymph, tissue fluid



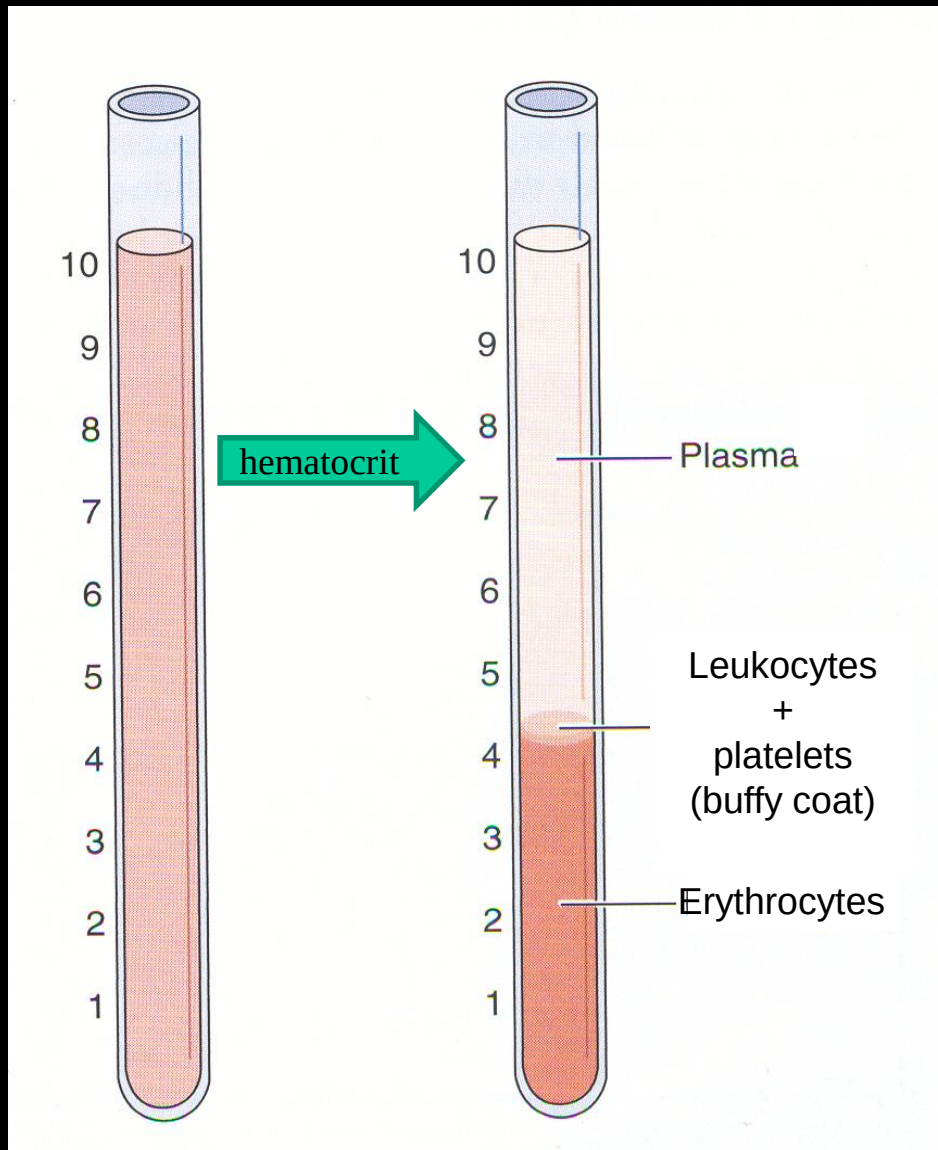
Blood plasma **– yellowish fluid**
90% water
9% *organic compounds* (proteins: albumin, α-,β-,γ- globulins, fibrinogen, complement, aminoacids, glucose, vitamins, hormons, lipids, lipoproteins etc.)
0.9% *inorganic salts*

plasma : formed elements ratio = *hematocrit*
0,35 - 0,47 women
0,40 - 0,50 men

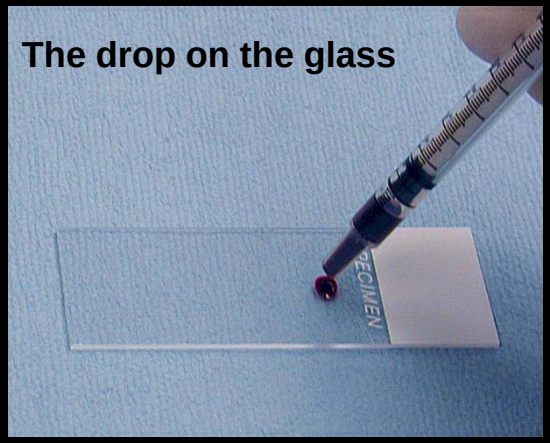
when sampled, blood forms *blood clot - coagulum* containing blood elements trapped within fibrin network and *blood serum*

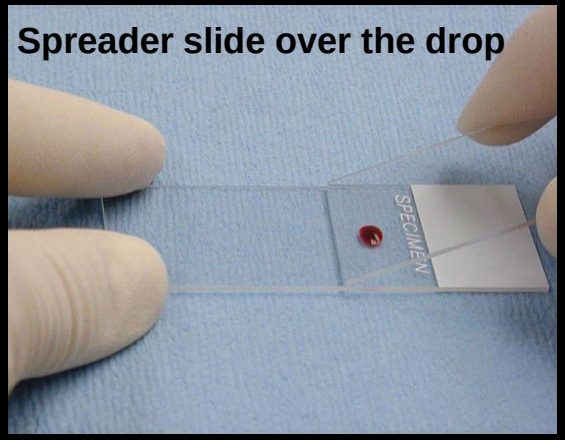
serum **= clear fluid**
plasma without fibrinogen **(protein converted into fibrin during blood clotting) and without *clotting factors***

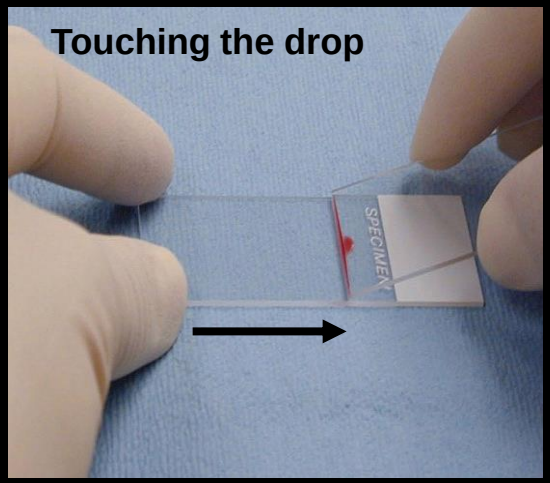
anticoagulants: heparin, sodium citrate, EDTA

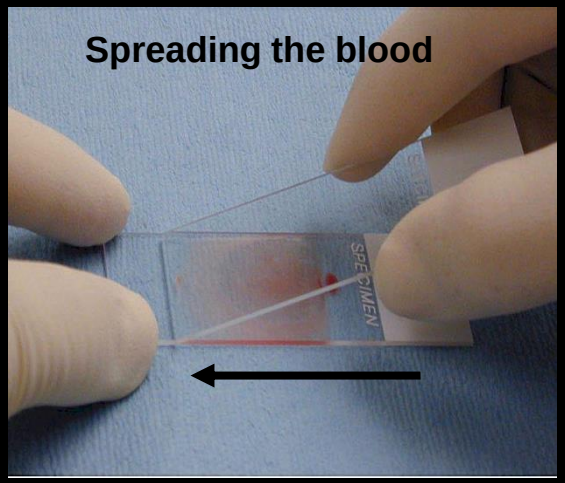


Spreading the blood drop over the glass



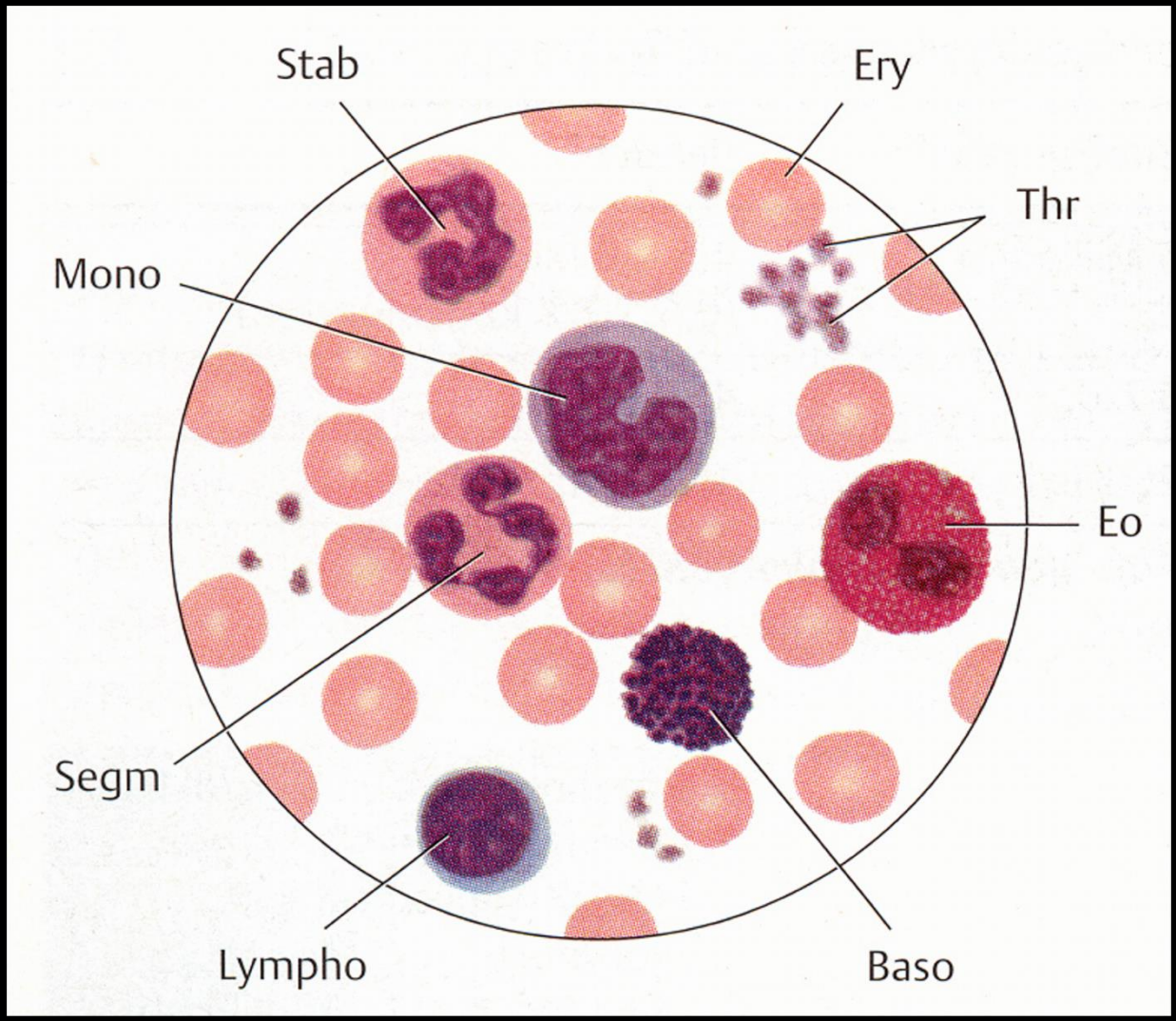






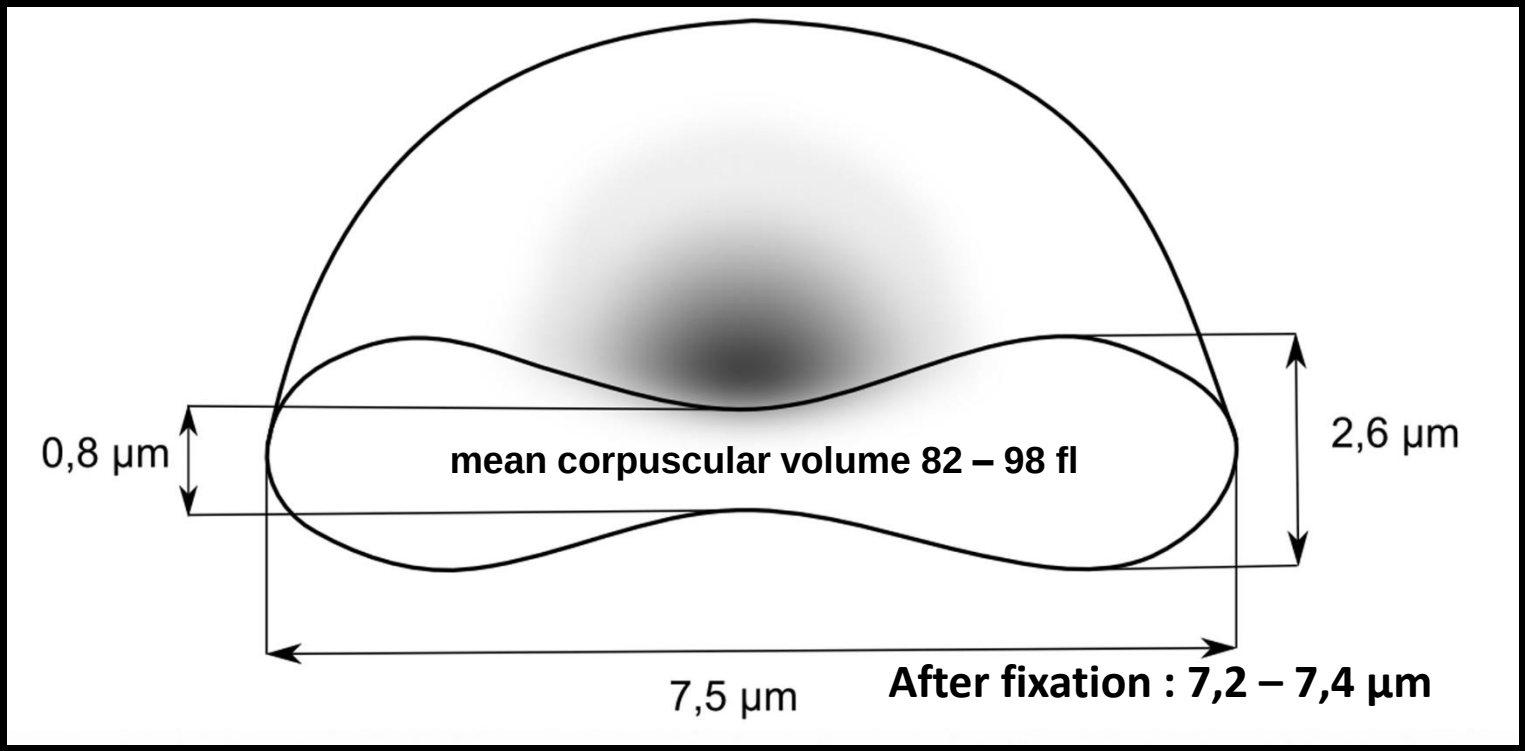
Staining of blood smear: Pappenheim panoptic method

- May-Grünwald solution = methanol (fixation) + methylene blue (basic, stains nuclei and granules of basophilic granulocytes) + eosin (acidic, stains hemoglobin and granules of eosinophilic granulocytes)
- Giemsa-Romanowsky solution = azur B (oxidative product of methylene blue, stains purple azurophilic granules) + eosin

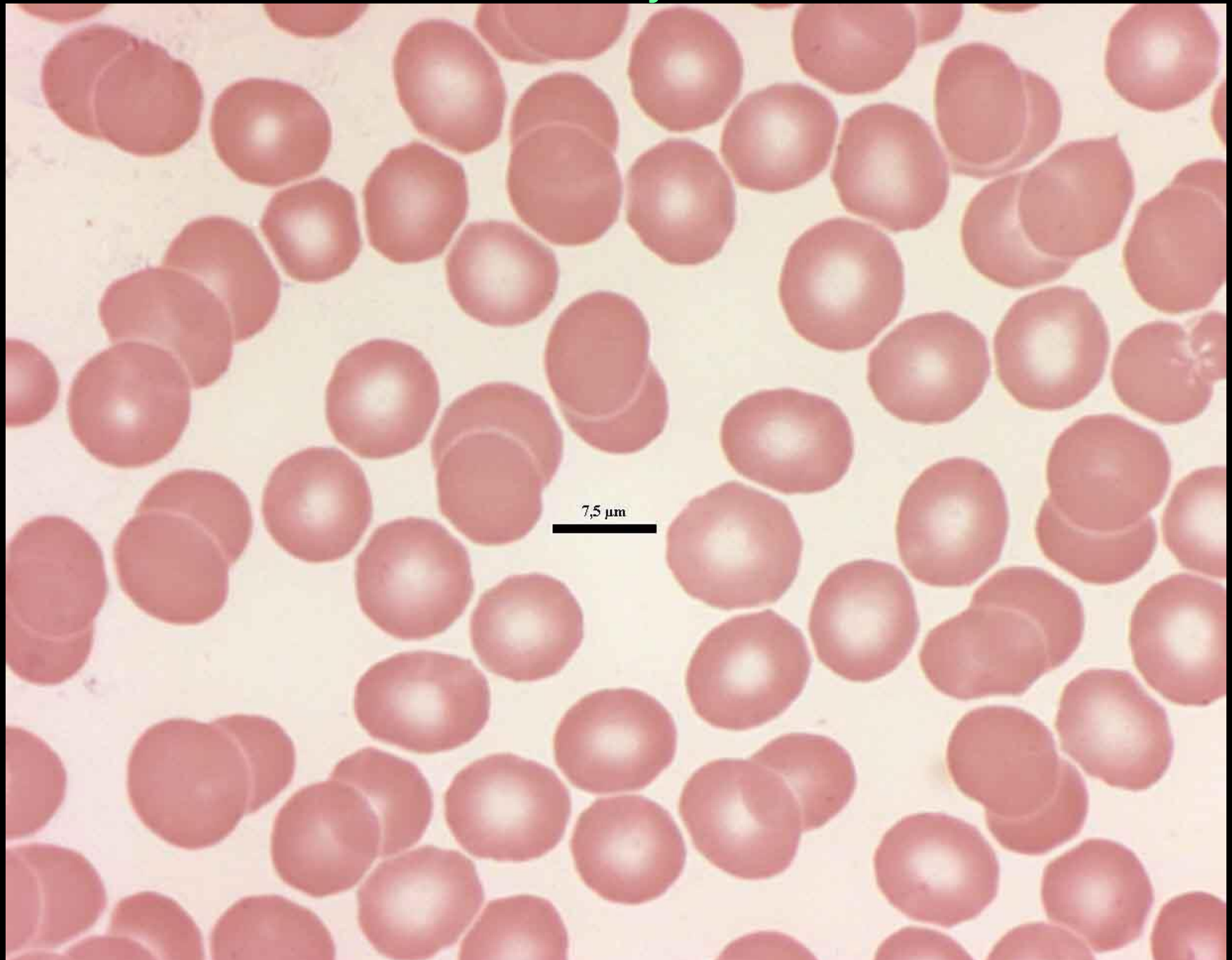


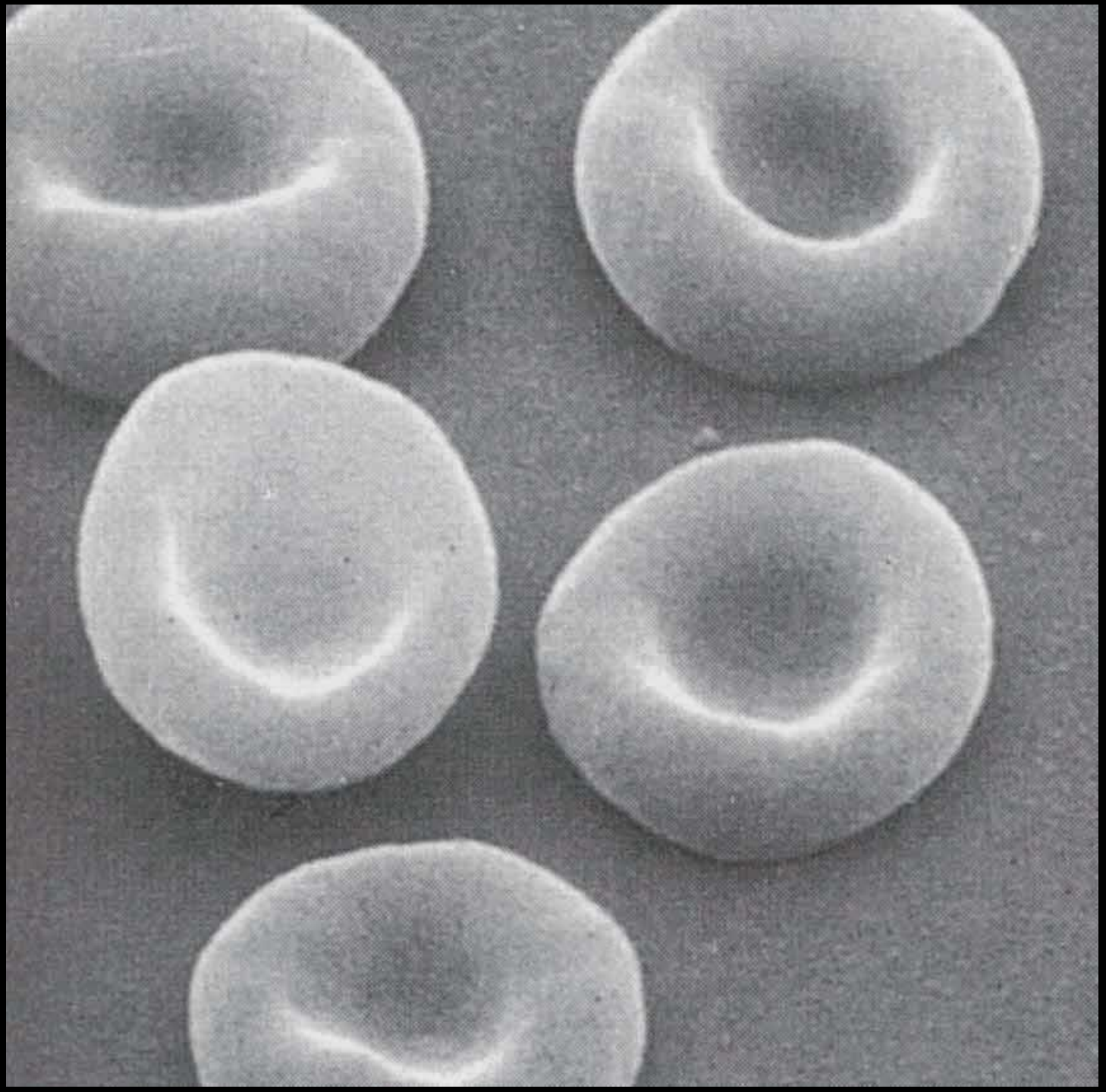
Red blood cells, erythrocytes

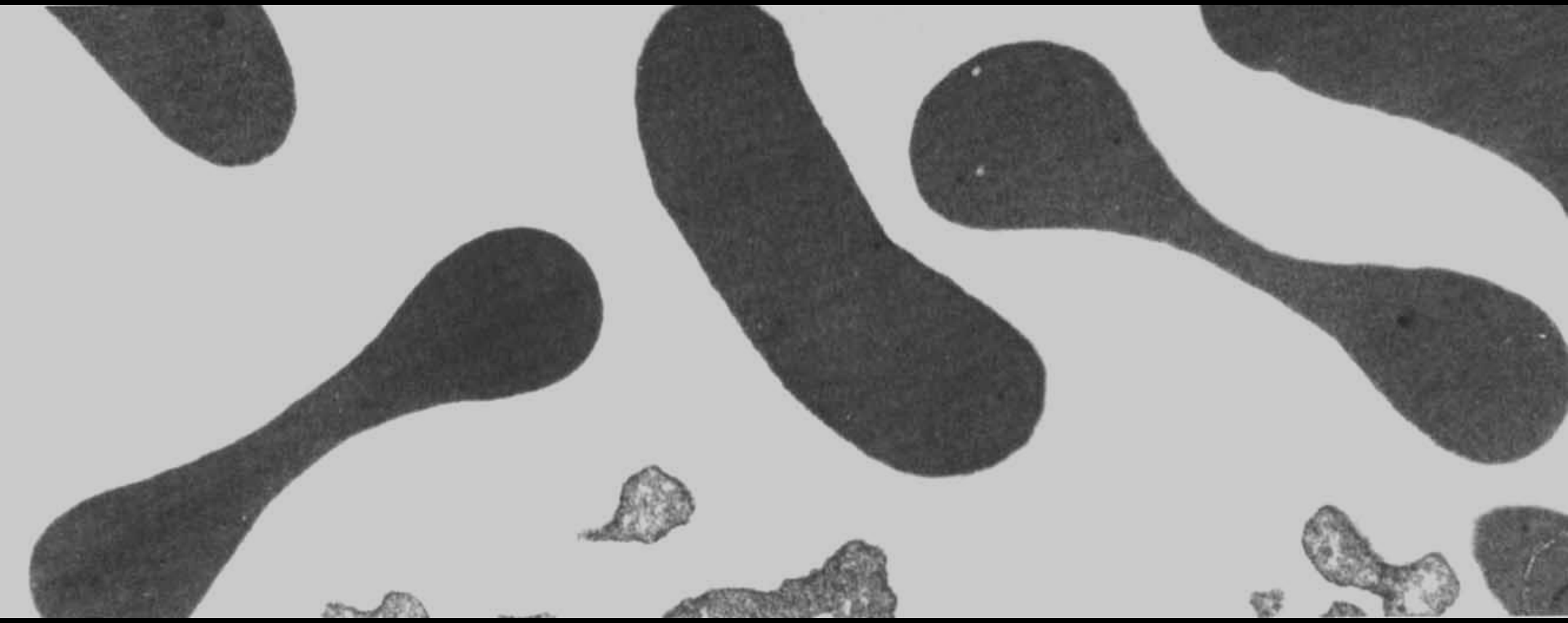
men 4.0 – 5.8 million/μl (mm^3)
women 3.8 – 5.2 million/μl (mm^3)

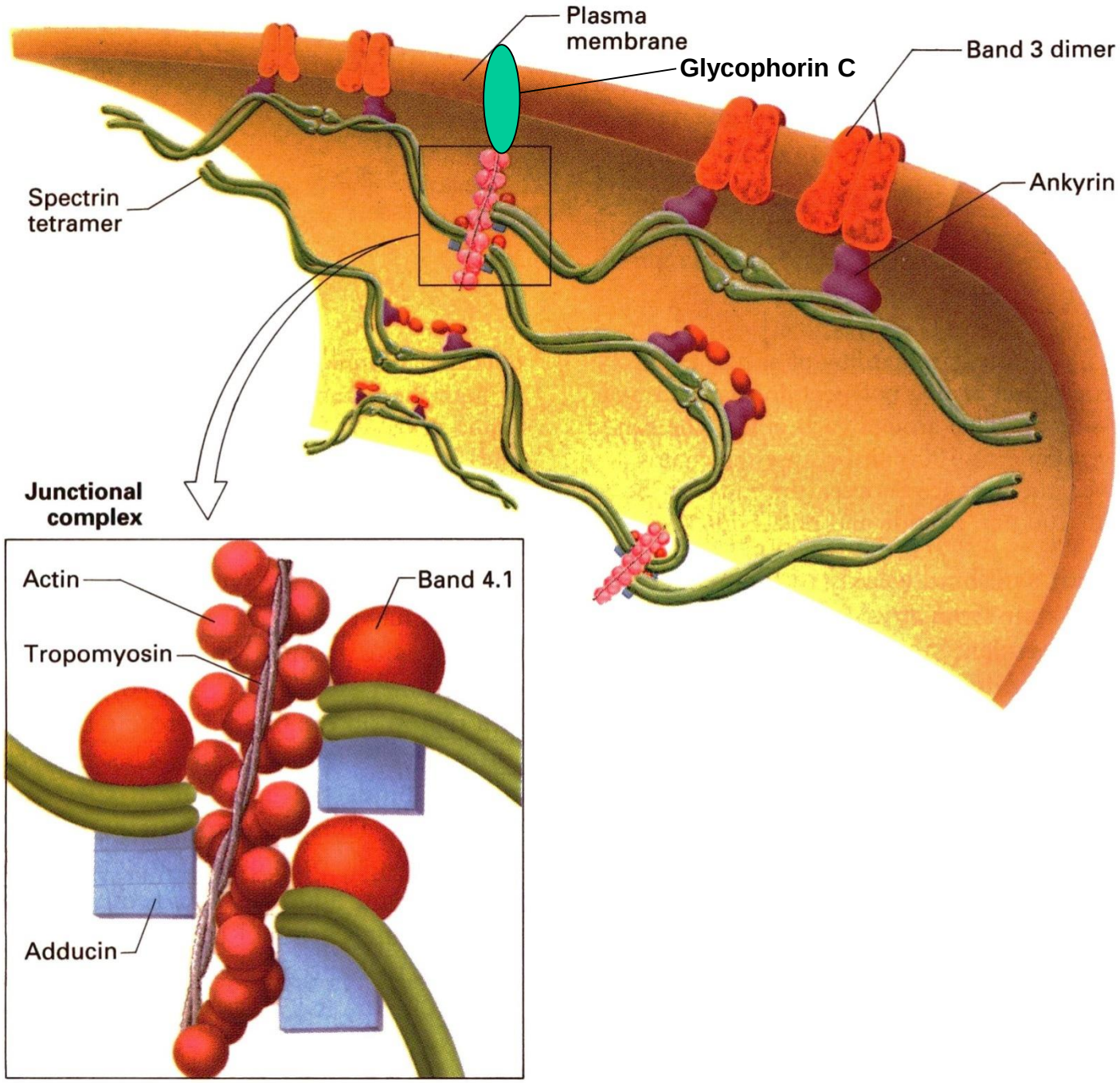


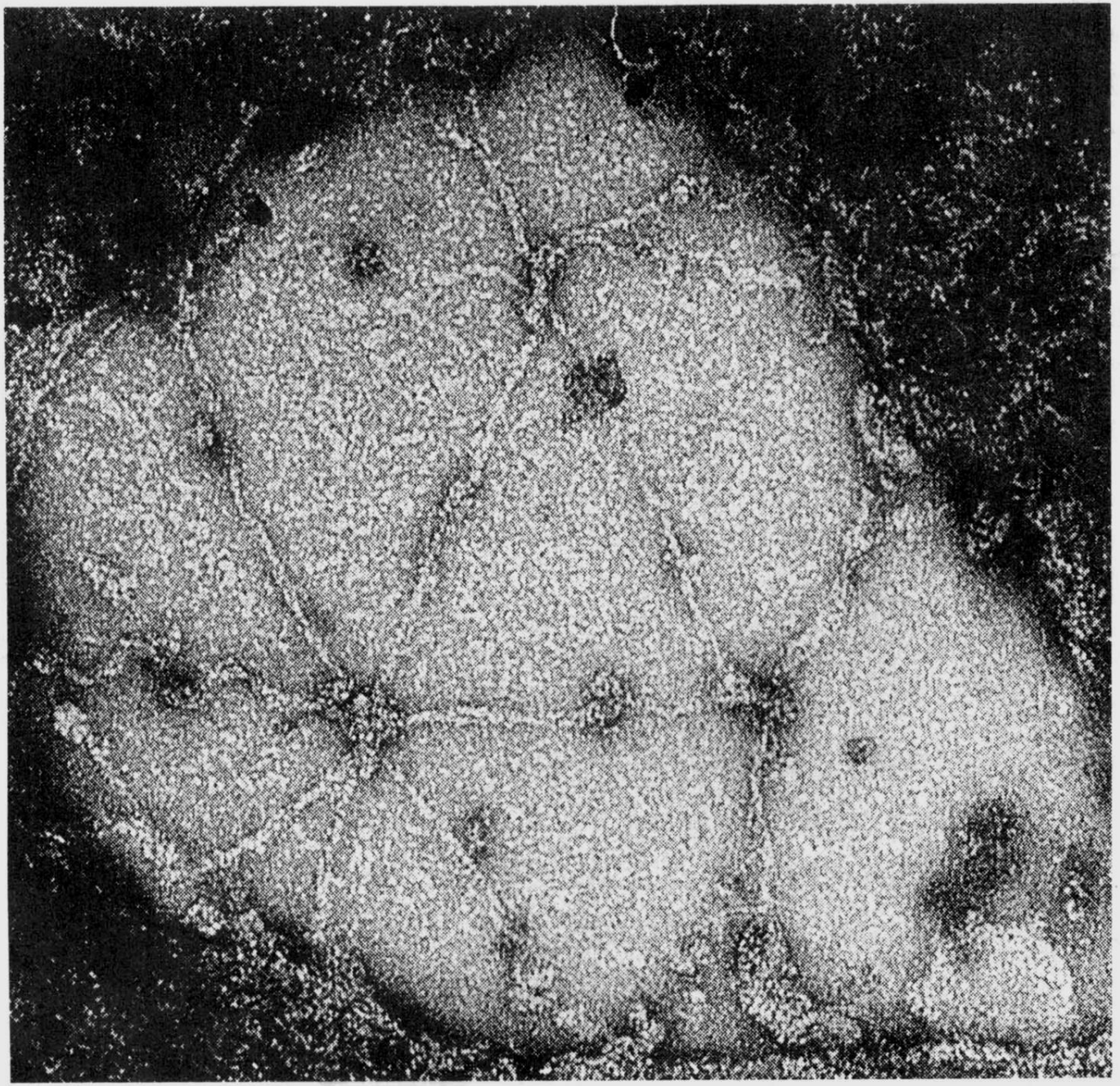
normocytes



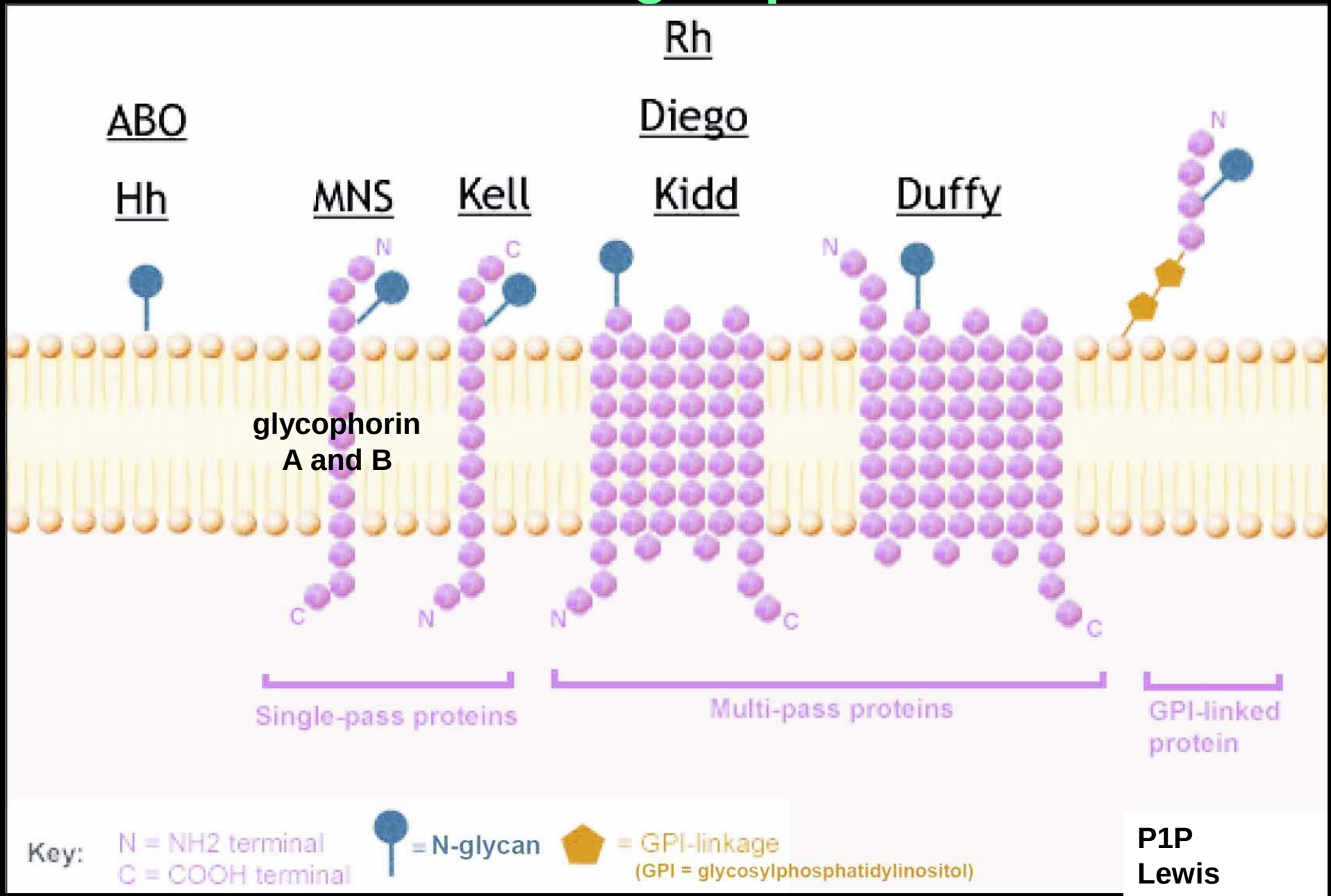





Plasma membrane
Glycophorin C
Band 3 dimer
Spectrin tetramer
Ankyrin
Junctional complex
Actin
Band 4.1
Tropomyosin
Adducin


0.1 μm

Blood groups



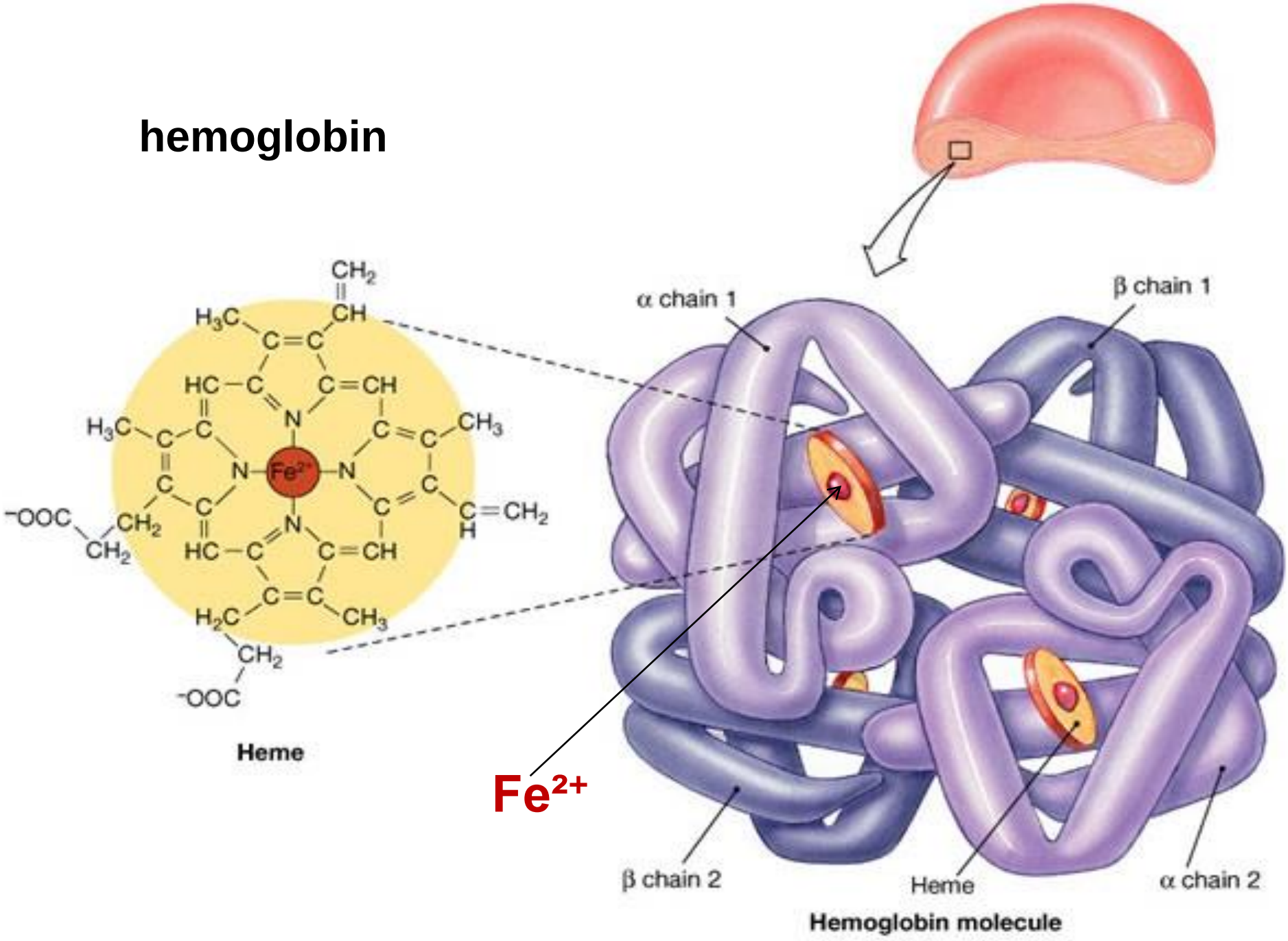
P1P
Lewis
Lutheran

Dean, L.: Blood Groups and Red Cell Antigens, NCBI Bethesda 2005

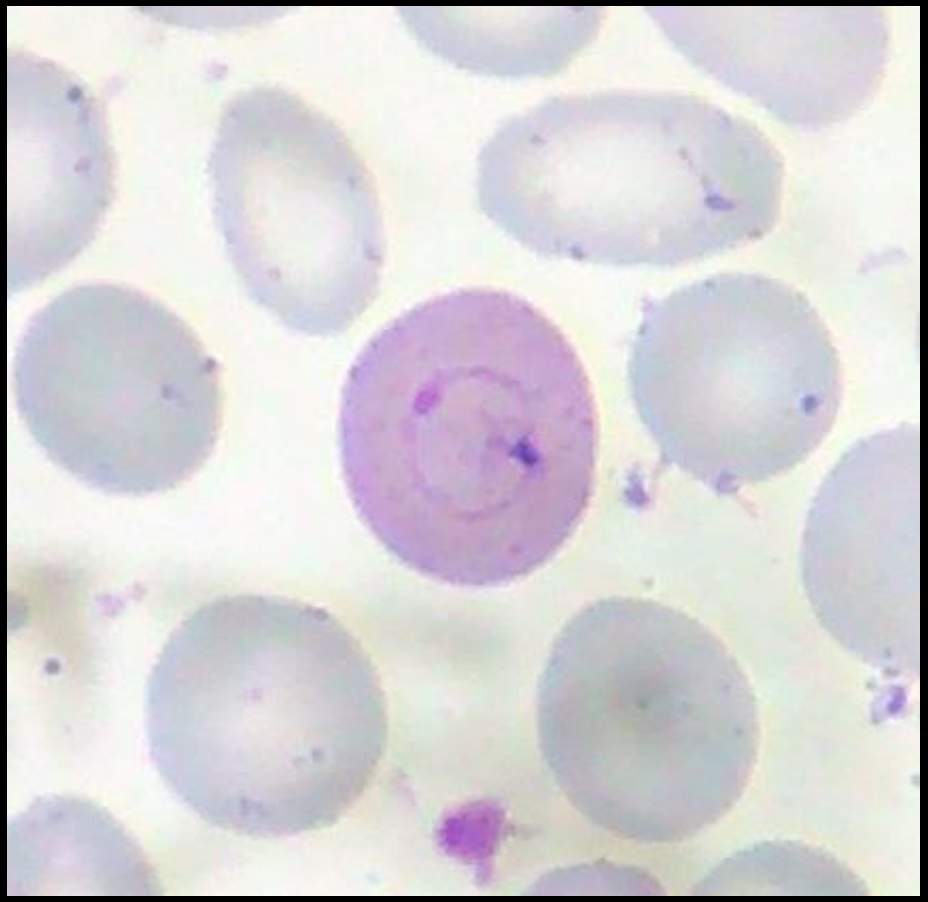
Hemoglobin

- eosinophilic
- 4 globin chains + 4 hemes (Fe^{2+})
- adults: A (2α, 2β) 95%, A_2 (2α,2δ) 3%,
  F (2α,2γ) 2%
- hemoglobin + O_2 = oxyhemoglobin
- hemoglobin + CO_2 = carbaminohemoglobin
- hemoglobin + CO = carboxyhemoglobin
- hemoglobin + NO_2^- = methemoglobin
- 32 - 36 % of erythrocyte volume
- 135 - 175 g/l in men, 120 - 160 g/l in women
- 28 - 34 pg in 1 erythrocyte

hemoglobin



Remnants of nucleus in erythrocyte



Cabot ring

Howell-Jolly body

Cessation of erythrocytes

- average life-span 120 days
- decrease of membrane transport, worsening of flexibility
- exposition of oligosaccharide chains, which were masked by sialic acid
- activation of mechanisms of so-called eryptosis, exposition of phosphatidylserine in outer leaf of membrane
- phagocytosis by macrophages, mainly in spleen

White blood cells, leukocytes

adults: 4,000 – 10,000/µl (mm^3)

1^{st} day: 9,000 – 38,000/µl

7^{th} day: 5,000 – 21,000/µl

10 – 15 years: 4,500 - 13,500/µl

- express membrane ligands for E- and P-selectins (cell adhesion molecules) of endothelial cells – marginal pool

  “rolling“ on a vessel wall

  diapedesis, emperipolesis, chemotaxis

Differential blood count

A) granulocytes

60 – 70 %  10-12 µm **neutrophilic granulocytes**

Polymorphonuclears

< 5 %  12-14 µm **eosinophilic** "

< 2 %  10 µm **basophilic** "

B) agranulocytes

20 – 45 %  6-8 µm **lymphocytes**

Mononuclears

2 – 10 %  12-20 µm **monocytes**

Differential blood count

Diagram

Special way of examination of the smear

cell	1	2	3	4	5	6	7	8	9	10	Σ (%)
NB	| || |	→									
NS	|										
EO	|										
BA											
LY	| | |										
MO	|										
	10	10	10	10	10	10	10	10	10	10	100

10 marks in each column ……..you determine 100 leukocytes , then count the marks in the row

Granulocytes (polymorphonuclear leukocytes)

Neutrophilic granulocyte

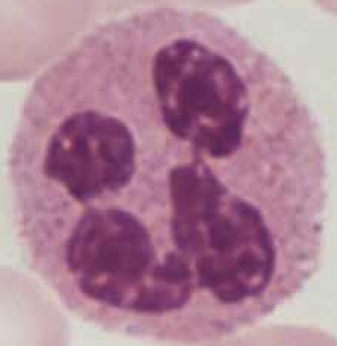
Eosinophilic granulocyte

Basophilic granulocyte

Neutrophilic granulocytes, neutrophils

Size : 10 – 12 µm

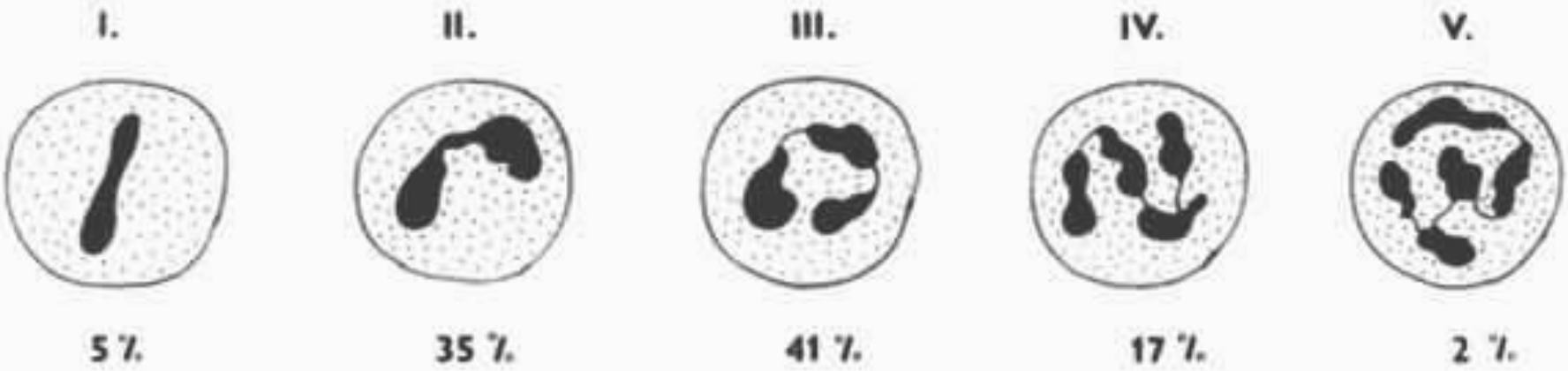
60 – 70 %



Specific granules (salmon pink)
and
azurophilic granules (purple red)

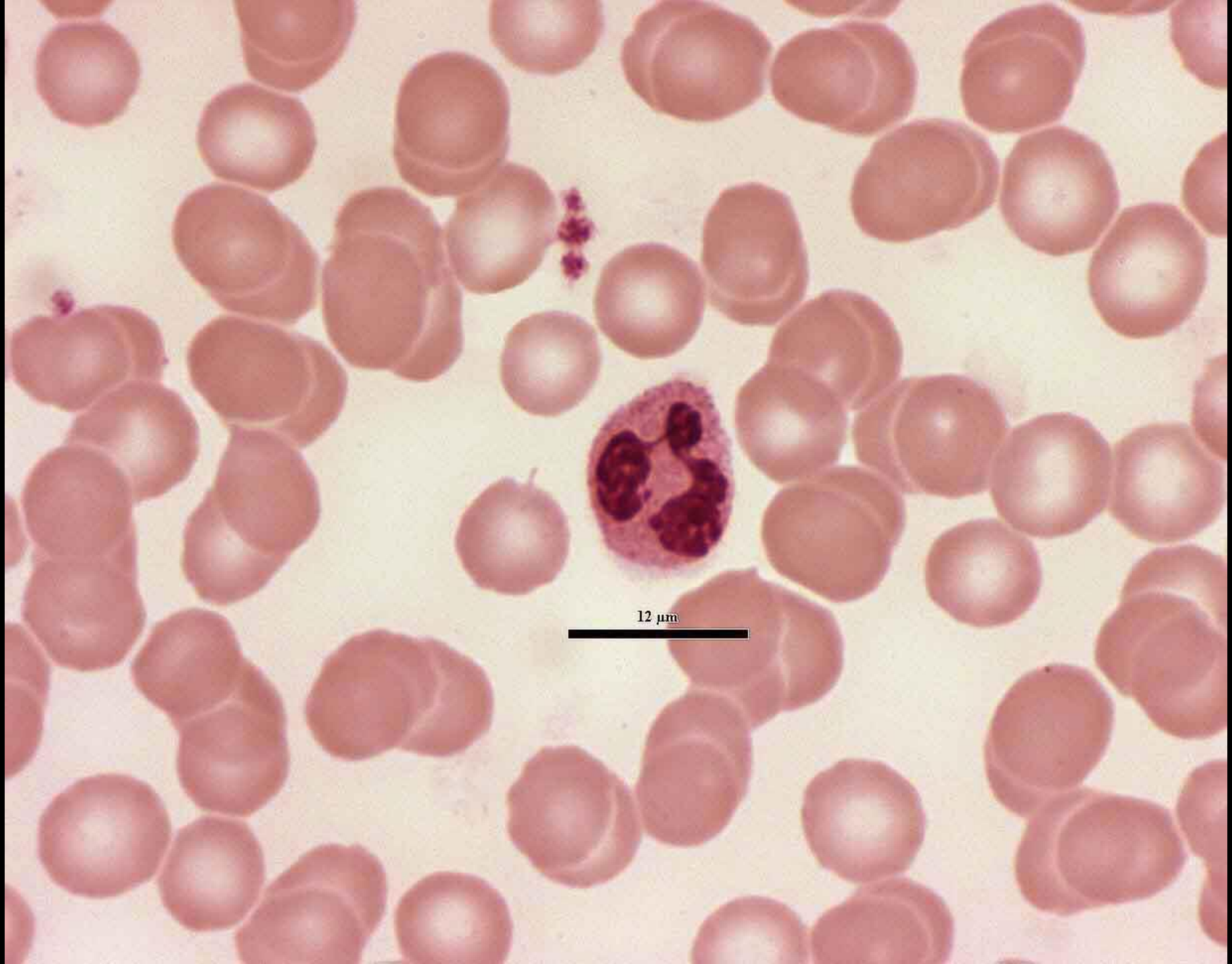
NEUTROPHILIC GRANULOCYTES

percentage of individual stages

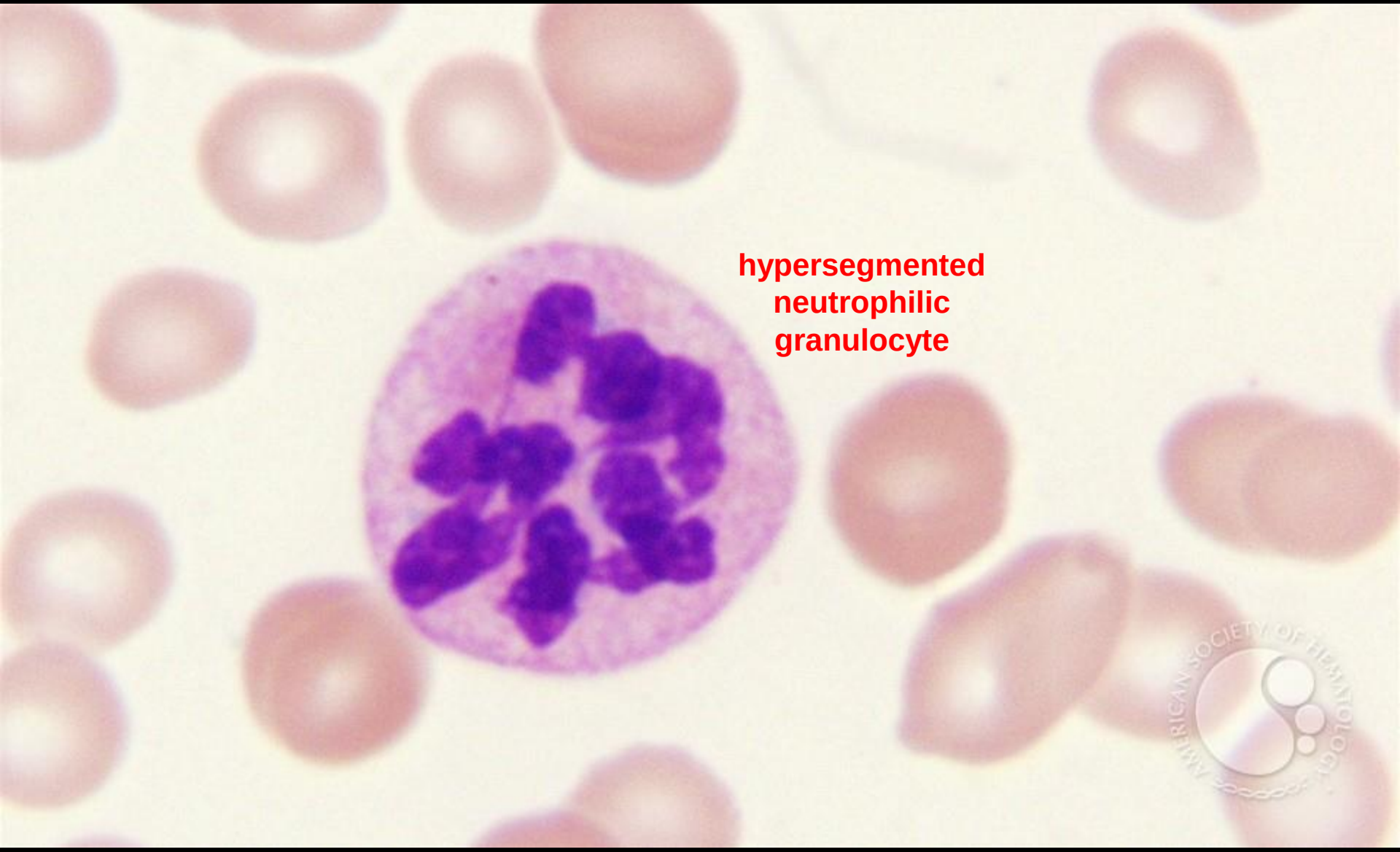


band

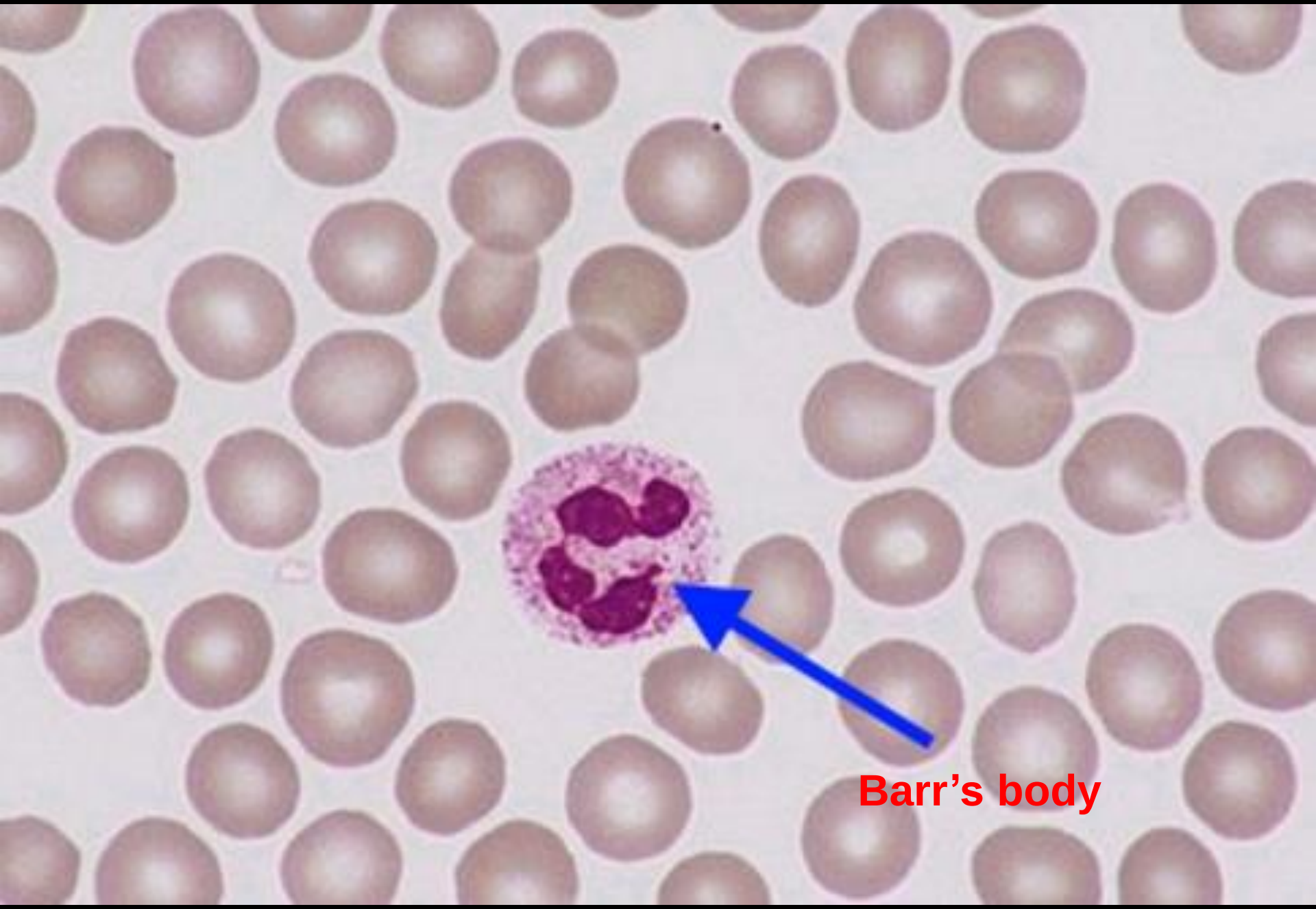
hypersegmented neutrophilic granulocyte


12 µm

https://www.labce.com/spg19934_definition_of_a__band_cell.aspx



https://imagebank.hematology.org/image/4051/hypersegmented-neutrophil--1

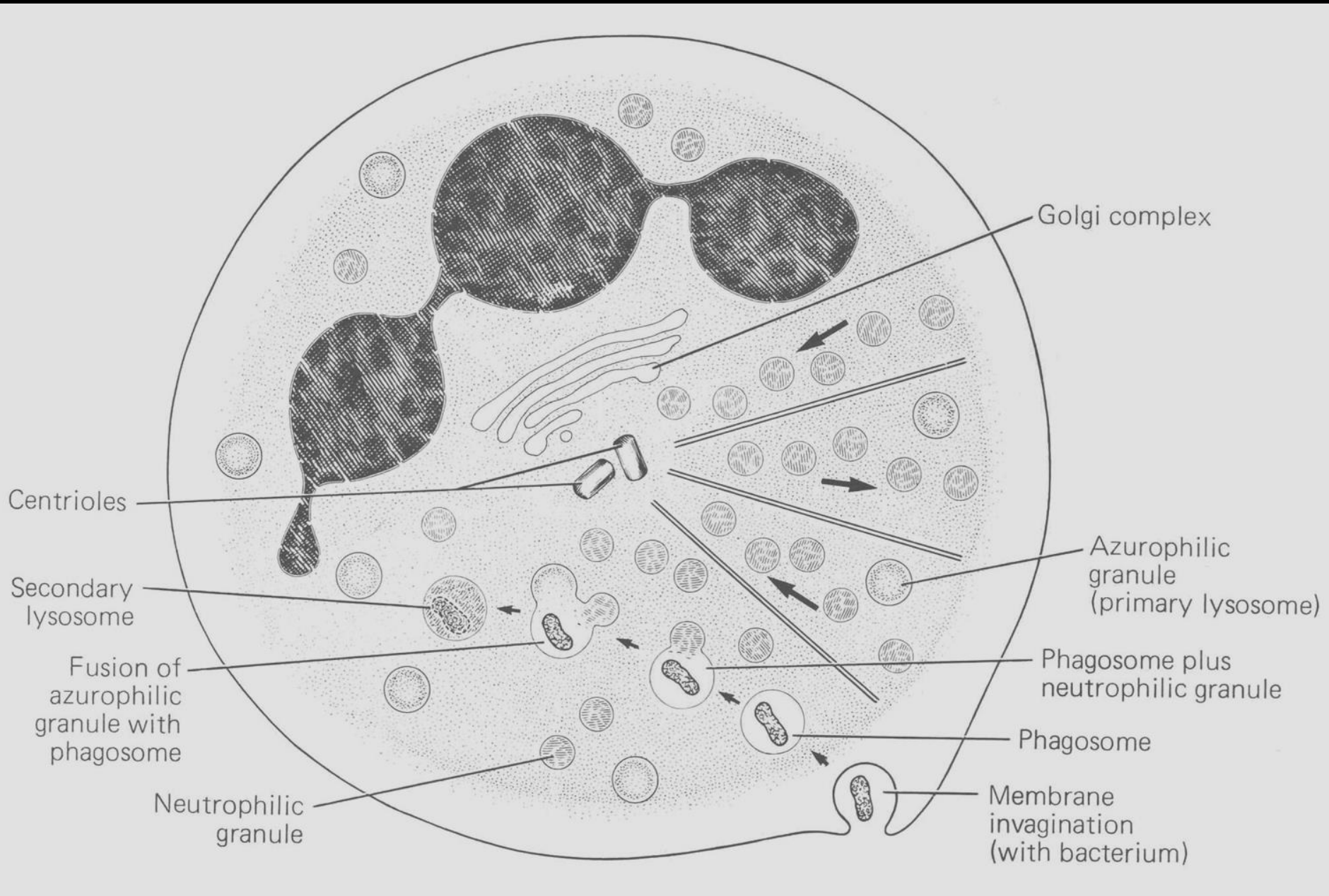


https://www.labce.com/spg28945_barr_body.aspx


Specific granules
Azurophilic granules

CELL TYPE	SPECIFIC GRANULES	AZUROPHILIC GRANULES
NEUTROPHIL	alkaline phosphatase	lysosomal enzymes
	collagenase	myeloperoxidase
	lactoferrin	lysozyme
	lysozyme	defensins
	fagocytins	
	phospholipase	
	complement activators	
	Neutrophilic granulocytes – tertiary granules (metalloproteinases, phosphatases)	
EOSINOPHIL	eosinophilic peroxidase (EPO)	lysosomal enzymes
	major basic protein (MBP)	
	eosinophilic cationic protein (ECP)	
	specific neurotoxin (EDN)	
	arylsulphatase	
	histaminase	
BASOPHIL	heparin	lysosomal enzymes
	histamine	
	chondroitin sulfate	
	slow-reacting substance of anaphylaxis (SRS-A)	
	eosinophil chemotactic factor	


G

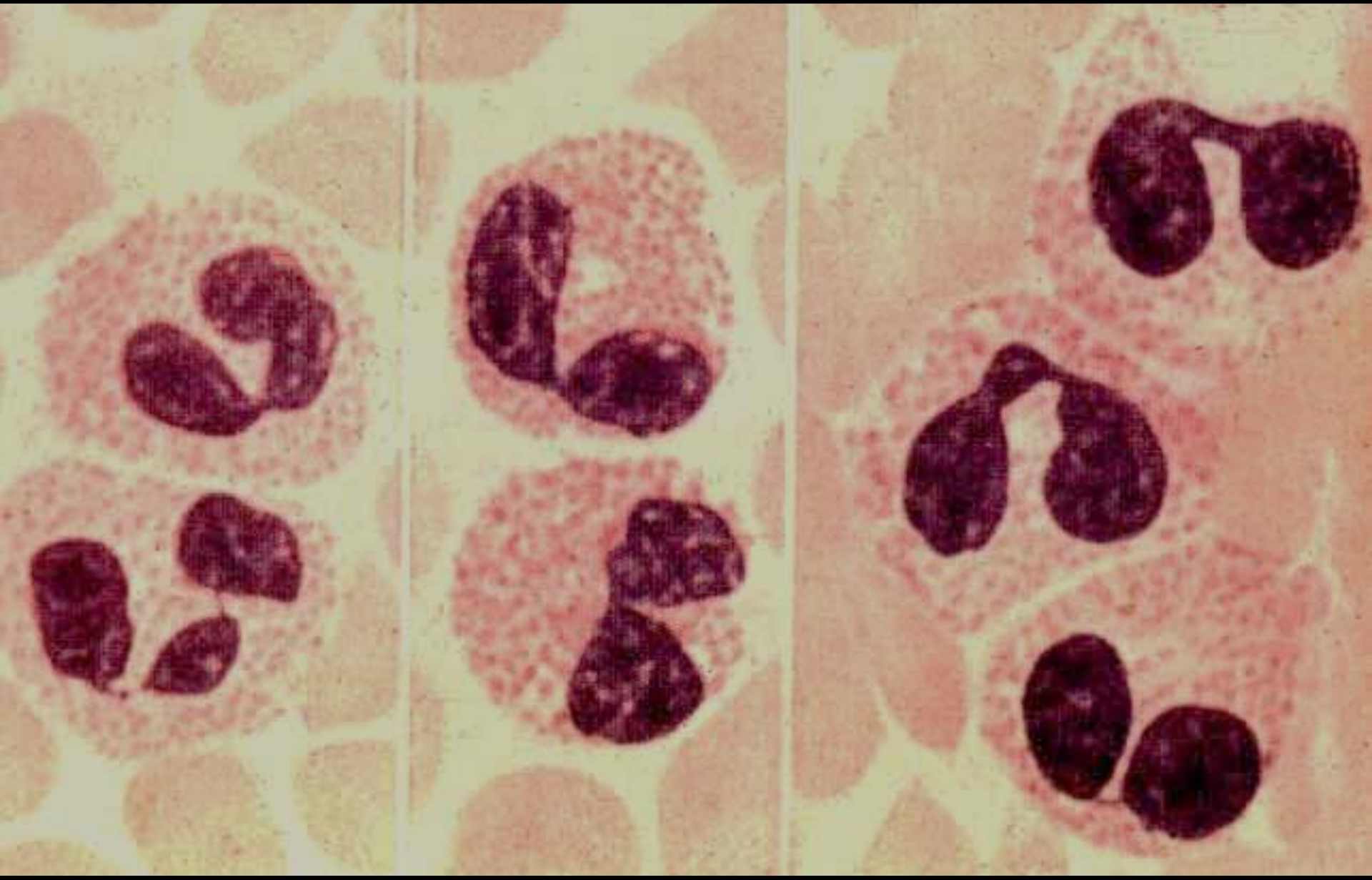

Golgi complex
Centrioles
Secondary lysosome
Fusion of azurophilic granule with phagosome
Neutrophilic granule
Azurophilic granule (primary lysosome)
Phagosome plus neutrophilic granule
Phagosome
Membrane invagination (with bacterium)

Eosinophilic granulocytes, eosinophils

Size : 12 – 14 µm

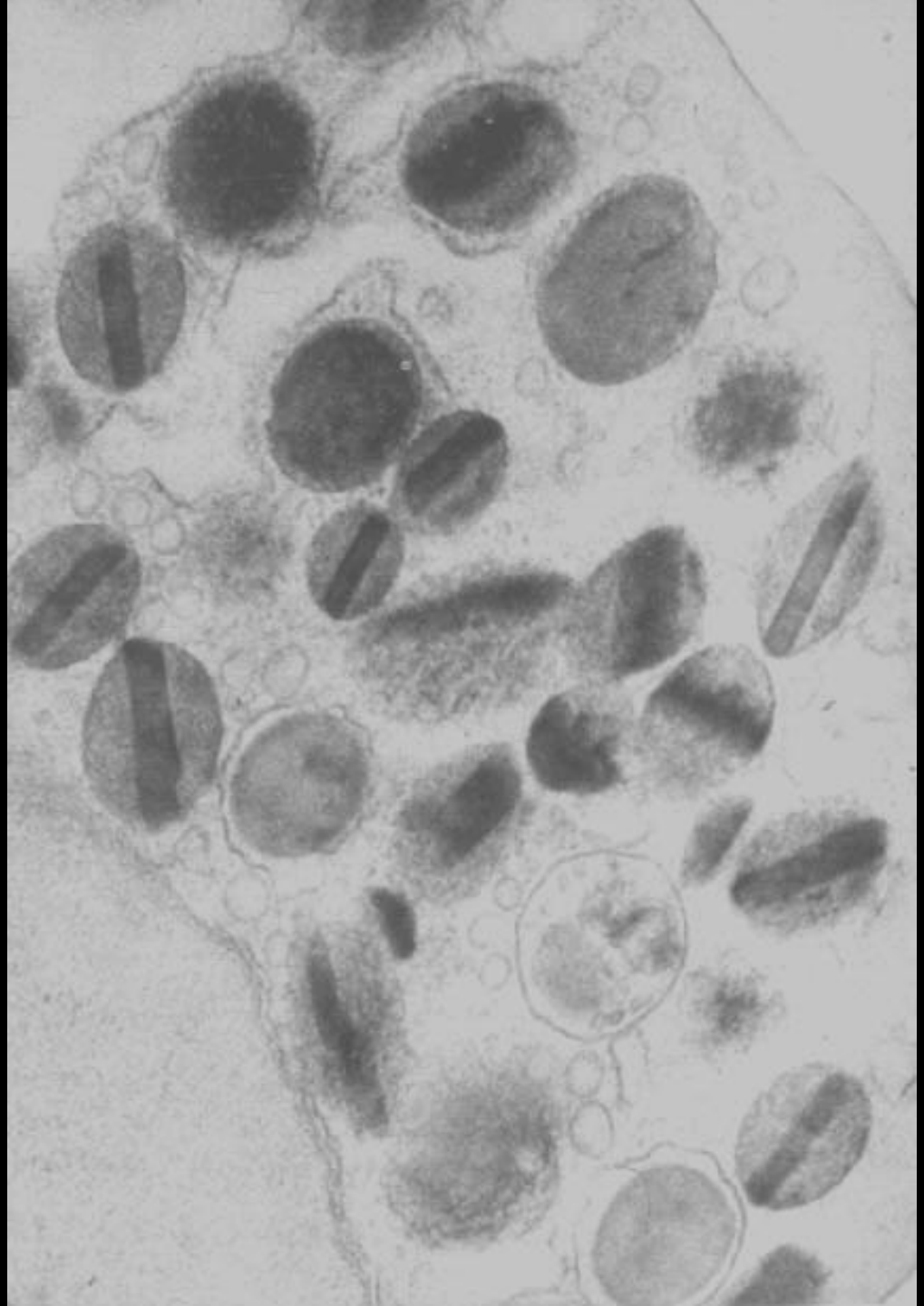
0 – 5 %

Specific granules
(brick red)
Azurophilic granules
(purple red)




EG
N
M

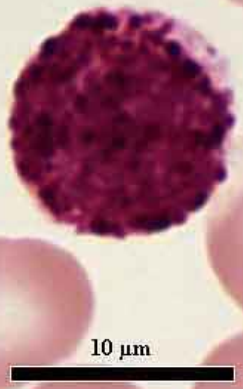
CELL TYPE	SPECIFIC GRANULES	AZUROPHILIC GRANULES
NEUTROPHIL	alkaline phosphatase	lysosomal enzymes
	collagenase	myeloperoxidase
	lactoferrin	lysozyme
	lysozyme	defensins
	fagocytins	
	phospholipase	
	complement activators	
	Neutrophilic granulocytes – tertiary granules (metalloproteinases, phosphatases)	
EOSINOPHIL	eosinophilic peroxidase (EPO)	lysosomal enzymes
	major basic protein (MBP)	
	eosinophilic cationic protein (ECP)	
	specific neurotoxin (EDN)	
	arylsulphatase	
	histaminase	
BASOPHIL	heparin	lysosomal enzymes
	histamine	
	chondroitin sulfate	
	slow-reacting substance of anaphylaxis (SRS-A)	
	eosinophil chemotactic factor	



Basophilic granulocytes, basophils

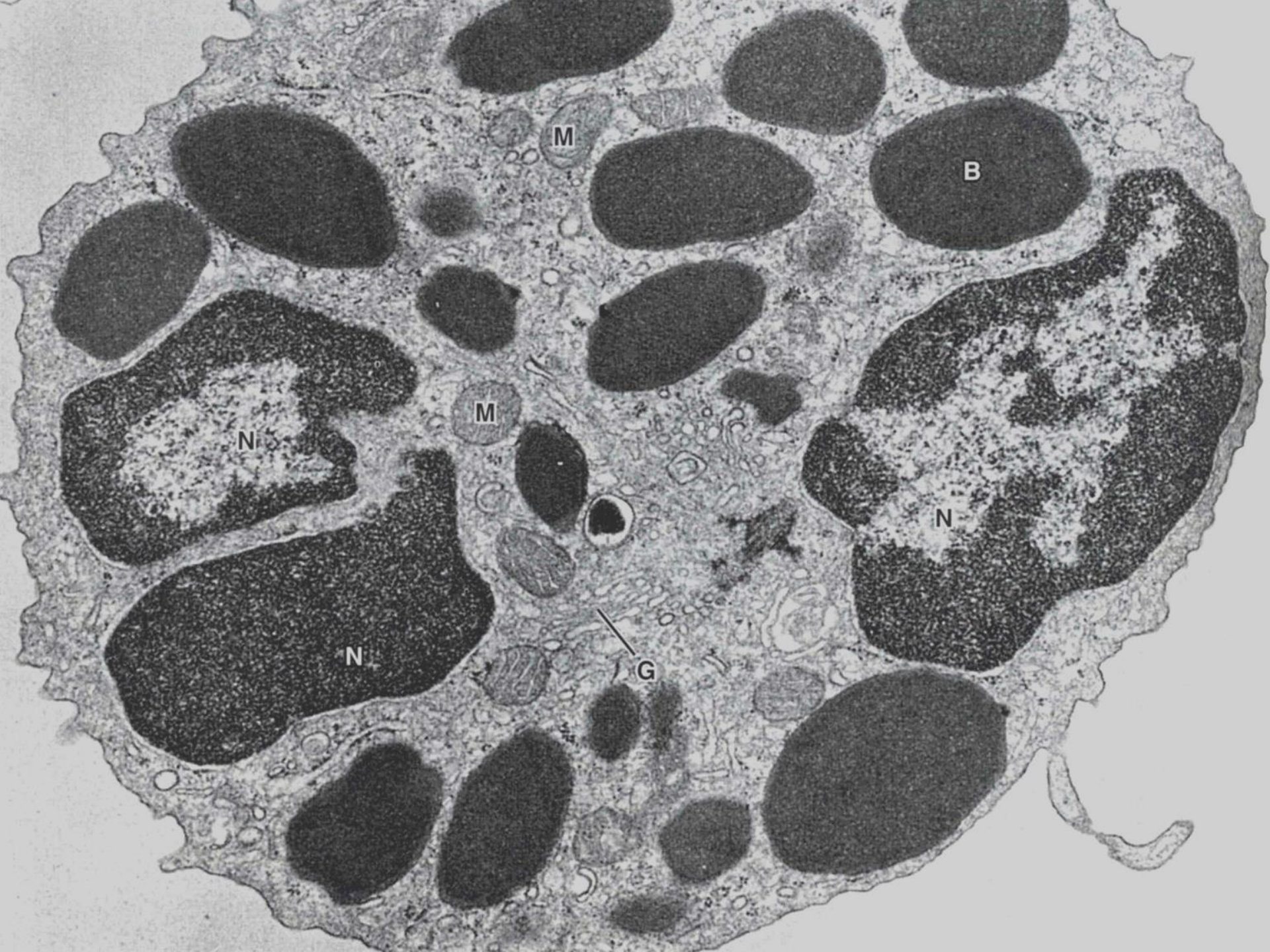
Size : 8 – 10 µm,

0 – 2 %



Specific granules
(0, 5 µm)
Dark blue – violet

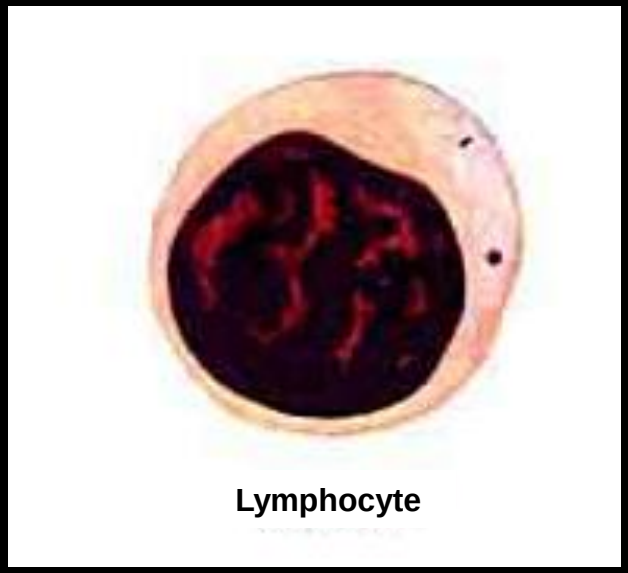
Azurophilic granules
Purple red


M
B
M
N
N
N
G


M
B
B
M
MF

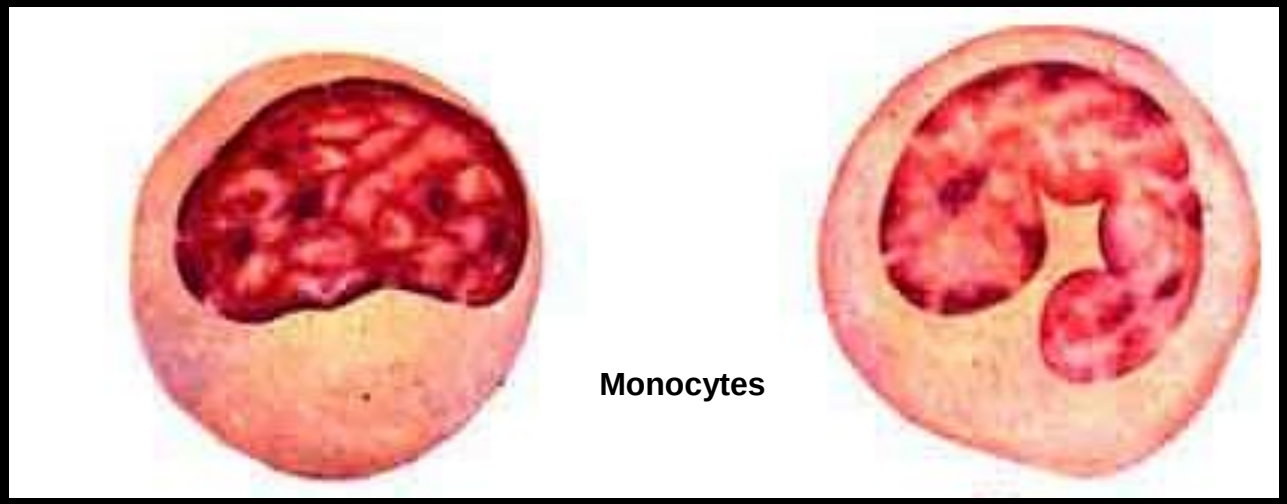
CELL TYPE	SPECIFIC GRANULES	AZUROPHILIC GRANULES
NEUTROPHIL	alkaline phosphatase	lysosomal enzymes
	collagenase	myeloperoxidase
	lactoferrin	lysozyme
	lysozyme	defensins
	fagocytins	
	phospholipase	
	complement activators	
	Neutrophilic granulocytes – tertiary granules (metalloproteinases, phosphatases)	
EOSINOPHIL	eosinophilic peroxidase (EPO)	lysosomal enzymes
	major basic protein (MBP)	
	eosinophilic cationic protein (ECP)	
	specific neurotoxin (EDN)	
	arylsulphatase	
	histaminase	
BASOPHIL	heparin	lysosomal enzymes
	histamine	
	chondroitin sulfate	
	slow-reacting substance of anaphylaxis (SRS-A)	
	eosinophil chemotactic factor	

Agranulocytes (mononuclear leukocytes)



Lymphocyte

20 – 45 %



Monocytes

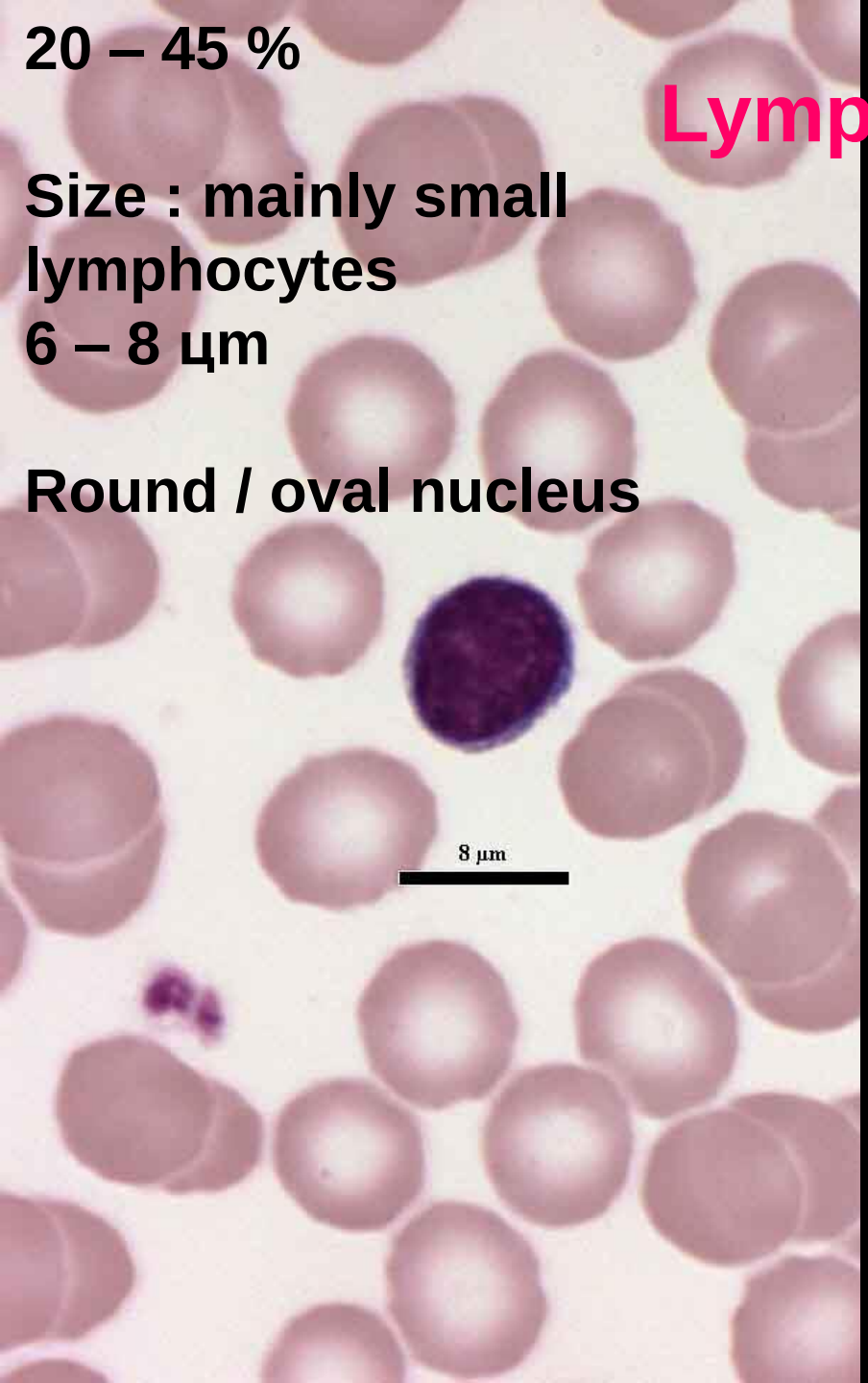
2 – 10 %

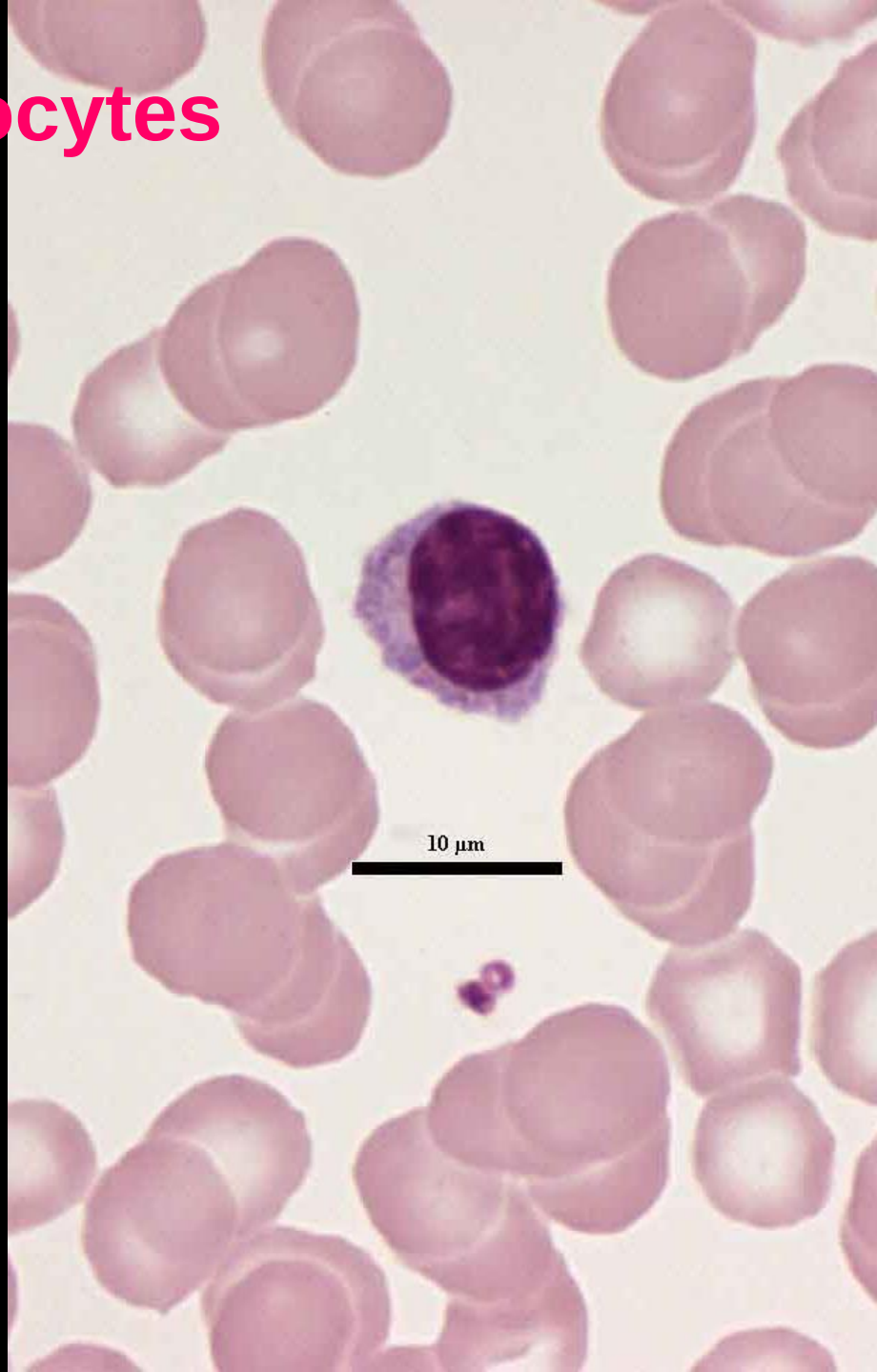
20 – 45 %

Lymphocytes

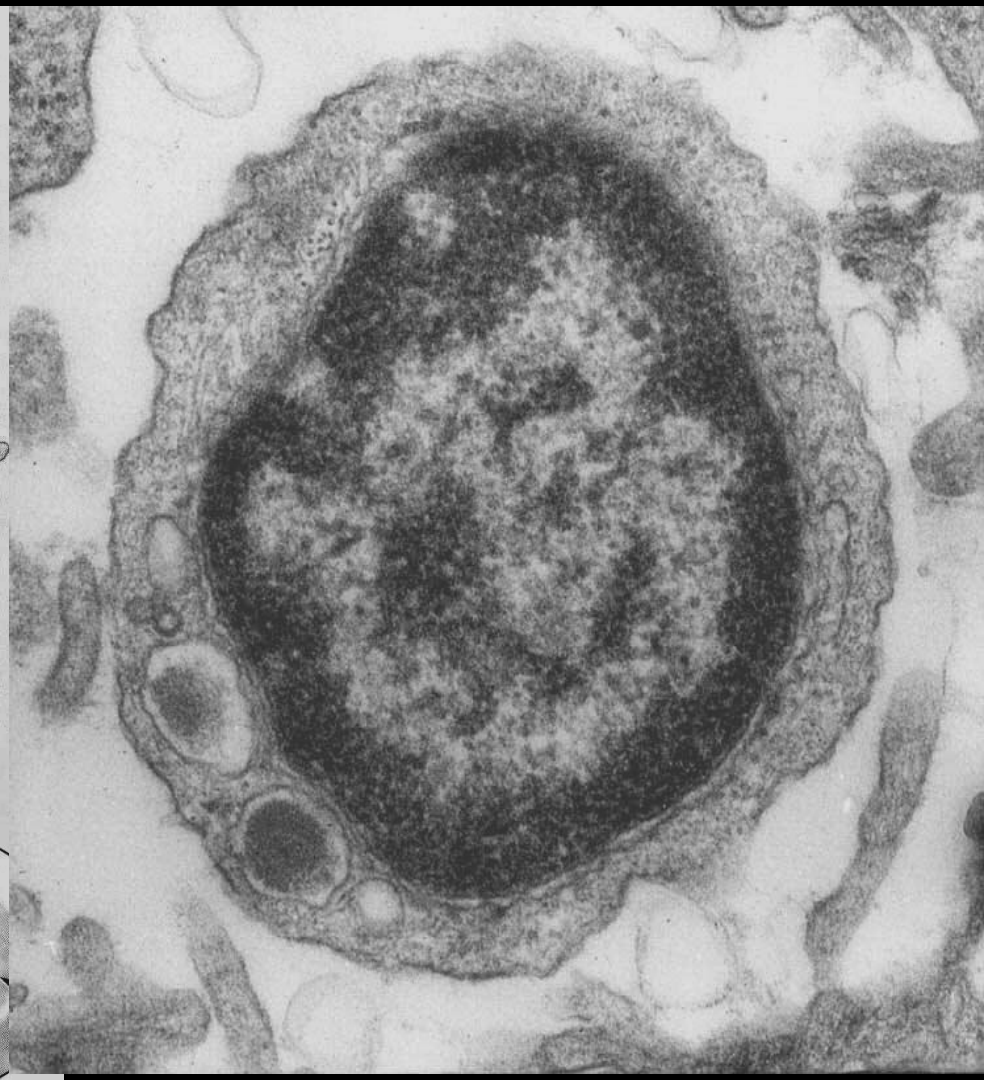
Size : mainly small lymphocytes
6 – 8 µm

Round / oval nucleus





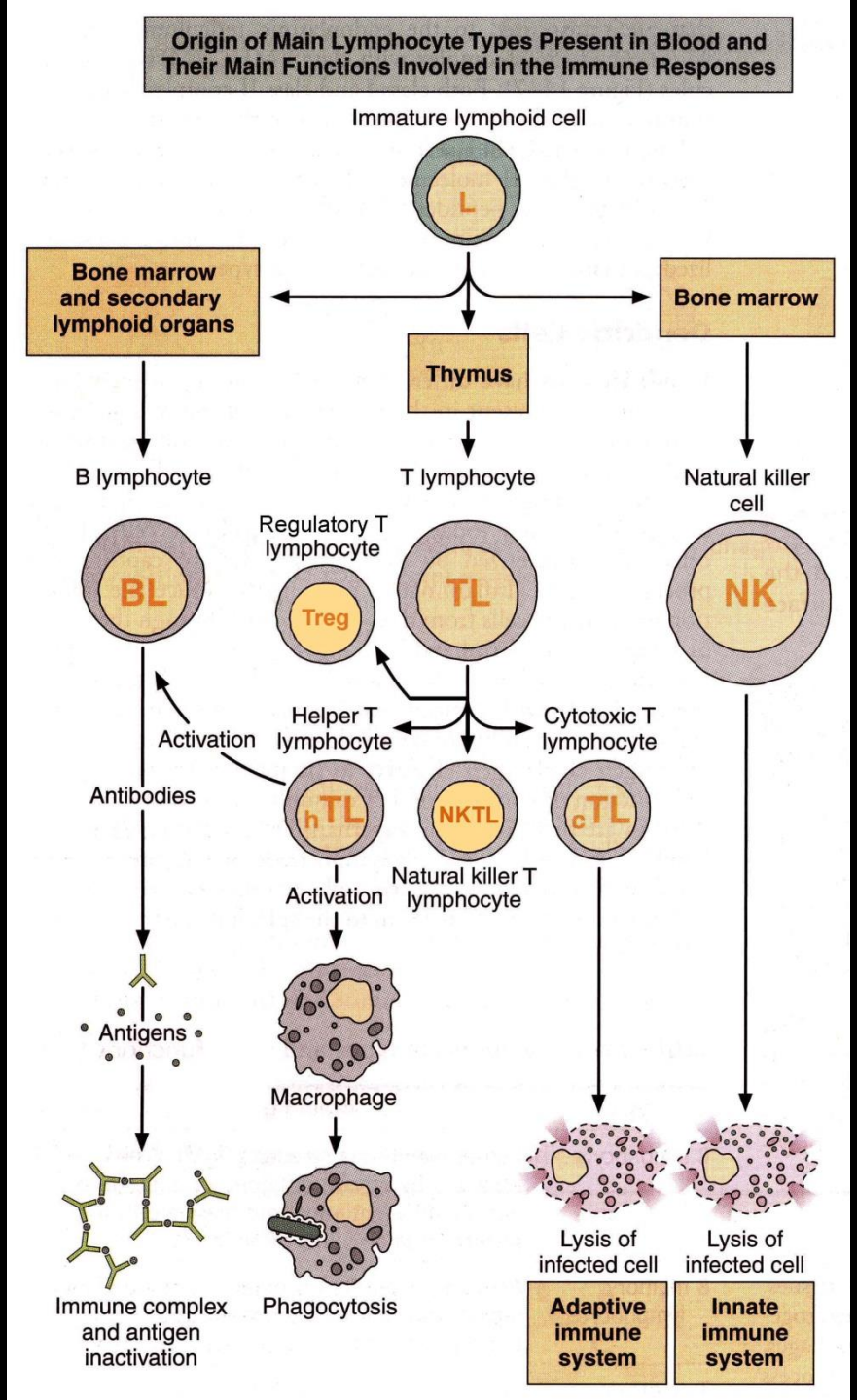

M
M
G
G
M



Lymphoblast (in bone marrow)

Places of maturation (immunocompetency acquirement)

Types of lymphocytes



SURFACE ANTIGENS

all B- lymphocytes
CD20,23,(19) BCR
MHC II

---

all T-lymphocytes
CD3 TCR

T_hL CD4

T_cL CD8

$T_{reg}L$ CD4 or CD8
CD25 and FOXP3

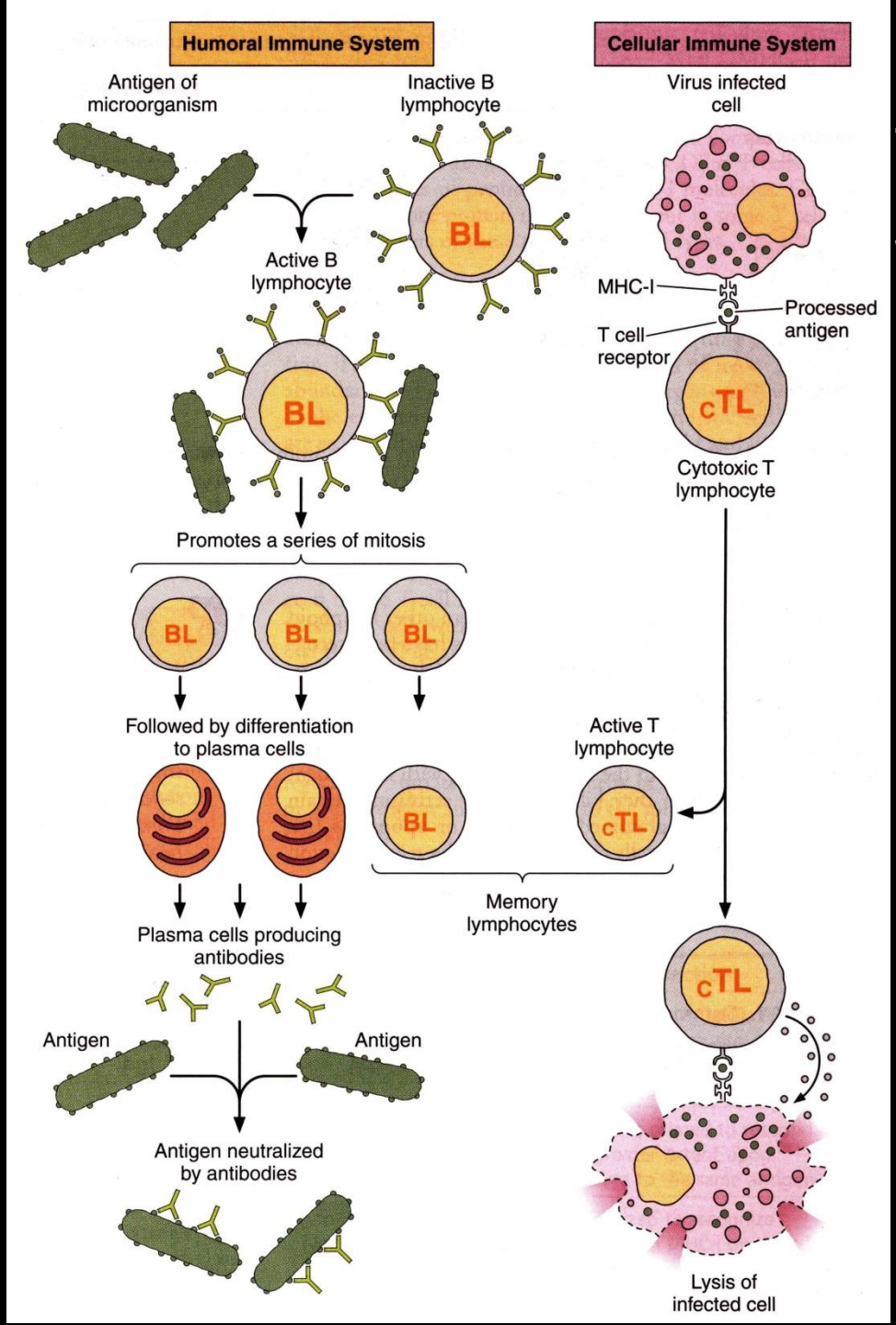
NKTL and other unconventional TL (MAIT)
CD1d CD16

NK-cells
CD16 CD56

antigen-specific stimulative signal

BL - free antigens

effector cells

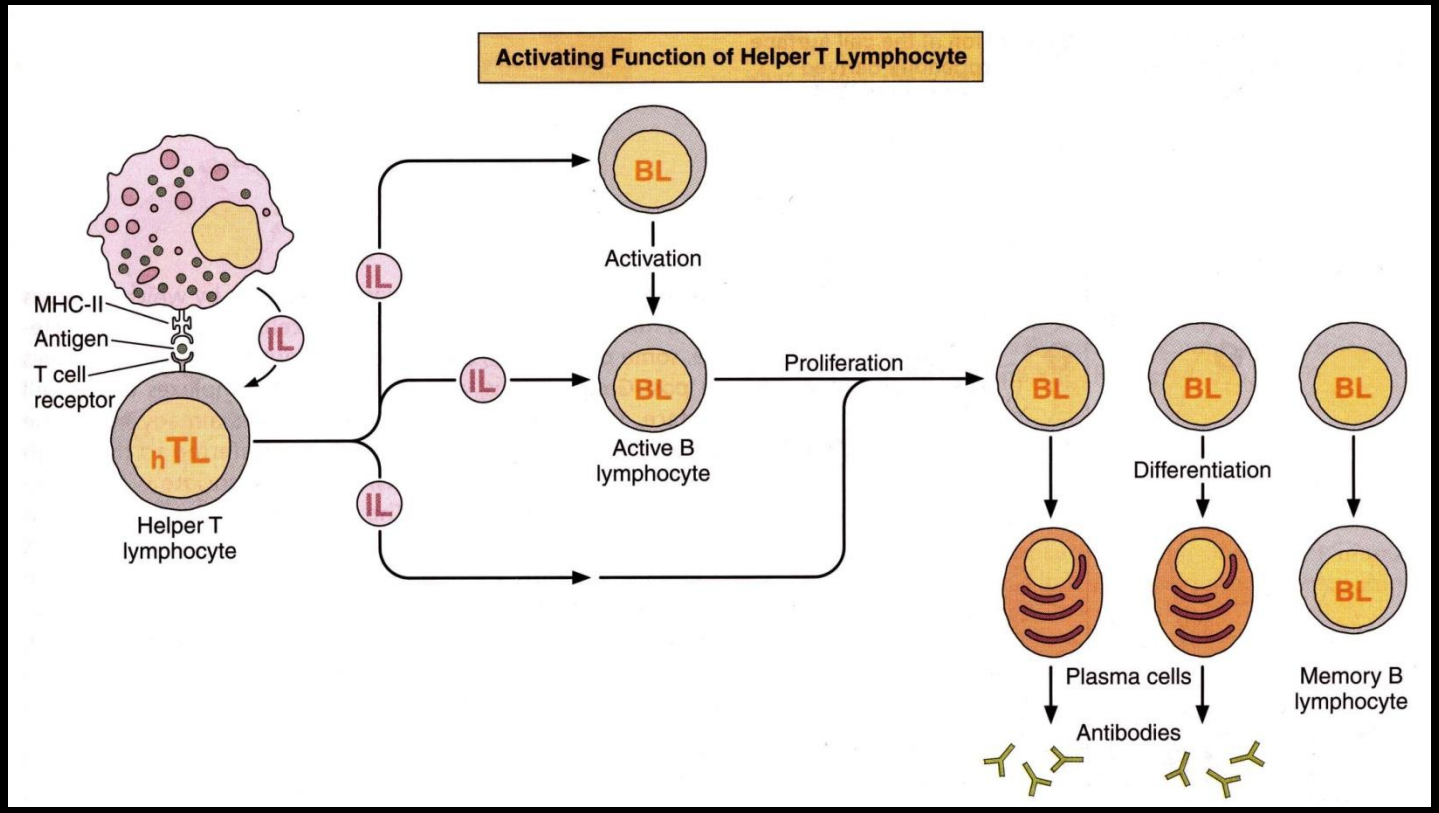


T_cL – recognize and bind cells with complexes MHC I and antigen

antigen is processed by proteasome digestion

perforins
granzymes

T_hL – recognize and bind cells with complexes MHC II and antigen antigen processed by lysosomal digestion



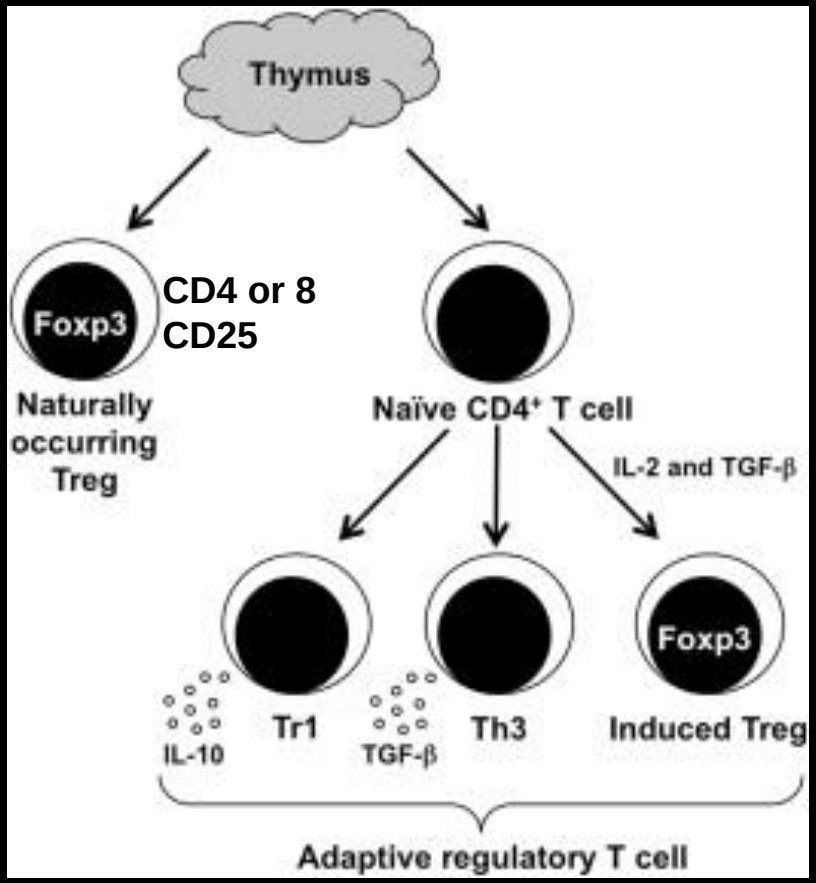
T_h1 activate macrophages with interferon-γ → phagocytosis (intracellular parasites)

T_h2 activate eosinophilic and basophilic granulocytes and mast cells with IL-4 and IL-13 → extracellular parasites

T_h17 activate neutrophilic granulocytes with IL-17

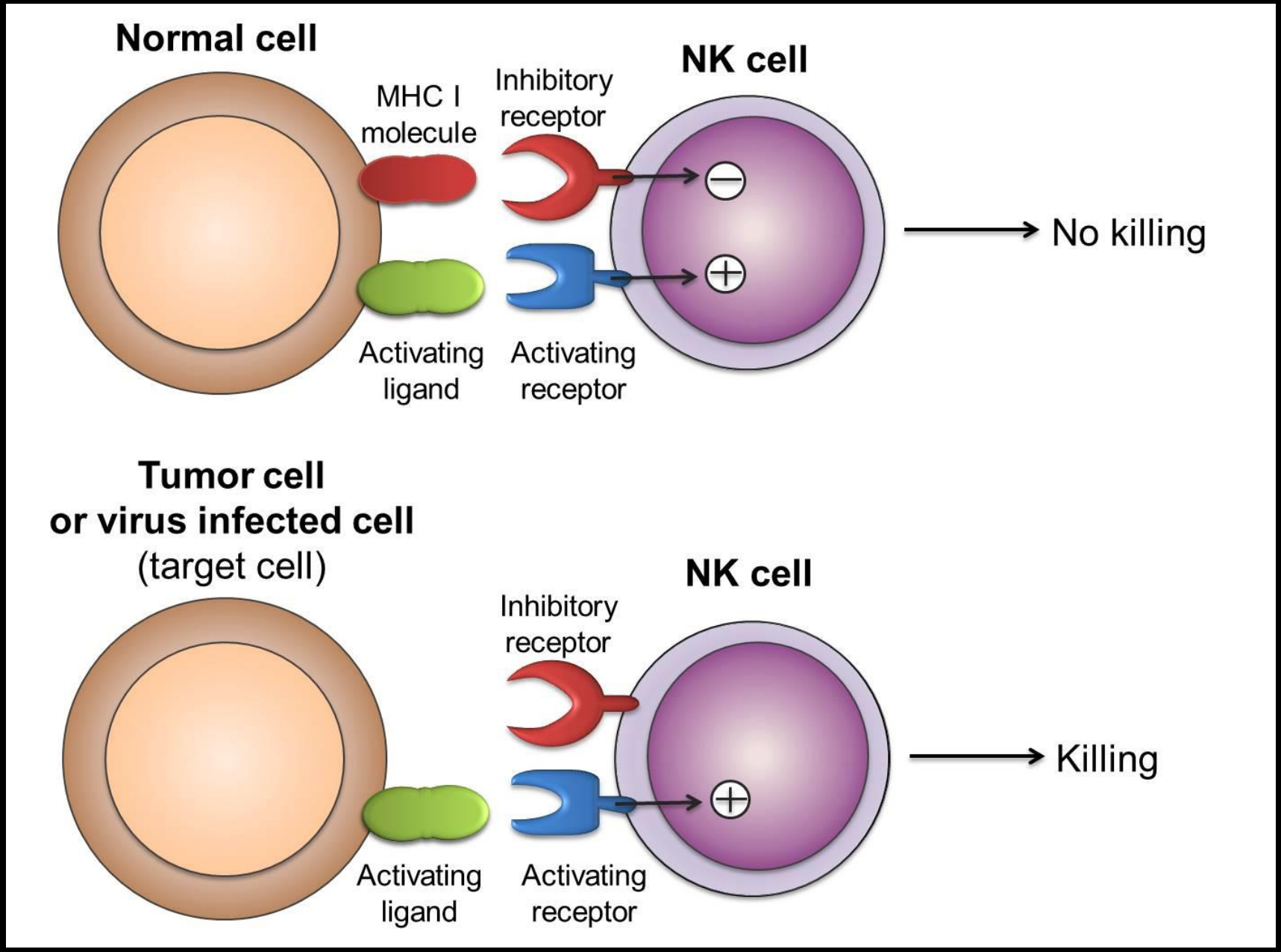
T_hf co-activate BL with IL-21 and IL-4 → proliferation and differentiation into plasma cells; decision of the isotype

$T_{reg}L$



natural inhibition of autoreactive T lymphocytes (Th and Tc)
induced suppression of exaggerated immune responses

NK – recognize and kill cells with incorrectly or insufficiently expressed MHC I

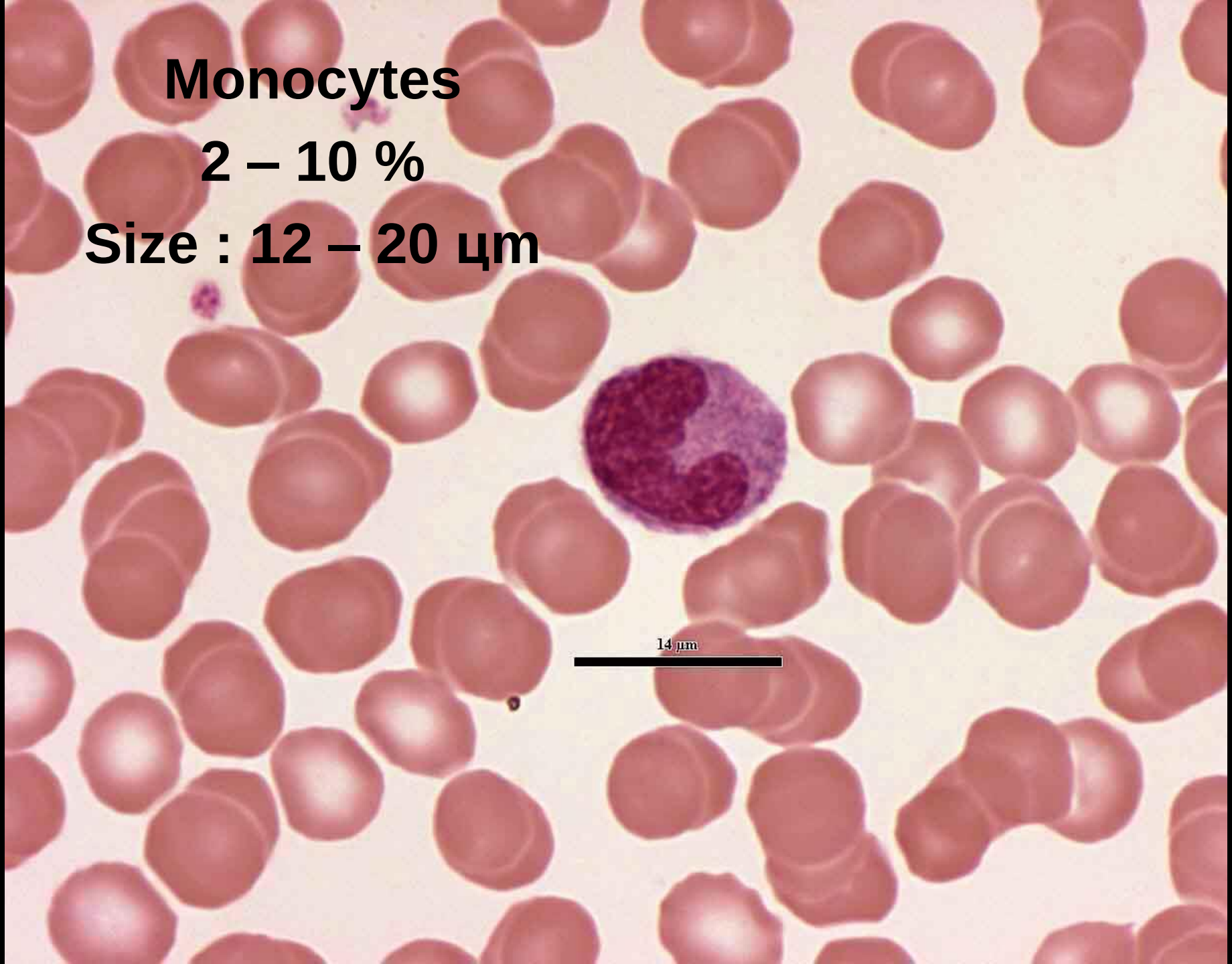


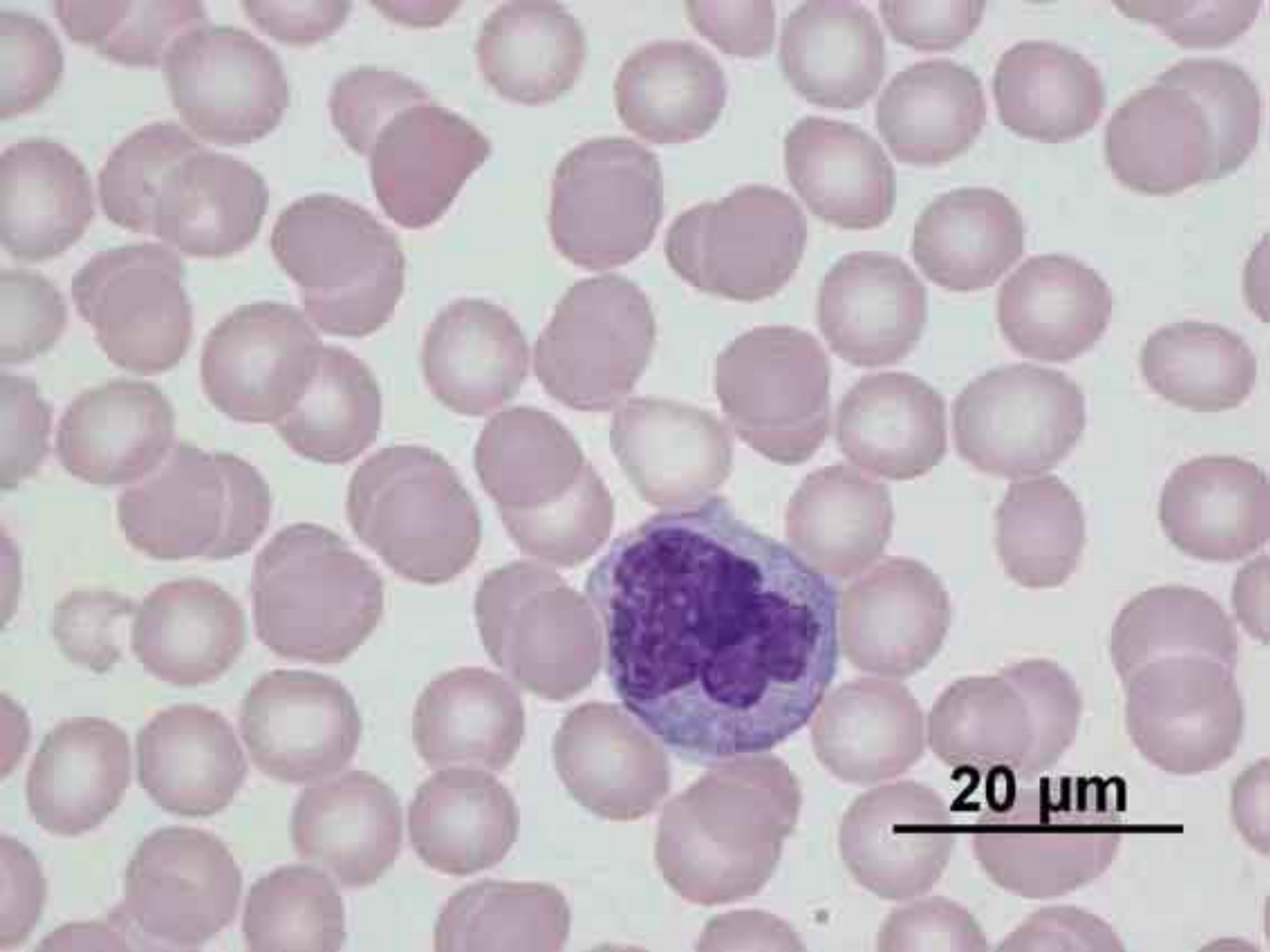
http://www.stream.wum.edu.pl/en/knowledge-base/96-nk-cells-applications-in-immuno-oncology

Monocytes

2 – 10 %

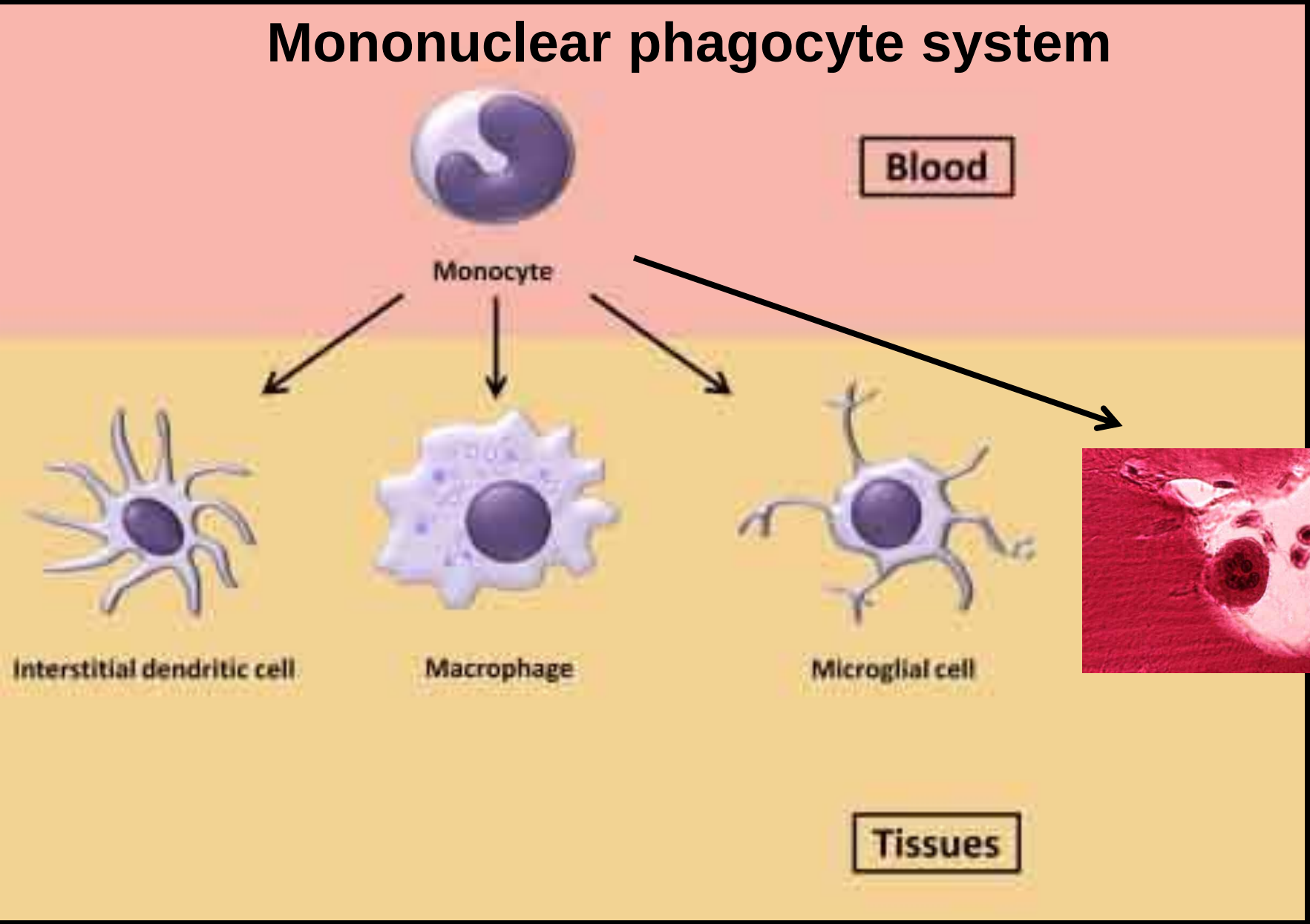
Size : 12 – 20 цm




20 µm


L
L
M
M
G
C

Mononuclear phagocyte system



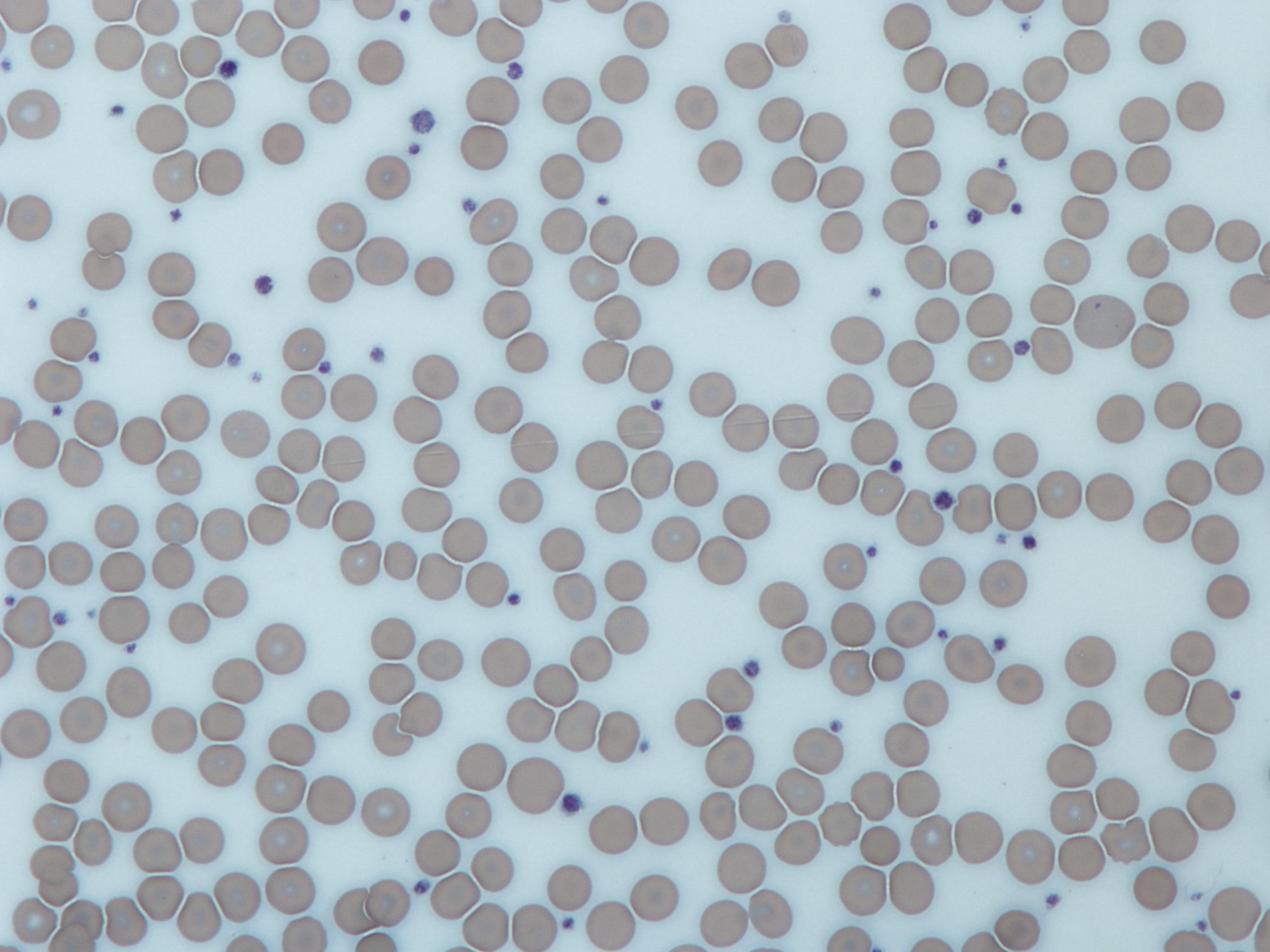
Blood platelets, thrombocytes

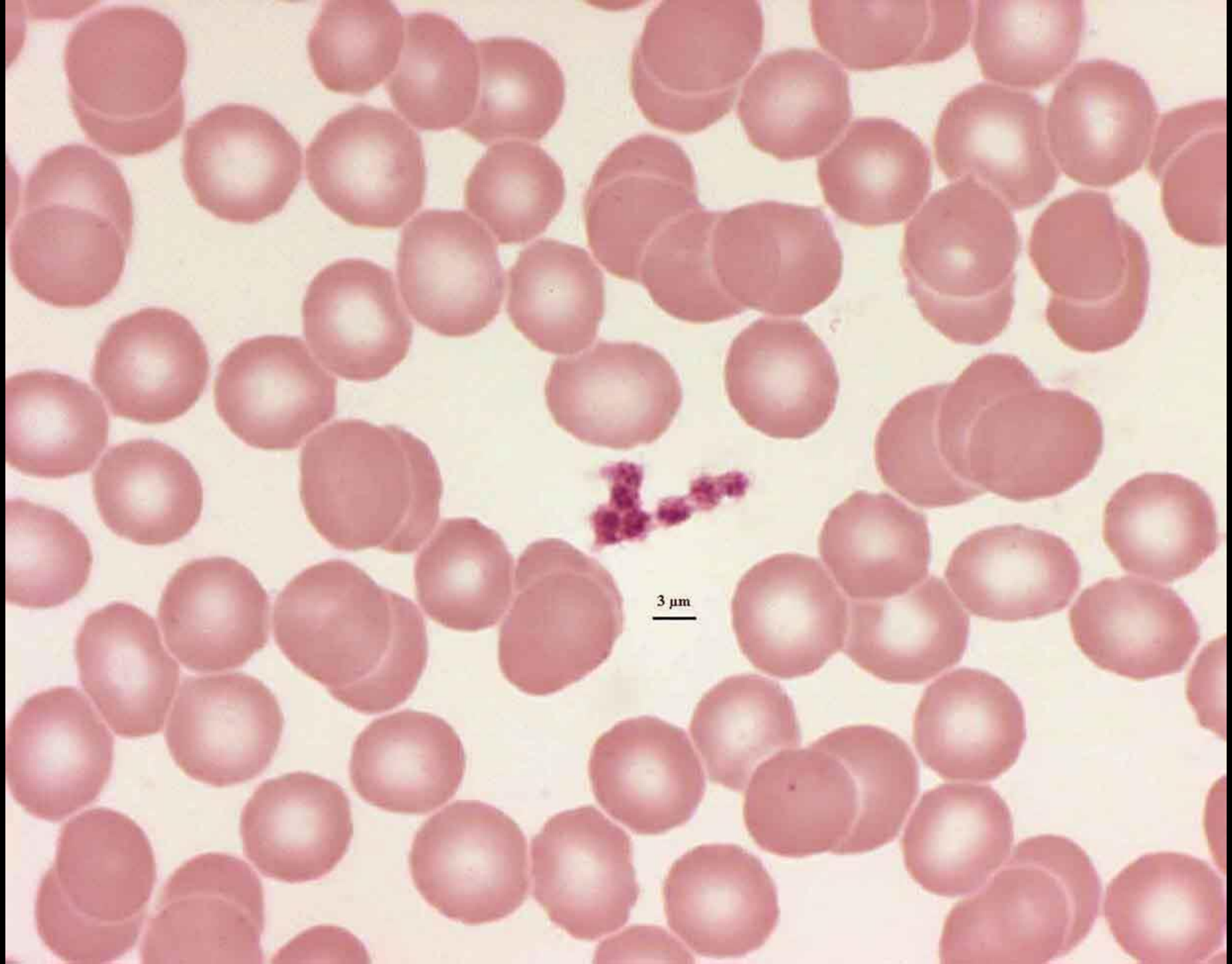
150 000 – 400 000/μl (mm^3)

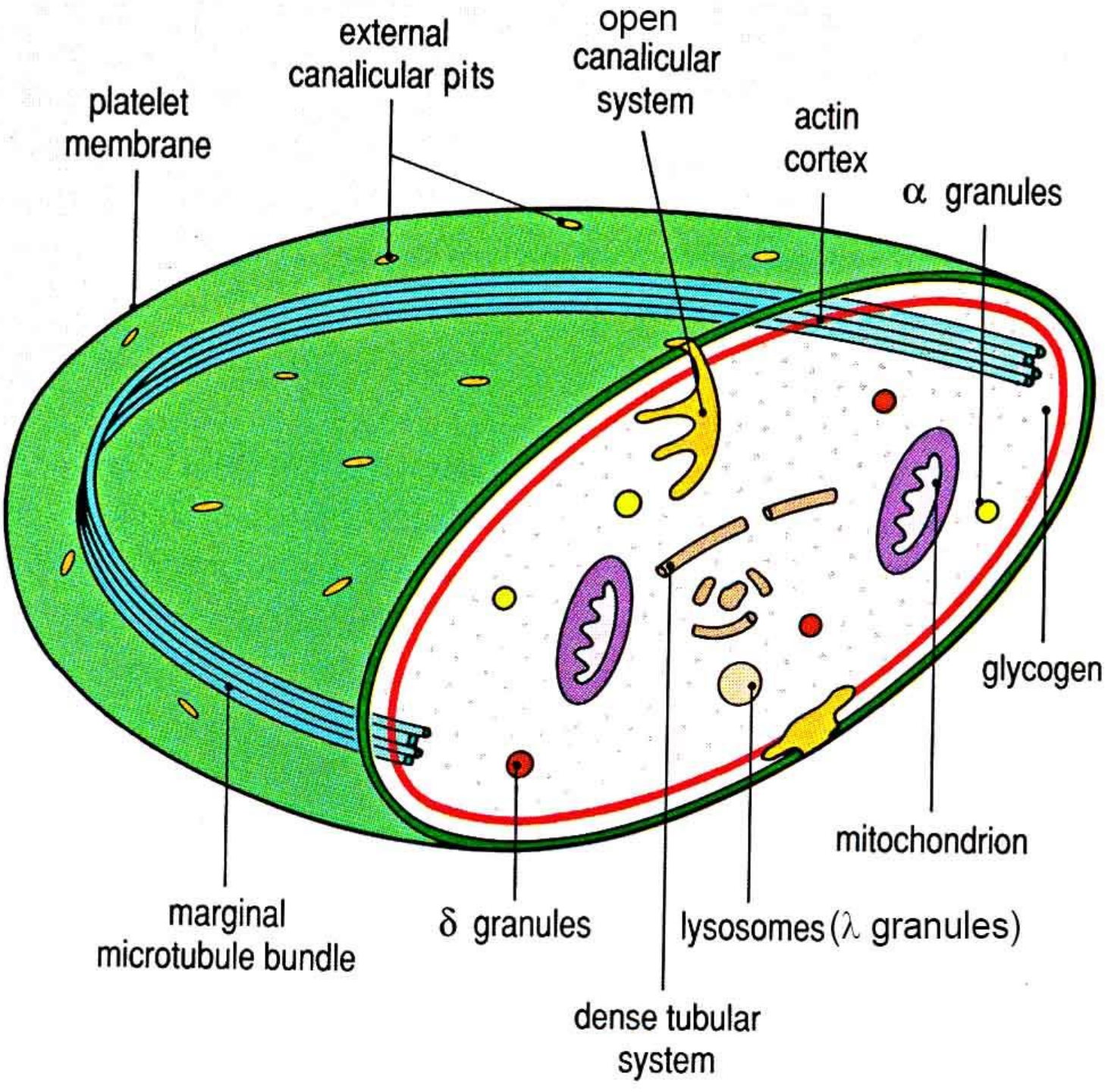
nonnucleated discoid fragments of megakaryocytes

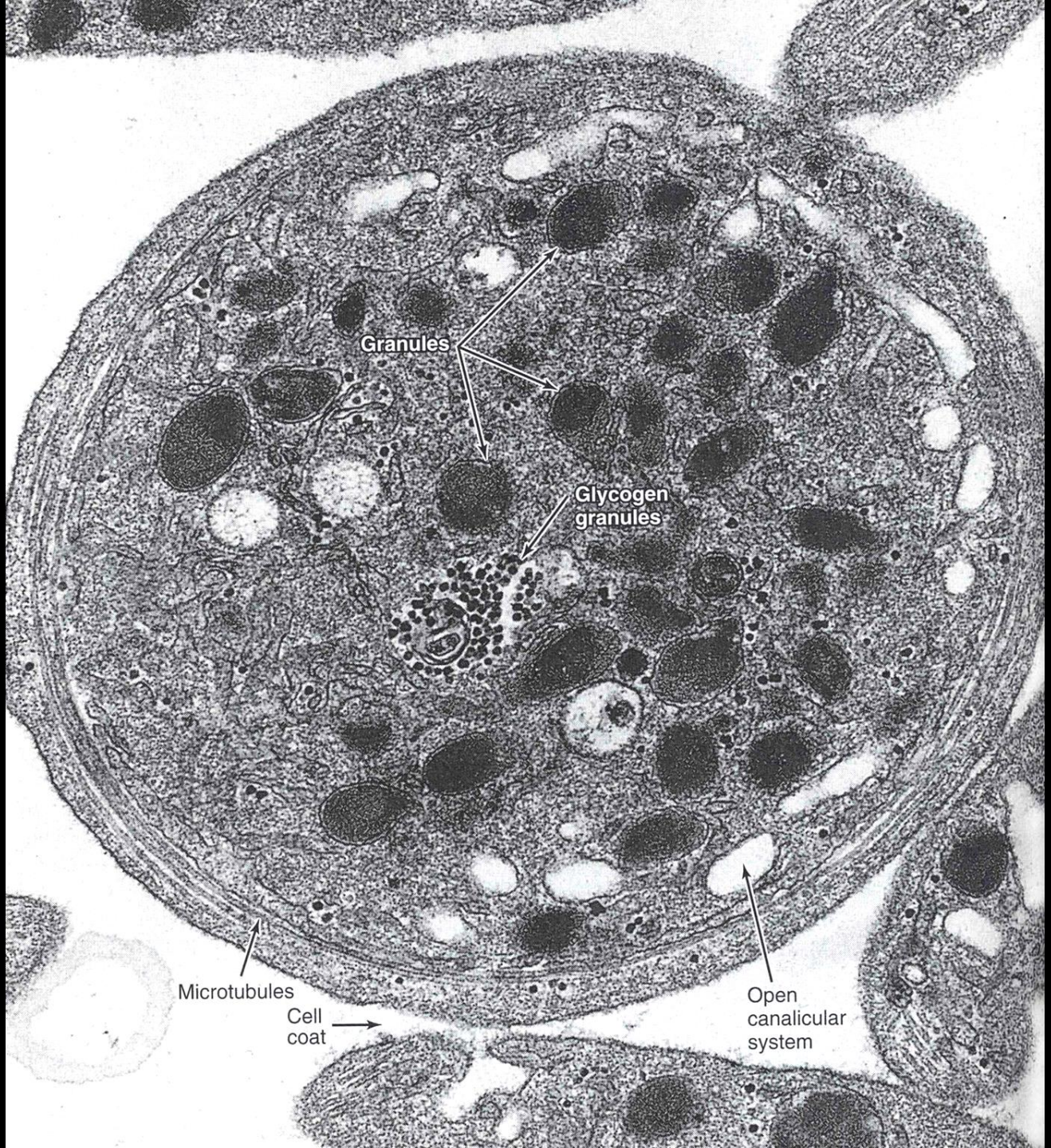
2 – 5 μm

blood clotting




3 µm


platelet membrane
external canalicular pits
open canalicular system
actin cortex
α granules
glycogen
mitochondrion
marginal microtubule bundle
δ granules
lysosomes (λ granules)
dense tubular system


Granules
Glycogen granules
Microtubules
Cell coat
Open canalicular system