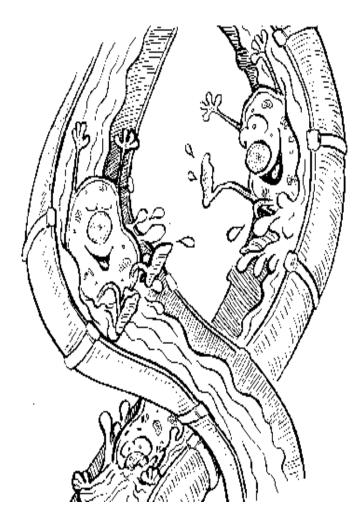
Blood

Warning

- please, do not manipulate the microscope,
- they are prepared for your study of blood smears after presentation,
- you will get instructions, how to study blood smears.



Blood

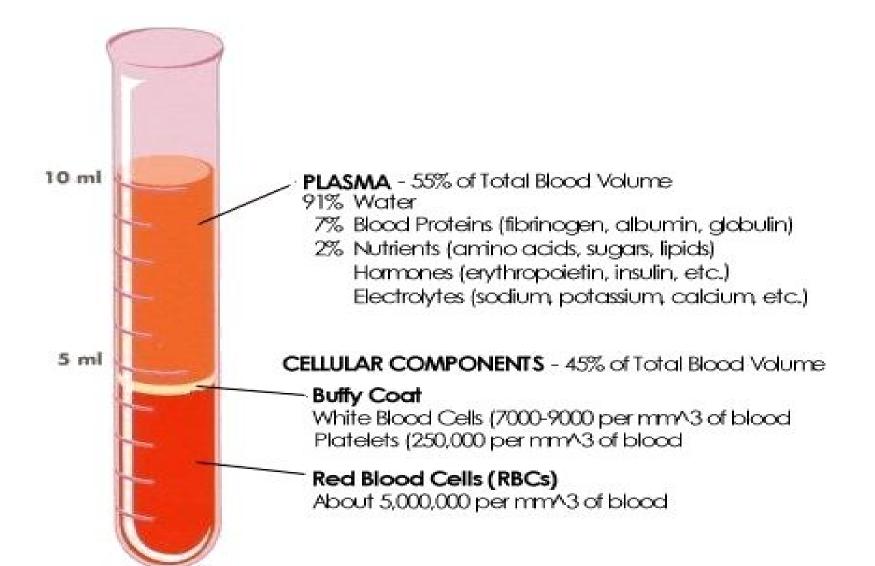
Plasma & Blood cells

Composition of plasma

- Water 90 %
- Proteins 7 % (albumins, globulins, fibrinogen)
- Other components 3 % (blood gasses, nutrients, hormones, enzymes, vitamins, electrolytes)

Hematocrit:

the volume of blood cells per unit volume of blood

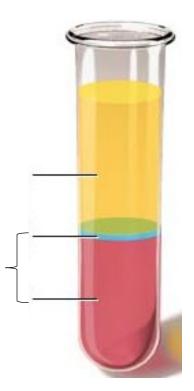


Blood

Hematocrit ♂ 42 - 52 % ♀ 37 - 47 %

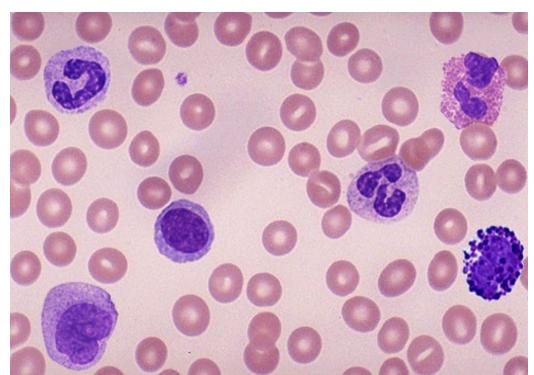
55 % plasma 90 % H₂O 7 % plasma proteins 3 % -AMAs, saccharids, lipids -hormones -electrolytes

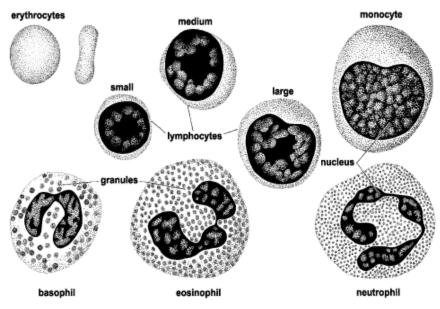
45 % blood corpuscles



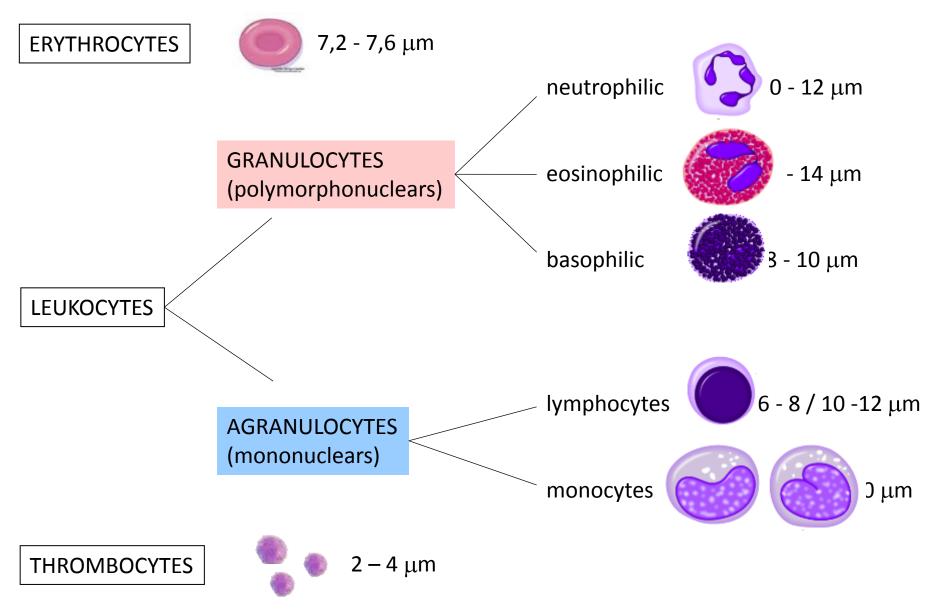
Blood cells (formed elements)

- Red blood cells erythrocytes
- White blood cells leukocytes
- Platelets thrombocytes









REMEMBER forever!

- Erythrocytes: 4 6 millions/ $1 \mu l$ of blood
- Leukocytes: 5,000 9,000 / 1 μl
- Thrombocytes: 150,000 250,000/ 1 μl

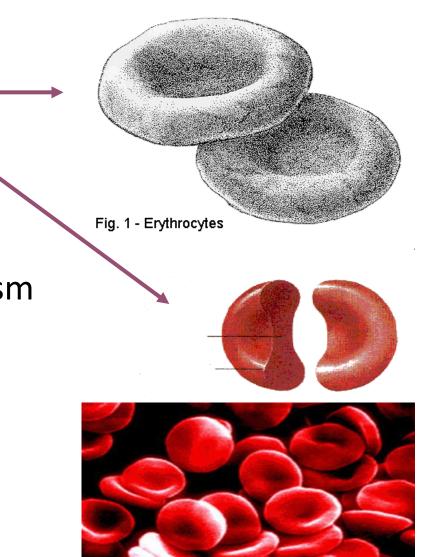
NEVER FORGET!

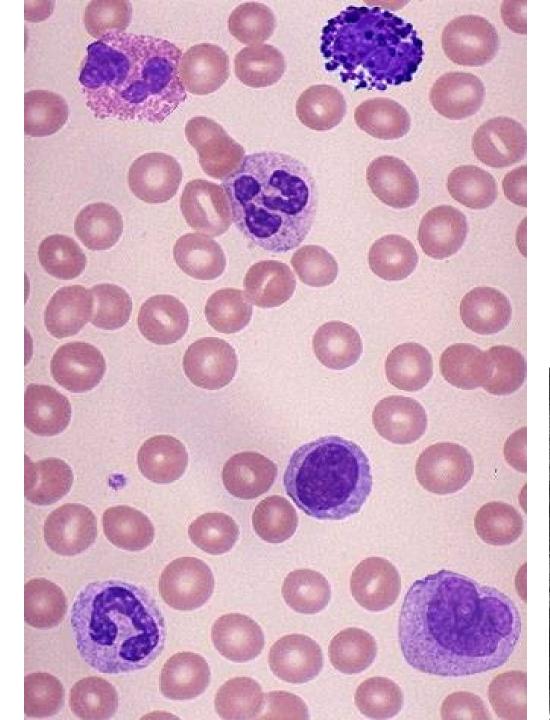
ERYTHROCYTES

- 4 6 million/µl
- Shape: biconcave disc,

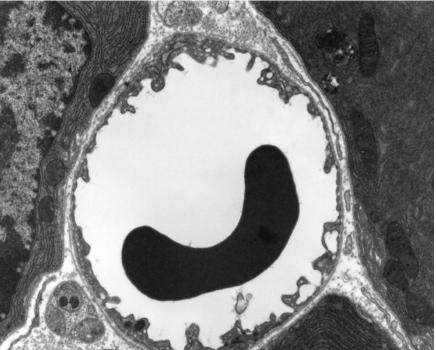
dumble-shaped (cross section)

- Size: 7.4 µm in diameter (= normocyte)
- Structure: plasmalemma, cytoplasm + hemoglobin 33 % absence! of the nucleus and cell organelles
- Lifespan: 120 days







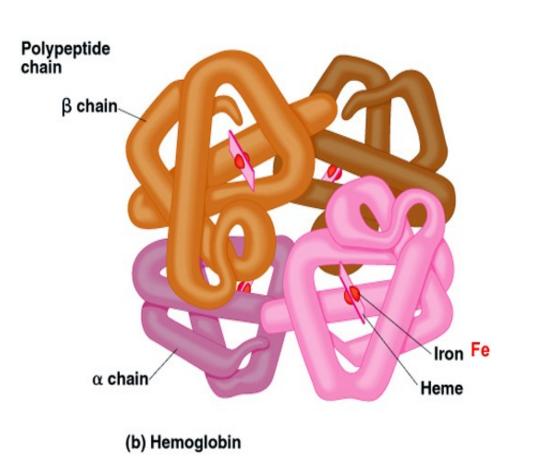


Hemoglobin

 a conjugated protein: polypeptide chains + heme groups = protoporphyrin ring with ferrous iron (Fe²⁺)

4

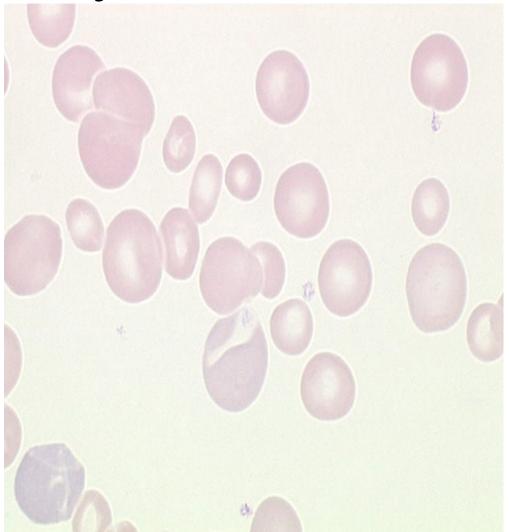
- Hb F (fetal)
- Hb A (adult)
- normochromatic ery: 32±2 picogramms (*hyper-, hypo-*)



<Important terms>

- **Polyglobulia** an increased number of ery
- Anemia a decreased number of ery
- Poikilocytosis an occurrence of different shaped ery (spherocytes, elliptocytes, drepanocytes = sickle cells, etc.)
- Anisocytosis an occurrence of different sized ery (microcytes, macrocytes)

Anisocytosis



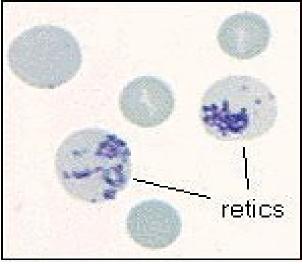
- Microcytes $\emptyset < 6.5 \ \mu m$
- Normocytes $\emptyset \pm 7.4 \ \mu m$
- Macrocytes
 > 8 μm

Ø

Reticulocytes

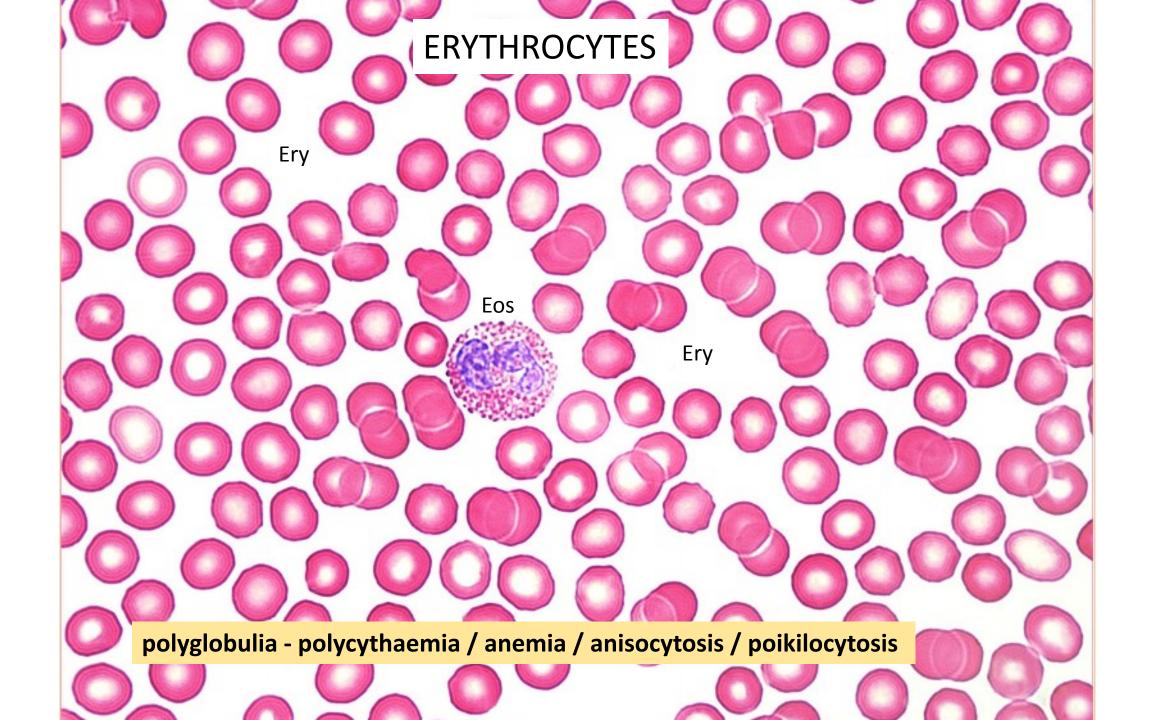


- Immature ery are released from the bone marrow into the peripheral blood (0.5 – 1.5 %)
- They contain the rests of organelles ribosomes, mitochondria – substantia reticulofilamentosa (brilantcresyl blue staining is used for detection)
- maturation into ery during 24 hours

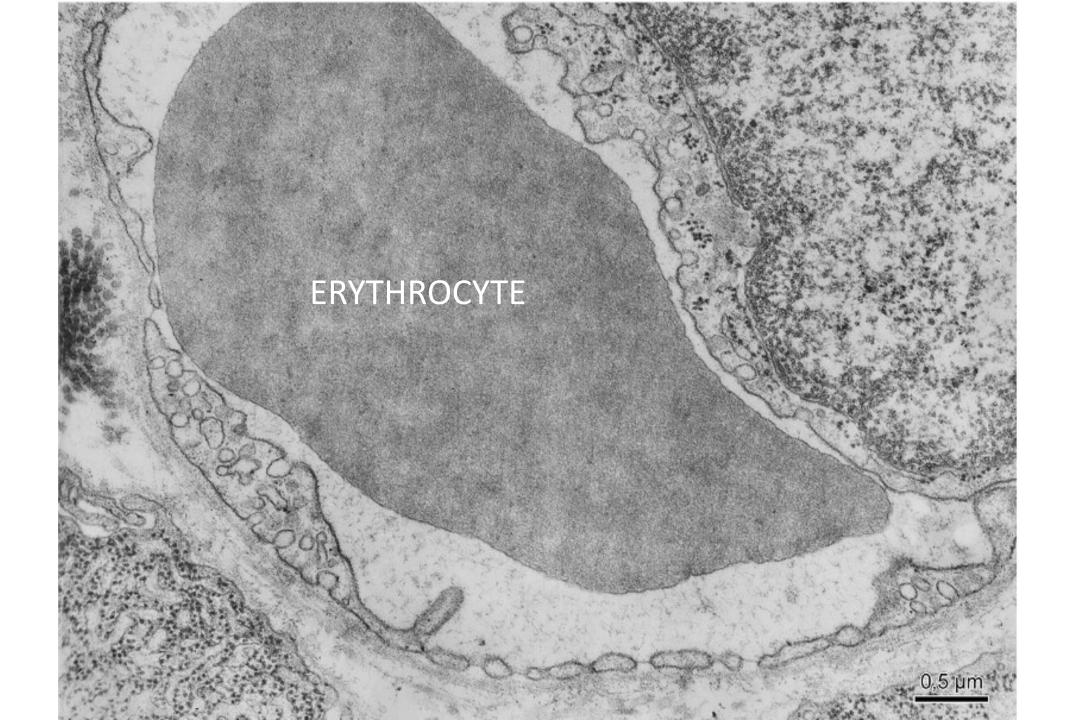


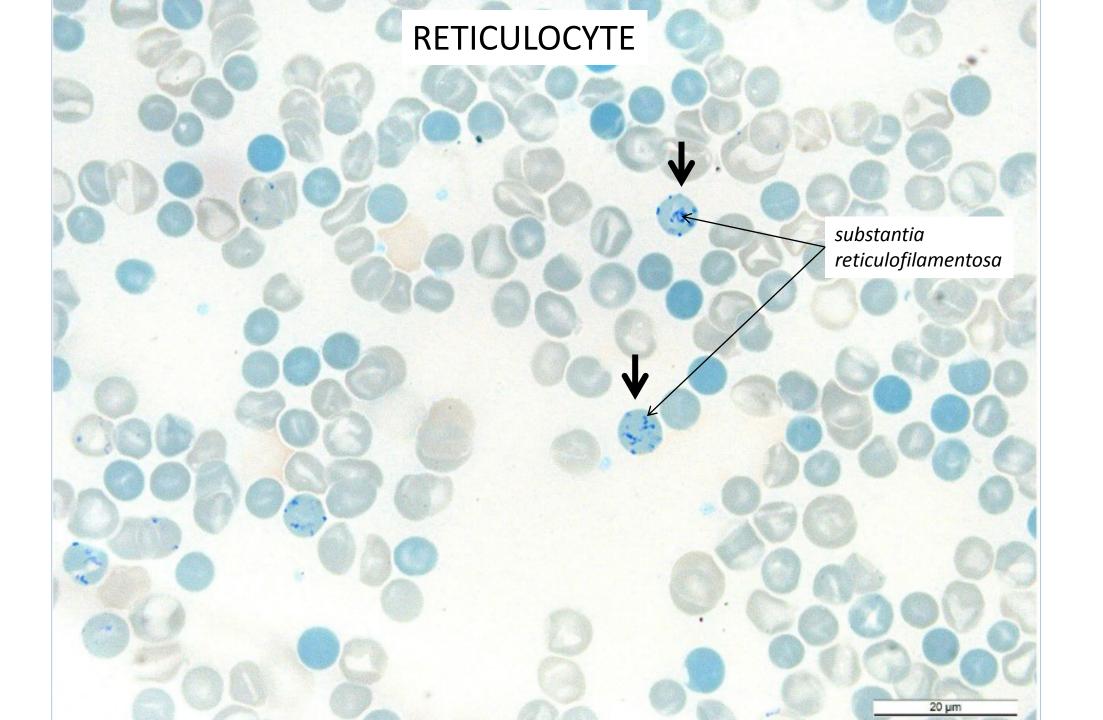
Functions of ery

- transport of oxygen from the lungs
- transport of carbon dioxide from the tissues







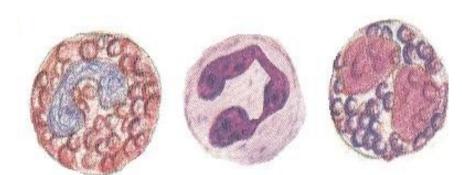


LEUKOCYTES

- Granulocytes:
 - neutrophils
 - eosinophils
 - basophils

General characteristic

Polymorphonuclears with acidophilic cytoplasm and Specific + azurophilic granules



- Agranulocytes
 - lymphocytes
 - monocytes

General characteristic Mononuclears with basophilic cytoplasm and azurophilic granules



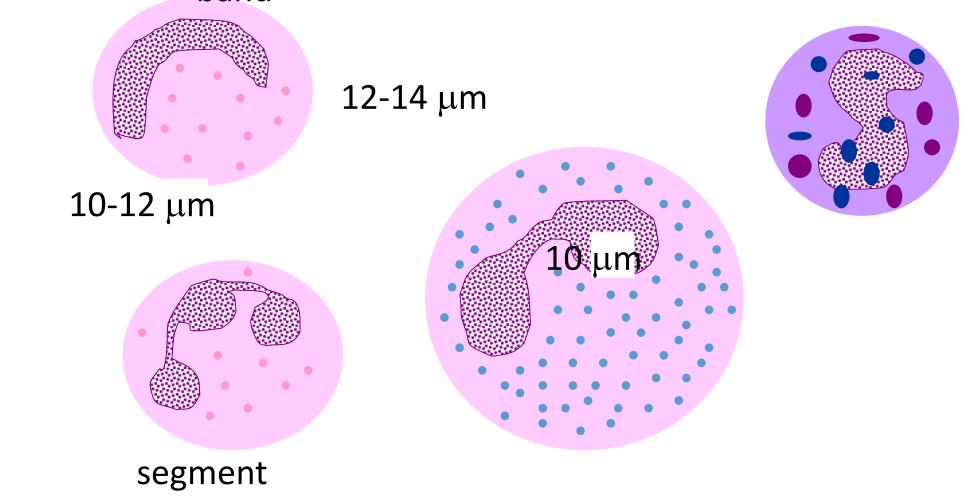
Granulocytes

- General charcteristic:
- polymorphonuclears different shape of nuclei



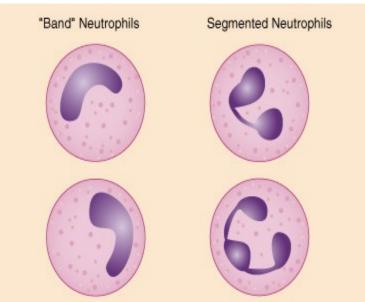
- acidophilic cytoplasm bright-pink
- specific granules with special enzymes
- azurophilic granules with lysosomal enzymes
- all granulocytes are able to migrate from the vessels and by diapedesis invade a site of inflamation

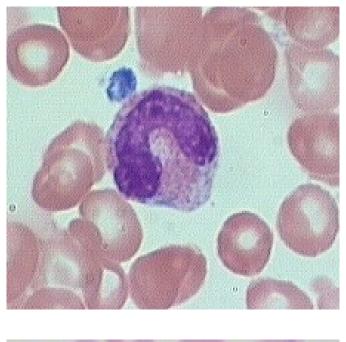
Granulocytes neutrophils – eosinophils - basophils band



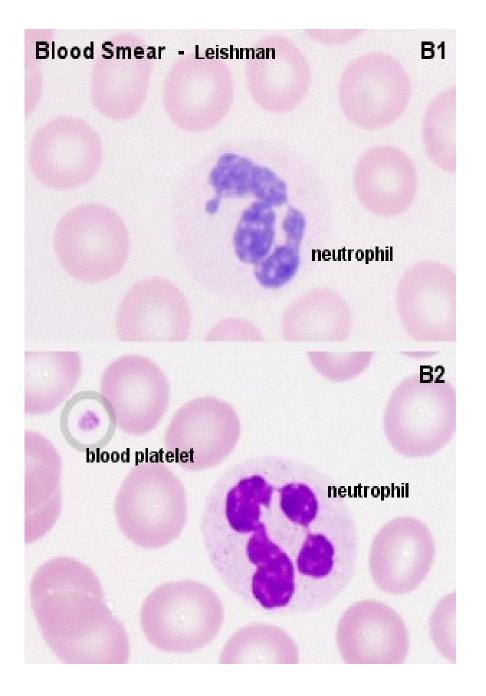
Neutrophil granulocytes (neutrophils)

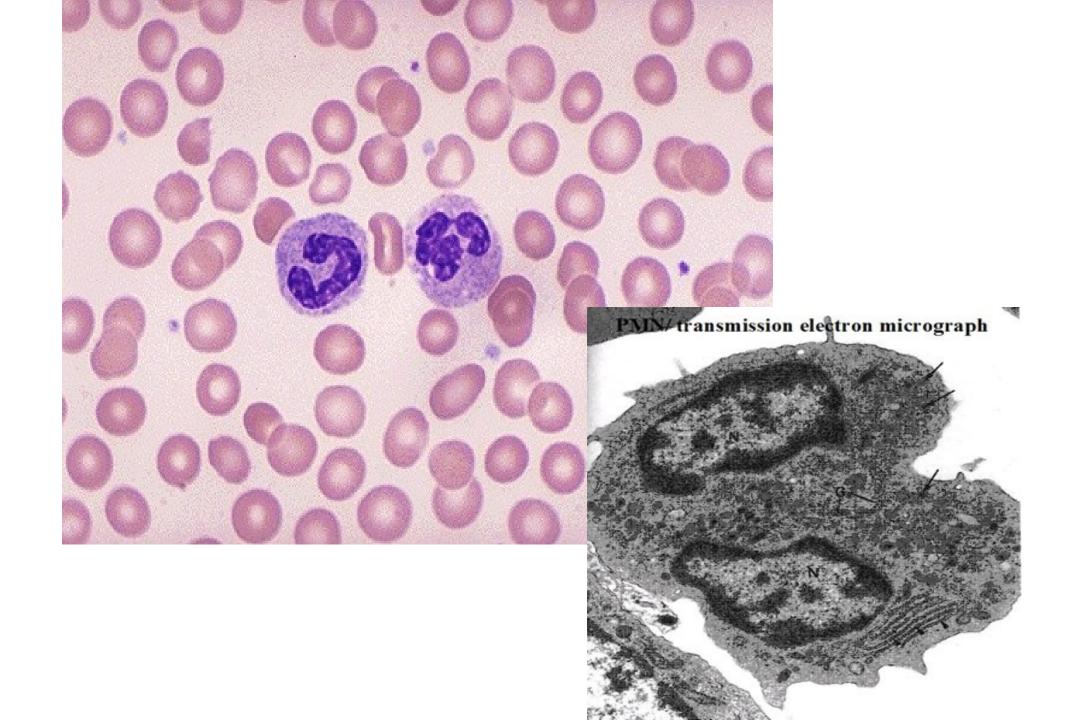
- 71 % of all white blood cells (DWCC)
- Ø 10 12 μ m
- Cytoplasm: bright pink (eosinophilic = acidophilic)
- Specific granules: neutrophilic (Ø 0.3 μm) (alcaline phosphatase, kolagenase, lysozyme, ...)
- Nucleus: shaped (4 %) segmented (67 %) 5 segments)











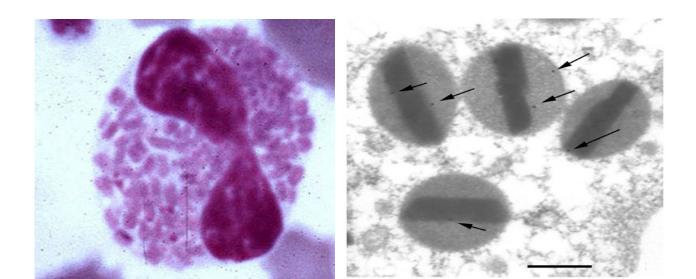
Functions of Neutrophils

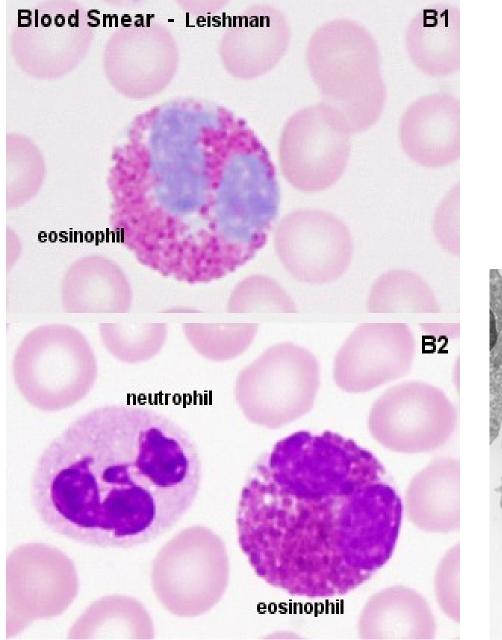
- a central role in inflammatory processes Neu invade, by diapedesis from the vessels, sites of infection in response to factors (e.g. cytokines) released by cells which reside at an infection site.
- cell membrane receptors allow Neu to recognise foreign bodies (bacteria, tissue debris), which they begin to phagocytose and destroy.

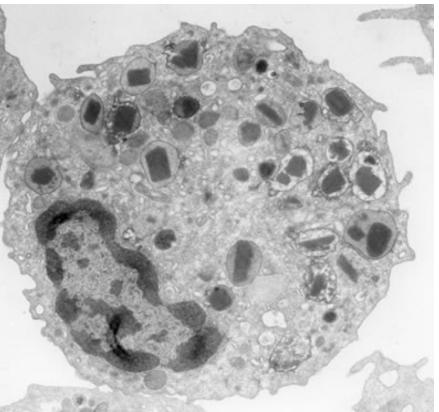
The Neu die once their supply of granules has been exhausted. Their lifespan is only about one week. Dead neutrophils and tissue debris are the major components of pus.

Eosinophil granulocytes (eosinophils)

- 1-4% of all white blood cells (DWCC)
- Ø 12 14 μm
- Cytoplasm: bright pink (eosinophilic = acidophilic)
- Specific granules: eosinophilic (Ø 0.5 1 μm) (enzymes: acid phosphatase, peroxidase, histaminase, arylsufatase ...)
- Nucleus: dumb-belt, segments)







Functions of Eosinophils

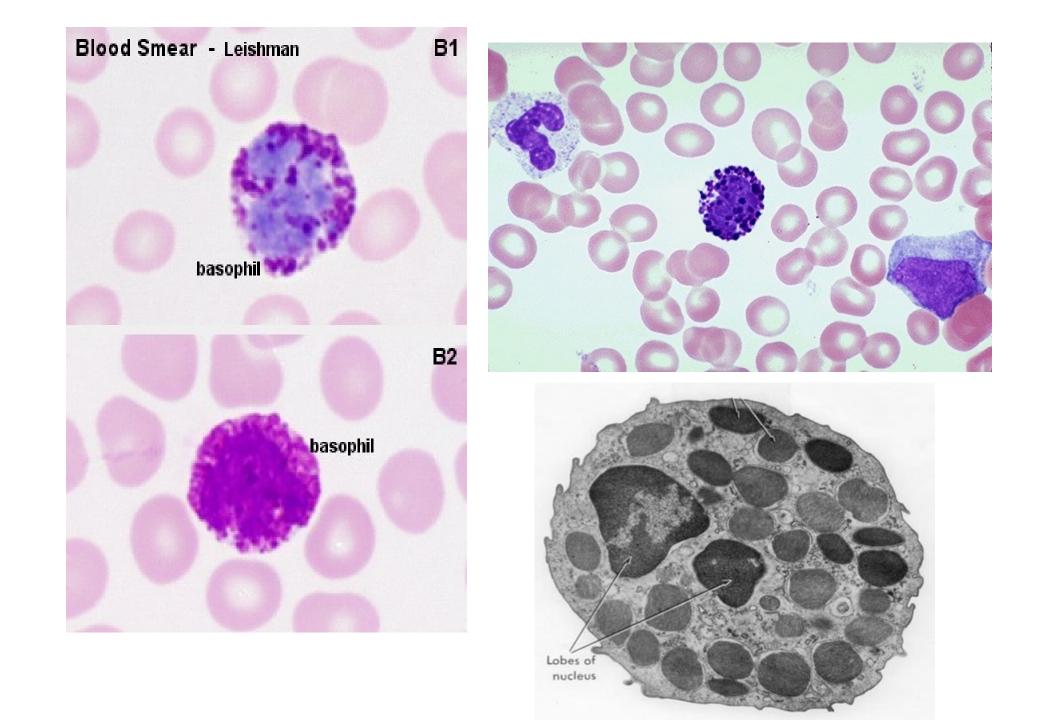
- phagocytosis of antibody-antigen complexes and prevention of the immune system from "overreacting,,
- eos are involved in the response of the body against parasitic infections, which are accompanied by an increase in the number of eosinophils.

Basophil granulocytes (basophils)

- up to 1 % of all white blood cells (DWCC)
- \bullet Ø up to 10 μm
- Cytoplasm: bright violet-pink (lightly basophilic)
- Specific granules: (Ø 2 μm)
- Nucleus:

(heparin, histamin, ...)

"shape of dick S"



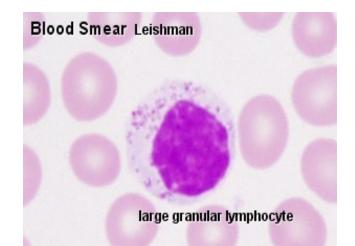
Functions of Basophils

- heparin and histamine are vasoactive substances. They dilate the blood vessels, make vessel walls more permeable and prevent blood coagulation. They facilitate the access of heparinocyte in a site of infection.
- antibodies produced by plasma cells (activated Blymphocytes) bind to the receptors on the plasma membrane of basophils. If these antibodies come into contact with antigens, they induce the release of the contents of the basophil granules.

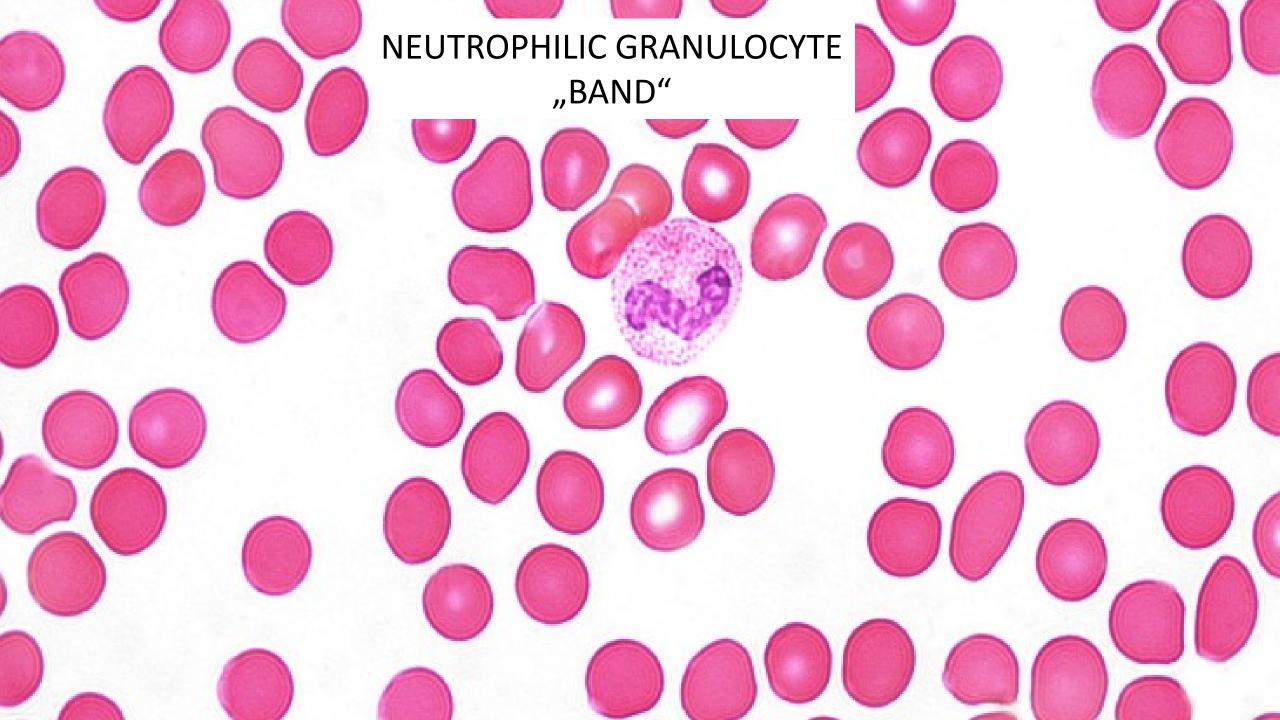
Agranulocytes

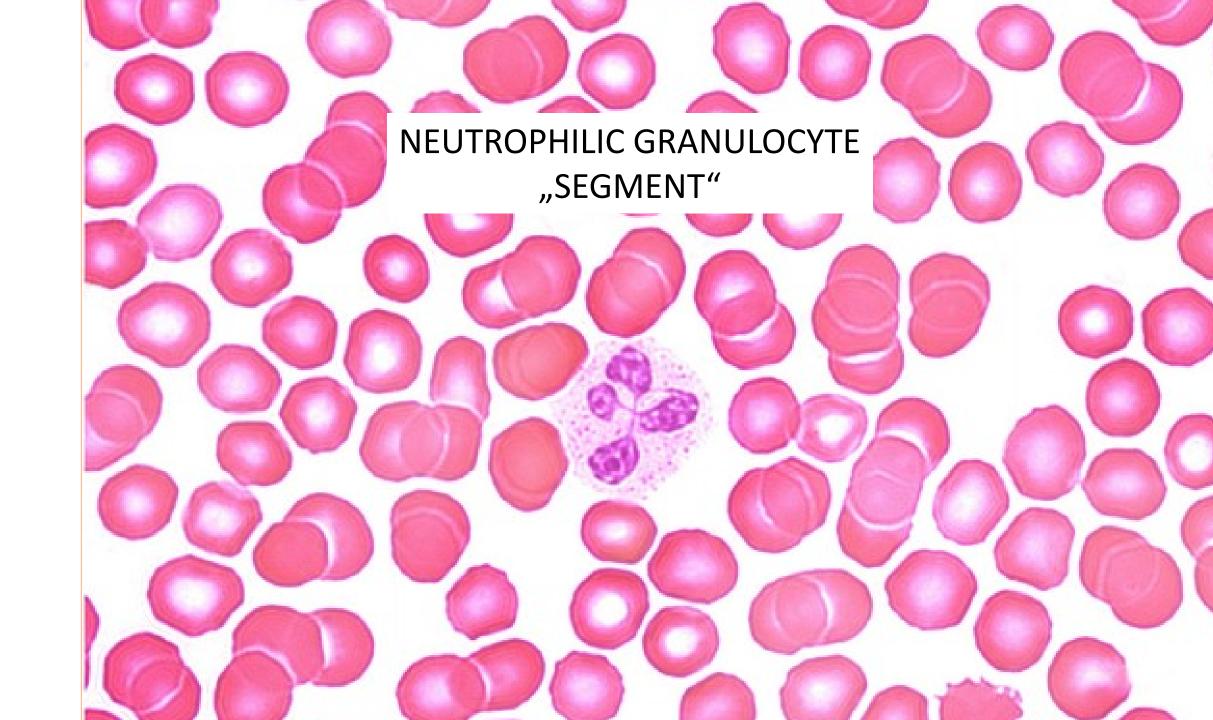
- <u>General charcteristic</u>:
- mononuclears shape of nuclei is spherical, oval or bean-shaped

- basophilic cytoplasm blue
- NO specific granules
- azurophilic granules lysosomal enzymes



NEUTROPHILIC GRANULOCYTES - bands, segments -Neu-B Neu-S 20 µm





EOSINOPHILIC GRANULOCYTE

Eos

Neu

EOSINOPHILIC GRANULOCYTE

EOSINOPHILIC GRANULOCYTE



BASOPHILIC GRANULOCYTE

LYMPHOCYTES

- Classification:
 - according to origin T-Ly (thymus), B-Ly

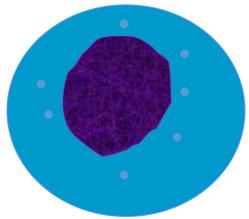
(bone marrow \cong bursa of Fabricius in birds)

- according to the size small (Ø 8 μm), medium (Ø 10-12 μm), large (Ø 16-18 μm),
- α according to the function matural killer calls
- according to the function natural killer cells, helper cells, memmory cells, supressor cells,
- according to life-span

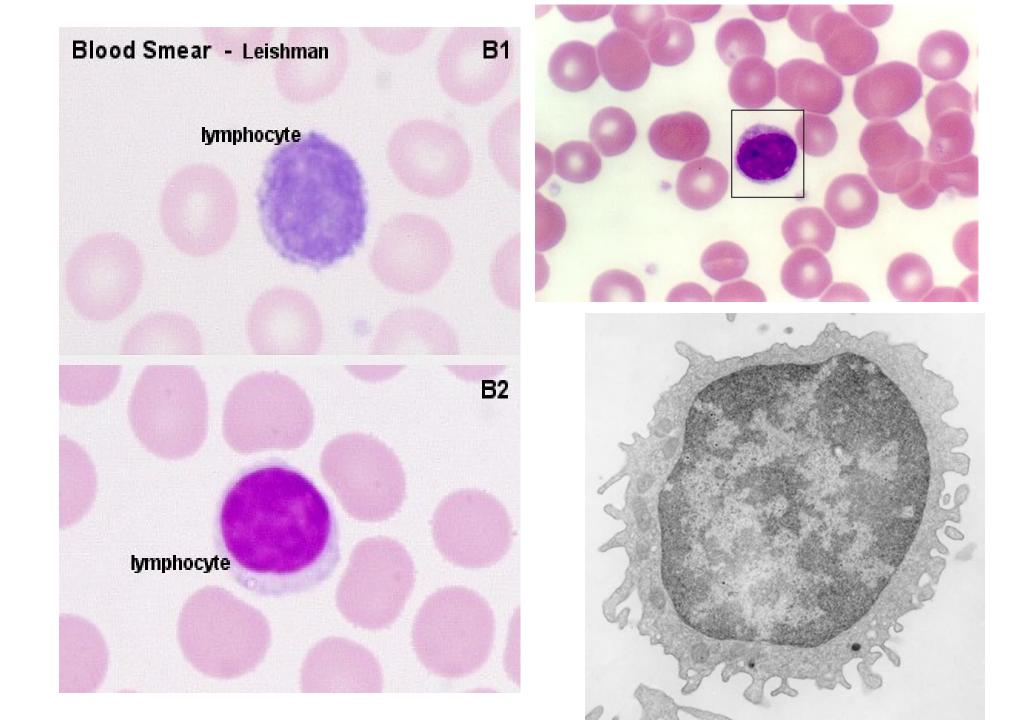
Lymphocytes - structure

• 20 % of all white blood cells (DWCC)



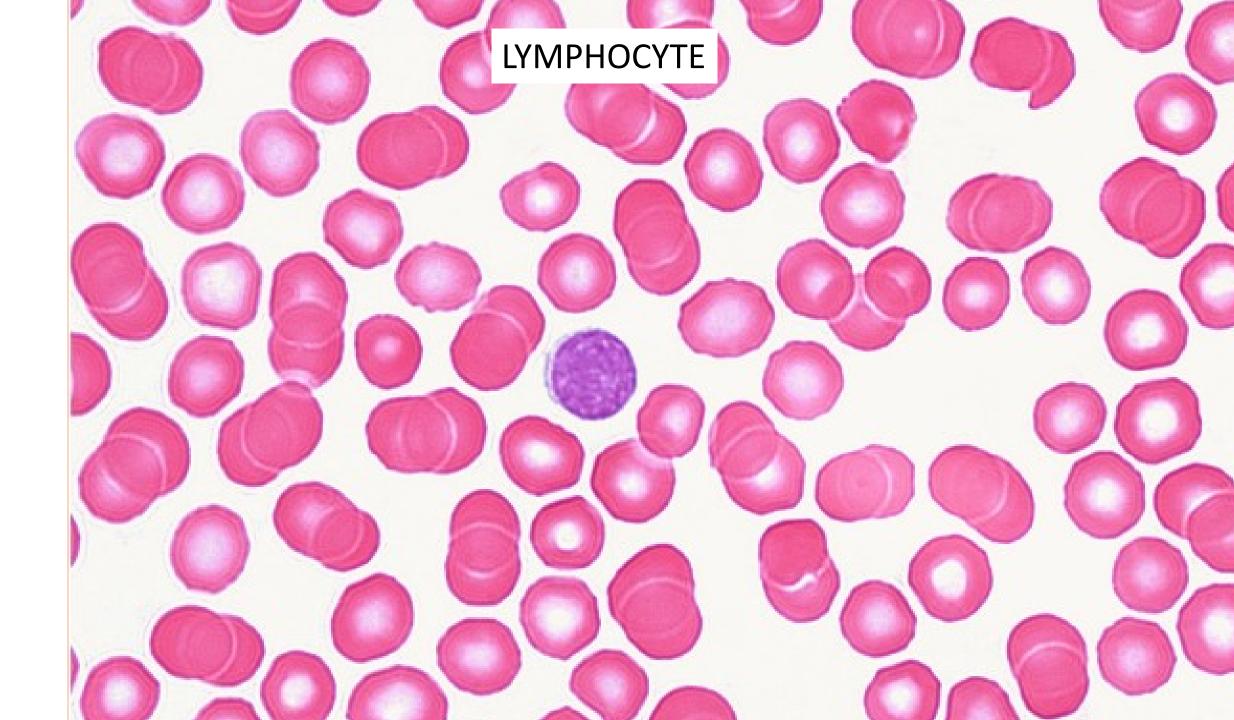


- small, medium-sized, large Ly
- cytoplasm dark blue, contains non-specific azurophilic granules with lysosomal enzymes (hydrolases) and numerous ribosomes
- nucleus round, hyperchromatic coarse grains of heterochromatin (*dark violet colour*)



Functions of Lymphocytes

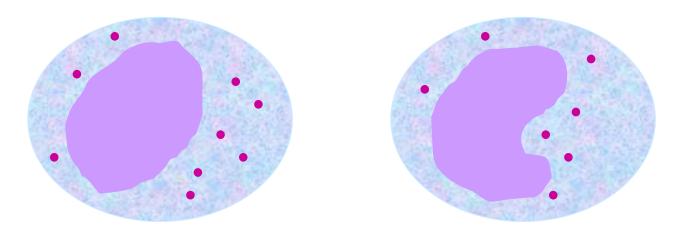
- B-lymphocytes differentiate into antibody producing plasma cells and so they represent "humoral immunity"
- T-lymphocytes represent the "cellular immunity" and may attack foreign cells, cancer cells and cells infected by e.g. a virus

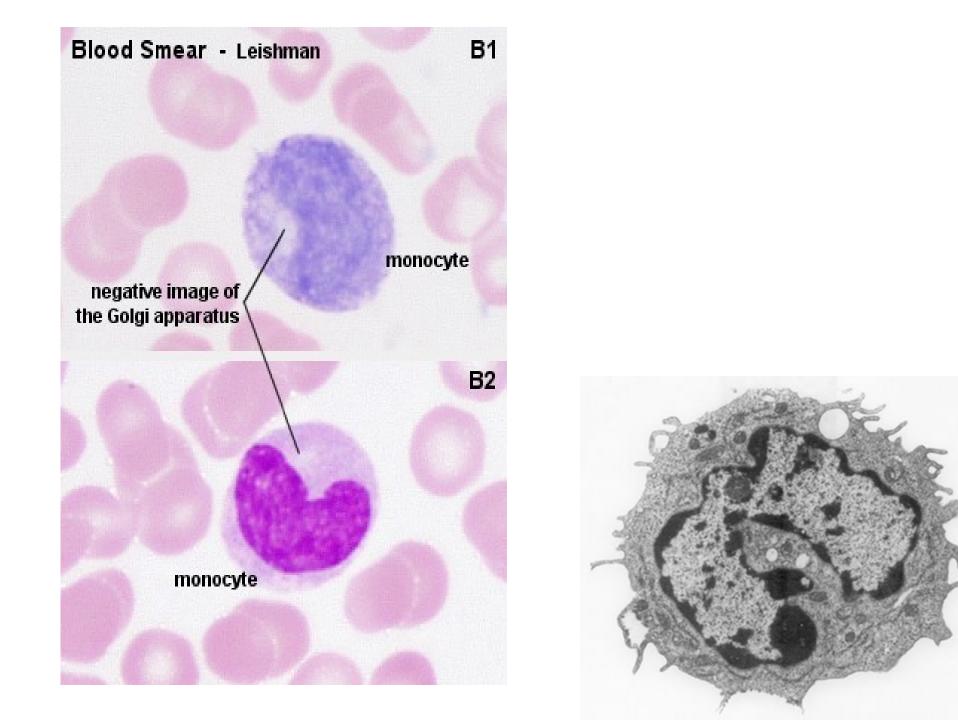




MONOCYTES

- 5 % (DWC), \varnothing 15 20 μm
- cytoplasm voluminous, bright blue, contains nonspecific azurophilic granules with lysosomal enzymes (hydrolases) and numerous ribosomes
- nucleus oval to bean-shaped, finely dispersed chromatin





Functions of Monocytes

- monocytes enter the connective tissue they differentiate into macrophages. At sites of infection macrophages are the dominant cell type after the death of the invading neutrophils.
- macrophages phagocyte microorganisms, tissue debris and the dead neutrophils.

• *mono also give rise to osteoclasts,* which are able to distroy bone. They are of importance in bone remodelling.

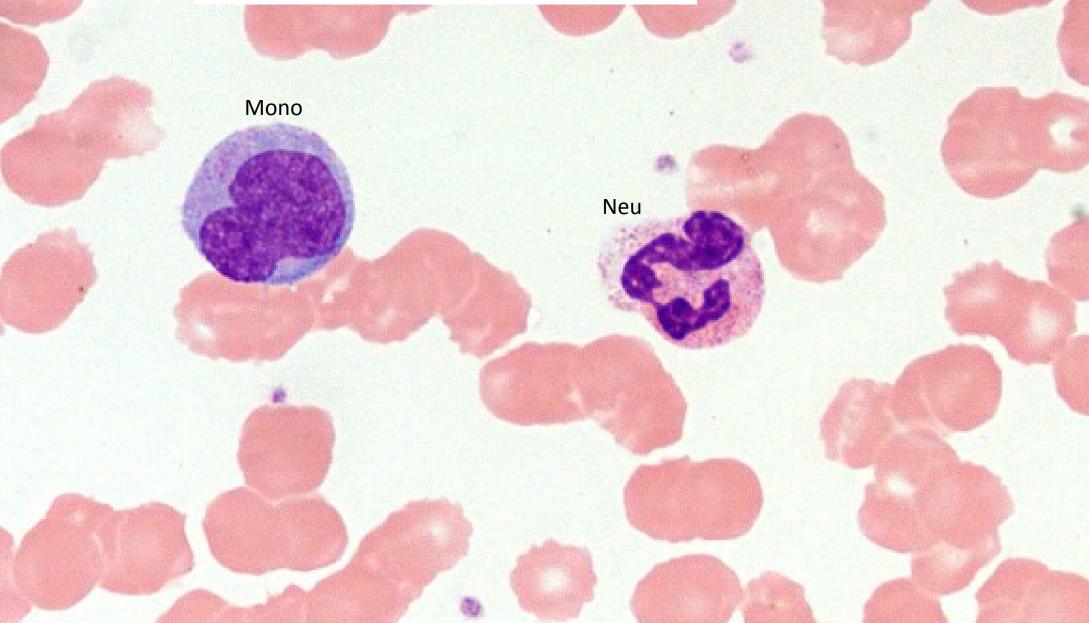
MONOCYTE and LYMPHOCYTE

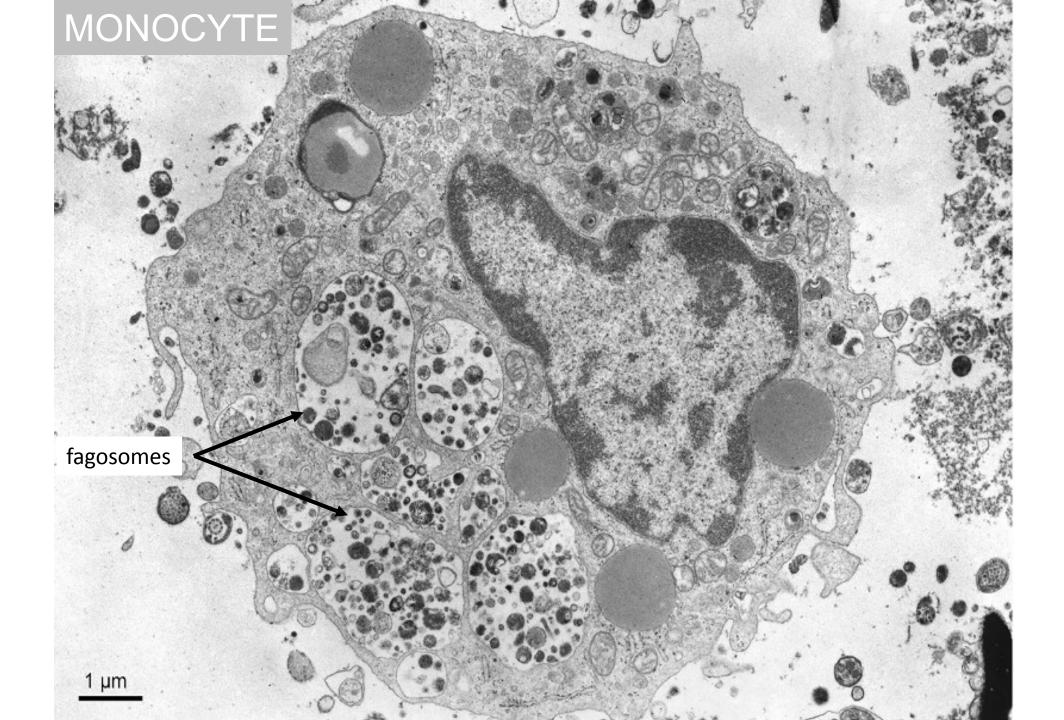
Ly

20 µm

Mono

MONOCYTE and "NEUTROPHIL"





THROMBOCYTES (blood platelets)

- + 150,000 300,000 / 1 μl of blood
- thrombocytosis X thrombocytopenia
- are not cells, but cytoplasmic fragments of large cell (megakaryocyte) in bone marrow
- shape: spindle-shaped discoid plate
- size: 2 4 µm
- cytoplasm basophilic (bright violet-blue), contains microtubules and α , δ and λ granules:

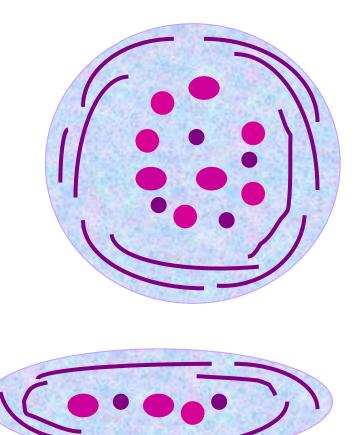
alpha granules – fibrinogen, ...

delta granules – serotonin, Ca ions, ATP and ADP,...

lambda granules – are small lysosomes

Platelet structure

- Hyaloplasm contains microtubules (on the periphery of platelet)
- Granuloplasm contains granules



Functions of thrombocytes

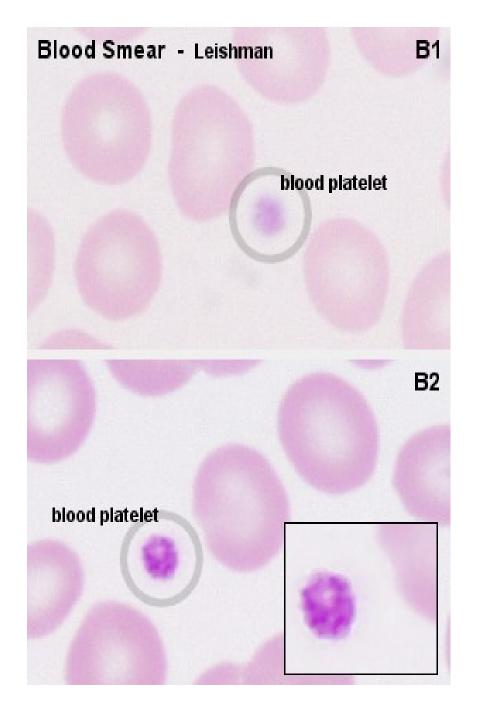
- Platelets assist in *haemostasis, the arrest of bleeding*.
- Serotonin is a vasoconstrictor. Its release from thrombocytes, adhering to the walls of a damaged vessels, is sufficient to close even small arteries. Platelets, which come into contact with collagenous fibers in the walls of the vessel, swell, become "sticky" and activate other platelets to undergo the same transformation. This cascade of events results in the formation of a *platelet plug (or platelet thrombus)*. Finally, activating substances are released from the damaged vessel walls and from the platelets. These substances mediate the conversion of the plasma protein *prothrombin* into *thrombin*. *Thrombin catalyzes* the conversion of fibrinogen into fibrin, which polymerizes into fibrils and forms a fibrous net in the arising blood clot. Platelets captured in the fibrin net contract leading to *clot retraction*, which further assists in haemostasis.

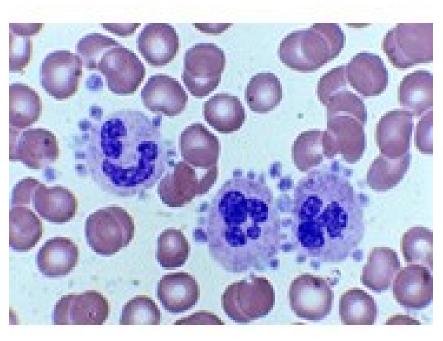
THROMBOCYTES BLOOD PLATELETES

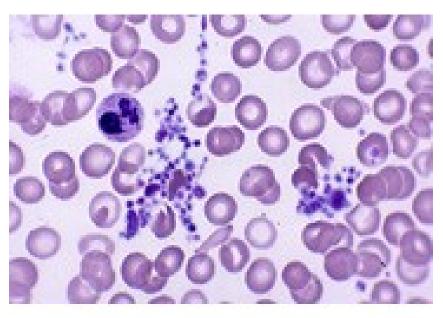
Neu

1

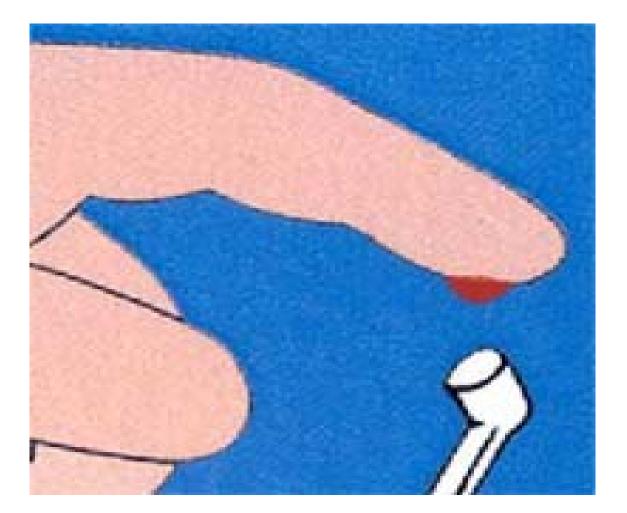
Ly





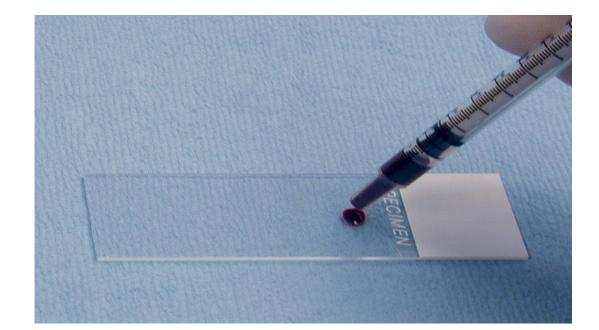


How to prepare blood smear?



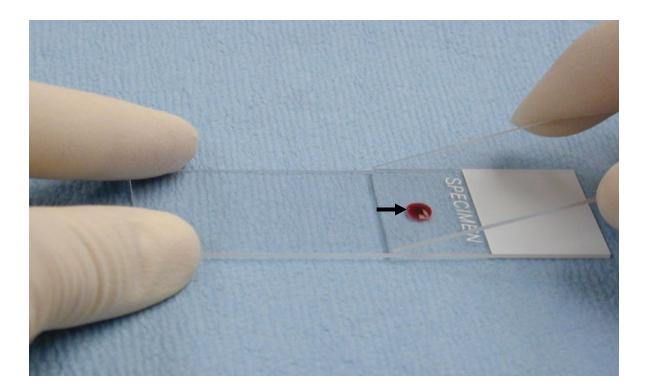
How to prepare blood smear - I

- Smears of peripheral blood must be made immediately.
- Step 1. Place drop of blood about 1cm from the frosted end of a clean slide.



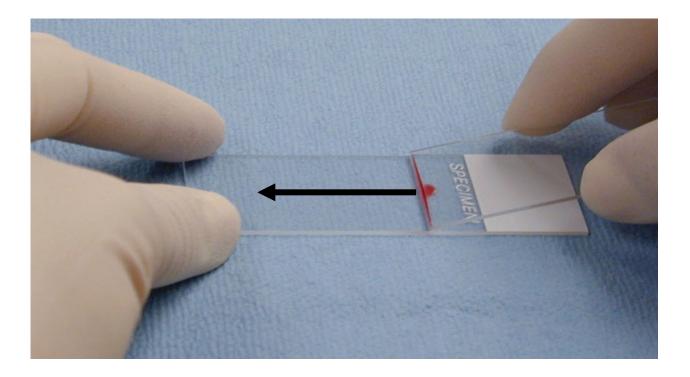
How to prepare blood smear - II

Step 2. hold the end of a second slide ("spreader") against the surface of the first slide at an angle of 30-45 degrees.



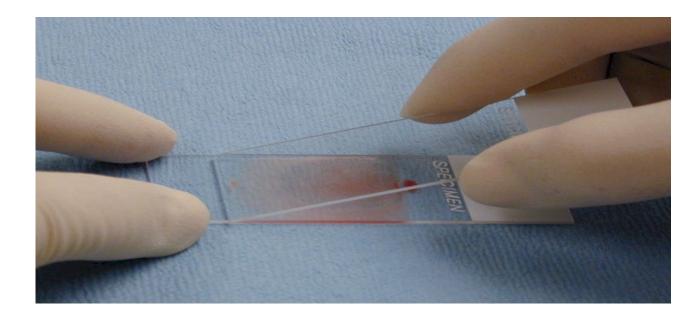
How to prepare blood smear - III

 Step 3. draw it back to contact the drop of blood. Allow the blood to spread and fill the angle between the two slides.



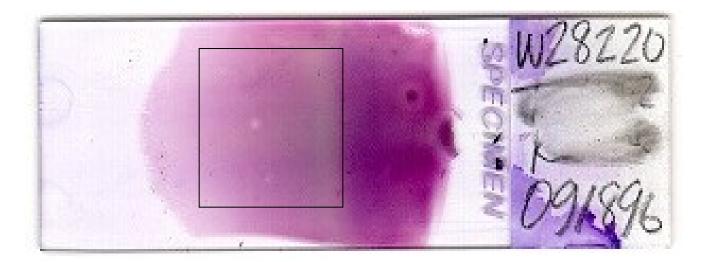
How to prepare blood smear - IV

• Step 4. Push the "spreader" slide at a moderate speed forward until all of the blood has been spread into a moderately thin film.



How to prepare blood smear - V

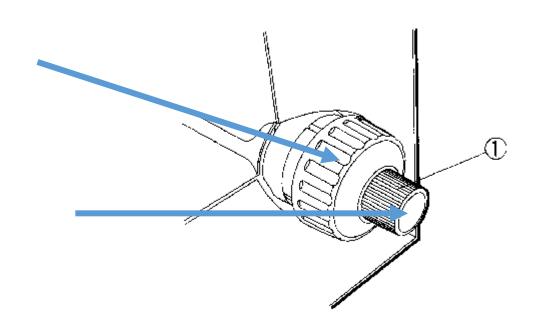
 prepared smear for fixation (methyl alcohol, 3-5 minutes) and staining (special panoptic method according to Pappenheim can be used)



Light microscope manipulation

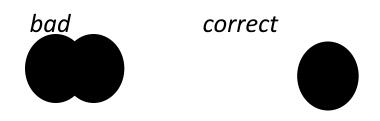
DO NOT USE TODAY

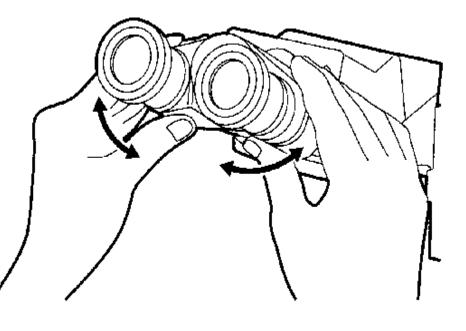
- Course adjustment knob
 ONLY
- Fine adjustment knob YOU CAN USE TODAY



Light microscope manipulation

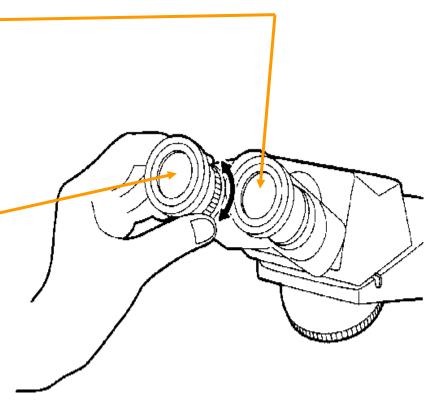
- focuse a picture in LM and look at it with both eyes
- regulate a distance between the eyepices so, you can see one focused circular field



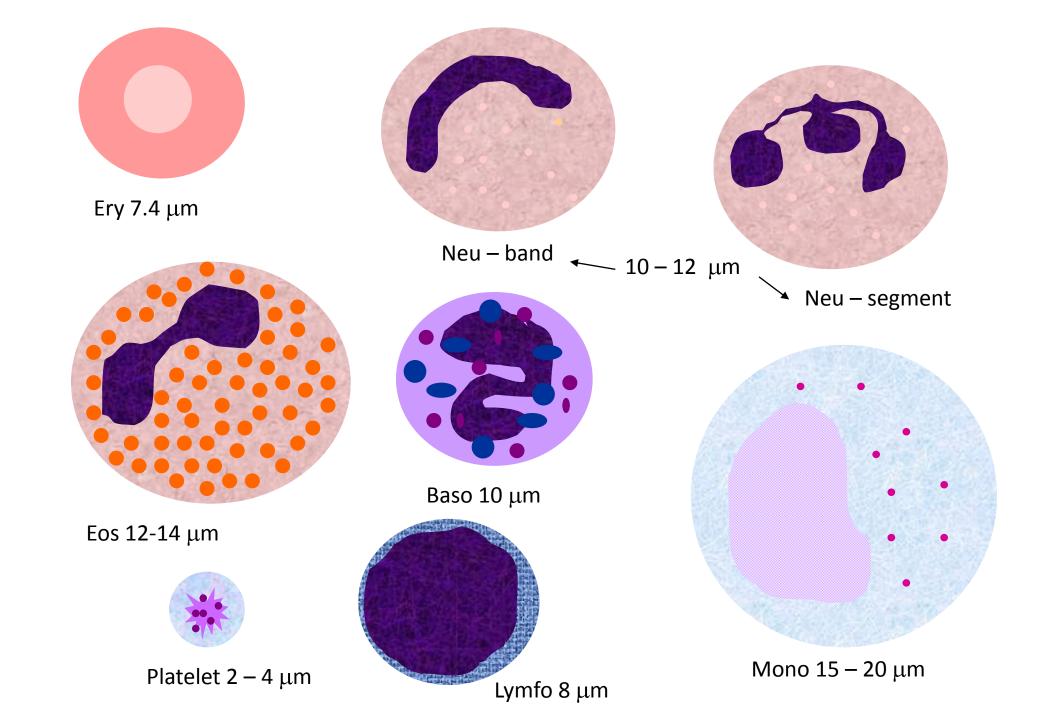


Light microscope manipulation

- Look at the slide only through the right eyepiece and focuse some point in the picture.
- Without refocusing, look at the left eyepiece.
- In doing so, screw the ring below the left eyepiece to focuse the same point.
- So, the dioptric correction is set up.



Now, you can start to study blood smear in your LM 🙂

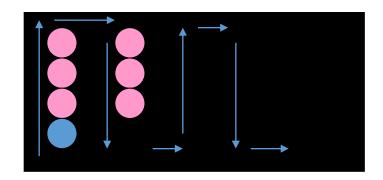


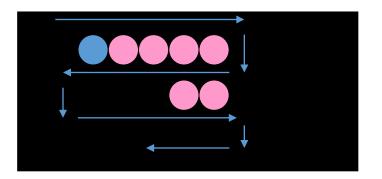
How to study blood smear in LM?

- Lens of <u>immersion objective</u> /magnifying <u>100x</u>/ is immersed into drop of <u>immersion oil</u> and blood smear is prepared for study.
- Swich on the microscope and check the picture in the microscope.
- If the image is not sharp, focus it using **microscrew!** If it is not possible, contact your teacher.

How to count leukocytes in blood smear?

 blood smear have to be systematically viewed (for fear to count some cells several-times)





How to count leukocytes in blood smear?

- differential white cell count (DWCC) is an important hematologic sreening which helps to diagnose
- leukocytes percentage is the result of this investigation
- 100 white cells must be count and registered in the table prepared for all types of leukocytes (Neu-bands, Neusegments, Eos, Baso, Ly, Mono)
- arithmetic sum of each type of leukocytes represents their percentage (%)

Table

	1	2	+
Neu bands	/		
Neu segments	+++++-	///	
Eos		/	
Baso			
Ly	//	////	
Mono		//	
	10 cells	10 cells	

9	10	results	norm
//			4 %
++++- /	///		67 %
/	//		3 %
	/		1 %
/	1111		20 %
			5 %
10 cells	10 cells	100%	100 %

Differential white cell count (DWC(



• Total number of leukocytes: normal values

Neutrophils - bands	4 %
- segments	67 %
Eosinophils	3 %
Basophils	1 %
Lymphocytes	20 %
Monocytes	5 %
	Σ = 100 %

Anomalies of DWCC

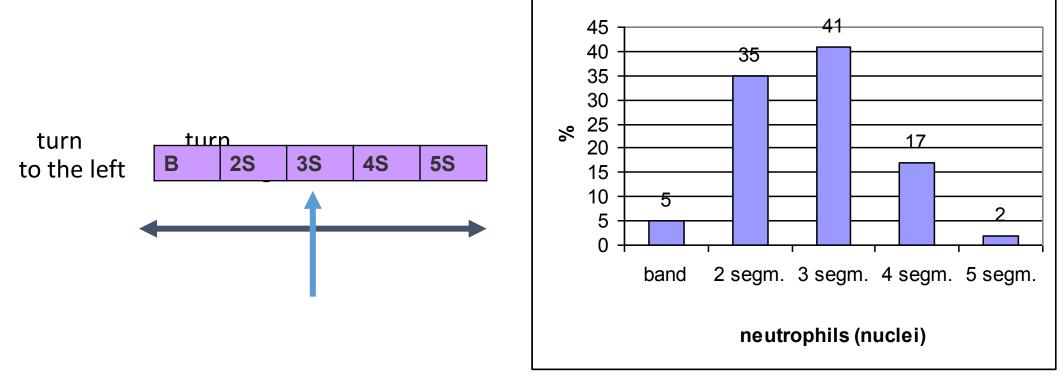
	♠ Increased number	✓ Decreased number
Neutrophils*	neutrophil granulocytosis	neutrophil granulocytopenia
Eosinophils	eosinophil granulocytosis	eosinophil granulo <mark>cytopenia</mark>
Basophils	basoophil granulocytosis	basoophil granulocytopenia
Lymphocytes	lymphocytosis	lymphocytopenia
Monocytes	monocytosis	monocytopenia

* sum total of bands and segments has to be compared with norm;

normal value is 71 % (4 % bans + 67 % segments)

Normal ratio neutrophil bands and segments

- (B : S) is 4 % : 68 % = 1 : 17
- Turn to the left bands are increased
- Turn to the right segments are increased in peripheral blood





BLOOD

Slide

Peripheral blood smear, panoptic staining (method of Pappenheim), immersion oil, magnif. 1000x

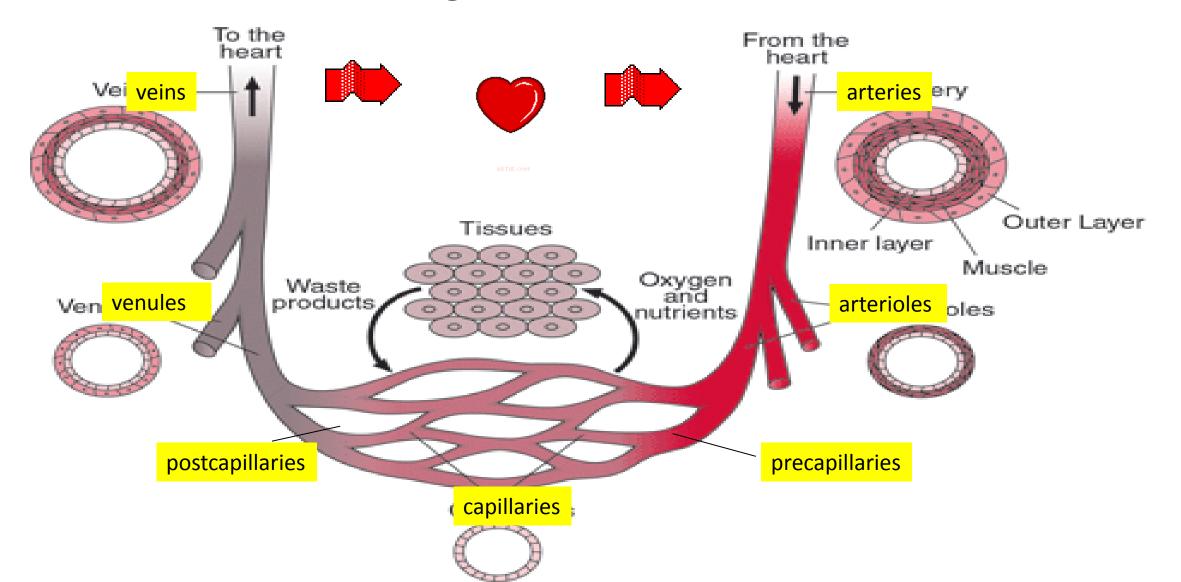
Blood vessels

are categorized by function :

- <u>Arteries</u> conduct blood away from the heart and have proportionately more smooth muscle and elastic tissue than veins of comparable size.
 - Arteries are commonly sub-categorized into elastic arteries (*the largest one*), muscular arteries (*middle-sized*), and arterioles.
- <u>Veins</u> return blood to the heart.

The composition of the wall varies among arteries and veins.

Bloodstream organization



Structure of blood vessel wall

• tunica interna (intima)

endothelium + subendothelial connective tissue

membrana elastica interna

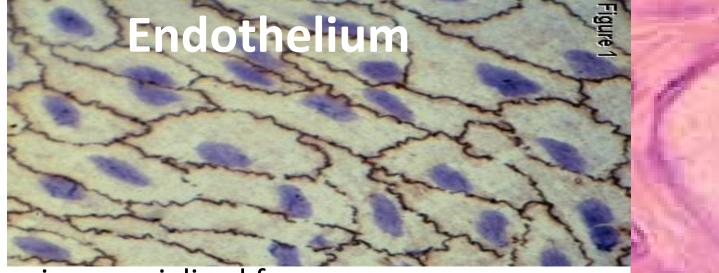
• tunica media

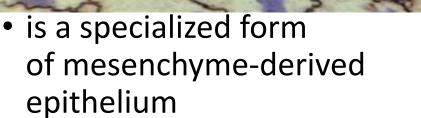
smooth muscle tissue - circularly oriented

membrana elastica externa

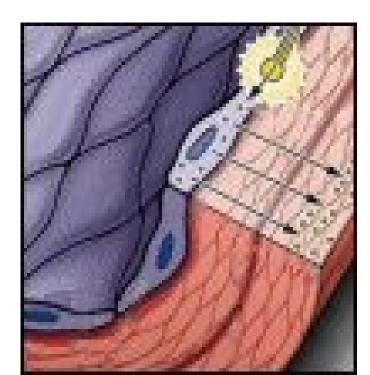
• tunica externa (adventitia)

loose connective tissue + nerves + vasa vasorum (+ longitudinal smooth muscle – <u>only</u> in veins)



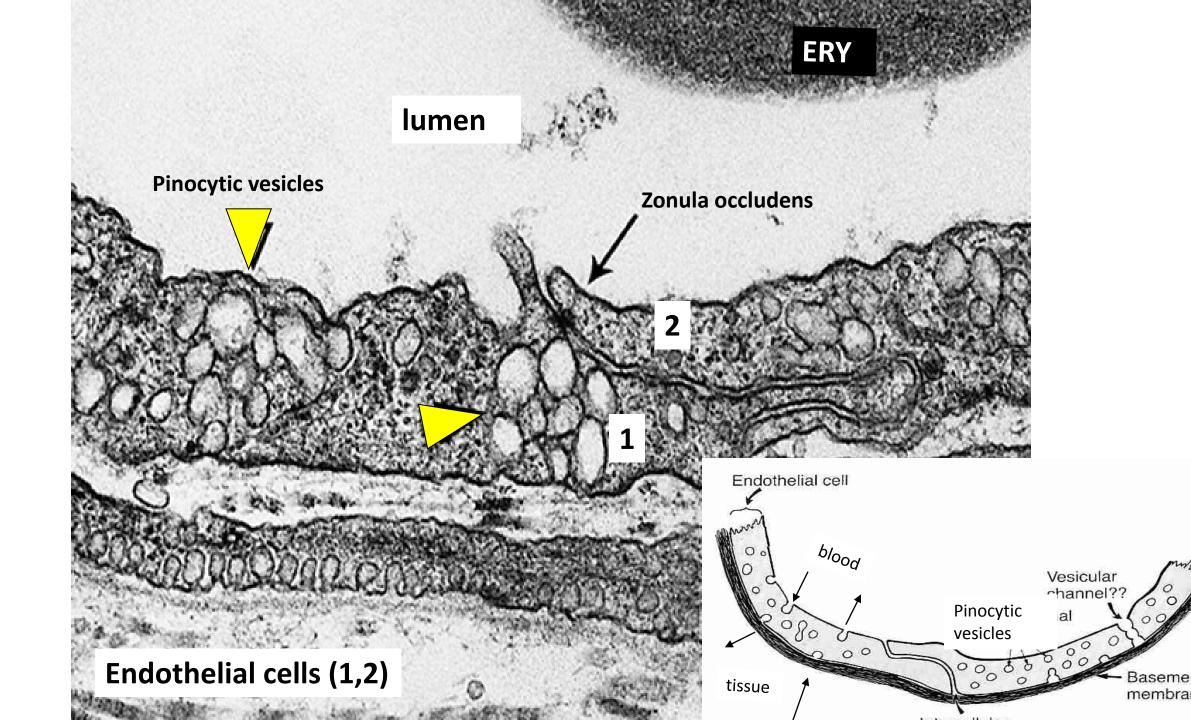


 simple squamous epithelium – 1 layer of flattened cells forms a thin, waterproof and antithrombogenic lining of all <u>blood vessels</u>, <u>heart</u> and <u>lymphatic</u> <u>vessels</u>

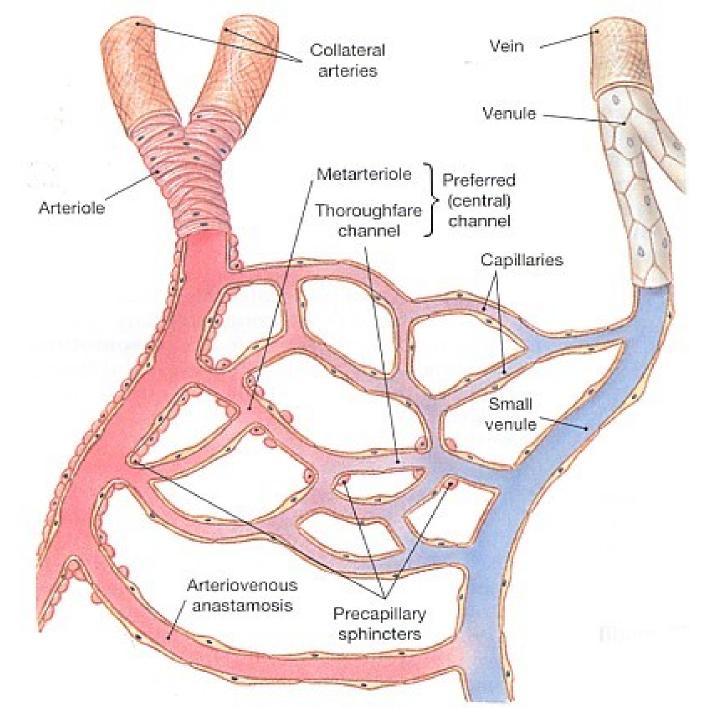


Function of endothelium

- the control of <u>blood pressure</u> by <u>vasoconstriction</u> and <u>vasodilation</u>,
- blood clotting,
- formation of new blood vessels (angiogenesis),
- control of the passage of materials and the transit of white blood cells into and out of the blood,
- in some organs, there are highly differentiated endothelial cells to perform specialized 'filtering' functions (renal glomerulus in kidney, blood-brain barrier, placental barrier).

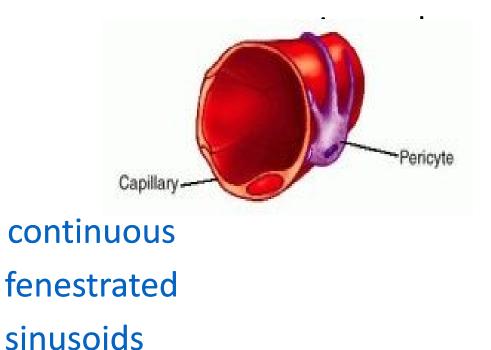


Blood capillaries

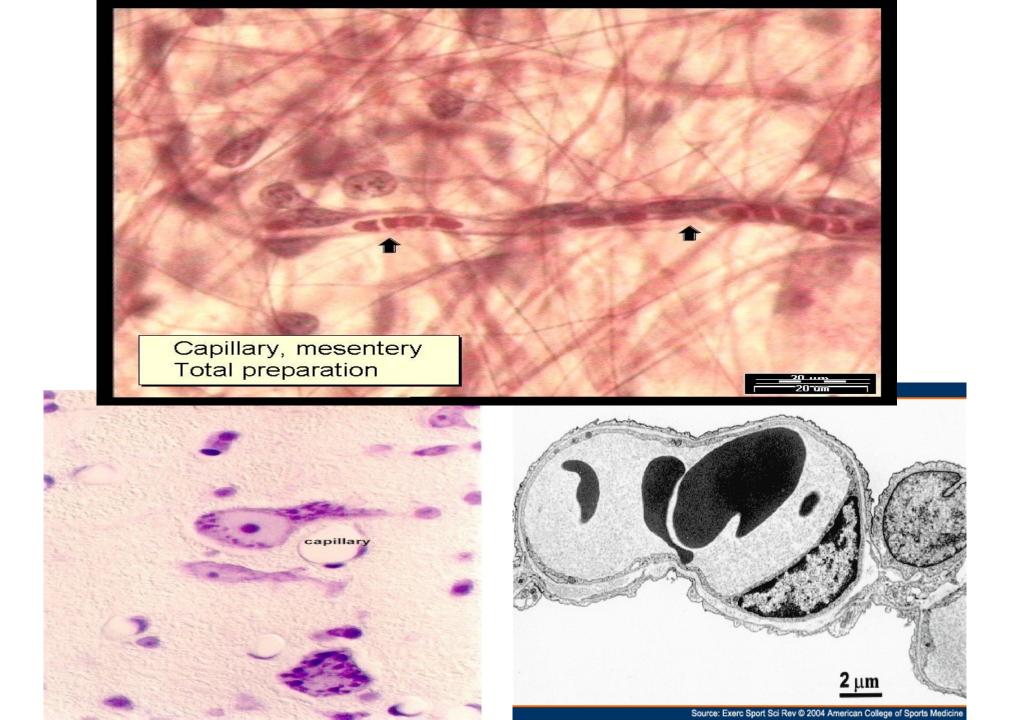


Blood capillaries

- diameter from about 8 μ m (to 30-40 μ m)
- lumen is lined by 1-2 endothelial cell
- reticular fibers surround the capillaries
- capillary bed between veins
- pericytes

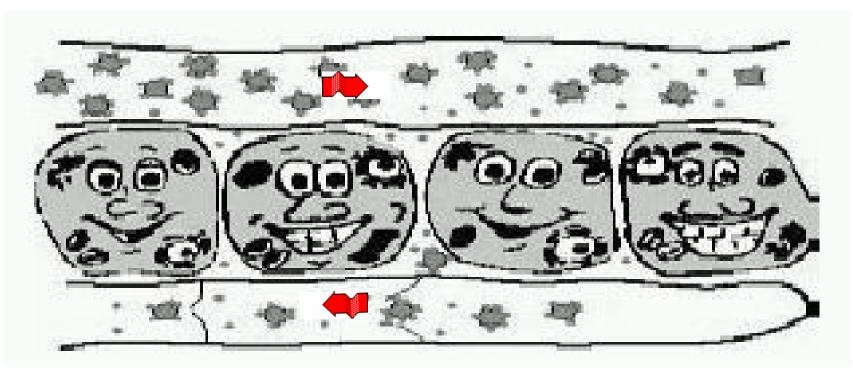


3 types of capillaries



Function of capillaries (1)

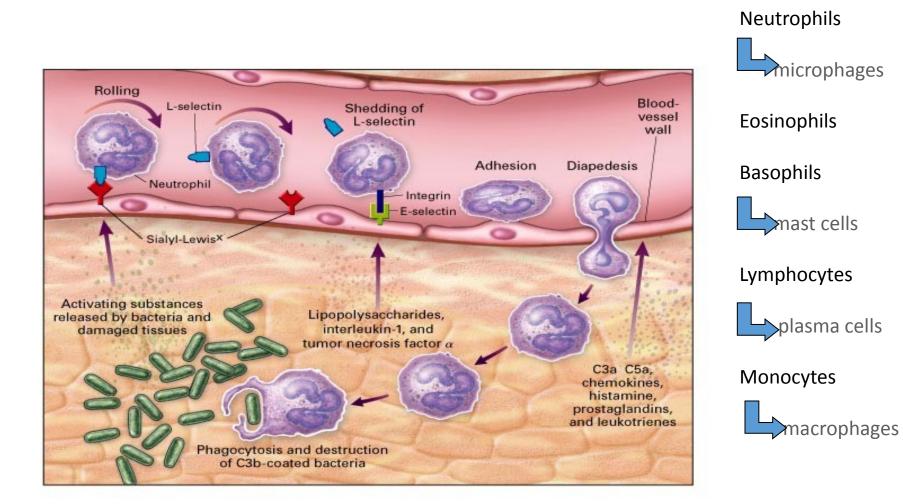
 respiratory gasses, nutrients and waste products change between blood and tissues



The illustration shows the healthy state of the cells in well vascularized tissue \odot

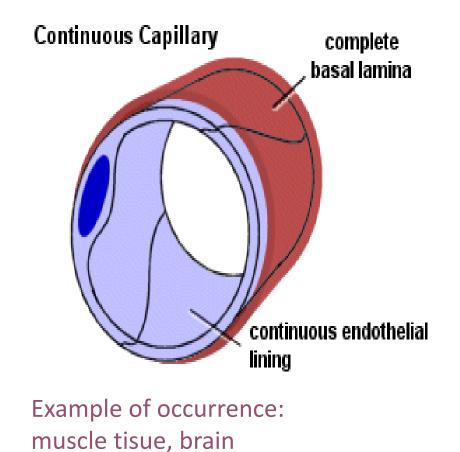
Function of capillaries (2)

• allow the blood cells to pass throughout their wall into the connective tissue (by diapedesis)



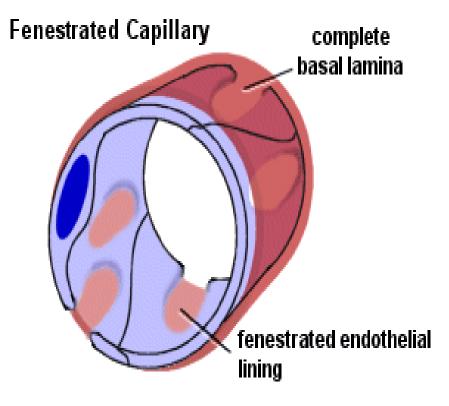
Continuous capillaries

- The smallest: cca 8 μ m
- The wall: endothelium – 1-2 cells
 - (zonulae occludentes and nexuses)
 - lamina basalis
 pericytes
 reticular fibers
- only allow small molecules, water and ions to diffuse



Fenestrated capillaries

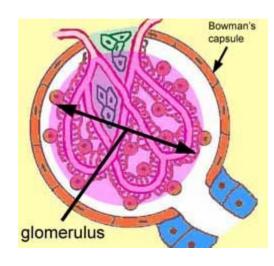
- Endothelial cells with fenestra ("windows") 70 nm Ø, diaphragm (thinner than plasma membrane) boards fenestrum
- continuous basal lamina
- in the organs with quic and intensive metabolism and substances change
- allow small molecules and limited amounts of protein to diffuse

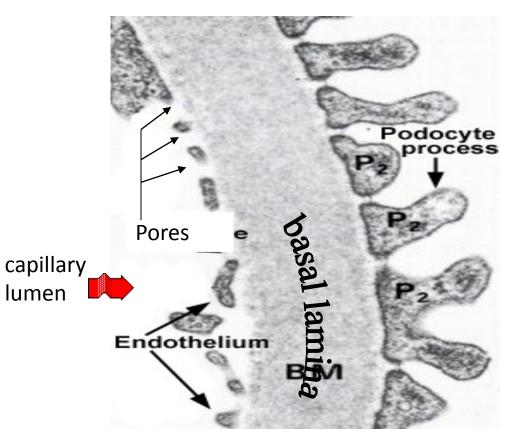


Exampl of occurrence: intestinal villi, endocrine glands

Capillaries with pores

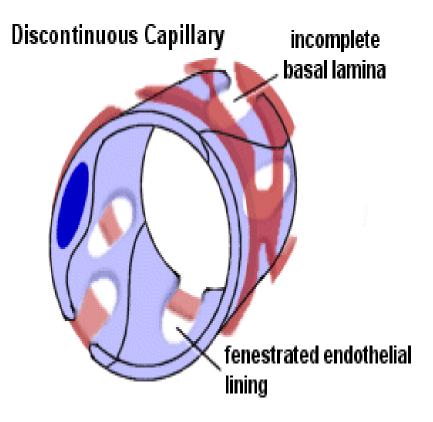
- special type of fenestrated capillaries
- not fenestra with diaphragm, but opened pores are in endothelium
- in glomeruli of renal corpuscles



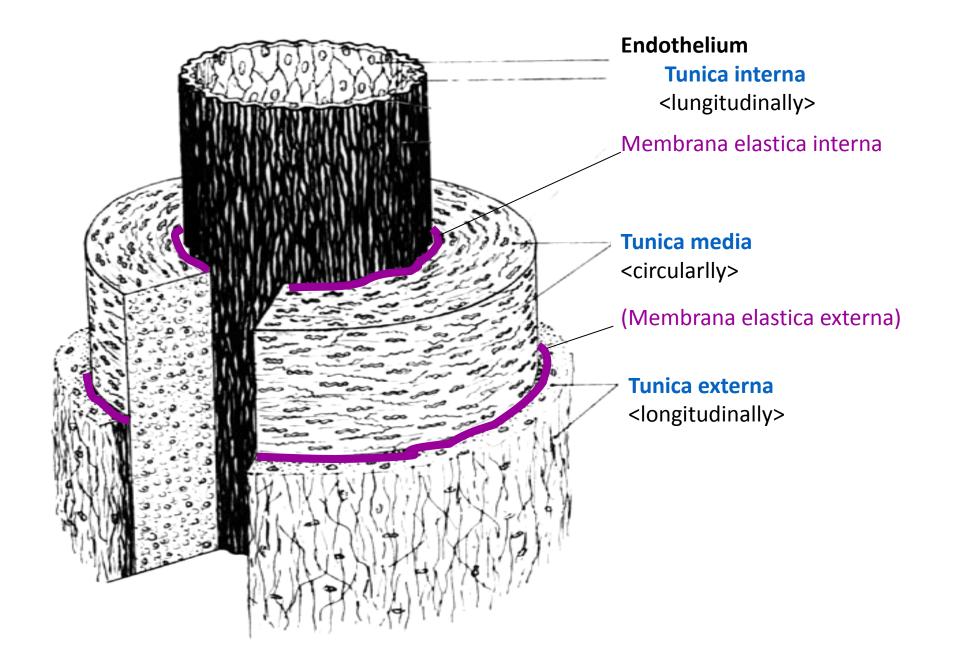


Sinusoidal capillaries (sinusoids)

- \emptyset from 8 to 40 μ m
- endothelium fenestra, pores and intercellular clefts; some cells are able to phagocyte
- incomplete basal lamina
- reticular fibers
- allow erytrhocytes and serum proteins to enter.

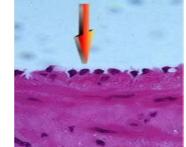


Example of occurrence: liver, spleen, bone marrow

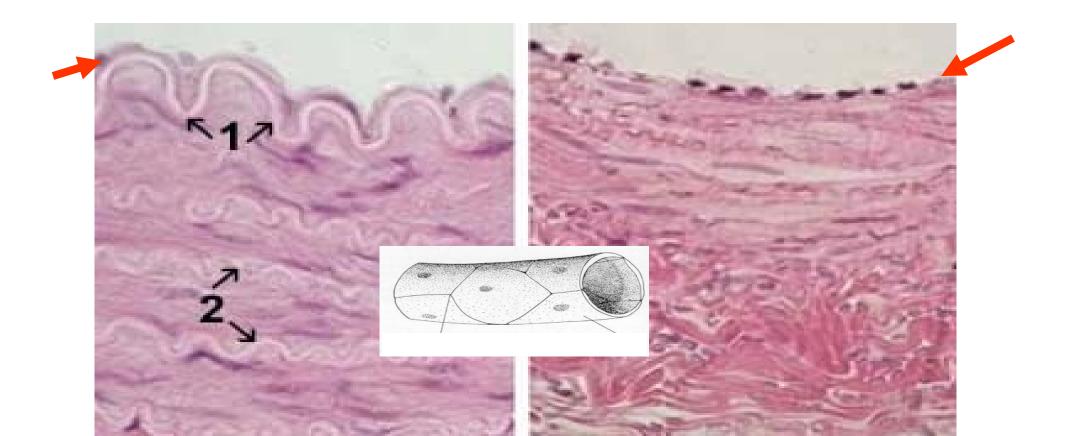


Tunica interna (*intima*) TI

• endothelium

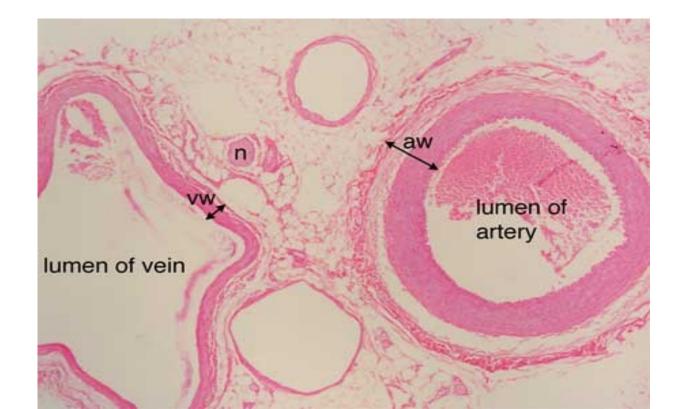


• **subendothelial connective tissue** – thin layer of elastic + collagen fibers *(longitudinally oriented)*



Tunica media TM

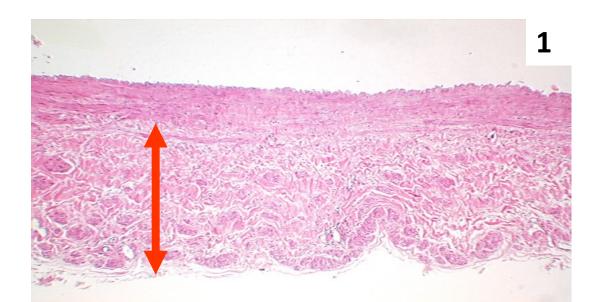
- consists of <u>smooth muscle cells</u> and <u>elastic membranes</u> in varying proportions (circularly oriented)
- is thicker in arteries than in veins

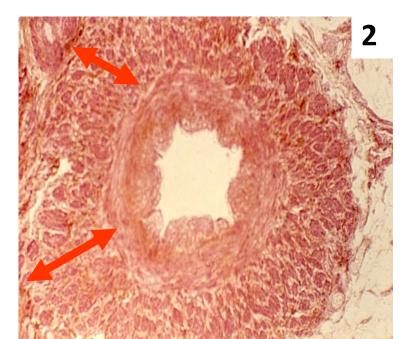


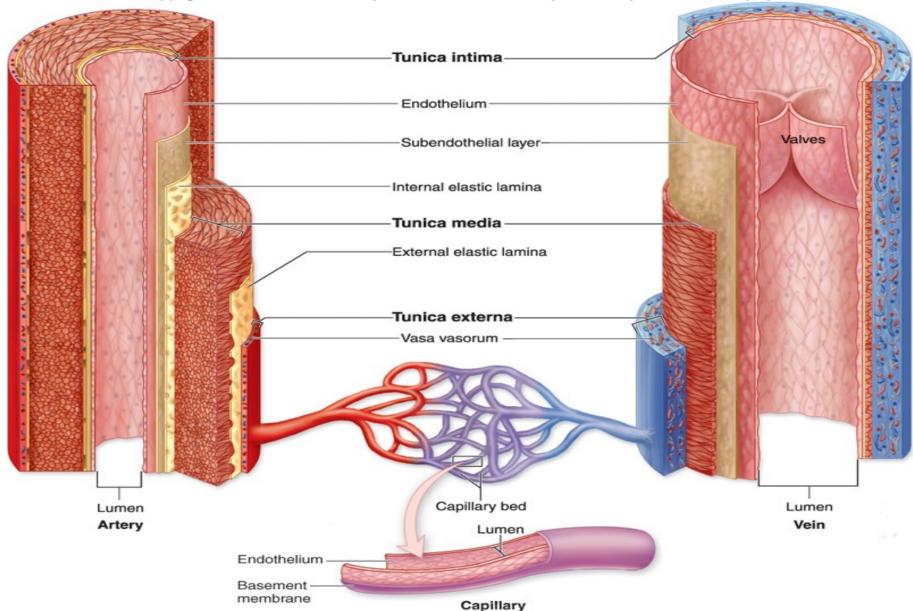
Compare aw – vw:

Tunica externa (adventitia) TA

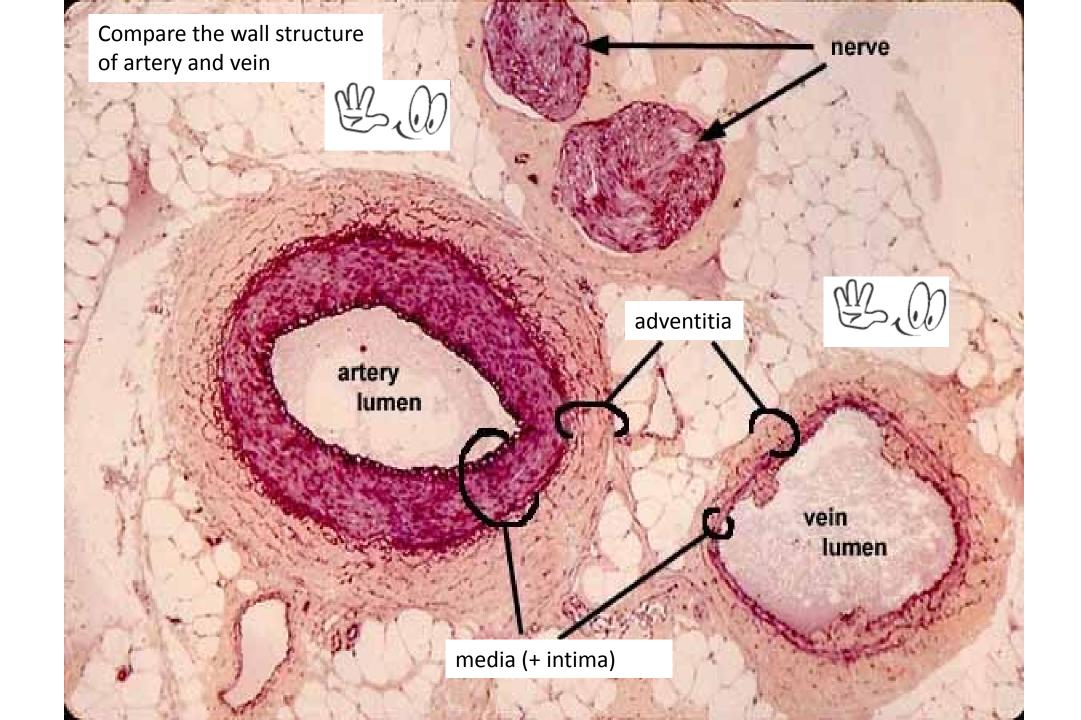
- <u>fibrous connective tissue</u> + smooth muscle cells in veins (*logitudinally*)
- is thicker in vein; is the thickest layer in large veins [1] and veins of low limbs [2]
- contains vessels and nerves (vasa et nervi vasorum) in large vessels







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Arterial part of bloodstream

According to diameter, morphological differences and ratio of elastic fibers and smooth muscle cells:

- Arterioles $\emptyset < 0.5 \text{ mm}$
- Muscular arteries (small and middle-sized)

 \emptyset 0.5 – 1 mm

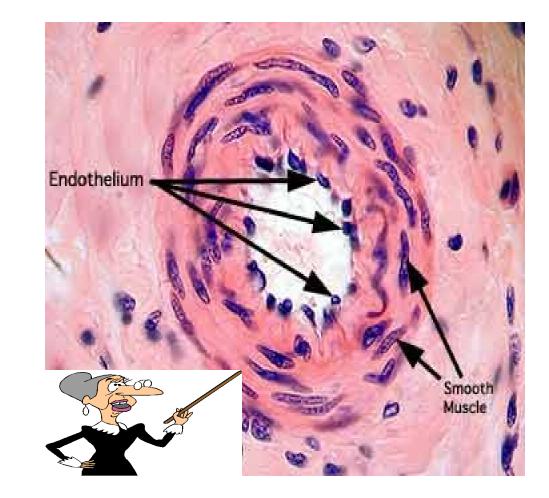
• Elastic arteries (large: aorta and arteries growing from aorta)

Arteriole

• Ø < 0.5 mm

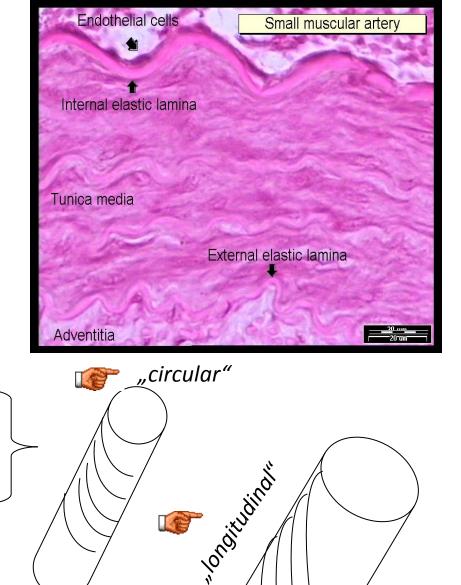
The wall

- TI: endothelium + subendothelium
- membrana elastica int.
- TM: smooth muscle cells (cca circular 5 layers)
- TA: fibrocytes, reticular (+collagen) fibers



Muscular artery

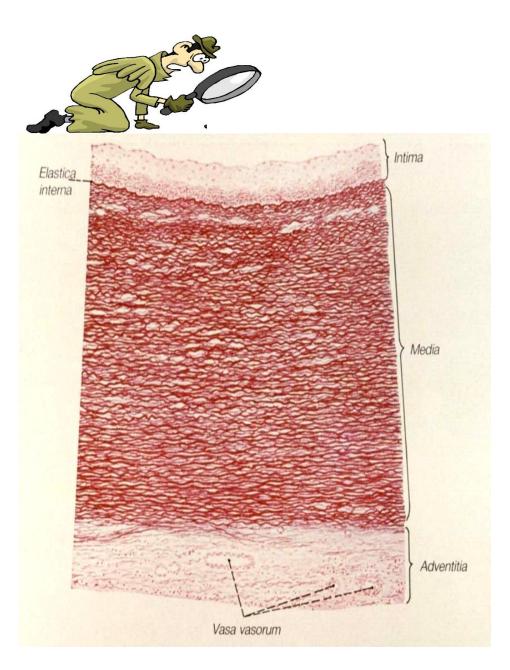
- TI: endothelium + subendothelium (with smooth muscle cells (longit.)
- membrana elastica int.
- TM: up to 40 layers of smooth muscle cells, elastic and collagen fibers
- membrana elastica ext.
- TA: loose connective tissue



TM+TA arrangement is spiral, but ...

Elastic artery

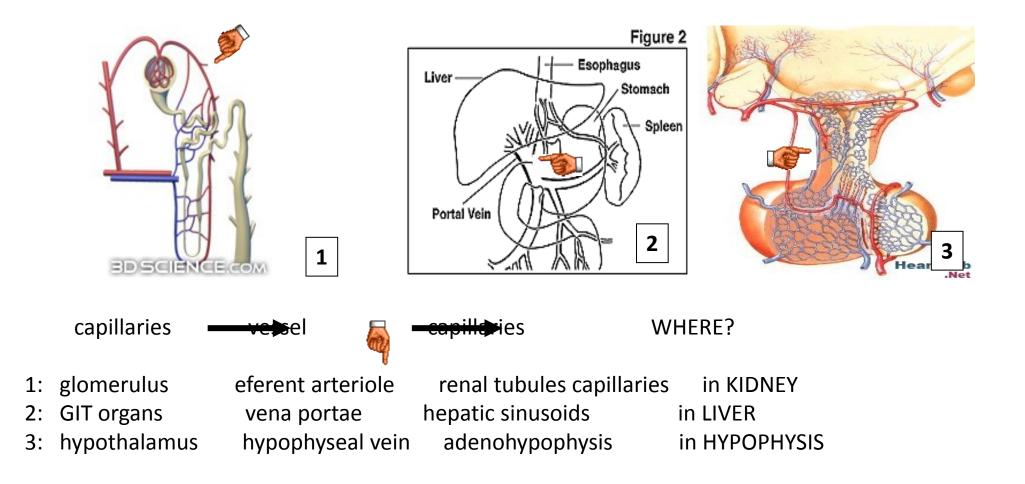
- TI: endothelium + subendothelium (100 μm wide layer of connective t.)
- TM: up to 40-60 layers of fenestrated elastic membranes, small amount of smooth muscle cells and reticular fibers
- TA: loose connective tissue (+ vasa et nervi vasorum)



Portal circulation:

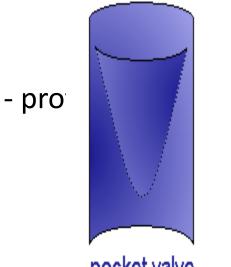
arterial or venous

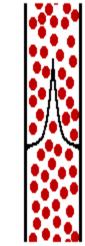
• two capillary systems side-by-side



Venous part of bloodstream

- Venules Ø 0.2 1 mm
- Small and medium sized veins \emptyset 1 9 mm
- Large veins (v. cava inf. et. sup. the largest vein)
- Valves
 like duplication of endothelium
 elastic c.t.
 reccurence



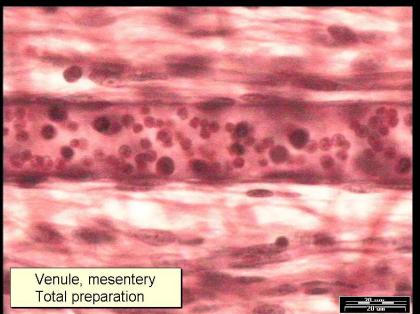


pocket valve

Venule

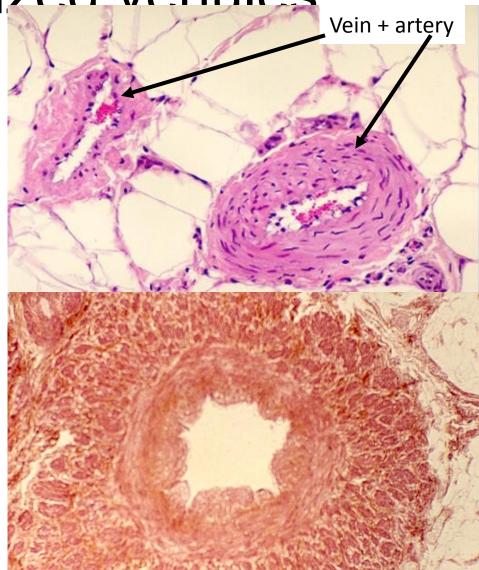
- Ø < 0.2 1 mm
- <u>The wall</u>
- TI: endothelium only
- TM: smooth muscle cells (cca circular 1-3 layers)
- TA: thick layer of loose connective tissue





Small and medium-sized venules

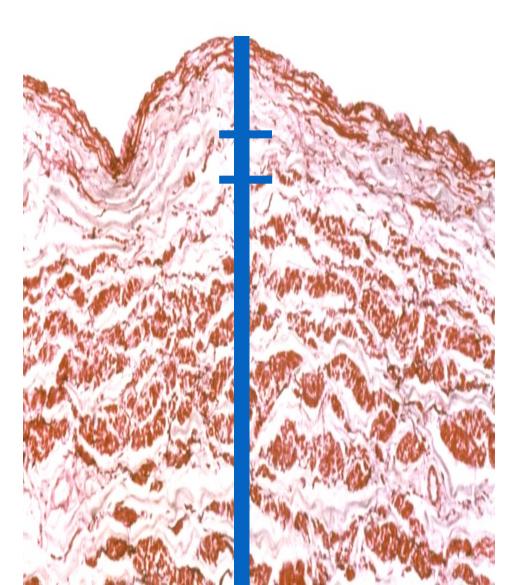
- Ø 1 9 mm
- TI: endothelium + irregular layer of subendothelium + valves
- TM: irregular, thin layer of smooth muscle cells, elastic and collagen fibers
- TA: thick layer of loose connective tissue with smooth muscle cells



Vein from lower part of body

Large veins

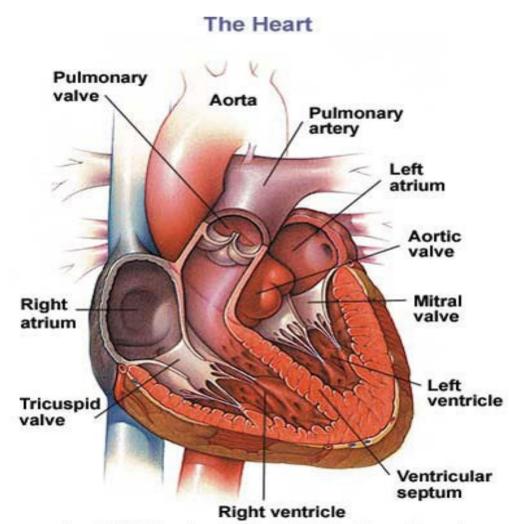
- TI: endothelium + subendothelium (+smooth muscle cells)
- TM: thin layer of connective tissue + reduced amount of smooth muscle cells
- TA: longitudinal bundles of smooth muscle cells in loose connective tissue (vasa et nervi vasorum)



The heart is the hardest working muscle in the human body.

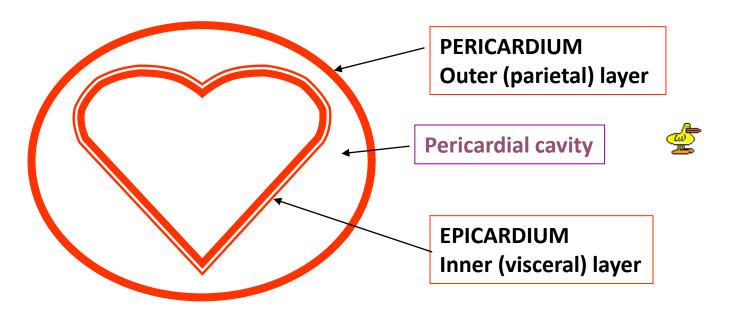


- Hollow muscular organ – blood pump
- Rythmic contraction
- Involuntary muscle



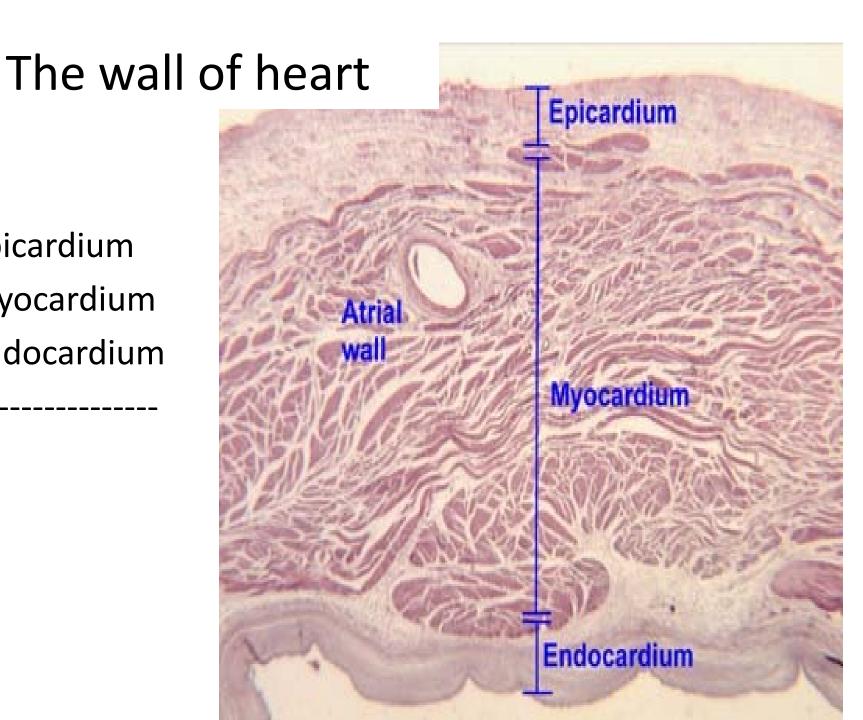
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Pericardial sac: pericardium + epicardium

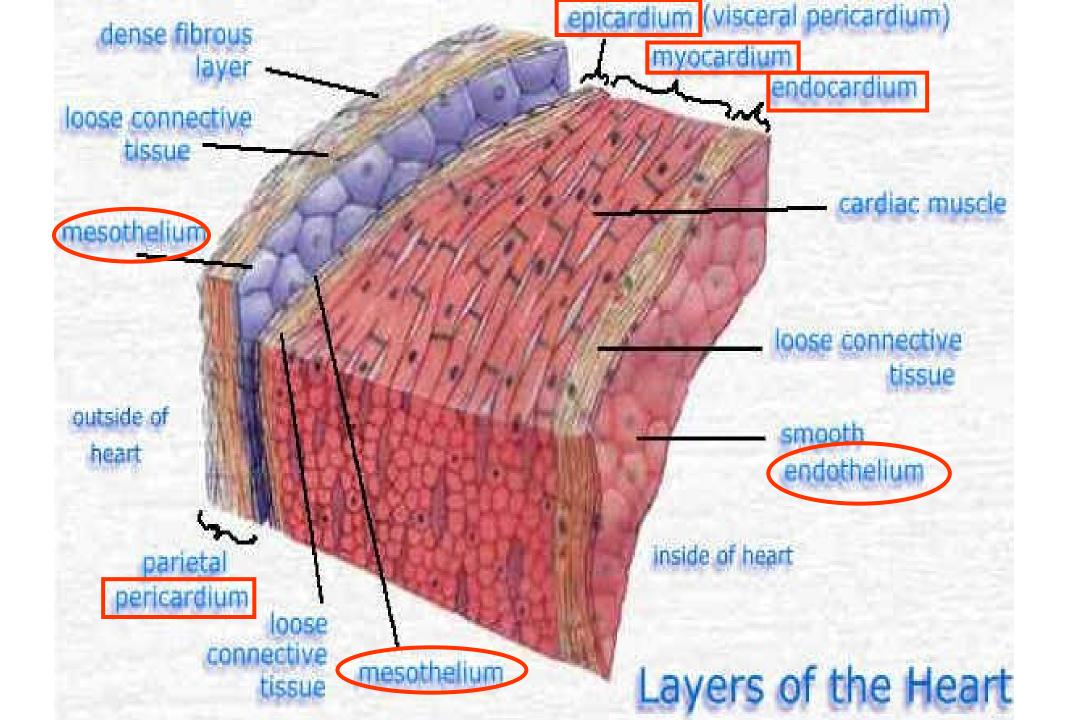


Pericardial cavity - contains 15 – 50 ml of serous fluid serves as lubricans; - is lined with mesothelium





- Epicardium
- Myocardium
- Endocardium



Endocardium

(homologous to intima of blood vessels)

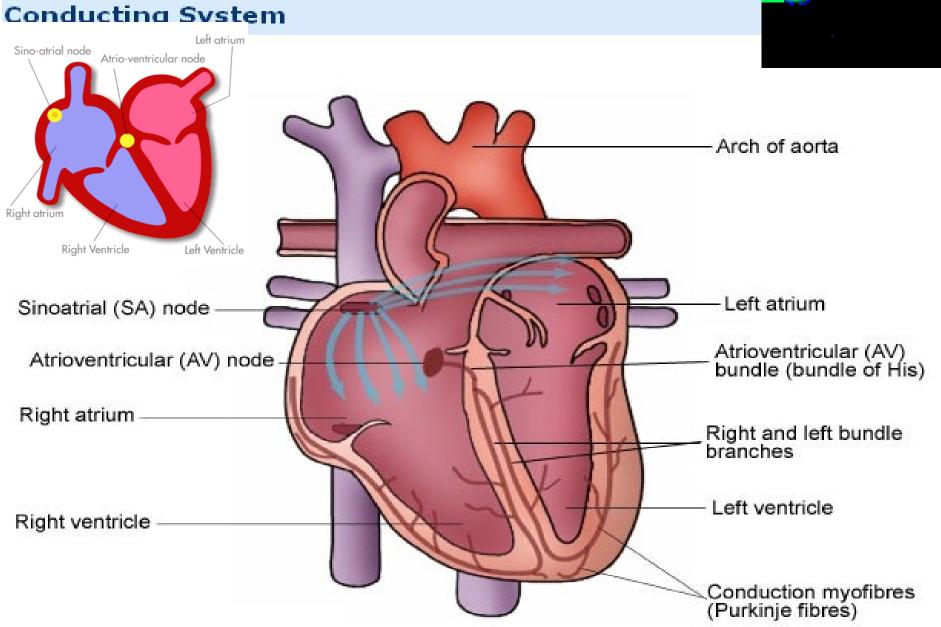
Consists of:

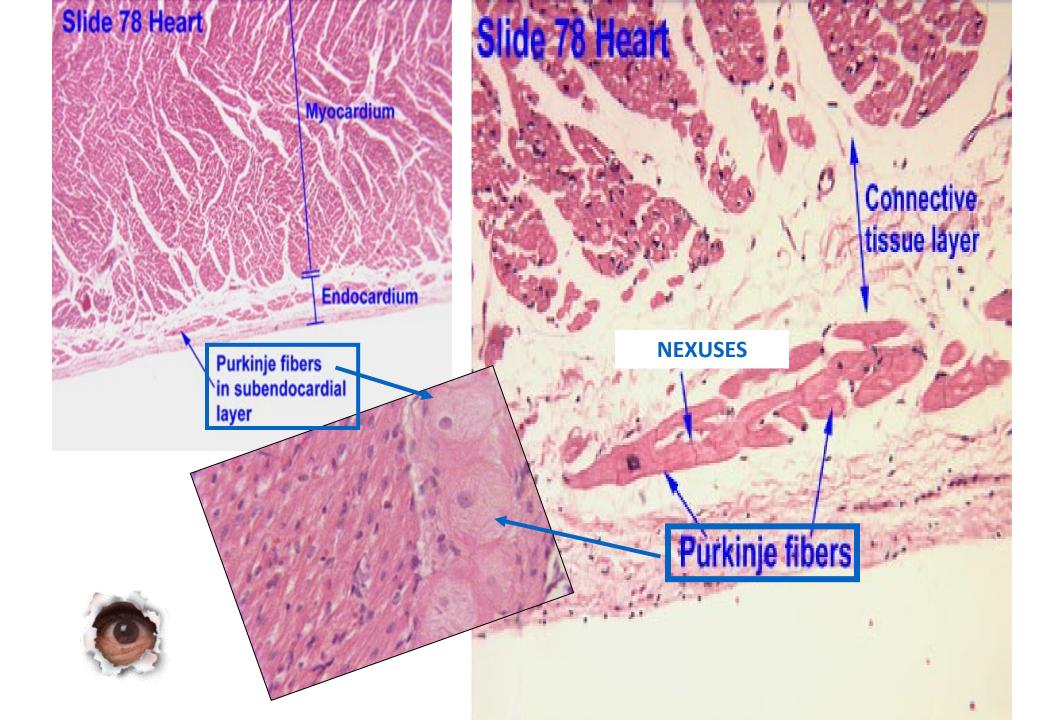
- Endothelium
- Subendothelium thin connective tissue layer
- Elastic-muscular layer dense c.t. (elastic fibers, smooth m. cells)
- Subendocardium c.t. + vessels, nerves and distal part of conducting system (ventricular bundles and Purkinje fibers)





Heart Structure

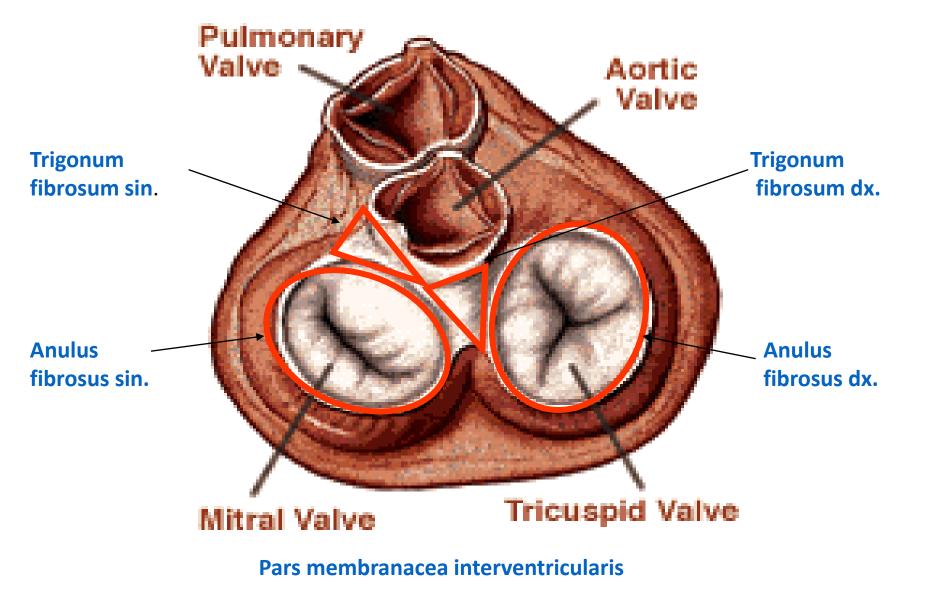




Myocardium

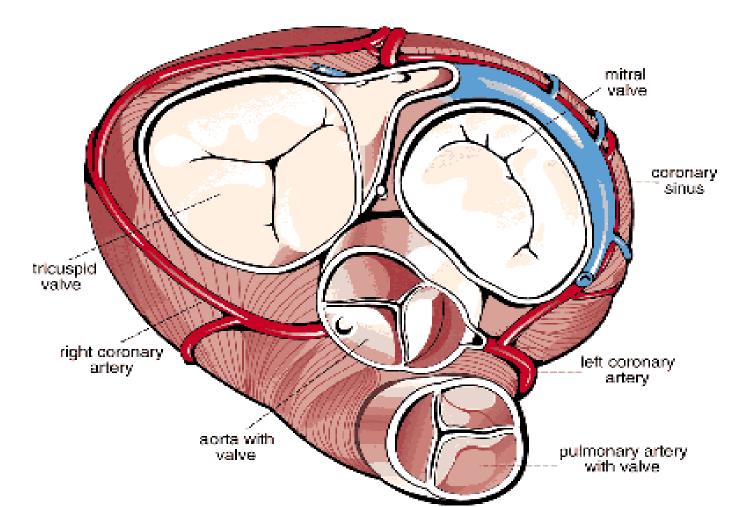
- cardiomyocytes "working" "conducting"
- cells in right ventricle natriuretic factor (when intravascular volume increases, this factor is released and causes natriuresis and diuresis in kidney)
- atrial myocardium is thinner than ventricular
- "left heart" myocardium is thinner than "right heart"
- cords of cardiomyocytes are ended on heart skeleton
- damage of myocardium infarction
- low regeneration of myocardium by scar (decreases function of heart muscle)

Heart skeleton



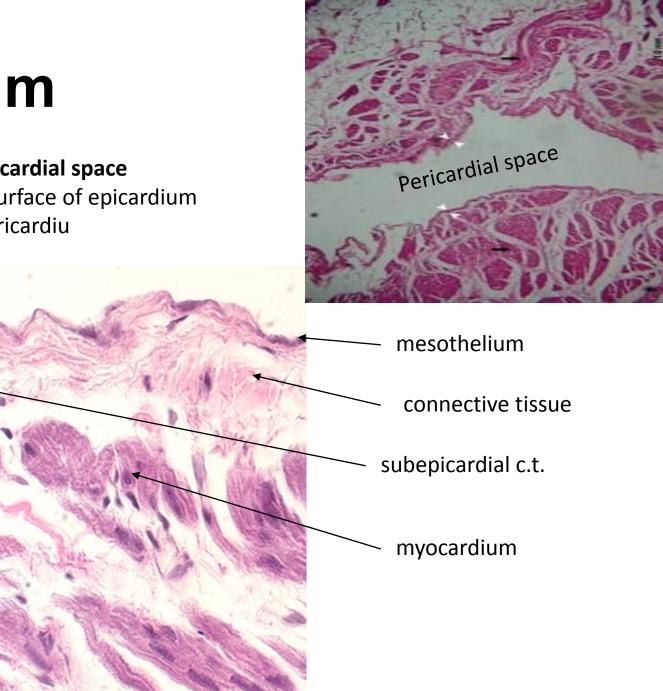
Endocardial valves

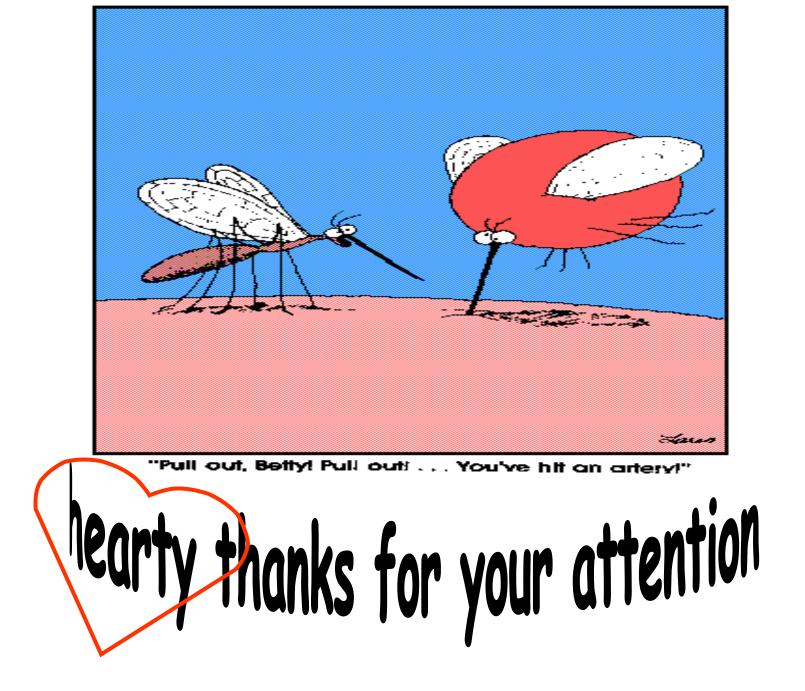
Plates of dense connective tissue (continuous with heart skeleton) covered with endocardium.



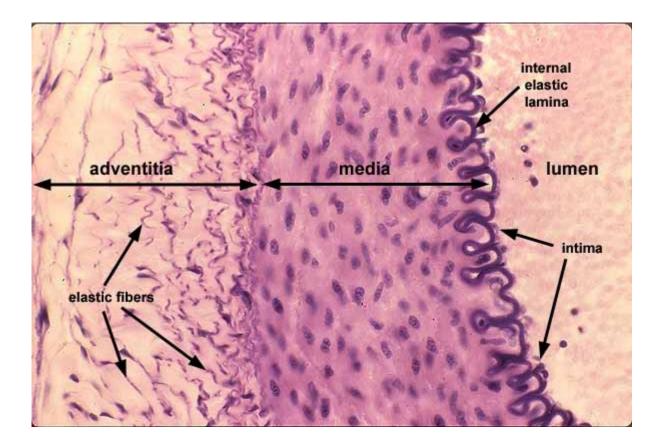
Epicardium

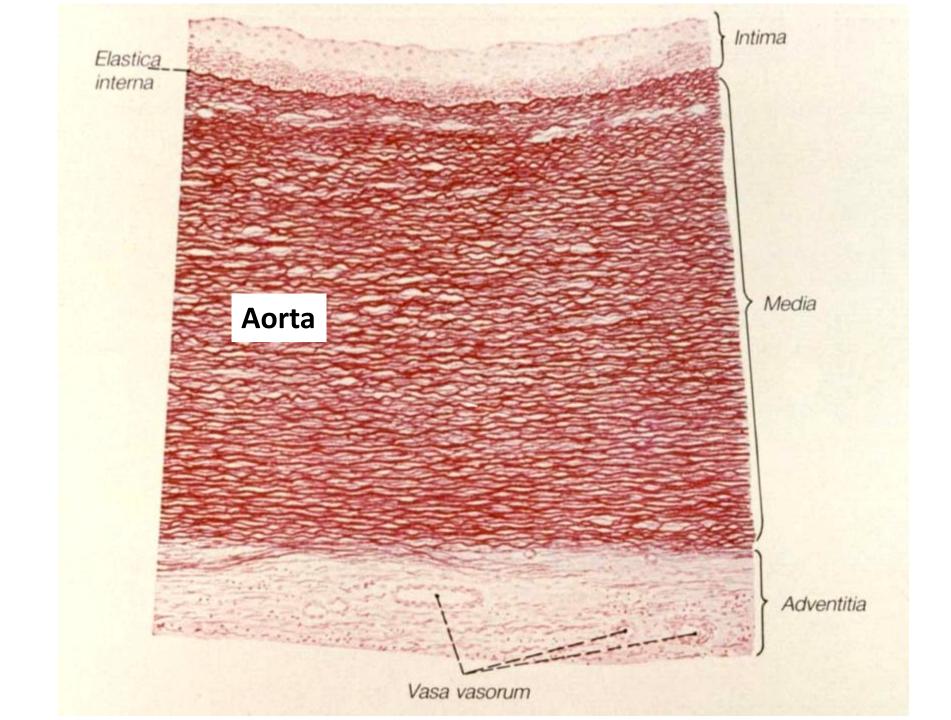
Mesothelium lines pericardial space and so it covers outer surface of epicardium and inner surface of pericardiu

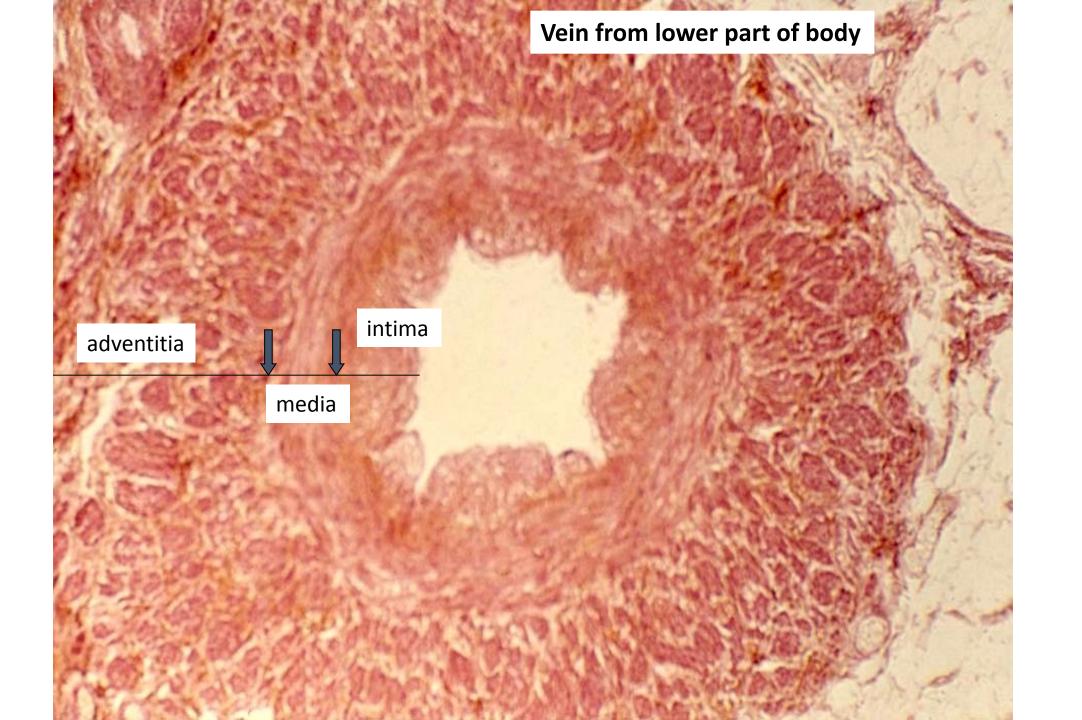


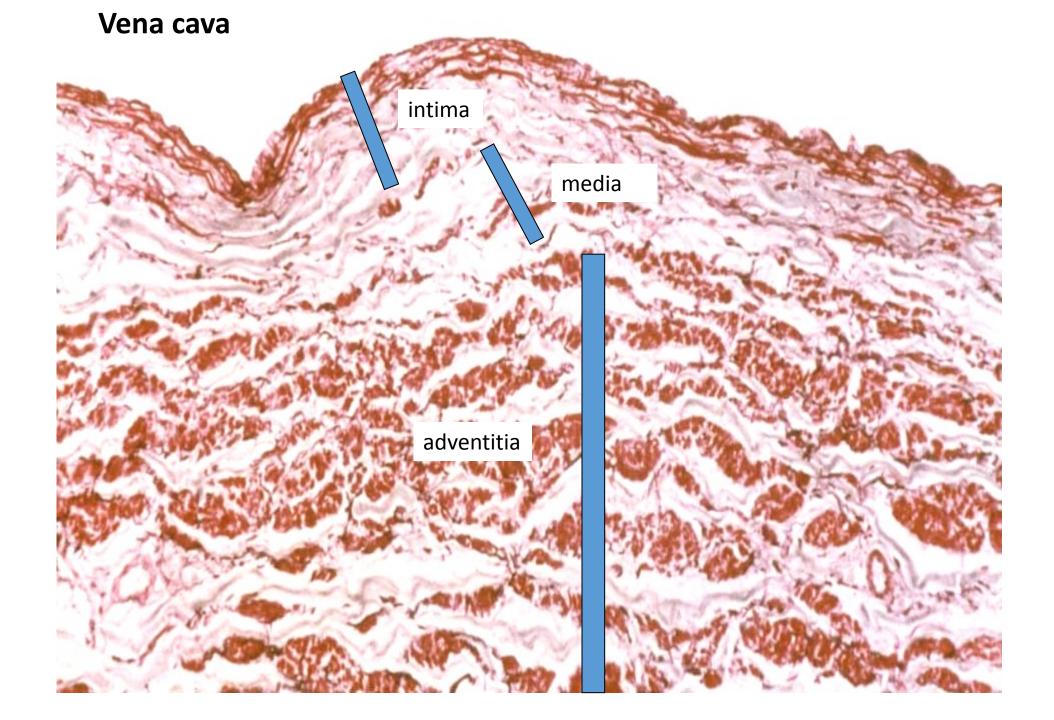


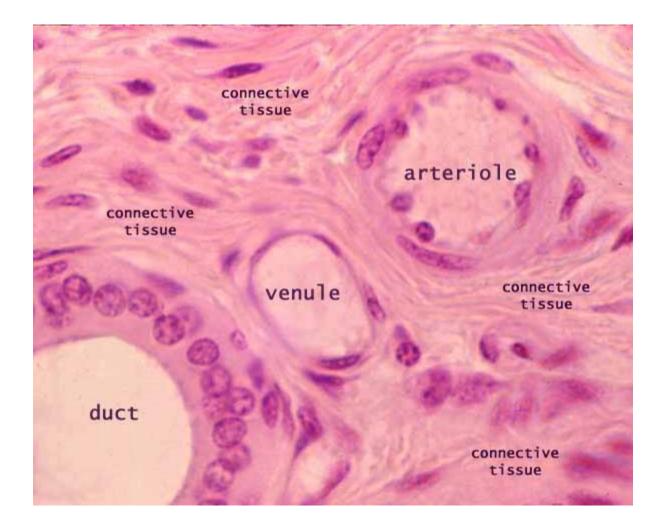
Muscular artery











Lymphatic vessells

- Lymphatic vessels (often just called *lymphatics*) are channels which drain excess fluid ("lymph") from tissues.
- In most peripheral tissues, some plasma seeps out of capillaries. A portion of this is taken back up in venules while the rest drains into terminal lymphatic channels, also called lymphatic capillaries. A shift in the balance between fluid entering and leaving tissues (e.g., increased vascular permeability due to inflammation) can result in accumulation of tissue fluid, or *edema*.
- All lymphatic vessels eventually lead "downstream" to the thoracic duct, which empties into the vena cava (a point where blood pressure is quite low; higher pressure would impede drainage).
- Lymphatic vessels resemble blood vessels with exceptionally delicate walls (and, of course, without red blood cells). Smaller lymphatic vessels consist of little more <u>endothelium</u>.