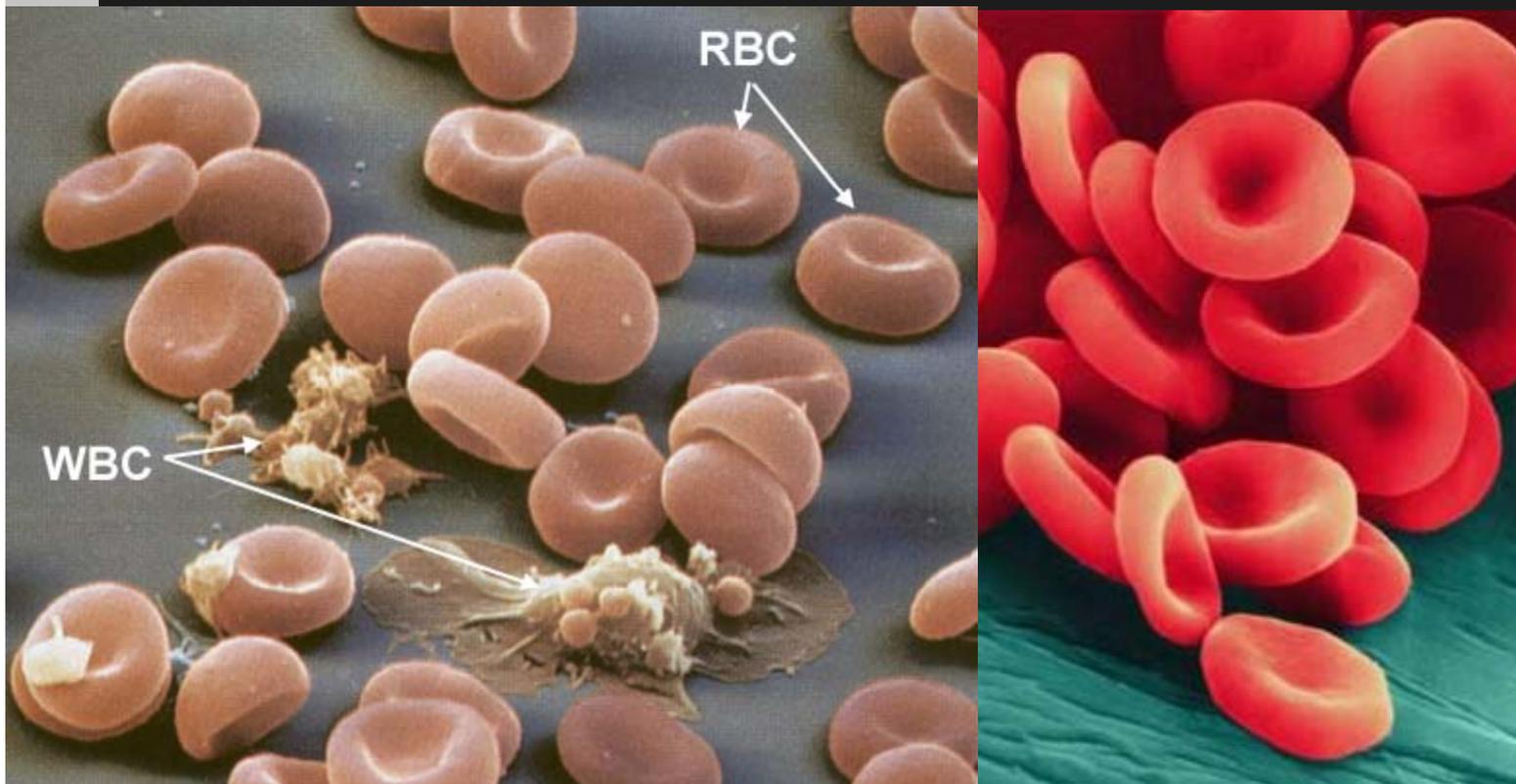
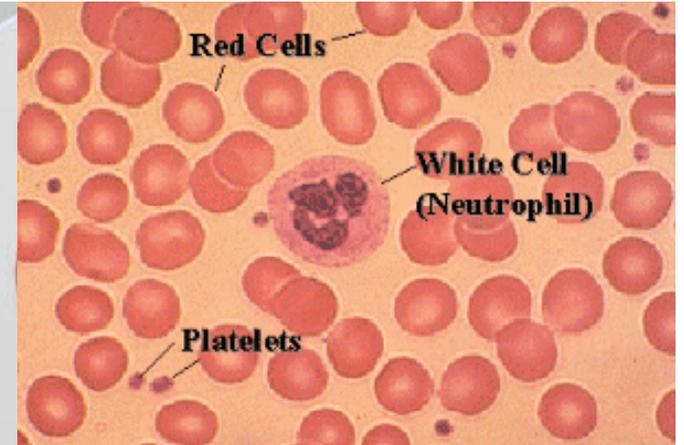


MICROSCOPY ANALYSIS OF BLOOD

Danil Hammoudi. MD



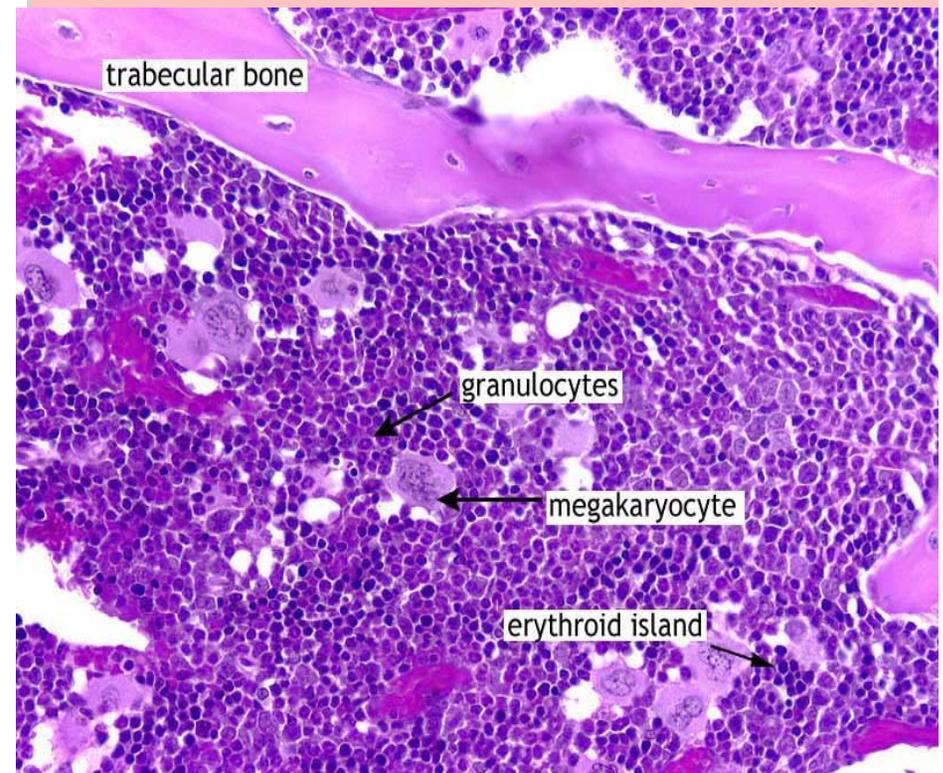
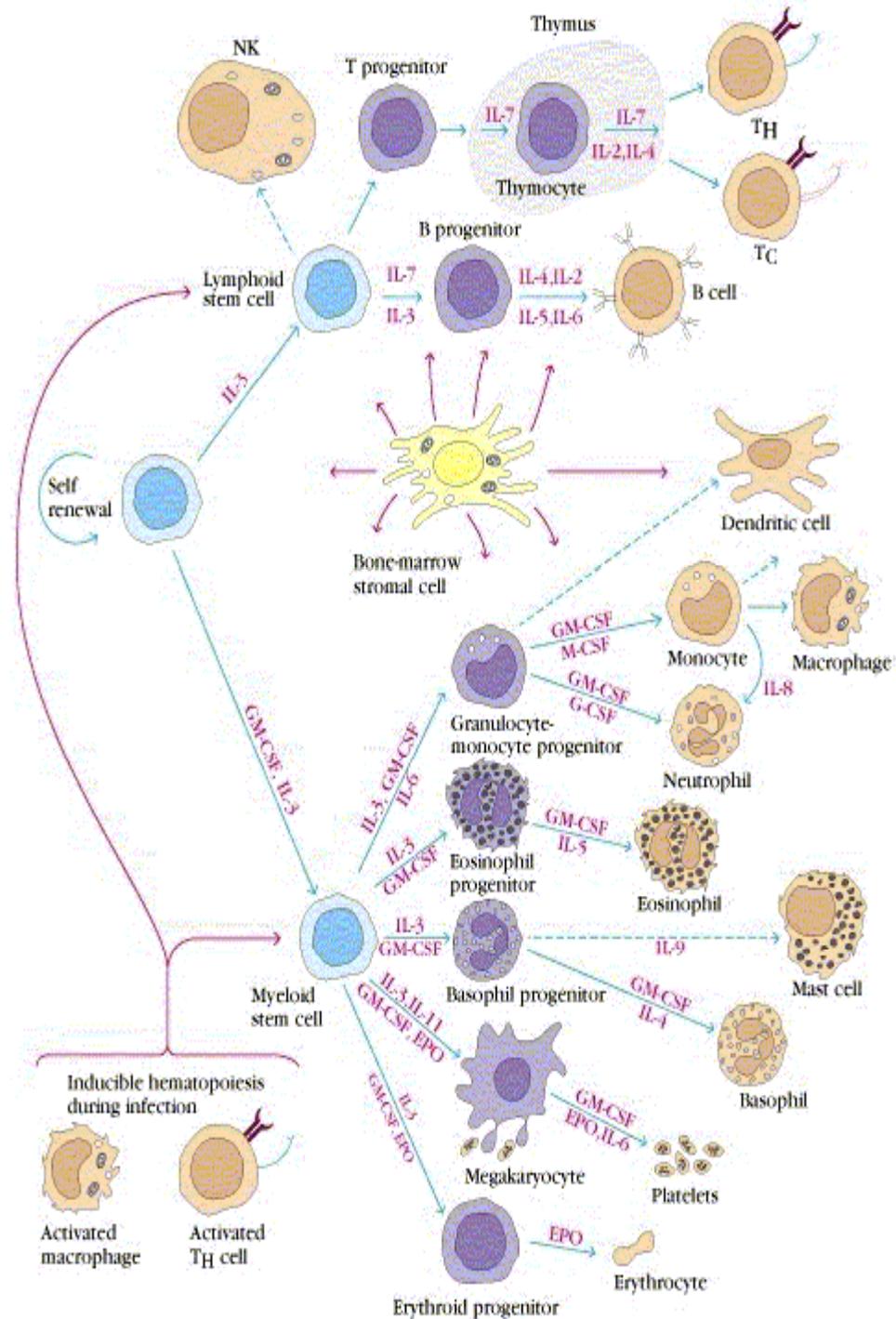
Components of Blood

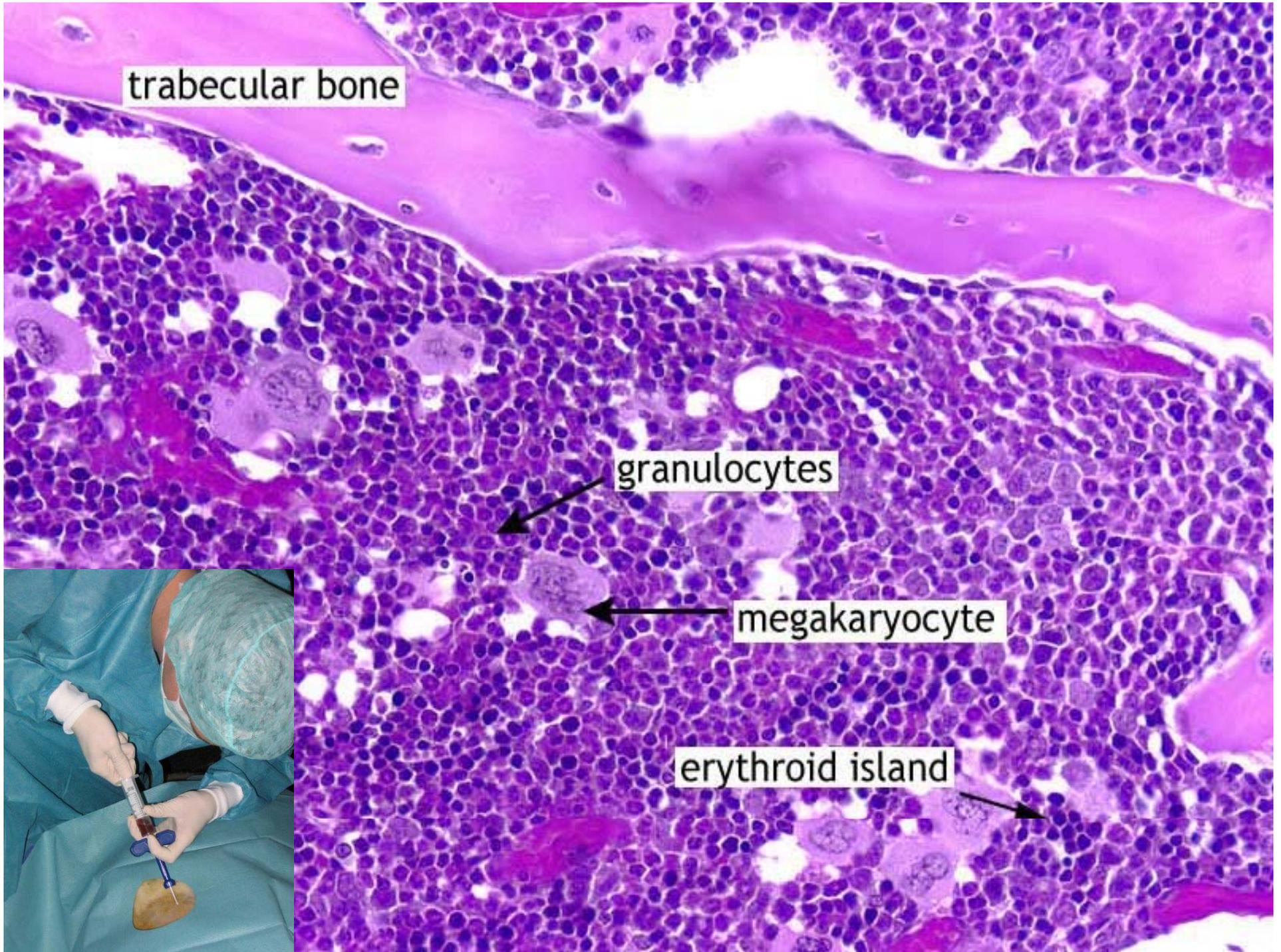


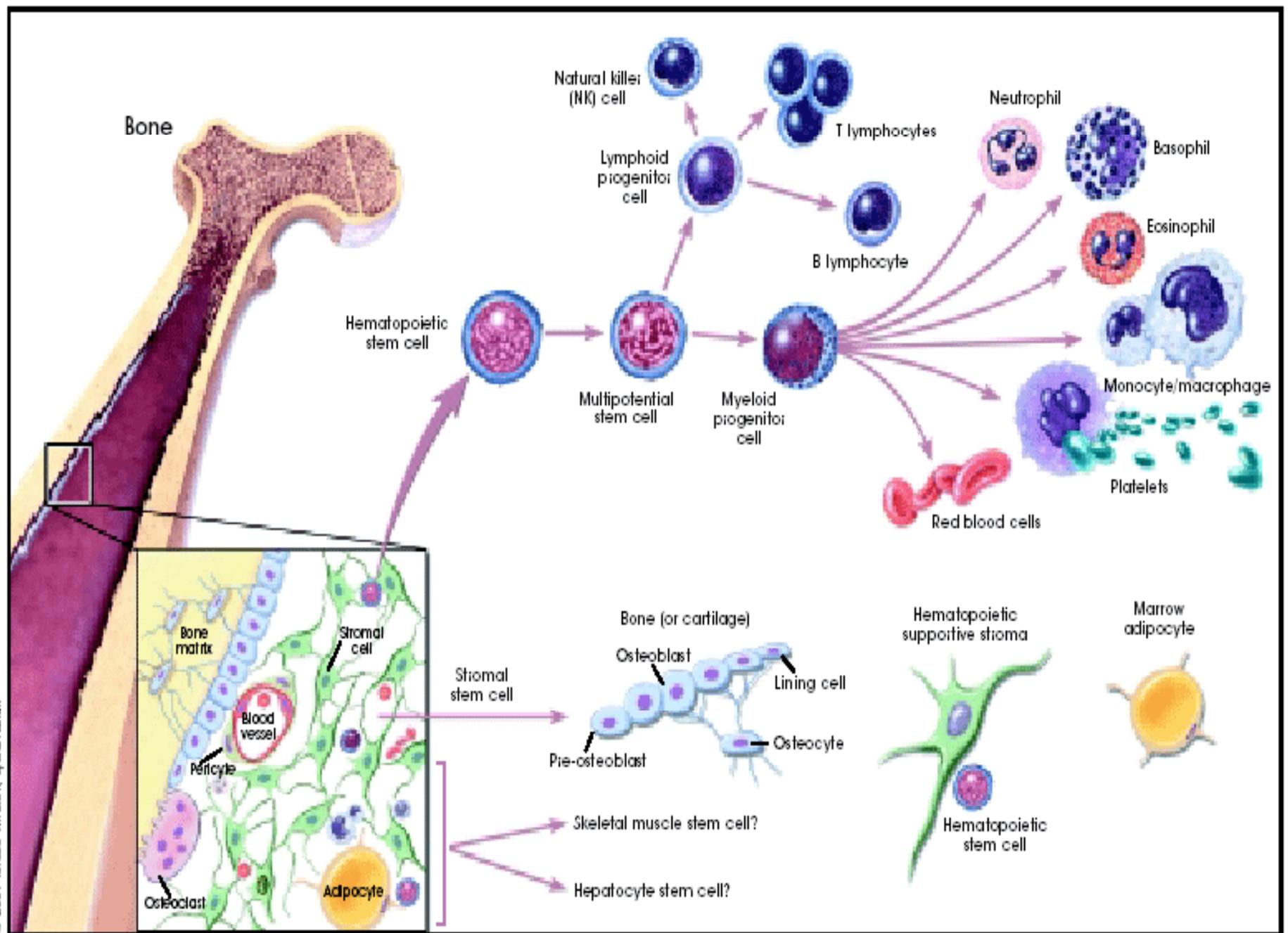
- - average adult has about 5 liters (about 5 qts):
- 1 - Formed elements:
 - Red blood cells (or erythrocytes)
 - White blood cells (or leucocytes)
 - Platelets (or thrombocytes)
- 2 - Plasma = water + dissolved solutes

Formed elements: ORIGIN

- RED Bone MARROW :
HEMATOPOIESIS

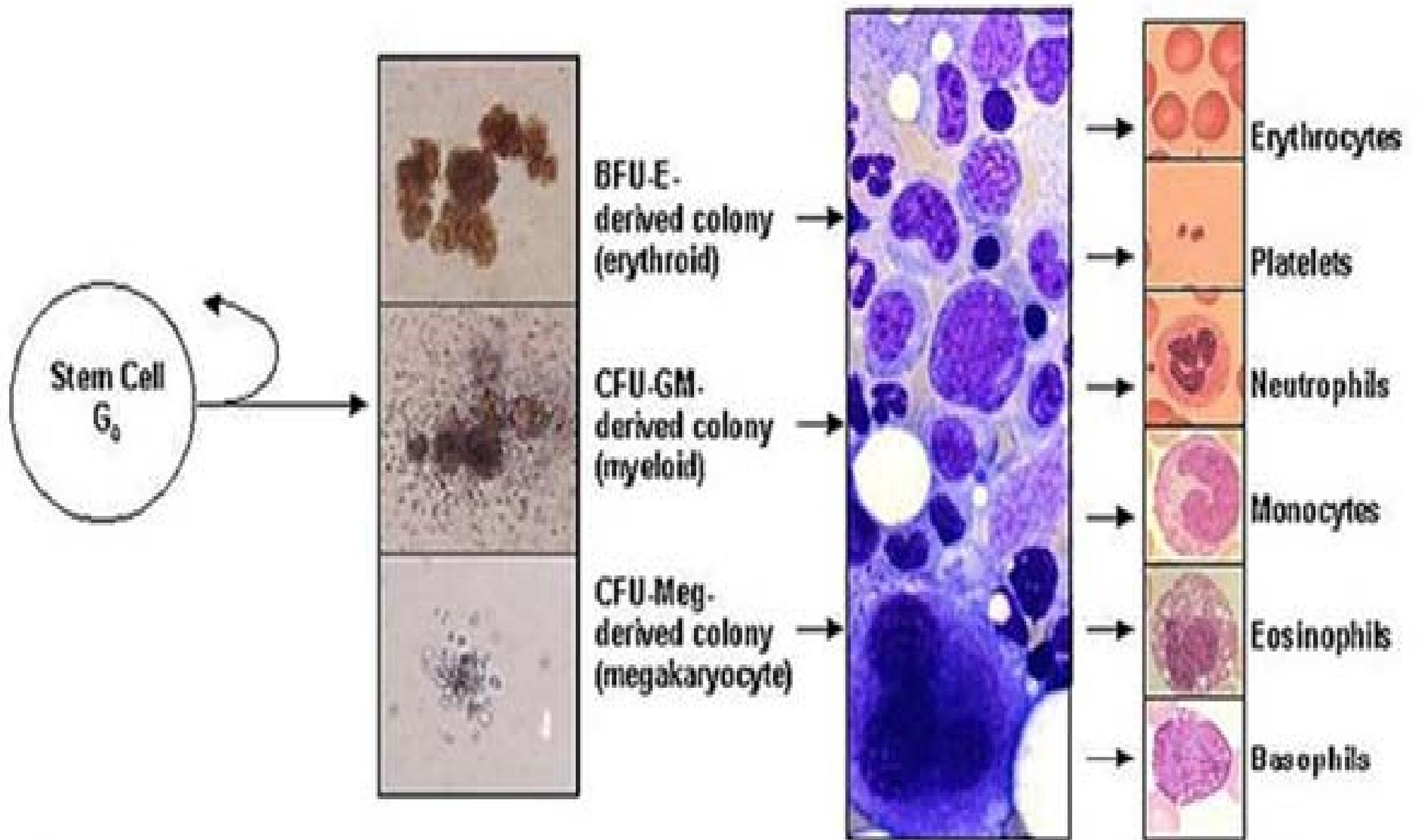






© 2001 Terese Winslow, Lydia Klauk

Figure 4.3. Hematopoietic and Stromal Stem Cell Differentiation.



Stem cell	Progenitor cells	Precursor cells	Mature cells
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Functions of Blood:

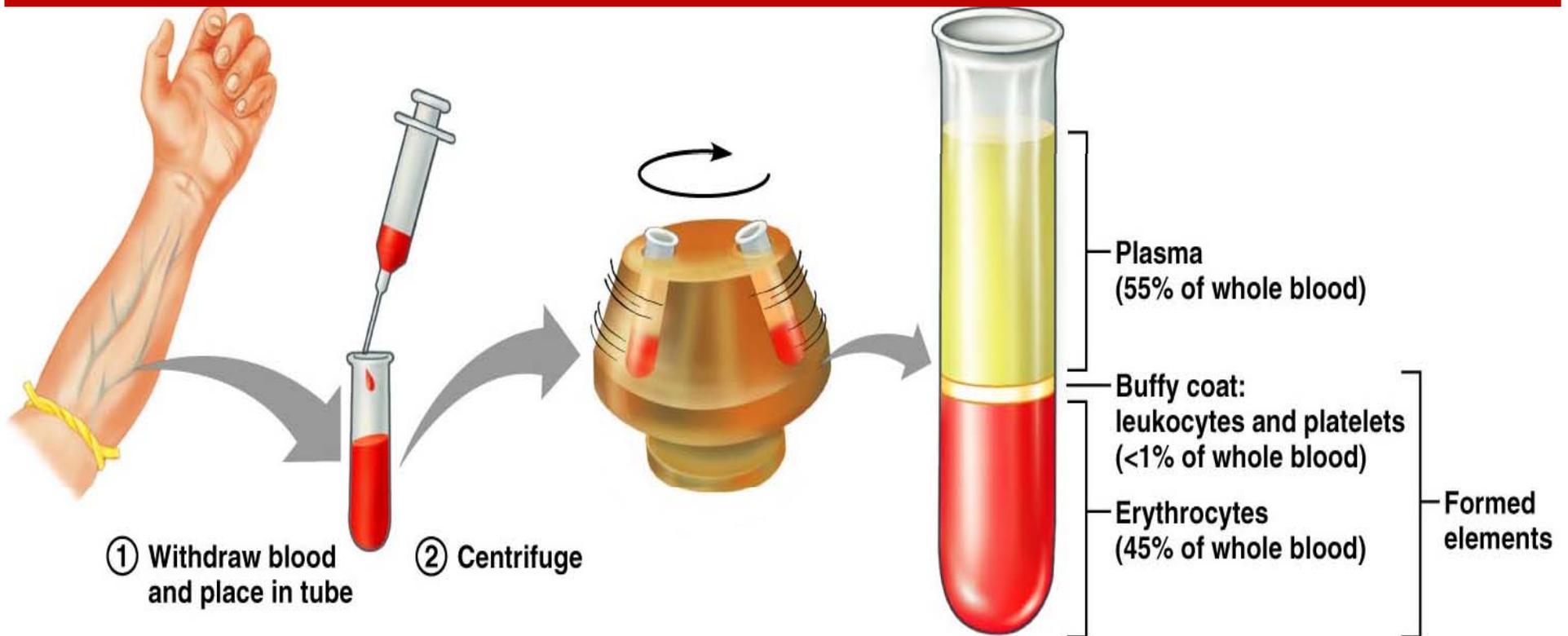
- 1 - Transportation:
 - oxygen & carbon dioxide
 - nutrients
 - waste products (metabolic wastes, excessive water, & ions)
- 2 - Regulation - hormones & heat (to regulate body temperature)
- 3 - Protection - clotting mechanism protects against blood loss & leucocytes provide immunity against many disease-causing agents

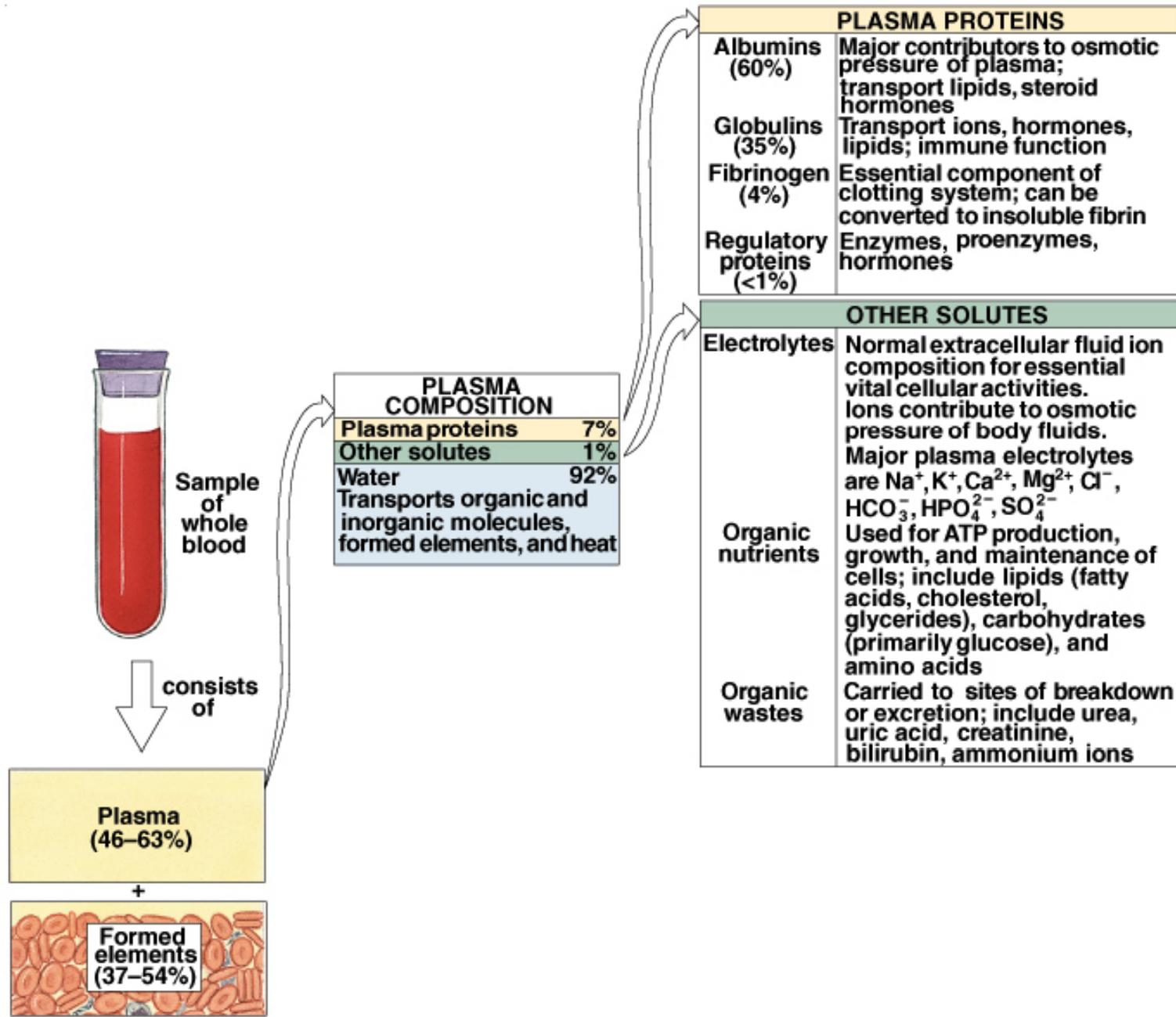
- In human adults about 5 liter of blood contribute 7-8 % to the body weight of the individual.
- The contribution of red blood cells (erythrocytes) to the total volume of the blood (hematocrit) is about 43%.
- Erythrocytes are the dominant (99%) but not the only type of cells in the blood.
- . Erythrocytes, leukocytes and blood platelets are also being referred to as the formed elements of the blood.
- Erythrocytes and blood platelets perform their functions exclusively in the blood stream.
- In contrast, leukocytes reside only temporarily in the blood.
- Leukocytes can leave the blood stream through the walls of capillaries and venules and enter either connective or lymphoid tissues.

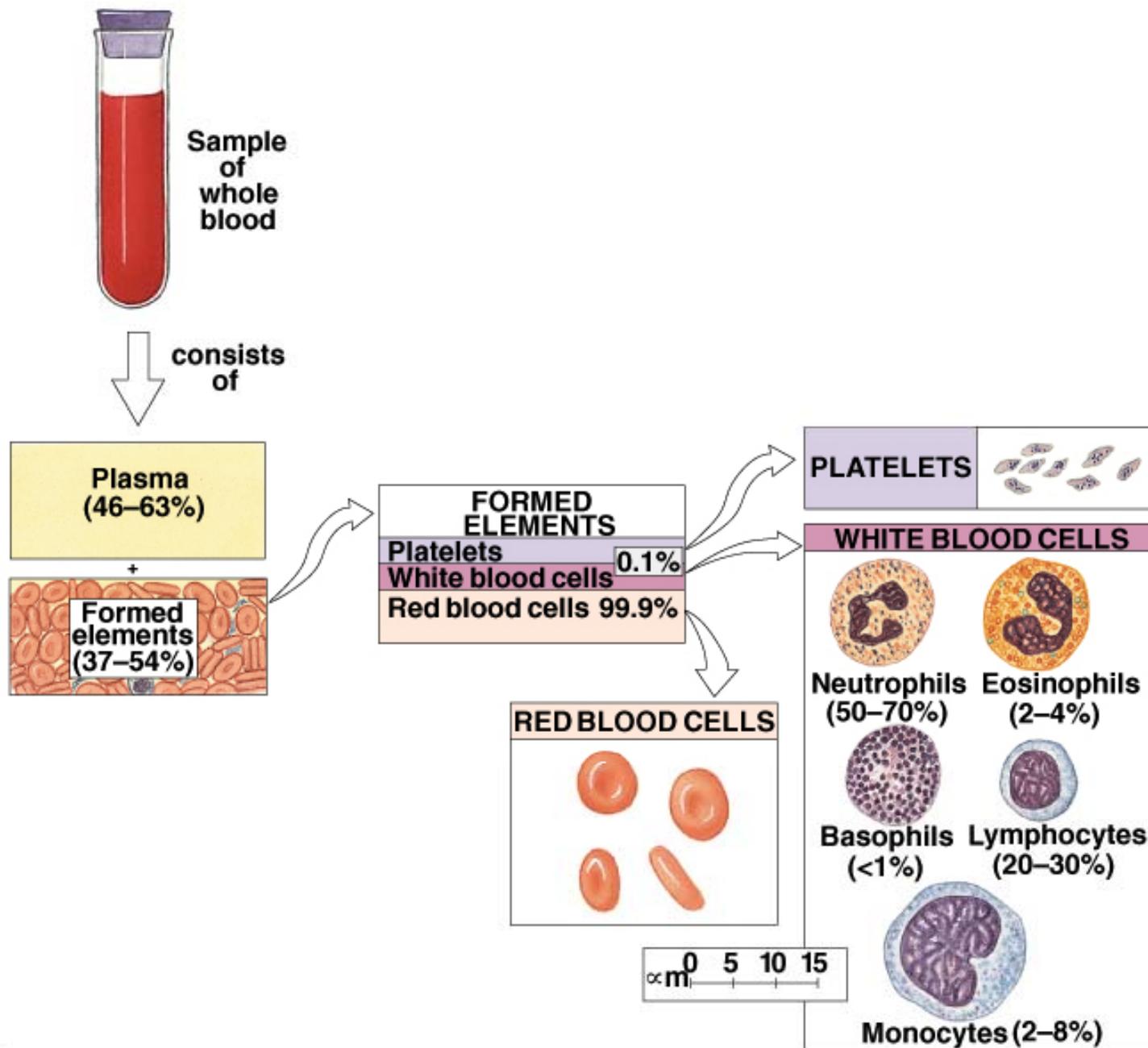
Components of Whole Blood

Whole blood is a living tissue that circulates through the heart, arteries, veins, and capillaries carrying nourishment, electrolytes, hormones, vitamins, antibodies, heat, and oxygen to the body's tissues.

Whole blood contains red blood cells, white blood cells, and platelets suspended in a fluid called plasma.







Blood Plasma

- **Blood plasma contains over 100 solutes, including:**
 - Proteins – albumin, globulins, clotting proteins, and others
 - Lactic acid, urea, creatinine
 - Organic nutrients – glucose, carbohydrates, amino acids
 - Electrolytes – sodium, potassium, calcium, chloride, bicarbonate
 - Respiratory gases – oxygen and carbon dioxide

Plasma

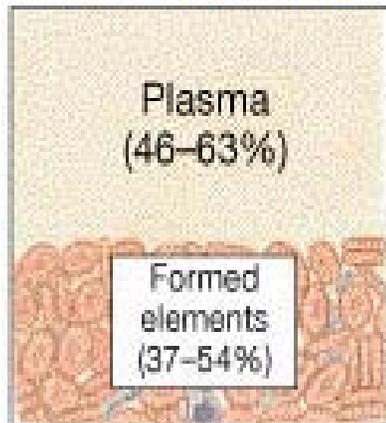
- it's a straw-colored, clear liquid that is 90 percent water, and it is an essential ingredient for human survival.
- It might seem like plasma is less important than the blood cells it carries. But that would be like saying that the stream is less important than the fish that swims in it. You can't have one without the other.
- Besides water, plasma also contains dissolved salts and minerals like calcium, sodium, magnesium, and potassium. Microbe-fighting antibodies travel to the battlefields of disease by hitching a ride in the plasma.
- Without plasma, the life-giving blood cells would be left floundering without transportation. Never underestimate the importance of plasma.



Sample of whole blood



Contains

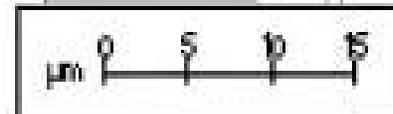
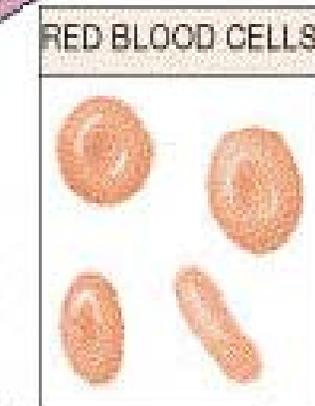
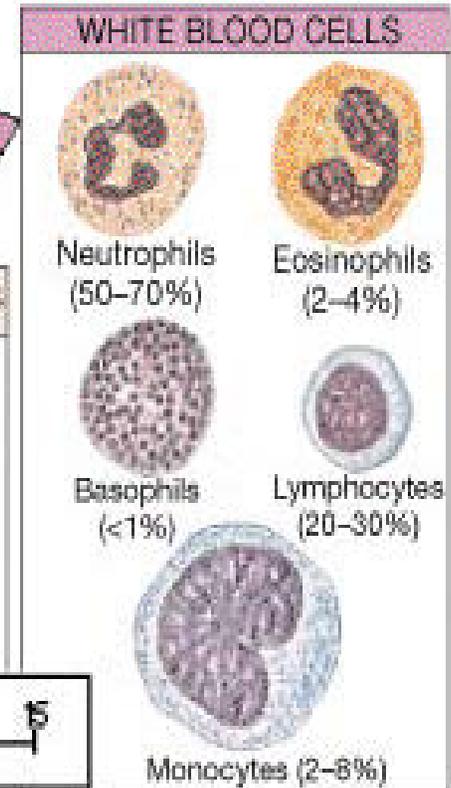


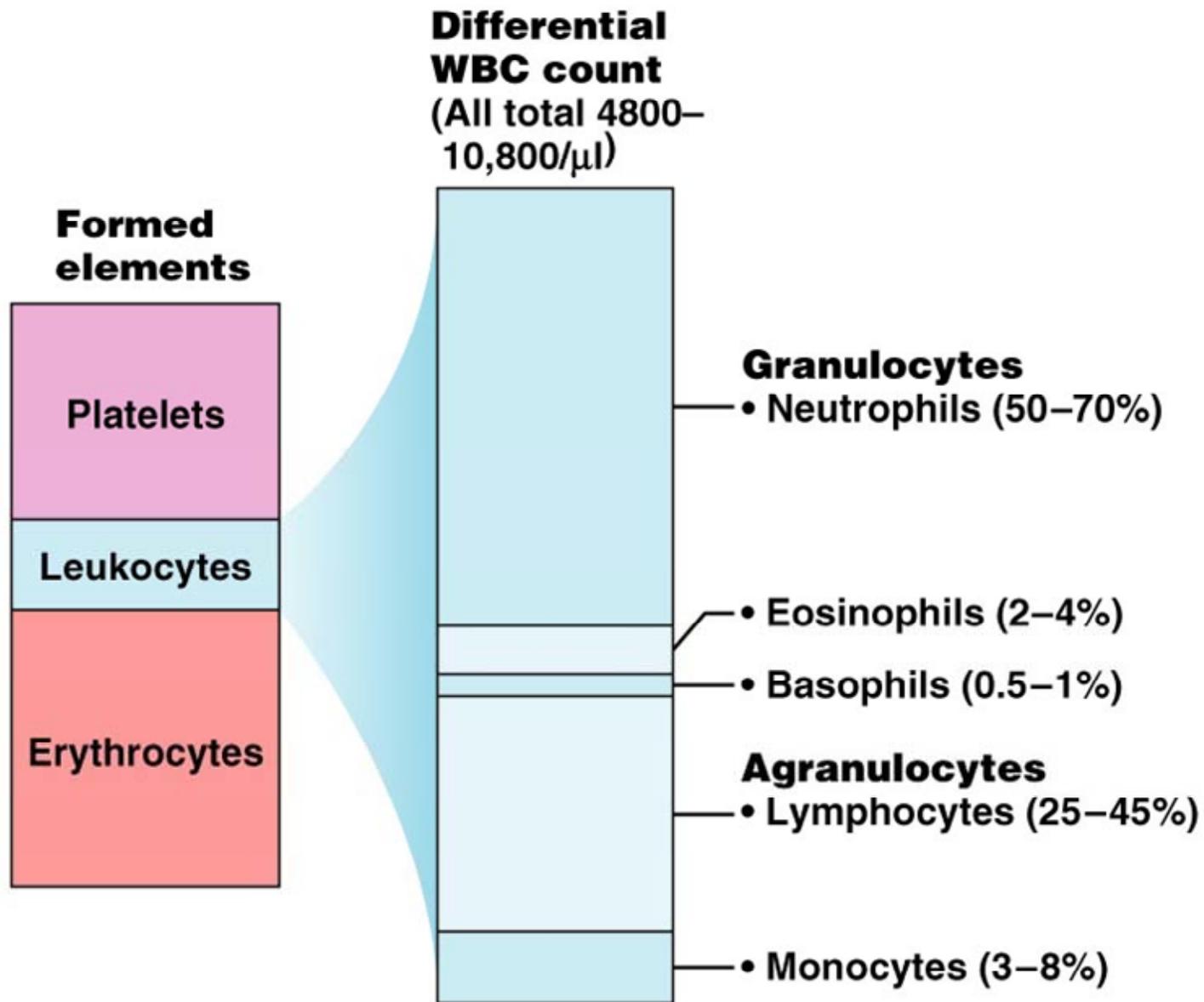
OTHER SOLUTES	
Electrolytes	Normal extracellular fluid ion composition essential for vital cellular activities. Ions contribute to osmotic pressure of body fluids. Major plasma electrolytes are Na^+ , K^+ , Ca^{2+} , Mg^{2+} , Cl^- , HCO_3^- , HPO_4^{2-} , SO_4^{2-}
Organic nutrients	Used for ATP production, growth, and maintenance of cells; include lipids (fatty acids, cholesterol, glycerides), carbohydrates (primarily glucose), and amino acids
Organic wastes	Carried to sites of breakdown or excretion; include urea, uric acid, creatinine, bilirubin, ammonium ions

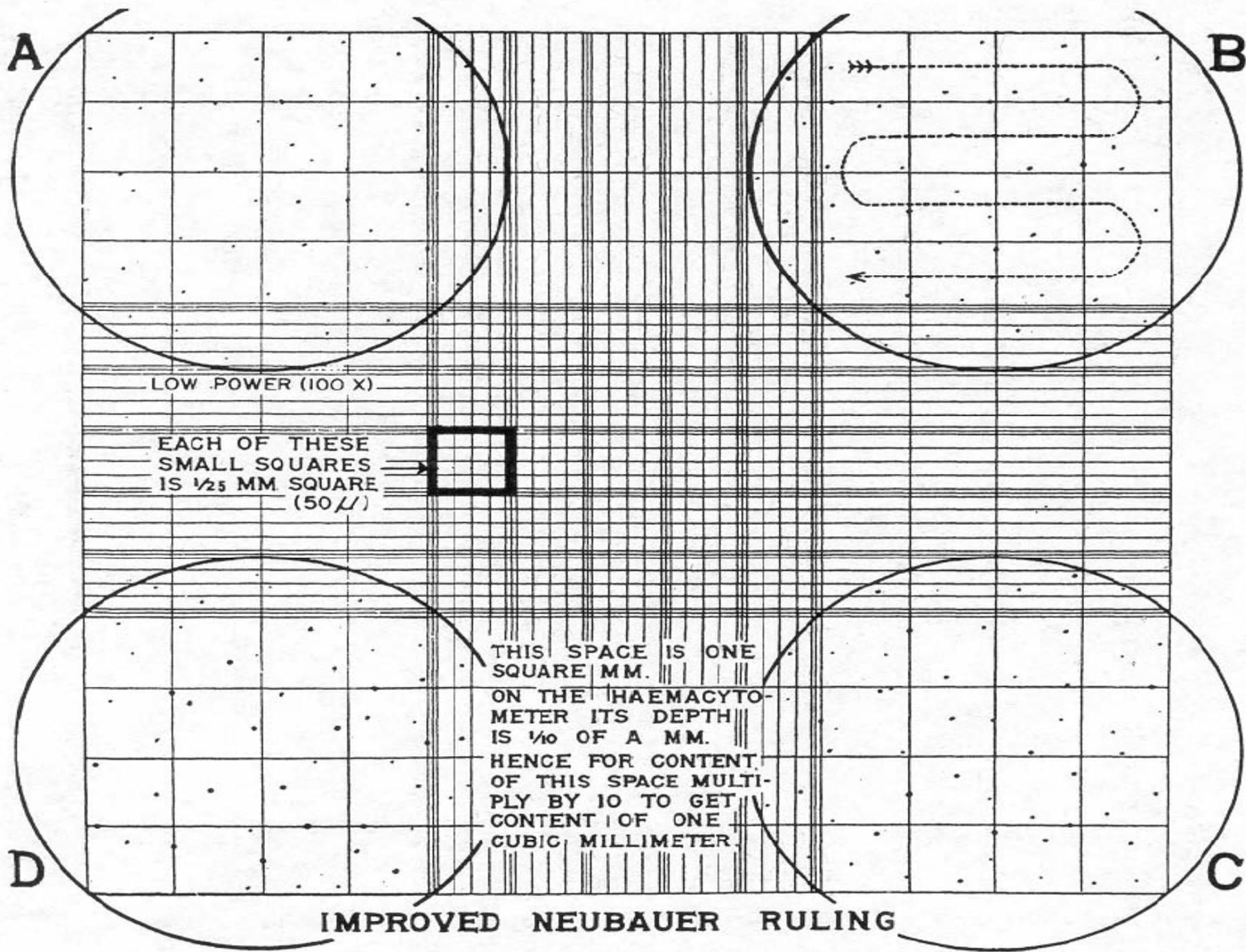
PLASMA PROTEINS	
Albumins (60%)	Major contributors to osmotic concentration of plasma; transport lipids, steroid hormones
Globulins (35%)	Transport ions, hormones, lipids; immune function
Fibrinogen (4%)	Essential component of clotting system; can be converted to insoluble fibrin
Regulatory proteins (<1%)	Enzymes, proenzymes, hormones

PLASMA COMPOSITION	
Plasma proteins	7%
Other solutes	1%
Water	92%
Transports organic and inorganic molecules, formed elements, and heat	

FORMED ELEMENTS	
Platelets	0.1%
White blood cells	
Red blood cells	99.9%







LOW POWER (100 X)

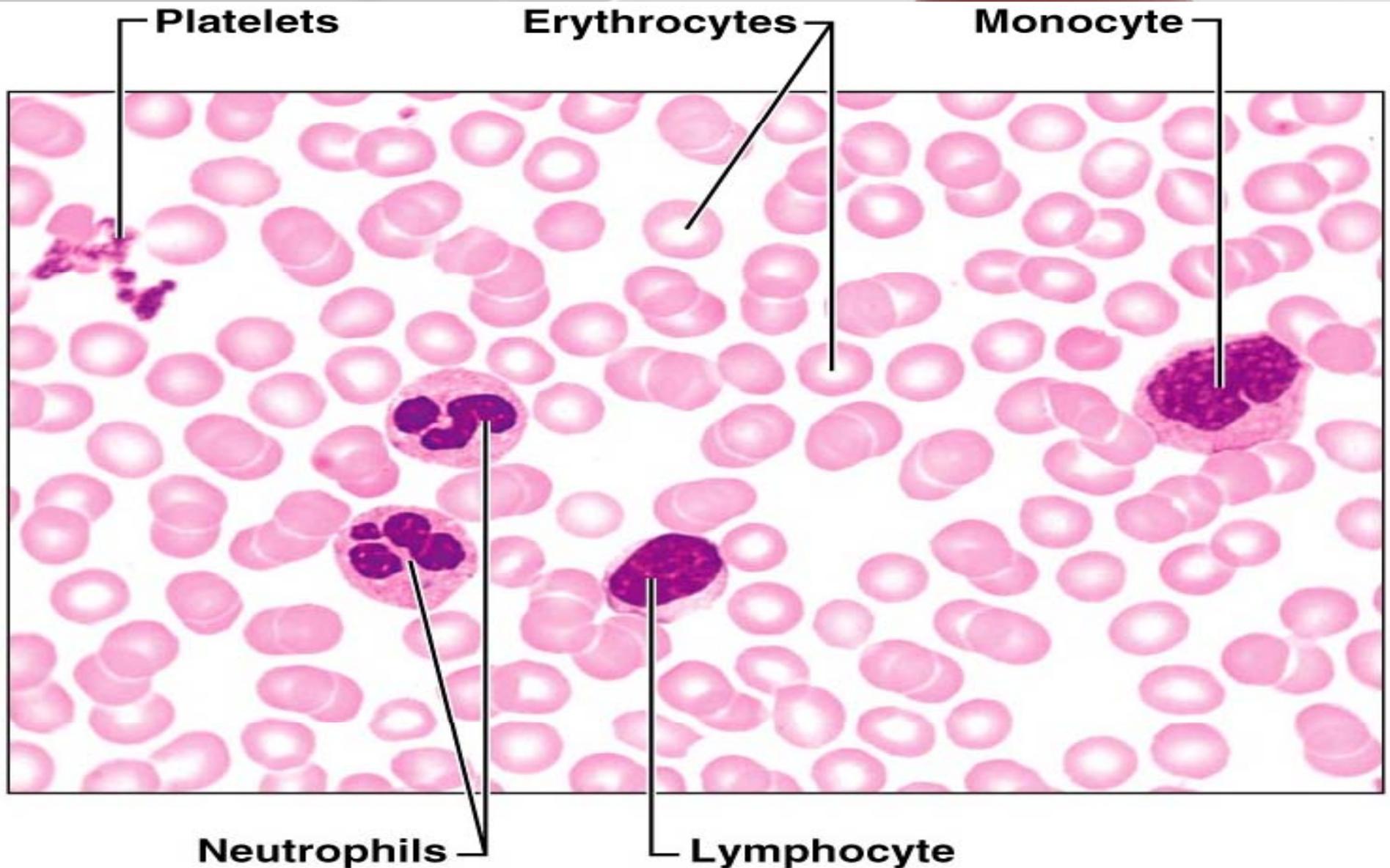
EACH OF THESE
SMALL SQUARES
IS $\frac{1}{25}$ MM SQUARE
(50 μ)



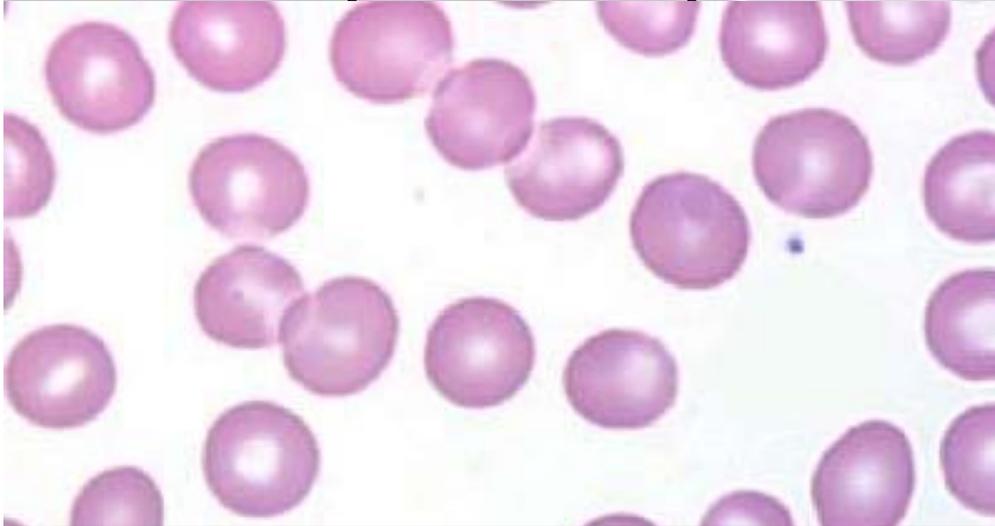
THIS SPACE IS ONE
SQUARE MM.
ON THE HAEMACYTO-
METER ITS DEPTH
IS $\frac{1}{10}$ OF A MM.
HENCE FOR CONTENT
OF THIS SPACE MULTI-
PLY BY 10 TO GET
CONTENT OF ONE
CUBIC MILLIMETER.

IMPROVED NEUBAUER RULING

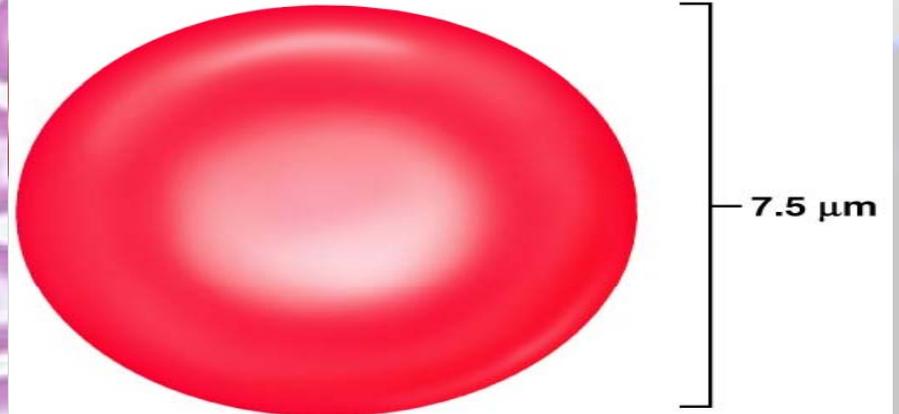
Components of Whole Blood



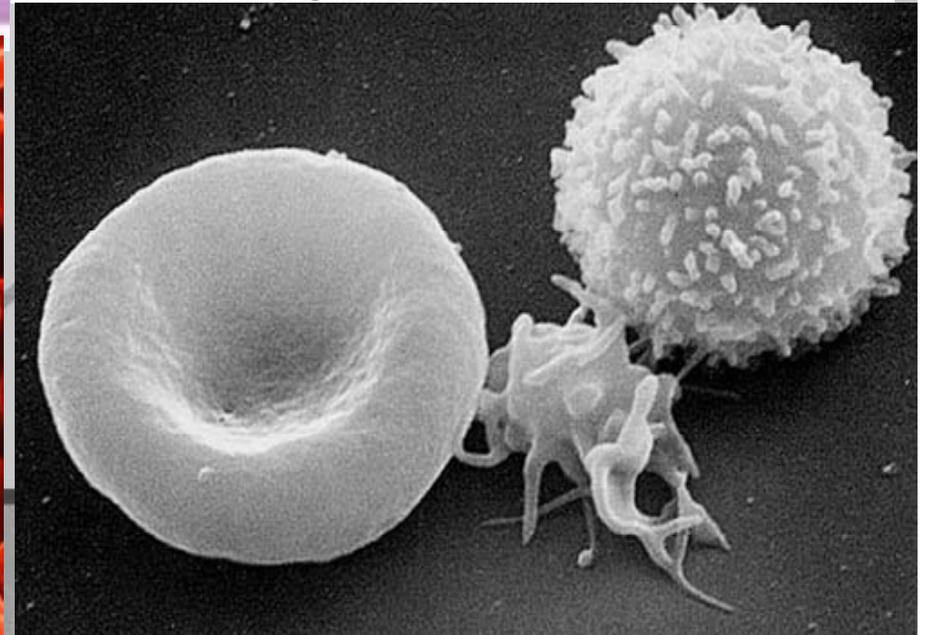
Erythrocytes (RBCs)

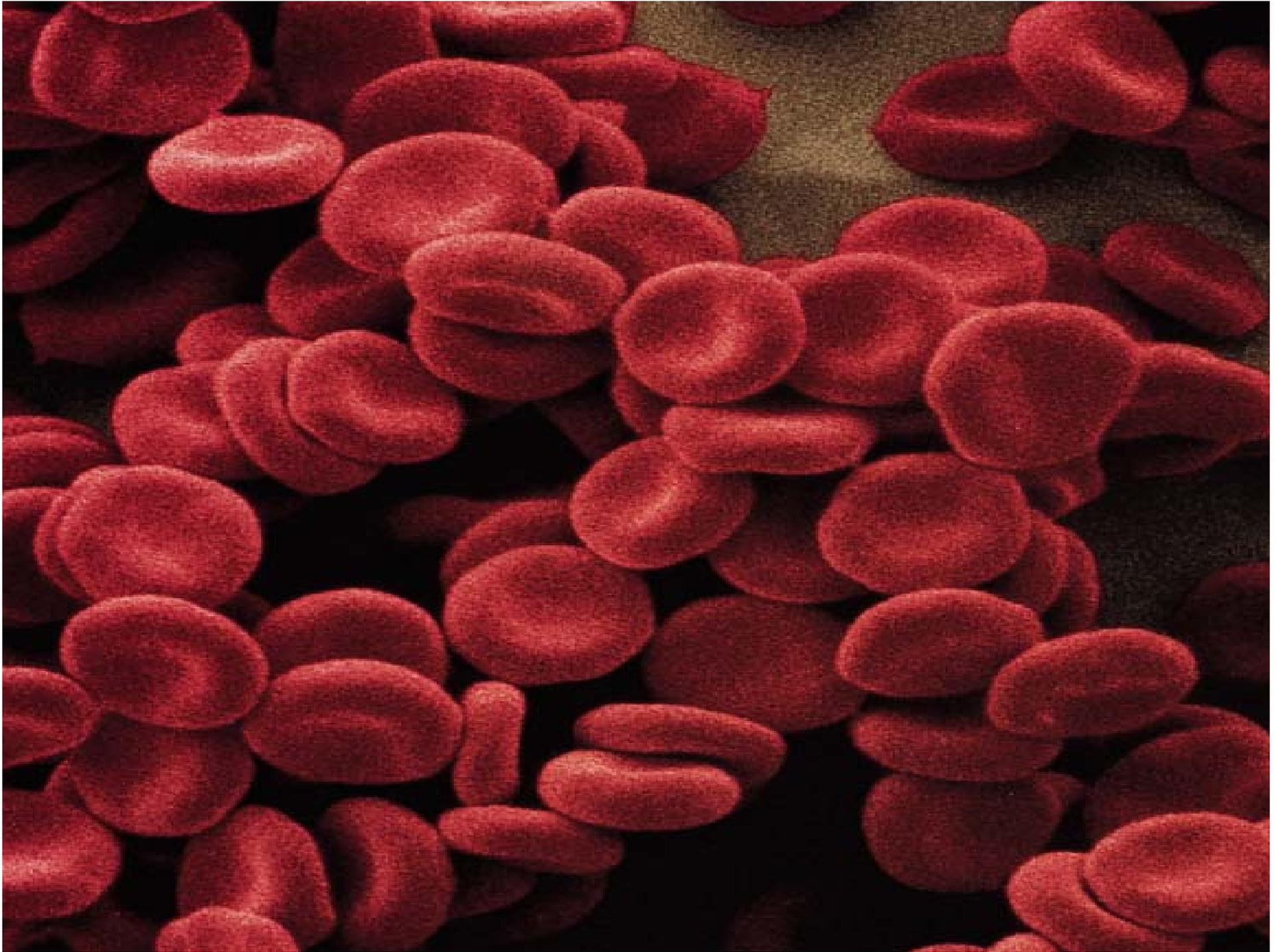


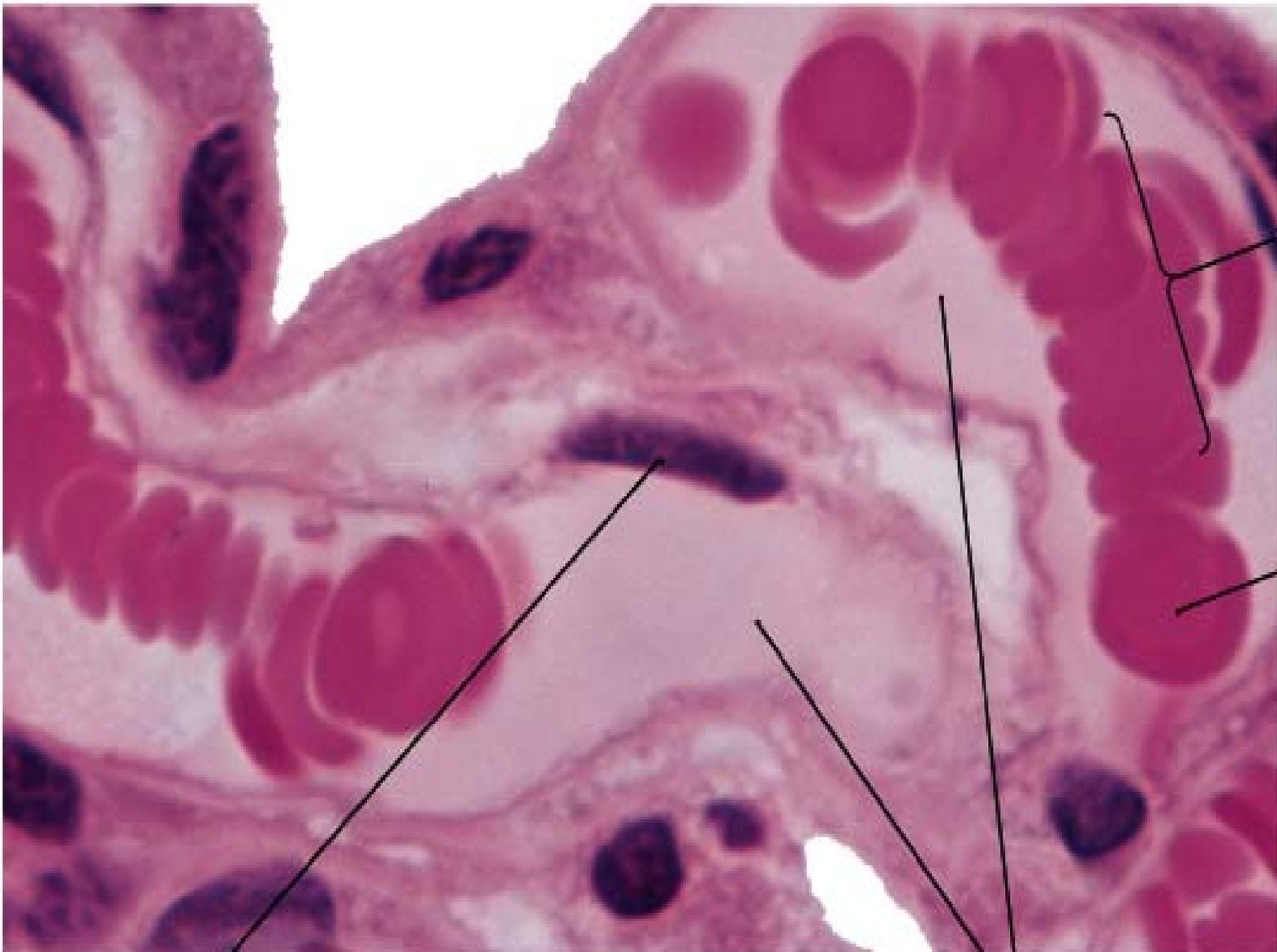
Side view



Top view





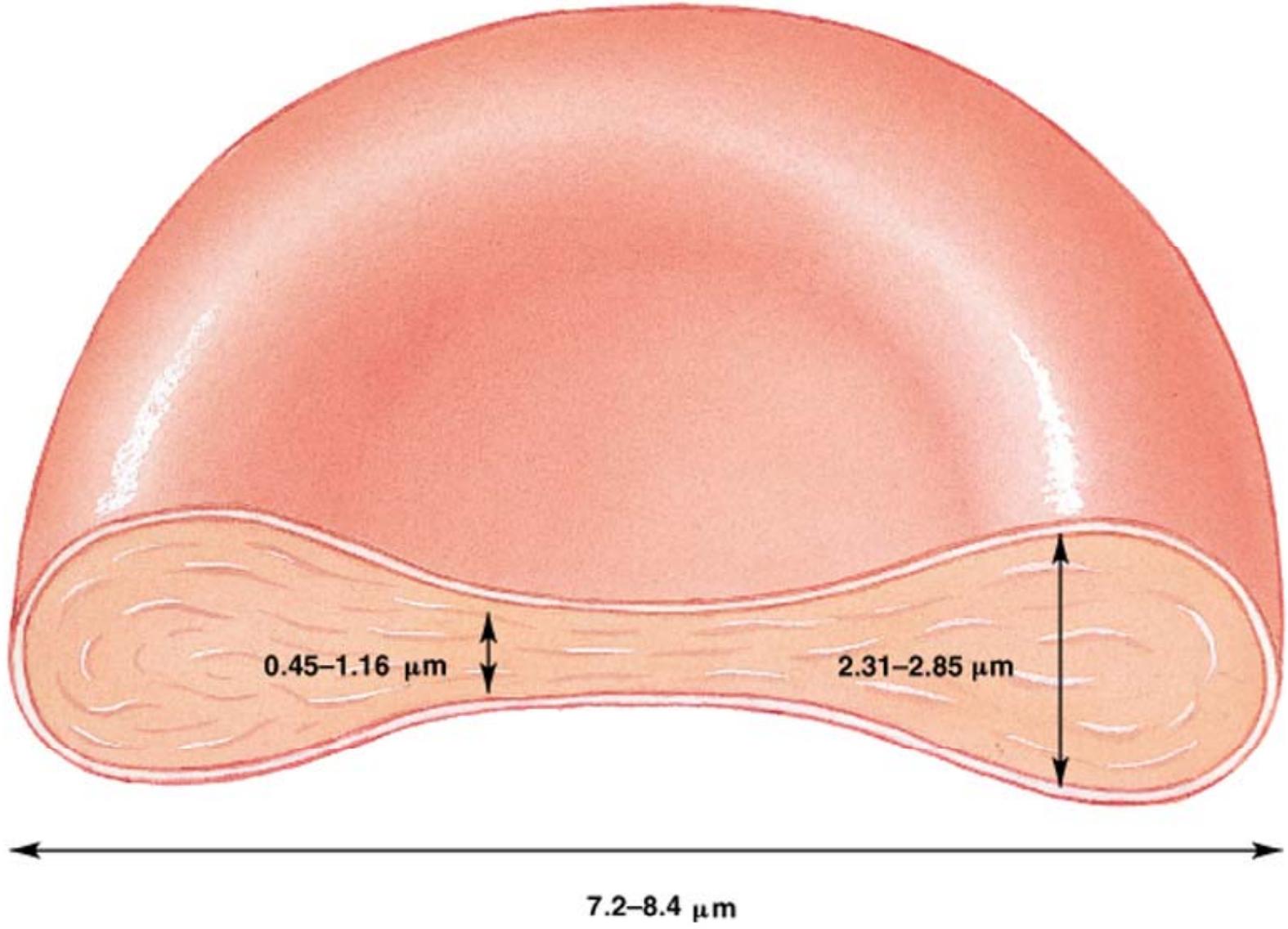


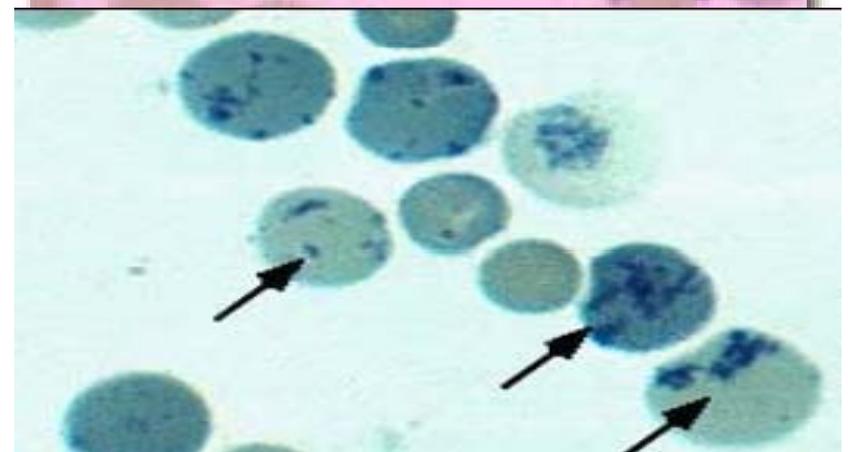
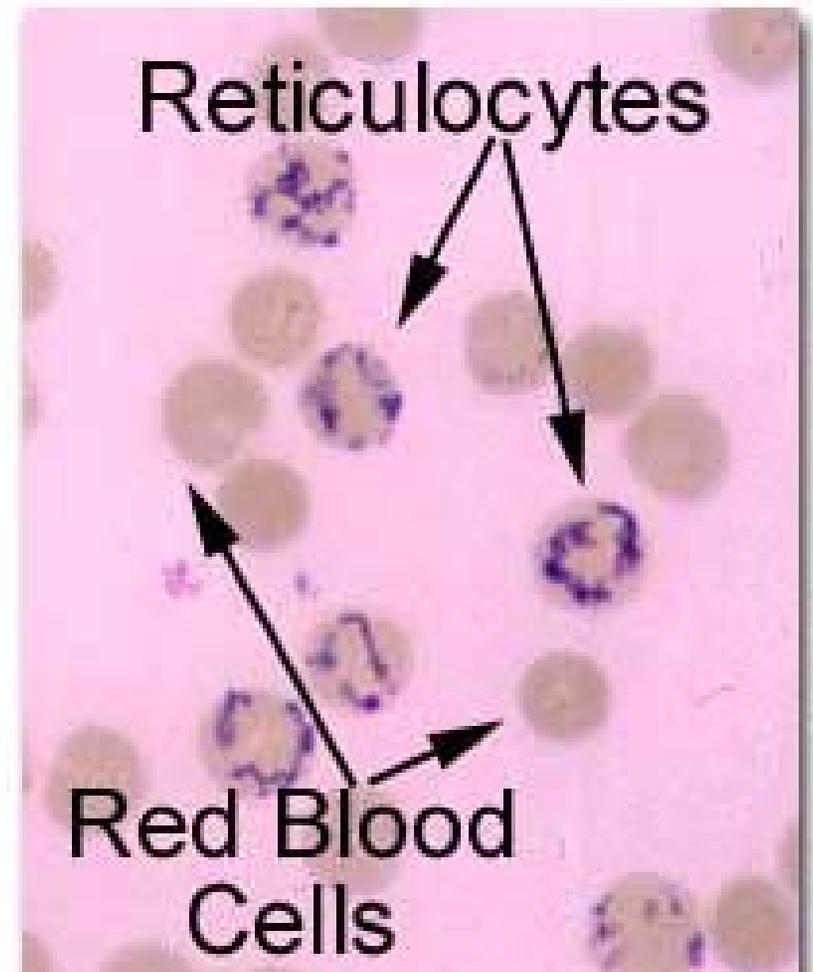
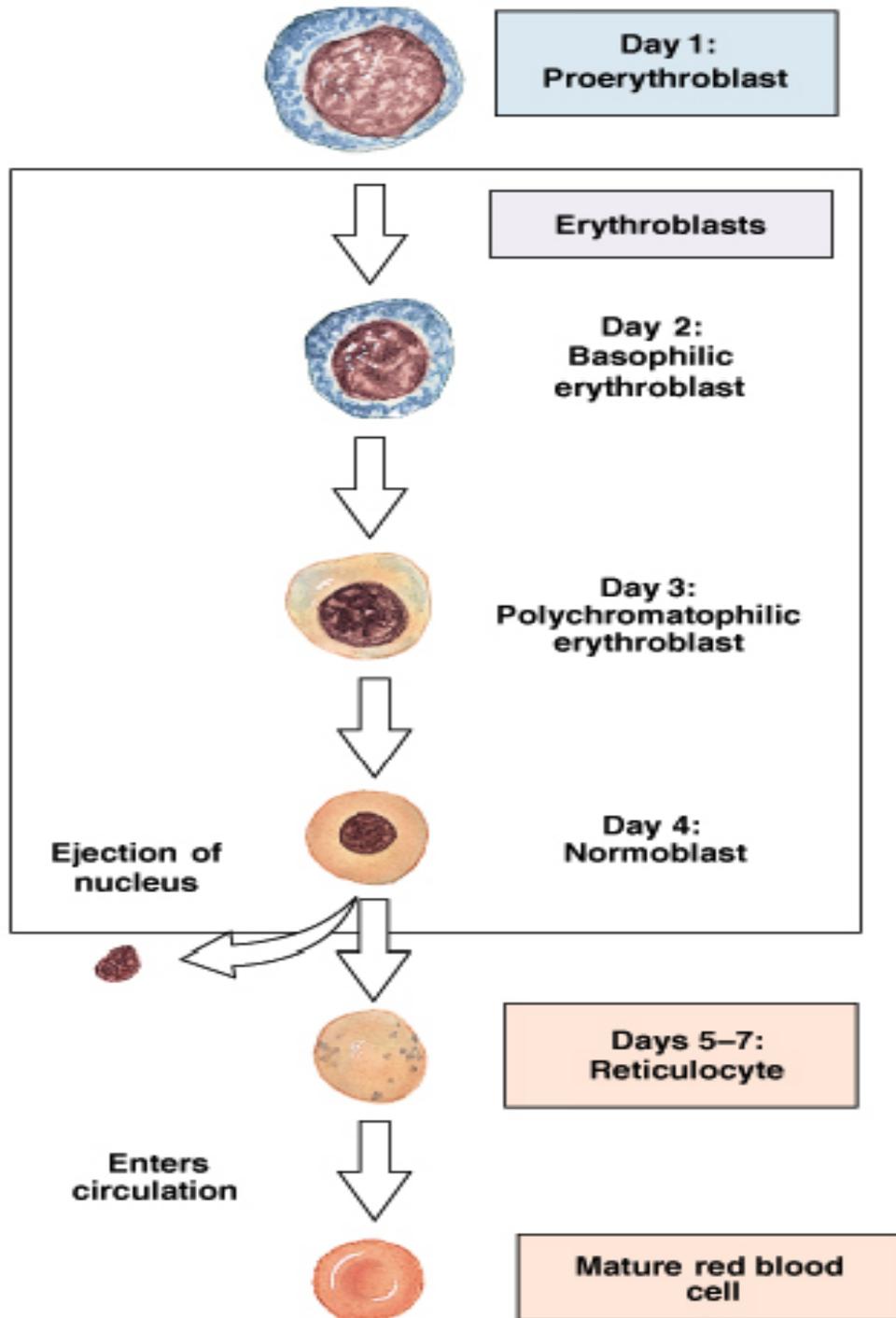
Stacked RBCs

Red blood cell (RBC)

Nucleus of endothelial cell

Blood vessels (viewed in longitudinal section)

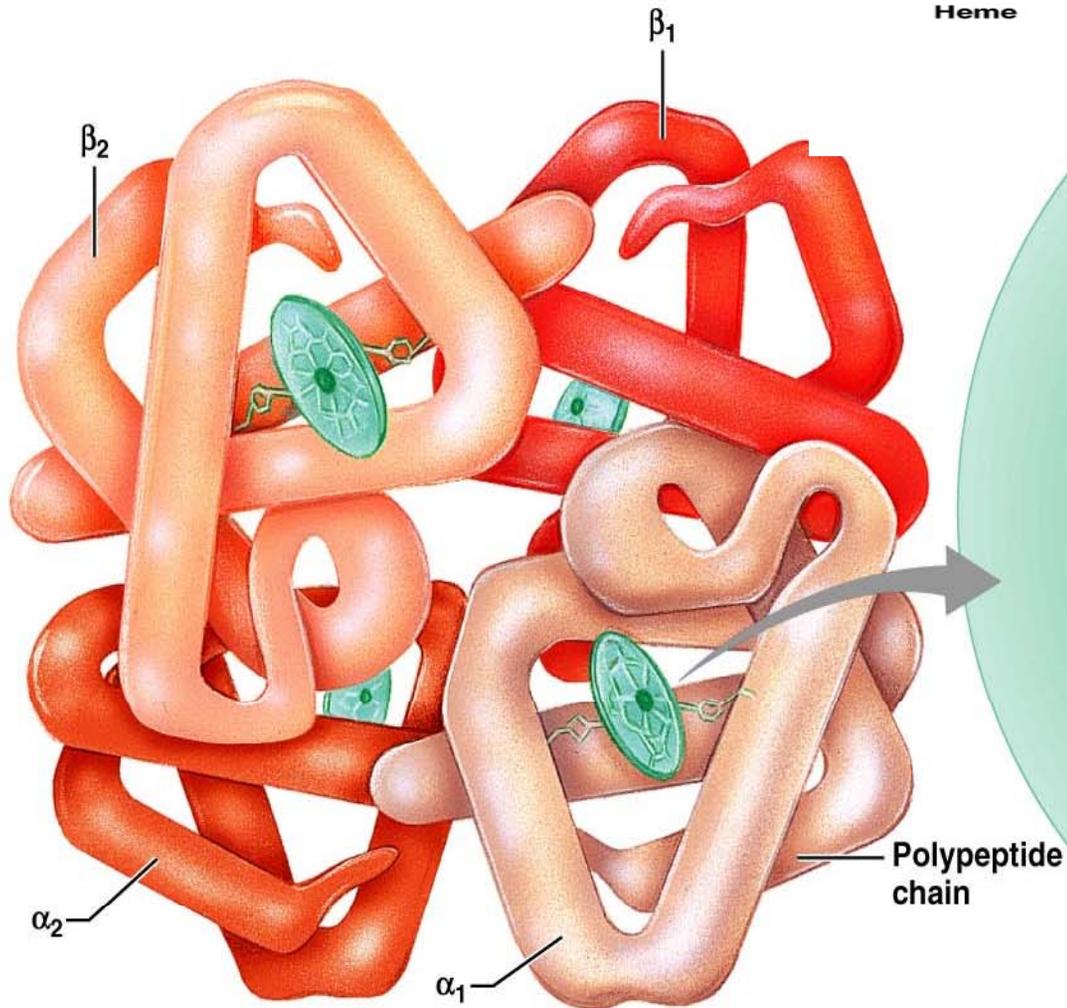
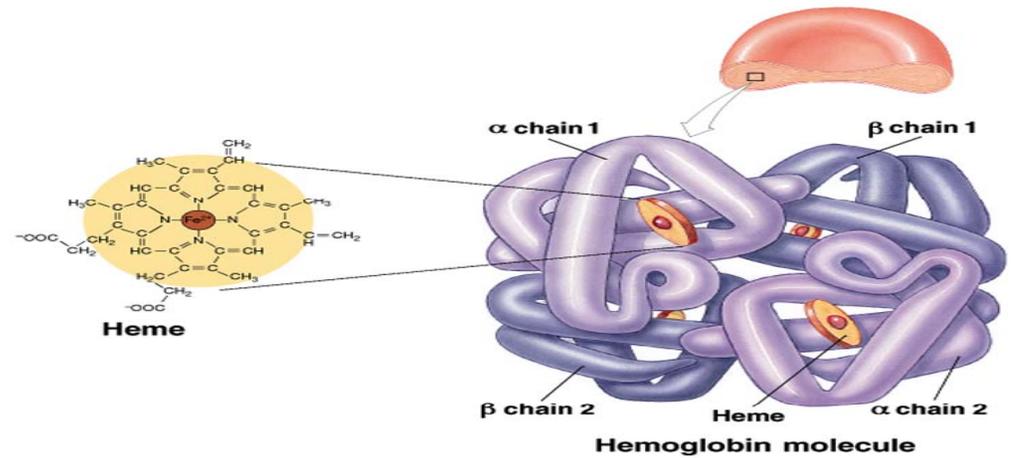




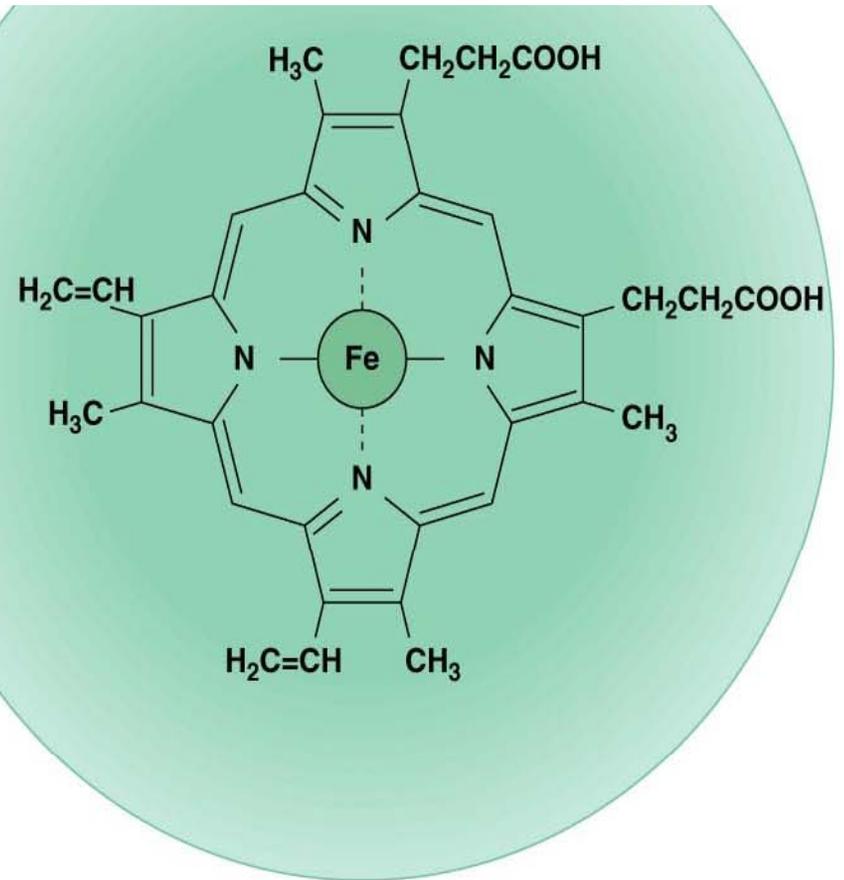
Erythrocyte Function

- RBCs are dedicated to respiratory gas transport
- Hb reversibly binds with oxygen and most oxygen in the blood is bound to Hb
- Hb is composed of the protein globin, made up of two alpha and two beta chains, each bound to a heme group
- Each heme group bears an atom of iron, which can bind to one oxygen molecule
- Each Hb molecule can transport four molecules of oxygen

Structure of Hemoglobin



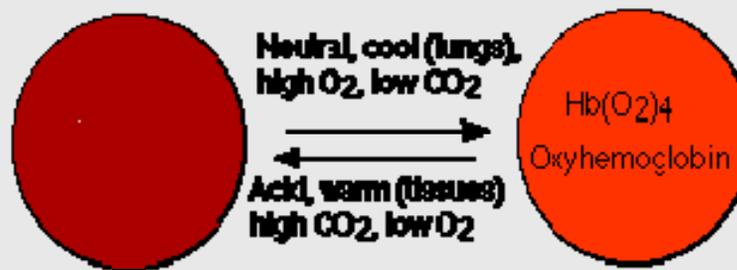
(a) Hemoglobin



(b) Iron-containing heme group

Hemoglobin (Hb)

- **Oxyhemoglobin** – Hb bound to oxygen
 - Oxygen loading takes place in the lungs
- **Deoxyhemoglobin** – Hb after oxygen diffuses into tissues (reduced Hb)
- **Carbaminohemoglobin** – Hb bound to carbon dioxide
 - Carbon dioxide loading takes place in the tissues



- Men: 13.5 to 16.5 g/dl
- Women: 12.1 to 15.1 g/dl
- Children: 11 to 16 g/dl
- Pregnant women: 11 to 12 g/dl

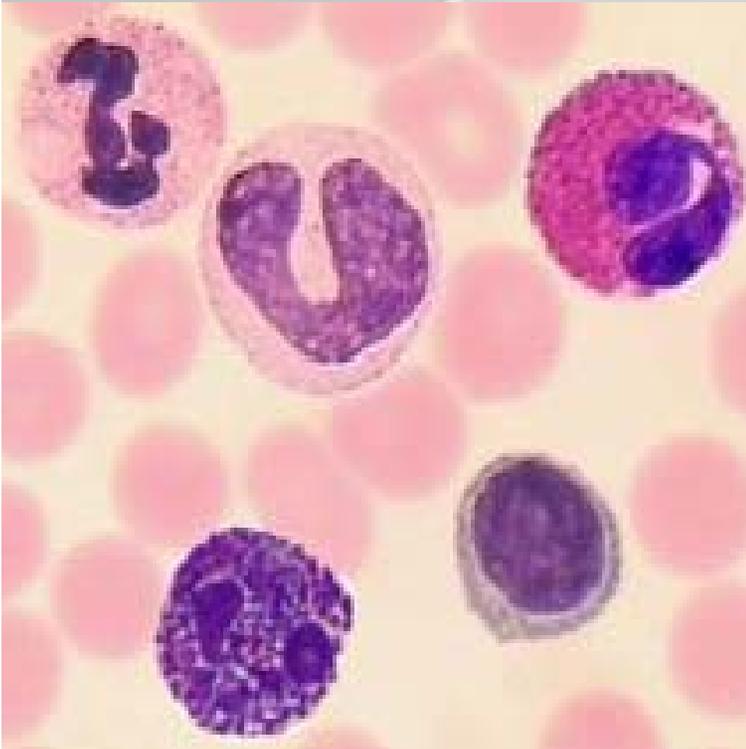
Name of Hemoglobin	Subunit Structure	Time of Expression
Hemoglobin Portland	$\zeta_2\gamma_2$	Embryonic
Hemoglobin Gower I	$\zeta_2\varepsilon_2$	Embryonic
Hemoglobin Gower II	$\alpha_2\varepsilon_2$	Embryonic
Hemoglobin F	$\alpha_2\gamma_2$	Fetal
Hemoglobin Barts	γ_4	Fetal (pathologic Hb secondary to absence of all 4 α globulin genes; fatal in utero)
Hemoglobin A₂	$\alpha_2\delta_2$	Minor adult hemoglobin
Hemoglobin A	$\alpha_2\beta_2$	Major adult hemoglobin

Granulocytes

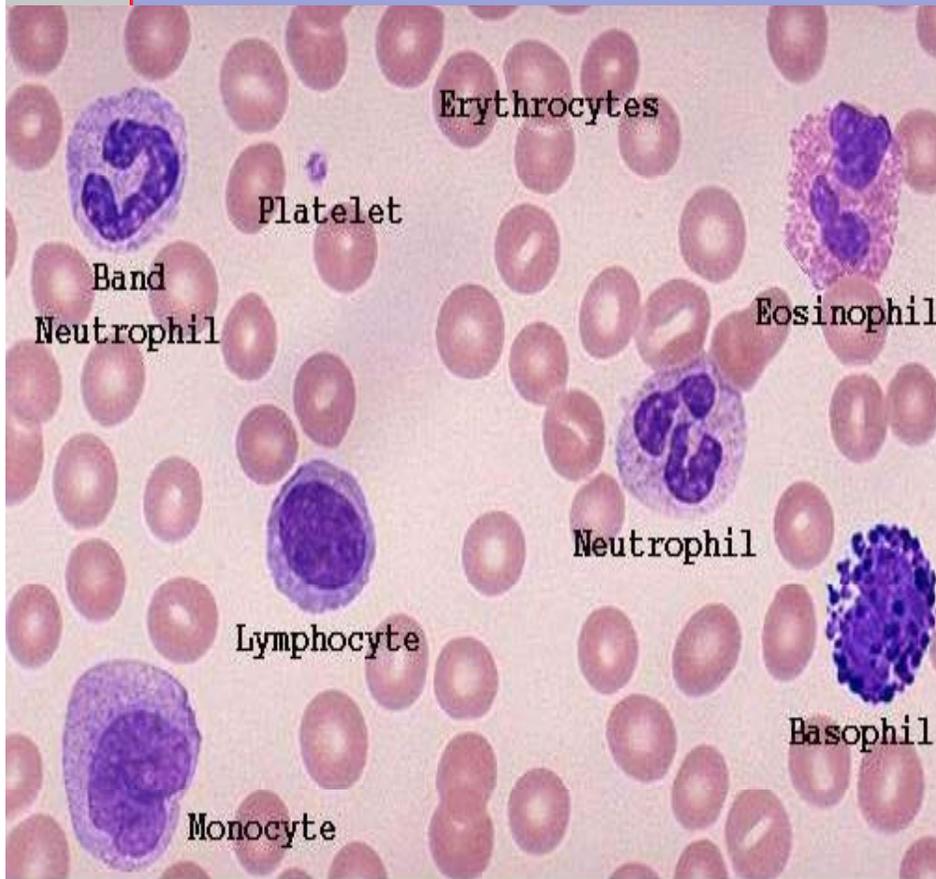
- Granulocytes –

- **neutrophils,**
- **eosinophils,**
- **and basophils**

- » Contain cytoplasmic granules that stain specifically (acidic, basic, or both) with Wright's stain
- » Are larger and usually shorter-lived than RBCs
- » Have lobed nuclei
 - » Are all phagocytic cells



White cells, leukocytes



Wright-stained smear of normal blood (x1000)
The RBC's are biconcave discs stained buff-pink, and the WBC's nucleus and cytoplasmic granules and platelet stain varying degrees of blue and pink.

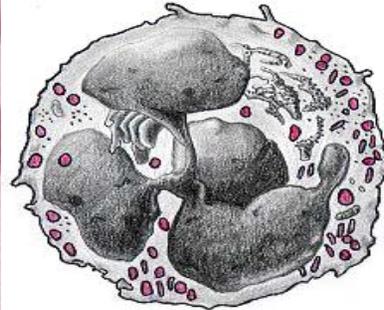


Fig. 8 - Neutrophil

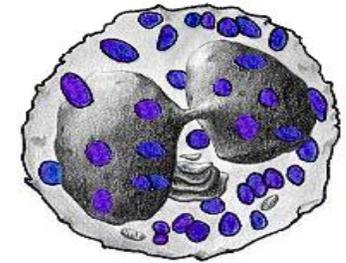


fig. 10 - Basophil

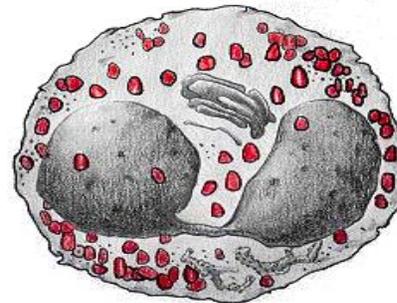


Fig. 11 - Lymphocyte

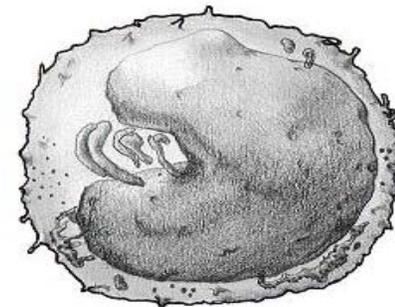
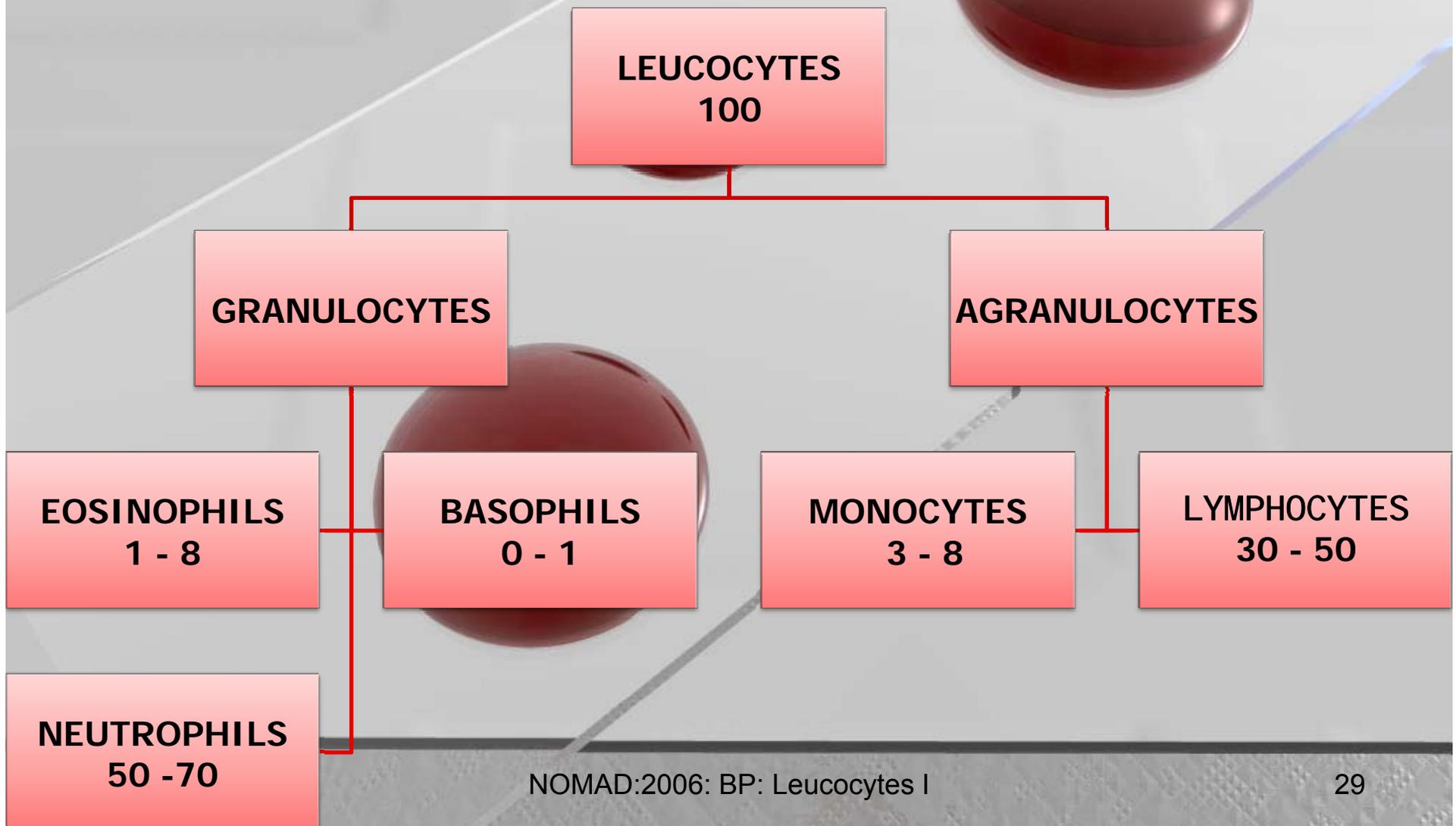


Fig. 12 - Monocyte

CLASSIFICATION OF LEUCOCYTES



WBC count: 4,500-11,000/ μ L

Polymorphonuclear neutrophils: 1800-7800/ μ L;
(50-70%)

Band neutrophils : 0-700/ μ L; (0-10%)

Lymphocytes : 1000-4800/ μ L; (15-45%)

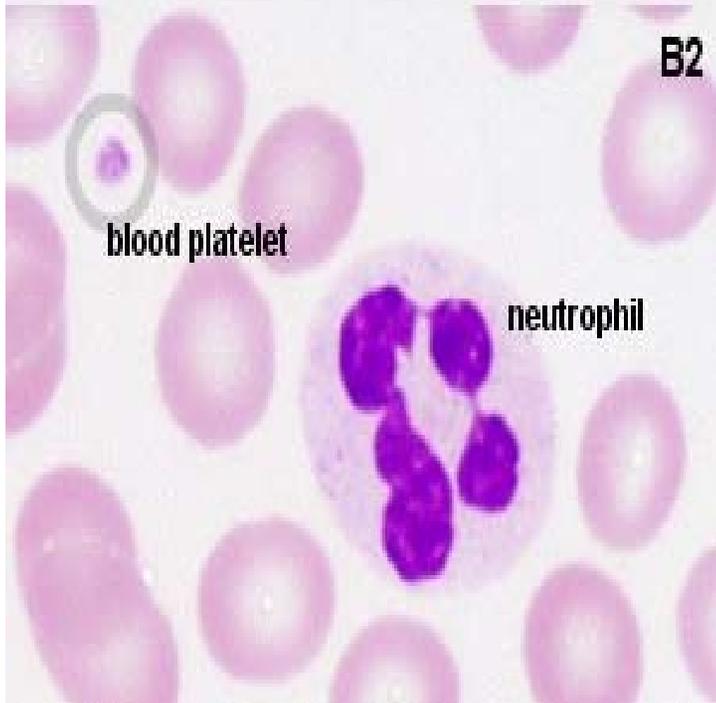
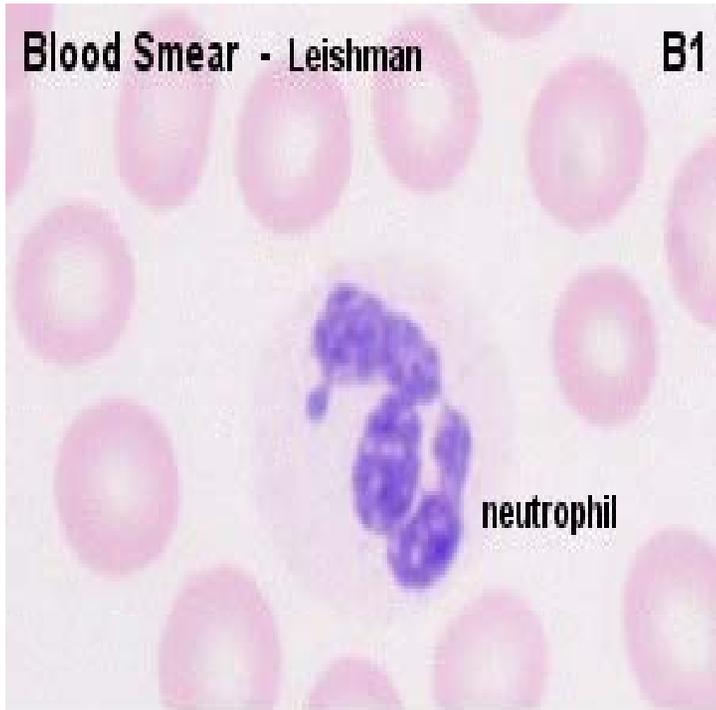
Monocytes : 0-800/ μ L; (0-10%)

Eosinophils : 0-450/ μ L; (0-6%)

Basophils : 0-200/ μ L; (0-2%)

Blood Smear - Leishman

B1



B2

Neutrophils



- Neutrophils have two types of granules that:
 - Take up both acidic and basic dyes
 - Give the cytoplasm a lilac color
 - Contain peroxidases, hydrolytic enzymes, and defensins (antibiotic-like proteins)
- Neutrophils are our body's bacteria slayers
- The most abundant wbc's
- Differential neutrophil count = $\frac{\# \text{neutro}}{\text{total \# wbc}} \times 100$

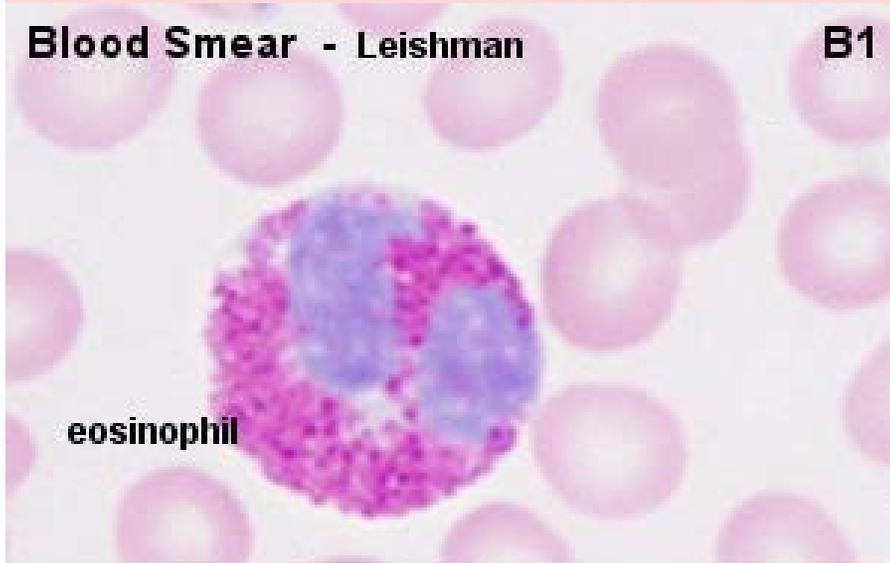
Eosinophils

Eosinophils account for 1–4% of WBCs

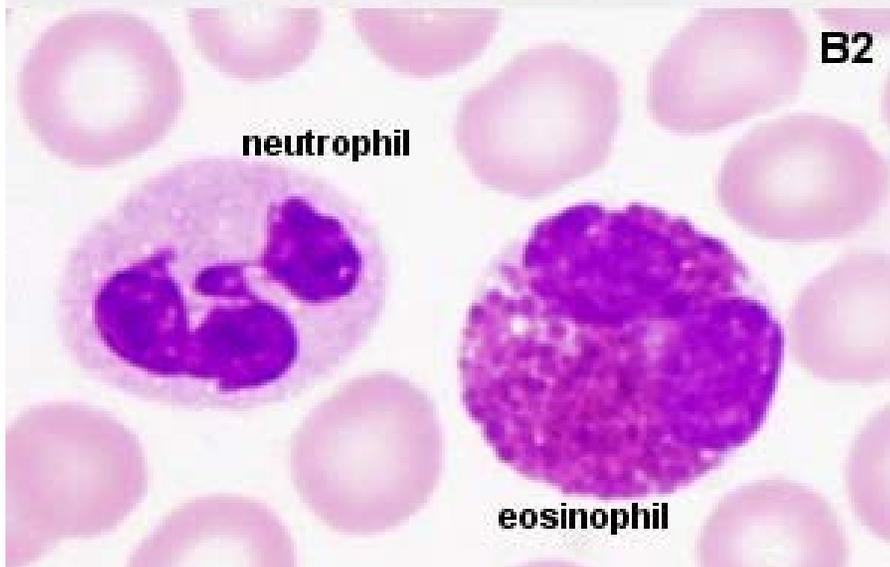
- Have red-staining, bilobed nuclei connected via a broad band of nuclear material
- Have red to crimson (acidophilic) large, coarse, lysosome-like granules
- Lead the body's counterattack against parasitic worms
- Lessen the severity of allergies by phagocytizing immune complexes

Blood Smear - Leishman

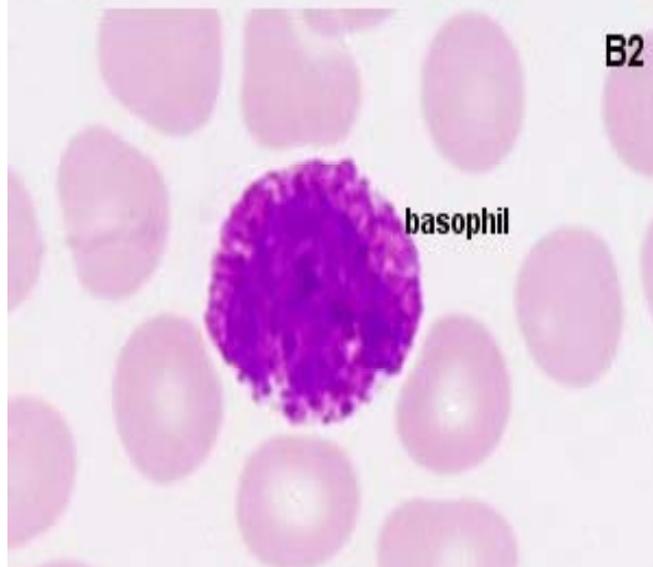
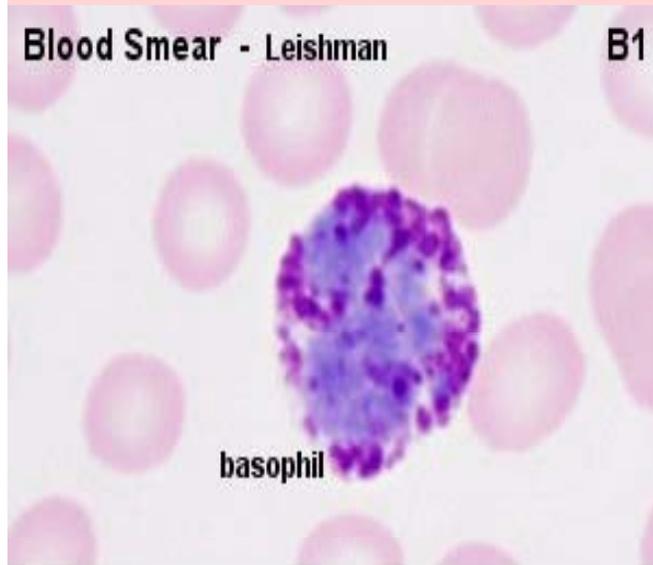
B1



B2



Basophils



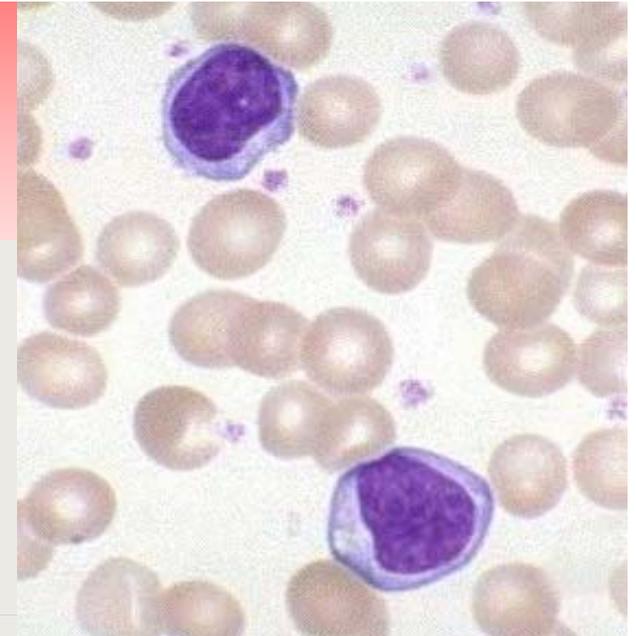
- Account for 0.5% of WBCs and:
 - Have U- or S-shaped nuclei with two or three conspicuous constrictions
 - Are functionally similar to mast cells
 - Have large, purplish-black (basophilic) granules that contain histamine
 - **Histamine** – inflammatory chemical that acts as a vasodilator and attracts other WBCs (antihistamines counter this effect)
 - **Heparin**

Agranulocytes

- Agranulocytes :
- **lymphocytes and monocytes:**
 - Lack visible cytoplasmic granules
 - Are similar structurally, but are functionally distinct and unrelated cell types
 - Have spherical (lymphocytes) or kidney-shaped (monocytes) nuclei

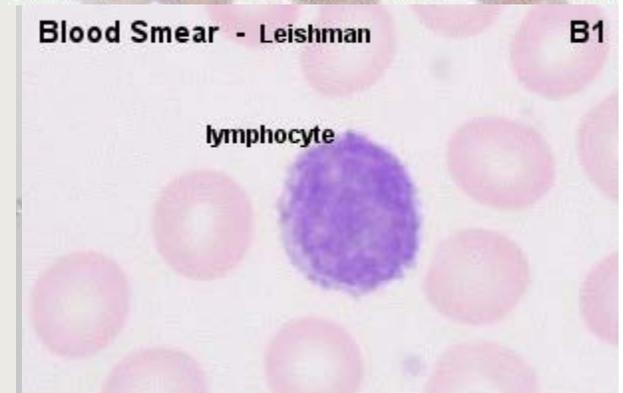
Lymphocytes

- Account for 25% -30% or more of WBCs and:
 - Have large, dark-purple, circular nuclei with a thin rim of blue cytoplasm
 - Are found mostly enmeshed in lymphoid tissue (some circulate in the blood)
- There are two types of lymphocytes: T cells and B cells
 - *T cells function in the immune response*
 - *B cells give rise to plasma cells, which produce antibodies*

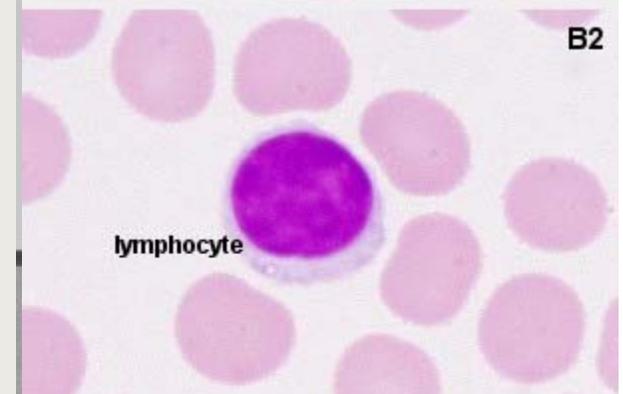


Blood Smear - Leishman

B1



lymphocyte



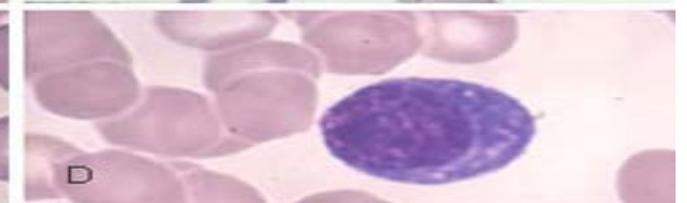
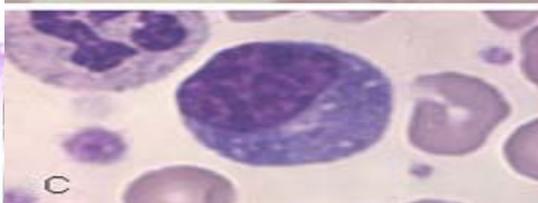
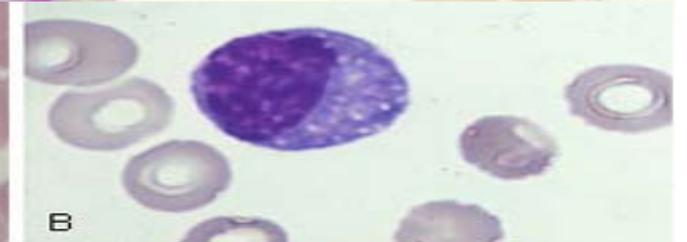
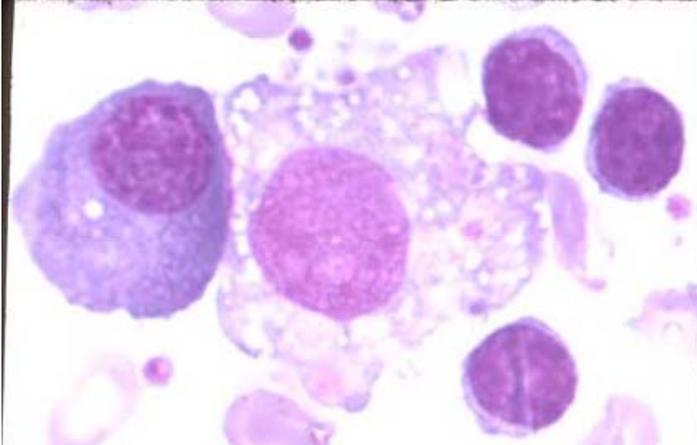
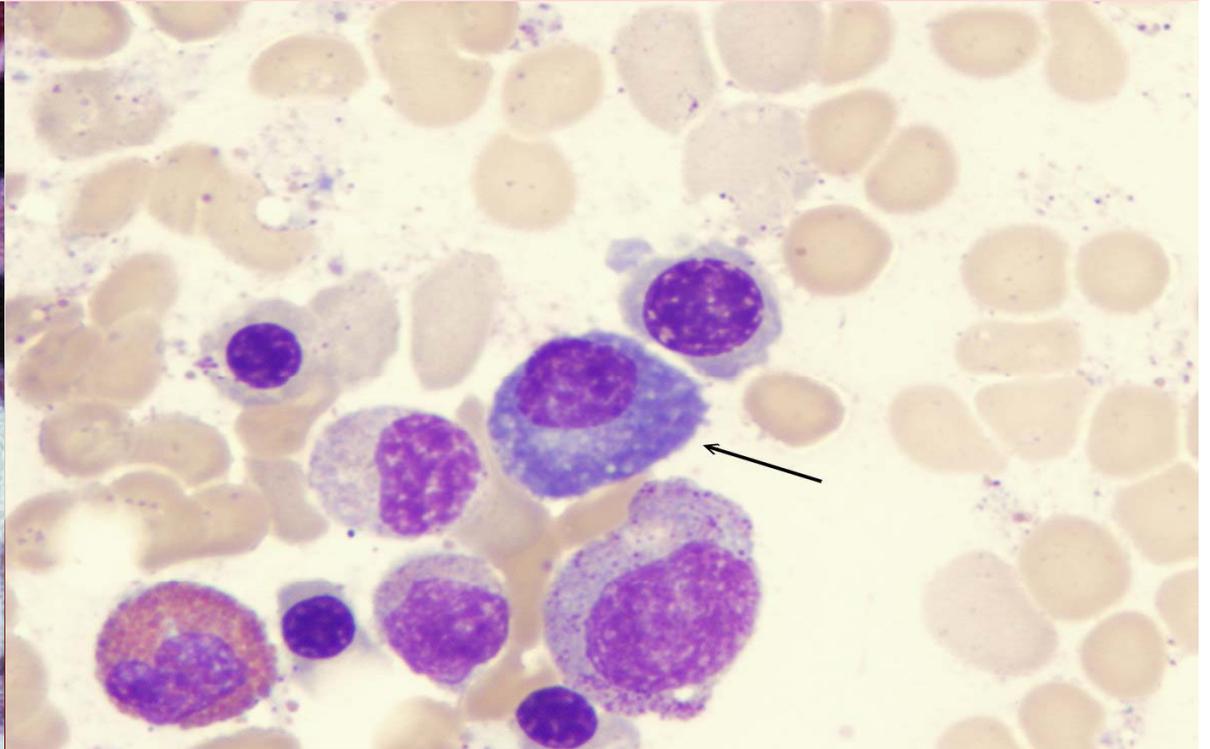
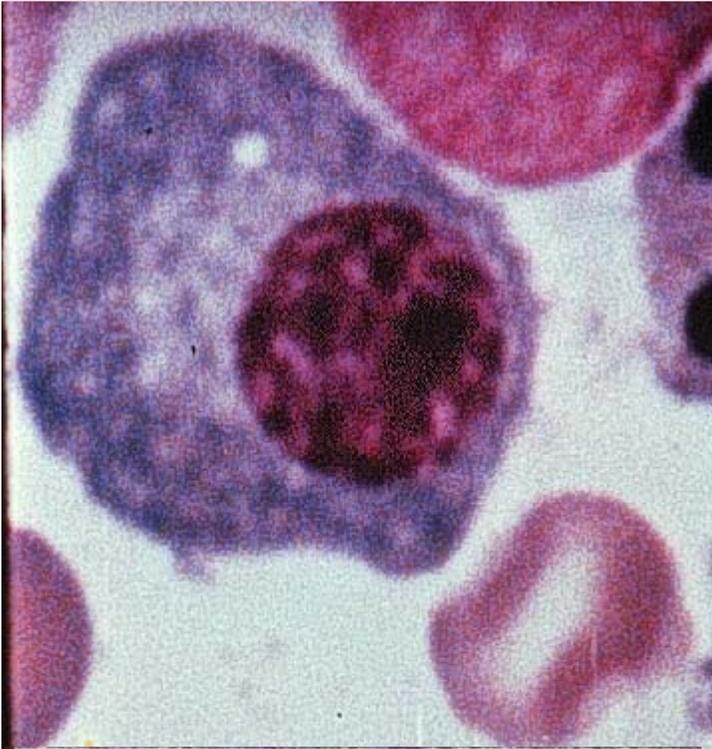
B2

lymphocyte

LYMPHOCYTES: IMMUNOCYTES

- **Physiological Classification: T and B**
 - **T' LYMPHOCYTES :**
 - **Thymus** trained or schooled cells
 - Responsible for **Cell mediated immunity.**
 - Provide protection against intracellular pathogens

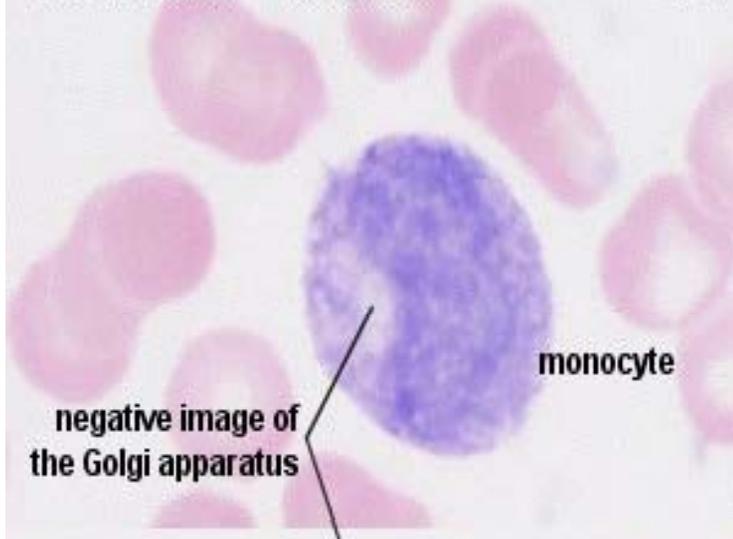
B lymphocytes , plasma cells



Monocytes

Blood Smear - Leishman

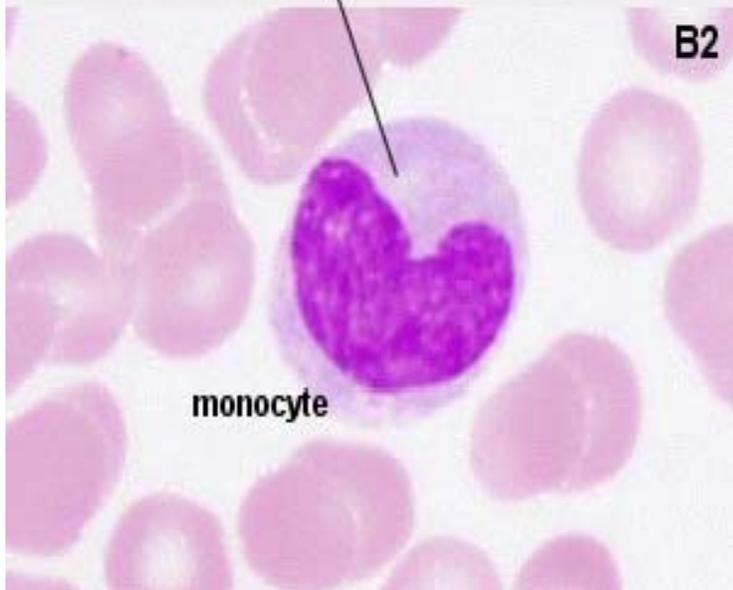
B1



negative image of
the Golgi apparatus

monocyte

B2

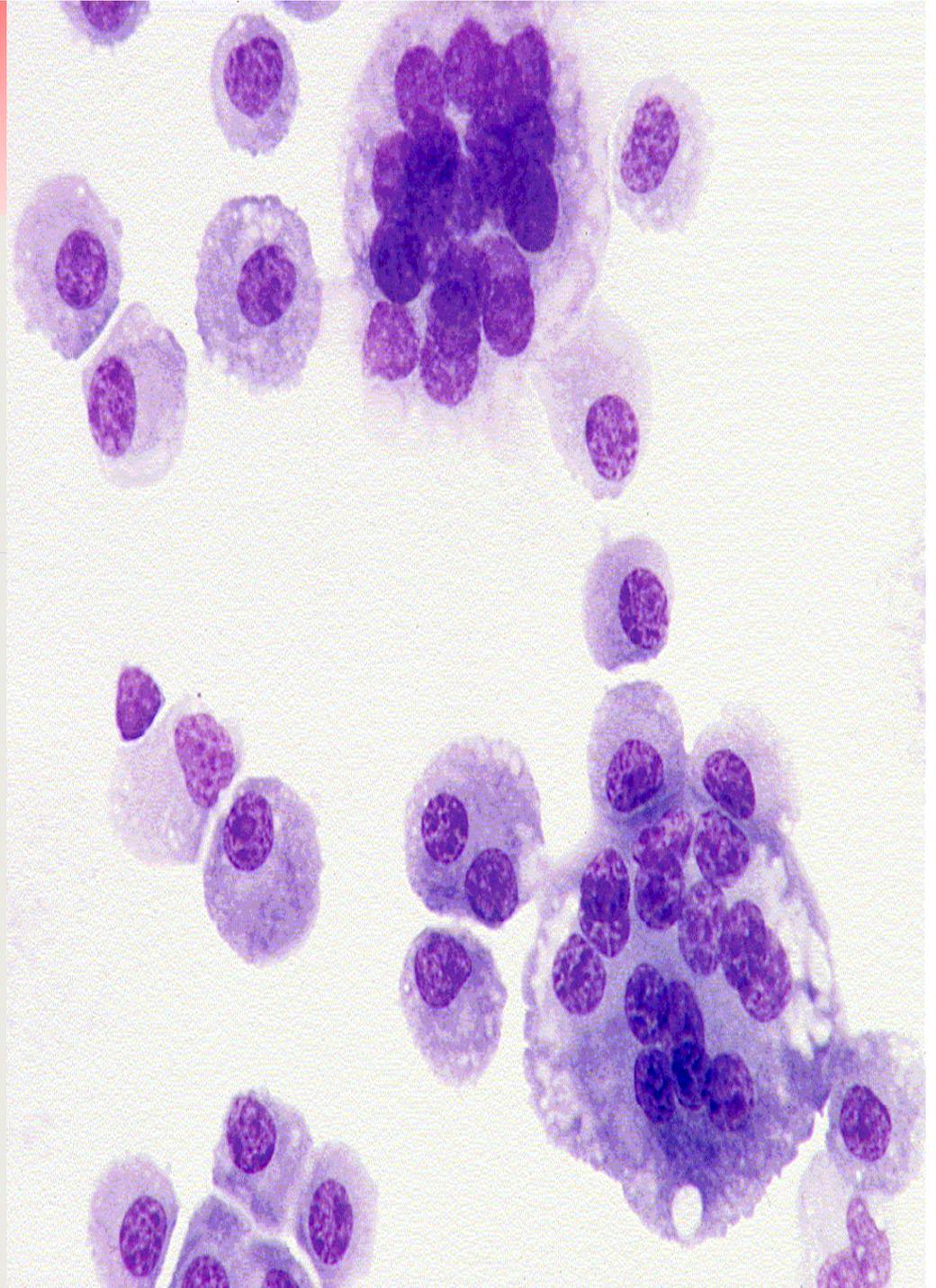


monocyte

- Monocytes account for 4–8% of leukocytes
 - They are the largest leukocytes
 - They have abundant pale-blue cytoplasm
 - They have purple-staining, **U- or kidney-shaped nuclei**
 - **They leave the circulation, enter tissue, and differentiate into macrophages and dendritic cells**

Macrophages

- Macrophages:
 - Are highly mobile and actively phagocytic
 - Activate lymphocytes to mount an immune response
 - Will have different names depending on the location



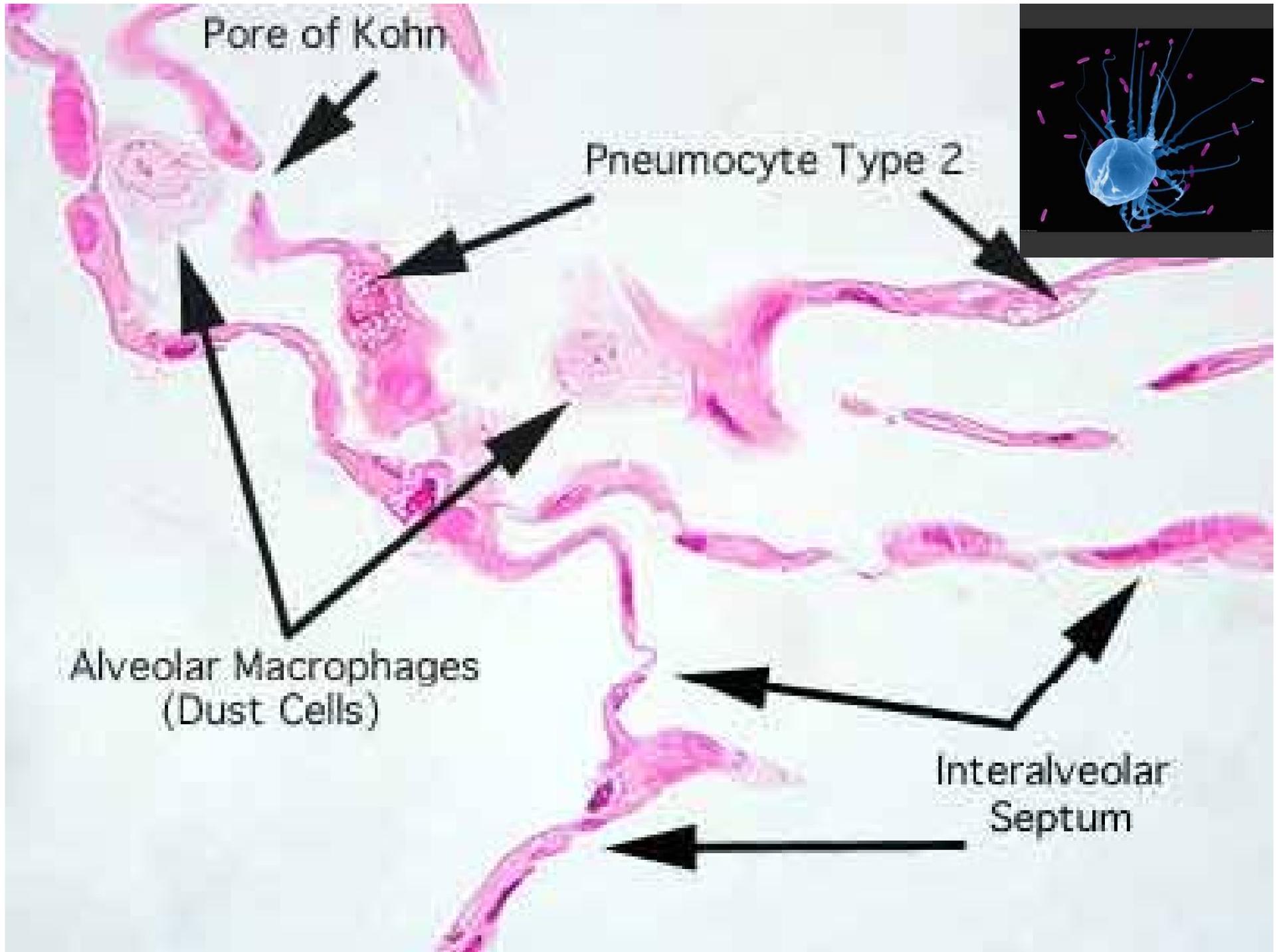
- Normal macrophages include macrophages located in tissues that include:
 - connective tissue – **histiocytes**
 - liver sinusoids – **Kupffer's cells**
 - lung – **alveolar macrophages[dust cells]**
 - lymph nodes – **free and fixed**

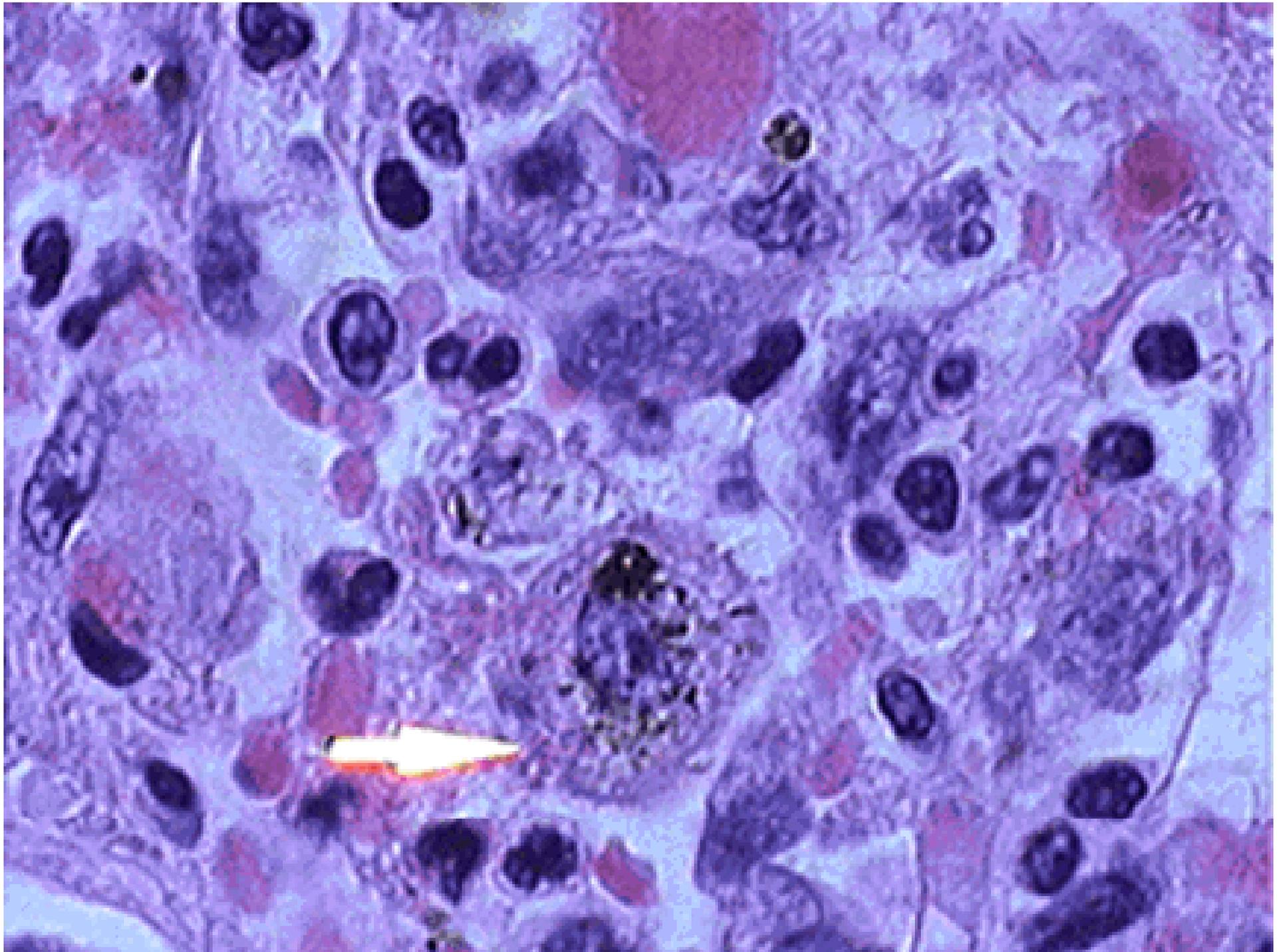
macrophages

- spleen – **free and fixed macrophages**
- bone marrow – **fixed macrophages**
- serous fluids – **pleural and peritoneal**

macrophages

- skin – **histiocytes, Langerhans's cell**

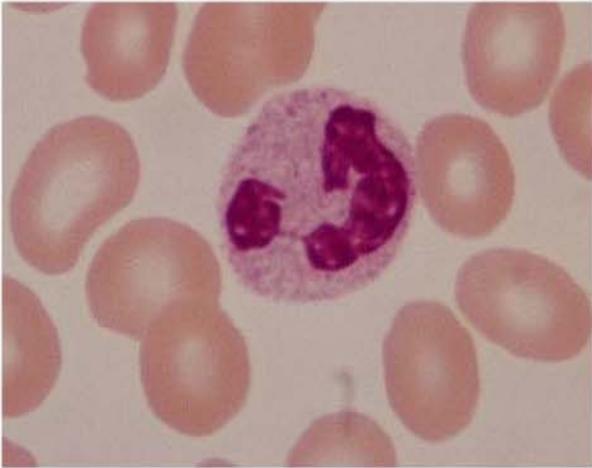




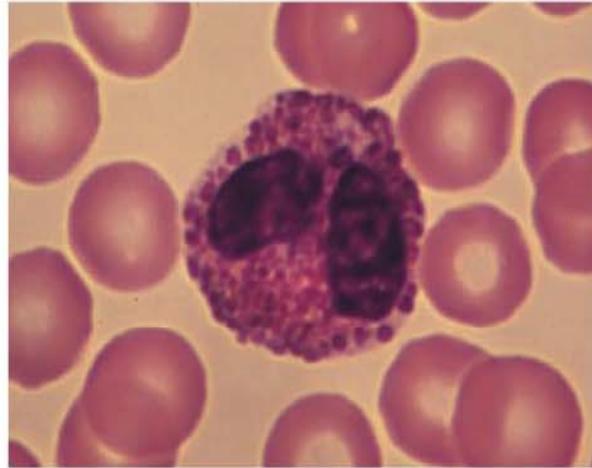
Neutrophils & Macrophages

- **Diapedesis:** They squeeze through the pores of the blood vessels.
- **Amoeboid movement:** They move at rates several times their own length!
- **Chemotaxis:** Directed movement – cells move towards infected areas.

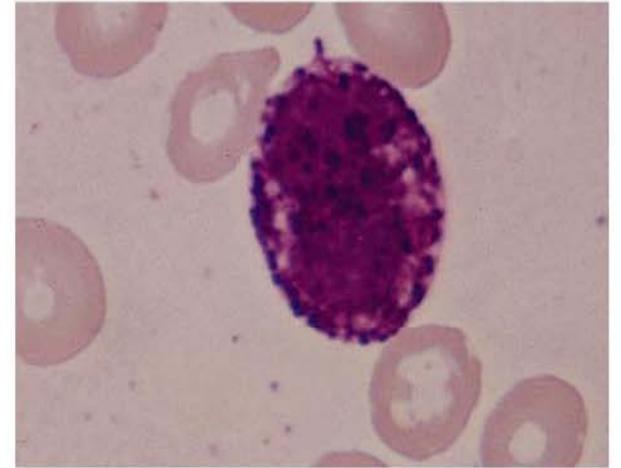
Leukocytes



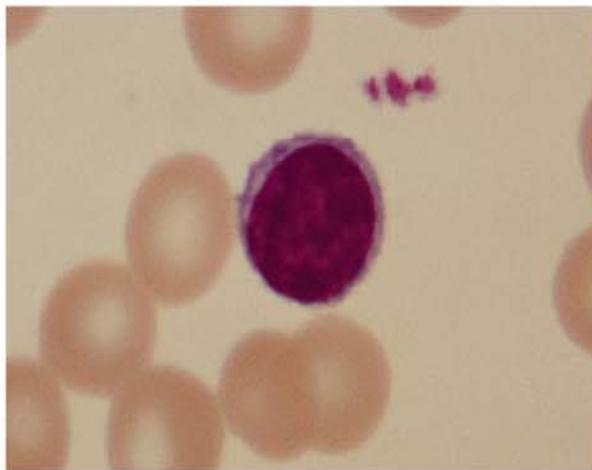
(a)



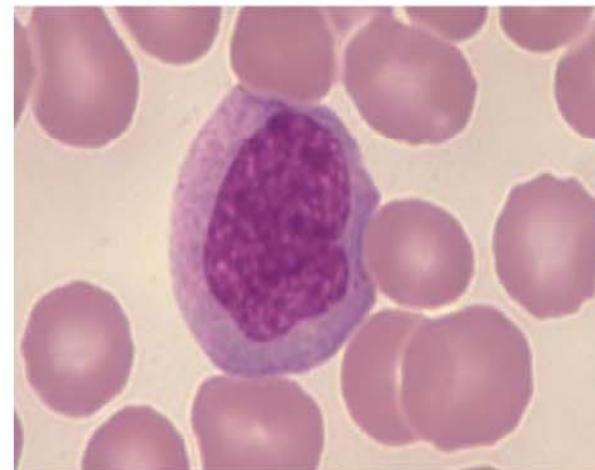
(b)



(c)



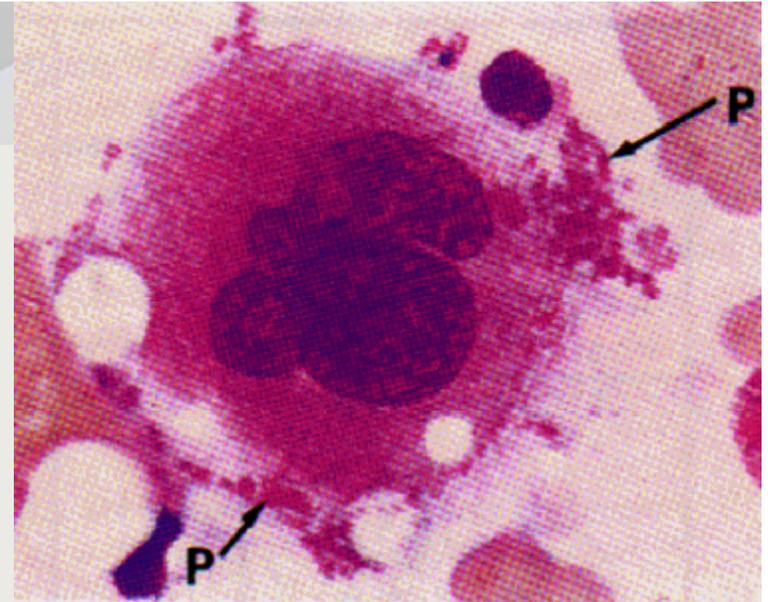
(d)



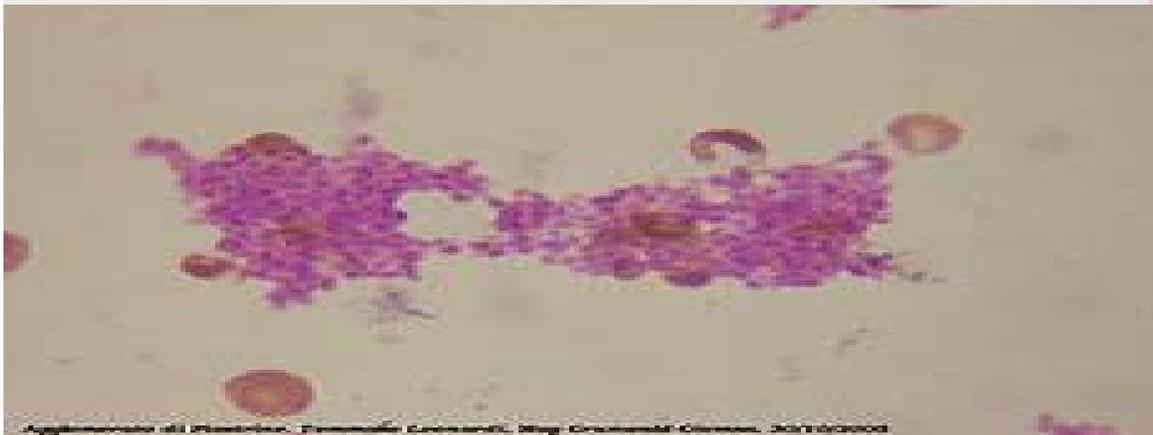
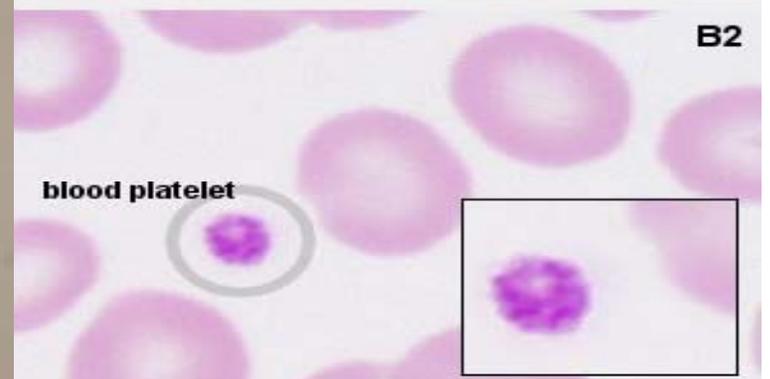
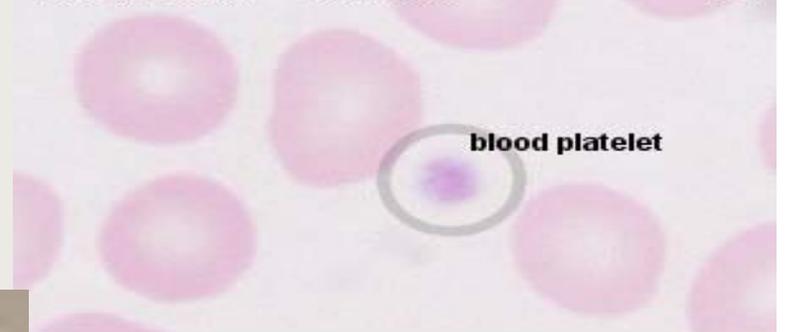
(e)

Platelets

- Platelets are fragments of **megakaryocytes** with a blue-staining outer region and a purple granular center
- Their granules contain
 - serotonin, Ca^{2+} ,
 - enzymes, ADP,
 - and platelet-derived growth factor (PDGF)
- Platelets function in **the clotting** mechanism by forming a temporary plug that helps seal breaks in blood vessels
- **Platelets not involved in clotting are kept inactive by NO and prostacyclin**



Megakaryocytes and platelet formation
Blood Smear - Leishman B1



Blood Smear - Leishman

B1

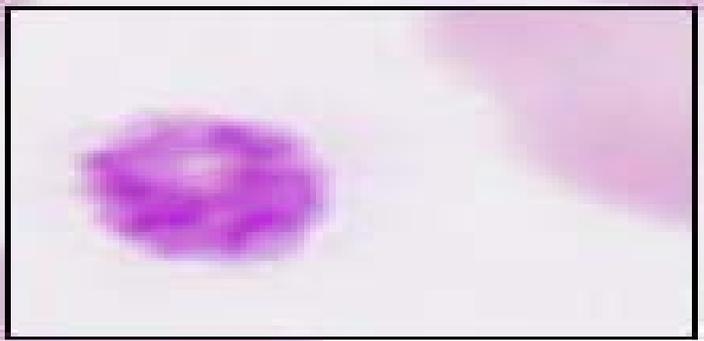


blood platelet

B2



blood platelet



Band cell—An immature neutrophil at the stage just preceding a mature cell. The nucleus of a band cell is unsegmented.

Basophil—Segmented white blood cell with large dark blue-black granules that releases histamine in allergic reactions.

Differential—Blood test that determines the percentage of each type of white blood cell in a person's blood.

Eosinophil—Segmented white blood cell with large orange-red granules that increases in response to parasitic infections and allergic reactions.

Lymphocyte—Mononuclear white blood cell that is responsible for humoral (antibody mediated) and cell mediated immunity.

Monocyte—Mononuclear phagocytic white blood cell that removes debris and microorganisms by phagocytosis and processes antigens for recognition by immune lymphocytes.

Neutrophil—Segmented white blood cell normally comprising 50-70% of the total. The cytoplasm contains both primary and secondary granules that take up both acidic and basic dyes of the Wright stain. Neutrophils remove and kill bacteria by phagocytosis.

Phagocytosis—A process by which a white blood cell envelopes and digests debris and microorganisms to remove them from the blood.