Histology - HLS

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Blood Cells

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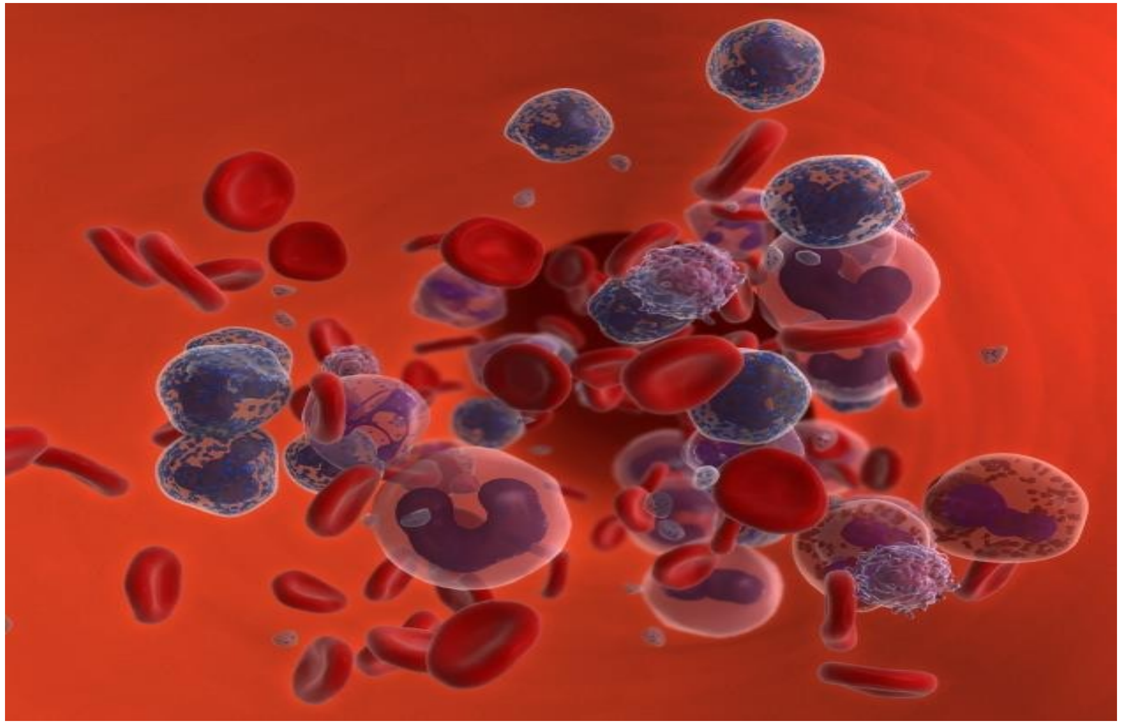
Blood

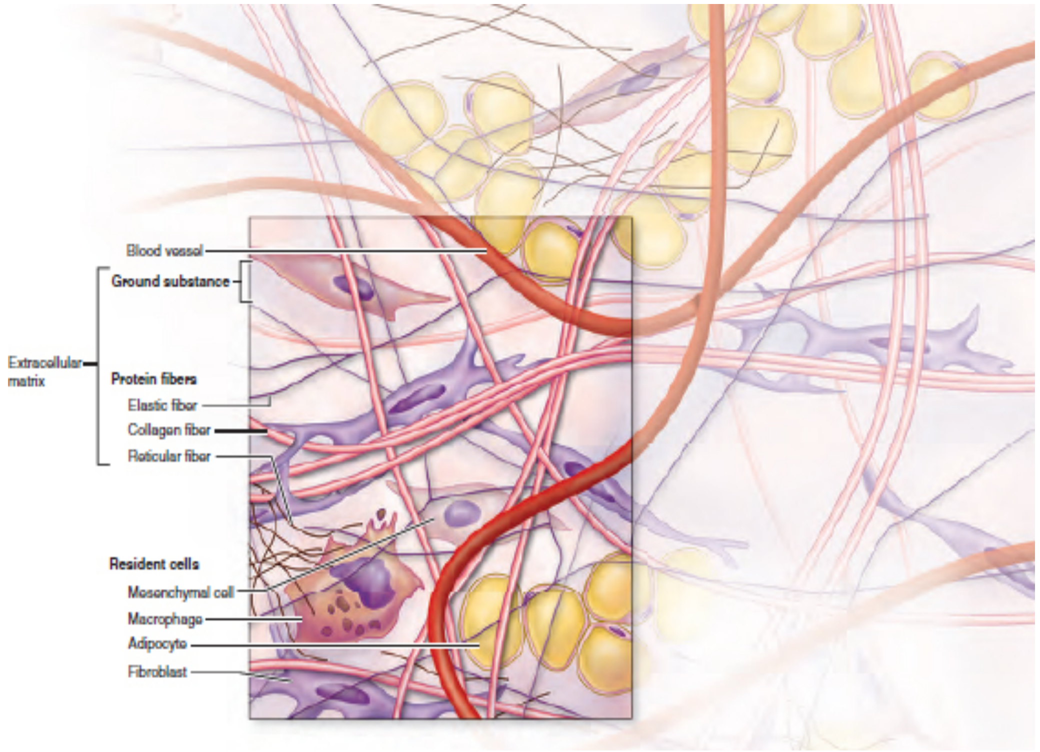
- Specialized form of **connective tissue** because it originates from the same origin, the mesenchyme, and it contains cells (RBCs, WBCs, platelets) + ECM (plasma).

- Components:
  - **Blood cells** (several types; RBCs, WBCs, and platelets)
  - **Plasma** (extracellular matrix)



Overview of loose connective tissue:

- Loose connective tissue has cells, different types of cells (mostly fibroblasts, we also have macrophages, and adipocytes), and it has ECM which contains fibers (collagen, reticular and elastic fibers) and ground substance (water stabilized by large macromolecules called proteoglycans which convert the fluid state of water into a viscous state).
- What characterizes the blood, is that its ECM is fluid and it's called plasma, it lacks fibers. It also lacks proteoglycans and that's why the blood is considered a special form of connective tissue.



Functions of Blood

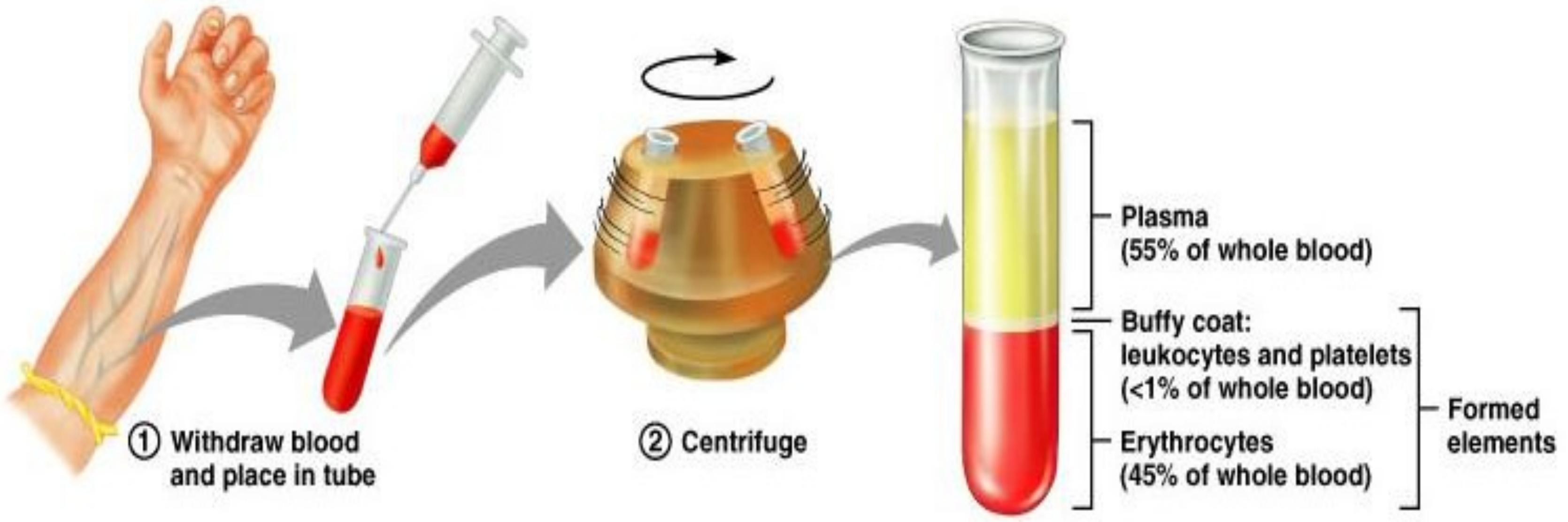
- **Transports nutrients and respiratory gases**
- **Transports waste products to organs and tissues where they can be recycled or released**
- **Transports hormones**. Hormones are released by the endocrine glands into the blood stream and by passing through the blood, they can reach their targets.
- **Transports immune cells throughout the body,** WBCs which we need to fight infections and by passing through the bloods, these cells can reach the sites of infection.
- **Helps regulate body temperature,** vasodilation and vasoconstriction of blood vessels controls heat loss from the body, increased vasodilation leads to increased heat loss from the body and vice versa.
- **Maintains of acid-base and osmotic balance**

- The pH of the blood is slightly alkaline.
- The blood flows through the cardiovascular system, starting and ending at the heart.
- The blood is always red in color and the degree of this redness depends on the amount of oxygen in this blood. The more the oxygen content of the blood, the brighter in red it is.

✓ pH of 7.4
✓ Color is dependent on amount of Oxygen
More oxygen = brighter the red
Less oxygen = duller the red

Blood is propelled mainly by rhythmic contractions of the heart

About **5-6 Liters** of blood in an average adult moves unidirectionally within the closed circulatory system



Collected blood in which clotting is prevented by the addition of **anticoagulants (eg, heparin , citrate or EDTA)** can be separated by centrifugation into layers that reflect its heterogeneity

The test tubes which we collect blood in are pre-treated by the anticoagulants.

After that we centrifuge the sample, spinning at high speed, to separate the different components of the blood according to their density. The denser components of the blood are going to precipitate at the bottom of the tube and the lighter components are going to form the supernatant.

Physical Characteristics

Fluid

- **Living 45%**
  - Cells (**formed elements**)
    - RBC **Erythrocytes** (carry oxygen)
      44% of blood volume
    - WBC **Leukocytes** (immune)
    - Platelets **Thrombocytes** (clotting)

BUFFY COAT (WBC + Platelets)

- **Non living (Matrix) 55%**
  - Plasma (pale yellow fluid)
    - 90% water
    - 10 % (electrolytes, nutrients, proteins (albumin), waste (CO_2, ammonia, urea), gases, hormones)

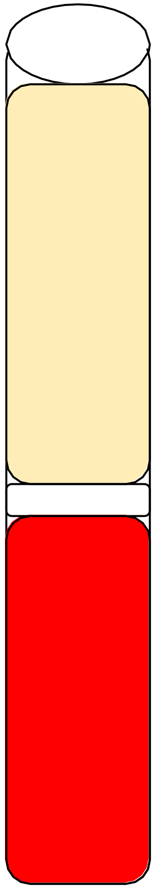
HEMATOCRIT: Ratio of the volume of RBCs to the volume of whole blood

Example: a hematocrit value of 40% means that there are 40 ml of RBCs in 100 ml of whole blood

Normal hematocrit:

Males=40-53%

Females= 36-48%



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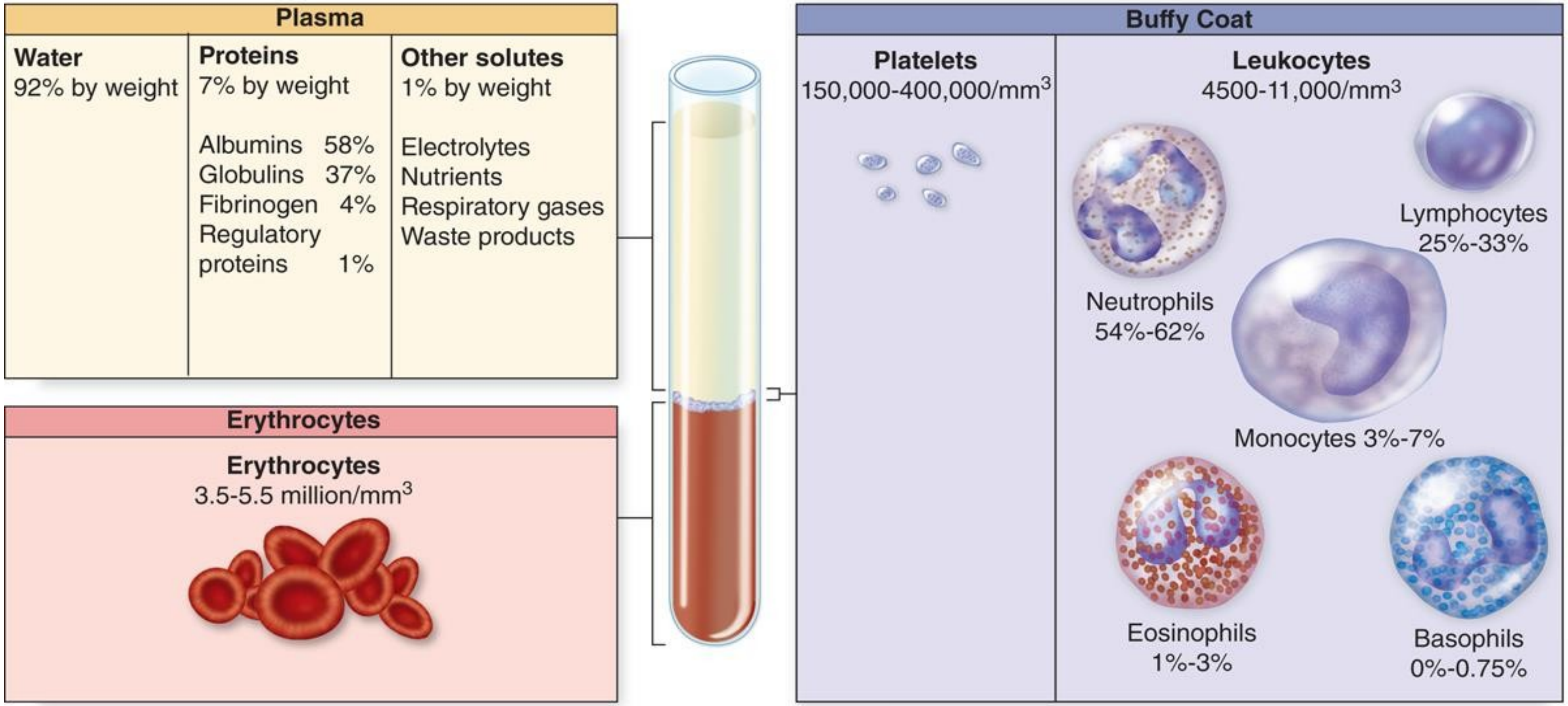
The blood cells are called **formed elements**, and this is because they are not formed within the blood vessels, they are formed in the bone marrow.
The cavities inside the bones are filled with red bone marrow and inside this marrow we have stem cells which divide and differentiate in order to form the different types of blood cells and this process is called **hematopoiesis**.
These cells are called **hematopoietic mesenchymal stem cells.**

The buffy coat is a thin layer, and it appears **whitish in color**, and that is why WBCs are called that, not because they are actually white, but because their condensation produces a white color

Females have lower value of hematocrit because:
1. Menstruation
2. The estrogen hormone is a suppressor for the activity of the bone marrow.

Values are those used by the US National Board of Medical Examiners



Serum = everything in plasma, minus the clotting factors

Albumin:

- ✓ The most abundant plasma protein
- ✓ Is made in the liver
- ✓ Helps maintain the osmotic pressure in capillaries
- ✓ Transports steroid hormones and fatty acids

Fibrinogen:

- ✓ The largest plasma protein
- ✓ Is made in the liver
- ✓ Important for clot formation

Plasma proteins are important to maintain the osmotic pressure inside cells. Inside the blood, we have two types of pressure, hydrostatic pressure and osmotic pressure, the hydrostatic pressure is caused by the pumping of the heart while the osmotic pressure by the plasma proteins.

Fresh plasma

Plasma cell

The importance of proteins inside the plasma is to prevent fluid loss and to create osmotic pressure (to keep the blood inside the blood vessels)

They are large proteins, and they stay inside the blood vessel in order to create an osmotic pressure which is necessary to keep the blood inside the blood vessels.

Globulins (α, β and or γ globulins):

α and β globulins

- ✓ Are made mainly by liver
- ✓ Transport fat soluble vitamins, lipids and iron

γ-globulins (Immunoglobulins (antibodies):

secreted by plasma cells

5 types: MAGED

Antibodies are synthesized by plasma cells, this cell when activated it secretes antibodies specific for a certain antigen. So, when the antibody bonds to its antigen, this complex is going to deactivate the antigen or facilitate its phagocytosis by the phagocytes inside our immune system.

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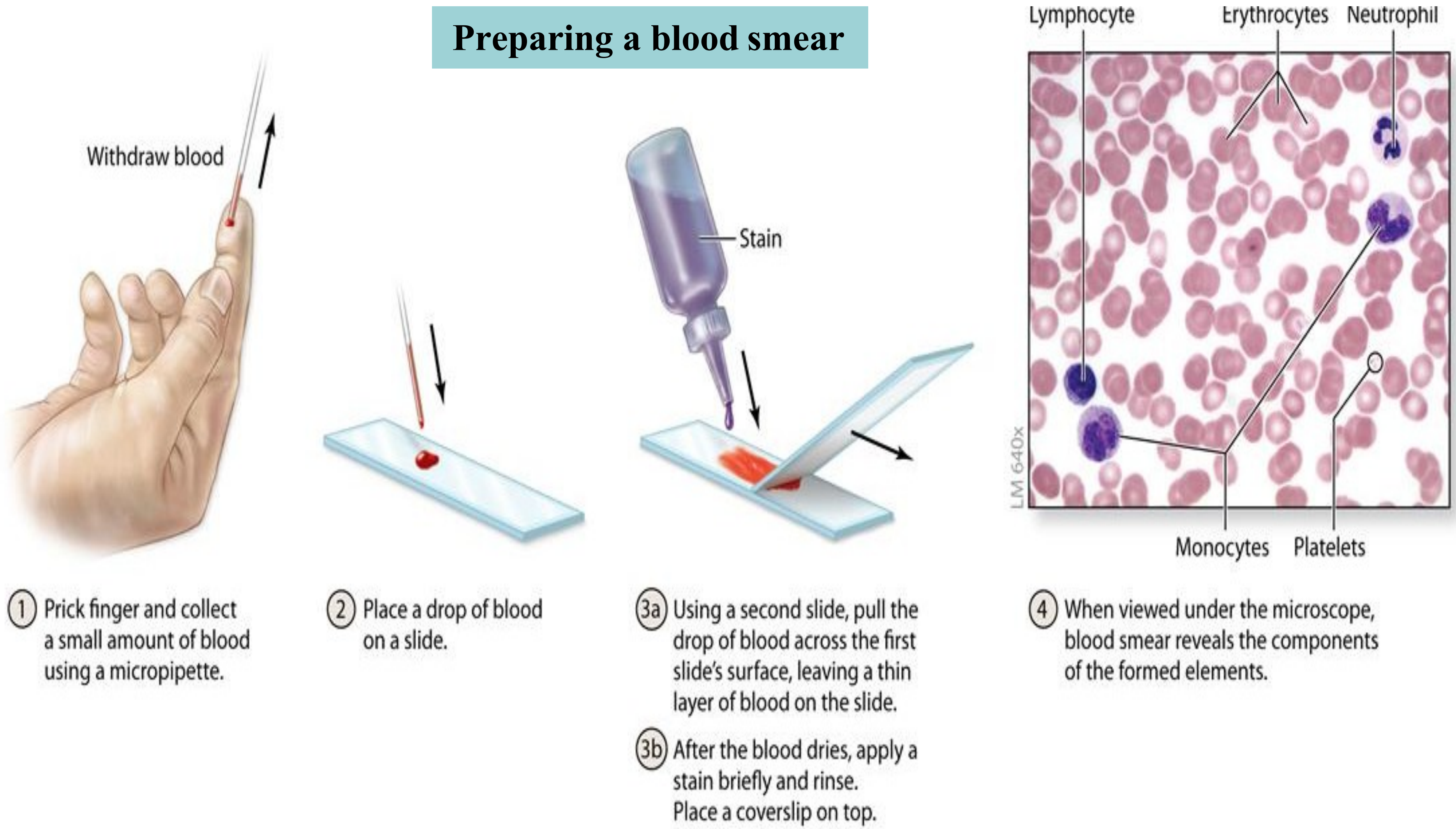
Preparing a blood smear



Polychromatic stains:
Giemsa
Wright
Leishman

→

Blood cells can be studied histologically in smears prepared by spreading a drop of blood in a thin layer on a microscope slide

Figure 12-3

The Staining of Blood Cells

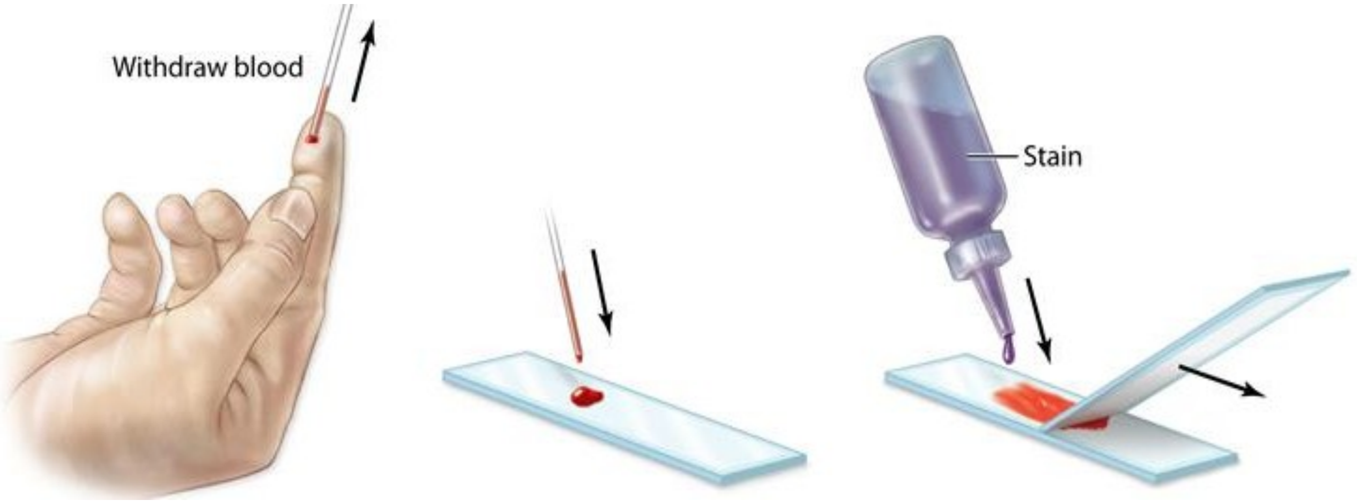
Blood film/ smear: a drop of blood is spread on a glass slide and left dry in air
Staining: with neutral stain e.g **Leishman's stain**

Leishman's stain: formed of a mixture of:

- **Eosin**, an acidic dye that stains pink to red
- **Methylene blue**, a basic dye that stains blue to purple

Dissolved in methyl alcohol (fixative)

Azure dye!!



Blood film/smear

How to prepare blood in order to view it under the light microscope:

1. we take a drop of blood
2. we put it on a glass slide
3. we use another glass slide to spread the drop of blood on the first slide. We call this glass slide the spreader.
4. what we have in the end, is called a blood film or blood smear.
5. In order to view these samples, we have to stain the blood film, we use many stains. The polychromatic stains (in the slide before) are all considered neutral stains, they are composed of both acidic and basic dyes (just like H&E).

- Hematoxylin: basic dye, stains acidic (negatively charged) components of our tissue blue
- Eosin: acidic dye, stains basic (positively charged) components of our tissues pink or red based on the concentration of eosin (if it was more, it would stain more red).

Before staining we have to **fix** these cells (usually this is done with formaldehyde or formalin) but in the blood, the thin film will break down because it is a very strong fixative and so we use a lighter fixative such as methyl alcohol which is already added to the stain.

Leishman stain: eosin+ methylene blue, dissolved in methyl alcohol.

Azure dye: the structures that are stained with this dye are called azurophilic structures.

6. after staining, we add a cover slip over our blood smear, and it is now ready to be viewed under the light microscope.

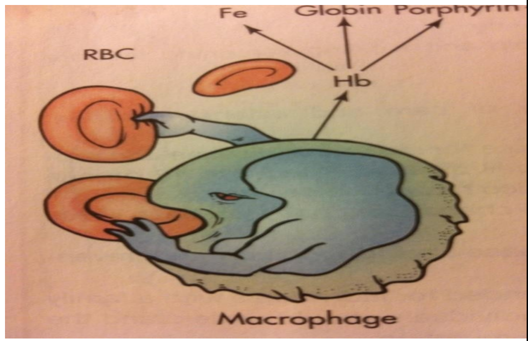
Erythrocytes (RBCs)

- **Small, biconcave discs**
- **Transport oxygen and CO_2, cytoplasm is full of hemoglobin molecules**
- **Have no nuclei or organelles**
- **Pick up O_2 at lung capillaries and release it at body tissue capillaries**

- **<u>Fate:</u>** Survive for ~100-120 days (4 months) in the circulation. Worn out RBCs are removed by macrophages of the spleen, bone marrow and liver.

During their maturation process, the erythrocytes extrude their nuclei, and the mature RBCs enter the bloodstream, without their nuclei

Male: 4.5-5.5 million/mm3
Female:4-5 million/mm3



- Erythrocytes are simply small bags of hemoglobin; this protein is important for the transport of oxygen and carbon dioxide.
- These cells have no nuclei and no organelles, the only organelle we find inside this cell is the plasma membrane.
- The process of the formation of RBCs is called **erythropoiesis**.
- The precursor cells of RBCs have organelles and nuclei, and the most important organelle in this precursor cell is the ribosomes which are needed for the formation of the protein hemoglobin. With the differentiation and maturation of these cells, the ribosomes produce more and more hemoglobin and the organelles inside the cell begin to disappear and the nucleus will be expelled from the cell.

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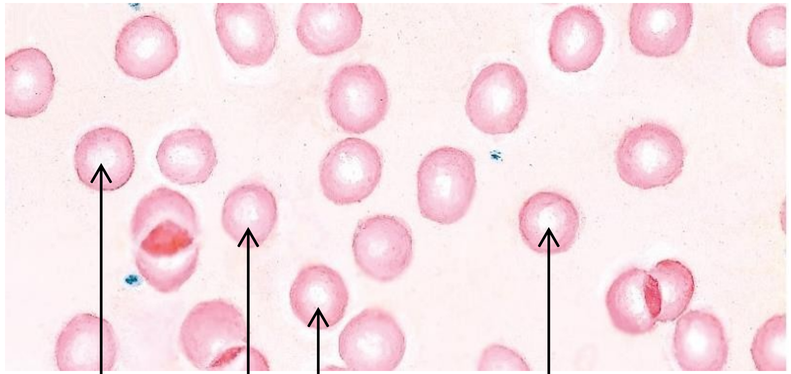
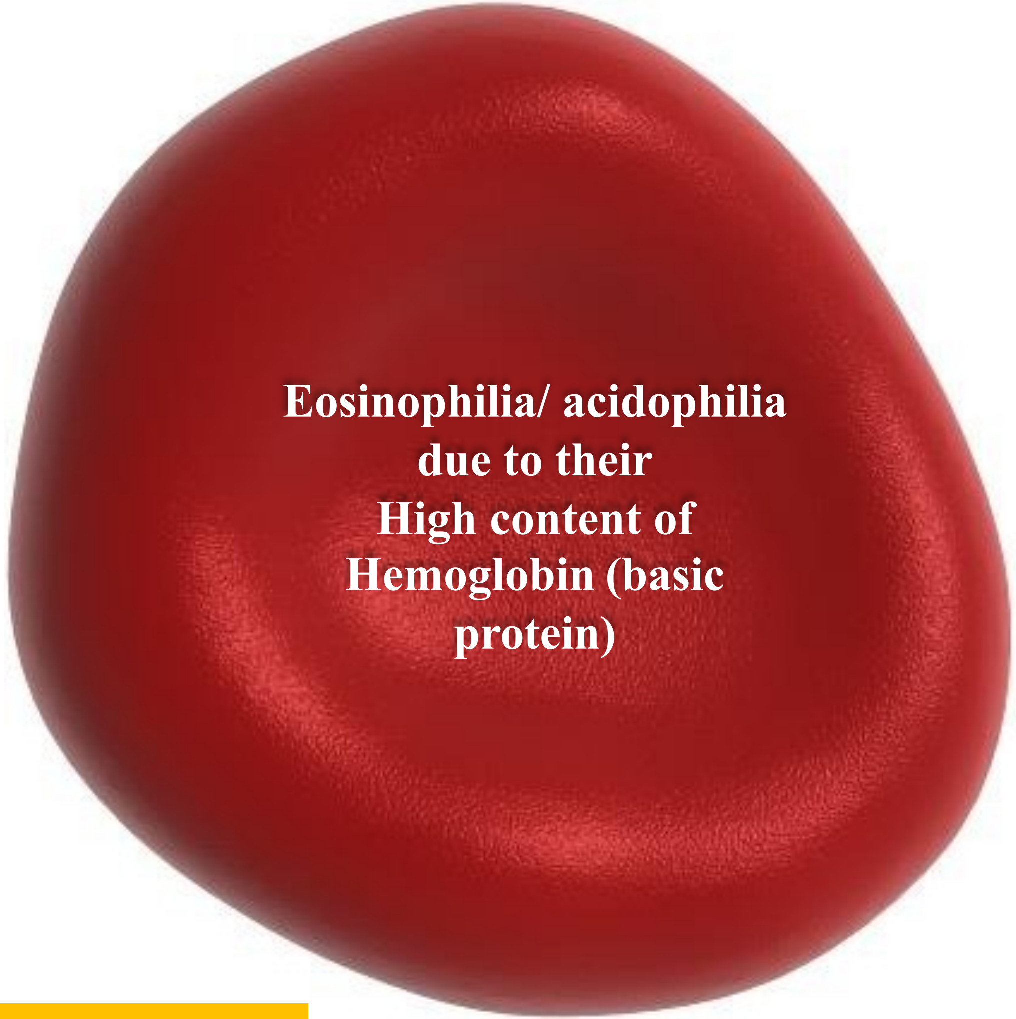
Biconcave shape provides 20-30% greater surface area than a sphere relative to cell volume, facilitating gas exchange

The biconcave shape along with the fluidity of the plasma membrane (50% proteins) permits erythrocytes to bend and adapt to the small diameters and irregular turns of capillaries

Erythrocyte consists of an **outer plasma membrane** enclosing hemoglobin and a **limited number of enzymes** necessary for maintenance of plasma membrane integrity and gas transport functions



The pale staining of the central region is a result of its biconcave disc shape ⟹ **Normochromic RBCs**



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Immediately beneath the plasma membrane is a **meshwork of proteins** (Spectrin and Ankyrin) forming a cytoskeleton

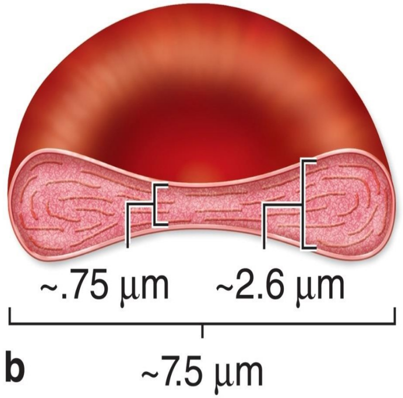
This submembranous meshwork stabilizes the membrane, maintains the cell shape, and provides the cell elasticity required for passage through capillaries

Top view

Size
6-9 um in diameter (7.5 um)
Thickness
2.6-µm thick at the rim, but only 0.75-µm thick in the center

!!!!! Erythrocytes can be used as a size reference for other cell types

Sectional view



(Important)

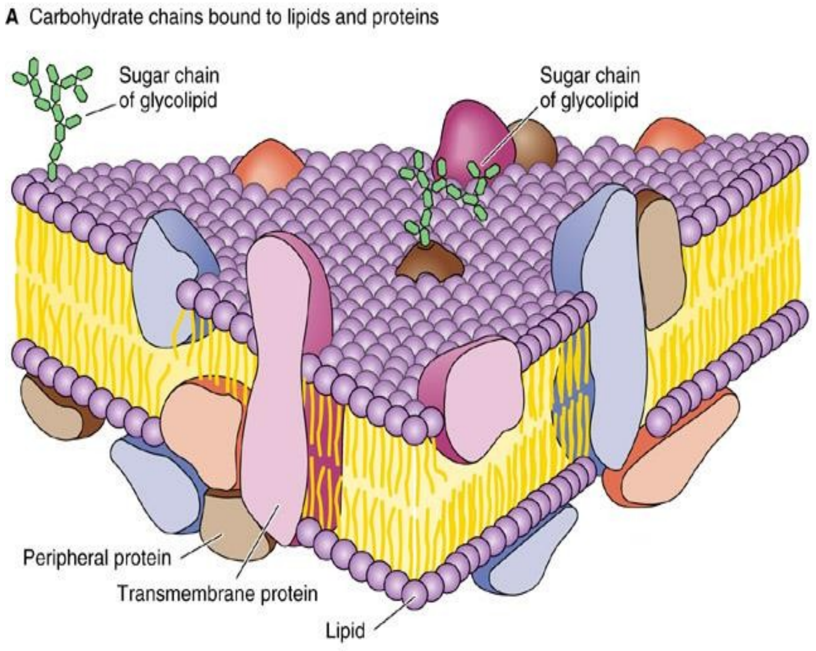
- This biconcave shape provides flexibility for the RBC, because this cell needs to pass through small capillaries.
- Small capillaries can reach up to 4 micrometer in diameter and the RBC average diameter is 7.5, so for it to pass through these tiny capillaries it would undergo cupping and this is possible because of its flexibility
- We can estimate the size of any cell by comparing its diameter to the diameter of a RBC.

- Another feature is that it has rounded edges, this is important to facilitate their movement within the blood vessels especially at the bifurcation area.
- If the cell had pointed edges, then that would mean that when this cells passes through tiny capilaries, they are going to stick to the wall of the blood vessels, and this will decrease the blood flow to the tissue resulting in hypoxia
- All the morphological features of RBCs facilitate their function of gas exchange.

Cell Membrane

Overview of the plasma membrane

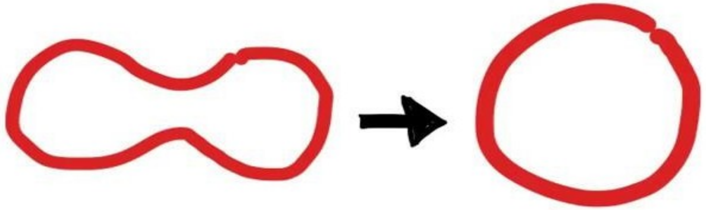
- Plasma membranes are a bilayer of phospholipids, and we have proteins spanning the plasma membrane and this protein is called a transmembrane protein or we can call it integral membrane protein.
- We have other proteins called peripheral proteins and some of them are attached to the extracellular surface of the cell membrane and some of them are attached to the intracellular (cytoplasmic) surface of the cell membrane.
- The plasma membrane also contains sugars, they can be attached to a protein (forming a glycoprotein) or to a lipid (forming a glycolipid).
- The sugars are located on the outer surface of the cell membrane, on the extracellular surface.
- In the erythrocyte, 50% of the plasma membrane is formed by proteins, 40% lipids and we have 10% sugar or carbohydrates. The high composition of proteins in the plasma membrane facilitates the flexibility of the cell and the cupping and the changing of their shape as they are passing through tiny capillaries.



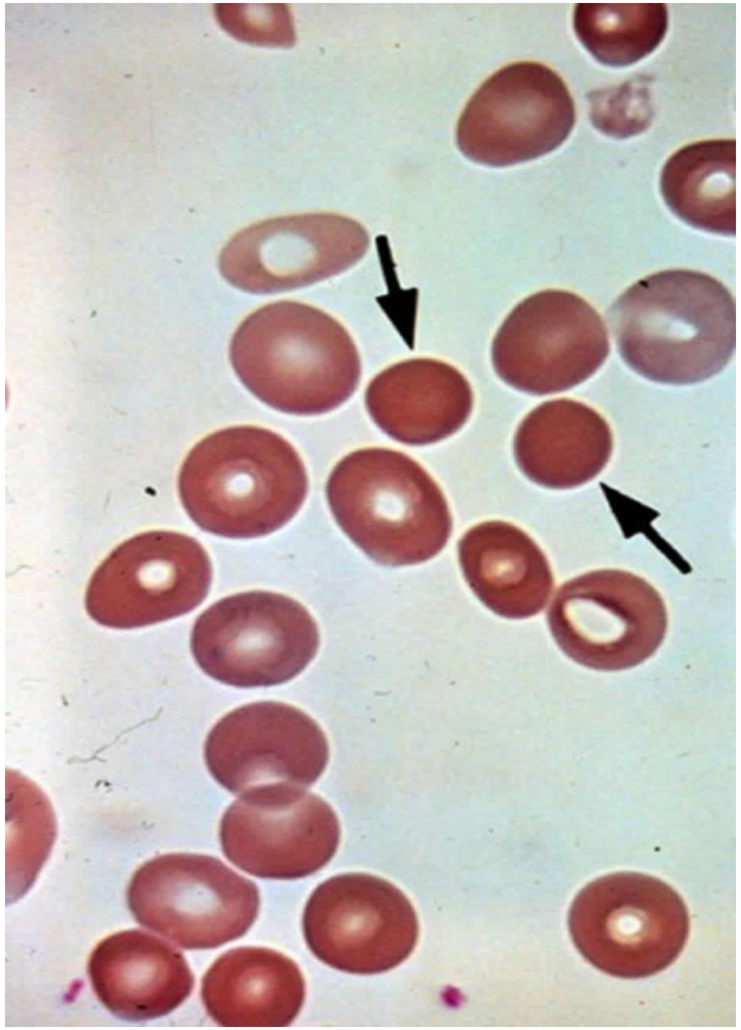
Since erythrocytes have larger plasma membranes compared to their substance, when these cells pass through tiny capilaries, the membrane will begin to break down, but because we have under the plasma membrane a network of submembranous proteins these proteins are going to hold the plasma membrane into the sunbstance of the RBC and stabilize this membrane and prevent its fragmentation especially when it passes through tiny capillaries.

The **glycocalyx (cell coat)** is a glycoprotein and glycolipid covering that surrounds the cell membranes

Hereditary Spherocytosis



Caused by mutations in genes relating to membrane proteins (mostly Spectrin and Ankyrin) that allow for the erythrocytes to maintain their biconcave shape



Most of the cells in the blood film, in the peripheral blood are spherical in shape, instead of having a biconcave shape of cells we have spherocytes.
The submembranous proteins are **not** able to hold the plasma membrane with the substance of the RBC and when these cells pass through tiny capillaries, we will have something called membranous fragmentation.

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- Erythrocytes are acidophilic anucleated cells, they appear acidophilic because we have hemoglobin in the cytoplasm and it is a basic protein so it is dyed by the acidic dye, producing red or pinkish color
- The central areas of RBCs are lightly stained and they are paler in color in comparison to the area around it and this is because of the biconcave shape of the erythrocytes, the amount of hemoglobin in the center of the cell is less than the peripheral part of the cell. This results in less acidophelia.
- When we have normal staining reaction of RBCS we call these cells normochromic.

- Under the TEM the erythrocyte appears as an electron dense cell, it only contains hemoglobin, it appears homogenous, there are no organelles inside the cell.

LM:

Blood film stained with Leishman:

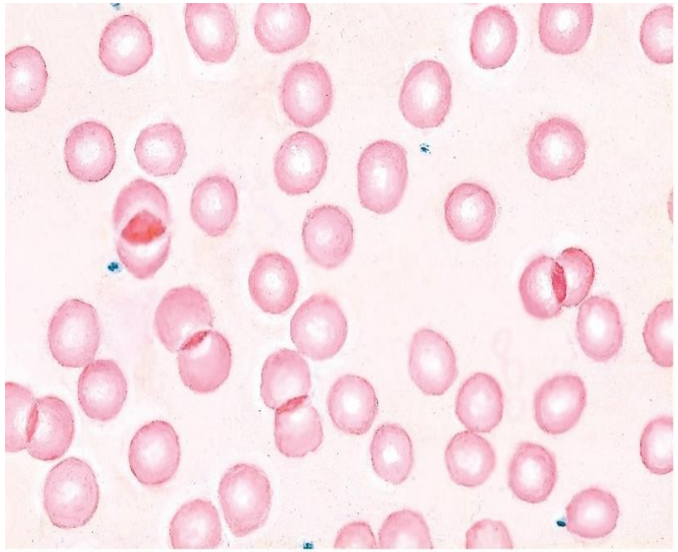
Rounded

Non nucleated

Acidophilic (with pale central area)

A zone of **central pallor** is about 1/3 the size of the RBC

LM



EM:

✓Have no nucleus or organelles

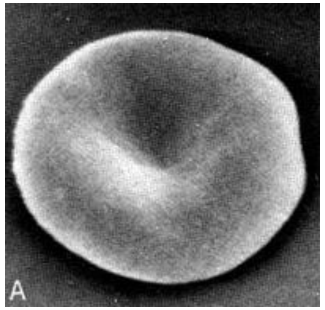
✓Filled with hemoglobin

Electron dense and homogenous

This is a cross section of a capillary

TEM

SEM



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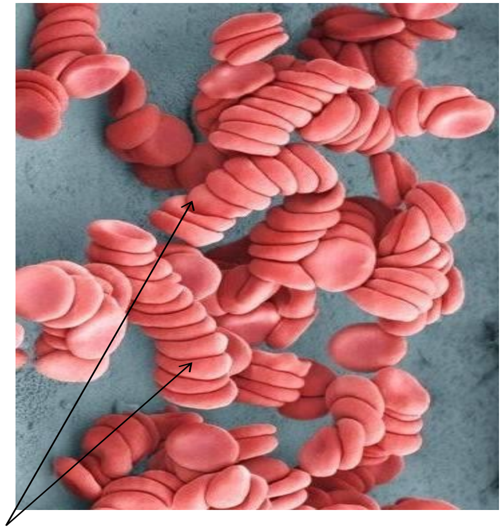
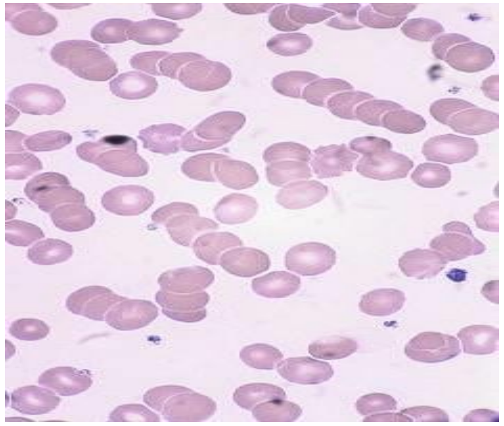
Rouleaux appearance occurs to some extent in all films

- **Roleaux formation**: RBCs from stacks on top of each other, the surface tension between them increases and they form piles of coins. This takes place only in slow blood flow or slow circulation. Normally erythrocytes do not stick to each other, but during slow circulation, they form these files.
- This appearance can be seen in normal blood films, because there it is considered an artifact, it is a result of histological preparation. But if it is significant in the blood film, this could be a nonspecific indication of pathology, such as in infection.
- They can also appear in certain types of cancers or when there is an increase in the viscosity of the blood. It can also take place in diabetic patients and in varicose veins where we have accumulation of blood in these dilated veins.





Rouleaux formation:

✓RBCs may adhere to one another loosely in stacks called Rouleaux (pile of coins)

✓In slow (not in normal) circulation

Due to surface tension caused by their biconcave surface (reversible)

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Abnormalities of Erythrocytes

Change from the normal **size**, **shape** or **staining properties** of erythrocytes are **important indicator of disease**. However, some of these abnormalities may be found in healthy individuals

Abnormal sizes:

Microcytes (<6um)

Macrocytes (>9um)

Anisocytosis (different sizes)

Normocytes: Normal size

Abnormal shapes:

Due to changes either in the cell membrane or Hb content

Abnormal staining:

Hypochromia: Denotes a decrease in the intensity of staining

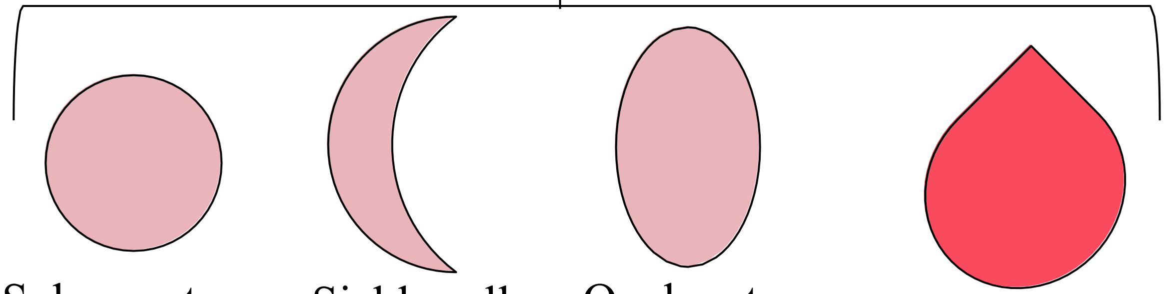
Indicates a decreased amount of hemoglobin

Frequently accompanies microcytosis

Hypochromic microcytic anemia

Poikilocytes

(Poikilocytosis)



Spherocytes

Sickle cells

Ovalocytes

Elliptocytes

Dacrocytes

One of the most sever changes in shape occurs during SICKLING of RBCs in sickle cell anemia where erythrocytes take on the form of crescents

Sickle shaped RBCS are formed because of a point mutation of hemoglobin.

Teardrop shaped cells

Cells that are tear-drop-shaped have a pointed edge and this edge sticks to the walls of the blood vessels and causes the slowing down of blood flow.

The consequences:

- The capacity of these cells to carry oxygen is less
- These cells are abnormal in shape, they will stick to the walls of the blood vessels and slow down blood flow and therefore oxygen supply to the tissues causing hypoxia.

Sickle cell anaemia results from abnormal hemoglobin

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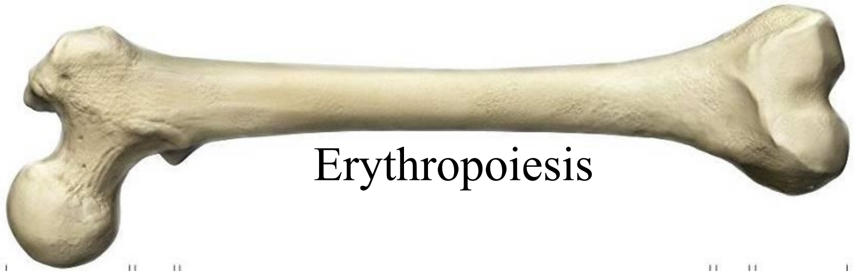

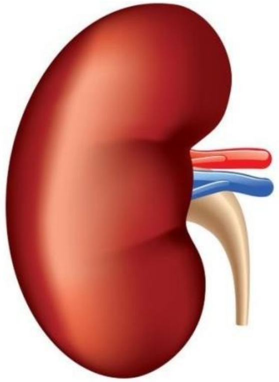

Production of erythrocytes in the bone marrow, is stimulated by erythropoietin

⇩

Erythropoietin is produced by the kidneys

⇩

When RBC count drops, such as during blood loss, the resulting oxygen-deficiency state, **hypoxemia**, is detected by the kidneys.

⇩

The kidneys respond by increasing their erythropoietin secretion, which leads to increased red blood cell production

Anemia: a decrease in the total number of RBCs (and/or hemoglobin)

Polycythemia: an increase in the total number of RBCs

Erythropoietin is produced by the endothelial cells of the blood vessels inside the kidney. When we have low oxygen, these cells will be stimulated to produce erythropoietin and then the erythropoietin is going to affect the precursor cells of the RBCS in order to increase their production

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Consequently

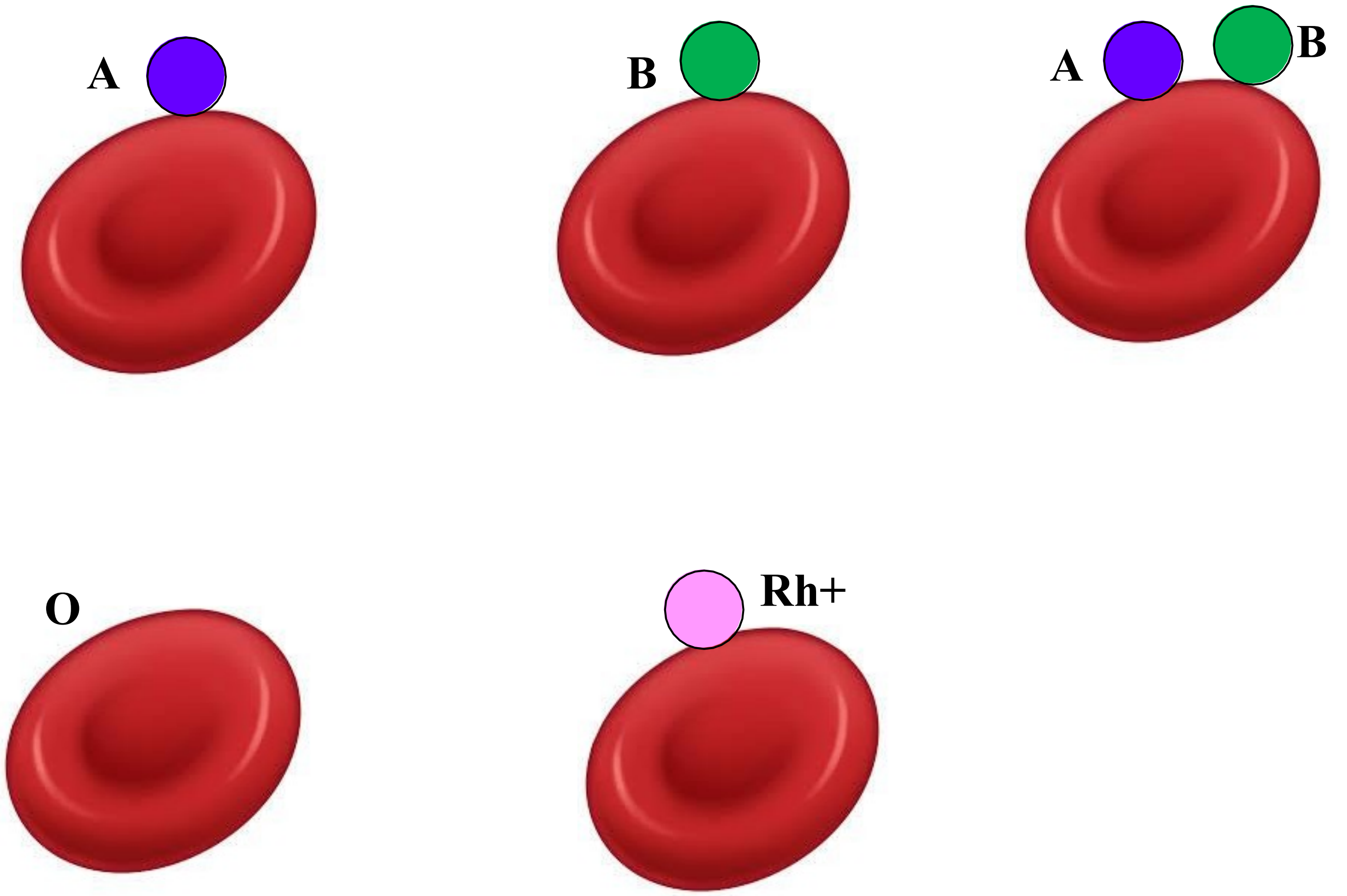
People living **at high altitudes** usually have higher RBC count as a response to lower oxygen levels.

Athletes whose demand for oxygen is more elevated, also have higher RBC counts.

This occurs after almost a week of living in high altitudes

RBC plasma membranes have glycoprotein antigens on their external surfaces

Glycophorin A is an integral membrane protein. The glycosylated extracellular domains of the glycophorins include antigenic sites that form the basis for the ABO blood typing system



On the plasma membrane of RBCs, we have an integral protein called glycophorin A, it is a glycoprotein. The sugar connected to this protein has antigenic sites and we use these antigenic sites for blood grouping.

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