Blood cells

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Blood

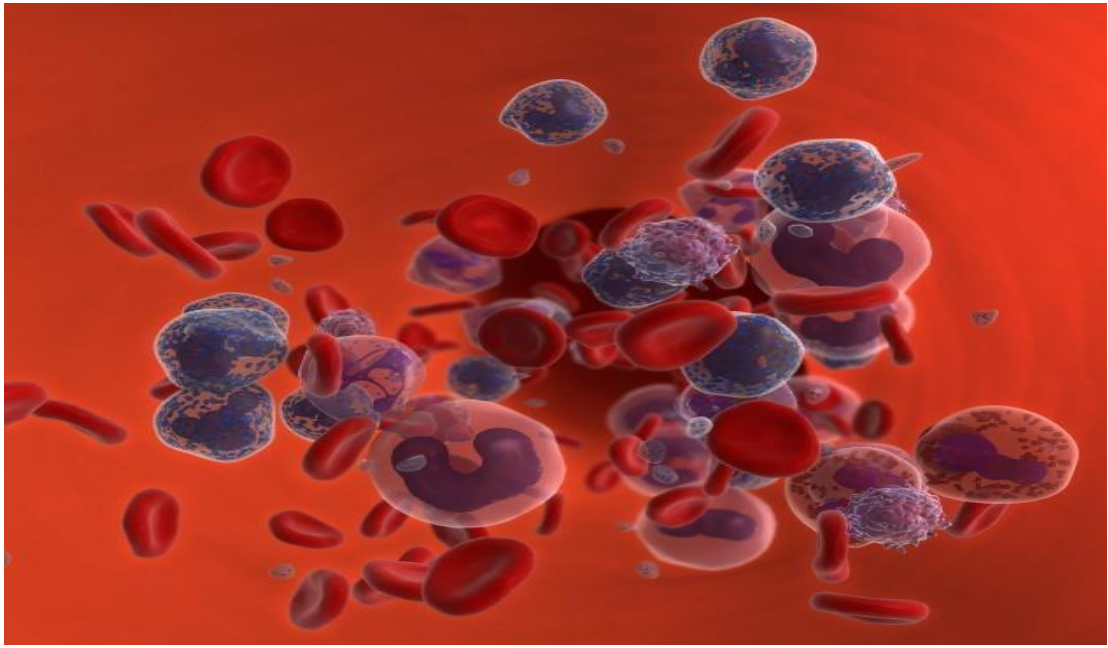
- Specialized form of **connective tissue**

Why?

1- it has the same origin of connective tissue which is the mesenchyme

2- it contains cells (RBCs, WBCs, Platelets) + ECM (plasma)

- Components:
  - Blood cells (several types)
  - Plasma (extracellular matrix)



Let's shortly revise the structure of the loose type of connective tissue:

Cells:
Most of them are fibroblasts + macrophages + adipocytes

ECM:
1- ***fibers***: collagen fibers, reticular fibers, elastic fibers
2- ***ground substance***: water stabilized by large macromolecules called proteoglycans [they convert the fluid state of water into viscous state]

What is special about blood is that the ECM is fluid and that's because the ECM of blood lacks fibers and proteoglycans

Functions of Blood

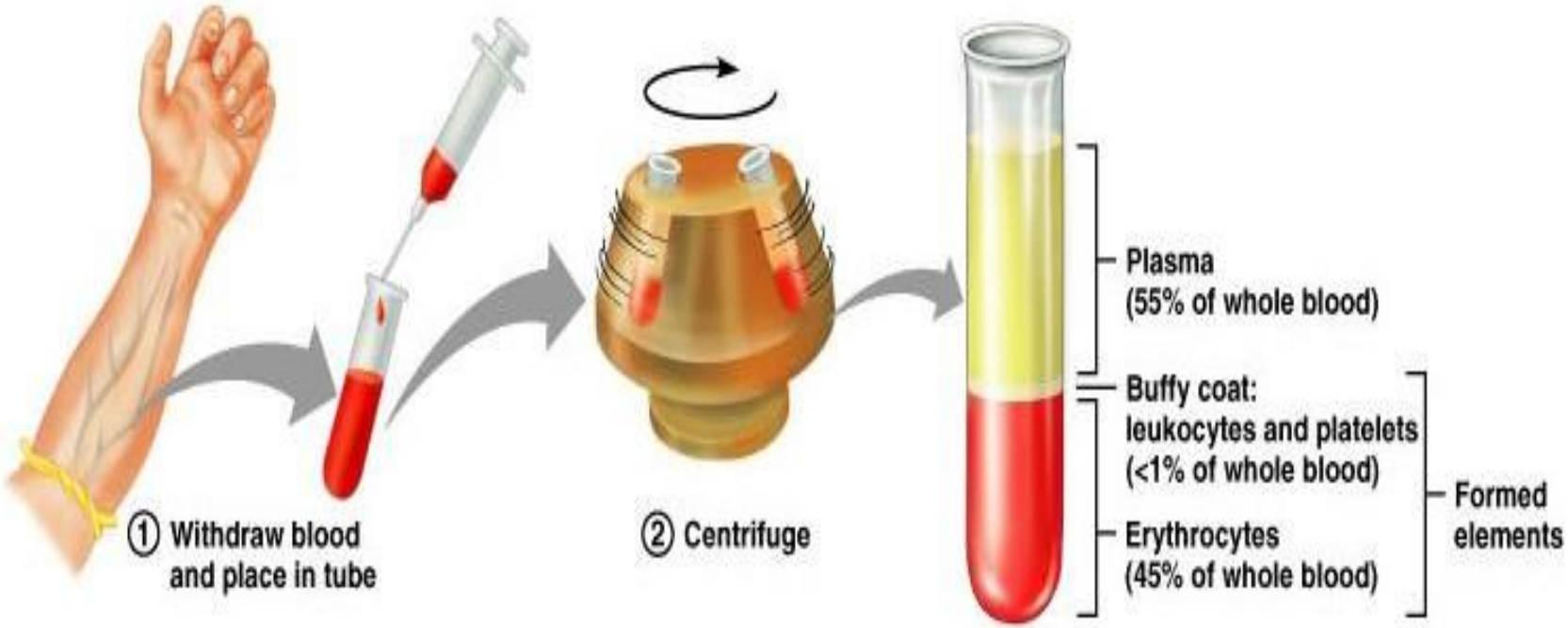
- Transports nutrients and respiratory gases
- Transports waste products to organs and tissues where they can be recycled or released
- Transports hormones so they can reach their targets
- Transports immune cells (WBCs) throughout the body so they can reach the site of infection
- Helps regulate body temperature through **vasodilation** (increases the lose of heat) and **vasoconstriction** (decreases the lose of heat)
- Maintains of acid-base and osmotic balance

✓ pH of 7.4 **Slightly alkaline**
✓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

In anatomy textbooks we draw arteries in red color and veins in blue color, but this is a MISCONSEPTION because the blood is always red, but the degree of redness depends on the amount of oxygen.



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

In order to study the different components of blood:

1- take a sample of blood
2- put it inside a test tube [must be pre-treated with anticoagulants to prevent blood coagulation]
3- centrifuge the sample [spinning it at high speed] to separate the components of blood *according to their density*

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Physical Characteristics

Fluid

- **Living 45%**

  Cells (**formed elements**)

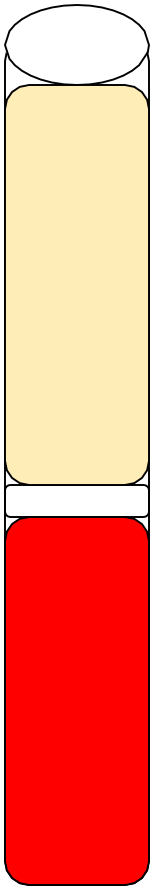
  - RBC **Erythrocytes** (carry oxygen) **forms 44% of blood volume**
  - WBC **Leukocytes** (immune)
  - Platelets **Thrombocytes** (clotting)

  BUFFY COAT 1% of blood volume

- **Non living (Matrix) 55%**

  Plasma (pale yellow fluid)

  - 90% water
  - 10 % (electrolytes, nutrients, plasma proteins (albumin), waste (CO_2, ammonia, urea), gases, hormones)



Top layer= plasma

Middle layer= buffy coat

Bottom layer=RBCs
(Packed Cell Volume)

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The cells are called **formed elements** because they aren't formed inside the blood vessels, they are formed inside the red bone marrow which contains stem cells that divide and differentiate in order to form the different types of blood cells.

The process of reproduction of blood cells is called **Hematopoiesis.**
And the stem cells that are inside the bone marrow are called **hematopoietic mesenchymal stem cells (HMSCs).**

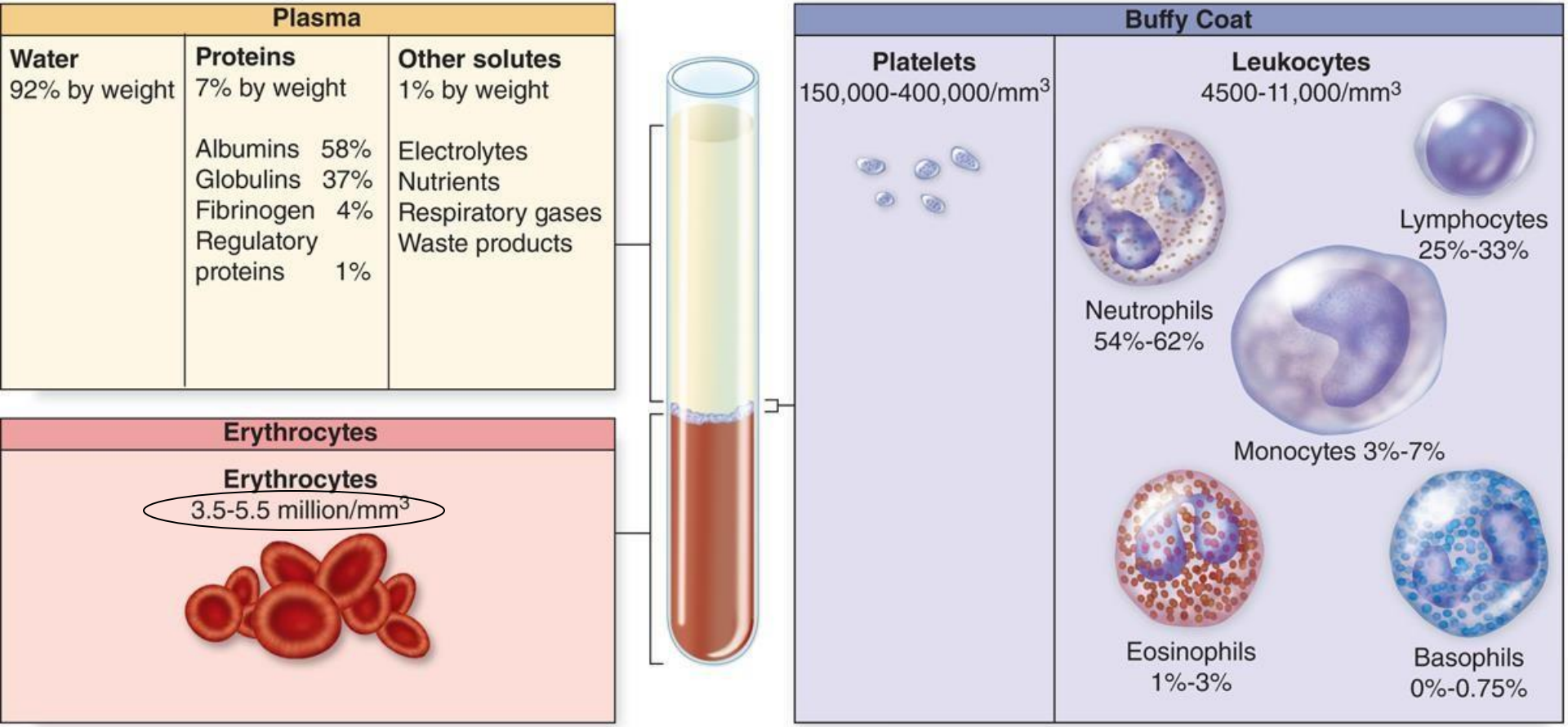
HEMATOCRIT: Ratio of the volume of RBCs to the volume of whole blood

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%

[females have lower values because of the menstruation each month + the estrogen suppresses 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

Figure 12-1

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

Inside the blood vessels we have two types of pressure
1- hydrostatic [created by the pumping force of the heart]
2- osmotic [by plasma protein]

Fresh plasma

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)

Plasma cell

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

There are five types: M, A, G, E, D

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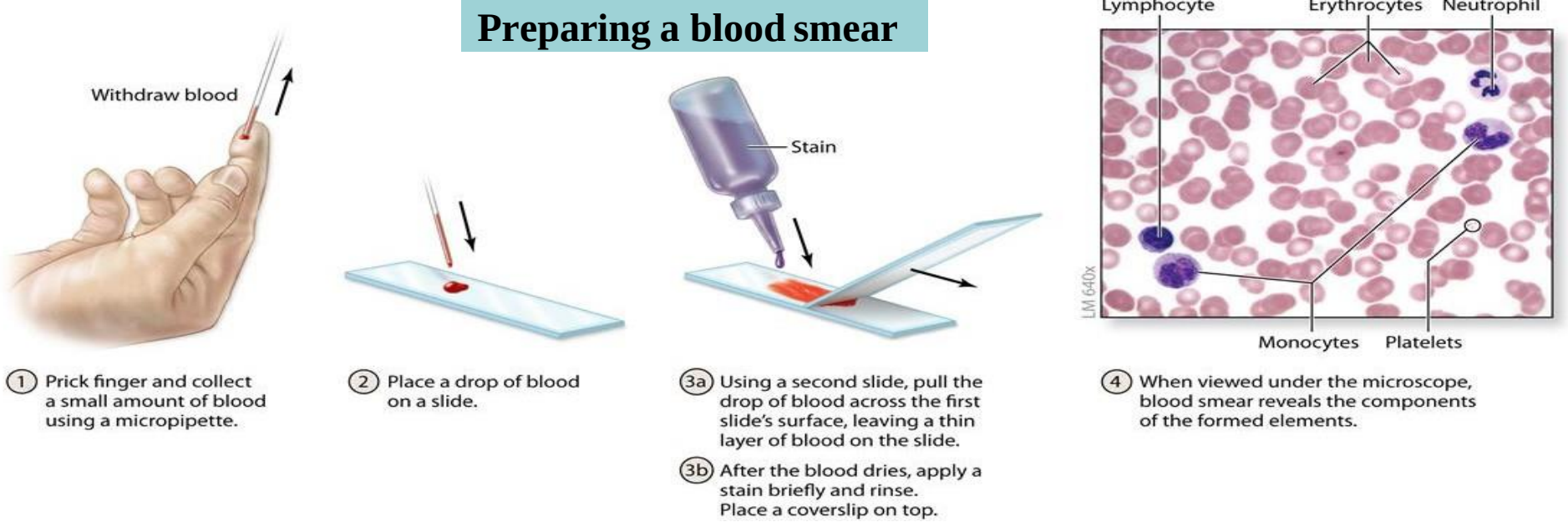
Preparing a blood smear



To prepare the blood to be viewed under the light microscope: take a drop of blood -> put it on a glass slide -> use another slide to spread this drop [spreader] >>> that's called **blood film/ blood smear** -> fix the cells [don't use formaldehyde because it is very harsh and it is going to break down the cells/ use a lighter type of stain like methyl alcohol] -> stain using one of **the neutral stains** [that contain acidic dye and basic dye / **the acidic dye will stain the basic components red/pink, while the basic dye will stain the acidic components blue**]

** usually the fixative is combined with the stain.

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

The Staining of Blood Cells

Blood film: 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:

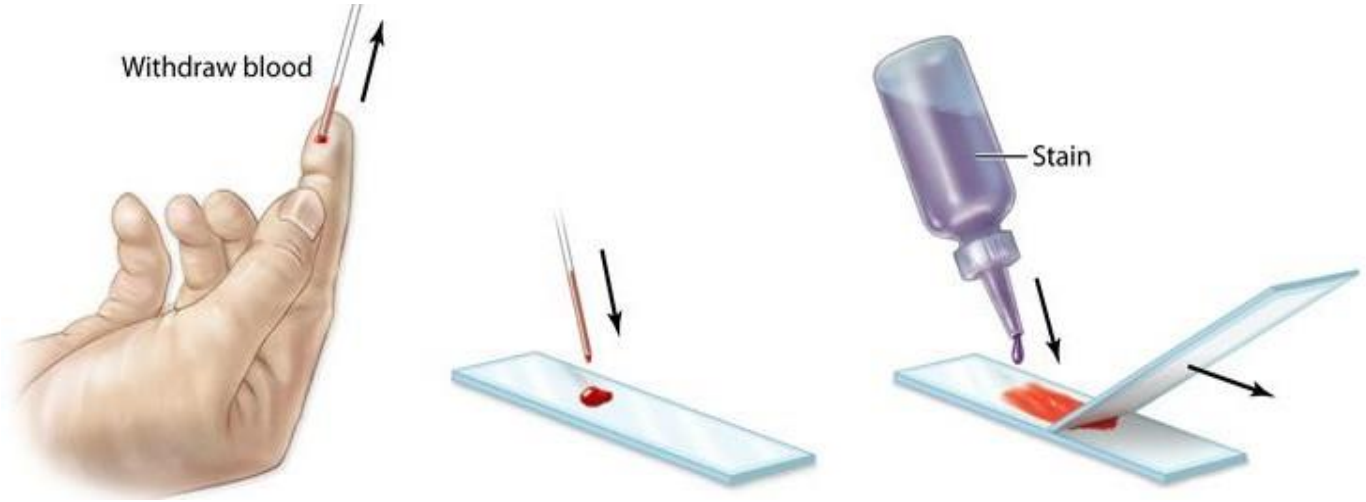
- **Eosin**, an acidic dye that stains pink to red
- **Methylene blue**, a basic dye that stains blue to purple

Dissolved in methyl alcohol (fixative)

-Some stains contain a third type of dye called **azure dye**

-Remember that:

the structures stained with acidic dye are called **acidophilic structures**, while the structures stained with basic dye are called **basophilic structures,** and the structures stained with azure dye are called azurophilic structures.



Blood film/smear

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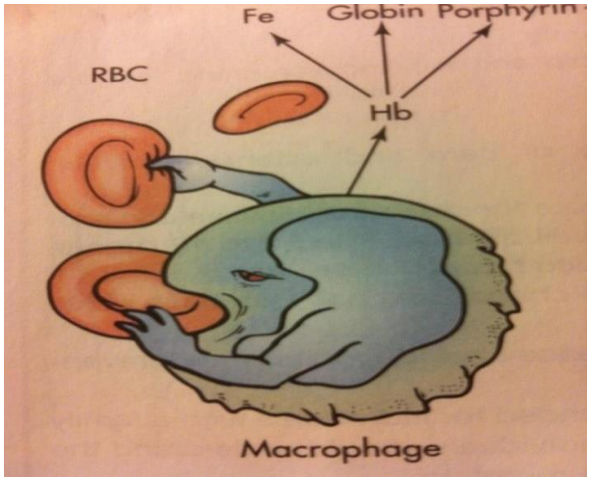
Erythrocytes (RBCs)

- Small bags of hemoglobin
- Small, biconcave discs
- Transport oxygen and CO_2, cytoplasm is full of hemoglobin molecules
- **Have no nuclei or organelles**
- The only organelle that can be found in RBCs is the plasma membrane and the cytoplasm is completely filled with hemoglobin → we should call them **Red Blood Corpusles**
- We call the process of RBCs formation **erythropoiesis**
- Pick up O_2 at lung capillaries and release it at body tissue capillaries

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



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

The precursor cells are nucleated and have organelles [mainly ribosomes for the synthesis of hemoglobin]

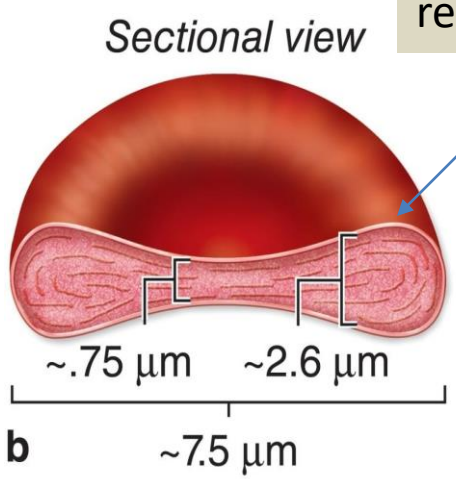
- **Fate:** Survive for ~100-120 days in the circulation. Worn out RBCs are removed by macrophages of the spleen, bone marrow and liver.

The importance of the biconcavity of RBCS:

1.Biconcave shape provides 20-30% greater surface of the plasma membrane compared to the substance of RBCs and this will increase the ability of these cells to exchange gases across their plasma membrane

2. Providing flexibility for RBCs to enable their passing through tiny capillaries because the diameter of the red blood cell is about 6-9 um in diameter (7.5 um) while the diameter of tiny capellaries is about (4 um) and while they're passing through these tiny capellaries they form something called cupping.

Erythrocytes have rounded edges to facilitate their movement within blood vessels.
If the edges were pointed this cell will stick to the wall of tiny blood vessels or bifurcation areas resulting in hypoxia.



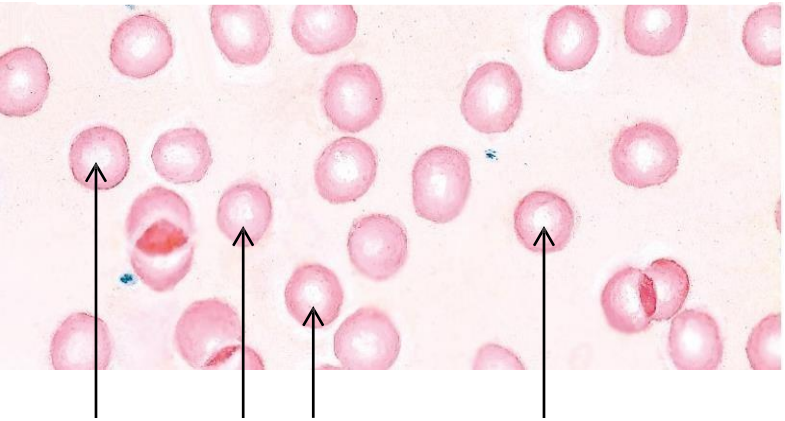
All morphological features Of RBCs facilitate their function in gas exchange

Note:
the spherical shape doesn't mean higher amount of haemoglobin because sphere shape will provide less surface area for the plasma membrane and the capacity to exchange gases will decrease

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

Erythrocytes are acidophilic and anucleated
The amount of haemoglobin in the centre of the cell is less than the peripheral part of it .



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

Eosinophilia/ acidophilia due to their High content of Hemoglobin (basic protein) producing pinkish or red color under the light microscope

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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 because they hold the plasma membrane with the substance of erythrocytes (the plasma membrane has surface area larger than erythrocyte itself) and prevent fragmentation of plasma membrane

Size

6-9 um in diameter (7.5 um)

Thickness

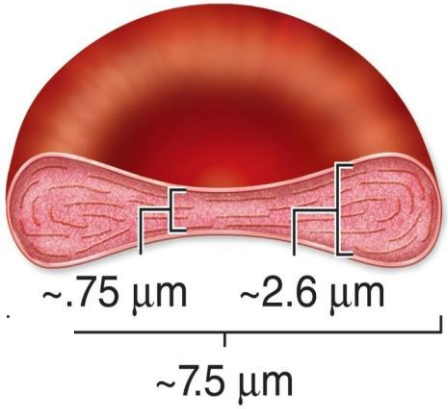
2.6-µm thick at the rim, but only 0.75-µm thick in the center

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

The plasma membrane is the only organelle within RBCs.

The cytoplasm of these cells is filled with haemoglobin. About 33% is occupied with haemoglobin and 66% occupied by water while only 1% of it is enzymes necessary for gases exchange

Sectional view

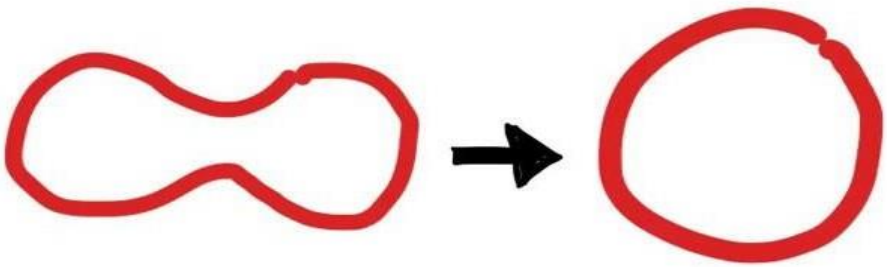


Top view

NOTE :

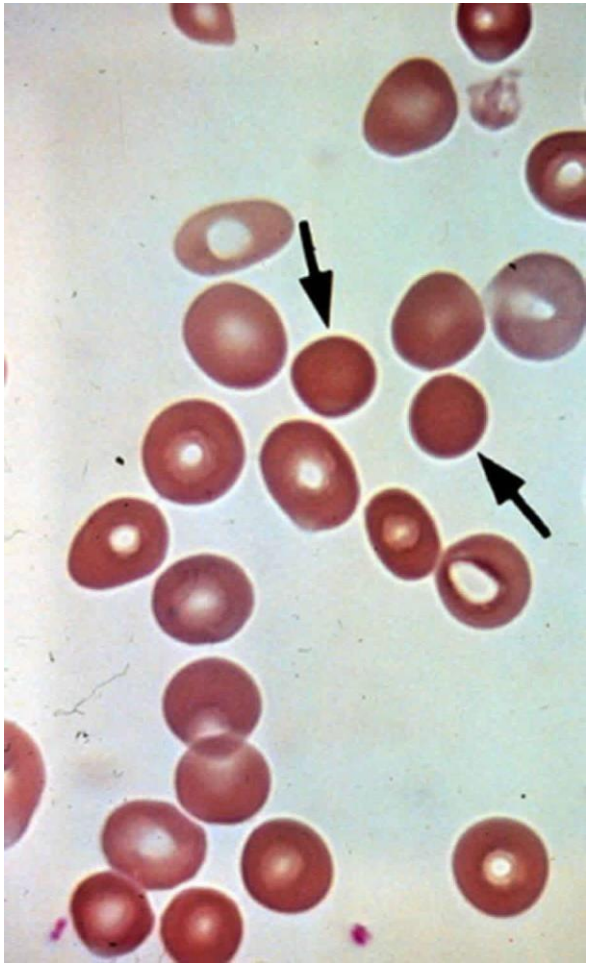
From top view the erythrocytes appear rounded in shape but in fact from sectional view they appear biconcave (it has a concavity from above and down)

Hereditary Spherocytosis



Spherocytosis :most of the cells in peripheral blood are spherical in shape instead of having a biconcave shape

Caused by mutations in genes relating to membrane proteins that allow the erythrocytes to maintain their biconcave shape disabling the submembranes proteins from holding plasma membrane with the substance of red blood cells →resulting in membranous fragmentation when cells pass through tiny capillaries and end up with spherical RBCs called spherocytes



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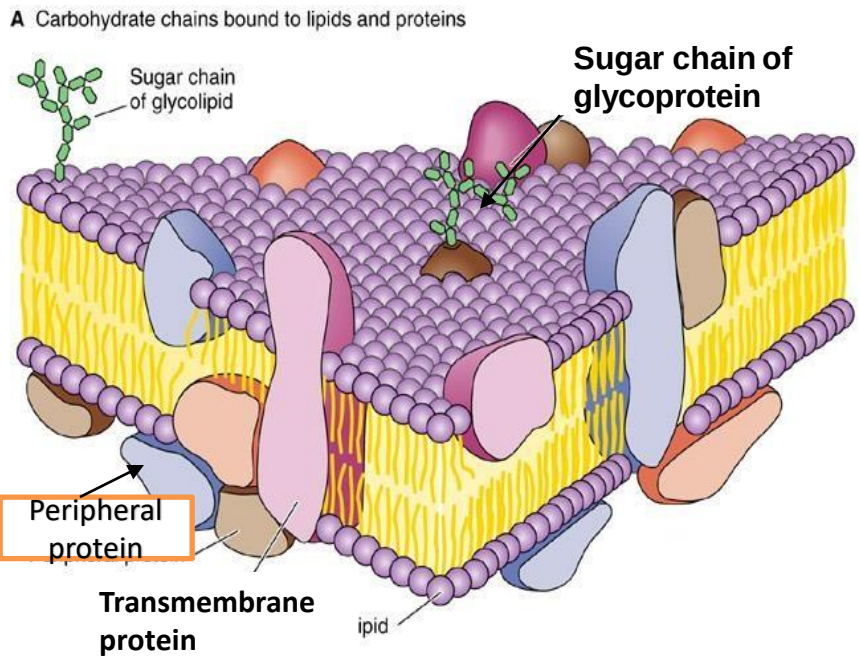
Cell Membrane



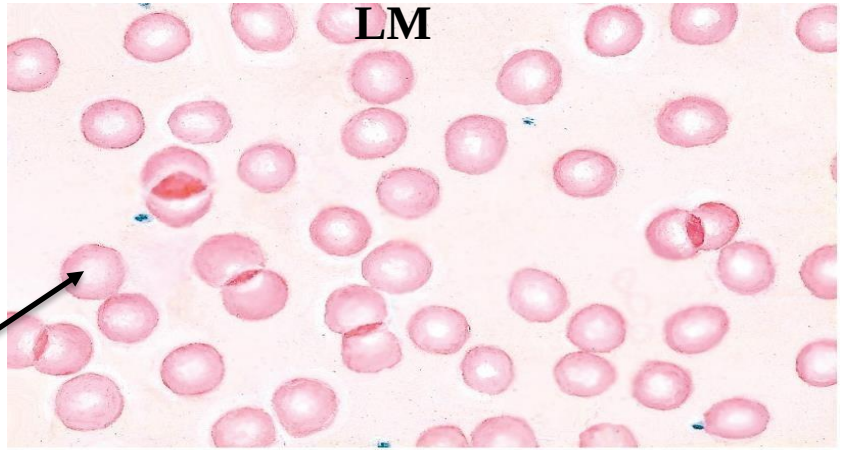
The cell membrane of erythrocytes is highly selective and flexible because 50% of plasma membrane is formed by proteins while 40% is lipids and 10% sugars or carbohydrates .
That is why the plasma membrane facilitates the fixability and cupping of RBCs while passing through tiny capillaries

Some peripheral proteins are attached to the cytoplasmic surface of the cell and others are attached to intracytoplasmic surface of the cell membrane

The plasma membrane has 2 types of sugar :
1.Glycoproteins
2.Glycolipid

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

LM:
Blood film stained with Leishman:
Rounded
Non nucleated
Acidophilic (with pale central area)



Rounded cells with pale central area and they are acidophilic because of haemoglobin

Under the transmission electron microscope they appear as electron dense cell because inside the cell there is only haemoglobin (no organelles or nucleus)so it appears homogeneous

Notice the cupping of the erythrocyte while it is passing through capillary

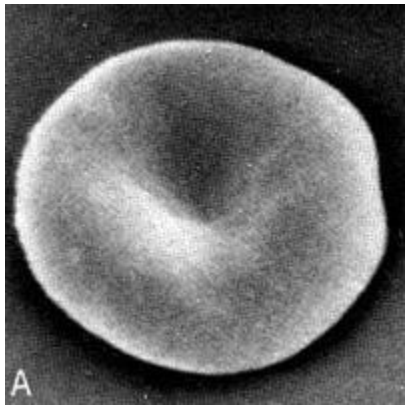
Scanning electron microscope scans the surface of the cells so we can see the biconcave shape.

EM:
✓Have no nucleus or organelles
✓Filled with hemoglobin

Electron dense and homogenous

TEM

SEM



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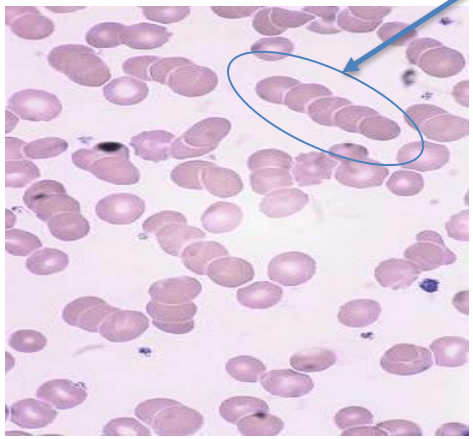
Rouleaux appearance occurs to some extent in all films



This is normal and considered an artifact resulting from histological preparation where the RBCs stick to each other. But if Rouleaux appearance is significant in the blood film it could be a non specific indication of pathology .
for example at the infection site we have increase in the viscosity of blood forming this appears also in certain types of cancers or in case of increased concentration of plasma proteins this will increase the viscosity of blood and the possibility that these RBCs stick to each other forming Rouleaux appearance it also can take place in diabetic patients and inside varicose veins where we have accumulation of blood inside the dilated veins

.

Rouleaux formation:

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

✓In slow (not in normal) circulation so they don't normally stick to each other .

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)

Abnormal shapes:

Due to changes either in the cell membrane or Hb content

if we have an abnormal haemoglobin or mutation in the submembranes proteins this could result in abnormal shapes of erythrocytes

Abnormal staining:

Hypochromia: Denotes a decrease in the intensity of staining
Indicates a decreased amount of hemoglobin
Frequently accompanies microcytosis

Hypochromic microcytic anemia

Sickle cell anaemia results from abnormal hemoglobin

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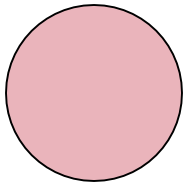
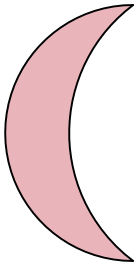
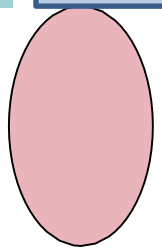


Spherocytes

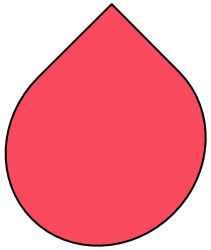


Sickle cells

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



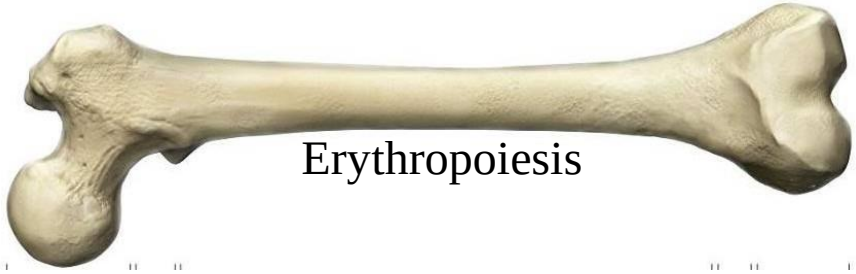
Ovalocytes

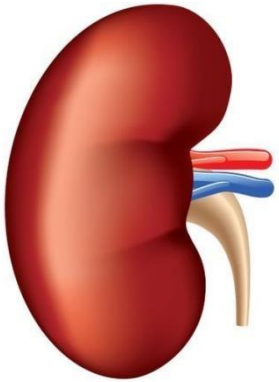


Poikilocytes

The cells may show blunt/ pointed projections from their surfaces

the consequences of this abnormal shape :
1.Less capacity to carry oxygen
2.Sticking to the wall of capillaries (hypoxia)





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

Erythropoietin is produced by the kidneys (the endothelial cells of the blood vessels inside the kidney)

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

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Consequently

People living at high altitudes usually have higher RBC count as a response to lower oxygen levels.because the air pressure there is less than air pressure inside our lungs so it is difficult for them to obtain oxygen as a result there will be lower oxygen inside our blood which will stimulate the kidney cells to secrete erythropoietin that will stimulate erythropoiesis inside the bone marrow .NEEDS 1 WEEK

PHYSIOLOGICAL CONDETION

Athletes whose demand for oxygen is more elevated to supply their muscles , also have higher RBC counts.

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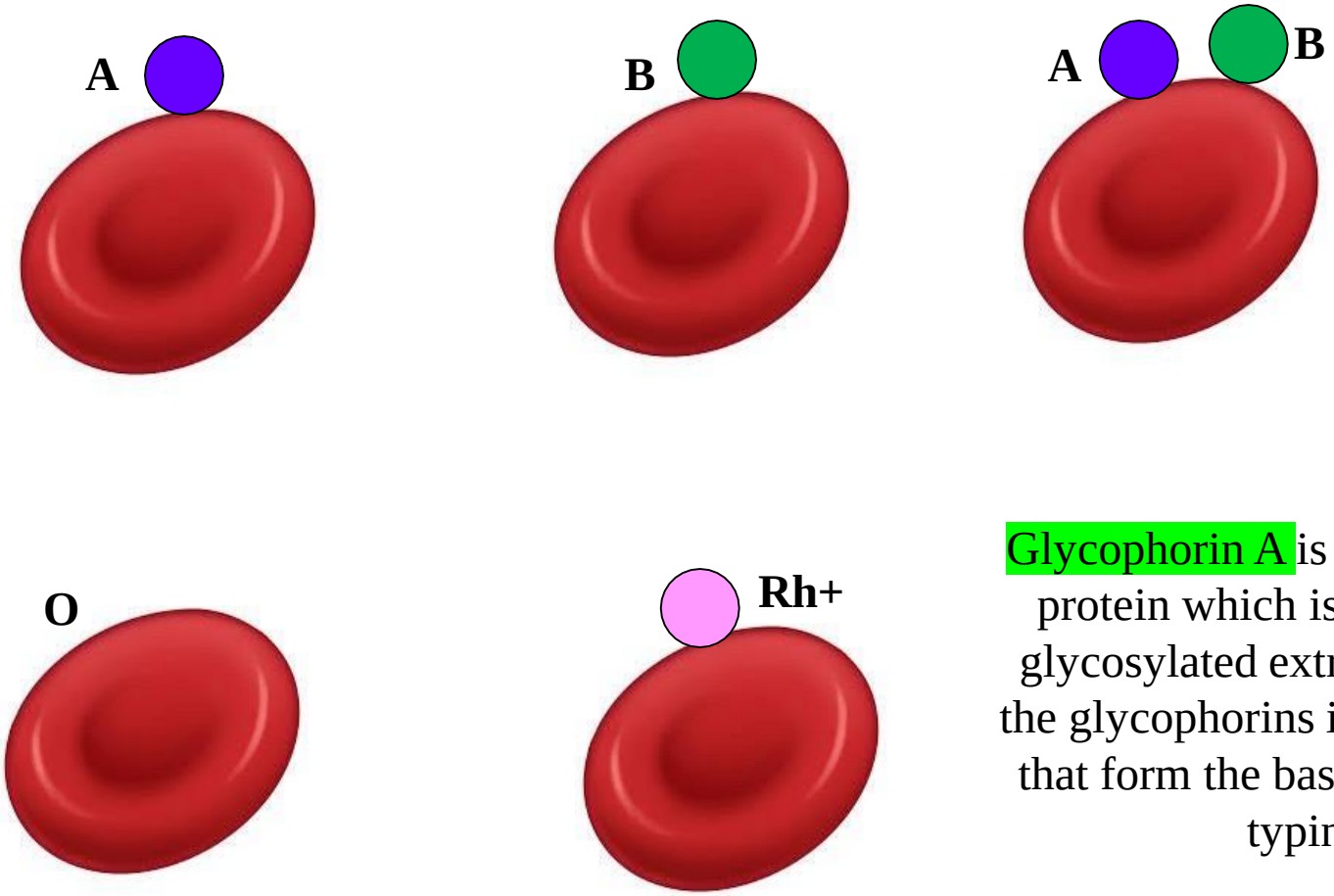
RBC plasma membranes have glycoprotein antigens on their external surfaces



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

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