Blood Cells

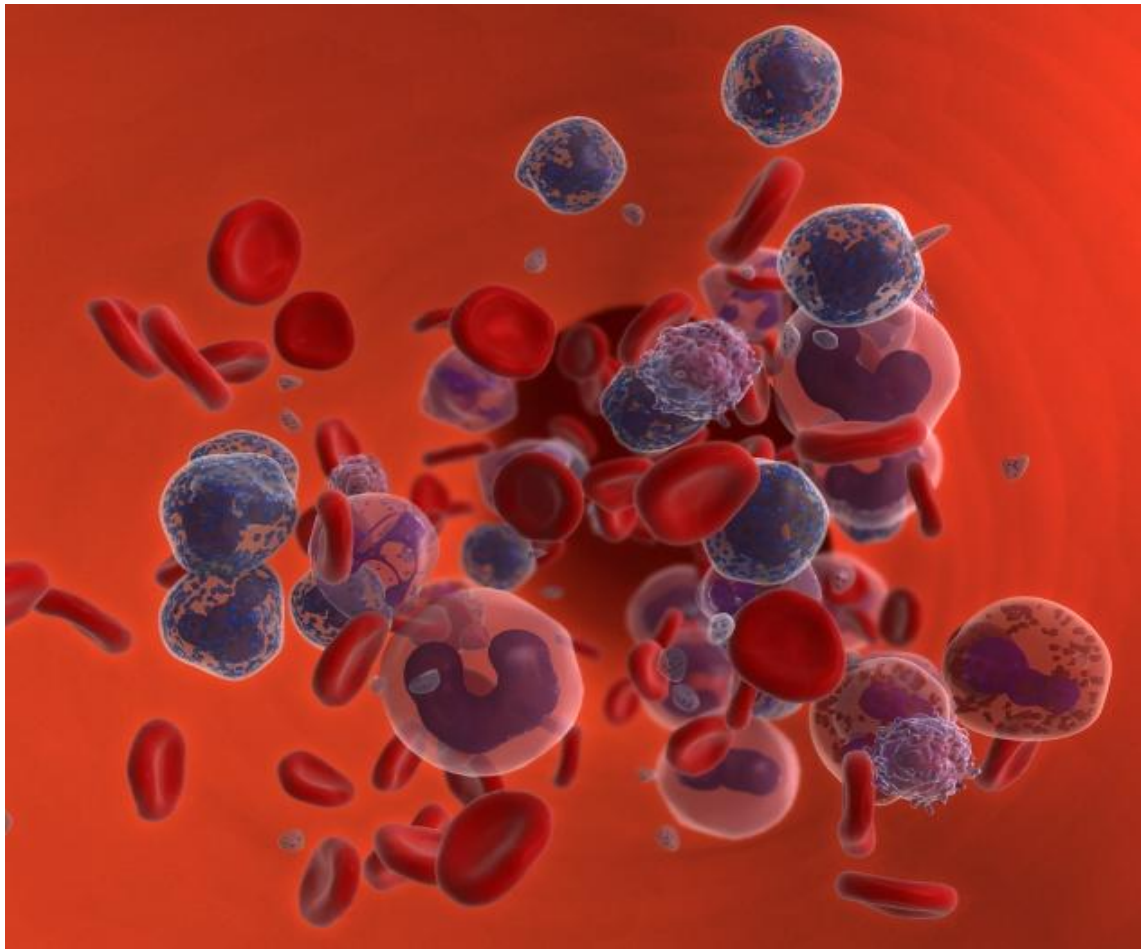
Dr. Heba Kalbouneh
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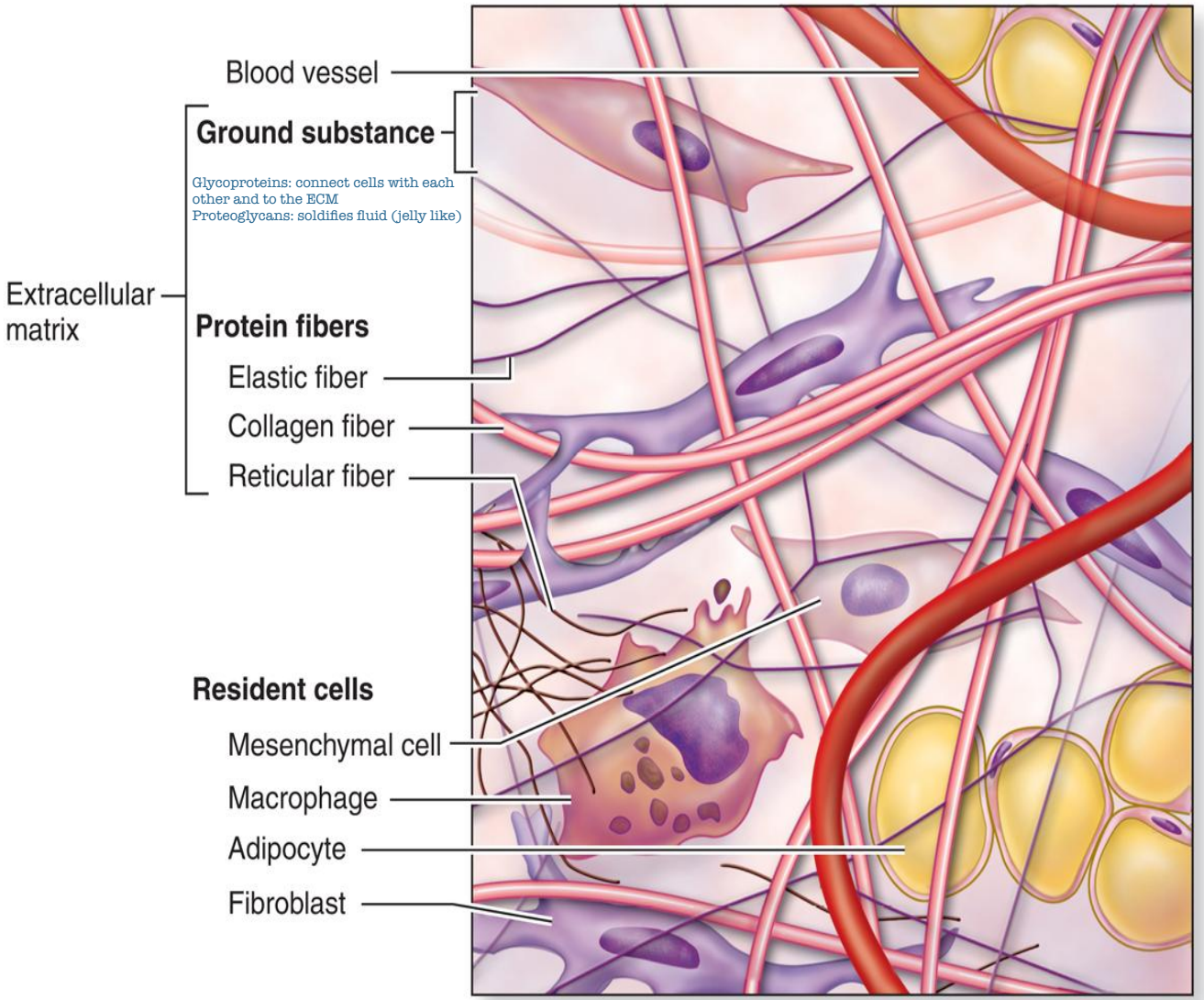
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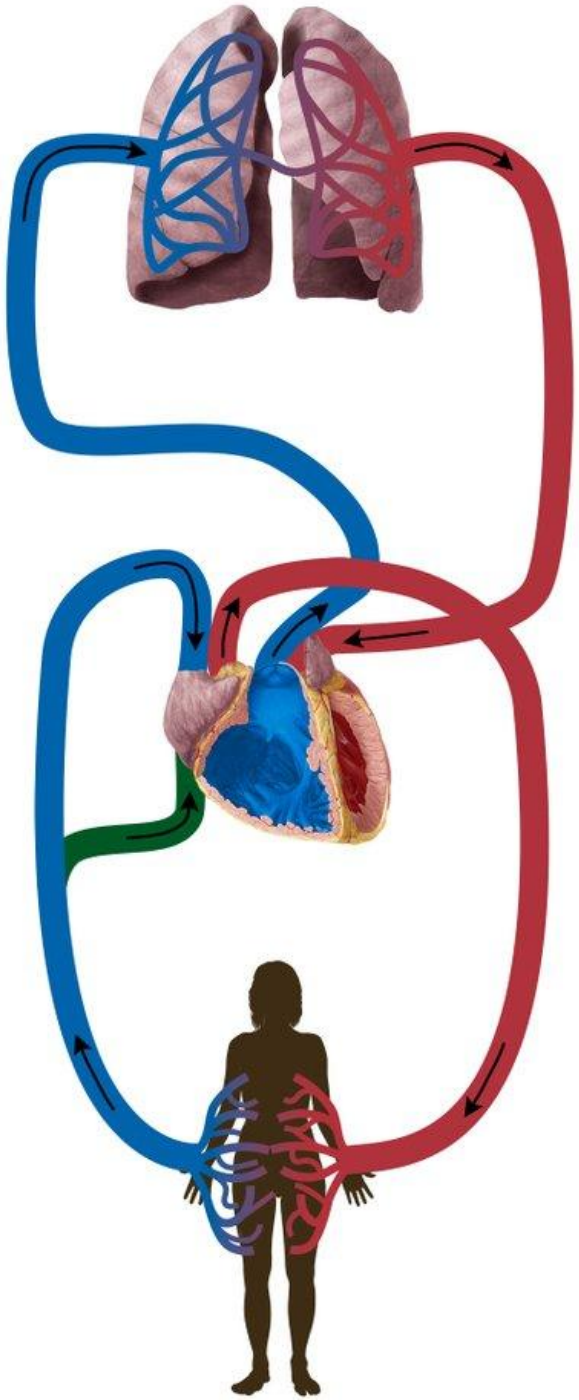
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Blood

- Specialized form of **connective tissue**
- Components:
  - Blood cells (several types)
  - Plasma (extracellular matrix)







Functions of Blood

- Transports nutrients and respiratory gases.
- Transports waste products to organs and tissues where they can be recycled or eliminated.
- Transports hormones.
- Transports immune cells throughout the body.
- Helps regulate body temperature.
- Maintains acid–base and osmotic balance.

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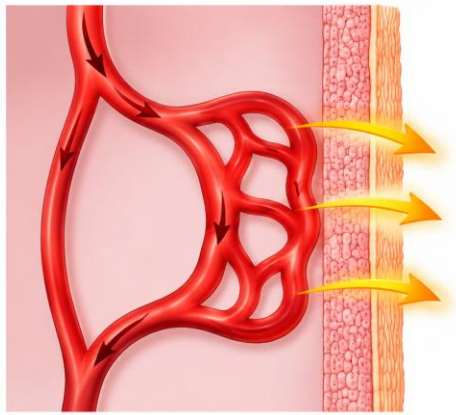
Characteristics of Blood

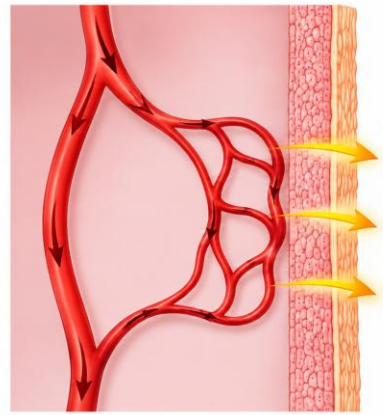
pH: Approximately 7.4

Color: Depends on the amount of oxygen:

–More oxygen → brighter red

–Less oxygen → darker/duller red





Blood Circulation

✓ Blood is propelled mainly by the rhythmic contractions of the heart.

✓ An average adult has approximately 5–6 liters of blood.

✓ Blood moves unidirectionally within the closed circulatory system.

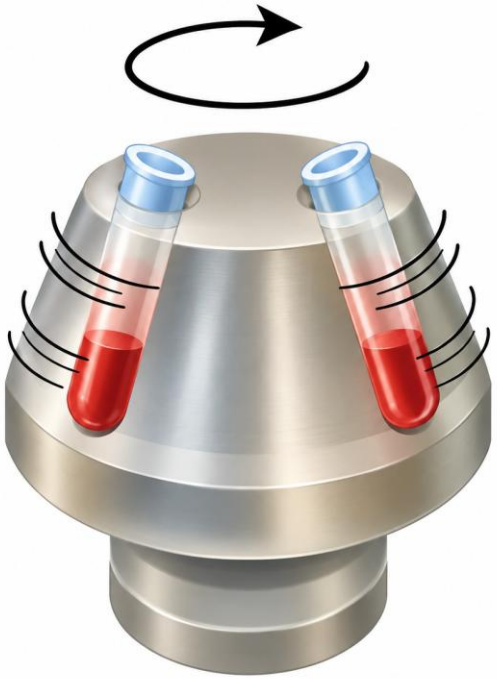
1

Withdraw blood and place in tube



2

Centrifuge





Blood collected with an anticoagulant (e.g., heparin, citrate, or EDTA) can be separated by centrifugation into distinct layers that reflect its heterogeneous composition.

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Composition of Blood

Formed Elements ~45%

- **Erythrocytes (RBCs):** transport oxygen
- **Leukocytes (WBCs):** immune defense
- **Platelets (thrombocytes):** involved in blood clotting

Plasma ~55%

- Pale-yellow fluid component of blood
- ~90% water
- ~10% dissolved substances, including:
  - Plasma proteins (e.g., albumin)
  - Electrolytes
  - Nutrients
  - Metabolic waste products
  - Dissolved gases
  - Hormones

Serum = Plasma − Clotting Factors



Top layer
Plasma

Leukocytes and platelets
Middle layer
Buffy coat yellowish-gray layer

Bottom layer
RBCs
(Packed Cell Volume)

Hematocrit (Hct)

Hematocrit: The percentage of the total blood volume occupied by red blood cells (RBCs).

Example:

A hematocrit of 40% means that 40 mL of every 100 mL of whole blood consists of RBCs.

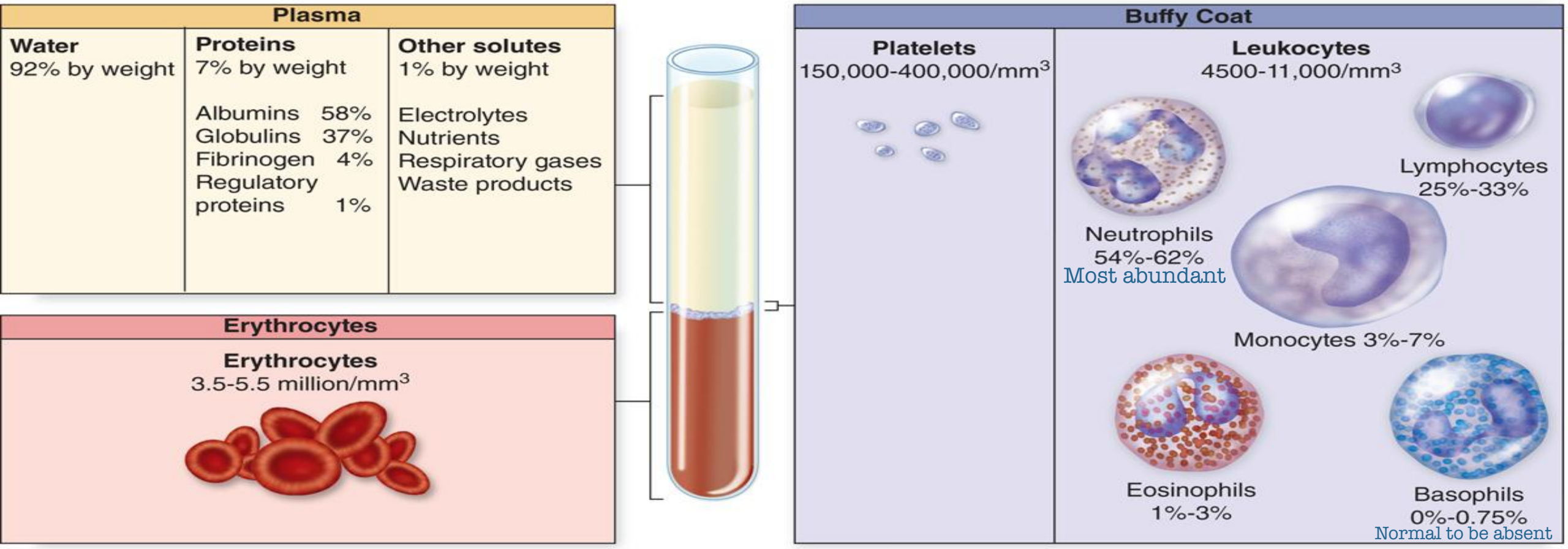
Normal hematocrit values:

- **Males: 40–53%** Testosterone stimulates hematopoesis
- **Females: 36–48%**

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NOTE: A cubic millimeter of blood is equivalent to a microliter (μL)

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



Total leukocyte count (TLC): Total number of leukocytes per μL of blood.
Differential Leukocyte Count (DLC): Percentage of each leukocyte type among 100 leukocytes counted.

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Albumin

- ✓ The most abundant plasma protein.
- ✓ Synthesized mainly by the liver.
- ✓ Helps maintain colloid osmotic (oncotic) pressure of the blood.
- ✓ Acts as a carrier protein, transporting substances such as: Steroid hormones and Fatty acids

Fibrinogen

- ✓ The largest major plasma protein.
- ✓ Synthesized by the liver.
- ✓ Plays an essential role in blood clotting.
- ✓ During clot formation, fibrinogen is converted into fibrin, which forms a mesh that helps stabilize the blood clot.

Fresh plasma



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Importance of Plasma Proteins

Plasma proteins help maintain the colloid osmotic (oncotic) pressure of blood, preventing excessive fluid loss from the blood vessels into the tissues.

→ help keep fluid within the blood vessels.

Globulins (α, β and or γ globulins):

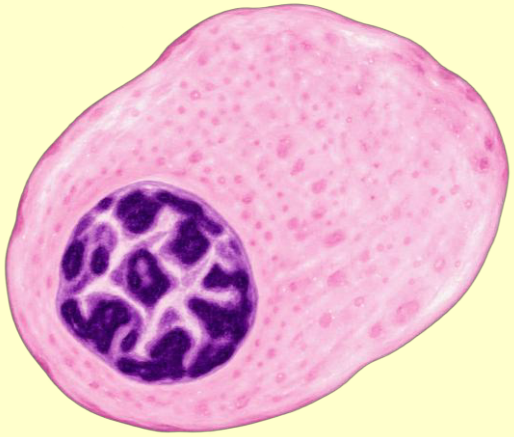
α- and β-Globulins

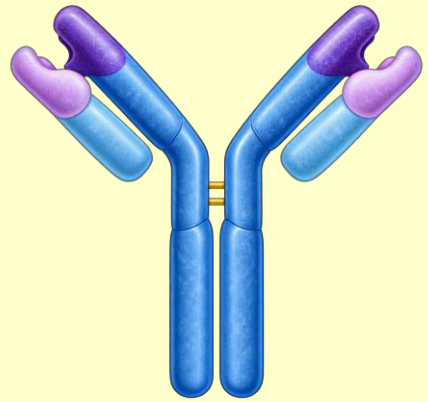
- ✓ Synthesized mainly by the liver.
- ✓ Function primarily as transport proteins.
- ✓ Transport substances such as: Lipids, Fat-soluble vitamins, and Iron → *Iron is transported mainly by transferrin, a β-globulin.*

γ-Globulins (Immunoglobulins/Antibodies)

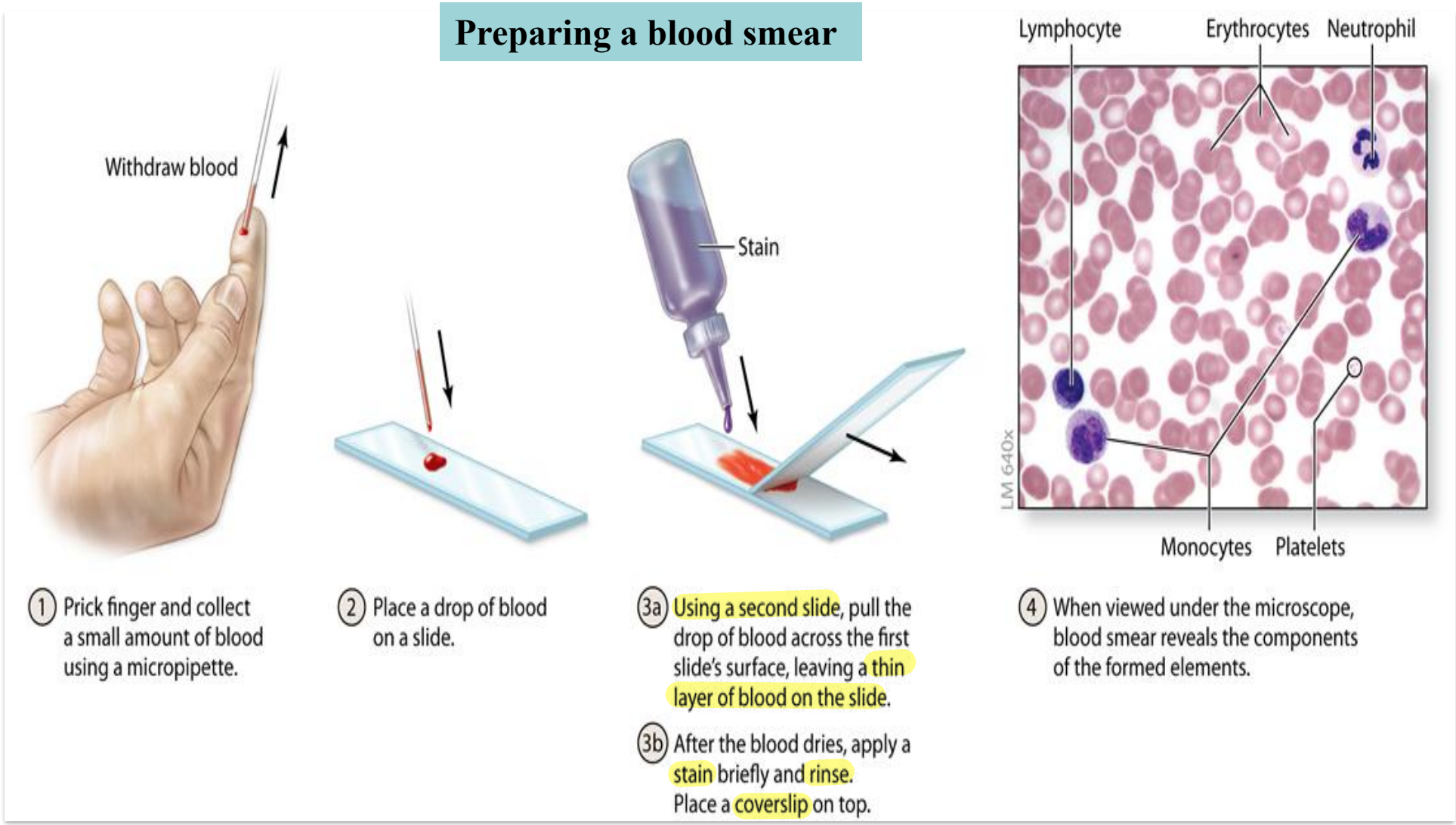
- ✓ Produced and secreted by plasma cells.
- ✓ Function as antibodies.
- ✓ Play an essential role in humoral immune defense.

Plasma cell





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

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The Staining of Blood Cells

Blood film/ smear: A drop of blood is spread into a thin layer on a glass slide and allowed to air-dry.

Staining: with polychromatic stain e.g **Leishman's stain**

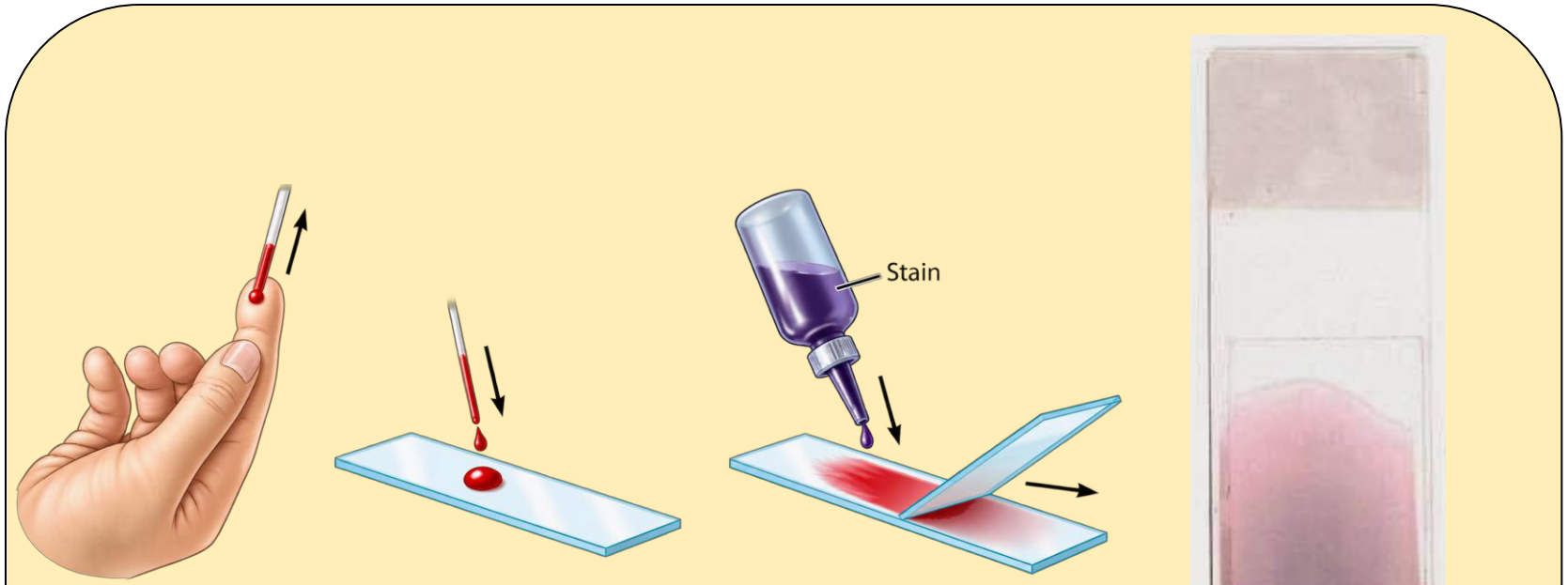
Leishman stain is a blend of eosin and polychromed methylene blue. When methylene blue is exposed to heat and alkali (before mixing it with eosin), it oxidizes, breaking down into methylene blue and its derivatives specifically the azure dyes like Azure B. Eosin, which is acidic, stains basic cell components pink or red, while the basic azure dyes bind to acidic components like DNA and RNA in cell nuclei, staining them blue or purple.

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

Polychromatic Stains

Commonly used for staining peripheral blood smears:

- Giemsa stain
- Wright stain
- Leishman stain

These are Romanowsky-type stains that use combinations of acidic and basic dyes to differentiate blood cells and their components.

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Erythrocytes (RBCs)

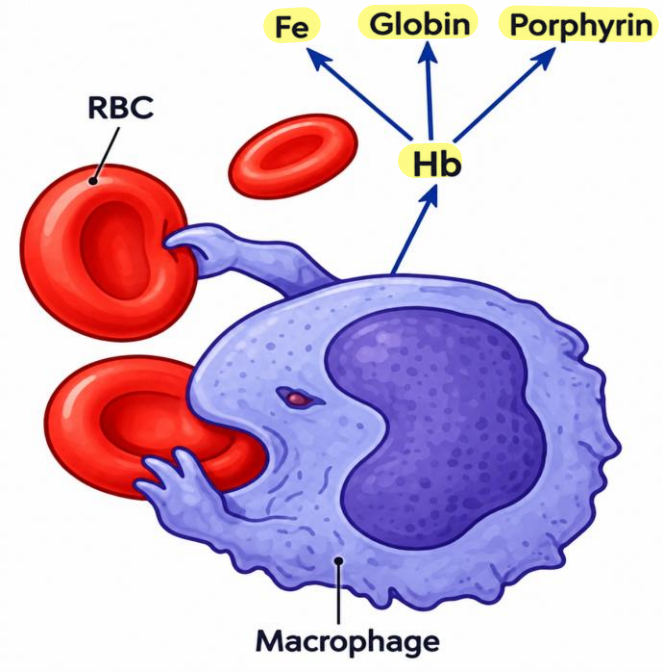
- Small, biconcave discs.
- Specialized for the transport of O_2 and CO_2.
- Cytoplasm is packed with hemoglobin.
- Mature RBCs have no nucleus or organelles.
- Pick up O_2 in the pulmonary capillaries and release it in tissue capillaries.
- Carry part of the CO_2 from tissues back to the lungs.

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



Life Span and Fate

- Circulate for approximately 120 days.
- Aged or damaged RBCs are removed by macrophages, mainly in the spleen, and also in the liver and bone marrow.
- Their components, particularly iron, are recycled.



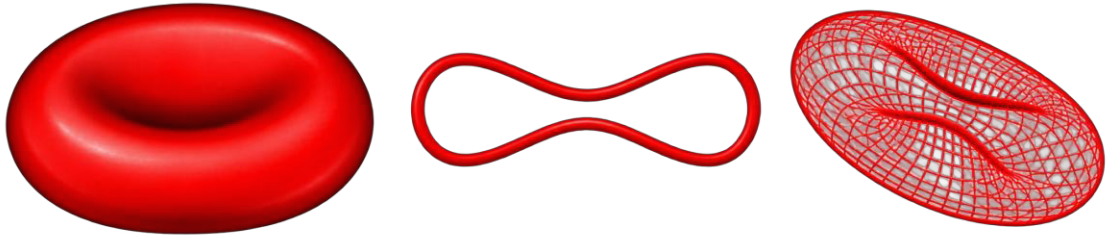
Note: During maturation, erythrocytes extrude their nuclei, progressively lose their cytoplasmic organelles and enter the bloodstream as anucleate cells.

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

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

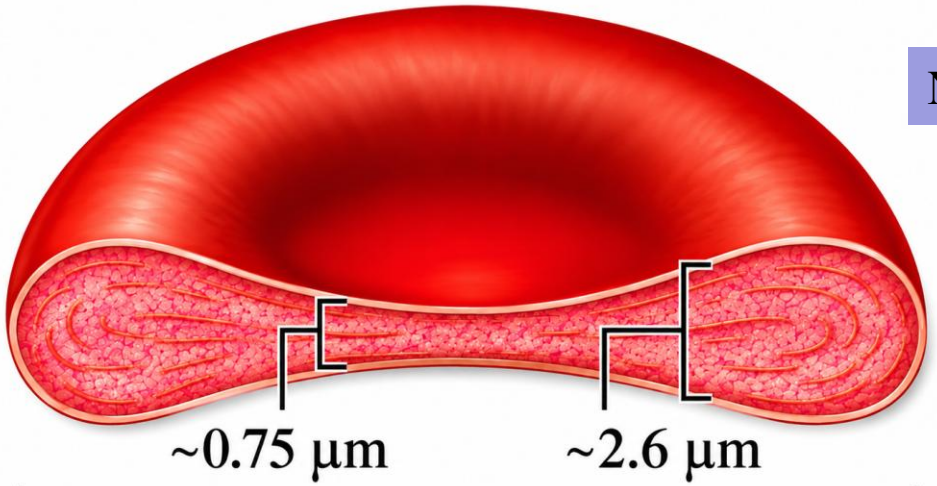
Prevents the fragmentation of membrane when passing through small capillaries



Top view



Sectional view



Normocytic RBCs

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

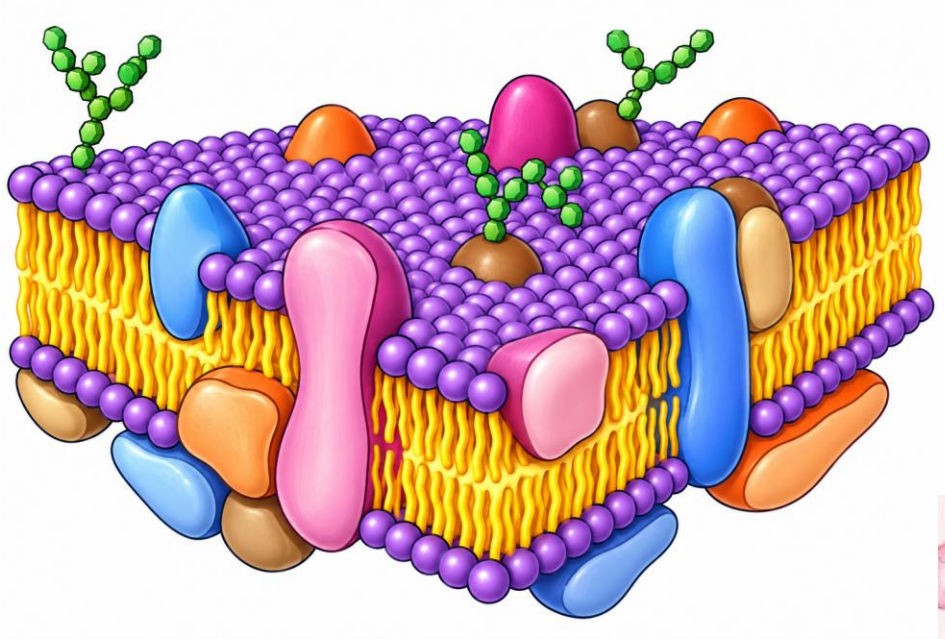
!!!!! Erythrocytes can be used as a size reference for other cell types

The Diameter of There are capillaries that are 4um in diameter

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

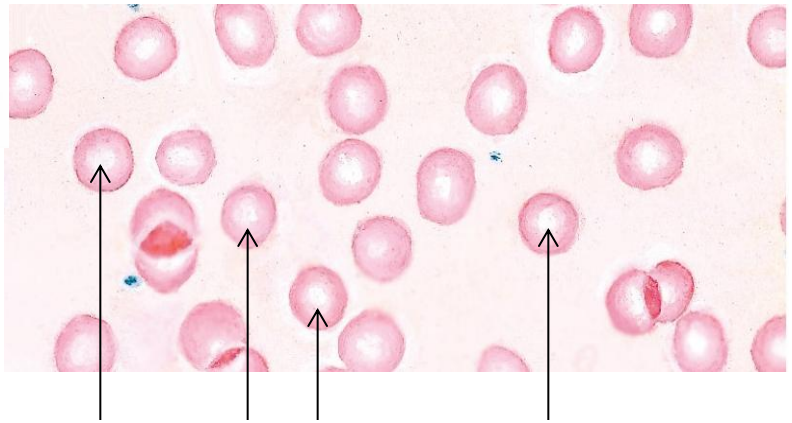
The biconcave shape, together with the flexibility of the plasma membrane and its underlying cytoskeleton, allows erythrocytes to deform and pass through narrow, tortuous capillaries.



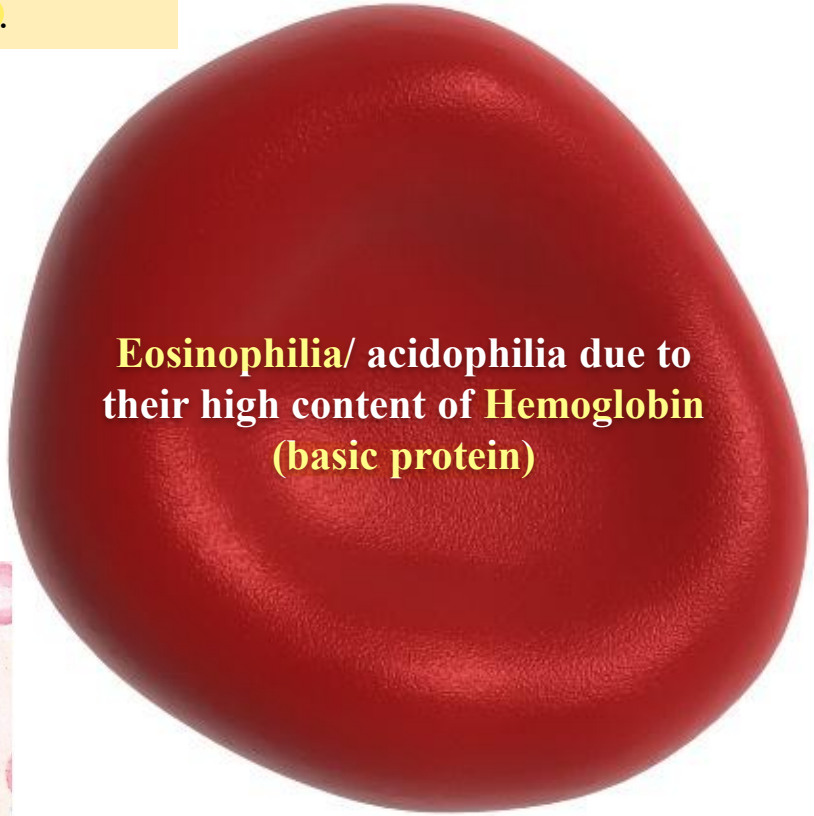
RBC membrane: approximately 50% protein, 40% lipid, and 10% carbohydrate by weight.

Lipid bilayer → provides fluidity

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



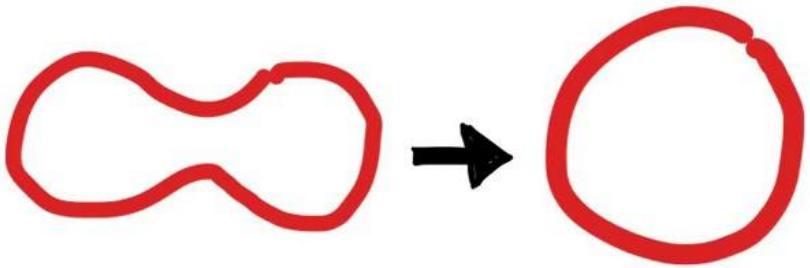
The pale staining of the central region is a result of its biconcave disc shape ⟹ **Normochromic RBCs**

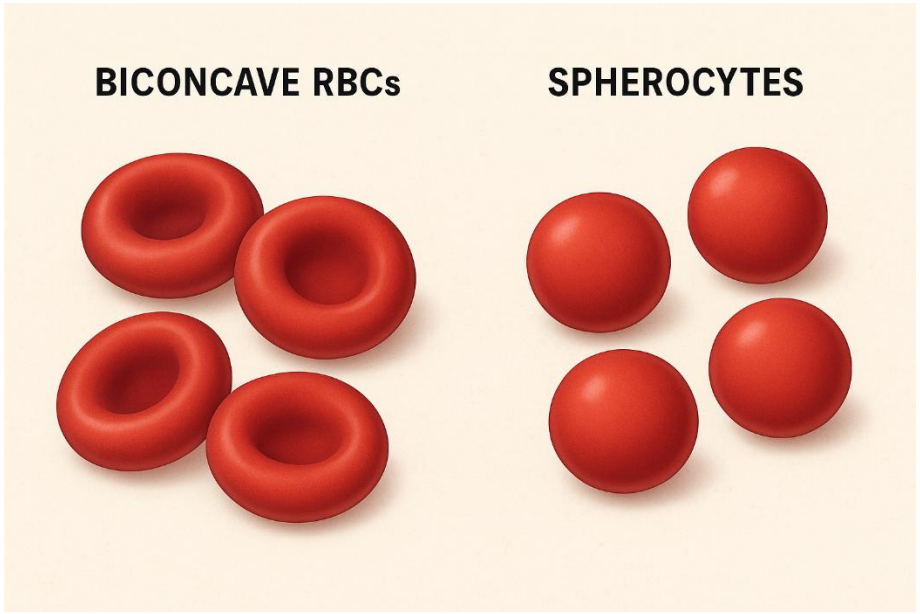


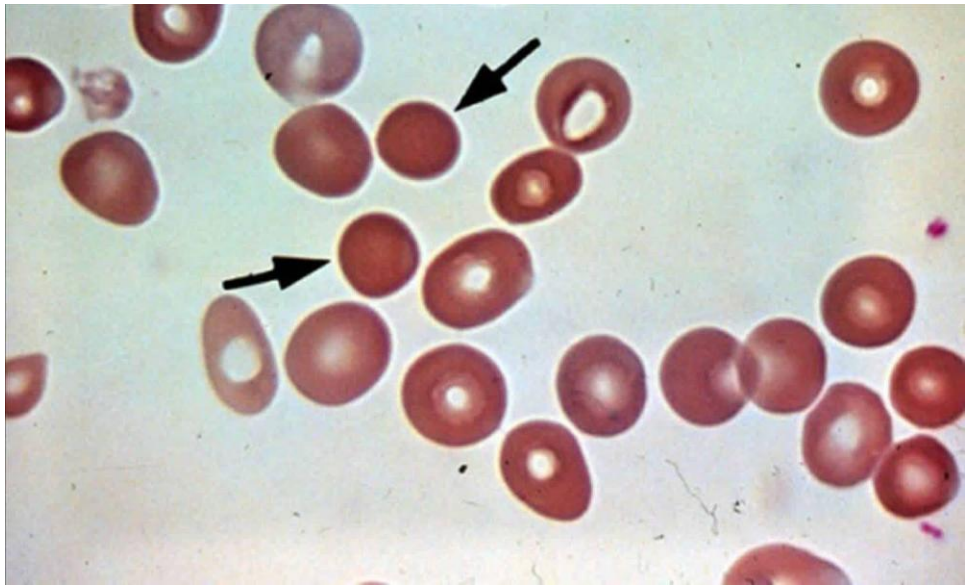
Eosinophilia/ acidophilia due to their high content of Hemoglobin (basic protein)

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Hereditary Spherocytosis







Hereditary spherocytosis: Caused by defects in RBC membrane cytoskeletal proteins (especially spectrin and ankyrin), resulting in loss of the normal biconcave shape and formation of spherical, less deformable RBCs.

In spherocytosis, membrane loss decreases the surface-area-to-volume ratio. The RBC becomes a sphere, with no central pallor

Spherocytosis → membrane loss → spherical RBC + loss of central pallor + increased splenic destruction.

Erythrocytes are removed by macrophages in the liver, spleen and bone marrow. Because in spherocytosis, the red blood cells are spherical they get trapped within the splenic capillaries leading to their destruction, which could lead to anemia.

LM:

Blood film stained with Leishman:

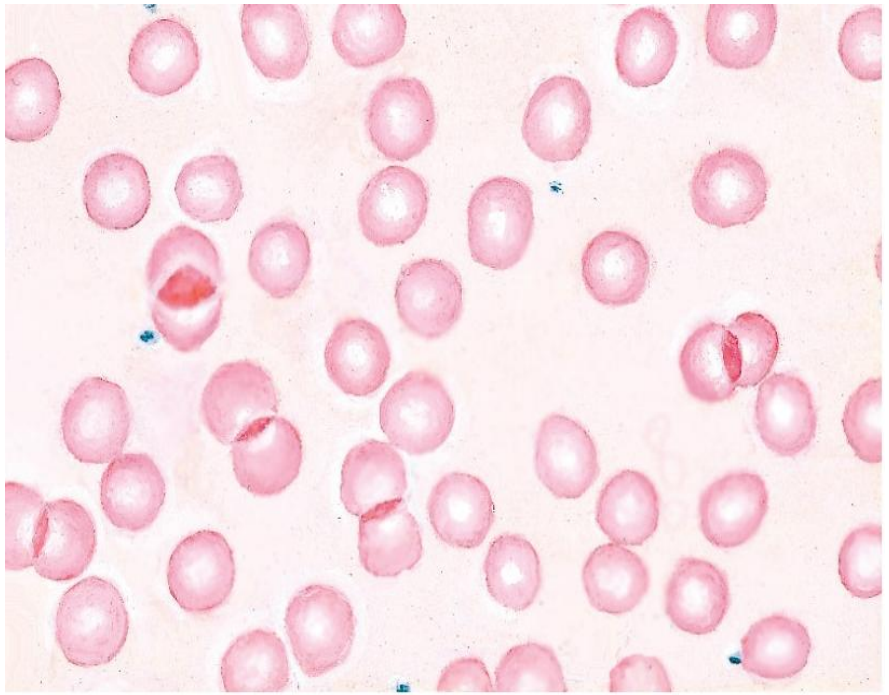
Rounded

Non nucleated

Acidophilic (with pale central area)

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

LM



TEM Of Erythrocytes Passing Through Capillary Wall



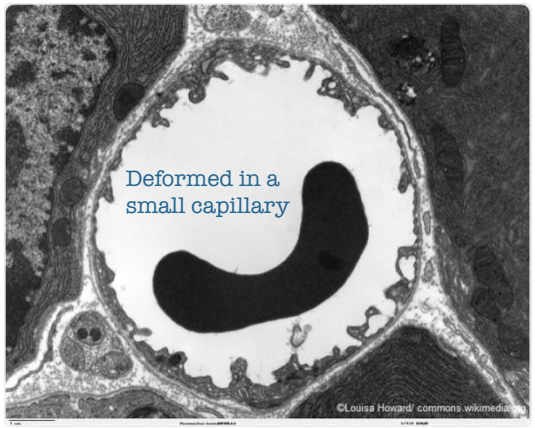
EM:

✓Have no nucleus or organelles

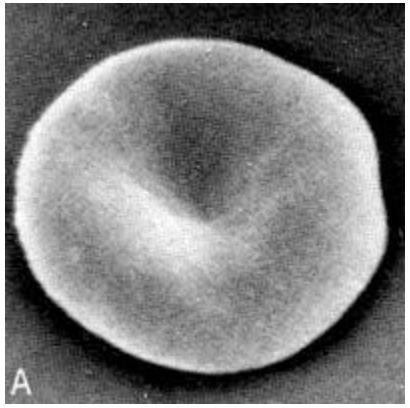
✓Filled with hemoglobin

Electron dense and homogenous

TEM



SEM

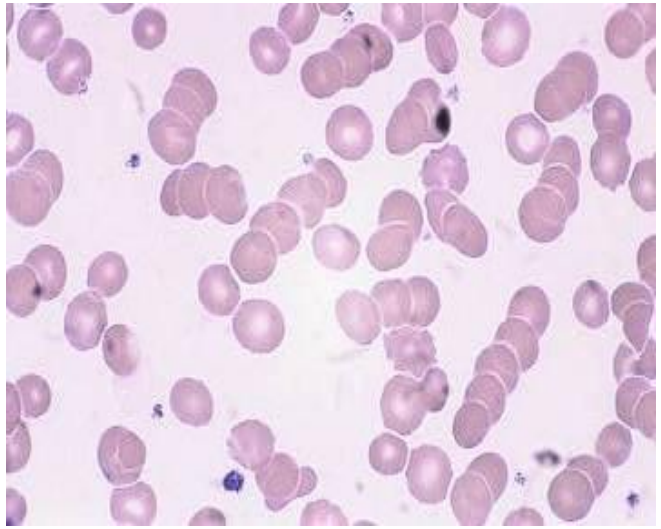


Single Erythrocyte squeezing through capillary



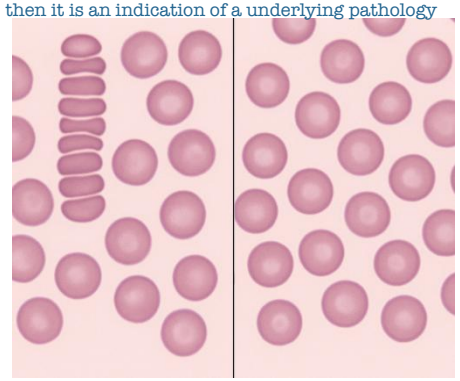
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Rouleaux appearance occurs to some extent in all films

Can be seen in a normal person but if it is too much then it is an indication of a underlying pathology







Normally, RBCs tend to repel each other because their surfaces carry a negative charge (zeta potential).

When plasma proteins increase, they reduce this repulsive force, allowing RBCs to come together and form rouleaux.

Rouleaux formation: is the arrangement of red blood cells in stacks resembling a stack of coins.

✓In slow (not in normal) circulation — An increase in plasma proteins can raise blood viscosity. Higher viscosity increases the resistance to blood flow which can result in slower circulation.

Due to reduced repulsive forces (zeta potential) between erythrocytes, especially in the presence of increased plasma proteins such as fibrinogen and globulins (reversible)

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

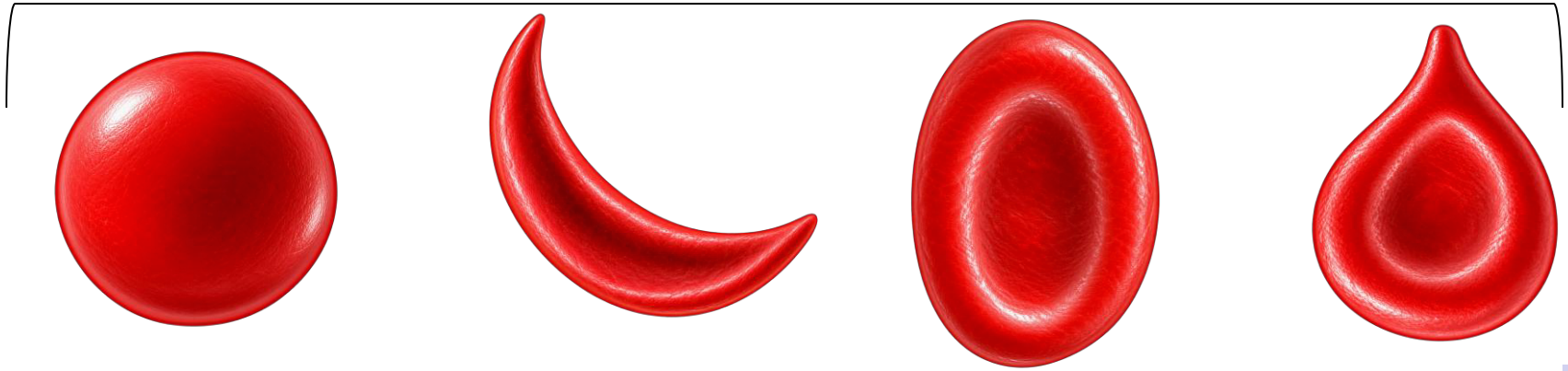
Abnormal sizes:
Microcytes (<6um)
Macrocytes (>9um)
Anisocytosis (different sizes)

Abnormal shapes:
Due to changes either in the cell membrane or Hb content
Poikilocytosis: variation in RBC shape

Abnormal staining:
Hypochromia: Decreased staining intensity of RBCs due to reduced hemoglobin, resulting in increased central pallor.
Hyperchromia: Increased staining intensity of RBCs with reduced or absent central pallor, typically due to altered RBC shape, as seen in spherocytes.

Poikilocytosis = abnormal RBC shape
Anisocytosis = abnormal RBC size

Poikilocytes
(Poikilocytosis)

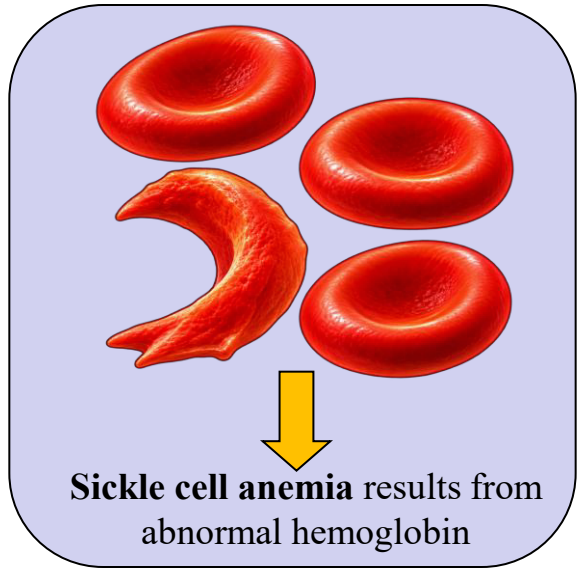


Spherocytes

Sickle cells

Ovalocytes
Elliptocytes

Dacrocytes → Teardrop shaped cells



Sickle cell anemia results from abnormal hemoglobin

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

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Based on Hemoglobin Content (color /chromasia):

Normochromic: RBCs have a normal hemoglobin concentration → exhibit normal pink staining with central pallor occupying approximately one-third of the cell diameter.

Hypochromic: RBCs have reduced hemoglobin concentration → appear paler with increased central pallor (commonly seen in iron deficiency anemia).

Hyperchromic: RBCs exhibit increased staining intensity with reduced or absent central pallor, often associated with increased hemoglobin concentration, as seen in hereditary spherocytosis.



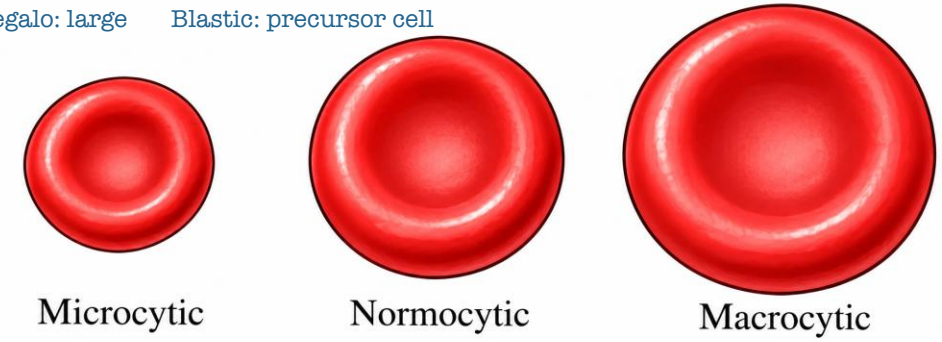
Together, these terms are used to describe and classify anemias (e.g., microcytic hypochromic anemia in iron deficiency, or macrocytic normochromic anemia in B12 deficiency).

Note: Hypochromia frequently accompanies microcytosis, resulting in **hypochromic microcytic anemia**, as commonly seen in iron deficiency anemia.

Explanation: In vitamin B12 deficiency and folate deficiency, DNA synthesis in red blood cell precursors in the bone marrow is impaired. Because these precursor cells cannot divide properly but continue to grow, they become abnormally large. These oversized cells eventually mature and enter the bloodstream as large, yet normochromic, red blood cells.

Cross-link with GI: Pernicious anemia is specifically an autoimmune form of Vitamin B12 deficiency, autoimmune attack on your stomach's lining, specifically destroying parietal cells. These cells produce intrinsic factor, a protein necessary for the absorption of vitamin B12 in the terminal ileum. Without enough intrinsic factor, vitamin B12 cannot be absorbed, leading to deficiency and the development of megaloblastic anemia, which is characterized by macrocytic normochromic appearance of RBCs.

Megalo: large Blastic: precursor cell



Based on Cell Size (cytometry):

Normocytic: RBCs are of normal size.
Microcytic: RBCs are smaller than normal (seen in iron deficiency anemia).
Macrocytic: RBCs are larger than normal (seen in vitamin B12 or folate deficiency anemia).

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When RBC count or blood oxygen-carrying capacity decreases, such as during blood loss, tissue hypoxia develops.

↓

Hypoxia is detected by the kidneys.

↓

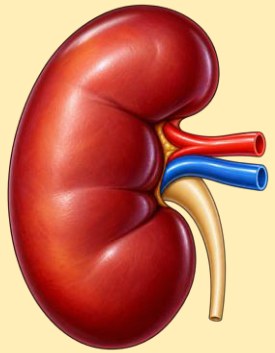
The kidneys respond by increasing the production and secretion of erythropoietin (EPO).

↓

Erythropoietin stimulates erythropoiesis in the red bone marrow.

↓

RBC production increases, improving the oxygen-carrying capacity of the blood.





At high altitudes, lower oxygen levels stimulate erythropoietin production, leading to an increased RBC count.



Increased oxygen demand

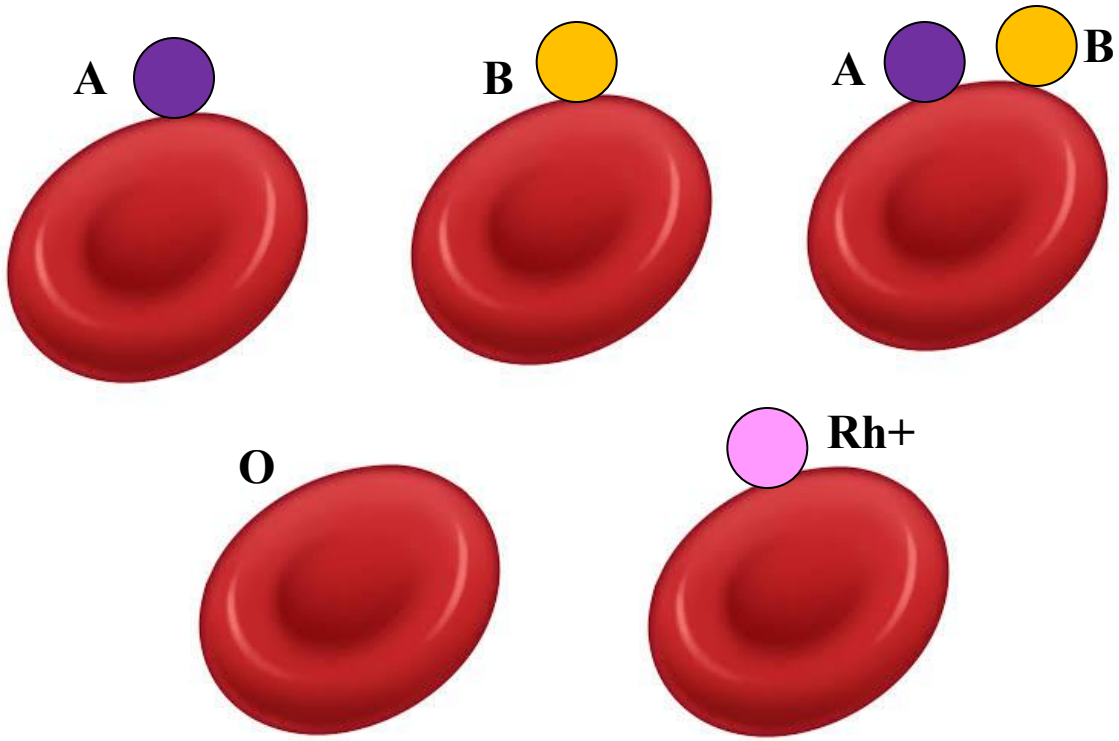
Endurance athletes may have an increased red blood cell production to support greater oxygen demands.



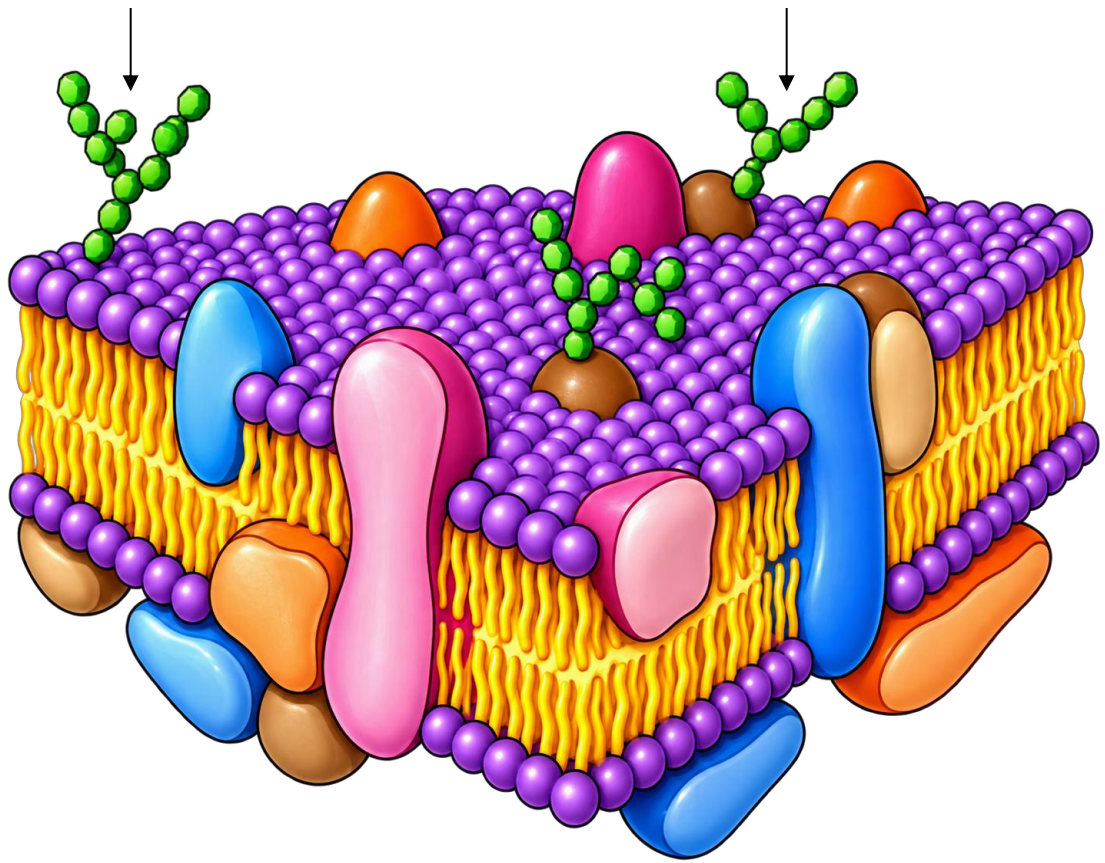
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RBC plasma membranes carry carbohydrate antigens (A and/or B antigens) on their external surface.

These carbohydrate antigens are attached to glycoproteins and glycolipids in the RBC membrane.



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The ABO blood group system consists of four main blood groups: A, B, AB, and O. These groups are determined by the presence or absence of specific carbohydrate antigens (A and B antigens) on the surface of RBCs.

	RBCs (Erythrocytes)	WBCs (Leukocytes)
Types	1 type	5 types
Nucleus	Anucleate	Nucleated
Number	Male: ~4.5–5.5 million/mm³; Female: ~4.0–5.0 million/mm³	~4,500–11,000/mm³
Diameter	~6–9 µm average ~7.5 µm	~7–20 µm, depending on type
Life span	~120 days	Hours/days to years, depending on type
Origin and maturation	Red bone marrow	Red bone marrow and lymphoid tissue
Shape	Biconcave discs	Variable; generally spherical in blood Changes in tissues
Function	Transport of O_2 and CO_2	Defense and immunity
Motility	Non-motile	Motile
Location/ function	Function exclusively within vascular system	Function mainly OUTSIDE blood vessels in the tissues