PHYSICAL PROPERTIES OF ERYTHROCYTES AND THEIR FUNCTION

Hematopoietic System and Disorders (MED202)

Assoc. Prof. Güvem GÜMÜŞ AKAY

Ankara University School of Medicine
Department of Physiology

Lecture outline

Physical properties of erythrocytes

- Morphology of erythrocytes
- Erythrocyte sedimentation
- Flexibility
- Deformability
- Fragility
- Structure of erythrocyte membrane

Physiological functions of erythrocytes

- Trasport of Hb
- Gas exchange
- Buffering
- Carbonic anhydrase
- Chloride shift
- Anti-oxidizing function
- Regulation of vascular tonus
- Immune defence

Erythrocyte count

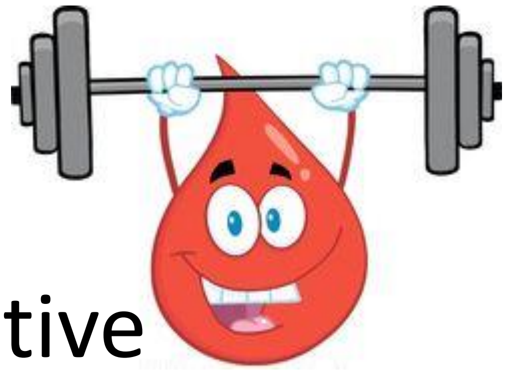
The number of erythrocytes per microliter of blood.

Normal ranges:

Male	4.7-6.1 million/µL
Female	4.2-5.4 million/µL
New born	6-7 million/µL

Morhology of Erythrocytes

- **Shape:** Biconcave disc
- **Diameter:** 7 – 8 um
- **Thickness:** Thickest → 2,5 um
  Center → 1 um
- **Volume:** 78-94 μm^3 :Normocyte
  < microcyte
  > macrocyte
- **Surface area:** 136 μm^2
- **Enucleated:** No nucleus



- ✓ **Life span:** 120 days
- ✓ No nucleus to direct regenerative processes
- ✓ No mitochondria available for efficient oxidative metabolism
- ✓ No ribosomes for regeneration of lost or damaged proteins
- ✓ No devo synthesis of lipids

Durability of erthrocytes is remarkable

Cytoplasm

- Stains pink
- The faint central area is 1/3rd of the cell diameter.
- This region lacks hemoglobin.

Rouleaux formation

- Erythrocytes sometimes form stacks on top of each other in large blood vessels.
- It is more common in cases where some plasma proteins are increased (eg. inflammation).

Erythrocyte sedimentation rate

- Specific weight (density) of the RBC is higher than that of the plasma
- In a stabilized blood, RBC slowly sink towards the bottom of the test tube: Sedimentation
- **Sedimentation rate:** The distance, in mm, the RBCs fall in 1 hr

- Males: 0-15 mm / hr
- Females: 0-20 mm/hr
- Non-specific test

Erythrocyte sedimentation rate

- ESR is determined by the interaction between factors that promote (fibrinogen) and resist (negative charge of RBCs that repel each other) sedimentation.
- Normal RBCs settle slowly as do they do not form rouleaux. Instead, they gently repel each other due to the negative charge on their surfaces
- Rouleaux are stacks of many RBCs that become hevaier and sediment faster.

Electrical properties of erythrocyte membrane

- Sialylated glycoproteins of the RBC membrane are responsible for a **negatively charged** surface which creates a repulsive electric zeta potential (ζ) between **cells** .

Mechanism of sedimentation

- Plasma proteins, especially fibrinogen, adhere to the erythrocyte membranes and neutralize the surface negative charges, promoting cell adherence and rouleaux formation
- ESR is directly proportional to the weight of the cell aggregate and inversely proportional to the surface area

Factors affecting ESR

Pasma factors		Cellular factors		Age/Gender	
Fibrinogen, globulin, and cholesterol	Rapid	Increased RBC mass	Slow	Infant	Slow
Plasma albumin	Slow	Decreased RBC mass	Rapid	Adult	Gradually increase
Lecithin	Slow	Decreased RBC size (microcyte)	Slow	Old	Rapid
		Increased RBC size (macrocyte)	Rapid	Women	Slightly rapid
		Sickle cell, spherocytic (hamper rouleaux formation)	Slow	Pregnancy	Begins to increase about 3rd month of gestation

Clinical significance of ESR

Increased ESR

- Pregnancy
- Anemia
- Macrocytosis
- Inflammatory disease
- Acute and chronic infection
- Multiple myeloma
- Rheumatic fever
- Rheumatoid arthritis
- Anemia
- Tuberculosis
- Systemic lupus erythematosus

Decreased ESR

- Hyperviscosity
- Decreased fibrinogen levels
- Polycythemia
- Sickle cell anemia
- Spherocytosis
- Microcytosis

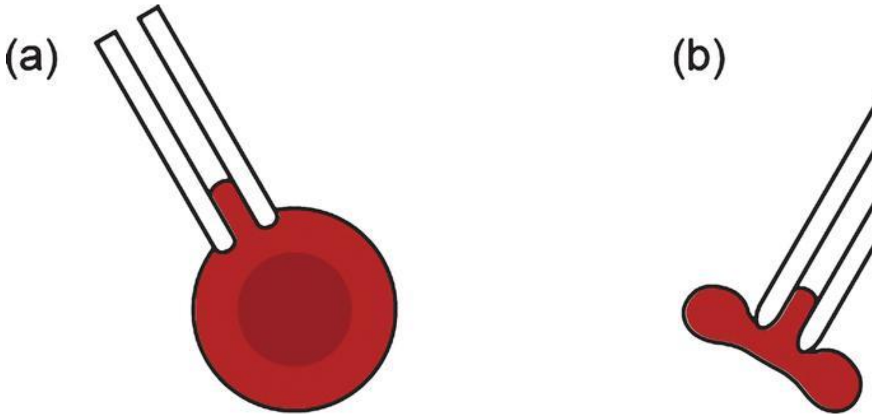
Amazing flexibility

- Shape of the erythrocytes can change significantly as they pass through the capillaries.
- Cells are sturdy yet flexible enough to squeeze through narrow capillaries
- Since their cell membrane is much wider compared to the their volume, erythrocytes does not rupture not during deformation.

https://jacobsschool.ucsd.edu/news/news_releases/release.sfe?id=484

Erythrocyte deformability

- It is the ability of the erythrocyte to change its shape without breaking (hemolysis) under an applied stress.
- It is particularly important under mechanical forces occurring during blood flow or during transitions in microcirculation.



The visco-elastic properties of erythrocytes are determined by 3 factors

- **Geometry of erythrocytes :** The biconcave-discoid shape provides the cell with an extra surface it can stretch. Thus, it can change its shape without the need to increase its surface. Also, less force is required for deformation.
- **Cytoplasmic viscosity:** Reflects the cytoplasmic Hb concentration of erythrocytes
- **Visco-elastic properties of the erythrocyte membrane:** It is mainly determined by the special membrane-cytoskeleton network of erythrocytes.

Erythrocyte plasma membrane

- The lifespan and survival of erythrocytes largely depends on their cell membranes.
- Chemical content of erythrocyte membrane
  - 50 % proteins
  - 40 % lipids (20% Phospholipids, 20% Cholesterol)
  - 10 % carbohydrates

Three basic components of RBC cell membrane

1. Lipid bilayer
2. Integral membrane proteins
3. A membrane cytoskeleton

Membrane lipids of erythrocytes

	Molar Concentration[351]		Weight Concentration[350]	
Lipid	μmol/10^{10} Cells	% of Total	mg/10^{10} Cells	% of Total
Phospholipids				
Phosphatidylcholine (lecithin)	1.3		1.0	
Phosphatidylethanolamine (cephalin)	1.2		0.9	
Sphingomyelin	1.0		0.8	
Phosphatidylserine	0.6		0.4	
Lysolecithin	0.04			
Others	0.07			
Total phospholipids	4.2	49.5	3.1 (1.7–3.2)[a]	69
Cholesterol	4.0	47.1	1.3 (1.1–1.4)[a]	29
Glycolipids (globoside)	0.21	3.4	0.1	2
Total lipids	8.41	100	4.5 (3.9–5.2)[a]	100

Membrane lipids of erythrocytes

The maintenance of an asymmetric phospholipid distribution in the bilayer is critical

- Outer monolayer:
  - Phosphatydilcholine (PC)
  - Sphingomyelin (SM)
- Inner monolayer:
  - Phosphatidylethanolamine (PE)
  - Phosphatidylinositol (PI)
  - Phosohatidylserine (PS)

PS can regulate mechanichal function of membrane via their interactions with skeletal proteins

An exposure of PS can potentiate adhesion of RBCs to vascular endothelial cells.

Premature destruction of thallassemic sickle red cells has been linked to disruptions of lipid asymmetry leading to exposure of PS

Membrane proteins of the erythrocyte membrane

Peripheral

- Spectrin
- Ankyrin (band 2.1)
- Aktin
- Protein 4.1 / 4.2 / 4.9
- Band 6, band 7 (tropomyosin)
- Adducin

Integral

- Glycophorins (A, B, C, D):
  - 75%
  - carry sialic acid in erythrocyte membrane.
  - Glyphoforin A and B contain some antigens.
  - Glycophorin C and D helps to preserve membrane deformability.
- Band 3 (chloride shift):
  - 25%
  - acts as a bridge to support the cell membrane by binding with ankyrin and beta spectrin.
  - has Cl^- and HCO_3^- anion channels.
  - It plays a role in ion and water exchange.

Names	Definition	Function
Peripheral protein		
1. Spectrin	cytoskeletal protein that lines the intracellular side of the plasma membrane	Responsible for biconcave shape of RBC
2. Actin	Abundant protein in cell membrane	participates in more protein-protein interactions
3. Ankyrin	are a family of adaptor protein	Interacts with band 3 protein and spectrin to achieve linkage between bilayer and skeleton.
4. Protein 4.1	is a major structural element.	Stabilises actin-spectrin interactions.
5. Protein 4.2	is an ATP-binding protein	regulate the association of protein 3 with ankyrin.
6. Trophomyosin	Heterodimeric protein	Stabilizing the actin filaments.

Names	**Definition**	**Function**
Integral protein		
1. Glycophorin	Sialic acid rich glycoproteins	imparts a negative charge to the cell, reducing interaction with other cells/ endothelium
2. Band 3 protein	anion exchanger 1	Exchanges bicarbonate for chloride (chlorine shift).

Cytoskeleton

- Formed by structural proteins
- Basic unit: hexagonal lattice with 6 spectrin molecules
- Tail end: tetramers linked to actin and protein 4.1
- Head end: β spectrin linked to ankyrin

Vertical interaction:

Stabilizes the lipid bilayer membrane

Horizontal interaction:

Maintain biconcave shape of RBC

Kim et al. 2017 10.5045/br.2017.52.2.84

The osmotic fragility test

- is a measure of the ability of the RBCs tot ake up fluid without lysing.
- is a test to measure RBC resistance to hemolysis when exposed to a series of increasingly dilute saline solutions.
- Factors affecting the osmotic fragility?

Functions of erythrocytes

A major function of RBCs is to **transport *hemoglobin***, which, in turn, carries oxygen from the lungs to the tissues.

- ✓ in some animals it circulates as free protein
- ✓ in humans within RBC- loss by fitration 3%

Hemoglobin must remain inside RBCs to effectively perform its functions in humans

Functions of erythrocytes

- Retaining oxygen-carrier proteins in these specialized cells (unlike oxygen carriers dissolved in body fluids) is an important step in the evolution of vertebrates.
- Less viscous, more oxygenated blood tissue
- More effective diffusion of oxygen between tissue and blood
- Erythrocytes are capable of concentrating Hb up to 34 g / dL
- Concentration does not exceed this value. Because this is the metabolic limit of the Hb synthesis of cells.

Functions of erythrocytes

- ✓ Because of their biconcave shape, erythrocytes optimize the flow properties of blood in large vessels.
- ✓ Maximize laminar flow
  - Minimize the aggregation of platelets
  - Inhibits the atherogenic activity in large vessels

Functions of erythrocytes

- Although the size of erythrocytes varies among vertebrates, it is approximately 25% wider than the capillary diameter.
- This is thought to facilitate the transfer of oxygen into tissues.
  - The size of a RBC forces it to slow down as it passes through a capillary.
  - The surface of the RBC is exposed to surface of the capillary and so gas exhange will efficiently happen

Functions of erythrocytes

- The RBCs have other functions besides transport of hemoglobin.
- They contain a large quantity of ***carbonic anhydrase***
  - an enzyme that catalyzes the reversible reaction between carbon dioxide (CO_2) and water to form carbonic acid (H_2CO_3)
  - increasing the rate of this reaction several thousand fold

Functions of erythrocytes

- ✓ The rapidity of this reaction makes it possible for the water of the blood to transport enormous quantities of CO_2 in the form of bicarbonate ion (HCO_3^-) from the tissues to the lungs,
- ✓ In the lungs it is reconverted to CO_2 and expelled into the atmosphere as a body waste product.

Instead of taking place within seconds or minutes in plasma, this reaction in erythrocytes reaches full equilibrium in a fraction of a second.

In this way, huge amounts of CO_2 can react in RBCs before the blood leaves the capillaries.

Functions of erythrocytes

- ✓ The carbonic acid formed in RBCs is dissociates into hydrogen and bicarbonate ions: H^+ and HCO_3^-
- ✓ The majority of H^+ then combine with Hb in erythrocytes
- ✓ The hemoglobin in the RBCs is an excellent ***acid-base buffer***, so the RBCs are responsible for most of the acid-base buffering power of whole blood.

Functions of erythrocytes

- Many of the HCO_3^- diffuse from the RBCs into the plasma, while
  Cl^- ions diffuse into the red blood cells to take their place in order to maintain electrical neutrality.
- This diffusion is made possible by the presence of a special ***bicarbonate-chloride carrier protein*** in the RBC membrane that shuttles these two ions in opposite directions at rapid velocities.
- Thus, the Cl^- content of venous RBCs is greater than that of arterial RBCs, a phenomenon called the ***chloride shift*.**

Mammalian erythrocytes are the only cell type that does not contain nuclei in their mature form.

- Although RBCs have nucleus in the early stages of their development, during maturation process they lose their nucleus to provide more space for Hb.
- RBCs then also loses other organelles such as mitochondria, golgi complex, and ER.

Metabolic profile of erythrocytes

- As a result of their lack of mitochondria, RBCs DO NOT use the oxygen that they carry to produce energy.
- **Embden-Meyerhof Pathway (EMP)** (90-95%)
  - They catabolize glucose anaerobically to lactic acid
    - **NADH**: Cofactor of methemoglobin reductase
    - **ATP**
    - **2,3-DPG**: regulates Hb function
- **Pentose Phosphate Pathway** (5-10%)
    - **NADPH**: It is not used to generate energy, but is used in the reduction of oxidized Glutathione (Glutathione is one of the most important molecules that protect the cell against oxidative attacks).

Functions of erythrocytes

- Like vascular endothelial cells, RBCs also synthesize NO from L-Arginine.
- Exposure of RBCs to shear stress at physiological levels increases NO production by activating the NO synthase enzyme.
- It has a regulatory effect on the vascular tonus.

Functions of erythrocytes

- RBCs also play a role in the body's immune response.
- When they are lysed by a pathogen, the released Hb causes the formation of free radicals.
- Free radicals cause to break down of the cell wall and membrane of the pathogen.