In the Name of ALLAH, Most Beneficent, Infinitely Merciful
Biochemistry of RBC Metabolism
Learning Objectives

1. Recognizing the main metabolic pathways occurring in RBCs with reference to their relations to functions.
2. Discuss the relation of characteristic features of structure of the red blood cell membrane.
3. Summarize the causes of the major metabolic disorders affecting red blood cells.
4. Recognizing changes occurring in aging of RBCs.
5. Understand the concept of stem cells and their importance.
Major functions of the RBC

RBC are small cell (6-8μm in diameter)

• Transport $O_2$ from lungs to the peripheral tissues.
• Disposal of $CO_2$ and $[H^+]protons$ formed during tissue metabolism.
• Carry $CO_2$, to lungs for elimination by exhalation.
Introduction to the Red Blood Cells (RBCs)

• The red blood cells (RBCs) are not true cells.

• Composed of Membrane surrounding a solution of hemoglobin (95%). Bag filled with hemoglobin.

• RBCs contain no nucleus or nucleic acids, and thus, can not reproduce.
Introduction to the Red Blood Cells (RBCs)

- RBCs contain no cell organelles (as mitochondria, Golgi, ER or lysosomes) and thus possess no synthetic activities (no protein biosynthesis, no lipid synthesis & no carbohydrate synthesis).

- RBCs must be able to squeeze through some tight spots in micro-circulation. For that RBCs must be easily & reversibly deformable.
Biochemical composition of the RBCs

- Red cells contain about 35% solids.
- Hemoglobin, the chief protein of the red cells.
- Other proteins are present in combination with lipids and oligosaccharide chains, forming the stroma and cell membrane.
- Potassium, magnesium, and zinc concentrations in red cells are much higher than in the plasma.
Hemoglobin
Introduction:

- RBCs contain **no mitochondria**, so there is **no** respiratory chain, **no** citric acid cycle, and **no** oxidation of fatty acids or ketone bodies.
- The RBC is highly dependent upon glucose as its energy source.
- Energy in the form of ATP is obtained **ONLY** from the glycolytic breakdown of glucose with the production of lactate (**anaerobic glycolysis**).
- ATP produced being used for keeping the biconcave shape of RBCs & in the regulation of transport of ions & water in and out of RBCs. (Na+-K+-ATPase and the anion exchange protein)
Glucose transport through RBC membrane:

Glucose is transported through RBC membrane by facilitated diffusion through glucose transporters (GLUT-1).

- Glucose transporters (GLUT-1) are independent on insulin i.e. insulin does not promote glucose transport to RBCs

- It functions by generating a gated pore in the membrane to permit passage of glucose;
2. **Glycolysis:**

- Glucose is metabolized in RBCs through **anaerobic glycolysis** (that requires **no** mitochondria and **no** oxygen)
- One molecule of glucose yields **2 molecules of ATP** by one anaerobic glycolytic pathway.
- In addition, **2 molecules of lactate** are produced.
- **Lactate** is transported to blood & in the liver it is converted to glucose.
Glucose metabolism in RBC

**Embden-Meyerhof glycolytic pathway**

- Glucose → Glucose-6-P → Fructose-6-P → Fructose-1,6-DP → Glyceraldehyde-3P → 1,3-DPG
- 1,3-DPG → Mutase → 2,3-DPG → Phosphatase → 3-PG → 2-PG
- 2-PG → Luebering-Rapoport shunt
- Luebering-Rapoport shunt → 3-PG → Pyruvate → Lactate
- Embden-Meyerhof glycolytic pathway

**Hexose monophosphate shunt pathway**

- Glucose → Glucose-6-P → Fructose-6-P → 6-PG
- 6-PG → Ribulose-5-P
- Lactate

**Luebering-Rapoport shunt**
Anaerobic Glycolysis

Importance of glycolysis in red cells:

**Energy production:** it is the only pathway that supplies the red cells with ATP.

**Reduction of methemoglobin:** glycolysis provides NADH for reduction of metHb by NADH- cytob5 reductase

**In red cells 2,3 bisphosphoglycerate** binds to Hb, decreasing its affinity for O$_2$, and helps its availability to tissues.
Metabolism of RBCs (cont.)

Genetic defects in enzymes of glycolysis:
Genetic defects of one of the enzymes of glycolysis in RBCs results in a reduce rate of glycolysis in RBCs & by this way deprive RBCs of the only means for producing energy.

As a result, hemolytic anemia will be a consequence as RBCs will not be able to keep the biconcave flexible shape which allows it to squeeze through narrow capillaries with an end result of hemolysis (destruction of RBCs).

95% of cases of genetic defects in glycolytic enzymes is caused by pyruvate kinase deficiency.
4% is caused by phosphoglucone isomerase deficiency.
Metabolism of RBCs (cont.)

3. Production of 2,3 bisphosphoglycerate (2, 3-BPG):

In RBCs, some of glycolysis pathways are modified so that 2, 3 bisphosphoglycerate is formed (by bisphosphoglycerate mutase).

2, 3 bisphosphoglycerate decreases affinity of Hb for O₂. So, it helps oxyhemoglobin to unload oxygen.

Storing blood results in decrease of 2,3-BPG leading to high oxygen affinity Hb. This leads to oxygen trap. 6-24 hours are needed to restore the depleted 2,3 BPG. Maximum storage time for RBCs is 21-42 days.
4. **Pentose phosphate pathway** (HMP-SHUNT)

- RBCs contain an active pentose phosphate pathway (PPP) for glucose that supplies **NADPH** (PPP is the only source for NADPH in RBCs)

- **NADPH** is important in keeping glutathione in the reduced glutathione.

- Reduced glutathione plays a very important role in the survival of the red blood cells. (prevents oxidation of membrane)
Glucose 6- phosphate dehydrogenase deficiency (G6PD Deficiency):

- Glucose 6-phosphate dehydrogenase is the first enzyme of pentose phosphate pathway & its deficiency leads to reduced production of NADPH ending in acute hemolytic anemia.
Metabolism of RBCs (cont.)

- The erythrocytes contain *carbonic anhydrase*. Carbon dioxide combines with water only after it enters the red cells where hemoglobin, the most important buffer for carbonic acid, is present.

\[
\text{CO}_2 + \text{H}_2\text{O} \rightarrow \text{HCO}_3^- + \text{H}^+
\]

- The red cell also contain *rhodanese enzyme* responsible for the detoxification of *cyanides*
Energy metabolism

Glycolysis

Pentose Phosphate Pathway

Rapoport–Luebering Shunt

Enzymes and Pathways:
- Glucose
- NADP
- NADPH
- 6-Phosphogluconate
- Ribulose-5-phosphate
- Xylulose-5-phosphate
- Sedoheptulose-7-phosphate
- Glyceraldehyde-3-phosphate
- 1,3-Diphosphoglycerate
- 3-Phosphoglycerate
- 2-Phosphoglycerate
- Enolase
- Pyruvate
- Lactate

Reactions:
1. Glucose → HK
2. Fructose-6-phosphate → PGI
3. Fructose-1,6-bisphosphate → FBP
4. Aldolase
5. TPI
6. Glyceraldehyde-3-phosphate
7a. PGD
7b. PGK
7c. PGM
8. 2-Phosphoglycerate
9. Enolase
10. PK
11. LDH
12. G6PD
13. Lactonase
14. NADP
15. EP
16a. TRK
16b. TRK
16c. TRK
17. TRA
18a. TRK
18b. TRK
18c. TRK

Other Reactions:
- ATP → ADP
- ADP → ATP
- NAD → NADH
- NADH → NAD
- 6-Phosphogluconate → Lactonase
- Ribulose-5-phosphate + CO₂ → EP
- Xylulose-5-phosphate → (Nucleotides)
- Sedoheptulose-7-phosphate + glyceraldehyde-3-phosphate → TRA
RBCs must be able to squeeze through some tight spots in microcirculation (capillaries). For that RBCs must be easily & reversibly deformable. Its membrane must be both fluid & flexible.

RED CELL MEMBRANE STRUCTURE
Membrane of the Human Red Blood Cell

- About 50% of membrane is **protein**, 40% is **fat** & up to 10% is **carbohydrate**.

- RBCs membrane comprises a **lipid bilayer** (which determine the membrane fluidity), **proteins** (which is responsible for flexibility) that are either peripheral or integral penetrating the lipid bilayer & **carbohydrates** that occur only on the external surface.
The major lipid classes in membrane are phospholipids and cholesterol;

**Major Phospholipids are**

- Phosphatidylcholine (PC), Phosphatidylethanolamine (PE), and Phosphatidylserine (PS) along with Sphingomyelin (Sph).

  - *The choline-containing phospholipids, predominate in the outer leaflet*
    - Phosphatidyl Choline and Sphingomyelin
  
  - *The amino-containing phospholipids predominate in the inner leaflet.*
    - Phosphatidyl Ethanolamine and Phosphatidyl Serine

**Glycosphingolipids (GSLs) 5–10%**

  - (neutral GSLs, gangliosides, and ABO blood group substances)
A schematic representation of the red cell membrane structure

- Sphinomyeline (SM)
- Phosphatidylcholine (PC)
- Phosphatidylethanolamine (PE)
- Phosphatidylserine (PS)
### TABLE I. Lipid Content of the Normal Human Red Cell

<table>
<thead>
<tr>
<th>Plasma Membrane&lt;sup&gt;a&lt;/sup&gt;</th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>μmol/ml RBC</strong></td>
<td><strong>TPL</strong></td>
</tr>
<tr>
<td>Phospholipid</td>
<td>4.2</td>
</tr>
<tr>
<td>Cholesterol</td>
<td>4.0</td>
</tr>
<tr>
<td>Free fatty acid</td>
<td>0.29</td>
</tr>
<tr>
<td>Glycolipid</td>
<td>0.11</td>
</tr>
<tr>
<td>Phospholipid/cholesterol, mole/mole</td>
<td>1.05</td>
</tr>
<tr>
<td>% of total membrane phospholipids</td>
<td></td>
</tr>
<tr>
<td>Phosphatidylcholine (PC)</td>
<td>25–30</td>
</tr>
<tr>
<td>Sphingomyelin (SM)</td>
<td>25–30</td>
</tr>
<tr>
<td>Phosphatidylethanolamine (PE)</td>
<td>25–30</td>
</tr>
<tr>
<td>Phosphatidylserine (PS)</td>
<td>10–15</td>
</tr>
<tr>
<td>Lyso-phosphatidylcholine (Lyso-PC)</td>
<td>1–2</td>
</tr>
<tr>
<td>Other (phosphoinositides, phosphatidic acid)</td>
<td>2–3</td>
</tr>
</tbody>
</table>

<sup>a</sup>Adapted, with permission, from Cooper [1978].
The major membrane Proteins

The membrane skeleton is four structural proteins that include α & β spectrin, ankyrin, protein 4.1 & actin.

Spectrin is major protein of the cytoskeleton & its two chains (α & β) are aligned antiparallel manner α & β chains are loosely interconnected forming a dimer, one dimer interact with another, forming a head to head tetramer.

Ankyrin binds spectrin & in turn binds tightly to band 3 securing attachment of spectrin to membrane.
Red Cell Membrane Structure (cont.)

Band 3 is anion exchange protein permits exchanges of Cl\(^-\) for HCO\(_3\)\(^+\).

**Actin** binds to the tail of **spectrin** & to **protein 4.1** which in turn binds to integral proteins, **glycophorins A, B & C**.

**Glycophorins A, B, C** are transmembrane glycoproteins;

**Defects of proteins may explain some of the abnormalities of shape of RBCs membrane as hereditary spherocytosis & elliptocytosis.**
The red cell membrane structure
Comparison of RBC membrane & other cell membrane

Carbohydrate  Band 3  Glycophorin

Ankyrin  Tropomyosin  Actin

Lipid bilayer  Spectrin dimer

Alpha-helix protein  Oligosaccharide side chain

Glycolipid  Globular protein

Phospholipid  Hydrophobic segment of alpha-helix protein  Cholesterol
Fate Of RBC

• When RBCs reach the end of their lifespan, the globin is degraded to amino acids (which are reutilized in the body), the iron is released from heme and also reutilized, and the tetrapyrrole component of heme is converted to bilirubin, which is mainly excreted into the bowel via the bile.
# Changes in RBCs due to aging

<table>
<thead>
<tr>
<th></th>
<th>Increased in old cells</th>
<th>Decreased in old cells</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Hb</strong></td>
<td>Glycosylated Hb</td>
<td>Bisphosphoglycerate (BPG)</td>
</tr>
<tr>
<td><strong>Membrane</strong></td>
<td>Osmotic fragility</td>
<td>Sialic acid</td>
</tr>
<tr>
<td></td>
<td>Na⁺</td>
<td>K⁺</td>
</tr>
<tr>
<td></td>
<td>Binding of IgG</td>
<td>Lipids and Proteins</td>
</tr>
<tr>
<td><strong>Enzymes</strong></td>
<td></td>
<td>G6PD</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Pyruvate dehydrogenase</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Others</td>
</tr>
<tr>
<td><strong>General</strong></td>
<td>Cell density</td>
<td>Deformability</td>
</tr>
<tr>
<td></td>
<td>Sphericity</td>
<td>Disc like shape</td>
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</tbody>
</table>
Protection of Blood Cells from Oxidative Stress & Damage

Reactive oxygen species (ROS)

Oxidants produced during metabolism, in blood cells and most other cells of the body include:

1. Superoxide ($\text{O}_2^\cdot$),
2. hydrogen peroxide ($\text{H}_2\text{O}_2$),
3. peroxyl radicals ($\text{ROO}^\cdot$), and
4. hydroxyl radicals ($\text{OH}^\cdot$)

Free radicals are atoms or groups of atoms that have an unpaired electron.
Protection of Blood Cells from Oxidative Stress & Damage

OH\textsuperscript{·} is a particularly reactive molecule and can react with proteins, nucleic acids, lipids, and other molecules to alter their structure and produce tissue damage.
Oxygen ($O_2$) reacts with haemoglobin to form oxyhaemoglobin. Oxyhaemoglobin is then converted to superoxide ($O_2^-$) by superoxide dismutase. Superoxide is further converted to hydrogen peroxide ($H_2O_2$) by catalase. Hydrogen peroxide can be decomposed back into water ($H_2O$) by catalase. Glutathione peroxidase converts hydrogen peroxide to water. Glutathione reductase uses NADPH to convert glutathione disulfide (GSSG) back to glutathione (GSH). This process is part of the pentose phosphate pathway. Methaemoglobin reductase converts methaemoglobin back to haemoglobin.
Protective systems in cells against Per-oxidative reactions

- Superoxide Dismutase
- Catalase
- Glutathione Peroxidase
- Glutathione Reductase
- Glutathione Synthetase
- Met Hb Reductase
- NADPH
- NADP
- Hexose Monophosphate Shunt (G6PD)
- G-6-P
- Amino Acid
- Fe³⁺
- Vit. E (oxid. Vit. E can be recycled by Vit. C)

Damage Cellular components leading to accelerated cell removal
Hemolytic Anemias Are Caused by Abnormalities Outside, Within or Inside the Red Cell Membrane

- **Intrinsic**
  - Mutations affecting membrane proteins
  - PNH
  - Enzymopathies
  - Abnormal hemoglobins
  - Parasites (e.g. plasmodia)

- **Extrinsic**
  - Hypersplenism
  - Antibodies (various)
  - Hemolysins (e.g. bacterial)
  - Snake venoms (some)
Hemolytic Anemia Are Caused by Abnormalities Outside the Red Cell Membrane

1. **Hypersplenism**, a condition in which the spleen is enlarged from a variety of causes and red blood cells become sequestered in it.

2. **Antibodies** (eg, transfusion reactions and anti-Rh antibodies, Plasma warm, and cold antibodies that lyse red blood cells.

3. **Hemolysins** released by various infectious agents, such as certain bacteria (eg, certain strains of *Escherichia coli* and clostridia).

4. **Snake venoms** that lyse the red cell membrane (eg, via the action of phospholipases or proteinases).
Hemolytic Anemia Are Caused by Abnormalities within the Red Cell Membrane

1. Abnormalities of proteins. (Spectrin structure abn)
   1. Hereditary spherocytosis and
   2. Hereditary elliptocytosis,

2. Paroxysmal nocturnal hemoglobinuria
Hemolytic Anemia Are Caused by Abnormalities Inside the Red Cell Membrane

1. **Hemoglobinopathies:**
   1. Sickle cell anemia and
   2. Thalassemia are the most prevalent hemoglobinopathies

2. **Enzymopathies**
   1. Abnormalities of enzymes in the pentose phosphate pathway
      1. Deficiency of glucose-6-phosphate dehydrogenase
   2. Abnormalities in glycolysis
      1. Deficiency of pyruvate kinase the mechanism appears to be due to impairment of glycolysis, resulting in decreased formation of ATP, affecting various aspects of membrane integrity.

3. **Parasitic infections** (eg, the plasmodia causing malaria) are also important causes of hemolytic anemias in certain geographic areas.
Laboratory Investigations that Assist in the Diagnosis of Hemolytic Anemia

General tests and findings
1. Increased unconjugated (indirect) bilirubin
2. Reticulocytosis
3. Low level of plasma haptoglobin

Specific tests and findings
1. Hb electrophoresis (eg, HbS)
2. Red cell enzymes (eg, G6PD or pyruvate kinase deficiency)
3. Osmotic fragility (eg, hereditary spherocytosis)
4. Coombs test
HAPPINESS IS IN GIVING