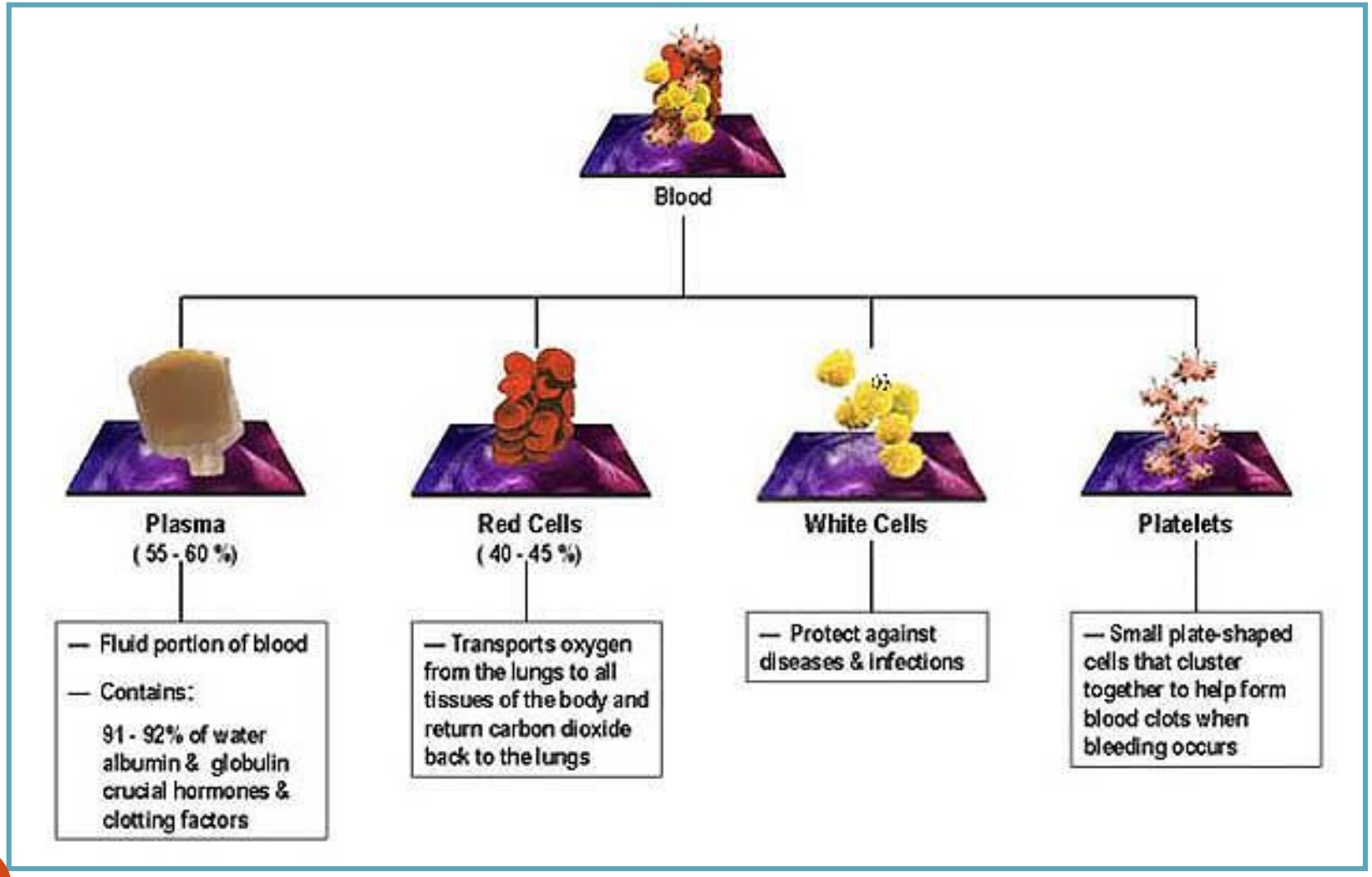
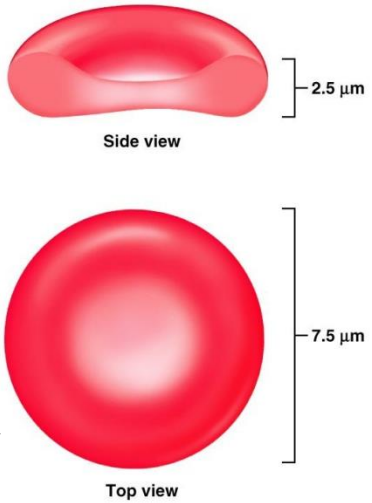
Erythrocytes (RBC)

Two major components of blood: liquid phase and formed elements



Erythrocyte Structure



- RBCs are flattened biconcave discs
  - Flexible structure
  - Shape provides increased surface area for diffusion
- 7.5 mm diameter, 2.5 mm thick
- Life cycle – 120 days
- Lack a nucleus and other organelles.
- 33% of weight is hemoglobin molecules (Each RBC contains 280 million hemoglobins).
- Other proteins include antioxidants and those to maintain RBC shape (spectrin)

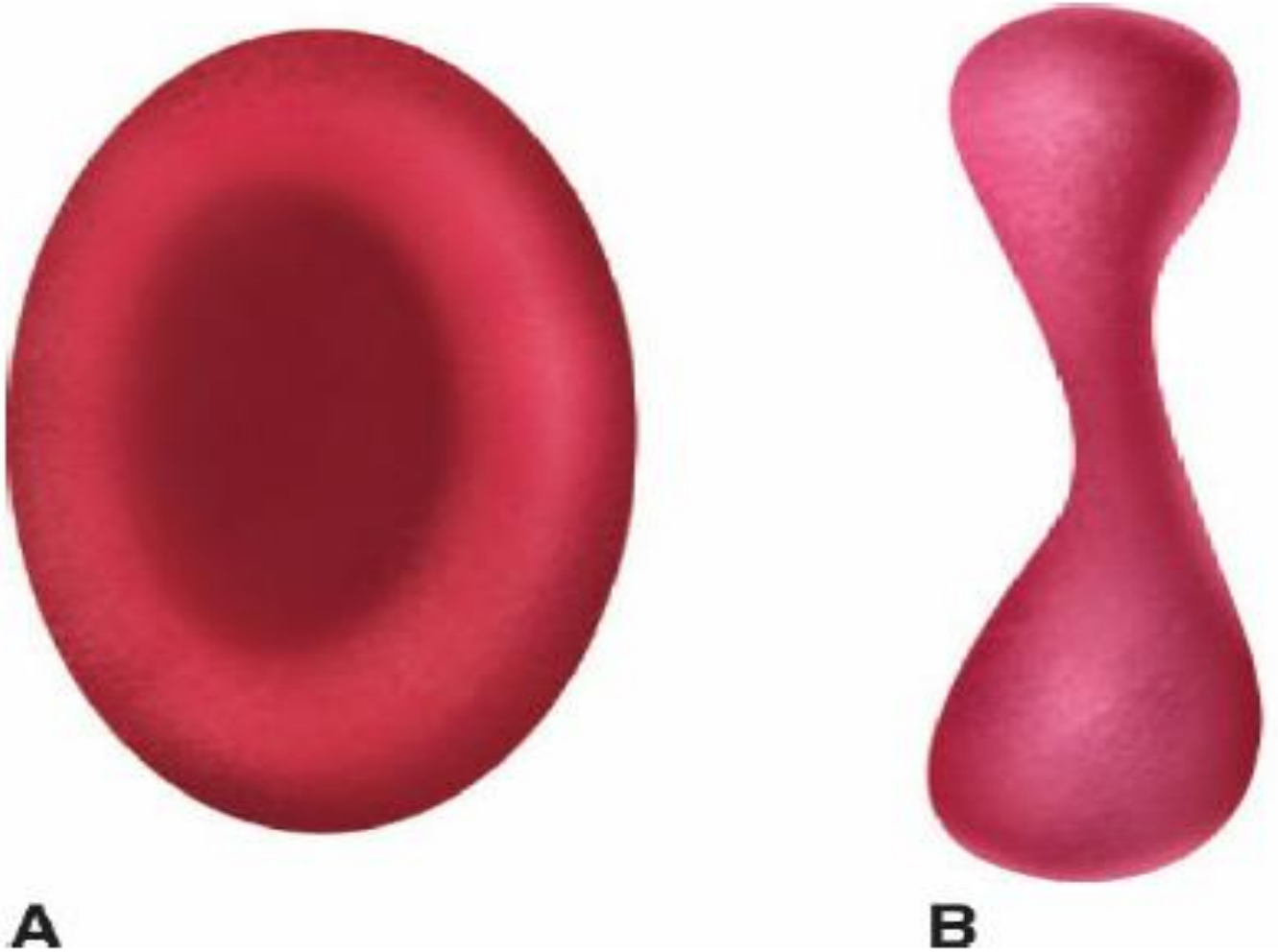


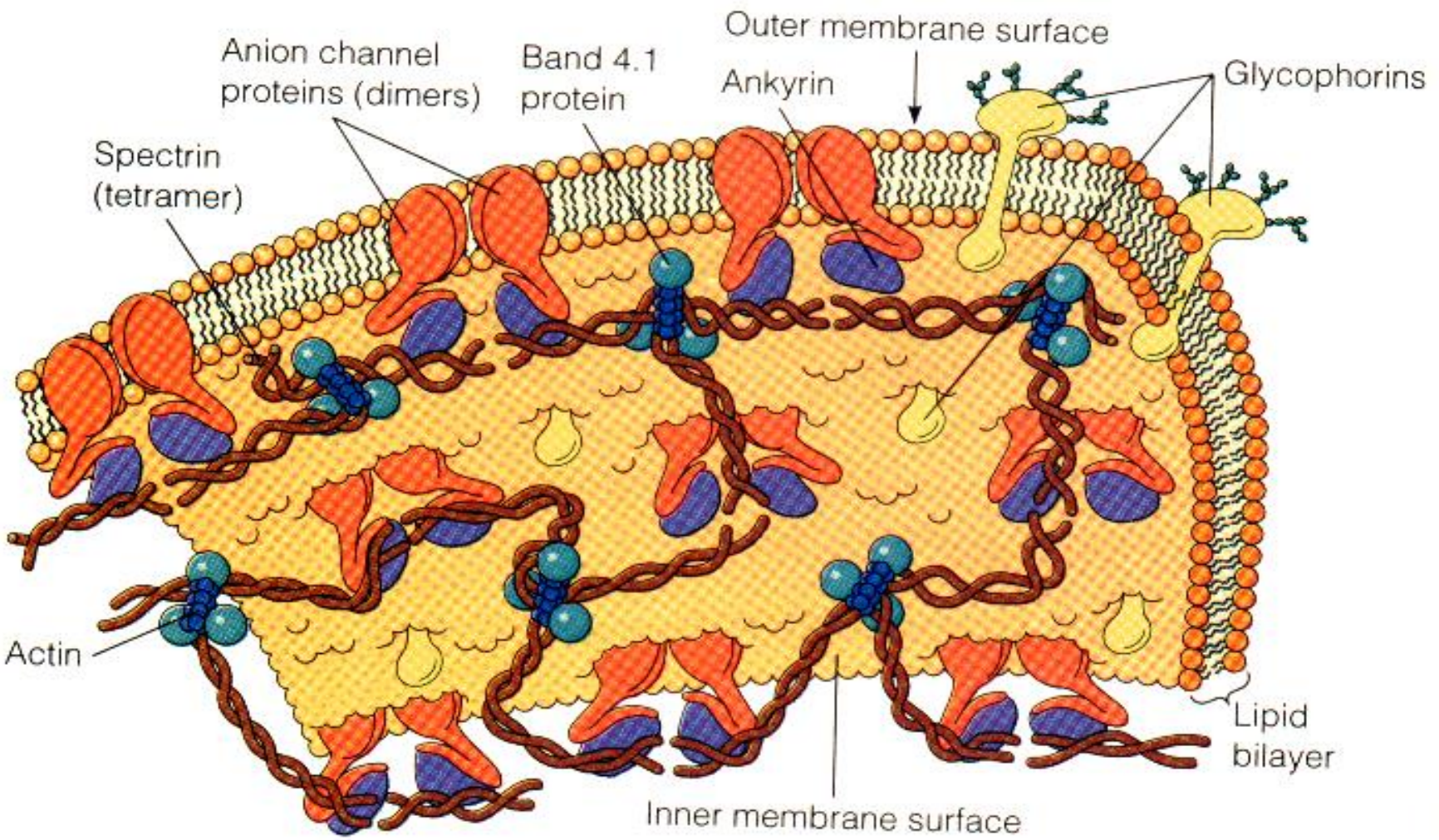
FIGURE 53–2 Red blood cells are shaped like biconcave disks. Shown are drawings of **(A)** a red blood cell, **(B)** a section through a red blood cell

Erythrocyte Function

- Erythrocytes are dedicated to respiratory gas transport (transport O_2 to tissues and CO_2 from tissues)

- Hemoglobin reversibly binds with oxygen and most oxygen in the blood is bound to hemoglobin

RBC's Membrane



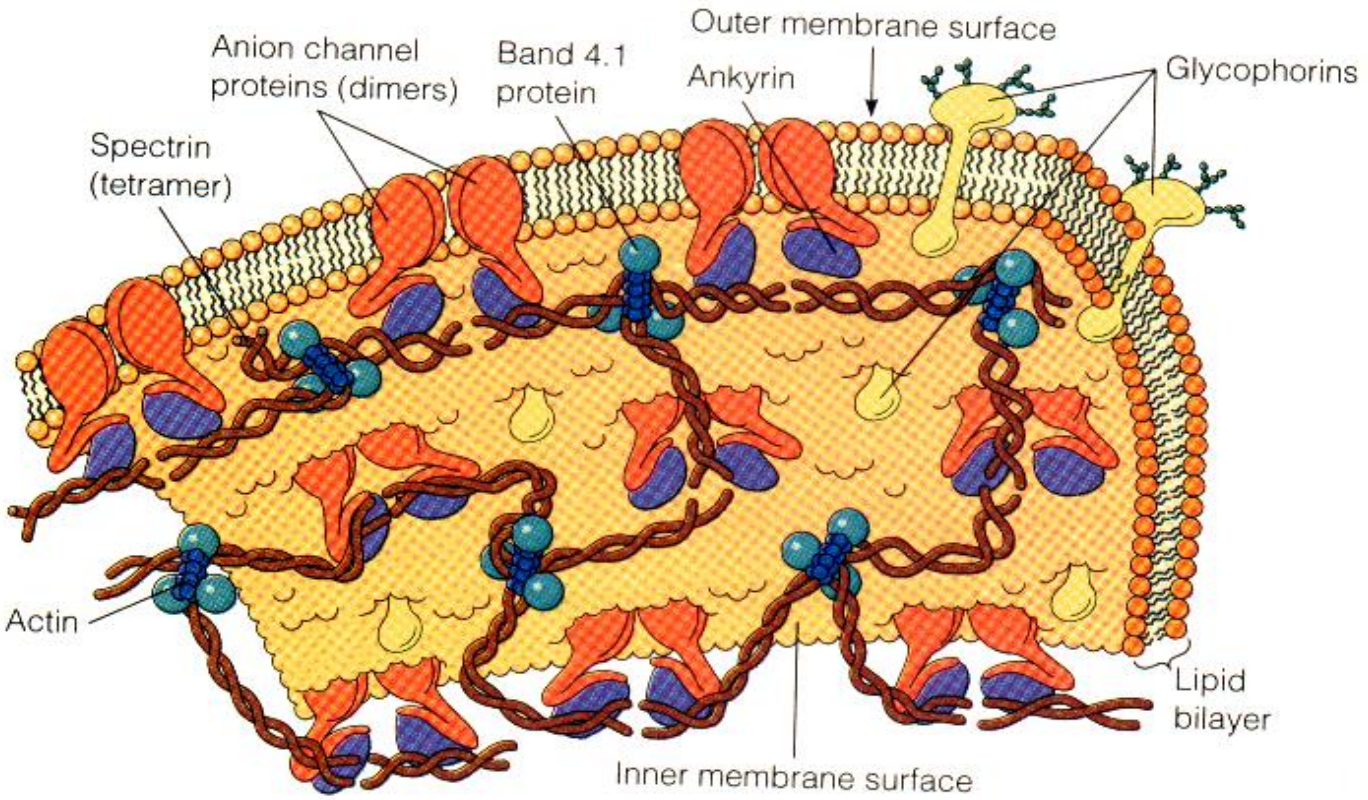
RBC's Membrane characterizations

- Is **very elastic**
- Is a **semi-permeable lipid bi-layer** supported by a mesh-like **cytoskeleton**
- Is a three-layer structure:

1. **An outer hydrophilic portion:** glycolipid, glycoprotein and protein
2. **A central hydrophobic layer:** protein, cholesterol and PL
3. **An inner hydrophilic layer:** containing protein

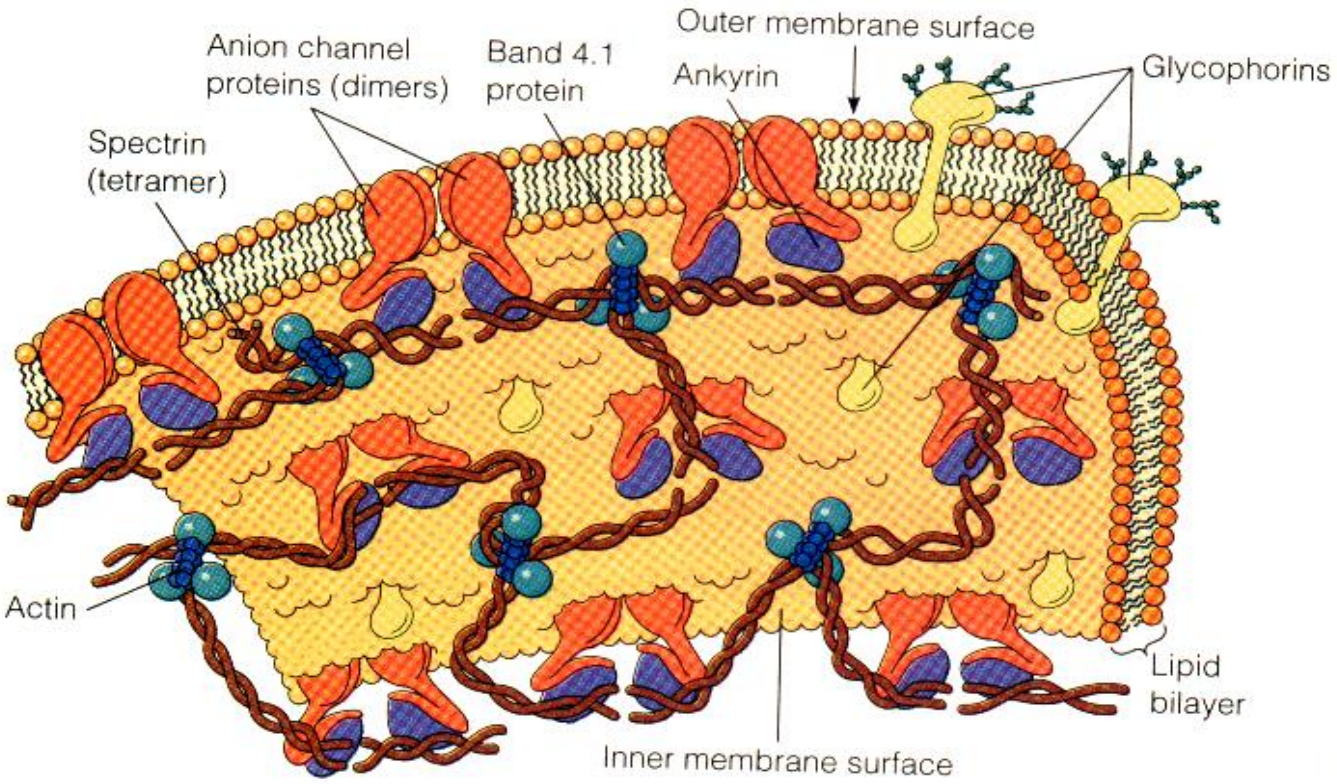
Components of RBC membrane

- Protein 50%
- Phospholipid 20%
- Cholesterol 20%
- Carbohydrate 10%



Components of RBC membrane (cont.)

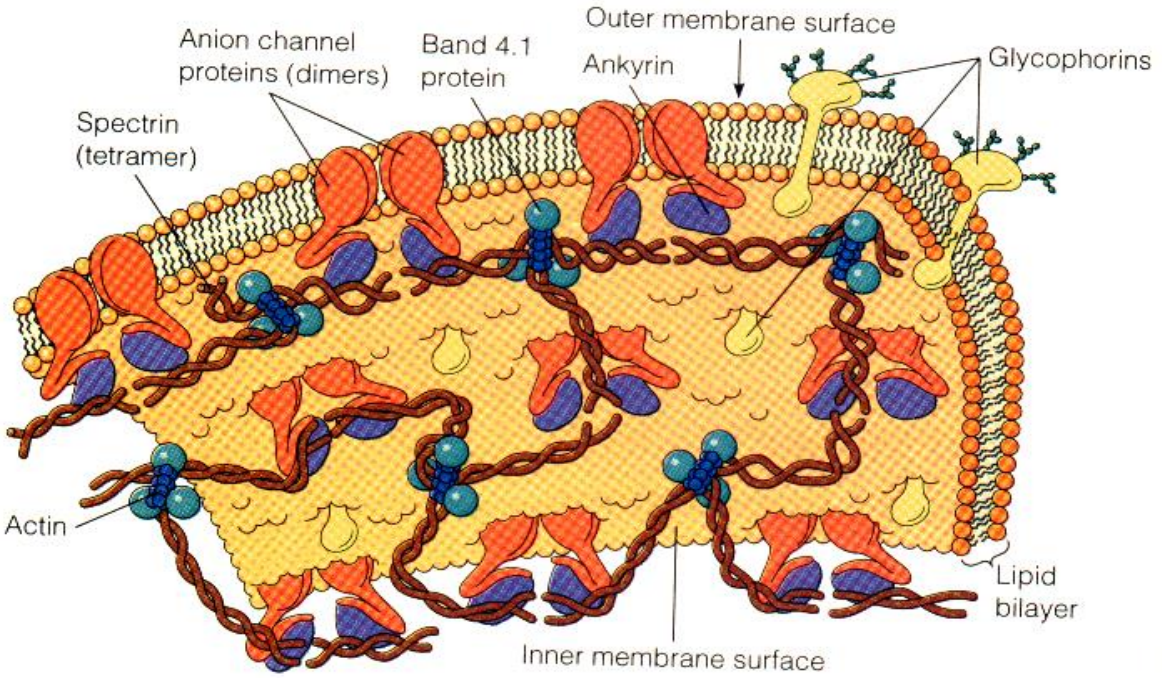
- Lipid bilayer
- Integral membrane protein
- Membrane cytoskeleton



Components of RBC membrane (cont.)

Lipid bilayer

- Phospholipid
- Cholesterol
- Glycolipid
- Integral protein
- Peripheral protein.



Components of RBC membrane (cont.)

Several proteins are present in the RBC's

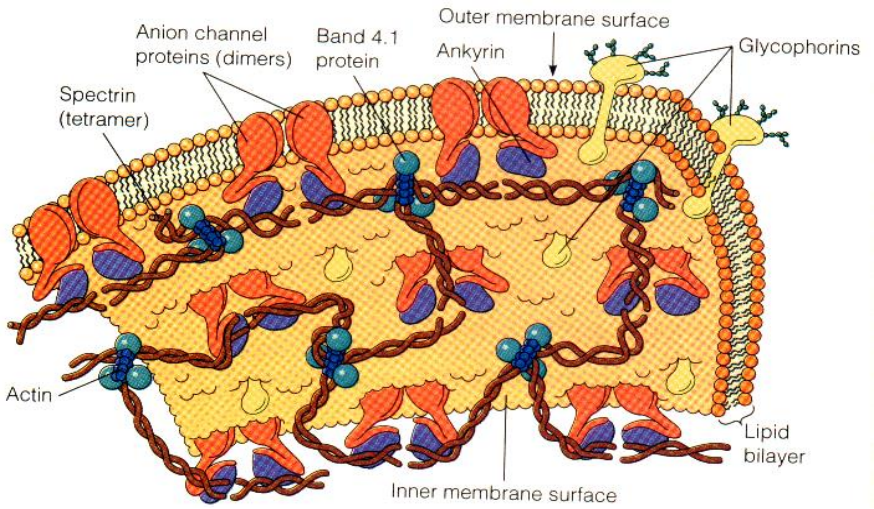
- **Peripheral proteins**:

  Limited to cytoplasmic surface of membrane and forms the RBC cytoskeleton.

- **Integral membrane proteins:**

  Extend from outer surface and transverse entire membrane to inner surface

Components of RBC membrane (cont.)



Peripheral Proteins	Integral Proteins
Tropomyosin	Glycophorin
Spectrin	Band 3 Protein
Actin	
Protein 4.1	
Ankrynin	
Protein 4.2	

Peripheral Proteins

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

Integral Proteins

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

Components of RBC membrane (cont.)

Cytoskeleton:

- Network of proteins on the inner surface of the plasma membrane, called the **peripheral membrane proteins**
- Provides **rigid support** and **stability** to lipid bilayer
- Responsible for maintaining **shape** and **deformability** of RBC .

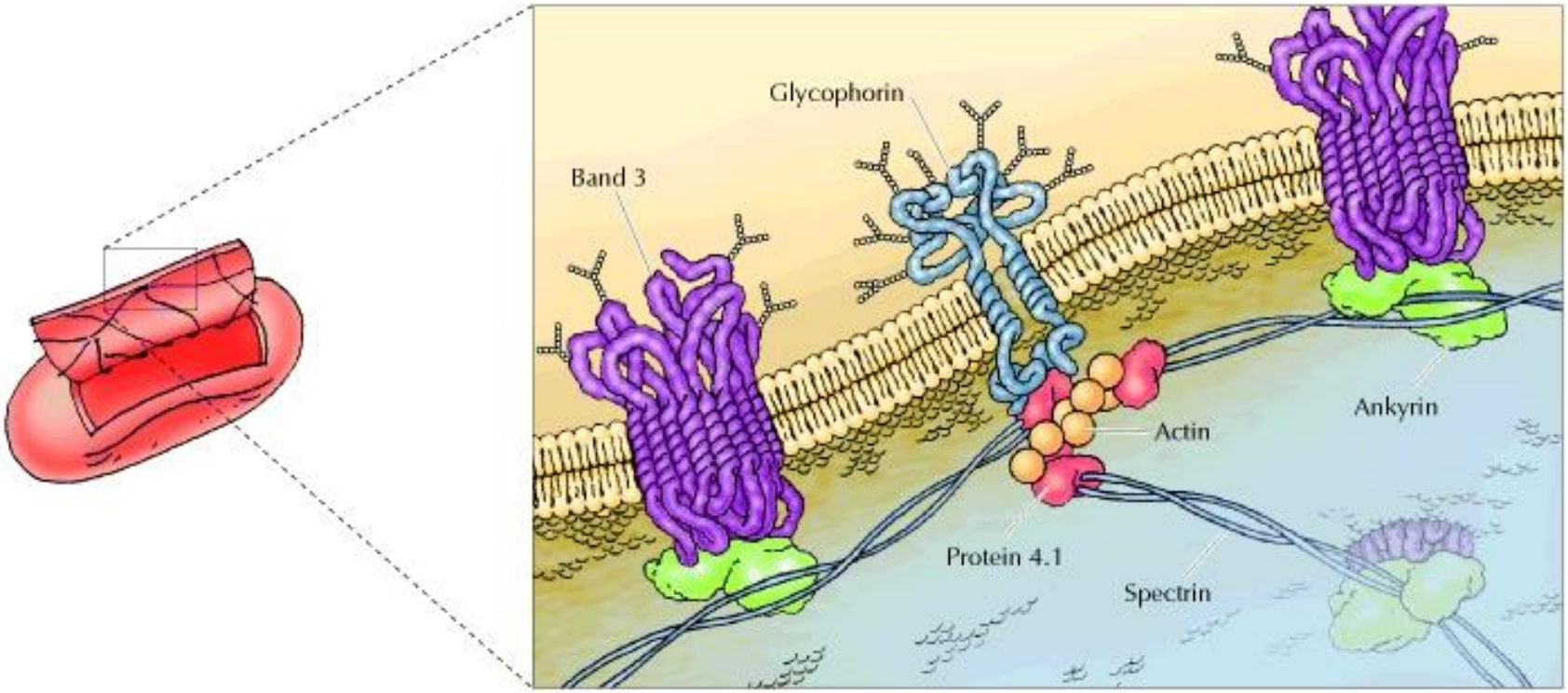
(Go back to slide 5)

Peripheral Membrane Proteins

1. Spectrin :

- The **most abundant** peripheral protein.
- Flexible, rod like molecule composed of an alpha helix of two polypeptide chains.
- Is an important factor in **RBC membrane integrity** because it binds with other peripheral proteins to form the skeletal network of microfilaments on the inner surface of RBC membrane.
- Microfilaments **strengthen** membrane, protecting cell from being broken.
- Controls **biconcave shape** and **deformability** of cell.

Peripheral Membrane Proteins



Peripheral Membrane Proteins

2. Ankyrin :

- Primarily anchors lipid bilayer to membrane skeleton

3. Protein 4.1:

- May link the cytoskeleton to the membrane by means of its associations with glycophorin

4. Actin:

- Responsible for contraction and relaxation of membrane

Integral Membrane Proteins:

Glycophorin:

- Is the principle RBC glycoprotein. Spans entire thickness of lipid bilayer and appears on external surface of RBC membrane
- Three types of glycophorins identified: **A**, **B**, and **C**
- All glycophorins carry RBC antigens and are receptors or transport proteins

Red Cell Membrane Lipids

- Erythrocyte membrane lipid consists of bilayer of phospholipids intermingled with molecules of cholesterol in nearly equal amounts. Also, small amounts of free fatty acids and glycolipids.

- Different types of phospholipids are found on the inside layer than on the outside layer.

- Abnormalities in the PL may result in decreased deformability and decreased red cell survival.

Red Cell Membrane Lipids (cont.)

- Most of glycolipids are located in outer half of lipid bilayer and interact with glycoproteins to form many of RBC antigens

- Cholesterol **equally distributed** on both sides of lipid bilayer (25% of RBC membrane lipid content)

- RBC membrane cholesterol is in **continual exchange** with plasma cholesterol

Red Cell Membrane Lipids (cont.)

- Cholesterol plays important role in **regulating membrane fluidity** and **permeability**

- Accumulation of cholesterol results in decreased deformability and may lead to hemolytic anemia

Red Cell Membrane Carbohydrates

- They occur only on the **external surface** of the red cell
- They occur as glycoproteins or glycolipids
- **The antigens of ABO** blood groups are examples of carbohydrate membranes

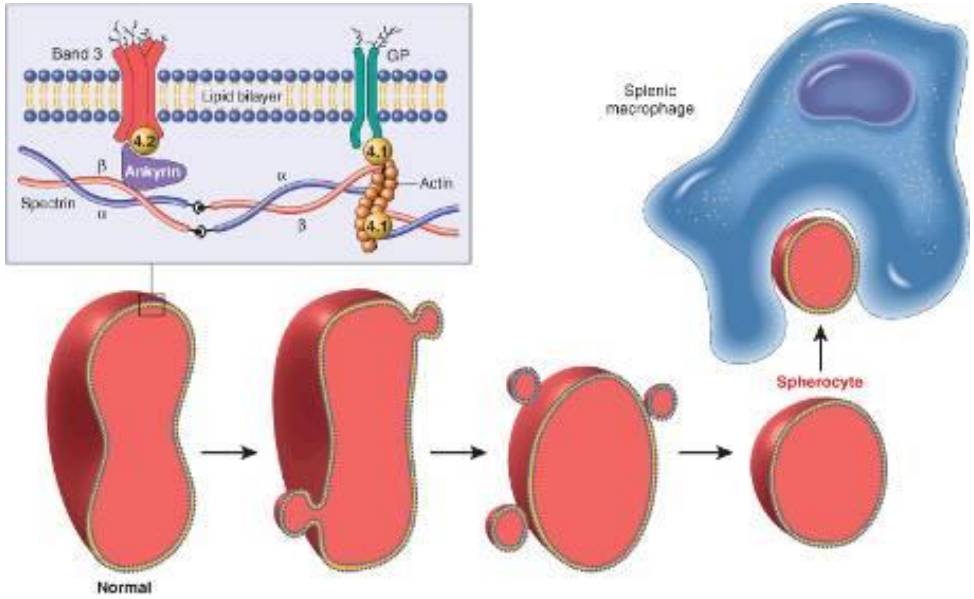
Abnormalities of the RBC membrane

1-Hereditary Spherocytosis (HS):

- The most common hereditary haemolytic anemia in north Europeans, autosomal dominant.
- Defective or absent of Spectrin\Ankrin molecule which involved in the vertical interactions between the membrane skeleton and lipid bilayer of the red cell.
- Leads to loss of RBC membrane, decreased deformability of cell, spherocytosis.
- Increased osmotic fragility

- Normal biconcave red cell **loses membrane fragments** and adopts a **spherical shape**

- Inflexible cells are trapped in the spleen cords, phagocytosed by macrophages

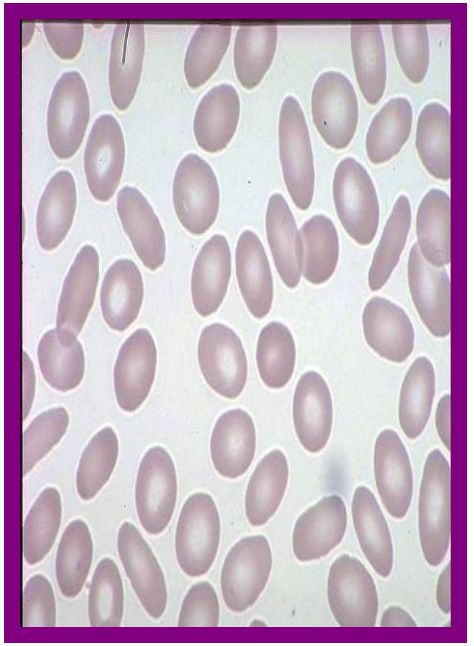


Hereditary Spherocytosis

Abnormalties of the RBC's membrane (cont)

2. Hereditary Elliptocytosis:

- The most common hereditary haemolytic anemia in South-East Asian ovalocytosis.
- Hereditary disorder of the RBCs (autosomal dominant trait)
- RBCs assume an elliptical shape, rather than the typical round shape.



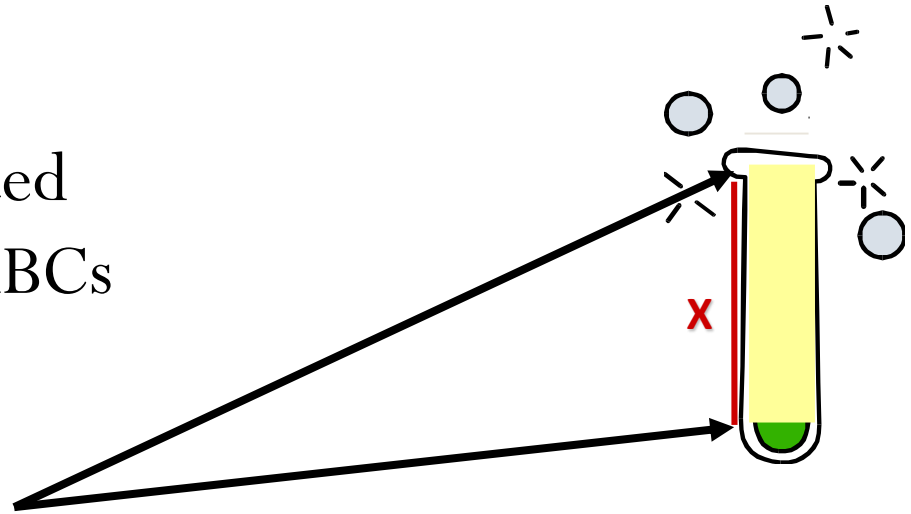
Abnormalties of the RBC's membrane (cont)

2. Hereditary Elliptocytosis:

- **Spectrin** abnormality or deficiency of **protein 4.1**
- RBC hemolysis occurs in the spleen, thus splenectomy corrects the hemolysis, **but not the RBC membrane defect**

Erythrocyte Sedimentation Rate (ESR)

- Nonspecific test for inflammatory process
- Anticoagulated blood in calibrated tube; rate of sedimentation of RBCs in 1h
- **Normal**: <15mm/h male;<20mm/h female; add 10 past age 60



Erythrocyte Sedimentation Rate (ESR)

- Any condition that **elevates fibrinogen** should elevate ESR.

- Dramatically ↑ with infection, malignancy, connective tissue disease. Also ↑ with pregnancy, inflammatory disease, and anemia.

- A ↓ ESR is associated with blood diseases in which red blood cells have an irregular or smaller shape that causes slower settling ie: **sickle cell anemia**

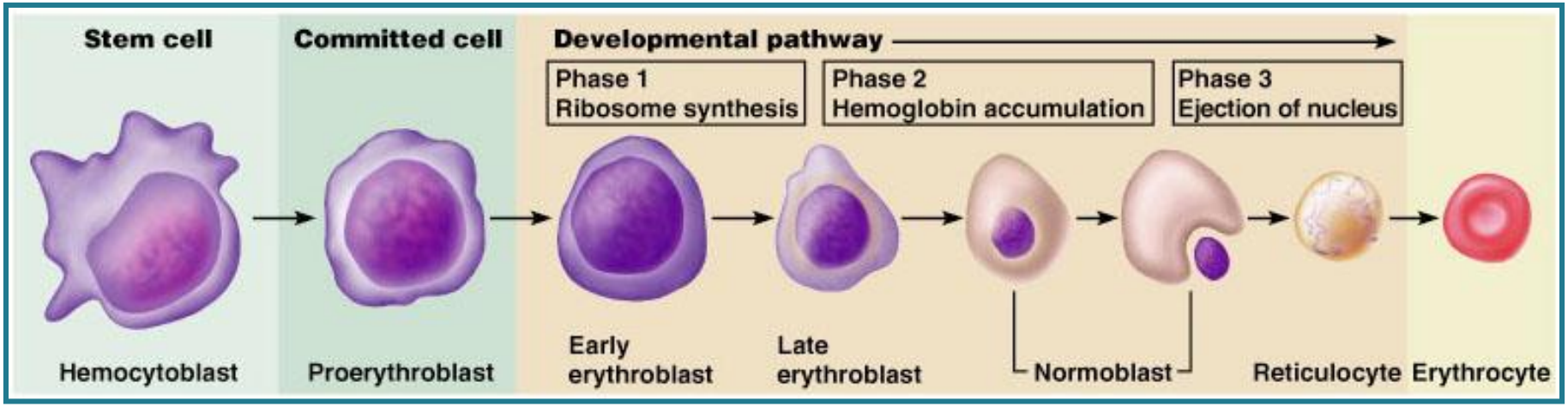
Erythropoiesis

- The term **erythropoiesis** (erythro = RBC, and poiesis = to make) is used to describe the process of RBC formation or production.

- In humans, erythropoiesis occurs almost exclusively in the **red bone marrow**

Erythropoiesis

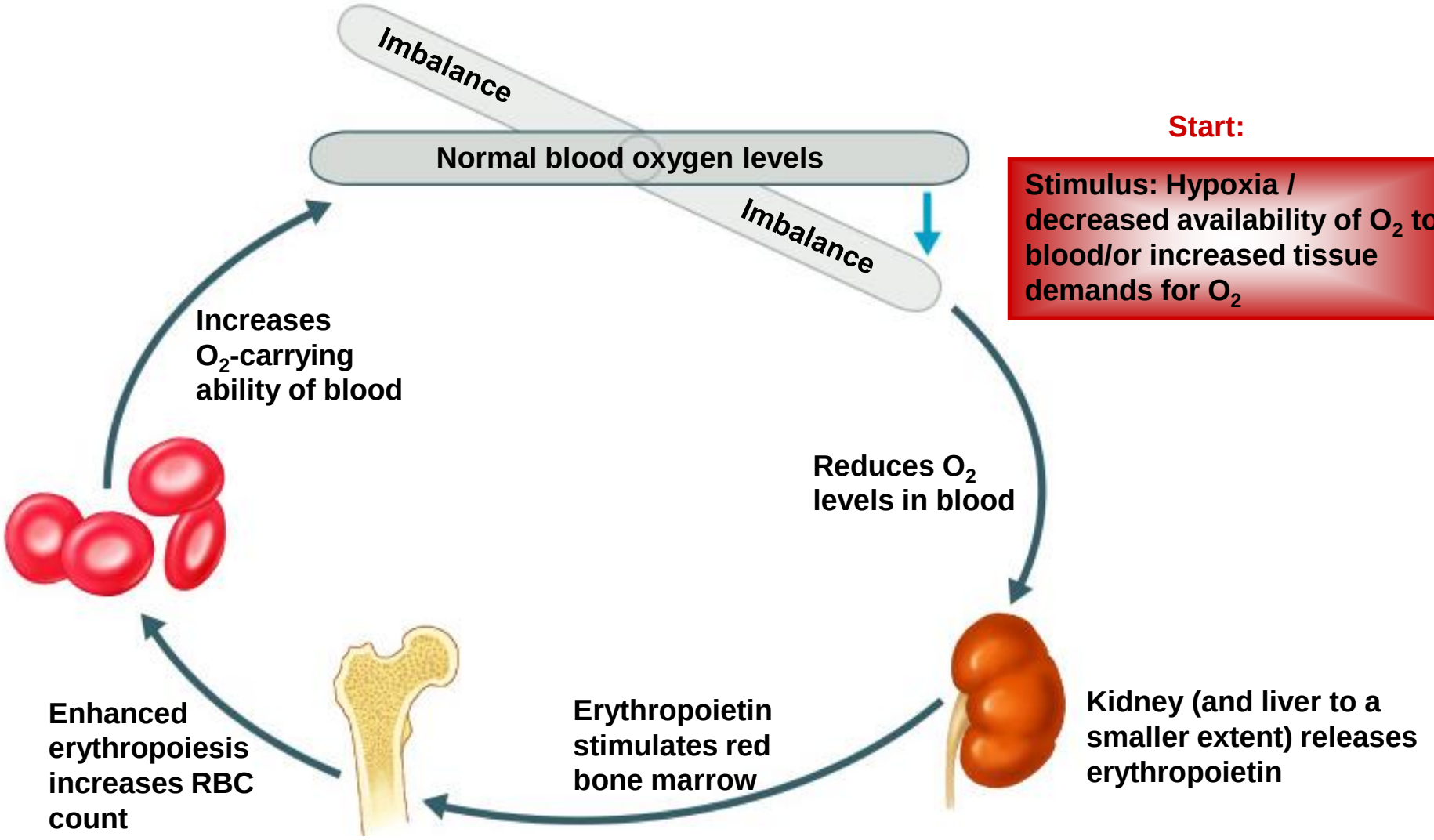
- A **hemocytoblast** is transformed into a committed cell called the **proerythroblast**
- Proerythroblasts develop into **early erythroblasts**
- The developmental pathway consists of three phases
  - **Phase 1:** ribosome synthesis in **early erythroblasts**
  - **Phase 2:** hemoglobin accumulation in **late erythroblasts** and **normoblasts**
  - **Phase 3:** ejection of the nucleus from normoblasts and formation of **reticulocytes**
- Reticulocytes then become mature **erythrocytes**



Role of B12 and Folic acid in the maturation of RBC

- Essential for DNA synthesis
- Deficiency leads to delay in maturation of nucleus

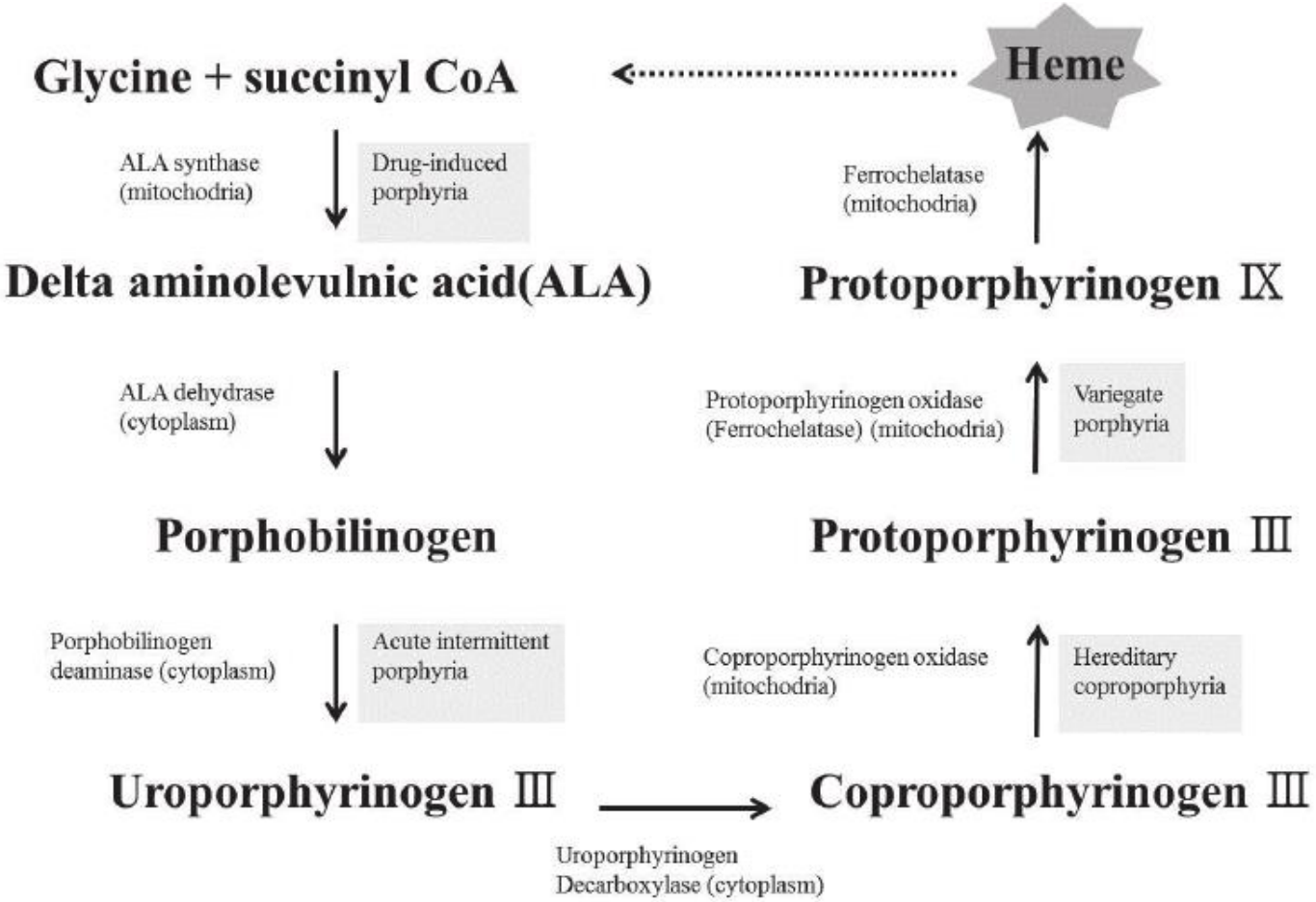
Erythropoietin Mechanism

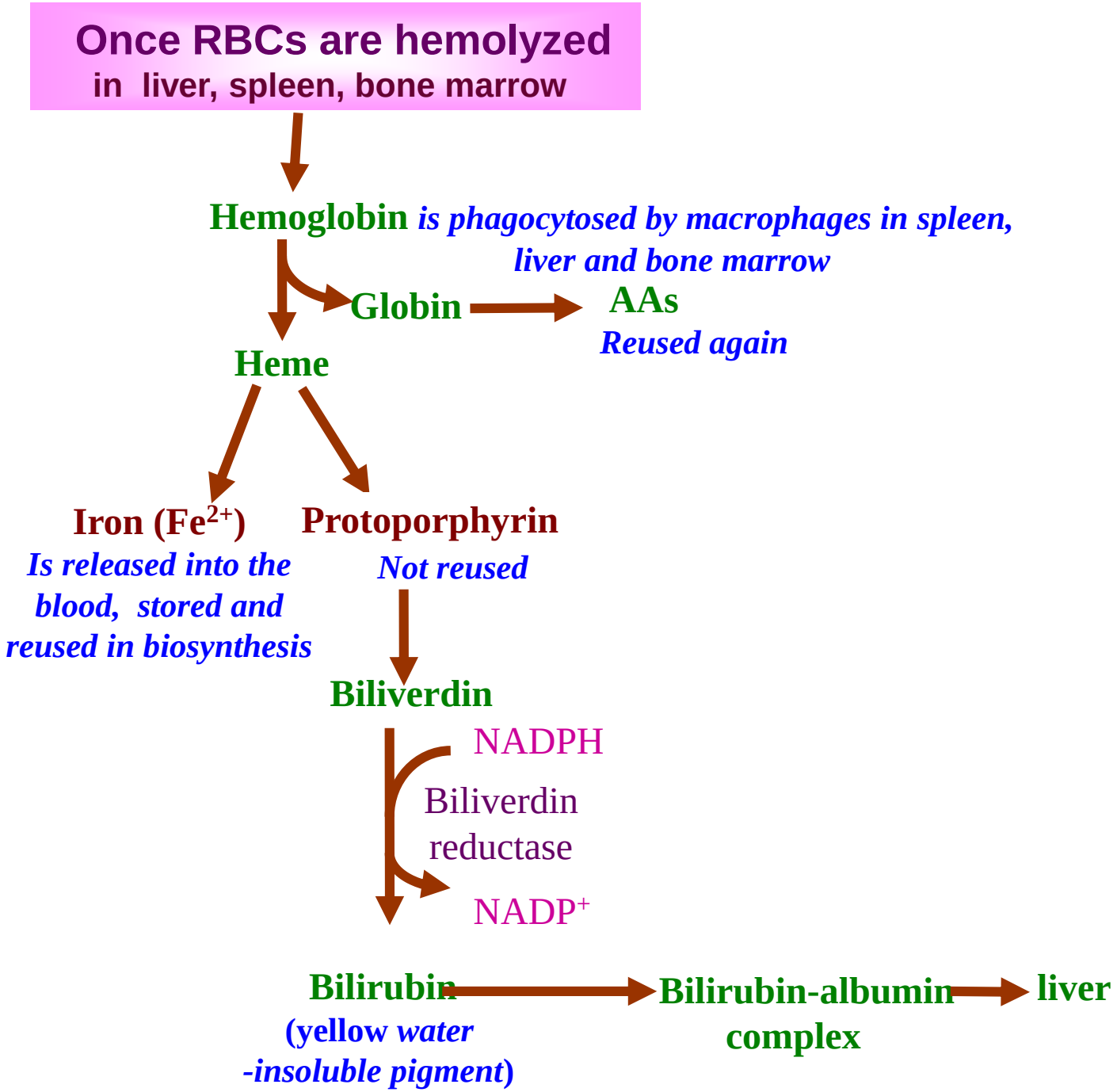


Formation of haemoglobin

- Begins in proerythroblast.
- Starting molecule: succinyl CoA with glycine forms a pyrrole.
- Four pyrroles form protoporphyrin IX
- Protoporphyrin IX combines with iron to form haem.
- Each haem combines with globin to form haemoglobin.

Heme biosynthesis




Once RBCs are hemolyzed
in liver, spleen, bone marrow
Hemoglobin is phagocytosed by macrophages in spleen, liver and bone marrow
Globin
AAs
Reused again
Heme
Iron (Fe^{2+})
Is released into the blood, stored and reused in biosynthesis
Protoporphyrin
Not reused
Biliverdin
NADPH
Biliverdin reductase
$NADP^+$
Bilirubin
(yellow water -insoluble pigment)
Bilirubin-albumin complex
liver

What Is Hemolysis?

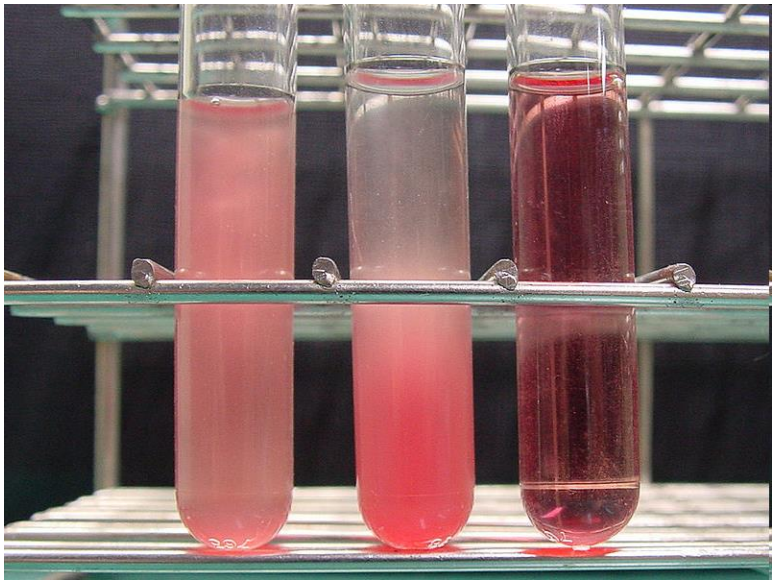
- Hemolysis is the breakage of the RBC's membrane, causing the release of the hemoglobin and other internal components into the surrounding fluid.

- Hemolysis is visually detected by showing a pink to red tinge in serum or plasma (The Hb of the lysed cells dissolves in plasma and makes it red)

- In-vivo hemolysis may be due to pathological conditions, such as autoimmune hemolytic anemia or transfusion, drugs and infections…

What Is Hemolysis?

- **(a)** without hemolysis: red blood cell suspension seems red and opaque.
- **(b)** without hemolysis: RBCs sedimented spontaneously for 60 min. Note that the supernatant is not colored.
- **(c)** hemolysis: RBC suspension become transparent by hemolysis.



(a) (b) (c)

RBC Metabolic Pathways

- No organelle – no mitochondria
- Generate energy through anaerobic breakdown of glucose (glycolysis) (2ATP+lactate)
- ATP is used as substrate for Na^+, K^+ or the Ca^{2+} dependant ATPases that maintain intracellular concentrations of these cations
- The glucose uptake by RBC's through high affinity glucose transporter is independent on insulin

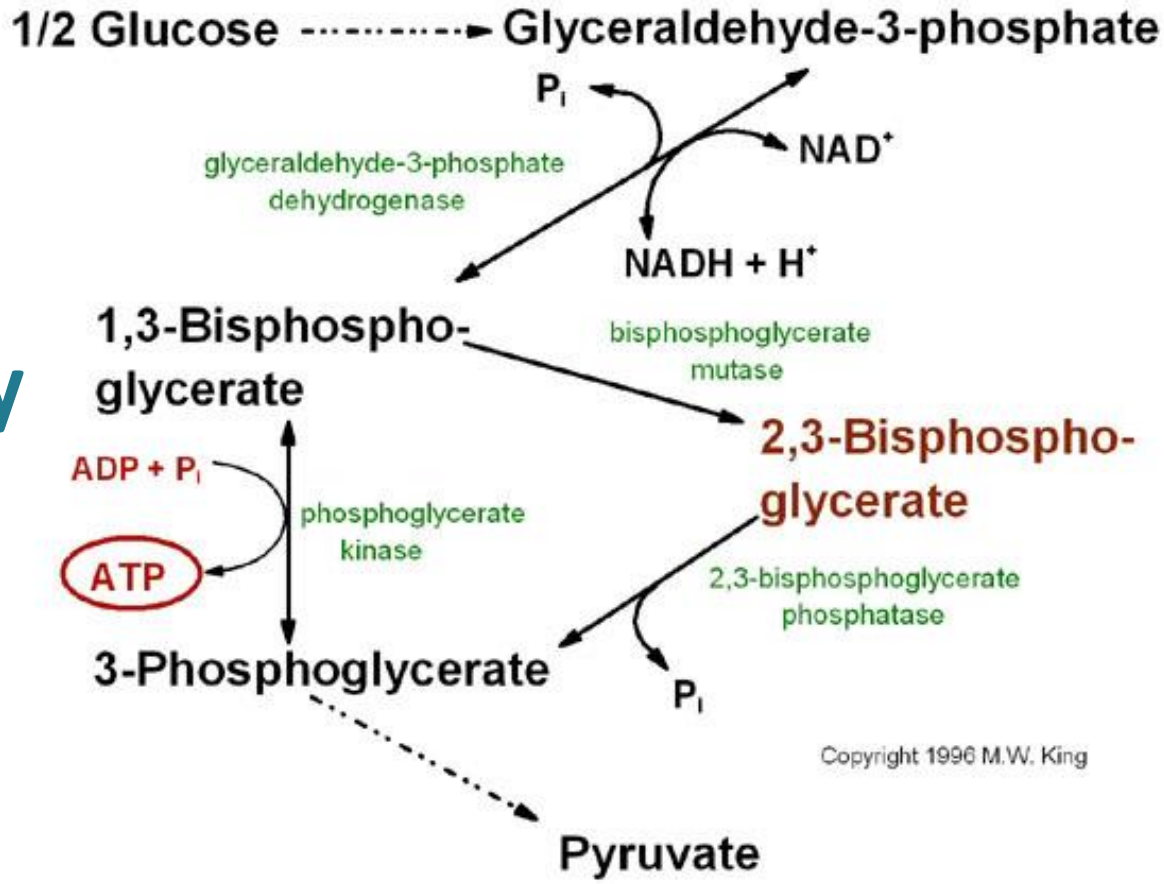
RBC Metabolic Pathways (Cont.)

Four pathways involved in RBC metabolism:

1. Pentose Phosphate Pathway
2. Glycolysis pathway
3. Methemoglobin reductase pathway
4. 2,3 BPG pathway is unique for RBC

- In red cells 1,3 BPG is converted to 2,3BPG which unites with oxy Hb and **helps release of oxygen at tissues**.
- The rate of synthesis and hydrolysis of 2,3 DPG are **very sensitive to pH**: a fall of pH inhibits mutase and stimulates phosphatase causing **decrease of 2,3 DPG**

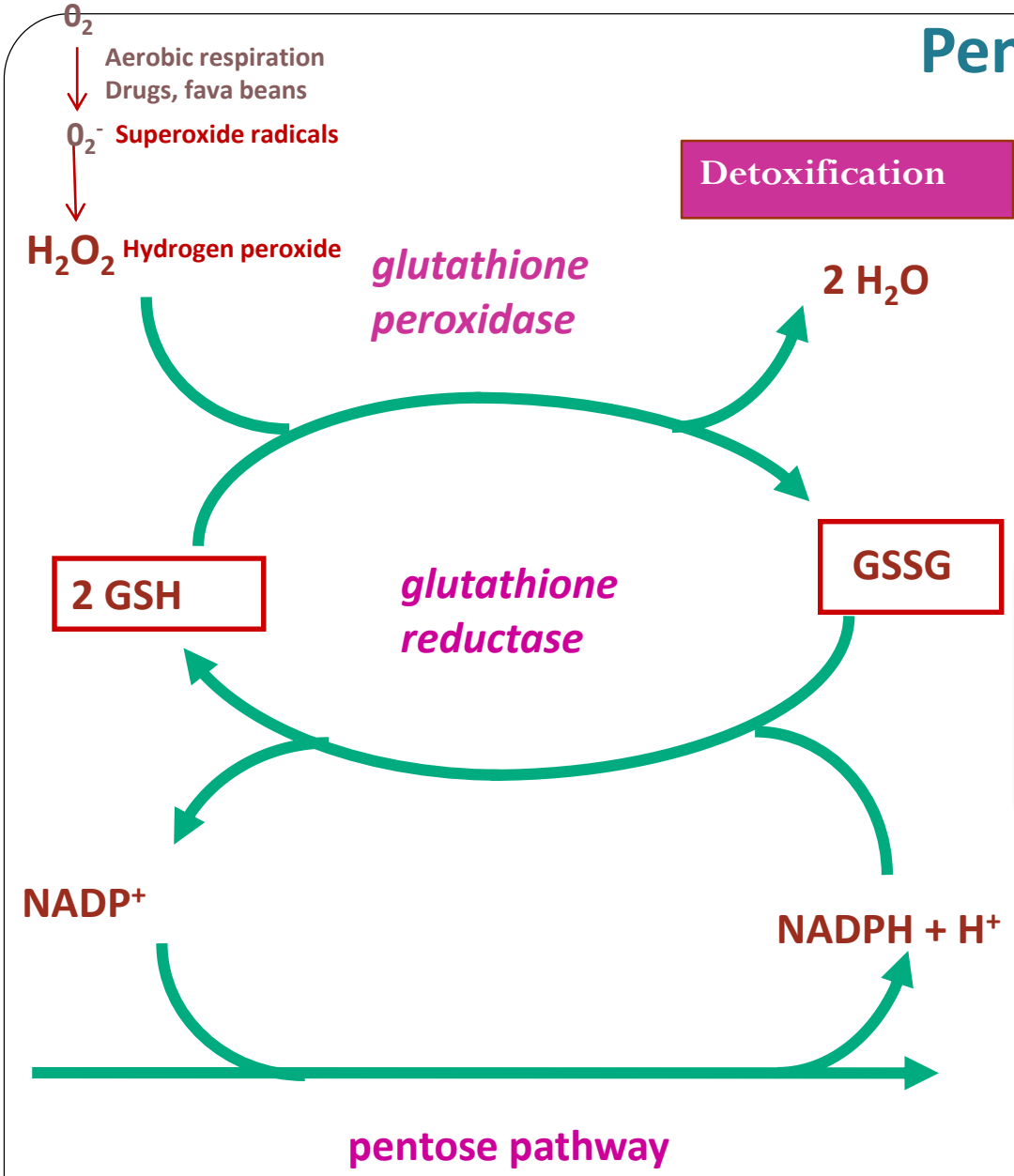
2,3 BPG pathway



Metabolism of glutathione

- RBC takes up glutathione from circulation.
  - Liver produces glutathione and releases into circulation.
- Glutathione keeps -SH groups of membrane lipids and proteins in reduced form.
- RBC may produce some amount of glutathione.

Pentose Phosphate Pathway



- When erythrocytes are exposed to chemicals that generate high levels of superoxide radicals, **GSH (Reduced Glutathione)** is required to reduce these damaging compounds

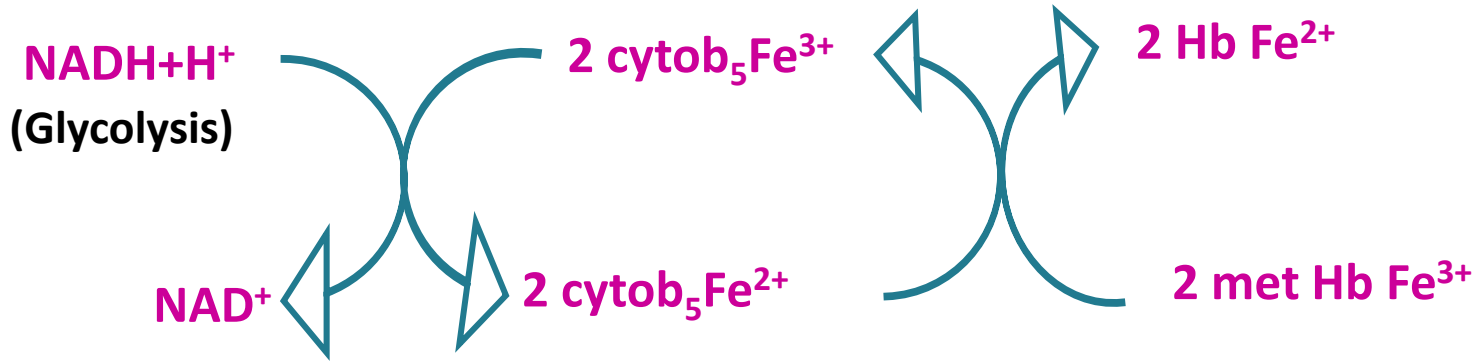
The major role of PPP in RBCs is the production of NADPH which protect these cells from oxidative damage by providing GSH for removal of H_2O_2

oxidized to a disulfide **GSSG**

- The PPP is responsible for maintaining high levels of NADPH in red blood cells for use as a **reductant** in the glutathione reductase reaction.

Methemoglobin Reductase Pathway

- Important in maintaining heme iron in the reduced or ferrous functional state
- The metHbFe^{3+} is reduced to deoxyHbFe^{2+} by the flavoprotein, MetHb reductase (MW 185Kda) (NADH cytochrome b_5 reductase).

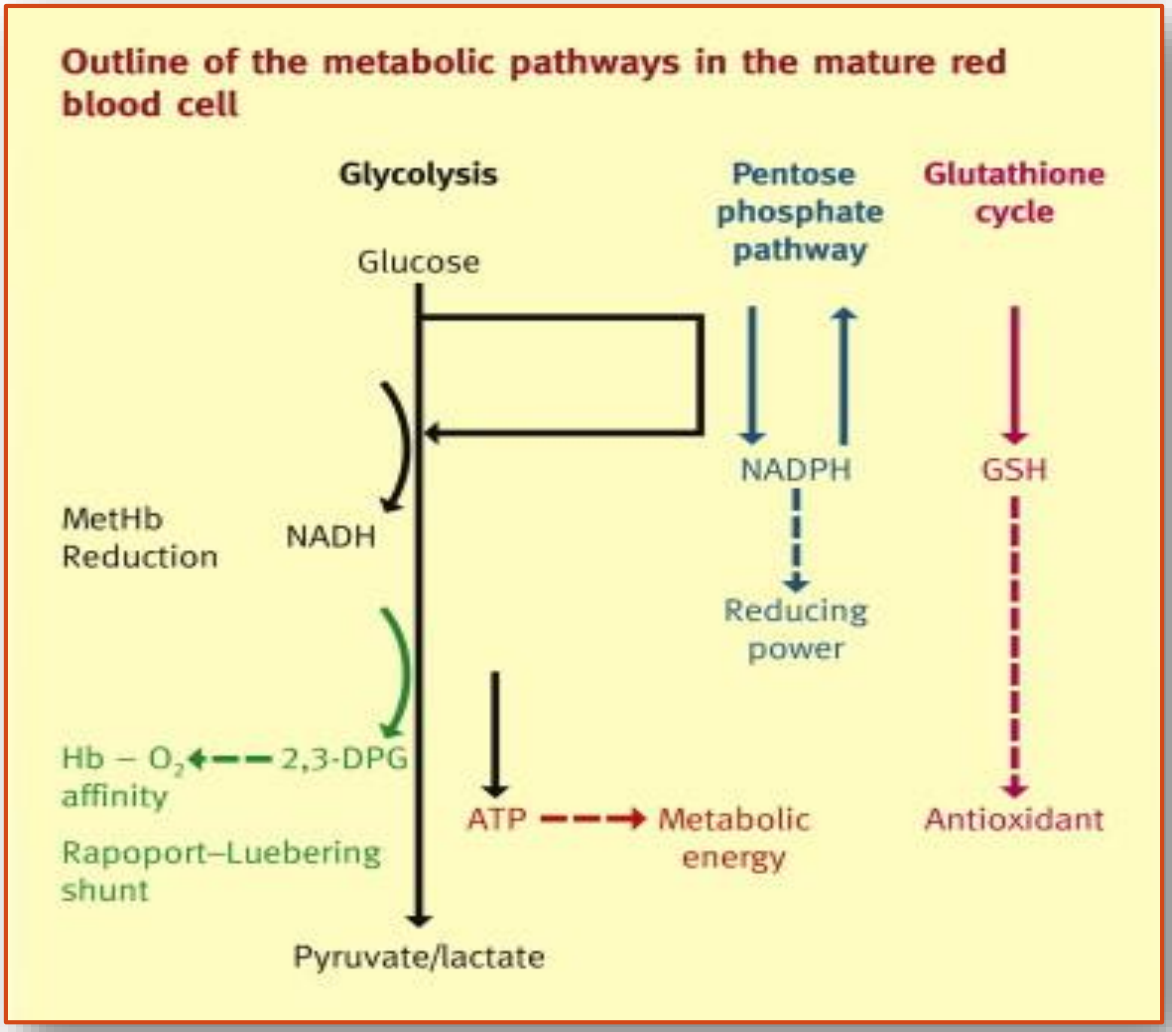


Antioxidant enzymes

- Superoxide dismutase, catalase, and peroxidase are antioxidant enzymes present in RBC.

- They protect erythrocyte membrane lipids and proteins from the deleterious effect of Reactive oxygen species (ROS).

Outline of the metabolic pathways in the mature red blood cell

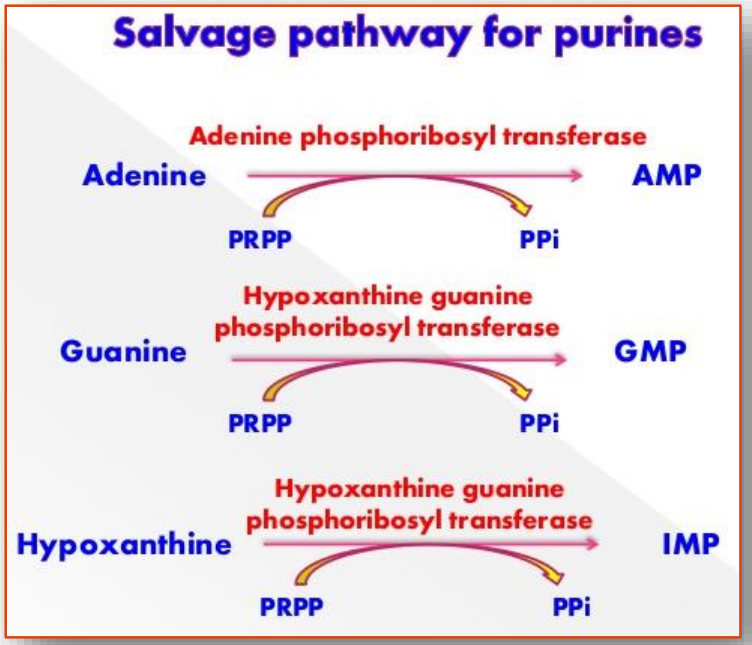


Salvage pathway synthesis of purine nucleotides

- The salvage pathway is particularly important in certain tissues such as erythrocytes where de novo (a new) synthesis of purine nucleotides is not operative.
- This pathway ensures the recycling of purines formed by degradation of nucleotides.

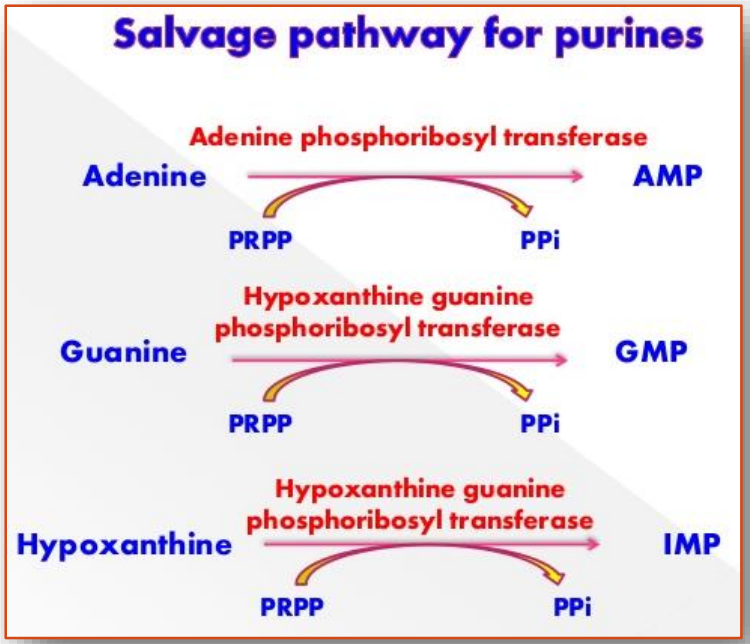
Salvage pathway synthesis of purine nucleotides (cont.)

- Phosphoribosyl pyrophosphate (PRPP) is the starting material in this pathway.
- The free purines are salvaged by two different enzymes.
    1. Adenine phospho ribosyl transferase (APRTase).
    2. Hypoxanthine guanine phosphoribosyl transferase (HGPRTase).



Salvage pathway synthesis of purine nucleotides (cont.)

- APRTase catalyses the formation of AMP from adenine.
- HGPRT converts guanine & hypoxanthine to GMP & IMP.
- PRPP is the donor of ribose 5-phosphate in the salvage pathway.



Glucose-6-phosphate dehydrogenase deficiency causes hemolytic anemia

- Mutations present in some populations causes a deficiency in glucose 6-phosphate dehydrogenase (X-linked), **with consequent impairment of NADPH production**

- Detoxification of H_2O_2 is inhibited, and cellular damage results - lipid peroxidation leads to erythrocyte membrane breakdown (hemolysis) and hemolytic anemia.

Glucose-6-phosphate dehydrogenase deficiency causes hemolytic anemia(cont.)

- Most G6PD-deficient individuals are asymptomatic - only in combination with certain environmental factors (sulfa antibiotics, herbicides, antimalarials, *divicine) do clinical manifestations occur.

*toxic ingredient of fava beans

Pyruvate Kinase Deficiency

This deficiency causes the entire glycolysis pathway to cease working so that little to no ATP is produced.

- Potassium and water leak from the cell, while calcium concentrations increase.

Pyruvate Kinase Deficiency (cont.)

Consequently, the defective red cells are destroyed in the spleen causing anaemia and the spleen enlarges (splenomegaly) because it is overworked.

The excessive destruction of red blood cells (hemolysis) results in the breakdown of hemoglobin stored in these cells.

Deficiencies are inherited as autosomal recessive traits, so both sexes are affected equally.

Hormonal Control of Erythropoiesis

- Erythropoiesis is controlled by a circulating hormone: **Erythropoietin (EPO)**

- EPO release by the kidneys is triggered by:
  - Hypoxia due to decreased RBCs (low O_2)
  - Decreased oxygen availability
  - Increased tissue demand for oxygen

- Enhanced erythropoiesis increases the:
  - RBC count in circulating blood
  - Oxygen carrying ability of the blood