

Red Cell Structure and Function

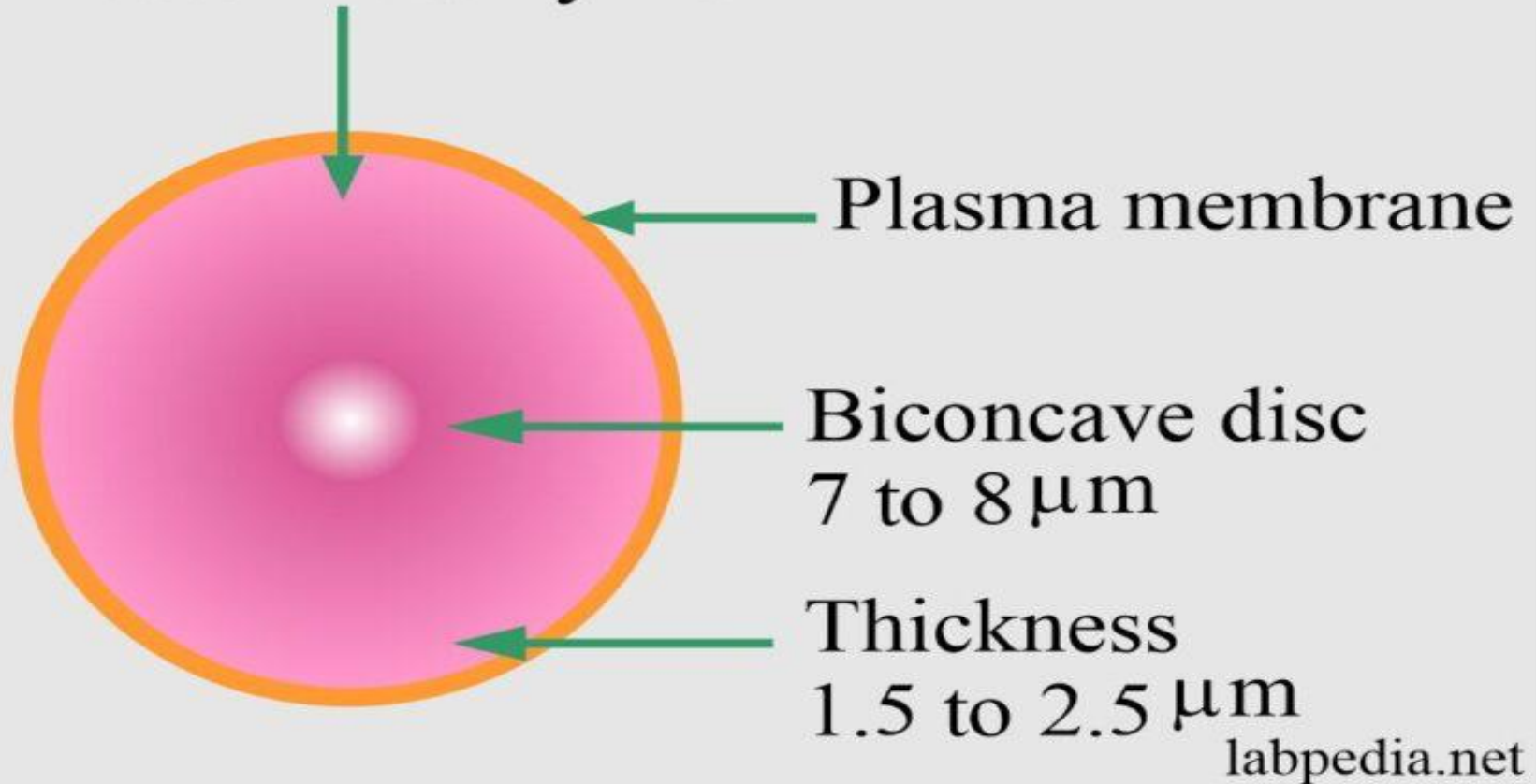
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Introduction to RBC Function

- ▶ **Three areas of RBC metabolism essential for survival and function:**
 - RBC membrane
 - Hemoglobin structure and function
 - Cellular energetics
- ▶ **Defects in any area results in impaired RBC survival (RBCs have normal 120 day life span).**

Red blood cell structure

Protein mainly Hb

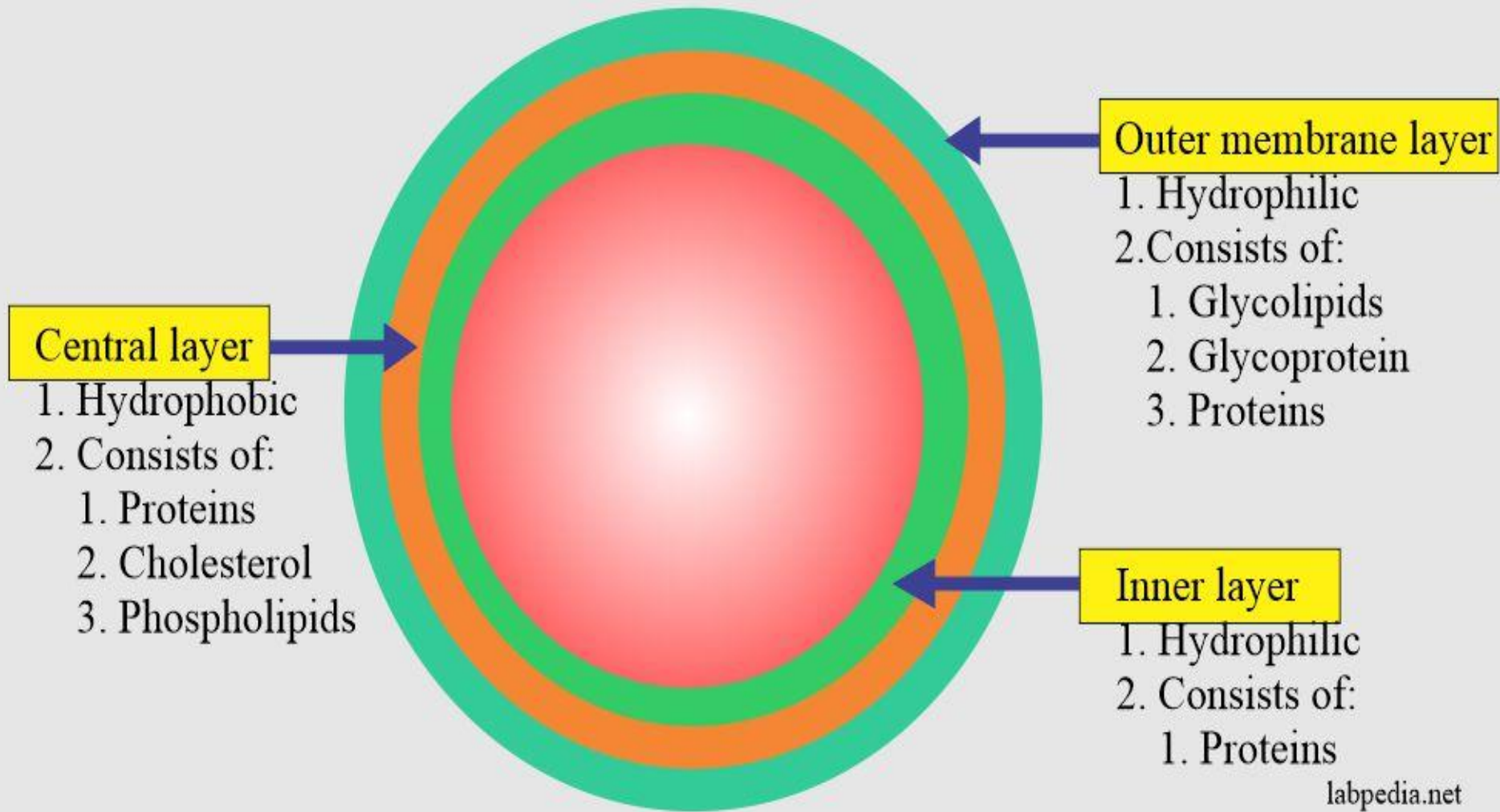


RBC Membrane

- ▶ RBC membrane is a three layer structure:
 - an outer hydrophilic portion composed of glycolipid, glycoprotein, and protein
 - a central hydrophobic layer containing protein, cholesterol, and phospholipid
 - an inner hydrophilic layer containing protein
- ▶ Membrane is very elastic.
- ▶ Membrane is a semi-permeable lipid bi-layer supported by a mesh-like cytoskeleton.

Red blood cell membrane

1. Highly elastic
2. Can have large membrane extensions without fragmentation



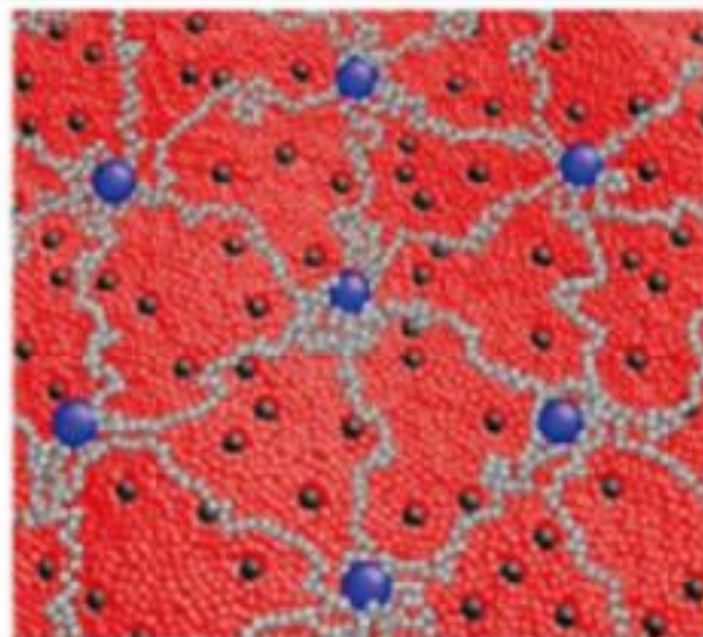
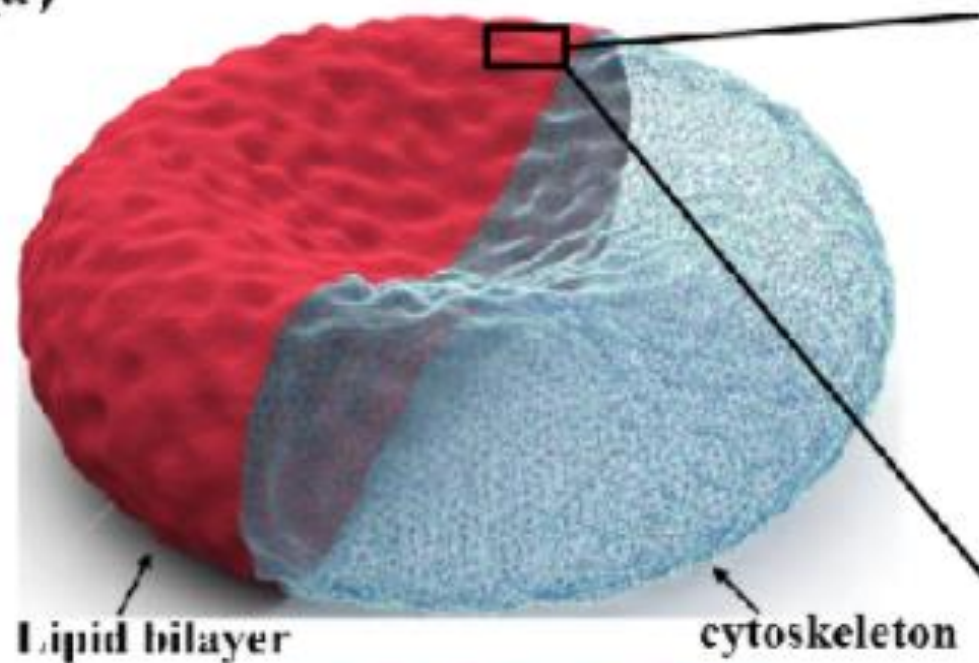
RBC Membrane

► Cytoskeleton:

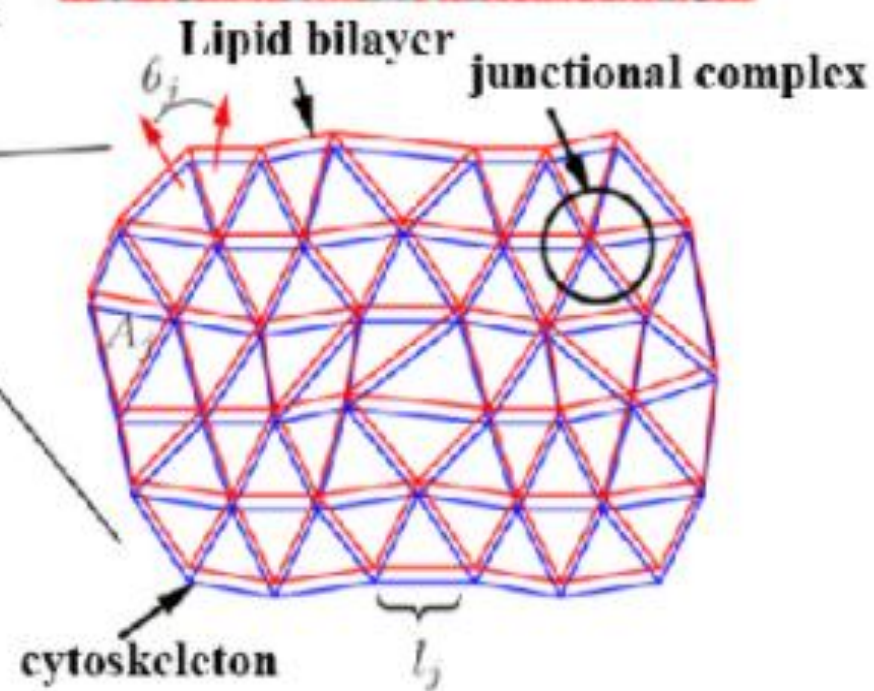
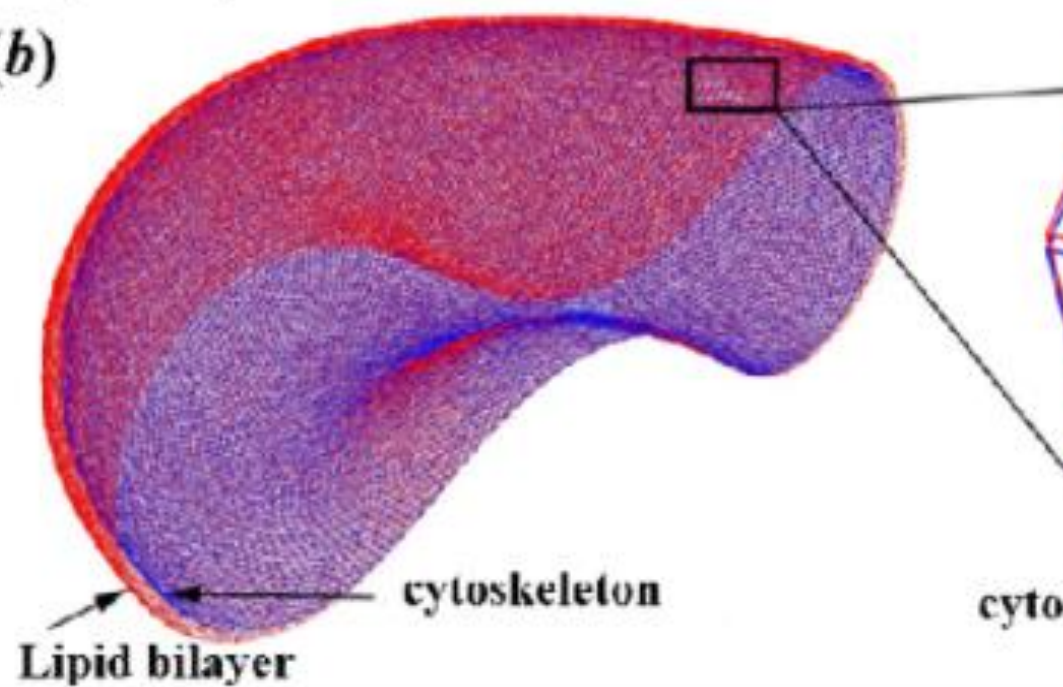
- Network of proteins on the inner surface of the plasma membrane, called the peripheral membrane proteins
- Responsible for maintaining shape, stability, and deformability of RBC

► Lipid bi-layer contains equal amounts of cholesterol and phospholipids with proteins scattered throughout.

(a)



(b)



Red Blood Cell Structure

G6PD enzyme

RBC

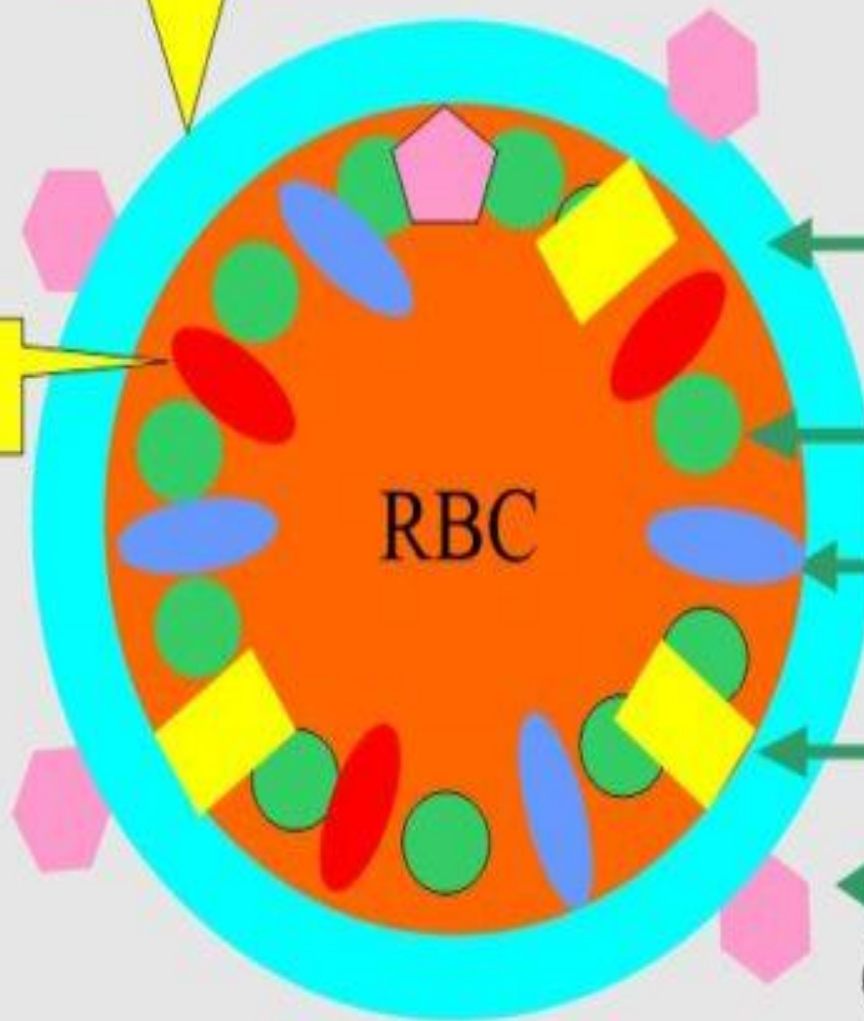
Lipid bilayer

50% Protein

20% Phospholipids

20% Cholesterol

10% Carbohydrate
(only on external surface)



RBC Membrane

► **Integral membrane proteins:**

- Extend from outer surface and transverse entire membrane to inner surface

► **Peripheral proteins:**

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

NOTE: They aren't really "peripheral" to the RBC, since they are within the RBC membrane

RBC Membrane Proteins

- ▶ The two most important protein constituents include:
 - **Glycophorin** (an integral membrane protein)
 - **Spectrin** (a peripheral membrane protein of the cytoskeleton)

Integral Membrane Proteins

- ▶ Glycophorin is the principle RBC glycoprotein. Spans entire thickness of lipid bilayer and appears on external surface of RBC membrane, accounting for location of many RBC antigens.
- ▶ Three types of glycophorins identified: A, B, and C.
- ▶ All glycophorins carry RBC antigens and are receptors or transport proteins.

Peripheral Proteins

- ▶ The major peripheral proteins that make up the cytoskeleton include:
 - Spectrin
 - Ankyrin
 - Protein 4.1
 - Actin

Peripheral Proteins

► **Spectrin:**

- The most abundant peripheral protein
- Flexible, rodlike 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
- Cytoskeletal network also provides stability to lipid bilayer.

Peripheral Proteins

► **Ankyrin:**

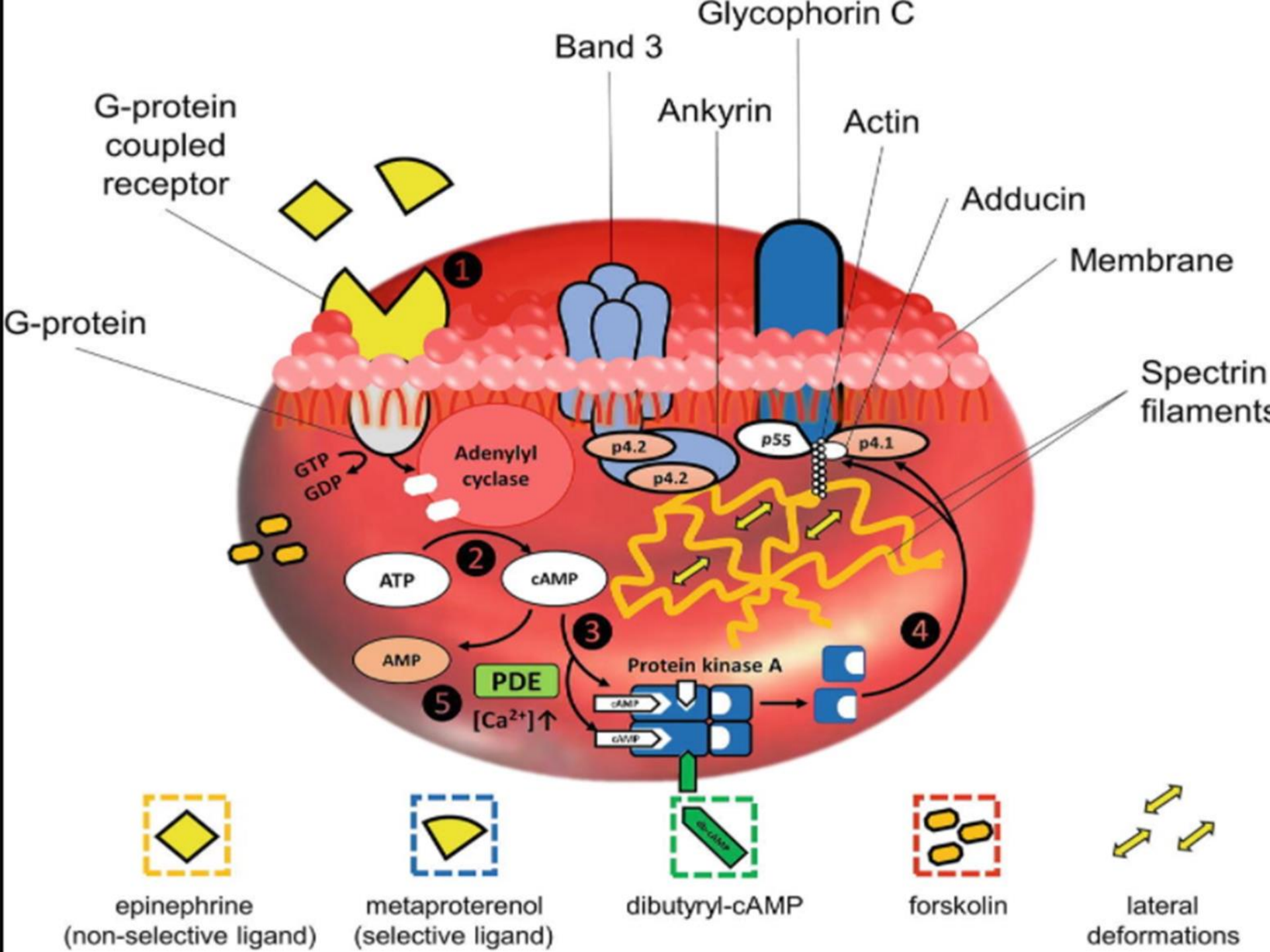
- Primarily anchors lipid bilayer to membrane skeleton

► **Protein 4.1:**

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

► **Actin:**

- Responsible for contraction and relaxation of membrane



Deformability

- ▶ Critical to RBC survival as it travels through microvasculature. Also essential for oxygen delivery.
- ▶ Loss of ATP (energy) leads to decrease in phosphorylation of spectrin, which, in turn, leads to loss of membrane deformability. Also leads to accumulation of calcium in membrane causing an increase in membrane rigidity and loss of pliability.

Deformability

- ▶ Cells lacking flexibility quickly removed from circulatory system. Spleen responsible for removal of RBCs.
- ▶ Rigid parts of cell membrane may be removed, resulting in malformed cells - spherocytes and "bite" cells.
- ▶ Lack of deformability shortens survival time.
- ▶ Sometimes deformability is reversible.
- ▶ Discoid shape most efficient form to maximize ratio of surface area to cell volume.

Permeability

- ▶ RBC membrane freely permeable to water and anions (chloride and bicarbonate).
- ▶ Exchange of anions between internal environment of cell and external environment facilitated by exchange channels.
- ▶ RBC membrane relatively impermeable to cations (primarily sodium and potassium). Is through control of sodium and potassium intracellular concentrations that RBC maintains its volume and water homeostasis.

Red Cell Membrane Lipids

- ▶ 1. Phospholipids
- ▶ 2. Glycolipids and Cholesterol

Phospholipids

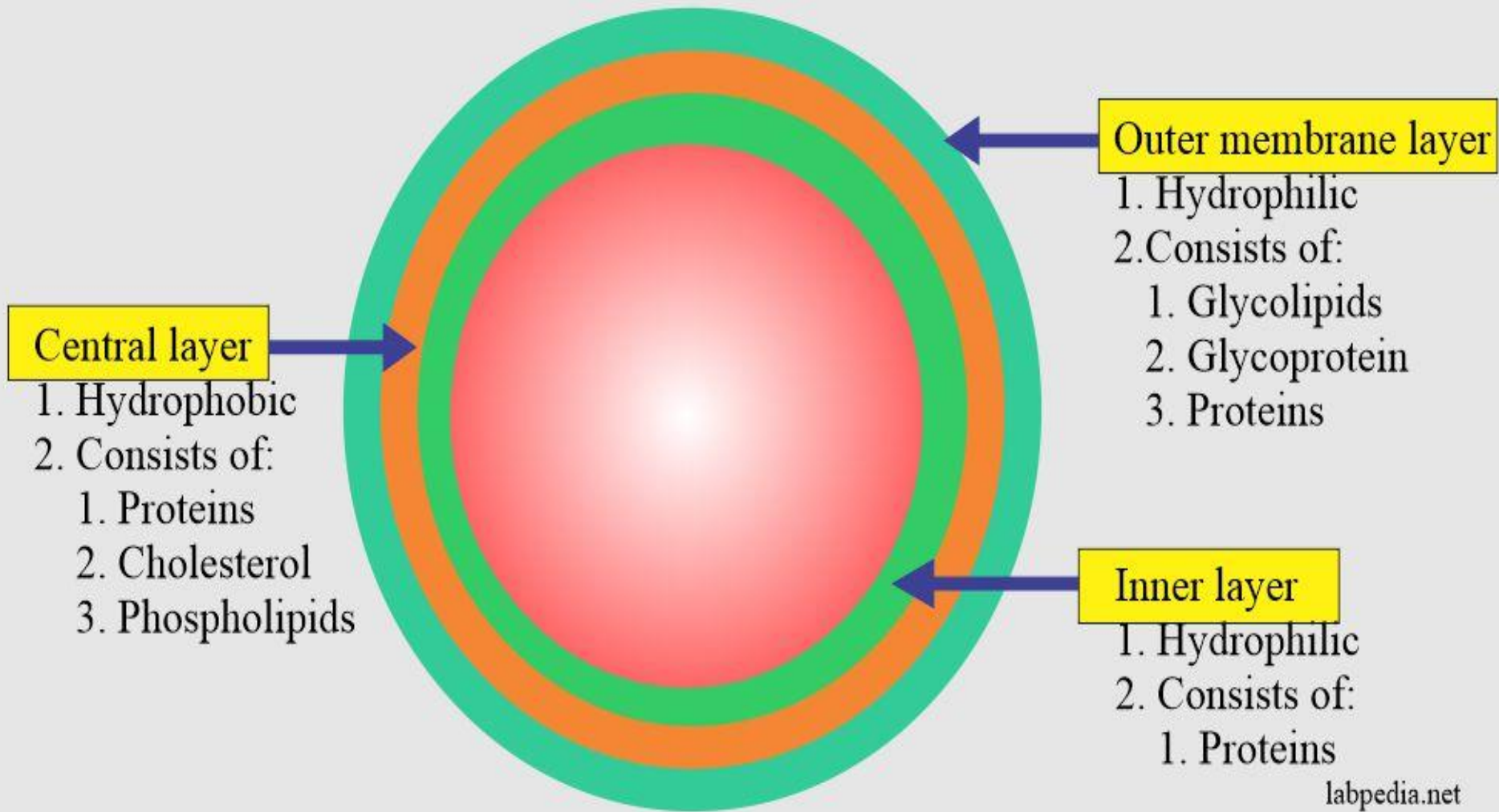
- ▶ Erythrocyte membrane lipid consists of bilayer of phospholipids intermingled with molecules of cholesterol in nearly equal amounts. Also small amounts of free fatty acids and glycolipids.
- ▶ Different types of phospholipids are found on the inside layer than on the outside layer. The orientation of these phospholipids, and ratios of them, are important to proper transport of substances in and out of the RBC. Abnormalities in the phospholipids may result in decreased deformability and decreased red cell survival (extravascular or intravascular hemolysis).

Glycolipids and Cholesterol

- ▶ 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. Is 25% of RBC membrane lipid content. RBC membrane cholesterol is in continual exchange with plasma cholesterol.
- ▶ Cholesterol plays important role in regulating membrane fluidity and permeability.
- ▶ Accumulation of cholesterol can result in formation of target cells, acanthocytes, bite cells, and spherocytes.
- ▶ Accumulation of cholesterol results in decreased deformability and may lead to hemolytic anemia.

Red blood cell membrane

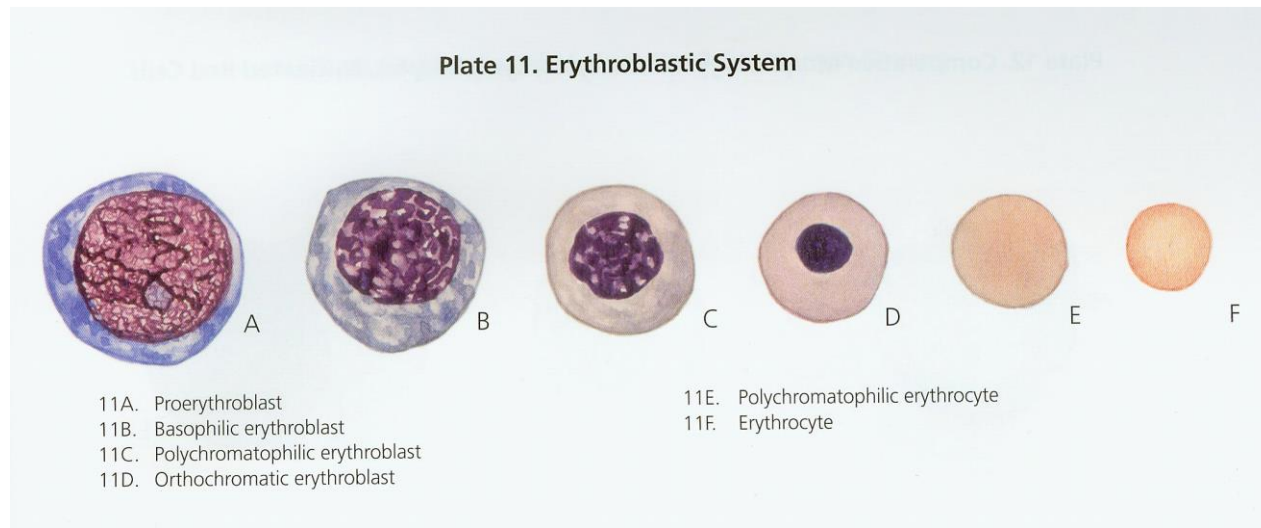
1. Highly elastic
2. Can have large membrane extensions without fragmentation



Hemoglobin Synthesis

Introduction to Hemoglobin Synthesis

- ▶ Hemoglobin is a conjugated globular protein.
- ▶ Constitutes about 95% of RBC's dry weight.
- ▶ About 65% of Hgb synthesis occurs during nucleated stages of RBC maturation, and 35% occurs during the reticulocyte stage.



Introduction to Hemoglobin Synthesis (cont.)

- ▶ Normal hemoglobin consists of **globin** (a tetramer of two parts of unlike globin polypeptide chains) and four **heme groups**, each of which contains a **protoporphyrin ring** plus **iron**. Also contains one molecule of **2,3-DPG** (2,3-diphosphoglycerate).

Glossary Definitions

► Globin:

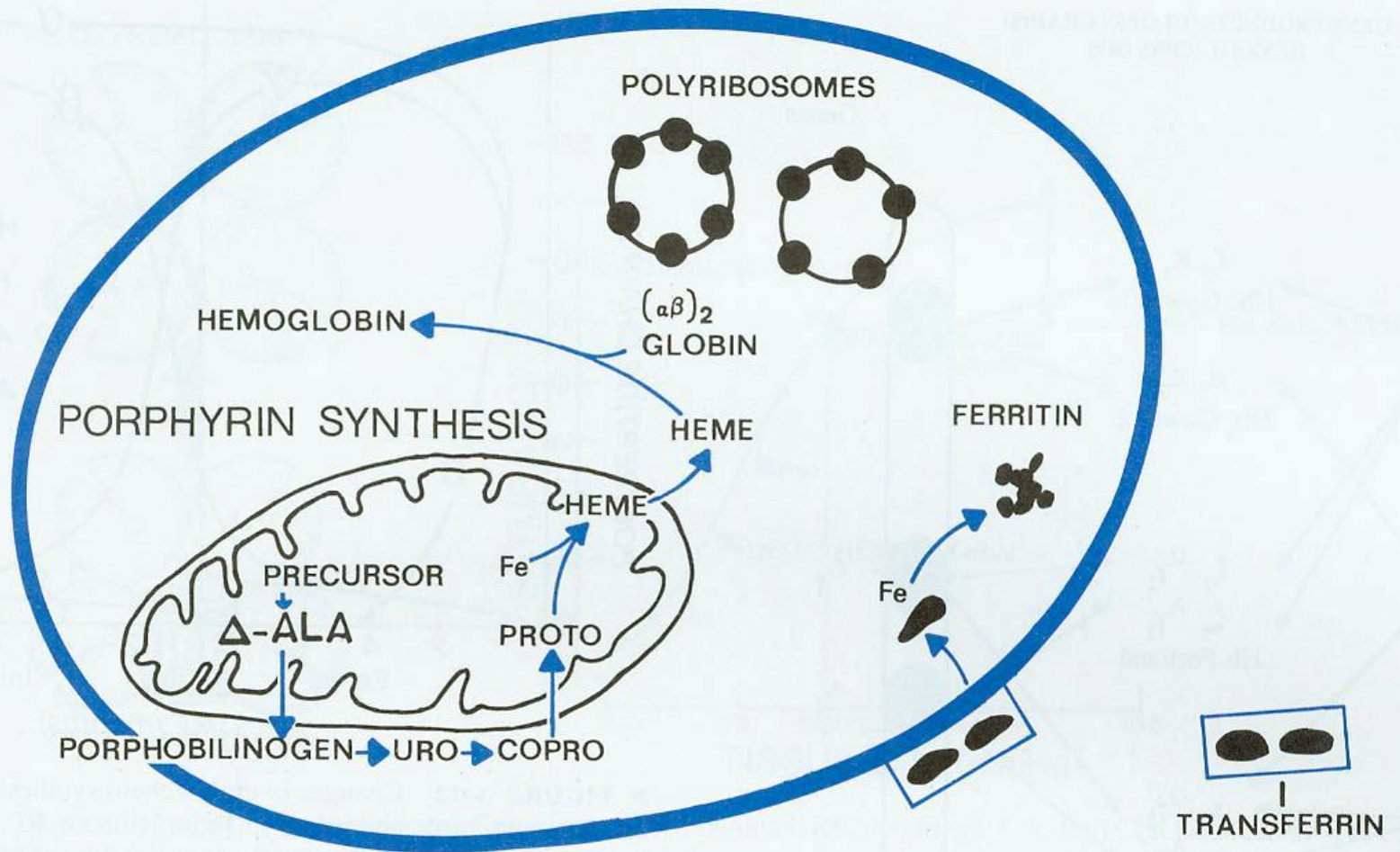
- A protein constituent of hemoglobin
- There are 4 globin chains in the hemoglobin (Hgb) molecule

► Heme:

- The iron-containing protoporphyrin portion of the Hgb wherein the iron is in the ferrous (Fe^{2+}) state

Hemoglobin Synthesis

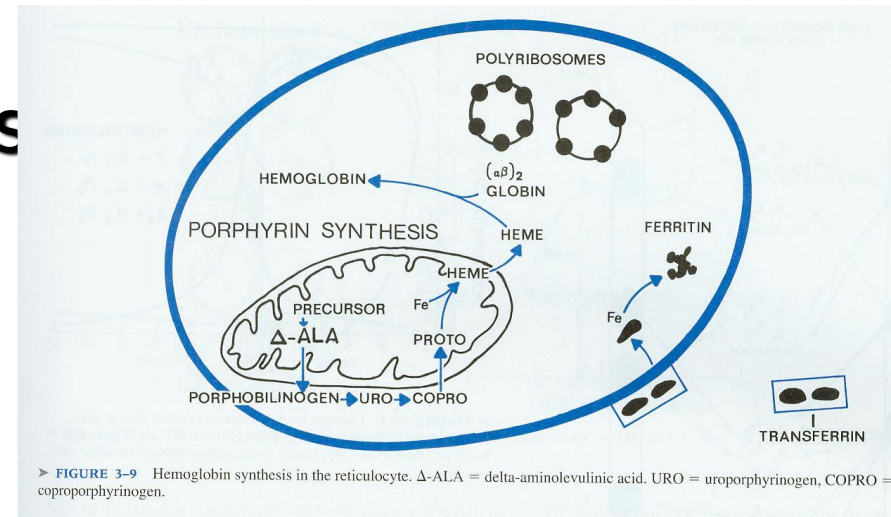
- ▶ **Depends on three processes:**
 - 1. **adequate supply and delivery of iron;**
 - 2. **adequate synthesis of
protoporphorins (heme precursor);**
 - 3. **adequate globin synthesis**



➤ **FIGURE 3-9** Hemoglobin synthesis in the reticulocyte. Δ -ALA = delta-aminolevulinic acid. URO = uroporphyrinogen, COPRO = coproporphyrinogen.

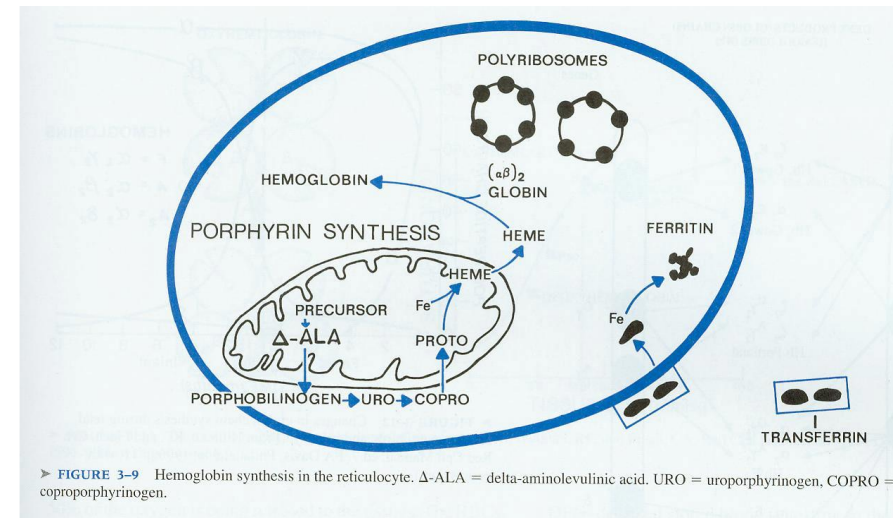
Iron Delivery and Supply

- ▶ Iron delivered to membrane of RBC precursor by protein carrier **transferrin**.
- ▶ Most of the iron that crosses membrane and enters cytoplasm of cell is committed to hemoglobin synthesis. Proceeds to mitochondria for insertion into **protoporphyrin ring** to form **heme**.

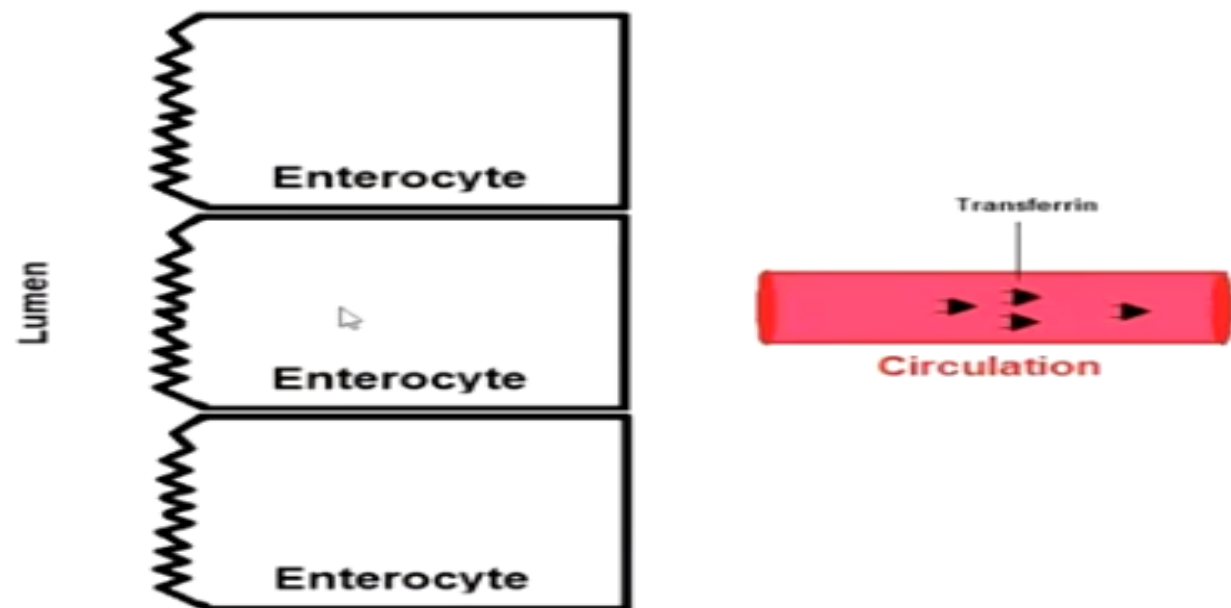


Iron Delivery and Supply

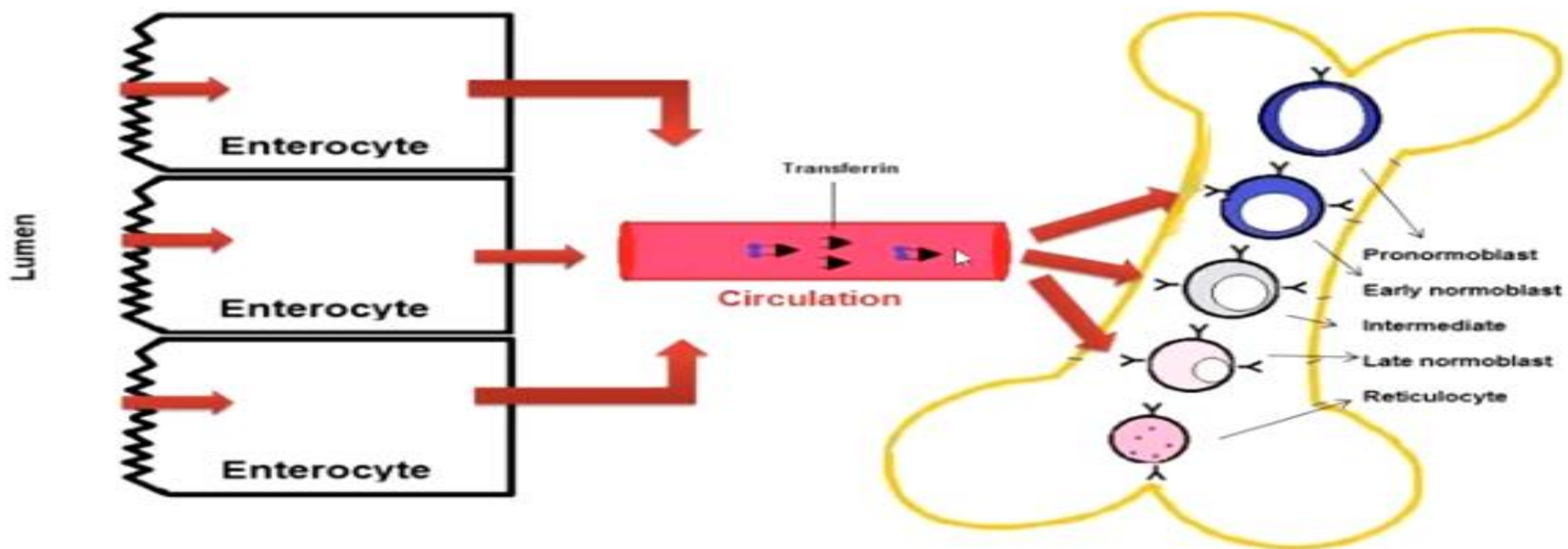
- ▶ Excess iron in cytoplasm aggregates as **ferritin**. Amount of ferritin stored depends on ratio between level of plasma iron and amount of iron required by erythrocyte for hemoglobin synthesis.
- ▶ Two-thirds of total iron supply is bound to heme in hemoglobin.



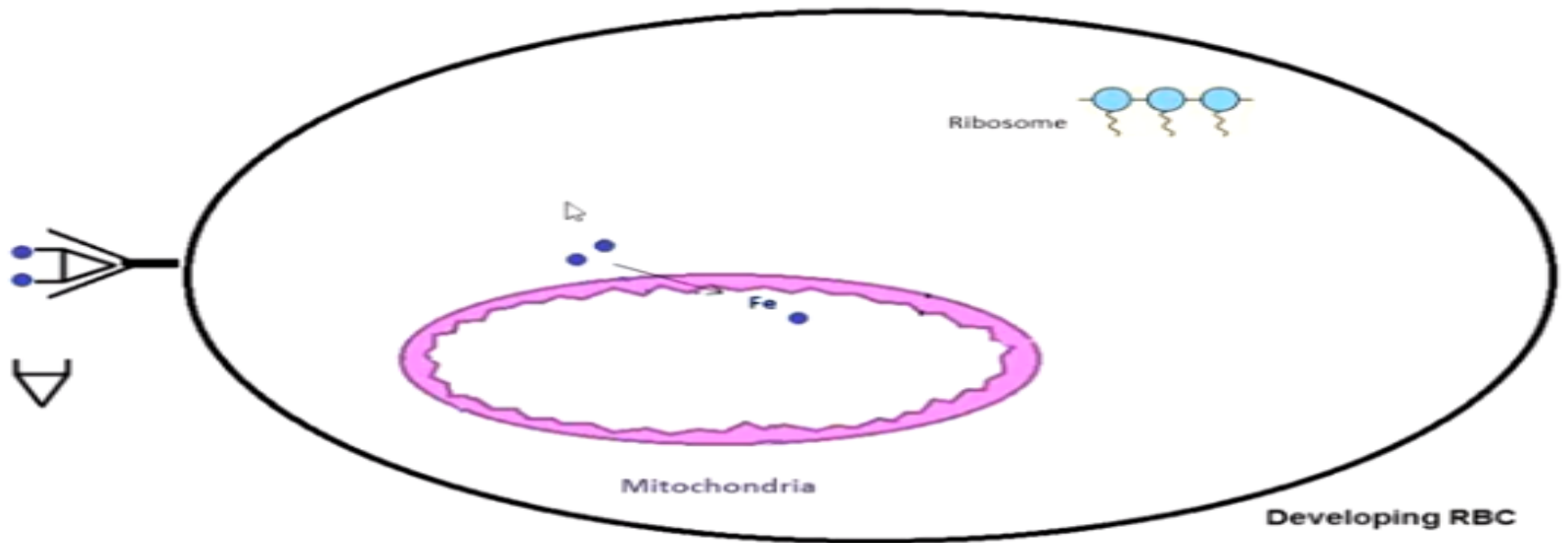
Iron Metabolism



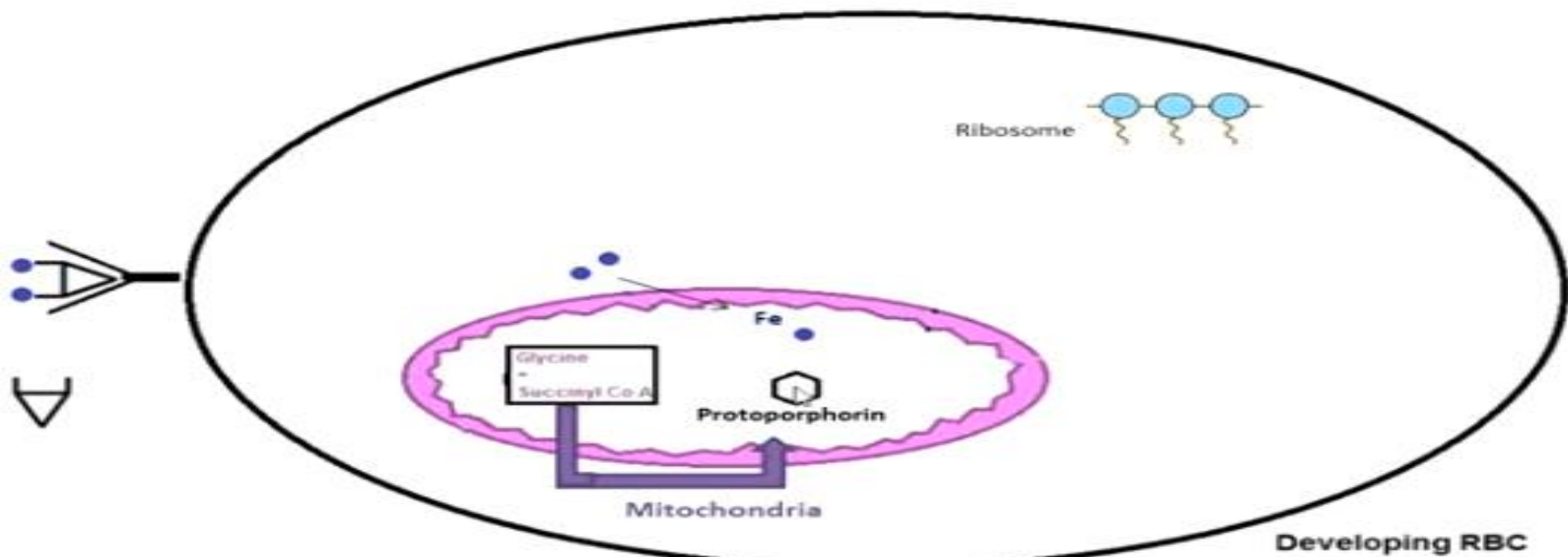
Iron Metabolism



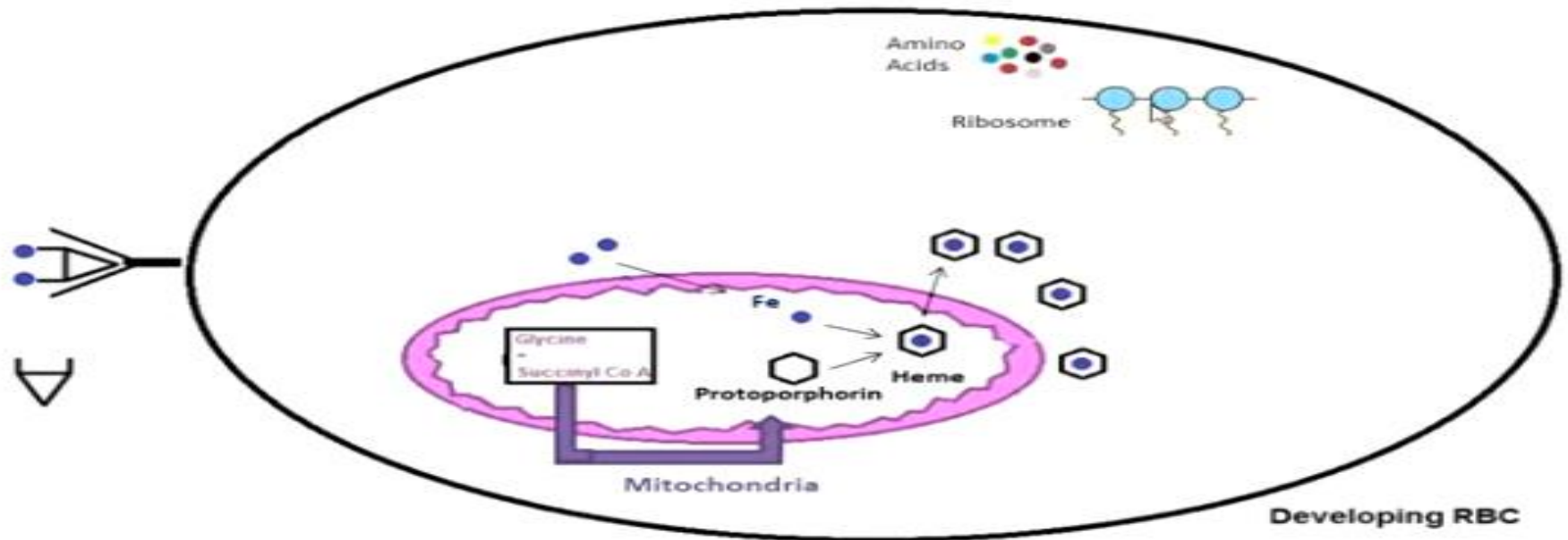
Hemoglobin synthesis



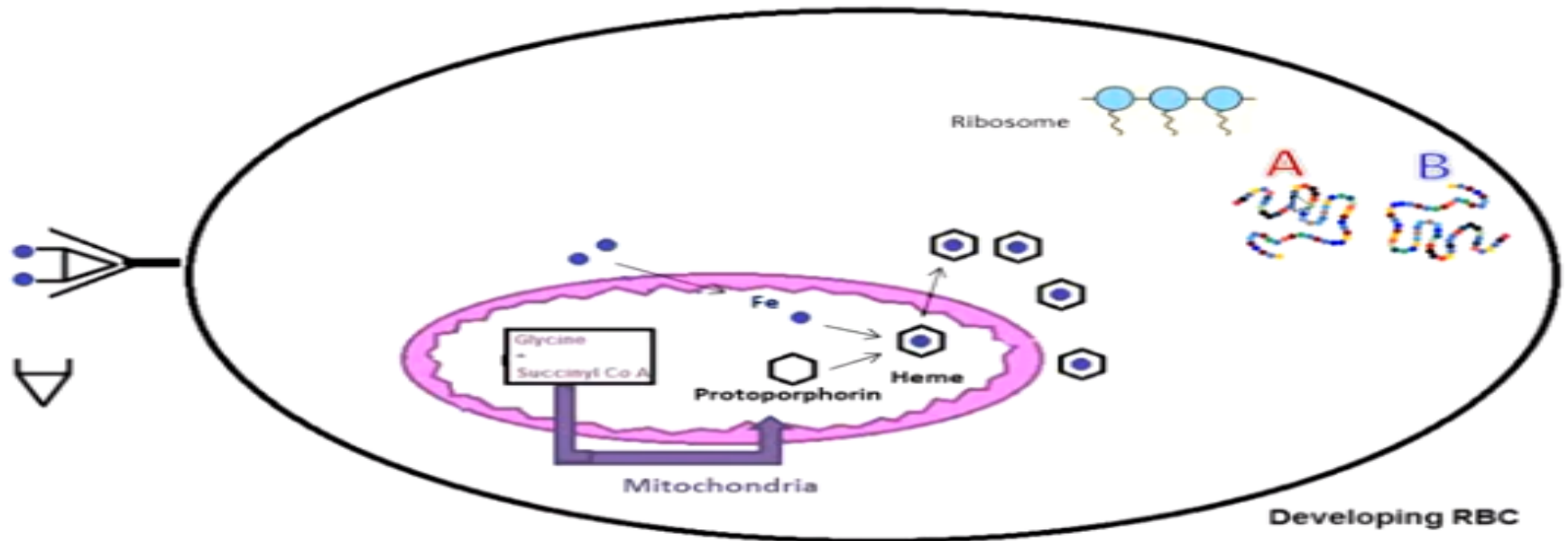
Hemoglobin synthesis



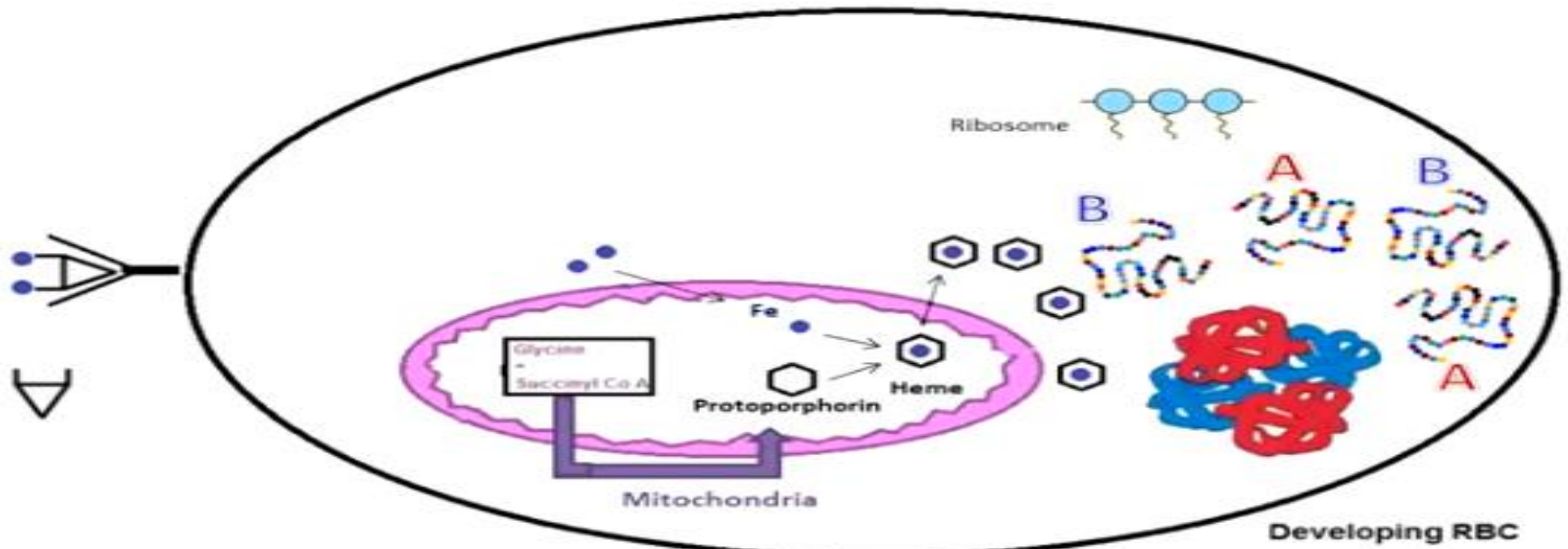
Hemoglobin synthesis



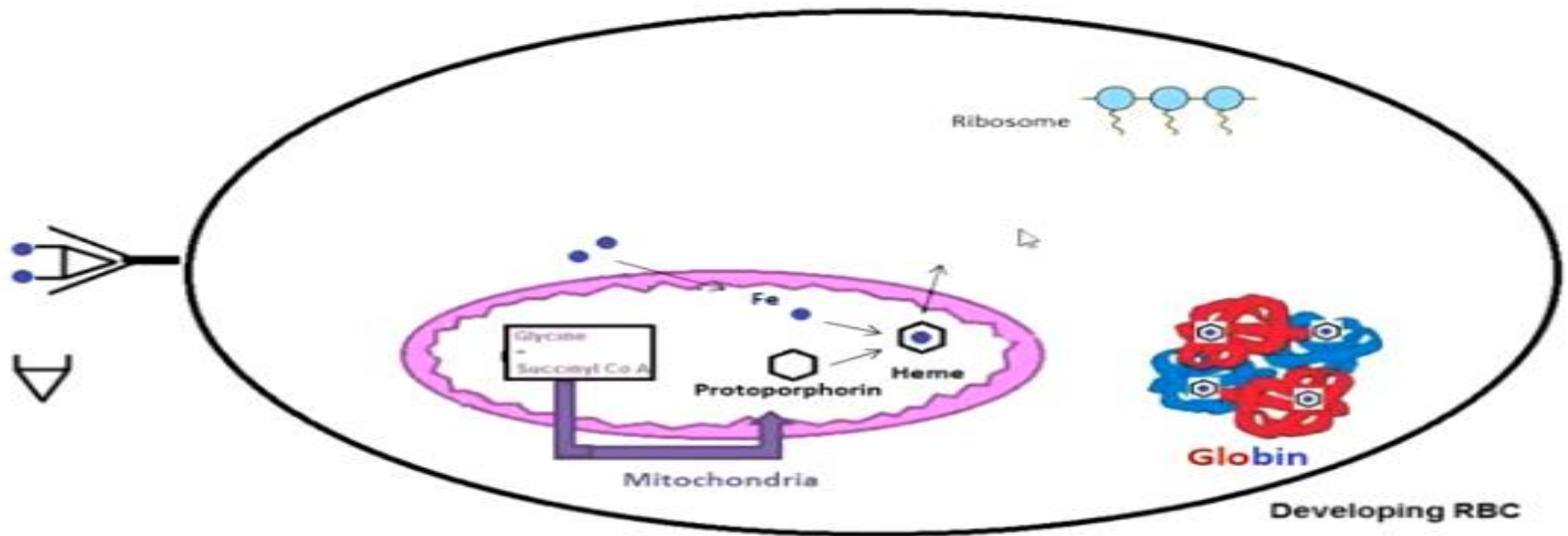
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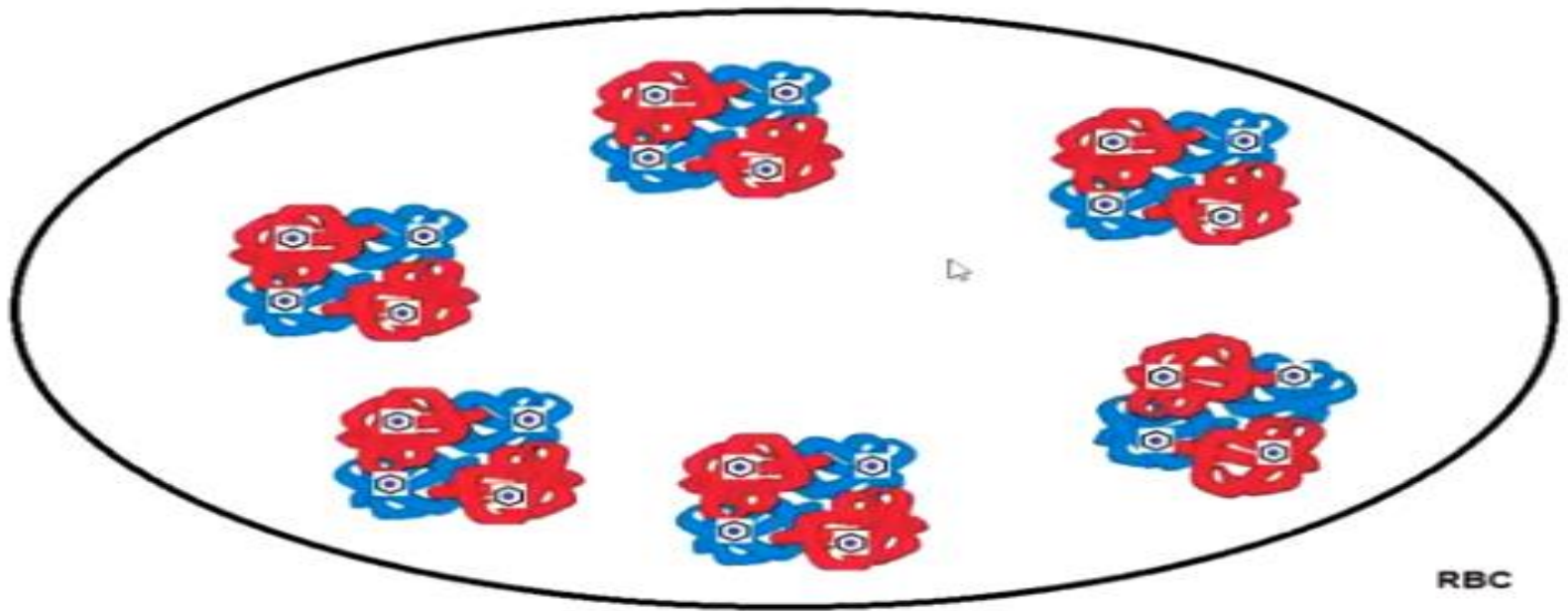
Hemoglobin synthesis



Hemoglobin synthesis



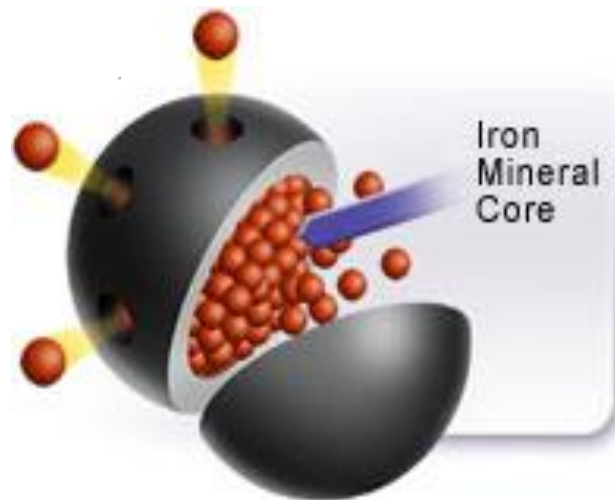
Hemoglobin synthesis



Iron Storage Forms

ferritin :

- consist of 24 polypeptide sub-unit cluster together to form hollow sphere of 5 nm in diameter.
- The stored iron form the central core of the sphere.
- ferritin contains about 25% of iron by weight.
- About 2/3 of body iron stores are present as ferritin.
- If the capacity for **storage of iron in ferritin** is **exceeded**, a **complex of iron with phosphate and hydroxide** forms (**hemosiderin**).
- Iron store in the liver and nearly all other cells.



Ferritin molecules store thousands of iron atoms within their mineral core. When excess dietary iron is absorbed, the body responds by producing more ferritin to facilitate iron storage

Hepcidin

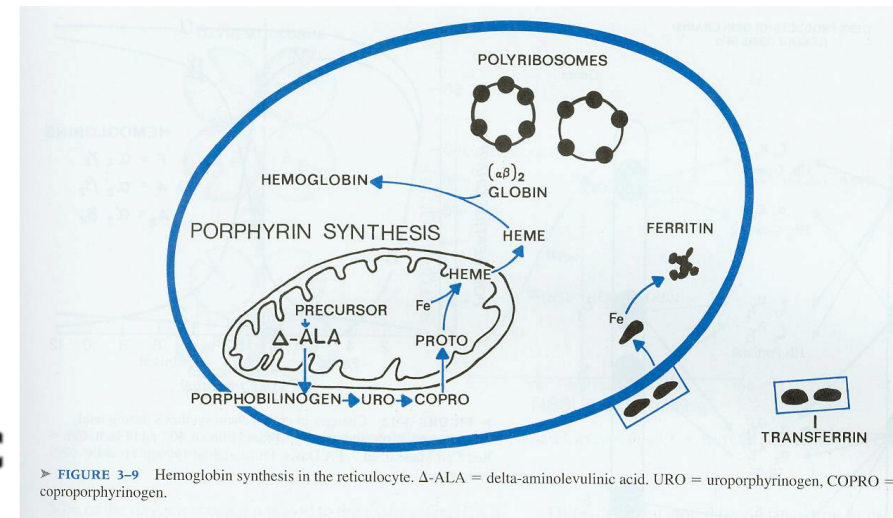
- 25 amino acid peptide , synthesised in the liver.
- Hepatic bacteriocidal protein
- Master iron regulatory hormone
- Hepcidin is an iron-regulatory hormone that maintains plasma iron levels and iron stores within normal range
- Hepcidin regulates the entry of iron into plasma from duodenal enterocytes
- Hepcidin controls gut iron absorption and therefore body iron stores

Transferrin& Receptors

- Collects iron from transferrin for uptake into cells
 - Recognises and binds transferrin
 - Receptor + transferrin Iron released into cell via Iron transporter
 - Receptor + transferrin return to cell surface
 - Transferrin released

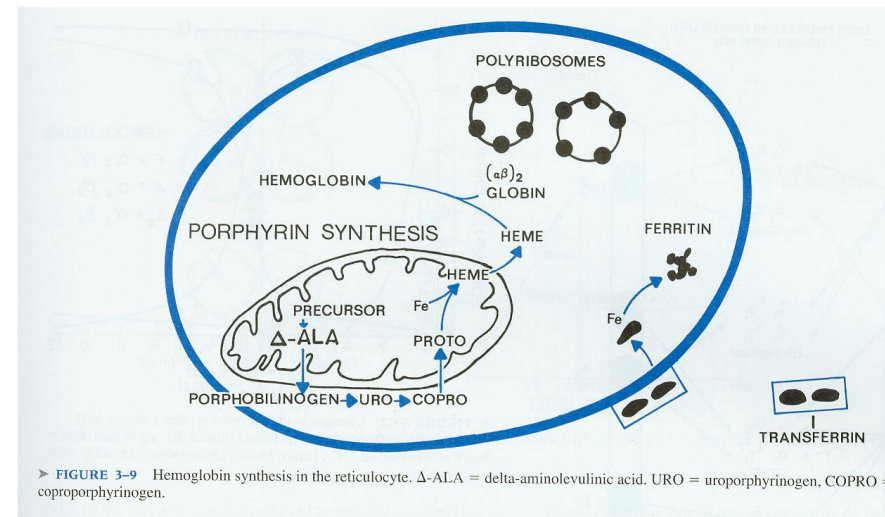
Synthesis of Protoporphyrins

- ▶ Begins in mitochondria with formation of **delta-aminolevulinic acid** from **glycine** and **succinyl coenzyme A (CoA)**. Is the major rate controlling step in heme biosynthesis.
- ▶ Enzyme, **delta aminolevulinic acid synthetase** (delta ALA) mediates this reaction. Amount of enzyme influenced by amount of erythropoietin and Vitamin B6 available.



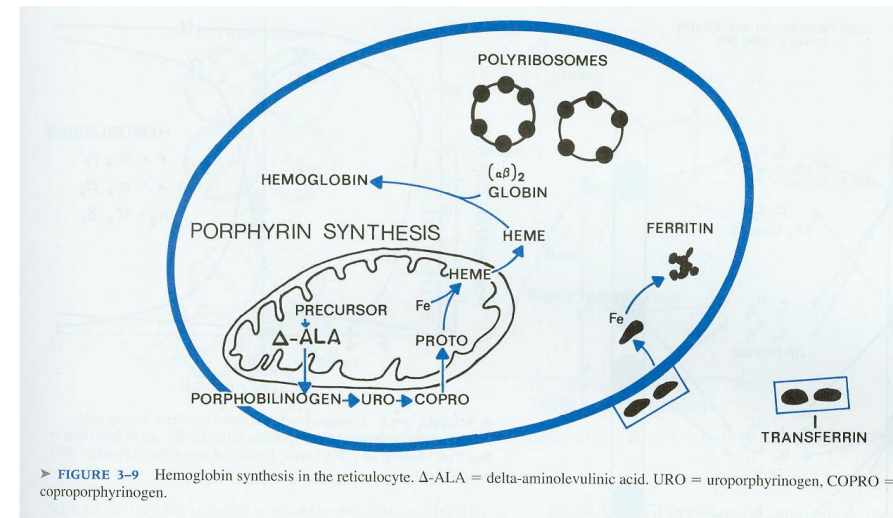
Synthesis of Protoporphyrins

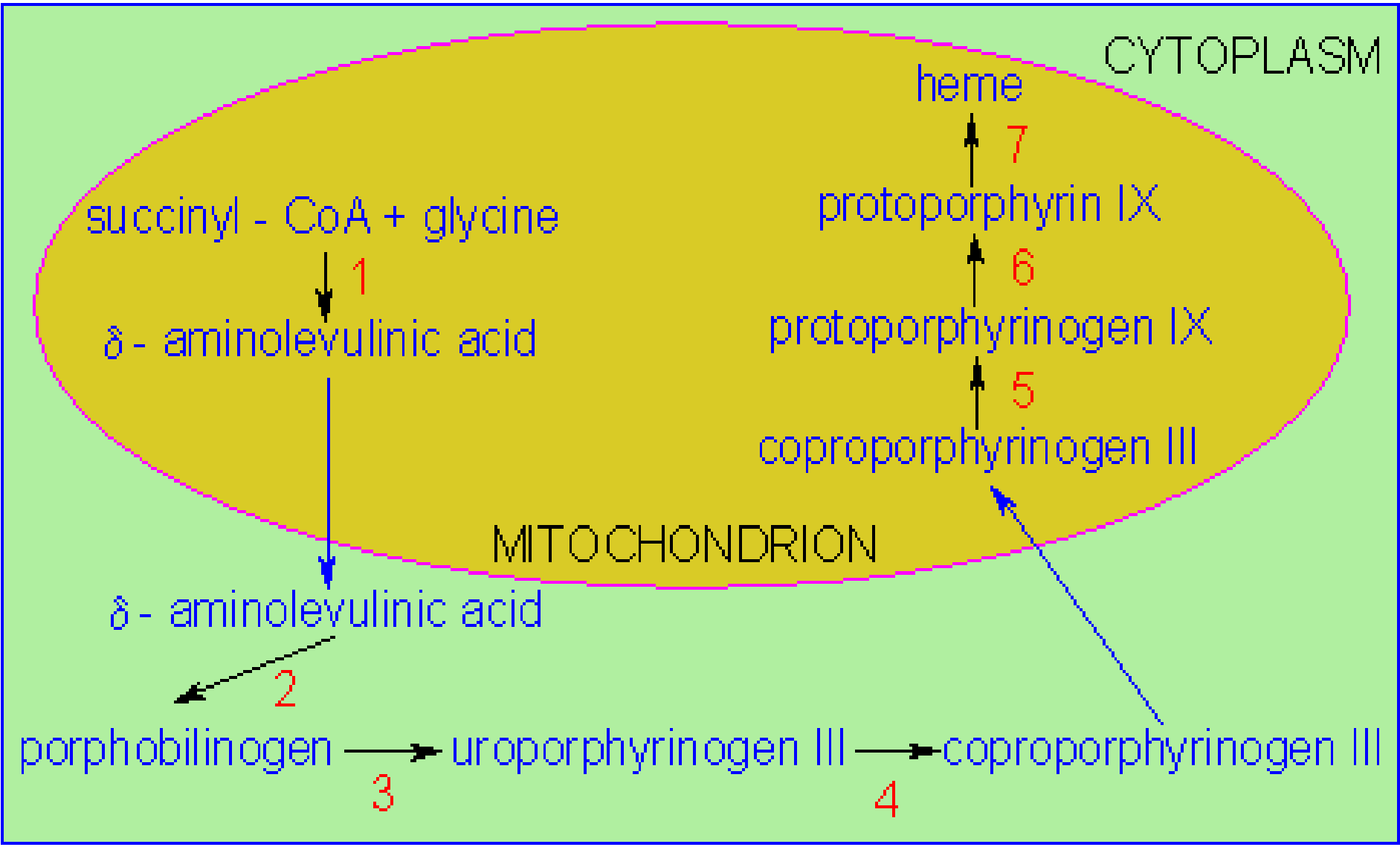
- ▶ **Porphyrinogens** are intermediate products in heme synthesis. If any one of normal enzyme steps in heme synthesis blocked, excessive formation of porphyrins can occur. Results in condition called **porphyria**.
- ▶ **Protoporphyrinogen IX** is last porphyrinogen formed. Becomes **Protoporphyrin IX**.



Synthesis of Protoporphyrins

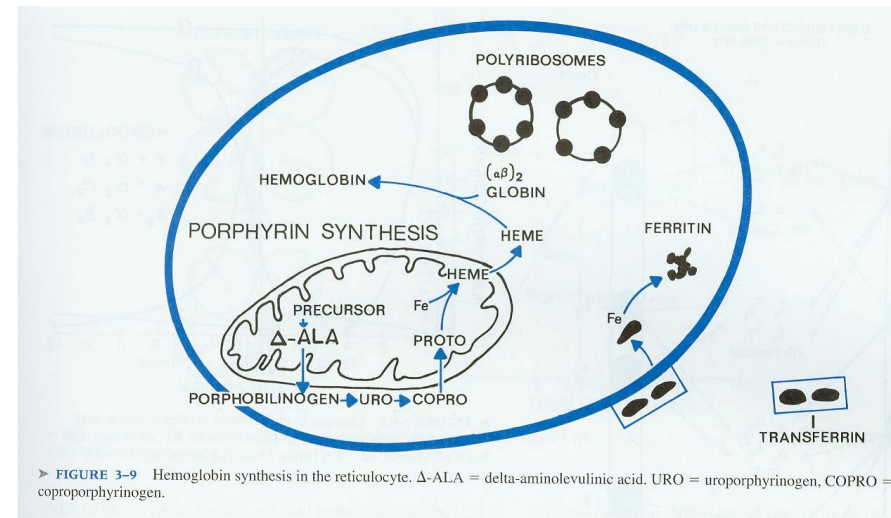
- ▶ Once Protoporphyrin IX formed, iron (Fe^{2+}) is inserted into its ring structure. Once iron has been inserted, a **HEME** molecule has been formed.
- ▶ At end of heme synthesis, have small amount of excess porphyrin in mitochondria. Is complexed to zinc. Excess is called **free erythrocyte protoporphyrin (FEP)**. FEP is elevated when iron supply depleted.





Globin Synthesis

- Occurs on RBC-specific ribosomes which are derived from inheritance of various structural genes. Each RBC contains four alpha, two zeta, two beta, two delta, two epsilon, and four gamma genes. Resulting gene products are alpha, zeta, beta, delta, epsilon, and gamma globin chains.



Globin Synthesis

Hemoglobin Chain	Greek Symbol
Alpha	α
Beta	β
Delta	δ
Epsilon	ϵ
Gamma	γ
Zeta	ζ

Globin Synthesis

- ▶ Throughout embryonic and fetal development, activation of globin genes progresses from zeta to alpha, and from epsilon to gamma to delta to beta.

zeta → alpha

epsilon → gamma → delta → beta

Globin Synthesis

Hemoglobin	Globin Chain Types
Gower I	2 zeta and 2 epsilon
Gower II	2 alpha and 2 epsilon
Portland	2 zeta and 2 gamma
Hemoglobin F (fetal Hgb)	2 alpha and 2 gamma
Hemoglobin A (Adult Hgb)	2 alpha and 2 beta

Globin Synthesis

► Table 3-6

COMPOSITION OF HEMOGLOBIN FOUND IN NORMAL HUMAN DEVELOPMENT

Globin Chains	Hemoglobin	Stage of Development
---------------	------------	----------------------

$\alpha_2\epsilon_2$	Gower 2	Embryo
$\zeta_2\epsilon_2$	Gower 1	
$\zeta_2\gamma_2$	Portland	
$\alpha_2^A\gamma_2$	F	Fetus
$\alpha_2^G\gamma_2$	F	
$\alpha_2\beta_2$	A	Adult
$\alpha_2\delta_2$	A ₂	

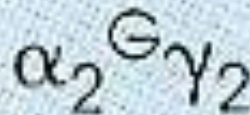
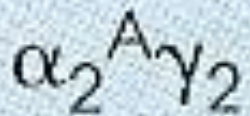
Globin Synthesis

- Epsilon and zeta globins usually appear only during embryonic development. **Gower I Hemoglobin** is two zeta and two epsilon chains. **Gower II Hemoglobin** is composed of two alpha and two epsilon chains. **Hemoglobin Portland** is composed of two zeta and two gamma chains.

Globin Chains	Hemoglobin	Stage of Development
$\alpha_2\epsilon_2$	Gower 2	Embryo
$\zeta_2\epsilon_2$	Gower 1	
$\zeta_2\gamma_2$	Portland	

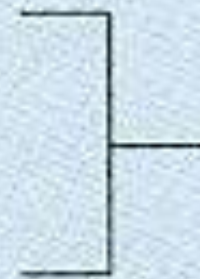
Globin Synthesis

- In fetus, major hemoglobin is **Hemoglobin F** (two alpha and two gamma chains). By age two, Fetal Hemoglobin (Hemoglobin F) comprises less than 2% of total hemoglobin.



F

F



Fetus

Globin Synthesis

- ▶ Beta chain production steadily increases after birth until adult percentages are reached between three months and six months of age.
- ▶ All normal adult hemoglobins (**Hemoglobin A**) consists of two alpha and two non-alpha globin chains.

Globin Synthesis

- ▶ Hemoglobin A has two alpha and two beta chains and comprises 95-97% of adult hemoglobin. Hemoglobin A₂ has two alpha and two delta chains and comprises 2-3% of total adult hemoglobin.

$\alpha_2\beta_2$

A

$\alpha_2\delta_2$

A₂



Adult

Globin Synthesis

- ▶ Each globin chain links with heme molecule (protoporphyrin ring with iron) to form hemoglobin.
- ▶ Entire hemoglobin molecule has two alpha chains, two beta chains, and four heme groups.
- ▶ Precise order of amino acids in globin chains critical to hemoglobin molecule's structure and function.

Globin Synthesis

- ▶ Rate of globin synthesis directly related to rate of porphyrin synthesis and vice versa. Is **NO** such relationship between iron uptake when either globin or protoporphyrin synthesis impaired.
- ▶ Iron accumulates in RBC cytoplasm as ferritin aggregates. Iron-laden RBC called **sideroblast** and anucleated form called **siderocyte**. (Will see iron clusters when cells stained with Prussian Blue stain).
- ▶ Completed hemoglobin molecule is three-dimensional structure. Has a globular shape. Is species specific. Called a dimer structure.

2,3-Diphosphoglycerate (2,3-DPG)

- ▶ Is an organic phosphate responsible for hemoglobin's affinity for oxygen.
- ▶ Is a product derived from Luebering-Rapaport shunt.
- ▶ Is located in the central cavity of hemoglobin molecule. Is bound to beta chains.

lumbering Rapaport shunt :metabolic pathway in mature erythrocytes involving the formation of 2,3-bisphosphoglycerate (2,3-BPG), which regulates oxygen release from hemoglobin and delivery to tissues

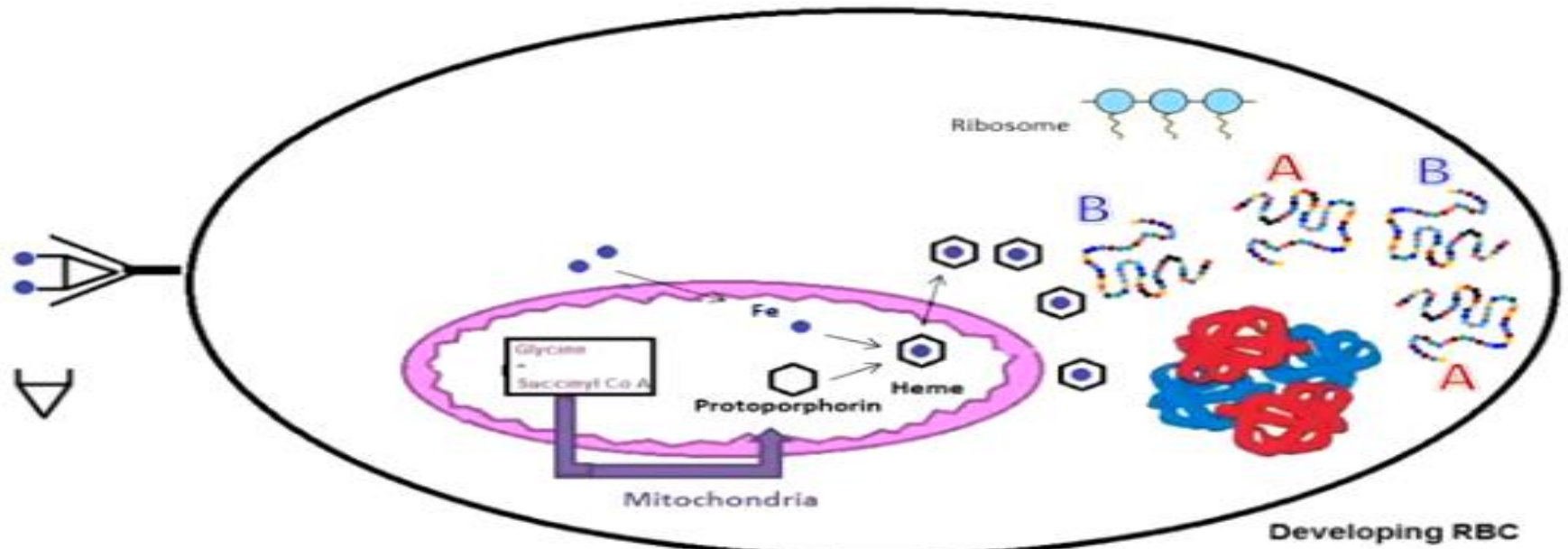
Assembly of Hemoglobin Molecule

- ▶ Formation of hemoglobin requires iron, globin chains, protoporphyrin IX, and 2,3-DPG.
- ▶ To assemble molecule, ferric iron (Fe^{3+}) must be obtained from ferritin. Iron is chemically reduced (Fe^{2+}), and then inserted as ferrous iron into center of protoporphyrin IX molecule.
 - Ferrous (2+) “reduced” - gained an electron
 - Ferric (3+) “oxidized” - lost an electron

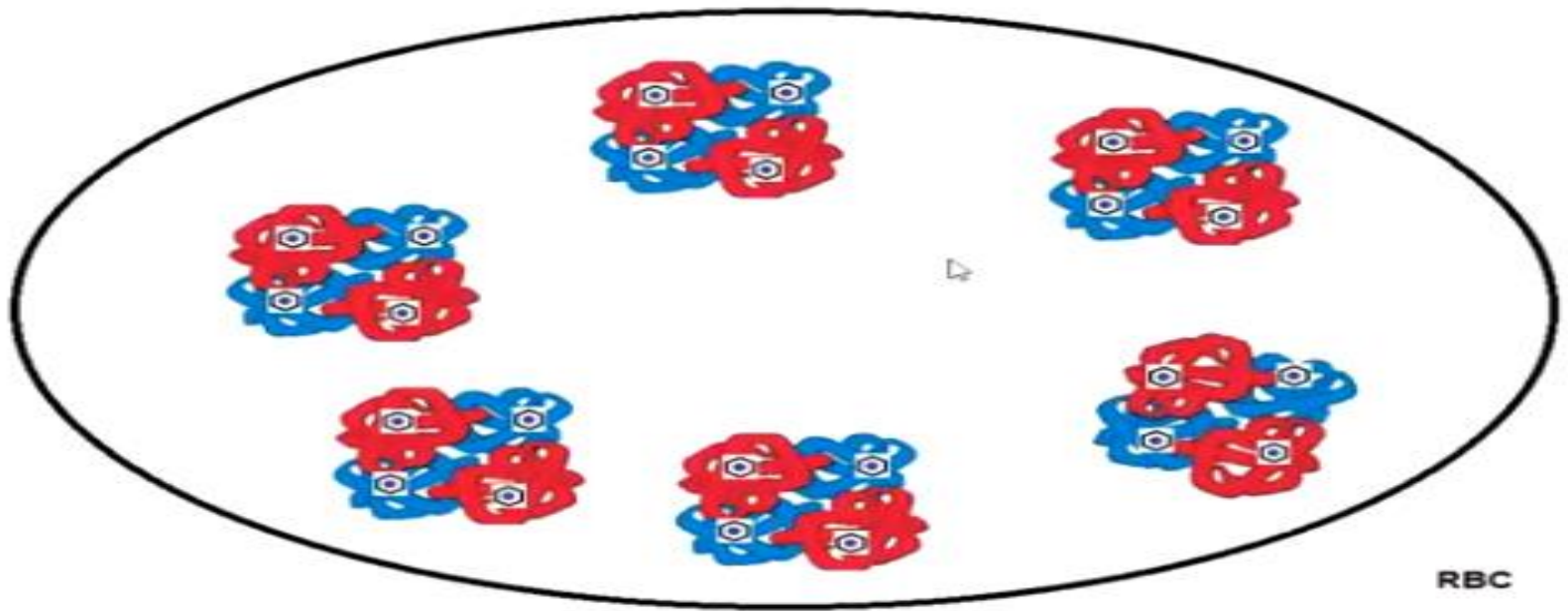
Assembly of Hemoglobin Molecule

- ▶ When globin chain completed on ribosome, it is released to cytoplasm. Individual alpha and beta chains quickly and spontaneously form alpha-beta dimers. Two heme molecules bind to each alpha-beta dimer. Two dimers quickly form a tetramer and assume final three dimensional shape.
- ▶ Last step is insertion of 2,3-DPG molecule into center cavity of hemoglobin molecule.

Hemoglobin synthesis



Hemoglobin synthesis



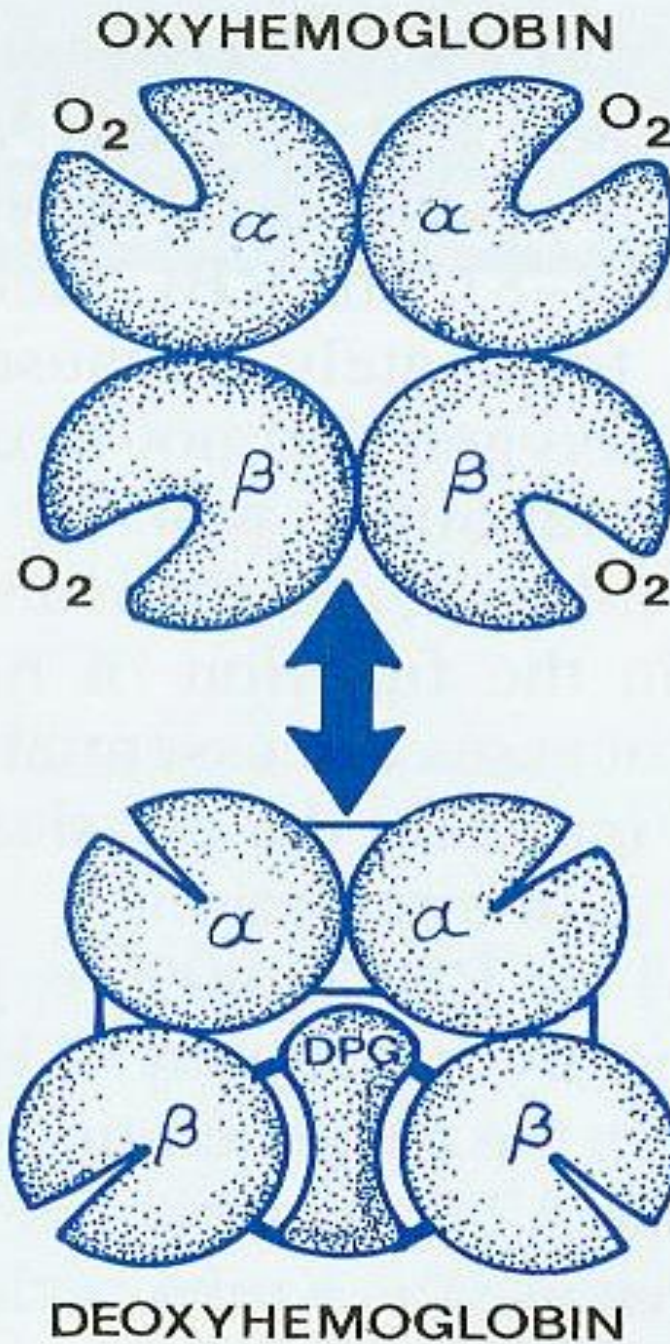
Hemoglobin Function

Hemoglobin Function

- ▶ **Primary function is delivery and release of oxygen to tissues and the facilitation of carbon dioxide excretion.**
- ▶ **One of most important controls of hemoglobin affinity for oxygen is RBC organic phosphate: 2,3-diphosphoglycerate (2,3-DPG).**

Hemoglobin Function

- Unloading of oxygen by hemoglobin accompanied by widening of space between beta chains and binding of 2,3-DPG to beta chains. Resulting hemoglobin molecule known as "**tense form**", which has a lower oxygen affinity. Also known as **deoxyhemoglobin**.

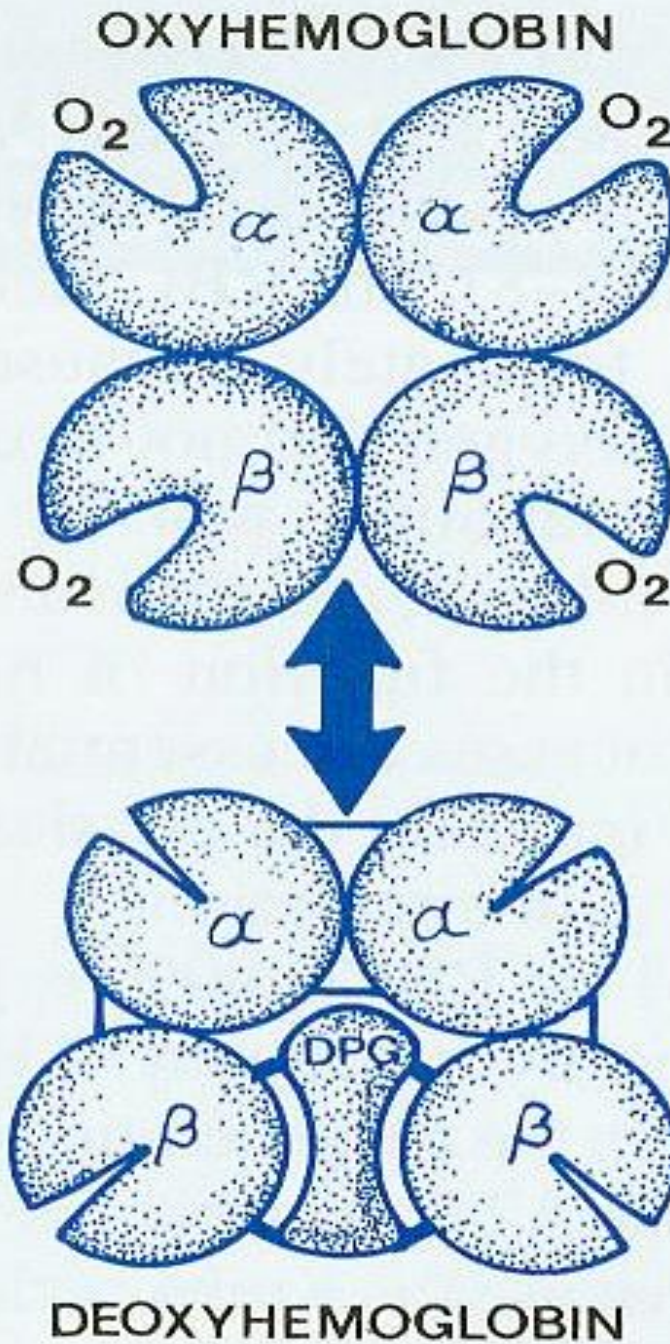


Tense Form

Hemoglobin Function

- ▶ When hemoglobin binds oxygen, 2,3-DPG and beta chain bonds break. Beta chains close up and 2,3-DPG expelled. Is **"relaxed form"** of hemoglobin. Has a high affinity for oxygen. Is also called **oxyhemoglobin**.
- ▶ Conversion between tense form and relaxed form referred to as respiratory movement.

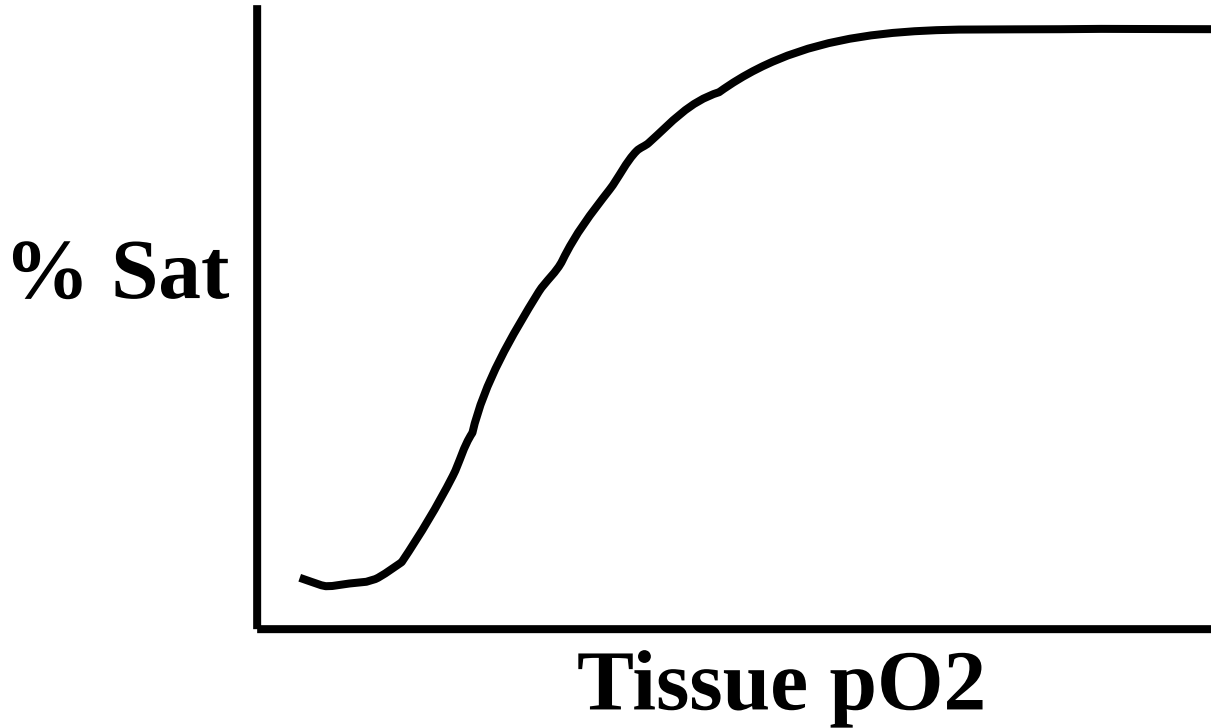
Relaxed Form



Hemoglobin Function

- Relationship between hemoglobin and oxygen has a sigmoid curve shape - is called the **oxygen dissociation curve**. Shape of curve means lots of oxygen can be delivered to tissues with small drop in oxygen tension.

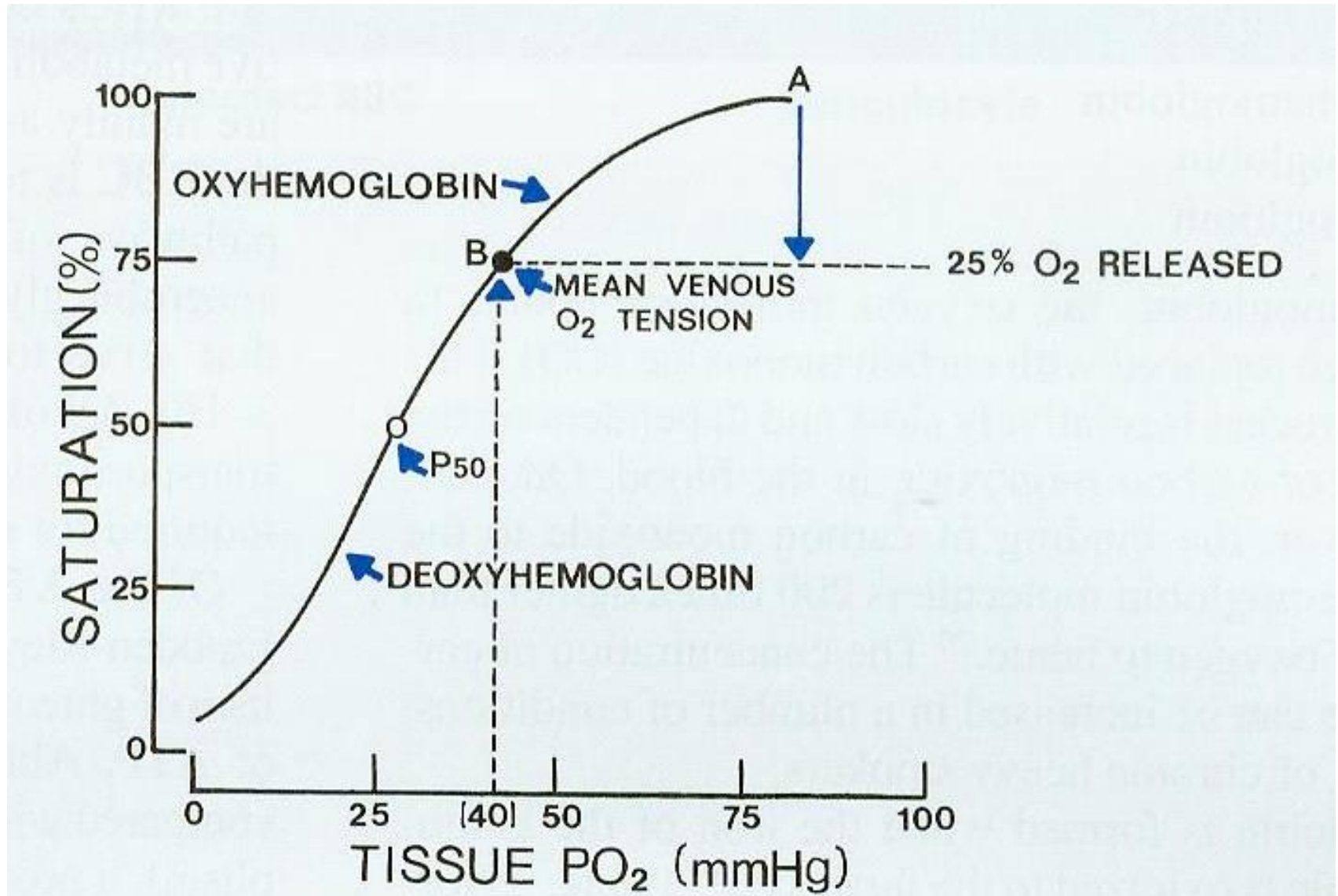
Oxygen Dissociation Curve



Hemoglobin Function

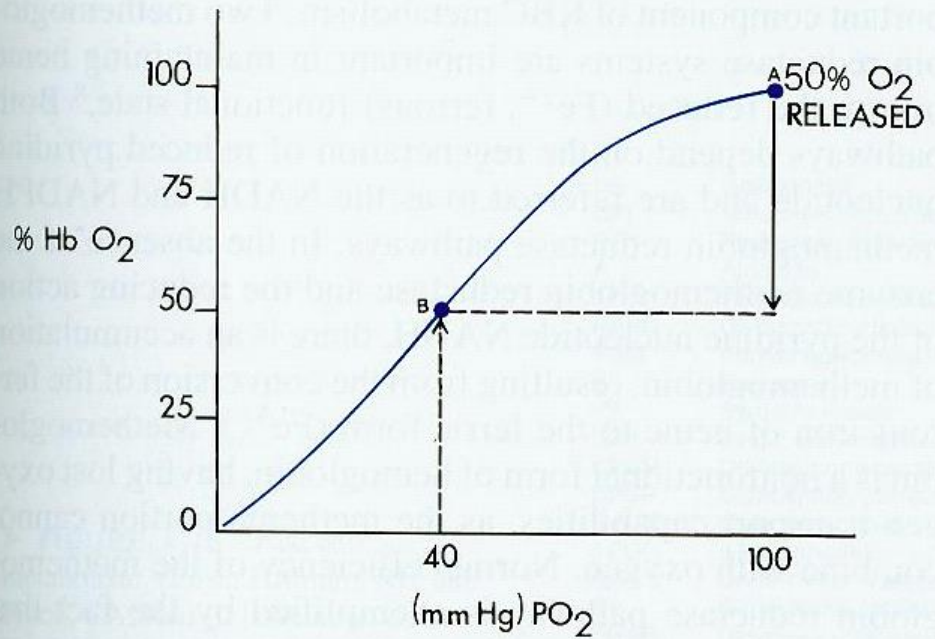
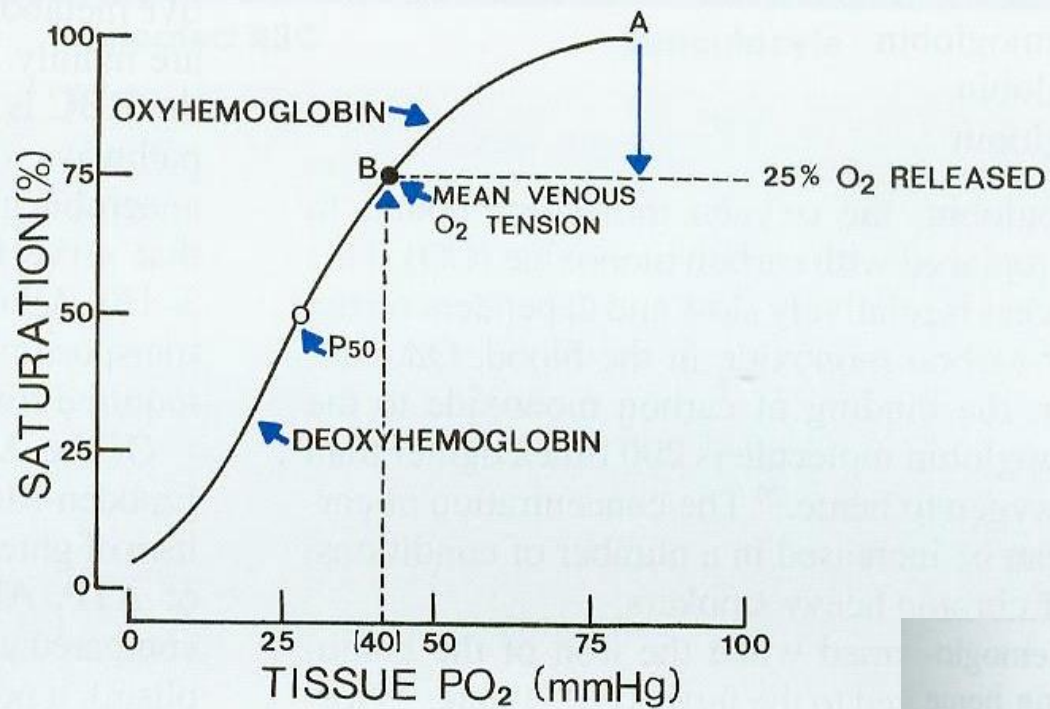
- ▶ In lungs, where pO_2 (oxygen tension) very high, hemoglobin almost 100% saturated with oxygen. As RBCs travel to tissues where oxygen tension drops, hemoglobin saturation drops to about 75%, releasing oxygen to tissues.
- ▶ Is normally occurring process.

Oxygen Dissociation Curve



Hemoglobin Function

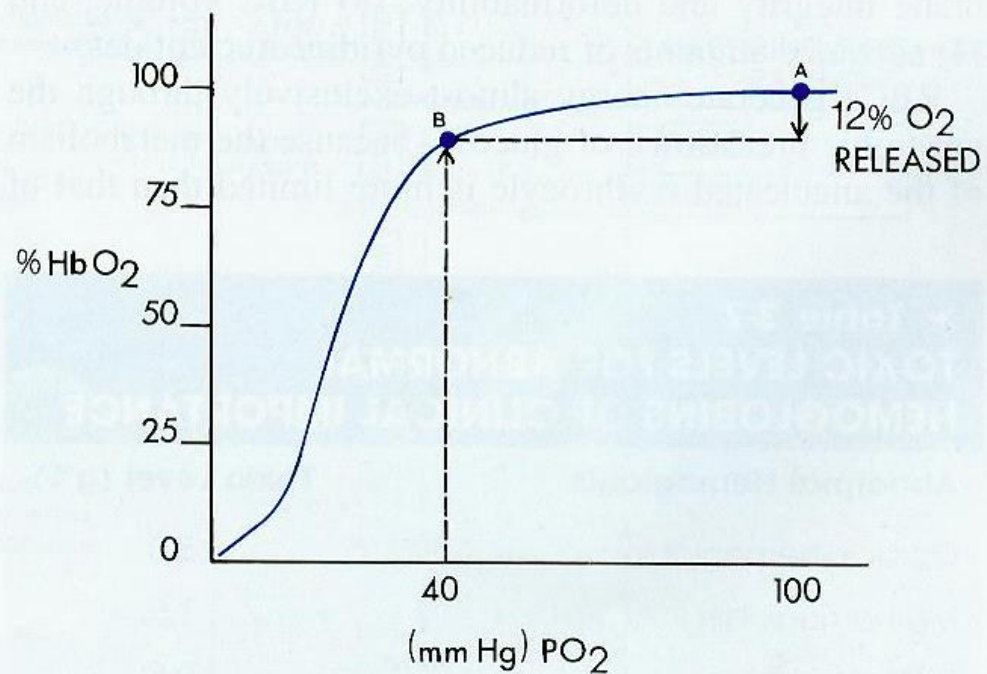
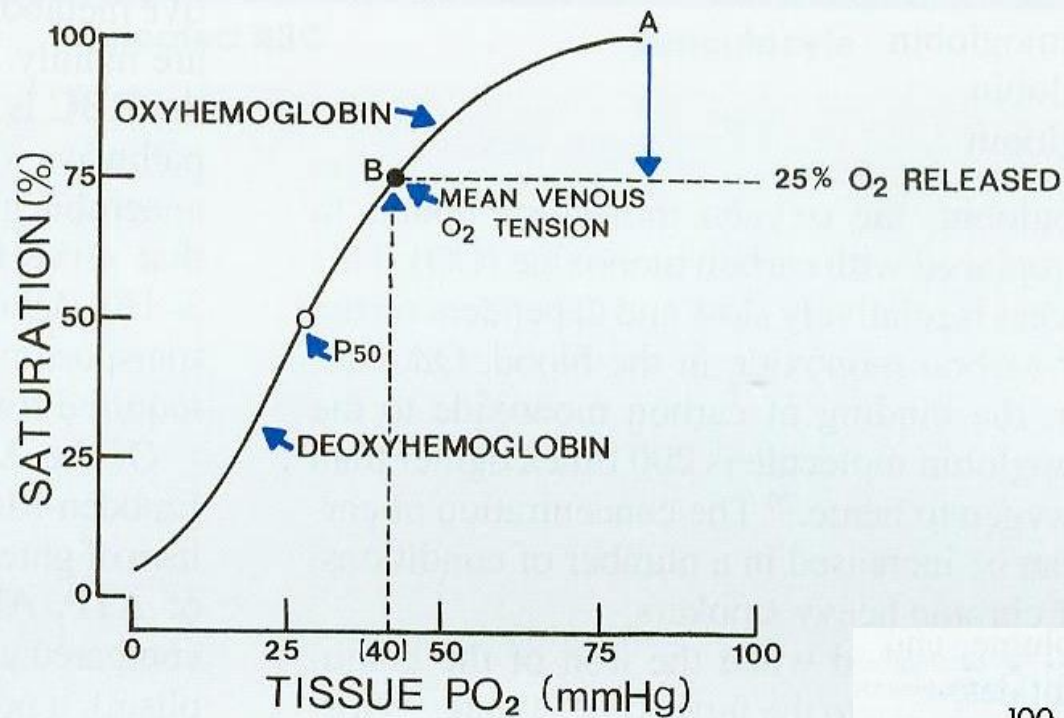
- ▶ In hypoxia, a compensatory **"shift to right"** of hemoglobin dissociation curve occurs to relieve tissue oxygen deficit. Right shift of curve, mediated by increase in 2,3-DPG, results in decrease in hemoglobin's affinity for oxygen and an increase in oxygen delivery to tissues.
- ▶ Shifts to right commonly occur in hypoxia, anemia, acidosis, and rise in body temperature.



► **FIGURE 3-14** Right-shifted hemoglobin-oxygen dissociation curve.

Hemoglobin Function

- ▶ In "shift to left" of hemoglobin dissociation curve, see an increase in hemoglobin-oxygen affinity and decrease in amount of oxygen being delivered to tissues.
- ▶ Conditions causing a "**shift to left**" include alkalosis, increase in the amount of abnormal hemoglobins (methemoglobin or carboxyhemoglobin), increased amount of Hemoglobin F, or multiple transfusions of 2,3-DPG depleted stored blood.



➤ **FIGURE 3-15** Left-shifted hemoglobin-oxygen dissociation curve.

Hemoglobin Function

	Shift to Right	Shift to Left
2,3-DPG	Increased	Decreased
O2 Affinity	Decreased	Increased
O2 Delivery to Tissues	Increased	Decreased
Body Temperature	Increased	Decreased
pH	Decreased (Acidosis)	Increased (Alkalosis)

Abnormal Hemoglobins

Abnormal Hemoglobins

- ▶ Are three clinically significant abnormal hemoglobins that are unable to transport or deliver oxygen:
 - **1.** Carboxyhemoglobin
 - **2.** Methemoglobin, and
 - **3.** Sulfhemoglobin

Carboxyhemoglobin

- ▶ Oxygen bound to hemoglobin replaced by carbon monoxide (CO)
- ▶ Replacement process relatively slow and dependent upon blood concentration of carbon monoxide
- ▶ Heme binds to carbon monoxide about 200 times tighter than it binds to oxygen
- ▶ Is a reversible condition (oxygen inhalation)

Methemoglobin

- ▶ Formed when iron of hemoglobin molecule oxidized to ferric state (Fe^{3+})
- ▶ Can occur as result of overload to oxidant stress (ingesting strong oxidant drugs) or to enzyme deficiency in RBC metabolic pathways
- ▶ Is a reversible condition (with administration of strong reducing substances)

Sulfhemoglobin

- ▶ Occurs when sulfur content in blood builds up (ingestion of sulfur-containing drug or chronic constipation)
- ▶ Is an irreversible condition (RBCs must be removed from circulation)

► **Table 3-7**

**TOXIC LEVELS FOR ABNORMAL
HEMOGLOBINS OF CLINICAL IMPORTANCE**

Abnormal Hemoglobin	Toxic Level (g%)
Carboxyhemoglobin	5.0
Methemoglobin	1.5
Sulfhemoglobin	0.5

RBC Metabolic Pathways

Introduction to RBC Metabolic Pathways

- ▶ **Necessary for generation of ATP (energy)**
- ▶ **Necessary for RBC to maintain:**
 - hemoglobin function
 - membrane integrity and deformability
 - RBC volume

Introduction to RBC Metabolic Pathways

- ▶ Generate energy through anaerobic breakdown of glucose
- ▶ Four pathways involved in RBC metabolism:
 - Phosphogluconate pathway
 - Embden-Meyerhof pathway
 - Methemoglobin reductase pathway
 - Luebering-Rapaport pathway

Phosphogluconate Pathway (or Hexose Monophosphate Pathway)

- ▶ Produces pyridine nucleotides - one of the main lines of defense for RBC against oxidative injury which may be caused by infections or oxidant drugs
- ▶ Deficiency in this pathway results in deficiency of glutathione which results in globin denaturation and precipitation as aggregates inside the RBC (Heinz bodies)

Embden-Meyerhof Pathway

- ▶ **90% of the ATP needed by the RBC is generated through this pathway**
- ▶ **Also generates NADH which is used in other metabolic pathways**

Embden–Meyerhof pathway, there are two main stages. The first one is the **conversion of the sugar to** a common intermediate, which is **glucose-6-phosphate** followed by the second stage including the **conversion of the intermediate into pyruvate.**

Methemoglobin Reductase Pathway

- ▶ Important in maintaining heme iron in the reduced or ferrous functional state
- ▶ Dependent on the hexose monophosphate pathway for production of pyridine nucleotides
- ▶ In absence of enzyme methemoglobin reductase, have an accumulation of methemoglobin (iron in the ferric or oxidized state)
- ▶ Methemoglobin is non-functional, having lost ability to transport oxygen

Leubering-Rapaport Shunt

- ▶ Causes an accumulation of RBC organic phosphate 2,3-DPG which is very important for hemoglobin's affinity for oxygen

► **Table 3-8**

**COMPARISON OF RED BLOOD CELL METABOLIC ACTIVITIES
DURING VARIOUS STAGES OF MATURATION**

	Nucleated RBC	Reticulocyte	Adult RBC
Replication	+	0	0
DNA synthesis	+	0	0
RNA synthesis	+	0	0
Lipid synthesis	+	+	0
RNA present	+	+	0
Heme synthesis	+	+	0
Protein synthesis	+	+	0
Mitochondria	+	+	0
Krebs' tricarboxylic acid cycle	+	+	0
Embden-Meyerhof pathway	+	+	+
Pentose phosphate pathway	+	+	+
Maturation and/or senescence	+	+	+

Erythrocyte Senescence

Introduction to Erythrocyte Senescence

- ▶ Average life span of RBC is 120 days.
- ▶ Are continually aging (senescence).
- ▶ Old RBCs removed by macrophages located in the **reticuloendothelial system** (RES) (**spleen** most important organ).
- ▶ As old RBCs removed, are replaced by younger RBCs from the bone marrow.
- ▶ Two major pathways for RBC removal:
 - Extravascular Hemolysis
 - Intravascular Hemolysis

Extravascular Hemolysis

- ▶ 90% of destruction of RBCs occurs here.
- ▶ Old or damaged RBCs phagocytized by RES cells and digested by lysosomes.
- ▶ Hemoglobin molecules disassembled and broken down into component parts.
- ▶ Iron returned by transferrin to bone marrow.
- ▶ Globin broken down into amino acids and returned to amino acid pool.

Extravascular Hemolysis

- ▶ Protoporphyrin ring disassembled into carbon monoxide, which is expelled. The remaining component, **biliverdin**, is converted to **bilirubin** and carried by albumin to liver. In liver, bilirubin conjugated to **bilirubin glucoronide** and excreted with bile into intestines. Bilirubin glucoronide converted by bacteria into **urobilinogen** and excreted in the stool. Small amount recycled back to liver and then excreted through kidneys in urine.

Extravascular Hemolysis

- ▶ Both unconjugated (prehepatic) and conjugated bilirubin (posthepatic) can be measured in plasma and used as an indicator of the amount of extravascular hemolysis occurring.

Intravascular Hemolysis

- ▶ Only 5-10% hemolysis occurs in this pathway.
- ▶ RBCs break down within lumen of blood vessel, releasing hemoglobin directly into bloodstream.
- ▶ Hemoglobin disassociates into globin dimers and picked up by protein carrier - haptoglobin.
- ▶ Hemoglobin-haptoglobin complex too big to be excreted through kidneys. Complex carried to liver where it is further catabolized.
- ▶ At this point, pathway in liver identical to extravascular pathway.

Plasma Proteins

By

Dr. Faten Allyan

Plasma Proteins

- About 500 proteins have been identified in plasma
- The plasma proteins include the immunoglobulins, enzymes, and enzyme inhibitors.
- Most plasma proteins, with the notable exception of immunoglobulins, are synthesized in the liver.
- Plasma proteins circulate in the blood and between the blood and the extracellular tissue spaces.

Functions of proteins

- Generally, proteins **do everything in the living cells**
- Functional classification of plasma proteins is useful in understanding the changes that occur in disease:
 - **Tissue nutrition**
 - **Proteins of immune defense**
 - Antibodies
 - **Acute phase proteins**
 - Proteins associated with inflammation
 - **Transport proteins (albumin, transferrin)**
 - Proteins used to bind and transport
 - **Hemostasis**
 - Proteins involved in forming clots and acting very closely with complement

Functions of proteins

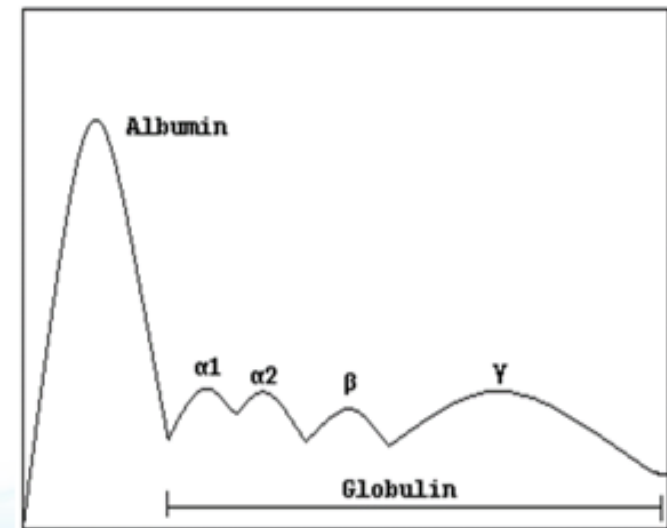
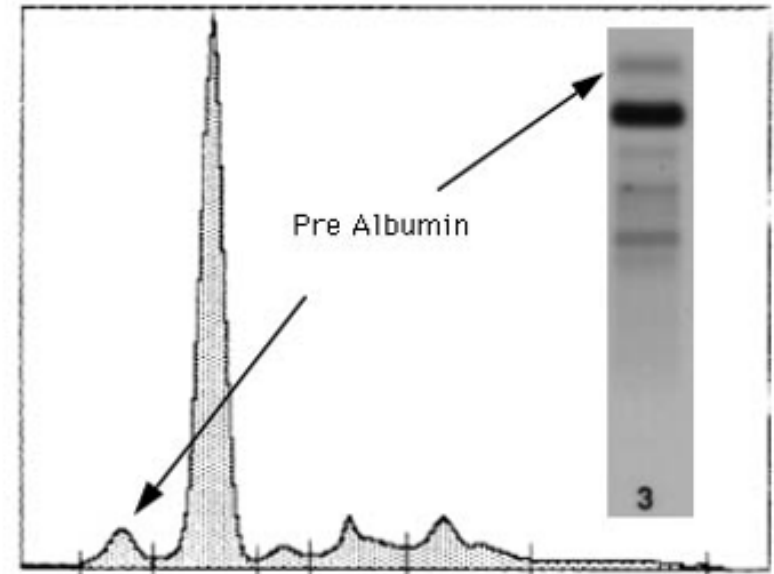
- Regulatory
 - (receptors, hormones)
- Catalysis,
 - enzymes
- Osmotic force
 - Maintenance of water distribution between cells and tissue and the vascular system of the body
- Acid-base balance
 - Participation as buffers to maintain pH
- Structural, contractile, fibrous and keratinous

Plasma Proteins

❑ Prealbumin	• α 2 Macroglobulin
❑ Albumin	• Transferrin (<u>Siderophilin</u>) β
❑ Globulins α 1, α 2, β , and γ	• <u>Hemopexin</u>
• α 1-Antitrypsin	• Acute phase proteins (CRP)
• α 1-Fetoprotein	• Immunoglobulins
• α 1-Acid Glycoprotein	■ <u>Myoglobin</u>
• <u>Haptoglobin</u> (α 2)	■ <u>Troponin</u>
• <u>Ceruloplasmin</u>	

Prealbumin (Transthyretin)

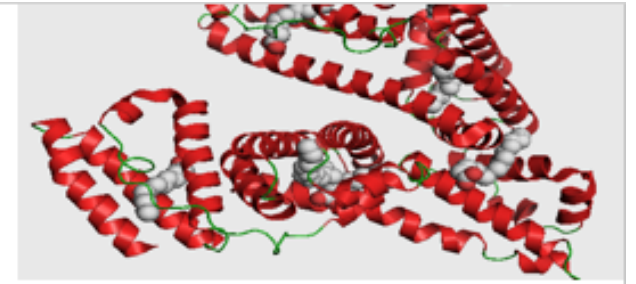
- Migrates just ahead of Albumin
- When a diet is deficient in protein, hepatic synthesis of proteins is reduced
 - Indicator of nutritional status (very short half life- 2 days)
- Transport of thyroid hormones & it also binds with retinol-binding protein to form a complex that transports retinol (vitamin A)



Prealbumin (Transthyretin)

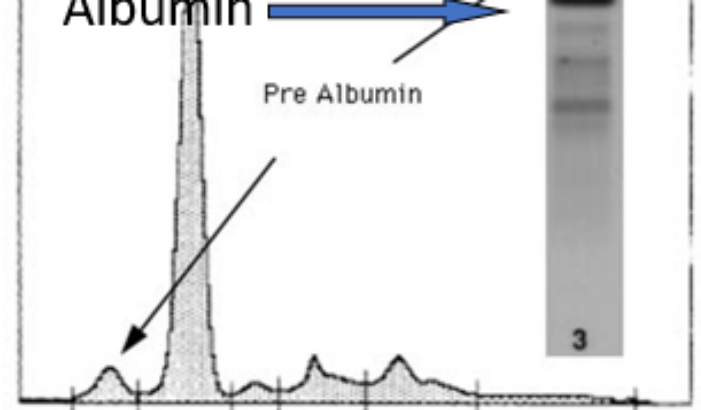
- **Low levels found in:**
 - Hepatic damage
 - Acute phase responses (-ve acute phase reactant)
 - Nutritional deficit – short half-life means decrease seen early in disease
- **Increased level found in:**
 - Steroid treatment
 - Alcoholism
 - Chronic renal failure (glomerular filtration rate decreased)

Albumin



- Synthesized in the liver from 585 amino acids at the rate of 9–12 grams per day with no reserve or storage
- Highest concentration plasma protein
- Albumin also exists in the extravascular (interstitial) space
- Two primary functions
 - Colloidal osmotic pressure (80%) serves to hold water within the vascular space
 - Bind and transport of numerous substances
 - Bilirubin, steroids, fatty acids, Ca^{++} , Mg^{++} ,
 - salicylic acid & other medications

Albumin

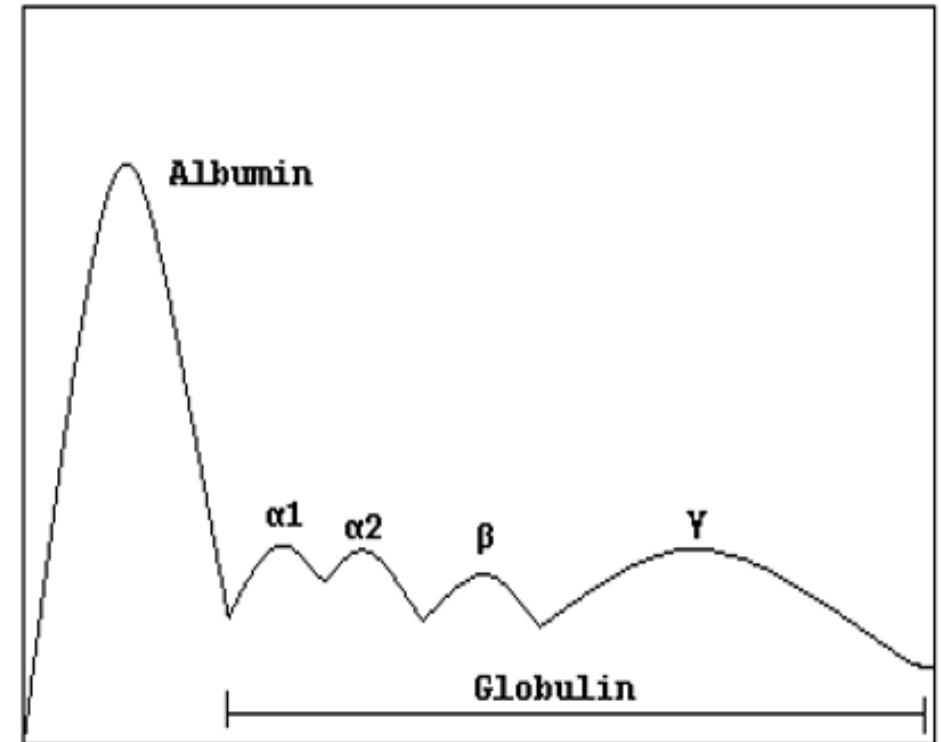


- **Decreased Albumin**

- Malnutrition & muscle wasting diseases
- Liver diseases – inability to synthesize
- GI loss due to inflammation or mucosal lining diseases
- Loss in urine due to renal disease
- Genetic analbuminemia (Mutation causes absence of albumin)
- Bisalbuminemia (genetically inherited metabolic defect characterised by an impaired synthesis of serum albumin)
 - results from two copies of different albumin genes, resulting in different charges.
- **Increased levels of Albumin**
 - Seen in dehydration

Globulins

- The globulin group of proteins consists of α_1 , α_2 , β , and γ fractions.
- Each fraction consists of a number of different proteins with different functions.



Globulins (α 1-Antitrypsin)

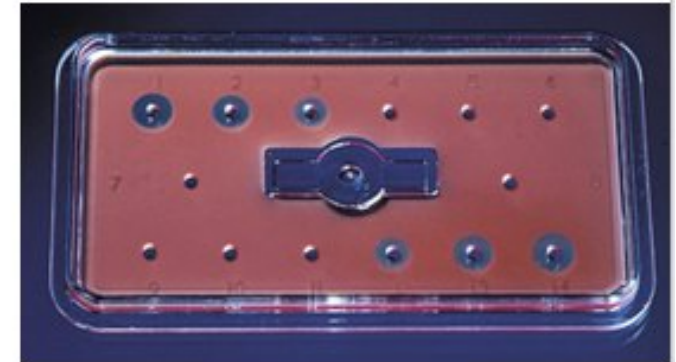
- Major component (90%) of α 1 fraction
- Acute phase reactant, synthesized in the liver
- Most important function the inhibition of the protease neutrophil elastase
 - Neutrophil elastase is released from leukocytes to fight infection, but it can destroy alveoli
- Mutations in the *SERPINA1* gene cause:
 - α 1-antitrypsin deficiency which is associated with
 - Severe degenerative emphysema
 - Abnormal form of α_1 -antitrypsin can also accumulate in the liver and can cause cirrhosis

α 1-Antitrypsin



- **Increased levels in:**

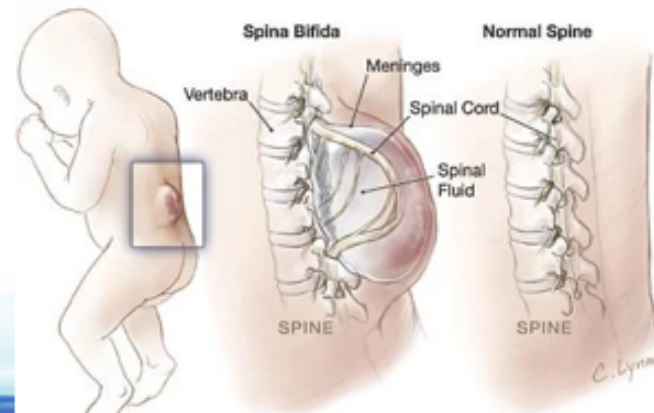
- Inflammatory reactions
- Pregnancy
- Contraceptive use



- Since major component of α 1 band – changes in levels apparent on protein electrophoresis
- Quantitative methods used to confirm electrophoresis findings are radial immunodiffusion

α 1-Fetoprotein (AFP)

- AFP synthesized by fetal yolk sac & later by parenchymal cells of liver
- Peaks in fetus at **13 weeks** – decreases gradually by birth
- Acts like a fetal “albumin”
- Maternal serum testing used to screen fetus for birth defects
 - $\updownarrow$ Neural tube defects & twins
 - $\downarrow$ Increase risk of Down's syndrome & trisomy 18



α 1-Fetoprotein (AFP)

- **In adults level is high in:**
 - 80% of hepatocellular carcinoma
 - Gonadal tumors in adults (testis or ovary)
- The methods commonly used for AFP determinations are enzyme-labeled immunoassay (EIA)

α 1-Acid Glycoprotein (AAG)

- Acute phase reactant, synthesized in the liver
- Regulates immune responses
 - **Increased in:**
 - inflammation,
 - cancer,
 - pneumonia,
 - Rheumatoid arthritis (RA).

Haptoglobin ($\alpha 2$)

- Synthesized in the liver
- Considered an acute-phase protein
- Binds free hemoglobin
- Prevents loss of Hemoglobin & Iron into urine
- Used to detect and evaluate hemolytic anemia and to distinguish it from anemia due to other causes

	<u>Haptoglobin</u>	Reticulocytes	Diagnosis
1	Decreased	Increased	Hemolytic anemia
2	Normal	Increased	RBC destruction may be occurring in organs such as the spleen and liver
3	Normal	Normal	Anemia present is not due to RBC breakdown

Haptoglobin (α_2)

- **Increased in**
 - Inflammations, burns & nephrotic syndrome due to fluid losses and lower-molecular-weight proteins have been lost
- **Decreased in**
 - with transfusion reactions, HDN
 - Thus, a low plasma haptoglobin concentration can be indicative of intravascular haemolysis.
 - decreased synthesis are seen in chronic liver disease
- Radial immunodiffusion has been used for the quantitative determination of haptoglobin

Ceruloplasmin (α_2)

- Synthesized in the liver
- Acute-phase reactant
- Copper carrying protein
- 90% of serum copper is bound to it
- In Wilson disease, the processes of incorporation of copper into ceruloplasmin and excretion of excess copper into bile are impaired
 - decreased levels of ceruloplasmin and excess storage of copper in the liver, brain, and other organs resulting in hepatic cirrhosis and neurologic damage.

Ceruloplasmin

- **Increased levels**
 - Pregnancy, inflammatory processes,
 - malignancies, oral estrogens & contraceptives
- **Low levels**
 - Malabsorption
 - Severe Liver Disease
 - Nephrotic syndrome
 - Menkes' Syndrome (decreased Cu absorption)
- sparse, kinky hair; failure to gain weight and grow at the expected rate (failure to thrive); and deterioration of the nervous system



α_2 Macroglobulin

- Tetramer of four identical subunits
- Synthesized by liver
- major component of the α_2 band in protein electrophoresis
- Primarily intravascular spaces
- Inhibits proteases trypsin, pepsin & plasmin

α 2 Macroglobulin

- **Increased levels**
 - Nephrosis (Large size prevents loss)
 - Oral contraceptives (high estrogens)
- **Decreased levels**
 - Pancreatitis

Transferrin (Siderophilin)

- The major component of the β -globulin.
- Glycoprotein synthesized by liver
- Carries 2 ferric iron molecules
- Normally 33% saturated
- Prevents loss of iron through kidneys
- Transports to storage sites where Iron is transferred to ferritin
- Transports to bone marrow for RBC synthesis
- **Negative acute-phase protein (The decrease of such proteins may be used as markers of inflammation)**

Transferrin (Siderophilin)

- Transferrin is abnormally high in iron deficiency anemia
- **Decreased in**
 - General protein deficiencies
 - Liver disease
 - Malnutrition
 - Inflammations (Negative acute phase protein)
 - Hereditary Disorders

Transferrin (Siderophilin)

- A transferrinemia is inherited as an autosomal recessive trait due to mutation of both transferrin genes with a resulting absence of transferrin.
- It is characterized by anemia and hemosiderosis (iron deposition) in the heart and liver.
- The iron damage to the heart can lead to heart failure.
- This disease can be effectively treated by plasma infusions of transferrin.

Hemopexin

- Beta globulin
- acute-phase reactant
- Purpose is to remove circulating Heme
 - Breakdown product of Hemoglobin & myoglobin
 - Carried to liver – broken down
- **Increased in**
 - pregnancy,
 - Some malignancies
- Low hemopexin levels are **diagnostic of a hemolytic anemia**
- Hemopexin can be determined by radial immunodiffusion

C-Reactive Protein

- Acute phase reactant
- CRP was so named because it precipitates with the **C substance**, a **polysaccharide of pneumococci**.
- Antibody-like reactivity for many bacteria
 - **Opsonization** (protein-coating process to enhance phagocytosis)
- It is elevated in acute rheumatic fever, bacterial infections, myocardial infarction, rheumatoid arthritis, etc...

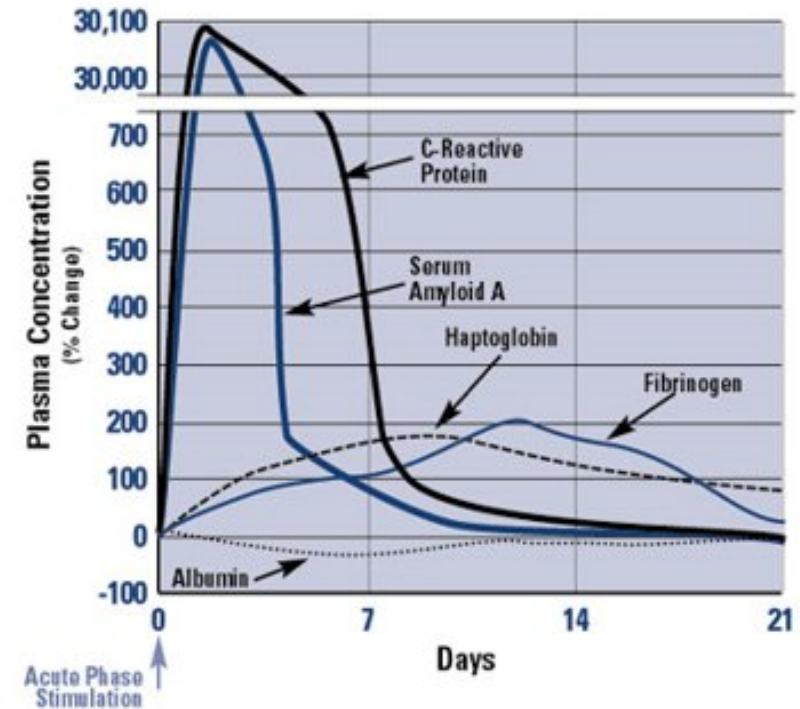
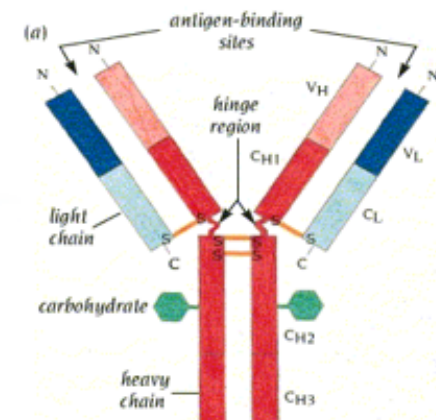


Fig. 1. Alterations in some acute phase reactants following tissue injury. Note that CRP levels rise the highest and the fastest.

Immunoglobulins

- IgA, IgD, IgE, IgG, IgM
- Consist of two identical heavy (H) and two identical light (L) chains
- Decreases seen in general protein deficiencies
- Increases
 - Chronic inflammatory processes - polyclonal
 - Malignancy (multiple myeloma) - monoclonal

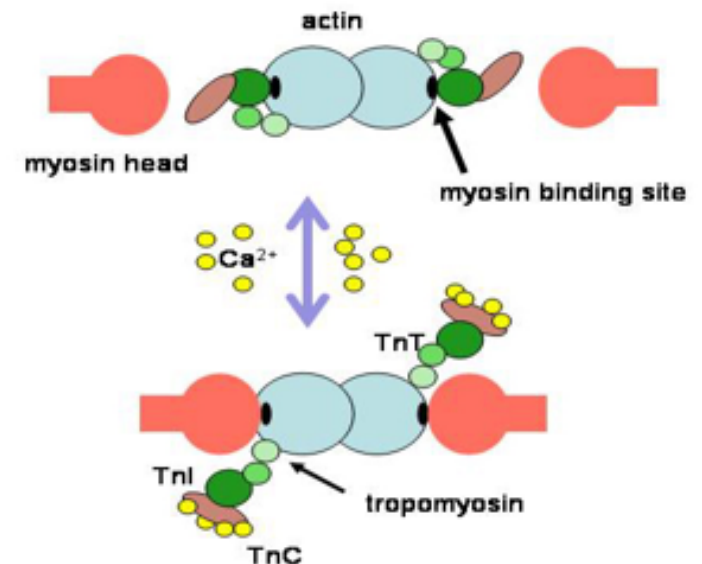


Myoglobin

- Heme protein of skeletal & cardiac muscle
- Single polypeptide chain transports oxygen to muscle tissue
- Released into blood when striated muscle is damaged
 - Cardiac injury (AMI)
 - Trauma or crush injuries
-

Troponin

- Complex of 3 proteins that bind to filaments of striated muscle (cardiac & skeletal)
 - Troponin T (TnT)
 - Troponin I (TnI)
 - Troponin C (TnC)
- The major function of troponins is to bind Calcium and Regulate muscle contraction
- Regulate muscle contractions
 - Calcium release → attaches troponin
- Increase indicative of myocardial injury

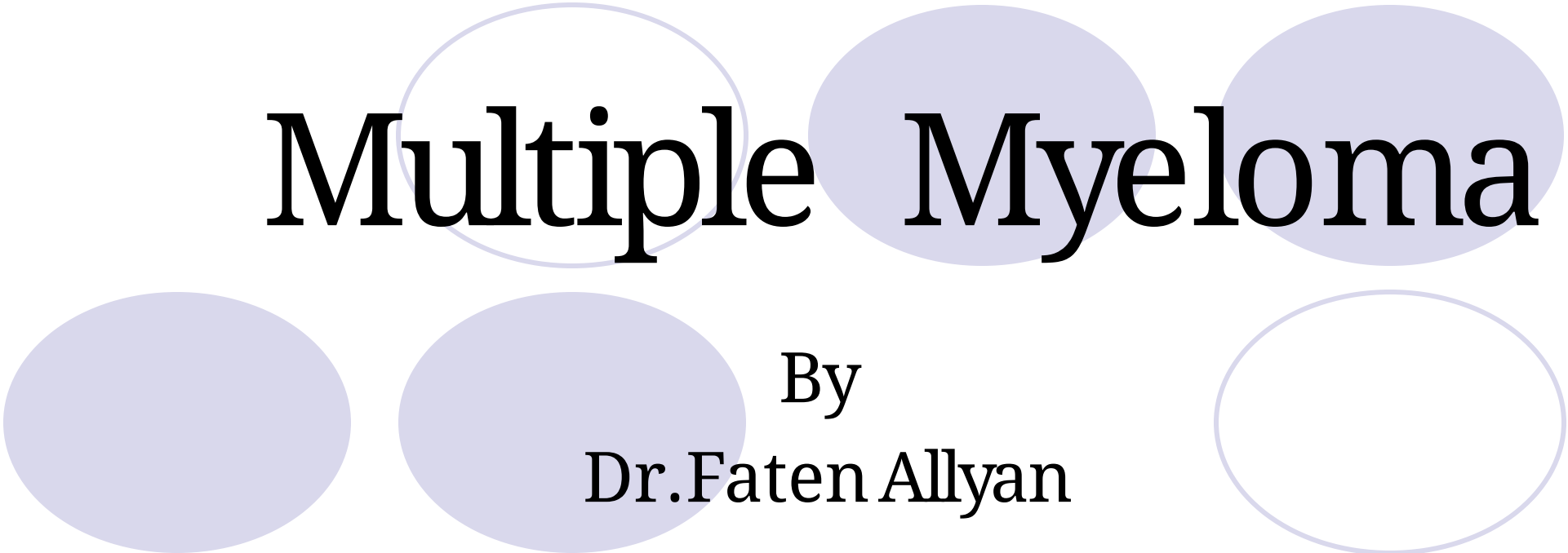


Hypoproteinemia

- Total protein level less than the reference interval, occurs in any condition where a negative nitrogen balance exists:
 - Malnutrition and/or malabsorption
 - Excessive loss as in renal disease, GI leakage,
 - excessive bleeding, severe burns
 - Excessive catabolism
 - Burns, trauma, shock
 - Liver disease
 - Primary site of protein

Hyperproteinemia

- Dehydration
 - Relative due to fluid decrease
 - Decreased intake or increased loss
 - All fractions remain normal ratios
- Monoclonal increases
 - Multiple Myeloma or related malignancies
- Polyclonal increases
 - Chronic inflammatory diseases



Multiple Myeloma

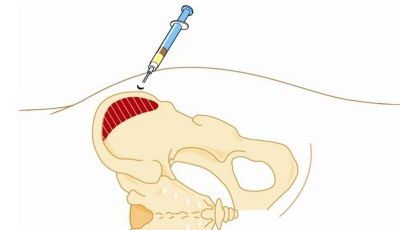
By
Dr.Faten Allyan

Definition

A malignant proliferation of plasma cells derived from a single clone derived from the same cell in the bone marrow

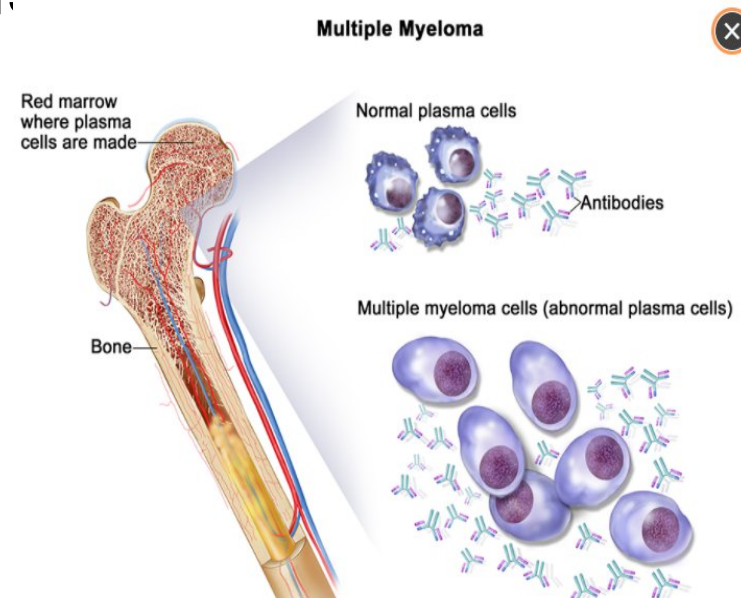
M.M is a defect of the plasma cells.

Plasma cells are white blood cells that make antibodies that protect us from infection

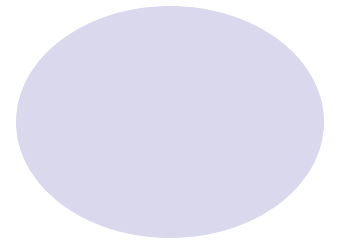
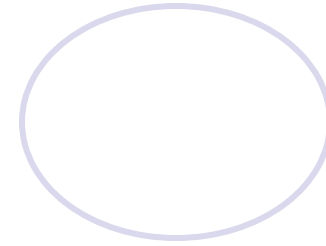
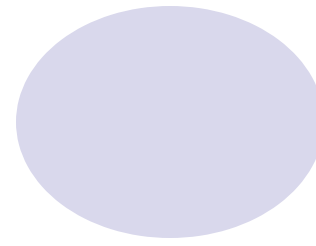
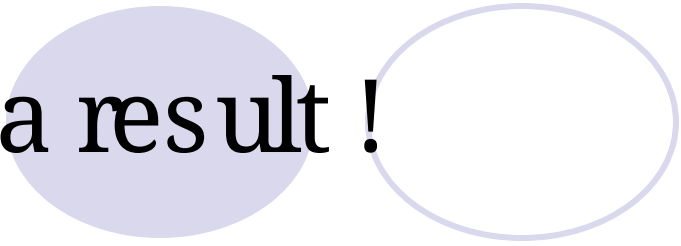


In myeloma, the cells **grow too much**, crowding out normal cells in the bone marrow that make red blood cells, platelets, and other white blood cell

The multiple myeloma cell produces **monoclonal immunoglobulins** that may be identified on serum or urine protein electrophoresis



As a result !



Malignant cell, its products, and the patients response to it, result in a number of **organ dysfunctions**

Fracture/bone pain

Renal failure

Susceptibility to infection

Anemia

Hypercalcemia

Clotting abnormalities

Vascular manifestations of hyperviscosity

Neurologic symptoms

Etiology



Cause not known !

More commonly than expected among

Low socioeconomic class

Farmers

Wood workers

Leather workers

Sheet metal workers

Nuclear industry workers

Those exposed to petroleum products (??benzene)

Exposed to the radiation of nuclear warheads in World War II after a 20-year latency

Incidence



Increases with age

Ages from 45 up to 68 years

Only 2 to 3 percent of cases are reported in patients < 30 years

Males > Females

Accounts for about

Blacks < Whites

13% of all hematologic cancers in whites

MULTIPLE MYELOMA: Pathophysiology

The pathological and clinical features of myeloma are due to:

1. **Tissue infiltration** (The material collected in those tissues or cells of **foreign substances** in amounts excess of the normal is called infiltrate)
2. **Production of large amount of paraprotein** (a monoclonal immunoglobulin or light chain present in the blood or urine, it is produced by B cells)
3. **Impairment of immunity**

Production of **large amount of paraprotein** results
in a **wide** variety of **abnormalities**

Raised serum globulin level

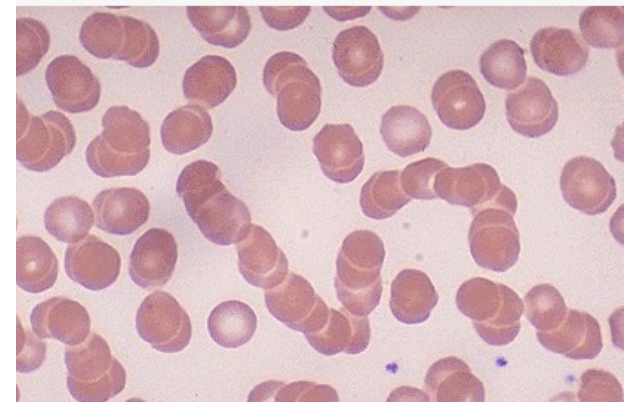
Hypoalbuminemia

Anemia

Raised ESR, rouleaux in blood film

**hyperviscosity, interference with platelets
function and coagulation pathway.**

Proteinuria, renal failure



Multiple myeloma cell clone produces an excess of:

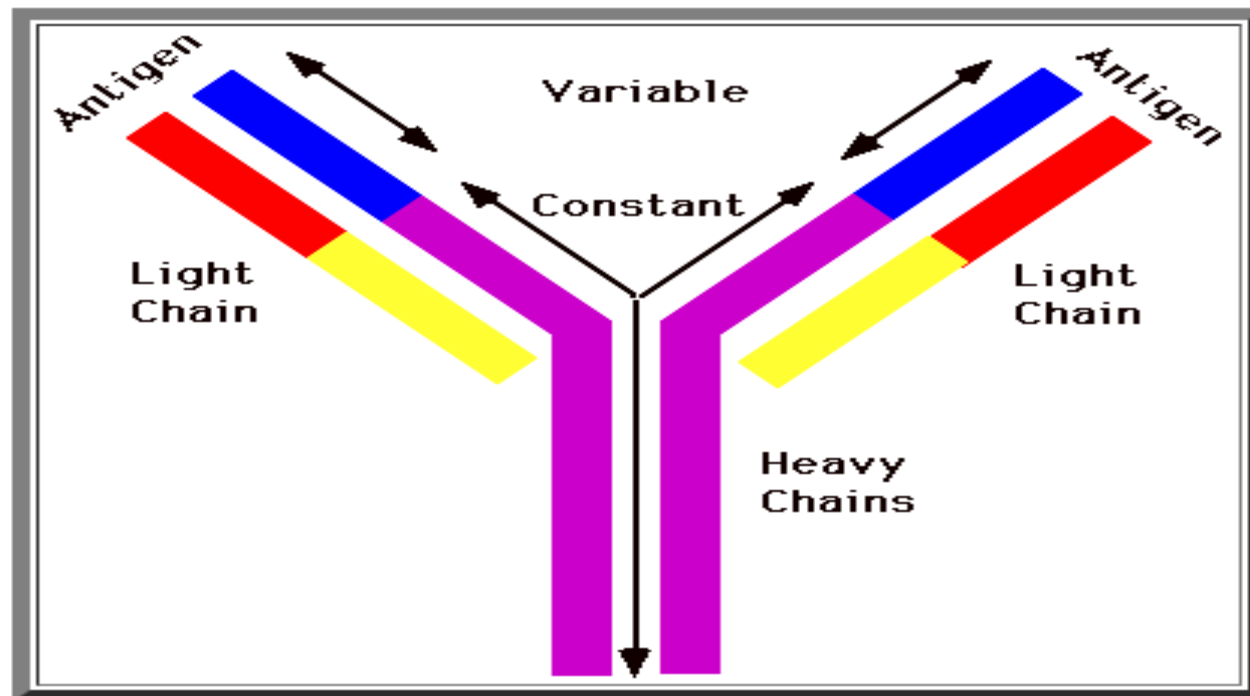
Monoclonal immunoglobulins that are nonfunctional and are called **paraproteins** recognized as IgA, IgD, IgG, IgE or IgM, depending on their heavy chain class

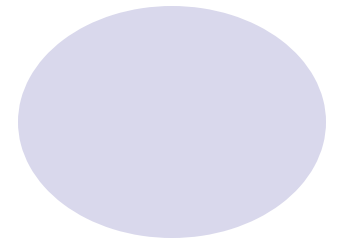
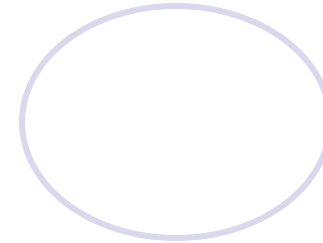
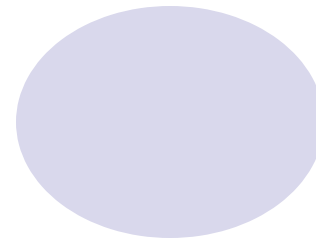
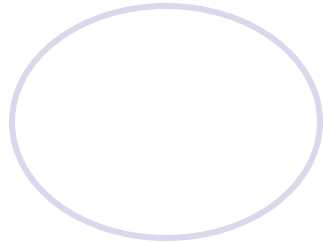
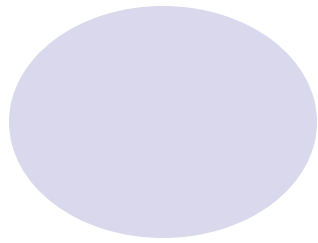
Responsible for **the hyperviscosity syndrome** which **interferes** with **fibrin aggregation** and **platelet function**.

Incomplete immunoglobulins as free light chain proteins (kappa or lambda) in 75% of cases (Bence Jones proteins)

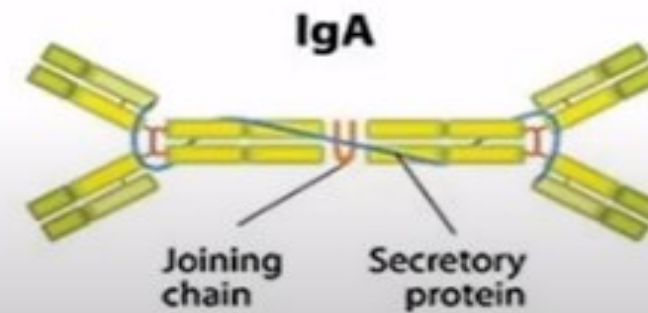
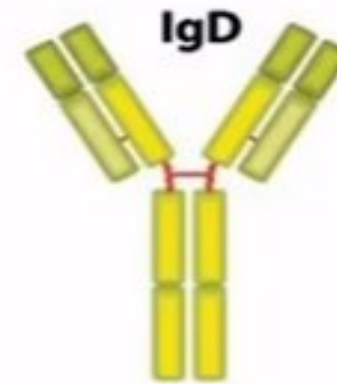
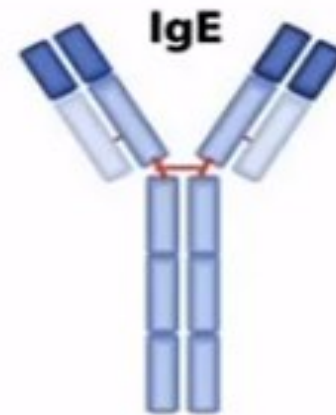
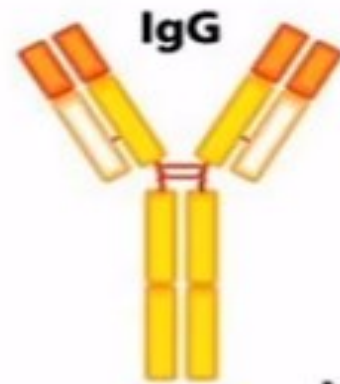
The humoral system

- Antibodies- Immunoglobulin, a tetramer -two identical "light" and "heavy" chains.
- Variable region, includes the N-terminal end of the heavy and light chains, and is responsible for antigen recognition.
- Constant region- C-terminal end of the heavy and light chains are the same for each class of antibody-light chains, and five major classes of heavy chain giving IgM, IgG, IgA, IgD, and IgE.





Immunoglobulins



Immunoglobulins

- **5 types:**
 - **IgM, IgG, IgA, IgD, IgE based on type of heavy chain**
 - **IgH locus: Chromosome 14q32**
- **Light chains: Kappa and Lambda**

Globulin fractions

Alpha1 globulins:

- alpha1 antitrypsin, alpha lipoproteins,

Alpha2 globulins:

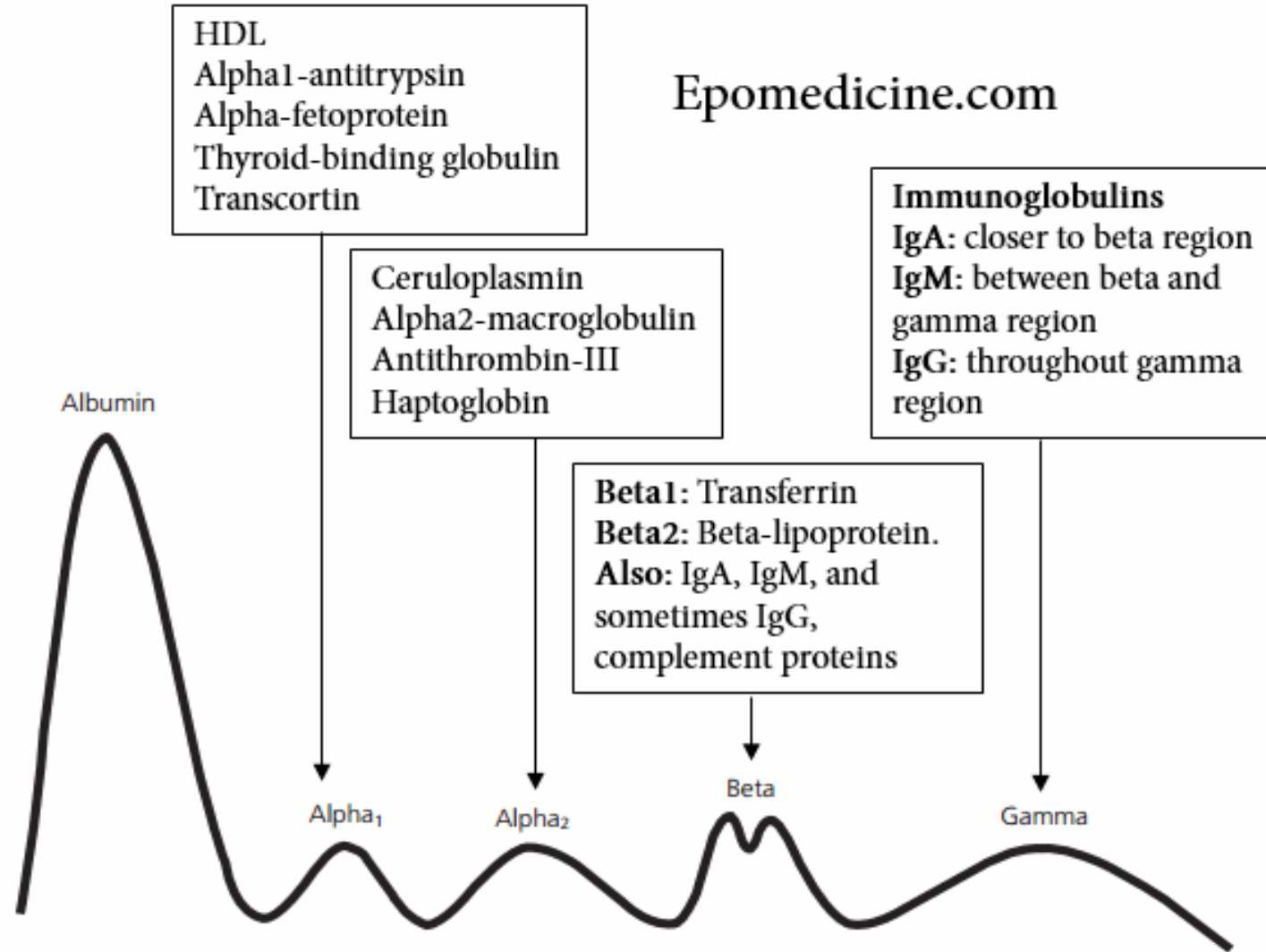
- caeruloplasmin, haptoglobins, alpha2 macroglobulin

Beta globulins:

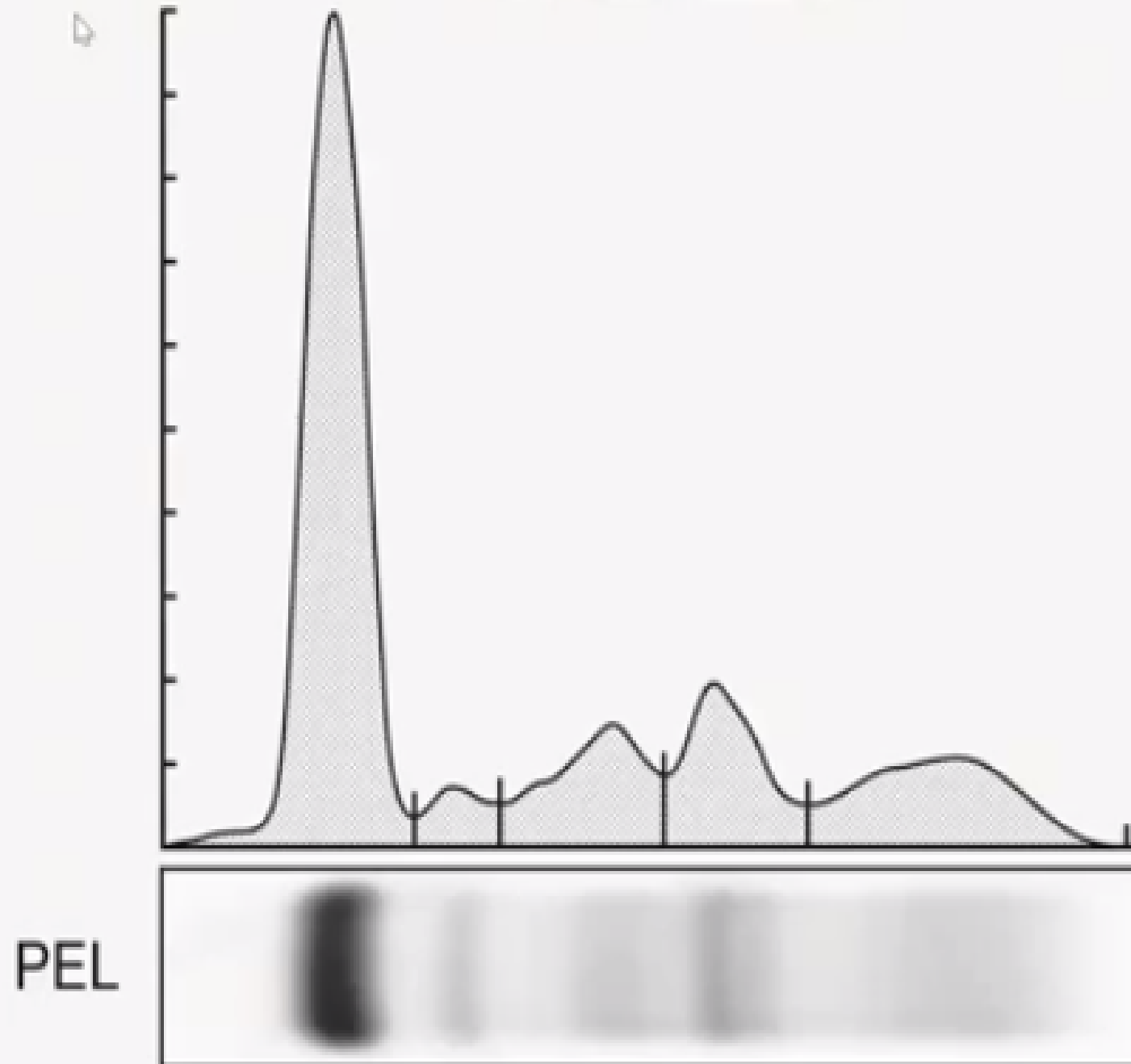
- beta lipoprotein, transferrin, fibrinogen

Gamma globulins:

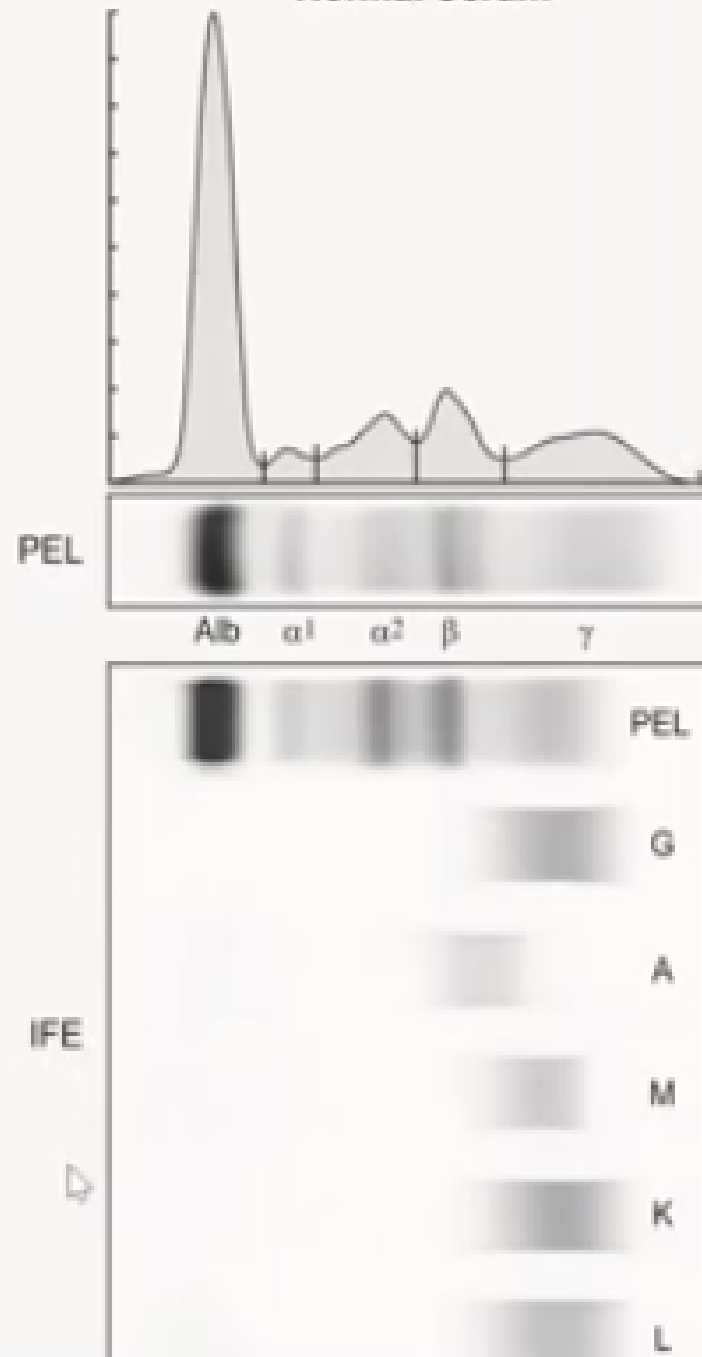
- immunoglobulins, CRP

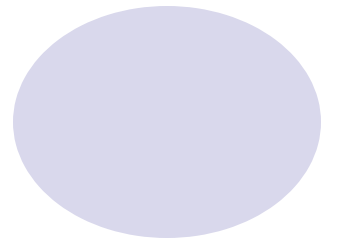
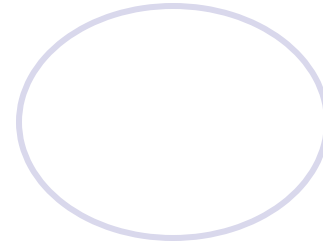
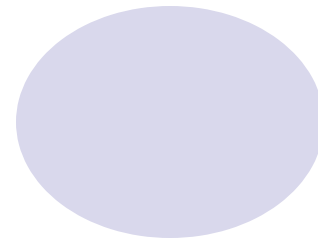
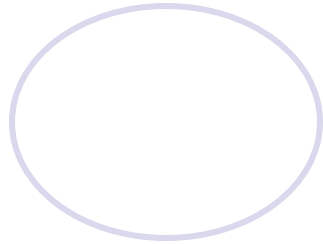
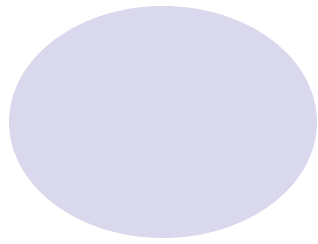


Normal Serum

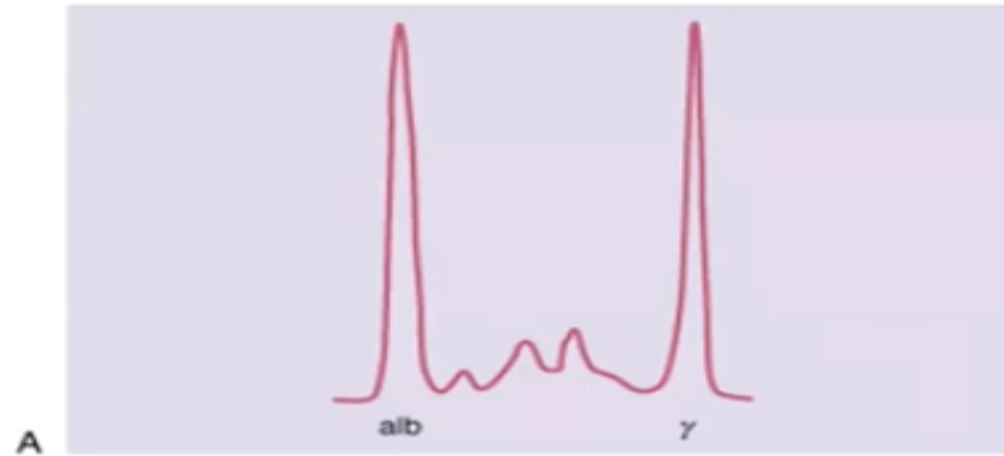


Normal Serum





MAYO CLINIC



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A patient's bone marrow is found to have increased numbers of plasma cells, lymphocytes

The patient's serum most likely has an **M shape** composed of:

IgA

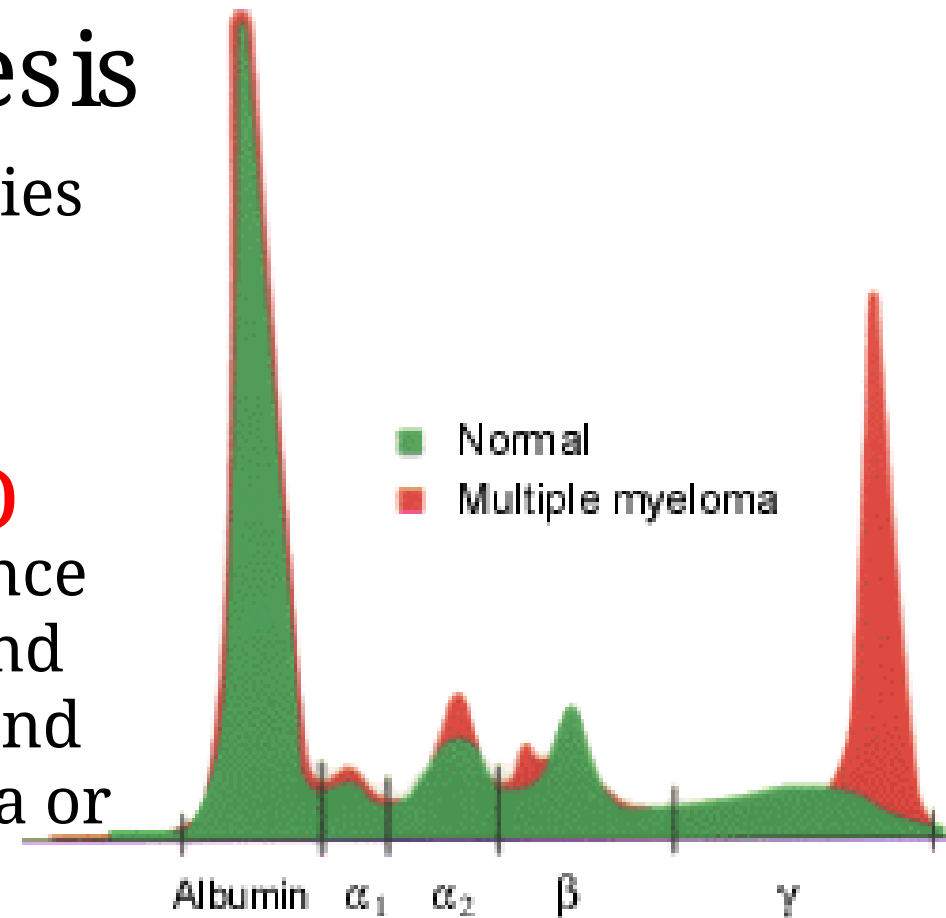
c. IgG

IgM

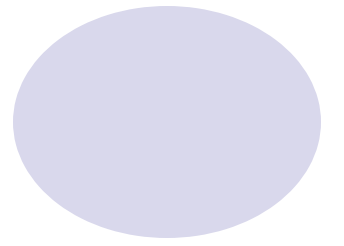
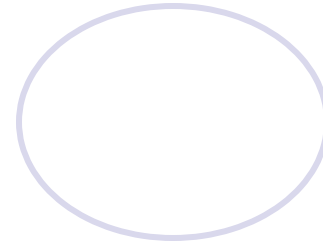
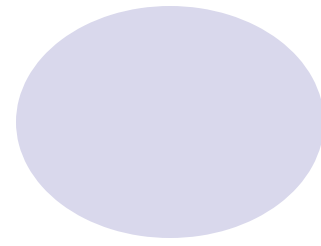
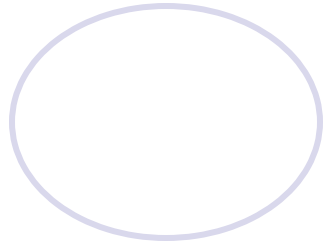
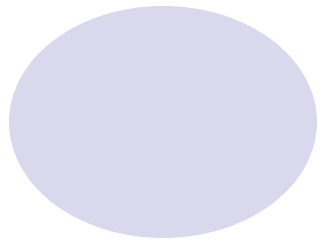
d. Heavy chain, only

Clinical evaluation Protein electrophoresis is

In Monoclonal gammopathies & Myeloma the single clone of plasma cells produce a homogeneous monoclonal immunoglobulin (M protein) characterized by the presence of a sharp, well-defined band with a single heavy chain and a similar band with a kappa or lambda light chain



The M protein is identified as a narrow peak or "spike" in the γ , β or α_2 regions



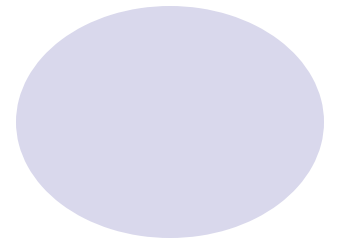
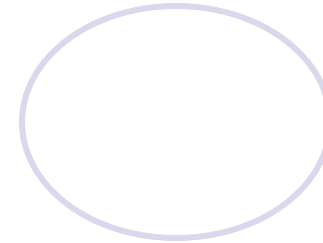
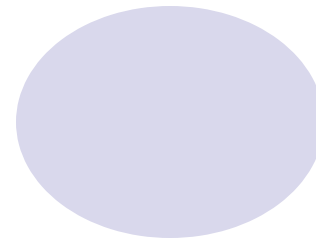
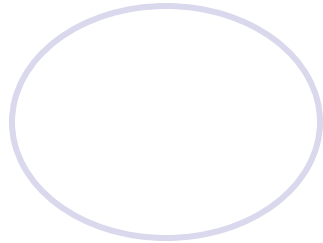
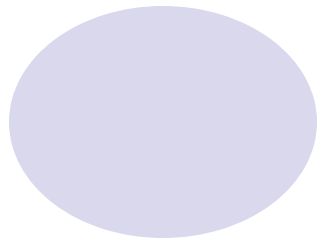
The abnormal protein frequently found in the urine of persons with multiple myeloma is:

Albumin

IgM

IgG

Bence Jones protein



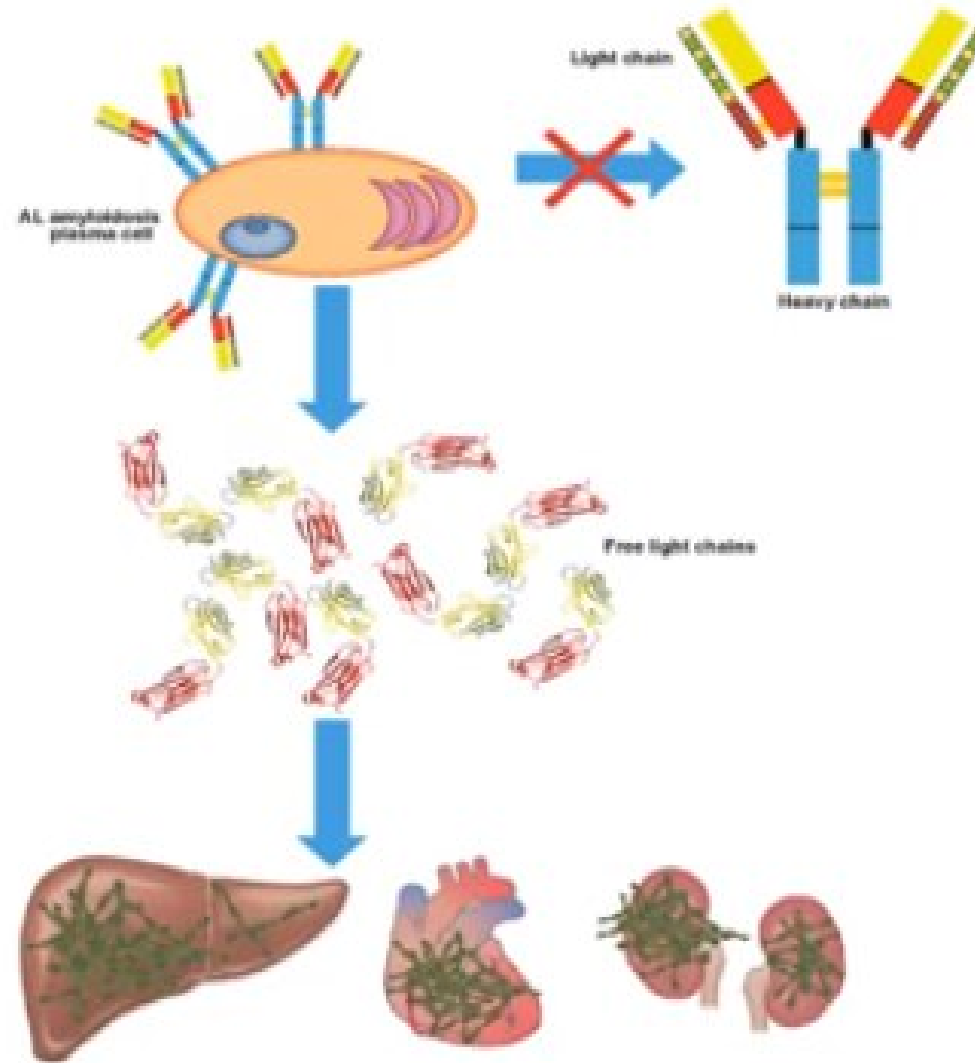
Bence Jones proteins

- These are monoclonal immunoglobulin light chains (kappa or lamda) synthesized by neoplastic plasma cells.
- seen in multiple myeloma, macroglobulinemias, primary amyloidosis.
- Test- Thermal method(waterbath):

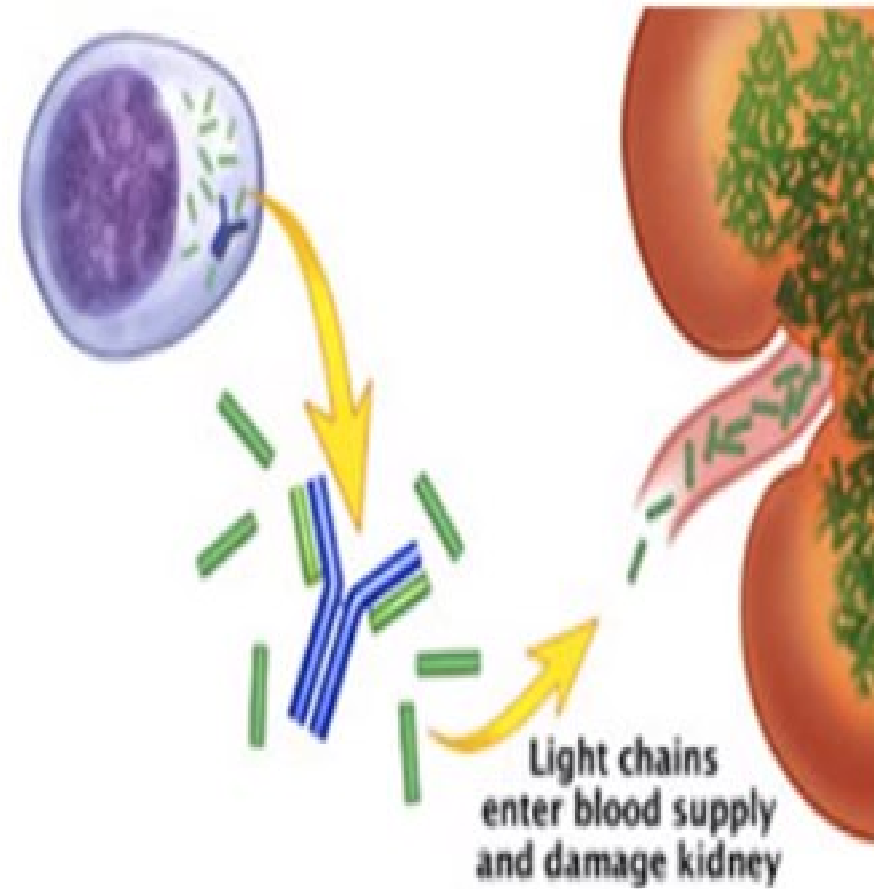
Proteins have unusual property of precipitating at 40° -60°c & then dissolving when the urine is brought to boiling(100°c) & reappears when the urine is cooled.

- Light chains precipitate in tissues causing amyloidosis.

Protein aggregation in kidney



- Light chain deposition in the renal tubules causing nephropathy.



Clinical evaluation



Blood studies

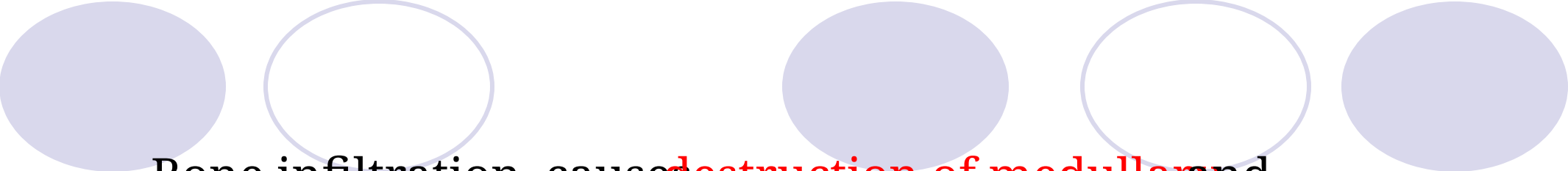
CBC (anemia, pancytopenia ,)

ESR (elevated)

Ca, BUN ,Cr and uric acid (elevated)

Serum alkaline phosphatase is usually normal (absence of osteoblastic activity)

Hypoalbuminemia



Bone infiltration, causes **destruction of medullary and cortical bone** due to the **stimulation of osteoclast activity** by a factor released by the myeloma cells, usually designated **osteoclast-activating factor**.

This leads to **osteoporosis** and more frequently to localized lytic lesions and pathological features.

Skeletal destruction results in the **release of bone salts**, **defect in calcium balance** and hypercalcemia **hypercalciuria** in virtually all cases.

Hypercalciuria Impairment of renal tubular reabsorption Dehydration diminished urine output

Pathogenesis and Clinical Manifestations of Multiple Myeloma

Clinical Finding	Underlying Cause	Pathogenic Mechanism
Hypercalcemia Pathologic fractures Lytic bone lesions Osteoporosis Bone pain	Skeletal destruction	Tumor expansion Production of osteoclast activating factors (OAF) by tumor cells
Renal failure	Light chain proteinuria Hypercalcemia Urate nephropathy	Toxic effects of tumour products Light chains DNA breakdown products

Clinical Manifestations of Multiple Myeloma

- Pain in the lower back, long bones or ribs
-
- Generalized malaise
-
- Infections
-
- Fever
-
- Bleeding
-
- Symptoms of hypercalcemia
 - Nausea
 - Fatigue
- Symptoms of hyperviscosity
 - Headaches
 - Bruising
 - Ischemic neurologic symptoms
- Other neurologic symptoms
 - Peripheral neuropathy
 - Meningitis

Pathogenesis and Clinical Manifestations

Bone Symptoms

Bone pain (most common symptom affecting 70% of patients)

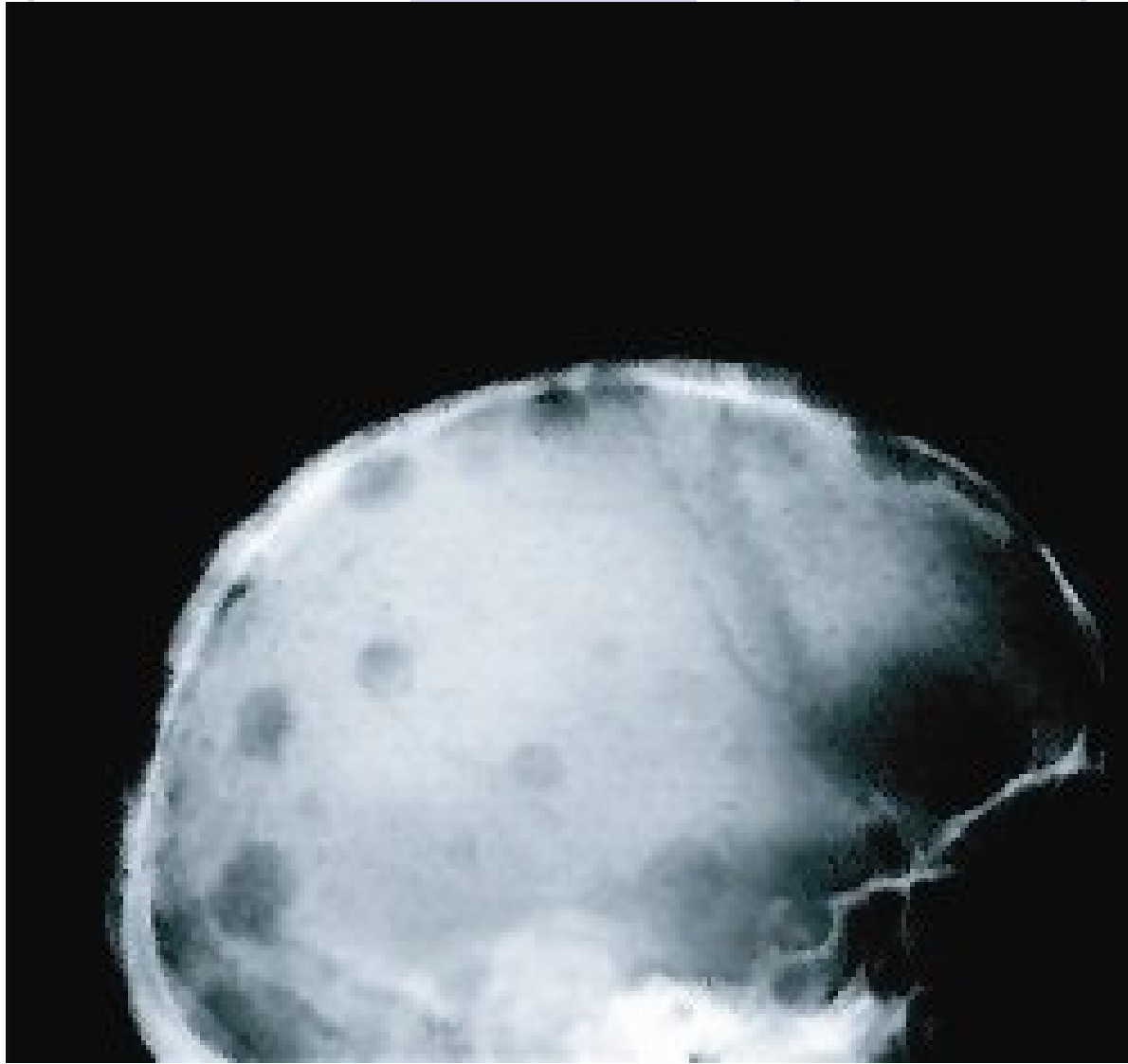
Involves back , ribs , long bones, skull and pelvis

Precipitated by movement (unlike the pain of metastatic carcinoma, which often is worse at night)

If persistent & localized usually signifies **pathologic fracture**







Pathogenesis and Clinical Manifestations Susceptibility to bacterial infections

Recurrent infections are the presenting features in 25% of patients

Most common infections are

Pneumonias (*Streptococcus pneumoniae*, *Staphylococcus aureus*, and *Klebsiella pneumoniae*)

Pyelonephritis (*Escherichia coli* and other gram-negative organisms)

Pathogenesis and Clinical Manifestations

Renal failure / pathology

Factors contributing to renal dysfunction

Hypercalcemia (most common cause of renal failure)

Glomerular deposits of amyloid (protein aggregation in kidney)

Hyperuricemia

Recurrent infections

Infiltration of the kidney by myeloma cells

Tubular damage associated with the excretion of light chains (almost always present)

Pathogenesis and Clinical Manifestations

Anemia

Occurs in 80% of myeloma patients

Types

Usually **normocytic and normochromic**

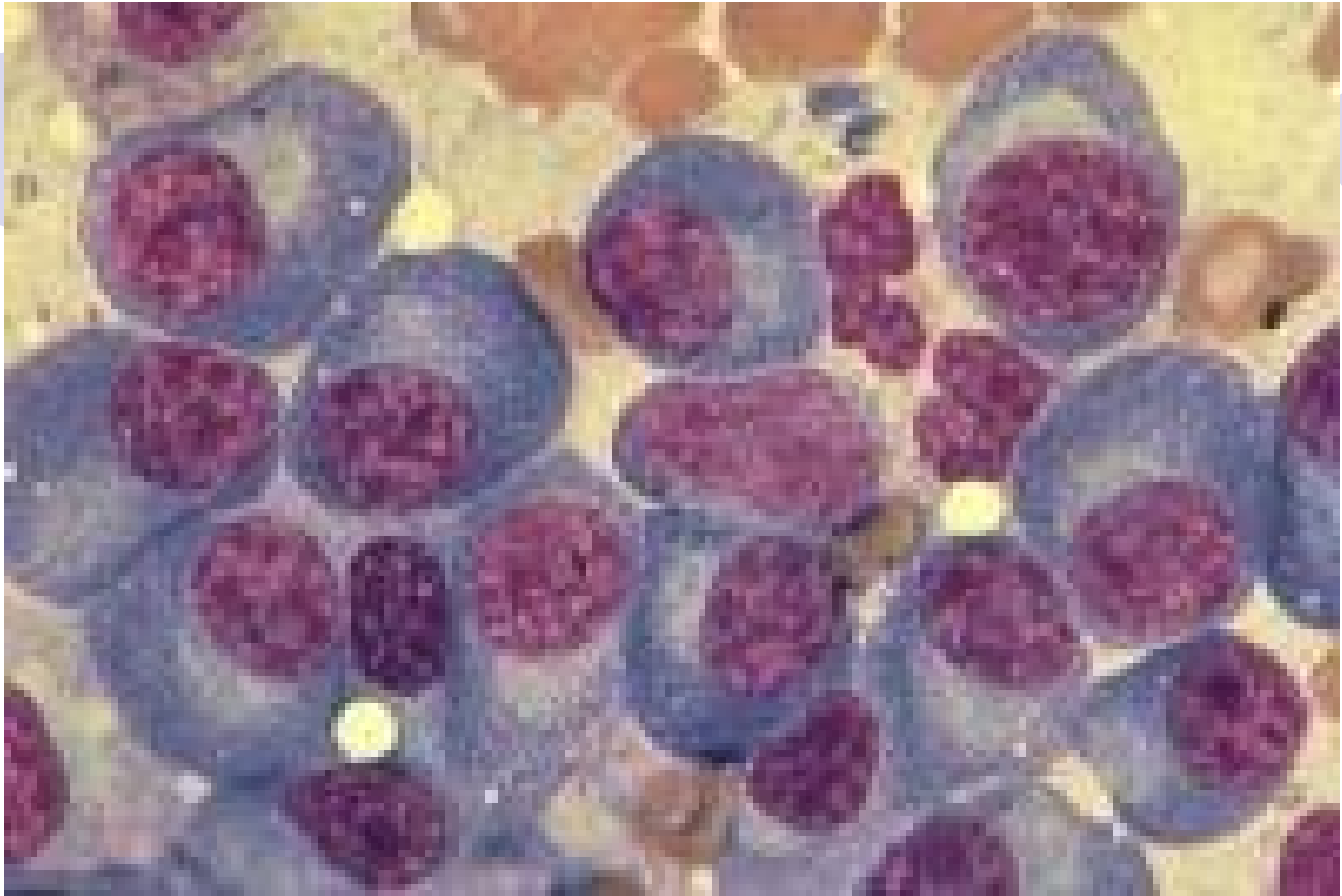
Can be megaloblastic due to either folate or vitamin B12 deficiency

Due to

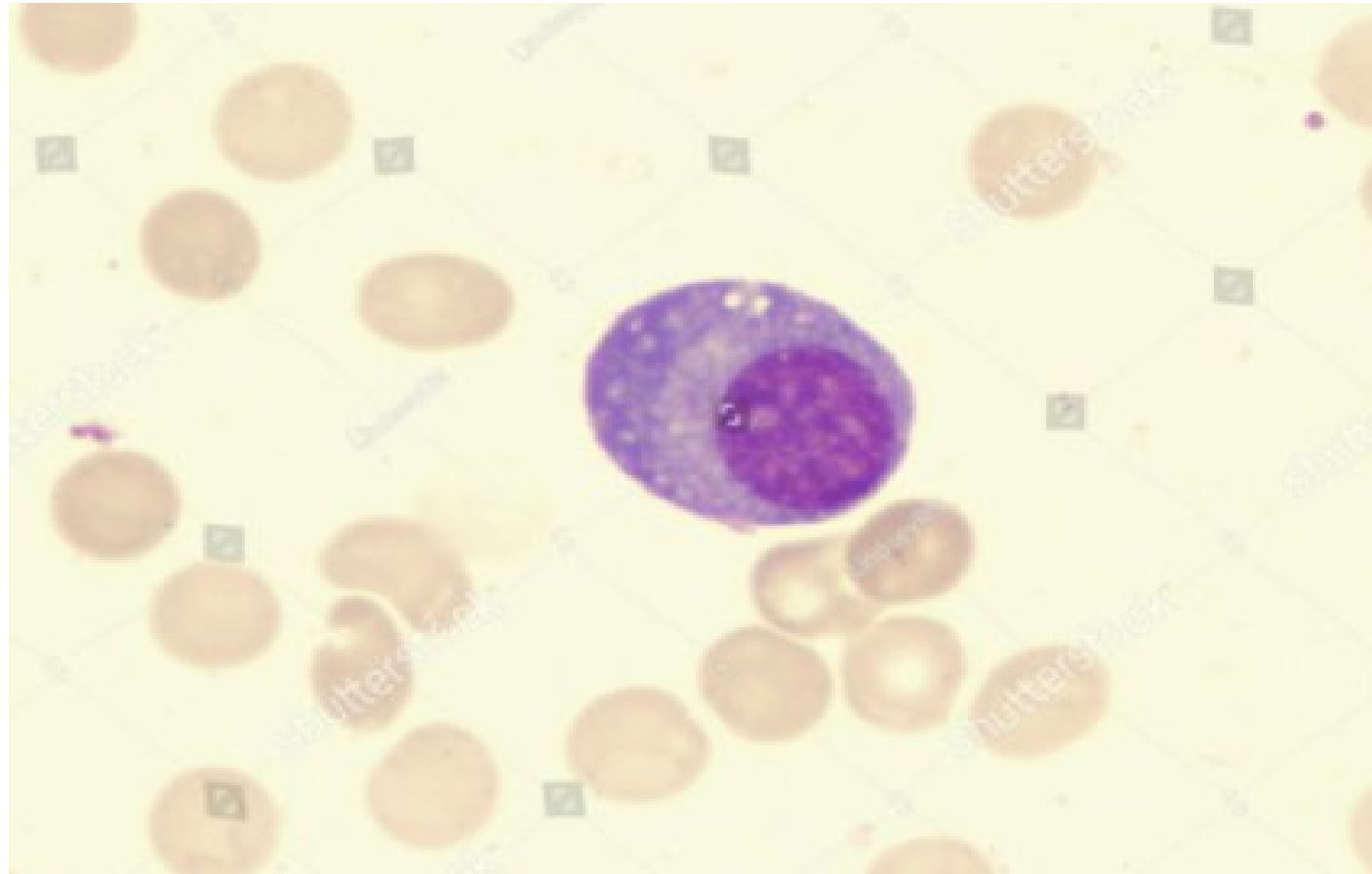
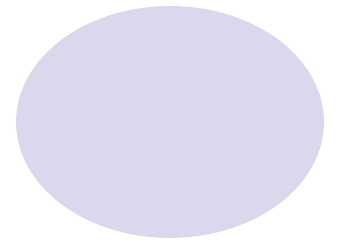
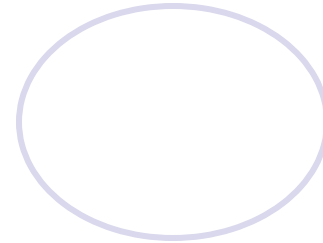
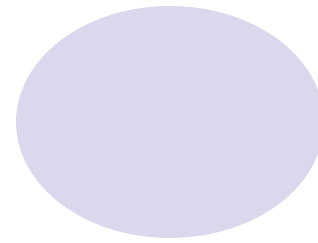
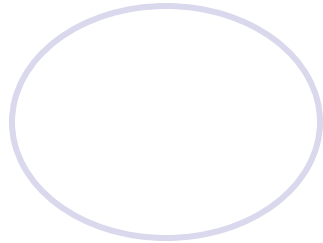
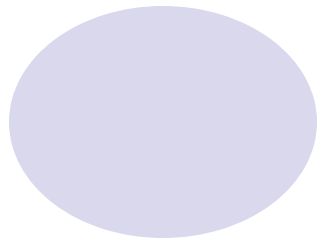
Replacement of normal marrow by **expanding tumor cells**

Inhibition of hematopoiesis by factors made by the tumour

Mild **hemolysis**



Bone marrow aspirate demonstrating plasma cells of multiple myeloma. Note the blue cytoplasm, eccentric nucleus, and perinuclear pale zone



The blood coagulation mechanism in multiple myeloma

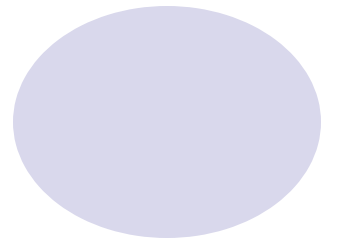
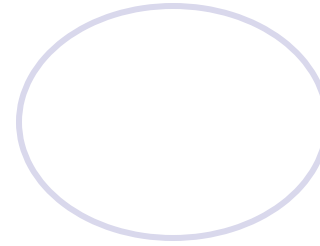
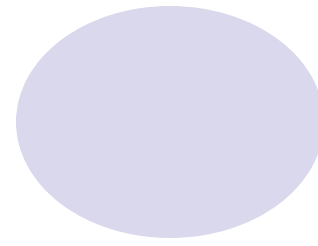
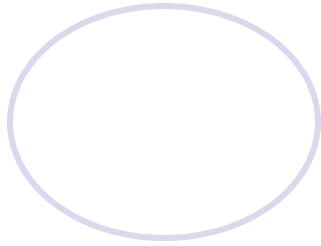
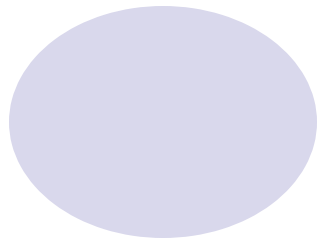
Multiple myeloma Hypercoagulability

At least four possible reasons for hypercoagulability have been described in myeloma patients:

- Interference of immunoglobulins on fibrin structure
- Procoagulant autoantibody production, effects of inflammatory on endothelium

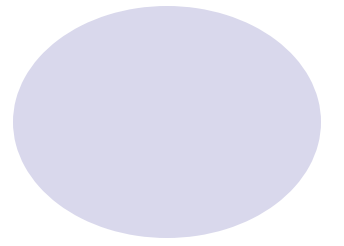
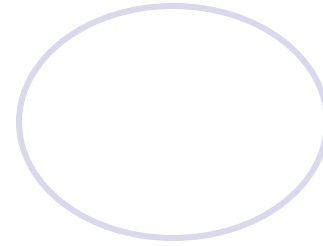
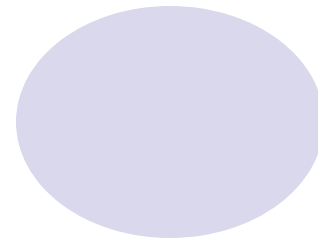
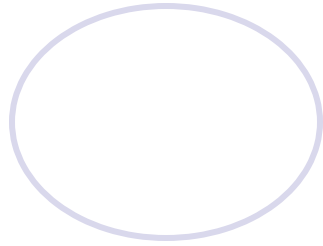
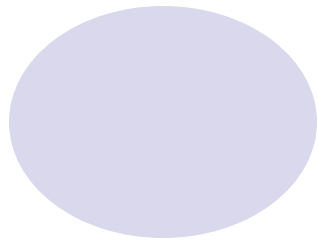


- Moreover, **injury to endothelium** by tumor cells may **predispose to thrombosis** by causing upregulation of adhesion molecules
- Allowing adhesion of blood cellular elements (platelets, lymphocyte, neutrophils, and tumor cells, which secrete thrombogenic substances).
- In most cases, the pathogenesis of a thrombotic complication in myeloma patients remains unexplained.



At the time of diagnosis, the peripheral blood film of a patient with multiple myeloma typically shows:

- A. Greater than 10% plasma cells
- b. Marked lymphocytosis
- d. Rouleaux formation



A patient is **suspected** to have multiple myeloma.

Serum protein electrophoresis appears to **be normal**.

The laboratory should

Examine the urine for Ig light chain

Repeat the serum protein electrophoresis

Measure plasma viscosity

Perform an erythrocyte sedimentation rate.

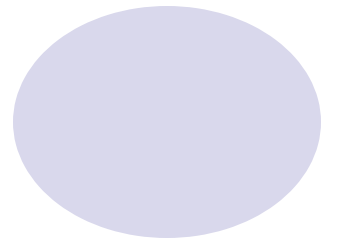
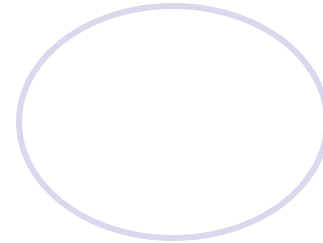
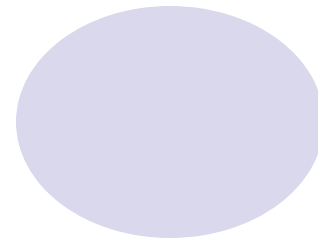
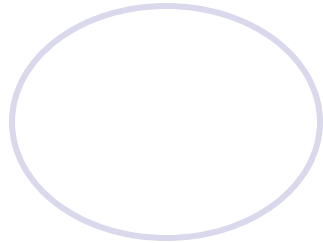
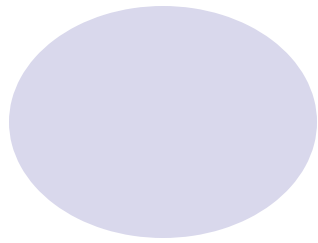


Diagnosis

30% of new cases are diagnosed incidentally during evaluation for seemingly unrelated problems (strokes)

In 30 % a pathologic fracture is the presenting feature

In 25% of patients recurrent infections are the presenting features



Thank you