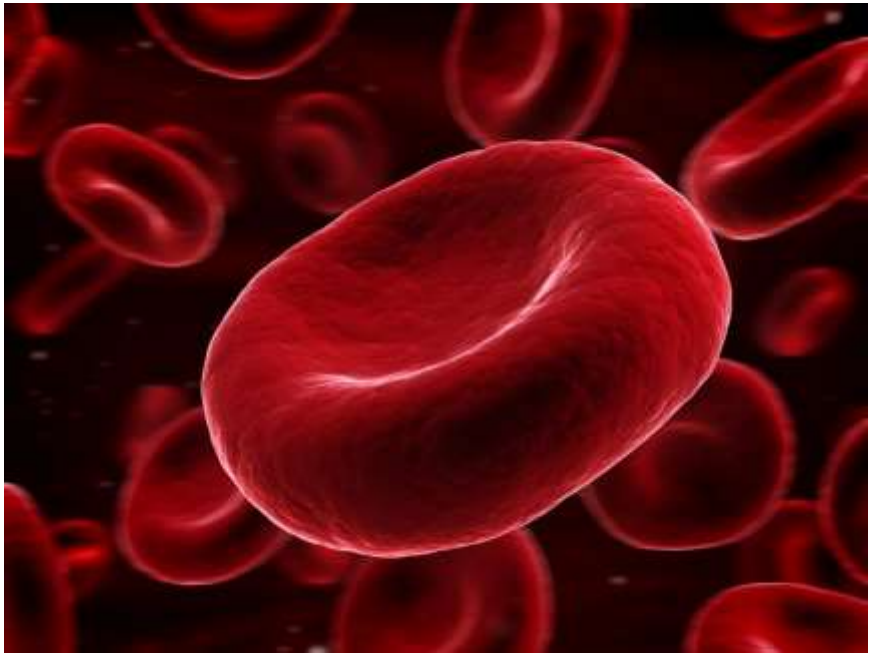
BLOOD AND BODY FLUIDS



SEMESTER -3

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PHYSIOLOGY HONOURS

Introduction

Blood is a body fluid in animals that delivers necessary substances such as nutrients and oxygen to the cells and transports metabolic waste products away from those same cells.

Blood is a connective tissue. Like all connective tissues, it is made up of cellular elements and an extracellular matrix.

Characteristics:

- Colour: Opaque red
- pH: 7.4 (7.35-7.45)
- Specific gravity: 1.050-1.060
- Volume: 5-6 liters
- Viscosity: 5 times more than water
- Blood makes up around 7-8% of the weight of a human body.
- The normal temperature of blood is slightly higher than normal body temperature—about 38 °C (or 100.4 °F),

Composition:

❑The cellular elements—referred to as the '**Formed elements'** **(Living blood cells)**—include **red blood cells (RBCs)**, **white blood cells (WBCs)**, and cell fragments called **platelets,** and this makes up **45% of our blood composition.**

❑The **non-living component** of our blood is known as the extracellular matrix, called **plasma**,and it makes up **55% of our blood composition.**

❑ blood **unique among connective tissues** because it is **fluid.** This fluid, which is mostly water, perpetually suspends the formed elements and enables them to circulate throughout the body within the cardiovascular system.

Functions of blood

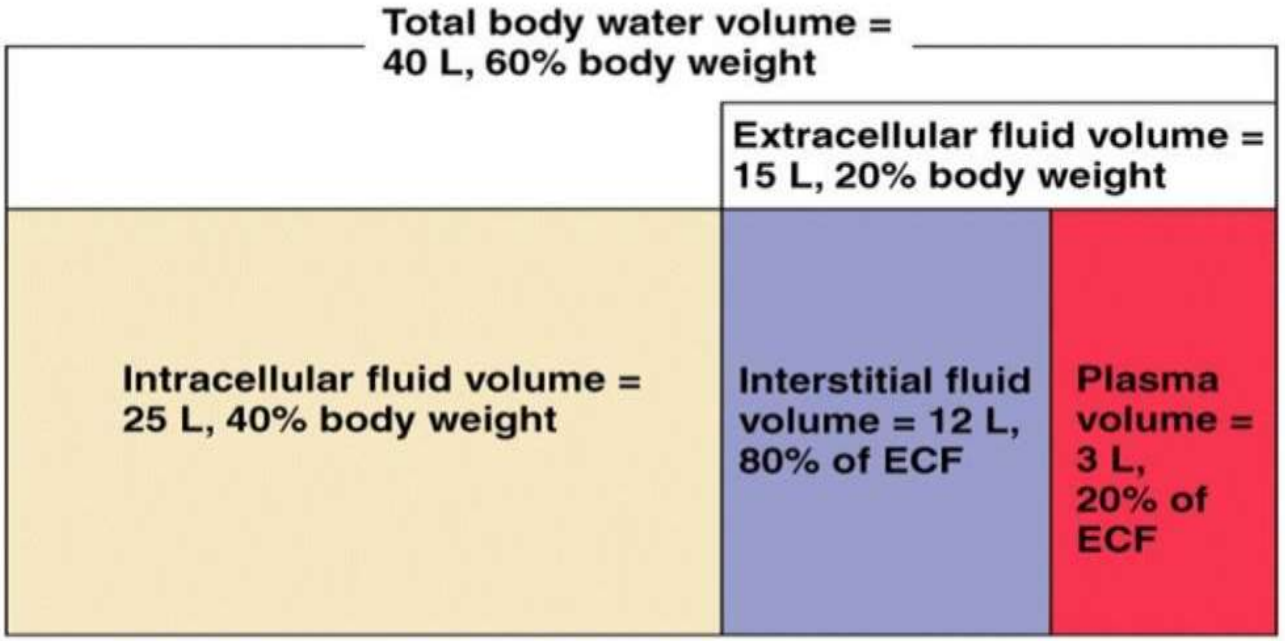
- **Transportation**
  - O2, CO2
  - metabolic wastes,
  - Nutrients
  - hormones
- **Regulation**
  - helps regulate pH through buffers
  - helps regulate body temperature
  - helps regulate water content of cells
- **Protection from disease/Defense**

Body Fluid Compartments

Total body water: 40-42 L (in a 70 kg adult)

It is distributed in 3 compartments:

1. **Intracellular fluid (25-28 L)**
2. **Extracellular fluid (Plasma-3.5 L + Interstitial fluid 10.5-12 L)**
3. **Trans-cellular fluid (1-2L)**

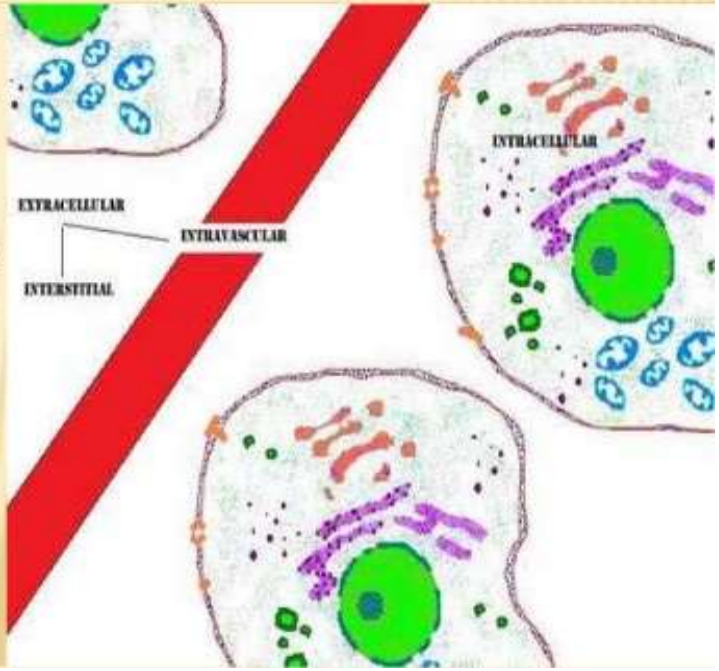


What is Trans-cellular fluid?

TRANSCELLULAR FLUID

Specialized ECF or another small compartment of fluid. It is the fluid separated from plasma by epithelium. This compartment includes:

- Synovial fluid
- Peritonial fluid
- Pericardial fluid
- Intraocular fluid
- Cerebrospinal fluid



BONEMARROW

A soft gelatinous tissue that fills the cavity of bones. It is either Red or yellow depending on the preponderance of hematopoietic (red) or Fatty (yellow) tissue. Adult has approx. 2.6 kg of bone marrow. Half is red. Bone marrow represent 4% of total body mass.

RED BONE MARROW (Medulla ossium rubra)

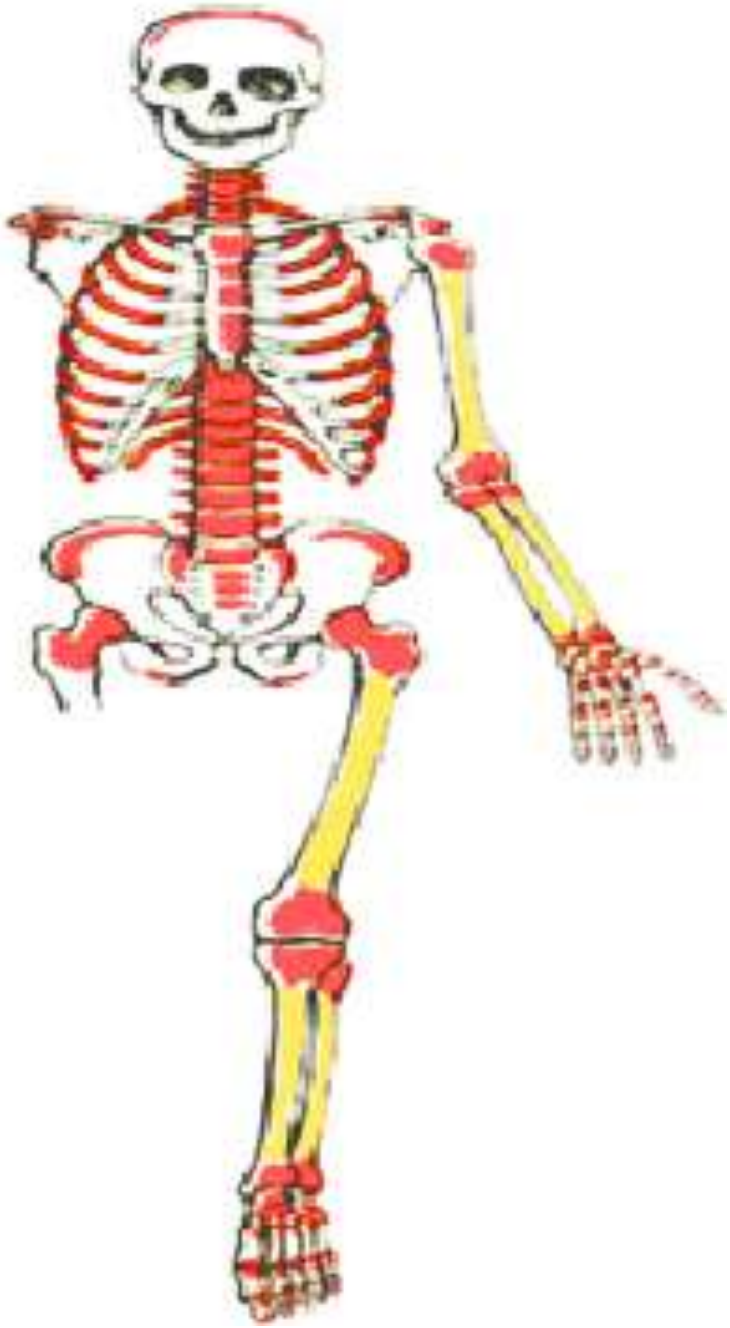
1. At birth and until 7 yrs of age, all human marrow is red.
2. In adult, red marrow is only found in flat bones like skull, vertebrae, hips, ribs, sternum etc. and in the spongy epiphyseal end of the long bones.
3. It contain hematopoietic stem cells which differentiate into various blood cells.

YELLOW BONE MARROW (Medulla ossium flava)

1. At birth, it is absent. After the age of seven, fatty cells (yellow in colour) gradually replaces red bone marrow.
2. In adult it is found in hollow cavity in the middle of long bones.
3. It contains fat cells that act as body's energy and blood reserve in case of emergency.

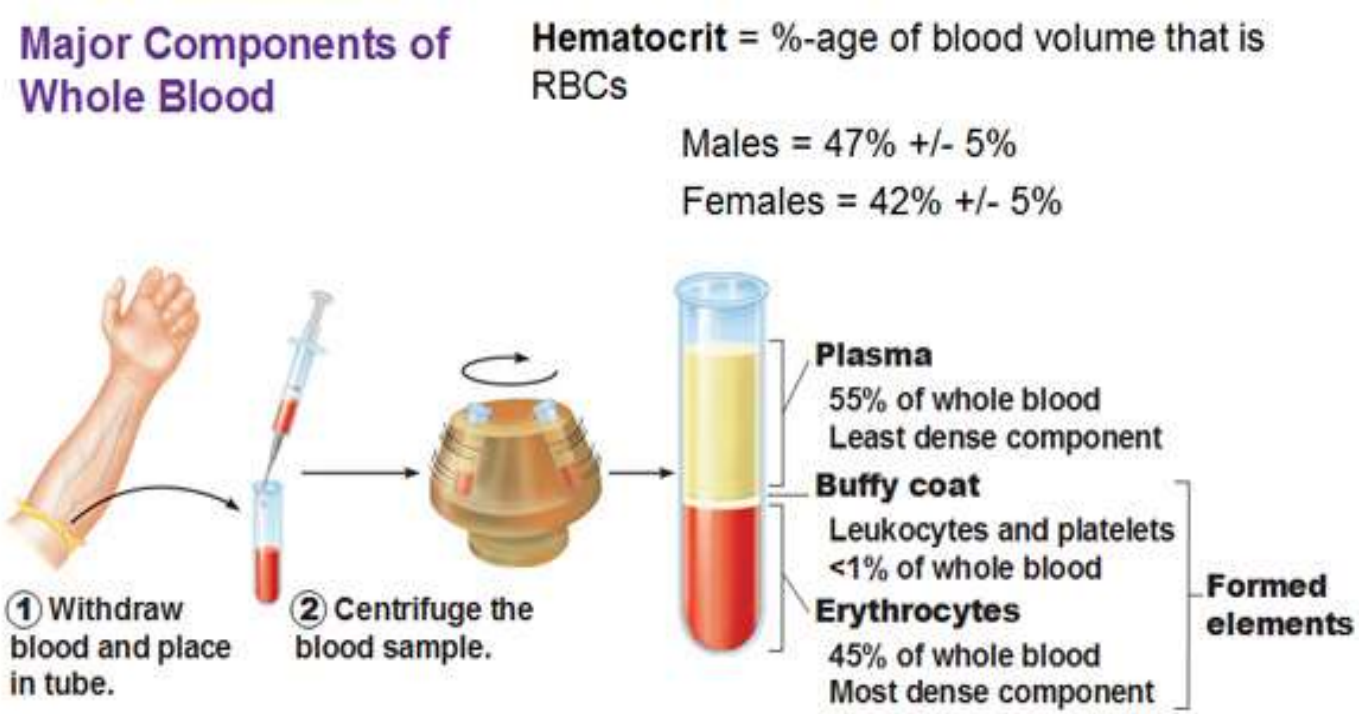
Areas with red marrow

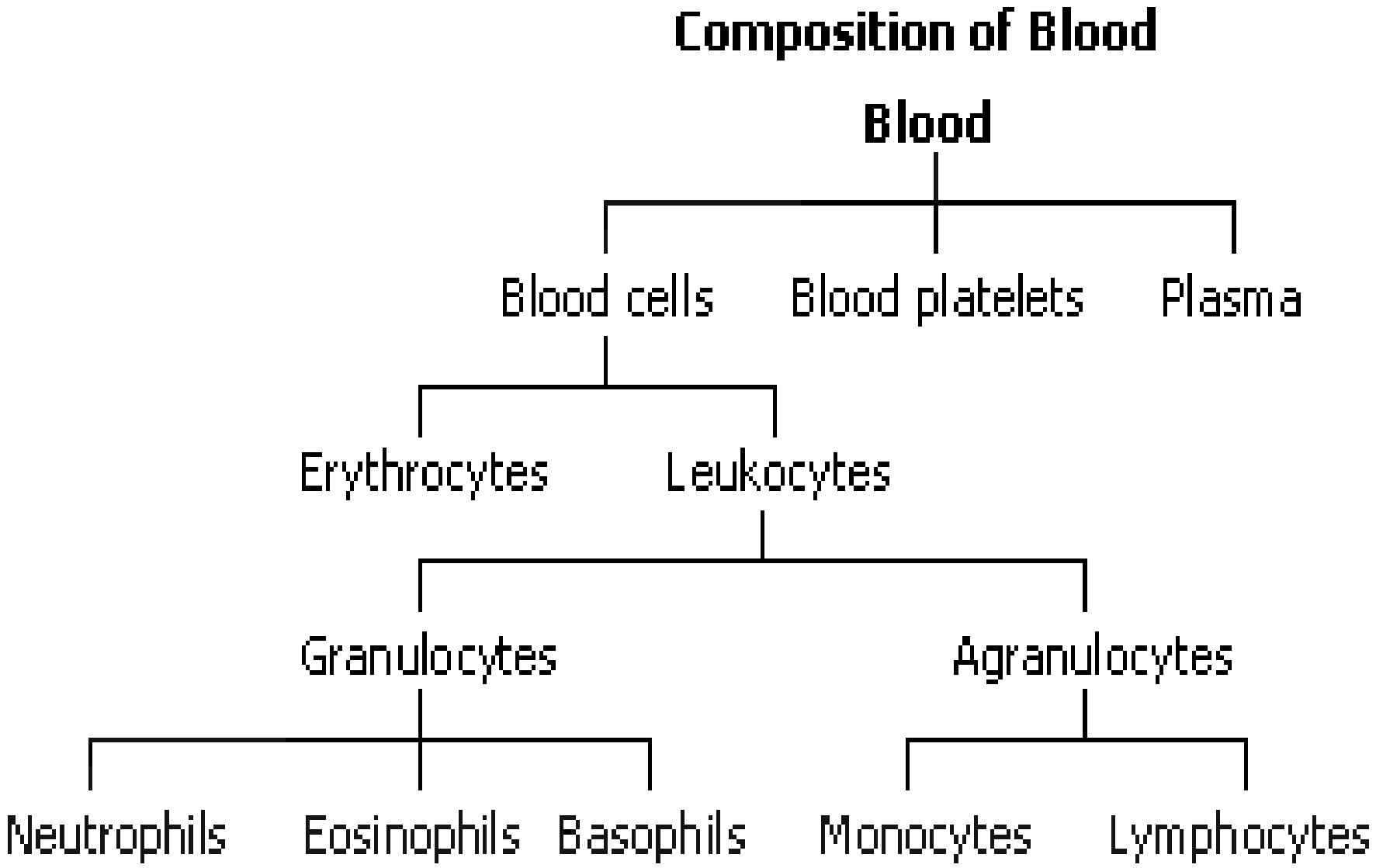
Areas with yellow marrow

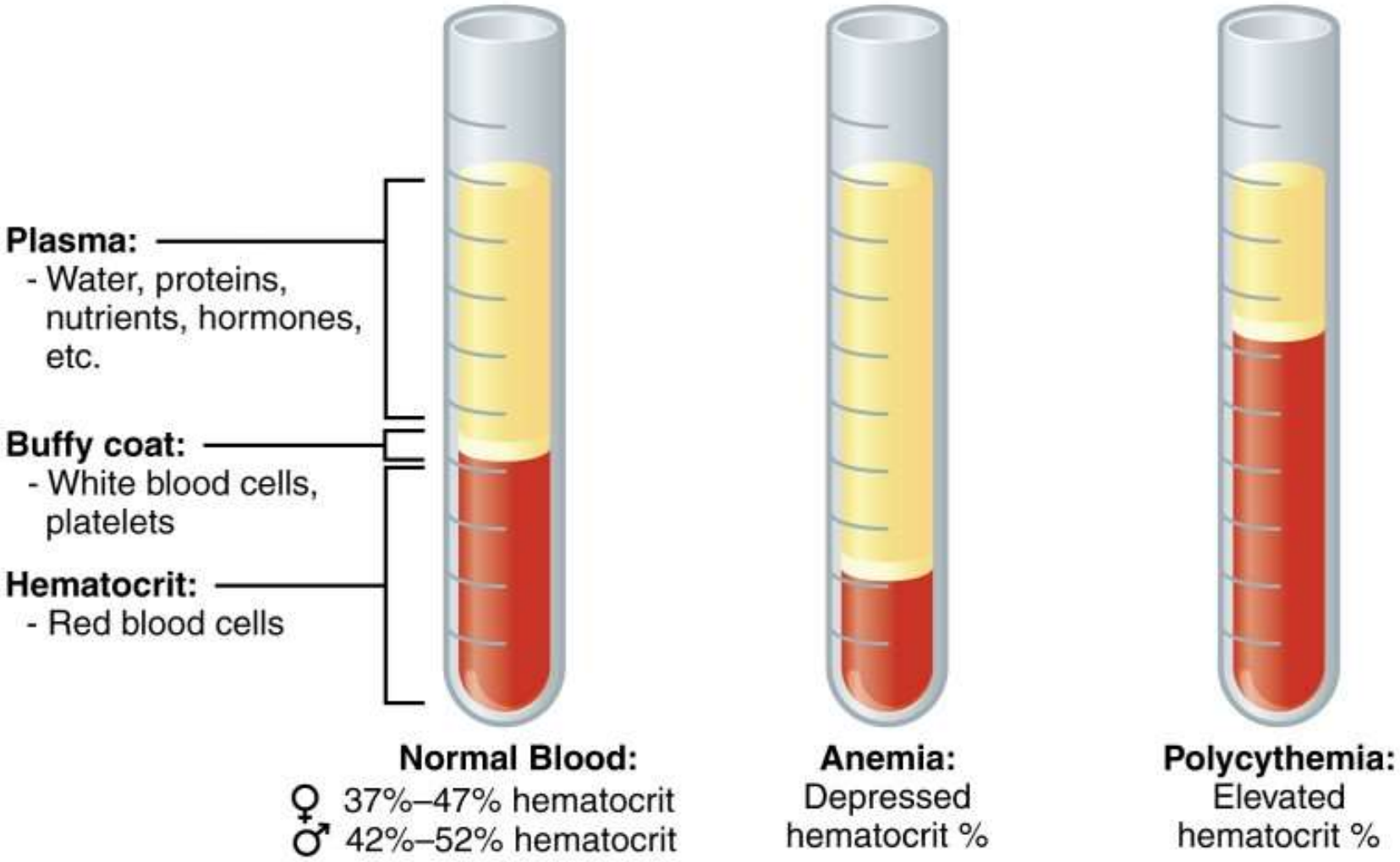


COMPOSITION OF BLOOD

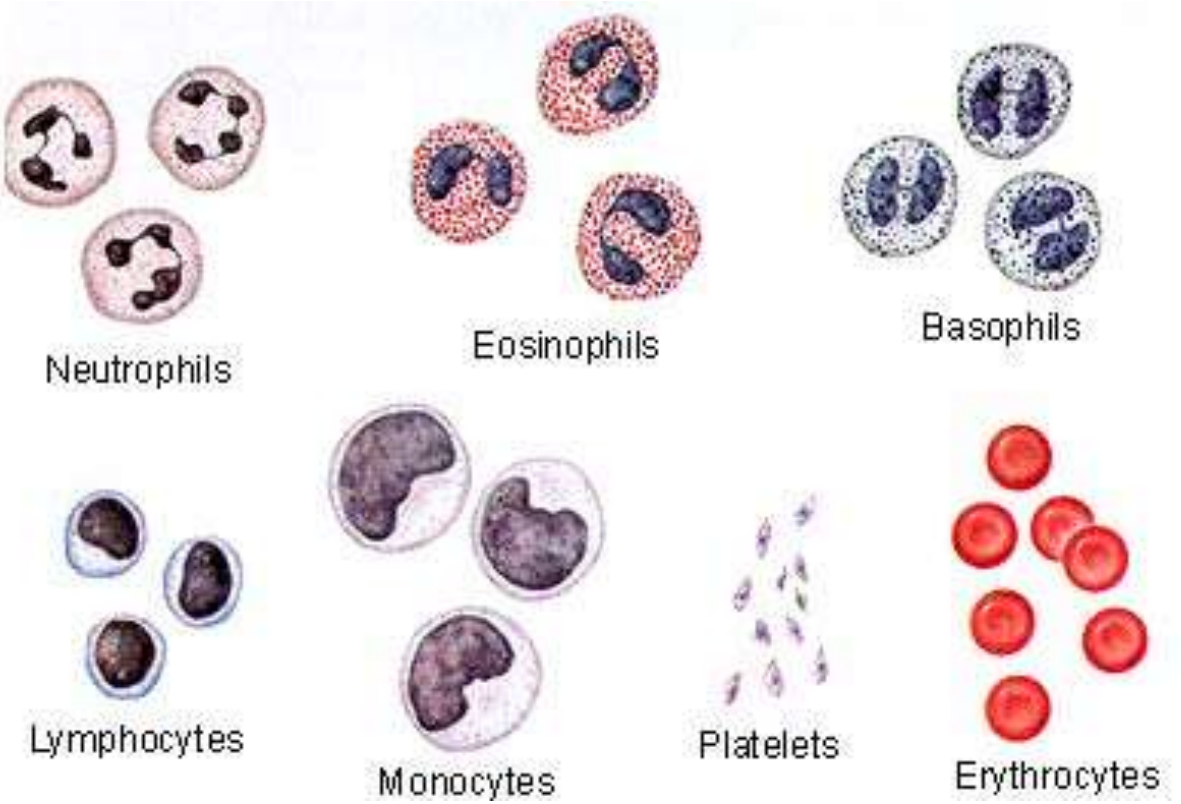
1. Blood consists of 55% **plasma** and 45% **formed elements.**
2. Blood plasma consists of 91.5% water and 8.5% solutes.
3. Formed elements contains Red blood cells (erythrocytes),White blood cells (leukocytes) and Platelets (special cell fragments).



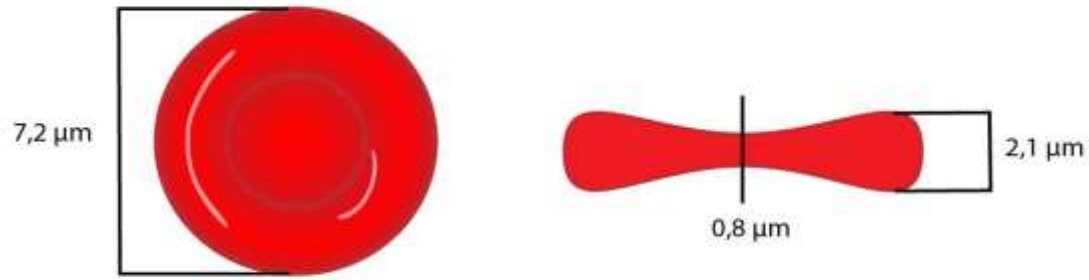

Composition of Blood
Blood
Blood cells
Blood platelets
Plasma
Erythrocytes
Leukocytes
Granulocytes
Agranulocytes
Neutrophils
Eosinophils
Basophils
Monocytes
Lymphocytes


Plasma:
- Water, proteins, nutrients, hormones, etc.
Buffy coat:
- White blood cells, platelets
Hematocrit:
- Red blood cells
Normal Blood:
♀ 37%–47% hematocrit
♂ 42%–52% hematocrit
Anemia:
Depressed hematocrit %
Polycythemia:
Elevated hematocrit %

Formed elements of Blood:



Red Blood Cells



Erythrocytes are anucleate cells of biconcave shape measuring about 7,2 µm – 7,5 µm in diameter and central thickness of 0.6 µm and edge thickness 2,6 µm.

Mean corpuscular volume of erythrocyte (MCV) is **84-96 fl**.

If the diameter of erythrocytes is larger than 8 µm and MCV larger than 96 fl, are these erythrocytes called **Macrocytes.** If the diameter is smaller than 6 µm and MCV lower than 84 fl are these cells called **Microcytes**.

Concentration of Red Blood Cells in the Blood

- Normal value Male
  52,00000

  Or 5.2 million per cubic millimeter
- Normal value Female
  47,00000

  Or 4.7 million per cubic millimeter

  ± 300000

40 to 45 % of the blood volume

One drop of blood = 1cmm

Life span

- The RBCs lack a nucleus so it has no power of repair & reproduction.
- It days are strictly numbered
- Average life span is 120 days
- It has been calculated that each RBC travels 175 miles in the course its comparatively short life

With time, they become less flexible, deformed and subsequently, they are degraded by splenic macrophages.

Other facts:

1. **Total surface area per RBC: 120-140 µm2**
2. **Total volume per RBC : 80 µm3**
3. **Shape: Biconcave**
4. **Average weight of HB per RBC: 30 pg.**

Biconcave shape of RBC

- **RBC membrane contains 4 structural proteins. Spectrin, Ankyrin, Protein 4.1 and Actin.**

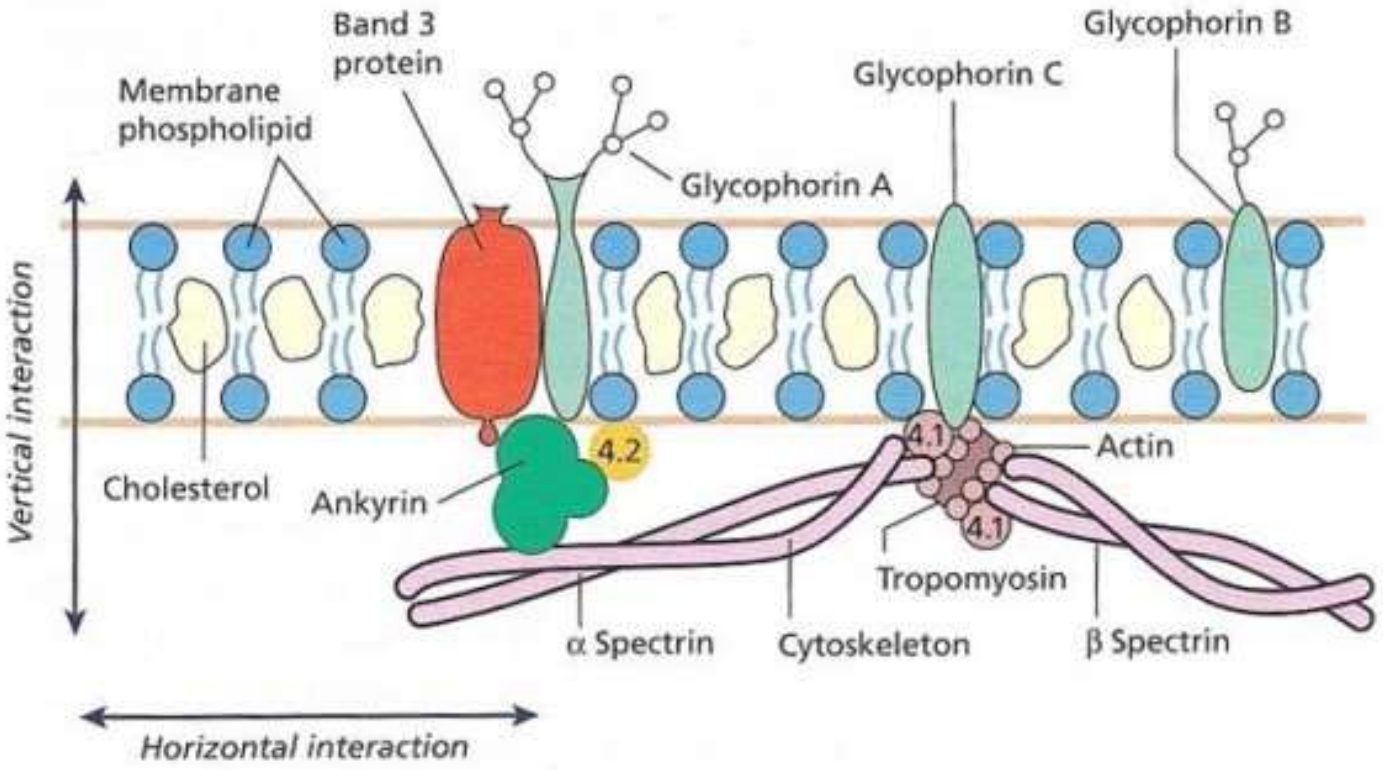
- **At first α- and β-chain of fibrous protein spectrin forms dimers and then tetramers binding to the actin molecule.**

- **Ankyrin anchors the spectrin to membrane via band -3 protein (anion excahnger protein)**

- **Actin binds to tails of spectrin and protein 4.1 which in turns bind to glycophorins A,B,C (transmembrane glycoproteins).**

- **Thus, on the inner site of erythrocyte plasma membrane some kind of 'scaffold' is formed.**

The red cell membrane structure



Questions asked:

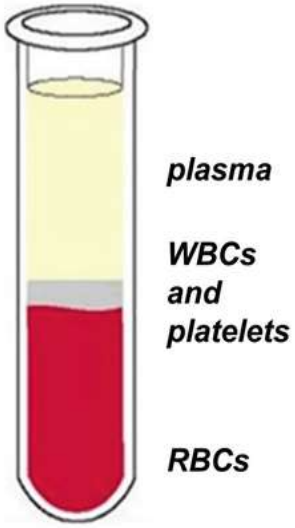
1. What is the advantage of having a bi-concave shape of RBC?
2. Why red blood cells have no nucleus?
3. Can a red blood cell divide?
4. Where do you find red bone marrow in adults?

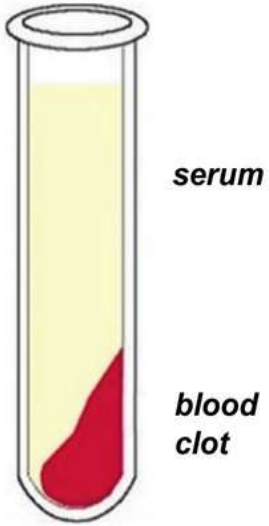
Plasma and Serum

❑ **Blood plasma** appears as light-yellowish or straw-colored liquid. It is the liquid portion of blood (55%) that remains when RBCs, WBCs, Platelets and other cellular elements are removed.

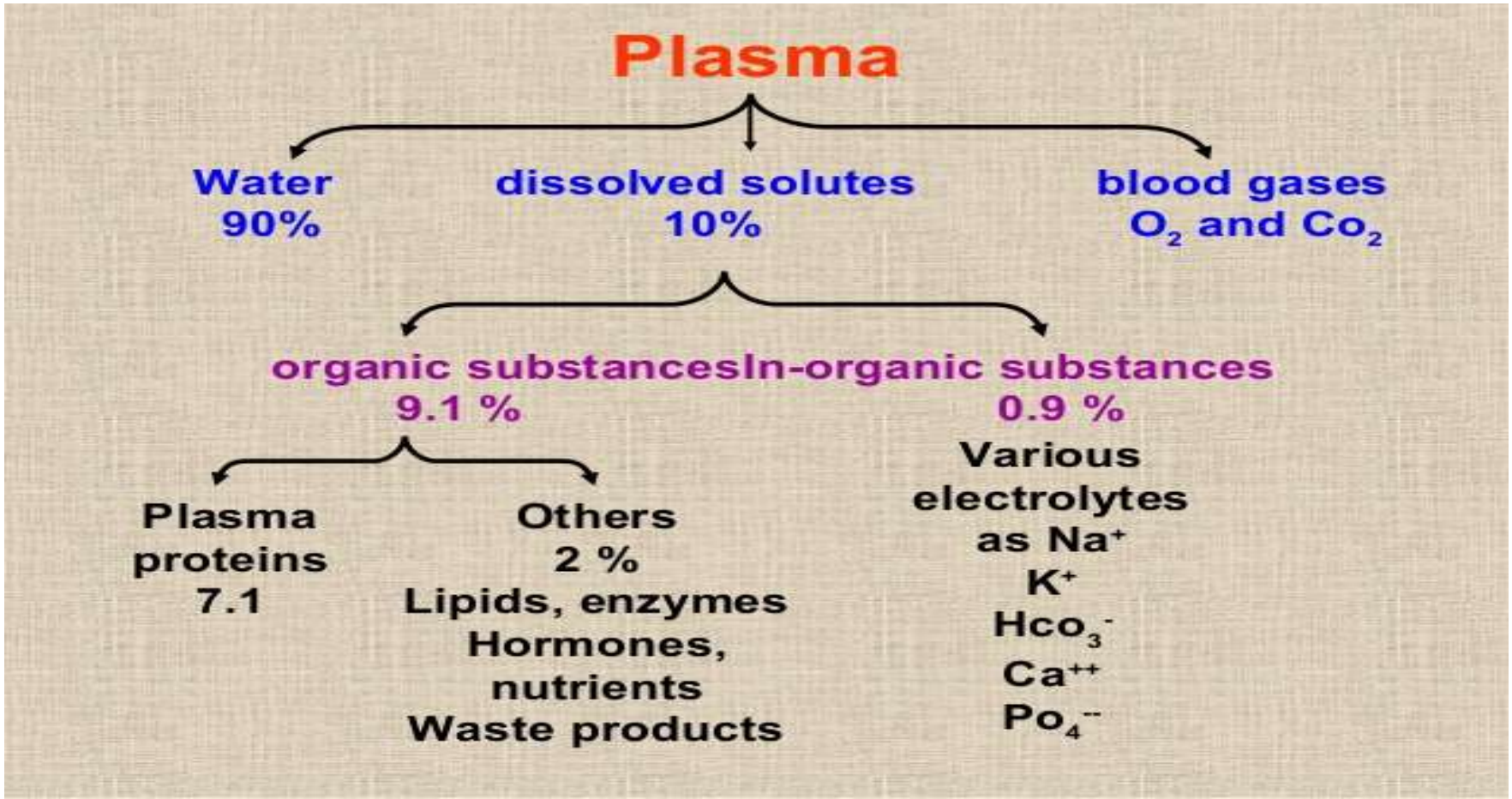
❑ **Serum** consists of plasma without fibrinogen.

PLASMA	SERUM
• anti-coagulants are needed for purification	• anti-coagulants are not needed
• it can be prepared as soon as it has been mixed thoroughly	• 30 minutes delay for a clot formation
• fibrinogen is present	• fibrinogen is absent
• platelets and cells (WBCs) can contaminate the liquid fraction	• cleaner sample, depleted of cells and cell remnants, but latent clotting can lead to fibrin formation
• composition of ions is representative of the circulating blood	• clot retraction elevates potassium level relative to its plasma value
• considered less stable (especially during longer storage)	• considered more stable – the gold standard for biobanking

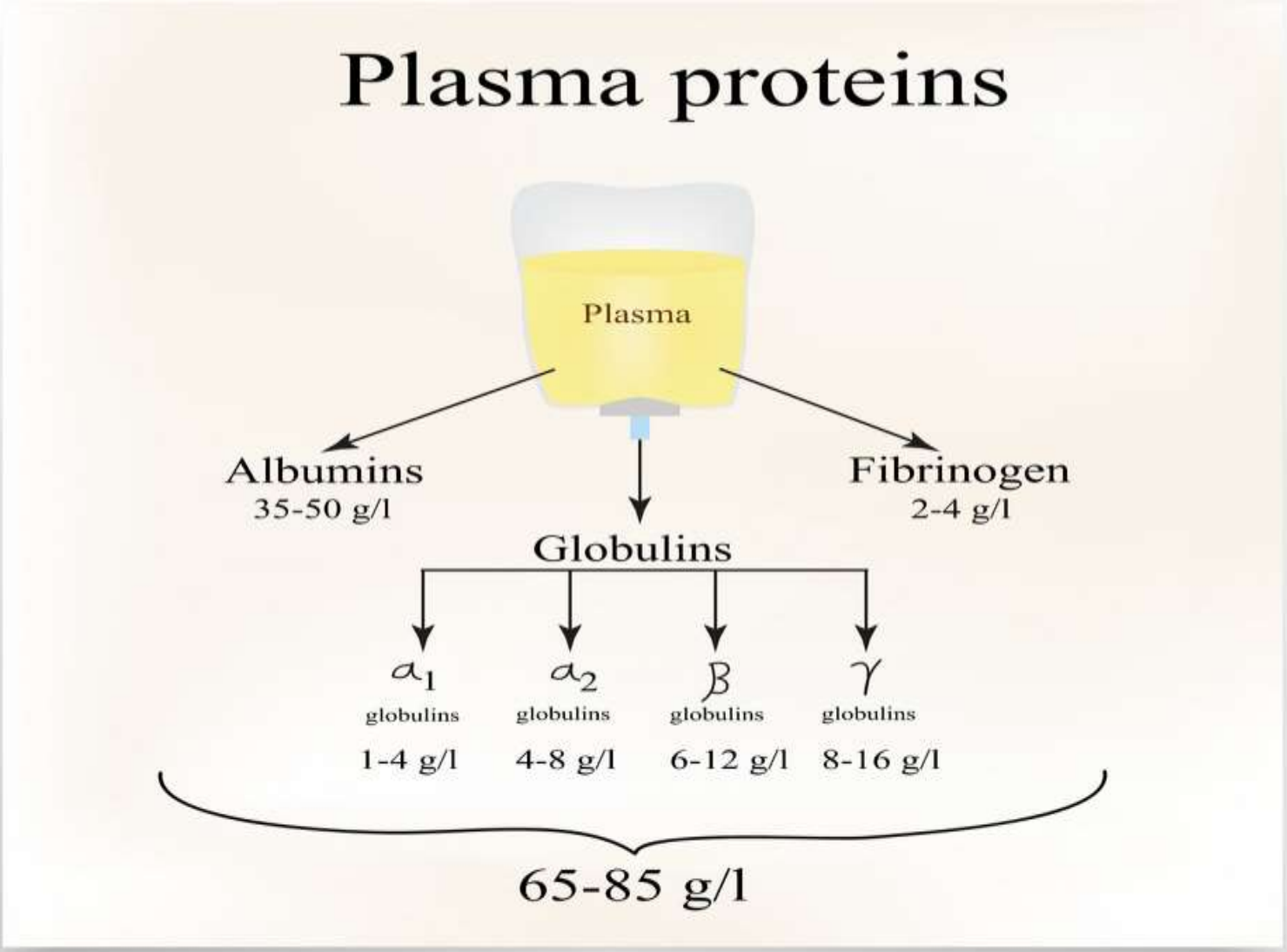




Components of Plasma



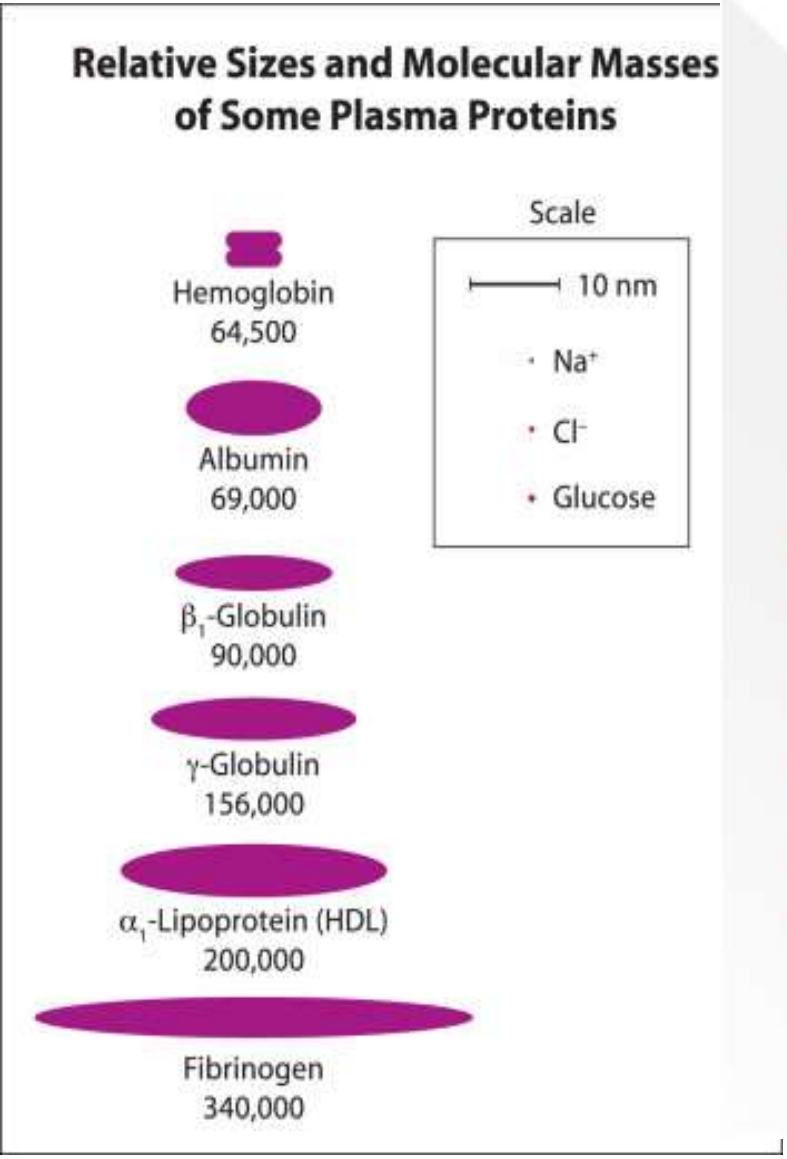
Plasma Proteins
Total concentration: 6-8 gm/dl



Plasma proteins fractions

Fractions	*Rel. amount (%)*	*c (g/l)*
Albumins: albumin pre-albumin (transthyretin)	52 – 58	34 – 50
α_1-globulins: thyroxin-binding globulin, transcortin, α_1-acid glycoprotein, α_1-antitrypsin, α_1-lipoprotein (HDL), α_1-fetoprotein	2,4 – 4,4	2-4
α_2-globulins: haptoglobin, macroglobulin, ceruloplasmin	6,1 – 10,1	5 – 9
β-globulins: transferrin, hemopexin, lipoprotein (LDL), fibrinogen, C-reactive protein, C3 and C4 components of the complement system	8,5 – 14,5	6 – 11
γ-globulins: IgG, IgM, IgA, IgD, IgE	10 – 21	8 – 15

Albumin



Albumin

- The normal range of albumin is 3.5 – 5 g/dl
- Human albumin has a molecular weight of 69,000 & consists of a single polypeptide chain of 585 amino acids with 17 disulfide bonds.
- Albumin is exclusively synthesized by the liver.
- Liver produces about 12 g albumin per day.
- Albumin has an half – life of 20 days.
- **Functions:**
- Maintenance of the osmotic pressure.
- Transport of steroid hormones, free fatty acids, bilirubin, drugs, (sulfonamides, aspirin), Ca^{2+}, Cu^{2+}

B. ***Globulin*** – This is the second largest fraction of the non-nutrient proteins. It accounts for about **36%** of the total. There are three primary types of globulin proteins:

1. Alpha globulin
2. Beta globulin
3. Gamma globulin

Alpha and **Beta** globulins are formed in the **liver** and aid in the transport of lipids and fat soluble vitamins in the blood.

Gamma globulins are produced by "**plasma cells**" located in the **lymph nodes** and are the proteins that function as immunoglobulins or antibodies.

Fibrinogen

- Plasma protein
- 100 – 700 mg/dl
- M.W. 340,000
- Formed in liver
- Liver diseases $\rightarrow \downarrow$fibrinogen $\rightarrow$ bleeding tendency
- Large molecule, does not leak into interstitial fluid which has very poor clotting property
- When it leaks into interstitial space in pathological conditions. Interstitial fluid clots

Albumin Globulin Ratio
(Ratio of Albumin to globulin in Blood)

$$\text{A/G Ratio} = \frac{\text{Albumin Level}}{\text{(Total protein - Albumin Level)}}$$

A-G Ratio

Direct:

- Albumin (A) & Globulins (G) in the serum sample are separated & determined separately

Indirect:

- Serum total protein: Biuret reaction
- Serum albumin: Bromocresol Green (BCG)
- Serum globulin = Serum total protein - Serum albumin

Albumin/Globulin (A/G) Ratio

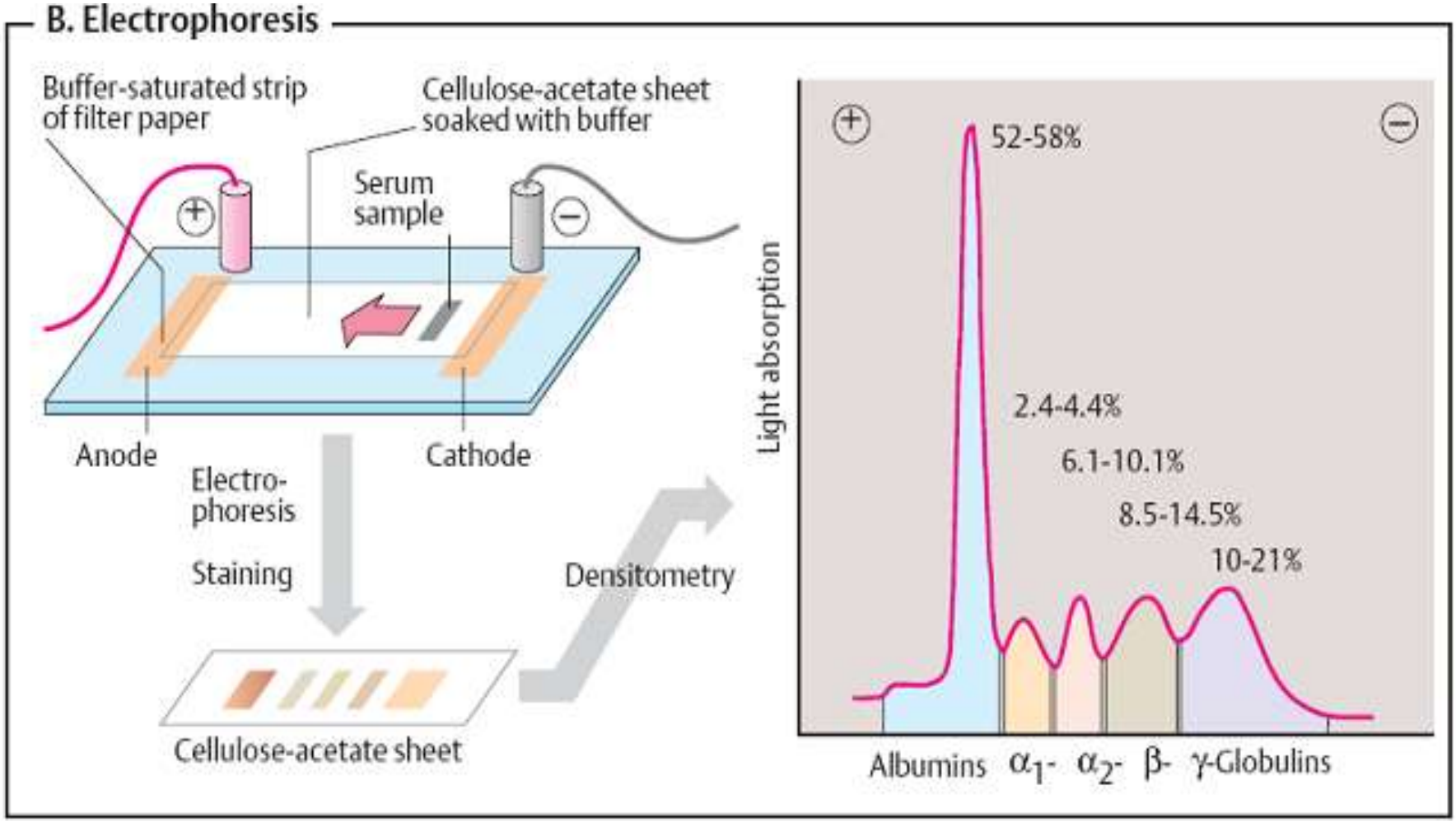
Normal Range: 1.5 to 2.5

- A low A/G ratio may reflect:
  - Overproduction of globulins, such as seen in multiple myeloma or autoimmune diseases, or
  - Underproduction of albumin, such as may occur with cirrhosis, or
  - Selective loss of albumin from the circulation, as may occur with kidney disease (nephrotic syndrome).
- A high A/G ratio suggests:
  - Underproduction of immunoglobulins as may be seen in some genetic deficiencies and in some leukemias.

The normal serum **protein** level is 6 to 8 g/dl. **Albumin** makes up 3.5 to 5.0 g/dl,

Separation /Fractionation of Plasma protein

Migration of charged particle in an electric field is called electrophoresis.
It is method of choice for fractionation of plasma proteins



Method

- A small amount of the serum or plasma **is adsorbed onto a cellulose acetate strip** equilibrated in an alkaline buffer.

- **Positive and negative electrodes are connected** to the ends of the strip and current is applied in order to **attract the proteins towards the positive electrode**. (The more negatively charged is the protein, and the smaller its dimension, the faster it moves. it should be remembered that at alkaline pH protein exist as anion).

- The proteins adsorbed on the strip are **fixed and stained** with a suitable dye and their concentration is measured using a reflectance **spectrometer**. The resulting graph can be used to measure the relative protein concentrations in the serum or plasma.

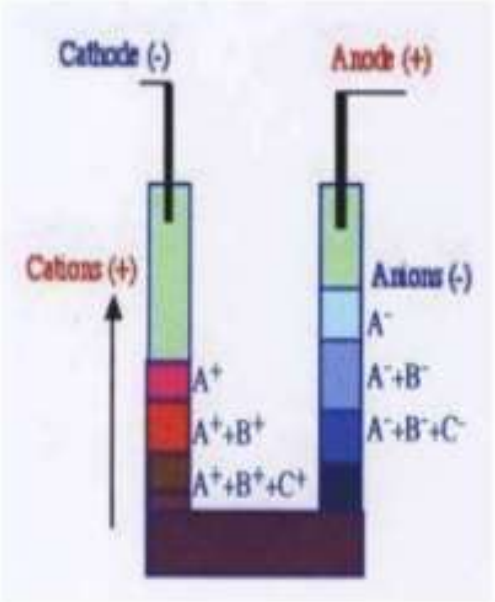
- **Albumin is the fastest moving protein** and forms a sharp band. The alpha1 and alpha2 globulin fractions are less mobile than albumin, followed by the beta globulin fraction. The slowest protein component is constituted by the gamma globulins which essentially corresponds to the antibodies.

- These fractions do not correspond to pure chemical species, and many different proteins co-migrate. Because of this reason quantification of these bands in the 'electropherogram' only gives a very generic information.

Moving boundary/frontal electrophoresis

- Protein solution is injected into a quartz U-tube.
- The arms of the U- tube are filled with buffers.
- When electricity is passed, different species of protein molecules separate in to bands of proteins.
- Discrete zones does not occur.

Tiselius in 1937 introduced this technique.



Physiological variability

- increased concentrations
  - plasma > serum (fibrinogen)
  - stand-up position (by 10-15 %)
  - increased muscle activity (by 12 %)
  - dehydration
- decreased concentrations
  - children, pregnant women
  - after starvation (albumin, transferrin, C3)

Regulation of synthesis

INCREASE	DECREASE
inflammation	liver damage with ↓ parenchymal tissue
hyperthyroidism	nutritional deficit
hypercortisolism	hypothyroidism
Growth hormone ↑	diabetes mellitus
iron deficiency	alcoholism
protein loss	
clonal production of Ig	

FUNCTIONS OF PLASMA PROTEINS:

1. **Transport of substances :**

albumin – fatty acids, bilirubin, calcium, drugs
transferin – iron
cerulplasmin – copper
transcortin – cortisol, cortikosteron
lipoproteins – lipids
haptoglobin – free hemoglobin
thyroxin binding globulin – thyroxin
retinol binding protein – retinol

2. Osmotic regulation:

Plasma proteins are colloidal and non-diffusable and exert a **colloidal osmotic pressure (25 mm of Hg)** which helps to maintain a normal blood volume and a normal water content in the interstitial fluid and the tissues.

Albumin content is most important in regulation of colloidal osmotic or oncotic pressure.

FUNCTIONS OF PLASMA PROTEINS:

3. **Catalytic function (enzymes):**

   E.g., Lipases for removal of lipids from the blood

4. **Protective function:**

   - **Immunoglobulin** combines with foreign antigens and remove them.
   - **Complement system** removes cellular antigens.
   - **Enzyme inhibitors** remove enzymes by forming complexes with them. e.g. α_1-antitrypsin combines with elastase, trypsin and protects the hydrolytic damage of tissues such as lungs.
   - Some proteins increase during **acute phase** and protect the body. E.g. α_1-antitrypsin, α_2-macroglobulins.

FUNCTIONS OF PLASMA PROTEINS:

5. **Blood clotting:**

Many factors are involved in clotting mechanism and prevent loss of excessive amount of blood; e.g. clotting factors IX, VIII, thrombin, fibrinogen etc.

An excess of deficiency leads to a disease; e.g. hemophilia, thrombus formation

6. **Anticoagulant activity (thrombolysis):**

Plasmin breaks down thrombin and dissolves the clot

7. **Buffering capacity:**

Proteins in plasma help to maintain acid-base balance

8. **Antioxidants:**Transferrin,Ferritin,Ceruloplasmin,haptoglobin, Hemopexin (binds heme and transfers it to the liver) act as antioxidants.

FUNCTIONS OF PLASMA PROTEINS:

9.Haptoglobin: **A** plasma glycoprotein that binds to extra-corpuscular hemoglobin in a tight complex and prevent the loss of Hb through kidney. This conserves the iron in body.

10. Maintains the **viscosity** of blood.

11. Act as **reservoir of proteins** in body.

12. **Role in ESR:** Fibrinogen, an acute phase reactant, increases during acute inflammatory conditions and contributes to increase in ESR which is used as prognostic and diagnostic tool.

SYNTHESIS OF PLASMA PROTEINS:

1. **IN EMBRYONIC STAGE:** Mesenchymal cells through secretion and dissolution.
2. **IN ADULTS**: Liver (albumins and fibrinogens, αand β globulins), plasma cell (γ globulins).

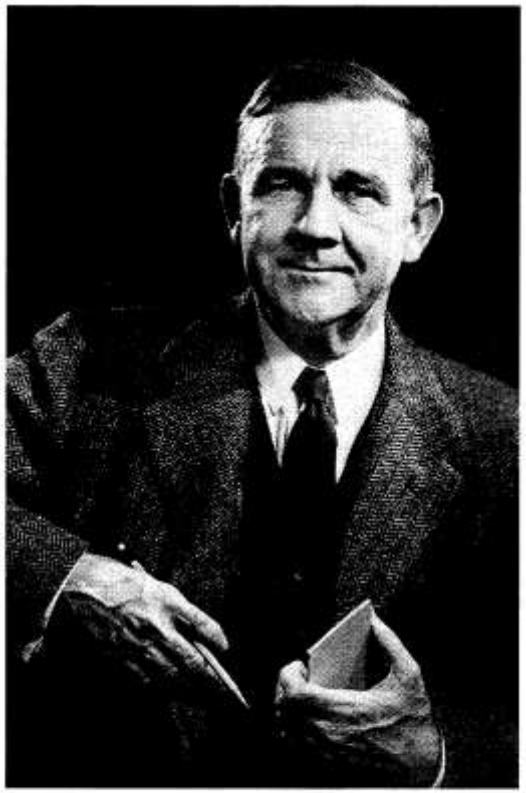
Diagnostic Enzyme Markers in Serum:

Certain tissue cells contain characteristic enzymes which enter the blood only when the cells to which they are confined are **damaged or destroyed**. The presence in the blood of significant quantities of these specific enzymes indicates the probable site of tissue damage.

Serum Enzymes used for diagnostics

Serum Enzyme	Major Diagnostic Use
Aminotransferases	
Aspartate aminotransferase (AST, or SGOT)	Myocardial infarction
Alanine aminotransferase (ALT, or SGPT)	Viral hepatitis
Amylase	Acute pancreatitis
Ceruloplasmin	Hepatolenticular degeneration (Wilson's disease)
Creatine kinase	Muscle disorders and myocardial infarction
γ-Glutamyl transpeptidase	Various liver diseases
Lactate dehydrogenase (isozymes)	Myocardial infarction
Lipase	Acute pancreatitis
Phosphatase, acid	Metastatic carcinoma of the prostate
Phosphatase, alkaline (isozymes)	Various bone disorders, obstructive liver diseases

PLASMAPHERESIS

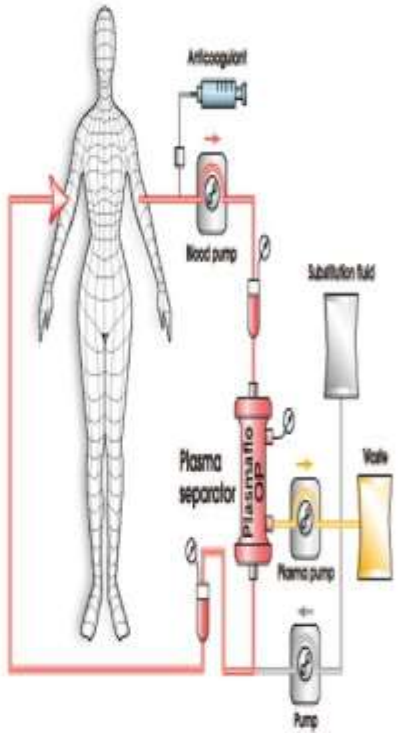


George H Whipple

Factors affecting synthesis of plasma proteins- Plasmapheresis (Whipple's Experiment)

▪Plasmapheresis: a method of removing blood plasma from the body by withdrawing blood, separating it into plasma and cells, and transfusing the cells back into the bloodstream. (Clinical use-It is performed especially to remove antibodies in treating autoimmune conditions).

Plasma Exchange (PE) treatment diagram



Plasmapheresis

Experiment: Dog bled daily . Blood withdrawn.
Cells re-injected daily with ringer-lock solution.

↓

Plasma protein level falls (up to 4 gm%)
Plasma protein level measured.

↓

Several standard protein diet introduced

↓

Plasma protein level measured again

Observation:

- ❑ Dietary protein essential for plasma protein synthesis.
- ❑ Resemblance of food proteins with plasma proteins.
- ❑ Animal-albumin. Plant- globulin
- ❑ Essential amino acid is needed for plasma protein synthesis.
- ❑ Take 14 days for synthesis.

Uses of plasmapheresis:

- Used to remove antibodies from blood.
- Temporary benefit of protecting the tissues from antibodies.
- Required repetitive session of the treatment.
- Diseases:
  - Mysthenia gravis.
  - Thrombocytopenia purpura.
  - Paraproteinimic peripheral neuropathy.
  - Chronic demylenating polyneuropathy.
  - Guillain barre syndrome.
  - Lambert Eaton myasthenic syndrome.

Haematopoiesis

❑ What is haematopoiesis?

- Blood cells die within hours, days or weeks. They have limited life span.
- The process of new blood cell formation is called **haematopoiesis** or haemopoiesis.

❑ Sites of Haematopoiesis:

- **In the embryo:**

  It occurs in **yolk sac, liver, spleen**, thymus, lymph nodes & red bone marrow.

- **In adult:**

  It occurs only in **red marrow of flat bones** like sternum, ribs, skull & pelvis and ends of long bones. The liver, thymus, and spleen may also resume their hematopoietic function, if necessary. This is called **'extramedullary-haematopoiesis'**.

❑ Some Definitions:

- **Erythropoiesis:** It is a process by which human ***erythrocytes*** are produced. It is triggered by erythropoietin.
- **Leucopoiesis:** Process of making ***leukocytes***, stimulated by various colony-stimulating factors (CSFs).
- **Thrombopoiesis:** It is a process of making ***platelets***, begins with the formation of megakaryoblasts**.**

Blood development in vertebrates involves two waves of haematopoiesis

Primitive wave

1. It occurs during **early embryonic development.** It involves an **'erythroid progenitor',** that gives rise to erythrocytes and macrophages during

2. Primary purpose of the primitive wave is **to produce red blood cells** that can facilitate tissue oxygenation as the embryo undergoes rapid growth.

3. The primitive wave is transitory, however, and these **erythroid progenitors are not pluri-potent** and do not have renewal capability.

Definitive Wave

1. It occurs **later in development**, notably at different time points in different species with production of progenitors called **'erythroid-myeloid progenitors' (EMPs).** In human, definitive haematopoiesis occurs in the bone marrow and thymus.

2. It can give rise to **all blood lineages of the adult organism.**

3. It involves Hematopoietic stem cells (HSCs), which are **pluri-potent.**

Common stages of Haematopoiesis (Blood Cell Development)

Haematopoietic stem cells

(Myeloid and Lymphoid stem cells,/ **Pluripotent**)

↓

Progenitor cells

(Colony forming units like CFU-E, CFU-GM etc./**Oligopotent**)

↓

Precursor cells

(Blast cells like erythroblast, myeloblast, lymphoblast etc/**unipotent**)

↓

↓

Formed elements of Blood

(Mature RBC, WBCs, Platelets etc)

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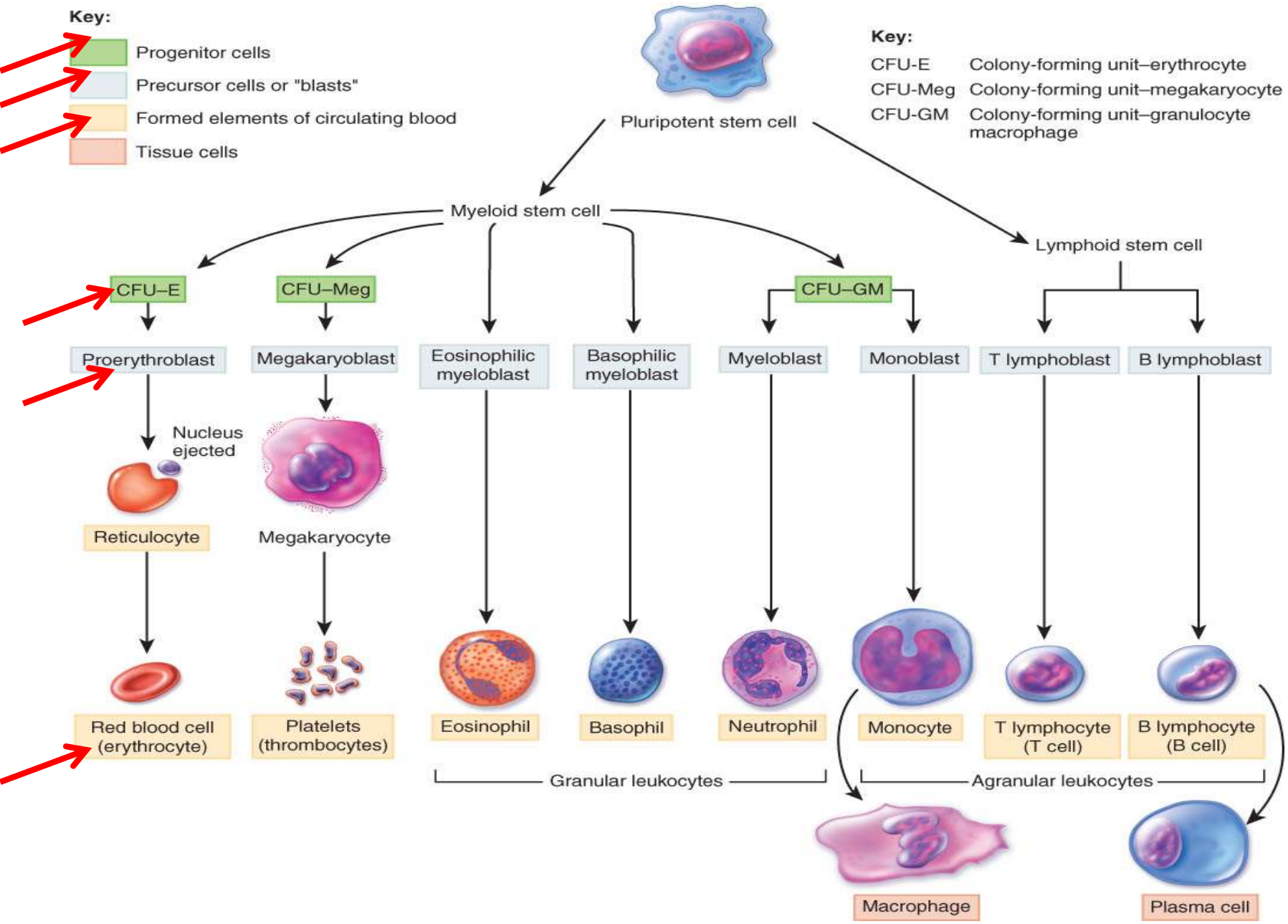
Sometimes further modified in to tissue cells

(Macrophages, plasma cells etc)


Key:
Progenitor cells
Precursor cells or "blasts"
Formed elements of circulating blood
Tissue cells
Pluripotent stem cell
Key:
CFU-E Colony-forming unit–erythrocyte
CFU-Meg Colony-forming unit–megakaryocyte
CFU-GM Colony-forming unit–granulocyte macrophage
Myeloid stem cell
Lymphoid stem cell
CFU–E
CFU–Meg
CFU–GM
Proerythroblast
Megakaryoblast
Eosinophilic myeloblast
Basophilic myeloblast
Myeloblast
Monoblast
T lymphoblast
B lymphoblast
Nucleus ejected
Reticulocyte
Megakaryocyte
Red blood cell (erythrocyte)
Platelets (thrombocytes)
Eosinophil
Basophil
Neutrophil
Monocyte
T lymphocyte (T cell)
B lymphocyte (B cell)
Granular leukocytes
Agranular leukocytes
Macrophage
Plasma cell

Hematopoietic Stem Cells

❑ What is a stem cell?

A stem cell is a special kind of cell in a multi-cellular organism with following few properties:

- They can **proliferate indefinitely** to produce more of similar cells
- They can **differentiate** into various types of cells. i.e., pluripotent.
- They are the **earliest type** of cells in a cell lineage.
- Found both in embryonic stage and adult stage.
- They are characteristically different from progenitor and precursor cells in a lineage.

❑ Haematopoietic stem cells:

- They are stem cells which **can differentiate into various blood cells** (pluripotent)
- They are capable unlimited self-replication **(self-renewal)**. They may have life-long self renewal ability (Long term HSC) or limited self renewal ability (Short term HSC).
- They are found in peripheral blood and bone marrow.
- They **undergo diversification** into myeloid and lymphoid cell lineage via MPP (Multipotent progenitors).
- These two lineages are separable at the progenitor level to produce either **CMP (common myeloid progenitor)** or **CLP (common lymphoid progenitor).**

❑ Progenitor Cells:

1. Progenitor cells **(CMP or CLP)** are can differentiate to **form specific cell types**. **But they can not divide indefinitely.**
2. Both CMPs and CLPs are **oligopotent** progenitor cells.
3. They gradually lose the differentiation potential resulting into commitment of progenitors into a specific type of blood cell.
4. They become **lineage-restricted** and continue to differentiate along cell lines to become precursor cells (Blast cells) and then to final mature blood cells.

Myeloid progenitor cells (CMP)

↓

CMPs differentiate in to RBCs, platelets, granulocytes, mast cells, monocyte- macrophages and dendritic cells.

Lymphoid progenitor cell (CLP)

↓

CLPs differentiate into rise to lymphocytes (T cell and B-cell), NK cells and dendritic cells.

❑ Precursor cells:

- Also called a **blast cells** or simply blasts (for example, erythroblast, lymphoblast etc.) are partially differentiated cells.
- They are usually **unipotent** to have lost most of their stem cell properties. They are incapable of self-renewal.

ERYTHROPIESIS

1. RBCs have limited life span of 120 days. They need to be replaced.

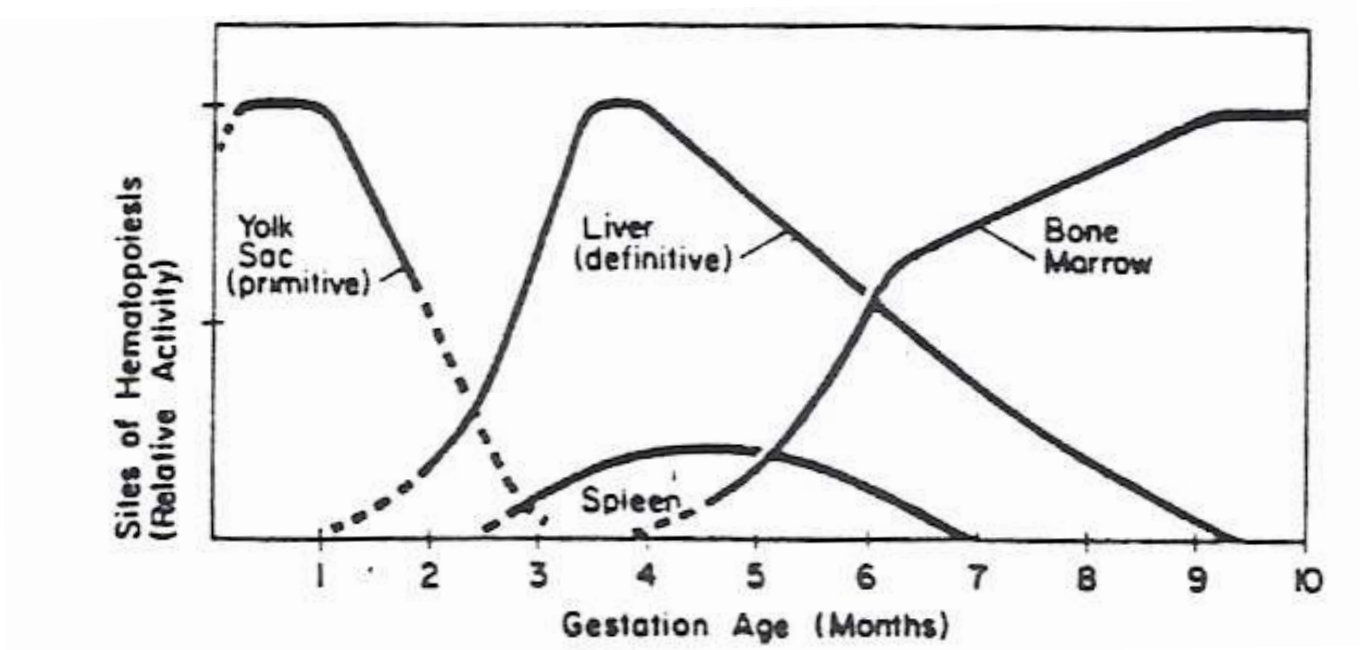
2. Erythropoiesis is a **complex physiological process** by which a fraction of primitive multi-potent HSCs become committed to the red cell lineage and finally become matures erythrocytes. The process involves highly specialised functional differentiation and gene expression.

3. During this process, a large basophilic pro-normoblast with volume of **900 fL** is converted to a small enucleated bi-concave disc with a volume of only **95 fL.**

4. Erythropoiesis is a **tightly regulated** process. A host of growth factors, iron (Fe) and erythropoietin (hormone) are necessary ingredients required for effective erythropoiesis.

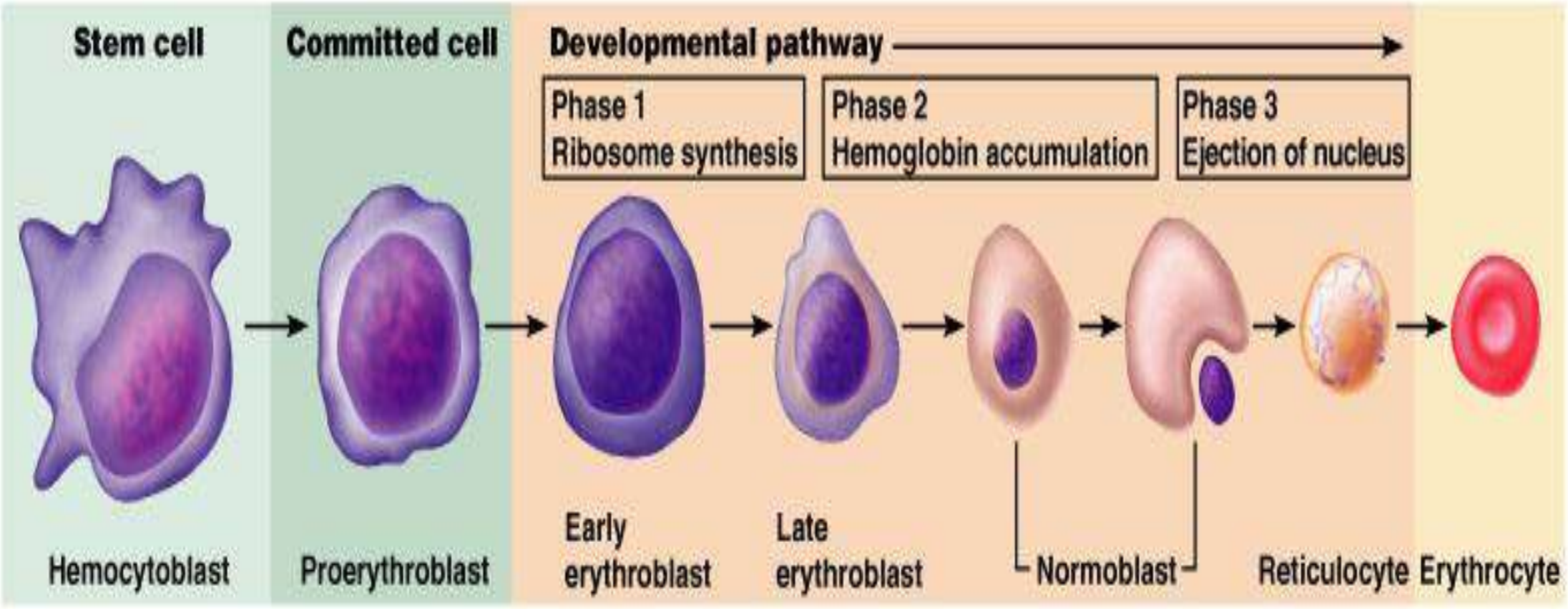
Organs for Primitive and definitive Erythropoiesis

Organ of erythropoiesis	Fetal period	Kinds of blood cells
Yolk sac	2~9 week	RBC
liver	9~24 week	RBC, myelocyte, platelet
spleen	10~24 week	RBC, myelocyte, platelet
Lymph node	8 week~after birth	lymphocyte
Bone marrow	10 week~after birth	RBC, myelocyte, platelet

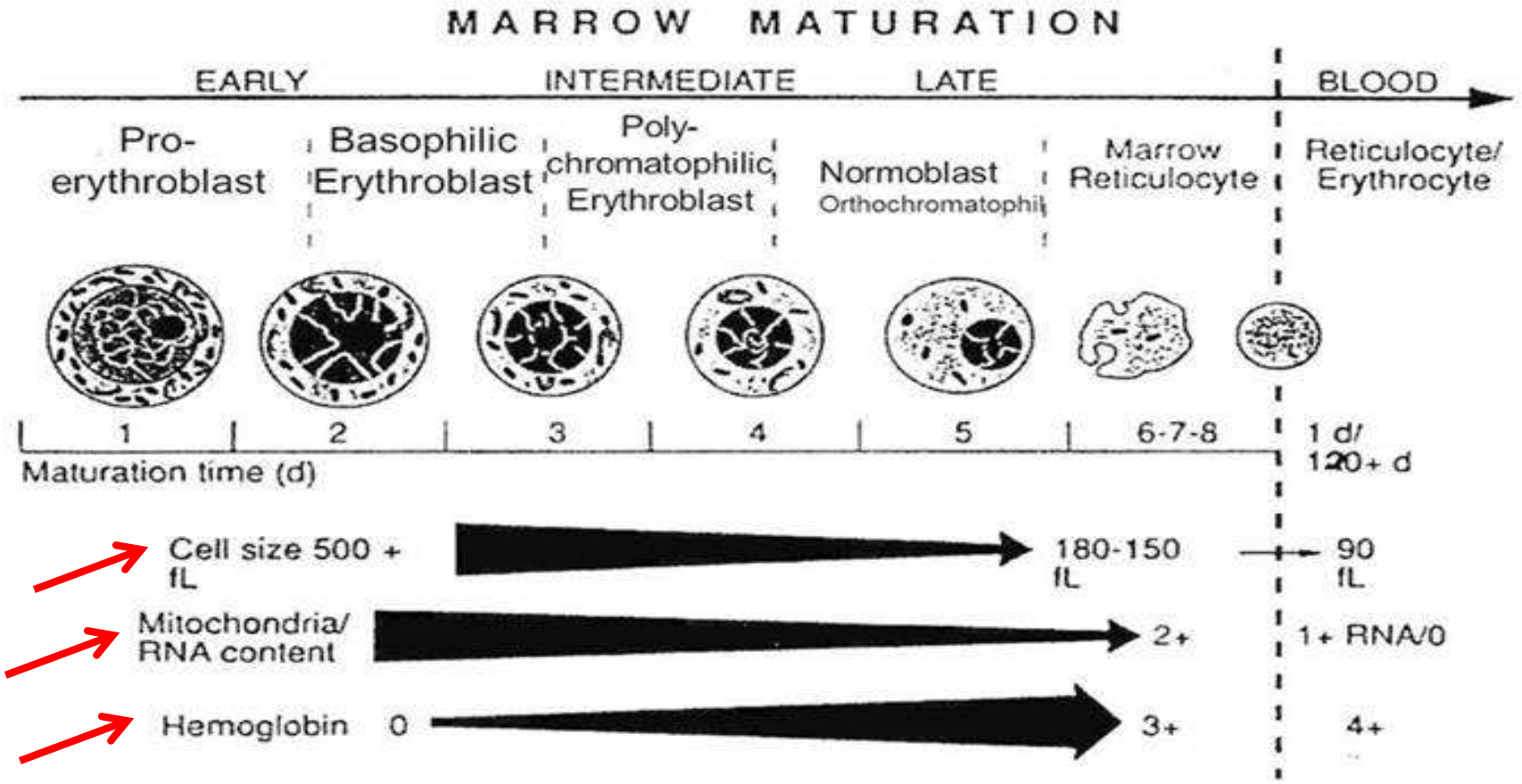


Biological processes involved in Erythropoiesis:

- Overall size of the precursor cell reduces
- Cytoplasm to nucleus ratio (C:N) increases
- Nuclear diameter decreases with condensation of chromatin.
- Colour of cytoplasm changes from blue to pinkish red
- Proliferation of cells occurs at each stage.



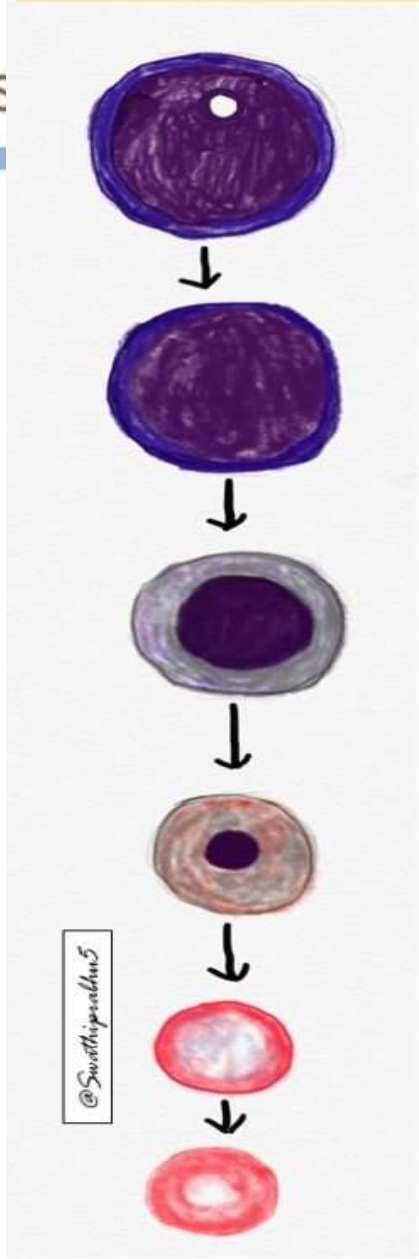
Recognizable Stages of Erythropoiesis



ERYTHROPOIESIS

STAGES OF ERYTHROPOIES

1. Proerythroblast.
2. Early normoblast.
3. Intermediate normoblast.
4. Late normoblast.
5. Reticulocyte.
6. Matured erythrocyte.



Proerythroblast: High nuclear to cytoplasmic ratio, immature nuclear chromatin,1-2 nucleoli, basophilic cytoplasm

Basophilic erythroblast (Early): High nuclear to cytoplasmic ratio, Chromatin condenses, no nucleoli, basophilic cytoplasm

Polychromatophilic erythroblast: Round nucleus with mature (clumped chromatin),no nucleoli, Cytoplasm is grayish in colour due to hemoglobin synthesis occurring at this stage.
Cell division ceases at this stage

Orthochromic erythroblast: Densly pyknotic nucleus with well hemoglobinized cytoplasm (reddish).

Reticulocyte: late erythroblasts extrude nuclei to become reticulocyte. Intermediate position between nucleated RBCs and mature red blood cells. Has bluish tinge due to RNAs.

Mature RBCs: Size of a mature lymphocyte nucleus. Lacks nucleus. Has central pallor 1/3rd the diameter of the cell

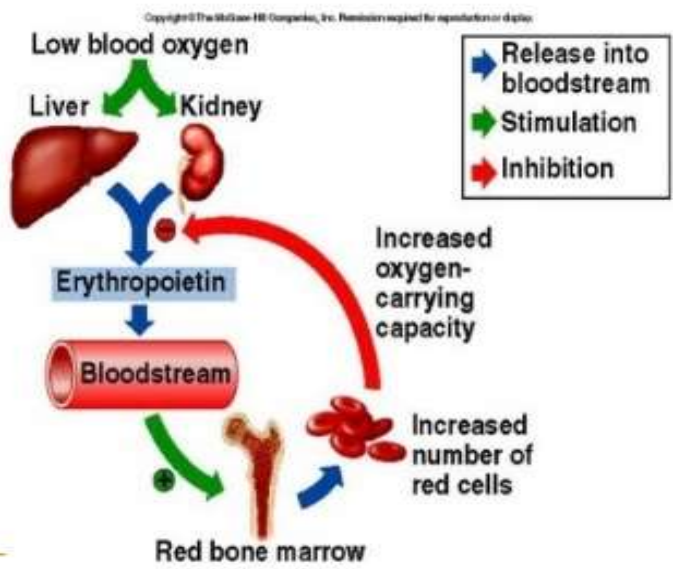
Regulation of Erythropoiesis

1. **Tissue oxygenation**
2. **Vitamins**
3. **Metals**
4. **Proteins**
5. **Hormones**
6. **Other factors/ conditions**

Tissue oxygenation: Anemia, exposure to high altitudes, heart and lung problems stimulate release of erythropoietin which stimulates erythropoiesis

Functions of Erythropoietin

- Erythropoietin increases RBC production in 3 ways:
  - Promotes pronormoblast production
  - Shortens the transition time through the normoblast stage
  - Promotes the early release of reticulocytes.



2. Vitamins: Vitamin B12, Folic acid, B6, Riboflavin, nicotinic acid, biotin, vitamin C and Vitamin E.

- Vitamin B12 important for DNA synthesis and maturation of RBC. Deficiency of B12 leads to failure of nuclear maturation & division, abnormally large & oval shaped RBC, Short life span of RBC, Reduced RBC count & and reduced Hb. The condition is known as **Macrocytic (megaloblastic) anemia.**

- Malabsorption of Vitamin B12 leads to **Pernicious anemia**. VB12 absorption needs intrinsic factor secreted by parietal cells of stomach. VB12 + intrinsic factor are absorbed in the terminal Ileum.

3. Metals: Iron, Copper. Cobalt, Zinc and Manganese.
4. Proteins: For the formation of globin in Hb.
5. Hormones: Testosterone, growth hormone, thyroid hormone, cortisol, ACTH

CONTROL OF ERYTHROPOIESIS:
Vitamin B12 and folic acid

- **Are required for maturation of the RBC**
  - ↑ Synthesis of DNA (synthesis of thymidine triphosphate – DNA building block) → rapid proliferation of the erythroblastic cells
- **Vitamin B12** (cyanocobolamin)
  - Is required for action of **folic acid** on erythropoiesis

Parietal/oxyntic cells of gastric mucosa produce intrinsic factor (IF)

B12 binds with the IF – protection from digestion by GIT secretions

↓

Complex of Vit B12 +IF complex binds to the mucosal receptors in the ileum → transport across mucosa

↓

Release of B12 into the portal blood freed of IF

↓

Binding to the plasma globulins (transcobolamin I, II and III) → red bone marrow or storage in the liver (very large quantities – 3-4 years reserve)

Haemoglobin

- Haemoglobin is a type of **globular protein** present in red blood cells (RBCs), which transports oxygen in our body through blood. It is a **tetrameric protein** and contains the **heme** prosthetic group attached to each subunit. It is a respiratory pigment and helps in transporting oxygen as oxyhaemoglobin from the lungs to different parts of the body.

- **Haemoglobin level:** The haemoglobin level is measured in g/dL of the blood. In a healthy individual, the level ranges from 12 to 20 g/dL. Generally Hb level in males is greater compared to females. The normal level in males is 13.5 to 17.5 g/dL and in females, it is 12 to 15.5 g/dL.

Types of Hemoglobin:

There are several different types of haemoglobin present in the population due to mutation in genes. Not all mutations lead to diseases. The three main types of haemoglobin are:

1. Haemoglobin A ($\alpha 2\beta 2$) – It accounts for 95-98% of haemoglobin in adults. It is composed of two alpha and two beta polypeptide chains.

2. Haemoglobin A2 ($\alpha 2\delta 2$) – It accounts for 2-3% of haemoglobin present in adults. It is composed of two alpha and two delta polypeptide chains.

3. Haemoglobin F ($\alpha 2\gamma 2$) – It is known as fetal haemoglobin. 2-3% of haemoglobin F is present in adults. It is composed of two alpha and two gamma chains. Fetal haemoglobin has more affinity for oxygen compared to HbA.

4. Embryonic Hb exists (Gower I & II) found in embryonic stage.

Structure of hemoglobin:

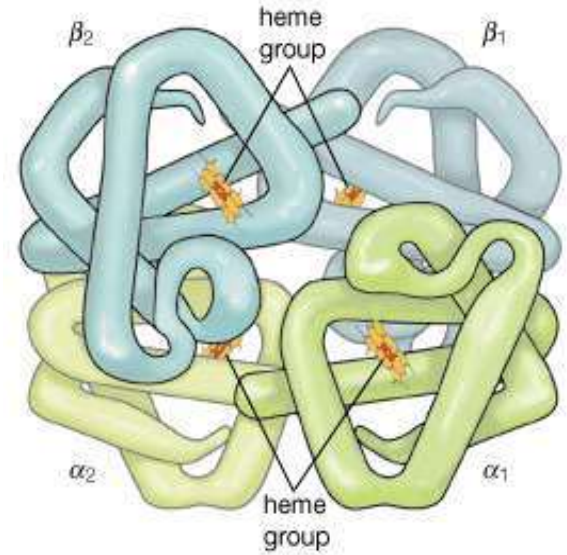
In 1937, Dr. G. S. Adair gave Dr. Max Perutz crystals of horse hemoglobin (personal communication, Max Perutz, 1966). This started Dr. Perutz on the path that led to the elucidation of the structure of hemoglobin. For this endeavor he was awarded **the Nobel Prize in chemistry in 1962.**

Mammalian hemoglobins have molecular weights of about 64,500.

In most vertebrates, Hb is a tetramer, consisting of **two α-subunits ($_{\alpha 1}$ and $_{\alpha 2}$) and two β-subunits ($_{\beta 1}$ and $_{\beta 2}$)** that are structurally similar and about the same size. The main type of haemoglobin in adults is made up of two subunits each of '***α***' and '**β**' polypeptide chains. Each polypeptide chain is linked to a heme prosthetic group.

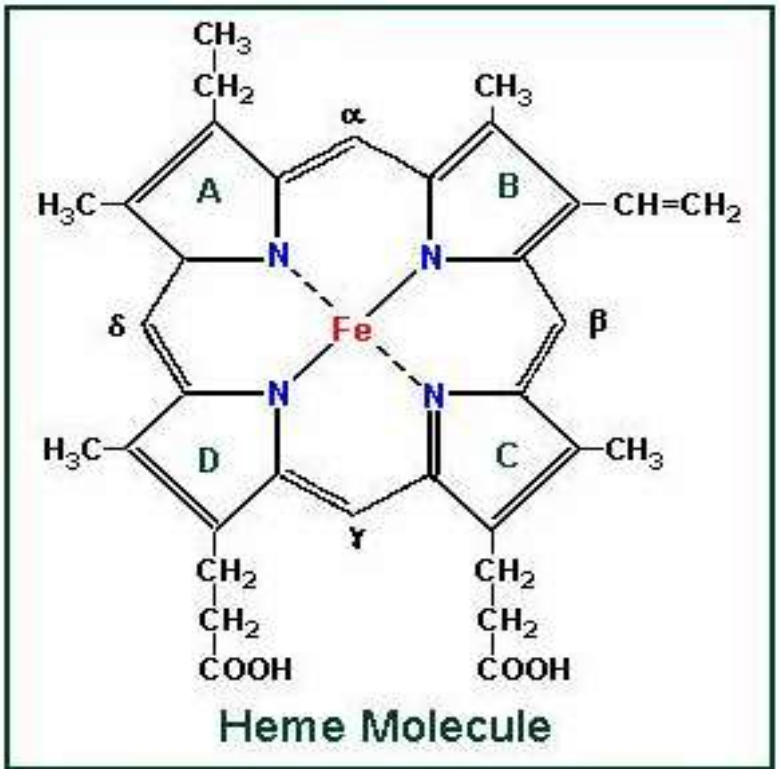
α subunit – It is made up of alpha polypeptide chain having **141 amino acid** residues.

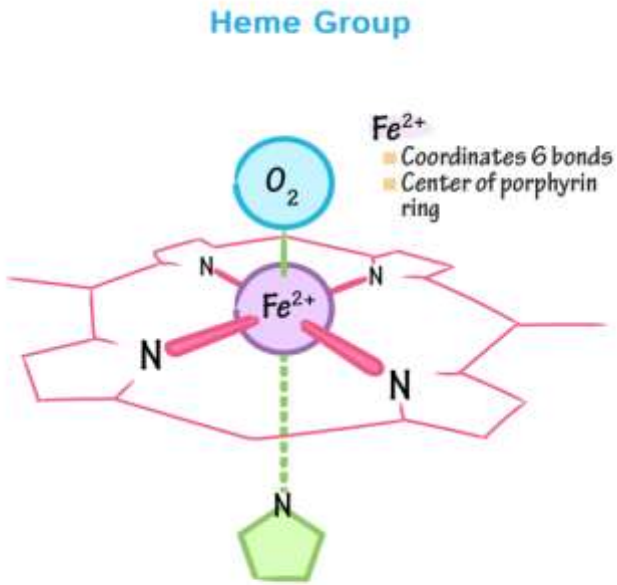
β subunit – It is made up of beta polypeptide chain having **146 amino acid** residues.



Structure of Heme:

1. A heme group consists of an iron (Fe) ion held in a heterocyclic ring, known as a **porphyrin**.
2. This porphyrin ring consists of **four pyrrole molecules** cyclically linked together (by methane bridges) with the iron ion bound in the center.
3. The iron ion may be either in the ferrous Fe^{2+} or in the ferric Fe^{3+} state, but ferrihemoglobin (methemoglobin) (Fe^{3+}) cannot bind oxygen.
4. **Iron is coordinated to four pyrrole nitrogens of protoporphyrin IX,** and to an imidazole nitrogen of a histidine residue from the globin side of the porphyrin. The sixth coordination position is available for binding with oxygen and other small molecules.



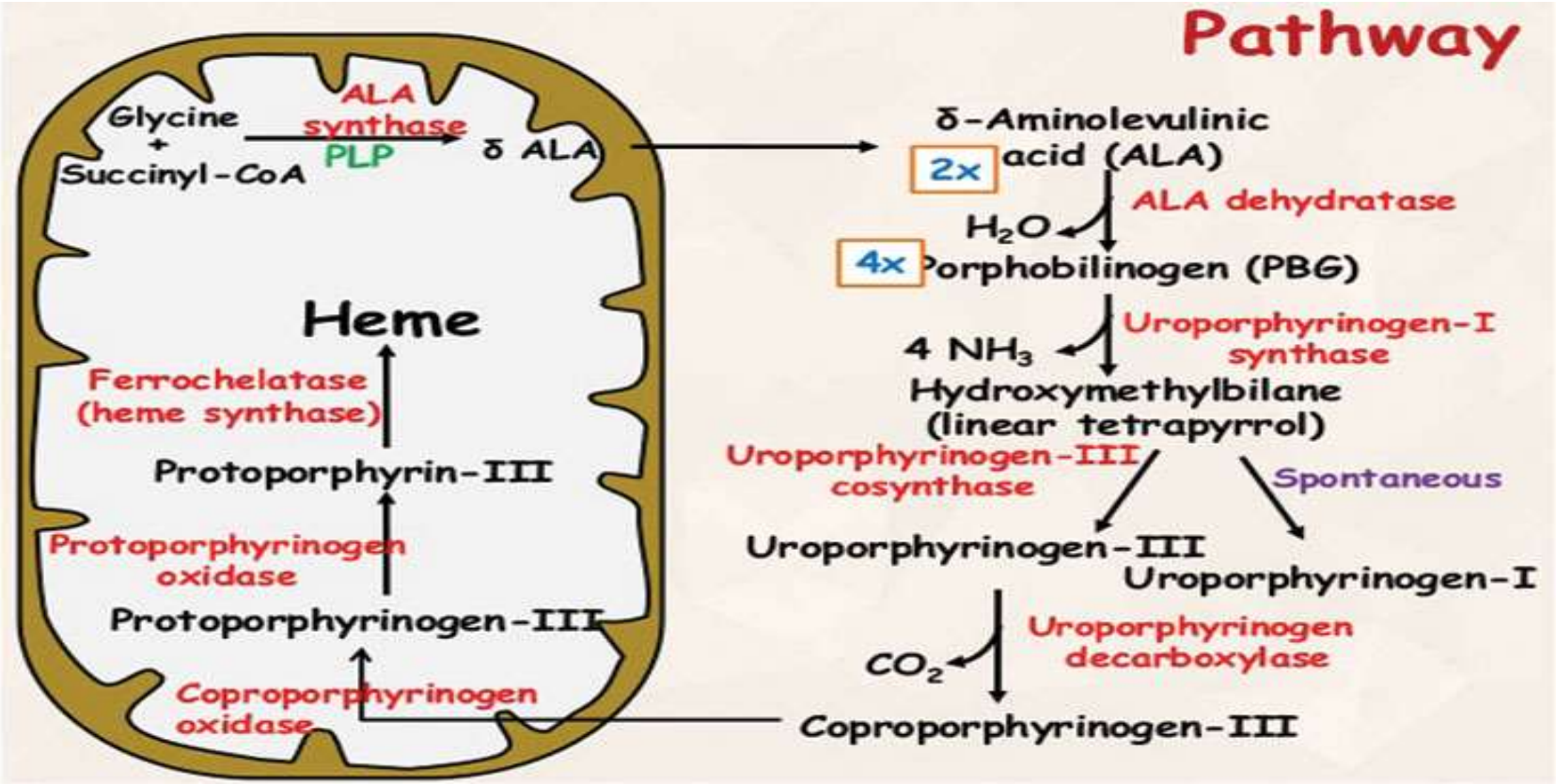


Biosynthesis of Hemoglobin:

❑The two main components of hemoglobin synthesis are globin production and heme synthesis.

❑Heme synthesis occurs in both the cytosol and the mitochondria of erythrocytes. It begins with glycine and succinyl coenzyme A and ends with the production of a protoporphyrin IX ring.

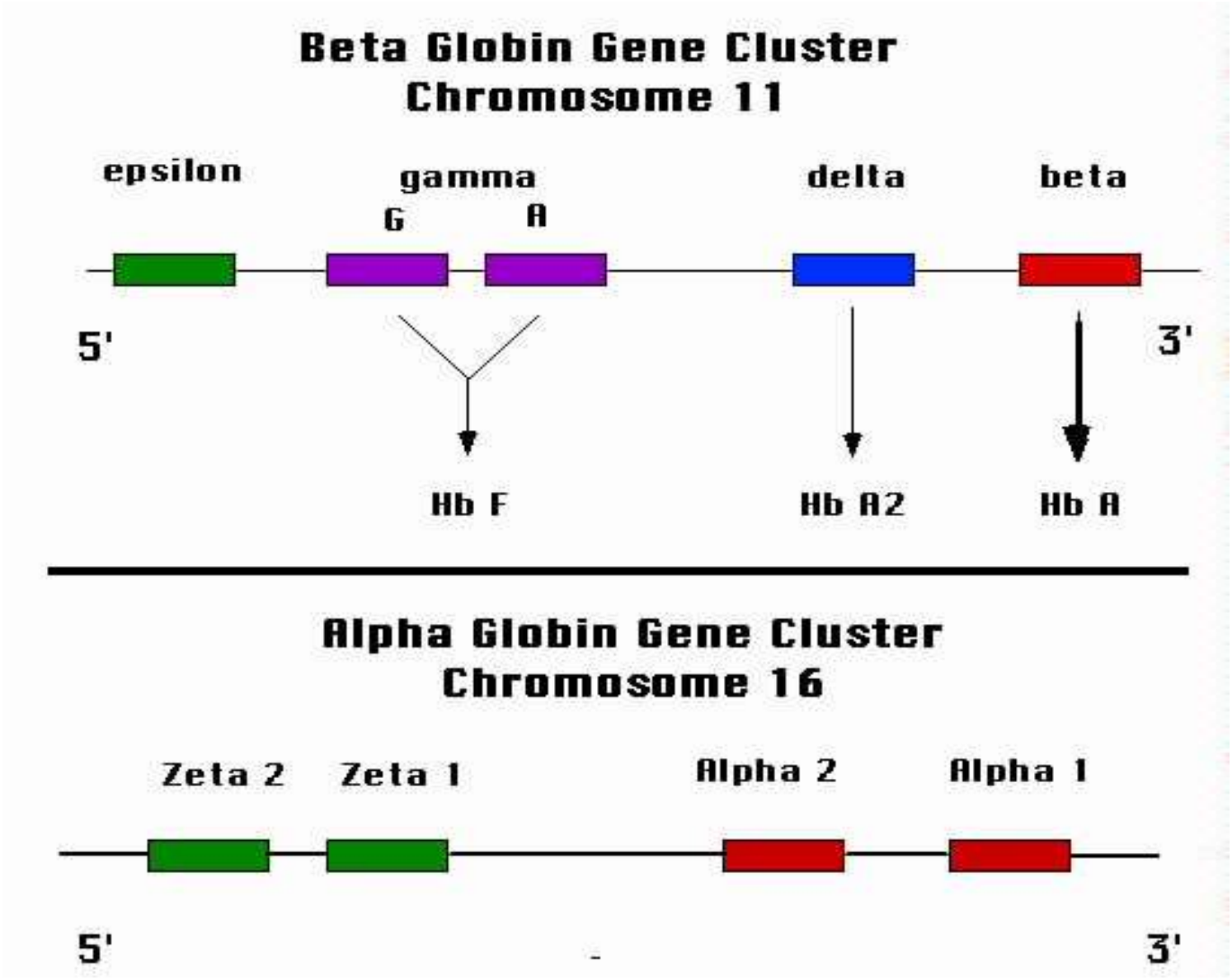
❑Globin chain production occurs in the cytosol of erythrocytes and occurs by genetic transcription and translation. Genes for the alpha chain are on chromosome 16 and genes for the beta chain are on chromosome 11.

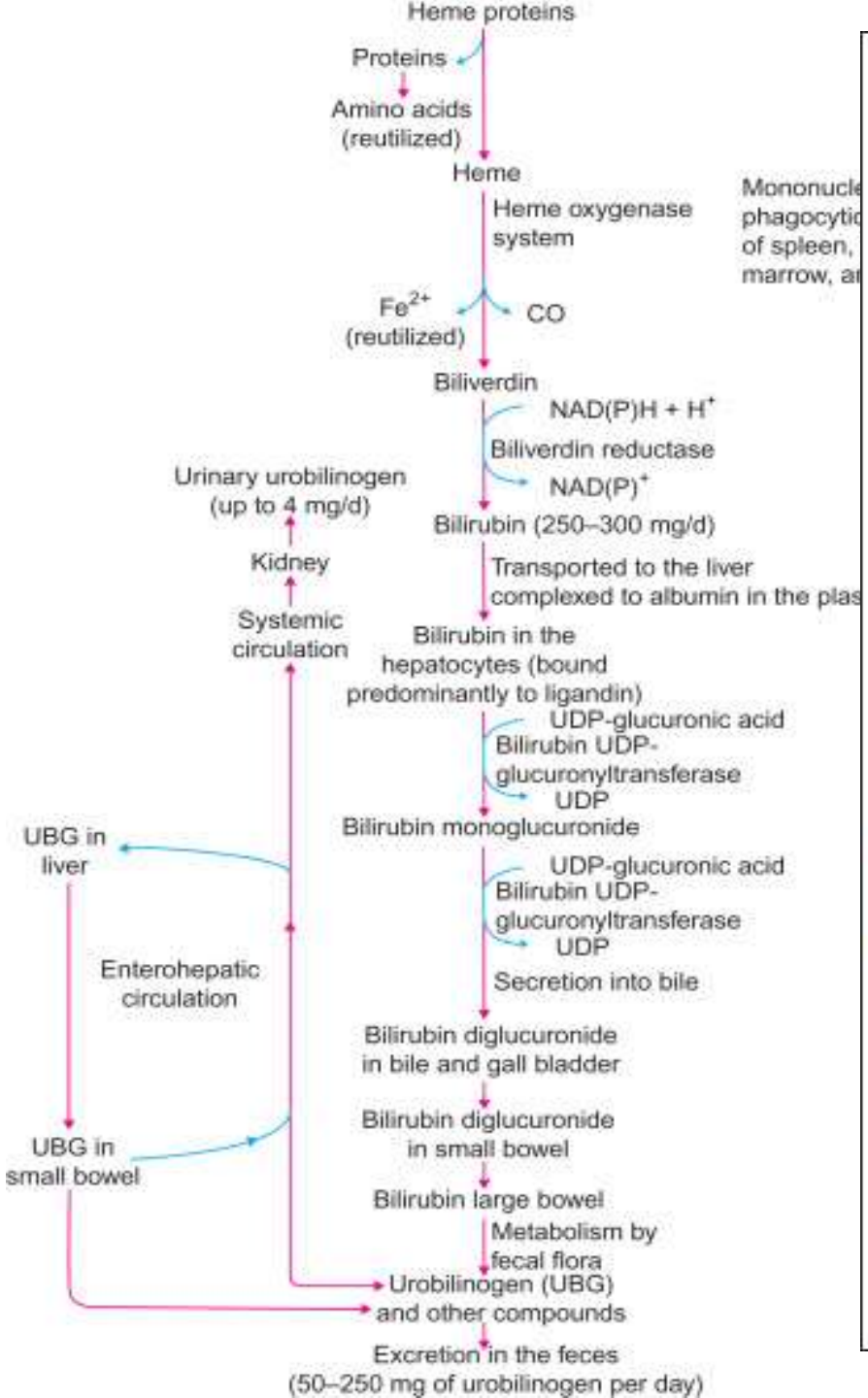


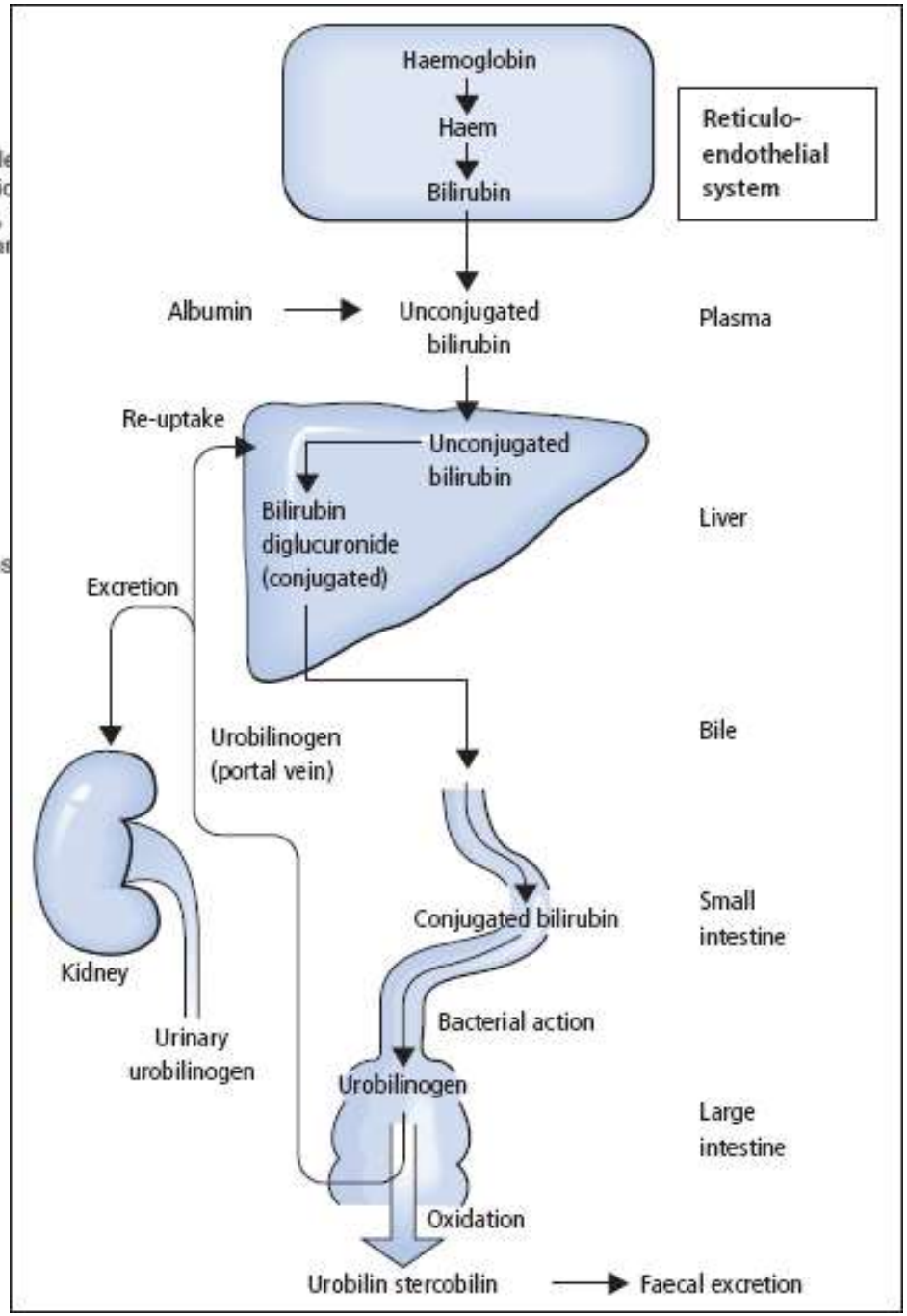
Globin Synthesis:

- The genes that encode the **alpha globin chains** are on **chromosome 16**. Each chromosome 16 has two alpha globin genes. The zeta gene of the alpha globin gene cluster is expressed only during the first few weeks of embryogenesis. Thereafter, the alpha globin genes take over.

- Those that encode the **non-alpha globin chains** are on **chromosome 11**. The genes in the beta globin locus are arranged sequentially from 5' to 3' beginning with the gene expressed in embryonic development (the first 12 weeks after conception; called epsilon). The sequence of the genes is: **epsilon, gamma, delta, and beta**. There are two copies of the gamma gene on each chromosome 11. The others are present in single copies.
- For the beta globin gene cluster, the **epsilon gene** is expressed initially during embryogensis. The **gamma gene** is expressed during fetal development. Around the time of birth, the production of gamma globin declines in concert with a rise in **beta globin** synthesis.
- The **delta gene**, which is located between the gamma and beta genes on chromosome 11 produces a small amount of delta globin in children and adults. The product of the delta globin gene is called hemoglobin A2, and normally comprises **less than 3%** of hemoglobin in adults.


Beta Globin Gene Cluster
Chromosome 11
epsilon
gamma
G
A
delta
beta
5'
3'
Hb F
Hb A2
Hb A
Alpha Globin Gene Cluster
Chromosome 16
Zeta 2
Zeta 1
Alpha 2
Alpha 1
5'
3'

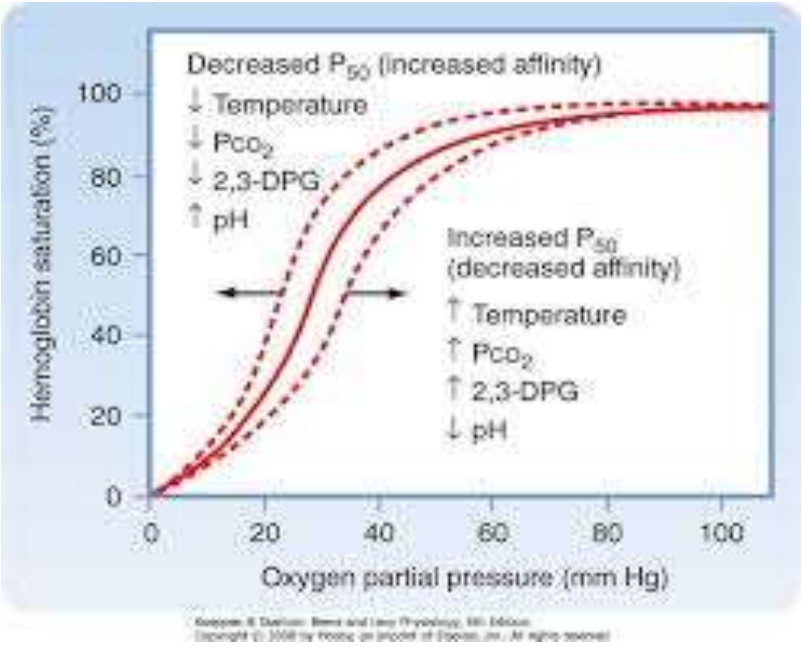

Heme proteins
Proteins
Amino acids (reutilized)
Heme
Heme oxygenase system
Fe^{2+} (reutilized)
CO
Biliverdin
NAD(P)H + H^+
Biliverdin reductase
$NAD(P)^+$
Bilirubin (250–300 mg/d)
Transported to the liver complexed to albumin in the plas
Bilirubin in the hepatocytes (bound predominantly to ligandin)
UDP-glucuronic acid
Bilirubin UDP-glucuronyltransferase
UDP
Bilirubin monoglucuronide
UDP-glucuronic acid
Bilirubin UDP-glucuronyltransferase
UDP
Secretion into bile
Bilirubin diglucuronide in bile and gall bladder
Bilirubin diglucuronide in small bowel
Bilirubin large bowel
Metabolism by fecal flora
Urobilinogen (UBG) and other compounds
Excretion in the feces (50–250 mg of urobilinogen per day)
Urinary urobilinogen (up to 4 mg/d)
Kidney
Systemic circulation
UBG in liver
Enterohepatic circulation
UBG in small bowel


Haemoglobin
Haem
Bilirubin
Reticulo-endothelial system
Albumin
Unconjugated bilirubin
Plasma
Re-uptake
Unconjugated bilirubin
Bilirubin diglucuronide (conjugated)
Liver
Excretion
Urobilinogen (portal vein)
Bile
Kidney
Urinary urobilinogen
Conjugated bilirubin
Small intestine
Bacterial action
Urobilinogen
Large intestine
Oxidation
Urobilin stercobilin
Faecal excretion

Derivatives of hemoglobin

- **Oxyhemoglobin** (oxyHb) = Hb with O_2
- **Deoxyhemoglobin** (deoxyHb) = Hb without O_2
- **Methemoglobin** (metHb) contains Fe^{3+} instead of Fe^{2+} in heme groups
- **Carbonylhemoglobin** (HbCO) – **CO binds to Fe^{2+}** in heme in case of CO poisoning or smoking. CO has 200x higher affinity to Fe^{2+} than O_2.
- **Carbaminohemoglobin** ($HbCO_2$) - CO_2 is non-covalently bound to globin chain of Hb. **$HbCO_2$ transports CO_2 in blood** (about 23%).
- **Glycohemoglobin** (HbA1c) is formed spontaneously by nonenzymatic reaction with Glc. People with DM have more HbA1c than normal (> 7%). Measurement of blood HbA1c is useful to get info about long-term control of glycemia.

Oxygen binds reversibly to haem molecule to produce oxyhemoglobin. **Each hemoglobin can carry 4 oxygen molecules.** The oxygen binding capacity of hemoglobin (BO_2) is defined as the amount of oxygen in milliliters carried by each gram of hemoglobin, also referred as **Hüfner's constant**. Average value of BO_2 is 1.39 ml/gm.



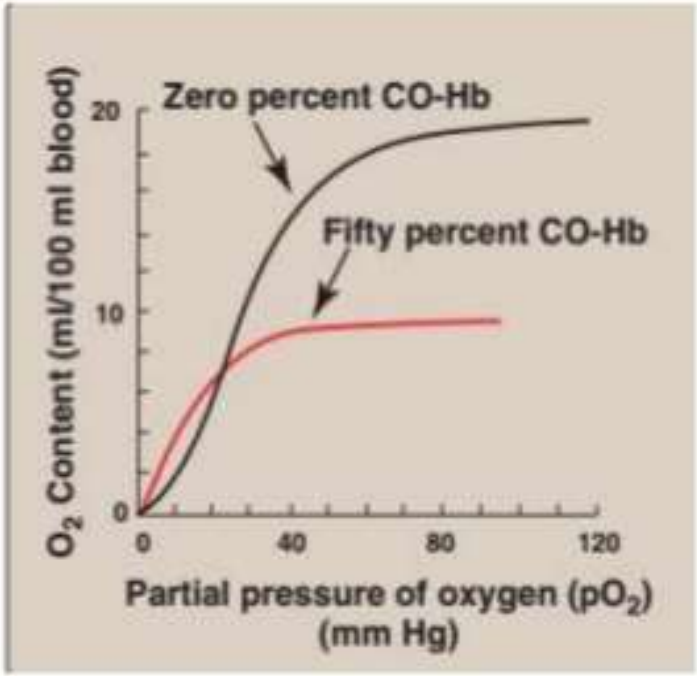
5. Binding of CO:

Carbon monoxide (CO) binds tightly (but reversibly) to the hemoglobin iron, forming carbon monoxyhemoglobin (or carboxyhemoglobin).

When CO binds to one or more of the four heme sites, hemoglobin shifts to the relaxed conformation, causing the remaining heme sites to bind oxygen with high affinity.

This shifts the oxygen dissociation curve to the left, and changes the normal sigmoidal shape toward a hyperbola. As a result, the affected hemoglobin is unable to release oxygen to the tissues

Carbon monoxide poisoning is treated with 100% oxygen at high pressure (hyperbaric oxygen therapy), which facilitates the dissociation of CO from the hemoglobin.



Effect of carbon monoxide on the oxygen affinity of hemoglobin.
CO-Hb = carbon monoxyhemoglobin.

Abnormal Hemoglobin:

Hemoglobin disorders (also known as hemoglobinopathies) are rare blood conditions that affect a person's hemoglobin. They are inherited conditions that may change the shape or amount of red blood cells in the body.

Hemoglobin disorders can be broadly classified into two general categories .

1. Those in which there is a **QUALITATIVE** structural defect in one of the globin chains. Also known as haemoglobinopathy. Eg., Sickle cell anaemia.
2. Those in which there is a **QUANTITATIVE** defect in the production of one of the globin subunits, either total absence or marked reduction. These are called the thalassemia syndromes.

Sickle Cell Anaemia:

○ Sickle hemoglobin **(HbS)** results from an amino acid substitution at the sixth residue of the β-globin subunit: β^6-Glu → Val.

○The basis of sickling is polymerization of deoxy-HbS resulting in the formation of **multistranded fibers that create a gel and change the shape of RBCs from biconcave discs to elongated crescents.** eventually causing **premature destruction of RBCs and a chronic hemolytic anemia.** Sickled RBCs are rigid, increase blood viscosity and obstruct capillary flow, causing **tissue hypoxia** and, if prolonged, cell death, tissue necrosis/infarction, and progressive organ damage. Acute episodes are often called vaso-occlusive pain crises.

THALASSEMIA SYDROME:

The thalassemia syndromes are inherited disorders characterized by absence or markedly decreased accumulation of one of the globin subunits of hemoglobin.

1. α–THALEASSEMIA:

In the alpha (α)-thalassemias, there is absent or decreased production of α-globin subunits. The α-thalassemia syndromes are usually caused by the deletion of one or more α-globin genes and are sub-classified according to the number of α-globin genes that are deleted (or mutated).

- If one α -gene deleted it is called **(α^{+}-thalassemia).**Often called silent carrier.
- If two genes are deleted on the same chromosome, it is called **(α^{0}-thalassemia)**. The clinical phenotype consists of mild hypochromic, microcytic anemia, without hemolysis.
- If three genes deleted it is called **(HbH disease)**.It is a compensated hemolytic anemia that usually does not require treatment by RBC transfusion. The basis of the hemolysis is the excess accumulation of β-globin subunits that self-associate to form β-chain tetramers or HbH.
- If four genes deleted it is called **(hydrops fetalis with Hb Bart's)**. The deletion of all four α-globin genes is usually fatal during late pregnancy or shortly after birth. Hb Bart's is a tetramer of four γ-globin subunits and is ineffective as an oxygen transporter: it has a very high oxygen affinity,

2. β-THALASSEMIA:

In the beta (β)-thalassemias, there is absent or reduced production of β-globin subunits. The β-thalassemias can be subclassified into two types.

- In first type, there is total absence of normal β-globin subunit synthesis or accumulation known as **β^0-thalassemias**,
- the second type, in which some structurally normal β-globin subunits are synthesized, but in markedly decreased amounts. They are known as **β^+-thalassemias**.

With over **200 different mutations** having been described for beta (β)-thalassemias. In general, the mutations causing β-thalassemia are **point mutations affecting a single nucleotide, or a small number of nucleotides in the β-globin gene.**

Common pathophysiology: deficiency of Hb-A tetramers and excess accumulation of free α-subunits incapable of forming hemoglobin tetramers because of deficiency of β-like globin subunits.

In heterozygotes **(β-thalassemia trait or β-thalassemia minor**), there is mild to moderate hypochromic microcytic anemia, without evidence of hemolysis. whereas in homozygotes or compound heterozygotes (**β-thalassemia major)**, there is usually a severe transfusion-dependent hemolytic anemia associated with marked ineffective erythropoiesis resulting in destruction of erythroid precursor cells in the bone marrow.