HEMATOLOGY

Oral Medicine and Oral Surgery Aspects

ESSENTIALS

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Dr. Amar Kumar

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This Book extensively covers physiology of Blood and its implications in Dentistry, specially focused on Oral Medicine and Surgery. This would be a great help for undergraduate and postgraduate students as well as for the Practioners.

Salient Features:

- Easy to understand and student-friendly book
- Hand-drawn illustrations
- Concise text in bullet form for easy review

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DEFINITION 1

DR SURENDRA KUMAR ACHARYA

"The fluid that carries oxygen and other elements to the tissues & carbon dioxide away from the tissues through the heart and vascular system in humans and other animals"

"The fluids that circulates in the principal vascular system of human beings & other vertebrates, in humans consisting of plasma in which the red blood cells, white blood cells and platelets are suspended".(Medical dictionary).

Normal total circulating volume:

- 5600 ml in a 70 kg man
- 6-8 % of body weight

(in man – 52-83 mL/kg; woman – 50-75 mL/kg), 55% of this volume is plasma

- Red, Opaque, Viscous fluid
- Under microscope – consists of fluid, the plasma & a number of different varieties of cells:

1) Red corpuscles – 5,000,000/cumm

2) Platelets – 250000/ml

3) White Corpuscles – 7500/ml

Granulocytes- Neutrophil – 5000, Eosinophil – 3000, Basophil – 30

Agranulocytes- Lymphocytes - 2000, Monocytes – 200

Specific Gravity

- Usually about **1.060 (**erythrocytes – 1.097 plasma – 1.027)
- Greater in man (1.052 to 1.063) – 1.059 than in woman 1.056 (1.050 to 1.058)
- Specific gravity of blood is conditioned mainly by number of **red blood cells**.

Viscosity

- Roughly 4 times more viscid than water
- Measured by Viscometer
- Relative viscosity on an average

  in men is 4.7 (4.3 to 5.3) &

  in woman is 4.4 (3.9 to 4.9)

- The viscosity of plasma or serum is lower than whole blood.
- Viscosity of plasma is in direct proportion to total **protein concentration** & especially to the concentration of **serum globulin**. (have large long & asymmetric molecule)
- Developed species have a constant osmotic pressure, they are called "**homoisomotic**" because the pressure of their blood plasma is maintained at constant level.
- Human plasma freezes at -0.56 degree celcius (-0.54 to -0.59) which corresponds to a 0.3 molar solution, & an osmotic pressure at 6.7 atmospheres.

  ** 75 % of this pressure is due to Nacl.

- Osmotic pressure developed by the plasma proteins is quantitavely much less than that of other plasmatic substances but it is at great importance. (**Starling**)
- Plasma protein develop an osmotic pressure **at 25 to 35 mm** Hg.
- The osmotic pressure of serum **albumin** is much greater than that of serum globulin.
- Serum albumin accounts for **80 %** of the total osmotic pressure developed by the plasma proteins.

The Proportion of Plasma and Corpuscles

- Uncoagulable blood is obtained by adding sodium oxalate to blood , sedimentation by centrifuge forms 3 layers

➢ Upper layer - Pale yellow fluid

➢ Middle Layer - thin, buff – colored (buffy coat) of white cells & Platelets

➢ Lowest layer – deep red & almost entirely at RBCs.

Suspension Stability

If a column of citrated blood is mounted vertically in a narrow graduated tube & allowed to remain undistributed, the cells sink slowly towards the bottom of the column & a layer of clear plasma appears at the top.

Under microscope: Red cells have become adherent to one another, formed ROULEAUX

Westergren technique: the sedimentation rate of normal blood is less than 10 mm in 1 hr, that is a layer of clear plasma less than 10 mm thick appears at the top of the column at blood in one hour.

Color

- Arterial oxygenated blood – **Scarlet**
- Venous less oxygenated blood – **dark red**

  - almost **black** in reflected light

  - **purple red** in transmitted light

Scarlet color - oxyhemoglobin

Dark Red - Hemoglobin or methemoglobin & other hemoglobin derivates in abnormal cases.

-more co2

When seen through skin blood vein has **blue** color.

- **Blood plasma & Serum** - *transparent* but can be cloudy or opalescent or even have a milky aspect, due to fine microscope particles of fat (hemokonia, chylomicrons)

- Blood plasma & serum have a more or less **intense yellow tinge**, mainly due to *bilirubin*.
- **Pink or red** - due to *faulty* technique in collecting the blood. Hemoglobin set free by destruction of few red blood cells.
- **- hemoglobinemia**
- **- hyperlipemia**

Opacity

- Normally opaque, because erythrocytes reflect light.
- When the erythrocytes are dissolved (hemolysis) blood becomes *transparent* (**laked** blood)
- CHEMICAL COMPOSITION

Constituents	*Blood*	*Plasma*	*Erythrocytes*
	Gm./100 cc.		
Water	78.0 (77–85)	90.7	66.0
Total solids	22.0 (18–23)	9.3	34.0
Organic substances	21.2	8.5	33.0
Salts	0.8 (0.6–1)	0.93	0.7
Total protein	18.5	7.0	30.0
Serum albumin	2.5	4.2	
Serum globulin	1.38	2.6	
Fibrinogen	0.25	0.3	
Hemoglobin	15.0 (13–17)	....	34.0
Total nitrogen	3.3	1.2	5.3

DR. SURENDRA KUMAR ACHARYA

	Mg./100 cc.		
Nonprotein N	33.0	25.0	44.0
Urea N	12.0	15.0	11.0
Amino-acid N	5.6	4.5	7.4
Undetermined N	13.0	3.0	25.0
Urea	20.0–35.0	26.0	2.0
Uric acid	2.0	3.0	2.0
Creatinine	1.1	3.3	0.7
Creatine	0.4	0.42	3.1
Ammonia	0.25		
Indican	.....	0.6	
Phenol	1.6	1.7	1.5
Bilirubin	.....	0.6	
Glucose	70.0	80.0	65.0
Lactic acid	6.0	8.0	5.0
Fatty acids	360.0	370.0	340.0
Lecithin	300.0	200.0	400.0
Cholesterol	200.0	180.0	200.0
Ketonic bodies	2.0		
Total phosphorus	45.0	10.0	75.0
Acid-soluble P	30.0	25.0	50.0
Inorganic P	5.0	3.5	6.0
Ester P	24.0	.....	24.0
Lipid P	13.0	7.0	18.0

1. Respiration:

It carries oxygen from the lungs to the tissues & the excess carbon dioxide from the tissues to the lungs.

2. **Nutrition**:

It carries food stuffs absorbed from the intestine or produced within the body to the cells, which use or store them.

3. **Excretion**:

It carries the waste products of cellular metabolism to the excretory organs where they are eliminated.

4. **Immunity**:

It transports leukocytes, antibodies and other protective substances.

5. Hormonal Correlation:

It carries nutritive and hormonal secretions from one organ to another ; these secretions regulate the functions of the organisms.

6. Water Balance of the body:

- Water content of the blood is freely interchangeable with interstitial fluid. This helps in the regulation of water content of the body.

7. Temperature regulation:

In this blood plays a part in several ways:

a) Because of the **high specific heat** of the water, body fluids can store a great quantity of the heat.

b) The blood **circulating rapidly** distributes this heat and tends to keep an even temperature throughout the body.

c) Blood carries heat to the **body surface** where the heat is eliminated by irradiation & evaporation.

d) Blood supplies the water for cutaneous & pulmonary evaporation.

8. Regulation of the osmotic pressure:

-maintained by plasma proteins esp albumin.

9. Regulation of acid –base equilibrium:

-Plasma proteins and hemoglobin act as buffers and help

in the regulation of acid-base balance

10. Regulation of the blood pressure by changes in the blood volume

11. Regulation of ionic equilibrium between cations and anions:

- between monovalent cations (Na + k+) & bivalent cations (ca++,mg++);
- between electrolytes & proteins.

12. STORAGE FUNCTION

- Water and some important substances like proteins,glucose, sodium and potassium are constantly required by the tissues.
- Blood serves as a readymade source for these substances.And, these substances are taken from blood during the conditions like starvation, fluid loss, electrolyte loss, etc.
- **This equilibrium is maintained by:**

a) The **rapidity** with which substances leave the blood when they are in excess or enter the blood when they fall below the normal concentration.

b) Certain mechanisms within the blood itself , such as those which neutralize base and acid.

c) The activity of the tissues and organs.

- The **excess** of a chemical substance of the blood cells is controlled by the following mechanisms:

a) passage into the interstitial fluid , if the substance is diffusible.

b) Storage or fixation in certain cells.

c) Destruction or transformation.

d) Elimination : excess or waste products are eliminated by the lungs (gas , water),the kidney (water ,salts , waste products foreign substances),the intestine (water , salts etc)

When on the contrary ,one of the normal constituents of the blood **diminishes** :

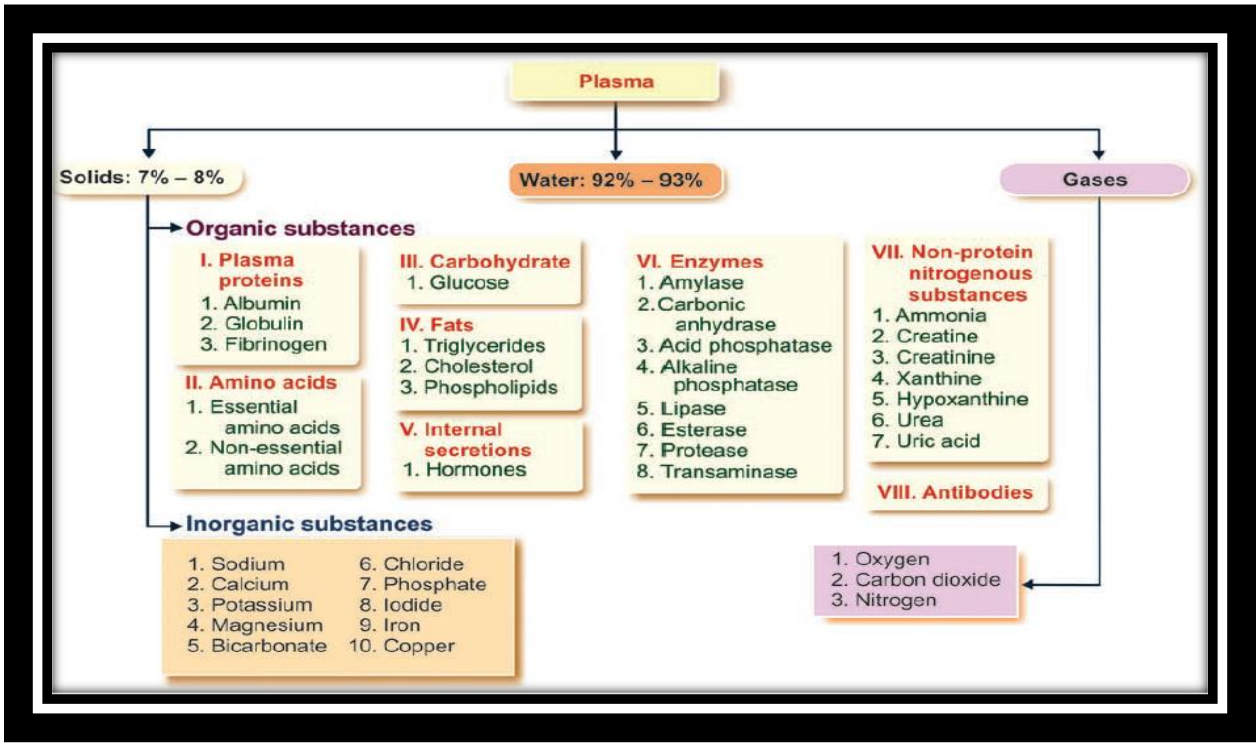
a) The tissue give up substances kept in storage such as water, salts,co2.

b) Certain organs produce necessary substances, such as glucose, proteins and other nutritive components

c) The blood cells by hemopoietic tissues - change in number of erythrocytes are compensated within a few days or weeks by stimulation of greater production of cells.

CHAPTER 6: PLASMA

- The fluid portion of the blood.
- **Pale yellow** made up of 91% water, 9% other
- Normal plasma volume- 5 % of the body weight roughly 3500 ml in 70kg man.
- Plasma clots on standing, remaining fluid only if an anticoagulant is added.
- If whole blood is allowed to clot & clot is removed the remaining fluid is SERUM



INORGANIC COMPONENTS

- Plasma contains 90% water
- Extracellular fluid i.e plasma & intertitial fluid has a prepondance of Cl , Na & Ca.
- The small quantities of Plasmatic K & Mg are of great physiologic significance.
- These elements together equilibrium which is of fundamental importance in the life & function of cells.

- 75 % of the molecules in blood plasma are electrolytes 75 % of the electrolytes is Nacl.
- **Na -** constitutes 92% of the base in plasma while makes up almost all the base in human erythrocyte.
- **Phosphorous** – found in 4 kinds of combination
  - Inorganic (orthophosphate) –less than 1/10 of total
  - Organic – ester phosphorous (glycerophosphates, hexosephosphates)
- **Nucleic Acid Phosphorous** – very little amount

PLASMA PROTEINS 6

DR. ABHISHEK GUPTA

The plasma protein consists of

Albumin

Globulin

Fibrinogen fractions

Globulin fraction – α 1, α 2,β 1 , β 2 and γ.

Total concentration 7.0 gram % (6to 8)

When salts are added proteins are precipitated in the following order:

a/c to molecular wt

Fibrinogen > Euglobulins > pseudoglobulins & serum albumin.

Protein with large molecules are less stable than those with smaller molecules.

- mol. Wt. Conc.

Fibrinogen – 500,000 0.2 -0.4 gm %

Globulins – 150,000 1.5 -3 gm %

Albumin – 69,000 4 -5.6 gm %

Albumin –globulin ratio varies from 1.5 to 2.

- Serum **albumin** can be obtained in crystalized form; it is the plasma protein with the highest osmotic pressure
- **Globulin** are most viscous of all the plasma proteins.
- The antibodies i.e the antitoxin activity of antitoxic serums are found in the **euglobulins**;

  - when these are precipitated & redissolved solution is called **antitoxin**.

- **Fibrinogen** (coagulation)

- changes from a hydrosol to gel called fibrin

- it is unstable & when plasma is heated it coagulates at 56 to 60 degrees centigrade.

- By **electrophoresis** , at an adequate pH the different proteins migrate at different velocities.

➢ The order of velocity for the plasma proteins is the following:

1) Serum albumin 55 %

2) Alpha globulins (α1,α2) 13 %

3) Beta globulins (β1,β2) 14 %

4) Fibrinogen 7%

5) Gamma globulin 11 % (lowest velocity)

i. **OSMOTIC PRESSURE**: Plasma Proteins, owing to their osmotic pressure, are important for retention of water within the capillaries.

ii. **SUSPENSION STABILITY OF THE ERYTHROCYTES & ESR :** This is dependent principally on fibrinogen, less on globulin & much less on albumin concentration.

iii. **IMMUNITY**: by euglobulins, more specifically with gamma globulins & partly with beta globulin.

iv. **ROLE IN PRODUCTION OF TREPHONE SUBSTANCES**
Trephone substances are necessary for nourishment of tissue cells.
These substances are produced by leukocytes from the plasma proteins.

v. **ROLE AS RESERVE PROTEINS**

- During fasting, inadequate food intake or inadequate protein intake, the plasma proteins are utilized by the body tissues as the last source of energy.
- Plasma proteins can be formed when the following are injected i.v :

1) Plasma
2) Serum
3) The product of digestion of the plasma or serum.
4) The product of advanced digestion of hemoglobin or of casein.
5) Or the amino acids indispensable for nitrogen equilibrium (**Elman**, **whipple**)

FIBRINOGEN
- is rapidly regenerated

- it is almost completely restored in 6 to 24 hrs but not if liver is removed.

- there is a small extrahepatic store of fibrinogen.

- There is a lower concentration of proteins & of serum albumin in **children** than in adults.
- During **pregnancy** albumin decreases & fibrinogen & in a lesser degree globulins increase.
- In many Acute infections **fibrinogen** increases.
- In chronic infections & in course of immunization **globulins** increases.
- In Nephrosis albumin decreases & edema occurs.

ORIGIN OF PLASMA PROTEINS

- In embryo- from **mesenchyme cells**.
- Circulating antibodies in the **gamma globulin** fraction of the plasma proteins are synthesised in the **plasma cells**.
- Most of the other plasma proteins are synthesised in reticuloendothelial cells of **liver**.
- Also in spleen, bone marrow and disintegrating blood cells and general tissue cells.
- In normal adults
- Plasma Albumin level – 3.5-5.0 g/dl
- Total exchangeable albumin pool – 4-5 g/kg body wt.
- 38-45% of this albumin is intravascular & much of it is in the skin.

HYPOPROTENEMIA

- Hypoproteinemia is accompanied by lesions in several organs, **delay in the healing of wounds & edema**.
- Plasma protein levels are maintained during starvation until body protein stores are markedly depleted.

Decreases in:

- Prolonged starvation
- malabsorption syndrome due to intestinal diseases such as sprue.
- Also in liver diseases & nephrosis.

- Congenital absence (rarely) eg: afibrinogenemia

NON PROTEIN NITROGEN

- After the plasma proteins have been completely removed, the plasma contains only **crystalloids** nitrogenous compounds.
- The nitrogen of these substances is known as non protein nitrogen
- Its concentration is from **25** to **35** mg per 100cc.
- **Urea** is the principal nonprotein nitrogenous substances; about ½ of the total non-protein nitrogen is in form of urea

Then

- Amino acids > uric acid > creatinine (as creatinine) > undetermined nitrogen

- THREE factors have an influence on the non-protein nitrogen.

1) Deficient renal excretion

2) Excess production

3) Increased fixation in the tissues.

- **Increases in following cases**

- Severe dehydration
- Shock
- Acute Adrenal insufficiency
- Hemorrhage into digestive tract
- After the ingestion of hemoglobin
- Some acute infectious diseases.

- **Decreases**

-only occasionally at the end of **pregnancy** & more markedly a few hours after injecting the growth promoting extract of the anterior hypophysis.

- Data could be expressed in weight but it is usually given in volume.
- Total blood volume
- Plasma volume &
- Erythrocyte volume.

DIRECT METHODS

Welker's method – still used as a standard of comparison when studying new methods

Subject to certain errors

a) Pigment in muscle is extracted

b) Cloudiness of the fluid makes the colorimetric comparison difficult.

c) Some erythrocytes can remain in the clots or in the small capillaries.

INDIRECT METHODS

-usually only the total plasma volume or total red cell volume is measured ; then the plasma cell ratio is determined by means of the hematocrit & thus the total blood volume can be approximately established.

Two types of indirect methods

a) A certain quantity of fluid is injected intravenously & dilution suffered by a normal constituent of blood.eg erythrocytes or proteins is measured.

b) A certain quantity of a foreign substance is introduced into the circulation & once it has been distributed throughout the blood, its concentration is measured- **only used ones now**.

- **Carbon monoxide method**

Based on the fact that CO makes a stable combination with the hemoglobin in the erythrocytes.

- O2 capacity is determined which is same as the CO – maximum quantity of o2 absorbed by 100 c.c of blood.

- **Radioactive iron method**

- radioactive iron given to donor (group o)

- Sample drawn & radioactivity measured

- A known quantity of this radioactive blood is injected into the blood stream of subject.

the dilution of the radioactivity is measured & the total blood volume can be calculated.

Dye method

- most frequently used, accurate & reliable method

- measured amount of a colloid dye is injected intravenously & by determining its dilution, the blood volume can be calculated

- **THE NORMAL BLOOD VOLUME**

can be measured in basal state or Physiologic conditions eg: excersise.

To measure in basal state

- fasting

- lying down &

- completely at rest both mentally & physically.

- Fairly constant in same subject
- Varies to some extent by different methods

CO method - 7.1 to 7.5 % of body wt.

Dye method - 8 to 10 % of body wt.

* Blood volume directly proportional to w 0.72 (w = body weight)

* Child has greater blood volume per kg wt of body than adult also man > women

Dye method

8.2 kg – 7.7 litres per 100 kg in man &

7 kg - 6.6 litres per 100 kg in women

- Blood volume is more closely proportional to **body surface** area than to body weight.
- **Plasma volume is more constant than cell volume & varies little in the same subject.

  Avg. per kg is 43 cc in men &

  41 cc in women.

DISTRIBUTION OF BLOOD IN THE BODY : (BAZETT)

❖ Total blood 5.2 liters

Heart – 250 cc,Pulmonary circuit– 1300 cc,Arteries - 550 cc,Capillaries – 300 cc,Major circuit (arteries, capillaries & veins),Veins – 2,250 cc 3,100 cc,Blood reservoirs (liver , spleen)-550 cc.

VARIATIONS AND REGULATION OF THE BLOOD VOLUME

Changes can take place in the **volume** of the whole blood or in that of only of the **blood constituents**

Rowntree's terminology- adopted to describe different alterations in blood volume.

Normovolemia- three state a/c to proportion of erythrocytes & plasma

Hypovolemia- Normocythemic – proportion normal, Polycythemic – erythrocytes proportion increase, Oligocythemic- erythrocytes proportion decrease

PHYSIOLOGIC VARIATIONS

1) **Rapid changes**: retention of erythrocytes in blood stores especially in the spleen or inversely by release of stored erythrocytes by contraction of spleen as in –

- Muscular excersise

- Emotional state

- Anoxia

2) **Slow variations** – increase in formation or destruction of red cells.

3) **Pregnancy** - increase has gradual onset & reaches a maximum of 23 % for whole blood & 25 % for plasma.

4) **After meals or ingestion of fluid-** very small & transitory increase in blood volume Taken up by tissues & eliminated by kidney.

5) An **increase** in **temperature** of environment

- increase erythrocytes due to **spleen contraction**

- increase in total plasma volume due to **reabsorption** of water from the tissue fluids

- cold decreases plasma volume because water passes out into the tissues ,esp the skin ,muscles & liver.

6) Prone to erect position

plasma volume can diminish by as much as 15 % in 30 min.

7) **Exercise**

first an increase in the total blood vol. & the red cell vol.,due to contration of spleen.

afterwards ,water diffuses from the blood plasma into the tissues because of vasodilation.

finally ,sweating cause loss of water & salts ,so plasma volume diminishes even more & hemoconcentration is more marked.

8) **Altitude**

aviation or climbing high mountain

-stimulation of erythrocyte production

PATHOLOGIC-VARIATIONS

1) Hemorrhage

- First a normocythemic hypovolmia

- but water is very rapidly reabsorbed from the tissues,blood is diluted & the plasma volume increases.

- Dilution is greater & last longer in proportion to amount of blood lost.

2) Traumatic & surgical shock , acute adrenal insufficiency & diabetic coma:

- water & salts diffuse from the blood vessels into the tissues

-There is a progressive decrease in plasma volume & hemoconcentration (increase in the concentration of erythrocytes & plasma proteins) – can be fatal

3) Anhydremia & hemoconcentration (Polycythemic hypovolemia)

- intense dehydration due to insufficient intake or excesssive loss of water .

- Diarrhea , polyuria, profuse sweating

- repeated vomiting, high intestinal obstruction etc

- present in cases of severe burning, mainly as a result of the loss of plasma (plasmorrhea) into damaged tissues.

4) **Myxedema-** by thyroid insufficiency

- both plasma & erythrocytes are diminished.

- Certain degree oligocythemia.

5) **Anemia**

- whole volume is only slightly diminished

- the decrease in erythrocyte volume is compensated in part by a moderate increase in plasma volume (oligocythemic hypovolemia)

6) **Chronic Nephritis**

- Blood volume may be normal
- Oligocythemia (erythrocyte decreased)

7) **Obesity**

- The blood volume per kg is reduced
- But regard to body surface it is normal

8) **Polycythemia vera**

- increase in whole blood volume

- erythrocyte volume increase more than plasma

volume (poylchemic hypervolemia)

9) Chronic Anoxia

- pulmonary or cardiac origin also causes polycythemia.

10) Acute congestive cardiac insufficiency

20-25 % increase in plasma volume

11) **Cirrhosis** of liver – increase in plasma volume

12) **Leukemia** – leukocyte & plasma volume increase

FORMATION OF BLOOD CELLS

- **Fetus**

Early few weeks of embroynic life (**Mesoblastic stage**)

- YOLK SAC

Middle trimester (**Hepatic Stage**)

- Liver (mainly), also by spleen & lymph nodes

Last months/ or gestation & after birth (**Myleoid Stage**)

- exclusively by bone marrow.

- In **children** : (5-6 yrs)

Blood cells are actively produced in marrow cavities of all the bones

- **BY age 20**:

The marrow in the cavities of long bones except for upper humerus & femur has become inactive.

- **Active** cellular marrow is called **Red** Marrow
- **Inactive** marrow - **yellow** marrow

MAJOR PLASMA PROTEINS, CONC. & FUNCTIONS:

s	CONC.	FUNCTIONS
ALBUMIN	4.5 TO 5 g/dl	• Binding & carrier protein for metals ,ions ,fatty acids, amino acids ,bilirubin , enzymes & drugs. • Osmotic regulator , so usde for transfusion to restore blood volume • Acid base balance

GLOBULIN	1.5 to 3 g/dl	• Most viscous , contributes to viscosity of blood. • An important factor in conditioning the work of the heat & in maintaining the blood pressure • The antibodies i.e the antitoxin activity of antitoxic serums are found in the euglobulins . Antibodies for measles ,mumps , diptheria . • Accelerates for roleaux formation - ESR
	CONC	**FUNCTION**
FIBRINOGEN	200 TO 450 mg/dl	• Precusor to fibrin in hemostasis ,changes from a hydrosol to gel • Determines suspension stability of erythrocytes • Accelerates roleax formation
ANTITHROMBIN-III	17 -30 mg /dl	• protease inhibitor of intrinsic coagulation system.
HAPTOGLOBIN	40 to 180 mg /dl	• Binding & transport of cell –free hemoglobin

HYPERVOLEMIA	**HYPOVOLEMIA**
1. HYPERTHYROIDISM	1. HYPOTHYROIDISM
2. HYPERALDOSTERONISM	2. ANEMIA

3. LIVER CIRRHOSIS	3. HEMOLYSIS
4. CONGESTIVE CARDIAC FAILURE	4. FLUID/BLOOD LOSS

FORMATION OF BLOOD CELLS 8

DR. SANDEEP KUMAR

HAEMATOPOIESIS

PLURIPOTENTIAL HEMATOPOIETIC STEM CELLS (HSCs)

- Bone marrow cells that are capable of producing all types of blood cells.
- HSCs are derived from uncommitted , **Totipotent stem cells**

  - form any cell in body
- Best source – **umbilical cord**
- Adults have few ; are more readily obtained from **blastocysts** of embryos.
- differentiate into one or other type of **committed stem cells** (progenitor cells)
- form the various differentiated types of blood cells.
- There **are separate pools of progenitor cells** for megakaryocytes ,lymphocytes ,erythrocytes , eosinophils & basophilis , neutrophils & monocytes arise from a common precursor.
- The bone marrow stem cells are also source of osteoclasts , kuffer cells, mast cells, dentritic cells & langerhans cells.

PRINCIPLES OF REGULATION AND DYSREGULATION (IN THE BLOOD CELL SERIES)

1. Compensatory increases in cell production

- induced by cell loss or increased cell demand

2. Increased cell counts in one series can lead to

- *suppression* of cell production in *another series*.

3. Metabolite deficiency as a pathogenic stimulus

- affects the *erythrocyte series* first and most frequently.

4. Toxic influences on cell production affect *all cell series*.

- toxic chemicals (including alcohol), irradiation, chronic infections, or tumor load.

5. Autoimmune and allergic processes may *selectively affect* a *single cell series*.

- "allergic" agranulocytosis, immunohemolytic anemia, thrombocytopenia.

6. Malignant dedifferentiation

- causing leukemias., Erythroblastosis, polycythemia, essential thrombocythemia.

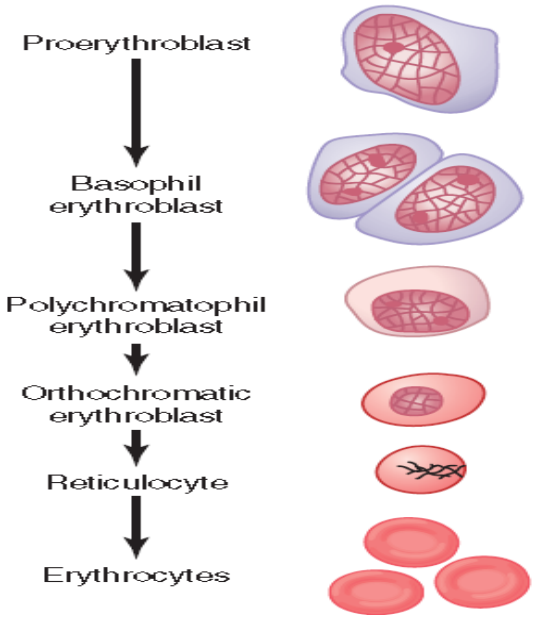
7. All disturbances of bone marrow function

- Quantitative and/or qualitative changes in the composition of *blood cells* or *blood proteins*.

RED BLOOD CELLS

SHAPE, SIZE & VOLUME

- **Biconcave disks.**
- A mean diameter of about **7.8 micrometers.**
- Thickness of **2.5 micrometers** at the thickest point and **1 micrometer** or less in the center.
- The average **volume** of the RBC is **90 to 95 cubic micrometers.**



Concentration of Red Blood Cells in the Blood.

- Men- 5,200,000 (±300,000);
- Women- 4,700,000 (±300,000)

Quantity of Hemoglobin in the Cells.

- 34 grams in each 100 milliliters of cells.
- Erythropoiesis

Stages of Differentiation of Red Blood Cells

1. Proerythroblast (Megaloblast) 20 μ

- The first cell derived from CFU-E.
- **nucleus** and occupies the cell almost completely.
- two or more nucleoli and a reticular network.
- does not contain hemoglobin.

2. *Early Normoblast (basophil erythroblast)* 15 μ.

- **nucleoli** disappear.
- **Condensation** of **chromatin** network occurs.
- Cytoplasm is basophilic**.**
- **Stages of Differentiation of Red Blood Cells**

3. *Intermediate Normoblast (*polychromatic erythroblast)10- 12 μ

- **nucleus** is still present.
- chromatin network shows further condensation.
- **hemoglobin starts appearing**.
- stains with both acidic as well as basic stains (**polychromophilic)**

4. *Late Normoblast (orthochromatic erythroblast).*8 to 10 μ.

- Nucleus: very much condensed chromatin network "**ink-spot"**
- hemoglobin increases.
- almost **acidophilic**.
- final stage:**nucleus disintegrates and disappears**.
- **Stages of Differentiation of Red Blood Cells**

5. *Reticulocyte (*immature RBC)

- slightly larger than matured RBC.
- cytoplasm contains the **reticular network or reticulum**, (remnants of disintegrated organelles).
- **Basophilic**: remnants of **disintegrated Golgi** apparatus, **mitochondria** and other organelles of cytoplasm.
- enter the blood capillaries.

6. *Matured Erythrocyte*

- **Reticular network disappears**
- decreases in size to 7.2 μ diameter.
- without nucleus.

Factors necessary for erythropoiesis:

1. General factors
2. Maturation factors
3. Factors necessary for hemoglobin formation.

GENERAL FACTORS

- i. Erythropoietin
- ii. Thyroxine
- iii. Hemopoietic growth factors
- iv. Vitamins

I.Erythropoietin (hemopoetin or erythrocyte stimulating factor)

- Glycoprotein with **165 amino acids**.
- Major quantity secreted by peritubular capillaries of kidney, also from liver and brain.

Actions :

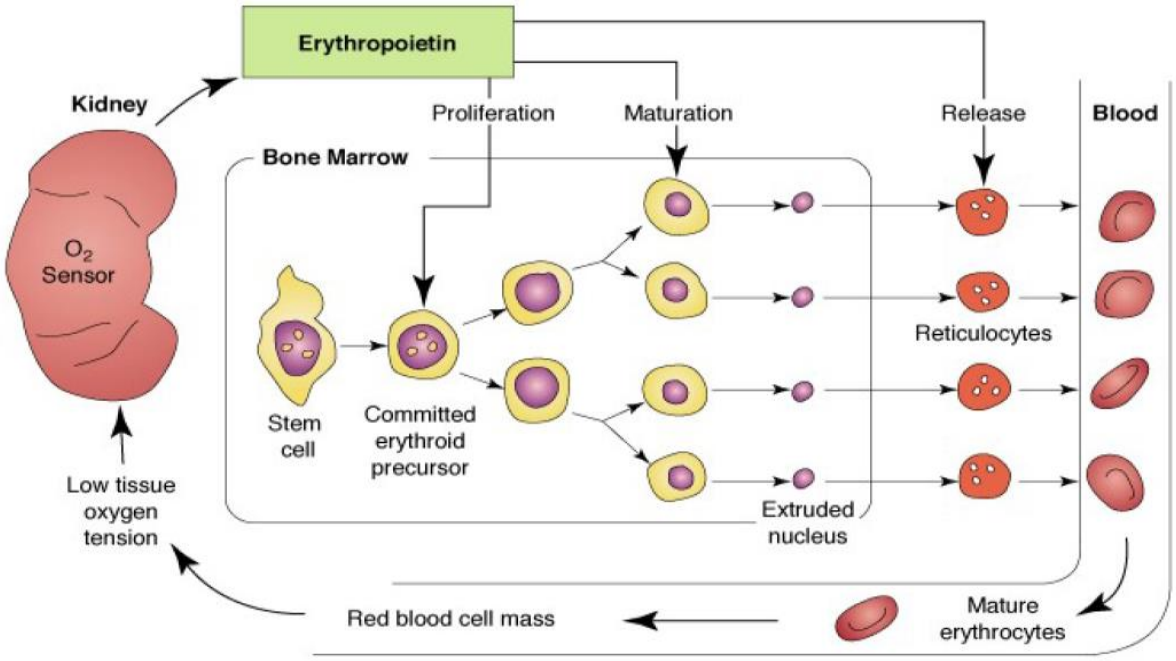
a. CFU-E to proerythroblasts.

b. Proerythroblasts into matured RBCs

c. Release of matured erythrocytes into blood.

FACTORS THAT DECREASE OXYGENATION:

1. low blood volume
2. anemia
3. Low hemoglobin
4. poor blood flow
5. pulmonary diseases



II. Thyroxine

- metabolic hormone,
- accelerates the process of erythropoiesis at many levels.
- So, hyperthyroidism and polycythemia together are common.

III. Hemopoietic Growth Factors

- growth inducers are the **interleukins** and stem cell factor .
- Interleukin (IL-3 , IL-6, IL-11)

IV. Vitamins

a. Vitamin B, C, D, E

2. MATURATION FACTORS

i. Vitamin B12,

ii. Intrinsic factor

iii. Folic acid

- Are required for the formation of **thymidine triphosphate.**
- se in vitamin B12 or folic acid causes abnormal and diminished DNA and, consequently, ***failure of nuclear maturation and cell division***.
- Failing to proliferate rapidly,
- ***macrocytes*** (larger than normal RBCs) are produce , has a flimsy membrane and is often irregular, large, and oval.
- fragility causes them to have a short life, ½ to 1/3.

FACTORS NECESSARY FOR HB

i. **First class proteins and amino acids**

ii. Iron, copper, cobalt, nickel

iii. Vitamins

First class proteins and amino acids:

- Proteins of high biological value.
- Amino acids derived from these proteins are required for the synthesis of - globin.

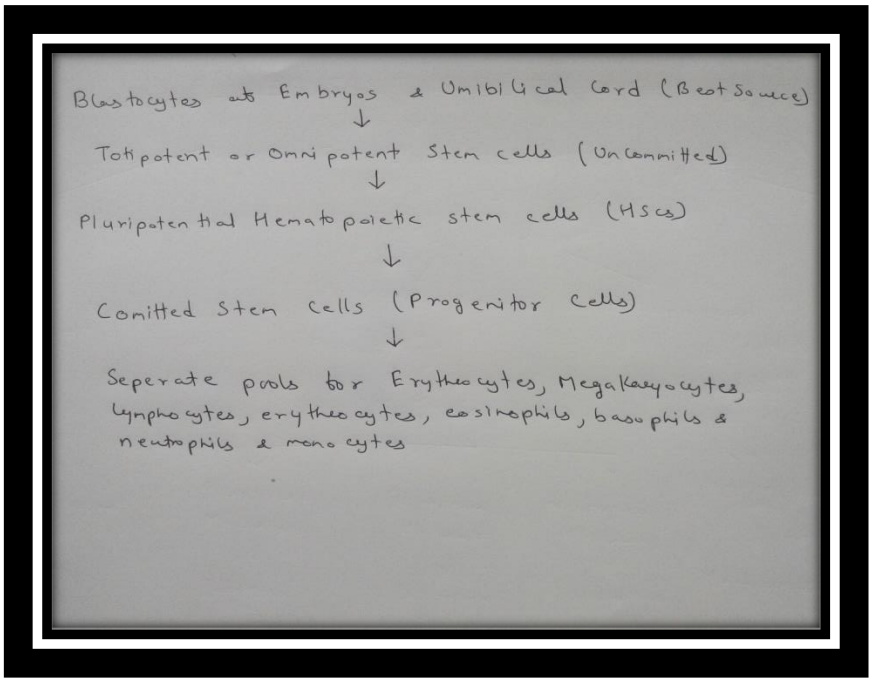
2. Iron*:* for the **formation** of - heme .

Copper*:* for the **absorption** of iron from the gastrointestinal tract.

Cobalt and nickel: These metals are essential for the utilization of iron during hemoglobin formation.

3. Vitamins*:* Vitamin C, riboflavin, nicotinic acid and pyridoxine are also essential for the formation of hemoglobin.

- **HAEMATOPOIESIS**



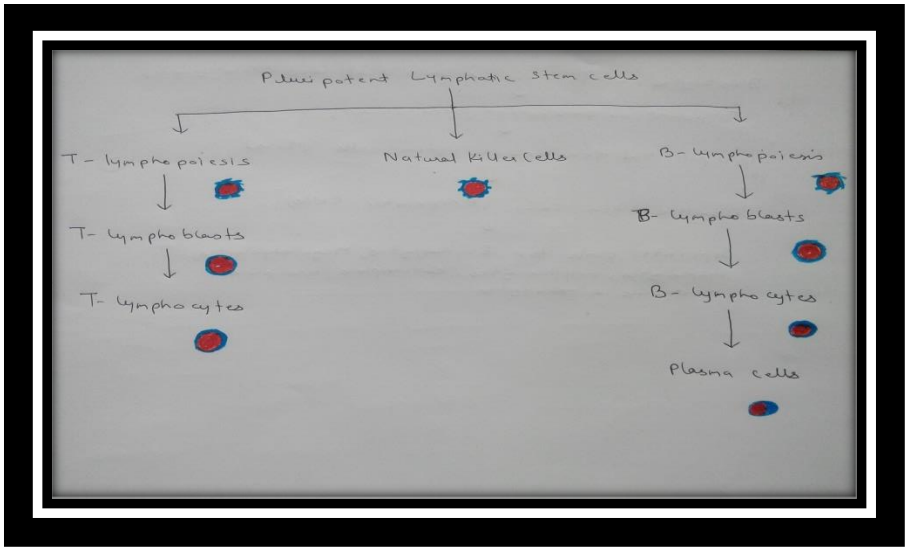
- **HAEMATOPOIESIS**
- Blood forming tissues divided into 2 groups:

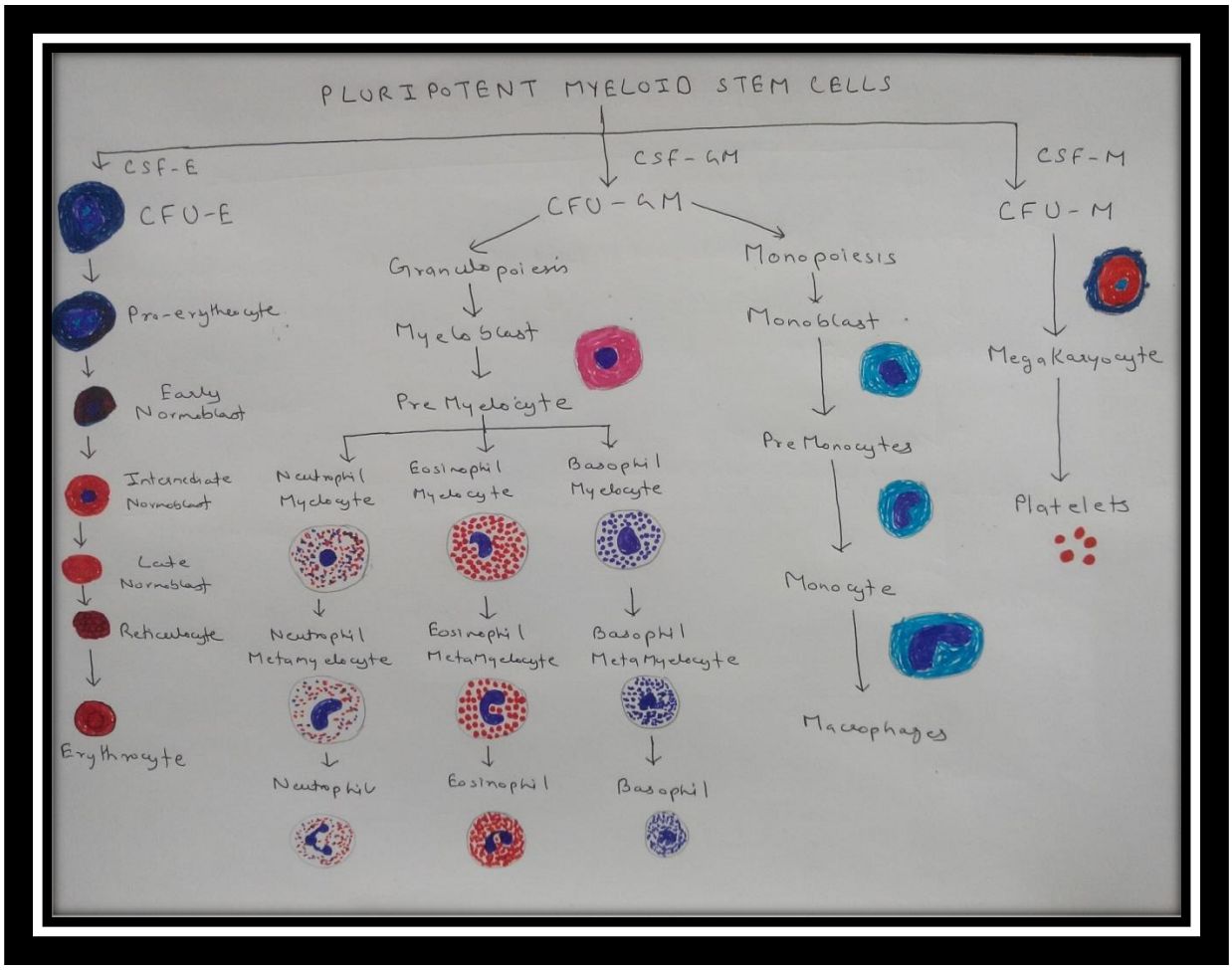
1. MYLEOID TISSUE – *red bone marrow*.

- it produces rbc , granulocytes (neutrophils, eosinophils , basophils),
- monocytes & platelets.

2. LYMPHOID TISSUE – includes *lymph nodes, thymus, & spleen*.

- it produces lymphocytes (T & B).


Pluripotent Lymphatic stem cells
T- lymphopoiesis
Natural Killer Cells
B- lymphopoiesis
T- lymphoblasts
B- lymphoblasts
T- lymphocytes
B- lymphocytes
Plasma cells


PLURIPOTENT MYELOID STEM CELLS
CSF-E
CSF-GM
CSF-M
CFU-E
CFU-GM
CFU-M
Granulopoiesis
Monopoiesis
Pro-erythrocyte
Myeloblast
Monoblast
MegaKaryocyte
Early Normoblast
Pre Myelocyte
Pre Monocytes
Intermediate Normoblast
Neutrophil Myelocyte
Eosinophil Myelocyte
Basophil Myelocyte
Platelets
Late Normoblast
Monocyte
Reticulocyte
Neutrophil Metamyelocyte
Eosinophil MetaMyelocyte
Basophil MetaMyelocyte
Erythrocyte
Macrophages
Neutrophil
Eosinophil
Basophil

- The proliferation and self-renewal of the **pluripotential** cells depend on their production of the protein formed by the ***scl* (stem cell leukemia) gene**.
- The proliferation and maturation of the cells are regulated by glycoprotein **growth factors** or **hormones** that cause cells in one or more of the committed cell lines to **proliferate** and **mature**.
- The factors stimulating the production of committed stem cells include **granulocyte-macrophage CSF (GM-CSF), granulocyte CSF (G-CSF),** and **macrophage CSF (M-CSF).**
- Interleukins **IL-1** and **IL-6** followed by **IL-3** act in sequence to convert pluripotential **uncommitted** stem cells to committed **progenitor** cells.
- IL-3 is also known as **multi-CSF.**
- they activate and sustain mature blood cells.
- Growth and reproduction of the different stem cells are controlled by ***growth inducers***.
- Differentiation of the cells by ***differentiation inducers***.
- Reason for rbc not having nucleus or organelles
- allows the rbc to contain more hemoglobin and, therefore, carry more oxygen molecules.
- also allows the cell to have its distinctive bi-concave shape which aids diffusion.
- No nucleus > no mitochondria > no o2 consumption

STANDARD HB LEVEL IN INDIAN POPULATION

- A/C TO NATIONAL HEALTH PORTAL
- The normal levels are:
- Women: 12.1 to 15.1 gm/dl
- Men: 13.8 to 17.2 gm/dl
- Children: 11 to 16 g/dl
- Pregnant women: 11 to 15.1 g/dl.

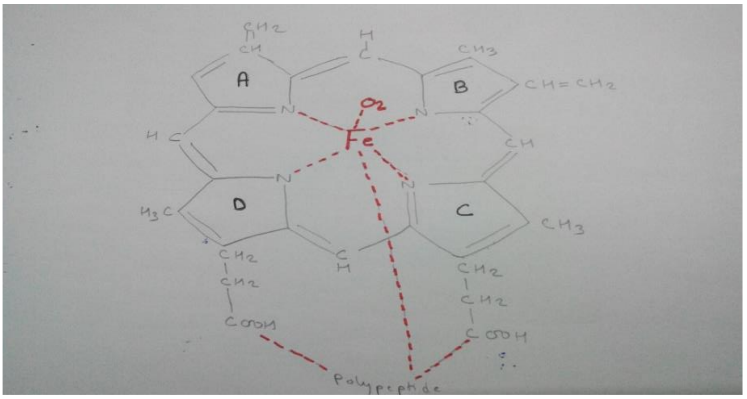
Apollo:

- female: 12.1757

- male: 14.6958
- Kolkota & eastern india

  male : 13.64 +_ 2.6
- Mumbai: 15.4 & 13.4

STRUCTURE OF HEMOGLOBIN:



- a conjugated protein, molecular weight 64,458.
- protein part - **globin** and the iron containing pigment - **heme.**

- **IRON:** ferrous (Fe2+) form, unstable or loose form.
- **PORPHYRIN:** pigment part.
- four pyrrole rings (tetrapyrrole) called, I, II, III & IV, attached by methane (CH4) bridges.
- The iron is attached to 'N' of each pyrrole ring and 'N'of globin molecule.
- **GLOBULIN:** 4 polypeptide chains
- **STRUCTURE OF HEMOGLOBIN**
- **TYPES**

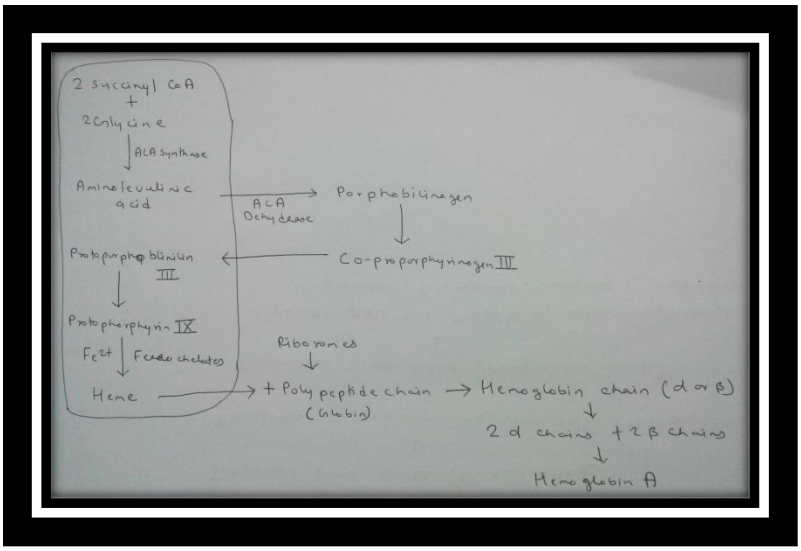
1. **Adult** hemoglobin – **Hb A**, 2 alpha & 2 beta chains,

   - less affinity for oxygen than fetal Hb

2. **Fetal** hemoglobin – **Hb F**, 2 alpha & 2 gamma chains

   - more affinity for oxygen

- Replacement of fetal hemoglobin by adult hemoglobin starts immediately after birth.
- It is completed at about **10th to 12th week** after birth
- **FORMATION:**



- **FUNCTION**

1. **TRANSPORT OF RESPIRATORY GASES:**

- Oxygen & carbondioxide binds loosely with one of the so-called **coordination bonds** of the iron atom.
- easily reversible.
- Affinity of hemoglobin for carbon dioxide is 20 times more than that for oxygen

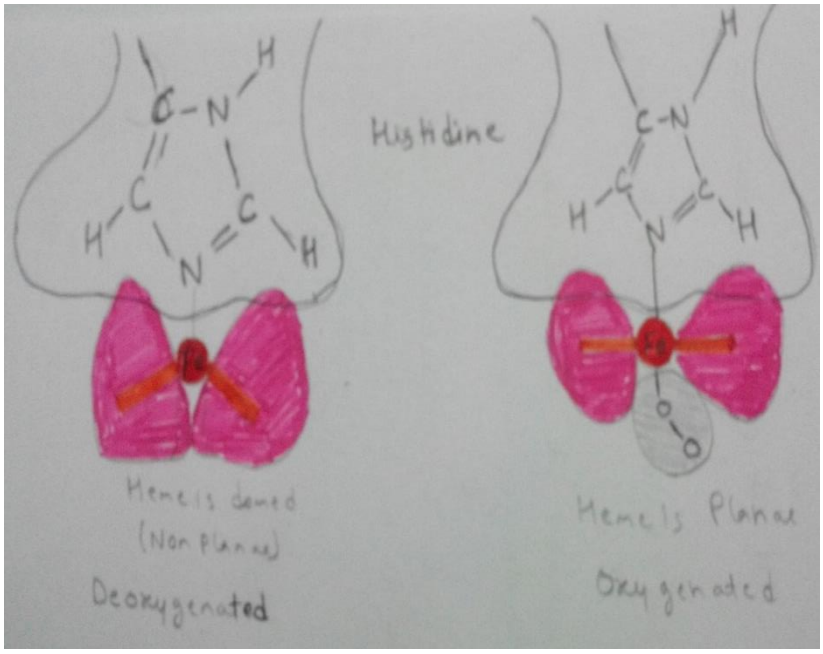
2. BUFFER ACTION

- acts as a buffer and plays an important role in acid-base balance
- Transport of gases

<u>Hemoglobin Combines Reversibly With Oxygen.</u>

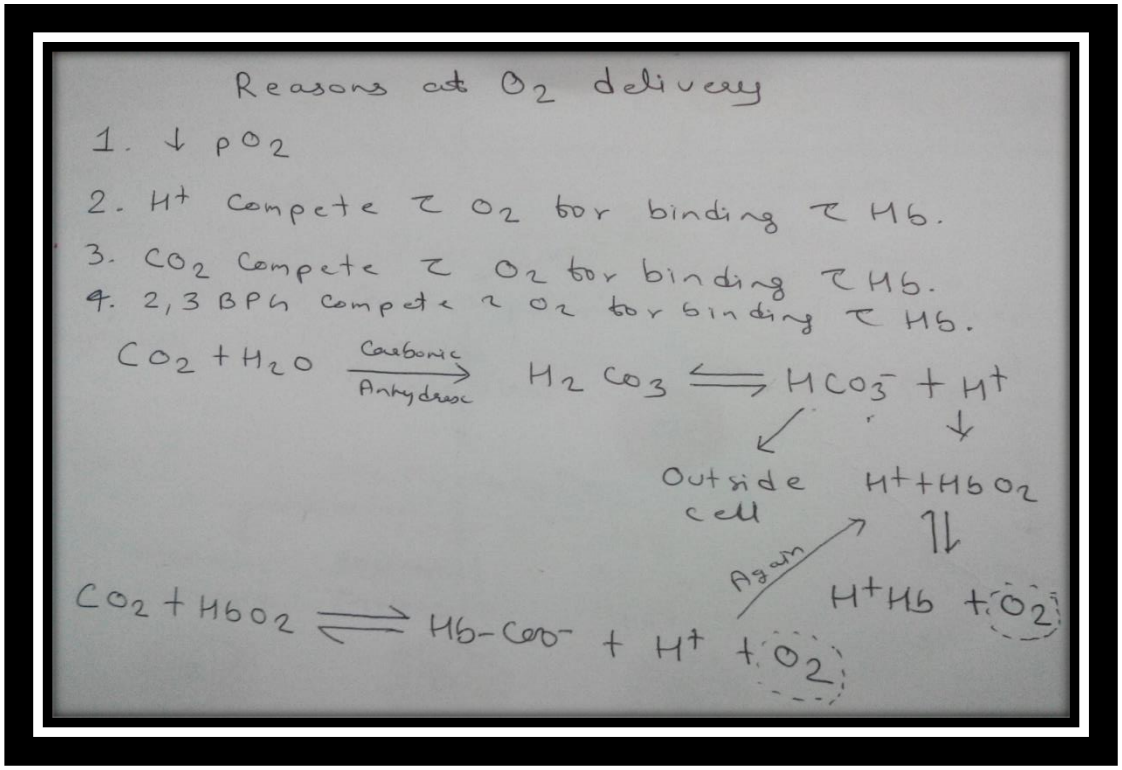
- most important feature - combine *loosely* and *reversibly* with oxygen.
- Oxygen *does not* combine with the two positive bonds of the iron in the hemoglobin molecule.
- It binds loosely with one of the so-called coordination bonds of the iron atom. (extremely loose, easily reversible).
- oxygen does not become ionic oxygen but is carried as molecular oxygen (composed of two oxygen atoms) to the tissues.

Conformational Changes Upon Binding of Oxygen



- The valence electrons in the atoms surrounding iron in the heme group and the valence electrons in the histidine residue form **"clouds" of electron density**.
- Because electrons repel one another, the regions occupied by the valence electrons in the heme group and in the histidine residue are pushed apart.
- porphyrin adopts the domed (nonplanar) configuration in which the **Fe is out of the plane of the porphyrin ring**.

- Hence, when a single heme group in the hemoglobin protein becomes oxygenated, the whole protein changes its shape.
- In the new shape, it is easier for the other three heme groups to become oxygenated.
- Thus, the binding of one molecule of O2 to hemoglobin enhances the ability of hemoglobin to bind more O2 molecules.
- This property of hemoglobin is known as "**cooperative binding**."



Transport of gases

- **Bohr effect :**

  - increase in CO2 and H+ in the blood causes O2 to be displaced

  from the hemoglobin (which is an important factor in increasing O2 transport)

- **Haldane effect :**

  - binding of O2 with hemoglobin tends to displace CO2 from the blood.

- **ABNORMAL HEMOGLOBIN:**
- Structural changes in the **polypeptide chains** caused by mutation in the genes of the globin chains.

1. Hemoglobinopathies

2. Hemoglobin in thalassemia and related disorders.

1.Hemoglobinopathies

β-chains are abnormal -

i. **Hemoglobin S**: Sickle cell anemia.

ii. **Hemoglobin C**:

iii. **Hemoglobin E**:

- mild hemolytic anemia and splenomegaly.

iv. **Hemoglobin M**:

- methemoglobin.
- mutation of genes of both in **α and β chains**,
- blue baby syndrome.

2. Hemoglobin in Thalassemia

- different types of abnormal hemoglobins are present.
- In **α-thalassemia**, the α-chains are decreased, absent or abnormal and Hb level 9-11gm/dl.
- total amount of normal adult haemoglobin A ($\alpha_2\beta_2$) is lowered because the *a* chains are not supplied in sufficient quantity
- in **β-thalassemia**,the β-chains are decreased, absent or abnormal and Hb level < 6 gm/dl.
- B thalassaemia is due to a defective regulation of the

production of normal messenger RNA

- **ABNORMAL HEMOGLOBIN DERIVATIVES**
- Abnormal hemoglobin derivatives are formed by

  - carbon monoxide (CO) poisoning or

  - drugs like nitrites, nitrates and sulphanamides.

- Abnormal hemoglobin derivatives are: **Normal percentage**

1. **Carboxyhemoglobin** 3 -5 %

2. Methemoglobin **< 3 %**

3. **Sulfhemoglobin**. Traces

- 1. **CARBOXYHEMOGLOBIN**
- combination of carbon **monoxide** with hemoglobin.
- hemoglobin has **200 times** more affinity for carbon monoxide than oxygen,
- it hinders the transport of oxygen resulting in **tissue hypoxia**.

- ***Sources of Carbon Monoxide:***

Charcoal burning

Coal mines

Deep wells

Underground drainage system

Exhaust of gasoline engines

Gases from guns and other weapons

Heating system with poor or improper ventilation

Smoke from fire

Tobacco smoking.

Signs and Symptoms

1. < 1% of CO, (Hb saturation is 15% to 20%)

- mild symptoms like **headache** and **nausea** appear.

2. > than 1% CO,(Hb saturation is 30% to 40%)

- causes severe symptoms like:
- i. Convulsions
- ii. Cardiorespiratory arrest
- iii. Unconsciousness and coma.

3. When Hb saturation increases above 50%,death occurs.

- CO-Hgb levels should be measured via a **co-oximeter**, measures :
- total hemoglobin concentration,
- oxyhemoglobin & deoxyhemoglobin and
- abnormal hemoglobins, such as **CO-Hgb** and **methemoglobin**, by differentiating wavelength absorbance values.
- arterial blood gas monitoring, electrolytes ,cardiac markers, blood urea nitrogen and creatinine, creatine phosphokinase,

chest radiograph, electrocardiogram, neuropsychometric testing, and neuroimaging studies.

2. METHEMOGLOBIN

formed when iron molecule of hemoglobin is oxidized from normal ferrous state to **ferric state**.

also called **ferrihemoglobin**. leads to tissue hypoxia, which causes cyanosis and other symptoms

- Causes

  Common factors of daily life:

  i. Well water contaminated with nitrates and nitrites

  ii. Fires

  iii. Laundry ink

  iv. Match sticks and explosives

  v. Meat preservatives (which contain nitrates and nitrites)

  vi. Mothballs (naphthalene balls)

  vii. Room deodorizer propellants

- **2. *Exposure to industrial chemicals such as:***
  - i. Aromatic amines
  - ii. Fluorides
  - iii. Irritant gases like nitrous oxide and nitrobenzene
  - iv. Propylene glycol dinitrate.
- 3. ***Drugs:***
  - i. Antibacterial drugs like sulfonamides
  - ii. Antimalarial drugs like chloroquine
  - iii. Antiseptics
  - iv. Inhalant in cyanide antidote kit

vi. Local anesthetics like benzocaine

- **. *Hereditary trait:***
  - Due to deficiency of **NADH-dependant reductase** or
  - presence of abnormal **hemoglobin M**.
  - blue baby syndrome
- - characterized by bluish skin discoloration (cyanosis),

  caused by congenital heart defect

3.SULF HEMOGLOBIN

- formed by the combination of hemoglobin with hydrogen sulfide.
- drugs such as phenacetin or sulfonamides.
- Sulfhemoglobin cannot be converted back into hemoglobin.
- Only way of elimination after destruction of RBC after their lifespan.
- level rises above 10 gm/dL, cyanosis occur.

DESTRUCTION OF HEMOGLOBIN

- After 120 days, the RBC is destroyed & the hemoglobin is released into plasma.
- hemoglobin is degraded in the reticuloendothelial cells and split into globin and heme.
- **Globin**: utilized for the re-synthesis of hemoglobin.
- **Heme** : degraded into iron and porphyrin.

  - **Iron** is stored as *ferritin* and *hemosiderin*, which are reutilized for the synthesis of new hemoglobin
  - **Porphyrin** is converted into a green pigment called *biliverdin* > bilirubin

IRON

- oxygen transport.
- Iron is important for the formation of hemoglobin and myoglobin.

NORMAL VALUE AND DISTRIBUTION

- Total quantity of iron in the body is about 4 g.
- **Approx Distribution:**
- hemoglobin : 65% to 68%
- myoglobin : 4%
- As intracellular oxidative heme compound : 1%
- transferrin : 0.1%
- Stored in the reticuloendothelial system : 25% to 30%

DIETARY IRON:

- ***1. Heme Iron***
  - fish, meat and chicken.
  - found in the form of heme.
  - easily from intestine.
- ***2. Non-heme Iron***
  - vegetables, grains and cereals. not absorbed easily as heme iron

ABSORPTION OF IRON

- mainly from the small intestine.
- Hydrochloric acid from gastric juice makes the ferrous iron soluble.
- it could be converted into ferric iron by the enzyme ferric reductase from enterocytes.
- Transported into blood by a protein called ferroportin.
- In the blood, ferric iron is converted into ferrous iron and transported.

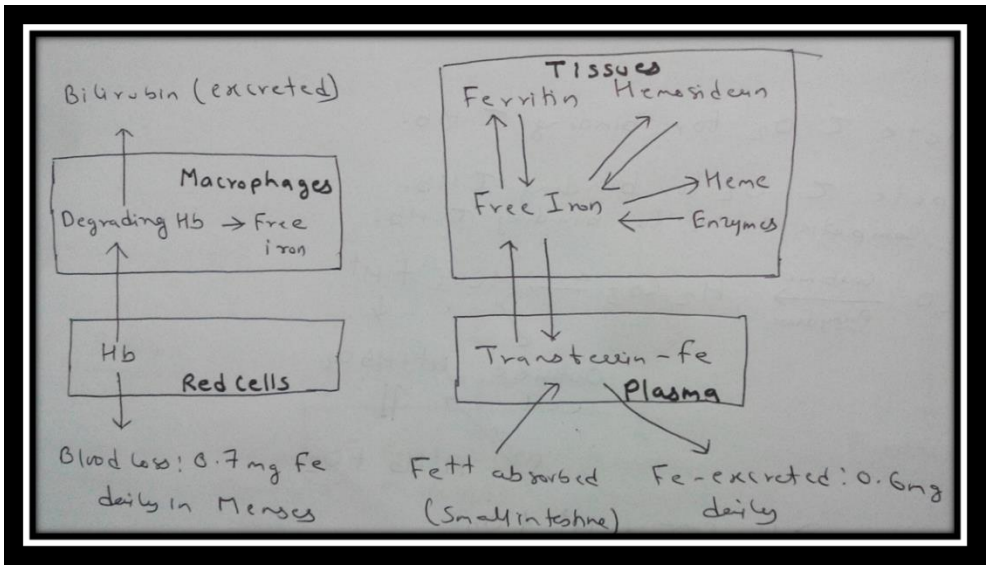
TRANSPORT OF IRON

- Immediately after absorption into blood,
- iron combines with a β-globulin called **apotransferrin**
- resulting in the formation of transferrin**.**
- And iron is transported in blood in the form of transferrin.

- Iron combines loosely with globin and can be released easily at any region of the body

DAILY LOSS OF IRON

- In males, about 1 mg of iron is excreted everyday through feces.
- In females, the amount of iron loss is very much high because of the menstruation.
- In females, during every menstrual cycle, about 50 mL of blood is lost by which 2.5 mg of iron is lost.



VARIATIONS IN NUMBER OF RBC

PHYSIOLOGICAL VARIATIONS

A. ***Increase in RBC Count (*polycythemia).**

1. *Age*

- At birth, 8 to 10 million/cu mm of blood.
- decreases within 10 days - **physiological jaundice**.

2. *Sex*

- Before puberty and after menopause in females , similar to that in males.
- During reproductive period of females, the count is less than that of males (4.5 million/cu mm).

3. *High altitude*

- above 10,000 feet, > 7 million/cu mm.

4. *Muscular exercise*

- temporary.
- mild hypoxia and contraction of spleen.

5. *Emotional conditions*

- emotional conditions such as anxiety.

6. *Increased environmental temperature*

- increases all the activities in the body

7. *After meals*

- need for more oxygen for metabolic activities.

B. *Decrease in RBC Count*

1. *High barometric pressures*

- When the oxygen tension of blood is higher.

2. *During sleep*

- decreases slightly during sleep.
- all the activities of the body are decreased.

3. *Pregnancy*

- a relative reduction in the RBC count.

Primary Polycythemia – Polycythemia Vera

- characterized by persistent increase in RBC count above 14 million/cu mm of blood.
- always a/w increased WBC above 24,000/cu mm.
- occurs in **myeloproliferative disorders** like malignancy of red bone marrow.

Secondary Polycythemia

- 1. Respiratory disorders like emphysema.
- 2. Congenital heart disease.
- 3. Ayerza's disease.
- 4. Chronic carbon monoxide poisoning.
- 5. Poisoning by chemicals like phosphorus and arsenic

- 6. Repeated mild hemorrhages.

VARIATIONS IN SIZE OF RBC

- 1. **MICROCYTES**
  - i. Iron-deficiency anemia
  - ii. Prolonged forced breathing
  - iii. Increased osmotic pressure in blood.
- 2. **MACROCYTES**
  - i. Megaloblastic anemia
  - ii. Decreased osmotic pressure in blood.
- **3. ANISOCYTES**
  - pernicious anemia.

VARIATIONS IN SHAPE OF RBC

1. Crenation*:* Shrinkage as in hypertonic conditions.

2. ***Spherocytosis****:* Globular form as in hypotonic conditions.

3. ***Elliptocytosis****:* Elliptical shape as in certain types of anemia.

4. ***Sickle cell****:* Crescentic shape as in sickle cell anemia.

5. ***Poikilocytosis****:* Unusual shapes due to deformed cell membrane.

The shape will be of flask, hammer or any other unusual shape.

VARIATIONS IN STRUCTURE OF RBC

1. PUNCTATE BASOPHILISM

Striated appearance of RBCs by the presence of dots of **basophilic materials** (porphyrin). eg. **lead poisoning.**

2. **RING IN RED BLOOD CELLS (Goblet ring)**

Ring or twisted strands of basophilic material appear in the periphery of the RBCs.

3. **HOWELL-JOLLY BODIES**

nuclear fragments present in the cytoplasm of the RBCs.

eg: B12 or folic acid deficiency

ANEMIAS 9

DR. SURENDRA KUMAR ACHARYA

Anemia is the blood disorder, characterized by the reduction in:

1. Red blood cell (RBC) count
2. Hemoglobin content
3. Packed cell volume (PVC).

SIGNS AND SYMPTOMS OF ANEMIA

- **SKIN AND MUCOUS MEMBRANE**
- Color of the skin and mucous membrane becomes **pale**.
- Paleness is more constant and prominent in **buccal** and **pharyngeal** mucous membrane, conjunctivae, lips, ear lobes, palm and nail bed.
- Skin - looses the elasticity and becomes thin and dry.
- Thinning, loss and early grayness of hair occur.
- The nails become brittle and easily breakable.

CARDIOVASCULAR SYSTEM

- There is an increase in heart rate (**tachycardia**) and cardiac output.
- Heart is dilated and cardiac murmurs are produced.
- The velocity of blood flow is increased.

RESPIRATION

- There is an increase in rate and force of respiration.
- breathlessness and dyspnea
- Oxygenhemoglobin dissociation curve is shifted to right.

DIGESTIVE SYSTEM

- Anorexia, nausea, vomiting, abdominal discomfort and constipation are common.
- In pernicious anemia, there is atrophy of papillae in tongue.

- In aplastic anemia, necrotic lesions appear in mouth & pharynx.

METABOLISM

- Basal metabolic rate increases in severe anemia.

KIDNEY

- Renal function is disturbed.
- Albuminuria is common.

PRODUCTIVE SYSTEM

- In females, the menstrual cycle is disturbed.
- There may be menorrhagia, oligomenorrhea or amenorrhea

NEUROMUSCULAR SYSTEM

- Common neuromuscular symptoms are increased sensitivity to cold, headache, lack of concentration, restlessness, irritability, drowsiness, dizziness or vertigo and fainting.
- Muscles become weak and the patient feels lack of energy and fatigued quite often and quite easily

- **MORPHOLOGICAL CLASSIFICATION:**

  depends upon the size and color of RBC.

  Size of RBC is determined by mean corpuscular volume (MCV).

  Color is determined by mean corpuscular hemoglobin concentration (MCHC).

  1. Normocytic Normochromic Anemia- Size (MCV) and color (MCHC) of RBCs are normal. But the number of RBC is less

  2. Macrocytic Normochromic Anemia-RBCs are larger in size with normal color.

  RBC count is less.

  3. Macrocytic Hypochromic Anemia-RBCs are larger in size. MCHC is less, so the cells are pale (less colored).

  4. Microcytic Hypochromic Anemia- RBCs are smaller in size & pale.

ETIOLOGICAL CLASSIFICATION:

1. Hemorrhagic anemia

2. Hemolytic anemia

3. Nutrition deficiency anemia

4. Aplastic anemia

5. Anemia of chronic diseases.

- **Types of anemia and their physiological causes:**

1. Hemorrhagic anemia.

rapid hemorrhage: low concentration of RBCs usually returns to normal within 3 to 6 weeks.

chronic blood loss: gives rise to microcytic, hypochromic anemia.

- ANEMIAS

2. Hemolytic Anemia.

- Different abnormalities of the RBCs, many of which are hereditarily acquired, make the cells fragile,
- so they rupture easily as they go through the capillaries, especially through the spleen.
- the life span of the fragile RBC is so short that the cells are destroyed faster than they can be formed, and serious anemia results.

3. Hemolytic Anemia-

- **Extrinsic : (normocytic normochromic)**

i. Liver & Renal disorder

ii. Hypersplenism

iii. Burns

iv. Infections – hepatitis, malaria and septicemia

v. Drugs – Penicillin, antimalarial drugs and sulfa drugs

vii. Poisoning by lead, coal and tar

viii. Presence of isoagglutinins like **anti Rh-** ***erythroblastosis fetalis***

xi. Autoimmune diseases – rheumatoid arthritis and ulcerative colitis.

Erythroblastosis fetalis

Rh-positive RBCs in the fetus are attacked by antibodies from an Rh-negative mother.

These antibodies make the Rh-positive cells fragile, leading to rapid rupture.

causing the child to be born with a serious case of anemia

Intrinsic: Hereditary disorders

A) Hereditary spherocytosis

B) Sickle cell anemia

C) Thalassemia

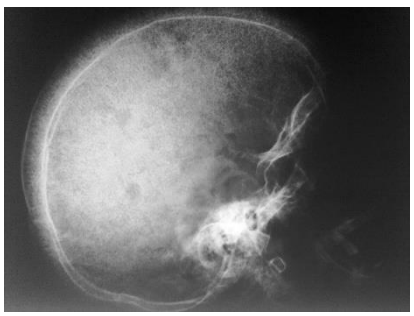
- **Hereditary spherocytosis**
- RBCs are very small and *spherical*.
- *Defect in ankyrin cytoskeletal protein*

  Upon passing through the splenic pulp and some other tight vascular beds, they are easily ruptured by even slight compression.

Sickle cell anemia-

- **B) Sickle cell anemia,**
- 0.3 to 1.0 percent of West African and American blacks,
- abnormal type of hemoglobin called *hemoglobin S*
- faulty beta chains in the hemoglobin molecule.
- replacement of glutamic acid by valine in position 6 at the N-terminus of the beta-chain of globin
- In children, hemolyzed sickle cells aggregate and block the blood vessels, leading to infarction.
- The infarcted small bones in hand and foot results in varying length in the digits.
- This condition is known as hand and foot syndrome.
- Jaundice also occurs in these children.

- **Radiographic Features**
- a decrease in the trabecular pattern of bone and
- a less prominent "hair-on-end" appearance of bone in a skull radiograph.



- **Clinical Features**
- Delayed eruption of teeth and dental hypoplasia,disorders of enamel and dentin mineralization, malocclusion, hypercementosis,
- and a degree of periodontitis
- Pallor of oral mucosa
- changes to the superficial cells of the tongue
- Bone pain
- Osteoporosis
- ↑ occurrence of osteomyelitis, asymptomatic pulpal necrosis and mandibular paresthesia

- **Dental considerations**
- no contraindications (stable condition).
- visits should be short and stress-free.
- It is wise to avoid long complex dental procedures due to an increased risk of acidosis.
- Prophylactic antibiotics should be used for major surgical procedures and if infection ensues, it should be treated aggressively.
- Clinicians should be prudent with their use of local anesthetics. If the patient is symptom-free, 2% lidocaine with 1:200,000 can be safely administered for a routine extraction.
- It is unadvisable to treat patients who are in a sickle crisis or have been having recurrent episodes.
- ***C) Thalassemia (*Cooley's anemia)**

  or Mediterranean anemia- characterized by abnormal hemoglobin.

  α-Thalassemia

  occurs in fetal life or infancy.

  α chains are less, absent or abnormal.

  Hb level 9-11gm/dl.

  leads to defective erythropoiesis and hemolysis.

infants may be stillborn or may die immediately after birth.

β-Thalassemia

- B chains are less in number, absent or abnormal
- defective erythropoiesis and hemolysis.
- Hb level < 6 gm/dl.
- due to a defective regulation of the production of normal messenger RNA
- **orofacial features:**
- Enlargement of the upper jaw (**chipmunk** face)
- Migration and spacing of upper anterior teeth
- Varying degrees of malocclusion (overbite, open bite)
- Teeth may be discoloured, with short crowns and roots
- Higher rate of dental decay
- Tooth bearing bone may have a '**chickenwire-like**' radiological appearance
- Pale gums and mucosa / lining of the mouth .
- Sore or burning tongue.
- Painful swelling of salivary glands and dry mouth
- Reduced salivary protection (reduced IgA)
- **Dental considerations:**
- splenectomy - risk of infection following any procedures associated with bacteraemia (extractions or scaling); antibiotics may be prescribed to prevent this.
- acute dental infections / abscesses should be treated at the earliest opportunity.
- extremely important to keep teeth and gums in as clean and healthy a state as possible.
- recurrent exchange transfusions – heparinisation, risk of carriage of blood-borne viruses
- cardiomyopathy (heart disease due to effects of iron deposition)
- medication related side effects.
- orthodontic treatment: faster movement, retention phase is also more difficult in these patients.

4. NUTRITION DEFICIENCY ANEMIA:

- deficiency of a nutritive substance necessary for erythropoiesis
  - ***A) Iron deficiency anemia***
  - ***B) Protein deficiency anemia***
  - ***C) Pernicious anemia or Addison's anemia***
  - ***D) Megaloblastic anemia***

decrease in vitamin B12 or folic acid causes abnormal and diminished DNA and, consequently, ***failure of nuclear maturation and cell division***.

- ***A) Iron deficiency anemia***

most common type of anemia.

due to inadequate availability of iron for hemoglobin synthesis.

RBCs are microcytic and hypochromic.

Features:

brittle nails, spoonshaped nails (koilonychias), brittle hair,

atrophy of papilla in tongue

dysphagia (difficulty in swallowing).

Causes of iron deficiency anemia:

i. Loss of blood

ii. Decreased intake of iron

iii. Poor absorption of iron from intestine

iv. Increased demand for iron in conditions like growth and pregnancy.

B) Protein deficiency anemia

Due to deficiency of proteins, the synthesis of hemo globin

is reduced. The RBCs are macrocytic and hypochromic.

C) Pernicious anemia or Addison's anemia

autoimmune destruction of parietal cells.

atrophy of the gastric mucosa

decreased production of intrinsic factor

poor absorption of vitamin B12(maturation factor).

RBCs are larger and immature (megaloblast)

- **Characteristic features**:

lemon yellow color of skin

and red sore tongue.

- **Neurological disorders**: (extreme conditions)
- paresthesia
- progressive weakness
- Ataxia
- ***D) Megaloblastic anemia***

Due to the deficiency of another maturation factor called folic acid.

The RBCs are not matured.

The DNA synthesis is also defective, so the nucleus remains immature.

The RBCs are megaloblastic and hypochromic.

Features of pernicious anemia appear in megaloblastic anemia also.

5. APLASTIC ANEMIA

high-dose radiation or chemotherapy for cancer treatment.

high doses of certain toxic chemicals, such as insecticides or benzene in gasoline,

In autoimmune disorders, such as lupus erythematosus

In about half of aplastic anemia cases the cause is unknown, a condition called idiopathic aplastic anemia.

6. ANEMIA OF CHRONIC DISEASES

second common type of anemia.

Characterized by short lifespan of RBCs,

caused by disturbance in iron metabolism or resistance to erythropoietin action.

Anemia develops after few months of sustained disease.

RBCs are normocytic and normochromic.

5. *ANEMIA OF CHRONIC DISEASES*

Common causes anemia of chronic diseases:

- **i. Noninfectious inflammatory diseases:** rheumatoid arthritis
- **ii. Chronic infections:** tuberculosis
- **iii. Chronic renal failure,**
- **iv. Neoplastic disorders:**

  Hodgkin's disease (malignancy involving lymphocytes)

  and cancer of lung and breast.

- in progressive disease a/w iron deficiency & *microcytic* and hypochromic anemia.

- Rate at which the erythrocytes settle down.
- **Sed rate** or **Biernacki reaction.**
- Erythrocyte sedimentation rate (ESR) is an easy, inexpensive and non-specific test, which helps in diagnosis as well as prognosis.
- **DETERMINATION OF ESR**

1. WESTERGREN METHOD

- Westergren tube is used to determine

ESR.

2. **WINTROBE METHOD**

- Wintrobe tube is used to determine
- ESR and PCV

NORMAL VALUES OF ESR

1. By Westergren Method

In males : 3 to 7 mm in 1 hour

In females : 5 to 9 mm in 1 hour

Infants : 0 to 2 mm in 1 hour

2. By Wintrobe Method

In males : 0 to 9 mm in 1 hour

In females : 0 to 15 mm in 1 hour

Infants : 0 to 5 mm in 1 hour.

VARIATIONS OF ESR :

- **PHYSIOLOGICAL VARIATION**
- 1. ***Age:*** less in children and infants because of more number of RBCs.

- 2. ***Sex:*** more in females than in males because of less number of RBCs.
- 3. ***Menstruation:*** The ESR increases during menstruation because of loss of blood and RBCs
- 4. ***Pregnancy:*** From 3rd month to parturition, ESR increases up to 35 mm in 1 hour because of hemodilution.

- **PATHOLOGICAL VARIATION:**

Increases in conditions:	Decreases in conditions:
1. Tuberculosis	1. Allergic conditions
2. All types of anemia except sickle cell anemia	2. Sickle cell anemia
3. Malignant tumors	3. Peptone shock
4. Rheumatoid arthritis	4. Polycythemia
5. Rheumatic fever	5. Severe leukocytosis.
6. Liver diseases.	

FACTORS AFFECTING ESR:

FACTORS INCREASING ESR	FACTORS DECREASING ESR
1. Increase in Specific Gravity of RBC	1. Increase in Viscosity of Blood

2. Rouleaux Formation	2. Increase RBC count
3. Increase in Size of RBC	

- **PACKED CELL VOLUME (PCV)**
- proportion of blood occupied by RBCs, expressed in percentage.
- It is the volume of RBCs packed at the bottom of a hematocrit tube when the blood is centrifuged.
- **hematocrit value** or **erythrocyte volume fraction (EVF).**
- In males = 40% to 45%
- In females = 38% to 42%
- **SIGNIFICANCE OF DETERMINING PCV**

Determination of PCV helps in:

1. Diagnosis and treatment of **anemia**
2. Diagnosis and treatment of **polycythemia**
3. Determination of extent of **dehydration** and recovery from dehydration after treatment
4. Decision of blood **transfusion**.

BLOOD INDICES 11

DR. AMAR KUMAR

- Blood indices are the calculations derived from RBC count, hemoglobin content of blood and PCV.
- Blood indices help in diagnosis of the type of anemia.
- **DIFFERENT BLOOD INDICES**

1.Mean Corpuscular Volume (MCV)

average volume of a single RBC.

Normal MCV is 90 cu μ (78 to 90 cu μ).

Normocyte, Macrocyte, microcyte.

2. Mean Corpuscular Hemoglobin (MCH)

MCH is the quantity or amount of hemoglobin present in one RBC.

Normal MCH is 30 pg (27 to 32 pg).

3. Mean Corpuscular Hemoglobin Concentration (MCHC)

concentration of hemoglobin in one RBC.

It is the amount of hemoglobin expressed in relation to the volume of one RBC.

Normal value of MCHC is 30% (30% to 38%).

Normochromic,hypochromic,

4. Color Index (CI)

ratio between the percentage of hemoglobin and the percentage of RBCs in the blood.

average hemoglobin content in one cell of a patient compared to the average hemoglobin content

Polychythemia vera

- manifest intraorally with

  - erythema of mucosa,

  - glossitis, and

- erythematous, edematous gingiva.

Spontaneous gingival bleeding can occur

- **Oral Health Considerations**
- Control of hemorrhage after dental surgery should be considered.
- Hemorrhage : may be associated with
  - high platelet counts,
  - acquired von Willebrand's disease,
  - high doses of antiplatelet drug therapy.
- Low-dose aspirin is rarely associated with hemorrhagic complications from dental extractions.
- Relation bet albumin and anemia
- In anemia Hypoxic, hyperosmolar renal medulla.
- Chronic renal diseases:

  Decrease erythropoiten

  also albuminuria is a sign of chronic renal disease

White blood cells (LEUKOCYTES)

mobile units of the body's protective system.

- They are formed

-partially in the **bone marrow** (*granulocytes* and *monocytes*

and a few *lymphocytes*) and

-partially in the **lymph tissue** (*lymphocytes* and *plasma cells*).

- 4-11000 /ml
- Specifically transported to areas of infection and inflammation,
- provide a rapid and potent defense against infectious agents.

Types of White Blood Cells

Polymorphonuclear neutrophils(62%),

Polymorphonuclear eosinophils(2.3%),

polymorphonuclear basophils(0.4%),

Monocytes(5.3%),

Lymphocytes(30%)

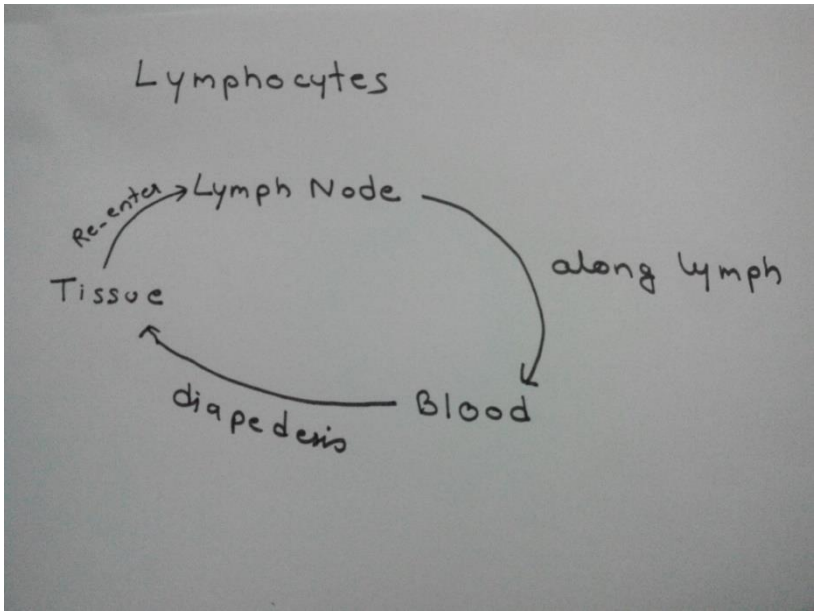
- **LIFE SPAN OF WHITE BLOOD CELLS**
- After being released from the bone marrow :

normally **4 to 8 hours** circulating in the blood and

another **4 to 5 days** in tissues where they are needed.

- The **monocytes** : **10 to 20 hours** in the blood, before wandering through the capillary membranes into the tissues.

- In the tissues-- swell to much larger sizes - ***tissue macrophages***, live for **months**.

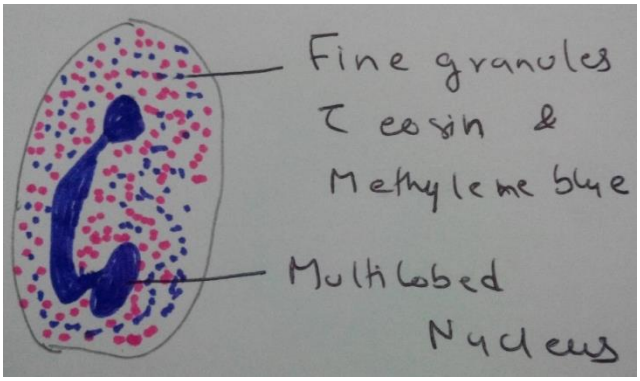


- The lymphocytes have life spans of **weeks or months**.
- NEUTROPHILS:
- Polymorphs, fine or small granules in the cytoplasm.
- Take acidic and basic stains.
- When stained with **Leishman's stain**

  the granules appear violet in color.

- Nucleus is multilobed,
- In younger: nucleus is not lobed.

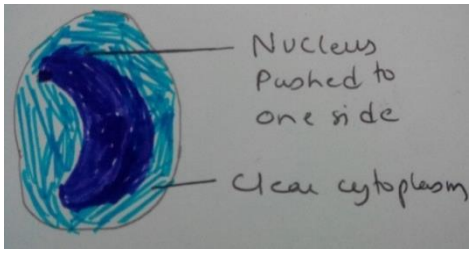
  older : nucleus has 2 to 5 lobes.
- Diameter:10 to 12 µ



- MONOCYTES
- largest leukocytes ,14 to 18 µ.
- cytoplasm is clear without granules.
- Nucleus:

- is round, oval and horseshoe , bean or kidney shaped &

- is placed either in the center of the cell or pushed to one side large amount of cytoplasm is seen.



- **NEUTROPHILS AND MACROPHAGES DEFEND AGAINST INFECTIONS**
- The **neutrophils** are mature cells that can attack and destroy bacteria even in the circulating blood.

- **monocytes**- immature cells in the blood and have little ability to fight infectious agents at that time.

- enter the tissues, begin to swell (fivefold) 60 to 80 micrometers , now called ***macrophages***, and they are extremely capable of combating disease agents in the tissues.

White Blood Cells Enter the Tissue Spaces by Diapedesis.

Neutrophils and monocytes can squeeze through the pores of the blood capillaries by *diapedesis.*

That is, even though a pore is much smaller than a cell, a small portion of the cell slides through
the portion sliding through is momentarily constricted to the size of the pore,

Both neutrophils and macrophages can move through the tissues by **ameboid** motion.

Many different chemical substances in the tissues cause both neutrophils and macrophages to move toward the source of the chemical, phenomenon is known as ***chemotaxis.***

Chemotaxis depends on the concentration gradient of the chemotactic substance.

- **PHAGOCYTOSIS**
- most important function of the neutrophils and macrophages
- which means cellular ingestion of the offending agent.

1. most natural structures - smooth surface,

rough surface- likelihood of phagocytosis is increased.

2. most natural substances - protective protein coats .

most dead tissues and foreign particles - **no protective coats**,

3. immune system of the body develops **antibodies** against infectious agents such as bacteria.

The antibodies then adhere to the bacterial membranes > susceptible to phagocytosis.

- This process by which a pathogen is selected for phagocytosis and destruction is called ***opsonization***

- **PHAGOCYTOSIS BY NEUTROPHILS.-**

The neutrophil first attaches itself to the particle and then projects pseudopodia in all directions around the particle.

The pseudopodia meet one another on the opposite side and fuse.

creates an enclosed chamber that contains the phagocytized particle.

chamber invaginates to the inside of the cytoplasmic cavity and breaks away from the outer cell membrane

to form a free-floating phagocytic vesicle (also called a phagosome) inside the cytoplasm

- **PHAGOCYTOSIS BY MACROPHAGES.**
- ability to engulf much **larger** particles, even whole RBCs or, occasionally, malarial parasites.
- Also, after digesting particles, macrophages can **extrude** the residual products and often survive and function for many more months.
- **Once Phagocytized, Most Particles Are Digested by**

Intracellular Enzymes.

- lysosomes and other cytoplasmic granules in the neutrophil or macrophage immediately come in contact with the phagocytic vesicle,

and their membranes fuse, dumping many **digestive enzymes** and bactericidal agents into the vesicle - *digestive vesicle*

- **Proteolytic** enzymes in both &
- **Lipases** in macrophages

Formation of Pus

- When neutrophils and macrophages engulf large numbers of bacteria and necrotic tissue (essentially all the neutrophil)many of the macrophages eventually die.
- After several days, a cavity is often excavated in the inflamed tissues.
- This cavity contains varying portions of necrotic tissue, dead neutrophils, dead macrophages, and tissue fluid.
- This mixture is commonly known as *pus.*

Neutrophils and Macrophages Can Kill Bacteria.

- ***oxidizing agents*** formed by enzymes in the membrane of the phagosome or by a special organelle called the ***peroxisome***.
- These oxidizing agents include large quantities of

1) *superoxide* (O_2^-),

2) *hydrogen peroxide* (H_2O_2), and

3) *hydroxyl ions* (OH^-),

- which are lethal to most bacteria, even in small quantities.

INFLAMMATION:

ROLE OF NEUTROPHILS AND MACROPHAGES

1. TISSUE MACROPHAGES - First Line of Defense.

- first effect is rapid enlargement of each of these cells.
- previously sessile macrophages break loose from their attachments and become mobile,
- forming the first line of defense against infection during the first hour.
- numbers are not much, but they are lifesaving.

2. NEUTROPHIL INVASION - Second Line of Defense.

- Within the first **hour** after inflammation begins,

- large numbers of neutrophils begin to invade the inflamed area from the blood.

- increases 4-5 times

- caused by inflammatory cytokines
  - (e.g., tumor necrosis factor and interleukin-1) and
  - other biochemical products produced by the inflamed tissues & initiate the reactions.

3. SECOND MACROPHAGE - Third Line of Defense.

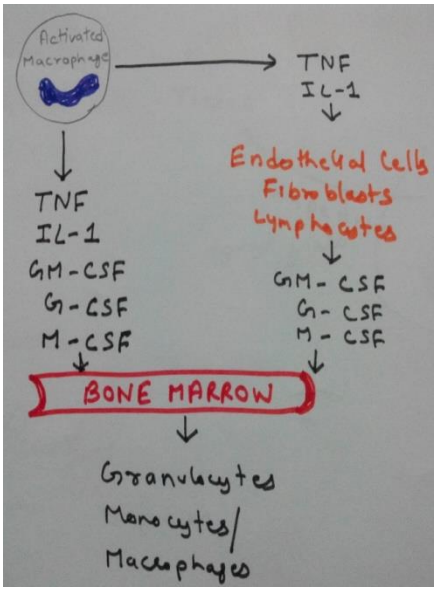
- Along with the invasion of neutrophils, monocytes from the blood enter the inflamed tissue and enlarge to become macrophages.
- the number of monocytes in the circulating blood is low.
- storage pool of monocytes in the bone marrow is much less than that of neutrophils.
- Therefore, the buildup of macrophages is slower than that of neutrophils, requiring **several days** to become effective.

4. GRANULOCYTES & MONOCYTES - Fourth Line of Defense.

- stimulation of the granulocytic and monocytic progenitor cells of the marrow.
- greatly increased production by the bone marrow.
- it takes **3 to 4 days** before newly formed granulocytes and monocytes reach the stage of leaving the bone marrow.
- can **continue** to produce these cells in tremendous quantities for months and even years,

Macrophage and Neutrophil Responses Feedback Control

- combination of TNF, IL-1, and colony-stimulating factors provides a powerful feedback mechanism that begins with tissue inflammation and
- proceeds to formation of large numbers of defensive WBCs that help remove the cause of the inflammation.



- **EOSINOPHILS**
- About 2 percent of leukocytes.
- Defense mechanism against parasites.
- Also in allergic diseases like asthma.
- Eosinophils are responsible for detoxification, disintegration and removal of foreign proteins.
- neither markedly motile nor phagocytic
- ***Mechanism of Action:***

1. ***Eosinophil peroxidase***:

- capable of destroying helminthes , bacteria and tumor cells.

2. ***Major basic protein (MBP):***

- very active against helminths.
- causes distension (ballooning) and detachment of the tegumental sheath (skin-like covering) of these organisms.

3**.** ***Eosinophil-derived neurotoxin:***

- destroys the nerve fibers particularly, the myelinated nerve fibers.
- ***Mechanism of Action:***

4. *Eosinophil cationic protein (ECP):*

- major destroyer of helminths
- about 10 times more toxic than MBP.
- destroys the parasites by means of complete disintegration.
- also a **neurotoxin**.

5. *Cytokines:*

- Cytokines such as IL-4 and IL-5 accelerate inflammatory responses by activating eosinophils.
- also kill the invading organisms.

BASOPHILS

- Similar to the large tissue *mast cells* located immediately outside many of the capillaries in the body.
- Play an important role in some types of allergy.
- because of the presence of **receptors for IgE** in basophil membrane.
- important role in healing processes.
- ***Mechanism of Action of Basophils***

1. ***Heparin:***

- prevent the intravascular blood clotting.

2. *Histamine, slow-reacting substances of anaphylaxis, bradykinin and serotonin:*

- produce the acute hypersensitivity reactions by causing vascular and tissue responses.

3. *Proteases and myeloperoxidase:*

- destroy the microorganisms.

4. *Cytokine:*

- Cytokine such as *interleukin-4* accelerates inflammatory responses and kill the invading organisms.

APPLIED ASPECT:

- PATHOLOGICAL VARIATIONS OF NEUTROPHILS

- Variations in the **count**.

- Variations in the **morphology**.

- Defective **functions**

- **VARIATIONS IN THE COUNT.**

DISODER	VARIATION	CONDITION
Neutrophilia	Increase in neutrophil count > 7500/microL	Acute infections Vaccine injections Ingestion of foreign bodies Metabolic disoders Poisionoing Acute hemorrhage
Neutropenia	Decrease in neutrophil < 2500/miroL	Autoimmune disease Bone marrow disorder Tuberculosis Typhoid

VARIATION IN MORPHOLOGY

- **Vacuoles**: in severe septicemias
- **Granules, Dohle** bodies: in bacterial infections
- **Nuclear abnormalities**:
- Sex chromatin: drum stick chromatin attached to chromatin in female sex chromosomes.

- **Pelger –Huet anomaly**: inherited disorder ,decrease in no of nuclear segments , corse staining chromatin.

  - Nucleus is spectacle/dumbbell shaped.

- **DEFECTIVE FUNCTIONS**

Defective chemotaxis:	**Defective Phagocytosis**	**Defective killing**
• Lazy-leukocyte syndrome • Steroid therapy • Aspirin ingestion • Alcoholism • Myeloid leukemia	• Hypogammaglobinemia • Hypocomplementemia • After splenectomy	• Chediak higashi syndrome • Myeloid leukemia • Chronic granulomatous diseases

- **Granulocytosis**

Causes

- **Infectious**

- Bacterial, viral, fungal, parasitic, rickettesia

- can present as abcesses, tonsillitis,otitis media, osteomyelitis, peritonitis.

- **Non infectious**

- burns, acute MI, attacks of gout, glomerulonephritis.

- **Metabolic conditions**

- diabetic ketoacidosis, uremia,preclamsia, Poisoning

- **Granulocytopenia**:

Clinical features	Oral manifestations
Fever	Ulceration without surrounding inflammation
Malaise	Extremely painful,foul smelling necrotic tissue
Acute pharnygitis	Advanced periodontal diseases
Mucosal ulcers	pericoronitis
lymphadenopathy	Pulpal infections
Varicella- zoster infection (common)	

Infection with minimal signs of immflammatory reactions

AGRANULOCYTOSIS:

Causes	Clincal features
Decreased production or increase destruction	**Fever**
drugs	**Stomatitis**
Congenital (kostamaan syndrome)	**Pharnygitis**

Condition with absence of neutrophils	**Skin abscesses**
	Lung infections

• A condition in which the cells of the granulocyte series, particularly neutrophils are absent.

- **CYCLIC NEUTROPENIA**
- oscillation after every 14 to 36 days .
- A rare disorder secondary to periodic failure of the stem cells of the bone marrow.
- Patient is healthy in b/w the neutropenic episodes.

c/f	**Oral manifestations**
Bacterial infections	Anorexia,pharyngitis,lymph node enlargement
Sore throat	Ulcerations on lip,tongue,buccal mucosa **without erythematous halo**
Fever	Fiery red gingiva, marked gingival recession
Chills	bone loss,
Bone pain	tooth mobility
Pneumonia	Advanced periodontitis

CLINICAL MANIFESTATIONS

• Bacterial infections

• Sore throat

• Swelling

• Fever

• Chills

• Bone pain

• Pneumonia

Oral manifestations

• Anorexia , pharyngitis,lymph node enlargement

• Oral ulcerations on lip, tongue,buccal mucosa without erythematous halo.

• Fiery red Gingiva,bone loss,marked gingival recession, tooth mobility

• Advanced Periodontitis

CHEDIAK HIGASHI SYNDROME

- Autosomal recessive disorder
- Large blue gray granules in the cytoplasm of **granulocytes** & **melanocytes**.
- Decreased chemotactic & bactericidal ability

Clinical features

- Progressive neurologic abnormalities
- Oculocutaneous albinism.
- Hypo pigmentation
- Recurrent bacterial infections
- Gingival & periodontal disease
- LAZY LEUKOCYTE SYNDROME
- Loss of chemo tactic function of neutrophils.
- Cells are **unable** to **migrate** from the marrow to the peripheral tissues.

Clinical features

- Gingivitis
- Stomatitis

- Bronchitis
- Total WBC count is in the normal range but absolute Neutrophil count low.
- Neutrophils show lack of response to epinephrine and piromen (pseudomonal polysaccharide)
- Leukocyte Adhesion Deficiency
- Autosomal recessive disorder.
- Defects in surface receptors Mac-1,LFA-1 & p150,95
- **c/f:**
- Fiery red gingiva
- Advanced bone loss
- Early exfoliation of deciduous teeth
- Prophylactic dental treatment should be carried in such patients

LYMPHOCYTES

- ***Types of Lymphocytes***
- ORIGIN AND DEVELOPMENT OF LYMPHOCYTES :
- stem cells differentiate into

1) **Pre-T** cells – that migrates through the blood to the **thymus** glands , Where they divide and preprocessed into -T-cells.

2) Other stem cells they produce **pre-B** cells which are processed in red bone marrow into the B-cells.

- Thymus glands produces hormones such as **thymosin** which stimulate T-cell maturation.
- MATURATION OF T-CELLS AND B-CELLS :
- T-cell and B-cell : make several distinctive proteins that are inserted into the plasma membrane.
- Some of these proteins functions as **antigen-receptors** – molecules capable of recognizing the specific antigen.
- T-cells exit the thymus as either **CD4+ or CD8+** cells.

- CD8+ T cells: proliferate into **cytotoxic T** cells that **directly** attack the invading antigen.
- CD4+T cells : become **helper** T-cells that aid both cell-mediated and antibody mediated immune response.
- B-cells transform into **plasma cells** which synthesize antibodies

Immunity

DEVELOPMENT OF CELL-MEDIATED IMMUNITY

- Immunity developed by **cell-mediated response**.
- also called **cellular** immunity or T cell immunity.
- involves several types of cells such as **T lymphocytes, macrophages** and **natural killer cells.**
- major defense mechanism against **infections** by viruses, fungi and few bacteria like tubercle bacillus.
- also responsible for **delayed allergic** reactions and the **rejection** of **transplanted** tissues.
- mainly by T lymphocytes
- it starts developing when T cells come in contact

with the antigens.

- usually, the invading microbial or non-microbial organisms carry the antigenic materials.
- These antigenic materials are released from invading

organisms and are presented to the helper T cells by

antigen-presenting cells.

- **ANTIGEN-PRESENTING CELLS**
- induce the release of antigenic materials from invading organisms and later present these materials to the helper T cells.
- ***Types of Antigen-Presenting Cells***
- Antigen-presenting cells are of three types:

1. Macrophages

2. Dendritic cells

3. B lymphocytes.

1. ***Macrophages***

- large **phagocytic** cells,
- digest the invading organisms to release the antigen.
- The macrophages are present along with lymphocytes

in almost all the lymphoid tissues.

2. ***Dendritic Cells***

- are nonphagocytic in nature.
- Based on the location:

i. Dendritic cells of **spleen**- trap the antigen in blood.

ii. Follicular dendritic cells in **lymph nodes**- trap the antigen in the lymph.

iii. Langerhans dendritic cells in **skin**: trap the organisms coming in contact with body surface.

3. ***B Lymphocytes***

- Recently, it is found that B lymphocytes also act as antigen-presenting cells.
- least efficient antigen presenting cells and
- need to be activated by helper T cells.
- ***Role of Antigen-presenting Cells***
- Invading foreign organisms are either engulfed by **macrophages** through phagocytosis or trapped by **dendritic** cells.
- Later, the antigen from these organisms is digested into small peptide products.
- These antigenic peptide products move towards the surface of the antigen-presenting cells and bind with human leukocyte antigen (HLA).
- Role of **B cells** as antigen-presenting cells is not fully understood.
- ***Presentation of Antigen***

- Antigen-presenting cells present their **class II MHC**

molecules together with antigen-bound HLA to the helper

T cells.

- This activates the helper T cells through series of events

Sequence of Events during Activation of Helper T cells

- ***Role of TH1 Cells***
- secrete two substances:

i. Interleukin-2, which activates the other T cells.

ii. Gamma interferon, which stimulates the phagocytic activity of cytotoxic cells, macrophages and natural killer (NK) cells.

- **ROLE OF CYTOTOXIC T CELLS**
- activated by helper T cells,
- circulate through blood, lymph and lymphatic tissues and destroy the invading organisms by attacking them **directly**.
- **Receptors** situated on the outer membrane of cytotoxic T cells bind the **antigens** or organisms tightly.
- release cytotoxic substances like the **lysosomal enzymes**.
- **destroy** the invading organisms
- **ROLE OF SUPPRESSOR T CELLS**
- also called **regulatory** T cells.
- **suppress** the activities of the killer T cells.
- preventing the killer T cells from destroying the body's

own tissues along with invaded organisms.

- „ RO**LE OF MEMORY T CELLS**
- Some of the T cells activated by an antigen do not enter

the circulation but remain in lymphoid tissue.

- These T cells are called memory T cells.
- In later periods, the memory cells migrate to various

lymphoid tissues throughout the body.

- When the body is exposed to the same organism for the second time,the memory cells identify the organism and immediately activate the other T cells.
- Response is very quick & also more powerful this time.
- **HUMORAL IMMUNITY**
- ***Transformation B Cells***
- Proliferated B cells are transformed into two types of cells:

1. Plasma cells - producing the **antibodies** (immunoglobulins)

2. Memory cells - occupy the lymphoid tissues throughout the body.

- second exposure, the memory cells are stimulated by the antigen and produce more quantity of antibodies at a faster rate, than in the first exposure (basic principle of **vaccination** against the infections).
- **ROLE OF HELPER T CELLS**
- Helper T cells are simultaneously activated by antigen.
- Activated helper T cells secrete two substances called **interleukin-2** and **B cell growth factor**, which promote:

1. Activation of more number of B lymphocytes.

2. Proliferation of plasma cells.

3. Production of antibodies.

- **NATURAL KILLER CELL**
- large granular cell with indented nucleus.
- Considered as the third type of lymphocyte (non-T, non-B cell).
- First line of defense in **specific** immunity, particularly against viruses.
- kills the invading organisms or the cells of the body **without prior sensitization**.
- granules contain hydrolytic enzymes such as **perforins** and **granzymes**.
- ***Functions***

1. Destroys the **viruses**

2. Destroys the **viral infected or damaged cells**, which might form tumors

3. Destroys the **malignant cells** and prevents development of cancerous tumors

4. Secretes **cytokines** such as interleukin-2, interferons , colony stimulating factor (GM-CSF) and tumour necrosis factor-α.

- **ANTIBODIES OR IMMUNOGLOBULINS**
- produced by B lymphocytes in response to the presence of an antigen.
- form 20% of the total plasma proteins.
- Antibodies enter almost all the tissues of the body.
- ***Types***
- 1. IgA (Ig alpha)
- 2. IgD (Ig delta)
- 3. IgE (Ig epsilon)
- 4. IgG (Ig gamma)
- 5. IgM (Ig mu).
- Among these antibodies, IgG forms 75% of the antibodies in the body.
- ***Structure of Antibodies***
- gamma globulins (mol wt 1.5- 9,00,000).
- formed by two pairs of chains
- - **heavy** chain consists of about 400 amino acids and
- - **light** chain consists of about 200 amino acids.
- two halves, which are identical

  - held together by disulfide bonds (S–S).

- Each chain of the antibody includes two regions:

1. *Constant Region*

- Amino acids: similar in number and placement (sequence) in all the antibodies of each type. So, this region is called constant region or **Fc** (Fragment **crystallizable**) region.

2. *Variable Region*

- smaller
- Amino acids: different in number and placement (sequence) in each antibody.
- Enables the antibody to **recognize** the **specific antigen** and to **bind** itself with the antigen.
- antigen-binding region or **Fab** (Fragment **antigen binding**) region.
- ***Functions of Different Antibodies***

1. IgA plays a role in localized defense mechanism in external secretions like tear

2. IgD is involved in recognition of the antigen by B lymphocytes

3. IgE is involved in **allergic** reactions

4. IgG, IgM is responsible for **complement fixation**

- ***Mechanism of Actions of Antibodies***

1. *Direct Actions of Antibodies*

- inactivate the invading organism by any one of the following methods:

i. **Agglutination**: foreign bodies like **RBCs** or **bacteria** with antigens on their surfaces are held together in a clump.

ii. **Precipitation**: the soluble antigens like **tetanus toxin** are converted into insoluble forms and then precipitated.

iii. **Neutralization**: During this, the antibodies cover the toxic sites of antigenic products.

iv. **Lysis**: by the most potent antibodies.

- rupture the cell membrane of the organisms and then destroy them.

2. *Actions of Antibodies through Complement System*

- activated in three ways:

a. *Classical pathway*

- C1 binds with the antibodies and triggers a series of events in which other enzymes are activated in sequence.
- enzymes or the byproducts formed during these events produce the following activities:

i. ***Opsonization:*** Activation of neutrophils and macrophages

ii. ***Chemotaxis***: Attraction of leukocytes to the site

iii. ***Lysis*** iv. ***Agglutination*** , v. ***Neutralization:***

vi. ***Activation of mast cells and basophils***

b. ***Lectin pathway***

- occurs when mannose-binding lectin binds with mannose or

fructose group on wall of bacteria, fungi or virus.

c. ***Alternate pathway***

- factor I.
- binds with polysaccharides present in the cell membrane of the invading organisms.
- This binding activates **C3** and **C5**, which ultimately attack the antigenic products of invading organism.
- **IMMUNIZATION**
- procedure by which the body is prepared to fight against a specific disease.
- used to induce the immune resistance of the body to a

specific disease.

- Immunization is of two types

1. **PASSIVE IMMUNIZATION**

- produced without challenging the immune system of the body.
- administration of serum or gamma globulins from a person who is already immunized (affected by the disease) to a non-immune person.
- either naturally or artificially.

Passive Natural Immunization

- short lived
- Before birth: form of maternal antibodies (mainly IgG) through placenta.
- After birth : antibodies (IgA) are transferred through breast milk.

Passive Artificial Immunization

- developed by injecting previously prepared antibodies using serum from humans or animals.
- Prophylactic or immediate protection against acute infections like tetanus,measles, diphtheria, etc. and for poisoning by insects,snakes

2. ACTIVE IMMUNIZATION

- activating immune system of the body.
- Body develops resistance against disease by producing antibodies following the exposure to antigens.
- ***Active Natural Immunization***
- achieved in both clinical and subclinical infections.

a . Clinical infection

b . Subclinical infection

- ***Active Artificial Immunization***
- administration of vaccines or toxoids.
- Applied aspect

Disoder	Variation	Condition
Lymphocytosis	Increase in lymphocytes	Diptheria Infectious hepatitis Mumps Syphlis Thyrotoxicosis tuberculosis
lymphocytopenia	Decrease in lymphocytes	Aids Hodgkins Steroid Radiation therapy

		malnutrition

LEUKOPENIA

bone marrow stops producing WBCs.

Bacteria from the ulcers rapidly invade surrounding tissues and the blood. Without treatment, death often ensues in less than a week after acute total leukopenia begins.

Ulcers may appear in the mouth and colon,or respiratory infection mighdevelop.

Causes:

- x-rays or gamma rays,
- drugs and chemicals (benzene or anthracene nuclei)
- chloramphenicol, thiouracil and Zven various barbiturate hypnotics on rare occasions cause leukopenia.

LEUKEMIAS

- characterized by greatly increased numbers of abnormal WBCs in the circulating blood.
- **Types:**

1. Lymphocytic:

- cancerous production of lymphoid cells
- beginning in a lymph node or other lymphocytic tissue and spreading to other areas of the body.

2. Myelogenous:

- Cancerous production of young myelogenous cells in the bone marrow and then spreads ,especially in the lymph nodes, spleen, and liver.
- Occasionally produces partially differentiated cells, resulting
- *neutrophilic leukemia,*
- *eosinophilic leukemia,*
- *basophilic leukemia,* or
- *monocytic leukemia.*

EFFECTS OF LEUKEMIA ON THE BODY

1. Metastatic growth of leukemic cells in abnormal areas of the body.

2. Invade the surrounding bone (pain and fracture easily).

3. Spread to the spleen, lymph nodes, liver, and other vascular regions.

4. Infection, severe anemia, and thrombocytopenia.

5. Excessive use of metabolic substrates by the growing cancerous cells.

   - Metabolic starvatio death.

PLATLETS AND CLOTTING 12

DR. ABHISHEK GUPTA

- formed elements of blood, fragments of cytoplasm.
- small colorless, non-nucleated and moderately refractive bodies.

Size:

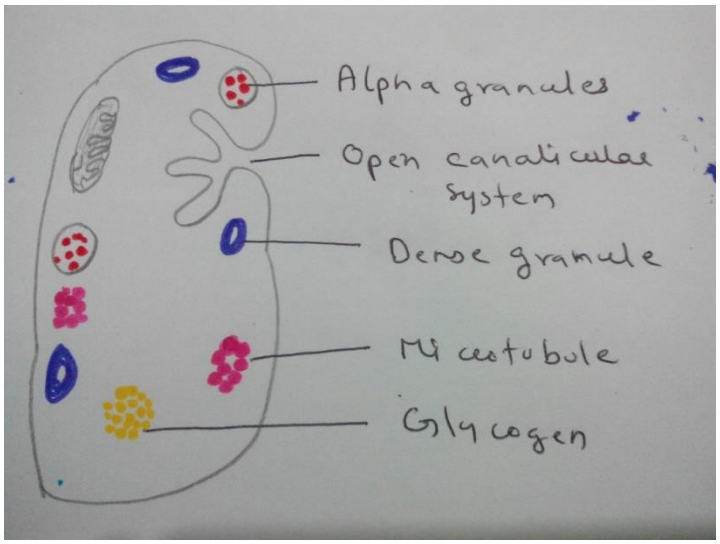
- Diameter : 2.5 μ (2 to 4 μ)
- Volume : 7.5 cu μ (7 to 8 cu μ).

Shape:

- several shapes:spherical or rod-shaped or oval or disk-shaped(inactivated.
- Sometimes, the dumbbell, comma shape, cigar shape.
- **Inactivated** : without processes or filopodia
- Normal platelet count is 2,50,000/cu mm of blood.
- It ranges between 2,00,000 and 4,00,000/cu mm of blood.
- **STRUCTURE COMPOSITION**

Platelet is constituted by:

1. Cell membrane or surface membrane
2. Microtubules
3. Cytoplasm.



1. CELL MEMBRANE

- 6 nm thick.
- Extensive invagination forms an open **canalicular system** .
- - a delicate tunnel system through which the platelet granules extrude their contents.
- contains lipids in the form of **phospholipids**, cholesterol and glycolipids, carbohydrates as glycocalyx and **glycoproteins** and proteins.
- ***Glycoproteins***
- prevent the adherence of platelets to normal endothelium, but accelerate the adherence of platelets to **collagen** and **damaged endothelium** in ruptured blood vessels.
- Glycoproteins also form the receptors for adenosine diphosphate (ADP) and thrombin.
- ***Phospholipids***
- accelerate the clotting reactions.
- form the precursors of thromboxane A2 and other prostaglandin-related substances.

2. MICROTUBULES

- form a ring around cytoplasm below the cell membrane.
- made up of polymerized proteins called **tubulin.**
- - provide structural support for the inactivated platelets to maintain the disklike shape.

3. CYTOPLASM

- contains the cellular organelles,Golgi apparatus, endoplasmic reticulum, mitochondria,microtubule, microvessels, filaments and granules.
- also contains some chemical substances
- such as **proteins**, **enzymes**, **hormonal substances**, etc.
- ***Proteins***

1. ***Contractile proteins***

- i. Actin and myosin: Contractile proteins, which
- are responsible for contraction of platelets.
- ii. Thrombosthenin: Third contractile protein, which
- is responsible for clot retraction.

2. ***von Willebrand factor*** *:* Responsible for adherence of platelets and regulation of plasma level of factor VIII.

3. ***Fibrin-stabilizing factor*** *:* A clotting factor.

4. ***Platelet-derived growth factor*** *(PDGF):* repair of damaged blood vessels and wound healing.

- potent mytogen (chemical agent that promotes mitosis) for smooth muscle fibers of blood vessels.

5. ***Platelet-activating factor*** *(PAF):* aggregation of platelets during the injury of blood vessels,resulting in prevention of excess loss of blood.

6. ***Vitronectin*** *(serum spreading factor):* Promotes adhesion of platelets and spreading of tissue cells in culture.

7. ***Thrombospondin****:* Inhibits angiogenesis (formation of new blood vessels from pre-existing vessels).

Enzymes

- 1. Adensosine triphosphatase (ATPase)
- 2. Enzymes necessary for synthesis of prostaglandins.

Hormonal Substances

- 1. Adrenaline
- 2. 5-hydroxytryptamine (5-HT; serotonin)

- 3. Histamine.
- ***Platelet Granules***
- *Alpha granules*
- 1. Clotting factors – fibrinogen, V and XIII
- 2. Platelet-derived growth factor
- 3. Vascular endothelial growth factor (VEGF)
- 4. Basic fibroblast growth factor (FGF)
- 5. Endostatin
- 6. Thrombospondin.
- *Dense granules*
- Dense granules contain:
- 1. Nucleotides
- 2. Serotonin
- 3. Phospholipid
- 4. Calcium
- 5. Lysosomes.

PROPERTIES OF PLATELETS

1. **ADHESIVENESS**

- Adhesiveness is the property of sticking to a rough surface.
- During injury of blood vessel, endothelium is damaged and the subendothelial collagen is exposed.
- While coming in contact with collagen, platelets are activated and adhere to collagen.
- Adhesion of platelets involves interaction between **von Willebrand factor** secreted by damaged endothelium and a receptor protein called glycoprotein Ib situated on the surface of platelet membrane.
- Other factors which accelerate adhesiveness are collagen, thrombin, ADP, Thromboxane A2, calcium ions, P-selectin and vitronectin.

2. „ **AGGREGATION**

- Adhesion is followed by activation of more number of platelets by substances released from **dense granules** of platelets.
- During activation, the platelets change their shape with elongation of long filamentous pseudopodia which are called **processes** or **filopodia** .
- Filopodia help the platelets aggregate together.
- Activation and aggregation of platelets is accelerated by **ADP, thromboxane A2 and platelet-activating factor**

3. **AGGLUTINATION**

- Agglutination is the **clumping** together of platelets.
- Aggregated platelets are agglutinated by the actions of

some platelet **agglutinins** and **platelet-activating factor**.

- **FUNCTIONS OF PLATELETS**

1. ROLE IN BLOOD CLOTTING

- Platelets are responsible for the formation of intrinsic prothrombin activator (onset of blood clotting)

2. ROLE IN CLOT RETRACTION

- In the blood clot, blood cells including platelets are entrapped in between the fibrin threads.
- Cytoplasm of platelets contains the **contractile proteins,** namely actin, myosin and thrombosthenin, which are responsible for clot retraction.

3. ROLE IN PREVENTION OF BLOOD LOSS (HEMOSTASIS)

- Platelets accelerate the hemostasis by three ways:

i. Platelets secrete **5-HT**, which causes the constriction of blood vessels.

ii. Due to the **adhesive** property, the platelets seal the damage in blood vessels like capillaries.

iii. By formation of **temporary plug**, the platelets seal the damage in blood vessels.

4. ROLE IN REPAIR OF RUPTURED BLOOD VESSEL

- Platelet-derived growth factor (PDGF) formed in cyto -plasm of platelets is useful for the repair of the endothelium and other structures of the ruptured blood vessels.

5. ROLE IN DEFENSE MECHANISM

- By the property of agglutination, platelets encircle the foreign bodies and destroy them.

ACTIVATORS OF PLATELETS

1. Collagen, which is exposed during damage of bloodvessels

2. von Willebrand factor

3. Thromboxane A2

4. Platelet-activating factor

5. Thrombin

6. ADP

7. Calcium ions

8. P-selectin: Cell adhesion molecule secreted from endothelial cells

9. Convulxin: Purified protein from snake venom.

INHIBITORS OF PLATELETS

1. Nitric oxide

2. Clotting factors: II, IX, X, XI and XII

3. Prostacyclin

4. Nucleotidases which breakdown the ADP.

DEVELOPMENT OF PLATELETS

- Platelets are formed from bone marrow.
- Pluripotent stem cell gives rise to the colony forming unit-megakaryocyte (CFU-M).
- This develops into megakaryocyte.
- Cytoplasm of megakaryocyte form **pseudopodium.**
- A portion of pseudopodium is detached to form platelet, which enters the circulation .
- Production of platelets is influenced by colony-stimulating factors and **thrombopoietin.**

LIFESPAN AND FATE OF PLATELETS

- Average lifespan of platelets is 10 days.
- It varies between 8 and 11 days.
- Platelets are destroyed by tissue macrophage system in spleen.
- So, **splenomegaly** (enlargement of spleen) decreases platelet count and
- **splenectomy** (removal of spleen) increases platelet count.
- **APPLIED PHYSIOLOGY**

PHYSIOLOGICAL VARIATIONS

1. ***Age:*** less in infants (1,50,000 to 2,00,000/cu mm) and reaches normal level at 3rd month after birth.

2. ***Sex:*** no difference normally

- reduced during menstruation.

3. ***High altitude:*** increases.

4. ***After meals:*** increases.

PATHOLOGICAL VARIATIONS

- Platelet disorders occur because of pathological variation in platelet **count** and **dysfunction** of platelets.

Platelet disorders are:

1. Thrombocytopenia

2. Thrombocytosis

3. Thrombocythemia

4. Glanzmann's thrombasthenia.

1. *Thrombocytopenia*

- Decrease in platelet count
- leads to thrombocytopenic purpura.
- occurs in the following conditions:

i. Acute infections

ii. Acute leukemia

iii. Aplastic and pernicious anemia

iv. Chickenpox

v. Smallpox

2. *Thrombocytosis*

- Increase in platelet count.
- occurs in the following conditions:

i. Allergic conditions

ii. Asphyxia

iii. Hemorrhage

iv. Bone fractures

v. Surgical operations

vi. Splenectomy

vii. Rheumatic fever

viii. Trauma (wound or injury or damage caused by external force).

3. *Thrombocythemia*

- Thrombocythemia is the condition with **persistent** and abnormal increase in platelet count.
- Thrombocythemia occurs in the following conditions:

i. Carcinoma

ii. Chronic leukemia

iii. Hodgkin's disease.

4. *Glanzmann's Thrombasthenia*

- inherited hemorrhagicdisorder,
- caused by structural or functional abnormality of platelets.
- the platelet count is normal.
- leads to **thrombasthenic purpura**

- characterized by normal clotting time, normal or prolonged bleeding time
    - but defective **clot retraction**.
- Hemostasis
- arrest or stoppage of bleeding.

STAGES

- the injury initiates a series of reactions, resulting in hemostasis.
- **VASOCONSTRICTION**
- **PLATELET PLUG FORMATION**
- **COAGULATION OF BLOOD**

Coagulation

- process in which blood loses its fluidity and becomes a jelly-like mass few minutes after it is shed out or collected in a container.
- occurs through a series of reactions due to the activation of a group of substances.
- Thirteen clotting factors are identified

SEQUENCE OF CLOTTING MECHANISM ENZYME CASCADE THEORY

- all the factors are present in the form of inactive **proenzyme.**
- Activation is carried out by a series of proenzyme-enzyme conversion reactions.
- Enzyme cascade theory : explains how various reactions, involved in the conversion of proenzymes to active enzymes take place in the form of a cascade.

Stages of Blood Clotting

STAGE 1: FORMATION OF PROTHROMBIN ACTIVATOR

- initiated by substances produced either within the blood or outside the blood.

i. ***Intrinsic Pathway for the Formation of Prothrombin Activator***

- initiated by platelets, which are within the blood itself

ii.***Extrinsic Pathway for the Formation of Prothrombin Activator***

- In this pathway, the formation of prothrombin activator

is initiated by the tissue thromboplastin, which is formed

from the injured tissues.

STAGE 2: CONVERSION OF PROTHROMBIN INTO THROMBIN

- Blood clotting is all about thrombin formation.
- Once thrombin is formed, it definitely leads to clot formation.

STAGE 3: CONVERSION OF FIBRINOGEN INTO FIBRIN

- The final stage of blood clotting involves the conversion of fibrinogen into fibrin by thrombin.

BLOOD CLOT

- mass of coagulated blood which contains RBCs, WBCs and platelets entrapped in fibrin meshwork.
- The external blood clot is also called scab.
- It adheres to the opening of damaged blood vessel and prevents blood loss.

CLOT RETRACTION

- After the formation, the blood clot starts contracting.
- after about 30 to 45 minutes, the straw-colored serum oozes out of the clot.
- Contractile proteins, namely actin, myosin and thrombosthenin in the cytoplasm of platelets are responsible for clot retraction.

FIBRINOLYSIS

- Lysis of blood clot inside the blood vessel.
- helps to remove the clot from lumen of the blood vessel.
- requires **plasmin** or fibrinolysin.
- Formation of plasmin
- **ANTICLOTTING**

1. *Physical Factors*

i. Continuous circulation of blood.

ii. Smooth endothelial lining of the blood vessels.

2. *Chemical Factors – Natural Anticoagulants*

i. heparin

ii. thrombomodulinotein

iii. All the clotting factors are in inactive state.

ANTICOAGULANTS

- prevent or postpone coagulation of blood
- Anticoagulants are of three types:

1. Anticoagulants used to prevent blood clotting inside the body, i.e. *in **vivo***.

2. Anticoagulants used to prevent clotting of blood that is collected from the body, i.e. *in **vitro***.

3. Anticoagulants used to prevent blood clotting **both *in vivo* and *in vitro***.

1. HEPARIN

- a conjugated polysaccharide.
- produced by **mast cells** ,also by Basophils.
- ***Mechanism of Action***

i. Antithrombin - directly suppresses the activity of thrombin

ii. Combines with antithrombin III (a protease inhibitor present in circulation) and removes thrombin from circulation

iii. Activates antithrombin III

iv. Inactivates the active form of other clotting factors like IX, X, XI and XII .

Uses of Heparin

- both *in vivo* and *in vitro.*
- *use*:

i. To prevent intravascular blood clotting during **surgery**.

ii. While passing the blood through artificial kidney for **dialysis**.

iii. During cardiac surgery, which involves **heartlung machine**.

iv. To preserve the blood before **transfusion**.

- also used as anticoagulant *in vitro* while collecting blood for various investigations.

COUMARIN DERIVATIVES

- Warfarin and dicoumoral are the derivatives of coumarin.

Mechanism of Action

- Coumarin derivatives prevent blood clotting by inhibiting the action of vitamin K.
- Vitamin K is essential for the formation of various clotting factors, namely II, VII, IX and X.

Uses

- commonly used **oral anticoagulants** (*in vivo*).
- Prevent myocardial infarction (heart attack), strokes and thrombosis.

3. EDTA

- Ethylenediaminetetraacetic acid (EDTA) ,a strong anticoagulant.
- i. Disodium salt (Na2 EDTA).
- ii. Tripotassium salt (K3 EDTA).

Mechanism of Action

- prevent blood clotting by removing calcium from blood.

Uses

- both *in vivo* and *in vitro*.
- i. i.v - lead poisoning.
- ii. Used in the laboratory .

4. OXALATE COMPOUNDS

- prevent coagulation by forming calcium oxalate, which is precipitated later.

Mechanism of Action

- Oxalate combines with calcium and forms insoluble calcium oxalate.

Uses

- only as *in vitro* anticoagulants
- Since oxalate is poisonous, it cannot be used *in vivo*.

5. CITRATES

- Sodium, ammonium and potassium citrates are used as anticoagulants.

Mechanism of Action

- combines with calcium in blood to form insoluble calcium citrate.

Uses

i. It is used to store blood in the **blood bank** as:

a. Acid citrate dextrose (ACD)

b. Citrate phosphate dextrose (CPD)

ii. laboratory : formol-citrate solution (Dacie's solution) for RBC and platelet counts.

PHYSICAL METHODS

COLD

- about 5°C postpones the coagulation of blood.

COLLECTING BLOOD IN A CONTAINER WITH SMOOTH SURFACE

- like a **silicon-coated** container prevents clotting.
- smooth surface inhibits the activation of factor XII andplatelets.

PROCOAGULANTS

- accelerate the process of blood coagulation.

THROMBIN

- **SNAKE VENOM**
- **EXTRACTS OF LUNGS AND THYMUS**
- **SODIUM OR CALCIUM ALGINATE**
- **OXIDIZED CELLULOSE**
- **TESTS FOR BLOOD CLOTTING**

diagnose blood disorders,

monitor the patients treated with anticoagulant drugs

1. Bleeding time

2. Clotting time

3. Prothrombin time

4. Partial prothrombin time

5. International normalized ratio

6. Thrombin time.

1. BLEEDING TIME

- time interval from oozing of blood after a cut or injury till arrest of bleeding.
- Duke method: using blotting paper or filter paper method.
- normal duration is 3 to 6 minutes.
- prolonged in purpura.

2. **CLOTTING TIME**

- time interval from oozing of blood after injury till the formation of clot.
- capillary tube method-3 to 8 minutes.
- prolonged in hemophilia.

3. **PROTHROMBIN TIME**

- time taken by blood to clot after adding tissue thromboplastin to it.
- Prothrombin time indicates the total quantity of prothrombin present in the blood.
- Normal duration: 10 to 12 seconds.
- It is prolonged in deficiency of prothrombin and other factors like factors I, V, VII and X.
- normal in hemophilia.

4. **PARTIAL PROTHROMBIN TIME OR ACTIVATED PROTHROMBIN TIME**

- time taken for the blood to clot after adding an activator such as **phospholipid** , along with calcium & surface activator to it.
- useful in monitoring the patients taking anticoagulant drugs.
- Phospholipid serves as **platelet substitute.**

- Commonly used surface activator is **kaolin.**
- Normal: 30 to 45 seconds.
- prolonged in **heparin or warfarin therapy** and deficiency

or inhibition of factors II, V, VIII, IX, X, XI and XII.

5. **INTERNATIONAL NORMALIZED RATIO**

- rating of a patient's prothrombin time when compared to an average.
- measures **extrinsic** clotting pathway system.
- monitoring impact of anticoagulant drugs such as warfarin and to adjust the dosage of anticoagulants.
- Normal INR : 1.
- INR > 4 : blood is clotting too slowly and there is a risk of uncontrolled blood clotting.

6. THROMBIN TIME

- time taken for the blood to clot after adding thrombin to it.
- done to investigate the presence of heparin in plasma or to detect fibrinogen abnormalities.
- Normal: 12 to 20 seconds.
- prolonged in heparin therapy and
- During dysfibrinogenemia

APPLIED PHYSIOLOGY

A. BLEEDING DISORDERS

- conditions characterized by prolonged bleeding time or clotting time.
- Bleeding disorders are of three types:

1. Hemophilia.

2. Purpura.

3. von Willebrand disease.

- **1. *Hemophilia***
- group of sex-linked inherited blood disorders,

- characterized by prolonged clotting time.
- Usually, it affects the males, with the females being the carriers.
- Easy bruising and hemorrhage in muscles and joints are also common in this disease.
- ***Causes***
- lack of formation of prothrombin activator.
- formation of prothrombin activator is affected due to

the deficiency of factor VIII, IX or XI.

Types of hemophilia

i. Hemophilia A or **classic hemophilia:** deficiency of factor VIII.

ii. Hemophilia B or **Christmas disease:** deficiency of factor IX

iii. Hemophilia C or factor XI deficiency: deficiency of factor XI.

***Symptoms** of hemophilia*

i. Spontaneous bleeding.

ii. Prolonged bleeding due to cuts, tooth extraction and surgery.

iii. Hemorrhage in gastrointestinal and urinary tracts.

iv. Bleeding in joints followed by swelling and pain

v. Appearance of blood in urine.

2. *Purpura*

characterized by prolonged bleeding time.

the clotting time is normal.

Characteristic feature: spontaneous bleeding under the skin from ruptured capillaries.

hemorrhagic spots in many areas of the body.

hemorrhagic spots under the skin are called **purpuric spots**

Sometimes **ecchymoses.**

Types:

- i. *Thrombocytopenic purpura*

- due to the deficiency of platelets.
- In bone marrow disease

- ii. *Idiopathic thrombocytopenic purpura*
  - unknown cause
  - believed that platelet count decreases due to the development of antibodies against platelets, which occurs after blood transfusion.
- iii. *Thrombasthenic purpura*
  - due to structural or functional abnormality of platelets. However, the platelet count is
  - defective clot retraction

3. *von Willebrand Disease*

- excess bleeding even with a mild injury.
- deficiency of von Willebrand factor

- a protein secreted by **endothelium** of damaged blood vessels and platelets.

- responsible for **adherence** of platelets to endothelium of

blood vessels during hemostasis after an injury.

- also responsible for the survival and maintenance of factor VIII in plasma.
- excess bleeding, which resembles the bleeding that occurs during platelet dysfunction or hemophilia.,,

B. THROMBOSIS

- coagulation of blood inside the blood vessels.
- ***Causes of Thrombosis***

1. *Injury to blood vessels*
2. *Roughened endothelial lining*
3. *Sluggishness of blood flow*
4. *Agglutination of RBCs*
5. *Toxic thrombosis*

6. *Congenital absence of protein C*

BLOOD GROUPS AND TRANSFUSION 13

DR. SURENDRA KUMAR ACHARYA

ABO BLOOD GROUPS

- discovered by the Austrian Scientist **Karl Landsteiner,** in 1901
- Determination of ABO blood groups depends upon the immunological reaction between antigen and antibody.
- Landsteiner found two antigens on the surface of RBCs and named them as A antigen and B antigen.

  - also called agglutinogens
- He noticed the corresponding antibodies or agglutinins in the plasma (anti-A or α-antibody and anti-B or β-antibody) „

LANDSTEINER LAW

Landsteiner law states that:

1. If a particular **agglutinogen** (antigen) is present in the RBCs, corresponding **agglutinin** (antibody) must be absent in the serum.

2. If a particular agglutinogen is absent in the RBCs the corresponding agglutinin must be present in the serum.

Though the second part of Landsteiner law is a fact, it is not applicable to Rh factor.

BLOOD GROUP SYSTEMS

- More than 20 genetically determined blood group systems are known today.
- But, Landsteiner discovered two blood group systems called the **ABO system** and the **Rh system**.

ABO SYSTEM

- Based on the presence or absence of antigen A and antigen B, blood is divided into four groups:

1.'A' group - antigen A

- β-antibody in the serum

2. 'B' group - antigen B

- α-antibody

3. 'AB' group - both the antigens are present,

- serum of this group does not contain any antibody

4. 'O' group – both antigens are absent

- both α and β antibodies are present in the serum.

IMPORTANCE OF ABO GROUPS IN BLOOD TRANSFUSION

- While transfusing the blood, **antigen** of the **donor** and the **antibody** of the **recipient** are considered.
- antibody of the donor and antigen of the recipient are ignored mostly.

1. RBC of 'O' group has no antigen and so agglutination does not occur with any other group of blood. So, 'O' group blood can be given to any blood group persons and the people with this blood group are called 'universal donors'.

2. Plasma of AB group blood has no antibody. This does not cause agglutination of RBC from any other group . People with AB group can receive blood from any blood group persons. So, people with this blood group are called 'universal recipients'.

MATCHING AND CROSS-MATCHING

- cross-matching is done after the blood is typed.
- done to find out whether the person's body will accept the donor's blood or not.
- For blood matching: **RBC** of the individual (**recipient**) and **test sera** are used.
- Cross-matching: is done by mixing the serum of the recipient and the RBCs of donor - **always done before blood transfusion**.

TRANSFUSION REACTIONS

- reactions may be **mild** causing only fever and hives (skin disorder characterized by itching) or may be **severe** leading to renal failure, shock and death.

- In mismatched transfusion, the transfusion reactions occur between **donor's RBC** and **recipient's plasma**.
- But, if recipient's **plasma** contains **agglutinins** against donor's RBCs, the immune system launches a response
- Donor RBCs are agglutinated resulting in transfusion reactions

Signs and Symptoms of Transfusion Reactions

Non-hemolytic transfusion reaction

- within a few minutes to hours .
- Common symptoms are fever, difficulty in breathing and itching.

Hemolytic transfusion reaction

- may be acute or delayed.
- **Acute:** within few minutes of transfusion.
- develops because of rapid hemolysis of donor's RBCs.
- Symptoms include fever, chills, increased heart rate, low blood pressure, shortness of breath,bronchospasm, nausea, vomiting, red urine, chest pain, back pain and rigor..
- **Delayed**: occurs from 1 to 5 days
- hemolysis of RBCs results in release of large amount of hemoglobin into the plasma causing:

1. ***Jaundice***
2. ***Cardiac Shock***
3. ***Renal Shutdown***

Rh FACTOR

- Rh factor is an antigen present in RBC.
- discovered by Landsteiner and Wiener in **Rhesus monkey** and hence the name 'Rh factor'.
- There are many Rh antigens but only the **D antigen** is more antigenic in human.

- The persons having D antigen are called ‘Rh positive’ and those without D antigen are called ‘Rh negative’.
- Among Indian population, 85% of people are Rh positive and 15% are Rh negative
- Rh group system is different from ABO group system because, the antigen D does not have corresponding natural antibody (anti-D).
- However, if Rh positive blood is transfused to a Rh negative person anti-D is developed in that person.
- On the other hand, there is no risk of complications if the Rh positive person receives Rh negative blood.

INHERITANCE OF Rh ANTIGEN

- Rhesus factor is an inherited dominant factor.
- It may be homozygous Rhesus positive with DD or heterozygous Rhesus positive with Dd.
- Rhesus negativeoccurs only with complete absence of D (i.e. with homozygous dd).

TRANSFUSION REACTIONS DUE TO Rh INCOMPATIBILITY

- **1st time**: not affected much, since the reactions do not occur immediately.
- Rh antibodies develop within one month.
- The transfused RBCs, which are still present in the recipient’s blood,are agglutinated.
- These agglutinated cells are lysed by macrophages.
- So, a delayed transfusion reaction occurs.
- **second time:** the donor RBCs are agglutinated and severe transfusion reactions occur immediately.
- These reactions are similar to the reactions of ABO incompatibility.
- **ERYTHROBLASTOSIS FETALIS**
- **HEMOLYTIC DISEASE OF FETUS AND NEWBORN**
- abnormal hemolysis of RBCs due to Rh incompatibility, i.e. the difference between the Rh blood group of the mother and baby.
- When a mother is Rh negative and fetus is Rh positive (the Rh factor being inherited from the father),usually the first child escapes the complications of Rh incompatibility.

- This is because the Rh antigen cannot pass from fetal blood into the mother's blood through the **placental barrier.**
- at the time of parturition the Rh antigen from fetal blood may leak into mother's blood because of **placental detachment.**
- During postpartum period, i.e. within a month after delivery, the mother develops Rh antibody in her blood.

- When the mother conceives for the second time and if the fetus happens to be Rh positive again, the Rh antibody from mother's blood crosses placental barrier and enters the fetal blood.
- Thus, the Rh antigen cannot cross the placental barrier, whereas Rh antibody can cross it.
- Rh antibody which enters the fetus causes **agglutination** of fetal RBCs resulting in **hemolysis**.
- Severe hemolysis in the fetus causes **jaundice**.
- To compensate the hemolysis of more and more number of

RBCs, there is rapid production of RBCs, not only from

bone marrow, but also from spleen and liver.

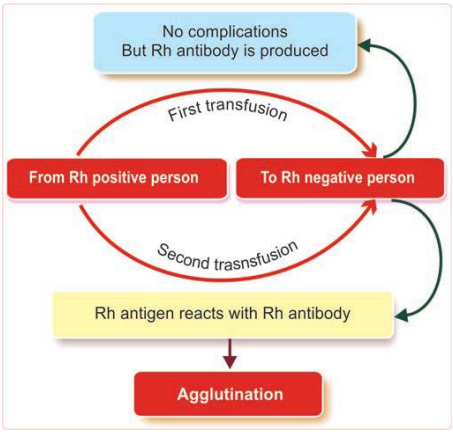
- Now, many **large** and **immature cells** in **proerythroblastic** stage are released into circulation.
- Because of this, the disease is called erythroblastosis fetalis.
- Ultimately due to excessive hemolysis severe complications develop.

1. *Severe Anemia*

- Excessive hemolysis results in anemia and the infant dies when anemia becomes severe.

2. *Hydrops Fetalis*

- Characterized by edema.
- enlargement of liver and spleen and cardiac failure.



- it may lead to **intrauterine death** of fetus.

3. *Kernicterus*

- **brain damage** in infants caused by severe jaundice.
- because of high bilirubin content.

LEWIS BLOOD GROUP

- first found in a subject named Mrs Lewis.
- The antibody that was found in this lady reacted with the antigens found on RBCs and in bodyfluids such as saliva, gastric juice, etc.
- The antigens,are formed in the tissues,released in the body secretions and then absorbed by the RBC membrane.
    - secretor antigens.
- Presence of Lewis antigens in children leads to some complications such as retarded growth.
- Sometimes,it causes transfusion reactions also.

MNS BLOOD GROUPS

- MNS blood groups are determined by their reactions with anti-M, anti-N and anti-S.
- these blood groups rarely cause any trouble like hemolysis following transfusion.

OTHER BLOOD GROUPS

i. Auberger groups

ii. Diego group

iii. Bombay group

iv. Duffy group

v. Lutheran group

IMPORTANCE OF KNOWING BLOOD GROUP

1. **Medically**, it is important during blood transfusions and in tissue transplants.

2. **Socially**, one should know his or her own blood group and become a member of the Blood Donor's Club so that he or she can be approached for blood donation during emergency conditions.

3. prevent the complications due to **Rh incompatibility** and save the child from the disorders like erythroblastosis fetalis.

4. **Judicially**, it is helpful in medico-legal cases to sort out parental disputes.

CONDITIONS WHEN BLOOD TRANSFUSION IS NECESSARY

1. Anemia

2. Hemorrhage

3. Trauma

4. Burns

5. Surgery.

PRECAUTIONS – BEFORE TRANSFUSION

1. Donor must be healthy, without any diseases like: syphilis,

   hepatitis, AIDS,etc.

2. Only compatible blood must be transfused

3. Both matching and cross-matching must be done

4. Rh compatibility must be confirmed.

PRECAUTIONS TO BE TAKEN WHILE TRANSFUSING BLOOD

1. Apparatus for transfusion must be sterile

2. Temperature of blood to be transfused must be same as the body temperature

3. Transfusion of blood must be slow. The sudden rapid infusion of blood into the body increases the load on the heart, resulting in many complications.

HAZARDS OF BLOOD TRANSFUSION

1.Reactions due to mismatched (incompatible) blood transfusion – transfusion reactions

2. Reactions due to massive blood transfusion

3. Reactions due to faulty techniques during bloodtransfusion

4. Transmission of infections.

REACTIONS DUE TO MASSIVE BLOOD TRANSFUSION

transfusion of blood equivalent or more than the patient's own blood volume. leads to

i. **Circulatory shock**

ii. **Hyperkalemia**

iii. **Hypocalcemia**

iv. **Hemosiderosis**

REACTIONS DUE TO FAULTY TECHNIQUES

DURING BLOOD TRANSFUSION

- Faulty techniques adapted during blood transfusion leads to:

i. **Thrombophlebitis** (inflammation of vein,

- associated with formation of thrombus).

ii. **Air embolism** (obstruction of blood vessel due to

- entrance of air into the bloodstream).

TRANSMISSION OF INFECTIONS

i. HIV

ii. Hepatitis B and A

iii. **Glandular fever** or infectious mononucleosis (acute infectious disease caused by **Epstein- Barr virus** and characterized by fever, swollen lymph nodes, sore throat and abnormal lymphocytes)

iv. **Herpes** (viral disease with eruption of small blister-like vesicles on skin or membranes)

v. Bacterial infections.

BLOOD SUBSTITUTES

- instead of whole blood :

1. Human plasma

2. 0.9% sodium chloride solution (saline) and 5% glucose

3. Colloids like gum acacia, isinglass, albumin and animal gelatin.

EXCHANGE TRANSFUSION

- procedure which involves removal of patient's blood completely and replacement with fresh blood or plasma of the donor known as **replacement transfusion.**
- life-saving procedure carried out in conditions such as severe jaundice, sickle cell anemia, erythroblastosis fetalis, etc.

PROCEDURE

CONDITIONS WHICH NEED EXCHANGE TRANSFUSION

1. Hemolytic disease of the newborn (erythroblastosis fetalis).

2. Severe sickle cell anemia.

3. Severe polycythemia (replacement with saline, plasma or albumin).

4. Toxicity of certain drugs.

5. Severe jaundice in newborn babies, which does not respond to **ultraviolet light therapy.**

Normally,neonatal jaundice is treated by exposure to ultraviolet rays.It breaks down the bilirubin which is excreted by liver.

AUTOLOGOUS BLOOD TRANSFUSION

- collection and reinfusion of patient's own The conventional transfusion of blood
- Autologous blood transfusion is used for planned surgical procedures.
- Patient's blood is withdrawn in advance and stored.
- Later, it is infused if necessary during surgery.
- prevents the transmission of viruses such as HIV or hepatitis B.
- eliminates transfusion reactions.
- Reason for hair on end appearance

1. widening of diploic space , and thinning of outer and inner table or virtual disappearance of the outer table

2. periosteal reaction manifesting as perpendicular trabeculations interspersed by radiolucent marrow hyperplasia along the skull vault (new bone formation at right angles to the inner table)

Bone changes due to marrow hyperplasia

- In sickle cell anaemia the erythroid hyperplasia affects the bone trabeculae and causes absorption, osteoporosis, softening and change in shape (Carroll and Evans 1949).
- The most obvious radiographic changes may occur in the skull.
- A loss of trabecular definition, giving a "ground glass" appearance, is seen, and the outer table appears thin and partly absorbed (Cole 1955).

Polychythemia vera

- manifest intraorally with - erythema of mucosa, - glossitis, and

  - erythematous, edematous gingiva.

- Spontaneous gingival bleeding can occur

PROCEDURE:

- Affected person's blood is slowly drawn out in small quantities of 5 to 20 mL, depending upon the age and size of the person and the severity of the condition.
- Equal quantity of fresh, prewarmed blood or plasma is infused through intravenous catheter. This is carried out for few minutes.
- Catheter is left in place and the transfusion is repeated within few hours.
- This procedure is continued till the whole or predetermined volume of blood is exchanged.

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