PHYSIOLOGY I 2018–2019

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Hematology

Blood as a system

Function of Blood-Blood is a type of liquid connective tissue and is specialized fluid of connective tissue.

- Contains cells suspended in a fluid matrix
- The major function of blood is transport.

Subfunctions

Respiration :-if oxygen and carbon dioxide are transported
Trophic : -when the nutrient materials are delivered to the tissues
Excretive : -when the metabolites are delivered from tissues to excretory organs
Regulative : if the hormones and BAS are transported
Homeostatic :maintenance of water content and acid-basebalance
Protective : immunity and non-specific resistance;
blood coagulation

- Maintenance of body temperature :-as a result of a redistribution of blood volume between skin and the internal organs at high and low temperature of external environment.

Blood (peripheral circulating)
Organs for haemopoiesis destruction of blood
Regulatory apparatus (nervous and humoral)

The total blood volume makes up about 6-8 percent (1/13) of the body's weight in human, 1/15 in horse, 1/12 in cow, 1/13 in dog and sheep, 1/22 in pig, 1/20 in cat, rabbit and guinea pig. Accordingly, a 70-kilogram person will have 5 to 6 litres of blood. Circulating blood volume will be lesser than total blood volume, because some amount of blood will be deposited in organs like liver.

General Characteristics of Blood

- 38°C (100.4°F) is normal temperature
- High viscosity
- Slightly alkaline pH (7.35–7.45)

Blood composition

Blood consists of
liquid **plasma** (volume-55-60%)
formed elements (cells) (volume-40-45%)
Formed elements include

1. Erythrocytes (red blood cells, RBCs);
   - transport oxygen
2. Leukocytes (White blood cells, WBCs):
   - part of the immune system
3. Platelets (Thrombocytes):
   - cell fragments involved in clotting

The hematocrit, also known as packed cell volume (PCV) or erythrocyte volume fraction (EVF), is the volume percentage (%) of red blood cells in theblood. It is normally about 40-48% for men and 36-42% for women.

If a portion of blood is centrifuged or allowed to stand for a sufficient long time, it will be found that the blood cells will settle towards the bottom of the test tube while the plasma remains on top. By this means the percentage of blood cells in whole blood can be determined.

ACID-BASE BALANCE

Learning Objectives

By the end of this section, you will be able to:

- Identify the most powerful buffer system in the body
- Explain the way in which the respiratory system affects blood pH

Proper physiological functioning depends on a very tight balance between the concentrations of acids and bases in the blood. Acid-balance balance is measured using the pH scale, as shown in Figure 1. A variety of buffering systems permits blood and other bodily fluids to maintain a narrow pH range, even in the face of perturbations. A buffer is a chemical system that prevents a radical change in fluid pH by dampening the change in hydrogen ion concentrations in the case of excess acid or base. Most commonly, the substance that absorbs the ions is either a weak acid, which takes up hydroxyl ions, or a weak base, which takes up hydrogen ions.

BUFFER SYSTEMS IN THE BODY

The buffer systems in the human body are extremely efficient, and different systems work at different rates. It takes only seconds for the chemical buffers in the blood to make adjustments to pH. The respiratory tract can adjust the blood pH upward in minutes by exhaling CO_2 from the body. The renal system can also adjust blood pH through the excretion of hydrogen ions (H^+) and the conservation of bicarbonate, but this process takes hours to days to have an effect.

The buffer systems functioning in blood plasma include plasma proteins, phosphate, and bicarbonate and carbonic acid buffers. The kidneys help control acid-base balance by excreting hydrogen ions and generating bicarbonate that helps maintain blood plasma pH within a normal range. Protein buffer systems work predominantly inside cells.

PROTEIN BUFFERS IN BLOOD PLASMA AND CELLS

Nearly all proteins can function as buffers. Proteins are made up of amino acids, which contain positively charged amino groups and negatively charged carboxyl groups. The charged regions of these molecules can bind hydrogen and hydroxyl ions, and thus function as buffers. Buffering by proteins accounts for two-thirds of the buffering power of the blood and most of the buffering within cells.

HEMOGLOBIN AS A BUFFER

Hemoglobin is the principal protein inside of red blood cells and accounts for one-third of the mass of the cell. During the conversion of CO_2 into bicarbonate, hydrogen ions liberated in the reaction are buffered by hemoglobin, which is reduced by the dissociation of oxygen. This buffering helps maintain normal pH. The process is reversed in the pulmonary capillaries to re-form CO_2, which then can diffuse into the air sacs to be exhaled into the atmosphere. This process is discussed in detail in the chapter on the respiratory system.

PHOSPHATE BUFFER

Phosphates are found in the blood in two forms: sodium dihydrogen phosphate ($Na_2H_2PO_4^-$), which is a weak acid, and sodium monohydrogen phosphate (Na_2HPO42-), which is a weak base. When Na_2HPO42- comes into contact with a strong acid, such as HCl, the base picks up a second hydrogen ion to form the weak acid $Na_2H_2PO_4^-$ and sodium chloride, NaCl. When $Na_2HPO42-$ (the weak acid) comes into contact with a strong base, such as sodium hydroxide (NaOH), the weak acid reverts back to the weak base and produces water. Acids and bases are still present, but they hold onto the ions.

$$HCl + Na_2HPO_4 \rightarrow NaH_2PO_4 + NaCl$$

(strong acid) + (weak base) → (weak acid) + (salt)

$$NaOH + NaH_2PO_4 \rightarrow Na_2HPO_4 + H_2O$$

(strong base) + (weak acid) → (weak base) + (water)

BICARBONATE-CARBONIC ACID BUFFER

The bicarbonate-carbonic acid buffer works in a fashion similar to phosphate buffers. The bicarbonate is regulated in the blood by sodium, as are the phosphate ions. When sodium bicarbonate ($NaHCO_3$), comes into contact with a strong acid, such as HCl, carbonic acid (H_2CO_3), which is a weak acid, and NaCl are formed. When carbonic acid comes into contact with a strong base, such as NaOH, bicarbonate and water are formed.

$$NaHCO_3 + HCl \rightarrow H_2CO_3 + NaCl$$

(sodium bicarbonate) + (strong acid) → (weak acid) + (salt)

$$H_2CO_3 + NaOH \rightarrow HCO_{3-} + H_2O$$

(weak acid) + (strong base)→(bicarbonate) + (water)

As with the phosphate buffer, a weak acid or weak base captures the free ions, and a significant change in pH is prevented. Bicarbonate ions and carbonic acid are present in the blood in a 20:1 ratio if the blood pH is within the normal range. With 20 times more bicarbonate than carbonic acid, this capture system is most efficient at buffering changes that would make the blood more acidic. This is useful because most of the body's metabolic wastes, such as lactic acid and ketones, are acids. Carbonic acid levels in the blood are controlled by the expiration of CO_2 through the lungs. In red blood cells, carbonic anhydrase forces the dissociation of the acid, rendering the blood less acidic. Because of this acid dissociation, CO_2 is exhaled (see equations above). The level of bicarbonate in the blood is controlled through the renal system, where bicarbonate ions in the renal filtrate are conserved and passed back into the blood. However, the bicarbonate buffer is the primary buffering system of the IF surrounding the cells in tissues throughout the body.

RESPIRATORY REGULATION OF ACID-BASE BALANCE

The respiratory system contributes to the balance of acids and bases in the body by regulating the blood levels of carbonic acid (Figure 2). CO_2 in the blood readily reacts with water to form carbonic acid, and the levels of CO_2 and carbonic acid in the blood are in equilibrium. When the CO_2 level in the blood rises (as it does when you hold your breath), the excess CO_2 reacts with water to form additional carbonic acid, lowering blood pH. Increasing the rate and/or depth of respiration (which you might feel the "urge" to do after holding your breath) allows you to exhale more CO_2. The loss of CO_2 from the body reduces blood levels of carbonic acid and thereby adjusts the pH upward, toward normal levels. As you might have surmised, this process also works in the opposite direction. Excessive deep and rapid breathing (as in hyperventilation) rids the blood of CO_2 and reduces the level of carbonic acid, making the blood too alkaline. This brief alkalosis can be remedied by rebreathing air that has been exhaled into a paper bag. Rebreathing exhaled air will rapidly bring blood pH down toward normal.

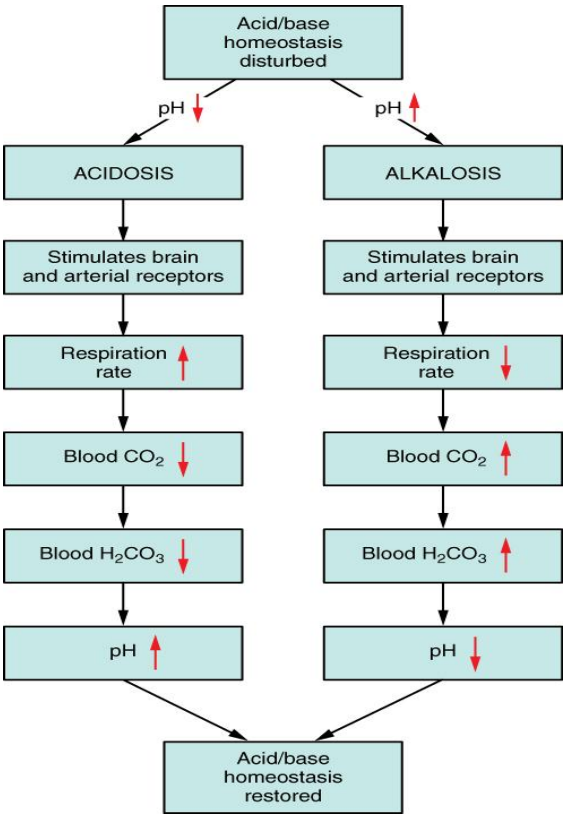


Figure 2. **Respiratory Regulation of Blood pH.** The respiratory system can reduce blood pH by removing CO2 from the blood.

The chemical reactions that regulate the levels of CO_2 and carbonic acid occur in the lungs when blood travels through the lung's pulmonary capillaries. Minor adjustments in breathing are usually sufficient to adjust the pH of the blood by changing how much CO_2 is exhaled. In fact, doubling the respiratory rate for less than 1 minute, removing "extra" CO_2, would increase the blood pH by 0.2. This situation is common if you are exercising strenuously over a period of time. To keep up the necessary energy production, you would produce excess CO_2 (and lactic acid if exercising beyond your aerobic threshold). In order to balance the increased acid production, the respiration rate goes up to remove the CO_2. This helps to keep you from developing acidosis.

The body regulates the respiratory rate by the use of chemoreceptors, which primarily use CO_2 as a signal. Peripheral blood sensors are found in the walls of the aorta and carotid arteries. These sensors signal the brain to provide immediate adjustments to the respiratory rate if CO_2 levels rise or fall. Yet other sensors are found in the brain itself. Changes in the pH of CSF affect the respiratory center in the medulla oblongata, which can directly modulate breathing rate to bring the pH back into the normal range.

Hypercapnia, or abnormally elevated blood levels of CO_2, occurs in any situation that impairs respiratory functions, including pneumonia and congestive heart failure. Reduced breathing (hypoventilation) due to drugs such as morphine, barbiturates, or ethanol (or even just holding one's breath) can also result in hypercapnia. Hypocapnia, or abnormally low blood levels of CO_2, occurs with any cause of hyperventilation that drives off the CO_2, such as salicylate toxicity, elevated room temperatures, fever, or hysteria.

A variety of buffering systems exist in the body that helps maintain the pH of the blood and other fluids within a narrow range—between pH 7.35 and 7.45. A buffer is a substance that prevents a radical change in fluid pH by absorbing excess hydrogen or hydroxyl ions. Most commonly, the substance that absorbs the ion is either a weak acid, which takes up a hydroxyl ion (OH^-), or a weak base, which takes up a hydrogen ion (H^+). Several substances serve as buffers in the body, including cell and plasma proteins, hemoglobin, phosphates, bicarbonate ions, and carbonic acid. The bicarbonate buffer is the primary buffering system of the IF surrounding the cells in tissues throughout the body. The respiratory and renal systems also play major roles in acid-base homeostasis by removing CO_2 and hydrogen ions, respectively, from the body.

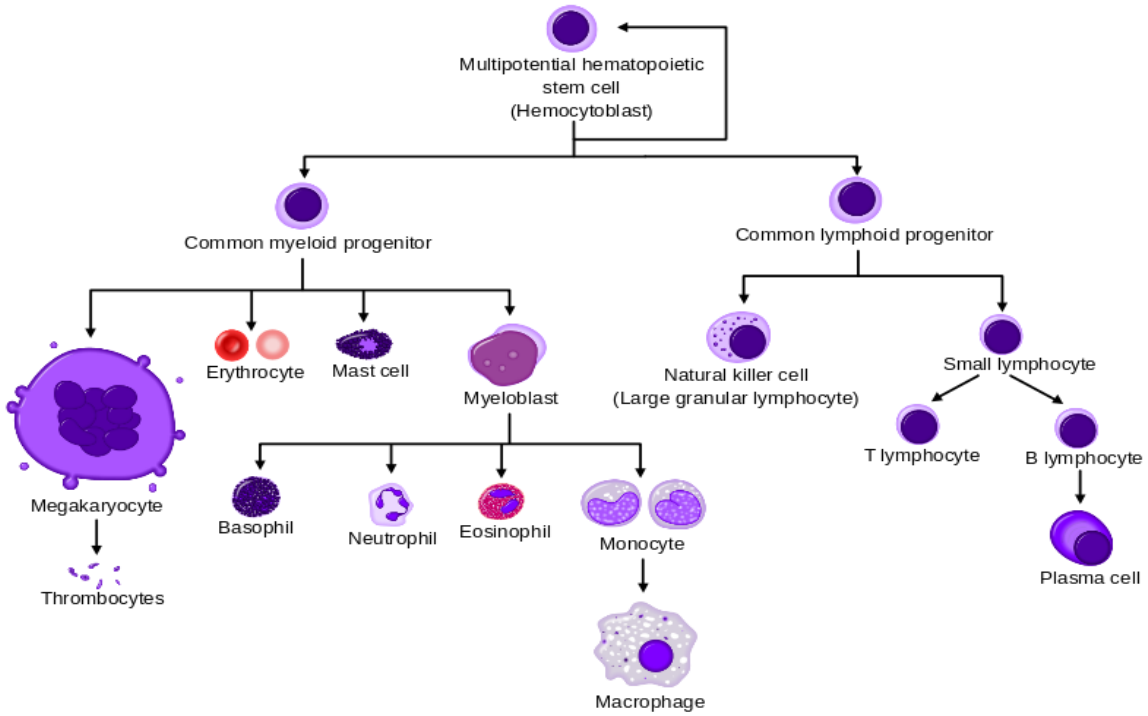
Haemopoiesis Haemopoiesis is the formation of blood cellular components and process of producing formed elements by myeloid and lymphoid stem cells. The All cellular blood components are derived from pluripotent haemopoietic stem cells which is present in the bone marrow.

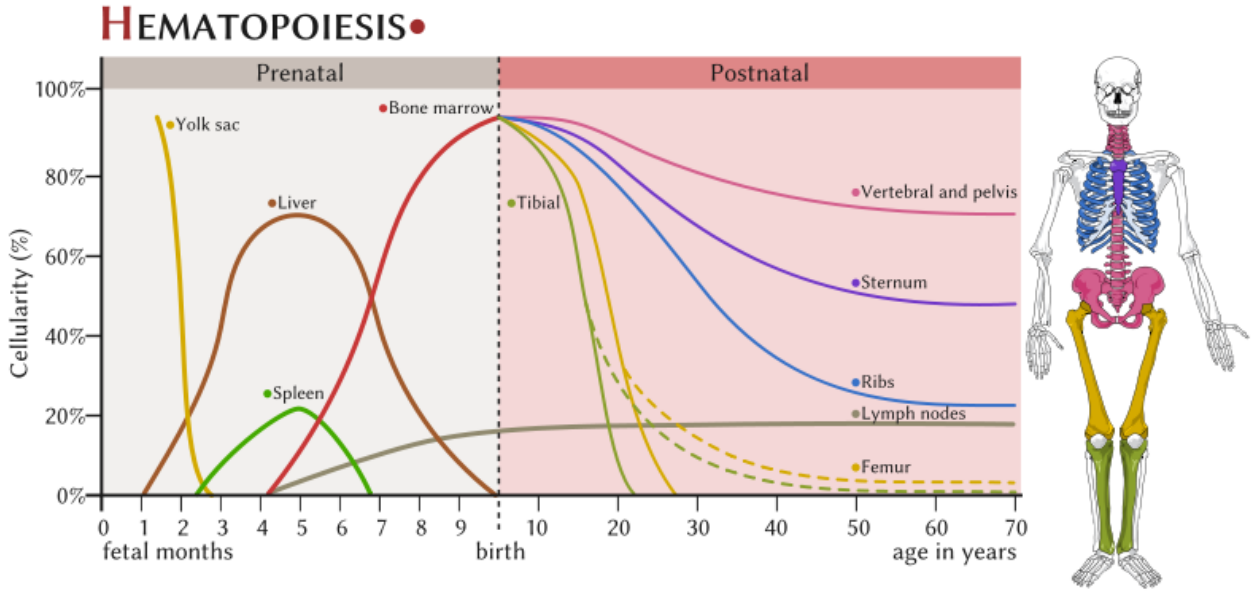
In a healthy adult person, approximately 1011–1012 new blood cells are produced daily in order to maintain steady state levels in the peripheral circulation.

Regulation of haemopoiesis

Humoral regulation by hormones: These hormones are produced by kidney and liver

- Erythropoietin
- Leucopoietin
- Thrombopoietin





2. **Blood plasma** Is similar to, and exchanges fluids with, interstitial fluid and is a matrix of formed elements

Composition : 90-92% of water
8-10% of dry substance mainly consisting from proteins (6-8%)
Dry substance includes : other solutes

- inorganic (mineral)
- organic components

- **Extracellular Fluids**

- **Interstitial fluid (IF) and plasma**

  Materials plasma and IF exchange across capillary walls:
  - **water**
  - **ions**
  - **small solutes**

Differences between Plasma and IF

- **Levels of O_2 and CO_2**
- **Dissolved proteins:** plasma proteins do not pass through capillary walls

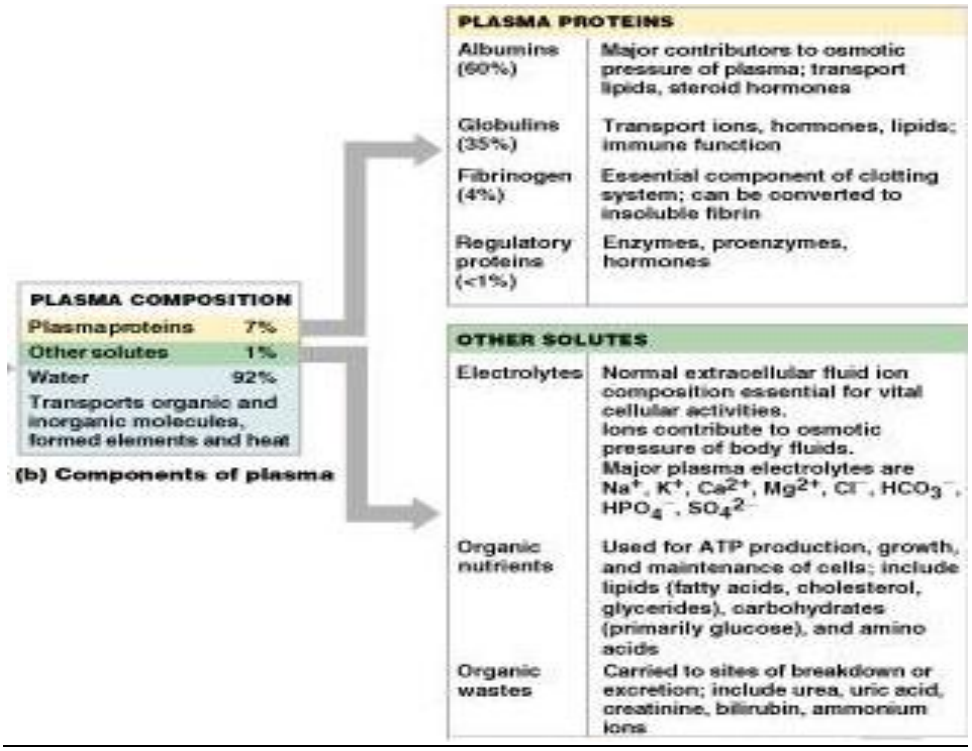
Blood plasma Plasma resembles interstitial fluid, but contains a unique mixture of proteins not found in other extracellular fluids.
The main (inorganic) mineral components :(0.9-1.5 %):

Cations : Calcium (Ca++),
Sodium (Na+),
Potassium (K+)
Magnesium (Mg++)

Anions : Phosphates ($PO4^-$)
Chlorides(Cl^-)
Bicarbonates($HCO3^-$)

A solution with the same salt concentration 0.9% is named **isotonic** solution. If salt concentration more than 0.9% such solution is called **hypertonic.**
 If salt concentration is less than 0.9% –**hypotonic solution.**

The organic components of plasma include : proteins
lipids
carbohydrates



Plasma proteins and their role Plasma proteins include :

- **Albumin** - (65-85 g/l):
  Transportation of fatty acids, thyroid hormones and steroid hormones
  Regulation of oncotic pressure
  Regulation of pH
- **Globulin** – (28 g/l); Antibodies, also called immunoglobulins. Globulins are small molecules that transport
  - hormone-binding proteins
  - metalloproteins
  - apolipoproteins (lipoproteins)
  - steroid-binding proteins

  ❖ **α** (**Alpha)globulins** are a group of globular proteins in plasma that are highly mobile in alkaline pH or the greatest electrophoretic mobility (electrically charged solutions) next to albumin.
  ❖ β **globulins** – Transportation
  ❖ γ **globulins** - Defense

- **Fibrinogen -** Blood clotting (haemostasis)(3 g/l) Molecules form clots and produce long, insoluble strands of fibrin.
- **Other Plasma Proteins** 1% of plasma, changing quantities of specialized plasma proteins, enzymes, hormones, and prohormones.

Origins of Plasma Proteins 90% made in liver. Antibodies made by plasma cells. Peptide hormones made by endocrine organs

Lipids and carbohydrates in plasma

- The major plasma carbohydrate is glucose (80-110 g/dL & 3.3-5.5 mmol/L) .
- Plasma normally contains varying amounts of hormones, enzymes, pigments, and vitamins.
- The composition of plasma varies with thebody's activity and different physiological states.

Serum

When fibrinogen is removed from plasma as a result of coagulation, such plasma without fibrinogen is called serum. (Liquid part of a blood sample, in which dissolved fibrinogen has converted to solid fibrin)

3.Physico-chemical constants of blood

- Osmotic pressure
- Oncotic pressure
- Blood pH
- Viscosity
- Specific gravity
- Erythrocyte sedimentation rate(ESR)

Osmosis -movement of water from higher_concentration to lower concentration.(or) movement of dissolved substancesfrom lower concentration to higherconcentration.

Osmotic pressure (7.6 atm)

- Osmotic pressure is defined as the minimum amount ofpressure needed to prevent osmosis. Dissolved particles maintain the osmotic pressure. Among them, the most active is-NaCl (0.9 % isotonic) and also glucose (5 % isotonic).

In hypotonic solution

- swelling and bursting occurs. (**Haemolysis**)

In hypertonic solution

- shrinkage occurs.

Oncotic pressure

Oncotic pressure, or colloid osmotic pressure,is a form of osmotic pressure exerted by blood plasma proteins. It usually tends to pull water (fluid) into the circulatory system (capillaries).

- It is the opposing force to hydrostatic pressure
- Its normal value is : 0.03-0.04 atm (or) 20-25 mm Hg

pH of blood

pH is a measure of the acidity or basicity of an aqueous solution. It is the negative decimal logarithm of hydrogen concentration. Normal pH of blood is : (arterial blood) 7.45 – 7.35 (venous blood). If pH is less than 7.3 , it is acidosis. If pH is more than 7.5, it is alkalosis

pH is maintained by :

- The excretion of carbon dioxide by the lungs
- The excretion of H+ or OH- by the kidneys.
- By the action of buffer system

Carbonate Phosphate Protein Haemoglobin (HA $H^+ + A^-$)

Viscosity

 Blood viscosity can be described as the thickness and stickiness of blood.

 It is a measure of the resistance of blood to flow.

 The viscocity of blood is 5 times more than that of water (based on time taken for the flow of both in a tube)

 It depends on : RBCs
Plasma proteins

Specific gravity

 Specific gravity is the ratio of the density of a substance to the density of a reference substance. Specific gravity is also called relative density.

 Blood normally has a specific gravity of : 1.05 - 1.06 g/L

Specific gravity depends on : RBCs Plasma proteins.The higher the concentration of RBCs and plasma proteins, higher will be the specific gravity. 1.03 1.04 1.05 1.06

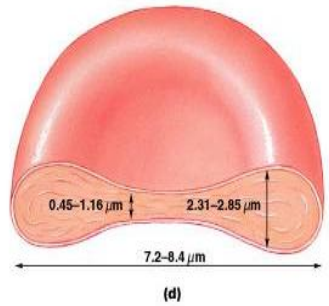
4. Erythrocytes

Red blood cells (RBCs) are the most numerous cells in the body. Red blood cells (RBCs) make up 99.9% of blood's formed elements,Red blood cells, or erythrocytes, are the most abundant type of blood cell. Approximately 2.4 million new erythrocytes are produced per second. Approximately a quarter of the cells in the human body are red blood cells. RBCs are lack of nuclei, mitochondria, and ribosomes. Lifespan of RBCs Live is about 120 days

- Red blood cell count reports the number of RBCs in 1 microliter whole blood
- Hematocrit (packed cell volume, PCV): percentage of RBCs in centrifuged whole blood

- RBC count Normal range : **3.5- 5.2 × 10^6/ mm^3**(million in cubic millimeter) or microliter
    - male: 4.5–6.3 **10^6/ mm^3** or **10^{12}/L**
    - female: 4.–5.5 **10^6/ mm^3** or **10^{12}/L**
- Hematocrit:
    - male: 52%
    - female: 47%

RBC count reflects bloods oxgen carrying capacity.



Erythrocytes –Structure

Mature red blood cells are oval biconcave disks and they are flexible. A typical erythrocyte is a small and highly specialized disc diameter of approximately 6.2–8.2 µm. Thin in middle and

thicker at edge. They lack a cell nucleus and most organelles like mitochondria, and ribosomes, in order to accommodate maximum space for haemoglobin. Since RBCs have a elastic membrane, they are able to change their shape when they pass through the capillaries. The cells develop in the bone marrow and circulate for about 100–120 days in the body before their components are recycled by macrophages. Human red blood cells take on average 20 seconds to complete one cycle of circulation.

Importance of RBC Shape and Size RBC Count

1. High surface-to-volume ratio:
    - quickly absorbs and releases oxygen
2. Discs form stacks:
    - smoothes flow through narrow blood vessels
3. Discs bend and flex entering small capillaries:
    - 7.8 μm RBC passes through 4 μm capillary

RBCs - Functions

The major function of these cells is a transport of haemoglobin, which in turn carries oxygen from lungs to the issues. Red blood cells contain carbonic anhydrase,which catalyzes the reaction between carbondioxide and water, that has a significance in transporting carbon dioxide (CO2) from tissues to lungs. The haemoglobin is an excellent acid-base buffer.

- Maintanence of acid-base balance.
- Blood group determination.

Erythropoiesis is the process by which red blood cells (erythrocytes) are produced. Red blood cell formation occurs only in red bone marrow (myeloid tissue). Stem cells mature to become RBCs. It is stimulated by decreased O2 in circulation, which is detected by the kidneys, which then secrete the hormone erythropoietin. The whole process lasts about 7 days. Through this process erythrocytes are continuously produced in the red bone marrow of large bones, at a rate of about 2 million per second in a healthy adult. Mature red blood cells live in blood circulation for about 100 to 120 days. At the end of their lifespan, they become senescent, and are removed from circulation by the macrophages. This process is termed eryptosis, erythrocyte programmed cell death.

The production of red cells involves the coordinate interaction of two organ systems in the body. The first is the bone marrow which produces red cells. The second is the kidney which produces the hormone erythropoietin. Hypoxia (low oxygen) of the kidney prompts synthesis and release of erythropoietin. The erythropoietin then travels to the bone marrow via the blood circulation where it activates new red blood production. This increases the blood's oxygen carrying capacity and corrects the hypoxia which was the primary stimulus to erythropoietin production. Erythropoietin therefore is part of a finely-tuned feedback circuit that controls red blood cell levels. The best analogy is a thermostat and a furnace. The thermostat senses the temperature and the furnace produces heat to eliminate the chill. Once the proper temperature is reached, the thermostat signals the furnace to cease heat production.

The distribution of body iron stores shows the importance of iron to red cell production. Normally, about 70% of iron is found in the circulating erythrocytes. Approximately 20% of iron is stored as ferritin, primarily in the liver. Smaller amounts of iron are coupled with enzymes, myoglobin and other proteins. The high iron content of erythrocytes reflects the fact that iron is an integral part of hemoglobin. Hemoglobin comprises over 95% of the protein in red cells. Low grade bleeding (for

instance, due to hookworm infection) is the most common cause of iron deficiency worldwide. No physiological means of iron excretion exists. Iron absorption from the gastrointestinal tract is the sole means of regulating iron stores.

Iron is an essential part of the diet and is absorbed from the duodenum and proximal jejunem. The iron is rapidly transferred to the carrier protein transferrin which delivers it to all the cells in the body. About 80% of absorbed iron is delivered to the bone marrow. A feedback exists such that iron absorption increases when more red cells are produced by the bone marrow. Ironically, some patients with extremely active marrow red cell production can develop iron overload. This occurs in some forms of thalassemia, for instance.

The body has no means of excreting iron. The obligatory daily iron losses are approximately 1-2 mg. The primary modes of iron loss are epithelial shedding from the GI and GU tracts. Epidermal descremation is another source of iron loss. In females, menstruation accounts for approximately 1 mg of iron loss per day on average.
Erythropoiesis involves the close interaction of iron and **erythropoietin**. Erythropoietin (EPO) is also called erythropoiesis-stimulating hormone:

- secreted when oxygen in peripheral tissues is low (hypoxia)
- due to disease or high altitude

In essence, erythropoietin is the accelerator that drives erythropoiesis. Iron is the fuel for the production of new red blood cells. When the two are coupled, red cell production moves briskly and efficiently. If one component is absent (e.g., iron deficiency) anemia results. Even when both components are available, they must be coordinately delivered to the bone marrow for proper action. For instance, if iron arrives on the scene 6 hours after erythropoietin reaches the bone marrow, red cell production will be suboptimal. The erythropoietin would have spent itself on cells that were unable to respond. Little or none of the erythropoietin would be left when the iron finally arrived.

RBC Maturation

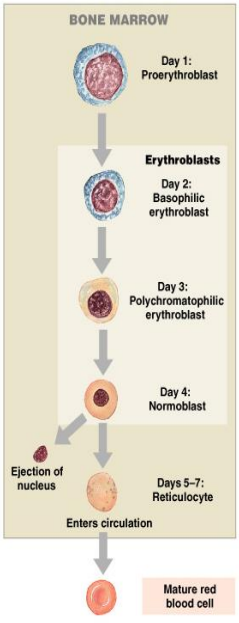
Stem cells in bone marrow divide to produce:
myeloid stem cells: become RBCs, some WBCs
lymphoid stem cells: become lymphocytes

Stages of RBC Maturation

- Myeloid stem cell
- Proerythroblast
- Erythroblasts
- Reticulocyte
- Mature RBC

Building red blood cells requires:

- amino acids
- iron
- vitamins B_{12}, B_6, and folic acid



According to size :

Normocytes - Normal sized RBCs
Microcytes - Small sized RBCs
Macrocytes - Large sized RBCs

According to colour :

Normochromia - Normal coloured RBCs
Hyperchromia - Darker,due to increased hemoglobin
Hypochromia - Paler, due to decreased hemoglobin

They are determined by measuring the :

Mean corpuscular volume (**MCV**)
Mean corpuscular haemoglobin (**MCH**)
Mean corpuscular haemoglobin concentration (**MCHC**)

Pathological forms of Red Blood Cells-

Erythrocytosis - (Polychythemia)

 If the erythrocyte count is more than normal, such state is called erythrocytosis.
 Erythrocytosis: Physiological Pathological Erythrocytosis
 Physiological Pathological

Absolute Primary

- In high altitude. -Bone marrow disorder.

Relative Secondary

- Exercises. -due to any CV or respiratory disease.

Erythropenia

 If the erythrocyte count is less than normal, such state is called erythropenia.
 A deficiency in number of RBCs or reduced haemoglobin levels in RBCs is known as anaemia.Erythropenia may be because of :Problems in production, excessive destruction (haemolysis) and blood loss.

Physiological Pathological

Absolute Primary

- Deficiency of -Bone marrow production disorder.

Relative Secondary

- Pregnancy -due to any kidney (RBC dissolves in fluid) disease.

5.**Erythrocyte Sedimentation Rate (ESR)** The erythrocyte sedimentation rate (ESR), is the rate at which red blood cells sediment in a period of one hour. RBC and plasma will be separated. It is a common hematology test.

Normal values :

Men - 2-10 mm/hr
Women - 2-15 mm/hr

Factors influencing the ESR :

Plasma proteins mainly fibrinogen and globulin negative charge of the erythrocytes (zeta potential).Increased ESR may be due to : Pregnancy, inflammation orcancer.

Decreased ESR may be due to : Polycythemia, Sickle cell anemia, Hereditary spherocytosis or congestive heart failure.

6.Haemolysis of RBCs, its types Haemolysis is the rupturing of erythrocytes and the release of theircontents (cytoplasm) into surrounding fluid (blood plasma). Hemolysis may occur in vivo or in vitro (inside or outside the body).

Haemolysis of RBCs

Causes :

- Inherited defects in the blood cells(e.g., Hereditary spherocytosis , Thalassemia)
- Chemicals, venoms The toxic products of microorganisms
- Transfusion of the wrong blood type or Rh incompatibility of fetal and maternal blood, a condition called erythroblastosis fetalis.

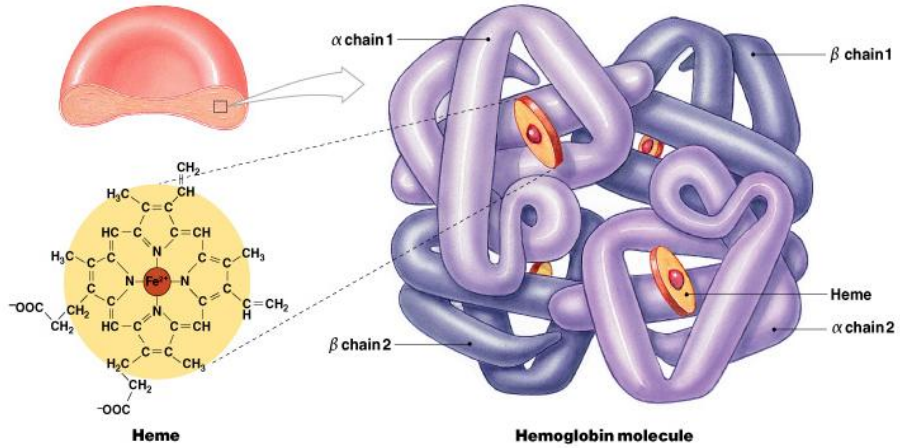
Types of haemolysis :

— Intrinsic - Due to problems within the RBC
- Physical - Radiation injury
- Osmotic - In hypotonic solution
- Mechanical - Due to pressure
- Thermal - Due to heat
- Biological - Blood transfusion, poison
- Chemical - Due to drugs

— Extrinsic - Antibodies against
- RBC(Immunological).

Osmotic resistance of RBCs; Concentration at which complete hemolysis of erythrocytes occurs. Normal value is 0.35 - 0.45% due to elasticity of erythrocytes membrane.

7.<u>Haemoglobin</u>



<u>Structure Content:</u> Protein molecule, transports respiratory gases. It is composed of the protein globin (a polypeptide), and the pigment heme. **Four globular protein subunits,each with 1 molecule of heme. Each heme contains 1 iron ion.** Iron ions easily associate with oxygen (oxyhemoglobin) or dissociate from oxygen (deoxyhemoglobin).

Normal range of hemoglobin concentration in adult male is : **14–18 g/dl** (140-180 g/L) of whole blood.20,5 in new born baby , 11-18 in horse, 8-14 in cow, 8-16 in sheep, 10,5-20 in dog, 8-15 in cat, 10-16 in pig, 8,5-15,5 in rabbit and 11-16 in guinea pig.

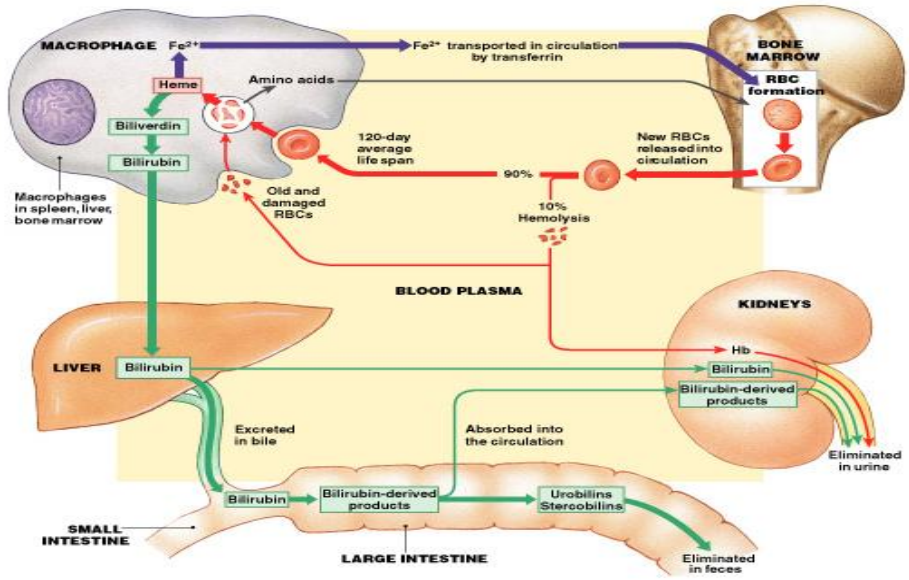
<u>Structure</u> : The haemoglobin has the ability to combine with oxygen is due to the four iron atoms associated with each heme group within the molecule.

<u>Physiological role:</u> The main function of erythrocytes is carried out by means of haemoglobin.

- Carbaminohemoglobin
  - binds carbon dioxide and carries it to lungs

Compounds of haemoglobin Physiological associations of haemoglobin :

- **Oxyhemoglobin** : - Oxygen combines weakly with the haemoglobin molecule. Such association is called oxyhemoglobin . It is formed in lungs.
- **Deoxyhemoglobin :** When the oxygen is released to the tissues of the body, the haemoglobin is called reduced haemoglobin or deoxyhemoglobin.
- **Carbaminohemoglobin** : In tissues with low oxygen (peripheral capillaries), hemoglobin releases oxygen and Hb combines with carbon dioxide and form carbaminohemoglobin.
- **Carboxyhemoglobin** or **carboxyhaemoglobin** (symbol **COHb** or **HbCO**) is a stable complex of carbon monoxide and hemoglobin (Hb) that forms in red blood cells upon contact with carbon monoxide (CO). Exposure to small concentrations of CO hinder the ability of Hb to deliver oxygen to the body, because carboxyhemoglobin forms more readily than does oxyhemoglobin (HbO_2). CO is produced in normal metabolism and is also a common chemical. Tobacco smoking (through carbon monoxide inhalation) raises the blood levels of COHb by a factor of several times from its normal concentrations.



Hemoglobin Recycling: 1% of circulating RBCs about 3 million RBCs per second wear out per day. Macrophages (phagocytes) of liver, spleen, and bone marrow:

- monitor RBCs
- engulf RBCs before membranes rupture (hemolyze)

Phagocytes break hemoglobin into components:

- globular proteins to amino acids
- heme to biliverdin
- To transport proteins (transferrin)
- To storage proteins (feritin and hemosiderin)

Iron recycles and transported by proteins (transferrin) and storage proteins for iron are feritin and hemosiderin.

Breakdown of Biliverdin

- Biliverdin (green) is converted to bilirubin (yellow).Bilirubin is excreted by liver (bile) and converted by intestinal bacteria to urobilins and stercobilins which account for the yellow brown to brown color of stool. Jaundice is caused by bilirubin buildup

Pathological combinations of haemoglobin :

Carboxyhemoglobin - is combination of hemoglobin and carbon monoxide. It is gas without smell and color that easily associates
with hemoglobin (more easily than oxygen).

Methemoglobin - is such hemoglobin in which iron has potential not 2++ as usually, but 3++. Such iron creates strong chemical connection and not able give oxygen to tissues.

Diagnosing Disorders

- Hemoglobinuria: hemoglobin breakdown products in urine due to excess hemolysis in blood stream
- Hematuria: whole red blood cells in urine due to kidney or tissue damage

Treatments for pathological associations

 Treatments for pathological associations of haemoglobin are :

 For carboxyhaemoglobin (HbCO) :

- Oxygenotherapy

 For methaemoglobin (HbMt) :

- Blood transfusion

But only if small amount of methaemoglobin is present (which is normal), then an enzyme called methhaemoglobin reductase which is present in the RBCs, act on them and actively eliminate them.

If a concentration of the pathological associations of hemoglobin is too high then, hypoxia (decrease of oxygen in tissues) can develop.

Types of haemoglobin

Types of hemoglobin :

- Adult hemoglobin (HbA) - (α2β2)
- Fetal hemoglobin (HbF) - (α2γ2) Strong form of hemoglobin found in embryos and takes oxygen from mother's hemoglobin
- Primitive hemoglobin (HbP) - (α2ε2) (Embryo)
- Fetal Hemoglobin

Types of haemoglobin:

- Embryo has HbP (primitive) - (α2ε2)
- Adults have HbA - (α2β2)
- Fetal hemoglobin (HbF)-Before birth (α2γ2)

The fetal hemoglobin (HbF) is different from the adult type (HbA). It has more affinity to oxygen and can be saturated with oxygen at a lower oxygen tension. In infants, the hemoglobin molecule is made up of 2 α chains and 2 γ chains. The gamma chains are gradually replaced by β chains as the infant grows.

Haemoglobin If a changing of amino acid order occurs inglobin part of hemoglobin molecule, they maylead to formation of pathological types of Hb.

 Anemia is developed due to formation HbS when only one amino acid change its place in globin chain of hemoglobin. At this state the erythrocytes change their forms and transport oxygen badly. But obtain a resistance to malaria

Colour index

The average content of hemoglobin in one erythrocyte is called color parameter of blood.

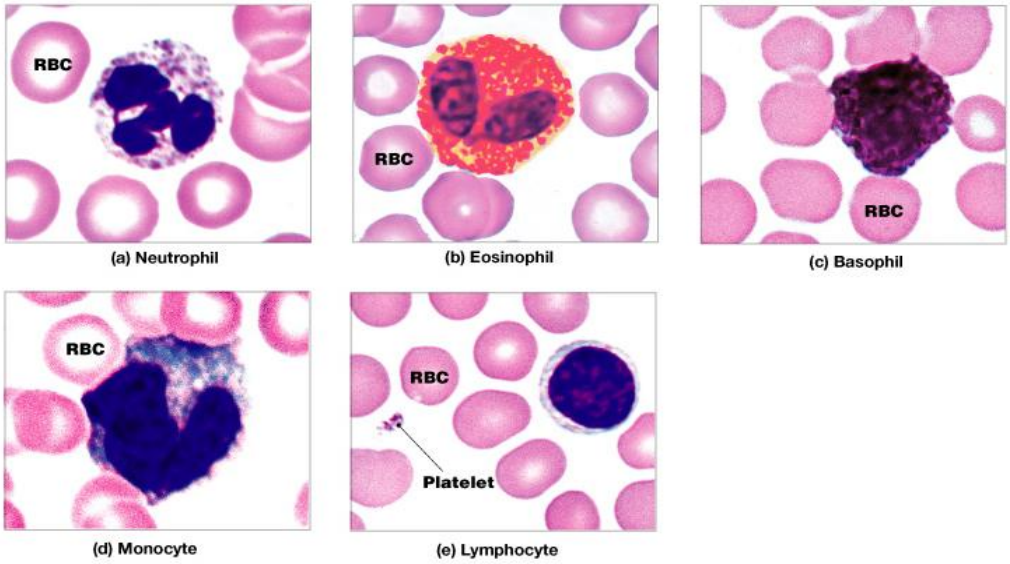
Formula :

Colour index = Haemoglobin content(g/L) × 3
First three numerals of RBC count. It fluctuates between 0.8 - 1 unit. If less than 0.8 - Hypochromia, If more than 1 - Hyperchromia.

Anemia : Hematocrit or hemoglobin levels are below normal Is caused by several conditions.

Pernicious Anemia Causes low RBC production. Due to unavailability of vitamin B_{12} (remember the intrinsic factor produced by the parietal cells in the gastric glands of the stomach, which is needed to absorb vitamin $B_{12)}$

Leukocytes (WBC)



White blood cells do not have hemoglobin. Have nuclei and other organelles Size 9-12 µk. They make up approximately 1% of the total blood volume in a healthy adult. They live for about three to four days in the average human body.

Normal count of WBC : 4-9 x 10^9/L or 4000 to 9000 per microliter
Types of WBCs

1. **Neutrophils**
2. **Eosinophils**
3. **Basophils**
4. **Monocytes**
5. **Lymphocytes**

Functions of Leukocytes- The major function of leucocytes is protective function. It provides immunity and thus defends the body against pathogens. Remove toxins and wastes and attack abnormal cells. Most WBCs can move in connective tissue proper and lymphatic system organs. Circulating WBCs can migrate out of bloodstream, have amoeboid movement, attracted to chemical stimuli (positive chemotaxis). Neutrophils, eosinophils, and monocytes.
Leukopoiesis

It is the production of leukocytes. It is produced from pluripotent haemopoietic stem cells, which is present in the bone marrow. Differentiation of lymphocytes - in the lymph tissue.
Leukopoiesis
 Granulocytes
 Myeloblast
 Promyelocyte
 Myelocyte (neutrophilic, eozinopilic,basophilic)
 Metamyelocyte (neutrophilic, eozinophilic, basophilic)
Monocyte
Monoblast
Promonocyte
monocyte
Lymphocyte
Lymphoblast
prolymphocyte
Lymphocyte The lymphocytes end differentiation in the lymphoid tissue (thymus)

Role of Leucopoietins are It is a hormone produced by liver and kidney. It provides humoral regulation of leucopoiesis.

Leucocytosis
Increased amount of leucocytes in blood.-It may be :
Physiological effects: Food intake, Exercises, Emotion
Pathological effects: Inflammation, Cancer or Stress
Leucopenia
Abnormally low concentration of leucocytes in blood.
Only pathological effects: Severe viral infections, Autoimmune disease, Chemotherapy Radiation injury
10. **Types of leukocytes**
Leucocytes are of 2 types :

Granulocytes :	Agranulocytes :
Neutrophil	Monocyte
Basophil	Lymphocyte
Eosinophil	

- **Neutrophils** Also called polymorphonuclear leukocytes. 50–70% of circulating WBCs (2.5–7.5 x 109/L) 47 - 72% in humanbeings, primats, canines and equidae. 15-45% in bovine, 30% in sheep, 30-40 % in goats, 35-40% in swine, 30% in chicken
  Juvenile(band) – 6% ,
  Immature(young) – 1% ,
  Segmented – 47-72% in humanbeings.

- Pale cytoplasm granules with:
  - lysosomal enzymes
  - bactericides (hydrogen peroxide and superoxide)

Functions : First line of defense (first cells that come to the area of inflammation). Multi functional cells that attack and destroy viruses and bacteria. Very active, first to attack bacteria

- Engulf pathogens
- Digest pathogens
- Release prostaglandins and leukotrienes
- Form pus

Neutrophils defends against antigenic molecules by Phagocytosis -cellular ingestion of bacteria with enzymes proteases, peroxidases, cationic proteins

Microphagocyte – up to 15 or 20 only. Respiratory burst – also called oxidative burst is the rapid release of chemicals from immune cells when they encounter with a bacteria or fungi. It is a crucial reaction that occurs in phagocytes to degrade internalized particles and bacteria.

Degranulation

- Removing granules from cytoplasm
- Defensins:
    - peptides from lysosomes
    - attack pathogen membranes

Basophils Are less than 1% of circulating WBCs (0.01-0.1 x 109/L) small cells and accumulate in damaged tissue

Basophils contain :

- Histamine – for vasodilation
- Heparin – anticoagulant
- Has IgE and thus participates in allergicreaction along with mast cells in tissues
- Promotes functions of other leucocytes

Eosinophils Also called acidophils. 2–4% of circulating WBCs (0.04-0.4 x 109/L)

Functions :

- Antiparasitic (kills parasites includingworms).Attack large parasites
- Excrete toxic compounds:
    - nitric oxide
    - cytotoxic enzymes

- They migrate to the site of infection.
- Weak phagocytes.
- Contains histaminase – and so it reducesallergic reaction.

Are sensitive to allergens Control inflammation with enzymes that counteract inflammatory effects of neutrophils and mast cells. **Eosinophilia** – increased level of eosinophilsin the blood.

Monocytes (0.2–0.8 x 109/L) 2–8% of circulating WBCs. Are large and spherical cells.Enter peripheral tissues and become macrophages.

Functions :

- They differentiate into macrophages which can phagocytose upto 100 bacteria.
- Antigen – presentation function.

 Monocytes In tissues Wandering, Kupffer cells goes to the site of Alveolar macrophages inflammation and in microglia. Engulf large particles and pathogens as amacrophage. Secrete substances that attract immune system cells and fibroblasts to injured area

Lymphocytes (1.5–3.5 x 109/L) Provides immunity. Are part of the body's specific defense system.20–30% of circulating WBCs. They are larger than RBCs. Migrate in and out of blood.Mostly in connective tissues and lymphatic organs. Are produced by lymphoid stem cells. Lymphopoiesis:is the production of lymphocytes. Some lymphoid stem cells migrate to peripheral lymphoid tissues (thymus, spleen, lymph nodes).Also produce lymphocytes.

Two types : B – lymphocytes and T- lymphocytes.

- B – lymphocytes provide humoral immunity.
- T – lymphocytes provides cell-mediated immunity.
- B – cells differentiate into plasma cells which furtherproduces 5 classes of antibodies that provides immunity

 T- cytotoxic cells aims to eliminate : Virus-infected cells Cancer cells and also causes graft rejection.

Diagnostic importance

- ↑Neutrophils – inflammation
- ↑Eosinophils – allergy, parasiticinfections
- ↓ Eozinophils – stress
- ↑ Lymphocytes – cancer (leukemias –cancerous production of lymphoid cells)

9.Leucogram

A blood leucocyte profile (leucogram) provides information on total leucocyte count, differential leucocyte count and leucocyte morphology.

Leucogram-

Normal count	Total no. of leuocytes
Eosinophils	0.5-5%
Basophils	0- 1%
Juvenil neutrophils	6%
Immatature neutrophils	1%
Segmented neutrophils	47-72%
Lymphocytes	19-37%
Monocytes	3-11%
Normal Range	4-9 $x10^9$/L (10^3/mm^3

The Differential Count of Circulating WBCs Detects changes in WBC populations. Infections, inflammation, and allergic reactions

Leucogram- variations

Total no. of leucocytes

Eosinophils	5%

Basophils	1%
Juvenil neutrophils	6%
Immatature neutrophils	4%
Segmented neutrophils	39%
Lymphocytes	35%
Monocytes	10%

Normal Range 9 x 10^9/L

Shift to left (regenerative shift):

If the amount of young neutrophil is normal but the amount of mature neutrophil is very low , then we conclude as : Infection by pathogen

The leucogram below is an example of this shift.

Leucogram- variations

Total no. of leucocytes	11 x10^9/L
Eosinophils	3%
Basophils	1%
Juvenil neutrophils	0%
Immature neutrophils	0%
Segmented neutrophils	70%
Lymphocytes	21%
Monocytes	5%

Shift to right (degenerative shift): If the amount of mature neutrophil is normal but the amount of young neutrophil is zero , then we conclude as : Problem in bone marrow
The leucogram below is an example of this shift.

Physiological decussation

- Normally in adults neutrophil level is higher than the level of lymphocytes
- At birth, the amount of neutrophils and lymphocytes are in the ratio as in adults
- At 3 - 5 days,lymphocytes increase and neutrophil decrease and remains same until 3– 5 years, and then again becomes normal. This is called the physiological cross of leucocytes in ontogenesis

11.Immunity

- Immunity is the capability to resist from pathogens, that tend to damage the tissues or organs, through biological defense.
- Leucocytes play a major role in providing immunity.

Types of immunity

Immunity Innate (non-specific)
Cellular
Humoral
Acquired (specific) (adaptive) Cellular
Cellular
Humoral

Types of immunity-Role of leucocytes
Neutrophils Lysozymes Provided by Basophils Interferon T- cells B-cells
Eosinophils Complement system
Macrophages Stomach acid Plasma cells
NK- cells Tear; saliva
Phagocytosis Skin Antibodies
Mucous membrane

Innate
Cellular Humoral
Acquired
Cellular Humoral

Types of immunity After an encounter with Eg;- Vaccination. a disease , the body Serum. produces own antibodies. Acquired and Active Passive

WBC Disorders

Leukopenia: abnormally low WBC count
Leukocytosis: abnormally high WBC count
Leukemia: extremely high WBC count

What is blood typing?

12.**Agglutination of RBCs**

ABO and Rh incompatibilities

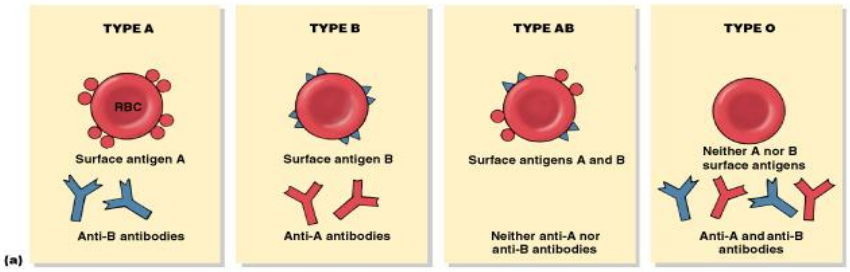
Surface Antigens are cell surface proteins that identify cells to immune system. Normal cells are ignored and foreign cells attacked. They are genetically determined. By presence or absence of RBC surface antigens **A, B, Rh**

Basic Blood Types

- A (surface antigen A)
- B (surface antigen B)
- AB (antigens A and B)
- O (neither A nor B)

Agglutinogens are antigens on surface of RBCs and screened by immune system. Plasma antibodies attack (agglutinate) foreign antigens.
Blood Plasma Antibodies

- Type A: type B antibodies
- Type B: type A antibodies
- Type O: both A and B antibodies
- Type AB: neither A nor B



The clumping of RBCs due to binding of antibody with the corresponding antigen is called haemagglutination.

Example : Anti -A binds A antigen and anti-B binds B antigen

It has two common uses : Blood typing and the quantification of virus dilutions.

- A (surface antigen A)
- B (surface antigen B)
- AB (antigens A and B)
- O (neither A nor B)

Agglutinogens Antigens on surface of RBC screened by immune system

Agglutination

 Agglutinogens (antigens) are proteins that exist on the surface of every red blood cell.

 This agglutinogen , which is present on the surface of RBCs, will stimulate the production of agglutinin (antibody) in the plasma in case of incompatible blood transfusion.

 Helps in determining the blood type of a person.

ABO blood group system

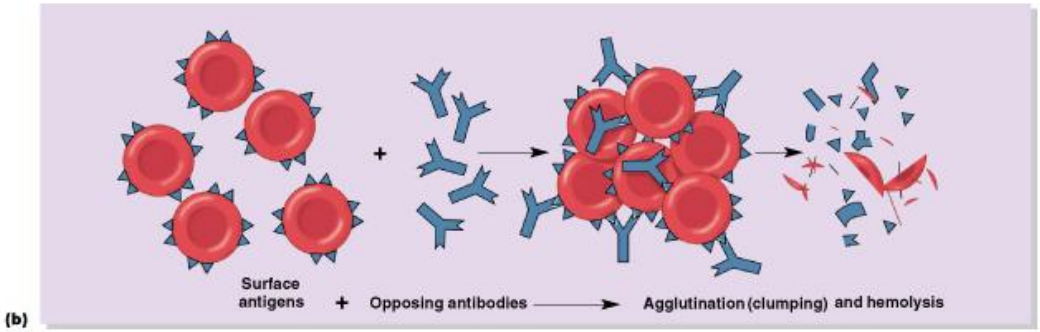
 According to the ABO blood typing system there are four different kinds of blood types: **O, A, B, AB.**

 I(O) - α,β (40%);

 II(A) - β (39%);

 III(B) - α (10-15%);

 IY(AB) - (5%).



CDE blood group system

- Out of C,D and E D is the strongest antigen. Also called Rhesus(Rh) system. It is determined by anti-Rh serum. 85% of the population is - Rh^+. If Rh-D antigen is present in blood(RBC) –

The Rh Factor Also called D antigen. Either Rh positive **Rh^+** (Rh^+) or Rh negative (Rh^-) .Only sensitized Rh^- blood has anti-Rh antibodies

If Rh-D antigen is absent in blood(RBC),- **Rh^-**

Rh incompatibility in blood transfusion : Rh^- person cannot receive blood from Rh^+ person, whereas Rh^+ person can receive blood from Rh^-.person without any problems. If a Rh^- person receive blood from Rh^+ person for the first time, due to this exposure, there will be formation antibodies(anti-RhD). So, if a second transfusion is done again with Rh^+ blood, then, the antibodies which are already presentcauses clumping.

Rh incompatibility **Erythroblastosis fetalis :** If a Rh^- mother carry a Rh^+ fetus, due toplacental barrier the blood doesn't mix. Howeverduring delivery some Rh^+ from fetus reaches mother. So,the mother will start producing antibodies against Rh^+ . During consecutive pregnancies, this may cause destruction of RBCs in the fetus causing haemolytic anaemia(erythroblastosis fetalis). So after each pregnancy, the mother will receive anti-RhD(prophylaxis)to prevent this incompatibility.

13.**Blood transfusion**

Blood transfusion is the process of receiving blood products into one;s circulation intravenously. Transfusions are used in a variety of medical conditions in order to replace the lost components of the blood. Early transfusions used whole blood, but modern medical practice commonly uses only components of the blood, such as red blood cells, white blood cells, plasma, clotting factors, and platelets.

Types of blood transfusion

- Direct
- Indirect
- Autohemotransfusion

Rules of blood transfusion

- Check for ABO blood group compatibility.
- Check for Rhesus (Rh factor) compatibility.
- Cross-match(individual tests):

By taking RBC from donor and plasma from recipient. Agglutination must be absent.

Biological test : Three times introducing donor's blood in small portions like -10-25 ml into the recipient and check for any complaints or deviation of physiological parameters.

Cross-Reaction Also called transfusion reaction

- Plasma antibody meets its specific surface antigen
- Blood will agglutinate and hemolyze
- If donor and recipient blood types not compatible

Cross-Match Test Performed on donor and recipient blood for compatibility. Animals have numerious blood types. Soif ıt need blood transfusion. Practically cross match test could be performed.

14.**Thrombocytes (platelets)**

Fragments of megakaryocytes (red bone marrow). They do not have a nucleus and 2–3 µm in diameter. Circulation in bloodis appox. 8-12 days

Normal range : 180-320 x 109/L

Platelets- functions

- The main function of platelets is the maintenance of hemostasis.

 Trophic (endothelium)
 Transport of BAS
 Immunity(Phagocytosis)
 Clot retraction
 Procoagulant
 Inflammation

Thrombopoiesis Platelets in bone marrow, by budding off from megakaryocytes. Megakaryocyte and platelet production is regulated by thrombopoietin, a hormone usually produced by the liver and kidneys. Each megakaryocyte produces between 5,000 and 10,000 platelets. Around 10^{11} platelets are produced each day by an average healthy adult. Reserve platelets are stored in the spleen, and are released when needed by sympathetically induced splenic contraction. Old platelets are destroyed by phagocytosis in the spleen and by Kupffer cells in the liver.

Haemostasis Haemostasis is a process which causes bleeding tostop, meaning to keep blood within a damaged blood vessel. It is the first stage of wound healing. Most of the time this includes blood changing from a liquid to a solid state. The opposite of hemostasis is hemorrhage. Hemostasis has three major steps:

Vasoconstriction,
Temporary blockage of a break by a platelet plug,
Blood coagulation, or formation of a clot that seals the hole until tissues are repaired.

Haemostasis - its types
 Two types :
 Vascular-thrombocytes hemostasis :
Stoppage of blood loss from the microcirculatory vessels having low blood pressure.
Finally – Platelet plug is formed
 Coagulation hemostasis :
Stoppage of blood loss from the large vessels having higher blood pressure.
Finally – Blood clot is formed

Vascular-thrombocytes hemostasis:Vessel is injured. Vasoconstriction due to nervous reflex
Von-Willebrand factor works adherence of platelets to collagen-Positive feedback
(platelets undergo shape change and release ADP and ATP ,Ionized calcium, Serotonin, Epinephrine, Thrombaxane A2 from granules)
(Primary) Temporary platelet plug
(Stable) Permanent platelet plug – more stable
Adhesion
Secretion
Aggregation

Cellular (platelet) clotting factors
 Cellular clotting factors are present in the granules of thrombocytes.
 These factors are released when the platelets undergo degranulation.
 They are :

ADP and ATP
Ionized calcium

Serotonin
Epinephine
Thrombaxane A2

15.Coagulatory haemostasis

Stages of coagulatory haemostasis

1. Activation of prothrombin activator
2. Prothrombin → Thrombin
3. Fibrinogen → Fibrin-monomer -

fibrin-polymer – cross-linked fibrin-polymer

Significance : Stoppage of blood loss from the large vesselshaving higher blood pressure by the formation of blood clot. The Coagulation Phase begins 30 seconds or more after the injury. Calcium ions (Ca^{2+}) and vitamin K are both essential to the clotting process.

Blood clotting (coagulation):

- Involves a series of steps
- converts circulating fibrinogen into insoluble fibrin

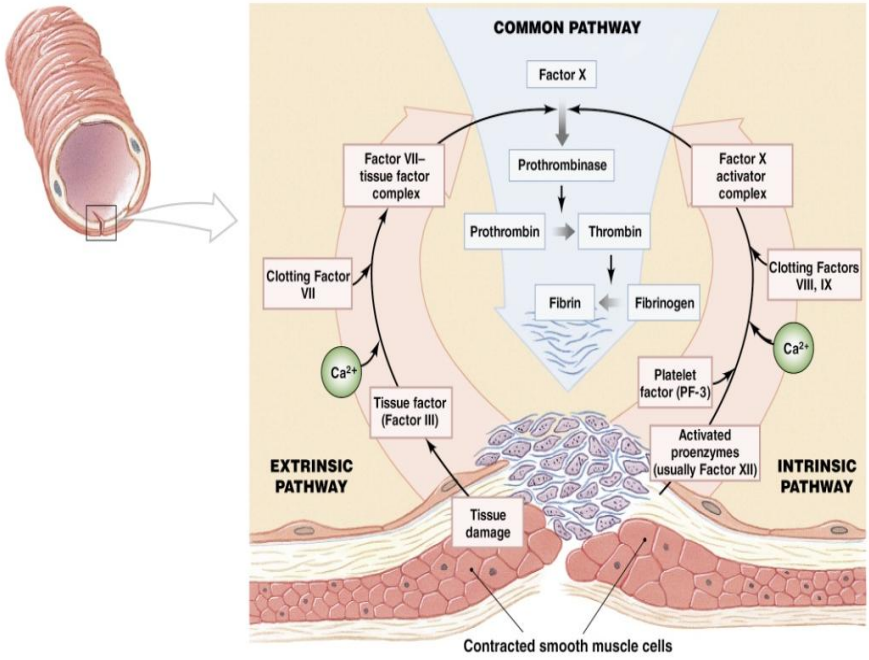
Activated Platelets Release Clotting Compounds

- Adenosine diphosphate (ADP)
- Thromboxane A_2 and serotonin
- Clotting factors
- Platelet-derived growth factor (PDGF)
- Calcium ions

There are 13 plasma clotting factors;

Factor number Name

- I - Fibrinogen
- II - Prothrombin
- III - Tissue Factor
- IV - Ca2+
- V a - Proaccelerin
- VII – Proconvertin
- VIII - Antihemophilic Factor
- IX - Christmas Factor
- X - Stuart Factor
- XI - Plasma thromboplastin antecedent
- XII - Hageman factor
- XIII - Fibrin Stabilizing Factor



(a) The coagulation phase

During coagulation phase

- Chain reactions of enzymes and proenzymes
- Form 3 pathways
- Extrinsic pathway: Damaged cells release tissue factor (TF)
    - TF + other compounds = enzyme complex
    - Activates Factor X
    - begins in the vessel wall
    - outside blood stream
- Intrinsic pathway: begins with circulating proenzymes within bloodstream
    - Activation of enzymes by collagen
    - Platelets release factors (*e.g.,* PF–3)
    - Series of reactions activate Factor X
- Common pathway: Enzymes activate Factor X
    - Forms enzyme prothrombinase
    - Converts prothrombin to thrombin
    - Thrombin converts fibrinogen to fibrin
    - where intrinsic and extrinsic pathways converge

Bleeding Time Normally, a small puncture wound stops bleeding in 1–4 minutes

<u>Clotting Restriction</u>

1. Anticoagulants (plasma proteins):
    - antithrombin-III
    - alpha-2-macroglobulin
2. Heparin
3. Protein C –stimulates plasmin formation an enzyme that breaks down fibrin strands (Protein C is activated by thrombomodulin)

Prostacyclin also inhibits platelet aggregation

16.**Fibrinolysis** Degradation products are Fibrin And α-2

Tissue plasminogen activator.Factor 12 kallikrein
Plasminogen ⟹ Plasmin
Streptokinase
Fibrin

Fibrinolytic system Slow process of dissolving clot:

- thrombin and tissue plasminogen activator (t-PA):
  - activate plasminogen
- Plasminogen produces plasmin:
  - digests fibrin strands

Function:
Lysis of blood clots (for a few days).
 Clot destroyed by plasmin (fibrinolysin)which formed from plasminogen (profibrinolysin).
 This reaction is activated by blood andtissue activators.

17.**System of anticoagulation**
 The pre-existing (primary) anticoagulants :
(natural) Heparin
Antithrombin III
(artificial) Dicumarin
Pelentan
 Inactive clotting factors (13)
System of anticoagulation
 Rapid blood flow.
 Smoothness of endothelium.
 Same charge on formed elements andwalls of vessels.
 Presence of glycoproteins and prostaglandins on its surface.

Regulation of haemostasis
Sympathetic nervous system –stimulation of coagulation.
 Parasympathetic nervous system –stimulation of anticoagulation system (according to some data).