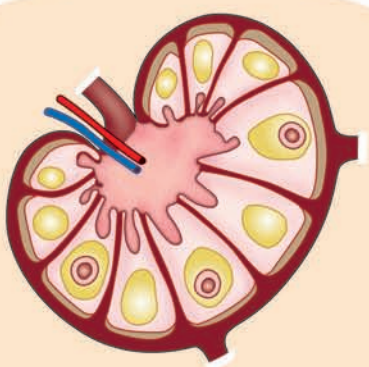
Section

2

Blood and Body Fluids



Chapter 6

Body Fluids

- INTRODUCTION
- SIGNIFICANCE
- COMPARTMENTS
- COMPOSITION
- MEASUREMENT
- CONCENTRATION
- MAINTENANCE OF WATER BALANCE
- APPLIED PHYSIOLOGY

INTRODUCTION

Body is formed by solids and fluids. Fluid part is more than two third of the whole body. Water forms most of the fluid part of the body.

In human beings, the total body water varies from 45% to 75% of body weight. In a normal young adult male, body contains 60% to 65% of water and 35% to 40% of solids. In a normal young adult female, the water is 50% to 55% and solids are 45% to 50%. In females, water is less because of more amount of subcutaneous adipose tissue. In thin persons, water content is more than that in obese persons. In old age, water content is decreased due to increase in adipose tissue. Total quantity of body water in an average human being weighing about 70 kg is about 40 L.

SIGNIFICANCE OF BODY FLUIDS

IN HOMEOSTASIS

Body cells survive in the fluid medium called **internal environment** or **'milieu interieur'.** Internal environment contains substances such as glucose, amino acids, lipids, vitamins, ions, oxygen, etc. which are essential for growth and functioning of the cell. Water not only forms the major constituent of internal environment but also plays an important role in homeostasis.

IN TRANSPORT MECHANISM

Body water forms the transport medium by which nutrients and other essential substances enter the cells; and unwanted substances come out of the cells. Water forms an important medium by which various enzymes, hormones, vitamins, electrolytes and other substances are carried from one part to another part of the body.

IN METABOLIC REACTIONS

Water inside the cells forms the medium for various metabolic reactions, which are necessary for growth and functional activities of the cells.

IN TEXTURE OF TISSUES

Water inside the cells is necessary for characteristic form and texture of various tissues.

IN TEMPERATURE REGULATION

Water plays a vital role in the maintenance of normal body temperature.

COMPARTMENTS OF BODY FLUIDS – DISTRIBUTION OF BODY FLUIDS

Total water in the body is about 40 L. It is distributed into two major compartments:

1. *Intracellular fluid (ICF):* Its volume is 22 L and it forms 55% of the total body water
2. *Extracellular fluid (ECF):* Its volume is 18 L and it forms 45% of the total body water.

   ECF is divided into 5 subunits:
   i. Interstitial fluid and lymph (20%)
   ii. Plasma (7.5%)
   iii. Fluid in bones (7.5%)
   iv. Fluid in dense connective tissues like cartilage (7.5%)
   v. Transcellular fluid (2.5%) that includes:
      a. Cerebrospinal fluid
      b. Intraocular fluid
      c. Digestive juices
      d. Serous fluid – intrapleural fluid, pericardial fluid and peritoneal fluid
      e. Synovial fluid in joints
      f. Fluid in urinary tract.

Volume of interstitial fluid is about 12 L. Volume of plasma is about 2.75 L. Volume of other subunits of ECF is about 3.25 L. Water moves between different compartments (Fig. 6.1).

■ COMPOSITION OF BODY FLUIDS

Body fluids contain water and solids. Solids are organic and inorganic substances.

■ ORGANIC SUBSTANCES

Organic substances are glucose, amino acids and other proteins, fatty acids and other lipids, hormones and enzymes.

■ INORGANIC SUBSTANCES

Inorganic substances present in body fluids are sodium, potassium, calcium, magnesium, chloride, bicarbonate, phosphate and sulfate.

ECF contains large quantity of sodium, chloride, bicarbonate, glucose, fatty acids and oxygen. ICF contains large quantities of potassium, magnesium, phosphates, sulfates and proteins. The pH of ECF is 7.4. The pH of ICF is 7.0. Differences between ECF and ICF are given in Table 6.1.

■ MEASUREMENT OF BODY FLUID VOLUME

Total body water and the volume of different compartments of the body fluid are measured by indicator dilution method or dye dilution method.

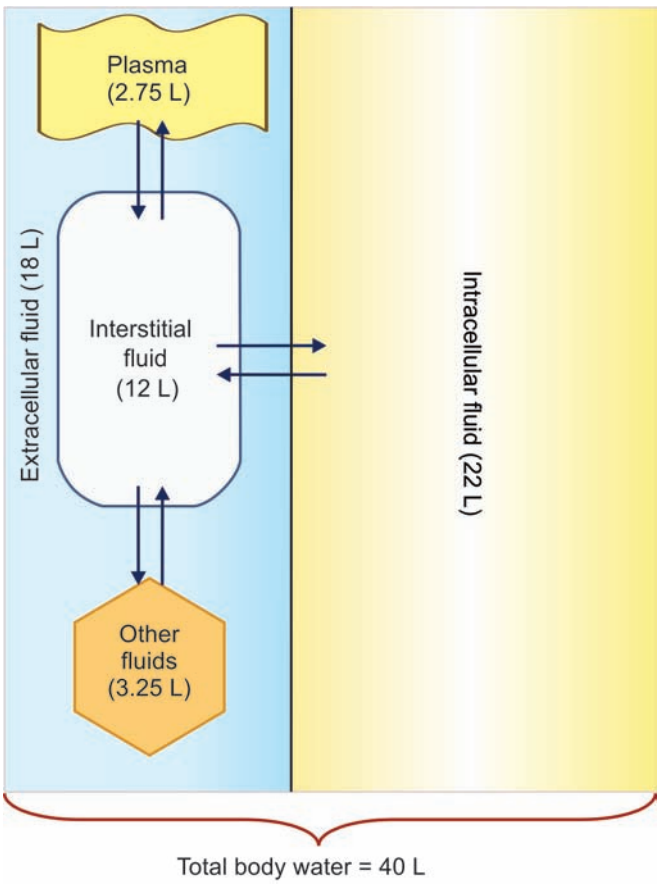


FIGURE 6.1: Body fluid compartments and movement of fluid between different compartments. Other fluids = Transcellular fluid, fluid in bones and fluid in connective tissue.

■ INDICATOR DILUTION METHOD

Principle

A known quantity of a substance such as a dye is administered into a specific body fluid compartment. These substances are called the marker substances or indicators. After administration into the fluid compartment, the substance is allowed to mix thoroughly with the fluid. Then, a sample of fluid is drawn and the concentration of the marker substance is determined. Radioactive substances or other substances whose concentration can be determined by using colorimeter are generally used as marker substances (Table 6.2).

Formula to Measure the Volume of Fluid by Indicator Dilution Method

Quantity of fluid in the compartment is measured using the formula:

$$V = \frac{M}{C}$$

V = Volume of fluid in the compartment.
M = Mass or total quantity of marker substance injected.
C = Concentration of the marker substance in the sample fluid

Correction factor

Some amount of marker substance is lost through urine during distribution. So, the formula is corrected as follows:

$$\text{Volume} = \frac{M - \text{Amount of substance excreted}}{C}$$

Uses of Indicator Dilution Method

Indicator dilution or dye dilution method is used to measure ECF volume, plasma volume and the volume of total body water.

Characteristics of Marker Substances

Dye or any substance used as a marker substance should have the following qualities:

1. Must be nontoxic
2. Must mix with the fluid compartment thoroughly within reasonable time
3. Should not be excreted rapidly
4. Should be excreted from the body completely within reasonable time
5. Should not change the color of the body fluid
6. Should not alter the volume of the body fluid.

TABLE 6.1: Differences between extracellular fluid (ECF) and intracellular fluid (ICF)

Substance	ECF	ICF
Sodium	142 mEq/L	10 mEq/L
Calcium	5 mEq/L	1 mEq/L
Potassium	4 mEq/L	140 mEq/L
Magnesium	3 mEq/L	28 mEq/L
Chloride	103 mEq/L	4 mEq/L
Bicarbonate	28 mEq/L	10 mEq/L
Phosphate	4 mEq/L	75 mEq/L
Sulfate	1 mEq/L	2 mEq/L
Proteins	2 g/dL	16 g/dL
Amino acids	30 mg/dL	200 mg/dL
Glucose	90 mg/dL	0-20 mg/dL
Lipids	0.5 g/dL	2-95 g/dL
Partial pressure of oxygen	35 mm Hg	20 mm Hg
Partial pressure of carbon dioxide	46 mm Hg	50 mm Hg
Water	15 to 20 L (18)	20 to 25 L (22)
pH	7.4	7.0

Marker Substances Used to Measure Fluid Compartments

Marker substances used to measure different fluid compartment are listed in Table 6.2.

■ MEASUREMENT OF TOTAL BODY WATER

Volume of total body water (fluid) is measured by using a marker substance which is distributed through all the compartments of body fluid. Such substances are listed in Table 6.2.

Deuterium oxide and **tritium oxide** mix with fluids of all the compartments within few hours after injection. Since plasma is part of total body fluid, the concentration of marker substances can be obtained from sample of plasma. The formula for indicator dilution method is applied to calculate total body water.

Antipyrine is also used to measure total body water. But as it takes longer time to penetrate various fluid compartments, the value obtained is slightly low.

■ MEASUREMENT OF EXTRACELLULAR FLUID VOLUME

Substances which pass through the capillary membrane but do not enter the cells, are used to measure ECF volume. Such marker substances are listed in Table 6.2.

These substances remain only in ECF and do not enter the cell (ICF). When any of these substances is injected into blood, it mixes with the fluid of all subcompartments of ECF within 30 minutes to 1 hour. Indicator dilution method is applied to calculate ECF volume. Since ECF includes plasma, the concentration of marker substance can be obtained in the sample of plasma.

Some of the marker substances like sodium, chloride, inulin and sucrose diffuse more evenly throughout all subcompartments of ECF. So, the measured volume of ECF by using these substances is referred as **sodium space, chloride space, inulin space** and **sucrose space.**

Example for Measurement of ECF Volume

Quantity of sucrose injected (Mass) : 150 mg
Urinary excretion of sucrose : 10 mg
Concentration of sucrose in plasma : 0.01 mg/mL

$$V = \frac{M}{C}$$

$$\text{Sucrose space} = \frac{\text{Mass} - \text{Amount lost in urine}}{\text{Concentration of sucrose in plasma}}$$

$$= \frac{150 - 10 \text{ mg}}{0.01 \text{ mg/mL}}$$

$$= 14{,}000 \text{ mL}$$

Therefore, the ECF volume = 14 L.

■ MEASUREMENT OF PLASMA VOLUME

The substance which binds with plasma proteins strongly and diffuses into interstitium only in small quantities or does not diffuse is used to measure plasma volume. Such substances are listed in Table 6.2.

(Measurement of plasma volume and blood volume is explained in chapter 23).

■ MEASUREMENT OF INTERSTITIAL FLUID VOLUME

Volume of interstitial fluid cannot be measured directly. It is calculated from the values of ECF volume and plasma volume.

Interstitial fluid volume = ECF volume – Plasma volume

■ MEASUREMENT OF INTRACELLULAR FLUID VOLUME

Volume of ICF cannot be measured directly. It is calculated from the values of total body water and ECF.

ICF volume = Total fluid volume – ECF volume.

■ CONCENTRATION OF BODY FLUIDS

Concentration of body fluids is expressed in three ways:

1. Osmolality
2. Osmolarity
3. Tonicity.

■ OSMOLALITY

Measure of a fluid's capability to create osmotic pressure is called osmolality or osmotic (osmolar) concentration of a solution. In simple words, it is the concentration of osmotically active substance in the solution. Osmolality is expressed as the number of particles (osmoles) per kilogram of solution (osmoles/kg H_2O).

TABLE 6.2: Marker substances used to measure body fluid compartments

Fluid compartment	Marker substances
Total body water	1. Deuterium oxide (D_2O) 2. Tritium oxide (T_2O) 3. Antipyrine
Extracellular fluid	1. Radioactive sodium, chloride, bromide, sulfate and thiosulfate. 2. Non-metabolizable saccharides like inulin, mannitol, raffinose and sucrose
Plasma	1. Radioactive iodine (^{131}I) 2. Evans blue (T-1824)

■ OSMOLARITY

Osmolarity is another term to express the osmotic concentration. It is the number of particles (osmoles) per liter of solution (osmoles/L).

Osmotic pressure in solutions depends upon osmolality. However, in practice, the osmolarity and not osmolality is considered to determine the osmotic pressure because of the following reasons:

i. Measurement of weight (kilogram) of water in solution is a difficult process
ii. Difference between osmolality and osmolarity is very much negligible and it is less than 1%.

Often, these two terms are used interchangeably. Change in osmolality of ECF affects the volume of both ECF and ICF. When osmolality of ECF increases, water moves from ICF to ECF. When the osmolality decreases in ECF, water moves from ECF to ICF. Water movement continues until the osmolality of these two fluid compartments becomes equal.

Mole and Osmole

A mole (mol) is the molecular weight of a substance in gram. Millimole (mMol) is 1/1000 of a mole. One osmole (Osm) is the expression of amount of osmotically active particles. It is the molecular weight of a substance in grams divided by number of freely moving particles liberated in solution of each molecule. One milliosmole (mOsm) is 1/1000 of an osmole.

■ TONICITY

Usually, movement of water between the fluid compartments is not influenced by small molecules like urea and alcohol, which cross the cell membrane very rapidly. These small molecules are called ineffective osmoles. On the contrary, the larger molecules like

sodium and glucose, which cross the cell membrane slowly, can influence the movement of water. Therefore, such molecules are called effective osmoles. Osmolality that causes the movement of water from one compartment to another is called effective osmolality and the effective osmoles are responsible for this.

Tonicity is the measure of effective osmolality. In terms of tonicity, the solutions are classified into three categories:

i. Isotonic fluid
ii. Hypertonic fluid
iii. Hypotonic fluid.

i. *Isotonic Fluid*

Fluid which has the same effective osmolality (tonicity) as body fluids is called isotonic fluid. Examples are 0.9% sodium chloride solution (normal saline) and 5% glucose solution.

Red blood cells or other cells placed in isotonic fluid (normal saline) neither gain nor lose water by osmosis (Fig. 6.2). This is because of the **osmotic equilibrium** between inside and outside the cell across the cell membrane.

ii. *Hypertonic Fluid*

Fluid which has greater effective osmolality than the body fluids is called hypertonic fluid. Example is 2% sodium chloride solution.

When red blood cells or other cells are placed in hypertonic fluid, water moves out of the cells (exosmosis) resulting in shrinkage of the cells (crenation). Refer Figure 6.2

iii. *Hypotonic Fluid*

Fluid which has less effective osmolality than the body fluids is called hypotonic fluid. Example is 0.3% sodium chloride solution.

When red blood cells or other cells are placed in hypotonic fluid, water moves into the cells (endosmosis) and causes swelling of the cells (Fig. 6.2). Now the red blood cells become globular (sphereocytic) and get ruptured (hemolysis).

■ MAINTENANCE OF WATER BALANCE

Body has several mechanisms which work together to maintain the water balance. The important mechanisms involve hypothalamus (Chapters 4, 149) and kidneys (Chapter 53).

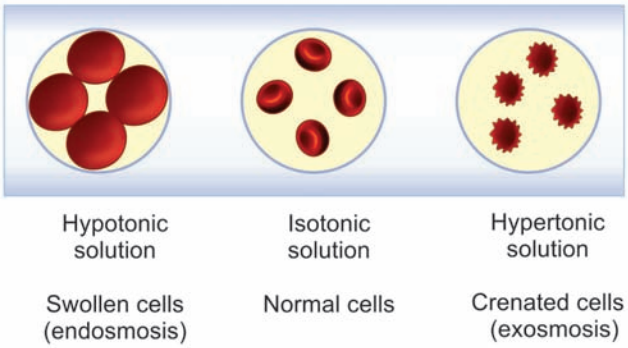


FIGURE 6.2: Effect of isotonic, hypertonic and hypotonic solutions on red blood cells

■ APPLIED PHYSIOLOGY

■ DEHYDRATION

Definition

Dehydration is defined as excessive loss of water from the body. Body requires certain amount of fluid intake daily for normal functions. Minimum daily requirement of water intake is about 1 L. This varies with the age and activity of the individual. The most active individuals need 2 to 3 L of water intake daily. Dehydration occurs when fluid loss is more than what is consumed.

Classification

Basically, dehydration is of three types:

1. *Mild dehydration:* It occurs when fluid loss is about 5% of total body fluids. Dehydration is not very serious and can be treated easily by rehydration.
2. *Moderate dehydration:* It occurs when fluid loss is about 10%. Dehydration becomes little serious and immediate treatment should be given by rehydration.
3. *Severe dehydration:* It occurs when fluid loss is about 15%. Dehydration becomes severe and requires hospitalization and emergency treatment. When fluid loss is more than 15%, dehydration becomes very severe and life threatening.

On the basis of ratio between water loss and sodium loss, dehydration is classified into three types:

1. *Isotonic dehydration:* Balanced loss of water and sodium as in the case of diarrhea or vomiting.
2. *Hypertonic dehydration:* Loss of more water than sodium as in the case of fever.
3. *Hypotonic dehydration:* Loss of more sodium than water as in the case of excess use of diuretics.

Causes

1. Severe diarrhea and vomiting due to gastrointestinal disorders
2. Excess urinary output due to renal disorders
3. Excess loss of water through urine due to endocrine disorders such as diabetes mellitus, diabetes insipidus and adrenal insufficiency
4. Insufficient intake of water
5. Prolonged physical activity without consuming adequate amount of water in hot environment
6. Excess sweating leading to heat frustration (extreme loss of water, heat and energy). Severe sweating and dehydration occur while spending longer periods on regular basis in the saunas
7. Use of laxatives or diuretics in order to lose weight quickly. This is common in athletes.

Signs and Symptoms

Mild and moderate dehydration

1. Dryness of the mouth
2. Excess thirst
3. Decrease in sweating
4. Decrease in urine formation
5. Headache
6. Dizziness
7. Weakness
8. Cramps in legs and arms.

Severe dehydration

1. Decrease in blood volume
2. Decrease in cardiac output
3. Low blood pressure
4. Hypovolemic cardiac shock
5. Fainting.

Very severe dehydration

1. Damage of organs like brain, liver and kidneys
2. Mental depression and confusion
3. Renal failure
4. Convulsions
5. Coma.

Dehydration in Infants

Infants suffering from severe diarrhea and vomiting caused by bacterial or viral infection, develop dehydration. It becomes life threatening if the lost body fluids are not replaced. This happens when parents are unable to recognize the signs.

Aging Effects on Dehydration

Elders are at higher risk for dehydration even if they are healthy. It is because of increased fluid loss and decreased fluid intake. In some cases, severe dehydration in old age may be fatal.

Treatment

Treatment depends upon the severity of dehydration. In mild dehydration, the best treatment is drinking of water and stopping fluid loss. However, in severe dehydration drinking water alone is ineffective because it cannot compensate the salt loss. So the effective treatment for severe dehydration is oral rehydration therapy.

Oral rehydration therapy

Oral rehydration therapy (ORT) is the treatment for dehydration in which a **oral rehydration solution (ORS)** is administered orally. ORS was formulated by World Health Organization (WHO). This solution contains anhydrous glucose, sodium chloride, potassium chloride and trisodium citrate.

In case of very severe dehydration, proper treatment is the intravenous administration of necessary water and electrolytes.

■ WATER INTOXICATION OR OVERHYDRATION

Definition

Water intoxication is the condition characterized by great increase in the water content of the body. It is also called overhydration, hyperhydration, water excess or water poisoning.

Causes

Water intoxication occurs when more fluid is taken than that can be excreted. Water intoxication due to drinking excess water is rare when the body's systems are functioning normally. But there are some conditions that can produce water intoxication.

1. Heart failure in which heart cannot pump blood properly
2. Renal disorders in which kidney fails to excrete enough water in urine
3. Hypersecretion of antidiuretic hormone as in the case of syndrome of inappropriate hypersecretion of antidiuretic hormone (SIADH)
4. Intravenous administration of unduly large amount of medications and fluids than the person's body can excrete

5. Infants have greater risk of developing water intoxication in the first month of life, when the filtration mechanism of the kidney is underdeveloped and cannot excrete the fluid rapidly
6. Water intoxication is also common in children having swimming practice, since they are more prone to drink too much of water while swimming
7. An adult (whose heart and kidneys are functioning normally) can develop water intoxication, if the person consumes about 8 L of water everyday regularly.

Signs and Symptoms

1. Since the brain is more vulnerable to the effects of water intoxication, behavioral changes appear first
2. Person becomes drowsy and inattentive
3. Nausea and vomiting occur
4. There is sudden loss of weight, followed by weakness and blurred vision
5. Anemia, acidosis, cyanosis, hemorrhage and shock are also common
6. Muscular symptoms such as weakness, cramps, twitching, poor coordination and paralysis develop
7. Severe conditions of water intoxication result in:
   i. Delirium (extreme mental condition characterized by confused state and illusion)
   ii. Seizures (sudden uncontrolled involuntary muscular contractions)
   iii. Coma (profound state of unconsciousness, in which the person fails to respond to external stimuli and cannot perform voluntary actions).

Treatment

Mild water intoxication requires only fluid restriction. In very severe cases, the treatment includes:

1. Diuretics to increase water loss through urine
2. Antidiuretic hormone (ADH) receptor antagonists to prevent ADH-induced reabsorption of water from renal tubules
3. Intravenous administration of saline to restore sodium.

Blood

Chapter 7

INTRODUCTION

Blood is a connective tissue in fluid form. It is considered as the **'fluid of life'** because it carries oxygen from lungs to all parts of the body and carbon dioxide from all parts of the body to the lungs. It is known as **'fluid of growth'** because it carries nutritive substances from the digestive system and hormones from endocrine gland to all the tissues. The blood is also called the **'fluid of health'** because it protects the body against the diseases and gets rid of the waste products and unwanted substances by transporting them to the excretory organs like kidneys.

PROPERTIES OF BLOOD

1. *Color:* Blood is red in color. Arterial blood is scarlet red because it contains more oxygen and venous blood is purple red because of more carbon dioxide.
2. *Volume:* Average volume of blood in a normal adult is 5 L. In a newborn baby, the volume is 450 ml. It increases during growth and reaches 5 L at the time of puberty. In females, it is slightly less and is about 4.5 L. It is about 8% of the body weight in a normal young healthy adult, weighing about 70 kg.
3. *Reaction and pH:* Blood is slightly alkaline and its pH in normal conditions is 7.4.
4. *Specific gravity:*
   Specific gravity of total blood : 1.052 to 1.061
   Specific gravity blood cells : 1.092 to 1.101
   Specific gravity of plasma : 1.022 to 1.026
5. *Viscosity:* Blood is five times more viscous than water. It is mainly due to red blood cells and plasma proteins.

COMPOSITION OF BLOOD

Blood contains the blood cells which are called formed elements and the liquid portion known as plasma.

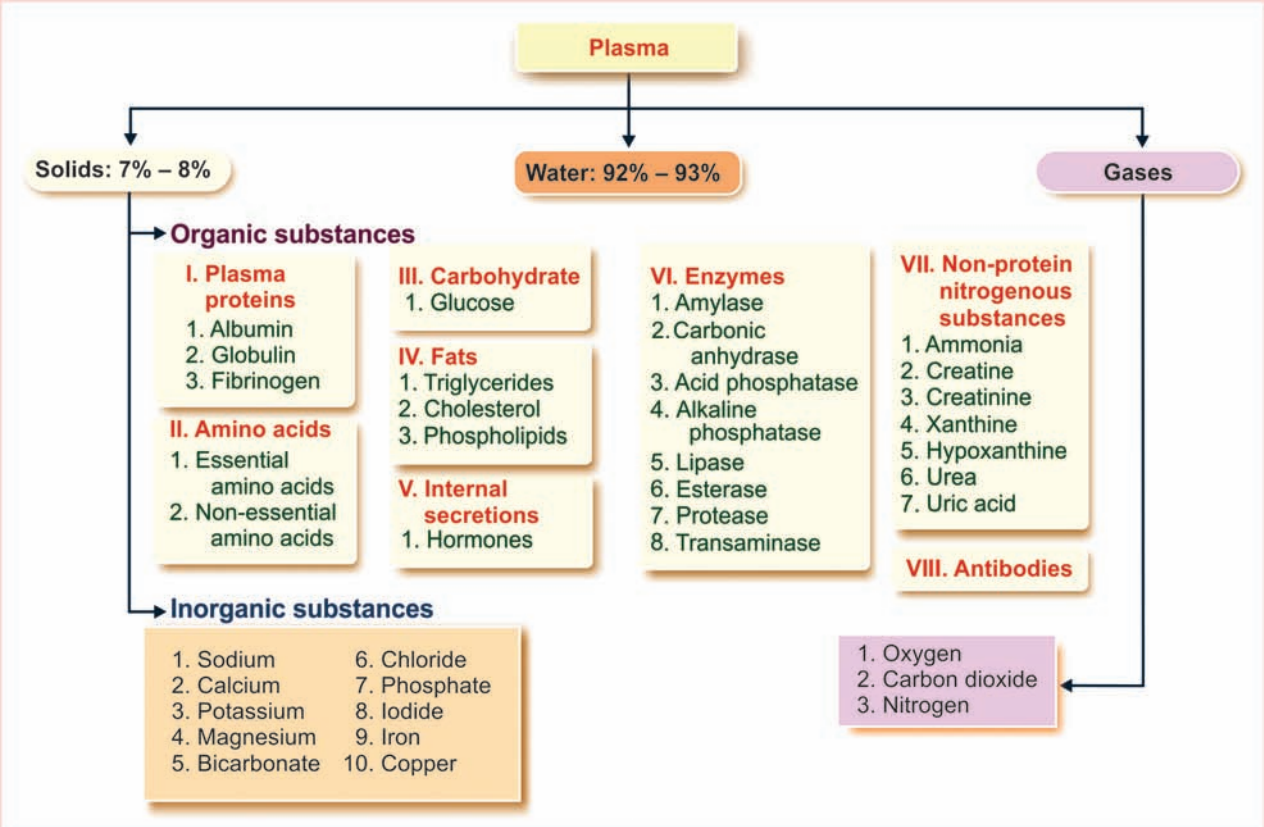


FIGURE 7.1: Composition of plasma

■ BLOOD CELLS

Three types of cells are present in the blood:

1. Red blood cells or erythrocytes
2. White blood cells or leukocytes
3. Platelets or thrombocytes.

Hematocrit Value

If blood is collected in a hematocrit tube along with a suitable anticoagulant and centrifuged for 30 minutes at a speed of 3000 revolutions per minute (rpm), the red blood cells settle down at the bottom having a clear plasma at the top. Plasma forms 55% and red blood cells form 45% of the total blood. Volume of red blood cells expressed in percentage is called the hematocrit value or packed cell volume (PCV). In between the plasma and the red blood cells, there is a thin layer of **white buffy coat.** This white buffy coat is formed by the aggregation of white blood cells and platelets (see Fig. 12.1).

TABLE 7.1: Normal values of some important substances in blood

Substance	Normal value
Glucose	100 to 120 mg/dL
Creatinine	0.5 to 1.5 mg/dL
Cholesterol	Up to 200 mg/dL
Plasma proteins	6.4 to 8.3 g/dL
Bilirubin	0.5 to 1.5 mg/dL
Iron	50 to 150 µg/dL
Copper	100 to 200 mg/dL
Calcium	9 to 11 mg/dL 4.5 to 5.5 mEq/L
Sodium	135 to 145 mEq/L
Potassium	3.5 to 5.0 mEq/L
Magnesium	1.5 to 2.0 mEq/L
Chloride	100 to 110 mEq/L
Bicarbonate	22 to 26 mEq/L

■ PLASMA

Plasma is a straw-colored clear liquid part of blood. It contains 91% to 92% of water and 8% to 9% of solids. The solids are the organic and the inorganic substances (Fig. 7.1). Table 7.1 gives the normal values of some important substances in blood.

■ SERUM

Serum is the clear straw-colored fluid that oozes from blood clot. When the blood is shed or collected in a container, it clots. In this process, the fibrinogen is converted into fibrin and the blood cells are trapped in this fibrin forming the blood clot. After about 45 minutes, serum oozes out of the blood clot.

For clinical investigations, serum is separated from blood cells and clotting elements by centrifuging. Volume of the serum is almost the same as that of plasma (55%). It is different from plasma only by the absence of fibrinogen, i.e. serum contains all the other constituents of plasma except fibrinogen. Fibrinogen is absent in serum because it is converted into fibrin during blood clotting. Thus,

Serum = Plasma – Fibrinogen

■ FUNCTIONS OF BLOOD

■ 1. NUTRITIVE FUNCTION

Nutritive substances like glucose, amino acids, lipids and vitamins derived from digested food are absorbed from gastrointestinal tract and carried by blood to different parts of the body for growth and production of energy.

■ 2. RESPIRATORY FUNCTION

Transport of respiratory gases is done by the blood. It carries oxygen from alveoli of lungs to different tissues and carbon dioxide from tissues to alveoli.

■ 3. EXCRETORY FUNCTION

Waste products formed in the tissues during various metabolic activities are removed by blood and carried to the excretory organs like kidney, skin, liver, etc. for excretion.

■ 4. TRANSPORT OF HORMONES AND ENZYMES

Hormones which are secreted by ductless (endocrine) glands are released directly into the blood. The blood transports these hormones to their target organs/tissues. Blood also transports enzymes.

■ 5. REGULATION OF WATER BALANCE

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

■ 6. REGULATION OF ACID-BASE BALANCE

Plasma proteins and hemoglobin act as buffers and help in the regulation of acid-base balance (Chapter 5).

■ 7. REGULATION OF BODY TEMPERATURE

Because of the high specific heat of blood, it is responsible for maintaining the thermoregulatory mechanism in the body, i.e. the balance between heat loss and heat gain in the body.

■ 8. 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.

■ 9. DEFENSIVE FUNCTION

Blood plays an important role in the defense of the body. The white blood cells are responsible for this function. Neutrophils and monocytes engulf the bacteria by phagocytosis. Lymphocytes are involved in development of immunity. Eosinophils are responsible for detoxification, disintegration and removal of foreign proteins (Chapters 16 and 17).

Plasma Proteins

Chapter 8

- INTRODUCTION
- NORMAL VALUES
- SEPARATION
- PROPERTIES
- ORIGIN
- FUNCTIONS
- PLASMAPHERESIS
- VARIATIONS IN PLASMA PROTEIN LEVEL

INTRODUCTION

Plasma proteins are:

1. Serum albumin
2. Serum globulin
3. Fibrinogen.

Serum (Chapter 7) contains only albumin and globulin. Fibrinogen is absent in serum because, it is converted into fibrin during blood clotting. Because of this, the albumin and globulin are usually called serum albumin and serum globulin.

NORMAL VALUES

Normal values of the plasma proteins are:

Total proteins	:	7.3 g/dL (6.4 to 8.3 g/dL)
Serum albumin	:	4.7 g/dL
Serum globulin	:	2.3 g/dL
Fibrinogen	:	0.3 g/dL

ALBUMIN/GLOBULIN RATIO

Ratio between plasma level of albumin and globulin is called albumin/globulin (A/G) ratio.

It is an important indicator of some diseases involving liver or kidney.

Normal A/G ratio is 2 : 1.

SEPARATION OF PLASMA PROTEINS

Plasma proteins are separated by the following methods.

1. PRECIPITATION METHOD

Proteins in the serum are separated into albumin and globulin. This is done by precipitating globulin with 22% sodium sulfate solution. Albumin remains in solution.

2. SALTING-OUT METHOD

Serum globulin is separated into two fractions called **euglobulin** and **pseudoglobulin** by salting out with different solutions. Euglobulin is salted out by full saturation with sodium chloride solution; half saturation with magnesium sulfate solution and one-third saturation with ammonium sulfate solution. It is insoluble in water. Pseudoglobulin is salted out by full saturation with magnesium sulfate and, half saturation with ammonium sulfate. It is soluble in water but it cannot be salted out by sodium chloride solution.

3. ELECTROPHORETIC METHOD

In this, the plasma proteins are separated depending on their differences in electrical charge and the rate of migration. It is done in a **Tiselius apparatus** by using

paper or cellulose or starch block. By this method, the proteins are separated into albumin (55%), alpha globulin (13%), beta globulin (14%), gamma globulin (11%) and fibrinogen (7%).

4. COHN'S FRACTIONAL PRECIPITATION METHOD

By this method, plasma proteins are separated into albumin and different fractions of globulin, depending upon their solubility.

5. ULTRACENTRIFUGATION METHOD

In this method, albumin, globulin and fibrinogen are separated depending upon their density. This method is also useful in determining the molecular weight of these proteins.

6. GEL FILTRATION CHROMATOGRAPHY

Gel filtration chromatography is a column chromatographic method by which the proteins are separated on the basis of size. Protein molecules are separated by passing through a bed of porous beads. The diffusion of different proteins into the beads depends upon their size.

7. IMMUNOELECTROPHORETIC METHOD

By this method, the proteins are separated on the basis of electrophoretic patterns formed by precipitation at the site of antigen-antibody reactions. This technique provides valuable quantitative measurement of different proteins.

PROPERTIES OF PLASMA PROTEINS

MOLECULAR WEIGHT

Albumin : 69,000
Globulin : 1,56,000
Fibrinogen : 4,00,000

Thus, the molecular weight of fibrinogen is greater than that of other two proteins.

ONCOTIC PRESSURE

Plasma proteins are responsible for the oncotic or osmotic pressure in the blood. Osmotic pressure exerted by proteins in the plasma is called **colloidal osmotic (oncotic) pressure** (Chapter 3). Normally, it is about 25 mm Hg. Albumin plays a major role in exerting oncotic pressure.

SPECIFIC GRAVITY

Specific gravity of the plasma proteins is 1.026.

BUFFER ACTION

Acceptance of hydrogen ions is called buffer action. The plasma proteins have 1/6 of total buffering action of the blood.

ORIGIN OF PLASMA PROTEINS

IN EMBRYO

In embryonic stage, the plasma proteins are synthesized by the **mesenchyme cells.** The albumin is synthesized first and other proteins are synthesized later.

IN ADULTS

In adults, the plasma proteins are synthesized mainly from **reticuloendothelial cells** of liver. The plasma proteins are synthesized also from spleen, bone marrow, disintegrating blood cells and general tissue cells. Gamma globulin is synthesized from B lymphocytes.

FUNCTIONS OF PLASMA PROTEINS

Plasma proteins are very essential for the body. Following are the functions of plasma proteins:

1. ROLE IN COAGULATION OF BLOOD

Fibrinogen is essential for the coagulation of blood (Chapter 20).

2. ROLE IN DEFENSE MECHANISM OF BODY

Gamma globulins play an important role in the defense mechanism of the body by acting as antibodies (immune substances). These proteins are also called immunoglobulins (Chapter 17). Antibodies react with antigens of various microorganisms, which cause diseases like diphtheria, typhoid, streptococcal infections, mumps, influenza, measles, hepatitis, rubella, poliomyelitis, etc.

3. ROLE IN TRANSPORT MECHANISM

Plasma proteins are essential for the transport of various substances in the blood. Albumin, alpha globulin and beta globulin are responsible for the transport of the hormones, enzymes, etc. The alpha and beta globulins play an important role in the transport of metals in the blood.

4. ROLE IN MAINTENANCE OF OSMOTIC PRESSURE IN BLOOD

At the capillary level, most of the substances are exchanged between the blood and the tissues. However,

because of their large size, the plasma proteins cannot pass through the capillary membrane easily and remain in the blood. In the blood, these proteins exert the colloidal osmotic (oncotic) pressure. Osmotic pressure exerted by the plasma proteins is about 25 mm Hg.

Since the concentration of albumin is more than the other plasma proteins, it exerts maximum pressure. Globulin is the next and fibrinogen exerts least pressure.

Importance of Osmotic Pressure – Starling's Hypothesis

Osmotic pressure exerted by the plasma proteins plays an important role in the exchange of various substances between blood and the cells through capillary membrane. According to Starling's hypothesis, the net filtration through capillary membrane is proportional to the hydrostatic pressure difference across the membrane minus the oncotic pressure difference (Chapter 27).

■ 5. ROLE IN REGULATION OF ACID-BASE BALANCE

Plasma proteins, particularly the albumin, play an important role in regulating the acid-base balance in the blood. This is because of the virtue of their buffering action (Chapter 5). Plasma proteins are responsible for 15% of the buffering capacity of blood.

■ 6. ROLE IN VISCOSITY OF BLOOD

Plasma proteins provide viscosity to the blood, which is important to maintain the blood pressure. Albumin provides maximum viscosity than the other plasma proteins.

■ 7. ROLE IN ERYTHROCYTE SEDIMENTATION RATE

Globulin and fibrinogen accelerate the tendency of rouleaux formation by the red blood cells. **Rouleaux formation** is responsible for ESR, which is an important diagnostic and prognostic tool (Chapter 12).

■ 8. ROLE IN SUSPENSION STABILITY OF RED BLOOD CELLS

During circulation, the red blood cells remain suspended uniformly in the blood. This property of the red blood cells is called the suspension stability. Globulin and fibrinogen help in the suspension stability of the red blood cells.

■ 9. ROLE IN PRODUCTION OF TREPHONE SUBSTANCES

Trephone substances are necessary for nourishment of tissue cells in culture. These substances are produced by leukocytes from the plasma proteins.

■ 10. 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 are split into amino acids by the tissue macrophages. Amino acids are taken back by blood and distributed throughout the body to form cellular protein molecules. Because of this, the plasma proteins are called the reserve proteins.

■ PLASMAPHERESIS

■ DEFINITION

Plasmapheresis is an experimental procedure done in animals to demonstrate the importance of plasma proteins. Earlier, this was called **Whipple's experiment** because it was established by George Hoyt Whipple.

■ PROCEDURE

Plasmapheresis is demonstrated in dogs. Blood is removed completely from the body of the dog. Red blood cells are separated from plasma and are washed in saline and reinfused into the body of the same dog along with a physiological solution called Locke's solution.

Due to sudden lack of proteins, the animal undergoes a state of shock. If the animal is fed with diet containing sufficiently high quantity of proteins, the normal level of plasma proteins is restored within seven days and the animal survives. The new plasma proteins are synthesized by the liver of the dog.

If the experiment is done in animals after removal of liver, even if the diet contains adequate quantity of proteins, the plasma proteins are not produced. The shock persists in the animal and leads to death.

Thus, the experiment 'plasmapheresis' is used to demonstrate:

1. Importance of plasma proteins for survival
2. Synthesis of plasma proteins by the liver.

■ CLINICAL SIGNIFICANCE OF PLASMAPHERESIS – THERAPEUTIC PLASMA EXCHANGE

Plasmapheresis is used as a blood purification procedure for an effective temporary treatment of many auto-

TABLE 8.1: Variations in plasma protein level

Plasma Protein	Conditions when increases	Conditions when decreases
Total proteins	Hyperproteinemia: 1. Dehydration 2. Hemolysis 3. Acute infections like acute hepatitis and acute nephritis 4. Respiratory distress syndrome 5. Excess of glucocorticoids 6. Leukemia 7. Rheumatoid arthritis 8. Alcoholism	Hypoproteinemia: 1. Diarrhea 2. Hemorrhage 3. Burns 4. Pregnancy 5. Malnutrition 6. Prolonged starvation 7. Cirrhosis of liver 8. Chronic infections like chronic hepatitis or chronic nephritis
Albumin	1. Dehydration 2. Excess of glucocorticoids 3. Congestive cardiac failure	1. Malnutrition 2. Cirrhosis of liver 3. Burns 4. Hypothyroidism 5. Nephrosis 6. Excessive intake of water
Globulin	1. Cirrhosis of liver 2. Chronic infections 3. Nephrosis 4. Rheumatoid arthritis	1. Emphysema 2. Acute hemolytic anemia 3. Glomerulonephritis 4. Hypogammaglobulinemia
Fibrinogen	1. Acute infections 2. Rheumatoid arthritis 3. Glomerulonephritis 4. Myocardial infarction 5. Stroke 6. Trauma	1. Liver dysfunction 2. Use of anabolic steroids 3. Use of phenobarbital
A/G ratio	1. Hypothyroidism 2. Excess of glucocorticoids 3. Hypogammaglobulinemia 4. Intake of high carbohydrate or protein diet	1. Liver dysfunction 2. Nephrosis

immune diseases. It is also called therapeutic plasma exchange.

In an autoimmune disease, the immune system attacks the body's own tissues through antibodies (Chapter 17). The antibodies that are proteins in nature circulate in the bloodstream before attacking the target tissues. Plasmapheresis is used to remove these antibodies from the blood.

Procedure

Venous blood is removed from the patient and blood cells are separated from plasma by the equipment called cell separator. This equipment works on the principle of a centrifuge. An anticoagulant is used to prevent the clotting of blood when it is removed from the body. After the separation of blood cells, the plasma is discarded. The blood cells are returned to the bloodstream of the patient by mixing with a substitute fluid (saline) and sterilized human albumin protein.

Uses of Plasmapheresis

Though plasmapheresis is used to remove antibodies from the blood, it cannot prevent the production of antibodies by the immune system of the body. So, it can provide only a temporary benefit of protecting the tissues from the antibodies. The patients must go for repeated sessions of this treatment.

Plasmapheresis is an effective temporary treatment for the following diseases:

1. Myasthenia gravis – autoimmune disease causing muscle weakness (Chapter 17)
2. Thrombocytopenic purpura – bleeding disorder (Chapter 20)

3. Paraproteinemic peripheral neuropathy – dysfunction of peripheral nervous system due to an abnormal immunoglobulin called paraprotein.
4. Chronic demyelinating polyneuropathy – neurological disorder characterized by progressive weakness and impaired sensory function in the legs and arms due to the damage of myelin sheath in peripheral nerves.
5. Guillain-Barré syndrome – autoimmune disease causing weakness, abnormal sensations (like tingling) in the limbs and paralysis.
6. Lambert-Eaton myasthenic syndrome – autoimmune disorder of the neuromuscular junction.

■ VARIATIONS IN PLASMA PROTEIN LEVEL

Level of plasma proteins vary independently of one another. However, in several conditions, the quantity of albumin and globulin change in opposite direction. Elevation of all fractions of plasma proteins is called **hyperproteinemia** and decrease in all fractions of plasma proteins is called **hypoproteinemia.** Variations in the level of plasma proteins are given in Table 8.1.

Red Blood Cells

Chapter 9

- INTRODUCTION
- NORMAL VALUE
- MORPHOLOGY
- PROPERTIES
- LIFESPAN
- FATE
- FUNCTIONS
- VARIATIONS IN NUMBER
- VARIATIONS IN SIZE
- VARIATIONS IN SHAPE
- VARIATIONS IN STRUCTURE

INTRODUCTION

Red blood cells (RBCs) are the **non-nucleated** formed elements in the blood. Red blood cells are also known as erythrocytes (erythros = red). Red color of the red blood cell is due to the presence of the coloring pigment called hemoglobin. RBCs play a vital role in transport of respiratory gases. RBCs are larger in number compared to the other two blood cells, namely white blood cells and platelets.

NORMAL VALUE

RBC count ranges between 4 and 5.5 million/cu mm of blood. In adult males, it is 5 million/cu mm and in adult females, it is 4.5 million/cu mm.

MORPHOLOGY OF RED BLOOD CELLS

NORMAL SHAPE

Normally, the RBCs are disk shaped and biconcave (dumbbell shaped). Central portion is thinner and periphery is thicker. The biconcave contour of RBCs has some mechanical and functional advantages.

Advantages of Biconcave Shape of RBCs

1. Biconcave shape helps in equal and rapid diffusion of oxygen and other substances into the interior of the cell.
2. Large surface area is provided for absorption or removal of different substances.
3. Minimal tension is offered on the membrane when the volume of cell alters.
4. Because of biconcave shape, while passing through minute capillaries, RBCs squeeze through the capillaries very easily without getting damaged.

NORMAL SIZE

Diameter	:	7.2 µ (6.9 to 7.4 µ).
Thickness	:	At the periphery it is thicker with 2.2 µ and at the center it is thinner with 1 µ (Fig. 9.1). This difference in thickness is because of the biconcave shape.
Surface area	:	120 sq µ.
Volume	:	85 to 90 cu µ.

NORMAL STRUCTURE

Red blood cells are non-nucleated. Only mammal, which has nucleated RBC is camel. Because of the

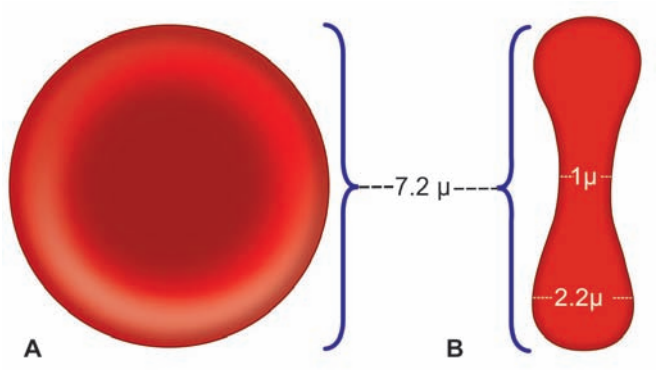


FIGURE 9.1: Dimensions of RBC.
A. Surface view, B. Sectioned view.

absence of nucleus in human RBC, the DNA is also absent. Other organelles such as mitochondria and Golgi apparatus also are absent in RBC. Because of absence of mitochondria, the energy is produced from glycolytic process. Red cell does not have insulin receptor and so the glucose uptake by this cell is not controlled by insulin.

RBC has a special type of **cytoskeleton,** which is made up of **actin** and **spectrin.** Both the proteins are anchored to transmembrane proteins by means of another protein called **ankyrin.** Absence of spectrin results in hereditary spherocytosis. In this condition, the cell is deformed, losses its biconcave shape and becomes globular (spherocytic). The spherocyte is very fragile and easily ruptured (hemolyzed) in hypotonic solutions.

■ PROPERTIES OF RED BLOOD CELLS

■ ROULEAUX FORMATION

When blood is taken out of the blood vessel, the RBCs pile up one above another like the pile of coins. This property of the RBCs is called rouleaux (pleural = rouleau) formation (Fig. 9.2). It is accelerated by plasma proteins globulin and fibrinogen.

■ SPECIFIC GRAVITY

Specific gravity of RBC is 1.092 to 1.101.

■ PACKED CELL VOLUME

Packed cell volume (PCV) is the proportion of blood occupied by RBCs expressed in percentage. It is also called hematocrit value. It is 45% of the blood and the plasma volume is 55% (Chapters 7 and 13).

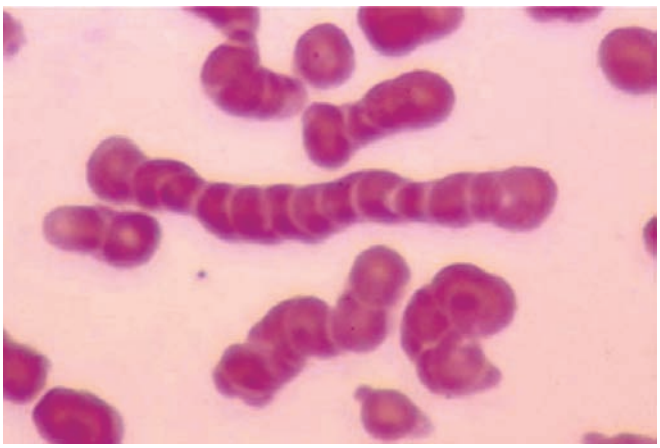


FIGURE 9.2: Rouleau formation
(*Courtesy:* Dr Nivaldo Medeiros)

■ SUSPENSION STABILITY

During circulation, the RBCs remain suspended uniformly in the blood. This property of the RBCs is called the suspension stability.

■ LIFESPAN OF RED BLOOD CELLS

Average lifespan of RBC is about 120 days. After the lifetime the senile (old) RBCs are destroyed in reticuloendothelial system.

Determination of Lifespan of Red Blood Cells

Lifespan of the RBC is determined by radioisotope method. RBCs are tagged with radioactive substances like radioactive iron or radioactive chromium. Life of RBC is determined by studying the rate of loss of radioactive cells from circulation.

■ FATE OF RED BLOOD CELLS

When the cells become older (120 days), the cell membrane becomes more fragile. Diameter of the capillaries is less or equal to that of RBC. Younger RBCs can pass through the capillaries easily. However, because of the fragile nature, the older cells are destroyed while trying to squeeze through the capillaries. The destruction occurs mainly in the capillaries of red pulp of spleen because the diameter of splenic capillaries is very small. So, the spleen is called **'graveyard of RBCs'.**

Destroyed RBCs are fragmented and hemoglobin is released from the fragmented parts. Hemoglobin is immediately phagocytized by macrophages of the body, particularly the macrophages present in liver **(Kupffer cells),** spleen and bone marrow.

Hemoglobin is degraded into iron, globin and porphyrin. Iron combines with the protein called apoferritin to form ferritin, which is stored in the body and reused later. Globin enters the protein depot for later use (Fig. 9.3). Porphyrin is degraded into bilirubin, which is excreted by liver through bile (Chapter 40).

Daily 10% RBCs, which are senile, are destroyed in normal young healthy adults. It causes release of about 0.6 g/dL of hemoglobin into the plasma. From this 0.9 to 1.5 mg/dL bilirubin is formed.

■ FUNCTIONS OF RED BLOOD CELLS

Major function of RBCs is the transport of respiratory gases. Following are the functions of RBCs:

1. *Transport of Oxygen from the Lungs to the Tissues*

Hemoglobin in RBC combines with oxygen to form **oxyhemoglobin.** About 97% of oxygen is transported in blood in the form of oxyhemoglobin (Chapter 125).

2. *Transport of Carbon Dioxide from the Tissues to the Lungs*

Hemoglobin combines with carbon dioxide and form **carbhemoglobin.** About 30% of carbon dioxide is transported in this form.

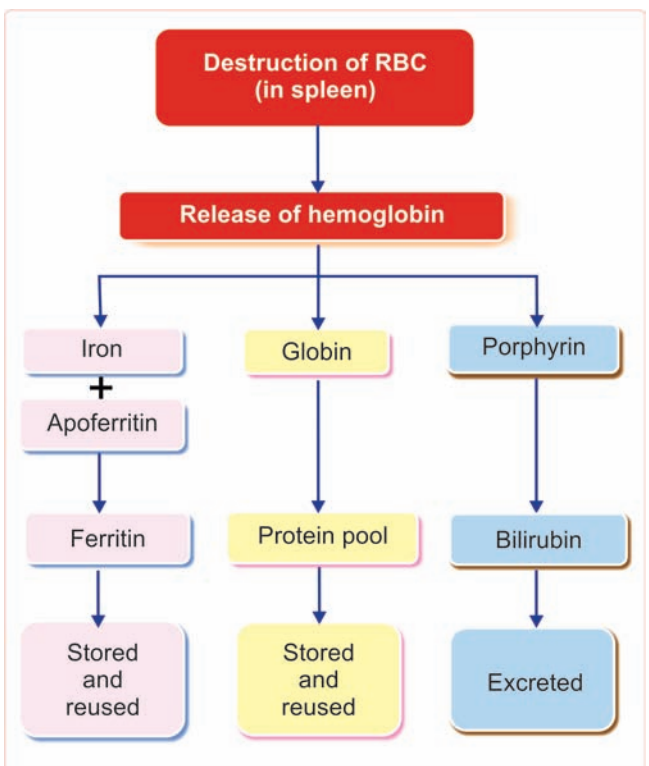


FIGURE 9.3: Fate of RBC

RBCs contain a large amount of the **carbonic anhydrase.** This enzyme is necessary for the formation of bicarbonate from water and carbon dioxide (Chapter 125). Thus, it helps to transport carbon dioxide in the form of bicarbonate from tissues to lungs. About 63% of carbon dioxide is transported in this form.

3. *Buffering Action in Blood*

Hemoglobin functions as a good buffer. By this action, it regulates the hydrogen ion concentration and thereby plays a role in the maintenance of acid-base balance (Chapter 5).

4. *In Blood Group Determination*

RBCs carry the **blood group antigens** like A antigen, B antigen and Rh factor. This helps in determination of blood group and enables to prevent reactions due to incompatible blood transfusion (Chapter 21).

■ VARIATIONS IN NUMBER OF RED BLOOD CELLS

■ PHYSIOLOGICAL VARIATIONS

A. *Increase in RBC Count*

Increase in the RBC count is known as **polycythemia.** It occurs in both physiological and pathological conditions. When it occurs in physiological conditions it is called physiological polycythemia. The increase in number during this condition is marginal and temporary. It occurs in the following conditions:

1. *Age*

At birth, the RBC count is 8 to 10 million/cu mm of blood. The count decreases within 10 days after birth due to destruction of RBCs causing **physiological jaundice** in some newborn babies. However, in infants and growing children, the cell count is more than the value in adults.

2. *Sex*

Before puberty and after menopause in females the RBC count is 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*

Inhabitants of mountains (above 10,000 feet from mean sea level) have an increased RBC count of more than 7 million/cu mm. It is due to **hypoxia** (decreased oxygen supply to tissues) in high altitude. Hypoxia stimulates kidney to secrete a hormone called **erythropoietin.** The

erythropoietin in turn stimulates the bone marrow to produce more RBCs (Fig. 9.4).

4. *Muscular exercise*

There is a temporary increase in RBC count after exercise. It is because of mild hypoxia and contraction of spleen. Spleen stores RBCs (Chapter 25). Hypoxia increases the sympathetic activity resulting in secretion of adrenaline from adrenal medulla. Adrenaline contracts spleen and RBCs are released into blood (Fig. 9.5).

5. *Emotional conditions*

RBC count increases during the emotional conditions such as anxiety. It is because of increase in the sympathetic activity as in the case of muscular exercise (Fig. 9.5).

6. *Increased environmental temperature*

Increase in atmospheric temperature increases RBC count. Generally increased temperature increases all the activities in the body including production of RBCs.

7. *After meals*

There is a slight increase in the RBC count after taking meals. It is because of need for more oxygen for metabolic activities.

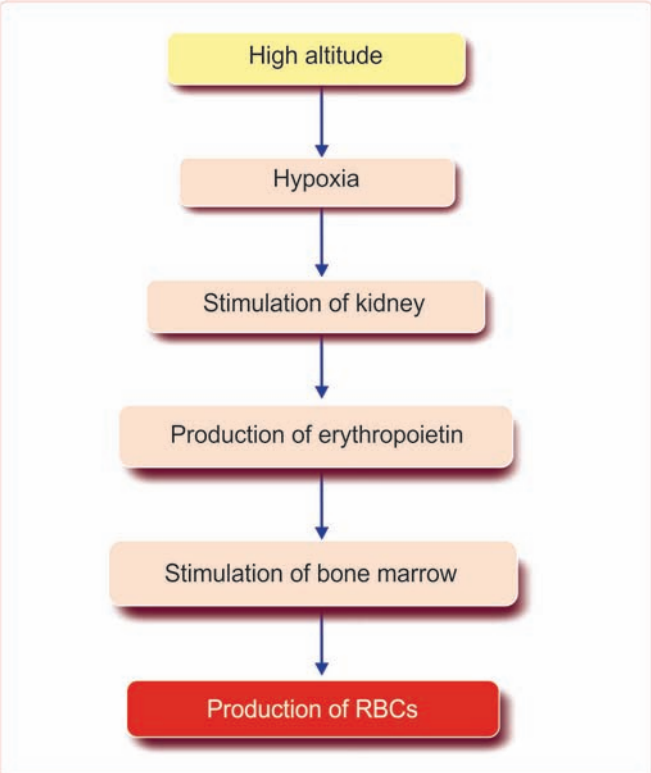


FIGURE 9.4: Physiological polycythemia in high altitude

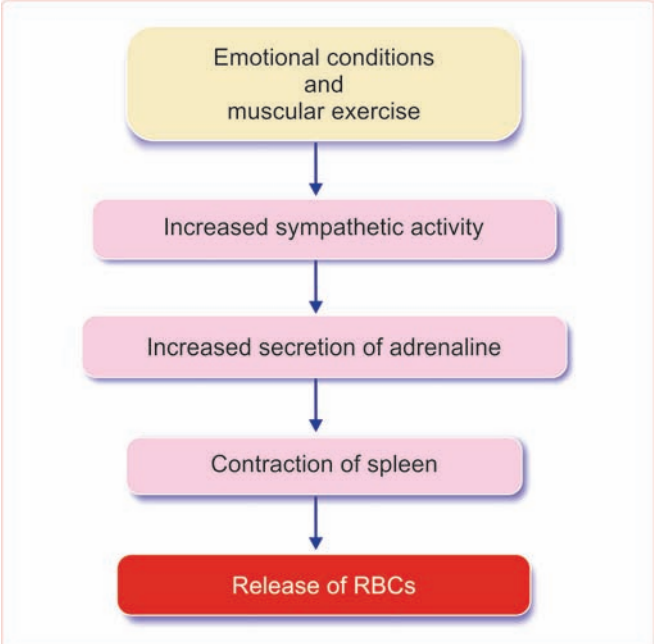


FIGURE 9.5: Physiological polycythemia in emotional conditions and exercise

B. *Decrease in RBC Count*

Decrease in RBC count occurs in the following physiological conditions:

1. *High barometric pressures*

At high barometric pressures as in deep sea, when the oxygen tension of blood is higher, the RBC count decreases.

2. *During sleep*

RBC count decreases slightly during sleep and immediately after getting up from sleep. Generally all the activities of the body are decreased during sleep including production of RBCs.

3. *Pregnancy*

In pregnancy, the RBC count decreases. It is because of increase in ECF volume. Increase in ECF volume, increases the plasma volume also resulting in hemodilution. So, there is a relative reduction in the RBC count.

■ PATHOLOGICAL VARIATIONS

Pathological Polycythemia

Pathological polycythemia is the abnormal increase in the RBC count. Red cell count increases above 7 million/cu mm of the blood. Polycythemia is of two types, the primary polycythemia and secondary polycythemia.

Primary Polycythemia – Polycythemia Vera

Primary polycythemia is otherwise known as polycythemia vera. It is a disease characterized by persistent increase in RBC count above 14 million/cu mm of blood. This is always associated with increased white blood cell count above 24,000/cu mm of blood. Polycythemia vera occurs in **myeloproliferative disorders** like malignancy of red bone marrow.

Secondary Polycythemia

This is secondary to some of the pathological conditions (diseases) such as:

1. Respiratory disorders like emphysema.
2. Congenital heart disease.
3. Ayerza's disease (condition associated with hypertrophy of right ventricle and obstruction of blood flow to lungs).
4. Chronic carbon monoxide poisoning.
5. Poisoning by chemicals like phosphorus and arsenic.
6. Repeated mild hemorrhages.

All these conditions lead to hypoxia which stimulates the release of erythropoietin. Erythropoietin stimulates the bone marrow resulting in increased RBC count.

Anemia

Abnormal decrease in RBC count is called anemia. This is described in Chapter 14.

■ VARIATIONS IN SIZE OF RED BLOOD CELLS

Under physiological conditions, the size of RBCs in venous blood is slightly larger than those in arterial blood. In pathological conditions, the variations in size of RBCs are:

1. Microcytes (smaller cells)
2. Macrocytes (larger cells)
3. Anisocytes (cells with different sizes).

■ MICROCYTES

Microcytes are present in:

i. Iron-deficiency anemia
ii. Prolonged forced breathing
iii. Increased osmotic pressure in blood.

■ MACROCYTES

Macrocytes are present in:

i. Megaloblastic anemia
ii. Decreased osmotic pressure in blood.

■ ANISOCYTES

Anisocytes occurs in pernicious anemia.

■ VARIATIONS IN SHAPE OF RED BLOOD CELLS

Shape of RBCs is altered in many conditions including different types of anemia.

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 RED BLOOD CELLS

■ PUNCTATE BASOPHILISM

Striated appearance of RBCs by the presence of dots of **basophilic materials** (porphyrin) is called punctate basophilism. It occurs in conditions like **lead poisoning.**

■ RING IN RED BLOOD CELLS

Ring or twisted strands of basophilic material appear in the periphery of the RBCs. This is also called the **Goblet ring.** This appears in the RBCs in certain types of anemia.

■ HOWELL-JOLLY BODIES

In certain types of anemia, some nuclear fragments are present in the ectoplasm of the RBCs. These nuclear fragments are called Howell-Jolly bodies.

Chapter 10

Erythropoiesis

DEFINITION

Erythropoiesis is the process of the origin, development and maturation of erythrocytes. Hemopoiesis or hematopoiesis is the process of origin, development and maturation of all the blood cells.

SITE OF ERYTHROPOIESIS

IN FETAL LIFE

In fetal life, the erythropoiesis occurs in three stages:

1. *Mesoblastic Stage*

During the first two months of intrauterine life, the RBCs are produced from **mesenchyme** of yolk sac.

2. *Hepatic Stage*

From third month of intrauterine life, **liver** is the main organ that produces RBCs. **Spleen** and **lymphoid organs** are also involved in erythropoiesis.

3. *Myeloid Stage*

During the last three months of intrauterine life, the RBCs are produced from red **bone marrow** and **liver.**

IN NEWBORN BABIES, CHILDREN AND ADULTS

In newborn babies, growing children and adults, RBCs are produced only from the red bone marrow.

1. *Up to the age of 20 years:* RBCs are produced from red bone marrow of all bones (**long bones** and all the **flat bones**).
2. *After the age of 20 years:* RBCs are produced from **membranous bones** like vertebra, sternum, ribs, scapula, iliac bones and skull bones and from the ends of long bones. After 20 years of age, the shaft of the long bones becomes yellow bone marrow because of fat deposition and looses the erythropoietic function.

In adults, liver and spleen may produce the blood cells if the bone marrow is destroyed or fibrosed. Collectively bone marrow is almost equal to liver in size and weight. It is also as active as liver. Though bone marrow is the site of production of all blood cells, comparatively 75% of the bone marrow is involved in the production of leukocytes and only 25% is involved in the production of erythrocytes.

But still, the leukocytes are less in number than the erythrocytes, the ratio being 1:500. This is mainly because of the lifespan of these cells. Lifespan of erythrocytes is 120 days whereas the lifespan of leukocytes is very

short ranging from one to ten days. So the leukocytes need larger production than erythrocytes to maintain the required number.

PROCESS OF ERYTHROPOIESIS

STEM CELLS

Stem cells are the primary cells capable of self-renewal and differentiating into specialized cells (Chapter 1). Hemopoietic stem cells are the primitive cells in the bone marrow, which give rise to the blood cells.

Hemopoietic stem cells in the bone marrow are called uncommitted pluripotent hemopoietic stem cells (PHSC). PHSC is defined as a cell that can give rise to all types of blood cells. In early stages, the PHSC are not designed to form a particular type of blood cell. And it is also not possible to determine the blood cell to be developed from these cells: hence, the name uncommitted PHSC (Fig. 10.1). In adults, only a few number of these cells are present. But the best source of these cells is the umbilical cord blood.

When the cells are designed to form a particular type of blood cell, the uncommitted PHSCs are called committed PHSCs. Committed PHSC is defined as a cell, which is restricted to give rise to one group of blood cells.

Committed PHSCs are of two types:

1. Lymphoid stem cells (LSC) which give rise to lymphocytes and natural killer (NK) cells
2. Colony forming blastocytes, which give rise to myeloid cells. Myeloid cells are the blood cells other than lymphocytes. When grown in cultures, these cells form colonies hence the name colony forming blastocytes.

Different units of colony forming cells are:

i. Colony forming unit-erythrocytes (CFU-E) – Cells of this unit develop into erythrocytes
ii. Colony forming unit-granulocytes/monocytes (CFU-GM) – These cells give rise to granulocytes (neutrophils, basophils and eosinophils) and monocytes
iii. Colony forming unit-megakaryocytes (CFU-M) – Platelets are developed from these cells.

CHANGES DURING ERYTHROPOIESIS

Cells of CFU-E pass through different stages and finally become the matured RBCs. During these stages four important changes are noticed.

1. Reduction in size of the cell (from the diameter of 25 to 7.2 μ)
2. Disappearance of nucleoli and nucleus

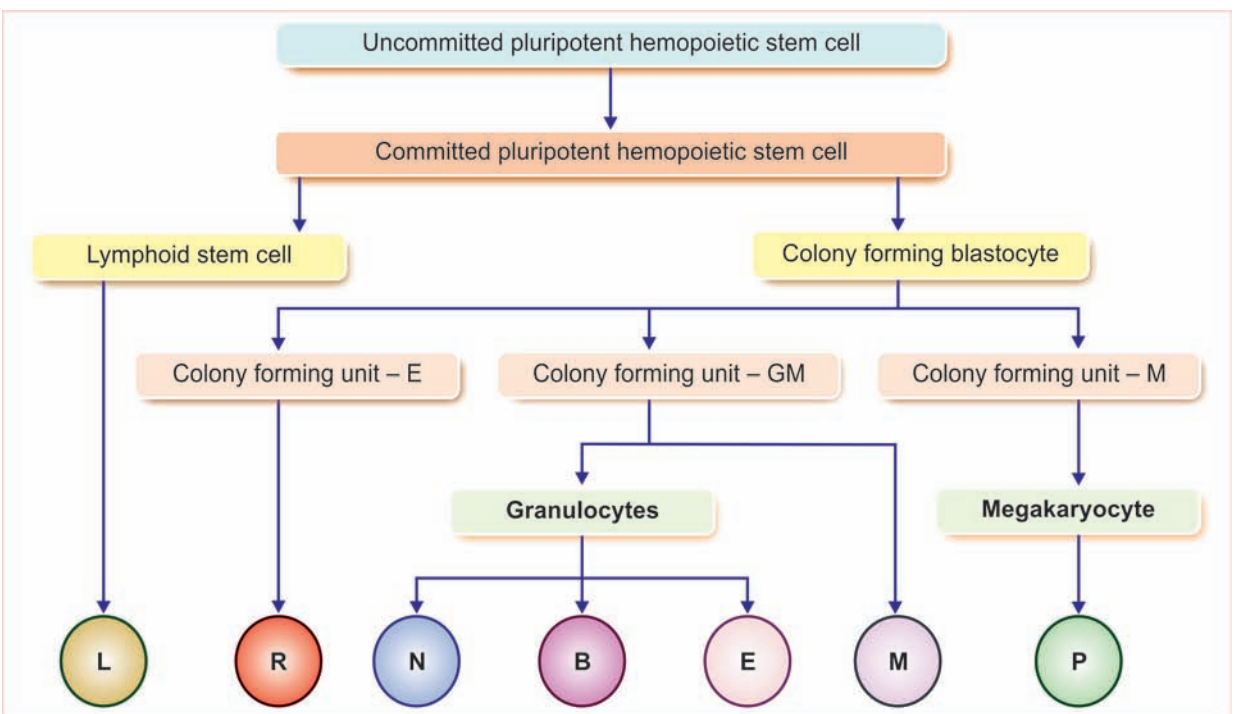


FIGURE 10.1: Stem cells. L = Lymphocyte, R = Red blood cell, N = Neutrophil, B = Basophil, E = Eosinophil, M = Monocyte, P = Platelet.

3. Appearance of hemoglobin
4. Change in the staining properties of the cytoplasm.

■ STAGES OF ERYTHROPOIESIS

Various stages between CFU-E cells and matured RBCs are (Fig. 10.2):

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

1. *Proerythroblast (Megaloblast)*

Proerythroblast or megaloblast is the first cell derived from CFU-E. It is very large in size with a diameter of about 20 µ. Its nucleus is large and occupies the cell almost completely. The nucleus has two or more nucleoli

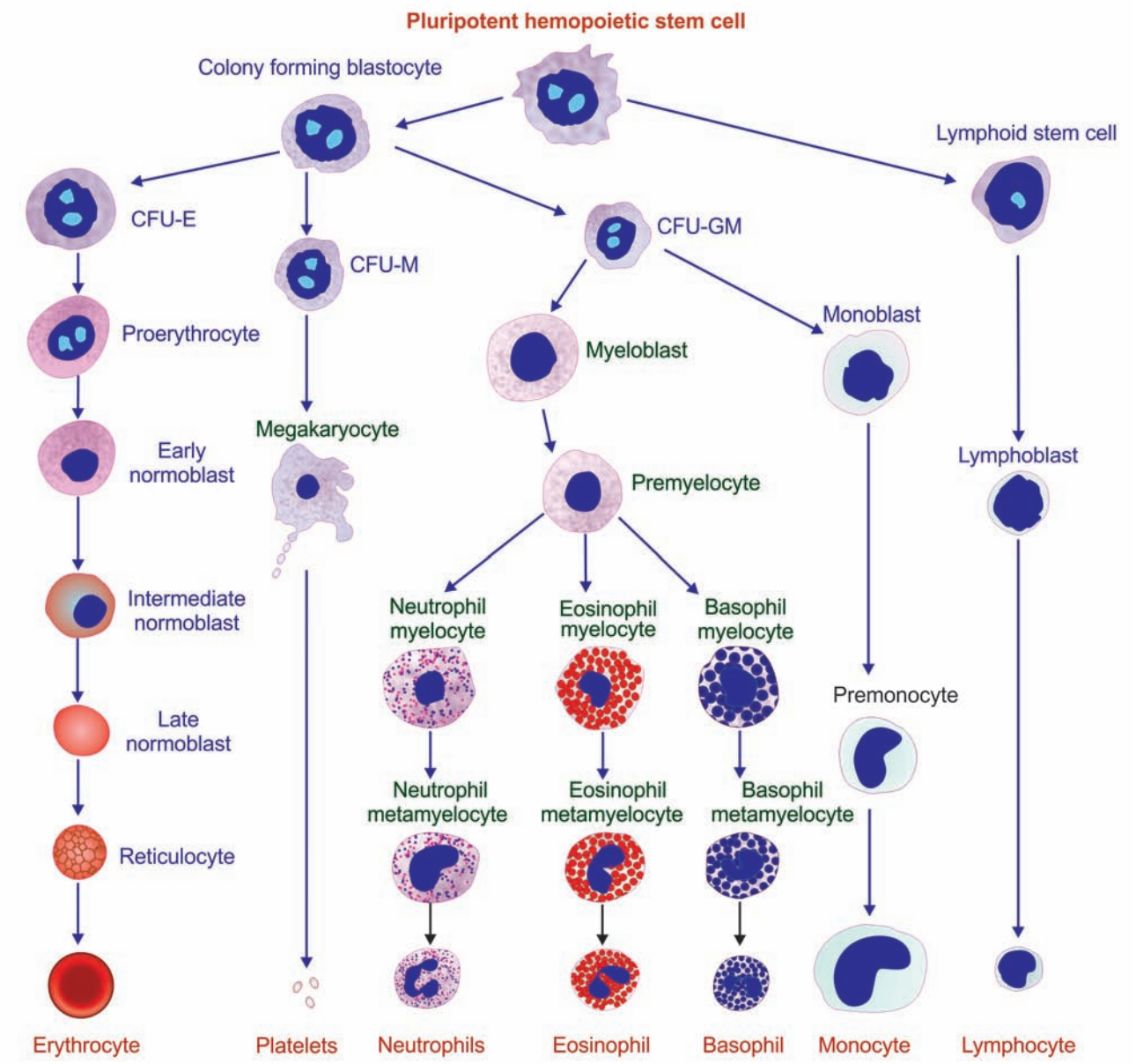


FIGURE 10.2: Stages of erythropoiesis. CFU-E = Colony forming unit-erythrocyte, CFU-M = Colony forming unit-megakaryocyte, CFU-GM = Colony forming unit-granulocyte/monocyte.

and a reticular network. Proerythroblast does not contain hemoglobin. The cytoplasm is basophilic in nature. Proerythroblast multiplies several times and finally forms the cell of next stage called early normoblast. Synthesis of hemoglobin starts in this stage. However, appearance of hemoglobin occurs only in intermediate normoblast.

2. *Early Normoblast*

The early normoblast is little smaller than proerythroblast with a diameter of about 15 µ. In the nucleus, the nucleoli disappear. Condensation of chromatin network occurs. The condensed network becomes dense. The cytoplasm is basophilic in nature. So, this cell is also called **basophilic erythroblast.** This cell develops into next stage called intermediate normoblast.

3. *Intermediate Normoblast*

Cell is smaller than the early normoblast with a diameter of 10 to 12 µ. The nucleus is still present. But, the chromatin network shows further condensation. The hemoglobin starts appearing.

Cytoplasm is already basophilic. Now, because of the presence of hemoglobin, it stains with both acidic as well as basic stains. So this cell is called polychromophilic or **polychromatic erythroblast.** This cell develops into next stage called late normoblast.

4. *Late Normoblast*

Diameter of the cell decreases further to about 8 to 10 µ. Nucleus becomes very small with very much condensed chromatin network and it is known as ink-spot nucleus.

Quantity of hemoglobin increases. And the cytoplasm becomes almost acidophilic. So, the cell is now called orthochromic erythroblast. In the final stage of late normoblast just before it passes to next stage, the nucleus disintegrates and disappears. The process by which nucleus disappears is called **pyknosis.** The final remnant is extruded from the cell. Late normoblast develops into the next stage called reticulocyte.

5. *Reticulocyte*

Reticulocyte is otherwise known as immature RBC. It is slightly larger than matured RBC. The cytoplasm contains the reticular network or reticulum, which is formed by remnants of disintegrated organelles. Due to the reticular network, the cell is called reticulocyte. The reticulum of reticulocyte stains with supravital stain.

In newborn babies, the reticulocyte count is 2% to 6% of RBCs, i.e. 2 to 6 reticulocytes are present for every 100 RBCs. The number of reticulocytes decreases during the first week after birth. Later, the reticulocyte count remains constant at or below 1% of RBCs. The number increases whenever production and release of RBCs increase.

Reticulocyte is basophilic due to the presence of remnants of disintegrated Golgi apparatus, mitochondria and other organelles of cytoplasm. During this stage, the cells enter the blood capillaries through capillary membrane from site of production by diapedesis. Important events during erythropoiesis is given in Table 10.1

6. *Matured Erythrocyte*

Reticular network disappears and the cell becomes the matured RBC and attains the biconcave shape. The cell decreases in size to 7.2 µ diameter. The matured RBC is with hemoglobin but without nucleus.

It requires 7 days for the development and maturation of RBC from proerythroblast. It requires 5 days up to the stage of reticulocyte. Reticulocyte takes 2 more days to become the matured RBC.

TABLE 10.1: Important events during erythropoiesis

Stage of erythropoiesis	Important event
Proerythroblast	Synthesis of hemoglobin starts
Early normoblast	Nucleoli disappear
Intermediate normoblast	Hemoglobin starts appearing
Late normoblast	Nucleus disappears
Reticulocyte	Reticulum is formed. Cell enters capillary from site of production
Matured RBC	Reticulum disappears Cell attains biconcavity

■ FACTORS NECESSARY FOR ERYTHROPOIESIS

Development and maturation of erythrocytes require variety of factors, which are classified into three categories:

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

■ GENERAL FACTORS

General factors necessary for erythropoiesis are:

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

i. *Erythropoietin*

Most important general factor for erythropoiesis is the hormone called erythropoietin. It is also called hemopoietin or erythrocyte stimulating factor.

Chemistry

Erythropoietin is a glycoprotein with 165 amino acids.

Source of secretion

Major quantity of erythropoietin is secreted by peritubular capillaries of kidney. A small quantity is also secreted from liver and brain.

Stimulant for secretion

Hypoxia is the stimulant for the secretion of erythropoietin.

Actions of erythropoietin

Erythropoietin causes formation and release of new RBCs into circulation. After secretion, it takes 4 to 5 days to show the action.

Erythropoietin promotes the following processes:

a. Production of proerythroblasts from CFU-E of the bone marrow
b. Development of proerythroblasts into matured RBCs through the several stages – early normoblast, intermediate normoblast, late normoblast and reticulocyte
c. Release of matured erythrocytes into blood. Even some reticulocytes (immature erythrocytes) are released along with matured RBCs.

Blood level of erythropoietin increases in anemia.

ii. *Thyroxine*

Being a general metabolic hormone, thyroxine accelerates the process of erythropoiesis at many levels. So, hyperthyroidism and polycythemia are common.

iii. *Hemopoietic Growth Factors*

Hemopoietic growth factors or growth inducers are the interleukins and stem cell factor (steel factor). Generally these factors induce the proliferation of PHSCs. Interleukins (IL) are glycoproteins, which belong to the cytokines family.

Interleukins involved in erythropoiesis:

a. Interleukin-3 (IL-3) secreted by T-cells
b. Interleukin-6 (IL-6) secreted by T-cells, endothelial cells and macrophages
c. Interleukin-11 (IL-11) secreted by osteoblast.

iv. *Vitamins*

Some vitamins are also necessary for the process of erythropoiesis. Deficiency of these vitamins cause anemia associated with other disorders.

Vitamins necessary for erythropoiesis:

a. *Vitamin B:* Its deficiency causes anemia and pellagra (disease characterized by skin lesions, diarrhea, weakness, nervousness and dementia).
b. *Vitamin C:* Its deficiency causes anemia and scurvy (ancient disease characterized by impaired collagen synthesis resulting in rough skin, bleeding gum, loosening of teeth, poor wound healing, bone pain, lethargy and emotional changes).
c. *Vitamin D:* Its deficiency causes anemia and rickets (bone disease – Chapter 68).
d. *Vitamin E:* Its deficiency leads to anemia and malnutrition.

■ MATURATION FACTORS

Vitamin B12, intrinsic factor and folic acid are necessary for the maturation of RBCs.

1. *Vitamin B12 (Cyanocobalamin)*

Vitamin B12 is the maturation factor necessary for erythropoiesis.

Source

Vitamin B12 is called **extrinsic factor** since it is obtained mostly from diet. Its absorption from intestine requires the presence of **intrinsic factor of Castle.** Vitamin B12 is stored mostly in liver and in small quantity in muscle. When necessary, it is transported to the bone marrow to promote maturation of RBCs. It is also produced in the large intestine by the intestinal flora.

Action

Vitamin B12 is essential for synthesis of DNA in RBCs. Its deficiency leads to failure in maturation of the cell and reduction in the cell division. Also, the cells are larger with fragile and weak cell membrane resulting in macrocytic anemia.

Deficiency of vitamin B12 causes **pernicious anemia.** So, vitamin B12 is called antipernicious factor.

2. *Intrinsic Factor of Castle*

Intrinsic factor of castle is produced in gastric mucosa by the parietal cells of the gastric glands. It is essential

for the absorption of vitamin B12 from intestine. In the absence of intrinsic factor, vitamin B12 is not absorbed from intestine. This leads to pernicious anemia.

Deficiency of intrinsic factor occurs in:

i. Severe gastritis
ii. Ulcer
iii. Gastrectomy.

Hematinic principle

Hematinic principle is the principle thought to be produced by the action of intrinsic factor on extrinsic factor. It is also called or **antianemia principle.** It is a maturation factor.

3. Folic Acid

Folic acid is also essential for maturation. It is required for the synthesis of DNA. In the absence of folic acid, the synthesis of DNA decreases causing failure of maturation. This leads to anemia in which the cells are larger and appear in megaloblastic (proerythroblastic) stage. And, anemia due to folic acid deficiency is called **megaloblastic anemia.**

■ FACTORS NECESSARY FOR HEMOGLOBIN FORMATION

Various materials are essential for the formation of hemoglobin in the RBCs. Deficiency of these substances decreases the production of hemoglobin leading to anemia.

Such factors are:

1. *First class proteins and amino acids:* Proteins of high biological value are essential for the formation of hemoglobin. Amino acids derived from these proteins are required for the synthesis of protein part of hemoglobin, i.e. the globin.
2. *Iron:* Necessary for the formation of heme part of the hemoglobin.
3. *Copper:* Necessary for the absorption of iron from the gastrointestinal tract.
4. *Cobalt and nickel:* These metals are essential for the utilization of iron during hemoglobin formation.
5. *Vitamins:* Vitamin C, riboflavin, nicotinic acid and pyridoxine are also essential for the formation of hemoglobin.

Hemoglobin and Iron Metabolism

Chapter 11

- INTRODUCTION
- NORMAL HEMOGLOBIN CONTENT
- FUNCTIONS
- STRUCTURE
- TYPES OF NORMAL HEMOGLOBIN
- ABNORMAL HEMOGLOBIN
- ABNORMAL HEMOGLOBIN DERIVATIVES
- SYNTHESIS
- DESTRUCTION
- IRON METABOLISM

INTRODUCTION

Hemoglobin (Hb) is the iron containing coloring matter of red blood cell (RBC). It is a chromoprotein forming 95% of dry weight of RBC and 30% to 34% of wet weight. Function of hemoglobin is to carry the respiratory gases, oxygen and carbon dioxide. It also acts as a buffer. Molecular weight of hemoglobin is 68,000.

NORMAL HEMOGLOBIN CONTENT

Average hemoglobin (Hb) content in blood is 14 to 16 g/dL. However, the value varies depending upon the age and sex of the individual.

Age

At birth	:	25 g/dL
After 3rd month	:	20 g/dL
After 1 year	:	17 g/dL
From puberty onwards	:	14 to 16 g/dL

At the time of birth, hemoglobin content is very high because of increased number of RBCs (Chapter 9).

Sex

In adult males	:	15 g/dL
In adult females	:	14.5 g/dL

FUNCTIONS OF HEMOGLOBIN

TRANSPORT OF RESPIRATORY GASES

Main function of hemoglobin is the transport of respiratory gases:

1. Oxygen from the lungs to tissues.
2. Carbon dioxide from tissues to lungs.

1. *Transport of Oxygen*

When oxygen binds with hemoglobin, a physical process called **oxygenation** occurs, resulting in the formation of oxyhemoglobin. The iron remains in ferrous state in this compound. Oxyhemoglobin is an unstable compound and the combination is reversible, i.e. when more oxygen is available, it combines with hemoglobin and whenever oxygen is required, hemoglobin can release oxygen readily (Chapter 125).

When oxygen is released from oxyhemoglobin, it is called reduced hemoglobin or ferrohemoglobin.

2. *Transport of Carbon Dioxide*

When carbon dioxide binds with hemoglobin, carbhemoglobin is formed. It is also an unstable compound and the combination is reversible, i.e. the carbon dioxide can be released from this compound. The affinity of

hemoglobin for carbon dioxide is 20 times more than that for oxygen (Chapter 125).

■ BUFFER ACTION

Hemoglobin acts as a buffer and plays an important role in acid-base balance (Chapter 5).

■ STRUCTURE OF HEMOGLOBIN

Hemoglobin is a conjugated protein. It consists of a protein combined with an iron-containing pigment. The protein part is globin and the iron-containing pigment is **heme.** Heme also forms a part of the structure of **myoglobin** (oxygen-binding pigment in muscles) and **neuroglobin** (oxygen-binding pigment in brain).

■ IRON

Normally, it is present in ferrous (Fe^{2+}) form. It is in unstable or loose form. In some abnormal conditions, the iron is converted into ferric (Fe^{3+}) state, which is a stable form.

■ PORPHYRIN

The pigment part of heme is called porphyrin. It is formed by four pyrrole rings (tetrapyrrole) called, I, II, III and IV. The **pyrrole rings** are attached to one another by methane (CH_4) bridges.

The iron is attached to 'N' of each pyrrole ring and 'N' of globin molecule.

■ GLOBIN

Globin contains four polypeptide chains. Among the four polypeptide chains, two are chains and two are α-chains (Refer Table 11.1 for molecular weight and number of amino acids in the polypeptide chains).

TABLE 11.1: Molecular weight and number of amino acids of polypeptide chains of globin

Polypeptide chain	Molecular weight	Amino acids
α-chain	15,126	141
β-chain	15,866	146

■ TYPES OF NORMAL HEMOGLOBIN

Hemoglobin is of two types:

1. Adult hemoglobin – HbA
2. Fetal hemoglobin – HbF

Replacement of fetal hemoglobin by adult hemoglobin starts immediately after birth. It is completed at about 10th to 12th week after birth. Both the types of hemoglobin differ from each other structurally and functionally.

Structural Difference

In adult hemoglobin, the globin contains two α-chains and two β-chains. In fetal hemoglobin, there are two α chains and two γ-chains instead of β-chains.

Functional Difference

Functionally, fetal hemoglobin has more affinity for oxygen than that of adult hemoglobin. And, the oxygen-hemoglobin dissociation curve of fetal blood is shifted to left (Chapter 125).

■ ABNORMAL HEMOGLOBIN

Abnormal types of hemoglobin or hemoglobin variants are the pathologic mutant forms of hemoglobin. These variants are produced because of structural changes in the polypeptide chains caused by mutation in the genes of the globin chains. Most of the mutations do not produce any serious problem. Occasionally, few mutations result in some disorders.

There are two categories of abnormal hemoglobin:

1. Hemoglobinopathies
2. Hemoglobin in thalassemia and related disorders.

1. *Hemoglobinopathies*

Hemoglobinopathy is a genetic disorder caused by abnormal polypeptide chains of hemoglobin.

Some of the hemoglobinopathies are:

i. *Hemoglobin S:* It is found in sickle cell anemia. In this, the α-chains are normal and β-chains are abnormal.

ii. *Hemoglobin C:* The β-chains are abnormal. It is found in people with hemoglobin C disease, which is characterized by mild hemolytic anemia and splenomegaly.

iii. *Hemoglobin E:* Here also the β-chains are abnormal. It is present in people with hemoglobin E disease which is also characterized by mild hemolytic anemia and splenomegaly.

iv. *Hemoglobin M:* It is the abnormal hemoglobin present in the form of methemoglobin. It occurs due to mutation of genes of both in α and β chains, resulting in abnormal replacement of amino acids. It is present in babies affected by hemoglobin M disease or blue baby syndrome. It is an inherited disease, characterized by methemoglobinemia.

2. *Hemoglobin in Thalassemia and Related Disorders*

In thalassemia, different types of abnormal hemoglobins are present. The polypeptide chains are decreased, absent or abnormal. In α-thalassemia, the α-chains are decreased, absent or abnormal and in β-thalassemia, the β-chains are decreased, absent or abnormal (Chapter 14). Some of the abnormal hemoglobins found in thalassemia are hemoglobin G, H, I, Bart's, Kenya, Lepore and constant spring.

■ ABNORMAL HEMOGLOBIN DERIVATIVES

'Hemoglobin derivatives' refer to a blood test to detect and measure the percentage of abnormal hemoglobin derivatives.

Hemoglobin is the only carrier for transport of oxygen, without which tissue death occurs within few minutes. When hemoglobin is altered, its oxygen carrying capacity is decreased resulting in lack of oxygen. So, it is important to know about the causes and the effects of abnormal hemoglobin derivatives.

Abnormal hemoglobin derivatives are formed by carbon monoxide (CO) poisoning or due to some drugs like nitrites, nitrates and sulphanamides.

Abnormal hemoglobin derivatives are:

1. Carboxyhemoglobin
2. Methemoglobin
3. Sulfhemoglobin.

Normal percentage of hemoglobin derivatives in total hemoglobin:

Carboxyhemoglobin : 3% to 5 %
Methemoglobin : less than 3%
Sulfhemoglobin : trace (undetectable).

Abnormally high levels of hemoglobin derivates in blood produce serious effects. These derivatives prevent the transport of oxygen resulting in oxygen lack in tissues, which may be fatal.

■ CARBOXYHEMOGLOBIN

Carboxyhemoglobin or carbon monoxyhemoglobin is the abnormal hemoglobin derivative formed by the combination of carbon monoxide with hemoglobin. Carbon monoxide is a colorless and odorless gas. Since hemoglobin has 200 times more affinity for carbon monoxide than oxygen, it hinders the transport of oxygen resulting in tissue hypoxia (Chapter 127).

Normally, 1% to 3% of hemoglobin is in the form of carboxyhemoglobin.

Sources of Carbon Monoxide

1. Charcoal burning
2. Coal mines
3. Deep wells
4. Underground drainage system
5. Exhaust of gasoline engines
6. Gases from guns and other weapons
7. Heating system with poor or improper ventilation
8. Smoke from fire
9. Tobacco smoking.

Signs and Symptoms of Carbon Monoxide Poisoning

1. While breathing air with less than 1% of CO, the Hb saturation is 15% to 20% and mild symptoms like headache and nausea appear
2. While breathing air with more than 1% CO, the Hb saturation is 30% to 40%. It causes severe symptoms like:
   i. Convulsions
   ii. Cardiorespiratory arrest
   iii. Unconsciousness and coma.
3. When Hb saturation increases above 50%, death occurs.

■ METHEMOGLOBIN

Methemoglobin is the abnormal hemoglobin derivative formed when iron molecule of hemoglobin is oxidized from normal ferrous state to ferric state. Methemoglobin is also called **ferrihemoglobin.**

Normal methemoglobin level is 0.6% to 2.5% of total hemoglobin.

Under normal circumstances also, body faces the threat of continuous production of methemoglobin. But it is counteracted by erythrocyte protective system called **nicotinamide adenine dinucleotide (NADH)** system, which operates through two enzymes:

1. Diaphorase I (nicotinamide adenine dinucleotide phosphate [NADPH]-dependent reductase): Responsible for 95% of the action.
2. Diaphorase II (NADPH-dependent methemoglobin reductase): Responsible for 5% of the action.

These two enzymes prevent the oxidation of ferrous iron into ferric iron.

Methemoglobinemia

Methemoglobinemia is the disorder characterized by high level of methemoglobin in blood. It leads to tissue hypoxia, which causes cyanosis and other symptoms.

Causes of methemoglobinemia

Methemoglobinemia is caused by variety of factors:

1. *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
   v. Local anesthetics like benzocaine.

4. *Hereditary trait:*

Due to deficiency of NADH-dependant reductase or presence of abnormal hemoglobin M. Hemoglobin M is common in babies affected by blue baby syndrome (a pathological condition in infants, characterized by bluish skin discoloration (cyanosis), caused by congenital heart defect).

■ SULFHEMOGLOBIN

Sulfhemoglobin is the abnormal hemoglobin derivative, formed by the combination of hemoglobin with hydrogen sulfide. It is caused by drugs such as phenacetin or sulfonamides.

Normal sulfhemoglobin level is less than 1% of total hemoglobin.

Sulfhemoglobin cannot be converted back into hemoglobin. Only way to get rid of this from the body is to wait until the affected RBCs with sulfhemoglobin are destroyed after their lifespan.

Blood Level of Sulfhemoglobin

Normally, very negligible amount of sulfhemoglobin is present in blood which is nondetectable. But when its level rises above 10 gm/dL, cyanosis occur. Usually, serious toxic effects are not noticed.

■ SYNTHESIS OF HEMOGLOBIN

Synthesis of hemoglobin actually starts in proerythroblastic stage (Fig. 11.1). However, hemoglobin appears in the intermediate normoblastic stage only. Production of hemoglobin is continued until the stage of reticulocyte.

Heme portion of hemoglobin is synthesized in mitochondria. And the protein part, globin is synthesized in ribosomes.

■ SYNTHESIS OF HEME

Heme is synthesized from succinyl-CoA and the glycine. The sequence of events in synthesis of hemoglobin:

1. First step in heme synthesis takes place in the mitochondrion. Two molecules of succinyl-CoA combine with two molecules of glycine and condense to form δ-aminolevulinic acid (ALA) by ALA synthase.
2. ALA is transported to the cytoplasm. Two molecules of ALA combine to form porphobilinogen in the presence of ALA dehydratase.
3. Porphobilinogen is converted into uroporphobilinogen I by uroporphobilinogen I synthase.
4. Uroporphobilinogen I is converted into uroporphobilinogen III by porphobilinogen III cosynthase.
5. From uroporphobilinogen III, a ring structure called coproporphyrinogen III is formed by uroporphobilinogen decarboxylase.
6. Coproporphyrinogen III is transported back to the mitochondrion, where it is oxidized to form protoporphyrinogen IX by coproporphyrinogen oxidase
7. Protoporphyrinogen IX is converted into protoporphyrin IX by protoporphyrinogen oxidase.
8. Protoporphyrin IX combines with iron to form heme in the presence of ferrochelatase.

■ FORMATION OF GLOBIN

Polypeptide chains of globin are produced in the ribosomes. There are four types of polypeptide chains namely, alpha, beta, gamma and delta chains. Each of these chains differs from others by the amino acid sequence. Each globin molecule is formed by the combination of 2 pairs of chains and each chain is made of 141 to 146 amino acids. Adult hemoglobin contains two alpha chains and two beta chains. Fetal hemoglobin contains two alpha chains and two gamma chains.

■ CONFIGURATION

Each polypeptide chain combines with one heme molecule. Thus, after the complete configuration, each

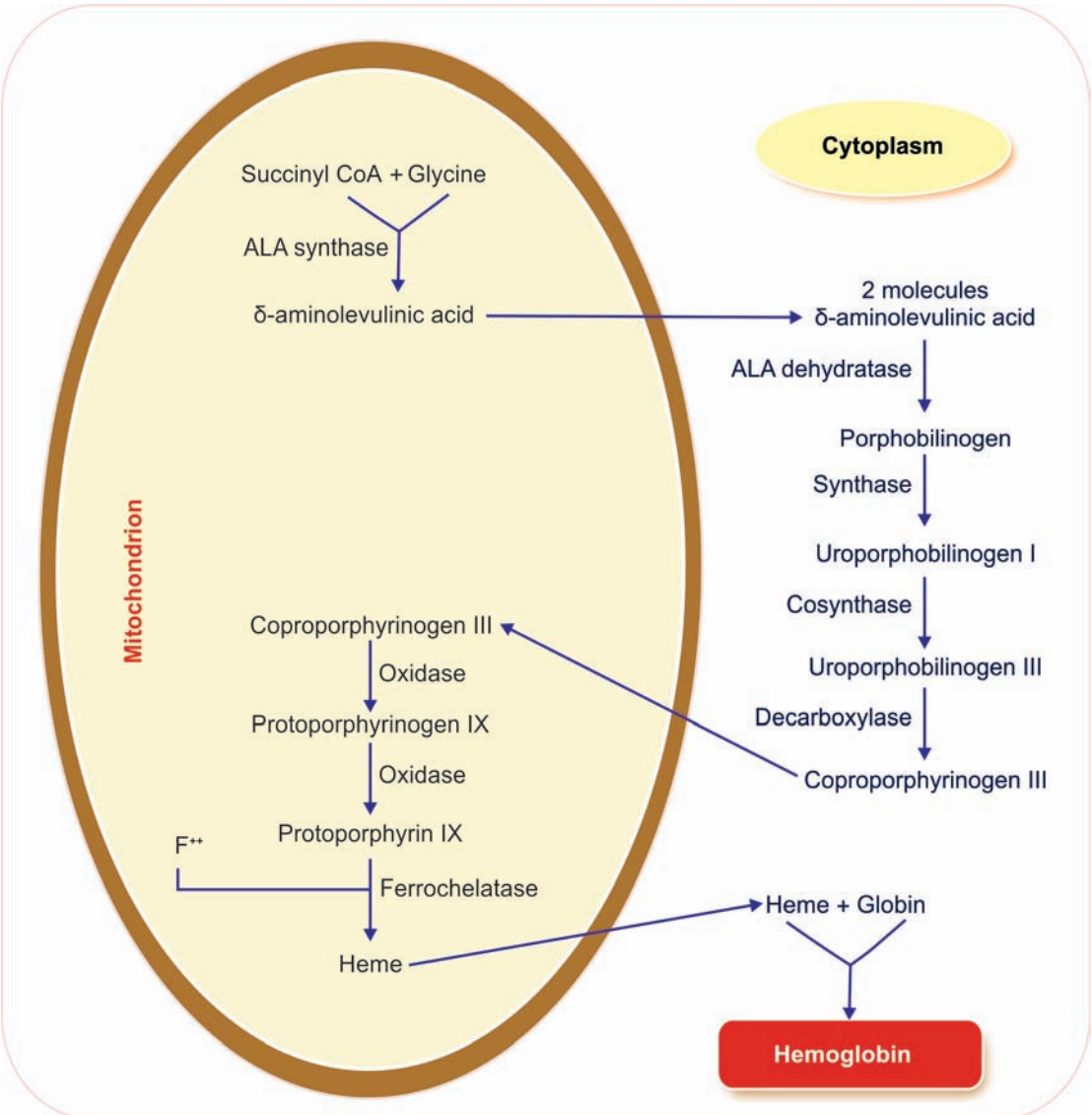


FIGURE 11.1: Synthesis of hemoglobin

hemoglobin molecule contains 4 polypeptide chains and 4 heme molecules.

■ SUBSTANCES NECESSARY FOR HEMOGLOBIN SYNTHESIS

Various materials are essential for the formation of hemoglobin in the RBC (Refer Chapter 10 for details).

■ DESTRUCTION OF HEMOGLOBIN

After the lifespan of 120 days, the RBC is destroyed in the reticuloendothelial system, particularly in spleen and the hemoglobin is released into plasma. Soon, the hemoglobin is degraded in the reticuloendothelial cells and split into globin and heme.

Globin is utilized for the resynthesis of hemoglobin. Heme is degraded into iron and porphyrin. Iron is stored in the body as ferritin and hemosiderin, which are reutilized for the synthesis of new hemoglobin. Porphyrin is converted into a green pigment called biliverdin. In human being, most of the biliverdin is converted into a yellow pigment called bilirubin. Bilirubin and biliverdin are together called the bile pigments (Details of bile pigments are given in Chapter 40).

■ IRON METABOLISM

■ IMPORTANCE OF IRON

Iron is an essential mineral and an important component of proteins, involved in oxygen transport. So, human

body needs iron for oxygen transport. Iron is important for the formation of hemoglobin and myoglobin. Iron is also necessary for the formation of other substances like **cytochrome, cytochrome oxidase, peroxidase** and **catalase.**

■ NORMAL VALUE AND DISTRIBUTION OF IRON IN THE BODY

Total quantity of iron in the body is about 4 g. Approximate distribution of iron in the body is as follows:

In the hemoglobin	: 65% to 68%
In the muscle as myoglobin	: 4%
As intracellular oxidative heme compound	: 1%
In the plasma as transferrin	: 0.1%
Stored in the reticuloendothelial system	: 25% to 30%

■ DIETARY IRON

Dietary iron is available in two forms called heme and nonheme.

Heme Iron

Heme iron is present in fish, meat and chicken. Iron in these sources is found in the form of heme. Heme iron is absorbed easily from intestine.

Non-heme Iron

Iron in the form of nonheme is available in vegetables, grains and cereals. Non-heme iron is not absorbed easily as heme iron. Cereals, flours and products of grains which are enriched or fortified (strengthened) with iron become good dietary sources of non-heme iron, particularly for children and women.

■ ABSORPTION OF IRON

Iron is absorbed mainly from the small intestine. It is absorbed through the intestinal cells (enterocytes) by pinocytosis and transported into the blood. Bile is essential for the absorption of iron.

Iron is present mostly in ferric (Fe^{3+}) form. It is converted into ferrous form (Fe^{2+}) which is absorbed into the blood. Hydrochloric acid from gastric juice makes the ferrous iron soluble so that it could be converted into ferric iron by the enzyme ferric reductase from enterocytes. From enterocytes, ferric iron is 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** (secreted by liver through bile) 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.

■ STORAGE OF IRON

Iron is stored in large quantities in reticuloendothelial cells and liver hepatocytes. In other cells also it is stored in small quantities. In the cytoplasm of the cell, iron is stored as ferritin in large amount. Small quantity of iron is also stored as hemosiderin.

■ 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. This is because of the menstruation.

One gram of hemoglobin contains 3.34 mg of iron. Normally, 100 mL of blood contains 15 gm of hemoglobin and about 50 mg of iron (3.34 × 15). So, if 100 mL of blood is lost from the body, there is a loss of about 50 mg of iron. In females, during every menstrual cycle, about 50 mL of blood is lost by which 25 mg of iron is lost. This is why the iron content is always less in females than in males.

Iron is lost during hemorrhage and blood donation also. If 450 mL of blood is donated, about 225 mg of iron is lost.

■ REGULATION OF TOTAL IRON IN THE BODY

Absorption and excretion of iron are maintained almost equally under normal physiological conditions. When the iron storage is saturated in the body, it automatically reduces the further absorption of iron from the gastro-intestinal tract by feedback mechanism.

Factors which reduce the absorption of iron:

1. Stoppage of apotransferrin formation in the liver, so that the iron cannot be absorbed from the intestine.
2. Reduction in the release of iron from the transferrin, so that transferrin is completely saturated with iron and further absorption is prevented.

Erythrocyte Sedimentation Rate

Chapter 12

DEFINITION

Erythrocyte sedimentation rate (ESR) is the rate at which the erythrocytes settle down. Normally, the red blood cells (RBCs) remain suspended uniformly in circulation. This is called suspension stability of RBCs. If blood is mixed with an anticoagulant and allowed to stand on a vertical tube, the red cells settle down due to gravity with a supernatant layer of clear plasma.

ESR is also called sedimentation rate, **sed rate** or **Biernacki reaction.** It was first demonstrated by Edmund Biernacki in 1897.

DETERMINATION OF ESR

There are two methods to determine ESR.

1. Westergren method
2. Wintrobe method

WESTERGREN METHOD

In this method, Westergren tube is used to determine ESR.

Westergren Tube

The tube is 300 mm long and opened on both ends (Fig. 12.1A). It is marked 0 to 200 mm from above downwards. Westergren tube is used only for determining ESR.

1.6 mL of blood is mixed with 0.4 mL of 3.8% sodium citrate (anticoagulant) and loaded in the Westergren tube. The ratio of blood and anticoagulant is 4:1. The tube is fitted to the stand vertically and left undisturbed. The reading is taken at the end of 1 hour.

WINTROBE METHOD

In this method, Wintrobe tube is used to determine ESR.

Wintrobe Tube

Wintrobe tube is a short tube opened on only one end (Fig. 12.1B). It is 110 mm long with 3 mm bore. Wintrobe tube is used for determining ESR and PCV. It is marked

on both sides. On one side the marking is from 0 to 100 (for ESR) and on other side from 100 to 0 (for PCV).

About 1 mL of blood is mixed with anticoagulant, ethylenediaminetetraacetic acid (EDTA). The blood is loaded in the tube up to '0' mark and the tube is placed on the Wintrobe stand. And, the reading is taken after 1 hour.

NORMAL VALUES OF ESR

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

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

SIGNIFICANCE OF DETERMINING ESR

Erythrocyte sedimentation rate (ESR) is an easy, inexpensive and non-specific test, which helps in diagnosis as well as prognosis. It is non-specific because it cannot indicate the exact location or cause of disease. But, it helps to confirm the diagnosis. Prognosis means monitoring the course of disease and response of the patient to therapy. Determination of ESR is especially helpful in assessing the progress of patients treated for certain chronic inflammatory disorders such as:

1. Pulmonary tuberculosis (Chapter 14)
2. Rheumatoid arthritis (Chapter 14)
3. Polymyalgia rheumatica (inflammatory disease characterized by pain in shoulder and hip)
4. Temporal arteritis (inflammation of arteries of head).

VARIATIONS OF ESR

PHYSIOLOGICAL VARIATION

1. *Age:* ESR is less in children and infants because of more number of RBCs.
2. *Sex:* It is 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.

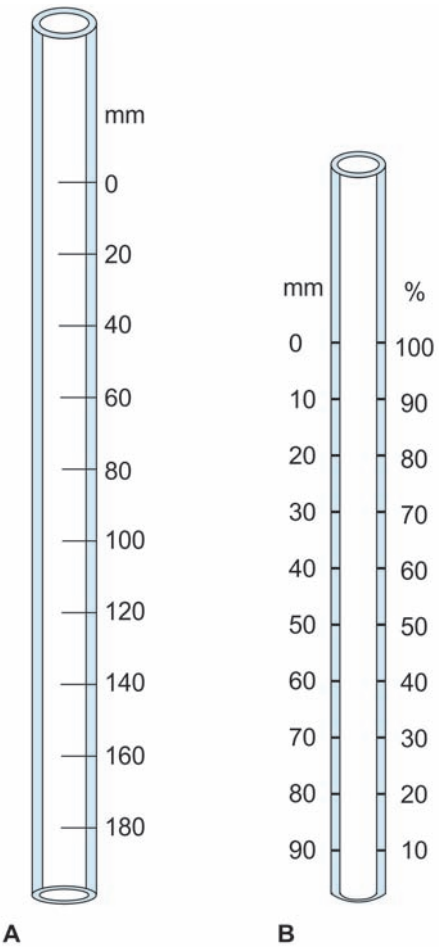


FIGURE 12.1: A. Westergren tube: This is used for determining ESR; **B.** Wintrobe tube: This is used to determine ESR and PCV.

PATHOLOGICAL VARIATION

ESR increases in diseases such as the following conditions:

1. Tuberculosis
2. All types of anemia except sickle cell anemia
3. Malignant tumors
4. Rheumatoid arthritis
5. Rheumatic fever
6. Liver diseases.

ESR decreases in the following conditions:

1. Allergic conditions
2. Sickle cell anemia

3. Peptone shock
4. Polycythemia
5. Severe leukocytosis.

FACTORS AFFECTING ESR

FACTORS INCREASEING ESR

1. *Specific Gravity of RBC*

When the specific gravity of the RBC increases, the cells become heavier and sedimentation is fast. So ESR increases.

2. *Rouleaux Formation*

Rouleaux formation increases the ESR. Globulin and fibrinogen accelerate the rouleaux formation.

3. *Increase in Size of RBC*

When the size of RBC increases (macrocyte), ESR also increases.

FACTORS DECREASING ESR

1. *Viscosity of Blood*

Viscosity offers more resistance for settling of RBCs. So when the viscosity of blood increases, the ESR decreases.

2. *RBC count*

When RBC count increases, the viscosity of blood is increased and ESR decreases. And when the RBC count decreases, ESR increases.

Packed Cell Volume and Blood Indices

Chapter 13

- DEFINITION
- METHOD OF DETERMINATION
- SIGNIFICANCE OF DETERMINING
- NORMAL VALUES
- VARIATIONS
- BLOOD INDICES
- IMPORTANCE OF BLOOD INDICES
- DIFFERENT BLOOD INDICES
- CALCULATION OF BLOOD INDICES

DEFINITION

Packed cell volume (PCV) is the 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. It is also called **hematocrit value** or **erythrocyte volume fraction (EVF).**

METHOD OF DETERMINATION

Blood is mixed with the anticoagulant ethylenediaminetetraacetic acid (EDTA) or heparin and filled in hematocrit or Wintrobe tube (110 mm long and 3 mm bore) up to 100 mark. The tube with the blood is centrifuged at a speed of 3000 revolutions per minute (rpm) for 30 minutes.

RBCs packed at the bottom form the packed cell volume and the plasma remains above this. In between the RBCs and the plasma, there is a **white buffy coat,** which is formed by white blood cells and the platelets (Fig. 13.1).

In the laboratories with modern equipments, hematocrit is not measured directly but calculated indirectly by autoanalyzer. It is determined by multiplying RBC count by mean cell volume. However, some amount of plasma is always trapped between the RBCs. So, accurate value is obtained only by direct measurement of PCV.

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.

NORMAL VALUES OF PCV

Normal PCV:

In males = 40% to 45%
In females = 38% to 42%

VARIATIONS IN PCV

INCREASE IN PCV

PCV increases in:

1. Polycythemia
2. Dehydration
3. Dengue shock syndrome: Dengue fever (tropical disease caused by flavivirus transmitted by mosquito *Aedes aegypti*) of grade III or IV severity.

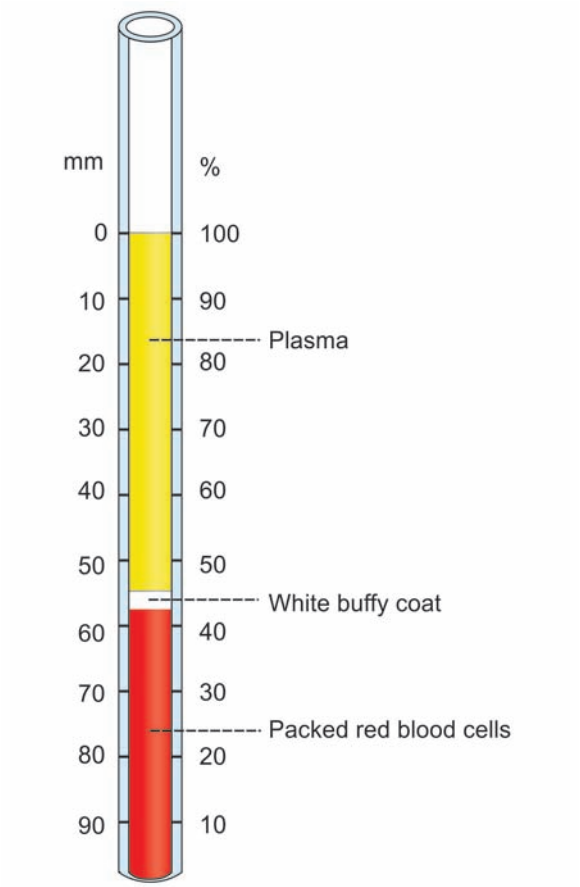


FIGURE 13.1: Packed cell volume

■ DECREASE IN PCV

PCV decreases in:

1. Anemia
2. Cirrhosis of liver (Chapter 40)
3. Pregnancy
4. Hemorrhage due to **ectopic pregnancy** (pregnancy due to implantation of fertilized ovum in tissues other than uterine wall), which is characterized by vaginal bleeding.

■ BLOOD INDICES

Blood indices are the calculations derived from RBC count, hemoglobin content of blood and PCV.

■ IMPORTANCE OF BLOOD INDICES

Blood indices help in diagnosis of the type of anemia.

■ DIFFERENT BLOOD INDICES

Blood indices include:

1. Mean corpuscular volume (MCV).
2. Mean corpuscular hemoglobin (MCH).
3. Mean corpuscular hemoglobin concentration (MCHC).
4. Color Index (CI).

1. *Mean Corpuscular Volume (MCV)*

MCV is the average volume of a single RBC and it is expressed in cubic microns (cu μ). Normal MCV is 90 cu μ (78 to 90 cu μ).

When MCV is normal, the RBC is called normocyte. When MCV increases, the cell is known as a macrocyte and when it decreases, the cell is called microcyte.

In pernicious anemia and megaloblastic anemia, the RBCs are macrocytic in nature. In iron deficiency anemia the RBCs are microcytic.

2. *Mean Corpuscular Hemoglobin (MCH)*

MCH is the quantity or amount of hemoglobin present in one RBC. It is expressed in micro-microgram or picogram (pg). Normal value of MCH is 30 pg (27 to 32 pg).

3. *Mean Corpuscular Hemoglobin Concentration (MCHC)*

MCHC is the concentration of hemoglobin in one RBC. It is the amount of hemoglobin expressed in relation to the volume of one RBC. So, the unit of expression is percentage. This is the most important absolute value in the diagnosis of anemia. Normal value of MCHC is 30% (30% to 38%).

When MCHC is normal, the RBC is normochromic. When the MCHC decreases, the RBC is known hypochromic. In pernicious anemia and megaloblastic anemia, RBCs are macrocytic and normochromic or hypochromic. In iron deficiency anemia, RBCs are microcytic and hypochromic. A single RBC cannot be hyperchromic because, the amount of hemoglobin cannot increase beyond normal.

4. *Color Index (CI)*

Color index is the ratio between the percentage of hemoglobin and the percentage of RBCs in the blood. Actually, it is the average hemoglobin content in one cell of a patient compared to the average hemoglobin content

in one cell of a normal person. Normal color index is 1.0 (0.8 to 1.2). It was widely used in olden days. However, it is useful in determining the type of anemia. It increases in macrocytic (pernicious) anemia and megaloblastic anemia. It is reduced in iron deficiency anemia. And, it is normal in normocytic normochromic anemia.

CALCULATION OF BLOOD INDICES

Blood indices are calculated by using different formula. These calculations require the values of RBC count, hemoglobin content and PCV.

For example, in the blood of a male subject:

RBC count = 4 million/cu mm.
Hemoglobin content = 8 g/dL
PCV = 30%

Color index, MCV, MCH and MCHC are calculated as follows:

COLOR INDEX

Color index is calculated by dividing the hemoglobin percentage by the RBC count percentage.

$$\text{Thus, color index} = \frac{\text{Hemoglobin \%}}{\text{RBC \%}}$$

Hemoglobin %

$$= \frac{\text{Hemoglobin content in the subject}}{\text{Hemoglobin content in normal persons}} \times 100$$

$$= \frac{8 \text{ g/dL}}{15 \text{ g/dL}} \times 100 = 53.3\%$$

RBC %

$$= \frac{\text{RBC count in the subject}}{\text{RBC count in normal persons}} \times 100$$

$$= \frac{4 \text{ million/cu mm}}{5 \text{ million/cu mm}} \times 100 = 80\%$$

By using these two values, CI is calculated

$$\text{Color Index} = \frac{\text{Hemoglobin\%}}{\text{RBC\%}} = \frac{53.3\%}{80\%} = 0.67$$

Thus, color index = 0.67

MEAN CORPUSCULAR VOLUME

$$\text{MCV} = \frac{\text{PCV in mL / 1,000 mL of blood}}{\text{RBC count in million/cu mm of blood}}$$

$$= \frac{\text{PCV in 1000 mL or in 100 mL} \times 10}{\text{RBC count in million/cu mm}} \text{ cu } \mu$$

$$\text{MCV} = \frac{30 \times 10}{4} = 75 \text{ cu } \mu.$$

Thus, MCV = 75 cu μ

MEAN CORPUSCULAR HEMOGLOBIN

$$\text{MCH} = \frac{\text{Hemoglobin in gram per 1000 ml of blood}}{\text{RBC count in million/cu mm}}$$

$$\text{MCH} = \frac{\text{Hemoglobin in gram per 100 ml of blood} \times 10}{\text{RBC count in million/cu mm}}$$

$$= \frac{80}{4} \text{ pg.}$$

Thus, MCH = 20 pg or micro-micro gram.

MEAN CORPUSCULAR HEMOGLOBIN CONCENTRATION

$$\text{MCHC} = \frac{\text{Hemoglobin in gram/100 mL of blood}}{\text{PCV in 100 mL of blood}} \times 100$$

$$= \frac{8}{30} \times 100$$

$$= \frac{800}{30} \%$$

Thus, MCHC = 26.67%.

RESULTS

CI	=	0.67	(Normal = 0.8 to1.2)
MCV	=	75 cu μ	(Normal = 78 to 90 cu μ)
MCH	=	20 pg	(Normal = 27 to 32 pg)
MCHC	=	26.67%	(Normal = 30% to 38%)

Results of these indices indicate that the person is suffering from microcytic hypochromic anemia, which commonly occurs during iron deficiency.

Chapter 14

Anemia

- INTRODUCTION
- CLASSIFICATION
- SIGNS AND SYMPTOMS

INTRODUCTION

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

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

Generally, reduction in RBC count, hemoglobin content and PCV occurs because of:

1. Decreased production of RBC
2. Increased destruction of RBC
3. Excess loss of blood from the body.

All these incidents are caused either by inherited disorders or environmental influences such as nutritional problem, infection and exposure to drugs or toxins.

CLASSIFICATION OF ANEMIA

Anemia is classified by two methods:

1. Morphological classification
2. Etiological classification.

MORPHOLOGICAL CLASSIFICATION

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). By this method, the anemia is classified into four types (Table 14.1):

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 with less color.

ETIOLOGICAL CLASSIFICATION

On the basis of etiology (study of cause or origin), anemia is divided into five types (Table 14.2):

1. Hemorrhagic anemia
2. Hemolytic anemia
3. Nutrition deficiency anemia
4. Aplastic anemia
5. Anemia of chronic diseases.

TABLE 14.1: Morphological classification of anemia

Type of anemia	Size of RBC (MCV)	Color of RBC (MCHC)
Normocytic normochromic	Normal	Normal
Normocytic hypochromic	Normal	Less
Macrocytic hypochromic	Large	Less
Microcytic hypochromic	Small	Less

1. *Hemorrhagic Anemia*

Hemorrhage refers to excessive loss of blood (Chapter 115). Anemia due to hemorrhage is known as hemorrhagic anemia. It occurs both in acute and chronic hemorrhagic conditions.

Acute hemorrhage

Acute hemorrhage refers to sudden loss of a large quantity of blood as in the case of accident. Within about 24 hours after the hemorrhage, the plasma portion of blood is replaced. However, the replacement of RBCs does not occur quickly and it takes at least 4 to 6 weeks. So with less number of RBCs, hemodilution occurs. However, morphologically the RBCs are normocytic and normochromic.

Decreased RBC count causes hypoxia, which stimulates the bone marrow to produce more number of RBCs. So, the condition is corrected within 4 to 6 weeks.

Chronic hemorrhage

It refers to loss of blood by internal or external bleeding, over a long period of time. It occurs in conditions like peptic ulcer, purpura, hemophilia and menorrhagia.

Due to continuous loss of blood, lot of iron is lost from the body causing iron deficiency. This affects the synthesis of hemoglobin resulting in less hemoglobin content in the cells. The cells also become small. Hence, the RBCs are microcytic and hypochromic (Table 14.2).

2. *Hemolytic Anemia*

Hemolysis means destruction of RBCs. Anemia due to excessive hemolysis which is not compensated by increased RBC production is called hemolytic anemia. It is classified into two types:

A. Extrinsic hemolytic anemia.
B. Intrinsic hemolytic anemia.

A. *Extrinsic hemolytic anemia:* It is the type of anemia caused by destruction of RBCs by external factors. Healthy RBCs are hemolized by factors outside the blood cells such as antibodies, chemicals and drugs. Extrinsic hemolytic anemia is also called **autoimmune hemolytic anemia.**

Common causes of external hemolytic anemia:

i. Liver failure
ii. Renal disorder

TABLE 14.2: Etiological classification of anemia

Type of anemia	Causes	Morphology of RBC
Hemorrhagic anemia	Acute loss of blood	Normocytic, normochromic
	Chronic loss of blood	Microcytic, hypochromic
Hemolytic anemia	Extrinsic hemolytic anemia: i. Liver failure ii. Renal disorder iii. Hypersplenism iv. Burns v. Infections – hepatitis, malaria and septicemia vi. Drugs – Penicillin, antimalarial drugs and sulfa drugs vii. Poisoning by lead, coal and tar viii. Presence of isoagglutinins like anti Rh xi. Autoimmune diseases – rheumatoid arthritis and ulcerative colitis	Normocytic normochromic
	Intrinsic hemolytic anemia: Hereditary disorders	Sickle cell anemia: Sickle shape
		Thalassemia: Small and irregular
Nutrition deficiency anemia	Iron deficiency	Microcytic, hypochromic
	Protein deficiency	Macrocytic, hypochromic
	Vitamin B12	Macrocytic, normochromic/hypochromic
	Folic acid	Megaloblastic, hypochromic
Aplastic anemia	Bone marrow disorder	Normocytic, normochromic
Anemia of chronic diseases	i. Non-infectious inflammatory diseases – rheumatoid arthritis ii. Chronic infections – tuberculosis iii. Chronic renal failure iv. Neoplastic disorders – Hodgkin's disease	Normocytic, normochromic

iii. Hypersplenism
iv. Burns
v. Infections like hepatitis, malaria and septicemia
vi. Drugs such as penicillin, antimalarial drugs and sulfa drugs
vii. Poisoning by chemical substances like lead, coal and tar
viii. Presence of isoagglutinins like anti-Rh
ix. Autoimmune diseases such as rheumatoid arthritis and ulcerative colitis.

B. *Intrinsic hemolytic anemia:* It is the type of anemia caused by destruction of RBCs because of the defective RBCs. There is production of unhealthy RBCs, which are short lived and are destroyed soon. Intrinsic hemolytic anemia is often inherited and it includes sickle cell anemia and thalassemia.

Because of the abnormal shape in sickle cell anemia and thalassemia, the RBCs become more fragile and susceptible for hemolysis.

Sickle cell anemia

Sickle cell anemia is an inherited blood disorder, characterized by sickle-shaped red blood cells. It is also called **hemoglobin SS disease** or **sickle cell disease.** It is common in people of African origin.

Sickle cell anemia is due to the abnormal hemoglobin called hemoglobin S (sickle cell hemoglobin). In this, α-chains are normal and β-chains are abnormal. The molecules of hemoglobin S polymerize into long chains and precipitate inside the cells. Because of this, the RBCs attain sickle (crescent) shape and become more fragile leading to hemolysis (Fig. 14.1). Sickle cell anemia occurs when a person inherits two abnormal genes (one from each parent).

In children, hemolyzed sickle cells aggregate and block the blood vessels, leading to infarction (stoppage of blood supply). The infarction is common in small bones. 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.

Thalassemia

Thalassemia is an inherited disorder, characterized by abnormal hemoglobin. It is also known as **Cooley's anemia** or **Mediterranean anemia.** It is more common in Thailand and to some extent in Mediterranean countries.

Thalassemia is of two types:
i. α-thalassemia
ii. β-thalassemia.

The β-thalassemia is very common among these two.

In normal hemoglobin, number of α and β polypeptide chains is equal. In thalassemia, the production of these chains become imbalanced because of defective synthesis of globin genes. This causes the precipitation of the polypeptide chains in the immature RBCs, leading to disturbance in erythropoiesis. The precipitation also occurs in mature red cells, resulting in hemolysis.

α-Thalassemia

α-thalassemia occurs in fetal life or infancy. In this α-chains are less, absent or abnormal. In adults, β-chains are in excess and in children, γ-chains are in excess. This leads to defective erythropoiesis and hemolysis. The infants may be stillborn or may die immediately after birth.

β-Thalassemia

In β-thalassemia, β-chains are less in number, absent or abnormal with an excess of α-chains. The α-chains precipitate causing defective erythropoiesis and hemolysis.

3. *Nutrition Deficiency Anemia*

Anemia that occurs due to deficiency of a nutritive substance necessary for erythropoiesis is called nutrition deficiency anemia. The substances which are necessary for erythropoiesis are iron, proteins and vitamins like C, B12 and folic acid. The types of nutrition deficiency anemia are:

Iron deficiency anemia

Iron deficiency anemia is the most common type of anemia. It develops due to inadequate availability of iron for hemoglobin synthesis. RBCs are microcytic and hypochromic.

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.

Features of iron deficiency anemia: Features of iron deficiency anemia are brittle nails, spoon-shaped nails **(koilonychias),** brittle hair, atrophy of papilla in tongue and **dysphagia** (difficulty in swallowing).

Protein deficiency anemia

Due to deficiency of proteins, the synthesis of hemoglobin is reduced. The RBCs are macrocytic and hypochromic.

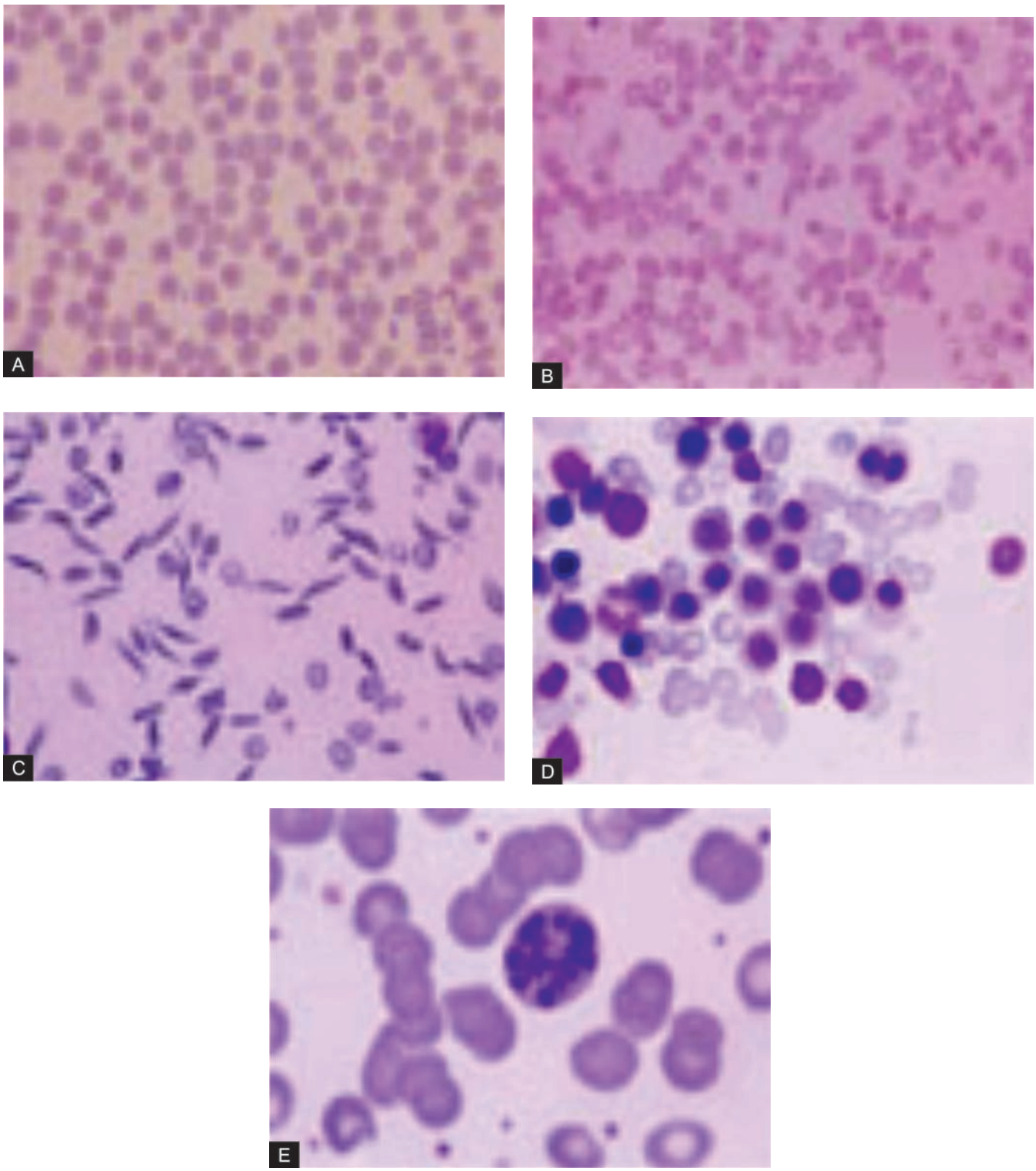


FIGURE 14.1: A. Normal RBC; **B.** Hypochromic anemia; **C.** Sickle cell anemia; **D.** Thalassemia; **E.** Megaloblastic anemia (*Courtesy:* Dr Nivaldo Medeiros).

Pernicious anemia or Addison's anemia

Pernicious anemia is the anemia due to deficiency of vitamin B12. It is also called Addison's anemia. It is due to atrophy of the gastric mucosa because of autoimmune destruction of parietal cells. The gastric atrophy results in decreased production of intrinsic factor and poor absorption of vitamin B12, which is the maturation factor for RBC. RBCs are larger and immature with almost normal or slightly low hemoglobin level. Synthesis of hemoglobin is almost normal in this type of anemia. So, cells are macrocytic and normochromic/hypochromic.

Before knowing the cause of this anemia, it was very difficult to treat the patients and the disease was considered to be fatal. So, it was called pernicious anemia.

Pernicious anemia is common in old age and it is more common in females than in males. It is associated with other autoimmune diseases like disorders of thyroid gland, Addison's disease, etc. Characteristic features of this type of anemia are lemon yellow color of skin (due to anemic paleness and mild jaundice) and red sore tongue. Neurological disorders such as **paresthesia** (abnormal sensations like numbness, tingling, burning, etc.), **progressive weakness** and **ataxia** (muscular incoordination) are also observed in extreme conditions.

Megaloblastic anemia

Megaloblastic anemia is due to the deficiency of another maturation factor called folic acid. Here, 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. However, neurological disorders may not develop.

4. *Aplastic Anemia*

Aplastic anemia is due to the disorder of red bone marrow. Red bone marrow is reduced and replaced by fatty tissues. Bone marrow disorder occurs in the following conditions:

i. Repeated exposure to X-ray or gamma ray radiation.
ii. Presence of bacterial toxins, quinine, gold salts, benzene, radium, etc.
iii. Tuberculosis.
iv. Viral infections like hepatitis and HIV infections.

In aplastic anemia, the RBCs are normocytic and normochromic.

5. *Anemia of Chronic Diseases*

Anemia of chronic diseases is the second common type of anemia (next to iron deficiency anemia). It is 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.

Common causes anemia of chronic diseases:

i. Non-infectious inflammatory diseases such as **rheumatoid arthritis** (chronic inflammatory autoimmune disorder affecting joints).
ii. Chronic infections like tuberculosis (infection caused by *Mycobacterium tuberculosis*) and abscess (collection of pus in the infected tissue) in lungs.
iii. Chronic renal failure, in which the erythropoietin secretion decreases (since erythropoietin is necessary for the stimulation of bone marrow to produce RBCs, its deficiency causes anemia).
iv. Neoplastic disorders (abnormal and disorganized growth in tissue or organ) such as **Hodgkin's disease** (malignancy involving lymphocytes) and cancer of lung and breast.

RBCs are generally normocytic and normochromic in this type of anemia. However, in progressive disease associated with iron deficiency the cells become microcytic and hypochromic.

■ 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. Sometimes, it leads to breathlessness and dyspnea (difficulty in breathing). Oxygen-hemoglobin dissociation curve is shifted to right.

■ DIGESTION

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 and pharynx.

■ METABOLISM

Basal metabolic rate increases in severe anemia.

■ KIDNEY

Renal function is disturbed. Albuminuria is common.

■ REPRODUCTIVE SYSTEM

In females, the menstrual cycle is disturbed. There may be menorrhagia, oligomenorrhea or amenorrhea (Chapter 80).

■ NEUROMUSCULAR SYSTEM

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

Hemolysis and Fragility of Red Blood Cells

Chapter 15

- DEFINITION
- PROCESS OF HEMOLYSIS
- FRAGILITY TEST
- CONDITIONS WHEN HEMOLYSIS OCCURS
- HEMOLYSINS
  - CHEMICAL SUBSTANCES
  - SUBSTANCES OF BACTERIAL ORIGIN OR SUBSTANCES FOUND IN BODY

DEFINITION

1. Hemolysis: Hemolysis is the destruction of formed elements. To define more specifically, it is the process, which involves the breakdown of red blood cells (RBCs) and liberation of hemoglobin.
2. Fragility: Susceptibility (to be affected) of RBC to hemolysis or tendency to break easily is called fragility (Fragile = easily broken).

   Fragility is of two types:

   i. **Osmotic fragility,** which occurs due to exposure to hypotonic saline

   ii. **Mechanical fragility,** which occurs due to mechanical trauma (wound or injury).

Under normal conditions, only old RBCs are destroyed in the reticuloendothelial system. Abnormal hemolysis is the process by which even younger RBCs are destroyed in large number by the presence of hemolytic agents or hemolysins.

PROCESS OF HEMOLYSIS

Normally, plasma and RBCs are in osmotic equilibrium. When the osmotic equilibrium is disturbed, the cells are affected. For example, when the RBCs are immersed in hypotonic saline the cells swell and rupture by bursting because of **endosmosis** (Chapter 4). The hemoglobin is released from the ruptured RBCs.

FRAGILITY TEST

Fragility test is a test that measures the resistance of erythrocytes in hypotonic saline solution. It is done by using sodium chloride solution at different concentrations from 1.2% to 0.2%. The solutions at different concentrations are taken in series of Cohn's tubes. Then one drop of blood to be tested is added to each tube. The sodium chloride solution and the blood in each tube are mixed well and left undisturbed for some time.

Results can be analyzed by observing the tubes directly or by centrifuging the tubes after 15 minutes.

Direct observations

1. If there is no hemolysis: Fluid in the tube appears turbid
2. If hemolysis is started: Turbidity is reduced
3. If hemolysis is completed: Fluid becomes clear.

Observations after centrifugation

1. If there is no hemolysis: Cells sediment at the bottom with clear colorless fluid above
2. If hemolysis is started: Cell sedimentation is less and the fluid becomes slightly reddish because of the release of small amount of hemoglobin from few hemolyzed RBCs
3. If hemolysis is completed: Fluid becomes more reddish without any sedimentation due to release of

more amount of hemoglobin from all the hemolyzed cells.

Index for Fragility

After 20 minutes:

No hemolysis = up to 0.6%
Onset of hemolysis = around 0.45%
Completion of hemolysis = around 0.35%

At 0.45%, only the older cells are destroyed because, their membrane is fragile. So, these cells cannot withstand this hypotonicity. But, younger cells are not affected. At 0.35%, even the younger cells are destroyed.

■ CONDITIONS WHEN HEMOLYSIS OCCURS

1. Hemolytic jaundice
2. Antigen-antibody reactions
3. Poisoning by chemicals or toxins
4. While using artificial kidney for hemodialysis or heart-lung machine during cardiac surgery (rare occasions).

■ HEMOLYSINS

Hemolysins or hemolytic agents are the substances, which cause destruction of RBCs. The hemolysins are of two types:

A. Chemical substances
B. Substances of bacterial origin or substances found in body.

■ A. CHEMICAL SUBSTANCES

1. Alcohol
2. Benzene
3. Chloroform
4. Ether
5. Acids
6. Alkalis
7. Bile salts
8. Saponin
9. Chemical poisons like:
   i. Arsenial preparations
   ii. Carbolic acid
   iii. Nitrobenzene
   iv. Resin.

■ B. SUBSTANCES OF BACTERIAL ORIGIN OR SUBSTANCES FOUND IN BODY

1. Toxic substances or toxins from bacteria:
   i. *Streptococcus*
   ii. *Staphylococcus*
   iii. Tetanus bacillus, etc.
2. Venom of poisonous snakes like cobra
3. Hemolysins from normal tissues.

White Blood Cells

Chapter 16

- INTRODUCTION
- CLASSIFICATION
- MORPHOLOGY
- NORMAL COUNT
- VARIATIONS
- LIFESPAN
- PROPERTIES
- FUNCTIONS
- LEUKOPOIESIS

INTRODUCTION

White blood cells (WBCs) or leukocytes are the colorless and nucleated formed elements of blood (leuko is derived from Greek word leukos = white). Alternate spelling for leukocytes is leucocytes.

Compared to RBCs, the WBCs are larger in size and lesser in number. Yet functionally, these cells are important like RBCs because of their role in defense mechanism of body and protect the body from invading organisms by acting like soldiers.

WBCs Vs RBCs

WBCs differ from RBCs in many aspects. The differences between WBCs and RBCs are given in Table 16.1.

1. Larger in size.
2. Irregular in shape.
3. Nucleated.
4. Many types.
5. Granules are present in some type of WBCs.
6. Lifespan is shorter.

CLASSIFICATION

Some of the WBCs have granules in the cytoplasm. Based on the presence or absence of granules in the cytoplasm, the leukocytes are classified into two groups:

1. Granulocytes which have granules.
2. Agranulocytes which do not have granules.

1. Granulocytes

Depending upon the staining property of granules, the granulocytes are classified into three types:

i. Neutrophils with granules taking both acidic and basic stains.
ii. Eosinophils with granules taking acidic stain.
iii. Basophils with granules taking basic stain.

2. Agranulocytes

Agranulocytes have plain cytoplasm without granules. Agranulocytes are of two types:

i. Monocytes.
ii. Lymphocytes.

MORPHOLOGY OF WHITE BLOOD CELLS

NEUTROPHILS

Neutrophils which are also known as polymorphs have fine or small granules in the cytoplasm. The granules take acidic and basic stains. When stained with **Leishman's stain** (which contains acidic eosin and basic methylene blue) the granules appear violet in color.

Nucleus is multilobed (Fig. 16.1). The number of lobes in the nucleus depends upon the age of cell. In younger cells, the nucleus is not lobed. And in older neutrophils, the nucleus has 2 to 5 lobes. The diameter

TABLE 16.1: Differences between WBCs and RBCs

Feature	WBCs	RBCs
Color	Colorless	Red
Number	Less: 4,000 to 11,000/cu mm	More: 4.5 to 5.5 million/cu mm
Size	Larger Maximum diameter = 18 µ	Smaller Maximum diameter = 7.4 µ
Shape	Irregular	Disk-shaped and biconcave
Nucleus	Present	Absent
Granules	Present in some types	Absent
Types	Many types	Only one type
Lifespan	Shorter ½ to 15 days	Longer 120 days

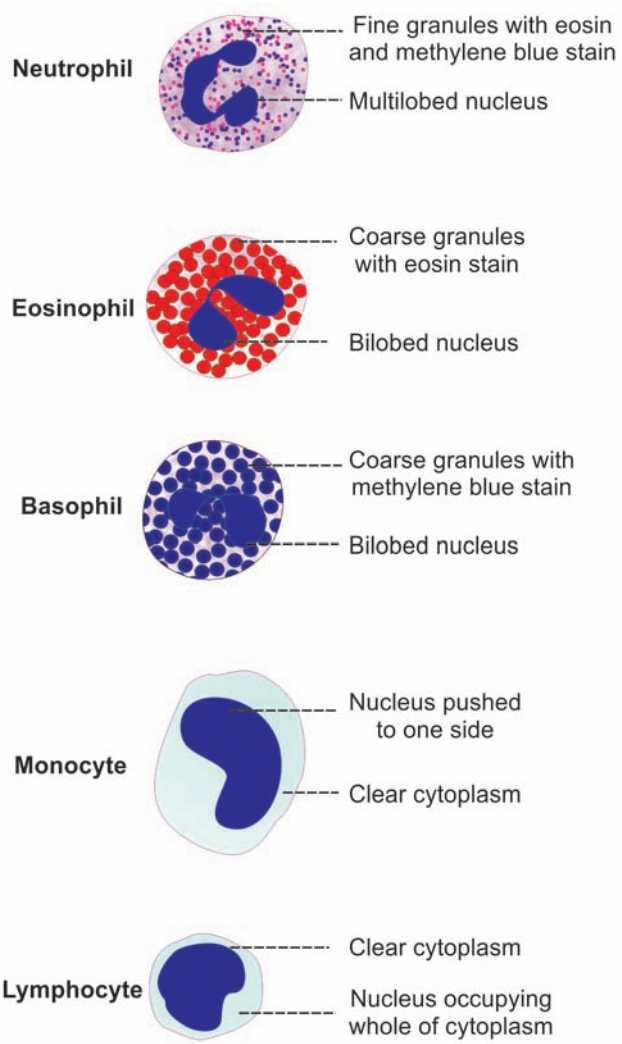


FIGURE 16.1: Different white blood cells

of cell is 10 to 12 µ (Table 16.2). The neutrophils are ameboid in nature.

■ EOSINOPHILS

Eosinophils have coarse (larger) granules in the cytoplasm, which stain pink or red with eosin. Nucleus is bilobed and spectacle-shaped. Diameter of the cell varies between 10 and 14 µ.

■ BASOPHILS

Basophils also have coarse granules in the cytoplasm. The granules stain purple blue with methylene blue. Nucleus is bilobed. Diameter of the cell is 8 to 10 µ.

■ MONOCYTES

Monocytes are the largest leukocytes with diameter of 14 to 18 µ. The cytoplasm is clear without granules. Nucleus is round, oval and horseshoe shaped, bean shaped or kidney shaped. Nucleus is placed either in the center of the cell or pushed to one side and a large amount of cytoplasm is seen.

■ LYMPHOCYTES

Like monocytes, the lymphocytes also do not have granules in the cytoplasm. Nucleus is oval, bean-shaped or kidney-shaped. Nucleus occupies the whole of the cytoplasm. A rim of cytoplasm may or may not be seen.

Types of Lymphocytes

Depending upon the size, lymphocytes are divided into two groups:

1. *Large lymphocytes:* Younger cells with a diameter of 10 to 12 µ.
2. *Small lymphocytes:* Older cells with a diameter of 7 to 10 µ.

Depending upon the function, lymphocytes are divided into two types:

1. *T lymphocytes:* Cells concerned with cellular immunity.
2. *B lymphocytes:* Cells concerned with humoral immunity.

TABLE 16.2: Diameter and lifespan of WBCs

WBC	Diameter (µ)	Lifespan (days)
Neutrophils	10 to 12	2 to 5
Eosinophils	10 to 14	7 to 12
Basophils	8 to 10	12 to 15
Monocytes	14 to 18	2 to 5
Lymphocytes	7 to 12	½ to 1

NORMAL WHITE BLOOD CELL COUNT

1. Total WBC count (TC): 4,000 to 11,000/cu mm of blood.
2. Differential WBC count (DC): Given in Table 16.3.

VARIATIONS IN WHITE BLOOD CELL COUNT

Leukocytosis

Leukocytosis is the increase in total WBC count. Leukocytosis occurs in both physiological and pathological conditions.

Leukopenia

Leukopenia is the decrease in total WBC count. The term leukopenia is generally used for pathological conditions only.

Granulocytosis

Granulocytosis is the abnormal increase in the number of granulocytes.

Granulocytopenia

Granulocytopenia is the abnormal reduction in the number of granulocytes.

Agranulocytosis

Agranulocytosis is the acute pathological condition characterized by absolute lack of granulocytes.

PHYSIOLOGICAL VARIATIONS

1. *Age:* WBC count is about 20,000 per cu mm in infants and about 10,000 to 15,000 per cu mm of blood in children. In adults, it ranges between 4,000 and 11,000 per cu mm of blood.
2. *Sex:* Slightly more in males than in females.
3. *Diurnal variation:* Minimum in early morning and maximum in the afternoon.
4. *Exercise:* Increases slightly.
5. *Sleep:* Decreases.
6. *Emotional conditions like anxiety:* Increases.
7. *Pregnancy:* Increases.
8. *Menstruation:* Increases.
9. *Parturition:* Increases.

TABLE 16.3: Normal values of different WBCs

WBC	Percentage	Absolute value per cu mm
Neutrophils	50 to 70	3,000 to 6,000
Eosinophils	2 to 4	150 to 450
Basophils	0 to 1	0 to 100
Monocytes	2 to 6	200 to 600
Lymphocytes	20 to 30	1,500 to 2,700

PATHOLOGICAL VARIATIONS

All types of leukocytes do not share equally in the increase or decrease of total leukocyte count. In general, the neutrophils and lymphocytes vary in opposite directions.

Leukocytosis

Leukocytosis is the increase in total leukocyte (WBC) count. It occurs in conditions such as:

1. Infections
2. Allergy
3. Common cold
4. Tuberculosis
5. Glandular fever.

Leukemia

Leukemia is the condition which is characterized by abnormal and uncontrolled increase in leukocyte count more than 1,000,000/cu mm. It is also called blood cancer.

Leukopenia

Leukopenia is the decrease in the total WBC count. It occurs in the following pathological conditions:

1. Anaphylactic shock
2. Cirrhosis of liver
3. Disorders of spleen
4. Pernicious anemia
5. Typhoid and paratyphoid
6. Viral infections.

Variation in Differential Leukocyte Count

Differential leukocyte count varies in specific diseases. Details are given in Table 16.4.

Neutrophilia

Neutrophilia or neutrophilic leukocytosis is the increase in neutrophil count. It occurs in the following conditions:

1. Acute infections
2. Metabolic disorders
3. Injection of foreign proteins

4. Injection of vaccines
5. Poisoning by chemicals and drugs like lead, mercury, camphor, benzene derivatives, etc.
6. Poisoning by insect venom
7. After acute hemorrhage.

Eosinophilia

Eosinophilia is the increase in eosinophil count and it occurs in:

1. Asthma and other allergic conditions
2. Blood parasitism (malaria, filariasis)

TABLE 16.4: Pathological variations in different types of WBCs

Disorder	Variation	Conditions
Neutrophilia or neutrophilic leukocytosis	Increase in neutrophil count	1. Acute infections 2. Metabolic disorders 3. Injection of foreign proteins 4. Injection of vaccines 5. Poisoning by chemicals and drugs like lead, mercury, camphor, benzene derivatives, etc. 6. Poisoning by insect venom 7. After acute hemorrhage
Neutropenia	Decrease in neutrophil count	1. Bone marrow disorders 2. Tuberculosis 3. Typhoid 4. Autoimmune diseases
Eosinophilia	Increase in eosinophil count	1. Allergic conditions like asthma 2. Blood parasitism (malaria, filariasis) 3. Intestinal parasitism 4. Scarlet fever
Eosinopenia	Decrease in eosinophil count	1. Cushing's syndrome 2. Bacterial infections 3. Stress 4. Prolonged administration of drugs like steroids, ACTH and epinephrine
Basophilia	Increase in basophil count	1. Smallpox 2. Chickenpox 3. Polycythemia vera
Basopenia	Decrease in basophil count	1. Urticaria (skin disorder) 2. Stress 3. Prolonged exposure to chemotherapy or radiation therapy
Monocytosis	Increase in monocyte count	1. Tuberculosis 2. Syphilis 3. Malaria 4. Kala-azar
Monocytopenia	Decrease in monocyte count	Prolonged use of prednisone (immunosuppressant steroid)
Lymphocytosis	Increase in lymphocyte count	1. Diphtheria 2. Infectious hepatitis 3. Mumps 4. Malnutrition 5. Rickets 6. Syphilis 7. Thyrotoxicosis 8. Tuberculosis
Lymphocytopenia	Decrease in lymphocyte count	1. AIDS 2. Hodgkin's disease (cancer of lymphatic system) 3. Malnutrition 4. Radiation therapy 5. Steroid administration

3. Intestinal parasitism
4. Scarlet fever.

Basophilia

Basophilia is the increase in basophil count and it occurs in:

1. Smallpox
2. Chickenpox
3. Polycythemia vera.

Monocytosis

Monocytosis is the increase in monocyte count and it occurs in:

1. Tuberculosis
2. Syphilis
3. Malaria
4. Kala-azar
5. Glandular fever.

Lymphocytosis

Lymphocytosis is the increase in lymphocyte count and it occurs in:

1. Diphtheria
2. Infectious hepatitis
3. Mumps
4. Malnutrition
5. Rickets
6. Syphilis
7. Thyrotoxicosis
8. Tuberculosis.

Neutropenia

Neutropenia is the decrease in neutrophil count. It occurs in:

1. Bone marrow disorders
2. Tuberculosis
3. Typhoid
4. Vitamin deficiencies
5. Autoimmune diseases.

Eosinopenia

Decrease in eosinophil count is called eosinopenia. It occurs in:

1. Cushing's syndrome
2. Bacterial infections
3. Stress
4. Prolonged administration of drugs such as steroids, ACTH, epinephrine.

Basopenia

Basopenia or basophilic leukopenia is the decrease in basophil count. It occurs in:

1. Urticaria (skin disorder)
2. Stress
3. Prolonged exposure to chemotherapy or radiation therapy.

Monocytopenia

Monocytopenia is the decrease in monocyte count. It occurs in:

1. Prolonged use of prednisone (immunosuppressant steroid)
2. AIDS
3. Chronic lymphoid leukemia.

Lymphocytopenia

Lymphocytopenia is the decrease in lymphocytes. It occurs in:

1. AIDS
2. Hodgkin's disease (cancer of the lymphatic system)
3. Malnutrition
4. Radiation therapy
5. Steroid administration.

■ LIFESPAN OF WHITE BLOOD CELLS

Lifespan of WBCs is not constant. It depends upon the demand in the body and their function. Lifespan of these cells may be as short as half a day or it may be as long as 3 to 6 months. Lifespan of WBCs is given in Table 16.2.

■ PROPERTIES OF WHITE BLOOD CELLS

1. *Diapedesis*

Diapedesis is the process by which the leukocytes squeeze through the narrow blood vessels.

2. *Ameboid Movement*

Neutrophils, monocytes and lymphocytes show amebic movement, characterized by protrusion of the cytoplasm and change in the shape.

3. *Chemotaxis*

Chemotaxis is the attraction of WBCs towards the injured tissues by the chemical substances released at the site of injury.

4. Phagocytosis

Neutrophils and monocytes engulf the foreign bodies by means of phagocytosis (Chapter 3).

■ FUNCTIONS OF WHITE BLOOD CELLS

Generally, WBCs play an important role in defense mechanism. These cells protect the body from invading organisms or foreign bodies, either by destroying or inactivating them. However, in defense mechanism, each type of WBCs acts in a different way.

■ NEUTROPHILS

Neutrophils play an important role in the defense mechanism of the body. Along with monocytes, the neutrophils provide the first line of defense against the invading microorganisms. The neutrophils are the free cells in the body and wander freely through the tissue and practically, no part of the body is spared by these leukocytes.

Substances Present in Granules and Cytoplasm of Neutrophils

Granules of neutrophils contain enzymes like proteases, myeloperoxidases, elastases and metalloproteinases (Table 16.5). These enzymes destroy the microorganisms. The granules also contain antibody like peptides called **cathelicidins** and **defensins,** which are antimicrobial peptides and are active against bacteria and fungi.

Membrane of neutrophils contains an enzyme called **NADPH oxidase** (dihydronicotinamide adenine dinucleotide phosphate oxidase). It is activated by the toxic metabolites released from infected tissues. The activated NADPH oxidase is responsible for bactericidal action of neutrophils (see below).

All these substances present in the granules and cell membrane make the neutrophil a powerful and effective killer machine.

Neutrophils also secrete **platelet-activating factor (PAF),** which is a cytokine. It accelerates the aggregation of platelets during injury to the blood vessel, resulting in prevention of excess loss of blood.

Mechanism of Action of Neutrophils

Neutrophils are released in large number at the site of infection from the blood. At the same time, new neutrophils are produced from the progenitor cells. All the neutrophils move by diapedesis towards the site of infection due to chemotaxis.

Chemotaxis occurs due to the attraction by some chemical substances called **chemoattractants,** which are released from the infected area. After reaching the area, the neutrophils surround the area and get adhered to the infected tissues. Chemoattractants increase the adhesive nature of neutrophils so that all the neutrophils become sticky and get attached firmly to the infected area. Each neutrophil can hold about 15 to 20 microorganisms at a time. Now, the neutrophils start destroying the invaders. First, these cells engulf the bacteria and then destroy them by means of phagocytosis (Chapter 3).

Respiratory Burst

Respiratory burst is a rapid increase in oxygen consumption during the process of phagocytosis by neutrophils and other phagocytic cells. Nicotinamide adenine dinucleotide phosphate (NADPH) oxidase is responsible for this phenomenon. During respiratory burst, the free radical O_2^- is formed. $2O_2^-$ combine with $2H^+$ to form H_2O_2 (hydrogen peroxide). Both O_2^- and H_2O_2 are the oxidants having potent bactericidal action.

Pus and Pus Cells

Pus is the whitish yellow fluid formed in the infected tissue by the dead WBCs, bacteria or foreign bodies and cellular debris. It consists of white blood cells, bacteria or other foreign bodies and cellular debris. The dead WBCs are called pus cells.

During the battle against the bacteria, many WBCs are killed by the toxins released from the bacteria. The dead cells are collected in the center of infected area. The dead cells together with plasma leaked from the blood vessel, liquefied tissue cells and RBCs escaped from damaged blood vessel (capillaries) constitute the pus.

■ EOSINOPHILS

Eosinophils play an important role in the defense mechanism of the body against the parasites. During parasitic infections, there is a production of a large number of eosinophils which move towards the tissues affected by parasites. Eosinophil count increases also during allergic diseases like asthma.

Eosinophils are responsible for detoxification, disintegration and removal of foreign proteins.

Mechanism of Action of Eosinophils

Eosinophils are neither markedly motile nor phagocytic like the neutrophils. Some of the parasites are larger in size. Still eosinophils attack them by some special type of cytotoxic substances present in their granules. When

TABLE 16.5: Substances secreted by WBCs

WBC	Substance secreted	Action
Neutrophil	Proteases	Destruction of microorganisms
	Myeloperoxidases	
	Elastases	
	Metalloproteinases	
	Defensins	Antimicrobial action Anti-inflammatory action Wound healing Chemotaxis
	Cathelicidins	Antimicrobial action
	NADPH oxidase	Bactericidal action
	Platelet-activating factor	Aggregation of platelets
Eosinophil	Eosinophil peroxidase	Destruction of worms, bacteria and tumor cells
	Major basic protein	Destruction of worms
	Eosinophil cationic protein	Destruction of worms Neurotoxic action
	Eosinophil-derived neurotoxin	Neurotoxic action
	Interleukin-4 and 5	Acceleration of inflammatory response Destruction of invading organisms
Basophil	Heparin	Prevention of intravascular blood clotting
	Histamine	Production of acute hypersensitivity reactions
	Bradykinin	
	Serotonin	
	Proteases	Destruction of microorganisms
	Myeloperoxidases	
	Interleukin-4	Acceleration of inflammatory response Destruction of invading organisms
Monocyte	Interleukin-1	Acceleration of inflammatory response Destruction of invading organisms
	Colony stimulation factor	Formation of colony forming blastocytes
	Platelet-activating factor	Aggregation of platelets
	Chemokines	Chemotaxis
T lymphocytes	Interleukin-2, 4 and 5	Acceleration of inflammatory response Destruction of invading organisms Activation of T cells
	Gamma interferon	Stimulation of phagocytic actions of cytotoxic cells, macrophages and natural killer cells
	Lysosomal enzymes	Destruction of invading organisms
	Tumor necrosis factor	Necrosis of tumor Activation of immune system Promotion of inflammation
	Chemokines	Chemotaxis

Contd...

B lymphocytes	Immunoglobulins	Destruction of invading organisms
	Tumor necrosis factor	Necrosis of tumor Activation of immune system Acceleration of inflammatory response
	Chemokines	Chemotaxis

released over the invading parasites from the granules, these substances become lethal and destroy the parasites. The lethal substances present in the granules of eosinophils and released at the time of exposure to parasites or foreign proteins are:

1. *Eosinophil peroxidase*: This enzyme is capable of destroying helminths (parasitic worms), bacteria and tumor cells.
2. *Major basic protein (MBP):* It is very active against helminths. It destroys the parasitic worms by causing distension (ballooning) and detachment of the tegumental sheath (skin-like covering) of these organisms.
3. *Eosinophil cationic protein (ECP):* This substance is the major destroyer of helminths and it is about 10 times more toxic than MBP. It destroys the parasites by means of complete disintegration. It is also a neurotoxin.
4. *Eosinophil-derived neurotoxin:* It destroys the nerve fibers particularly, the myelinated nerve fibers.
5. *Cytokines:* Cytokines such as interleukin-4 and interleukin-5 accelerate inflammatory responses by activating eosinophils. These cytokines also kill the invading organisms.

■ BASOPHILS

Basophils play an important role in healing processes. So their number increases during healing process.

Basophils also play an important role in allergy or acute hypersensitivity reactions (allergy). This is because of the presence of receptors for IgE in basophil membrane.

Mechanism of Action of Basophils

Functions of basophils are executed by the release of some important substances from their granules such as:

1. *Heparin:* Heparin is essential to prevent the intravascular blood clotting.
2. *Histamine, slow-reacting substances of anaphylaxis, bradykinin and serotonin:* Theses substances produce the acute hypersensitivity reactions by causing vascular and tissue responses.
3. *Proteases and myeloperoxidase:* These enzymes destroy the microorganisms.
4. *Cytokine:* Cytokine such as interleukin-4 accelerates inflammatory responses and kill the invading organisms.

Mast Cell

Mast cell is a large tissue cell resembling the basophil. Generally, mast cells are found along with the blood vessels and are prominently seen in the areas such as skin, mucosa of the lungs and digestive tract, mouth, conjunctiva and nose. These cells usually do not enter the bloodstream.

Origin

Mast cells are developed in the bone marrow, but their precursor cells are different. After differentiation, the immature mast cells enter the tissues. Maturation of mast cells takes place only after entering the tissue.

Functions

Mast cell plays an important role in producing the hypersensitivity reactions like allergy and anaphylaxis (Chapter 17). When activated, the mast cell immediately releases various chemical mediators from its granules into the interstitium. Two types of substances are secreted by mast cell:

1. *Preformed mediators:* These substances are already formed and stored in secretory granules. These substances are histamine, heparin, serotonin, hydrolytic enzymes, proteoglycans and chondroitin sulfates.
2. *Newly generated mediators:* These substances are absent in the mast cell during resting conditions and are produced only during activation. These substances are **arachidonic acid** derivatives such as leukotriene C (LTC), prostaglandin and cytokines.

■ MONOCYTES

Monocytes are the largest cells among the leukocytes. Like neutrophils, monocytes also are motile and phagocytic in nature. These cells wander freely through all tissues of the body.

Monocytes play an important role in defense of the body. Along with neutrophils, these leukocytes provide the first line of defense.

Monocytes secrete:

1. Interleukin-1 (IL-1).
2. Colony stimulating factor (M-CSF).
3. Platelet-activating factor (PAF).

Monocytes are the precursors of the tissue macrophages. Matured monocytes stay in the blood only for few hours. Afterwards, these cells enter the tissues from the blood and become tissue macrophages. Examples of tissue macrophages are Kupffer cells in liver, alveolar macrophages in lungs and macrophages in spleen. Functions of macrophages are discussed in Chapter 24.

■ LYMPHOCYTES

Lymphocytes play an important role in immunity. Functionally, the lymphocytes are classified into two categories, namely T lymphocytes and B lymphocytes. T lymphocytes are responsible for the development of cellular immunity and B lymphocytes are responsible for the development of humoral immunity. The functions of these two types of lymphocytes are explained in detail in Chapter 17.

■ LEUKOPOIESIS

Leukopoiesis is the development and maturation of leukocytes (Fig. 16.2).

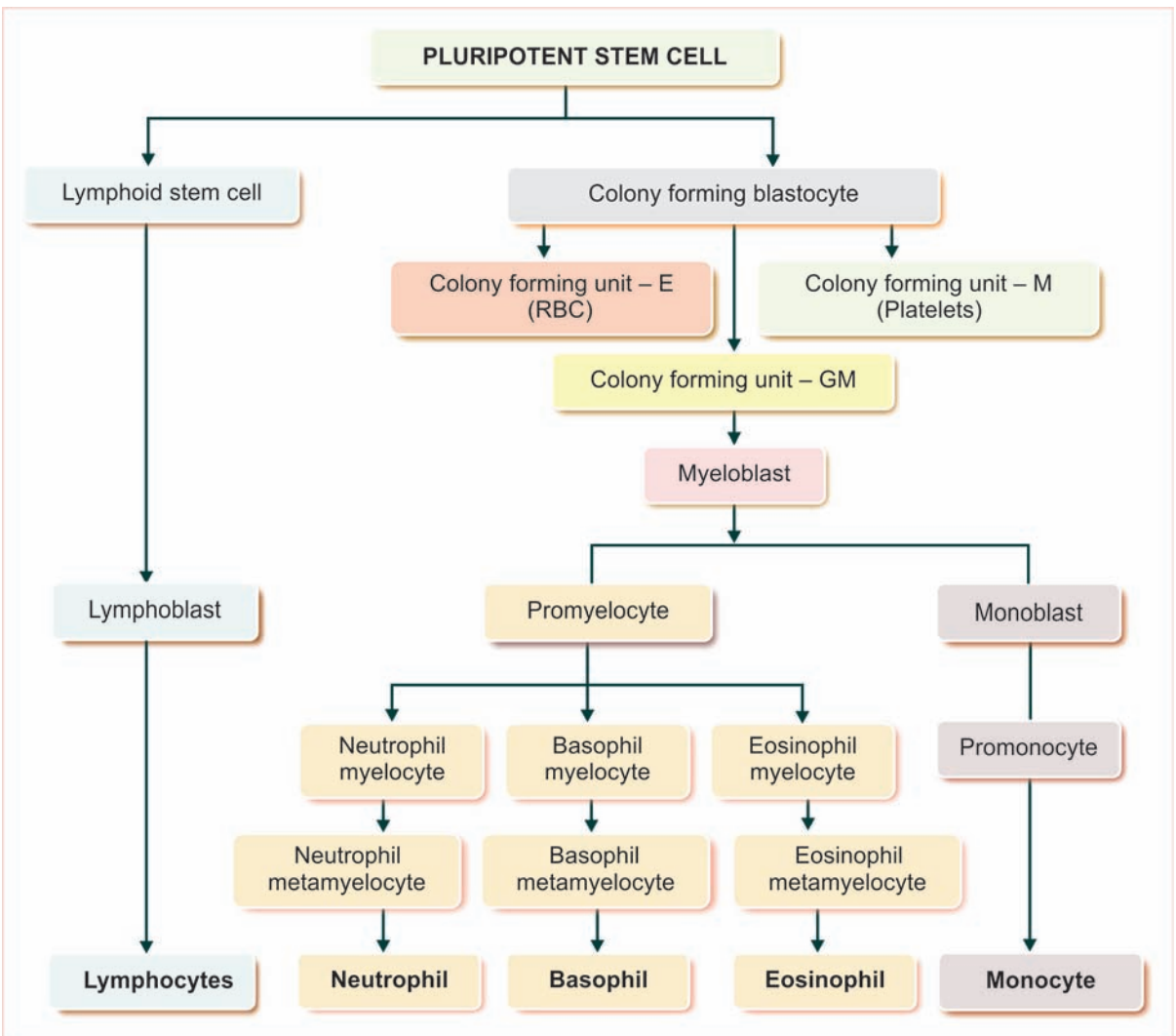


FIGURE 16.2: Leukopoiesis

■ STEM CELLS

Committed pluripotent stem cell gives rise to leukocytes through various stages. Details are given in Chapter 10.

■ FACTORS NECESSARY FOR LEUKOPOIESIS

Leukopoiesis is influenced by hemopoietic growth factors and colony stimulating factors. Hemopoietic growth factors are discussed in Chapter 10.

Colony stimulating Factors

Colony stimulating factors (CSF) are proteins which cause the formation of colony forming blastocytes. Colony stimulating factors are of three types:

1. Granulocyte-CSF (G-CSF) secreted by monocytes and endothelial cells
2. Granulocyte-monocyte-CSF (GM-CSF) secreted by monocytes, endothelial cells and T lymphocytes
3. Monocyte-CSF (M-CSF) secreted by monocytes and endothelial cells.

Chapter 17

Immunity

- DEFINITION AND TYPES OF IMMUNITY
- DEVELOPMENT AND PROCESSING OF LYMPHOCYTES
- ANTIGENS
- DEVELOPMENT OF CELL-MEDIATED IMMUNITY
- DEVELOPMENT OF HUMORAL IMMUNITY
- NATURAL KILLER CELL
- CYTOKINES
- IMMUNIZATION
- IMMUNE DEFICIENCY DISEASES
- AUTOIMMUNE DISEASES
- ALLERGY AND IMMUNOLOGICAL HYPERSENSITIVITY REACTIONS

DEFINITION AND TYPES OF IMMUNITY

Immunity is defined as the capacity of the body to resist pathogenic agents. It is the ability of body to resist the entry of different types of foreign bodies like bacteria, virus, toxic substances, etc.

Immunity is of two types:

I. Innate immunity.
II. Acquired immunity.

INNATE IMMUNITY OR NON-SPECIFIC IMMUNITY

Innate immunity is the inborn capacity of the body to resist pathogens. By chance, if the organisms enter the body, innate immunity eliminates them before the development of any disease. It is otherwise called the natural or non-specific immunity.

This type of immunity represents the first line of defense against any type of pathogens. Therefore, it is also called non-specific immunity.

Mechanisms of Innate Immunity

Various mechanisms of innate immunity are given in Table 17.1.

ACQUIRED IMMUNITY OR SPECIFIC IMMUNITY

Acquired immunity is the resistance developed in the body against any specific foreign body like bacteria, viruses, toxins, vaccines or transplanted tissues. So, this type of immunity is also known as specific immunity.

It is the most powerful immune mechanism that protects the body from the invading organisms or toxic substances. Lymphocytes are responsible for acquired immunity (Fig. 17.1).

Types of Acquired Immunity

Two types of acquired immunity develop in the body:

1. Cellular immunity
2. Humoral immunity.

Lymphocytes are responsible for the development of these two types of immunity.

DEVELOPMENT AND PROCESSING OF LYMPHOCYTES

In fetus, lymphocytes develop from the bone marrow (Chapter 10). All lymphocytes are released in the circulation and are differentiated into two categories.

TABLE 17.1: Mechanisms of innate immunity

Structures and Mediators	Mechanism
Gastrointestinal tract	Enzymes in digestive juices and the acid in stomach destroy the toxic substances or organisms entering digestive tract through food Lysozyme present in saliva destroys bacteria
Respiratory system	Defensins and cathelicidins in epithelial cells of air passage are antimicrobial peptides Neutrophils, lymphocytes, macrophages and natural killer cells present in lungs act against bacteria and virus
Urinogenital system	Acidity in urine and vaginal fluid destroy the bacteria
Skin	The keratinized stratum corneum of epidermis protects the skin against toxic chemicals The β-defensins in skin are antimicrobial peptides Lysozyme secreted in skin destroys bacteria
Phagocytic cells	Neutrophils, monocytes and macrophages ingest and destroy the microorganisms and foreign bodies by phagocytosis
Interferons	Inhibit multiplication of viruses, parasites and cancer cells
Complement proteins	Accelerate the destruction of microorganisms

The two categories are:

1. T lymphocytes or T cells, which are responsible for the development of cellular immunity
2. B lymphocytes or B cells, which are responsible for humoral immunity.

■ T LYMPHOCYTES

T lymphocytes are processed in thymus. The processing occurs mostly during the period between just before birth and few months after birth.

Thymus secretes a hormone called thymosin, which plays an important role in immunity. It accelerates the proliferation and activation of lymphocytes in thymus. It also increases the activity of lymphocytes in lymphoid tissues.

Types of T Lymphocytes

During the processing, T lymphocytes are transformed into four types:

1. Helper T cells or inducer T cells. These cells are also called **CD4 cells** because of the presence of molecules called CD4 on their surface.
2. Cytotoxic T cells or killer T cells. These cells are also called **CD8 cells** because of the presence of molecules called CD8 on their surface.
3. Suppressor T cells.
4. Memory T cells.

Storage of T Lymphocytes

After the transformation, all the types of T lymphocytes leave the thymus and are stored in lymphoid tissues of lymph nodes, spleen, bone marrow and GI tract.

■ B LYMPHOCYTES

B lymphocytes were first discovered in the bursa of Fabricius in birds, hence the name B lymphocytes. **Bursa of Fabricius** is a lymphoid organ situated near the cloaca of birds. Bursa is absent in mammals and the processing of B lymphocytes takes place in liver (during fetal life) and bone marrow (after birth).

Types of B Lymphocytes

After processing, the B lymphocytes are transformed into two types:

1. Plasma cells.
2. Memory cells.

Storage of B Lymphocytes

After transformation, the B lymphocytes are stored in the lymphoid tissues of lymph nodes, spleen, bone marrow and the GI tract.

■ ANTIGENS

■ DEFINITION AND TYPES

Antigens are the substances which induce specific immune reactions in the body.

Antigens are of two types:

1. Autoantigens or self antigens present on the body's own cells such as 'A' antigen and 'B' antigen in RBCs.
2. Foreign antigen s or non-self antigens that enter the body from outside.

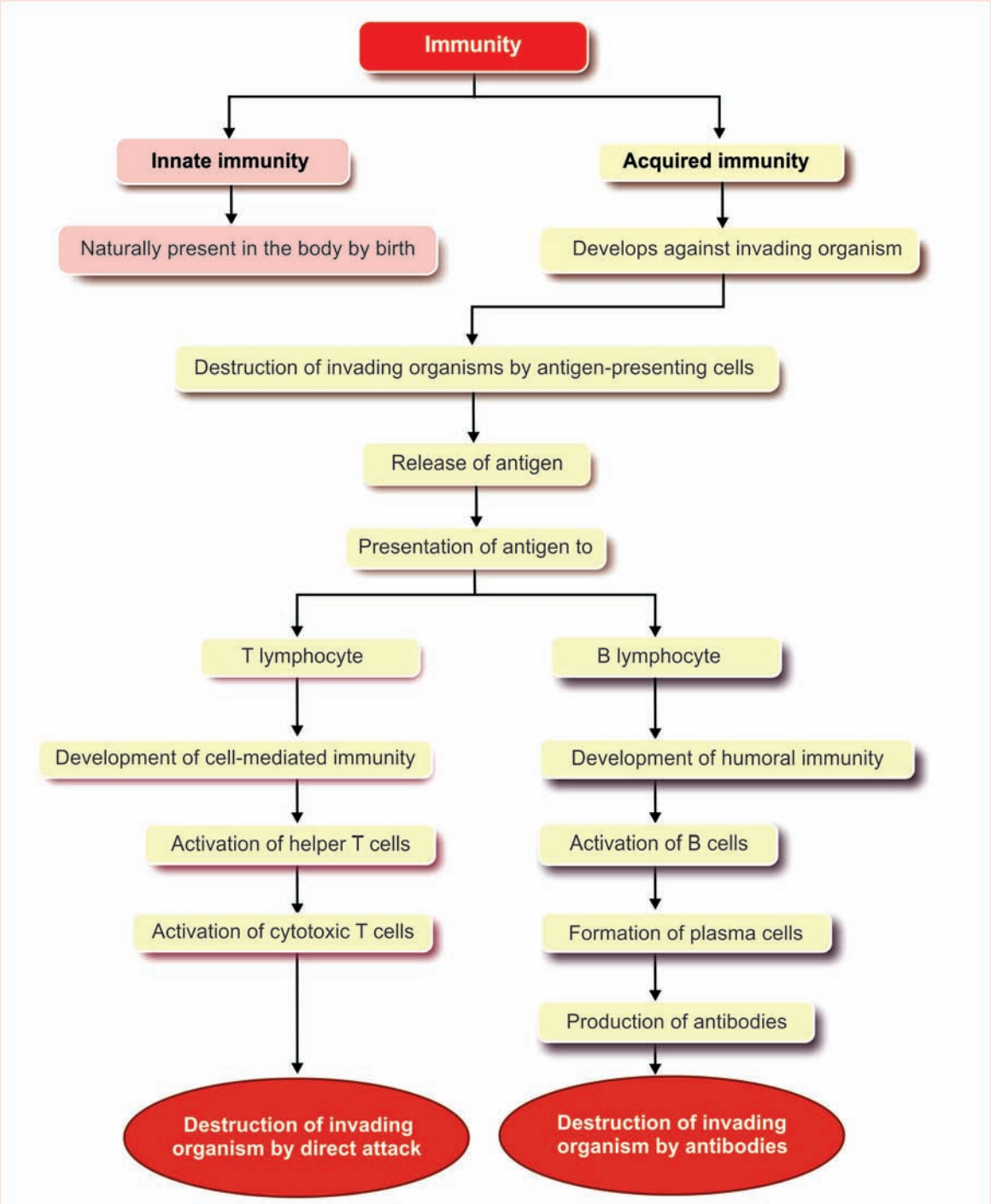


FIGURE 17.1: Schematic diagram showing development of immunity

■ NON-SELF ANTIGENS

Following are non-self antigens:

1. Receptors on the cell membrane of microbial organisms such as bacteria, viruses and fungi.
2. Toxins from microbial organisms.
3. Materials from transplanted organs or incompatible blood cells.
4. **Allergens** or allergic substances like pollen grains.

Types of Non-self Antigens

Non-self antigens are classified into two types, depending upon the response developed against them in the body:

1. Antigens, which induce the development of immunity or production of antibodies **(immunogenicity).**
2. Antigens, which react with specific antibodies and produce allergic reactions **(allergic reactivity).** (The allergic reaction is explained in the later part of this chapter).

■ CHEMICAL NATURE OF THE ANTIGENS

Antigens are mostly the conjugated proteins like lipoproteins, glycoproteins and nucleoproteins.

■ DEVELOPMENT OF CELL-MEDIATED IMMUNITY

■ INTRODUCTION

Cell-mediated immunity is defined as the immunity developed by cell-mediated response. It is also called cellular immunity or T cell immunity. It involves several types of cells such as T lymphocytes, macrophages and natural killer cells and hence the name cell mediated immunity. Cell-mediated immunity does not involve antibodies.

Cellular immunity is the major defense mechanism against infections by viruses, fungi and few bacteria like tubercle bacillus. It is also responsible for delayed allergic reactions and the rejection of transplanted tissues.

Cell-mediated immunity is offered by T lymphocytes and 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

Antigen-presenting cells are the special type of cells in the body, which 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.

Among these cells, macrophages are the major antigen-presenting cells.

1. *Macrophages*

Macrophages are the large phagocytic cells, which digest the invading organisms to release the antigen. The macrophages are present along with lymphocytes in almost all the lymphoid tissues.

2. *Dendritic Cells*

Dendritic cells are nonphagocytic in nature. Based on the location, dendritic cells are classified into three categories:

i. Dendritic cells of spleen, which trap the antigen in blood.
ii. Follicular dendritic cells in lymph nodes, which trap the antigen in the lymph.
iii. Langerhans dendritic cells in skin, which 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. Thus, the B cells function as both antigen-presenting cells and antigen receiving cells. However, B cells are the 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). HLA is a genetic matter present in the molecule of class II major histocompatiblility complex (MHC), which is situated on the surface of the antigen-presenting cells.

B-cells ingest the foreign bodies by means of pinocytosis. Role of B cells as antigen-presenting cells in the body is not fully understood.

MHC and HLA

Major histocompatibility complex (MHC) is a large molecule present in the short arm of chromosome 6. It is made up of a group of genes which are involved in immune system. It has more than 200 genes including HLA genes. HLA is made up of genes with small molecules. It encodes antigen-presenting proteins on the cell surface.

Though MHC molecules and HLA genes are distinct terms, both are used interchangeably. Particularly in human, the MHC molecules are often referred as HLA

molecules. MHC molecules in human beings are divided into two types:

1. Class I MHC molecule: It is found on every cell in human body. It is specifically responsible for presentation of endogenous antigens (antigens produced intracellularly such as viral proteins and tumor antigens) to cytotoxic T cells.
2. Class II MHC molecule: It is found on B cells, macrophages and other antigen-presenting cells. It is responsible for presenting the exogenous antigens (antigens of bacteria or viruses which are engulfed by antigen-presenting cells) to helper T cells.

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 (Fig. 17.2).

Sequence of Events during Activation of Helper T cells

1. Helper T cell recognizes the antigen displayed on the surface of the antigen-presenting cell with the help of its own surface receptor protein called T cell receptor.
2. Recognition of the antigen by the helper T cell initiates a complex interaction between the helper T cell receptor and the antigen. This reaction activates helper T cells.
3. At the same time, macrophages (the antigen-presenting cells) release interleukin-1, which facilitates the activation and proliferation of helper T cells.
4. Activated helper T cells proliferate and the proliferated cells enter the circulation for further actions.

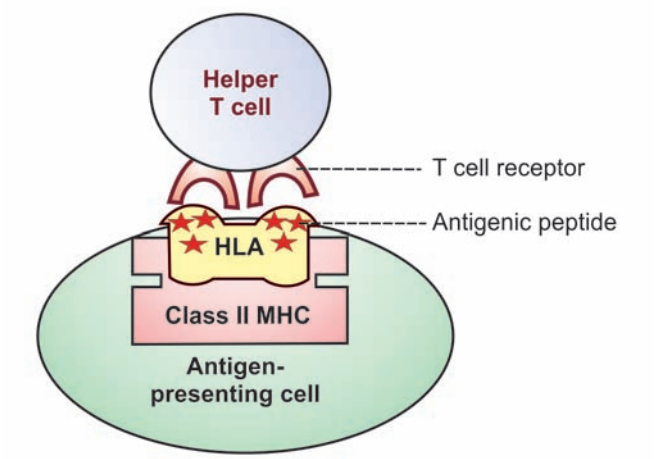


FIGURE 17.2: Antigen presentation. The antigen-presenting cells present their class II MHC molecules together with antigen-bound HLA to the helper T cells. MHC = Major histocompatiblility complex. HLA = Human leukocyte antigen.

5. Simultaneously, the antigen which is bound to class II MHC molecules activates the B cells also, resulting in the development of humoral immunity (see below).

■ ROLE OF HELPER T CELLS

Helper T cells (CD4 cells) which enter the circulation activate all the other T cells and B cells. Normal, CD4 count in healthy adults varies between 500 and 1500 per cubic millimeter of blood.

Helper T cells are of two types:

1. Helper-1 (TH1) cells
2. Helper-2 (TH2) cells.

Role of TH1 Cells

TH1 cells are concerned with cellular immunity and 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 TH2 Cells

TH2 cells are concerned with humoral immunity and secrete interleukin-4 and interleukin-5, which are concerned with:

i. Activation of B cells.
ii. Proliferation of plasma cells.
iii. Production of antibodies by plasma cell.

■ ROLE OF CYTOTOXIC T CELLS

Cytotoxic T cells that are activated by helper T cells, circulate through blood, lymph and lymphatic tissues and destroy the invading organisms by attacking them directly.

Mechanism of Action of Cytotoxic T Cells

1. Receptors situated on the outer membrane of cytotoxic T cells bind the antigens or organisms tightly with cytotoxic T cells.
2. Then, the cytotoxic T cells enlarge and release cytotoxic substances like the lysosomal enzymes.
3. These substances destroy the invading organisms.
4. Like this, each cytotoxic T cell can destroy a large number of microorganisms one after another.

Other Actions of Cytotoxic T Cells

1. Cytotoxic T cells also destroy cancer cells, transplanted cells, such as those of transplanted heart or kidney or any other cells, which are foreign bodies.

2. Cytotoxic T cells destroy even body's own tissues which are affected by the foreign bodies, particularly the viruses. Many viruses are entrapped in the membrane of affected cells. The antigen of the viruses attracts the T cells. And the cytotoxic T cells kill the affected cells also along with viruses. Because of this, the cytotoxic T cell is called killer cell.

■ ROLE OF SUPPRESSOR T CELLS

Suppressor T cells are also called regulatory T cells. These T cells suppress the activities of the killer T cells. Thus, the suppressor T cells play an important role in preventing the killer T cells from destroying the body's own tissues along with invaded organisms. Suppressor cells suppress the activities of helper T cells also.

■ ROLE 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. So, the invading organism is destroyed very quickly. The response of the T cells is also more powerful this time.

■ SPECIFICITY OF T CELLS

Each T cell is designed to be activated only by one type of antigen. It is capable of developing immunity against that antigen only. This property is called the specificity of T cells.

■ DEVELOPMENT OF HUMORAL IMMUNITY

■ INTRODUCTION

Humoral immunity is defined as the immunity mediated by antibodies, which are secreted by B lymphocytes. B lymphocytes secrete the antibodies into the blood and lymph. The blood and lymph are the body fluids (**humours** or **humors** in Latin). Since the B lymphocytes provide immunity through humors, this type of immunity is called humoral immunity or B cell immunity.

Antibodies are the gamma globulins produced by B lymphocytes. These antibodies fight against the invading organisms. The humoral immunity is the major defense mechanism against the bacterial infection.

As in the case of cell-mediated immunity, the macrophages and other antigen-presenting cells play an important role in the development of humoral immunity also.

■ ROLE OF ANTIGEN-PRESENTING CELLS

The ingestion of foreign organisms and digestion of their antigen by the antigen-presenting cells are already explained.

Presentation of Antigen

Antigen-presenting cells present the antigenic products bound with HLA (which is present in class II MHC molecule) to B cells. This activates the B cells through series of events.

Sequence of Events during Activation of B Cells

1. B cell recognizes the antigen displayed on the surface of the antigen-presenting cell, with the help of its own surface receptor protein called B cell receptor.
2. Recognition of the antigen by the B cell initiates a complex interaction between the B cell receptor and the antigen. This reaction activates B cells.
3. At the same time, macrophages (the antigen-presenting cells) release interleukin-1, which facilitates the activation and proliferation of B cells.
4. Activated B cells proliferate and the proliferated cells carry out the further actions.
5. Simultaneously, the antigen bound to class II MHC molecules activates the helper T cells, also resulting in development of cell-mediated immunity (already explained).

Transformation B Cells

Proliferated B cells are transformed into two types of cells:

1. Plasma cells
2. Memory cells.

■ ROLE OF PLASMA CELLS

Plasma cells destroy the foreign organisms by producing the antibodies. Antibodies are globulin in nature. The rate of the antibody production is very high, i.e. each plasma cell produces about 2000 molecules of antibodies per second. The antibodies are also called immunoglobulins.

Antibodies are released into lymph and then transported into the circulation. The antibodies are produced until the end of lifespan of each plasma cell, which may be from several days to several weeks.

■ ROLE OF MEMORY B CELLS

Memory B cells occupy the lymphoid tissues throughout the body. The memory cells are in inactive condition until the body is exposed to the same organism for the second time.

During the 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. The antibodies produced during the second exposure to the foreign antigen are also more potent than those produced during first exposure. This phenomenon forms the 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.

■ ANTIBODIES OR IMMUNOGLOBULINS

An antibody is defined as a protein that is produced by B lymphocytes in response to the presence of an antigen. Antibody is gamma globulin in nature and it is also called immunoglobulin (Ig). Immunoglobulins form 20% of the total plasma proteins. Antibodies enter almost all the tissues of the body.

Types of Antibodies

Five types of antibodies are identified:

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

Antibodies are gamma globulins with a molecular weight of 1,50,000 to 9,00,000. The antibodies are formed by two pairs of chains, namely one pair of heavy or long chains and one pair of light or short chains. Each heavy chain consists of about 400 amino acids and each light chain consists of about 200 amino acids.

Actually, each antibody has two halves, which are identical. The two halves are held together by disulfide bonds (S–S). Each half of the antibody consists of one heavy chain (H) and one light chain (L). The two chains in each half are also joined by disulfide bonds (S – S). The disulfide bonds allow the movement of amino acid chains. In each antibody, the light chain is parallel to one end of the heavy chain. The light chain and the part of heavy chain parallel to it form one arm. The remaining part of the heavy chain forms another arm. A hinge joins both the arms (Fig. 17.3).

Each chain of the antibody includes two regions:

1. Constant region
2. Variable region.

1. *Constant Region*

Amino acids present in this region are 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. Thus, the identification and the functions of different types of immunoglobulins depend upon the constant region. This region binds to the antibody receptor situated on the surface of the cell membrane. It also causes complement fixation. So, this region is also called the complement binding region.

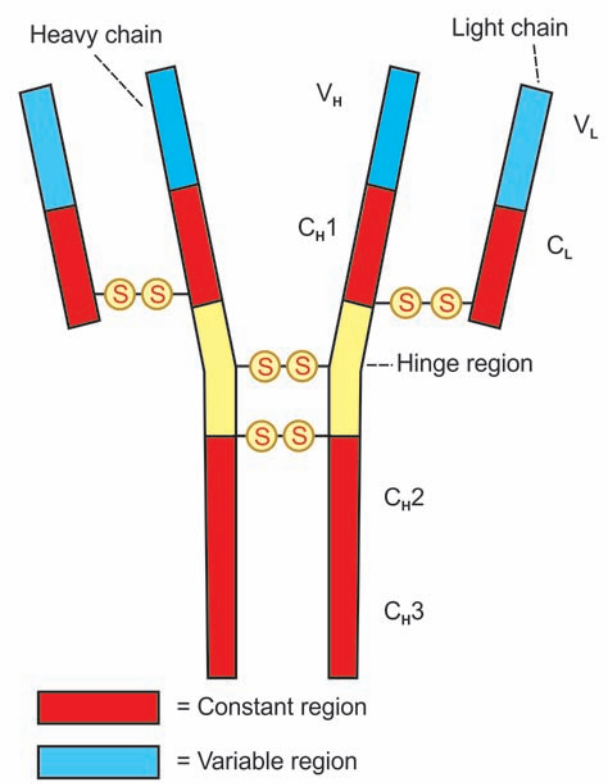


FIGURE 17.3: Structure of antibody (IgG) molecule. V_L = Variable region of light chain, V_H = Variable region of heavy chain, C_L = Constant region of light chain, C_H1, C_H2 and C_H3 = Constant regions of heavy chains.

2. *Variable Region*

Variable region is smaller compared to constant region. Amino acids occupying this region are different in number and placement (sequence) in each antibody. So, it is called the variable region. This region enables the antibody to recognize the specific antigen and to bind itself with the antigen. So, this region of the chain is called 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 is responsible for complement fixation
5. IgM is also responsible for complement fixation.

Mechanism of Actions of Antibodies

Antibodies protect the body from invading organisms in two ways (Fig. 17.4):

1. By direct actions
2. Through complement system.

1. *Direct Actions of Antibodies*

Antibodies directly inactivate the invading organism by any one of the following methods:

i. Agglutination: In this, the foreign bodies like RBCs or bacteria with antigens on their surfaces are held together in a clump by the antibodies.
ii. Precipitation: In this, 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: It is done by the most potent antibodies. These antibodies rupture the cell membrane of the organisms and then destroy them.

2. *Actions of Antibodies through Complement System*

The indirect actions of antibodies are stronger than the direct actions and play more important role in defense mechanism of the body than the direct actions.

Complement system is the one that enhances or accelerates various activities during the fight against the invading organisms. It is a system of plasma enzymes, which are identified by numbers from C_1 to C_9. Including the three subunits of C_1 (C_{1q} C_{1r} C_{1s}), there are 11 enzymes in total. Normally, these enzymes are in inactive form and are activated in three ways:

a. Classical pathway
b. Lectin pathway
c. Alternate pathway.

a. *Classical pathway*

In this the C_1 binds with the antibodies and triggers a series of events in which other enzymes are activated in sequence. These enzymes or the byproducts formed during these events produce the following activities:

i. *Opsonization:* Activation of neutrophils and macrophages to engulf the bacteria, which are bound with a protein in the plasma called opsonin.
ii. *Lysis:* Destruction of bacteria by rupturing the cell membrane.
iii. *Chemotaxis*: Attraction of leukocytes to the site of antigen-antibody reaction.
iv. *Agglutination:* Clumping of foreign bodies like RBCs or bacteria.
v. *Neutralization:* Covering the toxic sites of antigenic products.
vi. *Activation of mast cells and basophils, which liberate histamine:* Histamine dilates the blood vessels and increases capillary permeability. So, plasma proteins from blood enter the tissues and inactivate the antigenic products.

b. *Lectin pathway*

Lectin pathway occurs when mannose-binding lectin (MBL), which is a serum protein binds with mannose or fructose group on wall of bacteria, fungi or virus.

c. *Alternate pathway*

Complementary system is also activated by another way, which is called alternate pathway. It is due to a protein in circulation called factor I. It binds with polysaccharides present in the cell membrane of the invading organisms. This binding activates C_3 and C_5, which ultimately attack the antigenic products of invading organism.

Specificity of B Lymphocytes

Each B lymphocyte is designed to be activated only by one type of antigen. It is also capable of producing antibodies against that antigen only. This property of B lymphocyte is called specificity. In lymphoid tissues, the lymphocytes, which produce a specific antibody, are together called the clone of lymphocytes.

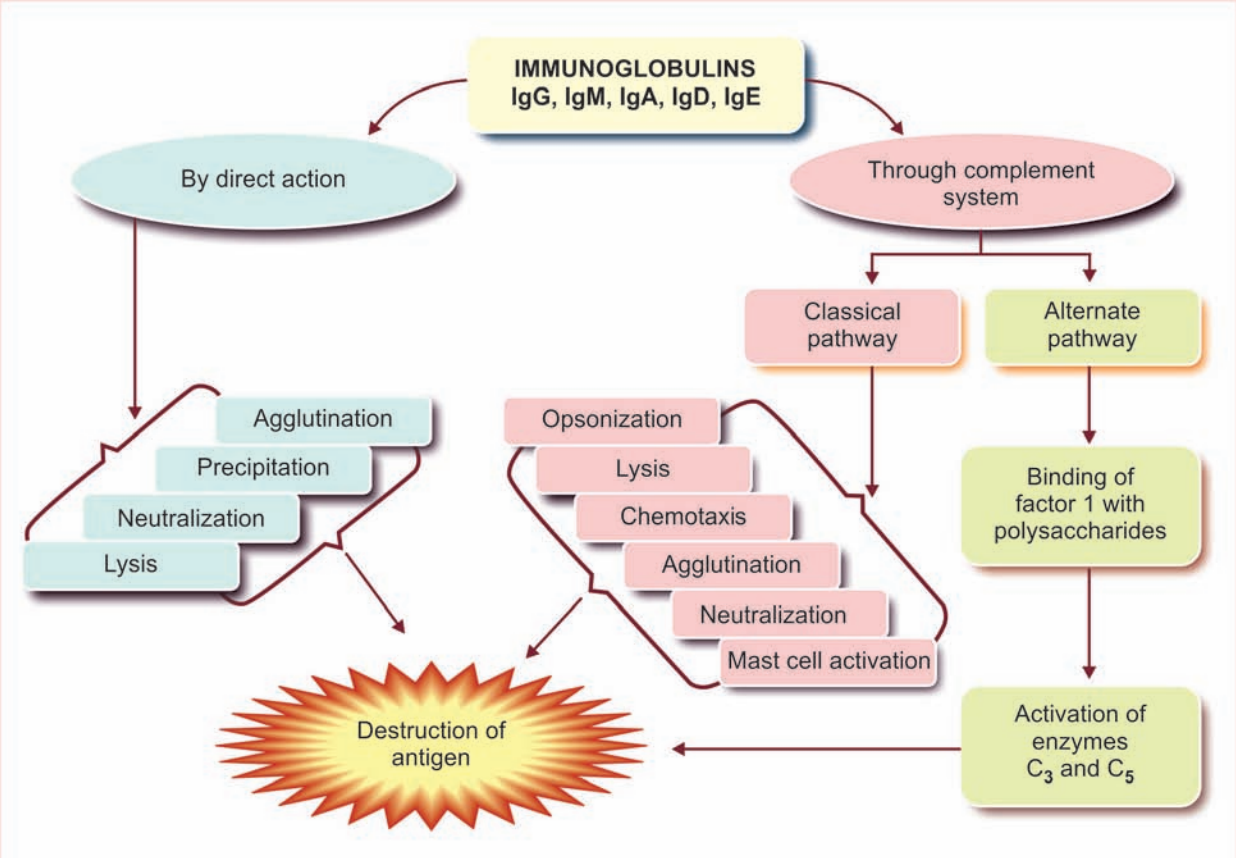


FIGURE 17.4: Mechanism of action of immunoglobulins

■ NATURAL KILLER CELL

Natural killer (NK) cell is a large granular cell that plays an important role in defense mechanism of the body. It has an indented nucleus. Considered as the third type of lymphocyte, it is often called the non-T, non-B cell. It is derived from bone marrow. NK cell is said to be the first line of defense in specific immunity, particularly against viruses.

NK cell kills the invading organisms or the cells of the body without prior sensitization. It is not a phagocytic cell but its granules contain hydrolytic enzymes such as perforins and granzymes. These hydrolytic enzymes play an important role in the lysis of cells of invading organisms.

Functions of Natural Killer (NK) Cell

Natural killer cell:

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 tumor necrosis factor-α. Cytokines are explained later in this chapter.

■ CYTOKINES

Cytokines are the hormone-like small proteins acting as intercellular messengers (cell signaling molecules) by binding to specific receptors of target cells. These non-antibody proteins are secreted by WBCs and some other types of cells. Their major function is the activation and regulation of general immune system of the body.

Cytokines are distinct from the other cell-signaling molecules such as growth factors (Chapter 1) and hormones (Chapter 65).

■ TYPES OF CYTOKINES

Depending upon the source of secretion and effects, cytokines are classified into several types:

1. Interleukins
2. Interferons
3. Tumor necrosis factors

4. Chemokines
5. Defensins
6. Cathelicidins
7. Platelet-activating factor.

Source of secretion and actions of these cytokines are given in Table 17.2.

1. *Interleukins*

Interleukins (IL) are the polypeptide cytokines which are produced mainly by the leukocytes and act on other leukocytes.

Types of interleukins

So far, about 16 types of interleukins are identified. IL-1, IL-2, IL-3, IL-4, IL-5, IL-6 and IL-8 play important role in the process of immunity. Recently IL-12 (otherwise called natural killer cell stimulatory factor) and IL-11 are also considered as important cytokines.

2. *Interferons*

Interferons (IFN) are the glycoprotein molecules. These cytokines are considered as antiviral agents.

Types of interferons

Interferons are of three types namely, INF-α, INF-β and INF-γ.

3. *Tumor Necrosis Factors*

Tumor necrosis factors (TNF) are of three types, TNF-α (cachectin), TNF-β (lymphotoxin) and TNF-γ.

4. *Chemokines*

Cytokines having chemoattractant action are called chemokines.

5. *Defensins*

Defensins are the antimicrobial peptides.

Types of defensins

Two types of defensins are identified in human:

i. α-defensins, secreted by neutrophils, macrophages and paneth cells in small intestine.
ii. β-defensins, secreted by airway epithelial cells (respiratory tract), salivary glands and cutaneous cells.

6. *Cathelicidins*

Cathelicidins are also the antimicrobial peptides which play an important role in a wide range of antimicrobial activity in air passage and lungs.

7. *Platelet-activating Factor*

Platelet-activating factor (PAF) accelerates agglutination and aggregation of platelets.

■ IMMUNIZATION

Immunization is defined as the procedure by which the body is prepared to fight against a specific disease. It is used to induce the immune resistance of the body to a specific disease. Immunization is of two types:

1. Passive immunization
2. Active immunization.

■ PASSIVE IMMUNIZATION

Passive immunization or immunity is produced without challenging the immune system of the body. It is done by administration of serum or gamma globulins from a person who is already immunized (affected by the disease) to a non-immune person.

Passive immunization is acquired either naturally or artificially.

Passive Natural Immunization

Passive natural immunization is acquired from the mother before and after birth. Before birth, immunity is transferred from mother to the fetus in the form of maternal antibodies (mainly IgG) through placenta. After birth, the antibodies (IgA) are transferred through breast milk.

Lymphocytes of the child are not activated. In addition, the antibodies received from the mother are metabolized soon. Therefore, the passive immunity is short lived. The significance of passive immunity that is obtained before birth is the prevention of Rh incompatibility in pregnancy.

Passive Artificial Immunization

Passive artificial immunization is developed by injecting previously prepared antibodies using serum from humans or animals. Antibodies are obtained from the persons affected by the disease or from animals, particularly horses which have been immunized artificially. The serum containing the antibody (antiserum) is administered to people who have developed the disease (therapeutic). It is also used as a prophylactic measure. Prophylaxis refers to medical or public health procedures to prevent a disease in people who may be exposed to the disease in a later period.

This type of immunity is useful for providing immediate protection against acute infections like tetanus, measles, diphtheria, etc. and for poisoning by insects, snakes and venom from other animals. It is also used as a prophylactic measure. However, this may result

TABLE 17.2: Cytokines

Cytokine	Source of secretion	Action
Interleukins	1. T cells 2. B cells 3. Eosinophils 4. Basophils 5. Monocytes 6. Mast cells 7. Macrophages 8. NK cells	1. Activation of T cells, macrophages and natural killer (NK) cells 2. Promotion of growth of hemopoietic cells and B cells 3. Acceleration of inflammatory response by activating eosinophils 4. Chemotaxis of neutrophils, eosinophils, basophils and T cells 5. Destruction of invading organisms
Interferons	1. WBCs 2. NK cells 3. Fibroblasts	1. Fighting against viral infection by suppressing virus multiplication in target cells 2. Inhibition of multiplication of parasites and cancer cells 3. Promotion of phagocytosis by monocytes and macrophages 4. Activation of NK cells
Tumor necrosis factors	1. T cells 2. B cells 3. Mast cells 4. Macrophages 5. NK cells 6. Platelets	1. Causing necrosis of tumor 2. Activation of general immune system 3. Production of vascular effects 4. Promotion of inflammation
Chemokines	1. T cells 2. B cells 3. Monocytes 4. Macrophages	Attraction of WBCs by chemotaxis
Defensins	1. Neutrophils 2. Macrophages 3. Paneth cells in small intestine 4. Airway epithelial cells 5. Salivary glands 6. Cutaneous cells	1. Role in innate immunity in airway surface and lungs 2. Killing the phagocytozed bacteria 3. Antiinflammatory actions 4. Promotion of wound healing 5. Attraction of monocytes and T cells by chemotaxis
Cathelicidins	1. Neutrophils 2. Macrophages 3. Airway epithelial cells 4. Macrophages	Antimicrobial activity in air passage and lungs
Platelet-activating factor	1. Neutrophils 2. Monocytes	Acceleration of agglutination and aggregation of platelets

in complications and anaphylaxis. There is a risk of transmitting HIV and hepatitis.

■ ACTIVE IMMUNIZATION

Active immunization or immunity is acquired by activating immune system of the body. Body develops resistance against disease by producing antibodies following the exposure to antigens. Active immunity is acquired either naturally or artificially.

Active Natural Immunization

Naturally acquired active immunity involves activation of immune system in the body to produce antibodies. It is achieved in both clinical and subclinical infections.

Clinical infection

Clinical infection is defined as the invasion of the body tissues by pathogenic microorganisms which reproduce, multiply and cause disease by injuring the cells, secreting a toxin or antigen-antibody reaction. During infection, the plasma cells produce immunoglobulins to destroy the invading antigens. Later, due to the activity of memory cells, body retains the ability to produce the antibodies against the specific antigens invaded previously.

Subclinical infection

Subclinical infection is defined as an infection in which symptoms are very mild and do not alert the affected subject. The disease thus produced may not be severe

to develop any manifestations. However, it causes the activation of B lymphocytes, resulting in production of antibodies.

Active Artificial Immunization

Active artificial immunization is a type of immunization is achieved by the administration of vaccines or toxoids.

Vaccines

Vaccine is a substance that is introduced into the body to prevent the disease produced by certain pathogens. Vaccine consists of dead pathogens or live but attenuated (artificially weakened) organisms. The vaccine induces immunity against the pathogen, either by production of antibodies or by activation of T lymphocytes.

Edward Jenner produced first live vaccine. He produced the vaccine for **smallpox** from **cowpox virus.** Nowadays, vaccines are used to prevent many diseases like measles, mumps, poliomyelitis, tuberculosis, smallpox, rubella, yellow fever, rabies, typhoid, influenza, hepatitis B, etc.

Toxoids

Toxoid is a substance which is normally toxic and has been processed to destroy its toxicity but retains its capacity to induce antibody production by immune system. Toxoid consists of weakened components or toxins secreted by the pathogens. Toxoids are used to develop immunity against diseases like diphtheria, tetanus, cholera, etc.

The active artificial immunity may be effective life-long or for short period. It is effective lifelong against the diseases such as mumps, measles, smallpox, tuberculosis and yellow fever. It is effective only for short period against some diseases like cholera (about 6 months) and tetanus (about 1 year).

■ IMMUNE DEFICIENCY DISEASES

Immune deficiency diseases are a group of diseases in which some components of immune system is missing or defective. Normally, the defense mechanism protects the body from invading pathogenic organism. When the defense mechanism fails or becomes faulty (defective), the organisms of even low virulence produce severe disease. The organisms, which take advantage of defective defense mechanism, are called opportunists.

Immune deficiency diseases caused by such opportunists are of two types:

1. Congenital immune deficiency diseases
2. Acquired immune deficiency diseases.

■ CONGENITAL IMMUNE DEFICIENCY DISEASES

Congenital diseases are inherited and occur due to the defects in B cell or T cell or both. The common examples are **DiGeorge syndrome** (due to absence of thymus) and severe combined immune deficiency (due to lymphopenia or the absence of lymphoid tissue).

■ ACQUIRED IMMUNE DEFICIENCY DISEASES

Acquired immune deficiency diseases occur due to infection by some organisms. The most common disease of this type is acquired immune deficiency syndrome (AIDS).

Acquired Immune Deficiency Syndrome (AIDS)

AIDS is an infectious disease caused by immune deficiency virus (HIV). A person is diagnosed with AIDS when the CD4 count is below 200 cells per cubic millimeter of blood.

AIDS is the most common problem throughout the world because of rapid increase in the number of victims. Infection occurs when a glycoprotein from HIV binds to surface receptors of T lymphocytes, monocytes, macrophages and dendritic cells leading to the destruction of these cells. It causes slow progressive decrease in immune function, resulting in opportunistic infections of various types. The common opportunistic infections, which kill the AIDS patient are **pneumonia (Pneumocystis carinii)** and malignant skin cancer **(Kaposi sarcoma).** These diseases are also called AIDS-related diseases.

After entering the body of the host, the HIV activates the enzyme called **reverse transcriptase.** HIV utilizes this enzyme and converts its own viral RNA into viral DNA with the help of host cell DNA itself. Now, the viral DNA gets incorporated into the host cell DNA and prevents the normal activities of the host cell DNA. At the same time, the HIV increases in number inside the host's body. The infected host cell ruptures and releases more number of HIV into the bloodstream. After exposure to HIV, no symptoms develop for several weeks. This is the incubation period. The patient develops symptoms only when sufficient number of infected cells is ruptured. The common symptoms are fatigue, loss of weight, chronic diarrhea, low-grade fever, night sweats, oral ulcers, vaginal ulcers, etc. This phase prolongs for about three years before the disease is diagnosed.

Mode of transmission

The HIV infection spreads when secretions from the body of infected individual come in contact with blood of the recipient through the damaged skin or mucous membrane. The most common ways of infection are

contaminated blood transfusion, contaminated needles or other invasive instruments, transmission from mother to fetus during pregnancy, transmission from mother to child during delivery or breastfeeding and vaginal sexual intercourse.

Prevention

Prevention of AIDS is essential because the authentic treatment for this disease has not been established so far. Progress in the development of effective treatment is very slow. Moreover, the maximum duration of survival after initial infection is only about 10 to 15 years. So, it is necessary to prevent this disease.

Following safety measures should be followed to prevent AIDS:

1. Public must be educated about the seriousness and prevention of the disease.
2. HIV infected persons should be educated to avoid spreading the disease to others.
3. Blood should be screened for HIV before transfusion.
4. Intravenous drug users should not share the needles.
5. Pregnant women should get the blood tested for HIV. If the mother is infected, the treatment with zidovudine may reduce incidence of infection in infants. The baby must be given zidovudine for 6 weeks after birth.
6. Young adults and teenagers must be informed about the safer sex techniques and use of condoms. The need for limitation of sexual partners must be emphasized.

■ AUTOIMMUNE DISEASES

Autoimmune disease is defined as a condition in which the immune system mistakenly attacks body's own cells and tissues. Normally, an antigen induces the immune response in the body. The condition in which the immune system fails to give response to an antigen is called tolerance. This is true with respect to body's own antigens that are called self antigens or autoantigens. Normally, body has the **tolerance** against self antigen. However, in some occasions, the tolerance fails or becomes incomplete against self antigen. This state is called autoimmunity and it leads to the activation of T lymphocytes or production of autoantibodies from B lymphocytes. The T lymphocytes (cytotoxic T cells) or autoantibodies attack the body's normal cells whose surface contains the self antigen or autoantigen.

Thus, the autoimmune disease is produced when body's normal tolerance decreases and the immune system fails to recognize the body's own tissues as 'self'.

Autoimmune diseases are of two types:

1. Organ specific diseases which affect only one organ
2. Organ nonspecific or multisystemic diseases, which affect many organs or systems.

■ HUMAN LEUKOCYTE ANTIGEN SYSTEM AND AUTOIMMUNE DISEASES

Human leukocyte antigen (HLA) is a group of genes on human chromosome 6. These genes encode the proteins which function in the cells to transport the antigens from within the cell towards the cell surface. HLA is the product of major histocompatilility complex.

HLA system monitors the immune system in the body (see above). The HLA molecules are recognized by the T and B lymphocytes and hence the name called antigens. HLA is distributed in almost all the tissues of the body. Antibodies are directed against the tissues possessing the HLA, leading to autoimmune diseases. Most of the autoimmune diseases are HLA linked.

■ COMMON AUTOIMMUNE DISEASES

Common autoimmune diseases are:

1. Insulin-dependent diabetes mellitus
2. Myasthenia gravis
3. Hashimoto thyroiditis
4. Graves disease
5. Rheumatoid arthritis.

1. *Insulin-dependent Diabetes Mellitus*

Insulin-dependent diabetes mellitus (IDDM) is very common in childhood and it is due to HLA-linked autoimmunity.

Common causes for IDDM

i. Development of islet cell autoantibody against β-cells in the islets of Langerhans in pancreas.
ii. Development of antibody against insulin and glutamic acid decarboxylase.
iii. Activation of T cells against islets.

Other details of IDDM are given in Chapter 69.

2. *Myasthenia Gravis*

This neuromuscular disease occurs due to the development of autoantibodies against the receptors acetylcholine in neuromuscular junction. Details of myasthenia gravis are given in Chapter 34.

3. *Hashimoto Thyroiditis*

Hashimoto thyroiditis is common in the late middle-aged women. The autoantibodies impair the activity of thyroid

follicles leading to hypothyroidism. Hypothyroidism is explained in detail in Chapter 67.

4. Graves Disease

In some cases, the autoantibodies activate thyroid-stimulating hormone (TSH) receptors leading to hyperthyroidism. The details of this disease are given in Chapter 67.

5. Rheumatoid Arthritis

Rheumatiod arthritis is the disease due to chronic inflammation of synovial lining of joints (synovitis). The synovium becomes thick, leading to the development of swelling around joint and tendons. The characteristic symptoms are pain and stiffness of joints. The chronic inflammation occurs due to the continuous production of autoantibodies called rheumatoid arthritis factors (RA factors).

■ ALLERGY AND IMMUNOLOGICAL HYPERSENSITIVITY REACTIONS

The term allergy means hypersensitivity. It is defined as abnormal immune response to a chemical or physical agent **(allergen).** During the first exposure to an allergen, the immune response does not normally produce any reaction in the body. Sensitization or an initial exposure to the allergen is required for the reaction. So, the subsequent exposure to the allergen causes variety of inflammatory responses. These responses are called allergic reactions or immunological hypersensitivity reactions.

Immunological hypersensitivity reactions may be innate or acquired. These reactions are mediated mostly by antibodies. In some conditions, T cells are involved. Common symptoms include sneezing, itching and skin rashes. However, in some persons the symptoms may be severe.

Common allergic conditions are:

1. Food allergy
2. Allergic rhinitis
3. Bronchial asthma
4. Urticaria.

■ ALLERGENS

Any substance that produces the manifestations of allergy is called an allergen. It may be an antigen or a protein or any other type of substance. Even physical agents can develop allergy.

Allergens are introduced by:

1. Contact (e.g.: chemical substance)
2. Inhalation (e.g.: pollen)
3. Ingestion (e.g.: food)
4. Injection (e.g.: drug).

Common Allergens

1. *Food substances:* Wheat, egg, milk and chocolate.
2. *Inhalants:* Pollen grains, fungi, dust, smoke, perfumes and disagreeable odor.
3. *Contactants:* Chemical substances, metals, animals and plants.
4. *Infectious agents:* Parasites, bacteria, viruses and fungi.
5. *Drugs:* Aspirin and antibiotics.
6. *Physical agents:* Cold, heat, light, pressure and radiation.

■ IMMUNOLOGICAL HYPERSENSITIVE REACTIONS

Immunological hypersensitive reactions to an agent give rise to several allergic conditions and autoimmune diseases.

Hypersensitive reactions are classified into five types:

Type I or anaphylactic reactions.
Type II or cytotoxic reactions.
Type III or antibody-mediated reactions.
Type IV or cell-mediated reactions.
Type V or stimulatory/blocking reactions.

Type I or Anaphylactic Reactions

Anaphylaxis means exaggerated reactions of the body to an antigen or other agents to which the body is sensitized already. It is also called immediate hypersensitive reaction because it develops within few minutes of exposure to an allergen. Anaphylactic reactions are mediated by IgE and other factors involved in inflammation (inflammation means the protective response of the tissues to the damage or destruction of cells).

When the body is exposed to an allergen, the IgE immunoglobulins are produced. Also called reagins or sensitizing antibodies, these immunoglobulins bind with the surface receptors of mast cells and circulating basophils. Mast cells are the granulated wandering cells found in connective tissue and beneath the mucous membrane in the throat, lungs and eyes.

During subsequent exposure of the body to the same allergen, the allergen IgE antibody reaction takes place. This leads to degranulation of mast cells and basophils, with the release of some chemical mediators such histamine. The chemical mediators produce the hypersensitivity reactions. Most serious reactions are fall in blood pressure (due to vasodilatation),obstruction of air passage and difficulty in breathing (due to bronchoconstriction) and shock (Chapter 116).

Type II or Cytotoxic Reactions

Cytotoxic reactions involve mainly the IgG antibodies, which bind with antigens on the surface of the cells, particularly the blood cells. The affected cells are destroyed. Sometimes, IgM and IgA antibodies are also involved. The diseases developed due to cytotoxic reactions are hemolytic diseases of newborn in case of Rh incompatibility and **autoimmune hemolytic anemia.**

Type III or Antibody-mediated Reactions

Excess amounts of antibodies like IgG or IgM are produced in this type. The antigen-antibody complexes are precipitated and deposited in localized areas like joints causing **arthritis,** heart causing **myocarditis** and glomeruli of kidney producing **glomerulonephritis.**

Type IV or Cell-mediated Reactions

This type of hypersensitivity is also called delayed or slow type of hypersensitivity. It is found in allergic reactions due to the bacteria, viruses and fungi. It is also seen in contact dermatitis caused by chemical allergens and during rejection of transplanted tissues. An example of type IV reaction is the delayed reaction after intradermal injection of tuberculin in persons who are previously affected by tuberculosis (tuberculosis skin test or **Mantoux test**). The important feature of delayed type of hypersensitivity is the involvement of T lymphocytes rather than the antibodies.

Type V or Stimulatory/Blocking Reactions

It is seen in autoimmune diseases like Graves' disease (stimulatory reactions) and myasthenia gravis (blocking reactions).

Graves' disease: Normally, TSH combines with surface receptors of thyroid cells and causes synthesis and secretion of thyroid hormones. The secretion of thyroid hormones can be increased by thyroid-stimulating antibodies (TSAB) produced by plasma cells (B lymphocytes). The excess secretion of thyroid hormone leads to Graves' disease.

Myasthenia gravis: It is due to the development of IgG autoantibodies (see above).

Chapter 18

Platelets

- INTRODUCTION
- STRUCTURE AND COMPOSITION
- NORMAL COUNT AND VARIATIONS
- PROPERTIES
- FUNCTIONS
- ACTIVATORS AND INHIBITORS
- DEVELOPMENT
- LIFESPAN AND FATE
- APPLIED PHYSIOLOGY – PLATELET DISORDERS

INTRODUCTION

Platelets or thrombocytes are the formed elements of blood. Platelets are small colorless, non-nucleated and moderately refractive bodies. These formed elements of blood are considered to be the fragments of cytoplasm.

Size of Platelets

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

Shape of Platelets

Normally, platelets are of several shapes, viz. spherical or rod-shaped and become oval or disk-shaped when inactivated. Sometimes, the platelets have dumbbell shape, comma shape, cigar shape or any other unusual shape. Inactivated platelets are without processes or filopodia and the activated platelets develop processes or filopodia (see below).

STRUCTURE AND COMPOSITION

Platelet is constituted by:

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

CELL MEMBRANE

Cell membrane of platelet is 6 nm thick. Extensive invagination of cell membrane forms an open **canalicular system** (Fig. 18.1). This canalicular system is a delicate tunnel system through which the platelet granules extrude their contents.

Cell membrane of platelet contains lipids in the form of phospholipids, cholesterol and glycolipids, carbohydrates as glycocalyx and glycoproteins and proteins. Of these substances, glycoproteins and phospholipids are functionally important.

Glycoproteins

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

Phospholipids accelerate the clotting reactions. The phospholipids form the precursors of thromboxane A_2 and other prostaglandin-related substances.

■ MICROTUBULES

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

■ CYTOPLASM

Cytoplasm of platelets contains the cellular organelles, Golgi apparatus, endoplasmic reticulum, mitochondria, microtubule, microvessels, filaments and granules.

Cytoplasm 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):* Responsible for repair of damaged blood vessels and wound healing. It is a potent mytogen (chemical agent that promotes mitosis) for smooth muscle fibers of blood vessels.
5. *Platelet-activating factor (PAF):* Causes 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.

Other Chemical Substances

1. Glycogen
2. Substances like blood group antigens
3. Inorganic substances such as calcium, copper, magnesium and iron.

Platelet Granules

Granules present in cytoplasm of platelets are of two types:

1. Alpha granules
2. Dense granules.

Substances present in these granules are given in Table 18.1.

Alpha granules

Alpha granules contain:

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.

■ NORMAL COUNT AND VARIATIONS

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.

■ PHYSIOLOGICAL VARIATIONS

1. *Age:* Platelets are less in infants (1,50,000 to 2,00,000/cu mm) and reaches normal level at 3rd month after birth.
2. *Sex:* There is no difference in the platelet count between males and females. In females, it is reduced during menstruation.
3. *High altitude:* Platelet count increases.
4. *After meals:* After taking food, the platelet count increases.

TABLE 18.1: Substances present in platelet granules

Alpha granules	Dense granules
Clotting factors: fibrinogen, V and XIII Platelet-derived growth factor Vascular endothelial growth factor Basic fibroblast growth factor Endostatin Thrombospondin	Nucleotides Serotonin Phospholipid Calcium Lysosomes

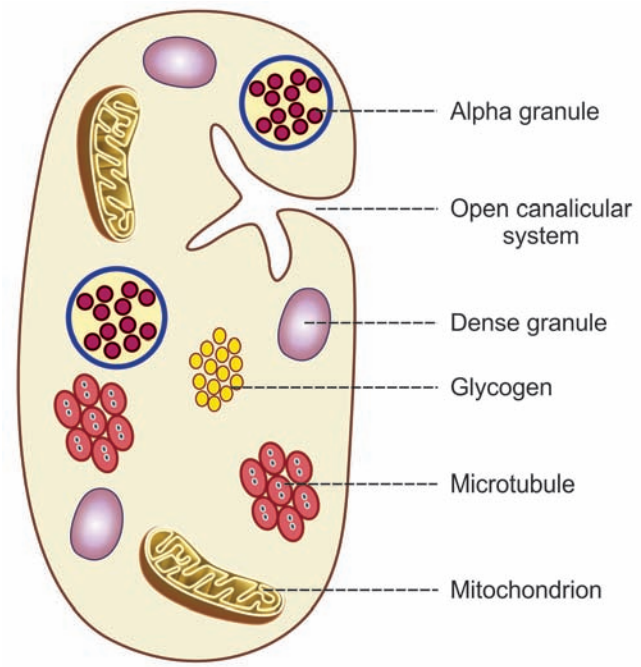


FIGURE 18.1: Platelet under electron microscope

■ PATHOLOGICAL VARIATIONS

Refer applied physiology of this chapter.

■ PROPERTIES OF PLATELETS

Platelets have three important properties (three 'A's):

1. Adhesiveness
2. Aggregation
3. Agglutination.

■ 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 A_2, calcium ions, P-selectin and vitronectin.

■ AGGREGATION (GROUPING OF PLATELETS)

Aggregation is the grouping of platelets. 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 (Fig. 18.2).

Filopodia help the platelets aggregate together. Activation and aggregation of platelets is accelerated by ADP, thromboxane A_2 and platelet-activating factor (PTA: cytokine secreted by neutrophils and monocytes; Chapter 16).

■ 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

Normally, platelets are inactive and execute their actions only when activated. Activated platelets immediately release many substances. This process is known as platelet release reaction. Functions of platelets are carried out by these substances.

Functions of platelets are:

■ 1. ROLE IN BLOOD CLOTTING

Platelets are responsible for the formation of intrinsic prothrombin activator. This substance is responsible for the onset of blood clotting (Chapter 20).

■ 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 (Chapter 20).

■ 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 (Chapter 19).

■ 4. ROLE IN REPAIR OF RUPTURED BLOOD VESSEL

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

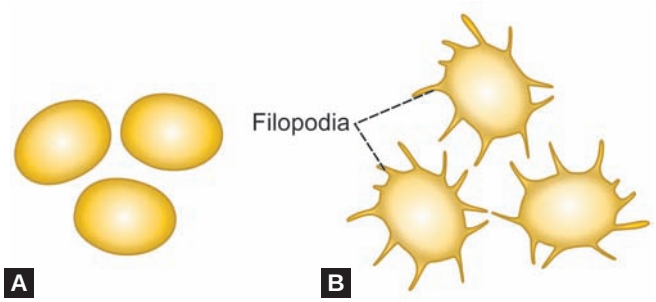


FIGURE 18.2: A. Inactive platelets. **B.** Activated platelets.

■ 5. ROLE IN DEFENSE MECHANISM

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

■ ACTIVATORS AND INHIBITORS OF PLATELETS

■ ACTIVATORS OF PLATELETS

1. Collagen, which is exposed during damage of blood vessels
2. von Willebrand factor
3. Thromboxane A_2
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 (Fig. 10.2).

Production of platelets is influenced by colony-stimulating factors and **thrombopoietin.** Colony-stimulating factors are secreted by monocytes and T lymphocytes. Thrombopoietin is a glycoprotein like erythropoietin. It is secreted by liver and kidneys.

■ 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 – PLATELET DISORDERS

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 is called thrombocytopenia. It leads to thrombocytopenic purpura (Chapter 20).

Thrombocytopenia occurs in the following conditions:

i. Acute infections
ii. Acute leukemia
iii. Aplastic and pernicious anemia
iv. Chickenpox
v. Smallpox
vi. Splenomegaly
vii. Scarlet fever
viii. Typhoid
ix. Tuberculosis
x. Purpura
xi. Gaucher's disease.

2. *Thrombocytosis*

Increase in platelet count is called thrombocytosis.

Thrombocytosis 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*

Glanzmann's thrombasthenia is an inherited hemorrhagic disorder, caused by structural or functional abnormality of platelets. It leads to **thrombasthenic purpura** (Chapter 20). However, the platelet count is normal. It is characterized by normal clotting time, normal or prolonged bleeding time but defective clot retraction.

Chapter 19

Hemostasis

DEFINITION

Hemostasis is defined as arrest or stoppage of bleeding.

STAGES OF HEMOSTASIS

When a blood vessel is injured, the injury initiates a series of reactions, resulting in hemostasis. It occurs in three stages (Fig. 19.1):

1. Vasoconstriction
2. Platelet plug formation
3. Coagulation of blood.

VASOCONSTRICTION

Immediately after injury, the blood vessel constricts and decreases the loss of blood from damaged portion. Usually, arterioles and small arteries constrict. Vasoconstriction is purely a local phenomenon. When the blood vessels are cut, the endothelium is damaged and the collagen is exposed. Platelets adhere to this collagen and get activated. The activated platelets secrete serotonin and other vasoconstrictor substances which cause constriction of the blood vessels. Adherence of platelets to the collagen is accelerated by von Willebrand factor. This factor acts as a bridge between a specific glycoprotein present on the surface of platelet and collagen fibrils.

PLATELET PLUG FORMATION

Platelets get adhered to the collagen of ruptured blood vessel and secrete adenosine diphosphate (ADP) and thromboxane A_2. These two substances attract more and more platelets and activate them. All these platelets aggregate together and form a loose temporary platelet plug or temporary hemostatic plug, which closes the ruptured vessel and prevents further blood loss. Platelet aggregation is accelerated by platelet-activating factor (PAF).

COAGULATION OF BLOOD

During this process, the fibrinogen is converted into fibrin. Fibrin threads get attached to the loose platelet plug, which blocks the ruptured part of blood vessels and prevents further blood loss completely. Mechanism of blood coagulation is explained in the next chapter.

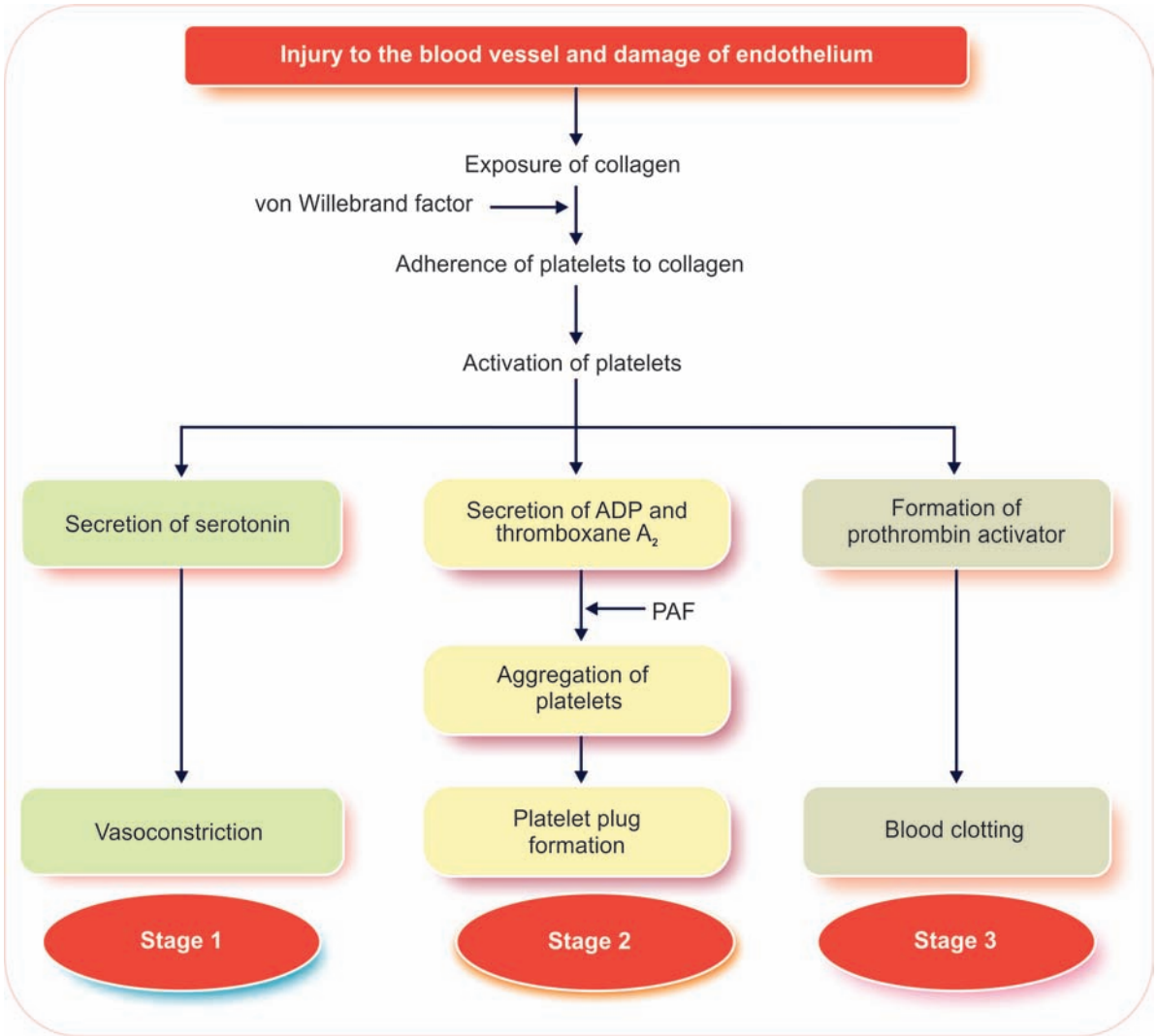


FIGURE 19.1: States of hemostasis. ADP = Adenosine diphosphate; PAF = Platelet-activating factor.

Coagulation of Blood

Chapter 20

- DEFINITION
- FACTORS INVOLVED IN BLOOD CLOTTING
- SEQUENCE OF CLOTTING MECHANISM
- BLOOD CLOT
- ANTICLOTTING MECHANISM IN THE BODY
- ANTICOAGULANTS
- PHYSICAL METHODS TO PREVENT BLOOD CLOTTING
- PROCOAGULANTS
- TESTS FOR BLOOD CLOTTING
- APPLIED PHYSIOLOGY

DEFINITION

Coagulation or clotting is defined as the 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.

FACTORS INVOLVED IN BLOOD CLOTTING

Coagulation of blood occurs through a series of reactions due to the activation of a group of substances. Substances necessary for clotting are called clotting factors.

Thirteen clotting factors are identified:

Factor I	Fibrinogen
Factor II	Prothrombin
Factor III	Thromboplastin (Tissue factor)
Factor IV	Calcium
Factor V	Labile factor (Proaccelerin or accelerator globulin)
Factor VI	Presence has not been proved
Factor VII	Stable factor
Factor VIII	Antihemophilic factor (Antihemophilic globulin)
Factor IX	Christmas factor
Factor X	Stuart-Prower factor
Factor XI	Plasma thromboplastin antecedent
Factor XII	Hageman factor (Contact factor)
Factor XIII	Fibrin-stabilizing factor (Fibrinase).

Clotting factors were named after the scientists who discovered them or as per the activity, except factor IX. Factor IX or Christmas factor was named after the patient in whom it was discovered.

SEQUENCE OF CLOTTING MECHANISM

ENZYME CASCADE THEORY

Most of the clotting factors are proteins in the form of enzymes. Normally, all the factors are present in the form of inactive **proenzyme.** These proenzymes must be activated into enzymes to enforce clot formation. It is carried out by a series of proenzyme-enzyme conversion reactions. First one of the series is converted into an active enzyme that activates the second one, which activates the third one; this continues till the final active enzyme thrombin is formed.

Enzyme cascade theory explains how various reactions, involved in the conversion of proenzymes to active enzymes take place in the form of a cascade. Cascade refers to a process that occurs through a series of steps, each step initiating the next, until the final step is reached.

Stages of Blood Clotting

In general, blood clotting occurs in three stages:
1. Formation of prothrombin activator
2. Conversion of prothrombin into thrombin
3. Conversion of fibrinogen into fibrin.

■ STAGE 1: FORMATION OF PROTHROMBIN ACTIVATOR

Blood clotting commences with the formation of a substance called prothrombin activator, which converts prothrombin into thrombin. Its formation is initiated by substances produced either within the blood or outside the blood.

Thus, formation of prothrombin activator occurs through two pathways:
i. Intrinsic pathway
ii. Extrinsic pathway.

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

In this pathway, the formation of prothrombin activator is initiated by platelets, which are within the blood itself (Fig. 20.1).

Sequence of Events in Intrinsic pathway

i. During the injury, the blood vessel is ruptured. Endothelium is damaged and collagen beneath the endothelium is exposed.
ii. When factor XII (Hageman factor) comes in contact with collagen, it is converted into activated factor XII in the presence of **kallikrein** and high molecular weight (HMW) **kinogen.**
iii. The activated factor XII converts factor XI into activated factor XI in the presence of HMW kinogen.
iv. The activated factor XI activates factor IX in the presence of factor IV (calcium).
v. Activated factor IX activates factor X in the presence of factor VIII and calcium.
vi. When platelet comes in contact with collagen of damaged blood vessel, it gets activated and releases phospholipids.
vii. Now the activated factor X reacts with platelet phospholipid and factor V to form prothrombin activator. This needs the presence of calcium ions.
viii. Factor V is also activated by positive feedback effect of thrombin (see below).

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.

Sequence of Events in Extrinsic Pathway

i. Tissues that are damaged during injury release tissue thromboplastin (factor III). Thromboplastin contains proteins, phospholipid and glycoprotein, which act as proteolytic enzymes.
ii. Glycoprotein and phospholipid components of thromboplastin convert factor X into activated factor X, in the presence of factor VII.
iii. Activated factor X reacts with factor V and phospholipid component of tissue thromboplastin to form prothrombin activator. This reaction requires the presence of calcium ions.

■ STAGE 2: CONVERSION OF PROTHROMBIN INTO THROMBIN

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

Sequence of Events in Stage 2

i. Prothrombin activator that is formed in intrinsic and extrinsic pathways converts prothrombin into thrombin in the presence of calcium (factor IV).
ii. Once formed thrombin initiates the formation of more thrombin molecules. The initially formed thrombin activates Factor V. Factor V in turn accelerates formation of both extrinsic and intrinsic prothrombin activator, which converts prothrombin into thrombin. This effect of thrombin is called **positive feedback** effect (Fig. 20.1).

■ STAGE 3: CONVERSION OF FIBRINOGEN INTO FIBRIN

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

Sequence of Events in Stage 3

i. Thrombin converts inactive fibrinogen into activated fibrinogen due to loss of 2 pairs of

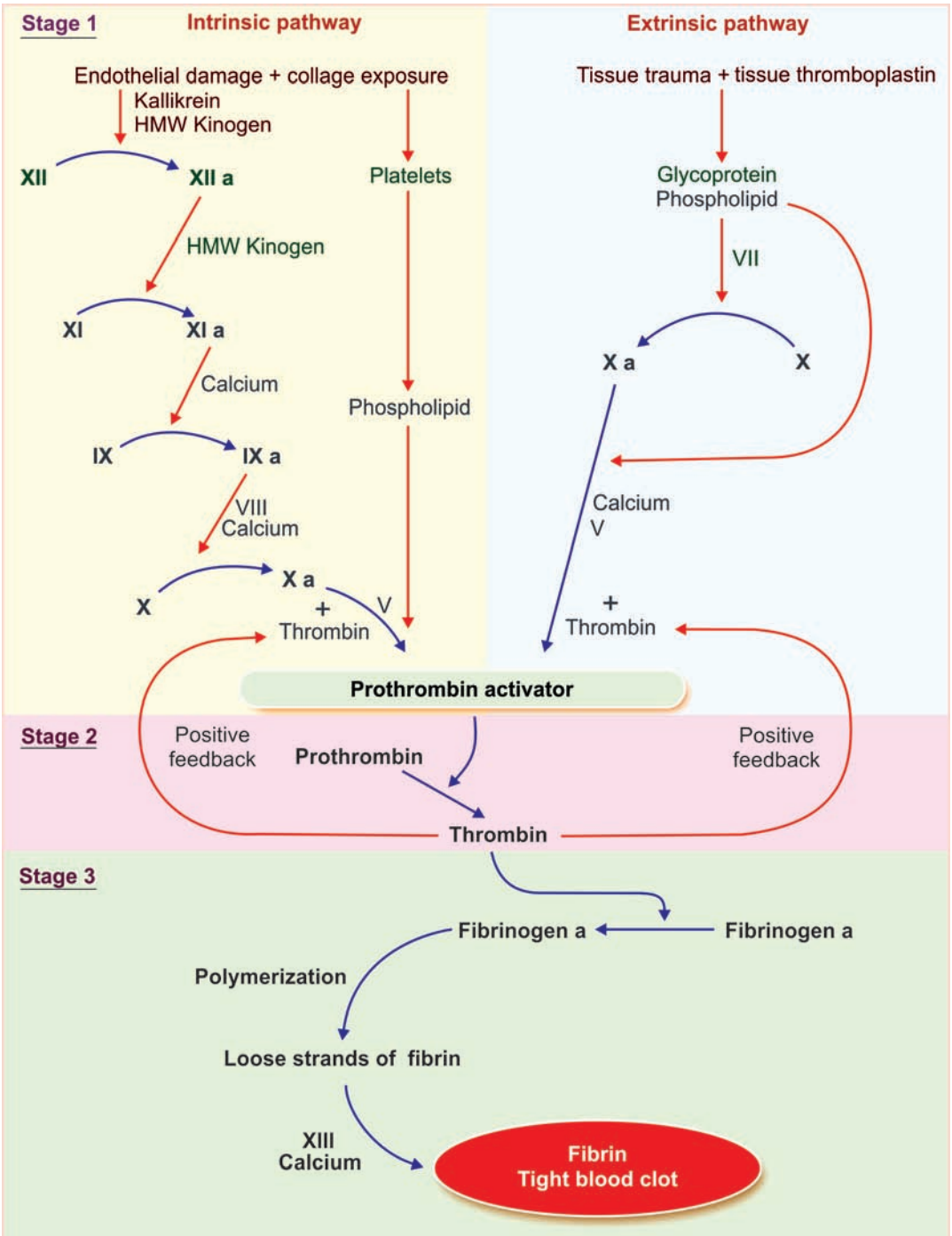


FIGURE 20.1: Stages of blood coagulation. a = Activated, + = Thrombin induces formation of more thrombin (positive feedback); HMW = High molecular weight.

polypeptides from each fibrinogen molecule. The activated fibrinogen is called **fibrin monomer.**

ii. Fibrin monomer polymerizes with other monomer molecules and form loosely arranged strands of fibrin.

iii. Later these loose strands are modified into dense and tight fibrin threads by fibrin-stabilizing factor (factor XIII) in the presence of calcium ions (Fig. 20.1). All the tight fibrin threads are aggregated to form a meshwork of **stable clot.**

BLOOD CLOT

DEFINITION AND COMPOSITION OF CLOT

Blood clot is defined as the mass of coagulated blood which contains RBCs, WBCs and platelets entrapped in fibrin meshwork.

RBCs and WBCs are not necessary for clotting process. However, when clot is formed, these cells are trapped in it along with platelets. The trapped RBCs are responsible for the red color of the clot.

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. And after about 30 to 45 minutes, the straw-colored serum oozes out of the clot. The process involving the contraction of blood clot and oozing of serum is called clot retraction.

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 is called fibrinolysis. It helps to remove the clot from lumen of the blood vessel. This process requires a substance called plasmin or fibrinolysin.

Formation of Plasmin

Plasmin is formed from inactivated glycoprotein called plasminogen. Plasminogen is synthesized in liver and it is incorporated with other proteins in the blood clot. Plasminogen is converted into plasmin by tissue **plasminogen activator (t-PA),** lysosomal enzymes and thrombin. The t-PA and lysosomal enzymes are released from damaged tissues and damaged endothelium. Thrombin is derived from blood. The t-PA is always inhibited by a substance called **t-PA inhibitor.** It is also inhibited by factors V and VIII.

Besides t-PA, there is another plasminogen activator called **urokinase plasminogen activator (u-PA).** It is derived from blood.

Sequence of Events Involved in the Activation of Plasminogen

1. During intravascular clotting, the endothelium of the blood vessel secretes a thrombin-binding protein, the **thrombomodulin.** It is secreted by the endothelium of all the blood vessels, except the minute vessels of brain.
2. Thrombomodulin combines with thrombin and forms a thrombomodulin-thrombin complex
3. Thrombomodulin-thrombin complex activates protein C
4. Activated protein C inactivates factor V and VIII in the presence of a cofactor called **protein S**
5. Protein C also inactivates the t-PA inhibitor
6. Now, the t-PA becomes active
7. Activated t-PA and lysosomal enzymes activate plasminogen to form plasmin. Plasminogen is also activated by thrombin and u-PA (Fig. 20.2).

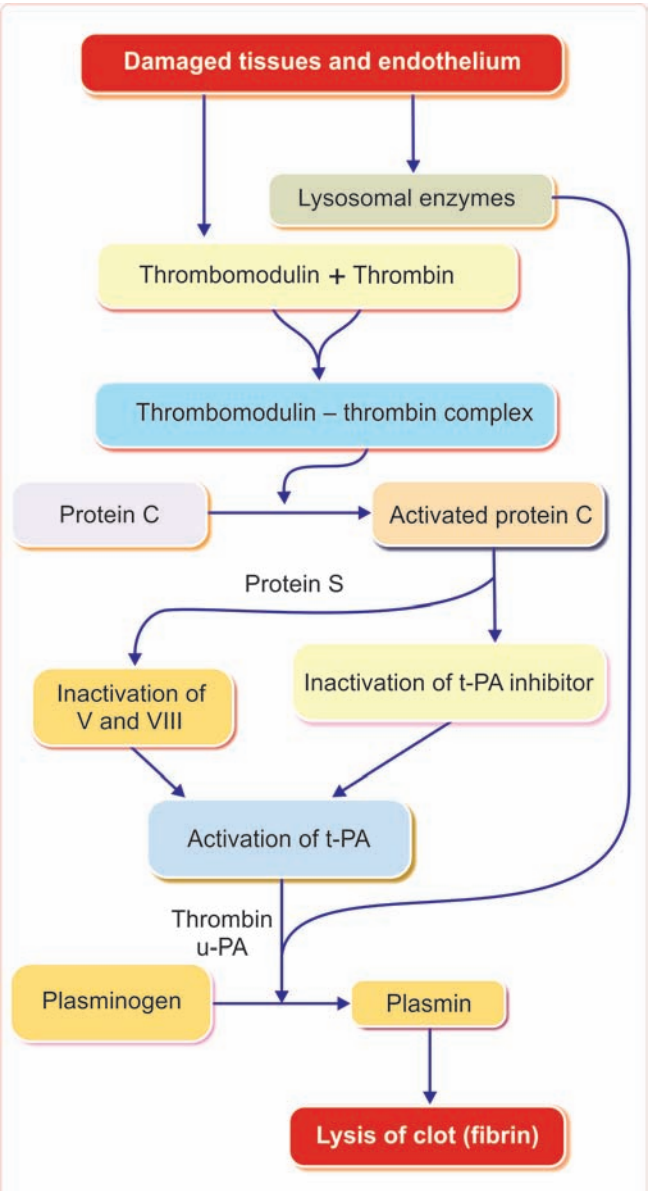


FIGURE 20.2: Fibrinolysis. t-PA = Tissue plasminogen activator, u-PA = Urokinase plasminogen activator.

■ ANTICLOTTING MECHANISM IN THE BODY

Under physiological conditions, intravascular clotting does not occur. It is because of the presence of some physicochemical factors in the body.

1. *Physical Factors*

i. Continuous circulation of blood.
ii. Smooth endothelial lining of the blood vessels.

2. *Chemical Factors – Natural Anticoagulants*

i. Presence of natural anticoagulant called heparin that is produced by the liver
ii. Production of thrombomodulin by endothelium of the blood vessels (except in brain capillaries). Thrombomodulin is a thrombin-binding protein. It binds with thrombin and forms a thrombomodulin-thrombin complex. This complex activates protein C. Activated protein C along with its cofactor protein S inactivates Factor V and Factor VIII. Inactivation of these two clotting factors prevents clot formation
iii. All the clotting factors are in inactive state.

■ ANTICOAGULANTS

Substances which prevent or postpone coagulation of blood are called anticoagulants.

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

Heparin is a naturally produced anticoagulant in the body. It is produced by **mast cells** which are the wandering cells present immediately outside the capillaries in many tissues or organs that contain more connective tissue. These cells are abundant in liver and lungs. Basophils also secrete heparin.

Heparin is a conjugated polysaccharide. Commercial heparin is prepared from the liver and other organs of animals. Commercial preparation is available in liquid form or dry form as sodium, calcium, ammonium or lithium salts.

Mechanism of Action of Heparin

Heparin:

i. Prevents blood clotting by its antithrombin activity. It 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 (Fig. 20.3).

Uses of Heparin

Heparin is used as an anticoagulant both *in vivo* and *in vitro.*

Clinical use

Intravenous injection of heparin (0.5 to 1 mg/kg body weight) postpones clotting for 3 to 4 hours (until it is destroyed by the enzyme **heparinase**). So, it is widely used as an anticoagulant in clinical practice. In clinics, heparin is used for many purposes such as:

i. To prevent intravascular blood clotting during surgery.
ii. While passing the blood through artificial kidney for dialysis.
iii. During cardiac surgery, which involves heart-lung machine.
iv. To preserve the blood before transfusion.

Use in the laboratory

Heparin is also used as anticoagulant *in vitro* while collecting blood for various investigations. About 0.1 to 0.2 mg is sufficient for 1 mL of blood. It is effective for 8 to 12 hours. After that, blood will clot because heparin only delays clotting and does not prevent it.

Heparin is the most expensive anticoagulant.

■ 2. 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.

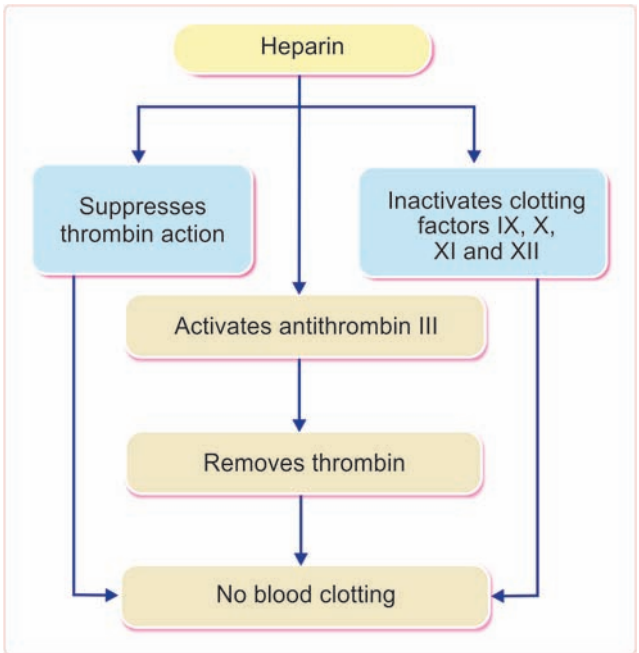


FIGURE 20.3: Mechanism of action of heparin

Uses

Dicoumoral and warfarin are the commonly used **oral anticoagulants** (*in vivo*). Warfarin is used to prevent myocardial infarction (heart attack), strokes and thrombosis.

■ 3. EDTA

Ethylenediaminetetraacetic acid (EDTA) is a strong anticoagulant. It is available in two forms:

i. Disodium salt (Na_2 EDTA).
ii. Tripotassium salt (K_3 EDTA).

Mechanism of Action

These substances prevent blood clotting by removing calcium from blood.

Uses

EDTA is used as an anticoagulant both *in vivo* and *in vitro*. It is:

i. Commonly administered intravenously, in cases of lead poisoning.
ii. Used as an anticoagulant in the laboratory (*in vitro*). 0.5 to 2.0 mg of EDTA per mL of blood is sufficient to preserve the blood for at least 6 hours. On refrigeration, it can preserve the blood up to 24 hours.

■ 4. OXALATE COMPOUNDS

Oxalate compounds prevent coagulation by forming calcium oxalate, which is precipitated later. Thus, these compounds reduce the blood calcium level.

Earlier sodium and potassium oxalates were used. Nowadays, mixture of ammonium oxalate and potassium oxalate in the ratio of 3 : 2 is used. Each salt is an anticoagulant by itself. But potassium oxalate alone causes shrinkage of RBCs. Ammonium oxalate alone causes swelling of RBCs. But together, these substances do not alter the cellular activity.

Mechanism of Action

Oxalate combines with calcium and forms insoluble calcium oxalate. Thus, oxalate removes calcium from blood and lack of calcium prevents coagulation.

Uses

Oxalate compounds are used only as *in vitro* anticoagulants. 2 mg of mixture is necessary for 1 ml of blood. Since oxalate is poisonous, it cannot be used *in vivo*.

■ 5. CITRATES

Sodium, ammonium and potassium citrates are used as anticoagulants.

Mechanism of Action

Citrate combines with calcium in blood to form insoluble calcium citrate. Like oxalate, citrate also removes calcium from blood and lack of calcium prevents coagulation.

Uses

Citrate is used as in vitro anticoagulant.

i. It is used to store blood in the **blood bank** as:
   a. Acid citrate dextrose (ACD): 1 part of ACD with 4 parts of blood
   b. Citrate phosphate dextrose (CPD): 1 part of CPD with 4 parts of blood
ii. Citrate is also used in laboratory in the form of formol-citrate solution (Dacie's solution) for RBC and platelet counts.

■ OTHER SUBSTANCES WHICH PREVENT BLOOD CLOTTING

Peptone, C-type lectin (proteins from venom of viper snake) and **hirudin** (from the leach Hirudinaria manillensis) are the known anticoagulants.

PHYSICAL METHODS TO PREVENT BLOOD CLOTTING

Coagulation of blood is postponed or prevented by the following physical methods:

COLD

Reducing the temperature to about 5°C postpones the coagulation of blood.

COLLECTING BLOOD IN A CONTAINER WITH SMOOTH SURFACE

Collecting the blood in a container with smooth surface like a **silicon-coated** container prevents clotting. The smooth surface inhibits the activation of factor XII and platelets. So, the formation of prothrombin activator is prevented.

PROCOAGULANTS

Procoagulants or hemostatic agents are the substances which accelerate the process of blood coagulation. Procoagulants are:

THROMBIN

Thrombin is sprayed upon the bleeding surface to arrest bleeding by hastening blood clotting.

SNAKE VENOM

Venom of some snakes (vipers, cobras and rattle snakes) contains proteolytic enzymes which enhance blood clotting by activating the clotting factors.

EXTRACTS OF LUNGS AND THYMUS

Extract obtained from the lungs and thymus has thromboplastin, which causes rapid blood coagulation.

SODIUM OR CALCIUM ALGINATE

Sosium or calcium alginate substances enhance blood clotting process by activating the Hageman factor.

OXIDIZED CELLULOSE

Oxidized cellulose causes clotting of blood by activating the Hageman factor.

TESTS FOR BLOOD CLOTTING

Blood clotting tests are used to diagnose blood disorders. Some tests are also used to monitor the patients treated with anticoagulant drugs such as heparin and warfarin.

1. Bleeding time
2. Clotting time
3. Prothrombin time
4. Partial prothrombin time
5. International normalized ratio
6. Thrombin time.

BLEEDING TIME

Bleeding time (BT) is the time interval from oozing of blood after a cut or injury till arrest of bleeding. Usually, it is determined by Duke method using blotting paper or filter paper method. Its normal duration is 3 to 6 minutes. It is prolonged in purpura.

CLOTTING TIME

Clotting time (CT) is the time interval from oozing of blood after a cut or injury till the formation of clot. It is usually determined by capillary tube method. Its normal duration is 3 to 8 minutes. It is prolonged in hemophilia.

PROTHROMBIN TIME

Prothrombin time (PT) is the time taken by blood to clot after adding tissue thromboplastin to it. Blood is collected and oxalated so that, the calcium is precipitated and prothrombin is not converted into thrombin. Thus, the blood clotting is prevented. Then a large quantity of tissue thromboplastin with calcium is added to this blood. Calcium nullifies the effect of oxalate. The tissue thromboplastin activates prothrombin and blood clotting occurs.

During this procedure, the time taken by blood to clot after adding tissue thromboplastin is determined. Prothrombin time indicates the total quantity of prothrombin present in the blood.

Normal duration of prothrombin time is 10 to 12 seconds. It is prolonged in deficiency of prothrombin and other factors like factors I, V, VII and X. However, it is normal in hemophilia.

PARTIAL PROTHROMBIN TIME OR ACTIVATED PROTHROMBIN TIME

Partial prothrombin time (PPT) is the time taken for the blood to clot after adding an activator such as phospholipid, along with calcium to it. It is also called activated partial prothrombin time (APTT). This test is useful in monitoring the patients taking anticoagulant drugs.

It is carried out by observing clotting time after adding phospholipid, a **surface activator** and calcium to a patient's plasma. Phospholipid serves as **platelet substitute.** Commonly used surface activator is **kaolin.**

Normal duration of partial prothrombin time is 30 to 45 seconds. It is prolonged in **heparin or warfarin therapy** (since heparin and warfarin inhibit clotting) and deficiency or inhibition of factors II, V, VIII, IX, X, XI and XII.

■ INTERNATIONAL NORMALIZED RATIO

International normalized ratio (INR) is the rating of a patient's prothrombin time when compared to an average. It measures extrinsic clotting pathway system.

INR is useful in monitoring impact of anticoagulant drugs such as warfarin and to adjust the dosage of anticoagulants. Patients with atrial fibrillation are usually treated with warfarin to protect against blood clot, which may cause strokes. These patients should have regular blood tests to know their INR in order to adjust warfarin dosage.

Blood takes longer time to clot if INR is higher. Normal INR is about 1. In patients taking anticoagulant therapy for atrial fibrillation, INR should be between 2 and 3. For patients with heart valve disorders, INR should be between 3 and 4. But, INR greater than 4 indicates that blood is clotting too slowly and there is a risk of uncontrolled blood clotting.

■ THROMBIN TIME

Thrombin time (TT) is the time taken for the blood to clot after adding thrombin to it. It is done to investigate the presence of heparin in plasma or to detect fibrinogen abnormalities. This test involves observation of clotting time after adding thrombin to patient's plasma. Normal duration of thrombin time is 12 to 20 seconds. It is prolonged in heparin therapy and during dysfibrinogenimia (abnormal function of fibrinogen with normal fibrinogen level).

■ APPLIED PHYSIOLOGY

■ BLEEDING DISORDERS

Bleeding disorders are the 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*

Hemophilia is a group of sex-linked inherited blood disorders, characterized by prolonged clotting time. However, the bleeding time is normal. Usually, it affects the males, with the females being the carriers.

Because of prolonged clotting time, even a mild trauma causes excess bleeding which can lead to death. Damage of skin while falling or extraction of a tooth may cause excess bleeding for few weeks. Easy bruising and hemorrhage in muscles and joints are also common in this disease.

Causes of hemophilia

Hemophilia occurs due to lack of formation of prothrombin activator. That is why the coagulation time is prolonged. The formation of prothrombin activator is affected due to the deficiency of factor VIII, IX or XI.

Types of hemophilia

Depending upon the deficiency of the factor involved, hemophilia is classified into three types:

i. Hemophilia A or **classic hemophilia:** Due to the deficiency of factor VIII. 85% of people with hemophilia are affected by hemophilia A.
ii. Hemophilia B or **Christmas disease:** Due to the deficiency of factor IX. 15% of people with hemophilia are affected by hemophilia B.
iii. Hemophilia C or factor XI deficiency: Due to the deficiency of factor XI. It is a very rare bleeding disorder.

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.

Treatment for hemophilia

Effective therapy for classical hemophilia involves replacement of missing clotting factor.

2. *Purpura*

Purpura is a disorder characterized by prolonged bleeding time. However, the clotting time is normal. Characteristic feature of this disease is spontaneous bleeding under the skin from ruptured capillaries. It causes small tiny **hemorrhagic spots** in many areas of the body. The hemorrhagic spots under the skin are called **purpuric spots** (purple colored patch like appearance). That is why this disease is called purpura. Blood also sometimes collects in large areas beneath the skin which are called **ecchymoses.**

Types and causes of purpura

Purpura is classified into three types depending upon the causes:

i. *Thrombocytopenic purpura*

Thrombocytopenic purpura is due to the deficiency of platelets (thrombocytopenia). In bone marrow disease, platelet production is affected leading to the deficiency of platelets.

ii. *Idiopathic thrombocytopenic purpura*

Purpura due to some unknown cause is called idiopathic thrombocytopenic purpura. It is believed that platelet count decreases due to the development of antibodies against platelets, which occurs after blood transfusion.

iii. *Thrombasthenic purpura*

Thrombasthenic purpura is due to structural or functional abnormality of platelets. However, the platelet count is normal. It is characterized by normal clotting time, normal or prolonged bleeding time but defective clot retraction.

3. *von Willebrand Disease*

von Willebrand disease is a bleeding disorder, characterized by excess bleeding even with a mild injury. It is due to deficiency of von Willebrand factor, which is a protein secreted by endothelium of damaged blood vessels and platelets. This protein is responsible for adherence of platelets to endothelium of blood vessels during hemostasis after an injury. It is also responsible for the survival and maintenance of factor VIII in plasma.

Deficiency of von Willebrand factor suppresses platelet adhesion. It also causes deficiency of factor VIII. This results in excess bleeding, which resembles the bleeding that occurs during platelet dysfunction or hemophilia.

■ THROMBOSIS

Thrombosis or intravascular blood clotting refers to coagulation of blood inside the blood vessels. Normally, blood does not clot in the blood vessel because of some factors which are already explained. But some abnormal conditions cause thrombosis.

Causes of Thrombosis

1. *Injury to blood vessels*

During infection or mechanical obstruction, the endothelial lining of the blood vessel is damaged and it initiates thrombosis.

2. *Roughened endothelial lining*

In infection, damage or arteriosclerosis, the endothelium becomes rough and this initiates clotting.

3. *Sluggishness of blood flow*

Decreased rate of blood flow causes aggregation of platelets and formation of thrombus. Slowness of blood flow occurs in reduced cardiac action, hypotension, low metabolic rate, prolonged confinement to bed and immobility of limbs.

4. *Agglutination of RBCs*

Agglutination of the RBCs leads to thrombosis. Agglutination of RBCs occurs by the foreign antigens or toxic substances.

5. *Toxic thrombosis*

Thrombosis is common due to the action of chemical poisons like arsenic compounds, mercury, poisonous mushrooms and snake venom.

6. *Congenital absence of protein C*

Protein C is a circulating anticoagulant, which inactivates factors V and VIII. Thrombosis occurs in the absence of this protein. Congenital absence of protein C causes thrombosis and death in infancy.

Complications of Thrombosis

1. *Thrombus*

During thrombosis, lumen of blood vessels is occluded. The solid mass of platelets, red cells and/or clot, which obstructs the blood vessel, is called thrombus. The thrombus formed due to agglutination of RBC is called agglutinative thrombus.

2. *Embolism and embolus*

Embolism is the process in which the thrombus or a part of it is detached and carried in bloodstream and occludes the small blood vessels, resulting in arrests of blood flow to any organ or region of the body. Embolus is the thrombus or part of it, which arrests the blood flow. The obstruction of blood flow by embolism is common in lungs (**pulmonary embolism**), brain (**cerebral embolism**) or heart (**coronary embolism**).

3. *Ischemia*

Insufficient blood supply to an organ or area of the body by the obstruction of blood vessels is called ischemia. Ischemia results in tissue damage because of hypoxia (lack of oxygen). Ischemia also causes discomfort,

pain and tissue death. Death of body tissue is called necrosis.

4. *Necrosis and infarction*

Necrosis is a general term that refers to tissue death caused by loss of blood supply, injury, infection, inflammation, physical agents or chemical substances.

Infarction means the tissue death due to loss of blood supply. Loss of blood supply is usually caused by occlusion of an artery by thrombus or embolus and sometimes by atherosclerosis (Chapter 67).

Area of tissue that undergoes infarction is called infarct. Infarction commonly occurs in heart, brain, lungs, kidneys and spleen.

Blood Groups

Chapter 21

INTRODUCTION

When blood from two individuals is mixed, sometimes clumping (agglutination) of RBCs occurs. This clumping is because of the immunological reactions. But, why clumping occurs in some cases and not in other cases remained a mystery until the discovery of blood groups by the Austrian Scientist **Karl Landsteiner,** in 1901. He was honored with Nobel Prize in 1930 for this discovery.

ABO BLOOD GROUPS

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. These antigens are also called agglutinogens because of their capacity to cause agglutination of RBCs. He noticed the corresponding antibodies or agglutinins in the plasma and named them anti-A or α-antibody and anti-B or β-antibody. However, a particular agglutinogen and the corresponding agglutinin cannot be present together. If present, it causes clumping of the blood. Based on this, Karl Landsteiner classified the blood groups. Later it became the 'Landsteiner Law' for grouping the blood.

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. These two blood group systems are the most important ones that are determined before blood transfusions.

■ ABO SYSTEM

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

1. 'A' group
2. 'B' group
3. 'AB' group
4. 'O' group.

Blood having antigen A belongs to 'A' group. This blood has β-antibody in the serum. Blood with antigen B and α-antibody belongs to 'B' group. If both the antigens are present, blood group is called 'AB' group and serum of this group does not contain any antibody. If both antigens are absent, the blood group is called 'O' group and both α and β antibodies are present in the serum. Antigens and antibodies present in different groups of ABO system are given in Table 21.1. Percentage of people among Asian and European population belonging to different blood group is given in Table 21.2.

'A' group has two subgroups namely 'A_1' and 'A_2'. Similarly 'AB' group has two subgroups namely 'A_1B' and 'A_2B'.

■ DETERMINATION OF ABO GROUP

Determination of the ABO group is also called blood grouping, blood typing or blood matching.

Principle of Blood Typing – Agglutination

Blood typing is done on the basis of agglutination. Agglutination means the collection of separate particles like RBCs into clumps or masses. Agglutination occurs if an antigen is mixed with its corresponding antibody which is called **isoagglutinin.** Agglutination occurs when

TABLE 21.1: Antigen and antibody present in ABO blood groups

Group	Antigen in RBC	Antibody in serum
A	A	Anti-B (β)
B	B	Anti-A (α)
AB	A and B	No antibody
O	No antigen	Anti-A and Anti-B

TABLE 21.2: Percentage of people having different blood groups

Population	A	B	AB	O
Indians	23	33	7	37
Asians	25	25	5	45
Europeans	42	9	3	46

A antigen is mixed with anti-A or when B antigen is mixed with anti-B.

Requisites for Blood Typing

To determine the blood group of a person, a suspension of his RBC and testing antisera are required. Suspension of RBC is prepared by mixing blood drops with isotonic saline (0.9%).

Test sera are:

1. Antiserum A, containing anti-A or α-antibody.
2. Antiserum B, containing anti-B or β-antibody.

Procedure

1. One drop of antiserum A is placed on one end of a glass slide (or a tile) and one drop of antiserum B on the other end.
2. One drop of RBC suspension is mixed with each antiserum. The slide is slightly rocked for 2 minutes. The presence or absence of agglutination is observed by naked eyes and if necessary, it is confirmed by using microscope.
3. Presence of agglutination is confirmed by the presence of thick masses (clumping) of RBCs
4. Absence of agglutination is confirmed by clear mixture with dispersed RBCs.

Results

1. *If agglutination occurs with antiserum A:* The antiserum A contains α-antibody. The agglutination occurs if the RBC contains A antigen. So, the blood group is A (Fig. 21.1).
2. *If agglutination occurs with antiserum B:* The antiserum B contains β-antibody. The agglutination occurs if the RBC contains B antigen. So, the blood group is B.
3. *If agglutination occurs with both antisera A and B:* The RBC contains both A and B antigens to cause agglutination. And, the blood group is AB.
4. If agglutination does not occur either with antiserum A or antiserum B: The agglutination does not occur because RBC does not contain any antigen. The blood group is O.

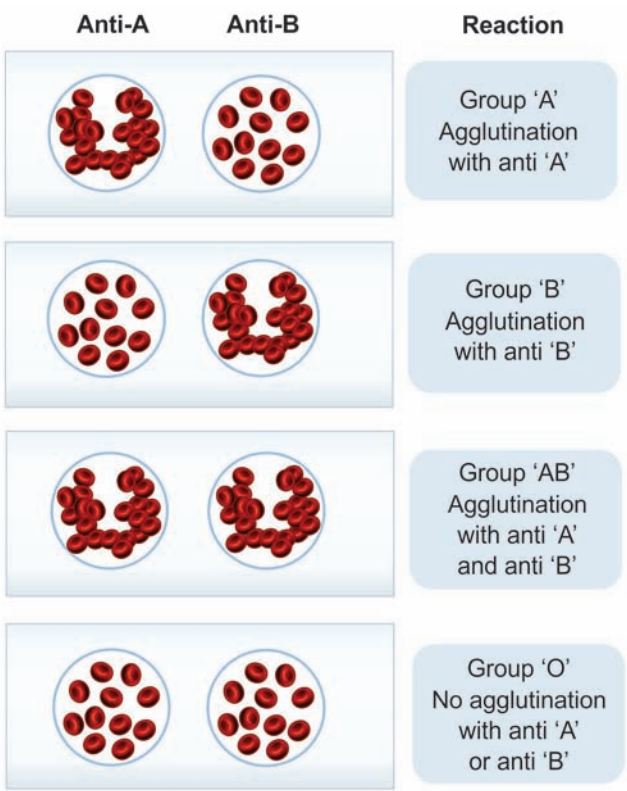


FIGURE 21.1: Determination of blood group

■ IMPORTANCE OF ABO GROUPS IN BLOOD TRANSFUSION

During blood transfusion, only compatible blood must be used. The one who gives blood is called the **'donor'** and the one who receives the blood is called **'recipient'.**

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

Thus, 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'.**

Plasma of AB group blood has no antibody. This does not cause agglutination of RBC from any other group of blood. 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

Blood matching (typing) is a laboratory test done to determine the blood group of a person. When the person needs blood transfusion, another test called cross-matching is done after the blood is typed. It is 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. Cross-matching is always done before blood transfusion. If agglutination of RBCs from a donor occurs during cross-matching, the blood from that person is not used for transfusion.

Matching = Recipient's RBC + Test sera.
Cross-matching = Recipient's serum + Donor's RBC.

■ INHERITANCE OF ABO AGGLUTINOGENS AND AGGLUTININS

Blood group of a person depends upon the two genes inherited from each parent. Gene A and gene B are dominant by themselves and gene O is recessive. Inheritance of blood group is represented schematically as given in Table 21.3.

Agglutinogens appear during the 6th month of fetal life. Concentration at birth is 1/5 of the adult concentration. It rises to the adult level at puberty. Agglutinogens are present not only in RBCs but also present in many organs like salivary glands, pancreas, kidney, liver, lungs, etc. The A and B agglutinogens are inherited from the parents as Mendelian phenotypes.

Agglutinin α or β is not produced during fetal life. It starts appearing only 2 or 3 months after birth. Agglutinin is produced in response to A or B agglutinogens which enter the body through respiratory system or digestive system along with bacteria.

Agglutinins are the gamma-globulins which are mainly IgG and IgM immunoglobulins.

■ TRANSFUSION REACTIONS DUE TO ABO INCOMPATIBILITY

Transfusion reactions are the adverse reactions in the body, which occur due to transfusion error that involves transfusion of incompatible **(mismatched)** blood. The 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. So, if the donor's plasma contains agglutinins against recipient's RBC, agglutination does not occur because these antibodies are diluted in the recipient's blood.

But, if recipient's plasma contains agglutinins against donor's RBCs, the immune system launches a response

TABLE 21.3: Inheritance of ABO group

Gene from parents	Group of offspring	Genotype
A + A A + O	A	AA or AO
B + B B + O	B	BB or BO
A + B O + O	AB O	AB OO

against the new blood cells. Donor RBCs are agglutinated resulting in transfusion reactions.

Severity of Transfusion Reactions

Severity of transfusion reactions varies from mild (fever and chills) to severe (acute kidney failure, shock and death). Severity depends upon the amount of blood transfused, type of reaction and general health of the patient.

Cause for Transfusion Reactions

Transfusion of incompatible blood produces hemolytic reactions. The recipient's antibodies (IgG or IgM) adhere to the donor RBCs, which are agglutinated and destroyed. Large amount of free hemoglobin is liberated into plasma. This leads to transfusion reactions.

Signs and Symptoms of Transfusion Reactions

Non-hemolytic transfusion reaction

Non-hemolytic transfusion reaction develops within a few minutes to hours after the commencement of blood transfusion. Common symptoms are fever, difficulty in breathing and itching.

Hemolytic transfusion reaction

Hemolytic transfusion reaction may be acute or delayed. The acute hemolytic reaction occurs within few minutes of transfusion. It 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. Some patients may develop pulmonary edema and congestive cardiac failure.

Delayed hemolytic reaction occurs from 1 to 5 days after transfusion. The hemolysis of RBCs results in release of large amount of hemoglobin into the plasma. This leads to the following complications.

1. *Jaundice*

Normally, hemoglobin released from destroyed RBC is degraded and bilirubin is formed from it. When the serum bilirubin level increases above 2 mg/dL, jaundice occurs (Chapter 40).

2. *Cardiac Shock*

Simultaneously, hemoglobin released into the plasma increases the viscosity of blood. This increases the workload on the heart leading to **heart failure.** Moreover, toxic substances released from hemolyzed cells reduce the arterial blood pressure and develop circulatory shock (Fig. 21.2).

3. *Renal Shutdown*

Dysfunction of kidneys is called renal shutdown. The toxic substances from hemolyzed cells cause constriction of blood vessels in kidney. In addition, the toxic substances along with free hemoglobin are filtered through glomerular membrane and enter renal tubules. Because of poor rate of reabsorption from renal tubules, all these substances precipitate and obstruct the renal tubule. This suddenly stops the formation of urine (anuria).

If not treated with artificial kidney, the person dies within 10 to 12 days because of jaundice, circulatory shock and more specifically due to renal shutdown and anuria.

Rh FACTOR

Rh factor is an antigen present in RBC. This antigen was discovered by Landsteiner and Wiener. It was first discovered 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. Percentage of Rh positive people is more among black people.

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 (Fig. 21.3). Rhesus negative occurs only with complete absence of D (i.e. with homozygous dd).

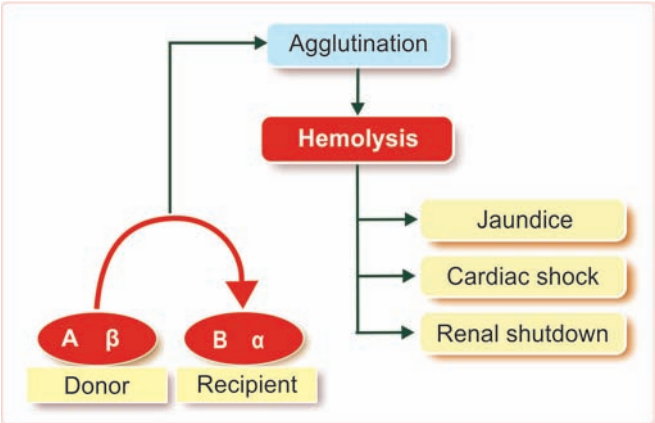


FIGURE 21.2: Complications of mismatched blood transfusion

TRANSFUSION REACTIONS DUE TO Rh INCOMPATIBILITY

When a Rh negative person receives Rh positive blood for the first time, he is not affected much, since the reactions do not occur immediately. But, the 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. But, it is usually mild and does not affect the recipient. However, antibodies developed in the recipient remain in the body forever. So, when this person receives Rh positive blood for the second time, the donor RBCs are agglutinated and severe transfusion reactions occur immediately (Fig. 21.4). These reactions are similar to the reactions of ABO incompatibility (see above).

HEMOLYTIC DISEASE OF FETUS AND NEWBORN – ERYTHROBLASTOSIS FETALIS

Hemolytic disease is the disease in fetus and newborn, characterized by abnormal hemolysis of RBCs. It is due to Rh incompatibility, i.e. the difference between the Rh blood group of the mother and baby. Hemolytic disease leads to erythroblastosis fetalis.

Erythroblastosis fetalis is a disorder in fetus, characterized by the presence of erythroblasts in blood. 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.**

However, at the time of parturition (delivery of the child), 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.

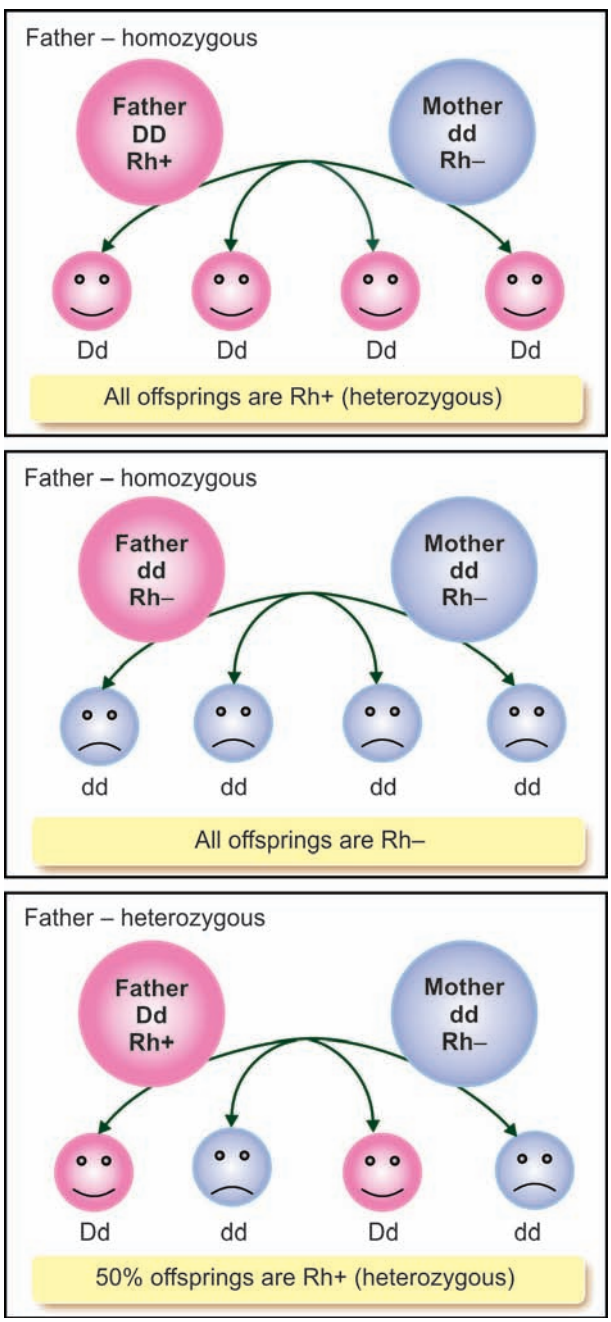


FIGURE 21.3: Inheritance of Rh antigen

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, viz.

1. Severe anemia
2. Hydrops fetalis
3. Kernicterus.

1. *Severe Anemia*

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

2. *Hydrops Fetalis*

Hydrops fetails is a serious condition in fetus, characterized by edema. Severe hemolysis results in the development of edema, enlargement of liver and spleen and cardiac failure. When this condition becomes more severe, it may lead to **intrauterine death** of fetus.

3. *Kernicterus*

Kernicterus is the form of **brain damage** in infants caused by severe jaundice. If the baby survives anemia in erythroblastosis fetalis (see above), then kernicterus develops because of high bilirubin content.

The blood-brain barrier is not well developed in infants as in the adults (Chapter 163). So, the bilirubin enters the brain and causes permanent brain damage. Most commonly affected parts of brain are basal ganglia, hippocampus, geniculate bodies, cerebellum and cranial nerve nuclei. The features of this disease are:

i. When brain damage starts, the babies become lethargic and sleepy. They have high-pitched cry, hypotonia and arching of head backwards.
ii. As the disease progresses, they develop hypertonia and opisthotonus (Chapter 155).
iii. Advanced signs of the disease are inability to suckle milk, irritability and crying, bicycling movements, choreoathetosis (Chapter 151), spasticity, (Chapter 34) seizures (Chapter 161), fever and coma.

Prevention or treatment for erythroblastosis fetalis

i. If mother is found to be Rh negative and fetus is Rh positive, anti D (antibody against D antigen) should be administered to the mother at 28th and 34th weeks of gestation, as prophylactic measure. If Rh negative mother delivers Rh positive baby, then anti D should be administered to the mother within 48 hours of delivery. This develops passive immunity and prevents the formation of Rh antibodies in mother's blood. So, the hemolytic disease of newborn does not occur in a subsequent pregnancy.
ii. If the baby is born with erythroblastosis fetalis, the treatment is given by means of exchange transfusion (Chapter 22). Rh negative blood is transfused into the infant, replacing infant's own Rh positive blood. It will now take at least 6 months for the infant's new Rh positive blood to replace the transfused Rh negative blood. By this time, all the molecules of Rh antibody derived from the mother get destroyed.

■ OTHER BLOOD GROUPS

In addition to ABO blood groups and Rh factor, many more blood group systems were found, such as Lewis blood group and MNS blood groups. However, these systems of blood groups do not have much clinical importance.

■ LEWIS BLOOD GROUP

Lewis blood group was 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 body

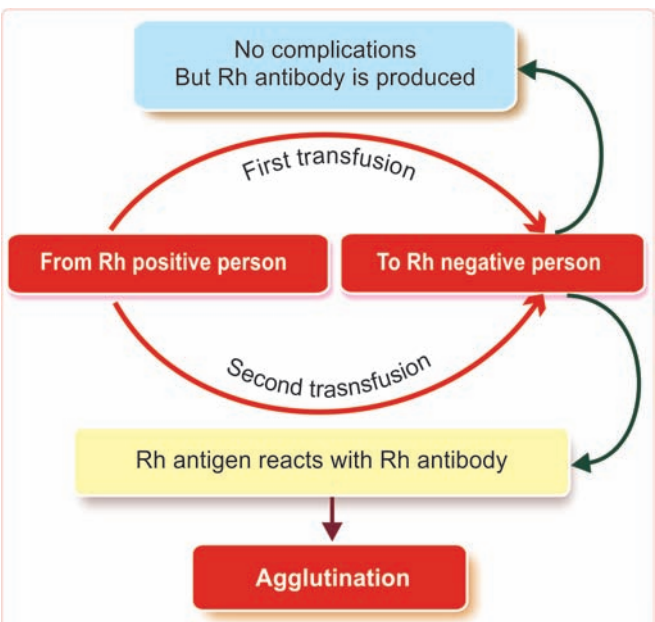


FIGURE 21.4: Rh incompatibility

fluids such as saliva, gastric juice, etc. The antigens, which are named Lewis antigens are formed in the tissues, released in the body secretions and then absorbed by the RBC membrane. Because of secretion along with body secretions, these antigens are also known as 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. However, these blood groups rarely cause any trouble like hemolysis following transfusion.

OTHER BLOOD GROUPS

Other blood groups include:

i. Auberger groups
ii. Diego group
iii. Bombay group
iv. Duffy group
v. Lutheran group
vi. P group
vii. Kell group
viii. I group
ix. Kidd group
x. Sulter Xg group.

IMPORTANCE OF KNOWING BLOOD GROUP

Nowadays, knowledge of blood group is very essential medically, socially and judicially. The importance of knowing blood group is:

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. It general among the couples, knowledge of blood groups helps to 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.

Blood Transfusion

Chapter 22

- INTRODUCTION
- PRECAUTIONS
- HAZARDS OF BLOOD TRANSFUSION
- BLOOD SUBSTITUTES
- EXCHANGE TRANSFUSION
- AUTOLOGOUS BLOOD TRANSFUSION

INTRODUCTION

Blood transfusion is the process of transferring blood or blood components from one person (the donor) into the bloodstream of another person (the recipient). Transfusion is done as a **life-saving procedure** to replace blood cells or blood products lost through bleeding.

CONDITIONS WHEN BLOOD TRANSFUSION IS NECESSARY

Blood transfusion is essential in the following conditions:

1. Anemia
2. Hemorrhage
3. Trauma
4. Burns
5. Surgery.

PRECAUTIONS

Certain precautions must be followed before and during the transfusion of blood to a patient.

PRECAUTIONS TO BE TAKEN BEFORE THE TRANSFUSION OF BLOOD

1. Donor must be healthy, without any diseases like:
   a. Sexually transmitted diseases such as syphilis
   b. Diseases caused by virus like 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

Hazards of blood transfusion are of four types:

1. Reactions due to mismatched (incompatible) blood transfusion – transfusion reactions
2. Reactions due to massive blood transfusion
3. Reactions due to faulty techniques during blood transfusion
4. Transmission of infections.

REACTIONS DUE TO MISMATCHED BLOOD TRANSFUSION – TRANSFUSION REACTIONS

Transfusion reactions due to ABO incompatibility and Rh incompatibility are explained in the Chapter 21.

REACTIONS DUE TO MASSIVE BLOOD TRANSFUSION

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

i. Circulatory shock, particularly in patients suffering from chronic anemia, cardiac diseases or renal diseases
ii. Hyperkalemia due to increased potassium concentration in stored blood
iii. Hypocalcemia leading to tetany due to massive transfusion of citrated blood
iv. Hemosiderosis (increased deposition of ion in the form of **hemosiderin,** in organs such as endocrine glands, heart and liver) due to iron overload after repeated transfusions.

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

Blood transfusion without precaution leads to transmission of blood-borne infections such as:

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

Fluids infused into the body instead of whole blood are known as blood substitutes.

Commonly used blood substitutes are:

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

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

PROCEDURE

Procedure involves both removal and replacement of affected blood in stages. Exchange transfusion is carried out in short cycles of few minutes duration, as follows:

1. 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.
2. Equal quantity of fresh, prewarmed blood or plasma is infused through intravenous catheter. This is carried out for few minutes.
3. Catheter is left in place and the transfusion is repeated within few hours.
4. This procedure is continued till the whole or predetermined volume of blood is exchanged.

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

Autologous blood transfusion is the collection and reinfusion of patient's own blood. It is also called **self blood donation.** The conventional transfusion of blood that is collected from persons other than the patient is called **allogeneic** or **heterologous blood transfusion.**

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.

This type of blood transfusion prevents the transmission of viruses such as HIV or hepatitis B. It also eliminates transfusion reactions.

Blood Volume

Chapter 23

- NORMAL BLOOD VOLUME
- VARIATIONS
- MEASUREMENT
- REGULATION
- APPLIED PHYSIOLOGY

NORMAL BLOOD VOLUME

Total amount of blood present in the circulatory system, blood reservoirs, organs and tissues together constitute blood volume. In a normal young healthy adult male weighing about 70 kg, the blood volume is about 5 L. It is about 7% of total body weight. It ranges between 6% and 8% of body weight. In relation to body surface area, blood volume is 2.8 to 3.1 L/sq M.

VARIATIONS IN BLOOD VOLUME

PHYSIOLOGICAL VARIATIONS

1. *Age*

Absolute blood volume is less at birth and it increases steadily as the age advances. However, at birth, the blood volume is more when compared to body weight and less when compared to the body surface area. At birth and at 24 hours after birth, the blood volume is about 80 mL/kg body weight. At the end of 6 months, it increases to about 86 mL/kg. At the end of one year, it is about 80 mL/kg. It remains at this level until 6 years of age. At 10 years, it is about 75 mL/kg. At the age of 15 years, the blood volume is about 70 mL/kg body weight, which is almost the adult volume.

2. *Sex*

In males, the blood volume is slightly more than in females because of the increase in erythropoietic activity, body weight and surface area of the body. In females, it is slightly less because of loss of blood through menstruation, more fats and less body surface area.

3. *Surface Area of the Body*

Blood volume is directly proportional to the surface area of the body.

4. *Body Weight*

Blood volume is directly proportional to body weight.

5. *Atmospheric Temperature*

Exposure to cold environment reduces the blood volume and exposure to warm environment increases the blood volume.

6. *Pregnancy*

During early stage of pregnancy, blood volume increases by 20% to 30% due to the increased fetal mass and sodium retention. However, it reduces in later stages.

7. *Exercise*

Exercise increases the blood volume by increasing the release of erythropoietin and production of more RBCs.

8. *Posture*

Standing (erect posture) for long time reduces the blood volume by about 15%. It is because the pooling of blood

in lower limbs while standing increases the hydrostatic pressure. This pressure pushes fluid from blood vessels into the tissue spaces; so blood volume decreases.

9. *High Altitude*

Blood volume increases in high altitude. It is because of hypoxia, which stimulates the secretion of erythropoietin. It induces the production of more RBCs, which leads to increase in blood volume.

10. *Emotion*

Excitement increases blood volume. It is because of sympathetic stimulation, which causes splenic contraction and release of stored blood into circulation.

■ PATHOLOGICAL VARIATIONS

Abnormal increase in blood volume is called hypervolemia and abnormal decrease in blood volume is called hypovolemia. Refer applied physiology for details.

■ MEASUREMENT OF BLOOD VOLUME

Blood volume is measured by two methods, direct method and indirect method.

■ DIRECT METHOD

Direct method is employed only in animals because it involves sacrificing the life. The animal is killed by decapitation and the blood is collected. The blood vessels and the tissues are washed thoroughly with known quantity of water or saline. And, this is added to the blood collected already. The total volume is measured. From this, the volume of water or saline used for washing the tissues is deducted to obtain the volume of the blood in the animal.

This method was first employed by Welcker, in 1854. Later, B'Schoff employed the same method on decapitated criminals, to determine the blood volume in human beings.

■ INDIRECT METHOD

Indirect method is advantageous because, it is used to measure the blood volume in human beings without causing any discomfort or any difficulty to the subject.

Measurement of total blood volume involves two steps:

1. Determination of plasma volume
2. Determination of blood cell volume.

Determination of Plasma Volume

Plasma volume is determined by two methods:

i. Indicator or dye dilution technique
ii. Radioisotope method.

i. Determination of plasma volume by indicator or dye dilution technique

Principles and other details of this technique are explained in Chapter 6. The dye which is used to measure plasma volume is Evans blue or T-1824.

Procedure

10 mL of blood is drawn from the subject. This is divided into 2 equal portions. To one part, a known quantity of the dye is added. This is used as control sample in the procedure. The other portion is used to determine the hematocrit value.

Then, a known volume of the dye is injected intravenously. After 10 minutes, a sample of blood is drawn. Then, another 4 samples of blood are collected at the interval of 10 minutes. All the 5 samples are centrifuged and plasma is separated from the samples. In each sample of plasma, the concentration of the dye is measured by colorimetric method and the average concentration is found.

The subject's urine is collected and the amount of dye excreted in the urine is measured.

Calculation

Plasma volume is determined by using the formula,

$$\text{Volume} = \frac{\text{Amount of dye injected} - \text{Amount excreted}}{\text{Average concentration of dye in plasma}}$$

ii. Determination of plasma volume by radioisotope method

Radioactive iodine (^{131}I or ^{132}I) is injected. After sometime, a sample of blood is collected. The radioactivity is determined by using appropriate counter. From this, the plasma volume is determined.

Determination of Blood Cell Volume

Blood cell volume is determined by two methods:

i. By hematocrit value
ii. By radioisotope method.

i. Determination of blood cell volume by hematocrit value

This is usually done by centrifuging the blood and measuring the packed cell volume (Chapter 13). Packed

cell volume (PCV) is expressed in percentage. If this is deducted from 100, the percentage of plasma is known. From this and from the volume of plasma, the amount of total blood is calculated by using the formula.

$$\text{Blood volume} = \frac{100 \times \text{Amount of plasma}}{100 - \text{PCV}}$$

ii. Determination of blood cell volume by radioisotope method

Volume of blood cell is measured by radioisotope method also. Radioactive chromium (Cr^{52}) is added with heparinized blood and incubated for 2 hours at 37°C. During this time, all the red cells in the blood are 'tagged' with Cr^{52}. Then, this is injected intravenously. After giving sufficient time for mixing, a sample of blood is drawn. Hematocrit value is determined by measuring the radioactivity in the blood sample. Radioactive iron (Fe^{59}, Fe^{55}) or radioactive phosphorus (P^{32}) is also used for determining the hematocrit value.

■ REGULATION OF BLOOD VOLUME

Various mechanisms are involved in the regulation of blood volume. The important ones are the renal and hormonal mechanisms. Hypothalamus plays a vital role in the activation of these two mechanisms during the regulation of blood volume.

When blood volume increases, hypothalamus causes loss of fluid from the body. When the blood volume reduces, hypothalamus induces retention of water. Hypothalamus regulates the extracellular fluid (ECF) volume and blood volume by acting mainly through kidneys and sweat glands and by inducing thirst. This function of hypothalamus is described in Chapter 149.

Hormones also are involved in the regulation of blood volume through the regulation of ECF volume. Hormones which are involved in the maintenance of ECF volume are:

1. Antidiuretic hormone (Chapter 66)
2. Aldosterone (Chapter 70)
3. Cortisol (Chapter 70)
4. Atrial natriuretic peptide (Chapter 72).

■ APPLIED PHYSIOLOGY

■ HYPERVOLEMIA

Increase in blood volume is called hypervolemia. It occurs in the following pathological conditions:

1. *Hyperthyroidism*

Blood volume increases because thyroxine increases the RBC count and plasma volume.

2. *Hyperaldosteronism*

In hyperaldosteronism, excess retention of sodium and water leads to increase in the ECF volume and blood volume.

3. *Cirrhosis of the Liver*

In this condition, the blood volume is more because of increase in the plasma volume.

4. *Congestive Cardiac Failure*

Retention of sodium occurs in this condition. Sodium retention leads to water retention and increase in ECF volume, plasma volume and blood volume.

■ HYPOVOLEMIA

Decrease in blood volume is called hypovolemia. It occurs in the following pathological conditions:

1. *Hemorrhage or Blood Loss*

Acute hemorrhage occurs due to cuts or accidents. The chronic hemorrhage occurs in ulcers, bleeding piles and excessive uterine bleeding in females during menstruation.

2. *Fluid Loss*

Fluid loss occurs in burns, vomiting, diarrhea, excessive sweating and polyuria.

3. *Hemolysis*

Excessive destruction of RBCs occurs because of the presence of various hemolytic agents and other factors such as, hypotonic solution, snake venom, acidity or alkalinity, mismatched blood transfusion, hemorrhagic smallpox and measles.

4. *Anemia*

Blood volume decreases in various types of anemia because of decrease in RBC count. In some cases, the quantity (volume) of blood remains the same but the quality of the blood alters. Blood becomes dilute (hemodilution) because of the entrance of fluid into the blood vessel.

5. *Hypothyroidism*

During hypothyroidism, the blood volume is decreased because of reduction in plasma volume and RBC count.

Reticuloendothelial System and Tissue Macrophage

Chapter 24

DEFINITION AND DISTRIBUTION

RETICULOENDOTHELIAL SYSTEM OR MACROPHAGE SYSTEM

Reticuloendothelial system or tissue macrophage system is the system of primitive phagocytic cells, which play an important role in defense mechanism of the body. The reticuloendothelial cells are found in the following structures:

1. Endothelial lining of vascular and lymph channels.
2. Connective tissue and some organs like spleen, liver, lungs, lymph nodes, bone marrow, etc.

Reticular cells in these tissues form the tissue macrophage system.

MACROPHAGE

Macrophage is a large phagocytic cell, derived from monocyte (Chapter 17).

CLASSIFICATION OF RETICULOENDOTHELIAL CELLS

Reticuloendothelial cells are classified into two types:

1. Fixed reticuloendothelial cells or tissue macrophages.
2. Wandering reticuloendothelial cells.

FIXED RETICULOENDOTHELIAL CELLS – TISSUE MACROPHAGES

Fixed reticuloendothelial cells are also called the tissue macrophages or fixed histiocytes because, these cells are usually located in the tissues.

Tissue macrophages are present in the following areas:

1. *Connective Tissue*

Reticuloendothelial cells in connective tissues and in serous membranes like pleura, omentum and mesentery are called the fixed macrophages of connective tissue.

2. *Endothelium of Blood Sinusoid*

Endothelium of the blood sinusoid in bone marrow, liver, spleen, lymph nodes, adrenal glands and pituitary glands also contain fixed cells. Kupffer cells present in liver belong to this category.

3. *Reticulum*

Reticulum of spleen, lymph node and bone marrow contain fixed reticuloendothelial cells.

4. *Central Nervous System*

Meningocytes of meninges and microglia form the tissue macrophages of brain.

5. *Lungs*

Tissue macrophages are present in the alveoli of lungs.

6. *Subcutaneous Tissue*

Fixed reticuloendothelial cells are present in subcutaneous tissue also.

WANDERING RETICULOENDOTHELIAL CELLS AND TISSUE MACROPHAGES

Wandering reticuloendothelial cells are also called free histiocytes. There are two types of wandering reticuloendothelial cells:

1. *Free Histiocytes of Blood*

i. Neutrophils
ii. Monocytes, which become macrophages and migrate to the site of injury or infection.

2. *Free Histiocytes of Solid Tissue*

During emergency, the fixed histiocytes from connective tissue and other organs become wandering cells and enter the circulation.

FUNCTIONS OF RETICULOENDOTHELIAL SYSTEM

Reticuloendothelial system plays an important role in the defense mechanism of the body. Most of the functions of the reticuloendothelial system are carried out by the tissue macrophages.

Functions of tissue macrophages:

1. *Phagocytic Function*

Macrophages are the large phagocytic cells, which play an important role in defense of the body by phagocytosis.

When any foreign body invades, macrophages ingest them by phagocytosis and liberate the antigenic products of the organism. The antigens activate the helper T lymphocytes and B lymphocytes. (Refer Chapter 17 for details).

Lysosomes of macrophages contain proteolytic enzymes and lipases, which digest the bacteria and other foreign bodies.

2. *Secretion of Bactericidal Agents*

Tissue macrophages secrete many bactericidal agents which kill the bacteria. The important bactericidal agents of macrophages are the **oxidants.** An oxidant is a substance that oxidizes another substance.

Oxidants secreted by macrophages are:

i. Superoxide (O_2^-)
ii. Hydrogen peroxide (H_2O_2)
iii. Hydroxyl ions (OH^-).

These oxidants are the most potent bactericidal agents. So, even the bacteria which cannot be digested by lysosomal enzymes are degraded by these oxidants.

3. *Secretion of Interleukins*

Tissue macrophages secrete the following interleukins, which help in immunity:

i. Interleukin-1 (IL-1): Accelerates the maturation and proliferation of specific B lymphocytes and T lymphocytes.
ii. Interleukin-6 (IL-6): Causes the growth of B lymphocytes and production of antibodies.
iii. Interleukin-12 (IL-12): Influences the T helper cells.

4. *Secretion of Tumor Necrosis Factors*

Two types of tumor necrosis factors (TNF) are secreted by tissue macrophages:

i. TNF-α: Causes necrosis of tumor and activates the immune responses in the body
ii. TNF-β: Stimulates immune system and vascular response, in addition to causing necrosis of tumor.

5. *Secretion of Transforming Growth Factor*

Tissue macrophages secrete transforming growth factor, which plays an important role in preventing rejection of transplanted tissues or organs by immunosuppression.

6. *Secretion of Colony-stimulation Factor*

Colony-stimulation factor (CSF) secreted by macrophages is M-CSF. It accelerates the growth of granulocytes, monocytes and macrophages.

7. *Secretion of Platelet-derived Growth Factor*

Tissue macrophages secrete the platelet-derived growth factor (PDGF), which accelerates repair of damaged blood vessel and wound healing.

8. *Removal of Carbon Particles and Silicon*

Macrophages ingest the substances like carbon dust particles and silicon, which enter the body.

9. *Destruction of Senile RBC*

Reticuloendothelial cells, particularly those in spleen destroy the senile RBCs and release hemoglobin (Chapter 9).

10. *Destruction of Hemoglobin*

Hemoglobin released from broken senile RBCs is degraded by the reticuloendothelial cells (Chapter 11).

Chapter 25

Spleen

STRUCTURE OF SPLEEN

Spleen is the largest **lymphoid organ** in the body and it is highly vascular. It is situated in left hypochondrial region, i.e. upper left part of the abdomen, behind the stomach and just below the diaphragm. About 10% of people have one or more **accessory spleens** which are situated near the main spleen.

Spleen is covered by an outer serous coat and an inner fibromuscular capsule. From the capsule, the trabeculae and trabecular network arise. All the three structures, viz. capsule, trabeculae and trabecular network contain collagen fibers, elastic fibers, smooth muscle fibers and reticular cells. The parenchyma of spleen is divided into red and white pulp.

RED PULP

Red pulp consists of venous sinus and cords of structures like blood cells, macrophages and mesenchymal cells.

WHITE PULP

The structure of white pulp is similar to that of lymphoid tissue. It has a central artery, which is surrounded by splenic corpuscles or **Malpighian corpuscles.** These corpuscles are formed by lymphatic sheath containing lymphocytes and macrophages.

FUNCTIONS OF SPLEEN

1. FORMATION OF BLOOD CELLS

Spleen plays an important role in the hemopoietic function in embryo. During the hepatic stage, spleen produces blood cells along with liver. In myeloid stage, it produces the blood cells along with liver and bone marrow.

2. DESTRUCTION OF BLOOD CELLS

Older RBCs, lymphocytes and thrombocytes are destroyed in the spleen. When the RBCs become old (120 days), the cell membrane becomes more fragile. Diameter of most of the capillaries is less or equal to that of RBC. The fragile old cells are destroyed while trying to squeeze through the capillaries because, these cells cannot withstand the stress of squeezing.

Destruction occurs mostly in the capillaries of spleen because the splenic capillaries have a thin lumen. So, the spleen is known as ‘graveyard of RBCs’.

3. BLOOD RESERVOIR FUNCTION

In animals, spleen stores large amount of blood. However, this function is not significant in humans. But, a large number of RBCs are stored in spleen. The RBCs

are released from spleen into circulation during the emergency conditions like hypoxia and hemorrhage.

■ 4. ROLE IN DEFENSE OF BODY

Spleen filters the blood by removing the microorganisms. The macrophages in splenic pulp destroy the microorganisms and other foreign bodies by phagocytosis. Spleen contains about 25% of T lymphocytes and 15% of B lymphocytes and forms the site of antibody production.

■ APPLIED PHYSIOLOGY

■ SPLENOMEGALY AND HYPERSPLENISM

Splenomegaly refers to enlargement of spleen. Increase in the activities of spleen is called hypersplenism. Some diseases cause splenomegaly resulting in hypersplenism.

Diseases which cause splenomegaly:

1. Infectious diseases such as malaria, typhoid and tuberculosis
2. Inflammatory diseases like rheumatoid arthritis
3. Pernicious anemia
4. Liver diseases
5. Hematological disorders like spherocytosis
6. Cysts in spleen
7. Hodgkin's disease
8. Glandular fever.

Effects of Splenomegaly

1. Hemolysis resulting in anemia
2. Leukopenia
3. Thrombocytopenia
4. Increase in plasma volume.

■ HYPOSPLENISM AND ASPLENIA

Hyposplenism or hyposplenia refers to diminished functioning of spleen. It occurs after partial removal of spleen due to trauma or cyst. Asplenia means absence of spleen. Functional asplenia means normal functions of spleen. It occurs in the following conditions:

1. Congenital absence of spleen function (congenital asplenia).
2. Acquired through surgical removal of spleen (splenectomy).
3. Acquired through some diseases, which destroy spleen to such an extent that it becomes non-functional. This process is called autosplenectomy. The diseases which cause autosplenectomy are sickle cell anemia and spherocytosis.

Lymphatic System and Lymph

Chapter 26

LYMPHATIC SYSTEM

Lymphatic system is a closed system of lymph channels or lymph vessels, through which lymph flows. It is a **one-way system** and allows the lymph flow from tissue spaces toward the blood.

ORGANIZATION OF LYMPHATIC SYSTEM

Lymphatic system arises from tissue spaces as a meshwork of delicate vessels. These vessels are called **lymph capillaries.**

Lymph capillaries start from tissue spaces as enlarged blind-ended terminals called **capillary bulbs.** These bulbs contain valves, which allow flow of lymph in only one direction. There are some muscle fibers around these bulbs. These muscle fibers cause contraction of bulbs so that, lymph is pushed through the vessels.

Lymph capillaries are lined by endothelial cells. Capillaries unite to form large lymphatic vessels. Lymphatic vessels become larger and larger because of the joining of many tributaries along their course.

The structure of lymph capillaries is slightly different from that of the blood capillaries. Lymph capillaries are more porous and the cells lie overlapping on one another. This allows the fluid to move into the lymph capillaries and not in the opposite direction.

DRAINAGE OF LYMPHATIC SYSTEM

Larger lymph vessels ultimately form the **right lymphatic duct** and **thoracic duct.** Right lymphatic duct opens into right subclavian vein and the thoracic duct opens into left subclavian vein. Thoracic duct drains the lymph from more than two third of the tissue spaces in the body (Fig. 26.1).

SITUATION OF LYMPH VESSELS

Lymph vessels are situated in the following regions:

1. Deeper layers of skin
2. Subcutaneous tissues
3. Diaphragm
4. Wall of abdominal cavity
5. Omentum
6. Linings of respiratory tract except alveoli
7. Linings of digestive tract
8. Linings of urinary tract

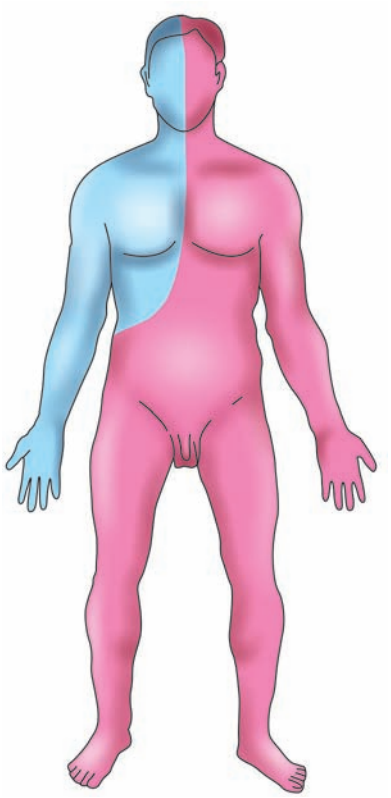


FIGURE 26.1: Lymph drainage. Blue area = Drained by right lymphatic duct; Pink area = Drained by thoracic duct.

9. Linings of genital tract
10. Liver
11. Heart.

Lymph vessels are not present in the following structures:

1. Superficial layers of skin
2. Central nervous system
3. Cornea
4. Bones
5. Alveoli of lungs.

■ LYMPH NODES

Lymph nodes are small glandular structures located in the course of lymph vessels. The lymph nodes are also called lymph glands or lymphatic nodes.

■ STRUCTUTRE OF LYMPH NODES

Each lymph node constitutes masses of lymphatic tissue, covered by a dense connective tissue capsule. The structures are arranged in three layers namely cortex, paracortex and medulla (Fig. 26.2).

Cortex

Cortex of lymph node consists of primary and secondary **lymphoid follicles.** Primary follicle develops first. When some antigens enter the body and reach the lymph nodes, the cells of primary follicle proliferate. The active proliferation of the cells occurs in a particular area of the follicle called the germinal center. After proliferation of cells, the primary follicles become the secondary follicle. Cortex also contains some B lymphocytes, which are usually aggregated into the primary follicles. Macrophages are also found in the cortex.

Paracortex

Paracortex is in between the cortex and medulla. Paracortex contains T lymphocytes.

Medulla

Medulla contains B and T lymphocytes and macrophages. Blood vessels of lymph node pass through medulla.

Lymphatic Vessels to Lymph Node

Lymph node receives lymph by one or two lymphatic vessels called **afferent vessels.** Afferent vessels divide into small channels. Lymph passes through afferent vessels and small channels and reaches the cortex. It circulates through cortex, paracortex and medulla of the lymph node. From medulla, the lymph leaves the node via one or two **efferent vessels.**

Distribution of Lymph Nodes

Lymph nodes are present along the course of lymphatic vessels in elbow, axilla, knee and groin. Lymph nodes

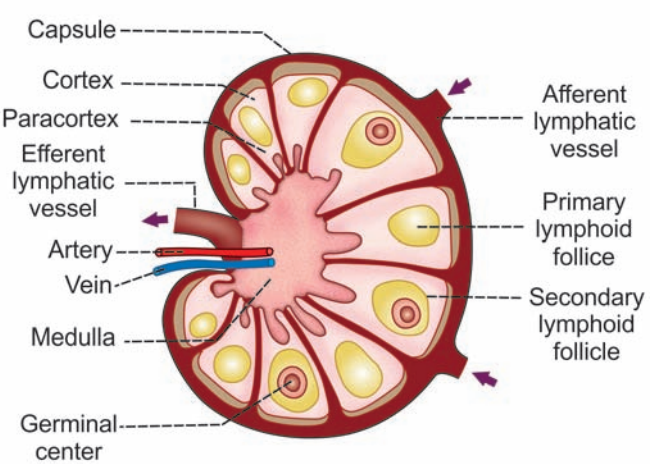


FIGURE 26.2: Structure of a lymph node

are also present in certain points in abdomen, thorax and neck, where many lymph vessels join.

■ FUNCTIONS OF LYMPH NODES

Lymph nodes serve as filters which filter bacteria and toxic substances from the lymph.

Functions of the lymph nodes are:

1. When lymph passes through the lymph nodes, it is filtered, i.e. the water and electrolytes are removed. But, the proteins and lipids are retained in the lymph.
2. Bacteria and other toxic substances are destroyed by macrophages of lymph nodes. Because of this, lymph nodes are called defense barriers.

■ APPLIED PHYSIOLOGY – SWELLING OF LYMPH NODES

During infection or any other processes in a particular region of the body, activities of the lymph nodes in that region increase. This causes swelling of the lymph nodes. Sometimes, the swollen lymph nodes cause pain.

Most common cause of swollen lymph nodes is infection. Lymph nodes situated near an infected area swell immediately. When the body recovers from infection, the lymph nodes restore their original size gradually, in one or two weeks.

Causes for Lymph Node Swelling

1. Skin infection of arm causes swelling of lymph nodes in armpit.
2. **Tonsillitis** or throat infection causes swelling of lymph nodes in neck.
3. Infection of genital organs or leg results in swelling of lymph nodes in groin.
4. Viral infections such as **glandular fever** which affect the whole body cause swelling of lymph nodes in various parts of the body.
5. Cancer in a particular region may spread into the nearby lymph nodes causing the swelling.

   Examples:

   i. **Throat cancer** may spread into lymph nodes in neck.

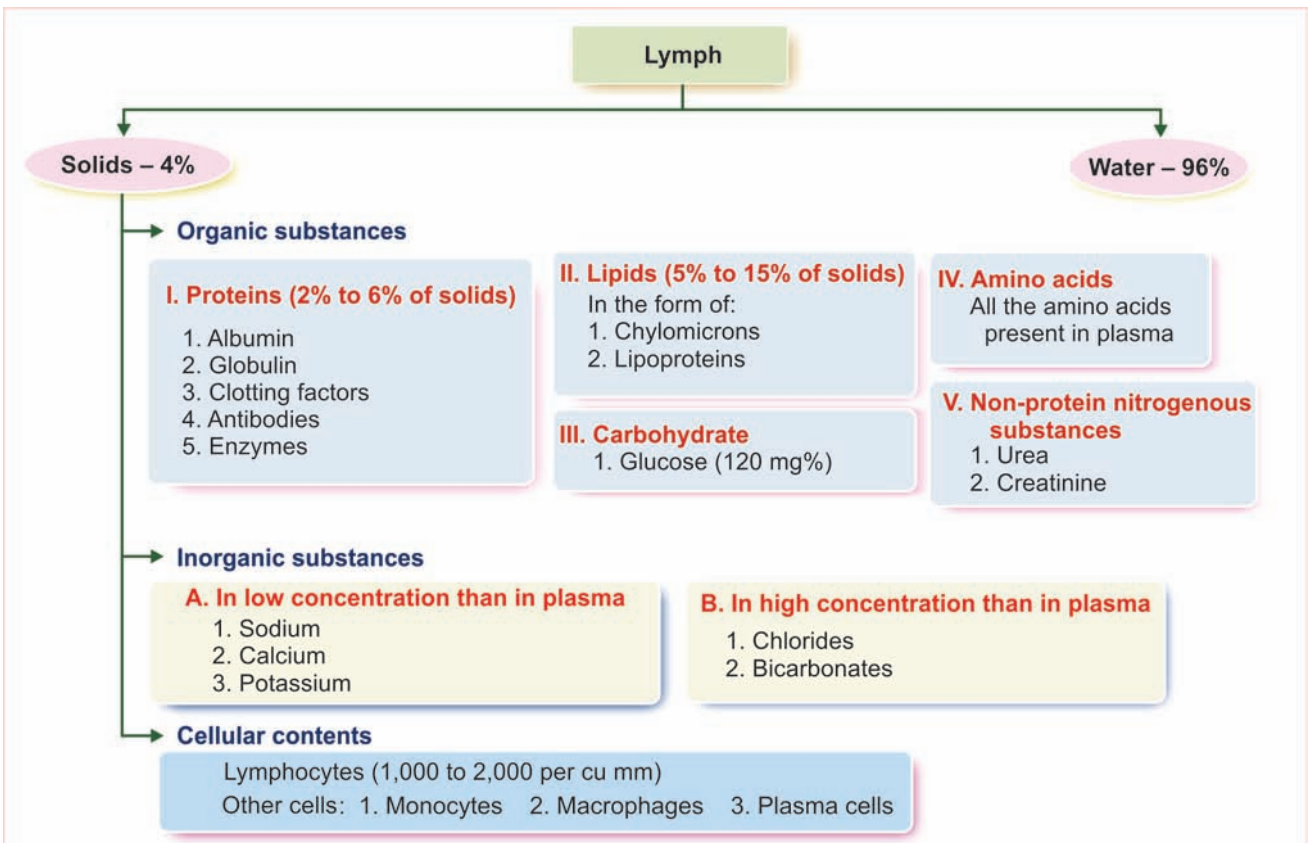


FIGURE 26.3: Composition of lymph

ii. **Lung cancer** may spread into lymph nodes in chest.
iii. **Breast cancer** may spread into lymph nodes in armpit.
iv. **Intestinal cancer** may spread into lymph nodes in abdomen.
v.. **Lymphomas** (cancer of lymphatic system) and leukemia cause swelling of lymph nodes in many parts of the body.

LYMPH

FORMATION OF LYMPH

Lymph is formed from interstitial fluid, due to the permeability of lymph capillaries. When blood passes via blood capillaries in the tissues, 9/10th of fluid passes into venous end of capillaries from the arterial end. And, the remaining 1/10th of the fluid passes into lymph capillaries, which have more permeability than blood capillaries.

So, when lymph passes through lymph capillaries, the composition of lymph is more or less similar to that of interstitial fluid including protein content. Proteins present in the interstitial fluid cannot enter the blood capillaries because of their larger size. So, these proteins enter lymph vessels, which are permeable to large particles also.

Addition of Proteins and Fats

Tissue fluid in liver and gastrointestinal tract contains more protein and lipid substances. So, proteins and lipids enter the lymph vessels of liver and gastrointestinal tract in large quantities. Thus, lymph in larger vessels has more proteins and lipids.

Concentration of Lymph

When the lymph passes through the lymph nodes, it is concentrated because of absorption of water and the electrolytes. However, the proteins and lipids are not absorbed.

RATE OF LYMPH FLOW

About 120 mL of lymph flows into blood per hour. Out of this, about 100 mL/hour flows through thoracic duct and 20 mL/ hour flows through the right lymphatic duct.

Factors Increasing the Flow of Lymph

Flow of lymph is promoted by the increase in:
1. Interstitial fluid pressure.
2. Blood capillary pressure.
3. Surface area of lymph capillary by means of dilatation.
4. Permeability of lymph capillaries.
5. Functional activities of tissues.

COMPOSITION OF LYMPH

Usually, lymph is a clear and colorless fluid. It is formed by 96% water and 4% solids. Some blood cells are also present in lymph (Fig. 26.3).

FUNCTIONS OF LYMPH

1. Important function of lymph is to return the proteins from tissue spaces into blood.
2. It is responsible for redistribution of fluid in the body.
3. Bacteria, toxins and other foreign bodies are removed from tissues via lymph.
4. Lymph flow is responsible for the maintenance of structural and functional integrity of tissue. Obstruction to lymph flow affects various tissues, particularly myocardium, nephrons and hepatic cells.
5. Lymph flow serves as an important route for intestinal fat absorption. This is why lymph appears milky after a fatty meal.
6. It plays an important role in immunity by transport of lymphocytes.

Tissue Fluid and Edema

Chapter 27

- DEFINITION
- FUNCTIONS
- FORMATION
  - FILTRATION
  - REABSORPTION
- APPLIED PHYSIOLOGY – EDEMA
  - DEFINITION
  - TYPES
  - INTRACELLULAR EDEMA
  - EXTRACELLULAR EDEMA
  - PITTING AND NON-PITTING EDEMA

DEFINITION

Tissue fluid is the medium in which cells are bathed. It is otherwise known as **interstitial fluid.** It forms about 20% of extracellular fluid (ECF).

FUNCTIONS OF TISSUE FLUID

Because of the capillary membrane, there is no direct contact between blood and cells. And, tissue fluid acts as a medium for exchange of various substances between the cells and blood in the capillary loop. Oxygen and nutritive substances diffuse from the arterial end of capillary through the tissue fluid and reach the cells. Carbon dioxide and waste materials diffuse from the cells into the venous end of capillary through this fluid.

FORMATION OF TISSUE FLUID

Formation of tissue fluid involves two processes:

1. Filtration.
2. Reabsorption.

FILTRATION

Tissue fluid is formed by the process of filtration. Normally, the blood pressure (also called **hydrostatic pressure**) in arterial end of the capillary is about 30 mm Hg. This hydrostatic pressure is the driving force for filtration of water and other substances from blood into tissue spaces. Along the course of the capillary, the pressure falls gradually and it is about 15 mm Hg at the venous end.

Capillary membrane is not permeable to the large molecules, particularly the plasma proteins. So, these proteins remain in the blood and exert a pressure called **oncotic pressure** or **colloidal osmotic pressure.** It is about 25 mm Hg.

Osmotic pressure is constant throughout the circulatory system and it is an opposing force for the filtration of water and other materials from capillary blood into the tissue space. However, the hydrostatic pressure in the arterial end of the capillary (30 mm Hg) is greater than the osmotic pressure. And, the net filtration pressure of 5 mm Hg is responsible for continuous filtration (Fig. 27.1).

Starling Hypothesis

Determination of net filtration pressure is based on Starling hypothesis. Starling hypothesis states that the net filtration through capillary membrane is proportional to the hydrostatic pressure difference across the

membrane minus the oncotic pressure difference. These pressures are called **Starling forces** (Refer Chapter 52 for more details).

■ REABSORPTION

Fluid filtered at the arterial end of capillaries is reabsorbed back into the blood at the venous end of capillaries. Here also, the pressure gradient plays an important role. At the venous end of capillaries, the hydrostatic pressure is less (15 mm Hg) and the oncotic pressure is more (25 mm Hg). Due to the pressure gradient of 10 mm Hg, the fluid is reabsorbed along with waste materials from the tissue fluid into the capillaries. About 10% of filtered fluid enters the lymphatic vessels.

Thus, the process of filtration at the arterial end of the capillaries helps in the formation of tissue fluids and the process of reabsorption at the venous end helps to maintain the volume of tissue fluid.

■ APPLIED PHYSIOLOGY – EDEMA

■ DEFINITION

Edema is defined as the swelling caused by excessive accumulation of fluid in the tissues. It may be generalized or local. Edema that involves the entire body is called generalized edema. Local edema is the one that occurs is specific areas of the body such as abdomen, lungs and extremities like feet, ankles and legs. Accumulation of fluid may be inside or outside the cell.

■ TYPES OF EDEMA

Edema is classified into two types, depending upon the body fluid compartment where accumulation of excess fluid occurs:

1. Intracellular edema
2. Extracellular edema.

■ INTRACELLULAR EDEMA

Intracellular edema is the accumulation of fluid inside the cell. It occurs because of three reasons:

1. Malnutrition
2. Poor metabolism
3. Inflammation of the tissues.

1. *Edema due to Malnutrition*

Malnutrition occurs because of poor intake of food or poor circulatory system, through which the nutritive substances are supplied. Due to the lack of nutrition, the ionic pumps of the cell membrane are depressed leading to poor exchange of ions. Especially, the sodium ions leaking into the cells cannot be pumped out. Excess

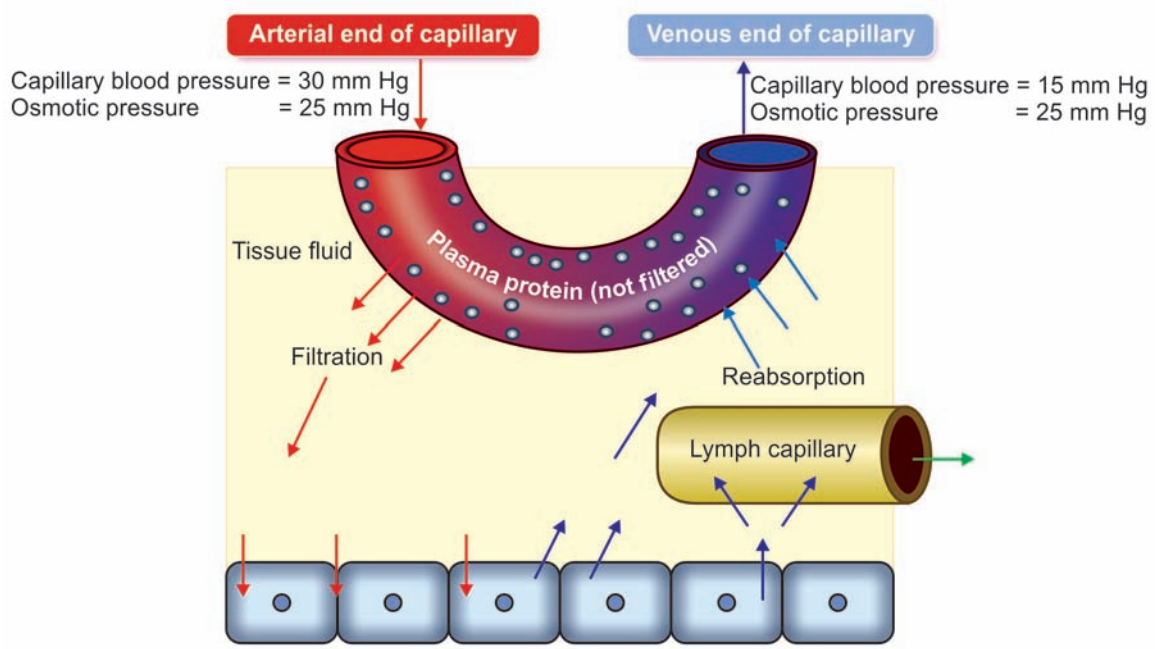


FIGURE 27.1: Formation of tissue fluid. Plasma proteins remain inside the blood capillary as the capillary membrane is not permeable to plasma proteins.

sodium inside the cells causes endosmosis, resulting in intracellular edema.

2. *Edema due to Poor Metabolism*

Poor metabolism is caused by poor blood supply. Poor blood supply leads to lack of oxygen. It results in poor function of cell membrane and edema, as explained above.

3. *Edema due to Inflammation of Tissues*

During inflammation of the tissues, usually the permeability of cell membrane increases. This causes the movement of many ions, including sodium into the cells resulting in endosmosis and intracellular edema.

■ EXTRACELLULAR EDEMA

Extracellular edema is defined as the accumulation of fluid outside the cell.

Causes for extracellular edema

1. Abnormal leakage of fluid from capillaries into interstitial space.
2. Obstruction of lymphatic vessels that prevents fluid return from interstitium to blood.

Conditions which lead to extracellular edema

1. Heart failure.
2. Renal disease.
3. Decreased amount of plasma proteins.
4. Lymphatic obstruction.
5. Increased endothelial permeability.

1. *Edema due to Heart Failure*

Edema occurs in heart failure because of various reasons such as:

i. Failure of heart to pump blood: Failure of the heart to pump blood from veins to arteries increases venous pressure and capillary pressure. This leads to increased capillary permeability and leakage of fluid from blood into interstitial fluid, causing extracellular edema.

ii. Fall in blood pressure during heart failure: It decreases the glomerular filtration rate in the kidneys, resulting in sodium and water retention. So, the volume of blood and body fluid increases. This in turn increases the capillary hydrostatic pressure. These two factors together increase the accumulation of fluid causing extracellular edema.

iii. Low blood supply to kidneys during heart failure: It increases renin secretion, which in turn increases aldosterone secretion. Aldosterone increases the reabsorption of sodium and water from renal tubules into ECF resulting in the development of extracellular edema.

Pulmonary Edema

Pulmonary edema is the accumulation of fluid in pulmonary interstitium. In left heart failure, the blood is easily pumped into pulmonary circulation by right ventricle. However, the blood cannot return from lungs to left side of the heart because of weakness of this side of the heart. This increases pulmonary vascular pressure leading to leakage of fluid from capillaries into pulmonary interstitium. It causes pulmonary edema which can be life threatening.

2. *Edema due to Renal Diseases – Generalized Edema*

In renal disease, the kidneys fail to excrete water and electrolytes particularly sodium, leading to retention of water and electrolytes. So, the fluid leaks from blood into interstitial space causing extracellular edema. Initially, the edema develops in the legs, but later it progresses to the entire body (generalized edema).

3. *Edema due to Decreased Amount of Plasma Proteins*

When the amount of plasma proteins decreases, the colloidal osmotic pressure decreases. Because of this, the permeability of the capillary increases, resulting in increased capillary filtration. So, more amount of water leaks out of the capillary. It accumulates in the tissue spaces resulting in extracellular edema.

Amount of plasma proteins decreases during the conditions like malnutrition, liver diseases, renal diseases, burns and inflammation.

4. *Edema due to Lymphatic Obstruction – Lymphedema*

Lymphedema is the edema caused by lymphatic obstruction. It is common in filariasis. During this disease, the parasitic worms live in the lymphatics and obstruct the drainage of lymph. Accumulation of lymph along with cellular reactions leads to swelling that is very prominent in legs and scrotum. Repeated obstruction of lymphatic drainage in these regions results in fibrosis and development of elephantiasis.

Elephantiasis

Elephantiasis is a disorder of lymphatic system, characterized by thickening of skin and extreme

enlargement of the affected area, most commonly limbs (legs), genitals, certain areas of trunk and parts of head.

5. *Edema due to Increased Endothelial Permeability*

The permeability of the capillary endothelium increases in conditions like burns, inflammation, trauma, allergic reactions and immunologic reactions, which lead to oozing out of fluid. This fluid accumulates leading to development of edema.

■ PITTING AND NON-PITTING EDEMA

Interstitial fluid is present in the form of a gel that is almost like a semisolid substance. It is because the interstitial fluid is not present as fluid but is bound in a proteoglycan meshwork. It does not allow any free space for the fluid movement except for a diameter of about a few hundredths of a micron.

Normal volume of interstitial fluid is 12 L and it exerts a negative pressure of about 3 mm Hg. It applies a slight suction effect and holds the tissues together. However, in abnormal conditions, where the interstitial fluid volume increases enormously, the pressure becomes positive. Most of the fluid becomes free fluid that is not bound to proteoglycan meshwork. It flows freely through tissue spaces, producing a swelling called edema. This type of edema is known as pitting edema because, when this area is pressed with the finger, displacement of fluid occurs producing a depression or pit. When the finger is removed, the pit remains for few seconds, sometimes as long as one minute, till the fluid flows back into that area.

Edema also develops due to swelling of the cells or clotting of interstitial fluid in the presence of fibrinogen. This is called non-pitting edema because, it is hard and a pit is not formed by pressing.

QUESTIONS IN BLOOD AND BODY FLUIDS

LONG QUESTIONS

1. What are the compartments of body fluid? Enumerate the differences between ECF and ICF and explain the measurement of ECF volume.
2. What is indicator dilution technique? How is it applied in the measurement of total body water? Describe dehydration briefly.
3. Give a detailed account of erythropoiesis.
4. Define erythropoiesis. List the different stages of erythropoiesis. Describe the changes which take place in each stage and the factors necessary for erythropoiesis.
5. Describe the morphology, development and functions of leukocytes.
6. Describe the development of cell-mediated immunity.
7. Describe the development of humoral immunity.
8. Define blood coagulation. Describe the mechanisms involved in coagulation. Add a note on anticoagulants.
9. Enumerate the factors involved in blood coagulation and describe the intrinsic mechanism of coagulation.
10. Give an account of extrinsic mechanism of coagulation of blood. Give a brief description of bleeding disorders.
11. What is normal blood volume and what are the factors regulating blood volume? Describe the measurement of blood volume and give a brief account of edema.
12. Give an account of tissue fluid. Add a note on edema.

SHORT QUESTIONS

1. Dye or indicator dilution technique.
2. Measurement of total body water.
3. Measurement of ECF volume.
4. Measurement of ICF volume.
5. Measurement of blood volume.
6. Measurement of plasma volume.
7. Dehydration.
8. Water intoxication.
9. Functions of blood.
10. Plasma proteins.
11. Plasmapheresis.
12. Functions of RBCs.
13. Fate of RBCs.
14. Lifespan of RBCs.
15. Physiological variations of RBC count.
16. Polycythemia.
17. Stem cells.
18. Factors necessary for erythropoiesis.
19. Destruction of hemoglobin.
20. Abnormal hemoglobin.
21. Abnormal hemoglobin derivatives.
22. Iron metabolism.
23. Pernicious anemia.
24. Erythrocyte sedimentation rate.
25. Packed cell volume or hematocrit.
26. Anemia.
27. Blood indices.
28. Hemolysins.
29. Types and morphology WBCs.
30. Functions of WBCs.
31. T lymphocytes.
32. B lymphocytes.
33. Role of macrophages/antigen-presenting cells in immunity.
34. Immunoglobulins or antibodies.
35. Immune deficiency diseases.
36. Autoimmune diseases.
37. Immunization.
38. Cytokines.
39. Platelets.
40. Hemostasis.
41. Fibrinolysis.
42. Tests for coagulation.
43. Anticoagulants.
44. Procoagulants.
45. Bleeding disorders.
46. Hemophilia.
47. Purpura.
48. Thrombosis.
49. ABO blood groups.
50. Rh factor.
51. Transfusion reactions.
52. Hemolytic disease of the newborn/ erythroblastosis fetalis.
53. Exchange transfusion
54. Blood volume.
55. Reticuloendothelial system or tissue macrophage.
56. Functions of spleen.
57. Lymph.
58. Lymph nodes.
59. Tissue fluid.
60. Edema.