

***SHRIMATI INDIRA GANDHI COLLEGE***

*(Nationally Accredited at 'A' Grade(3<sup>rd</sup> cycle) by NAAC)*

***TIRUCHIRAPPALLI – 02***

**DEPARTMENT OF HOSPITAL ADMINISTRATION**



**LEARNING MATERIAL  
BASIC BIOLOGICAL SCIENCE-I**



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# Basic Biological Science Part I

## List of Contents

<b>S.No</b>	<b>Particulars</b>	<b>Page. No.</b>
<b>1</b>	<b>Unit 1</b>	<b>1-13</b>
<b>2</b>	<b>Unit II</b>	<b>13-32</b>
<b>3</b>	<b>Unit III</b>	<b>33-94</b>
<b>4</b>	<b>Unit IV</b>	<b>94-139</b>
<b>5</b>	<b>Unit V</b>	<b>139-159</b>

# Basic Biological Science Part I

## **BASIC BIOLOGICAL SCIENCE – Part-I (Applied Anatomy, Physiology and Microbiology)**

### **UNIT I**

An introduction to basic Human anatomy and study of basic vital organs. Anatomy of Heart, Ear, Eye, Brain, Kidney.

### **UNIT II**

Physiology: Introduction to Human physiology & study of various systems – Circulatory system – Central Nervous System – Autonomous Nervous System, Reproductive System, Digestive System, Respiratory System – Sensory organs and their functions – Glands.

### **UNIT III**

Biochemistry of proteins – Fat – Amino acids – Carbohydrates metabolism – Enzymes – Vitamins – Hormones – Blood chemistry

### **UNIT IV**

Microbiology: Introduction to Classification & General Characteristics of various microorganisms - microbiology of food born diseases and food preservation relating.

### **UNIT V**

Introduction to Parasitology – commonly prevailing parasites – diseases – identification and treatment.

### **References:**

Anatomy and Physiology for Nurses.

Physiology : K. Madhavan Kutty

Microbiology : Ananthanarayanan

Phraseology : Chatterjee

# Basic Biological Science Part I

Entomology : Roy and Brown

## **Basic Biological Science –I**

### **Unit I**

#### **1. Anatomy:**

Anatomy is a branch of bio-chemical science dealing with normal structure ,shape, size, and location of various parts of the body.

A knowledge of these structures is necessary to understand their functions .

The subject matter of Anatomy includes :

Cytology - study of cells

Histology - study of tissues

Osteology –study of bones

Mycology study of muscles

Arthrology –study of joints

Splanchnology - study of organs

Neurology - study of the nervous system

Structures constituting human body

#### **Main Divisions of human body :**

Life is perhaps the most mysterious and marvelous fact in the universe.

One can see the life in different stages ranging from the unicellular

## Basic Biological Science Part I

amoeba to the highest evolved creature, **the human being**. The complexity of the organization of the organism progressively increases from unicellular, multi cellular, and multilayered animals to the worms and then from worms to vertebrates and finally the human beings. Although the human body has the most complex organization, fundamentally the cell remains the functional unit of life. The body as a whole, is built of cells. An aggregation or group of cells having similar origin, structure and functions forms the tissue. There are four primary types of tissues;

1. Epithelial
2. Connective
3. Muscular and
4. Nervous

### **Organ**

Different tissues combined together to perform a particular function or functions is called an organ. For example, Stomach is composed of epithelial, muscular and connective tissues, each has its own function. The epithelial system covers , the muscular system performs movements and connective system connects and support them.

# Basic Biological Science Part I

## **Organ system:**

Various organs working in co-ordination for the particular function or functions form an organ system. Various organ systems are osseous (skeletal,) auricular, muscular, digestive, circulatory, respiratory, nervous, excretory, reproductive and endocrine system. Their names are suggestive of their respective functions.

## **Some commonly used descriptive terms in Anatomy:**

The arrangement of various parts of the may be

- i. Symmetric e.g. limbs ,eyes, ears, and lungs. Their arrangement on the right side and left side are similar.
- ii. Asymmetric e.g. spleen and liver. The spleen lies entirely in the left side. Liver lies mostly on three right side.

The study of human body is done in an anatomical position. In this position, the body is erect, the head facing forwards, arms by the sides and palms of hand facing forward.

The following area few important terms which are used to describe the human body

## Basic Biological Science Part I

- ❖ Median line : the central plane which divides the body into two halves i.e. right and left.
- ❖ Medial : nearer to the median line
- ❖ Lateral : away from the median line
- ❖ Superior : nearer to the head
- ❖ Inferior : nearer to the foot
- ❖ Anterior : nearer to the front surface of the body
- ❖ Posterior : nearer to the back surface of the body
- ❖ Proximal : nearer to the origin of the structure
- ❖ Distal : away from the origin of the structure
- ❖ Superficial : nearer to the skin surface
- ❖ Deep : deeper from the skin surface

The following are a few descriptive terms used to convey the movements which occur at various joints:

- Flexion ; a movement where similar surfaces come nearer to each other. This reduces the angle between two bones. E.g bending the forearm at the elbow

## Basic Biological Science Part I

- Extension : movement where similar surfaces go apart. Here the angle between two bones is decreased. It is the opposite of the flexion. E.g. straightening of the bent forearm
- Adduction : A movement which brings the limb towards mid line.
- Abduction : It is the opposite of adduction where the limb is drawn away from the mid line
- Rotation : A movement around the central axis involving  $360^\circ$
- Medial rotation : A rotation towards medial direction
- Lateral rotation : A rotation towards lateral direction.

### 2. Muscles:

The muscular system consists of a large number of muscles ( more than 300). They bring about various movements in the body. Muscles are attached to bones, cartilages ,ligaments, skin, or other muscles by fibrous structures called *tendons (or) aponeurosis*

Tendon is a cord like structure whereas aponeurosis is a strong fibrous sheet . Muscles are richly supplied by blood vessels and nerves. Each muscle has an origin and an insertion. Origin is an end which remains stationary when the muscle contracts. The end which moves is called insertion. But it is not the same in all cases.



## Basic Biological Science Part I

### **Muscles of head, face and neck :**

They are classified into the following main groups

a) Muscles of scalp : *Occipito frontalis* is the muscle of the scalp.

It consists of two parts

- Occipital belly which is situated under the skin of occipital bone
- Frontal belly which is situated under the skin of frontal bone

b) Muscles of facial expression :

- Orbicularis oculi which are circular muscles around the eye. they produce closing of the eyes.
- Orbicularis oris which is present in the lips. It closes the lips.
- Buccinators the muscle of cheek. It is used in chewing and sucking.

c) Muscles of mastication :

- Temporal muscles arising from temporal fossa of skull.

## Basic Biological Science Part I

- Masseter arising from zygomatic arch of temporal bone. These muscles control the movements of lower jaw. They are involved in chewing.

### d) Muscles of neck :

They attach the head to trunk. They are

- Platysma which extends from lower jaw to deep fascia of chest. It depresses the jaw.
- Sterno-mastoid which extends from sternum and clavicle below to mastoid process of skull.

### Muscles of thorax :

- Pectoralis major which forms the anterior part of the chest and axilla.
- Pectoralis minor which lies deep into pectoralis major .
- Serratus anterior which extends from ribs to vertebral border of scapula.
- Intercostal muscles (11 pairs) which extend between the lower and upper border of two successive ribs.

### **Muscles of abdomen:**

**Anterior abdominal wall:** It is composed of

## Basic Biological Science Part I

- *Reticulas abdominis* which are two muscles lying one on each side.
- *External oblique* which arises from the lower ribs
- *Internal oblique* which arises from the iliac crest
- *Transverses abdominis* which runs horizontally across the anterior abdominal wall.

**Posterior abdominal wall :** It is made of

- *Quantum lumborum* which extends from the iliac crest to the 12<sup>th</sup> rib.
- *Iliacus* which arises from iliac fossa.
- *Psoas* which arises from lumbar vertebrae.

Muscles of lower limb :

Muscles of buttock : they are

1. *Gluteus maximums*
2. *Gluteus medius*
3. *Gluteus minimus*

**Muscles of upper limb :**

## Basic Biological Science Part I

### Muscles of arm :

They are i) biceps ii ) brachialis iii) triceps

### Muscles of forearm :

They can be classified into two types

- i) Anterior muscles a ) flexor digitorum sublimes b) flexor digitorum profundus iii) flexor carpi radialis iv) pronator teres.

Posterior muscles consists of i) *Extensor digitorum communis* ii) *Extensor muscles of fingers*, iii) *thenar eminence* iv) *hypothenar eminence*,

### Muscles of lower limb :

#### Muscles of thigh :

They are classified in

- a) Anterior muscles which include quadriceps femoris contains four muscles i.e . rectus femoris, vastus medialis, vastus intermedius, vastus lateralis.
- b) Medial muscles which contains *Adductor longus, Adductor brevis, Adductor magnus.*
- c) Posterior muscles consists of i) *biceps* ii) *semitendinosus* iii) *semimembranosus.*

## Basic Biological Science Part I

### **Muscles of leg :**

They are classified in

#### **a) Anterior muscles which include**

**i) *tibialis anterior* , ii) *Extensor digitorum longus***

#### **b) *Posterior muscles***

## **UNIT -- II**

### **BLOOD**

Blood is a specialized connective tissue which is fluid in nature. The total volume of blood in the body is about 6 liters. blood is slightly alkaline with a pH of about 7.4. The specific gravity of blood is about 1.055

#### **Functions of Blood :**

Blood serves the following important function:

1. It transports oxygen and nutrients to various tissues
2. It transports waste products to organs of excretion
3. It carries hormones from endocrine glands to various tissues.
4. It redistributes water from one part of the body to the other
5. It contains antibodies and white blood cells which protect the body from diseases.

## Basic Biological Science Part I

6. Clotting of blood protects against hemorrhage.

### **Composition of blood:**

Blood contains a fluid called plasma, in which the cellular elements of blood are suspended.

**PLASMA :** Plasma contains

1. Water to the extent of 91%
2. Protein (albumin, globulin and fibrinogen)
3. Other substances like glucose, sodium chloride, iron, urea, uric acid and cholesterol.

Serum is obtained from plasma after removing fibrinogen.  
(Serum = Plasma - Fibrinogen)

**Plasma Proteins :** Plasma proteins occur in blood to the extent of 7 to 8%. The plasma proteins are:

1. **Albumin :** It is present in very high concentration. It is responsible for osmotic pressure of blood. It is synthesized in the liver.
2. **Globulin :** It is of three types : alpha, beta and gamma. It is produced in lymphoid tissues. It produces antibodies and immune substances.
3. **Fibrinogen :** It is responsible for coagulation of blood. It is synthesized in the liver.

### **Functions of Plasma Proteins:**

1. They transport hormones, iron and other substances.

## Basic Biological Science Part I

2. They exert osmotic pressure and regulate blood volume.
3. They Provide viscosity to blood (Which helps in maintaining blood pressure)
4. Fibrinogen of plasma is necessary for clotting
5. Globulin of plasma is important for the synthesis of immune substances called antibodies.

### **CELLULAR ELEMENTS OF BLOOD :**

The cellular elements of blood are :

1. Red blood cells (Erythrocytes)
2. White blood cells (Leucocytes)
3. Platelets (Thrombocytes)

### **RED BLOOD CORPUSCLES (RBC) OR ERYTHROCYTES:**

They are circular biconcave, disc shaped cells. They do not have a nucleus. But they have a respiratory pigment called *hemoglobin*. The normal RBC count is 4.5 to 5 millions per cu.mm. RBCs serve important functions such as transport of oxygen and maintenance of acid base balance. They are synthesized in the bone marrow found at the ends of long and short bones. The average life span of RBC is about 120 days.

**HEMOGLOBIN:** It is the respiratory pigment of erythrocytes. The red colour of blood is due to hemoglobin. It contains globin, a protein which is conjugated with heme (hemoglobin = heme + globin).HEme molecule

## Basic Biological Science Part I

contains four pyrrole rings with iron in the centre. The hemoglobin content of body is about 15 G per 100 ml of blood, Anemia occurs due to a decrease in hemoglobin.

The functions of hemoglobin are:

1. Transport of oxygen and carbon dioxide
2. Maintenance of acid base equilibrium.
3. As a source for the formation of bilirubin (Bilirubin is formed from porphyrin fraction of hemoglobin).

Hemolysis is the escape of hemoglobin from RBC into blood. This is caused by hypotonic condition, certain drugs and toxins.

**WHITE BLOOD CELLS (WBC):** They are colourless cells containing a nucleus. They are larger in size than RBCs. Also their number is less when compared to RBCs (about 8000 per cu.mm of blood).

**Classification of WBCs:** WBCs are classified as:

1. Granulocytes which are of three types : neutrophils, eosinophils and basophils.
2. Agranulocytes which are of two types : Lymphocytes and monocytes.

## Functions of WBCs



## Basic Biological Science Part I

1. Protection against infection. This is done by neutrophils and monocytes which engulf bacteria. This process is called as phagocytosis.
2. To aid in the repair of injured tissues.
3. To produce immune substances which defend against diseases. This is done by Lymphocytes through the synthesis of gammaglobulin.
4. Basophils secrete an anticoagulant substance called heparin.

**PLATELETS OR THROMBOCYTES:** These are round or oval shaped cells with biconvex surface. They are roughly one fourth of the size of a RBC Normal platelet count is 2 to 5 lakh per cu.mm of blood. Platelets do not have a nucleus. But cytoplasm contains distinct granules. They are synthesized by megakaryocytes (giant cells) of bone marrow.

### Normal and average values of cellular Elements of blood

Blood elements	Normal value	Average value
Red blood cells(RBC's)	4.5 to 5.5 million	5 million
White blood cells (WBC's)	6,000 to 10,000	8,000

## Basic Biological Science Part I

Granulocytes	60 to 70%	66%
Eosinophils	1 to 2%	1%
Basophils	0.5 to 2%	1%
Lymphocytes (Large and small)	20 to 30%	25%
Monocytes	4 to 8%	5%
Platelets	2 to 5 lakhs	3.5 lakhs

### Function of platelets:

1. thromboplastin liberated from platelets is essential for clotting.
2. They close minute lesions in the walls of blood vessels.
3. They aid in body's defence mechanism against bacteria.
4. They contain histamine and serotonin.
5. They contain some antigenic substances also.

Thrombocytopenia : It is a condition where there is a decrease in platelet count.

### CLOTING OF BLOOD (Coagulation of blood)

Clotting of blood is a defence mechanism of the body. It prevents loss of blood from the site of injury. If a leak develops in blood vessels, a clot is formed and it plugs the leak. This prevents the loss of blood.

**Mechanism of clotting :** Clotting of blood occurs in the following stages:

1. Thromboplastin is liberated from disintegrated tissues and damaged platelets.

## Basic Biological Science Part I

2. Thromoplastin converts prothrombin into thrombin THIS OCCURS IN PRESENCE OF Calcium ions.
3. Thrombin converts fibrinogen to fibrin.
4. The insoluble fibrin threads The formed elements of blood get entangled in this and form the clot.

**BLOOD GROUPS :** In early times, transfusion of blood from one person to another was dangerous and unsuccessful. This to because, plasma of some individuals contain some factors. These factors produce agglutination or hemolysis of the erythrocytes of their persons These reactions occur due to the presence of agglutinins and agglutnogens in blood. Agglutinogens are present in erythrocytes. They are of two types : A and B. Agglutinins are present in plasma. They are of two types : a and b, Depending on the presence of these two substances, blood is grouped as blood isgrouped as follows:

Group A contains A agglutinogen and b agglutinin.

Group B contains B agglutinogen and a agglutinin.

Group AB contains AB agglutinogens and no agglutinins.

Group O contains no agglutinogen but a and b agglutinins.

**Rh factors:** It is another type of agglutinogen. It is called as Rhesus factor (Rh factors) since it was first seen in Rhesus monkey. Rh+ve individuals have this factors. But Rh-ve individuals do not have The corresponding agglutinin is never present in the body. But it is developed after the first exposure too the agglutinogen. If a Rh+ve blood is given to

## Basic Biological Science Part I

a Rh-ve person, no immediate reaction occurs. But during a second transfusion, the Rh-ve person develops Anti-Rh agglutinin. This further leads to agglutination.

**Thrombosis:** Intravascular clotting of blood is called thrombosis. Thrombosis may occur due to roughening or thickening of blood vessels (as in arteriosclerosis). Thrombosis can obstruct essential blood vessels (like coronary or cerebral vessels). This may lead to death.

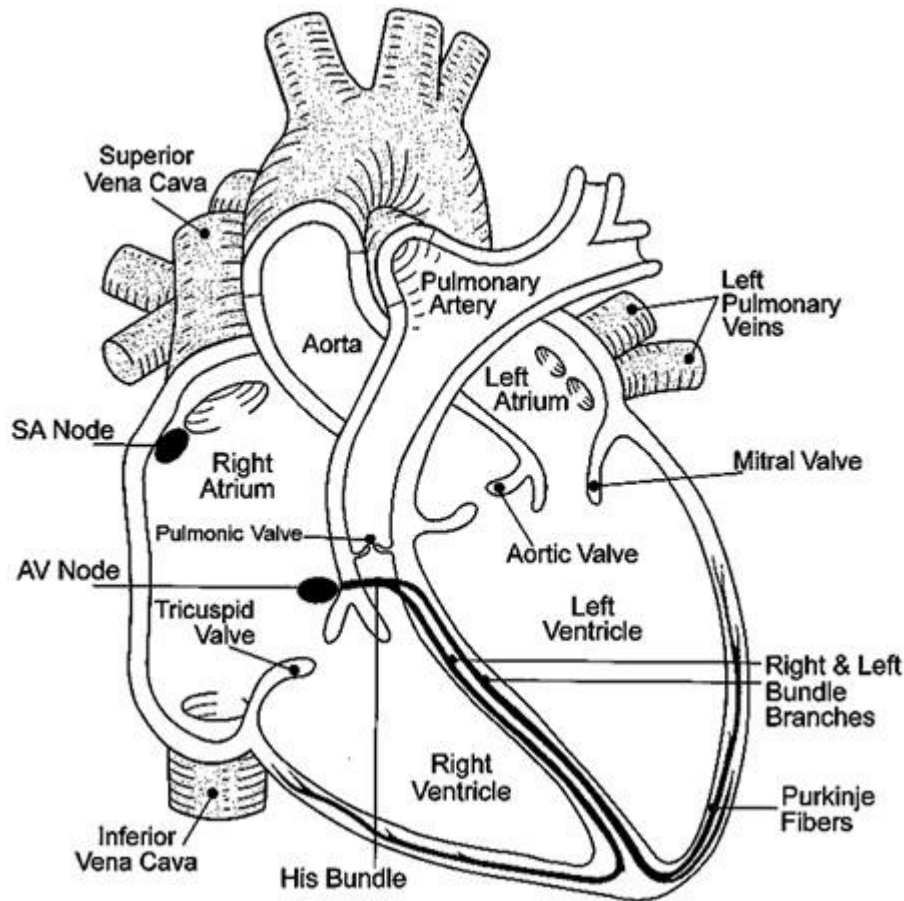
### **Circulation**

The cardiovascular system consists of heart and blood vessels. It is mainly a transport system. It transports respiratory gases, nutrients and excretory products to various parts of the body. Blood is the medium through which these substances are transported.

### **HEART**

Heart is a conical, hollow, musculotendinous organ. It lies in the thorax between the lungs and behind the sternum. It is about 10 cm long and weight about 300 grams. The base of the heart is above and apex is below.

# Basic Biological Science Part I



**1. Position of the heart:** The heart lies in the thorax between the lungs and behind the sternum. Two thirds of the heart is on the left side. It lies obliquely. It is directed more towards the left side than on the right side. The apex of the heart lies at the level of 5<sup>th</sup> intercostal space, 9 cm to the left of midline. The base extends to the level of second rib.

**2. Structure of the heart :**

- i) Heart is surrounded by an outer covering called pericardium. It contains two layers called visceral pericardium and parietal

## Basic Biological Science Part I

pericardium. Pericardial fluid is present between these two layers.

- ii) The middle layer is made of heart muscle fibres. It is called as myocardium.
- iii) The inner lining is called as endocardium.

**3. Chambers of the heart:** Heart is made of four chambers. The two chambers on the right side are known as 1) right atrium 2) right ventricle. The chambers on the left side are known as 1) left atrium 2) left ventricle. These four chambers are formed by two septa which divide the heart. They are inter atrial septum and interventricular septum.

**4. Valves of the heart:** The opening between right atrium and right ventricle is guarded by tricuspid valve. The opening between left atrium and left ventricle is guarded by mitral valve (bicuspid valve.) Some tendinous cords arise from the lower border of these valves. They are called chordate tendinae. The chordate tendinae in turn are attached to papillary muscles which arise from ventricular walls.

**5. Blood vessels attached to heart:**

- i) The right atrium receives superior vena cava and inferior vena cava. They carry venous blood to heart.
- ii) From the right ventricle, arises the pulmonary artery. It carries venous blood to lungs for oxygenation.
- iii) The left atrium receives four pulmonary veins. They carry oxygenated blood to heart.

## Basic Biological Science Part I

- iv) From the left ventricle, arises the aorta. It delivers our blood to all parts of the body.

### **6. Blood supply to heart:**

The heart receives blood supply through right and left coronary arteries. They are the first branches of aorta. Venous blood of heart is collected by coronary sinus. It opens directly into right atrium.

- 7. Nerve supply to heart:** Heart is supplied by sympathetic and vagus nerves. Branches from these nerves pass to the sino auricular node.

**BLOOD CIRCULATION:** Depending on the course of blood, circulation can be classified into:

1. Systemic circulation
2. Pulmonary circulation
3. Coronary circulation
4. Portal circulation

**1. Systemic circulation:** It is the circulation involving blood supply to all parts of the body except lungs. This circulation starts from aorta (which carries oxygenated blood from left ventricle).

**2. Pulmonary circulation:** It is the part of circulation involving the purification of blood in lungs. Impure venous blood is pumped by the right ventricle.

**3. Coronary circulation:** The circulation involves blood supply to the heart itself.

**4. Portal circulation:** It is the circulation of blood through the liver.

## Basic Biological Science Part I

**FUNCTIONS OF THE HEART:** The heart acts as a pump. It maintains a constant circulation of blood throughout the body. It is achieved as follows:

1. The superior vena cava and inferior vena cava bring venous blood from various part of the body to the heart. This venous blood fills the right atrium.
2. When it is full, the right atrium contracts sending blood to the right ventricle.
3. Now the right ventricle contracts This sends blood to the lungs through pulmonary trunk (which divides into right and left pulmonary arteries).
4. The blood gets oxygenated in the lungs. The oxygenated blood is carried by pulmonary veins to the left atrium.
5. Now, the left atrium contracts and sends blood to the left ventricle.
6. Now, the left ventricle contracts and sends blood into the acorta. This blood is circulated throughout the body.

**BLOOD PRESSURE (BP)** It is defined as the lateral pressure exerted by blood on blood vessels. The blood pressure which is normally expressed is arterial blood pressure. It has two phases.

1. Systolic blood pressure: It is the maximum blood pressure .This occurs during the systole of the heart.(range 100 to 120 mm Hg.)



## Basic Biological Science Part I

2. Diastolic blood pressure: It is the minimum pressure. It occurs during the diastole of the heart (range 60 to 80 mm Hg.)

Pulse pressure is the difference between systolic and diastolic blood pressure (It is nearly 40 mm Hg.)

**Measurement of blood pressure:** Blood pressure is usually measured by an instrument called sphygmomanometer. It consists of a mercury manometer, cuff and hand pump. The cuff is tied the cubital fossa of the individual. Then the hand pump is pressed so that air is inflated in the cuff. When the cuff is fully inflated, air pressure is more than blood pressure. So blood flow in the brachial artery is completely is obstructed. Now the hand pump is slowly released till the time the appearance of the first sound is heart (by means of a stethoscope put in the cubital fossa). The manometric reading is now noted. This reading is he systolic blood pressure.

Left the hand pump is slowly released till the time the sound becomes louder and louder. Later it stops. The manometric reading is noted when the sound disappears. This reading is the diastolic blood pressure.

**RESPIRATION:** Respiration is defined as the exchange of gases between body tissues and the external environment. Supply of oxygen to the tissues and excretion of carbondioxide occur only through respiration.

The functions of respiration are:

1. Transport of oxygen to tissues and excretion of carbon dioxide.

## Basic Biological Science Part I

2. Excretion of volatile substances like ammonia
3. Regulation of temperature through loss of heat in the expired air.
4. Maintenance of pH of blood.
5. Regulation of water balance through excretion of water vapour.

**RESPIRATORY SYSTEM:** The respiratory system consists of the following structures:

1. Nasal cavity
2. Pharynx
3. Larynx
4. Trachea
5. Bronchi
6. Bronchioles
7. Alveoli

**MECHANISM OF RESPIRATION:** Respiration involves two stages:

- 1) inspiration
2. Expiration.

**Inspiration (or breathing in):** It is an active process It is produced by the contraction of the following muscles:

1. Diaphragm the contraction of which enlarges the chest cavity vertically (i.e., from above downwards).
2. Intercostal muscles when contract produce elevation or ribs and sternum. This enlarges the chest cavity in all the other four sides.
3. The lungs expand at this stage and fill this increased space. Now the pressure in the lungs is less than atmospheric pressure. So air flows into the lungs.

## Basic Biological Science Part I

**Expiration (or breathing out):** It is a passive process It is produced by the relaxation of diaphragm an intercostals muscles. This produces by the reduction in the size of chest cavity. So the pressure in the lungs increases which forces the air out

The rate of respiration is 16 to 18 per minute in adults The rate is higher in children.

**REGULATION OF RESPIRATION:** Respiration is regulated by two controles: 1. Nervous control 2. Chemical control.

**1. Nervous control:** It is exerted by respiratory center present in the medulla oblongata of brain From this centre afferent impulses pass to:

1. Diaphragm through phrenic nerve.
2. Intercostal muscles through intercostals nerves.

These impulses cause rhythmic contraction of diaphragm and intercostal muscles.

Afferent impulses arise due to the distention of. Airsacs. They are carried by vagus to the respiratory centre.

2. **Chemical control:** This is effected through carbondioxide content of blood. An increase in the level of carbondioxide produces stimulation of the respiratory centre. A decrease in carbondioxide level produces the opposite effect.

## Basic Biological Science Part I

**EXCHANGE OF GASES:** It occurs in two stages:

1. Exchange between tissues and blood.
2. Exchange between alveoli and blood.

1. **Exchange between tissues and blood.** This is called as tissue or internal respiration. The oxygen tension of pure blood supplying the tissues is high (100 mm Hg.) But the oxygen tension of tissues is low (40 mm Hg.) So oxygen of blood goes to tissues. The carbondioxide tension is more in tissues than in blood. So carbondioxide goes out from the tissues to blood Now blood containing more carbondioxide is taken back to the heart by venous system.

2. **Exchange between alveoli and blood:** It is called as pulmonary or external respiration. The oxygen tension in the alveolar air is high (100 mm Hg). But oxygen tension of blood in the capillaries is low. Due to the pressure difference, oxygen of alveoli enters into blood similarly carbondioxide tension of capillary blood is higher than in alveoli. So carbondioxide enters into alveoli and it is breathead out through the expired air.

## Excretion

The urinary system is the main excretory system of the body. It consists of

1. two kidneys
2. Two ureters
3. An urinary bladder
4. An urethra.

## Basic Biological Science Part I

**KIDNEY:** They are two bean shaped organs lying on the posterior abdominal wall, on each side of the vertebral column.

### **Functions of the kidneys:**

1. Excretion of water and waste products of protein metabolism.
2. Excretion of excess salt.
3. Excretion of harmful substances, drugs and toxins.
4. Regulation of pH of blood.

**Structure of kidney:** Kidney is surrounded by an outer fibrous capsule.

Below this lies the substance of the kidney which consists of:

1. An outer cortex which is reddish – brown in colour.
2. Inner medulla which contains pyramids of the kidney.
3. An upper expanded end of ureter called pelvis.

Microscopically the kidneys are made of a number of structural and functional units called nephrons. There are about one million nephrons in each kidney. A nephron consists of two parts:

1. Malpighian bodies made of Bowman's capsule and glomerulus.
2. Renal tubules.

**FORMATION OF URINE:** The formation of urine by kidneys involves three processes:

1. Glomerular filtration
2. Tubular secretion

## Basic Biological Science Part I

### 3. Tubular reabsorption

- 1. Glomerular filtration** Filtration of water, salts and other substances occurs in the glomeruli. Glomerular filtrate is the fluid that is formed after filtration. About 10ml of glomerular filtrate is formed per minute. This filtrate passes into the proximal convoluted tubule.
- 2. Tubular secretion:** It is an active process which occurs in the convoluted tubules. Abnormal substances or normal substances present in excess in blood are eliminated by this process. Potassium, hydrogen and drugs like penicillin are excreted by tubular secretion.
- 3. Tubular reabsorption:** The rate of glomerular filtration is about 100 ml per minute. So about 6 litres of glomerular filtrate can be formed in one hour. But the volume of urine eliminated per day is only about 1.5 litres. It is so, because nearly 99 percent of the glomerular filtrate is reabsorbed. Reabsorption of water occurs in the convoluted tubules and collecting tubule. In addition to water some salts are also reabsorbed in the renal tubules.

Urine is the fluid that results from the above three processes. It enters the collecting tubules and then into the pelvis of kidney. From there, it enters the urinary bladder through ureter.

## Basic Biological Science Part I

**Composition of urine:** The volume of urine excreted in man varies from 1 to 2 litres daily. The colour of urine is pale amber, odour is aromatic and reaction is slightly acidic (pH 6). Specific gravity varies from 1010 to 1025. (Specific gravity of water and that of water is 1000).

Urine consists of :

- |                        |   |     |
|------------------------|---|-----|
| 1. Water               | - | 96% |
| 2. Urea                | - | 2%  |
| 3. Uric acid and salts | - | 2%  |

## Digestion

The digestive system consists of gastrointestinal tract (alimentary canal) and its glands. The functions of gastrointestinal tract are ingestion, digestion and absorption of food and excretion of waste products.

**PARTS OF DIGESTIVE SYSTEM:** Digestive system consists of the following parts:

1. Mouth
2. Pharynx
3. Oesophagus
4. Stomach
5. Small intestine
6. Large intestine
7. Rectum
8. Anus

## MOUTH ( Buccal Cavity)

It is the upper expanded portion which forms the beginning of alimentary canal. It can be divided into two parts:

## Basic Biological Science Part I

1. Vestibule an outer part. It lies between lips and inner lining of cheeks externally and gums and teeth internally.
2. Cavity of mouth an inner part. It is bounded by teeth and mastoid bone at the sides, palate above and tongue below.

Palate forms the roof of mouth cavity It consists of hard palate which is in front and

soft palate which is behind. Uvula is a conical process which hangs from the middle of soft palate. Two folds of mucous membranes called anterior and posterior pillars of fauces lie on either side of uvula. Tonils lie in between these folds.

**Structure of stomach:** Stomach consists of the following four coats:

1. Peritoneal coat (made of serous covering).
2. Muscular coat (made of longitudinal, circular and oblique fibres).
3. Submucous coat (made of areolar tissue).
4. Mucous coat (made of mucous membrane).

## UNIT -- III

### Atomic Structure

Atom consists of nucleus and planetary electrons. Nucleus of the atom consists of two types of nucleons called as protons and neutrons. The protons are positively charged particles while neutrons have no charge. The mass of the nucleus is equal to mass of neutrons plus mass of



## Basic Biological Science Part I

protons present in the nucleus. Nuclear force bind proton and neutron together.

The chemical nature of an atom is determined by the number of protons in its nucleus (Atomic number) and its mass of total number of nucleons (Mass number)

The atomic number (Z) is the number of protons in the nucleus It is always constant for and characteristic of a particular element and is conventionally indicated in the following way.



The mass number (A) is the total number of protons and neutrons in the nucleus.

**Nuclide :** A nuclide is a particular nuclear species characterized by its atomic number and mass numbers, e.g.  ${}_6\text{C}^{12}$ ,  ${}_{11}\text{Na}^{23}$  are nuclides.

Isotopes: These are nuclides. with the same atomic number but different mass number, i.e different numbers of neutrons in the nucleus. E.g.  ${}_1\text{H}^1$ ,  ${}_1\text{H}^2$ ,  ${}_1\text{H}^3$  are isotopes of hydrogen. Isotopes may be stable or unstable. If they are unstable. They undergo radioactive decay or disintegration and are known as radioactive isotopes or radionuclides. Carbon has five isotopes.

Two are stable  ${}_6\text{C}^{12}$  and  ${}_6\text{C}^{13}$

And three are unstable i.e. Radioactive  ${}_6\text{C}^{10}$ ,  ${}_6\text{C}^{11}$ ,  ${}_6\text{C}^{14}$

### Stable and Unstable nuclei

## Basic Biological Science Part I

The nucleus of the element having low atomic number is most stable if the number of neutrons is equal to or slightly greater (1 more) than the number of protons in it.

The nucleus is unstable if the number of neutrons is less or significantly greater (i.e. 2 or more) than the number of protons in it.

e.g.

Nuclei (Nuclides)	Protons	Neutrons
Stability		
${}^6_6\text{C}^{10}$	6	4
Unstable		
${}^6_6\text{C}^{11}$	6	5
Unstable		
${}^6_6\text{C}^{12}$	6	6
Stable		
${}^6_6\text{C}^{13}$	6	7
Stable		
${}^6_6\text{C}^{14}$	6	8
Unstable		

Unstable nuclei have excess energy. They discharge this energy in the form of particles or radiations and become stable.

## RADIOACTIVITY

## Basic Biological Science Part I

Act of spontaneous emission of rays which affect a photographic plate even when the plate is covered with black papers or by some substances is called radioactivity.

In other terms, the property of certain nuclides of emitting radiation by the spontaneous transformation of their nuclei into those of other nuclides is called as radioactivity of the nuclides.

The radioactivity of the preparation is the number of nuclear transformation per unit time in a given amount of the preparations.

The substances having such property of emitting rays or particles are called radioactive substances. These also cause discharge of electrified bodies. The elements are called radioactive because they are unstable and undergo spontaneous decomposition accompanied by emission of radiation or rays like, alpha, beta and gamma rays.

### **Types of radiation**

The radiation emitted by radioactive isotopes is in the form of charged particles of matter (alpha or beta particles) Sometimes after the emission of a particle, the nucleus still has too much energy. This is dissipated in the form of gamma rays, a type of electromagnetic radiation.

1. Alpha particles,
2. Beta particles
3. Gamma radiation

#### **1. Alpha particles,**

## Basic Biological Science Part I

They possess the following characteristics:

1. These are equivalent to helium nuclei containing 2 protons and 2 neutrons ( ${}^4_2\text{He}$ )
2. They are heavy and positively charged particles having very high ionizing power upon interaction with air or other media.
3. Their range in air is few centimeters and a fraction of millimeter in body tissues.
4. Alpha particles move at a relatively slow speed averaging about 0.1 the speed of light.
5. Alpha radiation is usually emitted only from elements having atomic numbers greater than 82.

Alpha emitted are not used in pharmaceutical preparations.

### 2. Beta particles

1. They have the same mass as in electron.
2. Their range in air is few metres and about a centimeter in body tissues.
3. Beta particles are sometimes called as negatrons and they are emitted by unstable nuclei having neutrons in excess of protons.
4. Some beta particles are positively charged but negatively charged beta particles are most common.

### 3. Gamma radiation

The gamma rays are of short wavelength ( $10^{-10}\text{X}10^{-8}$  CM) similar to x-rays and having the speed of light only difference between gamma rays and rays is that

## Basic Biological Science Part I

the gamma radiation is emitted from the nucleus, while X-radiation is produced from the planetary electrons.

1. Gamma ray is supposed as a photon of electromagnetic radiation.
2. They have high penetrating power because of very high energy.
3. As it is electromagnetic radiation it has no mass or charge and travels with the speed of light.

### **Biological effects of radiation**

The effect of radiation upon biological tissue depends upon number of factors such as

1. Ability of the radiation to penetrate tissue,
2. The energy of radiation
3. The particular tissue
4. Surface area of the tissue exposed
5. Does rate of the radiation

The radiation interacts with the molecules present in the tissue and forms abnormal chemical species like ions and / or free radicals.

The abnormal chemical species can alter the local pH in the tissues and initiate the undesirable free radical chain reactions producing peroxides and other compounds toxic to the tissue. This may lead to necrosis and ultimately, destroy the tissue or organ.

Water molecule in the tissue is the most probable reactive species in the path of ionizing radiation. Other biochemicals may also be involved in the interaction to some extent,

Free radicals and hydrogen peroxide formation.

## Basic Biological Science Part I

$x\text{H}_2\text{O}$  Radiation  $\text{XH}^+$   $\text{XHO}$  other products

$y\text{H}_2$   $y\text{H}_2\text{O}_2$

Free radicals formed from water can also abstract radicals from other molecules and produce various toxic species which can alter the DNA in cells and cause cross linking between certain amino acids in proteins Thus the particular tissue gets destroyed.

Alpha particles have a potential to produce a tremendous amount of ionization or free radicals but the range and penetration of these particles are so slight Therefore the isotopes emitting alpha particles must be close enough to the individual so as reach the radiation to the skin in order to observe biological effects.

Gamma rays have relatively low ionizing power even though the range and penetrating power of this type of radiations are high enough to produce significant damage of the particular tissue at distances of several metres from the source.

### **Unit of radioactivity**

The fundamental unit of radioactivity is 'Curie' (Ci) Millicurie (mCi) and Microcurie ( $\mu\text{Ci}$ ) and Becquerel (Bq) are also the consequent units of radioactivity.

Curie (Ci) : It is a fundamental unit of radioactivity and defined as  $3.7 \times 10^{10}$  transformations per second

Becquerel (Bq) : It is the SI unit of activity and defined as 1 transformation per second

## Basic Biological Science Part I

$$1\text{Ci} = 3.7 \times 10^{10}\text{Bq}$$

$$1\text{Bq} = 2.7 \times 10^{-11} \text{ Ci}$$

Half life : The time taken for half of the radioactive nuclei to disintegrate is known as 'half life'

It is also defined as the time in which the amount of radionuclide decays to half its initial value. It is related to the decay constant by the equation.

$$t \frac{1}{2} = \frac{0.693}{l}$$

**Decay constant (Disintegration constant or transformation constant):** It is the fraction of radioactive atoms that undergo transformation in unit time provided that the time unit is short compared with the half life.

**Specific activity or specific radioactivity:** The specific activity of a preparation of a radioactive material is the radioactivity of the radionuclide concerned per unit mass of the element or of the compound concerned.

It is the ratio of active atoms to inactive atoms in a sample.

### Measurement of radioactivity

Methods for detection and measurement of radioactivity depend on the effects the radiation has on the matter and particular property or properties of radiations such as

- (a) The property of radiation to effect ionisation (in a gas)

## Basic Biological Science Part I

- (b) The property of radiation to cause scintillation (fluorescence)
- (c) The property of radiation responsible for chemical changes (e.g. effect on photographic emulsion). Accordingly the methods are devised

For measurement of radioactivity the following used:

1. Ionisation chamber
2. Geiger-Muller counter
3. Scintillation counter
4. Proportional counter
5. Autoradiography

### Geiger Muller counter

The method of Geiger Muller counting is based on the ability of radiation to cause ionization in a gas. In G.M, counter easily ionisable gas, argon is used

It is useful to detect alpha, beta and gamma particles but most frequently used for beta particles. Its efficiency for beta particles is almost 100 per cent but for radiations it is only about 1 per cent.

Geiger Muller counter contains a central tungsten wire anode and a concentric conducting cathode

The space between the electrode contains argon gas at a pressure of a few millimeters of mercury. In a G.M counter the field is made so high to effect collision of ions formed.



## Basic Biological Science Part I

As shown in fig the radiation like beta particle enter the Geiger tube through the thin mica end window and causes ionization of some argon atoms The argon ions thus formed are attracted to the cathode the outer negative electrode by the substantial potential gradient present, Movement of these heavy charged ions gives rise to further ions which are in turn attracted to the anode causing further ionization. Thus the repeated ionization (avalanche effect) result into ionization of whole of the gas present in the tube.

The avalanche effect is equivalent to the flow of a pulse of current The current if necessary can be amplified can be amplified and recorded electronically.

## Radioisotopes

Unstable or radioactive isotopes are radioisotopes. Isotopes decompose or decay by emission of nuclear particles, into other isotopes of the same or different elements. The decay is characteristic for a particular radioisotopes.

### Uses of radioisotopes

Radioisotopes and radiopharmaceuticals are used widely in many branches of medicine and surgery.

Some of the uses are

1. Diagnostic application
2. Radiotherapy

## Basic Biological Science Part I

3. Sterilisation techniques
4. Research application
5. Analytical application

**1. Diagnostic application :** Radiation for diagnostic purpose must have sufficient energy to pass through tissues between the locus of radioactive isotope in the body and the detecting device.

Eg(a) Phosphorus –  $^{32}_{15}\text{P}$

Phosphorus – 32 as sodium phosphate ( $^{32}\text{P}$ ) is employed in diagnosis of malignant neoplasms especially those affecting eye, brain and skin.

(b) Chromium-51  $^{51}_{24}\text{Cr}$

Chromium-51, as sodium chromate ( $^{51}\text{Cr}$ ) employed in the form of sterile solution is used to label red blood cells so that red-cell survival and red blood cell volume can be measured.

Chromium-51 activity in the faeces can be used to estimate gastrointestinal blood losses.

(c) Cobalt-57  $^{57}_{27}\text{Co}$  and Cobalt -58  $^{58}_{27}\text{Co}$

Various preparations of cyanocobalamin labeled with either cobalt 57 or cobalt-58 are useful for measurement of absorption of vitamin B<sub>12</sub> and thus in the diagnosis of pernicious anemia and other malabsorption syndroms.

Other radioisotopes such as Gallium -67  $^{67}_{31}\text{Ga}$  Iodine -  $^{131}_{53}\text{I}$

## Basic Biological Science Part I

Mercury -197  $^{197}_{80}\text{Hg}$  Mercury-203  $^{203}_{80}\text{Hg}$  Selenium-75  $^{75}_{34}\text{Se}$  etc. are used for diagnostic purposes.

### 1. Radiotherapy

Certain isotopes are used for their therapeutic action in specific conditions. The principle of radiotherapy is to destroy diseased tissue without destroying healthy tissue.

A radiation is not sufficiently effective because of less penetrating power. B-beta radiation has sufficient penetration so used to treat surface lesions such as on the eye. Gamma radiation has the most penetrating power and is used for treating deep-seated tumors.

e.g. I-131  $\left(\frac{131}{53}\text{I}\right)$ , I-123  $\left(\frac{123}{53}\text{I}\right)$  are used in study of thyroid function and in the treatment of thyrotoxicosis and some of the thyroid carcinoma.

(b) Cobalt-60  $\left(\frac{60}{27}\text{Co}\right)$  is used for radiotherapy in the form of alloys in large sealed sources.

(c) Yttrium-90  $\left(\frac{90}{39}\text{Y}\right)$  in the form of colloidal suspensions of yttrium silicate  $^{90}\text{Y}$  is used in the treatment of arthritic conditions of joints.

(d) Phosphorus-32  $\left(\frac{32}{15}\text{P}\right)$ -Sodium phosphate ( $^{32}\text{P}$ ) is used in the treatment of polycythaemia vera by intravenous injection.

## Basic Biological Science Part I

### 3. Sterilisation

Radioisotopes are also employed in the radiation sterilizations of heat-labile drugs like hormones, vitamins and antibiotics surgical dressings, disposable syringes etc.

e.g. Cobalt -60 ( ${}^{60}_{27}\text{Co}$ ) is used as a radiation source for the sterilization by gamma-irradiation of disposable syringes, catheters, and surgical dressings and other surgical materials.

This facility is available at Bhabha Atomic Research Centre (BARC) Bombay.

### 4. Research applications

Radioisotopes have many valuable research applications. In bio-chemical research they may be used for determination of mechanism of reactions locus of action etc.

e.g. (a) Carbon-14 ( ${}^{14}_6\text{C}$ ) : Many organic compounds labeled with carbon -14 are used in research.

(b) Iodine – 131

A special grade of sodium iodohippuric ( ${}^{131}\text{I}$ ) is used for the determination

## Basic Biological Science Part I

of effective renal plasma flow. Other isotopes such as sodium – 24 ( $^{24}_{11}\text{Na}$ ) phosphorous -32 ( $^{32}_{15}\text{P}$ ) are useful in various research methods.

### 5. Analytical

Radioisotopes are valuable for analytical purposes, especially when dealing with very dilute solution.

## **DISORDERS OF PLASMA LIPOPROTEINS**

### Hypolipoproteinemias

Familial hypobetalipoproteinemia It is inherited as an autosomal dominant trait. LDL concentration is between 10 to 60% of normal but chylomicron formation occurs.

Abetalipoproteinemia A rare inherited disease characterized by the absence of  $\beta$ -lipoprotein (LDL) in plasma, low blood lipids, especially triacylglycerol. There is no formation of chylomicrons or VLDL. Triacylglycerol accumulates in intestine and liver. Defect is apo – B synthesis.

Familial  $\alpha$ -Lipoprotein deficiency It is characterized by the absence of HDL in plasma and accumulation of cholesteryl esters in tissues but there is no impairment of chylomicron formation and secretion of VLDL. Hypertriacylglycerolemia occur due to absence of apo C-II, which activates LPL.

# Basic Biological Science Part I

## Hyperlipoproteinemias

**Type I – familial LPL deficiency** It is a rare disorder due to deficiency of lipoprotein lipase, characterized by slow clearance of chylomicrons and VLDL, Low levels of LDL and HDL.

It is a fat induced condition. Variation of the disease is caused by deficiency of apo C-II.

**Type II – familial hypercholesterolemia** It is a common disorder due to defective LDL receptors. It is characterized by elevated levels of LDL due to reduced clearance, resulting into hypercholesterolemia. The clinical features exhibit premature cardiovascular diseases and atherosclerosis.

**Type III – familial hyperlipoproteinemia** It is an inherited disease characterized by increase in chylomicron and VLDL remnants. It causes hypercholesterolemia The defect is the clearance of remnants by liver due to abnormality in apo-E

**Type IV – familial hypertriglyceridemia** In this disease due to increase in VLDL, cholesterol level rises. LDL and HDL tend to be subnormal. The metabolic defect includes over production of VLDL. It is associated with coronary heart diseases non insulin dependent diabetes mellitus, obesity and alcoholism.

## Basic Biological Science Part I

Type V = familial hyperlipoproteinemia It is characterized by hypertriacylglycerolemia and hypercholesterolemia with low LDL and HDL.

Familial hyperalphalipoproteinemia It is a rare condition beneficial to health and characterized by increased concentrations of HDL.

Familial LCAT deficiency In this disease absence of LCAT results into block in reverse cholesterol transport. The plasma concentrations of cholesteryl esters and lysolecithin are decreased

### Atherosclerosis

A correlation exists between raised serum lipid levels and the incidence of coronary heart disease and atherosclerosis. Hypercholesterolemia and other abnormalities in lipid metabolism contribute a major risk factor in atherosclerosis Increase in the cholesterol level may be due to any one of the following abnormalities:

- Elevated concentrations of VLDL with normal concentration of LDL
- Elevated LDL with normal VLDL
- Elevated of both LDL and VLDL

Atherosclerosis is a disease primarily of the elastic arteries (e.g. aorta, carotid and iliac arteries) and large and medium sized muscular arteries (e.g. coronary and poplital arteries). The basis lesion-the atheroma or fibrofatty plaque-consists of a raised focal plaque within the intima, having a core of lipid (mainly cholesteryl ester of lipoproteins apo-B-100) and a covering fibrous cap. Atheromas are sparsely distributed at first but as the disease advances, they become more and

## Basic Biological Science Part I

more numerous, sometimes covering the entire circumference of severely affected arteries. As the plaques increase in size, they progressively encroach on the lumen of the artery as well as on the subjacent media. Consequently in small arteries, atheromas are occlusive compromising blood flow to distal organs and causing ischemic injury, but in large arteries they are destructive, weakening the affected blood vessel wall causing rupture or forming thrombosis.

Diseases in which prolonged elevated levels of VLDL, IDL, or LDL occur in the blood (e.g diabetes mellitus, lipid nephrosis, hypothyroidism and other conditions of hyperlipidemia) are often accompanied by premature or more severe atherosclerosis. In contrast serum levels of high density lipoproteins (HDL) are inversely related to risk, the higher the level, the lower the risk. Thus HDL is often called the good cholesterol.

Hereditary factors play the greatest role in determining individual blood cholesterol concentration. High dietary intake of cholesterol and saturated fats, such as those present in egg yolk, animal fats and butter, raises the plasma cholesterol level. Additional factors considered to play a part include hypertension. Cigarette smoking and diabetes. Other risk factors, which are sometimes referred to as minor, or 'soft' risk factors because they are associated with a less pronounced and difficult to quantitate risk. These include:

- (1) Insufficient regular physical exercise
- (2) Competitive stressful life style



## Basic Biological Science Part I

- (3) Obesity
- (4) The use oral contraceptives
- (5) Drinking soft as opposed to hard water
- (6) Hyperuricemia
- (7) High carbohydrate intake
- (8) Hyperhomocysteinemia

Nevertheless, some patients with atherosclerosis related diseases have no obvious risk factors. Premenopausal women appear to be protected against many of these deleterious factors. Because they have higher concentrations of HDL than do men and post menopausal women.

The substitution of polyunsaturated and monounsaturated fatty acids for some of the saturated fatty acids is most beneficial in reducing the risk of atherosclerosis. Naturally occurring oils that contain a high proportion of polyunsaturated fatty acids include sunflower, cotton seed corn and soyabean oil. Olive oil contains a high concentration of monounsaturated fatty acids. The cholesterol lowering effect of polyunsaturated fatty acids could be attributed to the stimulation of cholesterol excretion into the intestine and increases oxidation of cholesterol to bile acids. It is evidenced that cholesterol lowering effect is also due to a shift in distribution of cholesterol from plasma into tissues because of increased catabolic rate of LDL due to up-regulation of the LDL receptors by poly- and monounsaturated fatty acids.

## Basic Biological Science Part I

Significant reductions of plasma cholesterol can be effect by drugs  
The use of cholestyramine resin causes a block in reabsorption of bile acids. Thus conversion of cholesterol to bile acids is enhanced to maintain the pool of bile acids. Consequently LDL receptors in the liver are up-regulated causing increased uptake of LDL and lowering of plasma cholesterol. Sitosterol acts by blocking the absorption of cholesterol from the gastrointestinal tract.

Several drugs block the formation of cholesterol at varios stages in the biosynthetic pathway. Mevastatin and lovastatin inhibit the enzyme HMG CoA reductase, a rate limiting step in cholesterol biosynthesis and also reduce LDL cholesterol levels by up-regulation of the LDL receptors Clofibrate exetrts hypolipidemic effect by diverting the hepatic inflow of free fatty acids from esterification to oxidation, thus decreasing the secretion of triacylglycerol and cholesterol containing VLDL by the liver. Probucol increases LDL catabolism via receptor independent pathways. Nicotinic acid rduces the flux of FFA by inhibiting adipose tissue lipolysis and inhibiting VLDL production by liver.

### **Risk factors for Coronary Artery Diseases**

**Hypercholesterolemia:** Cholesterol level in serum/plasma should be preferably below 200 mg/100 ml. Values around 220 mg/100 ml indicate moderate risk and above 259 mg/100 ml high risk.

**LDL cholesterol:** Blood levels of LDL-cholesterol are desirable under 130 mg/100 ml. Level between 130-159 mg/100 ml indicate borderline and above 160 mg/100 ml high risk.

## Basic Biological Science Part I

**HDL Cholesterol:** Above 60 mg/100 ml protects against heart diseases. A level below 35 mg/100 ml increases the risk of CAD .For every 1 mg/100 ml drop in HDL-cholesterol the risk 3%

**LP (a):** It inhibits fibrinolysis. It level more than 30 mg/100 ml increase the risk 3 times. When Lp (a) increase is associated with increased LDL, the risk increases 6 times.

**Triacyleglycerol:** Increase in serum triglycerides more than 100 mg/100 ml is injurious to health

### COENZYMES

Coenzymes are heart stable, dialyzable, nonprotein organic molecules and are prosthetic group of enzymes. Coenzymes my be regarded as second substrate and function as group transfer agent.

**Classification:** Coenzymes are classified as:

- (1) Based on functional characteristics
  - (a) For transfer of group other than H. Examples are coenzyme A, thiamin pyrophosphate pyridoxal phosphate, folate coenzymes, biotin, cobamide coenzymes, and lipoic acid.
  - (b) For transfer of H Example are FAD, FMN, NAD<sup>+</sup>, NADP<sup>+</sup>, and coenzymes Q.
- (2) Based on chemical characteristics
  - (a) Containing an aromatic hetero ring. Examples are ATP, NAD, NADP, FMN etc.
  - (b) Containing a nonaromatic hetero ring. Examples are biotin, lipoic acid.

## Basic Biological Science Part I

(c) No hetero ring. Example is coenzyme Q.

Many coenzyme are derivatives of vitamin B complex group (Table 5-1)  
The details have been dealt separately with individual vitamin

**TABLE 5-1**

### Coenzymes related to Vitamins

Name	Abbreviation	Groups transferred	B-vitamin component
Nicotinamide adenine dinucleotide	NAD <sup>+</sup>	H <sup>+</sup> +2e	Nicotinamide
Nicotinamide adenine dinucleotide phosphate	NADP	H <sup>+</sup> +2e	Nicotinamide
Flavin mononucleotide	FMN	2H <sup>+</sup> +2e	Riboflavin
Flavin adenine dinucleotide	FAD	2H <sup>+</sup> +2e	Riboflavin
Coenzyme A	CoA	Acetyl group and acyl group	Pantothenic acid
Thiamin	TPP	Ketol group C <sup>2</sup>	Thiamin

## Basic Biological Science Part I

pyrophosphate lipothiamide pyrophosphate		aldehyde group	
Pyridoxal phosphate	$B_6PO_4$	Amino and keto group	Pyridoxine pyridoxal pyridoxamine
Biotin coenzyme		$CO_2$ fixation	Biotin
Folate coenzyme	$FH_4$	One C= transfer	Folic acid
Cobamide coenzyme		$-CH_2$ group and isomerisation	Cobalamin

Non vitamin coenzymes are ATP, VDP, UDP, S= adenosylmethionine and phosphoadenosine phosphosulphate.

## VITAMINS

Vitamins are the accessory, indispensable food factors, These are organic in nature, required in minute quantities and their deficiency results into diseased state.

## Basic Biological Science Part I

The vitamins are generally divided into two major groups. Water soluble and fat soluble. The vitamins of the B-complex and vitamin C comprise the water soluble group. The fat soluble vitamins are A, D, E and K.

### **WATER SOLUBLE VITAMINS**

#### **Vitamin C (l-Ascorbic Acid)**

Ascorbic acid is an enediol lactone resembling L-glucose (Fig.5.1). Vitamin C is readily oxidized to dehydroascorbic acid. Both forms are physiologically active.

**Properties** It is a white crystalline water soluble substance and sour in taste. It is a powerful reducing agent on account of its enediol structure. Vitamin C is stable in solid form and in acid solution but rapidly destroyed in alkaline solution.

**Sources** Richest sources are citrus fruits like amla, tomato, lemon, lime berries grapes etc. Other sources are leafy vegetables milk, and liver (destroyed during cooking).

In the body highest concentrations occur in adrenal cortex anterior pituitary, corpus luteum and thymus.

#### **Daily requirements**

Infants : 30 mg

Children : 40 gm

Adults : 50-70 mg

During pregnancy and lactation : 60-80 mg

#### **Functions**

## Basic Biological Science Part I

- (i) Vitamin C is a strong reducing agent with a hydrogen potential of +0.08 V, reduces compounds like molecular oxygen. Nitrate, cytochromes a and c. It is involved in oxidation-reduction reaction coupled with glutathione, flavin nucleotides etc.
- (ii) Ascorbic acid is required for hydroxylation of proline to form hydroxyproline, which is an important amino acid of collagen
- (iii) In tyrosine metabolism, the oxidation of p-hydroxyphenylpyruvate to homogentisate requires vitamin C.
- (iv) Vitamin C is required for the formation of bile acids
- (v) It is required in the conversion of folic acid to folinic acid
- (vi) Vitamin C is required in the absorption and mobilization of iron.
- (vii) Vitamin C has an established function in maintaining the normal intracellular material of cartilage, dentin and bone.
- (viii) Vitamin C also plays an important role in the state of stress, Adrenal cortex contains a large quantity of vitamin C, which is depleted on stimulation of adrenal gland by ACTH, suggesting its involvement in the reaction of body to physiological stress.

**Deficiency** Deficiency of vitamin C causes the disease scurvy. Normal stores of vitamin C are sufficient to last 6 for 3-4 months before signs of scurvy appear. Scurvy results from severe deficiency of vitamin C resulting into failure in the maintenance and formation of intercellular materials.

**Clinical manifestation of scurvy** In scurvy the following symptoms occur:

## Basic Biological Science Part I

- (i) Internal hemorrhages.
- (ii) Hyperkeratotic papules over upper arm, back, buttocks and calves.
- (iii) Pain on movement and swelling at the end of long bones.
- (iv) Swelling, sponginess, tenderness, bleeding of gums and loss of teeth.
- (v) Poor wound healing.
- (vi) Easy fracturability of bones.
- (vii) Susceptibility to infections.
- (viii) General weakness and anemia.

Infantile scurvy occurs in infants, fed on exclusively sterilized food. (Vitamin C content of which is destroyed) and is characterized by hemorrhages and anemia.

### Vitamin B Complex Group

The members of B-complex group are:

- (i) Thiamin (B<sub>1</sub>)
- (ii) Riboflavin (B<sub>2</sub>)
- (iii) Niacin (B<sub>3</sub>)
- (iv) Pyridoxine (B<sub>6</sub>)
- (v) Pantothenic acid
- (vi) Lipoic acid
- (vii) Biotin
- (viii) Folic acid
- (ix) Cyanocobalamin (B<sub>12</sub>)



## Basic Biological Science Part I

- (x) Inositol
- (xi) P-Aminobenzoic acid
- (xii) Choline

### Thiamin (Antiberiberi Factor, Aneurine)

Chemically it is substituted pyrimidine (2,5-dimethyl 6-aminopyrimidine) combined with substituted thiazole ring, joined by a methylene bridge

**Sources** Unrefined cereals, grains liver heart and kidney

### Daily requirements

Infants : 0.3-0.5 mg

Children : 0.7-1.2 mg

Adult

Male : 1.5 mg

Female : 1.2 mg

**Functions** Active thiamin is the coenzyme thiamin pyrophosphate (TPP) which is formed by an ATP dependent enzyme-thiamin diphosphotransferase, present in brain and liver.

TPP acts as coenzyme in the following reactions:

1. Oxidative decarboxylation of a-keto acids like a-ketoglutarate, pyruvate, a-keto analogue of branched chain amino acids.

Oxidative decarboxylation of pyruvate:

Pyruvate combines with TPP : Carbon atom between the N and S atoms in the thiazole ring of thiamine is highly acidic. It ionizes to form a carbon ion, which readily adds to the carboxyl group of pyruvate.

## Basic Biological Science Part I

Protonation then gives hydroxyl-ethyl thiamine pyrophosphate. Hydroxyethyl group is oxidized to form an acetyl group which is transferred from acetyl-lipoamide to coenzyme A to form acetyl-coA. Oxidised form of lipoamide is regenerated. NAD is oxidant catalysed by dihydrolipoyl dehydrogenase, FAD is the prosthetic group of the enzyme.

2. Transketolase reaction: The enzyme transketolase contains a tightly bound

TPP as its prosthetic group. The mechanism is similar in that an activated aldehyde unit is transferred to an acceptor. The acceptor is an aldolase whereas in pyruvate dehydrogenase reaction it is lipoamide

**Deficiency** Deficiency of thiamin causes beriberi.

Beriberi is of three Dry beriberi wet beriberi and infantile beriberi.

Early symptoms of dry and wet beriberi are:

- (i) Anorexia
- (ii) Heaviness and weakness of legs
- (iii) Palpitation and precordial pain
- (iv) Numbness in legs and complaints of pain and needles
- (v) Tenderness of calf muscles
- (vi) Tendon jerks are usually sluggish

In dry beriberi essential feature is polyneuropathy. Notable features of wet beriberi are oedema, pain in legs after walking, enlargement of heart, tachycardia and increase in pulse pressure.

## Basic Biological Science Part I

Infantile beriberi occurs in breast fed infants. It is acute and fatal. Infant becomes restless, cries a lot, passes less urine, puffiness, suddenly cyanosed, dyspnoea, tachycardia and dies within 24-48 hours.

Antivitamins Oxythiamin and pyriithiamin.

### **Riboflavin**

Chemically riboflavin consists of an isoalloxazine ring attached to ribity 5-phosphate in its structure

**Sources** Milk, liver, kidney, heart. Germinating seeds and green vegetables are the best sources of riboflavin.

### **Daily requirements**

Infants : 0.4-0.6 mg      Children : 0.8-1.2 mg

Adults : 1.2-1.7 mg

An additional amount of 0.3-0.6 mg is required during pregnancy and lactation.

**Functions** Active riboflavin is in the coenzyme forms i.e. flavin mononucleotide (FMN) and flavin adenine dinucleotide (FAD) which are formed as shown in fig 5-7

1. FMN and FAD as prosthetic groups of oxidoreductase enzymes. (fig. 5-8) These enzymes are known as flavoproteins. FMN and FAD serve as components of respiratory chain.

Some reactions catalysed by flavoproteins and metalloflavoproteins are

given in table 5-2

### **TABLE 5-2**

## Basic Biological Science Part I

### Some Reactions catalysed by Flavo and Metalloflavo-proteins.

Enzyme	Electron donor	Product	Coenzyme	Electron acceptor
D-Amino acid oxidase	D- Amino acid	a-Keto acid +NH <sub>3</sub>	FAD	O <sub>2</sub> -H <sub>2</sub> O <sub>2</sub>
L-Amino acid oxidase (liver)	D-Amino acid	a-Keto acid+NH <sub>3</sub>	FAD	O <sub>2</sub> -H <sub>2</sub> O <sub>2</sub>
L-Amino acid oxidase (Kidney)	D-Amino acid	a-Keto acid+NH <sub>3</sub>	FMN	O <sub>2</sub> -H <sub>2</sub> O <sub>2</sub>
Aldehyde oxidase (liver)	Aldehydes	Carboxylic acid	FAD Fe,Mo	Respiratory chain
Glycolic acid oxidase	Glycolate	Glyoxylate	FMN	O <sub>2</sub> -H <sub>2</sub> O <sub>2</sub>
Acyl-CoA dehydrogenase	Acyl-CoA	Enoyl-CoA	FAD	Electron transferring flavoprotein
Xanthine oxidase	Xanthine	Uric acid	FAD Mo, Fe	O <sub>2</sub>
Lipoyl dehydrogenase	Reduced lipoic acid	Oxidised lipoil acid	FAD	NAD
Cytochrome e reductase	NADH	NAD	FAD Mo	Respiratory chain

# Basic Biological Science Part I

## Deficiency Symptoms

- (i) Angular stomatitis (fissures at the angles of mouth)
- (ii) Cheilosis (a zone of red epithelium at the line of closure of lips)
- (iii) Glossities (inflammation of tongue)
- (iv) Dyssebacia (appearance of enlarged follicles around the sides of nose, plugged with dry sebaceous material)
- (v) Scrotal dermatitis
- (vi) Vascularisation of cornea leading to lachrymation misty vision and photophobia.

Antivitamins Galactoflavin and dichlororiboflavin.

Niacin (Pellagra Preventive Factor)

Chemically niacin or nicotinic acid is a pyridine derivative

**Sources** Yeast, unrefined cereals and grains, rice polishing, milk, egg, tomato, fruit and green leafy vegetables are good sources of niacin. Tryptophan is precursor of niacin (60 mg of tryptophan provides 1 mg of niacin)

### Daily requirements

Infant	:	5-8 mg	Adult Male	:	
		16-20 mg			
Children	:	9-16 mg	Female	:	12-16 mg
Adult Male	:	16-20 mg	Adult female	:	12-16 mg

During pregnancy additional 3 mg and during lactation additional 7 mg are required.

## Basic Biological Science Part I

**Functions** Nicotinamide adenine dinucleotide (NAD) and nicotinamide adenine dinucleotide phosphate (NADP) are the active forms of niacin. The pathway for synthesis of NAD<sup>+</sup> and NADP<sup>+</sup> is shown in

NAD is a component of respiratory chain. NAD and NADP are coenzymes for many oxidoreductase enzymes.

Some reactions catalysed by nicotinamide nucleotides enzymes are given in Table 5.3

### Some Reactions catalysed by NAD/NADP Dependent Enzymes

Enzyme	Substrate	Product	Coenzyme
Alcohol dehydrogenase	Ethanol	Acetaldehyde	NAD <sup>+</sup>
Isocitrate dehydrogenase	Isocitrate	$\alpha$ -Ketoglutarate+CO <sub>2</sub>	NAD <sup>+</sup>
Glycerol phosphate dehydrogenase	Glycerol 3-phosphate	Dihydroxyacetone phosphate	NAD <sup>+</sup>
Lacticdehydrogenase	Lactate	Pyruvate + CO <sub>2</sub>	NAD <sup>+</sup>
Malic enzyme	L-Malate	Pyruvate + CO <sub>2</sub>	NADP <sup>+</sup>
Glyceraldehyde 3-phosphate dehydrogenase	Glyceraldehyde 3-phosphate	1, 3 bisphospho glycerate	NAD <sup>+</sup>
Glucose 6-phosphate dehydrogenase	Glucose 6-phosphate	6-phospho-gluconic acid	NADP <sup>+</sup>

## Basic Biological Science Part I

Glutathione reductase	Oxidised glutathione	Reduced glutathione	NADPH <sup>+</sup>
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Nicotinic acid is used therapeutically for lowering plasma cholesterol. It inhibits flux of three fatty acids from adipose tissue resulting into less synthesis of cholesterol containing lipoproteins.

**Deficiency** Deficiency of niacin results into pellagra which is characterized by the following symptoms

- 1. Dermatitis:** Erythema (resembling severe sun burn), roughening , thickening and dryness of skin.
- 2. Dementia (delirium):** with other C.N.S. symptoms like weakness, anxiety, depression, irritability and failure of concentration.
- 3. Diarrhoea:** is common but not present always and is associated with anorexia, nausea, dysphagia and dyspepsia.

### Pyridoxine(B<sub>6</sub>)

Vitamin B<sub>6</sub> consists of three closely related naturally occurring pyridine derivatives and their phosphates All the three have equal vitamin like activity.

**Sources** Yeast, seeds, wheat, corn, liver, milk, egg and green leafy vegetables are good sources of pyridoxine.

**Daily requirement** 2 mg

### Functions

- (i) Pyridoxal phosphate (B<sub>6</sub>PO<sub>4</sub>) is the major coenzyme required for the

## Basic Biological Science Part I

activity of the enzymes transaminase decarboxylase, deaminase, transsulphurase, kynureninase and phosphorylase.

**Mechanism of transamination** Pyridoxal phosphate (PLP) enzymes form covalent Schiff-base intermediates with their substrate. In the absence of substrate the aldehyde group of PLP is in Schiff-base linkage with the  $\alpha$ -amino group of a specific lysine residue at the active site. The  $\alpha$ -amino group of the amino acid substrate displaces the  $\text{NH}_2$  group of the active site lysine. The amino acid-PLP Schiff-base that is formed remains tightly bound to the enzyme by non-covalent forces. This Schiff-base and the one between PLP and the active site lysine are aldimines. During catalysis, the double bond in amino acid-PLP Schiff-base linkage shifts position to form ketimine which is then hydrolysed to pyridoxamine phosphate enzyme and an  $\alpha$ -keto acid

- (ii) It is also required as coenzyme in the formation of  $\gamma$ -amino butyrate (regulator of neuronal activity) and serotonin (neurohormone).

**Deficiency symptoms** In the deficiency of  $\text{B}_6$ , the symptoms are:

- (i) Impaired growth
- (ii) Acrodynia (typical dermatitis)
- (iii) Edema of connective tissue layer of skin
- (iv) Convulsion
- (v) Hyperirritability
- (vi) Demyelination of peripheral nerves and degeneration of axons
- (vii) Severe microcytic hypochromic anemia



## Basic Biological Science Part I

### **Pantothenic Acid**

It consists of b-alanine joined to pantoic acid through a peptide bond

**Sources** Important sources are yeast, liver, rice polishing, wheat germ, milk, meat, egg, leafy vegetables and fruits.

#### **Daily requirements**

Infants : 1-2 mg

Children : 4-5 mg

Adults : 5-10 mg

**Functions** It is a constituent of CoA and acetyl CoA are involved in all cellular metabolism.

**Deficiency** Anemia frequently occurs in pantothenic acid deficiency.

### **Lipoic Acid**

Chemically it is 6,8-dithio octanoic acid. It exists in oxidised and reduced forms

**Function** It acts as coenzyme in oxidative containing sulphur

**Sources** Egg yolk, liver, kidney. Yeast, milk, fruits and vegetables.

**Daily requirement** 50-60 mg

#### **Functions**

1. Biotin acts as cocarboxylase. Is is required in the fixation of CO<sub>2</sub> in the conversions of pyruvate to oxaloacetate, acetyl-COA to malonyl-CoA, propionate to succinate and in purine synthesis.

**Deficiency** Biotin deficiency occurs by taking raw egg white which contains avidin. Avidin combines very tightly with biotin, preventing its

## Basic Biological Science Part I

absorption and inducing biotin deficiency or egg white injury. The symptoms are:

- (i) Retarded growth
- (ii) Loss of hair
- (iii) Depression
- (iv) Hallucination
- (v) Muscle pain
- (vi) Dermatitis

### **Folic Acid**

Chemically folic acid consists of the base pteridine, attached to p-aminobenzoic acid and glutamic acid. In liver it is present as pentaglutamate.

**Sources** Yeast, liver and leafy vegetables like cauliflower are the major sources of folic acid.

Daily requirements

Infants : 50mg

Children : 100-300 mg

Adults : 400 mg

During pregnancy and lactation : 600-800 mg

**Functions** Folic acid is very intimately involved in the metabolism of the carbon moieties in various ways. In such reactions, folic acid is first, reduced to tetrahydrofolic acid (FH<sub>4</sub>) in the presence of reduced NADP and vitamin C. The reaction is catalysed by folic acid reductase. Tetrahydrofolic acid can accept formate, formaldehyde (CHO)

## Basic Biological Science Part I

or hydroxymethyl group ( $-\text{CH}_2\text{OH}$ ) forming 5-formyl-5,6,7,8-or tetrahydrofolic acid or 5-hydroxymethyl-5,6,7,8-tetrahydrofolic acid. Serine is the major source of a carbon unit in the form of a methylenegroup, which it transfers reversibly to  $\text{H}_4$  folate to form glycine and  $\text{N}^5, \text{N}^{10}$ -methylene  $\text{H}^4$  folate which plays a central role in one carbon unit metabolism. These derivatives, later on act as donor of 'One carbon moieties' in various biotransformations, such as ,in the conversion of glycinamide ribotide to formylglycinamide ribotide in purine biosynthesis, conversion of 4-amino-5-imidazole carboxamide ribotide conversion of d-UMP into 5-hydroxymethyl-d UMP and finally into TMP, glycine to serine and homocystein to methionine. Conversion of homocysteine to methionine requires  $\text{N}^5$ -methyl- $\text{FM}^4$ , glycine into serine requires  $\text{N}^5, \text{N}^{10}$ -hydroxymethyl-FJ methionine  $\text{H}^4$  d-UMP to 5-hydroxymethyl d-UMP requires  $\text{N}^{10}$  hydroxymethyl- $\text{FH}^4$ , 4-amino-5-imidazole carboxamide ribotide to N-formyl-4-amino-5-imidazole carboxamide ribotide requires  $\text{N}^{10}$  formyl- $\text{FH}_4$  and glycinamide ribotide to formylglycinamide requires  $\text{N}^5, \text{N}^{10}$  anhydroformylfolic acid .

Formiminoglutamate (Figlu), a catbolite of histidine transfers its formimino group to  $\text{H}_4$  folate to form  $\text{N}^5$ -formimino  $\text{H}_4$ -folate In folate deficiency figlu will accumulate after oral challenge with histidine.

**Deficiency** Folic acid deficiency in man causes megaloblastic anemia.

**Anivitamins** Aminopterin and amethopterin.

**Cyanocobalamin (B<sub>12</sub>-antipernicious anemia factor)**

## Basic Biological Science Part I

The structure of vitamin B<sub>12</sub> consists of a central portion of 4 reduced and extensively substituted pyrrole rings surrounding a central cobalt atom. This central structure is referred as corrin ring system.

Below the corrin ring system there is 5,6-dimethyl benzimidazole riboside connected at one central cobalt atom and at the other end to ribose moiety through phosphate and aminopropanol to a side chain on ring IV of tetrapyrrole nucleus. A cyanide group is coordinately bound to CO atom which may be removed .

**Sources** Rich sources of vitamin B<sub>12</sub>, are liver, kidney, meat, fish and egg.

Daily requirements

Infants : 0.3 mg

Children : 1-2 mg

Adults : 3 mg

During pregnancy and lactation : 4 mg

**Functions** Deoxyadenosyl cobalamin (Cobamide) is the active form of vitamin B<sub>12</sub>, It acts as coenzyme in isomerisation of methyl malonyl CoA to succinyl CoA aid in the combined conversion of homocysteine to methionine and methyl tetrahydrofolate to tetrahydrofolate By this reaction stores of methionine are maintained in the body and tetrahydrofolate is made available to participate in purine and pyrimidine synthesis.

## Basic Biological Science Part I

**Deficiency** Vitamin B12 deficiency is caused due to reduced absorption because of lack intrinsic factor, resulting into pernicious anemia.

### **Inositol**

This Compound exists in the natural form as mesoinositol (myoinosito) which is biologically effective as a growth factor. There are nine stereo-isomers of which the biologically active form is the optically inactive form.

**Sources** Inositol is widely distributed in most natural foodstuffs of plants and animal origin. Yeast, milk, nuts and fruits are the best sources.

Phytic acid is inositol hexaphosphoric acid The calcium and magnesium phytins are present in corn.

**Functions** It acts as a lipotropic agent along with choline in experimental animals. It converts neutral triacylglycerols and phosphatidic acids to inositol phosphatides (lipositols) Some hormones use the latter as their second messengers to release  $Ca^{2+}$ . Deficiency of inositol results in alopecia and failure of growth Inositol probably forms a complex with tocopherols needed for the proper storage of creatine in the muscle.

**Choline** Choline is trimethyl-hydroxyethyl-ammonium hydroxide It is synthesized in the body from glycine and is related to one-carbon metabolism and folic acid. Some authors do not include choline

## Basic Biological Science Part I

under vitamins as it could be synthesized by the human system from serine through ethanolamine in required amounts.

**Sources** Meat, egg yolk, bread, cereals, beans and peanuts are good sources.

**Functions** Choline is a lipotropic substance Choline is a constituent of various phospholipids like the lecithins and sphingomyelins which have important physiological functions.

**Para-aminobenzoic acid** It was observed that a diet containing all the known vitamins like thiamin, riboflavin, niacin, pyridoxine and pantothenic acid was still lacking in a factor concerned with lactation in rats. The hair of black rats on such a diet turned grey and this factor was called anti-grey hair factor.

This is now identified as para-aminobenzoic acid. The compound is already present as a constituent part of folic acid and is believed to be necessary for the biosynthesis of folic acid by micro-organisms in the intestine.

## FAT SOLUBLE VITAMINS

### Vitamin A

Vitamin A is a complex primary alcohol. It is a polyisoprenoid compound with a  $\beta$ -ionone or cyclohexenyl ring. Carotenes are the precursor of vitamin A. These carotenes include  $\alpha$ ,  $\beta$  and  $\gamma$  carotenes. Out of these  $\beta$ -carotene has been found to be most effective precursor of vitamin A. (On cleavage 2 molecules of vitamin A are formed) whereas  $\alpha$  and  $\gamma$ -forms are about half effective (the two ionone rings are different

## Basic Biological Science Part I

so on cleavage 1 molecule of vitamin A is formed) (fig. 5-25). Vitamin A<sub>1</sub> (with one double bond) and A<sub>2</sub> (with two double bonds) are known to exist.

**Sources** Vegetable sources contain precursor form and include all yellow vegetables and fruits e.g. carrot, sweet potato, apricot, yellow peaches and leafy green vegetables.

### Daily requirements

Infants : 1500 IU

Children : 2000-3000U

Adults : 5000IU

During pregnancy and lactation : 6000-8000 Iu

(1 IU=activity caused by 0.34 mg of retinol ester / 0.3mg of retinol / 0.6 mg of B-carotene).

**Physiological functions** Compounds from animals sources possessing vitamin A like activity are retinol are retinol, retinal and retinoic acid These three have own unique physiological functions.

**Retinol** It acts like a steroid hormone. Retinol bound with cellular retinol binding protein binds to nuclear and thus involved in the control of expression of certain genes.

Various physiological and biochemical function of retinol are mainly due to its role in maintenance of normal integrity of epithelium. As such retinol plays an important role in normal growth. It is related to normal development of various epithelial tissues particularly salivary

## Basic Biological Science Part I

gland, tongue respiratory tract, genitor-urinary tract, glands of internal secretion and eyes.

**Skin** In deficiency of vitamin A skin becomes dry, rough and development of pappular eruptions occur (Tad's skin).

**Bones and teeth** Abnormal development of bones and teeth occur in deficiency of vitamin A.

**Urolithiasis** In deficiency person becomes more prone for urinary stone formation. The epithelium of urinary tract is keratinized resulting into bacterial invasion which causes alkalinuria. This favours precipitation of calcium and phosphate.

**Reproduction** In deficiency of vitamin A, in males atrophy of germinal epithelium occurs and in females normal estrous cycle is not maintained

**Anti infective vitamin** Vitamin A maintains resistance to infections

**Retinal** It plays an important role in night vision. Rhodopsin (ascis-retinal + opsin) is essential for vision in dark. In presence of light rhodopsin is converted to trans-retinal and opsin. For regeneration of rhodopsin cis-retinal is essential.

### Deficiency Diseases

**Night blindness** As retinal is required in the formation of rhodopsin, in deficiency of vitamin A the capacity to look into dark is decreased. This is referred to as nyctalopia or night blindness



## Basic Biological Science Part I

**Xerophthalmia and keratomalacia** Vitamin A deficiency, because of morphological changes in epithelial surface, results into Xerophthalmia. Earliest signs are lachrymal glands cease to produce tears, conjunctiva becomes dry, thick, pigmented and acquires a peculiar smoky appearance. Desquamated and thickened conjunctival epithelium forms glistening white plaques, which are triangular in shape and firmly adhered with underlying epithelium. These are called Bitot's spot. When dryness spreads to cornea, it takes a dull, hazy and lack luster appearance. Later cornea undergoes necrosis and ulceration (Keratomalacia).

### **Hypervitaminosis A**

Massive administration of vitamin A causes nausea, irritability, headache, abnormal calcification, pain and swelling over bones.

### **Vitamin D**

Vitamin D is a group of steroids (D1,D2,D3,D4 and D5) chiefly occurring in animals but also in plants and yeast. The physiologically active forms are D2 and D3 provitamins D are ergosterol (plants) and 7 dehydrocholesterol (in skin) These on UV-irradiation or sunlight exposure give rise ergocalciferol (D2) and cholecalciferol (D3) respectively. Vitamin D possesses cyclopentanophenanthrene ring in the structure

**Sources** Richest sources are liver and viscera of fish. Other sources are egg and cheese. Milk is poor source Its vitamin D content can be increased by UV irradiation.

### **Daily requirements**

## Basic Biological Science Part I

Infants and children : 400 IU

Adults : Do not require under normal conditions.

During pregnancy and lactation : 500-600 IU

(1 IU= activity caused by 0.025 mg of calciferol)

### Physiological functions

- (i) Vitamin D has a direct effect on calcification.
- (ii) It increases calcium and phosphorous absorption from intestine.
- (iii) It has a role in citrate metabolism as administration of vitamin D increases citrate contents of blood, bones, kidney, heart and small intestine.
- (iv) Vitamin D is involved in renal handing of phosphorous.

**Mode of action of vitamin D** Vitamin D acts as prohormone In liver

D<sub>3</sub> (or D<sub>2</sub>) is hydroxylated on position 25 to form 25-hydroxy cholecalciferol by the enzyme D<sub>3</sub>-25 hydroxylase In kidney 25-hydroxycholecalciferol 1 $\alpha$ -hydroxylase to form 1 $\alpha$ -25-dihydroxy cholecalciferol (calcitriol)-the most active metabolite of vitamin D.

25-Hydroxy D<sub>3</sub> can also be hydroxylated at 24 position to form 24,25 dihydroxy D<sub>3</sub> which is reciprocally related to the level of 1 $\alpha$ -25-dihydroxy D<sub>3</sub> The action of serum calcium and phosphorous concentrations on 1,25-dihydroxy D<sub>3</sub> formation is depicted in

Calcium effects activity of hydroxylase through parathormone whereas phosphate effects directly.

## Basic Biological Science Part I

Calcitriol enters the cell and binds to a specific cytoplasmic receptor molecule. This complex is translocated to nucleus where it effects synthesis of calcium binding protein, necessary for absorption of calcium.

### **Deficiency Diseases**

**Rickets in children** Rickets is characterized by faulty calcification of bones due to low vitamin D content of body a deficiency of calcium and phosphorous in diet or due to both. The features are : infant becomes restless fretful and pale with flabby and toneless muscles abdomen is distended and there is extension and widening of epiphysis at growing points.

**Osteomalacia in adults** Disease is limited to female sex. The bones specially pelvic girdle ribs and femora become soft, painful and deformed Softening of bones is primarily due to deficiency of vitamin D and to a lesser extent to the deficiency of calcium.

**Vitamin E (Antisterility Factor)** Vitamin E activity is attributed to a series of compounds –the tocopherols (Tokos-child birth, pherein-to bear). There are 4 such substances –a,B,Y and S All are isoprenoid substituted 6-hydroxychromanes D-a Tocopherol has widest natural distribution and greatest biological activity.

**Sources** Rich sources of vitamin E are milk, egg, muscle meat, fish, cereals and leafy vegetables.

**Daily required** Adults : 30 mg

**Functions**

## Basic Biological Science Part I

- (i) Vitamin E acts as natural antioxidant. It appears to be the first line of defence against peroxidation of polyunsaturated fatty acids contained in phospholipids of cellular and subcellular membranes and prevent the deleterious effects. The auto-oxidation of membrane lipids proceeds as a chain reaction. The primary event is the interaction of polyunsaturated fatty acid and free radical (OH) to produce a carbon centred radical. The carbon centred radical rapidly reacts with molecular oxygen molecule of polyunsaturated fatty acid.

Tocopherol acts as the most effective chain breaking naturally occurring antioxidant. It reacts with peroxy radical and converts it to inactive products.

- (ii) Vitamin E and selenium act synergistically and reduce requirement for each other in the other in the body.
- (iii) It prevents hepatic necrosis produced by lack of 'S' containing amino acids in dietary proteins.
- (iv) Vitamin E acts as cofactor in electron transfer system between cytochromes b and c.

**Deficiency symptoms** In human deficiency causes muscle weakness muscular dystrophy fragility of erythrocytes with mild anemia.

In animals deficiency causes resorption of foetus in females and atrophy of spermatogenic tissue leading to permanent sterility in males.

**Clinical use** In human vitamin E is used in cases of habitual abortion.

# Basic Biological Science Part I

## Enzymes

Enzymes are biocatalysts. They are protein in nature colloidal, thermolabile and reaction specific. Enzymes produced in the cells of a particular tissue and required by the same cells are known as intracellular enzymes whereas the enzymes produced in cells of a particular tissue and used by the cells of other tissue(s) are known as extracellular enzymes.

## Classification

According to International Union of Biochemistry (IUB) system, enzymes are classified into 6 major classes, depending on reaction type and reaction mechanism.

- 1. Oxide-reductases:** The enzymes catalyse oxidation reduction reactions between two substrates and require coenzymes like NAD<sup>+</sup>/NADP<sup>+</sup>, FAD etc. The common examples are cytochrome oxidase, alcohol dehydrogenase, glutamate dehydrogenase catalase peroxidase etc.
- 2. Transferases :**Enzymes which catalyse transfer of a group other than hydrogen from one substrate to another are known as transferases The important examples are transaminase transmethylases, acyltransferases. Etc.
- 3. Hydrolases:** These enzymes catalyse hydrolysis of ester, ether and peptide bonds with addition of water molecule. Examples are

## Basic Biological Science Part I

B-galactosidase, pseudo-cholinesterase, trypsin, chymotrypsin, lipase etc.

- 4. Lyases:** Enzymes which catalyse removal of groups from substrate by mechanism other than hydrolysis leaving double bond are known as lyases. Common examples are fumarase, citrate lyase and various decarboxylases.
- 5. Isomerases:** These enzymes catalyse interconversion of optical geometric or positional isomers. Examples are cis-trans isomerases, racemases and epimerases.
- 6. Ligases :** These enzymes catalyse linking together of two compounds, the energy required is derived from high energy phosphate bond like ATP and from pyrophosphate. Examples are succinyl-CoA synthetase, succinic, glutamine synthetase and acetyl-CoA carboxylase.

### Nomenclature

Each enzyme has a code number (EC) that characterizes the reaction type as to class (first digit) subclass (second digit) and subclass (third digit). The fourth digit is for specific enzyme. Thus EC 2-7-1-1 denotes class 2 (transferase) subclass 7 (transfer of phosphate) subclass (an alcohol as phosphate acceptor) and last 1 denotes the name of the enzyme hexokinase.

Some other examples are:

- 1.4.1.3.1.1 - Glutamic dehydrogenase
- 1.11.1.6 - Catalase

## Basic Biological Science Part I

1.1.1.1 - Alcohol dehydrogenase

2.4.1.1 - Phosphorylase

**4.1.2.7 - Aldolase**

### **Proenzymes or Zymogens:**

Many enzymes are secreted in the form of inactive precursor form. The precursor forms are termed proenzymes or zymogens. Conversion of a proenzyme to the mature or active form involves selective proteolysis. Examples of enzymes manufactured as proenzyme or zymogen include the digestive enzymes pepsin, trypsin and chymotrypsin (pepsinogen, trypsinogen and chymotrypsinogen zymogens respectively).

Proenzyme secretion is probably a protective mechanism to prevent autodigestion of the tissue of origin. Proenzyme also facilitates rapid mobilization of an activity in response to physiological demand.

### **Enzyme Specificity**

A given enzyme catalyses only a very few reactions (frequently only one). It is the most significant property of an enzyme to catalyse one specific reaction. For example, the reaction of hydrolysis of urea to ammonia and carbon dioxide is catalysed by urease. Urea is the only substrate for urease.

### **Group Specificity**

Many enzymes show group specificity and catalyse the same type of reactions. For example, maltase is specific for  $\alpha$ -glycosidic linkages and hydrolyses not only maltose but  $\alpha$ -methyl glucose also. Which of the

## Basic Biological Science Part I

possible reaction will occur in living organism depends on the relative concentration of alternative substrates in the cells and relative affinity of the enzyme for substrates.

### **Optical Specificity**

Enzymes generally show absolute optical specificity for at least a portion of a substrate molecule. Thus maltase catalyses the hydrolysis of a but not b-glucosides Human enzymes are specific for L-amino acids and D-carbohydrates.

### Mechanism of Enzyme Action

Recent view of the mechanism of enzyme action involves for at least a portion of a substrate molecule Thus maltase catalyses the hydrolysis of a but not B-glucosides. Human enzymes are specific for L-amino acids and D-carbohydrates.

### **Mechanism of Enzyme Action**

Recent view for the mechanism of enzyme action involves combination of enzyme with its substrate to form an intermediary enzyme substrate complex which dissociates to form product and the free enzyme. The free enzyme is re-utilised in the similar manner.

Enzyme + Substrate = Enzyme Substrate = Enzyme + product

(E)            (S)            (E.S)            (P)

Most of the enzyme catalysed reactions are reversible in nature The equilibrium of the reactions may be shifted towards either direction depending upon the concentration of substrates or products in the reaction medium.



## Basic Biological Science Part I

### Catalytic Site of Enzyme

According to Fischer's template or lock and key model. The active site of the enzyme already exists in proper conformation even in the absence of substrate and provides a rigid pre-shaped template, fitting with the size form and groups of the substrate. The active site binds with the substrate and catalyses the reaction without any change in its own three dimensional structure. A more general model is the induced fit model of Koshland. In induced fit model the substrate induces conformational changes in the enzyme which aligns amino acid residues or other groups on the enzyme in the correct special orientation for substrate binding and for catalysis. Substrate analogues may cause some of the correct conformational changes. On attachment of the true substrate (A) all groups are aligned correctly. But attachment of a substrate analogue (B and C) that may be big or small induces incorrect alignment. This flexibility of the active site explains the complex saturation kinetics, competitive inhibition and allosteric modulation of enzyme activity.

### **CELLULAR ELEMENTS OF BLOOD :**

The cellular elements of blood are :

4. Red blood cells (Erythrocytes)
5. White blood cells (Leucocytes)
6. Platelets (Thrombocytes)

## Basic Biological Science Part I

### **RED BLOOD CORPUSCLES (RBC) OR ERYTHROCYTES:**

They are circular biconcave, disc shaped cells. They do not have a nucleus. But they have a respiratory pigment called *hemoglobin*. The normal RBC count is 4.5 to 5 millions per cu.mm. RBCs serve important functions such as transport of oxygen and maintenance of acid base balance. They are synthesized in the bone marrow found at the ends of long and short bones. The average life span of RBC is about 120 days.

**HEMOGLOBIN:** It is the respiratory pigment of erythrocytes. The red colour of blood is due to hemoglobin. It contains globin, a protein which is conjugated with heme (hemoglobin = heme + globin). Heme molecule contains four pyrrole rings with iron in the centre. The hemoglobin content of body is about 15 G per 100 ml of blood, Anemia occurs due to a decrease in hemoglobin.

The functions of hemoglobin are:

4. Transport of oxygen and carbon dioxide
5. Maintenance of acid base equilibrium.
6. As a source for the formation of bilirubin (Bilirubin is formed from porphyrin fraction of hemoglobin).

Hemolysis is the escape of hemoglobin from RBC into blood. This is caused by hypotonic condition, certain drugs and toxins.

## Basic Biological Science Part I

**WHITE BLOOD CELLS (WBC):** They are colourless cells containing a nucleus. They are larger in size than RBCs. Also their number is less when compared to RBCs (about 8000 per cu.mm of blood).

**Classification of WBCs:** WBCs are classified as:

3. Granulocytes which are of three types : neutrophils, eosinophils and basophils.
4. Agranulocytes which are of two types : Lymphocytes and monocytes.

### Functions of WBCs

5. Protection against infection. This is done by neutrophils and monocytes which engulf bacteria. This process is called as phagocytosis.
6. To aid in the repair of injured tissues.
7. To produce immune substances which defend against diseases. This is done by Lymphocytes through the synthesis of gammaglobulin.
8. Basophils secrete an anticoagulant substance called heparin.

**PLATELETS OR THROMBOCYTES:** These are round or oval shaped cells with biconvex surface. They are roughly one fourth of the size of a RBC Normal platelet count is 2 to 5 lakh per cu.mm of blood.

## Basic Biological Science Part I

Platelets do not have a nucleus. But cytoplasm contains distinct granules.

They are synthesized by megakaryocytes (giant cells) of bone marrow

### Normal and average values of cellular Elements of blood

Blood elements	Normal value	Average value
Red blood cells(RBC's)	8.5 to 5.5 million	5 million 8,000
White blood cells (WBC's)	6,000 to 10,000	66%
Granulocytes	60 to 70%	1%
Eosinophils	1 to 2%	1%
Basophils	0.5 to 2%	25%
Lymphocytes (Large and small)	20 to 30%	5%
Monocytes	4 to 8%	3.5 lakhs
Platelets	2 to 5 lakhs	

### LABORATORY TECHNIQUES :

❖ ELECTROPHORESIS

❖ CHROMATOGRAPHY

❖ FLAME PHOTOMETRY

# Basic Biological Science Part I

## 1. ELECTROPHORESIS

The movement of charged particles towards the oppositely charged electrode, under the influence of an electric current is referred to as electrophoresis. The cations move towards Negative cathode while anions move towards positive anode.

The electrophoresis system consists of the electrophoresis tank to hold the buffer, fitted with electrodes and a power and a supply.

The different types of electrophoresis techniques commonly used are **zone electrophoresis, isoelectric focusing and immuno -- electrophoresis.**

### I )Zone Electrophoresis

(a) **Paper electrophoresis:** The support media is the Whatman No. 1 filter paper strip.

The sample is applied on the strip moistened with buffer, placed in the tank so that the free ends of the strip are dipped into the buffer contained in the chamber. An electric current is passed for suitable period so as to allow a good migration of the molecules. The paper is removed, dried and stained Identification or quantitative estimation is made by comparing with standard sets or using a scanner.

(b) **Gel electrophoresis:** The charged particles migrate through a gel by an electric field

## Basic Biological Science Part I

and are separated by their different electrophoretic abilities. The commonly used gels are cellulose acetate membrane gel, agarose gel, polyacrylamide gel and sodium dodecyl sulphate gel.

The gel is prepared in the buffer and spread over microscopic slides and allowed to solidify. A cut is made into the gel layer and sample is applied and electrophoresis is carried out for about 90 minutes. After the electrophoretic run, the slide is fixed using suitable fixative like acetone, methanol etc. and stained with suitable dye (Amidoschwartz, Ponceau S etc.) and scanned using a densitometer.

### **II ) Immunoelectrophoresis**

The technique involves both electrophoresis and immunological reactions. The sample is subjected to electrophoresis. The antibody is applied parallel to electrophoretic separation. The antibodies diffuse and precipitation occurs when they come in contact with antigens. The precipitation bands are then identified.

## **2.CHROMATOGRAPHY**

Chromatography is a collective term referring to a group of separation process whereby a mixture of solutes, dissolved in a common solvent, are separated from one another by a different distribution of the solutes between the two phases. One phases. One phases, the solvent is mobile and carries the mixture of solutes through the other phase, The fixed or Stationary phase.

## Basic Biological Science Part I

Chromatographic methods are generally classified according to the physical state of the solute carrier phase for example liquid Chromatography and gas Chromatography. The classification is also based on the interaction between the stationary phase and mobile phase. These interactions include the physico-chemical principles such as adsorption, partition, ion exchange, molecular sieving and affinity. The principle of some of the common – techniques are given below.

### 1. Partition Chromatography

The molecules of a mixture are partitioned between the stationary phase and mobile phase. It includes:

(a) **Paper Chromatography:** It is basically a type partition Chromatography between water absorbed onto the cellulose fibre of the paper and a liquid mobile phase in a closed tank. The paper is hung by means of clips or strings and the lower end is made to dip into the eluting solvent. The material under test is applied as a spot 2.5 cm or so above the lower end of the paper and marked with a pencil. Eluents are normally aqueous mixtures of organic Solvents, acids or bases. The descending technique has also been used and in this case the top of the paper dips into a trough containing eluent which travels downwards. The spots are applied at the top of paper close to the solvent front. A closed tank is necessary for these operations. Elution time varies from several hours to a day depending on the solvent system and paper. For more efficient separations the dried paper is eluted with a different solvent along a direction which is  $90^\circ$  from

## Basic Biological Science Part I

that of the first elution. This is referred as two dimensional paper Chromatography. In a third application (circular paper Chromatography) ordinary circular filter papers are used.

After the solvent have travelled the required distance in the above separations, the papers are air dried and the spots are revealed by their natural colours or by spraying with a reagent (for example ninhydrin reagent in case of amino acids) that forms a coloured product with spots. The migration of a substance is expressed as  $R_f$  value:

$$R_f = \frac{\text{Distance travelled by the substance}}{\text{Distance travelled by solvent front}}$$

The  $R_f$  value of each substance is characteristic of a given solvent system and helps in the identification of that substance.

### (b) Thin layer Chromatography (TLC): Thin layer

Chromatography is in principle similar to paper Chromatography. The adsorbent (e.g. silica, alumina, cellulose) is spread on a rectangular glass plate (or solid inert plastic sheet). Some adsorbents (e.g. silica ) are mixed with a setting material (e.g.  $\text{CaSO}_4$ ) which causes the film to set on drying . The adsorbent can be activated by heating at  $100-110^\circ$  for a few hours. The spots will need to be placed at such a distance as to ensure that when the lower end of the plate is immersed in the solvent, the spots are a few mm above the elution solvent. Elution is called out in a close tank. It requires less than three



## Basic Biological Science Part I

hours for the solvent to reach the top of the plate. Good separation at right angles to the first as in two dimensional paper chromatography. The spots or areas by spraying the reagent can be revealed or easily scraped off the plates and eluted with the required solvent and quantitatively estimated.

More recently plates of a standardised silica gel 60 are used. These have a specific surface of  $500\text{m}^2/\text{g}$ . They are so efficient that they have been called high performance thin layer Chromatography (HPTLC). In another variant of thin layer Chromatography the adsorbent is coated with an oil thus producing reverse phase thin layer Chromatography

### (c) **Gas-liquid Chromatography:**

It is used for the separation of volatile substances. The stationary phase is an inert material. Impregnated with a non-volatile liquid and packed in a narrow column. The mixture of volatile material is applied along with the mobile phase which is an inert gas like argon, helium or nitrogen. The separated products are identified and quantitated by a detector.

### **2. Adsorption Column Chromatography**

The adsorbents like silica gel, alumina, Charcoal and calcium hydroxyapatite are packed into a column in a glass tube. The mixture to be separated is applied in a solvent to the column. The individual substances are differentially adsorbed onto the solvent.

# Basic Biological Science Part I

## 2. Affinity Chromatography

Proteins bind non-covalently to other molecules specifically. The desired protein captured by the ligand can be eluted using free ligand molecules or by any suitable reagent which can break the binding. The technique is used for separation and purification of enzymes, vitamins, antibodies etc.

## 3. Ion Exchange Chromatography

It is based on the electric charges of the molecules and ion exchange resins are used for this purpose. The resins are either cation exchange resins or anion exchange resins. In this technique the resin is packed into a column. The mixture to be separated is passed through the column and eluted by using buffer of different pH. Different fractions so obtained are treated with ninhydrin reagent and quantitatively estimated. Certain ion exchange resins are, Dowex-1 DEAE (diethyl aminoethy) cellulose Amberlite IRC-50.

## 4. Gel Filtration Chromatography:

Gels like acrylamide, agarose, dextran are used for separation the solution mixture is applied to column containing gel beads and eluted with suitable buffer. The larger molecules cannot pass through the pores of gel and move faster. The smaller molecules enter the gel beads and are left behind and come out slowly.

Flame photometer is widely used in the clinical laboratory to determine sodium and potassium concentrations in biological fluids.

## Basic Biological Science Part I

### 3.FLAME PHOTOMETRY

Flame photometer is widely used in the clinical laboratory to determine sodium potassium concentrations in biological fluids.

**Principle** Atoms of some metals, when given sufficient heat energy as supplied by a hot flame will become excited and re-emit this energy at wave lengths characteristic for the element. The intensity of the characteristic wave length of radiant energy produced by the atoms in the flame is directly proportional to the number of atoms excited in the flame which is directly proportional to the concentration of the substance of interest in the sample.

The solution is sprayed as a fine mist of droplets into a non-luminous flame which becomes coloured by the characteristic emission of the metal. Light of a wavelength corresponding to the element being analysed is selected by a light filter or prism system and allowed to fall on a photocell. The signal from the photocell gives a measure of the concentration of the element.

# Basic Biological Science Part I

## UNIT --IV

### Micro biology

#### Bacteria

Bacteria are unicellular, microscopic, prokaryotic organisms devoid of chlorophyll-a. They constitute the major group of microorganisms. As they resemble with fungi in some aspects, they have usually been placed in the plant kingdom. But they have some unique features which are not seen in the plants. Therefore, they are treated as a separate group Bacteria. The study of bacteria is termed Bacteriology.

Bacteria are ubiquitous in distribution. They are found everywhere in the soil, water, air and in and on the living beings Rhizobium is a good example for soil bacteria. Escherichia coli is an example for bacteria living in waters contaminated with organic wastes. Streptococcus lactis is found only in the milk of infected mammals. Several bacteria infect plants and animals including man and cause severe diseases in them.

Bacteria are included in the order Schizomycetes of the division Protophyta Antony Van Leeuwenhock (1632-1723) was the first who observed certain Bacteria under his crude lens and described them scientifically Hence he is known as the father of Bacteriology.

## Basic Biological Science Part I

Bacteria can tolerate a wide range of temperatures. The that grow well between 25<sup>0</sup>C and 40<sup>0</sup>C are called mesophilic Bacteria. Those growing in temperatures no less than 45<sup>0</sup>C are known as thermophilic Bacteria The Bacteria growing at temperatures less than 25<sup>0</sup>C are called psychrophilic Bacteria

Certain Bacteria are growing well at pH around 7. They are called neutrophilic Those growing under acidic conditions are called acidophilic Bacteria. Bacteria growing well under alkaline conditions are known as alkaliphilic Bacteria.

### **Salient Features of Bacteria .**

The Salient features of Bacteria are the following

1. Bacteria are unicellular prokaryotic organisms.
2. Their size ranges from 0.5 micron to 3 micron.
3. They are in the form of rods, spheres, spirals or filaments
4. The cell is surrounded by a cell wall and a capsule. The cell wall has peptidoglycon.
5. The cell bears appendages called flagella and pili.
6. The cell may be gram positive or gram negative
7. The nuclear material is represented as a nucleoid without nuclear membrane

## Basic Biological Science Part I

8. One to many circular DNAs called plasmids present in the cell.
9. The cell contains 70S ribosomes alone. The other cell organelles are absent.
10. Bacteria reproduce by binary fission and endospores.
11. They show absorptive mode of nutrition.

### Classification of Bacteria

Lysenko (1959) broadly divided Bacteria into Gram positive Bacteria and Gram negative Bacteria. Those Bacteria which take Gram's stain is called Gram positive Bacteria. Those which do not take up the stain are called Gram negative Bacteria. Each of these groups is divided into genera on the basis of morphology, Catalase activity, growth activity, cell arrangement, oxidase activity, pigment production and fermentation characteristics of Bacteria.

Bergey in his Manual of Determinative Bacteriology divided all Bacteria into 19 groups. In the 9<sup>th</sup> edition of this manual, all Bacteria are divided into four major groups. Each major group is divided into many groups. There are 35 groups of Bacteria in this manual. Each and every group is divided into sub-groups and genera.

According to the Bergey's Manual of Determinative Bacteriology (9<sup>th</sup> edition). There are four major groups of Bacteria. They are-

1. Gram Negative Eubacteria that cell walls

## Basic Biological Science Part I

2. Gram Positive Eubacteria that have cell walls
3. Cell wall less Eubacteria
4. Archaeobacteria

### **1. Gram Negative Eubacteria that Have Cell Walls**

All Bacteria are gram negative and have cell walls. The cells may be coccoid, rods, spiral, helical or vibrioid. They are divided into 16 groups. They are :-

1. Spirochetes
2. Aerobic/ microaerophilic, motile, helical/ vibrioid gram negative Bacteria
3. Non motile, gram negative curved Bacteria.
4. Gram negative aerobic / microaerophilic rods and cocci.
5. Facultatively anaerobic gram negative rods
6. Gram negative anaerobic straight. Curved and helical rods
7. Sulfur reducing Bacteria
8. Anaerobic gram negative cocci.
9. Rickettsias and chlamydias
10. Anoxygenic phototrophic Bacteria

## Basic Biological Science Part I

11. Oxygenic phototrophic Bacteria
12. Aerobic chemolithotrophic Bacteria
13. Budding and/or Appendaged Bacteria
14. Sheathed Bacteria
15. Non-photosynthetic, non-fruiting gliding Bacteria
16. Myxobacteria

### **2. Gram Positive Eubacteria that Have Cell Walls**

This category includes true bacteria having thick cell walls. All bacteria are gram positive. The cells may be rods, cocci or in filaments. There are 15 groups in this category. They are-

1. Gram-positive cocci
2. Endospore-forming, gram-positive rods and cocci
3. Regular non-sporing gram-positive rods
4. Irregular, non-sporing gram positive rods
5. Mycobacteria
6. Actinomycetes

### **3. Cell Wall-less Eubacteria**

This category includes true Bacteria which are pleomorphic and devoid of cell walls. The Bacterial colonies appear as fried egg on agar.



## Basic Biological Science Part I

medium. The cells are facultatively anaerobic to obligately anaerobic. This category includes only one group, the Mycoplasmas or Mollicutes.

### 3. Archaeobacteria

This category includes a unique group of Bacteria which have certain distinct features not seen in any one of the true Bacteria. However the morphology and structural organization are the same. The distinct features of Archaeobacteria are-

1. Cell wall lacks murein.
2. Presence of glycerol isoprenyl ether lipids.
3. Absence of long chain fatty acids bound to glycerol by ester linkages.
4. Presence of pseudouridine in the common arm of tRNA.
5. Methionyl initiator tRNA is not formylated.
6. Translation is inhibited specially by anisomycin.

This category includes five groups of Bacteria. They are-

1. Methanogens
2. Archaeal sulfate reducers
3. Extremely halophilic archaeobacteria
4. Cell wall-less archaeobacteria

## Basic Biological Science Part I

5. Extremely thermophilic and hyperthermophilic sulphur metabolizers.

### **Structure of Bacteria**

Bacteria are microscopic unicellular plants. They lack chlorophyll-a cells are prokaryotic They range in size from 0.5 um to 600nm. They are ubiquitous in distribution.

The shape, arrangement of cells flagella and spores are seen under light microscopes. The detailed structures of different parts of the cells are examined with electron microscopes after sectioning the Bacteria.

Based on the shape, there are five types of Bacteria. They are Bacilli, Cocci, Vibrio, Spirillum and Filaments.

The spherical or round bacterium is called a coccus (pl.cocci) The cocci exist in different forms The cocci that exist as individual cells are called monococci. Those existing in pairs are knows as diplococcic. If they are found in fours, they are called tetracocci. If the cells are arranged in the form of chains, the cocci are called streptococci. If the cocci are in irregular clusters, they are called staphylococci, If 8 cells are found in clusters, they are called sarcinae (singular-sarcina).

The rod-shaped bacteria are called bacilli )singular-bacillus). The rod-shaped individual cells are called bacilli. Those occur in pairs are

## Basic Biological Science Part I

called diplobacilli. Those rod existing in chains are known as streptobacilli. Those rods existing in clusters are termed staphylobacilli.

The curved rods, the curve of which forms less than one complete spiral, are called vibrios (singular-vibrio). They are also called comma bacteria.. eg. *Vibrio cholera*.

The long rigid spirally coiled bacteria are spirilla (singular-spirillum).

Some bacteria grow as fungal hyphae. They are called filamentous bacteria.

Some bacteria are pleomorphic, ie. They exist in different forms depending upon the environmental conditions.

The bacteria may be motile or non-motile. The motility is mainly concerned with flagella. Flagella are absent in Cocci. So Cocci are non-motile. Bacilli and spirilla have flagella and hence they are motile.

On the basis of number and arrangement of flagella, the following types are recognised.

1. **Artichous:** Flagellum is absent. Eg. **Cocci**.
2. **Monotrichous:** A single flagellum at one end of the cell.  
Eg. **Vibrio**

## Basic Biological Science Part I

3. **Lophotrichous:** A tuft of flagella at one end of the cell. Eg. **Spirillum undula.**
4. **Amphitrichous:** A tuft of flagella at both ends of the cell. Eg. Spirilla.
5. **Peritrichous:** Many flagella arranged along all sides of the cell. Eg. Salmonella typhosa.

Electron microscopic studies show that the bacterial cell is bounded by a rigid cell wall which is protected by a capsule. Inner to the cell wall there is a plasma membrane that surrounds the protoplasm

The protoplasm consists of cytoplasmic matrix, ribosomes, mesosomes, nucleoid and plasmids. Many small hair-like appendages called pili and flagella arise from the surface of the bacterium.

1. **Bacterial Cell Wall:** The bacterial cell is bounded by a rigid layer of non-living materials called cell wall. It surrounds the plasma membrane. It consists of peptidoglycan. The peptidoglycan consists of alternating units of N-acetyl muramic acid and N-acetyl glucosamine units by 1-4 linkages. The adjacent polysaccharide chains are joined by tetrapeptide chains and pentaglycine chains. In the cell wall of gram positive bacterial the polysaccharides are joined by teichoic acids (polymers of glycerol or reibitol). The bacterial cell wall provides strength and rigidity to the cell.

## Basic Biological Science Part I

2. **Capule:** The cell wall in some bacterial is surrounded by a gelatinous envelope called capsule. It is closely attached to the cell wall. Those bacterial having a capsule are called capsulated bacterial. They form smooth colonies. They are more resistant to adverse conditions such as high temperature bactericides, bacteriophages and so on The capsule is made up of disaccharides and polypeptides.

Capsule is absent in some bacteria. Such bacteria are called non-capsulated bacteria. They produce rough colonies.

3. **Outer Membrane:** Outer membrane is present beneath the capsule. It is found only in gram negative bacteria. It is an unit membrane It is a three layered membrane. The outer and inner layers are composed of phospholipids and a middle layer is composed of protein. In the phospholipid, the polar heads are on the surface and the tails are in the interior. The protein components are embedded within the phospholipids The outers membrans acts as selective membrane for the membrane transport. It provide sites for phage infection and O-antibodies.

4. **Plasma Membrane (Inner Membrane):** Plasma membrane lies below the cell wall. It is unit membrane It is made up of proteins and phospholipids. They are arranged in three layers. The outer and inner layer are phospholipids and the middle layer is protein. In the phospholipid layer, the head face outwards and the tail face inwards. The proteins are embedded in the phospholipits.

## Basic Biological Science Part I

The plasma membrane-

- a) Acts as a selectively permeable.
  - b) Acts as a transporting system.
  - c) Act as the site of energy production.
  - d) Provides a specific site for the attachment of chromosome for replication.
5. **Cytoplasm:** The cell membrane encloses the cytoplasm. It is colloidal in nature. The cytoplasm does not show streaming movement. It contains ribosomes, mesosomes etc. Golgi body, mitochondria, lysosomes, endoplasmic reticulum, etc. are absent.
6. **Ribosomes:** Ribosomes are the centres of protein synthesis. They are smaller than the ribosomes of eukaryotes. They are called 70 S. The 70 S ribosome is made up of two sub-units, namely a large sub-unit 50 S and a small sub-unit 30S.

The ribosomes are present individually or in a linear series attached to mRNA. Such linear ribosomes are called polyribosomes.

7. **Mesosomes:** Mesosomes are intra-cytoplasmic membrane structures. They are vesicular, convoluted or multilaminated structures formed as invaginations of the plasma membrane into the cytoplasm. They contain vesicles. Tubules or lamellar whorls.

## Basic Biological Science Part I

Mesosmes participate in DNA splitting and cytokinesis during binary fission. They are believed to be involved in the transport of exocellular enzymes

8. **Chromatophores (Chlorosomes):** These are pigment bearing Membranous structures They are in the form of thylakoids They are involved in photosynthesis. They are found in photosynthetic bacteria (cyanobacteria).
9. **Magnetosomes:** Magnetosomes are cellular inclusions present in some bacteria. They are sensitive to magnetic Field. They help to orient the cell in a magnetic field.
10. **Nucleoid:** The bacterial chromosome remains in a part of cytoplasm. This is not surrounded by a nuclear membrane. The nuclear material without a nuclear membrane is called nucleoid.

The bacterial chromosome is made up of a single double stranded circular DNA proteins are not found associated with the DNA

11. **Plasmids:** Plasmids are small, circular and autonomously replicating double stranded. DNA molecules. These plasmids are found in the cytoplasm. Plasmids are extrachromosomal genetic elements. These vary in size from a few to several hundred kilobases in length The chromosome and the plasmids together constitute the bacterial genome A cell may contain 1 to 100 plasmids.

## Basic Biological Science Part I

Some plasmids are capable of integrating with bacterial chromosome. Such plasmids are called episomes.

The plasmids exist in a super coiled form, or open circle or linear duplex.

**12. Flagella:** Bacteria possess one or more long appendages called flagella. They are used for locomotion.

Each flagellum consists of three components, namely a basal body, a hook and a shaft.

The basal body consists of two sets of rings connected by a rod. Each set has rings and altogether there are four rings. They are named M ring (M=membrane), S ring (S= super membrane), P ring (Peptidoglycan ring), and L ring (L=lipopolysaccharide) from the inner to the outside. The M ring is embedded in the plasma membrane and outer membrane. The p ring is attached to the peptidoglycan. The L ring is attached to the lipopolysaccharide of the outer membrane.

The hook connects the basal body with the shaft.

The flagellum is made up of subunits called flagellin. They are helically arranged.

**13. Pili (Fimbriae):** pili are short hair-like appendages arising from the surface of bacteria. They are shorter than flagella. They are straight and not hooked. They are found in Gram negative bacteria.



## Basic Biological Science Part I

The cells which contain pili are called Fim<sup>+</sup> cells and the cells which do not contain pili are called Fim<sup>-</sup> cells.

Pili are made up of protein subunits called pilin or fimbrin. The pilin subunits are arranged in a helical manner.

Pili are divided into two types. Namely normal pili and sex pili.

Sex pili are hair-like structures present on the surface of some bacteria. They are longer than normal pili. They have an axial hole. They have a knob at the terminal end. They are determined by plasmids.

## Nutritional Types of Bacteria

Bacteria need enough suitable nutrients for its growth and reproduction. Enough moisture and temperature, suitable pH and enough nutrients such as a carbon source a nitrogen source. Electron donors and trace elements are necessary for the growing bacteria. Bacteria. Are extraordinarily diverse in their specific nutrient requirements. The various nutritional types of bacteria are discussed below.

On the basis of nutrition. Bacteria are divided basically into two groups. They are autotrophs and heterotrophs. The bacteria which utilize carbon.-di-oxide as the source of carbon are called autotrophs. The bacteria which use organic compounds as the source of carbon are known as heterotrophs.

## Basic Biological Science Part I

The autotrophic bacteria which use sunlight as the energy source are called photoautotrophs or photosynthetic bacteria

### **Structure of E.coli**

E.coli is Escherichia coli. It is a colon bacterium It lives in the colon of man It is harmless.

It is a prokaryote It is rod-shaped Hence it is a bacillus.

It is covered by a capsule Hence it is a capsulated bacterium

Below the cell wall there is a plasma membrane It is an unit membrane. It is composed of phospholipids protein and polysaccharides.

The plasma membrane encloses the cytoplasm. The cytoplasm is colloidal in nature.

The cytoplasm contains a circular double stranded DNA. It is the bacterial chromosome. It does not contain a nuclear membrane. The nuclear material without a nuclear membrane is called a nucleoid or incipient nucleus.

The cytoplasm contains 70s ribosomes. One or more membranous vesicles are attached to plasma membrane. They are called mesosomes.

Small circular stranded DNAs are also found in the cytoplasm They are called plasmids.

## Basic Biological Science Part I

*E. coli* is a Gram negative bacterium. It appears red in colour on Gram staining.

The surface of bacteria contain numerous flagella used for locomotion.

In between the flagella. There are short appendages called pili.

*E. coli* exhibits anaerobic respiration.

It reproduces asexually and sexually *E. coli* reproduces asexually by binary fission and endospore formation Sexual reproduction includes conjugation transformation and transduction

## Viruses

The viruses are sub-microscopic nucleo-protein particles that infect plants, animals and bacteria, and multiply only in these organisms. Viruses become active only when they are inside the living cells when they are remaining outside the cells, they seem to be non-living things. Hence they are called living chemicals. Viruses are intermediate between living beings and non-living chemicals. Eg. Tobaccomosaic virus (TMV), Adenovirus, T<sub>4</sub>-bacteriophage, etc.

In Greek ;'virus' means poison. They are neither prokaryotes nor eukaryotes They are neither prokaryotes nor eukaryotes. They have no cellur organization and are smaller than the smallest cell. Hence they are believed to be sub-microscopic particles. The viruses pass through the pores of filter papers so that they were once called filterable molecules.

## Basic Biological Science Part I

The Greek 'virus' means poison. They are neither prokaryotes nor eukaryotes. They have no cellular organization and are smaller than the smallest cell. Hence they are believed to be sub-microscopic particles. The viruses pass through the of filter papers so that they were once called filterable molecules.

The study of viruses is called virology. The specialists concerning with virology are called virologists.

Viruses were first observed by Carolus clusivs in 1576. The existence of viruses in infected plants was proved by Bomitrii Iwanowski in 1899. In 1933, Schelsinger described the chemical composition of some viruses. By 1935 Stanely isolated Tobacco Mosaic Virus and made them into crystals. Besides this he proved crystals have the ability to cause to cause mosaic disease in tobacco.

The viruses cause many diseases in man, animals and crop plants. Luria (1953) defined viruses as Sub-microscopic entities capable of being introduced into specific living cells and of reproducing inside such cells only.

### **Distinctive Features of Viruses**

1. Viruses are sub-microscopic particles that multiply tnt racellularly.
2. They are living chemicals
3. They infect plants, animals and microbes.

## Basic Biological Science Part I

4. They have no cellular organization.
5. Each virus consists of a nucleic acid surrounded by a protein coat.
6. The nucleic acid of animal and bacterial viruses is DNA. But the plant viruses contain only RNA.
7. Ribosomes, enzymes of energy metabolism and protein synthesis are altogether absent
8. Viruses can be made into crystals and stored for a long time
9. The crystallised viruses cause diseases when they are introduced into proper hosts.
10. Viruses remain lifeless outside the living cells.
11. They are not affected by antibiotics.

### Classification of Viruses

Holmes (1948) included all viruses in the order virales of the division Acaryota. The virales consists of three suborders-

- 1. Phaginae:** It includes all viruses that infect bacteria. These viruses usually contain DNA. They are commonly called bacterial viruses or bacteriophages. Eg. T<sub>4</sub> bacteriophage.
- 2. Phytophaginae:** It includes all viruses that infect plants. These viruses usually contain RNA. They are popularly known as plant viruses. Eg. Tobacco mosaic virus

## Basic Biological Science Part I

**3. Zoophaginae:** It includes all viruses that infect animals including man. These viruses may contain DNA or RNA as the genetic material. They are otherwise called animal viruses eg. Adenovirus.

The detailed classification of animal and bacterial viruses is beyond the scope of this book. The classification of plant viruses are discussed here under.

### **Classification of Plant Viruses**

Viruses that infect plants are called plant viruses. Most of the plant viruses contain RNA but a few contain DNA as their genetic material. They are usually named after their host and disease caused by them. For example, in the name of tobacco mosaic, 'tobacco' refers to the name of the host plant and 'mosaic' refers to the disease caused by the virus.

F. Contrat and R.P. Wagner (1974) classified all plant viruses into 19 groups. Of these 17 groups contain single stranded RNA (ss RNA), one group contains double stranded RNA (ds RNA) and yet another group contains double stranded DNA (ds DNA).

#### **RNA Viruses**

1. Tobamovirus group
2. Potecvirus group
3. Carlavirus group

## Basic Biological Science Part I

4. Potyvirus group
5. Tobravirus group
6. Barley stripe mosaic virus group
7. Lettuce necrotic yellow virus group
8. Tombusvirus group
9. Tobacco necrosis virus group
10. Tymovirus group
11. Comovirus group
12. Nepovirus group
13. Pea enation mosaic virus group
14. Bromovirus group
15. Alfalfa mosaic group
16. Satellite viruses
17. Tomato spotted wilt virus group

### **B. ds RNA Viruses**

18. Reovirus group

### **C. ds DNA virus group**

## Basic Biological Science Part I

### 19. DNA virus group

#### **Structure of Viruses**

Viruses are sub-microscopic nucleo-protein particles. They infect plants, animals and microbes, and multiply only within the living cells. They exist as non-living chemical outside the cells. They are ubiquitous in distribution.

The viruses that live in animal cells are called animal viruses eg. Poliovirus, Reovirus, AIDS virus, etc. The viruses living in plant cells are called plant viruses eg. Tobacco mosaic virus (TMV) potato Virus –X etc. bacteriophages eg. T<sub>4</sub>-phage

As the viruses are too small in size electron microscopes and x-ray different equipments are used to study their structures. They are 10-20 nm in diameter and 160-500 nm in length. The longest virus is Citrus tristeza, measuring 20,000 nm length.

The shape of viruses is variable. It may be-

1. Elongated rod-shaped (eg. TMV)
2. Short rods (eg. Alfalfa mosaic virus)
3. Spherical (eg. Retrovirus)
4. Bullet –shaped (eg. Lettuce necrotic yellow virus)
5. Cubical (eg. Bromovirus)



## Basic Biological Science Part I

### 6. Tadpole-shaped (eg. T<sub>4</sub> phage)

A virus consists of two major components, namely a protein coat and nucleic

acid. The protein coat is called capsid and it surrounds the nucleic acid. The capsid consists of many small sub-units consisting of polypeptides these sub-units are called capsomers. The capsomers are polygonal or helically coiled or rod-shaped. The capsid gives definite shape to the virus and protects the nucleic acid core. It has antigenic molecules.

Nucleic acid forms the central core of the virus particle. It may be a DNA or RNA. Plant viruses usually contain RNA. Animal viruses and bacteriophages usually contain DNA, but rarely RNA, The virus having RNA is called RNA virus The virus Containing DNA is called DNA virus.

The nucleic acid of viruses may be single stranded or double stranded Based on the nature of nucleic acid viruses are divided into four groups.

1. Viruses with single stranded DNA. Eg. Coliphage.
2. Viruses with double stranded DNA. Eg. Vaccinia virus
3. Viruses with single stranded RNA eg. TMV
4. Viruses with double stranded RNA. Eg. Reovirus.

## Basic Biological Science Part I

The fully formed mature virus is called virion. The nucleic acid core without capsid is called viroid, But it also can infect some host cells.

The structure of a plant virus, animal virus and bacteriophage is discussed below.

### Plant Viruses

Virus that infects plants are called plant viruses The nucleic acid is usually RNA but rarely DNA. It may be single stranded or double stranded Tobacco mosaic virus (YMV), Tomato ring spot virus. Potato virus-X Cauliflower mosaic virus etc. are plant viruses.

Tobacco mosaic virus is a typical plant virus. It is abbreviated as TMV. It causes a disease called tobacco mosaic tobacco plants acid and hence the name. The infected plants show yellow. And green mottling which appears a mosaic pattern.

The tobacco mosaic virus is a RNA virus It is a helical virus It is rods shaped It is about  $3000\text{\AA}$  long  $170\text{\AA}$  diameter

The TMV is made up of two components namely an outer capsid and an inner RNA molecule.

The capsid is the outer coat. It is formed of protein The capsid is made up of small subunits called capsomeres. There are about 2130 capsomeres in a TMV The capsomeres are helically arranged around the RA. There are about 129 complete spirals.

## Basic Biological Science Part I

The RNA forms the core of the virus. It is a single stranded nucleic acid. The RNA is also helically coiled like that of capsid. There are about 3 nucleotides in the RNA per capsomere. So there are about 6400 nucleotides in the RNA.

Viruses that infect animals are called animal viruses. They cause diseases in animals including man. Most of them however are non-pathogenic. Polio virus, Mumps virus, Adenovirus, Herpes virus, Rabies virus, etc. are animal viruses. Adenovirus is a typical animal virus. It infects eye and alimentary canal. It is a non-enveloped virus. It is a polyhedral virus. It is a DNA virus. It has the appearance of a space vehicle.

It is made up of two components namely an outer coat the capsid and an inner core the DNA. The capsid is made up of small sub-units called capsomeres.

There are about 252 capsomeres. The capsomeres are arranged in the form of an icosahedron, having 20 triangular facets and 12 vertices. The 12 capsomeres at the vertices have 5 neighbours and are called pentons. The remaining 240 capsomeres have six neighbours and are called hexons.

# Basic Biological Science Part I

## FOOD MICROBIOLOGY

### Introduction

Microorganisms are intimately associated with the availability, the abundance, and the quality of food for human consumption. Food items are easily contaminated with microorganisms in nature, during handling and in processing. After it is contaminated, food serves as a medium for the growth of the microorganisms. If they are allowed to grow, these microorganisms can change the physical and chemical characteristics of the food and may also be responsible for food poisoning and food-borne infections. Accordingly, much attention has been directed to develop methods for the preservation of food.

### Food Spoilage

Food spoilage refers to the process, where the food is made useless, bad and unfit for eating. It alters the chemical properties, appearance, texture, colour, taste, flavor, odour and stability of the food. The spoiled food becomes less palatable and toxic.

### Causes of food spoilage

Food is spoiled by many factors. They are the following:

- a. Microorganisms
- b. Insects
- c. Rough handling
- d. Transport
- e. Enzyme activity

# Basic Biological Science Part I

## Biochemical changes of food spoilage

During food spoilage, the following biochemical changes occur:

1. Putrefaction
2. Fermentation
3. Rancidity
4. Autolysis

**1. Putrefaction :** Putrefaction is the enzymetic decomposition of the protein present in the food with the production of foul smelling compounds such as, hydrogen sulphide, ammonia, etc. It is caused by *Pseudomonas putrescens*, etc.

**During putrefaction protein is converted into amino acids, amines, ammonia and H<sub>2</sub>S**

**Protein    Microbes    Amino acid + Ammonia + H<sub>2</sub>S**

**2. Fermentation :** Fermentation is the anaerobic enzymatic conversion of carbohydrates into ethyl alcohol. It is caused by *Streptococcus*, *Micrococcus*, etc.

**Carbohydrates    Fermenting    Alcohol + Cases.**  
**Microbes**

**3. Rancidity :** Rancidity is the decaying of fat.

**Fat            Fatty acids + Glycerol**

## Basic Biological Science Part I

**4. Autolysis :** Autolysis is the spontaneous disintegration of cells or tissues by enzymes

### **Spoilage of Meat**

Meat is the animal flesh used as human food. It includes sheep, goats, cattle, pig and chicken.

Meat contains 75% water, 19% protein, 2.5% lipid and 1.2% carbohydrate.

The spoilage of meat is due to chemical and biological processes.

The chemical processes of spoilage are due to putrefaction, fermentation, rancidity and autolysis.

The biological processes of spoilage are brought about by microbes. The microbes causing meat spoilage are bacteria and fungi.

The important bacteria spoiling meat are

Pseudomonas                      Streptococcus

Salmonella                      Micrococcus

Lactobacillus                      Escherichia

Bacillus                      Clostridium

The common fungi are

Mucor                      Penicillium

Chrysosporium                      Rhizopus

Cladosporium                      Sporotrichum

## Basic Biological Science Part I

The microbes enter meat from internal as well as external sources. During cutting the microbes of gut contents and the skin of the animal may infect the meat.

The microbes are also introduced during handling, processing, packaging and storage.

Meat undergo deep spoilage near the bone called bone taint. It is caused by Clostridium and Enterococcus

The fungi, Mucor and Rhizopus cause Whiskets on meat.

Cladosporium (Fungus) causes black spot.

The common bacterium spoiling meat is Pseudomonas.

### **Spoilage of Fish**

Fish is perishable food. It is a non – acid food because the pH is above 4.5.

The fish contains protein, carbohydrates and lipids.

The fish is spoiled by mechanical, chemical and quickly.

Normally flat fishes spoil quickly and round fishes spoil slowly.

The chemical processes of spoilage include putrefaction, fermentation, rancidity and autolysis.

The biological processes of spoilage is due to bacteria and fungi.

The bacteria causing fish spoilage are

Pseudomonas                  Flavobacterium                  Bacillus

Achromobacter      Micrococcus

Pseudomonas      causes discoloration

## Basic Biological Science Part I

Achromobacter causes putrefaction.

Examples of fungi

Albugo                      Mucor

Rhizopus                  Pilobolus

Aspergillus                Penicillium

Saccharomyces (Yeasts)

### **Spoilage of Milk**

Raw milk becomes ropy. Ropyness is caused by the bacterium *Alcaligenes viscolactis*. The ropy milk is slimy and pulled out into long threads. It is due to the synthesis of viscous polysaccharide in the milk.

Curdling is a spoilage of milk. In curdling lactose of milk is fermented into lactic acid. It is caused by *Streptococcus lactus*, *Lactobacilli*, etc.

Rapid fermentation of milk is called stormy fermentation. It is caused by *Clostridium*.

The milk becomes rancid when the milk lipids undergo lipolysis. Rancidity is caused by *Pseudomonas* and *Achromobacter*.

### **Spoilage of Egg**

Freshly laid eggs are sterile. Soon after laying. Micro –Organisms enter the eggs and spoil them.



## Basic Biological Science Part I

Common bacterial spoilage of egg is called rotting (decaying)

Green rot is caused by *Pseudomonas fluorescens*

Colourless rot is produced by *Pseudomonas*.

Black rot is produced by *Proteus*.

Red rot is produced by *Serratia*

Custard rot is produced by *Proteus vulgaris*

Mustiness is another bacterial spoilage of eggs caused by *Pseudomonas graveolens*.

Mold spoilage of egg is called pinspots. It is caused by *Penicillium*.

Certain microorganisms grow in milk and bring colour changes, *Pseudomonas synchyanea* gives blue colour. *Pseudomonas synxatha* gives yellow colour. The yeast *Torula* produces red colour.

In the milk foam is formed by the production of gas by *Clostridium*; *Bacilli*, *Yeast* etc.

### **Spoilage of Fruits and Vegetable**

The bacterial spoilage of fruits and vegetables is called bacterial soft rot. The fruits and vegetable become soft and bad smelling.

Grey mold rot is caused by the fungus *Botrytis*.

Soft rot is caused by *Rhizopus* leading to colony growth.

Souring and sliminess are produced by saprophytic bacteria.

The rot of potatoes is called black leg. It is caused by *Erwinia* sp.

## Basic Biological Science Part I

### **Spoilage of Bread**

The bacterial spoilage of bread is called ropiness. Ropy bread is caused by *Bacillus subtilis*. *Bacillus* gives the bread a soft and cheesy texture with long stringy threads.

Moldy bread is caused by *Rhizopus*.

Red bread is caused by *Neurospora*

### **Spoilage of Canned food**

Food preserved in cans is called canned food.

Normally canned food remains fresh as it is preserved under sterile condition.

The spoilage of canned food is caused by three factors

1. Chemical changes in the canned food
2. The spores or microbes surviving during processing
3. Microbes entering through leakage of cans

Canned – food spoilage is of the following types:

1. Putrefaction
2. Flat sour spoilage
3. Swelled can spoilage
4. Sulfide spoilage
5. Spoilage by thermophilic microbes
6. Spoilage by mesophilic microbes.

#### **1. Putrefaction**

## Basic Biological Science Part I

Putrefaction is the rottenness of the food. It produces putrid odour. It produces gas. Hence the can bulges at the ends and bursts. It is caused by bacterium. *Clostridium sporogenes*.

### **2. Flat Sour Spoilage**

In flat sour spoilage. Gas is not produced and hence the can does not swell.

The ends of the can remain flat. This spoilage can be observed only after opening the can. It is caused by *Bacillus coagulans*.

It causes souring and abnormal odour. A cloudy liquid-like substance is formed in the food.

### **3. Swelled Can Spoilage**

In swelling can spoilage the two ends of the can bulge out due to the accumulation of gas in the can. The ends do not remain flat.

In this spoilage hydrogen gas is produced. Hence it is called hydrogen swell.

Gas production is caused by *Clostridium* yeasts and molds.

Depending on the pressure of the gas, there are different stages of swells namely flipper, springer, soft swell, hard swell, etc.

In flipper both only one end of the can swells.

In springer both ends bulged. If pushed in. one or both ends remain concave.

In softswell both ends buldge out and that may be dented by pressing with fingers.

## Basic Biological Science Part I

In hard swell both ends are bulged out and that cannot be dented by hand. Hard swell is the hydrogen swell.

### **4. Sulfide spoilage**

Sulfide spoilage is caused by *Clostridium nigricans*. It produces hydrogen sulfide. As a result the food is blackened and it gives rotten egg odour.

### **5. Spoilage by Thermophilic Microbes**

Thermophilic bacteria survive well in temperature above 40°C.

## **FOOD POISONING**

Food poisoning refers to the toxicity introduced into food by microbes and their products. Ingestion of poisoned food causes a group of acute illness.

Food poisoning is of two types namely food intoxications and food infections.

### **1. Food Intoxication**

Food intoxication is due to the presence of bacterial exotoxin in the food

contaminated with bacteria. Food intoxications are of five types, namely

**Botulism,**

**Staphylococcal food poisoning,**

**Infantile gastroenteritis,**

**Travellers diarrhea,**

**Mycotoxicosis.**

# Basic Biological Science Part I

## 1. Botulism

❖ Botulism is a neuroparalytic disease. It is due to an exotoxin produced by the bacterium *Clostridium botulinum* in improperly canned or preserved foods.

❖ It is due to food poisoning.

❖ It is a food intoxication because the disease is caused by the toxin secreted by the bacteria and not by the bacteria themselves.

❖ The word botulism is derived from latin meaning sausage. In Germany during 1793. About 13 people shared uncooked smoked sausage and they became ill and later 6 persons died.

❖ *Clostridium botulinum* lives in the soil. It grows only in anaerobic condition. It grows in canned smoked or cured food. It does not grow in fresh food. It is a rod shaped bacterium. The food is easily contaminated by the spores of *Clostridium* In improperly canned or preserved food the spores grow and produce the toxin.

❖ When multiplying it releases a powerful exotoxin called botulinum. It affects the nervous system. Hence it is called a neurotoxin.

❖ This toxin is a protein and is easily destroyed by heat (70°C)

❖ The main sources of botulism are canned meat, fish and other protein foods. The infection mostly occurs when preserved food is eaten.

❖ A mere taste of the food containing the poison is enough to cause death.

❖ When the toxin is swallowed along with food it is rapidly absorbed

## Basic Biological Science Part I

into the blood stream It is carried in the blood stream to never endings in the muscles. It prevents acetylcholine production in the nerve endings. Hence impulse is not conducted cannot contract. They are paralysed.

- ❖ The first sign of the disease is the paralysis of muscles of eye lid,  
This symptom

appears in hours of eating the food.

- ❖ Next, the paralysis affects the muscles of speech. Swallowing becomes difficult.
- ❖ Finally the respiratory muscles stop their activity Suffocation occurs and death may result within a day.
- ❖ The symptoms of botulism occurs within 6 hours from the time of consumption of

contaminated food. Botulism is a neuroparalytic disease. It is characterized by the following symptoms.

Blurred vision or double vision,  
Dilated pupils  
Paralysis of eye muscles,  
Difficulty in speaking and swallowing due to paralysis of pharyngeal muscles.

- ❖ Prevention : Botulism can be prevented by the following methods.
  - a) The canned and preserved food must be heated before eating.  
Heating destroys botulinum.
  - b) Spoiled canned food must be rejected.
  - c) The food must be well cooked

## Basic Biological Science Part I

- ❖ Treatment : Botulism can be treated with polyvalent botulinum antitoxin

### 2) Staphylococcal food poisoning

Staphylococcal food poisoning is one of the most common types of food poisoning. This is caused by the bacterium *Staphylococcus aureus*. This is a Gram-positive coccus arranged in clusters. It produces an enterotoxin.

*S. aureus* usually resides in the nose and sometimes in the hands. When the hands are contaminated with nasal secretions.

Consumption of contaminated raw or cooked food and serving of not-properly refrigerated food may lead to the development of food poisoning.

The foods likely to be involved in this type of food poisoning are milk products, custards, processed meat, cream puffs, sandwich poultry stuffing and potato salad.

Symptoms of poisoning occur within 1 to 6 hours after consumption of food. The symptoms of staphylococcal food poisoning include nausea, vomiting and moderate diarrhea. But usually no fever. The disease usually lasts for less than 12 hours and is never fatal.

The best preventing measures are to use sanitary precautions when preparing all perishable foods and refrigerate the food at temperatures below 6 to 7°C. Food should not be allowed to stand for several hours at room temperature before serving.

### 3) Infantile Gastroenteritis and Traveller's diarrhea

## Basic Biological Science Part I

These are caused by the enterotoxin produced by *E. coli* and *Bacillus cereus*.

### 4) Mycotoxicosis

Mycotoxicosis is a food poisoning caused by the ingestion of fungal toxin. The toxin is called mycotoxin. It is produced in the food in which the fungus lives.

The fungus *Aspergillus flavus* produces a toxin called aflatoxin. It causes hepatoma and carcinoma (cancer). *Penicillium rubrum* produces rubratoxin which affects the liver.

The mushroom *Amanita phalloides* produces amatoxin which causes liver damage and hypoglycemia.

## 2. Food-Infections

Food infection refers to the illness produced by the eating of food containing living microbes. These microbes grow and sporulate within the intestine. After producing sufficient number they cause illness.

The following are the important food-borne infections

*Salmonella typhimurium* causes head-ache, chills, abdominal pain, nausea, vomiting, diarrhea and fever.

*Vibrio cholera* causes cholera.

*Shigella* causes bacillary dysentery

*Entamoeba histolytica* causes amoebic dysentery

*Giardia* causes giardiasis.



## Basic Biological Science Part I

Food infections can be prevented by the following sanitary methods.

### **Salmonellosis**

Salmonellosis is a disease caused by bacterial food infection. It is a food poisoning caused by the bacterium *Salmonella*.

Salmonellosis is a Gram negative rod-shaped bacterium It grows well in the food. Infection. Occurs through contaminated food or domestic animals. The *Salmonella* reaches the intestine through the ingested food. The bacterium multiplies in the intestine and causes the disease

Salmonellosis is of two types, namely enteritis and typhoid fever

Enteritis is due to existence of *Salmonella enteritis* in the intestine It produces a toxin called enterotoxin.

The symptoms of enteritis include chills, head ache nausea vomiting, abdominal pain and severe diarrhea. Symptoms persist for 2 to 3 days Mortality is low.

Typhoid fever is a kind of salmonellosis It is caused by intestine through contaminated food. Then they penetrate the intestinal cells and reach the blood They infect macrophages and gall bladder. They produce a cytotoxin

The symptom of typhoid fever include fever headache abdominal tenderness Constipation and appearance of rose red spots on body.

In later stages diarrhea with pea soup stools appear. In severe cases. There is haemorrhage in the intestine and perforation of the intestine leading to peritonitis.

## Basic Biological Science Part I

Salmonellosis can be prevented by the following methods.

1. Avoiding consumption of contaminated food.
2. Destruction of Salmonella by heat
3. The prevention of Salmonella growth by refrigeration

Salmonellosis can be treated by antibiotics like chloramphenicol.

Ampicillin amoxicillin etc.

### **Symptoms of food poisoning**

The most usual symptoms of food poisoning includes stomach pain vomiting and diarrhoea In acute cases death may occur.

### **Prevention of food poisoning**

The following precautions should be adopted by food handlers to prevent food poisoning.

2. Strict standards of hygiene must be maintained
3. Frequent washing of hands while processing the food.
4. Should not smoke during food preparation
5. Sneezing and coughing should be avoided.
6. Should wear clean clothes
7. Open cuts, boils and septic lesions be covered with a clean waterproof dressing.
8. Finger nails should be kept clean
9. Cooking utensils equipment's premises should be kept clean
10. Pests should be kept off from kitchen premises
11. The food should be stored in refrigerator.

### **Food Preservation**

# Basic Biological Science Part I

Food preservation is a technique to prevent food spoilage, food infection food poisoning and microbial contamination from the food.

## **Objectives**

Food preservation is done with the following objectives

- a) To prevent and to remove microbial contamination
- b) To arrest or inhibit microbial growth and metabolism.
- c) To kill contaminating pathogens.
- d) To minimize food spoilage food infection and food poisoning

## **Principle**

Any method of food preservation should aim at any one or more than one of the following

- (1) To prevent autolysis
- (2) To prevent microbial growth and metabolism
- (3) To inactivate the enzymes
- (4) To remove one or more than one of the requirements necessary for microbial growth
- (5) To create an anaerobic growth
- (6) To bring about change of pH
- (7) To kill microbial pathogens.

# Basic Biological Science Part I

## Methods of food preservation

The food is preserved by the following methods.

1. Pickling
2. Salting
3. Smoking
4. Aseptic processing
5. Canning
6. Bottling
7. Pasteurization
8. Refrigeration
9. Sterilization
10. Dehydration
11. Lyophilization
12. High osmotic pressure
13. Chemical additives
14. Radiation

### 1. Pickling

The food to be preserved is acidified in vinegar. It preserves vegetables, meat, etc., due to the action of acetic acid.

### 2. Salting

Fish can be preserved by the addition of salts.

### 3. Smoking

Smoking of fish and meat prevents spoilage by dehydration. The fish and meat are exposed to smoke produced by incomplete combustion of selected woods. Corn cobs or other materials.

The chemical preservatives of smoke contain cresols formic acid methyl alcohol formaldehyde etc. They are absorbed in small quantities over the surface of meat and fish which are then said to be cured. Smoked

## Basic Biological Science Part I

food develops an agreeable flavor. Smoked food can stay long till moisture is not allowed to come in contact.

### 4. Aseptic processing

It is a relatively new development in the food industry. The food item is first sterilized then dispersed into previously sterilized containers and sealed under aseptic conditions.

### 5. Canning

‘Canning’ is the preservation of food by putting it in a metal container and Sealing it air tight.

Nicholas Appert was the father of canning.

The food products such as meat, fish poultry products. Pumpkin spinach, tomatoes, berries fruits, vegetables, etc., are preserved through canning.

High temperature is applied for canning The objective of canning is to kill all the living organisms in the food stored in a can or a jar.

Canning involves 6 steps They are-

1. Cleaning
2. Blanching
3. Exhausting
4. Sealing
5. Autoclaving
6. Cooling

For canning the food materials are, first of all, cleaned to remove dirt and microbes.

## Basic Biological Science Part I

Then the food materials are immersed in hot water. This is called blanching This removes some microbes

Then the hot-food is filled in the jars or cans.

The filled can are passed through an exhaust box in which hot water or steam is used. A This is call exhausting.

Exhausting expands food drives out air and provides an atmosphere of steam at the top of the can.

Immediately after exhausting the cans are sealed This prevents recontamination of canned food.

After sealing the cans are autoclaved by keeping them in an autoclave for one hour.

The autoclaved cans are cooled immediately either in air or in cold water. sometimes the canned food may undergo spoilage. The spoilage of canned food may be due to chemical or biological reasons. The most important chemical spoilage is the hydrogen swell caused by the action of food acid on the metal of cans This leads to swelled cans with bulging ends.

Biological spoilage of canned food may be due to the surviving microbes in the processed food or due to leakage of the cans. The canned food is spoiled by either putrefaction. Acid production gas formation or blackening.

The *Bacillus coagulans* and *B. sterothermophilus* produce flat sour In flat sour, the organisms produce acid without gas. Hence the ends of

## Basic Biological Science Part I

the can remain flat and spoilage cannot be detected unless the can is opened.

The bacterium *Clostridium thermosaccharolyticum* produces thermophilic acid spoilage. In this spoilage the can swells due to the production of  $\text{CO}_2$  and  $\text{H}_2$ . *Clostridium nigrificans* produces sulphide spoilage. Spoilage is indicated by the presence of  $\text{H}_2\text{S}$  and blackening.

### 6. Bottling

In bottling the food products are preserved in bottles. It involves high temperature treatment as in canning. Liquid foods such as milk, wine, ghee, etc., can be preserved in bottles.

### 7. Pasteurization

Pasteurization is the process of heating milk below the boiling point to kill all non-sporing pathogenic bacteria but not to cause change in the composition, flavor, and nutritive value of milk. This process was originally introduced by Pasteur and hence the name.

There are two methods of pasteurization. They are:

1. Holding method
  2. Flash method
- 1) **Holding method:** In this method the milk is held in tanks for a long time (30 minutes) at a low temperature ( $62.8^\circ\text{C}$ ). This method is also called low temperature holding method (LTH).
  - 2) **Flash method:** In flash method the milk is subjected to high temperature ( $71.7^\circ\text{C}$ )

## Basic Biological Science Part I

for a short time (15 seconds) This method is also called high temperature short time method

Pasteurization stops fermentation. Pasteurized milk rarely sours Liquid egg

can also be pasteurized.

### **8. Refrigeration**

Refrigeration is keeping food in good condition by keeping and making it cold Here low temperature plays the main role. Food remain unspoiled in refrigerators at 5<sup>0</sup>C. In freezer at 5<sup>0</sup>C microbes are killed Deep freezers at 60<sup>0</sup>C reduce biochemical activities of microbes Deep freezers are useful for storing meat and fish.

### **9. Sterilization**

Sterilization is the removal of microbes from food. Milk is sterilized by boiling at 100<sup>0</sup>C . The sterilized milk can be stored in the refrigerator indefinitely.

### **10. Dehydration**

Dehydration is the removal of water from the food. It is the simplest and cheapest method. Drying in the sun is a simple method of dehydration. Fish is dried in the sun and can be stored.

A variety of fruits apricots, chillies, spices etc., are stored by dehydration.

### **11. Lyophilization**



## Basic Biological Science Part I

Lyophilization is a method of freeze-drying. The food is preserved by rapid freezing and dehydration of the frozen product under high vacuum. The dry product is then sealed in foil and is reconstituted with water. This method is very useful for storing, transporting and preserving bacterial culture.

### **12. High osmotic pressure**

High water is withdrawn from microbial cells by placing them in solution containing large amounts of dissolved substances such as sugar or salt. As a result of this water loss, microbial metabolism is halted. It also prevents microbial growth.

### **13. Chemical additives**

Organic acids such as benzoic acid, sorbic acid, acetic acid, lactic acid and propionic acids are used legally to preserve food. They inhibit microbial growth. Sorbic and propionic acids are used to inhibit mold growth in foods. Nitrates are used to preserve meat colour. They are also inhibitory to some anaerobic bacteria.

### **14. Radiation**

This is also recent development in food preservation. Non-ionizing and ionizing radiation are used to control microorganisms in food.

## Basic Biological Science Part I

Ultraviolet (non-ionizing) rays of sufficient intensity and time of exposure, is microbicidal.

Ionizing radiation such as, beta rays cathode rays and gamma rays are used extensive in food preservation This method is also called cold sterilization.

### UNIT -- V

Ecology is the study of relationship between organisms and their environment. A parasite's environment is primarily the host, of course, but transmission stages such as spores, eggs and often juveniles must also survive abiotic conditions. A host usually represents a rich and highly regulated supply of nutrients. Most body fluids of animals have a wide array of dissolved proteins, amino acids, carbohydrates, and nucleic acid precursors; virtually all animals have mechanisms for maintaining the chemical makeup and osmotic balance of their body fluids; and vertebrates such as birds and mammals control body temperature as well. We should expect parasites to exhibit traits that allow them to exploit such environments. A flea cannot live in the white-footed mouse's habitat, for example, unless the mouse itself is living there too.

But hosts are relatively small patches within the vast matrix that is their own habitat; that is, they are "islands" in the true sense of Taliaferro's quote as well as in the metaphorical sense. Thus, suitable parasite environments are dispersed in addition to being rich and

## Basic Biological Science Part I

regulated. For example, there is an enormous volume of water in lake compared to the volume of fish in that same lake. This seemingly trivial observation points to a major problem for monogenean flatworms' that must live on these fish: Unless the worms' reproductive stages are able to keep finding fish to infect, the parasite is likely to become locally extinct. In deed, many parasite control strategies are based on reducing the probability of host and parasite encounter, and conversely, many parasites possess traits that function to increase the probability of finding a host.

### **The Host as an Environment**

Host species include virtually the full spectrum of organisms, from human to protists. When viewed from a parasite's perspective, all organisms are complex environments with many separate habitats. For example, membrane-bound organelles of eukaryotic cells are distinct compartments with differing biochemical properties. Even the smallest insects and crustaceans offer many places, both internally and externally, that can be colonized by parasites. And larger animals, such as rodents, birds and human beings, provide dozens of microenvironments capable of supporting parasites.

### **Macro and Microparasites**

Large parasites that do not multiply (in the cycle stage of interest) in or on the host are called **macroparasites**. Examples of macroparasites are tapeworms, adult trematodes, most nematodes, acanthocephalans and

## Basic Biological Science Part I

arthropods such as ticks and fleas. Macroparasites often if not typically, occur in **aggregated populations**.

Small parasites that multiply within the host are called **microparasites**, and these include bacteria, rickettsia and protozoan infections such as those caused by malarial parasites, trypanosomes and amebas. Whereas in the case of macroparasites one can generally assume that one parasite reflects a single encounter between host and infective stage, that assumption is not valid for microparasites.

### **Multiple Species Infections**

A single host individual can be infected with a number of parasite species; that is, it can contain **a parasite community**. These communities can be extra ordinary rich, as illustrated by the intestinal parasites of some endothermic (warm blooded) vertebrates. In a series of typical studies by Holmes and his colleagues, 26 species of intestinal helminthes were reported from a sample of 31 eared grebes and 52 species, with “Slightly less than 1 million individuals”, were found in 45 scaup ducks. Mammals such as coyotes and black bears may also be heavily and frequently infected.

There is some evidence that parasites interfere with one another in various ways, especially in heavy intestinal infections. This evidence has led workers to postulate a variety of types of parasite communities, from interactive ones, in which competition may occur, to noninteractive ones,

## Basic Biological Science Part I

usually with few species, in which there appears to be little if any competition.

### **Classification of the Protozoan Phyla**

Classification of the eukaryotic microorganisms is a monumental task that has occupied scientists since the time of van Leeuwenhoek. Biologists who work with these organisms generally applaud each others efforts to achieve monophyletic groupings while admitting that such efforts are often in vain. Unicellular eukaryotes are exceedingly diverse. Only recently have advances in molecular biology and the growth of comparative ultrastructural information allowed us to resolve questions about homology and evolutionary significance of certain organelles. For example, it is doubtful that the various kinds of pseudopodia are homologous structure. Progress has been made, however, in terms of our altered perceptions of primitive (plesiomorphic) and derived (apomorphic) conditions. Thus, the presence of flagella (undulipodia) is now considered a plesiomorphic character of virtually all eukaryotes; in the past possession of a flagellum during much of the life cycle was used as a defining character for the subphylum Mastigophora.

### **Phylum Microspora**

Unicellular spores, each with imperforate wall, containing one uninucleate or dinucleate sporoplasm and a polar filament; sporoplasm injected into host cells through extruded polar filament; without

## Basic Biological Science Part I

mitochondria, peroxisomes, or hydrogenosomes; with 70S ribosomes; intracellular parasites in nearly all major animal groups.

### **Class Rudimicrosporea (Metchnikovellidea)**

Spherical to lenticular spores, with filament extruding laterally; polaroplast and posterior vacuole absent; sporulation sequence with dimorphism, occurring either in parasitophorous vacuole or in thick-walled cyst; hyperparasites of gregarines in annelids. Genera: *Amphiacantha*, *Metchnikovella*.

### **Class Microsporea**

Spore elongated, oval or tubular, with complex extrusion apparatus of Golgi origin, often including polaroplast and posterior vacuole; spore wall with three layers; sporocyst present or absent; often dimorphic in sporulation sequence.

### **Order Pleistophorida**

Sporulation sequence occurring within more or less persistent intracellular (in host cell) sporocyst (pansporoblastic membrane); often dimorphic, with another sporulation sequence not involving such membrane; spores uninucleate or binucleate, depending on thickness of wall and type of development or transmission; number of spores from sporoblasts variable. Genera: *Encephalitozoon*, *Glugea*, *Pleistophora*, *Thelohania*, *Amblyospora*.

# Basic Biological Science Part I

## Order Nosematida

Spores diplokaryotic, with paired nuclei dividing synchronously.

Genus: *Nosema*.

## Phylum Retortamonada

Mitochondria and dictyosomes absent, three anterior flagella and one recurrent flagellum, the latter lying in a cytostomal groove; intestinal parasites or free living in anoxic environments.

### Class Retortamonadea

#### *Order Retortamonadida*

Two pairs of kinetosomes, large, cytostome, intranuclear division spindle; cysts present. Genera: *Chilomastix*, *Retortamonas*.

### Class Diplomonadea

One or two karyomastigonts; individual mastigonts with one to four flagella, typically one of them recurrent and associated with cytostome or with organelles forming cell axis; mitochondria and Golgi apparatus absent; intranuclear division spindle; cysts present; free living or parasite.

#### **Order Enteromonadida**

Single karyomastigont containing one to four flagella; one recurrent flagellum in genera with more than single flagellum; frequent transitory

## Basic Biological Science Part I

forms with two karyomastigonts; all parasitic. General: *Enteromonas*, *Trimitus*.

### **Phylum Axostylata**

With an axostyle made of microtubules.

#### ***Class Oxymonadea***

One or more karyomastigonts, each containing four flagella typically arranged in two pairs in motile stages; one to many axostyles per organism; mitochondria and Golgi apparatus absent; division spindle intranuclear; cysts in some; sexuality in some; all parasitic in termites and wood-eating cockroaches.

#### ***Class Parabasalea***

With very large dictyosomes associated with karyomastigont; up to thousands of flagella.

### **Order Trichomonadida**

Typically atleast some kinetosomes associated with rootlet filaments characteristic of trichomonads; parabasal body present; mitochondria absent; division spindle extranuclear; karyomastigonts with four to six flagella, but only one flagellum in one genus and no flagella in another; pelta and noncontractile axostyle in each mastigont, except for one genus: hydrogenosomes present: no sexual reproduction; true cysts



## Basic Biological Science Part I

rare: all parasitic. Genera : *Dientamoeba*, *Histomonas*, *Monocercomonas*, *Trichomonas*.

### **Order Polymonadida**

Multinucleated, each nucleus associated with set of flagella, axostyles and parabasal bodies. Parasites of termites. Genera: *Calonympha*, *Coronympha*.

### **Order Hypermastigida**

Mastigont system with numerous flagella and multiple parabasal bodies; flagella-bearing kinetosomes distributed in complete or partial circle, in plate or plates or in longitudinal or spiral rows meeting in centralized structure; many with microtubule sheets or peltoaxostylar lamellae; one nucleus per cell; mitochondria absent; division spindle extranuclear; cysts in some; sexuality in some; all symbiotic in wood-eating insects.

### **Trichomonas vaginalis**

This species was first found by Donne in 1836 in purulent vaginal secretions and in secretions from the male urogenital tract. In 1837 he named it *Trichomonas vaginalis*, thereby creating the genus. It is a cosmopolitan species, found in the reproductive tracts of both men and women the world over. Donne thought the organism was covered with hairs, which is what prompted the generic name.

## Basic Biological Science Part I

### *Morphology*

*Trichomonas vaginalis* is very similar to *T. tenax* but differs in the following ways: It is somewhat larger, 6  $\mu\text{m}$  to 32  $\mu\text{m}$  long by 5  $\mu\text{m}$  to 12  $\mu\text{m}$  wide; its undulating membrane is shorter; and there are more granules along its axostyle and costa. In living and appropriately fixed and stained specimens, the constancy in presence and arrangement of the hydrogenosomes is the best criterion for distinguishing *T. vaginalis* from other *Trichomonas spp.* *T. vaginalis* frequently produces pseudopodia.

### *Pathogenesis*

Most strains are of such low pathogenicity that the infected person is virtually asymptomatic. However, some strains cause an intense inflammation, with itching and a copious white discharge (**leucorrhea**) that is swarming with trichomonads. They feed on bacteria, leukocytes and cell exudates and are themselves ingested by monocytes. Like all flagellates, *T. vaginalis* divides by longitudinal fission and like other trichomonads, it does not form cysts.

A few days after infection there is a degeneration of the vaginal epithelium followed by leukocytic infiltration. The vaginal secretions become abundant and white or greenish and the tissues become intensely inflamed. In vitro studies show that the flagellates attach to epithelial cells by means of numerous cytoplasmic extensions and microfilaments. An acute infection will usually become chronic with a lessening of

## Basic Biological Science Part I

symptoms, but will occasionally flare up again. It should be noted, however, that leucorrhoea is not symptomatic of trichomoniasis; indeed, at least half of the patients even with severe leucorrhoea are negative for *T.vaginalis*. In men the infection is usually asymptomatic, although there may be an irritating urethritis or prostatitis.

### ***Diagnosis and Treatment***

Diagnosis depends on recognizing the trichomonad in a secretion or from an in vitro culture made from a vaginal irrigation. Cultivation is recommended to detect low numbers of organisms. Culture of parasitic protozoa is often time-consuming and laborious, but plastic envelope methods have been developed for *T.vaginalis*, using dry ingredients that have a long shelf life and are reconstituted with water immediately before use. Dot-blot DNA hybridization assays have also been developed for *T.vaginalis* and in clinical trials these assays were more effective than was microscopic examination. However, cross-reactions with *Pentatrichomonas hominis* were observed.

Oral drugs, such as metronidazole, usually cure infection in about five days, but resistant strains occur. In vitro tests of such strains show that required minimum lethal concentration of the drug are up to 11 times the MLC of susceptible strains. Some apparently recalcitrant cases may be caused by reinfection by the sexual partner. Suppositories and douches are useful in promoting an acid pH of the vagina. Sexual partners should be treated simultaneously to avoid reinfection.

## Basic Biological Science Part I

### Order Trichurida

The Trichurida contains, among others, three genera of medical importance : *Trichuris*, *Capillaria*, and *Trichinella*.

### Family Trichuridae

Whipworms, members of the family Trichuridae are so called because they are threadlike along most of their body, and then they abruptly become thick at the posterior end, reminiscent of a whip with a handle. The name *Trichocephalus*, in widespread use in some countries, was coined when it was realized that the ‘hair’ was the anterior end rather than the tail, but the term *Trichuris* has priority. There are many species in a wide variety of mammalian hosts, and one is a very important parasite of humans.

Eggs of *Trichuris trichiura* have been found in a glacier mummy more than 5000 years old, and the worm has likely been with us for much longer; it probably coevolved with us as a parasite of our nonhuman ancestors.

### *Trichuris trichiura*

**Morphology.** *Trichuris trichiura* measures 30 mm to 50 mm long. With males being somewhat smaller than females. The mouth is a simple opening, lacking lips. The buccal cavity is tiny and is provided with a

## Basic Biological Science Part I

minute spear. The esophagus is very long, occupying about two thirds of the body length, and consists of a thin-walled tube surrounded by large, unicellular glands, the **stichocytes**. The entire structure often is referred to as a **stichosome**. The anterior end of the esophagus is somewhat muscular and lacks stichocytes. Both sexes have a single gonad, and the anus is near the tip of the tail. Males have a single spicule that is surrounded by a spiny spicule sheath. The ejaculatory duct joins the intestine anterior to the cloaca. In females the vulva is near the junction of the esophagus and the intestine. The uterus contain many unembryonated, lemon-shaped eggs, each with a prominent opercular plug at each end.

An excretory system is absent. The ventral surface of the esophageal region bears a wide band of minute pores, leading to underlying glandular and nonglandular cells. This **bacillary band** is typical of the order. Although the function of the cells in the bacillary band is unknown, their ultrastructure suggests that the gland cells may have a role in osmotic or ion regulation, and the nongland cells may function in cuticle formation and food storage.

### Diagnosis and Treatment

Specific diagnosis depends on demonstrating a worm or egg in the stool. The eggs, with distinctive bipolar plugs, are 50  $\mu\text{m}$  to 54  $\mu\text{m}$  by 22  $\mu\text{m}$  to 23  $\mu\text{m}$  and have smooth outer shells. Their structure and formation have been reported by Preston and Jenkins, Clinical symptoms may be

## Basic Biological Science Part I

confused with those of hookworm, amebiasis, or acute appendicitis. Worms can be demon started dramatically by colonoscopy.

Because of their frequent location in the cecum, appendix, or lower ileum, whipworms are difficult to reach with oral drugs or medicated enemas. Mebendazole and albendazole are effective drugs. Training of children and adults in sanitary disposal of feces and in washing of hands is necessary to prevent reinfection.

### **Hookworm Disease**

The distinction between hookworm infection and hookworm disease is important. Far more people are infected with the worm than exhibit symptoms of the disease. The presence and severity of the disease depend strongly on three factors: the number of worms present, the species of hookworm and the nutritional condition of the infected person. In general fewer than 25 *N.americanus* in a person will cause no symptoms, 25 to 100 worms lead to light symptoms, 100 to 500 produce considerable damage and moderate symptoms, 500 to 1000 result in severe symptoms and grave damage, and more than 1000 worms causes very grave damage that may be accompanied by drastic and often fatal consequences. Because *Ancylostoma* spp. Suck more blood than *N.americanus*, fewer worms cause greater disease; for example, 100 worms may cause severe symptoms. However, the clinical disease is intensified by the degree of malnutrition, corresponding impairment of the host's immune response, and other considerations.

# Basic Biological Science Part I

## Diagnosis

Demonstration of hookworm eggs or the worms themselves in feces is, as usual, the only definitive diagnosis of the disease. Demonstration of eggs in direct smears may be difficult, however, even in clinical cases and one of the several concentration techniques should be used. If estimation of worm burden is necessary, techniques are available that give reliable data on egg counts.

It is not necessary or possible to distinguish *N.americanus* eggs from those of *Ancylostoma* spp., but care should be taken to differentiate *strongyloides stercoralis* infections. This is not a problem unless some hours pass between time of defecation and time of examination of feces. Then hook worm eggs may have hatched and the juveniles must be distinguished from those of *S.stercoralis*.

## Treatment

Mebendazole is the drug of choice for treatment, as it removes both species of hookworm and also any concurrent infection with *Ascaris lumbricoides*. Single dose therapy is inexpensive, convenient and effective. Unfortunately, there is evidence for resistance of *N.americanus* to mebendazole in Africa.

Treatment for hookworm disease should always include dietary supplementation. In many cases, provision of an adequate diet alleviates

## Basic Biological Science Part I

the symptoms of the disease without worm removal, but treatment for the infection should be instituted, if only for public health reasons.

### Control

Control of hookworm disease depends on lowering worm burdens in a population to the extent that remaining worms, if any, can be sustained within the nutritional limitations of the people without causing symptoms. Mass treatment campaigns do not eradicate the worms but certainly lower the “seeding” capacity of their hosts. Education and persuasion of the population in the sanitary disposal of feces are also vital. Economic dependence on nightsoil in family gardens remain one of the most persistent of all problems in parasitology.

### Superfamily Ascaridoidea

#### Family Ascarididae

The ascaridids are among the largest of nematodes, some species achieving a length of 45 cm or more. Cervical, lateral, and caudal alae are present or absent, as are esophageal ceca and ventriculi. Three large rounded or trapezoidal lips are present; interlabia are absent. Spicules are simple and equal. This family contains one of the oldest associates of humankind: *Ascaris lumbricoides* the intestinal large roundworm.

#### **Ascaris lumbricoides and Ascaris sum**



## Basic Biological Science Part I

Because of their great size, abundance, and cosmopolitan distribution, these nematodes may well have been the first parasites known to humans. Certainly, the ancient Greeks and the Romans were familiar with them and they were mentioned in the Ebers Papyrus. It is probable that *A.lumbricoides* was originally a parasite of pigs that adapted to humans when swine were domesticated and began to live in close association with humans – or perhaps it was a human parasite that we gave to pigs. (The physiologies of people and swine are remarkably similar as, on occasion, are their eating and social habits). Today two populations of this parasite exist, one in humans and one in pigs. The two forms are so close morphologically that they were long considered the same species. Slight differences in the tiny denticles on the dentigerous ridges along the inner edge of the lips are consistent and clearer when the structures are viewed with the scanning electron microscope, therefore, we now consider the two separate species: *A. suum* from pigs and *A.lumbricoides* from humans.

### **Diagnosis and Treatment**

Accurate diagnosis of migrating juveniles is impossible at this time. Demonstration of juveniles in sputum is definitive, provided the technician can identify them. Most diagnoses are made by identifying the characteristic, mammillated eggs in the stool or by an appearance of the worm itself. So many eggs are laid each day by one worm that one or two direct fecal smears are usually sufficient to demonstrate at least one.

## Basic Biological Science Part I

*Ascaris lumbricoides* should be suspected when any of the previously listed pathogenic conditions are noted. Most light infections are asymptomatic and presence of worms may be determined only by spontaneous elimination of spent individuals from the anus.

Mebendazole is the drug of choice, with pyrantel pamoate as an alternative. Mebendazole binds to tubulin in the worm's intestinal cells and body wall muscles. No efficient treatment of migrating juveniles has been discovered. Nitazoxanide, a drug used to treat cryptosporidial diarrhea, appears to be effective against a variety of helminthes, including *A.lumbricoides* Ivermectin is effective against *A.lumbricoides*.

### Family Oxyuridae

#### **Enterobius Vermicularis and E.Gregorii**

Pinworms have infected *Homo sapiens* since the time of our species origin in Africa. In some ways they are paradoxical among nematode parasites of humans. They are not limited to the tropics and subtropics in their distribution, thriving well into temperature zones of the world. Furthermore, pinworms often are found in families at high socio economic levels, where, after introduction into the premises by one member, they rapidly become a “family affair”. It is fair to say, however, that the greatest pinworm problems are among institutionalized.

### **Diagnosis and Treatment**

## Basic Biological Science Part I

Positive diagnosis can be made only by finding eggs or worms on or in the patient. Ordinary fecal examinations are usually unproductive because few eggs are deposited within the intestine and passed in feces. Heavy infections can be discovered by examining the perianus closely under bright light. During the night or early morning. Wandering worms glisten and can be seen easily.

When adults cannot be found, eggs often can be, as they are left behind in the perianal folds. A short piece of cellophane tape, held against a flat, wooden applicator or similar instrument, sticky side out, is pressed against the junction of the anal canal and the perianus. The tap is then reversed and stuck to a microscope slide for observation. If a drop of xylene or toluene is placed on the slide before the tape, it will dissolve the glue on the tape and clear away bubbles, simplifying the search for the characteristic, flat-sided eggs. A physician can teach an infected child's parent how to prepare the slide, since it should be done just after awakening in the morning, certainly before bathing the child for a trip to the doctor's office.

The preferred drug is mebendazole. Treatment should be repeated after about 10 days to kill worms acquired after the first dose and sanitation procedures should be instituted concurrently. All members of the household should be treated simultaneously, regardless of whether the infection has been diagnosed in all.

## Basic Biological Science Part I

Although diagnosis and cure of enterobiasis are easy, preventing reinfection is more difficult. Personal hygiene is most important. Completely sterilizing the household is a difficult activity, albeit gratifying, but it is of limited usefulness. Nevertheless, at the time of treatment, all bed linens, towels and the like should be washed in hot water, and the household should be cleaned as well as possible to lower the prevalence of infective eggs in the environment. If all people in a household are undergoing chemotherapy while reasonable care is being taken to avoid reinfection, the family infection can be eradicated – until the next time a child brings it home from school.

### Family Onchocercidae

Members of the family Onchocercidae live in the tissues of amphibians, reptiles, birds, and mammals. Most are of no known medical or economic importance, but a few cause some of the most tragic, horrifying, and debilitating diseases in the world today. Of these, species of *Wuchereria*, *Brugia*, *Onchocerca* and *Loa* will be considered in some detail. Short mention will be made of others.

### **Wuchereria Bancrofti**

Perhaps the most striking disease of humans is the clinical entity known as **elephantiasis**. The horribly swollen parts of the body afflicted with this condition have been known since antiquity. Ancient Greek and Roman writers likened the thickened and fissured skin of infected persons

## Basic Biological Science Part I

to that of the elephant, although they also confused leprosy with this condition. Actually, elephantiasis is a non-sense word, since literally translated it means “a disease caused by elephants”. The word is so deeply entrenched, however, that it is not likely ever to be abandoned. Classical elephantiasis is a more unusual consequence of infection by *Wuchereria bancrofti* and by at least two other species of filaroids.

Infection of the lymphatic system by filarial worms is best referred to as **lymphatic filariasis** and is much more common than elephantiasis; recent estimates put the global prevalence at 119 million cases. **Bancroftian filariasis** is responsible for 90% of lymphatic filariasis, extending throughout central Africa, the Nile Delta, Turkey, India.

### **Diagnosis and Treatment**

Demonstration of microfilariae in the blood involves a simple and fairly accurate diagnostic technique, provided that thick blood smears are made during the period when the juveniles are in the peripheral blood. Technicians must be able to distinguish this species from others that could be present. A technique based on the polymerase chain reaction can detect as little as 1 pg of filarial DNA (just 1% of the DNA in one microfilaria). Because many infected people are amicrofilaremic, techniques to detect antigens from adult worms would be most useful. Some such techniques are easy to use in the field and are very promising. The vigorous movement of adults can often be detected by

## Basic Biological Science Part I

ultrasonography, a pattern of noises referred to as a “filarial dance sign. X-ray examination can detect dead, calcified worms.

The drug of choice for the past 40 years has been diethyl, carbamazine which eliminates microfilariae from the blood and with careful administration usually kills the adults. The standard treatment has been 6mg/kg, which has had some significant disadvantages.

Prevention primarily involves protection against mosquito bites in endemic areas. People temporarily visiting such places should use insect repellent, mosquito netting, and other preventive measures rigorously. Long-term protection requires mosquito control and mass chemotherapy of indigenous people to eliminate microfilariae from the circulating blood, where they are available to mosquitoes. To be successful, such measures require some education of people in endemic areas.

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