!!JAY AMBE!!

B. PHARMACY SEMESTER – I
HUMAN ANATOMY AND PHYSIOLOGY
PRACTICALS
ACCORDING TO PCI SYLLABUS

PREPARED BY

DR. NAITIK D. TRIVEDI,
M. PHARM, PH. D
LECTURER AT GOVERNMENT AIDED,
A. R. COLLEGE OF PHARMACY & G. H. PATEL INSTITUTE OF PHARMACY, VALLABH VIDYANAGAR, ANAND.
Mobile: +91 - 9924567864
E-mail: info@drnaitiktrivedi.com

&

DR. UPAMA N. TRIVEDI,
M. PHARM, PH. D
ASSOCIATE PROFESSOR & HoD (Pharm.D),
INDUBHAI PATEL COLLEGE OF PHARMACY AND RESEARCH CENTRE, DHARMAJ.
E-mail: ups.aasthu@gmail.com

CERTIFICATE

Class:_____ **Year:**_____

This is to certify that Mr./Miss. _____

of _____ at _____

Enrollment No. _____ & Roll No. _____ has satisfactory completed his/her _____ out of _____ experiments/practicals of the subject _____ for the academic year 20 __ to 20 __.

Signed by:

_____ _____ _____

Head of Department **External Examiner** **Subject Teacher**

Date of certified:

B. PH SEM – I: HUMAN ANATOMY AND PHYSIOLOGY PRACTICAL

INDEX

EXPERIMENT NO.: 1 **DATE:**

AIM: INTRODUCTION AND SCOPE OF ANATOMY AND PHYSIOLOGY

INTRODUCTION:

- Anatomy and physiology concern with the structures and functions of the human body.
- **Anatomy describes the structures of the body** -- their scientific names, composition, location, and associated structures. Anatomy ("a cutting open") is a plan or map of the body.
- **Physiology studies the function of each structure**, individually and in combination with other structures.
- Anatomy and physiology always work together. As we examine each part of the body, always consider both its structure and its function.

➢ **The study of anatomy is divided into 2 major fields:**

1. **Gross anatomy** is the study of large visible structures
2. **Microscopic anatomy** is the study of structures that are too small to see, such as cells and molecules.

1. **Gross anatomy**, also called macroscopic anatomy, is separated into 5 major divisions:
   A. **Surface anatomy** describes surface forms and marks.
   B. **Regional anatomy** describes the organization of specific areas of the body such as the head or hand. This approach is used mostly in professional schools: medical, dental, physical therapy.
   C. **Systemic anatomy** describes groups of organs that function together for a single purpose.
   D. **Developmental anatomy** describes the structural changes in an organism from fertilized egg to maturity. Embryology is the anatomical study of early development.
   E. **Clinical anatomy** describes various medical specialties, including medical anatomy (changes that occur during illness), and radiographic anatomy.

2. **Microscopic anatomy** is divided into two major divisions:
   A. **Cytology,** the study of cells and their structures.
   B. **Histology,** the study of tissues and their structures.

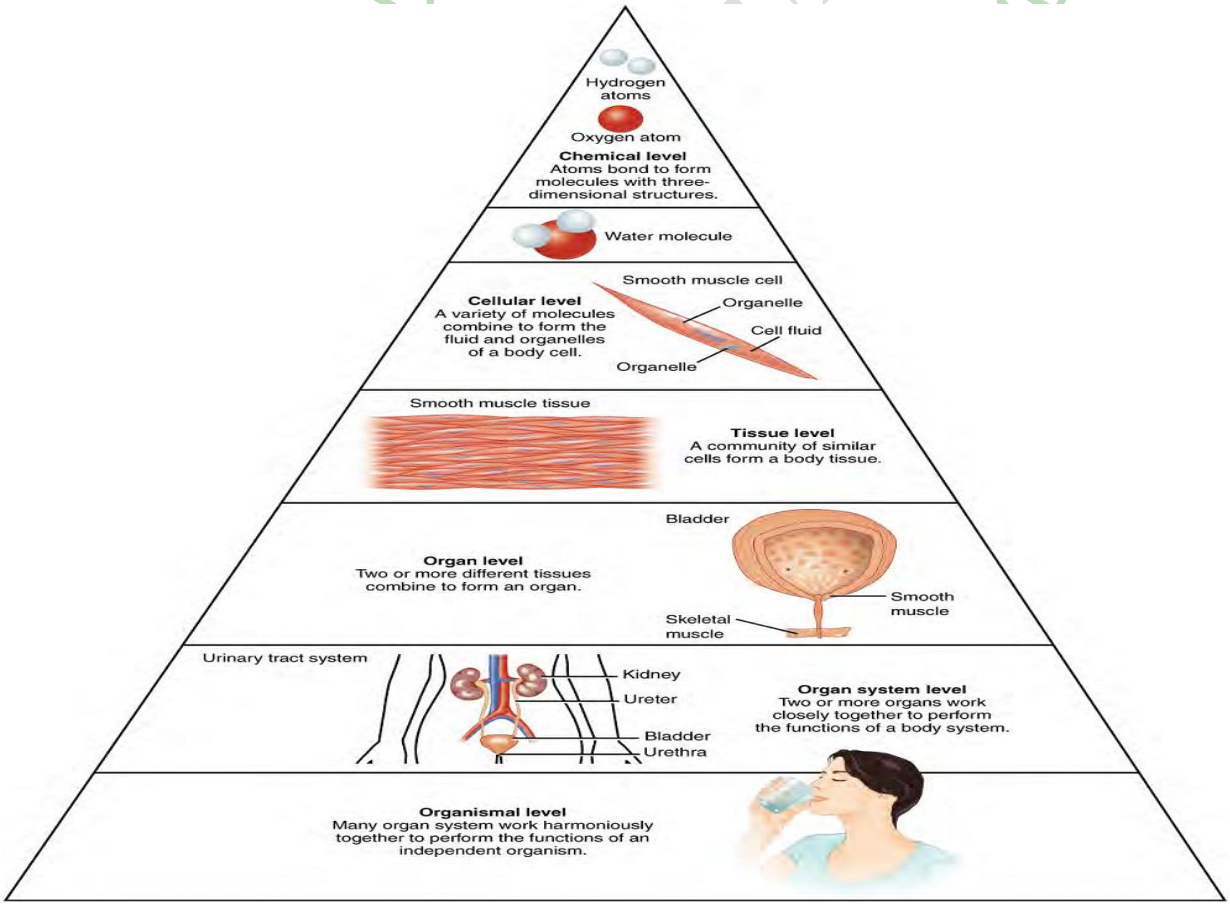
➢ **Physiology has many specialties. The 4 basic divisions are:**

1. **Cell physiology**, including chemical and molecular processes within and between cells.
2. **Special physiology**, the study of specific organs such as the heart.

3. **Systemic physiology,** the cooperative functions of all the organs in an organ system. We will use a systemic physiology approach in this class.
4. **Pathological physiology**, the effects of diseases on organs and organ systems.

➢ Levels of Organization

- Our bodies are organized at many different levels.
- The levels of organization of living things, from smallest to largest, are:
  1. **Atoms,** the smallest functional units of matter.
  2. **Molecules,** active chemicals.
  3. **Organelles**, specialized structures within a cell.
  4. **Cells,** the smallest living units.
  5. **Tissues,** a group of similar cells that work together.
  6. **Organs,** two or more tissue types working together.
  7. **Organ systems,** two or more organs working together.
  8. **Organism,** a single individual, including all of the above.



The human body is divided into 11 interconnected organ systems. All organ systems work together, and many organs function in more than 1 organ system.

1. **The Integumentary System:** includes the skin & derived structures, it protects internal organs & helps maintain body temperature.

2. **The Skeletal System:** includes the bones & joints, it provides support & protection to internal organs.
3. **The Muscular System:** includes skeletal muscle and it provides movement.
4. **The Nervous System:** includes the brain, spinal cord, and nerves. It provides regulation of body functions & sensory perception.
5. **The Endocrine System:** includes hormone-producing cells & glands. It regulates homeostasis, growth & development.
6. **The Cardiovascular System:** includes blood, heart, & blood vessels. It is responsible for delivery of oxygen & nutrients to the tissues.
7. **The Lymphatics & Immune System:** includes lymphatic vessels & fluid. It is involved in the defense against infection.
8. **The Respiratory System:** includes lungs & airways. It is involved in the absorption of oxygen & release of carbon dioxide.
9. **The Digestive System:** includes organs of the gastrointestinal tract. It is responsible for the absorption of nutrients.
10. **The Urinary System:** includes the kidneys, ureters, and bladder. It is responsible for electrolyte balance & waste removal.
11. **The Reproductive System:** includes the reproductive organs in males and females. It controls the biological process by which new individuals are produced.

HOMEOSTASIS:

- Ability to maintain relatively stable internal conditions despite a changing external environment. Dynamic state of equilibrium, or balance.
- The body is said to be in homeostasis when its cellular needs are adequately met and functional activities are occurring smoothly.
- Virtually every organ system plays a role in maintaining the internal environment.

A homeostatic regulatory mechanism consists of 5 parts:

1. **Receptors:** It act as a sensors/receiver that respond to a stimulus. It monitors change in control condition.
2. **Sensory Neurons:** It send the input information/message to control center, means information from cell/tissue/organ etc to integrated system i.e brain and spinal cord.
3. **Integrated System:** It analyze the incoming message received from the sensory neurons and sends out commands/messages. In the body there are hundred controlled

conditions. A few examples are heart rate, blood pressure, temperature and breathing rate.

4. **Motor Neurons:** The output information/message from integrated center (brain and spinal cord) to cell/tissue/organ etc are travelled by motor neurons.
5. **Effectors:** The cell/tissue/organ etc act as effector that responds according to output command of the control/integrated center.

Receptors, control center and effectors maintain the homeostasis by two mechanisms:

1. **Negative feedback:**

- When the response of effectors opposes the original stimulus, it is called negative feedback because it negates the stimulus.
- An example of negative feedback is the temperature thermostat in your home.
- Temperature sensors turn the air conditioner off and on to maintain air temperature within a specific, limited range.
- In the same way, the brain controls normal body-temperature homeostasis by negative feedback.
  - Some stimulus (Stress) disrupts homeostasis (control condition) by an increase in body temperature.
  - Due to this condition thermoreceptors (temperature sensitive receptors) in the skin and brain activate and send input message via nerve impulse to control center.
  - Control center analyze the input message and send output message to effectors (skin).
  - Effectors according to output message of control center increases sweating from sweat glands causes increased heat loss by evaporation.
  - Finally, decreases the temperature in the form of response and normalize the body temperature (control condition).

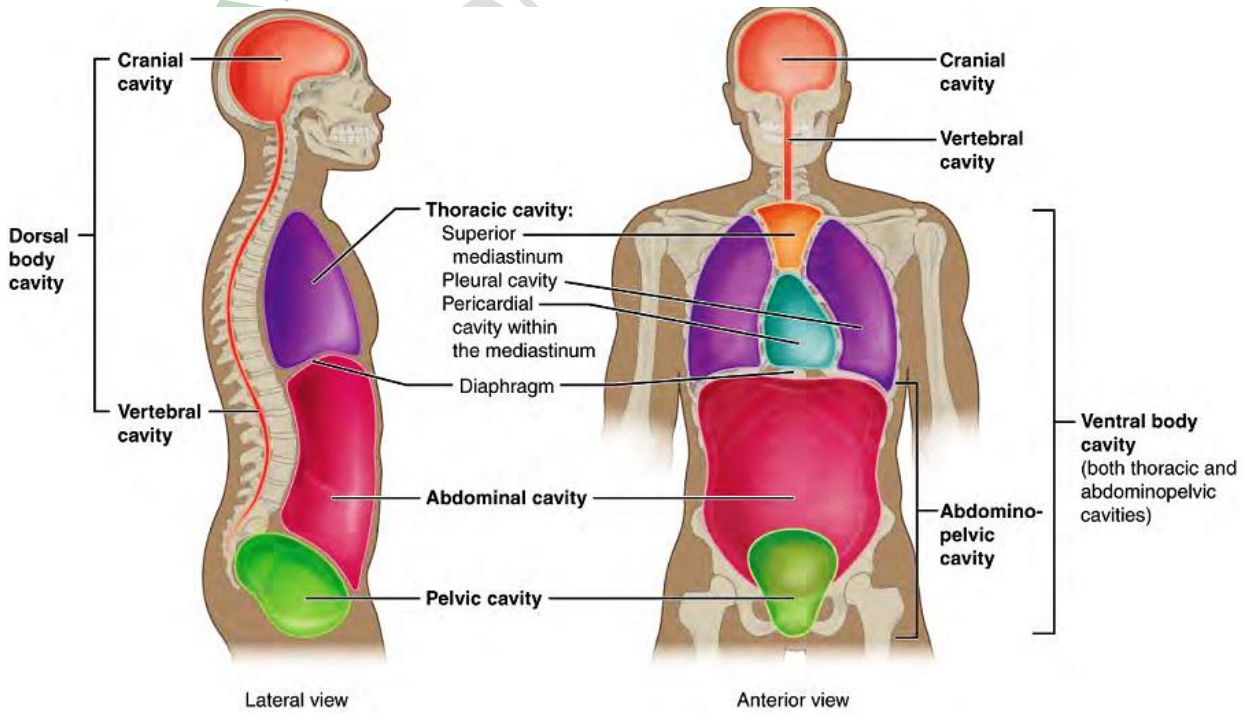
2. **Positive feedback:**

- The effector adds to the initial stimulus instead of negating it, speeding up the process.
  - Labor contraction is the example of positive feedback system.
  - Labor contractions force baby's head or body into birth canal.
  - It produces effect on control condition and increases distention of cervix of uterus.

- It activates the stretch receptors of cervix and send input message to control center via sensory nerve impulse.
- Control center activates the hypothalamus and pituitary gland and send the output message to increase oxytocin secretion in blood.
- Oxytocin produces their effect on to the effector (cervix of uterus) and cause distention of cervix of uterus than the normal value to push the baby further into birth canal.
- Birth of the baby decreases distention of cervix of uterus and interrupts positive feedback cycle.

BODY CAVITIES AND SEROUS MEMBRANES

- The body maintains its internal organization by means of membranes, sheaths, and other structures that separate compartments.
- The dorsal (posterior) cavity and the ventral (anterior) cavity are the largest body compartments.
- These cavities contain and protect delicate internal organs, and the ventral cavity allows for significant changes in the size and shape of the organs as they perform their functions. The lungs, heart, stomach, and intestines, for example, can expand and contract without distorting other tissues or disrupting the activity of nearby organs.



Subdivisions of the Posterior (Dorsal) and Anterior (Ventral) Cavities

- The posterior (dorsal) and anterior (ventral) cavities are each subdivided into smaller cavities.
- In the posterior (dorsal) cavity, the cranial cavity houses the brain, and the spinal cavity (or vertebral cavity) encloses the spinal cord.

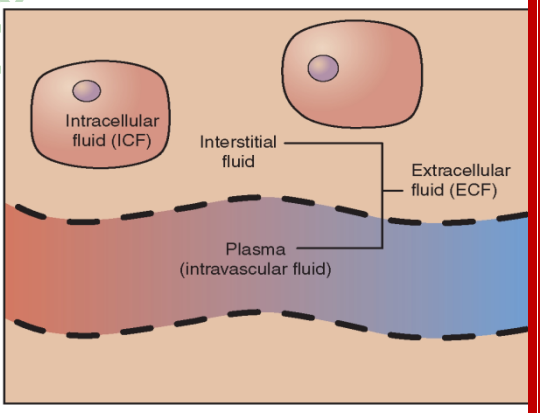
The anterior (ventral) cavity has divided by the diaphragm muscle into 2 parts:

1. **A superior thoracic cavity,** containing the
   A. **Pleural cavity (left and right, divided by the mediastinum) organs:** lungs membranes: visceral and parietal pleura
   B. **Pericardial cavity organs: heart membranes:** visceral and parietal pericardium
2. **Inferior abdominopelvic cavity**, containing the
   A. **Peritoneal cavity membranes:** visceral and parietal peritoneum
   B. **Abdominal cavity (superior peritoneal) organs:** liver, stomach, spleen, intestine
   C. **Pelvic cavity (inferior peritoneal) organs:** intestine, bladder, reproductive organs

Membranes of the Anterior (Ventral) Body Cavity:

- The walls of the ventral body cavity and the outer surfaces of the organs are covered with a thin, double layered membrane – serosa or serous membranes.
- Part of the membrane lining the cavity walls - parietal serosa -folds on itself to form the visceral serosa which covers the organs in the cavity.
  – Parietal - "parie"- means wall
  – Visceral - "viscus"- means an organ in a body cavity

BODY FLUIDS:



Water content of the body is divided into:

1. Intracellular compartment (67%) - Inside the cell
2. Extracellular compartment (33%) - Outside the cell

1. Intracellular Fluid (ICF)

- Comprises, 2/3 of the body water.
- If body has 60% water, ICF is about 40% of your weight.
- The ICF is primarily a solution of potassium and organic anions, proteins etc.
- The cell membranes and cellular metabolism control the constituents of this ICF.

2. Extracellular compartment (ECF):

- It is the remaining 1/3 of your body's water.

- ECF is about 20% of the body weight.
- The ECF is primarily a NaCl and NaHCO3 solution.
- The ECF is further subdivided into three sub-compartments:

A. Interstitial Fluid (ISF). B. Plasma. C. Transcellular fluid

A. Interstitial Fluid (ISF)

- Interstitial Fluid (ISF) surrounds the cells, but does not circulate.
- It is the main component of the extracellular fluid
- It comprises about 3/4 of the ECF.
- Interstitial fluid is found in the interstitial spaces, also known as the tissue spaces.

Composition of interstitial fluid:

- Water solvent amino acids
- Sugars
- Fatty acids
- Coenzymes
- Hormones
- Neurotransmitters
- Salts
- Waste products from the cells.
- Lymph is considered a part of the interstitial fluid

Function of interstitial fluid

- Intercellular communication.
- Interstitial fluid bathes the cells of the tissues.
- Removal of metabolic waste.

B. Plasma:

- It is the yellow liquid component of blood in which the blood cells in whole blood are normally suspended
- 55% of the total blood volume.
- It is the intravascular fluid part of extracellular fluid (all body fluid outside of cells)
- It makes up about 1/4 of the ECF.

Composition of plasma

- Water (90% by volume)
- Dissolved proteins
- Glucose
- Clotting factors
- Mineral ions
- Hormones
- Carbon dioxide.

Function of plasma

- Plasma is the main medium for excretory product transportation.

- Blood serum is blood plasma without fibrinogen or the other clotting factors (i.e., whole blood minus both the cells *and* the clotting factors).

C. Transcellular fluid

- Transcellular fluid is the portion of total body water contained within epithelial lined spaces.
- Smallest compartment.
- It is about 2.5% of the total body water.

  Examples
  - Cerebrospinal fluid
  - Ocular fluid (Aqueous humor)
  - Joint fluid (Synovial fluid)
  - Urine

Composition of transcellular fluid:

1. Cerebrospinal fluid:
   - The CSF is mainly produced by the choroid plexus.
   - The entire nervous system contains between 80-150 ml of CSF.
   - It is a clear colorless liquid that contains White blood cells, glucose, protein, lactic acid, urea, cations (Na^+, K^+, Ca^+ etc) and anions (Cl^-, and HCO_3^-).
2. Ocular fluid (Aqueous humor):
   - The aqueous humor is a transparent, gelatinous fluid similar to plasma.
   - It is located in the anterior and posterior chambers of the eye, the space between the lens and the cornea.
   - It contains Amino acids (transported by cilliary muscles), 98% water, Electrolytes, Ascorbic acid, Glutathione
3. Joint fluid (Synovial fluid):
   - Synovial fluid is clear, pale yellow, viscid, and does not clot.
   - The principal role of synovial fluid is to reduce friction between thearticular cartilage of synovial joints during movement.
   - It contains Normal 3–4 mg/ml hyaluronic acid, a polymer of disaccharides, WBC, RBC and proteins
4. Urine:
   - Urine is a typically sterile liquid by product of the body secreted by the kidneys through a process called urination and excreted through the urethra.

- It contains 95% water, Organic solutes like urea, creatinine, uric acid, and trace amounts of enzymes, carbohydrates, hormones, fatty acids, pigments, and mucins, and inorganic ions such as sodium (Na+), potassium (K+), chloride (Cl-), magnesium (Mg2+), calcium (Ca2+), ammonium (NH4+), sulfates (SO42-), and phosphates (e.g., PO43-).

SOME DEFINITIONS RELATED TO HUMAN ANATOMY AND PHYSIOLOGY SUBJECT:

CELL: It is living structural and functional units of body enclosed by membrane.

CYTOLOGY: It is the branch of science concern with the study of cells.

TISSUE: It is a group of cells that usually have common embryonic origin and function together for special activities.

BLOOD: It is a liquid connective tissue

LYMPH: It is a thin, watery, clear, modified tissue fluid formed by the passage of substance from the blood capillaries into the tissue space (interstitial space) and enters in to the closed system of lymphatic capillaries to lymphatic vessels and lymphatic sinus.

CARDIOVASCULAR SYSTEM: Cardiovascular is the system which includes the study of the heart, blood vessels and blood.

IMMUNE SYSTEM: It is the collection of cells, tissues and molecules that protects the body from numerous pathogenic microbes and toxins in our environment.

SIGNATURE OF TEACHER

EXPERIMENT NO.: 2 **DATE:**

AIM: TO STUDY THE USE AND CARE OF MICROSCOPE.

INTRODUCTION:

Types of microscope:

1. Microscopes used in clinical practice are **light microscopes**. They are called light microscopes because they use a beam of light to view specimens.
2. A **compound light microscope** is the most common microscope used in microbiology. It consists of two lens systems (combination of lenses) to magnify the image. Each lens has a different magnifying power. A compound light microscope with a single eye-piece is called monocular; one with two eye-pieces is said to be binocular.
3. Microscopes that use a beam of electrons (instead of a beam of light) and electromagnets (instead of glass lenses) for focusing are called **electron microscopes**. These microscopes provide a higher magnification and are used for observing extremely small microorganisms such as viruses.

PARTS OF MICROSCOPE:

The main parts of the microscope are the eye-pieces, microscope tube, nosepiece, objective, mechanical stage, condenser, coarse and fine focusing knobs, and light source.

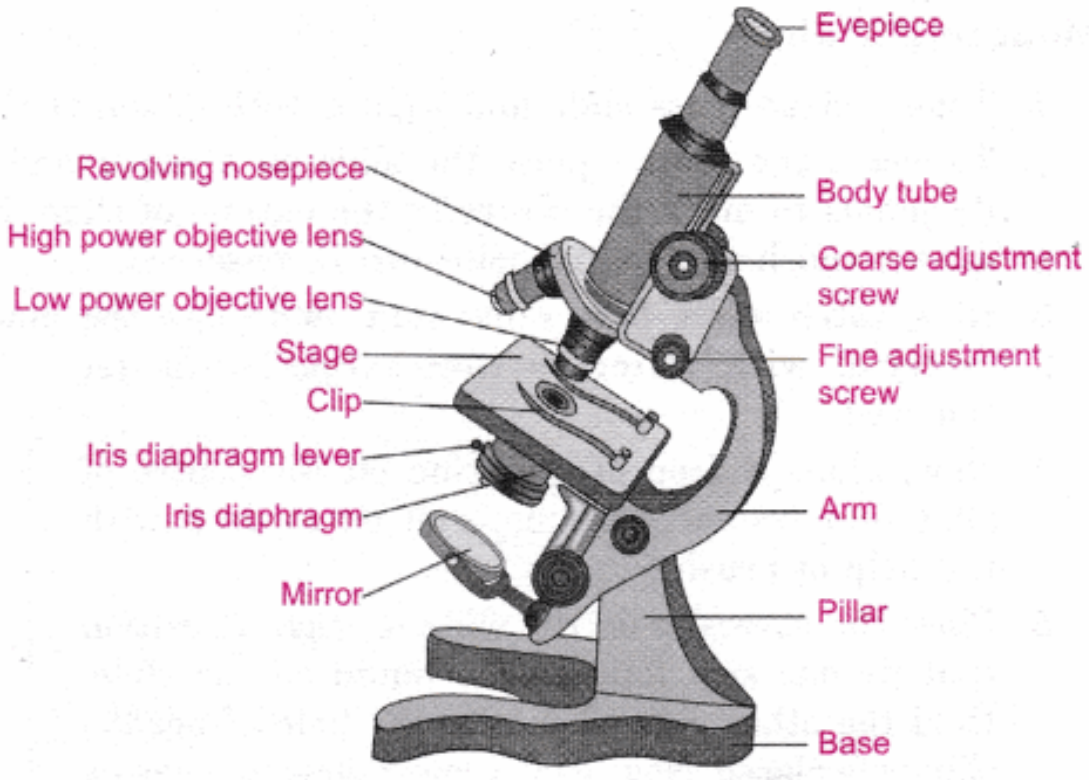


Fig. A compound microscope

COMPOUND MICROSCOPE PARTS

- A high power or compound microscope achieves higher levels of magnification than a stereo or low power microscope. It is used to view smaller specimens such as cell structures which cannot be seen at lower levels of magnification. These key microscope parts are illustrated and explained below.

A. STRUCTURAL COMPONENTS

1. **Base (foot):** It is U or horseshoe-shaped metallic structure that supports the whole microscope.
2. **Pillar:** It is a short upright part that connects to base as well as arm.
3. **Arm (Limb):** It is a curved metallic handle that connects with the arm by inclination joint. It supports stage and body tube.
4. **Inclination Joint:** It is used for tilting the microscope if required for observation in sitting position.
5. **Stage:** It is a metallic platform with a central hole fitted to the lower part of the arm. Microscopic slides held on the stage by either simple side clips or by a mechanical stage clip.
6. **Body tube:** It is meant for holding ocular and objective lenses at its two ends. The end holding ocular lens is called head while the end containing 3-4 objective lens is called nose piece. The body tube has an internal pathway for the passage of light rays which form the enlarged image or microscopic objects.
7. **Draw tube:** It is a small tube that remains fixed at the upper end of the body tube. It holds eyepiece or ocular lens.
8. **Rack and pinion:** The microscope has a rack and pinion attached either to body tube or the stage for bringing the object under focus.
9. **Adjustment screws:** There are two pairs of screws for moving the body tube in relation to stage, larger for coarse adjustment and smaller for fine adjustment. In fine adjustment the body tube or stages moves for extremely short distances. In coarse adjustment the body tube or stage can move up and distance. In coarse adjustment is meant for briefly objective lens at a proper distance from the object so as to form image of the same at the ocular end. Fine adjustment is required to obtain sharp image.
10. **Automatic Stop:** It is a small screw fitted at lower end or rack and pinion. It is meant for stopping the downward sliding of the body tube so as to prevent the damage of objective lens and the slide.

B. OPTICAL COMPONENTS

1. **Diaphragm:** It is flitted just below the stage for regulating the amount of light failing on the object. Diaphragm is of two types, disc and iris.
2. **Condenser:** It is attached below the diaphragm. Condenser can be moved up and down to focus light on the object.
3. **Reflector (Mirror):** It is attached just above the base. Both its surface bear mirrors, plane on one side and concave on other side. Plane side is used in strong light and concave side in weak light. Reflector directs the light on the object through the condenser and diaphragm system.
4. **Objective Lenses:** They are fitted over the nose piece. Objective lenses are of three types – low power (commonly 10X or 5X), high power (commonly 45X) and oil immersion (commonly 100X, can be more).
5. **Ocular Lens or Eyepiece:** It is lens through which image of the microscopic object is observed. It also takes part in magnification. Depending upon magnification, the eye piece is of four types-5X, 10X, 15X, and 20 X.

USE AND CARE OF THE MICROSCOPE

- Always keep the microscope clean, dust free and covered. Clear space on the bench before getting the microscope from the cabinet
- Grasp the microscope with two hands – one on the arm and the other under the base
- When you remove the microscope from the cabinet, do it slowly and carefully
- Remove the dust cover and store it in the scope cabinet
- Verify that the MIRROR is set for minimum light. Concave mirror is used while using low power lens and the plane mirror is used while using high power or oil immersion lens. Adjust the mirror such that the maximum and even illumination is obtained.
- Lower the stage (or raise head)
- Check that the **CONDENSER** is flush with the stage and the iris diaphragm is open
- Using the knurled nose ring, rotate and click the shortest.
- Load a slide, being sure it sits flat on the stage, held by the spring clip
- While looking into the eyepieces, slowly turn the coarse knob, moving lens closer to stage. As soon as you see a hint of color, switch to the small, fine focus knob and focus the object. Close one eye at a time to compare images.
- Once the slide is perfectly focused and the image is centered on low power, use the knurled nosepiece to click the next larger lens into place. **DO NOT USE THE**

COARSE FOCUS KNOB after increasing magnification. Only use the fine focus knob to focus with a higher power lens.

- If you cannot find the image when you increase the magnification, go back to 4X and start again.

SIGNATURE OF TEACHER

EXPERIMENT NO.: 3 **DATE:**

AIM: TO STUDY AND INTRODUCTION OF EPITHELIAL AND CONNECTIVE TISSUE.

DEFINITION: "It is a group of cells that usually have common embryonic origin and function together for special activities."

INTRODUCTION:

Body tissues can be classified in to four principal types according to their function and structure:

1) Epithelia tissue:

- It cover body surface, lines hollow organs, body cavity and ducts.
- It also forms glands.

2) Connective tissue:

- It provides the supports and protects the body and its organs.
- It binds organs together.
- It store energy as reserved fat.
- It provides immunity.

3) Muscle tissue:

- It is responsible for movements and generation of force.

4) Nervous tissue:

- It initiates and transmits action potential (Nerves Impulse) that helps coordinate body activities.

During the embryonic developments zygote produces three germ layers:

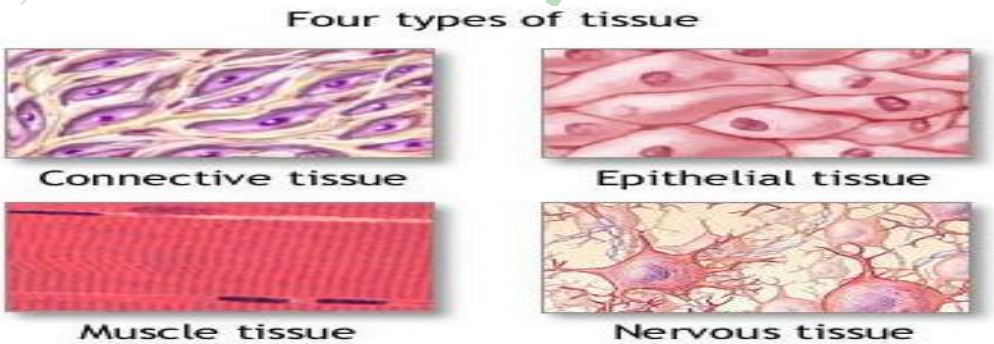
a) Ectoderm

b) Endoderm and

c) Mesoderm.

These three are embryonic tissue from which all tissues and organs of the body develop.

- Epithelium tissue derived from all three layers.
- Connective tissue and most muscles tissue derived from mesoderm.
- Nervous tissue derived from ectoderm

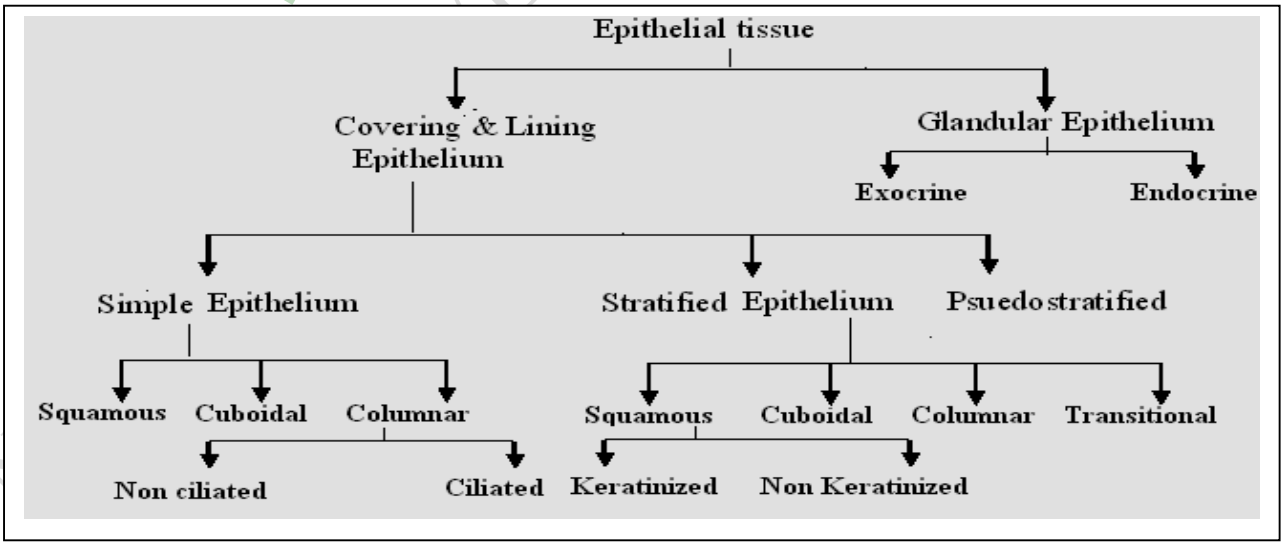


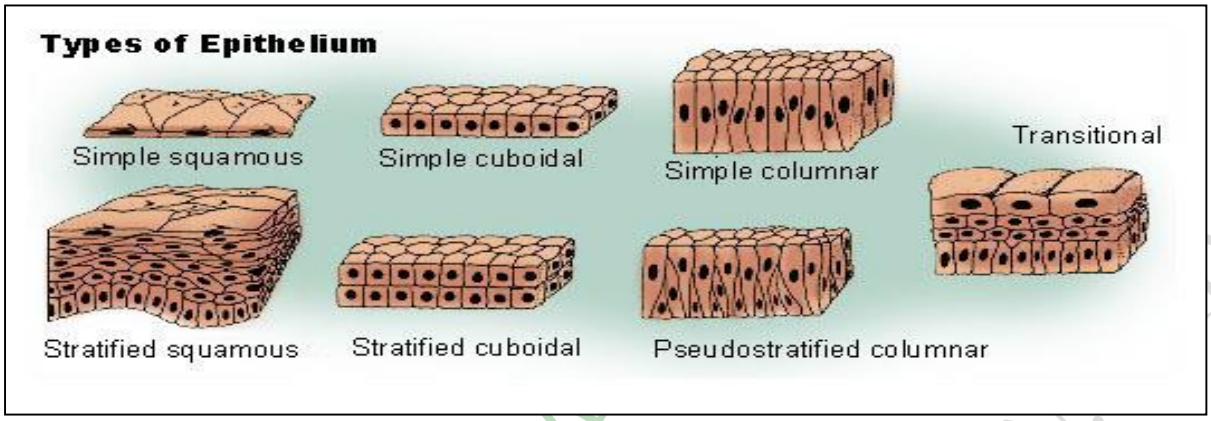
1. EPITHELIAL TISSUE:

General future of epithelial tissue:

- It consist large and closely packed cells with little extracellular material between adjacent cells.
- It's arrangement produce continues sheet which is either single layer or multi layers.
- Epithelial cells have an apical (free) surface, which produce the lining of internal organ so it exposed in to a body cavity.
- The basal surface of the epithelial cells attached with the basement membrane.
- Epithelial cells are avascular so the blood vessels that supply to nutrients and move wastes are located in adjacent connective tissue.
- The material exchanges take place in epithelium by the diffusion process.
- Epithelium cells are adheres to connective tissue which holds the epithelium in their position.
- The junction between the epithelium and connective tissue is known as basement membrane which consist two layers.
  1. **Basal lamina:** contain collagen, laminin and proteoglycan secret by epithelium.
  2. **Reticular lamina:** This contains reticular fibers, fibronectin and glycoproteins.
- The main function of epithelium is protection, filtration, lubrication, secretion, digestion, absorption, transportation, sensory reception and reproduction.

➢ CLASSIFICATION OF EPITHELIAL TISSUE:





1) Covering and lining epithelium:

- It forms the superficial layer of the skin and some internal organ.
- It forms the inner lining of blood vessels, ducts, body cavities and the interior of the respiratory, digestive, urinary and reproductive systems.

➢ According to **arrangements of layer** it is classified in to three types:

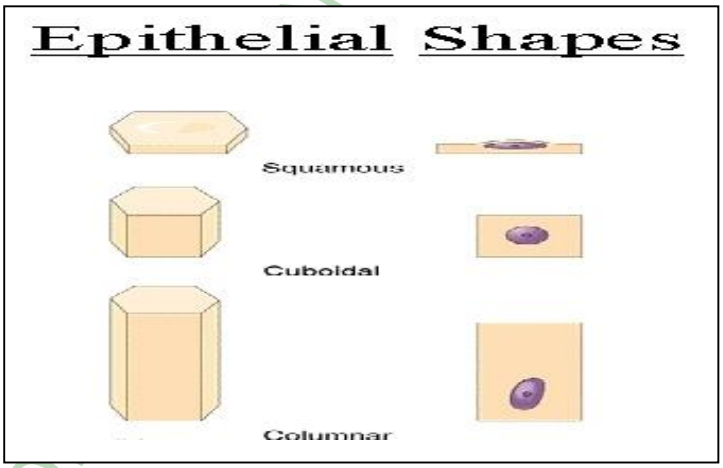
I) Simple epithelium:

- It is a single layer of cells.
- It founds where activities such as diffusion, osmosis, filtration, secretion and absorption occurs.

✓ **According to shape of the cells it is further divided in to:**

Epithelium is named according to shape, structure, and arrangement of cells.

- •squamous - thin and flat cells
- •cuboidal - cube shaped cells
- •columnar - column shaped cells
- •simple - single layer of cells
- •stratified - multilayered cells
- •pseudostratified - false stratified
- •transitional - stretchable
- •ciliated - cells possess cilia



a) Simple squamous Epithelium:

- It is a flat in shape.
- This consists of a single layer of flat cells.
- Their surfaces look like as tiles floor.
- The nucleolus of each cell is oval or spherical shape.

- It follows the osmosis or diffusion process.
- It found in the body where the little wear and tear is found.
- It lines the hearts, blood vessels and lymphatic vessels and also forms the wall of capillary known as endothelium.
- The cells which form the epithelial layer of serous membrane are known as mesothelium.

b) Simple cuboidal epithelium:

- It is cuboidal in shape.
- The nucleus of the cell is round.
- The main function of this tissue is absorption and secretion.

c) Simple columnar epithelium:

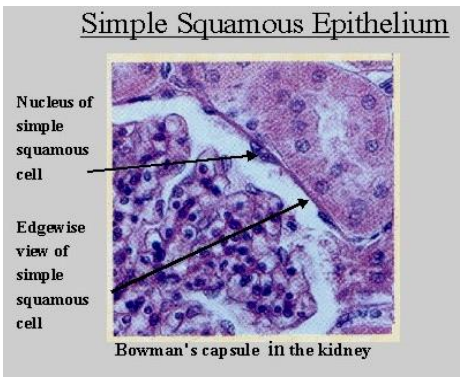
- It is rectangular in shape.
- It consist oval nuclei.
- It mainly produces two forms:

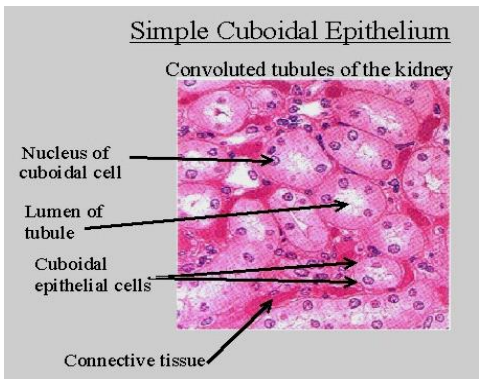
i) Nonciliated simple columnar epithelium:

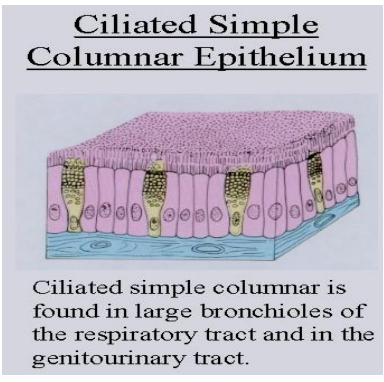
- It contains microvilli and goblet cells.
- Microvilli produce the microscopic fingerlike structure which increases the surface area of plasma membrane.
- Goblet cells secret mucus which is slightly sticky fluid.

ii) Celiated simple columnar cells:

- Celia produces the hairlike processes means it's movement gives the motion.
- Eg.: Secondary oocyte moves toward fallopian tube for fertilization by or fertile ovum down the uterin tube to the uterus help of celia.







II) Stratified Epithelium:

- It contains two or more layers.
- It protects the underline tissues from where there is considerable tear and wear.

➢ According **to shape it can** be further classified as under:

a) Stratified squemous epithelium:

- In the superficial layer this type of cells are flat whereas in the deep layers cells vary in shape from cuboidal to columnar.
- Here, the basal cells continuously replicate by cell division and produce new cells which shift upward toward the surface.
- So, they loss their blood supply from the connective tissue so they become dehydrated, shrunken and harder.
- These processes replace old cells by new cells.

✓ **Stratified squemous epithelium exists in two forms:**

i) Keratinized stratified squemous epithelium:

- It consist tough layer of keratin.
- It is a protein which is water proof and prevents us from several bacterial attacks.

ii) Non keratinized stratified squemous epithelium:

- It does not contain keratin and remains moist.

b) Stratified cuboidal epithelium:

- It consist two or more layer of cells in which superficial cells are cube-shaped.
- Duct of adult sweat glands and part of male urethra consist these cells.
- The main function is to give protection.

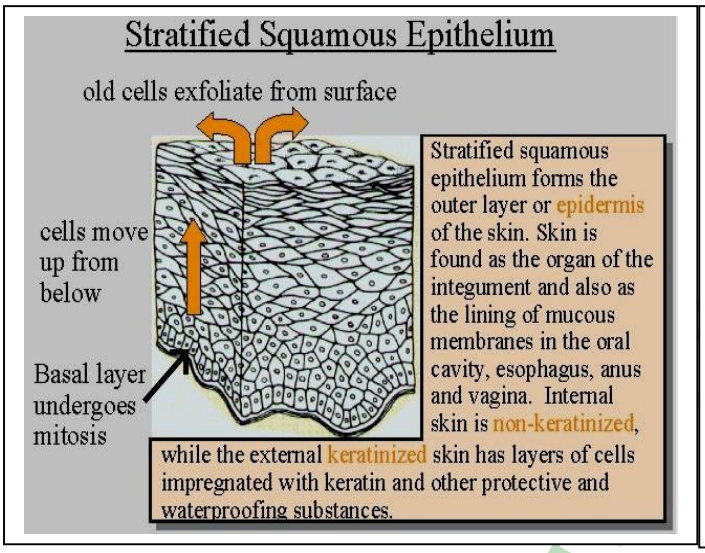
c) Stratified columnar epithelium:

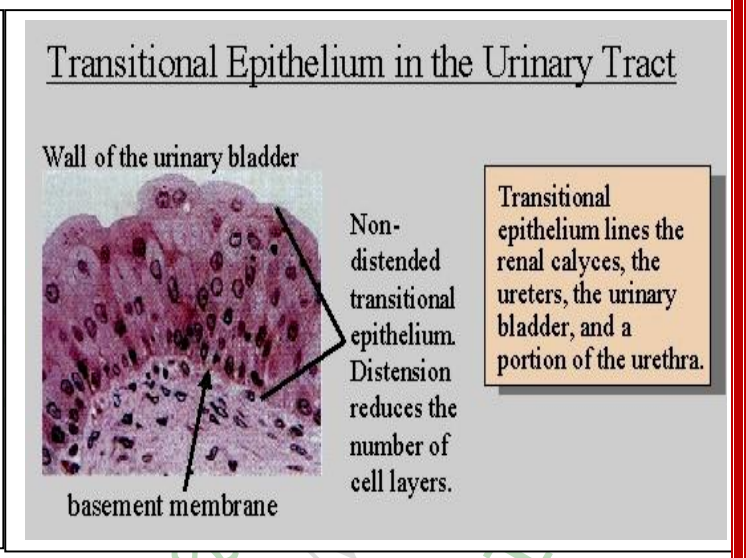
- It consist several layer of polyhedral cells.
- Only the superficial cells are columnar.
- Conjunctiva of eye, anal mucous membrane, urethra consist these cells.
- It gives protection and secretion.

c) Transitional epithelium:

- Its appearance is variable mainly it depends either it is stretched or relaxed.

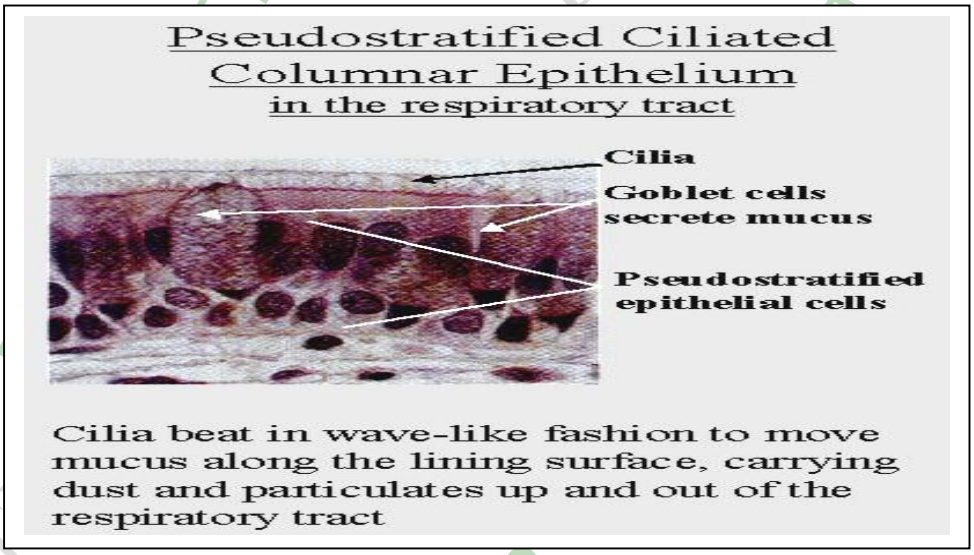
- Its line the urinary bladder and portion of ureters and urethra.





III) Pseudostratified epithelium:

- It contains mixture of cells in one layer.
- It produces multilayered tissue like appearance because all cells nuclei not reach to the surface of cells. These type of cells either ciliated or secrete mucus.



2) Glandular epithelium:

- These types of cells are mainly present in gland the main function of these cells is secretion.

➢ **There are two types of secretary gland:**

a) exocrine:

- It secret their product in to duct.

- The secretion includes mucus, perspiration, skin oil, ear wax and digestive enzymes.
- Eg.: Sweat glands, Salivary glands.

✓ **According to structure it is divided into two classes:**

i) unicellular

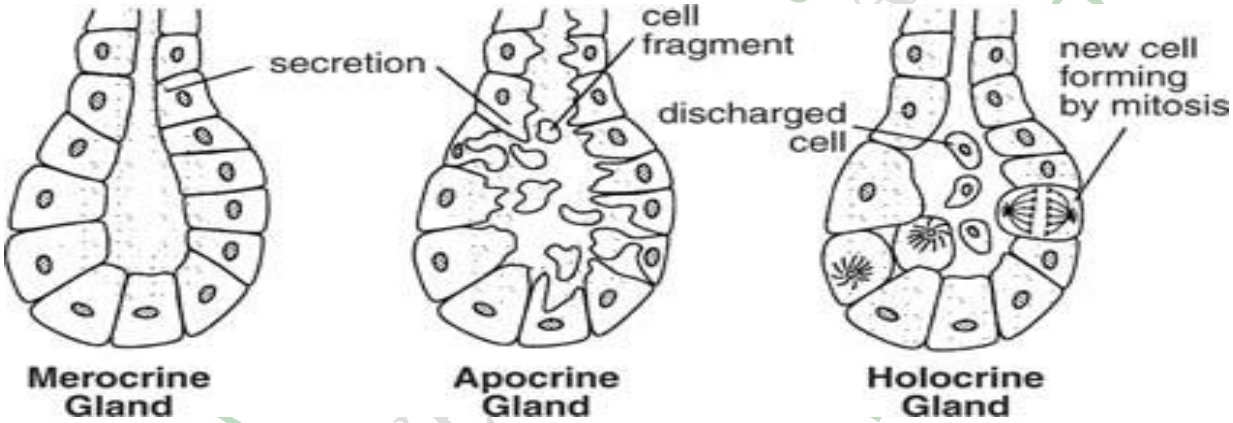
ii) multi cellular

✓ **According to function it is divided in to:**

i) **Merocrine glands:** it forms the secretary product and discharge it. (salivary glands)

ii) **Apocrine glands:** accumulate their secretary product on their apical surface. (mammary glands).

iii) **Halocrine glands:** accumulate secretary product in cytosol. (Sebaceous gland).



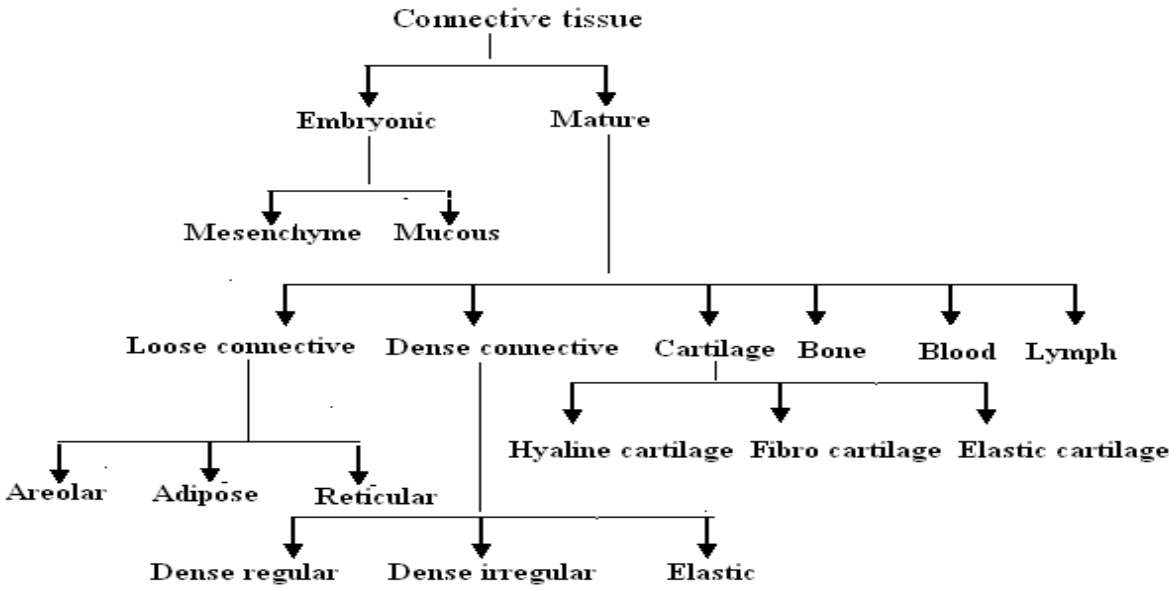
b) endocrine glands:

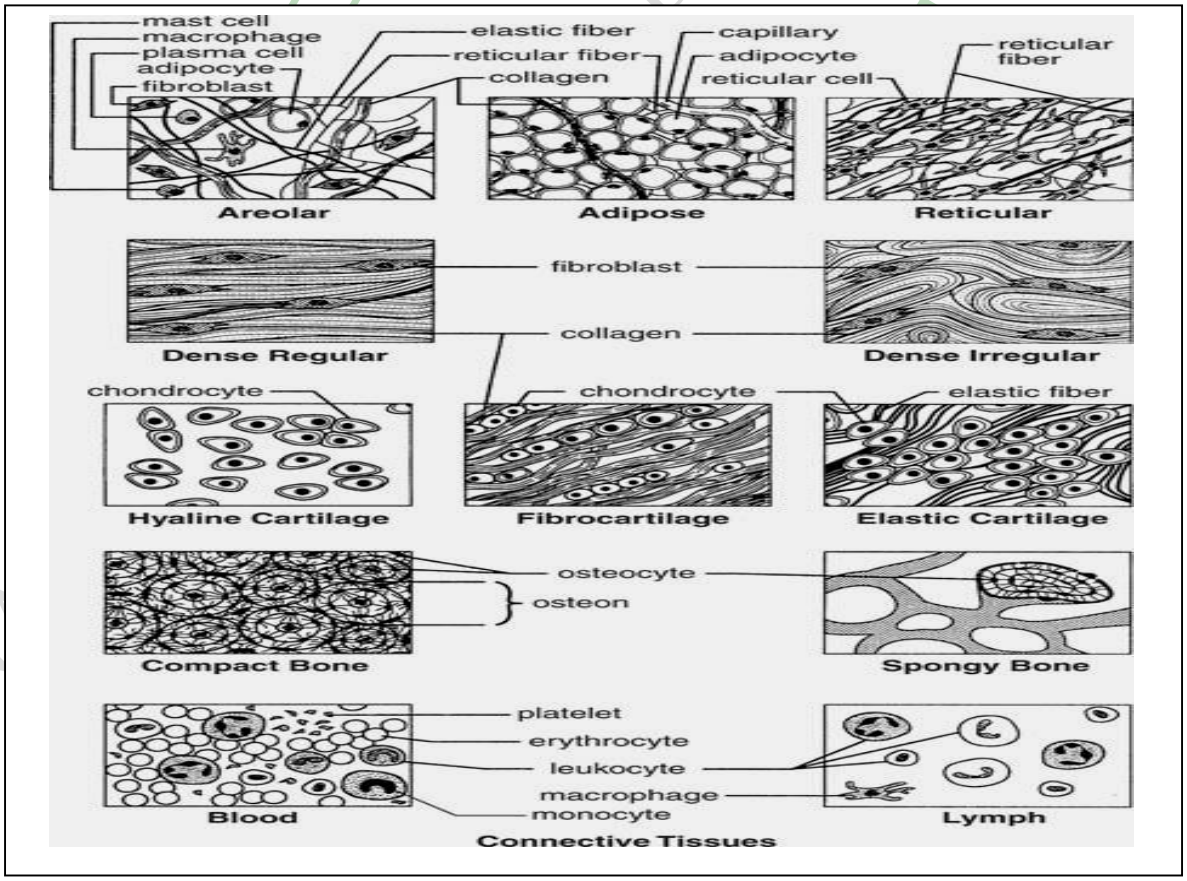
- Secret product in to blood.
- Eg.: pituitary glands, thyroid gland.

2. CONNECTIVE TISSUE:

"It is the tissue which provide supports and strength of the other body tissues, protect and insults internal organs also it binds the other cells or tissue together.

Classification of Connective tissue





CONNECTIVE TISSUE			
Tissue Type	**Cells Present**	**Fibers Present**	**Matrix Characteristics**
Loose Connective Tissue:			
Areolar	Fibroblasts macrophages adipocytes mast cells plasma cells	Collagen elastic reticular	Loosely arranged fibers in gelatinous ground substance
Adipose	Adipocytes	Reticular collagen	Closely packed cells with a small amount of gelatinous ground substance; stores fat
Reticular	Reticular cells	Reticular	Loosely arranged fibers in gelatinous ground substance
Dense Connective Tissue:			
Dense regular	Fibroblasts	Collagen (some elastic)	Parallel-arranged bundles of fibers with few cells and little ground substance; great tensile strength
Dense regular	Fibroblasts	Collagen (some elastic)	Irregularly arranged bundles of fibers with few cells and little ground substance; high tensile strength
Cartilage:			
Hyaline (gristle)	Chondrocytes	Collagen (some elastic)	Limited ground substance; dense, semisolid matrix
Fibrocartilage	Chondrocytes	Collagen (some elastic)	Limited ground intermediate between hyaline cartilage and dense connective tissue
Elastic	Chondrocytes	Elastic	Limited ground substance; flexible but firm matrix
Bone (osseous tissue):			
Compact (dense)	Osteoblasts osteocytes	Collagen	Rigid, calcified ground substance with (canal systems)
Spongy (cancellous)	Osteoblasts osteocytes	Collagen	Rigid, calcified ground substance (no osteons)
Blood & Lymph (vascular tissue):			
Blood	Erythrocytes leukocytes thrombocytes	“Fibers” are soluble proteins that form during clotting	“Matrix” is liquid blood plasma
Lymph	Leukocytes	“Fibers” are soluble liquid proteins that form during clotting	“Matrix” is blood plasma

1) Embryonic Connective Tissue:

- It is primarily present in the embryo or fetus.
- The term embryo used for developing human from fertilization through the first two months of pregnancy.
- The term Fetus used for developing human from the third month of pregnancy to birth.

➢ **It is mainly divided in to two types:**

a) Mesenchyme:

- It forms all kind of connective tissue.

- It is composed by irregularly shaped mesenchymal cells, a semisolid ground substance and delicate reticular fibers.

b) Mucous connective tissue:

- It is primarily found in umbilical cord of the fetus.
- It also forms from the Mesenchyme.
- It contains star shaped cells, a more viscous and jelly like ground substance and collagen fibers.

2) Mature connective tissue:

- It exists in new born baby.
- It, form from Mesenchyme and does not change after the birth.

➢ **It is sub divided in to:**

a) Loose connective tissue:

- Here the fibers are loosely woven.

It consists:

i) Areolar connective tissue:

- It is the most widely connective tissue.
- It consist several types of cells like as fibroblasts, macrophages, plasma cells, mast cells and a few white blood cells.
- All three type of fibers – collagens, elastic and reticular.
- The fluid, semi fluids or gelatinous ground substance contains hyluronic acid, chondrotin sulfate, dermatan sulfate and keratin sulfate.
- It located in subcutaneous layer of skin, papillary region of dermis of skin, mucous membrane, blood vessels, nerve and around body organ.
- It provides strength, elasticity and support.

ii) Adipose tissue:

- The cells of adipose tissue contain a fatty substance and they are large and round in shape.
- It consists adipocytes cells that are specialized to store triglyceride (Fat and oil). It is located in subcutaneous layer of skin, around heart, kidney, yellow bone marrow of long bone and behind the eye ball sockets.
- The main functions of these tissues are reduce heat loss through skin, serve as energy reserve, provides supports and protection.

iii) Reticular connective tissue:

- It consists fine interlacing reticular fibers and reticular fibers.
- It forms the framework of certain organs and helps to bind together certain cells.

b) Dense Connective tissue:

- It consists more numerous, thicker and densely packed fibers.
- It further divided in to:

i) Dense regular connective tissue:

- Here, the bundle of collagen fibers regular and parallel arrangements which gives great strength.
- The tissue is silvery white and tough.

ii) Dense irregular connective tissue:

- It consists collagen fibers that are usually irregularly arranged.
- Heart valves, pericardium consists this type of tissue.

iii) Elastic connective tissue:

- It consist branched elastic fibers.
- It provides strength and can be stretched.

c) Cartilage:

- It is hard but elastic in nature.
- It consist elastic and collagen fibers.

✓ **There are three types of cartilage:**

i) Hyaline cartilage:

- It provides the supports and flexibility, reduce the friction and absorb the shock at joints.
- Cartilage cells are large, arranged in group of 2 and 4.
- It's mostly found in bones and ribs.

ii) Fibro cartilage:

- It consists large cells which are arranged in groups.
- The collagen fibers are more than hyaline cartilage.
- It is found in inner vertebral discs, in knee joins.

iii) Elastic Cartilage:

- It consist elastic fiber in matrix.
- Mostly found in laryngeal cartilages, epiglottis and in Eustachian tube.

d) Bone tissue:

- Together cartilage, joints and bone comprise the skeletal system.

- It is the hardest connective tissue.
- It consists two types of bone cells osteoclasts and osteoblasts.
- Bone tissue is mainly divided in to two types compact bone and spongy bone.
- Long bones are the examples of compact bones and spongy bones are flats at the end of long bones.
- The main function of bones are it provide support, protection, assists in movement, site of blood cell production, storage of energy.

e) Blood (Vascular tissue):

- Blood is a liquid connective tissue.
- It consist mainly formed elements like platelets, leukocytes, erythrocytes and plasma consist protein, water and other solutes.
- The man function of blood tissue is transportation, regulation and protection.

f) Lymph:

- "Lymph is a thin, watery, clear, modified tissue fluid formed by the passage of substance from the blood capillaries into the tissue space (interstitial space) and enters in to the closed system of lymphatic capillaries to lymphatic vessels and lymphatic sinus known as lymphatic system."
- In short the lymphatic system consists the fluid is known as lymphatic fluid.
- Interstitial fluid and lymphatic fluid are basically same, only the different in their location. When it is located between tissue spaces it is known as interstitial fluid and when it goes in to lymphatic vessels it known as lymph.

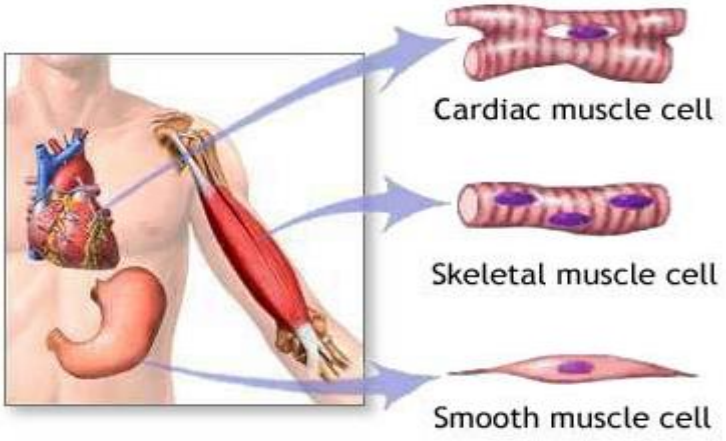
SIGNATURE OF TEACHER

EXPERIMENT NO.: 4 **DATE:**

AIM: TO STUDY AND INTRODUCTION OF MUSCULAR AND NERVOUS TISSUE.

3. MUSCULAR TISSUE:

- Muscles cells consist fibers that are beautifully constructed and generate force for constriction.
- As a result of constriction power it provides motion, maintains posture and generates heat.



- Based on location, function and structure it is **divided in to three types**:

1) Skeletal muscles tissue:

- Its name shows its location means attached to bone.
- It is strait in nature, fiber contain light and dark band which is known as striation which are visible in microscope.
- A single skeletal muscles fiber is very long, roughly cylindrical in shape and has more than one nuclei which are periphery of the cells.
- Skeletal muscles are voluntary in nature because it can be contracted and relaxed below the conscious level.

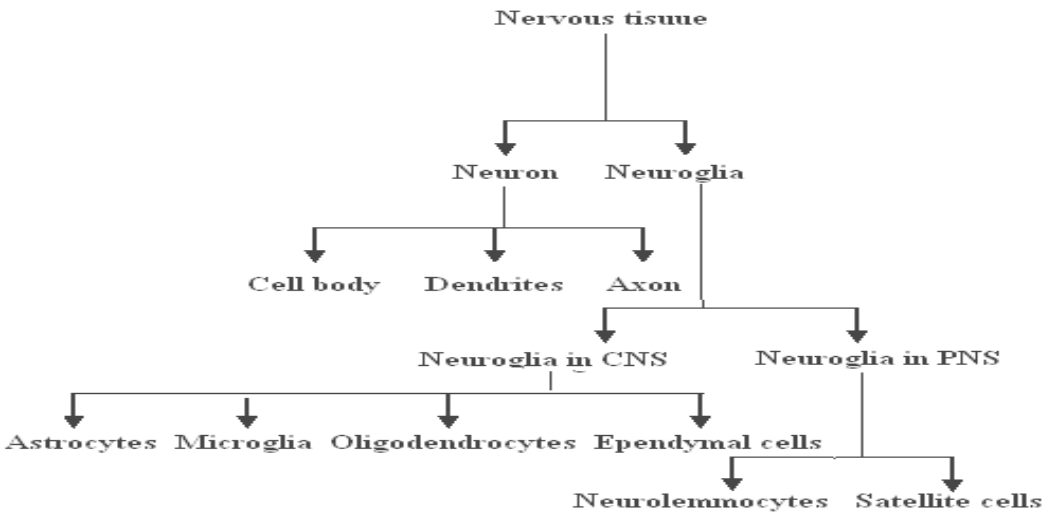
2) Cardiac muscles tissue:

- They are in bulk form and produce wall of the heart.
- Like skeletal muscles it is striated but it is involuntary in nature means constriction in not under the control of conscious level.
- The fibers are branched and cross sections are squares in shape.
- Centrally it contain one nuclei and cardiac muscles fibers attached end to end by one another and the joint is known as intercalated disc which form welding like spot between cells.

3) Smooth muscles tissue:

- It is located in the wall of hollow internal structures such as blood vessels, air ways to the lungs, intestines, gallbladders and urinary bladders.
- It provides help in breakdown of foods, elimination of wastage and move fluid and food throughout body.
- It is involuntary in control.

4. NERVOUS TISSUE



- **It consist of the two principle kinds of cells**

1) Neurons:

✓ The neurons consists of **three basic portion :**

a) Cell body:

- Cell body contains a nucleolus surrounded by cytoplasm that includes typical organelles such as lysosomes, mitochondria and Golgi complex.
- In the cytoplasm it also contains the **Chromatophilic substance (Nissl bodies)** which is ordinary arrangement of endoplasmic reticulum, the site of protein synthesis and it also contain **neurofibrils** which forms the cytoskeleton and provide the support and shape of the cells.

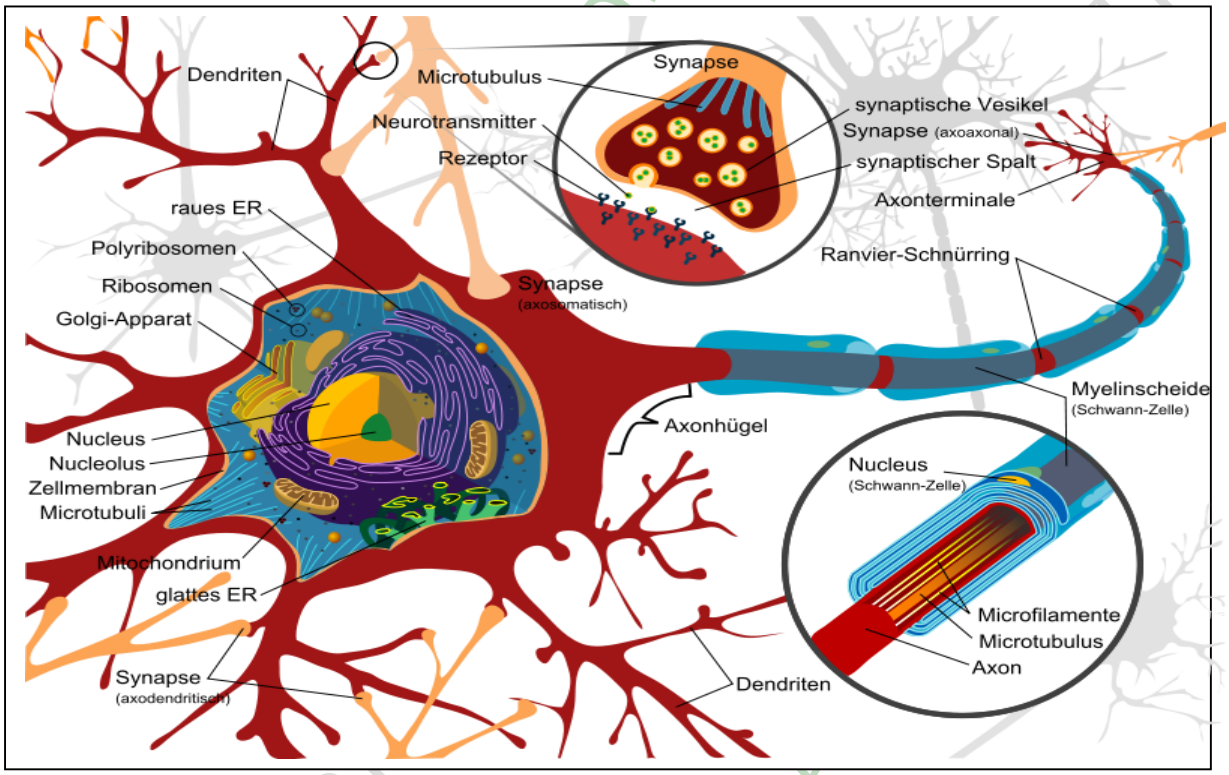
b) Dendrites:

- Dendrites are the receiving or input portion of the neurons.
- They are usually short, tapering and highly branched.
- Usually dendrites are not mylinated.
- Their cytoplasm contains chromatophilic substance, mitochondria and other organelles.

c) Axon:

- It is a long, thin and cylindrical in shape.
- It is joined with cell body by axon hillock.
- The first portion of axon is known as initial segment where the nerves impulse are arise.

- It also contains mitochondria, microtubules and neurofbrils but no rough endoplasmic reticulum so it does not synthesize protein.
- Its cytoplasm known as axoplasm which is surrounded by membrane known as axolema.
- The side branch of axon is known as axon collaterals.
- At the end of axon it divides branch like structure known as axon terminals.
- The tip of some axon terminals swell in to bulb shaped known as synaptic end bulbs.



➢ Classification of neurons:

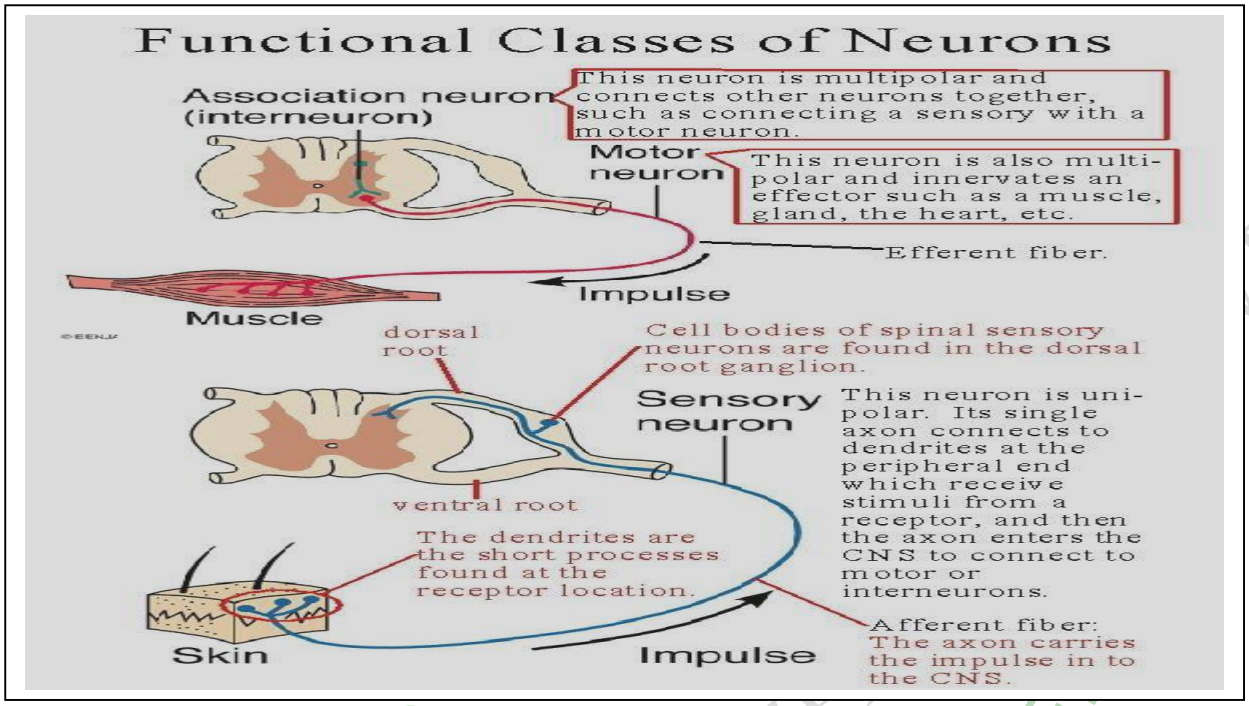
✓ According to functional classification it is divided in to:

i) Sensory neurons or afferent neurons:

- It transmits nerve impulse from receptors of skin, sense organ, muscles, and joints into the CNS.

ii) Motor or Efferent Neurons:

- It conveys motor nerve impulse from the CNS to the effectors which may be either muscles or glands.



✓ According to structural it can be classified in to:

i) Multi polar neurons:

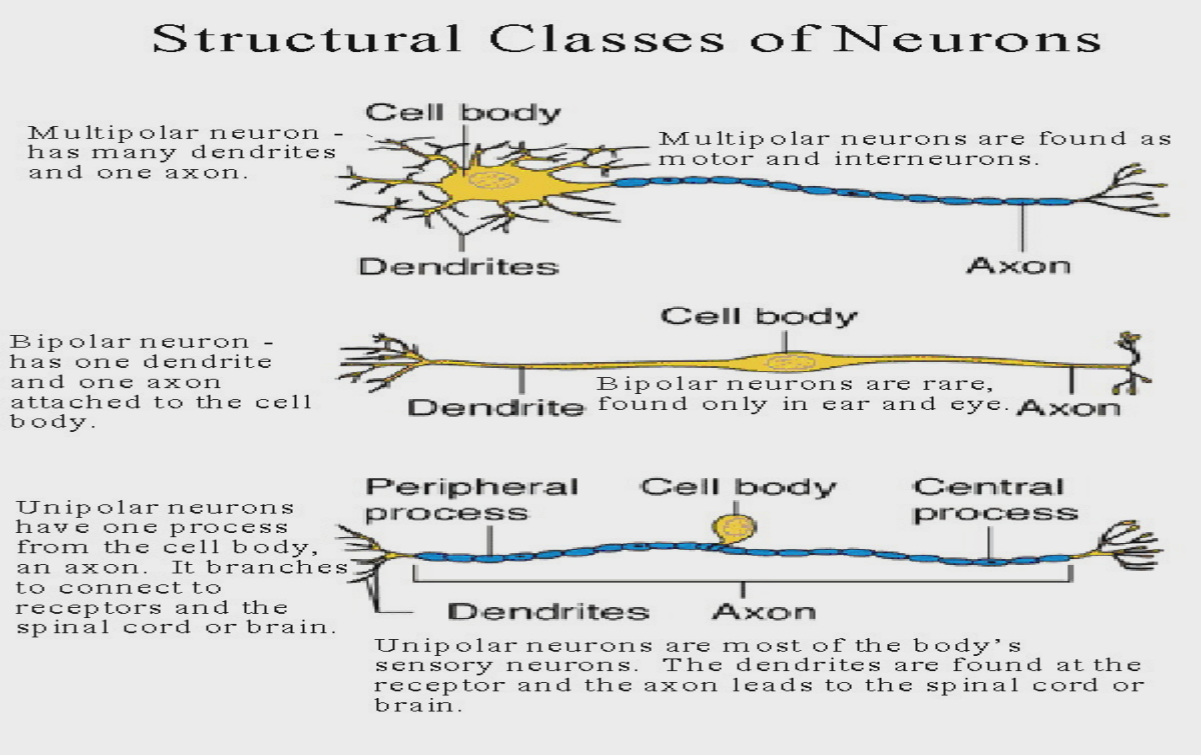
- It has several dendrites and one axon.
- Most neurons of brain and spinal cord are of this type.

ii) Bipolar neurons:

- It has one main dendrites and one axon.
- It is found in the eye, inner ear and olfactory areas of the brain.

iii) Unipolar neurons:

- It's originated as bipolar neurons in the embryo but during the development axon and body get fuse into a single process that divides in to two branch and consist one cell body.
- It is always sensory neurons.



2) Neuralgia:

- Neuroglia or glia fills about half of the CNS.
- Its have the glue like characteristics so it held nervous tissue together.
- Neuroglias are generally smaller than neurons.
- Neuroglia can multiply and divide in the mature nervous systems.

➢ **Classification of Neuroglia:**

- There are mainly six types of Neuroglia in which four astrocytes, olegodendrocytes, microglia and ependymal cells are **found in the CNS.**
- While neurolemmocytes (schwann cells) and satellite cells **found in peripheral nervous system.**

a) Neuroglia found in CNS:

i) Astrocytes:

- They are star shaped.
- It produces the metabolism of neurotransmitters, maintain the proper balance of K^+ for generation of nerves impulse, and participate in brain development.
- It forms the blood brain barrier which regulates entry of substance in to the brain.

ii) Olegodendrocytes:

- It is the most common glial Cells in the CNS.
- It is smaller than astrocytes.

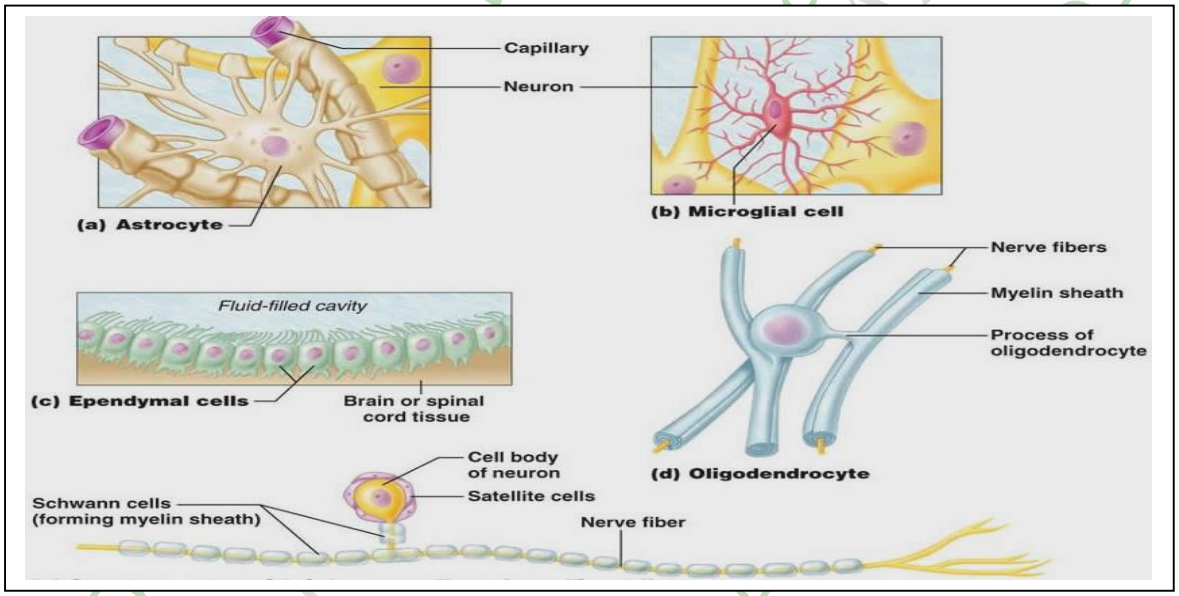
- They coil around neurons and produce supporting structure to the neurons.
- It produces protein and lipid covering known as myelin sheath.

iii) Microglia:

- It is the small and phagocytic Neuroglia derived from monocytes.
- They protect the CNS from the disease by engulfing invading microbes and clearing away debris from dead cells.

iv) Ependymal:

- It is the epithelial cells.
- The cells have different shaped from cuboidal to columnar and many are ciliated.
- Ependymal cells line the fluid filled ventricles, cavity within the brain and central canal means a narrow passage from spinal cord.
- It forms the fluid which is known as cerebrospinal fluids.



b) Neuroglia found in peripheral nervous system:

i) Neurolemmocytes (schwann cells):

- Each cell produces myelin sheath around PNS Neurons.

ii) Satellite cells:

- Which supports neurons in ganglia in PNS.

Myelination:

- The axons of most mammalian neurons are surrounded by a multilayered lipids and proteins of Neuroglia and this covering is known as myelin sheath and the axon with such a covering are said to be a myelinated.
- Whereas those without covering are known as unmyelinated axon.

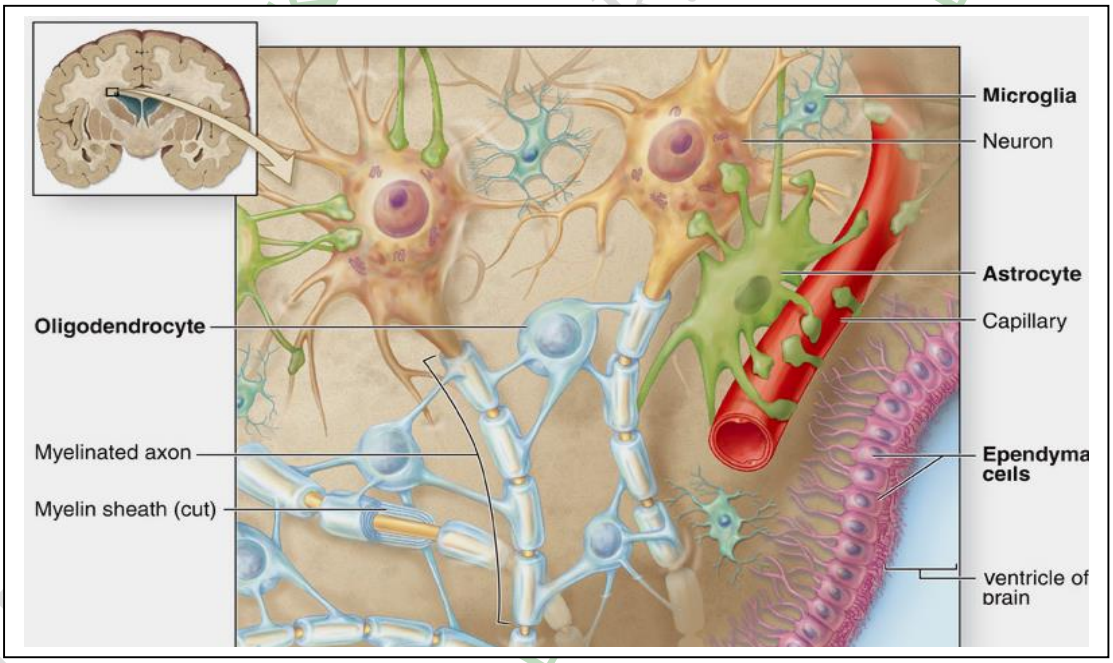
- The sheath electrically insulates the axon of neurons and increases the speed of nerve impulse conduction.

Two types of Neuroglia produce myelin sheath:

a) **Neurolemmocytes in PNS.**

b) **Olgodendrocytes in CNS.**

- Myelination and unmyelination produce Grey matter and white matter in brain and spinal cord.
- White matter refers to aggregations of myelinated process from many neurons. The whites colour of myelin gives white matter.
- The grey matters of nervous system contain either neuron cell bodies, dendrites and axon terminals or bundles of unmyelinated axons and Neuroglia.
- In spinal cord the white matter surrounds inner core of gray matter shaped like a butterfly or the letter H.
- In the brain grey matter surrounds the outer region while white matter surround inner region of brain exactly opposite to spinal cord.



➢ **Function of Nervous tissue:**

a) Sensory function:

- It sense certain changes both within body (the internal environment) such as stretching of your stomach or increase the acidity and outside the body (the external environment) such as rain drop landing on your arm or the aroma of rose.

b) Integrative Function:

- It analyzes the sensory information, store some aspect and make some decision regarding appropriate behavior.

c) Motor function:

- It may respond to stimuli by initiating muscular contraction or glandular secretion.

SIGNATURE OF TEACHER

EXPERIMENT NO.: 5 **DATE:**

AIM: TO STUDY AND INTRODUCTION OF AXIAL BONES.

INTRODUCTION:

Skeletal System

The skeletal system includes all of the bones and joints in the body. Each bone is a complex living organ that is made up of many cells, protein fibers, and minerals.

Components of Human Skeleton:

- Bones: Bone is a tough and rigid form of connective tissue. It is the weight bearing organ of human body and it is responsible for almost all strength of human skeleton.
- Cartilages: Cartilage is also a form of connective tissue but is not as tough and rigid as bone. The main difference in the cartilage and bone is the mineralization factor. Bones are highly mineralized with calcium salts while cartilages are not.
- Joints: Joints are important components of human skeleton because they make the human skeleton mobile. A joint occurs between "two or more bones", "bone and cartilage" and "cartilage and cartilage".

Divisions of human skeleton:

Axial skeleton - The axial skeleton (80 bones) is formed by the vertebral column (32–34 bones; the number of the vertebrae differs from human to human as the lower 2 parts, sacral and coccygeal bone may vary in length), a part of the rib cage (12 pairs of ribs and the sternum), and the skull (22 bones and 7 associated bones).

Appendicular skeleton - The appendicular skeleton (126 bones) is formed by the pectoral girdles, the upper limbs, the pelvic girdle or pelvis, and the lower limbs. Their functions are to make locomotion possible and to protect the major organs of digestion, excretion and reproduction.

Functions of bone and skeletal system

1. Support: The skeletal system is the structural framework of the body as well as for muscles and skin.

2. Protection: The skeletons protect the internal organs from any kind of external injury.

3. Movement: The skeletal system along with the muscular system and central nervous system helps the locomotion of the body as well as the purposeful movement of the body parts.

4. Blood cell formation: The blood cells are formed in the red bone marrow (connective tissue) within certain bones from the pluripotent stem cells.

5. Triglyceride storage: Triglycerides are stored as chemical energy reserve in the yellow bone marrow, present in the bone.

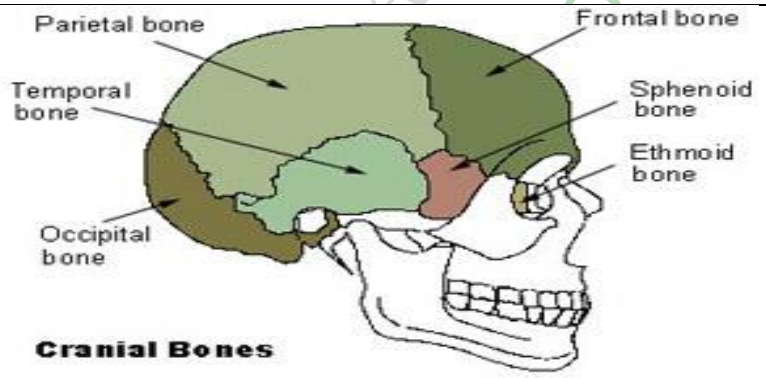
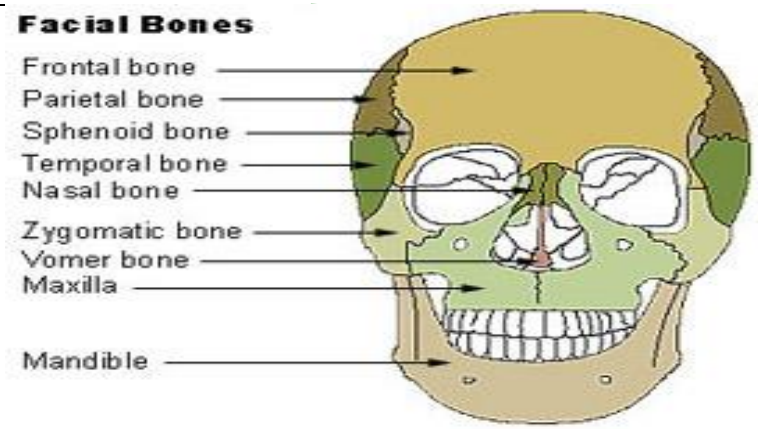
6. Bones provide attachment points to the muscles for smooth performing their activities like movements, contraction and relaxation of muscles.

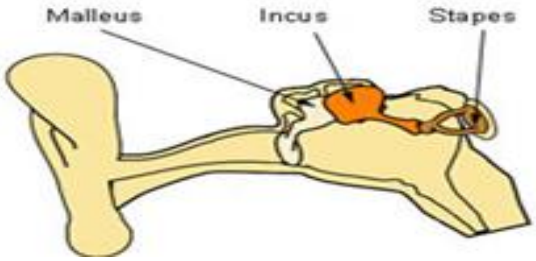
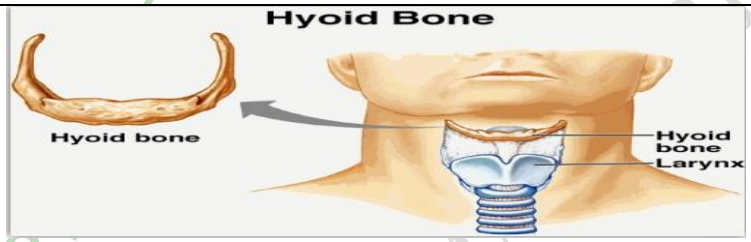
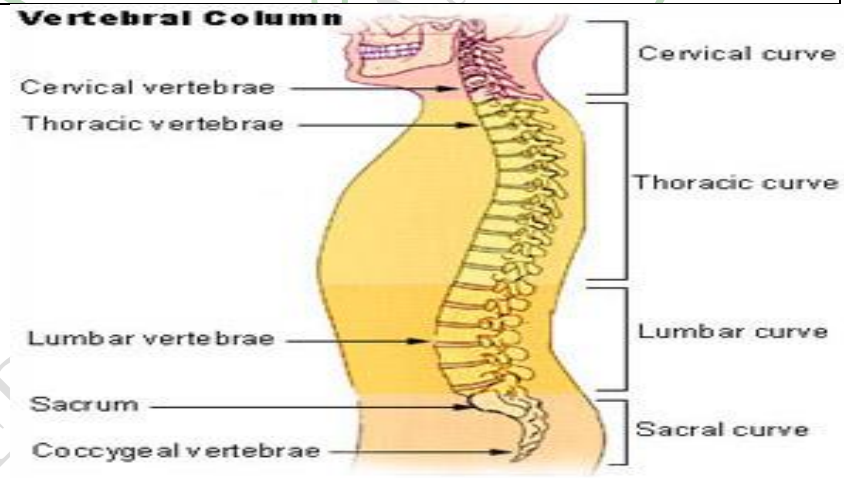
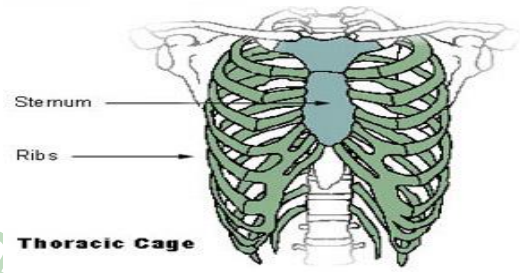
7. Axial skeleton of thorax assists in breathing.

8. Teeth help to disintegrate the foods.

9. Mineral homeostasis: Bone is the reservoir of calcium (Ca++). 99% of body calcium is stored in the bone and released in the plasma when required.

AXIAL SKELETON (80 BONES)

SR. NO	BONES	NUMBERS	DAIGRAM
	CRANIAL BONES (8)		
1	Parietal	2	
2	Temporal	2	
3	Frontal	1	
4	Occipital	1	
5	Ethmoid	1	
6	Sphenoid	1	
	FACIAL BONES (14)		
1	Maxilla	2	
2	Zygomatic	2	
3	Mandible	1	
4	Nasal	2	
5	Platine	2	
6	Inferior nasal concha	2	
7	Lacrimal	2	
8	Vomer	1	
	AUDITORY OSSICLES (6)		
1	Malleus	2	
2	Incus	2	

3	Stapes	2	
HYOID (1)			
1	Hyoid	1	
VERTEBRAL COLUMN (26)			
1	Cervical vertebrae	7	
2	Thoracic vertebrae	12	
3	Lumbar vertebrae	5	
4	Sacrum	1	
5	Coccyx	1	
THORACIC CAGE (25)			
1	Sternum	1	
2	Ribs	24	
Total axial bones		80	

The table below lists the location and function of the major bones of the axial skeleton:

Bone(s)	Location	Function	Major grouping of axial skeleton
Cranium	Head	Supports facial structures, encloses and protects the brain, provides muscle attachments for chewing and moving the head	Skull
Mandible	Lower jaw	Permits chewing	Skull
Vertebrae	Spine	Permit mechanical stability for the body and protect the spinal cord	Vertebral column
Ribs	Chest wall	Provide protection for the organs of the upper body	Thoracic cage
Sternum	Center of the chest	Provides attachment for many (not all) ribs	Thoracic cage

The skeletal system in an adult body is made up of 206 individual bones. These bones are arranged into two major divisions: the *axial skeleton* and the *appendicular skeleton*. The axial skeleton runs along the body's midline axis and is made up of 80 bones in the following regions: Skull, Hyoid, Auditory ossicles. Ribs, Sternum, Vertebral column

❖ **SKULL**

- The skull is composed of 22 bones that are fused together except for the mandible.
- The bones of the superior portion of the skull are known as the cranium and protect the brain from damage.
- The bones of the inferior and anterior portion of the skull are known as facial bones and support the eyes, nose, and mouth.

❖ **HYOID AND AUDITORY OSSICLES**

- The hyoid is a small, U-shaped bone found just inferior to the mandible. The hyoid is the only bone in the body that does not form a joint with any other bone—it is a floating bone.
- The hyoid's function is to help hold the trachea open and to form a bony connection for the tongue muscles.
- The malleus, incus, and stapes—known collectively as the auditory ossicles—are the smallest bones in the body.
- Found in a small cavity inside of the temporal bone, they serve to transmit and amplify sound from the eardrum to the inner ear.

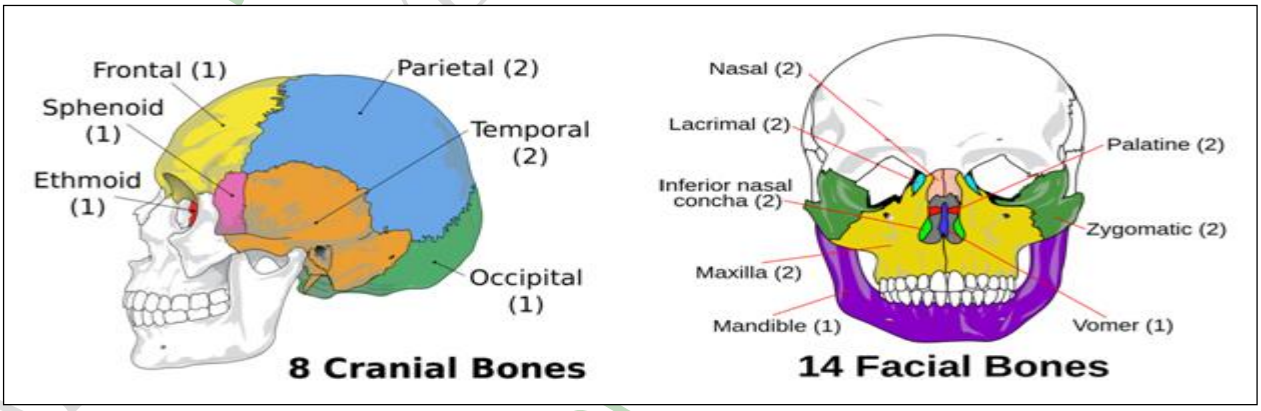
VERTEBRAE

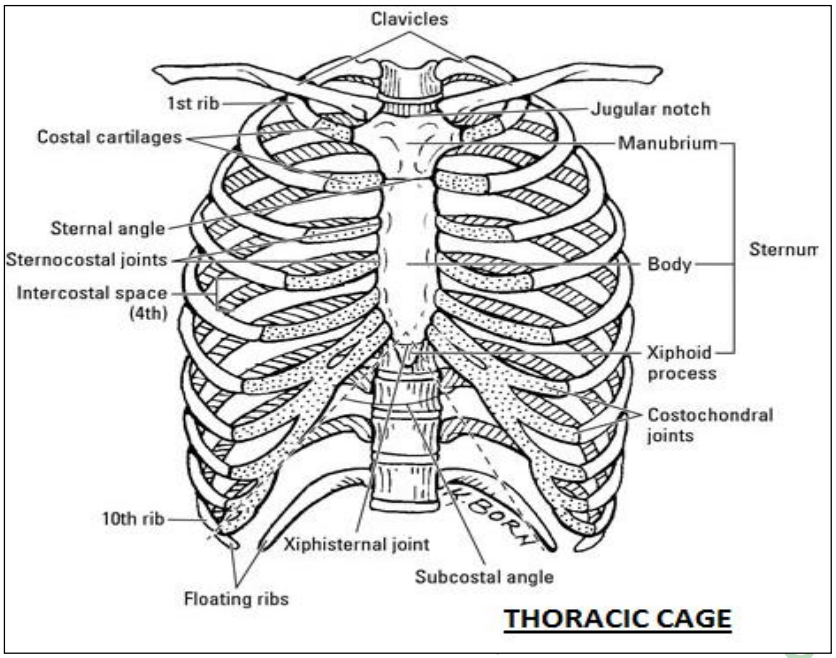
Twenty-six vertebrae form the vertebral column of the human body.

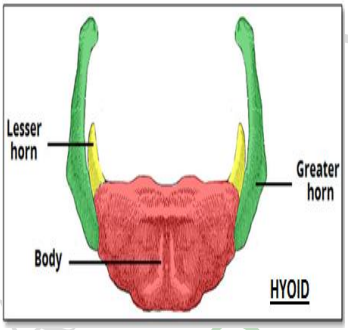
- Cervical (neck) - 7 vertebrae
- Thoracic (chest) - 12 vertebrae
- Lumbar (lower back) - 5 vertebrae
- Sacrum - 1 vertebra
- Coccyx (tailbone) - 1 vertebra
- With the exception of the singular sacrum and coccyx, each vertebra is named for the first letter of its region and its position along the superior-inferior axis.

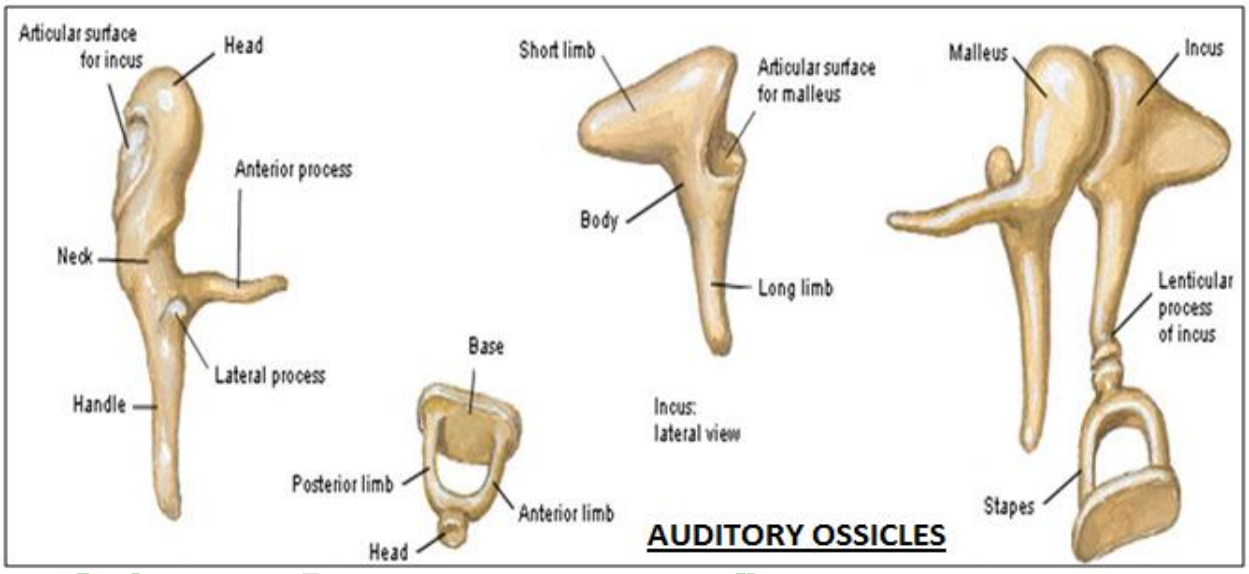
RIBS AND STERNUM

- The sternum, or breastbone, is a thin, knife-shaped bone located along the midline of the anterior side of the thoracic region of the skeleton. The sternum connects to the ribs by thin bands of cartilage called the costal cartilage.
- There are 12 pairs of ribs that together with the sternum form the ribcage of the thoracic region. The first seven ribs are known as “true ribs” because they connect the thoracic vertebrae directly to the sternum through their own band of costal cartilage. Ribs 8, 9, and 10 all connect to the sternum through cartilage that is connected to the cartilage of the seventh rib, so we consider these to be “false ribs.” Ribs 11 and 12 are also false ribs, but are also considered to be “floating ribs” because they do not have any cartilage attachment to the sternum at all.




Clavicles
1st rib
Costal cartilages
Sternal angle
Sternocostal joints
Intercostal space (4th)
Jugular notch
Manubrium
Sternum
Body
Xiphoid process
Costochondral joints
10th rib
Xiphisternal joint
Subcostal angle
Floating ribs
THORACIC CAGE


Lesser horn
Greater horn
Body
HYOID


Articular surface for incus
Head
Anterior process
Neck
Lateral process
Handle
Base
Posterior limb
Anterior limb
Head
Short limb
Articular surface for malleus
Body
Long limb
Incus: lateral view
Malleus
Incus
Lenticular process of incus
Stapes
AUDITORY OSSICLES

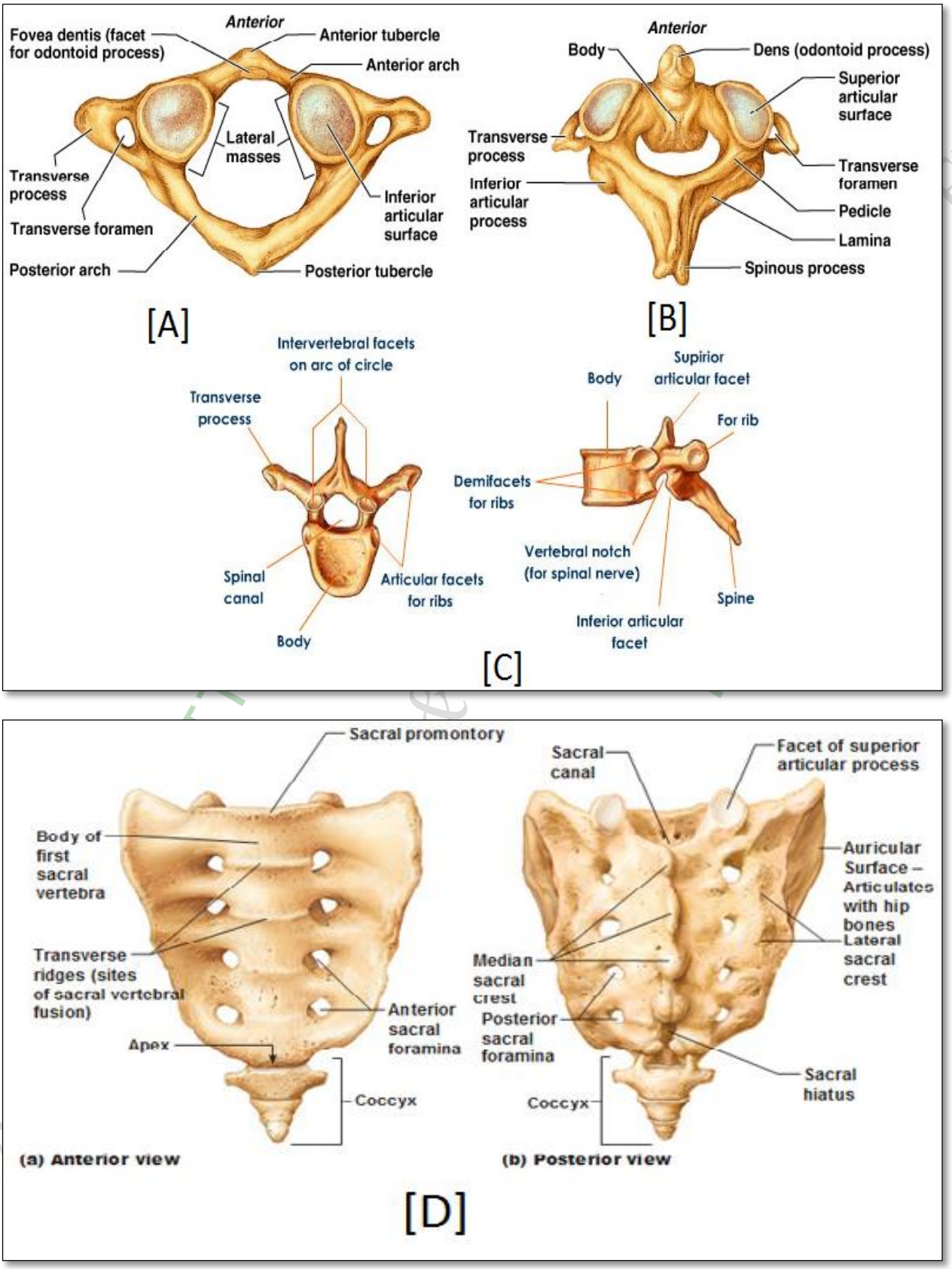


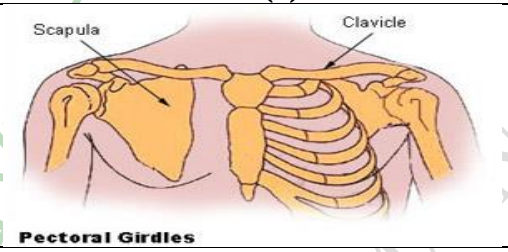
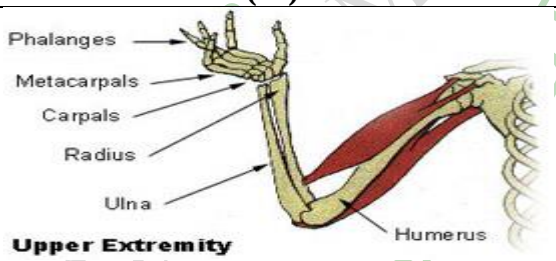
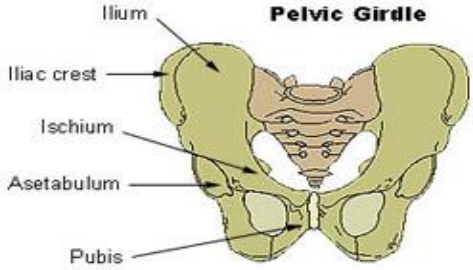
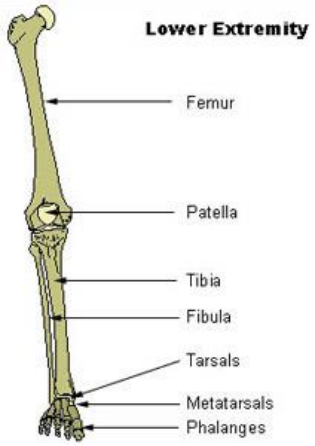
Fig: A) ATLAS B) AXIS C)THORACIC D) SACRUM

SIGNATURE OF TEACHER

EXPERIMENT NO.: 6 **DATE:**

AIM: TO STUDY AND INTRODUCTION OF APPENDICULAR BONES.

TOTAL APPENDICULAR BONES: 126

SR. NO	BONES	NUMBERS	DAIGRAM
PECTORAL GIRDLES (4)			
1	Clavicle	2	
2	Scapula	2	
UPPER EXTREMITY (58)			
1	Humerus	2	
2	Radius	2	
3	Ulna	2	
4	Carpals	16	
5	Metacarpals	10	
6	Phalanges	28	
PELVIC GIRDLE (4)			
1	Coxal,	2	
2	innominate, or hip bones	2	
LOWER EXTREMITY (60)			
1	Femur	2	
2	Tibia	2	
3	Fibula	2	
4	Patella	2	
5	Tarsals	14	
6	Metatarsals	10	
7	Phalanges	28	
Total bones		126	

APPENDICULAR SKELETON

The appendicular skeleton is composed of the 126 bones of the appendages and the pectoral and pelvic girdles, which attach the limbs to the axial skeleton.

A] UPPER LIMB

Thirty-two (32) separate bones form the bony framework of each upper limb.

PECTORAL (Shoulder)

- The paired pectoral girdles each consist of two bones, the anterior clavicle and the posterior scapula. The shoulder girdles function to attach the upper limbs to the axial skeleton.
- The pectoral girdle is exceptionally light and allows the upper limb a degree of mobility not seen anywhere else in the body. This is due to multiple factors including:
  - ✓ The sternoclavicular joints are the only site of attachment of the shoulder girls to the axial skeleton.

1. **Clavicle**: A slender, doubly-curved bone that joins the sternum to the scapula.
   - **Sternal end**: Rounded terminus; articulates with the sternal manubrium.
   - **Acromial end**: Flattened terminus articulates with the scapula to form part of the shoulder joint.
2. **Scapula**: Thin, triangular flat bone; lies on the dorsal surface of the rib cage serves as the attachment point for the arm.
   - **Superior border**: Short, sharp border that forms the upper margin of the scapula.
   - **Medial (vertebral) border**: Border which parallels the vertebral column when articulated with the axial skeleton.
   - **Lateral (axillary) border**: The thick border that abuts the armpit when articulated with the axial skeleton.
   - **Glenoid cavity (fossa)**: Small, shallow depression superior to the lateral border, articulates with humerus of the arm.
   - **Spine**: The upper posterior surface of the scapula; site of muscle attachment.
   - **Acromion**: Enlarged, roughened triangular structure of the lateral end of the scapular spine; articulates with the acromial end of the clavicle.
   - **Coracoid process**: Beak-like structure projecting anteriorly from the superior scapular border; site of muscle attachment.
   - **Suprascapular notch**: Shallow groove in the superior border of the scapula at the base of the coracoid process; passageway for nerves.

- **Supraspinous fossa**: Deep depression superior to the spine on the posterior surface of the scapula; site of muscle attachment.
- **Infraspinous fossa**: Shallow depression inferior to the spine on the posterior surface of the scapula; site of muscle attachment.
- **Subscapular fossa**: Shallow depression formed by the entire anterior scapular surface; site of muscle attachment.

ARM

The arm consists of a single bone, the humerus. The largest and longest bone of the upper limb, it articulates with the scapula at the shoulder and with the radius and ulna (forearm bones) at the elbow.

1. **Humerus**:
   - **Head**: Smooth, hemispherical projection at the proximal end of the humerus; articulates with glenoid cavity of scapula.
   - **Anatomical neck**: Slight constriction just distal to the head of the humerus.
   - **Greater Tubercle** [lateral surface] and **Lesser Tubercle** [medial surface] sites of muscle attachment.
   - **Intertubercular sulcus**: Shallow groove between lesser and greater tubercles; guides tendon.
   - **Surgical neck**: Constricted region of the humerus; common site of bone fracture.
   - **Deltoid tuberosity**: V-shaped rough region; site of muscle attachment.
   - **Radial groove**: nerve passageway.
   - **Trochlea**: Medial spool-shaped structure; articulates with ulna of the forearm.
   - **Capitulum**: Lateral ball-like structure; articulates with radius of the forearm.
   - **Medial epicondyle and Lateral epicondyle:** site of muscle attachment.
   - **Coronoid fossa**: receives process from ulna when elbow flexes / extends.
   - **Radial fossa**: receives head of radius when elbow flexes.
   - **Olecranon fossa**: receive process from ulna to form elbow joint.

FOREARM

Two parallel bones, the radius and the ulna, form the forearm.

1. **Ulna**: Long, slender bone with a hook at the proximal end that forms the elbow joint with the humerus; lies medially in the forearm when the body is in anatomical position.
   - **Olecranon process**: On the proximal end of the ulna; forms the upper portion of the hook that articulates with the trochlea of the humerus.

- **Coronoid process**: forms the lower portion of the hook that articulates with the trochlea of the humerus.
- **Trochlear notch**: Deep concavity found between the olecranon process and coronoid process; 'grips' trochlea to form elbow joint.
- **Radial notch**: Small depression of the coronoid process; articulates with head of radius.
- **Head**: Knob-like structure and the distal end of the ulna; articulates with wrist bone.
- **Styloid process**: Pointed process medial to the head of the ulna; site of ligament attachment.

2. **Radius**: Long bone that is thin at its proximal end and wide at its distal end; lies laterally in the forearm when the body is in anatomical position.
   - **Head**: Wheel-shaped proximal end of radius; articulates with capitulum of humerus and radial notch of ulna.
   - **Radial tuberosity**: Rough projection just inferior to the head of the radius; site of muscle attachment.
   - **Ulnar notch**: Medial shallow depression on the distal end of the radius; articulates with the ulna.
   - **Styloid process**: Pointed process lateral to the ulnar notch; site of ligament attachment.

HAND

- The skeleton of the hand includes the bones of the carpus (wrist); the bones of the metacarpus (palm), and the bones of the phalanges
- **Carpals (wrist):** Eight (8) marble-size short bones closely united by ligaments; quite flexible due to gliding movements between bones.
- **Metacarpals:** Five (5) small long bones radiating from the wrist like spokes; numbered 1 – 5 from the thumb to the little finger.
- **Phalanges:** Fourteen (14) miniature long bones that form the fingers; numbered 1 – 5 from the thumb (pollex) to the little finger.
- Proximal phalange (1 – 5), Middle phalange (2 – 5), Distal phalange (1 – 5)

B] LOWER LIMB

Like the upper limb, the lower limb is divided into three regions. The **thigh, leg** and **foot**. The lower limb contains 30 bones.

FEMUR

- The femur, or thigh bone, is the single bone of the thigh region. It is the longest and strongest bone of the body, and accounts for approximately one-quarter of a person's total height.
- The head of the femur, which articulates with the hip bone to form the hip joint. The fovea capitis is the site of attachment for the ligament of the head of the femur.
- The greater trochanter [the large, upward, bony] and lesser trochanter [is a small,] are the bony prominence.
- The trochanters are also connected on the posterior side of the femur by the larger intertrochanteric crest.
- The roughened area on the outer, lateral side of the condyle is the lateral epicondyle of the femur. The adductor tubercle is a small bump located at the superior margin of the medial epicondyle.

PATELLA

- The patella (kneecap) is largest sesamoid bone of the body. A sesamoid bone is a bone that is incorporated into the tendon of a muscle where that tendon crosses a joint.
- The patella is found in the tendon of the quadriceps femoris muscle.
- The patella articulates with the patellar surface of the femur and thus prevents rubbing of the muscle tendon against the distal femur.The patella does not articulate with the tibia.

TIBIA

- The tibia (shin bone) is the medial bone of the leg and is larger than the fibula, with which it is paired.
- The tibia is the main weight-bearing bone of the lower leg and the second longest bone of the body, after the femur
- The proximal end of the tibia is greatly expanded. The two sides of this expansion form the medial condyle of the tibia and the lateral condyle of the tibia. These areas articulate with the medial and lateral condyles of the femur to form the knee joint. The shaft of the tibia becomes triangular in shape. The anterior apex of this triangle forms the anterior border of the tibia.
- The large expansion found on the medial side of the distal tibia is the medial malleolus ("little hammer").
- On the lateral side of the distal tibia is a wide groove called the fibular notch, forming the distal tibiofibular joint.

FIBULA

- The fibula is the slender bone located on the lateral side of the leg. The fibula does not bear weight.
- The head of the fibula is the small, knob-like, proximal end of the fibula. It articulates with the inferior aspect of the lateral tibial condyle, forming the proximal tibiofibular joint. The distal fibula also articulates with the fibular notch of the tibia. The distal end form ankle joint with tallus

FOOT

1. **TARSAL BONES**

- The posterior half of the foot is formed by seven tarsal bones. The most superior bone is the talus.
- This has a relatively square-shaped, upper surface that articulates with the tibia and fibula to form the ankle joint. The cuboid bone and metatarsal Bones

2. **METATARSAL**

- The anterior half of the foot is formed by the five metatarsal bones, which are located between the tarsal bones of the posterior foot and the phalanges of the toes.
- These elongated bones are numbered 1–5, starting with the medial side of the foot. The first metatarsal bone is shorter and thicker than the others. The second metatarsal is the longest.
- Each metatarsal bone articulates with the proximal phalanx of a toe to form a metatarsophalangeal joint.

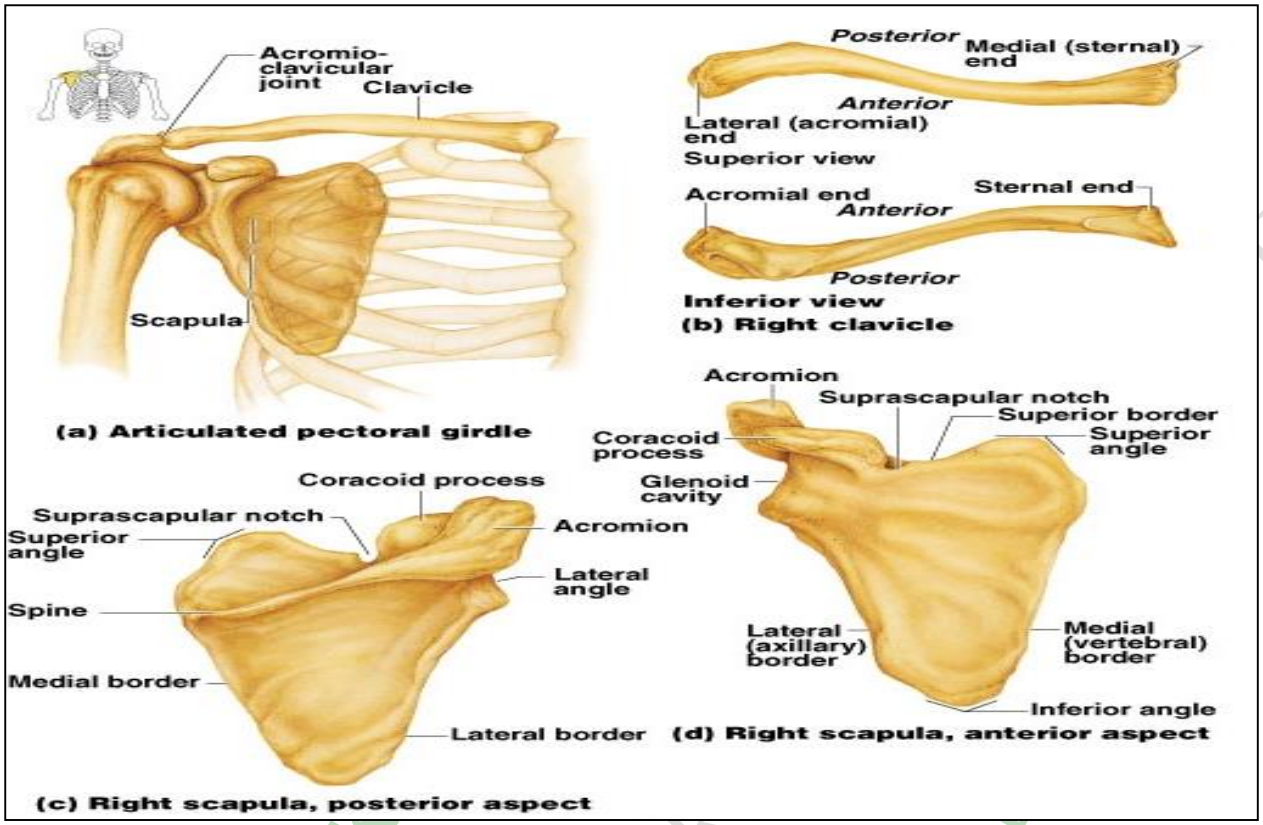
3. **PHALANGES**

- The toes contain a total of 14 phalanx bones (phalanges), arranged in a similar manner as the phalanges of the fingers.
- A joint between adjacent phalanx bones is called an interphalangeal joint.

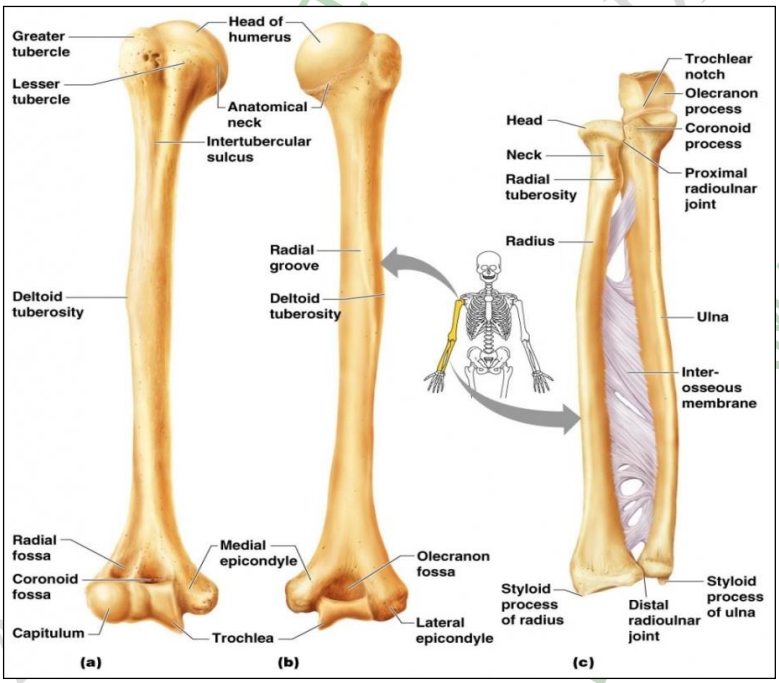
The table below lists the location and function of the major bones of the appendicular skeleton:

Bone(s)	Location	Function	Grouping
Scapula	Flat, triangular bone located on the posterior side of each shoulder	Articulates with the clavicle and humerus	Pectoral girdle
Clavicle	Located in each shoulder at the base of the neck	Helps to keep the shoulders in place; connects upper arm to the body	Pectoral girdle
Humerus	Extends from the scapula to the elbow	Provides attachments for muscles that move the shoulder and upper	Upper limbs

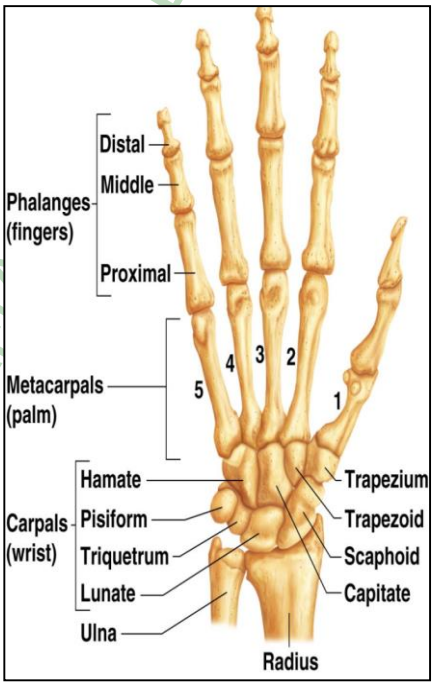
Bone(s)	Location	Function	Grouping
		arm at the proximal end; articulates with the radius and ulna at the distal end	
Radius	Located on the lateral side of the forearm between the elbow and wrist	Provides attachment for muscles that bend the arm at the elbow and muscles that allow movement of the wrist	Upper limbs
Ulna	Located on the medial side of the forearm between the elbow and wrist	Provides attachment for muscles that bend and straighten the arm at the elbow and muscles that allow movement of the wrist	Upper limbs
Ilium	Located on the superior portion of the coxal bone	Connects the bones of the lower limbs to the axial skeleton	Pelvic girdle
Femur	Extends from the hip to the knee	Provides attachment for muscles of the lower limbs and buttocks; distal end articulates with the tibia and patella	Lower limbs
Tibia	Located on the medial side of the leg between the knee and the ankle	Articulates with the femur, on its superior side, to form the knee joint; articulates with the fibula on the lateral side; articulates with the patella on the anterior side; and the tarsels to form the ankle joint	Lower limbs
Fibula	Located on the lateral side of the tibia between the knee and ankle	Forms the lateral part of the ankle joint	Lower limbs
Patella	Located on the anterior surface of the articulation between the femur and tibia	Supports movement of the knee joint	



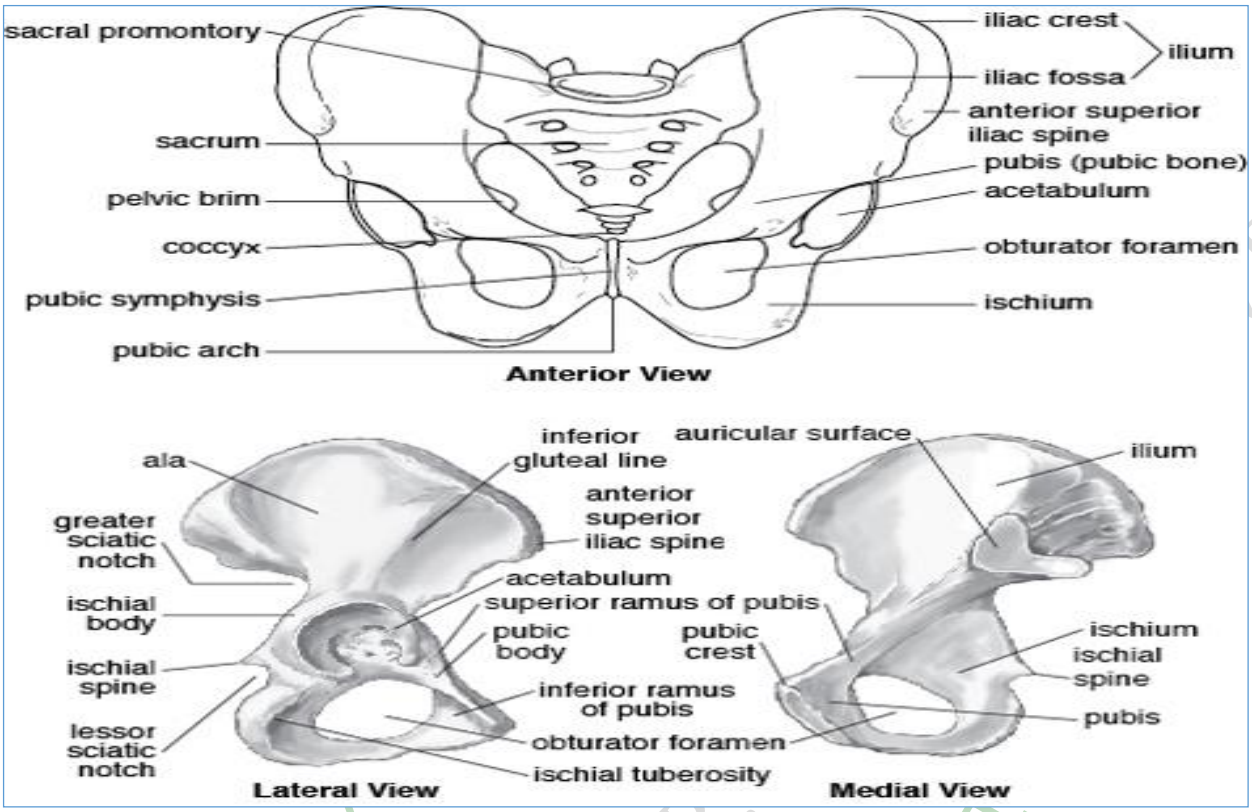
PECTORIAL GIRDLE



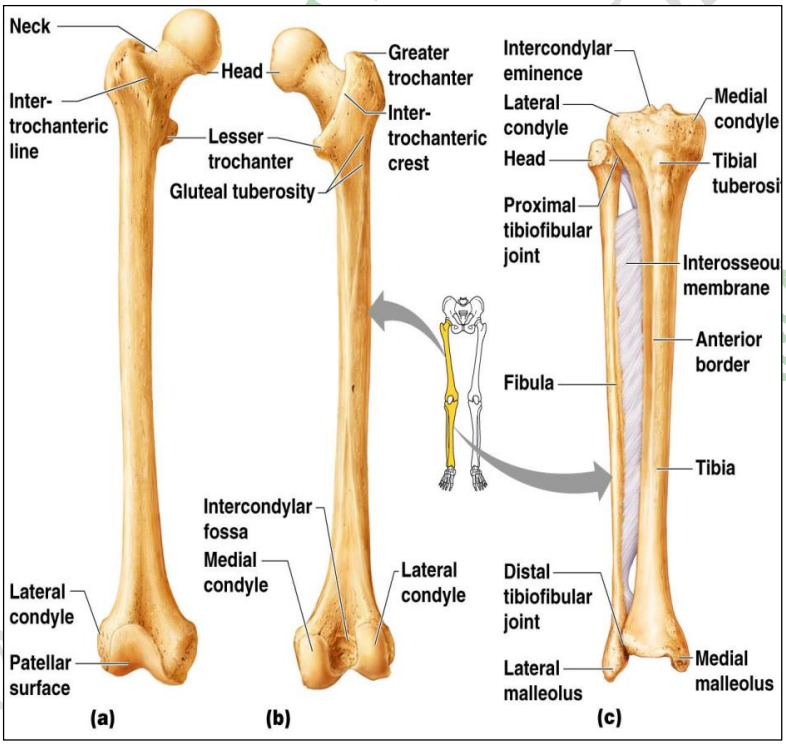
RADIOUS AND ULNA



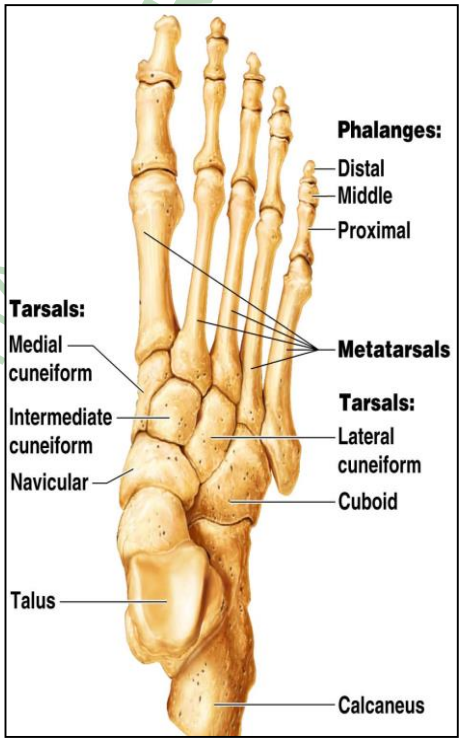
BONES OF HAND



PELVIC GIRDLE



TIBIA AND FIBULA



BONES OF FOOT

SIGNATURE OF TEACHER

EXPERIMENT NO.: 7 **DATE:**

AIM: TO STUDY AND INTRODUCTION OF THE HEMOCYTOMETER

REQUIREMENTS: Hemocytometer, cotton swab with spirit, pricking needle.

THEORY:

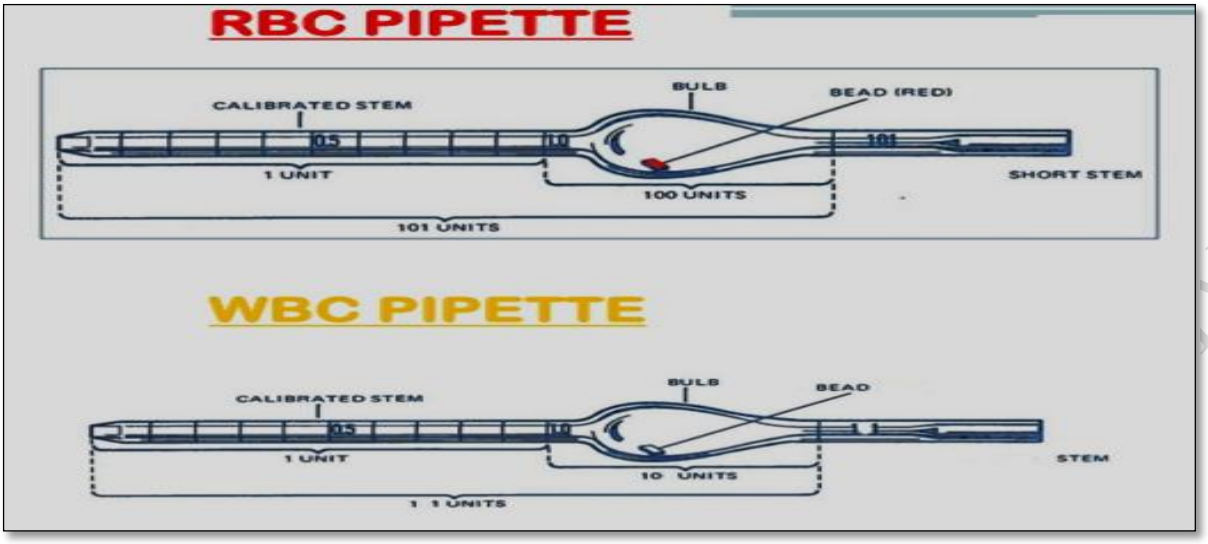
- Hemocytometer, Hemo, for blood; cyto, for cell; meter, for measuring. So altogether: measuring blood cells.
- The counting of cells [RBC and WBC] in blood using hemocytometer set is called hemocytometry.
- It is a device used for determining the number of cells per unit volume of a suspension is called a counting chamber. It is the most widely used type of chamber, since it was mainly designed for performing blood cell counts. It is now used to count other types of cells and other microscopic particles as well.
- The hemocytometer was invented by Louis-Charles Malassez.

Hamocytometer set consisting:

1. Dilution pipette
2. Counting chamber (Thomas or Neubauser's counting chamber) and Special coverslip (Thosmas cover slip)

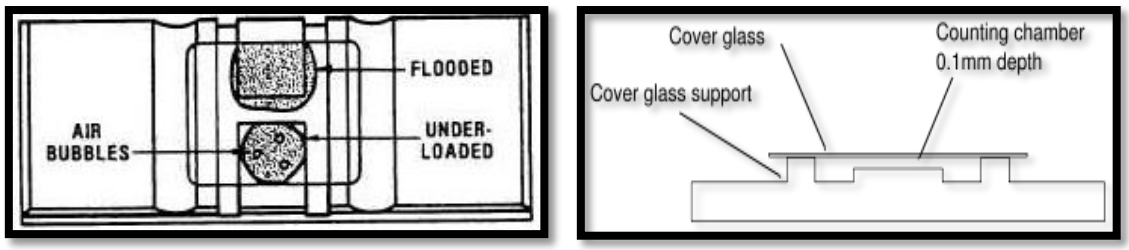
1. Dilution pipette

- It consisting 4 parts- the long stem, the blub, the short stem and the sucker.
- The long stem has a uniform capillary bore extending from a well ground conical tip and merging in the bulb.
- The long stem is divided into 10 equal parts from the tip to the mark '1' just near the bulb.
- The 5^{th} division is heavily marked and labeled as 0.5.
- This part is to measure exact amount of blood taken for counting.
- The fluid in this does not take part in dilution and hence this quantity must must be deducted while calculating the dilution factor.
- The bulb is part where dilution of blood takes place. It consists of while or red bead which helps in uniform mixing of blood with dilution fluid. The bulb ends in a short stem which have the mark '11' [in wbc piptte] or '101'[in RBC pipette]



2. Counting chamber

- The glass microscope slide has a rectangular indentation that creates an 'H' shaped chamber at the centre. Two counting areas with ruled grids are separated by the horizontal groove of the 'H'.
- There is also a very flat, reusable cover slip (Thosmas coverslip). The glass cover slip(Thosmas coverslip) is held at 0.1 mm above the surface of the counting areas by ground glass ridges on either side of the vertical grooves of the H shape.
- The device is carefully crafted so that the area bounded by the depth and lines of the chamber is also known. Because the height is constant, the volume of fluid above each square of the grid is known with precision.
- The hemocytometer is used by putting the cover slip on the device, and filling the space with a liquid containing the cells you want to count.
- There is a "V" or notch at either end which is the place where the cell suspension is loaded into the hemocytometer. The fluid is usually drawn into the space by capillary action.



The Neubauer's counting chamber

*Ruling area on The Neubauer's counting chamber

The total ruing area of each slide is 3mm in length and 3mm in breadth. It is divided into nine equal squares os 1sq. mm area. The boundary lines of these squares are triple linings. Four squares of the corners are used for WBC counting while the central square is for RBC counting. Each WBC square is divide into 16 equal squares by single lining. The area of each square is

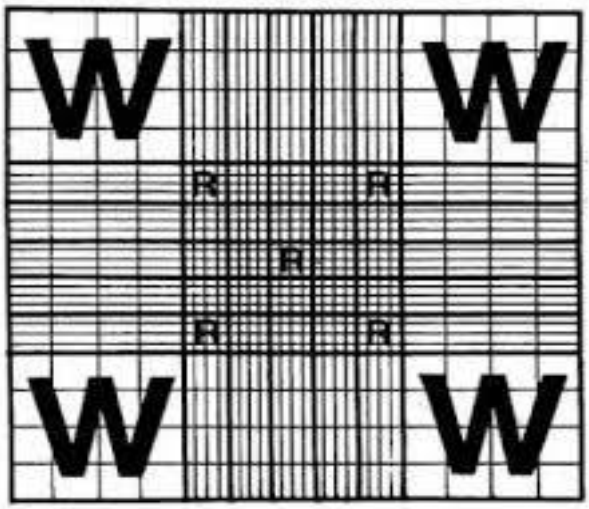
$$\frac{1}{4} \times \frac{1}{4} = \frac{1}{16} \text{ sq.mm.}$$

Each RBC square is divide by triple lines into 25 equal small RBC squares, and each of these 25 small RBC squares are further divided into 16 smallest squares by single lining. Thus whole Central Square is divided into 400 smallest squares the area of each is

$$\frac{1}{20} \times \frac{1}{20} = \frac{1}{400} \text{ sq.mm.}$$

Summary of Neubaur's counting chamber

	AREA	VOLUME OF FLUID
One small WBC square	1 sq.mm	0.1 cmm
One smallest WBC square	1/16 sq.mm	1/600 cmm
One small RBC square(16 smallest RBC squares)	1/25 sq.mm	1/250 cmm
One smallest RBC square	1/400 sq.mm	1/4000 cmm



The dilution fluids

Various dilution fluids are used in haemocytometry but the basic criteria for preparing the dilution fluid is that it should be isotonic to blood plasma. The composition of the dilution fluid depends on other requirements such as staining, fixation etc.

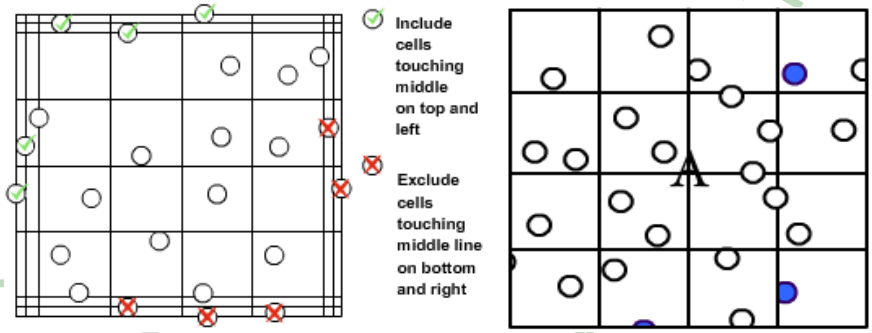
1. Haymen's RBC dilution fluid

SUBSTANCE	AMOUNT	PURPOSE
Sodium Chloride	1.0gm	Provides isotonicity Prevents hemolysis.
Sodium-sulphate	5.5 gm	Provides isotonicity
Mercuric – sulphate	0.5 gm	Causes fixation of the cells, Prevents bacterial growth.
Water	Up to 100 ml	Diluent

2. WBC dilution fluid

SUBSTANCE	AMOUNT	PURPOSE
Glacial acetic	2.0 ml	Destroys RBCs
Gentian or Methyl violet	1.0 ml	Stains nuclei of WBCs
Water	Upto 100 ml	Diluent

NOTE: DO NOT COUNT CELLS ON LININGS.



SIGNATURE OF TEACHER

EXPERIMENT NO.: 8 **DATE:**

AIM: TO ESTIMATION TOTAL WBC (LEUCOCYTE) COUNT OF OWN BLOOD SAMPLE

REQUIREMENTS:

Microscope, Haemocytometer, Thomas coverslip, WBC diluting fluid, Cotton swab, Pricking Needle/Lancet, Napkin, Plastic Sheet

PRINCIPLE:

The blood specimen is diluted 1:20 in a WBC pipette with the diluting fluid (water: glacial acetic acid: gentian violet = 97:2:1) and the cells are counted under low power of the microscope (10X) by using a counting chamber. The glacial acetic acid lyses the red cells while the gentian violet slightly stains the nuclei of the leukocytes to locate the WBC under microscope.

THEORY:

- White blood cells, present in plasma take part in body defense against invading micro-organisms.
- They are produced from the pluripotent stem cell in the bone marrow in adults. In case of foetus haemopoiesis occurs in liver and spleen.

Clinical Significances of total leukocyte count:

- Increase in total leukocyte count of more than 10,000/cu mm (µl) is known as leukocytosis and decrease of less than 4 000 cu mm (µl) as leukopenia.

Causes of leukocytosis:

- It is common for a transient period in infections (bacterial, protozoal (malaria), or parasitic),
- Leukocytosis is also observed in severe hemorrhage and in leukemia ii. High temperature
- Severe pain iv. Accidental brain damage.

Causes of Leucopenia:

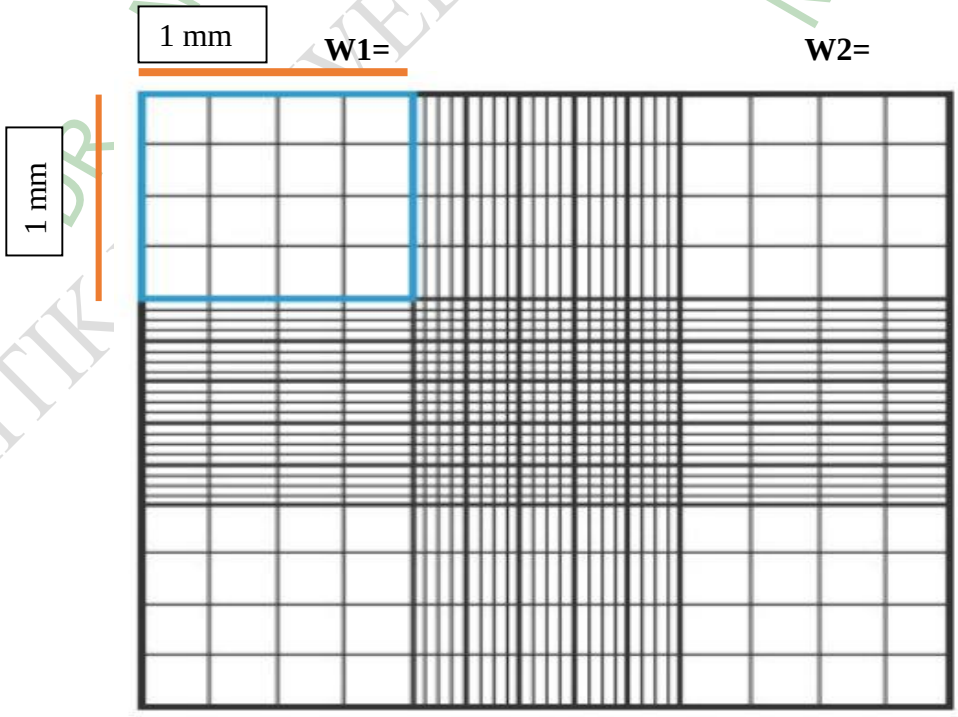
- Certain viral (hepatitis, influenza, measles, etc.), and bacterial (typhoid, paratyphoid, tuberculosis, etc) infections
- Primary bone marrow depression (aplastic anaemia)
- Secondary bone marrow depression (due to drugs, radiation, etc.) Iv. Anaemia (iron deficiency megaloblastic etc).

NORMAL RANGES:

About 4,000to 10,000 per µl or cubic mm of blood

PROCEDURE:

- Clean microscope, neubauer counting chamber and Thomas cover slip,
- Place the Thomas cover lip on Neubauer counting chamber and adjust the Neubauer counting chamber under low power objective lens i. e 10X.
- Make ready neat and cleaned WBC pipette to collect blood from the ring finger
- Sterilize the ring finger with 70% of alcohol and pricked boldly with the help of pricking needle.
- 1st drop discarded, then hold the WBC pipette slightly down position like tip of the Pipette touch the pricking site.
- Take the WBC pipette tube of the next end in the mouth and try to pull blood in capillary without AIR bubble till the making of 0.5.
- Then pill WBC dilution fluid upto the mark 11.
- Make a 1:20 Dilution.
- Give a node to the WBC pipette tube and mix the fluid gently for 1-2 minutes.
- Open the node of WBC Pipette and place the tip of WBC pipette like that the fluid portion enter between the gap of Thomas cover slip and Neubauer counting chamber.
- Allowed the fluid to spread on the counting.
- Try to count WBC as shown in below figure.



CALCULATIONS:

- Total Number of white blood cells in 16 small square[64 smallest square] = N

  N means = W1+W2+W3+W4 =
- Length (L) of 1 small WBC square = 1mm
- Width (W) of 1 small WBC square= 1mm

  So area of 1 small square (L X W) = l mm X 1 mm= 1 mm^2 or 1 sq. mm
- Height (Thickness) of the counting chamber 1/10 mm = 0.1 mm
- Volume of fluid in 1 small square (L X W X H) = l mm X 1 mm X 0.1 mm = 0.1 mm^3
- So, Volume of fluid in 4 small square = 0.1 X 4 = 0.4 cmm or 0.4 mm^3

  If,

  0.4 cmm or (4/10) cmm, of diluted fluid contains N WBC

  So, 4 cmm (4 X 1 cmm) of diluted fluid contains = N X 10 WBC

  1 cmm of diluted fluid contains = N/4 X 10 WBC
- Dilution factor is 0.5 in 10 or 1 in 20

  Therefore total number of WBC in undiluted fluid = N/4 X 10 X 20

  = N X 50 /cmm

  =

  =

RESULT: Total WBC count of my own blood is _____/cmms

(Normal WBC count 4000 to 11,000/cmm of blood)

CONCLUSION: Therefore, my blood is in **normal/ abnormal**

CLINICAL SIGNIFICANCES OF DIFFERENTIAL LEUKOCYTE COUNT:

Types of WBC, elevated in plasma	Significances
Neutrophils	Acute bacterial infections, hemorrhage, diabetic acidosis
Basophils	Increase in types of blood dyscrasias
Eosinophils	Increase in parasitic and allergic conditions, pernicious anemia
Monocyte	Hodgkin's disease, lipid storage disease, recovery from severe infections, monocytic leukemia
Lymphocyte	Viral and chronic bacterial infections, acute and chronic lymphocytic leukemia, antigen reaction

Increased WBC Count (Leukocytosis):

The count is more than 11,000/cmm.

1. Infections mostly acute bacterial give rise to an increase in the WBC count.
2. Trauma and stress.
3. Hemorrhage.
4. Dehydration.
5. Steroid therapy.
6. Inflammations.
7. Thyroid hormone increases.
8. Leukaemias or another myeloproliferative process.
9. Other malignancies may increase WBC count.

Decreased WBC Count (Leukopenia):

The count is less than 4000/cmm.

1. Drug toxicity causing bone marrow depression.
2. Cytotoxic drugs.
3. Bone marrow failure.
4. Severe infections.
5. Bone marrow infiltration by the tumors or myelofibrosis.
6. Dietary deficiency like vitamin B12 and iron deficiency.
7. Hypersplenism.
8. Autoimmune diseases.
9. Low TLC may be seen in typhoid fever.

SIGNATURE OF TEACHER

EXPERIMENT NO.: 9 **DATE:**

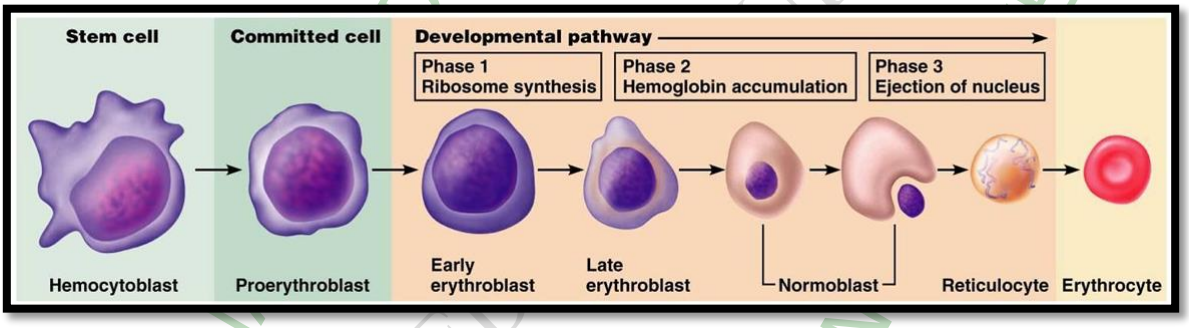
AIM: TO ESTIMATE TOTAL RBC (ERYTHROCYTE) COUNT OF OWN BLOOD SAMPLE

REQUIRMENT:

Microscope, Neubauer Chamber, Thomas Coverslip, Microscope, RBC Diluting Fluid, Cotton swab, Pricking Needle/Lancet, 70 % Methylated Alcohol (Spirit)

THEORY:

- Erythrocytes, also known as red blood corpuscles, contain the hemoglobin (Hb) which carries the oxygen to cells and tissues, is responsible for the red colour of blood. The life span of normal RBC is 120 days. The RBC is produced in red bone marrow in adult.
- The agents, required for the synthesis of blood cells are called hematinic agents (iron, folic acid, vitamin B12, erythropoietin, colony stimulating factors).



Steps of Erythropoiesis

Functions of RBC

1. Transport of oxygen and carbon dioxide.
2. Maintains of acid base balance, ionic balance and viscosity of blood.
3. Formation bile pigment as result of destruction of RBC.

Clinical significances:

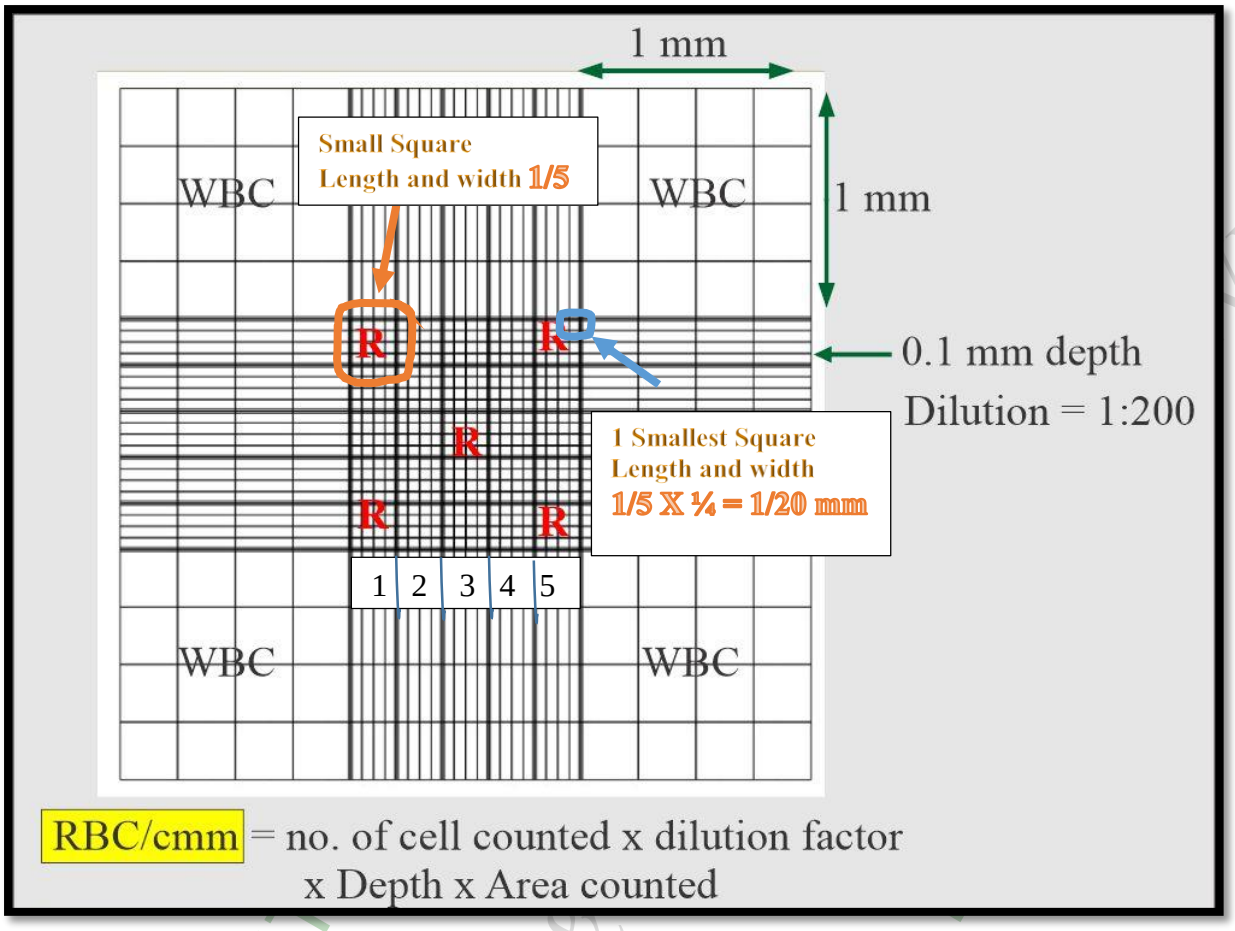
- The decreased count of RBC indicates several conditions like iron deficiency anemia, megaloblastic anaemia, pernicious anemia, thalassemia, sickle cell anemia, aplastic anemia, chronic renal failure etc.
- Increased RBC production (Polycythemia vera) occurs in burn, lack of oxygen during rock climbing, bone marrow cancer, respiratory disorders etc., when the viscosity of the blood increases.

NORMAL RANGES:

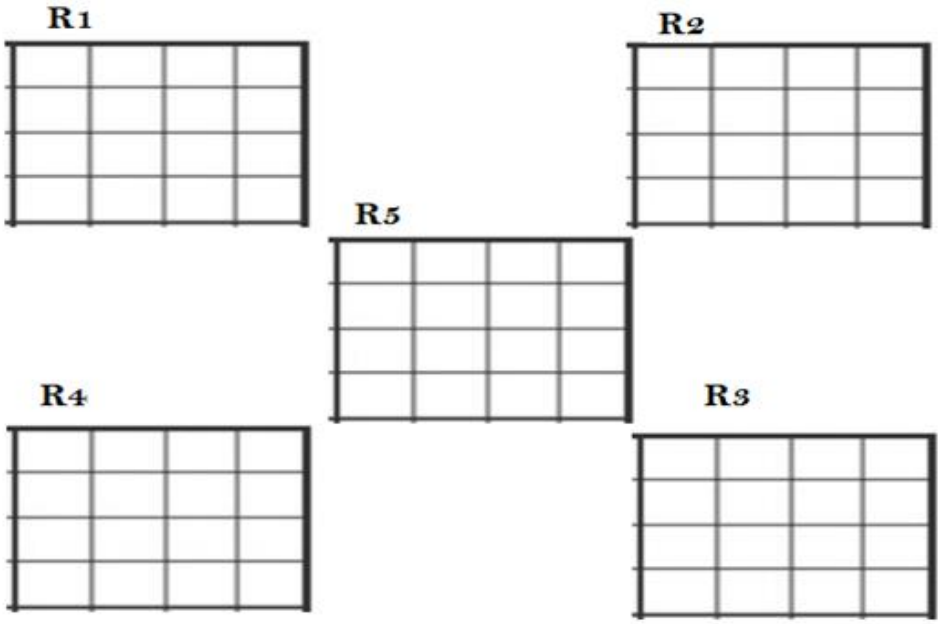
- **Male:** about 5-6 million per µl or cubic mm of blood
- **Female:** about 4-5 million per µl or cubic mm of blood
- Cord blood = 3.9 to 5.5 million/cmm
- Adult = 18 to 44 years :
  - Male = 4.7 to 6.1 million/cmm.
  - Female = 3.8 to 5.4 million/cmm
- 45 to 64 years :
  - Male = 4.2 to 5.6 million/cmm.
  - Female = 3.8 to 5.0 million/cmm
- 65 to 74 years :
  - Male = 3.8 to 5.8 million/cmm.
  - Female = 3.8 to 5.2 million/cmm

PROCEDURE:

- Clean microscope, neubauer counting chamber and Thomas cover slip,
- Place the Thomas cover lip on Neubauer counting chamber and adjust the Neubauer counting chamber under high power objective lens i. e 45X.
- Make ready neat and cleaned RBC pipette to collect blood from the ring finger
- Sterilize the ring finger with 70% of alcohol and pricked boldly with the help of pricking needle.
- 1st drop discarded, then hold the RBC pipette slightly down position like tip of the Pipette touch the pricking site.
- Take the RBC pipette tube of the next end in the mouth and try to pull blood in capillary without AIR bubble till the making of 0.5.
- Then pill WBC dilution fluid upto the mark 101.
- Make a 1:200 Dilution.
- Give a node to the RC pipette tube and mix the fluid gently for 1-2 minutes.
- Open the node of RBC Pipette and place the tip of RBC pipette like that the fluid portion enter between the gap of Thomas cover slip and Neubauer counting chamber.
- Allowed the fluid to spread on the counting.
- Try to count RBC as shown in below figure.



OBESRVATION TABLE



CALCULATION:

- Total Number of red blood cells in 5 small square (N= R1+R2+R3+R4+R5) is 80 smallest square
- Length (L) of 1 small sq. RBC is 1/5 mm part of the RBC counting chamber.
- So length of 1 smallest sq. RBC is 1/5 X 1/4 = 1/20 mm part of the RBC counting chamber.
- Width (W) of 1 small sq. RBC is 1/5 mm part of the RBC counting chamber.
- So width of 1 smallest sq. RBC is 1/5 X 1/4 = 1/20 mm part of the RBC counting chamber.
- Area of 1 smallest square = Length X Width = 1/20 mm X 1/20 mm = 1/400 sq. mm
- Height (Thickness) of the RBC counting chamber is 1/10 mm.
- So, Volume of fluid in:

  1 smallest square = Length X Width X Height

  = 1/20 mm X 1/20 mm X 1/10 mm

  = 1/4000 cmm

  We need to count RBC in R1+R2+R3+R4+R5 box so it consist total 80 smallest square.

  Consider 80 Smallest square consist = N RBCs

  If we wright 80 X 1 Smallest square consist = N RBCs

  Therefore 1 smallest square contain = N/80 RBCs
- 1/4000 cmm contain = N/80 RBCs (Put value of 1 smallest square is 1/4000 cmm)
- 1 cmm contains (N/80) X 4000 RBCs
- 1 cmm contains (N/80) X 4000 X 200 RBCs (200 is dilution factor)

  =_____RBCs/cmm (N = R1+R2+R3+R4+R5)

RESULT: Total RBC count of my own blood is _____millions/cmms

CONCLUSION: Therefore, my blood is **normal/ abnormal.**

Calculate color index: Hb% / RBC % **(**Color index normal range is 0.85 - 1.0)

Increased RBC Count Is Seen In:

1. Primary Erythrocytosis.
2. Polycythemia.
3. Erythremia (Erythrocytosis).
4. Secondary Erythrocytosis.
5. Vigorous exercise.
6. Hemoconcentration.
7. High Altitude.
8. Chronic obstructive pulmonary disease (COPD).
9. Severe dehydration.
10. Thalassemia trait.
11. Hemoglobinopathies.
12. Congenital heart disease.
13. Extra-renal tumors.
14. Tobacco use.

Decreased RBC Count is Seen In:

- Anaemias.
- Drugs that cause aplastic anemia.
- G-6 PD deficiency.
- Immune mechanism.
- Malignancy like Hodgkin's disease, lymphomas.
- Acute and chronic hemorrhage.
- Autoimmune diseases like SLE and rheumatoid arthritis.
- A chronic infection like subacute endocarditis.
- Cirrhosis.
- Dietary deficiency of iron, and vit B12.
- Pregnancy.
- Marrow failure, e.g., Bone Marrow fibrosis, leukemia infiltration, chemotherapy, and antiepileptic drugs.
- Drugs leading to bone marrow failures like quinidine, chloramphenicol, and hydantoin.
- Hemolysis is seen in spherocytosis, G6PD deficiency, and splenomegaly.
- The genetic abnormality is seen in thalassemia and sickle cell anemia.
- Hemorrhage, e.g., in GI tract or trauma.
- Chronic illness due to infections or malignancies.
- Organ failure as seen in renal diseases.

SIGNATURE OF TEACHER

EXPERIMENT NO.: 10 **DATE:**

AIM: TO STUDY AND DETERMINATION OF THE BLEEDING TIME OF OWN BLOOD SAMPLE.

REQUIRMENTS:

PRINCIPLE:

- A bleeding time test determines how quickly your blood clots to stop bleeding. The test involves making small punctures in your ring finger.
- The test is a basic assessment of how well your blood platelets work to form clots.
- Platelets are tiny cell fragments that circulate in your blood. They're the first cells to react to a blood vessel injury. They seal off the wound to prevent more blood from escaping.
- Abnormal results from a bleeding time test can be a sign platelet function defect.

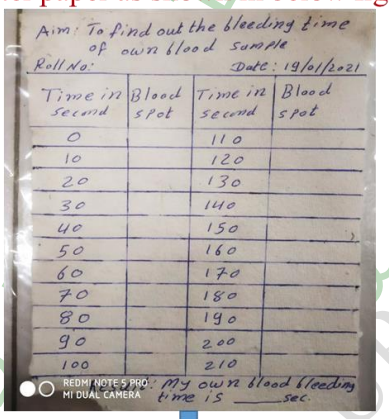
THEORY

- The time required for complete stopping of blood flow from the punctured blood vessels called the **bleeding time.**
- Normally it is 1-3 minutes for a normal human's blood.
- Normal clotting time and bleeding time values differ because bleeding time is the time for stopping bleeding by the formation of fibrin network on the surface of punctured skin known as surface phenomenon.

- But the clotting time is the time for clotting the whole blood, collected in the capillary tube; therefore it is a volume phenomenon.
- For this reason clotting time is more than the bleeding time, when determining by conventional methods.

PROCEDURE:

Write the aim, date and roll number on piece of filter paper and draw the pattern to take result on filter paper as shown in below figure



Take a new or unused Lancet/Pricking Needle and Sterilize it with 70 % of alcohol

Sterilize the tip of ring finger with 70% of alcohol

Rub the finger to increase the blood circulation then hold the finger using your thumb

Give the sharp Prick by the lancet on the tip of ring finger

Star to take result on filter paper at 10 sec interval, Take first result at 0 Second time means immediately after pricking, then at 10 Sec, 20 Sec, 30 Sec …..

Take the response till no blot appears on filter paper

Time from first appearance of the blood spot to no blot appears is your bleeding time

Normal Bleeding time of healthy person is 1-3 Minutes

Why We Use Left Hand Ring Finger ????

- It is least used as compared to other finger
- Synovial sheath of ring finger short of the hand and due to this infection do not spread exceed to their limit.

RESULTS

Bleeding time of my own blood is _____ minute _____ Seconds.

PRECAUTION

Following precautions should be enforced

i) Needle should be sterilized.

ii) A faint stain of blood should not be avoided.

iii) Time should be noted properly.

iv) Use fresh needle every time for the practical

CONCLUSION

My own blood bleeding time is _____ which lie in normal/abnormal range.

CLINICAL SIGNIFICANCE

- To study the hemorrhagic disorders.
- To study the coagulation defects
- To have an idea about the platelets count of the patient.
- Bleeding time is prolonged in few disorders like: vascular lesions, platelet defect, severe liver disease, uremia and anti-coagulant drug administration.

SIGNATURE OF TEACHER

EXPERIMENT NO.: 11 **DATE:**

AIM: TO DETERMINE THE CLOTTING TIME OF OWN BLOOD SAMPLE.

PRINCIPLE:

- Clotting prevents excessive bleeding during the time of injury. Coagulation/Clotting tests measure your blood's ability to clot, and how long it takes to clot.
- During coagulation solution/liquid form of the blood convert in to the gel/semisolid form.
- Note down the time required for this physical change.

THEORY

- **Clotting time** is the time taken to coagulate the blood after rupture of blood vessels. Normally it is **4-10 minutes** for a normal human's blood depending upon platelets count and other clotting factors inside the plasma.

▪ Clotting process is well described by three main pathways which are:
a) Extrinsic pathway b) Intrinsic pathway and c) Common pathways

a) Extrinsic pathways:

▪ It has fewer steps than the intrinsic pathway.
▪ It is a fast process than the intrinsic pathway usually takes few seconds.

During the injury
↓
Endothelial cells which lines the blood capillary get damaged
↓
So Factor VII leaves the circulation and comes into contact with tissue factor (TF)
↓
It activate the clotting factor VII
↓
Which combine with the factor X and activate factor X
↓
It combines with factor V in the presence of Ca++
↓
Which form the active enzyme prothrombinase

▪ Tissue factor (TF) is a complex mixture of lipoprotein and phospholipids, It release from the surface of damaged cells.

b) Intrinsic pathways:

▪ It is a more complex process than the extrinsic pathway.
▪ It is a slow process than the extrinsic pathway usually required several minutes

During the injury
↓
Endothelial cells which lines the blood capillary get damaged
↓
Blood come with contact of collagen in the surrounding basal lamina (Junction between Endothelial tissue and Connective tissue)
↓
Activate the clotting factor XII
↓

Clotting factor XII activates factor XI

↓

Factor XI activates the factor IX which is also activate by extrinsic pathway factor VII

↓

Factor IX by the help of factor VIII and platelet phospholipids activate factor X

↓

Activated factor X combine with factor V and Ca++ (same as extrinsic pathway processes)

↓

Which form the active enzyme prothrombinase

c) Common pathway:

- Once prothrombinase is form it start the common pathway.
- It this pathway:

Prothrombinase convert in to thrombin by the help of Ca++

↓

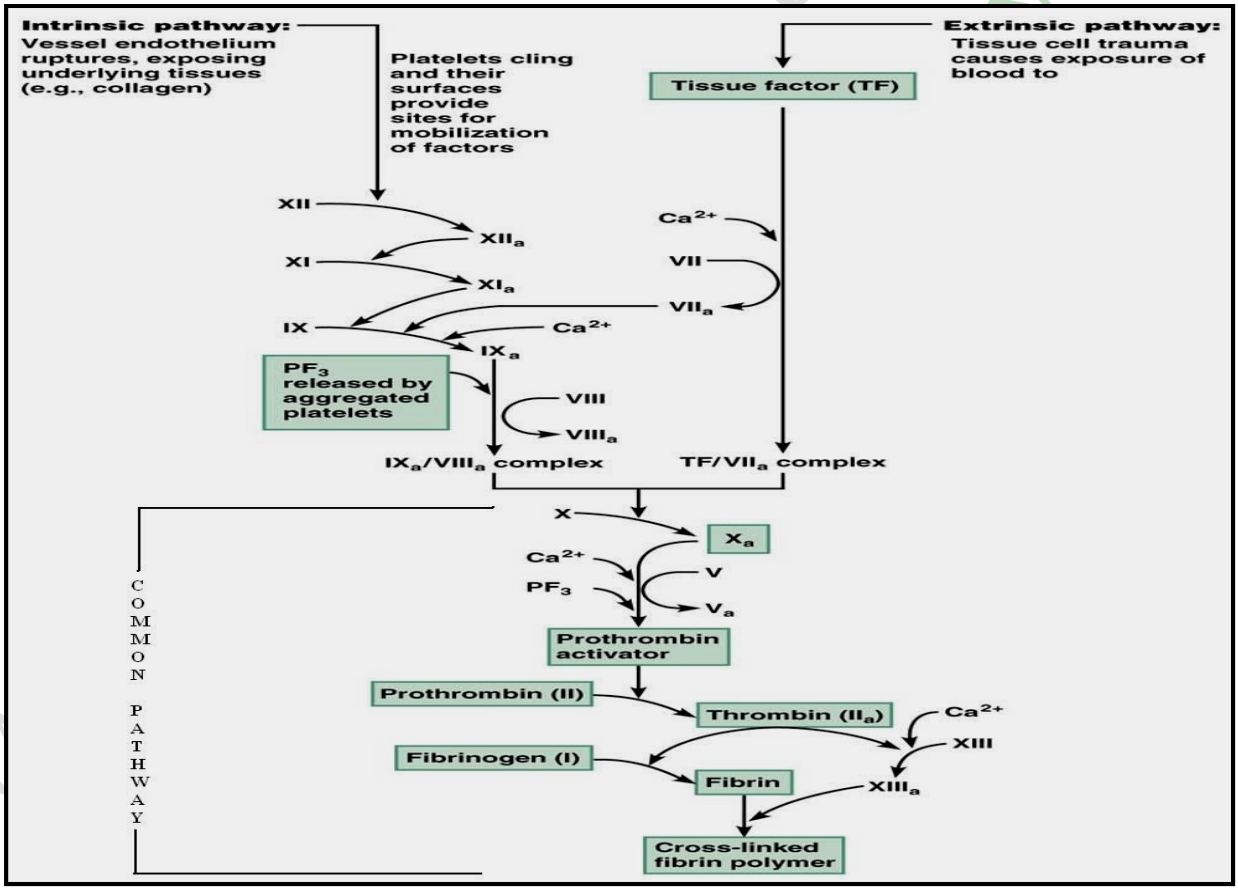
Thrombin activate factor XIII as well as convert in to Fibrinogen in the presence of Ca++

↓

Fibrinogen converts into soluble fibrin

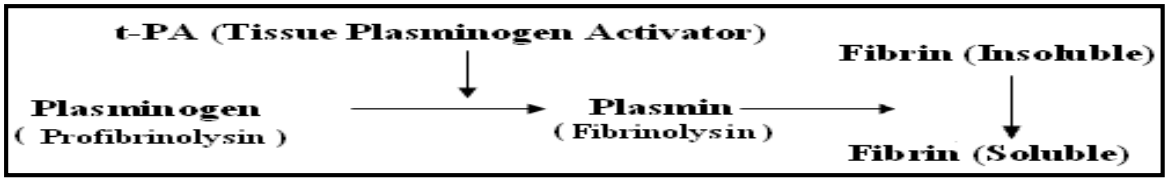
↓

Soluble fibrin converts in to insoluble fibrin by the help of activated factor XIII



Fibrinolysis:

- Once repair s over, the fibrinolysis system is activate. This process inhibit the clot formation in blood because in clot formation soluble fibrin is convert in insoluble while in fibrinolysis insoluble fibrin convert in to soluble fibrin.



Factor	Name(s)
Prekallikrein (PK)	Fletcher factor
High molecular weight kininogen (HMWK)	contact activation cofactor; Fitzgerald, Flaujeac Williams factor
I	Fibrinogen
II	Prothrombin
III	Tissue Factor
IV	Calcium
V	Proaccelerin, labile factor, accelerator (Ac-) globulin
VI (same as Va)	Accelerin
VII	Proconvertin, serum prothrombin conversion accelerator (SPCA), cothromboplastin
VIII	Antihemophiliac factor A, antihemophilic globulin (AHG)
IX	Christmas Factor, antihemophilic factor B,plasma thromboplastin component (PTC)
X	Stuart-Prower Factor
XI	Plasma thromboplastin antecedent (PTA)
XII	Hageman Factor
XIII	Protransglutaminase, fibrin stabilizing factor (FSF), fibrinoligase

Hemorrhageic condition:

If there is hemorrhage we can guess few possibilities:

- **Thrombocytopenia** Blood platelets count is below 150000/ cubic mm of blood.
- **Vitamin K deficiency** Vitamin K is essential for synthesis of factor II, VII, IX, and X. Syntheses of these factors are impaired and hemorrhagic disease is progressed due to Vit K deficiency.
- **Hamophilia** Bleeding disease that causes excessive bleeding especially in men.
- **Hemophilia A:** Factor VIII is abnormal **Hemophilia B or Christmas disease:** Factor IX is deficient.
- **Von Willebrand's disease** Factor VIII has two parts, a large component and a small component. The smaller component is more important in intrinsic pathway of blood clotting and loss of this component causes the classical haemophilia. Sometimes haemophilia is seen due to deficiency of the large component, called Von Willebrand's disease.

Thromboembolic condition

When the clotting time is lower than the normal range we can guess there might be **thromboembolic condition** inside the tissue:

- **Thrombus:** An abnormal blood clot developed inside the blood vessels.
- **Emboli:** Tiny parts of breakdown thrombi are called emboli.
- **Causes of thromboembolic condition:** i. Rough endothelial surface caused due to atherosclerosis, infection or trauma. ii. Slow blood flow.

PROCEDURE

There are mainly three methods to find out the Clotting time of own blood sample

1. Lee and White's method
2. Wright's Method
3. Duke's Method

1. Wright's Method:

PROCEDURE

Take a new or unused Lancet/Pricking Needle and Sterilize it with 70 % of alcohol

↓

Sterilize the tip of ring finger with 70% of alcohol using cotton swab

↓

Rub the finger to increase the blood circulation then hold the finger using your thumb

↓

Give the sharp Prick by the lancet on the tip of ring finger

↓

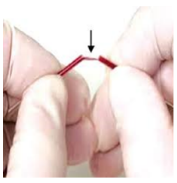
Place Capillary Tube in vertical position at pricking site of finger from where blood comes out (Blood move from higher pressure to lower pressure and entre in to the Capillary Tube)

↓

Fill the blood capillary at the 80-90 % of total volume

↓

After every 30 second. Cut/break about 1/10 portion of capillary tube until to get thread like structure (Coagulum) between two broken part of capillary tube



↓

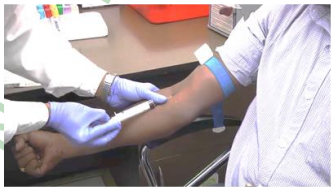
Note down this time and write in to the result.

Correction factor: If you take more than 10 sec to fill the capillary tube then add this time in result.

2. Lee and White's method

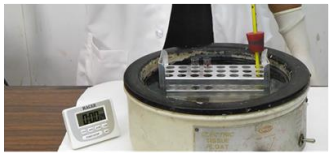
PROCEDURE

Collect the blood through the venepuncture / Venous Blood

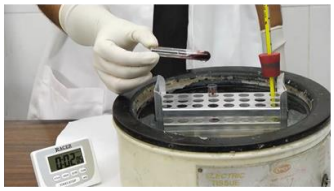


Pour the collected blood (Approximate 1mL) into the Standard Size Test tube.

Start the stopwatch and keep Test Tube in water bath at 37^{O}C Temp.



After 2 Min. tilt the tube for blood clot, Check for clotting in both tubes at every 30 sec. & Note the result



Note: Take the average time for clotting in both the tubes – Clotting Time

3. Duke's Method:

PROCEDURE

Take a new or unused Lancet/Pricking Needle and Sterilize it with 70 % of alcohol

↓

Sterilize the tip of ring finger with 70% of alcohol using cotton swab

↓

Rub the finger to increase the blood circulation then hold the finger using your thumb

↓

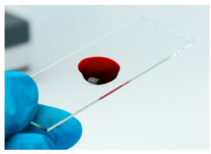
Give the sharp Prick by the lancet on the tip of ring finger

↓

Place the blood drop on the Glass Slide, approximate which produce 5 mm diameter

↓

Move the slide vertical at every 30 sec. to observe the movement of blood drop



↓

Note down the time when no movement/change in shape observe in the blood drop. This is your clotting time

OBSRVATION

The fibrin thread is formed at the breaking point after clot is formed

RESULTS

Clotting time of my own blood is _____ min_____seconds.

PRECAUTION

Following precautions should be enforced

i) Needle should be sterilized.

ii) A fain stain of blood should not be avoided.

iii) Time should be noted properly.

CONCLUSION

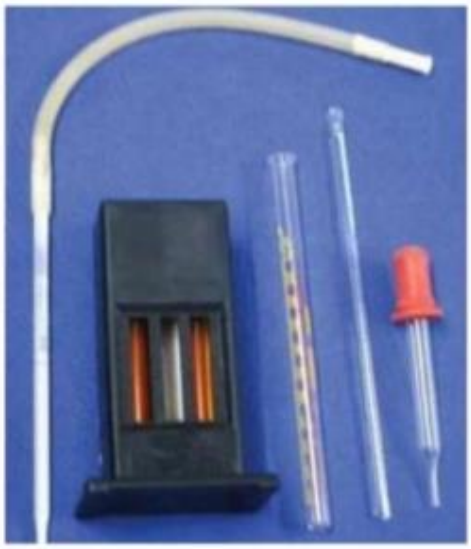
Clotting time of my own blood is _____ in normal/abnormal range.

SIGNATURE OF TEACHER

EXPERIMENT NO.: 12 **DATE:**

AIM: TO ESTIMATE HAEMOGLOBIN WITH THE HELP OF SAHLI'S HAEMOGLOBINOMETER AND COLOR INDEX

REQUIREMENTS: Sahli's hemoglobinometer, Hydrochloric acid, Distilled Water.

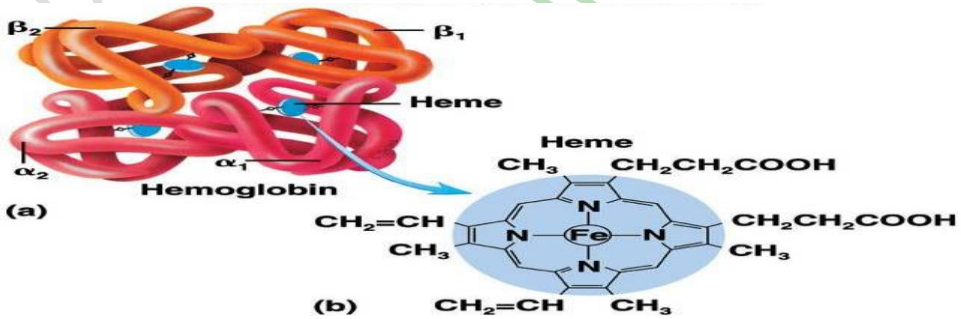


THEORY

- Hemoglobin is the molecules which are present in to the RBC.
- In Hemoglobin protein part is known as globin and non-protein part is known as heme.

  Globin molecules: Transport carbon dioxide and nitric oxide

  Heme molecules: Transport oxygen.
- Globin composed by four polypeptide chain 2α and 2β.
- Each hemes are associated with one polypeptide chain and iron ion (Fe^{+2}) that can combine reversibly with oxygen.



- Each hemoglobin has the capacity to carry four molecules of O_2 which release in to interstitial fluid from there in to cells.
- Each (RBC) one contains about 280 million hemoglobin molecules.
- It has no nucleolus because all space is available for oxygen transport.

- As well as RBC has no mitochondria so it generates ATP by anaerobically so they do not use O_2 which they transport.

NORMAL RANGE OF HAEMOGLOBIN:

- **At birth –** 13.5 to 19.5 gm/dl
- **Children (2-5 yrs) –** 11 to 14 gm/dl
- **Children (6 – 12 yrs) –** 11.5 to 15.5 gm/dl
- **Adult female –** 13 to 15 gm/dl
- **Adult male –** 14 to 17 gm/dl

CLINICAL SIGNIFICANCE OF HEMOGLOBIN ESTIMATION

1. **Anemia:**

Hemoglobin estimation below normal level indicates that the patient is anemic. In this condition there is not enough Hb available to carry sufficient oxygen from the lungs to tissue.

Types of anemia:

A. **Based on hemoglobin estimation**: if hemoglobin level is below 10gms/dl it is called mild anemia. If hemoglobin level goes below 8gms/dl, and 6gms/dl, the conditions are called moderate and severe anemia respectively.

B. **Based on etiology of anemia:** Deficiency of iron in diet (iron deficiency anemia), Vitamin B12 and Folic acid are deficient in diet or folic acid is destroyed due to drug's action (Megaloblastic anemia), Due to impaired absorption of Vitamin B12 from the small intestine, as the intrinsic factor is not synthesized by stomach or destroyed due to gastric surgery (Pernicious anemia), Synthesis of Hb is impaired due to bone marrow failure (Hypoplastic or aplastic anemia), destruction of Hb in circulation due to effect of drugs, free radicals or other toxic chemicals (Hemolytic anemia). Hemoglobin A is replaced by Hb S, Hb C, Hb D, and Hb E (hereditary haemoglobinopathies, Sickle cell anemia etc.), Hemoglobin Batrs (consists of four gamma chains) is present instead of normal Hemoglobin (Thalassemia).

2. **Polycythemia:**

When RBC count is elevated sometimes in disease condition like haemoconcentration due to burns, cholera, chronic heart disease, conditions of decreased lung function such as emphysema or due to climbing (as the oxygen level is less in the high altitude).

METHODS OF HAEMOGLOBIN COUNT:

1. Visual colour comparison method

i. Sahil's method
ii. Dares method
iii. Hadens method
iv. Wintrobes method
v. Haldanes method
vi. Tallquists method

2. Gasometric method

- Van Slyke method

3. Spectrophotometric method

i. Oxyhaemoglobin method
ii. Cyanmethemoblobin method

4. Automated haemoglobinometry

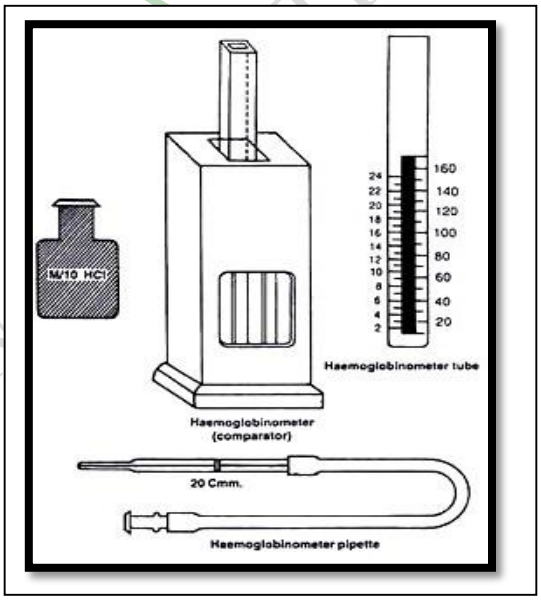
5. Non-automated haemoglobinometry

6. Other methods:

i. Alkaline-hematin method
ii. Specific gravity method
iii. Comparator method.

HB COUNT BY SAHLI'S HAEMOGLOBINOMETER (ACID HAEMATIN METHOD):

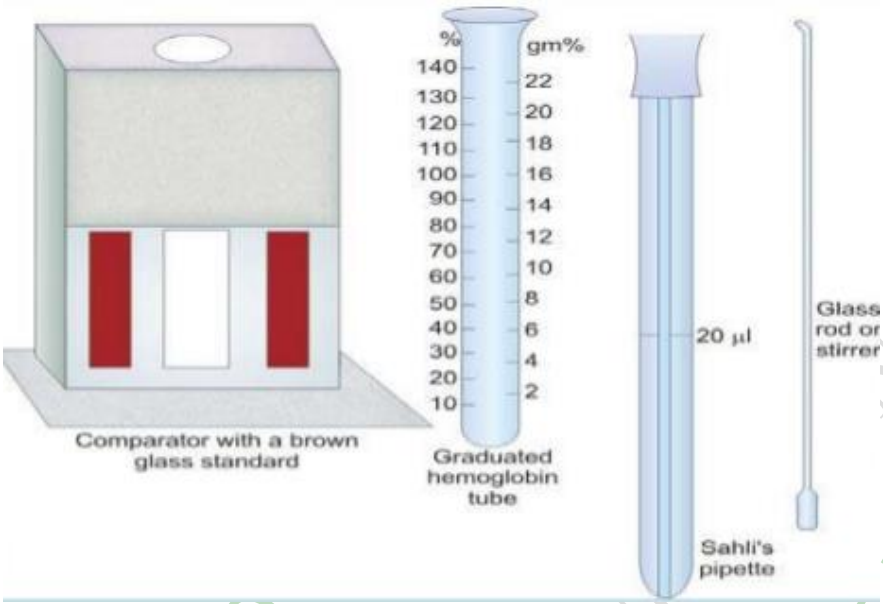
PRINCIPLE:



Anticoagulated blood is added to the 0.1 N HCl and kept for 5-7 minutes to form acid hematin, which give red color to the solution. The color of this acid hematin should be matched with the solution, present in the calibration tube (Reference Solution). Distilled water is added drop by drop to the acid hematin until the color matches and the final reading is directly noted from the graduation in the calibration tube. [Please note that 100 percent on the scale corresponds to 14.5gm % to 15gm %].

SAHLI'S HAEMOCYTOMETER

PROCEDURE:



- The graduated diluted tube and micropipette are clean thoroughly and dried.
- Place N/10 HCL (0.1 N HCL) in diluting tube up to the mark 2gm mark.
- Ring finger is sterilized with 70% alcohol and pricked boldly with the help of pricking needle.
- 1st drop discarded, then suck the blood from pricking site by the help of hemoglobin pipette up to 20-cubic-mm-mark.
- Transfer or blow it into diluting tube and rinse/stir well.
- To clean the pipette rinse it with HCl 2-3 times.
- The blood is immediately deposited at the bottom of graduated tube.
- The blood and HCL mixed properly by glass rod/Stirred and solution allowed to stand for 10-15 mins so all Hb converted into hematin.
- After 10 minutes add distilled water by using the dropper and mix the tube until it has exactly the same color as the reference solution.
- Note down the reading in G% and % of hemoglobin. (It is your observed Hb result)

CALCULATIONS

International value of Hb is 14.5 gm % = 100%

So _____ (Your Hb%) = ? (%)

Calculated Hb% = (Your Hb gm% X 100) /14.5

Oxygen carrying capacity

Amount of oxygen in cc. carried by 100ml of blood during one pulmonary circulation.

Hb% X 1.34 cc oxygen

1gm of Hb contains 1.34 cc oxygen

Therefor, N gm of Hb contains= N X 1.34 cc oxygen N = Your Hb gm% Value

Color index

The relative amount of Hb present in single RBC. It can be determine by finding ratio of Hb% and RBC%

Color index=Hb% / RBC%

RESULT

Observed:

My own blood observed Hb gm%=_____gm%

My own blood observed Hb %=_____%

Calculated:

My own blood calculated Hb %=_____%

Color Index

The Color index of the own sample is_____.

CONCLUSION

SIGNATURE OF TEACHER

EXPERIMENT NO.: 13 **DATE:**

AIM: TO FIND OUT BLOOD GROUP OF OWN BLOOD SAMPLE.

REQUIRMENT: Blood group detection kit [antisera kit], glass slides, Permanent Marker Pen, 70% alcohol, Cotton swabs.

THEORY

- The surface of the erythrocyte contains some glycoprotein and glycolipids that can act as antigen.These antigens are known as isoantigens or agglutinogens.
- Based on the presence or absence of various isoantigens blood is categorized in to different blood groups. More than 100 isoantigens that can be detected on the surface of red blood cells and according to that total of 35 human blood group systems are now recognized by the International Society of Blood Transfusion (ISBT).
- The two most important ones are: ABO and the RhD antigen; they determine someone's blood type (A, B, AB and O, with +, − or Null denoting RhD status).

➢ **ABO blood groups:**

- The ABO blood groups is based on two glycolipids isoantigens called A and B.

Blood Group	Antigens present	Antibodies present
A	A Antigen	Anti-B
B	B Antigen	Anti-A
AB	A and B Antigens	No antibodies
O	Neither Antigens	Anti-A and Anti-B

- The above four ABO bloods types results from the inheritance of various combination of three different genes known as ***I gene:***
    a) I^A codes for the A antigen
    b) I^B codes for the B antigen.
    c) ***i*** codes for neither A nor B antigen.
- Each inherits two I-genes alleles, one from mother side and one from father side.
- The six possible combinations genes of mother and father produce four blood types:
    i) $I^A I^A$ or $I^A i$ produces type A blood.
    ii) $I^B I^B$ or $I^B i$ produces type B blood.
    iii) $I^A I^B$ Produce type AB blood.
    iv) ***ii*** produce type O blood.

➢ **Child blood type estimate table**

		Father's Blood Type			
		A	**B**	**AB**	**O**
Mother's Blood Type	**A**	A/O	A/B/AB/O	A/B/AB	A/O
	B	A/B/AB/O	B/O	A/B/AB	B/O
	AB	A/B/AB	A/B/AB	A/B/AB	A/B
	O	A/O	B/O	A/B	O

➢ **Rh Blood groups:**

- Rh antigen first find out in to Rhesus monkey so it is known as Rh blood groups.
- People whose blood have Rh antigen is known as Rh positive (+).
- People whose blood have not Rh antigen is known as Rh negative (-).
- According to Rh positive and Rh negative ABO blood group further divided in to eight types:

1. A+ve blood group
2. B+ve blood group
3. AB+ve blood group
4. O+ve blood group
5. A-ve blood group
6. B-ve blood group
7. AB-ve blood group
8. O-ve blood group

- If Rh- person receive Rh+ blood, their immune system start to make anti-Rh antibodies that will remains in the blood and during the second transfusion the previous formed

anti-Rh antibodies will cause hemolysis of donated blood and cause severe reaction. Example:

BLOOD TYPE COMPATIBILITY:

		Donor Blood Type							
		A+	**A-**	**B+**	**B-**	**AB+**	**AB-**	**O+**	**O-**
Recipient Blood Type	**A+**	√	√	X	X	X	X	√	√
	A-	X	√	X	X	X	X	X	√
	B+	X	X	√	√	X	X	√	√
	B-	X	X	X	√	X	X	X	√
	AB+	√	√	√	√	√	√	√	√
	AB-	X	√	X	√	X	√	X	√
	O+	X	X	X	X	X	X	√	√
	O-	X	X	X	X	X	X	X	√

PRINCIPLE

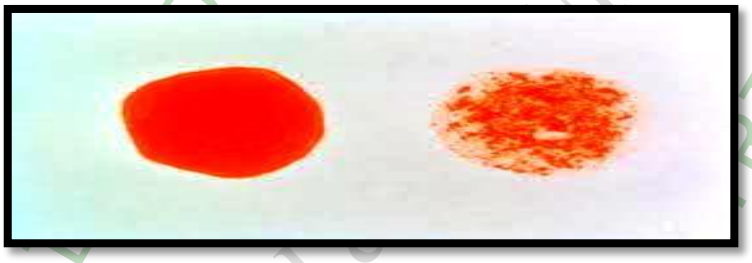
Compatibility between the blood groups of donor and recipient determines the success of a blood transfusion. The AB0 and Rh blood groups are looked at while conducting the test. In a diagnostic lab, Monoclonal antibodies are available for A, B and Rh antigen. Monoclonal antibody against Antigen A (also called Anti-A), comes in a small bottles with droppers; the monoclonal suspension being **BLUE** in color. Anti-B comes in **YELLOW** colour. Anti-D (monoclonal antibody against Rh) is **COLORLESS**. All the colour codes are universal standards. When the monoclonal antibodies are added one by one to wells that contain the test sample (blood from patient), if the RBCs in that particular sample carry the corresponding Antigen, clumps can be observed in the corresponding wells. A drop of blood is left without adding any of the antibodies; it is used as a control in the experiment. The monoclonal antibody bottles should be stored in a refrigerator.

PROCEDURE

1. Take neat and clean four glass slides, Mark A, B, and D on three slide top middle part respectively. Use fourth slide to mix the blood withantisera.
2. Sterilize the ring finger with spirit using cotton swab.
3. Prick the finger using lancet/pricking needle.
4. Discard the first drop of blood.

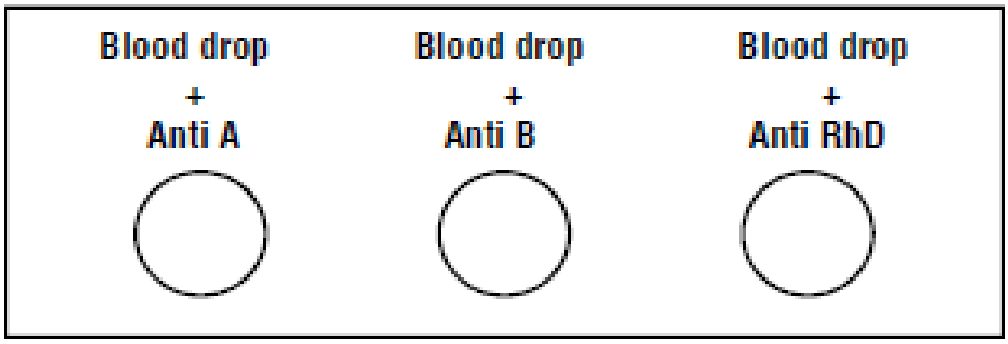
5. Put blood drop on Slide A, Slide B and Slide D at the center.
6. Take the Anti-A (blue) bottle, re-suspend the content and use the dropper to place a drop of the Anti-A in the Slide A. Place the bottle back in ice.
7. Take the Anti-B (yellow) bottle, re-suspend the content and use the dropper to place a drop of the Anti-B in the Slide B. Place the bottle back in ice.
8. Take the Anti-D (colorless) bottle, re-suspend the content and use the dropper to place a drop of the Anti-D in the Slide D. Place the bottle back in ice.
9. Take a fourth slide, mix the Slide A, Slide B and Slide D blood drops with Anti-A, Anti-B and Anti-D solution respectively using alternate corner of the fourth slide. So, it prevent the mixing of one slide solution with the other slide solution.
10. After mixing, wait for a while to observe clumps.

DIAGRAM



No clumping Clumping

OBSERVATION



Slide No. A:	**Blood+Anti serum A**	**Clump/No Clump**
Slide No. B:	**Blood+Anti serum B**	**Clump/No Clump**
Slide No. D:	**Blood+Anti serum D (Rh Factor)**	**Clump/No Clump**

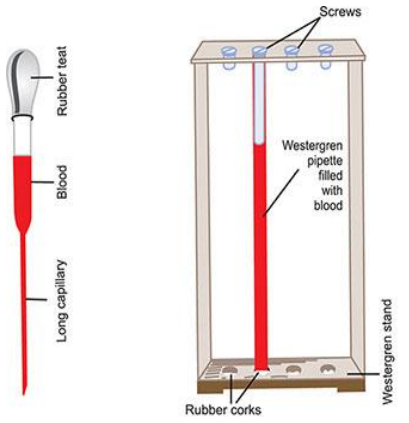
RESULT

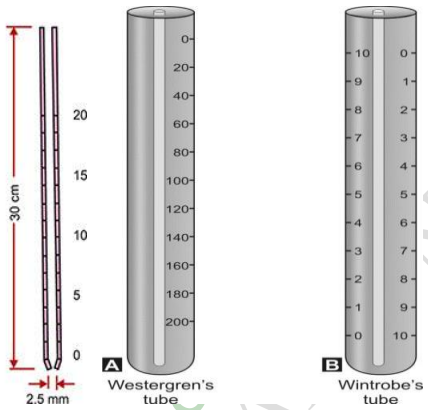
Blood group of own blood sample is _____

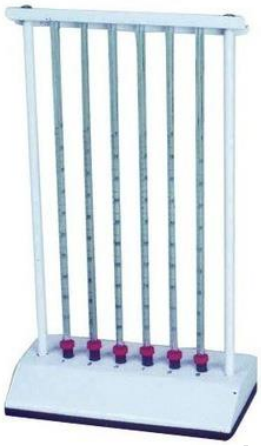
SIGNATURE OF TEACHER

EXPERIMENT NO.: 14 **DATE:**

AIM: TO DETERMINE ERYTHROCYTE SEDIMENTATION RATE BY WESTERGREN'S METHOD







Westergren

Wintrobe

WORKING PRINCIPLE:

When anticoagulated blood is allowed to stand in a narrow vertical glass tube, undisturbed for a period of time, the RBCs – under the influence of gravity- settle out from the plasma. The rate at which they settle is measured as the number of millimeters of clear plasma present at the top of the column after one hour (mm/hr). This mechanism involves three stages:

1. **Stage of aggregation:** It is the initial stage in which piling up of RBCs takes place. The phenomenon is known as Rouleaux formation. It occurs in the first 10-15 minutes.
2. **Stage of sedimentation:** It is the stage of actual falling of RBCs in which sedimentation occurs at constant rate. This occurs in 30-40 minutes out of 1 hour, depending upon the length of the tube used.

3. **Stage of packing:** This is the final stage and is also known as stationary phase. In this, there is a slower rate of falling during which packing of sedimented RBCs in column occurs due to overcrowding. It occurs in final 10 minutes in 1 hour.

THEORY:

- The erythrocyte sedimentation rate (ESR) is a common hematological test for nonspecific detection of inflammation that may be caused by infection, some cancers and certain autoimmune diseases. It can be defined as the rate at which Red Blood Cells (RBCs) sediment in a period of one hour.
- When anticoagulated blood is allowed to stand undisturbed for a period of time, the erythrocytes tend to sink to the bottom.
- Two layers are formed, the upper plasma layer and the lower one of red blood cells.
- The rate at which the red cells fall is known as the erythrocyte sedimentation rate.
- The first is the stage of aggregation when the red cells form rouleaux (RBCs cling together like coins in pile).
- This is followed by the stage of sedimentation in which the falling of the red cells takes place.
- The rate of falling of erythrocyte is directly proportional to the aggregation in first stage.

Clinical significance

- ESR is increased in all conditions where there is tissue breakdown or where there is entry of foreign proteins in the blood, except for localized mild infections.
- The determination is useful to check the progress of the infectious disease. If the patient is improving the ESR tends to fall.
- If the patient's condition is getting worse the ESR tends to rise. The ESR increases high in some chronic bacterial diseases like tuberculosis, typhoid, rheumatic diseases etc.

NORMAL RANGE:

- **Male:** 0-15 mm after 1st hour.
- **Female:** 0-20 mm after 1st hour.

FACTORS AFFECTING ESR:

i. The changed levels of plasma proteins such as fibrinogen and globulins tend to increase rouleaux formation. ESR is therefore increased in any condition causing an increase in fibrinogen (any cause of tissue breakdown such as tuberculosis and other chronic infections) or globulins (rheumatic fever, myeloma, kala-azar, etc.)

ii. Albumin retards sedimentation.

iii. High blood count however, tend to lower the sedimentation rate, while low blood counts tend to accelerate the rate of fall.

iv. ESR is greater in women than in men.

v. During pregnancy ESR gradually increases after 3rd month and returns to normal in about 3 to 4 weeks after delivery.

vi. ESR is low in infants and gradually increases up to puberty.

The Laboratory factors which influence ESR are as follows:

- **Time:** The test should be performed as early as possible after the collection of fasting specimen. There is progressive decrease in sedimentation in first four hours and after that there is a rapid decrease in sedimentation.
- **The length of the ESR tube:** ESR is greater with longer tubes (Westergren's tube) than with shorter tube (Wintrobe's tube). To ensure reliable results the column of blood should be as high as possible. The internal diameter of the tube should be more than 2.5 mm. The tubes should be kept in vertical position. Deviation of the tubes from the vertical position increases the ESR.
- **Temperature:** The red cell sedimentation is increased at higher temperature.

METHODS FOR ESR DETERMINATION:

There are two main methods to determine ESR :

1. Wintrobe’s method
2. Westergren’s method

Each method produces slightly different results. Mosely and Bull (1991) concluded that Wintrobe’s method is more sensitive when the ESR is low, whereas, when the ESR is high, the Westergren’s method is preferably an indication of patient’s clinical state.

PROCEDURE

1. Westergren's method

Requirements:

- Anticoagulated blood (0.4 ml of 3.13% trisodium citrate solution + 1.6 ml blood)
- Westergren tube
- Westergren stand
- Rubber bulb (sucker)

It is better method than Wintrobe's method. The reading obtain is magnified as the column is lengtheir. The Westregren tube is open at both ends. It is 30 cm in length and 2.5 mm in diameter. The lower 20 cm are marked with 0 at the top and 200 at the buttom. It contains about 2 ml of blood.

1. Fill the Westergren's tube exactly up to zero mark by means of a rubber bulb (avoid air bubbles).
2. Place the tube upright in the stand. It should fit evenly into the groove of the stand.
3. Note the time. Allow the tube to stand for exactly one hour.
4. Exactly after one hour, note the level to which the red cell column has fallen.
5. Report the result in terms of mm/after 1st hour.

Normal values

For males: 0-10 mm/hr

For females: 0-15 mm/hr

2. Wintrobe method:

Requirements:

- Anticoagulated blood (EDTA, double oxalate)
- Pasteur pipette
- Timer
- Wintrobe's tube
- Wintrobe's stand

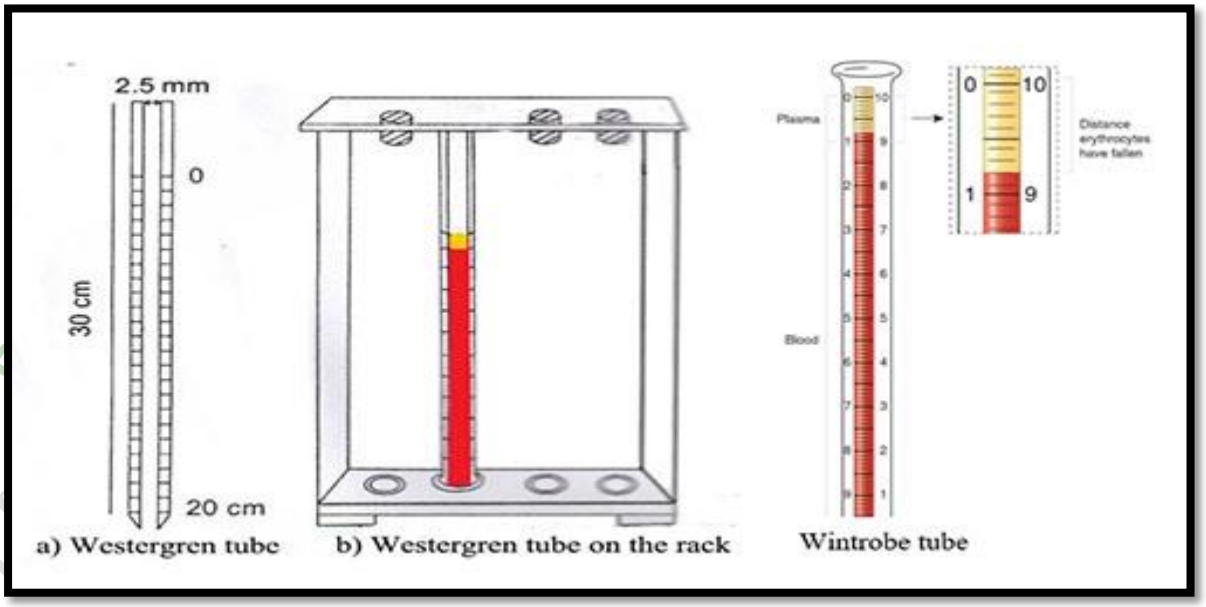
This method uses Wintrobe's tube, a narrow glass tube closed at the lower end only. The Wintrobe's tube has a length of 11 cm and internal diameter of 2.5 mm. It contains 0.7-1 ml of blood. The lower 10 cm are in cm and mm. The marking is 0 at the top and 10 at the bottom for ESR

1. The Wintrobe method is performed similarly except that the Wintrobe tube is smaller in diameter than the Westergren tube and only 100 mm long.
2. EDTA anticoagulated blood without extra diluent is drawn into the tube, and the rate of fall of red blood cells is measured in millimeters after 1 hour.
3. The shorter column makes this method less sensitive than the Westergren method because the maximal possible abnormal value is lower.
4. Fill the Wintrobe tube exactly up to zero mark by means of a rubber bulb (avoid air bubbles).
3. Place the tube upright in the stand. It should fit evenly into the groove of the stand.
4. Note the time. Allow the tube to stand for exactly one hour.
5. Exactly after one hour, note the level to which the red cell column has fallen.
6. Report the result in terms of mm/after 1st hour.

Normal values :

For males: 0-9 mm/hr

For females: 0-20 mm/hr



CLINICAL SIGNIFICANCE OF ESR

The erythrocyte sedimentation rate (ESR) is a non-specific test. It is raised in a wide range of infectious, inflammatory, degenerative, and malignant conditions associated with changes in plasma proteins, particularly increases in fibrinogen, immunoglobulins, and C-reactive protein. The ESR is also affected by many other factors including anaemia, pregnancy, haemoglobinopathies, haemoconcentration and treatment with anti-inflammatory drugs.

CAUSES OF A SIGNIFICANTLY RAISED ESR :

- All types of anemias except sickle cell anemia
- Acute and chronic inflammatory conditions and infections including:
  - – HIV disease
  - – Tuberculosis
  - – Acute viral hepatitis
  - – Arthritis
  - – Bacterial endocarditis
  - – Pelvic inflammatory disease
  - – Ruptured ectopic pregnancy
  - – Systemic lupus erythematosus
- African trypanosomiasis (rises rapidly)
- Visceral leishmaniasis
- Myelomatosis, lymphoma, Hodgkins disease, some tumours
- Drugs, including oral contraceptives

CAUSES OF REDUCED ESR :

- Polycythaemia
- Poikilocytosis
- Newborn infants
- Dehydration
- Dengue haemorrhagic fever
- Other conditions associated with haemoconcentration

OBSERVATION TABLE

Method	At the end of one hr		At the end of two hr	
	Male	Female	Male	Female
Westerngren				
Wintrobe				

CONCLUSION

- The ESR of the given sample is normal / abnormal
- If abnormal then is it higher/lower than normal.

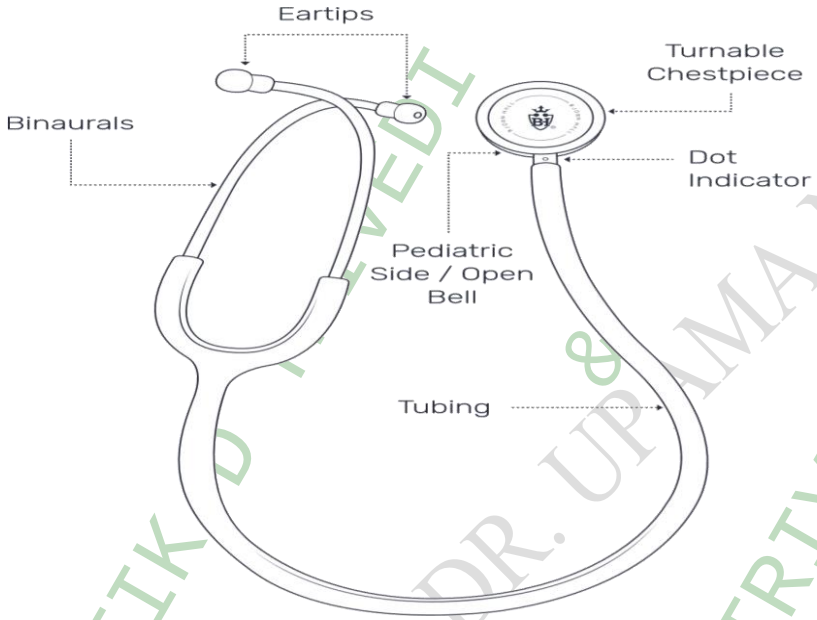
SIGNATURE OF TEACHER

EXPERIMENT NO.: 15 **DATE:**

AIM: TO STUDY AND DETERMINATION OF HEART RATE AND PULSE RATE

REQUIRMENTS

Stethoscope



THEORY

Heart rate and pulse rate are different because a heart rate measures the heartbeats of the heart, whereas a pulse rate measures the rate of blood pressure. A heartbeat pushes the blood through the body.

A normal resting heart rate for adults ranges from 60 to 100 beats per minute.

There are different techniques to measure the pulse rate and heart rate.

There are a few places on your body where it's easier to take your pulse:

- The insides of your wrists
- The insides of your elbows
- The sides of your neck
- The tops of your feet

Put the tips of your index and middle fingers on your skin. Press lightly until you feel the blood pulsing beneath your fingers. You may need to move your fingers around until you feel it.

Count the beats you feel for 15 seconds. Multiply this number by four to get your pulse per minute

What Things Affect Heart Rate and Pulse Rate?

Other than exercise, things that can affect your heart rate include:

- **Weather.** Your pulse may go up a bit in higher temperatures and humidity levels.
- **Standing up.** It might spike for about 20 seconds after you first stand up from sitting.
- **Emotions.** Stress and anxiety can raise your heart rate. It may also go up when you're very happy or sad.
- **Body size.** People who have severe obesity can have a slightly faster pulse.
- **Medications.** Beta-blockers slow your heart rate. Too much thyroid medicine can speed it up.
- **Caffeine and nicotine.** Coffee, tea, and soda raise your heart rate. So does tobacco.

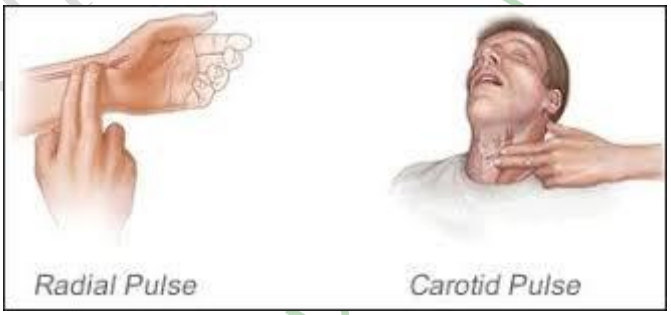
How to Lower Your Resting Heart Rate

In general, people who are more fit and less stressed are more likely to have a lower resting heart rate. A few lifestyle changes can help you slow it down:

- **Exercise regularly.** It raises your pulse for a while, but over time, exercise makes your heart stronger so it works better.
- **Eat right.** Losing weight may slow your resting heart rate. And studies have found lower heart rates in men who eat more fish.
- **Tackle stress.** Set aside time to disconnect from electronic devices and relax each day. Meditation, tai chi, and breathing exercises can also help.
- **Stop smoking.** It's one of the best things you can do for your overall health.

PROCEDURE:

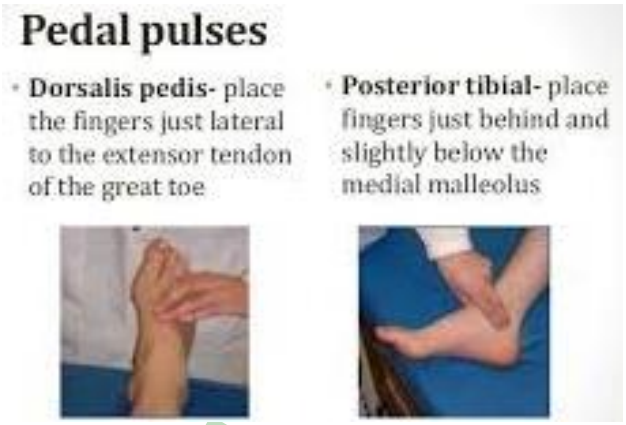
For Pulse rate:



1. **Radial Pulse Method:**
    - It check your pulse through wrist.
    - At your wrist, place two fingers between the bone and the tendon over your radial artery — which is located on the thumb side of your wrist.
2. **Carotid Pulse Method:**
    - It check pulse rate through windpipe.

- Place your index and third fingers on your neck to the side of your windpipe.

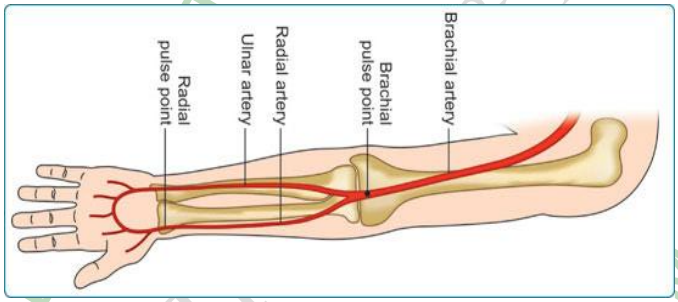
3. **Pedal pulse Method**

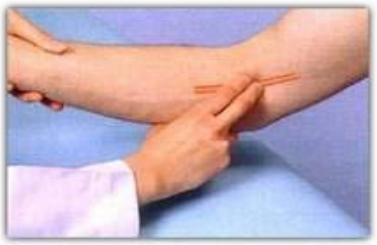


You can also find your pulse on the top of your foot. This is called the pedal pulse.

- Place your index and middle fingers above the highest point of the bone that runs along the top of your foot. You may have to move your fingers along the bone or slightly to either side to feel the pulse.
- Once you have found your pulse, count the beats for 15 seconds.
- Multiply by 4 to obtain your heart rate.

4. **Brachial pulse Method**





Another location for checking your pulse is the brachial artery. This method is used most commonly in young children.

- Turn your arm so it's slightly bent and your inner arm is facing up toward the ceiling.
- Place your index and middle fingers along the side of your arm between the crook of your elbow on the top and the pointy part of your elbow bone on the bottom. Then move your fingers an inch up your arm. You may have to press quite firmly to feel your pulse.
- Once you can feel the pulse, count how many beats occur in 15 seconds.
- Multiply this number by 4 to obtain your heart rate.

For Heart Rate:

- A stethoscope is a medical instrument which is useful to hear sounds made by the heart, lungs, and intestines. Using a stethoscope to hear sounds is called auscultation
- Procedure:
- Get a high-quality stethoscope.
- Adjust your stethoscope's earpieces.
- On the chest piece of stethoscope before use.
- Hold the diaphragm over the subject's heart. Position the diaphragm on the left upper part of the chest where the 4th to 6th ribs meet, almost directly under the breast. Hold the stethoscope between your pointer and middle fingers and apply enough gentle pressure so that you don't hear your fingers rubbing together.
- Listen to the heart for a full minute. Ask the subject to relax and breathe normally. You should hear the normal sounds of the human heart, which sound like "lub-dub." These sounds are also called systolic and diastolic. Systolic is the "lub" sound and diastolic is the "dub" sound.
  - The "lub," or systolic, sound happens when the mitral and tricuspid valves of the heart close.
  - The "dub," or diastolic, sound happens when the aortic and pulmonic valves close.
- Count the number of heartbeats you hear in a minute. The normal resting heart rate for adults and children over 10 years old is between 60-100 beats per minute. For well-trained athletes, the normal resting heart rate may only be between 40-60 beats per minute.
- Listen for abnormal heart sounds. As you count the heartbeats, you should also listen for any abnormal sounds. Anything that does not sound like lub-dub may be considered abnormal. If you hear anything abnormal, your subject may need further evaluation by a doctor.

RESULT:

1. My Pulse Rate is _____ Pulse/Min.
2. My Heart Rate is _____ beats/min.

DISCUSSION:

To measure your heart rate, simply check your pulse. When you feel your pulse, count the number of beats in 15 seconds. Multiply this number by four to calculate your beats per minute.

Normal heart rates and pulse rate at rest:

Age	Pulse rate (beats per minute)
Newborn (resting)	100-180
Infant (resting)	80-150
Child 26 years	75-120
Child 6-12 years	70-110
Adolescent-adult	60-90

-

Above 100 beats a minute indicate as tachycardia and resting heart rate is below 60 beats a minute indicate bradycardia

There are many factors that alter the heart rate and pulse rate:

- Age
- Fitness and activity levels
- Being a smoker
- Having cardiovascular disease, high cholesterol or diabetes
- Air temperature
- Body position (standing up or lying down, for example)
- Emotions
- Body size
- Medications

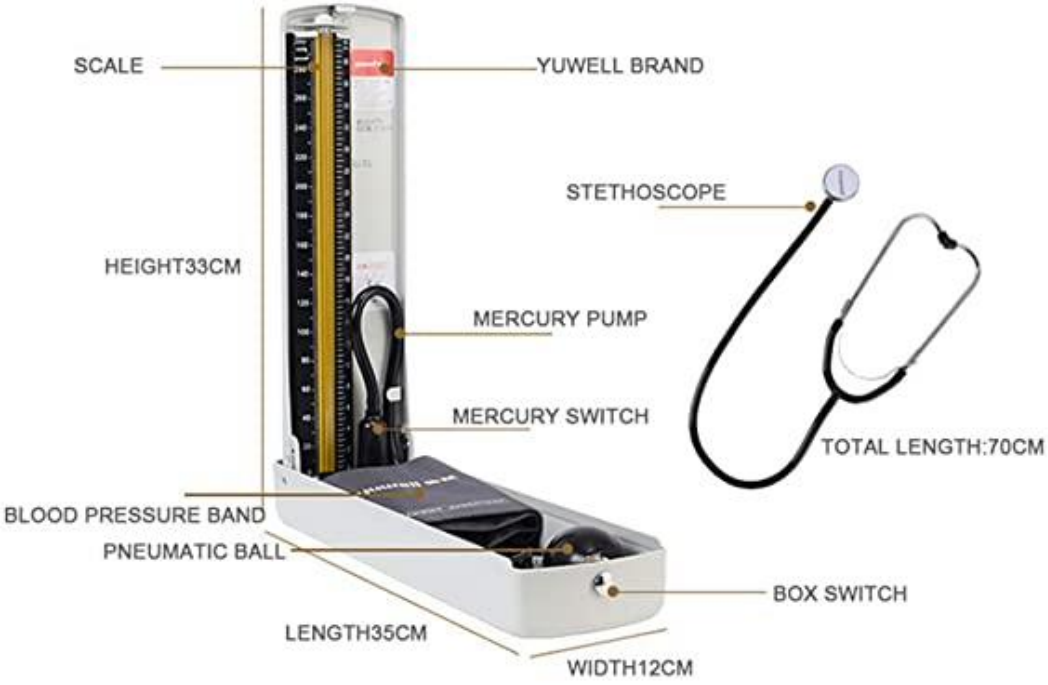
SIGNATURE OF TEACHER

EXPERIMENT NO.: 16 **DATE:**

AIM: TO MEASURE THE BLOOD PRESSURE USING SPHYGMOMANOMETER.

REQUIRMENTS

Sphygmomanometer and stethoscope.



THEORY

Blood Pressure (BP) is the force or pressure which the blood exerts on the wall of blood vessels. When the left ventricle contracts and pushes blood into the aorta the pressure produced within the arterial system is called systolic blood pressure and in the complete cardiac diastole stage, the heart is resting following the ejection of blood, the pressure within the arteries is called diastolic blood pressure. In adult normal systolic BP ranges about 110-130 mm Hg and the diastolic BP ranges about 70-85 mm Hg in adult.

Clinical significance:

Blood pressure, more than the normal range is called hypertension and blood pressure less than the normal is called hypotension.

Classification of hypertension in adults

Category	Systolic BP	Diastolic BP
Optimal	<120	<80
Normal	120–129	80–84
High Normal	130–139	85–89
Hypertension grade I (Mild)	140–159	90–99
Hypertension grade II (Moderate)	160–179	100–109
Hypertension grade III (Severe)	≥180	≥110
Isolated systolic hypertension	≥140	<90

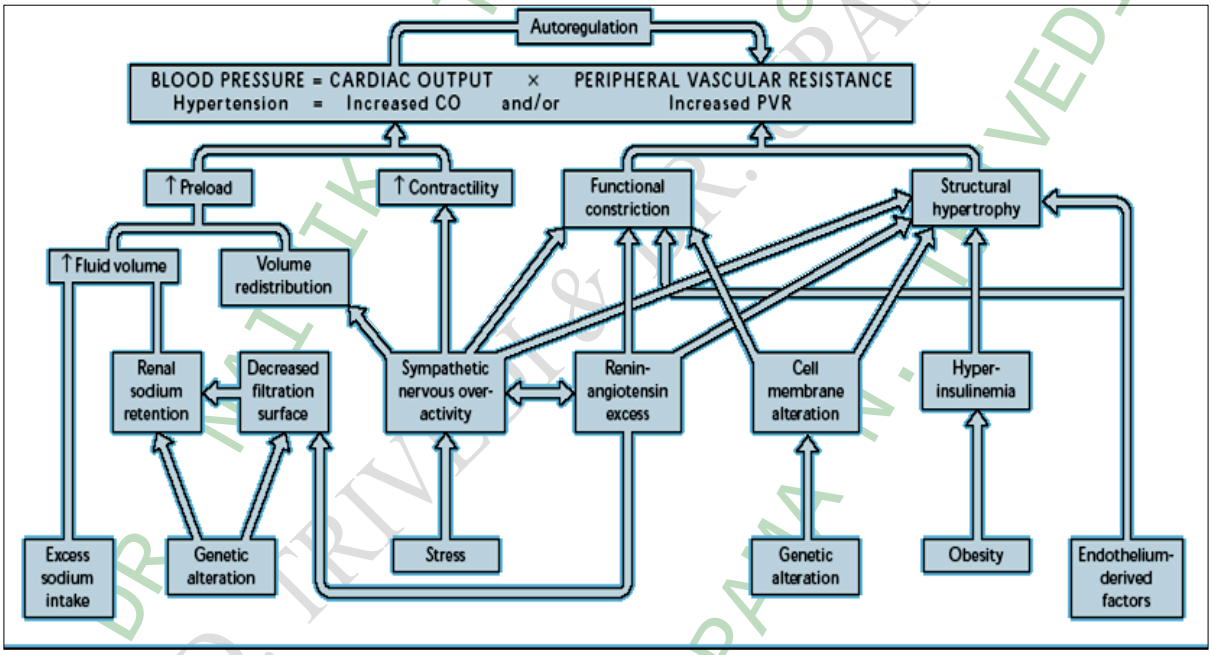
TYPES OF HYPERTENSION

1. **Essential/Primary/Idiopathic HT :** Primary HT in which no specific cause can be identified, constitutes more than 90% of all cases of systemic hypertension. The avg age of onset is about 35 years.
2. **Secondary Hypertension:** Secondary hypertension results from an identifiable cause, such as renal disorder or adrenal of hyper function, accounts for remaining 2%-5% of cases of systemic hypertension. The avg age of developing this HT is between 30 and 50 years.
3. **Labile hypertension (Sustain hypertension):** Patient having labile hypertension are those who has sometime but not always have arterial pressure in HT range. These patients are often considering as borderline HT.
4. **Malignant hypertension:** Sustained hypertension can become accelerated or enter a malignant phase. Patient with malignant HT often has a BP above 200\140 the condition is define by the presence of papilledema usually accompanied by the retinal haemorrhage and exhudate rather than by absolute pressure level.
5. **Accelerated HT:** It is define as significant increase over previous HT level associated with evidence of vascular damage **on Funduscopic examination** but without papilledema.
6. **Isolated systolic hypertension:**The hypertensive condition in which the Systolic pressure is ≥ 140 mmhg and diastolic pressure is <90 mmhg is called Isolated systolic hypertension (ISH).

CAUSES OF HYPERTENSION:

1. Genetic variation (overexpressed or underexpressed genes)
2. Hypertensinogenics (high alcohol intake, high salt intake, obesity, insulin resistance)
3. aging and perhaps
4. sedentary lifestyle
5. Stress
6. low potassium intake
7. low calcium intake. explain
8. Coarctation of aorta explain
9. Diabetes mellitus
10. Hypercholestrolemia

PATHOGENESIS OF HYPERTENSION:



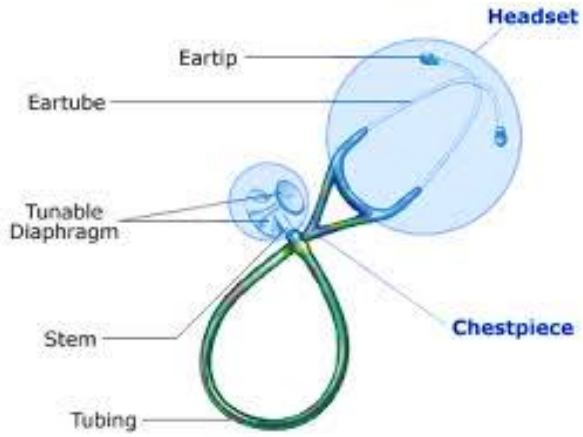
SPHYGMOMANOMETER

A **sphygmomanometer**, is a device used to measure blood pressure. It is consist an inflatable cuff to collapse and then release the artery under the cuff in a controlled manner, and a mercury or mechanical manometer to measure the pressure.

It is an instrument for measuring blood pressure, typically consisting of an inflatable rubber cuff which is applied to the arm and connected to a column of mercury next to a graduated scale, enabling the determination of systolic and diastolic blood pressure by increasing and gradually releasing the pressure in the cuff.

STETHOSCOPE

It amplify the sound and it is made up by 7 parts:



1. **Chestpiece**

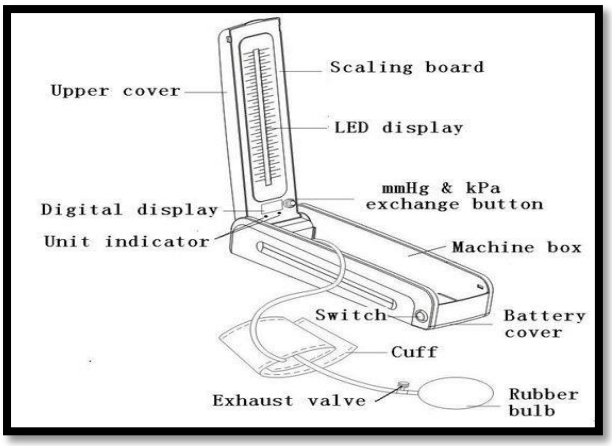
   "Stethos" means "chest," so this is the logical place to start. The chestpiece is the part of the instrument that you hold against the body of the patient.

2. **Diaphragm**

   It may be single-sided or double-sided.

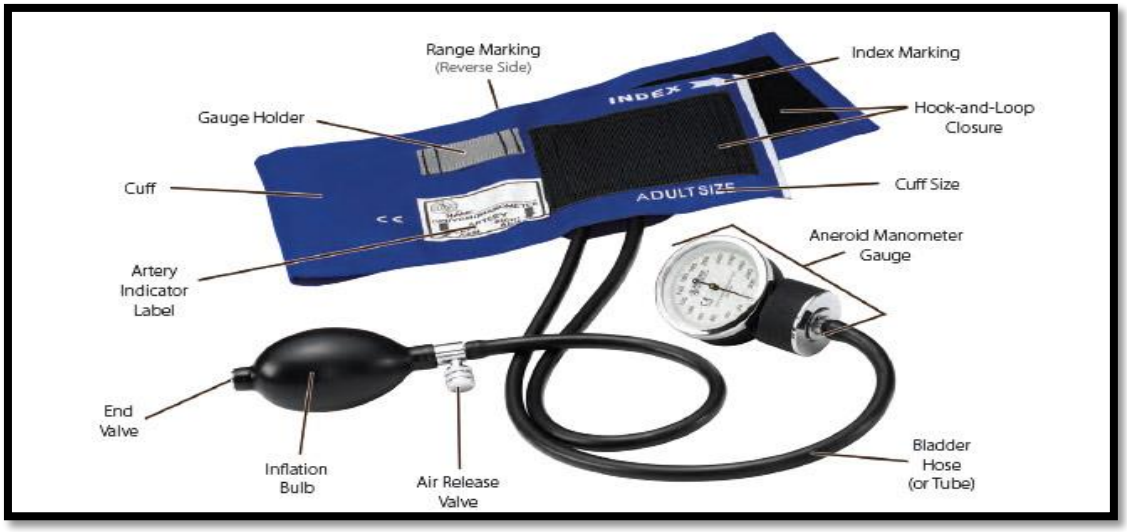
   - A two-sided chestpiece will typically have a diaphragm on one side and a deep cup-shaped side called the bell.
   - Both single-sided and double-sided pieces will usually have a flexible ring called a chill ring encircling them, which helps to make an airtight seal and buffer the patient against the coldness of the part.

3. **The stem** is what connects the chestpiece to the tubing.
4. **Acoustic tubes:** The acoustic tubes are the hollow rubber-like tubes that connect the chestpiece to the ear tubes.
5. **Headset:** This is typically the metal portion of the stethoscope.
6. **Ear tubes:** The ear tubes are hollow metal tubes that connect to the acoustic tubes on one end and the earpieces on the other.
7. **Earpieces:** Earpieces are the small tips on the ends of the ear tubes that fit into your ears. Some stethoscopes may have a choice of hard plastic or soft silicone earpieces.



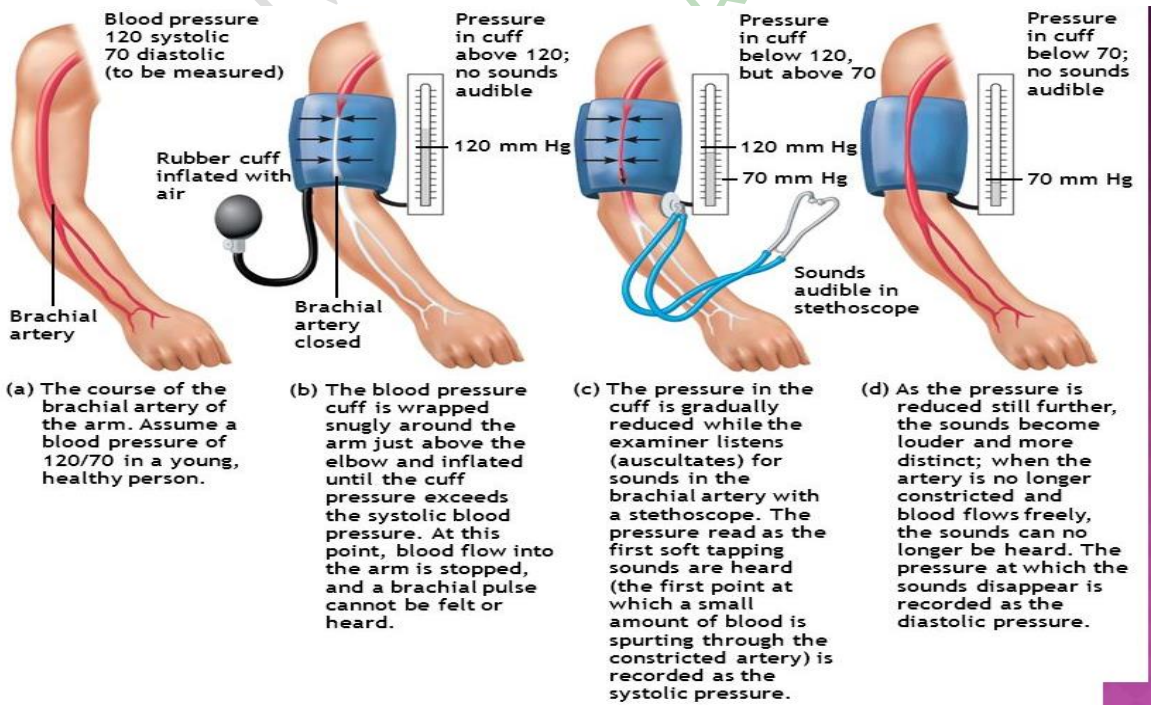
A] Manual sphygmomanometer

B] Digital sphygmomanometer



C] Aneroid sphygmomanometer

PRINCIPLE OF SPHYGMOMANOMETER



(a) The course of the brachial artery of the arm. Assume a blood pressure of 120/70 in a young, healthy person.

(b) The blood pressure cuff is wrapped snugly around the arm just above the elbow and inflated until the cuff pressure exceeds the systolic blood pressure. At this point, blood flow into the arm is stopped, and a brachial pulse cannot be felt or heard.

(c) The pressure in the cuff is gradually reduced while the examiner listens (auscultates) for sounds in the brachial artery with a stethoscope. The pressure read as the first soft tapping sounds are heard (the first point at which a small amount of blood is spurting through the constricted artery) is recorded as the systolic pressure.

(d) As the pressure is reduced still further, the sounds become louder and more distinct; when the artery is no longer constricted and blood flows freely, the sounds can no longer be heard. The pressure at which the sounds disappear is recorded as the diastolic pressure.

The flow through a large size artery is obstructed by means of air pressure exerted through a rubber bag wrapped around the limb.

The pressure is slowly released and the entry of the blood through the obstruction is studied by three methods:

1. Feeling of the pulse **(palpatory method)**
2. Observation of oscillations of the mercury level **(oscillatory methods)**
3. Hearing with the stethoscope the sound produced in the segment of the artery distal obstruction **(auscultatory method).**

The blood flow stops when pressure transmitted to the artery through the rubber bag is equal to or more the blood pressure. The first entry of blood through an obstruction indicates the blood pressure.

Usually arm or thigh is used because there is used because there is only one big blood vessel runs superficially in each of these parts.

PROCEDURE

- Place the Sphygmomanometer on a desk
- Ask the subject to seat calm on a table or chair and place lower hand on the desk with closed fist and uppersame line with the heart.
- Cuff is wrapped around the upper arm just above the elbow.
- The chest piece of the stethoscope is placed upon the brachial artery.
- The other ends of the stethoscope are connected with two ears.
- The bag of the cuff is filled by the pump up to 240 mm Hg. pressure.
- The pressure inside the cuff is released slowly by losing the air with the air adjustment screw. As the pressure is released sudden appearance and disappearance of a sound is heard and recorded. Also observe the movement of mercury inside the tube of sphygmomanometer.
- Sudden onset of trapping sound is systolic blood pressure and the sudden disappearance of sound is diastolic pressure.

MTHODS OD RECORDING OF BLOOD PRESSURE

1. Palpatory method

During the release of sphygmomanometer pressure fix eye at mercury level and finger at the pulse (At the start it is disappeared). When the pulses reappear gives the systolic pressure. This is palpalatory method. This method does not give any idea about diastolic

pressure. The systolic pressure measure by this method is about 5-10 mm lower than actual systolic pressure.

2. **Oscillatory methods**

When the pulse appears, it is also noted that mercury stars oscillating. The first oscillation gives systolic pressure read within 15 secs. On continuation of deflation the oscillation start increasing in magnitude and then slowly diminish. The level at which the oscillations are maximum is taken as the diastolic pressure which read within 30 secs. This is called oscillatory methods

3. **Auscultatory method**

After inflation of cuff, the chest piece of stethoscope placed over the brachial artery in the cubical fossa and deflation is started by slowly releasing the pressure. The level at which a sudden tap sound is heard is the systolic pressure. The sound suddenly gets muffled and disappears is the diastolic pressure. The sound heard are Korokorr's sound. These are due to interrupted flow of blood.

OBSERVATION TABLE

Sr. no	Systolic BP mmHg	Diastolic BP mmHg
1.		
2.		
3.		

RESULT

Systolic blood pressure: _____ mmHg

Diastolic blood pressure: _____ mmHg

PRECAUTIONS

The cuff should be wrapped tightly, the cuff bag should be air free, the apparatus should be kept at the level of heart, pumping and measuring should be done carefully.

CONCLUSION

- From the result my blood pressure is _____ which is in normal/abnormal range.
- It Indicates person is_____ [normal/hypertensive/hypotensive].

SIGNATURE OF TEACHER

ANSWER THE FOLLOWING QUESTIONS

PRACTICAL NO.: 1

1. **Define the following terms:**

Gross anatomy:

Microscopic anatomy:

Gross anatomy:

Surface anatomy:

Regional anatomy:

Systemic anatomy:

Developmental anatomy:

Clinical anatomy:

Microscopic anatomy:

Cytology:

Histology:

PRACTICAL NO.: 2 & 3

2. Draw the neat and labeled diagram of microscope.

3. **Write Classification of the tissue.**

4. **Differentiate between skeletal, cardiac and smooth muscular tissue.**

5. **Explain neurons according to their structure.**

PRACTICAL 5:

6. **Enlist the name and number of Axial Bone.**

7. **Draw the neat and labeled diagram of Atlas, Axial and Thoracic vertebrae.**

PRACTICAL: 6

8. **Enlist the name and number of appendicular bone.**

9. **Draw the neat and labeled diagram of Radius and Ulna bones.**

PRACTICAL: 7

10. Give a brief introduction about hemocytometry.

11. Write Substances, Amount and Purpose of Haymen's RBC dilution fluid

12. Write Substances, Amount and Purpose of WBC dilution fluid

PRACTICAL: 8

13. Write principle of total leucocyte (WBC) count practical.

14. Write procedure of total leucocyte (WBC) count practical.

15. Draw WBC counting chamber diagram.

16. Write calculation steps for total WBC count practical.

PRACTICAL: 9

17. Write principle of total leucocyte (WBC) count practical.

18. Write procedure of total leucocyte (WBC) count practical.

19. Draw WBC counting chamber diagram.

20. Write calculation steps for total WBC count practical.

PRACTICAL: 10

21. Write principle of bleeding time practical.

22. Write procedure of bleeding time practical.

PRACTICAL: 11

23. Write principle of Clotting time practical.

24. Enlist the methods for clotting time measurement .

25. Write steps of the duke's method.

26. Explain clotting mechanism through the diagram.

27. Enlist the clotting factor with their name.

PRACTICAL: 12

28. Write the normal range of Hb:

29. Enlist the methods for Hemoglobin count.

30. Explain the principle of Hb count practical.

31. Explain the procedure for Hb Count practical.

32. Write the calculation steps for Hb practical.

PRACTICAL: 13

33. Write short note on ABO blood group system .

34. Explain the working principle of Erythrocyte Sedimentation Rate (ESR).

35. Draw neat and labeled diagram of westergren and wintrobe pipette.

PRACTICAL 15:

36. Explain the procedure for Pulse Rate measurement.

37. Explain the procedure for Heart Rate measurement

PRACTICAL: 16

38. Explain parts of stethoscope.

39. Explain three methods for blood pressure measurement.

SIGNATURE OF TEACHER