East Point College of Pharmacy

East Point Campus, Jnana Prabha, Virgo Nagar Post
Bengaluru – 560049, Karnataka

Approved
by
Pharmacy Council of India, New Delhi

Affiliated
to
Rajiv Gandhi University of Health Sciences
Karnataka
Bengaluru – 560 041
India

LAB MANUAL

Human Anatomy and Physiology
PHARM D
1st Year

EAST POINT COLLEGE OF PHARMACY
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PROGRAM OUTCOMES
DOCTOR OF PHARMACY
PO1: Comprehensive Pharmaceutical Knowledge
Acquire and demonstrate a deep understanding of fundamental concepts in pharmacotherapeutics, clinical pharmacy, pharmacoepidemiology, clinical pharmacokinetics, therapeutic drug monitoring, as well as behavioral, social, and administrative pharmacy. Showcase advanced proficiency by designing and executing effective medication therapy management plans.
PO2: Strategic Planning Proficiency
Cultivate the capability to formulate and execute plans, proficiently organizing tasks to meet deadlines. Demonstrate adeptness in time management, resource allocation, delegation, and organizational skills. Exhibit competence in clinical decision-making by seamlessly integrating pharmaceutical knowledge with patient-specific factors to enhance healthcare outcomes.
PO3: Problem Solving Proficiency
Apply the principles of scientific inquiry, engaging in analytical, clear, and critical thinking to address challenges and make informed decisions in routine clinical practice. Graduates will demonstrate adept communication skills, counseling patients on medication usage, potential side effects, and lifestyle adjustments, fostering patient comprehension and adherence.
PO4: Leadership and Entrepreneurship
Demonstrate leadership skills and entrepreneurial spirit, contributing to the growth and development of the pharmaceutical profession and industry.
PO5: Professional Identity
Exhibit a strong professional identity including a commitment to ethical practice, effective communication, and leadership in advocating for optimal patient care, continuous professional development, and active engagement with the broader healthcare professionals, promoters and stakeholders.
PO6: Adherence to Ethical Standards
Uphold the highest ethical standards in pharmaceutical practice, adhering to the Pharmacy Council of India's code of ethics and promoting patient welfare.
PO7: Communication
Demonstrate effective communication skills, sustaining clear and empathetic interactions with patients, healthcare professionals, and diverse stakeholders. They will proficiently convey pharmaceutical information, contributing to collaborative and patient-centered care. This emphasis on communication ensures graduates are well-equipped to navigate complex healthcare scenarios and advocate for optimal therapeutic outcomes.
PO8: Community Engagement
Participate in community engagement activities, applying pharmaceutical knowledge to address healthcare needs and improve the overall well-being of the community.
PO9: Environment and Sustainability
Demonstrate a profound understanding of environmental issues in the pharmaceutical domain, applying sustainable practices in research, development, and clinical settings. They will champion eco-friendly approaches, fostering a commitment to minimizing ecological impact and promoting responsible stewardship of natural resources.
PO10: Clinical Research Skills
Proficient in conducting clinical research, applying ethical principles, and contributing to advancements in pharmaceutical sciences and healthcare.
PO11: Continuous Professional Development
Embrace a commitment to lifelong learning, staying abreast of advancements in pharmaceutical sciences, healthcare policies, and technological innovations.

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PROGRAM SPECIFIC OUTCOMES	
DOCTOR OF PHARMACY	
PSO1	Acquire a thorough foundational knowledge in pharmaceutical sciences, including pharmacology, pharmaceutics, pharmaceutical chemistry, pharmacognosy and pharmaceutical analysis to excel in further academic pursuits
PSO2	Acquire and apply the pharmacotherapeutic concepts for better patient care enhancing employability across various sectors including clinical research organizations, academic and hospitals
PSO3	Equip with entrepreneurial skills and knowledge of pharmacoepidemiological studies and regulatory aspects to initiate and run successful ventures in the healthcare sector

Course:	**Code: 1.1T** **Human Anatomy and Physiology**
CO1	Describe the structure (gross and histology) and functions of various organs of the human body
CO2	Describe the various homeostatic mechanisms and their imbalances of various systems
CO3	Identify the various tissues and organs of the different systems of the body
CO4	Perform the hematological tests and record BP, heart rate, pulse and respiratory volumes
CO5	Appreciate coordinated working patterns of different organs of each system and interlinked mechanisms in the maintenance of normal functioning of the human body

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11	To perform pregnancy diagnosis test.
12	Study of appliances used in experimental physiology
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15	To record simple effect of temperature using gastroenemius sciatic nerve preparation.
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17	To record simple fatigue curve using gastroenemius sciatic nerve preparation.

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Experiment No.1

Study of tissues of human body (a) Epithelial tissue. (b) Muscular tissue.

AIM: To study the histology of Epithelial Tissue and Muscular Tissue

EPITHELIAL TISSUE It is made up of one or more layers of cells that provide covering or lining of body and cavities. It is classified as

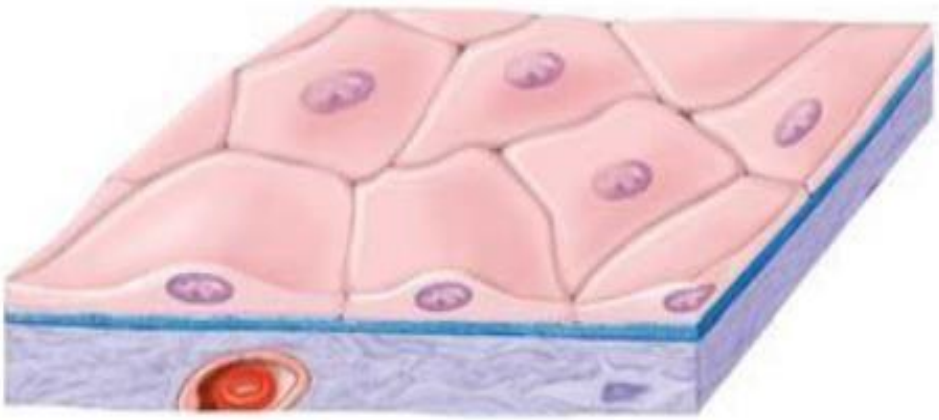
1) Simple Epithelium
2) Pseudo stratified Epithelium
3) Compound Epithelium

Simple epithelium tissue :-

Squamous epithelium: -It is made up of single layer. Nature of cells: Flat polygonal in surface view centrallylocated nucleus.

Location :- Lungs, Bowman's capsule, Henle's loop of kidney inner wall of blood vessels, smooth inner lining of heart, blood vessels, lymphatic vessels, lymph vessels as endothelium.

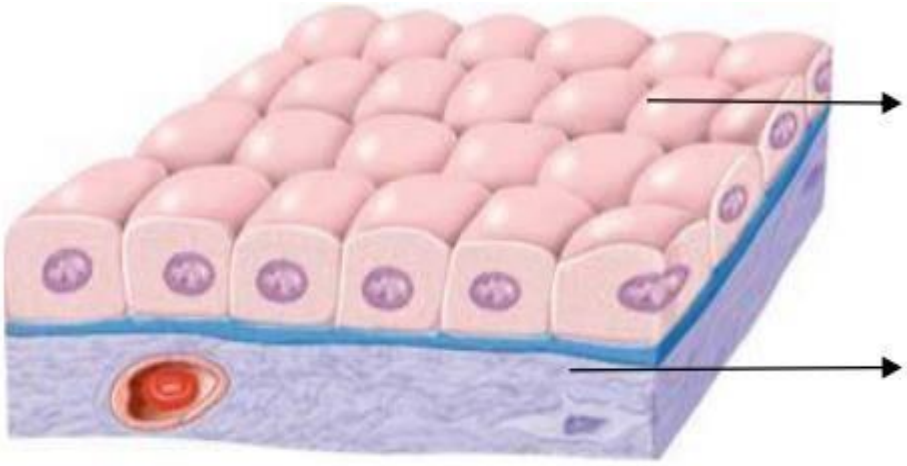
Functions:- Excretion, protection, secretion, absorption, filtration



Cuboidal epithelium:- It's made up of single layer o cubical cells arranged on basement membrane.Nature of Cells:- Cube like cells, polygonal in surface view and elongated Nucleus.

Location: - Stomach, small intestine, large intestine, Gall bladder.

Functions: - Secretion, absorption.

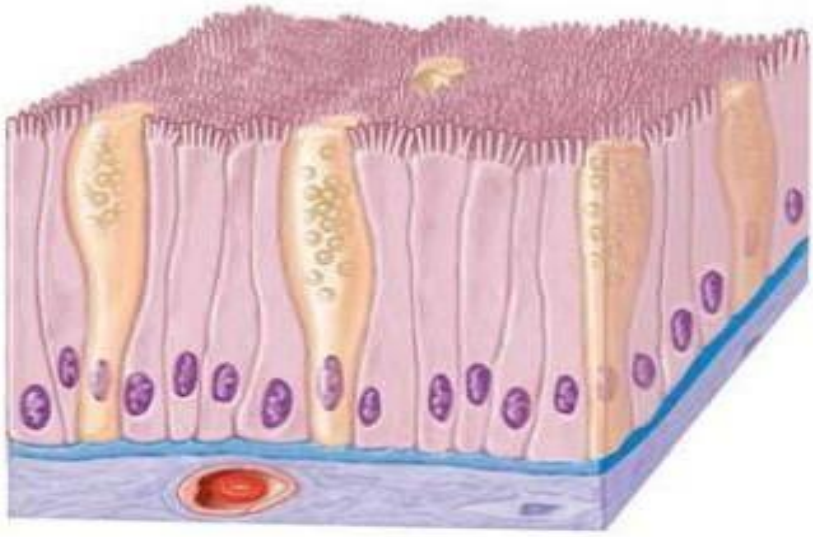


Columnar epithelium: - Made up of single layer of pillar shaped cells.

Nature of Cells: - Elongated cells, polygonal in surface view and elongated nucleus.

Location: - Stomach, small intestine, large intestine, Gall bladder.

Function: - Secretion, Absorption.

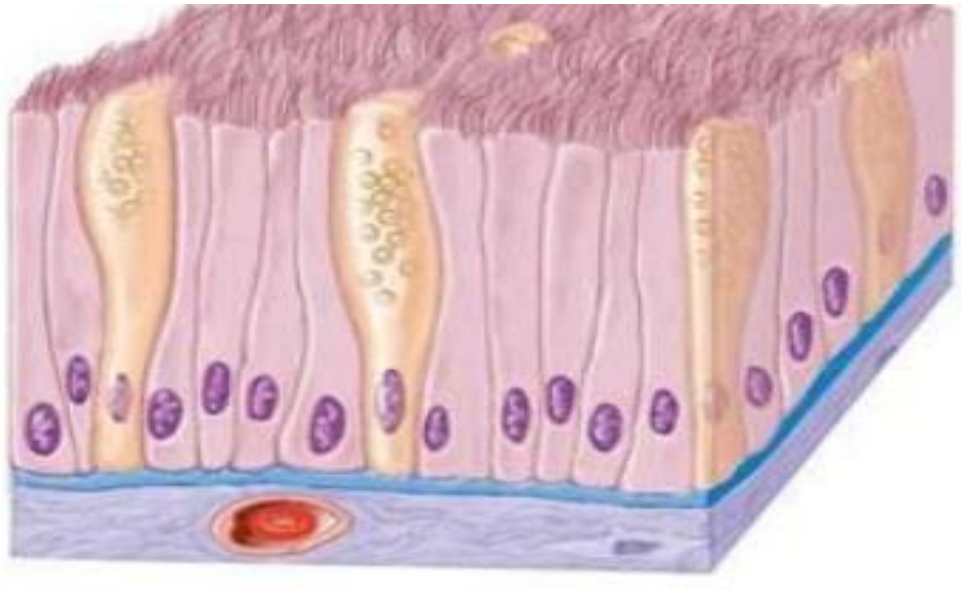


Ciliated epithelium: - It's made up of single layer.

Nature of Cells: - The cells may be Cuboidal (or) columnar. The cells have hair like structures called cilia on itsborder (or) free surface area. The wave like movement of cilia propels the contents of the tube

Location:- a) Cuboidal ciliated- urinary tubules.

b) Columnar ciliated – Fallopian tube, Bronchioles.



Glandular epithelium: -It forms the lining of alveoli and portion of ducts in the glands. It's made up of cubical cells(or) short columnar cells (or) sometimes polyhedral cells.

Functions: -Secretion, lubrication, dilution of irritants.

MUSCULAR TISSUE

The muscle tissue originates from embryonic mesoderm. Themuscle cell is called myocyte. They areof 3 types.

1) Skeletal Muscle.
2) Smooth Muscle.
3) Cardiac Muscle.

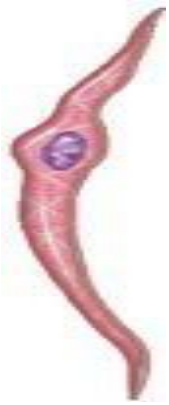
1) Skeletal Muscle:

- It's also called striated muscle.
- Shapes cylindrical, Multinucleate.
- Striations (alternate light and dark bands) are present
- Sarcoplasmic reticulum is well developed
- t's voluntary in function and gets fatigue soon.

- Intercalated discs are absent.
- They are innervated by motor nerves.
- Blood supply is abundant. Location:- Limbs, biceps and body wall.

1) Smooth Muscle:

- It's also called visceral and involuntary muscle.
- Shape-Spindle, uninucleate, nucleus at the centre striations is absent.



- Sarcoplasmic reticulum is less developed.
- Involuntary in function and don't get fatigue soon.
- They contract slowly for a long time.
- They are innervated by ANS

  Location:- Hollow visceral organs like GIT, blood vessels urinary bladder,biliary body, respiratory system etc.

2) Cardiac Muscle:-

- This tissue forms 3-D network.
- Shape-short, cylindrical and branched.
- Uninucleate –nucleus at the centre.

- Sarcoplasmic reticulum is less developed.
- Intercalated dieses are present
- Rhythmic contractions.
- Involuntary in function.
- Myofibrils distinct with faint light and dark band.
- They are innervated by ANS

Location**:** Heart -Blood supply is abundant.

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Experiment No.2

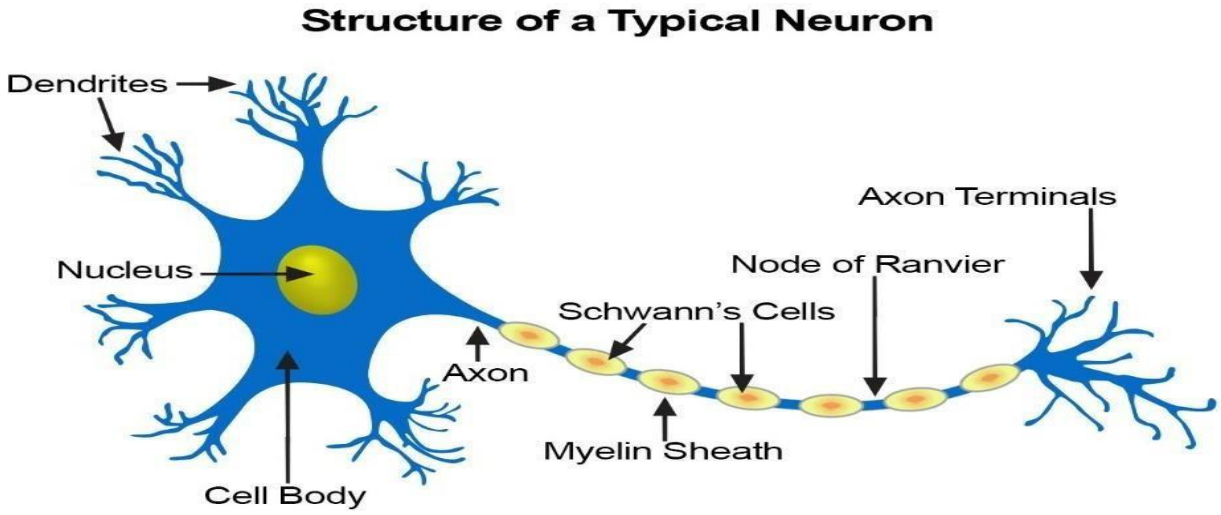
Study of tissues of human body (a) Connective tissue. (b) Nervous tissue.

AIM: To study the histology of Connective tissue and Nervous tissue

NERVOUS TISSUE

- Nerve cells are called Neurons.
- It's structural and functional unit of nervous system.
- Cell body (Cyton) consists of neuroplasm,nucleus, mitochondria andGolgi bodies.

The cell process is two types:



A) Dendron's(Dendrites): These are much branched process for receiving impulses.

B) Axon: A single long cylindrical process for conducting impulses away from cyton.

- The nerve fibres are of two types i.e., Myelinatedand Non-Myelinated.
- The Myelinated nerve fibre has nodes of Ranvierwhich helps in rapid transmission of nerveimpulses.
- The neurilemma consists of Schwann's cells which produce myelin sheath around the neurons.Neuroglia: - It is supporting and packing cells found in brain, spinal cord and Ganglia

Functions: - To receive, discharge and transmit impulses. Co-ordination and integration of the various activities of the body.

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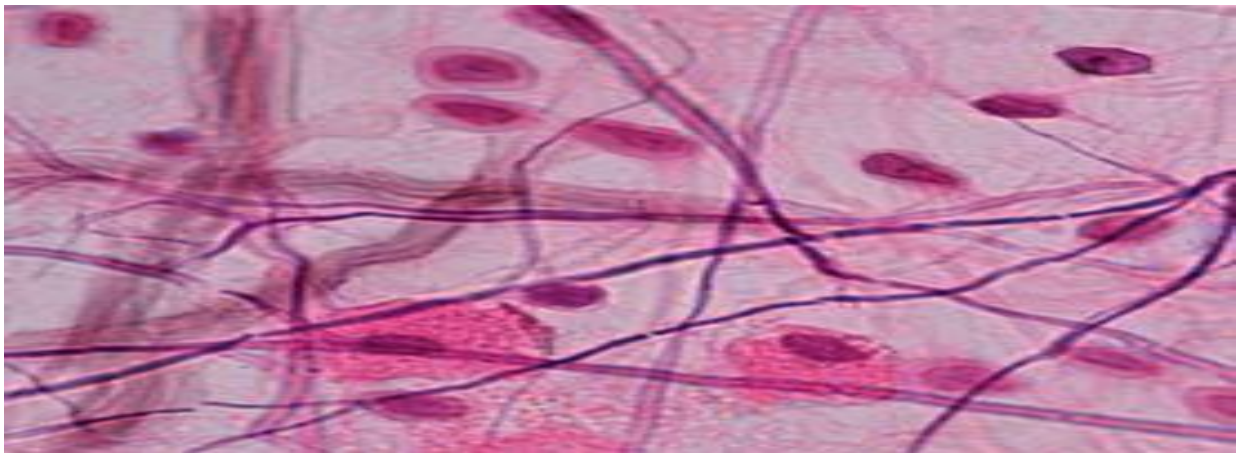
CONNECTIVE TISSUE

It's developed from mesoderm. They are of many types.

a) Areolar Tissue:- Transparent jelly like matrix is found. It contains various of cells like fibroblasts, histocytes, basophiles cells, plasma cells, most cells, pigment cells,



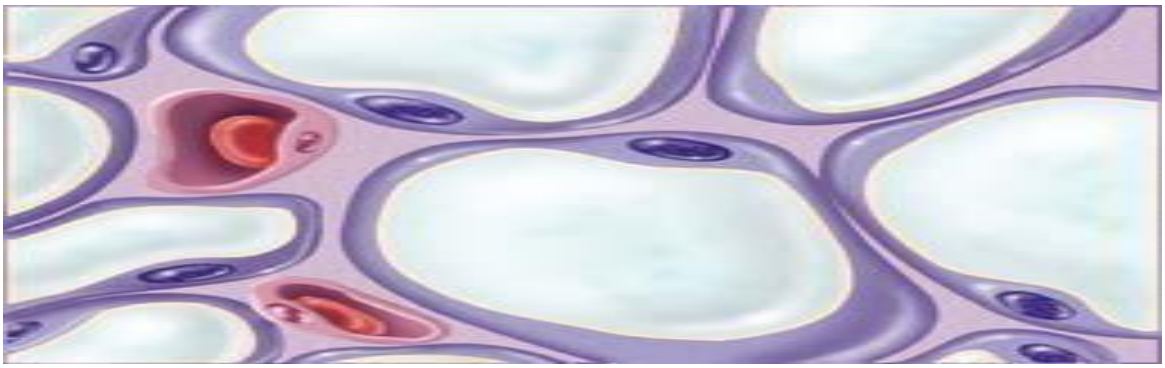
Two type's fibres are present:

White fibre-Fine, wary flexible and un branched made up of collager proteins.

Yellow fibre- They are thick, straight, flexible, elastic and branched made up of elastic protein.

Function:- It connects the skin with muscle, blood vessels and nerves with the surrounding tissue, serve as packing material.

b) Adipose tissue:- It's made up of large round (or) oval flat cells containing fat droplets and fat globules. It's of 2 types.
    a. White adipose tissue (ii) Brown adipose tissue. Matrix contains fibroblasts,macrophages and fibres.



Location:- Sub-Cutaneous areas, mesentery.

Function:- Stores energy in the form of fat, gives shape to the limbs and Body. Regulation of body Temperature.

c) White fibrous tissue:- It consists of white collagen fibres. The tissue is tough and vinelastic due to presence of protections called collagen.

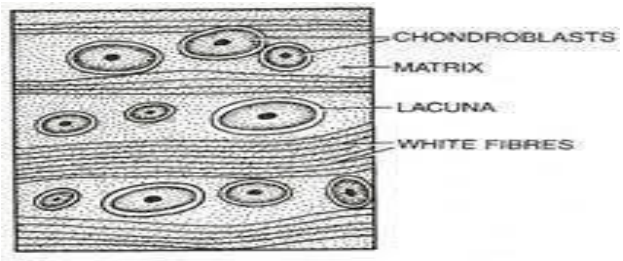


Fig. 7.20. White fibrous cartilage

Location:- It forms tendons, ligaments, articular capsule, capsuleetc.,

d) Yellow elastic tissue:-It's atype of proper connective tissue.

- Fibres are straight, flexible, elastic and occur single and madeup of elastic protein.
- They are thicker, branched and yellow in colour.

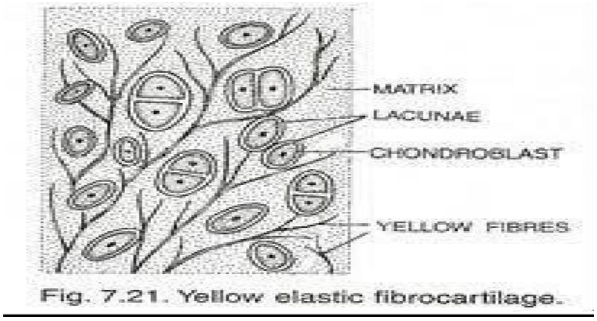
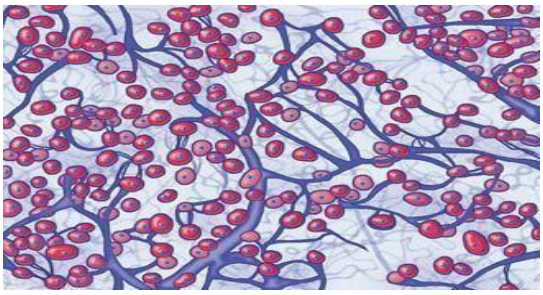


Fig. 7.21. Yellow elastic fibrocartilage.

(a)



(b)

Location:- Lungs, walls of blood vessels, bronchioles,etc.

Function:- Provides strength, movement of organs and alsoin expiration.

a) Reticular tissue:- It consists of reticular cells and reticular fibres.

- Reticular fibres are thinner than white fibres and branched.
- It's a member of reticular endothelial systems made up ofreticular protein.

Location:- Spleen, lymph gland, liver, bone marrow, etc.

Function:- The cells are phagocytic and provide defence to the body.

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Experiment No.3

Study of appliances used in hematological experiments.

AIM: Studying appliances used in hematological experiments involves understanding the specializedequipment and instruments crucial for analyzing blood and its components**.**

a. **Microscope**: Essential for examining blood cells, including red blood cells, white blood cells, andplatelets. Microscopes in hematology labs are often equipped with specialized staining techniques to differentiate various cell types.

b. **Hematology Analyzer**: Automated machines that analyze blood samples to provide detailed information on cell counts and characteristics. They can differentiate between different types of whiteblood cells, red blood cells, and platelets.

c. **Centrifuge**: Used to separate blood components based on density, such as isolating plasma or buffy coat from whole blood. Different types of centrifuges (e.g., microcentrifuge, refrigerated centrifuge) areused depending on the specific requirements of the experiment.

Flow Cytometer: Utilized for analyzing characteristics of individual cells in a heterogeneous population. It can be used to study surface markers on white blood cells, detect abnormal cells, and quantify cell populations.

d. **Incubator**: Maintains a controlled environment of temperature, humidity, and CO2 levels

necessaryfor culturing cells or performing certain hematological assays.

e. **Hemocytometer**: Used for manual cell counting, particularly for determining cell concentration in ablood sample. It consists of a grid etched onto a glass slide, and cells are counted under a microscope.

f. **Electrophoresis Equipment**: Used for separating proteins or nucleic acids based on their size andcharge, which can be useful in certain hematological studies such as hemoglobin electrophoresis.

g. **Spectrophotometer**: Measures the absorbance of light by substances in a solution, useful forquantifying hemoglobin levels or conducting other biochemical assays.

h. **Coagulation Analyzer**: Measures the blood's ability to clot, providing information aboutcoagulation factors and screening for bleeding disorders.

i. **Pipettes and Micropipettes**: Essential for accurately measuring and transferring small volumes ofliquids, critical for preparing samples and reagents in hematological experiments.

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Experiment No. 4

Determination of W.B.C. count of blood.

AIM: To find out the number of White blood cells in one cubic millimeter of blood.

APPARATUS: WBC Diluting fluid (Turk's Fluid) Composition: Each 100 ml of fluid contains 3ml (tolyse/destroy the◊•Glacial acetic acid membrane of WBC, RBCs and platelets) 1ml (to stain the nuclei of◊•Gentian violet (1%) WBCs deep violet black so that they can be identified easily) to make 100ml (solvent)◊•Distilled water.

PRINCIPLE • Since the normal WBC count runs into thousands, the count is made possible by dilutingthe sample of blood before counting and subsequently multiplying the count by the dilution factor • Thedilution employed is 1:20.

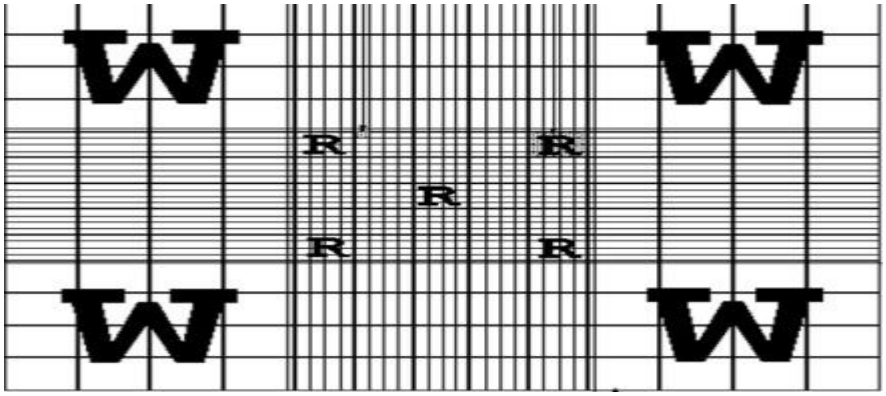
PROCEDURE

- Take WBC diluting fluid (Turk's Fluid) in a watch glass • After pricking finger, suck the second dropof blood into the WBC pipette exactly up to 0.5 mark and dilute it with WBC diluting fluid by suckingthe fluid up to 11mark (dilution 1 in 20)
- Gently rotate the pipette at least 3-4 minutes in the palm of the hand to ensure the proper mixing of theblood an the fluid www.indiandentalacademy.com
- Discard first few drops of WBC fluid in the stem of the pipette, charge the counting chamber (a smalldrop of fluid is allowed to form at the tip of the pipette and gently brought into contact with the edge ofthe cover slip that is already placed on the chamber) and allow time for settling of the cells
- Under low power objective, identify and check the distribution of WBCs in the 4 corner squares. Recharge the chamber if distribution is not uniform (WBCs are seen as regular nucleated, rounded bodies with a clear refractivity around them) www.indiandentalacademy.com
- Count the number of WBCs in each WBC square preferably under low power objective.
- Count the WBCs in 4 corner WBC squares and enter your observations in the corresponding squares.

Calculation

Count the number (N) of cells in 4 large squares located at the four corners of the chamber. The size 4 large squares in which "N" numbers of cells are found is: 1x1x1/10x4= 4/10 mm3 Where 1mm:- is thesideline of the large square 1/10 :- is the depth of the counting chamber between cover slip and the ruling 4:- is the number of large squares used to count

The total numbers of cells in 1mm3 are = Nx10/4 (before dilution of the sample) The actual totalnumbers of cells after dilution should be= Nx10/4x20=Nx50



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Experiment No.5

Determination of R.B.C. count of blood.

AIM: To find out the number of red blood cells in one cubic millimeter of blood

PRINCIPLE: The number of RBC in a known volume of diluted blood is counted and the number ofcells in one cmm of undiluted blood is calculated from this.

APPARATUS: Hemocytometer, RBC diluting fluid, compound microscope, sterile lancet, watchglass,cotton, rectified spirit

HAEMOCYTOMETER: This includes a counting chamber, a special cover slip, and RBC dilutingpipette and a WBC diluting pipette.

The improved Neu-Bauer's double counting chamber:
This is a thick rectanguar glass with a polished transverse bar in the centre, separated from the rest of the slide by two parallel grooves on either side. The polished bar is divided into two equal platforms bya groove in the middle resulting in 'H' shaped depression (moats). The surface of the platforms is 1/10 mm below the surface of the rest of the slide. So if a cover glass is placed over the surface of the counting chamber, the under surface of the cover glass remains 1/10 mm above the polished surface of the platform. The counting area is in the form of a central ruled area on the polished surface of each platform. It is a square of 3 mm side, divided into 9 equal squares of 1 mm side. Of these, the four corner squares are used for WBC counting. Each WBC square of 1 mm side is again divided into sixteen smaller squares each of 1/4mm side. The central 1 mm square is divided into 25 equal small squares of 1/5mm side, by means of triple lines of which the 4 corner ones and the central one are usedfor RBC counting. Each of these squares is subdivided into 16 smallest squares each of 1/20mm side.

Procedure:
Clean and dry the counting chamber and put on the special cover slip provided. Focus under the high power objective and identify the RBC counting area. Clean the RBC pipette first with distilled water, then with absolute alcohol and finally with ether and keep it dry. Take a small quantity of diluting fluidin a watch glass and keep aside. Clean the finger tip using rectified spirit and make a deep prick with a sterile lancet, so that blood comes out freely without squeezing.

Wipe off the first drop which may contain tissue fluid also. Allow a good sized blood drop to form hanging drop and keep the pointed tip of the pipette touching the drop. Suck in blood up to the 0.5 mark carefully, without any air bubble.

Excess blood at the tip of the pipette is removed using a bloating paper or piece of cotton. Immediately, diluting fluid from the watch glass is sucked in upto the 101 mark with out any air bubble by keeping the pipette in vertical position. Then thoroughly mix the blood and diluting fluid in the pipette by gentlyrolling the pipette held horizontally between the palms and keep aside. Mixing takes place only in the bulb of the pipette. The column of diluting fluid contained in the stem of the pipette does not enter into the dilution (i.e. 101-1 = 100). So that the blood sucked upto 0.5 mark will have a dilution of 0.5 in 100 or 1 in 200. Now take out the counting chamber for charging discard first few drops from the pipette, asthe stem contains only diluting fluid. Bring one small drop of diluted blood at the tip of the pipette, to the edge of the cover slip on the counting chamber at an angle of about 450 The fluid enters by capillaryaction under the cover slip and fills the counting chamber. Both areas are filled.

Focus the RBC counting area under high power. Keep the counting chamber undisturbed about 3 minutes for the cells to settle down in the counting area, and start counting. At least 5 squares, each having 16 smallest squares (preferably 4 corner and 1 central) should be counted to obtain a satisfactoryaverage and a better dispersal value. While counting each small square, cells touching the top and left margin of each square should be omitted and cells touching bottom and right margin of each square should be counted. Draw a chart of the counting squares in the record and enter the number of cells in each square and when counted.

Calculation:

Let the number of cells counted in (5x16) 80 smallest squares be “N”

Number of cells in 1 smallest square is N/80

Side of 1 square = 1/20mm

Area of 1 square = 1/400mm2Depth of fluid film in counting chamber is 1/10mm

Volume of diluted blood in 1 square=1/400x1/10=1/4000mm3

Number of cells in 1/4000mm3 diluted blood = N

80 Numberof cells in 1 mm3 of diluted blood N 80x1/4000

= Nx4000 80

The dilution factor is 1 in 200

(Total diluted volume in bulb of the pipette is 100 parts, out of which 0.5 is blood. So dilution is 0.5 in 100)

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Experiment No.6

Determination of differential count of blood.

A differential white cell count (leukocyte formula) consists of an examination of blood to determine the presence and the number of different types of white blood cells. It is obtained examining a blood film ora peripheral blood smear. A peripheral blood smear is a microscope slide made from a drop of blood, which allows the cells to be examined microscopically. Blood films are made by placing a drop of bloodon one end of a slide, and using a spreader slide to disperse the blood over the slide's length. The aim is to get a region where the cells are spaced far enough apart to be counted and differentiated. The slide is left to air dry, after which the blood is fixed to the slide by immersing it briefly in methanol. The fixative is essential for good staining and presentation of cellular detail. After fixation, the slide is stained using the May-Grünwald-Giemsa method to distinguish the cells from each other: the basophilic structures (which take up basic dyes) are colored in blue; the acidophilic or eosinophilic structures (which take up acid dyes) are colored in red whereas neutrophilic structured take up both dyes and are colored brownish- purple. There are several different types of white blood cells. A major distinguishing feature of some leukocytes is the presence of granules in their cytoplasm; white blood cells are often characterized as granulocytes or agranulocytes.Granulocytes (polymorphonuclear leukocytes) are subdivided according to the specific coloration of granules:

► Neutrophils account for the largest percentage of leukocytes found in a normal blood sample. On astained blood smear, the cytoplasm of a neutrophil has numerous fine lilaccolored granules and a darkpurple nucleus. The diameter of a neutrophil is10-15 μm.

- Neutrophils are subclassified according to their age or maturity, which is indicated by changes inthe nucleus (Figure no. 7)
- metamyelocyte – is the youngest neutrophil generally reported, the nucleus is large,round orbean-shaped, the cytoplasm is abundant, pale blue
- neutrophilic band or stab – the nucleus is elongated and curved (horseshoe or Sshape),cytoplasm is abundant, pink
- segmented neutrophil – is a mature neutrophil, the nucleus is separated into 2-5 segments orlobes, the cytoplasm is pale red.

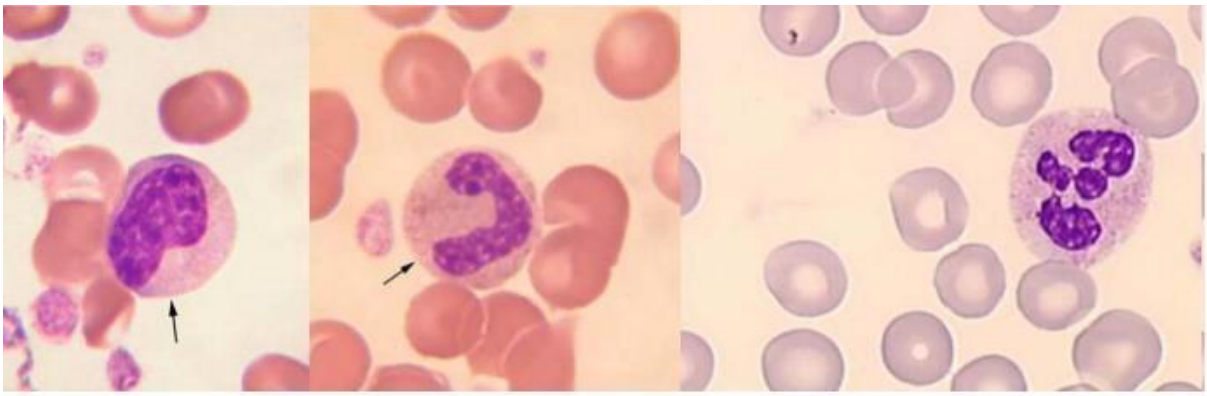

Figure no. 7. Neutrophil granulocytes with different maturation degree: (from left to right) metamyelocyte, neutrophilic band and segmented neutrophil.

- Eosinophils have a diameter of 12-14 μm, the nucleus usually large, typically a bilobate(two-lobed), the cytoplasm is eosinophilic (pale red) with coarse round granules of uniform size which appear brick-red after staining with eosin (Figure no.
- Basophils have a diameter of 11-13 μm. Their nucleus is usually large, irregular, sometimes having three lobes, the cytoplasm is basophilic, containing scattered large,dark-blue granules which may overlay the nucleus (Figure no. 9).

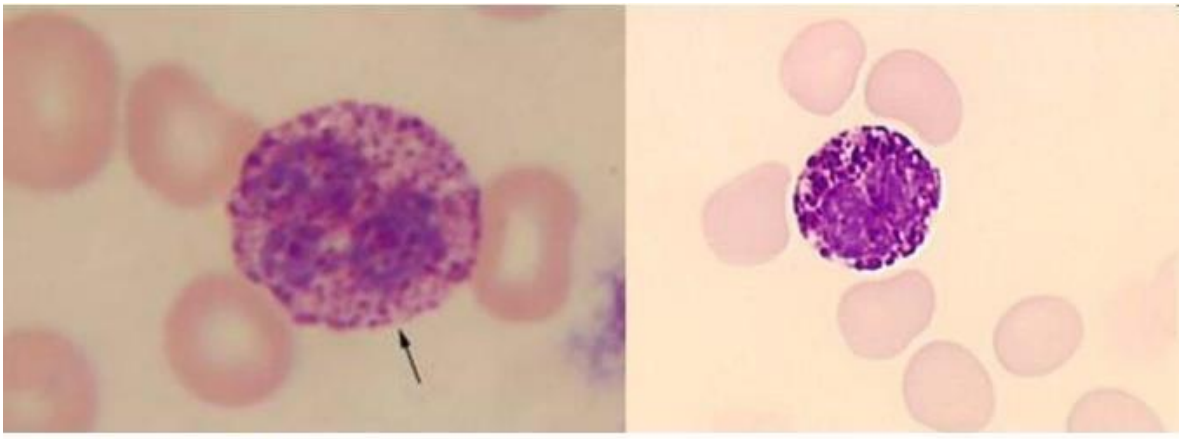

Figure no. 9. Basophil granulocytes

- Agranulocytes (mononuclear leucocytes) are characterized by the apparent absence of granulesin their cytoplasm. Although the name implies a lack of granules these cells do contain non- specificazurophilic granules.

- Lymphocytes have a diameter of 8-10 μm. The nucleus is large, generally round, oval, or slightly indented. The cytoplasm of a lymphocyte is basophilic, scanty, (in circle or "halfmoon" around the nuclues) (Figure no. 10, left).Monocytes are the largest of the normal white blood cells (the diameter is 15-25 μm). The nucleus is large, round, indented or lobulated, the cytoplasm ismuddy gray-blue (Figure no.10, right).

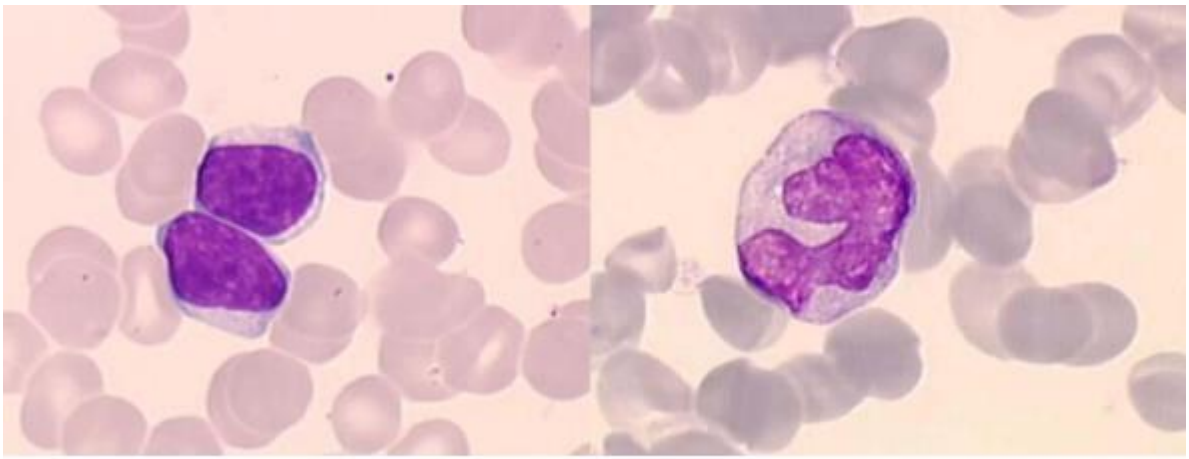

Figure no. 10. Agranulocytes: left: two lymphocytes, right: monocyte.

MATERIALS – stained blood films – light microscope with oil immersion objective (90x) – immersionoil (cedar tree oil)

PROCEDURE:

Focus at low power (10x) on a region of a blood smear. Do not alter focus for the following steps. Partially rotate turret so that 10x and 90x objectives straddle the specimen. Place a small drop of oil onthe slide in the center of the lighted area. Rotate turret so that the 90x oil immersion objective touches the oil and clicks into place. Focus only with fine focus. Scan the blood film in crisscross examining both the center and the sides of the smear. Search for 100 successive white blood cells and identify them. Note the cells in Schilling's table (Table 1): mark 10 white blood cells in every column.

cell type											total (%)
metamyelocytes											
band neutrophils											
segmented neutrophils											
eosinophils											
basophils											
lymphocytes											
monocytes											
total	10	10	10	10	10	10	10	10	10	10	100

Table 1. Schilling's table

RESULTS :

Sum the number of different cell types by rows in Schilling's table. Report the ratio of different whiteblood cells in percents.

DATA INTERPRETATION

Normal distribution of different white blood cell types:

metamyelocytes	< 1 %
band neutrophils	1-2 %
segmented neutrophils	55-65 %
eosinophils	2-4 %
basophils	0-1 %
lymphocytes	23-35 %
monocytes	4-8 %

Alteration of the ratio of different white blood cells:

cell type	increase	decrease
neutrophil	neutrophilia	neutropenia
eosinophil	eosinophilia	eosinopenia
basophil	basophilia	basopenia
lymphocyte	lympocytosis	lymphopenia
monocytes	monocytosis	monocytopenia

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Experiment No.7

a. Determination of Erythrocyte Sedimentation Rate

AIM: To report ESR of your blood.

Erythrocyte Sedimentation Rate (ESR)

The ESR is the rate at which erythrocytes settle down or produce amillimetre of clear plasma at the topof a vertical column in an hour. In this procedure, the blood is mixed with anticoagulant and is allowedto stand in a vertical tube.

There are two commonly used methods for calculating the ESR that are:

1) Westergren's Method: This tube should be opened at both ends and labelled in mm up to the 20mmmark.

2) Wintrobe's Method: This tube should be opened at the top and closed at the bottom and labelled inmm up to the 100mm mark. The ESR can be used to diagnose a wide range of illnesses and physiological problems.

Increased Sedimentation

Tuberculosis, cancer, rheumatic fever anaemia, menstruation,pregnancy and Jokaemia.

Decreased ESR

Congestive heart failure, polycythaemia, congenital diseases.

Procedure

1) Anticoagulant solution (1/5 of blood), ie, oxalate or citrate should be taken in a clean dry vial
2) Then two ce of blood should be extracted out by vein puncture
3) The blood should be mixed properly with anticoagulant solution.
4) Then the blood should be sucked into Westergren tube upto 200 mark.
5) should be placed in the stand.
6) The Westergern tube should be held vertically in the stand.
7) The reading should be note after one hour.

Normal Range:

Average values in healthy men are: <15mm/hr; in healthy females, they are somewhat higher: <20mm.

b)Determination of Hemoglobin content of Blood

AIM: To determine the hemoglobin content in 20µl of blood sample.

PRINCIPLE: A hemoprotein composed of globin and heme that gives red blood cells their characteristiccolor; function primarily to transport oxygen from the lungs to the body tissues. The red blood cells are broken down with hydrochloric acid to get the hemoglobin into a solution. The free hemoglobin is exposed for a while to form hemin crystals. The solution is diluted to compare with a standard colour.

MATERIALS: Hemometer, Single mark pipette, Distilled water, Needle, Spirit, Cotton, HCl.

PROCEDURE:

- Take 1/10 HCl in the Hb tube upto the lowest mark '2'.
- Prick the finger with needle and collect 20µl of blood sample with single mark pipette.
- Place the Hb tube on working table for five minutes for the formation of hemin crystals.
- Place the Hb tube in the compater/hemometer and add drop by drop of distilled water into it until thecolour of the solution in the Hb tube coincides with the glass plates of the compater.
- If the colour coincides with the glass plates of the compater, observe the reading in the Hb tube. Thepercentage of Hb can be calculated from the reading.

DATA ANALYSIS:

Hb content in grams X 100 / 14.5

NORMAL VALUES:

Males = 14 to 18 grams

Females = 13 to 14 grams Children = 10 to 13 grams

RESULT: The hemoglobin content present in 20µl of blood sample is ----

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a) Determination of Bleeding Time

AIM : To determine the bleeding time of a patient.

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 offibrin network on the surface of punctured skin; that is it is the 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.

Clinical significance

It plays a significant role

i. to study the haemorrhagic disorders.

ii. to study the coagulation defects

iii. 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-coagulantdrug administration.

Materials: Sterilized needle, filter paper, cotton, spirit, and stop watch.

Procedure (Duke's method)

Finger of a subject is sterilized with spirit and pricked with sterilized needle. Time of pricking is noted.Take the stain of the punctured point on a filter paper on 30 second and keep taking stain of blood in 20second intervals until the bleeding stops. The time of no stain has come is noted properly; it is the bleeding time of the subject.

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.

b) Determination of Clotting Time

AIM : To determine the clotting time of a subject.Requirements:

Fine capillary glass tubes of about 10 mm length, cotton, rectified spirit, lancet, stop watch.

Procedure:

Capillary tube method: (Wright's method)

Under sterile precautions make a sufficiently deep prick in the finger tip. Note the time

when bleeding starts (start the stop watch). Touch the blood drop at the finger tip using one endof the capillary tube kept tilted downwards. The tube gets easily filled by capillary action. Afterabout two minutes start snapping off small lengths of the tube, at intervals of 15 seconds, each time noting whether the fibrin thread is formed between the snapped ends. Note the time (stop the stop watch) when the fibrin thread is first seen.

Discussion:

Clotting time is the interval between the moment when bleeding starts and the momentwhen the fibrin thread is first seen.
Normal value is 3to 10 minutes.

Bleeding time and clotting time are not the same. Bleeding time depends on the integrity

of platelets and vessel walls, whereas clotting time depends on the availability of coagulation factors. In coagulation disorders like haemophilia, clotting time is prolonged but bleeding timeremains normal.
Clotting time is also prolonged in conditions like vitamin K deficiency, liver diseases, disseminated intravascular coagulation, overdosage of anticoagulants etc.

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Experiment No.8

a) Determination of Blood group

AIM: of Blood group.

ABO blood group system, the classification of human blood based on the inherited properties of red blood cells (erythrocytes) as determined by the presence or absence of the antigens A and B, which are carried on the surface of the red cells. Persons may thus have type A, type B, type O, or type AB blood.The A, B, and O blood groups were first identified by Austrian immunologist Karl Landsteiner in 1901.See blood group.

Blood containing red cells with type A antigen on their surface has in its serum (fluid) antibodies against type B red cells. If, in transfusion, type B blood is injected into persons with type A blood, thered cells in the injected blood will be destroyed by the antibodies in the recipient's blood. In the same way, type A red cells will be destroyed by anti-A antibodies in type B blood. Type O blood can be injected into persons with type A, B, or O blood unless there is incompatibility with respect to some other blood group system also present. Persons with type AB blood can receive type A, B, or O blood.

The ABO and Rh groups in transfusion

system	recipient type	donor red cell type	donor plasma type
ABO	A	A* or O	A or AB
ABO	B	B or O	B or AB
ABO	O	O only	O, A, B, or AB
ABO	AB	AB*, A*, B, or O	AB
Rh	positive	positive or negative	positive or negative
Rh	negative	negative or positive**, ***	negative or positive**

*Not if the patient's serum contains anti-A1 (antibody to common type A red cell in subgroup A patients).
**Not if the patient is a female less than 45 years old (childbearing possible), unless life-threatening hemorrhage is present and transfusion of Rh-positive blood is lifesaving.
***Not if the patient's serum contains anti-D (antibody to positive red cells), except under unusual medical circumstances.

Blood group O is the most common blood type throughout the world, particularly among peoples of South and Central America. Type B is prevalent in Asia, especially in northern India. Type A also is common all over the world; the highest frequency is among the Blackfoot Indians of Montana and in theSami people of northern Scandinavia.

The ABO antigens are developed well before birth and remain throughout life. Children acquire

ABO antibodies passively from their mother before birth, but by three months of age infants are making their own; it is believed that the stimulus for such antibody formation is from contact with ABO-like antigenic substances in nature. ABO incompatibility, in which the antigens of a mother and her fetus aredifferent enough to cause an immune reaction, occurs in a small number of pregnancies. Rarely, ABO incompatibility may give rise to erythroblastosis fetalis (hemolytic disease of the newborn), a type of anemia in which the red blood cells of the fetus are destroyed by the maternal immune system. This situation occurs most often when a mother is type O and her fetus is either type A or type B.

b) Determination of Blood

Pressure. Definition of Blood Pressure Arterial blood pressure is the force exerted by the blood on the wall of a blood vessel as the heart pumps (contracts) and relaxes. Systolic blood pressure is the degree of force when the heart is pumping (contracting). Thediastolic blood pressure is the degree of force when the hearts relaxed.

Method of Measuring Arterial Blood Pressure

In the measurement procedure a cuff is wrapped around a person's arm with an inflatable rubber bag inside the cuff centered over the brachial artery. Enough air pressure is pumped into the cuff to close theartery. Air pressure is then released by opening the thumb valve. When the pressure in the cuff is equal to the pressure on the artery, the artery opens and the blood begins to return to the part of the artery that was closed.

As the blood returns to the artery, pulse sounds begin. These sounds can be heard through a stethoscopeplaced over the brachial pulse point. The sounds continue for a time while the cuff is deflated slowly, eventually becoming too faint to hear.

The cuff is connected by tubing to a manometer, which shows the amount of pressure on the artery.When the first pulse sounds are heard, the reading on the manometer measures the systolic blood pressure. The last sound heard is the diastolic blood pressure. In children, the muffling of sound or fourth sound is often used as the diastolic blood pressure rather than the disappearance of sound.

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BLOOD PRESSURE CATEGORY	SYSTOLIC mm Hg (upper number)	and/or	DIASTOLIC mm Hg (lower number)
NORMAL	LESS THAN 120	and	LESS THAN 80
ELEVATED	120 – 129	and	LESS THAN 80
HIGH BLOOD PRESSURE (HYPERTENSION) STAGE 1	130 – 139	or	80 – 89
HIGH BLOOD PRESSURE (HYPERTENSION) STAGE 2	140 OR HIGHER	or	90 OR HIGHER
HYPERTENSIVE CRISIS (consult your doctor immediately)	HIGHER THAN 180	and/or	HIGHER THAN 120

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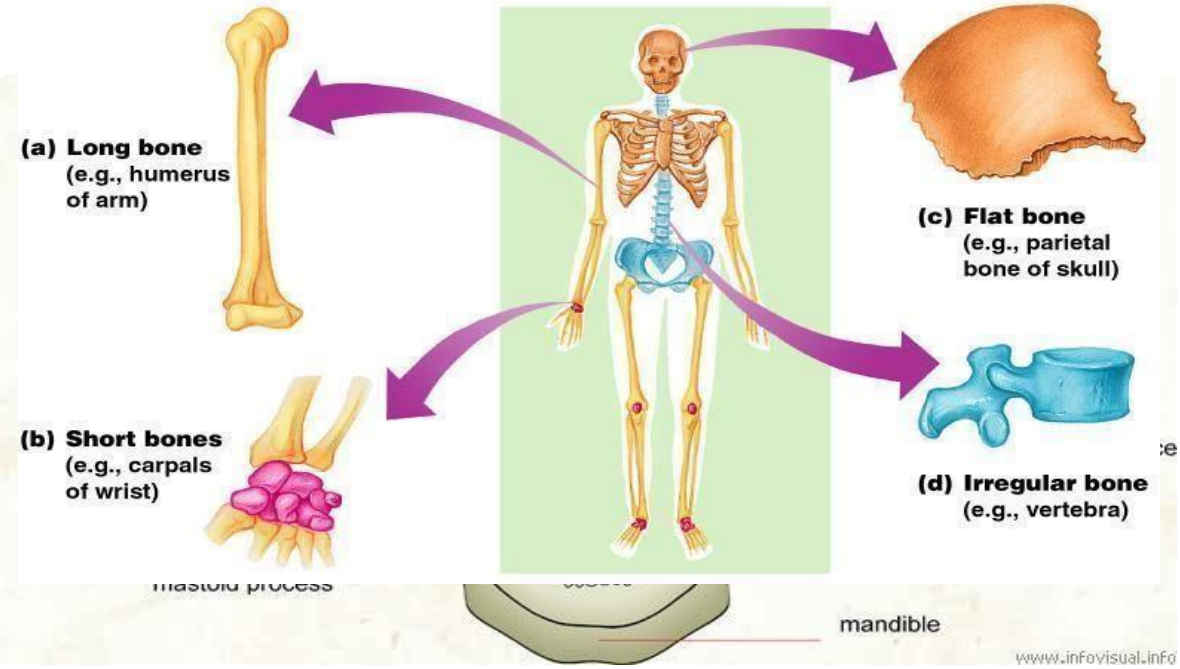
Experiment No -9

Study of various systems with the help of charts, models & specimens

a. Skeleton system part I-axial skeleton



b) Skeleton system part II- appendicular skeleton.

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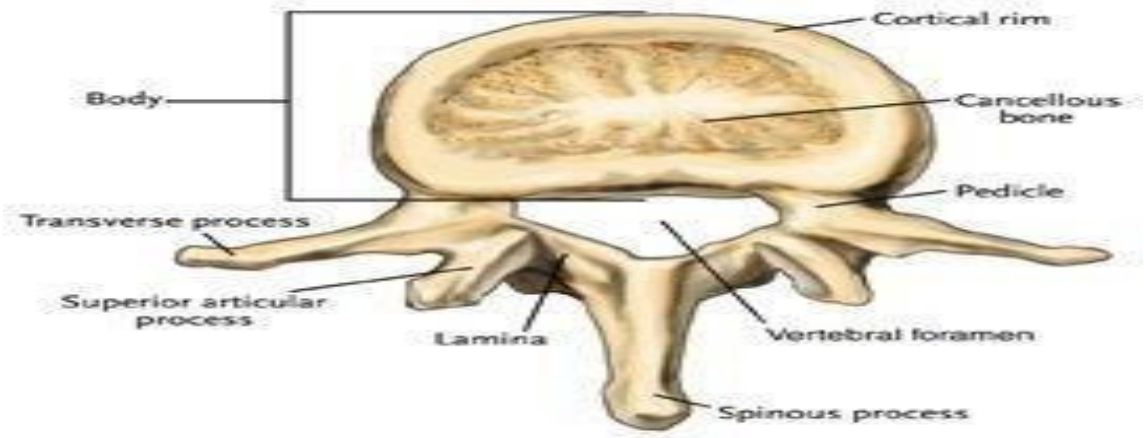

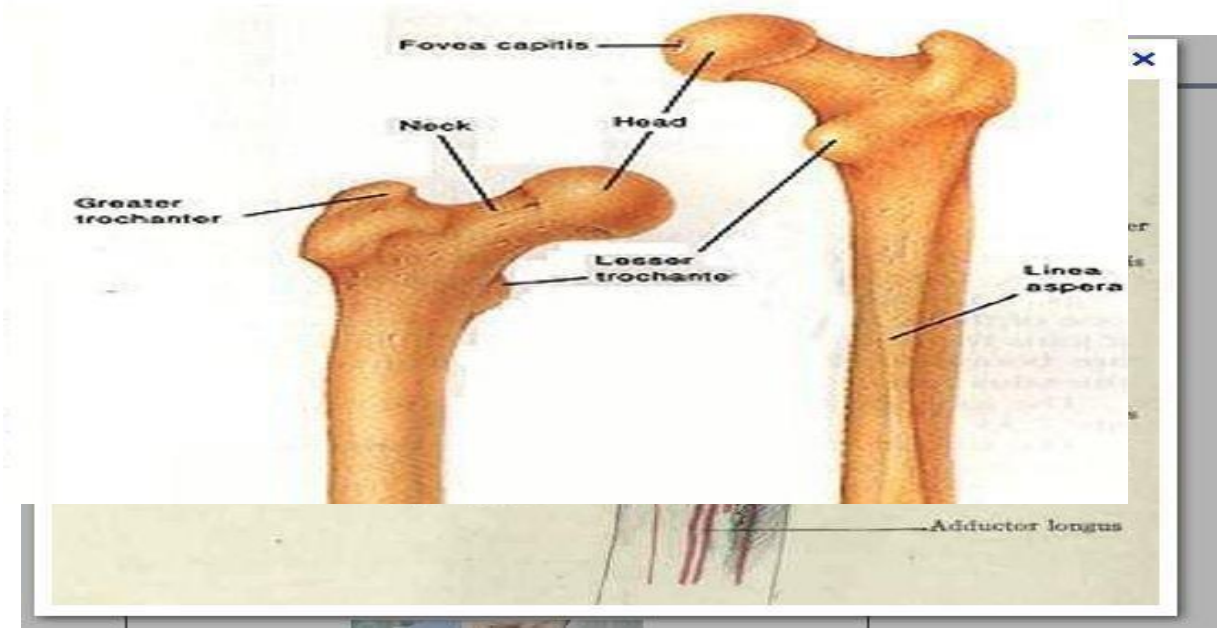

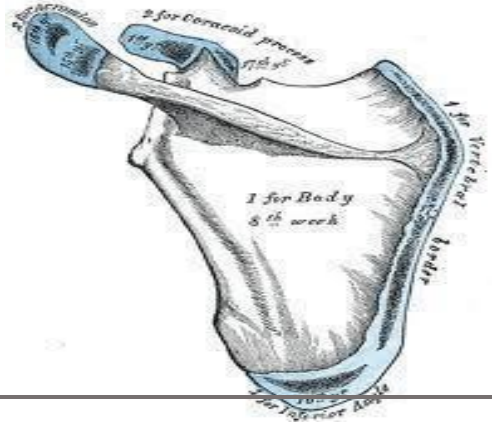

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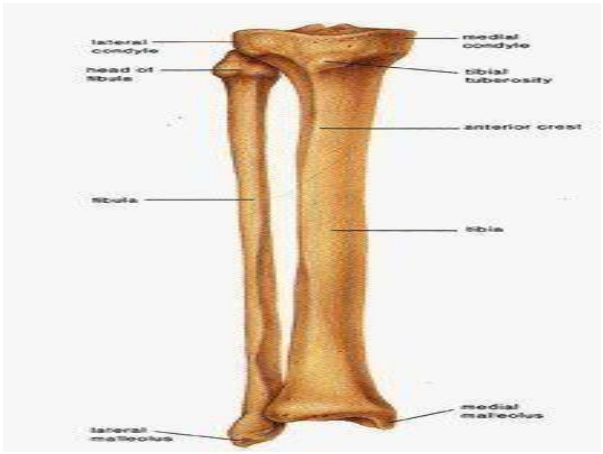

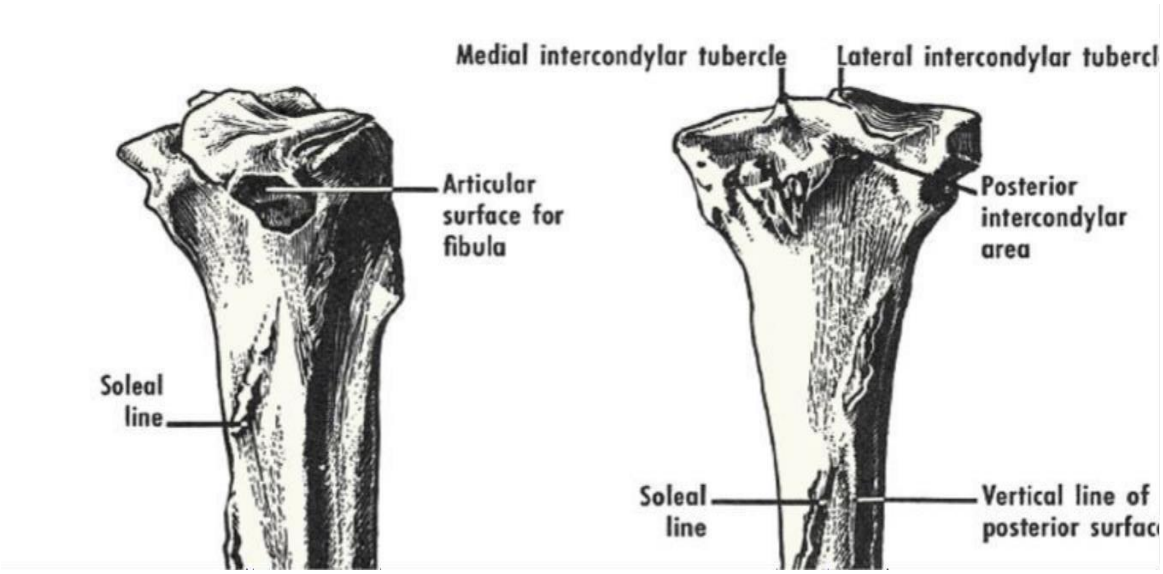

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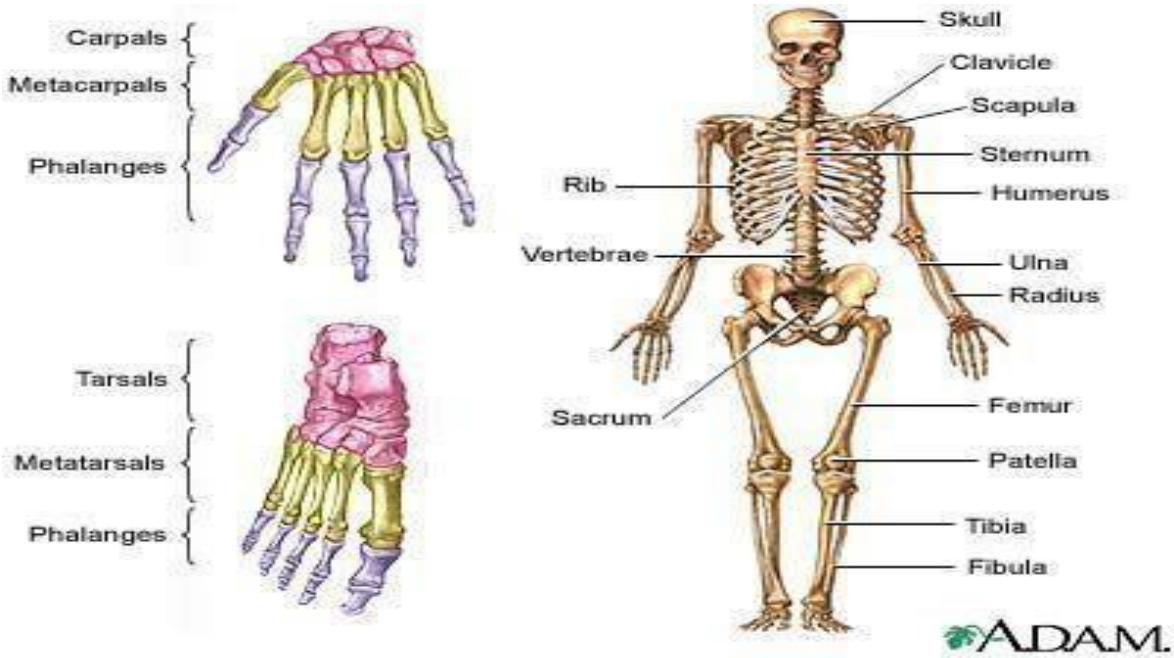
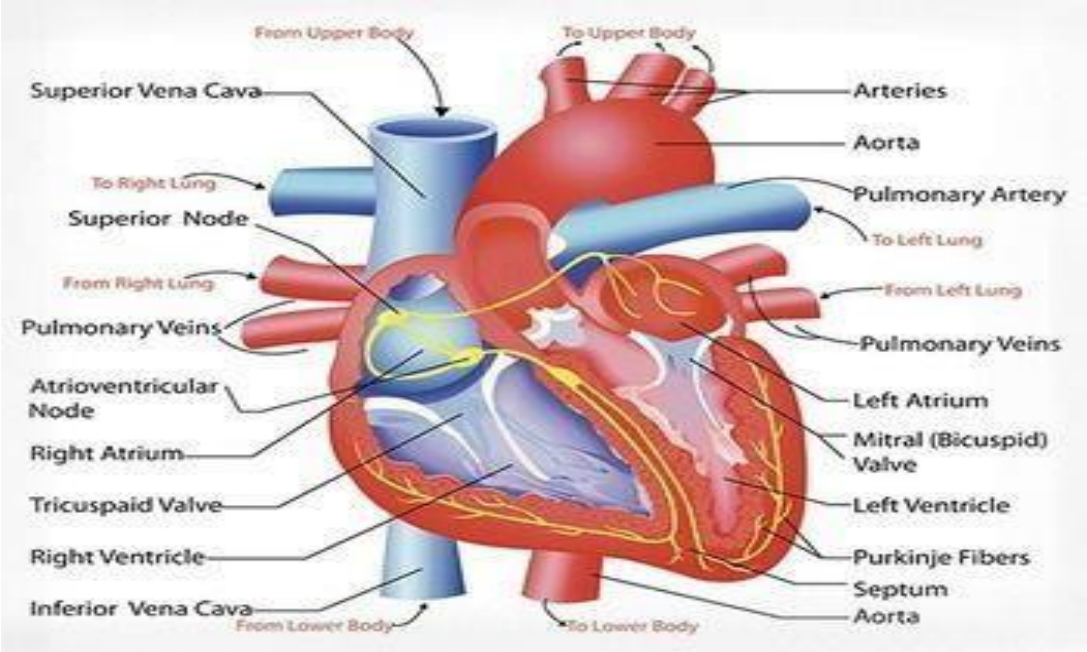


c. Cardiovascular system.



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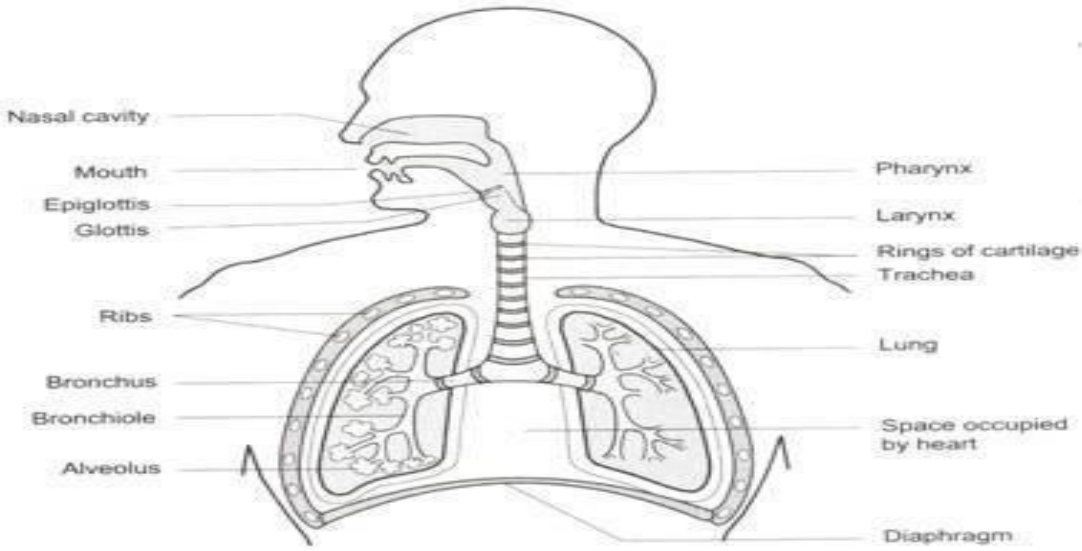
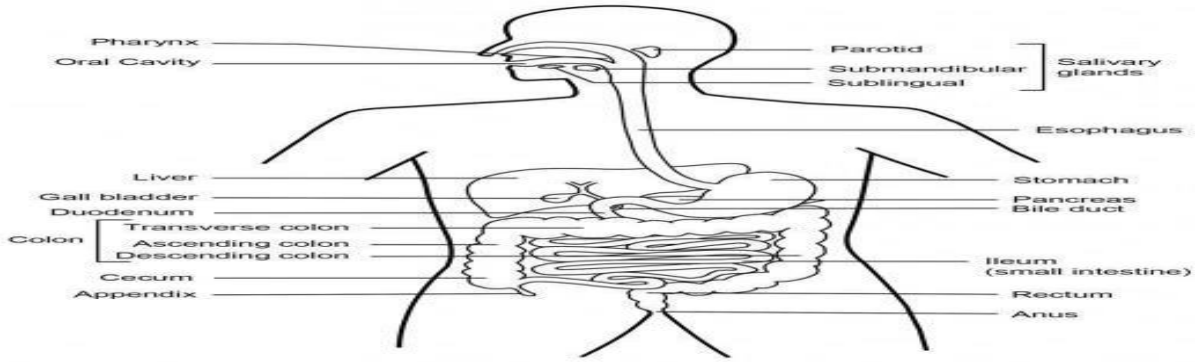
d. Respiratory system.

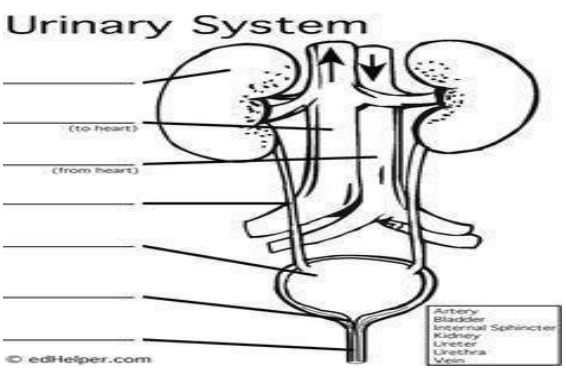


Human respiratory system

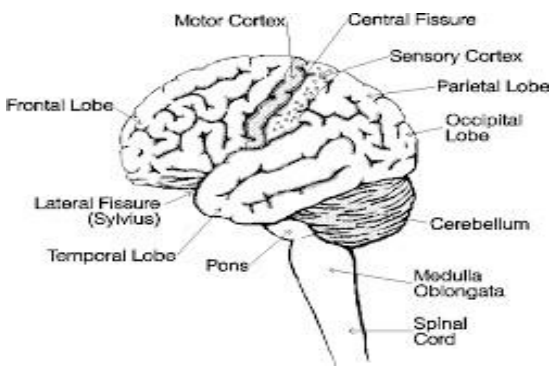
e.Digestive system



f.Urinary system



g. Nervous system



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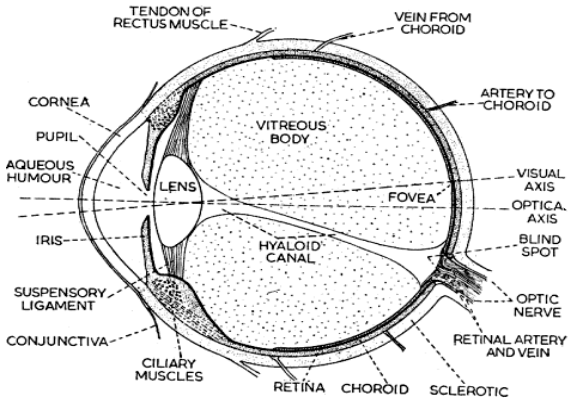
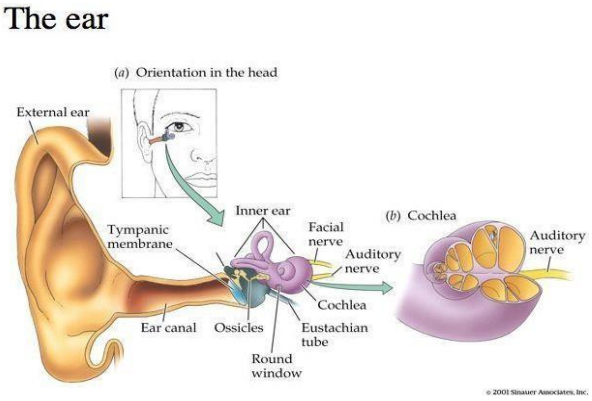
h) Special senses.

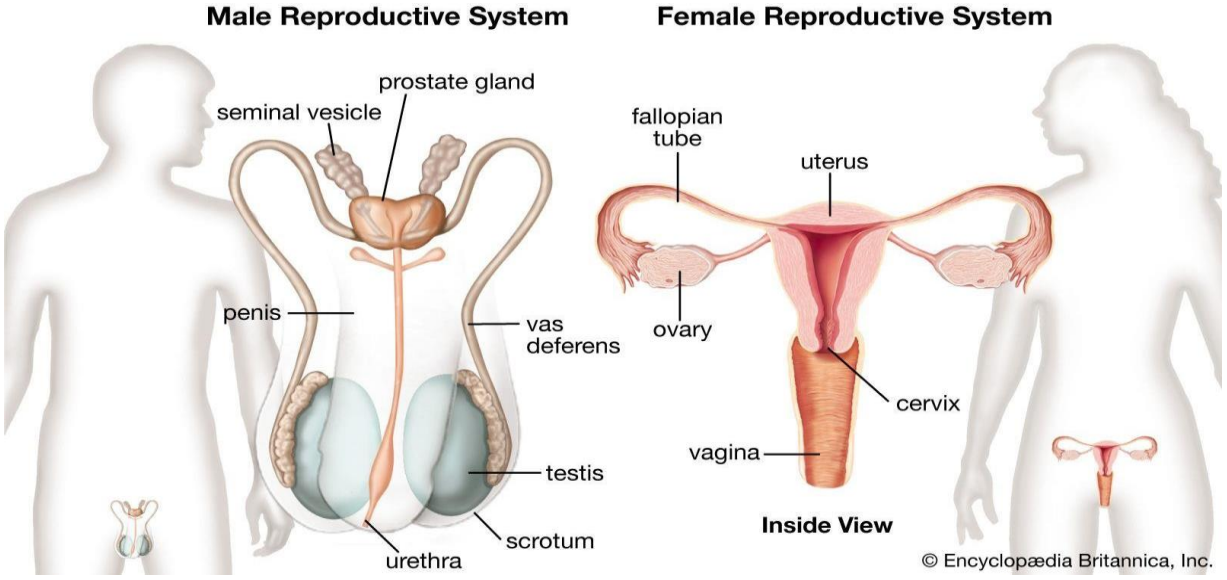
EYE



EAR



i)Reproductive system



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Experiment No 10

Study of different family planning appliances.

AIM: To study the family planning devices.

Theory:

Family planning e birth control is the process to prevent fertilization of ovum and sperm and thereby avoid pregnancy using various devices or medicines. Looking at long term effect withminimum side effects use of devices is more common over consumption of medicines,

The various family planning devices are as follows:

- Barrier devices
- Intrauterine devices (TUDs)
- Implants

I. Barrier Devices

These devices are designed to prevent access of sperms to the female reproductive tract

(a) Male Barrier Devices:

Male Condom

The condom is the most widely used device by the males. It is a non-porous elastic rubber sheath worn over the erected penis prior to start of physical intercourse which physically blocks ejaculated sperm from entering the female reproductive tract. In addition, it gives the protection against sexually transmitted diseases (STDs) like AIDS, syphilis and gonorrhoea. It is very cheap and highly effective contraceptive device.

(b) Female barriers devices:

1. Diaphragm: It is the rubber dome structure which is fitted over the cervix by the female with or without spermicidal jelly. It is made from latex, silicone, or natural rubber. It works either by blockingaccess of sperms to cervix or by killing them with spermicide.

2. Female Condom: It is also called as vaginal pouch and made up of two flexible rings connected by nitrile, latex or polyurethane sheath. One ring lying inside the sheath is inserted to fit in the cervix andother ring remains outside the vagina and covers the external genitals. Use of appropriate creams, jellies, foams, suppositories, etc. in the female before intercourse, may be combined with barriers.

3. Sponge: It is a soft foam containing spermicide which is inserted into the vagina to cover the cervix.It must be moistened with water to activate spermicide that kills sperms and prevent their entry into cervix. It should not be kept in vagina more than 30 hrs.

4. Cervical Cap: It is made up of silicone which fits over the cervix and blocks sperm fromentering the uterus through the external orifice of the uterus.

II. Intrauterine Devices (IUDs):

IUD is made up of either plastic or metal that is placed in the uterus. IUDs are of two types viz.non-hormonal copper IUDs and hormonal IUDs. Copper IUDs (E.g. Copper T) work by dsperm motility anddamaging sperms by its spermicidal action. The hormonal IUDs containprogesterone which act by blocking the endometrial proliferation making it unsuitable forfertilization and preventing the sperm penetration by increasing the viscosity of cervical mucus.

IUDs have a major advantage of long term use (6-10 years) with flexibility to remove when desired.

III. Implants: These are small tubes which are placed under the skin of upper arm. Hormones from thesetubes prevent sperms from approaching egg and inhibit release of egg.disrupting

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AIM : To perform pregnancy diagnosis test.

To study pregnancy diagnosis test. Requirements: Any one marketed single use pregnancy detection kit(which contains 1 test device and 1 disposable dropper, vial), urine sample.

Principle:

Pregnancy test helps to determine whether a woman is pregnant or not. The fertilized egg secretes the hormone called Human Chorionic Gonadotropin (HCG) which is found in urine during early pregnancy. When urine sample of pregnant women is reacted with specific HCG antibodies precipitation, haemagglutination or complement fixation like reaction occur which is used as diagnostic test for pregnancy.

These tests can be performed using immunological, biological and radiological techniques L.

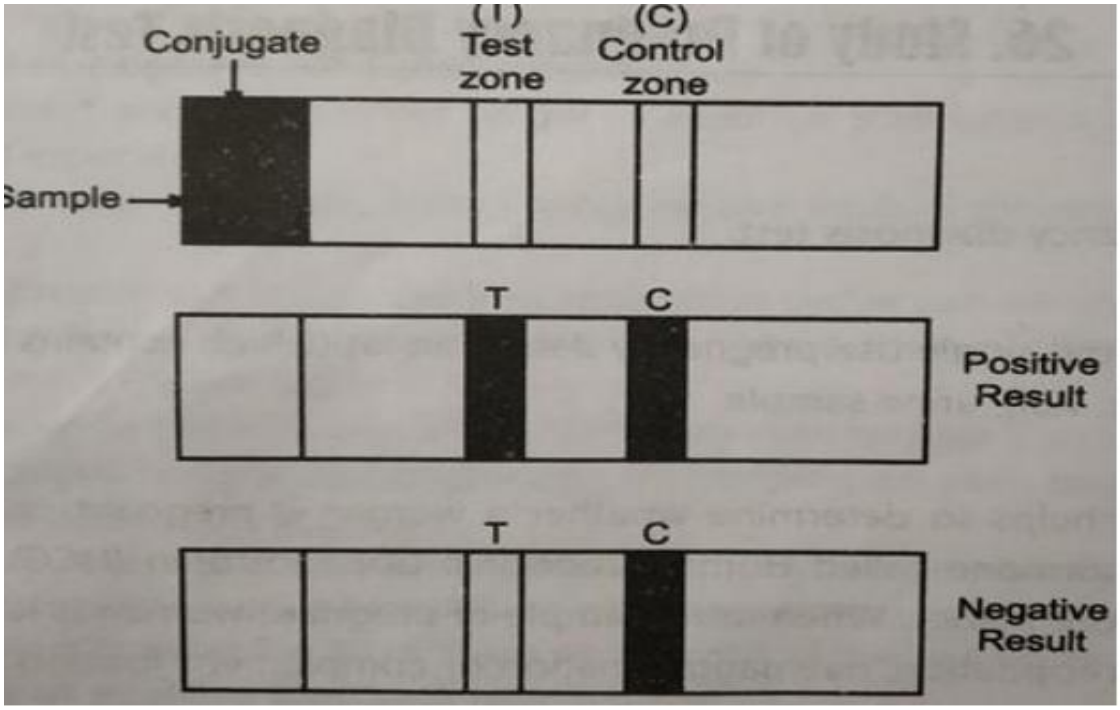
I. Immunological tests: These are commonly used and performed with the help of readily available marketed kits based on the reaction between urine HCG and specific HCG antibodies.

   Working Principle of Immunological Test: This test gives qualitative detection of HCG inthe urine. It is based on the combination of urine HCG with monoclonal antibody-dye conjugate and polyclonal HCG antibodies present on the strip of a diagnostic kit. A urine sample is applied to the test zone of the strip. If HCG is present, antibody-HCG-antibody dye complex will be formed and a pink-purple coloured band develops. A control zone is provided to check the potency of the test reagents, flow and volume of urine added.

II. Biological tests involve injection of urine sample into various animals. They are accurate buttime consuming and costly.

III. Radiological tests are performed by Radioimmunoassay (RIA) and Enzyme linked immune sorbent assay (ELISA) techniques using radio-labelled HCG and its specific antibodies.

IV. Ultrasonography is a reliable method for detecting pregnancy which uses pulses of ultrasonicwaves at high frequency on various parts of uterus. The echoes (reflected waves) are displayed on the ultra sound screen and thereby confirms pregnancy. The pregnancy can be evident as early as 5th week.



Procedure:

1. Collect the first urine sample in the morning in clean container (First few ml of urine shall bediscarded and then collected).
2. Add 2-3 drops of urine on strip and observe the colour change of the bands, if any, on the test andcontrol zones.
3. Read the results as positive (i.e. development of pink or purple colour on both control and test zone)or negative (i.e. development of pink or purple colour only on control zone).
4. Note: False positive (test is positive but the female is not pregnant) or false negative (test is negative butfemale is pregnant) result is the major limitation of this test.

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AIM : Study of appliances used in experimental physiology.

The study of appliances used in experimental physiology involves understanding various instruments and equipment essential for conducting experiments in the field of physiology. These appliances are designed to measure, monitor, manipulate, and record physiological parameters and responses in livingorganisms. Here are some key appliances commonly used in experimental physiology:

1. **Electrocardiograph (ECG)**: Used to record the electrical activity of the heart over time. It is crucial for studying cardiac function and detecting abnormalities.
2. **Electromyograph (EMG)**: Measures the electrical activity produced by skeletal muscles. It helps in studying muscle function, movement, and disorders affecting muscle control.
3. **Electroencephalograph (EEG)**: Records the electrical activity of the brain. EEG is essential for studying brain function, sleep patterns, and diagnosing neurological disorders.
4. **Blood Pressure Monitor**: Measures blood pressure, which is critical for understanding cardiovascular function and assessing the effects of drugs or interventions.
5. **Spirometer**: Measures lung volumes and capacities, providing information about respiratory function and efficiency.
6. **Microscope**: Used for examining tissues, cells, and cellular structures to study physiological processes at the microscopic level.
7. **Centrifuge**: Separates components of blood or other fluids based on density gradients, essential for studying cellular components and biochemical properties.
8. **Sphygmomanometer**: Measures blood pressure non-invasively, typically using an inflatable cuff and a pressure gauge.
9. **Thermometer**: Measures body temperature, crucial for monitoring thermoregulation and responses to heat or cold stress.
10. **Calorimeter**: Measures heat production or energy expenditure in organisms, important for studying metabolism and energy balance.
11. **Stimulators and Recorders**: Devices used to stimulate nerves or muscles and record their responses, allowing researchers to study neural and muscular physiology.
12. **Gas Analyzer**: Measures the concentration of oxygen and carbon dioxide in exhaled air, important for studying respiratory physiology and metabolic rate.

13. **Pulse Oximeter**: Measures oxygen saturation in blood, providing insights into respiratory and cardiovascular function.
14. **Perfusion Systems**: Used to maintain blood flow or fluid circulation through organs or tissues ex vivo, enabling studies on organ function and response to drugs or stimuli.
15. **Data Acquisition Systems**: Collect and process data from various sensors and instruments, allowing researchers to analyze and interpret physiological responses.

Experiment No.13

~~To record simple muscle curve using gastrocnemius sciatic nerve preparation.~~

Recording a simple muscle curve using a gastrocnemius sciatic nerve preparation typically involves stimulating the sciatic nerve and recording the resulting muscle contraction (muscle twitch). Here's abasic outline of how you can conduct and record such an experiment:

Equipment Needed:

1. **Experimental Setup:**
    - Animal preparation (anaesthetized and positioned for surgery)
    - Surgical instruments for exposing and isolating the sciatic nerve and gastrocnemius muscle
    - Suture materials for ligatures
2. **Physiological Recording Equipment:**
    - Stimulator (constant current or voltage) to stimulate the sciatic nerve
    - Electrodes (stimulating electrode for the sciatic nerve and recording electrodes for the muscle)
    - Amplifier and oscilloscope or data acquisition system to record muscle responses
    - Grounding electrodes or clips
    - Electrical isolation equipment (to ensure safety and prevent electrical interference)

Experimental Procedure:

1. **Animal Preparation:**
    - Anesthetize the animal according to ethical guidelines and institutional protocols.
    - Secure the animal in a supine or prone position.
    - Expose the sciatic nerve and gastrocnemius muscle through a surgical procedure, ensuring minimal trauma to surrounding tissues.
2. **Stimulation Setup:**
    - Place the stimulating electrode near the exposed sciatic nerve. Ensure proper placement and secure it with grounding electrodes.
    - Connect the stimulating electrode to the stimulator output.
    - Set the stimulator parameters (e.g., frequency, pulse duration, intensity) based on experimental requirements and safety limits.
3. **Recording Setup:**
    - Place recording electrodes (typically needle electrodes) into the belly of the

gastrocnemius muscle.

- Connect the recording electrodes to the input of an amplifier or data acquisition system.
- Ensure proper grounding and electrical isolation to minimize noise and artifacts.

4. **Experiment Execution:**
    - Apply a single electrical stimulus to the sciatic nerve using the stimulator.
    - Observe and record the resulting muscle twitch response on the oscilloscope or data acquisition software.
    - Adjust stimulus parameters (intensity, duration) if necessary to obtain optimal muscle responses.
    - Repeat the stimulation multiple times to obtain averaged responses and ensure reproducibility.
5. **Data Collection and Analysis:**
    - Record and analyze the muscle twitch responses, measuring parameters such as latency, amplitude (peak force), and duration of contraction.
    - Plot the muscle curve (stimulus intensity vs. muscle response) to visualize the relationship between nerve stimulation and muscle contraction.
6. **End of Experiment:**
    - After completing the experiment, euthanize the animal according to ethical guidelines and dispose of biological waste appropriately.
    - Clean and sterilize surgical instruments and experimental setup for future use.

Notes:

- **Ethical Considerations:** Ensure that all procedures comply with animal welfare regulations and ethical guidelines.
- **Safety Precautions:** Use appropriate anesthesia and handle electrical equipment safely to prevent harm to the animal and researchers.
- **Data Interpretation:** Analyze and interpret the recorded data to draw conclusions about nerve-muscle physiology and responses to stimulation.

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AIM: To record simple effect of temperature using gastroenemius sciatic nerve preparation.

To record the simple effect of temperature on the gastrocnemius sciatic nerve preparation, you can follow these general steps:

Materials Needed

- Frog or other suitable model organism
- Dissection tools (scalpel, scissors, forceps)
- Physiological saline solution
- Recording apparatus (oscilloscope or computer-based data acquisition system)
- Temperature control setup (water bath or temperature-controlled chamber)
- Muscle stimulation apparatus
- Thermometer or thermocouple
- Electrodes

Procedure

1.Preparation of the Specimen:

- Anesthetize the frog using an appropriate anesthetic.
- Pin the frog ventral side up on a dissection tray.
- Expose the sciatic nerve and the gastrocnemius muscle by carefully removing the overlying skin and connective tissue.
- Isolate the sciatic nerve and carefully cut it to free it from surrounding tissues while ensuring it's still connected to the gastrocnemius muscle.

2. Setting up the Recording Apparatus:

- Attach stimulating electrodes to the proximal end of the sciatic nerve.
- Attach recording electrodes to the gastrocnemius muscle.
- Connect the electrodes to the recording apparatus (oscilloscope or computer-based system).

3. Temperature Control:

- Prepare a temperature-controlled environment using a water bath or chamber.
- Ensure the physiological saline solution used for bathing the preparation can be temperature-controlled.
- Place the preparation in the saline solution and use a thermometer to monitor the temperature.

4. Baseline Recording:

- Record the baseline activity of the muscle at room temperature (typically around 22°C).
- Apply a standard stimulus to the sciatic nerve and record the muscle response.

5. Temperature Variation:
   - Gradually increase the temperature of the saline solution in small increments (e.g., 2-5°C).
   - Allow the preparation to equilibrate at each temperature increment before recording the muscle response.
   - Record the muscle response at each temperature increment.
6. Data Analysis:
   - Compare the amplitude, latency, and other characteristics of the muscle response at different temperatures.
   - Plot the muscle response characteristics as a function of temperature.

Points to Consider

- Ensure that the temperature changes are gradual to avoid thermal shock to the preparation.
- Maintain consistent stimulus intensity and duration throughout the experiment to ensure reliable comparisons.
- Be mindful of the possible effects of prolonged exposure to high temperatures on the viability of the preparation.

Safety and Ethical Considerations

- Follow appropriate ethical guidelines for the use of animals in research.
- Ensure all personnel involved are trained in proper handling and care of the animals.
- Dispose of biological waste according to institutional and legal guidelines.

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To record simple effect of load & after load using gastroenemius sciatic nerve preparation.

To record the simple effects of load and afterload using a gastrocnemius-sciatic nerve preparation, you can follow these steps:

Materials Needed

1. Frog or other suitable model organism
2. Dissection tools (scalpel, scissors, forceps)
3. Physiological saline solution
4. Recording apparatus (oscilloscope or computer-based data acquisition system)
5. Muscle stimulation apparatus
6. Force transducer
7. Weights (for load and afterload)
8. Electrodes
9. Thread or fine wire (for attaching weights
10. Stand and clamp (to hold the preparation in place)

Procedure

1. **Preparation of the Specimen:**
    - Anesthetize the frog using an appropriate anesthetic.
    - Pin the frog ventral side up on a dissection tray.
    - Expose the sciatic nerve and the gastrocnemius muscle by carefully removing the overlying skin and connective tissue.
    - Isolate the sciatic nerve and carefully cut it to free it from surrounding tissues while ensuring it is still connected to the gastrocnemius muscle.
2. **Setting up the Recording Apparatus:**
    - Attach stimulating electrodes to the proximal end of the sciatic nerve.
    - Attach the gastrocnemius muscle to a force transducer using thread or fine wire.
    - Connect the electrodes and the force transducer to the recording apparatus (oscilloscope or computer-based system).
3. **Baseline Recording:**
    - Record the baseline activity of the muscle without any load.
    - Apply a standard stimulus to the sciatic nerve and record the muscle response.
4. **Applying Load:**

- Attach a known weight (load) to the gastrocnemius muscle.
- Ensure the load is applied in a way that mimics the physiological conditions (e.g., the weight should pull the muscle downwards).
- Apply the same standard stimulus to the sciatic nerve and record the muscle response under load conditions.

5. **Applying Afterload:**
    - After recording the response to the initial load, increase the weight incrementally to simulate afterload conditions.
    - Record the muscle response at each incremental weight.
    - Ensure the muscle is given enough time to return to baseline tension between each afterload condition.
6. **Data Analysis:**
    - Compare the amplitude, duration, and other characteristics of the muscle response under no load, load, and various afterload conditions.
    - Plot the muscle response characteristics as a function of the applied load and afterload.

Points to Consider

- Ensure consistent stimulus intensity and duration throughout the experiment for reliable comparisons.
- Allow the muscle to rest and recover between different load and afterload conditions to avoid fatigue.
- Use physiological saline to keep the preparation moist and viable throughout the experiment.

Safety and Ethical Considerations

- Follow appropriate ethical guidelines for the use of animals in research.
- Ensure all personnel involved are trained in proper handling and care of the animals.
- Dispose of biological waste according to institutional and legal guidelines.

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AIM: To record simple fatigue curve using gastroenemius sciatic nerve preparation

1. Frog or other suitable model organism
2. Dissection tools (scalpel, scissors, forceps)
3. Physiological saline solution
4. Recording apparatus (oscilloscope or computer-based data acquisition system)
5. Muscle stimulation apparatus
6. Force transducer
7. Electrodes
8. Stand and clamp (to hold the preparation in place)

Procedure

1. Preparation of the Specimen:
    - Anesthetize the frog using an appropriate anesthetic.
    - Pin the frog ventral side up on a dissection tray.
    - Expose the sciatic nerve and the gastrocnemius muscle by carefully removing the overlying skin and connective tissue.
    - Isolate the sciatic nerve and carefully cut it to free it from surrounding tissues while ensuring it is still connected to the gastrocnemius muscle.
2. Setting up the Recording Apparatus:
    - Attach stimulating electrodes to the proximal end of the sciatic nerve.
    - Attach the gastrocnemius muscle to a force transducer using thread or fine wire.
    - Connect the electrodes and the force transducer to the recording apparatus (oscilloscope or computer-based system).
3. Baseline Recording:
    - Record the baseline activity of the muscle without any stimulation to establish a control.
    - Apply a single stimulus to the sciatic nerve and record the muscle response to ensure proper setup.
4. Fatigue Protocol:
    - Apply repetitive stimulation to the sciatic nerve at a constant frequency (e.g., 1 Hz or another appropriate frequency) for a prolonged period to induce muscle fatigue.
    - Record the muscle response continuously throughout the stimulation period.
    - Monitor and record the decline in muscle force production over time.

5. Data Analysis:

- Measure the amplitude of the muscle contractions at different time points throughout the stimulation period.
- Plot the muscle contraction amplitude against time to create a fatigue curve.
- Analyze the rate of decline in muscle force and identify any plateau or recovery phases.

Points to Consider

- Ensure the stimulus intensity and duration are consistent throughout the experiment to maintain reliable comparisons.
- Allow the muscle to rest and recover between different experimental runs to avoid cumulative fatigue effects.
- Use physiological saline to keep the preparation moist and viable throughout the experiment.
- Be mindful of the potential for muscle damage or overstimulation, and adjust the protocol accordingly if signs of excessive stress are observed.

Safety and Ethical Considerations

- Follow appropriate ethical guidelines for the use of animals in research.
- Ensure all personnel involved are trained in proper handling and care of the animals.
- Dispose of biological waste according to institutional and legal guidelines.

Vision and Mission of the Institution

Vision

The East Point College of Pharmacy aspires to be a globally acclaimed institution, **recognized** for **excellence in** pharmaceutical education, research and nurturing students for **holistic development.**

Mission

M1 Create pharmacy graduates through **quality education**

M2 Promote innovation, **creativity**, and excellence **in teaching**, learning, and **research**

M3 **Inspire** integrity, teamwork, critical thinking**, personal** development, and ethics in **students** and lay **the** foundation for lifelong learning

M4 **Serve** the **healthcare, technological, scientific**, and **economic** needs of then **society**.