THE TAMIL NADU Dr. M.G.R. MEDICAL UNIVERSITY, GUINDY, CHENNAI – 32

FIRST MBBS

ANATOMY

PRACTICAL RECORD BOOK

CERTIFICATE

CERTIFIED THAT THIS IS THE BONAFIDE ANATOMY PRACTICAL RECORD BOOK OF MS/MR._____WHO HAS UNDERGONE FIRST YEAR MBBS COURSE, DURING THE PERIOD FROM_____ TO _____. HIS/HER WORK HAS BEEN SATISFACTORY / GOOD.

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Professor & Head,

Department of Anatomy

INDEX

Serial Number	DATE	TOPIC	Page No.

Page 1 to 150….

List of Gross Anatomy diagrams to be drawn by I MBBS students

Upper Limb	**Marks**	**Thorax**	**Marks**
1.Typical spinal nerve		1.Subdivisions of mediastinum	
2.Lymphatic Drainage of breast		2.T.S. of thorax at T3 level	
3.Brachial Plexus		3.T.S. of thorax at T4 level	
4.Axillary Artery		4.Arterial supply of heart	
5.Anastomosis around the scapula		5.Venous drainage of heart	
6.Dermatomes of the upper limb		6.Medial surface of lungs	
7.Anastomosis around the Elbow		7.Bronchi and bronchopulmonary segments	
8.Flexor Retinaculum of Wrist		**Head and Neck**	
9.Fascial Spaces of Palm		1.Layers of the scalp	
10.Extensor Retinaculum of wrist		2.Vessels & nerves of the scalp	
Lower Limb		3.Muscles of facial expression	
1.Femoral sheath		4.Cutaneous innervation of head and neck	
2.Adductor canal		5.Posterior triangle	
3.Cutaneous innervation of the dorsum of foot		6.Cavernous sinus	
4.Superior surface of the tibia		7.Superior orbital fissure and common tendinous ring	
5.Anastomosis around the knee		8.Ciliary ganglion and its connections	
Abdomen		9.Carotid triangle - boundaries	
1.Regions of abdomen		10.Carotid arteries and nerves in the neck	
2.Superficial veins of anterior abdominal wall		11.Structures related to hyoglossus	
3.Lymphatic drainage of skin of abdominal wall		12.Nasal septum	
4.Rectus sheath		13.Arteries & nerves of nasal cavity	
5.Lesser sac		**CNS**	
6.Blood supply, Nerves supply of stomach		1.Internal structure of spinal cord showing position of tracts	
7.Lymphatic drainage of stomach		2.Interpeduncular fossa and attachment of cranial nerves to the anterior surface of the brain stem	
8.Thoracolumbar fascia		3.Arteries on the base of the brain	
9.Anterior & posterior relations of kidney		4.Floor of fourth ventricle	
10.Portosystemic anastomosis		5.Functional areas of cerebrum	
11.Peritoneal reflections in male		6.Blood supply of cerebrum	
12.Peritoneal reflections in female			

List of Histology slides to be drawn by I MBBS students

General Histology

Epithelial Tissue	**Marks**	**Blood vessels**	**Marks**
1 - Simple squamous epithelium		21- Elastic artery	
2- Simple cuboidal epithelium		22- Medium sized artery and vein	
3- Simple columnar epithelium		23- Large vein	
4- Pseudostratified ciliated columnar epithelium		**Glands**	
5- Stratified squamous epithelium		24- Unicellular gland (goblet cell)	
6- Transitional epithelium		25- Simple tubular gland	
Connective Tissue		26- Serous gland	
7- Areolar tissue		27- Mucous gland	
8- Adipose tissue		28- Mixed gland	
9- Tendon L.S		29-Sebaceous and sweat gland	
Cartilage		30 -Mammary gland	
10- Hyaline cartilage		**Lymphoid Tissue**	
11- Elastic cartilage		31- Lymph node	
12- Fibro cartilage		32- Spleen	
Bone		33- Thymus	
13- Compact bone L.S.		34- Tonsil	
14- Compact bone C.S.		**Nervous Tissue**	
15- Spongy bone		35- Nerve C.S	
16- Developing bone		36-Nerve L.S (osmicated)	
Muscle		37- Spinal ganglion	
17-Skeletal muscle L.S.		38- Sympathetic ganglion	
18-Skeletal muscle C.S.		**Integumentary System**	
19- Smooth muscle		39- Hairy skin	
20- Cardiac muscle		40- Non-hairy skin	
		41- Nail	

Special Histology

Gastrointestinal system	**Marks**		**Marks**
42- Oesophagus		75- Cornea	
43- Cardio-oesophageal junction		76- Irido-corneal junction	
44- Stomach - fundus		77- Retina	
45- Stomach - pylorus		78- Optic nerve	
46- Duodenum		79- Macula	
47- Jejunum		80- Crista	
48- Ileum		81- Organ of Corti	
49- Large intestine		82- Pituitary gland	
50- Appendix		83- Pineal gland	
51- Liver		84- Thyroid gland	
52- Gall bladder		85- Parathyroid gland	
53- Pancreas		86- Tongue – fungiform and filiform papillae	
Genitourinary system		87- Tongue – circumvallate papilla	
54- Kidney		88- Epiglottis	
55- Ureter		89- Olfactory epithelium	
56- Urinary bladder		**CNS**	
57-Adrenal gland		90- Motor end plate	
58-Testis		91- Muscle spindle	
59-Epididymis		92- Meissner's corpuscle and Pacinian corpuscle	
60-Vas deferens		93-Spinal cord - cervical	
61-Seminal vesicle		94-Spinal cord - Thoracic	
62-Prostate		95-Spinal cord - Lumbar	
63-Ovary		96-Spinal cord - Sacral	
64-Fallopian tube		97- Medulla - Motor Decussation	
65-Uterus		98- Medulla - Sensory Decussation	
66-Cervix		99- Medulla – Mid-olivary Level	
67-Placenta		100- Pons – Lower pons	
68-Umbilical cord		101- Pons - Upper pons	
Respiratory System		102- Midbrain – Inferior colliculus	
69-Trachea		103- Midbrain - Superior colliculus	
70- Lung		104- Cerebrum – Typical cortex	
Head & Neck		105- Cerebellum	
71- Eyelid			
72- Lip			
73- Adult tooth			
74- Developing tooth –Bell stage			

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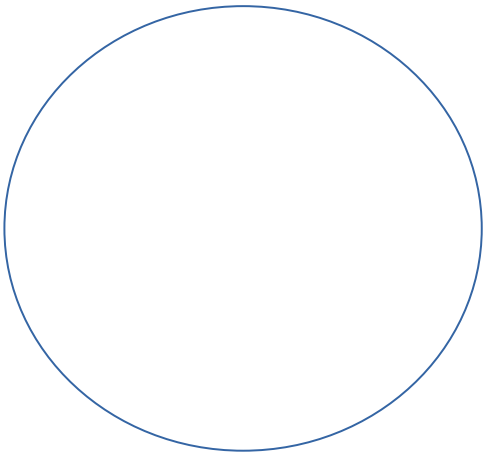

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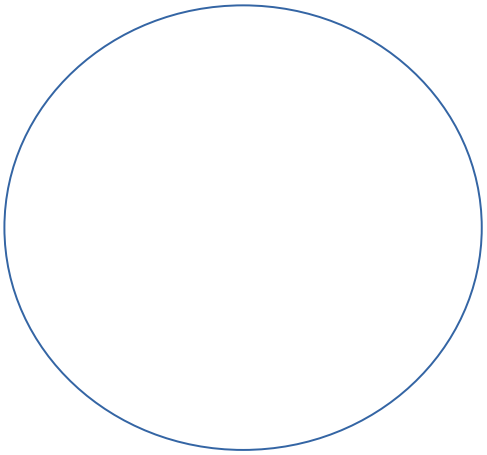

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ROLL NO :

INDEX

EXPT. NO.	DATE	NAME OF THE EXPERIMENT HAEMATOLOGY	SIGNATURE OF THE MENTOR
		MICROSCOPE	
		HEMOCYTOMETER	
		ESTIMATION OF TOTAL RBC COUNT	
		ESTIMATION OF TOTAL WBC COUNT	
		ABSOLUTE EOSINOPHIL COUNT	
		DIFFERENTIAL COUNT	
		HEMOGLOBIN ESTIMATION	
		BLOOD GROUPING & TYPING	
		BLEEDING TIME & CLOTTING TIME	
		ERYTHROCYTE SEDIMENTATION RATE	
		PACKED CELL VOLUME	
		OSMOTIC FRAGILITY	
		SPECIFIC GRAVITY	

NAME : **YEAR OF STUDY :**

ROLL NO :

INDEX

EXPT. NO.	DATE	NAME OF THE EXPERIMENT HAEMATOLOGY	SIGNATURE OF THE MENTOR
		GENERAL EXAMINATION	
		EXAMINATION OF RS	
		RESPIRATORY EFFICIENCY TEST	
		SPIROMETRY	
		STETHOGRAPH	
		EXAMINATION OF PULSE	
		EXAMINATION OF CVS	
		EXAMINATION OF BP	
		EFFECT OF POSTURE & EXERCISE ON BP	
		EXAMINATION OF SENSORY SYSTEM	
		EXAMINATION OF MOTOR SYSTEM	
		EXAMINATION OF REFLEXES	
		CEREBELLAR FUNCTION TEST	
		EXAMINATION OF 1-6 CRANIAL NERVES	
		EXAMINATION OF 7-12 CRANIAL NERVES	

Haematology

Experiment No. **MICROSCOPE** **Date**

A microscope is an optical instrument which magnifies the image of an object. There are various types of microscope which use different types of lens and different principles of optics. Compound microscope is one of the most frequently used equipments in a medical laboratory.

Physical terms:

1. Resolution

It is the ability to reveal closely adjacent structural details as separate and distinct. The limit of magnification of a microscope is set by its resolving power.

2. Numerical aperture

It is the ratio of the diameter of the lens to its focal length. Greater the numerical aperture greater the resolving power.

3. Working distance

It is the distance between the objective and the slide.

PARTS OF THE COMPOUND MICROSCOPE

I. SUPPORT SYSTEM

1. Base

It supports the microscope on the working table.

2. Pillars

Two upright pillars project upwards from the base.

3. Handle

Handle is hinged to the pillars. It supports the magnifying and adjusting systems. It is the handle by which the microscope must be carried. It is curved and the microscope can be tilted at the hinged joint.

4. Body tube

The eyepiece fits into the top of the body tube. The nose piece with the objective lenses fits into its lower end. It is the part through which the light passes to the eyepiece. It actually conducts the image.

5.Stage

Fixed stage is the horizontal platform on which the object is placed. It has a central opening through which the illuminating system focuses the light on the object. Mechanical stage has a spring mounted clip to hold the slide or counting chamber in position. It has two screws to move the mounted object from side to side and for wards and backwards.

6. Nose piece

Fixed nose piece is attached to lower end of body tube. Revolving nose piece carries objective lenses of different magnifying powers.

II. ADJUSTING SYSTEM

It consists of the coarse and fine adjustment screws mounted in the handle by a double sided micrometer mechanism.

1. Coarse adjustment screws

It consists of rack and pinion which moves the tube rapidly through a large distance when the screw is rotated clockwise or anticlockwise. It is used to obtain an approximate focus of the object.

2. Fine adjustment screws

Similar to coarse adjustment screw, but several rotations will move the tube through a very small distance. It is used to obtain exact focus of the object.

III. ILLUMINATION SYSTEM

1. Source of illumination

Light source may be internal or external.

Internal source –In modern microscopes, there is an in-built light source with an electrical tungsten lamp, which is placed directly under the stage.

External source – This can be from an electric lamp housed in a lamp box with a window or from the sun. The rays of light are reflected by a mirror towards the object. The mirror is located at the base of the microscope which are plane and concave.

2. Condenser

It focuses the rays of light reflected from the mirror onto the object under observation and helps in resolving the image. It is mounted below the stage of the microscope. Position of the condenser has to be adjusted according to the objective lens used.

3. Iris diaphragm

It is located at the bottom of the condenser. It has a central aperture. The size of the aperture can be altered to regulate the amount of light that passes through the con denser onto the object under observation.

IV. MAGNIFICATION SYSTEM

1. Eye piece

This is a magnifying lens inserted into the upper end of the body tube. Each eyepiece has two lenses, an eye lens mounted at the top and a field lens at the bottom. I t has a magnification power of 5and 10. It magnifies the primary image to give a virtual image which is observed through the eye piece.

2. Objective lens

Three objective lenses are fitted to the lower end of the body tube in the revolving nose piece. They are the low power, high power and oil immersion objective lenses. The desired objective lens is placed close to object on the stage and it produces a real magnified and inverted primary image. When the oil immersion objective is used, the space between the object and the lens is filled with cedar wood oil which has the same refractive index as that of glass and hence prevents refraction of light.

Objective	Working distance	Numerical aperture	Magnification
Low power	5 - 15 mm	0.3	10
High power	0.5 - 4 mm	0.65	40/45
Oil immersion	0.15 – 1.5 mm	1.3	100

Adjustments for low power objective

1.Concave mirror is used.
2.Condenser is lowered.
3.Iris diaphragm is slightly opened to decrease the intensity of illumination.

Adjustments for high power objective

1.Concave mirror is used.
2.Condenser is slightly raised.
3.Iris diaphragm is partially opened to increase the intensity of illumination.

Adjustments for oil immersion objective (OPR)

1.Open the Iris diaphragm fully to get maximum intensity of illumination.
2.Plane mirror is used.
3.Raise the Condenser.

Precautions

1.Objectives and eyepiece should be free from dust.
2.The mirror, the position of the condenser, and the aperture of the iris should be checked in order to get proper illumination.
3.While changing the objective it should be noted that the objective clicks into its proper position.
4.Do the necessary microscopic adjustments before using each objective.
5.While focusing, lower the objective close to the slide and focus the object by slowly raising the objective.
6.Never bring down the objective with the coarse adjustment screw while looking into the microscope.
7.Examine the slide under low power and high power before examining it under oil immersion objective.
8.After using oil immersion objective, clean the lens with filter paper and xylol.

QUESTIONS

1. Name the oils used for oil immersion objective.
2. How will you calculate the total magnification power of the microscope for each objective?
3. Name the other types of microscope.

Experiment No.	HEMOCYTOMETER	Date

The formed elements of blood are counted by Hemocytometry. The apparatus is called as hemocytometer. It consists of diluting pipettes and counting chamber.

The counting chamber in common use is the improved Neubauer's counting chamber. This is a thick glass slide divided into two central platforms by a 'H' shape d groove. The central platform is slightly lower than the sides. When a cover slip is pl aced over the central platforms, resting on the side platforms, a space of 1/10 mm dept h will be present between the cover slip and the central platform. This area is used f or charging the chamber with the diluted blood for cell counting.

The central platforms have ruled squares which are used for cell counting. The ruled area is a square measuring 3mm X 3mm. This area is divided into 9 large equal squares each having an area of $1mm^2$. The four large corner squares are used for W BC count. The central square is used for RBC count. All nine squares are used for Absolute eosinophil count.

WBC counting squares

1. The four large corner squares are used for the WBC count and each has 16 medium squares (16X4=64 medium squares).
2. Side of each large square is 1 mm.
3. Area of each large square is 1 x 1 = 1 sq.mm.
4. Volume of each large square = area x depth = 1 x 1/10 = 1/10 cu.mm.
5. Volume of each medium square = 1/4 x 1/4 x 1/10 = 1/160 cu.mm.

RBC counting squares

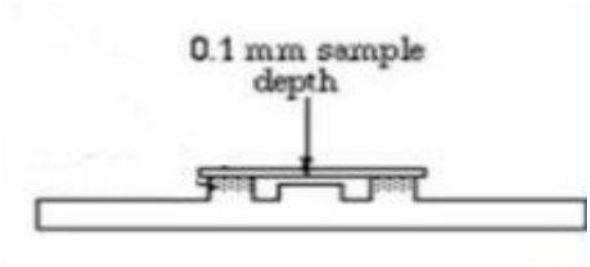
1. The 1mm2 central RBC square is divided into 25 medium sized squares by triple lines. The four corner and central medium sized squares are used for RBC count.
2. Each medium sized square is further divided into 16 small squares. (5 x 16 = 80 small squares).
3. Side of each medium sized square is 1/5 mm.
4. Area of each medium sized square is 1/5 x 1/5 = 1/25 sq.mm.
5. Volume of each medium sized square is 1/250 cu.mm.
6. Volume of each smallest square = 1/20 x 1/20 x 1/10 = 1/4000 cu.mm.

Pipettes

The pipettes are used to dilute the blood to a known dilution.
Two types of pipettes are used – RBC pipette, WBC pipette .

NEUBAUER CHAMBER

NEUBAUER CHAMBER - SIDE VIEW



RBC & WBC PIPETTE

The parts of a pipette are

The stem:

The long narrow stem has a capillary bore and a well-grounded conical tip. It is divided into 10 equal parts with two numbers etched on it – 0.5 in the middle and 1.0 at the junction of stem and the bulb.

The bulb:

The bulb contains a free-rolling bead. The bead helps in identifying the pipette and mixing the diluents with blood in the bulb. Free rolling of the bead in the bulb indicates whether the pipette is dry or not.

Rubber tube and mouthpiece:

The narrow rubber tube attached to the bulb, facilitates filling of the pipette by gentle suction. There is a marking just above the bulb. This marking is 11 in WB C pipette and 101 in RBC pipette. The graduations do not indicate absolute or definite amounts in terms of cubic mm .They only indicate relative volumes in relation to each other. The markings indicate relative parts in the pipette.

RBC pipette:

1. Markings are 0.5, 1.0 and 101
2. The capillary bore is narrow
3. Bulb is larger and has a red bead
4. Volume of the bulb is 100 parts

WBC pipette:

1. Markings are 0.5, 1.0 and 11
2. The capillary bore is wider
3. Bulb is smaller and has a white bead
4. Volume of the bulb is 10 parts

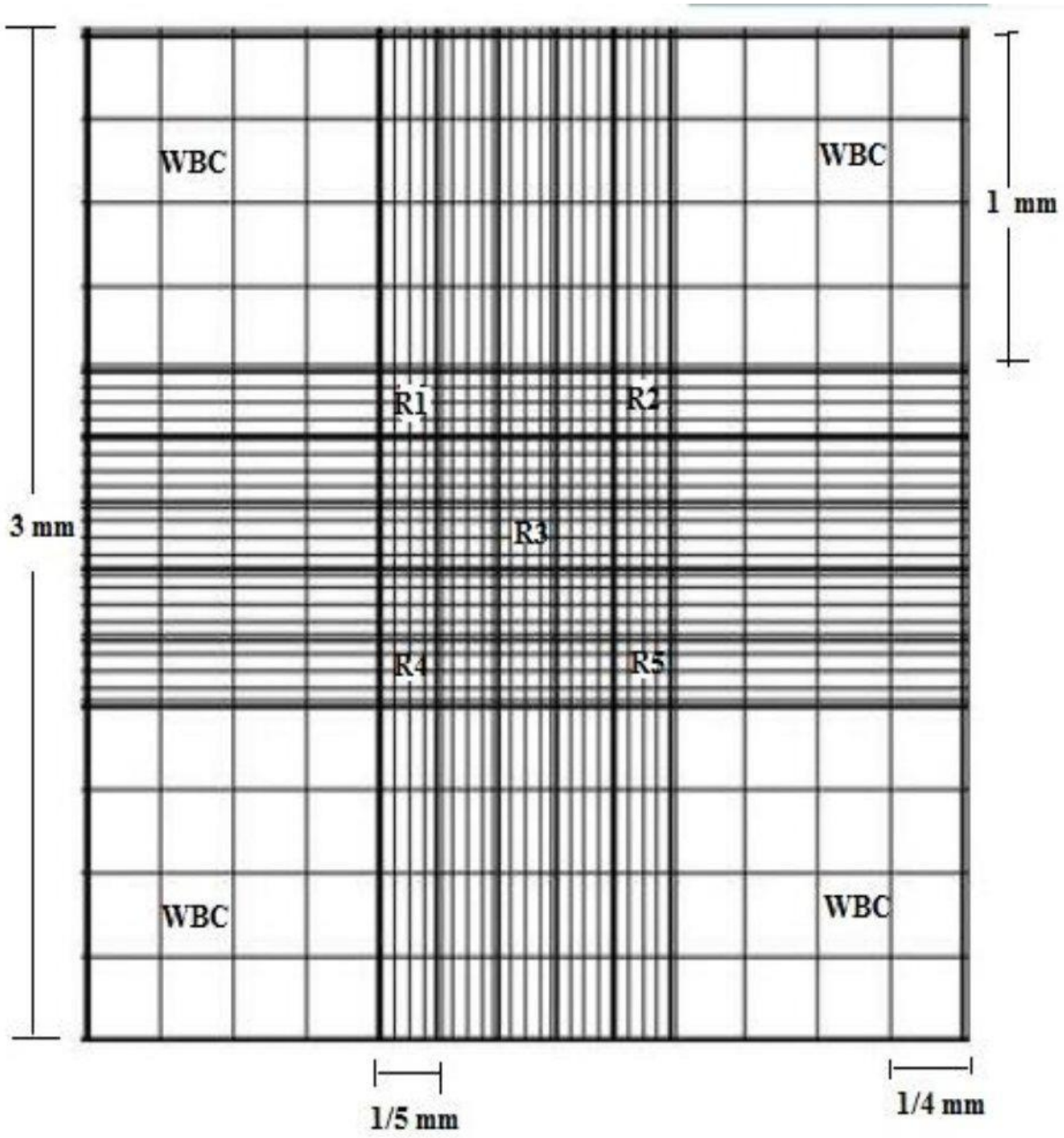
FINGER PRICK

1. Clean the tip of the finger with spirit and allow the area to dry.
2. Prick the tip of finger with the lancet, deep enough to get a good drop of blood.
3. Don't squeeze the finger pulp after pricking as this leads to the seepage of tissue fluid resulting in dilution of the blood.
4. The prick is usually made on middle or ring finger.

FILLING THE PIPETTE

1. Under aseptic precautions prick the finger and wipe away the first drop and allow the flowing blood to form a good sized drop.
2. Hold the pipette horizontally and dip its end into the blood drop. Gently suck blood upto 0.5 or 1.0 mark depending on the dilution required.

NEUBAUER CHAMBER - COUNTING GRID



3. If the blood overshoots 0.5 or 1.0 mark, remove the excess blood by gently tapping the tip of the pipette on to the palm. Do not use cotton or any absorbent material as it might absorb water content of blood and concentrates blood.
4. Place the tip of the pipette into the diluting fluid and suck upto the 11 mark in case of WBC pipette or 101 mark in case of RBC pipette without any air bubble.
5. Place the pipette horizontally between the palms of both hands with the rubber tube folded parallel to it and roll the pipette for 1-2 minutes, for thorough mixing of blood with the fluid in the bulb.

Precautions:

1. The pipette must be dry and free from clotted blood and the bead must roll freely in the bulb.
2. The tip must not press against the finger or be lifted out of the blood drop or else air will enter it.
3. The blood must be diluted immediately, or else it may clot.
4. Always hold the pipette horizontally to avoid leakage of fluid from the pipette while mixing.

FOCUSSING THE COUNTING GRID:

Focus the counting grid with low power and then high power objective. The lines of the squares must be seen clearly.

CHARGING THE CHAMBER:

1. Place the coverslip on the central platform of the chamber covering the ruled squares.
2. Discard the stem fluid before charging the chamber as it contains only the diluting fluid.
3. Form a good drop of diluted blood at the tip of the pipette, by squeezing the rubber tube, while closing its mouthpiece or by gently blowing through the rubber tube.
4. Hold the pipette at 45 degree inclination and touch the chamber with the tip of the pipette between the cover slip and the central platform.
5. A thin layer of the fluid spreads under the coverslip on the central platform by capillary action.
6. Avoid overcharging the chamber which is recognized by fluid in the trenches.
7. Wait for 2 minutes for the cells to settle down.
8. Focus the squares under the desired objective and start counting.

Precautions:

1. The chamber and the coverslip should be properly cleaned.
2. The contents of the bulb must be thoroughly mixed before charging.
3. 2-3 drops of fluid must be discarded from the pipette before charging as the stem contains only diluents.
4. Air bubbles should not enter the platform of the chamber while charging.
5. The chamber should not be overcharged (gives false low results) or undercharged (the cells may not be found in peripheral squares).

CELL COUNTING:

1. Count the cells in the respective squares.
2. Care should be taken not to count the same cells again by following L rule. (Count the cells present inside the square and those on the left and lower lines. Ignore those on the right and upper lines).

QUESTIONS.

1. What are the other types of cell counting chambers ?
2. What are the other cells that can be counted using Neubauer's chamber?
3. Mention the differences between RBC and WBC pipettes.

Experiment No.	RBC COUNT	Date

Aim: To enumerate the number of erythrocytes in 1 cubic mm of blood.

Apparatus required: Microscope, Hemocytometer (RBC diluting pipette and counting chamber), RBC diluting fluid (Hayem's fluid), spirit, cotton and lancet.

Hayem's fluid - Composition and functions

Sodium chloride - 0.5 g - Maintains isotonicity
Sodium bi sulphate - 2.5 g - Prevents aggregation of RBCs (Rouleaux formation)
Mercuric per chloride - 0.25 g - Acts as preservative, antifungal and antibacterial
Distilled water - 100 ml - Acts as solvent

Procedure: Make a sterile finger prick and discard the first drop of blood. Draw blood upto 0.5 mark and Hayem's fluid upto 101 mark with the pipette. Mix the contents thoroughly. Discard the first few drops and then charge the Neubauer chamber. Allow the cells to settle for 3-4 minutes.Count the RBCs in the 4 medium sized corner squares and in the central medium sized square of the RBC counting area (total of 16 x 5 = 80 smallest squares) under high power objective.

Calculation:

Number of RBCs in 5 medium sized RBC squares = n
Area of 1 medium sized RBC square =1/5 × 1/5 = 1/ 25 sq.mm
Volume of 1 medium sized RBC square = 1/25 × 1/10 = 1/250 cu.mm
Volume of 5 medium sized RBC squares = 1/250 × 5= 1/50 cu.mm
Number of cells in 1/50 cu.mm of diluted blood = n
Number of cells in 1 cu.mm of diluted blood = 50 n
Dilution factor = 1 : 200
Number of cells in 1 cu.mm of un diluted blood = n × 50 × 200
= n × 10000

Result :

RBC count in the given blood sample is _____ cells / cu.mm

QUESTIONS

1. Name the other diluting fluids used for red cell count.
2. How will you identify the RBC counting squares?
3. What is the normal RBC count in males and females?
4. Why is the RBC count high in males?
5. Mention the physiological and pathological causes for anemia and polycythemia?

Observation:

Calculation

Result:

Experiment No.	**TOTAL LEUCOCYTE COUNT**	**Date**

AIM:

To enumerate the number of leucocytes (white blood cells) in 1 cubic millimetre of blood.

APPARATUS REQUIRED:

Microscope, Hemocytometer, WBC pipette, Turk's fluid, Spirit, Cotton, Lancet

Turk's fluid : Composition and function:

1% Glacial Acetic Acid	-	1.5 ml -Lyses RBCs without affecting WBCs
Gentian Violet	-	1.5 ml -Stains nuclei of WBCs
Distilled Water	-	100 ml -Acts as solvent

PROCEDURE:

Make a sterile finger prick and discard the 1st drop of blood. Draw blood upto 0.5 mark and Turk's fluid upto 11 mark in the WBC pipette. Mix the contents thoroughly. Discard the first few drops and charge the Neubauer chamber. Allow the cells to settle for 3 to 4 minutes. Count the WBCs in the 4 corner large squares (WBC counting area) under high power objective.

CALCULATION:

Number of cells in 4 WBC squares			=	**n**
Area of 1 WBC square	=	1× 1	=	1 mm^2
Volume of 1 WBC square	=	1× 1 / 10	=	1 /10 mm^3
Volume of 4 WBC squares	=	4× 1 / 10	=	4 / 10 mm^3
Number of cells in 4/10 mm^3 of Diluted blood			=	**n**
Therefore, Number of cells in 1 mm^3 of Diluted blood			=	n × 10 / 4
Dilution Factor			=	1 **:** 20
Therefore, Number of cells in 1 mm^3 of Undiluted blood			=	n × 10 / 4 × 20
			=	n × 50

RESULT:

Total WBC count in the given blood sample = _____ cells/ cu.mm

QUESTIONS:

1. What is the normal RBC **:** WBC ratio ?
2. In which condition is RBC pipette used for counting WBCs ?
3. Why is blood diluted only 20 times in WBC counting?
4. Mention the physiological and pathological causes of high and low WBC count.
5. What is Leucocytosis ?
6. What is Leukemia?

OBSERVATION :

CALCULATION :

Experiment No.	ABSOLUTE EOSINOPHIL COUNT	Date

AIM:

To determine the number of eosinophils per cu mm of blood

APPARATUS REQUIRED:

Microscope, Hemocytometer, Dunger's fluid, spirit, cotton and lancet.

Composition and functions of the diluting fluid (Dunger's Fluid)

1% of solution of eosin in water (5ml)- Eosin stains the eosinophilic granules.

Acetone (5ml) - Acetone lyses the cell membrane of all other cells.

Distilled water (90ml) - Distilled water to make up to 100ml.Acts as solvent.

Procedure :

Make a sterile finger prick and discard the first drop of blood. Draw blood upto 1 and the Dunger's fluid upto mark 11 in a WBC pipette. Mix the contents thoroughly. Cover the pipette with a petri dish lined by moistened filter paper. Wait for 15 minutes. Discard the first few drops and charge the Neubauer chamber. The eosinophils are identified by the pinkish orange stained coarse granules in the cytoplasm. Count the Eosinophils in all the 9 large squares of the Neubauer chamber. Count within 30 minutes of charging .

Calculation:

Number of cells counted in 9 large squares = n

Area of 1 large square = 1 x1 = $1mm^2$

Volume of 1 large square = 1 x 1/ 10 = $1 / 10mm^3$

Volume of 9 large squares = 9 x 1 / 10 = $9/10mm^3$

Number of cells in $9/10mm^3$ of diluted blood = n

Number of cells in $1mm^3$ of diluted blood = nx10 /9

Dilution factor = 1:10

Therefore, number of cells in $1mm^3$ of undiluted blood = nx10/9 x10

= nx100/9

RESULT:

Number of eosinophils in the blood is = ———————— cells/cu.mm.

Questions:

1. What is the normal value of Absolute Eosinophil Count ?
2. What is the difference between the Differential Count and the Absolute Eosinophil Count ?
3. What are the other diluting fluids used for Absolute Eosinophil Count?
4. What are the contents of eosinophilic granules ?
5. What are the functions of eosinophils?
6. What are eosinopenia and eosinophilia?

Experiment No.	DIFFERENTIAL COUNT	Date

Aim:

To determine the differential count of White Blood Cells.

Apparatus required:

Grease free and dry glass slides, Leishman's stain, distilled water, lancet, spirit, cotton.

Leishman's stain – Composition and Functions:

Methylene blue (basic) - Stains acidic granules in cytoplasm especially granules of basophils and Nuclei of leucocytes.

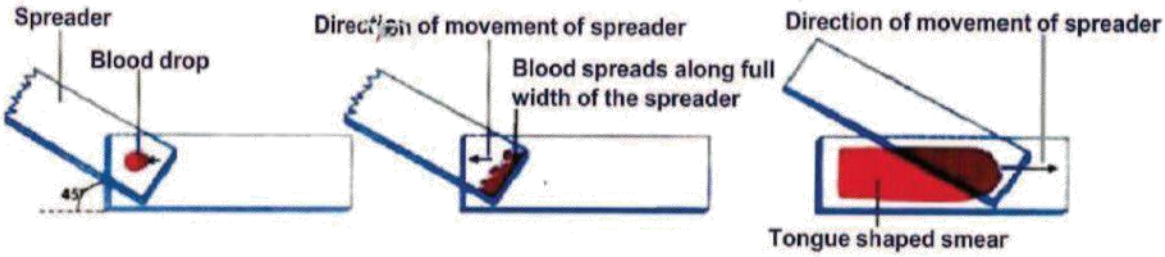
Eosin (acidic) - Stains cytoplasm, basic granules in cytoplasm, Hemoglobin of RBCs.

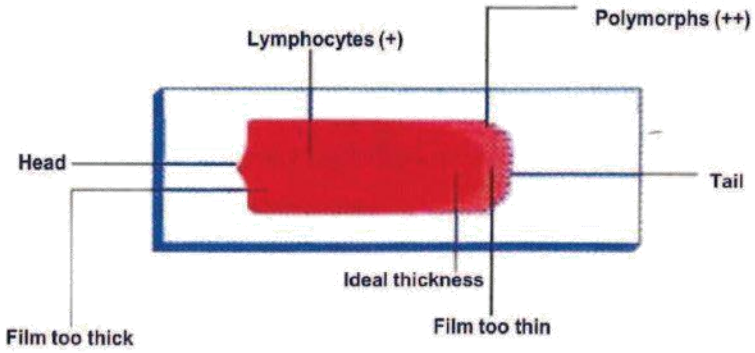
Acetone free methyl alcohol - Fixes the cells (Acetone free methyl alcohol is used as acetone is a lipid solvent that lyses cell membrane).

Procedure:

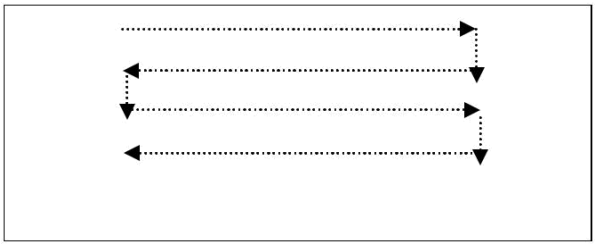
Under aseptic precautions, prick the finger. Discard the first drop of blood. Place the slide on the table and support with left hand. Place the blood drop on the right end, one cm away from the edge. Place the spreader slide just infront of the blood drop. Draw the spreader slide backwards to touch the drop. The blood spreads across the edge of the spreader. Draw the spreader slide forward at an angle of 45° with a smooth, fast and firm movement to make a thin tongue shaped blood smear. Too thick, thin or a patchy smear is to be avoided. Air dry the smear quickly.

Place the glass slide with the smear on a tray and add Leishman's stain, drop by drop till the entire smear is covered with the stain. Count the number of drops added. Note the time and wait for 2 minutes **(Fixation time)**. After 2 minutes, add double the quantity of distilled water over the film using a dropper. See to that the distilled water uniformly covers the entire surface of the slide and dilutes the stain homogenously. Gently blow the stain and the distilled water from one end of the slide to the other for uniform mixing. Wait for about 8-10 minutes for the smear to take up the stain uniformly **(Staining time)**.





Making an ideal blood smear



Flush the slide under a gentle stream of tap water to remove the excess stain. Dry the slide. Scan the film under low & high power objective. Make necessary microscopic adjustments for oil immersion objective (100X). Add a drop of cedar wood oil over the smear at the junction between the body and the tail, as the smear will be of one cell thickness with uniform staining here. Cedar wood oil has the same refractive index as that of glass and minimizes refraction. Examine in a zig-zag manner as shown in the figure.

Draw a table with 100 squares to count 100 WBCs and enter the type of cell as identified while examining the film.

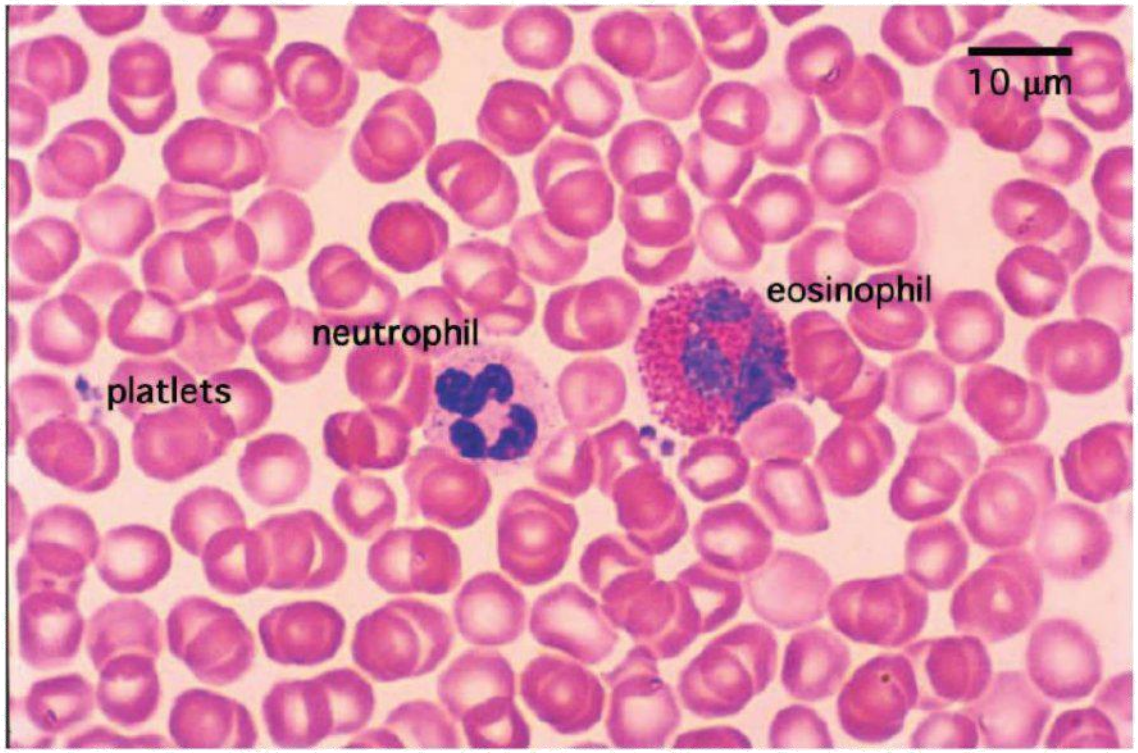
Result:

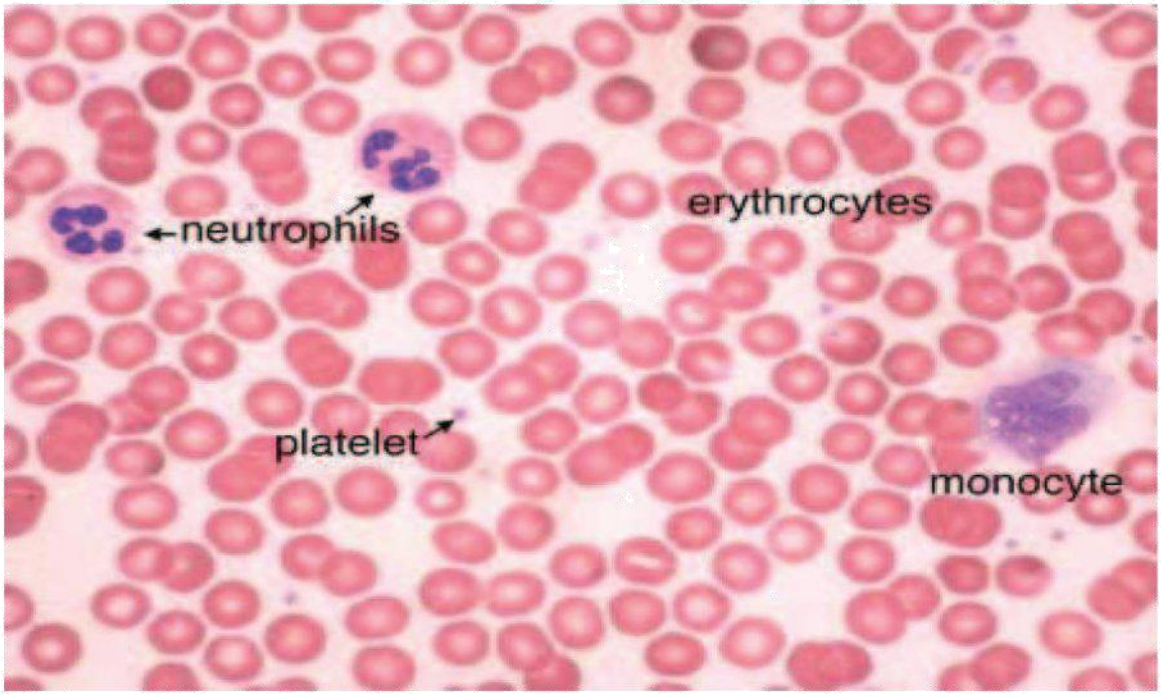
The differential count of WBCs in the blood sample is as follows.

Neutrophil	=	_____ %
Eosinophil	=	_____ %
Basophil	=	_____ %
Lymphocyte	=	_____ %
Monocyte	=	_____ %

QUESTIONS:

1. Draw the different WBCs using appropriate colours.
2. What other cells can you visualize in the smear?
3. Enumerate the criteria of a good blood smear.
4. Can tap water be used for dilution? why?
5. Mention the functions of various types of WBCs and their abnormalities in count.
6. Mention the clinical importance of peripheral blood smear.


10 µm
eosinophil
neutrophil
platlets


neutrophils
erythrocytes
platelet
monocyte

IDENTIFICATION OF THE CELLS:

A leukocyte is identified by its size, nucleus, cytoplasm and granules.

Cell type	Size	Nucleus	Cytoplasm	Normal values
Neutrophil	10 – 14 μm	2-5 lobes connected by narrow strands of chromatin	Fine violet-pink granules	60-70%
Eosinophil	10 - 15 μm	Often bi-lobed connected by thick strands of chromatin (spectacle shaped nucleus)	Coarse brick-red to orange granules	2-8%
Basophil	10 - 15 μm	Irregularly shaped (S shaped) nucleus masked by the granules	Very coarse deep purple granules	0-1%
Small lymphocyte	7-9 μm	Single, round, almost fills the cell	Thin cresent of clear, light blue cytoplasm. No visible granules.	20-30%
Large lymphocyte	10 - 15 μm	Single, round, almost fills the cell. May be central or eccentric.	Large cresent of clear, light blue cytoplasm. No visible granules.	
Monocyte	12 - 20 μm	Horse-shoe shaped nucleus Indented	Abundant, muddy blue in appearance. No visible granules.	1-5%

Experiment No.	HEMOGLOBIN ESTIMATION	Date

Aim:

To estimate the hemoglobin content of the blood by Sahli's acid hematin method.

Apparatus required:

Sahli's Hemoglobinometer, Hemoglobin pipette, N/10 HCl, Distilled water, Glass stirrer, Dropper, Lancet, Spirit and Cotton.

Principle:

The amount of hemoglobin in the blood can be estimated by converting a known volume of blood into acid hematin solution and matching the color of the acid hematin solution with that of the standard colour.

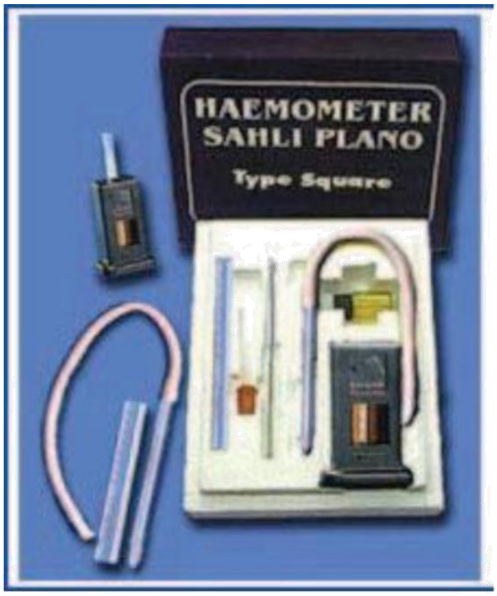
Description of the apparatus:

The hemoglobinometer is a rectangular cubic box consisting of a central compartment to accommodate the 'Hemoglobin tube' and two yellow brown coloured cylindrical rods on either side as comparators. The Hb tube is graduated in percentage on one side and in gram percentage on the other side.

The hemoglobin pipette has a single mark on the stem which corresponds to 0.02 ml or 20 cubic mm. A glass stirrer is provided for thorough mixing while diluting acid hematin solution.

Procedure

1. Fill the hemoglobin tube with N/10 HCl upto its lowest mark (2 gm%).
2. Prick the finger under aseptic precautions to form an adequate drop of blood and suck blood into the hemoglobin pipette upto 20 cu.mm.mark.
3. Gently wipe exterior of the tip of the pipette.
4. Insert the pipette into the hemoglobin tube containing N/10 HCl and blow out the blood. Rinse the pipette 2 or 3 times with the acid present in the tube.
5. Wait for 10 minutes for the formation of acid hematin.
6. Then, dilute the acid hematin by adding distilled water drop by drop and mix with the stirrer.
7. Continue dilution till its color matches with that of the standards on either side.
8. While matching, always take care to raise the stirrer above the level of the solution. Never take the stirrer out of the tube.
9. Note down the final reading. (Lower meniscus).



Add drops of water until colors matched

Record as $\frac{g\ Hb}{100\ mL\ blood}$

Result:

The Hb content of the given sample of blood is _____gms%.

QUESTIONS:

1. What are the other methods used to estimate Hb content of blood?
2. Which is the most reliable method for estimation of Hb?
3. What are the different types of normal Hb in adults?
4. Mention the names of abnormal hemoglobin.
5. What are the differences between adult Hb & fetal Hb?
6. What are the different RBC indices? What are their clinical significance?

Experiment No.	DETERMINATION OF BLOOD GROUP	Date

Aim: To determine the ABO blood group and Rh type of the given blood sample.

Apparatus required:

Lancet, spirit, cotton, normal saline, clean white porcelain tile, glass marking pencil, anti-A serum, anti-B serum, anti-D serum, small sticks for mixing, glass slides, microscope.

Principle:

Determination of blood group is done by using specific agglutinins (antibodies), to confirm the presence or absence of corresponding agglutinogen (antigens) on the surface of the red blood cells.

Procedure:

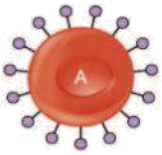
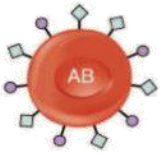
1. Divide the porcelain tile into four columns with a marking pencil.
2. Mark the columns as A, B, Rh and Control
3. Take1ml of normal saline in a test tube.
4. Prick the finger with the lancet under aseptic conditions.
5. Mix 3-5 drops of blood with the saline to obtain a suspension of red blood cells.
6. Add a drop each of anti-A, anti-B, anti-D sera and saline to the respective columns.
7. Place a drop of the red cell suspension adjacent to the anti-sera in the respective columns.
8. Mix the anti-sera and red cell suspension by using separate sticks.
9. Wait for few minutes and observe the agglutination (clumping).
10. Compare it with the saline standard.
11. Record your findings.
12. If there is doubt regarding agglutination, confirm it under the microscope.

Result:

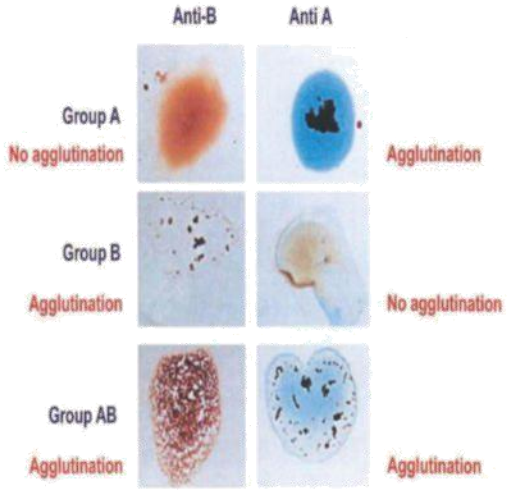
The blood group of the subject is _____

QUESTIONS:

1. State Landsteiner's law.
2. What is cross-matching of blood?
3. What is the preservative used to store blood in the blood bank?
4. What are the clinical applications of blood grouping and Rh typing?
5. What are the minor blood groups?
6. What is the concept of universal donor/ universal recipient?
7. What are the indications and hazards of blood transfusion?
8. What are the differences between ABO system and Rh system?

	Blood Type			
	A	B	AB	O
Red Blood Cell Type				
Antibodies in Plasma	 Anti-B	 Anti-A	None	 Anti-A and Anti-B
Antigens in Red blood Cell	A antigen	B antigen	 A and B antigens	None
Blood Types Compatible in an Emergency	A, O	B, O	A, B, AB, O (AB^+ is the universal recipient)	O (O is the universal donor)

Schema of agglutinogens and agglutinins



Blood groups showing agglutination

Experiment No. **DETERMINATION OF BLEEDING TIME** **Date**

Aim:

To determine the bleeding time by Duke's method.

Apparatus Required:

Filter paper, lancet, spirit, cotton swabs & stop watch.

Principle:

The time interval between skin puncture and spontaneous, unassisted stoppage of bleeding is called bleeding time. It is a test for assessing the function of platelets and integrity of capillaries.

Procedure:

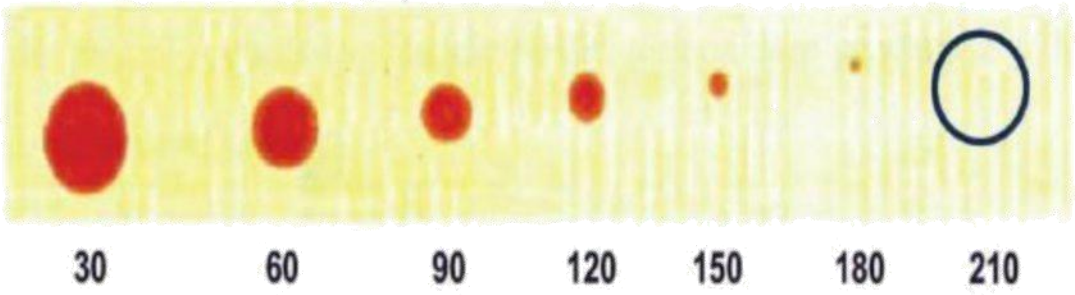
1. Clean the tip of the finger with spirit and cotton and allow the finger to dry.
2. Make a good deep finger prick with the lancet to get free flowing blood.
3. Do not squeeze the finger.
4. Immediately start the stopwatch.
5. Gently touch the puncture site with a clean filter paper every 30 seconds.
6. Repeat this step until no further blood spot appears on the filter paper.
7. Observe that successive spots are smaller in size.
8. Count the number of blood spots including the dry spot on the filter paper and divide it by 2 to get the bleeding time in minutes.
9. Normal bleeding time by Duke's method is 2-5minutes.

Result:

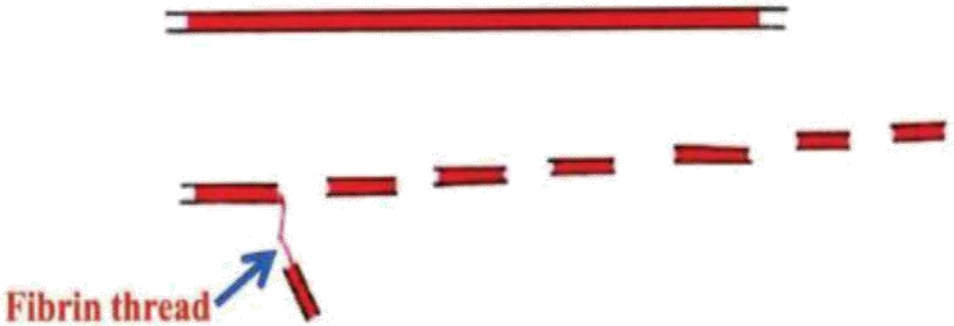
The bleeding time determined by Duke's method is ----------

QUESTIONS:

1. Define bleeding time.
2. What are the other methods to determine the bleeding time?
3. What is hemostasis?
4. What is the role of platelets in hemostasis?
5. What is the normal platelet count? What do you mean by thrombocytosis?
6. Name few conditions where bleeding time is prolonged?
7. What is Thrombocytopenic purpura? Comment on the clotting time in this condition.



Determination of Bleeding time



Determination of clotting time

Experiment No. **DETERMINATION OF CLOTTING TIME:** **Date**

Aim:

To determine the clotting time of blood by Wright's capillary glass tube method.

Requirements:

Capillary glass tube, lancet, spirit, cotton swabs & stop watch.

Principle:

When blood comes in contact with glass surface, the coagulation pathway gets activated. The time taken for the formation of insoluble fibrin thread(blood clot) is called clotting time.

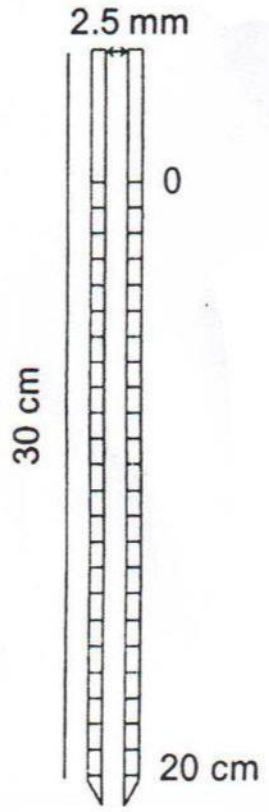
Procedure:

1. Clean the tip of the ring finger with spirit and allow the finger to dry.
2. Make a good deep finger prick with the lancet to get free flowing blood.
3. Do not squeeze the finger.
4. When a blood drop of optimum size has formed, gently place the end of the capillary tube in the drop such that the other end of the tube is at a lower level.
5. Blood enters readily into the tube by capillary action.
6. Start the stop watch.
7. Hold the capillary tube with blood between the palms to maintain it at body temperature.
8. After 2 minutes, break a small bit of capillary tube at its end and check for the formation of fibrin thread.
9. Repeat it every 30 seconds until the appearance of insoluble fibrin thread between the broken ends of capillary tube and note the time.
10. The appearance of the fibrin thread indicates that the blood has clotted.
11. The total time taken for the formation of fibrin thread is recorded as the clotting time.
12. Normal clotting time by this method is 2-8 minutes.

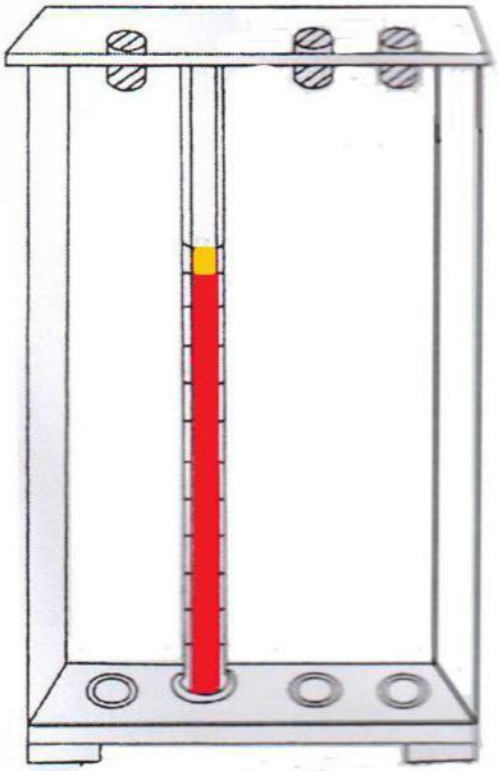
QUESTIONS:

1. Define clotting time.
2. What are the other methods used to determine the clotting time?
3. Name the conditions in which clotting time is prolonged.
4. What is haemophilia? Comment on the clotting time in this condition.
5. What is clot retraction time?
6. Name the Vitamin K dependent coagulation factors.
7. What is an anticoagulant? Mention some invivo and invitro anticoagulants.
8. Name the proteins involved in fibrinolytic system.

ESTIMATION OF ERYTHROCYTE SEDIMENTATION RATE (ESR)



a) Westergren tube



b) Westergren tube on the rack

Experiment No. **ESTIMATION OF ERYTHROCYTE SEDIMENTATION RATE (ESR)** **Date**

Aim:

To determine the Erythrocyte Sedimentation Rate of the given blood sample.

Apparatus required:

Westergren's pipette and stand, syringe with needle and 3.8% sodium citrate solution (anticoagulant)

Principle:

If blood treated with anticoagulant is allowed to stand in a tube placed vertically, the RBCs settle down gradually to the bottom since their specific gravity (1.093) is greater than that of the plasma (1.030).

The rate at which the RBCs settle down is called as Erythrocyte Sedimentation Rate.

Procedure:

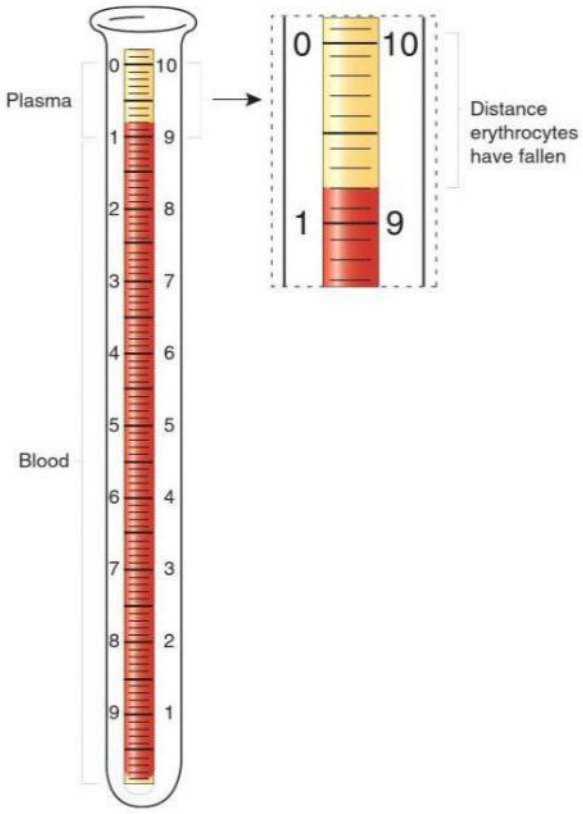
Westergren's method:

- Westergren's pipette (tube) which is used for this procedure is open at both ends and is graduated in mm from 0-200 with a bore diameter of 2.5mm.
- A sterile solution of 3.8% sodium citrate is used as an anticoagulant.
- In a clean dry syringe, draw 2ml of blood from the antecubital vein under aseptic precautions and mix with 0.4ml of 3.8% sodium citrate solution in a plastic container with its lid closed.
- Fill the Westergren's pipette with blood by sucking, after placing the tip of the finger over the top of the pipette to control the flow of blood into and out of it, or with a rubber bulb.
- Bring the blood column to exact zero mark.
- Keeping the finger (or the rubber bulb) over the pipette, transfer it to the Westergren stand by firmly pressing its lower end into the rubber cushion. Now slip the upper end of the pipette under the screw cap.
- After an hour, note the mm of clear plasma above the red cells.

Result:

Erythrocyte Sedimentation Rate of the given blood sample is _____ mm in first hour.

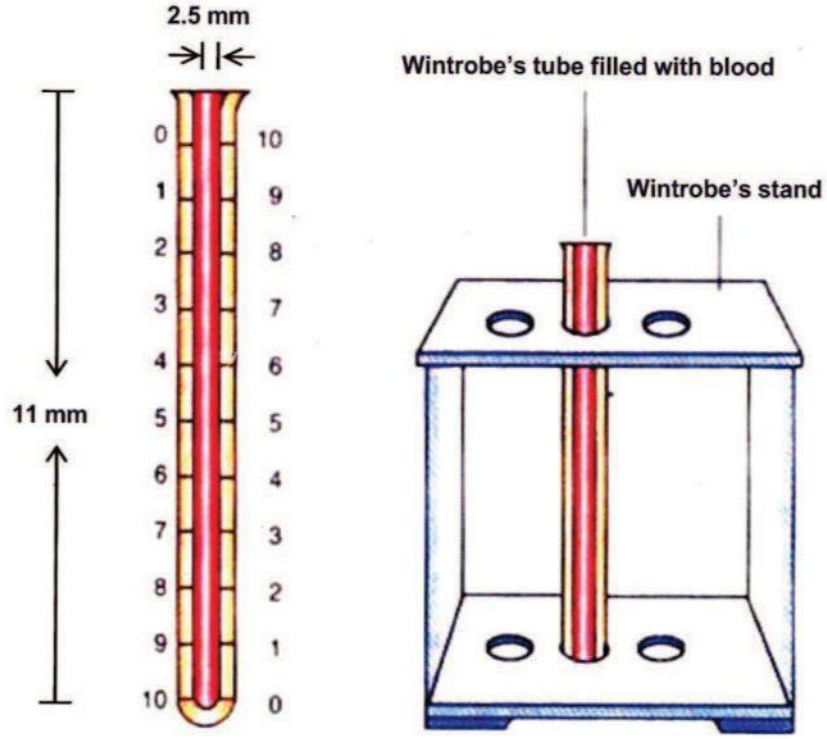
Wintrobe tube



QUESTIONS:

1. What is ESR?
2. What are the 3 stages by which sedimentation of red cells occur?
3. What are the other methods of estimating ESR?
4. What are the advantages and disadvantages of Westergren method?
5. What are the advantages and disadvantages of Wintrobe method?
6. Can you use oxalate mixture in Westergren method and citrate in Wintrobe method?
7. What are the factors determining ESR?
8. What is rouleaux formation?
9. Why is ESR reading taken after one hour?
10. What is the normal ESR in males and females?
11. Why is the ESR higher in females than that of males?
12. What is the clinical significance of ESR?
13. Mention some physiological and pathological conditions in which ESR is increased / decreased?
14. What is zeta potential?

PACKED CELL VOLUME



Packed cell volume

Experiment No.	DETERMINATION OF PACKED CELL VOLUME / HEMATOCRIT	Date

Aim:

To determine the packed cell volume of the given blood sample.

Apparatus required:

Centrifuge, Hematocrit tube (Wintrobe tube), Pasteur pipette, syringe with needle and double oxalate or EDTA (anticoagulant).

Principle:

When the blood is mixed with an anticoagulant and centrifuged in a hematocrit tube, the red blood corpuscles settle down at the bottom. The ratio of the volume of the settled red blood cells to that of whole blood in the hematocrit tube is called the packed cell volume or the hematocrit. A thin grey-white layer of white cells at the top of the red blood cell column is called the buffy coat layer.

Hematocrit measures the percentage of volume of the packed red cells. It is used to diagnose and classify the various types of anemia, along with other red blood cell indices.

Procedure:

Wintrobe's method:

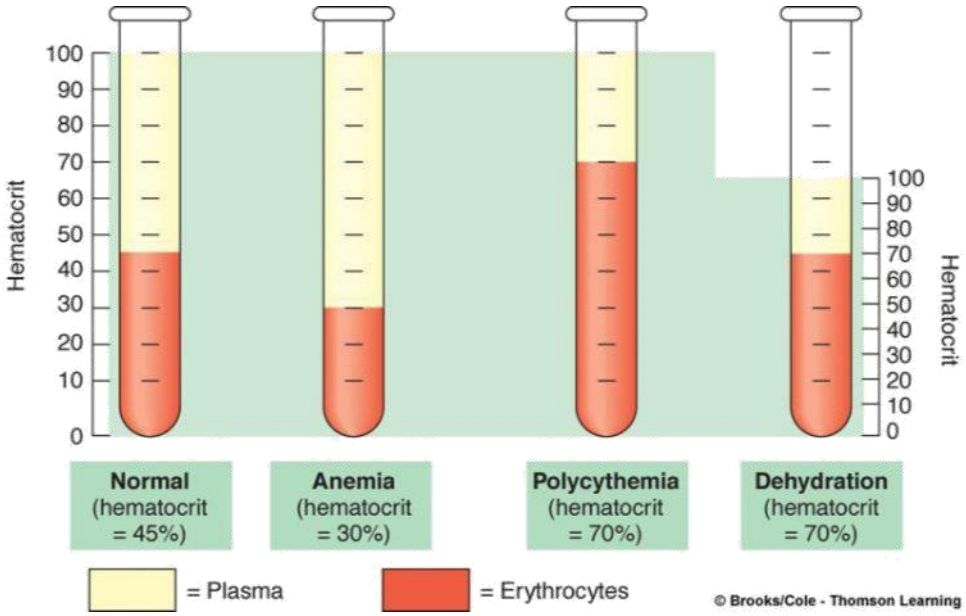
- Wintrobe's tube is a thick walled cylindrical tube, 11cm in length with an internal bore of 3mm. The tube is graduated from 0 to 10cm(100 mm) both in the ascending and descending order on either sides. The marking 0 – 10 from above downwards is used for ESR and the marking 0-10 from below upwards is used for reading PCV.

- In a clean dry syringe, 2ml of blood is drawn from the antecubital vein under aseptic precautions and transferred to a container with anticoagulant.

- The anticoagulated blood is then filled in the hematocrit tube from below upwards upto the mark 10 using the Pasteur pipette.

- The tube is centrifuged at a rate of 3000 rpm for a period of 30 minutes.

- At the end of 30 minutes, take the reading of upper level of packed red cell column.


Hematocrit
100
90
80
70
60
50
40
30
20
10
0
Hematocrit
100
90
80
70
60
50
40
30
20
10
0
Normal (hematocrit = 45%)
Anemia (hematocrit = 30%)
Polycythemia (hematocrit = 70%)
Dehydration (hematocrit = 70%)
= Plasma
= Erythrocytes
© Brooks/Cole - Thomson Learning

Result:

The packed cell volume or the hematocrit value of the given blood sample is _____ %

QUESTIONS:

1. Define PCV.
2. What is the clinical significance of PCV?
3. What is the normal range of PCV in males and females?
4. What is the ideal anti-coagulant used and why?
5. What is the difference between arterial and venous blood hematocrit?

Experiment No.	OSMOTIC FRAGILITY OF RED BLOOD CORPUSCLES	Date

Aim:

To determine the osmotic fragility of red blood cells in the given sample of blood.

Apparatus required:

Test tubes with rack, anticoagulated blood, NaCl, distilled water.

Principle:

The normal red blood cells can remain suspended in 0.9% sodium chloride solution (normal saline) for hours without any change in their size & shape. But when they are placed in decreasing strengths of hypotonic sodium chloride solutions they imbibe water due to osmosis and finally burst releasing the hemoglobin pigment in the medium.

Procedure:

1. Sodium chloride solution of 1% tonicity is prepared by dissolving 1 gram of NaCl in 100ml of distilled water.
2. Arrange the test tubes in the rack and number them serially from 1 to 12.
3. Prepare solutions of increasing hypotonicity by mixing required number of drops of 1% NaCl solution and distilled water in the test tubes as given in the table.

Test tube no.	1	2	3	4	5	6	7	8	9	10	11	12
No. of drops of 1%NaCl	22	16	15	14	13	12	11	10	9	8	7	0
No. of drops of Distilled water	3	9	10	11	12	13	14	15	16	17	18	25
Strength of saline solution (%)	0.9	0.64	0.60	0.56	0.52	0.48	0.44	0.40	0.36	0.32	0.28	0

Tonicity of NaCl in % 0.9 0.64 0.56 0.52 0.48 0.44 0.40 0.36 032 0.0

Start of hemolysis – 0.48
Complete hemolysis – 0.36

Findings of blood drop after one hour in increasing hypotonicity of NaCl solution and distilled water (only 10 tubes shown)

4. Use separate droppers for saline solution and distilled water.

5. Note that the tube No.1 contains normal saline (0.9% approximately) – Isotonic with plasma while tube No.12 contains distilled water.

6. Draw 2ml of venous blood and treat it with anticoagulant in a test tube.

7. Add one drop of blood into each of the above 12 tubes.

8. Invert each tube gently once to mix blood with saline.

9. Leave the test tubes undisturbed for one hour. Then observe the extent of hemolysis in each tube by holding the rack at eye level, with a white paper sheet behind it.

Interpretation

- Test tube with partial hemolysis shows a supernatant fluid with pink colour proportionate to the degree of hemolysis and a lower layer of sedimented red cells at the bottom of the tube.
- Test tube with complete hemolysis shows a clear homogeneously pink solution with no cells at the bottom.
- Test tube with no hemolysis shows a clear colourless supernatant solution with a layer of sedimented red cells at the bottom of the tube

Result:

Hemolysis begins in_____% of NaCl solution

Hemolysis is complete in _____% of NaCl solution.

QUESTIONS:

1. What is the normal range of osmotic fragility of red cells?
2. What is osmosis?
3. What do you mean by hypo/hyper tonicity?
4. Define fragility.
5. What happens to red cells when they are placed in isotonic, hypotonic and hypertonic solutions?
6. Name some conditions which increase / decrease the osmotic fragility of the RBC.
7. What are the advantages of the shape of red blood cells?

Experiment No.	DETERMINATION OF THE SPECIFIC GRAVITY OF BLOOD	Date

Aim:

To determine the specific gravity of the given blood sample by using "Copper sulphate falling drop method".

Apparatus required:

Test tubes with rack, distilled water, anticoagulated blood, copper sulphate crystals ($CuSO_4.5H_2O$), beaker, measuring jar, Pasteur pipette

Principle:

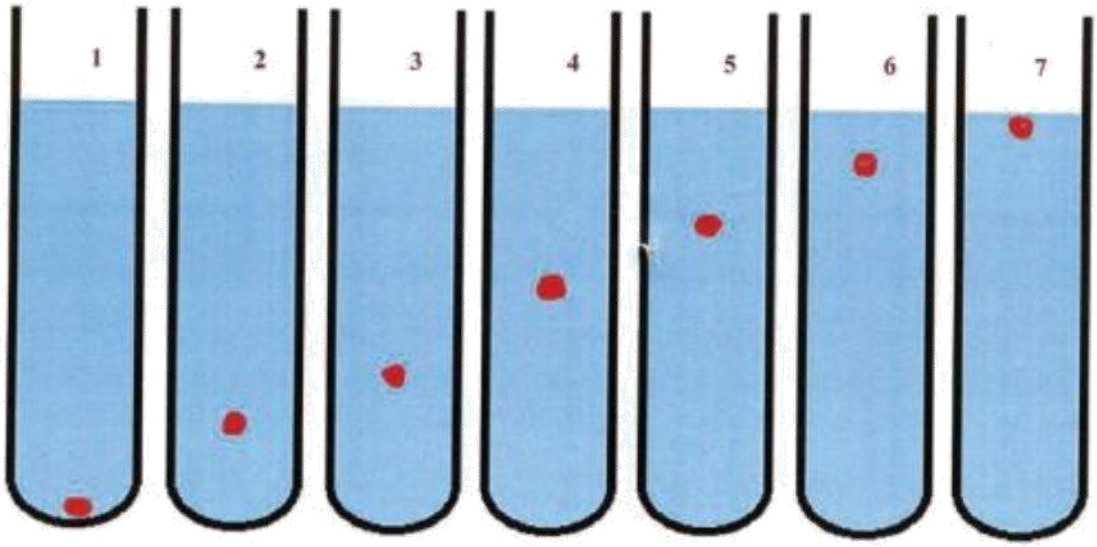
The specific gravity of blood is determined by comparing the specific gravity of one drop of blood with that of copper sulphate solution of known specific gravity.

Procedure:

1. Stock solution of copper sulphate is prepared by dissolving 159 gm of $CuSO_4.5H_2O$ in 1 liter of distilled water. The specific gravity of this stock solution is 1100.

2. Standard copper sulphate solutions of known specific gravity are prepared by mixing specific quantity of stock solution and distilled water as shown in table and label the test tubes.

Test tube No.	1	2	3	4	5	6	7
Stock solution (ml)	49	51	54	57	59	61	64
Distilled water (ml)	51	49	46	43	41	39	36
Specific gravity	1.050	1.052	1.055	1.058	1.060	1.062	1.065



Position of blood drop in $CuSO_4$ solution of Sp. gr. ranging from 1050 to 1065

8.5	10.5	12.5	13.5	14.5	15.5	17.0

Specific gravity of blood and the corresponding hemoglobin content in g%

3. Arrange the test tubes in rack in order of increasing specific gravity from left to right in test tube rack.
4. Draw 2ml of venous blood and treat it with the anticoagulant in a test tube.
5. Using Pasteur pipette a drop of blood is delivered into the middle test tube (no.4) from a height of about 1cm above the surface of the copper sulphate solution so that it doesn't touch the walls of the test tube.
6. The drop sinks to about 2-3cm, and loses its momentum in 3-4 seconds. The drop then behaves according to its specific gravity; observe its behaviour during the next 15 seconds.
7. If the specific gravity of blood drop is greater than that of the solution, the drop sinks to the bottom; if it is less than that of the solution, it rises to the surface and if it is the same as that of the solution, it becomes stationary and floats in the middle of the solution.
8. If the drop continues to sink in test tube no.4, try with higher specific gravity solution; if it begins to rise, try with the lower specific gravity solution till you come to a solution where the drop remains in the middle of the solution.
9. The whole observation has to be made in each step within 15 seconds.
10. The tube in which the blood drop remains suspended in the middle of the tube for at least 15 seconds is noted and the specific gravity mentioned on the tube is read and recorded.

Result:

The specific gravity of the given blood sample is _____.

QUESTIONS:

1. Define specific gravity.
2. What is the normal specific gravity of blood, plasma, serum and red blood cells?
3. Why is copper sulphate solution used in this experiment?
4. Why should the observation be made within 15 seconds?
5. Enlist the physiological and pathological conditions in which the specific gravity of blood is increased and decreased.
6. What are the clinical applications of this method?

Experiment No.	GENERAL EXAMINATION	Date

A thorough general examination is done before any systemic examination. Vital information such as name, age, sex, height, weight, occupation and address of the individual are recorded. The subject is comfortably seated. The room is well illuminated. It is always preferable to examine under good day light. The examiner should always be on the right side of the subject while examining.

THE FOLLOWING OBSERVATIONS ARE RECORDED:

LEVEL OF CONSCIOUSNESS

The level of consciousness can be clear sensorium, drowsiness, stupor, semicoma and coma.

ORIENTATION TO TIME, PLACE AND PERSON

Ask about the day, date, month, year and time of day. Subject should know where they are (e.g. home or hospital) Similarly test his orientation towards person.

BODY BUILD AND NOURISHMENT

Build refers to skeletal frame work and nourishment refers to muscular bulk. It should be observed whether he is well / moderately / thin built and nourished.

TEMPERATURE

Recorded by a clinical thermometer

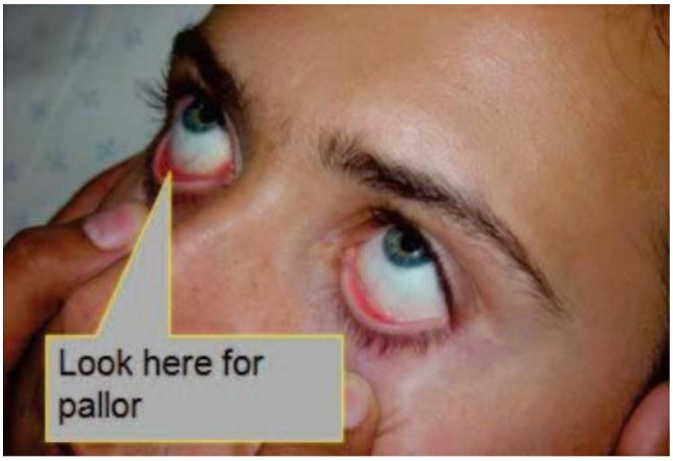
Method of recording temperature

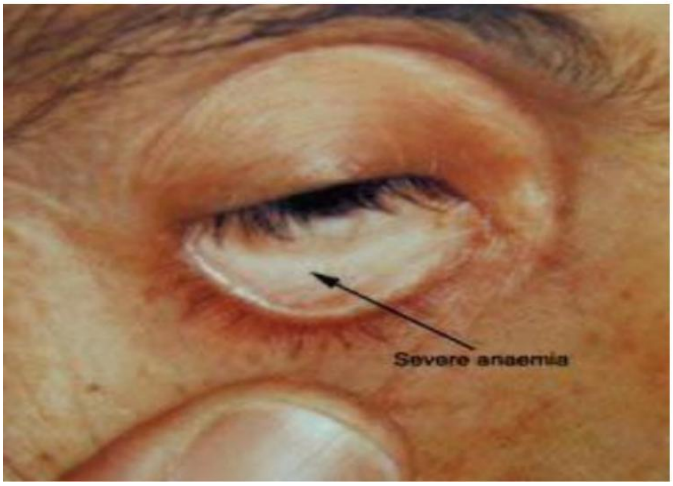
Do not touch the bulb of the thermometer. Shake down the mercury column into the bulb. Keep it under the tongue with the mouth closed for one minute before reading the temperature.

Sites of recording temperature

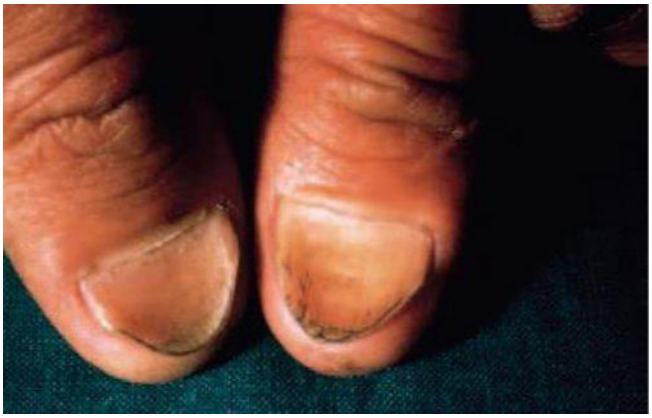
1 Mouth (36.6°C to 37.2°C)
2 Axilla (0.5°C lower than oral temperature)
3 Rectum (0.5°C higher than oral temperature – Closer to core temperature)

Evert the lower lid to inspect the palpebral conjunctiva for pallor





KOILONYCHIA



5. PALLOR

Pallor of the skin and mucous membrane.

Areas to look for pallor

1. Lower palpebral conjunctiva

   Instruct the subject to look up and retract the lower lid to see the palpebral conjunctiva
2. Dorsum of tongue
3. Mucous membrane of oral cavity
4. Nail bed
5. Skin over palm and sole

Conditions where pallor is seen

1 Anemia – Reduced count of RBCs / Hb content in blood
2 Shock

6.JAUNDICE (ICTERUS)

Yellowish discoloration of the sclera, skin and the mucous membrane due to presence of excess bilirubin in blood of more than 2mg%

Areas to look for jaundice

1. Bulbar conjunctiva of both eyes – Upper sclera

   Instruct the subject to look down and retract the upper lid to view the sclera
2. Mucus membrane of oral cavity

   (especially the undersurface of tongue and floor of mouth)
3. Nail bed
4. Skin

Causes of yellowish discoloration of skin

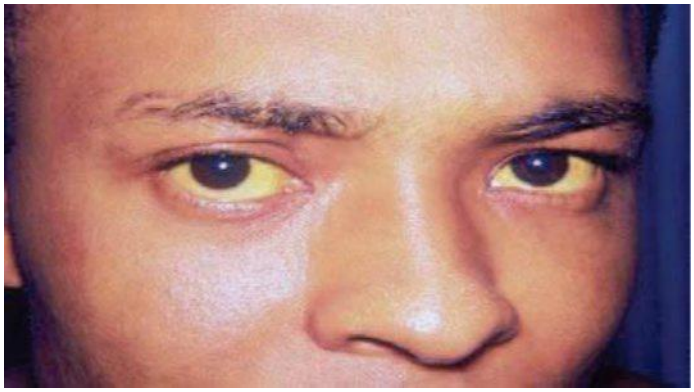
Jaundice, Carotenemia, Hemochromatosis

Hypercarotenemia occurs in

1. People who eat large quantity of raw carrots and tomatoes

2. Hypothyroidism

JAUNDICE



Cyanosis

Low oxygen levels in the blood cause the lips, fingers, and toes to look blue (cyanotic)

Children with Tetralogy of Fallot exhibit bluish skin during episodes of crying or feeding.

7.CYANOSIS

It is the bluish discoloration of the skin and mucous membrane due to an increased quantity of reduced hemoglobin concentration of more than 5 gm in 100 ml of blood.

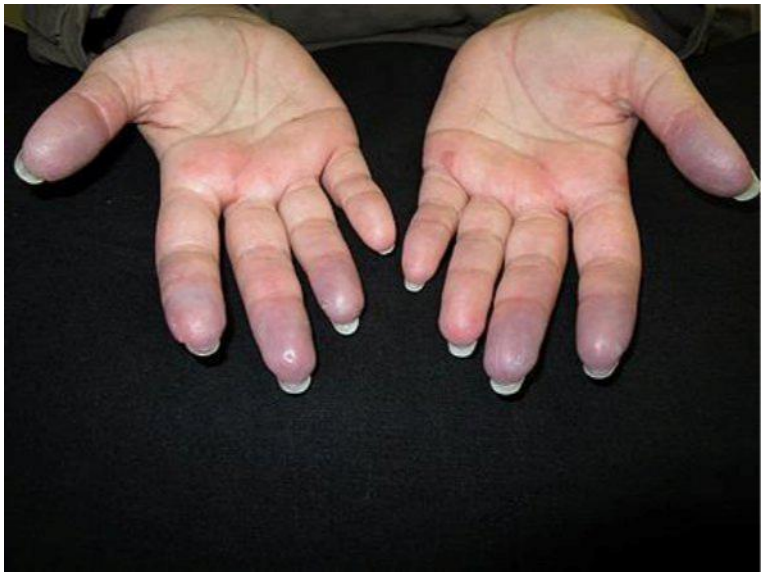
Areas to look for cyanosis:

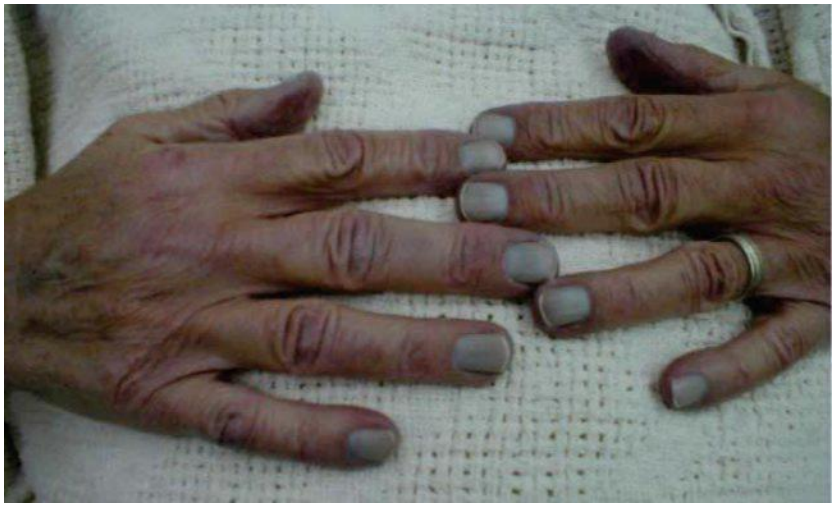
1. Lips,
2. Tongue
3. Mucous membrane over the Palate
4. Conjunctiva
5. Tip of the nose and ear lobules
6. Extremities (fingers, toes and nail beds)

Types of Cyanosis:

1. **Central cyanosis**
2. **Peripheral cyanosis**
3. **Differential cyanosis**

FEATURES	**CENTRAL CYANOSIS**	**PERIPHERAL CYANOSIS**
1. MECHANISM	Right to left shunts or abnormal Hb or inadequate O_2 saturation	Peripheral stasis of blood
2. SITE	Whole body (Central parts of the body such as tongue plus peripheral parts)	Nail beds, nose tip, ear lobe, extremities (tips of fingers and toes)
3. ASSOCIATED WITH	Clubbing and polycythemia	Not associated with any other symptoms
4. EXTREMITIES	Warm	Cold
5. ON WARMING EXTREMITIES	No change	Disappears
6. O_2 INHALATION	Slight improvement	No change
7. ARTERIAL PaO_2	< 85%	Normal





8. CLUBBING

Bulbous enlargement of soft parts of the terminal phalanges with overcurving of the nails both longitudinally and transversely.

Schamroth's sign:

When two fingers of both hands are held together with their nails facing each other, a diamond shaped space is seen. This space is lost in clubbing (Positive Schamroth's sign).

Clubbing is seen in

- Bronchopulmonary diseases like bronchiectasis, lung abscess, empyema, chronic bronchitis and bronchogenic carcinoma
- Cardiac diseases like congenital cyanotic heart disease and bacterial endocarditis
- Gastro intestinal diseases like ulcerative colitis and liver cirrhosis
- Endocrine disorders like acromegaly, thyrotoxicosis
- Hereditary

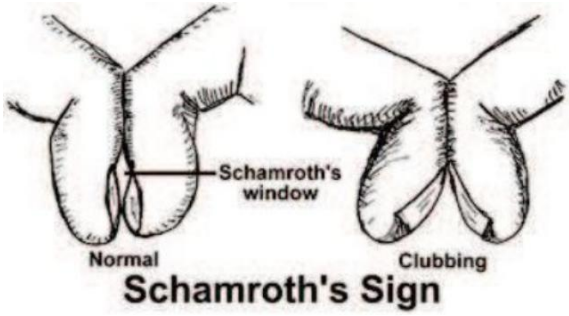
9.OEDEMA

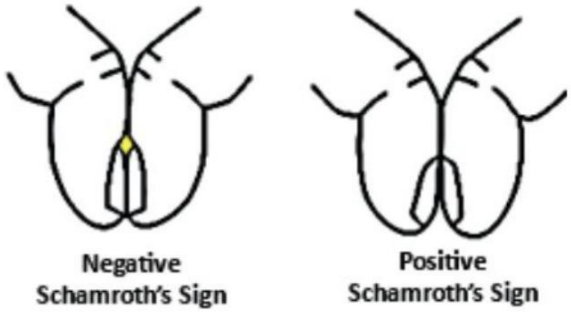
It is the condition where there is accumulation of free fluid in the interstitial space. Pedal oedema is examined by pressing the skin and the tissue against the tibial bone just above the medial malleolus, sustaining the pressure for at least 15 seconds and releasing the pressure to look for pitting, if any. In bed ridden subjects oedema is examined by pressing over the presacral region.

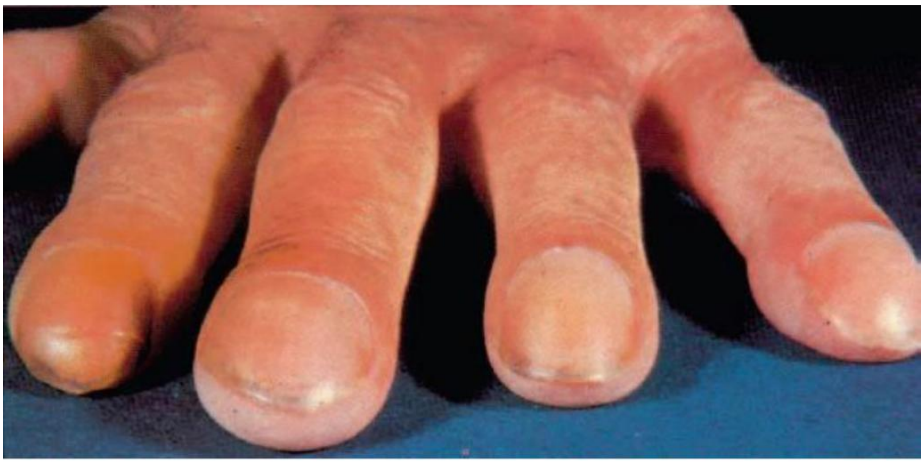
Types of oedema:

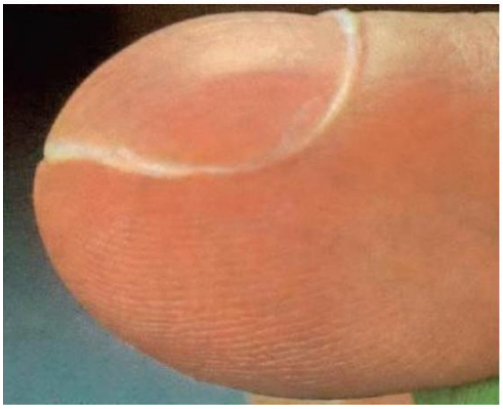
1. Pitting oedema – Heart failure, renal disease, hypoproteinemia
2. Non-pitting oedema – Myxoedema, filariasis

Clubbing

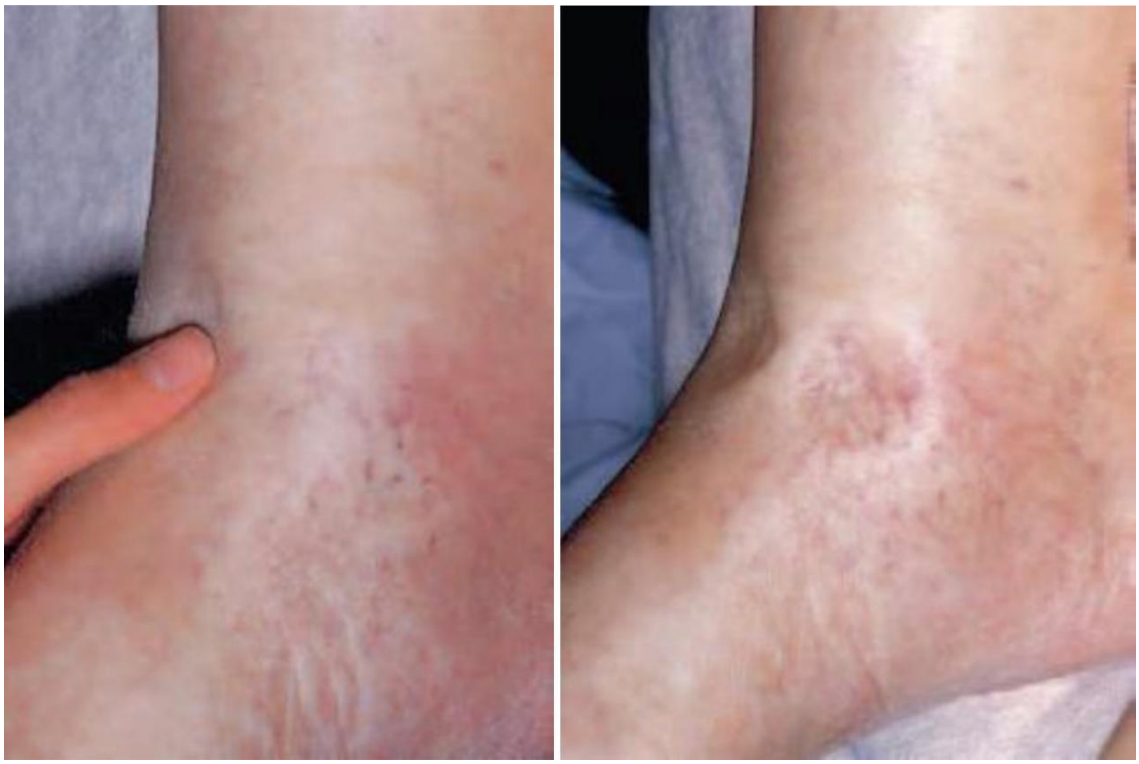

Schamroth's window
Normal
Clubbing
Schamroth's Sign


Negative Schamroth's Sign
Positive Schamroth's Sign





Pitting pedal edema



10. LYMPHADENOPATHY

Group of nodes to be examined:

Submental, submandibular, cervical, posterior auricular, occipital, supraclavicular, axillary, inguinal and popliteal nodes.

Points to be noted while examining lymph nodes:

Site, size, number, consistency (soft, firm or hard), tenderness, mobility, matted or discrete, fixity to skin, generalized or localized.

Examination of lymph nodes:

Examiner should stand behind the sitting subject- Submental, submandibular, deep cervical nodes in the anterior triangle of neck

Examiner should stand in front of the sitting subject – deep cervical nodes in the posterior triangle of neck, posterior auricular and occipital nodes

Axillary nodes – sitting posture with abducted arm

Inguinal nodes – supine position with thigh flexed to 10°

Popliteal nodes – Flex the knees and palpate deep into popliteal fossa

QUESTIONS:

1. Where will you look for pallor?
2. What is cyanosis?
3. What are the types of Jaundice?
4. How do you detect clubbing?
5. What are the types of oedema and what are their causes?
6. What are the vital signs?

GENERAL EXAMINATION OF THE SUBJECT

NAME: AGE :

SEX: OCCUPATION:

Consciousness and orientation

Build and nourishment

Afebrile / Febrile

Pallor

Jaundice

Cyanosis

Clubbing

Pedal edema

Lymphadenopathy

Vital signs:

- Temperature
- Pulse rate
- Respiratory rate
- Blood pressure

INFERENCE:

Experiment No.	EXAMINATION OF THE RESPIRATORY SYSTEM	Date

Anatomical Landmarks

1. Midclavicular line – a vertical line that extends downwards from the midpoint between the middle of the suprasternal notch and the tip of the acromion.
2. Anterior axillary line – a vertical line extending downwards from the anterior axillary fold.
3. Posterior axillary line – a vertical line extending downwards from the posterior axillary fold.
4. Midaxillary line – a vertical line originating at a point midway between the anterior and posterior axillary line.

Examination of the respiratory system is carried out by,

Inspection

Palpation

Percussion

Auscultation

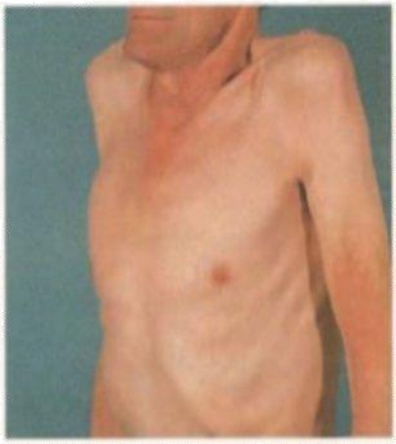
INSPECTION:

Ask the subject to sit on a stool with the chest and upper abdomen fully exposed.

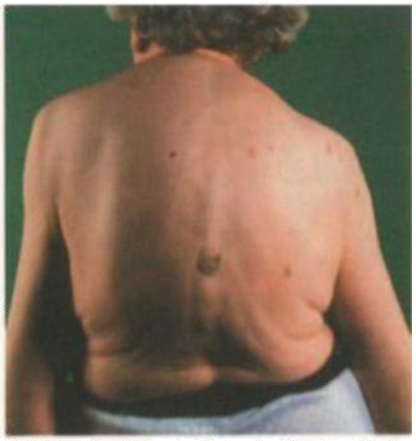
1. Shape and symmetry of the chest
2. Position of Trachea
3. Apical impulse
4. Movement of the chest
5. Rate, rhythm and type of respiratory movements
6. Abnormal pulsations, dilated veins, scars and sinuses over the chest wall

1.Shape and symmetry of the chest:

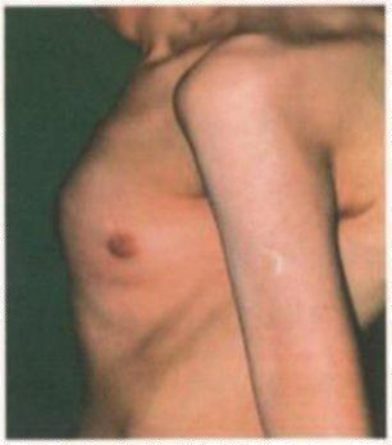
- Examine both the front and back of the chest.
- Look for any skeletal deformities and drooping of shoulder.
- Normal chest is bilaterally symmetrical and elliptical in cross section.
- The transverse diameter is greater than the anteroposterior diameter with a ratio of 7:5 (Hutchinson's index).



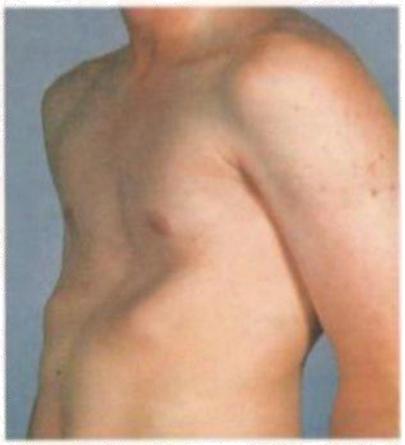
Hyperinflated chest



Kyphoscoliosis



Pectus carinatum



Pectus excavatum

Abnormal chest shapes

Abnormal findings:

- ❖ Barrel shaped chest - anteroposterior diameter is more than the transverse diameter. It is seen in patients with severe COPD.
- ❖ Pectus carinatum (pigeon chest) is a localized prominence of the sternum and adjacent costal cartilages. Occurs in rickets.
- ❖ Pectus excavatum (funnel chest) is a developmental deformity with a localized depression of the lower end of sternum
- ❖ Kyphosis is forward bending of the spine and Scoliosis is lateral bending. Kyphoscoliosis involves both deformities.

2. Tracheal Position:

Stand in front of the subject. Ask the subject to look straight. Observe for any deviation of trachea. Normally, it is in the midline or slightly deviated to the right.

Look for any prominence of the sternocleidomastoid muscle. If it is prominent on one side, it indicates tracheal deviation to that side. This is TRIAL'S sign.

3. Apical Impulse*:*

Look for apical impulse over the precordium. (Refer CVS Examination)

4. **Movement of the chest*:***

The subject is instructed to take deep breaths. Observe, if the respiratory movements are equal on both sides.

5. Respiratory Rate*:*

Count the respiratory rate (breaths/min) for one minute, while you divert his attention by palpating the radial pulse. Normal rate ranges from 12-16 breaths / min. Increased rate of respiration is known as Tachypnoea and decreased rate is known as bradypnoea.

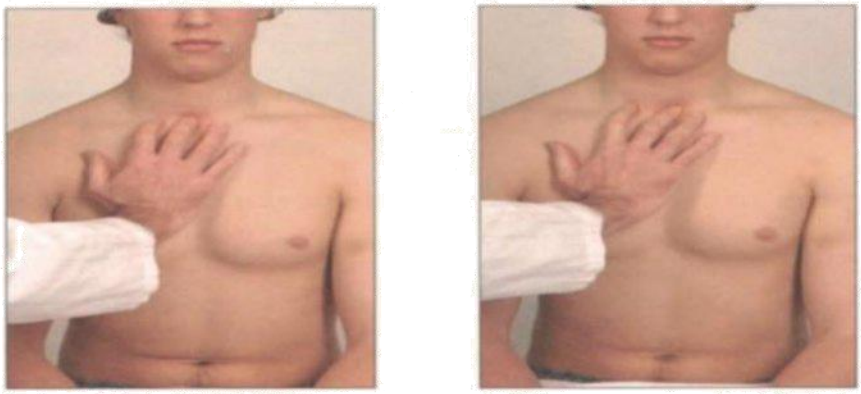
Respiratory rhythm:

Observe, if the rhythm of respiration is regular or irregular.

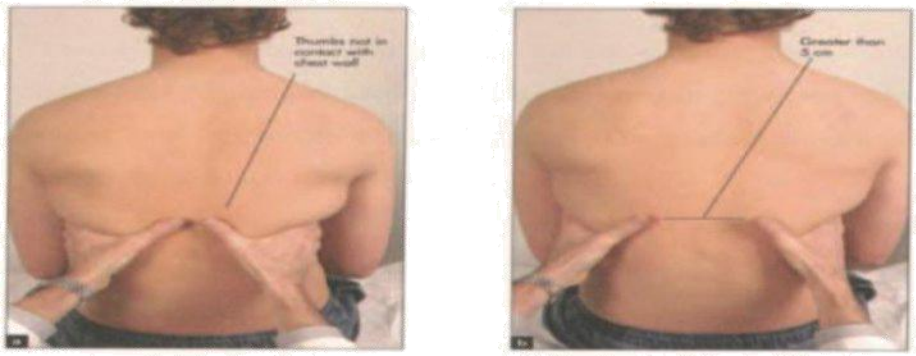
Type of Respiratory movements:

Observe, if the respiration is predominantly thoracic or abdominal. During normal respiration, women use the intercostal muscles more than the diaphragm, and their respiratory movements are predominantly thoracic. Men rely more on the diaphragm and their respiratory movements are predominantly abdominal.

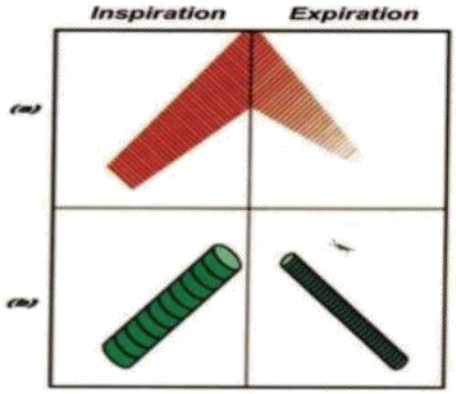
6. Look for any abnormal pulsations, dilated veins, scars of previous heart or lung surgery and sinuses over the chest wall..



Examining for tracheal shift



Palpation for lower lobe expansion
(a) expiration (b) inspiration



Breath sounds (a) Vesicular (b) Bronchial

PALPATION:

1. Determine the position of mediastinum by examining the tracheal position and apical impulse.

Tracheal Position:

- Ask the subject to sit erect. Keep your index and ring finger of the right hand on medial ends of clavicle. Using the middle finger, gently feel for the trachea and assess whether it is in midline or deviated to one side.
- Then, insinuate your middle finger into the space between the trachea and the sternocleidomastoid muscle on either side to assess the degree of yielding or resistance.

Common causes of Tracheal deviation

Towards the side of the lung lesion (pull) - Upper lobe collapse, Upper lobe fibrosis, Pneumonectomy

Away from the side of the lung lesion (push) - Tension pneumothorax, Massive pleural effusion

Apical impulse / Apex beat:

- Palpate the precordium first with palm and then with ulnar border of hand to locate the apical impulse.
- Place the index finger on the apical impulse and using the other hand, count the intercostal spaces with reference to sternal angle.
- The normal apical impulse is felt at the left 5^{th} intercostal space, half an inch medial to the left mid clavicular line.

Shift of mediastinum:

Shift of the upper mediastinum causes tracheal deviation. Displacement of apical impulse may indicate shift of lower mediastinum. Displacement of the cardiac impulse without tracheal deviation is usually due to left ventricular enlargement but can occur in scoliosis, kyphoscoliosis or severe pectus excavatum.

2.Respiratory Movements:

- Assess expansion of the lower lobes by placing your hands firmly on the anterior chest wall. Extend your fingers around the sides of the subject's chest. Your thumbs should almost meet in the midline and hover just off the chest so that they can move freely with respiration.
- Ask the subject to take deep breaths. Observe, whether your thumbs move equally on both sides.
- To examine the movement of apical lobes, place the hands over the shoulders on both sides from behind. The degree of lift of the fingers indicates the degree of expansion of the apical lobes.

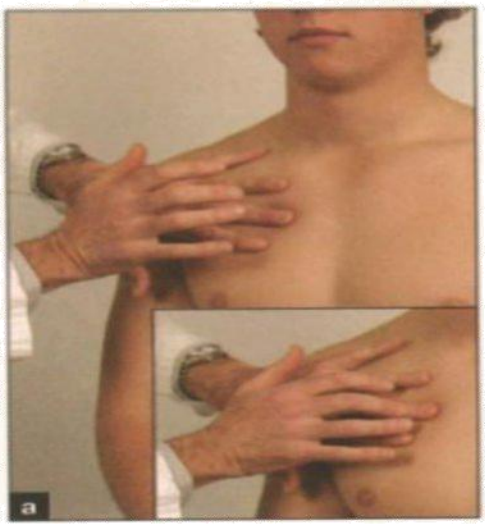
- Examine the lower lobes of the lungs as explained before by placing the hands in the infrascapular area.
- Reduced expansion on one side indicates abnormality on that side such as pleural effusion, lung collapse, pneumothorax and fibrosis

3.Chest Expansion:

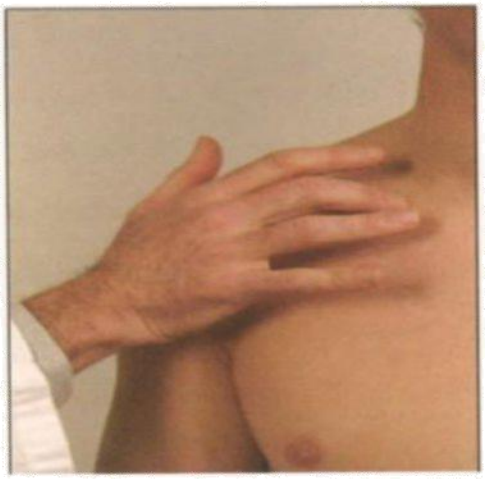
- The chest expansion is measured quantitatively by using a measuring tape.
- Hold the measuring tape around the chest wall just below the level of nipples.
- The difference in expansion after a full expiration and a deep inspiration is the total chest expansion.
- The normal expansion ranges from 5 to 8 cm.
- It is decreased in restrictive lung diseases.

4.Vocal fremitus / Tactile vocal fremitus:

- Ask the subject to repeat the word "ONE" or "NINETY NINE" in a clear voice.
- Feel the vibrations using ulnar border of the hand in each intercostal space and compare it with the other side consecutively.
- VF is increased in consolidation of lungs.
- VF is decreased in pleural effusion and empyema.



Percussing (plexor) finger poised
Inset: plexor finger strikes pleximeter finger



Direct percussion of the clavicle
for upper lobe resonance

PERCUSSION:

Percussion allows you to hear the pitch and loudness of the percussed note. Percussion is done in sequence over corresponding areas on both sides of the chest.

To Percuss

Lateral chest wall*:* Ask the subject to keep the hands over the head.

Posterior chest wall: Ask the subject to fold the arms across the front of the chest which will move the scapulae laterally.

Rules of percussion:

- Place the middle finger of the left hand (Pleximeter finger) firmly against the chest, aligned in the intercostal space.
- Strike the centre of the middle phalanx of Pleximeter finger with the tip of your right middle finger (Percussing finger). Use a loose swinging movement of the wrist and not the forearm.
- The long axis of the pleximeter finger should be parallel to the border of the organ being percussed.
- Percussion is performed from a more resonant to less resonant area.

Methods of percussion:

- Direct percussion - Percuss directly over the medial third of clavicles without an intervening finger.
- Percuss the lung apices by placing the finger in the supraclavicular fossa.
- Continue the percussion downwards on the anterior chest wall, axillae and posterior chest wall covering all the lung areas.
- Always compare the percussion note on the two sides of the chest systematically, moving from one side to the other side. Do not percuss all the way down one side and then down the other.
- Normal lung produces a resonant note.

Percussion Notes

Resonant - Normal lung
Hyperresonant – Pneumothorax
Dull - Lung Consolidation, lung collapse, severe pulmonary fibrosis Stony dull - Pleural effusion

AUSCULTATION:

- The stethoscope is used to auscultate the breath sounds, added sounds and vocal resonance.
- Instruct the subject to take deep breaths with open mouth.
- With the diaphragm of the stethoscope, auscultate all areas of the lung.
- As with percussion, listen to the sounds in comparable positions on either side alternately, switching back and forth from one side to the other side.
- Normal breath sounds auscultated are known as Vesicular breath sounds. They are low pitched and have a rustling quality. There is no distinct pause between the end of inspiration and the beginning of expiration. Sound is heard throughout inspiration and only during one third of expiration.
- Abnormal breath sounds are known as bronchial breath sounds.

Bronchial breath sounds:

- It is a high-pitched breath sound with a hollow or blowing quality. The breath sounds are heard throughout inspiration and expiration. There is a distinct pause between the inspiration and expiration.
- Bronchial breath sounds are heard in pulmonary consolidation (pneumonia) and in fibrosis.

Causes for absence of breath sounds

Pleural effusion, Pneumothorax

Vocal Resonance:

- It is the auscultatory component of vocal fremitus.
- Ask the subject to repeat "ONE" and auscultate in all the lung areas consecutively on both sides to assess the quality and amplitude of vocal resonance.
- Vocal resonance is increased in consolidation and decreased in pleural effusion, pneumothorax and fibrosis.

ADVENTITIOUS OR ADDED SOUNDS:

These are abnormal sounds that arise in the lung or in the pleura.

Wheeze/ Rhonchi:

Wheeze is a continuous musical sound, heard due to narrowing of airways. It is louder and more persistent during expiration. It is heard in bronchial asthma and COPD.

Crackles/ Rales/ Crepitations:

Crackles are short, explosive sounds often described as bubbling sounds. Crackles may result from sudden opening of previously closed small airways. It may also be heard when air bubbles through secretions in major bronchi. It is heard in bronchiectasis and pulmonary edema.

Pleural rub:

It is a creaking sound produced when inflamed parietal and visceral pleurae slide over one another. It is heard in pleuritis.

Note: Examination of respiratory system is carried out in the following lung areas.

1. Supraclavicular
2. Infraclavicular
3. Mammary
4. Inframammary
5. Axillary
6. Infraaxillary
7. Suprascapular
8. Interscapular
9. Infrascapular

QUESTIONS:

1. What is the normal Respiratory rate?
2. What is Tachypnoea?
3. What is dyspnoea?
4. Name the muscles of inspiration and expiration.
5. Name one condition in which vocal fremitus is increased and decreased.
6. What are the normal breath sounds?
7. What are Wheezes and Crackles?

Experiment No.	RESPIRATORY EFFICIENCY TEST	Date

Aim:

To assess the efficiency of the respiratory muscles.

Apparatus:

Inch tape, BP apparatus, Peak flow meter, Match stick, Candle and Stop watch.

The following tests are performed:

1. Breath holding time
2. Expiratory blast test
3. Snider's test
4. Respiratory endurance test
5. Peak expiratory flow rate

1.Breath holding time (BHT):

Ask the subject to sit quietly for a few minutes breathing normally. Ask the subject to pinch his nostrils with the thumb and index finger and to hold the breath after a normal inspiration and start the stop watch. The time duration for which the subject is able to hold the breath is noted. Make three such observations at an interval of five minutes. Similarly, record the breath holding times after quiet expiration, deep inspiration and deep expiration.

Breath holding at the end of...	Time (sec)			Best value
	1st	2nd	3rd	
Quiet inspiration				
Quiet expiration				
Deep inspiration				
Deep expiration				

2. Expiratory blast test:

BP apparatus is required for this test. The rubber tube leading from the mercury reservoir to the cuff is disconnected. Ask the subject to take a deep inspiration and blow into the tube to raise the mercury column to the highest level possible. A normal subject can raise the mercury column to 65-100 mmHg or more during a single forceful expiration.

3. Snider's test:

A normal adult should be able to blow out a burning match stick or candle held at a distance of 30 cms in front of his face, with a single forceful expiration

4. Respiratory endurance test:

Ask the subject to take a deep breath, close his nostrils and blow into the rubber tubing to raise the mercury column to 40 mmHg level in the manometer. He is instructed to maintain the mercury level at 40mmHg as long as possible. Normal person can hold it at the same level for 40-70 seconds or more.

5. Peak expiratory flow rate:

Wright's Peak flow meter measures the maximum flow rate which is achieved during a single forced expiration. It does not measure the volume of air exhaled.

Procedure:

Ask the subject to take a deep breath and then to blow hard into the mouth piece of the flow meter forcefully with his nostrils closed. The reading on the dial is the PEFR in litres/min. Repeat the procedure thrice at an interval of 1-2 minutes. Normal range is 300-500 litres/minute.

QUESTIONS:

1. What is the normal breath holding time?
2. What is expiratory blast test?
3. Define PEFR? What is the normal value?
4. What are the uses of Wright's peak flow meter in clinical practice?

Experiment No.	SPIROMETRY	Date

AIM:

To measure Tidal Volume, Expiratory Reserve Volume and Vital Capacity in the given subject.

APPARATUS REQUIRED:

Student's Spirometer

SPIROMETER :

Student's Spirometer is also known as Vitalograph or Simple Spirometer. It consists of a double-walled metal cylinder. The outer container is filled with water in which a light-metal gas bell of 6 litre capacity floats. The water acts as an air-tight seal. The bell is attached to a counter-weight by a chain passing over a graduated frictionless pulley. The pulley bears a spring-mounted indicator needle that moves with the pulley and indicates the volume of air present in the bell. A corrugated rubber tube with a mouthpiece acts as the inlet tube.

PROCEDURE :

All the procedures should be done in standing posture.

Tidal Volume: The volume of air inspired or expired during quiet respiration

- Set the pointer at zero
- Ask the subject to take a few normal breaths with nostrils closed
- Ask the subject to breathe out into the mouth piece at the end of normal inspiration
- Record the reading as Tidal Volume

Expiratory Reserve Volume: The volume of air that can be expired forcefully after a normal expiration

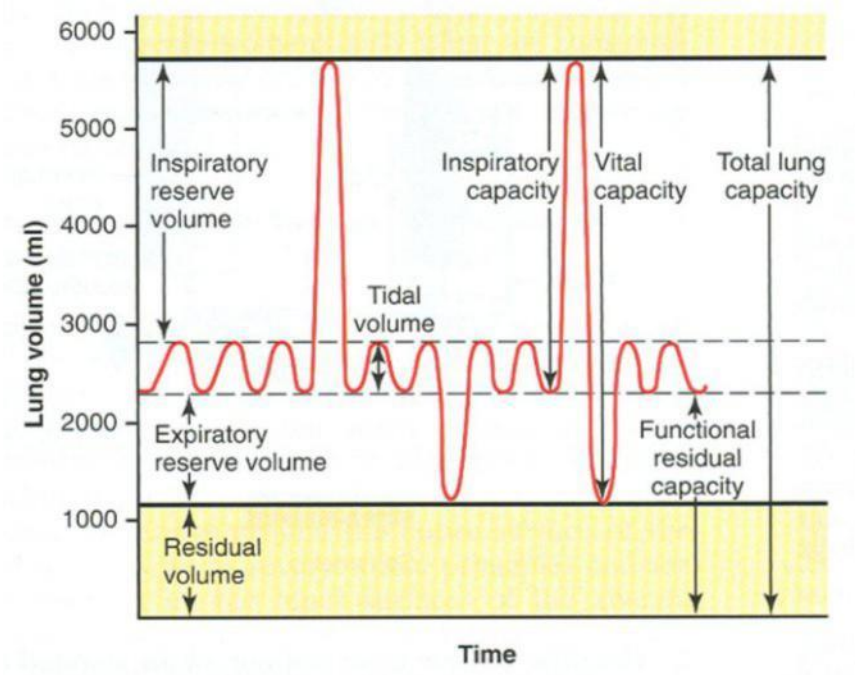
- Set the pointer at zero
- Ask the subject to take a few normal breaths with nostrils closed
- At the end of normal expiration ask the subject to breathe out forcefully into the mouthpiece to the maximum
- Record the reading as Expiratory Reserve Volume

Vital Capacity : The maximum volume of air that can be forcibly expired after maximum inspiration

- Set the pointer at zero
- Ask the subject to take a few normal breaths with nostrils closed
- Ask the subject to inspire to the maximum and then to expire forcefully and completely into the mouthpiece
- Record the reading as Vital Capacity

STUDENT'S SPIROMETER

LUNG VOLUMES AND CAPACITIES



RESULT:

Tidal Volume = _____
Expiratory Reserve Volume = _____
Vital Capacity = _____

QUESTIONS :

1. What are the factors affecting Vital Capacity ?
2. Name the volumes and capacities that cannot be measured directly using Student's Spirometer.
3. What is Breathing Reserve ?
4. What is Dyspnoeic Index ?
5. Give the normal values of Tidal Volume, Vital Capacity & Expiratory Reserve Volume in adult men and women.
6. Comment on FVC and FEV_1 % in Restrictive and Obstructive lung disorders.
7. Define Dead space. Write a note on Anatomical and Physiological Dead space.

OBSERVATIONS:

Name : Age :
Sex : Occupation :

Lung Volumes & Capacities	**Reading 1**	**Reading 2**	**Reading 3**
Tidal Volume			
Expiratory Reserve Volume			
Vital Capacity			

Experiment No.	STETHOGRAPHY	Date

AIM:

To record Respiratory movements using Stethograph in the given subject.

APPARATUS REQUIRED:

Stethograph , Marey's tambour, Kymograph, Stop watch, A glass of drinking water .

Stethograph consists of a corrugated rubber tube with one end sealed and the other end connected to Marey's tambour. Marey's tambour has a small metal cup with a rubber diaphragm. When the Stethograph is connected to the tambour, the pressure change in the Stethograph is transmitted to it. Pressure changes are recorded on a moving drum using a lever placed on the rubber diaphragm. During chest expansion, the volume of the stethograph increases and pressure decreases. This causes downward movement of the rubber diaphragm on the Marey's tambour. The lever on the Marey's tambour moves down. So inspiration is recorded as a downstroke.

PROCEDURE :

- Ask the subject to sit with his back towards the recording setup
- Tie the Stethograph at mid-chest level (4^{th} intercostals space)
- Connect the Stethograph to the Marey's tambour. Bring the writing lever in contact with the smoked drum
- Check for the movement of lever with movement of chest wall

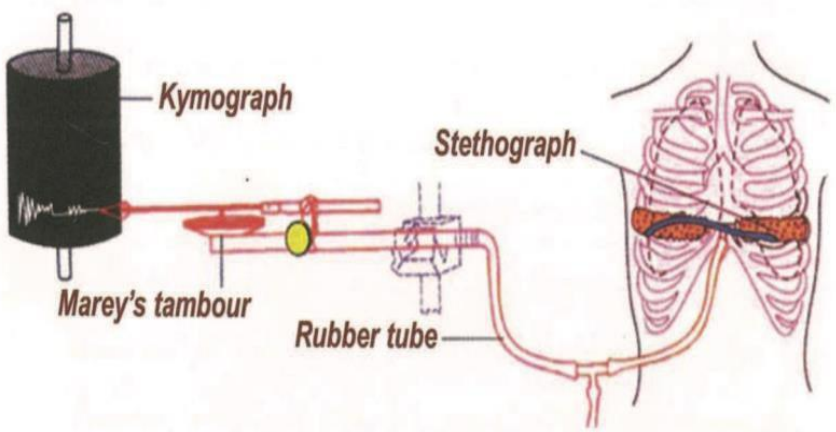
Normal Respiration :

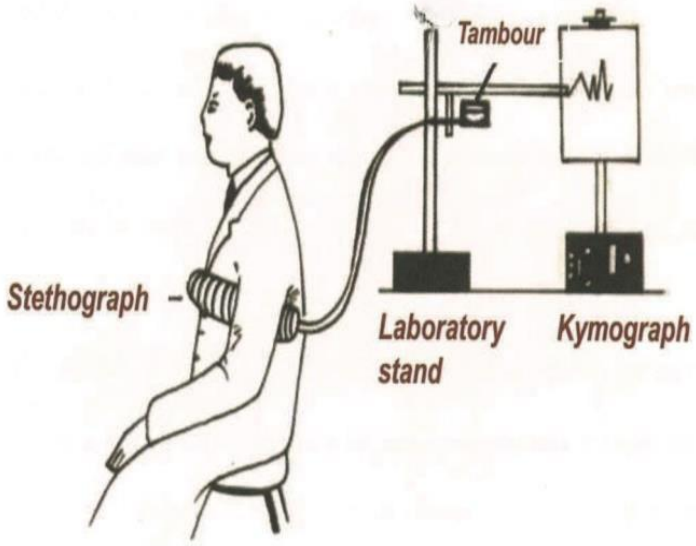
- Record few normal respiratory movements
- Upstroke of pointer corresponds to expiration and down stroke to Inspiration

Effect of Hyperventilation :

- Record few normal respiratory movements
- Ask the subject to hyperventilate for 2 minutes
- Record the findings
- Hyperventilation is followed by a short period of Apnoea. Apnoea is recorded as a straight line on the drum

STETHOGRAPHY





Stethograph

Effect of Swallowing :

- Record few normal respiratory movements
- Instruct the subject to drink from a glass of water continuously and record the effects
- Respiration stops during swallowing. It is recorded as a straight line. This is known as Deglutition Apnoea

Breath Holding time :

- Record few normal respiratory movements
- Instruct the subject to hold his breath for as long as possible at the end of normal Inspiration and record the Breath holding time. It is recorded as a straight line. This period is followed by respiratory excursions. This is called Breaking point
- Repeat the procedure at the end of Normal expiration, Maximum Inspiration and Maximum expiration and record the Breath holding time for each

Effect of Exercise :

- Record few normal respiratory movements
- Detach the tube and ask the subject to exercise for 3 minutes (sit ups)
- Re-attach the tube and record the respiratory movements
- Hyperpnoea –i.e. increased rate and depth of respiration is seen. Slowly the respiration returns back to normal

QUESTIONS :

1. What is Deglutition Apnoea ?
2. What is the effect of Exercise on respiration ?
3. What is the normal Breath-holding time in adults?
4. What is the physiological basis for Breaking point ?
5. Mention the changes in respiration following hyperventilation.
6. What is Periodic breathing ? Give few examples.

OBSERVATIONS: (Graphical observations)

Experiment No. EXAMINATION OF THE ARTERIAL PULSE Date

Definition:

Arterial pulse is the rhythmic expansion of the arterial wall due to transmission of pressure waves along their walls during each systole of the heart.

Procedure:

I . Examination of the arterial pulse is done to assess the following parameters:

1.Rate

2.Rhythm

3.Volume

4. Character

5.Condition of the vessel wall

6. Radiofemoral delay

7.Examination of other peripheral pulses.

Radial pulse is examined for Rate, Rhythm and Volume

Hold the subjects right arm in the semi prone position with slight flexion at the wrist. The radial pulse is palpated using the right index, middle and ring fingers by gently compressing the artery against the radial styloid process. Count for one full minute.

1. **Rate:**

Normal rate is 60─100 /min.

Bradycardia : When the pulse rate is less than 60/min

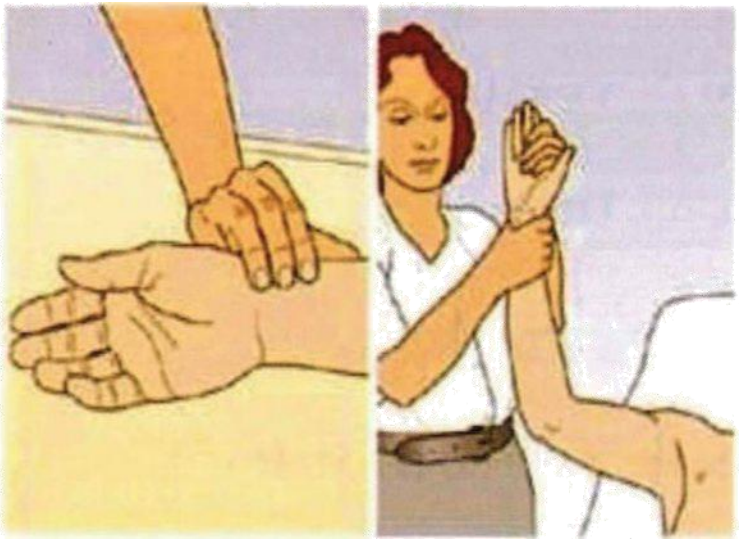
Physiological causes: Sleep, old age, trained athletes

Pathological causes : Heart blocks, Myxoedema, increased intracranial tension

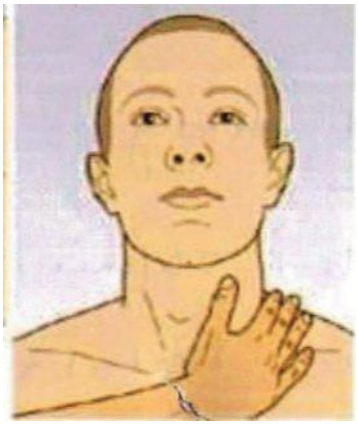
Tachycardia: When the pulse rate is more than 100/min .

Physiological causes: exercise, emotion, excitement and in newborn

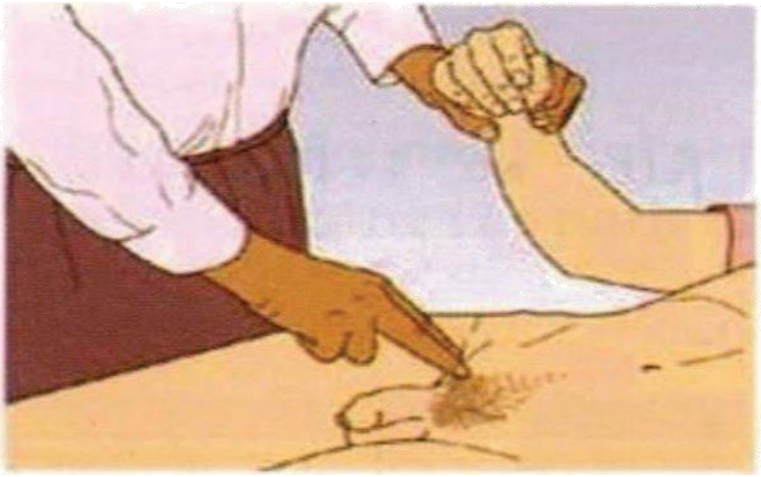
Pathological causes : fever ,anemia, thyrotoxicosis.



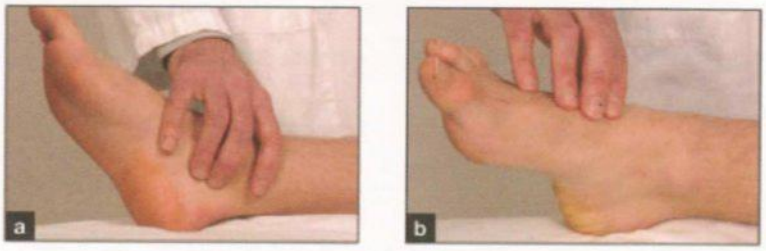
LOCATING AND PALPATING THE RADIAL PULSE , FEELING FOR COLLAPSING PULSE.



LOCATING THE CAROTID PULSE.



EXAMINING THE FEMORAL ARTERY AND SIMULTANEOUSLY CHECKING FOR RADIOFEMORAL DELAY



Feeling
(a) the posterior tibial artery
(b) the dorsalis pedis artery

2.Rhythm: When the interval between the pulse wave is constant, the pulse is said to be regular in rhythm .When the spacing is not constant the pulse is irregular .

Sinus arrhythmia : This is a normal finding .The pulse rate increases during inspiration and decreases during expiration.

Abnormal Rhythms (**Arrhythmias)**

Regularly irregular rhythm: Extrasystoles or ventricular premature contractions

Irregularly irregular rhythm: Atrial Fibrillation.

3.Volume:

It is the degree of expansion of arterial vessel wall during the passage of the pulse wavefront. The pulse volume depends on the stroke volume and arterial compliance .

High volume pulse or pulsus magnus is observed in aortic incompetence, beriberi, anemia and fever.

Low volume pulse or pulsus parvus is observed in aortic stenosis and shock.

4.Character: Character of the pulse wave is best appreciated by palpating the carotid artery in the neck.

Abnormal character of pulse:

Collapsing pulse: Also known as water hammer pulse, is characterized by rapid upstroke and rapid downstroke. It is observed in aortic regurgitation.

Pulsus alternans : A pulse waveform showing alternating strong and weak beats .It is observed in left ventricular failure.

5.Condition of the vessel wall:

It is assessed in the radial artery .The artery in the young individual is elastic and compliant .It becomes thickened like a cord due to atherosclerosis and calcification in old age. Hence it becomes palpable in them.

6.Radiofemoral delay :

Both radial and femoral artery are palpated simultaneously by the examiner .Normally, there is no delay between the appearance of pulse in the radial and femoral artery
Radiofemoral delay is seen in Coarctation of Aorta, where radial pulse is felt before femoral pulse.

EXAMINATION OF OTHER PERIPHERAL PULSES :

Radial pulse : Palpated against the radial styloid process.

Brachial pulse: Palpated in the antecubital fossa medial to the biceps tendon.

Carotid pulse: Palpated between the thyroid cartilage and anterior border of the sternocleidomastoid muscle .Do not examine the carotid pulse simultaneously on both sides.

Popliteal pulse :The subject's knees are flexed at an angle of 120° .The artery is palpated with the finger tips of both the hands placed in the popliteal fossa with the thumb resting on his patella.

Posterior tibial pulse: Palpated 1cm below and behind the medial malleolus .

Dorsalis pedis pulse: Palpated just lateral to the extensor hallucis tendon .

EXAMINATION OF THE VENOUS PULSE :

Measurement of venous waves in the neck helps to determine the right atrial pressure,which is transmitted to the right internal jugular vein .

- Ask the subject to lie down in a couch ,and raise the head end of the couch to 45°, so that the back of the subject is supported and the neck muscles are relaxed.
- Ask the subject to turn to the left and look for venous pulsations in between the two heads of sernocleidomastoid on the right side of his neck.

- In normal persons in this position, the venous pulse appears just at the upper border of the clavicle.
- Venous pulse above the clavicle in this position is considered as raised JVP.

It is measured in centimeter as follows:

Place a scale at the level of the top of the pulse wave in the neck horizontally Measure the vertical distance from sternal angle to the horizontal scale, by using another scale.

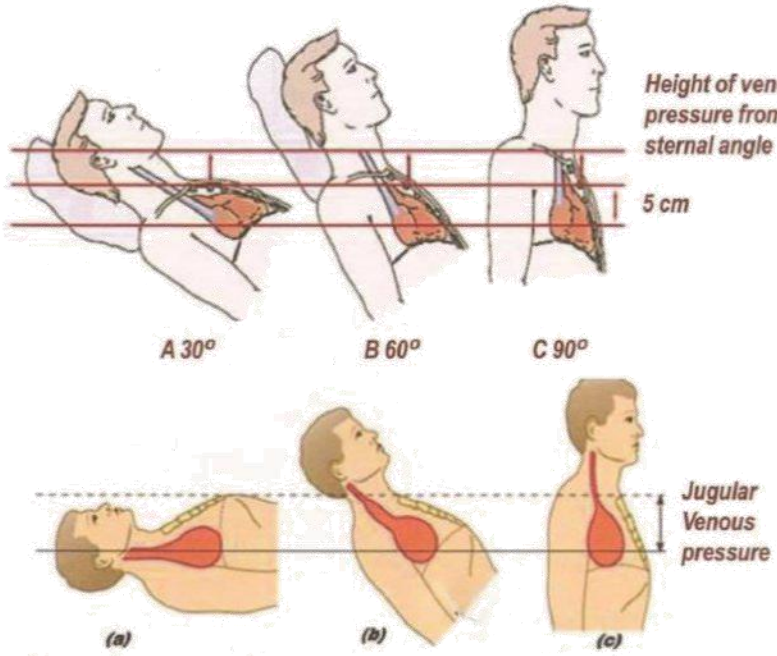
Pulsation in the internal jugular vein is more reliable as it directly reflects the pressure changes in the right atrium .

External jugular veins are not reliable because

1. External jugular veins has many valves.
2. External jugular vein passes through fascial planes ,in the neck.

The venous pulse can be differentiated from arterial pulse in the neck by

1. Venous pulse is better seen than felt .Arterial pulse is better felt.
2. Venous pulse has a definite upper level, which falls during inspiration.
3. Gentle pressure on the right hypochondrium will raise the venous pulse level due to a transient increase in venous return (Hepato jugular reflex).
4. By exerting moderate pressure above the clavicle with a finger, venous pulse can be obliterated.
5. Two to three waves can be seen in venous pulse.

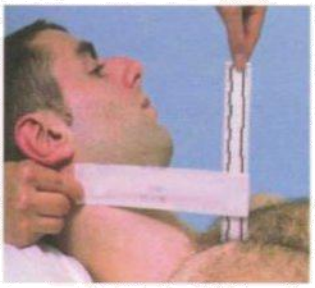


Jugular venous pressure

(a) Supine : Jugular vein distended, pulsations not visible

(b) Reclining at 45 angle : Point of transition between distended and collapsed, vein can usually be seen to pulsate just above the clavicle

(c) Upright: upper part of vein collapsed and transition point obscured.



Measurement of Jugular venous pulse and jugular venous pulse tracing

QUESTIONS:

1. Define pulse.
2. What is the cause for sinus bradycardia in athelets?
3. How will you examine for collapsing pulse? Name a condition producing collapsing pulse?
4. What is pulse deficit?
5. What is pulsus alternans?
6. What is pulsus paradoxus?
7. What are the waves seen in JVP?

Experiment No. EXAMINATION OF CARDIOVASCULAR SYSTEM Date

The cardiovascular system is examined to assess the functions of the heart and blood vessels as follows:

1. General Examination
2. Examination of Arterial Pulse
3. Examination of the Jugular Venous Pulse / Pressure
4. Examination of Blood Pressure
5. Examination of Precordium

1.GENERAL EXAMINATION (Refer chapter on General examination)

2.EXAMINATION OF ARTERIAL PULSE (Refer chapter on Arterial Pulse)

3.EXAMINATION OF JVP (Refer chapter on Venous Pulse)

4. EXAMINATION OF BLOOD PRESSURE (Refer chapter on Blood pressure)

5. EXAMINATION OF PRECORDIUM

Precordium is the anterior aspect of the chest wall overlying the heart. Examination of precordium is done under the following headings.

INSPECTION:

- Examine the subject in sitting posture in good day light , stripped to the waist so as to expose the precordium fully.
- Shape and Symmetry of the chest wall
- Tracheal position
- Apical Impulse

- Abnormal pulsations over the precordium
- Skeletal deformities - Abnormal bulge/ depression over the precordium
- Dilated veins over the precordium

    - Look for the apical impulse in relation to the left nipple. Apical impulse is usually seen just below and medial to the nipple.
    - Look for pulsation in the other areas of the precordium, especially pulmonary, aortic and left parasternal area.
    - Inspect for any bulge in the precordium and any dilated and engorged superficial veins in the precordium. Precordial bulge is seen in congenital heart disease. Engorged veins indicate superior or inferior venacaval obstruction.

PALPATION:

(Refer Respiratory system for inspection and palpation of Tracheal position)

- Confirm the tracheal position
- Confirm the apical impulse
- Palpate for any thrill
- Palpate for parasternal heave
- Palpate for any abnormal pulsations

Apical Impulse / Apex beat:

Apical impulse is defined as the lowermost and outermost definite cardiac Impulse. It is normally located in the left fifth intercostal space half an inch medial to mid clavicular line.

First, place the palm on the precordium to feel the apical impulse and the n place the ulnar border of the palm horizontally in the intercoastal space where the pulsation is felt. Finally the apex beat is localised by the tip of the index finger C count the intercostal spaces with reference to the sterna angle (Angle of Lewis) – the most prominent point on the sternum. The second rib joins the manubrium sternum. The space below the second

rib is the second intercostal space and accordingly other intercostals spaces are counted. Note the position of the apex in the intercostals space with relation of midclavicular line.

If the apex beat is not palpable in the supine posture, ask the subject to sit and lean forward. If apex beat is still not palpable, palpate the corresponding area of the chest on the right side. Despite all efforts apex beat may still not be palpable for following reasons.

a) Located behind a rib

b) Chest wall may be thick due to fat or muscle.

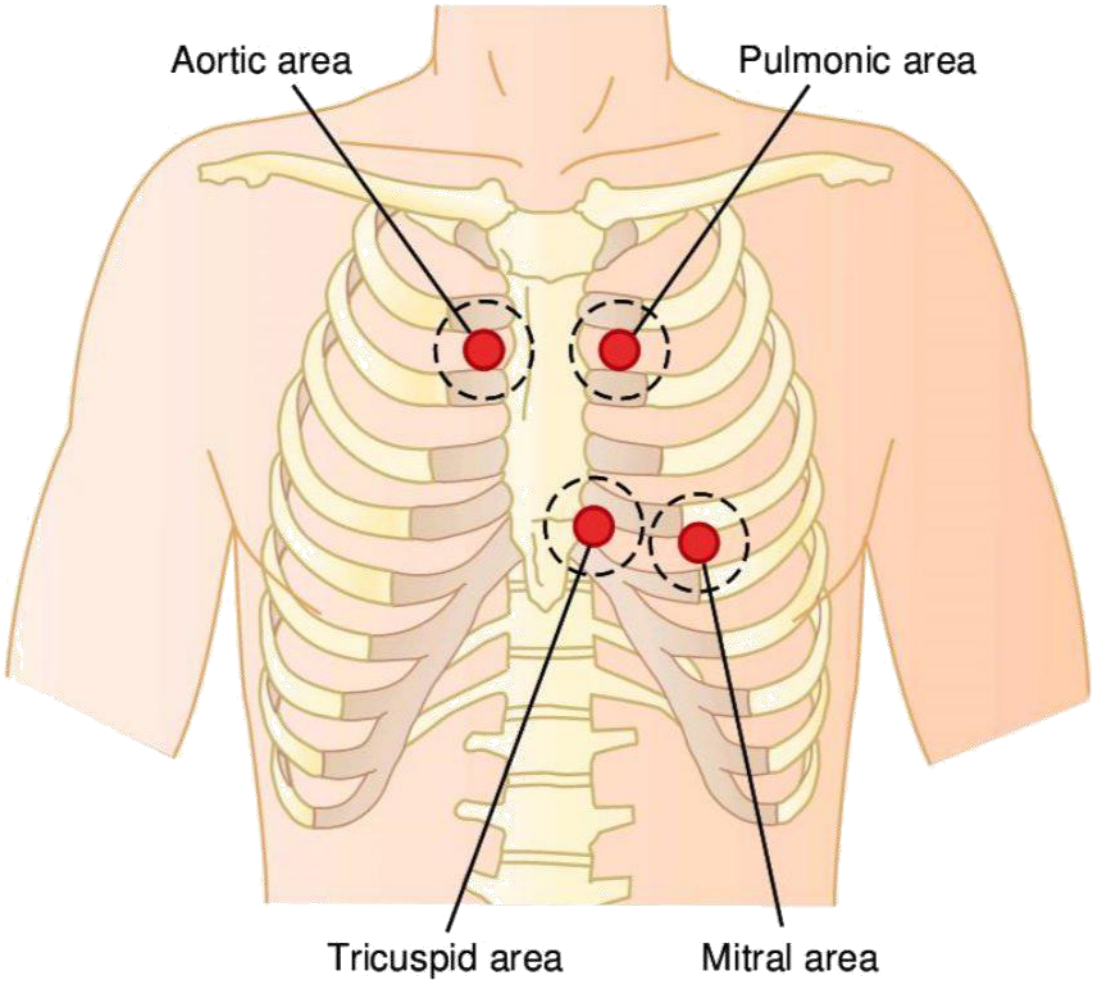
c) Emphysematous lung may cover part of the heart.

d) In females, the breast may be pendulous.

Normally the apical impulse just touches and lifts the examining finger. The abnormal characters are : Tapping , Hyperdynamic and Heaving apical impulse.

Thrill:

Palpate all over the precordium for thrill with the palm of the hand. A palpable murmur is called as thrill which is due to vibrations from heart or the great vessels. It is due to abnormal flow through a normal valve or a normal flow through an abnormal valve. Thrill is felt as a feeling similar to the purring of the cat.


Aortic area
Pulmonic area
Tricuspid area
Mitral area

Parasternal Heave

The ulnar border of the palm is kept along the left parasternal border vertically. A definite sustained lift felt for a while is which is called Parasternal heave. It is caused by Right ventricular hypertrophy.

PERCUSSION:

Follow the rules of percussion as explained inrespiratory system Right border:

Percuss anteriorly in the mid-clavicular line on the right side downwards till the liver dullness is made out. Move one space above. Then percuss from right anterior axillary line medially till dullness is heard. Similarly percuss from lateral to medial in all the spaces above one by one and connect all points by an imaginary line which gives the right border of heart.

Left border:

Start percussion from the midaxillary line in the intercostals space where apical impulse is felt. Percuss medially in the intercostals space till you reach the dullness. Usually it coincides with apical impulse. Similarly percuss in all spaces above from mid axillary line medially and note all the points of dullness and connect. This gives the left border of the heart.

AUSCULTATION:

Auscultate with diaphragm of the stethoscope in the following area.

Mitral area: Corresponds to Apical impulse (left 5^{th} intercostals space half an inch medial to midclavicular line.

Tricuspid area: It lies close to lower end of the left border of sternum.

Aortic area: It lies in the right 2^{nd} intercostals space close to sternum.

Pulmonary area: It lies in the left 2^{nd} intercostals space close to sternum.

While auscultating an area, feel for the carotid pulse in the neck simultaneously. The sound which coincides with the carotid pulse is the first heart sound. The heart sound that occurs in between two successive pulsations of the carotid artery is the second heart sound.

1st heart sound (S1) is produced by closure of AV valves.

2nd heart sound (S2) is produced by closure of semilunar valves.

3rd heart sound (S3) is due to vibrations set up by rapid ventricular filling.

4th heart sound (S4) is due to atrial systole.

First and second heart sounds are heard normally. Third heart sound is physiological in children and young adults. Fourth heart sound is not heard and can be recorded only with phonocardiogram.

The second heart sound may be split. Split S2 is better appreciated in thin individuals, in pulmonary and aortic area. The Aortic component appearing before the pulmonary component.

Added sounds : Murmurs, Opening snap, Ejection click, Pericardial rub.

Murmur: These are abnormal sounds which are produced due to turbulence in blood flow at or near a valve or an abnormal communication within the heart. It may be systolic or diastolic or continuous.

QUESTIONS:

1. Define Apical impulse.
2. Mention the conditions in which the apex beat is abnormally placed.
3. Mention few causes for systolic and diastolic murmurs.
4. What are the characteristics of S1 and S2?
5. What is pericardial effusion?

Experiment No.	**RECORDING OF BLOOD PRESSURE**	**Date**

Definition:

Blood pressure is the lateral pressure exerted by the column of blood on the walls of the arteries.

Aim:

To record the blood pressure of the given subject at rest.

Apparatus required :

Sphygmomanometer and Stethoscope.

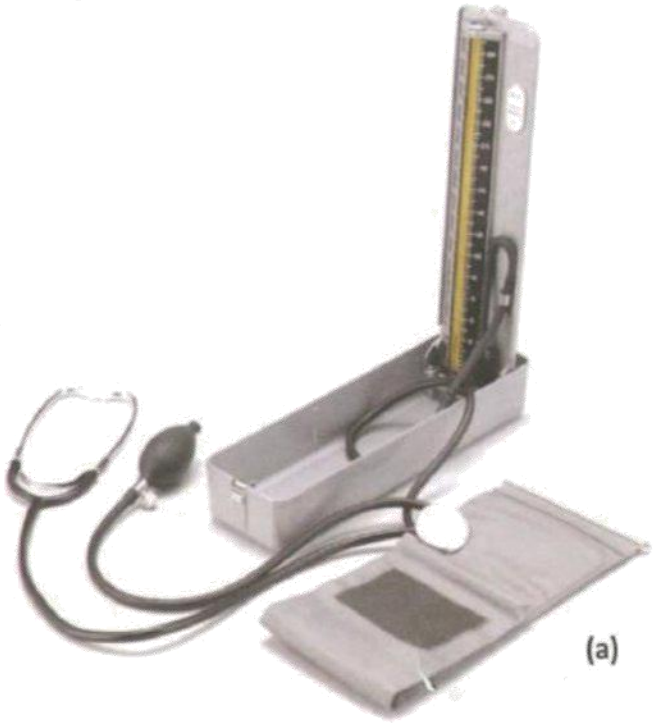
Description of Sphygmomanometer:

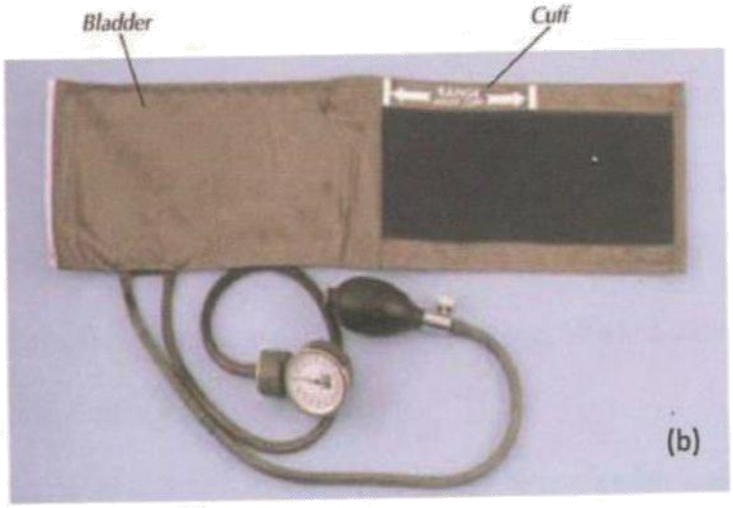
The Sphygmomanometer consists of mercury manometer, cuff and air pump.

Mercury manometer : It has two limbs. The broader limb is the reservoir and narrow one is graduated from 0 to 300mm.Mercury reservoir is connected to the Riva Rocci cuff through a rubber tube.

Riva Rocci cuff: It is an inflatable rubber bag .Two tubes are attached to the bag .One transmits air pressure to mercury manometer and other is connected to the air pump. The length of the rubber bag should be two thirds and its width should be one third of the mid arm circumference of the subject.

Airpump: It is a rubber hand bulb provided with a one way valve and a leak valve arrangement. A rubber tube connects the air pump to the Riva Rocci cuff .The cuff can be inflated and deflated using the handpump.





a)**MERCURY MANOMETER**

b)**ANEROIDMETER**

Principle:

The arterial BP is balanced against the pressure in the Riva Rocci cuff. Whenever the cuff pressure is raised above the systolic BP, the artery is occluded. When the cuff pressure becomes equal to or just below the Systolic BP, flow is restored through the artery. But the flow is turbulent. This is the reason for Korotkoff sounds. When the cuff pressure is lowered below Diastolic BP, once again streamline flow is restored and hence korotkoff sound disappears.

Procedure :

Palpatory Method:

Subject is made to sit or lie down at ease on a couch. He is allowed to rest for few minutes .The Riva Rocci Cuff is wrapped around the middle of the arm,so that the lower edge of the cuff is two finger breadths above the antecubital fossa. It should neither be too loose nor too tight. Zero level of the manometer should be checked. The cuff around the arm and the manometer are kept at the level of the heart to avoid the effect of gravity. Palpate the radial artery pulse.

The pressure in the cuff is raised gradually until the radial pulse is no longer felt .The pressure at which the radial pulse disappears is noted .Now the pressure in the cuff is raised further and then slowly brought back by deflating the cuff. The pulse now reappears. The reading at which the pulse reappears is also noted .The disappearance and reappearance of the pulse coincides .This value is the approximate Systolic blood pressure.

Auscultaory method:

The chest piece of the stethoscope is placed on the brachial artery in the antecubital fossa .The pressure in the cuff is raised to 20 to 30 mmHg above the systolic blood pressure determined by the palpatory method. While auscultating the brachial

artery, the pressure in the cuff is lowered gradually .The reading in the manometer at which faint tapping sound is heard, is noted down. This is the systolic blood pressure.

As the pressure in the cuff is further lowered, the sound undergoes a series of changes both in quality and intensity. Note down the reading at which the auscultatory sounds disappear. This is the diastolic blood pressure .Sometimes muffling of sounds also can be taken as diastolic BP. The ausculatory sounds heard during measurement of Blood pressure are called korotkoff's sounds.

Phases of koratkoff sounds :

Phase I : Faint ,tapping sound .

Phase II : Sound becomes murmurish in quality .

Phase III: Sound becomes clearer and louder .

Phase IV: Sound becomes muffled. The sound then disappears.

Palpatory method has to be done prior to auscultatory method because,

It gives a rough value of systolic blood pressure, It helps to avoid missing of Auscultatory gap.

Systolic BP by palpatory method (mmHg)	Systolic BP by auscultatory method (mmHg)	Diastolic BP by auscultatory method (mmHg)	Pulse pressure (SBP─DBP) mmHg	Mean arterial pressure (DBP+1/3PP) mmHg

Experiment No. **EFFECTS OF POSTURE AND EXERCISE ON BLOOD PRESSURE** **Date**

Aim:

To record the effects of changes in posture and physical exercise on blood pressure.

Apparatus required :

Sphygmomanometer and stethoscope

Procedure

Effects of changes in posture on BP

- The subject should be mentally and physically relaxed.
- Record resting blood pressure of the subject in supine posture by palpatory and ausculatory method.
- The deflated cuff is still wrapped around his arm.
- Now the subject is instructed to stand up swiftly from the lying posture and to lean against a support (the couch).
- Immediately record his blood pressure by auscultatory method .Record blood pressure again at the end of 2min and 5min on standing.

Discussion:

- When an individual stands up immediately from recumbent posture, there is pooling of blood in the legs.

- ❖ The venous return to the heart is reduced thereby reducing the cardiac output. Hence, the systolic blood pressure will fall, which may be recorded within 15seconds. This will be brought back to normal by Baroreceptor reflex within 15 to 30 seconds .Hence, a fall in systolic BP is usually difficult to record in normal subjects.
- ❖ This is a function of autonomic nervous system (ANS).

Effect of change in posture on blood pressure:

Posture	Systolic BP by palpatory method (mmHg)	Systolic BP by auscultatory method (mmHg)	Diastolic BP by auscultatory method (mmHg)
Supine			
Immediately on standing	Not to be done		
2min	Not to be done		
5min	Not to be done		

Effect of exercise on blood pressure:

Procedure:

- Record resting blood pressure in sitting posture by palpatory and ausculatory method.
- The Riva Rocci cuff is disconnected from the BP apparatus.
- The cuff is still wrapped around the subject's arm.
- The subject is instructed to perform physical activities like on the spot jogging for five minutes or ten sit ups
- Immediately after the exercise, BP is recorded by auscultaory method without any time delay.

Discussion:

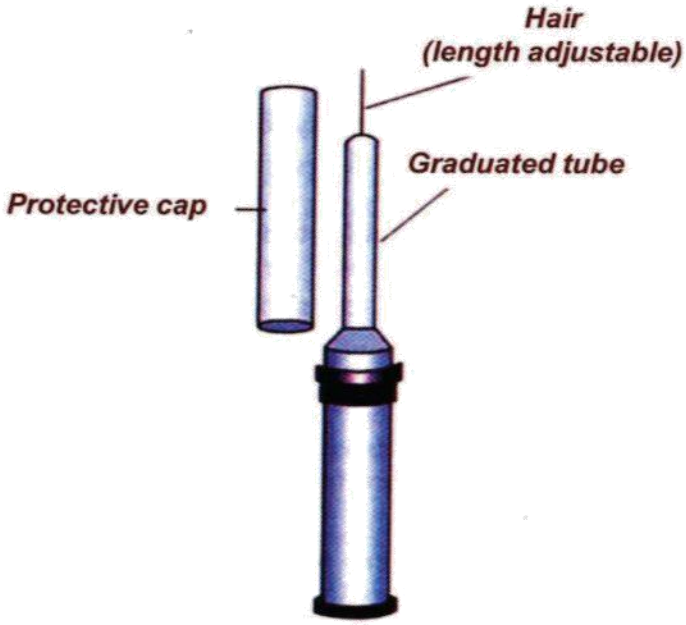
Systolic BP will show a rise due to increase in the heart rate and cardiac output. This is due to increased activity of sympathetic nervous system .Diastolic BP will not change in mild to moderate exercise. In severe exercise, diastolic BP may fall due to local vasodilatation in working muscles which will decrease the peripheral resistance.

Effect of exercise on blood pressure:

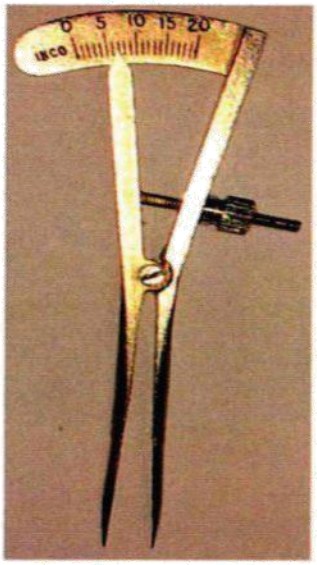
	Systolic BP by palpatory method (mmHg)	Systolic BP by auscultatory method (mmHg)	Diastolic BP by auscultatory method (mmHg)
BP at rest			
Immediately after exercise	Not to be done		
2 min after exercise	Not to be done		
5 min after excercise	Not to be done		

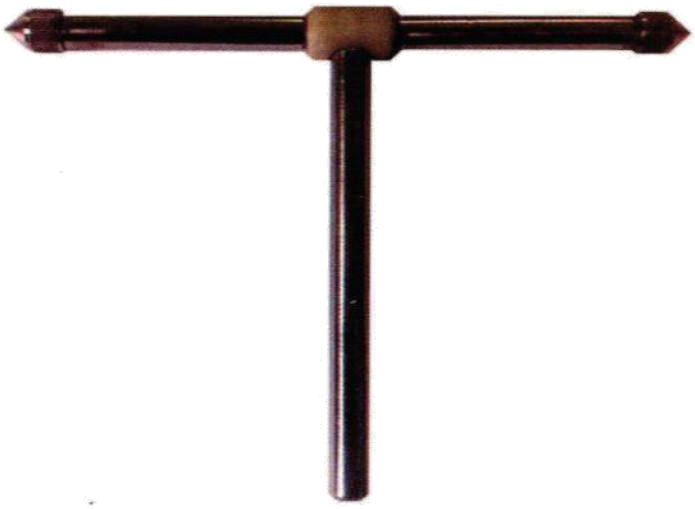
QUESTIONS:

1. Define Blood pressure.
2. What is pulse pressure ?
3. How will you calculate Mean arterial pressure.?
4. What is the importance of palpatory method ?
5. What is an auscultatory gap?
6. What are korotkoff's sounds?
7. What is postural hypotension?



Hair aesthesiometer





Compass aesthesiometer **Thermal aesthesiometer**

Experiment No. EXAMINATION OF THE SENSORY SYSTEM Date

Pre-requisites for the examination

- Before starting the procedure explain the nature of the test to be performed. Ask the subject to close the eyes or turn the face to the other side whenever necessary.
- Apply uniform sensory stimulus and examine dermatome wise on both sides.

Following sensations are tested

1. Tactile sensation - light touch, crude touch, tactile localisation and discrimination
2. Position sense:
3. Vibration sense
4. Pain – superficial and deep
5. Temperature sense- warmth and cold
6. Stereognosis:
7. Romberg's sign

1.TACTILE SENSATION: (Tested with eyes closed)

a) Fine touch (Light (touch):

a) Ask the subject to say 'yes' or raise his finger when he perceives the sensation of touch.
b) With a wisp of cotton wool, lightly touch the different parts of the body and compare with the corresponding area on the opposite side dermatome wise
c) Also enquire whether he perceives it normally or differently.
d) It can also be elicited by Von frey's hair aesthesiometer.

b) Crude touch (Pressure sense):

a) Pressure is the sustained touch sensation.

b) Elicit the pressure sensation by pressing with your finger tip on corresponding areas of both sides dermatome wise

c) Tactile localisation:

a) It is the ability to localise the touch with a ball pen tip.

b) Measure the distance between the two points (*localisation distance*).
Tested in all dermatomes on both sides.

Localisation distance varies in different parts of the body. It corresponds with the density of touch receptors.

d) Two point discrimination:

a) Instruction is given to the subject to say whether he feels the touch as one point or two points when he is touched by a compass aesthesiometer

b) Start with minimum distance of 1mm, touch the skin of the subject lightly with the two points simultaneously.

c) Ask the subject to say whether he is being touched by one or two points.

d) If the subject says one, increase the distance gradually till two separate points are appreciated by him. This is the *minimum separable distance.*

e) Record the minimum separable distance on different parts of the body on both the sides.

(Minimum separable distance varies in different parts of the body - about 2mm on finger tips, 5mm on hands and maximum on the back of the trunk which is about 5cm. This is due to the difference in distribution of sensory receptive fields.)

Anaesthesia – loss of all sensations

Hypoaesthesia – Reduced touch sensation

Hyperaesthesia - When the response to the sensory stimulus is exaggerated.
eg.thalamic lesion

Paraesthesia - Perverted touch sensation (Touch may produce unpleasant sensation)

2. SENSE OF POSITION: (to be done with eyes closed)

The appreciation of passive movement

Hold the finger and fix the interphalangeal joint. Passively flex and extend the finger holding the joint, and leave it in some definite position . Ask the subject to keep the corresponding finger in similar position .Perform the test on all the upper and lower limb small and large.

(Movements of less than 10° are appreciated at all the normal joints).

3. VIBRATION SENSE: (to be done with eyes closed)

a) Place the base of a vibrating tuning fork (frequency 128Hz) over bony prominences like styloid process of the radius ,the lower end of the tibia, and medial or l ateral malleolus.

b) Ask the subject if he perceives vibrations and instruct him to raise his hand when he ceases to feel the vibration.

c) Immediately place tuning fork on the corresponding bony prominence of the examiner. If the examiner can still perceive it, the subject's perception of vibration is impaired.

4. PAIN SENSATION (to be done with eyes open)

Superficial pain:

a) Explain the procedure
b) Elicit superficial pain by gently pricking the skin with a pin
c) Ask the subject if he perceives the pain
d) Test pain in all the dermatomes on both sides.

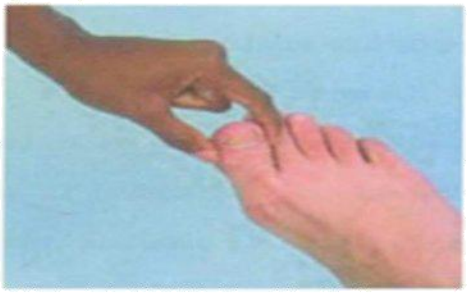
Deep pain:

Elicit Deep pain by squeezing the deeper structures such as the muscle or the tend on. It can also be quantified using Algometer.

Analgesia is loss of pain sensation

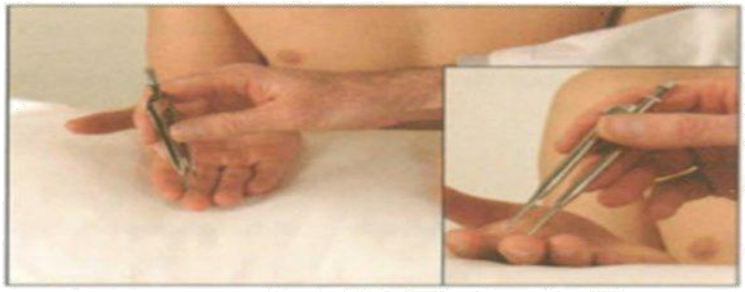
Hypoalgesia is partial loss of pain sensibility

Hyperalgesia is a condition of exaggerated sensibility to pain



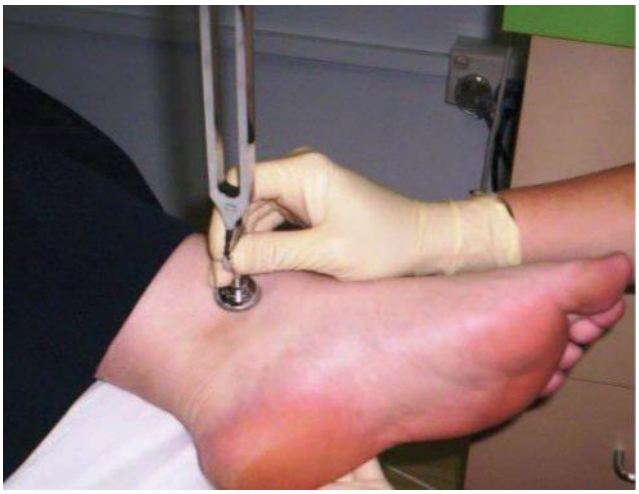
Position sense

Pain sense



Two point discrimination

VIBRATION SENSE



5. THERMAL SENSE:

a) Take two test tubes containing warm and cold water separately
b) Give proper instructions to the subject (to say whether he feels cold or warm when touched with different glass tubes)
c) Place the bottom of the test tubes on the skin of the subject each 'in turn or randomly' and ask the subject to say what he feels
d) Test and compare all the dermatomes.

6. STEREOGNOSIS: (to be done with eyes closed)

a) Place a familiar object in subject's hand
b) The subject should identify the object by its shape,texture, size, etc. and should tell what it is
c) Repeat the procedure with 4 to 5 familiar objects
d) Repeat the procedure in the other hand

Ability to recognize the known objects purely from the feel of its shape and size with eyes closed is called *stereognosis.*

Tactile localization, tactile discrimination and stereognosis are known as *cortical sensations* as intact parietal association area is needed.

7. ROMBERG'S SIGN:

Ask the subject to stand with his feet close together and then to close his eyes. Swaying of the body from side to side will occur if the posterior column is affected. The position sense from the legs is lacking, hence the subject becomes unsteady on closing the eyes.

This is a test for the loss of position sense (*sensory ataxia*) in the legs. It is not at a test for cerebellar function. This is a test to differentiate sensory ataxia from cerebellar ataxia. In *cerebellar ataxia*, the subject is unsteady with his feet together even with eyes open.

S.No.	SENSES	CARRIED BY THE TRACTS
1.	Fine touch: Tactile localization Tactile discrimination, position sense, vibration sense and stereognosis	Dorsal / posterior column
2.	Crude touch	Anterior spinothalamic tract
3.	Pain & Temperature	Lateral spino-thalamic tract

CLINICAL EXAMINATION OF SENSORY SYSTEM

NAME: AGE:

SEX: OCCUPATION:

SENSATION	**CERVICAL**		**THORACIC**		**LUMBAR**		**SACRAL**	
	Right	**Left**	**Right**	**Left**	**Right**	**Left**	**Right**	**Left**
Fine touch								
Crude touch								
Tactile localisation								
Two point discrimination								
Joint & position								
Vibration								
Superficial pain								
Deep pain								
Temperature Cold Warm								
Stereognosis								
Romberg's sign								

INFERENCE:

QUESTIONS:

1. What is a dermatome?
2. Name the receptors for pain.
3. What are the types of pain?
4. Name the sensations lost in posterior column lesions of the spinalcord.
5. What are cortical sensations?.
6. What are Brodmann's area numbers for primary and secondary sensory area?
7. What is the frequency of the tuning fork used to test vibration sense?
8. What is astereognosis?

Experiment No. EXAMINATION OF THE MOTOR SYSTEM Date

Examination of the motor system is carried out by assessing,

A Nutrition or bulk of the muscle
B Tone of the muscle
C Power or strength of the muscle
D Co-ordination of movements
E Reflexes
F Gait
G Involuntary movements

A NUTRITION OR BULK OF THE MUSCLE:

A Inspect the different groups of muscles of the body on both sides for any wasting or contractures.

B Palpate the muscles and feel for the consistency. Atrophic muscle appears smaller and feels soft and flabby. Hypertrophic muscle appears bulky and are firm in consistency.

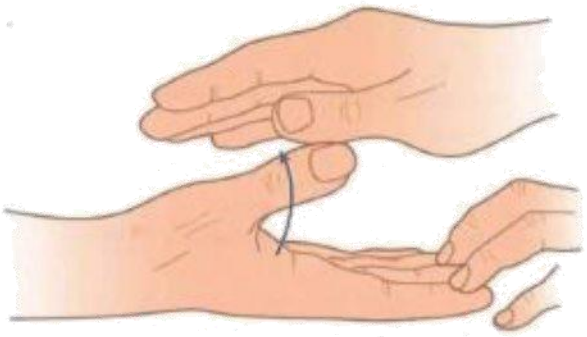
C Bulk of the muscle is assessed by measuring the circumference of the limbs at point of maximum bulk with reference to a definite bony landmark using an inch tape.

D Bony landmarks

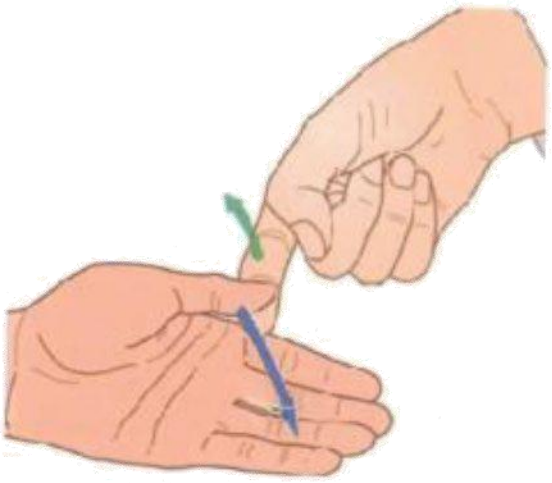
Upper limb – Arm circumference with reference to acromian or olecranon process.Forearm circumference with reference to olecranon process.

Lower limb – Thigh and leg circumference with reference to tibial tuberosity. e.

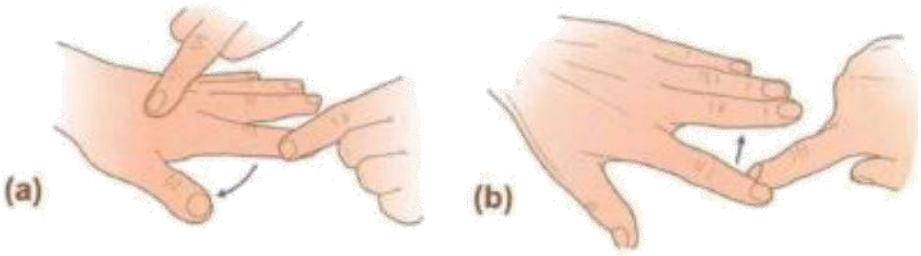
Compare the bulk of both limbs at the same reference point.



Testing the abductor pollicis brevis.



Testing the opponens pollicis



Testing the first
(a) Dorsal interosseous muscle
(b) Palmar interosseous muscle

2. TONE:

Muscle tone is a state of partial contraction of muscle at rest.

A. Ask the subject to keep the limb relaxed. Hold the limb and do passive movements on various joints and observe the degree of resistance offered by the muscle.

B. The degree of resistance offered by the muscle indicates the tone of the muscle.

C. Compare the tone of the muscles on both sides.

Hypertonia is increase in muscle tone. It is seen in upper motor neuron lesions. It is of two types: spasticity and rigidity.

A ***Spasticity:*** Hypertonia of the corticospinal tract lesion is of Clasp knife type and it involves one group of muscles either the agonist or the antagonist muscles. It is maximum in the flexors of the upper limb and extensors of the lower limb.

B ***Rigidity:*** Hypertonia of the extrapyramidal tracts lesion, involves both the agonist and the antagonist muscles. In Cog wheel rigidity, the resistance offered is intermittent. In Lead pipe rigidity, the resistance is felt throughout the range of movement.

Hypotonia is decrease in muscle tone. It is seen in lower motor neuron lesions. Eg: Poliomyelitis and cerebellar lesions.

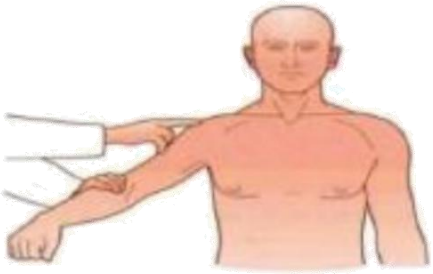
6. POWER OR STRENGTH OF THE MUSCLES:

a. Expose the muscle to be tested. Ask the subject to perform the movement as instructed and offer resistance. Observe the contraction of the muscle. Compare strength of same group of muscle on the other side

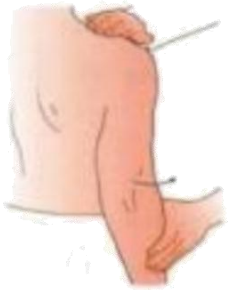
b. The strength of the muscle is assessed by comparing the strength of the examiner.

Testing elbow flexion

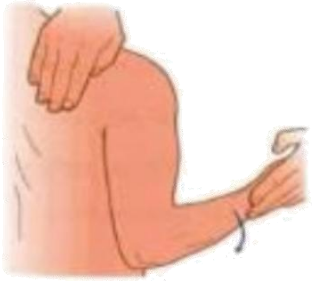
Testing elbow extension
(Triceps)



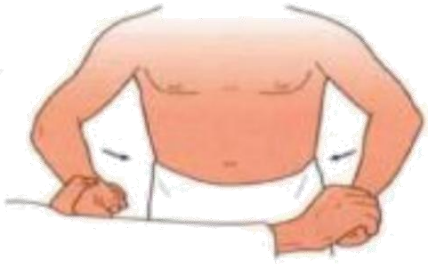
Testing the deltoid



Testing the
supraspinatus



Testing the
infraspinatus



Testing the pectoralis major

Grading of power:

Grade 0 : Complete paralysis. Grade 1 : Flicker of contraction only.

Grade 2 : Muscle movement only when gravity is eliminated

Grade 3 : Muscle movement against the force of gravity, but not against resistance. Grade 4 : Some degree of weakness, usually described as poor, fair or moderate strength. Grade 5 : Normal power.

a) Testing muscles of the upper limb:

1. Abductor pollicis brevis*:*

Ask the subject to abduct the thumb against resistance in a plane at right angle to the palmar aspect of the index finger.

2. Opponens pollicis:

Ask the subject to touch the tip of his little finger with the point of his thumb against resistance without moving the little finger.

3. **First dorsal interosseous:**

Ask the subject to abduct his index finger against resistance.

4. Interossei and lumbricals: Dorsal interossei:

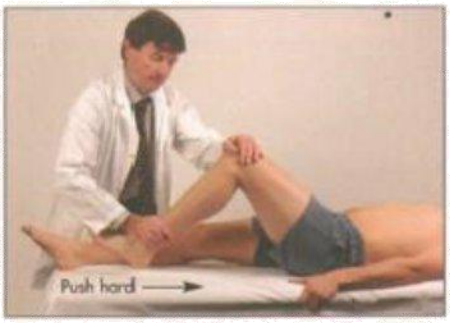
Ask the subject to abduct the fingers against resistance. The dorsal interossei causes the abduction of the fingers at the metacarpophalangeal joint.

Palmar interossei:

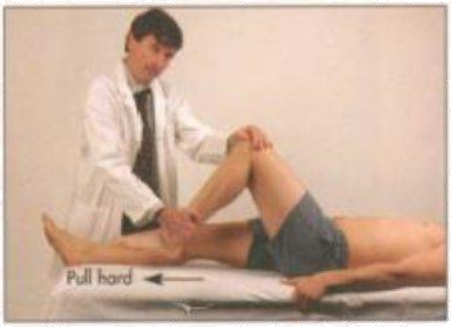
Place the paper between the fingers and ask the subject to hold it. Try to pull the paper out. The palmar interossei causes adduction of the fingers at the metacarpophalangeal joint.

Lumbricals:

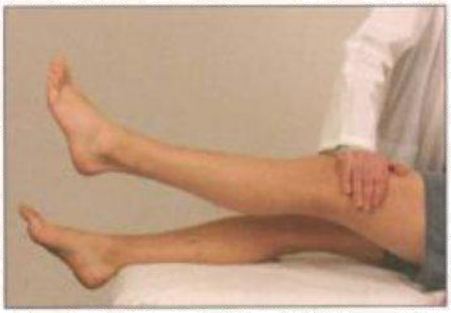
Ask the subject to flex the metacarpophalangeal joints and to extend the distal interphalangeal joints against resistance.



Testing knee extension:
'Straighten your knee and don't let me bend it'



Testing knee flexion:
'Bend your knee and don't let me straighten it'



Testing hip flexion:
'Lift your leg up and don't let me push it down'

5. Flexors of the fingers:

Ask the subject to squeeze the examiner's index and middle fingers on both sides and assess the force of grip.

6. Flexors of the wrist:

Ask the subject to flex the wrist against resistance.

7. Extensors of the wrist:

Ask the subject to make a fist and hold the hand with palm facing downwards. Offer resistance when the subject is asked to extend the wrist.

8. Brachio-radialis:

Ask the subject to place the forearm in mid prone position, and flex the forearm. Offer resistance by grasping the hand.

9. Biceps:

With the forearm in full supination, ask the subject to flex the forearm against resistance.

10. Triceps:

The subject is asked to extend the flexed forearm against resistance.

11. Supraspinatus:

Ask the subject to keep the arm by the side of the body and to lift his arm at right angles to his side against resistance. The first $30^0$of abduction is carried out by supraspinatus. The remaining 60^0 is by the deltoid.

12. Deltoid*:*

Ask the subject to make forward and backward movement of the abducted arm against resistance.

13. Infraspinatus*:*

Ask the subject to flex the forearm at right angle and tuck the elbow to his side. Ask to rotate his forearm outwards against resistance. The elbow has to be tucked to the side throughout.

14. Pectoralis:

Ask the subject to stretch his arms out in front of him and to clasp his hands together while the examiner attempts to hold them apart.

15. Serratus anterior:

Ask the subject to push forward his hands against a wall. If there is paralysis of serratus anterior, winging of scapula occurs on that side.

16. Lattisimus dorsi:

Ask the subject to clasp his hands behind his back while the examiner stands behind him and offers passive resistance.

D. **Muscles of the trunk: 1.**

Abdominal muscles*:*

With the subject in supine position, ask the subject to lift up his head against resistance.

In paralysis of lower abdominal segment, umbilicus moves up. When the upper segment is paralysed umbilicus is pulled down. This is called **Beevor's sign.**

In weakness of the abdominal muscles, the subject will not be able to sit up in bed from the supine position without the aid of his hands**(Babinski's rising up sign).**

2. Muscles of the back: (Erector spinae*).*

Ask the subject to lie with his face down and to raise his head from the bed by extending his neck and the back. Muscles of the back stand out prominently.

E. **Muscles of lower limb:**

1. Muscles of foot and toes*:*

Dorsi-flexors and plantar flexors of the feet and toes:

Ask the subject to dorsiflex and plantarflex the distal foot against resistance.

Evertors and invertors:

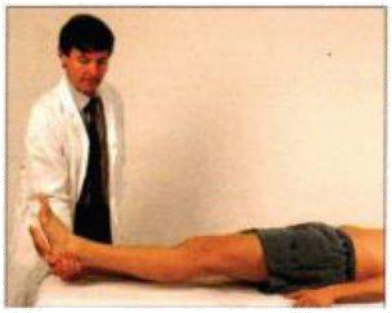
Ask the subject to evert and invert against resistance. Eversion and inversion takes place at the subtalar joint.

2. Extensors of knee:

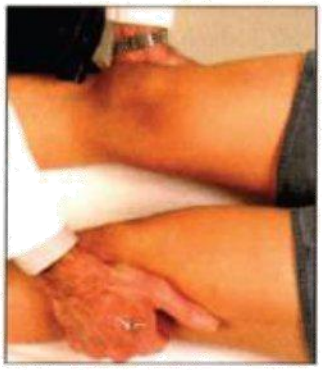
In supine position, ask the subject to flex his knee and to extend the leg while offering resistance on the shin just above the ankle.

3. Flexors of knee:

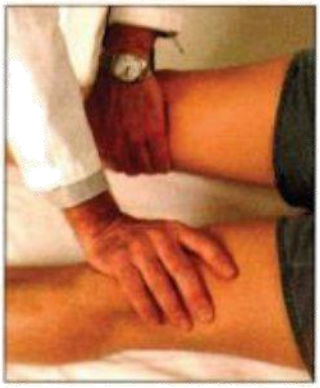
Raise the leg up from the bed, supporting the thigh with your left hand and holding the ankle with your right hand, ask the subject to bend his knee



Testing hip extension:
'Push your heel down and don't let me pull it up'



Testing power-hip abduction:
'Don't let me push your knees together'



Testing power-hip adduction:
'Don't let me push your knees apart'

4. Extensors of hip*:*

With the limb fully extended, lift the subject's foot off the bed, and ask him to bring the limb down to the bed against resistance.

5. Flexors of thigh:

With the leg extended, ask the subject, to raise his leg off the bed against resistance without bending the knee. Resistance is given just above the ankle joint.

6. Abductors of thigh:

With the legs placed together in the midline, ask the subject to abduct his legs against resistance.

7. Adductors of thigh*:*

With the legs kept apart, ask the subject to bring the legs to midline against resistance.

***8*. Rotators of the thigh or hip:**

With the lower limbs extended on the bed, ask the subject to rotate the limb outwards or inwards against resistance.

a) CO-ORDINATION: (refer experiment- Cerebellar function test)

b) REFLEXES:(refer experiment- Reflexes)

c) **GAIT:**

Ask the subject to walk barefoot in a straight line, and to turn round at a given point and then to come back. While walking, observe whether he walks in a straight line and if he deviates in any direction. Observe for automatic associated movements. .

1. Festinant gait in Parkinson's disease.
2. Waddling gait in muscular dystrophy and myopathies.
3. High stepping gait in common peroneal nerve palsy.

b) **INVOLUNTARY MOVEMENTS:**

a) Tremor*:*

Tremor is a regular, rhythmic, purposeless to and fro movement of a part of the body due to alternate contraction and relaxation of the agonist and antagonist muscles. It is of two types. Fine tremor and coarse tremor

Causes of tremor;

e)Physiological: Anxiety, old age and exposure to cold

f) *Pathological:* Parkinsonism, cerebellar disorders, thyrotoxicosis, hypoglycaemia, alcoholism and heavy metal poisoning.

b) Myoclonus*:*

Sudden shock like contraction of a single muscle or a group of muscles. **c) Athetosis*:***

It is characterized by slow, worm like writhing movements of the extremities affecting chiefly the fingers and the wrist. Seen in lesion of Globus pallidus.

d) Chorea:

It is characterized by brisk, jerky, purposeless and graceful movements of the distal part of the extremities and is usually associated with twitching of the face. Seen in Huntington's disease and Rheumatic heart disease.

e) Ballismus:

It is a rare involuntary movement characterized by a sudden violent movement of the body. Occurs in lesion of subthalamic nucleus. When it occurs in one half of the body it is called hemiballismus.

f) Ticks: Sudden, rapid, repeated, coordinated and purposeless movements that occur in the form of blinking of the eyes or wriggling of the shoulders.

QUESTIONS:

1. Define muscle tone.
2. Define hemiparesis, hemiplegia and paraplegia.
3. Name the descending tract that controls voluntary motor movements?
4. Name the extra-pyramidal tracts.
5. What are the differences between UMN lesion and LMN lesion?
6. List out the differences between spasticity and rigidity.

INFERENCE:

Experiment No. **REFLEXES** **Date**

DEFINITION:

Reflex is an automatic (involuntary) response to a stimulus. The response depends on the integrity of the reflex arc and is influenced by the higher centres.

COMPONENTS OF REFLEX ARC:

A. Receptor

B. Afferent

C. Centre

D. Efferent

E. Effector organ

TYPES OF REFLEXES:

←Superficial reflexes

←Deep reflexes

←Visceral reflexes

AIM:

To elicit superficial, deep and visceral reflexes in the subject.

APPARATUS REQUIRED:

Knee hammer, cotton swab.

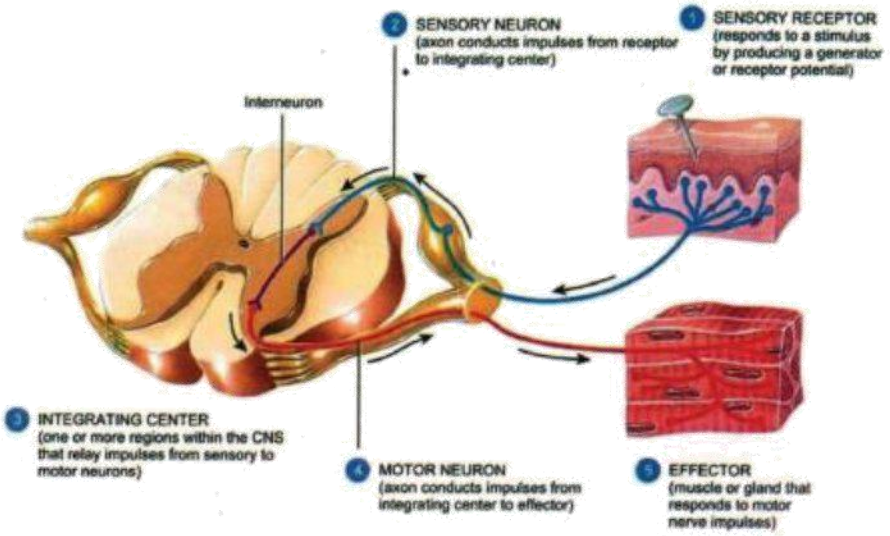
A. SUPERFICIAL REFLEXES: These are **'polysynaptic reflexes'.**

• *Stimulus:* Superficial structure. (skin / mucous membrane)

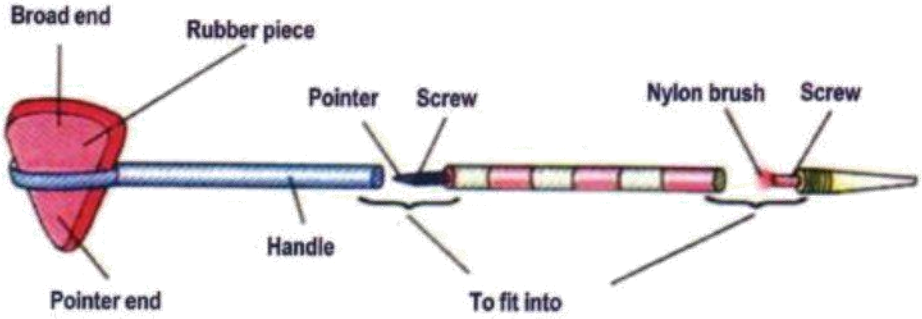
B *Response:* Contraction ofunderlying muscles.

C *Pathway*:

Afferent - Sensory nerve (component of mixed nerve) Center -
Spinal cord or brainstem or forebrain.
Efferent - Motor nerve (component of mixed nerve) to underlying muscles.



The reflex arc



The knee hammer

Conjunctival reflex:

Ask the subject to look at a distant object. Touch the bulbar conjunctiva with a fine wisp of sterile cotton brought from the side. Test both the eyes.

Response : Closure of both eyes. Afferent – 5^{th} cranial nerve. Efferent – 7^{th} cranial nerve.

Corneal reflex: Ask the subject to look at a distant object. Then touch the limbus (sclerocorneal junction) with a sterile cotton wool brought from the side of the subject. Test both the eyes.

Response: Closure of both eyes.. Afferent – 5^{th} cranial nerve. Efferent – 7^{th} cranial nerve.

Abdominal Reflex: (T7- T12)

Ask the subject to lie in supine position with abdomen uncovered.

Stimulus: With the blunt end of the knee hammer, stroke lightly and briskly on each quadrant of abdomen above and below the umbilicus from outer aspect towards midline.

*Response:*Contraction of abdominal muscles in that quadrant.

Plantar Reflex: (L5, SI)

Ask the subject to lie in supine position with the muscles of lower limbs completely relaxed.

Stimulus: With the blunt end of the knee hammer, outer edge of the sole of foot is stroked gently along the lateral border starting from heel towards little toe and then along the bases of other toes medially.

Normal *Response:* "Flexor plantar response" is manifested by

☞ Plantar flexion of all the toes at metatarsophalangeal and inter phalangealjoints.

G. Inversion and plantar flexion of ankle.

Abnormal response*:* "Extensor plantar response" manifested by

C Dorsiflexion of great toe with abduction and fanning out of other toes.

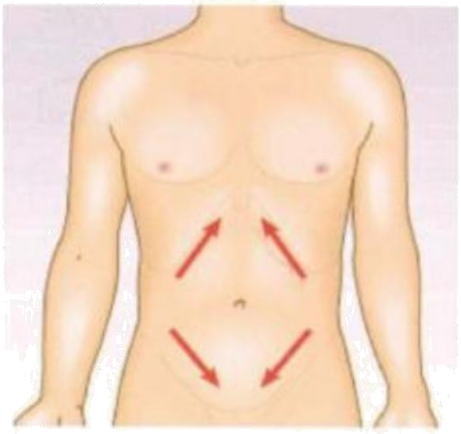
D Eversion and dorsiflexion of ankle.

E Also called as Babinski's sign.

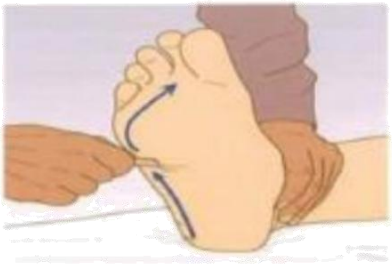
F The diagnostic feature of UMN lesion especially corticospinal tract lesion.

G Normally seen in newborns and infants (primitive reflex), sleep and deep anaesthesia

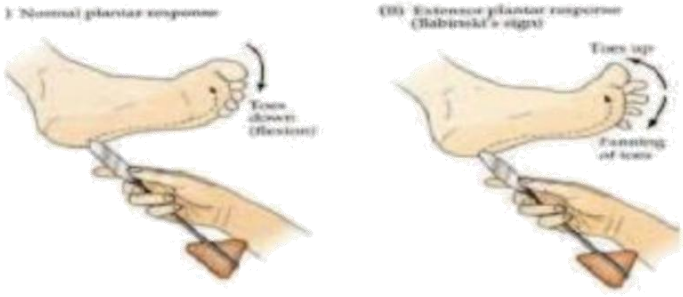
Testing the corneal reflex



Abdominal reflex

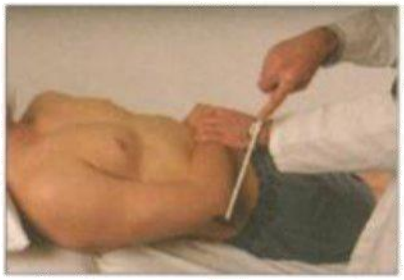


Eliciting plantar reflex

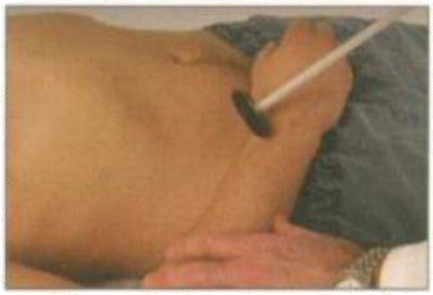


Babinski sign – Dorsiflexion of great toe and fanning of other toes

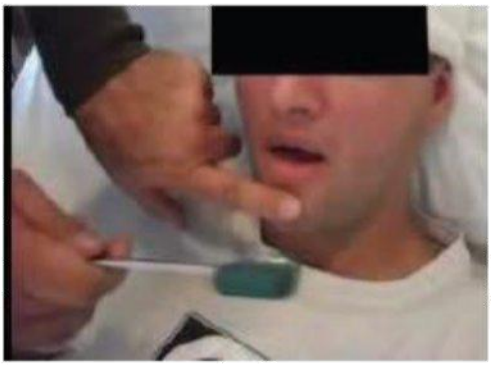
The Biceps jerk examination



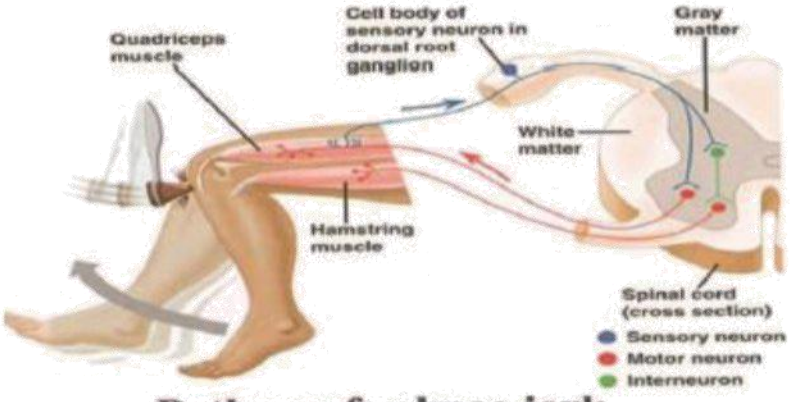
The Triceps jerk examination



The Supinator jerk examination



The jaw jerk



Pathway for knee jerk

Other reflexes:

7. Scapular reflex: C5-T1.
8. Anal reflex: S3-S4.
9. Cremasteric reflex: LI - L2.
10. Bulbo-cavernal reflex: S3, S4.
11. Cilio-spinal reflex: C8,Tl-T2.

B. DEEP REFLEXES:

These are "monosynaptic reflexes", also called as "stretch reflexes".

H. Stimulus: Sudden stretch of muscle at the tendinous region

I. Receptor: Muscle spindle

J. Afferent: Sensory nerve component of mixed nerve

K. Centre: Spinal cord

L. Efferent: Motor nerve component of mixed nerve.

When the tendon of a lightly stretched muscle, is struck by a single sharp blow with soft rubber hammer, the muscle contracts briefly. This is tendon or stretch reflex.

Before performing the deep tendon reflexes, instruct the subject to keep the muscles relaxed. Expose the corresponding muscles to see for the contraction.

1. Jaw jerk: (Afferent- V, Efferent- V, Centre-Pons)

Ask the subject to let his jaw sag open slightly. Place the left thumb over the chin and tap with the knee hammer.

Response: Closure of mouth.

2. Biceps jerk: (C5, C6)

Keep the elbow of the subject partially flexed and place the fore arm in a semi prone position, relaxing on the examiner's fore arm. Place the thumb over the biceps tendon and strike the thumb with the knee hammer.

Response: (contraction of the biceps) flexion of the elbow.

3. Triceps jerk: (C6, C7)

Flex the elbow of the subject and rest the forearm across the subject's chest. Tap the triceps tendon just above the olecranon process gently with the knee hammer.

Response: (contraction of the triceps) Extension of the elbow.

4. Supinator jerk/Brachio-radialis jerk: (C5, C6)

Flex the elbow of the subject and keep the forearm in a semiprone position. Tap gently the tendon overlying the radial styloid process with the knee hammer.

Response: (contraction of brachio-radialis muscle) Supination of the forearm.

5. ***Knee jerk: (L1,L2, L3)***

Ask the Subject to lie in a supine position with the Quadriceps femoris muscle relaxed. Support the knee to be tested with the hand underneath the knee joint. Strike sharply the Quadriceps tendon (patellar tendon) with a knee hammer.

Response:(contraction of quadriceps muscle) extension of leg at the knee. Reflex may also be elicited in a sitting posture. The subject is seated on a stool with one leg crossed over the other with the leg hanging loosely. Examiner's hand is placed over the quadriceps muscle and the patellar tendon is gently tapped with the knee hammer.

6. Ankle jerk: (S1-S2)

Ask the subject to lie in a supine position. Flex the lower limb at the knee joint. Stretch the calf muscles by dorsiflexion at the ankle joint. Strike sharply at the achille's tendon.

Response: Contraction of calf muscles with plantar flexion.

This reflex can also be elicited when the subject is kneeling on a chair.

Clonus: It is a sign of increased reflex activity characterized by repetitive muscular contraction produced by stretch

Grading of reflexes:

Grade 0: Absent
Grade 1: Present (as a normal ankle jerk)
Grade 2: Brisk (as a normal knee jerk)
Grade 3: Very brisk
Grade 4: Clonus

Reinforcement technique:

The Jendrassik's maneuver is applied to those subjects in whom deep tendon reflexes are difficult to elicit. Reinforcement technique acts by increasing excitability of anterior horn cells and by increasing the sensitivity of muscle spindle to stretch by increasing the gamma motor discharge.

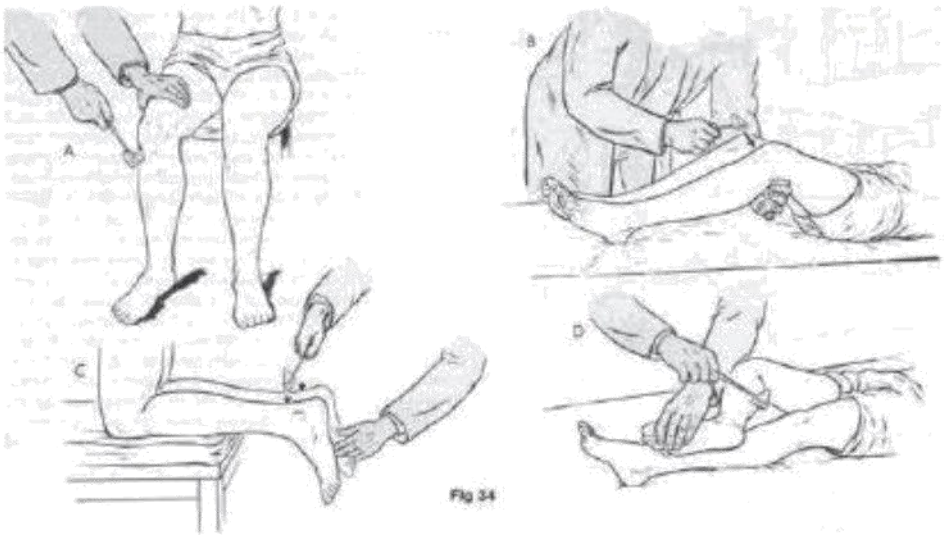
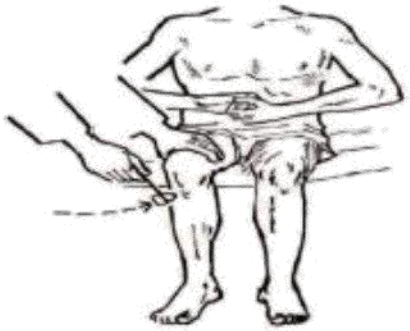


Fig 34

ELICITING KNEE JERK AND ANKLE JERK

KNEE JERK ELICITED WITH JENRASSIK'S MANOUVRE



The knee jerk with reinforcement:
'Grip your fingers and pull your hands apart'

For eliciting upper limb reflexes:

Ask the subject to clench the teeth while the tendon reflexes are being elicited.

For eliciting lower limbs reflexes:

Ask the subject to interlock the flexed fingers and to attempt to pull them apart at the time the tendon is being struck ***(Jendrassik's maneuver).*** Ask the subject to tighten immediately before striking the tendon and to relax thereafter.

Abnormal tendon reflexes:

Deep tendon reflexes are absent in LMN lesion. Eg: tabesdorsalis, poliomyelitis

Hyperreflexia:

Deep tendon reflexes are exaggerated in UMN lesion, anxious individuals, thyrotoxicosis, tetanus. In cerebellar diseases the reflexes have pendular (oscillatory) quality.

Superficial reflexes are absent both in UMN and LMN lesion except plantar, which is exaggerated in UMN lesion.

3. VISCERAL REFLEXES:

1.Defaecation reflex:

The subject is questioned about his bowel habits to rule out incontinence.

2. Micturition reflex:

The subject is questioned about his micturition habits to rule out bladder incontinence.

3. Swallowing reflex: (Deglutition reflex)

The subject is questioned about any difficulty in swallowing, nasal regurgitation of food during swallowing.

QUESTIONS:

d) Describe the reflex arc.
e) What is a stretch reflex?
f) What is babinski sign and what is its significance?
g) Why are deep tendon reflexes exaggerated in UMN and lost in LMN lesion?
h) What is Jendrassik's sign?

Experiment No. **Date**

CEREBELLAR FUNCTION TESTS

The functions of the cerebellum are tested under the following headings:

H Tone
I Coordination
J Reflexes
K Speech
L Gait
M Involuntary movements.

1.TONE

Muscle tone is the state of partial contraction found in healthy muscles at rest. It is the degree of tension present in a muscle at rest. It is assessed by passive movements

Method of eliciting tone:

Instruct the subject to keep his limbs relaxed. Move the limbs passively at the joints and assess the resistance offered by the muscles.

2.COORDINATION

The coordination of movements means smooth recruitment, interaction and cooperation of a group of muscles to carry out a precise and definite motor act.

Tests of coordination in the upper limbs:

a) The finger-nose test:

Ask the subject to keep his right upper limb outstretched and then to touch the tip of his nose with his forefinger. Ask him to do this few times and check if he is able to perform it smoothly. Repeat this in the opposite limb.

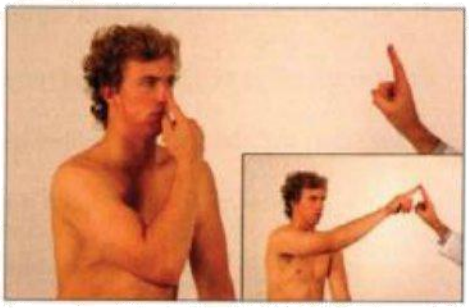
In cerebellar lesions, there will be overshooting of the finger ('past pointing). This is known as Dysmetria.

b) The finger-finger-nose test:

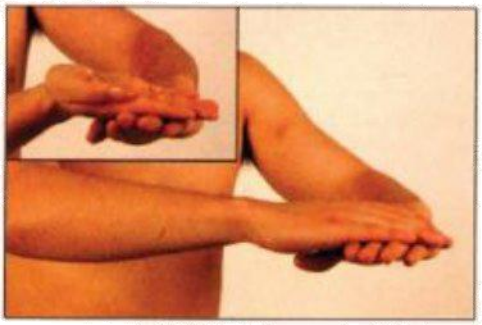
The subject is instructed to touch the examiner’s finger first and then touch his nose. Ask him to do this few times.Repeat this in the other limb.

c) Drawing a circle in the air:

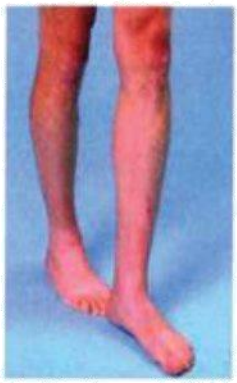
Ask the subject to draw a circle in the air with his index finger.



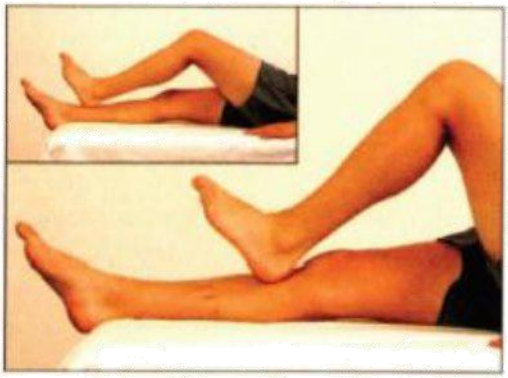
The finger nose test and finger - finger nose test



Testing for dysdiadochokinesis in the upper limbs



Tandem walking



Performing the heel shin test

d) Diadochokinesia:

Ask the subject to carry out rapid alternating movements of supination and pronation of one forearm and hand over the palm of the other hand. Difficulty in performing such rapid alternate movements is called dysdiadochokinesia.

Rebound Phenomenon:

Ask the subject to flex his forearm against resistance offered by the examiner. Withdraw the resistance suddenly and check for rebound phenomenon. Repeat the test on the other side.

Normally he should be able to put a brake on the movement. In cerebellar lesions, there is inability to terminate the movement all of a sudden due to which he may even hit his own face.

Tests of coordination in the lower limbs:

a) Tandem walking:

Ask the subject to walk in a straight line heel to toe.

In cerebellar lesions, he will deviate to one side or the other depending on the side of lesion.

b) Heel knee test:

In supine position, ask the subject to place the heel of one leg on the opposite knee and then to slide it down along the shin of the tibia towards the ankle. Ask him to repeat the procedure few times in quick succession. Repeat the test for the other leg also.

In cerebellar lesions, the subject will have difficulty in performing the test smoothly.

c) Drawing a circle in the air:

Ask the subject to draw a circle in the air with his great toe. Test the other leg also.

3. DEEP REFLEX – KNEE JERK

Perform knee jerk on both sides in the sitting position with legs hanging down freely.

In cerebellar lesion, pendular knee jerk response is observed.

4. SPEECH:

Ask the subject to pronounce “RAJAGOPALACHARI”/“RHINOCEROUS”

In cerebellar lesion, there will be dysarthria. (scanning speech/ staccato speech)

5. GAIT:

Ask the subject to walk barefoot for at least 9 meters and then turn around and walk back to the starting point. Observe the gait.

In cerebellar lesion, the gait is broad based with feet apart, staggering or reeling towards the side of the lesion.This is called, 'Ataxic or Reeling or Drunken gait'.

6. INVOLUNTARY MOVEMENTS:

Tremor:

Tremor may be defined as involuntary, regular, rhythmic, purposeless movements due to alternate contraction and relaxation of the agonist and antagonist muscles.

In cerebellar lesion, coarse tremor occurs when the subject intends to do something or attempts to pick up an object. This is called intention tremor.

7.Look for nystagmus**.** Nystagmus is involuntary, jerky, to and fro, often rhythmical oscillations of the eyeball. In cerebellar lesion, nystagmus is seen towards the side of the lesion.

QUESTIONS:

1. What are the functions of cerebellum?
2. What is dysdiadochokinesia?
3. Name the gait seen in cerebellar lesion.
4. Name few other abnormal gaits.
5. What is Resting tremor?

Experiment No. **Date**

EXAMINATION OF 1 TO 6 CRANIAL NERVES

1st CRANIAL NERVE – OLFACTORY NERVE (purely sensory)

It serves the sense of olfaction.

Test:

Make sure that the subject does not have any upper airway congestion or obstruction. To examine use familiar substances like clove oil, peppermint oil,coffee,soap,asafoetida etc. Avoid using irritants like ammonia as it stimulates the fifth cranial nerve.

Test each nostril separately with eyes closed after giving proper instructions to the subject, to indicate if he recognizes the particular substance or not. Tabulate the results.

Discussion:

A normal subject can recognize the substance with each nostril.

Anosmia means complete absence of smell.Parosmia means perverted sensation of smell Hyposmia means reduction in the sense of smell. All these conditions develop due to damage to the olfactory pathways by trauma or disease. Olfactory hallucinations occur in temporal lobe epilepsy.

2nd CRANIAL NERVE – OPTIC NERVE (purely sensory)

It serves the sense of vision. The functions to be tested are:

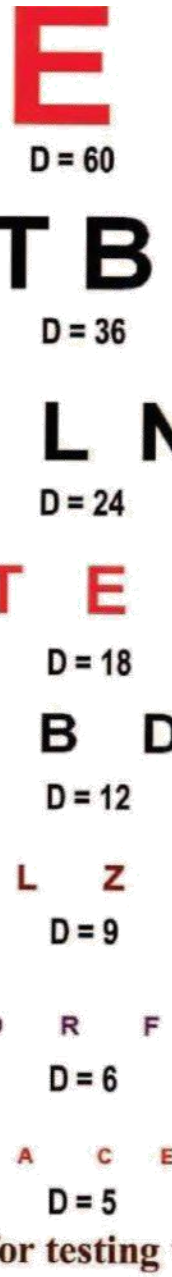
N Visual acuity

O Field of vision

P Color vision

Q Pupillary light reflex.

D Accommodation reflex.



Snellen's chart for testing visual acuity

1.Visual acuity:

It is defined as the resolving power of the eyes i.e. the extent to which the eye can perceive the details and contours of an object clearly. It is explained in terms of mi nimum separable distance ie, the shortest distance by which two lines can be separated and still can be perceived as two lines. Visual acuity is the function of cones.

M. Test for distant vision

N. Test for near vision.

a) Test for distant vision:(Snellen's chart)

A series of letters of varying size are constructed in such a way that the top letter is visible to normal eyes at 60 metres and the subsequent lines at 36 , 24, 18, 12, 9 , 6, 5 meters respectively. Visual acuity, V=d/D , where d is the distance at which the letters are read (6m) and D is the distance from which the letters can be read normally.

The subject is seated at a distance of 6 m from a well illuminated Snellen's chart and each eye should be tested separately. The line upto which the subject is able to read is noted.If the subject wears glasses , visual acuity should be tested with and without them.

Observation:

If the person is able to read upto the third line, his visual acuity is 6/24. A normal person can read upto the 7^{th}line ie. Visual acuity is 6/6. If the subject is not able to read even the first line, ask him to move towards the chart until he can read the top letter.

For ex. If he is able to read at 2 meters then his visual acuity is 2/60. If the visual acuity is less than 1/60, then proceed with

A. Finger counting test
B. Hand movement test
C. Light Perception test

12. **Test for near vision : (Jaegar’s chart)**

The subject is seated in a well illuminated room comfortably. The visual acuity for near vision is tested by asking the subject to read Jaeger's chart at reading distance(25-30cm).This chart consists of letters of various size based on the principle of Printer’s point system. The smallest point is N5 and the largest point is N36.The near vision is recorded as the smallest type which the subject can read comfortably.

2. Field of vision: (Confrontation Test)

This method is useful for testing the peripheral field of vision.The examiner must have a normal visual field as field defects in the subject are detected by comparing with that of the examiner. The examiner sits at one arm distance from the subject at the same level. Each eye is tested separately .

To test the subject's right eye, the subject's left eye and the examiner's right eye are closed. The subject is asked to fix his gaze at the examiner's left eye. The subject is instructed not to turn his head or shift his gaze. The examiner holds out his left arm to its full extent and then moves his finger from periphery to the centre, kept midway between him and the subject in all the four quadrants (temporal, nasal, superior and inferior) .

Ask the subject to say 'yes' when he sees the finger tip.

If the subject's field of vision is normal, he will be able to see the moving finger at the point where the examiner sees it. Repeat the test in the other eye.

Discussion: The field of vision is maximum in the temporal quadrant and minimum in the superior quadrant.

Visual Field defects- Scotoma and Hemianopia

Perimeter is used to accurately map the peripheral field of vision

3. Colour vision

O. Ishihara's chart: The Ishihara's chart consists of series of lithographic colour plates which are printed with figures or designs in coloured circles on a background of differently coloured circles of the same size. The subject is asked to read numbers or trace the lines in each plate of the book.A person with defective colour vision will read a different number from the colour plate.

P. Holmgren's wool test: The subject is asked to perform a series of colour matching from a collection of wools of different colours. A colour blind subject will not be able to match the colours.

As Optic nerve is a component of papillary reflexes, light reflex and accommodation reflex are tested.

4. Pupillary light reflex:

a. Direct light reflex: Examine each eye separately.Ask the subject to fix the gaze at a distant object. Shine the light from the side with a pen torch into the eye. Observe the pupilary reaction.

Response: A brisk constriction of
the pupil is noted. Afferent – 2nd
cranial nerve.
Efferent – 3rd cranial nerve.

b. Indirect/ Consensual light reflex: Subject is instructed to place his hand over the nose as a partition between his eyes.This will prevent the light entry into the other eye. Now while shining the light into one eye, observe the pupillary response in the other eye. The response of pupillary constriction in the left eye when light is shown on the right eye is indirect light reflex.

Response:

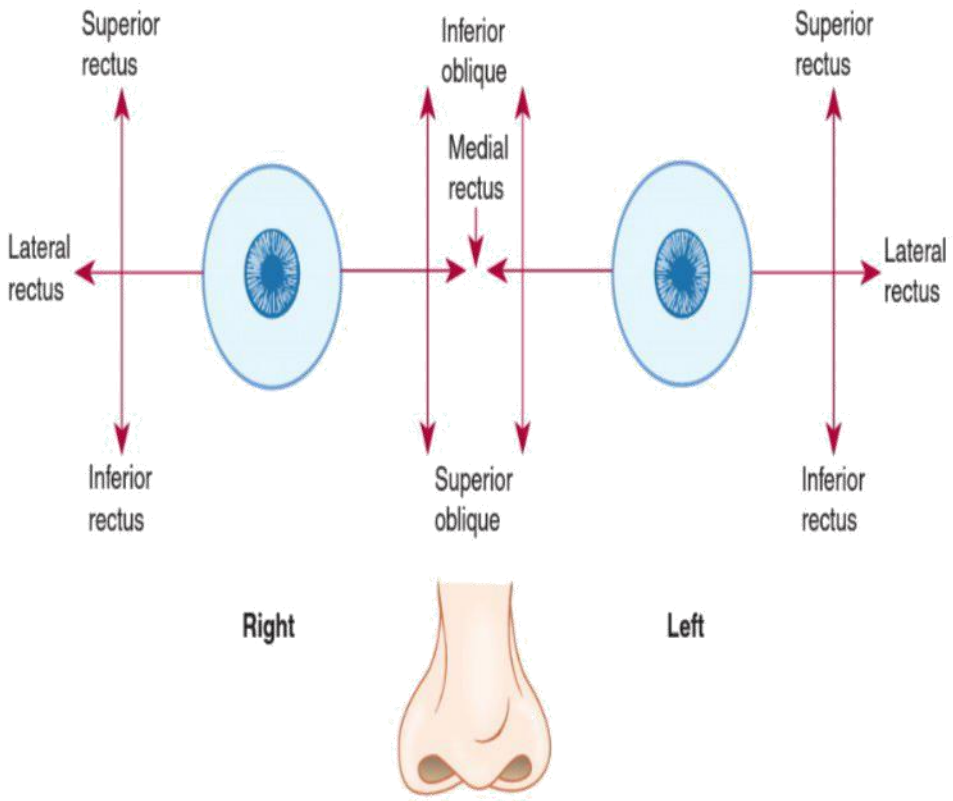
Showing the light into the testing eye causes constriction of
pupil in both eyes. Afferent – 2nd cranial nerve.
Efferent – 3rd cranial nerve. -
Consensual light reflex is due to the involvement of Edinger-Westphal nucleus (3^{rd} nerve nucleus) on both sides.

5. Accommodation reflex:

Test: Ask the subject to fix the gaze at a distant object. Then,ask him to look at the examiner's finger which is held close to the subject's nose .

Response: ["3Cs"]

i) Convergence of both the eyeballs by medial recti
j) Constriction of pupil by sphincter pupillae.
k) Increase in anterior curvature of lens by contraction of ciliary muscle. Afferent – 2nd cranial nerve.
Efferent – 3rd cranial nerve.


Superior rectus
Inferior oblique
Superior rectus
Medial rectus
Lateral rectus
Lateral rectus
Inferior rectus
Superior oblique
Inferior rectus
Right
Left

Discussion:

In lesions of 3rd cranial nerve, accommodation reflex is absent on the affected side. Argyll-Robertson Pupil:In neurosyphilis the pretectal region of the midbrain is damaged. Here pupillary light reflex is absent and the accommodation reflex is preserved.

3,4 & 6 CRANIAL NERVES –OCCULOMOTOR,TROCHLEAR & ABDUCENT NER VES (Purely motor)

They supply the extra ocular muscles which move the eyeball.

♍☽ Trochlear nerve supplies the superior oblique.

♎☽ Abducent Nerve supplies the lateral rectus.

♏☽ Occulomotor nerve supplies Superior rectus, Inferior rectus, Medial rectus and Inferior oblique.

It also supplies Sphincter pupillae, Ciliary muscle and Levator palpebrae superioris.

Tests:

g) Examine the eyes of the subject for the presence of ptosis.

h) Examine the size and shape of pupil.

(c) Test for conjugate movements of eye

Ask the subject to sit comfortably in front of the examiner at one arm distance. Ask the subject to follow the movements of the examiner's finger with his eyes, without moving his head. First, move your finger laterally to the right of the subject, from there vertically upwards and downwards. Then, move your finger laterally to the left , from there vertically upwards and downwards. Observe if the subject is able to follow the finger movements and look for any limitation of movement in any direction .

e) Pupillary Light reflex : (refer 2nd cranial nerve)

f) Accommodation reflex: (refer 2nd cranial nerve)

5^{th} CRANIAL NERVE: TRIGEMINAL NERVE (MIXED NERVE)

It has sensory (Ophthalmic, Maxillary and Mandibular divisions carry the sensations of face) and motor components (Muscles of mastication).

a) Tests for sensory function:

The areas of face supplied by the three divisions of 5^{th} nerve are tested.

1) Touch 2) Pain 3) Temperature

4) Pressure 5) Tactile localization 6) Two point discrimination.

Refer the sensory system examination for methods to elicit the above sensations.

2. Tests for motor functions:

To test the muscles of mastication : Masseter, Temporalis and Pterygoids.

8. Ask the subject to clench his teeth and palpate the prominence of the Temporalis and Masseter muscles on both sides over the temple and upper part of the cheek. If there is paralysis on one side , the muscles on that side will fail to become prominent.
9. Ask the subject to open his mouth and look for deviation of jaw. If the is paralysis on one side, on opening the mouth , the jaw will deviate towards the paralysed side due to unopposed action of the lateral pterygoid muscle of healthy side.

(c) Ask the subject to open his mouth and move the jaw from side to side.

3.Reflexes:

a. Conjunctival reflex:

Ask the subject to look at a distant object. Touch the bulbar conjunctiva with a fine wisp of sterile cotton brought from the side. Test both the eyes.

Response : Closure of both eyes. Afferent – 5^{th} cranial nerve. Efferent – 7^{th} cranial nerve.

b. Corneal reflex: Ask the subject to look at a distant object. Then touch the limbus (sclerocorneal junction) with a sterile cotton wool brought from the side of the subject . Test both the eyes.

Response: Closure of both eyes..

Afferent – 5^{th} cranial nerve.

Efferent – 7^{th} cranial nerve.

c. Jaw Jerk: Ask the subject to let his jaw sag open slightly. Place the left thumb over t he chin and tap with the knee hammer.

Response: Closure of mouth.

Afferent & Efferent – 5^{th}

cranial nerve.

INFERENCE:

QUESTIONS:

1. Trace the pathway for smell.
2. What are the factors that affect the visual acuity?
3. Name the refractory errors. How do you correct them?
4. What is the clinical significance of tests for colour vision?
5. What are the other methods for testing colour vision?

(i) Define field of vision.

(ii) What is blind spot?

(iii) Mention the visual field defects produced by lesions at various levels in the visual pathway.

(iv) What is ptosis?

(v) Trace the pathway for light reflex.

Experiment No. **Date**

EXAMINATION OF 7 -12 CRANIAL NERVES

7th CRANIAL NERVE – FACIAL NERVE (Mixed nerve)

The seventh nerve consists of,

R Motor fibres supply the muscles of facial expression, muscles of the scalp and ear, platysma, posterior belly of digastrics, stylohyoid and stapedius.

S Parasympathetic fibres supply nasal, palatine, lacrimal, submaxillary and sublingual glands.

T Special sensory fibres carrying taste from anterior two third of the tongue.

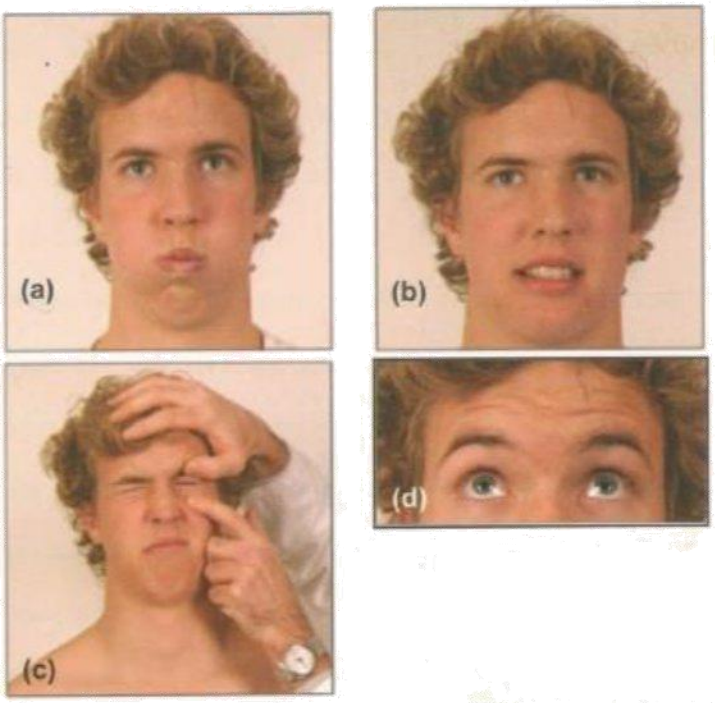
EXAMINATION OF FACIAL NERVE

1. Motor component:

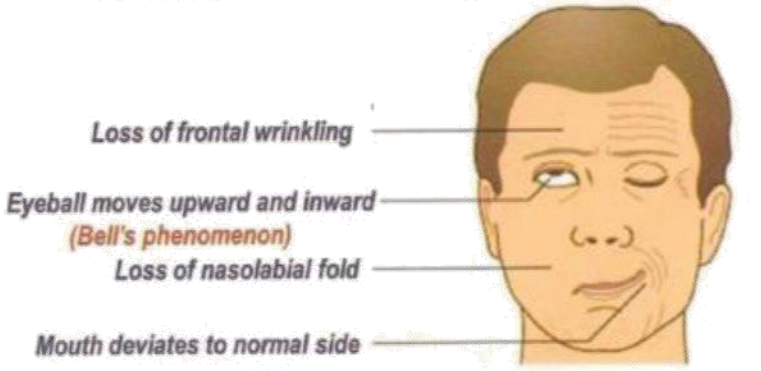
Muscles of facial expression

- Look for the symmetry of face, loss of facial expression and obliteration of nasolabial folds
- Ask the subject to raise the eyebrows and look for wrinkling of the forehead, (Frontal belly of occipitofrontalis).
- Ask the subject to frown (Corrugator supercilii).
- Ask the subject to shut his eyes tightly and attempt to open his eyes while the subject tries to keep them closed . (Orbicularis oculi).
- Ask the subject to smile or show his teeth and look for deviation of the angle of mouth. In lesions of the facial nerve, the angle of mouth is deviated to the healthy side.
- Ask the subject to whistle,(orbicularis oris and buccinators)

K Ask the subject to inflate his mouth with air and blow out the cheeks. Tap with the finger on either side and see if air escapes easily from the mouth.



Testing the motor function of the facial nerve
Ask the patient to
(A) blow out the cheeks
(B) show the teeth
(C) close the eyes against resistance
(D) raise the eyebrows and wrinkle the fore head



Bell's Palsy – right side

- Ask the subject to clench his teeth and look for the prominence of platysma muscle in the neck.

- Bell's phenomenon: Ask the subject to close his eyes. On closing the eyes the eyeballs roll upwards, which is not seen in a normal person since the eyelids close properly. In Bell's palsy (LMN type of facial nerve palsy), this phenomenon is seen on the affected side as he cannot close the eyelids.

Q.**Sensory component:**

Test the taste sensation in anterior two third of the tongue

- Use solutions of sugar, common salt, citric acid and quinine to test sensations of sweet, salt, sour and bitter respectively

- Ask the subject to protrude the tongue out and apply the solution on the surface of one half of the tongue with a swab.

- The subject is asked to indicate perception of the taste by finger signs without taking the tongue back into the mouth.

- After each test, the mouth must be rinsed.

- The test is repeated with each solution and then on the other half of tongue.

- The bitter quinine should be applied last, as its effect lasts longer.

H **Reflexes:**

- Corneal reflex (Refer 1-6 cranial nerve)

14. Conjunctival reflex (Refer 1-6 cranial nerve)

4. Ask for any history suggestive of hyperacusis (stapedius)

8th CRANIAL NERVE- VESTIBULO-COCHLEAR NERVE (Purely sensory)

Eighth nerve consists of 2 components – Cochlear component innervates cochlea and subserves hearing.Vestibular component supplies the labryrinth and semicircular canals which subserves equilibrium and balance.

TESTS FOR COCHLEAR FUNCTION

Assessment of Hearing

R. Watch test

S. Whisper test

T. Tuning fork tests

U. Audiometry

1. Watch test:

Ask the subject to close his eyes. The examiner should stand to the side of the ear to be tested and the other ear is masked by tragal movement. A ticking watch is brought from a distance and note the distance at which its ticking is heard. Repeat the same procedure on the opposite ear. The results of the two ears are compared with that of the normal subject.

2. Whisper test:

The test starts with a whispered voice at 60cm (approximate intensity 15dB) from the test ear and proceeds with a whispered voice at 15cm (35dB). If there is no response, a conventional voice at 60cm (50dB) is used. Repeat the same procedure on the opposite ear.

3. Tuning fork tests:

A tuning fork of 256 Hz or 512 Hz is used.

l) Rinne's test:

This test compares hearing by air and bone conduction

After giving proper instructions to the subject, vibrate the tuning fork by holding the stem and strike it against the hypothenar eminence

Immediately place the base of the vibrating fork on the mastoid process behind the ear and ask the subject to raise the finger when he ceases to hear the sound.

4. Once he stops hearing, hold the fork in line with the external ear canal and ask if he hears the sound. When he stops hearing, bring the fork close to your ear to confirm whether the vibration has actually stopped.

Interpretation:

In normal subjects, air conduction is better than bone conduction – Rinne positive .

Rinne positive response is also found in patients with partial sensorineural deafness.

Rinne negative – Bone conduction is better than air conduction, found in patients with conduction deafness

i) In total or complete nerve deafness – no sound is heard either by way of air or bone conduction.

b. Weber's test:

This test compares bone conduction of both the ears.

j) Place the base of the vibrating tuning fork in midline i.e. centre of the forehead(Glabella)or vertex of the head and ask the subject to indicate, whether the sound is heard equally in both ears or lateralized to one ear

Interpretation:

♑◑ In normal subjects – the sound is heard in midline or equally in both ears.

♒◑ Sensorineural deafness – the sound is heard louder in the normal ear.

i) Conduction deafness - the sound is heard louder on the side with conduction deafness.

c. Absolute bone conduction (Schwabach test):

This test compares the bone conduction of the subject with that of the examiner.

The bone conduction is made absolute by occluding the external auditory meatus. Place the vibrating tuning fork on the mastoid process of the subject first and when he ceases to hear the sound, place it on the examiner's mastoid process.

Interpretation:

b) Normal response: Absolute bone conduction should be equal for the subject and the examiner i.e. the subject ceases to hear the sound at the same time as the examiner.

4. AUDIOMETRY:

Audiometry is an objective and accurate method to asses the type and degree of deafness and frequency range at which it manifests.

TESTS FOR VESTIBULAR COMPONENT

🗁🗀📣 Ask the subject for history of vertigo.

11. Look for Nystagmus(Involuntary, jerky, to and fro rhythmic movements of the eyeball).

9th CRANIAL NERVE – GLOSSOPHARYNGEAL NERVE (Mixed nerve)

It consists of 3 components:

j) Motor fibres supply stylopharyngeus muscle and middle constrictor of the pharynx which helps in swallowing.

k) Sensory fibres carry taste sensation from posterior 1/3rd of the tongue and general sensations from mucous membrane of pharynx.It innervates the baroreceptors in the carotid sinus.

l) Secretomotor fibres supply the parotid gland.

1. TEST FOR MOTOR FUNCTION

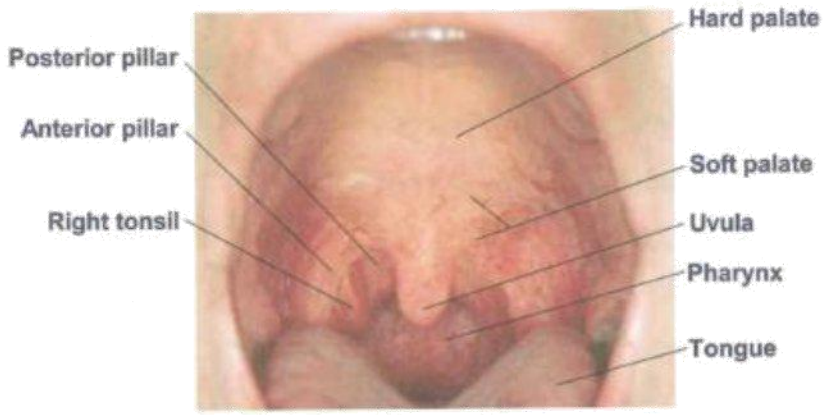
Pharygeal reflex (Gag reflex):

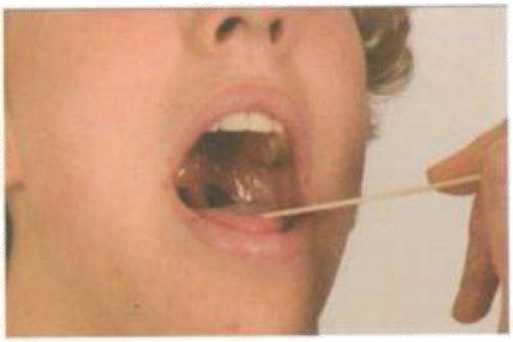
Ask the subject to open his mouth wide and tickle the back of the pharynx with the help of a cotton swab stick and observe the contraction of posterior pharyngeal wall, (9thand 10thcranial nerve)

2. TESTS FOR SENSORY COMPONENT

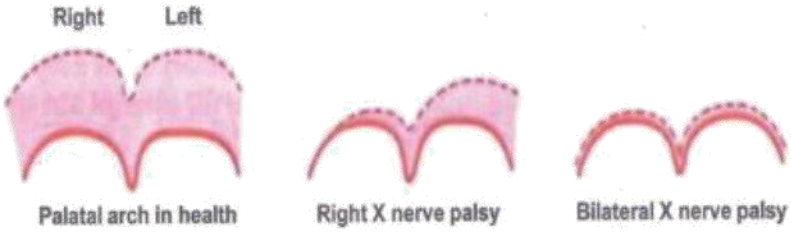
☎❖♓◑ Test the taste sensation (as explained in the 7th cranial nerve) on the posterior 1/3rd of the tongue.

(vii) Baroreceptor function-Record the blood pressure of the subject in the supine position and immediately (within 15 seconds) on standing. A fall in systolic BP of more than 20mmHg is suggestive of orthostatic hypotension.





Palatal reflex



Examination of soft palate movements during phonation

10th CRANIAL NERVE – VAGUS NERVE (Mixed nerve)

It has motor and sensory components.

- Motor fibres supply muscles of soft palate, pharynx and larynx.
- Sensory fibres supply pharynx, larynx, trachea and most of the thoracic and abdominal viscera.
3. Parasympathetic fibres supply the heart, bronchial muscle and glands, smooth muscle and glands of most of GIT.

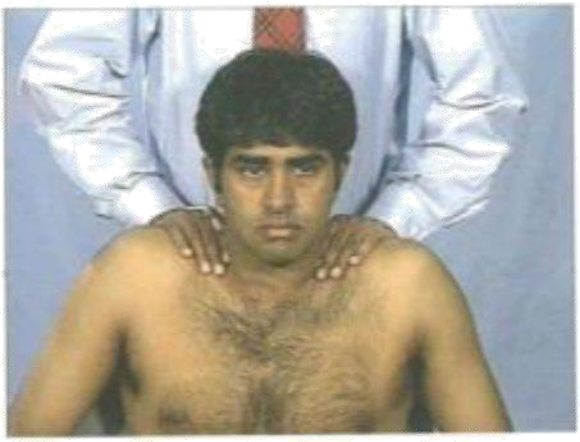
TESTS

16. Ask the subject if there is any history of regurgitation and difficulty in swallowing (dysphagia).
17. Ask the subject to pronounce the words that require complete closure of nasopharynx such as egg, rub etc. 'Egg' is sounded as 'eng' and 'rub' as 'rum' in paralysis of soft palate.
18. Examination of soft palate:

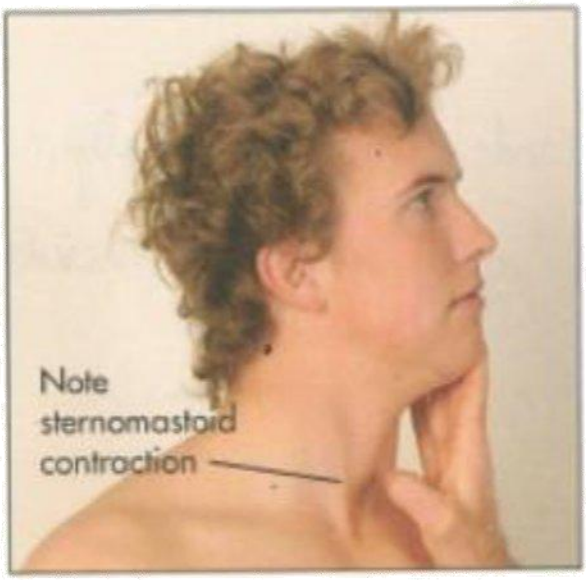
    Ask the subject to open his mouth and depress the tongue with a tongue depressor Ask him to say 'ah' and observe whether both sides of the soft palate arch equally upwards.

    In unilateral paralysis, paralysed side will remain flat and immobile.
19. Elicit Gag reflex : Refer 9th cranial nerve.
20. Check for hoarseness of voice
21. Baroreceptor function - Test for postural hypotension (refer 9th cranial nerve).

(a)



(b)



Testing

(a) Trapezius muscle

(b) Right sternocleidomastoid muscle

11th CRANIAL NERVE – SPINAL ACCESSORY NERVE (Purely motor)

The spinal part of the accessory nerve supplies sternocleidomastoid and trapezius muscle.

1.Test for Sternocleidomastoid muscle:

- Ask the subject to turn the chin towards left side and offer resistance with your hand over the left side of his face. Look for the prominence of the muscle. Repeat the procedure for testing the left side muscle by asking the subject to turn the chin towards right side against resistance

5. Ask the subject to depress his chin and offer resistance, to test muscles on both sides simultaneously.

2.Test for Trapezius muscle:

1. Stand behind the subject and ask the subject to shrug the shoulders while the examiner presses them downward

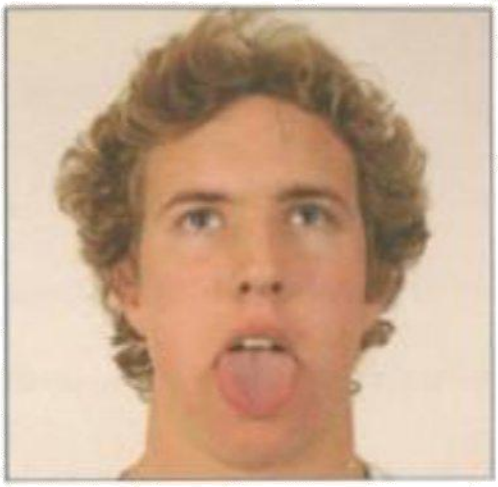
12th CRANIAL NERVE – HYPOGLOSSAL NERVE (Purely motor)

It supplies all the muscles of the tongue and depressors of the hyoid bone.

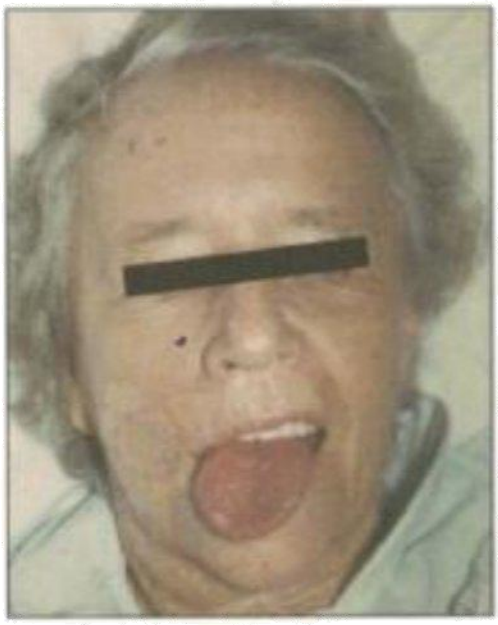
Test:

1. Ask the subject to put out his tongue and look for any deviation, wasting, tremor or fasciculations of the tongue

2. Ask the subject to move the tongue from side to side and push against the cheeks, while the examiner offers resistance from outside.



Examination of tongue



Right hypoglossal (XII) nerve palsy
Lower motor neuron lesion

Interpretation:

- In unilateral paralysis, the tongue will be deviated towards the paralysed side on protrusion. The muscles of the healthy side push the tongue to the paralysed side.

- In bilateral paralysis, the person will not be able to protrude the tongue.

- In LMN type of paralysis, wasting, fasciculation and flaccidity of the tongue are present.

4. In UMN type of paralysis, the tongue may be small and spastic.

QUESTIONS:

1. What is Bell's palsy?
2. What is Bell's phenomenon?
3. Differentiate between UMN and LMN palsy of facial nerve.
4. What are the differences between UMN and LMN paralysis of twelfth cranial nerve?
5. What are the two types of deafness? Give examples for each.
6. What is Rinne's positive?
7. How do you differentiate nerve deafness from conduction deafness by using Weber's test?
8. What is Barany's caloric test?

THE TAMIL NADU Dr.M.G.R.MEDICAL UNIVERSITY, GUINDY, CHENNAI – 32

FIRST MBBS

BIOCHEMISTRY

PRACTICAL RECORD BOOK

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Name of student:

Certified to be a bonafide record of work done in the Biochemistry laboratory of this college by the candidate with university register number

_____

Name and signature of Professor and Head of the Department of Biochemistry

Date:

CONTENTS

I. CARBOHYDRATES

A. Reactions of monosaccharides

Glucose: Glucose is an aldohexose. It is known as “grape sugar” because of its presence in ripe grapes. It is also called dextrose because it causes dextro-rotation of plane-polarized light. It is widely distributed in nature. All starch in our diet is converted into glucose before absorption from the intestine.

Fructose: Fructose is a ketohexose. It is sweeter than glucose. It is found in fruits. It is also known as laevulose because it causes laevorotation of plane-polarized light.

Galactose: Galactose is an aldohexose. Galactose and glucose are products of hydrolysis of lactose (found in milk). It is a constituent of glycolipids of neural tissue.

1. Molisch's test for carbohydrates

This is a universal test for carbohydrate. Strong acids cause dehydration of carbohydrates to yield furfural derivatives. These furfural derivatives condense with phenolic compounds (such as alcoholic α-naphthol) to give a purple-coloured complex.

2. Benedict's test for reducing sugars

Reducing sugars form enediols in an alkaline medium. Enediols are powerful reducing agents and reduce cupric ions to cuprous ions that form a red precipitate of cuprous oxide.

Benedict's test is a semi-quantitative test. The amount of reducing sugar in the sample can be graded as given below.

Appearance	Report	Approximate conc. (g/dL)
Blue	0	0
Green with yellow precipitate	1+	~0.3
Olive green	2+	~1.0
Brownish-orange	3+	~1.5
Brick red	4+	>2.0

Benedict's reagent contains copper sulphate, sodium citrate and sodium carbonate.

3. Barfoed's test for reducing monosaccharides

This is a test to distinguish between monosaccharides and reducing disaccharides. Monosaccharides retain their reducing property in mildly acidic conditions, while disaccharides do not. Therefore, monosaccharides reduce cupric ions to a red precipitate of cuprous oxide in the presence of dilute acetic acid, which is a component of Barfoed's reagent. Disaccharides

may also give a positive test if the sample is boiled for a long time, due to degradation of disaccharides to yield monosaccharides.

4. Seliwanoff's test for ketoses and ketose-containing sugars

Ketoses and sugars containing them form furfural derivatives rapidly, when heated with hydrochloric acid. The furfural derivatives condense with resorcinol to give a cherry red complex. Aldoses may react slightly to produce a faint pink colour.

Date:

Perform the following tests with glucose and fructose solutions provided.

Experiments	Observation	Inference
1. Molisch's test To 5 ml of sugar solution in a test tube, add 4 drops of Molisch's reagent. Mix thoroughly. Incline the tube and allow about 2 ml of concentrated sulphuric acid to flow down the side of the tube, thus forming a layer of acid beneath the sugar solution. Record your observation. a) Glucose b) Fructose		

2. Benedict's test for reducing sugars:

To 5 ml of Benedict's reagent in a test tube, add exactly 8 drops of sugar solution. Mix well. Heat the mixture until it begins to boil.

(Ensure that the contents do not boil out of the tube and that the mouth of the tube is not directed towards any person, during the heating).

Place on a test tube rack to cool for 1-2 minutes. Record your observation.

m) Glucose

n) Fructose

3. Barfoed's test:

To 5 ml of Barfoed's solution in a test tube, add 10 drops of sugar solution and heat the solution till it begins to boil; take it off the flame and cool it by leaving on a test tube rack for a few minutes. Examine the bottom of the test tube. Record your observation.

j) Glucose

k) Fructose

4. Seliwanoff's test: To 5 ml of Seliwanoff's reagent in a test tube, add 5 drops of sugar solution and heat the mixture to boiling. Place the test tube in the rack and allow it to cool for 1-2 minutes. Record your observation. k) Glucose l) Fructose		

B. Reactions of disaccharides (demonstration)

The disaccharides of importance in humans are maltose (formed during the digestion of starch), lactose (found in milk) and sucrose (table sugar). Each is built up of two monosaccharides as follows: Maltose Lactose Sucrose

Maltose and lactose are reducing sugars, because they have a free aldehyde group. On the other hand, in the case of sucrose, the aldehyde group of glucose is linked with the ketone group of fructose. Therefore, sucrose is a non-reducing sugar.

Date:

The following reactions of disaccharides (lactose and sucrose), will be demonstrated. Record your observations and inferences.

Experiments	Observation	Inference
1. Molisch's test To 5 ml of sugar solution in a test tube, add 4 drops of Molisch's reagent. Mix thoroughly. Incline the tube and allow about 2 ml of concentrated sulphuric acid to flow down the side of the tube, thus forming a layer of acid beneath the sugar solution. Record your observation. a) Lactose b) Sucrose		

2. Benedict's test for reducing sugars: To 5 ml of Benedict's reagent in a test tube, add exactly 8 drops of sugar solution. Mix well. Heat the mixture until it begins to boil. Take the tube off the flame and allow to cool on a test tube rack. Record your observation. m) Lactose n) Sucrose 3. Barfoed's test: To 5 ml of Barfoed's solution in a test tube, add 10 drops of sugar solution and heat the solution till it begins to boil, and then cool it by leaving on a test tube rack. Examine the bottom of the test tube. Record your observation. c) Lactose d) Sucrose		

C. Reactions of polysaccharides (demonstration)

Starch is built up of a large number of glucose molecules, which are connected by α-1:4 and α-1:6 glycosidic linkages. It is insoluble in cold water, but when boiled with water it forms a colloidal solution. It is completely precipitated by half saturation with ammonium sulphate. It gives a blue colour with iodine due to the formation of an adsorption compound. Though it is made up of glucose units, it has no reducing properties, because all the aldehyde groups are bound up in the 1:4 glycosidic linkages.

Dextrins are intermediary products formed during the hydrolysis of starch. Depending upon the colour formed with iodine, they are called amylodextrin (violet colour), erythrodextrin (red colour) and achrodextrin (no colour). They are only partially precipitated by full saturation with ammonium sulphate. Dextrin shows slight reducing action because of a free aldehyde group at the end of the chain in the large molecule.

Glycogen is the storage polysaccharide found in animals; it corresponds to starch which is found in plants.

Date:

The following reactions will be demonstrated. Record your observations and inferences.

Experiments	Observation	Inference
1. Molisch's test To 5 ml of starch solution in a test tube, add 4 drops of Molisch's reagent (1% solution of α-naphthol in alcohol). Mix thoroughly. Incline the tube and allow about 2 ml of concentrated sulphuric acid to flow down the side of the tube, thus forming a layer of acid beneath the sugar solution. Record your observation. 2. Iodine test Take 2 ml of starch solution in a test tube and add a drop of N/50 iodine. Record your observation. 3. Benedict's test for reducing sugars: To 5 ml of Benedict's reagent in a test tube, add exactly 8 drops of sugar solution. Mix well. Heat the mixture until it begins to boil. Cool by placing the test-tube on a rack. Record your observation.		

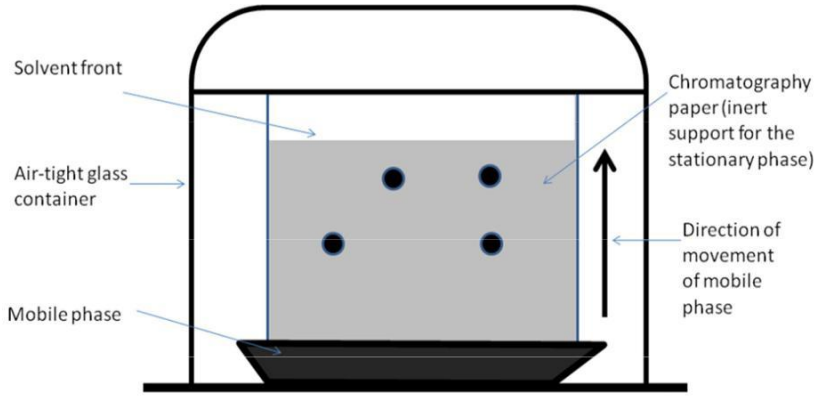
D. Paper chromatography of carbohydrates (demonstration)

Chromatography was first used for the separation of coloured substances; hence the name. It is one of many effective techniques for separating, identifying and purifying biomolecules. It is based on the way in which substances distribute themselves between two immiscible phases. The mixture of substances to be separated (called solutes) is allowed to interact with two phases - a mobile phase and a stationary phase. The mobile phase, which may be a gas or a liquid, moves the substances (solutes) through a support containing a solid or liquid stationary phase. The components of the mixture distribute themselves differentially between the mobile and the stationary phases, and will thus be separated.

There are various types of chromatographic techniques, such as paper chromatography, thin layer chromatography, gas-liquid chromatography, adsorption chromatography, affinity chromatography, ion exchange chromatography and high performance liquid chromatography (HPLC).

In paper chromatography, paper serves as an inert support. When the mobile phase moves along the paper, the mixture of substances (e.g. carbohydrates) applied as a spot on the paper partitions between the mobile solvent and the stationary phase embedded in the interstices of the paper. The rates of migration of carbohydrates depend on their affinity for the two phases. Different carbohydrates migrate at different rates and thus get separated.

The mixture of sugars to be separated and their corresponding standards (5% for monosaccharide standards, 7% for disaccharide standards and 10% for the test mixtures) are dissolved in isopropanol and water (in the ratio 4:1). A pencil line is drawn about 2 cm from the bottom of a sheet of standard chromatogram paper (11 x 19 cm in size). Fifty microlitres of standard carbohydrate and test mixture solutions are spotted individually along the line. The spotting is done using a capillary tube, at sites at least 2 cm apart along the line.



The solvent system (mobile phase) is prepared by mixing isopropanol and water in the ratio of 4:1. A trough containing the mobile phase is placed in such a way that the lower end of the paper is dipped

in the mobile phase. The solvent will travel up the paper by capillary action. This is allowed

to take place for 3 hours. Care must be taken to make sure that the spots are not submerged in the solvent. The whole set-up is kept in an airtight enclosure by inverting a glass jar over it and sealing the edges with vaseline.

At the end of 3 hours, the paper is taken out and the distance to which the solvent has migrated (solvent front) is marked on the paper immediately, using a pencil. The paper is then air-dried. Once dry, it is sprayed with 0.5% of 3, 5-dinitrosalicylic acid in 1N NaOH solution. The paper is kept in a hot air oven at 110°C, for 5-10 minutes. The carbohydrates that have moved along the paper appear as brown spots. The center of each spot is marked. The distance between the centre of each spot and the point of application of the carbohydrate/mixture is measured.

The R_f value (ratio of fronts) is calculated as follows:

R_f = Distance traveled by the solute (carbohydrate) / Distance traveled by the solvent

The R_f is constant for a particular carbohydrate for a given solvent system.

Date:

Questions:

12. Calculate the R_f values for the given sugars. Explain why calculating R_f value is more useful than simply reporting the distance travelled by the spot on the chromatogram.
13. List the disorders of carbohydrate metabolism where paper chromatography is used for diagnosis.

Note: Questions at the end of each experiment should be answered on the left-hand pages of the record note book.

II. COLORIMETRY (DEMONSTRATION)

Introduction:

A colorimeter is an instrument used to measure the intensity of colour in a given solution, with reference to a similarly coloured standard solution. In this way, if the concentration of the colour-producing substance in the standard solution is known, its concentration in the unknown solution can be readily ascertained.

Principle of colorimetry:

A coloured solution maximally absorbs light corresponding to its complementary colour. When such light is passed through a coloured solution, a certain percentage of this light is absorbed and the rest is transmitted. The absorbance (A) or optical density (OD) of this solution is defined as the logarithm of the ratio of the incident to transmitted light

$$\text{Optical density, OD} = \log \frac{\text{Incident light}}{\text{Transmitted light}} = \log \frac{100}{T}$$

where, incident light is taken as 100% and 'T' refers to the percent of light transmitted (transmittance).

OD = log 100 - log T

o) log 10^2 - log T

p) 2 log 10 - log T

OD = 2 - log %T

The transmitted light falls on a photo-detector, resulting in conversion of light energy to electrical energy that is recorded on a meter or is displayed digitally.

Two laws of photochemistry explain the relationships involved in the principles of colorimetry. These are the laws of Beer and Lambert. Beer's law states that the optical density (OD) of a coloured solution is directly proportional to the concentration of the coloured substance in the solution. Lambert's law states that the OD (absorbance) of a coloured solution is directly proportional to the distance through which the light has to pass in the solution. These statements comprise Beer-Lambert's law.

If the distance through which the light passes in the coloured solution is held constant (usually 1 cm), it follows that the ratio of the OD of the unknown solution to that of the standard solution is equal to the ratio of the concentration of the coloured substance in the unknown solution to that in the standard solution, since OD is directly proportional to the concentration of the substance.

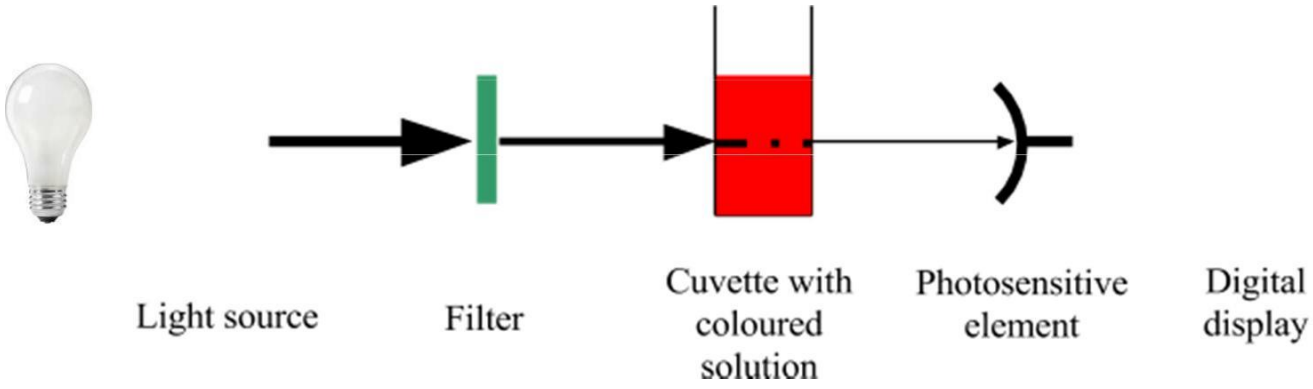
$$\frac{\text{Concentration of substance in unknown solution}}{\text{Concentration of substance in standard solution}} = \frac{\text{OD of unknown solution}}{\text{OD of standard solution}}$$

$$\text{Concentration of unknown} = \frac{\text{OD of unknown}}{\text{OD of standard}} \times \text{Concentration of standard}$$

Individual photoelectric colorimeters differ with respect to the mode of calibration of the galvanometer in the instrument. Most of them display calibration both in optical density (absorbance) and percent transmission scale. If the readings are taken in percent transmission, then for calculation purposes, it has to be converted into optical density by adopting the formula OD = 2 - log %T.

The photoelectric colorimeter:

This instrument measures the intensity of colour of a solution by registering the quantity of light absorbed by the given solution. Hence, a photoelectric colorimeter is better known as absorptiometer. Several types of absorptiometers are available and they essentially consist of the following components.



(viii) Light source

(ix)A filter to select a narrow wave band of light from the light source:

In most colorimeters, the narrow wave band of light is achieved by placing a suitable filter in the pathway of light. This facilitates the resolution of light of desired wavelength.

(iii) Cuvettes:

These are made of glass, which is inert. The solution, the OD of which is to be determined, is poured into a cuvette and placed in the colorimeter, for measurements to be made. An important point to be observed is that all cuvettes used in a particular instrument should possess the same absorptive capacity and hence should be interchangeable. Cuvettes usually have a constant internal diameter of 1cm.

(iv) Photosensitive element:

In most absorptiometers, "barrier layer" cells are used for the photosensitive element. These cells are furnished with a layer of selenium. When light falls on the selenium layer, it is activated and emits electrons. The emission is proportionate to the light falling on it. If a galvanometer is attached, electron flow can be measured, which in turn will indicate the amount of light falling on the cells.

(v) A highly sensitive galvanometer:

This measures the output of the photosensitive element.

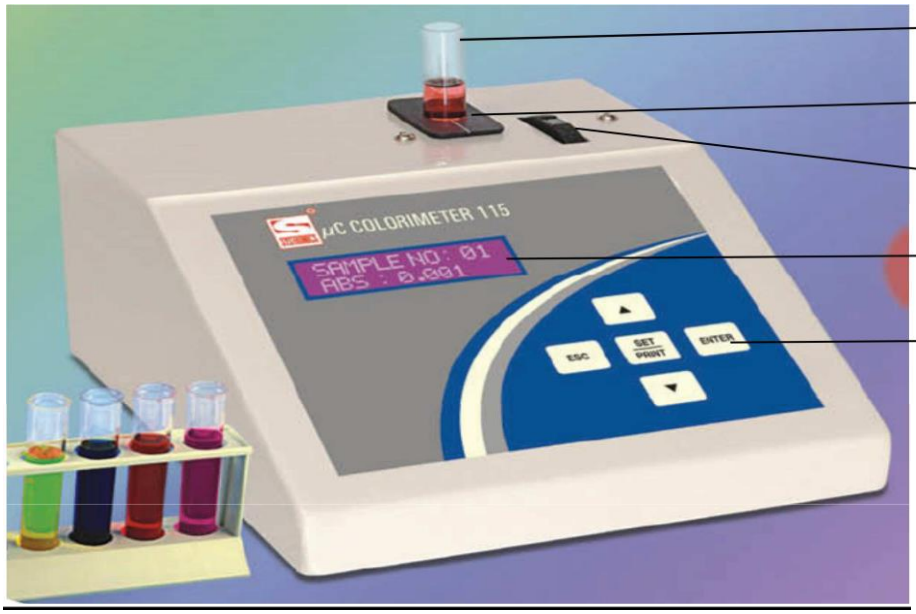
Significance of blank solutions

In all colorimetric analyses, preparation of a 'blank' solution is necessary. This is because of the fact that some colour in the coloured solution (of interest) is, inevitably, due to the colour of the reagents themselves. Hence, if a blank solution is prepared using all the reagents, except the substance which is being measured, and its OD value is subtracted from the OD of the unknown and standard, the resulting value represents the true optical density.

Choice of filters

This is another key feature in colorimetric estimations. If the relationship of concentration and optical density is to be linear, monochromatic light is necessary. Invariably, in every case, the precise wavelength selected is that of the light which is most strongly absorbed by the coloured solution.

Picture of colorimeter in the laboratory



Instructions for using the colorimeter:

The colorimeter that you will use is a Systronics colorimeter 115. The following instructions are specific to this model.

1. Turn the instrument on, by putting on the switch at the back of it. Wait for a few seconds till the digital display on the instrument reads 'Busy'.
2. Use the knob for adjusting the filter to set the filter required for the estimation.
3. After a few more seconds, the display will read "Measuring mode". At this point, press the "Enter" button on the front of the instrument.
4. The display will now read "Transmission". Press the button on the panel that shows the down arrowhead. The display will now read "Absorbance". Press the "Enter" button.
5. The display will now read "Put reference". Pour the blank solution into the cuvette provided. Take care to keep the outside of the cuvette dry. Wipe dry the outside of the cuvette, in case of any spills. Place the cuvette into the sample holder. Press the "Enter" button.
6. The display will first read "Busy". This will be followed by "Put sample".
7. Pour out the blank solution back into its test tube. Replace it with either the test or standard solution (see note below). Press the "Enter" button.
8. The display will first read "Busy". This will be followed by a value for absorbance.
9. Write down this value.
10. Press the "Esc" button on the panel on the instrument.
11. The display will read "Put sample".
12. Replace the solution in the cuvette with the remaining solution (test or standard solution, as the case may be) (see note below). Press the "Enter" button.
13. The display will first read "Busy". This will be followed by a value for absorbance.
14. Write down this value.
15. You will have now completed taking readings for your "standard" and "test" solutions.

16.Take out the cuvette and pour back the solution into its test tube.
17.Press the “Esc” button thrice for instrument to revert to “Measuring mode”.
18.Rinse the cuvette and leave it for the next student to use.
19.The next student to take readings will need to follow instructions from step 2 to 17.

Note:

When taking readings for many solutions, using the same cuvette, it is best to first take the reading of the solution with the least intensity of colour, followed by the rest of the solutions of increasing colour intensity. This is to avoid carry-over of the substance being measured from one solution to another and will avoid errors in readings. Drain out the fluid in the cuvette (onto a piece of tissue) as much as possible, before pouring in the next solution.

Date:

Question:

1. Explain the concepts of “blank” and “standard” solutions used for colorimetric estimations.

Note: Questions at the end of each experiment should be answered on the left hand pages of the record note book.

Date:

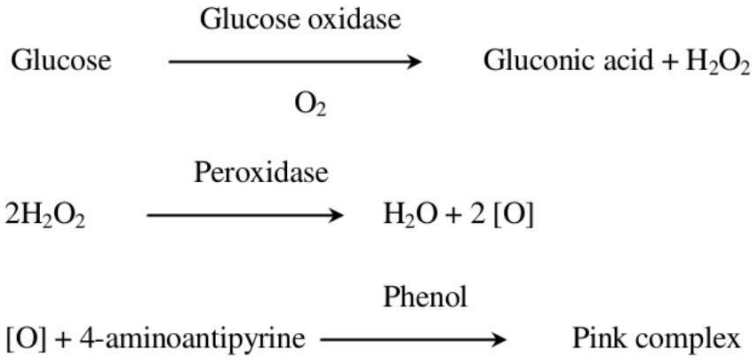
III. ESTIMATION OF PLASMA GLUCOSE

Introduction:

Glucose estimations used to be performed using whole blood, often with relatively non-specific methods, which also measured other reducing substances such as glutathione, uric acid and creatinine present in whole blood. In recent years, glucose is estimated in plasma by a colorimetric method using a glucose oxidase/peroxidase system. This measurement is specific for glucose. The reference range for fasting plasma glucose is 70-100 mg/dL, while 2-hour post-prandial levels should be less than 140 mg/dL (as per the criteria set out by the American Diabetes Association [ADA], 2016). A fasting sample of blood is one that is collected after 8-12 hours of zero calorie intake; this is usually done most conveniently after an overnight fast.

Principle: (Glucose oxidase/peroxidase method)

Glucose is oxidized by glucose oxidase (GOD) to gluconic acid, with liberation of hydrogen peroxide. The hydrogen peroxide is acted upon by peroxidase (POD) to yield nascent oxygen. In the presence of phenol, nascent oxygen reacts with 4-aminoantipyrine to form a pink-coloured complex. The intensity of the coloured solution is read at 540 nm, using a colorimeter.



Procedure:

Step 1:Dilution of plasma and standard glucose solution:

Label 2 clean test tubes as 'P' (for plasma) and 'DS' (for diluted standard).Add the following reagents

Reagents	Plasma (ml)	Standard (ml)
Distilled water	3.8	3.8
Glucose standard (200mg/dL)	-	0.2
Plasma	0.2	-

Mix the contents of each tube well.

Step 2:
Label 3 clean test tubes as B (blank), T (test) and S (standard) and add the following reagents to each:

Reagents	B (ml)	T (ml)	S (ml)
Distilled water	0.2	-	-
Diluted plasma	-	0.2	-
Diluted standard	-	-	0.2
Colour reagent	2.5	2.5	2.5

The colour reagent contains phosphate buffer pH 7.0 (KH_2PO_4/Na_2HPO_4), glucose oxidase, peroxidase, 4-aminoantipyrine, phenol and Tween-20 (a detergent).

Mix the contents of each test tube well. Place them in a rack in a water bath maintained at 37°C, for 15 minutes. At the end of this incubation, use the colorimeter provided to determine the absorbance (optical density) of the test and standard solutions, at 540 nm.

Calculation

OD of test solution =

OD of standard solution =

Concentration of glucose standard provided (mg/dL) =

Concentration of glucose in the plasma sample (mg/dL) =

$$\frac{\text{OD of test}}{\text{OD of standard}} \times \text{concentration of standard}$$

Result

Interpretation of result

Questions:

6. Interpret the plasma glucose value in the sample you have been provided with, assuming that the sample was obtained from a patient after an overnight fast.
7. List criteria of the American Diabetes Association (ADA) and World Health Organization (WHO) for diagnosis of diabetes mellitus. What are some of the other biochemical investigations that are to be done to monitor a diabetic patient who has come for a check-up?
8. What is glycated haemoglobin? What is the clinical importance of measuring this analyte?

Note: Questions at the end of each experiment should be answered on the left hand pages of the record note book.

Date:

IV. PROTEINS

A. Paper chromatography of amino acids (demonstration)

The procedure for paper chromatography for separation of amino acid mixtures is similar to that for separation of carbohydrate mixtures (refer to page 11). The mobile phase and the stationary phase are prepared by mixing n-butanol, acetic acid and water in the ratio of 4:1:5 in a separating funnel. The mixture is allowed to stand in the separating funnel for a minute. Two layers will separate out. The upper layer is butanol saturated with water. It is used as the mobile phase. The lower layer is water saturated with butanol. It is used as the stationary phase.

A pencil line is drawn about 3 cm from the bottom of a sheet of Whatman chromatogram paper (11 x 19 cm). About 40 microlitres of the standard amino acid solution (approximately 40-45 mg, dissolved in 5 ml of 10% isopropanol in 0.1N HCl) is applied on the paper as a spot, using a capillary tube. The mixture of amino acids to be identified is applied similarly. The samples applied are allowed to dry.

A trough containing the mobile phase is placed so that the lower end of the paper dips into the mobile phase. Care must be taken to make sure that the spots are not submerged in the solvent. The whole setup is kept in an airtight enclosure by inverting a glass jar over it and sealing the edges with vaseline. The solvent will travel up the paper by capillary action. This is allowed to take place for 3 hours. At the end of 3 hours, the paper is taken out and the solvent front is marked immediately, using a pencil. The paper is then air-dried. It is sprayed with 0.1% ninhydrin in acetone, and dried in a hot-air oven at 110°C, for 5-10 minutes. The amino acids that have migrated to various points on the paper react with ninhydrin and produce purple-coloured spots.

Ninhydrin reaction:

Ninhydrin (2,2-dihydroxyindane-1,3-dione), a powerful oxidizing agent, reacts with free amino acids (between pH 4 and 8) to give hydrindantin, which reacts with another molecule of ninhydrin to give a purple-coloured complex (Ruhemann's purple). Ninhydrin also reacts with primary amines and ammonia, but without the liberation of CO_2. The imino acids, proline and hydroxyproline, also react with ninhydrin, but in this case a yellow colour is obtained. The reaction is very sensitive and ideal for the detection of amino acids on chromatograms and for their quantitative determination.

The R_F value is calculated using the formula given below.

$$R_f = \frac{\text{Distance traveled by the solute (amino acid)}}{\text{Distance traveled by the solvent}}$$

Question: What are aminoacidurias? Classify them and give examples.

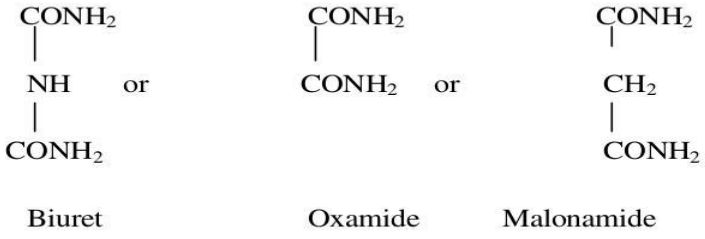
Note: Questions at the end of each experiment should be answered on the left hand pages of the record note book.

B. Colour reactions of proteins (demonstration)

The term "protein" is derived from the Greek word "proteios" meaning primary or holding first place. Proteins are complex nitrogen-containing organic compounds, which are found in all animal and plant cells. Protein molecules are built up of a large number of alpha amino acids joined together by peptide linkages.

Proteins undergo certain reactions producing coloured compounds/complexes, which are characteristic of the side chains (functional groups) of the amino acids they contain.

The biuret reaction is commonly used to detect the presence of proteins, as molecules containing two or more peptide linkages give a positive reaction. It is, however, not a specific test for proteins because it is also answered by substances that contain two carbamyl (-$CONH_2$) groups, joined either directly together or through a single atom of nitrogen or carbon. Examples of such molecules are shown below.



Aldehyde test (Hopkins Cole test) indicates the presence of the indole ring of tryptophan.

Sulphur test indicates the presence of the amino acids that contain labile sulphur (cysteine or cystine, but not methionine).

A protein, which does not contain one or more of these particular amino acids, will not test positive in the corresponding reactions. A great majority of proteins, however, do contain most of them.

Date:

Perform the following tests using albumin solution.

Experiments	Observation	Inference
1. Biuret test To 2 ml of albumin solution in a test tube, add 2 ml of 5% NaOH and mix. Add two to three drops of 1% copper sulphate and mix. Do not add an excess of copper sulphate because the characteristic deep blue colour of cupric		

hydroxide will mask the colour produced in the test. Record your observation. 2. Aldehyde test To 1 ml of albumin solution in a test tube, add 1 drop of 1 in 500 formalin followed by a drop of 10% mercuric sulphate in 10% sulphuric acid (Millon's reagent). Mix well and gently layer 3 to 4 ml of conc. sulphuric acid at the bottom of the tube by adding it along the side of the tube. Gently swirl the tube so that the fluids slowly mix at the interface. Record your observation. 3. Sulphur test To 1.5 ml of the albumin solution, add 1.5 ml of 40% NaOH and boil for at least a minute. Then add 0.5 ml of 10% lead acetate solution. Record your observation.		

C. Precipitation reactions of proteins (demonstration)

Proteins form colloidal solution called emulsoids or hydrophilic colloids. One of the most characteristic properties of emulsoids is that such systems have two stability factors, charge and hydration, either or both of which keep proteins in solution. Factors that neutralize the charge or remove the shell of hydration precipitate proteins from solution.

Individual proteins show marked differences in the hydration of particles in solution. Most proteins are soluble in dilute acids and alkalies because the particles acquire a positive or negative charge, depending upon the pH of the solution. For every protein, there is a characteristic pH, known as isoelectric pH, at which the particles are electrically neutral and hence, cease to migrate to the poles of an electrical field.

Proteins can be precipitated from solution by alkaloidal reagents (e.g., sulphosalicylic acid and phosphotungstic acid) or concentrated salt solutions (e.g., ammonium sulphate, sodium sulphate and sodium chloride) or heat.

In acidic solution, proteins are positively charged (in the form of cations) and will combine with negatively charged moieties of alkaloidal reagents to precipitate out of solution. A white precipitate indicates presence of protein.

Proteins are precipitated by concentrated salt solutions, such as ammonium sulphate, sodium chloride and sodium sulphate, which remove the hydration shell of proteins. The concentration of salt required for the precipitation of a protein depends on the particular protein and on the pH of the solution. The precipitation of proteins by salt solution is called "salting out" process. The protein precipitated by the salting out methods is unaltered (native) and usually re-dissolves when treated with fresh portions of the original solvent. Thus, the process of salting out finds wide application in the purification of proteins.

Date:

Perform the following tests:

Experiments	Observation	Inference
2. Precipitation of albumin by alkaloidal reagents To 2 ml of the albumin solution, add 3 to 4 drops of 3% sulphosalicylic acid reagent. Record your observation. 3. Precipitation of albumin by concentrated salt solutions Take 3 ml of the albumin solution in a test tube. Add small quantities of solid ammonium sulphate and mix thoroughly to dissolve the salt. Continue adding ammonium sulphate until the solution is fully saturated (until some ammonium sulphate crystals settle at the bottom of the tube and cannot be dissolved). Record your observation. 4. Heat coagulation test for albumin Take 5 ml of albumin solution in a test tube and heat it. Record your observation. Now add about 5 drops of 1% acetic acid, drop by drop. Record your observation.		

V. LIVER FUNCTION TESTS

The liver function tests are a group of tests that assess the various functions of the liver and hence, help in evaluation and diagnosis of liver diseases. They are also useful in assessing prognosis of liver diseases and monitoring response to therapy.

These tests include measurement of total serum proteins, serum albumin, serum bilirubin, prothrombin time and activity of enzymes [alanine transaminase (ALT), aspartate transaminase (AST), alkaline phosphatase (ALP) and gamma glutamyltransferase (GGT)].

Date:

A. Estimation of serum total proteins

Introduction:

Measurement of serum total protein levels provides information on the functioning of many organ systems, especially the liver and kidney. The biuret method is the most widely used method for determination of total protein levels. The reference range for serum total protein in healthy adults is 6.7 to 8.6 g/dL.

Principle: (Biuret method)

In an alkaline medium, peptide bonds complex with cupric ions (Cu^{2+}) to form a violet-coloured complex, the absorbance of which can be measured at 540 nm.

The biuret test is not a specific test for proteins. Cupric ions will react with any compound that has two carbamyl groups, joined either directly together or through a single atom of nitrogen or carbon. One such compound, biuret, combines with cupric ions in this manner and the method derives its name from this reaction.

Procedure:
Label three test tubes as B (blank), T (test) and S (standard). Add reagents to these as follows:

Reagents	B (ml)	T (ml)	S (ml)
0.9% Saline	2.5	2.4	2.4
Standard protein solution	-	-	0.1
Serum	-	0.1	-
Biuret reagent	3.0	3.0	3.0

Mix the contents of the tubes thoroughly. Incubate at room temperature for 15 minutes. Read absorbance of the solutions at 540 nm.

(The biuret reagent consists of copper sulfate, potassium tartrate and potassium iodide in an alkaline medium. Potassium tartrate complexes with cupric ions and prevents their precipitation in the alkaline medium. Potassium iodide acts as an antioxidant.)

Calculation:

OD of test =

OD of standard =

Concentration of standard protein (g/dL) =

$$\text{Concentration of serum total protein (g/dL)} = \frac{\text{OD of test}}{\text{OD of standard}} \times \text{concentration of standard}$$

Result

Interpretation of result

Questions:

3. List the biochemical tests for evaluation of the various functions of the liver.
4. Classify and list the causes of hypoproteinemia and hyperproteinemia.
5. Where are plasma proteins synthesized? Classify and list the plasma proteins.
6. List the functions of the major plasma proteins.

Note: Questions at the end of each experiment should be answered on the left hand pages of the record note book.

Date:

B. Estimation of serum albumin

Introduction:

Albumin has a molecular weight of approximately 69,000 Da. It is the major protein of human plasma. It is synthesized in the liver and comprises about 60% of the total plasma protein. In the laboratory, albumin in serum is commonly estimated by a dye-binding method, using bromocresol green (BCG) dye. The reference range for serum albumin in healthy adults is 3.7 to 4.9 g/dL.

Principle: (Bromocresol green [BCG] method)

Albumin binds, with great affinity, to bromocresol green dye at pH 4.15, resulting in the formation of a green colour, which shows maximum absorbance at 630 nm.

Procedure:

Step 1: Pipette out 0.2 ml of serum into a test tube. Add 1.8 ml distilled water to it to achieve a dilution of 1 in 10. Dilute the standard protein in a similar way.

Step 2: Add the diluted solutions to individual test tubes marked blank (B), standard (S) and test (T) as indicated in the table.

Reagents	B (ml)	S (ml)	T (ml)
Distilled water	0.2	-	-
Diluted standard protein	-	0. 2	-
Diluted serum	-	-	0.2
BCG reagent	5.0	5.0	5.0

Mix the contents of each tube well. Let them stand for 10 minutes at room temperature and then take the reading of the solutions at 600 nm.

Calculation:

OD of test =

OD of standard =

Concentration of standard albumin solution (g/dL) =

$$\text{Concentration of albumin in serum (g/dL)} = \frac{\text{OD of test}}{\text{OD of standard}} \times \text{concentration of standard}$$

Result

Interpretation of result

Questions

5. What are the functions of serum albumin?
6. What are the major causes of hypoalbuminemia?
7. What is meant by an A:G ratio? Calculate the A:G ratio from the values that you have obtained in your estimations. What does “reversal” of the A:G ratio mean? Name a few conditions where you would find a reversed A:G ratio.

Note: Questions at the end of each experiment should be answered on the left hand pages of the record note book.

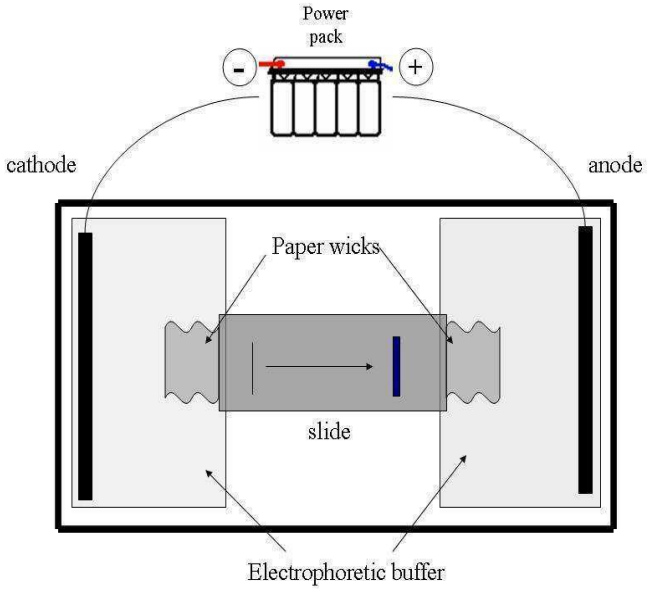
VI. SEPARATION OF SERUM PROTEINS BY ELECTROPHORESIS (demonstration)

Introduction:

Electrophoresis can be defined as the migration of charged particles under the influence of an electric field. Proteins in serum can be separated on the basis of their differential electrophoretic migration, due to differences in their molecular sizes and charges. All serum proteins carry negative charge at a pH of 8.6, which is alkaline to the isoelectric pH of the proteins, and therefore move towards the anode.

Apparatus for electrophoresis:

The electrophoretic tank has two buffer compartments, one each for the anode and the cathode. The electrodes run the full length of their respective compartments. The electrodes are connected to a power pack, which maintains constant current/voltage across the electrodes. There is a bridge on which a slide on which electrophoretic separation takes can be placed. The slide is coated with agarose gel. Filter paper wicks connect the gel and the buffer in the buffer compartments.



Reagents:

1. Electrophoretic buffer (Tris buffer pH 8.6)
2. Acid-ethanol mixture (50 ml): a mixture of ethanol, water and acetic acid in the ratio 70:25:5 (v/v/v)
3. Dehydrating solution (100 ml): acetone and water in the ratio 90:10 (v/v)
4. Staining reagent - amido black IOB (Amidoschwarz IOB)
5. Destainer: 2% acetic acid (100 ml)

Procedure:

1. Preparation of the agarose gel-coated slide: Weigh 100 mg of agarose and transfer into a boiling tube. Add 10 ml of electrophoretic buffer. Place the tube in a boiling water bath, until the agarose dissolves. Pour 2.5 ml of the solution onto a glass slide. Allow it to set for 3 min.
2. Application of the serum: A small volume of the serum is mixed with a marker dye, bromophenol blue. Dip a cover slip in the sample and apply 2 to 3 µl of the sample by cutting a slot in the gel, across one end of the slide.
3. Separation of proteins in serum: Place the slide on the bridge of the tank, with the point of application being at the cathodic end of the electrophoretic tank. Connect the ends of the slide to their respective buffer compartments, using wicks of Whatman No.1 filter paper. The chamber is closed and a current of 120 V or 6-7 mA is applied, until the bromophenol blue marker has reached the other end of the slide. Switch off the current and remove the slide.
4. Fixation of separated proteins and dehydration step: Place the slide in cold dilute acid-ethanol mixture for 30 min. The slide is then dehydrated by replacing it in the acetone-water mixture for 2 hrs. Dry the slide at 37°C.
5. Staining of proteins: Immerse the slide for 10 min in amido black IOB dye solution, and then wash it by immersing three times in 2% acetic acid for 5 min each. Rinse it with water and dry at $37^{O}C$ for 10 min.

Date:

Questions:

1. Define electrophoresis. List the names of different types of electrophoresis.
2. Draw the normal serum protein electrophoretic pattern.
3. Draw the serum protein electrophoretic pattern seen in multiple myeloma.

Note: Questions at the end of each experiment should be answered on the left hand pages of the record note book.

VII. RENAL FUNCTION TESTS

Introduction:

Renal function tests are a group of tests used for the evaluation of kidney function. These include:

a) measurement of non-protein nitrogenous (NPN) metabolites, like urea and creatinine, in blood
b) estimation of glomerular filtration rate (GFR)
c) evaluation of tubular secretory capacity
d) evaluation of tubular reabsorptive capacity for water and electrolytes
e) physical and chemical analysis of urine (urinalysis)

Date:

A. Estimation of serum urea

Introduction:

The reference range for blood urea is 15 to 40 mg/dL. This represents a balance between its production in the liver and excretion by the kidney. The concentration of blood urea rises if excretion is impaired; this frequently occurs in renal diseases. Apart from this, pre- and post-renal conditions can also cause elevations in blood urea. Hence, serum urea is not a specific marker of renal dysfunction.

Principle: (Diacetylmonoxime-thiosemicarbazide method)

Urea reacts with diacetylmonoxime, under acidic conditions, to form a yellow condensation product, diazine. This is called the Fearon reaction. The reaction is intensified by the presence of ferric ions and thiosemicarbazide. The intensity of the resulting red-coloured complex is proportional to the urea concentration. The absorbance of the solution is obtained at 540 nm.

Procedure:

Step 1: Dilution of serum and standard urea solution:

Label 2 clean test tubes 'serum' and 'standard' and add the following reagents:

Reagents	Serum (ml)	Standard (ml)
Distilled water	1.9	1.9
Urea standard (50 mg/dL)	-	0.1
Serum	0.1	-

Mix the contents of the test tubes well.

Step 2: Label 3 clean test tube as B (blank), T (test) and S (standard) and add the following:

Reagents	B (ml)	T (ml)	S (ml)
Distilled water	0.1	-	-
Diluted serum	-	0.1	-
Diluted standard	-	-	0.1
Colour reagent	3	3	3

Place a glass marble over the mouth of each test tube (to prevent evaporation of the contents); place all the tubes in a rack in a boiling water bath, for 15 minutes. Remove the tubes and cool them to room temperature by placing them in a beaker of tap water. Read the absorbance of the solutions at 540 nm.

Note: The colour reagent contains diacetylmonoxime, thiosemicarbazide and ferric chloride in a strongly acidic medium.

Calculation:

OD of test =

OD of standard =

Concentration of urea standard (mg/dl) =

Concentration of serum urea (mg/dL)

= ~~OD of test x~~ concentration of standard

OD of standard

Result

Interpretation of result

Questions:

1. What is the clinical importance of estimating blood urea levels?
2. What is azotemia? Classify and list the causes of azotemia.
3. What are the sources of ammonia in the body? How is ammonia detoxified in the brain and liver?
4. What is blood urea nitrogen (BUN)? Calculate the BUN value in the sample provided.
5. What is hyperammonemia? Classify causes of this condition and give examples of such causes.

Note: Questions at the end of each experiment should be answered on the left hand pages of the record note book.

Date:

B. Estimation of serum creatinine

Introduction:

Creatinine is formed by spontaneous non-enzymatic dehydration of creatine. Since it is excreted by the kidneys, its levels in blood increase in renal diseases. Blood levels of creatinine are fairly constant and is unaffected by the various factors that affect blood urea levels. Due to this, creatinine is considered a more specific marker of renal function than urea. The reference range for serum creatinine is 0.6 - 1.2 mg/dL in males and 0.5 – 0.9 mg/dL in females.

Principle: (alkaline picrate method)

Creatinine in serum is measured by using the Jaffe's reaction. Creatinine reacts with picric acid, in an alkaline medium, to yield a yellow-red (orange) colour. The intensity of the colour is a measure of the concentration of creatinine present in the sample.

Procedure:

Step 1: Preparation of protein-free filtrate:

Pipette out 4 ml of serum into a clean and dry boiling tube. To this, add 6 ml of water, followed by 2 ml of 10% sodium tungstate and 4 ml of 2/3N H_2SO_4. Mix the contents of the boiling tube thoroughly; let it then stand for 10 minutes. Filter the contents of the tube through a Whatman No.1 filter paper; use the filtrate obtained for creatinine estimation.

Step 2: Assay of creatinine

Label three test tubes as B (blank), T (test) and S (standard) and add the following reagents.

Reagents	B (ml)	T (ml)	S (ml)
Filtrate	-	3	-
Standard (creatinine -1 mg/dL)	-	-	3
Water	3	-	-
Picric acid (0.04 M)	1	1	1
NaOH (0.75 M)	1	1	1

Mix the contents of all the tubes properly and let stand for 10 minutes. Take the OD readings of the solutions at 540 nm.

Calculation

OD of test =

OD of standard =

Concentration of creatinine standard (mg/dl) =

Dilution factor =

(Note: In step 1, during preparation of the protein-free filtrate, 4 ml of the sample was diluted to 16 ml, therefore the dilution factor is 4.)

Serum creatinine (mg/dL) = $\frac{\text{OD of test}}{\text{OD of standard}}$ x concentration of standard x dilution factor

Result

Interpretation of result

Questions:

1. What is the clinical significance of estimating serum creatinine in a patient?
2. How is creatinine formed in the body?
3. Why is serum creatinine a better indicator of renal function than blood urea?
4. What is meant by acute kidney injury (AKI) or acute renal failure (ARF)? Classify and list the causes of this condition.
5. What is the blood urea nitrogen (BUN): creatinine ratio? What is its significance?

Note: Questions at the end of each experiment should be answered on the left hand pages of the record note book.

Date:

C. Creatinine clearance

Introduction:

Estimation of glomerular filtration rate (GFR) is an important aspect of assessing renal function. The clearance of creatinine is a valid measure of glomerular filtration rate, since it is freely filtered and neither reabsorbed nor secreted by the renal tubules.

The clearance value is obtained mathematically using serum creatinine concentration and creatinine concentration in urine excreted for a given period of time, usually 24 hours. Specimen collection therefore includes both a 24-hour urine specimen and a serum sample, ideally collected at the midpoint of the 24-hour urine collection.

Collection of 24-hour urine sample:

1. The patient is given free access to water. S/he must avoid tea, coffee and alcohol while the urine collection is being made. S/he should also avoid vigorous exercise during this period.
2. The patient is asked to void completely. Urine voided from this point onwards is collected in a clean, dry container free of contaminants and preservatives. This is done for a period of 24 hours. The urine collected should be stored in a refrigerator till laboratory analysis is performed.
3. A sample of blood is collected at the midpoint of the 24-hour urine collection.

NB. Improper collection of urine is the commonest source of error in the estimation of creatinine clearance.

Measure serum creatinine (as above) and urine creatinine (as below), using Jaffe's method. The value of the total volume of urine produced in 24 hours will be provided.

Estimation of urine creatinine

Introduction

Estimation of urine creatinine is important for the calculation of creatinine clearance, which is indicative of the GFR. Urinary creatinine concentrations vary widely, depending upon the volume of urine produced. However, the total creatinine excreted per day for a particular person is relatively constant as it is dependent on the muscle mass of the person. An average adult excretes 1 to 2 grams of creatinine in 24 hours.

Since the total daily excretion of creatinine in given person does not change on a day to day basis, estimation of total urinary excretion of creatinine can be used to check the completeness of a 24-hour urine collection.

Principle

Creatinine in urine is determined using the Jaffe's reaction. Creatinine reacts with picric acid, in an alkaline medium, to yield a yellow-red (orange) colour. The intensity of the colour is a measure of the concentration of creatinine present in the sample.

Procedure

Step 1: Dilute urine 1 to 20. For this, add 1 ml of urine to 19 ml of distilled water. This step is required to dilute the concentration of creatinine to a level that falls within the linear range of measurement of intensity of colour by the colorimeter.

Step 2: Label three test tubes as B (blank), T (test) and S (standard) and add the following reagents, as indicated.

Reagents	B (ml)	T(ml)	S (ml)
Water	3	-	-
Urine (diluted 1 in 20)	-	3	-
Creatinine standard (5 mg/dL)	-	-	3
Picric acid (0.04 M)	1	1	1
NaOH (0.75 M)	1	1	1

Mix contents of all the tubes well; let them stand for 10 minutes. Take the OD readings of the solutions, at 540 nm.

Calculation

OD of test =

OD of standard =

Concentration of creatinine standard (mg/dl) =

Dilution factor =

$$\text{Concentration of serum creatinine (mg/dL)} = \frac{\text{OD of test}}{\text{OD of standard}} \times \text{concentration of standard} \times \text{dilution factor}$$

(Note: In step 1, 1 ml of urine was diluted to 20 ml. Therefore the dilution factor is 20.)

Result

Calculation of creatinine clearance

Calculate the creatinine clearance using the following formula.

$$\text{Creatinine clearance (ml/min)} = \frac{U \times V}{P}$$

Where,

U = concentration of creatinine in urine (expressed as mg of creatinine in 100 ml of urine) V = volume of urine formed / minute.

P = concentration of creatinine in serum (mg of creatinine in 100 ml of serum)

Result

Interpretation of result

Reference ranges:

Creatinine clearance is approximately equal to GFR.

Creatinine clearance in males = 105 ± 20 ml/min

females= 95 ± 20 ml/min

Questions:

1. Calculate the creatinine clearance in the patient who samples were provided. Interpret the results obtained
2. Define clearance.
3. What are the characteristics of an ideal substance the clearance of which is representative of the GFR?
4. What are the advantages of estimation of creatinine clearance over other clearance tests?
5. What is the gold standard for estimation of GFR?
6. What is meant by estimated GFR (eGFR)?

Note: Questions at the end of each experiment should be answered on the left hand pages of the record note book.

Date:

VIII. ESTIMATION OF SERUM URIC ACID

Uric acid is the end product of purine catabolism. It is transported in plasma to the kidney, where it is excreted. The reference range for uric acid in blood is 3-7 mg/dL in men and 2-6 mg/dL in women. When blood uric acid level rises above the normal range, the condition is called hyperuricemia.

Principle: (Caraway method)

For the estimation of serum uric acid, serum is deproteinized. The uric acid in the protein-free filtrate reduces phosphotungstic acid in alkaline medium to form a blue complex, tungsten blue. The intensity of blue colour that develops is measured at 700 nm.

Procedure:

Step 1: Preparation of a protein-free filtrate of serum

Take 4.5 ml of 2/3 N sulphuric acid and 4.5 ml of 10% sodium tungstate in a boiling tube. Add 1.0 ml of serum. Mix the contents well and let stand for 10 minutes. Filter the contents through a Whatman no. 1 qualitative filter paper. Use the clear filtrate for the estimation.

Step 2: Label three test tubes as B (blank), T (test) and S (standard). Add the reagents as indicated.

Reagents	B (ml)	T (ml)	S (ml)
Distilled water	3.0	-	-
Protein-free filtrate	-	3.0	-
Uric acid standard - 2 mg/dL	-	-	3.0
Phosphotungstic acid	1.0	1.0	1.0
10% sodium carbonate	1.0	1.0	1.0

Mix contents of all the tubes well and allow to stand for 10 minutes at room temperature. Read the absorbance of the solutions, at 700 nm.

Calculation:

OD of test =

OD of standard =

Concentration of uric acid standard (mg/dl) =

Dilution factor =

$$\text{Serum uric acid (mg/dL)} = \frac{\text{OD of test}}{\text{OD of standard}} \times \text{concentration of standard} \times \text{dilution factor}$$

(Note: In step 1, 1 ml of serum was diluted to 10 ml. Therefore the dilution factor is 10.)

Result

Interpretation of result

Questions:

1. Classify and list the causes of hyperuricemia.
2. What is gout? How is it treated? Explain the mechanism of action of drugs used in the treatment of gout.

Note: Questions at the end of each experiment should be answered on the left hand pages of the record note book.

IX. URINE ANALYSIS

A. Examination of normal urine (demonstration)

Physical characteristics:

Appearance

Normally urine that is freshly voided is clear. Turbidity, due to the precipitation of phosphates, is normally noted in urine passed after a heavy meal. Urinary tract infections (UTI) can produce turbid urine, due to the presence of white blood cells.

Volume

The normal volume of urine excreted per day by an adult is in the range of 1 to 2 litres. It is influenced by a number of factors such as

(1) amount of fluids ingested

(2) climatic conditions, with less urine output in summer (due to loss of fluid by sweating) compared to winter

(3) loss of body fluids through other channels, such as excessive perspiration (sweating), vomiting and diarrhea. To conserve body fluids in such conditions, the kidneys excrete less urine with a higher concentration of solutes

(4) use of diuretics (drugs that increase urine output)

(5) pathological states, such as uncontrolled diabetes mellitus and diabetes insipidus, are associated with an increased volume of urine excreted. In acute and chronic renal failure, urine volume decreases.

Colour:

Normal urine is usually pale yellow in colour. The depth of the colour depends on the concentration of solutes in urine. The colour of normal urine is due to a pigment called urochrome (derived from urobilin).

Certain abnormal constituents and some drugs, when excreted in urine, may impart colour to the urine. Excretion of conjugated bilirubin in urine, which occurs as a consequence of an obstruction in the biliary passage, results in dark-coloured urine. In alkaptonuria, urine becomes black on standing, due to the excretion of homogentisic acid. Red-coloured urine is seen as a result of haematuria or due to intake of the anti-tuberculosis drug, rifampicin.

Odour:

Normal urine has a faint aromatic odour. The substances responsible for this odour are not well known. It may be due, at least in part, to the presence of minute amounts of certain volatile organic acids. An unpleasant ammoniacal odour evolves when urine is allowed to stand for a prolonged period of time. This is due to the release of ammonia from urea present in urine. Presence of ketone bodies in urine gives it a fruity odour.

Specific gravity:

The specific gravity of urine reflects its solute load. Normal urine has a specific gravity that varies between 1.015 and 1.025. Following copious drinking of water, the specific gravity may fall to 1.003 or lower (hyposthenuria), whereas in cases of excessive perspiration, it may rise as high as 1.040 (hypersthenuria). In acute renal failure, the urine is concentrated and of a high specific gravity, whereas in chronic renal failure, the specific gravity of urine becomes fixed at 1.010 (isosthenuria). In uncontrolled diabetes mellitus, the volume of urine is large and the specific gravity high, owing to the glucose excreted in the urine. In diabetes insipidus, urine volume is large and has low specific gravity.

Determination of specific gravity of a sample of urine using a urinometer:

Fill three-quarters of the cylinder provided with the given urine. Insert the urinometer into the urine and let it float in it. The urinometer should not touch the sides of the cylinder. Note the reading on the scale on the urinometer, which corresponds to the upper meniscus of the level of urine. The numbers found on the scale represent the second and third decimal places of the specific gravity value. Thus, if the upper meniscus of the urine level is at the number 20 on the scale, this reading of 20 is recorded as a specific gravity 1.020. Note the temperature of the specimen, using a thermometer. The urinometer is calibrated at 15°C. For every 3 degrees centigrade above 15°C, add 0.001 to the value of the specific gravity and for every 3°C below it, deduct 0.001.

Questions:

1. Calculate the specific gravity of the given sample of urine.
2. What is the significance of measuring specific gravity of urine in a patient?

pH:

The pH of urine can range from 4.5 to 8.0.Freshly passed urine from a normal individual is usually mildly acidic. The kidney regulates the acid-base balance in the body by controlling the excretion of acid or alkali in the urine. In acidosis, the kidneys excrete more acid. In alkalosis, they excrete less acid.

The composition of one's diet is an important factor in determining the pH of urine excreted. On a protein-rich diet, urine becomes acidic due to the production of sulphuric acid and phosphoric acid from the sulphur and phosphorus of amino acids, respectively. Urine may become alkaline in reaction as a result of ingestion of organic acids like citric acid (present in citrus fruits), which ultimately yield bicarbonate in the body.

Determination of pH:

Test the reaction (pH) of urine with universal pH paper.

Questions:

1. What is the normal pH of freshly-passed urine?
2. What will be the pH of urine if it is allowed to stand exposed to air for a long time? Explain your answer.

Chemical characteristics:

Normal urine varies widely in its chemical composition, with the components being influenced by diet and other factors. The following table represents the typical composition of urine from a normal person on a relatively high protein diet (about 100 grams of protein per day).

Composition of normal urine

Constituent	Daily excretion in grams	Constituent	Daily excretion in grams
Water	1200.00	Chloride	12.00
Solids	60.00	Sodium	4.00
Urea	30.00	Potassium	2.00
Uric acid	0.70	Calcium	0.20
Hippuric acid	0.70	Magnesium	0.15
Creatinine	1.20	Sulphur, total	1.00
Indican	0.01	Inorganic sulphates	0.80
Oxalic acid	0.02	Neutral sulphur	0.12
Allantoin	0.04	Conjugated sulfates	0.08
Amino acid nitrogen	0.20	Phosphate	1.10
Purine bases	0.01	Ammonia	0.70
Phenols	0.20		

The various constituents of normal urine are broadly classified as follows:

1. Inorganic constituents: These are soluble phosphates, sulphates and chlorides of sodium, potassium, calcium, magnesium and ammonia.
2. Organic constituents: These are urea, creatinine, uric acid, ethereal sulphates, urinary pigments and hippuric acid.

Date:

Inorganic constituents normally found in urine:

Experiments	Observation	Inference
1. Test for chlorides To 3 ml of urine in a test tube, add 1 ml of concentrated nitric acid and 1 ml of 3% silver nitrate solution. Record your observation.		
2. Tests for phosphates and calcium To 5 ml of urine add 0.5 ml of ammonium hydroxide and boil. Calcium and magnesium phosphates are precipitated. Filter. Discard this filtrate. Pour warm dilute acetic acid (1 ml glacial acetic acid and 4.5 ml of water) on the filter paper and collect the resultant solution in a test tube.		
a. To 3 ml of the filtrate obtained, add 1 ml of potassium oxalate solution. Record your observation.		
b. Take equal volumes of the filtrate and strong nitric acid. Add 3 ml of ammonium molybdate. Heat. Record your observation.		

Organic constituents normally found in urine:

Experiments	Observation	Inference
1. Hypobromite test for urea To 5 ml of urine, add 1 ml of alkaline sodium hypobromite. Record your observation.		
2.Specific urease test for urea Take 5 ml of urine in a test tube and 5 ml of water in another test tube. Add 2 drops of phenol red indicator to each tube. If the solution is purple in colour, add a drop or 2 of 1% acetic acid, till the solution just turns yellow. Then add 2 ml of urease suspension (from horse gram) to each of the tubes. Observe for a few minutes. Record your observation.		
3. Test for uric acid To 2 ml of urine, add 1 ml of phosphotungstic acid and 1 ml of 20% sodium carbonate solution. Record your observation.		
4. Jaffe's test for creatinine Take 5 ml of water in a test tube and 5 ml of urine in another test tube. To each add about 1 ml of saturated solution of picric acid and 10 drops of 10% NaOH. Record your observation.		
5. Weyl's test for creatinine Take 5 ml of urine in a test tube and add 3 drops of 5% sodium nitroprusside followed by 2 drops 5% NaOH solution. Record your observation.		

B. Detection of abnormal constituents in urine

Physical characteristics:

These have to be determined, as described earlier under examination of normal urine.

Chemical characteristics:

Many substances, considered as pathological constituents of urine, may be present in normal urine in very small amounts. The usual qualitative tests to detect these substances are not sensitive enough to pick up these small quantities. They give positive results readily when increased amounts of such substances are present. Abnormal substances that may be present in urine, as a consequence of disease processes include proteins, reducing sugars, blood, bile salts, bile pigments and ketone bodies.

(i) Tests for proteins:

Normal urine contains traces of protein (<150 mg over 24 hours) but the amounts present are not detectable by routine tests. The following proteins have been detected in the urine under pathological conditions.

(1) Albumin – nephrotic syndrome, glomerulonephritis
(2) Albumin and globulins – chronic renal failure
(3) Bence-Jones protein – multiple myeloma
(4) Hemoglobin – intravascular haemolysis (for example, haemolytic anemias), haematuria
(5) Myoglobin – rhabdomyolysis

Date:

Experiment	Observation	Inference
1. Sulphosalicylic acid test: To 3 ml of urine add 3 ml of 3% sulphosalicylic acid solution. Mix well. Warm gently. Record your observation.		

In the sulphosalicylic acid test, proteins that have a positive charge in acidic medium combine with anions like sulphosalicylate (or other alkaloidal reagents like phosphotungstic acid, trichloroacetic acid, tannic acid etc.) to give a white precipitate. This is a semi-quantitative test and proteinuria can be graded according to the following description:

Appearance	Report	Approximate protein concentration
No turbidity	Clear	~ <5 mg/dL
Perceptible turbidity	Trace	~ 20 mg/dL
Distinct turbidity but no granulation	1+	~ 50 mg/dL
Turbidity with granulation but no flocculation	2+	~ 200 mg/dL
Turbidity with granulation and flocculation	3+	~ 500 mg/dL
Clumps of precipitated protein or solid precipitation	4+	~ 1 g/dL

(Henry's Clinical diagnosis and management by laboratory methods; Chapter 28; Page 453; 22ND edition; 2011)

Experiment	Observation	Inference
2. Heat coagulation test: Take 10 ml of urine in a test tube and heat the top of the column. Record your observation comparing the top half of the column with the bottom half. Now add about 5 drops of 1% acetic acid, drop by drop. Record your observation.		

In the heat coagulation test, a coagulum, which becomes more flocculent upon the addition of the dilute acetic acid, indicates the presence of heat-coagulable proteins. The upper part of the column of urine is heated so that the lower part of the column will serve as a control, for purposes of comparison. Albumin and globulins are heat-coagulable proteins and give rise to a cloudy white coagulum when heated in mildly acidic conditions. Turbidity that disappears on addition of 1% acetic acid is due to the presence of phosphates in urine. If the precipitation is due to the presence of proteins, it will intensify on addition of acetic acid.

None of the tests described above is specific for albumin. However, it is common clinical practice to interpret a positive result in these tests as indicating the presence of albumin, because albumin is the protein that is most commonly found in cases of proteinuria. If it is necessary to identify the protein or proteins more specifically, further tests are necessary.

The biuret test is not recommended for the detection of protein in urine. This is because a biuret-like reaction can be obtained in protein-free urine samples due to the presence of certain urochrome pigments. However, it can be used to estimate the quantity of protein present provided the protein is first precipitated with trichloroacetic acid or sodium sulphate, and the biuret reagent is added to the re-dissolved precipitate.

Questions:

1. What is proteinuria? Classify and list the causes of proteinuria
2. What is microalbuminuria? What is its clinical significance?
3. List the proteins excreted in urine in various pathological conditions.

(ii) Test for reducing sugars:

Normal urine is devoid of reducing sugars. If they are present, they may be due to the presence of glucose (in diabetes mellitus), fructose (in essential fructosuria or hereditary fructose intolerance), galactose (in galactosemia), lactose (during pregnancy or lactation), glucuronate (which is normally present in very small amounts but which can increase on administration of drugs like aspirin and sulphanilamide) or pentoses (after ingestion of fruits rich in pentoses like prunes, cherries, grapes etc., and in essential pentosuria).

The commonest reducing sugar present in urine is glucose. The presence of each of these sugars can be confirmed by colour reactions for carbohydrates. Enzymatic methods are available for positive identification of glucose. Other reducing substances in urine which are non-carbohydrate in nature and can give a false positive Benedict's test include homogentisic acid (in alkaptonuria), ascorbic acid (after ingesting large amounts as in vitamin tablets) and certain drugs (like salicylates, levodopa, X-ray contrast media etc.).

Experiment	Observation	Inference
3. Benedict's test for reducing sugars: To 5 ml of Benedict's solution in a test tube, add exactly 8 drops of urine. Mix well and heat the solution till it begins to boil. Record your observation.		

Reducing sugars form enediols in an alkaline medium. Enediols are powerful reducing agents and reduce cupric (blue) ions to cuprous ions which are precipitated as insoluble red cuprous oxide. Benedict's reagent contains sodium carbonate, sodium citrate and copper (II) sulfate. Benedict's test is a semi-quantitative test for reducing sugars and can be graded according to the following description:

Appearance	Report	Approximate glucose concentration
Blue to green, no precipitate	0	0 - 0.1 g/dL
Green with yellow precipitate	1+	~ 0.3 g/dL
Olive green	2+	~ 1.0 g/dL
Brownish-orange	3+	~ 1.5 g/dL
Brick red	4+	> 2.0 g/dL

Questions:

1. What is the principle of Benedict's test?
2. Name the substances that can give a false positive Benedict's test.
3. Define glycosuria and list its causes.
4. Define renal threshold for a substance. What is the normal value for renal threshold of glucose?

(iii) Test for haemoglobin:

Pathological conditions in which haemoglobin may be found in urine may be classified as haematuria and haemoglobinuria. Haematuria occurs in acute glomerulonephritis or due to bleeding from a lesion in the kidney or of the urinary tract below the kidney. Haemoglobinuria occurs as a result of intravascular haemolysis, which may occur in incompatible blood transfusions, and some diseases like malaria and typhoid fever. In haematuria, plasma proteins may be present as well as haemoglobin.

Experiment	Observation	Inference
4. Ortho-tolidine test for blood in urine To 2 ml of urine add 3 drops of the ortho-tolidine reagent. Mix well. Add 3 drops of 20% hydrogen peroxide.		

Heme contained in haemoglobin catalyses the release of nascent oxygen from hydrogen peroxide (due to pseudoperoxidase activity of heme). Nascent oxygen oxidizes ortho-tolidine to give a green colour.

In urinary tract infections, peroxidase present in white blood cells (WBCs) in urine can also release nascent oxygen from hydrogen peroxide, resulting in a false positive test. However, the test will be

negative if the urine is boiled and cooled prior to doing the test, as the peroxidase in the WBC will be denatured by the heat and will lose its enzymatic activity. Another method to differentiate between the conditions would be to repeat the test with the supernatant obtained after centrifuging the sample. The centrifugation would cause any cells present to settle down and the resulting supernatant would be cell-free. Myoglobin, if present in urine, can also give a positive ortho-tolidine test.

A more specific, but less sensitive, test for haemoglobin would be to examine the urine spectroscopically for presence of haemoglobin.

Question:

1. Classify and list the conditions where haemoglobin may be found in urine.

(iv) Test for bile salts:

Bile salts are absent in normal urine. They can appear in urine as a result of biliary tract obstruction.

Experiment	Observation	Inference
5. Hay's test for bile salts: Add 3 ml of the given sample of urine to a test tube marked 'urine' and 3 ml of water to another test tube marked 'control'. Sprinkle a spatula full of finely powdered sulphur on the fluid surface in each test tube. Record your observation.		

The presence of bile salts reduces the surface tension of urine. Therefore, sulfur will float on the surface of normal urine and sink to the bottom of the test tube when bile salts are present. Pettenkofer's test can also be done to detect bile salts in urine.

Question:

1. Name the primary and secondary bile acids.

(v) Test for bile pigments:

Bile pigments found in urine are mainly bilirubin and biliverdin. Bile pigments appear in the urine when there is an intra-hepatic or extra-hepatic obstruction to the flow of bile. As a result, these substances enter the general circulation and get excreted in urine.

Experiment	Observation	Inference
6. Fouchet's test for bilirubin: Take 10 ml of urine in a boiling tube. Add a level spatula of magnesium sulphate and mix the contents, till it dissolves. Now add about 10 ml of 10% $BaCl_2$. A precipitate of $BaSO_4$ is formed. Allow the tube to stand for 10 minutes and then filter the contents of the tube. Discard the filtrate. Open out the filter paper and remove the excess moisture by placing dry folded filter paper below. In this way get the precipitate as dry as possible. Add a drop or two of Fouchet's reagent to one half of the precipitate on the filter paper. Record your observation.		

Bilirubin present in urine is adsorbed on to the surface of the $BaSO_4$ precipitate formed. Ferric chloride present in Fouchet's reagent oxidizes bilirubin to biliverdin (green) and bilicyanin (blue) to give a bluish-green colour.

Note:

Fouchet's reagent is 1% ferric chloride in 25% trichloroacetic acid.

Gmelin's test can also be done to detect bile pigments in urine.

Question:

Which form of bilirubin (conjugated or unconjugated) is found in urine, in a patient with an obstructive cause of jaundice? Explain why this is so.

(vi) Test for ketone bodies in urine:

The ketone bodies are acetoacetic acid, β-hydroxybutyric acid and acetone. Acetoacetic acid and β-hydroxybutyric acid are found in very small quantities in urine under normal conditions. Increased amounts of these substances are excreted in urine in uncontrolled diabetes mellitus, starvation and hyperemesis gravidarum (excessive vomiting during pregnancy).

Experiment	Observation	Inference
7. Rothera's test: Saturate 2 ml of urine with solid ammonium sulphate. Add 2 to 3 drops of freshly prepared 5% sodium nitroprusside, followed by 1 to 2 ml of ammonium hydroxide solution, to form a layer at the top.		

Sodium nitroprusside condenses with acetoacetate, under alkaline conditions, to give a purple coloured complex. Proteins, if present in the sample, can also give a positive test. They are, therefore, removed by full saturation with solid ammonium sulphate.

Gerhardt's test can also be done to detect ketone bodies in urine. This test is based on the reaction of ferric chloride with acetoacetate, producing a wine-red colour. It is non-specific, and other compounds, such as salicylate and phenol give similar colour. Therefore Rothera's test is more specific test for the detection of ketone bodies in urine.

Questions:

1. What is the metabolic origin of ketone bodies?
2. What are the metabolic consequences of ketonemia?

(vii) Use of dipsticks

Dipsticks can be used to determine the glucose and protein content in urine. It is a dry chemistry technique.

Experiment	Observation	Inference
8. Dipstick tests: Dip the reagent strip into freshly collected urine in a beaker. Tap the strip against the rim of the beaker to remove excessive urine. Match the colour of the reagent areas of the strip to the corresponding colour charts printed on the label of the bottle containing the dipsticks, as specified. The color for glucose should be read at 30 seconds after dipping in the urine; the colour for protein can be read immediately after dipping.		

Glucose: The strip is impregnated with the enzymes, glucose oxidase and peroxidase, and the indicator O-tolidine. Glucose is oxidized by glucose oxidase to gluconic acid and hydrogen peroxide. Peroxidase generates nascent oxygen from hydrogen peroxide. The O-tolidine is oxidized by nascent oxygen to a blue-green substance; varying shades of colour will develop depending on the glucose concentration in the sample. The colour that develops is compared with the standard chart provided to report the approximate level of glucose present in the urine.

Protein: This test is based on the protein error of a pH indicator. The test area of the reagent strip is impregnated with an indicator, tetrabromophenol blue, buffered to pH 3.0. At this pH, it is yellow in the absence of protein. Protein (especially albumin) will take up H^+ ions, thus increasing the local pH and turning the colour of the dye to green or bluish green. The colour that develops indicates the concentration of protein in the sample.

X. APPENDIX

Reference ranges for common biochemical analytes (in adults)

(Ref: Tietz Fundamentals of Clinical Chemistry, 5TH edition (2012), Harper's Illustrated Biochemistry, 30TH edition (2015) and Harrison's Principles of Internal Medicine 19TH edition (2015)

Blood:

1. Arterial blood gas analysis (ABG)	
a. pH	7.35 – 7.45
b. Bicarbonate	22-30 mEq/L
c. pCO_2	32-45 mmHg
d. pO_2	72-104 mmHg
2. Electrolytes	
a. Sodium	136 - 146 mEq/L
b. Potassium	3.5 - 5.0 mEq/L
c. Chloride	102 -109 mEq/L
d. Calcium (total)	8.7-10.2 mg/dL
e. Phosphorus (inorganic)	2.5 - 4.3 mg/dL
f. Magnesium	1.5 – 2.3 mg/dL
3. Glucose	
a. Fasting (normoglycemia)	70-100 mg/dL
b. 2-hour post-prandial (normoglycemia)	< 140 mg/dL
4. Glycated haemoglobin (HbA_{1C})	4.0-5.6% of total haemoglobin
5. Ketone bodies	< 3 mg/dL
6. Kidney function tests:	
a. Serum creatinine	
i. Male	0.6 - 1.2 mg/dL
ii. Female	0.5 – 0.9 mg/dL
b. Serum urea	15–40mg/dL
c. Blood urea nitrogen (BUN)	7 - 20 mg/dL
7. Serum amylase	31 – 107 U/L
8. Lipid profile:	
a. Total cholesterol	
Recommended (desirable)	< 200 mg/dL
Borderline high	200 – 239 mg/dL
High-risk	> 240 mg/dL
b. LDL cholesterol (optimal)	< 100 mg/dL

c. HDL cholesterol	40 - 59 mg/dL
d. Triglycerides (fasting)	< 150 mg/dL
9. Liver function tests:	
a. Total protein	6.7 - 8.6 g/dL
b. Albumin	3.7-4.9 g/dL
c. Bilirubin	
i. total	0.3-1.3 mg/dL
ii. direct	0.1-0.4 mg/dL
iii. indirect	0.2-0.9 mg/dL
d. Alanine transaminase (ALT/SGPT)	7-41 IU/L
e. Aspartate transaminase (AST/SGPT)	12-38 IU/L
f. Alkaline phosphatase (ALP)	33-96 IU/L
g. Gamma-glutamyltransferase (γGT / GGT)	9-58 IU/L
10. Serum osmolality	275-295 mOsmol/kg serum water
11. Thyroid profile:	
Thyroid stimulating hormone (TSH)	0.34 – 4.25 µIU/mL
Thyroxine	
i. Free	0.7 – 1.24 ng/dL
ii. Total	5.4 – 11.7 µg/dL
12. Uric acid	
Male	3.1 - 7.0 mg/dL
Female	2.5 – 5.6 mg/dL

CSF:

1. CSF chloride	116-122 mEq/L
2. CSF protein	15-50 mg/dL
3. CSF glucose (Usually 2/3rd of plasma glucose)	40-70 mg/dL

Urine:

1. Total protein	20-150 mg/day
2. Albumin	< 30 mg/day
3. Microalbuminuria	30 - 300 mg albumin/day
4. Creatinine	1.0-1.6 g/ day
5. Ketone bodies	< 1 mg/day
6. Sodium	100-260 mEq/day
7. Vanillylmandelic acid	<6.0 mg/day