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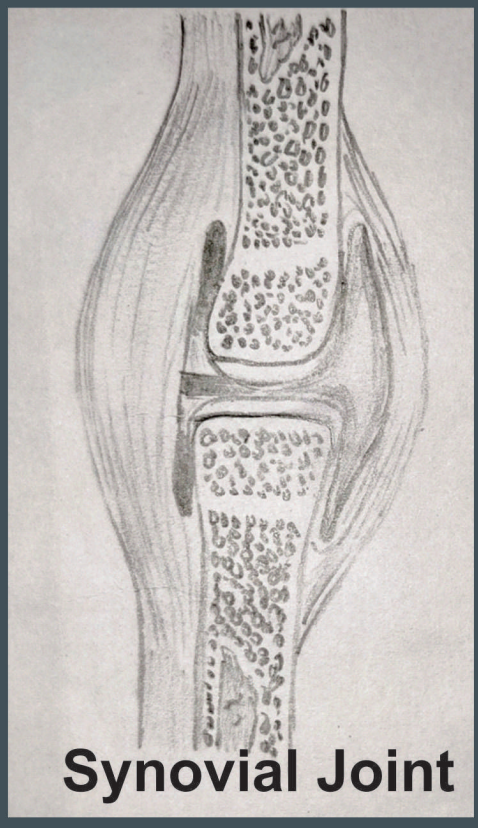
Practical Manual for Experimental Pharmacology

Prof. (Dr.) Dharmendra Ahuja



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Practical Book of

Human Anatomy & Physiology-II

Author: Prof. Dr. Dharmendra Ahuja

Practical: 01

Aim: - Introduction to hemocytometry.

HAEMOCYTOMETRY:

It is an instrument used to list the total cell count in the blood or other biological fluids of the deadbody. This can be accomplished with either a haemocytometer or an electronic cell counter.

PURPOSE:

The value of different types of cells can change in some pathological conditions. Thusby cou nting the cells in the blood or body fluids, it can be determined whether a person isnormal or not broadly, the cell count is mostly done: To figure out the normal and irregular cell count t o help and validate the patient's clinical diagnosis to find out the patient's reaction to the medication.

PRINCIPLE OF CELL COUNTING:

For a sufficient known amount of diluting fluid, the blood is filtered and then counted using a haemocytometer.

HAEMOCYTOMETER¬

This is an instrument used in blood or fluid to count the cells. It consists of a special methodc alled a counting chamber, a cover glass, a pipette to dilute the blood rubber tube and a plastic mouth piece to pull blood or fluid into the pipette

1. Counting Chamber: It is a dense glass slide divided by empty space two similar monitored areas and two raised ridges on both sides. For counting the cells, either of the ruled areas is used.
2. There are various forms of chamber counting, i.e. Old neubauer counting chamberupg raded neubauer counting chamber, rosenthal counting chamber with burker and fuch.

OLD NEUBAUER COUNTING CHAMBER:

The central platform is set 0.1 mm below the level of the two sides, which allows a width of 0 .1 mm for the chamber. An region of 9sq.mm divided into 9 squares of 1 sq.mm is protected by law. Each more the four corner squares, each with anarea of 1/16 of a sq, are subdivided in to 16 squares. Mm. 1 sq.mm of the central regulated area. The set of triple lines is broken into 16 broad squares. These wide squares are further sub divided
by single lines into 16 small squares.

IMPROVED NEUBAUER COUNTING CHAMBER:

The counting square here has an area of 9 sq.mm (3mm x 3mm). Four square corners with an area of 1 sq.mm. Every one (1 mm x 1 mm) is used to count white blood cells. In this, the trip le lines that divide the wide central square are far closer to each other. The central regulated r egion is broken into 25 broad squares. Such squares are subdivided into 16 smaller squares wi th an area of 1/400 of 1 sq.mm each. For red blood cell count, the four corner and one middle square are use.

The diameter of the strengthened neubauer chamber in equivalent to 0.1

Practical:- 02

AIM-Enumeration of white blood cell (WBC) count

REQUIREMENTS: Neubaur chamber, WBC pipette, Cover slip, WBC diluting fluid, Needle, spirit, cotton.

PROCEDURE: 1. Sterilize the finger tip with a 70 percent alcohol-soaked cotton plug and let it dry.

3. To get unrestricted blood supply, take a bold poke and draw up to 0.5 mark of blood i n a WBC pipette. 3. Dip the WBC pipette up to 11 marks in the WBC diluting fluid an d spin the pipette evenly in your hands to blend the solution by stirring well.

4. Take the hemocytometer and position it on the work bench's flat surface. Place the co ver slip on the chamber for counting.

5. Enable a tiny drop of diluted blood to sweep into the counting chamber through capillary motion,hanging from the pipette. Making sure that the air bubble is not present and that there is no overfilling outside the regulated area.

6.Leave the counting chamber on the bench for 3 minutes to allow the cells to relax. Observe the cellsby positioning the counting chamber at the mechanical level of the microscope. Foc us on one of the corner squares of the counting chamber and count the white cells schematica lly, starting from the small, upper left square of each square. In all the four corners of the cha mber, repeat the count. Apply the laws of the margin, that is, count the cells on two opposite margins and discard those on the other two margins.

DATA ANALYSIS: No. of cells X Dilution factor X Depth factor Area count Where: Dilution factor = 20, Depth factor = 10, Area count = 4 RESULT: The number of white blood cells present in one µl of blood specimen is_____.

Practical: 03

Aim: Enumeration of total red blood corpuscles (RBC) count

PRINCIPLE:

In a known amount of diluted blood, the number of RBCs is counted and the number of cells in one cmm of undiluted blood is determined from this.

Apparatus:

Microscope, Watch Glss, Cotton, Hamocytometer,RBC diluting fluid.

HAEMOCYTOMETER:

This consists of a counting chamber, a separate cover slip a diluting pipette for RBC and a diluting pipette for WBC. The strengthened double counting chamber of the NeuBauer: This is a thick rectangular glass with a transverse polished bar in the middle, divided on each side by two parallel grooves from the rest of the slip.

The polished bar is separated by a groove in the centre into two identical platforms leadin g to a 'H' shaped depression (moats). The platform surface is 1/10 mm below the surface of the rest of the slide. So, if the cover glass is placed o ver the counting chamber top, the cover glass under the surface stays 1/10 mm above the polished surface of the platform. The counting area on the polished surfaceof eachplatform is in the shape of a central managed zone. It is a side square of 3mm, divided into 9 equal squa res.

R.B.C.Pipette

(Thomaglasspipette): This is a bulb pipette with a pointed tip having a long stem. The bulb inside holds a red bead. For sucking blood and fluid into the pipette, a small rubber tube eq uipped with a mouth piece is linked to small narrow section above the lamp. There are three marks on the pipette, 0.5 in the centre of the stem, 1at the intersection between the stem and t he bulb, and 101 above the bulb. The complete pipette volume is 101 parts, one part of which is in the stem and 100 parts of which are in the bulb.Other uses of R.B.C. pipette:

1. WBC count in leukemias

2. Platelet counting

Uses of the bead in the bulb

1. For proper mixing

2. To know whether the pipette is dry

3. To identify the pipette

R.B.C diluting fluid: Hayem's fluid is the commonly used diluting fluid.

Composition:

Sodium chloride 0.5 Gm

Sodium sulphate 2.5 Gm

Mercuric perchloride 0.25 Gm

Distilled water 100 ml Sodium chloride and sodium sulphate together keeps the isotonicity of fluid. Sodium sulphate also prevents clumping of red cells. Mercuric perchloride fixes the cells and acts as a preservative.

Other diluting fluids:

Gower's fluid
Toison's fluid
Formol Citrate solution

Procedure:

Clean and dry the counting chamber and put on the supplied special cover slip. Focus and def ine the RBC counting field under the high power goal. First, with pure water, then with absol ute alcohol and eventually with ether, rinse the RBC pipette and keep it dry. In a watch bottle, take a small amount of diluting fluid and hold back.

Using rectified spirit to disinfect the finger tip and render a deep prick with a sterile lancet, so the blood flows out without pressing freely. Wipe out the first drop that could also contain t issue fluid. Enable the hanging drop to form a nice sized blood drop and hold the pointed tip of the pipette touching the drop.

Carefully draw in blood up to the 0.5 level, without any air bubbles. Using bloating paper or a sheet of cloth, extra blood at the tip of the pipette is eliminated. Immediately, by holding the pipette in vertical position.bulb, diluting fluid from the watch glass is sucked up to the 101 l evel with every air bubble.Then the blood and diluting fluid in the pipette are thoroughly com bined by softly rolling and

holding aside the pipette kept horizontally between the hands. Mixing happens only in the pipette bulb. The column of diluting fluid found in the pipette stem does not join (i.e. 1011 = 100) into the dilution.

There would be a dilution of 0.5 in 100 or 1in 200 with the blood sucked up to 0.5. Now pull out the counting chamber and discard the first few drops from the pipette for charging, since

the stem only contains diluting fluid. At the tip of the pipette, bring one tiny drop of diluted b lood to the edge of the cover slip at an angle of around 450 in the counting chamber. The flui d reaches the counting chamber through capillary action
under the cover slip and fills it. There are both places filled.Emphasis on high power in the RBC counting region.

For the cells to calm down in the counting field, keep the counting chamber undisturbed for a round 3
minutes and begin counting. To achieve a satisfactory average and a better dispersal meaning, at least 5 squares, each with 16 smaller squares (preferably 4 corners and 1 central) should be counted. Cells touching the top and left margin of each square should be omitted when mea suring each tinysquare and cells touching the bottom and right margin of each square should be counted. Draw the counting squares map in the record and enter the number of cells in eac h square and when the counting squares are counted.

Precautions:

1. The chamber and pipette for counting should be clean and dry.
2. Fingertip and lancet pricking can be sterile.
3. Blood, without pressing, can flow out easily.
4. To avoid blood clotting within the pipette, be patient.
5. There should not be an air bubble when loading the pipette and starting the counting chamber.
6.Blood can only be brought up to the 0.5 mark and sucked up to the 101 mark only by diluti ng fluid.
7. Blood should be combined with the diluting solution correctly.
8. Until setting, discard the first few drops because they will not produce RBC5.
9. Over-filling and spilling can be stopped when charging the counting chamber.
10. Until numbering, cells should be settled down and spread more or less equally. 11. Do not place the microscope in a tilted way.
12. Count from left to right to keep the same cell from counting.

Calculation: Let the number of cells counted in the smallest squares of (5x16) 80 be 'N'
The number of cells is N/80 in 1 smallest square 1 square hand = 1/20mm 1 square area = 1/ 400mm2 The fluid film depth in the counting chamber is 1/10mm2. 1square amount of filtere d blood=1/400x1/10=1/4000mm33 Cell count in 1/4000mm3 diluted blood =N

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

N = Nx4000

80 80= The dilution factor amounts to 1 in 200 (There are 100 sections of the gross diluted a mount in the pipette bulb, of which 0.5 is blood. Thus the dilution is 0.5 in 100i.e.1 in 200)So number of cells in 1 mm3 of undiluted blood =Nx 4000x200 = Nxl000080

Discussion: (Millions/mm3 expressed)

Standard Count of RBC Adult Male: 5 million-5.5 million/mm3
Female adults: 4.5 - 5 million/mm3
Count Variations: Increase in numbers Drop in numbers
Physiological disparities
1. Diurnal -
2. Age
3. Sex -
4. Elevated altitude –
5. Exercising muscles -

The Polycythemia (Erythrocytosis) Anaemia (Erythropenia) Less through the night, minimu m in the early morning, rises steadily during the day High counts are used in newborns, More from males Lower count due to hypoxia Increases counts.

Pathological increase in count:
1. Lung diseases like emphysema, pulmonary tuberculosis
2. Congenital heart disease
3. Carbon monoxide poisoning
4. Primary polycythemia (Polycythemia rubra vera)

Secondary polycythemis is due to hypoxia resulting from any cause, physiological or pathological.

Pathological decrease in count:
1. Increased destruction of RBC
2. Decreased production of RBC

Practical:- 04

Aim- To find out or know about the bleeding time of a body

DEFINATION: -

The time needed for bleeding is the time required for a minor cut to avoid bleeding. Blood comes out for a time after a blood artery is injured, and then it ceases because of platelet plug formation. Bleeding time is the length of bleeding. For bleeding time, the usual value is 1-3 minutes.

Significance: Bleeding time is used primarily in the management and diagnosis of hemorrhagic diseases. Just prior to procedures such as tonsillectomy, the bleeding time is also helpful. InSuch cases, an irregular bleeding mechanism can be found out.This will inform the doctor to take sufficient care.

REQUIREMENTS:

Replaceable hygenic sticking sting, fliter file, stopwatch, cotton swab, ethanol 70% v/vv

Principle-

Blood pressure is freshly struck on the upper arm to normalise pressure and then to normalise pressure. Small cut on the forearm (radius,ulna,
antbrachium) right up to the time of bleeding Fully measured stop Bleeding time the time lag for full blood flow stoppage from ruptured blood This is called bleeding time for vessels: 1-4 minutes

PROCEDURE:- Duke Length Bleeding Process

1 method. Next, stick a sterile or germ-free sting on Victtim's finger

2. Sticking time will be carefully noted, Ruptured point

3. Stain will take on filter point for 30 seconds and retain blood stain in 20 seconds. Minute Cycle to Avoid the Bleeding Time

4. The time at which the blood stain stopped will
carefully mention the time and that is the time Period of the victim's bleeding.

Precaution—

1. Time should be carefully noted.

2. Needle should be clear or clean of germs.

Practical:- 05

Estimation of hemoglobin content

The key ingredient in red blood cells is haemoglobin and it helps to transport O2 and CO2. H emoglobin is a conjugated protein that consists of globin and haeme, a simple protein. Each haemoglobin molecule consisting of one globin molecule and four haeme molecules, each co ntaining an iron molecule.

Aim:- Use the hemo metre to calculate the haemoglobin content in your blood.

Necessary apparatus and reagents:- Sahli-
Hellige Hemometer, N/10 HCl, distilled water, cotton swabs, alcohol, dropper, lancet.

Apparatus description:- The Sahli-Hellige Hemo metre has the following components:

Comparator: - It accommodates a contrast glass tube that is graduated
in gramme percent of Hb (2-24) and percentage of Hb (2-24) (20-140).

This tubing is used to hold hematin acid. Non-
fading regular brown tinted glass plates are supplied with the comparator. Hemoglobin pipett e: A single labelled pipette indicating 20 cu mm. The volume is given.

Glass stirrer:- Used for the contents of the reference tube to be stirred.

Procedure:-

Add N/10 HC1 up to the 20 percent level to the reference tube. Since brushing with cotton so aked in alcohol, poke the ball of a digit to extract blood using a sterile lancet. Wipe out one o r two drops of blood first. Suck the blood into the haemoglobin pipette up to the 20 cu. mm l evel as a goodsized blood drop forms. With cotton, brush away the remaining blood adhering to the top of the pipette. Move the contents of the pipette directly into the tube containing the HCl solution. Suck the pipette with a small volume of HCl acid and blast it into the drain . Mix and give 10 minutes for the tube to stand. Through a glassstirrer, apply a few drops of d istilled water and stir the contents. Continue to apply drop by drop water and stir the contents each time until the solution's colour is only darker than the normal colour.

Note, against diffused light, the reading. Again, apply one or two drops of purified water & r emember the reading again when the colour corresponds precisely with the norm.

Practical: - 06

Aim -

Knowing our blood group and form of ABO blood group system and knowing it. Materials

Mandatory: Toothpick,Blood sample,Alcohol swabs,Lancet,Sterile cotton balls,Biohazard di sposal,Clean glass slide Container,

Antibodies Monoclonal (Anti-A, B, and D)

Assumption: Take a slide of clean glass and draw three circles**.**

• Unpack the kit with monoclonal antibodies (MAB).

Add Anti-A to the firstcircle, and the second circle Add Anti-
B and, with the aid of a dropper, add AntiD to the third circle. Keep the slide securely aside w ithout upsetting it. Clean the ring finger with the alcohol swabs now andmassage softly near t he fingertip, where the alcohol swabs are positioned.

A sample of blood will be obtained.

With the lancet, lift the ring fingertip and wipe out the first drop of blood. Cause it to collapse softly on the three circles of the glass slide as blood continues to discharge out. Pressing the tip of the finger. Apply pressure to interrupt the blood supply
at the spot where it was pricked.
When you use a cotton ball, use the Needed. Mix the blood sample softly with the aid of a too thpick and examine the results at the end for a minute.

Interpreting: This graph displays the various kinds of
blood groups together with the Rh element.

Form of Blood

A B O AB

Rh-positive A+ B+ O+

AB+ A+ O+ AB+

Rh-negative of A-

B- O- AB-

Approvision:

After their use, extract the alcohol swabs,lancet, cotton balls and toothpick. Upon noticing the As a consequence, lower all items into the disposal tub, including the glass slide. There are f our main classes of blood and eight separate forms of blood, collectively called the ABO, Sys tem of Blood Community. Two specific antigens and antibodies are presence or absence1. Gr oup A- Antigen A and Antibody B, respectively.

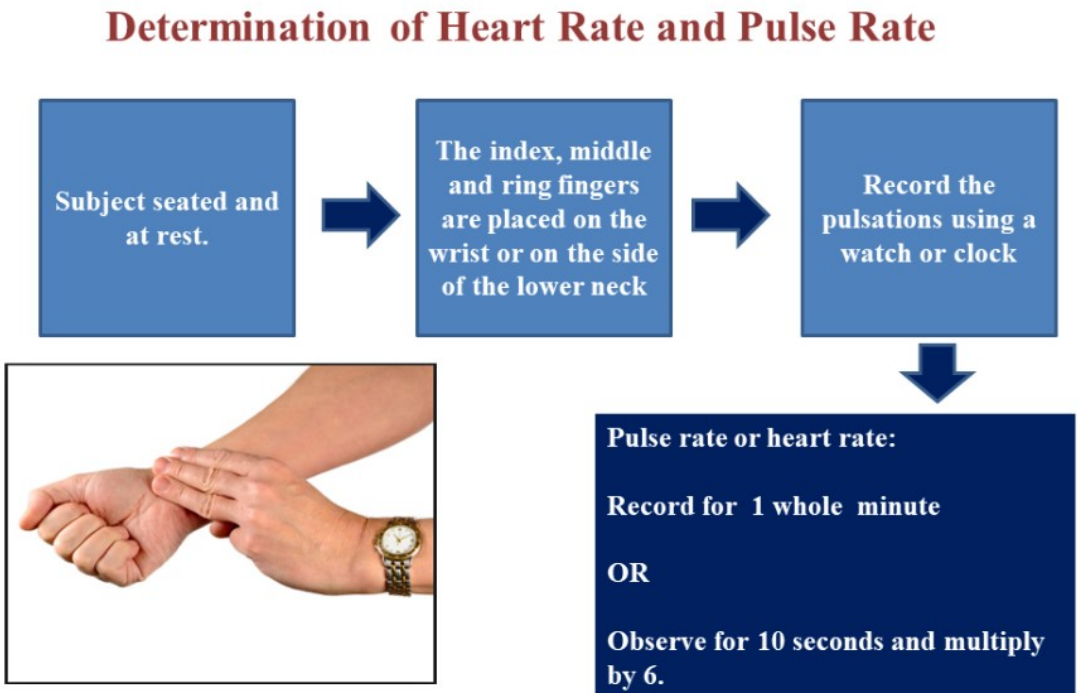
2. Group B- Antigen B and Antibody A, respectively.
3. Community AB- All Antigen A and B and no Antibodies
4. Community O- No antigens and Antibodies to both A and B.

The name of this antigen is the Rh element. There is a third type of antigen. The four classes of the blood are Classified into eight distinct forms of blood on the basis of Rh factor involve ment or absence:

1. A positive- Rh + presence
2. A bad- Rh-presence-
3. B positive- Rh ++ involvement
4. B unfavorable- Rh existence-
5. Positive AB- Involvement of Rh+
6. AB negative- Rh appearance-
7. O positive- Rh++ involvement
8. O negative- Presence of Rh-

Practical: - 07

Determination of heart rate and pulse rate.



Practical: - 08

Aim : To verify the temperature of the human body.

Requirement: antiseptic material, thermometer, antiseptic substance.

1-Body temperature Introduction:-

To preserve natural physiological cycles, body temperature is closely regulated. The hypothalamus achieves this power. The temperature of the body depends on the equilibrium between the development and lack of heat and the atmospheric

environment. Measuring the temperature is an essential aspect of the patient's gen eral evaluation. It is measured using the scale of centigrade (Celsius), but many c ountries use the scale of Fahranheit.

The natural temperature range for the body is 36.6-37.2 C.

Heat is created by:-

1. Phase of Metabolics.
2- Consumption of food (dynamic action).
3- Exercise (contraction of skeletal muscles**).**
4- Hot, sun-like climate, heater.Heat is wasted by: 1- Skin 97%(radiation, conduction, convection and evaporation) 2- 2% expired air . 3- 1% urine and faeces.

The temperature of the body is controlled by the hypothalamic temperature core If the body temperature increases so high, the anterior hypothalamus temperatuecentre is trig gered and the following processes take place I- The pathways triggered by heat are:-

A- Raise the loss of heat:-

1- Blood vessels in the skin grow to transmit internal heat to the skin.
2- 2- 2-Sweating from the nervous system with sympathy (SNS).
3.3- 3- Raises the rate of respiration.

4- B- Minimize the output of heat:- 1- Anorexia. 2- Inertia and apathy. Temperature measuring routes: -

1. Mouth route, the most widely used route, used by aware adults and children over 5 years o f age.

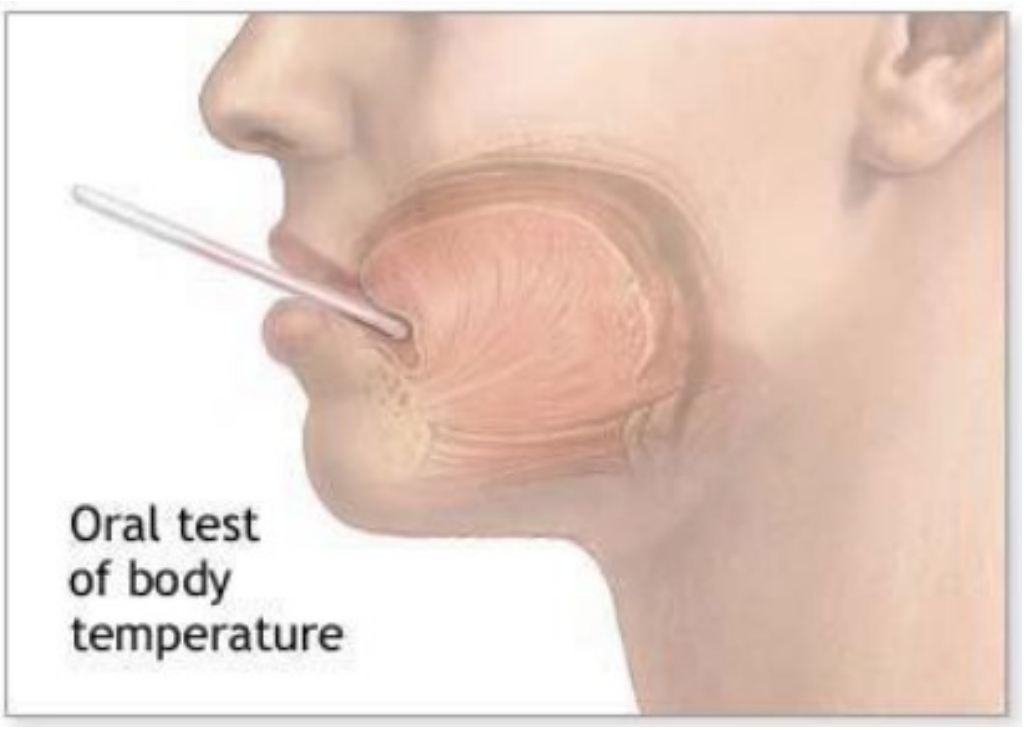
2- For unconscious adults and infants, the Axilla route is used.

3- For babies and younger ones, the groyne route is used.

5- Today, the rectal direction is scarcely followed.

Procedure:

1. Wash it with an antiseptic solution before sticking the thermometer into the mouth.
2- Keep the thermometer fingers and watch the mercury level, shake it
down to get mercury below this level if it is more than 35C.
3- Bring it under the tongue in your mouth for 2 minutes.
4- The mouth should be tightly closed, and airflow should be carried by the nose.
5- No hot or cold liquid is put in the mouth before reading, and no gum chewing



PRACTICAL-09

Aim:- Recording the body temperature.

Apparatus :-Medical and clinical thermometer, antiseptic solution.

Theory:-Before using the thermometer wash the thermometer by antiseptic solution.firstly watch the level of mercury if the temperature is above than 35degree celsius shake it down to bring mercury below the level. Put the thermometer in mouth under the tongue for 2 mintue Breathing is done through nose and the mouth is closed. After 2 minute finally check the reading of thermometer.

Procedure:-

FEVER: The suddenly increase in body temperature is called fever. Fever is present if the oral temperature is > 37.7 degree Celsius. Infection is the most common cause of fever but in elderly patients and those with weak immune function (due to primary disease, e.g. human immunodeficiency virus (HIV), e.g. oral steroids, immune suppressants), infection may not produce fever.

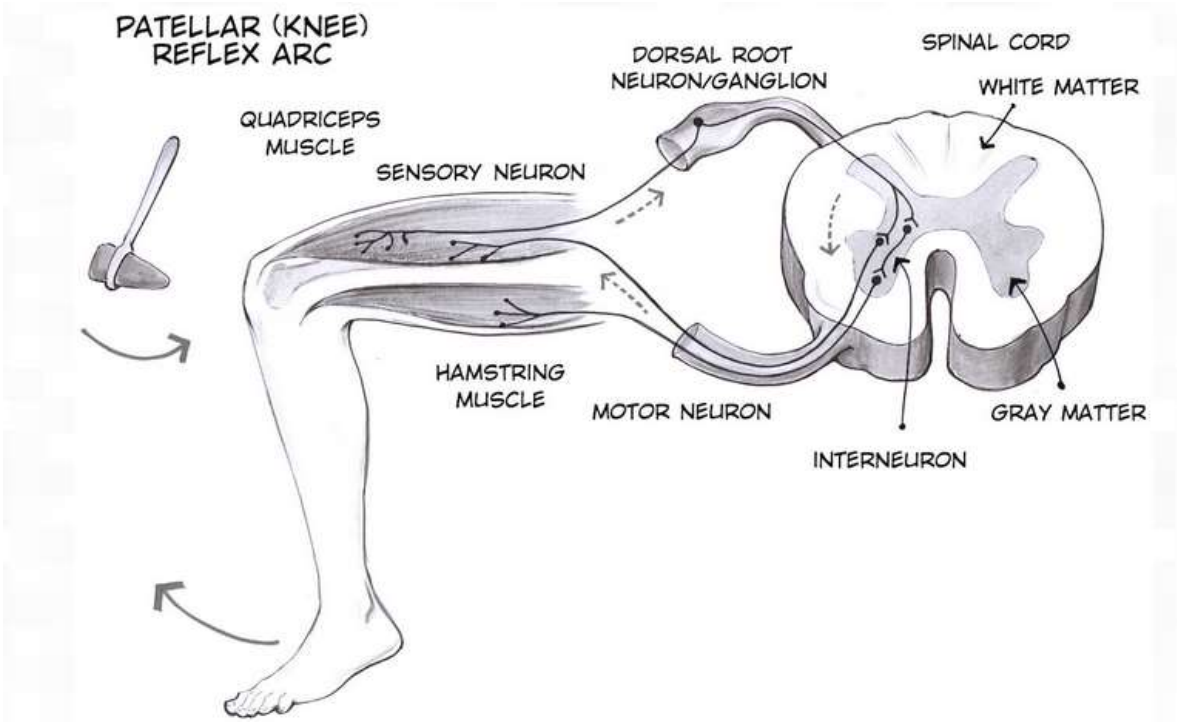
HYPOTHERMIA: It is a potentially dangerous drop in body temperature usually caused by long time exposure to cold temperature. As the winter month started the problem of hypothermia patient increase. Normal body temperature average 98.6 degree but the hypothermia patient has lower temperature is 82 degree.

Practical:10

Aim: To demonstrate and know about the reflex activity.

Reflex Activity

When we do a no thought action that is knownas reflex action. This action is automatic fast, and human being react to this action so quick. As we allknow milionsof neurons are present inour brain and they do the processing but we also need to dolot of things automatically. As we all know our thinking process is so slow so for some painfull reaction we need the action we called as reflex action.patellar stretch reflex is the example of reflex action. There are sensor in our muscle called it as muscle spindle.In this our spinal cordis join together. So the reflex pathway is when our spinal cord is connected to the sensors. As we all know one signalis nessasary for the muscle contraction from sensory neuron to the motor neurons.only because of this signal the reflex action is so fast. In a healthy human being it takes 15-25 sec for stretch and then muscle contract. But we blink our eyes in 5-15 times in that time.



Practical:11

Aim: To Know about the Homeostatic State

Homeostatic is a situation in which body remain in equilibrium . This state is maintain by all the body system as we all know body system is made up of organs and organs are made up of tissue and tissue is made up of cell. So to mainatin equilibrium it is the duty of all the cell to function properly. So that the internal environment of the body remains stable and maintained.

Set Point: Set points are the physiological value of body which must be match with human body system. like temperature, pressure, and all. As we know the normal body temperature is 37 degree Celsius.

Negative Feed Back

Body Temperature Regulation:

PRACTICAL-12

Aim:-To study the Olfactory Nerve.

Assumption:-Human have important bodies olfactory nerve is present , which in the first cranial nerves and conveys special sensory information related to smell. This nerve is considered as shortest cranial nerves and it passes its receptors from the nasal mucosa to the forebrain. Through the cribriform plate of the ethmoid bone it enters into the skull. nerve is the The sense of smell can be altered due to a variety of conditions referred to as hyperosmia, hypoosmia, anosmia, and dysosmia. The most common pathology to affect the olfactory common cold.

Olfactory nerve is the first cranial nerve (CN1).it is a sensory nerve. It carries the sensory information for the sense of smell.The olfactory cell are bipolar neurons. It lies in the olfactory part of the nasal mucosa ,serve as both receptor and first neurons in the olfactory pathway.

The pathway of the olfactory nerve is :-

Olfactory epithelium of nose then it goes olfactory rootlets and then goes olfactory bulb and then goes lateral and medial olfactory striae. Lateral olfactory stria is a pyriform lobe and medial olfactory stria is septal nuclei.

Purpose :-

To localize wheather the loss of smell if any is due to pathology of the nose or is due to neural lesion .

To determine wheather any impairment of the sense of smell is unilateral or bilateral.

Fuction of Olfactory Nerve:-

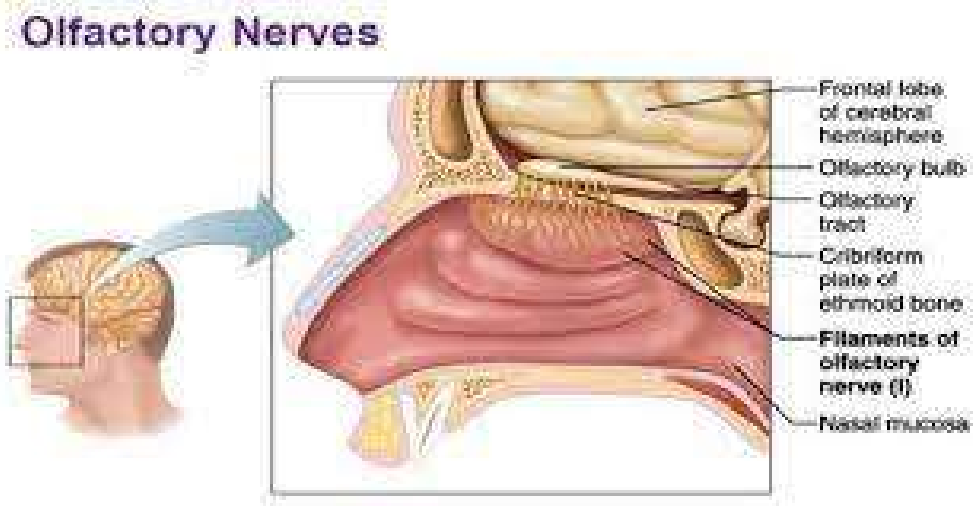
1.The olfactory afferents synapse within the olfactory bulbs.

2.Sensory carry visual information from special sensory receptors in the eyes.

3.Motor are the primary source of in- nervation for four of the extrinsic eye muscles.

4.The smallest cranial nerves, innervate the superior oblique muscles of the eyes.

5.It relays sensory data to the brain, and it is responsible for the sense of smell.



PRACTICAL-13

Aim – To study the structure and function of skin

Requirment –

Principle – Skin is the largest organ of the human body . it cover the whole body .it is the protective covering .

Function of skin –it protect us from microbes and regulate body tempreture and also allow to senses of touch, heat ,cold etc

Three layer of persent in skin

(1) Epidermis

(2) Dermis

(3) Hypodermis

Epidermis- it is the upper and outer most layer of the skin .it is made up of a type of cell that is squamous cell the are round cell which is called basal cells. The deepest part of the epidermis is melonocytes

Epidermis has five layer they are –

(1) Stratum Corneum

(2) Stratum lncidum

(3) stratum granulosum

(4) startum spinosum

(5) startum basal

(A**) Stratum corneum**- It is upper part of the epidermis which is made up of keretain . it is the protective outer barrier which control the entry of germs , bacteria ,or unwanted thing

(**B) startum lncidum**- it is the thin and clear region of dead cells of epidermis which is not visible by naked eyes .it can be seen by microscope . it also help in protection at specific place genrally it is found in palms and in feet .

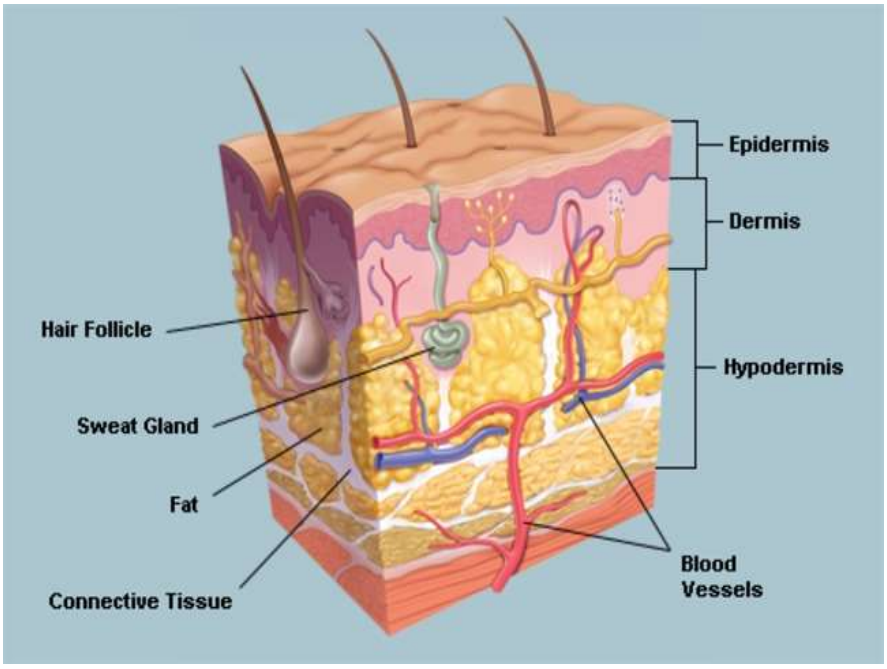
(C) startum granulosum-it is of dense bashophilic karetainohylline granules .and this granules is made up of lipid which are with desmosole connection which help in making waterproof barrier which function in fluid loss from body .

(D)Stratum spinosum-this layer is made up of polyhrdral karetenocytes . these are also joined by desmosomes connection .it is responsible for skin falexibility , strength

(E) startum basal- it bound epidermis to dermis by collegen fiber .it can be called as bassement membran

(2)Dermis layer- It is the lower and inner layer of skin. It is between epidermis and hypodermis.It contain blood vessels,lymph vessels,hair follicles,and gland.It has sebum (an oily substance the help in keeping skin from drying).It provide elasticity to skin. It has sebaceous gland,sweat gland.

(3) Hypodermis- It is also called fat gland.The inner most gland called a subcutaneous layer .It consist of fat .And provide structural support of skin.



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