JHARKHAND
Rai University

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PRACTICAL LAB MANUAL

LAB MANUAL HUMAN ANATOMY AND PHYSIOLOGY

(B. Pharm Ist Year)

EXPERIMENT – I

COMPOUND MICROSCOPE

Objects which are ordinarily not visible by naked eye are seen with microscope. Generally an object smaller than 0.1 mm cannot be seen by our eyes. Therefore, to observe an object smaller than this, compound microscope is very helpful. Hand lens (magnifying lens) is also a type of microscope but its magnifying capacity is very low. Dissecting microscope is also used to visualize tiny things, but it has only one lens. Compound microscope is generally used in the laboratories. Therefore, description, use and maintenance of ordinary compound microscope is mentioned here.

1.1 Parts of a Compound Microscope

Take out the microscope from the box holding the arm by one hand and supporting the base by another hand. Place carefully on the table and study the names and functions of the parts as mentioned in the figure. The parts of a compound microscope can been divided into 4 main parts:

(a) Base : This is U-shaped lower portion of the microscope on which the other parts of the microscope lie. Above the U-shaped portion, there is a perpendicular portion known as the pillar. On the top of this, another arm is fixed. This is known as inclination joint. This can be used to tilt the microscope at a desired angle.

(b) Arm : It supports the body tube and base of the microscope. This portion is used to hold or carry the microscope. On the base of this, stage is fixed. On the top of the arm body tube of the microscope is fixed and two knobs are fitted. One is for the coarse adjustment and the other for the fine adjustment. These are used for focussing the body tube.

(c) Body Tube : This is attached to the knob of the arm. It has one lens on the upper end known as eye piece. This lens can be changed according to the required magnificaton. On the bottom of this tube there is a nose piece. Two to four lenses can be fitted in this nose piece. Because the lenses are fitted on the objective, these are known as objective lenses. These are fitted in the body tube, known as objective lens body. The objective lens body is fitted into the noise piece.

(d) Stage : It is a platform having a circular hole in the centre to allow the passage for light from below. It is fixed to the base by the stand. One mirror is fixed to the stand. It is known as reflecting mirror. Below the stage is a condenser through which concentrated beam of light passes. Iris diaphragm is also attached to the condenser. The reflecting mirror reflects the light upward through the iris and diaphragm. This beam of light passes through the hole in the stage and provide light to the object kept on the slide. There are two clips for holding

the slide above the hole on the stage.

Operation (Use) : Keep a clean prepared slide in the centre of the stage. Use clips to fix the slide on the stage so that it does not move. Now move the body tube by the help of coarse adjustment knobs. Bring the slide in focus under the objective lens. Focussing should be made sharp by the use of fine adjustment knobs. When the focus is sharp then study the slide. The specimen is viewed by keeping one eye on the eye piece and the second eye should be kept open. This type of compound microscope is known as monocular compound microscope.

Some compound microscopes have two body tubes. So there are 2 eye pieces and specimen can be viewed by both the eyes. Such type of compound microscope is known as binocular compound microscope. In the research work generally bioncular compound microscope is used.

1.2 How to use a Compound Microscope

To use the microscope first of all rotate the nose piece until the low power objectives is in line with the body tube and clicks into position. Open the iris diaphragm. Look through the eye piece, adjust the mirror and diaphragm to set a complete field of vision. Place the slide you want to examine on the stage of the microscope and by the help of the clips fix it. Move the slide till the object comes roughly to the centre of the hole or the stage. Bring the object into focus using the coarse adjustment knob. Turn the fine adjustment knob to bring the object into sharp focus.

How much magnification the object needs will be learnt through experience. Eye lenses of 5x, 10x or 15x are available. Some way objective lenses of 4x, 10x & 40x are also available. The multiplication of magnification of eye piece and nose piece denotes the size of the object under observation.

Maintenance of Microscopes:

Microscope is a costly equipment. Therefore, it should be handled carefully. Always keep the microscope in an upright position while taking it from one place to another. As far as possible don't tilt the arm. Clean the lenses of the microscope with the lens paper or muslin cloth, never with the filter or any other kind of paper. If you are using the high power objective lens then after the observation is over, turn the nose piece and bring low power objective lens in line with the hole in the stage. Objective lens should be kept atleast 1 cm above the stage. After using the microscope always keep it in the box. Take care to see that the stage of microscope, the eye piece, the objective lens are dry and clean. No chemical should stick to these. Adjustment knobs and joints should be protected from rusting by applying vaseline.

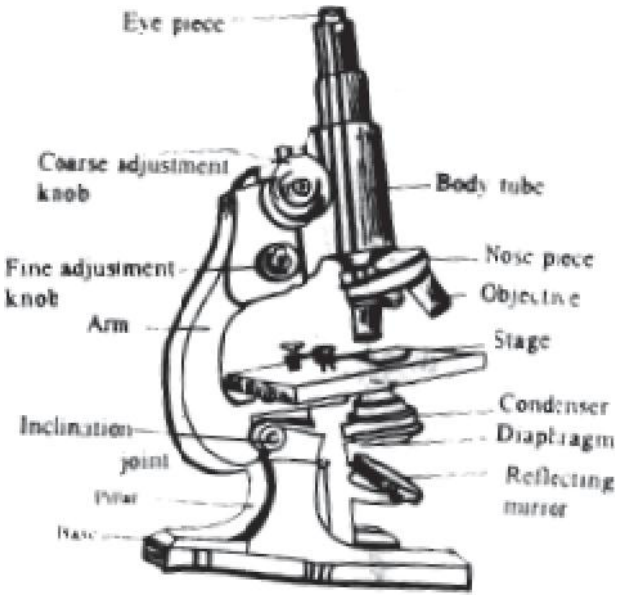
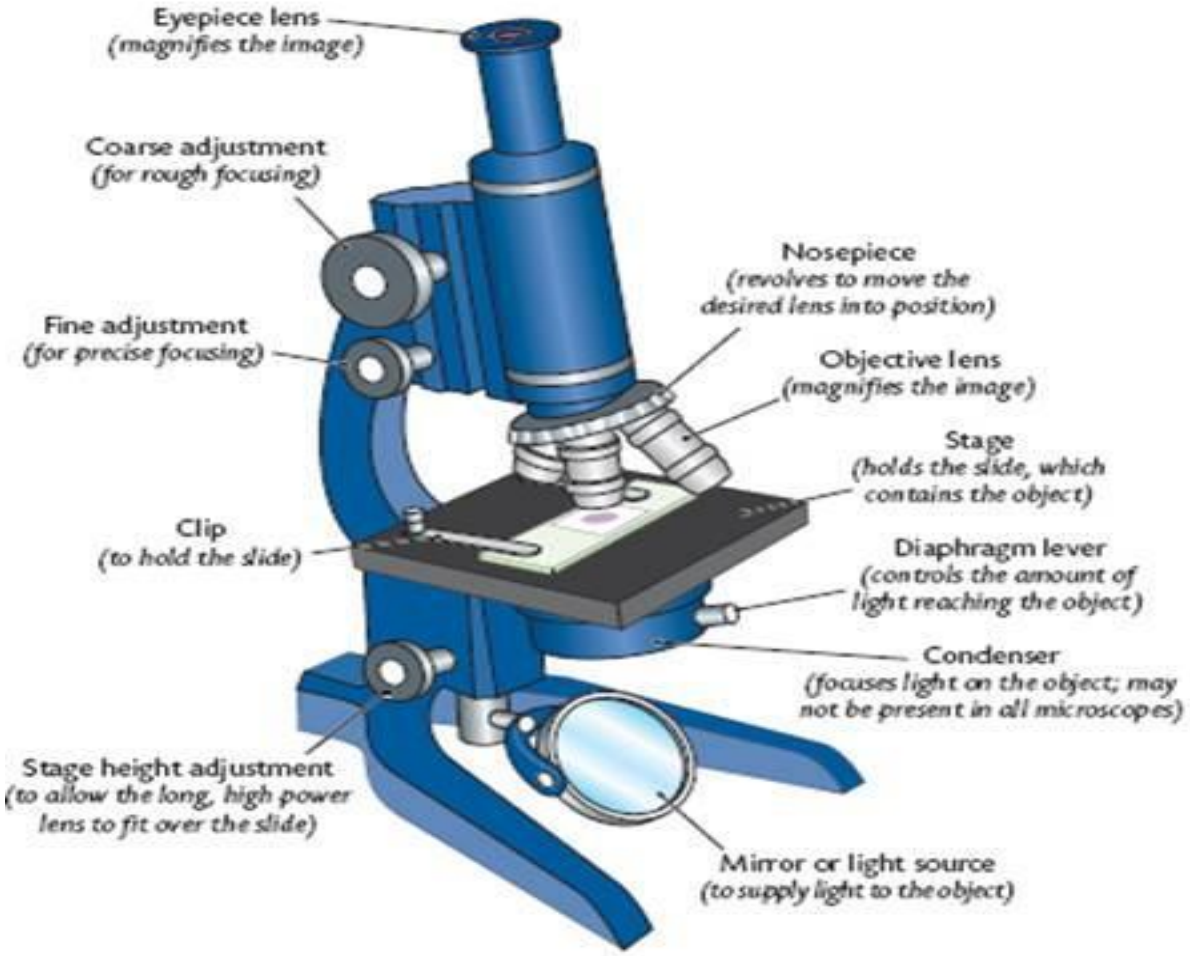
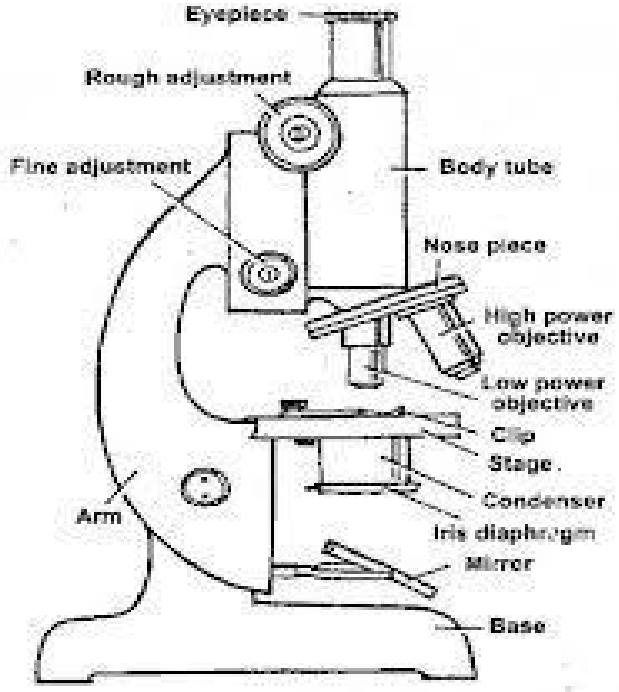


Fig. 1.1 A compound microscope


Eyepiece lens
(magnifies the image)
Coarse adjustment
(for rough focusing)
Nosepiece
(revolves to move the desired lens into position)
Fine adjustment
(for precise focusing)
Objective lens
(magnifies the image)
Stage
(holds the slide, which contains the object)
Clip
(to hold the slide)
Diaphragm lever
(controls the amount of light reaching the object)
Condenser
(focuses light on the object; may not be present in all microscopes)
Stage height adjustment
(to allow the long, high power lens to fit over the slide)
Mirror or light source
(to supply light to the object)

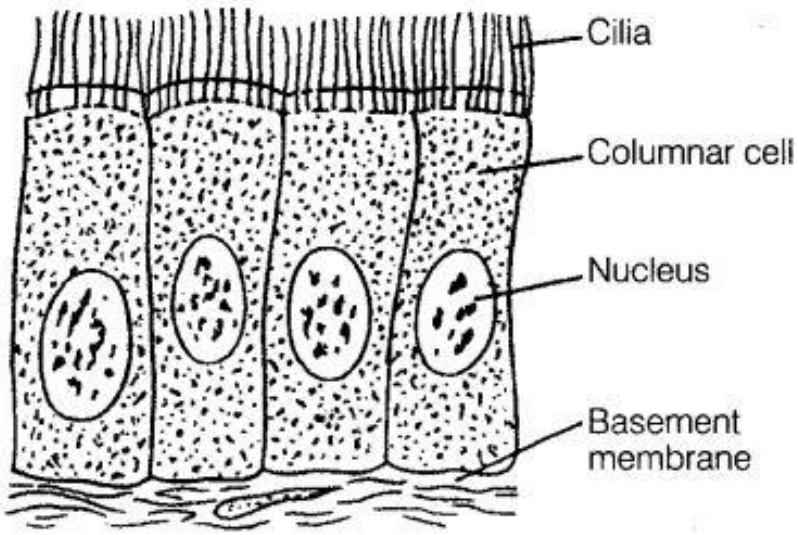

Eyepiece
Rough adjustment
Fine adjustment
Body tube
Nose piece
High power objective
Low power objective
Clip
Stage
Condenser
Iris diaphragm
Mirror
Arm
Base

EXPERIMENT – II

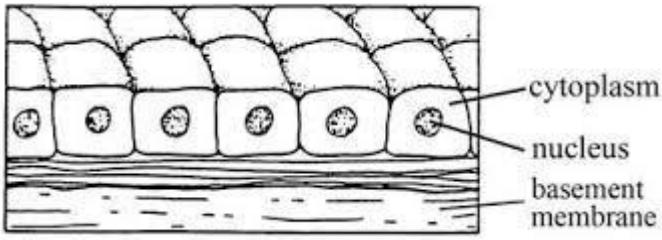
HISTOLOGY

1. Ciliated Epithelium tissue :

Ciliated columnar epithelial cells are rectangular in shape and have between 200 to 300 hair-like protrusions called cilia. The mitochondria are found toward the apical region of the cell while the cell nuceli are found towards the base and are often elongated. Cells are interconnected via desmosomses and tight junctions, creating a semipermeable membrane that is more selective that membrane found in other types of cell.

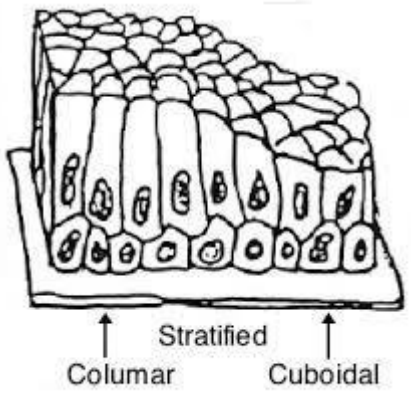


2. Cuboidal Epithelium :



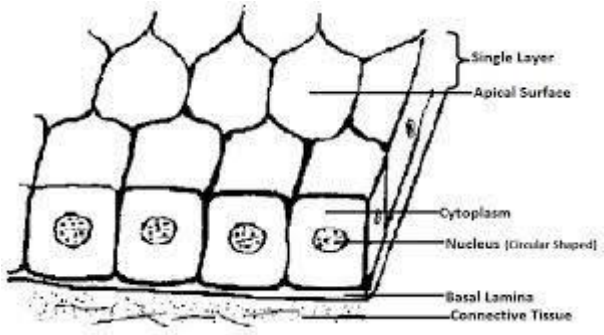
Cuboidal epithelia are epithelial cells having a cube-like shape; that is, their width is approximately equal to their height. They may exist in single layers (simple cuboidal epithelium) or multiple layers (stratified cuboidal epithelium) depending on their location (and thus function) in the body.

3. Stratified Columnar Epithelium :



Stratified columnar epithelia are found in the ocular conjunctiva of the eye, in parts of the pharynx and anus, the female's uterus, the male urethra and vas deferens. Also found in Lobar ducts in salivary glands. The cells function in secretion and protection. In simple terms, we can say that the upper and lowermost layer of cells are columnar in shape. The middle layer contains cuboidal cells. It forms the lining of respiratory tract, ureter, ovi duct ,etc.

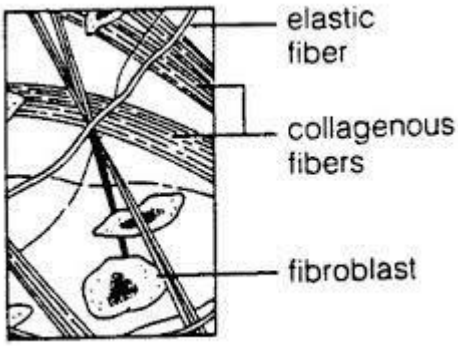
4. Stratified Cuboidal Epithelium :



Stratified cuboidal epithelium is a type of epithelial tissue composed of multiple layers of cube-shaped cells.

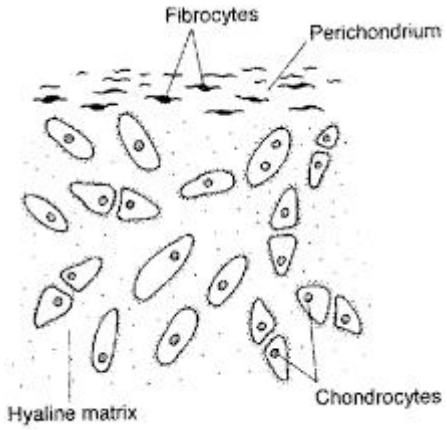
Only the most superficial layer is made up of cuboidal cells, and the other layers can be cells of other types. This is because, conventionally, naming of stratified epithelium is based on the type of cell in the most superficial layer.

4.Areolar connective tissue :



Loose connective tissue is the most common type of connective tissue in vertebrates. It holds organs in place and attaches epithelial tissue to other underlying tissues. It also surrounds the blood vessels and nerves. Cells called fibroblasts are widely dispersed in this tissue; they are irregular branching cells that secrete strong fibrous proteins and proteoglycans as an extracellular matrix. The cells of this type of tissue are generally separated by quite some distance by a gelatinous substance primarily made up of collagenous and elastic fibers.

5. Hyaline cartilage :



Hyaline cartilage

Hyaline cartilage is covered externally by a fibrous membrane, called the perichondrium, except at the articular ends of bones and also where it is found directly under the skin, i.e. ears and nose. This membrane contains vessels that provide the cartilage with nutrition.

Hyaline cartilage matrix is mostly made up of type II collagen and chondroitin sulfate, both of which are also found in elastic cartilage.

Hyaline cartilage exists on the ventral ends of ribs; in the larynx, trachea, and bronchi; and on the articular surface of bones

6. Tendon :

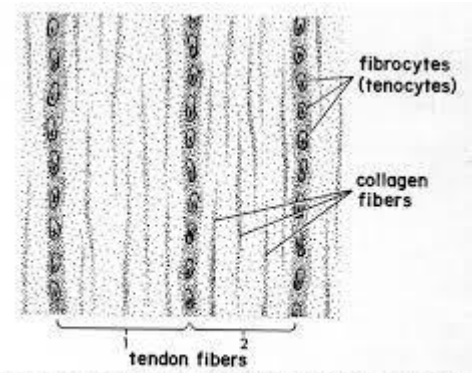
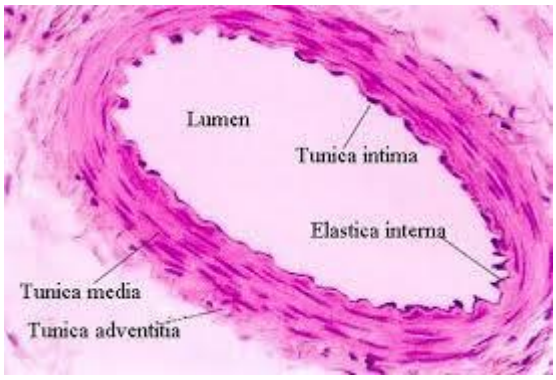


FIG. 69-2 A ligament is a band of relatively nonelastic tissue that unites two or more bones and is composed predominantly of long parallel collagenous fibers.

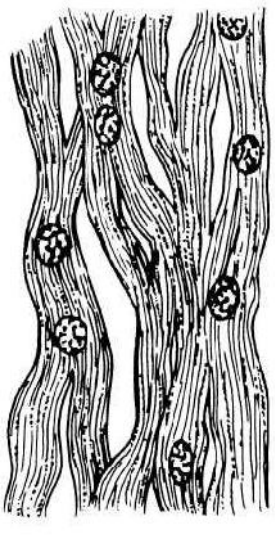
A **tendon** (or **sinew**) is a tough band of fibrous connective tissue that usually connects muscle to bone and is capable of withstanding tension. Tendons are similar to ligaments and fasciae; all three are made of collagen. Ligaments join one bone to another bone; fasciae connect muscles to other muscles. Tendons and muscles work together to move bones.

7. Human Vein :



Veins (from the Latin *vena*) are blood vessels that carry blood toward the heart. Most veins carry deoxygenated blood from the tissues back to the heart; exceptions are the pulmonary and umbilical veins, both of which carry oxygenated blood to the heart. In contrast to veins, arteries carry blood away from the heart. Veins are less muscular than arteries and are often closer to the skin. There are valves in most veins to prevent backflow.

8. Cardiac Muscle :



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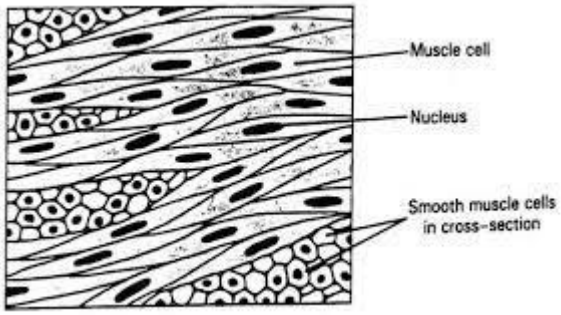
Cardiac muscle (**heart muscle**) is involuntary, striated muscle that is found in the walls and histological foundation of the heart, specifically the myocardium. Cardiac muscle is one of three major types of muscle, the others being skeletal and smooth muscle. These three types of muscle all form in the process of myogenesis. The cells that constitute cardiac muscle, called cardiomyocytes or myocardiocytes, contain only three nuclei. The **myocardium** is the muscle tissue of the heart, and forms a thick middle layer between the outer epicardium layer and the inner endocardium layer.

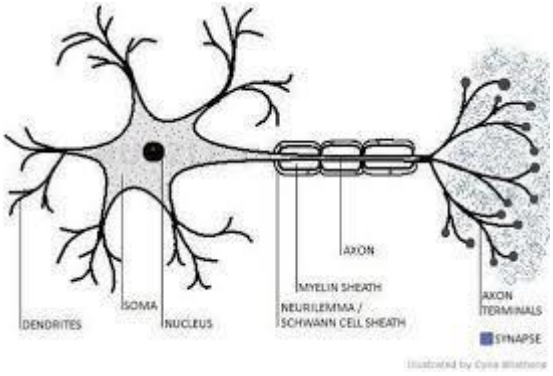
9. Smooth Muscle :



Smooth muscle is an involuntary non-striated muscle. It is divided into two subgroups; the single-unit (unitary) and multiunit smooth muscle. Within single-unit cells, the whole bundle or sheet contracts as a syncytium (i.e. a multinucleate mass of cytoplasm that is not separated into cells). Multiunit smooth muscle tissues innervate individual cells; as such, they allow for fine control and gradual responses, much like motor unit recruitment in skeletal muscle.

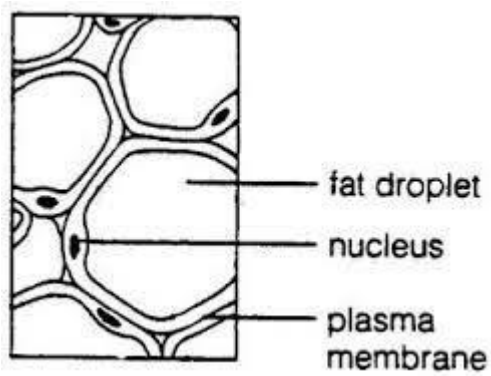
Smooth muscle is found within the walls of blood vessels (such smooth muscle specifically being termed vascular smooth muscle) such as in the tunica media layer of large (aorta) and small arteries, arterioles and veins. Smooth muscle is also found in lymphatic vessels, the urinary bladder, uterus (termed uterine smooth muscle), male and female reproductive tracts, gastrointestinal tract, respiratory tract, arrector pili[1] of skin, the ciliary muscle, and iris of the eye. The structure and function is basically the same in smooth muscle cells in different organs, but the inducing stimuli differ substantially, in order to perform individual effects in the body at individual times. In addition, the glomeruli of the kidneys contain smooth muscle-like cells called mesangial cells.

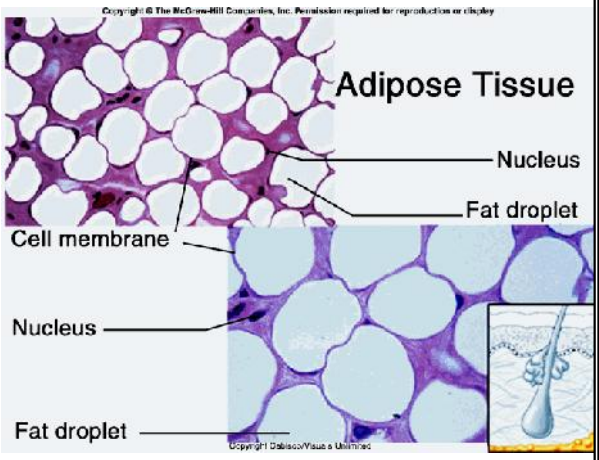
10. Neuron :



Nervous tissue is the main component of the two parts of the **nervous** system; the brain and spinal cord of the central **nervous** system (CNS), and the branching peripheral **nerves** of the peripheral **nervous** system (PNS), which regulates and controls bodily functions and activity.

11. Adipose tissue :





Adipose tissue or **body fat** or just **fat** is loose connective tissue composed mostly of adipocytes. In addition to adipocytes, adipose tissue contains the stromal vascular fraction (SVF) of cells including preadipocytes, fibroblasts, vascular endothelial cells and a variety of immune cells (i.e., adipose tissue macrophages [ATMs]). Adipose tissue is derived from preadipocytes. Its main role is to store energy in the form of lipids, although it also cushions and insulates the body. Far from hormonally inert, adipose tissue has, in recent years, been recognized as a major endocrine organ,[1] as it produces hormones such as leptin, estrogen, resistin, and the cytokine TNFα. Moreover, adipose tissue can affect other organ systems of the body and may lead to disease. The two types of adipose tissue are white adipose tissue (WAT), which stores energy, and brown adipose tissue (BAT), which generates body heat.

EXPERIMENT - III

HEAMATOLOGY

ESTIMATION OF HEMOGLOBIN BY SAHALI'S METHOD

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

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

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

PROCEDURE: Take 1/10 HCl in the Hb tube upto the lowest mark '2'.

2. Prick the finger with needle and collect 20µl of blood sample with single mark pipette.

3. Place the Hb tube on working table for five minutes for the formation of hemin crystals.

4. Place the Hb tube in the compater/hemometer and add drop by drop of distilled water into it until the colour of the solution in the Hb tube coincides with the glass plates of the compater.

5. If the colour coincides with the glass plates of the compater, observe the reading in the Hb tube. The percentage of Hb can be calculated from the reading.

DATA ANALYSIS: Hb content in grams X 100 / 14.5

NORMAL VALUES: Males = 14 to 18 grams
Females = 13 to 14 grams
Children = 10 to 13 grams

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

EXPERIMENT – IV

Determination of Bleeding Time

Aim

To determine the bleeding time of a patient.

Theory

The time required for complete stopping of blood flow from the punctured blood vessels called the bleeding time. Normally it is 1-3 minutes for a normal human's blood. Normal clotting time and bleeding time values differ because bleeding time is the time for stopping bleeding by the formation of fibrin network on the surface of punctured skin; that is it is the surface phenomenon. But the clotting time is the time for clotting the whole blood, collected in the capillary tube; therefore it is a volume phenomenon. For this reason clotting time is more than the bleeding time, when determining by conventional methods.

Clinical significance

It plays a significant role
i) to study the haemorrhagic disorders.
ii) to study the coagulation defects
iii)to have an idea about the platelets count of the patient. Bleeding time is prolonged in few disorders like: vascular lesions, platelet defect, severe liver disease, uremia and anti-coagulant drug administration.

Materials

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

Procedure (Duke's method)

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

Precaution

Following precautions should be enforced
i) Needle should be sterilized.
ii) A fain stain of blood should not be avoided.
iii) Time should be noted properly.

EXPERIMENT – V

Determination of Clotting Time

AIM

To determine the clotting time of a subject.

Requirements:

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

Procedure:

Capillary tube method: (Wright's method)

Under sterile precautions make a sufficiently deep prick in the finger tip. Note the time when bleeding starts (start the stop watch). Touch the blood drop at the finger tip using one end of the capillary tube kept tilted downwards. The tube gets easily filled by capillary action. After about two minutes start snapping off small lengths of the tube, at intervals of 15 seconds, each time noting whether the fibrin thread is formed between the snapped ends. Note the time (stop the stop watch) when the fibrin thread is first seen.

Discussion:

Clotting time is the interval between the moment when bleeding starts and the moment when the fibrin thread is first seen.

Normal value is 3to 10 minutes.

Bleeding time and clotting time are not the same. Bleeding time depends on the integrity of platelets and vessel walls, whereas clotting time depends on the availability of coagulation factors. In coagulation disorders like haemophilia, clotting time is prolonged but bleeding time remains normal.

Clotting time is also prolonged in conditions like vitamin K deficiency, liver diseases, disseminated intravascular coagulation, overdosage of anticoagulants etc.

Other method:

Modified Lee and White method:

Under aseptic precaution venepuncture is done and one ml. of blood is collected in each 3 small test tubes. Note the time when blood is taken. Keep the test tube in a water bath maintained at 37°c. Tilt the tubes every 30 seconds and see whether the blood is flowing. Repeat this till the tube can be inverted without the blood flowing out. Nore the time. Average value of the results in the 3 test tubes gives the clotting time.

Normal value is2 to 7 minutes

EXPERIMENT – VI

Identification of Blood Groups

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

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

The ABO and Rh groups in transfusion

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

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

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

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

EXPERIMENT – VII

RBC Count

AIM

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

PRINCIPLE:

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

APPARATUS:

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

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

The improved Neu-Bauer's double counting chamber:

This is a thick rectanguar glass with a polished transverse bar in the centre, separated from the rest of the slide by two parallel grooves on either side. The polished bar is divided into two equal platforms by a groove in the middle resulting in 'H' shaped depression (moats). The surface of the platforms is 1/10 mm below the surface of the rest of the slide. So if a cover glass is placed over the surface of the counting chamber, the under surface of the coverglass remains 1/10 mm above the polished surface of the platform.

The counting area is in the form of a central ruled area on the polished surface of each platform. It is a square of 3 mm side, divided into 9 equal squares of 1 mm side. Of these, the four corner squares are used for WBC counting. Each WBC square of 1 mm side is again divided into sixteen smaller squares each of 1/4mm side. The central 1 mm square is divided into 25 equal small squares of 1/5mm side, by means of triple lines of which the 4 corner ones and the central one are used for RBC counting. Each of these squares is subdivided into 16 smallest squares each of 1/20mm side.

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 mlSodium 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 special cover slip provided. Focus under the high power objective and identify the RBC counting area. Clean the RBC pipette first with distilled water, then with absolute alcohol and finally with ether and keep it dry. Take a small quantity of diluting fluid in a watch glass and keep aside. Clean the finger tip using rectified spirit and make a deep prick with a sterile lancet,so that blood comes out freely without squeezing. Wipe off the first drop which may contain tissue fluid also. Allow a good sized blood drop to form hanging drop and keep the pointed tip of the pipette touching the drop. Suck in blood upto the 0.5 mark carefully, without any air bubble. Excess blood at the tip of the pipette is removed using a bloating paper or piece of cotton. Immediately, diluting fluid from the watch glass is sucked in upto the 101 mark with out any air bubble by keeping the pipette in vertical position.

Then thoroughly mix the blood and diluting fluid in the pipette by gently rolling the pipette held horizontally between the palms and keep aside. Mixing takes place only in the bulb of the pipette. The column of diluting fluid contained in the stem of the pipette does not enter into the dilution (i.e. 101-1 = 100). So that the blood sucked upto 0.5 mark will have a dilution of 0.5 in 100 or 1 in 200. Now take out the

counting chamber for charging discard first few drops from the pipette, as the stem contains only diluting fluid. Bring one small drop of diluted blood at the tip of the pipette, to the edge of the cover slip on the counting chamber at an angle of about 450 The fluid enters by capillary action under the cover slip and fills the counting chamber. Both areas are filled.

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

Precautions:

1. Counting chamber and pipette should be clean and dry.

2. Fingertip and pricking lancet should be sterile.

3. Blood should freely come out without squeezing.

4. Be careful to prevent clotting of blood inside the pipette.

5. While filling the pipette and charging the counting chamber, no air bubble should enter.

6. Blood should be taken only upto the 0.5 mark and diluting fluid sucked only upto 101 mark.

7. Blood should be properly mixed with the diluting fluid.

8. Discard first few drops before charging because it will not contain RBC5.

9. While charging the counting chamber, over filling and spilling should be avoided.

10. Cells should be settled down and more or less evenly distributed before counting.

11 Don’t keep the microscope in tilted position.

12. Count from Left to Right and avoid counting of the same cell.

Calculation:

Let the number of cells counted in (5x16) 80 smallest squares be “N”
Number of cells in 1 smallest square is N/80
Side of 1 square = 1/20mm
Area of 1 square = 1/400mm2Depth of fluid film in counting chamber is 1/10mm

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

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

80 Numberof cells in 1 mm3 of diluted blood N 80x1/4000 = Nx4000 80

The dilution factor is 1 in 200

(Total diluted volume in bulb of the pipette is 100 parts, out of which 0.5 is blood. So dilution is 0.5 in 100 i.e.1 in 200)

So numberof cells in 1 mm3 of undiluted blood =Nx 4000x200 = Nxl0000 80

Discussion:

(Expressed in

millions/mm3)

Normal RBC Count

Adult male: 5- 5.5

millions/mm3

Adult female: 4.5 - 5 millions/mm3

Variations in Count:

Increase in count
Decrease in count

Physiological variations

1.Diurnal -

2.Age -

3.Sex -

4. High altitude -

5. Muscular exercise -

- Polycythemia (Erythrocytosis)

- Anaemia (Erythropenia)

Lessduring night, minimum in early morning, gradually increasesduring day

In newborn, high count is seen More in males Count higher due to hypoxia
Count increases

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

Total Leucocyte Count

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

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

 PROCEDURE • Take WBC diluting fluid (Turk's Fluid) in a watch glass • After pricking finger, suck the second drop of blood into the WBC pipette exactly up to 0.5 mark and dilute it with WBC diluting fluid by sucking the fluid up to 11mark (dilution 1 in 20) • Gently rotate the pipette at least 3-4 minutes in the palm of the hand to ensure the proper mixing of the blood an the fluid
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Recharge the chamber if distribution is not uniform (WBCs are seen as regular nucleated, rounded bodies with a clear refractivity around them)

 Count the number of WBCs in each WBC square preferably under low power objective. • Count the WBCs in 4 corner WBC squares and enter your observations in the corresponding squares

 CALCULATIONS • Calculation of diluting factor Dilution factor:Final volume achieved (10 parts) Original volume taken (0.5 parts).

 • Calculation of volume fluid examined: Area of 4 WBC squares = 4 X 1mm X 1mm = 4 sqmm Depth of the chamber = 0.1mm Therefore, volume of fluid in the 4 WBC squares = 4sq.mm X 0.1mm = 0.4 cu.mm

 • Calculation of Total Leukocyte count or Total WBC count:Let 'N' be the total number of WBCs in 4 WBC squares i.e. in 0.4 cu.mm of diluted blood Then total number of WBCs in 1 cu.mm of undiluted blood : N x Dilution factor (20) 0.4 = N x 50.

 PRECAUTIONS • Prick should be bold enough to give free flowing blood. At no stage finger should be squeezed to take out the blood • Both the chamber and the cover slip should be dry and free from grease • Use only dry pipette • Never use a broken cover slip • Before charging the chamber the fluid from the stem of the pipette should be discarded

 The cover slip should be placed symmetrical so as to cover the ruled area completely • There should be no under or over charging of the chamber (The count will be low in both the cases) • After charging the chamber time should be given for the cells to settle down, but counting should be started before the fluid in the chamber starts drying up. • While counting the cells the stage of the microscope should not be tilted. www.indiandentalacademy.com

ESTIMATION OF DIFFERENTIAL LEUCOCYTE COUNT (D LC)

APPARATUS • • • • • 4-5 glass slides Pricking apparatus Compound microscope Cedar – wood oil Leishman's stain.

 Leishman's Stain:It belongs to the Romanowsky group of stains containing an acidic and a basic dye. The unique property of this stain is •Making clear distinction in the shades of staining •Stains granules differentially 1. Eosin :- It is an acidic dye (negatively charged) and stains positively charged (basic) particles such as RBCs and granules of Eosinoplhils 2. Methylene blue:- It is a basic dye (positively charged) and stains negatively charged (acidic) particles such as cytoplasm, nuclei of WBCs and granules of basophils.

 Acetone free methyl alcohol:- It is a fixative It preserves the cell in whatever chemical and metabolic state they are at the time of staining The blood smear also gets fixed to the slide (precipitation of protein in it, by alcohol) so that it can not be washed off (Methyl alcohol should be acetone free as it causes shrinkage of cells and even lysis. It should be also water free as water may affect appearance of the film causing rouleaux formation).

 PROCEDURE A. Preparation of Blood Smear – Take 4-5 grease free slides with smooth edges; 3-4 to be covered by the blood film and one as a spreader – Lay the slides on the table, prick the finger, touch the bleeding point on the centerline of the slide at about 1 to 2 cm from one end of the slide, on each of the slides – Place the narrow edge of the spreader on the surface of slide just in front of the drop of the blood at an angle of 45 deg.

 Draw the spreader backwards so as to touch the drop and hold it there till the blood runs along the width of the spreader at the line of junction – The spreader is then moved to the other end of the slide slowly and smoothly maintaining the 45 deg angle – Allow the film to dry in air and repeat the procedure with the other slides.

 Criteria of a good blood smear:1. It should cover almost the entire width and about 3-4 cm length of the slide 2. It is tongue shaped with no tail at the end 3. It is of uniform thickness, not too thick or too thin (A thick film appears red when placed on a white back ground) 4. There should not be longitudinal striations or cross striations or air gaps in the smear.

 Fixing and staining the Blood smear :1. Fixing the blood smear: i) Place the blood smear across 2 parallel supports on the glass rods, to make them horizontal ii) Pour 8-12 drops of the Leishman's stain on the slides so as to cover the smear, leave it on for 2 mins (fixation time) 2. Staining the smear: i) At the end of 2 mins add an equal no. drops of buffered (pH 6.8) water over the stain so that water is not spilled over ii) Mix the stain and water evenly by gently blowing air intermittently with the help of a dropper.

 If the dilution of the stain is correct, the fluid will be covered by a thin greenish scum. Leave the stain to for 10 mins. (staining time) 3. At the end of 10 mins, pour off the stain hold the slide in a slanting position below the tap and the water is allowed to flow over the smear 4. Wash the slide (up to 2 mins) with tap water gently and thoroughly till the film gets a pinkish tinge. Make sure that :i) none of the greenish scum settles on the surface of the blood film ii) water stream should not strike the blood stream directly or else it may be washed off. 5. Wipe clean the back of the slide and set upright to dry.

 Assessment of the quality of the stained smear: A well stained smear has the following characteristics:1. The smear is single thick, with cells uniformly distributed 2. At least 1 WBC is seen per high power field (X 100) 3. The RBCs are stained light pink 4. In an over stained smear, RBCs look bluish black and WBCs will take up more methylene blue, hence look totally purple 5. In an under stained smear, RBCs appear very pale and WBCs look almost colorless.

 Examination of the stained smear under oil immersion:- Identification of the different types of blood cells is done only under oil immersion 1. Place a drop of cedar- wood oil on the blood film. Focus the oil immersion objective such that it just touches the oil drop. Now raise the objective and focus with a

fine adjustment. 2. Study the distribution and characteristics of different types of cells in the different parts of the smear.

- The following is observed:i) The most abundant cell type foun anywhere in the smear is RBCs, as light pink non nucleated dics of uniform size. ii) At the head end end of the smear the RBCs are crowded and superimposed, and the WBCs are poorly stained iii) At the extreme tail end, the cells are wide apart and the WBCs are distorted. iv) At the upper and lower edges of the smear, the WBCs are found in plenty but are poorly stained and abnormally rich in granulocytes

- Identification of different types of Leucocytes: The WBCs can be differentiated from RBCs by the presence of nucleus and Large size. While identifying the WBCs, keep 4 factors in mind:1. Size of the cells (compare with the surrounding RBCs which are of uniform size, 7.2µm) 2. Features of the nucleus (colour, number of lobes) 3. Features of the cytoplasmic granules (pink or blue, fine or coarse) Nuclear / Cytoplasmic ratio.

- Counting of the different types of leucocytes: A minimum of 100 WBCs are identified in a systematic manner and counting is made using the tally- bar method. Ideally all the cells should be counted in a single strip running the length of the smear, proceeding from the base to the apex. Use the mechanical stage to traverse the full length of the film. Move the slide along vertically by a distance of 2mm and again traverse the full length of the film this time in the opposite direction . This method ensures that the cells are not counted more than once.

- Alternatively a box containing 100 squares can be made in which entry of each one of identified cells can be made.

- precautions 1.The slides should be claen and greese free therefore , these should be cleaned troughly with soap and water immediately 2.The glass spreader should be carefully selected 3.Make about 4-5 blood films so that at least one is stisfactory

- Mark smeared surface with glass marking pencil 5.Donot dry or heat or blot dry smear 6.Store blood must not be used 7.The smear should be stained not later than 2 hours after it has been prepared www.indiandentalacademy.com

- Assess the quality of the smear, both grossly and microscopically before staining it 9.Only properly well stained slide should be examined under the oil-immersion lens.

EXPERIMENT – IX

Differential Leucocyte Count

DIFFERENTIAL WHITE BLOOD CELL COUNT

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

Granulocytes (polymorphonuclear leukocytes) are subdivided according to the specific coloration of granules:

► **Neutrophils** account for the largest percentage of leukocytes found in a normal blood sample. On a stained blood smear, the cytoplasm of a neutrophil has numerous fine lilac-colored granules and a dark purple nucleus. The diameter of a neutrophil is 10-15 μm. Neutrophils are subclassified according to their age or maturity, which is indicated by changes in the nucleus (Figure no. 7):
– metamyelocyte – is the youngest neutrophil generally reported, the nucleus is large, round or bean-shaped, the cytoplasm is abundant, pale blue;
– neutrophilic band or stab – the nucleus is elongated and curved (horseshoe or S- shape), cytoplasm is abundant, pink;
– segmented neutrophil – is a mature neutrophil, the nucleus is separated into 2-5 segments or lobes, the cytoplasm is pale red.

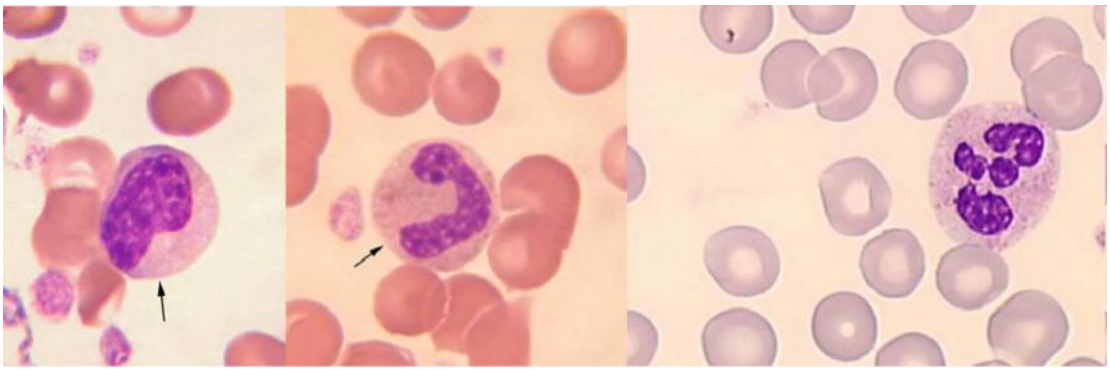


Figure no. 7. Neutrophil granulocytes with different maturation degree: (from left to right) metamyelocyte, neutrophilic band and segmented neutrophil.

► **Eosinophils** have a diameter of 12-14 μm, the nucleus usually large, typically a bilobate (two-lobed), the cytoplasm is eosinophilic (pale red) with coarse round granules of uniform size which appear brick-red after staining with eosin (Figure no.

► **Basophils** have a diameter of 11-13 μm. Their nucleus is usually large, irregular,sometimes having three lobes, the cytoplasm is basophilic, containing scattered large, dark-blue granules which may overlay the nucleus (Figure no. 9).

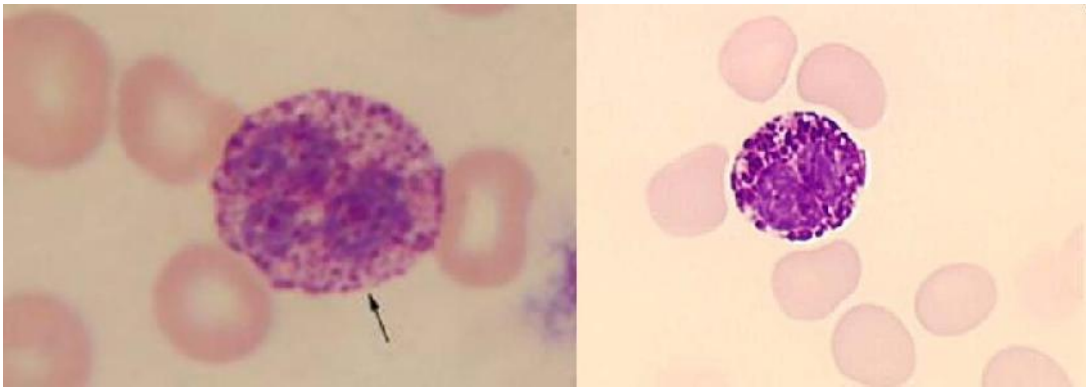


Figure no. 9. Basophil granulocytes

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

► **Lymphocytes** have a diameter of 8-10 μm. The nucleus is large, generally round, oval, or slightly indented. The cytoplasm of a lymphocyte is basophilic, scanty, (in circle or "half moon" around the nuclues) (Figure no. 10, left).

Monocytes are the largest of the normal white blood cells (the diameter is 15-25 μm). The nucleus is large, round, indented or lobulated, the cytoplasm is muddy gray-blue (Figure no. 10, right).

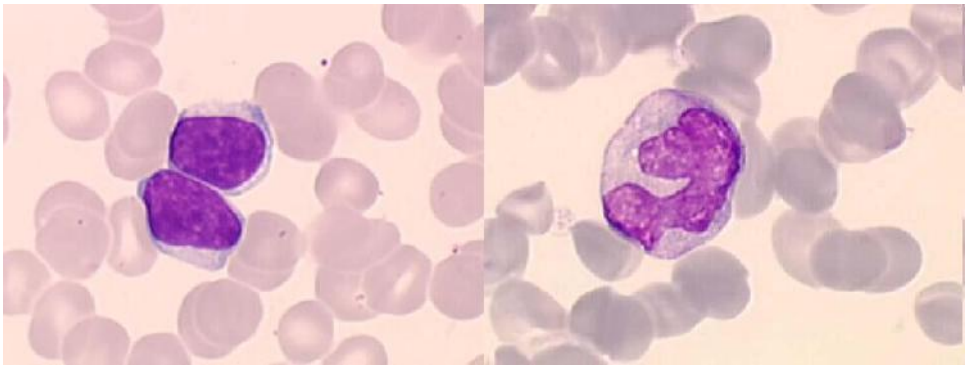


Figure no. 10. Agranulocytes: left: two lymphocytes, right: monocyte.

MATERIALS

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

PROCEDURE

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

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

Table 1. Schilling's table

Clean up! Cleanse microscope stage and workspace if any oil has spilled on it. Recap the immersion oil container securely. DO NOT wipe clean the 90x oil immersion objective nor the slide; this will be done by the assistant!

RESULTS

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

DATA INTERPRETATION

Normal distribution of different white blood cell types:

Metamyelocytes band neutrophils	< 1 % 1-2 %
Segmented neutrophils	55-65 %
Eosinophils	2-4 %
Basophils	0-1 %
Lymphocytes	23-35 %
Monocytes	4-8 %

Alteration of the ratio of different white blood cells:

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

Neutrophilia refers to a higher number of neutrophils in the peripheral blood. The most common cause of neutrophilia is a bacterial infection. Neutrophils are also increased in any acute inflammation and might also be the result of a malignancy.

A "**left shift**" refers to the presence of increased proportions of younger, less differentiated neutrophils in the blood. This generally reflects early or premature release of myeloid cells from the bone marrow, the site where neutrophils are generated. A "**right shift**" refers to the presence of older, hypersegmented neutrophils in the blood, reflecting the inadequate release or insufficient production of neutrophils.

Neutropenia refers to an abnormally low number of neutrophils. It can be caused by several conditions, including decreased production in the bone marrow or viral infections.

EXPERIMENT – X

Erythrocyte Sedimentation rate

There are two main methods used to measure the ESR: the Westergren method and the Wintrobe Method. Each method produces slightly different results. Most laboratories use the Westergren method.

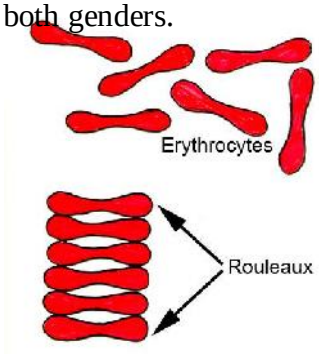
Westergren method:
The Westergren method requires collecting 2 ml of venous blood into a tube containing 0 .5 ml of sodium
citrate. It should be stored no longer than 2 hours at room temperature or 6 hours at 4 °C. The blood is drawn into a Westergren-Katz tube to the 200 mm mark. The tube is placed in a rack in a strictly vertical position for 1 hour at room temperature, at which time the distance from the lowest point of the surface meniscus to the upper limit of the red cell sediment is measured. The distance of fall of erythrocytes, expressed as millimeters in 1 hour, is the ESR.

Wintrobe method:
The Wintrobe method is performed similarly except that the Wintrobe tube is smaller in diameter than the Westergren tube and only 100 mm long. EDTA anticoagulated blood without extra diluent is drawn into the tube, and the rate of fall of red blood cells is measured in millimeters after 1 hour. The shorter column makes this method less sensitive than the Westergren method because the maximal possible abnormal value is lower. However, this method is more practical for demonstration purposes.

Average values in healthy men are: <15mm/hr; in healthy females, they are somewhat higher: <20mm. The values are slightly higher in old age, in both genders.

Theoretical considerations

The RBCs sediment because their density is greater than that of plasma; this is particularly so, when there is an alteration in the distribution of charges on the surface of the RBC (which normally keeps them separate form each other) resulting in their coming together to form large aggregates known as rouleaux.



Rouleaux formation is determined largely by increased levels of plasma fibrinogen and globulins, and so the ESR reflects mainly changes in the plasma proteins that accompany acute and chronic infections, some tumors and degenerative diseases. In such situations, the ESR values are much greater than 20mm/hr. Note that the ESR denotes merely the presence of tissue damage or disease, but not its severity; it may be used to follow the progress the diseased state, or monitor the effectiveness of treatment.

Some interferences which increase ESR:

- increased level of fibrinogen, gamma globulins.
- technical factors: tilted ESR tube, high room temperature.

Some interferences which decrease ESR:

- abnormally shaped RBC (sickle cells, spherocytosis).
- technical factors: short ESR tubes, low room temperature, delay in test performance (>2 hours), clotted blood sample, excess anticoagulant, bubbles in tube.

EXPERIMENT – XI

Recording of Pulse Rate

The heart, the blood, and the blood vessels make up the circulatory system. The heart pushes the blood through the blood vessels to vitals organs and tissues; the blood carries the oxygen they need to function, removes waste products that are a byproduct of metabolism, and carries them to the lungs for elimination.

The heart acts like a pump, and each heartbeat has two phases, a resting phase (*diastole)* and a pumping phase (*systole*). During diastole, the chambers of the heart fill with oxygenated blood. During systole, the walls of these chambers contract and send out a "wave" of blood to the lungs, brain, kidneys, muscles, etc. Taken together, systole and diastole make up the heartbeat. *The pulse is a measurement of the number of times the heart beats in one minute.*

In certain areas of the body, the blood vessels are close to the surface of the skin and this wave that represents a heartbeat can be felt. The pulse can also be measured by listening to heart with a stethoscope. Measuring the pulse rate is like measuring the body temperature; it is a quick, reliable, and easy way to determine someone's basic state of health or to determine if he/she is sick. The heart rate speeds up or slows down in response to stress, injury, infection, activity level, changes in the environment, drugs, etc.

As with the other vital signs there is a range of pulse are that is considered to be normal.

- Babies up to the age of 1: 100-160 beats per minute.
- Children of the ages 1 to 10: 60 to 120 beats per minute
- Children of the ages 11-17: 60 to 100 beats per minute.
- Adults: 60 to 100 beats per minute.
- Athletes: 40-60 beats per minute.

These values are considered to be normal for the given ages. But just as important as the rate, however, is the *rhythm*. *A normal heartbeat will be regular like the ticking of a clock. The amount of time between each beats will be the same and if it is not, this is considered abnormal.* Measuring and recording the pulse is simple. It can be done in many places in the body, but the two most common sites used to check the pulse are a) the chest, directly over the heart, using a stethoscope, and b) on the side of the wrist using the radial artery.

- Radial artery: The radial artery is located on the wrist (on the side *opposite* the back of the hand) just below the base of the thumb. You can find it taking two fingers and placing them in this area: you should easily feel a rhythmic pulse. Count the pulse for at least 30 seconds and then multiply times by two; the result will be the heart rate. Do *not* use your thumb to count the pulse. Many people have a strong pulse in their thumbs and this can interfere with accurately feeling someone's pulse.
- The chest: The heart is located on the left side of the chest, *approximately* midway between the waist and the shoulder. Place your stethoscope in that area and listen for the heartbeat. Count for 30 second and then multiply times two; the result will be the pulse rate.

- The pulse is a measurement of the number of heartbeats in one minute.
- The pulse can be increased or slowed down by illness, injury, infection, drugs, the environment, or activity level.
- The normal pulse for an adult should be regular and between 60 and 100 beats per minute.
- A pulse rate that is abnormally slow is called bradycardia.
- A pulse rate that is abnormally fast is called tachycardia.
- The two most accurate places to measure the pulse are the radial artery and the chest.
- *Notify the R.N. or your supervisor if the patient's heart rate bradycardic, tachycardic or is unusually slow or fast for that patient.*

EXPERIMENT – XII

Recording of Arterial Blood Pressure

Definition of Blood Pressure

Arterial blood pressure is the force exerted by the blood on the wall of a blood vessel as the heart pumps (contracts) and relaxes.

Systolic blood pressure is the degree of force when the heart is pumping (contracting). The Diastolic blood pressure is the degree of force when the hearts relaxed.

Method of Measuring Arterial Blood Pressure

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

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

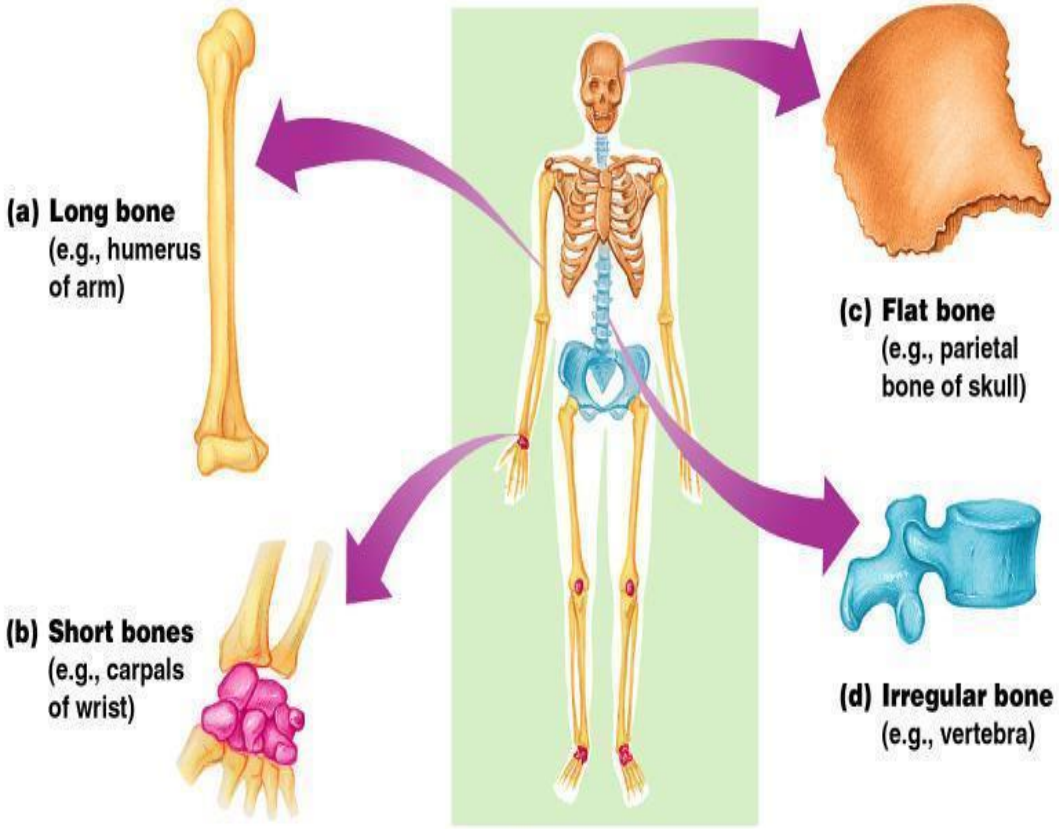
The cuff is connected by tubing to a manometer, which shows the amount of pressure on the artery. When the first pulse sounds are heard, the reading on the manometer measures the systolic blood pressure. The last sound heard is the diastolic blood pressure. In children, the muffling of sound or fourth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

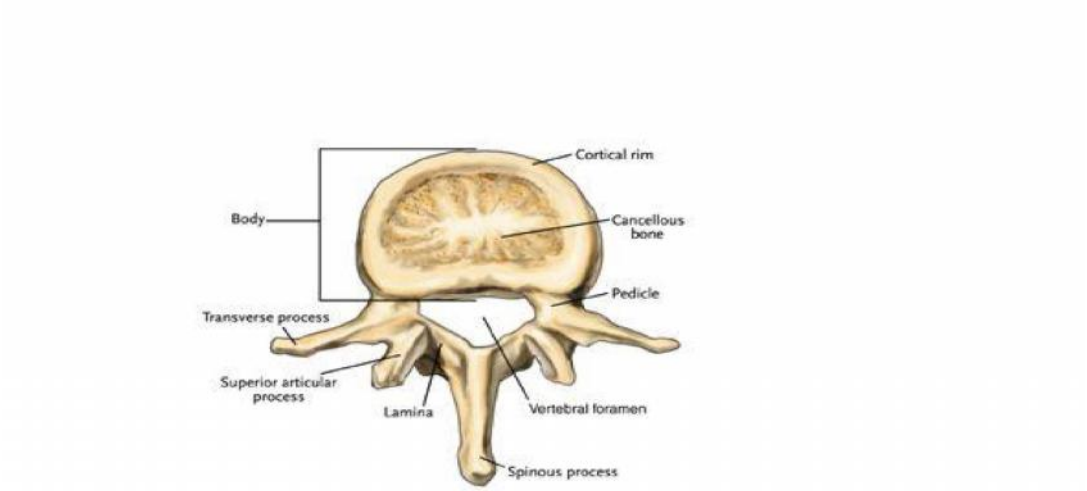
Reference ranges for blood pressure in children			
Stage	**Approximate age**	**Systolic**	**Diastolic**
Infants	1 to 12 months	75–100	50–70
Toddlers and preschoolers	1 to 5 years	80–110	50–80
School age	6 to 12 years	85–120	50–80
Adolescents	13 to 18 years	95–140	60–90

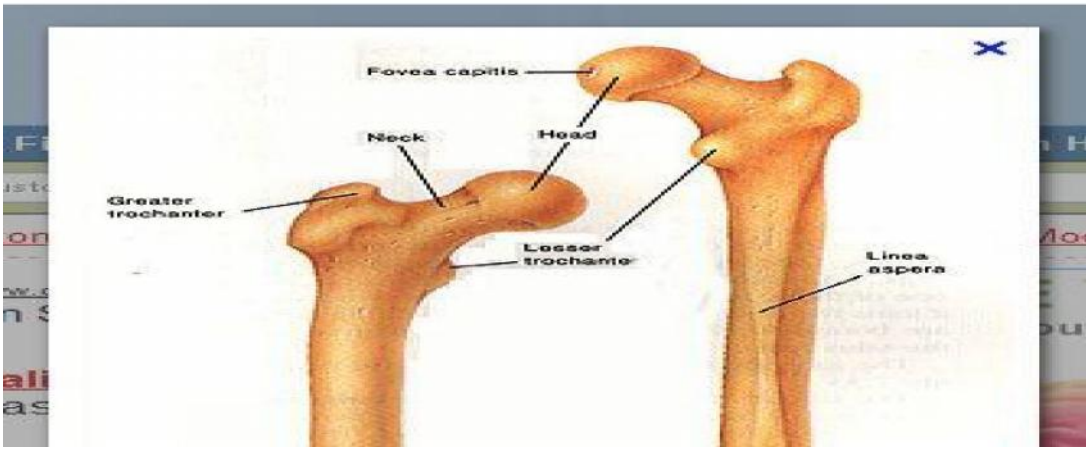
OSTEOLOGY

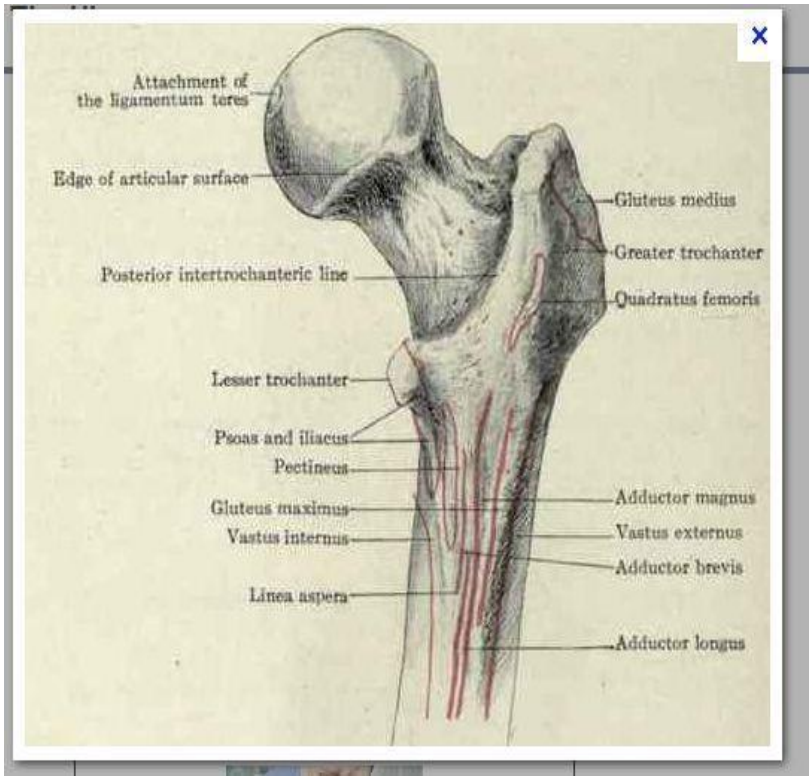
EXPERIMENT – XIII

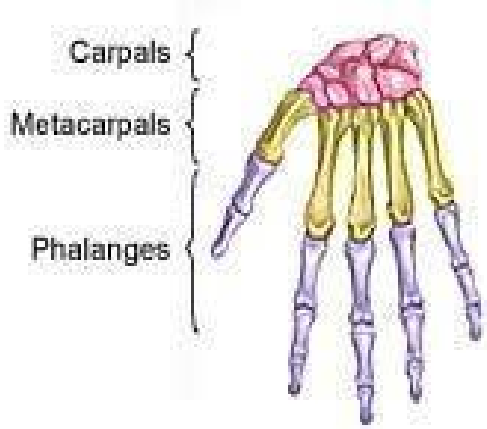
Classification of Bones

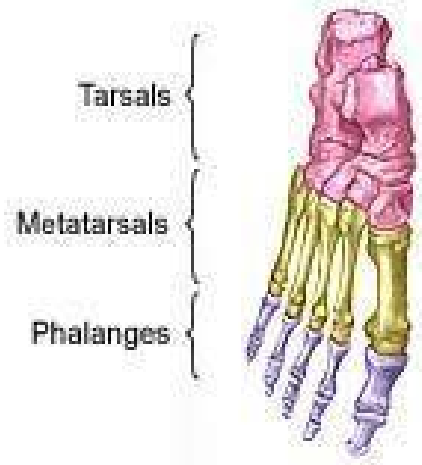


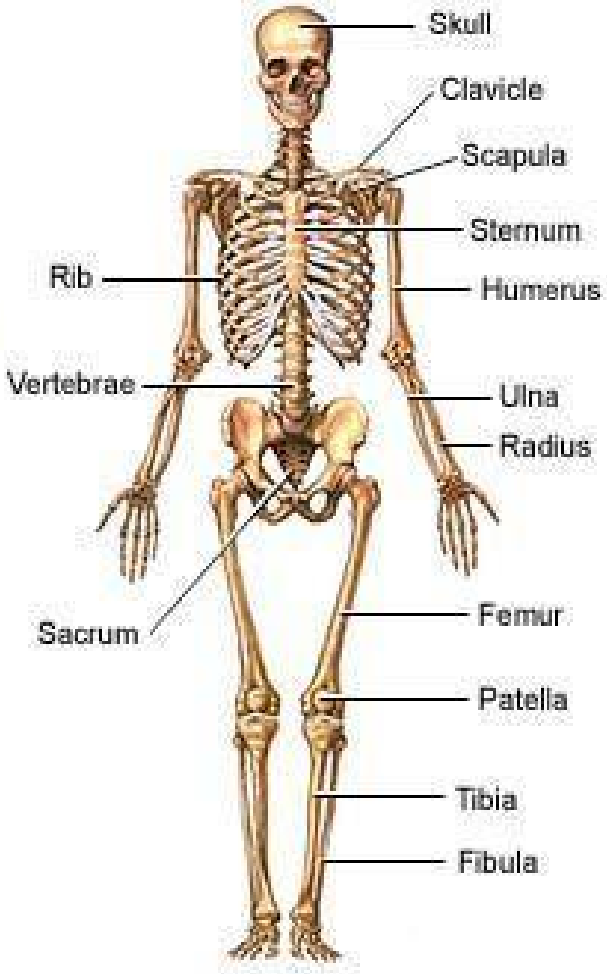

Cortical rim
Body
Cancellous bone
Pedicle
Transverse process
Superior articular process
Lamina
Vertebral foramen
Spinous process


Fovea capitis
Neck
Head
Greater trochanter
Lesser trochanter
Linea aspera


Attachment of the ligamentum teres
Edge of articular surface
Posterior intertrochanteric line
Lesser trochanter
Psoas and iliacus
Pectineus
Gluteus maximus
Vastus internus
Linea aspera
Gluteus medius
Greater trochanter
Quadratus femoris
Adductor magnus
Vastus externus
Adductor brevis
Adductor longus


Carpals
Metacarpals
Phalanges


Tarsals
Metatarsals
Phalanges


Skull
Clavicle
Scapula
Sternum
Rib
Humerus
Vertebrae
Ulna
Radius
Sacrum
Femur
Patella
Tibia
Fibula

XPERIMENT – IV

Sense Organ

1) EYE

Lecture notes 1: The human eye

The human eye is not much in use as a professional tool of astronomy. On the other hand, it is of great interest to understand how it works and by doing so we may illustrate many of the principles and problems that we will meet later in the course.

The eye and brain work together, and the brain can correct for many of the aberrations suffered by the eye. Thus the brain compensates for the fact that the image on the retina is inverted and for chromatic aberration.

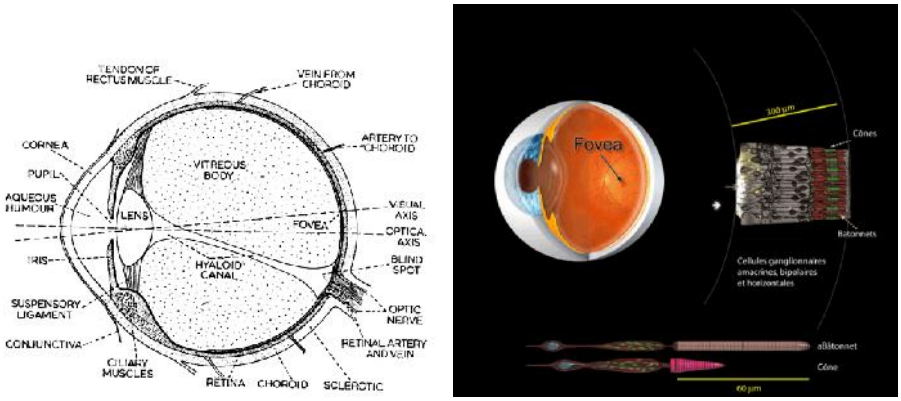


Figure 1: Cross section of the human eye (left), illustration of the eyes receptor cells; cones, used for color vision with the iodopsin layers arranged to the rigth, and rods with rhodopsin layers. Light enters these cells from the left before being absorbed by either iodopsin or rhodopsin.

Light is focussed on the retina, where there are two types of receptors: rods and cones. Cones for color reception, rods for black and white with higher sensitivity.

In the rods a pigment known as rhodopsin absorbs radiation. A protein with a weight of some 40 000 amu, arranged in layers 20 nm thick and 500 nm wide. Under influence of light a small fragment, a chromophore, will will split off. The chromophore is a vitamin A derivative called retinal (or retinaldehyde) with a molecular weight of 286 amu. The portion left behind is a colorless protein called opsin. The moment of visual excitation occurs during this break off process as the cell's electrical potential changes. This change in potential can then propagate along nerve cells to the brain. The rhodopsin molecule is hen (slowly) regenerated.

The response of cones is similar, but in this case the pigment is known as iodopsin which also contains the retinaldehyde group. Cone cells come in three varieties with different spectral sensitivities (see figure 2).

In bright light much of the rhodopsin is broken up into opsin and retinalde- hyde, and the rod sensitivity is much reduced so that vision is primarly provided by the cones, even though their light sensitivity is only of order 1% of the rods.

The three varieties of cones combine to give color vision. At low light levels only rods are triggered by the ambient radiation and vision is then in black and white.

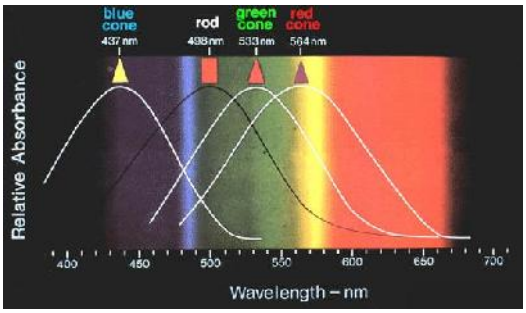


Figure 2: Absorption curves for the various types of cones and for rhodopsin.

Upon entering the dark from a brightly lit region rhodopsin will build up over a period of roughly 30 min, thus dark-adaptation takes this long and is based on rod cells. Somewhere between 1–10 photons are necessary to trigger an individual rod. However, several rods must be triggered in order to result in a pulse being sent to the brain as many rods can be connected to a single nerve fibre. The total number of rods is of order 10^8, of cones 6×10^6, these must share some 10^6 nerve fibres. Thus there are roughly 100 visual receptors per nerve fibre, note that there can be many cross connections between groups of receptors. Cones are concentrated towards the fovea centralis which is the region of most acute vision, while rods are most plentiful towards the periphery of the field of view. Weak objects are thus most easily visible with averted vision, ie when it is not looked at directly. In sum with all these effects the eye is usable over a range of illuminations differing by a factor $10^9 - 10^{10}$.

The Rayleigh limit of the eye, roughly given by λ/D where λ is the wave- length of the observed light and D is the size of the observing aperature, is of order 20 arcsec when the iris has its maximum diameter of 5–7 mm. However, for two separate images to be distinguished, they must be separated by at least one unexcited receptor cell, so even on the fovea centralis resolutin is limited in practice to between 1 arcmin an 2 arcmin. This is much better than elsewhere on the retina, since the fovea centralis is populated by small, tightly packed, singly connected cones. The average resolution of the eye lies between 5 arcmin and 10 arc min. The effect of granularity of the retina is countered by rapid scillations of the eye through a few 10 arc sec with a frequency of a few Hz, so that several receptors are involved in the detection when averaged over time.

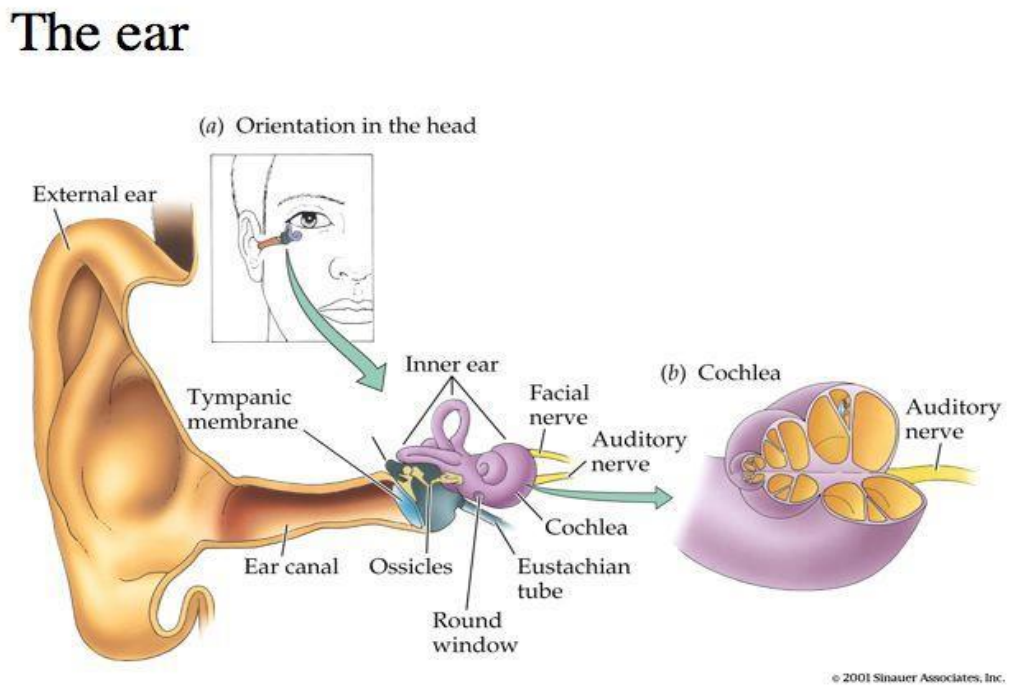
The response of the eye to changes in illumination is logarithmic; if two sources A and B are observed to differ by a given amount, and a third source C is seen to lie midway between them, then the energy from C will differ from A by the same factor as it differs from B. The faintest stars visible at a good site (magnitude 6^m) corresponds to a detection of approximately 3×10^{15} W. Sensitivity will vary between individuals and decreases with age, the retina of a 60 year old person will receive some 30% of the light seen by a person of 30-years.

The system used by astronomers to measure the brightness of stars is a very old one, and is based on the sensitivity of the eye. Hipparchos' catalogue of stars divided the stars into six classes from the brightest, of the first rank or magnitude, to the dimmest of the sixth magnitude. The present day system is based on this after the work of Norman Pogson put the magnitude scale on a firm basis in 1856. Pogson suggested a logarithmic scale that approximately agreed with earlier measurements: the difference between stars of magnitude m_1 and m_2 are given by

$$m_1 - m_2 = -2.5 \log \frac{E_1}{E_2}$$

where E_1 and E_2 are the energies per unit area at the surface of Earth for the two stars

2) EAR



Outer ear, middle ear, inner ear

Outer ear: Pinna - external part, Auditory canal

Middle ear: Eardrum - hard membrane, Ossicles

Inner ear: Oval window, Cochlea, Auditory nerve

The Pinna's function is to help in direction finding at high frequencies and helps funnel high frequencies into the auditory canal. The auditory canal acts as a resonant cavity in the range of 3,400 Hz, and causes our hearing to be more sensitive in this range.

Middle ear: Eardrum - hard membrane, Ossicles

The eardrum is a little more then 0.5 cm in diameter. Its function, along with the ossicles (hammer, anvil, stirrup), is to convert the sound waves into mechanical vibrations. Remember that sound is pressure waves and that pressure is force/area. The mechanical vibrations carried through the lever action of the ossicles presses on the oval window in the cochlea. The cochlea is very small, in part, to be able to detect very small vibrations. The middle ear enhances the sound wave energy two ways. First, through a reduction in size of the oval window relative to the size of the eardrum. The area of the oval window is 20-25 times smaller then the eardrum, Second, through a level action of the ossicles, which increases the force imparted on the oval window by 30%. The net effect is to increase the pressure up to 30 times on the much smaller oval window of the cochlea.
Eardrum - changes pressure fluctuations into mechanical vibrations.
Ossicles - transfer these vibrations to the *oval window* of the *cochlea. hammer, anvil and stirrup*

The function of the inner ear

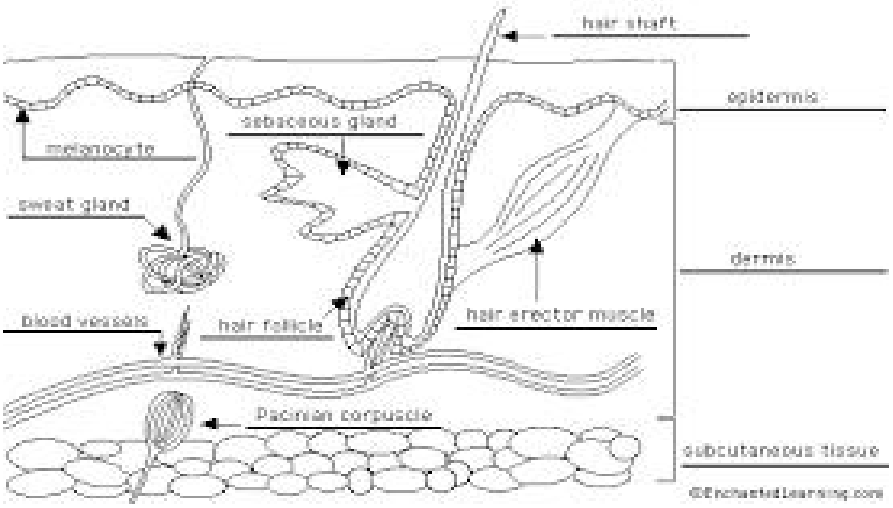
Cochlea - tapered, coiled tube, snail shaped, size of a pea! Stretched out it would be about 3 cm long. The cochlea is a very complex, very small device that converts the mechanical vibrations into neural impulses.

How the Cochlea works

Two tubes connected at the end of the cochlea (scala vestubuli and scala timpani) are filled with fluid called perilymph. Vibrations pass from the oval window to the round window. When the oval window pushes in the round window pushes out and visa-versa. The basilar membrane and related structures (cochlear duct, organ of corti), vary along the channel from thin and stiff to thick and loose. The cochlear duct is filled with viscous fluid called endolymph that is like spinal fluid. This causes the cochlear duct to be acoustically inactive. The vibrations of the basilar membrane primarily determine what we hear. The organ of corti rides along as the basilar membrane vibrates and senses its motion. High frequencies tend to vibrate the front end of the basilar membrane and low frequencies vibrate the back end.

Hair cells (approx. 24,000) sense the vibration of the basilar membrane and excite nerve cells that send nerve impulses down the individual fibers of the auditory nerve. The nerve cells fire more frequently when the vibrations are large amplitude. In fact, the hairs themselves vibrate when stimulated causing a positive feedback mechanism (this is a fairly recent research result). The location of the vibrations and the associated nerve impulses crudely determines the frequency of the sound. The auditory cortex in the brain does much more "signal processing," helping us to better distinguish musical sounds.

3) SKIN



The first, topmost, or superficial, layer of the skin the sun's rays hit is called the **epidermis**. Again, the **epidermis** is the outermost layer of the skin. The epidermis is itself made up of several layers. From outer to innermost, they are the:

- Stratum corneum
- Stratum lucidum
- Stratum granulosum
- Stratum spinosum
- Stratum basale

Do note, however, that the stratum lucidum is typically only found in places like the soles of your feet or the palms of your hand. Regardless, it's pretty easy to remember the exact order of the layers of the epidermis. Since we're on the topic of possible sunburns, the coolest mnemonic to remember the layers of the epidermis from top to bottom, or superficial to deepest, is:

'Come, Let's Get Sun Burn'.

Each word's first letter represents the first letter of each layer. In case you were wondering, the **epidermis** is actually the layer of skin that is primarily affected in most cases of sunburn and begins to peel off if damaged by the light's dangerous UV rays.

Types of Skin Cells

However, your skin isn't a weakling, and does have a defense mechanism that tries to fight off dangerous ultraviolet rays found in sunlight. In the deepest layer of the epidermis, the stratum basale, which is also sometimes called the basal layer, are cells called **melanocytes**. These are cells that produce the pigment melanin. It is this substance, melanin, which determines the skin color of an individual. Those with larger amounts of melanin in their skin have darker skin, or their skin darkens with more exposure to sunlight.

Melanocytes in the basal layer of the epidermis produce the pigment melanin

Basically, as the sunlight hits your skin, the light rays stimulate the production of melanin by melanocytes. Since the majority of melanin is called eumelanin, which is a brownish black color, your skin begins to darken as more melanin is produced. Keep in mind that this melanin isn't produced to give you a nice tan for aesthetic reasons, but instead, helps protect you from cancer-causing ultraviolet radiation found in the sunlight that is baking and peeling your skin off at the beach. At least the pale vampires who come out after twilight don't have to worry about this.

Pale vampires aside, your epidermis has other cells that are quite important. One of these cells are called **keratinocytes**. **Keratinocytes** are cells that eventually die in order to comprise the majority of the stratum corneum. The keratinocytes actually originate in the stratum basale, but as they mature and age, they move from the deepest to the most superficial layer of the epidermis.

Once the really old keratinocytes reach the stratum corneum they are known as 'corneocytes'. The corneocytes are basically the cells that are shed off your skin and become part of the dust floating around you. Disgusting, isn't it? When you inhale dust, you also inhale dead human skin cells.

As yucky as that might sound, the keratinocytes do play a lot of important roles. One of these roles actually involves the melanin produced by melanocytes. The keratinocytes take up and store some of the melanin produced by the melanocytes, and this gives your skin an extra layer of protection from the damaging ultraviolet radiation of the sun's light rays.

Keratinocytes store melanin, giving skin an extra layer of protection from UV rays

In addition to housing young keratinocytes and melanocytes, the basal layer of your skin also contains other cells, such as **Merkel cells**, which are cells that are important in the sensation of touch.

With all of that in mind, I do have an important point to make. The topmost layer of your skin we are going over, the epidermis, is made up of something called 'squamous' cells, which are basically a bunch of really flat cells. Bearing those squamous cells in mind, the 'basal' layer of the epidermis where the 'Merkel' cells and 'melanocytes' are located, it should come as absolutely no shocker that:

- Squamous cell carcinoma
- Basal cell carcinoma
- Merkel cell carcinoma
- Melanoma

are just some of the types of skin cancer you can get due to overexposure to damaging ultraviolet radiation from the sun's light rays. The next time you're frying at the beach, remember, your tan may be pretty, but skin cancer looks really nasty.