

ANATOMY & PHYSIOLOGY PRACTICAL NOTEBOOK

Pharmacy Technician (Category – B) PART – 1



Subscribe for more Lectures:
www.youtube.com/healthcysio

Anatomy & Physiology Practical Notebook

CONTENTS:

ANATOMY

PRACTICAL NO	NAME OF PRACTICAL
1)	STUDY OF HUMAN SKELETON
2)	HISTOLOGICAL EXAMINATION OF SLIDES; EPITHELIUM, CONNECTIVE TISSUES AND MUSCLES.

PHYSIOLOGY

PRACTICAL NO	NAME OF PRACTICAL
3)	DETERMINATION OF CONCENTRATION OF HEMOGLOBIN IN BLOOD (SAHLI'S APPARATUS)
4)	DETERMINATION OF RBCS COUNT
5)	DETERMINATION OF LEUCOCYTES COUNT
6)	DETERMINATION OF BLEEDING TIME
7)	DETERMINATION OF CLOTTING TIME
8)	DETERMINATION OF RADIAL PULSE
9)	DETERMINATION OF BLOOD PRESSURE
10)	DETERMINATION OF ERYTHROCYTE SEDIMENTATION RATE (ESR)
11)	DETERMINATION OF BLOOD GROUP SYSTEM
12)	TESTING OF VISUAL ACTIVITY
13)	RECORDING AND INTERPRETATION OF ECG

Practical-1

Study of human skeleton

The human skeleton is the internal framework of the body. It is composed of around decreases to around 270 bones at birth and decreases to around 206 bones by adulthood because some bones get fused together.

The bone mass in the skeleton reaches maximum density around age 21.

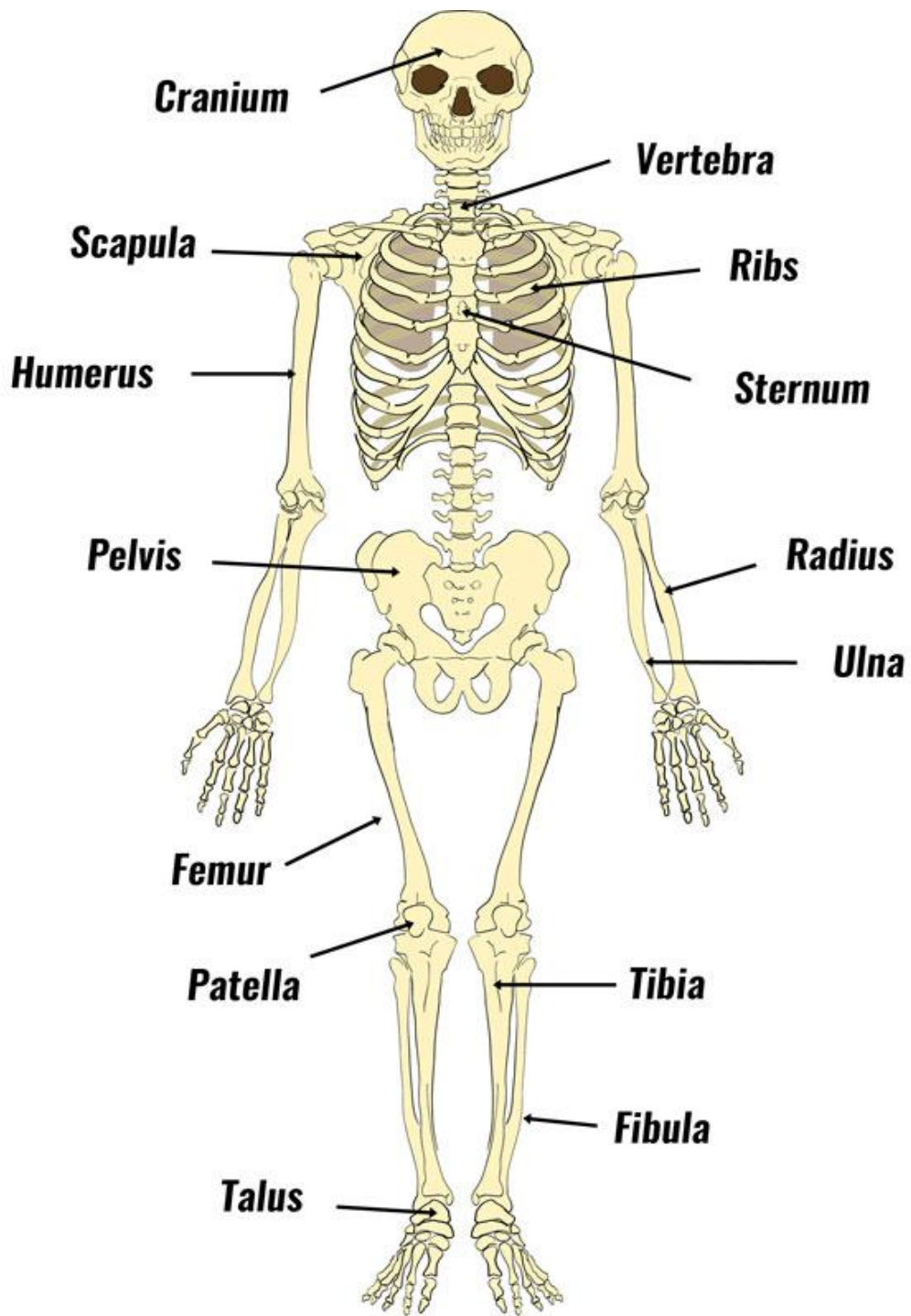
Division of the human skeleton:

The human skeleton can be divided into

- 1: Axial skeleton
- 2: Appendicular skeleton

1) Axial skeleton:

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



The upright posture of human is maintained by the axial skeleton, which transmits the weight from the head, the trunk, and the upper extremities down to the lower extremities at the hip joints. The bones of the spine are supported by many ligaments.

2) **Appendicular Skeleton:**

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

Functions of the human skeleton:

The human skeleton performs six major functions.

Support, movement, protection, production of blood cells, storage of minerals, and endocrine regulation.

1) Support:

The skeleton provides the framework which supports the body and maintains its shape. The pelvis associated ligaments and muscles provide a floor for the pelvic structures. Without the rib cages, costal cartilages, and intercostal muscles, the lungs would collapse.

2) Movement:

The joints between bones allow movement, some allowing a wider range of movement than others, e.g. the ball and socket joint allows a greater range of movement than the pivot joint at the neck. Movement is powered by skeletal muscles, which are attached to the skeleton at various sites on bones. Muscles, bones, and joints provide the principle mechanics for movement, all coordinated by the nervous system.

3) Protection:

The Skeleton helps to protect our many vital internal organs from being damaged.

The Skull protects the brain

The vertebrae protect the Spinal cord.

The rib cage, Spine, and Sternum protect the lungs, heart and major blood vessels.

4) **Blood cell production:**

The Skeleton is the site of hematopoiesis, the development of blood cell that takes place in the bone marrow.

In children, hematopoiesis occurs primarily in the marrow of the long bones such as the femur and tibia.

In adults, it occurs mainly in the pelvis, Cranium, vertebrae and Sternum.

5) **Storage:**

The bone matrix can store calcium and is involved in calcium metabolism, and bone marrow can store iron in ferritin and is involved in iron metabolism. However, bones are not entirely made of calcium, but a mixture of chondroitin sulfate

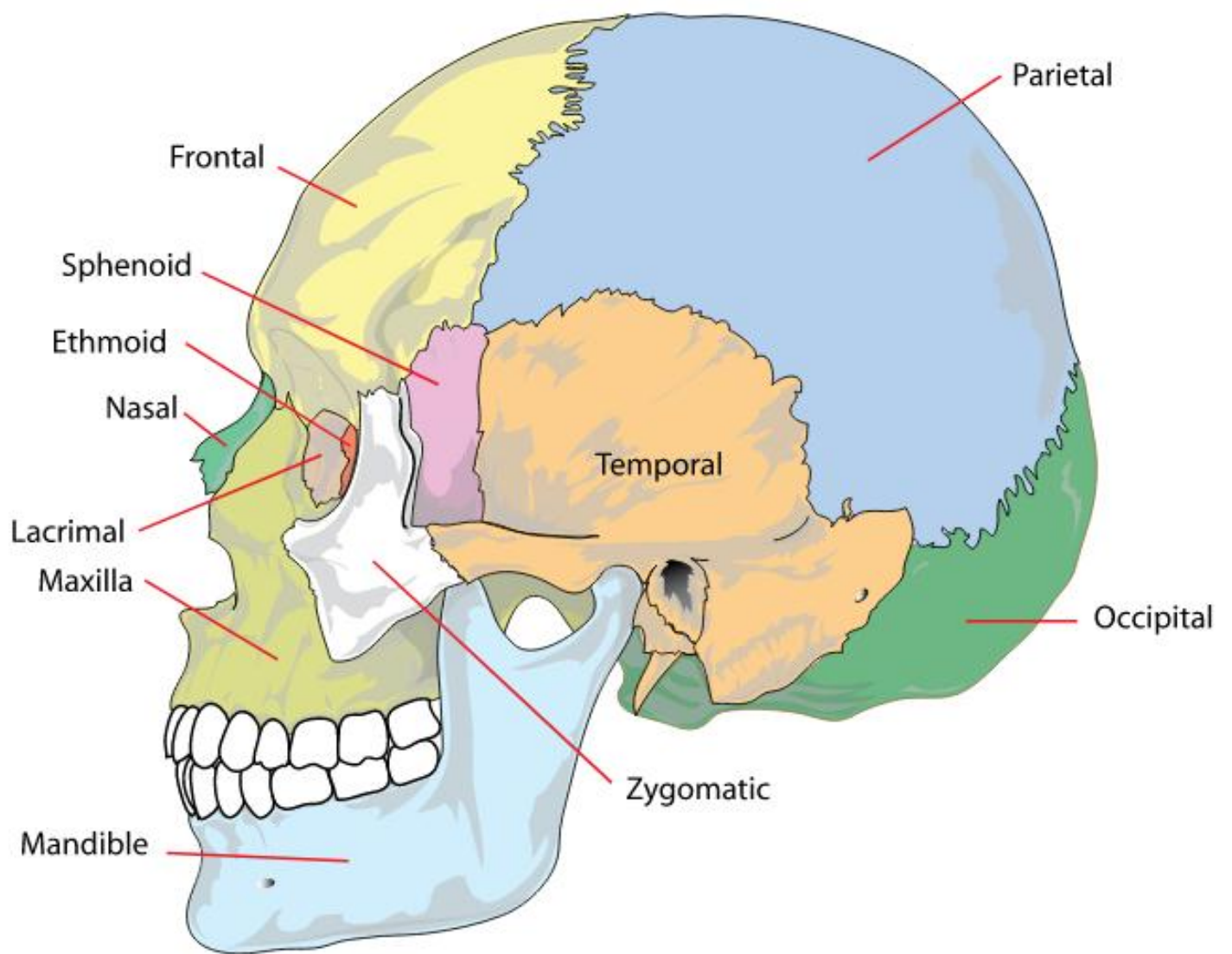
and hydroxyapatite, the latter making up 70% of a bone. Hydroxyapatite is in turn composed of 39.8% of calcium, 41.4% of oxygen, 18.5% of phosphorus, and 0.2% of hydrogen by mass, Chondroitin Sulphate is a sugar made up primarily of oxygen and carbon.

6) Endocrine regulation:

Bone cells release a hormone called osteocalcin, which contributes to the regulation of blood sugar (glucose) and fat deposition. Osteocalcin increases both the insulin secretion and sensitivity, in addition to boosting the number of insulin-producing cells and reducing stores of fat.

Human Skull anatomy

The skull is a bony structure that forms the head in vertebrates. It supports the structures of the face and provides a protective cavity for the brain. The skull is composed of two parts: the cranium and the mandible.



In the human these two parts are the neurocranium and the viscerocranium or facial skeleton that includes the mandible as its largest bone. The skull forms the anterior most portion of the skeleton and is a product of cephalization—housing the brain, and several sensory structures such as the eyes, ears, nose and mouth. In humans these sensory structures are part of the facial skeleton.

Function of the skull:

It include protection of the brain, fixing the distance between the eyes to allow stereoscopic vision, and fixing the position of the ears to enable sound localization of the direction and distance of sounds. In some animals such as horned ungulates, the skull also has a defensive function by providing the mount (on the frontal bone) for the horns.

Upper limb :

Sternum:

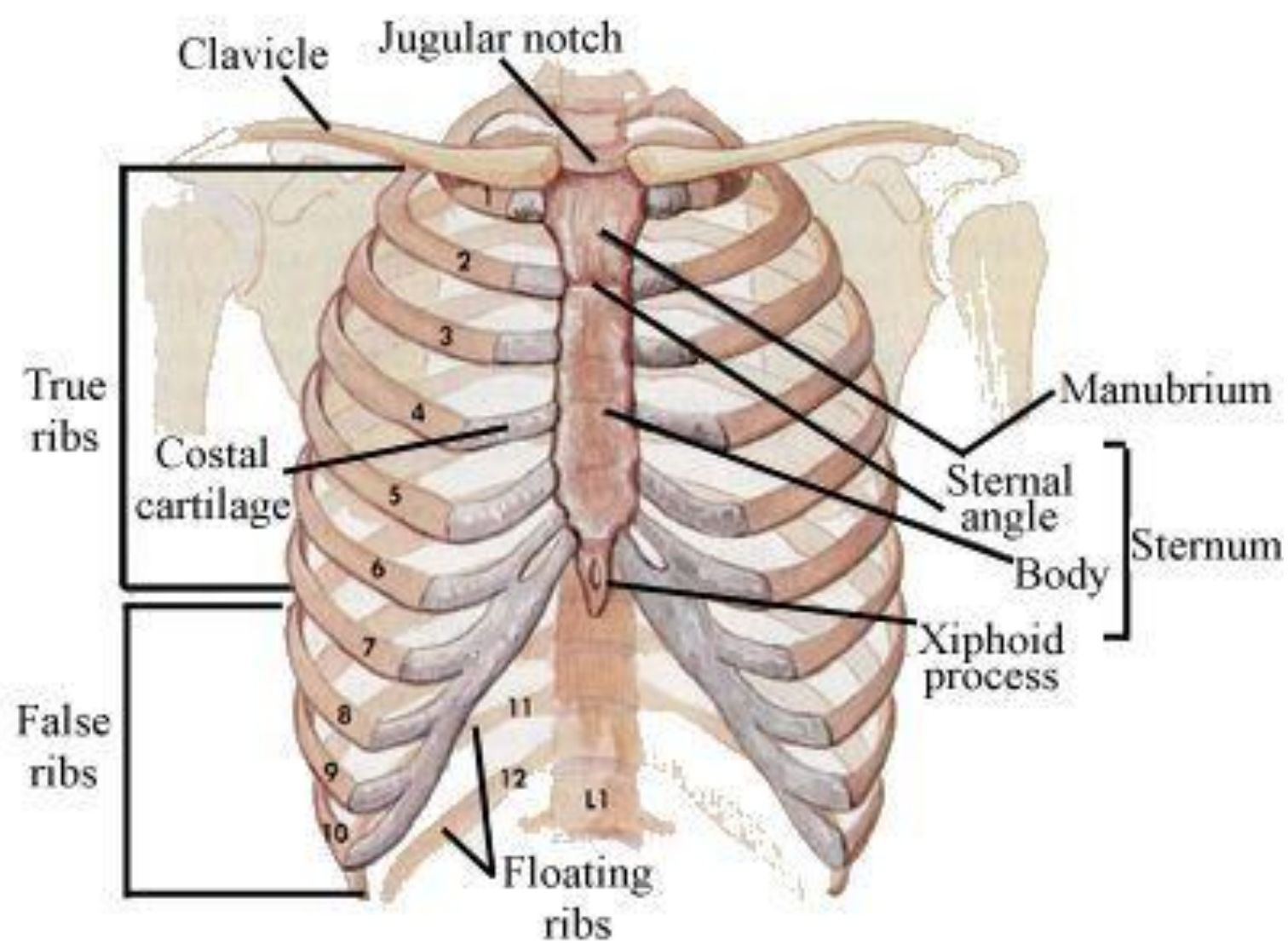
The Sternum is a flat, dagger shaped bone located in the middle of the chest. Along with the ribs, the sternum forms the rib cage that protects the heart, lungs, and major blood vessels from damage. The Sternum is composed of three parts:

- a) Manubrium
- b) Gladiolus
- c) Xiphoid process

Ribs:

The ribs are thin, flat, curved bones that form a protective cage around the organs in the upper body. They are comprised bones arranged in pairs. These bones are divided into three categories.

- a) True ribs
- b) False ribs
- c) Floating ribs.



Vertebral Column:

The vertebral column (also called the back bone, Spine, or Spinal column) consists of a series of irregularly shaped bones called vertebrae. These 33 bones are divided into five categories depending on where they are located in the back bone.

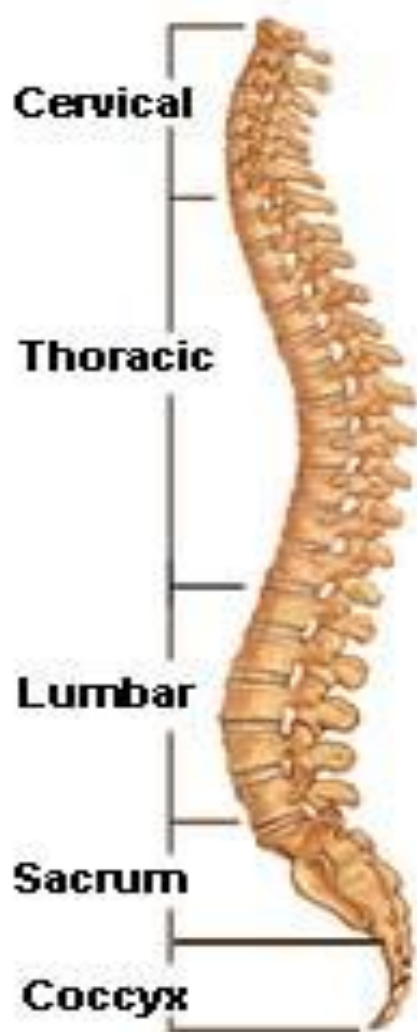
The upper Extremities:

The upper Extremities consists of three parts: arm, The forearm, and the hand.

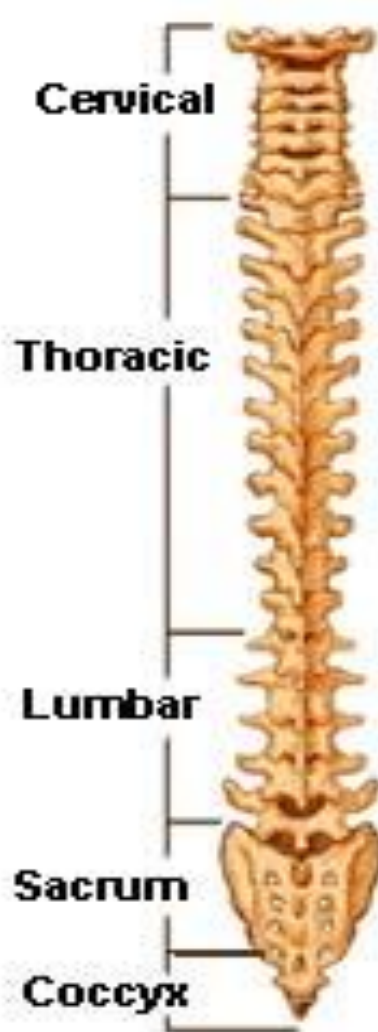
The arm:

The arm, or brachium, the technically only the region between the shoulder and elbow. It consists of a single long bone called the humerus. The humerus is the longest bone in the upper extremity. The top or head is large smooth, rounded; and fits in to the scapula in the shoulder. On the bottom of the humerus, are two depressions where the humerus connects to the ulna and radius of the forearm. The radius is

**Lateral (Side)
Spinal Column**



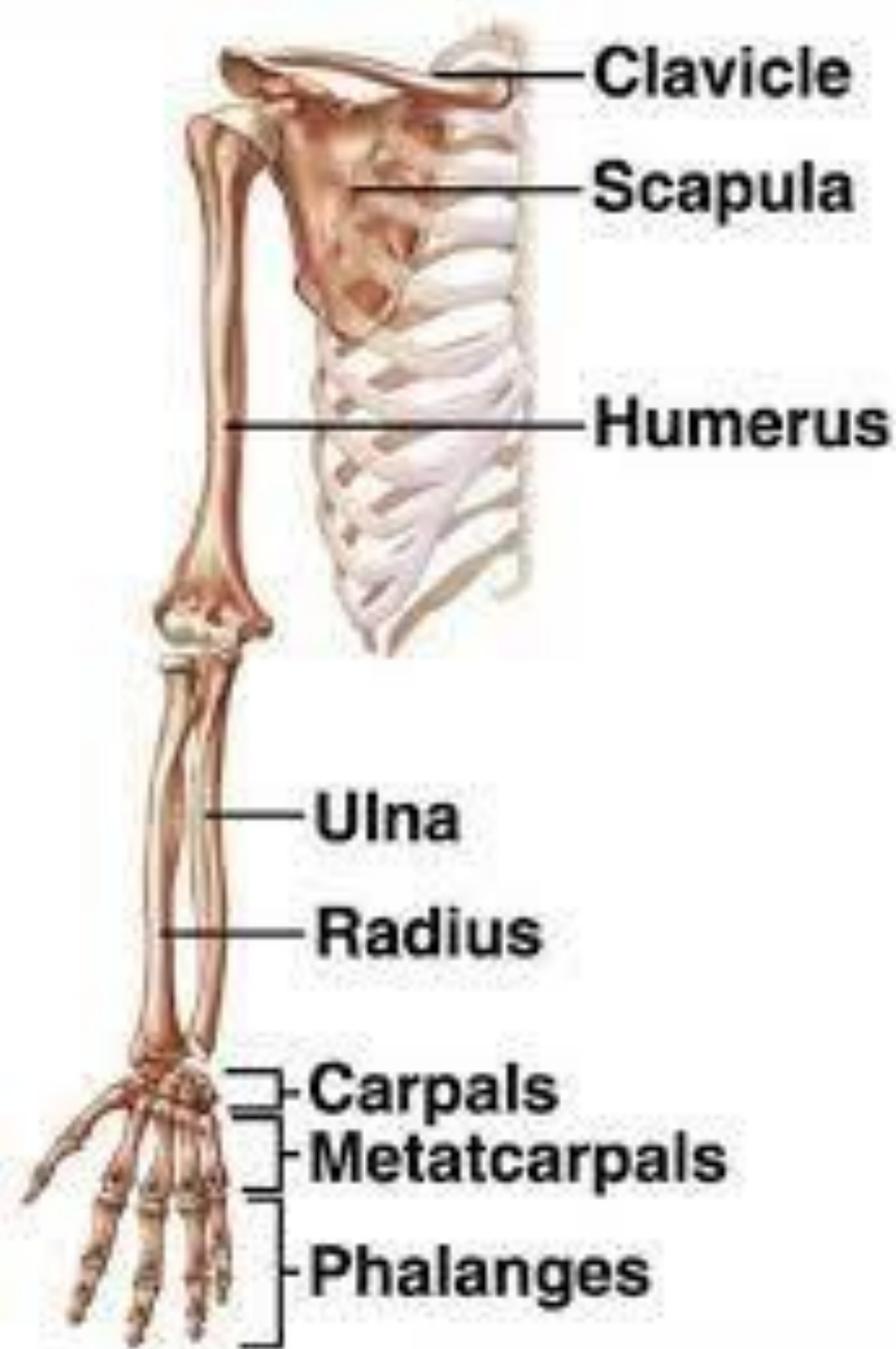
**Posterior (Back)
Spinal Column**



connects to the ulna and radius of the forearm. The radius is connected on the side away from the body (lateral side) when standing in the anatomical position. Together, the humerus and the ulna make up the elbow. The bottom of the humerus protects the ulna nerve and is commonly known as the "funny bone" because striking the elbow on a hard surface stimulates the ulna nerve and produces a tingling sensation.

The forearm:

The forearm is the region between the elbow and the wrist. The radius on the lateral side and ulna on the medial side forms it when the forearm is viewed in the anatomical position. The ulna is longer than the radius and connected more firmly to the humerus. The radius, however, contributes more to the movement of the wrist and hand than the ulna. When the hand is turned over so that palm is facing downwards, the radius crosses over the ulna. The top of each bone connects to the humerus of the arm and the bottom of each connects to the hand.

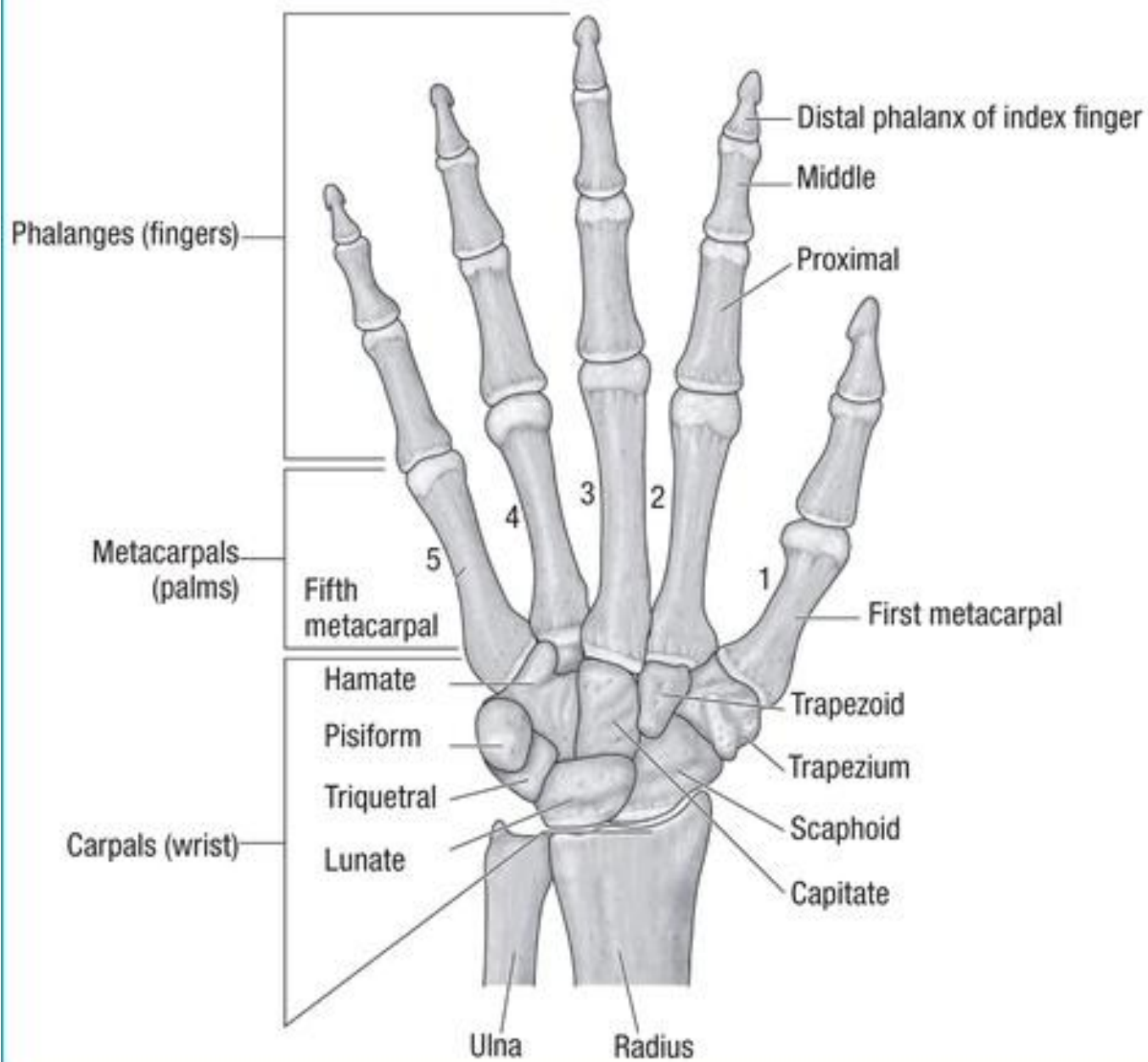


The Hand:

The hand consists of three parts (the wrist, palm and five fingers) and 27 bones.

The wrist, or carpus, consists of 8 small bones called carpal bones that are tightly bound by ligaments. These bones are arranged in two rows of four bones each. The top row (the row closest to the forearm) from the lateral (thumb) side to the medial side contains the Scaphoid, lunate, triquetrum & pisiform bones. The second row from lateral to medial contains the trapezium, trapezoid, capitate, and hamate. The Scaphoid and lunate connect to the bottom of the radius. The palm or metacarpus consists of five metacarpal bones. One aligned with each of the fingers. The metacarpal bones are not named but are numbered to V starting with the thumb. The bases of the metacarpal bones are connected to the wrist bones and the hands are connected to the bones of the fingers. The heads of the metacarpals form the knuckles of a clenched fist.

The fingers are made up of 4 bones called phalanges. A single finger bone is



called a phalanx. The phalanges are arranged in three rows. The first row (the closest to the metacarpals) is called the proximal row; the second row is the middle row. And the farthest row is called distal row. Each finger has a proximal phalanx, middle phalanx and a distal phalanx, except the thumb (also called the pollex) which does not have a middle phalanx. The digits are also number 1 to 5 starting from the thumb.

Shoulder Girdle:

The Shoulder girdle, also called the pectoral girdle, is composed of four bones: two clavicles and two Scapulae. The clavicles and two Scapulae. The clavicle, commonly called the collarbone, is a slender S-shaped bone that connects the upper arm to the trunk of the body and holds the shoulder joint away from the body to allow for greater freedom of movement. One end of the clavicle is connected to the sternum and end is connected to the scapula.

The scapula is a large, triangle, flat bone on the backside of the rib cage.

commonly called the shoulder blade. It overlays the second through seventh rib and serves as an attachment for several muscles. It has a shallow depression called the glenoid cavity that the head of the humerus (upper arm bone) fits into.

Usually, a "girdle" refers to something that encircles or is a complete ring. However, the shoulder girdle is an incomplete ring. In the front, the clavicles are separated by the sternum. In the back there is a gap between the two scapulae.

The primary function of the pectoral girdle is to provide an attachment point for the numerous muscles that allow the shoulder and elbow joints to move. It also provides the connection between the upper extremities (the arms) and the axial skeleton.

Lower Limb:

The lower extremity is composed of the bones of the thigh, leg, foot and the patella (commonly known as knee cap).

The Thigh:

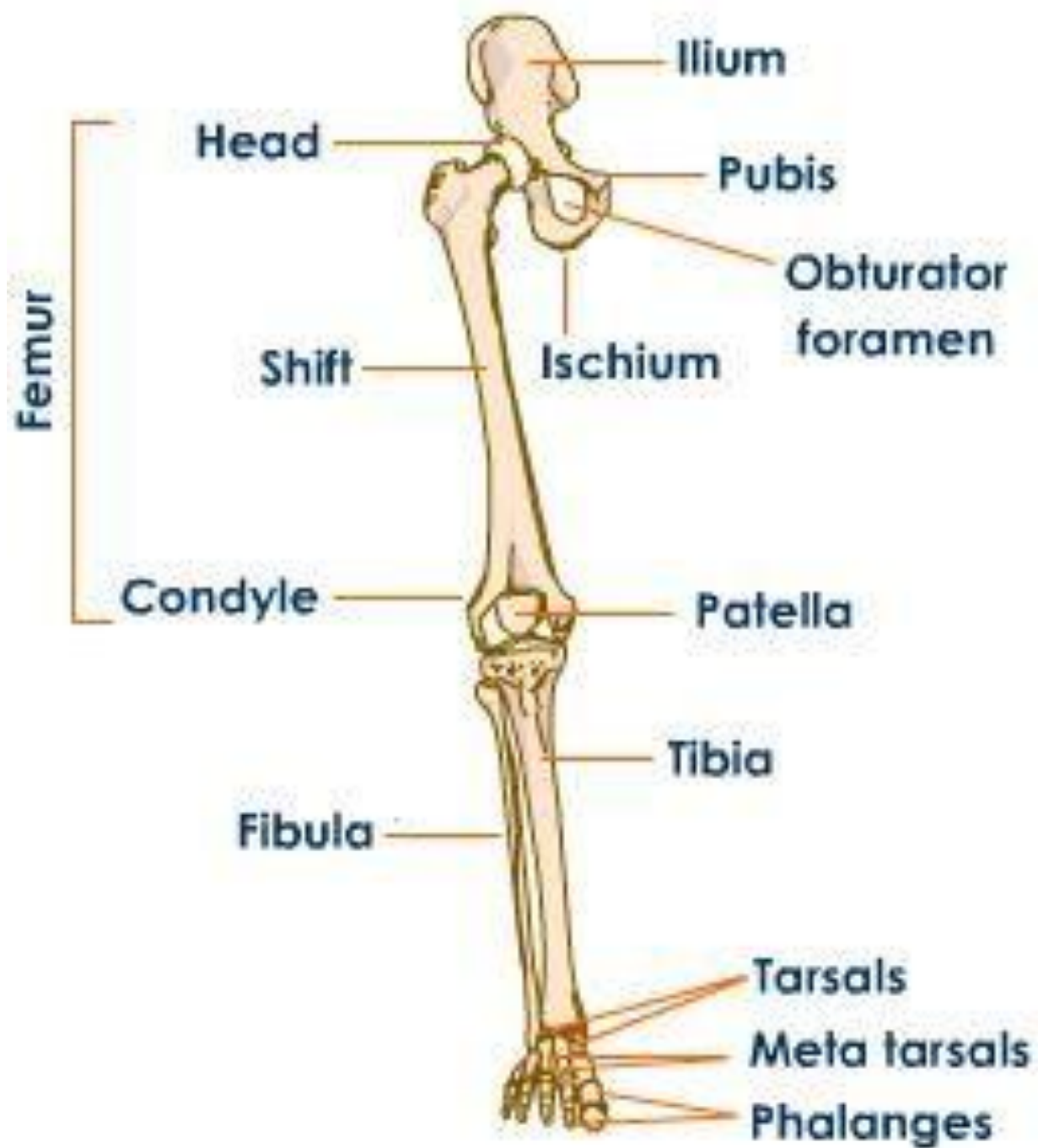
The thigh is the region between the hip and the knee and is composed of a single bone called the femur or thigh bone. The femur is the longest, largest, and strongest bone in the body.

The Leg:

The leg is technically only the region from the knee to the ankle. It is formed by the fibula on side away from the body (lateral side) and the tibia also called the shinbone, on the side nearest the body (medial side). The tibia connects to the femur to form the knee joint and with the talus, a foot bone, to allow the ankle to flex and extend. The tibia is larger than the fibula because it bears most of the weight, while the fibula serves as an area for muscle attachment.

The Foot:

The foot, or pes, contains the 26 bones of the ankle, instep and five toes. The ankle,



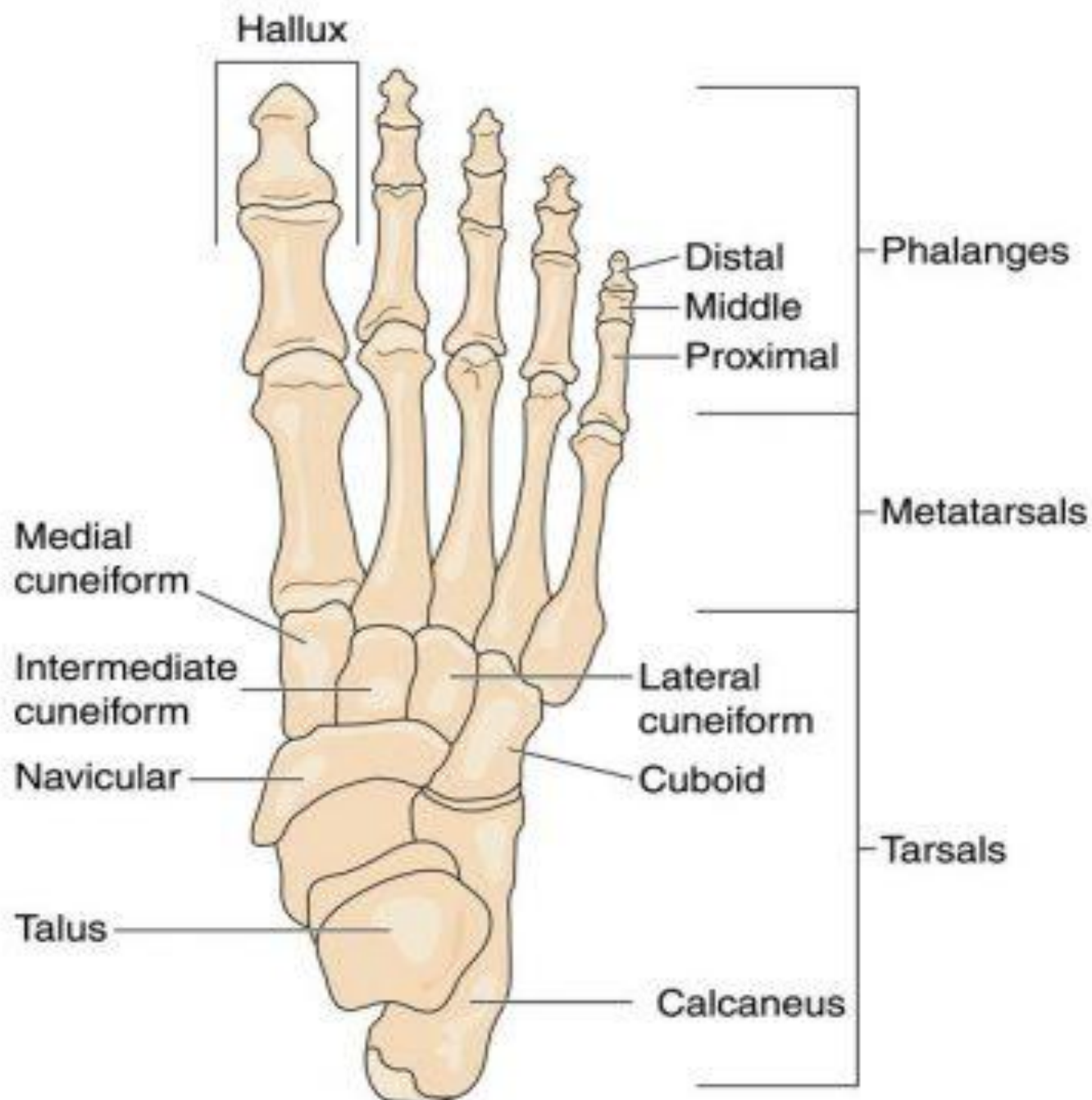
or tarsus, is composed of the seven tarsal bones. The remaining bones from medial to lateral are the medial, intermediate, the lateral cuneiform bones and the cuboid bones.

The metatarsal and phalanges bone of the foot are similar in number and position to the metacarpal and phalanges bone of the hand. The five metatarsal bones are numbered I to V starting on the medial side with the big toe.

The first metatarsal bone is longer than the others are because it plays a major role in supporting the body weight. The 14 phalanges of the foot, as with the hand, are arranged in a proximal row, a middle row, and a distal row with the big toe or hallux, having only a proximal and distal.

Pelvic girdle:

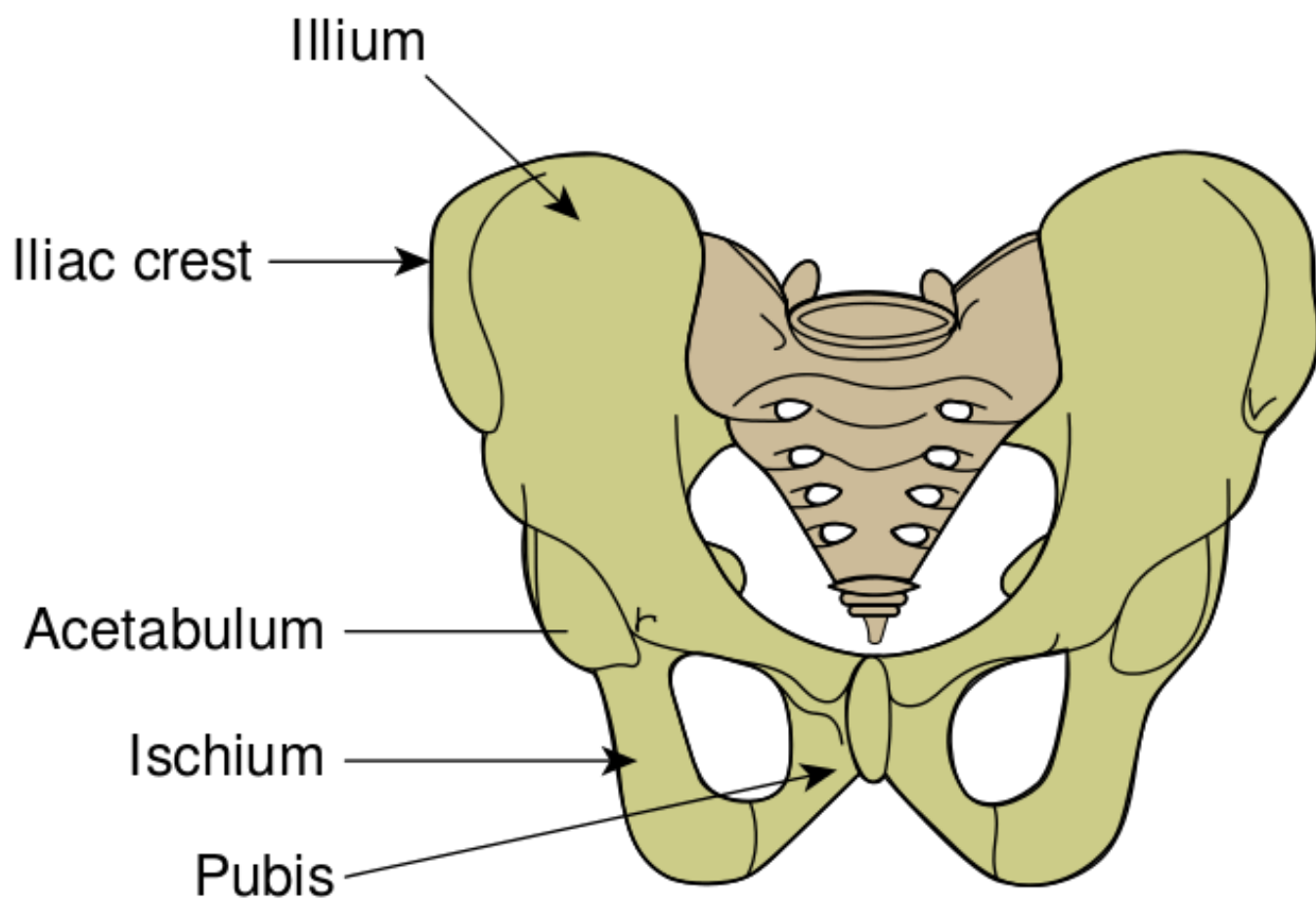
The pelvic girdle, also called the hip girdle, is composed of two coxal (hip) bones. The coxal bones are so called the *os coxae* or innominate bones. During childhood, each coxal bone consists of three separate parts: the



ilium, the ischium, and the pubis. In an adult, these three bones are firmly fused into a single bone. In the picture, the coxal bone on the left side has been divided into its component pieces while the right side has been preserved. In the back, these two bones meet on their side of the sacrum. In the front, they are connected by a muscle called the pubic symphysis.

The pelvic girdle serves several important functions in the body. It supports the weight of the body from the vertebral column. It also protects and supports the lower organs, including the urinary bladder, the reproductive organs and the developing fetus in pregnant women.

The pelvic girdle differs between man and women. In a man the pelvis is more massive and the iliac crests are closer together. In a woman, the pelvis is more delicate and ilium crests are farther apart. These differences reflect the woman's role in pregnancy and delivery of child. When a child is born, it must pass through mother pelvis, if the opening is too small. A cesarean section may be necessary.



Viva VOCE

Q) What bones are the parts of the upper limb?

The bones of the upper limb

- 1) clavicle
- 2) Scapula
- 3) Humerus
- 4) Radius
- 5) ulna
- 6) carpus
- 7) Metacarpus
- 8) Phalanges.

Q) what are the four parts of the upper limb?

The upper limb consists to four major parts: a girdle formed by the clavicles and Scapulae, the arm, the forearm, and the hand. Although very mobile, the limb is supported and stabilized by muscles connected to the ribs and vertebrae.

Q) what are the bones of lower limb?

There are bones found in the lower limb:

- 1) Hip bone
- 2) Femur
- 3) Patella
- 4) Tibia
- 5) Fibula
- 6) Tarsal
- 7) Metatarsals
- 8) Proximal phalanges
- 9) Intermediate phalanges
- 10) Distal Phalanges.

Practical-2

Histological Examination of Slides

Study of Epithelium

Epithelial cells cover or line all body surfaces, cavities and tubes. So, these are called covering epithelia.

Epithelial cells form the functional units of secretory glands. So, these are called glandular epithelia.

1) Simple Squamous epithelium:

Simple squamous epithelium cells are flat in shape. Arranged in a single layer. This single layer is thin enough to form a membrane that compounds can move through via passive diffusion. This epithelial type is found in the walls of capillaries, linings of the pericardium, and the linings of the alveoli of the lungs.

2) Simple cuboidal epithelium:

Simple cuboidal epithelium consists of a single layer cells that are as tall as they



Simple squamous epithelium



Simple cuboidal epithelium

are wide. The important functions of the simple cuboidal epithelium are secretion and absorption. This epithelium type is found in the small collecting ducts of the kidneys, pancreas, and salivary glands.

3) Simple columnar epithelium:

Simple columnar epithelium is a single row of tall, closely packed cells, aligned in a row. Found in areas with high secretory function (such as the wall of the stomach), or absorptive areas (as in small intestine). They possess cellular extensions (e.g., microvilli in the small intestine, or the cilia found almost exclusively in the female reproductive tract).

4) Pseudo Stratified:

These are simple columnar epithelial cells whose nuclei appear at different heights, giving the misleading (hence pseudo) impression that the epithelium is stratified when the cells are viewed in cross section. Pseudo stratified epithelium can also possess fine hair-like extensions of their apical (luminal) membrane.



Columnar Epithelium



Pseudostratified columnar epithelium

called cilia. Ciliated epithelium is found in the airways (nose, bronchi), but is also found in the uterus and fallopian tubes of females, where the cilia propel the ovum to the uterus.

5) Stratified Epithelium:

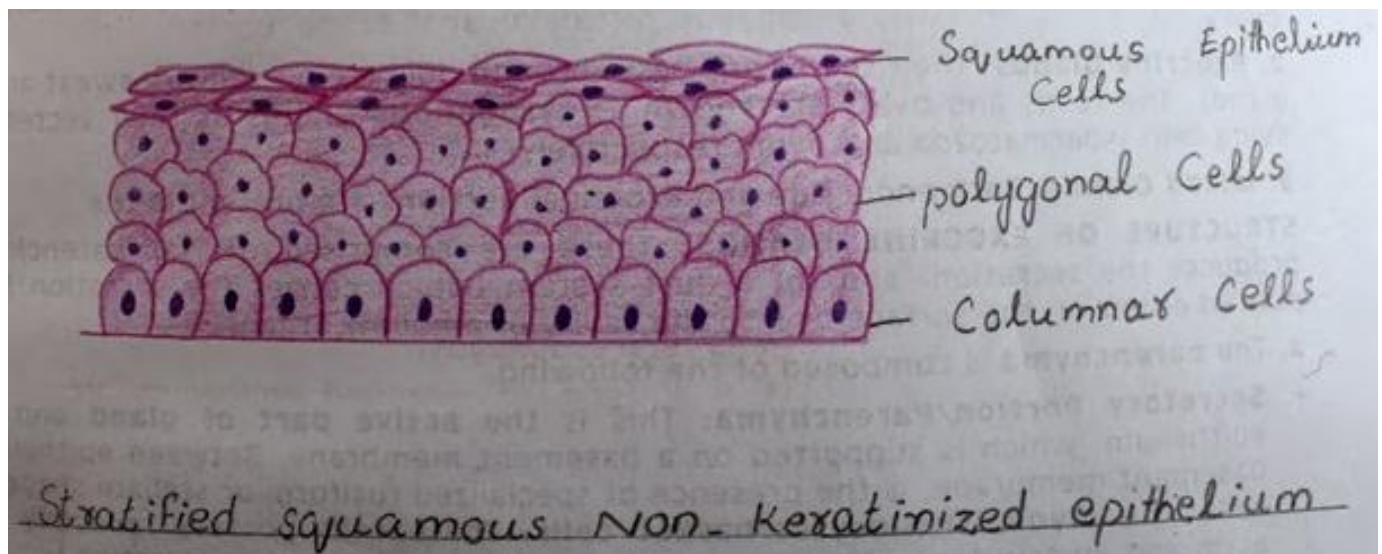
Stratified Epithelium differs from simple epithelium by being multilayered. It is therefore found where body linings have to withstand mechanical or chemical insults. Stratified epithelia are more durable and protection is one of their major functions. Since stratified epithelium consists of two or more layers, the basal cells divide and push towards the apex, and in the process flatten the apical cells. Stratified epithelia can be columnar, cuboidal, or squamous type.

6) Keratinized Epithelia:

In keratinized epithelia, the most apical layers (exterior) of cells are dead and lose their nucleus and cytoplasm. They contain a tough, resistant protein called keratin. This specialization



Stratified Cuboidal Epithelium



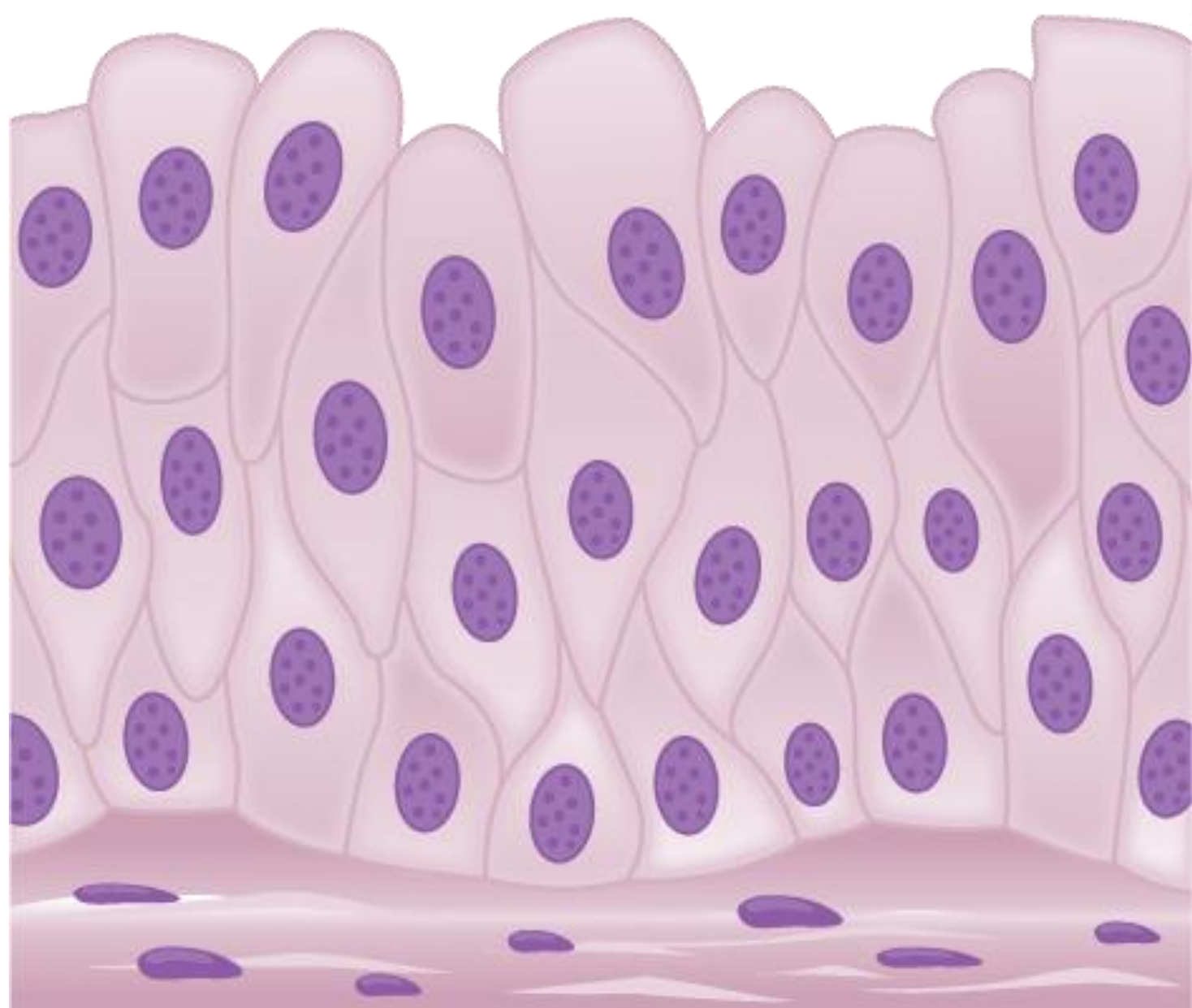
makes the epithelium waterproof, and it is abundant in mammalian skin. The lining of the esophagus is an example of a non-keratinized or moist stratified epithelium.

7) Transitional Epithelia:

Transitional Epithelia are found in tissues that stretch and it can appear to be stratified cuboidal when the tissue is not stretched, or stratified squamous when the organ is distended and the tissue stretches. It is sometimes called the urothelium since it is almost exclusively found in the bladder, ureters, and urethra.

Study of connective tissues

The tissues that connect the different parts of the body together are called connective tissues. The connective tissue is characterized by the presence of relatively few cells but a large amount of inter cellular substance (compare this with epithelium which consists of a large number of cells with very small amount of inter cellular substance).



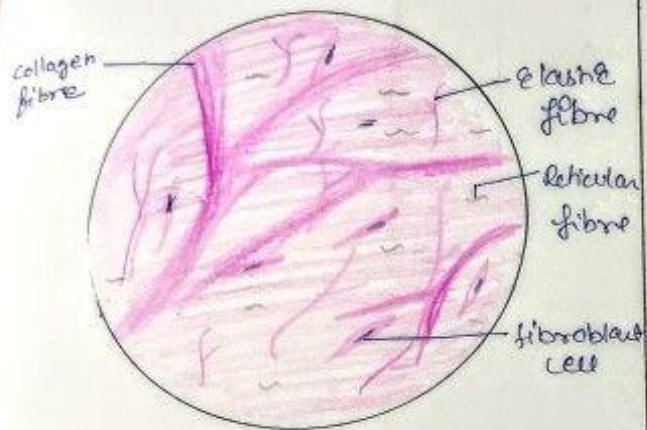
Study of Muscles tissue

Muscle is one of our 4 tissue types and muscle tissue combined with nerves, blood vessels, and various connective tissues.

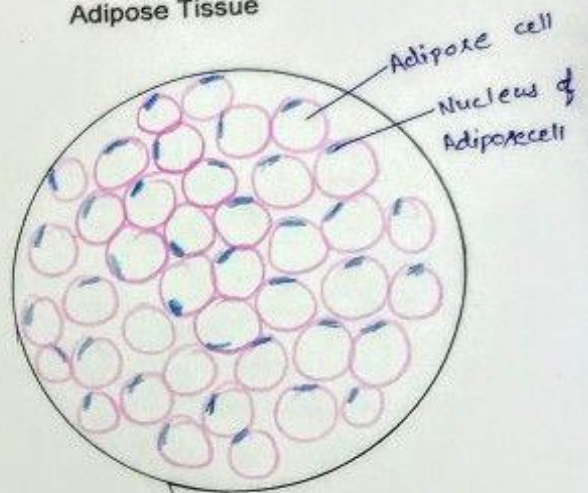
Muscle Tissue is a specialized tissue found in animals which functions by contracting, thereby applying forces to different parts of the body.

Muscle tissue consists of fibers of muscle cells connected together in sheets and fibers.

Loose Areolar Tissue

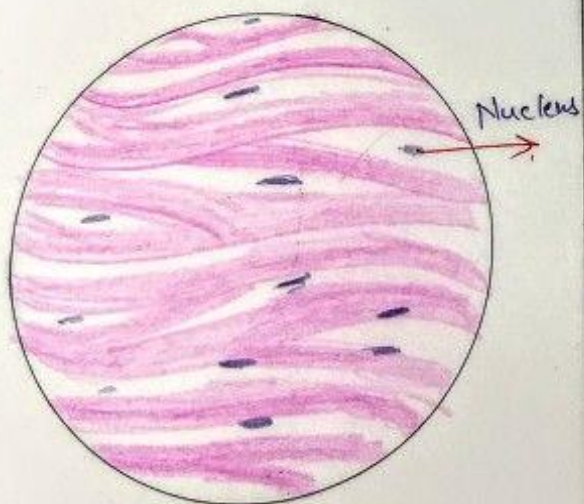


Adipose Tissue



Reticular Tissue

Dense regular connective tissue



Mucoid Tissue



✓

TH

Practical-3

Determination of concentration of Hemoglobin in blood.

(Sahil's Apparatus)

Haemoglobin is a protein pigment in blood that carries oxygen from lungs to the body tissues and returns carbon dioxide back from tissues to lungs. After the normal lifespan is over the RBCs are destroyed by macrophages in liver, spleen and red bone marrow into haem moiety and globin protein.

The amount of Hemoglobin in the blood is measured by destroying the red cell membrane (hemolysis) so that the pigment is released into the plasma and can be estimated colorimetrically. Determination of Hemoglobin in blood is an important clinical measurement which helps in the diagnosis of anemia.

Apparatus:

- Sahil's hemoglobinometer
- Diluting tube Stirrer
- Hb pipette
- 0.1 N HCl Solution
- Dropper

Observation & Calculation

NORMAL RANGES

Males	14-18g/100ml
Females	12-16g/100ml
Fetus/Newborn	23g/100ml
Pregnancy	10-14g/100ml

The average value of Hb:-

A 4/4/19

Procedure:

- 1) Take a hemoglobin tube and add HCl into it upto the mark 20 of red graduation.
- 2) Clean the middle finger with Spirit Swab to avoid the risk of any infection.
- 3) Take a lancet and prick the finger. Discard the first drop of blood.
- 4) Allow the hemoglobin pipette to fill with capillary action by simple touching the blood drop upto the mark 20.
- 5) Expel the blood into the Sahil's tube already containing HCl Solution
- 6) Mix the content gently with the stirrer and allow it to stand
- 7) wait for 10 minutes at least.
- 8) Now add water drop by drop, stirres well.
- 9) Match the colour with the colour of the Standard tube.

Precaution:

- Prick the finger carefully
- In diluting tube put the blood sample carefully to avoid clotting.
- There should no air bubble present in the pipette.

- The matching of color should be seen in good light.

Viva Voce

Q1) What are the normal values of hemoglobin?

Range in males: 14-18 gm/100ml

Range in females: 12-16 gm/100ml

Q2) What are physiological variations of hemoglobin quantity?

At high altitude, Hb increases due to hypoxia

After exercise Hb increases

During pregnancy Hb decreases due to hemodilution.

Q3) What are the pathological conditions of Hb variation?

- In anemia, Hb decreases
- In hemorrhage, Hb decreases
- In chronic renal failure, Hb decreases
- In dehydration due to vomiting, diarrhea or sweating, Hb increases.

Practical-4

Determination of RBC count

The red blood cells, one of the formed elements of the blood, transport oxygen and carbon dioxide to and from the body tissues. The number of cells in a small volume of accurately diluted blood is counted in a glass counting chamber (hemocytometer).

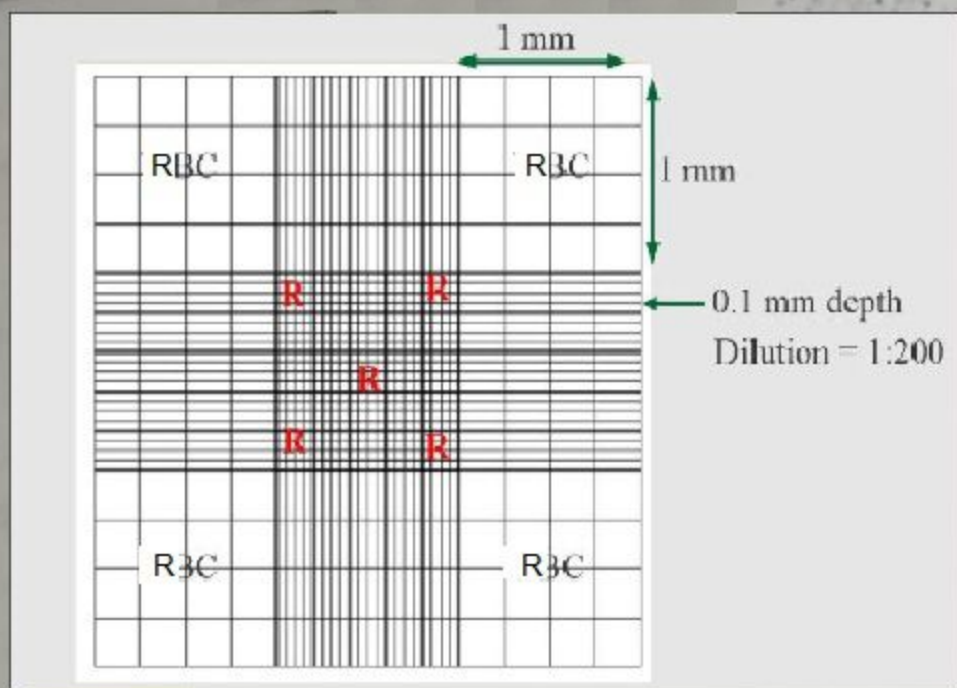
Anemia often results from an abnormal decrease in the number of erythrocytes, so that insufficient oxygen is carried to the tissue and they become oxygen starved.

For accurate diagnosis of the cause of anemia, the complete status of the RBCs must be examined i.e. hematocrit, hemoglobin, RBCs count, cell size, hemoglobin in each cell and other factors.

Apparatus:

- Blood Sample
- Lancet R.B.C pipette
- Cover Slip
- Neubauer's chamber
- Microscope
- Spirit swab
- Hayem's fluid

DETERMINATION OF RBCs COUNT



Number of RBCs in 80 small squares = 550

Number of RBCs in one small square = $550/80 = 6.8$

Volume of 1 small square = $1/4000 \text{ mm}^3$

Number of RBCs in $1/4000 \text{ mm}^3 = 550/80 = 6.8$

Number of RBCs in $1 \text{ mm}^3 = 550/80 \times 4000 = 27500$

Dilution = 200

Number of RBCs in diluted sample = $550/80 \times 4000 \times 200$
 $= 5500000$

Number of RBCs in diluted sample = 5.5 million/mm³

Result:

The total number of R.B.Cs in given sample is 5.5 million/mm³.

20

Composition of Hayem's Fluid:

- Sodium chloride 0.5g (prevent hemolysis)
- Sodium Sulphate 2.5g (prevent clumping)
- Mercuric chloride 0.25g (Disinfectant)
- Distilled water 100ml.

Procedure:

- 1) cleans the hemocytometer with distilled water and dries it.
- 2) Focus the Neubauer's chamber under the microscope first at low power and then at high power to focus the smallest square of 5 small squares.
- 3) Placed the coverslip upon the chamber and refocus it with the help of fine adjustment knob.
- 4) Clean your finger with spirit swab and allow drying.
- 5) Prick it with the lancet and discard the first drop of blood.
- 6) Suck the blood with R.B.C pipette upto mark 0.5. Care should be taken that no air bubble is formed in blood. Wipe off excess blood on the outer surface of R.B.C pipette.

Now suck the Hayem's fluid upto mark 101.
Secure the two end of pipette between the
finger and thumb. Rotate it for some time
for mixing blood with Hayem's fluid.
Discard first few drops of dilute fluid then
charge to Neubauer's chamber by putting two
drops of diluted blood on the junction of
cover slip present on the hemocytometer.
Wait to settle the R.B.Cs in four corners and
one small central square chamber, count them
inside smallest division.

Precautions:

- Select the correct position of Neubauer's chambers.
- Filling of the pipette with blood and then with diluting fluid should not have bubbles of air.
- No air bubble should be present in chamber area.
- For a good distribution, the mixing and charging of the chamber should be very careful and thorough.
- Dilution should be correct.
- Pipette should be clean and dry.
- While counting the RBCs use proper illumination,

- magnification and follow the thomas rule.
- There should be no overflowing of fluid in the H groove of chamber.

Viva Voce

Q1) What are the normal values of R.B.Cs?

- The normal RBC range for men is 4.7 to 6.1 million cells per microliter (mcl).
- The normal RBC range for women who aren't pregnant is 4.2 to 5.4 million mcl.
- The normal RBC range for children is 4.0 to 5.5 million mcl.

These ranges may vary depending on the laboratory or doctor.

Q2) why R.B.C count is more in males?

RBC count is more in male because of the normal sex hormone testosterone which cause increase metabolic rate.

Q3) What is the major Functions of R.B.Cs?

Carriage of oxygen.

Practical-5

Determination of leucocyte count (TLC).

The white blood cells (WBCs) or leucocytes are one of the three specialized classes of the blood cells. White blood cells combat inflammatory process and invading organism. WBCs must be present in a sufficient number to carry out these functions properly but not in an abnormal excess. Thus, counting of total number of WBCs is an important clinical measurement which helps to establish the capacity of blood for performing their defensive function. In this technique, the pipette used contains white blood cells and dilute the cells 20 times.

Apparatus:

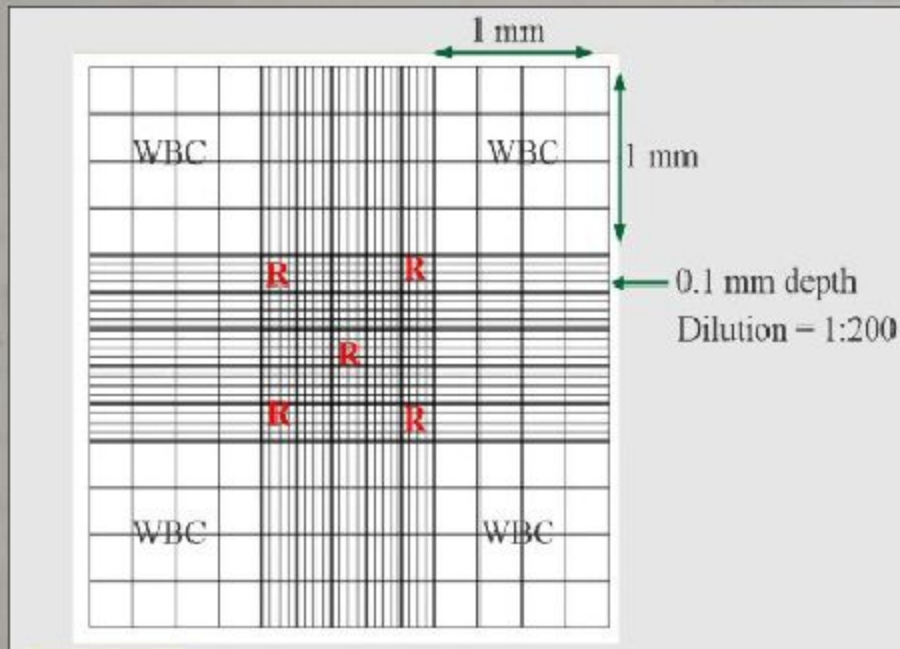
- Hematocytometer
- W.B.C Pipette
- Lancet
- Spirit swab
- coverslip
- Microscope.

Chemicals:

Turk's Solution.

DETERMINATION OF LEUKOCYTES COUNT

OBSERVATION AND CALCULATION



- Number of WBCs in square $W_1 = 35$
- Number of WBCs in square $W_2 = 40$
- Number of WBCs in square $W_3 = 44$
- Number of WBCs in square $W_4 = 39$
- Total number of WBCs = $x = 158$

Now

- Volume of 1 small square = $1/160 \text{ mm}^3$
- Volume of 64 small squares = $64/160 \text{ mm}^3$
- $1/160 \text{ mm}^3$ contain WBCs: Total number of WBCs / 64
- " " " = $158/64 = 2.5$
- 1 mm^3 contain WBCs = Total WBCs / 64 $\times 160 \text{ mm}^3$
- " " " = $158/64 \times 160 \text{ mm}^3 = 395 \text{ mm}^3$
- Dilution = 20

No. of diluted sample = $395 \times 20 = 7900 \text{ mm}^3$

Result:

The blood sample contain 7900 mm^3 WBCs

Composition of Turk's Solution:

- Glacial acetic acid _____ 1.5ml
- Gention violet _____ 5 drops
- Distilled water _____ 98.5ml

Procedure:

- 1) Clean the finger with spirit swab and dry it to avoid the risk of infection.
- 2) Prick the finger with lancet to obtain the blood.
- 3) Discard the first drop of blood.
- 4) Clean and dry the W.B.C's pipette. Insert the tip of pipette, keeping it in horizontal position into the drop of blood.
- 5) Suck gently to draw blood up to mark 0.5 on pipette.
- 6) Now placed the pipette in the Turk's Solution, add up to the mark 11 exactly.
- 7) After dilution blow out 3-4 drops to remove the diluting fluid from the stem of the pipette.
- 8) Place the cover slip over the counting chamber of hemocytometer.

9) Count the number of W.B.Cs in the four corner square.

Precautions:

- 1) Select the corner position of Neubauer's chambers.
- 2) Filling of the pipette with blood and then with diluting fluid should not have bubbles of air.
- 3) An appropriate WBCs pipette (with white bead) must be selected.
- 4) Care should be taken to suck blood exactly up to the mark 0.5.
- 5) The hemocytometer must be cleaned only with distilled water.
- 6) The WBCs pipette must be cleaned before and immediately after using it with proper solutions.
- 7) While counting the RBCs use proper illumination, magnification and follow the Thomas rule.

Viva Voce

Q1) What are the normal range of W.B.Cs?

The normal number of W.B.Cs in the blood is 4,500 to 11,000 WBC per microliter blood.

Q2) Is Low white blood count serious?

Underlying causes for a low white blood cell count can range from benign disorders, such as vitamin deficiencies, to more serious blood diseases, such as leukemia or lymphoma. A truly low white blood cell count also puts you at higher risk for infections - typically bacterial infections.

Q3) What does a high number of white blood cells indicate?

A high white blood cell count usually indicates: An increased production of white blood cells to fight an infection.

A disease of bone marrow, causing abnormally high production of white blood cells. An immune

System disorder that increases white blood cell production.

Q4) Can stress and anxiety cause high white blood cell count?

Severe physical or emotional stress can also cause an increased WBC count, including stress caused by overexertion and anxiety ... Smoking can also affect the increasing the white blood cell count.

Practical-6

Determination of Bleeding time by Duke's method

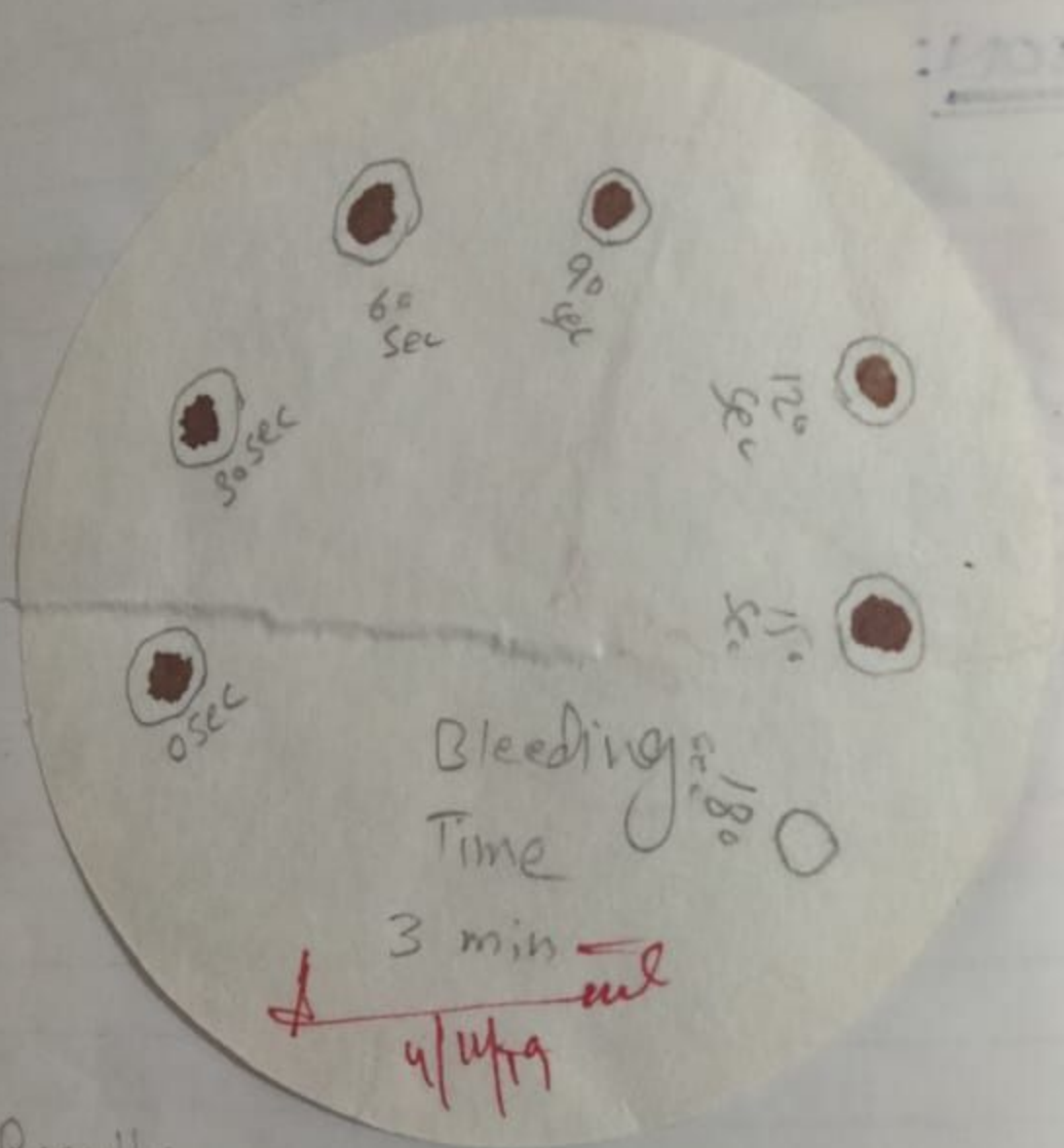
The Bleeding time is taken as the time for a small sharp incision to stop Bleeding. This test help in the diagnosis of various hemorrhagic disorders of vascular or platelets origin.

Apparatus:

- Lancet
- Filter paper
- Spirit Swab
- Stop watch

Procedure:

- 1) Clean the puncture site with an antiseptic to minimize the risk of infection.
- 2) Place a pressure cuff around your upper arm and inflate it.
- 3) Make small cut on ear lobe with lancet.
- 4) using a stopwatch or timer, blot the cut with filter paper, take it as zero second as reference.
- 5) After every 30 seconds blot the filter



⇒ Result:

The bleeding time is 3 minutes

11/4/19 ent

paper until the bleeding stops. Record the time and then bandage the cut.

Normal bleeding time is 3-5 minutes

usually, if the cuts continue to bleed after 20 minutes, the health care provider notes that the bleeding time was over 20 minutes.

Precautions:

- 1) Pricking should be done carefully under aseptic conditions to avoid the risk of infections.
- 2) Do not touch finger tip or ear lobe when wiping the blood away.

Viva Voce

Q1) Define bleeding time?

Bleeding time is a time taken for the stoppage of blood from punctured skin wound as a result of function hemostatic blood.

Q2) What are the factors to control the bleeding?

Numbers of platelets
Functions of platelets
Condition of blood vessels

Q3) What are the conditions in which bleeding time is increased?

Decrease in platelet count
capillary fragility

Q4) What is normal bleeding time?

Bleeding normally stops within 1-9 minutes but may be longer in children (1-13 minutes) and tends to take slightly longer in females than in males.

Practical-7

Determination of clotting Time by capillary tube method.

The coagulation time is the required for a sample of blood removed from the blood vessel to clot.

Apparatus:

- Lancet
- Spirit swab
- Capillary tube

Procedure:

- 1) Clean the fingertip with spirit swab and allow it to dry.
- 2) Puncture it with lancet and discard the first drop of blood.
- 3) Hold the capillary tube horizontally and dip its one end into oozing blood.
- 4) Allow the tube to fill by capillary action.
- 5) After every 30 seconds break the small portion of capillary tube until blood is converted into semi solid jelly like mass.

OBSERVATION & CALCULATION

- Time of each breaking tubes = 30 sec
- Number of breaking = 6
- Clotting time of blood = 6×30
- Clotting time = 180 sec

$$= \frac{180}{60}$$

$$= 3 \text{ minutes}$$

• **RESULT:**

Clotting time is 3..

Precautions:

- 1) Pricking should be done carefully under aseptic conditions to avoid the risk of infection.
- 2) Avoid air bubble in capillary tube.
- 3) keep the other end of capillary tube open during filling it with oozing blood.
- 4) The method should be repeated for 3 capillary tubes to record accurate timing.

Viva Voce

Q1) what are the methods for determining the coagulation time?

- a) capillary tube method
- b) Drop method
- c) Lee and white test tube method

Q2) Enumerate the condition in which clotting time is increased?

Decrease in platelets count
Factor V and VIII deficiency.

Q3) Define clotting or coagulation time?

clotting time is the time required for a sample of blood to coagulate in vitro under standard condition.

Q4) what is the normal clotting time?

Normal value of clotting time is 8 to 15 minutes.

Practical - 8

Determination of radial Pulse

Apparatus:

Stop watch
calculator

Procedure:

Step 1: Prepare volunteer

Straighten the volunteer's elbow and face the inside of his or her wrist upward.

Step 2: Position Fingers

Position the index and middle fingers of your dominant hand so the tips of the two fingers align.

Step 3: locate the hollow space

Ask the volunteer to form a fist and bend the wrist. In this position, you should see a ligament elevated in skin place your fingers next to this ligament on the same side as the

OBSERVATION AND CALCULATIONS

Number of Beats count in 30 sec : 38

Number of Beats convert in minute : 38×2

" " " = 76

Number of Beats per minute = 76 BPM

RESULTS:-

Radial Pulse : 76 BPM

(Ideal value : 72 BPM)

~~A~~ 2/2/19. ~~End~~

volunteer's thumb. Here, you should feel a hollow, soft space.

If your fingers are on the hard surface of the wrist bones, move them down and along the ligament until they reach a softer area.

Step 4: locate the radial artery

keeping your fingers in place, ask the volunteer to relax the hand, press your fingers into the hollow space to feel the radial artery beneath the skin.

Step 5: Count Radial Pulse

Start the time and count the beats of the pulse.

If the pulse is regular and strong, measure the pulse for 30 seconds.

Step 6: Convert to beats per minute

Using a calculator, multiply the number you counted by 2 to convert it to bpm.

Double the number to give to beats per minute (e.g.: 32 beats in 30 seconds means the pulse is 64 beats per minute).

Precautions:

- 1) In a quiet room, make sure the volunteer is sitting down and relaxed. If not, ask the volunteer to sit down and wait a couple of minutes to ensure his or her heart rate is at its resting rate.
- 2) If you noticed changes in rhythm or strength, you must measure the pulse for a full minute.

viva voce

Q1) what is a radial pulse?

The pulse of the radial artery (felt at the wrist) type of: heart rate, pulse, pulse rate. The rate at which the heart beats; usually measured to obtain a quick evaluation of a person's health.

Q2) why is the radial pulse most commonly used?

It is easier to maintain contact with a person's wrist for an extended period than it is to keep your hand on their neck.

Q3) what is the easiest way to find a radial pulse?

- a) Gently place 2 fingers of your other hand on this artery.
- b) Do not use your thumb because it has its own pulse that you may feel.
- c) Count the beats for 30 seconds; then double the result to get the number of beats per minutes.

Practical-9

Determination of blood pressure

"Blood pressure (B.P) is defined as the blood pressure exerted by the flowing blood against the walls of arteries"

Arterial blood pressure has two important components;

- 1) Systolic blood pressure
- 2) Diastolic blood pressure

Systolic blood pressure:

The highest pressure exerted by the blood on the walls of arteries produced by the ventricular contraction.

It ranges between 100-140 mmHg in normal healthy adult.

Diastolic blood pressure:

The lowest blood pressure exerted by the blood on the walls of arteries during ventricular relaxation.

Its normal value 80 mmHg.

Observation & Calculation

⇒ Systolic reading : 110 mmHg

⇒ Diastolic reading : 70 mmHg

Result:

The blood pressure of a subject: 110/70
mmHg

25/11/19

Apparatus:

- Stethoscope
- An appropriately sized blood pressure cuff
- A blood pressure measurement instrument such as an aneroid or mercury column sphygmomanometer or an automated device with a manual inflate mode.

Procedure:

- Make sure the patient is relaxed by allowing 5 minutes to relax before the first reading.
- Locate the brachial artery and position the BP cuff so that the artery marker points to the brachial artery.
- Wrap the BP cuff snugly around the arm.
- On the same arm where placed the BP cuff, palpate the arm at the antecubital fossa (crease of the arm) to locate the strongest pulse sounds and place the bell of the stethoscope over the brachial artery.
- Begin pumping the cuff bulb to listen the pulse sounds. When the BP cuff has inflated enough to stop blood flow, no sounds hear through the stethoscope. The gauge should read

30 to 40 mmHg above the person's normal BP reading. If this value is unknown you can inflate the cuff to 160-180 mmHg. (If pulse sounds are heard right away, inflate to a higher pressure).

- Deflate the cuff slowly, 2-3 mmHg in second.
- Listen for the systolic reading. The first occurrence of rhythmic sounds heard as blood begins to flow through the artery is the patient's systolic pressure.
- Listen for the diastolic reading, continue to listen as the BP cuff pressure drops and the sounds fade.
- Note the gauge reading when the rhythmic sounds stop. This will be the diastolic reading.

Precautions:

- 1)** The subject should sit upright with their upper arm positioned so it is level with their heart and feet flat on the floor.
- 2)** Remove excess clothing that might interfere with the BP cuff or constrict blood flow in the arm.

Viva voce

Q1) Define blood pressure?

Blood pressure is the pressure of circulating blood on the walls of blood vessels.

Q2) what is the range of normal blood pressure?

Normal resting blood pressure in an adult is approximately 120 millimeters of mercury (16 kPa) Systolic, and 80 millimeters of mercury (11 kPa) diastolic, abbreviated "120/80 mmHg".

Practical- 10

Determination of erythrocyte Sedimentation rate (ESR) By westergren's method

Erythrocyte Sedimentation Rate (ESR) is a rate at which Erythrocytes settle down.

Normal the Erythrocytes remain suspended uniformly in circulation. This is called Suspension stability of RBCs

Determination of ESR:

There are 2 methods to determine ESR.

- 1) westergren method
- 2) wintrobe method

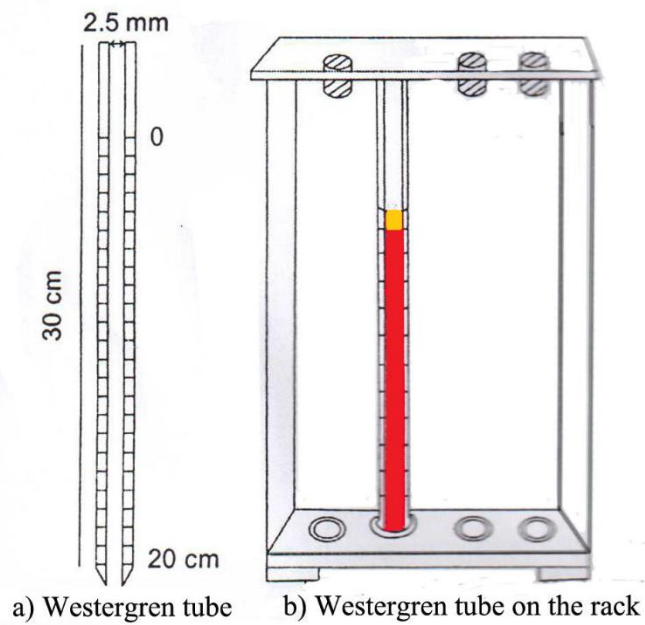
Apparatus:

- westergren's tube
- westergren's stand
- Disposable syringe
- Spirit swab

chemicals:

3.8 % Sodium citrate solution

OBSERVATIONS AND CALCULATIONS



Normal values of ESR:

In males 3-7 mm/hour

In female 5-9 mm/hour

In infant 0-2 mm/hour

Result:

Values of ESR: ----- mm Hg

Procedure:

- 1) Clean the antecubital area with spirit swab and allow it to dry. puncture the skin and vein and draw blood directly into syringe.
- 2) 1.6ml of venous blood is directly added to 0.4ml 3.8% Sodium citrate solution and mixed it by Gentle rotation.
- 3) Take the westerger's tube and fill it with blood by Sucking and placing the tip of index finger gently over the one end up to the mark '0'.
- 4) Fix the tube in westerger's stand and allow it to stand for 1 hour. At the end of 1 hour take the reading in mm of clear plasma column above the red cells.

Signification of Determined ESR.

- 1) ESR helps in diagnosis as well as prognosis. ESR is increased in inflammation, anemia, rheumatic fever, Some kidney disease and some cancer.
- 2) The ESR decrease in sickle cell anemia, leukamia and congestive heart failure.

Precautions:

- ESR should be performed within 2 hours after collecting blood.
- Dilution of blood should be accurate.
- Test should be performed at room temperature. Higher temperature result in increase in ESR.
- No air bubble should be present in column of blood.

Viva voce

Q1) what is ESR?

It is the rate at which R.B.Cs are settle down as a result of rouleaux formation and a clear column of plasma obtained in mmHg.

Q2) what are the normal values of ESR?

In males : 3-5 mmHg

In females : 4-7 mmHg

Q3) why we use sodium citrate solution?

Because sodium citrate solution act as anticoagulant.

Q4) what does it mean if your ESR is high?

Moderately elevated ESR occurs with inflammation but also with anemia, infection, pregnancy, and with aging. A very high ESR usually has an obvious cause, such as a severe infection,

marked by an increase in globulins, polymyalgia rheumatica or temporal arteritis.

Q5) what is the cause of low ESR?

The ESR decreased in polycythemia, hyperviscosity, Sick cell anemia, leukemia, low plasma protein (due to liver or kidney disease) and congestive heart failure.

Practical- 11

Determination of blood Group system

There are various methods for the determining of blood group but OAB system and Rh system is the most important one that assures safe blood transfusion.

Apparatus:

- Glass Slides
- Lancets
- Tooth pick

Chemicals:

Anti A anti Serum
Anti B anti Serum
Anti D anti Serum

Procedure:

- 1) Take a clean glass slide and divide the slide into two halves mark as A and the other side B.
- 2) Take another clean glass slide mark as D.
- 3) Pricks the finger tip using a lancet under

OBSERVATIONS AND CALCULATIONS

Agglutination at A =

Agglutination at B =

Agglutination at D =

Anti A serum	Anti B serum	Blood group	
+	-	A	+ Rh factor
-	+	B	
+	+	AB	- Rh factor
-	-	O	

RESULT:

The blood group is_____

the aseptic conditions.

4) Place a drop of blood on side A and one drop on the other side which is marked as B. also place a drop of blood on the slide mark as D.

5) Add one drop of Anti A anti Serum to the slide marked as A.

Add one drop of Anti B anti Serum to the slide marked as B.

Add one drop of Anti D anti Serum to the slide marked as D.

6) Mix the anti serum and blood with each other by using tooth pick. wait for 6-8 minutes.

7) Observe the slide:

if agglutination occur only on slide A, then the blood group is A.

if agglutination occurs only on slide B, then the blood group is B.

if agglutination occurs on both slides, then the blood group is O.

Now observe the other slide marked as D either agglutination occur or not, if agglutination occur, it is positive and if agglutination does not occurs means it is Rh negative.

Interpretation :

- 1) If agglutination occur with Anti-Serum A contains A antibody.
The agglutination occurs if it RBCs contain A antigen so the blood group is A.
- 2) If the agglutination occurs anti-Serum-B the blood group is B.
If the agglutination occurs with both anti-Serum A and B, the blood group is AB.
- 3) If the agglutination does not occur with both anti-Serum A and B the blood group is O.
- 4) For the determination of Rh factor (if the agglutination occurs with anti-Serum D, the blood group is +ve and if it the agglutination does not occur with anti-Serum D the blood group is -ve). Agglutination.

Precautions:

- 1) Make sure the anti-Sere are not expired.
- 2) The slides should be clean and dry.
- 3) Mixing of blood and serum should be quick to avoid coagulation.
- 4) Use different tooth picks on each slide to avoid mixing of anti A anti Sera and anti B antisera.

Practical- 12

Testing of visual Activity

"visual acuity is the degree to which the details and contours of the objects are perceived."

Usually defined in term of the minimum separable by which 2 lines can be separated and still be perceived 2 lines.

Clinically, visual acuity is often determined by use of the familiar Snellen's letter chart viewed at distance of 20ft (6 meter) of letter of vary sizes constructed that top letter is visible at 60 meters and subsequent lines at 36, 24, 18, 12, 9, 6 and 5.

The patient reads down the chart as far as he can.

Apperatus:

Snellen's chart and Subject.

Procedure:

For Distant vision

- 1) Subject being test is 20 feet away from

OBSERVATIONS AND CALCULATIONS

Name:

The vision of subject is:

Right eye:_____

Left Eye:_____

Snellen's chart and reads smallest line distinguishable loudly.

2) Snellen's charts are designed so that a normal individual can read, the letters in the smallest (7th) line at 20 feet or 6m. (Subtends a visual angle of 5 min).

3) A person visual acuity is started as $V = d/D$ in which "d" is the distance at which the patient can read the letters and D is the distance at which a normal eye can read the letters. Normal visual acuity 20/20 or 6/6.

4) If vision is recorded 10/20 it is subnormal because one must approach to within 10 feet to read letters that one readable at 20 feet by the normal eyes.

For Near Vision

visual acuity at the ordinary distance is assessed by using reading test types of vary sizes, the notation being based on the printer's point system. The smallest print used is N5. The smallest print is N5. The near vision is recorded as the smallest type which is the patient can read comfortably.

Precautions:

- 1) Examiner must have 6/6 (with or without glasses) vision and no colour blindness.
- 2) Each eye should be tested separately.
- 3) One eye should remain close while the other is being examined.

Practical-13

Recording and interpretation of ECG.

The electrocardiogram is a graphical recording of the action potential of the heart. It is recorded with an electrocardiograph and the study of this cardiac electrical activity is called electrocardiograph (ECG).

Apparatus:

- ECG machine
- Electrode gel
- Subject

Procedure:

- 1) Connect all the limbs and chest electrodes properly using contact gel. Conventionally all the electrodes are marked with codes and have specific colors. Make sure that the subject is lying comfortably having no metallic object on.
- 2) Check the amplitude on QRS complex in lead I, II and III. According to the Einthoven's law.
- 3) Examine the initial strip of the record and check that there should be no artifact.

4) Record in sequence, first for standard limb leads followed by augmented limb leads and finally for chest leads.

5) After having recorded the ECG, calculate the heart rate first and then observe the various components of ECG carefully. There are many different ways to calculate heart rate, a simple way is to divide 300 by the number of large squares between 2 consecutive beats (R-R interval) if the rhythm is regular.

Precautions:

- 1) Relax the patient before taking a record.
- 2) Use proper voltage and handle the electrical recorder gently.
- 3) There should be no metallic objects on the subject.
- 4) Contact of the electrodes with the skin should be made with electrolyte gel.

OBSERVATION & CALCULATION

NORMAL DURATION/ VOLTAGE

COMPONENTS	DURATION (Sec)	VOLTAGE (mv)
P wave	0.08 – 0.1	0.2 – 0.3
QRS complex	0.08 – 0.12	1 – 3
T wave	0.16 – 0.27	0.3 – 0.4