NESAMONY MEMORIAL CHRISTIAN COLLEGE,
MARTHANDAM

DEPARTMENT OF ZOOLOGY

SEMESTER V

ANIMAL PHYSIOLOGY AND BIOCHEMISTRY

MANUAL

FOR

UG ZOOLOGY PRACTICALS

ZOOLOGY ISN'T JUST A SCIENCE

IT'S ALIFE STYLE

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A word with you…

Dear students,

Hope you would enjoystudying Zoology through self-learning materials provided by the Department of Zoology, N.M.C.C. But you know, the true spirit of science lies in experiments. The experiments are not only the roots of growth and development of science, they are also essential for the learning of science. What you learn by doing activities and experiments indeed becomes a part and parcel of your personality. The Department of Zoology N.M.C.C kept laboratory work as an integral part of B.Sc. Zoology curriculum which is provided to you in the form of list of experiments. We know majority are resourceful enough to do most of the listed experiments by your own. some may require some guidance or help. so, keeping this in view, we have prepared this laboratory manual. The major objectives of this laboratory manual are:

- *To familiarize the students with some of the apparatus, tools and techniques used by scientists in their work.*
- *To develop the habit of taking keen observations, making plan for doing a work, working systematically and thinking logically.*

We are sure you will understand your experiments and mould yourself fully in the culture of science. In case if you have any doubt or difficult, feel free to consult your teacher.

Wishing you all success

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Head of the Department

SAFETY IN SCIENCE LABORATORY

Science laboratory is an exclusive work place for sincere workers. Go there with a sense of devotion and work honestly.A little careless can lead to accidents by which you may harm yourself and your neighbours. Proper handling of apparatus, chemicals and other materials in the laboratory can prevent majority of accidents. Remember the following points while working in a science laboratory.

- Use all chemicals carefully.
- Replace the reagent bottles in their respective place after use.
- Do not mix chemicals unless required.
- Do not taste chemicals.
- Before using a chemical, make sure whether it is the right chemical.
- Put off the gas to extinguish the flame of burner. Do not use any solid or liquid for this purpose.
- While pouring acids in the sink after use, do not forget to keep tap-water running so that they are completely flushed out.
- Do not keep volatile liquids such as alcohol, ether, acetone, etc. near the flame, as these are highly inflammable.
- Do not throw broken glasswares in the sink. Such things should be thrown into the dust bin.
- Do not talk to other students in the laboratory while performing the experiment. In case you have any difficulty, consult your teacher directly.
- Never point a test tube containing a reaction mixture or a mixture, which is being heated, towards your neighbor or yourself.
- Before leaving the laboratory wash your hands properly.

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RATE OF OXYGEN CONSUMPTION IN A FISH

AIM

To find out the rate of oxygen consumed by the fish in one hour

MATERIALS REQUIRED

Burette, burette stand, pipette, reagent bottle, conical flask, measuring cylinder, one liter jar with a cap and a fish

REAGENTS REQUIRED

Manganoussulphate, Alkaline Iodide, 0.025N Sodium Thiosulphate, concentrated Sulphuric acid and 1% Starch solution.

PRINCIPLE

The amount of oxygen present in the water is determined by **Iodometric method** (Winkler's method).When **ManganousSulphate** ($MnSO_4$) is added to the sample of water,**ManganousHydroxide** is produced due to oxidation of $MnSO_4$ to$Mn(OH)_2$ in the presence of dissolved oxygen in water.Addition of **Alkaline Iodide** along with **Sulphuric acid**(H_2SO_4) liberates Iodine equal to that of oxygen which is used for the conversion of $MnSO_4$ to $Mn(OH)_2$.Hence the amount of Iodine liberated is a measure of the amount of dissolved oxygen in a given water sample.The liberated Iodine can be estimated by titrating it against 0**.025N of Sodium Thiosulphate**($Na_2S_2O_3$) solution and 0.1% Starch as an indicator.

watersample + $MnSO_4$ $\longrightarrow$ $Mn(OH)_2$ (ManganousHydroxide)

$Mn(OH)_2 + O_2$ in the sample $\longrightarrow$ $Mn(OH)_3$(Manganic Hydroxide)

$Mn(OH)_3 + 2KI + H_2SO_4 \longrightarrow MnSO_4 + K_2SO_4 + I_2$

$2Na_2S_2O_3 + I_2 \longrightarrow 2NaI + Na_2S_4O_6$

PROCEDURE

A fish was weighed and introduced into the water filled conical flask.The conical flask was closed tightly with the stopper and without any air bubbles inside.A control was also setup with another conical flask without the fish.The two flasks were kept side by side for one hour at room temperature.The oxygen content of water in the control and experimental flasks were determined by Winkler's method. The difference in oxygen content gives the oxygen consumed by the fish.The rate of oxygen consumption of the fish is determined by dividing the oxygen consumed by the fish by the weight of fish.

ESTIMATION OF OXYGEN BY WINKLER'S METHOD

Reagent bottle was filled with water and closed tightly with the stopper. The volumes of the reagent bottles were found out by filling the bottle with water and measuring the volume of that with measuring jar. These reagent bottles were filled with the water from the control and experimental flasks. The bottles were tightly stopper immediately to avoid entry of air. To each bottle 1ml of ManganousSulphate solution was added and followed by 1ml of Alkaline Iodide solution.

The bottles were stoppered with care so as to exclude any air bubble present inside and mixed well by inverting the bottle for few minutes. When the precipitate settles down the stopper was removed carefully and 1ml of Sulphuric acid was added and stopper immediately and mixed well till the formation of homogenous straw colouredsolution. 25 ml of the sample was pipetted out into a conical flask and titrated against 0.025N Sodium Thiosulphate using 0.1% Starch as indicator and the titration was continued till the disappearence of blue colour.Titration was repeated to get concordant

value.From the volume of Sodium Thiosulphate,the dissolved oxygen content was calculated by using the formula

$$\text{Oxygen content} = \frac{\text{Kx 200 x 0.698 xVolume of Sodium thiosulphate}}{Volume\ of\ the\ sample}$$

Where,

$$\text{K} = \frac{\text{Volume of the reagent bottle}}{\text{Volume of the reagent bottle} - \text{Volume of the reagents added}}$$

200 =Equivalent weight of oxygen x Normality of sodium thiosulphate x 1000

0.698 = Conversion factor to convertmg/litre to ml/litre

Volume of the reagent = 2ml (1ml $MnSO_4$ +1ml KI)

OBSERVATION

Sample	**Volume of sample (ml)**	**Burette reading**		**Volume of $Na_2S_2O_3$ used (ml)**	**Concordant value**	**Indicator**
		Initial	**Final**			
Control						1% starch
Experiment						

CALCULATION

CONTENT	FORMULA & EXPANSION	CALCULATION
Oxygen content of control	$O_2 = \frac{\text{Kx 200 x 0.698 xVolume of } Na_2S_2O_3}{\text{Volume of Sample}}$	
Oxygen content of the Experimental sample	$O_2 = \frac{\text{K x 200 x 0.698 x Volume of } Na_2S_2O_3}{\text{Volume of Sample}}$	
Oxygen consumed by the fishin one hour	Oxygen content of control − Oxygen content of Experimental sample	
Rate of oxygen consumed by the fish	$\frac{\text{Oxygen consumed by the fish in one hour}}{\text{Weight of the fish}}$	

RESULT

Oxygen consumed bythefish in one hour = ml/l/hr

Rate of oxygen consumption = ml/l/gm/hr

INFERENCE

Oxygen is necessary for living organisms. Aquatic animals like fishes use oxygen which is dissolved in water. In fishes water enters through the mouth and goes out through gill slits.

In the above experiment it is found out that there is difference in the oxygen content between control water and experimental water in which the fish is placed. Oxygen content of control water is found as ---------- ml/l. Oxygen content of experimental water as ---------- ml/l. It proves that oxygen present in the water is consumed by the fish for its metabolic activities.

Various factors such as body weight, surface area, age of fish, size of fish, pH, temperature and salinity of water influences the rate of consumption of oxygen, Smaller the fish, greater will be the rate of consumption and larger the fish, lesser will be the rate of oxygen consumption.

EFFECT OF TEMPERATURE ON THE OPERCULAR MOVEMENT OF A FISH - CALCULATION OF Q_{10}

AIM

To study the rate of opercular movement with an increasing temperature in order to calculate the Q_{10} value

PRINCIPLE

The increase in the biological activity for a 10°C rise in temperature is referred as temperature coefficient or Q_{10}. According to **VantHaff's rule** the rate of biological reaction is doubled for every 10°C rise in temperature Q_{10} value is calculated using the formula.

$$Q_{10} = \frac{K2x\ 10}{K1\ (t_2 - t_1)}$$

K_1 = the role of activity at t_1°C

K_2 = the role of activity at t_2°C

t_1 = the initial temperature

t_2 = the final temperature

Materials required

Beaker, glass rod, thermometer, stop watch, conical flash, fish and hot water.

PROCEDURE

A live fish is taken gently without rubbing the mucous membrane and is placed in a conical flask containing 300ml of water. The temperature of water is noted. After five minutes, when fish was fully recovered from the stress due to change of medium, the opercular movement of the fish in noted. The time

taken for 10 such opercular movement is recorded with the help of a stop watch. The observation is repeated for three times and the average is calculated. The reciprocal of time gives the rate of opercular movement. Then by adding hot water slowly the temperature of the water is gradually increases and the time taken for 10 opercularmovement is recorded for 2°C rise in temperature. Care is taken to maintain the temperature at constant when the reading will be taken.

Temperature	**Time taken for tenopercular movements in seconds**		**Average time 't' (sec)**	**Rate of activity (1/t)**
28°C	1			
	2			
	3			
30°C	1			
	2			
	3			
32°C	1			
	2			
	3			
34°C	1			
	2			
	3			
36°C	1			
	2			
	3			
38°C	1			
	2			
	3			

PRECAUTIONS

i. Temperature changes in the medium should be gradated because animal cannot tolerate sudden change in temperature.

ii. Addition of hot water should not be made directly on the animal.

iii. Allow the animal for acclimatization to the temperature for some time.

RESULT

The Q_{10} value of opercular movement of fish = ----------

INFERENCE

Temperature is an ecological factor that influences various physiological activities of the body. According to Vant Hoff's rule, increasing of temperature of $10°C$ speeds up the velocity of chemical reaction to double in a constant proportionate rate up to an optimum.

In this experiment it is found out that the physiological activity, namely the opercular movement is accelerated by the increase in temperature. The rate of activity (Q_{10} value) is found to be doubled for every $10\,°C$ raise in temperature above the normal temperature up to an optimum temperature.

ACTION OF SALIVARY AMYLASE IN RELATION TO ENZYME CONCENTRATION

AIM

To study the action of human salivary amylase in relation to its concentration

MATERIALS REQUIRED

Test tubes, stand, pipette, 1% starch, 1% sodium chloride and 0.01% Iodine

PROCEDURE

About 3ml of human saliva was collected in a test tube. 5 test tubes each with 1ml of different concentration of enzyme (20,40,60,80 and 100%) were taken, and were labeled as 1,2,3,4 and 5.

The different enzyme concentrations were prepared by taking 0.2ml, 0.4ml, 0.6 ml, 0.8 ml and 1 ml of saliva in 5 test tubes and mixed with 0.8ml, 0.6ml, 0.4 ml, 0.2ml and 0ml of water respectively.

In a separate test tube 1ml of 1% **Sodium Chloride**, 1ml of 1% **Starch** and 2 drops of **Iodine** solution was taken.

The contents of this test tube (the mixture of reagent) were mixed with the content of the first test tube (20% saliva). A blue colour appeared. The time taken for the disappearance of blue colour (achromic point) was noticed represent as ‘t’. The same procedure was repeated for all the other test tubes also.

From this time ‘t’ the rate of salivary amylase activity was calculated using the formula

Rate of activity = 1/t,

Where,

't' is the time required for complete digestion of starch by amylase enzyme.

The rate of activity was then plotted on a graph by taking the enzyme concentration of X axis and rate activity on Y axis.

1. **Table showing the enzyme concentration**

Sl.No.	Quantity of saliva in (ml)	Quantity of water in (ml)	Enzyme concentration in (%)
1	0.2	0.8	20
2	0.4	0.6	40
3	0.6	0.4	60
4	0.8	0.2	80
5	1	0	100

2. **Rate of salivary amylase activity in relation to enzyme concentration**

Sl.No.	Concentration of the enzyme (%)	Time in seconds 't'	Rate of enzyme activity = 1/t (gm/sec)
1.	20		
2.	40		
3.	60		
4.	80		
5.	100		

RESULT

Rate of amylase activity at 20% of enzyme concentration = gm/sec

Rate of amylase activity at 40% of enzyme concentration = gm/sec

Rate of amylase activity at 60% of enzyme concentration = gm/sec

Rate of amylase activity at 80% of enzyme concentration = gm/sec

Rate of amylase activity at 100% of enzyme concentration= gm/sec

DISCUSSION

Saliva is a viscous fluid secreted by salivary glands. Saliva contains an important carbohydrate digesting enzyme called salivary amylase (ptyalin). When salivary amylase acts on starch, maltose is slowly produced. It passes though stages like **soluble starch, erythrodextrine, amylodextrin, achrodextrin** etc.

With iodine, starch first produce blue colour. When starch is in the form of erythrodextrine it becomes violet. As hydrolysisproceeds, erythrodextrin become achrodextrin, hence violet colour is no longer produced and it becomes colourless.

The above results clearly prove that, the rate of salivary amylase activity depends upon the enzyme concentration. When the enzyme concentration increases the quantity of salivary amylase also increases. The increase quantity of salivary amylase acts over the starch quickly, hence the time taken for the digestion of starch is less. This decreased time factor shows an increase in the rate of enzyme activity as the enzyme concentration increases.

QUALITATIVE TEST FOR CARBOHYDRATE (GLUCOSE)

AIM

To detect the presence of glucose in the given sample

Name of the Test	Observation		Inference
	Sample A	Sample B	
1. Benedict's test To 1ml of the test solution add 2ml of **Benedict'ssolution.** Boil for about 2 minutes. Allow it to cool and note the colour	Appearance of yellow or brownish red precipitate	No colour precipitate formation	Presence of glucose in sample A
2. Fehling's Test To 2ml of **Fehling's solution** (1ml of Fehling's A & 1ml of Fehling's B) add few drops of test solution. Boil for about few minutes and observe the colour change.	Appearance of yellow or brownish red precipitate	No colour precipitate formation	Presence of glucose in sample A
3. Picric acid test To 1ml of the sample solution add 2ml of standard **picric acid** and 2ml of 10% **sodium carbonate** solution. Warm the mixture gently, and notice the colour change.	Appearance of mahoganycolour	No colour change	Presence of glucose in sample A

Result : Glucose is present in sample A

QUALITATIVE TEST FOR PROTEIN

AIM

To detect the presence of Protein in the given sample

Name of the Test	Observation		Inference
	Sample A	Sample B	
1. Biuret test To 1ml of the test solution add 1ml of 10%**Sodium Hydroxide** and mixed thoroughly.To that add 7 drops of 5% **Copper Sulphate** solution drop by drop.Keep it for few minutes and note the colour change	Appearance of purple colour or pinkish violet colour	No colourappearance	Presence of protein in sample A
2. Xanthoproteic Test To 2ml of the testsolution carefully add 1ml of conc.**Nitric acid**. Boil the solution,cool and add 2ml of 20% **Sodium Hydroxide** and notice the colour change.	Appearance ofwhite precipitate on addition of nitric acid,change of white colour to yellow precipitate on boiling, and appearance of orange colour on addition of Sodium Hydroxide	No colourchanges	Presence of protein in sample A

3.Heller's test To 1ml of the test solution slowly add few drops of conc.**Nitric acid**.It is then mixed slowly by rotating between the two palms	Formation of **whitish ring** at the junction of the acid the sample	No ring formation	Presence of protein in sample A

Result :Protein is present in sample A

QUALITATIVE TEST FOR LIPID

AIM

To detect the presence of lipid in the given sample

Name of the Test	Observation		Inference
	Sample A	Sample B	
1. Grease spot test Put a drop of sample over a piece of ordinary writing paper and notice the change	Appearance of translucence spot on the paper	No translucent spot formation	Presence of lipid in sample A
2. Saponification Test To 10 drops of the sample in a test tube add a small amount of **Sodium Hydroxide** solution and gently boil for 3 minutes and observe the change	Appearance of soapy nature	No soapy appearance	Presence of lipid in Sample A
3. Picric acid test To the test solution add few drop of water and mixed well. Observe the change	Appearance of uniformly distributed oil droplets	No droplets formation	Presence of lipid in sample A

Result : Lipid is present in sample A

DEMONSTRATION OF BLOOD PRESSURE USING SPHYGMOMANOMETER

AIM

To study the blood pressure in man

MATERIALS REQUIRED

Sphygmomanometer, Stethoscope

DESCRIPTION OF THE INSTRUMENT

Sphygmomanometer has a vertical mercury column graduated from **0-300mm mercury**. The pressure sleeve opens into a mercury reservoir and depending upon the blood pressure, the mercury is pushed into the column and the pressure reading can be read out directly from the scale.

The range of normal blood pressure is:

1. Systolic : 100 – 120mm Hg
2. Diastolic : 60 – 80mm Hg

PROCEDURE

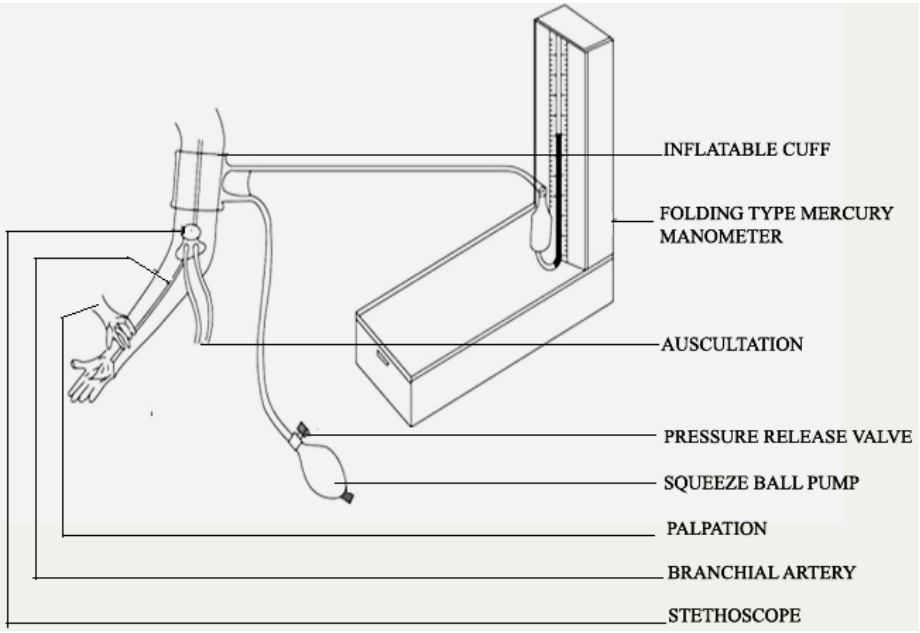
The pressure sleeve is placed snugly on the upper left arm without much pressure around the arm and tucked under the pressure sleeve. Now, the pressure sleeve is inflated upto 150mm mercury (mm-Hg) and by using the pressure release valve, the pressure sleeve is deflated very slowly, at a certain point a sound is heard with each heart beat. This shows that the pressure in the brachial artery is capable of overcoming the outside pressure and the blood has stated flowing through the artery. The highest point at which the heart beat can be heard is the systolic pressure.

Then, the pressure sleeve is gradually deflated and the diastolic pressure is measured. The change from sharp and clear clicking to murmuring sound of

the blood flow is noted. The sound is similar to the continuous flowing of river water. The pressure is reduced further and the lowest pressure at which the sound can still be heard in the diastolic pressure.

RESULT

The blood pressure measured is …… mm Hg.



ESTIMATION OF HAEMOGLOBIN (DEMONSTRATION ONLY)

AIM

To estimate Haemoglobin content by using Sahli'sHaemometer in gram percentage

MATERIALS REQUIRED

Sahli'sHaemometer, 0.1N Hydrochloric acid (HCl), distilled water, spirit, cotton wool and needle.

PRINCIPLE

Haemoglobin content is determined by **Acid Haematin method**. This method consists of conversion of blood to Haematin by using **HCl** and matching the colour of this acid Haematin solution with that of standard solution.

SAHLI'S HAEMOMETER

Sahli'sHaemometer is a frame with three compartments for test tubes. Two test tubes are fixed and have **haematic solution** of known concentration. The third test tube in the centre in graduated and is provided with two scales. One scale has **140 graduations** and the other scale on the opposite side of the test tube gives the reading of the haematic content in gram per 100cc of whole blood. The Haemometer reading may be expressed in percentage or in gram per 100 cc of whole blood. Haemometers are provided with a graduated Sahli's pipette with $20mm^3$ mark.

PROCEDURE

Sahli's pipette is filled with blood upto or slightly above the 20 mark. By touching the tip with filter paper the volume is adjusted exactly to the 20 mark. The blood is expelled into the haemometer tube containing **0.1N HCl**upto the 2

mark. The contents are mixed by a glass stirrer and allowed to stand for 10 minutes. Afterwards, the mixture is diluted with distilled water and mixed thoroughly with the stirrer until the colour of the solution matches with that of the standard solution provided in the Haemometer. The haemoglobin content is directly read in gram percentage from the graduated Haemometer tube.

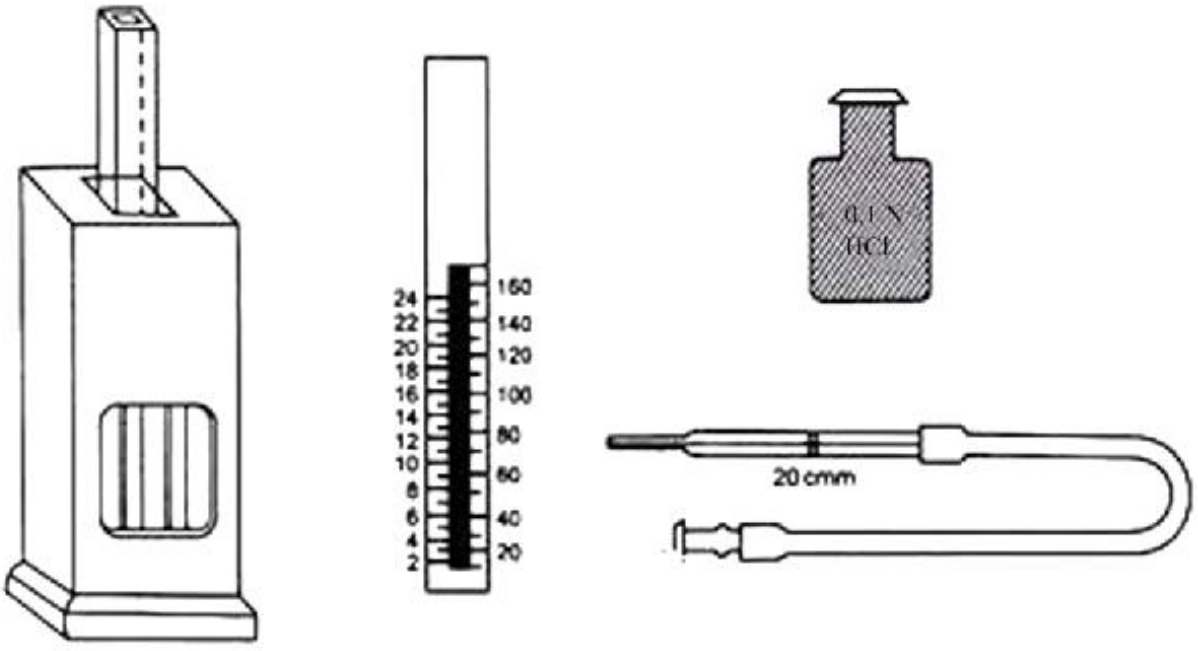
NORMAL HAEMOGLOBIN VALUES:

Men	:	15.51 $\pm$ 2.5/ gm/dl or 14 – 18 gm%
Women	:	14.0 2.5 gm/dl or 11.5 – 16.5gm%

RESULT

Haemoglobin content of the tested blood is ……. gm%

Sahli'sHaemoglobinometer



Haemoglobinometer (Comparator)

Haemoglobinometer tube

Haemoglobinometer pipette

COUNTING OF DIFFERENT KINDS OF BLOOD CELLS USING HAEMOCYTOMETER – DEMONSTRATION ONLY

HAEMOCYTOMETER

The Haemocytometer includes two graduated pipettes in which dilution of blood is done and a Neubauer's counting chamber.

RBC PIPETTE

The RBC pipette has a **red bead** for identification. The graduations in the pipette are **0.5, 1 and 101**. Blood is taken upto the 0.5 mark and diluted upto the 101 mark using RBC diluting fluid.

WBC PIPETTE

The WBC pipette has a **white bead** for identification. The graduations in the pipette are **0.5, 1 and 11**. Blood is taken upto the 0.5 mark and diluted upto the 11 mark using WBC diluting fluid.

NEUBAUER'S COUNTING CHAMBER

It is 3 inches long and 1.5 inches wide. It has two counting chambers with a cover slip. The slide bears one central platform separated by two grooves. The lateral platforms are further demarcated by a lateral groove. The central platform is divided by a central horizontal groove. Each counting chamber is divided into 9 squares of 1 x 1mm each. Of the 9 squares the central square is the RBC counting square and it is divided into 25 smaller squares. Each of these squares is further divided into 16 squares, so that there are 400 smallest squares, each within the area of 1/400 square mm.

The four corner larger squares are the WBC counting squares. Each corner square has an area of 1 square mm. Each corner square has 16 smaller

squares. Hence, WBC counting has to be done in 4 x 16 = 64 smaller squares having an area of 1 x 4 or 4mm.

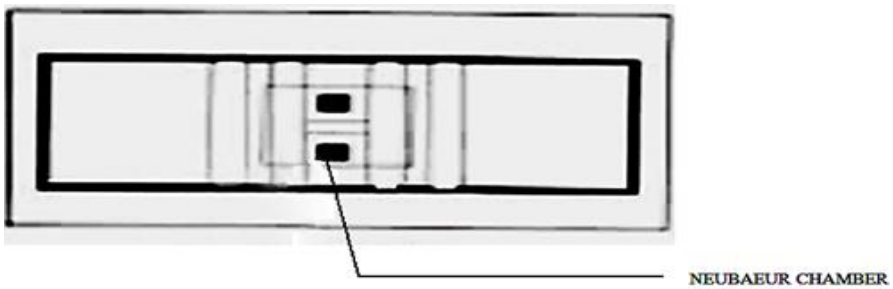
RBC counting is done in the four corners and the middle of the RBC counting area.

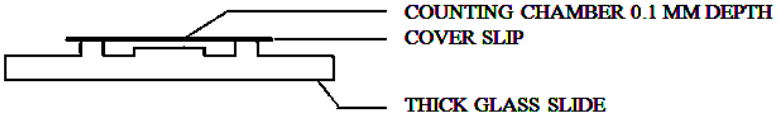
RESULTS

The total number of RBC's in 1mm^3 of blood is ……. millions

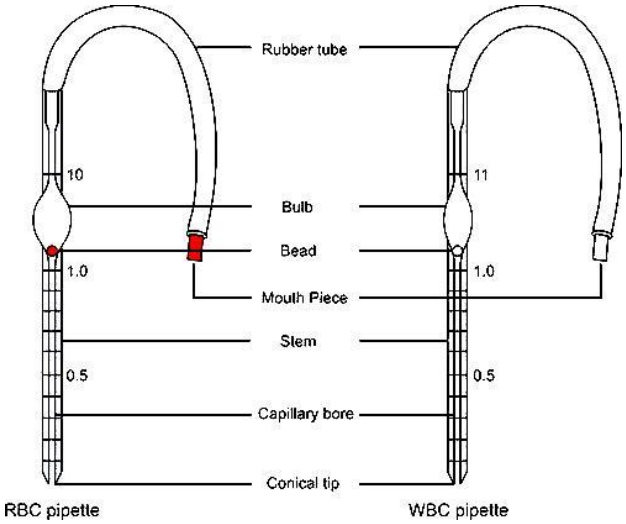
The total number of WBC's in 1mm^3 of blood is ……. millions

NAUBAEUR'S COUNTING CHAMBER

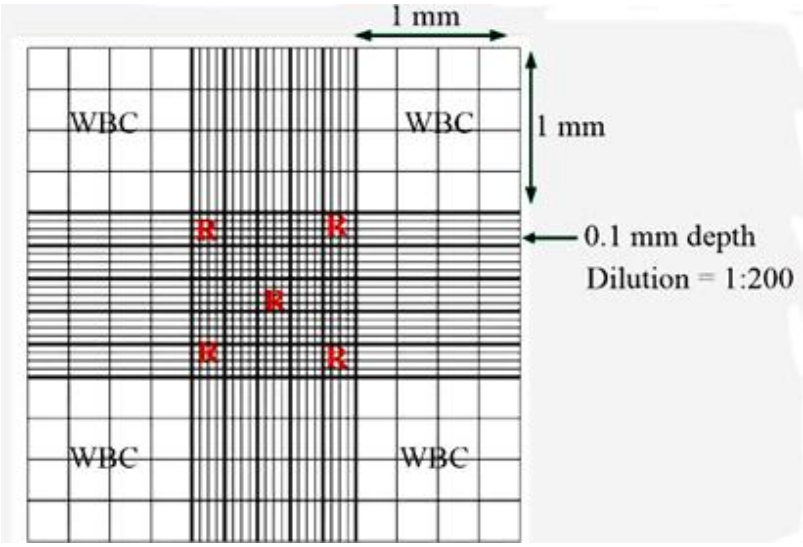




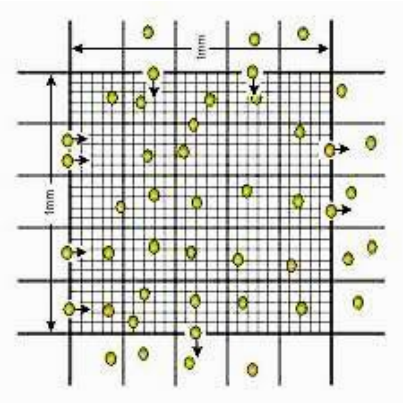
SIDE VIEW



Counting Chambers



Small squares in the 1 mm square for red cell count



QUALITATIVE TEST FOR AMMONIA,UREA AND URICACID

DETECTION OF AMMONIA

MATERIALS REQUIRED

Test tube and pipette

REAGENTS

Nessler's reagent

PROCEDURE

To about 2ml of water taken from a trough in which fish was kept for more than 5 hours,few drops of Nessler's reagent is added.

OBSERVATION

A reddish-brown precipitate is formed which indicates the presence of ammonia.

DETECTION OF UREA

MATERIALS REQUIRED

Test tube and pipette

REAGENTS

5%Sodium Hydroxide (NaOH),1% Copper Sulphate($CuSO_4$)

PROCEDURE

A small amount of diluted mammalian urine is taken in a test tube and 1ml of 5% sodium hydroxide and 2 drops of 1% copper sulphate solution are added.

OBSERVATION

There is formation of a blue precipitate. The blue precipitate indicates the presence of urea.

DETECTION OF URIC ACID

MATERIALS REQUIRED

Test tube, spirit lamp, mortar and pestle, glass slide, microscope.

REAGENTS

5% Sodium Carbonate(Na_2CO_3), dilute Hydrochloric acid (HCl).

PROCEDURE

White portion of excreta of bird is powdered and taken in a test tube. It is boiled with few drops of 5% **Sodium Carbonate** and cooled. Subsequently, a few drops of diluted **Hydrochloric acid** is added and shaken well. The uric acid crystals formed are examined under microscope.

OBSERVATION

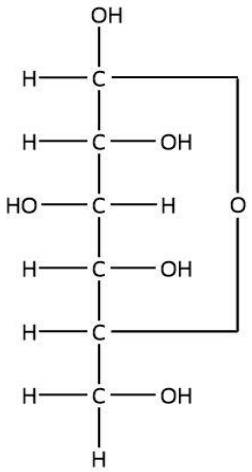
Uric acid crystals are seen in large variety of forms: wedge shaped, dumb bell shaped, rhomboid, irregular, triangular and hexagonal shaped.

Slides, Models and Charts

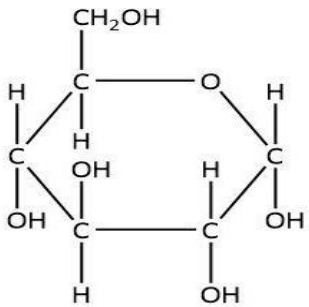
Glucose

1. It is a monosaccharide.
2. It is a simple hexose sugar with a molecular formula of **$C_6H_{12}O_6$**
3. It is an aldose sugar with aldehyde (CHO) group.
4. It is a white, crystalline, solid, highly soluble in water and sweet in taste.
5. It is mostly seen in sweet fruit like grapes, honey, etc hence called grape sugar.
6. Blood contains 85 to 100 mg of glucose in 100 ml of blood. So glucose is also called blood sugar. When glucose level exceeds than the normal level, glucose begins to appear in urine. This condition is called glycosuria.
7. It has **4 asymmetric carbon atoms** hence there are **16 possible isomers**.
8. It shows optical activity and rotates the plane of polarized light to the right hence called **dextrose**.
9. The structure of glucose is either represented as straight chain or as ring structure. The ring may be a 5 membered furanose ring or 6 membered pyranosering.
10. Glucose with another molecule of glucose forms maltose with one molecule of fructose forms sucrose with one molecule of galactose forms lactose.

Glucose + glucose → Maltose
Glucose + fructose → Sucrose
Glucose + galactose → Lactose



Open chain



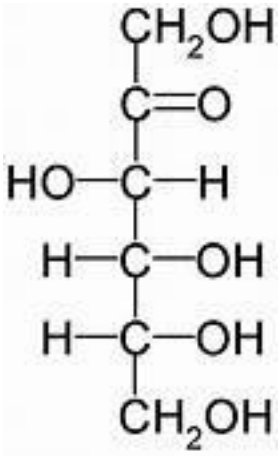
Ring structure

FRUCTOSE

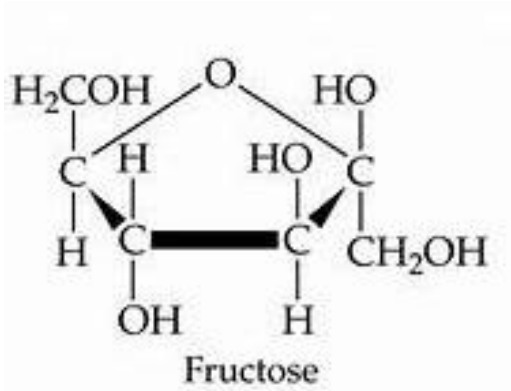
1. It is a monosaccharide and sweet in taste.
2. It is a hexose sugar with the molecular formula **$C_6H_{12}O_6$.**
3. It is a crystalline form readily soluble in H_2O.
4. It is **ketose sugar** having the functional group called keto (C=O) group.
5. It is seen in fruits, honey hence called fruit sugar.
6. It is a reducing sugar.
7. It is an optically active compound, rotates the plane light to the left. So it is called **laevulose** (levosugar)
8. The structure is represented either as straight chain or ring structure.
9. The ring structure may be 5 membered fructofuranose or 6 memeberedfructopyranose ring.
10. One molecule of fructose combines with one molecule of glucose and forms sucrose several units of fructose form inulin.

Glucose + Fructose ⟶ Sucrose

Fructose + Fructose ⟶ Inulin



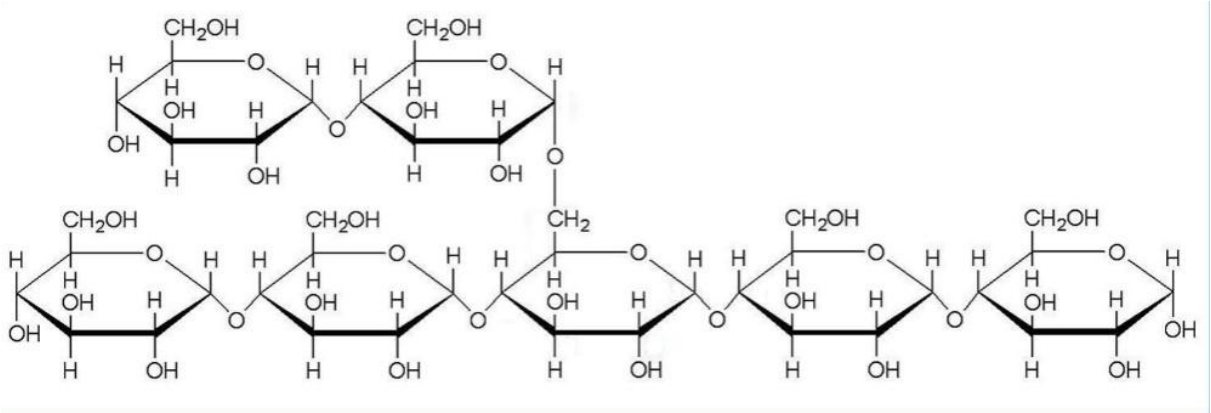
Open chain



Ring structure

GLYCOGEN

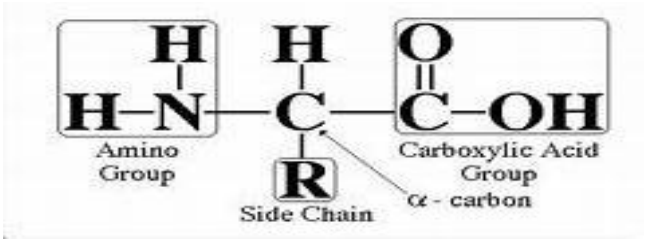
1. It is a homo polysaccharide formed of hundreds of **α D glucose** units hence called glucon.
2. It is a non-sugar because of its tasteless nature.
3. It is a branched polymer in which glucose units are linked by **α 1,4 and α 1,6 glycoside linkage.** The glucose units of the straight chains are linked by α 1,4 linkages but the glucose units at the point of branching is linked by α 1,6 linkages.
4. It is similar to the **amylopectin** to starch chemically except its molecular weight and degree of branching.
5. It is the major carbohydrate reserve in animals and is hence called animal starch. It is stored in the liver and muscles of all animals.
6. When the blood glucose level is lower than the normal level, the glycogen is converted into glucose by a process of **glycogenolysis**.
7. When the blood sugar level is more than the normal, then the glucose is transformed into glycogen through a process of glycogenesis.
8. It is white powder readily dissolved in water.
9. It has a molecular weight of 5 lakhs
10. It is a non-reducing sugar.



Ring structure

AMINO ACIDS

The amino acids are the building blocks of protein. There are about 22 to 25 amino acids.Each amino acid is an organic nitrogenous compound having an acidic **carboxyl (COOH)** group and a basic **amino (NH_2)** group. so the amino acid is called **amino carboxylic acid**. It is represented as



where,

COOH represents carboxylic group

NH_2 represents amino group

R represents the side chain. It has a group other than COOH and NH_2 group Carbon of COOH group is the first carbon.The carbon in which NH_2groupattached is called α carbon (2^{nd} carbon). All amino acid have the same arrangement (NH_2) at α carbon) and so the amino acids are called α amino acids.

Since the amino acids have both acidic and basic group, they react both with acid and base and form salts. So they are called amphoteric compounds or Zwitterions.

Out of the 22 to 25 amino acids, some are synthesized in the body itself and need not be supplied to the body. They are called non-essential amino acids. Some of the amino acids are not synthesized by the body and have to be supplied along with the food. They are called essential amino acids.

All the 22 amino acid are used for the synthesis of protein in a way similar to the formation of several millions of words from 26 alphabets.

They are linked with each other by peptide bonds and forms dipeptide, tripeptide and a polypeptide. One or more polypeptide chain will form a protein molecule.

CHOLESTEROL

Cholesterol means **solid bile alcohol**, as it was first isolated from human gall stones. It is a **derived lipid**.

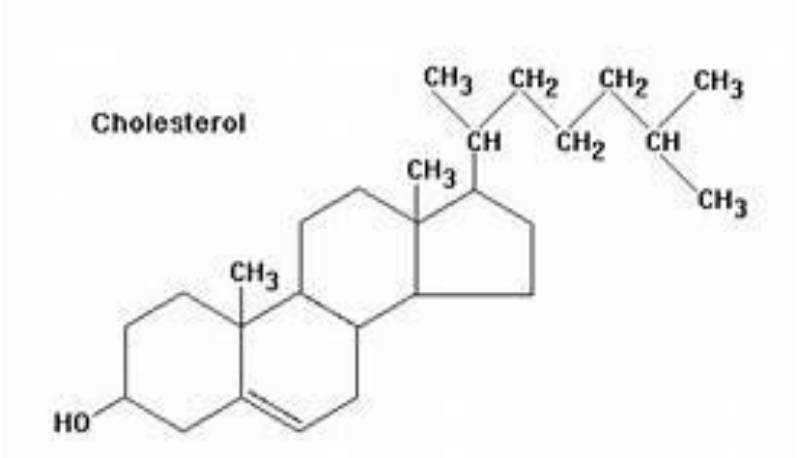
It is present only in animal fats and is absent in plant fats. It is abundant in the brain, nervous tissue, liver, skin, Adrenal gland, Corpus luteum etc.

It is composed of **cyclopentanoperhydrophenantherene ring**system which has a total of 17 carbon atoms. It has 8 asymmetric carbons in the position of 3,5,8,9,10,13,14 and 17.

The molecular formula of cholesterol is C_{27} $H_{46}O$

1. Cyclopentanoperhydrophenanthrene ring system.
2. An aliphatic 8 carbon side chain attached to C_{17}.
3. A 'OH' group attached to C_3.
4. A double bond between C_5 and C_6
5. It has **2 methyl groups** one attached to C_{10} and the other attached to C_{13}.

Cholesterol is considered to be an unsaturated alcohol due to the presence of double bond at C_5 and C_6. It is tasteless and it has ahigh melting point of 150°C. It is insoluble in water, but soluble in solvents like ether, benzene, chloroform etc. It is a poor conductor ofheat.



INTESTINAL VILLI

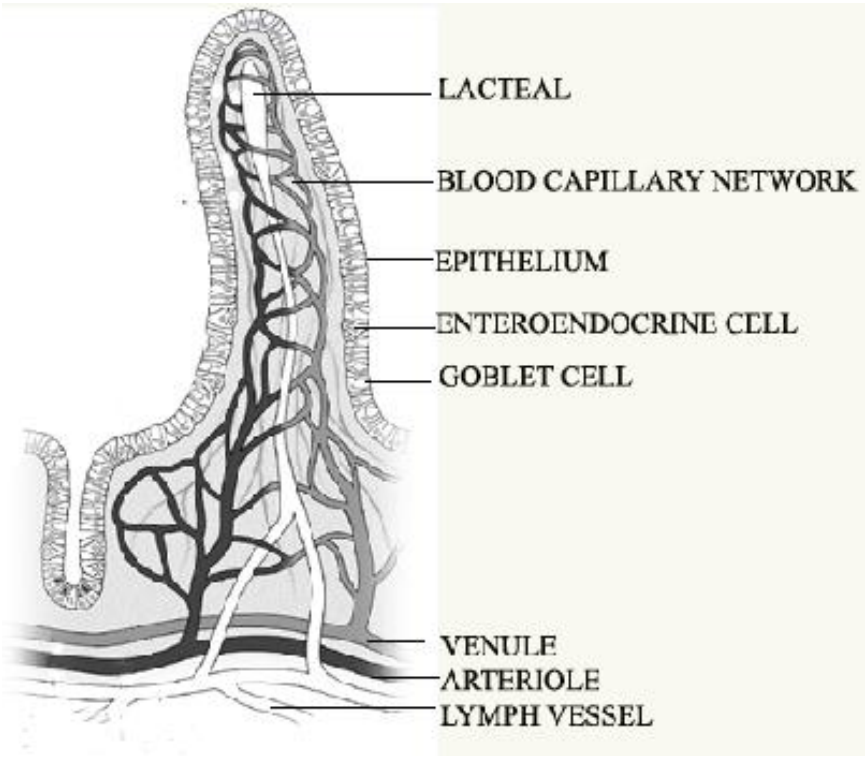
The mucous membrane of intestine is produced into numerous fingers like projections called villi (Sl. villus). The number of villi is about 20 to 40 per sq.mm. There are about **50 lakhs of villi** in human intestine.

- Each villus is lined by a layer of mucous epithelial cells containing tall columnar **epithelial cells** and few **goblet cells**.
- The free end of each columnar cell has a characteristic brush border.
- Each villus encloses a lymph vessel called the lacteal in the center. It contains a milky fluid rich in fat
- The lacteal is surrounded by a network of capillaries.
- The end products of carbohydrates and proteins digestion such as glucose and amino acid are absorbed by the blood capillaries and are transported through the portal system.
- The end products of fat digestion such as fatty acids and glycerol and absorbed by the lacteals and are transported through the lymphatic vessels.

Functions of villi

1. These intestinal villi are essential for the absorption of digested food.
2. They increase the area of absorption
3. They also enhance the rate of absorption

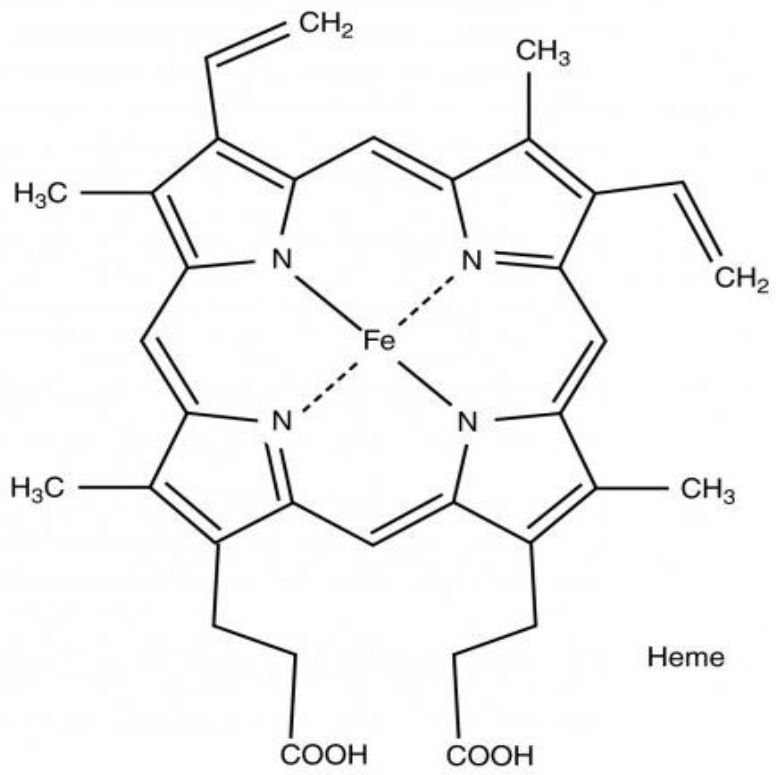
INTESTINAL VILLI



HAEMOGLOBIN

1. Haemoglobin is the most widely distributed red colour respiratory pigment.
2. It is present in all vertebrates,few holothurians, several crustaceans, some insects, many annelids etc.
3. In vertebrates haemoglobin is present in the erythrocytes (RBC) while in invertebrates Hb is distributed in the blood plasma.
4. In a normal person each 100 ml of blood contains **14 to 15 gms** of Hb. The total haemoglobin content in man is approximately 700 gms.
5. It is a globular protein, spherical in shape and has molecular weight of 68000 daltons.
6. It is a **conjugated chromoprotein** formed of two components namely haem (4%) and globin (96%).
7. Haem is the prosthetic group and globin is the protein component.
8. Each haemoglobin has four **haem** and a single **globin**.
9. Each haem is formed of a porphyrinand **iron**.
10. Since there are 4 haem, there are four irons and 4 porphyrin.
11. Each globin is formed of **4 polypeptidechain.**
12. Of the 4 polypeptide chains, 2 are identical called$\boldsymbol{\alpha}$ **chains** and the other two are identical called$\boldsymbol{\beta}$ **chain**.
13. Each α chain is formed of 141 amino acids, and each β chain is formed of 146 amino acids. A whole globin contain 574(2x141+2x146) amino acids.
14. Haemoglobin found in the muscles of birds and mammals are called myoglobin.

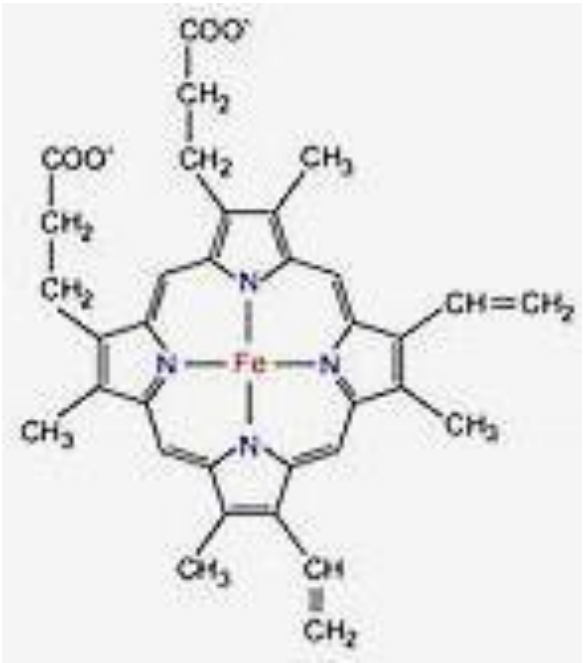
Structure of Haemoglobin



Heme

MYOGLOBIN

1. It is a low molecular weight oxygen – binding **heme protein**
2. It is one of the most important proteins in the human body
3. It is present predominantly in the sarcoplasm of skeletal and cardiac muscles.
4. Myoglobin is synthesized inside muscle cells. It '**stores' oxygen** therefore use at times of high metabolic demand.
5. Myoglobin has more affinity for oxygen as compared to haemoglobin. As a result, it can acquire oxygen from haemoglobin, hence transferring it from the blood to the muscle tissues.
6. In skeletal muscle it serves to transport oxygen from the cell membrane to the mitochondria.
7. Diving mammals such as seals and whales are able to remain submerged for long periods because they have greater amounts of myoglobin in their muscles.



Myoglobin Structure

ELECTROCARDIOGRAM (ECG)

The **rhythmic contraction** and **relaxation** of the auricles and ventricles of the heart is called heart beat.

The sequence of events occur during the heart beat is called **cardiac cycle**.

The record of the electric potential changes that occurs in the heart during cardiac cycle is called **electrocardiogram** (ECG). The instrument used to record the ECG is called **Electrocardiography**. ECG is recorded from the surface of the body by placing the instrument opposite to the heart.

Einthoven explains that ECG consists of 4 components namely.

i. P – wave
ii. QRS complex
iii. T wave
iv. Two isoelectric intervals

i. P wave

It is the first upward wave of ECG. It is a small wave with a round top. It is an atrial wave occurs in the **auricle**, due to the spreading of impulse in the auricles. Its duration is **0.1 second.**

ii. QRS waves

It is the combination of Q,R and S waves

a. Q wave

It is a small **negative downward** wave. It is mostly indistinct. In this stage impulse arrives at theinter ventricular septum and the septum gets activated.

b. R wave

It is the **prominent positive wave** with tallest amplitude. It is the second upward deflection. It represents that activity of **right ventricle**.

c. S wave

S is the **second downward negative** wave. It represents the activity of the **left ventricle**. The duration of QRS complex is about 0.08 second and it does not exceed **0.1 second**. The amplitude of the wave is about **1 mv.**

iii. T wave

Following S wave, there is an isoelectric interval. T wave begins after this isoelectric interval. T wave is the **third upward** wave. It is a broad, smooth rounded wave. It represents the contraction of the basal part is the ventricle. It's duration is **0.27 second** and an amplitude of **0.15 to 0.5 mv**. The average duration of QRST is 0.43 second.

iv. Isoelectric interval Periods

There are two isoelectric periods. They are

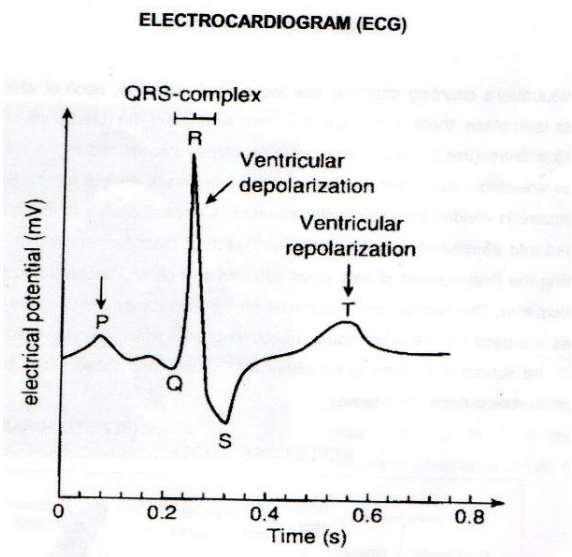
a. First isoelectric interval
b. Second isoelectric interval

a. First isoelectric interval

It lies in between Pand Q. It is short and flat. It represents the time taken for the impulse to travel over the auricle to reach auriculo ventricular node and muscles of ventricles.

b. Second isoelectric interval

It lies between S and T. It is a long period. It has duration of 27 second to 0.45 second. It represents the time taken for the impulse to come to the basal part of the ventricle.



SPHYGMOMANOMETER

Sphygmomanometer is a device used to measure systolic and diastolic blood pressures. It was developed by Riva – Rocci in 1896.

Parts of a sphygmomanometer are the following

a. Pressure cuff
b. Hand pump
c. Mercury manometer
d. Rubber tubes

a. Cuff

i. The cuff consists of a rubber bladder inside an inelastic fabric covering.
ii. It is to wrap around the upper arm while measuring blood pressure and fastened with either hooks or fastener.
iii. The cuff is normally inflated manually with a rubber bulb and deflated slowly through a needle value.

b. Hand pump

i. It is a small rubber pump with a screw at one end.
ii. It is used to raise or release the pressure by tightening or loosening the screw.

c. Manometer

It has graduations from **0 – 300**. Blood pressure can be measured from the level of mercury of this manometer.

d. Rubber Tubes

i. There are two rubber tubes.
ii. One end of these tubes is fixed to the cuff.

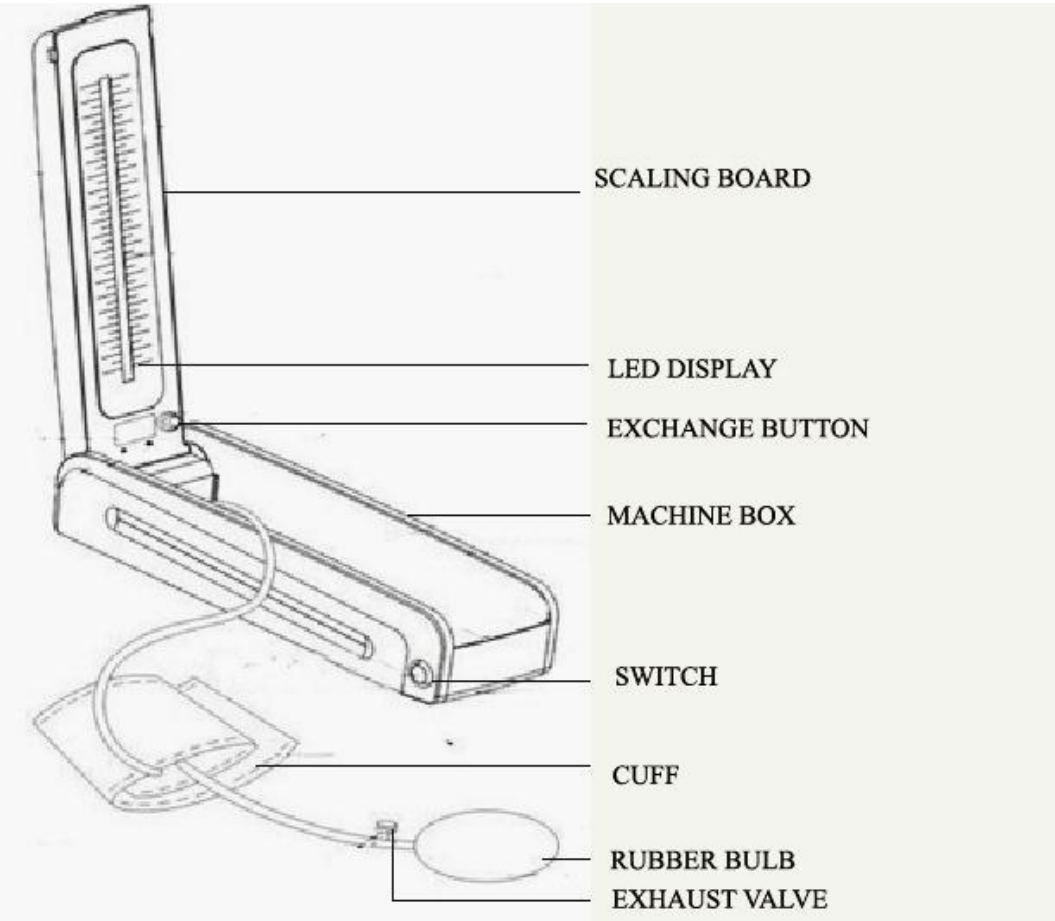
iii. The other end of the rubber tube is connected to the hand pump and the other rubber rube is connected with the manometer.

Normal blood pressure of a healthy adult body is **120/80mm Hg.** The average systolic pressure falls between 110-135mm Hg. Diastolic pressures is between 70-90 mm Hg.

USES OF SPHYGMOMANOMETER

i. It is a very important device to diagnose the diseases.

ii. It is also used to determine the progress of many diseased conditions by way of measuring the blood pressure.



HAEMOGLOBINOMETER

It is a device used to measure the haemoglobin content of the blood in grams or in percentage.

Haemoglobinometer has three components namely.

1. Comparator
2. Graduated tube
3. Micropipette of $20\mu l$ capacity.

In addition, it also contains a glass rod (stirrer), brush, a dropper, and a small bottle to contain deci normal acid solution.

1. Comparator

i. It is a rectangular plastic chamber.
ii. On one side of it, there is a white glass plate.
iii. On the other side, there are two sealed comparison tubes containing acid haematin. At the centre there is a slit to place the graduated tube.
iv. The graduated tube is placed in this slit of the comparator through a hole seen on the top.
v. The graduated tube of the comparator has marking on the side.

2. Graduated tube

i. It is a glass tube.
ii. The graduated tube has marking on the opposite sides.
iii. The markings on one side indicate the percentage (%) of haemoglobin. The markings on the other side indicate the quantity of haemoglobin in grams (gms).

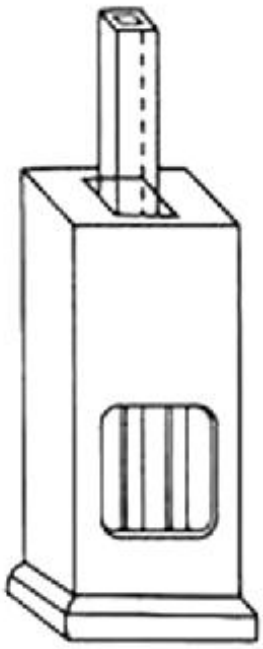
3. Micropipette

It has a marking at the top as 20 cubic mm. It is used to suck the blood from the finger tip.

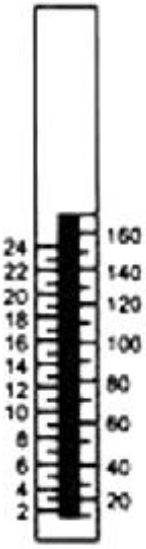
USES

It is used to determine the quantity of haemoglobin present in the blood in grams or in percentage.

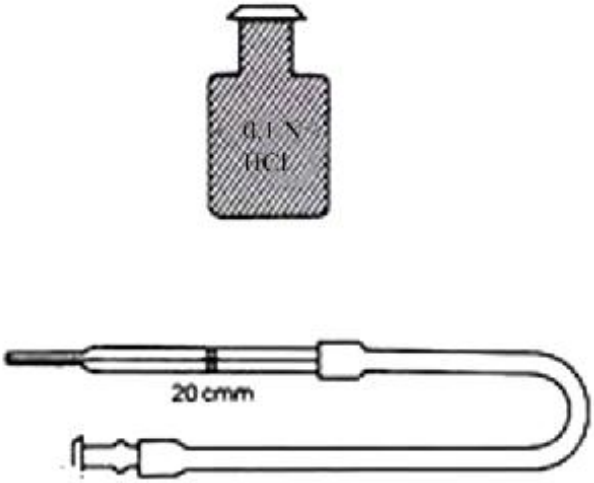
Sahli'sHaemoglobinometer



Haemoglobinometer
(Comparator)



Haemoglobinometer
tube



Haemoglobinometer
pipette

HAEMOCYTOMETER

1. Haemocytometer is a device used to count the number of RBC, WBC, microbes, etc. The common type of Haemocytometer used is NeubaeurHaemocytometer.
2. It consists of 2 graduated dilution pipettes, a rectangular glass slide and a cover glass. For counting the bacterial cell, only the glass slide is used. The glass slide consists of 2 counting chambers.
3. The glass slides is in the form of transverse bar. The bar is divided in the centre by a groove to have 2 counting chambers. 2 samples can be loaded at the same time for blood cell counting.
4. Each counting chamber is a square ruled area of $9mm^2$ and a side length of 3mm.
5. The depth of the counting chamber is 0.1 mm.
6. Each counting chamber is divided into 9 large squares. Each square has an area of 1 mm^2 and a volume of 0.1 mm^3.
7. The four corner squares are meant for WBC counting (WBC counting area)
8. The central square is meant for RBC counting (RBC counting area).
9. Each WBC counting square is further divided into 16 small sized squares.
10. Hence there are 64 small sized squares in the WBC counting area.
11. The central RBC counting square is further divided into 25 small sized squares.
12. Each small sized square of the central large square is again divided into 16 smaller squares.

13. Hence there are 400 smaller squares in the RBC counting area.
14. Small amount of blood is loaded in both the counting chamber.
15. RBC is counted from the central smaller squares, WBC is counted from the corner square.
16. Corpuscles on the line should not be counted twice.
17. Normal range of RBSs in men is 4.5 to 5.9 million / cu mm, and that of women is 4.1 to 5.1 million/cu mm. Normal range of WBCs in adult is 4,500 t0 11,000/cu mm.

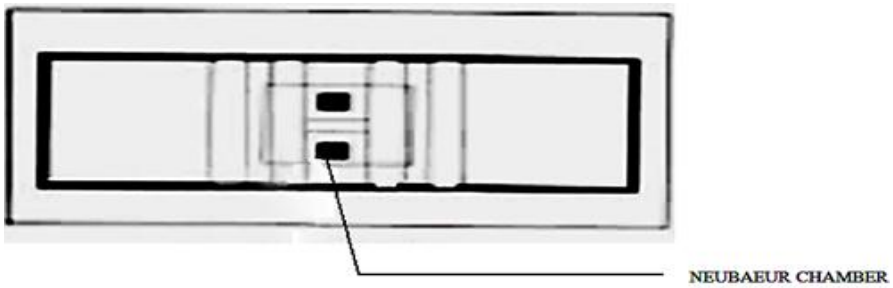
RBC PIPETTE

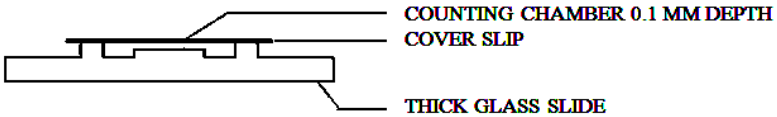
1.It is nothing but a glass tube.
2.One end of the pipette is attached to a rubber tube for sucking the blood.
3.The glass tube contains a graduated capillary tube.
4.The capillary tube opens into a bulb.
5.The bulb contains a red bead.
6.The graduations on the capillary tube are 0.5 below the bulb and 101 just above bulb.

WBC PIPETTE

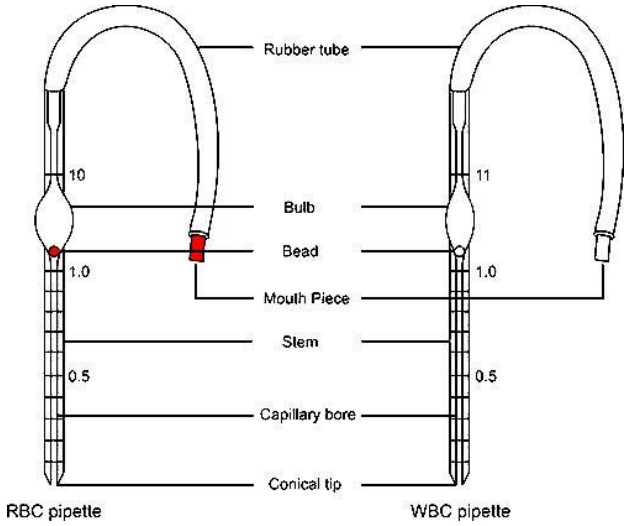
1.It is nothing but a glass tube
2.It is attached to a rubber tube for sucking the blood.
3.The glass tube contains a graduated capillary tube.
4.The capillary tube opens into a bulb.
5.The bulb contains a white bead.
6.The graduation on the capillary tube is 0.5 just below the bulb and 11 just above the bulb.

NAUBAEUR'S COUNTING CHAMBER

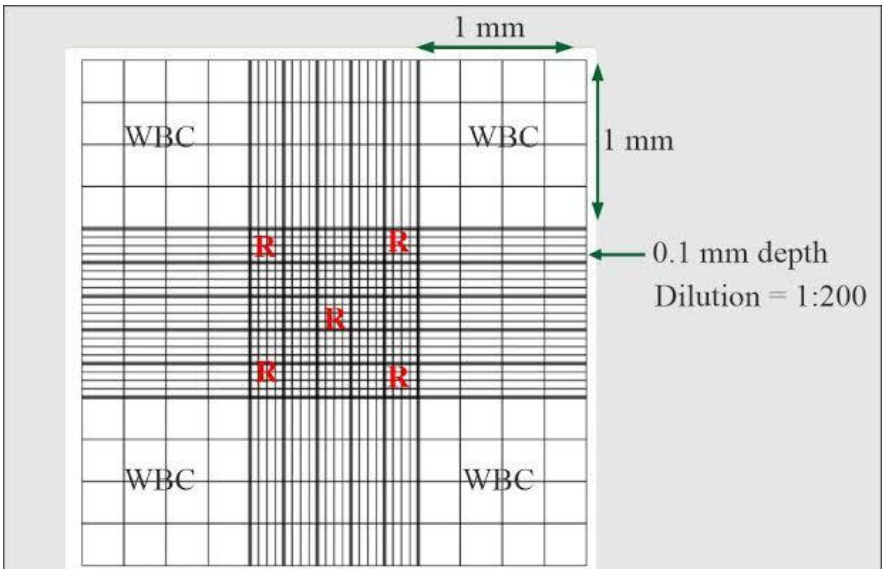




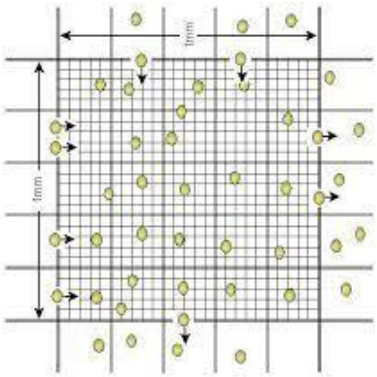
SIDE VIEW



Counting Chambers

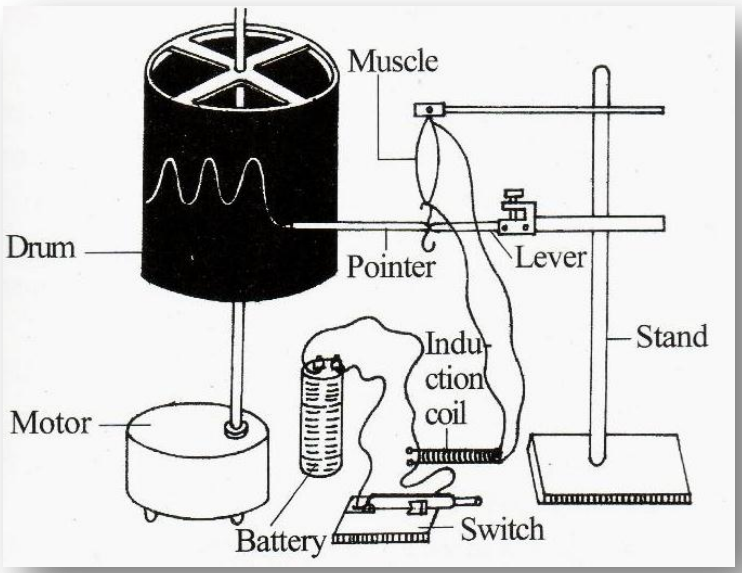


Small squares in the 1 mm square for red cell count



KYMOGRAPH

1. Kymograph is a physiological instrument used to record **muscle twitch.**
2. The muscle twitch is recorded in the form of a curve called muscle curve
3. The instrument consists of a motor, a central rod, a rotating drum, a stand, a battery and an induction coil.
4. The muscle is suspended from the clamp of the sand.
5. The free end of the muscle is tied with a lever. The lever has a pointer which is directed towards the rotating drum.
6. The speed of the drum can be adjusted by a gear system.
7. The muscle selected for studying muscle twitch is frog's gastrocnemius muscle (thigh muscle).
8. The two terminals of the wire coming out from the induction coil are connected to the ends of the muscle.
9. When an electric shock is given, the muscle contracts and hence shortens.
10. As a result the pointer moves up and makes a line of the smoked paper of the moving drum.
11. When the muscle relaxes, the pointer goes down and draws the next line. Both these lines form a muscle curve. The muscle curve is recorded on the paper.



CARDIAC MUSCLES

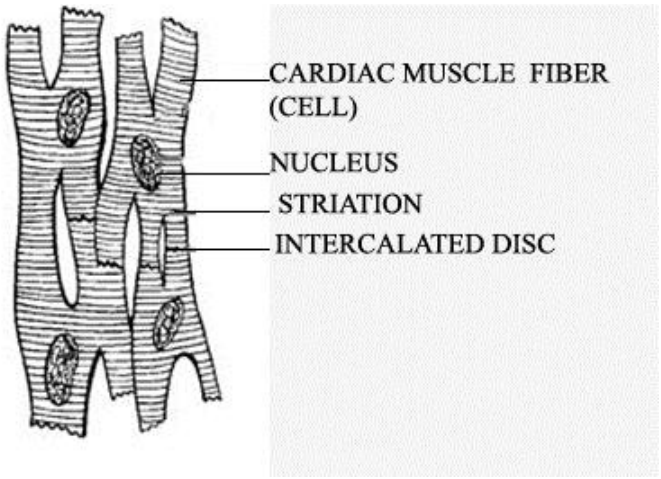
These muscles are found in the heart of animals (vertebrates). The fibres of these muscles are **involuntary** in nature as their activities are not under the control of will. The fibres of these muscles are both longitudinally and transversely striated. The mechanism of contraction is essentially the same as those in skeletal muscles but these muscles differ histologically from striated muscle of vertebrates in some ways.

i) These muscles are **uninucleated** whereas striated muscles are multi-nucleated.

ii) These are automatically innervated, while striated muscles are innervated by peripheral fibres whose cell bodies lie within the central nervous system and they inter-digitate to form what are known as **intercalary discs**.

The cardiac muscle fibres are very much branched and the branches unite with each other forming a lace work or network. Sarcolemma is absent.

CARDIAC MUSCLE TISSUE



STRIATED MUSCLES

Striated muscles are **voluntary** muscles.

They obey the will of the individual

They are found in association with skeletal system. Hence they are called **skeletal** muscles.

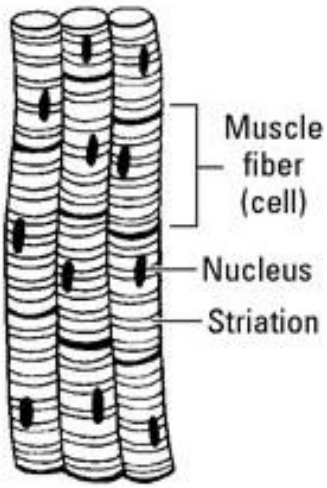
They have longitudinal and cross striations hence called **striated** muscles.

Each muscle is formed of many **muscle fibres**. A single muscle fibre consists of an outer sarcolemma, an inner sarcoplasm and many myofibrils. Each myofibril contains alternative dark bands (A bands) and light bands (I bands). The bands of the myofibril contain two types of proteins such as action and myosin.

Individual fibres are multinucleate. Their activity is under the control of central nervous system such as brain and spinal cord.

Eg.Biceps muscles, triceps muscles.

SKELETAL MUSCLE TISSUE



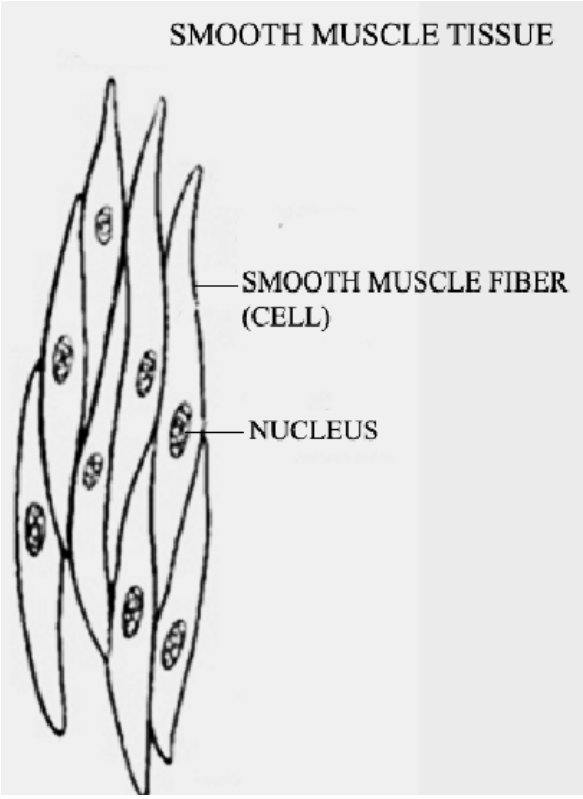
NON STRIATED MUSCLES

It is also known as **involuntary** muscles because the activities of these muscle are not under the control of our will.

They have no connection with the skeletal parts of the body. They do not have cross striations hence called non striated muscles.

The muscle is formed of many muscle fibres. Each muscle fibre is spindle shaped having an outer sarcolemma and inner sarcoplasm and nucleus. The individual muscle fibres are uninucleate.

They generally form the walls of the four great tracts of hollow organs such as respiratory, digestive, urinary and genital tracts.



SIMPLE MUSCLE TWITCH

Muscle has the ability to respond to stimulus and become active.The stimulus may be mechanical or chemical or thermal or electrical.Electrical stimulus is mostly used in the laboratory experiment.

In the laboratory experiment when the skeletal muscle is stimulated it contracts.The contraction is followed by relaxation.The contraction followed by relaxation due to a single stimulus is called as muscle twitch.

The muscle twitch can be recorded by means of an apparatus called **kymograph.**The record of the muscle twitch on a moving drum of kymograph will produce a curve called simple muscle curve.

Each muscle curve has 4 points ABCD and 3 phases.The three phases are latent period,period of contraction and period of relaxation.

A-Represents the time at which the stimulus is applied

B- Represents the starting point of muscle contraction

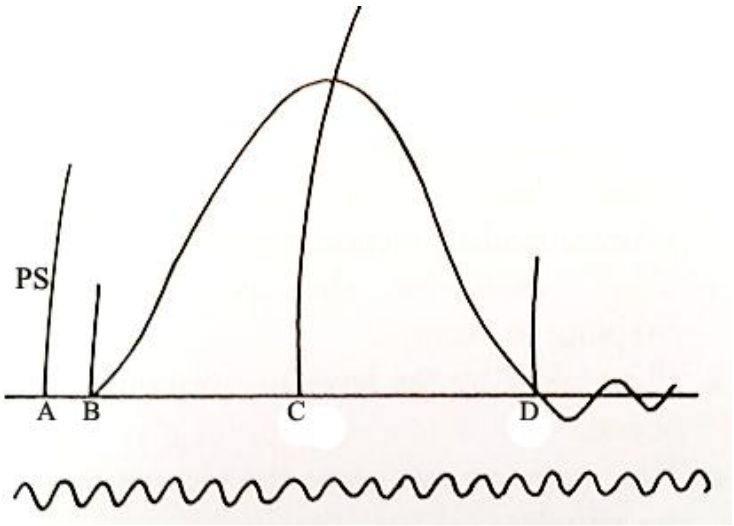
C- Represents the point at which relaxation starts

D-Represents the point at which relaxation comes to halt

Latent period (A-B) is the interval between the application of a stimulus and the beginning of contraction.It lasts for 0.01 second

Period of contraction (B-C) is the period from the beginning of contraction up to the maximum contraction.The contraction phase lasts for 0.04 second

Period of relaxation (C-D) is the period from the summit to the base.The relaxation phase lasts for 0.05 second.



Simple muscle twitch with time-trace below the curve.

PS: Point of Stimulation; AB: Latent period; BC: Contraction period; CD: relaxation period

OVARY T.S.

(i) A mammalian ovary is a solid structure bounded germinal epithelium followed by a thick layer of fibrous tissue called **tunica albuginea**.The ovary consists of the outer cortex and inner medulla.Inside that you will observe ovum.

(ii) In the section of ovary,there is a cell surrounded by one to several layers of **follicular cells**.As the ovum matures,the number of surrounding follicular cell layer increases.

(iii) In the later stage of follicular development a cavity called antrum appears.

(iv) The cavity gets further enlarged and the follicle grows bigger.This is the stage of **Graafian follicle** ready to release the ovum(ovulation)

(v) In the next stage,you may notice a corpus luteum or corpus albicans, which differ from each other and also from Graafian follicle in their features.