Qualitative Tests for Identification of Carbohydrates:

MOLISCH TEST:

AIM: Identification of carbohydrates in the given A, B samples

Principle: Molisch test is a specific test for the detecting the presence of carbohydrates. Acid hydrolysis (Con.H2SO4) of carbohydrates (glycosidic bonds) yields monosaccharide which are then dehydrated to furfural and its derivatives. On reaction with sulphonated alpha naphthol (Molisch agent), furfurals produce purple or brick reddish violet complex between acid and sugar layer.

Reagents: Molisch reagent, 5% glucose solution

Procedure: Pippet out 5 ml of sample solution A into one test tube and sample B into another test tube. Add few drops of Molisch reagent in to the test tubes. Hold the test tube in a slanting position and 3 ml of con H2SO4 gently down the sides of test tube.

Observation: observe the purple/ reddish-violet coloured complex at the junction of the two solutions.

Result:

S.No	Sample	Experiment	Inference	Result
1	Sample A	Added the few drops of Molisch reagent and 3 ml of con H2SO4 to the sample	Purple colored complex formed at the juncture of twosolutions	The carbohydrates are present
2	Sample B	‘’	No color is observed	Carbohydrates are absent.

BENEDICTS TEST:

Aim: identification of carbohydrates (reducing sugars) in the given samples

Principle: Benedict's reagent contains metallic cupric ions and is good oxidising agent. When Benedict's reagent is added it oxidises the sugar and reduces itself from the cupric to cuprous form. This reaction is indicated by the formation of yellow to red cuprous oxide precipitation.

Procedure: take 5 ml of Benedict's reagent in two test tubes and add the given A,B samples to Reagent containing test tubes. Boil the contents for few minutes in the hot water bath.

Observation: observe the colour changes of the contents from green to yellow to orange to red colour in the samples

Result:

S.No	Sample	Experiment	Inference	Result
1	Sample A	Added the 5ml of Benedict's reagent and heat the test tube in the water bath.	Yellow to brick red colour ppt is observed	The carbohydrates are present
2	Sample B	''	No colour is observed	Carbohydrates are absent.

BARFOED TEST:

AIM: Identification of sugars(common sugar/sucrose) in the given samples

Principle: Barfoed reagent is weak acid and is only reduced by monosaccharides.As sucrose is a disaccharide it is first hydrolysed by prolonged boiling with the barfoed reagent which contains acetic acid. It is also a copper reduction test but differ with other tests in that it reduces the sugars in the acedic medium.

Reagents: Barfoed reagent, sample solutions

Procedure: pipette out 5 ml of Barfoed reagent into two test tubes and add 0.5 ml of sample solutions to the test tubes. Heat the mixture to boiling.

Observation: observe the development of orange red coloured precipitate within 5 minutes. Continue the boiling of the mixture for 15 minutes if no colour is produced within 5 minutes

Result:

S.No	Sample	Experiment	Inference	Result
1	Sample A	Added the 5ml of Barfoed reagent and heat the test tube in the water bath.	Orange red colour ppt is observed	The carbohydrates(sugars)are present
2	Sample B	‘’	No colour is observed	Carbohydrates(sugars) are absent.

Iodine test:

Aim: identification of carbohydrates in the given sample solutions.

Principle: this is a specific test for a polysaccharide starch or animal glycogen. Starch as a polysaccharide insoluble in water and forms opalescent solution in water on boiling. With iodine starch forms blue coloured adsorption compound of iodide of starch.

Procedure: take 2 ml of sample solutions in to the two test tubes and few drops of iodine solution to the test tubes.

Observation: you can infer the blue colour that the given sample contains starch.

Result:

S.No	Sample	Experiment	Inference	Result
1	Sample A	Added the 5ml of Benedict's reagent and heat the test tube in the water bath.	Yellow to brick red colour ppt is observed	The carbohydrates are present
2	Sample B	''	No colour is observed	Carbohydrates are absent.

Identification of carbohydrates by special sucrose test:

Aim: identification of carbohydrates by special sucrose test

Apparatus and chemicals: Test tubes, spirit lamp,sucrose solution, fehlings reagent

Principle: Sucrose is a disaccharide, which means that it is a molecule that is derived from two simple sugars (monosaccharides). In the case of sucrose, these simple sugars are glucose and fructose. Inverted sugar is a mixture of glucose and fructose. It is obtained by splitting sucrose into these two components. The splitting of sucrose is a hydrolysis reaction which can be induced simply by heating an aqueous solution of sucrose. Acid also accelerates the conversion of sucrose to invert.

The sugar is treated with Fehlings solution(alkaline copper) cupric ions are reduced to cuprous ions and appear the red precipitate of cuprous hydroxide.

Procedure:

Add 5 mL of sucrose solution to two test tubes.

- Add 5 drops of conc. HCl to one testtube.
- Heat both tubes in boiling water bath for 10min.
- Cool and neutralize with diluted NaOH (use litmuspaper)
- Test both solutions for the presence of reducing sugar with Fehling'sTest.

-1 mL of Fehling's solution A (aqueous solution of CuSO4) add 1 mL of Fehling solution B (solution of

potassium tartrate).

- mix well andboil.

Try to see the red precipitate of cuprous oxide that forms at the end of the•reaction

Observation: appearance of the red colour is the indication for the presence of the

sugar. Result:

S.No	Sample	Experiment	Inference	Result
1	Sample A	Added the 5ml of sucrose solution and add 3 drops of HCl then heat and neutralize with NaOH AFTER cooling add Fehlings solution and boil.	red colour ppt is observed	The carbohydrates are present
2	Sample B	''	No colour is observed	Carbohydrates are absent.

Qualitative tests for identification of Proteins:

Identification of proteins by Millon's test :

Aim: Identification of proteins by Millon's test

Apparatus and chemicals: weighing balance, centrifuge, homogeniser, test tubes, pippets and glass rods, sample solutions and Millon's reagent.

Principle: Millon's test is a test specific for tyrosine, the only amino acid containing a phenol group, a hydroxyl group attached to a benzene ring. In Millon's test, the phenol group of tyrosine is first nitrated by nitric acid in the test solution. Then the nitrated tyrosine complexes mercury (I) and mercury (II) ions in the solution to form a red precipitate or a red solution, both positive results. Proteins that contain tyrosine will, therefore, yield a positive result.However, some proteins containing tyrosine initially forms a white precipitate that turns red when heated.

Procedure: take 2 test tubes and transfer the sample solutions 1ml into each test tube. Now add few drops of Millon's reagent to the test tube and boil in hot water bath for few minutes.

Observation: observe the white ppt produced and changing to red on heating.

Result:

S.No	Sample	Experiment	Inference	Result
1	Sample A	Take 1ml of sample into the test tube and few drops of Millon's reagent and heat on water bath for few minutes	red colour ppt appeared	The proteins are present
2	Sample B	''	No colour is observed	Proteins are absent.

Identification of proteins by Lead Acetate test:

Aim: Identification of proteins by lead acetate test

Apparatus and chemicals: weighing balance, centrifuge, homogeniser, test tubes, pippets and glass rods, sample solutions and lead acetate.

Principle:

This is a specific test sulphur containing amino acids like cysteine and Cystine. When these proteins are dissolved in NaOH the sulphur of aminoacids combines with Na ions and form sodium sulphide. This in addition of Lead Acetate by double decomposition reaction black coloured lead sulphide is (PbS) produced.

Procedure: take 2 test tubes and transfer the sample solutions 1ml into each test tube. Now add 1 ml of NaOH to each test tube and boil it for few minutes then add few drops lead acetate.

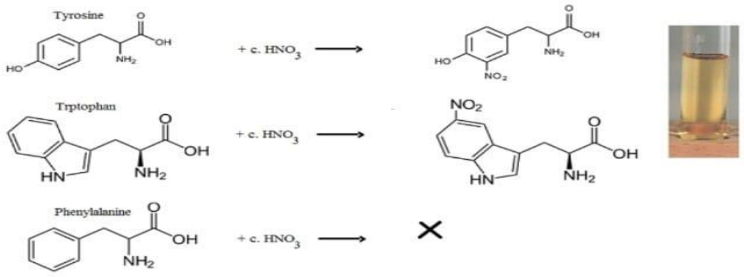
Observation: observe the formation of black or brown colour ppt.

Result:

S.No	Sample	Experiment	Inference	Result
1	Sample A	Take 1ml of sample into the test tube and 1ml of NaOH boil for few minutes and add lead acetate.	black colour ppt appeared	The proteins are present
2	Sample B	‘’	No colour is observed	Proteins are absent.

Identification of proteins by Xantho proteic test:

Aim: To identify the aromatic amino acids in the given sample
Apparatus and chemicals: test tube, Beakers, Centrifuge, Homogeniser, Test sample containing albumin, Nitrirc acid, 40% NaOH
Principle: The aromatic benzene ring underegoes nitration to give yellow color product on heating with concentrated HNo3. Phenyl alanine gives negative or weakly positive reaction as it is difficult to nitrate under normal condition. On adding alkali to these nitro derivatives the color changes from yellow to orange.



Procedure:1. take 1 ml test solution in dry test tube.
2. Similarly 1ml distilled water in another test tube as control
3. Add 1ml conc.HNO3 in both test tubes and mix well then it will turn in to yellow color.
4. Heat the test tubes in a water bath
5. Cool the solution under the tap water
6. Now add 2ml of 40% NaOH to both test tubes. Now the test tube containing aromatic amino acids will turns to orange color.

Result:

S.No	Sample	Experiment	Inference	Result
1	Sample A	Take 1ml of sample into the test tube and 1ml of Con HNO3 boil for few minutes and cool the test tube	Yellow color turns to orange color	The proteins with aromatic amino acids are present
2	Sample B	‘’	No colour change observed	The proteins with aromatic amino acids are not present

Qualitative test for identification of Lipids:

Identification of lipids by solubility test:

Aim: identification of lipids in the given samples.

Apparatus and chemicals: centrifuge, homogeniser, test tubes, pippets and glass rods,vegetable oil, water,chloroform, benzene

Principle:lipids are insoluble in water but soluble in non polar solvents like benzene and alcohol. Based in the solubility of the lipids this test is conducted.

Procedure: the sample(vegetable oil) is taken into 5 test tubes and add ether, benzene, chloroform, hot alcohol and water to the sample serially and shake the contents thoroughly. Due to the nature of insolubility of sample in water we observe the result.

Observation: the sample donot dissolve in the water.

Result:

S.No	Test tube	Experiment	Inference	Result
1	A	Take few drops of oil and ether solution.	The sample is dissolved	The sample is lipids.
2	B	Take few drops of oil and chloroform solution.	The sample is dissolved	The sample is lipids.
3	C	Take few drops of oil and benzene solution.	The sample is dissolved	The sample is lipids.
4	D	Take few drops of oil and hot alcohal solution.	The sample is dissolved	The sample is lipids.
5	E	Take few drops of oil and water	The sample not dissolved	The sample is lipids.

IDENTIFICATION LIPIDS BY SUDAN-IV TEST:

Aim: identification of lipids in the given samples.

Apparatus and chemicals: centrifuge, homogeniser, test tubes, pippets and glass rods,vegetable oil, water,chloroform,benzene

Principle: Sudan IV is a far soluble dye and is insoluble in water. Due to this property of dye the lipids are detected. Lipids from dispersion medium in water and to this when sudan IV dye is added, lipids produce a dark redcolour.

Procedure: take two test tubes and sample A to test tube one and take few drops of vegetable oil (sample B)into the test tube two and add 3ml of water to thesr and shake the contents well. As the fats are insoluble in water two separate layers of fat and water are formed. To the contents add a pinch of sudan-IV dye. Then shake the test tubes thoroughly and allow it to stand for 5 minutes.

Observation: observe the distribution of dye in the oil and a dark red colour is formed.

Result:

S.No	Sample	Experiment	Inference	Result
1	Sample A	Take 1ml of sample into the test tube and 3ml of water and shake the contents and a pinch of Sudan IV dye. Then shake the test tube well.	A red colour dispersion is observed.	The lipids are present in the sample.
2	Sample B	‘’	No colour is observed	lipids are absent.

Qualitative test for identification of Ammonia:

IDENTIFICATION OF AMMONIA BY NESSLERS'S TEST:

Aim: identification of ammonia in the given sample

Apparatus: test tubes, test tube stand, test tube holders, Bunsen burner, sample solutions, Nessler's reagent.

Principle: when a solution of potassium mercuric iodide containing excess of potassium hydroxide (Nessler's reagent) is added to a sample containing traces of ammonia, produces a brown precipitate of ammonium mercuric iodide.

$2KHgI_3+NH_3 \longrightarrow NH_2HGI+2KI+HI$

Procedure: take two test tubes and transfer 2ml of the samples into the test tubes. Then add the nessler's reagent to the test tubes and wait.

Observation: observe the formation of reddish brown precipitate.

Result:

S.No	Sample	Experiment	Inference	Result
1	Sample A	Take 2ml of sample into the test tube and few drops of Nessler's reagent and wait	A red brown colour ppt is observed.	The ammonia is present in the sample.
2	Sample B	''	No colour is observed	Ammonia is absent.

IDENTIFICATION OF UREA IN THE GIVEN SAMPLES:

Aim: identification of urea in the given sample

Apparatus: test tubes, test tube stand, test tube holders, Bunsen burner, sample solutions, urease enzyme, Nessler's reagent.

Principle: when a sample containing urea is treated with urease enzyme, it hydrolyses urea to ammonia and carbon dioxide. This when treated with Nessler's reagent it produces the brown red colour ppt.

Procedure: take the samples A,B in to two test tubes. And boil sufficiently to expel ammonia that may be present in the sample. Cool the contents and add a little pinch of urease powder. Shake the contents well and add 1ml of Nessler's reagent.

Observation: a brown red colour ppt is observed.

Result:

S.No	Sample	Experiment	Inference	Result
1	Sample A	Take 2ml of sample into the test tube and boil it then cool it and urease powder and few drops of Nessler's reagent and wait	A red brown colour ppt is observed.	The urea is present in the sample.
2	Sample B	''	No colour is observed	urea is absent.

IDENTIFICATION OF URIC ACID IN THE GIVEN SAMPLE

Aim: identification of uric acid in the given sample

Apparatus: test tubes, test tube stand, test tube holders, Bunsen burner, sample solutions, sodium carbonate solution, Follin's reagent.

Principle: when phospho tungstic acid (Follin's reagent) is added to the sample containing uric acid, the acid reduces phospho tungstic acid. The reduction is indicated by the formation of a blue colour.

Procedure: take two test tubes and 2 ml of sample solution to each test tube. Add 1ml of saturated sodium carbonate solution to neutralise the medium. Then add 1ml of Follin's reagent to the contents in the test tube and shake it thoroughly.

Observation: observe the appearance of blue colour.

Result:

S.No	Sample	Experiment	Inference	Result
1	Sample A	Take 2ml of sample into the test tube and add sodium carbonate solution then add Follin's reagent.	blue colour is observed.	The uric acid is present in the sample.
2	Sample B	''	No colour is observed	Uric acid is absent.

Zonation of gut in cockroach:

The alimentary canal starts from mouth and it consists of the preoral cavity, pharynx, oesophagus, crop and gizzard forming the foregut or stomodaeum; the mesenteron forming the midgut and the ileum, colon and rectum constituting the hindgut or proctodaeum.

The stomodaeum and proctodaeum are ectodermal in origin and lined internally by the continuation of the exoskeletal cuticle, while the mesenteron is endodermal in origin and without cuticular lining.

Foregut:

The so-called mouth is situated at the base of the pre-oral cavity or buccal chamber, also known as cibarium. The buccal chamber is a space in front of the mouth into which the food is received. This cavity is bounded in front by the labrum, posteriorly by the labium and on each side by a mandible and a maxilla. Inside this cavity a large tongue-like hypo pharynx is present.

The mouth opens behind into a short tubular pharynx which passes vertically upwards, then it bends backward into an oesophagus. The oesophagus is a narrow tubular passage which passes through the neck and gradually expands in the thorax, finally taking the shape of sac-like structure in abdomen. This sac-like structure is called crop which is thin-walled and muscular. The crop is followed by a gizzard or pro-ventriculus.

The gizzard is a round, thick-walled bulb-like structure. Structurally, it has an outer thick layer of circular muscles and its lumen gets considerably reduced due to the in-folding of its wall. The gizzard can be divided into anterior armarium and posterior stomodaeal valve.

The cuticular lining of armarium is thickened to form six highly chitinised plates called teeth. Behind the teeth, there are thin less chitinised plates which bear cushion-like pads covered with backwardly directed bristles. The deep grooves are also provided with fine bristles.

The teeth help in grinding the food, while bristles help in straining the food to allow only well crushed food to pass on. The hind part of the gizzard projects into the midgut as a funnel, called stomodaeal valve which prevents the passage of food from midgut into the gizzard.

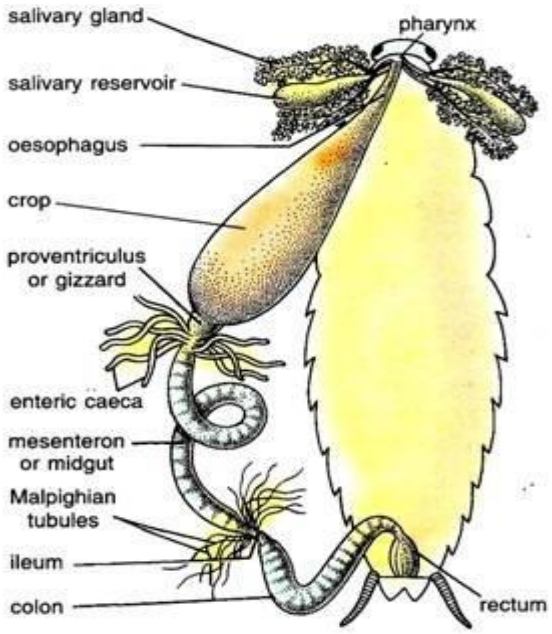


Fig. 73.13. *Periplaneta.* Digestive system.

Midgut:

The gizzard is followed by a narrow tube of uniform diameter representing the midgut or mesenteron. Its junctional region with the gizzard, which actually surrounds the stomodaeal valve, is called cardia. From this region, arise eight finger-like tubular blind processes called hepatic caeca or enteric caeca or mesenteric caeca.

The midgut is formed of tall columnar endodermal cells which are glandular in nature. The internal lining of the midgut is thrown out into small but several folds forming villi and covered by a very thin layer of transparent peritrophic membrane.

The peritrophic membrane is secreted by the anterior end of the cardia and it is permeable for enzymes and for the end products of digestion. It also protects the wall of the midgut. The process of digestion is completed in this region of the alimentary canal and the digested food is also absorbed in it.

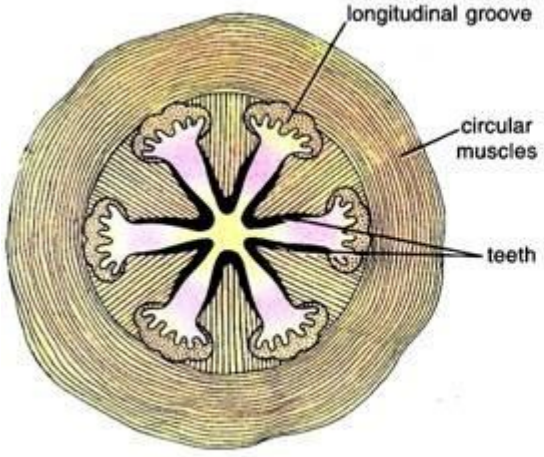


Fig. 73.14. *Periplaneta.* T.S. of gizzard.

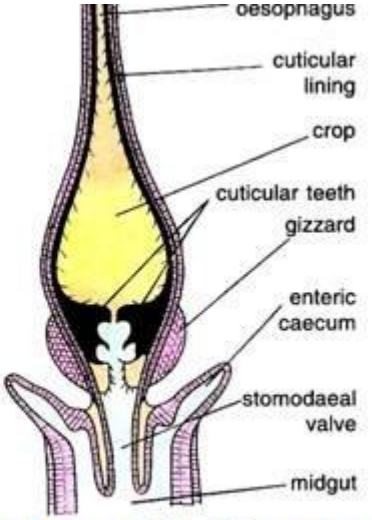


Fig. 73.15. *Periplaneta.* L.S. of foregut and anterior part of midgut.

Hindgut:

The junction of midgut and hindgut is marked by the presence of nearly sixty to one hundred fifty long, filamentous and blind Malpighian tubules which are not related with digestion but with excretion. The hindgut is relatively broader than the midgut. It is ectodermal and lined

internally with the cuticle. Its anterior region following the midgut is called ileum which is followed by a long and coiled colon.
The colon ends in a broad rectum which opens by an anus lying posteriorly below the 10th tergum. The lining of the colon is wrinkled and that of the rectum forms six thick longitudinal folds called rectal papillae. The cuticle covering the papillae is very thin but its underlying epithelium is thick, this is perhaps an adaptive device for absorbing maximum water from the passing out faeces.

EFFECT OF TEMPERATURE AND pH ON SALIVARY AMYLASE ACTIVITY:

Effect of Temperature:

AIM: To find out the effect of temperature on salivary amylase activity

Apparatus and chemicals: test tubes, beaker, droppers, Bunsen burner and thermometer, 1% starch solution, 1% NaCl solution, Amylase, Iodine.

Principle: Enzymes are bio catalysts and they are active at a particular temperature and they are denatured at lower or higher levels of the temperature.

Procedure: Take 3 test tubes and add 5ml of 1% starch solution and l ml of 1% of NaCl solution to each test tubes. Add 1 ml of salivary amylase to each test tube and mark them as A,B &C. Keep the A test tube in ice cubes, B test tube in water bath of 37˚C and C test tube in the water bath 50˚C. After few minutes add these contents the Iodine solution containing test tubes. Do the same with the changed times of exposure to the temperature.

Observation: the test tube A and test tube C are at the lowest and highest temperature so the amylase activity is prevented so they give blue color will be formed against iodine but the test

tube C is at active temperature so it convert the starch into maltose sugar it will not give the colour.

Reseult:

S.No	Test tube	Test	Observation
1	A	Add 5ml of 1% starch solution, 1ml of 1% NaCl and 1ml of salivary amylase. Keep it in the ice cubes. After some time transfer it to the iodine solution.	The blue color appeared because the enzyme is not active.
2	B	Add 5ml of 1% starch solution, 1ml of 1% NaCl and 1ml of salivary amylase. Keep it in the water bath at 37˚C. After some time transfer it to the iodine solution.	No color is seen because the enzyme is active and converted the starch into maltose sugars.
3	C	Add 5ml of 1% starch solution, 1ml of 1% NaCl and 1ml of salivary amylase. Keep it in the water bath at 50˚C. After some time transfer it to the iodine solution.	The blue color appeared because the enzyme is not active.

Effect of pH:

AIM: To find out the effect of temperature on salivary amylase activity

Apparatus and chemicals: test tubes, beaker, droppers, Bunsen burner and thermometer, 1% starch solution, 1% NaCl solution, pH capsules ,Amylase, Iodine.

Principle: Enzymes are bio catalysts and they are active at a specific pH and they are denatured at lower(acidic) or higher levels(base) of the pH.

Procedure: Take 3 test tubes and add 5ml of 1% starch solution and l ml of 1% of NaCl solution to each test tubes. Add 1 ml of salivary amylase to each test tube and mark them as A,B &C. Add pH 5 tablet to A test tube ,6.8 pH tablet to B test tube and pH 8 tablet to C test tube . immediately add these contents to the Iodine solution containing test tubes. Do the same with the time intervals.

Observation: the test tube A and test tube C are at the acidic and basic medium so the amylase activity is not shown at this pH so blue colour will be formed against iodine but the test tube C is at 6.8 pH at which the enzyme is active so it convert the starch into maltose sugar it will not give the blue colour.

Result :

S.No	Test tube	Test	Observation
1	A	Add 5ml of 1% starch solution, 1ml of 1% NaCl and 1ml of salivary amylase. And pH 5 tablet. Immediately transfer it to the iodine solution.	The blue colour appeared because the enzyme is not active.
2	B	Add 5ml of 1% starch solution, 1ml of 1% NaCl and 1ml of salivary amylase. And pH 6.8 tablet. Immediately transfer it to the iodine solution.	No colour is seen because the enzyme is active and converted the starch into maltose sugars.
3	C	Add 5ml of 1% starch solution, 1ml of 1% NaCl and 1ml of salivary amylase. And pH 8 tablet. Immediately transfer it to the iodine solution.	The blue colour appeared because the enzyme is not active.

Study of permanent Histological sections of Mammalian endocrine glands:

T.S OF PITUTORY GLAND:

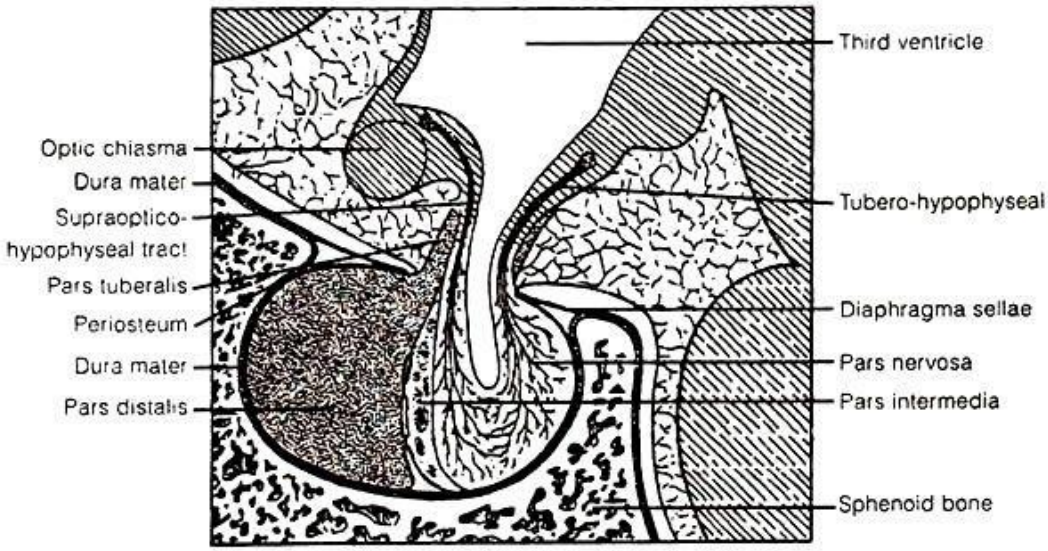


Fig. 7.11 : Diagrammatic sagittal section of human hypophysis

1. The entire pituitary gland is ectodermal inorigin.
2. Beneath the hypothalamus, near the optic chiasma, the pituitary gland lies at the base ofthe skull in a portion of the sphenoid bone called the sellaturcica.
3. The human pituitary is composed of an adenohypophysis (glandular or epithelial hypophysis) and aneurohypophysis.

A.ADENOHYPOPHYSIS

It consists of the following parts

1. Parsdistalis:

The pars distalis of adenohypophysis is composed of irregular masses and cords of epithelial cells separated by sinusoids and supported by a loose framework of connective tissue.

2. Parstuberalis:

This thin sleeve-like region of adenohypophysis is only 25-60 µm in thickness (Fig. 7.11), with its thickest portion on its anterior aspect.

3. Parsintermedia:

This lobe is rudimentary in adult human, but in other vertebrates like rat and in poikilotherms, the intermediate lobe is composed of polygonal cells that take basic dyes and in which secretory granules with diameter of 200-250 nm have been observed.

B. Neurohypophysis:

Neurohypophysis consists of median eminence of tuber cinereum, the infundibular stem and pars nervosa. The pars nervosa portion of neurohypophysis consists of branching cells, called pituicytes (P), thick network of fine un-myelinated nerve fibres that are the termination of the hypothalamohypophyseal tract

THYRIOD GLAND T.S:

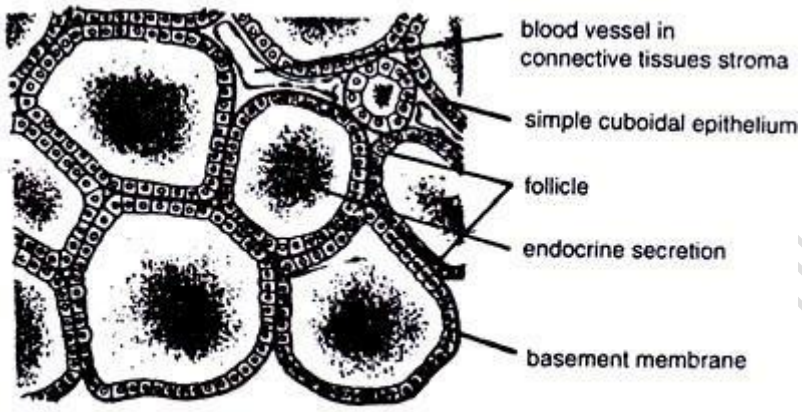


Fig. 41.15 : Thyroid gland. Mammalia. Transverse section

1. The thyroid gland is enclosed in a two layered capsule, the outer and inner. Septa of fibro-elastic tissue extend inwards from the inner capsule and divide the gland intolobules.
2. The glandular tissue (parenchyma) consists of groups of irregularly sphericalfollicles.
3. The follicles are supported by connective tissue carrying blood vessels, lymphatic's and nerves.
4. The follicles are lined by cuboid cells with spherical nucleus and supported by a thin basementmembrane.
5. Microvilli present on the apex of thecells.
6. Cytoplasm contains granules anddroplets.
7. Golgi apparatus well developed and the apical cytoplasm contains numerous membrane bound densegranules.

T.S OF PANCREASE:

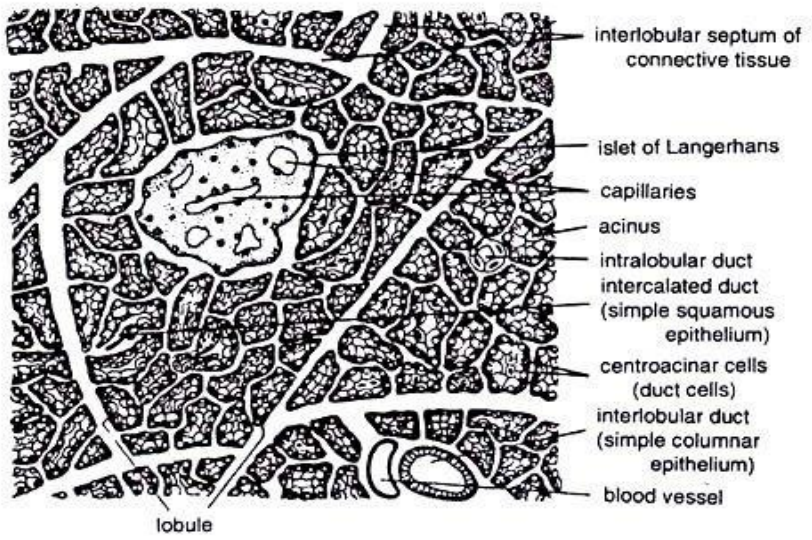


Fig. 41.9 : Pancreas. Mammalia. Section

1. Consists of a number of secretory alveoli, the exocrine component. Endocrine components or islet of Langerhans, are scattered amongst the exocrinetissues.

2. Exocrinecomponent:

a. Most of the alveoli are tubular and surrounded by alamina.

b. Cells lining the lumen of the alveolus are somewhat conical with a granular cytoplasmand a sphericalnucleus.

c. In a resting alveolus, the lumen is small and the cells are packed with zymogen granules except for a narrow outerzone.

d. In an active alveolus, the lumen is distended and the granules restricted only to the innermost part of thecell.

3. Endocrinecomponent:

a. Solid groups of lightly staining cells, marked off from the alveolar portion by a thin reticularmembrane.

b. Blood supply extremelyrich.

c. The cells contain granules and are of fourtypes,

i. coarsely granular α cells, less innumber.

ii. β cells containing small granules, much morenumerous.

iii. δ and F cells contain small granules and are less innumber.

ADRENAL GLAND T.S:

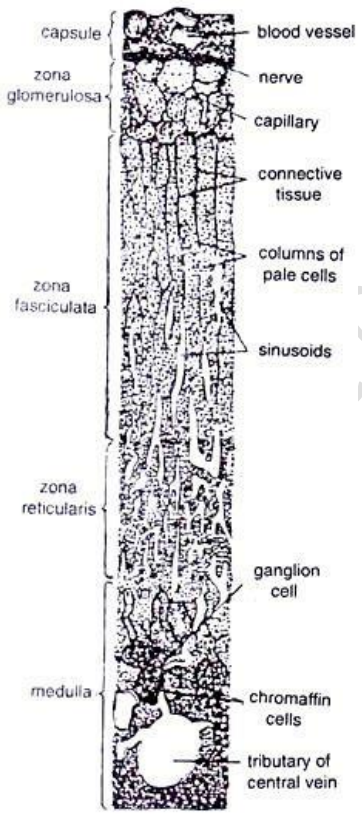


Fig. 41.16 : Adrenal gland.
Mammalia. Transverse section

1. The gland is enclosed in a thick capsule of fibrous connective tissue containing afew musclefibres.
2. Gland consists of a firm, outer cortex and a soft, centralmedulla.
3. Cortex consists of three layers, somewhat vaguely differentiated and merge into one another.
4. The outer zona glomerulosa is a narrowzone.
a. The cells are small, columnar and closely packed in roundedgroups.
b. The free surface of each cell adjoins acapillary.
c. The nuclei round, stain deeply and the cytoplasm isbasophil.
5. The middle zona faciculata isthickest.
a. Cells are large, polyhedral with vesicular nucleus and rich inlipid.
6. The inner zona reticularis is thicker than zonaglomerulosa.
a. The cords of cells are arranged as an anastomosing network round thesinusoids.
b. Cells are large, polyhedral, with vesicular nucleus and lesslipid.

7. The medulla consists of polyhedral cells arranged in groups and in contact with venous sinuses.
a. Cells are supported by a meshwork of fine fibres and contain small granules.

Estimation of haemoglobin by sahli method:

AIM: To estimate the haemoglobin in the given blood sample

Principle (Sahli Method): The haemoglobin per cent in vertebrate blood is estimated by acid-haematin method.Haemoglobin is converted to brown acid haematin with the addition of N/10 (0.1 N) hydrochloric acid. The brown colour is compared with standard brown glass plate .

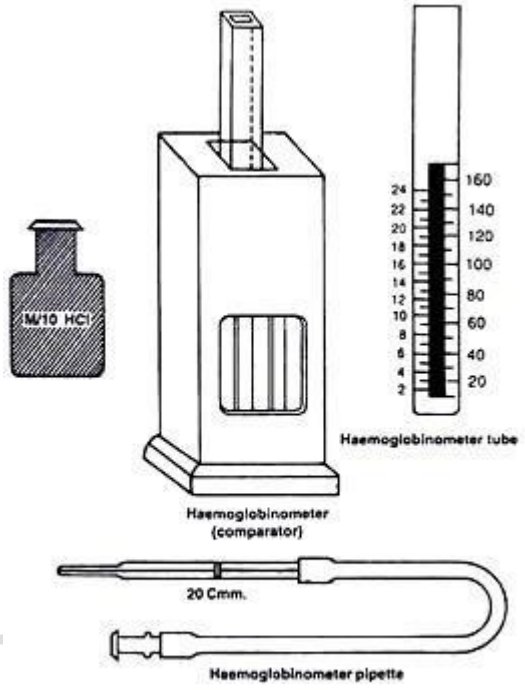


Fig. 51.9 : Sahli haemoglobinometer with accessories

Requirements:

i. Haemoglobinometertube:

It is a square graduated tube marked on both the sides in ascending order. The marks on the left side show g of Hb per 100 ml and on the right side indicate percentage.

ii. Haemoglobin comparator:

A mounted standard brown glass plate.

iii. Haemoglobinpipette:
A special slender pipette with a single mark 20 c mm.

iv. Vertebrateblood.

v. Decinormal (N/10) HCl solution. (1.2 ml concentrated HCI is diluted to 100 ml with distilledwater.)
Procedure:
i. The haemogiobinometer tube is filled to the lowest mark (20) with N/10HCI.

ii. Draw blood up to 20 c mm of the pipette from finger prick (p. 533) or from some other vertebrate. Wipe off the blood on outside the pipette with gauge or tissuepaper.

iii. Empty the pipette in the tube by touching the point of the pipette to the bottom of the tube and gently blowing off the blood without causing bubble. Rinse the pipette at least three times by drawing in and discharging the blood- acidmixture.

iv. Withdraw the pipette half way up the tube and rinse the outside of the pipette with a few drops of theacid.

v. Mix the acid-haematin solution with a glass rod and keep the tube in the comparator for at least 10 minutes. The brown color of acid-haematindevelops.

vi. The brown solution is diluted drop wise with distilled water and stirred with a stirreruntil the color matches with the standard brown glass plate in thecomparator.

vii. The matching of color should be done only against naturallight.

Reading:
The mark tallying with the upper level of diluted acid-haematin indicates the level of haemoglobin and is expressed in terms g per 100 ml blood.

Normal level of Hb:
Human–Male….....14.16 g %.

Female..... 13.15g%.

Newbornbaby..... 16.18 g %.

Toad .....6.9 g %

Estimation of blood clotting time:

The methods are:
1. Capillary Tube Method
2. Lee and White Method.

1. Capillary Tube Method:

Procedure:

i. Clean the tip of a finger with spirit.
ii. Puncture it upto 3 mm deep with a disposable needle.
iii. Start the stopwatch.
iv. Fill two capillary tube with free flowing blood form the puncture after wiping the first drip of blood.
v. Keep these tubes at body temperature.
vi. After 2 minutes, start breaking the capillary tube at 1 cm distance to see whether a thin fibrin stand is formed between the two broken ends.
vii. Stop the watch and calculate the time from average of the tow capillary tubes.

Disadvantages:

(i) Method is insensitive.
(ii) Method is unreliable.

Advantages:

It can be performed when venous blood cannot be obtained.
Normal clotting time is 1-5 minutes.

2. Lee and White Method:

Procedure:

i. After cleaning the forearm, make a venepuncture an draw 3 ml of blood in a silicon-sided glass or plast syringe.
ii. Start the stopwatch.
iii. Transfer 1 ml of blood each into 3 glass tubes which at kept 37° C in a water bath (Fig. 31.1)
iv. After 3 minutes tilt the tubes one by one every 30 second.
v. The clotting time is taken when the tubes can be title without spilling of their contents.
vi. Calculate the clotting time by average of 3 tube.

Advantages:

(i) More accurate and standard method.

(ii) Test can be run with control.

Disadvantages:

(i) It is also a rough method.

(ii) There can be contamination of syringe or tube.

Normal clotting time is 5-10 minutes.

Clinical Application of Clotting Time:

Clotting time is prolonged in following conditions:

(i) Sever deficiency of coagulation factors.

(ii) Afibrinogenaemia.

(iii) Administration of heparin.

(iv) Disseminated intravascular coagulation (DIC).

(v) Administration of drugs such as anticoagulants.

Identification of proteins by BIURET test:

Aim: Identification of proteins by BIURET test

Apparatus and chemicals: weighing balance, centrifuge, homogeniser, test tubes, pippets and glass rods, sample solutions and biuret reagent.

Principle: The biuret test for proteins positively identifies the presence of proteins in solution with a deep violet colour. Biuret, $H_2NCONHCONH_2$, reacts with copper (II) ions in a basic solution to form a deep violet complex. The peptide linkages in proteins resemble those in biuret and also form deep violet complexes with basic copper (II) ions in solution.

Procedure: take 2 test tubes and transfer the sample solutions 1ml into each test tube. Now add 1ml of biuret reagent to the test tube and shake the contents and allow it to stand for few minutes.

Observation: observe the development of purple to pink or violet colour in the protein sample.

Result:

S.No	Sample	Experiment	Inference	Result
1	Sample A	Take 1ml of sample into the test tube and 1 ml of biuret reagent and shake the content and allow them to stand.	Violet colour appeared	The proteins are present
2	Sample B	‘’	No colour is observed	Proteins are absent.

ESTIMATION OF UNIT OXYGEN CONSUMPTION BY FISH WITH REFERNCE TO BODY WEIGHT:

AIM: To estimate the unit oxygen consumption by fish with reference to its body weight.

Apparatus and chemicals: BOD bottles, burret, burret stand, pippet, beakers, Winkler's reagent A&B, sodium azide, Sulphuric acid, starch solution, hypo.

Principle:

in this Iodometry free o2 is liberated by the action of water with manganese sulphate and then alkali iodide and sulphuric acid. Liberated iodine is estimated by titration with sodium thiosulphate. Starch is used as indicator.

$2MnSO_4+2KOH \longrightarrow Mn(OH)_2+K_2SO_4$ ----------1

$2Mn(OH)_2+H_2O+1/2O_2 \longrightarrow 2Mn(OH)_3$---------- 2

$2Mn(OH)_3+2NaI+3H_2SO_4 \longrightarrow 2MnSO_4+Na_2SO_4+6H_2O+I_2$----------3

$2I_2+4Na_2S_2O_3 \longrightarrow 4NaI+2Na_2SO_4(O_6)$

Procedure:

Collect the water samples from the lake at a standard depth with the BOD bottles. For the controlled sample fix the bottles with winklers reagent. Then take the glass trough and fill
¼ with collected lake water. Then keep a fish with 200 gr weight in the glass trough and after 30 minutes take the water sample and estimate the O_2 in it by the following procedure for both the controlled and experimental samples.

Carefully fill a 300-mL glass Biological Oxygen Demand (BOD) stoppered bottle brim-full with sample water.

2. Immediately add 2mL of manganese sulfate to the collection bottle by inserting the calibrated pipette just below the surface of the liquid. (If the reagent is added above the sample surface, you will introduce oxygen into the sample.) Squeeze the pipette slowly so no bubbles are introduced via thepipette.
3. Add 2 mL of alkali-iodide-azide reagent in the samemanner.
4. Stopper the bottle with care to be sure no air is introduced. Mix the sample by inverting several times. Check for air bubbles; discard the sample and start over if any are seen. If oxygen is present, a brownish-orange cloud of precipitate or floc will appear. When this floc has settle to the bottom, mix the sample by turning it upside down several timesand let it settleagain.

5. Add 2 mL of concentrated sulfuric acid via a pipette held just above the surface of the sample. Carefully stopper and invert several times to dissolve the floc. At this point, the sample is "fixed" and can be stored for up to 8 hours if kept in a cool, dark place. As an added precaution, squirt distilled water along the stopper, and cap the bottle with aluminum foil and a rubber band during the storageperiod.
6. In a glass flask, titrate 201 mL of the sample with sodium thiosulfate to a pale straw color. Titrate by slowly dropping titrant solution from a calibrated pipette into the flask and continually stirring or swirling the samplewater.
7. Add 2 mL of starch solution so a blue colorforms.
8. Continue slowly titrating until the sample turns clear. As this experiment reaches the endpoint, it will take only one drop of the titrant to eliminate the blue color. Be especially careful that each drop is fully mixed into the sample before adding the next. It is sometimes helpful to hold the flask up to a white sheet of paper to check for absence of the bluecolor.
9. The concentration of dissolved oxygen in the sample is equivalent to the number of milliliters of titrant used. Each mL of sodium thiosulfate added in steps 6 and 8 equals 1 mg/L dissolvedoxygen.

Result:

$$\text{DO concentration (ppm)} = \frac{\text{Normality of thiosulphate} \times \text{Vol. of thiosulphate used}}{\text{Vol. of sample taken}} \times 200 \qquad (5.66)$$

For the controlled sample = DO_1

For experimentalsample =DO_2

The O2 consumed by the fish =DO_1-DO_2

Prepared By:
Dr.T.Mahesh
Asst.Professor Sr.Gr. Of Zoology
SRR GOVT. ARTS & SCIENCE COLLEGE KARIMNAGAR