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QUALITATIVE TESTS FOR IDENTIFICATION OF CARBOHYDRATES PROTEINS AND LIPIDS

PROTEINS

Proteins are the building blocks of the body. They are the nitrogen containing organic compounds found in the plant and animals cells where they constitue the major part of the protoplasm. All the proteins contain Carbon, hydrogen, Oxygen and nitrogen. Some of these also contain either Sulphur (or) phosphorus elements. Biochemically proteins are the polymers of aminoacids linked together by peptide are the polymers of peptide bonds. Proteins serves as the component of the cell and cytoplasm. Biologically they are very significant as structural components of food and bio catalyst protein may be seen often as peptones proteoses albumins and globuline. In the test for proteins some reactions indicate only the presence of proteins while others confirm the type of proteins present in the sample.

a) QUANTITATIVE TESTS FOR THE IDENTIFICATION OF PROTEINS

i) By Millons Reagent

Aim :- Identification of proteins using million reagent.
Apparatus :- Test tube, Spirit lamp
Procedure :- Take 2 ml of sample into the tube. Add 2 drops of million reagent mix thoroughly till the two

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Substances are completed mixed. Boil the test tube over the bunsen flame.

Result :—

The mixture turns to violet colour indicating the presence of proteins in the given sample.

ii) BIURET TEST

Aim :— To identify the presence of protein in the given sample fluid using biuret reagent.

Apparatus :— Test tube

Reagents :— Biuret reagent

Procedure :— 2 ml of given sample solution is taken into the test tube. Add one ml of biuret reagent and mix the two solutions thoroughly. Heat the mixture is heated for 10 min at 37°C

Result :—

The mixture turns to violet colour indicating the presence of protein in the given sample.

iii) Trichloroacetic acid test

Aim :— To identify the presence of proteins in the given sample through TCA test.

Principle :— To acidic medium, protein act as cation and react with acids negativitly forming into an insoluble white precipitate. It indicates the presence of proteins.

Apparatus :— Test tube

Reagents :— Trichloroacetic acid reagent [TCA reagent].

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Procedure :- 2 ml of sample solution is taken into the test tube. Add 5 ml of 10% TCA

Result :-

Appearance of white precipitate indicates presence of protein in the given sample.

iv) Anthoprotein test

Aim :- To identify the presence of proteins in given sample.

Apparatus :- Test tube

Reagents :- Con. HNO_3 and dil. NaOH

Procedure :- 2ml of solution is taken into the test tube and add 1-2 drops of Con. HNO_3. Heat the mixture and then add 1-3 drops of dil. NaOH after cooling it to room temp. Orange red colour appears indicating the presence of phenyl group in the proteins.

Result :-

The mixture turns into light yellow colour after heating. This turns to orgone-red after cooling & addition of dil. NaOH indicating the presence of protein in given sample.

v) Nitric acid test

Aim :- To identify the presence of proteins in given sample.

Apparatus :- Test tube

Reagents :- Conc. HNO_3

Procedure :- Take 2 ml of con. HNO_3 into a test tube & add few drops of sample solution along the sides of white coloured ppt ring is formed at the junction of the two fluids.

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Result :- Formation of white ring indicates the presence of proteins.

I(b) IDENTIFICATION OF CARBOHYDRATES.

Carbohydrates are widely distributed in plants and animals. They may be defined chemically as aldehydes ketone derivative of polyhydric alcohol (or) as a compound that yields these derivatives upon hydrolysis Carbohydrates are made up of C, H and O in the ratio of 1:2:1 Carbohydrates provides the required energy to the organism besides available to the organism as reserve food materials. Carbohydrates Combine with proteins to form glycoproteins & proteoglucoses.

Carbohydrates are available in two forms viz sugar and polysaccarides.

Sugars are the small molecules with low molecular weight. They dissolve easily in water and occur as crystals and taste sweet. These may be monosaccharides like glucose, fructose and glactose (or) disaccharides such as Sucrose, Maltose, & high lactoses polysaccarides are the large molecules with high molecular weight. They neither dissolve in water nor occur in crystal form. They are not sweet to taste.

Ex :- Starch cellulose and chitin.

1) Bendicts test

Aim :- To identify sugars present in the given Sample.

Principle :- Aldehydes, ketones and monosaccarides reduce $CuSO_4$ to Cu_2O being red in colour indicates the presence of monosaccharides.

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Procedure :— Add 2 ml of bendicts reagent to the sample solution taken into a test tube and boil for 2 min.
Result :— formation of green colour precipitate to brick red indicates the presence of monosaccarides.

2) Iodine test

Aim :— To identify sugars present in the given liquid.
Principle :— Iodine (I_2) is known to form coloured compounds with polysaccarides giving characteristics blue colour
Procedure :— To 1 ml of sample add 4 ml of HCl & 1 ml of I_2
Result :— A blue coloured solution is formed indicating the presence of polysaccarides.

3) Picric acid test

Aim :— To identify Carbohydrates present in given sample.
Principle :— The monosaccarides will reduce to form a red coloured precipitate.
Procedure :— Add 1 ml of Saturated picric acid solution to the sample and 1 ml of 40% NaOH
Result :— Appearence of the red coloured precipitate indicates the presence of Carbohydrates.

4) Molish Test

Aim :— To identify the presence of Carbohydrates
Apparatus :— Test tuble, Sample solution, molish reagent.
Procedure :— Take 2 ml of sample solution into a test tube

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and add equal quantity of molish reagent. To this few drops of conc. H_2SO_4 are added along the sides of the test tube leave the test tube for few mins.

Result :— A violet colour ring is formed at the junction of the two fluids after few mins indicates the presence of Carbohydrates.

I. (c). IDENTIFICATION OF FATS

Fats are the organic compounds which are insoluble in water, but soluble in organic solvents like ether, chloroform, Benzene etc. Their molecular structure is constitute by long chain hydro, Carbon lipids are non polar and hydrophobic in nature. They contribute for the formation of cellular membranes chromones and ketamins. They also provide rich energy to the biological systems. These are in fluid state at room temperature. They are commonly known as oils in fluid state and fats in solid state. Following tests are conducted to test the presence of fats.

i) Methyl red test

Aim :— To identify fats present in the given Sample.

Apparatus :— Test tube

Reagents :— Methyl red reagent

Procedure :— 7 ml of sample solution is taken into the test tube to this 30 x 4 drops of methyl red reagent is added.

Result :— Apperance of orgone red colour indicates the presence of fats.

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ii) Emulsification of fats

When an oil is mixed with water large bubbles are formed upon thoroughly making fats emulsify into fine droplets, emulsification plays an important role in the process of digestion.

Aim :— To identify fats present in the given sample.

Apparatus :— Test tube

Reagents :— H_2O, Na_2CO_3 solution, test tube

Procedure :— 1 ml of sample solution is taken into the test tube. To this 5 ml of water and 1 ml of Na_2CO_3 are added upon thoroughly mixing fine droplets of oil are formed indicating the presence of fats.

Result :— Appearence of fine droplets indicates the presence of fats.

iii) Sudan test

Aim :— To identify fats present in the given sample.

Apparatus :— Test tube

Reagent :— Sudan - 3 - solution.

Procedure :— 1 ml of given sample is taken into the test tube. To it few drops of 1% Sudan-3-solution is added and shaken the contents thoroughly.

Result :— Appearence of the blue colour indicates the presence of lipids.

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iv) Potassium Hydroxide Test

Aim :- To identify the fats present in the given sample.
Apparatus :- Test tube and bunsen burner
Reagents :- 20% KOH, distilled water and Conc. HCl
Procedure :- 5 ml of solution is taken into the test tube. To this 5 ml of KOH solution and 5 to 6 drops of Conc. HCl are added.
Result :-
Appearance of foamy white precipitate over the surface of the fluid indicates the presence of fats.

Few drops of the given solution is placed over a paper upon drying a transparent spot is formed indicates the presence of fats.

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QUALITATIVE TESTS FOR IDENTIFICATION OF AMMONIA, UREA AND URIC ACID [NITROGENOUS EXCRETORY PRODUCTS]

QUALITATIVE ANALYSIS OF EXCRETORY PRODUTS

Excretion of nitrogenous waste products may differ from animal to animal and the pattern may change with life cycle availability of water, nutrition and of the environmental factors. Nitrogenous excretory products are formed by the degradation of proteins during this metabolism as NH_3 urea and uric acid. Basing on the nature of excretory products the animals are identified as 1) Ammonotelic animals excreting ammonia 2) Ureotelic animals excreting urea and 3) uricotelic animals excreting uric acid.

The animals being classified and called with particular names basing on the type of excretory product produced and excreted. These wastes can be identified by using the following tests.

Tests for Ammonia

Aim :- To estimate Ammonia in a given Sample.

Apparatus :- Test tube

Reagents :- Nessler's reagent

Procedure :- Take 5 ml of Sample into the test tube and add 0.5 ml of Nessler's reagent.

Result :-

Appearence of brown coloured precipitate indicates the presence of NH_3.

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Test for Urea.

Aim :- To identify the urea in a given Sample.

Apparatus :- Test tube

Reagents :- Urease enzyme, Nessler's reagent.

Procedure :- Take 5 ml of sample into a test tube and 0.5 ml urease enzyme solution. After few min add 0.5 ml of Nessl reagent.

Result :- Appearance of brown coloured precipitate indicates the presence of urea in the given sample.

Test for uric acid

Aim :- To identify the uric acid.

Apparatus :- Test tube

Reagents :- Standard sodium carbonate (Na_2CO_3) Follin's uric acid rea

Procedure :- Take 5 ml of sample into a test tube. Add 1 ml of Standard sodium Carbonate & follin's reagent solution mix thoroughly after few mins a blue coloured ppt is formed indicat the presence of uric acid in the given sample.

Preparation of Nessler's reagent

Dissolve 8.5 g of KI in 20 ml of water prepare 25 ml of Saturated $HgCl_2$ solution. Add to the above solution. Dissolve 12 g of NaOH in 15 ml of distilled water and add the solution to the above mixture and then make up to 100 ml for using the solution.

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STUDY ON EFFECT OF PH AND TEMPERATURE ON SALIVARY -AMYLASE -ACTIVITY.

Salivary amylase or the phyalin is the enzyme secreted by the salivary glands in mammals. It promotes the digestion of Carbohydrates in alkaline medium. In mammals Carbohydrates are first digested in the buccal cavity. This can be demonstrated in the laboratory by conducting a small experiment.

Aim :- To digest Carbohydrates using Salivary amylase.

Apparatus :- Beaker, Boiling test tubes and Stongs.

Reagents :- Starch solution, Salivary amylase, Tamarind/sugar.

Procedure :- First collect your own saliva into a dish by keeping the mouth open. Tamarind/sugar infront of our eyes causes psychic stimulus releasing saliva. This is collected into a flask. Five different test tubes are taken are labelled as ABCDE of these one (A) is Control. Take 10 ml of starch solution into each test tube. Saliva is added B, C, D & E and left for incubation to about one hour. Then the solution is tested by adding iodine solution.

i) Test tube A :- only starch solution enzyme is not added used as Control.

ii) Test tube B :- Saliva with a ptl of 5 (Add lime juice (or) citric acid to lower the pH and test on ptl at 5 by dipping the ptl paper is added to the starch solution.

iii) Test tube C :- Saliva @ a ptl of 8.85 (by adding a pinch of salt ptl can be increases adding the salt. Check for ptl to find out the salt quantity to be added.

iv) Test tube D :- Saliva @ a ptl of 10 (By adding more salt ptl can be further be increased).

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Test tube E :- Saliva with normal pH of 8.0 but temperature is increases to see its action.

Inference

Test tube A :- Solution in test tube A turn blue indicating the presence of starch. Since on Salivary amylase is added this is the control.

Test tube B :- In test tube B blue colour is produced indicating the Salivary amylase is inactive.

Test tube C :- No colour is produced and hence amylase @ a pH 8-85 has digested the starch completely.

Test tube D :- At pH-10, enzyme doesn't act because of high alkalinity and hence again blue colour is produced indicating the presence of starch.

Test tube E :- At normal temperature the action of amylase is quick and immediate but rise in temp denature the enzyme and hence starch is not digested. So blue colour is formed indicating that enzyme is inactive at high temperature.

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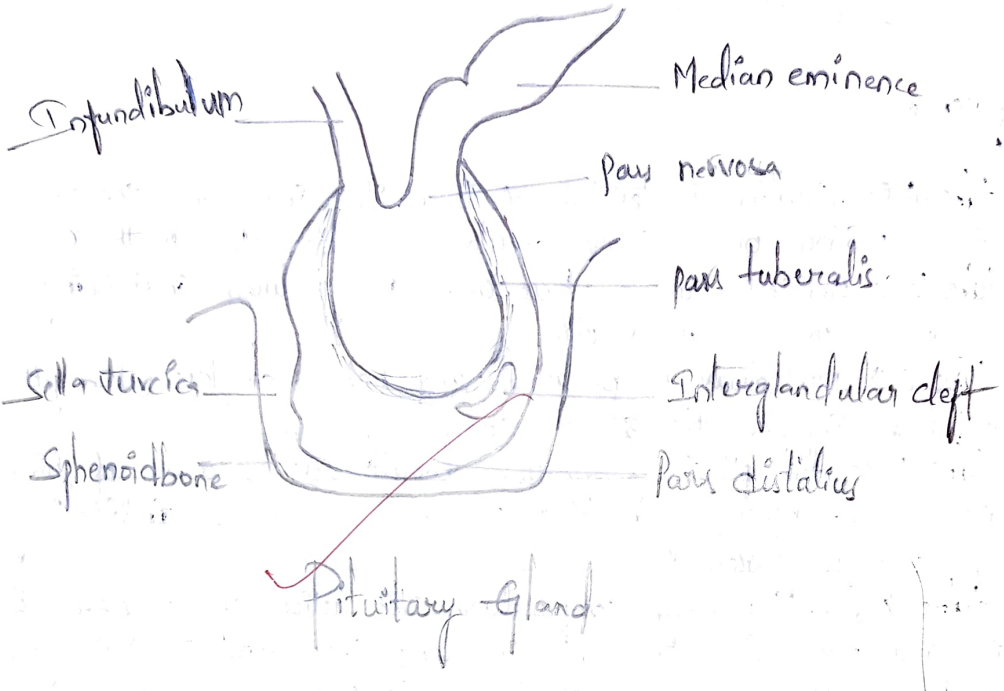
Infundibulum
Median eminence
Pars nervosa
Pars tuberalis
Sella turcica
Intermediate deft
Sphenoid bone
Pars distalis
Pituitary Gland

STUDY OF PERMANENT HISTOLOGICAL SECTION OF MAMMALIAN ENDOCRINE

TRANSVERSE SECTION OF PITUITARY GLAND.

1) A small nut like gland situated on the ventral side of the diencephalon of the brain of mammals.
2) Attached with the hypothalamus by a stalk like infundibulum.
3) Hormones secretes by the gland regulates the functions of many other endocrine glands and also play a vital role in the chemical coordination of the body.

S.No	Hormone	function	Disease.
A.	Anterior Adenohypophysis		
a)	Somatotrophic hormone (STH) (or) growth hormone (GH)	Protein metabolism & growth of skeleton	Gigantism, dwarfism acromegals.
b)	Thyroid stimulating hormone (TSH)	Stimulates thyroid gland	Thyroid malfunction.
c)	Adrenocorticotrophic hormone (ACTH)	stimulates adrenal cortex	Malfunctioning of adrenal cortex.
d)	Gonadotrophic hormone like follicle stimulating hormone (FSH) luteinizing hormone (LH) and luteotrophic hormone (or) prolactin.	Development of gonads formation of gametes production of sex hormone from gonads stimulation of mammary gland to secrete milk.	Non production of gametes & sterility, Non-production & release of milk.
e)	Diabetogenic hormone (or) pancreotropin	Reverse metabolism of carbohydrates where glycogen is converted to glucose	Diabetes.

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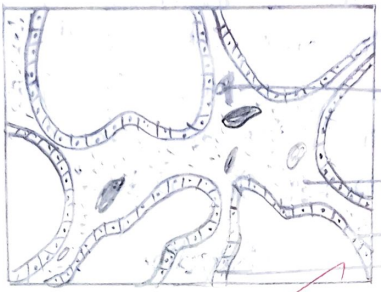

Thyroid gland

B. Infundibulum		
Melanocyte stimulating (MSH) or chromotrophid hormone (CTH) or intermedin	pigmentation of the body	Discolouration of the body.
C. Posterior Neurohyphysis		
a) Oxytocin	Uterine Contraction & secretion of milk	Abortion.
b) Vasopressin or Antidiuretic hormone (ADH)	Reabsorption of water from renal tubules	Diabetes incipidus (over urination).

4) Gland is also known as master endocrine gland or badmaster of endocrine Orchestra because of its overall control on all the other endocrine gland.

5) Gland consist of three lobes of different embryological origin.
i) Anterior and intermediate lobes arise from the roof of the mouth together they constitute the anterior adenohypophysis.
ii) Posterior lobe develops from hypothalamus of diencephelon and is called neurohypophysis.

(B) TRANSVERSE SECTION OF THYROID GLAND

1) Largest and bilobed endocrine gland situated on either side of the trachea below the larynx.
2) Both the lobes of thyroid are connected together by a connective tissue called isthmus.
3) Histologically it consist of a number of small thyroid vesicles or follicles in the form of closed sacs separated from one another by connective tissue intervened with blood & lymph vessels.

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T.S. of pancreas of a mammal.

4) Each follicle is lined by the cuboidal epithelial cells. These cells are richly provided with active secretory granuels secreting yellow colloidal secretion into the cavities of follicles.
5) Hormones produced is a colloidical globular protein called thyroglobulins (or) iodothyronines (or) thyroid hormones.
6) The mixture consist of nearly four most important chormones of which thyroxine is one.
7) Thyroxine regulates general metabolism of the body and controls the rate of growth.
8) Under-secretion of this hormone during childhood results in feeble mindeness retardation of growth lowered heart rate and sexual development.
9) Deficiency of thyrocine containing iodine in adults stimulates the formation of new gland tissue resulting in an enlargment of the thyroid called goitre.
10) Hypothyroidium caused myxodema in which for person becomes fatty and sluggish due to poor rate of oxidation.
11) Over. Secretion of this hormone increases food consumption bulging of eyeballs and irregular heart rate etc. It is also causes swelling of the thyroid gland resulting in the formation of goitre.

(C). TRANSVERSE SECTION OF PANCREAS GLAND

1) Pancreas lies inferior to the stomach in the loop of duodenum.
2) It is both an exocrine and an endocrine gland.
3) A large pancreatic duck runs through the gland carrying enzymes and other exocrine digestive secretions from the pancreatic acinar cells to the small intestine.

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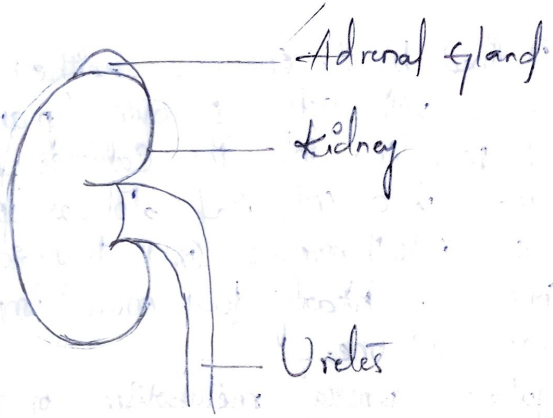
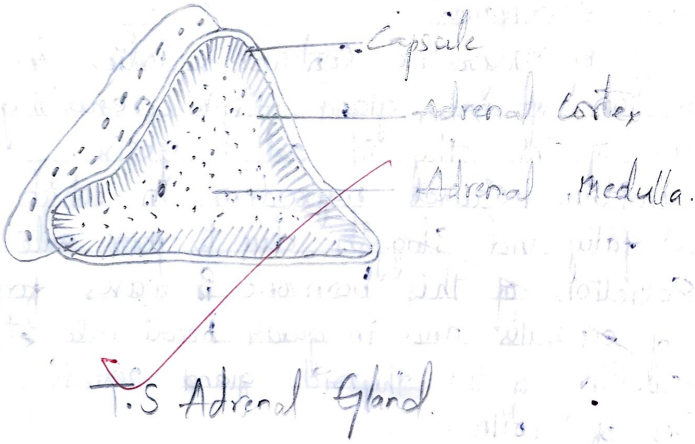

Adrenal Gland
Kidney
Ureles

(A)


Capsule
Adrenal Cortex
Adrenal Medulla.
T.S Adrenal Gland.

(B)

4) The tissue of the pancreas has in addition to the acinar cells group of cells called Islets of langerhans producing endocrine Secretions.

5) Four kinds of cells have been identified the islets
i) Alpha cells (about 15%) produce glycogen Alpha (α) cells.
ii) Beta cells (about 65%) produce insulin beta (β) are called β-cells.
iii) Delta cells are δ cells (about 5%) produce stomatostation (SS)
iv) Pancreatic polypeptide cells (or) pp cells (or) F cells (15%) produce pancreatic polypeptide (pp).

6) β cells are usually found towards the middle of the Islet α cells towards the periphery of the Islets and Delta (δ) & F cells are found scattered.

7) It promotes protein synthesis in tissue from amino acids.

8) Insulin reduces catabolism of protein. It is an anabolic hormone.

9) It increase the synthesis of fat in the adipose tissue from fatty acids.

10) Insulin reduces the breakdown and oxidation of fat. Insulin act on the cells of the liver muscles and adipose tissue.

11) Somatostatin (SS) :- The same substance as growth Inhibitory hormone to supress the release of other chromones from the pancreas and digestive track.

12) Pancreatic polypeptide (pp) :- It inhibits the release of digestive Secretion of the pancreas.

(D) TRANSVERSE SECTION OF ADRENAL GLAND

1) A pair of small ovoid white and yellowish glands situated just above each kidney. These are also known as suprarenal glands.

2) Each lobe has an outer cortex derived from mesoderm and

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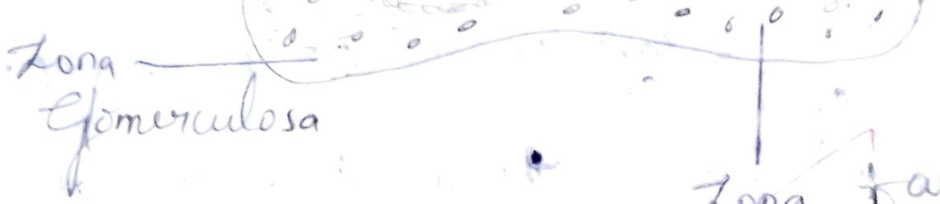

T.S Adrenal Gland

inner medulla derived from ectoderm.

ii) Cortex :- The cortex secretes as group of steroid hormones. Synthesised from cholesterol. This has three regions.

i) Zona Glomerulosa.

a) Cells are comparatively smaller than other zones.
b) Plasma membrane shows small microvilli.
c) Well developed smooth ER present.
d) Golgi apparatus present in very close association with nucleus.
e) Rich in mitochondrea which poses shelf like Cristae.
f) Few lipid droplets are found in the cytoplasm.

ii) Zona fasciculata

a) Cell are relatively larger and possess huge amount of lipid droplets in the cytoplasm.
b) Golgi complex distinct.
c) Mitochondria are many & tubular.
d) ER much more in concentration.
e) Microvilli are well apparent on the surface membrane.
f) Few cells are spongy in appearence.

iii) Zona reticulata

a) i) Cells are comparitively larger
ii) Lipid droplets are few & scattered in cytoplasm.
iii) Elongated mitochondria are well appeared
iv) ER & Golgi apparatus are distinct
v) Secretory Vesicles present
vi) Microvilli distinct.

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B) Medulla

a) The medulla produces a very important chormone known as adrenaline.
b) It is responsible for controlling the involuntary muscles and blood pressure.
c) Increase the heat beat, blood sugar level, metabolic level and delation of the branchs and pupil.
d) Used in the treatment of asthma & similar respiratory troubles.
e) Noradrenline regulates the blood pressure under normal Conditions & constructs the blood vessels.

Teacher' Signature : Mayank

Date

Expt. No. 05 Page No. 19

ESTIMATION OF HAEMOGLOBIN BY SAHLI'S METHOD

Introduction :- Haemoglobin is the major and principal constituent of the blood. It is present in the cytoplasm of the RBC. It accounts for approximately 90% of the dry weight of the mature cells. It is composed of heme and globin.

Structure of Hemoglobin

The Hemoglobin molecule is a tetramer consisting of two parts. pairs of similar polypeptide chains called globin chains. Every chain is attached to a heme molecule formed by iron in ferrous form and protoporphyron. The major (96%) type of haemoglobin present in adults is called HbA and it has 2 α globin chain and 2 β globin chains ($\alpha_2 B_2$).

Aim :- Estimation of Haemoglobin in the given sample by Sahli's acid hematin method.

Principle :- Blood is mixed with N/10 HCl resulting in the conversion of Hb to acid hematin which is brown in colour. The solution is diluted till it's colour matches with the brown coloured glass of the comparator box. The concentration of Hb is read directly.

Equipmed required :-

Hemoglobinometer consisting of comparator box having brown coloured glass on either side.

Hb pipette which is marked up to 20 mm (0.02 ml blood)

Tube with marking of Hb on one side.

Glass rod, Dropper.

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Reagents :—
N/10 HCl
Distilled water
Blood Sample, Venous blood, collected in EDTA.

Procedure :—
→ Add N/10 HCl into test tube upto mark 2g%.
→ Mix the EDTA and Sample by gentle inversion and fill the pipette with 0.02 ml blood. wipe the external Surface of pipette to remove any excess blood.
→ Add the blood into the tube containing HCl. wash out the contents of the pipette by drawing in and blowing out the acid two - three times. Mix the blood with the acid thoroughly.
→ Allow to stand undisturbed for 10 min.
→ Place the haemoglobinometer tube in the comparator & add distilled water to the solution drop by drop. stirring with the glass rod till its colour matches that of comparator glass.
→ Remove the glass rod & Compare the colour with the one in haemoglobinometer.
→ Take the reading directly by noting the height of the diluted acid hematin & express on g%.

Advantages :—
* Easy to perform
* Quick
* Inexpensive
* Can be used as a beside procedure.
* Does not require technical expertise.

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Disadvantages :—

* Less accurate
* The value of Hb obtained is less than the actual value.
* The colour of hematin develop slowly.
* Individual variation in matching the colour is seen.
* Colour of glass in the comparator box tends to fade with time.
* Lack of a true standard.

Teacher' Signature : nayak

Date

Expt. No. 06 Page No. 22

ESTIMATION OF BLOOD CLOTTING TIME

Introduction

This test was the first functional Plate. Evaluation test introduced by Duke in 1900. Clotting time of the blood is the time required for the blood to clot after it has came out of the vessel.

It is primarily used
To detect in primary hemostasis.
As a screening test for diagnosing Vascular disorders & platelet functioning.

Principle

- A standard incision is made at an appropriate place on the body to draw the blood.
- Initial time of bleeding from the incision is noted using stop watch.
- Stopping of bleeding indicates the formation of haemostasis plug.
- This depends on the adequate no. of platelets & on the ability of the platelets to adhere to the sub endothelium.

METHODS

Four methods are described here under

1) Slide method
2) Duke method
3) Copley & Kalitch method and
4) Capillary method.

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S.No	Name	Clotting time.
1	Anusha	3 min 26 sec
2	Abhinaya	5 min 1 sec 50 sec
3	Vivek	4 min
4	Pravalika	3 min 18 sec

36 mins

SLIDE METHOD

Equipment :—
Glass slide, stop watch, pin/ needle, lancet, alcohol, Cotton.

Procedure :—
1) Wipe the ball of your finger with cotton dipped in alcohol. Allow to dry.
2) Prick your finger with the lancet
3) Start the stop watch as soon as the flow of the blood starts.
4) Place 2-3 drops of blood on the slides.
5) At every 15 sec draw a pin/ niddle through the drop of blood until it picks up a fibrin thread.
6) Stop the watch & note the time.

End point :— The moment at which fibrin threads appear is end point.
Inference :— The time interval from the start of the blood flow to the appearence of fibrin threads denotes the clotting time.

Result :—
The clotting time is reported in 3 min 28 seconds 18 mill sec minutes & seconds.

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