DNR COLLEGE (A) BHIMAVARAM

DEPARTMENT OF ZOOLOGY

PRACTICAL MANUAL

PAPER- IV
ANIMAL PHYSIOLOGY, CELLULAR METABOLISMAND EMBRYOLOGY

D.N.R. COLLEGE (AUTONOMOUS), BHIMAVARAM

(Affiliated to Adikavi NannayaUniversity)
II B.Sc.Zoology practical syllabus (w.e.f 2020-21) admitted batch

Semester-IV
Paper IV- ANIMAL PHYSIOLOGY, CELLULAR METABOLISM AND EMBRYOLOGY

Total Hours-24 **Hours per week-02**
Total credits-01

I. ANIMAL PHYSIOLOGY

1. Qualitative tests for identification of carbohydrates, proteins and fats
2. Study of activity of salivary amylase under optimum conditions
3. T.S. of duodenum, liver, lung, kidney, spinal cord, bone and cartilage
4. Differential count of human blood

II. CELLULAR METABOLISM

1. Estimation of total proteins in given solutions by Lowry‟s method.
2. Estimation of total carbohydrate by Anthrone method.
3. Qualitative tests for identification of ammonia, urea and uric acid
4. Protocol for Isolation of DNA in animal cells

III. EMBRYOLOGY

1. Study of T.S. of testis, ovary of a mammal
2. Study of different stages of cleavages (2, 4, 8 cell stages)
3. Construction of fate map of frog blastula

D.N.R. COLLEGE (AUTONOMOUS), BHIMAVARAM
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II B.Sc. Zoology practical Examination (w.e.f 2020-21) admitted batch

Semester-VI

Paper IV- ANIMAL PHYSIOLOGY, CELLULAR METABOLISM AND EMBRYOLOGY

Model question paper and scheme of valuation

Duration :3 hrs
Max.Marks:50

1. Identify the presence of fats in the given sample(Physiology) 1x12=12M

Principle -2 ; Procedure -8; Result -2

2. Write about Isolation of DNA in animal cells (Cellular Metabolism)1x13=13M

3. Identify the following slides/ spotters (Embryology & Physiology) 3x5=15 M

Identification-1M; Diagram-1M; Comments-3M

A) **B)** **C)**

4. Vivavoce 5M

5. Record 5M

IDENTIFICATION OF CARBOHYDRATES IN THE GIVEN SAMPLE

Carbohydrates provide the required energy to the organisms besides available to the organisms as reserve food materials. They may be identified chemically as aldehydes, ketone derivatives of polyhydric alcohol (or) as a compound that yields these derivatives upon hydrolysis. Carbohydrates are made up of C, H & O in the ratio of 1:2:1. Carbohydrates provide the required energy to the organisms besides available to the organisms as reserve food materials. Carbohydrates are available in two forms, sugars and polysaccharides.

IDENTIFICATION TESTS

BENDICT'S TEST

Aim: To identify the presence of Mono saccharides in the given sample

Apparatus: Test tubes

Reagents: Bendict's reagent

Procedure: 5 ml of sample taken into a test tube, add 2ml of Bendict's reagent and boil for 2 minutes.

Inference: Green colour precipitate forms and turns to brick red colour.

Result: In test tube "A", red colour ppt is formed indicate the presence of carbohydrates in the sample.

In test tube "B" red colour ppt is not formed indicate the absence ofcarbohydrates in the Sample.

IODINE TEST

Apparatus: Test tubes

Reagents: Iodine solution.

Procedure: 5 ml of sample taken into a test tube, add 2 drops of Iodine into it.

Inference: Blue colour solution is formed.

Result: In test tube "A", blue colour solution is formed indicate the presence of carbohydrates

In test tube"B", blue colour solution is not formed indicate the absence of carbohydrates

PICRIC ACID TEST

Apparatus: Test tubes

Reagents: Picric acid, 40%NaOH

Procedure: 5 ml of sample taken into a test tube, add 1ml of picric acid and 1ml of 40%NaOH

Inference: Red colour ppt formed.

Result: In test tube "A", red colour ppt is formed indicate the presence of carbohydrates in the sample.

In test tube"B", red colour ppt is not formed indicate the absence of carbohydrates in the sample.

MOLISH TEST

Apparatus: Test tubes

Reagents: Molish solution.

Procedure: 5 ml of sample taken into a test tube, add equal quality of molish reagent. To this, few drops of con.H2So4 added along the sides of the testtube. Leave the test tube for few minutes.

Inference: AViolet colour ring is formed.

Result: In test tube "A", violet colour ring is formed indicate the presence of carbohydrates

In test tube"B" violet colour ring is not formed indicate the absence of carbohydrates in the sample.

IDENTIFICATION OF FATS IN THE GIVEN SAMPLE

Fats contribute for the formation of cellular membranes, hormones and vitamins. They also provide rich energy to the biological systems. Their molecular structure is constituted by long chain hydrocarbons. These are fluid state (oils) in room temperature and fats in solid state.

IDENTIFICATION TESTS

METHYL RED TEST

Aim: To identify the presence of fats in the given sample

Apparatus: Test tubes

Reagents: Methyl Red

Procedure: 1 ml of sample taken into a test tube, add 3 or 4 drops of Methyl red reagent .

Inference: Orange red colour is formed.

Result: In test tube "A",orange red colour is formed indicate the presence of fats in the sample.

In test tube"B"orange red colour is not formed indicate the absence of fats in the sample.

EMULSIFICATION OF FATS

Apparatus: Test tubes

Reagents: H_2O, Na_2CO_3

Procedure: 1 ml of sample taken into a test tube.To this, add 5ml of water and 1ml of Na_2CO_3. Mixed it thoroughly, fine droplets of oil are formed.

Inference: Fine droplets are formed around the test tube.

Result: In test tube "A"appearance of droplets around the test tube indicate the

presence of fats.

In test tube"B" fine droplets are not formed indicate the absence of fats in the sample

SUDAN TEST

Apparatus: Test tubes

Reagents: Sudan-3-solution

Procedure: 1 ml of sample taken into a test tube.To this, add few drops of 1% Sudan 3 solution added and shaken the contents thoroughly.

Inference: Blue colour appeared.

Result: In test tube "A"appearance of blue colour in the test tube indicate the presence of lipids.

In test tube"B" blue colour not appeared indicate the absence of lipids in the sample.

POTASSIUM HYDROXIDE TEST

Apparatus: Test tubes

Reagents: 20% KOH, distilled water, Con Hcl

Procedure: 5 ml of sample taken into a test tube. To this, 5ml of KOH solution, 5 to 6 drops of Con Hcl are added.

Inference: White foamy ppt formed.

Result: In test tube "A"foamy ppt is formed indicate the presence of lipids.

In test tube"B" foamy ppt is not appeared indicate the absence of lipids in the sample.

POTASSIUM HYDROXIDE TEST

Apparatus: Test tubes

Reagents: 20% KOH, distilled water, Con Hcl

Procedure: 5 ml of sample taken into a test tube. To this, 5ml of KOH solution, 5 to 6 drops of Con Hcl are added.

Inference: White foamy ppt formed.

Result: In test tube "A"foamy ppt is formed indicate the presence of lipids.

In test tube"B" foamy ppt is not appeared indicate the absence of lipids in the sample.

IDENTIFICATION OF PROTEINS IN THE GIVEN SAMPLE

Proteins are the building blocks of the body. They are the nitrogen contain organic compounds, found in the plant and animal cell where they constitute 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 amino acids linked together by peptic bonds. Proteins serve as the components of the cell and cytoplasm. Proteins may be seen often as peptones, proteoses, albumins and globulins.

IDENTIFICATION TESTS

MILLIONS TEST

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

Apparatus: Test tubes, spirit lamp

Reagents: Millions reagent

Procedure: 2 ml of sample taken into a test tube, add 3 drops of Millions reagent. Mix thoroughly till the two substances are completely mixed. Boil the test tube over Bunsen flame.

Inference: *Red precipitate* formed.

Result: In test tube "A",red colour ppt is formed indicate the presence of proteins in the sample.

In test tube"B" red colour ppt is not formed indicate the absence of proteins in the sample.

BIURET TEST

Apparatus: Test tubes, Spirit lamp

Reagents: Biuret reagent

Procedure: 2ml of sample taken into a test tube, add 1ml biuret reagent and mixed thoroughly. Heat the mixture for 10 min at 37_o C

Inference: Violet colour is formed.

Result: In test tube "A",orange violet colour is formed indicate the presence of proteins in the sample.

In test tube"B" violet colour is not formed indicate the absence of proteins.

TRICHLORO ACETIC ACID TEST

Apparatus: Test tubes.

Reagents: Trichloro acetic acid

Procedure: 2ml of sample taken into a test tube, add 5ml of 10%TCA

Inference: White precipitate is formed.

Result: In test tube "A",white ppt is formed indicate the presence of proteins in the sample.

In test tube"B" white ppt is not formed indicate the absence of proteins in the sample.

XANTHOPROTEIN TEST

Apparatus: Test tubes, Spirit lamp

Reagents: Con HNO3, dil NaOH

Procedure: 2ml of sample taken into a test tube, add 1 or 2 drops of Con.HNo3. Heat the mixture and add 2 to 3 drops of dil NaOH after cooling it to room temperature.

Inference: Orange red colour appears after cooling.

Result: In test tube "A",orange red colour is formed indicate the presence of proteins in the sample.

In test tube"B" orange red colour is not formed indicate the absence of proteins in the sample.

NITRIC ACID TEST

Apparatus: Test tubes

Reagents: Con. HNO3

Procedure: Take 3ml of Con.HNO3 into a test tube and add few drops of sample solution along the sides of the test tube slowly with the help of pipette.

Inference: A white colour ring is formed at the junction of two solutions.

Result: In test tube "A",White ring formed indicate the presence of proteins in the sample.

In test tube"B" white colour ring is not formed indicate the absence of proteins in the sample.

IDENTIFICATION OF THE AMYLASE ACTIVITY IN SALIVA

The saliva of man contains Salivary amylase enzyme. This enzyme digest carbohydrates in alkaline medium.

Aim: To identify the activity of salivary amylase enzyme.

Apparatus: Beaker, Test tubes.

Chemicals: Starch, Iodine.

Procedure: Collect few ml. of saliva into a beaker. Take two test tubes and labeled them as A & B. Add 10 ml of starch into both the test tubes, then add 5 ml of saliva to test tube B.

Take test tube "A" and add two drops of Iodine. Keep test tube "B" for one hour under 37_oC temperature. Later add 2 drops of Iodine.

Inference: Appearance of blue colour in test tube "A".

The solution in the test tube "B" will not turn into Blue colour after adding Iodine.

Result: In test tube A Blue colour appeared indicate the presence of carbohydrates.

In test tube B the sample did not turn into Blue colour indicate that carbohydrates are digested by salivary amylase enzyme.

T.S. OF MAMMAL DUODENUM

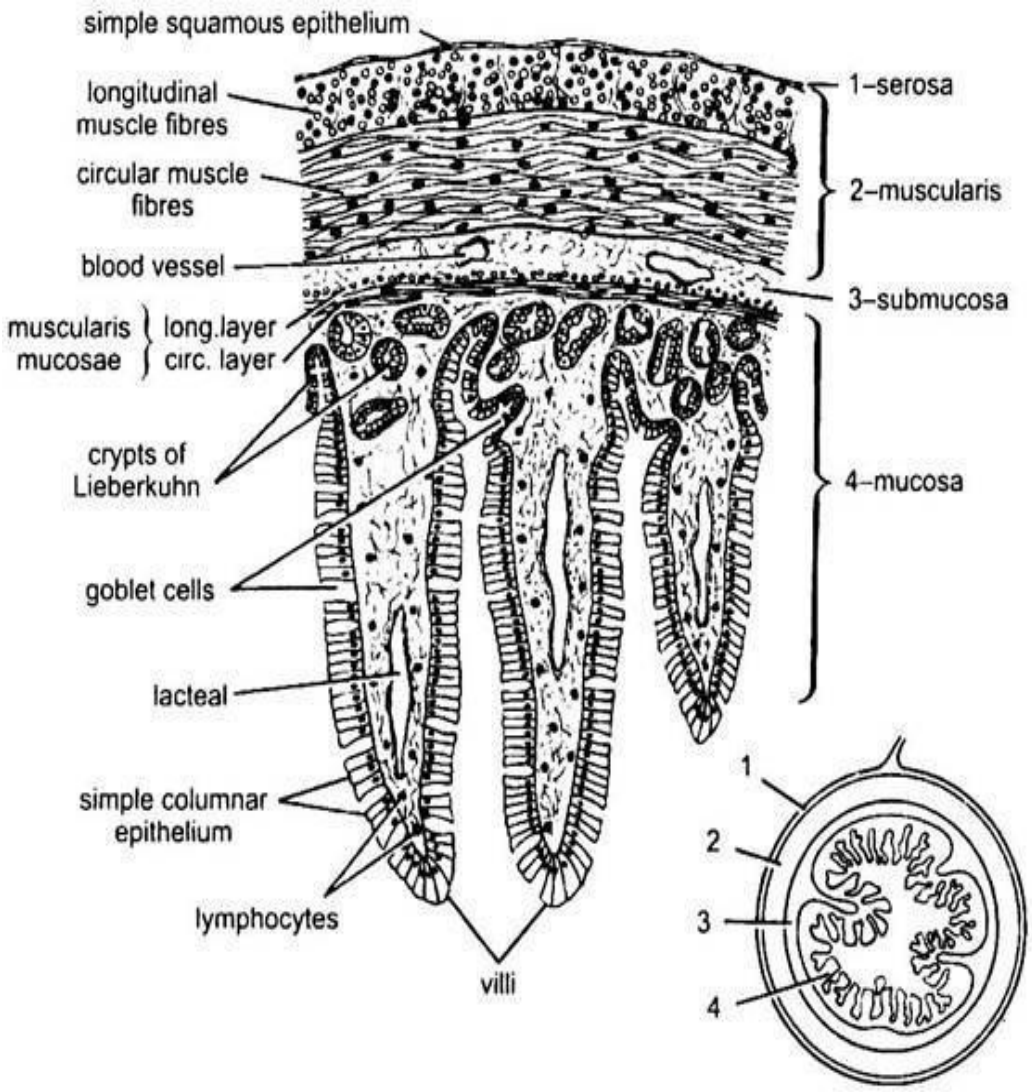
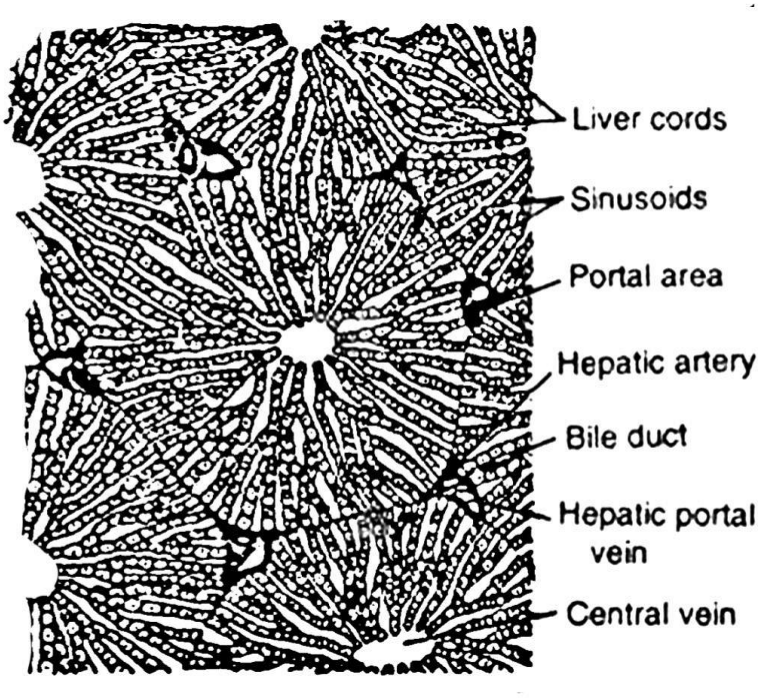


Fig. 29.31. Rabbit. T. S. of duodenum.

COMMENTS:

- Outer most layer is Serosa which is usually consists of simple sqamous epithelium.
- The Muscularis consists of longitudinal muscle fibres to the outside and circular muscle fibres inside.
- The sub mucosa consists of connective tissue holding blood vessels, nerves and lymphatic vessels.
- The muscularis mucosa is thin and double layered consisting of outer layer of longitudinal fibres and inner layer layer of circular fibres.
- The mucosa is thrown into numorous large and small finger like folds called villi which are all covered by simple columnar epithelium with scattered goblet cells.
- Each villus contains blood vessels, lymphocytes and a lacteal.
- At the base between the villi , crypts of Lieberkuhn present.
- The crypts of Lieberkuhn lead into Brunner's gland.
- The secretions of Lieberkuhn and Brunner's gland form the intestinal juices.

T.S. OF MAMMALIAN LIVER



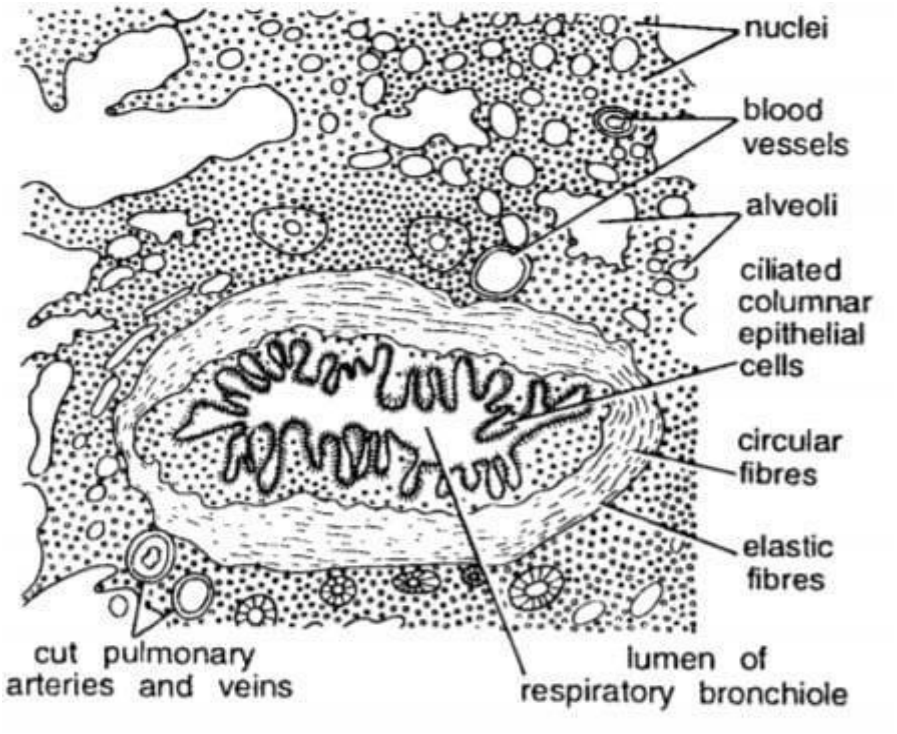
COMMENTS:

- The liver is composed of polygonal lobules containing a central vein in the centre and portal canals at the corners.
- Each portal canal consists of connective tissue strand and contains a branch of portal vein, hepatic artery, bile duct and lymph vessel.
- The liver cells are polyhydral and arranged in single celled long chains extending radially from the central vein to the periphery of the lobule.
- Each cell has granular cytoplasm and a prominent nucleus.
- The sinusoids are formed from branches of the hepatic portal veins and empty into central veins.

FUNTIONS:

- It produces bile which plays an important role in the digestion of food
- It stores the soluble products of digestion and metabolise them for assimilation.
- Oxidation of sugars takes place.
- Toxic substances are detoxicated in the liver.

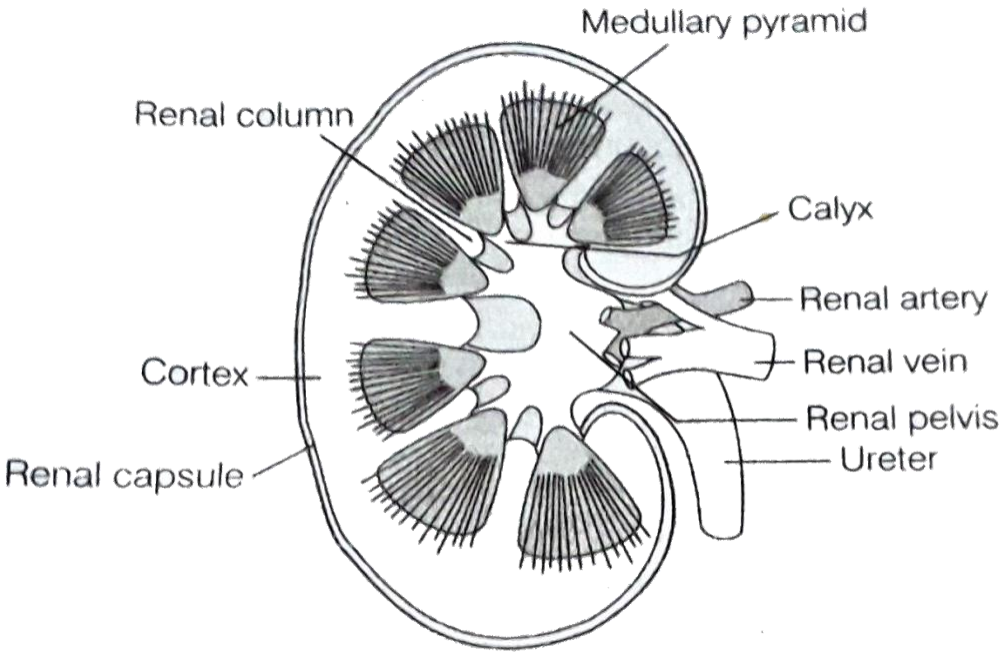
T.S. OF MAMMALIAN LUNG



COMMENTS:

- Histologically it consists of numerous alveoli.
- The alveoli communicate with one another by apertures in their walls.
- Around each alveolus in a network of capillary blood vessels in connection with pulmonary artery or vein of the lung.
- Numerous alveoli form clusters which open in a alveolar duct.
- Each bronchus as it enters the lungs, divides and subdivides into finer and finer branches, the bronchioles.
- The bronchioles are subdivided into respiratory bronchioles.
- The respiratory bronchiole gives rise to several alveolar ducts which open into alveoli or air sacs.
- Alveoli are richly supplied with blood vessels
- The air is taken into the alveoli by the respiratory bronchioles through alveolar ducts which get it from bronchioles which in their turn get it from the bronchus.

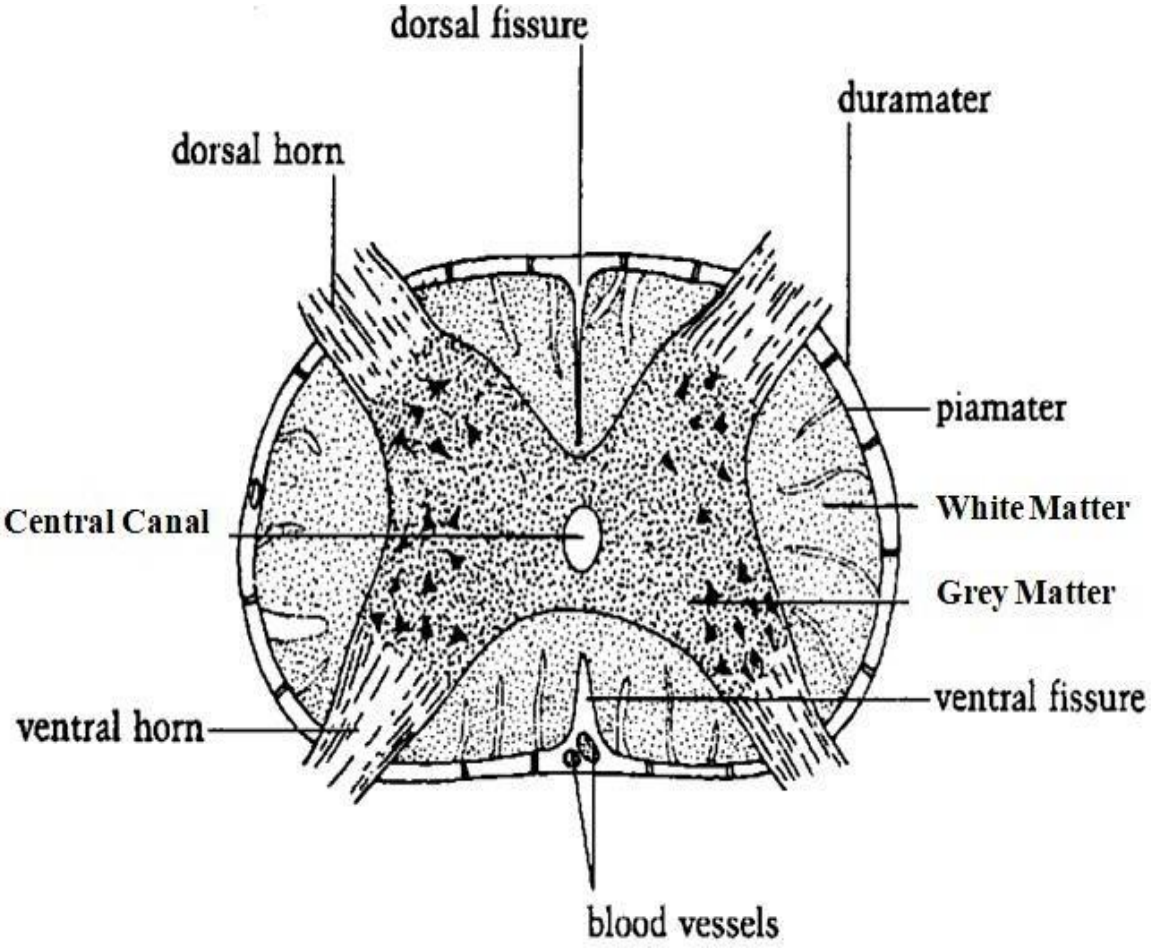
L.S. OF HUMAN KIDNEY



COMMENTS:

- The kidney is surrounded by a capsule of dense connective tissue.
- The glandular part of kidney composed of outer cortex and inner medulla.
- The cortex contains numerous uriniferous tubules, malpighian capsules having bowman's capsule and glomerulus scattered throughout.
- The medulla is composed of several renal pyramids, medullary rays, columns of Bertini, tubules of medulla and connective tissue.
- The depression found in the middle of the inner concave region is known as hilus.
- A slender muscular tube known as ureter takes its origin at the hilus and runs backwards to join the urinary bladder.
- The renal artery and renal vein are in and out at the hilus.
- The renal pelvis comprises uriniferous tubules which include the proximal portion of ureter, major renal calyces and minor renal calyces.

T.S.OF SPINAL CORD OF MAMMAL



COMMENTS:

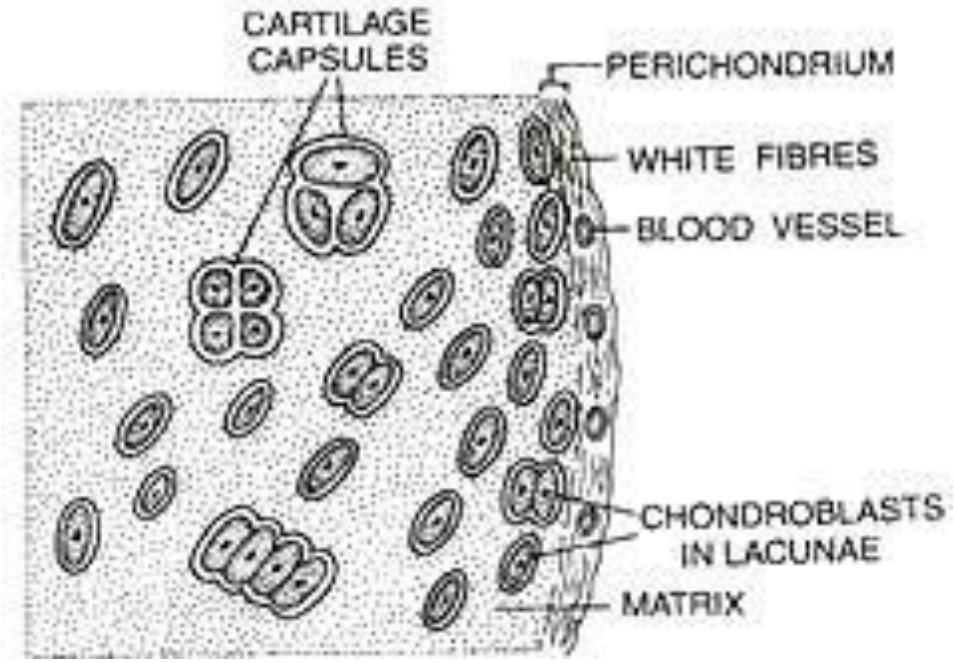
- The thin layer of Piameter surrounds the spinal cord.
- In the mid- dorsal surface is a dorsal fissure or septum and in the mid ventral surface is a ventral fissure which is slightly wider.
- In the centre there is a small cavity known as central canal. It is lined by simple epithelial cells.
- The substance of the cord is differentiated into two zones *i.e* the central zone surrounding the central canal and is called grey matter and a peripheral zone called the white matter.
- The grey matter is H-shaped projecting dorsally into two dorsal horns andventrally into two ventral horns.
- The grey matter shows the presence of bodies of neurons with tree-like branching of their dendrons and neuroglial cells.
- The white matter is composed of obliquely running medullated nerve fibres supported by prolongations of the neuroglia.
- The bands of fibres which extended transversely, one dorsal and other ventral to the central canal, are known as dorsal and ventral commissures respectively.

T.S. OF MAMMALIAN BONE

Transverse section of Bone exhibits the following features:

-
- Each consisting of a central Haversian canal surrounded canal surrounded by rings of osteocytes lying each in a lacuna.
- The lacunae are connected together by five canaliculi
- Among the rings of lacunae lie very thin concentric layers of bone lamellae which compose the matrix of tissue.
- Some bone lamellae, bone lacunae and canaliculi are present among the Haversian system but are not arranged around Haversian canals. These are called interstial lamelle.
- Haversian canals are about 22-110 microns in diameter.

T.S. OF MAMMALIAN CARTILAGE



Comments:

- Cartilage is the soft bone having flexibility seen in external ear, nose etc.,
- In transverse section, cartilage is encircled at its periphery by connective tissue membrane called perichondrium.
- Central part is filled with gelatinous matrix containing chondrin as its principal protein componrnt.
- Just beneath the perichondrium, chondroblast cells are arranged in annular layers.
- Chondroblasts divide mitotically to poduce free chondrocyte cells into the matrix.
- Chondrocytes are arranged in pairs inside the vacuoles.

TOTAL COUNT OF BLOOD CELLS

AIM: To count the total number of RBC and WBC in human blood.

REQUIREMENTS: Haemocytometer, sterilized needles, microscope, cover glass, cotton, spirit,RBC diluting fluid, WBC diluting fluid, etc.

HAEMOCYTOMETER

- The haemocytometer is in the form of a microscopic slide used for counting blood cells. It is also used for counting any cells, particles and microorganisms. It was invented by Louis Charles Malassez.
- The haemocytometer consists of a heavy glass slide with two counting chambers called Neubauer counting chambers.
- The two chambers are separated by a 'H' shaped ridge.
- A cover slip is placed on the ridge. Each counting chamber has a total ruled area of 9 sq.mm. and 0.1 mm depth.
- It consists of 9 squares of 1mm size.
- The central square is divided into 25 small squares and each square is sub divided into 16 squares.
- The four corner squares (W) are further divided into 16 squares. For total RBC counts, central 1mm square is used.
- For total WBC counts, the four large corner squares are used.

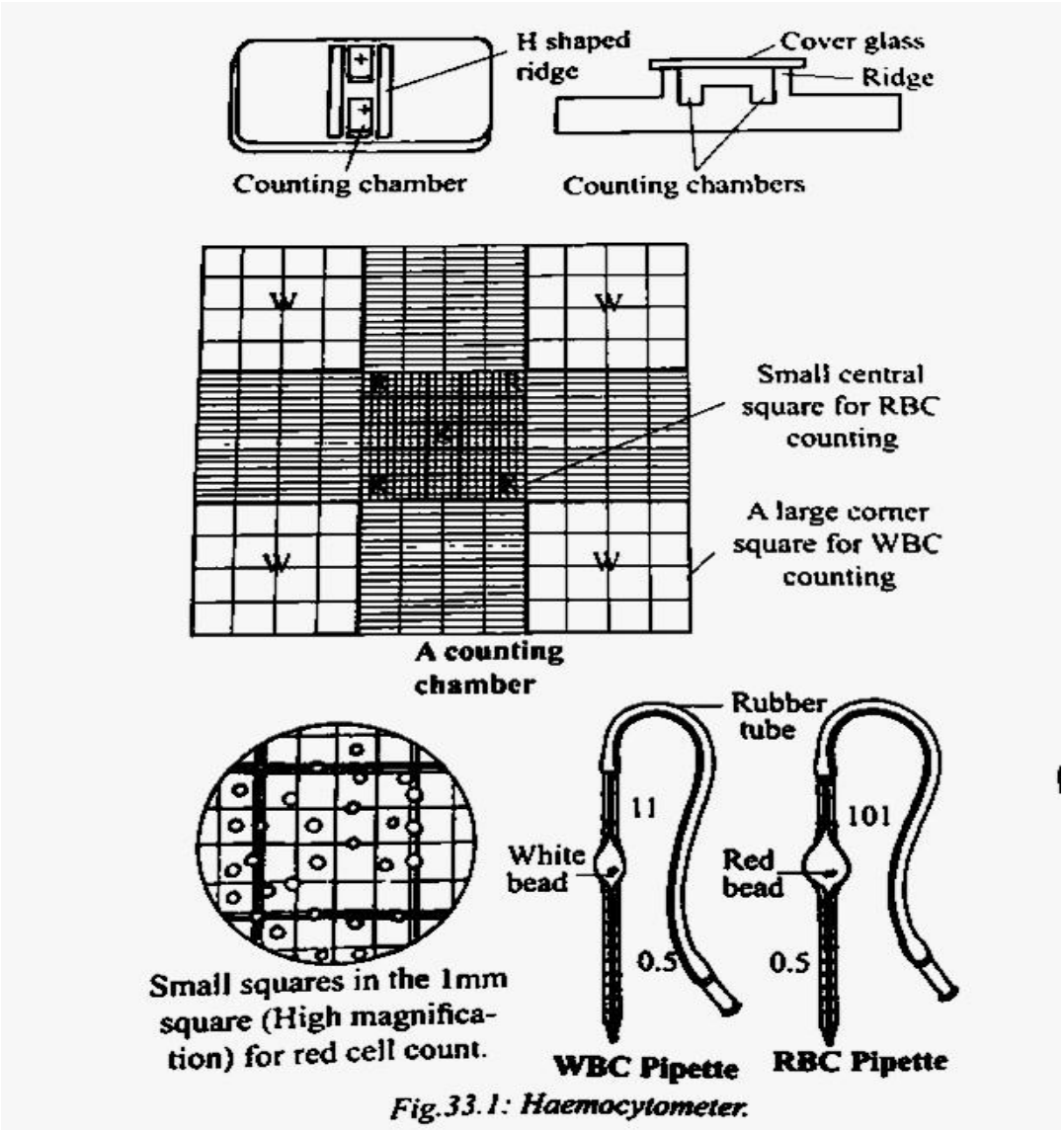


Fig.33.1: Haemocytometer.

RBC Pipette

The RBC pipette has a capillary tube and a mixing bulb with a red bead. It has two markings, namely 0.5 and 101.

WBC Pipette

The WBC pipette has a capillary tube and a mixing bulb with a white bead. has also two marking, namely 0.5 and 11.

RBC Diluting Fluid

There are two fluids and any one can be prepared and used.

a.Formal Citrate Solution

Trisodium citrate - 3 gm , Distilled water - 99 ml, Formalin - 1 ml

b. Hayem's Fluid Sodium chloride

Sodium Chloride - 0.5 g, Sodium sulphate - 2.5 g ,

Mercuric chloride-0.25Distilled water - 100 ml

WBC Dilution Fluid (Truck's Fluid)

Glacial acetic acid - 100 ml

Distilled water - 97 ml

Add gentian violet to give a pale violet colour.

Procedure for Red Cell Counts

The tip of the index finger is sterilized by rubbing with a cotton soaked inspirit.

Make a gentle prick with the help of a sterilized pin or needle.

The tip of the finger is pressed and blood oozes out.

The first drop is wiped out with the help of cotton.

Then the blood is aspirated into the RBC pipette exactly upto the 0.5 mark.

Immediately RBC diluting fluid is loaded upto the 101 mark.

The pipette is rotated between the thumb and forefinger. This will give a dilution of 1:200.

Clean the counting chamber and cover glass thoroughly.

Place the cover glass in position over the ruled area, using gentle pressure.

Mix the suspension thoroughly by rotating the pipette for about a minute holding it in horizontal position, and finally shake sidewise.

Expel the fluid from the stem of the pipette and without loss of time, fill the chamber by holding the pipette at an angle of 45 degrees and lightly touching the tip against the edge of the cover glass.

Care should be taken to ensure that the suspension does not flow into themoats on either side, nor should any bubble form under the cover glass.

Allow two to three minutes for the red corpuscles to settle. Place the slide under the microscope.

Count the number of RBC's in 80 small squares. (4 squares at the four

corners and one at the centre of central area).

Do not count the cells touching the lower and right hand lines, but count the cells touching the upper and left hand lines.

Calculation

a. Short-cut Method

Total number of cells in 80 small squares = 423

Add four zeroes = 4230000

Therefore total RBCs per c.mm is = 4230000

= 4.23 million per c.mm.

b. Detailed Method

Total number of cells in 80 small squares = 423

The area of a small square is = 1/400 sq.mm.

The depth of the counting chamber is = 1/1 mm

Therefore, the volume of a small square is = 1/ 400 x 1/10 c.mm.

The dilution of the blood is = 1/200

Total RBC's= 423 x 4000 x 200 / 80 x 1x1 = **42,30,000 per c.mm**

Normal

Men 4.5 to 6.5 millions per c.mm. **Women** 3.9 to 5.6 millions per c.mm.

Procedure for White Blood Cell (Leukocytes) Counts

Draw the blood upto the 0.5 mark in WBC pipette and dilute upto the mark 11 with WBC fluid as described in RBC counting and fill the counting chamber in the same manner.

Allow 3 minutes for cells to settle.

Count the cells in the four corner blocks by using the low power objectivelens of the microscope.

Count the cells touching on the inner lines on the right and top, but do not count the cells touching the lines on the left and bottom.

The difference between the two square millimeter areas should not be more

than 10 WBCs.

Calculation

a. Short-cut Method

Total number of cells counted in 4 squares = 104 cells

Divide it by 2 = 104/2

= 52

Add two zeros = 5200 cells per c.m.

b. Detailed Method

No. of WBCS counted in 4 squares = 104 cells

The volume of a square is = 10 c.mm.

The blood was diluted to = 1/20

Therefore, the number of cells per c.mm. of undiluted blood

= 104 × 10 × 20/

4x1x1

= 5200 cells per c.mm.

Normal

WBC's per c.mm of blood 4,000 to 10,000 per c.mm.

ESTIMATION OF PROTEIN BY LOWRY'S METHOD

AIM: To estimate the amount of Protein present in given unknown solution.

PRINCIPLE:

Alkaline $CuSo_4$ catalyses the oxidation of aromatic amino acids with subsequent reduction of sodium potassium molybdate tungstate of Folin's reagent giving a purple colour complex the intensity of the colour is directly proposition to the concentration of the aromatic amino acid in the given sample solution.

REAGENTS REQUIRED:

1. **Stock Solution:** Bovine Serum albumin of 100mg is weighed accurately and dissolved in 100ml of distilled water in a standard flask (concentration 1 µg /ml).

2.**Working Standard:** The Stock Solution of 10 ml is distilled to 100ml with distilled water ina standard flask (concentration 100 mg/ml).

3.**Folin's Phenol Reagent:** Folin's Phenol Reagent is mixed with distilled water in the ratio 1:2.

4.Alkaline copper reagent:

Solution A: 2% sodium carbonate in 0.1 N sodium hydroxide.

Solution B: 0.5% copper sulphate

Solution C : 1% sodium potassium tartarate.

Solution A, B and C are mixed in the proportion of 50:1:0.5.

Unknown Preparation:

The unknown protein is made upto 100 ml with distilled water.

PROCEDURE:

Working standard of 0.2 -1ml is pipette out into clean test tube and labeled as S1-S5. Test solution of 0.2ml is taken into test tube and labeled as T1. The volume is made

upto 1ml of distilled water. Distill water of 1ml serve as blank. To all the test tube 4.5ml of alkaline $CuSO_4$ reagent is added and incubated at room temperature for 10 minutes. All the test tube 0.5ml of folin's phenol reagent is added. The contents are mixed well and the blue colour developed is read at 640 rpm after 15 minutes. From the standard graph the amount of protein in the given unknown solution is calculated.

RESULT:

The amount of protein present in the given unknown soluion is -------mg (μg of protein).

ESTIMATION OF CARBOHYDRATE BY THE ANTHRONE METHOD

PRINCIPLE:

Carbohydrates are dehydrated by conc.H_2SO_4 to form furfural. Active form of the reagent is anthranol,the enol tautomer of anthrone,which reacts by condensing with the carbohydrate furfural derivative to give a green colour in dilute and a blue colour in concentrated solutions, which is determined colorimetrically. The blue - green solution shows absorption maximum at 620 nm.

REACTION:

(i) Hydrolysis to monosaccharides

Disaccharide ⇨ Monosaccharide

(ii) Dehydration---product is a furfural Monosaccharide Furfural

(iii) Reaction of furfural with anthrone

Furfural + Anthrone reagent Blue green complex

METHODOLOGY:

(A) Materials required:

Equipments:

- UV Spectrophotometer
- Vortex mixer
- Mantle heater/Water Bath.

(i) Chemicals/Reagents:

- Anthrone Reagent
- Glucose
- Other carbohydrates if desired

(ii) Glass wares and others:

Test tube, Test tube stand, Pipettes, Beaker, IceTest tube caps, Tissue paper, Wash bottle.

(B)REAGENTS:

(i) **Anthrone reagent**: Dissolve 2g of Anthrone in 1 litre of concentrated H_2SO4. Use freshly prepared reagent for the assay

(ii) **Glucose stock solution**: 200µg glucose per mL distilled water.

Note: Can include other carbohydrates of the same concentration if desired.

PROCEDURE:

1. Weigh 100 mg of the sample into a boiling tube.
2. Hydrolyse by keeping it in a boiling water bath for three hours with 5 mL of 2.5 N HCl and cool to room temperature.
3. Neutralise it with solid sodium carbonate until the effervescence ceases.
4. Make up the volume to 100 mL and centrifuge.
5. Collect the supernatant and take 0.5 and 1 mL aliquots for analysis.
6. Prepare the standards by taking 0, 0.2, 0.4, 0.6, 0.8 and 1 mL of the working standard.'0' serves as blank.
7. Make up the volume to 1 mL in all the tubes including the sample tubes by

adding distilled water.

8. Then add 4 mL of anthrone reagent.
9. Heat for eight minutes in a boiling water bath.
10. Cool rapidly and read the green to dark green colour at 630 nm.
11. Draw a standard graph by plotting concentration of the standard on the X-axis versus absorbance on the Y-axis.
12. From the graph calculate the amount of carbohydrate present in the sample tube.

CALCULATION:

Determine the amount of glucose in the unknown sample by plotting a standard curve of A620 on Y-axis and µg of Glucose on X-axis,

QUALITATIVE ANALYSIS OF EXCRETORY PRODUCTS

Excretion of nitrogenous waste products may differ from animal to animal and the pattern may change with life cycle, availability of water, nutrition and the environmental factors. Nitrogenous excretory products are formed by the degradation of proteins during their metabolism as NH3, 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 tested by using the following tests.

TEST FOR AMMONIA

AIM:

To estimate Ammonia in a given sample.

APPARATUS:

Test tubes

REAGENT:

Nessler' reagent.

PROCEDURE:

Take 5ml of sample into the test tube and add 0.5ml Nessler'reagent.

INFERENCE:

Brown colour precipitation formed.

RESULT:

In test tube "A" brown coloured precipitation is formed indicate the presence of

Ammonia in the sample.

In test tube "B" brown coloured ppt is not formed indicate the absence of Ammonia in the sample.

TEST FOR UREA

Aim:
To estimate urea in a given sample.

Apparatus:
Test tubes

Reagent:
Urease enzyme, nessler' reagent.

Procedure:
Take 5ml of sample into the test tube and add 0.5ml of urease enzyme. after few minutes, add 0.5ml nessler' reagent.

Inference:
Brown colour precipitation formed.

Result:

In test tube "a" brown coloured precipitation is formed indicate the presence of urea

in the sample.

In test tube "b" brown coloured ppt is not formed indicate the absence of urea in

the sample.

TEST FOR URIC ACID

AIM:

To estimate uric acid in a given sample.

APPARATUS:
Test tubes

REAGENTS:

Standard Sodium carbonate ($NaHCO_3$), Follin's uric acid

PROCEDURE:
Take 5ml of sample into the test tube and add 1ml of saturated sodium carbonate solution and mix thoroughly. Immediately after few minutes, blue colour is formed.

INFERENCE:
Blue colour precipitation formed.

RESULT:

In test tube "A" blue coloured precipitation is formed indicate the presence of Uric acid in the sample.

In test tube "B" blue coloured ppt is not formed indicate the absence of Uric acid in the sample.

Apparatus: Test tubes

Reagent: Urease enzyme, Nessler' reagent.

Procedure: Take 5ml of sample into the test tube and add 0.5ml of urease enzyme. After few minutes, add 0.5ml Nessler' reagent.

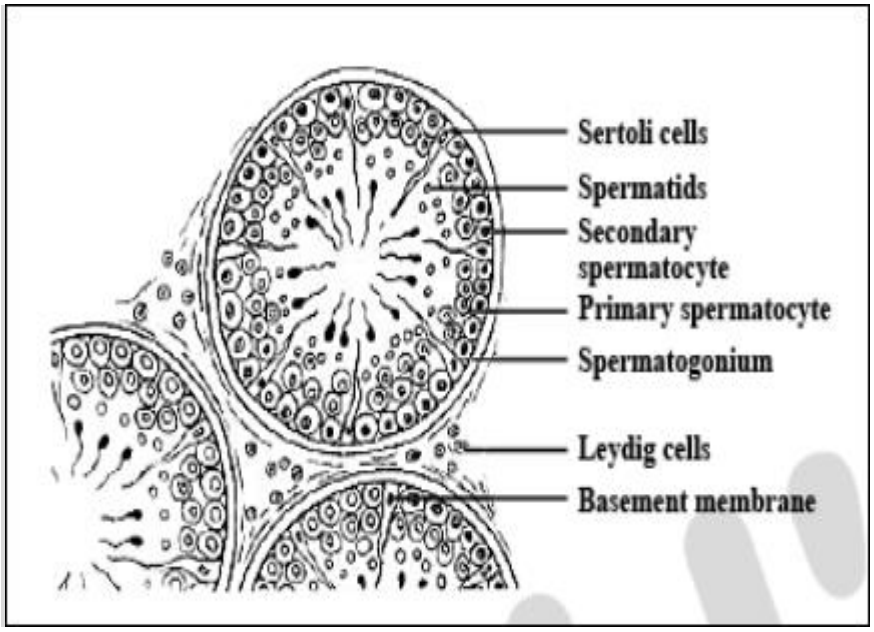
Inference: Brown colour precipitation formed.

Result:

In test tube “A” brown coloured precipitation is formed indicate the presence of urea in the sample.

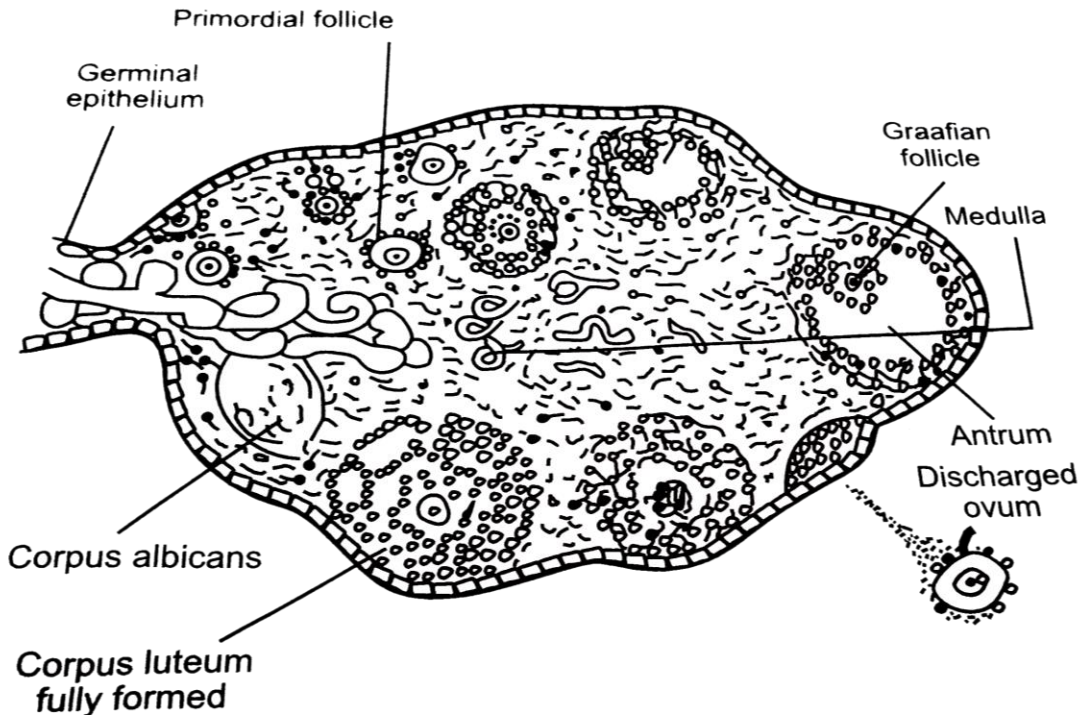
In test tube “B” brown coloured ppt is not formed indicate the absence of urea in the sample.

T.S. of Mammalian Testis



- Each testis contains about 200-300 tubules called seminiferous tubules.
- These are lined by a single layer of cuboidal germinal epithelium which undergo spermatogenesis.
- In the germinal epithelium, various stages of spermatogenesis such as spermatogonia, primary spermatocytes, secondary spermatocytes, spermatids and sperms are seen.
- Between these cells, few large and pyramidal cells called nurse cells or sertoli cells are present.
- Bundles of sperms are seen attached to Sertoli cells.
- These cells provide nourishment to the sperms till maturation.
- In between seminiferous tubules, connective tissue containing blood vessels, nerves, lymph vessels and groups of interstitial cells (Cells of leydig) is present.
- Interstitial cells produce male hormone testosterone.

T.S. of Mammalian Ovary

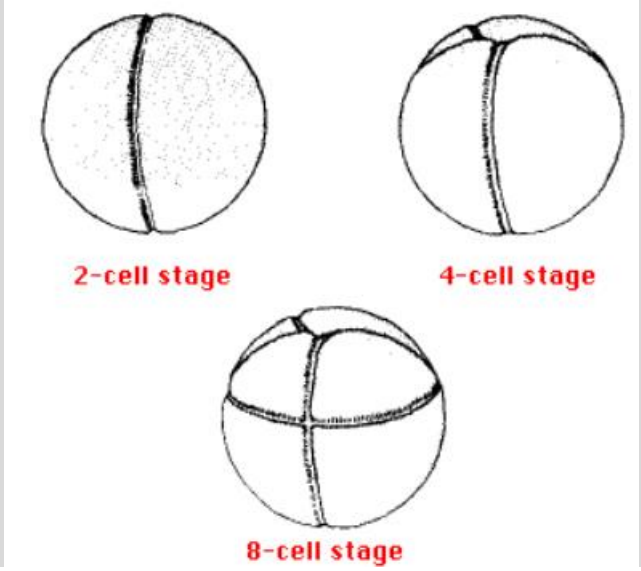


(i) In the section of ovary, there is a mass of tissue lined with germinal epithelium. Inside that you will observe an ovum, which is a cell surrounded by one to several layers of follicular cells. As the ovum matures, the number of surrounding follicular cell layer increases

(ii) In the later stage of follicular development a cavity called antrum appears.
(iii) The cavity gets further enlarged and the follicle grows bigger. This is the stage of Graffian follicle ready to release the ovum (ovulation).

(iv) In the next stage, you may notice a Corpus luteum, and/or Corpus albicans, which differ from each other and also from Graffian follicle in their features.

Different stages of cleavages (2, 4, 8 cell stages)



2-CELL STAGE:

The first cleavage plane is **meridional**. Initially, a furrow appears at the animal pole. It gradually extends towards the vegetal pole of the egg. It cuts the egg through its median animal-vegetal polar axis and results in two equal-sized blastomeres.

4- CELL STAGE:

The second cleavage furrow is again **meridional**. It bisects the first cleav-age furrow at right angles. It is a holoblastic cleavage affecting both the blastomeres of the first cleavage. It results in the formation of four blastomeres.

8- CELL STAGE:

In the next stage a **latitudinal** furrow is formed above the horizontal fur-row nearer to the animal pole. Such a furrow is due to the influence of yolk concentration in the vegetal pole. The latitudinal furrow uniformly affects all the blastomeres. It results in the formation of eight blastomeres. Four of them remaining in the vegetal pole are large. They are named as **macromeres**.

Another four blastomeres remain in the vegetal pole. They are named as **micromeres**. The micromeres are smaller in size than the macromeres.