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(A State University Established by the Government of Tamilnadu)**

KARAIKUDI – 630003

DIRECTORATE OF DISTANCE EDUCATION

M.Sc., (Zoology)

LAB – III: 35034

ANIMAL PHYSIOLOGY, IMMUNOLOGY, ENVIRONMENTAL BIOLOGY

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SYLABI BOOK MAPPING TABLE

ANIMAL PHYSIOLOGY, IMMUNOLOGY, ENVIRONMENTAL BIOLOGY

Syllabi	Mapping in Book

CONTENTS

III - ENVIRONMENTAL BIOLOGY

I - ANIMAL PHYSIOLOGY

1.1 QUANTITATIVE ESTIMATION OF AMYLASE ACTIVITY

1.1.1 AIM

To estimate the amount of amylase present in the human saliva.

1.1.2 PRINCIPLE

Amylase in the saliva hydrolyses the starch into smaller units called dextrin and finally to maltose. 3, 5 – Dinitro salicylic acid reagent (DNS) combines with maltose to give orange red coloured compound. The intensity of the colour is proportional to the amount of Maltose formed from the hydrolysis of starch. The activity of salivary amylase can be quantified from this value.

1.1.3 MATERIAL REQUIRED

Test tube, beaker, water bath and Spectrophotometer.

1.1.4 REAGENTS

1. Dinitro salicylic acid reagent: 1 gm of 3,5 Dinitro salicylic acid was dissolved in 20 ml of 2N,NaOH (4gm of NaOH dissolved in 100 ml distilled water) to the above solution. 30 gm of sodium potassium tartrate was added and then made up to 1000 ml with distilled water.

2. 0.2 M Sodium phosphate buffer preparation (pH 6.8): 0.2M sodium hydroxide (NaOH): 800 mg of NaOH dissolved in 100 ml distilled water 0.2sodium dihydrogen orthophosphate (NaOH3PO4): 3.2 gm of NaH3PO4 dissolved in 100 distilled water.

24 ml of NaOH solution was mixed with 76 ml of NaH3PO4 solution for 0.2M Phosphate buffer (pH6.8) preparation.

3. Starch solution: 2 gm dissolved in 100 ml distilled water.

4. Standard maltose: 100 mg dissolved in 100 ml distilled water.

1.1.5 PROCEDURE

Three test tubes were taken and were labelled as sample, standard and blank. 1 ml of saliva, 1 ml of 0.2 M Phosphate buffer, 0.5 ml of 2 % starch solution was taken in the sample test tubes. In the

standard test tube 1 ml of standard Maltose solution was taken. 1 ml of distilled water was taken in the blank test tube. The sample test tube alone incubated at 37°C for 10 minutes. 2 ml of Dinitro salicylic acid reagent was added to the sample, standard and blank test tubes. All the three test tubes were kept in boiling water bath for 5 minutes and then allowed to cool. The content in each test tube was made upto 10 ml with distilled water then the optical density of the sample, standard was measured at 540 nm using a spectrometer.

1.1.6 CALCULATION

$$\text{Amount of urea present in the given sample} = \frac{\text{O.D. of the sample}}{\text{O.D. of the standard}} \times \frac{\text{Conc. Of the standard}}{\text{Volume of the sample}} \times \text{Dilution factor}$$

1.1.7 RESULT

Amylase present in the human saliva = _____ µg maltose released / min.

1.1.8 DISCUSSION

Saliva is one of the digestive juices having the enzyme, Amylase (or) ptyalin. It is slightly acidic having pH 6.3 to 6.8. The optimum temperature for salivary amylase is 37°C. Ptyalin acts on starch and converts the starch into maltose. The secretion of saliva is under the reflex action. The physiological stimulus of saliva is the presence of food in the mouth. The food induces the mucous membrane of the mouth which in turn stimulates the salivary centre of the brain.

1.2 QUANTITATIVE ESTIMATION OF AMMONIA

1.2.1 AIM

To estimate the amount of ammonia present in the given sample.

1.2.2 PRINCIPLE

Sodium hypochloride combines with ammonia in the sample to produce chloride and hydroxyl ions. Sodium nitroprusside acts as catalyst for this reaction. Ammonium chloride in the presence of hydroxyl ions combines with phenol to form a complex. This complex combines with phenol molecule to form yellow phenol, which is a coloured compound. This colour thus formed is directly proportional to the amount of ammonium present in the sample.

1.2.3 MATERIAL REQUIRED

Test tube, measuring cylinder, pipette, fish tank water, boiling water bath and Spectrophotometer.

1.2.4 REAGENTS

1. Reagent – A: 2 gm of phenol and 10 mg of sodium nitroprusside dissolved in 100 ml of water.

2. Reagent – B: 1 ml of sodium hydroxide is added to 2 ml sodium hypochloride and make up to 100 ml with distilled water.

3. Reagent – C: Ammonium standard solution: Dissolve 38 mg of anhydrous ammonium chloride in 100 ml of distilled water. This was stock solution. From this solution working solution was prepared. 1 ml of stock solution was made up to 100 ml with distilled water (1 ml of solution = 0.00122 mg of ammonia).

1.2.5 PROCEDURE

Three test tubes were taken in one test tube 1 ml of sample was taken in the second test tube 1 ml of distilled water was added and it was marked to blank. In the third test tube 1 ml of standard ammonium solution was taken to it and 2.5 ml reagent A was added to all the test tubes. After 5 minutes, 2.5 ml of reagent B added to all test tube, sample, standard and blank. Then the test tube were incubated at 37°C for 20 minutes and allowed to cool for 30 minutes. The OD of the sample and the standard were measured at 625 nm by using spectrometer.

1.2.6 CALCULATION

$$\text{Amount of ammonia present in the given sample} = \frac{\text{O.D. of the sample}}{\text{O.D. of the standard}} \times \frac{\text{Conc. of the standard}}{\text{Volume of the sample}} \times \text{Dilution factor.}$$

1.2.7 RESULT

The amount of ammonia present in the 1 ml of given sample = _____mg/ml.

1.2.8 DISCUSSION

Ammonia is constantly produced in all organisms by the deamination of amino acids. It is highly soluble, toxic and injurious to cells. In aquatic animals it is quickly eliminated into surrounding water. The ammonia cannot be excreted by terrestrial animals as such, because

water from a valuable commodity. So ammonia is quickly converted less harmful components and excreted. Fishes excrete their chief nitrogenous waste product in the form of ammonia and therefore called asammonotellic animals.

1.3 QUANTITATIVE ESTIMATION OF UREA

1.3.1 AIM

To estimate the amount of urea present in the given sample.

1.3.2 PRINCIPLE

Urea when heated with diacetyl monoxein in acidic solution produce yellow colour, in the presence of orthophosphoric acid, and sulphuric acid. The intensity of the colour thus formed is directly propositional to the amount of urea present in the given sample.

1.3.3 MATERIALS REQUIRED

Test tube, 10 ml of measuring cylinder, 1 ml pipette, boiling water bath and spectrophotometer.

1.3.4 REAGENTS

1.2.5 % Diacetylmonoxein: 2.5 gm of Diacetyl monoxein is dissolved in 100 ml of distilled water.

2. Standard urea: 100 mg of anhydrous urea crystal is dissolved in 100 ml of distilled water.

1.3.5 PROCEDURE

2 ml of sample, 2 ml of standard urea and 2 ml of distilled water were taken in three separate test tubes and were labelled accordingly. To each test tubes, 3 ml of colour reagent was added. All the test tubes were kept in boiling water for 20 minutes and then cooled to room temperature. The OD of sample and standard were measured 520 nm in a spectrophotometer by using the blank.

1.3.6 CALCULATION

$$\text{Amount of urea present in the given sample} = \frac{\text{O.D. of the sample}}{\text{O.D.of the standard}} \times \frac{\text{Conc. Of the standard}}{\text{Volume of the sample}} \times \text{Dilution factor}$$

1.3.7 RESULT

The amount of urea present in the given sample = _____mg/ml

1.3.8 DISCUSSION

Urea is characteristics nitrogenous excretory product of mammals including monotremes. The composition of the urine may vary from species to species and within the same species depending upon the diet. From 60 – 65 % of all fluid taken in to the body is excreted as urine. The major constituent of the urine is urea. It is noted that high concentration of urea won't create any problem as it is less toxic. In 1904, the ammino acid arginine was identified as precursor of urea in mammals and the enzyme arginase was shown to be essential for the conversion of urea in the liver. The accumulation of urea in the liver is higher than those body tissues. It was also found that the major pathway of urea excretion is only through urine. So the biosynthesis of urea and its elimination from all sources including ornithine cycle is found to be the urine. Thus urea is important in maintaining homostatic machinery.

1.4 ESTIMATION OF BLOOD CHLORIDE

1.4.1 AIM

To estimate the amount of chloride salt present in sheep's blood.

1.4.2 PRINCIPLE

Mercuric nitrate reacts with chloride to form mercuric chloride. When excess of Mercuric nitrate is added, the mercuric ions become clumped and form a violet blue colour in the presence of an indicator Diphenyl carbazone.

1.4.3 MATERIALS REQUIRED

Centrifuge, Test tube, 1 ml pipette, conical flask (125 ml) and micropipette.

1.4.4 REAGENTS

1. Mercuric nitrate: 834 mg of Mercuric nitrate was dissolved in 3 ml of conc. HNO_3 and was made up to 1000 ml with distilled water.

2. Diphenyl carbazon: 100 mg of Diphenyl carbazone was dissolved in 100 ml of Ethanol. It should be stored in brown bottle.

3. Standard chloride: 585 mg of Sodium chloride was dissolved in 100 ml of distilled water.

4. Anticoagulant: 3 gm Potassium oxalate and 1 gm Sodium fluride powder were first dissolved. Then 300 mg mixed powder was dissolved in 100 ml of distilled water.

1.4.5 PROCEDURE

3 ml of blood was centrifuged at 3000 rpm for 10 minutes. Two test tubes were taken and were labelled as sample and standard. 9.9 ml of distilled water was added to both the test tubes. 0.1 ml of sheep's blood serum (supernatant) was added to the sample test tubes. 0.1 ml of standard chloride solution was added to the standard test tubes. 5 ml of diluted serum of sample test tube was taken in conical flask, to it 2 drops of diphenylcarbazone solution was added and mixed well. This solution was titrated against Mercuric nitrate solution taken in a 5 ml pipette. The appearance of violet blue colour was noted as end point. The same procedure was repeated for diluted standard chloride solution.

1.4.6 CALCULATION

$$\text{Amount of chloride present in the sheep's blood} = \frac{\text{Reading of the sample solution}}{\text{Reading of the standard solution}} \times 100.$$

The amount chloride present in the sheep's blood= _____ m Eq/llit.

1.4.7 RESULT

The amount chloride present in the sheep's blood= _____ m Eq/llit.

1.4.8 DISCUSSION

Chloride forms one of the main constituents of the blood plasma. It is very essential for normal healthy life. Chloride occurs as sodium chloride and Potassium chloride. Concentration of Sodium chloride is higher in plasma and is responsible for "salt's taste" of the blood. The chloride level in whole blood is 250mg %chloride is essential for water balance, osmotic pressure and acid-base balance. Chloride deficiency in diet produces retardation of growth and failure of reproduction.

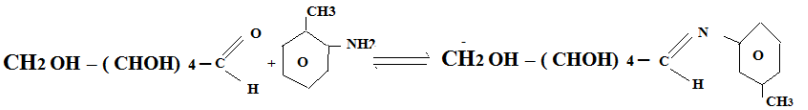
1.5 ESTIMATION OF GLUCOSE

1.5.1 AIM

To estimate the amount of glucose in the given sample by ortho-toluidine method.

1.5.2 PRINCIPLE

The aldehyde group of glucose condenses with ortho-toluidine in glacial acetic acid on heating blue-green colour in developed, which is due to formation of N-glucosylamine. The intensity of blue – green is proportional to the amount of glucose present. The optical density values are read in a spectrophotometer at 630nm and the amount of glucose present in 100ml of blood is calculated.



N-glucosylamine (Blue green complex)

1.5.3 MATERIALS REQUIRED

Test tubes with stand, pipette, centrifuge, cotton, syringe, water bath,spectrophotometer etc.

1.5.4 REAGENTS REQUIRED

1. Ortho-toluidine reagent:

Add 90ml of ortho-toluidine with 5 gm of thiourea and diluted to 1 liter with glacial acetic acid in brown bottle and keep in refrigerator.

2. 10% Trichloroacetic acid (TCA)

3. Glucose standard solution

Dissolve 10mg of glucose in 100ml of distilled water ie. 0.1ml of solution contains 10µg of glucose.

1.5.5 PROCEDURE

• Collect the blood sample using sterilized syringe and allowed to clot.

• Centrifuge the clotted blood at 10,000rpm for 10 minutes and collect the serum.

• Take 0.1ml of serum add 0.5ml of 10% TCA solution and 1ml of distilled water in a clean test tube and marked at test (T).

• Take 0.1ml of known concentration of standard glucose solution and add 1ml of distilled water and 0.5ml of 10% TCA solution in a test tube and marked as standard.

• Take 0.1 of distilled water and 0.5ml of 10% TCA solution in a clean test tube and marked as Blank (B).

• All three test tubes (B, S, T) are mixed well and leaves it for 5 minutes and then centrifuge it at 3500 rpm for 5 minutes.

• 0.5ml of supernatant is collected from three test tubes (B, S, T) and label each it as Blank, Standard and Test.

• Add 3ml of ortho-toluidine reagent in all three test tubes.

• Cover all the test tube with cotton. The mix all the solution well and put all the test tube into a boiling water bath for 10 minutes.

• After that, cool the test tube under running water and then mix again gently.

• Finally, read the absorbance at 630nm in spectrometer.

1.5.6 CALCULATION

$$\text{Estimation of glucose in given sample} = \frac{\text{OD value of test sample}}{\text{OD value of standard sample}} \times \frac{\text{Concentration of standard sample}}{\text{Volume of sample}} \times \text{Dilution Factor}$$

1.5.7 RESULT

The concentration of blood glucose in a given sample is ……µg.

1.6 ESTIMATION OF GLYCOGEN

1.6.1 AIM

To estimate the amount of glycogen in the given sample by the method of Hassid and Abraham, 1957.

1.6.2 PRINCIPLE

The tissue sample containing glycogen is digested with 30% potassium hydroxide, and then precipitated with ethanol. The

precipitate is treated with anthrone reagent and glucose in the hydrolysate is determined colorimetrically as reduced sugars

1.6.3 REAGENTS

1. **Potassium hydroxide (KOH) 30%:** 30g KOH was dissolved in

100ml of double distilled water.

2. **95% Ethanol (v/v):** 94.905ml of 99.9% ethanol was made up to 100ml with 5.095ml double distilled water.

3. **Sulphuric acid (H2SO4) 95%:** 95ml of concentrated sulphuric acid was made up to 100ml with 5ml of distilled water.

4. **Anthrone reagent 0.2%:** 0.2g anthrone was dissolved in 100ml of 95% sulphuric acid.

5. **Standard glucose (Stock standard):** 100mg d-glucose was dissolved in 100ml distilled water.

6.Working standard: 0.025, 0.05, 0.1, 0.15, 0.2ml stock solutions were made up to 5ml to get concentrations of 25, 50, 100, 150, 200µg, respectively.

1.6.4 PROCEDURE

Five mg tissue was digested with 1ml of 30% KOH for 20min in a boiling water bath. The contents were cooled in an ice bath and 1.25ml of 95% ethanol was added, thoroughly mixed and gently brought to boil in a hot water bath. This was cooled and centrifuged for 15 min at 750 x g. The supernatant was decanted and the tubes were allowed to drain on a filter paper for few min. The precipitate was redissolved in 1ml of distilled water, reprecipitated with 1ml of 95% ethanol, centrifuged and drained as stated before. The precipitate was dissolved in 5ml distilled water and 10ml of 0.2% anthrone reagent was added under ice-cold conditions. 5ml of distilled water and series of standards with a final volume of 5ml were treated with anthrone reagent and subjected to the same procedure. The tubes were covered with glass marbles and heated for 10min, in a boiling water bath. The contents were cooled immediately and the color was read at 680nm.

1.6.6 CALCULATION

Draw a standard graph by plotting concentration of the standard on the X-axis versus absorbance on the Y-axis. From the graph calculate the amount of glycogen present in the sample tube.

1.6.7 RESULT

The amount of glycogen is expressed as mg/g wet tissue.

1.7 ESTIMATION OF OXYGEN CONSUMPTION OF FISH

1.7.1 AIM

To estimate the oxygen consumption by the fish.

1.7.2 MATERIALS REQUIRED

Burette, pipette, reagent bottle, conical flask, respiration camber, procelin tile, kerosene, rubber tube, fish etc.

1.7.3 REAGENTS REQUIRED

Manganous sulphate, Alkaline iodine, 0.025 N sodium thio sulphate, conc. Sulphuric acid, starch etc.

1.7.4 PRINCIPLE

The following reaction takes place in Winker's method used for estimating the dissolved oxygen in the water.

Manganous sulphate reacts with potassium hydroxide. This results in the formation of manganous hydroxide

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

In the presence of oxygen a brown precipitate is formed as a consequence of transferring oxygen

$$2Mn(OH)_2 + O_2 \longrightarrow 2MnO(OH)_2$$

On addition of sulphuric acid the brown precipitate dissolves forming manganous sulphate.

$$Mn(OH)_2 + 2H_2SO_4 Mn(SO)_2 + 3H_2O$$

There is an intermediate reaction between manganeicsulphate and potassium iodide. This results in the liberation of iodine.

$$Mn(SO_4)_2 + 2KI \longrightarrow K_2SO_4 + MnSO_4 + I_2$$

The quantity of iodine liberated by the this reaction is proportional to the oxygen present in the sample and then it is determined by litrating known quantity of the sample against standard sodium thio sulphate solution (0.025N)

2 $Na_2S_2O_3Na_2$ S_4 ~~O_6~~ → NaI

1.7.5 PROCEDURE

A 1000 cc specimen bottle is taken as a respiration chamber and is filled with tap water. The initial oxygen content of respiration chamber is estimated by a collecting a sample into a narrow mouthed glass tapered reagent bottle of known volume.

A healthy fish is introduced into the respiration chamber and it is closed tightly. After 30 minutes the sample from the respiration chamber is taken into the reagent bottle of known volume.

1.7.6 DETERMINATION OF OXYGEN OF THE SAMPLE

The initial oxygen content of the water is determined by collecting sample into a narrow mouthed glass stoppered reagent bottle of known volume. To this 1 ml of manganous sulphate solution is added followed by the addition of 1 ml of alkaline iodine solution keeping the tap of the pipette well below the surface of the water. The bottle is stoppered and shaken vigorously and kept in a dark to prevent and photochemical reaction for about 15 minutes. The 1 ml of conc. sulphuric acid is completely dissolved by shaking vigorously 50 ml of the sample is taken in a conical flask and the liberated iodine is titrated against sodium thiosulphate using 2 or 3 drops of starch as an indication. The first disappearance of blue colour is takes as end point. The burette reading is noted and tabulated.

The final oxygen content of the respiration chamber is also determined in the same manner. From the difference of oxygen consumption by a fish is determined.

1.7.7 RESULT

Oxygen consumption by a fish = _____ml O_2/live fish/ hr.

a. OXYGEN CONTENT IN THE INITIAL SAMPLE

S.No	Volume of the sample	Burette reading		Volume of $Na_2S_2O_3$
		Initial	Final	
1	50 ml			
2	50 ml			

Oxygen content inthe K x 200 x Volume of $Na_2S_2O_3$ x 0.0698

given water sample = ----------

50

Where,

Initial O_2 content of the water = _____ml O_2 / lit.

b. OXYGEN CONTENT IN THE FINAL SAMPLE

S.No	Volume of the sample	Burette reading		Volume of $Na_2S_2O_3$
		Initial	Final	
1	50 ml			
2	50 ml			

Oxygen content in the final water sample = $\frac{K \times 200 \times \text{Volume of } Na_2S_2O_3 \times 0.0698}{50}$

Where, $K = \frac{\text{Volume of sample bottle}}{\text{Volume of sample bottle} - \text{Volume of reagent}}$

200 is obtained by normality of $Na_2S_2O_3$ x equivalent weight of O_2 x 1000

$0.698 = \frac{\text{Density of } H_2O}{\text{Density of } O_2}$

Final O_2 content of the water = _____ml O_2 / lit.

Oxygen consumed by the fish in 30 minutes = Initial O_2 content – Final O_2 content

Weight of fish = _____gm

Oxygen consumed by the gm fish in one hour = _____ x 2

Oxygen consumed by the One gm fish in one hour = $\frac{O_2 \text{ consumed in one hour}}{\text{Weight of fish}}$.

= _____ $ml O_2$ / gm. live fish/ hr.

II – IMMUNOLOGY

2.1 STUDY OF LYMPHOID ORGANS

2.1.1 AIM

To familiarize the primary and secondary lymphoid organ organs.

2.1.2 LYMPHOID ORGANS

As in other mammals, mouse also has primary lymphoid organs and secondary lymphoid organs.

2.1.3 PRIMARY LYMPHOID ORGANS

2.1.4 THYMUS

Thymus develops from third pharyngeal pouch. Later it migrate into thorax to a final position near the heart. It is called thymus because it resembles the leaf of thymus plant. Thymus is the first organ to become lymphoid in character. Thymus is a bilobed organ. Each lobe is divided into many compartments by septa and it contains loosely arranged cells. The cortical lymphocytes are immature cells. The medullary lymphocytes are mature T- lymphocytes. These mature T – lymphocytes enter the blood system. Thymus is the lymphoid organ where T – lymphocytes are formed. The T – lymphocytes are involved in cell mediated immunity (CMI).

2.1.5 BONE MARROW

Bone marrow is a tissue and not an organ. It can also called as myeloid tissue. Bone marrow is the site for the formation of all the cellular element of the body namely erythrocytes, granulocytes, lymphocytes and megakaryocytes in mouse and also all mammals. The B – lymphocytes develop and mature in the bone marrow (in birds the B – lymphocytes from the bursa of fabricus). The B – lymphocytes are involved in the humeral immunity (H1) by the production of antibodies.

2.1.6 SECONDARY LYMPHOID ORGANS

2.1.7 SPLEEN

It is the secondary lymphoid organ that lies in the peritoneum below the diaphragm and behind the stomach. The spleen has an outer area called the red pulp and inner area called the white pulp is the region where the RBC's are degraded. The white pulp contains lymphocytes, monocytes, macrophages and lymphoietic stem cells.

2.1.8 LYMPH NODE

Lymph node is bean shaped structure distributed throughout the body. They are connected by a lymphatic vessel. The main lymph nodes of mouse are cervical node, thoracic node, axillary node, brachial node, mesenteric node, lumbar node, caudal node and sciatic node. The lymph node has a outer cortex containing T – lymphocytes and an inner medulla containing plasma cells (antibody producing cells). The main functions of lymph node are to trap foreign matter (a microorganism). Inside the lymph nodes the lymphocytes mature.

2.1.9 GUIDELINES FOR THE DISSECTION OF VARIOUS LYMPHOID ORGANS IN MOUSE

The following guidelines are followed for the dissection of various lymphoid organs in mouse.

2.1.10 DISSECTION OF LYMPH NODE

The lymph nodes are encapsulated bean shaped cream coloured organ found throughout the body some of them are found simultaneously while some between muscles and some near viscera in the body cavity.

A. Thymus	**B. Spleen**
Pancreatic node	Lumbar node
Renal node	Caudal node
Mesenteric node	Sciatic node

2.2 HAEMAGGLUTINATION ASSAY

2.2.1 INTRODUCTION

Haemagglutination is a specific form of agglutination that involves red blood cells as antigens. It is the agglutination of red cells by antibodies

specifically directed to the antigens that are part of the red cell surface. It has two common uses in the laboratory: blood typing and to determine whether the quantity of antibodies against a particular infectious agent in a host blood is increasing.

2.2.2 PRINCIPLE

When antibodies react with antigenic epitopes on Red blood cells, they can cause cross linking of the RBCs resulting in agglutination, or clumping together of the cells. The quantity of antibodies is expressed as the antibody titre.

2.2.3 MATERIALS REQUIRED

1. Micro titre plate (96 well "V" bottom)

2. Micropipette – 50µl range

3. Micropipette tips

4. Physiological saline (0.15M)

5. 1% Human red blood cells (HRBC) from A or B group individuals in physiological saline

6. Anti-sera (Anti A and Anti B) (50µl/sample)

2.2.4 COMPOSITION OF ALSEVER'S SOLUTION

Dextrose- 2 g Tri-sodium citrate dehydrate- 0.8 g Citric acid monohydrate - 0.055 g Sodium chloride- 0.42 g Distilled water- 100 ml. The above listed ingredients are dissolved in 100ml of distilled water and the pH is adjusted to 6.1 with 10% citric acid solution and autoclaved at 15 pounds in a pressure cooker for 7 minutes.

2.2.5 PROCEDURE

1. Draw approximately 3ml of blood from A or B group individuals.

2. Mix it well with 9ml of Alsever's solution or collect blood in Detail coated tubes.

3. Centrifuge the solution at 1500 rpm and discard the supernatant.

4. Dissolve the cell pellet in to 1% of physiological saline.

5. Fifty microtitre of physiological saline is added into all the wells of a clean "V- bottom" microtitre plate.

6. Fifty micro titre of the antiserum (anti-A/anti-B) is serially double diluted from the first the well of the first row till the 11th well of the same row of the micro titre plate leaving the 12th well as the negative control.

7. Similarly other antiserum samples are also diluted serially in each row of the micro titre plate.

8. Add fifty microlitre of the antigen (HRBC) is added to all the wells of the microtitre plate.(Add HRBC from A group individual if u have used anti-A serum or vice versa)

9. The microtitre plate is gently shaken for efficient mixing of the reagents.

10. The plate is incubated at room temperature for an hour.

The highest dilution of the serum samples which shows detectable agglutination (matt formation) is recorded and expressed as Log2 antibody titre of the serum.

2.3 STUDY OF ANTIBODY TITRE VALUES

2.3.1 AIM

To study the antibody titre values.

2.3.2 PRINCIPLE

The antibody titer is a test that detects the presence and measures the amount of antibodies within a person's blood. The amount and diversity of antibodies correlates to the strength of the body's immune response. Antibody titration is a determination of the concentration of a specific antibody in the patient's serum or to determine the strength of antigen expression on different red cell samples. If the concentration of the specific antibody is being determined, the cells must contain the known antigen and the procedure should be performed under the optimal conditions for that antibody.

This test is usually performed to estimate the antibody activity of an all immunized pregnant female, to determine if there is any specificity to an autoantibody or to characterize antibodies that may have high titer with low avidity. Titration of ABO antibodies allows clinical assessment of the feasibility of ABO mismatched transplant, and monitoring of treatment to reduce antibody titre in preparation for ABO mismatched transplant.

Sample: Plasma or serum can be titrated.

2.3.3 PROCEDURE

1. Label 10 tubes according to the dilution: 1, 2, 4, 8, 16, 32, 64, 128, 256 and 512.

2. The first tube will be undiluted serum.

3. Tube 2 will be a 1/2 dilution, 4 will be a 1/4 dilution and so on.

4. Add 0.3 ml of saline to tube 2 to tube 512.

5. Saline is not added in tube 1

6. Add 0.3 ml of serum to both tubes 1 and 2.

7. Use a clean pipette to mix the 1/2 dilution (tube 2) several times and then transfer 0.3 ml to tube 4.

8. Use a clean pipette to mix the 1/4 dilution several times and then transfer 0.3 ml to tube 8.

9. Continue the process for all dilutions (512).

10. Remove 0.3 ml from tube 10 (512).

11. Add one drops of specific red blood cells to all the tubes.

12. Mix well and test by the appropriate technique for the specific antibody.

13. Examine test results macroscopically, grade and record the reactions.

2.4 SINGLE IMMUNODIFFUSION (RADIAL)

2.4.1 AIM

To perform radial immunodiffusion for the given sample.

2.4.2 PRINCIPLE

In this method, antibody is incorporated before the gel is made. Thus the antiserum is uniformly distributed throughout agar gel. Antigen is then allowed to diffuse from wells into the agar gel. Initially as the antigen diffuse out of the well, where its concentration becomes equivalent to that of the antibody in the gel, antigen-antibody precipitates and a precipitin ring is formed. Greater the concentration of antigen greater is the diameter of precipitin ring.

2.4.3 MATERIALS REQUIRED

Agarose, Borate saline buffer (pH 8.65), standard antigen, test antigen, antiserum, gel punch with syringe, glass plates, template, Moist chamber, assay buffer etc.

2.4.4 PROCEDURE

• The glass plate should be wiped grease free with cotton.

• Dissolve one gram of agarose in 100m1 of Borate saline buffer by heating.

• Allow the solution to cool to 55°c and mix 120 µL of antiserum with 6m1 of the solution.

• Pour the agarose solution containing the antiserum on to a grease free glass plate that had previously been set on a horizontal level.

• Allow the gel to form by cooling.

• Cut the wens of 4 mm diameter into the gel using a gel punch.

• Arrange four 1.5m1 micro centrifuge tubes in rack.

• Add 50 µL of buffer to all tubes

• Add 50 µL antigen to the first tube (1:2).

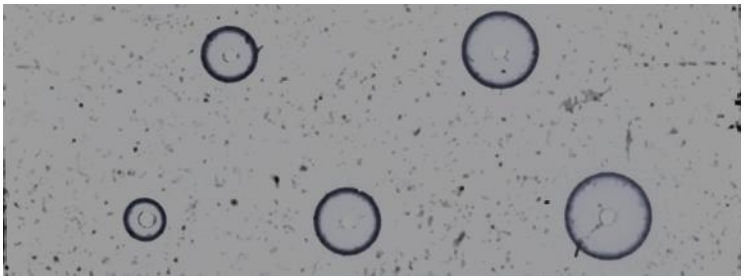
• Transfer 50 µL antigen from 1st tube to 2nd tube (1:4) and continue the same procedure to remaining tube (1:8 and 1:16).

• Add 20 µL, of diluted antigen to respective wells. Keep the gel plate in a box containing wet cotton and incubated overnight at room temperature.

• Measure the diameter of the precipitation disc by marking the edges of the circle.

2.4.5 OBSERVATION

Precipitation zone indicates the presence of specific antibodies in the serum to the test antigen added. The standard graph is drawn for the ring diameter.



2.5 DOUBLE IMMUNODIFFUSION

2.5.1 AIM

To check antisera for the presence and specificity of antibodies for a particular antigen.

2.5.2 PRINCIPLE

Double immunodiffusion technique was first described by Ouchterlony. Antigen and antibody placed in adjacent wells in agar gel diffuse radially. Initially as the antigen and antibody diffuses out of the respective wells, the concentrations are relatively high nearer to the well, however as they diffuse further from the wells, the concentration decreases at one point where the concentration become equivalent and antigen-antibody complex precipitate to form a precipitant line.

2.5.3 MATERIALS REQUIRED

Agarose, Veronal buffer, antigen, test antisera, glass plates, gel-punch with syringe, template, micropipettes, tubes, distilled water, a box to keep the gel plate in moist chamber.

2.5.4 PRECAUTION

The glass plate should be wiped grease free with cotton for the even spreading of the agarose. Ensure that the chamber has enough wet cotton to keep the atmosphere humid.

2.5.5 PROCEDURE

• Prepare 1% agarose solution in lx vernoal buffer by heating dissolved agarose completely.

• Cool the solution to about 55 - 60°C and pour required volume on to a grease free glass plate that had previously been set on horizontal level surface.

• Allow the gel to set for 20 - 30 minutes.

• Keep the gel plate on the template provided.

• Punch wells in the gel with the help of a gel punch corresponding to the marking on the template with gentle process.

• Add 20 μL of the antigen in the side wells and

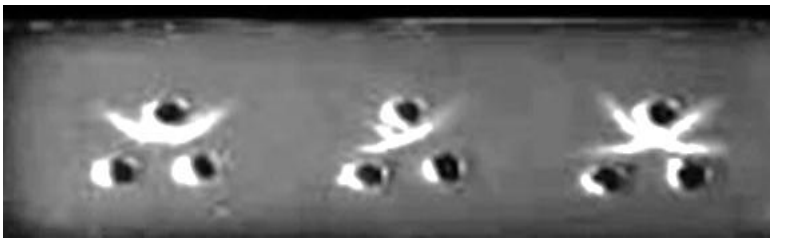
• Add 20 μL of antiserum to the center well

• Keep the plate in the moist chamber overnight at room temperature.

• Observe the plate for an opaque precipitant line between the antigen and antisera wells.

2.5.6 INTERPRETATION

Precipitant line indicates the presence of antibody in the antiserum to the antigen added.



2.6 IMMUNOELECTROPHORESIS

2.6.1 AIM

To perform the technique of immunoelectrophoresis.

2.6.2 PRINCIPLE

The immunoelectrophoresis technique combines electrophoresis and immunodiffusion. It is based on the principle of electrophoresis of antigens in agarose gel.Antigen gets separated according to their acquired change size and shape by migrating to different location. Antibody (antiserum) is then placed in a through which is cut parallel to the direction of antigen migration. Antigens thus resolved are subjected to the immunodiffusion with antibody added in a through cut in the agarose gel. The formation of a precipitin line indicates the presence of antibody specific to that of antigenic component. The

formation of only one precipitin line indicates the homogeneity of antibody. The presence of more than one precipitin line indicates the heterogeneity of antibody, while the antibody absence of precipitin line indicates that the antibody does not have any specificity towards the antigen component separated by electrophoresis.

It is widely used in chemical laboratory to separate the major blood proteins in serum. Immunoelectrophoresis is powerful analytical technique with great resolving power as the combines separation of antigen by electrophoresis followed by immunodiffusion against antibody.

2.6.3 MATERIAL REQUIRED

Agarose 1 x running buffer, antigen, antiserum – A, antiserum – B and immunoelectrophoresis apparatus

2.6.4 PROCEDURE

1. Take 5 ml of 1 x running buffer and add 50 milli gram of agarose to prepare 1 % agarose solution.Heat the solution in a boil water path till agarose dissolves completely. After completely dissolving the molden agarose solution has to be brought down to around 40° C - 50° C

2. The moltend agarose on to the glass microscopic slide that kept on a leveled surface. The agarose would be about 3 – 4 mm in thickness.

3. Allowed the agarose to get solidified before cutting the wells keep the slide at 2 – 8° C for a short period, so as to the cut edges of the wells do not break down when the agarose plugs are removed.

4. The agarose slide can also be stored at 4°C in case if it is not used immediately

5. Cut the well on the agarose slide using gel punch as per the template provided the well is 3 mm is diameter and should be at the negative end.

6. Cut two troughs on either slide with cuter but do not remove the agarose plug from it.

7. The agarose plugs can be removed from the wells either by using a fine gauze glass (or) metal cannula attached to a line add 10 µm antigen to the respective well by using a micropipette.

8. Place the slide on the bridges of the electrophoresis tank that has been filled with 1 x running buffer. The antigen well should be placed at the cathode (-ve) side of the electrophoresis tank (Black terminal).

9. Anode the slide to the running buffer by using filter paper (wicks) to the respective chamber of the tank.

10. electrophoresis at 50 – 100 volts for about 2 – 3 hours. This would aid in the separation of different antigenic components in the antigen sample. Make sure that heat is not generated during the run , as it would denature the protein antigen.

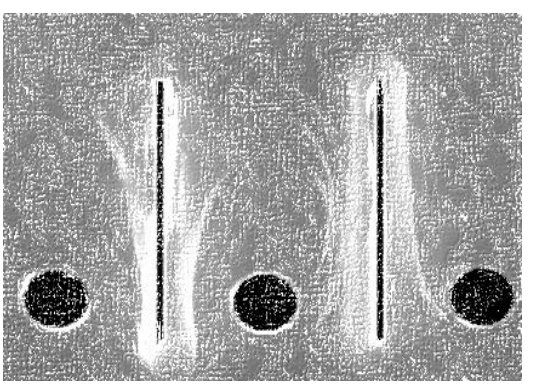
11. Remove the agarose plugs from the trough and equilibrate the slide at room temperature for about 10 min add 100 µl of antigen and in antibody (B) provided into the respective troughs.

12. Place the slide in a petri plate the petri plate should be kept moist by heating a tem paper dowel or wet cotton.

13. observed for the precipitin line are not clearly visible, dip the slide in 4 % enhancer solution. Add about 30 µl of the enhancer solution into a 90 mm petri dish keep the slide for 5 – 10 minutes to observe precipitate line after enhancer treatment the band would be clearly seen.

2.6.5 RESULT AND INTERPRETATION

The formation of only one precipitin line indicates the homogenicity of antibody presence of more than one precipitin line indicates the heterogenicity of antibody. While the absence of precipitin line indicates that line antiserum thus not have antibody to any of the antigenic components separated by electrophoresis.



2.7 BLOOD GROUPING

2.7.1 AIM

To determine the blood group by haemagglutination technique.

2.7.2 BACKGROUND INFROMATION

Human being possesses four types of blood groups namely A, B, AB, and O in the ABO blood group system. The four types of blood group is identified by following characteristics features.

2.7.3 A GROUP:

Persons with A group have A antigen in the RBC and anti B (antibody) in the plasma.

2.7.4 B GROUP:

Persons with B group have B antigen in the RBC and anti A (antibody) in the plasma.

2.7.5 AB GROUP:

Person with AB group have both A and B antigen in the RBC and no antibody in the plasma. Hence, they are called universal recipient, as they can receive blood from A, B, AB and O group persons.

2.7.6 O GROUP:

Persons with O group have no antigen in the RBC, but they anti A in their plasma. They are called universal donor and can give blood to any other blood group persons.

2.7.7 PRINCIPLES OF ABO BLOOD TYPING

It is based on the principle of haemagglutination. When antigen reacts with the corresponding antibody antigen – antibody complex is formed. Since the blood antigen are located on the RBC, the antibody makes RBC to agglutinate and this can be seen with the naked eye or under the microscope.

2.7.8 MATERIALS REQIURED

Microscopic glass slide, Anti ´A' serum, Anti ´B' serum, Sterilized needle.

2.7.9 PROCEDURE

- Take clean glass slide and mark the left side as anti A and right side as anti B
- Place a drop of anti A and anti B on left and right side of the slide
- Prick a finger and place a drop of blood near the anti A serum and on the anti B serum
- Then with the help of sterilized needle or stick mix the blood with the anti A serum and with the another stick mix the blood with anti B serum
- Observe the slide for agglutination of blood cells.

2.7.10 OBSERVATION

A GROUP: The agglutination is noticed on the left slide (with "anti – A"). The person is said to have **"A group".**

B GROUP: If agglutination is noticed on the right side (with "anti – B"). The person is said to have **"B group".**

AB GROUP: If agglutination is noticed on the both side (with "anti A and anti B"). The person is said to have **"AB group".**

O GROUP: If no agglutination is noticed on both left and right side, the person has **"O group".**

2.7.11 RESULT

Haemagglutination test shows the _____.

So my blood group is _____.

2.7.1.1 RHESUS FACTOR TYPING

2.7.1.2 AIM

To detect the rhesus antigen by haemagglutination technique.

2.7.1.3 BACKGROUND INFORMATION

Some persons have an Rh antigen in their blood cell called Rh positive. There is no corresponding antibodies in the plasma. Rh factor

has application in child birth. If a Rh negative mother has a Rh positive foetus the Rh antigen may enter the mothers blood and the mother produced anti Rh antibodies (anti O) which will agglutinate the foetusblood. This shows a disease called erythroblastosisfoetalis or haemolytic disease of new born.

2.7.1.4 MATERIAL REQIURED

Anti – Rh serum (anti – D), Microscopic glass slide, sterilized needle, microscope etc.

2.7.1.5 PRINCIPLE

The Rh factor typing uses the haemagglutination technique. When the Rh antigen is mixed with anti Rh serum, agglutination occurs. This is seen as clumping of the red blood corpuscles.

2.7.1.6 PROCEDURE

Place a drop of anti Rh serum on a clean microscopic slide with a sterilized needle makes a puncture in the finger and place a drop of blood near the anti Rh serum. Mix the blood thoroughly with the anti Rh serum using an applicator stick gently and observe the agglutination.

2.7.1.7 OBSERVATION

Rh POSITIVE: If a person is Rh positive agglutination is seen with anti Rh serum.

Rh NEGATIVE: If a person is Rh negative agglutination is not observed.

2.7.1.8 PRECAUTIONS

The following precaution should be observed in Rh typing

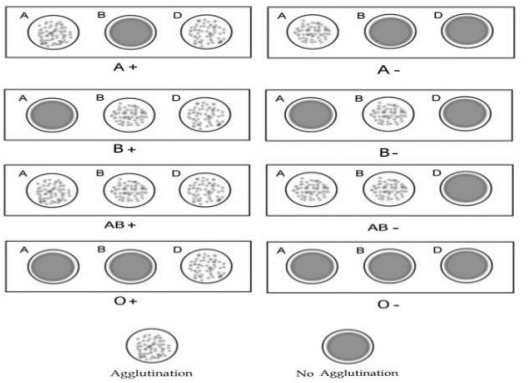
a) Adding of very small amount of blood and too much of antiserum is a common error.

b) Partial or complete drying up of the mixture must be avoided.

2.7.1.9 RESULT

Agglutination is_____.

So my blood group is _____.



2.8 HUMAN CHRONIC GONADOTROPHIN (HCG) TEST

2.8.1 AIM

To detect HCG in urine for the diagnosis of Pregnancy.

2.8.2 PRINCIPLE

Human chronic gonadotrophin (HCG) is a hormone produced by placenta very early in pregnancy. It's a glycoprotein and consist of two subunits a and b. The isolated sub units lose their biological activity but are able to generate antibodies. Sub unit a structure of HCG is identical with a chain of TSH, LI I, FSH, therefore b HCG forms the most acceptable marker for diagnosis of pregnancy. The secretion of HCG in urine approximately 20 days after the last menstrual period and increase rapidly. The test is based on antigen (b HCG in urine sample) and antibody (anti b HCG antisera) reaction. b HCG, present in urine sample neutralizes anti b HCG antibody and hence does not allow agglutination with HCG agglutinating antigen, however when b HCG is absent in urine sample, free anti b HCG antibody reacts with HCG agglutination antigen and results in agglutination. Thus a negative sample shows agglutination in less than minutes. Levels of HCG equivalent to 0.5mL / mL and above can be detected in urine specimens. This principle is known as latex agglutination inhibition assay.

2.8.3 MATERIALS REQUIRED

b HCG antibody, dropper bottle, HCG agglutination antigen, test sample, Disposable droppers, Reusable glass slides, Disposable

applicator sticks.

2.8.4 SPECIMEN COLLECTION

For optimal results its best to use the 1st urine voided in the morning as it contain the greatest concentration of HCG. Specimens could be stored at 2 to 8 °C for 72 hours.

2.8.5 PROCEDURE

• Bring HCG agglutinating antigen and b HCG antibody to room temperature and shaken well for some seconds before use.

• Wash the glass slide and dry with paper towel to remove cell traces of specimens and reagents previously tested. The slide must be clean and dry.

• Place one drop of clear urine sample on the clean slide.

• Add b HCG antibody onto the slide

• Mix the sample and the reagent well.

• Add 1 drop of HCG agglutinating antigen and mix well.

• Gently rock the slide for 2 minutes and observe the results.

NOTE:

- Urine specimen which contains blood and high bacterial contamination shall not be used.
- Positive results form very early pregnancy; it is recommended that weak positive result should be re tested with the fresh urine sample 48 hours later.
- Improper mixing of the specimens with the reagent can lead to incorrect results.

2.8.6 RESULT

Absence of agglutination shows positive result in sample A, it is due to the presence of HCG in urine. Hence sample A is positive to pregnancy test.

III - ENVIRONMENTAL BIOLOGY

3.1 ESTIMATION OF SALINITY

3.1.1 AIM

To estimate the amount of salinity in the given sample.

3.1.2 REQUIRMENT

0.1 N silver nitrate (Ag No3), Potassium chromate solution, Burette, Pipette, conical flask etc.

3.1.3 PRINCIPLE

The chloride content in the sample gets precipitated as silver chloride by titrating against 0.1 N silver nitrate with potassium chromate as indicator. The chlorinity of any given of water has definite relationship to the salinity and hence the salinity can be calculated by determining the chlorinity.

3.1.4 PROCEDURE

Take 20 ml of given water sample in a conical flask and add few drops of potassium chromate as indicator. Titrate the sample against 0.1 N Ag NO3. The appearance of brick red color should be taken as end point. Note down the volume of Ag NO3 used

3.1.5 CALCULATION

$V_1N1 = V_2N_2$

Where, V1 = Volume of AgNO3

V2 = Volume of sample

N1 = Normality of Ag NO3 (0.1N)

N2 = Normality of the sample

V1N1V1 x 0.1

N2 = ---------- = ----------

V2 25

Chlorinity = Normality of the sample (N2) X Equivalent weight of chloride (35.45)

Salinity of the sample = 0.03 + (1.805 x chlorinity)

S.No	Sample	Burette reading		Volume of AgNo3 Used	Indicator
		Initial	Final		
1	Tap water (A)				
2	Distilled water (B)				

3.1.6 RESULT

Salinity of given water sample A (Tap water) = _____‰

Salinity of given water sample B (Distilled water) = _____ ‰.

3.2 ESTIMATE OF DISSOLVED OXYGEN CONTENT OF WATER SAMPLES

3.2.1 AIM

To estimate the amount of dissolved oxygen present in the given samples using Winkler's method.

3.2.2 PRINCIPLES

The following reaction takes place in Winkler's method used for the estimation of dissolved O2 in the sample of water.

1. Manganoussulphate reacts with potassium hydrate in the alkaline potassium iodide. This results in the formation of white precipitate of manganous hydroxide.

$$Mn\ SO_4 + 2KOH \longrightarrow Mn(OH)_2 + K_2SO_4$$

2. Manganous hydroxide reacts in the oxygen present in the sample, forming a brown precipitate of manganous basic oxide.

$$2Mn(OH)_2 + O_2 \rightarrow 2MnO(OH)_2$$

3. On the addition of sulphuric acid the brown precipitate dissolves forming manganic sulphate

$$2MnO(OH)_2 + 2H_2SO_4 \rightarrow Mn(SO_4)_2 + 3H_2O$$

4. There is an immediate reaction between manganic sulphate and the potassium iodide previously added to the sample and result in the liberation of iodine and accounts for the typical iodine coloration of the samples.

$$Mn(SO_4)2KI \longrightarrow MnSO_4 + K_2 SO_4 + I_2$$

5. The quantity of the iodine liberated by the reaction is equivalent to the quantity of O2 present in the sample and is determined by titration a known quantity of sample against standard Na2 S3 O3 solution.

3.2.3 PROCEDURE

Collect the sample of water whose O2 content is to be estimated in a narrow mouthed glass stoppered bottle of known volume. To this add one 1ml of manganoussulphate solution and then 1 ml of alkaline iodide solution keeping the tip of the pipette well below the surface the water. Then cover the bottle with glass stopper shake vigorously and keep the bottle in a dark place to prevent any photochemical reaction. After 10 minutes take out the bottle and add 1 ml of Conc. Sulphuric acid in order to dissolve completely by shaking the well. Take 50 ml of sample which now contains the liberated iodine in a conical flask. Titrate this against 0.025N Na2S2O3 solution using starch as an indicator. The first disappearance of blue colour should be taken as end point. Titrate three such samples and take average value. Tabulate the burette values and calculate the O2 content of the sample by using the following formula,

$$O_2 \text{ in mg/lit of the sample} = \frac{K \times 200 \times \text{volume of } Na_2S_2O_3 \times 0.698}{\text{Volume of sample taken}}$$

$$\text{Where, } K = \frac{\text{Volume of sample bottle}}{\text{Volume of sample bottle} - \text{volume of reagent}}$$

200 is obtained by normality of $Na_2 S_2O_3$ x equivalent weight of O_2 x 1000

Density of H_2O

0.698 = ----------

Density of O_2

Table 1: Dissolved oxygen content of sample 1.

S.No	Volume of sample	Burette reading		Volume of $Na_2S_2O_3$ Used	Indicator
		Initial	Final		
1					Starch
2					
3					

Table 2: Dissolved oxygen content of sample 2.

S.No	Volume of sample	Burette reading		Volume of $Na_2S_2O_3$ Used	Indicator
		Initial	Final		
1					Starch
2					
3					

3.2.3 RESULT

Amount of the O2 present in the sample A = _____ml/lit.

Amount of the O2 present in the sample B = _____ ml/ lit.

3.3 MOUNTING OF PLANKTON

The term plankton is applied to all the organisms which live free in the water, being enclosed with limited power of locomotion at the mercy of wind and water currents. However many planktonic organisms are strong swimmers. Planktonic organisms differ in their size. Plankton which are larger them 1 mm are referred to as macroplankton and smaller than 1 mm are called microplankton, smaller than microplankton are called nanoplankton.

3.3.1 LUCIFER

3.3.2 CLASSIFICATION

PHYLUM : ARTHROPODA

CLASS : CRUSTACEA

3.3.3 COMMENTS

1. Lucifer is a pelagic Malacostracan.

2. It is found in India, Asia and Europe.

3. It is the most common member of Decapoda.

4. The animal is minute, slender and delicate.

5. The body is divided into cephalothorax, abdomen and telson.

6. The cephalothorax is smaller than abdomen.

7. The head is extremely elongated and bears long antennae and eyes with stalk.

8. The thoracic legs are non – chelate and the last two thoracic somites are without legs.

9.The embryonic life leads to the egg nauplius and passes by successive moults to a protozoae, zoae stage with segmented abdomen.

3.3.4 INDENTIFICATION

By the presence of long, slender body with stalked eye.

3.3.5 MYSIS

3.3.6 CLASSIFICATION

PHYLUM : ARTHROPODA

CLASS : CRUSTACEA

3.3.7 COMMENTS

1. Mysis is a small, transparent, marine pelagic shrimp like and laterally compressed malacostracan.

2. It is minute, slender and delicate.

3. It is frequently found in India and North Atlantic.

4. The body is divided into cephalothorax and abdomen.

5. A carapace is present which leaves two thoracic segments uncovered.

6. A thoracic segment bears 8 pairs of biramous appendages.

7. Two anterior appendages are small and form maxillipedes.

8. Posterior thoracic appendages bears a brood pouch with in which the young ones develop.

9. The bronchium or gills are absent and respiration occurs through the lining of the carapace.

3.3.8 IDENTIFICATION

By the presence of brood pouch.

3.3.9 NAUPLIUS

3.3.10 CLASSIFICATION

PHYLUM : ARTHROPODA

CLASS : CRUSTACEA

3.3.11 COMMENTS

1. It is free swimming, minute, conical and microscopic creature with brood anterior and narrow posterior ends.

2. The body is divided into indistinct head, trunk and bilobed anal region.

3. It consists of three pairs of appendages namely biramous antennules, biramous antennae and biramous mandibles which assist in swimming.

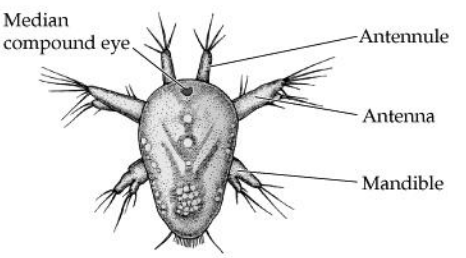
4. The nauplius contains a median eye.

5.The larva is unsegmented without ventral nerve cord and heart.

6. The larva has great phylogenetic significance and it is supported to be Arthropod an trochophora larva.

3.3.12 IDENTIFICATION

By the presence of conical, median eye and biramous antennae.



3.3.13 ZOEA LARVA

3.3.14 CLASSIFICATION

PHYLUM : ARTHROPODA

CLASS : CRUSTACEA

3.3.15 COMMENTS

1. Zoea larva is a crustacean larva.

2. The metanauplius directly hatches into zoea larva.

3. Body is differentiated into cephalothorax and abdomen.

4. Carapace covering of cephalothorax is protruded into rostrum anteriorly and median spine dorsally.

5. The head contains large compound eye, antennules, antennae etc.

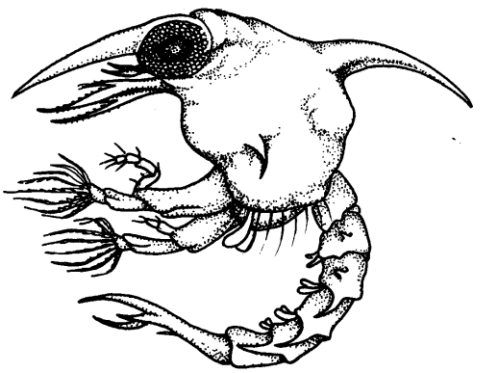
6. Thoracic appendages develop as buds the abdomen has sis segments.

7. The last abdominal segments contain caudal fork.

8. Zoea larva changes into metazoea during development.

3.3.16 IDENTIFICATION

By the presence of rostrum, large compound eye, median dorsal spine etc.



3.3.17 CALANUS

3.3.18 CLASSIFICATION

PHYLUM : ARTHROPODA

CLASS : CRUSTACEA

3.3.19 COMMENTS

1. It is marine pelagic plankton.

2. The body is more or less depressed.

3. Body is divided into head, thorax and abdomen.

4. Antennae are elongated with many joints.

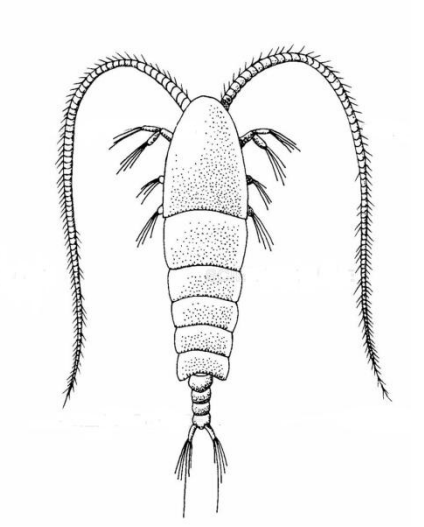
5. Fifth thoracic segment is firmly attached to the forth segment and forms movable articulation with the sixth segment.

6. Appendages are absent in the abdomen exist the caudal style.

7. Eggs are carried by the female in one or two ovisacs which is attached to the ventral surface of the genital segment.

3.3.20 IDENTIFICATION

By the presence of depressed body absence of median eye and the absence of abdominal appendages.



3.4 ANIMAL ASSOCIATIONS

3.4.1 SUCKER FISH AND SHARK (COMMENSALISM)

The given animal association is commensalism without continuous contact. eg:Sucker fish and shark

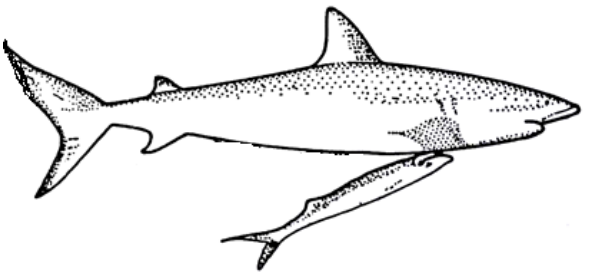
3.4.2 COMMENTS

1. The given relationship is interspecific symbiotic association present between the suckerfish (Echenis) and shark. It is a kind of beneficial association called co-operation. This type of association is called symbiosis. In this type of association one partner alone gets benefitted and the one partner is not harmed. The sucker fish is an ectocommensel attached to the body of the shark.

2. The sucker fish has a sucker which is modified dorsal fin for the attachment with the body of the shark. In this type of association the suckerfish echenis is benefitted by the shark in 2 ways.

- To transport from the one place to another.
- It shares the food captured by the shark.

- The sharks provide benefits to the sucker fish without getting itself affected.



3.4.3 PARASITISM

3.4.4 SACCULINA AND CRAB

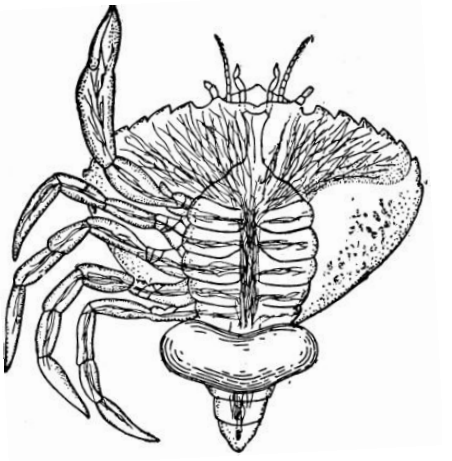
The given animal association is parasitism eg: sacculina and crab

3.4.5 COMMENTS

1. It is on antagonistic association. It is a kind of harmful association. There is no cooperation but only .exploitation. Hence it is known as parasitism

2. In this type of association the parasite sacculina exploits the host crab. The sacculina has no appendages armour at the ventral side of the abdomen of the host it absorbs the nutrients from the host by its roots like peduncle passing it into the host body.

3. The presence of sacculina affects the sexual character of the host leading castration when a male crab (host) is affected by this parasite change its character from maleners to female.



3.4.6 PREDATION

The given animal association is predation eg: cobra and frog.

3.4.7 COMMENTS

1. Predation is an interspecific relationship between two animals, where one of the animals its beneficial and other is affected this phenomenon is called antagonism.

2. In predation the animal which kills the prey is called predator, the animal which is killed by predator is called prey.

3. Predators are always bigger than the prey the predator always exploits the prey for its own benefits so predation is always one sided relationship and its known as exploitation.

4. A successful predator has high hunting ability.

5. Cobra lives in a holes under stones and thick vegetation and feeds on the prey (frog, rat, lizard etc.) it strikes the prey and swallow the prey.

3.4.8 MUTALISM

The given animal association is called mutalism eg: sea anemone and hermit crab

3.4.9 COMMENTS

1. It is a kind of beneficial association called cooperation i.e. mutalism.

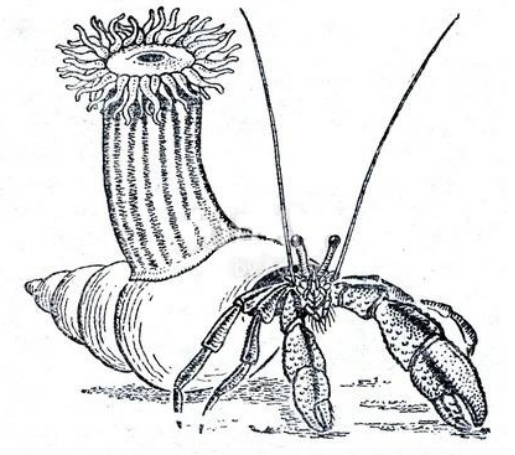
2. In this type, both the species get benefitted from each other.

3. The hermit crab lives inside the empty shell of gastropod.

4. The outer surface of the shell is completely colonized by the sea anemone

5. The hermit crab obtains the sea anemone from the rock and places it on the back to the shell in few cases the sea anemone completely covers the shell and in some cases the original shell is characterized.

6. Hence the entire crab is covered by the base of the sea anemone by the presence of stinging cells .sea anemone helps to prevent the approach of the predation fish .which usually feeds on the hermit crab. So the crab is protected while the sea anemone is benefitted in two ways by the hermit crab. i.e. to move one place to another and it sharing the food.



3.5 THE INTERTIDAL FAUNA

The intertidal area also known as the littoral zone is where the land and sea meet, between the high and low tide zones. This complex marine ecosystem is found along coastlines worldwide. It is rich in nutrients and oxygen and is home to a variety of organisms. Much of this inhospitable environment is washed by the tides each day, so organisms that live here are adapted to huge daily changes in moisture, temperature, turbulence (from the water), and salinity. The littoral zone is divided into vertical zones. The zones that are often used are the spray zone, high tide zone, middle tide zone, and low tide zone. Below these is the sub-tide zone, which is always underwater.

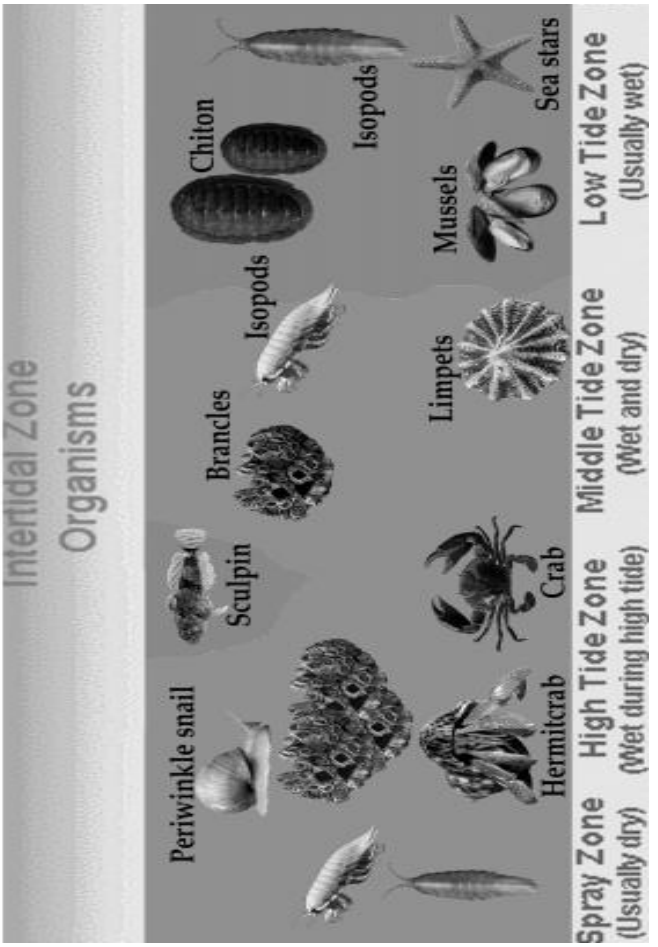
3.5.1 Spray Zone: Also called the Upper Littoral, the Supra littoral Fringe, the Splash Zone, and the Barnacle Belt. This area is dry much of the time, but is sprayed with salt water during high tides. It is only flooded during storms and extremely high tides. Organisms in this sparse habitat include barnacles, isopods, lichens, lice, limpets, periwinkles, and whelks. Very little vegetation grows in this area.

3.5.2 High Tide Zone: Also called the Upper Mid-littoral Zone and the high intertidal zone. This area is flooded only during high tide. Organisms in this area include anemones, barnacles, brittle stars, chitons, crabs, green algae, isopods, limpets, mussels, sea stars, snails, whelks and some marine vegetation.

3.5.3 Middle Tide Zone: Also called the Lower Mid-littoral Zone. This turbulent area is covered and uncovered twice a day with salt

water from the tides. Organisms in this area include anemones, barnacles, chitons, crabs, green algae, isopods, limpets, mussels, sea lettuce, sea palms, sea stars, snails, sponges, and whelks.

3.5.4 Low Tide Zone: Also called the Lower Littoral Zone. This area is usually under water - it is only exposed when the tide is unusually low. Organisms in this zone are not well adapted to long periods of dryness or to extreme temperatures. Some of the organisms in this area are abalone, anemones, brown seaweed, chitons, crabs, green algae, hydroids, isopods, limpets, mussels, nudibranchs, sculpin, sea cucumber, sea lettuce, sea palms, sea stars, sea urchins, shrimp, snails, sponges, surf grass, tube worms, and whelks.



3.6 CONSTRUCTION OF FOOD WEB

- Food chains are not an isolated segment, but inter connected with one another.
- Predator eats several types of food, and every kind of food is eaten by different organisms.
- The interlocking pattern of several food chains is known as food web.
- Solar energy is the primary source of energy it is trapped by the green plants and plankton.
- The plants are called primary producer and the trapped energy flow from producer to consumer- the primary and secondary successively
- Grass land ecosystem is taken as a model ecosystem for the construction of food web.
- In grass land ecosystem grasses of the family Graminae, forbs, shrubs and scattered tree constitute the primary producers.
- Herbivores such as grazing animals such as ruminants, rabbits and rodents, insects, and some annelids are the primary consumers.
- Animals like frogs, lizards, snakes, birds even the fox are carnivore feeds on herbivores constitutes the secondary consumers.
- The hawks occupy the top of the tropic level it feeds on the secondary consumers
- There are different linear food chains are established, which are interconnected to from food web.
- The stability of food chain and food web are very much essential for the continued existence of an ecosystem.

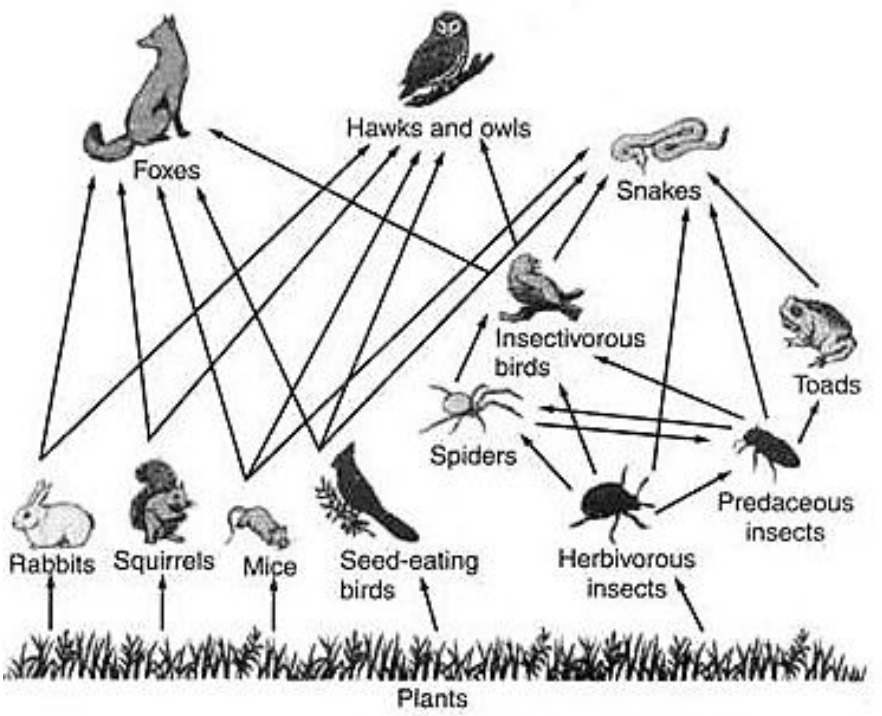
The food chains are:

1. Plants--- rabbit---fox----hawks
2. Plants---rodents---fox----hawks
3. Plants--- birds---fox----hawks
4. Plants--- rodents---snakes---hawks
5. Plants----insects--- frog --- birds----hawks
6. Plants----insects--- frog --- snakes----hawks

7. Plants--- insects--- birds--- hawks

8. Plants --- ruminants--- fox

9. Plants – rabbits – fox



3.7 MEASUREMENT OF LIGHT INTENSITY IN WATER BODIES USING SECCHI DISC

1. Secchi disc is a plain white circular disc of 30 cm in diameter used to measure water transparency or turbidity in the aquatic system.

2. The disc is mounted on a pole or line. It has a hook in the centre from which a rope arises.

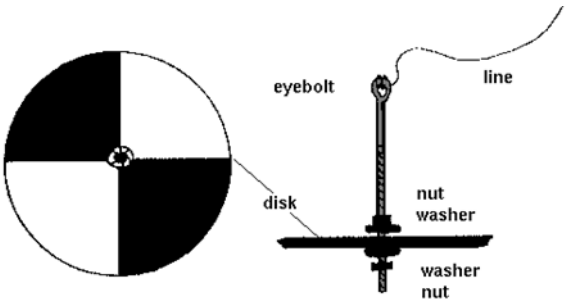
3. To measure transparency the disc is slowly dropped into water. The depth at which the disc disappears and no longer visible is noted.

4. Similarly the disc is slowly lifted. The depth at which appears is also noted. The average of the two readings is the transparency of water.

5. Transparency is the property of water by which it allows the light to pass through so that the object in the depth can be seen.

6. In freshwater transparency is decreased by suspended materials like clay, planktons bloom, water plants etc. in prevent the penetration of

light into the water. This reduces photosynthesis and productivity of water body.



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