EXERCISE 2 PROTOZOA-HI: PREPARATION OF SOME PROTOZOAN CULTURES AND MAKING PERMANENT SLIDES

Structure

2.1 INTRODUCTION

In the earlier exercise you observed permanent slides of some representative protozoans and learnt to identify and place them in different protozoan groups according to their characteristic features. In this exercise you will learn to prepare suitable cultures of *Amoeba, Paramecium, Vorticella* and *Euglena.* You will also take out parasitic protozoans from the rectum of frog and observe these protozoans alive as well as prepare their permanent mounts.

Objectives

After performing this exercise you should be able to:

- describe how a culture is prepared for the genera *Amoeba, Paramecium, Vorticella* and *Euglena,*
- prepare temporary slides of protozoans to study them alive,
- prepare permanent mounts of above mentioned protozoans,
- make cultures of any two of the above listed protozoans,
- prepare permanent mounts of rectal ciliates from frog to study parasitic protozoans,
- compare the structures of free living and parasitic protozoans.

2.2 MATERIAL REQUIRED

1. A glass trough and some petri dishes
2. Dry hay and some wheat grains
3. 2-3 living frogs
4. A few eggs
5. Glass slides and cover slips
6. Glass dropper and normal saline
7. Ascending grades of ethanol (ethyl alcohol)
8. Albumen glycerin
9. Absolute alcohol
10. Xylene
11. DPX mountant
12. Aceto-carmine and acetic acid

13. Watch glass
14. Dissection box

2.3 CULTURE METHODS

Free living protozoans can be best collected in the scum on ponds or among plants and debris at the edge of shallow ponds, marshes, or backwaters of streams or among tide pools along the seashores. However, it is also easy to culture these organisms in the labpratory for a regular supply of live samples. You will find it easier to start your culture during the summer months of the year.

In order to maintain a successful culture it is important that:

1. there be an abundant supply of food
2. adequate inorganic nutrients be present
3. suitable temperature and chemical conditions be maintained and
4. the environment is without any enemies.

2.3.1 Preparation of *Amoeba* Culture

Using the following method you can culture Amoeba:

- Take about 500 ml of water in a flask and add 25 –30 grains of wlieat and some. hay.
- Boil this to get the starch extracted.
- Allow the water to cool down. This provides the most satisfactory culture medium.
- Collect some pond water along with some decaying weeds; filter it through a muslin cloth.
- Now mix the residue with the starcli solution in a petri dish (the residue contains a large number of Amoeba).
- Cover the petri dish and leave it for a week or so.
- *Amoebae* will multiply rapidly, and can be obtained as and when required.

2.3.2 Preparation of *Paramecium* Culture

Paramecium can be cultured by the following method:

- Boil in a flask 500 ml water wit11 20-25 grains of wlieat and some hay.Allow it to cool down. This is your culture medium.
- Collect some water from a pond which has submerged leaves and some *Paramecia* (ascertain the presence of the protozoans by examining under the microscope).
- Mix this pond water with the culture solution prepared as above in a petri dish, cover and leave for about a week.

  In a few days paramecia will appear along with some bacteria in the petri dish.
- It is best to culture paramecia at 22-25' C.

2.3.3 Preparation of *Euglena* Culture

Euglena can *be* cultured by the following method:

- Take a glass trough and fill it about three-fourth with water. Add about 70-80 gms of wheat grains and some hay.
- Keep this trough at the base of a window so that the sun rays do not fall directly on it. Leave it for about seven days.
- Now add to it some pond water containing *Euglena.*
- Within 14-15 days the water will become greenish and the surface of the water will be covered with a scum.

- Examine this scum under the microscope for *Euglena.*

2.3.4 Preparation of *Vorticella* Culture

Vorticella culture can be prepared by tlie following method:

- Take about lgm of mashed liard boiled egg yolk and add to it a liter of distilled water.
- Allow it to stand for two days and then filter it through cotton.
- Take about 100 ml of this filtrate in a petri dish and introduce into it a few *Vorticella* specimens along with some weeds.
- Within 14-15 days , *Vorticella* multiply and will be available in plenty in the culture.

2.4 OBSERVING LIVING PROTOZOA

Once you have learnt to prepare protozoan cultures, you should also be able to observe living specimens under tlie microscope.

- Using a glass pipette or a dropper put one or two drops of water from tlie culture on a clean slide.
- Disperse the water evenly with the help of a glass rod or tip of the dropper.
- Place a clean coverslip gently over the drop. Too much water will make the coverslip float and too little water will dry up fast and you will not be able to observe any thing for long. With a little practice you will learn to put tlie right amount.
- To observe protozoans for some time, scrape some petroleum jelly from a thin layer applied to the palm of your hand onto each edge of the coverslip (see Fig. 2.1).
- Place tlie coverslip side coated witli jelly on the drop of water on the slide and press gently to seal it over tlie drop.
- Now you can observe tlie slide under the microscope.

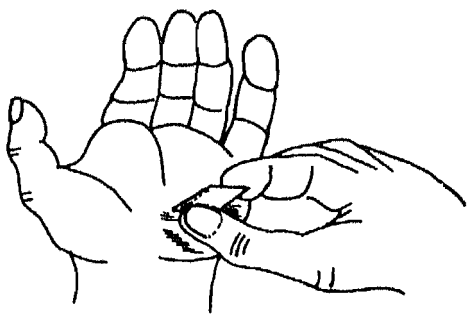

Fig. 2.1: Applying vaseline to the edges of a coverslip.

Many protozoans move very rapidly so that it becomes difficult to see them. A viscous solution of methyl cellulose slows them down without much distortion. Make a ring of methyl cellulose on the slide and put the drop of culture within the ring and cover with the coverslip (Fig. 2.2). As the organisms swim from tlie center into the mixing methyl cellulose and water, they slow down.

When you observe the live protozoans you will note that stained permanent slide of a protozoan usually looks much different from a live active, swimming specimen. Therefore, it is very important to make accurate drawings of live specimens and then compare them with the permanent mounts.,

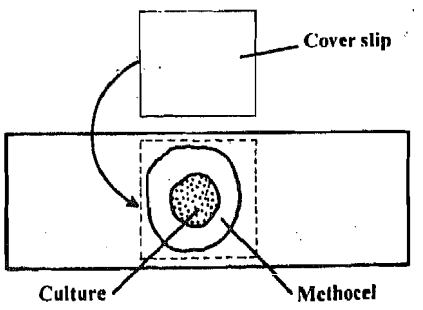

Fig.2.2: Methyl cellulose ring on a slide.

2.5 PREPARATION OF PEMANENT SLIDES OF SOME PROTOZOANS

In this part of the exercise you will learn to make a permanent mount of (i) *Paramecium* and (ii) rectal ciliates taken from a frog. Let us start with tlie method of mounting *Paramecium.*

2.5.1 Permanent Mounting of *Paramecium*

- Take a clear dry glass slide, put a very small drop of albumen glycerin in tlie center of the slide and witli tlie tip of your fore finger, spread it to form a thin film.
- Using a dropper put 1-2 drops of *Paramecium* culture on the film and observe under tlie low power of tlie microscope. You will see a large number of paramecia moving rapidly in the medium.
- Let tlie culture become dry, you can put the slide under an electric lamp.
- Now open a bottle of acetic acid, and quickly pass the slide (dried culture side downwards) over tlie mouth of tlie bottle. Tliis will fix the protozoans.
- Keep this slide in a larger petri dish (6" diameter) with tlie paramecia bearing side facing upwards. Put 3-4 drops of aceto-carmine so as to cover tlie culture fully.
- Stain tlie slide for 5-7 minutes.
- Drain off tlie extra stain from tlie slide with a sheet of blotting paper.
- Wash the slide with 30%, 50%, 70% and 90% alcoliol in ascending order, keeping tlie petri dish covered with another petri dish so tliat no atmospheric moisture enters, at tlie same time moisture is gradually removed from tlie culture. Tliis process is known as dehydration.
- Now cover tlie slide with absolute alcoliol. Give two treatments witli absolute alcohol, this will ensure complete dehydration.
- Remove the extra alcoliol, put a few drops of xylene on tlie culture film. This will make the protozoans transparent (a process known as clearing) so that they are visible better under the microscope.
- Any turbidity with xylene indicates improper dehydration. If any turbidity is seen
- then repeat the treatment with absolute alcohol followed by treatment with xylene.
- Put a drop of DPX mountant over the culture film and lower the coverslip gradually and carefully over it. Ensure that no air bubbles are trapped in the preparation.
- Keep tlie slide inside the incubator overnight for drying. Your permanent mount is ready.

2.5.2 Permanent Mounting of Rectal Protozoans from Frog

The rectal parasitic protozoans of frog are *Opalina,* (Fig. 2.3) *Balantidium* (Fig. 2.4) and *Nyctotherus* (Fig. 2.5). To obtain rectal ciliates tlie following procedure is used:

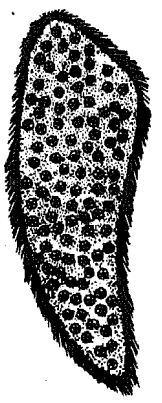

Fig. 2.3: *Opalina.*

- Take a freshly chloroformed frog, cut it open and remove its rectum. Make a longitudinal cut in the rectum and empty its contents in a petri dish containing 0.75% sodium chloride.
- Take a few drops of this solution on a clean slide and observe under the microscope to ascertain whether tlie rectum is infected by protozoans.
- Spread a thin film of albumen glycerine on another clean slide and put a few drops of the solution containing rectal protozoans.

  Fix the protozoans by passing tlie side of the slide with the solution over the open mouth of an acetic acid bottle or by putting a drop of ethanol on the slide.
- Let tlie slide dry and then follow the procedure of dehydration with alcohol series, clearing with xylene and mounting in DPX or Canada balsam as given in subsection 2.5.1.

  Observe under tlie microscope. Note the characteristic features and identify the type of protozoans present with the help of your counsellor.

  You may be able to observe *Opalina, Nyctotherus*, *Balantidium*. Draw and labe the important features as you observe them, in your note book.

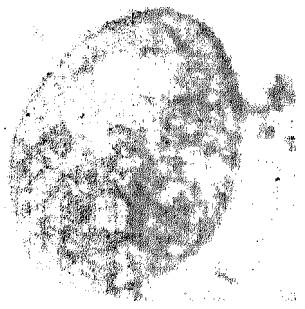

Fig. 2.4: ***Balantidium***

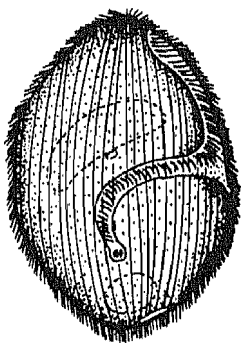

Fig. 2.5: ***Nyctotherus***

2.6 TERMINAL QUESTIONS

1. Why do we have to empty tlie contents of the rectum in 0.75% NaCl before making a slide of rectal parasitic protozoans?

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2. Why is it recommended that a culture of protozoans be prepared in tlie summer months?

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3. What difference did you note between the structure of free living and parasitic ciliates?

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