LABORATORY EXERCISE 2

KINGDOM PROTISTA

Today you will have the opportunity to observe several species of live protists (AKA, protozoans). Your task is to study the morphology, physiology, and behavior of these beasts as carefully as you are able, using the notes below as guides. The animals are available as pure cultures of one species of Protista. It is essential that you do not mix the different cultures as some species or their excretions are incompatible with others. Use only the specific pipette that is placed with each culture to extract animals, and be sure to return the pipette promptly to the proper dish. Squeeze the rubber bulb slightly before you insert the pipette into the culture medium; place the end of the pipette against the side or bottom of the dish; draw up a small drop of culture fluid. Do not stir the culture or blow bubbles into it. Place the drop of medium on a thoroughly clean microscope slide. When it is necessary for you to get a new culture, wash the slide thoroughly under running water and polish it dry and spotless.

PHYLUM SARCOMASTIGOPHORA; SUBPHYLUM SARCODINA --*Amoeba proteus*

Amoebas are gray, jelly-like, microscopic, single celled animals that are found in many different places (habitats), such as fresh water, the sea, the soil, and even within more complex animals, including man, where they exist as parasites. One parasitic species, *Entamoeba* burrows into the intestinal wall of men, usually causing diarrhea, which may be fatal. Today you will study the freshwater amoeba which, while relatively simple, is nevertheless much modified from the ancient organisms that evolved into all the other forms of animal life. In working with this animal you have an excellent opportunity to study living protoplasm. Observe the culture with a dissecting microscope which will enable you to appreciate the 3-dimensional configuration of the animal. Then obtain a specimen in the manner indicated by your instructor, and observe it under a compound microscope.

BEHAVIOR AND PHYSIOLOGY. With the light much reduced, look for a small, irregular, grayish body, which is very slowly but constantly changing shape. Observe the culture under low power, moving the slide systematically back and forth to cover all material under the cover glass, always focusing upon every object found. If a specimen is not found after patient search, do not throw the material away but ask for assistance. When an amoeba has been located, change to the next higher power (10X) and study as follows. Observe its shape and the apparent flowing of the granular material. Observe that it moves by thrusting out projections which are called pseudopodia. Watch the formation of pseudopodia and the flowing of the granular mass into them. This is called amoeboid movement which is produced by the shortening of contractile protein fibers within the cytoplasm. Make four outline drawings of a moving amoeba at ten second intervals. Is there a discernable direction of movement? If so, indicate it on your drawing.

STRUCTURE. Under high power (40^{+}x), note the clear outer region of the protoplasm, the ectoplasm. The surface of the ectoplasm in contact with the water constitutes the cell membrane, the familiar plasma membrane that shows as two electron dense layers around a less dense area in electron micrographs. The inner granular material is called endoplasm. It is not sharply separated from the ectoplasm, but is distinguishable, due to its more granular nature. The nucleus, a round or ovoid finely granulated body, may be found in the endoplasm. It is lighter in color and clearer than the surrounding protoplasm. The nucleus is difficult to observe in small specimens. Sometimes it is concealed by other material, and a stained slide must be studied in order to observe it. In what general region is it found? Near the nucleus may be found a small, spherical, transparent vesicle which upon reaching a certain size contracts and disappears. This is the contractile vacuole. Its primary function is to eliminate the excess water which continuously diffuses into the organism, and secondarily to excrete some waste substances. Observe its formation and note the interval between successive contractions. A food vacuole is composed of a food particle surrounded by water, into which have been secreted enzymes from the surrounding cytoplasm. The food soon begins to undergo digestion, owing to the action of enzymes on it. The cytoplasm includes everything except the nucleus. Draw an amoeba approximately 2-3 inches in length and label all structures.

REPRODUCTION. The amoeba reproduces by fission, a simple process of dividing into two amoebas. The nucleus divides, and the cytoplasm constricts until the parent animal is divided into two "daughter" amoebas.

PREPARED MATERIAL. Obtain stained preparations of amoebae, and identify all the structures

PHYLUM CILIOPHORA --*Paramecium caudatum*

Ciliates are distinguished by the presence of cilia, which are hair like outgrowths of protoplasm extending from the surface of the cell. They serve in locomotion and feeding. These minute structures are also found in man, in nasal passages, in the trachea, and in other parts of the body. While some of the ciliates such as *Paramecium caudatum* are perfectly harmless animals, there are others which are endoparasites of man. The ciliate *Balantidium coli* lives in the human intestines and may produce dysentery severe enough to cause death.

The paramecium not only serves as a good type for the study of a ciliate, but also it is a good example of a more complex protozoan. It is a favorable form with which to illustrate cyclosis and other physiological processes of single-celled animals.

BEHAVIOR. Place a drop of paramecium culture on a slide and observe the animals swimming freely. Can you distinguish anterior from posterior in this animal? Dorsal from ventral? Do they move in any one direction more than another? Turn off the power illuminator on the microscope and place a light on one side of the drop. How does this affect

the behavior? Add a few fibers stripped from the edge of a sheet of lens paper to the drop. How do paramecia react when they encounter such barriers?

STRUCTURE. On a clean slide make a ring of distilled water—methylcellulose solution which is 1/8 inch wide and whose diameter is a little less than the width of a cover glass. Place a drop of paramecium culture in the center of the ring. Then dip a dissection needle not more than l/4 inch into a mixture of yeast and Congo red dye, and add a small mass of this mixture to the ringed culture. Do not tease out the clump of yeast which you have added to the culture. Lay on a cover glass.

The outer layer of the paramecium is called ectoplasm, the surface of which is differentiated as a firm membrane, the pellicle. The endoplasm is the inner, granular, and more fluid portion of the cytoplasm, and contains a variety of organelles, such as nuclei and contractile vacuoles located near each end. When they become filled, they discharge their liquid contents to the exterior. Do they contract alternately, at the same time, or irregularly? What is the time interval between successive contractions of the same vacuole? In some preparations, the radiating canals which are associated with the development of a contractile vacuole may even be seen.

Most species of paramecia have two nuclei; the large oval macronucleus is located near the middle of the body, and controls nonreproductive functions. The much smaller micronucleus lies close by the side of the macronucleus, and mediates reproductive processes.

On the oral surface and running obliquely from the anterior end to about the middle of the body is a concave, somewhat funnel-shaped, depression, the oral groove. The oral groove leads into the cell gullet by way of the cell mouth. Constantly moving cilia sweep food particles down the oral groove and into the gullet. The mouth is the entrance to the gullet, and is located at the posterior end of the oral groove. Food vacuoles form at the inner end of the gullet; they appear as drops of water containing food particles. The cell "anus" is located about midway between the mouth and the posterior end of the body. It is a temporary opening which forms only when undigested particles are discharged. Nitrogenous wastes, chiefly ammonia, pass out of the cell by diffusion.

DIGESTION. Food is digested by the action of enzymes secreted from the cytoplasm into the food vacuoles. Different enzymes act best in an acid, or an alkaline, or a neutral medium; consequently, the paramecium must not only produce enzymes for the digestion of food, but also it must also furnish these enzymes with the optimal pH. In the food vacuoles of the paramecium, the reaction is at first acid, but later it becomes alkaline. One of the most important enzymes, the protein-digesting enzyme of the paramecium, acts only in an alkaline medium.

Congo red becomes blue in acid solutions (pH 3.0 or below) and red in less acid solutions (pH 5.0 or above). What color is the food vacuole when it is first formed? What color does it change to? What are the subsequent color changes? What is the significance of these changes in color of the food vacuole?

After about one-half hour, observe the pattern the food vacuoles made in the body of the paramecium. Can you distinguish between the younger and older food vacuoles? Do the food vacuoles decrease in size as they travel in the body of the paramecium? How do they change in position with time? Explain!

WATER BALANCE. With the people at each side of a table acting as a research team, set up the following demonstration: Make four different preparations of paramecium culture and methylcellulose and observe the behavior of the contractile vacuole simultaneously, recording the number of contractions per minute in each. The preparations are:

A. 1 drop culture & 1 drop methylcellulose solution.
B. 1 drop culture & 1 drop methylcellulose solution with 1% NaCl.
C. 1 drop culture & 1 drop methylcellulose solution with 2% NaCl.
D. 1 drop culture & 1 drop methylcellulose solution with 4% NaCl.

You should try to keep the drop sizes approximately equal in all parts of this demonstration. Why? Does this constitute a scientific experiment? Why? How does the behavior of the vacuole in these media help explain its function? Would you expect marine protozoa to possess a contractile vacuole? Why?

TRICHOCYSTS. Embedded in the ectoplasm just beneath the pellicle, are oval shaped bodies called trichocysts; these are best seen in a prepared slide. If the paramecium is irritated, these trichocysts are discharged in the form of long threads. They may serve as instruments of defense or for attachment while the animal is feeding. Add a drop of ink to a fresh culture on a slide. Observe the discharged trichocysts under high power. Prepared slides are also available showing these structures.

REPRODUCTION. Paramecia divide asexually in a manner similar to amoebae; and in addition, they possess a means for sexual reproduction termed conjugation. On prepared slides, observe the paramecia undergoing fission and conjugation and understand what is occurring in each case. Why should an animal have both a sexual and an asexual means of reproduction? What is the significance of each type?

PREPARED MATERIAL. Obtain stained preparations of paramecia, and identify all the structures. Draw a composite sketch of a paramecium showing and identifying all of the structures you observed in living and prepared specimens.