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Culture of Protozoa in the Classroom*

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Introduction

Protozoa are among the more interesting forms that may be used in the classroom at almost any level of biological study. They show such a wide variation in form and structure with so many specialized structures that they instantly attract the interest of even the most lethargic student. Their wide use in many fields of basic biological research, their growing importance as test organisms for many recent advances in vitamin metabolism, photosynthesis, and pharmacology—to name only a few areas of importance—offer many opportunities to awaken student interest in biology.

Collection of protozoa is possible from almost any conceivable habitat. Any hay infusion set up in the laboratory will yield many different types of protozoans. Mud collected from ponds, streams, and even transient bodies of water will produce forms after being kept for a short period of time in the laboratory. Plant debris, vegetation, and other materials may be collected and brought into the laboratory. The plankton protozoa may be collected by use of special collecting nets, and water may be filtered or centrifuged to obtain many minute forms.

In all of the above mentioned collections the protozoa may not become very abundant unless some additional food or nutrient material is added, and in any case, an impure culture consisting of many mixed species is obtained. A considerable amount of time is required to develop pure cultures of any desired species from such a basic culture source, and even then any pure culture will not remain in that condition for any length of time. Therefore, most instructors find that it is desirable to purchase cultures of needed species.

The purpose of the brief directions on culturing that follows is to acquaint the instructor with simple methods that can be followed in maintaining the common laboratory species for relatively long periods of time for class study. The methods presented have been picked because of their ease of operation, relatively satisfactory application to a wide range of forms, and because they are generally suited to the minimum conditions under which instructors can carry on the cultures.

General Considerations

In all of the culture methods described below certain general considerations must be kept in mind. The application of these general principles will go far to assure success with any of the proposed culture methods.

1. Temperature is a very vital factor in determining the length of life of any culture. Almost all free-living protozoa can be maintained satisfactorily over the average range of room temperatures; i.e., between 65-76° F. Specific optimum temperatures or deviations from this average are noted in specific cases. At temperatures below 53° F. growth ceases and the culture deteriorates; above 86° F. rapid destruction of the culture is evident.

2. Light is not necessary to most protozoa. This is particularly true of Paramecium (except *bursaria*) and the amoebas. These may be grown in darkness, and diffuse light is far more satisfactory than direct light. Cultures should be placed either in darkness or on the side of the room farthest away from windows. In the case of chlorophyll bearing forms such as *Paramecium bursaria*, *Volvox*, *Euglena*, and others, light from a north window is satisfactory. They should not receive direct light from windows facing other directions. In the

*A contribution from Ward's Teachers' Workshop. In September, 1959, Clarence Evaul, Science Supervisor of the Rochester City Schools, proposed a cooperative program between Ward's Natural Science Establishment and biology teachers in the high schools of Rochester, New York, and vicinity. As a result of this proposal, a schedule of regular meetings was drawn up, including lectures, demonstrations, and free discussion, permitting the exchange of ideas and experiences between persons in the scientific supply industry and those faced with the practical realities of classroom teaching. The first "Teachers' Workshop" was held in February, 1960, and covered the use of live cultures in the high school laboratory and their care and maintenance. This article is a summary of the material offered at that meeting.

absence of adequate or proper light, a light source such as a desk fluorescent lamp placed between six and eighteen inches from the cultures may be used. Only experience will determine the proper amount of light necessary for individual species.

3. The pH of the culture medium should approach neutrality. Acid conditions are to be avoided and the optimum pH should range from 7.0 to 7.6.

4. The water source is of paramount importance for success. In most areas, the use of distilled water will be found to be advantageous. Boiled pond or spring water or artificial spring water is best for cultures.

5. Sterilization of glassware and equipment is essential to avoid rapid contamination. Do not use detergents, or chemical cleaning solutions, because of possible contamination of the glassware. If possible, autoclave all glassware before use. If this is not practical, wash glassware thoroughly with a 10% solution of nitric acid, rinse with distilled water, and allow to dry in an inverted position. Glassware used should never have been in contact with formalin, fixatives, or strong chemicals. Formalin fumes in the same room will destroy most cultures.

6. Glassware used for cultures may be of various sorts and range from finger bowls through any available type of container to battery jars and gallon jugs. Finger bowls measuring 4½ inches in diameter represent a most convinent container. They are of sufficient size, easily manipulated, may be stacked to prevent contamination, and furnish a satisfactorily sized culture for most purposes. For larger cultures a one or two quart size battery jar is recommended. These may be covered with a glass plate to prevent contamination. In some special cases, erlenmeyer flacks of 250 or 500 ml. capacity are recommended.

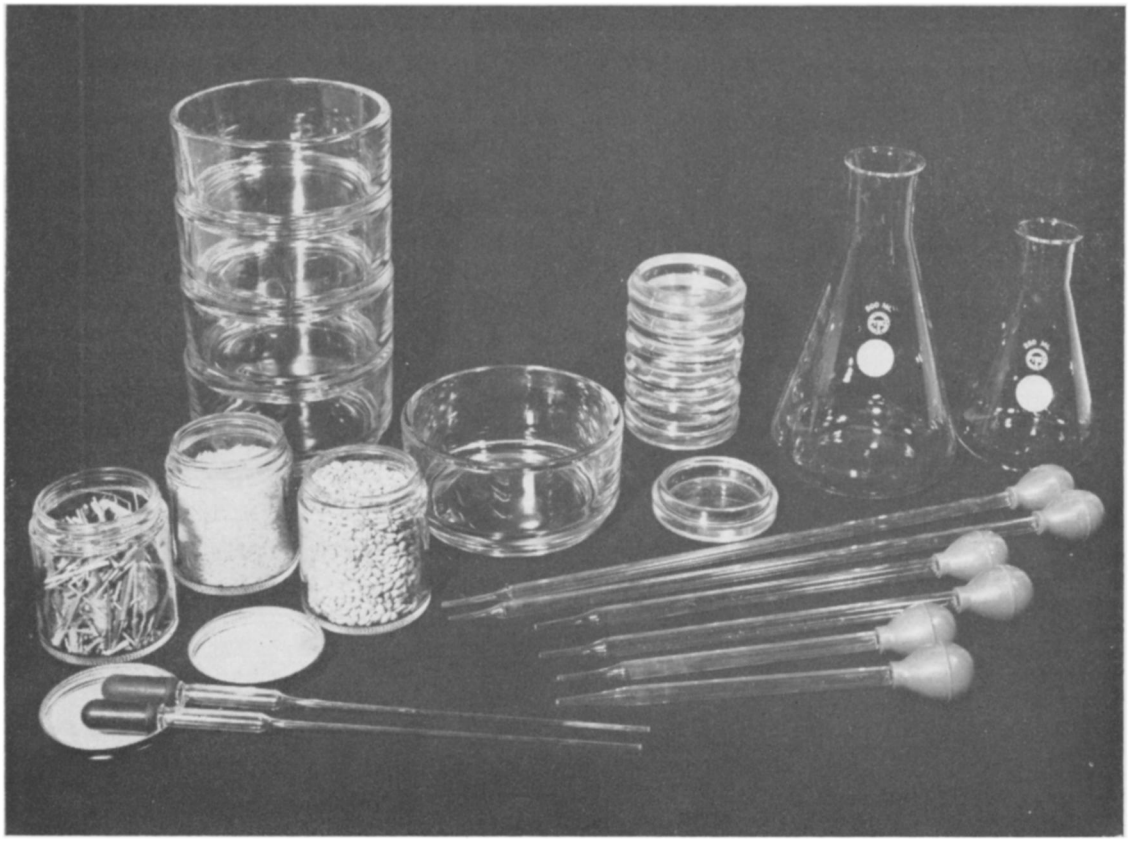

Figure 1. Materials used in culture of protozoa: finger bowls, syracuse dishes, and erlenmeyer flasks. Bottles contain timothy hay, polished rice, and wheat grains. Transfer pipettes and three sizes of pipettes are used in subculturing.

Figure 2. Finger bowl cultures stacked on shelves. Amoeba cultures on the left. Paramecium on the right. Stacking prevents entrance of dirt and contamination as well as aiding in maintaining uniform conditions.

7. In all cultures a source of food for the organism under culture must be maintained. For "impure species cultures" this may be other smaller species of protozoans such as *Chilomonas*, *Colpidium*, and in some cases *Paramecium*. In some cases, the culture media is devised so that a rapid growth of bacteria will take place. This is true of many ciliates such as *Paramecium*, *Blepharisma*, and *Spirostomum*.

Impure species cultures for our purposes may be defined as those cultures that have the desired species as the predominant form with other organisms present as food. In order to maintain this desired balance, subculturing is necessary at regular intervals, and grossly contaminated cultures must either be re-cultured or discarded.

Amoeba proteus

Amoeba is best cultured in finger bowls 4½ x 2 inches. These may be stacked and the top bowl allowed to remain empty or covered with a glass plate. Each bowl is filled with 150 ml. of distilled water and four grains of polished rice (minute rice as purchased in any grocery store) are added. Add approximately 2 ml. of *Chilomonas* from a well developed culture and inoculate with 50 *Amoeba proteus*.

At the end of two weeks, the culture should be ready for subculturing. This may be done by dividing the initial culture into a total of four bowls. Bring the volume up to 150 ml. in each bowl with distilled water and add more *Chilomonas* for food unless the growth of this species has been excessive. If subculturing is not desired, pour off half of the original solution at the end of two weeks and add fresh distilled water. Add more *Chilomonas* if necessary. Growth of mold on the rice grains is not detrimental to *Amoeba*. In heavily populated cultures, the *Amoeba* will be found around the mold on the rice. New cultures may be initiated by placing a rice grain with amoebae in a new culture dish. Examination of the culture may be made under a binocular dissecting microscope.

Another very satisfactory and universally used medium for *Amoeba* is a follows: Heat spring water to boiling and pour 200 ml. into each finger bowl while hot. Add to each 200 ml. of water 4 wheat grains that have been previously boiled for 5 minutes. When cooled to room temperature inoculate as above. Distilled water may be used in this method instead of spring water.

Chalkley's medium also serves as a widely used and more refined medium for culture of *Amoeba*. This consists of NaCL, 0.1 g.; KCL, 0.004 g.; $CaCl_2$, 0.006 g. in 1,000 ml. of distilled water. Place 200 ml. of this solution in finger bowls and add either rice or wheat grains as described above. Inoculate as given above and continue culture method.

Pelomyxa carolinensis

Pelomyxa carolinensis, often referred to as Chaos, is the largest of the amoebas and highly desirable for class study. In shipping it often tends to fragment and arrives as a large number of small amoebae. The same condition may be observed in cultures that are subject to rough handling in feeding and observation. Allowing cultures to remain relatively undisturbed for a period of three or four days will result in the individuals attaining a large size.

For proper culture of this species abundant food in the form of *Paramecium* and/or other ciliates must be available. This species should be reared in laboratory finger bowls as for *Amoeba*. Place 200 ml. of distilled water into each bowl and add 2 to 4 uncooked rice grains as for *Amoeba*. Feed daily if necessary or at least several times a week with *Paramecium* or other ciliates.

Chalkley's medium may also be used with rice grains added. Food may consist of

Chilomonas or other ciliates. A simple method that is widely used is to inoculate the *Pelomyxa* into finger bowls that contain rich cultures of *Paramecium*. Within about four weeks the *Pelomyxa* will need to be subcultured.

Pelomyxa is very susceptible to environmental conditions. A temperature of 68° F. is optimum for growth. A slight elevation of temperature to as low a figure as 72° F. generally results in deterioration of the culture. Excess concentration of organic matter or any great change in pH from 7.4 is also detrimental. Following the method outlined, using distilled water and rice grains, subcultures usually can be made every two weeks.

Other Sarcodina

Other members of the class Sarcodina that are usually cultured in the laboratory include *Arcella*, *Difflugia*, *Actinosphaerium*, *Actinophrys*, *Centropyxis*, and various amoebas and related forms that may be obtained from collection.

In all of these cases the use of laboratory finger bowls are recommended because of the reasons aforestated. In some situations the larger sized finger bowls may prove just as useful provided the depth of the fluid medium remains approximately the same as in the smaller bowls.

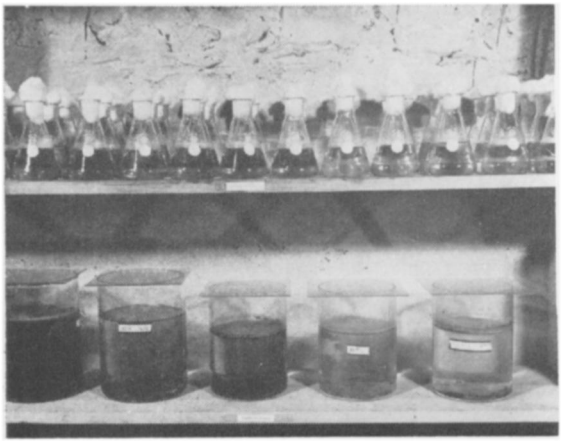

Figure 3. Top row: Cultures of *Paramecium bursaria* in 250 ml. erlenmeyer flasks. Contents of flasks are sterilized after stoppering and cooled to room temperature before inoculation. Bottom row: Series of battery jar cultures of Paramecium. These containers are ideal for typical "hay-infusion" cultures.

Arcella is commonly cultured in finger bowls having only 100 ml. of fluid medium. The shallow water level is one of the important factors in proper cultivation of either *Arcella vulgaris* or *Arcella discoides*. To 100 ml. of boiled pond water or boiled spring water add ½ gram of boiled timothy hay and two grains of boiled wheat. The timothy hay should be cut into one inch pieces and boiled for 5 minutes. Introduce *Chilomonas* as food.

Difflugia is often used as an example of the Sarcodina because of the variation in structure presented by the appearance of the shell or "test." In this genus the test is formed of cemented quartz-sand, diatoms, and other foreign bodies. Cultivation of this species is not as easy as some other forms; however, the method of Hegner usually produces satisfactory results. This method consists of shaking pond water with weeds violently and then filtering through eight thicknesses of cheesecloth. The filtrate is distributed in petri dishes, and when the suspended particles have settled, the *Difflugia* are introduced.

Another excellent method is to add one gram of fine clean sand to a finger bowl. Add spring or pond water to the finger bowl to a shallow depth, 150 ml. in a 4½ inch finger bowl. Place a considerable amount of green algae such as *Spirogyra* or *Zygnema* in the finger bowl. Usually growth will be quite satisfactory by the use of this method. Another good culture for this genus is the use of hay-wheat as described under *Arcella* or rice as described under *Amoeba*. In each case add one gram of sand to the bottom of the bowl. The sand is used by the organisms to construct the test.

Actinosphaerium and *Actinophrys* may be cultured very successfully by the use of Knop's solution. The formula is as follows:

Magnesium sulphate	0.25 g.
Calcium nitrate	1.00 g.
Potassium phosphate	0.25 g.
Potassium chloride	0.12 g.
Iron chloride (see directions below)	trace
Distilled water	1000 ml.

In mixing the solution divide the liter of distilled water into four parts of approximately 250 ml. each. Combine the solutions mixing thoroughly each time to avoid precipitation. Add the calcium nitrate last. To make the iron

chloride solution, make a 1% solution of ferric chloride fresh each time solution is used. Add one drop of this 1% solution to each liter of volume.

Another satisfactory method is to add 4 boiled wheat grains to 100 ml. of boiled pond or spring water in a culture bowl. If desirable, rice grains may be substituted for the wheat grains. In either case, as well as with Knop's solution, either of these species must be fed some other protozoan. For *Actinosphaerium*, it is recommended that *Paramecium* be used for food adding a large number of individuals as needed. For *Actinophrys*, a smaller ciliate such as *Colpidium* is desirable. Subcultures of *Actinosphaerium* should be made every two weeks, and in the case of *Actinophrys*, subcultures should be made monthly.

Centropyxis is best reared in a hay-wheat medium as recommended for *Arcella*. In some cases better results may be obtained by increasing the amount of hay to 1 g. and the number of wheat grains to 3 or 4. A slight amount of clean sand on the bottom of the bowl will also aid in the development of the test that contains fine sand grains or diatom shells.

Chilomonas

This colorless flagellate has a saprozoic form of nutrition and is widely distributed in stagnant water and hay infusions. Because of its small size, and ease of cultivation in large numbers, it is used extensively as a food organism for many other protozoans under culture. It can be obtained by bringing in water and vegetation and letting this material stand in the laboratory. This wild culture may be enriched by adding wheat grains or some other form of nutriment to the water-vegetation material.

The simplest method for culturing this species is the use of a wheat infusion made by adding 4 to 6 grains of wheat to 100 ml. of boiled pond water. Another very simple method for those who prefer the typical infusion technique is to add 10 g. of timothy hay to 1000 ml. of distilled or spring water. Boil for 10 minutes, allow to cool, and inoculate with *Chilomonas*.

Euglena

The members of this genus are widely distributed in freshwater ponds and streams that contain considerable organic debris. They may be found in abundance in stagnant pools and in the scum covering the surface of ponds. During the year they show their greatest abundance in June, July, and August. While almost all species may be cultured readily, the one usually used for classroom work is the common *Euglena gracilis*.

The simplest method for culturing *Euglena* that is applicable to many species is that given by Johansen (1940). The split pea infusion is made by boiling 40 split peas (yellow seem to give best results) in one liter of tap water. Add enough citric acid to prevent excessive bacterial growth. We have found that distilled, spring, or pond water works equally well, and in some cases, better than tap water. In addition, the citric acid is often unnecessary. Cool to room temperature and place in large clean battery jars and cover. Inoculate with *Euglena*. Keep the culture in a window sill or some other place where direct sunlight is available. This type of culture will last indefinitely if additional peas are added at monthly intervals and the water lost by evaporation or use is replaced by distilled water. Temperature requirements are not critical as *Euglena* can tolerate a wide range of temperatures. Subcultures may be made as and when needed.

Another simple method that is quite useful particularly for *Euglena gracilis* is as follows: To one gallon of boiled spring or pond water add 30 grains of boiled wheat and 30 grains of rice. Add 50 ml. of boiled and cooled skim milk. Place in battery jars and handle as above. Subculture once a month or add fresh medium every week or ten days. Normal length of life of individual cultures is approximately 3 months if fresh medium is added regularly.

Phytomonadina

The phytomonads are small, green flagellates that are more or less rounded. The vegetative cells are flagellated and usually are actively motile during the vegetative phases of the life cycle. They are closely related to the algae and are considered members of the order Volvocales by the algologist. While culture directions for these forms more properly come under a discussion of culturing algae, sufficient information is included here to enable the instructor to culture those genera such as *Chalmydomonas*, *Pandorina*, *Eudorina*, *Gonium*, and *Volvox* that are often considered

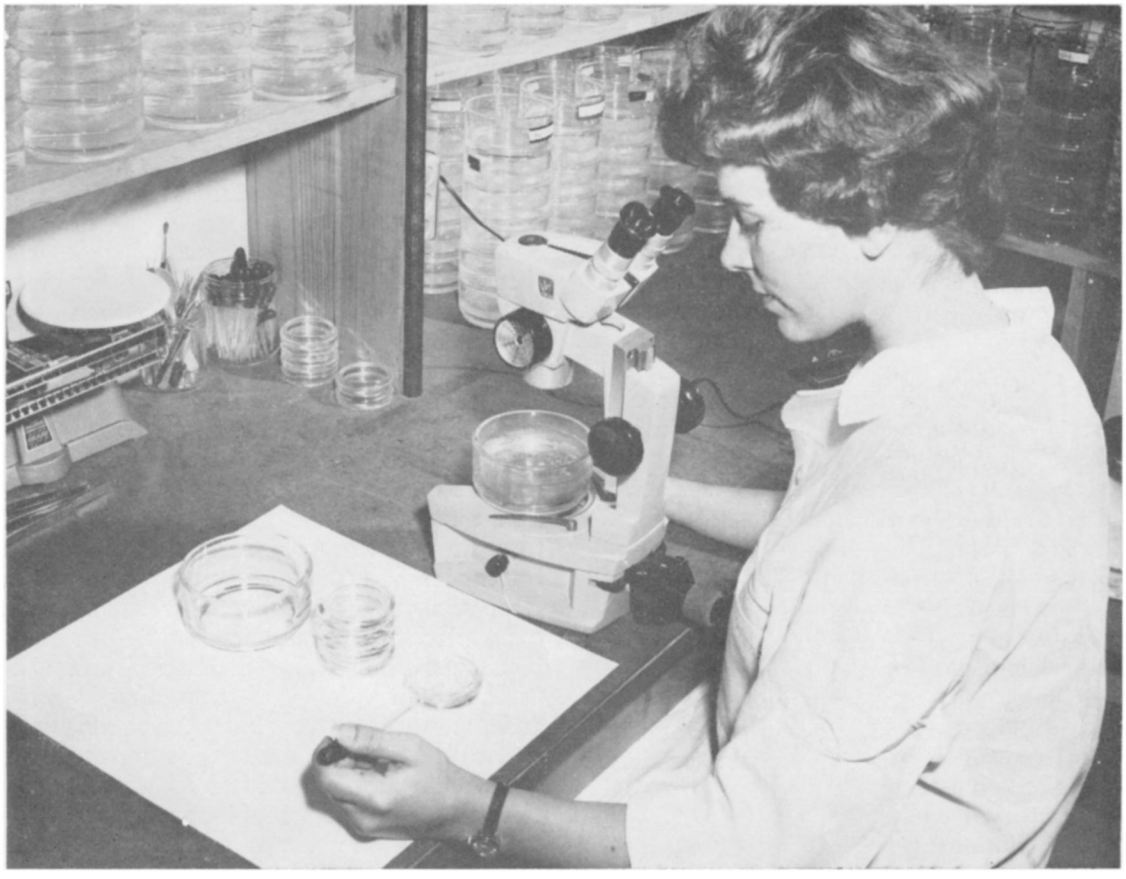

Figure 4. In subculturing, selected organisms are transferred to syracuse dishes in as small an amount of medium as possible. From these containers they are transferred to finger bowls. This method offers better control of possible contamination of cultures.

as protozoa and studied in conjunction with the more typical animal organisms.

One of the more commonly used culture mediums is made by adding 1 gram of commercial fertilizer, 4-10-4 or 5-10-5, to one liter of spring water. This material is then heated to between 80 and 90° C., filtered, and placed in culture bowls. For *Volvox*, it is best to use 0.2 g. of fishmeal and proceed as above. After filtering add 0.5 ml. of a 1% solution of ferric chloride. Place the bowls where they will receive a moderate amount of light and an even temperature.

The inorganic medium of Uspenski and Uspenskaja (1925) has been used with considerable success in the cultivation of all of these forms. The formula is as follows:

Potassium nitrate.....0.25 g.
Magnesium sulphate.....0.25 g.
Calcium nitrate.....1.00 g.
Monobasic potassium phosphate
.....0.25 g.
Potassium carbonate .....0.345 g.
Ferric sulphate.....0.0125 g.
Distilled water.....1 liter

In making this solution the ferric sulphate should be sterilized separately and added to the medium after both units have been sterilized. The pH of this solution should be about 7.6 at 20° C. For use the solution has to be diluted with distilled water. In most cases a 1:9 dilution is recommended; however, other dilutions may prove more practical in individual cases. In any case, for use pour 100 ml. of the solution in flasks of 200 ml. capacity and inoculate with a few colonies of the desired species. Keep the inoculated flasks in a cool place with artificial illumination. The flasks should be plugged with cotton to avoid contamination or otherwise covered. It is essential that the flasks that are used for this work be made of

alkali-free glass as otherwise the soluble alkalies will leech out into the solution and cause considerable trouble. If glass containers of this type are not available, wash the containers thoroughly with sulphuric acid and then rinse out the acid with distilled water. The rinsing must be so effective that all of the acid is removed from the glass.

One of the best all-round nutrient solutions for growing the members of this group is Knop's modified solution. The formula is as follows:

Magnesium sulphate	0.1 g.
Potassium phosphate	0.2 g.
Potassium nitrate	1.0 g.
Calcium nitrate	0.1 g.

To prepare this solution, divide one liter of distilled water into four parts of approximately 250 ml. each. After dissolving each of the chemicals noted above in one portion of the water, combine into one volume adding the calcium nitrate last. Mix thoroughly after combining each part. To the combined solution add one drop of a 1% ferric chloride solution that has been freshly made. This solution may be used in any dilution that is most satisfactory from full strength to 1:10. The lower dilutions appear to be the most satisfactory.

As a general rule, the addition of iron to these nutrient solutions should be made every ten days in summer and once a month during the winter. Our experience has been that *Chlamydomonas* will do best either in the commercial fertilizer solution or in a 1:10 Knop's solution. *Volvox* grows very well in the solution of Uspenski and Uspenskaja and in Knop's solution. The other genera mentioned grow equally well in any of these solutions.

Paramecium

Paramecium, like most of the ciliates, are bacterial feeding protozoans. The basic requirements of a good culture medium for this species is one that will enable a sufficient quantity of bacteria to develop so as to support optimum growth of the desired population. For containers either small finger bowls or battery jars are recommended. Again it should be pointed out that cultures in finger bowls are easier to handle and can be better regulated than those in battery jars.

The most widely used culture medium is made by adding 4 to 6 grains of previously boiled wheat to 200 ml. of boiled pond or spring water. Distilled water may be substituted if desired. The use of a hay infusion made from timothy hay is one of the best and at the same time readily used methods for culturing *Paramecium*. The method consists of placing 6 g. of timothy hay cut into one inch pieces in a liter of distilled water. Boil for 20 minutes or until the water becomes brown. Allow to cool, ripen for 24 hrs., and add *Paramecium*. Modifications of this method allow the use of finger bowls or erlenmeyer flasks. In the first case, divide the solution and the hay between finger bowls; in the second case divide the material between 250 ml. capacity erlenmeyer flasks. For *Paramecium aurelia* and *Paramecium bursaria* it is best to reduce the amount of hay to 4 g. Cultures may be kept for months by adding a few pieces of hay to the culture each month.

For *Paramecium bursaria* one of the best methods is that of a lettuce infusion. This is made by taking clean leaves of lettuce and drying slowly in an oven until they are crisp and brown but not black. Burned leaves should be discarded and the rest of the material ground with a mortar and pestle. If this ground lettuce is placed in a glass-stoppered bottle it may be kept indefinitely. Place 1½ g. of the ground lettuce in 1 liter of boiling water and boil for 5 minutes. Filter while hot into 250 ml. erlenmeyer flasks. Stopper with cotton and let stand over night. If "parafilm" is used to seal the flasks they may be stored for relatively long periods of time. For use take 2 parts lettuce infusion and 1 part sterile distilled water. The addition of an excess of calcium carbonate to maintain the pH at 7.2 is often helpful. Culture in 250 ml. erlenmeyer flasks that are stoppered and placed in diffuse light. The light is necessary for the growth of the symbiotic alga that produces the green color in *Paramecium bursaria*. Instead of using lettuce, a powder known as "Cerophyl" produced by the Cerophyl Laboratories, Kansas City, Missouri, can be used very successfully. In this case add 2 g. of Cerophyl to 1 liter of distilled water and heat to boiling. Allow the solution to stand after filtering for 24 hours at room temperature. Inoculate with *Paramecium bursaria*. Culture as given above.

Demonstration of Conjugation in Paramecium bursaria

In order to demonstrate this reaction it is necessary to start with two pure-line cultures of *Paramecium bursaria*. A drop of individuals from each of the two pure-lines may be mixed in depression slides, deep-well slides, or watch glasses. The mixing of the cultures must be carried out in the light, sunlight if possible, and around noon. The reason for the choice of time lies in the fact that the mating reaction is more intense at this hour than at any other time. All pipettes used must be kept clean, and the two stock cultures must not be contaminated by each other.

Study under the binocular dissecting microscope or the low power of the compound microscope. Clumping of the cultures takes place on mixing, and the clumps become larger and larger until clumps of many individuals are formed. After six hours, the only thing that will be observed are masses of individuals. After 16 hours conjugating paramecia may be observed. The number increases so that at 24 hours numbers of conjugating pairs are present. They remain as conjugants for approximately 24 to 48 hours, after which time little, if any, conjugation may be observed.

If purchased cultures are used for this demonstration they should be used within 24 hours of delivery. Two days should be allowed for the demonstration as conjugating pairs will usually show up to best advantage on the second day. The culture dishes used for this demonstration should be kept in the sunlight or where they receive some sunlight. In order to prevent evaporation, the use of some type of moist chamber is recommended. In mixing the cultures be sure to come as close to equal proportions of the two cultures as possible and use separate dropping pipettes for each culture so as to avoid contamination.

Other Ciliates

Most other ciliates that are of interest in the school laboratory may be cultured by modifications of either the wheat or the hay infusion. In order to obtain cultures that are rich in bacterial growth and capable of supporting rapid growing populations of *Paramecium* and other forms, a common method is to place 200 ml. of distilled water into a finger bowl and add 4 grains of wheat and 12 to 15 pieces of timothy hay. Both the wheat and hay having been previously boiled as described above. This medium is ready for immediate inoculation with the desired organism.

Blepharisma, the peach colored ciliate, may be cultured in 200 ml. of distilled water to which 10 to 12 grains of wheat have been added. Subculture in 4 to 6 weeks. *Colpidium*, *Colpoda*, *Euplotes*, *Spirostomum*, *Stentor*, and *Vorticella* may all be cultured by use of the wheat medium as given for *Paramecium*. In the case of *Spirostomum* the addition *of Chilomonas* as a food organism is recommended. Also *Stentor* will grow exceptionally well in a mixed protozoan culture either in finger bowls or battery jars. It may also be cultured by feeding regularly with *Paramecium*, *Euplotes*, or *Chilomonas*.

The culture of *Didinium* can best be done by feeding this carnivorous ciliate on *Paramecium*. The *Didinium* should be cultured in fresh pond or spring water and fed *Paramecium* that have been collected from the top of battery jar cultures so as to avoid the introduction of too much culture fluid. Subculture every 2 weeks. *Didinium* quite often encysts, and the dried cultures are usable.

References

The methods for culturing protozoa are many and varied, depending upon the use one wishes to make of them and the requirements of the situation. The methods briefly outlined here are those that are relatively simple to use and easy to prepare. Following the directions carefully should enable the instructor to maintain impure species cultures for relatively long periods of time under average laboratory conditions.

For those who wish to prepare more carefully controlled cultures, or for those who need to be more specific in their requirements, the following list of references is appended.

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Nominations for Regional Directors

NABT members should vote for *one* candidate for *each* of the regions listed. Biographical background for other officer candidates may be found in the November, 1960 issue. Duties of the Regional Directors will be to represent their regions on the Board of Directors, to promote regional meetings, to initiate teacher award programs, to promote membership, to promote activities of affiliated societies, and to work actively with other scientific and educational organizations in their regions.

Region I (Connecticut, Maine, Massachusetts, New Hampshire, Rhode Island, and Vermont)

Dorothy T. Harlow

Present Position: Biology teacher, Rockville High School, Rockville, Connecticut.

Degrees: B.A., Smith College. M.A., University of Connecticut.

Experience: Teacher, University of Connecticut–High School Cooperative Course. Assistant General Chairman, North Connecticut Science Fair Steering Committee. Member, General Committee, National Science Fair, 1959.

NABT Activities: State Membership Chairman.

Organization Membership and Activities: Member, CSTA, CEA, and AAAS. Winner, Elizabeth Thompson Award for outstanding science teaching in New England, 1958.

Irving Keene

Present Position: Biology teacher, Weston High School, Weston, Massachusetts.

Degrees: B.S., Middleburg, College. M.Ed., Boston University.

Experience: Biology teacher in secondary schools for over 30 years. Graduate study at Harvard and Cornell. Organized first Science Fair in Massachusetts. Speaker at various meetings. Developed Plant and Wildlife Conservation Center, Brookline, Massachusetts.

NABT Activities: Local chairman, AAAS meetings in Boston, 1944 and 1950. Managing Editor, ABT, 1947-49. Regional membership chairman since 1955. Planning Committee, North Central Conference on Biology Teaching, 1955. Member, Nominating Committee, 3 years. Second Vice-President, 1958.

Organization Membership and Activities: Member, AIBS. President, New England Biological Association. Chairman, New England area, Natural Areas for School Grounds, sponsored by Nature Conservancy.

Publications: Articles in ABT.

Region II (New Jersey, New York, and Pennsylvania)

Glenn C. Ball

Present Position: Chairman, Biology Department, Carthage Central High School, Carthage, New York.

Degrees: A.B., Houghton College. M.S., University of Michigan.

Experience: High school teacher for 9 years. State sponsored science program for teachers, 1958-1959. Conducted Science In-Service Institute at State University, Potsdam, New York, 1959-1960. Research Department, St. Regis Paper Company. Recipient, NSF Summer Fellowship, Rutgers University.

NABT Activities: State Membership Chairman.

Organization Membership and Activities: Member, New York Science Teachers Association, North Central Zone Science Fair Committee, Awards Committee of Carthage High School. Secretary-Treasurer, North Central Science Teachers Association. Chairman, Carthage Central Science Fair Committee, In-Service Training Committee, and Professional Advancement Committee of Carthage High School.