CONTRIBUTIONS FROM THE ZOÖLOGICAL LABORATORY OF THE MUSEUM OF COMPARATIVE ZOÖLOGY AT HARVARD COLLEGE UNDER THE DIRECTION OF E. L. MARK. No. 124.

SOME METHODS FOR USE IN THE STUDY OF INFUSORIA.

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The Yarn Siphon.

In accurate experimental work with Protozoa it often becomes desirable to separate them from the culture water in which they have grown and also from the solid débris, zoögloea, etc., contained in it. For one or several organisms this may be done by means of the "wash drop," as recommended by Eyferth (*Einfachste Lebensformen*, Braunschweig, 1900).

To obtain clean specimens in larger numbers, the following method has proved efficient for many kinds of Infusoria. From the culture jar a quantity of liquid containing the organisms and the débris naturally occurring there is removed with a pipette to a Stender dish. In this the organisms are well distributed by sucking up the liquid into, and forcing it out of, the pipette a few times. This is occasionally repeated during the subsequent procedure. A few pieces of woolen yarn about 10 cm. long are then laid parallel in a single strand, held in water, and pressed together (not twisted) until thoroughly wet. This yarn siphon is then placed with one end in the Stender dish, now elevated, the other end hanging down on the outside, a receiving vessel being placed underneath. Soon ciliated organisms pass over the siphon and are received into the lower vessel. The yarn acts as a filter as well as a siphon, keeping back solid matter and likewise dead organisms. From time to time fresh water is added to the Stender dish to replace that lost by siphoning. The process thus far yields the Infusoria in a large quantity of diluted culture water.

THE TUBE FILTER.

Numerous tests of the usual process of downward filtration with ordinary funnel and filter have shown that Infusoria (Paramœcia) can be removed from the inside of the filter only with the loss of a large proportion of their number, unless the filter be repeatedly rinsed. This results in a dilution, sometimes undesirable, and is at best an uncertain way of preventing the loss of organisms. To obviate these difficulties, I have employed another method.

To concentrate the organisms into a small amount of water, to remove the culture fluid entirely if desired, and to change the medium at will, I have devised the following apparatus, which may be called a "tube filter." One end of a short piece of wide glass tubing is closed by a piece of filter paper held in position by means of a rubber band binding it to the outer circumference of the tube. The process depends essentially upon the quality and area of the filter paper employed. For rapid work with a quantity of about 50 cc. contained in a Stender dish I have used a tube approximately 3 cm. in diameter and 6 cm. in length. This tube is held in a vertical position by a clamp fastened upon a ring stand. Under the tube, upon an elevated support, is placed the Stender dish, or preferably a deeper vessel, with the organisms. The tube is lowered until its paper diaphragm comes within a few millimeters of the bottom of the Stender dish. In the tube is hung a filled glass siphon with the lower end of its outer arm bent upward to prevent its running empty. As the water rises through the filter paper and into the tube it is removed by the siphon. More culture water with organisms, or any other fluid desired as medium, is then added to that in the Stender dish. The addition of the former effects concentration, as does also the final withdrawal of most of the liquid. This process of upward filtration leaves nearly all the organisms in the Stender dish when the tube is removed. By means of a supplying bottle, described below, carrying an air tube and a siphon, water may be added to the Stender dish as fast as it is withdrawn by the tube filter. This secures continuous renewal of the medium with practically no current.

The U-Cell for Renewable Media.

Another device, which I shall call the U-cell, serves much the same purpose as the tube filter, but on a smaller scale. It has the advantage of facilitating microscopic observation and of permitting more rapid change of medium.

To make this U-cell (Fig. 1) there are necessary two slides (best thin), a few rubber bands, and darning cotton of large diameter and close fibre. A piece one and a half times the length of the slides used is held at one end with the forceps and dipped into water until thoroughly wet, care being taken not to loosen its fibres or to make its diameter uneven by rough handling, although after dipping it may be drawn lightly between thumb and fingers to insure complete wetting. This is then laid lengthwise upon one slide (which is best placed across the top of an open Stender dish) in the form of a long U, and the other slide laid upon it. The ends of the U barely project beyond the parallel ends of the slides at the open end of the cell.

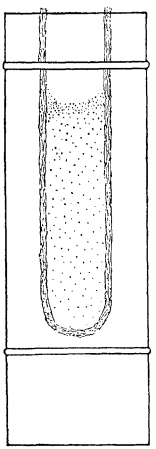

Fig. 1.

Two or three rubber bands — doubled so as to exert more pressure, if the smaller Infusoria are to be kept in it; otherwise not — are passed around the slides crosswise. This arrangement constitutes the U-cell. The darning cotton used should be of such a size as will cause the slides to be about 0.5 mm. apart when the rubber bands have been applied. This dimension and also the length of the U should be so regulated as to admit of the convenient use of a capillary pipette for the withdrawal of organisms. A shorter U should be obtained by the use of shorter slides, not by altering the proportions given above. Under the magnifier any selected individuals can be taken out. To fill the cell, water containing Protozoa is injected with a small pipette into the open end of the U, while the cell stands nearly vertical. A portion of the water will flow out through the cotton yarn, but capillary attraction will keep

sufficient water in it, even if the cell lies horizontal, as for microscopic examination. Moreover, this outflow affords a convenient method of removing the culture water and of renewing the medium at will without the loss of any Protozoa, if the cell is never permitted to overflow at its open end. A large number of organisms may be filled into it by repeated use of the pipette. Another method of filling the cell is to prepare a siphon consisting of a single wetted piece of woolen yarn, one end of which is inserted with a needle into the opening of the U to a depth of about 5 to 10 mm., the other end being put into the supplying Stender dish, elevated to permit the siphon to act. Over the single strand a continuous stream of Infusoria passes into the cell. These increase in numbers as the water passes through the U and escapes.

When it is desired to use higher magnification without removing organisms from the cell, an oblong cover-glass — *e.g.*, 22 × 44 mm. — may be substituted for the upper slide. Cover-glasses being too flexible, they must be braced in order to produce the even pressure upon the underlying thread necessary to retain small and active Infusoria. For this purpose slides are cut transversely into pieces about 5 mm. in width. At each end of the cover-glass one of these is laid across and a rubber band passed over it. At the open end of the U, as before, the ends of the slide and cover-glass must lie directly opposite each other. Such preparations can be conveniently preserved for a long time by standing them in an inclined position inside a low cylindrical vessel, the open ends of the U-slide projecting above the vessel and the lower ends resting against a bottle or a beaker, somewhat smaller than the vessel, placed in its center to serve as a stop.

The vessel may be filled with water to any desired depth. This method of preservation is applicable to organisms whose natural habitat is standing water.

THE U-CELL FOR CIRCULATING MEDIA.

The U-cell may also be used for a circulating medium, as shown in Fig. 2. The cells are placed in a cylindrical glass dish, with their lower ends resting in the angle of the dish.

They are inclined towards an inner vessel placed in the center of the first. The dimensions of the two vessels should be so selected that the upper ends of the cells come in contact with the inner vessel at about 5 mm. below its open end. From the inner vessel water is led by cotton-yarn siphons, *S''*, of appropriate size, into the cells. A constant-level glass siphon, *S'*, is hung over the wall of the outer vessel. This prevents both overflow from the cells and the complete exhaustion of their water. The inner vessel is supplied with water from an elevated bottle placed near by and stoppered with a two-hole cork. One hole carries an air tube, *A*, extending to the bottom. The other carries a siphon tube, *S*, whose outer arm dips below the surface of the water in the

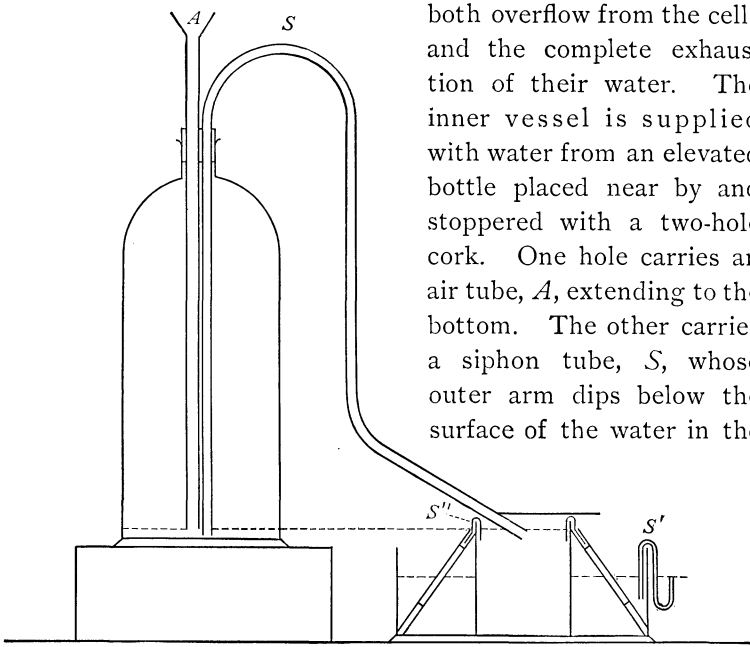

FIG. 2.

inner vessel. With the bottom of the bottle placed a little lower than the level desired for the liquid in the inner vessel, this level can be kept constant by raising or lowering the air tube of the supplying bottle. Then water will pass over the siphon only when the cells withdraw it from the inner vessel. The air tube may conveniently consist of a funnel tube, to be used also for filling the bottle with water. The inner vessel and its yarn siphons should be protected from dust by being covered with a glass plate whose edge is notched to admit the siphon tube. In siphoning, woolen yarn has been used wherever a rapid flow was desired, cotton yarn where a slower rate was needed.

USE OF ABSORBENT COTTON IN MAKING MICROSCOPIC PREPARATIONS.

Temporary or permanent preparations, permitting the frequent change of fluids under the cover-glass that is often required in micro-chemical work, can be made successfully by means of absorbent cotton. This method is very well adapted to the preparation of entire Infusoria. With the forceps a very small quantity of dry absorbent cotton, free from thick masses, is placed in position upon a well-cleaned slide. With a pipette a drop or two of water containing the Infusoria is placed upon the cotton. No more water should be used than can be absorbed by the cotton, which is then spread apart with two needles until the desired thinness of distribution of the fibres is reached. The cotton should occupy about the area of the cover-glass to be used. Both quantity and distribution must be learned by experience. A cover-glass is then lowered horizontally upon the preparation. If a hanging drop is to be transferred to the slide, the cotton is distributed while dry and the cover-glass lowered in the same manner. Two rubber bands, of such size as to exert some but not much pressure, are then passed around the cover-glass, one at each end. Fluids as desired are now passed under the cover-glass by adding them in drops at its upper end when the slide is placed in a more or less slanting position. The fluids emerging from the lower end of the cover-glass are permitted to run down the slide freely, or are guided down by means of a strip of filter paper. When the latter device is used, and with the larger Infusoria, the rubber bands may be removed after the passage of the fixing fluid, and all subsequent fluids slowly added in drops and entirely removed with filter paper, the slide in this case being kept in a horizontal position. In most cases the whole slide, held vertically and with rubber bands in position, may be alternately dipped into and raised out of the fluids to be applied; but balsam had better be added in drops, as above described.

Finally the rubber bands are removed. The cotton properly used is a sufficient mechanical obstruction to prevent the

washing away of any organisms once placed within its meshes. Far less time and care, consistent with the safety of the preparation, are necessary than in the common method of making preparations "under the cover-glass" with the object lying free. At any stage in the process examination is convenient. The preparations can also be stored in the alcohols, etc., if desired. Owing to the use of dry cotton and the horizontal lowering of the cover-glass, the organisms are caught *in the meshes* of the cotton, seldom under or over its fibres. But few if any organisms need be lost, either in this procedure or subsequently. Success depends upon a proper adjustment to each other of the size and quantity of the materials used, and this can be accomplished after a few trials.

CAMBRIDGE, May, 1901.