ALUM COCHINEAL AS A STAIN FOR PARAMOECIA*

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In experimenting with some stains alum cochineal was incidentally used as a stain for *Paramoecium caudatum*. The results were so pleasing it appeared that it might be worth while to pass the method on to others who might be interested. The method is as follows:

Concentrate numerous specimens into a few drops of water either by filtering or by centrifuging.

Fix in warm Bouin's (45° C.) and let stand at room temperature overnight.

Wash out the Bouin's fluid to 70% alcohol. All picric acid should be removed.

Carry through 50% and 30% alcohols to water.

Stain in alum cochineal for 24 hours or longer.

Rinse in distilled water with several changes.

Carry through a series of alcohols, 30%, 50%, 70% acid alcohol (1cc. Hcl to 100 cc. alcohol) for one to two minutes to remove any alum crystals present. Rinse in 70% alcohol, then 80% alkaline alcohol (saturated with sodium bicarbonate) for 10 minutes or more, 95% alcohol for 1 minute, absolute alcohol three changes at 2-minute intervals. Absolute alcohol containing light green may be used for the second change. This will stain the cilia and cause some of the other parts to stand out more clearly. Add xylene ¼, ½, and ¾ of the volume of alcohol present at 5-minute intervals; remove alcohol-xylene mixture and add pure xylene making one change. The specimens are now ready to mount in balsam.

In mounting the specimens care should be taken to get numerous specimens in a very small drop of xylene. Let the specimens settle to the bottom of the drop against the slide and drain or blot away the surplus xylene. Place a drop of thin balsam over the specimens so that it spreads well beyond the area occupied by the specimens. Put the cover glass in place.

At the beginning of the process the specimens were placed in a small tall shell vial. In most cases the specimens would settle to the bottom and the fluids could be easily drawn off by means of a small pipette. No attempt was made to remove the last drop at each change. If one starts with plenty specimens a loss of a few will make no difference.

The main advantage of this method is the ease with which a large number of specimens can be prepared at one time without danger of overstaining with the alum cochineal. There is danger of overstaining with the light green if exposed too long or if the solution is too concentrated. The purpose of the light green is to lightly stain the cilia.

If the whole procedure is successful the shape of the animals will be very well preserved. The micronucleus and the meganucleus will usually show clearly and can be readily distinguished from each other. The cilia show clearly in reduced light, also the striations are observable in some cases. The gullet, contractile vacuoles, food vacuoles and some cytoplasmic details are clear in many specimens. The undischarged trichocysts can usually be observed.

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