



## NOTES ON THE DESENSITIZATION OF PARAMOECIA WITH SOME OTHER MICROTECHNICAL OBSERVATIONS

George Moore

Oklahoma A. and M. College

Since paramoecia can be so easily secured in great numbers, they make very fine objects for a protozoan example in microtechnique classwork. But they have presented quite a problem because of the fact that fixation invariably causes more or less distortion, most fixatives completely obliterating the oral groove and producing also some distortion of the macronucleus.

Accordingly, during the summer and fall of 1931 a study of the effects of certain chemicals on paramoecia was carried out. Various fixatives and

modifications of them were tried but with poor results. However, it was noted that cobalt nitrate in dilute aqueous form (one to two per cent) would slowly kill the organisms, but after a time they appeared to shrink. At first, they seem to be desensitized because their rate of locomotion is lowered.

Paramecia can be very successfully killed with each of several good fixatives if they are first treated with cobalt nitrate. Hot fixatives (about 50 degrees C.) appear to give better results than cold ones. The following procedure yields excellent fixation. A measured quantity of concentrated culture is secured; to this, an equal quantity of two per cent aqueous cobalt nitrate is added. When the animals have slowed down until they have become quite sluggish, about four volumes of the selected fixative is added to the mixture.

Good results have been obtained with Tellyesniczky's fluid, Bouin's fluid, and Zenker's fluid. The best results, however, were procured with the following mixture:

Phenol, five per cent aqueous (five grams phenol crystals dissolved in a very small amount of ninety-five per cent alcohol and added to ninety-five ml. of distilled water.)	5 ml.
Saturated solution of picric acid	15 ml.
Formalin, forty per cent	5 ml.
Formic acid	½ ml.

Wash in water which has stood in a jar for some time to allow all air to escape. Otherwise small bubbles cling to the paramecia and they rise to the surface instead of settling. Then upgrade to seventy per cent alcohol for storage or stain as desired. Very pleasing results can be had by the use of borax carmine counter stained with indulin.

Hydras can be anaesthetized by the use of chloroform vapor. The addition of chloroform to the surface of the water causes the animals to contract and die in a contracted state, but if the container is placed under a bell jar and a small dish of chloroform is also placed under the cover, the hydras will die nicely extended when fixed with hot Bouin's or corrosive acetic. The fixative must be at hand and when the hydras fail to contract when struck gently with a needle the fixative is poured quickly over them. They can then be left in the fixative an appropriate length of time and preserved or mounted as desired.

Microstoma can be anaesthetized in the same manner if they are previously treated with two per cent aqueous cobalt nitrate for a few minutes then placed under a bell jar with chloroform vapor. As soon as the animals have become nearly immobile they may be fixed. Fixation was accomplished by using a concentrated solution of sulphate of iron suggested by Delage in 1886 (Lee. *The Microtometist's Vade-Mecum* 9th ed., p. 525). Sulphate of iron causes some swelling; but if used with an equal amount of physiological salt solution, to which excesses of picric acid and corrosive sublimate have been added very good results may be had. Due to the presence of a precipitate in the mixture it should be filtered. Specimens should remain in this fixative for about thirty minutes, washed in water several times and upgraded to seventy per cent alcohol rendered acid by the addition of a drop of hydrochloric acid. One drop to five ml. of alcohol is about the right proportion. The yellow color due to the iron sulphate will soon disappear.

Rotifers (*Asplanchna* and *Hydatina*) can be fixed with trochal discs extended by forcibly shooting them from a pipette into corrosive acetic or Gilson's fixative, either near the boiling point. Corrosive sublimate is removed in the usual manner by the use of tincture of iodine.