Staining Methods for General Use in Studying Protozoa

W. P. Allyn, Indiana State Teachers College

The study of Protozoa has presented some difficulties in the past because of the necessary time and skill involved in the preparation of stained mounts and of certain injuries suffered by the organisms during the fixing and staining processes. It is believed that the suggestions offered here will prove helpful to those interested in a general and more or less elementary phase of this study, the observation of both the external and internal structures of the organisms, including the nuclear patterns.

Certain problems were encountered in an attempt to devise simple, yet effective, combined fixing and staining techniques for general use. It was found that the different species of Protozoa do not respond alike in all cases to the same fixing and staining procedures. Moreover, in some cases members of the same species show noticeable variations. The differences in staining reactions seem to be attributed not only to such factors as the quantities and compositions of inclusions present but also to the age and general physiological state of the species at the time of staining.

Combined Fixative and Differential Stain for Temporany Mounts: 10cc. glycerine; 2cc. phenol (crystals reduced to a liquid by heat); 2 cc. glacial acetic acid; 100 milligrams methyl green; 1 milligram crystal violet; 100 cc. water. Note: A stock solution of crystal violet is prepared by dissolving 100 milligrams of crystal violet in 10 cc. of alcohol and diluting it to 100 cc. with water. Hence 1 cc. of stock solution contains 1 milligram of the dye. Also 2-4 cc. of 40% formaldehyde improves the above reagent for many protozoa, excluding the Hypotrichida.

The combined fixative and differential stain is introduced directly into a living suspension of the organisms on a microslide. An amount of stain approximately equal to that of the suspension of organisms was found to give satisfactory results. It requires some 10 minutes or longer for the Protozoa to absorb the stain, and the process may be observed conveniently under the microscope. The methyl green exhibits an affinity for the nuclear materials, while the crystal violet is absorbed in the main by the cytoplasm of the animal, thus giving a blue-green cast to the nucleus and a faint violet hue to the endoplasm.

It is noted that if an adequate amount of the staining reagent is used, the mount may be permitted to air-dry at room temperature for several days without any appreciable injury to the organisms. Since the glycerine contained in the stain is not volatile at ordinary temperatures, it remains incorporated in and around the specimens and acts as a preservative. After several days standing preparations have been rejuvenated with a drop of water, or preferably with a drop of the original reagent (without the dye), and the organisms were found to be preserved in excellent condition.

It is observed also that the density of the dye in the organisms can be controlled within certain limits. If it be desirable to intensify the dye, more of the original reagent may be added from time to time; but if too much of the dye has been absorbed and the organisms are becoming opaque, a drop of water, or preferably a drop of the original reagent without the methyl green and crystal violet, will tend to clear the specimen and render the internal structures visible. If further dilution is desired, the slide may be tilted slightly, the excess reagent floated off or absorbed by a filter paper, and more of the diluting reagent added until a dye-density best suited for the species under observation is attained.

If the organisms are laden with food vacuoles or other inclusions which absorb sufficient dye to obscure the nuclear patterns, it may be found expedient to omit the crystal violet entirely from the original staining reagent.

Satisfactory mounts of many of the common forms can be obtained with the above procedure. The various species of Amoebae, also *Paramecium caudatum, aurelia,* and *bursaria, Euplotes patella, Stylonychia mytilis* and *pustulata, Gastrostyla steinii,* and other representatives of the Sarcodina, Infusoria, and Mastigophora seem to respond in a very favorable manner.

Combined Fixative and Flagella Stain for Temporary Mounts: 10 cc. glycerine; 2 cc. glacial acetic acid; 5 cc. phenol (crystals reduced to a liquid by heat); 25 milligrams crystal violet; 100 cc. water.

The combined fixative and flagella stain is introduced also directly into a suspension of the organisms on a slide in a similar manner to that just described for the differential stain. The dye is usually absorbed within a few minutes, and the flagella, cilia, or cirri become rather easily discernible.

The reagent submitted here is a modification of a formula presented by Noland[1]. It is believed, however, that the immediate preparation possesses some advantages over that described by Noland. The Noland reagent, when introduced into a suspension of the organisms, was observed to react rather violently with the latter, the organisms were swept about in the medium by certain vortical currents set in motion, and many of the flagella, hardened by the formaldehyde, were broken off. On the other hand, by omitting the formaldehyde of the Noland reagent and adding 2% glacial acetic acid and also by decreasing the amount of phenol to 5% and increasing the glycerine content as indicated above, no appreciable damage to the flagella is produced during the preparation of mounts.

A Fixative for Permanent Mounts of Amoeba: 2 cc. glacial acetic acid; 2 to 4 cc. formalin (40% formaldehyde); 10 cc. glycerine; 100 cc. distilled water.

The reagent just described proved very satisfactory for killing and fixing Amoebae on slides preparatory to staining with iron-hematoxylin in the preparation of permanent mounts.

[1] Noland, Lowell E., 1928. A combined fiixative and stain for demonstrating flagella and cilia in temporary mounts, Science. 67:535.

A suspension of the Amoebae on a slide is observed with the low-power objective in order to determine when the organisms have the pseudopodia extended and in contact with the slide. At the proper time, the reagent, equal in quantity at least to that of the suspension of organisms, is added. After some three to five minutes have elapsed, the fixative and killing solution may be drained off gently by tilting the slide. Then by adding more reagent and repeating the process, much of the debris, which would otherwise obscure the Amoeba, can be floated off.

Amoeba, treated as described, remain intact and fixed sufficiently to the slide to endure rather rigorous treatment during the staining process. Following fixation, the preparations may be plunged immediately into 2% iron alum, then to hematoxylin, differentiated in ½ to 1% iron alum, and carried up with a very low casualty through the alcohols and xylols into balsam (Fig. 1).

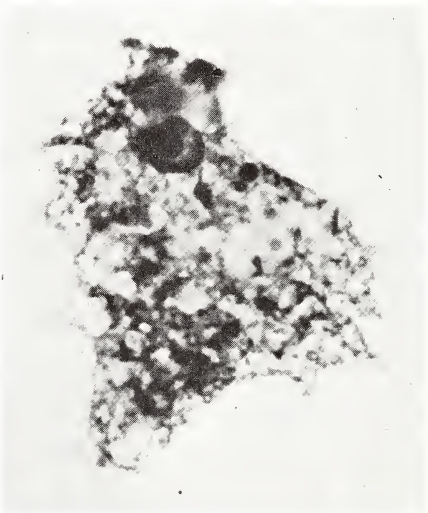

Fig. 1. Amoeba killed and fixed by the method for permanent mounts; stain, iron haematoxylin. x850.

It is well recognized that the suggestions set forth here are in need of many revisions and much refinement, but it is hoped that a preliminary step has been made at least in developing simpler and more convenient methods for studying these minute forms of animal life.