SCIENTIFIC APPARATUS AND LABORATORY METHODS

MOUNTING AMPHIBIA BY PARAFFIN INFILTRATION

CERTAIN difficulties seem to be inevitable in following the procedure for mounting Amphibia by paraffin infiltration as set forth by Noble and Jaeckle in The American Museum Novitates No. 233. A few changes have been found to give better results.

The terpineol, which is used as a clearing agent, has a tendency to cause the abdominal walls of most salamanders to collapse, and to harden the tissue so that it is almost impossible to renew the plumpness of the animal by a later injection of warm paraffin. Melted paraffin may be injected, however, before the animal is put in the terpineol, and the lifelike form will be maintained throughout the remainder of the procedure. In some of the smaller salamanders it is not necessary to repeat the injection after the paraffin bath.

Not only does this early injection of paraffin preserve the shape of the animal, but it shortens the length of the time required for the paraffin bath. The injected paraffin melts and passes into the viscera, so that all the paraffin which infiltrates the animal does not have to pass through the skin. This is important as the brittleness of the final mount seems to be proportional to the length of the time the animal is in the hot paraffin.

With some of the small salamanders, like the red-backed (*P. cinereus erythronotus*) and the two-liner (*E. bislineatus*), good results have been obtained by omitting the terpineol. The small animals clear quickly in xylol, and the week in terpineol which Noble suggests is eliminated. Of course absolute alcohol must be used in dehydrating if the terpineol is omitted.

Noble and Jaeckle state that in mounting the red elf, (the land stage of *T. viridescens*) tinted paraffin may be used. But they give no definite statement as to how this is done. All attempts to find tinted paraffin which would both keep its original color when heated and pass through the tissues of the animal easily have been of no avail in this laboratory. This particular animal may be colored very nicely by staining in eosin. In place of the 90 per cent. alcohol for the final dehydration use a saturated solution of eosin in 90 per cent. alcohol. The best results were obtained by staining overnight. It seems best to over-stain a bit because the finished mount is slightly duller than when taken out of the stain.

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SPECIAL ARTICLES

THE GROWTH OF HOOKWORM LARVAE ON PURE CULTURES OF BACTERIA[1]

THE exact food that can be utilized by hookworm larvae in developing to the infective stage has never been determined. In his monograph on the free-living stages of the hookworm,[2] Looss makes the statement, "The normal food of the Ancylostoma larvae consists of the finest solid constituents of the feces, perhaps also of certain substances contained in them in solution." He further observes that "bacteria have not a sufficient nutritive value for the larvae." Other opinions as to the food of hookworm larvae have been very indefinite, such as "the fine organic detritus of feces," and so on. In fact, all observations have been made upon growth in fecal cultures under conditions where the factors are so numerous that it would be practically impossible to determine with certainty which substances were necessary for growth. In the present study, ova of the dog hookworm, *Ancylostoma caninum,* have been obtained free from feces and sterilized. These sterile ova have been inoculated onto agar cultures of various bacteria, and the larvae have hatched normally and grown to the infective stage with bacteria as their sole source of food.

The method employed for freeing the ova from the feces consists in thoroughly mixing up about 25 grams of freshly passed feces from a heavily infested dog in 500 cc of water. The mixture is then washed through a series of copper-wire sieves ranging up to a mesh of 100 wires to the inch. The larger particles in the feces are caught in the sieves but the ova readily pass through with the filtrate. This filtrate is allowed to stand in a large sedimenting cone for about an hour while the ova and heavy debris settle to the bottom. The supernatant fluid is then poured off; the sediment is transferred to a 50 cc centrifuge tube and repeatedly washed with water, the solid matter being thrown to the bottom each time by centrifuging at a speed of 1,000 revolutions per minute. After the supernatant fluid from the washing has become practically clear, saturated salt solution is poured into the tube and the contents are again centrifuged at the same speed. This time the ova come to the surface and may be collected by removing the surface film with the open end of a piece of large glass tubing.

[1] From the Department of Helminthology, School of Hygiene and Public Health, the Johns Hopkins University. The work was aided by a grant from the International Health Division of the Rockefeller Foundation and was carried out under the direction of Dr. W. W. Cort.

[2] A. Looss, 1911, "The Anatomy and Life-history of *Anchylostoma duodenale* Dub." Part II, "The Development in the Free State." Ministry of Education, Egypt, *Records of the School of Medicine,* 4: 163–613.