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.

If the material is centrifuged four or five times, a majority of the ova present can be recovered. If much solid material comes to the surface with the ova, it may be necessary to refloat the ova in saturated salt solution a second or even a third time in order to get rid of the foreign material. This method is tedious and time-consuming, but if the feces of a *heavily* infested dog are used, large quantities of ova can be obtained almost entirely free from fecal material.

Ova collected by this method can be sterilized by treatment with a 5 per cent. antiformin solution in 10 per cent. formalin. From 10 to 50 per cent. of the ova remain viable after this treatment. The ova are washed several times with sterile distilled water and are then ready for inoculation onto the agar cultures. During the process of sterilizing and washing, the ova are best kept in a sterile 50 cc centrifuge tube closed with a cotton plug.

Cultures were made up in 250 cc Erlenmeyer flasks stoppered with cotton plugs, and consisted of 20 cc of ordinary bacteriological agar which had been diluted with three parts of water. The flasks were autoclaved and inoculated with bacteria twenty-four hours before the ova were introduced. Since at ordinary room temperature the ova do not hatch for an additional thirty-six hours, there was a heavy growth of bacteria in the cultures by the time the larvae were ready to begin feeding. The sterile ova were introduced into the flasks in 1 cc portions of an aqueous suspension, several thousand ova usually being put in each flask. In every experiment, 1 cc portions of this suspension were also inoculated into control broth cultures both before and after the inoculation of the experimental flasks. In this way the sterility of the suspension of ova could be checked. In addition, at the end of each experiment, in order to detect any possible contamination during the process of inoculating the flasks, subcultures in broth were made from the growths in the experimental flasks and after twenty-four hours examined by a Gram stain. When proper precautions were observed, only occasional contaminations occurred, and these flasks were discarded from the experiment.

In the experiments so far carried out larvae have grown to the infective stage in the normal period of about seven days on pure cultures of *Bacillus coli, B. subtilis, B. prodigiosus, B. lactis aerogenes, Staphylococcus aureus, Spirillum metchnikovi, S. rubrum* and *Micrococcus citreus.* Ova which were put on plain agar without bacteria hatched normally and lived for as long as ten days, but did not grow. If bacteria were then introduced into the flasks, the larvae grew to the infective stage. These experiments demonstrate that hookworm larvae in growing to the infective stage are able to utilize bacteria as their sole source of food.

Not all bacteria are suitable food for hookworm larvae, since they failed to grow on cultures of *Bacillus pyocyaneus* and *Sarcina lutea,* and growth was very much retarded on cultures of *B. cereus* and *B. megatherium.* Larvae, however, grew normally on a mixed culture of *Bacillus cereus* and *B. coli.* In all experiments quantitative methods were employed for counting the number of ova which were introduced into each flask and the number of larvae which developed.

The recovery of hookworm ova free from fecal material according to the procedure outlined above can be applied to obtain material not only for experiments on the food of the larvae but also for exact studies of the factors influencing their development. Almost all observations on the biology of the free-living stages of the hookworm have been made upon eggs and larvae in fecal cultures. Studies of the eggs and larvae free from feces in a controlled environment will make possible more definite knowledge concerning the influence of the various factors upon their development.

The conclusions drawn from the experiments in this report do not exclude the possibility that other substances than bacteria may serve as food for hookworm larvae. In fact, it is quite probable that other organic material may contribute to their nourishment. Various substances are being tested in this respect. But the experiments do demonstrate that certain bacteria may alone furnish adequate food for the growth of hookworm larvae to the infective stage.

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THE EXCITATION OF LUMINESCENCE BY THE AGITATION OF MERCURY IN GLASS AND TRANSPARENT FUSED SILICA TUBES AND VESSELS

IT is well known that when an exhausted glass tube containing a small quantity of mercury (leaving a partial vacuum, which must not contain even a trace of moisture) is held horizontally and given a reciprocating motion, static electricity is generated by the contact of the mercury against the glass, and the electrical discharges through the residual gas produce light. This is one of the most direct methods of producing electrical light by the expenditure of mechanical energy. As explained by Professor Elihu Thomson in an exceptionally valuable contribution,[1] "the electrification of the glass, by the running of the mercury over the surface, would, in neutralizing itself, together with the charge which the mercury acquired, illuminate the interior of the tube in much the same way that the Geissler tube is illuminated by the passage of an electric discharge."

Tubes of this kind have sometimes been designated as "mercurial phosphorus" and "Geissler's shaking

[1] "The Nature of Tribo-electricity, or Electricity of Friction, and Other Kindred Matters," *General Electric Review,* 25: 418–421, 1922.