The trustees of the Elizabeth Thompson Science Fund meet during the last ten days of the months of February, May and November. Applications for grants should be sent in well in advance of the meeting to the secretary of the fund, Dr. Edwin B. Wilson, 55 Van Dyke Street, Boston, Mass.

SCIENTIFIC APPARATUS AND LABORATORY METHODS

METHOD FOR CONCENTRATING AND SECTIONING PROTOZOA

DIFFICULTY in securing sufficient concentration and satisfactory orientation of protozoa for making paraffin sections is frequently encountered. Partial control of the degree of concentration of the protozoa in one area of the block which is to be sectioned as well as a "natural" or "self-orientation" of the individuals is to be desired. By the use of the method described below the protozoa may be satisfactorily prepared for sectioning.

For the best results in the final step (sectioning) every trace of the fixative used must be removed. Iodine may be used to remove mercuric bichloride or lithium carbonate to remove picric acid if either has been employed in the fixative.[1]

It is convenient to use a vial with a wide mouth and a round bottom for the entire procedure of infiltration, as described by Wenrich.[2] The lowest fourth cut from an ordinary sized test-tube is satisfactory for the purpose. The vial is placed in a mound of molding clay or in a hollowed cork which will serve as a base. A pipette with a rather large mouth is best for removing the fluids.

Following each change of fluids the protozoa are allowed to settle to the bottom of the vial, after which the next change is made by pipetting off the fluid to within 4 mm of the level of the protozoan mass. This method requires more frequent changes of the fluids, for as in the case of alcohol, the fluid remaining each time lowers the percentage of the newly added alcohol. This gradual change, however, prevents a great loss of specimens.

The protozoan mass may be carried through the entire process of dehydration and clearing in the same vial. A mixture of absolute alcohol and xylol and finally pure xylol is used to clear the specimens. After pipetting off most of the xylol, melted paraffin is quickly added. During several changes of melted paraffin the vial is kept under a heat bulb attached to a suspended light socket with a shade, to insure infiltration and complete removal of the xylol.

When infiltration is finished most of the paraffin may be removed by the use of a warm pipette, leaving only enough in the vial to cover the specimens completely. The vial is removed from under the lamp and the remaining paraffin containing the protozoa is allowed to solidify on the tip of a large dissecting needle. This is accomplished by moving the needle about gently in the mass as the paraffin cools. The result is, therefore, that the solid paraffin mass containing all the protozoa which were in the vial is now on the needle tip. This step may be referred to as the "temporary imbedding." This last step should be accomplished with the use of as little paraffin as is possible. The mass is now ready for permanent imbedding.

A small paper box of the size desired for the final block (4–10 mm) is made according to the diagram

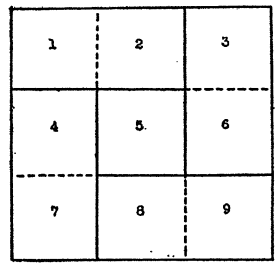

Fig. A

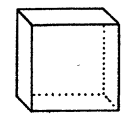

Fig. B

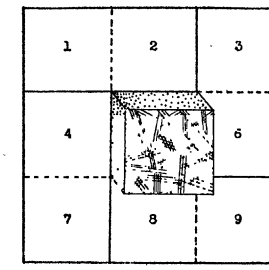

Fig. C

and accompanying directions and submerged in a watch crystal of melted paraffin, also under the heat bulb. The "temporary block" is now lowered into the paper box. The paraffin is allowed to melt and fuse completely with the surrounding paraffin and the specimens are allowed to settle to the bottom of the box. If the "temporary imbedding" has sufficiently concentrated the protozoa they fall from the block as it melts, into one small spot at the bottom of the box. Excess heat from the lamp will cause rapid diffusion of the protozoa and defeat the purpose. The box is lifted from the watch crystal, held long enough in the

[1] An alcoholic solution of iodine may be added drop by drop to the specimens in 70 per cent. alcohol until an amber color will persist for an hour. One drop of a saturated aqueous solution of lithium carbonate may be added to each 10 cc of alcohol containing specimens.

[2] D. H. Wenrich, "McClung's Handbook of Microscopical Technique," Hoeber, New York, 1929, p. 403.

air for the paraffin to seal to the sides and then the box with the contents is lowered into ice water.

After complete hardening, the paper may be peeled off. If the sides and corners of the box have been made in sharp creases, the block is now ready for sectioning without the usual trimming. In general, however, it is better to lose a few specimens by trimming, because accurate trimming aids in securing good serial sections. Frequently the specimens are so well concentrated that by mounting and sectioning the block at right angles to its position in the box only a small portion need be sectioned, to include all specimens. In cases where the protozoa used have one longer axis they will be likely to settle with that side against the bottom of the box, and so by orientating the block the majority of the individuals may be secured in a definite plane of section.

Concentration of both free-living and endozoic protozoa may be successfully carried out in this way.

Explanation of diagram. A square piece of paper is ruled into nine equal sized squares and cut along the dotted lines as indicated in Fig. A. Squares 7 and 8 are folded up over 4 and 5; 6 and 9 are folded to the left; then 2 and 3 are folded downward; squares 3 and 9 are then folded under the center square; the folds are next opened up and 2 and 3 are folded down over 5 and 6; 1 and 4 are folded to the right and 7 and 8 are folded upwards; the two ends, 1 and 7, are folded under the center square. If all the folds are now opened up, a box may easily be made with 1 outside of 2, 3 outside of 6, 9 outside of 8 and 7 outside of 4. The corners may be secured with gummed paper (Fig. B). This method of cutting and folding results in a box which has the four corners open at the bottom to facilitate the exchange of paraffin in the final imbedding. Fig. C shows the paraffin block secured by imbedding in this box.

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SPECIAL ARTICLES

INSULAR BIOTA AND DISPERSAL AGENTS

WORKING on a group of mites which are non-parasitic but common on vegetation, living or dead, the question of dispersal of these animals has often been brought to my attention. Sellnick[1] records two Oribatids from Krakatau. Halbert[2] records five from the Bills, sea-swept rocks off the west coast of Ireland. Praeger[3] summarizes the biota of these rock islands and endeavors to explain its origin. After suggesting every possibility that has been offered by former writers on distribution, he seeks refuge in the amplitude of another land bridge—this in spite of the fact that his collaborators have given him the clue. The majority of the animals listed were found in old nests of puffins and great black-backed gulls. These latter are builders of large nests, bringing material from the mainland.

A careful perusal of nesting habits of marine birds as brought together in the U. S. National Museum bulletins by Bent reveals that the gulls, more than any other marine birds, use considerable material for their nests which they place on islands to escape foxes (not man). This, coupled with their wide distribution and commonness, is enough to account for a great deal of transport of grasses, straws, plant material of all kinds even to sticks of wood.

Although Oribatid mites may be carried on sticks as adults, as many as thirty-three specimens representing three species having been taken from a single stick some two feet long within the limits of New York City, other larger animals may be transported on sticks, in straws and plant stems (or glued to them) *as eggs* or seeds. Many of the islands used by birds have no beach on which drift may accumulate. This necessitates carriage from the mainland—like Noah's dove. A catalogue of the spores, seeds and eggs that may be carried on nesting material will destroy a large number of land bridges. Certainly most of the hundreds of Oribatids described, many Collembolans and not a few beetles (especially wingless ones) may be carried thus as adults. Ernst[4] records gulls on Krakatau in 1906.

An extension of this type of carriage would include dispersal from place to place on land. An Oribatid traveling at the rate of an inch a minute on white paper, over a woodland or even a meadow floor may require twelve hours to advance three feet (especially if it has to crawl over a log), but a bird may carry it or its eggs thirty feet in a minute. On land, hawks, crows and squirrels are our chief distributors of dead wood. Although streams may check the latter, the first two would know but few boundaries. Vireos distribute lichen-dwellers, swallows disperse mud-dwellers, the majority of which tide over periods of desiccation in an encysted stage. The possibilities are as numerous as the habits of nest-builders, and the speed of dispersal per annum will be measured by the distance a bird will carry nesting material. The fauna of birds' nests has already been touched on by

[1] 1924, *Treubia*, 5: 371.
[2] 1915, "Clare Island Survey," pt. 39–ii, *Proc. Royal Irish Acad.*, vol. 31.
[3] 1915, *op. cit.*, pt. 68.
[4] "New Flora of the Volcanic Island of Krakatau," Cambridge University Press.