XIV.—*On an Improved Method of Preparing Embryological and other Delicate Organisms for Microscopical Examination.*

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DURING the progress of microscopical science, that department which relates to the preparation, either temporarily or permanently, of the various objects required for investigation has naturally received a very large share of attention; whilst some workers have devoted the whole of their time and attention to the careful study of this or that particular branch of natural history, others have on their part been the means of bringing the Microscope and its apparatus to its present high state of perfection, whilst others again have sought out the best method for preparing and preserving the countless subjects of microscopical examination.

It is more particularly to the latter branch that my paper refers, although the process which I shall describe was merely the result of a strong desire on my part to preserve the beautiful forms I so constantly met with during my study of the Stalk-eyed Crustacea of the English Channel.

The methods of permanent preparation for the Microscope have until recently been unsatisfactory, except for certain objects, namely, sections and specimens having no thickness; but the old system of mounting whole insects crushed, flattened, bleached and contorted was calculated to lead to error upon error, and it is fortunate that a general wish to mount "without pressure" is rapidly being carried into effect, not only in Canada balsam, but also in various fluids. For minute examination of parts, however, with high powers, the flattened method is of course necessary, though very misleading for general outline, structure, and form.

There are many objects to which Canada balsam could not be applied, and which owing to their great delicacy would never dry for the purpose of being examined as opaque objects, simply because an attempt to dry them would result in their almost complete disappearance, their structure being supported, so to speak, by the water in which they existed.

I refer to the delicate organisms of our seas and more especially to their embryological stage, which renders them still more delicate and fragile.

On the 27th of April, 1877, I collected in Mounts Bay, Cornwall, some egg-capsules of a mollusc *Nassa reticulata*, these I placed direct into Haentsche's fluid and mounted in a sunk cell, using marine glue as a cement and finishing with asphaltum and zinc white.

It was not, however, a success, and for some time I did not attempt the experiment of preserving marine ova. But three or four years later I was brought into constant contact with quantities of marine material, and as I overhauled the so-called "refuse" of scallop-boats for the purpose of selecting the crustacea already referred to, I regretted that some means could not be found for preserving the many beautiful objects that I was obliged to throw away.

Besides this there were so many points of interest connected with the crustacea themselves that required microscopic investigation, that it was absolutely necessary to do something to try and retain them.

Without attempting to go into details, apart from the immediate subject of my paper, I should like here to mention that as regards the crustacea alone, not only is it possible to identify a species by an examination of its ova, but even the habits and mode of life can be in most cases determined by such examination.

As regards fish embryos, the importance of being able to preserve them easily for the Microscope cannot be overestimated, whilst for less important forms the bare fact that the zoœa of crustacea were once thought to form a separate order of animals, shows how necessary a knowledge of such comparatively unfamiliar objects is.

With the desire therefore to retain these ova for microscopical examination, I tried many methods, all of which resulted more or less in failure; the means I now adopt, though perhaps not perfect, is at any rate suitable for the purpose, chiefly I believe owing to the strength of the cement employed. This cement is composed of 2 parts carbonate of lead or white lead, 2 parts red oxide of lead or minium, and 3 parts of litharge or oxide of lead. These parts should be ground very finely, mixed dry, and kept so in a wide-mouth bottle; when required for use, a little of this powder should be mixed in a small china pan with gold size to the consistency of ordinary paint: care being taken that no trace of grit or unground matter exists, for the slightest atom of this will prevent the cover-glass from settling down firmly to the cell, and cause an influx of air at once.

The first operation is to fix a cell to a glass slip by means of this cement; as soon as it is set apply a coat of the cement to the upper surface of the cell and to the outside angle of the same; this done, the slide should be put aside in a dry place to harden, and I consider it best to allow this to occupy a fortnight at least before further use, by which time there will be no fear of any leakage or fault; in fact, the cell will have become so firmly fixed that it can be entirely filed off, if a metal one, without breaking it away from the cement.

The cell having been duly constructed, I scrape round the inner edge with a pointed knife, which removes any foreign matter from the side of the cell and clears away any of the cement which may show itself on the inner angle; after cleaning carefully with a dry cloth, the cell is ready for the reception of the object. Before proceeding further with this stage, I will describe the preparation of the object about to be mounted.

I have already referred to the medium used in this work, which is on the basis of what is known as Haentsche's fluid; namely, a composition of 3 parts absolute alcohol, 2 parts pure glycerine, and 1 part distilled water. I say it is on this basis, for it is necessary to alter the density for certain objects, and I never think of using these proportions for mounting, but simply for preparing for that operation.

Now as regards the necessity for altering the density of the above formula, I find that the proportions mentioned answer very well for parts of the young of Crustacea; for zoëa; for young fish, if more than three or four days old; for nearly all hard ova; for young Echinoderms; for most insects, and for plants excepting the most delicate tissues: but I have found that it often crushes such organisms as the delicate ova of some fishes, the ova of the Nudibranch Mollusca and such fragile substances; so that I reduce the glycerine, and in some cases make the proportions 3 parts distilled water to 1 part each of alcohol and glycerine; but as experience is the best master, it is always as well to find out by practice the proportions best suited to the particular class of object under consideration.

Should the fluid be too dense, the objects will show it by becoming crushed; should, on the other hand, the fluid be too weak to preserve the specimens properly, a tendency to disintegrate and part company will be noticed on their part.

Having provided a number of small corked glass tubes, with numbers on the corks for the purpose of keeping the particulars of their contents in a book of reference—for I must here urge upon marine zoologists the value of keeping a proper record of the date and locality as well as the names of their specimens, for much depends on this—having then prepared the tubes of fluid, the specimens should be dropped in alive, or at any rate in a perfectly fresh condition; this is absolutely necessary with marine organisms, where the decomposition and breaking-up of minute bodies takes place in an incredibly short space of time after death. In shore work or in dredging or trawling a number of these tubes should be at hand, for, however conversant the naturalist may be with the objects of his search, it would be most rash to place all his specimens into one receptacle, with the intention of sorting them out at a more convenient season.

The tubes, having received their occupants, should be kept in a place where they can be easily examined. I find it a good system to rig up a box into divisions by means of double cross strings; by this I can store about five dozen tubes of ordinary size in a box 10 inches by 4 inches, and by means of the number on the cork can abstract any particular tube at pleasure. The reason for being able to obtain easy access to the specimens during their preparation is this: in a short time the fluid will become more or less discoloured, the mature ova of Crustacea are especially liable to cause this; that of fishes gives off a milkiness which would be simply ruinous to a slide, and plants would obviously stain the fluid with chlorophyll. These various discolorations can however be overcome by repeated washings. The tubes should be overhauled occasionally and their contents washed as follows: pour off the discoloured fluid gently, so as not to lose any of the objects, then fill up the tube with distilled water; the specimens, owing to their being charged with a heavier fluid, sink rapidly, and thus allow the tainted water to be poured off; repeat this operation until the water carries off no more extraneous matter, and then pour in some fresh fluid as before. It will be found necessary to allow some specimens to remain in preparation, with periodical washings, for six or eight months, but I have many that have been so treated for two years, with the result that they are exceedingly clear and beautiful. The majority of objects may however be finally mounted in less time, although I do not consider that marine objects would remain clear with less than at least three months of such preparation.

Having thus referred to the construction of the cell and the preparation of the object, I will describe the final stage of mounting.

A cell having been thoroughly cleaned, a rim of cement should be applied to its upper edge, and upon its becoming nearly set, which can readily be noticed by its losing its gloss and becoming dull, the cell should be filled to a convexity with fluid. The fluid I use for this part of the operation differs considerably in density from that used in the preparation of the objects; for instead of 3 parts alcohol, 2 parts glycerine, and 1 part distilled water, I use a fluid as weak in some cases as 6 parts distilled water to 1 part each of alcohol and glycerine. Here again, however, discretion must be exercised, for such proportions are only safe to use when the specimen to be mounted is thoroughly well preserved in the stronger fluid first, and if such be not the case, a stronger mounting medium than this must be made.

Having then filled the cell with fluid, the object should be placed in it and a few seconds allowed for the mingling of the two fluids of different density, which will often set up rotatory motions of a remarkable character amongst the objects. When this motion

ceases, take a clean cover-glass and breathe upon the side intended to come into contact with the fluid, thus enabling it to flow evenly without inclosing air-bubbles; press the cover-glass very gently into the cement, which will cut through the fluid, and attach the cover firmly. Then remove the superfluous fluid by means of a large camel's-hair brush, and lay aside for a few minutes to set, when a stiff coat of the same cement should be applied to the junction of the cell to the cover-glass.

In about a week this structure will have become so hard and tough, that nothing short of actual unfair treatment will damage it, and it may be finished off with such varnishes as fancy may dictate.

I have submitted specimens of my work to the most severe tests, with certainly more satisfactory results than would have been the case with an ordinary balsam or even dry mount.

I inclosed some slides, prepared in this manner, in a tin box, and buried them in frozen snow for twelve hours, from which I had to cut the box out with a chisel; I then placed them in an oven and submitted them to a dry temperature of 140° F. for four hours, at the end of which time the slides exhibited no change or deterioration. The cement itself, when once hard, is so exceedingly powerful, that, as before stated, a metal cell affixed by it to a polished slip can be filed completely away with a heavy coarse file without loosening the cement in the slightest degree: its advantage in being insoluble in the fluid used is also obvious, and I am surprised that it has not been more generally adopted by microscopists, considering its vast superiority over marine glue, or, in fact, over any cement in ordinary use.

The fluid itself, by judicious admixture of various proportions of alcohol, glycerine, and water, can be used satisfactorily for the preparation and final mounting of Fishes, Mollusca, Crustacea, Echinodermata, Insects, Arachnida, and Plants; many of the most delicate of which could not retain any form whatever in a dried state, and would be simply ruined in balsam.

I hope, therefore, that I have been able to show how such delicate organisms may be preserved for microscopic examination in a comparatively simple and easy manner; and as so much attention is just now directed to the very important question of our food-supply as derived from the ocean, there can be little doubt that the study of the early forms of life of our edible fish, mollusca or crustacea, is one of the most useful and valuable to which the microscopist can possibly devote his attention.