The Use of Unsaturated Polyester Resins for Embedding Biological Material

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A process for the volume production of biological materials embedded in plastic has been developed, utilizing an unsaturated polyester resin[1] found to be especially suited for soft and delicate forms such as embryos, jellyfish, cartilaginous skeleton parts, and flukes.

Several experimenters have published reports on successful methods for embedding biological materials (*1*), but material suitable for quantity production has not been a significant factor in this research.

Thus, the problem was twofold: (1) to find a plastic material suitable for quantity production, and (2) to provide plastic preparations of clarity and durability for educational purposes.

In methods described by other experimenters most of the work had been done with monomeric methyl or ethyl methacrylate and some with polymeric powders and combinations of monomeric liquids and polymeric powders. Monomeric styrene also has been used. The methacrylates, although unsurpassed in clarity, have properties which are serious drawbacks to their application.

After considerable work our efforts were concentrated on Selectron, which was found to be the most practicable from the standpoint of handling soft and delicate forms. Moreover, the plastic clears the specimens, making them transparent and the internal structure visible.

The process following was adapted from the technique employed in preparing microscope slides, to the point of bringing together the specimens and plastics.

(1) The specimens may be fixed in Bouin's fluid, formaldehyde, or any of the usual fixatives. All fixatives, regardless of the type used, must, however, be completely removed. In the case of Bouin's, which is customarily used on embryos, the picric acid must be eliminated by washing in several changes of 70 per cent alcohol. The first few changes should contain a small amount of saturated aqueous lithium carbonate. After all the yellow color has disappeared, the specimens should be transferred to water. Formaldehyde may be removed by washing in water.

(2) After the specimens are washed in water, they are stained in acid carmine to a point where internal structures are completely stained. Destaining is then carried to a point where the specimen is pink, since

FIG. 1. Necturus pelvic girdle and limbs.

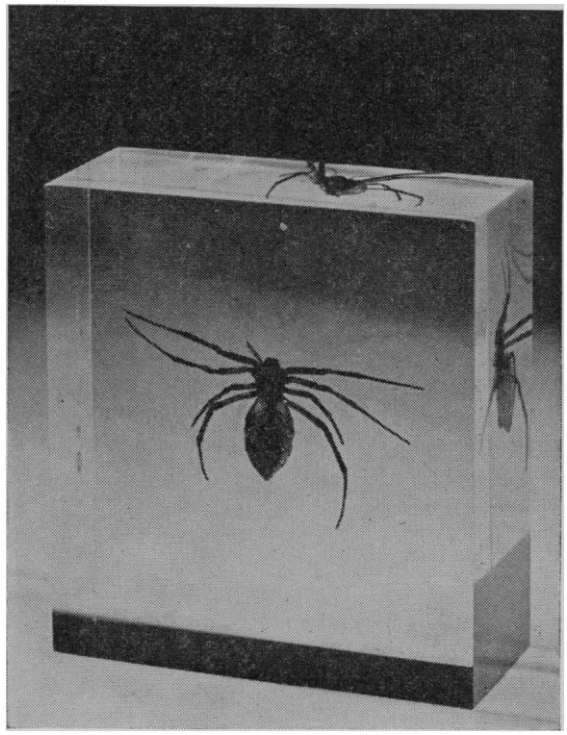

FIG. 2. Garden spider.

[1] "Selectron"—Pittsburgh Plate Glass Company.

the plastic intensifies the color. Hematoxylin has been tried, but has not produced a satisfactory stain. The difficulty lies in adjusting the intensity of the stain, which is increased by the plastic.

(3) The specimens are dehydrated in a series of alcohols (70, 83 and 95 per cent and absolute), the time depending upon the size of the specimens.

(4) The next stage of the process—removing the alcohol—presented many difficulties. At first, various compatible solvents were tried, but these proved unsuccessful. The plastic in and around the specimens was diluted, producing internal strains resulting in cleavages and fractures in the blocks. To overcome this, the specimens are transferred from absolute alcohol to anhydrous ether. Here again the time is varied from an hour for early chick embryos to several hours for larger specimens. The anhydrous ether displaces the alcohol and removes any traces of water from the specimen.

(5) The specimens are next transferred into the uncatalyzed plastic and evacuated in a vacuum desiccator to draw off the ether and allow the plastic to impregnate the specimen. This must be done very slowly and carefully until a vacuum is produced (500–700 mm. of Hg.) and left to stand until all the ether has evaporated. Too rapid evacuation causes the ether to boil, destroying the specimens.

(6) A quantity of the monomer is weighed and the catalyst (tertiary butyl hydroperoxide) added in the proportion of 0.1–0.5 per cent, depending upon the thickness of the block. The lesser percentages of the catalyst are used as the thickness of the block increases. The catalyst is stirred completely into the monomer and allowed to stand until all bubbles rise to the surface and break.

(7) A supporting layer of the catalyzed monomer is poured into a Pyrex dish or tray and allowed to gel at room temperature, until it is firm enough to support the specimens. This requires from 1 to 3 hours.

(8) The second layer is prepared in the same manner and poured onto the first.

(9) The specimens are transferred from the uncatalyzed monomer to the trays, care being taken to carry over as little as possible, and are then oriented on the first layer.

(10) The trays are left to stand at room temperature until the second layer has gelled.

(11) After the gelation is complete, the trays are placed in an oven to cure for approximately 2–4 hours. The heat is raised slowly from 100° F. to 250° F. and maintained at 250° F. for ½–1 hour.

(12) After cooling, the blocks are easily removed by merely inverting the trays and allowing the blocks to drop out.

The plastic, being thermosetting, does not require any elaborate or special equipment for cutting or polishing. A fine-toothed band saw is used for trimming and cutting, while polishing is done on a soft-rag wheel using tripoli and rouge.

The process, as outlined above, has worked successfully on chick embryos, pig embryos, jellyfish, flukes, and other similar forms. While no extensive experimental work has been done on forms which may present different problems, we feel confident that in time these other forms can be embedded in this type of resin.

In general, the unsaturated polyester resins are a light-yellowish color, but some of these are available in a purified form; while not as water-white as the methacrylates or styrene, these have the advantage of a much simpler curing schedule. Also, the polyesters are more resistant to abrasion than the methacrylate. Occasionally, the blocks will have to be repolished. This can be done easily on a soft-rag wheel or by hand, using pumice paste, finishing with rouge, and then wiping with a waxed cloth. It will be found that by using a little care in handling, much unnecessary work can be avoided.

Reference

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A New Spectrophotometric Method for the Determination of Vitamin A[1]

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The spectrophotometric determination of vitamin A is not generally applicable to materials low in vitamin A and containing appreciable amounts of other nonsaponifiable materials (*2*). In an attempt to develop a method which would be suitable for the determination of vitamin A from tissues of vitamin A-depleted animals, we have examined the possibility of purifying the nonsaponifiable fraction by chromatographic methods before spectrophotometry. We wish to report briefly on the difficulties encountered in chromatography, the use of floridin in vitamin A analyses, and the application of this technic to margarine.

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