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EXPERIMENTS IN DEHYDRATION TECHNIQUE

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INTRODUCTION

One of the most inconvenient aspects of plastination is its requirement for the use of hazardous solvents. A major purpose of solvents in plastination is dehydration. Water must be removed so that it will not interfere with polymer formation and crosslinking. A second major purpose is degreasing. The need to eliminate lipid varies with the nature of the specimen and the plastination technique to be employed. When impregnation is carried out immediately after dehydration, the amount of lipid removed is negligible since acetone, at freezer temperatures, has a greatly reduced solvent capacity for fat.

With regard to dehydration, we have found that many specimens, especially those that are rigid when finished, plastinate well after having been dehydrated by freeze-drying. Even some specimens that should be flexible will retain their flexibility after being processed in this way.

The third purpose of a solvent in plastination is its function as a volatile intermediary agent. The purpose of the volatile intermedium is to evaporate, dissolve through the entering plastic and be evacuated so that a vacuum will develop within the specimen. In practice, we find that the air occupying the interstitial spaces of a freeze-dried specimen can be made to perform this same function, at least in several of the tissues that have been investigated. Further, freeze-drying has been found to impart a rigidity to certain types of specimens, especially whole organisms, which greatly inhibits the shrinkage that often occurs when such specimens are dehydrated and plastinated by conventional means.

MATERIALS AND METHODS

In the experiments reported here, specimens were dehydrated by various means, subjected to certain, standard, post-dehydration treatments and plastinated with silicone rubber. Specimens from each group were then subjected to visual and tactile comparison by faculty and students who were unaware of how they had been prepared. Tissues were chosen to represent a selection of those commonly plastinated and grouped by tissue type.

Within each group, one specimen was dehydrated by freeze substitution and impregnated, one was freeze-dried and immersed in acetone for two hours before impregnation (so that the acetone could serve as a volatile intermedium) and one was freeze-dried, and impregnated directly.

RESULTS

1. CAT BRAIN:
The most obvious difference among the three plastinated cat brains was that the color of the two freeze-dry-dehydrated (FDD) organs became tan/yellow while that of the freeze-substitution-dehydrated (FSD) organs remained off-white to ivory. Also noted was a slightly greater opening of the longitudinal and transverse fissures in the FDD brains. Although this would suggest

greater shrinkage during FDD, the volume of both were determined and no significant difference was found (Table 1)

Acetone saturation did not seem to improve silicone impregnation. In fact, the difference in density among the three plastinated brains suggests that impregnation was most complete in the organ that was never exposed to acetone (Table 1.). The brains were sectioned transversely at the level of the pseudosylvian fissure and all three showed silicone in the small sub-arachnoid space. Close inspection of the FDD brains revealed a few tiny cracks on the surface which were not present on the FSD brain.

One student preferred the FDD brains for study, due to their more open fissures. The same student felt that more detail (contrast) was visible on the FDD brains. Overall, however, the FSD brain was strongly favored by both students and faculty (including the author) because of its better general appearance.

2. PELVIC LIMB PROSECTIONS:
Three cat pelvic limb prosections (hemipelvises with attached femurs, joint capsules and ligaments) and three stifle prosections (cruciate, collateral ligaments plus portions of the rectus femoris and peroneus longus muscles) were used. After dehydration and plastination, no differences were noted by either students or faculty in flexibility or greasiness. The muscle tissue in the FDD specimens was noticeably darker than that of the FDD counterpart. This was an unexpected finding in that freeze-drying usually results in some degree of pigment bleaching. The more natural color of the FSD stifle specimens was preferred. But, other than this, no appreciable difference was noted by either students or faculty.

3. DISTAL HORSE LIMBS:
Mid-sagittal sections of distal horse limbs, taken through the distal half of MC3, (each specimen 16 inches in length) were plastinated and compared. Greater shrinkage was noted in the FSD limb, as manifested by small "pull-apart" separations of ligaments and the appearance of interphalangeal spaces. Neither of these was seen in the FDD limbs. The FDD limbs were also judged to have retained a more natural color. Both students and faculty felt that the FDD limbs were greasier, a finding not unexpected. Opinion was divided almost equally as to which technique produced the most desirable specimen.

4. TRANSVERSE DOG SECTIONS:
Whole, transverse sections of a dog were treated the same as above. As with the cat brains, the most apparent difference was that the FDD sections were significantly darker. No difference in shrinkage or greasiness was noted. Students and faculty both preferred the lighter, more natural color of the FDD sections, often commenting that muscle groups and other structures were easier to distinguish. Neither FSD nor FDD sections were bleached, a step that would be particularly appropriate with FDD body sections. Sections to be impregnated with epoxy resin would have to spend a few days in room-temperature acetone after FDD for degreasing, a step that would likely alleviate some of the darkening.

5. WHOLE-ANIMAL SPECIMENS:
Although less useful than other types of specimens, it is sometimes desirable to plastinate whole organisms or large, integument-covered parts. Fish, turtles, whole fetuses and laboratory rodents are just a few examples of animals that have been plastinated intact. The integument covering such specimens limits polymer penetration and requires special provisions. Polymer infiltration by syringe or the placement of needles during impregnation becomes necessary to control shrinkage. Despite such measures, shrinkage remains a problem and several specimens have had to be discarded for this reason alone. A few years ago, as part of an experiment, the author freeze-dried a 30-inch water moccasin (which happened to be stored in the freezer). It was noted that this imparted stiffness, but resulted in no detectable shrinkage. The specimen was then immersed for two hours

in acetone and impregnated with silicone, using no needles or other special measures. After impregnation, the snake (which was dried in the shape of an "S" and which had four 1/4 inch ventral incisions, through which viscera had been removed) was gas-cured by the fast-cure method. The finished specimen showed no shrinkage and was still quite rigid. It could be grasped by the head and tail and extended and contracted about two inches like a spring. Everything considered, it was the nicest such specimen prepared in this laboratory.

As a continuation of the experiment reported here, a scarlet king snake and a copperhead were prepared by FSD, while a water moccasin was prepared by FDD and impregnated without exposure to acetone. All three were plastinated with silicone rubber and the three freshly prepared specimens compared with the moccasin that had been plastinated earlier.

Despite our best efforts to the contrary, both FSD animals showed some shrinkage and distortion. The FDD animal prepared without acetone had the same overall appearance as the snake plastinated earlier, except that it might have been slightly less flexible.

DISCUSSION

The author recognizes that freeze-drying as a means of dehydration will neither be available to most plastinators, nor is it one that most would need. It is, however, an interesting technique and offers unique advantages for some specimens. Its greatest benefit is that it eliminates shrinkage in those specimens where loss of dimension during dehydration is a prominent problem. For example, it results in only negligible shrinkage (less than 1%) of whole, integument-covered animals, even under the most difficult of circumstances. Also, it imparts a degree of flexibility not seen with other dehydration methods.

A second major advantage of FDD is that it is solvent-free. Although a two-day immersion in room-temperature acetone is recommended for degreasing specimens with a high lipid content, FDD still reduces solvent use to an absolute minimum. Table 2. is a summary of the effect of acetone exposure on silicone impregnation of FDD specimens. The most striking result was that acetone saturation had no measurable effect on the impregnation of smaller specimens and only a limited effect on larger examples. It might be. suspected that less uniform impregnation might result without a volatile intermedium but no such difference was noted by any student or faculty member reviewing the specimens.

In summary, although it would not be economically feasible to purchase a freeze-dryer for dehydration in routine silicone plastination, FDD is helpful in preparing specimens where shrinkage is a problem. It is much easier than FSD and results in shrinkage of less than 1%. Also, it reduces solvent use to an absolute minimum, a feature that may prove important if further restrictions are placed on waste solvent generation and disposal.

Table 1. - VOLUME AND DENSITY OF FSD CAT BRAIN SPECIMENS
(Before and after Plastination)

TREATMENT	VOLUME BEFORE	VOLUME AFTER	% ORIGINAL VOLUME	DENSITY Gm/cm^3
FSD	25.5 cm^3	16.5 cm^3	64.7	0.64
FDD+2 hour acetone	24.4 cm^3	16.0 cm^3	65.3	0.63
FDD Only	28.5 cm^3	19.5 cm^3	68.4	0.71

Table 2. - EFFECT OF ACETONE SATURATION ON SILICONE IMPREGNATION

SPECIMENS/ TREATMENT	DEHYDRATED WEIGHT (g)	CURED WEIGHT (g)	% INCREASE
Brain DFF+2hr acetone (Cat)	5.4	15.5	287
FDD only	6.9	20.1	291
Hemi-pelvis FDD+2hr acetone (Cat)	14.4	18.4	128
FDD only	13.6	17.4	128
Transverse FDD+2hr acetone Sect	46.8	121.2	259
(Dog) FDD only	131.3	322.1	245
Distal Limb FDD+2hr acetone Horse	511.3	729.2	143
FDD only	430.7	563.6	131