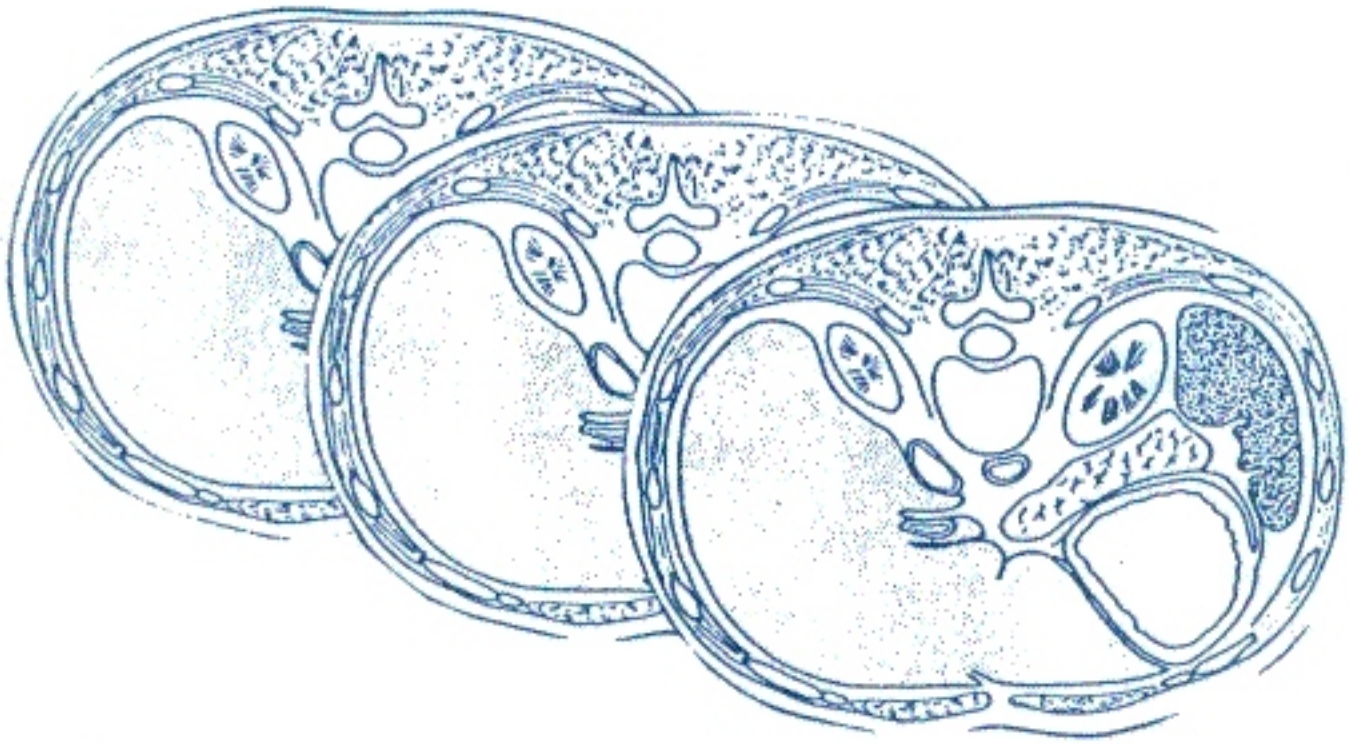
Journal of the

INTERNATIONAL SOCIETY FOR PLASTINATION



Volume 2 - - - - Number 2

July, 1988

JOURNAL OF THE INTERNATIONAL SOCIETY FOR PLASTINATION

Volume 2, Number 2
July, 1989

Table Of Contents

EDITOR'S PAGE

PERILS OF PUBLISHING

One of the advantages of operating as an editorial autocrat in a loosely structured organization like the ISP is that one **could** simply adjust the publication schedule to one's convenience. And it may seem like that's exactly what I'm doing -- but I'm not. Believe it or not, the date on which this journal gets mailed has depended more on when Nancy has time to complete the formatting than it does on almost anything else. (Those of you who attended the last international conference will remember Nancy.)

But now, as if our schedule wasn't arbitrary enough, Nancy has found another job, outside the university, and we have had to find someone else to perform this intricate step. Our difficulty in getting this issue arranged in proper format accounts for it being so late.

It seems certain that we will have to change our policy from publishing two issues of this journal per year, to publishing just one. As of now, we have only one or two acceptable manuscripts, no where near enough to think of publishing. Also, in July, the editorship will be assumed by Bob Henry and publication will be carried out at the University of Tennessee. Therefore, I suggest we discontinue the January number and plan to publish future issues on or about July first of each year. If you have strong feelings about this, please contact Bob or me.

MEMBERSHIP NEWS

One of the original intentions of the Editor's Page was to be able to keep the membership apprised of what was going on in plastination. Announcements of meetings, job opportunities, new products and revisions of technique were examples of the grist for this mill. Well, we are also out of grist! Any of you who have any for the next issue, please send it to Bob Henry.

As I relinquish the editorship, I would like to thank the membership for its confidence and apologize for the somewhat erratic publication schedule. I have enjoyed serving as editor and feel that our journal is becomeing a valuable addition to the scientific literature.

See you at the Heidelberg Conference!

J. Int. Soc. Plastination Vol. 2, No. 2, July, 1988 2

DEHYDRATION OF MACROSCOPIC SPECIMENS BY FREEZE SUBSTITUTION IN ACETONE

Klaus Tiedemann and Dubravka Ivic-Matijas

Anatomical Institute I
University of Heidelberg
Im Neuenheimer Feld 307
D-6900 Heidelberg
Federal Republic of Germany

INTRODUCTION

Dehydration of bulky specimens has attracted little research interest and has been performed mainly with a graded series of alcohols. Dehydration by freeze-substitution in acetone is recommended for plastination (1)(2), a method for preserving biological material by impregnation with curable polymer. In this process, the acetone also accomplishes defatting and serves as an intermediary solvent.

Freeze-substitution is based on the same principle as cryosubstitution, a dehydration procedure used in histochemistry and electron microscopy (3). Tissue to be dehydrated is removed from the fixative and placed in -25°C acetone. Water within the specimen freezes immediately upon immersion, providing an internal scaffold that tends to hold its shape and dimension. Slow substitution of ice by acetone then results in only minor shrinkage. Crystal formation will distort microscopic morphology, however, if the specimen is larger than 2 mm.

The few protocols published for freeze-substitution of whole organs recommend a minimum of three changes of an acetone bath, having a volume ten times that of the specimen. Dehydration time of up to five or six weeks is usually stipulated (4)(5). The present investigation was performed to arrive at a procedure that was more economical in both time and solvent. Water exchange was monitored daily in an effort to determine how many changes of what volume and grade of acetone is required to reach the minimum time of exposure for complete dehydration.

MATERIALS AND METHODS

All specimens were human tissue. Bone with attached muscle, uterus with bladder and rectum, prostate with bladder and rectum, tongue with larynx, as well as brain halves, were used. Most of these organs had been stored in a freezer prior to fixation and dissection. All were fixed in formalin, rinsed with tap water, pre-cooled to +5°C and blotted prior to immersion in acetone at -25°C.

Ten fixed hearts with epoxy-injected coronary arteries were exposed to -25°C acetone under hydrostatic pressure (via cannulated vessels). This was done in an effort to freeze and partially dehydrate them in an expanded state. These hearts were then used as part of this investigation. As expected, they showed a lower amount of water extraction during immersion, however they were considered valuable because they were easily monitored for shrinkage artifact.

A total of more than 55 specimens were used in 22 separate dehydration exercises. In most of these, two similar organs were weighed, placed in closed containers, filled with acetone at -22 to -25°C and maintained at this temperature for the duration of the procedure. Technical-grade acetone (99.9% by volume) was used throughout. For convenience, this is listed as 99% in all graphs. All unspecified solvent changes refer to this almost-absolute grade of acetone. Each bath consisted of 2 to 5 liters of acetone. This amounted to 5 to 12 times the sample volume. Both organs in any one series were treated in an identical

manner. In experiments that started with dilute acetone, 99% solvent was purposely diluted; used acetone was not employed.

Organs were turned every 24 hours, at which time hollow specimens were emptied and refilled. In calculating the percentage of water removed, the acetone concentration of a bath was measured, using a densitometric spindle graded in 1/10 degrees. In some cases, water determination was performed using the Karl Fischer, titrimetric method. After freeze-substitution, specimen containers were transferred to a cold room at +5°C for one or two days and then removed to ambient temperature.

RESULTS

The water content of fresh tissue is known from the literature. To compare this value to that of the formalin-fixed tissue used in these experiments, we allowed samples of fixed tissue used in pilot experiments to dry up, weighing them before and after. By this method, samples of gut (with mesentery) lost 72% water. Brain, 73-75%, embalmed liver, 74% and liver fixed via the portal vein, 89%.

Shrinkage of 7mm brain slices was determined as a result of exposure to an initial bath of 60%, 70%, 80%, 90% and 95% cold acetone (-25^{C}C). It was observed that concentrations of 60 to 80 percent do not lead to shrinkage. In these dilute solvents, a swelling appeared during the first few days, expanding 100 mm slices to 101, 102 or 103 mm. After 4-8 days, this swelling receded to almost the original dimension. In 90 and 95% cold acetone (-25°C), this swelling was less apparent or absent. After 9 days, 100 mm slices measured 97.5, 99, 100 and 101 mm.

The rate of penetration of -22°C acetone into 4 cm tissue cubes was checked using hard, fixed liver from the dissection laboratory and softer, fresh-frozen liver. The depth of penetration of 70% acetone was no more than 3 mm after 24 hours and only 4-8 mm after 9 days (in regions devoid of large vessels). The core of all specimens was still frozen. The serosal surface acted as a diffusion barrier. The softer sample was penetrated slightly more rapidly than the harder by this concentration of acetone.

In 98% acetone, both kinds of tissue were penetrated faster. One day resulted in 5 mm penetration into the soft block and 4 mm into the hard block. The frozen core of the soft block had been replaced after 4 days. The hard block was penetrated 10 mm in 4 days and its ice core was negligible after eight days.

The tissue-to-solvent volume ratio was of no critical importance. The recommended ratio of 1:10 was found unnecessary. Experiments showed that a ratio of 1:5 is sufficient (Fig. 5) and that, at this ratio, the first bath equilibrates at about 12% water. Thus, doubling the volume of specimens in a dehydration bath would be the first means of reducing expense.

The gravity-induced concentration gradient in deep cylinders filled with old acetone is remarkable. At the bottom, the water content may be as much as 3.5% higher than at the surface. Specimens must be kept off the bottom by suspension or elevation on a grid.

Graphs provide an interesting means of following a dehydration experiment. For example, it can be seen quite clearly that time is wasted by waiting too long before an acetone change. Once the water content of the bath equilibrates with that of the specimens, no further exchange of water takes place (Figs 2,5). Acetone change during a steep part of the curve, on the other hand, would lead to a waste of solvent since the potential for exchange into the old bath will not have been fully utilized.

Our first experiments showed that rewarming is the most critical factor with respect to tissue shrinkage. If tissue, still containing more than 10% water, is moved from -22°C

to room temperature, it will suffer severe shrinkage because 5-8% of its original water will be extracted in one day. We therefore introduced a +5° step (dashed line on graph) between the freezer phase (solid line) and ambient temperature (dotted line). Changes into fresh acetone at +5°C were not performed before the container had assumed the same temperature. Circles, triangles and squares that are filled in indicate not only a measured value but also a change of acetone.

Figure 1 demonstrates that similar organs, dehydrated under similar conditions, will manifest pproximately the same dehydration curve. Further, the reproduceability of these results is rather high.

In the experiment shown in Figure 1, dehydration of a heart in a short period of time with only a small amount of shrinkage was achieved, but at the expense of 25 liters of acetone for only 500 grams of tissue. The first acetone bath (up to the end of the second day) removed approximately 35% of the organ's weight as water. The first acetone change in the heart represented by circles was made too early (on the steep part of the slope). Both acetone changes after three days came too early and the second bath removed far too little water. The third bath also was inefficient.

The heart experiment shown in Figure 2 was performed with a graded series of cold acetone. More appropriate timing of changes was conservative of solvent and only three baths were employed. The heart that was started in 80% acetone (circle) showed no further water extraction in this first bath during the second day. The heart started in 90% acetone (triangle) still lost 9% water during the second day. On days two and four, the inclination of the curve indicates that it would have been more economical to change the solvent about one day later. Gradual rewarming of the specimens at this stage, even without an acetone change, enhances dehydration but still holds shrinkage to a minimum. Since the curve of the specimen represented by a circle is not yet horizontal by day 9, a third change, into a smaller acetone volume, would have been necessary to get below 1% residual water.

As a general rule, the proper use of a graded dilution series of cold acetone extracts more water than starting with pure acetone (Figs 2, 3, 5, 6). Figure 3 shows that the third day is most advantageous for the first change. The second change should be two or three days later (Figs 3, 4). Warming the specimen to +5° should take one or two days (Figs 4, 5, 6). The second day at this temperature without an acetone change (Figs 3, 5) removes less than 2.5% water. The rate of water extraction will, of course, be influenced by the shape and consistency of the specimen. In Figures 4 and 5, for example, pelvic organs show alargepercentage of water (up to 60%) extracted in the first bath.

Dehydration of bulky specimens, in which water and solvent exchange must take place through a considerable mass of tissue, requires much more time than the process described above. Brain halves with water trapped below the meningeal membranes (Fig 6) required 24 days in cold acetone for near-complete extraction. This resulted in shrinkage of 2%. The many acetone changes certainly were not necessary. The ice core must have remained intact for more than one week. The sudden onset of shrinkage (5.2-6%) coincides with the specimens' being warmed to ambient temperature, at which time fat extraction begins. This can be avoided and will be discussed in a separate paper.

DISCUSSION

The purpose of this investigation was to establish a dehydration procedure that was most conservative of solvent and time but still resulted in minimal dimensional change and adequate dehydration. Water-extraction characteristics of various organs were examined and several principles defined. These should be considered recommendations rather than inviolable rules, however,

These should be considered recommendations rather than inviolable rules, however, because certain inherent limitations of measurement precluded complete accuracy.

One such measurement difficulty centers around determination of water actually extracted. For example, it is assumed that the amount of water leaving the specimen equals that of the acetone entering. In fact, the total specimen volume diminishes by about 5% during the first days and increases again later in the process. Also, use of the densitometric spindle is very temperature - dependent, as well as sensitive to the lipid content of the acetone bath. And the Karl Fischer titrimetric method is not absolutely reliable in the presence of 20-30% water. Although exact determinations of water extraction and shrinkage can be done on small, homogeneous tissue cubes (6), these will not accurately predict changes occurring in large specimens during this same process.

Using freeze-substitution, shrinkage can be kept below 10% (7), much less than that experienced with a graded series of alcohols at room temperature and quite acceptable for macroscopic specimens. In systematic experiments intended to define shrinkage (8), temperatures between -28 and -30°C were found optimal. Brain shrinkage increased noticeably at higher temperatures, beginning as low as -25°C.

Gradual rewarming is absolutely required for control of specimen dimension. Abrupt warming from -25°C to room temperature can lead to massive shrinkage from sudden water extraction. Thus, the dehydrated specimen should always be kept for one or two days at +5°C to permit slow water equilibration at this intermediate temperature before restoring it to ambient temperature.

The rate of p on large liver cubes and brain halves during freeze-substitution (mentioned above). Thus, when working with thick, bulky specimens, an acetone change after three days may be too early since penetration has been too sluggish to reach the central ice core. This notwithstanding, even large specimens (subjected to three bath changes) will have reached near-complete dehydration within 9 to 11 days, after which the final bath will not contain more than 1% water.

It seems, then, that dehydration time can be significantly reduced from the six weeks recommended in an earlier publication (10). Three changes of solvent remains the rule and an intermediate warming to +5°C should be considered essential. Given a tissue-acetone volume ratio of 1-5 and these revised recommendations regarding exposure time, acetone consumption can be safely reduced to almost one half and time to about one third when compared to previously accepted minimum standards (10X11).

An additional economy can be realized by re-using acetone. Although used acetone causes no damage, once it becomes diluted to 70% it is ineffective, except as a first bath. Based on our experience, we feel that the previously recommended dilution limit of 95% for an initial bath (11) is far to high. Cost can be further reduced if the final bath from a previous dehydration is re-used as a second bath.

REFERENCES

1. Bickley, HC ; Donner, RC ; Walker, AN ; Jackson, RL
Preservation of tissue by silicone rubber impregnation
J Int. Soc. Plastination 1:30-38, 1987.

2. Bullivant, J
Freeze-substitution and supporting techniques
Lab. Investl4:440-457, 1965.

3. v Hagens, G ; Tiedemann, K ; Kriz, W
The current potential of plastination
Anat. Embryol
175:411-421, 1987.

4. v Hagens, G ; Schwab K
In preparation.

5. Lischka, M ; Prohoda, M
Plastination of whole-body slices with polymerizing emulsion
J.Int. Soc. Plastination
1:17-22, 1987.

6. Sasse, D ; Matthaei C
Improvement of freeze- substitution by programmed rewarming
Stain Technol. 52:299-300, 1977.

7. Schwab, K ; v Hagens, G
Freeze substitution of
macroscopic
specimens for plastination
Acta Anat. 111:139-140, 1981.

8. Seki, M
Untersuchungen mit nichtwassrigen Flussigkeiten.
1. Fixierung Gewebe in nichtwassrigen Flussigkeiten.
Z. Zellforsch.
26:305-320, 1936

9. Sitte, H ; Neumann, K ; Edelmann, L
Cryofixation and cryosubstitution for routine work in transmission electron microscopy.
In: Muller, MR ; Becker, B ; Boyde, A; Wolosewick, JJ (eds.)
The science of biological specimen preparation.
SEM Inc., AMF O'Hare, IL 60666,
103-118, 1986

10. Tiedemann, K ; v Hagens, G ;
The technique of heart plastination
Anat. Rec.
204:295-299, 1982

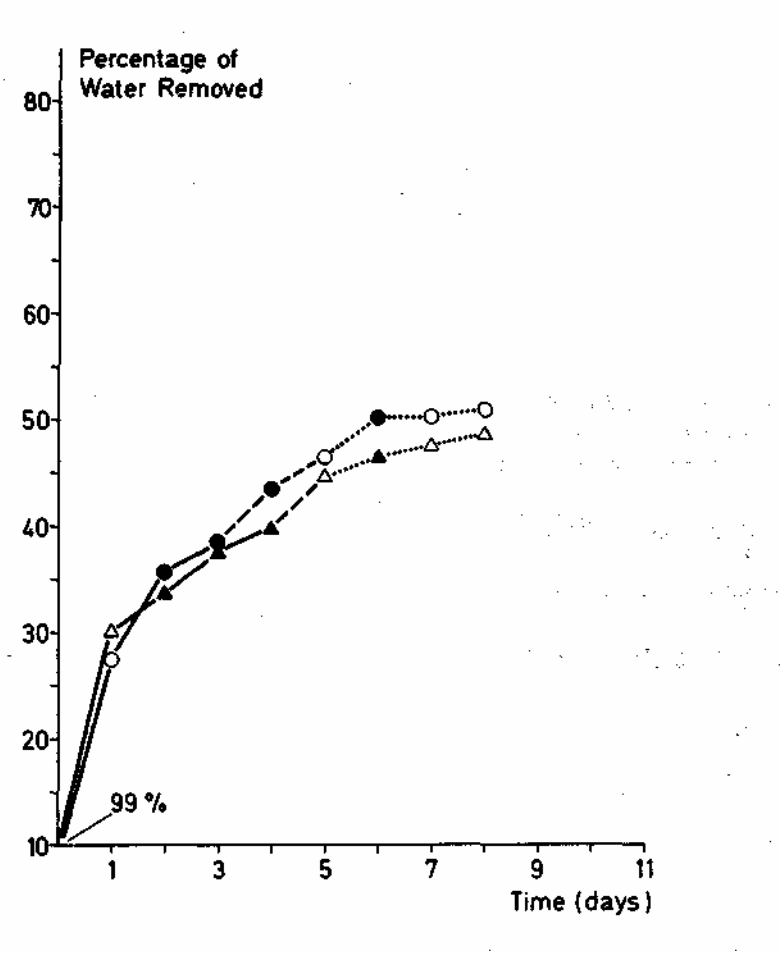

Percentage of
Water Removed
80
70
60
50
40
30
20
10
99 %
1
3
5
7
9
11
Time (days)

Figure 1

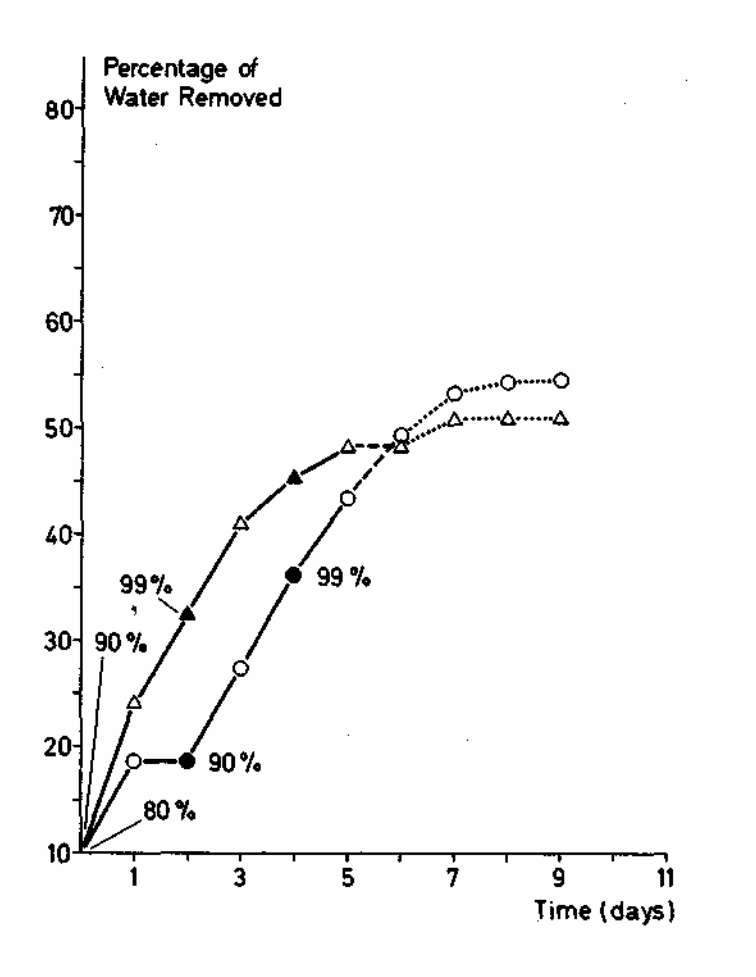

Percentage of
Water Removed
80
70
60
50
40
30
20
10
99%
90%
90%
99%
80%
1
3
5
7
9
11
Time (days)

Figure 2

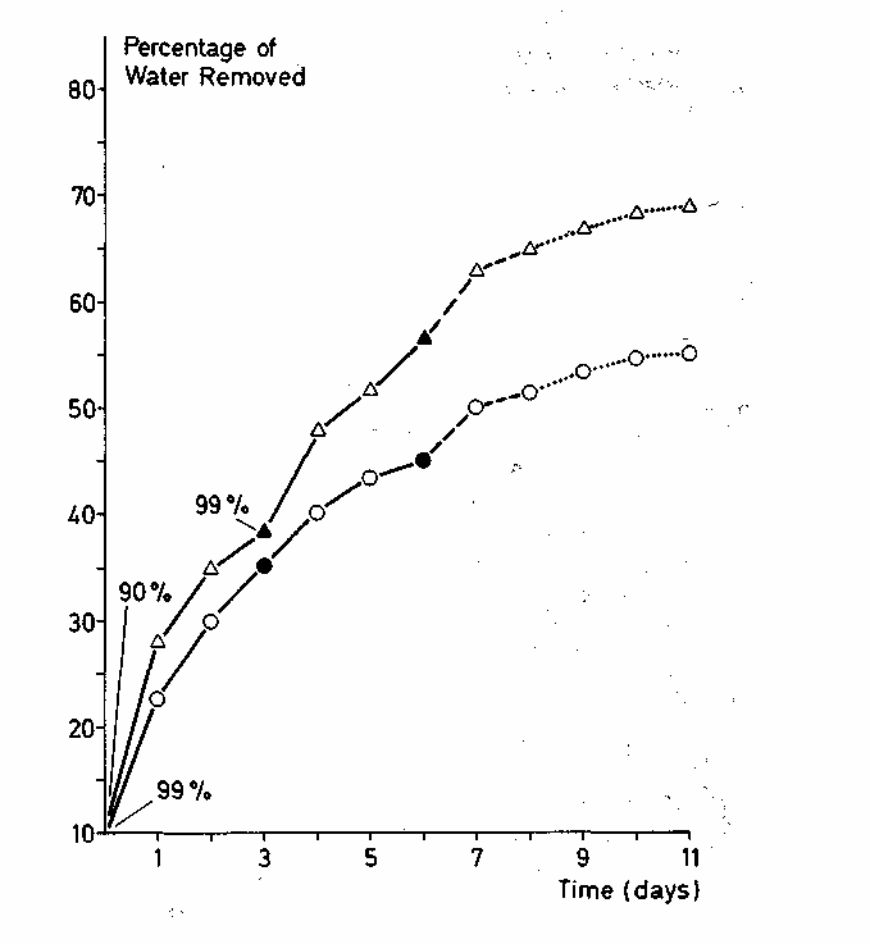

Percentage of
Water Removed
80
70
60
50
40
30
20
10
99 %
90 %
99 %
1
3
5
7
9
11
Time (days)

Figure 3

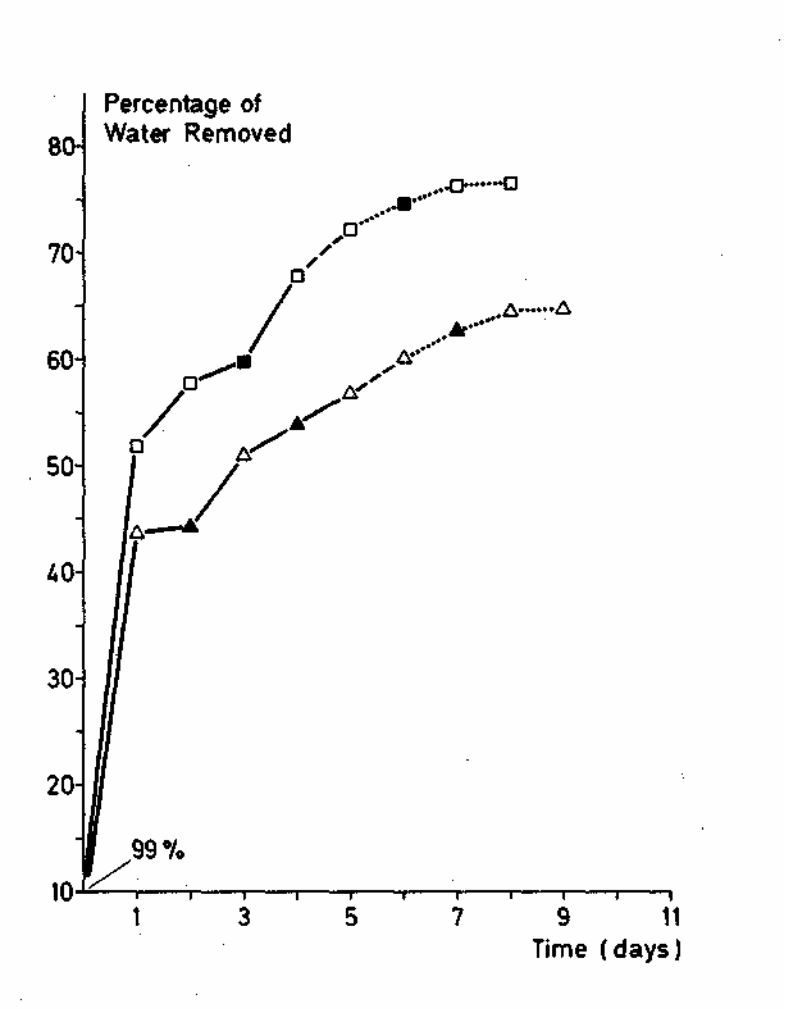

Percentage of
Water Removed
80
70
60
50
40
30
20
10
99 %
1
3
5
7
9
11
Time (days)

Figure 4

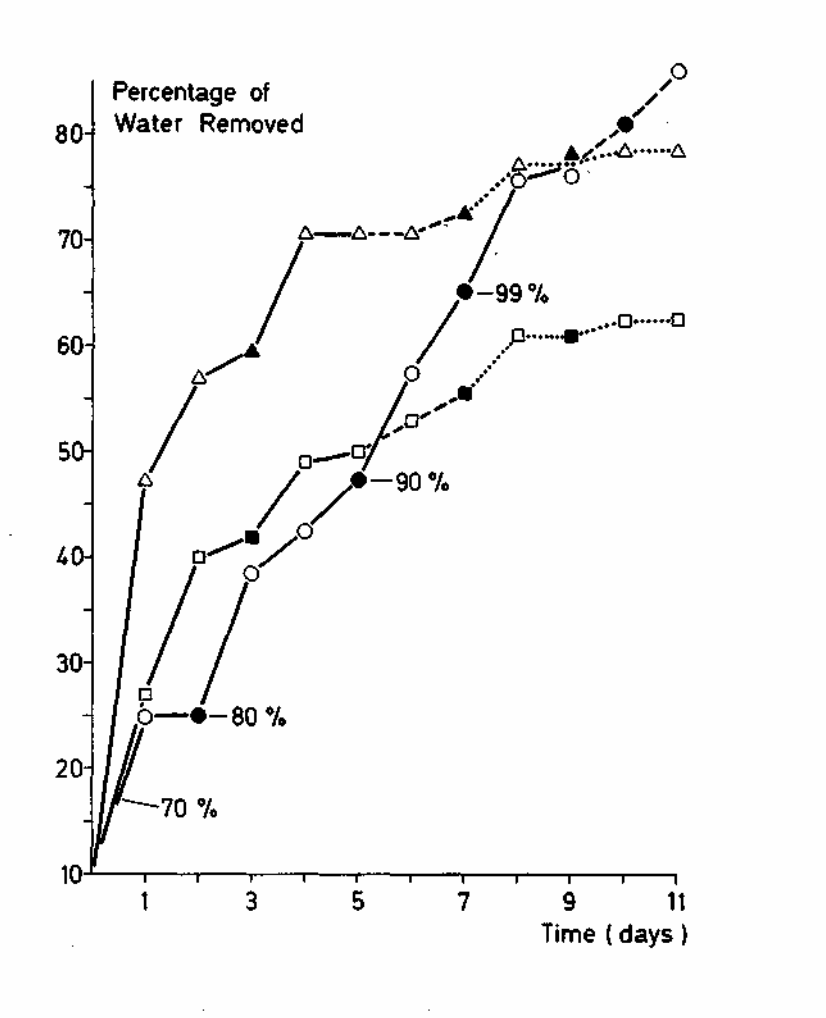

Percentage of
Water Removed
80
70
60
50
40
30
20
10
99 %
90 %
80 %
70 %
1
3
5
7
9
11
Time (days)

Figure 5

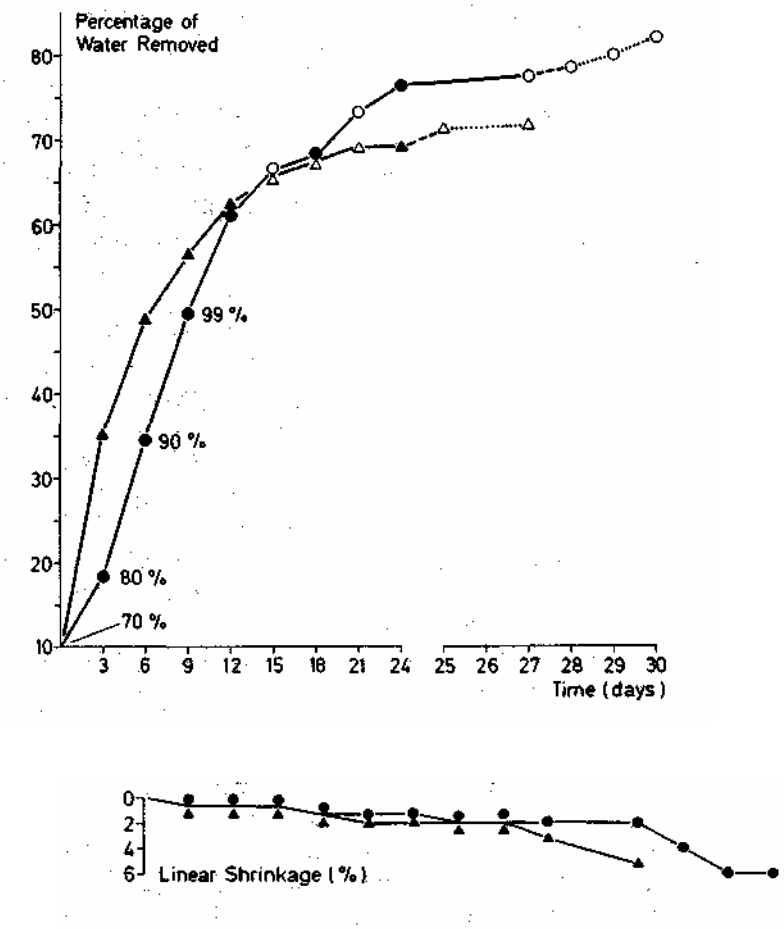

Percentage of
Water Removed
80
70
60
50
40
30
20
10
99 %
90 %
80 %
70 %
3 6 9 12 15 18 21 24 25 26 27 28 29 30
Time (days)
0
2
4
6
Linear Shrinkage (%)

Figure 6

J. Int. Soc. Plastination Vol. 2, No. 2, July, 1988 13

TECHNICAL COMMUNICATION: DNA PLOIDY ANALYSIS OF PLASTINATED TISSUE

H Frierson, AN Walker, RL Jackson, S Powell

University of Virginia Medical Center
Department of Pathology
Charlottesville, Virginia

and

Mercer University School of Medicine
Department of Pathology
Macon, Georgia

INTRODUCTION

DNA ploidy analysis by flow cytometry is gaining widespread use in the study of neoplastic disease. Although we have observed satisfactory preservation of light microscopic detail in plastinated tissue, we questioned whether plastinated neoplasms were still suitable for ploidy analysis.

MATERIAL AND METHODS

A representative sample was taken from silicone rubber-impregnated squamous carcinoma of the esophagus and a silicone rubber-impregnated adenocarcinoma of the colon. The samples were immersed in 5% sodium methoxide dissolved in methanol for 48 hours to remove the silicone polymer. They were then washed in fresh methanol and divided in half. One half of each sample was submitted for DNA ploidy analysis without further processing. The other respective halves were placed in cassettes and subjected to routine tissue processing on an automated tissue processor, whereafter they were embedded in paraffin. A 50 micron section from each of the two paraffin blocks was placed into separate glass centrifuge tubes and deparaffinized according to the technique of Hedley et al (1). Briefly, sections were dewaxed with xylene and rehydrated with decreasing concentrations of ethanol (100, 95, 70, 50%). Afterwashing the cells in distilled water, 1 ml of 0.5% pepsin (Sigma P-7012, St. Louis, MO) in 0.9% NaCl adjusted to pH 1.5 was added. Tubes were incubated in a waterbath at 37° for 45 minutes and vortexed intermittently. $IxlO^6$ cells were then placed into a DMSO-citrate buffer, frozen rapidly, and stored at -80°C,

The deparaffinized cells were prepared for cytometric analysis using the detergent-trypsin method of Vindelov (2). After staining with propidium iodide, nuclei were passed through a 70 um nylon filter and analyzed with an Epics C flow cytometer (Coulter Electronics, Inc., Hialeah, FL) equipped with a 5-watt argon laser. Excitation of propidium iodide occurred at 488 nm and the fluorescent emission was measured above 590 nm. Twenty thousand nuclei from each specimen were analyzed.

Non-neoplastic nuclei present in the paraffin blocks served as an internal diploid standard. Tumors were considered to be DNA aneuploid when another GO-G1 peak was present in addition to the diploid peak.

The DNA index was calculated as the ratio of the mean channel number of the GO-G1 peak for the neoplastic nuclei to the mean channel number of the GO-G1 peak for the diploid nuclei. The coefficient of variation (CV) was calculated for each GO-G1 peak using the half maximum-peak height.

RESULTS AND CONCLUSIONS

Both deplastinated, non-processed sections were unsuitable for flow cytometric analysis as the constituent cells resisted the disaggregating procedures. The deplastinated, processed and paraffin embedded material gave much more satisfactory results.

Figure 1 is a histogram of the nuclei from the esophageal squamous carcinoma, showing only a diploid peak. Figure 2 is a histogram of the nuclei from the colonic adenocarcinoma, showing both aneuploid and diploid peaks; the DNA index is 1.27. Both histograms contain abundant debris concentrated in the hypodiploid region. The coefficient of variation for each of the DNA diploid peak is less than 10%.

Figure 3 is a control histogram of the nuclei from fresh peripheral blood lymphocytes showing a single narrow diploid peak.

From this preliminary study we concluded that it is possible to perform subsequent ploidy analysis on plastinated tissues. Plastinated tissue does not appear to be optimal material for ploidy analysis, but can be used successfully if no other portion of a specimen is available.

REFERENCES

1. Hedley DW, Friedlander ML, Taylor IW, Rugg CA, Musgrove EA. Method for analysis of cellular DNA content of paraffin-embedded pathological material using flow cytometry. J Histochem Cytochem 31:1333-1335, 1983.

2. Vindelov LL, Christensen IJ, Nissen NI. A detergent-trypsin method for the preparation of nuclei for flow cytometric DNA analysis. Cytometry 3:323-327, 1983.

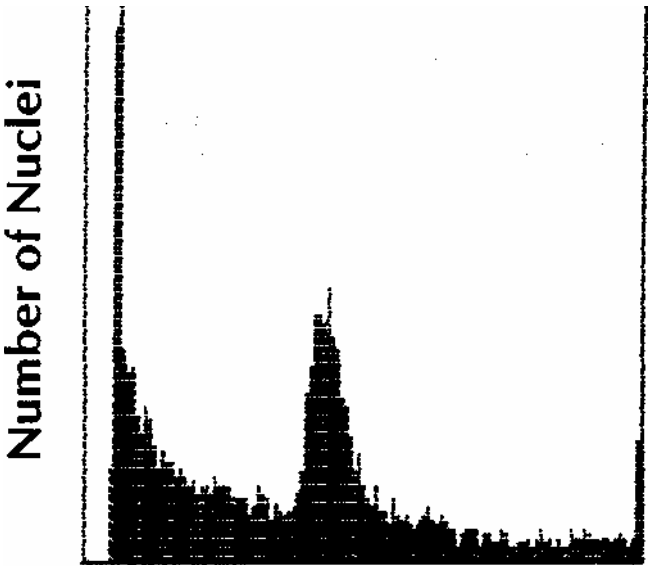


Figure 1

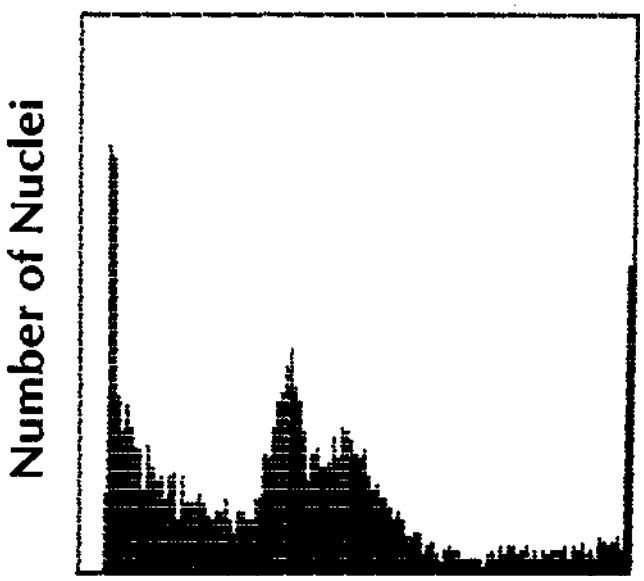


Figure 2

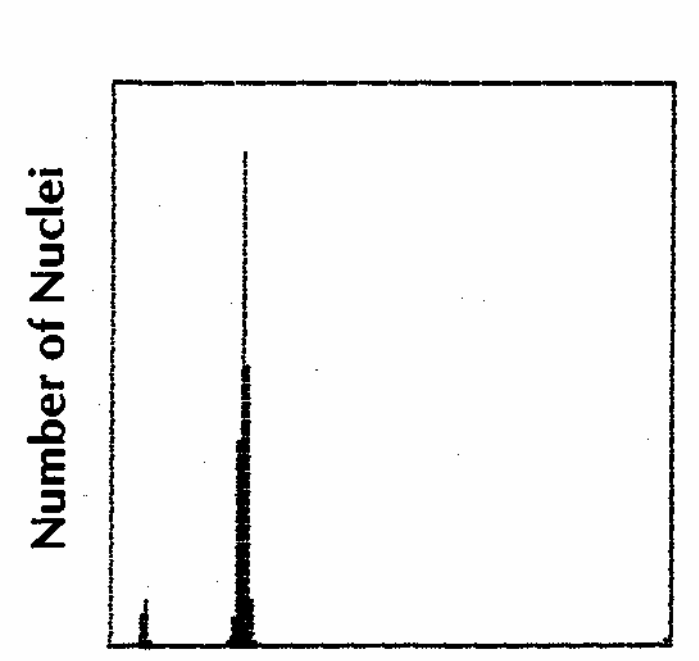


Figure 3

J. Int. Soc. Plastination Vol. 2, No. 2, July, 1988 17

EXPERIMENTS IN DEHYDRATION TECHNIQUE

Steven D. Holladay

Department of Anatomy, Physiology and Radiology
North Carolina State University
College of Veterinary Medicine
Raleigh, North Carolina 27606, USA

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

PLASTINATION OF THE HUMAN KIDNEY

Karine Oostrom
University of Utrecht
The Netherlands

and

Gunther von Hagens
Anatomical Institute I
University of Heidelberg
West Germany

INTRODUCTION

Plastination of the kidney is a moderately difficult undertaking. Perhaps not as challenging as the heart but somewhat more tricky than the placenta. In actual fact, each kidney seems to have its own way of tormenting you.

We will structure this paper as a "how-to-do-it" set of directions. Each step will be addressed in the order in which it is done. This should permit anyone wanting to undertake this project to simply follow the discussion and arrive at an acceptable specimen.

REMOVAL OF THE KIDNEY AND CANNULATION OF VESSELS

Let's start the whole procedure by taking the kidney out of the corpse properly. This is very simple. Just remember to be generous, liberal and careful, all at the same time. Kidneys prefer that their renal arteries and veins stay as long as possible. So cut these as close to the abdominal aorta and inferior vena cava as you can manage. Also, they prefer a generous ureteral remnant, ideally about 20 cm in length. Incidentally, do not deprive them of the shelter of their fat capsule, at least not yet.

Having done all this, we must prepare the kidney for removal of blood. To do so, it will be necessary to cannulate every artery you see. This may amount to just one vessel, if you are lucky. But two, or more, are not uncommon. We would advise that you cannulate the ureter and one of the veins while you're at it. These cannulas will be of no use yet but, since you'll require them later, you might as well get it over with. Try to do all of this without damaging the fat-capsule.

In Heidelberg we make our own cannulas from tubing of different diameter, all of which are derived from infusion systems. We cut these into pieces of about 5 centimeters. At the end of each piece we create a fine, rounded tip by cutting across the tubing obliquely and then trimming it round with a pair of scissors. This facilitates its introduction into a vessel.

REMOVAL OF BLOOD

We are now prepared to rinse out the blood. To do so, we connect all arterial tubing to the water tap. Don't attach the venous and/or ureter tubes by mistake. These cannulas will not be employed until color injection and fixation. Rinsing pressure is limited to 2 meters by the use of an overflow device. It is probably unnecessary to say this, but never, never rinse with warm water. This would only speed up deterioration and weaken the specimen. As rinsing progresses, the kidney will turn extremely pale, proof that the rinse is doing its job.

After about two hours of rinsing it will be really easy to remove the loosened fat-capsule from the kidney. This can be done best by "blunt dissection"; that is: with your fingers. Work from the hilus to the convex margin. Be sure to leave the underlying fibrous capsule intact and be alert for interesting structures hidden in the fat, like cysts. If, upon removing the fat capsule, you discover a pole artery or other accessory artery, you will find that the area of the kidney supplied by this vessel is not being rinsed. At this point, you must stop, cannulate this artery, connect it to the tap and continue. If you fail to do so, this part of the kidney will not receive proper color injection.

After two hours of rinsing via the main renal artery, these accessory arteries can be detected more easily. The problem is not to find them, it's how to get them cannulated. Most of them are too small to fit the infusion tubing. In Heidelberg we insert a piece of tubing with a smaller diameter into the infusion tubing, and tie a ligature around the whole thing to keep this insertion in place. A problem of this kind may demand patience and skill. It may even jeopardize your good mood. As everyone knows since Amundsen: poles are not easy to conquer. Anyway, once you have triumphed over the pole arteries, connect their cannulas to the water tap and rinse until the kidney is appropriately pale.

Another criterion to apply, in addition to the kidney's paleness, is the clearness of the rinse water coming out of the veins. It should not appear the least bit red. It will take about 4 hours to accomplish this. Also, it will save you a lot of blue resin spilling in later stages, if you take this opportunity to detect any leaking veins. Tie off all water-spraying veins except of course the cannulated one, and you'll be just fine.

COLOR INJECTION

And now, we have arrived at the most demanding, but also the most exciting, part of kidney plastination, color injection. Instruments and materials that will be needed for this are as follows:

- assuming that all cannulas have been inserted, we will not include them in this list (or did we just include them by not including them?)
- small hemostats for use in stopping leaks and occluding the cannulas at the end of injection
- syringes
- thread
- scissors
- red and blue epoxy injection mixtures
- acetone (for use as a cleaning agent)

Begin by preparing the blue mixture and injecting it into the vein. The constituents should be added in the sequence listed below and stirred thoroughly for at least 5 minutes. About 10-15 ml of this mixture will be needed per kidney.

BIODUR E 20 blue	100 parts per weight
BIODUR AT 10	20 ppw
MEK	10 ppw (methyl ethyl ketone)
BIODUR E 2	45 ppw

Although this mixture resembles the one used for the placenta, note that they are NOT the same. It is very important to be exact when you weigh these components. Also, be sure to wear gloves because the resin, especially the HARDENER E 2, is a rather strong allergen. It will turn your hands into large, fingered, red-pickled strawberries if you don't take precautions to protect them.

The injection should be carried out with a light, constant pressure in order to avoid extravasation of the resin. When about 5 ml of the blue resin has been injected, the kidney begins to show a blue tint. Now press the kidney softly and squeeze it a little bit, so as to massage the blue resin through the veins. Inject some more and massage it again. When you see some small, star-shaped venous patterns at the surface, you probably have injected sufficient blue polymer. A good rule of thumb is to quit

when you can count about 5 of these so-called "stellata" veins at the surface. At this point, you should bend the cannula double and clamp it with one of the hemostats.

Next, of course, is arterial injection. But don't forget to protect your valuable kidney from drying whilst you mix the red resin. You might submerse it in water and cover it with a wet cloth. The red injection mixture consists of:

BIODUR E 20 red 100 parts per weight 20
BIODUR AT 10 ppw 20 ppw 45 ppw
MEK BIODUR
E 2

You'll need about 25 to 30 ml of this mixture per kidney. Again, it is important to weigh the components accurately, add them in the order given, and mix them thoroughly.

If you have detected any accessory arteries, you ought to inject them first. There are three reasons for this:

> Accessory arteries are difficult to inject. In fact, you may fail to get any resin into them at all. If so, you should know this at the beginning so you can decide whether to continue.
>
> You'll have to watch only a small area to arrive at the right reddish color; it's easier to adjust the rest of the color of the kidney to this area than the other way around.
>
> There may be more, yet-undiscovered pole-arteries. It is easier to discover these if you inject the pole arteries you know about first, and the rest of the kidney later.

The arteries should be injected with red resin until the kidney surface shows uniformly distributed red spots. The most serious problem encountered while injecting a kidney with more than a single artery is that the area supplied by one artery is difficult to match to the area supplied by another. It's rather troublesome to get both areas equally red. You'll need to employ a lot of "Fingerspitzengefuehl" to get the balance just right. Once we have finished arterial injection we bend the tube and clamp it. Only then is it permissible to lean back and admire the beautiful kidney you have just created.

FIXATION

And so we've arrived at fixation! It is important to carry out fixation, without delay, after color injection. In fact, it should be done the same day. The reason for this is that fixation should start before the colored resin becomes cured within the kidney or it will be impossible to use fixation as a means of dilating the renal pelvis. Fixation of the kidney is done in two steps, dilation-fixation and immersion.

DILATATION-FIXATION: This is performed with 20% formalin, flushed through the kidney via the ureter. To do so we place a container, filled with this rather strong solution, about 2 meters above the specimen, thus creating a constant hydrostatic pressure for dilatation. The advantage of using such a concentrated solution of formalin is that it will quickly provide a very firm fixation. We can use a percentage this high only in the dilatation technique, i.e., the organ needs to be hollow and must be under pressure. If we were to use 20% formalin for simple immersion fixation, we would create a thoroughly fixed outer layer that would act as a diffusion barrier for the rest of the fixative. Penetration would be inhibited and the inner tissue would not become fixed.

IMMERSION: After 1 day of dilatation-fixation, we begin immersion. Put the kidneys in a 5% solution of formalin and diffusion from the inside and the outside will take care of the rest. Since we have created a concentration gradient between the inner tissue, where we deposited 20% formalin, and the outer tissue, which is surrounded by 5% formalin, fluid will be propelled from the immersion bath into the kidney.

There are two places in the process of kidney plastination, at which one might divide the kidney into halves. The one you will choose depends on the equipment you have to accomplish this. If you do NOT have a band saw, and will have to do this with a knife, your perfect moment has arrived. One day after the start of fixation the tissue will be firm enough to retain its shape, but not too firm to cut. Immersion fixation will take about 3 weeks. If you want to store the kidneys for a while, there are no objections to leave them in the 5% formalin slightly longer.

DEHYDRATION

By the time a kidney is colored and fixed, it usually gets very eager to become dehydrated. Since we have grown fond of this kidney, we will subject it to a sophisticated process called, freeze substitution. Rather than repeat what will be contained in another paper, We refer the reader to the article by Prof. Klaus Tiedemann that will appear in this very same issue.

FORCED IMPREGNATION

Actually, forced impregnation is what plastination is all about. If you are familiar with the Heidelberg Plastination Folder, you'll know how to do it. If you are not, you should plan to get a copy and read all about this step. Impregnating one kidney will consume between 100 and 250 grams of resin. But, of course, you will need much more than that to immerse it properly.

CURING

Curing of the kidney is carried out in much the same way as curing of any other organ impregnated with S 10. We can do no better than to recommend the Heidelberg Plastination Folder for the details of this step as well.

You will remember that we mentioned that the proper time for cutting the kidney into halves with a knife is one day after the beginning of immersion fixation. If you use a band saw, the proper time is after about 9 or 10 days of curing. Having cut the kidney, you will see the result of your unflagging industry. The splendor and charm of the renal pelvis will shine upon you and your collegues will flock to admire your artistry.

ACKNOWLEDGEMENTS

The first author would like to acknowledge that without:

- Prof. Pirn van Doorenmaalen
- Dr. Gunther von Hagens
- the financial support of
  Fonds Dr Catharine van Tussenbroek
- Prof. Harmon Bickley
- and my husband Bas

we would never have been able to bring you this paper and many kidneys would go unplastinated. Thank you, one and all.

J. Int. Soc. Plastination Vol. 2, July, 1988 25

SPECIAL FEATURES AND ADVANTAGES OF FREEZE-DRYER PLASTINATORS

Steve D. Holladay

Department of Anatomy, Physiology and Radiology
North Carolina State University
College of Veterinary Medicine
Raleigh, North Carolina 27606, USA

INTRODUCTION

Anatomists at the College of Veterinary Medicine at North Carolina State University first came into contact with plastinated specimens in late 1983 and immediately decided to incorporate the technique into their teaching program. As has been the case with many other plastinators, the first Step was to attend the next, regularly scheduled International Conference on Plastination (San Antonio, 1984). It was soon evident that the greatest difficulty to overcome for any beginning plastinator is the building of the actual plastination unit.

Following the Conference, Dr Harmon Bickley, of (at that time) The University of Texas Health Science Center at San Antonio, was called and asked a few questions about the unit being used there. Probably the most influencing bit of Information passed on by Dr Bickley was the comment that he would try to find an old freeze-dryer vacuum chamber if he was starting again to assemble such a unit. This was reasonable in that a freeze-dryer chamber is specifically built for vaccum. It is cylindrical (for greatest strength), well-iealed and fitted with a gasket and a heavy door.

It was then realized that the cooling System of a freeze-dryer could be used since it is specifically intended to maintain the temperatures needed for plastination. Also, a freeze-dryer comes equipped with a vacuum pump, capable of reaching the 5 microns of pressure desirable in plastination.

It gradually became apparent that there would be no need to disassemble a freeze-dryer to build a plastinator. All that was necessary to convert one to the other was to simply add a valve that could be used for vacuum cpntrol. A bonus not anticipated at the time was that, having done this, the freeze-drying capability of the machine would still be intact.

Having used a commercial freeze-dryer (Virtis Co. Model 220-PR) as a plastinator for the past three years, the author feels that scientists at this institution have an unusual opportunity to communicate Information that might be helpful to others who are either starting plastination or considering a change of equipment. The purpose of this article is to discuss the Special features and advantages of the freeze-dryer plastination apparatus.

OBSERVATIONS

1. Commercially available freeze-dryers come equipped with vacuum pumps of sufficient strength and capacity for plastination and have refrigeration Systems intended to hold the temperatures needed.

2. The only modification needed to give a freeze-dryer plastinating capability is the addition of a valve to the vacuum line. (Our

plastinator was "built" in 1/2 hour at a cost of $23.00).

3. Many individuals planning to start plastination have access to freeze-dryers and need not purchase a new set of equipment. For example, plastination was begun without delay by the Animal Science Department of this institution because a freeze-dryer was available and not being used. State and university property storage agencies often are able to provide surplus freeze-dryers (sometimes without charge). These may be as good as new or in need of minor repair. Even vacuum pumps are common surplus items.

4. Freeze-dryers (and plastinators) are are commercially available. These units are professionally assembled and guaranteed. Such machines cost about the same as might be spent assembling a more makeshift apparatus and often have a larger vacuum chamber. Acquiring a commercially available machine often results in a substantial savings of time and effort. Table 1 is provided as a means of comparing cost and capacity.

5. Commercially available freeze-dryers are easily modified to a top-load position if desired or, alternatively, it is not expensive to fabricate an aluminum box to fill the available space. The box used at this institution cost $80.00 and has a volume of about 2 cubic feet. It, has proven quite serviceable.

6. Most commercially available plastination devices have vacuum chambers of 2-4 cubic feet. Although fully capable,of serving as a, primary infiltration apparatus, such units also make ideal secondary, plastinators that can be devoted exclusively to an advanced process such as sheet plastination.

7. Freeze-dryer-derived plastinators take up little floor space, a major concern in most laboratories. The unit used by the author occupies a space only 24x34 inches (less than 6 square feet) and still has a 3.5 cubic-foot vacuum chamber (in the form of a cylinder 18 inches in diameter and 24 inches deep). Another consideration is that they also are equipped with wheels and can be easily moved.

8. A converted freeze-dryer serves not only has an exceptional plastination unit (The one used by the author has operated continuously for 3 years without a single problem.) but also provides a second major function for no additional cost. The author has freeze-dried many anatomical specimens, as well as several rattlesnakes, turtles, birds and small mammals WHILE plastinating laboratory specimens.

9. A major advantage of the converted freeze-dryer; is that the ice sludge which tends to form on the surface of the impregnation polymer is no longer a problem. A freeze-dry unit removes and traps moisture. This is due to the only real difference between a freeze-dryer and a plastinator, the incorporation of a condensing chamber. This condenser usually operates at -55 to -60 degrees Centigrade and accumulates the sublimated water. Impregnation is usually begun with the condenser turned off and the freeze-dryer functioning as any other plastinator. If turned on early during impregnation, the condenser will accumulate acetone, unless pressure is reduced very slowly. After the first few days, the condenser is turned on and begins to scavenge water. Not only does this keep the silicone impregnation mixture cleaner it also permits impregnation with less perfect specimen dehydration.

10. Even the least expensive freeze-dryers have lighted vacuum chambers, making bubble monitoring very easy. ,,, There is no need to open a freezer to view the impregnation since the heavy acrylic door is completely transparent. Due to the depressed solvent activity of acetone at this very low temperature, the door is not affected by its vapor. The unit used by the author shows no sign of solvent damage after 3 years of continuous use.

SUMMARY

The typical freeze-dryer can be considered a plastinator with a condenser added. The presence of the condenser, although not essential for plastination, actually improves the performance of the unit as a plastinator. In fact, with the addition of a valve for vacuum control, the freeze-dryer becomes a superior plastination apparatus. Freeze-dryers are often available in departments considering the establishment of a plastination laboratory. They also may be found in state or university surplus warehouses. The author and his colleagues would not hesitate to recommend the purchase of a new freeze-dryer or plasatinator in lieu of collecting and assembling separate components. This may amount to a considerable savings in money, time and effort.

TABLE 1.
SPECIFICATIONS OF COMMERCIALLY AVAILABLE
FREEZE-DRYER/PLASTINATORS*

Chamber Size (dia x length, inches)	Chamber Volume (cubic feet)	New Cost ($ American)
15 x 14	1.4	3,415
24 x 24	6.3	7,870
36 x 66	38.9	16,700

The first unit is abit larger than some in current use which cost almost twice as much to assemble. The second is suitable for general use. The third has a chamber large enough for four standing adults and would probably tempt only a few beginning plastinators.

J. Int. Soc. Plastination Vol. 2, No. 2, July, 1988 28

A STATE-OF-THE-ART EMBALMING AND AUTOPSY STATION

Arthur Rathburn, LFD

Department of Anatomy and Cell Biology The
University of Michigan
3626 Medical Science II Ann
Arbor, Michigan 48109-0010

INTRODUCTION

As part of a complete renovation of the anatomical morgue facility at The University of Michigan, a new embalming station was planned by the author and a colleague. The unit was built by the Lipshaw Corporation of Detroit, Michigan. It was intended to do the following:

1. protect the operator from formaldehyde fumes liberated by embalming fluid
2. allow rapid disposal of waste materials, tissue and fluids
3. permit interchange of bodies with minimum effort

In addition, it was felt that certain other features were desirable:

1. a hydro-aspirator to remove fluids
2. a hand sink for convenient wash-up
3. a platform to hold the embalming machine in a convenient location
4. distilled water and embalming fluid, piped in from bulk storage containers
5. an instrument drawer and instrument stand
6. water-proof electrical outlets at both the head and foot of the body
7. adequate lighting

Routine use has shown that this system fulfils the basic requirements and accommodates all of the additionally desirable features.

RINSING AND DRAINAGE

The station is composed of two sinks with an intervening platform for the embalming machine and a tall splash shield to deliver supporting utilities. The left sink receives drainage from a specially-designed body tray. During embalming, the walls of this tray are continually rinsed by encircling water jets. Drainage is conducted to a heavy-duty waste grinder in the sink. A hydro-aspirator is mounted at the left of the sink and drains into it via polyethylene tubing.

The right sink is a deep handsink. An eye-wash station is installed on the splash shield above it and a power spray hose is mounted in front. A removable, stainless steel perforated panel (24" x 36") is included and can be placed over this sink if additional work area is needed.

Another panel of identical dimensions is located between the sinks and supports the embalming machine. Located on the splash shield above the embalming machine are two special taps. One is connected to a stainless steel line that delivers embalming fluid from bulk storage in another room. The other is connected to a distilled water line. Embalming fluid can thus be mixed directly and conveniently in the reservoir of the embalming machine. A waterproof electrical outlet is located in this area of the splash shield to supply power to the machine. Directly beneath the embalming machine is the instrument drawer. This location has proven quite convenient for the operator.

FUME PROTECTION

The second major component of the embalming station is the tray support. This is essentially a stainless steel rack installed perpendicular to the sinks. It incorporates five rollers on each side, intended to receive a body tray from a mobile carrier. The edges of the tray are formed into a reinforced flange and support the weight of the body and the tray on the rollers.

Two exhaust ducts, 1 inch in width, are installed along the entire length of the tray support and join beneath the tray. Contaminated air is conducted under and behind the sinks, where it is vented to the outside via the exhaust system for the building. Because formaldehyde vapors are more dense than air (formaldehyde = 1.067 x air), the intake openings were positioned lateral to the body. Vapors are thus drawn off before they drift past the face of the operator. This is particularly important considering the possible carcinogenic effect of formaldehyde.

WATER AND ELECTRIC SERVICE

Also installed in the tray support are two cold-water tubes extending the entire length of the body tray. These tubes, one on each side, are equipped with a series of water jets, spaced 4 inches apart. They are supplied through flexible hoses from the sink, equipped with quick-disconnects. The water tubes are hinged and may be operated in a raised or lowered position, as needed.

Electrical outlets are located on both sides of each end of the tray support for operation of hair clippers, Stryker saws and other electrical instruments. An instrument stand is mounted on the tray support near the sink and swings out, over the body for easy access. A large fluorescent light fixture, positioned directly over the body, provides illumination.

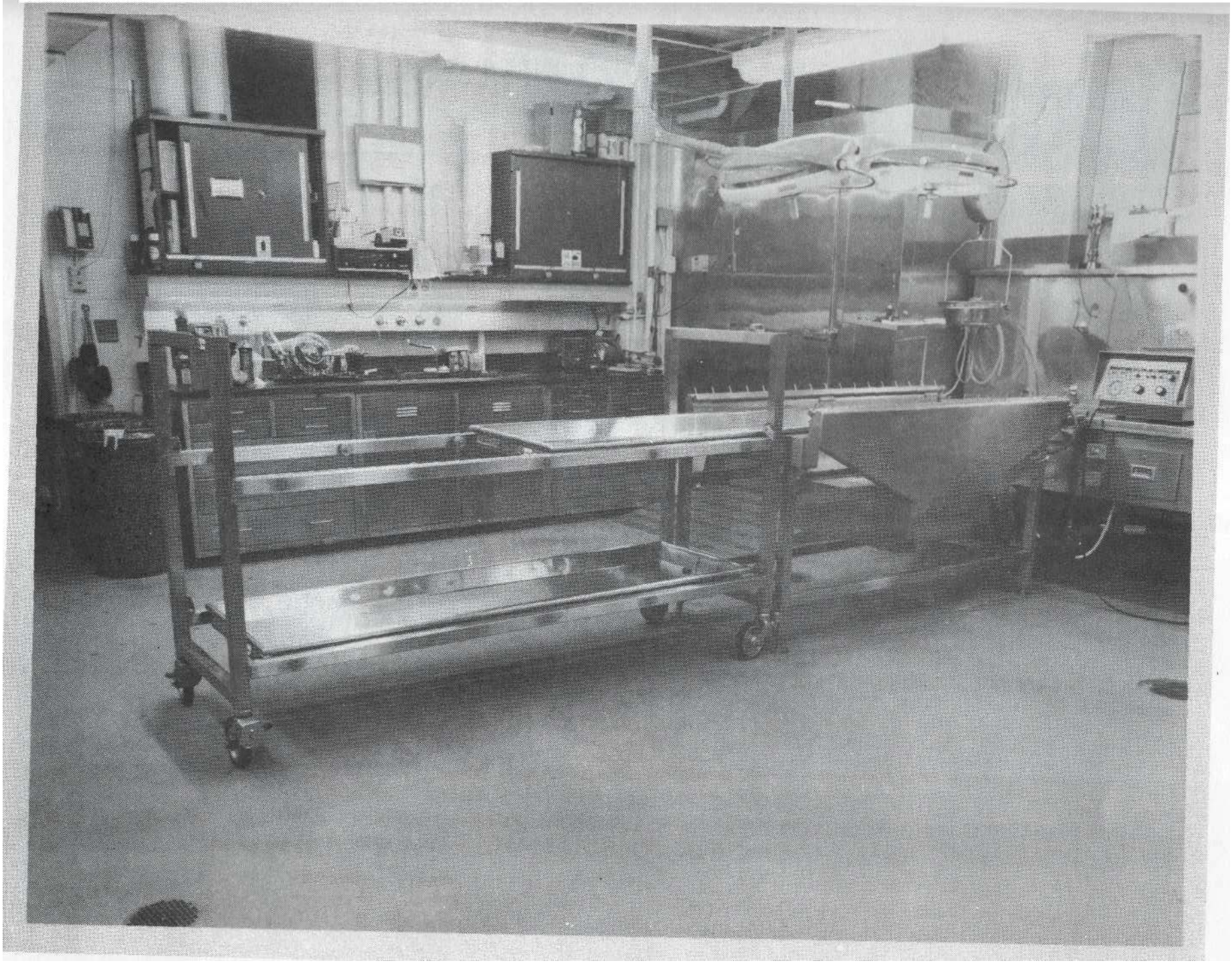
EMBALMING PROCEDURE

A two-body, mobile carrier is used to transport the body to the tray support. The water jets are raised and the tray, containing the body, is rolled onto the stand and clamped in place. The water jets are lowered, taking care that none of their openings are blocked by the body. Rinse water is then turned on for the duration of the procedure.

The body is cleaned, shaved and prepared for embalming. The embalming fluid tap is opened and a measured amount of fluid is run into the embalming machine. This is followed by an appropriate amount of distilled water. The body is then embalmed and allowed to drain overnight with the rinse water running in both the tray and the sink. The following day, latex is injected and the body tray rolled back onto the mobile carrier and taken to the storage room. Here, it is rolled onto the tray support of the cadaver lift, moved to an appropriate position in our mortuary rack and rolled into place for storage.

CONCLUSIONS BASED ON OUR EXPERIENCE

The University of Michigan processes about 300 cadavers per year to supply the needs of the medical and dental teaching programs, as well as other local academic institutions and hospitals. Before acquiring this new equipment, autopsy and embalming was considerably more difficult and inconvenient. Also, the institution of adequate safety procedures was virtually impossible. Three features of this new station have proven most important in alleviating these difficulties. The new transport system permits the body to be positioned on the same tray from the time it is received until it is placed in storage. This represents an enormous reduction in physical labor. The hinged, bilateral rinse-jet system permits continual flushing, thereby keeping the work area clean and sanitary. And, finally, the laterally positioned fume-exhaust ducts remove formaldehyde and other noxious vapors before they rise to expose the operator.



MEMBERS OF THE INTERNATIONAL SOCIETY FOR PLASTINATION

Dr. Santiago Aja-Guardiola
Department of Anatomy
Facultad De Med. Vet. y Zoo.
Univ. Nacional Autonoma de Mexico
C.P. 04510 Mexico, D.F.

Barbieri Maria Antonietta
c/o Guerci
University La Sapienza
Via Berengario 11
00162 Rome, Italy
(0039-984) 862673

Carlos A.C. Baptista, M.D.
Medical College of Ohio
Department of Anatomy
C.S. 10008
Toledo, Ohio 43699 USA
(419) 381-5256

Timothy R. Barnes
Ohio Univ Coll of Osteopathic Med
#135 Grosvenor Hall
Athens, Ohio 45701 USA
(614) 593-1800

Russell Barnett
University of Otago Medical School
Department of Anatomy
P.O. Box 913
Dunedin, New Zealand
740-062 ext. 669

Andrea Bassi
University of Rome "La Sapienza"
Via Alfonso Borelli
50 - 00161 Roma
Italy
ROME 492413

Al Batata, M.D.
Wright State Univ Sch of Medicine
Department of Pathology
Dayton, Ohio 45435 USA
(513) 873-2037

Harmon Bickley, PhD
Mercer University School of Medicine
Department of Pathology
1550 College Street
Macon, Georgia 31207 USA
(912) 741-2390

Dr. Colin M. Bloor
Department of Pathology M-012
UCSD School of Medicine
LaJolla, California 92093 USA
(619) 534-4324

Dr. Wernfried Boese
Findorffstrasse 5
D-2862 Worpswede
West Germany
04792-1489

Ewald O. Brune
Anatomy Department
Faculty of Medicine, Univ of Pretoria
P.O. Box 2034
Pretoria 0001, South Africa
011-3254000

Alan W. Budenz, DDS
Univ of the Pacific Sch Dentistry
Department of Anatomy
2155 Webster Street
San Francisco, California 94115 USA
(415) 929-6573

Claus D. Buergelt, DVM, PhD
Dept Comparative/Exp. Pathology
College of Veterinary Medicine
University of Florida
Box J-145, JHMHC
Gainesville, Florida 32610 USA
(904) 392-1221

Lily P. Cabellon, M.D.
Los Angeles College of Chiropractic
P.O. Box 1166
Whittier, California 90609-1166
(213) 947-8755 ext. 222

William R. Collins
UCSD School of Medicine, M-011
OLR-Anatomical Prep.
LaJolla, California 92093-0611
(619) 534-4126

Philip B. Conran, DVM, PhD
Medical College of Ohio
Department of Pathology
C.S. 10008
Toledo, Ohio 43699 USA
(419) 381-5256

Leo Cosio
Medical College of Pennsylvania
Department of Anatomy
3200 Henry Avenue
Philadelphia, Pennsylvania 19129 USA
(215) 842-4645

Grant Dahmer
University of Texas Health Science Center at San Antonio
7703 Floyd Curl Drive
San Antonio, Texas 78284-7762
(512) 567-3900

Gilles Desraisses
26 rue Santerre
75012 Paris, France
F-Paris-43460227

James F.P. Dixon
Department of Pathology
USC School of Medicine
2025 Zonal Avenue
Los Angeles, California 90033 USA
(213) 224-7479

Robert S. Donner, M.D.
Mercer University School of Medicine
Department of Pathology
Macon, Georgia 31207 USA
(912) 744-2560

Paul C. Engen, DDS
Loma Linda University
School of Medicine
Department of Anatomy
Loma Linda, California 92350 USA

Dean S. Folse
Department of Pathology
University of Texas Medical Branch
Galveston, Texas 77550 USA
(409) 761-2870

Larry E. Freeman, D.V.M.
Department of Veterinary Biosciences
Virginia-Maryland Regional College of Veterinary Medicine
Blacksburg, Virginia 24061
(703) 951-7447

Terry Frick, M.D.
305 S. Glover
Urbana, Illinois 61801 USA
(217) 244-1326

Dr. Paul O. Garcia-Torres
Professor of Anatomia Humana
U.N.A.M.
Corregidora #5 Dept. 24
Colonia Allpes
C.P. 01010 San Angel, D.F.
Mexico

Yvonne W. Goh
University of Rochester Medical Ctr
Department MDL, Box 709
575 Elmwood Avenue
Rochester, New York 14642 USA
(716) 275-4555

Alan R. Greenhalgh
Univ Birmingham Medical School
Department of Anatomy
Vincent Drive
Edgbaston, Birmingham
B15 2TJ England

Gilles Grondin
Dept of Anatomy & Cell Biology
Faculty of Medicine
University of Sherbrooke
Sherbrooke, Quebec
Canada J1H 5N4
(819) 564-5271

Dr. M.R. Haffajee
P.O. Box 201
Desainagar 4405
South Africa, 031-8202195

Lee C. Haines, Jr.
University of Washington
H.S.B., S-B, 56
Seattle, Washington 98195 USA
(206) 545-1188

Robert W. Henry,DVM, Ph.D.
P.O. Box 1071
University of Tennessee
College of Veterinary Medicine
Knoxville, Tennessee 37901-1071
(615) 546-9230 ext. 328

Steven D. Holladay
North Carolina State University
School of Veterinary Medicine
Department of Anatomy
4700 Hillsborough Street
Raleigh, North Carolina 27606 USA
(919) 829-4381

John H. Holliman, M.D.
Department of Pathology
Biomedical Sciences Bldg, Rm 434
P.O. Box 26901
Oklahoma City, Oklahoma 73190 USA
(405) 2717-2693

Godwin Nwachukwu Isitor
Department of Veterinary
Preclinical Sciences
Faculty of Medical Sciences
The University of West Indies
Trinidad, West Indies
809-645-2942

R. Lamar Jackson, Path. Asst.
Mercer University School of Medicine
Department of Pathology
Macon, Georgia 31207 USA
(912) 744-4062

Bharat S. Jadon
Division of Anatomy &
Experimental Morphology
McMaster University
1200 Main Street West
Hamilton, Ontarion Canada L8N 3Z5
(416) 525-9140 ext. 2271

Robin R. Jones, M.D.
Slot 517 - Dept of Pathology
Univ of Arkansas Medical Sciences
4301 W. Markham
Little Rock, Arkansas 72205 USA
(501) 661-5115

Geoffrey David Keye, MB, FRCSI
Anatomy Department
Trinity College Dublin
Dublin 2 Ireland 772941

Michele A. Keeling
Department of Anatomy, Cell
Biology and Neuroscience
Oral Roberts University
School of Medicine
7777 South Lewis Avenue
Tulsa, Oklahoma 74171

Kirksville Coll Osteopathic Medicine
Department of Pathology
800 W. Jefferson
Kirksville, Missouri 63501
(816) 626-2361

Hans-Ulrich Klatt
Ringstr. 6
D-5483 Heimersheim
West Germany

Carlos Kordjian
Flinders University of S.A.
School of Medicine
Depts. Anatomy & Histology
Bedford Park 5042
South Australia
275-9911 ext. 4261

Doug Krauskopf
University of Texas Health Science
Ctr at San Antonio
7703 Floyd Curl Drive
San Antonio, Texas 78284
(512) 567-4000

Richard Otto Krumins
Murdoch University
Sch of Veterinary Studies, Anatomy
South Street, Murdock
Western Australia, 6150
332-2134

Dr. Alex Lane
Triton College
2000 N. 5th Avenue
River Grove, Illinois 60171
(312) 456-0300

Arthur J. Lazik, M.D.
18350 Roscoe Blvd.
Northridge, California 91325 USA
(818) 993-8115

Dr. Martin Lischka
Institute of Anatomy
University of Vienna
Währingerstrasse 13
A-1090 Wien Austria
0222 (Vienna) 43-15-26

Jacob Malin, M.D.
Dept of Pathology and Lab Medicine
University of Nevada Sch of Medicine
124 Savitt Medical Sciences Bldg
Reno, Nevada 89557 USA
(702) 784-4068

Dr. Gilbert C. Mangubat
Dept of Anatomy, Faculty of Medicine
Unversity of Calgary
3330 Hospital Drive, NW
Calgary, Alberta
Canada T2N 4N1
(403) 270-7188

Dr. Jorge R. Martinez-Galindo
Calle Tecai #45
Col. San Buenaventura
14610 Tlalpan D.F.
Mexico
573-67-74

William F. McNary, Jr., PhD
Department of Anatomy
Boston University School of Medicine
80 E. Concord Street
Boston, Massachusetts 02118 USA
(617) 638-4166

Lawrence M. McNiesh, M.D.
14705 Lake Terrace
Rockville, Maryland 20853 USA
(301) 774-3400

Prof. P.P.C. Nel
Department of Anatomy
Faculty of Medicine
University of the Orange Free State
P.O. Box 339
Bloemfontein, 9300 South Africa
051-4707441 x 400

John Nettum
Pathology and Laboratory Medicine
208 Medical Sciences Building
Texas A&M University
College Station, Texas 77843-1114
(409) 845-3666

Karine Oostrom
Oisterwyksebaan 86
5018 CC Tilburg
The Netherlands

Mr. Gerhard Penz
University of Toronto Banting Inst.
Department of Pathology
100 College Street
Toronto, Ontario M5G 1L5
Canada
(416) 978-2545

Peggie L. Phillips
Life Chiroporactic College - West
Dept Chair, Anatomy and Chemistry
2005 Via Barrett
San Lorenzo, California 94580
(415) 276-9013

Richard A. Pollock, M.D.
1938 Peachtree Road, NW, Suite 601
Atlanta, Georgia 30309 USA
(404) 843-0960

Stephan M. Probst
Langenbergstrasse 23
D-6799 Haschback/RMBG
Federal Republic of Germany
06381/ 2717

P.T. Purinton, DVM, PhD
College of Veterinary Medicine
University of Georgia
Athens, Georgia

Arthur L. Rathburn
Professional Mortuary Service
P.O. Box 657
Ann Arbor, Michigan 48105
(313) 483-4653

Alfred Riepertinger
Institute of Pathology
Städtisches Krankenhaus
München-Schwabing, Koelner Platz 1
8000 München 40
West Germany
089-3068-484

Maurizio Ripani, M.D.
University of Rome "La Sapienza"
Via Alfonsa Borelli
50 - 00161 Roma Italy
ROME 492413

Michael Smith
Colorado State University
Dept of Anatomy & Neurobiology
Ft. Collins, Colorado 80523
(303) 491-5532

Dr. Elinor Spring-Mills
SUNY Health Science Center
Dept. of Anatomy & Cell Biology
Syracuse, New York 13078 USA
(315) 473-4844

Susan B. Stearns, PhD
SUNY Health Science Center
Dept. of Anatomy & Cell Biology
Syracuse, New York 13078 USA
(315) 473-5126

Elizabeth Tancred, Ph.D.
The University of New South Wales
School of Anatomy
P.O. Box 1, Kensington
New South Wales 2033
Australia
Phone: 02-6972474

Kenneth J. Tenedini
The Virtis Company
Route 208
Gardiner, New York 12525 USA
(914) 255-5000

Professor Klaus Tiedemann
University of Heidelberg
Institute of Anatomy
D-6900 Heidelberg
West Germany

Jerome P. Tift, M.D.
Mercer University School of Medicine
Department of Pathology
Macon, Georgia 31207 USA
(912) 741-2405

Dale Ulmer, Path.
Dept. of Pathology
University of South Alabama
College of Medicine
2451 Fillingim Street
Mobile, Alabama 36617
(205) 460-7000

Karin Varchmin-Schultheiss, Dr. med.
Institute of Forensic (Legal) Medicine
Am Schlossplatz 3
5300 Bonn 1
West Germany
0228/ 738317

Virginia-Maryland Regional College
of Veterinary Medicine
Multidiscipline Laboratory
c/o Pamela Orcutt
Blacksburg, Virginia 24061
(703) 961-7666

Gunther von Hagens, Dr. med.
University of Heidelberg
D-6900 Heidelberg
West Germany

Anna N. Walker, M.D.
Mercer University School of Medicine
Department of Pathology
1550 College Street
Macon, Georgia 31207 USA
(912) 744-4067

Dorothy Walton-Luglan
University of Rochester Med School
Department MDL, Box 709
601 Elmwood Avenue
Rochester, New York 14642 USA
(716) 275-3285

Erika Walz-Sembler, Path. Asst.
Yale University School of Medicne
Department of Pathology
310 Cedar Street
New Haven, Connecticut 06510
(203) 785-6013

David F. Warren
Memorial University of Newfoundland
Faculty of Medicine, Health Sciences Complex
St. John's, Newfoundland
Canada A1C 5S7
(709) 737-6726

Alexandra M. Whelan
Department of Pathology
Royal College of Surgeons in Ireland
St. Stephen's Green
Dublin 2
Ireland
Phone: 780200 Ext. 136

Glen Yarneau
Dept of Cell Biology & Anatomy
University of Alabama at Birmingham
Box 317
University Station
Birmingham, Alabama 35294

Joseph S. Zawadowski
Duke University Medical Center
Department of Anatomy, Box 3011
Durham, North Carolina 27710
(919) 471-3079

Hans-Jürgen Zobel
med. Präparator
Dept. of Neuropathology
University of Göttingen
Robert-Koch-Str. 40
D-3400 Göttingen, FRG
Phone: 0551-398465