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Effects of Dehydration Mediums and Temperature on Total Dehydration Time and Tissue Shrinkage

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Abstract: To assess the efficacy of current popular plastination dehydration techniques, a variety of organs were dehydrated to: 1. Determine the minimal length of time necessary to thoroughly dehydrate specimens for impregnation and 2. Measure tissue shrinkage during dehydration. Dehydrating agents commonly used for plastination (both room and cold temperature acetone and room temperature methanol) were evaluated. Cold acetone dehydration produced the least amount of tissue shrinkage. Shrinkage was greatest in the graded methanol series. Minimal length of time necessary for acetone dehydration was five days for both cold and room-temperature acetone dehydration.

*Key **words:*** **acetone; dehydration; plastination; shrinkage**

Introduction

Dehydration has been an integral part of the plastination procedure since its inception in 1977 (von Hagens and Whalley, 2000). Cold acetone has been the standard dehydration agent used in plastination since then. However, dehydration for preservation of biological material and its principals predate plastination 8,000 years. At least a partial dehydration was carried out in the mummification process by the ancient Chinese (Qing, 1996) and by the ancient Egyptians (Strub and Frederick, 1967). During the period of Egyptian embalming, 6,000 BC to 600 AD, an estimated 400,000,000 human mummies were prepared. Egyptian embalming reached its pinnacle during the 21st Egyptian dynasty, 1090-945 B.C. One of the steps in the most expensive methods of embalming utilized alcohol preservation and dehydration (Strub and Frederick, 1967). After other treatment and prior to bandaging the body, the body was exposed to the sun to complete the dehydration (Gale, 1961; Strub and Frederick, 1967).

The 20th century witnessed the rise and continued use of alcohol as an effective dehydrant for biological tissues (Deegener and Berndt, 1915; Berg, 1920; Hochstetter and Schmeidel, 1924; Ball, 1928; Guyer, 1930; Fessenden 1938; Bensley and Hensley, 1947; Jones, 1950; Boyde and Wood, 1969; Jones and Hamer, 1975; Gusnard and Kirshner, 1977; Fredenburgh, 1990). Alcohol may also be used as an efficient dehydrant for plastination (von Hagens, 1979a; von Hagens, 1979b; Bickley et al., 1981; Schwab and von Hagens, 1981; O'Sullivan and Mitchell, 1995). Variations of alcohol dehydration for plastination have been suggested and found to work (von Hagens et al., 1987; Kularbkaew et al., 1996). An inherent problem with alcohol dehydration and to some degree with other dehydrants is the reported and experienced high percent of tissue shrinkage (Schwab and von Hagens, 1981; von Hagens et al., 1987; Henry, 1990, 1992, 1995; Kularbkaew et al., 1996).

Acetone is another effective dehydrant that was not reported as such until the late 19th century (Pfitzer, 1895; Gatenby and Cowdry, 1928). Since then,

numerous accounts of acetone's dehydrating prowess have been reported (Mahon, 1938; Weidemann, 1938; Armed Forces, 1957; Ashworth et al., 1966; Knudsen, 1966; Lutz, 1969; Thijssen, 1972; Glauert, 1974; von Hagens 1980, 1981a, 1981b, 1982). However, acetone is also capable of causing tissue shrinkage of varying degrees. Cold acetone is supposed to cause the least amount of shrinkage.

Cold acetone dehydration (freeze substitution), was first used to obtain enhanced preservation in the field of electron microscopy (Fernandez-Moran and Dahl, 1952; Fernandez-Moran, 1959; Bullivant, 1965; Schwab and von Hagens, 1981; Tiedemann and von Hagens, 1982; Hangay and Dingley 1985). For many years, the classic protocol for acetone dehydration has been: 1, Pre-cooled specimens are submerged into cold (-25°C) 100% acetone and let equilibrate for three weeks. 2. The specimens are placed in the second cold, 100% acetone bath for two more weeks. 3. Finally, the specimens are placed in a final cold, 100% acetone bath for 1 week. In all three acetone changes, the recommended tissue to acetone ratio is 1:10 v/v (Schwab and von Hagens, 1981; Tiedemann and von Hagens, 1982; von Hagens, 1985; Bickley et al., 1987; Lischka and Prohoda, 1987; von Hagens et al. 1987; Nicaise et al., 1990). Other protocols call for 2-4 changes of cold acetone at weekly intervals (Tiedemann and Ivic-Matijas, 1988; Ripani et al., 1994). In recent years, both 100% room temperature acetone and graded series of cold or room temperature acetone have been shown to be effective means of dehydration even at lower tissue/fluid ratios (1:5 v/v) (Tiedemann and Ivic-Matijas, 1988; Zheng et al., 1996, 1998; Henry et al., 1998). Any of the above protocols have been shown to produce thoroughly dehydrated specimens for plastination. The method which produces the least amount of tissue shrinkage has not been determined. The recommended length of time that specimens are to be submerged in acetone has decreased over the years (Tiedemann and Ivic-Matijas, 1988; Henry, 1995, 1998) yet no one has standardized a shortened procedure.

The purpose of this project was: 1. To determine the minimum length of time necessary for specimen tissue fluid to be replaced with acetone in preparation for impregnation with a silicone polymer reaction-mixture and 2. To measure tissue shrinkage caused by various dehydrating agents that are routinely used in the plastination process.

Materials and methods

Two experiments were carried out on a variety of organs including kidney, heart, liver and testicle. After collection, organs were cleaned and submerged in 5% formalin for minimal fixation overnight. The next day, specimens were flushed in running tap water for six hours to remove formalin. The specimens were then submerged in different dehydrating solutions: Room temperature acetone (ra), Cold (-15°C) acetone (ca) or Room temperature methanol (rm), according to trial protocol.

***Experiment** one:* To determine minimal length of time for proper dehydration for impregnation with a silicone reaction-mixture, the fixed organs were submerged into either room-temperature or cold (-15°C) acetone. Seven groups (1, la, 2, 2a, 3, 4, 5,) of smaller specimens each containing: 2 dog hearts, 2 dog kidneys and 1 cat kidney and four groups of larger specimens containing either: 2 porcine kidneys and a heart (6L, 8L), 2 bovine hearts (7L) or 2 bovine livers (9L) were used. The tissue to dehydrant ratio was approximately 1:10 v/v and one of the following protocols was followed for each group of tissues. As specimens completed dehydration, they were stored in pure acetone for up to 7 days. This was necessary so that all of the dehydrated specimens (whether dehydration time was 5 or 12 days) could be impregnated at the same time.

Protocol A: Daily, small animal specimens (groups 1, la, 2, 2a) were placed in new 99.9% pure acetone either at room temperature (1, la) or cold temperature (-15°C) (2, 2a) until 99.9% purity was maintained. Dehydrant purity was measured using an appropriate acetonometer [1-100% (Fisher) or 90-100% (Biodur™ # HD 02)] calibrated at 15°C and 20 °C respectively.

Protocol B: Small animal (groups 3,4, 5) and large animal (groups 6L, 7L, 8L, 9L) specimens were placed in new pure room temperature (groups 4, 5, 6L, 7L, 8L, 9L) or cold temperature (group 3) acetone every other day until 99.9% purity was maintained. Dehydrant purity was measured every other day using a 90-100% acetonometer calibrated at 20°C (Biodur™ # HD 02). After dehydration, all specimens were plastinated using the standard cold-silicone technique (von Hagens, 1985). *Experiment two:* To quantify the amount of shrinkage encountered during each of the three dehydration regimes classically used for dehydration in preparation for impregnation with a silicone reaction-mixture, specimen volume was accessed by fluid displacement pre and post dehydration. A tissue fluid ratio of 1:10 v/v was used to dehydrate four small animal specimen types (cat hearts, cat & dog kidneys, cat livers, and dog testicles) in room-temperature methanol or acetone, or cold (-15°C) acetone. Three changes of 100% acetone were carried out at weekly

intervals. The methanol series (70, 80, 90, 95, 100, 100%) was updated weekly. To determine the initial volume of the specimen by fluid displacement, the fixed, water-flushed organs were submerged in water in an appropriate vessel (graduated cylinder or beaker). Specimens were rotated to free any trapped air. Specimen volume was measured via fluid displacement and recorded. Each specimen type was divided into three groups (ca, ra, rm) and submerged in one of the three dehydrants (ca, ra, rm). After the three acetone changes or six methanol changes, the volume of the specimen was determined again by fluid displacement using the appropriate vessel and dehydrating fluid and recorded.

After dehydration, ports were cut into the right and left ventricles and atria prior to impregnation to facilitate submersion of the hearts in the viscous silicone polymer. The specimens were impregnated using the cold-silicone reaction-mixture technique Biodur™ (von Hagens, 1985). Prior to curing, one half of the kidney specimens were sliced either longitudinally or transversely and ports in the hearts were enlarged to determine if the silicone reaction-mixture had penetrated the depths of the tissue.

Results

Experiment one (minimal dehydration time):

For protocol A, specimen dehydration time in cold acetone and room-temperature acetone was 5 to 7 days and 5 to 6 days respectively. The purity of the used acetone dehydration bath is recorded in Table 1. Specimens that completed dehydration first were held in pure acetone for up to 7 days. For protocol B, specimen dehydration time in cold acetone and room-temperature acetone ranged from 8 to 10 days for small animal organs and 10 to 12 days for large animal organs. These results are presented in Table 2 as percent of acetone purity after 2 days of immersion and prior to immersion into pure acetone. After impregnation, sliced organs revealed the silicone reaction-mixture oozing from the entire plane of the cut surfaces.

	24hrs	48hrs	72hrs	96hrs	120 hrs	144 hrs	168 hrs
#l,ra	95.5	98.2	99.0	99.6	100	-	-
#la, ra	94.2	98.0	99.1	99.5	99.8	100	-
#2, ca	97.0	90.5	93.5	99.1	100	-	-
#2a, ca	94.0	96.2	98.2	99.2	99.6	99.7	100

Table 1. Cold (ca) and room-temperature (ra) acetone dehydration by using daily changes of pure acetone. Percent used acetone purity is recorded for each specimen group (1, la, 2, 2a) at daily intervals.

	48 hrs	96 hrs	144 hrs	192 hrs	240 hrs	288 hrs
#3, ca	87.0	94.3	98.7	99.5	100	-
#4, ra	96.6	98.3	99.0	100	-	-
#5,ra	86.0	98.7	99.5	100	-	-
#6L, ra	96.3	99.2	99.8	99.8	100	-
#7L, ra	95.3	98.2	9.5	99.8	100	-
#8L, ra	95.4	97.4	99.4	99.8	100	-
#9L, ra	95.3	98.2	99.8	100	99.8	100

Table 2. Cold (ca) and room (ra) temperature acetone dehydration by using every other day changes of pure acetone. Percent used acetone purity is recorded for each group (3, 4, 5, 6L, 7L, 8L, 9L) at 48 hour intervals.

	cold acetone	room temp, acetone	room temp, methanol
Cat Heart	10.7 +/- 2.41	19.6 +/- 7.01	23.0 +/- 8.20
Cat & Dog Kidney	12.8+/-3.29	18.2+/-3.95	21.4+/-7.32
Cat Liver	24.6 +/- 6.03	29.7 +/- 7.77	29.2 +/- 6.66
Dog Testis	8.8+/- 1.13	13.2+/-0.71	14.9 +/- 0.85
Average	14.5 +/- 7.01	20.2 +/- 7.26	22.6 +/- 7.68

Table 3. Average percent shrinkage of specimens as determined volumetrically.

Experiment two (shrinkage):

The average shrinkage of cold temperature acetone dehydrated specimens was 14.5% with a range of 8.2% to 29%. The average shrinkage of room temperature acetone dehydrated specimens was 20.2% with a range of 11.1% to 34.3%. The average shrinkage of room temperature methanol dehydrated specimens was 22.6% with a range of 14.3% to 34.5%. The results for shrinkage for all three dehydration methods are displayed in Table 3. The average percent shrinkage for specimens with the liver data removed was 11.2% (ca), 17.9% (ra) and 20.8% (rm).

Discussion

Minimal length of time to dehydrate carnivore hearts and kidneys was five days, which was achieved by daily changes of cold and room temperature acetone. These findings seem to correlate with the findings of Tiedemann and Ivic-Matijas (1988) who reported cold acetone penetration of tissue blocks to be 4-5 mm the first day with a maximum of 10mm in 4 days. Their experiments used tissue blocks while these carnivore specimens were entire organs whose maximum tissue thickness was about 2.5cm. Ripani et al. (1994) suggested a markedly decreased dehydration time from the classic six weeks for parenchymatus organs (liver, spleen, kidney). They changed the cold acetone every other day and recommended a minimum of 3 to 4 baths for the best results. Their minimal length of dehydration time of six to ten days seems to correlate with our times. As well, Ripani et al. (1996) dehydrated rat organs, which are smaller than dog organs, in 48 hours.

After dehydration was complete, the carnivore organs were held in 100% acetone for 3 to 5 days to allow all specimens to be completely dehydrated. This allowed all specimens to be impregnated together as a group. Therefore, it is possible that if residual fluid was present in tissues after the 5 day dehydration period, it would have been removed during this holding period. All organs were impregnated and appeared normal twelve months post impregnation. All depths of incised tissue were impregnated with the polymer reaction-mixture.

The largest organs, cow heart and liver, took the longest time for dehydration, 12 and 14 days respectively. It was interesting that dehydration time for room temperature acetone dehydrated specimens was one or two days quicker than cold acetone dehydrated specimens. This could be a slight advantage for the room temperature dehydration procedure.

Daily changes of small specimens resulted in dehydration times of five to seven days, while every other day changes had dehydration times of eight to ten days. These findings indicate that dehydration is most rapid the first day in the dehydrant and dehydration rates slow slightly thereafter. This would suggest that optimum tissue penetration by acetone is via pure acetone with slowing of penetration upon dilution by tissue fluid. Tiedemann and Ivic-Matijas (1988) proposed that after 3 three days was the most efficient time to change the first acetone bath. Our findings, as well as the above, certainly seem to antiquate the old dehydration regime of three weeks, two weeks and then one week (Schwab and von Hagens 1981; Tiedemann and von Hagens, 1982; von Hagens 1985). Recall, this dehydration regime has produced beautiful specimens for 25 years. For economy and quality assurance, three or four days minimum in the dehydrating solution would seem to be prudent.

Shrinkage was only 14.5% in cold temperature acetone while room temperature acetone was 20.2% and room temperature methanol was 22.6%. These room temperature shrinkages were lower than the 35% reported by Holladay (1988) and the 44% and 53% reported by Schwab and von Hagens (1981). Part of the difference likely lies in the fact that their tissue included nervous tissue. Nerve tissue is known to have a higher percent shrinkage at room temperature than other tissues (von Hagens, 1985). Small animal livers had the highest percent shrinkage of the four organs tested. If liver tissue data is removed from all dehydrated specimen groups, the shrinkage percent is lowered across groups by 1.7 to 3.3%. This represents a similar decrease among groups. This finding was somewhat surprising since the liver is a parenchymatous organ. One might surmise that a densely cellular organ would shrink less than one with a lot of intercellular space because of the compactness of the cells resisting collapse. These results may indicate that less silicone enters the cells and more is extracellular, since liver tissue has minimal intercellular tissue. It would be interesting to look at this plastinated tissue histologically and see if there was a significant decrease in cell size.

Another factor that could affect results is the method and utensils used to measure shrinkage. Volume displacement seems to be an appropriate method to determine shrinkage. However, it is difficult to obtain precise measurements with conventional lab ware especially with larger specimens. This is because of the courser calibration of lab ware as the container gets larger.

The shrinkage observed using room temperature dehydration is likely large enough to alter data in quantitative studies. However, for routine gross anatomy demonstrations, it probably would not be

perceived. Certainly shrinkage can be reduced by using freeze substitution. However, the ease of a room temperature dehydration operation (no lifting of 50L or large containers of acetone in and out of the freezer), as well as, the need of fewer deep freezers may be worth the added 10% decrease in the size of the specimen. This decreased size may be a benefit when considering the storage of large specimens. Our findings confirm past speculation and reports about the shrinkage associated with dehydration using acetone and alcohol.

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