Preservation of Ruminant and Equine Anatomical Specimens by Silicone Plastination

Abdelhay M. Ali and Thnaian A. Al-Thnaian

Department of veterinary anatomy, College of Veterinary Medicine and Animal resources
King Faisal University, Al-Hassa, Saudi Arabia

Abstract:

Plastination is a method of preserving biological specimens by replacing the tissue water and lipid with a curable plastic polymer. In this study this technique was used to preserve gross specimens from sheep, ox, horse and camel. The specimens were fixed in 10% buffered formalin, dehydrated in cold -25° C acetone and impregnated with silicone at $-25C^{o}$ under vacuum. The final step involved drainage of excess fluids and exposure of specimens to the curing agent (Biodour^{TM} S6). The plastinted specimens obtained by this method were dry, durable, non-toxic, odourless and could be stored at room temperature.

Key words: plastination, silicone, anatomical specimens , sheep, ox, camel, horse.

Introduction:

Formaldehyde has been in use for over a century as a disinfectant and preservative. Medical students including veterinary students usually have a first-hand experience with formaldehyde in their early days of study of practical anatomy. However, there has always been a concern to the health hazards of exposure to formaldehyde. Students exposed to formaldehyde showed symptoms of irritation and cytogenic changes in epithelial cells of the mouth and in blood lymphocytes (Kriebel *et al*, 1993; Suruda *et al,* 1993).

It has been reported that regular exposure of technicians of histology and histopathology to formaldehyde induces irriation of the eye and upper respiratory tract (Main and Hogans, 1983; Chang and Gershwin, 1992; Giordano, *et al*, 1995; Manuel, 1999) , reduction in pulmonary function (Kilburn, *et al*, 1989) and as well increased the risk of nasal and lung cancer (Sterling and Weinkam, 1989, Hansen and Olsen, 1996). In females, a significant association was noted between exposure to formaldehyde and delayed conception and increased the risk of spontaneous abortion; both reflecting an adverse effect on fertility (Taskinen, *et al,*1999). Animal studies indicated that formaldehyde is carcinogenic (Brown, 1985; McLauglin, 1994).

In 1978, Dr. Gunther Von Hagens developed a unique technique of tissue preservation known as plastination (Tiedemann and von Hagens, 1982). In this process, water and lipids in biological tissues are replaced by curable polymers (silicone, epoxy resins, polyester), which are subsequently hardened, resulting in dry, odourless, non-toxic and durable specimens.

Plastination is carried out in many institutions worldwide and obtained great acceptance particularly because of the durability and the high teaching value of plastinated specimens (Tiedemann and von Hagens, 1982; von Hagens and Tiedemann, 1987; Dawson *et al,* 1990, Pond *et al,* 1992; O'sullivan and Mitchell, 1995; Sittel *et al* 1997;Weiglein,1997).

As teaching aids, plastinated organs offer advantages over models and organs preserved in formaldehyde, the traditional method.

The plastination techniques originally developed for macroscopic specimens are also modified for preparation of plastinated sections for microscopic studies (Fritsch and Hegemann, 1991, Grondin, *et al* , 1994). Preservation by plastination has so far been applied for human anatomy (Bickley,et al, 1981; (Tiedemann and von Hagens, 1982; Bickley,et al, 1987; Frenz, *et al,* 2000). The objective of this study is to establish the technique of plastination suitable for sheep, ox, horse,and camel, which are often used for research and teaching in departments of veterinary anatomy.

Materials and methods:

Materials:

Different organs (heart, lung, kidneys, muscles of forelimb, testes, liver, spleen, stomach ….etc.) from 6 sheep, 3 oxes, 2 horses and 4 camels, of either sex and their ages ranged from 2 to 8 years, were used in this study.

Methods:

The plastination process consists of four steps:

1. Fixation was achieved using 10% buffered formalin at room temperature. The organs were discected and were then perfused and / or immersed in the fixative for 4-10 days.
2. Dehydration was achieved by a process known as Freeze substitution where the specimens were placed into three bathes of cold –25° C solvent, acetone over a period 4-6 weeks.
3. Forced impregnation of dehydrated specimens was achieved by submerging the specimens in the liquid polymer (silicone rubber, Biodour^{TM} S10) mixed with a 1% of the silicone hardener (Biodour^{TM} hardener S3) and placed

under vacuum. The dehydration and forced impregnation steps were carried out in Plastination deep-freezer type HL04.

4. Curing was achieved by exposing the polymer filled specimens to a gaseous curing agent (Biodour^{TM} hardner S6) in a gas curing unit (tightly closed chamber) for 6- 8 weeks. The gas hardened the the polymer through the specimen.

Results:

Of the different results of fixation used in this study, it was found that large organs (e.g. liver of camel) that were first perfused with the fixative and then immersed in it, need less time (4 days) to be well fixed as compared to those which were fixed by immersion (up to 10 days).

Dehydration: at least, three bathes of cold –25° C solvent, acetone for 4-6 weeks is essential to remove the water and insure good dehydration.

Impregnation and curing: impregnation of the specimens in the silicone under the vacuum for 4-6 weeks and hardening in a curing agent for 6-8 weeks gave a good result.

The plastinated organs (Figs.1- 8) were dry, smooth in texture, clean, odorless and most of them maintained their original shape and natural look. The immersed fixed organs retained their close colour to the original after plastination, while the perfused fixed ones showed slight paleness. Some organs i.e. testes and kidneys showed slight decrease in size and some degree of shirnkage (Fig. 2,8). The shrinkage was remakable in the testes. The plastinated speciemens were easy to handle and could be stored at room temperature.

Disscusion:

Formaldehyde which has been in use for over a century as preservative ; is unpleasant, toxic (Main and Hogans, 1983; Chang and Gershwin, 1992; Giordano, et al, 1995; Manuel, 1999) and organs deteriorate quickly when taken out of the liquid. This leads the research workers to look for anthoer methods of preservations to minimized the use of formaldehyde. Plastination which is a method of preserving biological specimens by replacing the tissue water and lipid ; was carried out in many institutions worldwide and obtained great acceptance particularly because of the durability and the high teaching value of plastinated specimens (Tiedemann and von Hagens, 1982; von Hagens and Tiedemann, 1987, Pond *et al,* 1992; O'Sullivan and Mitchell;1995, Sittel *et*

al 1997). Plastination also allow the handling and examination of specimens without the burden of gloves and toxic fumes e.g. formalin.

In this study the technique of silicone plastination was applied on several organs from ruminants and horses. The optimal requrements for each step of plastination were established and saticfactory resuts were obtained.The plastinated specimens obtaianed were dry, clean, durable, odourless and non-toxic. They can be written on, and dissected to highlight specific structure and allow the study of anatomical function, textures and other properties of the tissue which are lost with typical preservation technique. The results were in confirmity with the previous studies (Dawson *et al,* 1990; O'Sullivan and Mitchell, 1995,)

Although most of the organs maintained their original shape, however some of the organs showed slight decrease in size and some degree of shrinkage. This may be attributed to incomplete dehydration of these organs and when cured they dried and shrink, or it may be due to the type of silicone used. Since the parenchyma of most of the organs that showed remarkable shirnkage is formed of very fine tubules i.e seminiferous tubules, the type of silcone used (S10) may be not suitable to enter and fill them so they shrink when cured resulting in reduced size of organs. It has been reported that some specimens need certain type of polymer, and the class of polymer used determines the optical and mechanical properties of the impregnated specimen (Bickley, *et al*, 1981; Weiglein,1997). Further investigation by different types of polymers (silicone, epoxy or polyester resin) is needed in future studies to determine the type of polymer suitable for each specimen.

Acknowledgment:

This study was supported by research grant No. 2028 from the Deanship of Scientific Research, King Faisal University.

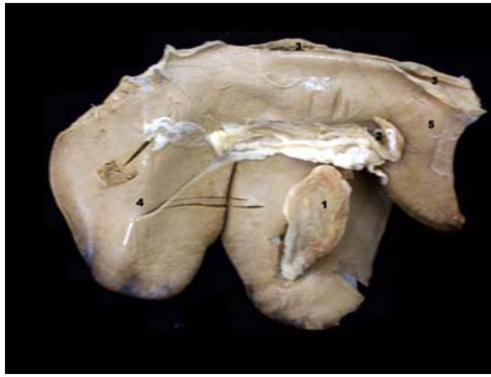

Fig. 1. Visceral surface of plastinated liver of sheep.
1. gallbladder 2. hepatic vein 3. caudal vena cava 4. left lobe of kidney. 5. caudate lobe of kidney.

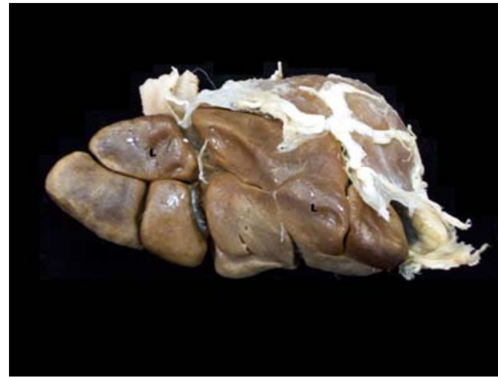

Fig. 2. plastinated left kidney of ox.
L. lobules of the kidney.

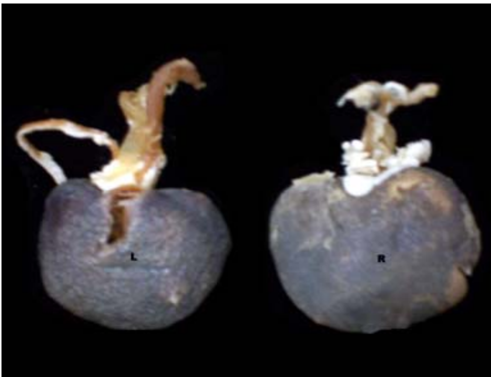

Fig. 3. plastinated kidneys of sheep.
L. left kiney R. right kidney.

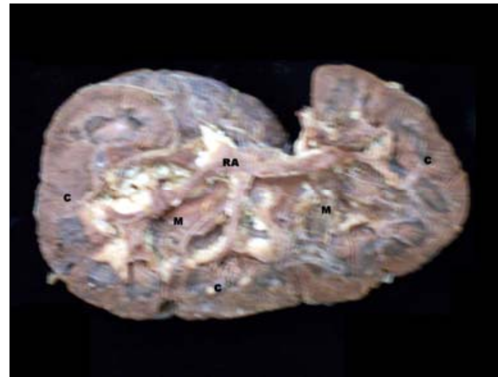

Fig. 4. plastinated longitudinal section of right kidney of ox showing the interior structures.
C. cortex. M. medulla. RA. Hepatic artery.

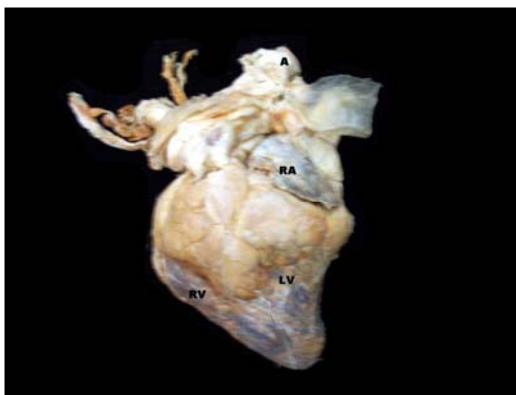

Fig. 5. plastinated heart of sheep.
LV. left ventricle. RV. Right ventricle.
LV.left atrium.

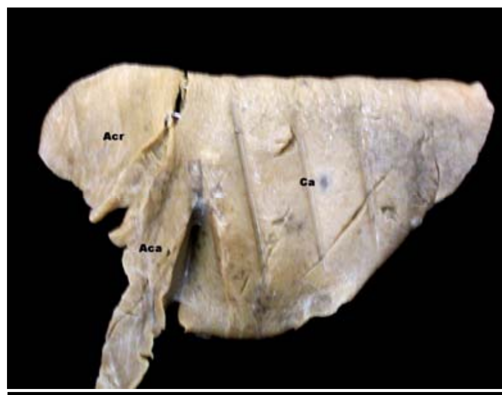

Fig. 6. lateral view of plastinated left lung of sheep. Acr. Cranial part of apical lobe. Aca. Caudal part of apical lobe. Ca. caudal lobe.

Fig. 7. plastinated transverse sections through the ventricles of heart of sheep. LV. left ventricle. RV. Right ventricl. SM. Septomarginal trabecula.

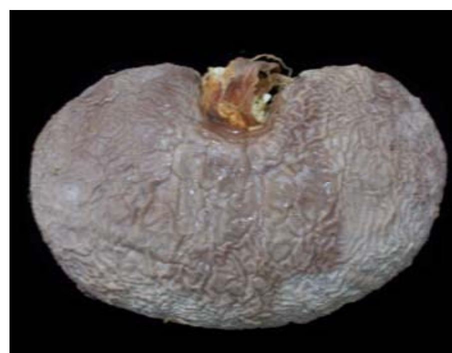

Fig. 8. plastinated kidney of camel (showing shrinkage).

References:

1. Bickley HC, von Hagens G and Townsend FM (1981). An improved method for the preservation of teaching specimens. Arch Pathol Lab Med 105(12): 674-676.
2. Bickley HC and Walker AN, Jackson RL and Donner RS (1987). Preservation of pathology specimens by plastination innovative adjunct to pathology education. Am J Clin Pathol 88(2): 220-223.
3. Brown KG (1985). Risk assessment of laboratory rats and mice chronically exposed to formaldehyde vapors. Risk Anal 5(3): 171-180.
4. Chang CC and Gershwin ME (1992). Perspectives on formaldehyde toxicity: separating fact from fantasy. Regul Toxicol Pharmacol 16(2): 150-160.
5. Dawson TP, James RS and Williams GT (1990). Silicone plastinated pathology specimens and their teaching potential.J Pathol 162(3): 265-272.
6. Fenz C, Fritsch H and Hoch J (2000). Plastination histologic investigations on the inserting pars terminalis aponeurosis dorsalis of three-sectioned fingers. Anat Anz 182(1): 69-73.
7. Fritsch H and Hegemann L (1991). Simplification of the production of plastination of histologic preparations through the use of a grinding machine. Anat Anz 173(3): 161-165.
8. Giordano C, Siccardi E, Fedrighini B, Romano C, Sulotto F, Coscia GC and Verganano P (1995). Nasal patency patterns observed during working hours in a group of technicians habitually exposed to formaldehyde. Acta Otorhinolaryngol Ital 15(5): 335-344.
9. Grondin G, Grondin GG and Talbot BG, (1994). A study of criteria permitting the use of plastinated specimens for light and electron microscopy. Biotech Histochem 69(4): 219-234.
10. Hansen J and Olsen JH (1996). Occupational exposure to formaldehyde and risk
11. Kilburn KH, Warshaw R and Thornton JC (1989). Pulmonary function in histology technicians compared with women from Michigan: effects of chronic low dose formaldehyde on a national sample of women. Br J Ind Med 46(7): 468-472.
12. Kriebel D, Sama SR and Cocanour B (1993). Reversible pulmonary responses to formaldehyde. A study of clinical anatomy students. Am Rev Respir Dis 148 (6 pt 1) 1509-1515.
13. McLaughlin JK (1994). Formaldehyde and cancer: a critical review. Int Arch Occup Environ Health 66(5): 295-301.
14. Main DM and Hogan TJ (1983). Health effect of low-level exposure to formaldehyde. J Occup Med 25(12): 896-900.
15. Manuel J (1999). Published erratum appears in Environ Health Perspect 1999 Nov, 107(11): A548 A healthy home environment? Environ Health Perspect 107(11): A352-357.

16. Pond KR, Holladay SD and Luginbuhl JM (1992). Technical note: preservation of tissues and gastrointestinal tract portions by plastic coating or plastination. J Anim Sci 70(4): 1011-1014.
17. O'Sullivan E and Mitchel BS (1995). Plastination for gross anatomy teaching using low cost equipment. Surg Radiol Anat 17(3): 277-281.
18. Sittel C, Eckel HE, Ricks S and Stennert E (1997). Sheet plastination of the larynx for whole-organ histology. Acta Anat (Basel) 158(3): 185-189.
19. Sterling TD and Weinkam JJ (1989). Reanalysis of lung cancer mortality in a National Cancer Institute Study on "Mortality among industrial workers exposed to formaldehyde ". Exp Pathol 37(1-4): 128-132).
20. Suruda A, Schulte P, Boeniger M, Hayes RB, Livingston GK, Steenland K, Stewart P, Herrick R, Douthit D and Fingerhut MA (1993). Cytogenetic effects of formaldehyde exposure in students of mortuary science. Cancer Epidemiol Biomarkers Prev 2(5): 453-260.
21. Taskinen HK, Kyyronen P, Sallmen M, Virtanen SV, Liukkonen TA, Huida O, Lindobohm ML and Anttila A (1999). Reduced fertility among females wood workers Exposed to formaldehyde. Am J Ind Med 36(1): 206-212.
22. Tiedemann K and von Hagens G (1982). The technique of heart plastination. Anat Rec 204 (3): 295-299.
23. von Hagens G and Tiedemann K (1987). The current potential of plastination. Anat and Embryol (Berl) 175(4): 411-421.
24. Weiglein,AH (1997). Plastination in the neurosciences. Acta Anat 158(1): 6-9.

حفظ النماذج التشريحية التدريسية من المجترات والخيل بوساطة التطويع اللدائني بالسليكون

عبدالحي محمد علي و ثيان الثيان

قسم التشريح – كلية الطب البيطري والثروة الحيوانية
جامعة الملك فيصل – الأحساء – المملكة العربية السعودية

المخلص :

التطويع اللدائنى طريقة لحفظ العينات الحيوية يتم فيه استبدال الماء والدهون الموجودة فى الانسجة باللدائن المتبلمرة. استخدمت في هذه الدراسة تقنية التطويع اللدائنى لحفظ أعضاء مختلفة تم جمعها من الغنم والبقر والإبل والخيل . أولا ، تم تثبيت العينات في ١٠٪ فورملين ثم تم تجفيفها (انكازها) بواسطة الأستون في درجة حرارة - ٢٥ درجة مئوية. بعد ذلك تم طمر العينات في السليكون في درجة حرارة - ٢٥ درجة.وكانت الخطوة الأخيرة هي تجفيف هذه العينات بواسطة عامل مجفف. تم بهذه الطريقة الحصول على عينات جافة، ونظيفة، وخالية من المواد السامة. ويمكن التعامل معا بسهولة كما يمكن حفظها على الأرفف في درجة حرارة الغرفة.