Final report for the Project No. 2028 sported by the Deanship of Scientific Research, King Faisal University.

Title: Establishment of the plastination technique for the preservation of the anatomical specimens.

Introdution:

The formaldehyde has been in use for over a century as a disinfectant and preservative agent. It is a substance with which most of the medical students including the veterinary students has a first-hand experience in their early days of study in the anatomy laboratory course.

However, there has been a concern in recent years as to the health hazards of exposure to formaldehyde. Students exposed to formaldehyde showed irritant symptoms and cytogenic changes in epithelial cells of the mouth and in blood lymphocytes (Kriebel *et al*, 1993; Suruda *et al*, 1993).

Professional formaldehyde exposure typical of that experienced by technicians working in histology and pathology departments induced an irritant effect on the eye and upper respiratory tract(Main and Hogans, 1983; Chang and Gershwin, 1992; Giordano, *et al*, 1995; Manuel, 1999) , reduced pulmonary function (Kilburn, *et al*, 1989) and increased the risk of nasal and lung cancer (Sterling and Weinkam, 1989, Hansen and Olsen, 1996). It is also significantly associated with delayed conception and increased the risk of spontaneous abortion, that means has an adverse effect on fertility (Taskinen, *et al*,1999). Animal studies indicated that formaldehyde is a widespread animal carcinogen (Brown, 1985; McLauglin, 1994).

Recently, Dr. Gunther Von Hagens developed plastination in 1978, a unique technique of tissue preservation. 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, Osullivan and Mitchell, 1995, Sittel *et al* 1997, Weiglein AH,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 light and electron microscopic studies (Fritsch and Hegemann, 1991, Grondin,*et al* , 1994).

The objectives of this study: -

1. To establish the technique of plastination in the department.
2. To apply the technique in the preparation of specimens for teaching.
3. By establishing this technique the use and exposure to the formaldehyde will be minimized.

Materials and methods: -

Materials: -

Different organs from sheep, ox, horse and camel (heart, lung, kidneys, muscles of forelimb, testes liver, spleen….ect.) were used in this study.

Equipments used: -

1. Plastination deep-freezer HL04.
2. Small plastination unit.
3. Stainless Steel Basket H101.

4. Stainless Steel Drums.
5. Acetonometer.
6. Gas curing Unit.
7. Vacuum pump.

Methods: -

The plastination process consists of four steps: -

1. Fixation or tissue preparation: for this process 10% buffered formalin was used tabilizes the tissue and prevents autolysis. The organs were discected and perfused and / or immersed fixed in the fixative for 4-10 days.
2. Dehydration: This is achieved by a process known as Freeze Substitution where the specimens are placed into three bathes of cold –25^{o} C solvent, usually acetone over a period 4-6 weeks. The tissue water is slowly replaced by the acetone.
3. Forced impregnation: The dehydrated specimens are submerged into the liquid polymer (silicone rubber,Biodour™ S10) mixed with a 1% of silicone hardener (Biodour™ hardener S3) and placed under vacuum. The vacuum draws out the acetone and the polymer takes its place. The dehydration and forced impregnation steps were carried out in Plastination deep-freezer type HL04.
4. Curing: The polymer filled specimens were hardened by exposing them to a gaseous curing agent (Biodour™ hardner S6) in a gas curing unit (tightly closed champer) for 6- 8 weeks. The gas will harden the the polymer throught the specimen.

Results:

Fixation: from the different trails used in this study it was found that organs which were first perfused with the fixative and then immersed in it, need less time (4 days) to be will fixed than those which only immeresed fixed (up to 10 days). However, the immeresed fixed

organs retain their close colour to the original after plastination, while the perfused fixed ones show slight plaeness.

Dehydration: at least thee bathes of cold –25^{o} C solvent, acetone for 4-6 weeks is essential to remove the water and insure good dehydration. Some of the incompletely dehydrated specimens showed a degree of shirnkage after the impegnation and curing.

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 except some sotf organs like testes and kidneys which showed some degree of shirnkage. the plastinated organs (Fig.1- 8) were dry to touch, clean, non-toxic, odorless and most of them maintains their original shape and natural look. They are easy to handle and can be stored at room temperature indifinitely.

Conclusions:

In this study the standard technique of silicone plastination was establishe in the department of veterinary anatomy and applied on some organs of different animals. Saticfactory resuts were obtained and they are inconfirmity with the previous studies (Dawson *et al*, 1990 , Osullivan and Mitchell, 1995,). The optimal requrements for each step was established. The plastinated specimens obtaianed were dry, durable, non-toxicand odourless. They can be written on, and dissected to highlight specific structure. Although most of the organs maintain their original shape and size , however some of soft organs showed slight degree of shrinkage. This may atributed to incomplete dehydration or it may be due to the type of silicone used. Since some specimens need certain type of polymer and the class of polymer used determines the optical and mechanical properties of the impregnated specimen. further

investigation by different types of polymers (silicone, epoxy or polyester resin) is needed in future studies.

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, Pond *et al*, 1992, Osullivan and Mitchell, 1995, Sittel *et al* 1997). Plastination permits preservation of anatomical specimens in physical state approaching that of the living condition , stable and easy to handle. Formaldehyde 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. On the other hand, plastinated organs are permanent, clean, non-toxic, dry and allow the study of anatomical function, textures and other properties of the tissue which are lost with typical preservation technique. Plastination also allow the handling and examination of specimens without the burden of gloves and toxic fumes e.g. formalin. In addition plastinated organs can be written on and dissected to highlight specific structures

References

1. Brown KG (1985). Risk assessment of laboratory rats and mice chronically exposed to formaldehyde vapors. Risk Anal 5(3): 171-180.
2. Chang CC, Gershwin ME (1992). Perspectives on formaldehyde toxicity: separating fact from fantasy. Regul Toxicol Pharmacol 16(2): 150-160.
3. Dawson TP, James RS and Williams GT (1990). Silicone plastinated pathology specimens and their teaching potential.J Pathol 162(3): 265-272.

4. Fritsch H, 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.
5. Giordano *et al* (1995). Nasal patency patterns observed during working hours in a group of technicians habitually exposed to formaldehyde. Acta Otorhinolaryngol Ital 15(5): 335-344.
6. Grondin , *et al* (1994). A study of criteria permitting the use of plastinated specimens for light and electron microscopy. Biotech Histochem 69(4): 219-234.
7. Hansen J, Olsen JH (1996). Occupational exposure to formaldehyde and risk
8. Kilburn et *al* (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.
9. Kriebel *et al*(1993). Reversible pulmonary responses to formaldehyde. A study of clinical anatomy students. Am Rev Respir Dis 148 (6 pt 1) 1509-1515.
10. McLaughlin JK (1994). Formaldehyde and cancer: a critical review. Int Arch Occup Environ Health 66(5): 295-301.
11. Main DM and Hogan TJ (1983). Health effect of low-level exposure to formaldehyde. J Occup Med 25(12): 896-900.
12. 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.
13. Pond *et al* (1992). Technical note: preservation of tissues and gastrointestinal tract portions by plastic coating or plastination. J Anim Sci 70(4): 1011-1014.

14. O, sullivan E and Mitchel BS (1995). Plastination for gross anatomy teaching using low cost equipment. Surg Radiol Anat 17(3): 277-281.
15. Sittel *et al* (1997). Sheet plastination of the larynx for whole-organ histology. Acta Anat (Basel) 158(3): 185-189.
16. 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).
17. Suruda *et al* (1993). Cytogenetic effects of formaldehyde exposure in students of mortuary science. Cancer Epidemiol Biomarkers Prev 2(5): 453-260.
18. Taskinen *et al* (1999). Reduced fertility among females wood workers Exposed to formaldehyde. Am J Ind Med 36(1): 206-212.
19. Tiedemann K, von Hagens G (1982). The technique of heart plastination. Anat Rec 204 (3): 295-299.
20. von Hagens G and Tiedemann K (1987). The current potential of plastination. Anat and Embryol (Berl) 175(4): 411-421.
21. Weiglein,AH (1997). Plastination in the neurosciences. Acta Anat 158(1): 6-9.

Legends of figures

Fig. 1. plastinated liver of sheep.

Fig. 2. plastinated kidney of ox.

Fig. 3. plastinated kidneys of sheep.

Fig. 4. plastinated cross section of ox.

Fig. 5. plastinated heart of sheep.

Fig. 6. plastinated lung of sheep

Fig. 7. plastinated cross sections of heart of sheep.

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