JUST AGRICULTURE
multidisciplinary e-Newsletter

PREPARATION OF ANATOMICAL MUSEUM

Dr P. J. Kapadnis, Dr S. K. Karmore, Dr S. K. Gupta and Dr Alka Suman

College of Veterinary Science and Animal Husbandry, Mhow

ARTICLE ID: 012

Introduction

A museum is defined as an institute that displays a collection of artifacts of scientific or artifacts significance to a specific group of visitors (Alexander & Alexander, 2008). The concept of anatomy museum was first conceived by the Edinburg surgeon during 1700-1763 A.D. The discovery of formalin in 1859 by Alexander Mikhailovich Butleror and in subsequent isolation in 1868 by August Wilhelm Von Holfman was a great milestone in anatomical history (Venketash *et. al.*, 2016). The museums have metamorphosed substantially from a collection of models and art to express anatomy in 18th century to the use of formalin preserved specimens in 19th century to plastinates in late 20th century (Kamath *et. al.*, 2014). The various techniques used for the preparation of museum specimens are:

- Preparation of dry specimens of bones.
- Preparation of wet specimens
- Preparation of freeze dried specimens
- Preparation of opaque specimens
- Preparation of transparent specimens
- Corrosion cast preparation
- Preparation of mummies / manikins
- Preparation of plastinated specimens

1. Preparation of dry specimens of bones: For osteological study, the bones are collected from carcass and duely prepared. The skin and the possible amount of flesh adhering to bones are removed by cutting with knife and gentle scrapping. Such bones are called wet bones. Wet bones are macerated to get rid of the animal matter adhering to them. It can be affected by burying them in the ground at a depth of not less than two feet. This is possible in dry climate and in fine weather.

JUST AGRICULTURE
multidisciplinary e-Newsletter

When the bones are required at quick intervals, the wet bones are boiled in a big container on slow free. Sodium hydroxide may be added in the boiling water to speed up the process of removal of flesh from the bones. After preliminary boiling is over, the bones are scrapped with the blunt knife and scrubbed with dandy brush. Then they are steeped overnight in water to which lime is added and reboiled next day with soap for shorter while. After complete clearing of bones, they are allowed to dry at room temperature.

For general osteological work, the wet bones are steeped in cold water band left over for days in a container covered with lid. By this process the soft tissue adhering to the bones purify. In gradual stages, the tissues are detached and collected at the top. The water in the container is changed at weekly intervals. The bones are periodically examined and shreds of tissues are scraped and brushed. At the last change, lime is added to the water.

The adult bones contain large amounts of fat in their medullary cavity and some fat is associated with their surface. The fat can be removed by soaking the bones in chloroform for 12 hrs. Then a second change with the fresh chloroform is given (Siddiqui, 1990) for another 12 hrs. After removing the bones from chloroform they are allowed to dry at room temperature for 24 hrs.

- The macerated bones can be kept on racks, or articulated into a skeleton on a platform.
- The macerated bones should be dusted with the insecticides occasionally (as parasite may still thrive on the traces of animal matter lying hidden in the bones.
- A cont of varnish gives a finishing touch and removes all traces of smell.

2. Preparation of wet specimens: For the preparation of wet specimens, the cadaver is collected and fixed properly. Then the specimens are prepared by fine dissection of different body parts/organs. The dissected specimens are kept in a room and passive drying is done for one or two days to make the surface of the structures to be coloured like vessels, nerves, ligaments, muscles, ducts, glands dry enough to apply colour. The vessels nerves etc are raised from the underlying muscles by putting swab of cotton under them which resulted in early drying of structures. The vessels and nerves dry earlier than other structures. Therefore the colour is applied first on them than on others. The standard colour coding should be used for different anatomical structures; specimens are kept overnight to make the paint dry. The glass jar is filled with mounting fluid which consists of 25 parts by volume of pure glycerine,

JUST AGRICULTURE
multidisciplinary e-Newsletter

75 parts by volume of distilled water and 5 parts of formalin. Glycerine has clearing effect on the tissue and improves the optical properties of mounting fluid. Finally the jars are covered with lid, sealed by cello tape and kept in museum (Kaur *et. al.*, 2017).

3. Preparation of freeze dried specimen: The freeze dried specimen preparation is quick and cheap method of producing dry specimens for teaching and learning anatomy. Taxidermists have developed techniques for freeze drying whole small animals as a method of long term preservation (Metcalf, 1981). In the same way the specimens of organs and dissected body parts can be freeze dried and used in anatomy teaching.

Method:

- First of all cadaver is fixed properly.
- Then prosection is prepared as required.
- Specimen is thoroughly washed to remove debris.
- The specimen is packed with non-absorbent cotton wool to retain the natural shape of the specimens as it dries.
- Freeze specimen to between -20^0C and 29^0C for 24 hrs.
- Remove specimen from freezer and place in freeze drier. Freeze dry the specimen until process is complete.
- It takes about 5-6 days for prosected laryax (Sullivan and Stewart, 1999). Remove any packing from the specimen.
- The specimen is then ready for use. When it is not in use, it should be stored in air tight container with silica gel to absorb any moisture.

4. Preparation of opaque specimens: For the preparation of opaque specimens any of the following method can be used.

I. Method provided by ICI plastic limited (Pulvertuft, 1950)

Materials required

- Methyl methacrylate monomer
- Benzoyl peroxide
- Dibutyl phatlate
- Sheet "perpex' 1/16 or 1/8 inches in thickness
- Caustic soda
- Perspex cement

JUST AGRICULTURE
multidisciplinary e-Newsletter

Method:

Washing of monomer: first of all the methyl methacrylate monomer is stabilized by the addition of hydroquinone and is then known as "Kellodoc" liquid. Before thickening, the stabilizer must be removed by washing with 5% caustic soda solution. For washing equal quantities of monomer and caustic soda is used and washing is carried out in a separation funnel, where the discoloured washing, which forms a layer below the monomer, can easily be run off. Two washings are sufficient and should be followed by washing with water until the liquid run off is no longer alkaline. The monomer forms the top layer in separating funnel. It is then run off and allowed to stand for 24 hrs. with slaked calcium chloride for its complete dehydration. Filter it through the filter paper, after which it is ready for thickening.

Syrup preparation: a mixture containing 85 parts by volume of washed methyl methacrylate monomer, 0.02%. Benzoyl peroxide (by weight) and 15 parts dibutyl phathalate is placed in flask, which should be half filled with an air condenser. Thickening takes place in approximately 15 minutes using an oil bath at 130^0C (or in 30 minutes using boiling water bath). Three to four shakings are necessary in each case during the process. As a precautionary measure a sink full of cold water should be at hand, so that if reaction becomes violent during shaking the flask, can be cooled immediately. The monomer is thickened to the maximum convenient viscosity and stored in a refrigerator until required for use.

Preparation of specimen:

- Dehydrate the fixed specimen thoroughly.
- Soak the specimen in unthickened monomer or in 10% solution of benzoyl peroxide in chloroform overnight before embedding.
- Hollow specimens and soft tissues are impregnated with monomer of slightly less viscosity than that used in embedding. A mould can be quickly made by joining suitably shaped pieces of glass with "cenmentum" to form a fox. The mould is either left overnight or may be completely dried and hardened in a 40^0C oven in a few hours.
- Embedding is done in layers. First a layer of about ¼ inch thick is poured into the mould and polymerized at 40^0C for few days to form a supporting layer. Second layer of syrup is poured into the mould and the specimenis placed in position in it. Further layers may be added if required to cover the specimen adequately if it is thick. A glass

JUST AGRICULTURE
multidisciplinary e-Newsletter

lid should be bound on to the mould with cellophane tape to form an air tight cover during polymerization to prevent evaporation.

When polymerization is complete the block can be shaken out of the mould or glass broken away. The block is cut and trimmed to size and shape and finally polished by hand or on finishing machine and finished with a good metal polish.

II. Embedding in Ward's "bio-plastic"

Material required:

- Ward's "bioplastic" (selectron)
- Tertiary butyl hydroperoxide (Wards catalyst)

The specimen is dehydrated with alcohol and then placed in anhydrous ether. Then it is transferred to uncatalysed monomer and the ether is removed in a dessicator by slowly reducing the pressure. The above technique is followed.

Catalysed bioplastic is prepared by adding 0.1-0.5% of "Ward's" catalyst to the monomer. The supporting layer is poured in to the mould and allowed to gel at room temperature for 1-3 hrs again a layer of catalysed monomer sufficient to cover the specimen is poured. The specimen is carefully placed in position so that no air bubbles are trapped. Gelling is allowed to occur at room temperature. Final layer is poured and allowed to occur at room temperature. Final polymerization is done in oven starting at 37^0C and gradually rising to but not exceeding to 60^0C. Finally the block is removed from the mould and shaped and polished.

III. Embedding in "macro S.B. 26 C" requires the following materials.

- Macro S.B. 26 Cresin
- "Monomer C"
- H.C.H. catalyst (I-hydroxycyclohexyl hydroperoxide-I)
- Accelerator "E" (solution of cobalt naphthanate in "monomer C"
- Plastic is prepared by
- Embedding in "macro S.B. 26 C" resin – 100 parts

(A) "monomer C" – 20 parts

(B) Catalyst – 2 parts

JUST AGRICULTURE
multidisciplinary e-Newsletter

- And adding to mixture 10 parts of plasticizer. This mixture is then filtered through glass wool. It is stable for 1-2 days.
- "Accelerator E" (1 part) is added and the plastic is ready for polymerization.

5. Preparation of transparent specimens: (Pulvertuft : 1950).

The mounting of transparent specimens depend on the replacement of tissue fluids by fluids of a high refractive index. Oil of wintergreen, glycerine and paraffin are being used in the preparation of transparent specimens. The important step is that dehydration must be complete. The longer is the process, better the result.

Method: (S Paltheolz, 1911)

- Specimens are fixed in 10% Formal saline. It is bleached in hydrogen peroxide.
- Specimen is gradually dehydrated as followed
  - 50% alcohol 2 weeks
  - 60% alcohol 2 weeks
  - 70% alcohol 2 weeks
  - 80% alcohol 2 weeks
  - 95% alcohol 2 weeks
- Finally, the dehydration is done in absolute alcohol. Two changes are made each of two weeks. Second change with a layer of anhydrous copper sulfate on the bottom of container covered by five layers of filter paper. Two changes of benzol are given, each of two weeks followed by two changes in benzyl benzoate each of two weeks.
- Mounting of specimen is done in equal parts of benzyl benzoate and oil of wintergreern. The specimen is mounted in glass jars as, the fluid is "Perspex solvent". Glass jars are sealed with cement.
  - Powdered gum Arabic - 50 g
  - Sugar - 50 g
  - Sodium silicate - 02 g
  - Formalia - 01 ml
- Thick paste of these ingredients is made by mixing these ingredients with water and lid is affixed leave the jar undisturb for 48 hrs.
- It 5% gelatin with finely ground chrome yellow is a good injection mass for vessels in transparencies, 0.4% formalin is added just before use. Vessels are first perfused with

JUST AGRICULTURE
multidisciplinary e-Newsletter

warm isotonic saline solution containing amyl nitrate to prevent vasoconstriction. After injecting the gelatin the specimen is placed in refrigerator for 24 hrs.

6. **Preparation of corrosion east:** the objective of preparation of corrosion cast is to preserve the internal conformation of one or several ducts of an organ or entire body, then eliminating all the surrounding tissue and finally obtaining a tridimensional polymeric specimens which shows internal conformation of the initial piece, without any biological risk (Estaben, 2017).

Materials which can be used for injection are – Acrylic, Epoxy resin, Polyester resin, RTV silicone.

Method

Weak formalin solution is usually preferred for fixing the tissues except lungs. Usually 4% formalin is preferred for general use and 2% for fixing tissues which becomes excessively hardened in the stronger solution (Tompesett: 1956). As per Estaban *et. al.* (2017) fresh specimens show better results in injection process. A thorough wash with water for several minutes removes clots and other debris and improves the visualization of the structures of interest. The process of injection is divided into three steps :

- Dissection and identification
- Isolation and cannulation
- Injection

Dissection and identification: it is very important to identify the duets / artery / veins which is to be injected. A colour code is to be established to differentiate structures e.g. red-artery, blue – vein white – airways, yellow – urinatry duets and green biliary ducts.

Isolation and cannulation : (Hill and Mckinney, 1981). The small vessels and ducts are dilated using the tube of correct size and material, which permits the injection of the polymer. After introducing the tube in the duct, it must be fixed in place to avoid any leakage of material injected under pressure. Cleaning of pathway (duct/vessels etc) can be performed by injecting each stones or any possible blockages that may interfere with polymer entry. If hydrogen peroxide is used, it is important to wash with water afterwards because the foam produced can affect adequate injection.

JUST AGRICULTURE
multidisciplinary e-Newsletter

Injection: Finally polymer of choice is injected directly in each tube independently using positive pressure pump. The pumping can be done with a compressor and a polymer container of manually with different sizes of syringes. After complete filling of ducts, all the tubes are sealed with different clamps. Injected organs are kept for few hours to allow the completion of process of polymerization.

Corrosion: Chemicals e.g. sodium hydroxide (NaOH) or hydrochloric acid (HCL) can be used for corrosion. In NaOH hydrolysis of tissue is slow at room temperature. The process of hydrosis can be accelerated by heating the NaOH solution to a point in which complete hydrolysis can be achieved within a few hours. After complete corrosion the cast is cleaned by putting it in luke warm water.

7. Preparation of plastinated specimens (plastination) : It is novice technique used in anatomy to preserve the body parts / organs. The water and fat are replaced by certain plastics yielding specimens that can be touched donot decay and even retain most microscopic properties of the original samples. The technique was discovered by Dr. Gunther Von Hagens in 1978. The process of plastination involves five steps viz; fixation, dehydration, forced impregnation in a vaccum and hardening. Water and lipid tissues are replaced by curable polymers e.g. silicone, epoxy resins and polyester – copolymer.

REFERENCES:

Hartman, M. J. and Groenewald, H. B. (2014) Silicone case in situ: A technique to demonstrate the arterial supply of the female reproductive organs of an African lion (*Panthera leo*). *J. Plastination* 26(2): 11-15.

Ramkrishna, V. and Leelavathy, N. (2017) Corrosion Cast of bronchopulmonary segments. *J. Plastination*. 29:5-7.

Bandari K., Acharya S., Srivastava, A. K., Kumari, R. and Nimmagadda, H. K. (2016) Plastination a new model of teaching anatomy. *Int J. Anant Res*. 4(3) : 2626-29.

Ramkrishna, V. and Leelavathy, N. (2017) New innovative method to prepare dry hollow organs and lungs. *J. Dental Med Sci.* 16:81-83.