Article

Production and use of plastinated anatomical specimens as teaching and learning tools in veterinary gross anatomy in the Caribbean

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Abstract: Veterinary Anatomy is considered as the backbone subject in the veterinary medicine programme. Formalinized specimens were not very much accepted by the veterinary students due to their wetness, bad smell and potential harmful effects. Plastication has evolved as a new technique for prolonged preservation of specimens by replacing the water and fat by a curable plastic polymer. The process of plastination involved fixation, dehydration, impregnation and curing. Plastinated specimens were appreciated by the staff and students as they were odorless, non-toxic and durable, needed less space for storage and they were used in teaching the veterinary anatomy courses. Plastinated specimens were used for studying not only in the dissection hall but also in the lecture room, both individually and in groups. The plastination technique was sensitive and time consuming and needed expensive chemicals. The plastinated specimens could not completely replace the traditional dissection because students learn best by exploratory and hands-on dissection. Moreover, the wet specimens provided a more accurate visual representation of the body parts and skills to aid in dealing with actual clinical cases in the future. Therefore, the plastinated and wet specimens were used simultaneously depending on the topic being taught at the time.

Key words: Plastinated specimens; Production; Teaching; Veterinary anatomy

1. Introduction

The animal cadaver and its organs are an integral part of learning veterinary anatomy in the DVM programme. The specimens used in teaching may be fresh or preserved [1]. However, decaying of the fresh specimens in a short time is a serious problem, so the specimen must be preserved to stay for prolonged period by both traditional methods such as mummification and embalming or modern methods such as plastination.

Non-plastinated specimens fixed with formaldehyde are toxic, allergenic and possibly carcinogenic. Concentrations of the formaldehyde above 0.1ppm in air can irritate the mucous membrane and eye causing watery eye. Moreover, inhalation of formaldehyde at the same concentration may cause difficult breathing, burning sensation in the throat, headache and may even lead to asthma. Also, non-plastinated specimens do not facilitate close interaction and manipulation of specimens and there is deterioration over time [2].
There were problems that occurred for both instructors and students during the teaching of practical gross anatomy such as handling of gastrointestinal tracts of equines and ruminants due to the size and flaccidity of the walls of the stomachs and intestines. In addition, these tracts were difficult to be demonstrated topographically and their ingesta needed to be evacuated in order to study the internal structures.

Preservation of the specimens and tissue by plastination technique was first made by Dr. Gunther von Hagens in Heidelberg, Germany, in 1978. The process was based on replacement the fat and water by polymeric materials to produce hard, dry and odourless specimens [3].

2. Materials and Methods

2.1. Materials

The specimens were selected from the different sources. Whole dogs and their fresh organs were removed from euthanatized dogs from the Trinidad and Tobago society for the prevention of cruelty to animals (TTSPCA). The organs of sheep, pig and ox were obtained from field station and abattoirs in Trinidad and Tobago.

2.1.1. Dissection

The whole animals were dissected by making a median incision was made between the spaces from the mandibular space caudally to the anus. The digestive and urogenital organs as well as brain were collected. The limbs were dissected. The domestic birds were slaughtered in a humane method and their abdominal cavities were opened to show the viscera in situ. The digestive tract was washed carefully of all its contents by making small incisions at various points and flushing with a steady stream of water from a small pipe. Hollow areas were dilated to maintain shape and assure proper dilation during curing

2.2. Methods:

The S10/S15 Plastination Technique [4] was used in the present study for the plastination of the organs, chicken and limbs.

2.2.1. Fixation:

All the specimens were then placed in 5% formalin solution for three (3) weeks to fix properly.

2.2.2. Dehydration:

The digestive and urogenital tracts specimens as well as heart and brain were then washed for 24 32 in tap water. Excess water was drained from the specimens. Hollow spaces in the digestive tract was filled with cold acetone and arranged in an anatomical position in the specimen basket. Specimens were then submerged into an aliquot of -25oC acetone (90-100%) and placed in the freezer at -25oC. After six days the purity of the acetone was checked and
recording. This was done by filling a two (2) litre measuring cylinder with the used acetone. The temperature was
monitored and an acetonometer inserted and read. On day seven the purity of the acetone was again checked and
recorded. Specimens were then placed in a new aliquot of -25°C acetone and placed back into the freezer. This
procedure was repeated weekly two more times.

Table 1. Shows a summary of the Dehydration Schedule

<table>
<thead>
<tr>
<th>Day 1</th>
<th>Wash specimens for 24 hrs in tap water</th>
</tr>
</thead>
<tbody>
<tr>
<td>Day 2</td>
<td>Place specimens in an aliquot of -25°C acetone (&gt;90%) 1:10 (specimen: acetone) ratio</td>
</tr>
<tr>
<td>Day 9</td>
<td>Measure and record purity of used acetone. Place the specimens into a fresh aliquot of new (99 – 100%) acetone (-25°C)</td>
</tr>
<tr>
<td>Day 16</td>
<td>Measure and record purity of used acetone. Place specimens into a fresh aliquot of acetone (-25°C)</td>
</tr>
<tr>
<td>Day 23</td>
<td>Measure and record purity of used acetone. Place specimens into a fresh aliquot of acetone (125°C)</td>
</tr>
<tr>
<td>Day 30</td>
<td>Check acetone purity and colour as well as fat colour: If purity is &gt; 98% and fat is negligible or opaque. Place specimens into cold impregnation mix</td>
</tr>
</tbody>
</table>

2.2.3. Impregnation:

For impregnating the specimens the instruments viz., Deep freezer, Vacuum chamber, Vacuum pump, Vacuum
gauge, tubing, fine adjustment needle-valve and Bennert mercury manometer were used. The impregnation reaction-
mixture Biodur S10/S3 mixture is prepared.

2.2.4. Impregnation Procedure:

Day 1 The dehydrated specimens were taken from the acetone and excess solvent drained. The dehydrated solvent
filled specimens were then placed in the cold polymer reaction-mixture. The specimens were submerged
immediately to prevent solvent evaporation from the specimens and drying of their surface. The hollow parts of the
digestive tract were filled with the cold reaction mixture. A glass lid was then placed on the vacuum chamber and
left to equilibrate overnight.

Day 2 The vacuum pump was then turned on and run for 10 minutes to warm to operational temperature. The
vacuum chamber was then sealed by closing the needle valves and applying the vacuum. When the seal was
accomplished the pressure was allowed to lower slowly to 22cm Hg. The Bennert mercury manometer was now
ready to commence reading at this pressure level. The pressure was then stabilized by opening the needle valves
incrementally and slowly. Small bubbles begun to rise through the polymer-mix at this point.

Day 3 Pressure was slowly decreased 1/3 of the current daily value 22cm to 14cm Hg.
Day 4  Pressure was slowly decreased 1/3 the current daily value to 9cm Hg

Day 5  Pressure was decreased slowly 1/3 the current daily value to 6cm Hg

Daily decrease of pressure was continued until 1cm bubbles continually rise to the polymer surface and burst.

This process was continued for approximately four weeks. Impregnation was completed when no more bubbles appeared at the surface of the reaction-mixture for several hours and near zero pressure was maintained for a few days. This phase took approximately four weeks

**Table 2. Shows a summary of the Impregnation Schedule**

<table>
<thead>
<tr>
<th>Day</th>
<th>Description</th>
</tr>
</thead>
<tbody>
<tr>
<td>Day 1</td>
<td>Load specimens and allow to equilibrate overnight</td>
</tr>
<tr>
<td>Day 2</td>
<td>Start pump, slowly decrease pressure to atmosphere to: 22cm Hg</td>
</tr>
<tr>
<td>Day 3</td>
<td>Slowly decrease pressure, 1/3 current pressure to: 14cm Hg</td>
</tr>
<tr>
<td>Day 4</td>
<td>Slowly decrease pressure, 1/3 current pressure to: 9cm Hg. Air bubbles form but not continually rising</td>
</tr>
<tr>
<td>Day 5</td>
<td>If no bubbles rising, slowly decrease pressure 1/3 current pressure to 6cm Hg</td>
</tr>
<tr>
<td>Day 6</td>
<td>If bubbles actively rising to the surface and bursting, do not decrease pressure 1/3 current pressure to: 4cm</td>
</tr>
<tr>
<td>Day 7</td>
<td>Active bubbles, do not decrease pressure</td>
</tr>
<tr>
<td>Day x</td>
<td>When bubbles cease or slow dramatically, decrease pressure 1cm Hg</td>
</tr>
</tbody>
</table>

**2.2.5. Specimen Removal**

Vacuum pump was now turned off. Valves were opened and vacuum chamber and specimens were allowed to return to atmosphere pressure slowly. Specimens were left for 24 hrs in the reaction-mixture to equilibrate.

Day 1  Specimens were removed from the reaction-mixture and excess polymer-mix was allowed to drain into the vacuum chamber. Specimens were turned a few times to allow more polymer-mix from the crevices and hollow parts of the specimen

Day 2  Specimens were removed from the freezer and allowed to continue draining at room temperature

Day 3  Specimens were placed on paper towels and continued to drain

Day 4 or x Once the specimen no longer oozed silicone it was placed for gas curing for two weeks.
2.2.6. Curing

The equipments used for curing are Curing chamber, Aquarium pump and Desiccant.

2.2.7. Curing Procedure

Specimens were placed into the curing chamber together with a desiccant (CaSO4). Normal anatomical shape and position of the specimens were assumed. A small container of S6 gas cure was also placed into the curing chamber. The chamber was closed and S6 was vaporized using an aquarium pump. Specimens were wiped every 2-3 hours and turned on the first day. The specimens were removed after six days and placed in an air tight bag for further curing.

22.8. Teaching

Cadaveric and produced plastinated specimens of the same specimens were used in teaching veterinary gross anatomy courses for year II veterinary students, School of Veterinary Medicine, Faculty of Medical Sciences, The University of the West Indies, St. Augustine, Trinidad and Tobago

3. Results

The plastination unit at the School of Veterinary Medicine, University of West Indies, Trinidad and Tobago has produced good quality plastinated specimens such as gastrointestinal and reproductive organs of dog and pig, limbs of horse and dog, heart of ox and dog, brain of ox and sheep, spleen of dog and pig and chicken (Figures 1, 2, 3) since 2008. The thoracic and abdominal cavities and limbs of the dog were demonstrated in relation to their various structures of muscles, organs, nerves and blood vessels. These specimens were used as new anatomical models for teaching and studying veterinary gross anatomy courses. The plastinated specimens were stored occupying less space in the anatomy laboratory than the formalin preserved specimens which needed more space, containers and formalin. Also the formalin needed to be changed regularly to prevent mold growth.

Plastinated specimens have replaced some of the formalin preserved specimens. The use of plastinated organs decreased the euthanization of dogs and sheep which are usually required in each academic year since the plastinated specimens will last for a longer time. Many students expressed relief that the use of the plastinated specimens allowed them to study veterinary gross anatomy without exposure to the formalin hazard and that it also enhanced their knowledge and understanding. Further, plastinated specimens helped the students to understand the complicated structures of anatomical specimens and the topographic relations of the organs and were utilized not only in the dissection hall but also in the lecture room, both individually and in groups.

The produced plastinated specimens were smooth in texture, clean and odorless with realistic shape but with slight difference in the colour in some cases. On the other hand, testes showed slight shrinkage and a variation in texture and appearance (Figure 2/N).
There were many advantages for the plastinated specimens as a tool for veterinary anatomy teaching, however the plastination technique was sensitive and time consuming. Although the plastinated specimens were easier to work with, they could not completely replace the traditional dissection because students learn best by exploratory and hands-on dissection. Moreover, the wet specimens provided a more accurate visual representation of the body parts and skills to aid in dealing with actual clinical cases in the future. Therefore, the plastinated and wet specimens were used simultaneously depending on the topic being taught at the time. We look for increasing the number and quality of the plastinated specimen for different animal species.
Figure 1. A photograph showing the plastinated specimens of the left thoracic cavity of dog (A); stomach and spleen of dog (B); Stomach and spleen of pig (C); heart of dog (C); longitudinal section of heart of horse (E); thoracic limb (F).
**Figure 2.** A photograph showing the plastinated specimens of uterus of bitch (G); uterus of sow (H); right kidney of horse (I); longitudinal section in right kidney of horse (J); right kidney of ox (K); kidney of pig (L); kidney of dog (M); Testis of horse (N); Accessory genital glands and penis of boar.
Figure 3. A photograph showing the plastinated specimens of tongue of pig (P); visceral surface of pig liver (Q); cross sectional slices of ox brain (R); visceral surface of ox liver (S); plastinated chicken (T).
4. Discussion

Specimens are normally fixed with 10% formalin. There are many disadvantages in the formalin embalming like deterioration of specimens over time, Shrinkage of tissues, short term exposure limit (STEL) working with formaldehyde. In addition, formaldehyde is a carcinogen, butagen, mutagen and a teratogen which limits teaching and research. Storage of specimens also poses a challenge as there was no more space in the lab to facilitate anymore. Overtime the formaldehyde turns to formic acid. With the above considerations the present study was taken to prepare the specimens which are durable, free of offensive odour and dry which can be used for teaching aids both in the classroom and in the clinical setting [5,6].

The plastinated specimens were prepared by using the S10/S15 technique as a superior standard than the silicone plastinated specimens as well as specimens preserved by other mechanisms. Unlike formalin fixed specimens, surface clarity was very good. The plastinated exhibited more flexibility. They were durable and free of offensive odour. These reports were concurrent with the findings of [4] in the specimens of domestic animals.

There was a natural progression from the gross dissected specimens to the final plastinated product. Specimen preparation was a key factor when starting the plastination process in current study. The specimen was well planned before starting the research with the consideration of how the final product should look and proceeded with dissection to carry out the plan. More flushing with water in the digestive tract was done which is necessary to remove contents. Plastination is a lengthy process and considering the time frame for the project, dilation of hollow structures in the digestive tract was done for a short time. Rinsing with water for 24 hours to remove the formalin is important because if this is not carried out, formalin can be leaked out of the specimen into the acetone. All the above processes are concurrent with the reports of [7]. Though, the short time given for the dilation of the digestive tract in the current study, it did not have any adverse effect on the final product.

The time for fixation with formaldehyde is depending on the organ and species of the animal in the plastination process. In this study, fixation was done for 3 weeks with 10% formaldehyde which is found to be more plastination friendly.

Dehydration was the major stage in the plastination process. The principles behind dehydration was that it replaced tissue fluid/water (both inter and intra-cellular) with an organic solvent. This solvent must be miscible with water and preferably volatile enough to serve as a volatile intermediary solvent which is the key to the impregnation step. Common dehydration and intermediary solvents were acetone, alcohols and methylene chloride. Acetone was the universal dehydrating solvent for the plastination process because alcohol vapour pressure at -150°C is too low to be extracted gradually and continually and methylene chloride is not miscible with water. For tissues with a lot of fat methylene chloride can be used as a degreaser or defatting agent [2].

The principles behind the impregnation stage or forced impregnation were the replacement of the volatile solvent in a biological specimen with a curable polymer. For this to occur, the volatile intermediary solvent must be miscible with the silicone polymer and must have a sufficiently high vapour pressure at -15°C to be gradually,
continuously and completely extracted during the impregnation step of the plastination process. As the principle of dehydration was to replace tissue water/fluid with acetone, the impregnation principle was to replace the volatile intermediary solvent (acetone) with the reaction mixture of S10/S3 [2]. The reaction mixture is too viscous to come to equilibrium with the solvent. Therefore, a forced vacuum is used to get the reaction-mixture inside the specimen in the current study.

The solvent filled (acetone) specimens were submerged in the liquid silicone reaction-mixture in the plastination chamber which was in a -15°C deep freezer. Applying vacuum to the reaction-mixture and the specimens caused the solvent to vaporize/boil (at a known pressure) and left the specimen, passed through the reaction-mixture and finally the vaporized solvent was pumped out through the vacuum pump exhaust. This is manifested by bubbles rising throughout the reaction mixture. Vaporization of the acetone from the specimen leaves a tissue void or negative pressure inside the specimen and the reaction-mixture is drawn into the specimen. For this exchange to take place there must be enough time for the viscous polymer-mixture to enter the cells of the specimen. Therefore, if the pump speed is too great and generates excess vacuum too quickly, too much acetone will leave and the tissue will collapse. The collapsed tissue will not allow the viscous reaction-mixture to enter. This will result in shrinkage and desiccation of the specimen [4]. Two fine adjustment needle-valves were used for delicate control of pressure in the present study.

Preparation of the reaction-mixture was important step in the process of plastination. The S10 or S15 polymer was mixed with S3 (catalyst with chain extender) at 100:1 to prepare the reaction-mixture. Thorough stirring was necessary; a normal stick can be used. During mixing, the reaction-mixture first turned opaque then clear. This polymer-catalyst-mixture was deaerated by placing the mix in a vacuum chamber and reducing pressure to 30 cm Hg. The air was boiled out and the reaction-mixture was ready to be used at -15°C for impregnation or to be stored in the deep freezer preferably less than -25°C. In the present study the readymade mixtures were used to ensure proper curing.

Monitoring of bubbles in the impregnation stage of the plastination process was very important. The too quick evacuation of acetone/solvent result in incomplete impregnation of the specimen with the polymer-mix and shrinkage [2]. To avoid the shrinkage pressure was reduced slowly (1/3 of the current reading) over a four to six day period to the point where the intermediary solvent began to vaporize/boil and leave the tissue at a slow steady rate. This allowed the reaction-mixture to enter the tissue void created by the vaporizing and exiting acetone. The pressure at which the solvent reach its boiling point depends on the temperature and the solvent used. When at boiling point, 1 cm bubbles rose continually to the surface and burst. Before this point a few sporadic bubbles rose but likely sit on the polymer surface. For acetone, this steady extraction of solvent at -15°C occurs around 3 cm Hg pressure or 1.5 cm Hg pressure at -25°C. If bubbles were actively rising to the top of the polymer and bursting, do not decrease pressure. It was found that decreasing pressure slowly to be effective than at a faster rate. Acetone removal and have polymer impregnation at cold temperature will take 3 to 5 weeks depending on volume of specimens and pump speed. When bubbles ceased to rise, slowly and incrementally the needle valve was closed to decrease pressure until active bubbles started to rise again. Usually it is necessary to lower pressure only 1-2 mm Hg.
to continue active bubble production. It took a few minutes after incremental valve closure before bubble production was observed. All these steps were performed as [2].

The impregnation reaction-mix within the specimen is cross-linked and the specimen made dry during curing. This is a two-step process consisting of chain extension and cross-linkage of polymer. Chain extension of the silicone molecules is an end to end alignment, thus forming longer chains using the chain extender portion of the S3 impregnation-mixture which is now in the specimens. This first reaction, known as pre-curing, results from the reaction of the S3 with the S10. This is known as slow cure. It is advantageous to pre-cure for at least two or three days. Longer pre-curing is even better and may yield more flexible specimens [2]. In the current study the curing was done as fast cure which also yielded good results.

The Specimens were drained for few days before curing. Fast cure at this timing will likely take at least one week to complete. The specimens were allowed to at room temperature for a few days to allow maximum chain extension. During this period, the specimens kept in anatomically correct position and dilated to assure correct position as chain extension progresses. Theoretically, chain extension starts when the S3 (catalyst and chain extender) and S10 polymer were mixed. This reaction slowed down dramatically by cold temperature (less than -15°C). At room temperature, the elongation occurred at an increased rate. The above findings are in concurrence with the reports [4].

Veterinary anatomy is a vast subject which has very extensive contents and needs more time for its understanding and memorizing, so that teaching of veterinary gross anatomy using different methods such as books, CD and dissection as well as different types of specimens such as cadaveric and plastinated was important for the students to understand anatomy. Using the traditional teaching methods such as didactic lectures and dissection of the cadavers for certain topics such as gastrointestinal and reproductive organs as well as brain and heart were not enough for better understanding, so such organs were plastinated and demonstrated for students for better understanding of these topics [7, 8].

Handling specimens preserved by traditional methods are discouragingly difficult [9]. The plastination process was used for a variety of species for producing plastinated specimens which are used as teaching aids in veterinary schools. However the limitation was the size of the impregnation chamber [10-15]. Plastination allowed specimens to be durable, dry, odorless, non-irritant, and easy to hold in the hand, nontoxic, prevent the exposure to formalin hazard and may even have a beautiful colour [16]. Minimal technical skills were needed to get plastinated specimens but the problem was the costs of purchasing the chemicals such as polymers, curing agents and building the chambers [17]. Plastination process as a new tool helped the better understanding the complex structure of the animal body but didn’t replace the traditional method of dissection [18, 19].

Anatomical relationship between the different structures of the thoracic and abdominal cavities and limbs of the dog were demonstrated, similar to [20] who demonstrate the relationship between the structures of the neck, abdominal region and lateral thoracic wall of the whole goat kid cadaver.
Most of the plastinated specimens kept their original shape, however, some specimens showed shrinkage and
decreasing in size such as testes and may be due to incomplete dehydration of the organ or the type of silicon used
and this will recommend trying other polymers [3, 21).

Conclusions

Plastination of well dissected specimens provided plastinated specimens which were a perfect tool for prolonged
preservation. Plastinated specimens were odorless, dry, non-irritant, nontoxic and easy to hold in the hand. The
plastinated specimens were appreciated by the staff members and students. Plastinated specimens gave a new
teaching method and tool for the veterinary gross anatomy teaching and learning as well as better understanding of
anatomy of the animals. Plastination specimens were not completely replaced the traditional guided animal
dissection. Some problems were occurred such as time consuming, high cost of the chemicals and limited size of the
impregnation chamber.

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Conflict of interest

The authors declare that they have no conflict of interest.

Author contributions

The first author dissected the specimens and wrote the manuscript. The second author prepared the specimens. The
two authors revised manuscript.

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