VETERİNER HEKİMLER DERNEĞİ DERGİSİ
JOURNAL OF THE TURKISH VETERINARY MEDICAL SOCIETY

ISSN: 0377-6395
e-ISSN: 2651-4214
Dergi ana sayfası:
Journal homepage:
http://dergipark.org.tr/vetheder

doi: 10.33188/vetheder.998978

Derleme / Review

From toxic cadavers to biosafe specimens: a brief history of plastination in veterinary anatomy

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MAKALE BİLGİSİ / ARTICLE INFORMATION:

Geliş / Received:
30 Eylül 21
00 December 00

Revizyon/Revised:
16 Aralık 21
16 December 21

Kabul / Accepted:
23 Aralık 21
23 December 21

Keywords:
History,
Plastination,
Veterinary Anatomy

Anahtar Sözcükler:
Plastinasyon,
Tarihçe,
Veteriner Anatomi

ABSTRACT:

The teaching of veterinary anatomy was started in line with the establishment of the first veterinary school in Lyon in 1762. During this time fewer tools and techniques were available for displaying and visualization of organs in order to teach anatomy. Over the years, many methods, tools, and techniques that are essential for veterinary students and anatomists were developed. Teaching materials like cadaver which preserved by formalin has being using for a long time. Instead of preserved cadavers, various teaching materials have also been using in different parts of the world. These alternative techniques were not hopeful to meet the professional challenges in the medical and veterinary sciences. In 1977, Dr. Gunther von Hagens came up with an exclusive method, for the preservation of biological materials, called plastination. In the process of plastination body fluids and lipids in biological tissues replace with epoxy silicone and polyester polymers. Plastinated specimens are an excellent alternative to formalin-fixed specimens. Plastination brings not only three-dimensional and cross-sectional specimens for teaching and research, but also durable, clean, non-toxic materials for students. Plastinates are also convenient to handle, transport, and store. Three major methods used in plastination are silicone, sheet plastination with epoxy method and sheet plastination with polyester method. Silicone plastination is the most adaptable technique for cadavers, whole body or organ slices. Relative to the other plastination methods, it is widely used for teaching veterinary anatomy. Sheet plastination with polyester resin has been used for the production of opaque brain slices, while sheet plastination with epoxy resins are used for transparent body or organ slices. Using of anatomic specimens after plastination can be quite efficient for both theoretical and practical courses.

Toksik kadavralardan, biyolojik güvenli örneklere plastinasyonun veteriner anatomideki kısa tarihçesi

ÖZET:

Veteriner anatomi eğitimi, 1762 yılında Lyon'da ilk veteriner okulunun kurulması ile başladı. O dönemlerde anatomi öğretimi için organların sergilenmesi ve görülmesi amacıyla oldukça sınırlı yöntemler ve teknikler bulunmaktaydı. Yıllar içerisinde, veteriner hekimliği öğrencileri ve anatomistler için vazgeçilmez olan birçok yöntem teknik ve ekipman geliştirildi. Kadavralar gibi formalin ile hazırlanmış eğitim materyalleri çok uzun zaman kullanıldı. Dünyanın farklı yerlerinde kadavraların muhafaza edilmesi yerine çeşitli öğretim materyalleri de kullanılmaktadır. Bu alternatif teknikler bile, tıp ve veteriner hekimlik bilimlerindeki mesleki zorluklarla başetme konusunda çok umut vaad etmiyordu. 1977'de Dr. Gunther von Hagens; biyolojik materyallerin korunması için plastinasyon adı verilen özel bir yöntem geliştirdi. Plastinasyon sürecinde, biyolojik dokulardaki vücut sıvıları ve lipidlerin, epoksi silikon ve polyester polimerler ile değiştirmesi sağlanmaktadır. Plastine edilmiş örnekler, formalinle tespit edilmiş numunelere göre mükemmel bir alternative olarak görülmektedir. Plastinasyon, sadece öğretim ve araştırma için üç boyutlu ve kesit örnekler değil, aynı zamanda öğrenciler için dayanıklı, temiz, toksik olmayan materyaller de sağlar. Plastinatların işlenmesi, taşınması ve depolanması da oldukça kolaydır. Plastinasyonda kullanılan başlıca üç yöntem; silikon, epoksi yöntemiyle kesitsel plastinasyon ve polyester yöntemiyle kesitsel plastinasyondur. Silikon plastinasyonu; kadavra, tüm vücut veya organ kesitleri için en uygun tekniktir. Diğer plastinasyon yöntemlerine göre veteriner anatomi öğretiminde yaygın olarak kullanılmaktadır. Opak beyin dilimlerinin üretiminde polyester polimer ile kesit plastinasyonu, şeffaf gövde veya organ dilimleri için de epoksi resinlerle kesit plastinasyonu kullanılabilmektedir. Anatomik örneklerin plastine edildikten sonra kullanılması; hem teorik hem de uygulamalı dersler için oldukça verimli olabilmektedir.

How to cite this article: Yunus HA, Ekim O, Bakıcı C, Batur B, Çakır A. From toxic cadavers to biosafe specimens: a brief history of plastination in veterinary anatomy. Vet Hekim Der Derg 2022;93(2): 158-165. DOI: 10.33188/vetheder.998978.

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1. Introduction

Study of veterinary anatomy was started as comparative anatomy with the help of Hippocratic teachings. Hippocrates (460–370 BCE) studied on the brain of humans and other animals. He stated that the brain is divided equally into two with a thin vertical membrane (1). The early Italian scientist Alcmaeon further improved anatomy by animal dissections and observing dissimilarities between arteries and veins (2). Later on, the Greek anatomist Galen founded several anatomical theories, through animal dissection (1). The teaching of veterinary anatomy was started in line with the foundation of the first veterinary school in Lyon in 1762 (3). During these periods, there was a scarcity of teaching tools. Through time, many methods and techniques that are essential for veterinary students and anatomists were developed (4). Veterinary education is the foundation of every veterinarian, with its ultimate goal to produce highly skilled, and practically equipped professionals who give priority to diseased animals (5).

Practical-learning assists students in identifying their real interests and choose a career accordingly. Teaching materials like cadavers have been using for such purposes. Without preservation, using of cadavers for long period is impossible. In normal situation organs decompose within few days. Decomposition is essentially a biological and biochemical phenomenon, caused by enzymes that are already present in the body, by digestive enzymes and the activities of exogenous flora and fauna colonizing the remains (6, 7). Tissues, organs, and body parts are also kept for various reasons, including research, teaching, record-keeping (museum specimen), and forensics. Therefore, specimens can be prepared with various chemicals such as alcohol, Bouin's solution and widely formalin solution (8). Even though formalin has been using for a long period of time in different parts of the world, still, is not promising to overcome current problems in the veterinary sciences (9).

There are several disadvantages with the use and handling of formalin preserved specimens. Loss of colour associated with long term use, and difficulty in retaining the three dimensional orientation of hollow and branching specimens are the most prominent ones (10). On the other hand, there are serious health concerns for the use of formalin mainly for anatomists, technicians, medical or veterinary students, and embalmers. Inhalation of even small quantities of formaldehyde can cause respiratory symptoms and eye, nose, and throat irritation, and could result in pneumonia and bronchitis. Furthermore, there is a report that indicates formaldehyde could cause nasal cancer in rats (11, 12). Following this, in 2011, the National Toxicology Program, an interagency program of the Department of Health and Human Services, named formaldehyde as a known human carcinogen in its 12th Report on Carcinogens (13). In order to overcome the problem other techniques for teaching purposes have been explored (14). Dr. Gunther von Hagens of Germany developed a unique technique for tissue preservation called plastination. From this point of view authors aimed to prepare a comprehensive review about this unique preservation technique.

2. Plastination

In 1977, while working as a research assistant at the University of Heidelberg's Institute of Pathology and Anatomy, Dr. Gunther von Hagens observed medical students struggle to work with cadavers that quickly decomposed and invented a specific method used for long term preservation of tissues (15). Dr. von Hagens published his first paper describing his invention in 1979. His finding got acceptance not only from academicians and researchers in the anatomy area but also from a wide range of communities all over the world (16). Moreover, von Hagens established the BIODUR®, a commercial company for plastination materials and equipment, and held the Body Worlds® exhibitions to reach easily different parts of the world. In the meantime, Dr. Harmon Bickley played a major role for the establishment of International Society for Plastination (ISP) and Journal of ISP (17, 18, 19). Plastination has led to a major expansion in providing anatomical specimens for teaching. But exhibitions of plastinated human bodies or parts of human bodies raised ethical concerns. Especially using them as an anatomical art raised ethical debates among scholars (20). Although such kind of ethical concerns for human body plastination, nowadays there are more than 400 laboratories in 40 countries around the world using plastination to prepare specimens for academic and research purposes.

Organic tissue with its natural state cannot stay more than a day without decomposition. The decomposition process occurs by two mechanisms, autolysis and putrefaction (6, 7). Both types occur in the presence of body fluid. Autolysis, self-digestion, is a cellular self-destruction process caused by hydrolytic enzymes that were originally found within cells (21) while putrefaction is the destruction of the soft tissues by the action of micro-organisms (bacteria, fungi, and protozoa) (22). The principle behind plastination is to intervene in the natural ongoing process of decomposition (10). Body fluids, even lipids, that are naturally found in the tissues substitute by polymers commonly epoxy, silicone, and polyester. Following this process, the specimen becomes hardened but maintains its natural look (19), and the decomposition is intercepted. Overall, the plastinated tissues are dry, non-sticky, odorless, chemical-free, and harmless, to some extent flexible, with detectable morphological structure, and almost retain their natural form (23).

Standard steps of plastination include fixation, dehydration, forced impregnation, and hardening (24). Fixation aims to stop autolysis and preserve cell/tissue form as original as possible (25). Fixation converts proteins of the body to a longer-lasting substance by forming cross-linkages between adjacent protein molecules. The formation of this high molecular cross-linked lattice causes loss of ability to retain water, thus preventing decomposition (26). The second step is dehydration. This step is necessary because polymers cannot directly replace lipids and water. They have to be removed and replaced by the medium of dehydrating solvents like acetone, alcohols, and methylene chloride (27). Under freezing conditions, the acetone draws out all the water and replaces itself inside the cells (28) through prolonged exposure and diffusion (26). Forced impregnation is performed by a variety of curable polymers which replace acetone, taking advantage of differences in their chemical properties (19). Since the acetone has a higher vapor pressure and low boiling point than the polymer mixture, the acetone evaporates while the polymer mixture will not when the vacuum is applied (26, 29). Hardening is the last step for plastination and can be performed with a gaseous liquid, ultraviolet light, or heat (30). After drying of specimen surface, it is placed in an air-tight bag and this provides the ongoing internal hardening of the polymer (31). During impregnation, chain extender should be applied for better results. Chain extension causes long-chained silicone molecules and this also provides better cross linkage during curing-hardening process. Qualified chain extension and cross-linkage enables the silicone polymer molecules and specimens eventually to be view as 3-D lattice (32).

3. General Advantages and Disadvantages of Plastination

Plastination has led to a major expansion in the range of anatomical specimens available for teaching general gross anatomy, sectional anatomy, neuroanatomy. Due to problems associated with formalin preserved specimens, students are reluctant to examine or handle such materials (28). Plastinated specimens are an excellent alternative to formalin-fixed specimens. Plastination technique provides durable, non-biohazardous, non-toxic specimens for students and researchers. Besides, plastinates are clean, dry, touchable, odorless anatomical materials not only for the anatomy labs, but also for the clinical and preclinical courses (19). Since specimens preserved by plastination look natural, it offers relatively more detailed features of the organs (33). They are also convenient to handle, transport and store (34). Plastination can preserve a specimen for more than 40 years. Therefore, this reduces the number of cadavers needed for the academic purpose of teaching, and research (35). It also produces an ideal specimen for museums and exhibitions (36). Thin sections of the specimen made by sheet plastination preserve the microscopic structure of the tissues (19) and therefore, studying topographical anatomy is much more efficient (37).

However, there are several disadvantages for plastination technique and the most prominent one is the financial cost. Although there are several techniques with reasonable costs which seem to be alternative for plastination, most of those cannot provide nature identical specimens in terms of anatomical details, colour or texture. Health hazards, physical hazards and biohazard are the other disadvantages which may occur during the plastination steps but not after the end of the plastination process. Occupational exposure to hazards can occur in the processing of specimens. Organic vapors, like acetone or other flammable chemicals, have a risk of causing fire and explosion is used in plastination laboratory (38). A considerable amount of evidence suggests that long-term occupational exposure to organic vapors has detrimental effects on the central nervous system (39). Using the combination of measures like proper laboratory

furnishings, strict laboratory safety regulations, and well designing of the equipment used for plastination are suggested to prevent such risks (38).

4. Special Advantage of Plastination for Veterinary Anatomy

A numerous number and variety of animals have been sacrificed worldwide during attempts to teach practical skills or to demonstrate scientific principles (40). The vast majority of animals used in the field of education are used for dissections. In some cases, healthy animals are euthanized to teach veterinary anatomy and surgery to students (41) and this brings serious ethical concerns. Eventhough recorded data for every country is not available, there are some data for some countries which indicate the number of animals sacrificed every year for teaching purposes. To give some examples, an estimated 10-12 million animals are euthanized per year in North America for education (42, 43) while, every year, an estimated 1,000 calves are killed in India to teach veterinary anatomy and surgery to students (44). As a result, society concerning for animal rights are organizing effective protests. Considerable number of researchers are discouraged from several studies in which animal dissection and related practices are handled. The severity of killing and dissection of animals for the teaching of veterinary anatomy is high in an underdeveloped and developing countries in which the teaching method was not well supported by non-invasive preparation methods, such as computer simulations, high-quality videos, ethically-sourced cadavers and formalin preserved specimens. Currently, a growing number of educators are choosing practical teaching with the ethical use of a minimum number of animals. There are other reasons which made educators look for other alternative practical teaching methods. For the veterinary anatomy practices, the cadavers of large animals like horses, or large ruminants can be compelling due to the size of these species. Removing of large specimens from a fixative solution for displaying can be somehow a challenging process for lab staff. In addition, there may be difficulties in identification of the formalin preserved organs of different animal species for the students due to the negative effects of formalin fixation. All of these problems can be overcame by a dry, odorless, nature identical and durable specimens by employing long-term preservation technique of the biological tissues, plastination.

4. Discussion and Conclusion

The three major methods are silicone plastination, sheet plastination with epoxy polymer, and sheet plastination with polyester polymer. Silicone plastination is the most versatile technique which can be used for cadavers, whole body or organ slices (45, 31). A study reported by Pendovski et al. (19) indicated that the S10 technique may be used for producing at least 2-3 mm organ slices. This study was conducted on kidney of pig (19).

A study conducted to validate the use of silicone plastination techniques for the preparation of long-lasting anatomical specimens for teaching of Veterinary Anatomy, showed that silicone plastinates are utilized as a teaching aid and anatomical museum models than formalin fumed specimens. In this study freshly collected liver, testis, tongue of adult goat and spleen of adult horse were plastinated. Except for it slight shrinkage of the rough surface of the liver of goat, the other plastinated organs were as good as fresh organs (46). The heart and kidneys of a cow, the lungs and attached trachea of a ram, and the penis and attached testicles of a ram were plastinated with silicone and except for the lungs, which discoloured and lost their natural shape, the others are satisfactory for use as teaching aids (47). Anatomical relationship of various structures of superficial muscle layers of various body parts were well demonstrated in the whole goat cadaver preparation (46). Similarly, longitudinal sections of horse limbs were plastinated with silicone by Menaka and Chaurasia, (48) was more pleasant to touch and easy handling for demonstration of superficial and deep layers of tendons, ligaments, muscles and bones as well.

Gastrointestinal and reproductive organs of dog and pig, limbs of horse and dog, the heart of ox and dog, brain of ox and sheep, spleen of dog and pig, and chicken were plastinated at the School of Veterinary Medicine, University of the West Indies. The plastinated specimens were displayed for staff and students, and it was appreciated (49). Bakici et al. (50) conducted silicone plastination on formalin fixed and non-fixed dog stifle joint. Their findings indicated that

the fixed plastinated specimens were quite elastic in respect to morphological features, and this fixed plastinated specimens were relatively good in terms of education and research purposes.

A report from the University of Córdoba showed that plastinated specimens of male genital organs were well accepted by the students. Not only for students it can also be exhibited in anatomy museums. The Biodur S10 technique of plastination was useful in understanding and learning the male genital organs in Veterinary Anatomy (51). Similarly, internal genital organs of adult cows were plastinated by Akgün et al. (52) and indicated that the plastinated specimens look like a natural genital organ and their normal anatomy was well preserved. They also suggested that specimens preserved by plastination can be effectively used in anatomy and clinical educations. Relatively, from the three major methods used in plastination, silicone plastination is commonly used in veterinary anatomy for purpose of teaching, anatomical museum demonstration, and research purposes (53).

Sheet plastination is a type of plastination that is considered to be a dynamic tool in the improvement and clarification of concepts of sectional anatomy (54). The introduction of sheet plastination has provided us an opportunity to combine modern cross-sectional imaging techniques with corresponding slices of human and animal tissues (55). Sheet plastination and the classic silicone plastination techniques utilize similar basic principles. There are two types of sheet plastination, sheet plastination with the epoxy method and sheet plastination with the polyester method. Sheet plastination with polyester resin is used for the production of opaque brain slices, while sheet plastination with epoxy resin is used for transparent body or organ slices.

Transparent plastinated slices (E12 technique) are important illustrative materials and learning tools for veterinary students and allow them a better and more accurate understanding of complex anatomical structures. Five intact equine toes were processed by using the standard E12 technique. On plastinated slices, synovial structures, as the synovial cavity is fully transparent. The bursa podotrochlearis as well as its surrounding structures were explored and details were investigated. Especially at the mesoscopic level of the equine toe, which connects the macroscopic and microscopic levels, plastinated slices can be used successfully to identify anatomic structures (56). Ottone et al. (57) obtained 2 mm thick coronal sections of two fresh rabbit heads, with high anatomical quality and definition, and strong colour contrast among several morphological structures. There was no shrinkage of tissues, including the brain, in conserving the sections of the original tissue shape. Plastinated slices with E12 technique have a great impact, not only for teaching purposes, but also for giving training programs in sectional topography, for cross-checking and training specialists in computed tomography and magnetic resonance imaging (58).

In the polyester plastination method, the tissue fluid is removed and it is replaced with a durable polyester resin. This method can be used for head slices, brain slices, and body slices (31). It is suitable to study the anatomical formation of 4 - 8 mm slices of nervous tissue (59) and for producing 3-4 mm semitransparent brain slices (60). A female cape dolphin was plastinated with Hoffen polyester (P45) resin by cutting the head into 43 and trunk into 348 with a thickness of 3.0 mm slices. A modified polyester sheet plastination technique was performed in this study. The tissue sections produced using this technique exhibit clear delineation between different tissues. The sections provide visually detailed information about the morphology of the dolphin (61). On the other study, horse legs were plastinated with Hoffen® polyester (P45) resin to assess the student's satisfaction with the use of P45 sheet plastinated slices in the teaching of Anatomy, Pathology, and Radiology. The result indicated that the P45 sheet plastinated specimens were clear, dry, odourless, durable, easy to handle and there were non-hazardous associated with their uses. Of the participated students, 90% of them were satisfied depending on plastinated models in medical classes (62). An unembalmed feline cadaver, after freezing, is divided into five regions (head, neck, abdomen, thorax, and pelvis) for sheet plastination with BIODUR® P40 polyester resin. Plastinated body slices displayed excellent anatomical detail of all tissues that were observed and they were good aids for teaching and research in classroom and laboratories (63).

Plastinated specimens are definitely the perfect way of preserving biological specimens. It produces a long-lasting, non-hazardous, and almost natural-looking specimens which helps veterinary anatomy. Furthermore, using of plastinated samples can reduce the sacrification of animals and other problems associated with using cadavers. Plastinated specimens are also better options to be used in anatomy museums and related exhibitions. However, plastinated specimens could not completely replace the traditional preservation and dissection methods. Therefore, the plastination and other anatomical preservation techniques can be used alternatively in anatomy lab and courses.

Conflict of Interest

The author declared no conflict of interest.

Funding

No financial resource has been received during the execution of the study.

Authors' Contributions

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Ethical Statement

An ethical statement was received from the authors that the data, information and documents presented in this article were obtained within the framework of academic and ethical rules and that all information, documents, evaluations and results were presented in accordance with scientific ethics and moral rules.

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