ADVANTAGES OF USING SEVERAL METHODS OF PRESERVING ANATOMICAL SPECIMENS
(SUMMARY OF Ph.D. THESIS)

SCIENTIFIC COORDINATOR:
Prof. DAMIAN AUREL, PhD

Ph.D. STUDENT:
DUMITRU IOANA-MIHAELA

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INTRODUCTION

The preservation of anatomical pieces is a topical subject for various reasons, starting with the observance of certain religious precepts, in the case of humans, to the production of didactical material for anatomy studies. Along with the evolution of science, the prevention of tissue, organ or cadaver decay has become a necessity for numerous domains of research in biology and medicine but also for the teaching institutions related to them.

Currently multiple techniques of anatomical pieces preservation are applied in anatomy laboratories, all aiming to meet certain quality standards such as: the processing and maintenance of the pieces must be as simple as possible, the characteristics of live tissue must be preserved as closely as possible and the items obtained must not jeopardize the health of people handling them.

Considering the need of a laboratory for this type of anatomical pieces, there are 4 tissue, organ and cadaver processing and preservation techniques. These can be classified according to the preservation degree in techniques for the preservation of osseous pieces, boiling, maceration and techniques for the preservation of soft tissues, mummification and plastination.

From a historical perspective, mummification is the oldest tissue preservation technique, dating back to the age of ancient Egypt.

Although anatomical pieces processing techniques are relatively simple and accessible, there are very few studies offering information with scientific value on the process of obtaining them.

**Key words:** anatomical pieces, preservation techniques, boiling, macerations, formalin preservation, mummification, plastination
AIM, MOTIVATION AND OBJECTIVES

The main aim of this research is to bring new scientific data on techniques for obtaining anatomical pieces.

Given the lack of a standard protocol for obtaining anatomical pieces, and that research addressing this area are relatively brief, this study aims to make a comparative assessment of the existing techniques and improving them.

A secondary aim was to evaluate certain adjuvant substances for the preservation process, such as: technical glycerin, acrylic paint, fixative spray, commercial whitener and sodium bicarbonate.

The elements of originality of this study consist of using and improving techniques briefly described in literature such as boiling and maceration; offering scientific data meant to help establish standard procedures for obtaining osseous pieces by boiling and maceration. Another originality element is the use of various combinations of preservative substances and evaluating their results on the final product, for the mummification and the formalin preservation techniques. This research also stands out by its descriptive approach of the newest methods in the field of anatomical exhibits, respectively by applying and implementing the plastination technique for the processing of veterinary anatomical pieces. We believe this study can be used as a practical guide for anatomy laboratories and also but also has a high practical scientific value in the field of processing and preserving anatomical pieces. Our research brings new knowledge in a field that, while being familiar, has been less studied from a scientific point of view, opening the way for new studies based on the results presented by this thesis. We hope that the elements comprised in our research will provide valuable information to those who venture in this field interlacing science and art.

Main objectives:

The present research brings new data on the applicability of the techniques of obtaining anatomical pieces by evaluating the results obtained by applying them on different species.

The investigations included in this paper are meant to highlight in a comparative manner the efficacy of the existing techniques from literature and of the modified ones, and, to eventually establish a standard of the technique application according to the desired type of piece.

The major objectives of this research are:
1. Comparative evaluation of the osseous pieces processing techniques, in view of improving the equality of the pieces by adding various degreasing and bleaching substances, dyes and fixative sprays;
2. Application of the mummification technique on various animal species and introducing dying agents and ventilation in the process of obtaining the mummies.
3. Evaluation of the formalin preservation technique, on various corporal regions and viscera;
4. Implementing the plastination technique and evaluating its results.
STRUCTURE OF THE THESIS

The doctoral thesis titled „The advantages of using various anatomical pieces preservation methods” extends over 148 pages, being composed of 2 parts, structured in 5 chapters each. The latter have been complied in compliance with the norms of the Doctoral School of USAMV Cluj-Napoca.

The I\textsuperscript{st} part, composed of 5 chapters extending over a number of 28 de pages, presents the state of the art in the domain of obtaining anatomical pieces, as well as the chronological evolution of tissue preservation methods. The literature study constitutes a solid base for the experimental investigations comprised in the original research.

The II\textsuperscript{nd} part, structured in 5 chapters, over a number of 94 pages, is comprised of my own research in the field of obtaining and preserving anatomical pieces. The own research chapters are structured according to the requirements of the Doctoral School of the USAMV-Cluj-Napoca, respectively: objectives, materials and methods, results, discussions and partial conclusions.

The last chapter, named „General conclusions and recommendations”, illustrates the information obtained following the research carried out in this study. The thesis then presents the reference list with a number of 173 literature titles selected according to their relevance for the conducted studies.

Finally, the summary of the thesis can be found, in Romanian and in English.

METHODOLOGY AND RESEARCH RESULTS

The methodology of the own research conducted in the domain of obtaining and preserving anatomical pieces is in agreement with the usual practices of anatomy laboratories. The main parameters, on which the evaluation of the results was based, were comprised of qualitative criteria, respectively: aspect, preservation degree, durability.

In the following, we will present the main aspects of the original own research.

Chapter II.1 presents the application of the boiling technique for obtaining bone. This chapter has as main objective the evaluation of the efficiency of this technique for obtaining osseous pieces from birds and pigs. The original element of this chapter is the use of pressure containers to shorten the duration of the boiling. The results obtained after applying the boiling technique have offered information with practical applicability for obtaining osseous pieces in the case of the studied species.

The boiling technique is easy to apply, due to its low cost, but it demands patience and attention from the person doing the maneuvers. After the boiling of the skeleton, the muscle and cartilage residues were removed, to clean the bones, and then the latter were immersed in hydrogen peroxide for bleaching and in acetone for degreasing, allowing 24 hours for each process, which, if not correctly carried out, deteriorate the bones, leading to an unsatisfactory final result. Applying this technique on bird cadavers has revealed the fact that it is not efficient on skulls, due to their small size (Fig. 1; Fig. 2).

The fine and fragile skull bones have been deteriorated visibly by both methods. Although more efficient in obtaining the osseous pieces, the use of a pressure recipient has deteriorated the delicate structures of the bird’s skull (Fig. 1).

The bird skull obtained by the classic technique has well maintained its integrity, although it was unsatisfactory from a qualitative point of view (Fig. 2).
In the case of the thoracic zonoskeleton (scapula, coracoids and clavicle), the scapulo-coracoid joint was preserved intact, but the clavicle detached itself and the segment articulating with the scapula was destroyed. Except the thoracic wall, the skeleton of the thoracic cavity was adequately preserved. The best results of applying this technique were obtained for the thoracic and pelvic limbs (Fig. 3).
In the case of the thoracic autopodium, we noted that it took 2 hours and 30 minutes until the remains of musculature and tendons started to detach off the bones. After the completion of the process, the bones were removed, as much as possible muscle and tendon remains were cleaned off with the help of dissection instruments, and the bones were immerged in water with a commercial detergent for degreasing, a process which has lasted 12 hours.

In the case of the autopodium obtained by means of the classic technique, we noticed that the bones presented a greasier aspect, with visible grease spots and a higher degree of muscle and tendon retention, necessitating ulterior processing (Fig. 4).

Fig. 4. Pig autopodium obtained through the classic boiling technique

In the case of the autopodium obtained through the pressure boiling technique, we noticed that the obtained pieces have a superior quality when compared to those obtained through the classic technique (Fig. 5). Using the pressure recipient resulted in a piece with a better degreasing degree, with a smaller number of grease spots and with a better detachment of adjacent tissues. Furthermore, we have noticed that the adherence of the adhesive we have used has been much improved in the case of the piece boiled under pressure. The pressure boiling was also more efficient from the point of view of the duration of the process, the latter being reduces by 30 de minutes.

Fig. 5 Osseous pieces mounted on a base
The literature indicates that the boiling technique consists of 5 essential steps: boiling, degreasing, drying, bleaching and mounting on a base (Allouch, et al., 2014), other authors considered only the first 4 steps as being essential (Allouch and Sheikh, 2008), steps which deteriorate the pieces if they are not correctly carried out.

The studies concluded in this field (Hussain, et al., 2007) indicate that the necessary maneuvers for obtaining quality pieces may vary, according to the type of the bone and the species of the animal.

<table>
<thead>
<tr>
<th>Bone type</th>
<th>Large animal</th>
<th>Small animal</th>
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<tr>
<td></td>
<td>Boiling (h)</td>
<td>Degreasing (days)</td>
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<tr>
<td>Long bone</td>
<td>6</td>
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<tr>
<td>Vertebras</td>
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<td>Skull</td>
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<tr>
<td>Sesamoid bones, phalanges</td>
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Regarding the maneuvers, through this research we have concluded that a prior dissection of the cadaver leads to shortening the working time, by reducing the quantity of tissue adjacent to the bones.

Taking into account the boiling steps mentioned in literature (Allouch, et al., 2014), respectively: boiling, degreasing, drying, bleaching and mounting, our research indicate the introduction of the dissection in this process as an initial step.

As an originality element, this research proposes the use of pressure recipients (cookers) to shorten the processing time. Pressure cookers also offer the advantage of higher quality pieces.

Using a preliminary dissection of the anatomical pieces facilitates the processing of a larger number of specimens and shortens the boiling time.

In 2007, Hussain et al. tested this technique by applying and comparing it on horses, bovines, dogs and poultry, obtaining good results at an average boiling time of 6 hours for large animals and 4 hours for small animals. Using the pressure cooker has shortened the actual boiling time by 30 minutes.

The advantages of the boiling technique are that it is easy to apply and its cost is low, but it demands patience and attention when performing the maneuvers. Its application on bird cadavers has revealed that it is not a good method for skulls, because of their fragility.

Chapter II.2 had as main objective the investigation of the maceration technique as described in literature (warm water maceration and insect maceration). The purpose and motivation of the research comprised in this chapter was the application of the maceration techniques on different species and making a comparative evaluation of the results.

The maceration is defined as a controlled form of putrefaction, a decomposing stage in which the proteins are anaerobically consumed by bacteria (Sommer and Anderson, 1974).

Following the application of the warm water maceration and cold water maceration we have registered notable differences regarding the processing time and the quality of the pieces.

By applying the cold water maceration technique we have obtained in 6 days a satisfying product regarding the quality, the anatomical integrity and the color of the pieces. The only inconvenient was the prolonged maceration time which led to the destruction of the alveolo-dental ligaments, resulting in the detachment of the teeth.

The warm water maceration only took 3 days of working time. Thanks to this, the alveolo-dental ligaments were preserved intact, resulting in a superior quality of the final product.

Comparatively, both techniques have produced similar items, the only inconvenient being the reattachment of the teeth necessary in the case of the piece macerated in cold water (Fig. 6 and Fig. 7).
By applying the sun heated water maceration technique on the roe deer skull (Fig. 8) we have obtained a piece of a satisfactory quality. However, it must be mentioned, that compared to the other techniques, the long waiting time necessary in this one led to the production of a soft and brittle piece. Because of this, the nasal bones, the maxilla bines and a part of the body of the mandible broke after a simple handling of the piece. Furthermore, the sun heated water maceration technique is especially not recommended for young specimens, where as the ossification process is not complete and thus the obtained pieces will be even more fragile and will not be resistant to handling.

The result of the maceration process with help of insects was an anatomically whole item. The inconvenience of this technique is that of color modifications of the piece (Fig. 9). This was not corrected by the bath with the commercial detergent, as the latter acted as a degreasing agent only, while the piece maintained its altered colors. Disregarding the esthetical changes due to the color modification, the obtained piece can be considered suitable to be used in osteology studies, as the anatomical characteristics have been adequately maintained.
The *Dermetidae* maceration was applied on the chicken body for 5 days, with remarkable results on the thoracic and pelvic zonoskeletons, with an excellent preservation of the anatomical characteristics of the small and brittle bones comprised in these regions (Fig. 10). The substances used for degreasing and bleaching have acted adequately as well, producing a well degreased and well bleached item.

Unlike its use on the chicken cadaver, applying this process on rabbits has revealed differences of quality of the obtained pieces, namely that the small bones of the sternal, costal and thoracic zonoskeleton regions detached themselves (Fig. 11). Both acetone and gasoline have adequately degreased the pieces. The hydrogen peroxide has yielded remarkable results in bleaching the pieces, compared to the sodium bicarbonate, which has not efficient. The applied dyes used for bone coloring were also efficient. The fixative spray presented the advantage of maintaining the colors even after frequent handling.
In the case of the tortoise (Fig. 12), following the use of the *Dermestidae* insects maceration technique, we have obtained very good results, taking into consideration the fact that the cadaver was only summarily dissected, by removing the plastron and removing the viscera.

Regarding the kangaroo cadaver (Fig. 13), despite the incomplete stage of ossification and the fact that bleaching and degreasing were not possible, it is worthy of notice that we have obtained an anatomically complete skeleton, with a satisfying quality of the bones. Assembling this skeleton would not have been possible without deteriorating the bones, but we can mention that it can be used for separate study of each bone.

The use of the *Dermestidae* on skulls yielded pieces of a higher quality than those produced by the warm and cold water maceration techniques. Both in the case of mammals and in the case of birds, this technique offers advantages from the point of view of the necessary steps and of the integrity of the
final products. Unlike the boiling technique, the *Dermestidae* insect maceration techniques have preserved the fine structures of bird skulls intact. In mammal cadavers, the insect maceration presented an unexpected inconvenient represented by the tainting of the bones when surpassing the optimal maceration processing time. Still, this inconvenient was surpassed by applying a bleaching process (Fig. 16).

Using various degreasing processes on skulls has offered us valuable information on obtaining qualitatively superior pieces. The degreasing process was able to compensate for an incomplete maceration. Using various substances has revealed the fact that acetone, ammonia and gasoline offered the best degree of degreasing, followed by the mixture of formalin and technical ethylic alcohol and the commercial detergent, these two offering adequate but not extraordinary results. When choosing the degreasing agent, we recommend taking into account the following factors: species, age, maceration degree and brittleness. If the process is incorrectly applied, the pieces will deteriorate, becoming unusable. Furthermore, the choice of the degreasing agent requires a careful evaluation of the piece, because structural differences between species indicate a different usability of agents. Thus, even though it hasn’t produced adequate results on mammal skulls, the formalin and technical ethylic acid mixture yielded spectacular results in birds. We noticed that the bleaching applied on the skulls will yield pieces of superior quality if certain factors are taken into account such as: the species, the animal’s age, respecting the optimal duration of the maceration and of the degreasing, as well as respecting the optimal duration for bleaching.

Taking into account the fact that bleaching is a chemical process relatively aggressive on the piece, not respecting the optimal time of exposure to the bleaching agents will deteriorate the pieces, modifying their aesthetic aspect, if the overexposure is short, and even their physical integrity, if the overexposure is prolonged. The fixative spray along with the acrylic dyes have offered improved aesthetics for the finite pieces.

Bird skulls (Fig. 14) are obtained in a short time by maceration with the *Dermestidae*, insects, by comparison to mammal skulls. Acetone degreasing and hydrogen peroxide bleaching were efficient on chicken skulls. During the hydrogen peroxide bleaching we noticed the tendency of chemical deterioration of the skull that becomes evident is the duration of the process is prolonged. Furthermore, the piece thus obtained has attracted insects from the environment which have contributed to its deterioration. In the case of the crow skull, the degreasing process has performed with a formalin solution mixed to technical ethylic alcohol. Due to the formalin’s insecticide action, this also had a repelling effect on the insects. Compared to the hydrogen peroxide, using a commercial detergent yielded much better results in the case of this piece.

The degreasing of the rabbit skulls (Fig. 15) obtained by *Dermastidae* insect maceration, we used acetone and gasoline. Bleaching with hydrogen peroxide yielded an excellent result, compared to the use of sodium bicarbonate, in which case the result was only adequate. Even if the sodium bicarbonate whitening was not efficient, we can mention that in both cases, the skulls were correctly preserved.

The pig skull (Fig. 16) was degreased using and ammonia solution, which yielding good results. Aesthetical deficiencies resulting from the prolongation of the maceration process, namely brown colored spots on the bones of the splanchnocranium, were partly remedied by prolonging the bleaching process from 4 to 7 days.

In the case of this piece it is worth noting that even if the maceration process was prolonged, the skull remained greasy, necessitating a prolonged degreasing time. Even so, the final result was a piece of adequate quality, useful for osteology study.
Using gasoline as a degreasing agent has yielded exceptional results in the case of the jackal skull. After applying this process for 6 days, we obtained a completely degreased piece, which, after bleaching with commercial bleach for 4 days, was ready to be put on display or to be used for teaching.

We have also obtained good results in the case of the wild cat skull (Fig. 18) which we have immersed in acetone for 4 days and then bleached with hydrogen peroxide for another 4 days.
The kangaroo skull (Fig. 19) resulted in a qualitatively acceptable item, even though its incomplete ossification made it too delicate and did not permit degreasing or bleaching treatments. Even so, the obtained piece allows the study of the bones of the cranium.

We have also obtained an adequate result in the case of the lion skull (Fig. 20). Although the maceration process lasted 30 days (a lot longer by comparison with other pieces), the degreasing with ammonia, 9 days and the bleaching with a commercial agent, 6 days, the result has good. We have obtained a well degreased and bleached piece and the fixative spray has improved its aesthetical aspect.

The use of the hot and cold water, local insects and Dermestidae insects maceration techniques produced notably different results in terms of specimen quality.

Applying the warm and the cold water maceration techniques indicated that the maceration time is shorter in the case of the first one and it yielded a piece of a better quality, than the cold water technique. Shortening the maceration time from 6 days (cold water technique) to 3 days (warm water technique) presents advantages like preserving alveolo-dental ligaments.
The sun heated water maceration technique applied on the roe deer skull yielded acceptable results. However, when compared to the other techniques, this one tends to produce brittle pieces because of the long processing time needed. This makes it especially not suitable for cadavers of young animals, who’s ossification process is incomplete as the resulting pieces will be very sensible to direct handling.

The local insects maceration technique used on the pig autopodium, with a long processing time of 12 weeks, has the inconvenient of yielding spotted bones, even though they are anatomically intact. This aesthetic disadvantage limits its usability, even more so as it also generates an unpleasant odor that can attract other animals that can deteriorate the piece.

Using the maceration technique with the *Dermestidae* insects on both skeletons and skulls, we have obtained good results, and a variable processing time, varying according to cadaver or skull size.

The degreasing process can compensate for certain deficiencies of the maceration process, especially in the case of the *Dermestidae* insects maceration technique, which takes place in a dry environment.

Comparative use of different degreasing substances has revealed that this step requires special attention, being influenced by factors such as: maceration time, cadaver species, and nature of the degreasing agents.

When choosing acetone as a degreasing agent one must take into account the fact that it has a high flammability potential and thus it requires careful manipulation. According to http://www.collectioncare.org/MSDS/Acetonemsds.pdf, it is a substance that may endanger the health of those exposed to it and it is also a costly product.

Although it presents certain disadvantages, the biggest advantage of this substance is it excellent degreasing properties, the time required for the degreasing varying widely according to its size from a few hours to a few days.

Aside the fact that it is an excellent degreasing agent, although a volatile substance, it can be recycled by freezing at -18°C, (grease freezes and it can be removed, and the acetone can be reused) (http://www.skullsite.com/misc/macerationmanual.htm).

As mentioned by Greene et al., 1993, when choosing a degreasing for the bones, gasoline produces remarkable results, but with the same inconveniences, namely its high price and flammability. Compared to acetone, it can be stored in closed plastic recipients, to prevent evaporation (http://www.skullsite.com/misc/macerationmanual.htm).

In 1997, Nawrocki S., in his studies of using ammonia as degreasing agent for osseous pieces, mentions that this is a very good degreasing agent, especially for large cadavers. A great disadvantage of this substance is that it cannot be reused. It may be used undiluted, but also diluted in water in a concentration of 50%. It is a very volatile substance, that necessitates cautious manipulation and which forms highly irrigative (http://archlab.uindy.edu).

According to http://www.skullsite.com/misc/macerationmanual.htm, commercial detergent, being cheap and not presenting that many risks, can be used with remarkable results, with the condition that the used water not to be boiled, but heated at 80°C, and also being used only on skulls or bodies of large animals, because it might destroy those of small size.

By carrying out experiments in which the degreasing was performed with a commercial detergent, in water at room temperature, we can mention that the detergent must not necessarily be dissolved in water at 80°C, because it is efficient at lower temperatures of 25-30°C.

Furthermore, to choose the degreasing agents, one must take into account the present state of the piece obtained by maceration. Thus we recommend a prior assessment of the state of the piece, before choosing the degreasing substances.

In fact, by the comparative use of different bleaching substances, it can be mentioned also that this stage requires special attention, being also influenced by factors such as maceration time, species, and the nature of the bleaching agents.

As mentioned by Gram, 2006, Husain et al., 2007, Allouch G.M., 2014, the degreased osseous pieces are whitened using hydrogen peroxide. Its concentration may vary from 37% to 4%, paying special attention to high concentrations (37%) usable for a very short bleaching time (4-6 hours), and the lower ones (4 %) used during several days (2-3 days), because this substance, as bleaching agent, presents the risk of destroying the pieces, if case of a prolonged exposure at inadequate concentration levels.
In the experiments using hydrogen peroxide for bleaching we have obtained satisfying results, on large size pieces, while small pieces presents in time signs of corrosion. The use of sodium bicarbonate has not yielded relevant results.

In literature, we found no data mentioning the use of a commercial whitener to achieve the bleaching process, therefore the use of this substance can be considered an element of originality of this chapter.

Chapter II.3 addresses the use mummification to obtain the anatomical pieces. This process differs from the previously presented methods, as it preserves the soft tissues, namely the muscles and the viscera. The mummification is in essence a dehydration process that, although visibly reducing and hardening the musculature or the viscera involved in the process, it maintains with precision the topography and certain anatomical particularities. This chapter had as main objective the evaluation of the mummification technique on different animal species, as well as its improvement by adding dyeting agents.

After applying this technique on a pony and rabbits, we have noted certain characteristics which ensure an adequate mummification. These were later applied on the following specimens prepared through this technique. Thus, when mummifying the second rabbit cadaver, removing the skin 3 days after the injection with the 10% formalin solution was an advantage, as keeping the skin permitted a better solution penetration and fixation of the tissues. Although, in the case of the pony mummy, where keeping the skin was a factor that has led to its degradation, in the case of the rabbit, keeping the skin on for 3 days favored the penetration of the formalin into the tissues. Moreover, unlike the pony, where the skin was not subsequently removed from the body, in the rabbit, this step of injection and subsequent removal of the skin was an element that contributed to obtaining a piece satisfactory in terms of quality.

Also, the steps of mechanical degreasing, of fixative solutions application, such as technical ethyl alcohol and formalin, as well as freezing the piece, have facilitated the replacement of the tissular fluids with the preservative substances and thus facilitated the preservation of the piece. Using acrylic dyes have improved the aesthetics of the piece, giving it a more natural texture, also having a higher degree of penetration of the pigment.

In the case of the cat mummies, applying the mummification technique has presented advantages linked to the quality of the piece. Applying the mentioned steps in the work protocol has ensured the production of an anatomical piece adequate in terms of maintenance of anatomical characteristics. Using the ventilator has offered the advantage of shortening the processing time from 90 to 30 days.

The mummification technique applied on the rabbit gastro-intestinal tract has yielded spectacular results. From the point of view of the anatomical particularities, injecting of acrylic dyes, followed by the formalin treatment helped maintain the aspects of the vascular supply, by highlighting it. In this case too, the ventilator has facilitated the drying process, reducing the processing time.

In case of applying the mummification techniques on reptile corpses, using formalin and technical ethyl alcohol mixture, as well as the application of acrylic dyes, yielded very good results.

In terms of the protocol used, except the pony mummy, all cases yielded remarkable results.

By adding the technical glycerin, in the case of the cat cadaver, we have noticed a more intense luster, when compared to the rabbit cadaver that was not treated with this substance.

With the exception of the pony, all pieces have maintained the anatomical characteristics of didactical interest, and they presented no deteriorating signs.

Regarding the processing time, we can state that this procedure is meticulous, demands patience when performing the maneuvers and the pieces are obtained after a rather long time interval.

In all the cases of using a ventilator to help dry the pieces, the device has shortened the mummified pieces processing time.

Comparatively to the classical mummification methods, which lead to pieces losing their original colors (Dumitru Ioana et al., 2012), applying this technique has helped preserve a large proportion of them.

Chapter II.4 aimed to evaluate the efficiency of the technology of preservation with formalin. This chapter intended to rate the comparative applicability of this technique for obtaining anatomical pieces from birds and mammals.

Following the completion of our experiments, we can assert that by using this technique the pieces lose their original colors, which will degrade in different shades of gray.
Regarding the duration of exposure to formalin, the obtained data indicate that the formalin start to work on the organs as early as in the first 15 minutes, by decolorizing them. Exposure to a concentration of 20% formalin, leads to a marked discoloration of the pieces associated with hardening due to water loss. By performing dissection, it was macroscopically observed that formalin and started to penetrate into the parts, causing discoloration.

Regarding skulls, during the experiment we have registered a changing of the color in different shades of gray, while maintaining with high fidelity the anatomical features.

If the case of the calf skull we have noted an adequate maintenance of all anatomical characteristics, with the exception of the original color of the fresh piece.

In the case of the pig head, applying this preservation technique allowed us to observe the particular aspects of the species. In this species, the lips are markedly uneven, the upper one being better developed than the lower one, although their muscles are well developed, the mobility being reduced. The mucosa lining the cheeks is smooth and presents at the level of the second molar, the molar papilla. The palate is long and narrow, with a broadening in between the canine teeth. At this level, the fresh piece lining is pink. Due to the conservation, the color turns into gray. The palatine ridges vary in number between 20 and 23, depending on the individual; they are smooth, divided by a prominent median groove. The incisive papilla is visible and bordered by two fissures, where the vomero-nasal ducts and laterally to them a tubercle is visible on each side. The soft palate is 5-6 cm long; on its oral side the median raphe can be seen as an extension of the raphe of the hard palate. The sublingual vestibule is narrow and small, with a prominent concavity. The lingual frenulum is placed vertically. The tongue is approximately 17-20 cm long, with a smooth rounded tip, with a shallow median longitudinal groove. The ventral side of the tongue shows a smooth mucosa that extends into the mouth floor by the aforementioned frenulum. The lining of the dorsal side of tongue has a velvety appearance due to the filiform papillae. In the fixed segment of the tongue, there are fungiform papillae, which are more numerous on the edges. There are only 2 goblet buds and they are located symmetrically on the dorsal side of the tongue base. The foliate papillae are located on both sides of the tongue base, at lingual extremity of the glosso-palatine crease (Popovic I. et al., 2003).

Concerning the horse thoracic and pelvic limbs, respectively, we registered that the anatomical pieces presented a satisfactory degree of preservation, associated with significant changes of texture and color.

The color changes consisted of the total loss of the characteristic nuances of the fresh piece, turning into grey shades. The consistency was also changed, namely an almost complete hardening of the muscular mass.

The anatomical pieces thus prepared, although presenting color and texture changes, have kept with fidelity the regional topography, respectively the positioning of the muscles, the vessels and the nerves.

The pieces thus prepared have been adequately preserved for 14 weeks, used as teaching material. To eliminate the irritating effects of the formalin, the pieces initially preserved in a 10% formalin solution, were immersed in successive baths with a solution with a low concentration (5%) of formalin.

Chapter II.5 stands apart due to the investigation of the plastination technique, a new and modern method of preserving tissues that decompose easily and have high water content.

The standard method of plastination uses Forced Impregnation with the silicon rubber BIODUR S10 and the hardener BIODUR S3. The specimen is finally cured with BIODUR S6. The standard technique BIODUR S10 becomes relatively affordable if the Forced Impregnation is performed at -25°C, because the polymer can be reused indefinitely.

This method is called standard because is the most frequently applied, currently, it covers the majority of anatomical pieces preservation needs, it always yields satisfactory results, it is the technique the easiest to perform and it necessitates a minimum of equipment. The fixation of the pieces intended for plastination employs usual techniques such as formalin solutions. The dehydration of the pieces is a necessary step for the polymer impregnation. The simplest method is that of freezing substitution. The piece is immersed in acetone at -25°C for several weeks.

The forced impregnation process is the most important step of the plastination process. It must be applied at a slow pace, in order to allow the polymer solution to penetrate to the core of the piece, where the acetone becomes gas and is removed. This process takes between 4 and 14 days, depending on
the size of the piece, on the density of the tissue and on the viscosity of the polymer solution. The polymer solution used for (BIODUR S 10) can be reused by simply adding a new quantity of polymer.

The curing of the impregnated specimen occurs in the presence of the BIODUR S6 reactive in a gaseous form, which is put in contact with the piece to conclude the polymerization.

The polymers used for plastination must have the following properties: low viscosity to facilitate tissue penetration, unlimited reusability, capacity to preserve the biological material, mechanical and optical adequate properties (e. g.: resistance to yellowing, adequate refractive index) and not degrading the pieces during processing.

To prevent shrinking, the pieces must be kept at a temperature -25°C and -30 °C during dehydration.

If the pieces are left too long in acetone, they lose their flexibility, become hardened and tend to brake, leading to the following disadvantages:

- They will be very sensitive to mechanical forces such as pressure during the polymer impregnation.
- The flexibility of the specimens impregnated with silicon rubber (BIODUR S) decreases. This is harmful for the pieces with thin walls (intestine), with a fragile structure, with filament (nerves) and with membranes (kidneys with intact renal pelvis).

For this reasons, all pieces impregnated with silicone rubber that must remain flexible after the plastination process, must not the submerged in acetone longer than necessary.

Mechanical properties of dehydrated specimens play no role for the mechanical properties of the final specimens, if a non flexible, hardened product is desired. Such pieces can be kept indefinitely in acetone. However, they are fragile and susceptible to mechanical pressure during processing.

The disadvantages of an insufficient dehydration are white spots or shrinking of the specimen; they can be more severe than those induced by an excessive dehydration.

Before the Forced Impregnation it is advised to always measure the water content of the Volatile Intermedium (acetone).

The water content of the Volatile Intermedium must be under 1%. Water content higher than 2% will lead to inferior quality products.

If one is inexperienced in carrying out the dehydration process, one should measure again the density of the dehydrating liquid after three days to see if the water content has remained constant.

The pieces must be only pre-cooled, because direct freezing leads to the formation of ice crystals, deteriorating the pieces due to several reasons:
- The pieces will blacken due to the ice crystals and the surface cell layers will dry in certain places, leading to white spots on the final products, the so called „freeze burning marks”
- If one is inexperienced in carrying out the dehydration process, one should measure again the density of the dehydrating liquid after three days to see if the water content has remained constant.
GENERAL CONCLUSIONS AND RECOMMENDATIONS

Out of the pool of general conclusions formulated through the analysis of the obtained results we have highlighted the following:

1. The boiling preservation technique has a higher degree of applicability due to the fact that it does not involve special equipment and it is low cost, but the risk of bone specimens degradation is present in a various degree according to the species.
2. The maceration technique is simple and when applied it yields good results in obtaining osseous pieces from reptiles, mammals and birds.
3. The acetone and the gasoline have produced a satisfying degreasing of the specimens;
4. The hydrogen peroxide was more efficient than the sodium bicarbonate in the process of bone bleaching.
5. The technique applied on the pony cadaver was not adequate and the applied protocol needs a revision.
6. The addition of the technical glycerin in the mummification process reduces its duration and helps maintaining the consistency of the original live tissue.
7. The cold air ventilator helps accelerate the mummification process.
8. Technical ethylic alcohol has a toxic effect on the insects that are potentially harmful to the specimens.
9. The mummification technique offers multiple advantages to the didactical process such as: specimen durability, accuracy of the anatomical particularities and the possibility of directly handling the items.
10. The formalin acts on the tissues as early as in the first 15 minutes.
11. The standard plastination technique S10 is complex and when applied it yields remarkable results, producing anatomical specimens of a quality superior to those obtained though the classical techniques.
12. We consider that the foundation of a plastination laboratory would represent a strong asset in obtaining anatomical specimens of superior quality for both student use and research in the veterinary medicine domain.

The following recommendations have been formulated from our results:

1. In order to obtain osseous pieces through the boiling technique, it is advisable to take into account their origin, as an inadequate boiling time leads to the destruction of the specimens.
2. We recommend the use of degreasing and drying, because, if they are not adequately applied, the assembling of the pieces becomes impossible, no matter the choice of adhesive.
3. When applying the mummification process, it is advisable to take into account the size of the cadaver and the degree of its decomposing state.
4. The mummification technique using simple formalin is not adequate for the preservation of anatomical specimens, at least not for those of large size such as intact large animal cadavers.
5. We recommend the use of technical glycerin during mummification, because it speeds up the process and helps maintaining the original consistency of live tissue.
6. Seeing the outage of the specimens obtained by formalin preservation, we recommend their incineration when signs of decay appear or when they lose their utility.
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