

Cryodehydration Technique Applied to Anatomical Segments

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Abstract

Introduction The present study describes a variation of the cryodehydration technique, applied to prepare biological tissues by repetitions of section freezing and thawing (SFT). First developed for muscles and then viscera, the aim of this process as presented here is to obtain anatomical segments to be used in anatomy classes, general exhibitions and museums.

Materials and Methods The animal, previously fixed in 10% formalin, must be frozen and then sectioned in longitudinal, transverse and horizontal slices of the body-axis. It has two steps: the “burning phase,” in which ice crystal formation and water dilatation cause micro ruptures in the tissue; and the “dehydration phase,” developed to dry out the slices segments slowly and, at the same time, to impregnate tissues with wood glue.

Results The obtained slices present several advantages, such as being dry segments, of light weight, and being rapidly used, easily stored and promptly studied. Also, it is possible to study the different organs of one segment and, by apposition, remount an entire animal, enabling a dynamic study.

Conclusion This very simple and inexpensive technique produces anatomical preparations with high durability and resistance, which are very helpful in practical and theoretical classes.

Keywords

- anatomical technique
- cryodehydration technique
- anatomical slices

Introduction

Anatomical studies require practical classes supported by numerous techniques to offer different views of the same structure. Details not revealed when a certain technique is used may clearly appear when another method is adopted.

Several techniques may be used to preserve biological tissues. In the case described here, besides fixation, dehydration by means of repetition of section freezing and thawing (SFT) is used to preserve anatomical segments prepared as slices in transversal (metameric), longitudinal (antimeric) and horizontal (pachymeric) planes.

According to studies about industrial flesh conservation, the adequate process to achieve cryoconservation is the fast freezing of sections, which promotes the formation of small ice crystals without tissue damage. Slow freezing results in the formation of large ice crystals, which appear either inside the cell or between the muscle fibers, resulting in cell disruption, fluid release and the consequent loss of flesh quality.^{1,2}

The method developed and described in the present manuscript can be divided in two steps.

In the first phase, the objective is to burn tissues by cooling. The “burn” process includes the period in which the low temperature produces discolored spots on the surface of the

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organs, called “pale places” and, at the same time, produces micro ruptures in cells and tissues due to ice crystal formation and water dilatation. These micro lesions will facilitate fluid release during the second phase and will reduce future retractions over the preparations.

The purpose of the second phase is the slow removal of interstitial fluids and water resulting from cell rupture, and, at the same time, the impregnation of the organs with water-soluble wood glue, which will provide resistance and durability to the specimens.

This procedure has already been described for the preservation of muscles,³ and for viscera.⁴ A variation of this cryodehydration technique, now applied to segments, was first described in 1994.⁵

The present study describes the use of the cryodehydration technique to obtain dry anatomical segments for several different uses.

Material and Methods

The specimens prepared for this technique may be obtained from veterinary hospitals or pathology laboratories and, therefore, there is no need of euthanasia. The specimens are fixed in 15% or 20% formalin, or in any other fixative that hardens tissues as formalin does. Perfusions to wash vascular lumens and previous fixations are not required.

The vessels selected for cannulation and formalin injection are distant from the area to be prepared. For example, the femoral artery or vein are indicated to prepare sections of the head, neck and trunk, as the use of the carotid artery or jugular vein may compromise the preparation of the neck.

During or soon after fixation, formalin should be injected in the digestive cavities, such as the stomach (specially ruminants), colon (particularly horses) and rectum, to stop gas production due to bacterial fermentation and prevent dilations, changing internal position or reductions of the thickness of the organ wall. The solution should be injected with a long needle inserted through the abdominal wall exactly over the organ.

Also, to avoid position changes or organ dislocation, special attention should be paid to body position during the fixation process. Not only large or heavy animals tend to present organ dislocation to the side when lying on the flat surface of the dissection table, thus, this position should be avoided.

Fixation should continue for 48 hours and, after that, the cadaver should be placed in a freezer for a period of time that depends on its size. For a medium-sized dog, the recommendation is not less than 1 week (temperature $\sim -10^{\circ}\text{C}$ to -15°C).

After this initial preparation, sectioning may be performed according to the desired plan to obtain longitudinal (antimeric), transversal (metameric) or horizontal (pachymeric) segments, using a manual or electric saw.

Each section is obtained at a 2.0 to 3.0 cm thickness and, immediately after sectioning, they should be labeled and put back into the freezer, lying alone or side by side on a flat surface, avoiding adhesions to each other.

Once the slices are ready, each segment should be placed individually under a low water flow, and tissue remains should



Fig. 1 Head section of a dog being rinsed with a brush under a low water flow.

be removed from both surfaces (►**Fig. 1**) using a smooth paintbrush. During rinsing, the segment should not defrost, and attention should be paid to avoid losing small pieces of the organs. If the ice starts to melt rapidly while rinsing, the segment should be returned to the freezer immediately, and the procedure should continue after it is frozen again.

Especially in the case of herbivores, organ content should be kept in the stomach and intestines. From a teaching point of view, it is very interesting to demonstrate the degree of physiological digestion inside each of these chambers. In case of museums, it is useful to demonstrate the differences in the amount of food inside each compartment.

It is only after this initial preparation that the cryodehydration process starts.

During the first phase, or “burning process,” the segment must lay on a tray, alone or side by side, without any cover, and be kept in the freezer until completely frozen at a temperature of -10°C to -15°C .

To start thawing, the trays should be filled with water, which is kept there until thawing is complete. The time depends on the size of the segment, but thawing should happen at room temperature and without using any heat source.

Both sides of the anatomical segment should be submitted to the effects of the frozen temperature directly. To ensure this, each section should be turned after each SFT session, which will be easier when done as soon as the segment is removed from the freezer, while it is still hard. This procedure should be repeated every 24 hours and, to establish a routine, it is recommended first to remove the sections from the freezer in the morning, turn them and, once defrosted, return them to the freezer until the next morning. After defrosting, almost all of the water is removed from the tray, and only a small amount of it remains.

The number of SFT repetitions before beginning the second phase (dehydration) depends on the size of the section, and the smallest are prepared most rapidly. The end of the burning phase and the beginning of the dehydration

phase is reached when the tissues, especially muscles, change their flesh appearance to a fibrous texture, similar to a sponge. When pressed, the muscle releases fluids through its fibers, as a sponge, and once the fluid is released, the pressed tissue returns to its previous form. Also, in parenchymatous viscera, such as liver and kidney, burning results in the formation of light areas on the surface, which indicate that the technique is developing accordingly. As a general recommendation, not fewer than 25 to 30 SFT repetitions should occur before the next phase begins.

To establish the dehydration routine to this phase, defrosting must occur at room temperature without using water or heat, and freezing should begin immediately after thawing. The anatomical segments do not need to dry after each repetition, just defrost.

The procedure during this phase depends on the performance of each section, as there are different organs in the same segment, and heart, lungs, liver and stomach tissues react differently to the process. The fact that one section has reached the end of the process does not mean that the process has also been completed for the other sections.

During the entire dehydration phase, attention should be paid to some special procedures:

1. In the first phase, as soon as thawing is complete, the anatomical segment must be put back into the freezer.
2. During this phase, the surfaces should not be parched, because this would produce retractions and tissue darkness.
3. The slower the freezing, thawing and drying (water evaporation) processes happen, the better the final result.
4. If substantial retractions occur in an organ, the segment should be placed in a tray full of water until the former shape is regained, and then a new SFT cycle should begin, as in the first phase.
5. When the drying process is almost ready and only humidity remains, it is time to start impregnating with 20% wood glue solution in water.
6. A brush should be used to spread and press the glue solution into the tissues on both sides of the segment.
7. At the end of the process, glue alone should be applied to the segment using a brush to form a protective surface by filling spaces and linking the different anatomical structures.
8. After impregnation, the anatomical segments do not have to be frozen again.
9. There are many types of wood glue, and the one to be used should be selected according to water solubility and the capacity to produce very little or no retractions.

If a section bends, it can be pressed between two hard flat surfaces (but not wood directly, to avoid that slices are glued together) for 1 or 2 days, until it becomes straight again.

Finally, both surfaces should be sanded to improve appearance, and the organs may be painted using material that reproduces the original color of each organ.

In case there is shrinkage or retractions, and to provide support between structures, a paste prepared with glue and sanding dust should be used to fill the spaces between organs.

Results

The slices obtained are very easy to be used, readily available and, after they are dry, there is no requirement of chemical reagents. As dehydrated anatomical segments, the conditioning is facilitated, they become light and, also, do not exude any smell. In the classroom, it is possible to study isolated segments, which mirror images of computed tomography (CT) or magnetic resonance imaging (MRI). Moreover, these segments allow the student to reconstruct the entire animal's body by affixing the corresponding pieces through apposition and reasoning about the location of each identified organ. Each segment allows the study and identification of organs relating them both within the systems to which they belong and shows the relationship among the various systems. As the cuts are from whole animals, it is still possible to study the positioning of organs relating them to stratigraphy, skeletopia, among others. Thus, the cryodehydration technique allows a differentiated, proper, and comprehensive dynamics of the study of anatomy.

Discussion

This technique is a variation of the procedures first applied in muscles³ and viscera⁴, but now used to obtain dehydrated anatomical segments, also organized to prepare longitudinal, transversal or horizontal slices of whole-body sections.

Alternating SFT, the so-called cryodehydration technique, is a very simple and inexpensive process to produce morphological segments to be used in theoretical and practical classes, exhibitions and museums.

According to the literature, in industrial flesh conservation, fast freezing sections is the best process to achieve cryoconservation because of the formation of small ice crystals without tissue damages.^{1,2} Otherwise, due the repetition of several slow freezing sections, large ice crystals are formed either inside the cell, or between connective tissue and muscle fibers, which leads to structural disruption and fluid release, the aim of this process. At the same time, fluid dilatation causes micro ruptures in superficial and deep fascia, tissues, organs and capsules. This is the so-called burning phase, during which thawing should be achieved by using running water.

Micro ruptures facilitate the subsequent dehydration phase, exactly when fluids are slowly released, avoiding substantial shrinkage. Only in this subsequent stage should the thawing sections be conducted in a dimly lit area, at room temperature and slowly.

Some advantages of this process are: 1) weight loss, which is particularly important in veterinary medicine, in which large animals are studied; 2) it is very simple to keep anatomical segments in cabinets or lockers, discarding large formalin trays; 3) the anatomical segments are ready to be rapidly used and stored again; 4) the costs of production and maintenance are low, and, at the same time, the results have long-term durability.

Perhaps the main advantage of preparing segments is their use for teaching purposes, because they can be studied



Fig. 2 Head of a dog: The segments produced using this technique reconstruct an animal and clearly demonstrate organ position and relations, as well as other anatomical data.

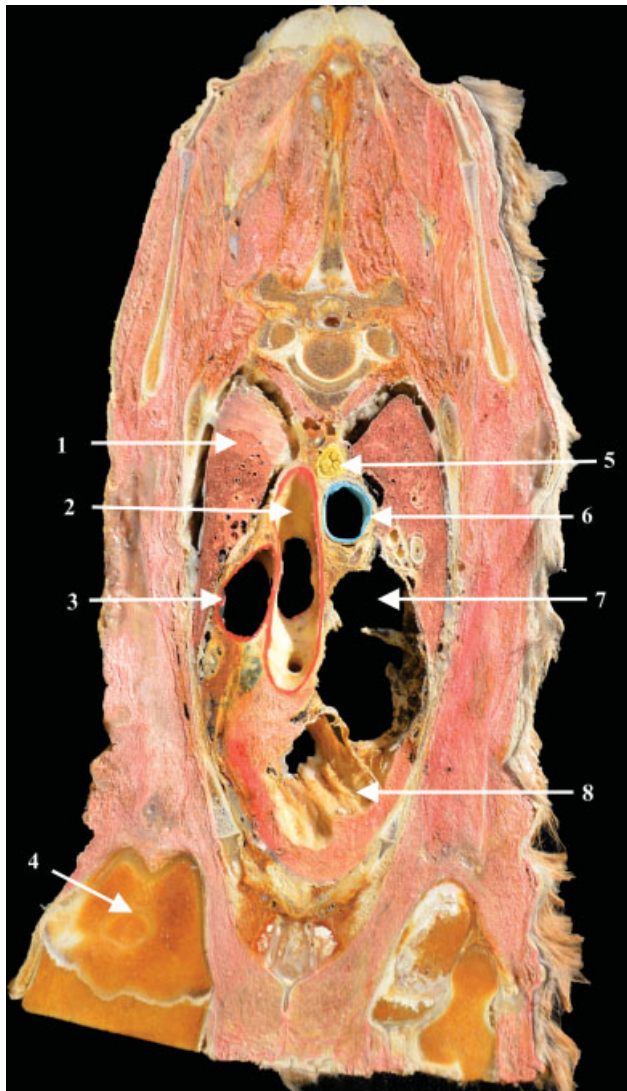


Fig. 3 A caudal-cranial view of the thorax in an anatomical segment of a horse: 1) left lung; 2) aorta; 3) pulmonary artery; 4) head of the humerus; 5) esophagus; 6) trachea; 7) right atrium; 8) right ventricle.

in different ways. As slices, they reproduce CT, introducing and facilitating future interpretation.

It is also possible to reconstruct an animal by placing one segment on top of the other and, virtually open an animal to show all the organs in each segment (► **Fig. 2**), so that some structures of the body can be analyzed spatially.

Moreover, students can very easily recognize and understand the positions of the organs in surgical, clinical or radiological procedures. As a whole, position, relations, organ morphology and volume may be studied in any segment, as each one provides a general view of the animal body (► **Fig. 3**).

Conclusion

As conclusions, it is possible to say that the cryodehydration technique allows the preparation of anatomical segments with the following characteristics:

1. The slices are very helpful during the theoretical and practical classes.
2. Dried, they become light, the conditioning in cabinets or lockers is facilitated, discarding large formalin trays.
3. The weight loss is particularly important in veterinary medicine, in which large animals are studied.
4. The slices mirror images of computed tomography or magnetic resonance.
5. By apposition of the segments the student can “reconstruct” the entire body of the animal.
6. It is possible to visualize the organs disposition inside the body.
7. The costs of production and maintenance are low.
8. The results have long-term durability.

Conflicts of Interests

The authors have no conflict of interests to declare.

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