

A Curation Technique: Groundwork of Bone from Embalmed Human Cadaver

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ABSTRACT

Soft tissue is removed from bones before they are macerated, bleached, and labelled. As most medical colleges do not handle bones after the dissection of human cadavers, a significant storehouse of human bones is lost in the absence of a regulated approach. Therefore, the goal of the current investigation was to govern the least time-consuming and most efficient way to prepare bones from embalmed and wet specimens.

Bony parts have different nature like shaft of long bones require less time to dissolve and end part of long bone require more time to dissolve. The procedure utilised included a maceration step, which includes removing soft tissue before boiling the bones (according to porous nature of bones) in 60 litres of water for two hours. After 30 minutes of starting the boiling process, potassium hydroxide pellets (caustic potash, mol. wt. 56.11), weighing 200–250 gm for male bones and 150–200 gm for female bones, were added to speed up the maceration process. After the bones had finished macerating, they were bleached by immersing them in a 30–35 litre solution of hydrogen peroxide (30% w/v) (mol. wt. 34.01) for 12–14 hours. The bleached bones were then thoroughly cleaned with water before being degreased for 12 hours by soaking in 30 to 35 litres of extra pure acetone (boiling point 55.5° to 56°C). lacquer thinner after they had naturally dried by being spread out on blotting paper. lacquer thinner re. This study found that the aforementioned technique was quick, easy, odourless, and produced high-quality human bone. This investigation came to the conclusion that the above approach was efficient, quick, odourless, and produced good quality human bones for anatomical examination. the excision of soft tissues Bleaching, degreasing, maceration, and potassium hydroxide.

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INTRODUCTION

Anatomy has changed over time as a base for medical education. . Osteology, or the study of bones, is a crucial and vital component of the anatomy curriculumⁱ. The capacity to teach osteology in three dimensions using human bones is unmatched. Bones can also be used to understand where soft tissue will attach and how the local neurovascular bundles will travel. The most effective way to study the crucial component of bone anatomy is by combining a dried bone with books, atlases, and anatomy dissectionⁱⁱ. Removal of soft tissues, bone bleaching, bone articulation, and labelling are the main components of

bone preparation. Depending on the size of the human dead corpse or the Caracas in the case of an animal, these procedures can take a variety of timesⁱⁱⁱ. Insect consumption and cold- or warm-water maceration have been used as standard methods for preparing bones, can take from two days to eight weeks, depending on the amount of bacteria present, the size of the material being macerated, and the temperature of the environment during the maceration. Skeletal material may also be boiled before being mechanically cleaned^{iv}. To extract soft tissue from bones, solutions of organic and inorganic chemicals

are also utilised. Antiformin, ammonium hydroxide, sodium hydroxide, and other alkaline solutions are inorganic compounds that are employed. The process of maceration with organic substances can be carried out using enzymes like papain or pepsin, as well as washing powders containing enzymes^v and burial in soil^{vi}. Due to the government's policy of not granting any merchant a licence to deal in human bones, original human bones are not readily available in the market. As most medical colleges do not process the bones following the dissection of human cadavers, a sizable repository of human bones is lost in the absence of a defined procedure of bone retrieval. Original human bones are overpriced and sold by unlicensed individuals without a proper invoice. The morphological details of commercially available artificial bones made of plaster of Paris or resins are subpar compared to those of real bones. This article presents the process for removing bones from cadavers (or animals) using cleaning techniques after trying various approaches and standardising their own method.

Technical Details and Method of Use

The steps involved in recovering bones from human cadavers are as follows:

Step 1: Removal of soft tissue. Using a scalpel, open up all of the joints in the extremities (Limbs), and carefully separate the bones, removing any loose muscles, ligaments, and soft tissues. Articulated hands and feet, the spinal column, and the pelvis should all be maintained together at this point because doing otherwise could result in harm.

Step 2: Maceration: To soften the bones, soak them in tap water for 24 hours. For one cadaver's bones, around 30 to 40 litres of water are needed, depending on the size and weight of the body.



- Use a soft scraper to gently remove any remaining soft tissues.
- Place the bones in a suitable container with water in it, making sure they are thoroughly submerged (approximately 80 to 100 litres of water), and boil

for two hours. The bones should be properly boiled in water, and the process should be monitored frequently. When feasible, boiling should be done outside. Skulls can be harmed by vigorous boiling. The bones should be gently simmered after the boiling has begun to prevent injury.



Add 200–250 gm of potassium hydroxide (KOH) pellets (caustic potash, molecular weight 56.11) for male bones and 150–200 gm for female bones (Figure 5). Half a kg (1/2 Kg) of common salt should be added to the solution, which should boil for 112 to 2 hours. Continue to look for bones. Take out as much flesh as you can. Separate the vertebrae and remove the spinal column. The hand and foot can also be disarticulated to remove additional tissue in a similar manner. Using pliers, a scalpel, or a knife, the flesh can be easily removed.

- Remove the skull, remove more tissue, and continue the procedure until the skull is thoroughly cleaned. To make it easier to remove the skull from the boiling water, tie a string to it. The brain must be dissected with a scalpel and scooped out with a duramater through the Foramen magnum. It's crucial to periodically check the boiling skull. Long-term boiling can destroy bone tissue and harm skulls.
- Use room temperature tap water to clean and rinse the bones. With a scalpel, examine the bones and cut away any soft tissue that is still clinging to them (Figure 6).
- Let the bones soak in room-temperature tap water for a minimum of 12 hours.



Step 3: Bleaching: Rinse and wash each bone. Make sure that every bone is soaked with hydrogen peroxide (H₂O₂) 30 percent w/v solution (M.W. 34.01) by soaking all the bones from a single cadaver in 30-35 litres of the solution. For 12 to 14 hours, keep covered with a lid. Keep checking since too much bleaching will weaken the bones.

Step 4: Degreasing: Use tap water to thoroughly wash the bleached bone. After exposing the bones to the bleach, make sure to properly rinse them in fresh water right away because the chlorine will continue to deteriorate the surface of the bones even after they have dried. Reboiling the item right away in clean or soapy water will also get rid of the bleach. Let them sit in extra-pure acetone M.W. 58.08 Boiling point (95%) at 55.5–56 °C for 12 hours, until all the bones are submerged (about 30–35 litres).

Step 5: Drying: Take the bones out of the acetone and wash them under running water. The bones should be spread out on blotting paper and allowed to dry for 4-5 days at room temperature (ambient: 36-44 degrees Celsius).



Step 6: Finishing: After the bones have dried fully, finish them by painting them with Johnson Touchwood® or a mixture of half a litre of lacquer and half a litre of lacquer thinner. By doing this, the ends of the bones won't erode.

To protect oneself against bacteria, chemicals, and standard safety procedures to prevent blood-borne pathogens, disposable latex gloves should be used. In order to avoid breathing in the fumes or steam during boiling to remove the bones from specimens preserved in formalin or formaldehyde, facemasks must be worn at all times. During processing, exposed skin should be covered, and it must be guaranteed that the disposal of the generated fluid and soft tissues complies with local laws.

IMPORTANT FEATURES

➤ The technique makes use of chemicals that are easily accessible in any medical college's anatomy department or from any vendor who sells chemicals.

- The method's chemical inputs are reasonably priced.
- Chemical solutions used to dissolve soft tissue have been shown to be the most efficient method of doing so because they quickly and odourlessly macerate bone. Additionally, unlike other cleaning methods, which take a long time and produce strong odours when used on human cadavers in particular, has a less damaging effect on bone DNA.
- Since insects don't chew into formalin-fixed tissue, the approach can be used on cadavers that have been fixed in formalin because it doesn't need insects to clean the bones. Additionally, thousands are needed to achieve quick cleaning, and if left with bones for an extended period of time, they will devour and consume them.
- Degreasing is part of the procedure since grease needs to be eliminated because it will smell, soak into the bone, and draw dirt and filth.
- No harm is done to the bone, and all of the morphological characteristics of the bones are preserved.

Discussion

Osteology, or the study of bones, is a crucial and vital component of the anatomy curriculum^{vii}. The capacity to teach osteology in three dimensions using human bones is unmatched. Bones can also be used to understand where soft tissue will attach and how the local neurovascular bundles will travel. The most effective way to learn about the crucial feature of bone anatomy was by combining a dried bone with books, atlases, and anatomy dissection^{viii}. The main steps in bone preparation include the removal of soft tissues, bone whitening, bone articulation, and labelling. The length of time needed for these procedures varies depending on the size of the human or animal corpse^{ix}. Insect consumption and cold- or warm-water maceration have been used as standard methods for preparing bones, and they can take anywhere from two days to eight weeks, depending on the number of bacteria present, the size of the material being macerated, and the temperature of the environment during the maceration. Skeletal material may also be boiled before being mechanically cleaned^x. Soft tissue from bones is also removed using chemical solutions, both organic and inorganic. Antiformin, ammonium hydroxide, sodium hydroxide, and other alkaline solutions are utilised as inorganic compounds. Enzymes like papain or pepsin, as well as washing powders with enzymes^{xi}, burying in soil, can all be used for organic chemical maceration.

Maceration

To study the effects of altering fresh water pH on bone, bones were macerated in solutions with various pH levels. Collagen and cartilage were further broken down by simmering in borax, and any remaining lipids were then removed by soaking in xyol. Extreme pH values have the potential to be harmful, whilst intermediate pH values have more subtle but nonetheless substantial consequences. The pH 7 and pH 10 solutions have negligible impact on bone, but the other solutions have different degrees of impact^{xii}.

Megat Maceration:

It has been demonstrated that dermestid beetles are effective for cleaning bones, particularly for the portions of the skeleton that are challenging to dissect by hand. In order to establish a reference collection, researchers conducted an experiment with the harvester termite *Trinervitermes trinervoides* (Isoptera: Termitidae) in the Sterkfontein Valley of South Africa. The results revealed that after six months, all bones had approximately half of their surface covered in a dark residue, had an etched appearance, and had recorded boreholes and destruction, especially of less dense elements and epiphyses. This study has shown that *T. trinervoides* is capable of destroying bone in all phases of preservation, favouring young, spongy, thin-cortical bone that contains meat and marrow^{xiii}. Chemicals: Human skulls are crucial for osteology because they help to understand the locations where soft tissues implantation and the intricate path of neurovascular structures in the base of the skull take place. The acquisition of human skull specimens has become extremely challenging due to recent geopolitical changes in Asia. It has been documented how to prepare dried human skulls from fresh and frozen cadavers using readily available chemicals^{xiv}. The process involves accelerating maceration with a number of enzymes, followed by defatting, washing, and bleaching, and takes around 8 weeks to complete. The created skulls lack any preparation artefacts and are of great quality and durability.

Now that an affordable source of skulls has been restored, it will be easier to understand the complex interactions at the base of the skull. African giant To test the effects of three different bone preparation techniques on the bones of the African Giant Rat (*Cricetomys gambianus*), 12 pouched rats of both sexes were utilised. Sodium hydroxide was used as a chemical preparation agent. This approach was determined to be the best in terms of the amount of time needed to finish the process, the quantity of bones retrieved, the colour of the bones, and the preparation's smell. The chemical approach, on the

other hand, has the drawback of dissolving, breaking the bones if a large concentration is utilised, and neglecting the preparation promptly^{xv}.

Detergent Maceration

Osteological evaluation of human remains is a crucial component of forensic work, particularly when looking at severely decayed, dismembered, or burned victims. It was determined in a study to determine the efficacy of detergents for the removal of soft tissue from animal derived specimens that such a method is comparable to enzymatic maceration, but with fewer health and safety concerns and greater advantages regarding transportation and availability of materials when an investigator is in a fieldwork scenario^{xvi}. When visual examination of skeletal remains is insufficient for identification, a forensic biologist is brought in to perform DNA testing. When skeletal preparation techniques are used to deflesh human remains, the likelihood of downstream DNA testing needs to be taken into account because they could have a significant impact on genetic analysis and eventual identification.

In a study, the impact of 10 maceration techniques on the preservation of DNA in the ribs of 12 pigs (*Sus scrofa*) and overall bone structure was assessed. This study demonstrates that the most effective ways to extract DNA from skeletal tissue are not necessarily the conventionally "conservative" maceration approaches^{xvii}. Rennick et al. (2005) tested the effects of three cleaning methods on the recovery of nuclear and mtDNA from a variety of human and non-human bones by boiling bones in water, bleach, and powdered detergent/sodium carbonate. Non-human bones treated with bleach saw a statistically significant drop in DNA yields, and DNA degradation was visible electrophoretically. The yields from bleach cleaning of human bones were likewise significantly reduced, whereas the detergent/carbonate approach allowed the biggest DNA segments to amplify, suggesting that it may not be as bone DNA-degrading as other cleaning methods^{xviii}.

Among anthropologists and anatomists, there has been much discussion about the ideal process for macerating the remains. Many people think boiling is a method that causes undue harm. However, after putting this strategy to the test, we feel that carefully applied boiling is far better than no-heat or low-heat treatments, insects, and caustics (bleach, ammonia), and that it also works more quickly. The best techniques for eliminating soft tissues and lipids are boiling and simmering. The majority of the work will be done by boiling, therefore dissecting tools should

be used with care to remove the skin, big tendon bundles, muscle, and ligaments^{xix}.

Conclusion

We come to the conclusion that our method for combining several bone-cleaning processes is quite

effective at retrieving bones for anatomical studies. In terms of morphological details, the yield of the bones thus recovered by this procedure is of very high quality and can be used for anthropological and morphometric studies.

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