Preparation of the Human Skull for Skull Base Anatomic Study

Abstract—Human skulls are unsurpassed in the ability to provide three-dimensional instruction in osteology as well as understanding the sites of soft tissue insertion and the intricate course of neurovascular structures in the skull base. Recent geopolitical developments in Asia have led to extreme difficulty in obtaining human skull specimens. The purpose of this article is to present a method for the preparation of dried human skulls from fresh and frozen cadavers using commonly available chemicals. The technique, requiring about 8 weeks total time and basic equipment, consists of maceration accelerated by several enzymes followed by defatting, washing, and bleaching. The skulls produced are of excellent quality and durability with no preparation artifacts. An economical source of skulls has now been reestablished to facilitate learning of the intricate relationships of the skull base. (*Skull Base Surgery*, 3(1):1–6, 1993)

Human skulls have long been used for the study of skull base anatomy. They are unsurpassed in the ability to provide three-dimensional instruction in osteology as well as understanding the sites of soft tissue insertion and the course of neurovascular structures in the region. The important aspects of this anatomy can be most efficiently learned by utilizing a dried skull in combination with textbooks and atlases as well as laboratory dissection.

In the past, Asia has been the source for almost all human skeletal material for anatomic study. Recent geopolitical developments in the region have led to extreme difficulty in obtaining these materials. Although skulls can infrequently be procured through commercial channels, often the quality is poor and the cost exorbitant. The purpose of this article is to present a method for the preparation of dried human skulls from fresh and frozen cadavers using commonly available chemicals.

MATERIALS

Unfixed whole heads were obtained from the Department of Anatomy at UCLA, which were either fresh or frozen soon after death. Chemicals and equipment required for the preparation procedure are listed in Table 1.

The preparation was performed in a closed 20 liter plastic container with a valve at the top and bottom of the container facilitating drainage and addition of solutions during the process. The fittings were designed to maintain a closed system to control foul odors produced during maceration. Brass fittings and polyethylene tubing obtained from a local hardware store were combined with 6.3 mm (outside diameter) brass tubing and silicone sealant to make a watertight system. A tube style thermostatically controlled immersion aquarium heater was suspended on the side of the container. Temperature was thermostatically controlled at 39° to 40°C at the top of the vessel. The procedure was performed with the apparatus in a fume hood because the odors produced, particularly during the maceration process, were quite noxious. In addition, the carbon tetrachloride (Chem Lab C255, Chem Lab Co, Placentia, CA) used in defatting the bone produces toxic fumes and was not allowed to contact the skin or mucous membrane of the individual preparing the skull.

METHODS

The method of skull production can be divided into four parts: preparation; maceration; defatting, washing,

Table 1. Chemicals and Equipment

Chemicals, technical grade
Sodium hydroxide (Chem Lab S350) granules, 250 gm
Hyaluronidase (Wyeth), 150 U
Pancreatin (3× USP, Sigma P1625), 2 gm
100% carbon tetrachloride (Chem Lab C255), volume for submersion
35% hydrogen peroxide (Chem Lab H2151), diluted with water to make 6% solution, volume for submersion
PEX laboratory soap
Equipment
Vessel of sufficient capacity to allow complete submersion of skeletal material. Large bore drainage outlet.
Immersion heater, thermostatically controlled
Glass or plastic concentric shell for heater tube

and bleaching; and articulation. The entire process requires about 8 weeks.

Preparation

All soft tissue was removed down to the attachments of the periosteum. No attempt was made to elevate or remove the periosteum, nor was tissue dissection performed intranasally or in other recesses of the skull. A skull at the conclusion of the preparation process is shown (Fig. 1). The brain and other intracranial structures were removed via the foramen magnum using curettage and suction, leaving the cranium intact. Much of the dura mater could not be removed because of adherence to the inner table of the calvarium.

Maceration

The maceration process was begun by submerging the skulls in a solution of 20 liters of tap water, 150 U of hyaluronidase (Wyeth), and 198 g of sodium hydroxide granules (Chem Lab S350). During the first week of the maceration process, a clear yellow lipid material collected on the surface of the water and was removed on a daily basis. This volume was replaced with tap water as needed. At the end of the first week, the water was completely drained and replaced. The skulls were noted to have a foul odor with gelatinous soft tissue firmly adherent to the periosteum of the bone. At the beginning of the second and third weeks, 1 g of pancreatin (Sigma P1625, 3× USP, Sigma Chemical, St. Louis, MO) was added. During the first 3 weeks of the process, the pH of the solution was maintained at 8 by addition of sodium hydroxide granules as needed to maximize the enzymatic activity. The pH was measured on an aliquot of solution using a standard laboratory pH meter. The solution was allowed to remain undisturbed, other than removal of the adipocere (a water-insoluble degradation product collected from the tank surface), for the remainder of the maceration period. At the end of 2 weeks, the soft tissue was quite necrotic and by 4 weeks most gross tissue had become loose. After 6 weeks, all bits of soft tissue from the recesses of the skull had been macerated away and the process halted by placing the skull in cold water. All teeth became loose during the maceration of the skulls, and this finding was used as a guide to the completion of this step. At this point, refrigeration of the skull was helpful in retarding growth of mold on the specimen.

Figure 1. Skull at conclusion of preparation stage.

Defatting, Washing, Bleaching

Defatting, washing, and bleaching of the skulls was performed using sequential solutions of 100% carbon tetrachloride, PEX laboratory soap (Peck Products), and 6% hydrogen peroxide (Chem Lab H2151). Initially, the skulls were immersed in carbon tetrachloride and left for 2 weeks in a closed container to control evaporation of the solvent. Subsequently, they were cleaned with laboratory soap to remove a thick water-insoluble greasy substance that remained on the bone at the conclusion of maceration and defatting. Gentle mechanical cleansing with a soft bristle brush was required. The skulls were then submerged in 6% hydrogen peroxide for 5 days. At the conclusion of these steps, the skull was white in all areas and without evidence of yellow lipid discoloration.

Articulation

After defatting, washing, and bleaching, the teeth were cemented into anatomic position with a small amount of 5-minute epoxy cement applied to the ends of the roots. The cranium was sectioned with a bandsaw to allow visualization of the interior of the cranial vault. Stainless steel pins (20 × 1.5 mm diameter) were cemented into pilot holes previously drilled into the calvarium in the midline anteriorly and posteriorly. These pins engaged holes drilled in the complementary half of the cranium. The mandible was articulated to the skull using two springs threaded through bilateral holes made in the alveolar ridge posterior to the 3rd molar and holes drilled in the floor of each middle cranial fossa.

Figure 2. Finished skull with white bone and no lipid discoloration. No soft tissue or residue is present in the skull recesses. Note the presence of the entire fine bony structure of the turbinates.

RESULTS

The skulls produced by this technique were of a uniform high quality with no identifiable preparation artifacts (Fig. 2). There was no difference between the use of frozen or fresh cadaver heads using this technique of preparation.

DISCUSSION

The need for quality anatomic material for study of the intricate relationships at the skull base has required us to reexamine the historical processes used for the preparation of skeletal parts. These procedures have not been widely appreciated in the Western world because the previously available Asian skeletal material was imported as a finished product. The result of our investigations has led to the development of a process to produce dried skulls of a caliber that meets or surpasses what was previously commercially available.

Historical Review

Our process employed to prepare skulls was adapted from a method first described in 18th century sources.¹ In this era material for anatomic study was frequently obtained from the practice of grave robbing and other nefarious means. Principles of supply and demand ultimately led two Scottish “resurrectionists,” William Hare and William Burke, to engage in an enterprise designed to obtain bodies for anatomic dissection at the medical school at Edinburgh. They obtained their material by murdering 16 citizens whose bodies were then sold to the school anatomy department. This crime, for which Burke was ultimately hanged, brought about the passage of the anatomy laws in the 1830’s.² The Commonwealth of Massachusetts passed a law in 1831 that provided that the bodies of “deceased persons . . . required to be buried at public expense” could be used by medical schools for anatomic studies. Subsequent to this episode, the whole process surrounding the preparation and procurement of specimens became much more publicized as well as honorable.

The process of skeletal material preparation consists of three basic steps: maceration or removal of the soft tissues, defatting, and bleaching. One of the first public accounts of skeletal preparation was that of the method of Pole.¹ The process began by immersion of body parts in water of sufficient depth to allow for complete cover. Since these accounts took place in the premicrobial era, little mention is made of the temperature requirements, but early anatomists did note that summer was the best season for maceration of anatomic material. At the conclusion of destruction of the "white textures," this soft tissue was scraped off the surface of the bones, and they were again placed in water for a few days. The bones were subsequently submerged in a solution of lime water and left for 1 week. Drying the bones was the next step, and the seashore was recommended as an ideal place to leave the bones because there they would be washed daily by seawater and then bleached by the sun. Modifications of this technique soon followed. Cloquet and Bogros in Parsons,³ recommended a change that they thought would lead to whiter bones in addition to shortening the time required for maceration. This was accomplished by placing the specimen into a watertight box into which a small amount of water was introduced. This produced a high humidity condition in the box, which was thought to be a "more active solvent than water," allowing maceration periods as short as 6 weeks to 2 months to destroy the soft tissues. A later source refers to the two main methods to produce reduction or, ultimately, complete removal of the soft tissues: maceration, much the same as already outlined, and boiling.⁴ Boiling was reserved for use when the specimen was "firm and solid" to avoid heat-induced damage to delicate bony structures. This boiling of the material was then followed by an accelerated maceration step. Most of the changes over the years in the processes used by early anatomists produced conditions that were more beneficial to the bacteria that perform the work of maceration of soft tissue.

Modern methods, as presented by Tompsett,⁵ basically followed the same procedures outlined herein. Unfixed material was superior to fixed because, in the latter material, the soft tissue was difficult to remove. The soft tissue should be preliminarily excised to reduce the amount of adipocere formed, since this material is extremely difficult to remove. The preferred method is to gently warm the material at 37°C, but a harsher boiling process (60 to 65°C) is employed when the bony features of the material can withstand it. The process is complete when the periosteum can be removed with gentle scraping and often this requires up to 3 months' time. After cleaning, the bones are soaked in 2% caustic soda (sodium hydroxide) for 1 to 4 hours to cause any remaining soft tissue to swell and become gelatinous. The bone is then scrubbed with a stiff brush dipped in a paste of quicklime (calcium oxide) and then subsequently rinsed in cold water. Bleaching of the specimen is accomplished by submersion for up to a week or longer in a dilute solution of

hydrogen peroxide with a "few" drops of ammonia added. The bones are then dried and the grease that appears on the surface is removed by soaking in a solvent such as trichloroethylene or benzene for 24 hours. This is repeated until grease is no longer detected. This approach requires a good deal of manual labor and time-intensive effort with brushes and is not suitable for the intricate features of the human skull.

The Dermestidae family of beetles is widely employed by natural history museums to produce rapid debridement of soft tissue from animal specimens.⁶ The larvae stage has a voracious appetite and indeed performs an excellent debridement of even the smallest cavities, but several thousand beetles are required to produce rapid cleaning. If the beetles are left with the bones for an extended period of time while deprived of other food, they will even eat the bone. However, the main problem in using these insects is involved in maintaining the colony between debridements. The beetles are quite difficult to contain and have a proclivity to bore into any wood, plastic, and even styrofoam insulation in the vicinity as well as dead animal material.^{7,8} Thus, for the casual, infrequent user, storage and maintenance overcome the benefits of efficient debridement.

Present Method

The quality of the final product is similar using both frozen and fresh heads and both are adequate sources of raw material for dried skull production. The use of embalmed heads may be possible as well; however, the changes in soft tissue produced by the fixation process generally makes the tissue resistant to maceration by natural means. Historically, embalmed material has been utilized primarily for sturdy skeletal material because of the boiling and harsh chemicals that must be employed to accomplish maceration after tissue fixation. The aggressive maceration procedures as well as the prior tissue fixation adversely affect the quality of the finished material.

The present method (summarized in Figure 3) differs from earlier descriptions primarily in the maceration process. Beneficial changes in the process during the premicrobial era were made in ignorance of the importance of bacteria in the maceration process. Our modifications are designed to encourage the bacterial activity, which accomplishes the maceration. Vigorous bacterial activity produces a clean specimen as well as a rapid process since maceration is the most time-consuming step in the procedure. In addition, a process that requires limited mechanical cleaning is necessary given the delicate nature of structures at the skull base. We utilized hyaluronidase and pancreatin with pH control to increase the surface area of the tissue available for bacterial digestion.⁹ Hyaluronidase is an enzyme that hydrolyzes the mucopolysaccharidoses of the intercellular ground substance.¹⁰ Pancreatin is com-

PREPARATION OF A DRIED SKULL

Figure 3. Flow sheet showing overall process. See text for further details.

mercially prepared from porcine pancreas and contains primarily amylase, trypsin, and lipase, which act to degrade polysaccharides, proteins, and fats, respectively.¹¹ Because of the pH-dependent action of these enzymes, the solution is ideally kept at pH 8 during the early period of maceration when these enzymes are at maximum activity. The alkaline pH of the solution is maintained with periodic additions of sodium hydroxide. During maceration, the putrefactive processes tends to produce acidic byproducts, which, if not neutralized, produce unfavorable conditions for enzymatic action. After a period of time for the enzymes to work (3 weeks), the solution pH was allowed to decrease naturally to provide an acidic environment for the putrefaction process to begin. The solution should be left undisturbed to allow for the rapid establishment of an anaerobic environment suitable for putrefaction. Destruction of the soft tissues ensues at a rapid pace once conditions conducive to the anaerobic putrefactive bacteria are present. The temperature sensitivity of this process is evident from the much slower putrefaction that takes place in the lower portion of the vessel where temperatures at times are only 2 to 4°C cooler. Future maceration vessels will employ a concentric glass tube around the heater element to promote convection mixing of fluids in the vessel and thus more uniform temperatures throughout the vessel.

The process of bleaching and defatting is simple and comparable to earlier methods. The best defatting solvent was found to be carbon tetrachloride after an informal test of several commonly available chemicals, including benzene, chloroform, and xylene. A long period of defatting is needed to remove all traces of grease, otherwise a

residual yellow area of bone is left that persists even after bleaching. The specimen should be air dried periodically to detect this condition. At the conclusion of defatting, a water-insoluble residue of lipid material is left on many surfaces of the skull. This substance is resistant to removal by several detergents as well as other solvents (including benzene, trichloroethylene, and xylene) and ultimately yields only to mechanical removal using PEX laboratory soap. Typically, only the broad surfaces of the specimen need be brushed because the material does not collect in the small recesses of the skull. The finished appearance of the skull prepared by this technique is excellent without discolored areas, a white appearance to the bone, no objectionable odor, and excellent preservation of even the most delicate bony areas. At this writing, approximately 2 years have elapsed without appreciable change in the finished appearance of the skulls.

CONCLUSIONS

A method has been developed to produce dried skulls for anatomic study easily from readily obtainable cadaver material using modifications of processes first described in the late 18th century. The technique, requiring about 8 weeks' time, consists of maceration accelerated by several enzymes followed by defatting, washing, and bleaching. The use of beetles for debridement, with the attendant problems of maintenance and containment, were not required. The skulls produced are of excellent quality and durability with no preparation artifacts. The cadaver material readily available in North America will allow production of skulls with features more in common with the endogenous population. Most importantly, an economical source of skulls has now been reestablished to promote the learning of skull base anatomy.

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The authors most regretfully report the recent passage of Dr. David S. Maxwell. His superb knowledge and teaching of head and neck anatomy and particularly his interest in skull base anatomy will be missed at UCLA.