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R. G. Snyder,[1] *Ph.D.; A. Burdi,*[2] *Ph.D.; and G. Gaul,*[3] *B.S.*

A Rapid Technique for Preparation of Human Fetal and Adult Skeletal Material

Medical examiners, forensic pathologists, physical anthropologists, and others working with cadaveric remains often need to remove soft tissues and prepare skeletal material for further examination and retention. Currently several techniques for accomplishing this are available, including a five-step anatomical technique consisting of maceration, cleaning, degreasing, bleaching, and varnishing the bones with Alvar® to preserve them [*1*]. Another technique often used is to allow a colony of Dermestidae beetles to clean the tissues. However, both of these techniques are time-consuming, and we have found that an antiformin technique provides a much better and faster preparation. This technique has resulted from refinement of the methods described by Ternak [*1*] and Schmitt [*2*] and developed in skeletal preparation instruction in the Department of Anthropology during the past two years [*3*]. Independent techniques have evolved in the Developmental Anatomy Laboratory of the Medical School for Preparation of fetal or infant osseous and cartilaginous materials, and these have been included to provide for the full range of human materials which may be encountered. The following instructions are intended to provide the user with a rapid method for skeletal preparation which we have found to be an improvement over previous methods.

Materials and Methods

The antiformin technique can be used to prepare skeletal materials from either fresh or fixed cadavers. There are two major advantages of this method over other techniques available. First, clean specimens may usually be obtained within an hour from any type of material. Second, the process is equally applicable to fresh materials, to formalin- or alcohol-preserved materials, and to partially decomposed materials, as often encountered in forensic situations. The fixed materials provide the best results, although they require more time for preparation than do fresh tissues. Fixed materials also show a greater resistance to the chemical action. The tendency for surface flaking, which may be

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[1] Professor, Department of Anthropology and Head, Biomedical Department, Highway Safety Research Institute, The University of Michigan, Ann Arbor, Mich.

[2] Professor, Department of Anatomy, Medical School and Senior Scientist, Center for Human Growth and Development, The University of Michigan, Ann Arbor, Mich.

[3] Research Assistant, Biomedical Department, Highway Safety Research Institute, The University of Michigan, Ann Arbor, Mich.

observed in fresh specimens, is not experienced with fixed materials. Flaking can be avoided by using a more dilute solution of antiformin, and correspondingly increasing the time of exposure of the bone to the solution.

Antiformin solution is prepared by combining 150 grams of sodium carbonate ($NaCO_3$) and 100 grams of bleaching powder with 1 litre of water. The sodium carbonate should be added to 250 ml of H_2O, while the bleaching powder is added to the remaining 750 ml, and then the two solutions are combined and mixed. This solution should be stirred for three to four hours, as necessary, and add to this filtrate an equal quantity (1 litre) of a 15% solution of sodium hydroxide.

Preparation of Materials

1. Cut away the main masses of soft tissue surrounding the bone. There is no need to waste time attempting to do more than this.
2. Place the specimen in a bath of antiformin, using sufficient solution to cover the specimen. For fresh tissues use 1 *part* of antiformin to 8 to 10 *parts* of water. Heat the solution to just short of boiling and maintain at this temperature.
3. Examine the specimen from time to time to see how quickly it is being denuded. The time required varies from a few minutes to perhaps an hour, depending upon the state of the material (fresh or fixed), the size of the specimen, and the amount of soft tissue left attached.
4. When almost clean, remove from the bath; any fragments of soft tissue may easily be removed with a brush. Do not leave the specimen in the bath too long or the bone will begin to disintegrate.
5. Wash in running water and dry.

The antiformin should be filtered and may be used several times, especially if it is reinforced by the occasional addition of a little fresh antiformin.

Bleaching the Bone Specimen

After the above treatment the bone is clean and almost free of fat; however, if a white specimen is required for museum or other purpose, the bone must be further treated as follows.

1. Degrease in benzol for 24 to 48 h. Benzol is a most satisfactory solvent.
2. After the bones have been degreased, rinsed, and thoroughly dried, they should be placed in either a 3% solution of hydrogen peroxide or potassium hydroxide (KOH) (reagent) for bleaching. This solution will bleach the bones to an off-white color. These chemicals must be handled with care, using rubber gloves and goggles, since the KOH in particular can cause severe burns. Bones should remain in this bath for at least 8 to 20 h, although embalmed material may require a longer period. Use the peroxide as it comes from the container, which is generally about 3% strength. The time required for this procedure will depend upon the bone material (fresh versus embalmed), its condition, and the strength and freshness of the peroxide solution. New peroxide can do a complete job in 8 h, while a used solution will take longer. The same solution can be used three to five times before discarding.
3. Once the bones have been satisfactorily bleached, rinse them under running tap water and allow them to dry thoroughly.

An Alternative Technique

When the time is not critical in preparing a specimen, the following departures from the outlined method result in reduced actual work time for the preparer.

1. After stripping away the main masses of tissue from the bones, place them in a room-temperature bath of 15% KOH for up to four days. This soak has no adverse effect on the strength or appearance of the final product, but reduces the boiling time drastically, sometimes to as little as 5 min. Since repeated boiling and scraping of the specimen may cause cracking and degradation, this method is especially desirable for use with thin and delicate structures (for example, scapulae and skulls).
2. The tenacity of intervertebral disks poses a problem in separating and cleaning vertebrae. To minimize the difficulty, remove all the external muscles and ligaments by soaking and boiling, then place the entire spinal column in a degreasing bath of trichloroethane for two to three days. After rinsing the spine in water, place it in 3% hydrogen peroxide for another two to three days. The intervertebral disks should by then be sufficiently softened to be easily removed. The vertebrae, having been whitened in the process, are then ready for mounting.

Varnishing the Bone Specimen

The final procedure for the lasting protection of the bone involves application of a varnish-like finish. To prepare this solution, 500 to 600 cm^3 of dry Alvar® flakes (available from Shawnigan Chemicals Ltd., Montreal, or Resins Corp., Springfield, Ill.) should be mixed per gallon of acetone. This should be stirred until dissolved and stored in glass containers. Note that this mixture is extremely flammable. It is a good idea to ventilate the room or use a ventilation fume hood during preparation.

The bone should be immersed in the Alvar® solution, which may be dumped into a large glass vat. Use long-handled forceps in handling the bones. Specimens should be left in the Alvar® solution about 24 h, or until bubbles cease to come out of the bones. Small bones, such as metacarpals and metatarsals, can be placed in a wire mesh basket and dipped in the Alvar® solution. Once bubbles cease, remove the bones, drip off the excess fluid, and place on a screen to dry. The bones should be turned or they will "spot," or if the Alvar® drips off, bumps will be left at the drip spot.

As soon as all bones are varnished, pour the solution back into the glass container. The Alvar® solution is reusable.

Special Techniques for Preparation of Fetal or Early Infant Materials

Demonstrations of fetal, neonatal, and infant human skeletons must take into account the extreme fragility of the skeleton at these times. What would be the adult bone is, in these young specimens, a unit of disjointed bony parts connected only by cartilage. It is this connecting cartilage which is usually removed by most maceration procedures, using even the weakest (1 to 2%) solutions of KOH, as would be used in older specimens.

The preparation of prenatal or early postnatal skeletons is best done using (1) controlled maceration and tissue clearing and (2) a combination of the previous methods with bone dyes. The first method is best for perinatal specimens and infants up to two years, after which procedures described earlier can be used. This calls for fresh or partially decomposed specimens which can be placed in a weak (2 to 5%) solution of commercial, household-quality meat tenderizer (for example, papain) in warm water for intervals of 2 to 6 h. Timing is critical. Younger specimens are best

observed every 2 h, while older specimens will require longer periods (2 to 6 h). Use of tenderizer is more suitable than KOH since the papain slowly breaks down connective tissue and muscle surrounding the fragile skeleton. Maceration effectiveness can be monitored by observing a change in soft tissue appearance from opaque to transluscent, after which the tissues will fray and can be removed by carefully teasing away. Once the majority of soft tissues is removed and skeleton is discernible, the specimen must be floated in a water bath with a gradual removal of water. This allows positioning of the intact specimen and eventual drying. In the youngest specimens the drying procedure should be on a fine gauze sheath to collect any bone parts which become disjointed. A preparation of only bony parts from young specimens is difficult, while maintaining the identity of the many bone parts is even more difficult.

Prenatal skeletons, especially the least developed, are best prepared as intact, whole-mount specimens. Bones can be demonstrated by staining with a dilute solution of alizarin red S (sodium alizarin sulfonate) which has an affinity for osseous tissue [*4*]. It does not stain cartilage for our purposes. Fresh, partially decomposed, or fixed specimens (95% alcohol) carefully washed in water can be treated either with a dilute papain solution or a 1 to 2% solution of KOH until the skeleton begins to show through overlying soft tissues, which become progressively transparent but not destroyed. As with most steps of this classical technique, timing is dependent on the size and fragility of the specimen. Once soft tissues are translucent to transparent, the specimen should be placed in a solution (0.1%) of alizarin red S in a 1% KOH vehicle. Existing bone will progressively appear as deep red to purple. This is the end point. Any dye in the soft tissues can be removed by a short term in 1% KOH. Finished products can be stored, studied, or further treated in a mixture of glycerine and alcohol (1:1). If it is necessary to demonstrate a combination of red-staining bone and cartilage, then the intact specimen can be counterstained with toluidine blue dye prior to the storage procedure.

In any event the demonstration of bones for age determination in the prenate and young infant skeleton is possible, but only with careful technical controls. Whole-mount, intact specimens are best for early prenatal specimens with progressive option toward the demonstration of individual, isolated bones for older specimens of the neonatal and early infancy period.

Summary

The techniques described above have been found to provide a rapid method of human skeletal preparation, with advantages of speed and applicability to fresh, fixed, or partially decomposed skeletal materials. While other techniques which can be used include the traditional use of Dermestidae beetle colonies, a five-step anatomical procedure, and other combinations of chemical solvents, the antiformin technique appears to have advantages useful to those involved in forensic medicine.

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Department of Anthropology
Angell Hall
The University of Michigan
Ann Arbor, Mich. 48104