CLEANING BONES

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GENERAL GUIDELINES

- Biohazard procedures may be required when cleaning bones, particularly if they are from a recent forensic case or animal roadkill.

- Whether preparing an animal specimen for curation or a forensic case for analysis, ALL bone surfaces must be cleaned completely. Adhering soft tissues must be removed because they will attract insects and may obscure perimortem trauma. Grease must be removed because it will smell, seep through the bone, and attract dust and grime.

- Careless processing can damage the bone, obscuring or removing morphological traits and/or creating 'pseudotrauma' that can be mistaken for real perimortem trauma. Stop earlier rather than later.

- It is preferable to take a DNA or toxicology sample from forensic cases before boiling. Remove and freeze an entire bone (such as a clavicle or a rib) before any processing is started. However, it should be noted that recent research has suggested that boiling may actually increase the eventual DNA yield, and so retroactive sampling after cleaning has already occurred is feasible.

DIRTY BONES

If no soft tissues are present and the bones are of archeological (ancient) origin, the risk of biohazard is generally small. They may be cleaned with a soft-bristled brush (such as a toothbrush) and clean warm (not hot!) water. Large clumps of dirt can be picked off first with barbecue sticks or sharpened chopsticks (wood is softer than a metal pick and should not damage the bone if you are careful).

Do not hold bones under the water and do not thoroughly soak them. Water molecules actually accelerate the breakdown of the collagen/mineral bonds and lead to more rapid disintegration of the specimens. Additionally, soaked bone may crack upon drying. Even a large bone drying in a fume hood with the fan on can crack because of the strong temperature differentials induced by the rapid air movement. Therefore, let wet bones dry naturally and slowly on newspaper or on screens. A simple alcohol (ethanol) bath can also remove water rapidly from fragile bones that are otherwise clean. Let them soak in a 50% solution for a few moments, then a 75% solution, then a 95% solution.

BIOHAZARDOUS SPECIMENS

Remains with soft tissues attached should always be treated as potentially biohazardous, particularly if blood, pink muscle, organs, or brain tissue is present. The simplest and most effective way of dealing with them is to use a bleach solution for boiling and cleaning. One cup of bleach to a gallon of water is fine, and detergent may still be added if it is a boiling solution. The bleach-water

solution should be used for cleaning pots, utensils, and processing surfaces afterwards, as an antibacterial agent. However, always make a fresh solution each time you need it because it will lose its potency after 24 hours. Use disposable latex gloves to protect yourself from both the bleach and any bacteria.

CAUTION: DO NOT OVER-SOAK OR OVER-BOIL BONES IN BLEACH!!! Bleach can etch and destroy the bone surfaces, particularly those of juveniles and small animals. Only use bleach during the FIRST boil. Make sure to thoroughly rinse the bones in fresh water immediately after subjecting them to the bleach, because the chlorine will continue to degrade the bone surfaces even after they are allowed to dry. Bleached bones should be placed in a plastic tray under a stream of running tap water for at least 15 minutes. Immediate reboiling in fresh or soapy water will also remove the bleach.

If you are concerned about infection prior to boiling (such as during transport of a recent roadkill), spray it well with Lysol. If it is very fresh (e.g., still warm), you should worry about fleas and ticks. Wear gloves and place it in a plastic garbage bag, filling it with Lysol spray. Smelly remains can be stored in a freezer with no fear of harm to the bones. If any blood is present, you should use standard safety precautions to avoid blood-borne pathogens.

It should be noted that human skeletal remains interred in historic graves in the 19th century may contain high levels of arsenic, mercury, or other heavy metals. These elements were used in the embalming process and could potentially poison archeologists in the field or osteologists in the lab. One should consult the local Department of Health for protective guidelines in these circumstances.

Autopsy specimens are frequently preserved in formalin or formaldehyde. These specimens may still be prepared by boiling as described in this document, although care should be taken not to breathe in the fumes or steam. Cover exposed skin while processing, and make sure that the disposal of the resulting fluid and soft tissues conforms with local regulations.

BOILING

There has been considerable debate among anthropologists as to how forensic remains should be macerated. Many feel that boiling is much too destructive a process. However, we have been experimenting with this method for years and now feel that boiling, when carefully applied, is far superior to no-heat or low-heat methods, beetles, and caustics (bleach, ammonia), and is also quicker.

Soft tissues and grease are best removed through simmering and boiling. First carefully remove skin, large bundles of tendon, muscle, and ligaments with dissecting tools. Try not to damage the bone surfaces -- the boiling will be doing most of the work for you. Put the bones in a metal pot with enough water to cover them with an extra inch or two (try to arrange them so that they fit flat on the bottom of the pot -- less water means a more rapid boiling time). Then add up to a cup of powdered enzyme-active borax and bring it to a boil. Let them boil with a loosly-placed lid (to keep the kettle from boiling over) for 30 minutes to an hour and check a few to see how the soft tissues are doing. Even the dense ligaments and cartilage should begin to degrade as the proteins break down. Scrape the soft tissues off with a wooden chopstick or popsicle stick as they loosen, and return them to the pot if necessary. Disarticulate all joints as soon as is possible. Boiling hands or paws separately in different pots will make later identification and analysis much easier.

Remove the teeth and clean the sockets, if possible. It is important not to repeatedly boil and then cool the teeth, because they will crack as the temperature fluctuates. Try to remove them after the first boil is complete. Staining of the tooth enamel is common (particularly in carnivorous animals and rodents) and should not be worried about. It is not necessary for the bones to be white either. Color is irrelevant for analytical purposes as long as the bone is free of soft tissues, grease, and odor.

The time required for boiling will depend on the condition of the remains at the start and the size of the specimen. DO NOT OVERBOIL, particularly small or immature specimens. Try to limit boiling time to an hour or two at the most, and then turn down the heat to a light simmer for as many as 6 to 8 hours. The lower temperature (75 to 80°C) is actually the optimum operating level for the enzyme-active borax and should produce the best results. Check the edges and ends of the bones frequently for mechanical erosion. If you can see flaking or exposure of the spongy bone, pull it out. Very fragile or small bones can be held in the pot in a metal strainer, to keep them from eroding as they rub on the bottom. Pans purchased at restaurant supply stores sometimes come with insertable straining pans that are large enough to hold even the long bones. Change the water when you see fatty scum collecting on the surface. It's usually a good idea to dump dirty water through a strainer to keep from losing small bones and teeth.

Boiling releases the fats held within the marrow cavities. It is important to remove as much fat as possible or else it will leach out and stain the bone in years to come. For this reason you may still want to boil bones that are greasy but otherwise devoid of soft tissues. Drilling holes in the ends of large animal long bones or formalin-fixed specimens will allow the fats to escape more quickly from the marrow cavities. A wire coat-hanger can be poked in the hole to break up the spongy bone first.

Whether in the field or in the lab, never package wet bones in plastic or any other wrapping that will hold in moisture (such as plastic bags or aluminum foil) unless they are very odorous or still have potentially biohazardous soft tissues attached**.** The moisture softens fragile bones, making it particularly susceptible to further damage. Bones that have been submerged in water and/or trapped within adipocere for a long time before recovery are almost always in poor condition and bend or break easily. They must be processed with extreme care -- boiling tends to erode the ends very rapidly. In these cases we usually stop the processing before the bones are completely cleaned, and degrease using chemical methods.

CHEMICAL DEGREASING

Sometimes the boiling process will not be able to remove the fats before the bone surfaces begin to mechanically degrade, or the specimens may be too large or too numerous to justify the close attention demanded for hours of boiling. In these cases, we soak the bones for a few days or weeks in various degreasing solutions. The cheapest of these is 2 or 3 cups of household ammonia per gallon of water. The bones can be sealed in 5 gallon plastic pails and left in the solution at room temperature. Small traces of adhering soft tissues will tend to liquefy during the soak.

If the remains are particularly tenacious or very fragile, a xyol solution (60% industrial grade alcohol + 40% xylenes) is superb. If the bones are wet, allow them to air-dry first for at least 24 hours (48 is frequently necessary). Then place them in a sealable glass container and pour in the xyol solution, using just enough to cover the top of the bones. Seal the container with silicone stopcock grease and place it in a fume hood. The alcohol in the xyol moves in and removes the water from the bone and also serves as a thinner for the xylenes, while the xylenes dissolves the fats in the bone.

However, the solution will not break down soft tissues. The xyol can be reused until it is completely filled with fats (i.e., when it takes on a very dark amber color, darker than apple juice, and a thick layer of grease floats to the surface). After soaking, the bones can be air-dried on paper. It will take about two days for them to dry. If you still smell xyol, they aren't dry yet. **CAUTION: xyol is extremely noxious -- it should only be mixed and used in a fume hood!** Never pour xyol down a sink -- only dispose of these chemicals in a fashion consistent with local regulations.

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