PREPARING SKELETONS FOR RESEARCH AND TEACHING

FROM PRESERVED HUMAN SPECIMENS

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ABSTRACT

As the need for access to human bone exponentially increases in forensics and academics, supply is decreasing through repatriation of remains and restricted access to collections. Currently underutilized sources of human skeletal material from preserved tissue offer a solution. Earliest preservation and skeletonization techniques followed guidelines that were very different from the present. Most focus today has been on non-preserved tissue; there is a practical need for research with fixed specimens. Producing dry skeletons without bone modification from preserved tissue must be exact, as they do not macerate easily. For this study three complete preserved human specimens were tested to determine which process would produce the most viable dry bone.

Commercially available emulsifying products were used for maceration: laundry and dish detergent, baking soda, meat-tenderizer, and ammonia. These were compared to texture, ease of tissue removal, and bone quality were compared based on the work of Steadman et al. (2006). Our results show that combinations of solutions provide very similar results; sodium bicarbonate consistently scored highest in all categories evaluated. Due to the incredible variations between skeletal elements in size, shape, and structure, a reproducible method for an entire skeleton with limited chemistry must be undertaken. In future studies, the most important rate-limiting factor will be removing preserved marrow without altering bone quality. These trials showed what not to do in future work as much as what direction to take.
DEDICATION

To my son Jake

Thank you for making me a better person and for teaching me what is most important in life
PREPARING SKELETONS FOR RESEARCH AND
TEACHING FROM PRESERVED HUMAN SPECIMENS

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The amount of work contained in this thesis, while substantial, is an initial foray into the expansive multi-disciplinary work required to create dry bone from preserved tissue. The introduction serves as a needs analysis for the continued production and preservation of dry bone from preserved tissue. Political and social issues of repatriation and human rights frame the managing of this resource. Scientific advancement in forensics and anthropology both demand that this work be carried out.

A thorough literature review provides a detailed summary of past documented work in this field. Comparing the relatively large number of osteological collections in the United States alone with the few acknowledged reports of process and outcomes points up the discrepancy. Those listed here at times both support and contradict other authors’ work. Appraising previous efforts shows clearly that a reliable and reproducible method for skeletonization from fixed tissue has yet to be discovered.

The scope of this research project encompasses investigative trials in the initial maceration and degreasing of preserved cadaveric specimens. Resulting analysis of processing solutions through Gas Chromatography Mass Spectrometry is also included here as another step in determining best practices. Clarifying tone, color, and fixing of the final product are the topic highlights of a next step in bone production, and as such are not included in this thesis. The work presented here represents extensive, ongoing efforts to determine the most effective and efficient methods for future bone production and preservation.
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INTRODUCTION AND NEEDS ASSESSMENT

At no time in history has the study and understanding of skeletal material been as valuable and pertinent as it is today. So many diverse endeavors in education as well as research require unfettered access to this valuable resource. To the average person, bones are most associated with such cultural standards as Halloween skeletons, a pirates’ flag, even ghoulish remnants of war and pestilence. They have ‘otherworldly’ characteristics that most scientists have been desensitized to. Only a few laypersons have considered their scientific value as a vital part of anatomical study. From a full skeleton hanging in a biology class or an anatomy laboratory to individual bones used in comparative and forensic contexts, study of the human condition would be impossible without the use of skeletal material. The ability to definitively identify fragments and severely damaged structures, as well as teeth and dental remains are a few of the extraordinary skills required.

The need for the use of authentic osteological specimens for students as well as professionals in established fields and in new burgeoning disciplines has rapidly increased over the past several decades. Philip Tobias published an “incomplete” list of 15 major reasons for investment in curating skeletal material. (1991: 278-280) He stated that: “The existence of human skeletons in such collections is crucial for the teaching of human anatomy, including dental anatomy, students of medicine, dentistry, physiotherapy, occupational therapy, nursing and pharmacy; as well students of comparative anatomy, primatology and general morphology.” He also states that comparative studies of diversification in Homo sapiens, immature versus mature
skeletons, sex differences, obstetrics, mechanical properties, prevalence of congenital
variants and acquired anomalies are but a few specific example uses. The number of new
fields that rely on access to this learning material has astronomically increased and
expanded in the twenty years since Tobias wrote the above statement. Forensics, DNA
research, and molecular biology are but a few worth mentioning. Others are on the
horizon.

Part 1: The History, Ethics and Politics of Skeletal Collections

Historically, bone collections that are used for anatomical study have been
procured in various legitimate and clandestine ways. Most academic institutions with
collections of skeletal material will have some bone that is well documented and others
where the provenance is somewhat unclear. Skeletal material amassed before the mid 19th
century usually consisted of human remains that were not preserved. Remains of
executed criminals and indigents have often been sources of bone for interested
individuals (Dalley et al., 1993: 248-249). As often as not these would have been
obtained through purposeful scavenging or excavation. “Most bodies were stolen
between November and March, when medical schools were in session and the cold
helped preserve the corpses…ten days was usually the limit between death and burial
(Hulkower, 2011: 2).” If the ‘owner’ of the specimen had the means after dissection, they
could allow nature to take care of defleshing and skeletonizing the body. Otherwise a
hasty burial could be provided.

Such practices were not relegated solely to the past. When stealing bodies on
Kodiak Island in 1929, Hrdlicka speaks of opening graves where the remains were “still
too fresh,” and to encountering an irate widow as he made off with her dead husband. He notes that native children and babies were especially prized as they could be used to determine the racial differences he was so intent on finding (Dumont, 2003: 117-118). Ultimately, Hrdlicka and his colleague’s genetic theories have been debunked, and a movement towards more honorable methods of procuring materials has paralleled the slow decline of colonialism and changing definitions of empire. Not in spite of, but because of this move towards social equality, a solution must be found to meet demand as supply dwindles and the inevitable change happens in the human race as mirrored in our own skeletal material.

Ultimately this work will show how it is possible to acquire and curate human bone material with transparency and respect for the living and the dead. It is disingenuous and likely racist when scientists conveniently forget how dead ancestors came to be in their ‘collections.’ Within this scenario, it is of utmost importance that those living relations to the bones in question be considered in decision-making. What would happen if the Republic of Vietnam refused to return the remains of American service men and women killed there? What if they said, “we want to keep these remains and study them, they have much scientific value?” (Dumont, 2003: 112-113). A policy that will continue to build collections and improve them for the educational demand that will be placed on them must be made. Continuing respectful access and increasing the numbers of available comparative samples is an ultimate goal.

Morals, ethics, and politics notwithstanding, virtually every skeletal collection through the first half of the twentieth century were comprised of specimens with suspect
origins. In 1868 the surgeon general of the United States issued an order to troops that a craniological collection was being undertaken for the Army Medical Museum and those stationed in Indian country or near burial mounds or cemeteries were urged to promote this undertaking (Quigley, 2001: 102-103). This practice of seizing and claiming remains and artifacts from conquered peoples is of course not condoned in contemporary society, though both Douglas Ubelaker and Lauryn Grant (1989: 249) argued that “it would be racist not to have large collections of aboriginal New World remains in New World Museums since that would imply lack of interest in the history of those people.” After all, Robert Mallouf (1996: 206) maintains, “scientific findings…have proven so important in dispelling the prejudiced European concepts of the ‘barbarous savage.’ Today archaeologists legally procure access prior to investigation that not only furthers scientific understanding but cultural access to bones and artifacts through open channels of communication.

Recent Indian burials were not placed off limits until 1979, with the passage of the ARPA. This legislation specifically states, “No item shall be treated as an archaeological resource … unless such item is at least 100 years of age.” (Dumont, 2003: 118) While not always followed, one can see a burgeoning conscience within anthropological circles regarding best practices for dealing with remains and artifacts belonging to the people that they were scrutinizing. Anthropology’s ultimate goal is to further a better existence for all humankind; working to achieve this while facing the moral dilemma of proper procurement and curating of human remains. Increasing modern collections will ensure that as time goes on the number of protected and documented
skeletons will multiply. (Dirkmaat et al., 2008: 33) With as extensive documentation as possible, these newer skeleton collections would help fill the daily increasing demand for comparative bone samples.

Reburial is a problem, Patricia Landau and D. Gentry Steele assert, because “physical anthropologists have an interest in learning just who humans are.” (1996: 223-224). What at first appears as such a straightforward situation quickly becomes convoluted and not at all simple. It is diminishing access to these priceless bones that helps to drive the need for new acquisitions in the world’s collections. Treatment of human remains is an evolving topic outside science, demanding greater sensitivity and respect for cultural heritage but leaving many historic collections and much of physical anthropology and archaeology in disarray. Repatriation legislation recognizes that scientific rights do not automatically take precedence over religious and cultural beliefs (McGowan & LaRouche, 1996: 109-121). When considered from a more objective side, it is clear that anthropologists wanting to access skeletal variation only serve to give more agency to marginalized persons they may observe and study.

Dr. William Bass stated, “From the viewpoint of a skeletal biologist (reburial) is similar to burning the books in our libraries.” (McGowan & LaRouche, 1996: 114). His attitude is in direct opposition to the beliefs of the Northwestern Plains/Missouri River Indians whose ancestors he collected and studied; “respect for the dead is more important than any knowledge of the past that might be gained by digging up graves.” He said that the tribal activists gave him the dubious title of ‘Grave Robber Number One.’ (Bass, 2004: 31) The net result of 20 plus years of territorial struggle is the NAGPRA and an
amendment to the NHPA. The African American community is the most conspicuously not represented by NAGPRA, as the legislation requires cultural affiliation, which descendants of former slaves cannot provide. On the other hand specific areas of Jamestown, Virginia have been preserved undisturbed where descendant community has historically retained power and control (McGowan & LaRouche, 1996: 116). Contemporary scientists with interest in these osteological remains of course would like to see policies and procedures that would allow equal access across the board.

In contrast to the unpreserved bone samples acquired through usurping the rights of others, the trend towards creating legitimate skeletal collections expanded logically from the development of tissue preservation techniques. The earliest evidence of embalming comes from Europe in the mid 17th to 18th centuries with the Work of Swammerdam (discoverer), Ruysch (refiner of technique), and Blanchard (openly published method (Mayer, 2006: 468-470). By the time of the Civil War in the United States, these methods had advanced enough to provide preservation of the deceased on an unprecedented scale. These events combined with an increasing use of human cadavers for anatomy dissection and experimentation and provided the specimens necessary for acquiring skeletal material of known clinical history.

Decreasing religious beliefs in dissection being a fate worse than death led to a phenomenon whereby citizens could donate their bodies to science without fear of damnation (Hulkower, 2011: 3). Grave robbing and other less-than-transparent means of specimen procurement diminished as legal methods continued to develop well into the 20th century. A major milestone occurred in 1968 with the passage of the UAGA, making
body donation a right, morally based on free choice and volunteerism. Further clarification occurred with the signing of an addendum act in 1987 that established the human body as property (Garment, 2007: 1002-1003). Although today, laws that affect the living such as the HIPAA also have repercussions for deceased donors. Where in the past, often pages of useful demographics and health history might be included with the donor, presently only specific information is available to the scientists: sex, age, known co-morbidities, and cause of death. (Stubblefield, 2011: 11)

**Part 2: Skeletal Collections in the Modern Era**

A process whereby skeletal material could be harvested from preserved human remains naturally progressed along with increasing interest and understanding of its value. Doing justice to the entire oeuvre of important osteological collections worldwide would take much more space than allotted in this work. Therefore the majority of skeletal collections referenced here will be from the United States of America. Generally speaking the most critically recognized have come directly from the work of George S. Huntington, Robert J. Terry, Mildred Trotter, Carl August Hamann & T. Wingate Todd, and W. Montague Cobb, all of whom are referenced below. It should be noted that Dart, the Mutter, the US Army Museum, and others (Quigley, 2001: 125-136) are but a few excellent representatives not detailed here.

Each of these pioneers served to forward the valuable work of human skeleton collecting and curating, providing insight into trends and taphonomy. First introduced in the Pan-American Geologist Journal by Ivan Efremov in 1940, taphonomy has come to represent the study of any antemortem, perimortem or postmortem changes represented in
bone before discovery of fossilization. Development of this most valuable concept continues to evolve as newer empirical analysis techniques are discovered (Stuart et al., 2006: 133-136). Therefore all bone is valuable. Along this line, most skeletons were obtained from medical school dissection room specimens, accompanied by at least some demographic data, depending on what may have been available at procurement. Even with the cultural shift allowing for ease of body donation, each collection represents either the milieu of the place it was established or those places where specimens were procured. Today, the Smithsonian Museum of Natural History, the Hamann-Todd, the Cobb at Howard University, the Peabody at Harvard, and several others curate major skeletal collections, continuing to evolve with the ever-changing landscape of repatriation and scientific needs and protocols.

Mentored by George Huntington (Collection at Museum of Natural History, New York City.) and Sir William Turner at Edinburgh University, Scotland, Robert J Terry championed not only processes for dissection room collections but also cataloguing and curating. Twice specimen collections he amassed were destroyed by fire and mismanagement (Hunt & Albanese, 2005: 407) Ultimately through his protégé Mildred Trotter, one of the most well appointed and documented collections of provenanced human skeletal material in the world is being curated at the Smithsonian Institute in Washington, D.C. Her work on estimating stature from long bones is well-placed in forensics and comparative anatomy (Trotter, 1952: 511-513).

As an example, the Hamann-Todd Collection in Cleveland, Ohio consists of more than 3,100 complete human skeletons gathered from the last decade of the 19th century
through 1938. Representing a population of individuals who were either indigent or otherwise unclaimed at death, each specimen is accompanied by extensive information about the person: name, age, sex, ethnicity, cause of death and over 70 measurements. After complete anatomical dissection at the Western Reserve Medical School, skeletal remains were harvested and used as teaching aides. The sheer number of specimens and detailed data in the Hamann-Todd Collection makes it a truly priceless resource for those who undertake anatomical study. “Since 1990 alone, more than 140 scholarly publications (roughly one every two weeks) have been based on research conducted at the Museum” (Jones-Kern & Latimer, 1996: 1-2). While this impressive testament to the collections scientific significance is undeniable, placing it in a contemporary context is imperative.

A classic example of an anthropologist with forward thinking ideals is the collection of W. Montague Cobb at Howard University in Washington, DC. An African-American doctor and activist, he realized early on that amassing data from his skeletons (they were mostly his black patients) would go a long way to providing empirical data to combat racism (Cobb, 1942: 115-120). Available notes from his process indicate that specimens were preserved in 2% phenol and that they were macerated, rinsed, and processed with wire brushing (Hunt & Albanese, 2005: 408). No further mention is made of chemistry or process than that found in his early dissertations. A quote by Cobb sums up one of the many purposes of this detailed research offering: “When our present system of collecting skeletal material was instituted, local mores were such that the ground had to be carefully prepared in respect to the value of such collections and the care which
must be observed in its conservation if it is to find the greatest use.” At this juncture there are many people who have already donated themselves for anatomical study. There is no shortage. (A. Corson USF Willed Bodies Program and C. Wacker UC Davis Body Donation Program, personal communication).

In effect, without diminishing the value of the skeletons, they do represent a specific time and place in cultural history. Because the Hammond-Todd collection was assembled prior to the introduction of antibiotics, many of the specimens show examples of the effects of tuberculosis and syphilis on bone. Rarely seen today, periostitis, a formerly common malnutrition disorder is well represented in the Cleveland collection. Skeletal remains also evidence medical treatments that have gone by the wayside, such as a thoracoplasty. As it was once thought to allow the lungs to heal in tuberculosis patients, removal of several ribs is often noted in the specimens. A fracture that has become almost “extinct” since the invention of the electronic ignition is noted in large numbers. All too often mishaps with the starting crank would happen if the handle slipped out of the hand and literally break the humerus in half at mid-shaft (Jones-Kern & Latimer, 1996: 5).

These are but a few examples of acquired anomalies reflecting time and place in skeletal collections; they bring up the need for more contemporary collections. To the untrained eye a bone may be a bone, but to an anthropologist it is very clear that each skeletal specimen will represent not only the individual’s life, but the cultural milieu in which they lived.

In the Yearbook of Anthropology (2008: 36-37), Dirkmaat, Ousley, and Symes focus their attention on the need for more contemporary sample populations. Their
*Fordisc* application uses well-established methods of multivariate analysis that were developed in the 1930s, and the focus rightfully belongs to the samples used in comparisons. More samples are needed for finer grained analyses that may well be of increasing importance in human identification, independent of the time period considered (Ousley and Jantz, 2012: 97). In the last 20 years, forensic anthropology has profiled extensive empirical results to refuel the typological concept in examining variation within groups such as American “Whites,” African groups, and East Asian groups (Willey & Leach, 2003: 184). So many advances have been made in how bone may be used to establish facts with increasing reliability. Access to viable samples for students of forensics to learn is crucial to this endeavor.

The *Daubert Guidelines* reinforced the need for modern samples as a basis for testing traditional analytical methods as well as developing new ones, and the evolution from experience-based analyses to replicable methods, often involving statistical analysis (Wiersema et al., 2009:83-84). Statistics is the science of prediction and certainty, and *Daubert* demands estimate of scientific certainty in conclusions. As the one tissue that is most likely to remain in any context, increasing bone availability in a more life like provenance still stands as the best solution to the steep learning curve for forensic evaluators. Key to this improvement is the identification and compilation of more appropriate contemporary samples (Dirkmaat et al., 2008: 36-37). Without representative samples of modern populations, the value of comparative anatomy is severely limited, possibly moot.
The Native American Graves Protection and Repatriation Act (NAGPRA) of 1994 in the United States (as well as similar laws in several other countries) have irreversibly reduced the inventory of what is available for study. Once remains have been buried or ceremonially destroyed, they are no longer available to science. All public and private academic institutions must open their laboratories for investigation and scrutiny. “This is especially important now, as the last two decades have provided prominent examples of native peoples seeking to recast the public—ultimately political—basis of their native identity in ways other than the reproduction, often fanciful, even fictional, pasts. Our hope is that we can help change the public perception of native struggle—allowing people to see that native cultures and societies are very much ongoing (and to a surprising extent on their own terms) and that the issues they confront carry important practical and theoretical implications for a more general understanding of cultural and political processes (Fine-Dare, 2002:ix).”

While it may appear that once bones are first studied they lose their scientific “value,” nothing could be further from the truth. Two major studies regarding progress in osteological research underscore this fact (Lovejoy et al., 1982: 330). reported that of the 2239 articles published in the American Journal of Physical Anthropology from 1930 to 1980, 45% pertained to osteology. Work by Buikstra & Gordon (1981: 451-452) showed clearly that skeletons collected during the 1930s were used most during the 1970s and they were just as useful for technical research. According to Rose et al. (1996: 85-86), “these two studies clearly demonstrate four important points: (a) osteology is a popular research endeavor; (b) skeleton collections have contributed significantly to the total
research effort of biological anthropologists; (c) skeleton collections have current 
research value regardless of excavation date; and (d) skeleton collections are repeatedly 
restudied, especially when new techniques become available.

**Part 3: Advancing Technology Increases Need for Bone**

Almost daily, methods are being discovered that would allow for gathering of 
new important information that is relevant to the descendants of those long-ago deceased 
as well as for the greater human population. The creation of the *Joint POW/MIA 
Accounting Command-Central Identification Laboratory* (J-PAC-CIL) in 1947 has helped to successfully identify numerous human remains dating back to the early 20th 
century and sometimes earlier. “As of March 2008, the CIL has identified more than 
1,400 MIA U.S. service members and civilians from previous wars. The CIL, as part of a 
broader effort, has achieved the U.S. government’s goal of finding, recovering, and 
identifying its MIAs by employing search and recovery teams wherever necessary in the 
world. (Farrell et al., 2013:11)”

These teams are supervised on-site by highly experienced archaeologists and 
forensic anthropologists who are trained not only in anthropology, but in the handling of 
evidence and the conduct of investigation. “By employing state-of-the-art equipment and 
adhering to strict scientific and legal principles, these teams find and recover America’s 
MIAs from some of the most hazardous and remote areas in the world…methods of 
recovery and identification used by the CIL echo the MIA herald, ‘You Are Not 
Forgotten.’ (Mann et al., 2003:139)”
Atrocities and “acts of nature” where proof of identity is necessary and yet extremely difficult from evidence that has been altered beyond recognition demand experts with the highest qualifications. Undoubtedly the most significant event that underscores the priceless value of anthropologists with specific expertise in all areas of anatomy, and particularly in small fragment identification, were the attacks of September 11, 2001 in Pennsylvania, Washington, DC, and New York City. Taphonomically, the conditions were very similar—fragmented, commingled, and burned remains. Each site had its own unique challenges. Both Flight 93 at Shanksville and Flight 77 at the Pentagon were considered “closed populations,” as there were a known number of named individuals. On the other hand, the World Trade Center site was labeled as an “open” population, with the numbers and identities of the victims as an unknown. (Sledzik et al., 2009:289-290)

At the site of Flight 93, the forensic anthropologists trained in skeletal biology, human anatomy, and fragmentary human osteology strategized and created a triage system, whereby human remains could be sifted from aircraft debris, personal effects, botanical items, animal remains, and other non-human material. Their team consisted of a dentist, a pathologist, a DNA specialist, and a Federal Bureau of Investigation (FBI) agent. The anthropologists guided their specialists through evaluation of sets of remains for potential identification. This sorting process focused efforts on remains that led to the most conclusive identification of the largest number of victims in the shortest period of time. Obviously without the expertise of the forensic anthropologists small pieces of tissue, severely burned or decomposed remains, and small bone fragments would not
have been identifiable. What is just as important to relate is that large pieces of bones with joints, skin with dermal ridges, and dental remains were often first correctly identified by those experts with the highest sense of human anatomical structure—the anthropologists (Sledzik et al., 2009:290).

A similar system was set up at the Pentagon in the wake of the terrorist attacks of September 11, 2001, although due to the site location the FBI oversaw procedures. Some of the most difficult and time-consuming cases, especially for the anthropologists, were those that consisted of thousands of pieces of bones and teeth, the majority of which were smaller than a fingernail. The role for forensic anthropology increased as the condition of the bodies received at the mortuary changed from complete to fragmentary, especially when the fingerprints and teeth were absent, unusable, or otherwise unavailable. In those cases, identification specialists relied more on the skills of the forensic anthropologist to help sort and identify remains. At any given time, a military pathologists might ask the anthropology team to assess an x-ray of a fragmented hip, a tray with hundreds of bone fragments, or a disarticulated foot to determine which bones were present, the side, and any biological information (such as sex or age). Anthropologists also helped separate human from animal bones and objects such as melted plastic that sometimes resembled bone (Rodriguez, 2003:H72). This skill alone saves countless dead ends, frustrations, and distraught family members.

A dramatically more problematic endeavor in area complexity and sheer amount of material was noted at the World Trade Center site. Tons of dirt and debris were mechanically sifted through multiple sized screens and carried on conveyor belts. NYPD
and FBI personally monitored these conveyor belts in two 12-hour shifts, recovering thousands of tissue and bone fragments, personal effects, and other evidence. (Sledzik et al., 2009: 300-301). Anthropologists analyzed the remains, and if determined to be human, they were transported to the New York City Office of the Chief Medical Examiner (OCME) mortuary and placed into the morgue stream as a case requiring further analysis. Anthropologists proved particularly adept at the triage station, where the fragmented remains and anatomic landmarks obscured with soft tissue made a detailed knowledge of osteology advantageous in classifying the elements.

There were a substantial number of non-human remains recovered from the destroyed restaurants also in the vicinity. Anthropologists specializing in identifying bone versus non-osseous material in addition to species identification were invaluable at this site. Their significant involvement prevented many false interpretations. When inexperienced personnel assisted in recovery at the WTC site, remains were frequently commingled. Body bags examined at triage rarely contained a single piece of human remains and unassociated parts within each bag were separated. Remains not attached to another by hard or soft tissues were removed individually, placed into a new bag and treated as a new case. This effort was made to maximize the number of individuals that could be identified (Sledzik et al., 2009:297).

Forensic anthropologists and archaeologists ensured that the large group of workers with varying degrees of expertise conducted their work appropriately and thoroughly, while focusing on accurate forensic methodology. Archaeologists directed heavy equipment use at the World Trade Center location while interpreting site formation
processes and soil stratigraphy. The OCME’s forensic anthropology unit supervised hand sifting and interpreted small bone fragments, distinguishing them first from non-skeletal, then non-human remains. Due to the number of restaurants in the densely populated area, commingling of human with animal bones was very often a finding (Budimlija, 2003:259-260). This simple process alone saved an unbelievable amount of time and effort. Where time is limited and at a premium, expert forensic anthropologists are best suited for the task. Without these very specially trained individuals, the recovery and reconciliation efforts would have been almost non-existent.

Lesser-known areas at or near to Ground Zero are excellent examples of why a need for students and scientist’s access to bone study is crucial and self-evident. The Deutsche Bank Building at 130 Liberty Street was seriously damaged from the collapse of both towers. During deconstruction work on the roof, laborers found many small bone fragments intermingled with the roof gravel. Staff anthropologists confirmed that they were consistent with human skeletal remains. Further DNA tests revealed that the individuals were aboard American Airlines Flight 11, which struck the North Tower of the WTC (Sledzik et al., 2009:297-298). The parcels of land in and around the fallen buildings, which contained manholes and subterranean structures, revealed many fragments of almost microscopic, yet identifiable, human skeletal material. Without the expertise of the bone specialists, this detailed forensic work could not happen. Ultimately, the scrutinizing methods of the forensic anthropologists at the WTC, the Pentagon, and in Pennsylvania at each of the staging and sifting facilities helped to
identify and conjoin, or re-associate remains of single individuals, which minimized the DNA testing required by those experts. (Mundorff, 2003:743-744)

A unique example of how further scientific advancement can radically change the lives of those family members who remain, is the case of The Tomb of the Unknown (formerly referred to as the Tomb of the Unknown Soldier) in Arlington National Cemetery. Mitochondrial DNA testing done on the remains of a Vietnam soldier buried there in 1998 provided a positive identification, allowing him to be returned to his family. (Arlington National Cemetery website, 2014:1) Without the initial bone identification that allowed for the placement of the individual at the tomb, it would have been impossible to take the DNA analysis step. Otherwise, substantial samples of human bone are needed for students of these vocations to learn from, further advances in identification and provenance reconstruction are severely limited. Skeletal experts only become so by repeated experience with their materials. It is clear that human skeletal material must be available for education and advancement to occur.

The United States does not hold a monopoly on the ethical, moral, and legal rights to bone access. A case in point regarding the ramifications of repatriation in Europe is evidenced with the 2012 discovery of the remains of King Richard III (1452-1485) in Leicester England. As the last monarch of the Plantagenet dynasty, he reigned for the remaining two years of his life and was killed at the final battle in the War of the Roses in what has come to represent the end of the Middle Ages in England. Stories regarding his demise and the subsequent disposal of his remains at or near the Black Friars Priory are legendary. Expert opinion relates that he was buried in a less-than-dignified state in a
shallow, small grave. Armed with historical event records, archaeologists soon
determined that the remains they found were indeed the deceased monarch. Skeletal
evidence of an idiopathic scoliosis and fatal wounds he received greatly increased the
likelihood that this was Richard III. A positive match of his DNA with a descendant
provided concrete evidence (Buckley et al., 2013:536-537).

Most often with discoveries of this kind, special interest groups surface, claiming
rights and demanding control over artifacts and remains. In this case, collateral (non-
direct) descendants of the Plantagenet monarchy called “The Plantagenet Alliance” laid
claim to all aspects of this archaeological find, citing their relationship to the deceased
king as evidence of their rights to determine what should be done with the discovery.
Their primary mandate is to have him reinterred in York rather than in Leicester
Cathedral (Watson, 2013:1-3). The New York based Richard III Foundation provided
additional pressure to this end. While in theory this circumstance may appear benign, in
fact their securing of a judicial review to determine where he should be reinterred
diametrically opposes the greater good. The archaeologists had legally procured access
prior to initiating the investigative dig, thus securing rights for the scientific community
to advance understanding of specific historical events and society as a whole.

As of this writing, final judgment as to whether Richard III will be curated or
interred is undecided, though one thing is clear. If his remains are held in a situation
without access, they will no longer be available to scientists. While all skeletal remains
are of value, those with well-documented provenance are crucial to the advancement of
our understanding of the human condition. In this specific case a sizable body of
knowledge regarding time, place, and characters is known. As new scientific methods of evaluation are developed, more discoveries may be made. A more open and shared appreciation for skeletal material, curated with respect and care is the best use of this precious resource.

**Part 4: No Replacement for Authentic Human Bone**

While modes such as computer applications, textbooks, and plastic models help students to learn how the human body works, there is virtually no substitute for the use of authentic human tissue. The normal variations in human anatomy cannot be adequately reproduced in software, illustrations, or models. Even photographs, while useful as documentation, cannot replace the tactile appreciation of bone. Identifying remains range from whole parts to the smallest increments and can only be learned through study of authentic materials. One of the first lessons a student of osteology must learn is how to distinguish between bone and non-bone. With the advent of petroleum-based plastics mid-20th century and continued development of faux materials, this skill alone is worth its weight in gold, or rather, bone. While non-human remains are readily available, without access to human skeletal resources, comparative study is impossible.

A resource not tapped since the early 20th century (Hunt & Albanese, 2005: 407, 416) exists in anatomy laboratories where human donor specimens are utilized for educational and research purposes. Traditionally, whatever tissue remains are returned to the Willed Bodies Program and cremated or interred. Essentially the individuals who have freely given their bodies for learning and research end their “term of service” once they are returned. The agreement, which they willingly sign, stipulates that as long as the
donor can be used for furthering education, their remains may be curated at whichever institution they are assigned. (A. Corson, USF Willed Bodies Program, personal communication) Utilizing these specimens as a solution to the morally correct and politically challenging decrease in bone availability is an idea that must be seriously considered. The purpose of this work is to identify and present a best-practice method for producing dry bone from preserved human donor tissue, thus creating a more sustainable educational resource. The examples enumerated in this needs assessment clearly point out the practical and justifiable rationale for this work to be done.
An in depth dissertation on skeletal acquisition and preparation could easily be referenced from a cultural history perspective. Suffice it to say that the contextual range for human skeletal remains through history is as varied as the physical and cultural milieu in which they are found. Initially imbued solely with spiritual and cultural significance, the modern era has witnessed their rise from a curiosity to a crucial component in empirical inquiry. Along with changing significance, manners of obtaining bone have shifted through various legitimate and clandestine means. Regardless, previous to preservation for dissection, natural tissue decomposition left little more than time as a factor in dry bone production. Indeed, one has to only browse through a catalogue of Leonardo da Vinci’s anatomical studies to evidence the fact that his dissections included the production of numerous full skeletons. He used his observations of bones to explain several of his hypotheses regarding physics and physiology, not the least of which was the mechanics of the human body. (McMurrich, 1906: 342-343)

As the Age of Enlightenment dawned, secular scientific education demanded increasing numbers of human remains for study. With this growing move away from negative religious connections to the body during the 18th century and the advent of tissue preservation for dissection in the 19th, a new source of skeletal material developed in the 20th century (Quigley, 2001:99-101). The challenge became how to remove chemically bonded preservatives without losing bone integrity. Pioneers tried several different methods, some documented, and some lost forever. All were in some way successful at
producing viable bone in the short term. Present observations show that some processes are more successful than others, with variable amounts of long-term integrity.

**Part 1: Earliest Processing and Research**

This literature review examines existing evidence for human bone preparation found in the public domain from the late 19th century to present day. The vast majority of early historical comparative skeletons from preserved tissue could be deemed “dissection room collections” (Hunt & Albanese, 2005: 407) and as such have a direct bearing on our present research and publication. Three of the most substantial assemblages are discussed here in order to point out similarities and differences in procurement, documentation and preparation. First, the Hamman-Todd Collection is now curated at Case Western Reserve University in Cleveland, Ohio. Secondly the Robert J. Terry collection established at Washington University is now held at the Smithsonian Museum in Washington, D.C. Finally, the Cobb Collection resides at Howard University in Washington DC. (Katzenberg and Saunders, 2000: 10-12; Rankin-Hill and Blakey, 1994: 75). There are several others, including such priceless curations as the Peabody at Harvard or the Mutter in Philadelphia. Various authors, however, point to the first three as most representative of teaching collections, and therefore they are reviewed here (Quigley, 2001: 129-136,148-149).

Publications detailing dry bone production from preserved specimens during this era were informative, if at times contradictory. Given the variables involved in macerating, de-greasing, and fixing bone, the discrepancies between Hamann Todd, Terry, and Cobb must be placed in the context of a greater picture. Embalming formulas
have changed dramatically over the past hundred or so years; this has a direct bearing on relating our work to previous trials. Past processes may act as guidelines, though their chemistries and procedures cannot be followed to the letter. Pre-existing bone conditions (ie Osteoporosis versus Ankylosing Spondylitis) and actual skeletal part (ie skull versus humerus) are crucial factors to consider in processing. None of these considerations were mentioned in any works reviewed here regarding Hamann-Todd, Terry, or Cobb.

Unresolved issues and conflicting data

A conflicting issue regarding the maceration process of the H-T specimens remains unresolved at this time. In her book Skulls and Skeletons (2001:111-115), Christine Quigley references a source from the now defunct Northern Ohio Live Magazine, indicating that the specimens were macerated through the use of dermestid beetles. Attempted contact with both the curators and past editors to clarify or reconsider this statement was not productive, though continued attempts at contact are being pursued. The thrust of our research indicates that this is not possible, as these dermestid insects will not ingest preserved tissue (personal conversation Andrew Corson & Eric Bartlelink). If dermestids are introduced to preserved tissue they will not survive (Steadman, 2006: 12, Taxidermy.net, 2012:1-2). Further reporting by Jones-Kern and Latimer (1996:2) gives no indication of processing details as the dermestid versus preservative question is not addressed. Short of direct perusal of the H-T or Northern Ohio Live archives, a question of how these skeletons may have been produced remains. This is yet another reason for our research to proceed with full and honest documentation of procedures and results.
At the time that Hammond-Todd, Terry, and Cobb were gathering skeletal specimens there was no precedent set for successful production of both legal and ethically viable bone collections. Nor were there observations of the long-term ramifications of processes used, such as Sodium hypochlorite, a universal bleaching agent (Steadman, 2007:12). Still, these collections provide exceptional insight into skeletal demographics as well as methods for the time from which they produced. Not only were strides made in skeletonization procedures, a huge leap forward was made in understanding human skeletal similarities and variations. For any conflicting reporting of data or processes in the literature, the overall value is priceless. Seeing what follows as a natural progression of these pioneers groundbreaking work is the most ethnographically sound direction to take.

Following the initial groundbreaking work of Hammond-Todd, Terry, and Cobb, scientists through the 20th century began looking for methods that were least harmful to specimen, scientist, and environment. We will see that with the exception of the above individuals, virtually none of the sources acquired and reviewed here focuses on acquiring dry bone from preserved skeletal material. Their statement that it will simply “take longer to produce” has proven naïve at best (Skinner, 1926: 327; Snyder, 1975: 576,579; Mooney, 1982: 125-126). Later scientists will acknowledge the definite difference in strategies required. “Far more difficult to reduce are embalmed remains where the fats have undergone chemical changes and have permeated the bone itself.” (Fenton, 2003: 2) This crucial detail is one major factor in this review and research endeavor. That there is a paucity of evidence for dry bone preparation from prepared
tissue is notable. Not understanding the changes in preservation formulas over time cautioned us against application of outdated formulas to present day procedures. Pure concentrations of phenol and/or formaldehyde from the past warranted a different approach than the newer formulas in use today. (Mayer, 2006: 41-44)

The most fully documented historical account of skeleton production is that of the Smithsonian Institute (NMNH), which now houses the Robert J. Terry Collection (Hunt and Albanese, 2005: 406; Steadman, 2006: 11). Begun in the early 20th century, his formula of 72-hour hot water soaking, brushing, and degreasing with pressurized benzene vapors at the time was a ‘best method’ utilized well into mid-century. Benzene, however, has proven to be highly toxic and was not used in our study (Smith, 2010: 133-134). Nevertheless, Terry’s groundbreaking idea that some grease should be left in the bone to keep it from degrading over time was a major consideration in this phase of our work (Hunt & Albanese, 2005: 11).

Terry’s suggestion to put smaller bones in cotton bags to equalize exposure to reagents was supported through discussions with the Universidad de Mexico Antropologia y Anatomía laboratorio (Baez-Molgado, 2011: 28). Terry’s accumulation of thousands of specimens allowing for detailed and reliable references for sex determination, and his age of death estimation was a first. Paleopathological interpretation and comparative modern human reference for evolutionary studies resulted from his work. Additional use of skeletal material as a basis for medical and dental training and research in normal skeletal variation for biomedical implants has insured its viability (Katzenberg and Saunders, 2000: 12-18).
Upon his retirement, Terry’s most gifted student, Mildred Trotter, continued his work, amassing even more skeletons while creating a more rounded sex, age, and race demographic. She also enhanced his detailed cataloging system; color coding right and left sides as well as tracking replacement specimens over time. Mentors George Huntington of the NMNH, and Sir William Turner of the Edinburgh University Anatomy Museum provided strategies for meticulous record keeping. Simultaneous work by Hamann-Todd, Cobb, Marsh and Raymond Dart added significant insight into the processing and curating of skeletons from preserved remains. Into the mid 20th century, most dissecting specimens were unclaimed bodies, as religious beliefs and class stigma highly discouraged body donation. In fact, in 1912, 200 New York physicians publicly pledged to donate their bodies after death in an effort to remove the stigma associated with dissection (Garment et al., 2007: 1002) Trotters’ diligence greatly improved public opinion regarding body bequests; donors soon surpassed indigent and unclaimed bodies as the primary source for dissection (Hunt and Albanese, 2005: 408-409).

In “A method for preparation of skeletons preserved by phenol,” HR Skinner (1926: 328) reported a method for preparation of skeletons from specimens thus preserved. He recommended macerating at 80° C followed by brushing. He details a formula that heats specimens in a weak solution of sodium hydroxide to remove tissue and fat from the bones. Repeated sodium hydroxide baths, rinsing and brushing, followed again by rinsing, then soaking in salt water for 4 to 6 weeks. Compressed air was discharged into the bottom of the tank several times a week to stir and provide oxygen (Skinner, 1926: 329). Noting the success of the simmering temperature was helpful in
determining a strategy for our process. Skinner’s specimens were preserved in an almost pure phenol solution (ours contains only 2% phenol). Differences in embalming solutions and procedures have a direct impact on the dry bone production model used. Correlation with our experiment is limited for this reason. Also, Skinner cautions against excessive use of alkali as it may easily injure bone integrity, thus ruling it out as a processing agent for our purposes. (Thacker, 2004:10)

At this point, this review focuses on more contemporary preservation formulas. Our specimens are preserved in a formulation that has a far lower concentration of phenol. The recipe used to fix our material is 61% water, 20% ethanol, 10% glycerol, 5% formaldehyde, 2% Lysol and just 2% phenol (Andrew Corson, personal communication). Preservation since the mid-20th century reflects an increasing understanding of chemistry that will stabilize previously living tissue with less compromise. Buffers, humectants and inorganic salts have been added to predominantly pure formalin and phenol concentrations in order to account for uneven and toxic conditions (Mayer, 2006: 126). The subsequent chemical composition of the human tissue of our cohort could easily react differently to processes and chemistry applied in any one of the studies reviewed here.

In “Methods of Processing Osteological Material for Research Value and Long-Term Stability” Williams states that:

Studies have demonstrated that the soaking and washing of osteological material with any kind of aqueous solution is destructive because of the hydroscopic and anisotropic nature of bone. The solutions that are often used for cleaning bones
are also potentially deleterious to proteinaceous materials. With regard to the incorporation of toxic chemicals for pest control, it is known that at least some fumigants degrade collagen and lipids. Although it is recognized that various treatments may compromise long-term stability of bone, it must be realized that these treatments may also compromise research potential, particularly for biochemical and genetic investigations (1991: 15).

In addition to his impressive body of work with regards to producing viable bone specimens, Williams references four extensively researched publications that support his emphasis on the destructive nature of water with regards to protein bonds. He references Balfe’s work with collagen reactions to acids, bases, and salts (1946: 86-94) and Gustavson’s work observing the reactivity of collagen (1956: ix). He further cites Rose’s overview of protein chemistry for conservators (1984: 122). Perhaps the most applicable to our work is his reference to Shelton & Buckley’s observations on enzyme preparations on skeletal bone (1990: 76-81). Simply recognizing that bone absorbs moisture from air and has different physical properties when measured in different directions and places (i.e. dense matrix and open spaces containing marrow) profoundly affects our materials, methods, and outcomes. (See discussion below)

Snyder, Burdi, and Gaul introduced a method of skeletal preparation that involved a quick acting formula they named anti-formin, prepared by combining sodium carbonate and bleaching powder (1975: 577). The authors note that other effective means are time consuming. They state that “the five-step method of maceration, cleaning, degreasing, bleaching, and fixing as well as the use of dermestid beetles are, in effect, not necessary.
They claim that their method is a refinement of others and will provide for the full range of human materials that may be encountered. “This technique is efficient because clean specimens can be obtained within an hour from any type of material, including formalin preserved materials, though they require more time for preparation and show greater resistance to the chemical action.” The authors imply that even though preserved specimens may take longer than an hour, they won’t take much longer. Our experience contradicts Snyder and his colleagues.

The authors mention tissue-eating insects as a viable, though time consuming way to deflesh tissue in comparison to theirs, and that it may be used with both fixed and non-preserved tissue. As reported previously, first-hand sources report that all attempts with dermestids on preserved tissue have failed, thus they were ruled out. They will not survive ingestion, even if specimens were first treated with fresh animal blood (A. Corson and E. Bartelink, personal conversations; Steadman, 2006: 12). Recall that documentation of the Hamann-Todd Collection reports dermestid beetles for defleshing when the preponderance of evidence negates this claim.

Without further understanding of Snyder’s materials and methods, his process is questionable. The use of bleaching powder in skeletal processing has been shown to destabilize bone structure well after removal from the solution (de Wet, 1990: 76; Fenton, 2003: 1; Rennick, 2005: 1; and Steadman, 2006: 12). Degreasing with benzol has been shown to be very effective, though not without a high risk of side effects for scientist, specimen, and environment. (Snyder, 1975: 577) The recommendation of either
hydrogen peroxide or potassium hydroxide as a further step in releasing grease from bone is well taken, with the added benefit of providing a more even visual tone to specimens (deWet, 1990: 39; A. Corson, personal conversation). This even tone has been obtained in the past by using sodium hypochlorite, mentioned above as a highly risky agent for bone production.

Snyder notes that hydrogen peroxide and potassium hydroxide are especially useful for bones that may crack or degrade from repeated boiling and scraping, such as skulls and scapulae. He suggests meat tenderizer (bromelain) for other delicate tissues where bone is replaced by cartilage such as in fetal and infant skeletal material. Somewhat toxic trichloroethane is suggested for tenacious intervertebral discs. A fixing method by adding Alvar® flakes to acetone is highly flammable. The element of exposure time is crucial for best outcomes. Even what appears to be the most innocuous solution can produce unwanted results if left unchecked (Snyder, 1975: 578). Indeed, as a universal solvent, it would not be out of the question for water alone to demineralize bone if given enough time, producing a useless skeletal specimen (Trueman and Martill, 2002: 371 and 380).

Although his specimens are not preserved due to their being part of the San Francisco Medical Examiner-Coroner’s Office work log for body identification, Stephens’ (1979: 660) work is useful for this review in that it points out how valuable skeletal remains have become today. It points to the need for uniform skeletal preparation methods that are quick and effective regardless of context. He states that he “uses household bleach for macerating and bleaching of his (unpreserved) specimens.” Acetone
is used for defatting, and sodium hydroxide is also required. Another method discussed that may help to avoid decalcification is macerating uncovered in clear water for a few days to weeks, followed by immersion in a detergent solution and simmered. The author refers to Snyder’s use of anti-formin as a reference for his work (Stephens, 1979: 660). While this direction in materials and methods is not ultimately followed in our work, it was nevertheless helpful in making decisions regarding best processes.

Mooney, Bardach, and Snodgrass (1982: 125) conducted a study specifically looking at less toxic materials and methods for producing dry skulls. Specimens were introduced into solutions that were made of 10% commercially available enzymatic detergent and water and heated to 75-80°C. Simmering time was proportional to skull size, fixation state, amount of soft tissue, and absence or presence of the calvarium. Their report that formalin fixed human skulls took 8 hours per day for 3 to 5 days is notable. Insect colonies and volatile compounds such as caustic soda, quick lime, hydrogen peroxide, trichloroethylene, and benzene are reviewed with similar cautions as mentioned by previous authors. They point out that the enzyme-active detergent method virtually eliminates the problems previously enumerated, as none of the skulls were over-bleached, though one was damaged through over-heating. (Mooney et al., 1982: 126)

Without tangible evidence of the work done, it is impossible to speculate on how specific findings may be used as a measurement for success. Compared to other articles reviewed, Mooney’s work is worthwhile for our purposes as it details how delicate skull processing is. Contrasting the authors’ use of laundry detergent for all stages with other reagents leaves questions regarding levels of grease remaining and final tone of the
specimens. On the other hand, isolating chemistry and process that simplifies while meeting all criteria is a goal. Surfactant chemistry such as that found in commercial laundry detergent (alkyl benzene sulfonate) stood out as a very good trial candidate for our study. Not mentioned in this or any other study is the serious consideration that commercial products rarely have a pure chemical make up, as the formula usually contains undisclosed agents that break down grease and soften water among several other proprietary actions (see discussion below).

**Part 2: Contemporary Processing and Research**

The articles compared and contrasted in the second half of this review come from the last 10 years prior to this submission. In each article, there is mention of work done by previous scientists, at times from a historical perspective and at others referencing findings. Experimental reports detail positive uses for detergents and enzymes, with some conflicting evidence on which is most effective and least toxic. While Mairs et al. (2004: 278) swears by the convenience of tablet form laundry detergent as a best choice for macerating and degreasing, Simonson et al. (2001: 480) state that “Detergent maceration makes use of the enzymes present in the cleaning agent, and an increased speed of maceration and removal of bad smell have been observed.” However, key here is that the exact composition of commercial detergents is often proprietary and not directly available. Besides various kinds of enzymes, the detergents also contain tensides, builders (inorganic complexing agents), additives, bleaching agents, and corrosion inhibitors. The aggressive mixture in detergents may cause damage to specimens, and decalcification, softening, and transparency of detergent-macerated bones have thus been noted.”
An example of another contradiction to be found between works is when Yin et al. (2010: 1) warn of the adverse effects of enzymatic maceration on hardness of bone, and a year later Simonsen et al. (2011: 481) apply varying concentrations of lipase and protease, with successful results reported. Several studies were carried out on non-preserved non-human tissue specimens with varying amounts of scouring, scrubbing and brushing involved (Mairs 2004: 276; Yin et al. 2010: 1; and Simonsen 2011: 480). In addition, these authors did not utilize a scoring system (see Steadman below), though the caveats in their work had a direct bearing on our choices with regards to materials and methods. As is often a finding, previous work as often steered us away from a processing choice as towards it.

Having said that, the materials and methods of four other works reviewed associate in a similar mindset as each experiment builds on those whose research came before. In turn, the most recent Lee in 2010 refers to Steadman in 2006, who refers to Rennick in 2005, who refers to Fenton, the earliest of the group in 2003. Each scientist modifies previous other authors’ work. Reducing bone modifications as well as increasing potential genetic material have become primary goals. While summary reviews are enumerated below, the one point that all agree on is the negative effect of bleaching, particularly with sodium hypochlorite. All caution against its use, warning that resultant bone will be flaky and at times crumbling quickly to dust. They instead suggest hydrogen peroxide or potassium hydroxide. This important step was utilized in our experimental process with some surprising results (see below).
When compared for consistency, of the four articles reviewed in this section, only Fenton et al. (2003: 1) performed trials where both fixed and non-preserved tissues were used. He does not mention preservation of mitochondrial DNA as a goal, though his unique trial materials and methods serve as a bridge from the past to this most recent body of work. He suggests a 3-stage procedure that evolved over a 30-year period, stating that it is effective for fresh to formalin-fixed specimens. Remains are simmered in a solution of household detergent and baking soda, degreased in water-based ammonia and fixed with Vinac/acetone solution. The authors note here that for preserved remains several repetitions of the process must be performed if a truly dry bone is to be produced. No comparisons are made between processing times for non-preserved and preserved remains. They warn against heating and cooling too quickly, most especially with teeth that may fracture.

Fenton et al. (2003: 2-3) compare four basic categories of skeletal preparation: cooking, cold or warm maceration, chemical maceration, and carrion insects. They warn that over-cooking and bleaching can ruin specimens while beetles will only work in the case of non-preserved tissue. Listing acetone, trichloroethylene, hydrogen peroxide, sodium perborate, papain, and a variety of dishwashing products, they caution that bleaching agents have a distinctly destructive effect on bone that continues past when the dry skeletal material has been fixed. They claim that their best choice for processing bone can provide a fully defleshed and degreased bone within a few days. The major strengths of this method are safety of ingredients, both to scientist and specimen (Fenton et al., 2003: 3).
The suggestion to use water-based ammonia solutions to continue degreasing specimens was a point well taken. After noting its efficacy in our first trial, we moved to a water-ammonia stock solution for processing of all subsequent experiments (see discussion below). Previous circumstances warranting the use of commercially available chemicals introduced “sudsy ammonia which is slightly diluted ammonia with a surfactant added to aid in breaking lipid bonds. Indeed the commercial use of this product is used widely for its cleaning and disinfecting qualities (Rosen & Kunjappu, 2012: 4-5). While there are some differences in solution strengths and process parameters between this work and ours, evidence of Fenton’s positive results helped to determine our starting amounts of chemical compounds.

The remainder of the articles reviewed here reference non-preserved tissue. Rennick, Steadman, and Lee used either animal or human bones or both in their experiments. Therefore application of their findings to our work must exist as extrapolation. There of course is inherent collective value in their work for application to our study, though exposure times and outcomes likely being dissimilar, findings must be approached cautiously. In 2005 (pp 2-3) Rennick, Fenton, and Foran aimed to isolate nuclear and mtDNA from a variety of human and non-human bones using 3 cleaning techniques: boiling in water, in bleach, and in powdered detergent/sodium carbonate. Their study points out that the physical as well as physiological effects of bleach on skeletal material are notable; bleached bone yielded a dramatically lower amount of viable mtDNA than either the control or the detergent/carbonate trials.
Fascinating outcomes revealed: “There were also indications that detergent/carbonate cleaning is preferable to even water, as only bones cleaned in detergent/carbonate generated the largest mtDNA aplicon (1064bp). It is possible that the carbonate in the cleaning solution acts as a buffer, helping to maintain a non-acidic pH as the cooking temperature is raised, thus protecting the DNA. If this is the case it would constitute yet one more advantage of this simple cleaning technique (Rennick, 2005: 2).”

While preserving DNA strands was not the focus of our study, noting differences in chemical reactions even with non-fixed specimens is helpful in appreciating modifications in processing parameters between preserved and non-preserved bone.

Rennick et al. point out that more accelerative defleshing processes are quite harsh, potentially dangerous, and can easily cause bone damage. He underscores the universal finding that while sodium hypochlorite is effective, it is far too caustic for skeletal processing. He chooses to use Fenton’s recent method of powdered detergent and baking soda as “safe, effective, and inexpensive (Rennick, 2005: 1)” Post-cleaning Sodium hypochlorite bleached samples were brighter white and detergent/carbonate produced a beige color; importantly, the latter were less greasy feeling. The outer layer of bleached bone flaked off easily. Due to its effective degreasing qualities, the detergent/carbonate method appeared as an excellent procedure for creating most intact dry bone, and thus was added to our reagent samples.

With each successive work reviewed, additional useful information was added to our understanding of processes as possible parameters for our own trials. In 2006, Steadman et al. (p 11) tested the effects of 10 maceration methods for their effect on
gross bone structure and DNA preservation. Samples were non-preserved pig ribs and as stated previously could have only so much direct relevance to our work, though the thoroughness alone with which the study was conducted is of great importance to our experiment. Recognizing that all the comparative skeletal collections culled from dissection room specimens are similar in their triad of flesh removal, degreasing and drying, the authors begin and end with the idea that a less toxic process was desirable. Robert Terry warned, “Any method that alters the chemical properties and morphological appearance of bone should be avoided.” (Steadman et al., 2006: 11)

In order to further identify the most effective chemical and heat maceration techniques specifically for recovery of DNA material, Steadman et al. (2006: 12) classify six maceration categories. Cold and warm water bacterial maceration, cooking, chemicals, enzymes, and invertebrates. Cold water is considered the safest as no heat or chemicals that may disrupt bone integrity are used while warm water is more conducive to bacterial maceration. Cooking refers to any method that requires additional heat, such as boiling or microwaving and has natural anti-bacterial properties. Alkaline solutions break down collagen and other proteins. A warm potassium hydroxide solution macerates and degreases bones simultaneously, though thorough rinsing is required. Hydrogen peroxide removes lipids and is considered to be less caustic to bone than household bleach. Prolonged exposure to any chemical can result in brittle, fragile bones and loss of cortical detail. Flammability and carcinogenic qualities of all these chemicals require protective equipment and the use of a fume hood.
Enzymatic preparation may include laundry detergent, pure papain, pepsin, or trypsin in warm or boiling water with or without chemical additives. Reference is made to Fenton et al. (2003: 1) using heated laundry detergent, baking soda and ammonia solutions as an effective method for preparation. Dermestid beetles are discussed with the caveat that non-preserved tissue is their only interest. Perhaps the simplest and most profound words are stated by Steadman et al. (2006: 12) in their observation, “there is no silver bullet solution to this process.” Depending on the outcome desired, different methods would be best chosen. Bleach cleans and whitens yet will likely cause cortical exfoliation over years, while hydrogen peroxide and enzymatic laundry detergent can do the same without cautious processing. Boiling can split teeth and scorch bones, especially if it is too rapid or extensive. While the authors’ goal is DNA preservation, it had a two-fold effect: those processes that would attend to genetic material salvage also consider integrity of bone features.

Each of Steadman’s (2006: 12-13) 10 experiments were repeated a minimum of 3 times using fresh ribs from different pigs. During each process, loose soft tissue was gently removed when solutions were changed, though mechanical removal was kept to a minimum. The following trials were conducted:

1) mechanical removal of meat and periosteum as control,

2) room temperature bath at 22°C,

3) hot water bath at 90°C,

4) boiling water at 100°C, microwave on high 1 minute intervals,

5) sodium hypochlorite solution room temperature,
6) hydrogen peroxide solution room temperature,
7) EDTA and papain solution 45°C,
8) meat tenderizer/dish soap solution 90°C,
9) detergent/ sodium carbonate solution 90°C,
10) detergent/sodium carbonate solution 90°C followed by ammonia degreasing.

Steadman devised a scoring system whereby odor, soft-tissue texture, ease of soft-tissue removal, and bone quality could be more quantifiable. (See chart 3)

Results of Steadman’s methods were first evaluated based on efficiency of the process and gross quality of the bone, followed by analysis of DNA quality and quantity. (2006: 14) Notably, the procedures that were not heated or slightly heated were slower than other techniques, more malodorous, and did not necessarily produce better quality bone. Elevated temperatures expedited soft-tissue removal and were complete within hours. At the same temperature, the detergent/carbonate produced faster results than meat tenderizer/dish soap though it scored slightly lower in bone density and cortex exfoliation. Note that this issue is one of the main points of our discussion and conclusion (see below). The meat tenderizer/dish soap also offered some advantage in soft tissue breakdown, ease of removal and cleanup. The microwave technique resulted in bone with good gross structure, though the odor of cooking meat necessitated the use of the fume hood for processing, and the specimen had to be kept moist.

The ideal techniques would quickly and efficiently remove soft tissue, provide degreased bone and leave intact mtDNA and/or nuclear DNA. The quickest techniques that provided the best quality bone also provided the best quality DNA. (Steadman et al.,
2006: 15-16) Of these best materials and methods, the use of boiling water at 100°C and microwaving were ruled out as processes both from a practical and experiential standpoint. Boiling water would risk modifying and denaturing the bone. The specimen size limitations and malodorous smell of microwaving deemed this process unsuitable (Steadman et al., 2006: 14). Recommended methods that included 90°C, detergent/sodium carbonate, sudsy ammonia, and meat tenderizer/dish soap were chosen to become part of the initial trials for our work (See discussion). Again it is important to stress that the author’s work was performed with non-preserved tissue so accommodations would be expected with regards to length of processing. Most notable is the chart designed to instill more objective comparisons between trial outcomes. Steadman’s careful detailing of reasonable conclusions put this work as one of the most critical for our purposes.

Lee et al. (2010: 4) modified previous methods and measurement techniques set out by Steadman. Though closer in composition to our skeletal material through the use of human leg bones, their non-preserved state ultimately did not alter our experimental preparations. As with Steadman, Lee established a control with a manually removed tissue specimen. Hot water bath, boiling, and microwave without chemicals were used. Sodium hypochlorite, hydrogen peroxide, and EDTA/papain all in simmering water were prepared. Combinations similar to Steadman were tried; meat tenderizer with dish soap, detergent with sodium carbonate, and ammonia degreasing. Outcomes of these trials provided data on cross-reactions and synergistic affects. Protocols regarding rinsing and tissue removal strategies were noted as improvements on previous work. Lee’s
description of measuring and controlling timing approaches proved useful as a template for our subsequent trials.

Three variables—odor, texture of soft tissue, and ease of flesh removal were scored before and at regular intervals during trials as in Lee et al. (2010: 4) Following their final extraction, bones were dried for a minimum of 72 hours and then assessed for bone quality. A table listing maceration results in increasing order of temporal duration of the experiment showed interesting results. From shortest to longest they were; microwave, laundry detergent and sodium carbonate, meat tenderizer and dish soap, boiling water, hot water, EDTA and papain, bleach, laundry detergent, sodium carbonate, and ammonia, and hydrogen peroxide. Notable findings included better bone quality with quicker processing although Lee et al. (2010: 9) claim that degreasing with ammonia resulted in weakened bone. For most experiments, tissue remained tightly adhered to bone until all the tissue came off about the same time. Essentially, mixed formulas at simmering temperatures for a shorter amount of time were found to be most effective. While differing amounts of DNA were extracted from each sample, the same general rules applied as when aiming for a bone that is of diagnostic quality on a physical level.

When compared to other studies reviewed here, there appears to be some conflicting data with regards to which chemistry and in which time frame the best results will be obtained. Having no doubts that Lee’s (2010: 1032-1038) work is of high caliber (indeed, Steadman serves as a co-researcher and author on the paper), the evidence for the most effective and efficient process for non-preserved tissue is inconclusive. Discussion below will show that our trials do not always agree with the amount of time
for processing. As one outstanding example, for Lee, laundry detergent appeared to rapidly speed up the process of maceration and degreasing. Our results revealed the exact opposite finding in both time and bone detail. With best efforts by previous researchers, numerous questions regarding process and outcomes have been raised. Although the literature on skeletal production is solid and insightful, it is not extensive. The variables that must be taken into account for a dependable and reproducible process have not been exhaustively discerned. There appears to be plenty of room for our work to find its place.
MATERIALS AND METHODS

The study was conducted at the Life Chiropractic College West Anatomy Laboratories (LCCW). Life West’s Institutional Review Board (IRB) approved our work and appropriate paperwork was filed (See Appendix 1). Permission and support from the University of California San Francisco Willed Bodies Program (WBP) for developing this formula for further use on future donor specimens was obtained. The large, well-ventilated laboratories at LCCW were perfectly suited to our needs. Maceration, degreasing, toning, drying, and fixing require substantial space for each individual process. Still, the cubic feet required for 3 entire skeletons to be completed and catalogued were easily accommodated. Ample room remains for ongoing trials and projected follow up studies. This paper represents 6 trials in 5 stages carried out for all 3 complete skeletal specimens, as well as outlining plans for further work.

Research specimen identification

Three donor specimens curated through the WBP were chosen for methods testing. Age, sex, recorded cause of death and limited co-morbidities were provided. Cross-referencing the WBP donor number (WBP#) with an internal identifying code (ID#) helped to insure anonymity (see Chart Two). For obvious ethical reasons, further demographic information was not held by the WBP, limiting possibilities for comparative soft tissue and skeletal findings. Increased access to additional demographic and health history information was not available for this particular study, though it may prove helpful in the future (see discussion). Without volumes of background and life history data, it was far easier to focus more on what the specimens revealed without attachment
to personal history. However, when additional discoveries were noted during maceration, degreasing, toning, and fixing processes, they were duly noted and extrapolations regarding their relationship to the individual’s habitus were made (see discussion below).

All donor specimens were fully dissected for chiropractic educational purposes, avoiding instrument contact with bone surfaces. After this stage the remaining tissue was removed as close to periosteal membranes as possible before disarticulating involved joint structures. Steps were taken to remove internal organs without damaging the rib cage and vertebral column. A craniotomy was performed on each specimen; brain tissue and visceral cranial structures were removed. Precautions offered by Mooney (1982: 125-126) regarding skull processing were noted and cranial bone was treated accordingly. Skulls were immersed into solution before temperature was raised to avoid and/or reduce scorching and cracking of delicate structures. Holes were drilled into the proximal and distal posterior surfaces of the humeri and femurs (de Wet, 1990: 38). This allowed for more complete release of preservative marrow-bonded tissue for further Gas Chromotography Mass Spectrometry (GCMS) testing (see below).

In order to properly facilitate the 6 trials to be conducted and 5 process stages, an alphanumeric system was structured to represent specimens and phase discussed. Each full skeleton was divided into 6 components: (1) shoulder girdle and upper extremity, (2) lower extremity minus feet, (3) feet, (4) skull, (5) pelvis and sacrum, and (5) spine (see Chart 3). Each of these elements was taken into account; alterations in process and results were duly noted (see discussion). Separating the whole skeleton into parts provided increased opportunities to evaluate tissue characteristics as well as different chemical
formulas and processing strategies (Steadman et al., 2006: 12). The long bones, flat bones, skull, and smaller, more fragile bones required special attention. Trial A stands for maceration. Trial B denotes further degreasing with acetone, Trial C represents a 30% hydrogen peroxide toning bath. Trial D indicates further steps taken (if any). Trial E represents fixing the bones. Collating, labeling, documenting, and curating course followed the six stages of trials 1 through 6 (see Chart 4).

A Max Burton Induction Stove was used as a consistent heat source along with a 16-quart OPTIO Induction ready stainless steel container with cover for 1A, 2A, and 4A. During initial skull processing, it appeared the Max Burton would not be sensitive enough for our needs, and the VWR was a much more reliable heat source and efficient means of processing. Our team decided to switch solely to using the VWR for the remainder of skulls 002 and 003 while noting results with 001 while using the Burton. It quickly became apparent that the skull process was more sensitive than other skeletal bones as noted by previous authors (Mooney, 1982: 125-126). Trials 3A, 4A, 5A, and 6A (skulls, pelvis, sacrum, spine, and ribs) were accommodated with a 6.8 gal (26 liter) VWR unstirred water bath (See Chart Three). All trials were conducted underneath a Lab Conco Basic 47 funnel hood, which had been inspected and cleared for effective ventilation.

Previous researchers agreed that regardless of the specimens’ preservation state, increased time of processing dramatically amplifies risk of fractures and denaturing of bone (Mooney, 1982: 125-126; Fenton, 2003: 2; Rennick, 2005: 2; and Steadman, 2006: 14). For this reason it was decided that samples would be checked each hour for tissue
quality and bone integrity. Authors were also in unanimous agreement that lower
temperatures work over a very long time and that boiling may help to quicken maceration
while risking damage to specimens. Heating at a simmering temperature appeared as the
quickest and safest set point. It was decided to keep the processing temperature as close
to 90°C (194°F) as possible for the duration of each trial sample. Both an internal
thermometer and external thermometer were used to monitor temperature during each
experiment. Ultimately the VWR was far superior to the Burton in this regard, with a
maximum temperature just over our desired processing temperature of 90°C.

Several earlier works recommended brushing to remove excess tissue. Fenton
(2003: 2) suggests the use of a metal chemical handling instrument, specifically a
Scoopula®, which is common to all labs. The nature of our work is to determine which is
the quickest and most efficient formula without using instruments that may damage or
create bone modification. Therefore de-fleshing at timed intervals was facilitated
predominantly by hand, not only to avoid altering bone features, but also to gain a better
tactile understanding of process effects. The only exception to this rule was during skull
and vertebral processing; small tweezers were used at times to avoid contact with smaller
delicate features that could not be accessed with fingertips. Dull edged probes were used
to remove tissue from foramen and intricate skeletal structures, such as the sacrum. At the
end of maceration the bones were rinsed under running water. Tough tissues such as
portions of cartilage that were not easily removed by hand were allowed to remain.
Special attention to odor, texture, ease of removal, and bone quality (as referenced in
Steadman et al., 2006: 2) were noted.
The maceration scoring system of Steadman (SP) was utilized (Chart 5), with additional data parameters. Hereafter referred to as Modified Steadman Protocols (MSP), odor, tissue texture, and ease of removal categories were further delineated to create an even number of components in each category of the rubric (See Chart 6). Keeping in mind our goal of tactile as well as empirical data to inform our decisions, we expanded on the excellent work of Dr. Steadman (2006: 13) regarding over all odor, texture, ease of removal, and bone quality. Our goal was to delineate every process variable possible for a truly informed decision regarding best practices. It was apparent after the very first trials (1A through 6A) to get a best result we would need more categories to describe processing of preserved skeletal tissue. Visual and tactile descriptions were so different from our experience with each consecutive trial that expanding the categories was our best choice. In the future creating new categories with descriptors targeting only preserved tissue would be a best step (see discussion).

Latex gloves and rubber-coated instruments were used to handle bones in process to avoid contamination. In order to provide a more even exposure to heat and chemistry, the carpals, metacarpals, and manual phalanges were processed in muslin bags, as were the metatarsals and pedal phalanges (Baez-Molgado, 2008: 28; Hunt & Albanese, 2005: 408; and deWet, 1990: 38). Tissue inspection, rating, and removal were carried out in aluminum foil trays for ease of handling. After the final process for each set these trays were lined with paper towels to absorb and allow observation of fat leakage prior to further degreasing. Visual description of amounts and patterns of additional grease
absorbed on paper towels after maceration is noted in the discussion below. Process and skeletal part appeared to determine these variables.

Compounds were obtained through commercial products containing the ingredients needed for each trial. Procuring pure compounds without additives was challenging, as it was cost prohibitive and local suppliers were wary of participation due to our use of human cadaver tissue. However, comparison of the active ingredients in previous works guided choices of the commercial substrates that were chosen. An exhaustive search of product labels in order to pick the best agent for the necessary chemicals was conducted. Contact with manufacturers was fruitless for further clarification on ingredients and additives. Their proprietary formulas and laws governing disclosure made obtaining more detailed descriptions difficult if not impossible. This disparity was taken into account as trials proceeded and conclusions were drawn. Chemicals were selected that appeared to have the fewest additives; added chemistry such as water softeners in detergent might help to drive or hinder reactions and enzyme substrate formation (See Chart 7).

Brands that did not fully disclose contents were disqualified along with those that contained bleach. High levels of enzymes, fragrances, dyes, and water softeners were avoided. Those ingredient labels that were straightforward and as ecologically sound as possible were chosen. For purposes of continuity through the entire series of trials, identical brand names of products were used. The targeted goals in choices were known chemical formulas that would neutralize and separate the preservative from the bone tissue efficiently. Distilled water (DW) was used as a solute for all reactions. A ratio
following Fenton (2003: 1) was used to determine chemical amounts in all trials. With subsequent experiments, some additives were eliminated and some recombined, depending on preceding outcomes.

Our choices of chemistry baths were most closely correlated with Fenton, Rennick, Steadman, and Lee. Although processing of preserved human bone is not documented in any of these works, the authors in turn state that in these situations, a longer time is necessary for creating bone from fixed tissue. Ultimately, for the macerating step commercial agents were chosen that contained predictable amounts of Alkyl benzene sulfonate, Sodium laureth sulfate, Sodium bicarbonate, Bromelain, and Hydrogen Nitride. (See Chart 7) All of the above authors reported good results for these five substrates, though perspectives varied whether they were predominantly useful for maceration, degreasing, or both. These compounds were scrutinized for their ability to remove connective tissue and/or emulsify lipid components from the bone matrix. Timing of results was crucial to evaluate their efficacy and it was closely monitored.

Finding previous research materials and methods that matched our procedures and rationales posed an additional challenge. Skeletonization of preserved human specimens is only documented for early 20th century dissection room collections, where solutions and techniques were far too toxic to both researcher and the bones. For example, Terry reports using benzene vapors following immersion in carbon tetrachloride, both of which are extremely toxic substances (Hunt & Albanese, 2005: 408). Indeed, the embalming formulas today are far less noxious than even through the mid to late 20th century, when formulas were almost entirely formaldehyde, ethyl alcohol, and phenol (Mayer, 2006:}
The majority of more recent work focused on non-human and non-preserved remains. Regardless of parameters, the body of work available for replication left very few, if any examples to follow. In all works, mixtures chosen to macerate and degrease bone have been used interchangeably and in place of each other. A methodical assessment of each additive by itself and in combination with others was provided data to determine best strategies.

Prior to each trial, specimens were fully dissected to the periosteal layer, leaving tissue that was difficult to remove intact. Joints were carefully disarticulated to provide quickest maceration through increased surface exposure to chemical reagents. With long bones, a hole was drilled into the posterior distal and proximal shaft to extract bone marrow for further testing. Each specimen was weighed and photographed previous to the first bath as well as before and after the hydrogen peroxide phase of degreasing. A separate solution was prepared for each day and at predetermined timed intervals, bones were removed and inspected for positive and negative processing effects. At the end of each day, excess tissue was removed by hand only and stored for possible further testing and subsequent return to the WBP.

**Part 1: Maceration**

**Trial 1A: Chart 8.1.**

The shoulder girdle and upper extremity were processed with separate agents, including plain distilled water as a control. In turn, each of five commercial products was added to distilled water; laundry detergent (Alkyl benzene sulfonate), dish soap (sodium laureth sulfate), baking soda (sodium bicarbonate), meat tenderizer (bromelain), and
sudsy ammonia (hydrogen nitride). At the end of each day during trial one, the Steadman Chart was used to score odor, texture, ease of removal, and bone integrity. After the first experiment it became apparent solely from visualization and palpation of the tissue that more detailed observational information would be best. Therefore we added parameters to Steadman’s chart, which hereafter will be referred to as “Modified Steadman”. Tissue descriptions in Steadman’s work were referring to non-preserved specimens; ours were fixed and likely would look and feel differently (see discussion).

Other technique related issues came up and were dealt with such as not allowing bones to cool to ambient air temperature makes ease of removal more expedient. The most challenging areas of course were joint surfaces; at times cartilage and ligament attachments were easier to remove with a gloved finger nail as opposed to just the tactile rubbing of surfaces. Although time was very prolonged for this specimen trial, the bones after the initial maceration were free from tissue remnants. Preservative bonded grease continued to exude from the bony structures as apparent dried blood and marrow was evident (Vahlensieck et al., 1995: 490-491).

**Trial 2A: Chart 8.2.**

Due to processing time and appearance of bone, Alkyl benzene sulfonate was dropped from Trial Two (tarsals, metatarsals, and toe phalanges). Having the highest percentage of additives such as water softeners and chemical additives, ABS was the most likely chemical compound to eliminate. Hydrogen nitride was purported by previous authors Fenton and Lee to have both macerating and degreasing properties. On visual inspection, the right shoulder girdle specimen 003 appeared as the cleanest and
most even in tone in relation to the other 5 specimens, having been the only specimen to be processed in this chemistry. From this point forward hydrogen nitride (household ammonia) was added to distilled water as a stock solution for all further experiments. In turn, Lauryl sulfate, Sodium bicarbonate, and Bromelain were added to each of the three specimens.

The right side of 001 was pretreated with reagent for 24 hours before processing while chemical agents were mixed into solution for the left side (see Chart 7.2). This process was repeated for each days work on each of the specimens. Given the known effects of pretreating excessively dirty laundry (Alkyl benzene sulfonate), dishes (lauryl sulfate), even tough, uncooked beef (bromelain) this pretreatment became a logical step to try (Ashie et al., 2002: 2140-2141). Given the shape and arrangement of the specimens, applying the macerating chemical directly to the tissue appeared to help slough off tissue more evenly (see discussion below). This technique was employed regardless of which chemical was used.

A variety of pre-existing conditions could be responsible for any process finding, not the least of which might be a reaction due to unknown underlying factors. Awareness of these possibilities required increasing scrutiny as testing proceeded, since findings at each trial were used to determine next steps. With incomplete demographics and unknown donor variables, choices made were often based on conjecture. Process data garnered from the literature review continuously was challenged by our findings. An example of this situation shaping process was with the apparent disintegration of some of the smaller tarsal bones in specimen number 002. Continued care was taken with each of
the bones, noting if and when similar deterioration occurred. X-ray analysis helped to further understanding the nature of osseous findings not listed on the individual’s demographics sheet (see discussion below).

**Trial 3A: Chart 8.3.**

In Trial 3A, femurs, tibia, fibula, and patellae were macerated. Again DW combined with HN was utilized as a base solution. Combinations of 2 ingredients for each specimen were used: BS and BR for 001, SLS and BR for 002, and SLS and BRS for 003. As in trial 2, the right side was pretreated for 24 hours and reagents were added to the base solution for the left sides. Fresh amounts of chemical reagents were used with each processing, whether pre-treating or adding to water and ammonium hydride processing solution. With the stability of VWR water bath unit, we allowed specimens to stay in solution over night, simmering at 90°C for up to 24 hours. Overall this proved to be a positive step, as it had already appeared that longer times in solution were a best strategy (see discussion below). Finding the proper time for each skeletal portion already was appearing to be one of the most crucial factors for a best formula. The ease and predictability of the VWR unit clarified the importance of heat source and its regulation.

**Initial observations with Sodium laureth sulfate**

Similar deterioration to that which occurred with the feet bones of specimen 002R was noted at the medial femoral condyle and fibular tail of 002R. Proceeding with caution, a similar reaction was noted with 002L At this point it was still difficult to determine the cause of this reaction, though it was noted that Sodium lauryl sulfate was present in baths for 002 in part of Trial 1, and all of Trial 2. Given the nature of our
experiment it was necessary to continue until all 3 skeletons are complete, therefore we proceeded with restraint, taking much care to keep any fragments remaining after simmering was completed. The sudsy ammonia base solution has an amount of surfactant present, therefore supporting the rationale for leaving out dish soap in subsequent trials if the ammonia with surfactant formula is used. With comparison to the MSP as well as our own developing skills of evaluation, a more focused emphasis on timing was used.

**Trial 4A: Chart 8.4.**

Processing of the skulls of all 3 specimens proved to be challenging and frustrating. Realizing that too many variables might create a weaker outcome and less viable predictions for the present study and future work, each skull was pretreated with only one of the 3 chemistries remaining. In turn, they were 001-Lauryl sulfate, 002-Bromelain, and 003-Sodium bicarbonate. The stock solution of hydrogen nitride dissolved in distilled water remained the same as the previous trials. The initial condition of each varied, as craniotomies had been performed, all with some manner of destruction to the cranial bones and posterior elements of the upper cervical spine. Differences in layers and thicknesses of bone were noted as hand defleshing occurred under low force ambient temperature water. The pterygopalatine fossa was a particularly challenging area to deflesh and macerate as spaces and foramen are so small that only prolonged exposure to chemistry and heat would slough off tissue. Lessons learned with 001 were applied to processing of both 002 and 003. There was no comparison with elements processed before or after.
It will be discussed below, yet it is notable that the skull processed with lauryl sulfate 001 took almost five times longer (121 hours) than either the sodium bicarbonate or bromelain specimens (24 hours each). In order to decrease chances of fracturing and disintegrating bony elements the skulls were submerged in solution before the heat source was applied and increased. With 001 not only did it soak in ambient temperature solution between heating, at one point it was stored in the freezer to accommodate a necessary break in processing. As previous trials were also refrigerated between specimen baths, it is most likely not a factor in the time discrepancy between skull maceration times. With skull 002 the hydrogen nitride concentration was dramatically increased from 5.67l to 18.92l to see if notable maceration and possible increased degreasing could occur (See discussion).

Initial processing with the Max Burton heater proved disadvantageous, as it needed to be more closely watched than was possible. The unit shuts off after an hour to avoid overheating, thus creating a wide variability in processing temperatures. The more robust bones of the upper and lower extremity (excluding hand and foot bones), while different in shape and size are not as fragile and thin as several of the cranial bones. The hyoid bones disintegrated during processing with complete destruction of the oropharynx. Indeed the cranium is invested with many more varieties of tissue composition and tensile strength than the arms and legs. With the delicate bones of the interior skull as well as teeth integrity to consider, it was obvious that the VWR heat source would be more controllable. Previous authors caution against this fluctuation in temperatures even
outside of the bath, as radical changes can not only weaken, but crack bones and teeth (Mooney, 1982: 125).

With all of the skeletal bones processed there was some degree of tough tissue removal necessary; ligaments and cartilage, specifically. Observances made during skull maceration clearly indicated that there would be a need for some instrument assistance in order to fully de-flesh a skeleton. Removing non-contractile tissue types of varying thicknesses in several small and relatively inaccessible areas was at times impossible with running water and using fingers only. Smaller tools such as the Scoopula® mentioned in Fenton et al., (2003: 2) would be required. A tool that proved to be the most useful and non-invasive was a pair of tweezers with roughened pincer surfaces. Even with these useful instruments, not all tissue responded favorably to maceration and manual removal. Clearly, the macerating process did not affect some areas, as tissue remained firmly fixed to some surfaces. Joint articulations such as skull sutures held firm.

**Trial 5A. Chart 8.5.**

The 3 sets of pelvic bones were pretreated overnight in the same fashion as the skull. A single chemical was used for each: 001 Laureth sulfate, 002 Sodium bicarbonate, and 003 Bromelain. For all 3 sets of bones, processing went very smoothly. It appeared that the relative bulk of each bone being large and solid (except the coccyx) allowed for slow but even heating. At first immersion the pubic symphysis protruded slightly from the solution though within hours as the sacroiliac joints disarticulated, the whole specimen became immersed, yet another indication for full skull processing. Skulls were submerged in the ambient temperature bath designated for their corresponding pelvic
girdles; the ilia and sacrum were added when solution reached 90°C. While there was relative stability during heating of these elements with skull parts present, the overall effect of pretreating bones again was notable. (See discussion below)

Given the amount of ligaments and dense connective tissue in foramina and other small uneven surfaces it was decided that more expedient cleaning of the macerated bone could be accomplished under running water. The lipid components had been so emulsified that it was next to impossible to see how much had actually sloughed off the bone surface without it. Dry hand cleaning served only to push the tissue with its chemistry (soap, baking soda, or tenderizer) from one surface to another. Periosteal layers were unusually thick and required more vigorous removal. Both 001 and 002 sacral elements showed evidence of collapse at the lateral aspect of their bases. Specimen 003 was softer and more pliable though no visible bone destruction was evident after maceration. Numerous congenital variants and possible acquired anomalies were noted such as a distinct lumbarization of the 003 sacrum (See discussion below).

The increased ammonia in solution also dramatically elevated fume production and therefore the exhaust fan was left on overnight to allow for continued processing. The goal here was to observe if the increased levels of hydrogen nitride from the sudsy ammonia have pulled more glycerol from the bone (see GCMS discussion below). In an effort to note if levels detected are in line with processes used, a reduction over 104 hours was created and tested from the solution used to macerate 002 the pelvic girdle. Earliest testing in previous specimens showed very small levels of glycerol remaining and mostly with marrow draws rather than in solution. This test was undertaken to see if reduction
would elevate notable levels in solution only and appear elevated in GCMS testing, not to extrapolate to what might be contained in any given specimen. The increase in ammonia may have had an influence on color, porosity, and bone firmness in trial 5A.

**Trial 6A: Chart 8.6.**

As with Trial 5A, an increased amount of hydrogen nitride was added to the distilled water solute. At certain points during process, additional clear dilute ammonia was added to the solution as the use of a new stainless steel cover for the VWR appeared to create a great deal of evaporation and condensation. The ribs were intact with spinal column though soft tissue between them was removed. The anterior cartilaginous portion was left above the solution line and remained intact until the ribs were disarticulated from the spine. At intervals vertebral segments were checked to see which would easily disarticulate. Timing for each spine trial became gradually less, though specimen reactions such as in 001 Lauryl sulfate determined was halted at 81 hours when the vertebral bodies appeared blackened and brittle. An additional ‘candle wax’ drip effect on the anterior bodies began to dissolve therefore processing was suspended to preserve this artifact (See discussion below).

The spine was the notable portion of our specimens that challenged our degreasing materials and protocols. Many of the vertebral motor units became so fragile during the maceration phase that further heating was discontinued in anticipation of possible changes afforded by degreasing with acetone. Sizable amounts of disc material remained at the vertebral body endplates and required separation with a scalpel to expose them to solution. This required disarticulation of the vertebral bodies and even facet
articulations pointed out a need for further investigation into more effective methods and materials for these types of tissue (See discussion below).

Snyder et al., (1975: 578) spoke of using trichloroethylene in order to soften discs and free them from the end plates. It was considered as a possible solution additive until further investigation revealed that even if it was to work for our experiment it might not be part of recommendations due to its potential for toxic exposure to humans and specimens (Lash et al., 2000: 177). Specimen 002 Bromelain appeared to speed disarticulation though processing was halted at 51 hours due to the bone appearing overly porous and fragile. Sodium bicarbonate seemed to drive the reaction at a lower temperature and to more readily denature disc material (See discussion below).

Part 2: Degreasing

Trial B: Chart 9.

This phase of our experiment targeted removing those lipid molecules that remained chemically bonded with the bone matrix after maceration. Much of this material appeared as a visible film on the long bones and with a distinct “oily” sensation when palpated. After macerating was completed, specimens were air dried for several months in a dark temperate well-ventilated space. The grease absorbed into paper was noted at 1-month intervals until all 3 skeletons were completely processed. As some of the more delicate elements still had remaining tissue it was also necessary to watch for mold growth. Among its many properties, bleach is extremely effective at removing fungus and mildew (Clausen, 2000: 234). As we were not using bleach in our process, increased awareness of issues such as spore appearance had to be monitored. The small
amount of mold that appeared on spine 002 within days of ending the maceration phase stopped growing and appeared to be a self-limiting event. Immersion in acetone removed all visible mold.

Degreasing of specimens was facilitated through submerging each full skeleton in an acetone bath for extended periods of time (see Chart 9). Time was increased with each specimen to evaluate length of exposure to acetone as a possible process variable. A large, covered non-corrosive plastic container was used for this phase of the experiment. The amount of acetone used was determined by the total size of the skeletal elements being processed. At calculated intervals, the bones were removed and inspected, and samples were taken for further testing using Gas Chromatography Mass Spectrometry (See discussion below). Gentle agitation with refreshing of solution was carried out at timed intervals in an effort to further accelerate processing. Plans for another acetone bath and possible maceration for some of the more stubborn elements were considered after the toning phase if it appeared that it might be useful.

The total amount of acetone required for complete immersion and refreshing of solution for all trials was as follows; 001: 38 liters, 002 and 003: 27 liters each. The acetone bath became increasingly discolored, turning a deep yellow brown with a notably oily texture by process’ end. Sensory evaluation alone pointed to the success of acetone in degreasing bone. While some tissue still remained in more difficult areas, as the bone became dry, areas that would needed additional attention become more visible than prior to the acetone trial. One month after removal from the acetone bath, some surfaces (long bones particularly) displayed what appeared to be spotty denaturing. Preserved blood in
the flat surfaces of the scapula was removed during the acetone processing. As with macerating procedures, the bones were left to dry over several months to better determine long term results of acetone.

Three months after removal from the acetone bath, the portion of long and flat bones with continuous surface area show the driest areas. While the shafts were perhaps too dry, from diaphyseal funnels to the end of proximal and distal epiphyses has remaining tissue and excess lipid that will need to be addressed (see discussion below). These elements clearly have been altered by the acetone, while more complicated bone surfaces such as the calcaneus and talus show less lipid removal. The skulls clearly the will require more intimate handling and thoughtful processing due to their irregular component shapes and delicate structures.

Part 3: Gas Chromatography/Mass Spectrometry

Samples of processing solutions were drawn off at the end of each days work in 3 cc syringes and stored for GCMS testing. Additional samples were taken from marrow cavities through pre-drilled openings in all humeri and femurs using small disposable paintbrushes. While the soft tissue takes up the embalming fluid, the bone also absorbs the preservative; having been living tissue it is also susceptible to deterioration and decay (see Chart 9). Virtually all of the preservatives come off the solution during the heating process except glycerol, making it the ideal marker for measurement of remaining preservative. (D. LeDuc, personal communication, 2013) The Varian CP-3800 Gas Chromatography Mass Spectrometry (GCMS) unit at California State University East
Bay (CSUEB) was utilized to measure molecular content of glycerol remaining in the processing solution or marrow draw. (Mittlebach, 1993: 1).

In order to create an internal standard solution, 5 mg of 1,4 butanediol was dissolved in 50 ml dimethylformamide (DMF). This was used in every sample and standard. Different known percentages of glycerol were used to make a series of standards through serial dilution. Bistrimethylsilyl trifluoroacetamide (BSTFA) was used to derivatize samples and standards to lower the temperature at which the glycerol would become gaseous, facilitating analysis by GC-MS. The measured ingredients in this formula were: 600 µl of DMF, 200 µl of BSTFA, 100 µl of Butantediol (DMF), and 100 µl of standard or sample for a total of 1000 µl. Samples were kept refrigerated until GCMS analysis was performed. The 100 µl otherwise used in each standard and liquid sample was replaced by the marrow soaked fabric for the humeri and femurs.

Several attempts were made to find the best method for analysis. Our ultimate choice was labeled “Esters starting conditions; 40 minute change” (D. LeDuc, personal communication, 2013). Standards and samples representing each of the 6 skeletal macerating sequences (upper extremity, ankles and feet, lower extremities, skull, spine, and pelvis) were made. In turn they were run through the GCMS evaluation process and resulting graphs were printed for each standard and sample. Samples evaluated were thus: 24 - upper extremity, 12 - ankle foot, 14 - lower extremity, 2 - skulls, 3 - spines, and 6 - pelvises. These included the marrow draws described above. Four standards were created for each of the 6 sets of bones.
Additional samples were taken during degreasing with acetone in an effort to identify any remaining glycerol that may be present in this all-important phase of the process. Two samples each were drawn from 001, 002, and 003. Each of the three skeletons was processed in one unit as opposed to the 6 assembled for the degreasing method. The first acetone specimen was drawn after 24 hours and the second at the end of processing. Unfortunately 5 of the 6 samples evaporated before standards and samples could be made. Further examination of future acetone baths may be undertaken in the future with consideration for the quick loss of acetone once it has been exposed to air (see discussion below).
FUTURE TESTING AND DEVELOPMENT

The macerating and degreasing trials included in this study provided an abundance of data from which next trials may be delineated. Discussion below will focus on the results of these two steps. Maceration results will require further testing on additional ‘just-dissected’ specimens to determine a best practice in this phase. Exposure of skeletal material to acetone provided information that was at once useful and confusing (See discussion below). With that in mind, trials are continuing with specimens 001, 002, and 003 beyond the publication of this thesis. Processing with hydrogen peroxide and other possible agents continues as results are gathered from work previously accomplished, each result determining the next step. The overall results point to a process with may more factors to consider than previous authors imply. Attempts at acetone evaluation may be reinstated along with other trials that may prove useful as continued research proceeds.

Previous experience has shown that while bleaching agents will effectively lighten the color of bone and perhaps aid in at least some lipid removal, the side effects are noteworthy, as the bone will visibly continue to denature after the chemical agent has been removed. Indeed, personal conversations with experts in the area (A.Corson and E. Bartelink, personal communication) as well as every authority referenced in this paper caution against the denaturing qualities of Sodium hypochlorite (Fenton, 2003: 1; Rennick, 2005: 1; and Steadman et al.,2006: 12). Lee went so far as to use it as one of the substrates in order to compare it to a variety of macerating and degreasing agents. Following a similar thought process here allowed the smaller tougher tissue to remain,
looking to Hydrogen peroxide as another possible agent of tissue removal as well as for its degreasing properties. References differ on which strength to use, varying from commercially available 3% (Steadman et al., 2011: 12) to industrial strength at 30% (A. Corson, personal communication, 2014).

Lee, Steadman, de Wet, and Snyder report substantial bleaching effects when immersing bones in Hydrogen peroxide or Potassium hydroxide to further degrease without bone destruction. Varying concentrations of Hydrogen peroxide will be used for the 3rd step for all 3 skeletons. 001 will be immersed in 3%, 002 in a 50/50 mixture of 3% and 30%, and 003 in 30% only. A large covered plastic rectangular tub is being used to hold an entire disarticulated skeleton and a rubber mesh screen to keep all skeletal elements submerged during degreasing. Every effort is being made to have elements arranged so that they can be easily accessed during the hydrogen peroxide phase of our process.

For the smaller bones, a screen bag system is utilized to keep them from becoming lost in solution. As the hydrogen peroxide solution decomposes rapidly at increasing temperatures, lower thermal conditions range from cool to ambient temperatures. Each full skeleton is submerged in solution, comparing differences and similarities of results against macerating chemistry, time and average temperature. Rates of refreshment of solutions may need to be varied to determine which concentration and soaking parameters might be best utilized. Per conversations with Andrew Corson the hydrogen peroxide is refreshed each time residue is skimmed off from the top of the bath.
Additional cleaning of tissue that had not been removed through the previous steps is undertaken as needed. Tweezers and other small stainless steel instruments are used to facilitate stubborn cartilage end plates and other connective tissue. Frequent rinsing during processing of elements helps to insure preservation of smaller landmarks, foramina, and prominences. The skulls and spinal segments are the most stubborn elements to clean. This step, while tedious and time consuming is necessary as only specimens that are devoid of all soft tissue may be identified, stored, displayed, and used as dry bone. With continuing development and further refinement of the process, this step will eventually be deemed unnecessary. This is of course, the premise of the research (see discussion below)

After all skeletal elements are fully processed, they were allowed to air dry for several weeks to months before the fixing stage. Each component is cataloged with a black ink pen and then covered with a thin coat of Vinac® to seal all surfaces. An indelible non-smearing is used for labeling according to the accepted system along the same nomenclatural tradition of the Terry collection (Hunt & Albanese, 2005: 408) All elements are photographed, highlighting notable congenital variants and acquired anomalies.
DISCUSSION

Removing tissue from a specimen and carefully reducing it until there is only bone remaining is most challenging when it has been preserved. The inherent differences between non-fixed and chemically maintained cadaver tissue are so dramatic that relying on non-preserved methods as a guide for skeletonization of fixed specimens is almost moot. Exposing non-preserved tissue to a natural process of decomposition through air, water, and naturally occurring organisms will, over time, produce a completely defleshed skeleton. None of these rules holds true for cadaveric specimens. Previously living tissue has been altered to a completely new chemical matrix, formulated to keep the specimen from decomposing. Other than changes that occur through exposure to ambient air and the expected process of dissection, it does this quite well. Practically speaking, preserved specimens will change their consistency over time, but the soft tissue structures will remain unless they are treated. While previous work cited is informative, very little of it is prescriptive for our methods. With that said it is possible to create dry bone from preserved tissue. How to do this is the purpose of this thesis.

Specific reference to creating dry skeletal bone from preserved tissue was made by several authors as a process that simply required more time, without clearly citing measured methods and outcomes. According to Fenton et al (2003: 1), their technique developed over a 30-year period; documentation included detailed notes on ingredient rationales, amounts and processing parameters (time and temperature). While we used their ratios of ingredients as our chemistry guide, as with other authors, their report on timing was not transferable to preserved tissue. They claimed that non-preserved tissue
could be processed within an hour and that others would just take longer. With so many variables to consider, this claim may be true; however, our trial results did not support a short macerating time.

Snyder et al., (1975: 576) claim that their anti-formin technique can produce clean specimens from any type of material within an hour. Their compound is a combination of sodium carbonate (NaCO₃) and bleaching powder. The presence of a bleaching agent may very well be all or part of the reason for the authors’ claim that preserved tissue takes such a short amount of time to macerate. Having stated that, there were no specific time comparisons noted in the their work. Our research purposely avoided bleaching agents due to its caustic nature even after processing is discontinued (Steadman et al., 2006: 12). Additional use of brushes and other tools to remove tissue may also add to a shorter maceration time. These variables along with other known differences such as pre-existing specimen condition and proprietary preservation formulas make it necessary to be cautious about comparisons and conjectures.

While traditional methods such as the use of dermestid beetles were considered, they were quickly ruled out; they won’t eat fixed tissue (A. Corson and E. Bartelink, personal communication). This first-hand insight from two separate individuals with different professional backgrounds directly contradicts our literature review. Previous authors have suggested their feasibility. Mooney et al. (1982: 126) and even Fenton et al. (2003: 2) recommend beetles for de-fleshing their preserved specimens though they caution against them due to time constraints rather than as a non-viable approach. Ambiguous statements such as these in previous work occur far too often, making it
difficult if not impossible to rely on procedures suggested by others. With that in mind, future possibilities include pre-treating the tissue and testing it to see if it may be more digestibly desirable to beetles. One idea would be to pretreat the specimens so that there is a tissue breakdown where mold and fungus could form, which would possibly indicate viability of tissue for insect ingestion.

The challenge lies in the quality of the specimen as it was preserved and its viability over the long-term. Proprietary preservation formulas, condition and type of skeletal elements, even the donor’s demographics and co-morbidities are pre-existing variables that may affect processing ease and outcomes. Personal conversation with other anatomy lab professionals and personnel supports this finding. The variability in odor, texture, and flexibility (such as it is in cadaveric tissue) of specimens in the dissection laboratory is noted by anyone who has had long-term lab experience. (A. Corson WBP, Dave Straub LCCW lab manager, Steven James LCCW chief dissector personal communication).

Time, temperature, and even the size of the container used for macerating, degreasing, and toning as well as other potential inconsistencies become critical factors. While there are several chemical formulas available, it is unclear which formula combination is best. It appears that the recipes used may not be as important as when and in what combination they are added (See discussion below). Each macerating, degreasing, toning, and fixing strategy has its own risk to benefit ratio to consider. All of this is underscored by literature that for the most part does not fully address concerns
inherent to preserved tissue. Each of these issues is of major importance when skeletonizing non-preserved specimens

With so many inconsistencies, it appeared only logical to control temperature as well as time parameters if usable data were sought. All 13 journal references in the literature review included in this thesis suggest not heating specimens above “simmering” (about 90°C). Some talk of room temperatures and slightly warmer though it must be stressed here that none of these trials were performed on fixed specimens. Diligent observance of process would help to minimize damage and keeping production time as short as possible. Controlling rate of temperature change as well as processing heat level reduces fracturing of teeth enamel and thinner more delicate bones. Attention to these two basic parameters was a critical step. Without it other factors and findings would be minimal if useful at all. Here the use of the Burton and VWR heat sources had different effects on the evenness of temperature, even though the VWR surpassed the Burton in controlling the solution at 90°C.

Trials referenced in the literature review suggested the use of several de-fleshing chemistries. Our work compares and contrasts the effects of the 5 most cited macerating and degreasing solutions: Alkyl benzene sulfonate, Lauryl sulfate, Sodium bicarbonate, Bromelain, and Ammonium hydroxide. In order to address the variety of compounds to be tested, each skeleton was divided into 5 parts: upper extremity, foot, lower extremity, skull, pelvis, and spine. This approach was useful for inquiry, affording the opportunity to observe reactions and outcomes from varying solutions. However, the drawback of treating different elements with dissimilar chemistries was the dramatic increase in
variables to manage that in turn complicated findings and conjectures. While our results on each of the maceration chemistries are interesting and somewhat helpful, they are not definitive. Future studies would do well to limit maceration chemistry to one per skeleton. Only one reagent will be used per specimen: detergent, enzymes, bicarbonates, or ammonia.

While treating the entire skeleton in the same bath simplifies procedures, it would not be without complications. As mentioned above, some bony elements are so different that they require longer processing time and special methods to reduce time while maintaining efficacy. As an example, macerating a skull is far different than processing a femur. Obvious inaccessible skull components would require a craniotomy and great care to de-flesh and degrease without causing damage to the bone. Long bones require drilled holes to allow solution to access marrow tissue for complete skeletonization. An in depth understanding of cartilaginous tissue locations such as in the anterior rib cage would allow for possible preservation of the entire costovertebral mechanism. Our materials and methods were purposely employed to macerate, degrease and evenly tone the specimens in the least invasive way possible. Our final results produced specimens with flaws that will be avoided in the future. This is the nature of our experiment.

Intervertebral discs

Our first hand observation of difficulty with removal of intervertebral discs and facet articulations verified previous authors suggestions for the use of more invasive methods to accomplish this end. Virtually all of the skeletal musculature along with the more superficial connective tissue such as the anterior longitudinal ligament virtually fell
away form the bone as with other skeletal components. The discs on the other hand, softened but did not come away from the bone without each vertebral segment being gently pulled apart from the ones adjoining it. At this point exposure to further heating in solution softened them so that most could be rubbed off the body with finger and hand pressure.

The challenge here was to not resort to highly toxic chemicals such as those used in the past (Hunt & Albanese, 2005: 407-408). Despite our imperfections in chemistry, timing and equipment, progress was made in uncovering a better trajectory for producing dry bone from preserved tissue. For minimizing variables a best approach would allow a full body to be processed simultaneously. The ability to move and remove skeletal parts in progress is essential for oddly shaped and articulated bones. The vertebral column proved this point and the skulls underscored the challenge. A way to control temperature in an exacting way is also crucial to protect other odd shapes and thicknesses as they are macerated and degreased. While chemical reagents are important and should be investigated further, it is clear that a process which as quickly as possible removes connective tissue and lipid from the bony matrix is the key.

An overview of preservation chemistries cited in the literature offers very little insight into their composition. Other than authors referencing known chemicals such as formalin (Mooney, 1982: 125) and phenol (Skinner, 1926: 327) as fixing agents, no further formula recipes are described in these works. Other references to processing of preserved human tissue involved those with undisclosed formulas that may or may not have matched the one used with our specimens. Utilizing the known formula of our
specimens as a starting point (See Chart 10), phenol (2%) and glycerol (10%) were found to be the only ingredients that would not likely be simmered off in the macerating process. The water (61%), alcohol (10%), formaldehyde (5%), and Lysol (2%) would be removed solely through the heating portion of our process (D. LeDuc, personal communication 2014). Because Fenton et al reported processing preserved specimens for over 30 years with numerous parameters we looked to their work for choice of macerating chemistry.

Knowing that limiting variables would be crucial to the value of outcomes, we scrutinized specimens with each trial to determine which chemistry appeared to have the most benefit with the least risk. The five ingredients listed here (laundry soap, dish soap, enzymes, bicarbonate, and ammonia) demonstrated notable macerating effects during processing. While conjectures can be made about which may have been better or best, so many inconsistencies in other aspects of our trials made it difficult if not impossible to pick a best chemistry. Hypothetically each would have a unique chemical effect on preserved tissue. In our observations this was not the case. It was clear when observing the process that something more than just simmering water was reacting with our specimens. There were minor differences noted between macerating reagents yet none were clearly unique. Perhaps under more controlled conditions through fewer variables, discernable differences would be observed. Indeed, the water only control took longer to macerate tissue. Though with the pure water control, the acetone bath appeared to actually “make up” for the macerating qualities of the five substrates used in the other trials.
Previous trials referenced in the literature review utilized commercial laundry detergent for its’ surfactant qualities (Mooney et al., 1982: 125; Fenton et al., 2003: 1; and Mairs et al., 2004: 278). Earliest attempts at obtaining pure concentrations of detergent were frustrating, with suppliers unwilling to work with us due to our testing human tissue specimens. In order to obtain surfactant chemistry, commercial products were employed (See Materials and Methods above). Using Arm and Hammer® laundry detergent for sensitive skin as a macerating agent was effective though it also contained sodium bicarbonate and trace enzymes (see Chart 7). Both of these additives would be used as the primary macerating agents for other skeletal elements. These additional chemicals defeated the purpose of using a pure macerating agent. The unavoidable presence of non-corrosive and suds-removing agents present in clothes washing detergents made it the most likely chemistry to remove from our list of macerating agents. It was only used for processing of the upper extremities of all 3 skeletons.

Background searching continued as treating of skeletal elements was under way. We were not aware of Alconox Powdered Precision Cleaner® as a viable macerating agent when we began our trials. The work of Rennick, Fenton, and Foran (2005: 1-3) was not discovered until our macerating trials were almost complete. They used this pure cleaning agent (Alconox, 2015) for their skeletal preparation trials, which targeted maintenance of viable mitochondrial DNA simultaneous with skeletal bone preparation. The authors claim that it acts as a buffer to rising temperatures, which aligns with our ultimate goal of minimal alteration to bony landmarks and matrix. Although this chemistry appears to be a better choice than the one we chose, the authors used household
bleach for their maceration methods, which contradicts our findings from the majority of our references.

It is significant that Fenton, one of the chief researchers published an earlier work in 2003 where Alconox® was not used. This earlier publication reports a process that evolved over 30 years, using household detergent and baking soda. Apparently something about Alconox® was notable in processing for the authors to publish their work. Clearly, while experts in the field have isolated good methods, best chemistries are still not known. These often confusing findings are a common theme when attempting a comparative analysis of previous authors works. My list of previous macerating materials and methods (Chart One) attempts to simplify the reagents used. As Steadman (2010: 16) states, “there is no cook book approach”. Therein lies the purpose of our research, to approximate a most reproducible method.

Bright green® biodegradable plant based dish washing liquid was used for its surfactant qualities. The active ingredient sodium laureth sulfate macerates effectively and it is far less irritating and corrosive than laundry soap. The non-toxic quality of dish soap on bare skin versus laundry detergent with its non-c corrosive and de-sudsing additives points up the differences. While both are adequate lipid emulsifiers, for our purposes achieving this end without further potential of over processing is best. Until the discovery of Alconox®, further testing would have involved sodium laureth sulfate rather than alkyl benzene sulfonate based solely on the amount of additives in laundry detergent versus dish soap. In the future, the more predictable uses and outcomes of this critical
anionic surfactant cleaner seem preferable to commercially available products, all who have proprietary formulas not available to the public.

The active ingredient in meat tenderizer (bromelain and papain) is very effective in breaking the peptide bonds that give proteins their form and substance. While emulsification breaks the lipid component of the matrix, enzyme chemistry will soften the tissue and make it easier to remove. Other authors recommend the use of enzymes for removing tissue from such challenging elements as skulls and fetal tissue (Mooney et al., 1982: 126). In contrast to these findings, Yin et al. (2009: 1) showed that enzymatic maceration of compact bone resulted in a significant reduction in micro-harness when compared with water maceration. It should be noted that their work was on non-preserved and non-human tissue that may easily alter process and findings. In our work there was some observable difference between meat tenderizer and the other reagents. With that in mind, it cannot be ruled out as potential chemistry particularly with certain elements such as skulls and vertebrae. In breaking down and removing thicker connective tissue such as intervertebral discs, possible use of bromelain in further trials will be considered, but not as a first choice.

Sodium bicarbonate may be used to soften proteinaceous materials. It is for this action that it was considered in our experiments. When visually comparing its effects with other agents used (surfactants and enzymes) it does not appear to have the same overall outcome. During several of our trial segments, a white residue was noted on the specimens and in the solution. It did not appear to mix evenly in solution or on the uneven surfaces of the specimens. It was not easily applied during the pretreatment phase
prior to maceration. Efficacy of pretreatment strongly supported its application in our
trials and in future work. Given that our experience has consistently pointed to a more
parsimonious approach for further trials, we would consider removing this reagent from
subsequent work. Perhaps its most positive affect in maceration is its tendency to reduce
or remove odors. At times this would be most beneficial, particularly when larger
specimens with larger amounts of tissue are being macerated.

Ammonia is a very effective degreasing agent. We chose commercially available
“sudsy ammonia” for the added surfactant effect of the detergent contained in the
solution. Pure ammonia does not fit our choice to be as “environmentally friendly” as
possible. It is somewhat dangerous, being volatile with a very intense odor. Reports of
ammonia and detergent having different effects on various mineral contents and chemical
bonds make a combination of the two a best choice (Perlman, 1956: 1). Upon removal
from processing during trials, tissue on the bones treated with the sudsy ammonia mixture
appeared to slough off more easily. This notable effect urged a new direction in our final
two maceration trials of the vertebral column and pelvis.

Comparison of standard spikes from the serial dilution was crucial in our effort to
look for evidence of remaining glycerol chemistry. An upward surge of this standard
solution appeared between 2.5 and 5 minutes. When present, comparative sample spikes
were noted at the 10 - minute mark. Of the 6 bone assemblages evaluated, only numbers
1, 2, and 3 (upper extremity, ankles/feet, and lower extremity showed any evidence of
remaining glycerol (see figures 1 through 9). The remaining samples 4, 5, and 6 did not
register any glycerol levels at the anticipated site on the graph; therefore they are not
Specimen 2 (ankles/feet) produced a small but detectable upsurge on the graph at the 10 minute mark. Curiously, these bones did not include a marrow draw, and yet glycerol was still detected; evidence of the molecule was noted at the 9 and 18-hour marks (first and last samples taken) of sample 002R.

Sample 3 included the lower extremity minus ankle and foot bones, including a marrow draw from the distal and proximal femur. Unfortunately the standards that were created showed a spike on the graph, but it did not change as the serial dilution became less concentrated. With this in mind, the only evidence that could be extrapolated from this part of the experiment was whether glycerol was detected or not. In fact, the 10-minute graph spikes, while evident, were so small that even if the standards had progressively spiked as is desired, further evidence of amounts and concentrations would not be available anyway. Having stated that, it is telling that the only spikes noted were in the marrow, not the processing solution.

Finally, the best results came with the 1st sample, that of the upper extremity. Allowing for human error in preparation and in processing, the standard readings were clear and graded with a larger spike as concentration increased. Of all 24 samples evaluated for group 1 (upper extremity), the marrow draw graphs each showed some amount of glycerol remaining. Further evaluation showed that concentration did decrease from the first to the last sample taken for the following specimens: 001R, 002R, 002L, 003R, and 003L (see figures 1 through 9). While the evidence is not definitive for the amounts remaining, it was clear from this initial GCMS evaluation that glycerol amounts can be measured. Further development of this assessment method is warranted.
This decision to increase ammonium hydride in the macerating baths for the spine and pelvis (Trials 5A and 6A) may have had a direct influence in their color change and increased porosity. Regardless of issues that may have occurred with Trials 1A through 4A, there was no darkening of bone to this extent. This phenomenon was noted in all three specimens. If no other data can be collected from this processing choice, it would be that there is a limit on how concentrated a macerating solution should be. Finding what that is will be a challenge that will best be addressed in a more controlled study using perhaps only one or two of the chemistries used in our experiment.

A cautionary note with virtually all the research cited for this paper was that regardless of chemistry or specimen size, the shortest amount of exposure to heat is a best choice. For this reason, steps that begin the maceration before heat is added would be appropriate. Pre-treating of specimens created a notable effect on ease of tissue removal, regardless of which reagent was used. Particularly difficult elements such as those with intricate joint surfaces responded very well to this technique. Surfactants, enzymes, bicarbonate and ammonia all have qualities that do not need heat to drive reactions. This was an easy, effective way to reduce time in heated solution. It will be utilized in all future trials.

Steadman’s Maceration Chart (Chart Five) assessing odor, texture, removal ease, and bone integrity was very helpful as a starting place with one major exception; her specimens were not preserved. As we began our trials it became obvious quickly that we would need to expand the chart to have it more accurately fit our work. Our modified version of this chart (Chart Six) helped to organize and delineate our findings. With that
in mind, our observations did not clearly fit into her descriptions. Descriptive language used by the authors did not always fit our observable findings, particularly with regards to texture and ease of removal. Determining intensity and quality of odor was not a critical evaluation point, though for our purposes we added two classifications to provide an equal number of protocol categories.

Steadman’s soft tissue texture analyses were very straightforward and comparable to our work with one exception. At no time did we have tissue that was floating on the surface with little or no connection to the bone. The ease of removal category is a visual testament to the difference between preserved and non-preserved tissue. Fixed tissue is at times easy to remove but it always takes some effort. Joint structures and surfaces required hands on removal of tissue. Steadman et al describe tissue as more rubbery at times. At no time did we see or feel what could be described as rubbery tissue. The bone quality descriptions fit our specimens well. At times there were bone changes that were obvious to see and to feel. Whether they were due to preexisting specimen conditions or processing strategies was not easy to ascertain.

Robert J Terry insisted that not all the fat be taken from the bone lest it lose its resilient and notably macro-hard cellular matrix. (Hunt & Albanese, 2005: 406) It is critical to note that these skeletons were produced from anatomy lab specimens and therefore were closer to our work. As noted earlier, though, preservation formulas today are far different than those used by Terry and his colleagues, making comparative conjectures difficult at best. His suggestion for using benzene vapors as the vehicle for this process would not work today; they are highly toxic and detrimental to scientists and
specimen alike. He does not indicate the amount of lipid content necessary to maintain viable bone, nor does he suggests how much to remove nor how to measure it. This fact is yet another aspect of our work that makes it somewhat unprecedented. It is part of our goal to determine as closely as possible exactly how much lipid removal is appropriate. Visualization and palpation using the Modified Steadman Chart (See Chart 6) as well as GCMS testing of the glycerol content remaining in bone combined to inform our decisions about best formulas and approaches for this outcome.

After maceration it was not clear on how much fat needed to be removed from the bone. Though it was very apparent that the specimens were still not at a ‘dry’ state. It was obvious simply from physical inspection of our specimens after trials 1A through 6A that more work would need to be done to produce viable bone. An oily residue continued to absorb into the paper where the specimens were placed to dry. This continued for several months. A fatty sheen accumulated on the bones during this period that was slippery to palpation, indicating the lingering presence of preservative in the bony matrix. Clearly a method for truly extracting the preservative remaining in the bone had to be applied.

Again anecdotal evidence from the Willed Bodies Program provided insightful. During conversation with Andrew Corson, he indicated that at times acetone would be used to further pull grease out of non-preserved bones. Stephens (1979: 660) indicated its use along with bleach for quick preparation of non-preserved bone as a macerating agent, yet another example of materials and methods gathered in the literature search that does not fit our trial goals and parameters. Having said that, its uses for degreasing proved
very helpful for our process; obvious lipid extraction occurred (See conclusion below). It will be included as a step in future works.

All sources cited for this research project unanimously agree that using bleaching agents will effectively remove lipids and lighten the color of bone (A. Corson and E. Bartelink, personal communication) while cautioning against its denaturing qualities. The pioneers highlighted in the introduction (Hamann-Todd, Terry, and Cobb) used bleaching agents to whiten bone. After maceration with scrubbing, further degreasing was accomplished through the use of whitening agents. Contemporary scholars caution against the guaranteed destructive properties of bleaching products (Fenton et al. 2003: 2; Rennick et al. 2005: 1; Steadman et al. 2006: 12; and Lee et al. 2010: 3). Sodium hypochlorite is effective at removing fat and whitening specimens though the bones will continue to breakdown after processing is finished. While some loss of detail may be acceptable, the long-term affect of continuous denaturing is obviously not acceptable.

As an alternative to Sodium hypochlorite, Hydrogen peroxide has been suggested (Andrew Corson WBP) and supported by the most recent literature Steadman et al. 2006: 16). Commercially available hydrogen peroxide is available at 3% strength and recommended by Steadman while the Willed Bodies Program reports best results with a much more concentrated 30% solution. Both are being used separately and in combination in this phase of our trials. It is important here to recognize that a white tone is not expected but more of a natural color that will signal a non-threat to the integrity of the bone matrix.
From observations and conversations through almost 30 years in the anatomy lab it appears that other factors affect the preservation state of the donor specimen. Life situations (ie cancer, chemical and radiation therapy) clearly affect how the specimen takes up the embalming fluid. Even applications of preserving fluids have variables that may complicate processing such as time and temperature. All evidence illustrates that ante-mortem, peri-mortem, and post-mortem events easily have a role in processes and outcomes. With so many possible variables, we chose 3 specimens for our experiment, and a 106 year old male (001), and two females, one 89 year old (002) and one 65 year old (003), respectively (See Chart 2). With minimal demographic information available, unreported or previously unknown congenital variants and other life conditions became apparent during the skeletonization process. Questions and conjectures regarding pre-existing conditions of patient, as well as possible proprietary embalming methods and materials must be considered. A scenario where the researcher can track and control variables is crucial for best outcomes.

Overall findings revealed that each of the three specimens had notable degenerative joint disease, as one would expect. Osteoarthritis being the most common skeletal disease of aging, each specimen displayed notable osteophytes at the joints. The weight bearing joints of 002 and 003 displayed this acquired anomaly. Number 003 displayed a notable lumbarization of the L5 vertebra. Specimen 002 appeared to have extensive osteoporosis through visual inspection that was confirmed with radiographic evidence. In addition to extensive osteoarthritis, extensive evidence of Diffuse Idiopathic Skeletal Hyperostosis throughout the thoracic spine was noted.
CONCLUSION

The need for access to dry bone skeletons for teaching and research is paramount, more today than ever before. Fields of study in both medical and forensic arenas are steadily increasing while access to human skeletal materials are being depleted. Ethical and moral justifications for repatriation not withstanding, a solution must be reached. The one source for this precious material that has not been fully utilized comes from those generous individuals who choose to donate their bodies to science after they pass away. A best method for effectively producing dry skeletal bone from these specimens has yet to be discovered and utilized.

While several valuable trials have been carried out and published, none has specifically targeted production of full skeletons from human specimens preserved with contemporary formulas. All the articles in the literature review were helpful but none of them matched our specimens or process. Numerous variables in preservation methods as well as in choices of processing materials make finding a best course extremely difficult. Processes that claim success with dermestid beetles and bleach do not our experience. Acknowledged experts (A. Corson and C. Wacker personal communication) agreement along with their previous attempts have met with many frustrations leading to their discontinuing trials that might skeletonize preserved tissue.

More thorough access to individuals’ habitus and co-morbidities will alert the scientist to possible challenges and outcomes. The congenital variants and acquired anomalies discovered during dissection and skeletonization could easily have an affect on preservation and maceration. The overall health of the donor at time of death could also
affect the specimen prior to and following processing. Donor specimens have different visual look, texture, odor, and resiliency; the causes for these differences cannot be isolated easily. These differences in sensory experience are marked. There is no reason to assume that soft tissue preservation state would not also affect bone tissue. At this point no studies can be found that would compare this important data, leaving yet another avenue for investigation.

The total time to remove tissue from bone using heated solution and tissue removal by hand shows notable differences. The most reliable data comes from comparison within similar skeletal elements; upper extremity, ankles feet, lower extremity, skull, spine, and pelvis. Early evidence pointed to the varying characteristics between long, short, flat, round, and irregular shaped bones measurably affecting process. Differences in architecture and bone histology (especially with regards to bone marrow) are notable. The only way to minimize side effects and control variables will be to process a full skeleton at the same time in similar solution.

As a “universal solvent”, water alone was successful at removing tissue from the bones. In fact, the control trial specimen (left side upper extremity) was identical in time to the same specimen (right side upper extremity) macerated in surfactant via laundry soap. Both were processed in 70 hours each. Clearly the surfactant used for the other side was no more or less effective than pure water. It is notable that the times of all other specimens from this trial took just over half the time of the control. The outcome is inconclusive. Future attempts would need to macerate an entire skeleton in water rather than parts so that each trial would have this comparison.
Overall evidence is lacking for whether pretreatment might have a pronounced effect. In one trial it appeared to make the process faster and in another it appeared to slow it down. As all specimens in Trials 4, 5 and 6 were pretreated no comparisons can be made. Comparing a full skeleton pretreated to a full skeleton not pretreated would be a best future choice. While focused comparison of outcomes will have one choice negate another, an overall consideration of solutions reveals a hierarchy of those most associated with short times. Sodium bicarbonate is most linked with quick maceration times in 4 out of 6 trials; upper extremity, lower extremity, skull, and spine. It came in second place with the ankles, feet, and pelvis. Bromelain followed with 3 out of 6 trials; upper extremity, lower extremity, and pelvis.

As the primary active agent in laundry soap and dish soap as well as being a co-factor in sudsy ammonia, surfactant is included in some amount in all 6 trials. There is no clear correlation with which surfactant is best for successful maceration as they all contain other ingredients that will react with the tissue. Conclusively, if a surfactant agent is to be used in the future it will need to be in pure forms such as Alconox Cleaner or pure ammonia. As before it would need to be used on an entire skeleton.

With some expansion of parameters (see Modified Steadman Chart 6), odor, tissue texture, ease of removal, and bone quality were scored from 1 to 5 with 1 indicating strong odor, tough tissue, and poor bone quality. Processing odor appeared to be the least intense when sodium bicarbonate was used even in the presence of ammonia. This finding matches well with the finding that baking soda also drove lowest processing
times. Bone quality results appeared best with substantial bones such as the femur and very low with more delicate components such as the skull and vertebral motor units.

The modified Steadman protocols proved to be very helpful in determining which chemistry would drive the safest and most effective process. When comparing which specimen proved the easiest to deflesh in the shortest amount of time, sodium bicarbonate came in first place in all 6 trials. According to the rubric baking soda created the most liquefied tissue texture which was helpful though not as critical as removal. In the ease of removal category sodium bicarbonate scored even higher without question. Their was no clear correlation with texture and ease of removal in regards to any other substrate.

Successfully removing bone marrow without causing bone modification is the one rate-limiting factor that must be overcome to produce dry bone from preserved material. Both the red and yellow marrow will break down to some degree during maceration as evidenced by visual and tactile inspection. GCMS shows that some preservative is still present in the marrow even after processing. Because of their location inside the bone cavity, complete removal is at this point, almost impossible to accomplish. Future investigation will determine what amount remains and if any, whether it is inert or active within the bone.

The trials resulted in finding what not to do in future work as much as what direction to take; a very valuable lesson. Each macerating agent is effective in some way. This is extremely complicated by variations found in antemortem specimen states and preservation processes. Further trials with less variables and more controlled parameters are required to determine how to most effectively and efficiently create a full dry skeleton.
from preserved tissue. The next trial of full skeleton comparisons between plain water, sodium bicarbonate, bromelain, and detergent surfactant will illuminate the best formula for creating dry skeletons from preserved cadaver tissue.
REFERENCES


Baez-Molgado, S. et al. (2010) “Técnicas de Laboratorio; Desccarnado Limpieza y Embalje” Universidad de Mexico; Ciudad de Mexico.


## CHARTS AND GRAPHS

### Chart 1: Literature Review

<table>
<thead>
<tr>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>Skinner, HR. (1926) “A method for the preparation of skeletons from cadavers preserved by phenol.” Anatomical Record 33: 327-30. [no references]</td>
<td>Human-preserved; phenol</td>
<td>Repeat methods &amp; brush ad lib, compressed air ~ 3 x week stirs solution, age &amp; disease may complicate, don't use strong alkali, 2nd heating NaOH removed odors [T&amp;T: About 80°C, 45 minutes (soak 4 to 6 weeks)]</td>
<td>Weak alkali solution (NaOH 1%) brushing &amp; scarping some cartilage (steam doesn't work) [No bleach reported]</td>
<td>NaCl H2O room temperature soak 4 to 6 weeks [R&amp;F: Tap H2O flora also macerate at 37°C. No fix reported]</td>
</tr>
<tr>
<td>Snyder PhD, RG, A Burdi PhD, and G Gaul BS.</td>
<td>Human-both; unknown</td>
<td>Refresh anti-formin solution after filtering,</td>
<td>Anti-formin [Na2CO3 &amp; Ca(ClO)2] &amp; C14H10O4, IVDs soaked in trichlorethane</td>
<td></td>
</tr>
</tbody>
</table>

[1] Terry Collection
<p>| (1975) “A Rapid Technique for Preparation of Human Fetal and Adult Skeletal Material.” Journal of Forensic Science 20:3 576-580. [no references] | formula | weak KOH or papain good for skull, fetal, &amp; early infant [T&amp;T: Just below 100°C, Minutes to to 1 hour] | brush (only main tissue cut away) [bleach: soak &amp; refresh with anti-formin, H2O2 or KOH over several days] | &amp; H2O [R&amp;F: Running H2O Alvar® flakes &amp; acetone ] |
| Mooney, Mark P., Eric M. Kraus, Janusz Bardach, and John I. Snodgrass. (1982) “Skull Preparation Using the Enzyme-Active Detergent Technique.” The Anatomical Record. 202: 125-129. [no references] | Human-both; preserved in formalin | Reviews use of bugs, toxic chemistry, caustic soda, quick lime, H2O2, trichlorethulene, benzene [T&amp;T: 75-80 °C, 8 hours a day for 3-5 days] | lab oven or calibrated hot plate, 10% commercial enzymatic detergent &amp; H2O, craniotomy with preserved tissue [no bleaching reported] | more 10% commercial enzymatic detergent &amp; H2O [no rinsing &amp; fixing reported] |
| De Wet, E., P. Robertson, and I. Plug. (1990) “Some Non-human-species not specified; | Drilling holes helps remove marrow &amp; grease (even with bugs), some manual removal (except with dermestids) trichloroethylene | trichlorethulene is hazardous, H2O2 less hazardous &amp; |</p>
<table>
<thead>
<tr>
<th>Techniques for Cleaning and Degreasing Bones and a Method for Evaluating the Long-Term Effects of these Techniques.” In Natural History Collections: Their Management and Value. Elizabeth M. Hertholdt, ed. Transvaal Museum, Pretoria, South Africa. [no references]</th>
<th>not preserved</th>
<th>H2O2 may decalcify, dish soap not not degreased may smell [T&amp;T: 1st to 90 °C then soak in 60 °C, 1 to 4 hours then soak at room temp]</th>
<th>, H2O2, commercial dish soap, cloth bags for small bones [Bleach: Mild (mustard powder cuts odor), NaBO3]</th>
<th>little smell, dishwashing soap not dangerous must be fully processed [no rinsing &amp; fixing reported]</th>
</tr>
</thead>
<tbody>
<tr>
<td>Human-both; unknown formula</td>
<td>Evolved over 30 yr period, with preserved tissue several repetitions must be performed, review NaBO3, H2O2, H2O only, anti-formin, dermestids, acetone, trichloroethylene, etc. [T&amp;T: Simmer, 1 hour]</td>
<td>H2O solution, household detergent, baking soda, Scoopula® [Bleach: says they don't but used H2O2]</td>
<td>H2O based ammonia is least destructive [ R&amp;F: None reported Vinac® &amp; acetone]</td>
<td></td>
</tr>
<tr>
<td>Nonhuman-pig; not preserved</td>
<td>Water, papain enzymes,&amp; 9 commercial detergents, after trials 2 human</td>
<td>Scouring pad, fresh smell of detergent, easy to use outside of lab</td>
<td>Papain potential damage to human respiratory</td>
<td></td>
</tr>
<tr>
<td>Rennick BS, Stephanie L, Todd W Fenton PhD, and David R Foran PhD. (2005) “The Effects of Skeletal Preparation Techniques on DNA from Human and Non-Human Bone.” <em>Journal of Forensic Science</em> 50:1-4. [Fenton]</td>
<td>Human &amp; non-human (cow, sheep &amp; pig); not preserved</td>
<td>Highest yields of DNA with detergent acting as buffer as temperature is raised [T&amp;T: Just below boil, 4 hours]</td>
<td>H2O softened &amp; purified by reverse osmosis, household bleach, powdered Alconox &amp; N2CO3 [Bleach: from none to 25% bleach (with 3% NaClO) to 20 cc each Alconox &amp; N2CO3]</td>
<td>H2O &amp; household bleach are greasy, powdered Alconox &amp; N2CO3 are less greasy [no rinsing &amp; fixing reported]</td>
</tr>
<tr>
<td>---</td>
<td>---</td>
<td>---</td>
<td>---</td>
<td>---</td>
</tr>
<tr>
<td>Steadman PhD, Dawnie Wolfe, Lisa L diAntonio, BS, Jeremy J Wilson, MA, Kevin E Sheridan, MA, and Steven P Tarramiello, Phd. (2006) “The Effects of Chemical and Heat Maceration Techniques on Non-human (pig); not preserved?</td>
<td>No silver bullet, leave some fat, best processes: hot H2O @ 90°C, boiling H2O, microwave, meat tenderizer &amp; dish soap @ 90°C, detergent &amp; Sodium carbonate @ 90°C [T&amp;T: 37 °C (warm]</td>
<td>10 methods &amp; 6 categories, cold &amp; warm H2O, bacterial, cooking, chemical, enzymes, invertebrates, alkali breaks down proteins (KOH), scoring system: odor, soft tissue texture, ease of</td>
<td>KOH R&amp;F: KOH rinsing required No fixing reported]</td>
<td></td>
</tr>
<tr>
<td>Source</td>
<td>Organism</td>
<td>Sample Size</td>
<td>Methodology</td>
<td>Preservation</td>
</tr>
<tr>
<td>-------</td>
<td>----------</td>
<td>-------------</td>
<td>-------------</td>
<td>-------------</td>
</tr>
<tr>
<td>Hammond-Todd, Terry, Cobb, Fenton</td>
<td></td>
<td></td>
<td>maceration) to 90 °C, hours to days</td>
<td></td>
</tr>
<tr>
<td>Lee, Esther J., Jennifer G. Luedkte, Jamie L. Allison, Caroilyn E. Arbor, D. Andrew Merriwether, Dawnie Wolfe Steadman</td>
<td>Human; not preserved?</td>
<td>1 tibia &amp; 1 fibula for each, scoring hourly for 2 days, 5x a day thereafter</td>
<td>microwave, detergent, Na2CO3, EDTA/Palmolive, boil, hot H2O, EDTA, bleach, detergent Na2CO3, ammonia, H2O2 [T&amp;T: room temp not used]</td>
<td>9 trials as in Steadman, deflesh by hand and with forceps, tap H2O, NaClO, H2O2, EDTA, used Steadman chart [Bleach: 1 trial only]</td>
</tr>
<tr>
<td>Yin, Venkatesan, Kalyanasundaram &amp; Qin (2010)</td>
<td>Non human-lamb; not preserved?</td>
<td>H2O enzyme maceration used, no change in porosity, enzymes affect bone microstructures &amp; mechanical properties</td>
<td>Fume cupboard not covered; rod, cooking, knife &amp; brush [Bleach: 1 in trypsin enzyme, 4 in water only]</td>
<td>None reported R&amp;F: None reported Isotonic phosphate buffer &amp; sodium azide preservative fix</td>
</tr>
<tr>
<td>microhardness of compact bone. <em>Biomedical Materials</em>, 5(1), 015006. [Mairs, Mooney, Rennick, Steadman]</td>
<td>[T&amp;T: Room temp, 5 days]</td>
<td></td>
<td></td>
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</tr>
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</table>
### Chart 2: Donor Specimen Demographics

<table>
<thead>
<tr>
<th>WBP #</th>
<th>ID#</th>
<th>AGE</th>
<th>SEX</th>
<th>CAUSE OF DEATH</th>
<th>MED HX</th>
</tr>
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<tbody>
<tr>
<td>8050</td>
<td>001</td>
<td>106</td>
<td>Male</td>
<td>Pneumonia</td>
<td>Bladder operation</td>
</tr>
<tr>
<td>8056</td>
<td>002</td>
<td>89</td>
<td>Female</td>
<td>Upper GI bleed, renal failure</td>
<td>Mastectomy</td>
</tr>
<tr>
<td>8073</td>
<td>003</td>
<td>65</td>
<td>Female</td>
<td>Adrenal carcinoma</td>
<td>Breast cancer</td>
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</table>

### Chart 3: Maceration Trial Specimen Groupings

<table>
<thead>
<tr>
<th>TRIAL</th>
<th>1</th>
<th>2</th>
<th>3</th>
<th>4</th>
<th>5</th>
<th>6</th>
</tr>
</thead>
<tbody>
<tr>
<td>SKELETAL PARTS</td>
<td>Shoulder girdle &amp; Upper extremity</td>
<td>Ankles &amp; feet</td>
<td>Femur, patella, tibia, &amp; fibula</td>
<td>Skull girdle &amp; sacrum</td>
<td>Vertebral column &amp; rib cage</td>
<td></td>
</tr>
<tr>
<td>HEAT SOURCE</td>
<td>Max Burton Induction Stove</td>
<td>Max Burton Induction Stove</td>
<td>VWR unstirred water bath</td>
<td>Max Burton &amp; VWR Stove</td>
<td>VWR unstirred water bath</td>
<td>VWR unstirred water bath</td>
</tr>
</tbody>
</table>
Chart 4: Stages of Trials

<table>
<thead>
<tr>
<th>TRIAL</th>
<th>MACERATION</th>
<th>DEGREASING</th>
<th>TONING</th>
<th>ADDED MEASURES</th>
<th>FIXING</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>1A</td>
<td>1B</td>
<td>1C</td>
<td>1D</td>
<td>1E</td>
</tr>
<tr>
<td>2</td>
<td>2A</td>
<td>2B</td>
<td>2C</td>
<td>2C</td>
<td>2E</td>
</tr>
<tr>
<td>3</td>
<td>3A</td>
<td>3B</td>
<td>3C</td>
<td>3D</td>
<td>3E</td>
</tr>
<tr>
<td>4</td>
<td>4A</td>
<td>4B</td>
<td>4C</td>
<td>4D</td>
<td>4E</td>
</tr>
<tr>
<td>5</td>
<td>5A</td>
<td>5B</td>
<td>5C</td>
<td>5D</td>
<td>5E</td>
</tr>
<tr>
<td>6</td>
<td>6A</td>
<td>6B</td>
<td>6C</td>
<td>6D</td>
<td>6E</td>
</tr>
</tbody>
</table>

Where A indicates the detergent, meat tenderizer, baking soda, and/or ammonia, B indicates acetone, C represents hydrogen peroxide, D indicates any extra steps taken to prepare bone, and E represents fixing.
**Chart 5: Steadman Protocols for Evaluating Tissue Maceration**

<table>
<thead>
<tr>
<th>ODOR (OD)</th>
<th>SOFT TISSUE TEXTURE (TT)</th>
<th>EASE OF SOFT TISSUE REMOVAL (TR)</th>
<th>BONE QUALITY (BQ)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1. Strong smell permeates space &amp; dissipates rapidly</td>
<td>1. Firm &amp;/or solid; may feel tougher &amp; more rubbery than first submerged</td>
<td>1. Adherence to bone is quite strong; little or no flesh removal is possible without bone damage</td>
<td>1. Brittle, fragile, easily broken</td>
</tr>
<tr>
<td>2. Moderate smell in the immediate vicinity of the fume hood dissipates dissipation slowly</td>
<td>2. As malleable as when originally observed</td>
<td>2. Adherence to bone is moderately strong; large portions can be easily removed; the core of flesh close to the bone is still adherent</td>
<td>2. No cortical erosion but lighter in weight &amp; porous</td>
</tr>
<tr>
<td>3. Little to no smell; slight odor possible around/under fume hood</td>
<td>3. Considerably softer than when the experiment began; very malleable</td>
<td>3. Adherence minimal to none; flesh falls off as bones are removed from solution or easily removed with fingertips</td>
<td>3. Softer, more pliable than normal no cortical damage.</td>
</tr>
<tr>
<td></td>
<td>4. Nearly liquefied floats on the surface with little or no connection to bone</td>
<td></td>
<td>4. Cortex eroding and/or flaking but not easily fractured</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>5. Strong normal texture &amp; quality</td>
</tr>
</tbody>
</table>
### Chart 6: Modified Steadman Protocols For Evaluating Tissue Maceration

<table>
<thead>
<tr>
<th>ODOR (OD)</th>
<th>SOFT TISSUE TEXTURE (TT)</th>
<th>EASE OF SOFT TISSUE REMOVAL (TR)</th>
<th>BONE QUALITY (BQ)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1. Strong smell permeates space</td>
<td>1. Firm &amp;/or solid; may feel tougher or more rubbery than first submerged</td>
<td>1. Firm and/or rubbery; removal more difficult if not impossible without damaging bone</td>
<td>1. Brittle, fragile, easily broken</td>
</tr>
<tr>
<td>dissipates rapidly</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>2. Strong smell permeates space</td>
<td>2. As malleable as when originally observed</td>
<td>2. Adherence to bone is quite strong; little or no flesh removal is possible without bone damage</td>
<td>2. No cortical erosion bone lighter in weight and porous</td>
</tr>
<tr>
<td>dissipates slowly</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>*</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>dissipation rapidly</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>*</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>4. Moderate smell in the immediate</td>
<td>4. More rubbery than when first submerged; apparent liquefaction and lifting of tissue</td>
<td>4. Adherence to bone is moderately strong, large portions are easily removed; the core of</td>
<td>4. Cortex eroding and/or flaking but bone will not easily fracture</td>
</tr>
<tr>
<td>vicinity of the fume hood</td>
<td>from bone *</td>
<td>flesh close to the bone is still adherent</td>
<td></td>
</tr>
<tr>
<td>dissipation slowly</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>5. Little to no smell; slight odor</td>
<td>5. Nearly liquefied; floats on the surface with little or no connection to bone</td>
<td>5. Adherence is minimal to none; flesh falls off as bones are removed from solution or easily</td>
<td>5. Strong, normal bone texture and quality</td>
</tr>
<tr>
<td>possible around or under fume hood</td>
<td></td>
<td>removed with fingertips</td>
<td></td>
</tr>
</tbody>
</table>

Expanded evaluation parameters create a more even comparison scale.
## Chart 7: Macerating Chemistry and Source

<table>
<thead>
<tr>
<th>SUB-STRATE</th>
<th>Alkyl benzene sulfonate (ABS)</th>
<th>Sodium laureth sulfate (SLS)</th>
<th>Sodium bicarbonate (SB)</th>
<th>Bromelain (BR)</th>
<th>Ammonium hydroxide (HN)</th>
</tr>
</thead>
<tbody>
<tr>
<td>PRODUCT</td>
<td>Arm &amp; Hammer laundry detergent for sensitive skin (no perfumes or dyes)</td>
<td>Bright green Concentrated Dish Washing Liquid (dye and fragrance free)</td>
<td>Arm &amp; Hammer Pure Baking Soda</td>
<td>McCormick Unseasoned Meat Tenderizer</td>
<td>Nice! Lemon-Fresh “Sudsy” Ammonia</td>
</tr>
<tr>
<td>ACTIVE FORMULA</td>
<td>Biodegradable anionic &amp; cationic surfactants</td>
<td>Sodium lauryl sulfate (a plant based surfactant)</td>
<td>Sodium bicarbonate</td>
<td>Bromelain</td>
<td>Hydrogen nitride</td>
</tr>
<tr>
<td>OTHER PARTS</td>
<td>Sodium bicarbonate, may contain trace enzymes</td>
<td>Biodegradable preservatives</td>
<td>None</td>
<td>Salt, dextrose, &amp; Calcium silicate2</td>
<td>Surfactant, fragrance, dye”</td>
</tr>
</tbody>
</table>
Chart 8.1: Macerating Solution & Treatment Protocols
Shoulder girdle and upper extremity

<table>
<thead>
<tr>
<th>SPECIMEN</th>
<th>DW (L)</th>
<th>PRETREAT</th>
<th>ABS (cc)</th>
<th>SLS (L)</th>
<th>SB (cc)</th>
<th>BR (ml)</th>
<th>HN (ml)</th>
</tr>
</thead>
<tbody>
<tr>
<td>001 Left</td>
<td>13.2</td>
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<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>001 Right</td>
<td>12.6</td>
<td>no</td>
<td>120</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>002 Left</td>
<td>12.25</td>
<td>no</td>
<td>0</td>
<td>34.5</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>002 Right</td>
<td>12.6</td>
<td>no</td>
<td>0</td>
<td>120</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>003 Left</td>
<td>11.0</td>
<td>no</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>31</td>
<td>0</td>
</tr>
<tr>
<td>003 Right</td>
<td>11.0</td>
<td>no</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>900</td>
</tr>
</tbody>
</table>

**Chart 8.2: Macerating Solution & Treatment Protocols**
Tarsals, metatarsals, & pedal phalanges

<table>
<thead>
<tr>
<th>SPECIMEN</th>
<th>DW (L)</th>
<th>PRETREAT</th>
<th>ABS (cc)</th>
<th>SLS (L)</th>
<th>SB (cc)</th>
<th>BR (ml)</th>
<th>HN (ml)</th>
</tr>
</thead>
<tbody>
<tr>
<td>001 Left</td>
<td>5.5</td>
<td>yes</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>31</td>
<td>900</td>
</tr>
<tr>
<td>001 Right</td>
<td>5.5</td>
<td>no</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>31</td>
<td>900</td>
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<tr>
<td>002 Left</td>
<td>6.3</td>
<td>yes</td>
<td>0</td>
<td>35.5</td>
<td>0</td>
<td>0</td>
<td>1030</td>
</tr>
<tr>
<td>002 Right</td>
<td>6.3</td>
<td>no</td>
<td>0</td>
<td>35.5</td>
<td>0</td>
<td>0</td>
<td>1030</td>
</tr>
<tr>
<td>003 Left</td>
<td>6.3</td>
<td>yes</td>
<td>0</td>
<td>0</td>
<td>120</td>
<td>0</td>
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## Chart 8.3: Macerating Solution & Treatment Protocols
Femur, patella, tibia, & fibula

<table>
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<th>DW (L)</th>
<th>PRETREAT</th>
<th>ABS (cc)</th>
<th>SLS (L)</th>
<th>SB (cc)</th>
<th>BR (ml)</th>
<th>HN (ml)</th>
</tr>
</thead>
<tbody>
<tr>
<td>001 Right</td>
<td>18.92</td>
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<td>0</td>
<td>360.38</td>
<td>106.64</td>
<td>3.1</td>
</tr>
<tr>
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<td>18.92</td>
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<td>0</td>
<td>0</td>
<td>360.38</td>
<td>106.64</td>
<td>3.1</td>
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<td>18.92</td>
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<td>3.1</td>
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<td>0</td>
<td>1.06</td>
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<td>106.64</td>
<td>3.1</td>
</tr>
<tr>
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<td>1.06</td>
<td>360.38</td>
<td></td>
<td>3.1</td>
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<td>0</td>
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<td>360.38</td>
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### Chart 8.4: Macerating Solution & Treatment Protocols
Skull, mandible, & hyoid

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<th>SB (cc)</th>
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<td>500</td>
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<td>500</td>
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<td>5.67</td>
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### Chart 8.5: Macerating Solution & Treatment Protocols
#### Ilia, sacrum & coccyx

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<th>ABS (cc)</th>
<th>SL (ml)</th>
<th>SB (cc)</th>
<th>BR (ml)</th>
<th>HN (L)</th>
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</thead>
<tbody>
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<td>500</td>
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<tr>
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<td>500</td>
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<tr>
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<td>500</td>
<td></td>
<td>18.92</td>
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### Chart 8.6: Macerating Solution & Treatment Protocols
#### Cervical, thoracic, lumbar vertebrae, ribs, & sternum

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<th>SPECIMEN</th>
<th>DW (L)</th>
<th>PRETREAT</th>
<th>ABS (cc)</th>
<th>SLS (L)</th>
<th>SB ml</th>
<th>BR ml</th>
<th>HN (L)</th>
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</thead>
<tbody>
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</tr>
<tr>
<td>002</td>
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<td></td>
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Chart 9: Degreasing with Acetone: Amount & Time of Immersion

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<th>FULL SKELETON</th>
<th>ACETONE</th>
<th>TIME IN SOLUTION</th>
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<tbody>
<tr>
<td>001</td>
<td>38 liters</td>
<td>216 hours</td>
</tr>
<tr>
<td>002</td>
<td>27 liters</td>
<td>336 hours</td>
</tr>
<tr>
<td>003</td>
<td>27 liters</td>
<td>456 hours</td>
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</tbody>
</table>

Chart 10: Preservative formula for specimens in this study

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<thead>
<tr>
<th>Reagent</th>
<th>Water</th>
<th>Ethanol</th>
<th>Glycerol</th>
<th>Formaldehyde</th>
<th>Phenol</th>
<th>Lysol</th>
</tr>
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<tbody>
<tr>
<td>Percentage</td>
<td>61%</td>
<td>20%</td>
<td>10%</td>
<td>5%</td>
<td>2%</td>
<td>2%</td>
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</table>
## Chart 11: Comparison of Processing Times and Chemistry

<table>
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<th>TRIAL*</th>
<th>CHEMISTRY**</th>
<th>TIME (hrs)</th>
<th>NOTES</th>
</tr>
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<tr>
<td>1</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>001L</td>
<td>H2O ONLY</td>
<td>70</td>
<td>Hole in infraspinatus fossa</td>
</tr>
<tr>
<td>001R</td>
<td>H2O/ABS</td>
<td>70</td>
<td>Ulnar styloid disintegrated, lighter than pure H2O, texture rubbery</td>
</tr>
<tr>
<td>002L</td>
<td>H2O/LS</td>
<td>31</td>
<td>Eroded and fragile, less rubbery &amp; easier to remove</td>
</tr>
<tr>
<td>002R</td>
<td>H2O/BS</td>
<td>21</td>
<td>Distinct odor, cortical erosion, tissue slippery &amp; liquefied as with laundry soap</td>
</tr>
<tr>
<td>003L</td>
<td>H2O/BR</td>
<td>25</td>
<td>Slow to remove, stiff not rubbery, relatively easy to remove</td>
</tr>
<tr>
<td>003R</td>
<td>H2O/SA</td>
<td>30</td>
<td>Tissue similar to previous specimens, appears cleaner at end with easier removal of cartilage &amp; connective tissue</td>
</tr>
<tr>
<td>2</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>001L</td>
<td>H2O/SA/BR (P)</td>
<td>26</td>
<td>Smell noted, quicker to remove tissue</td>
</tr>
<tr>
<td>001R</td>
<td>H2O/SA/BR (S)</td>
<td>24</td>
<td>Less smell, tissue comes off more slowly</td>
</tr>
<tr>
<td>002L</td>
<td>H2O/SA/BS (P)</td>
<td>22</td>
<td>Distal metatarsals &amp; phalanges eroding</td>
</tr>
<tr>
<td>002R</td>
<td>H2O/SA/BS (S)</td>
<td>18</td>
<td>Extensive erosion of metatarsals &amp; phalanges</td>
</tr>
<tr>
<td>003L</td>
<td>H2O/SA/LS (P)</td>
<td>30</td>
<td>Tissue liquefies easily, not cartilage</td>
</tr>
<tr>
<td>003R</td>
<td>H2O/SA/LS (S)</td>
<td>27</td>
<td>Tissue removed easily, not cartilage</td>
</tr>
<tr>
<td>3</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Trial</td>
<td>Chemistry</td>
<td>Time</td>
<td>Notes</td>
</tr>
<tr>
<td>-------</td>
<td>-----------</td>
<td>------</td>
<td>-------</td>
</tr>
<tr>
<td>001L</td>
<td>(H20/SA) BS/BR (S)</td>
<td>14</td>
<td>Color change in solution-baking soda &amp; bromelain? Tissue easy to remove except ligaments &amp; cartilage</td>
</tr>
<tr>
<td>001R</td>
<td>(H20/SA) BS/BR (P)</td>
<td>22</td>
<td>Color change in solution-baking soda &amp; bromelain? Tissue easy to remove except ligaments &amp; cartilage</td>
</tr>
<tr>
<td>002L</td>
<td>(H20/SA) LS/BR (S)</td>
<td>24</td>
<td>Bones fragile &amp; disintegrating</td>
</tr>
<tr>
<td>002R</td>
<td>(H20/SA) LS/BR (P)</td>
<td>35</td>
<td>Distal medial femur shows aggressive deterioration</td>
</tr>
<tr>
<td>003L</td>
<td>(H20/SA) LS/BS (S)</td>
<td>25</td>
<td>Tissue easy to remove except ligaments &amp; cartilage</td>
</tr>
<tr>
<td>003R</td>
<td>(H20/SA) LS/BS (P)</td>
<td>25</td>
<td>Tissue easy to remove except ligaments &amp; cartilage</td>
</tr>
<tr>
<td>Trial* 4</td>
<td>CHEMISTRY** (P) pretreat</td>
<td>TIME (hrs)</td>
<td>NOTES</td>
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<tr>
<td>001</td>
<td>(H20/SA) LS (P)</td>
<td>127</td>
<td>Extensive cracking, strong ammonia smell, all tissue removed except fossae and cranial depressions</td>
</tr>
<tr>
<td>002</td>
<td>(H20/SA) BR (P)</td>
<td>24</td>
<td>Major destruction of orbit, all tissue removed except fossae and cranial depressions</td>
</tr>
<tr>
<td>003</td>
<td>(H20/SA) BS (P)</td>
<td>24</td>
<td>Complete disarticulation of structures, all tissue removed except fossae and cranial depressions</td>
</tr>
<tr>
<td>Trial* 5</td>
<td>CHEMISTRY** (P) pretreat</td>
<td>TIME (hrs)</td>
<td>NOTES</td>
</tr>
<tr>
<td>001</td>
<td>(H20/SA) LS (P)</td>
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<td>Sacral ala collapsed bilaterally</td>
</tr>
<tr>
<td>Trial</td>
<td>CHEMISTRY*</td>
<td>TIME</td>
<td>NOTES</td>
</tr>
<tr>
<td>-------</td>
<td>------------</td>
<td>------</td>
<td>-------</td>
</tr>
<tr>
<td>001</td>
<td>(H20/SA) LS (P)</td>
<td>84</td>
<td>Tissue removes easily, discs fully intact, need to be cut and exposed for solution to macerate them. Vertebrae blackened and brittle</td>
</tr>
<tr>
<td>002</td>
<td>(H20/SA) BR (P)</td>
<td>51</td>
<td>Yellow fatty residue, discs still intact, need to be cut and exposed for solution to macerate them. DISH noted</td>
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<tr>
<td>003</td>
<td>(H20/SA) BS (P)</td>
<td>32</td>
<td>Discs appear easiest to remove with this solution, though still difficult.</td>
</tr>
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Chart 12: Graph: Macerating Solution versus Time

Trial 1

Scapula, humerus, ulna, radius, carpals, metacarpals, & phalanges

Trial 2

Tarsals, metatarsals, & phalanges
Femur, patella, tibia, & fibula

Trial 3

001L 001R 002L 002R 003L 003R

Skull

Trial 4

001L 001R 002L 002R 003L 003R

time (in hours)
## Chart 13: Modified Steadman Rubric Results

<table>
<thead>
<tr>
<th>Shoulder girdle &amp; upper extremity</th>
<th>PROCESSING ODOR</th>
<th>TISSUE TEXTURE</th>
<th>EASE OF REMOVAL</th>
<th>BONE QUALITY</th>
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<tbody>
<tr>
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<table>
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<th>Ankles &amp; Feet</th>
<th>PROCESSING ODOR</th>
<th>TISSUE TEXTURE</th>
<th>EASE OF REMOVAL</th>
<th>BONE QUALITY</th>
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<tbody>
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<td>2.66</td>
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<th>Femur, Patella, Tibia &amp; Fibula</th>
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<th>TISSUE TEXTURE</th>
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<th>BONE QUALITY</th>
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### Chart 14: Gas Chromatography Mass Spectrometry

<table>
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<th>TRIAL</th>
<th>SPECIMEN #</th>
<th>SAMPLE #</th>
<th>ID#</th>
<th>M/S*</th>
<th>TIME (hrs)</th>
<th>*SPIKE/NONE (S/N)</th>
</tr>
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<tr>
<td>1</td>
<td>002R</td>
<td>M242913</td>
<td>1M</td>
<td>M</td>
<td>9 First</td>
<td>S</td>
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<td>N5913</td>
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<td>M</td>
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<td>O5913</td>
<td>1O</td>
<td>M</td>
<td>8 First</td>
<td>S</td>
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<td>S5913</td>
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<td>M</td>
<td>9 First</td>
<td>N</td>
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<td>T5913</td>
<td>1T</td>
<td>M</td>
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<td>M</td>
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*Results indicate samples that spiked at 10 minutes*
RIC Merged 5-01-2013-d.sms 2000 CENTROID RAW

8056R
Final 2 hr normal

N
MS Data Review Active Chromatogram Plot - 6/4/2013 3:38 PM

MCounts

RIC Merged 5-01-2013-d002.sns  2000 CENTROID RAW

Final 29.5 hr

Marrow
RIC Merged 5-01-2013-d004.sms 2000 CENTROID RAW

\( \text{R}^2 8056R \)

FINAL 60.5min

MARNOW
MS Data Review Active Chromatogram Plot - 8/10/2013 12:25 PM

RIC Merged 3-21-13003.sms  2000 CENTROID RAW

W

8073L
Figs 6.29
Marrow