Author: FP McManamon at NP-WASO-DCA
Date: 9/14/00 10:01 AM
Priority: Normal
TO: Michael.K.Trimble@mvs02.usace.army.mil at NP--INTERNET,
Rhonda.R.Lueck@mvs02.usace.army.mil (Rhonda R. Lueck) at NP--INTERNET
CC: FP McManamon, Jason Roberts
Subject: RE: final report on Kennewick taphonomy

Sonny--I am sending you the final draft of the taphonomy report for your review and comments. Would you be able to get me any comments by next Monday, 18 September? I also am sending this to Rhonda for her information and in case you are out of email touch, she can let you know about it.

Please let me know if you can do this by Monday. Also, we can include in any comments a request that Phil provide the information you were concerned about this summer, related, I think, to accurate captions for the photos and molds.

Hope all else is well. FPM

---------- Forward Header ----------
Subject: RE: final report on Kennewick taphonomy
Author: "Phillip Walker" <tomal@gte.net> at np--internet
Date: 9/6/00 2:29 PM

Hi Frank,

Here is the final version of our report. As you will see, it doesn't contain any surprises, we simply included a lot of empirical data to reinforce our previous conclusions. As Clark mentioned in his message, I am leaving for two weeks in Italy on Sunday, so if there are any major changes that you would like us to make, we will need to know what they are right away. Otherwise, after I leave, just send any changes you want in the report to Clark and he will make the.

I hope you have had a good summer!

Cheers,

Phil

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Final Report on the Physical Examination and Taphonomic Assessment of the Kennewick Human Remains (CENWW.97.Kennewick) to Assist with DNA Sample Selection

Report to the Department of Justice and the Department of Interior
Phillip L. Walker, Clark Spencer Larsen, and Joseph F. Powell

September 5, 2000

Introduction

To obtain information for use in selecting bone samples suitable for DNA analysis, we conducted a physical examination and assessment of the Kennewick human remains at the Burke Museum, Seattle, Washington, on April 25-26, 2000. Dr. Powell made some additional observations for use in this report on April 27, 2000. As part of our analysis, we examined the entire skeleton and made both macroscopic and microscopic observations of its condition.

In this report we present a description of the methods we used and our conclusions regarding the taphonomic history of the skeleton with special reference to conditions that are likely to have affected the suitability of specific skeletal elements for DNA analysis. Our approach to the problem of assessing the condition of the skeleton involved a careful examination of each bone for clues to its ante-mortem, post-mortem, and post-recovery history. This information, along with documentary evidence and discussions with individuals responsible for the analysis and curation of the skeleton, provides the basis for our recommendations concerning the skeletal elements that are most likely to prove useful for biomolecular studies. Our recommendations are not based solely on our judgments concerning the potential of specific skeletal elements for DNA analysis. Instead, we have attempted to balance the research value of specific specimens in non-molecular studies (metrical, histological, paleopathological, and so on) against their potential as sources of molecular data.

DOI 09760

Methods and Organization of Research

During the afternoon of April 24, we met at the Burke Museum with representatives of the Burke Museum, the Corp of Engineers, the National Park Service, and other Department of Interior personnel. At the meeting we discussed the goals of the next day's activities and the rules that had been established to protect the skeletal remains. On the morning of April 25 we arrived at the Burke Museum and began our taphonomic analysis.

First, those of us who had not previously worked with the skeleton examined it to familiarize ourselves with the range of variation in its condition. Based on a discussion of these preliminary observations, we created a database containing the Corp of Engineers designations for each skeletal element and fields for recording relevant taphonomic variables. We developed a coding scheme for each variable that allowed us to efficiently record key characteristics of each specimen. These codes were designed to provide information relevant to reconstructing the taphonomic history of each skeletal element and determining its suitability for DNA extraction. We recorded the following taphonomic variables: 1) degree of degradation of cortical bone, 2) presence of adherent material, 3) divergence of the unfractured cortical surfaces of the bone from the dominant light-brown surface color that characterized most of the skeleton, and 4) probable time of fractures (ante-mortem, peri-mortem, or post-mortem) based on the characteristics of the fractured surface. The database also includes information on photographs we took of specific skeletal elements including comments on the features of special interest and information on the bone surfaces we replicated for further microscopic analysis using a high-resolution vinyl polysiloxane dental impression material.

To document the coding system we used and specific aspects of the Kennewick skeleton that are relevant to our analysis, we photographed features of special interests with a 35mm camera using both a 55 mm Micro-Nikkor macro lens

DOI 09761

and an Olympus SZ40 dissecting microscope. These images were subsequently digitized for inclusion in this report.

Taphonomic History

Strictly speaking, the taphonomic history of the Kennewick skeleton encompasses all events from the death of this person to the discovery of his skeleton. Owing to their relevance to DNA preservation and the research value of specific skeletal elements, we expanded the scope of our taphonomic analysis to encompass ante-mortem changes such as those associated with pathological conditions, and the post-recovery treatment of the skeleton during curation and research activities.

Ante-Mortem Changes

The Kennewick skeleton exhibits several minor osseous changes that are clearly of ante-mortem origin. These have been discussed in considerable detail elsewhere (Chatters, 2000; Powell and Rose, 1999) and we will only briefly mention them here. Although the skeleton shows some evidence of localized infections and traumatic injuries (Figure 1), there is no clear evidence of osteoporosis or other systemic conditions that affect bone density and thus might influence the preservation of DNA. A few bones show indications of osteoarthritis but, for the most part, these changes are very minor. Traumatic injuries include a projectile wound in the right ilium that resulted in a secondary infection and small, 6mm in diameter, well-healed depressed fracture to outer table of the left frontal bone (Figure 1). The left radius shows evidence of localized trauma and the olecranon fossa is partially filled with reactive bone indicative of an inflammatory process (Figure 1). There is also a 15mm in diameter area of abnormal bone in the external surface of the greater wing of the left sphenoid.

Some changes in the ribs that have previously been described as

DOI 09762

well healed, antemortem factures appear to us to be post-mortem changes in the skeleton. Chatters (Chatters, 2000) identified at least seven fractures of at least six ribs on both sides of the sternum that he believes resulted from a single antemortem traumatic episode. He argues that at least three and possibly four ribs failed to heal together, and formed pseudarthroses (false joints). Although Powell and Rose (1999) concurred with Chatter's diagnosis of antemortem rib fractures resulting in pseudarthroses, they only identified two right ribs as having this condition.

We carefully examined all of the rib fragments with a dissecting microscope. Based on this examination we conclude that no clear evidence of rib pseudarthroses is present in the Kennewick skeleton. Several of the rib fragments have ends that appear, based on cursory visual examination, to show in vivo remodeling (97.I.12a.1, 97.I.12a.3, 97.I.12a.7, 97.I.12a.9), but upon closer examination with a dissecting microscope show no evidence of new bone formation along the edge of the fracture (Figures 2 and 3). These broken ribs differ markedly from those seen in modern forensic cases with undoubted pseudarthroses (Walker et al., 1997; Figure 4). The differences between the Kennewick rib fractures and the pseudarthroses in modern forensic cases include: 1) the absence of any evidence of subperiosteal new bone or callus formation, 2) the fact that the fracture is perpendicular to the rib, instead of hinged as is typical for *in vivo* fractures, 3) the contrast between the straight fracture line of the Kennewick ribs and the "frayed" edges often seen in *in vivo* rib fractures, 4) the presence of what appears to be calcite along the edge and within the fractured edge of the Kennewick ribs, 5) the fact that the cortical surfaces of the Kennewick ribs have an unusual "pinched" configuration that causes them to closely approximate each other without any reduction in the thickness of the cortex. Healed *in vivo* fractures that have formed pseudarthroses, in contrast, typically have cortical surfaces that approximate each other only through the addition of new bone within the callus, and 6) there are

longitudinal cracks in the cortical bone adjacent to the fracture lines, which suggests the ends of the ribs were deformed by a mechanical post-mortem process that pinched their ends together after the loss of considerable collagen. Based on this evidence, we conclude that the condition previously diagnosed as rib pseudarthroses in the Kennewick skeleton is a result of a post-mortem process perhaps resulting from ground pressure or some other mechanical process operating within the depositional environment.

Peri-Mortem Changes

A few features of the Kennewick skeleton may possibly be the result of processes operating around the time of death. Based on a similarity in surface appearance to the surrounding bone-on-bone, Chatters (Chatters, 2000) suggests that a defect in the glenoid fossa of the right scapula is a peri-mortem fracture in which a small chip of bone was driven off the posterior edge of the glenoid fossa.

A left rib fragment is the only specimen with surface modifications possibly resulting from carnivore activity around the time of death (Figure 5). The surface of this bone has two roughly triangular dents in it with about 1 mm of intervening cortical bone that has been depressed into the medullary cavity. Although the dents resemble damage seen in bones chewed by carnivores, other features such as the straight edges of the fractured cortical bone are more consistent with a post-mortem process operating after the collagen content of the bone had been significantly reduced. The preservation of almost every element of the Kennewick skeleton and the fact that no other bones exhibit similar damage further reduce the likelihood that these are carnivore tooth marks. Although conceivable, it also seems unlikely, based on scavenging behavior studies (cf. Andrews and Fernandez, 1997; Selvaggio, 1998) that carnivores who had access to the body would leave an essentially intact skeleton with tooth marks on only a single rib.

DOI 09764

Our research reinforces Chatters' (Chatters, 2000) conclusion that, given the currently available evidence, the issue of whether or not this individual was intentionally buried remains unresolved. Since the skeleton eroded out of the riverbank, we do not know the details of its original stratigraphic context. The fact that the skeleton was essentially complete (only the hyoid, part of the sternum, and a few small additional bones are missing) suggests two alternative scenarios: 1) either this person was intentionally buried, or 2) the body was incorporated into the fluvial deposit through some catastrophic hydrologic process at the time of, or very soon after, death. If carnivores were present in the area at the time of burial, which seems likely, such a rapid burial would be necessary to prevent the damage and loss of skeletal elements through scavenging (Andrews and Armour-Chelu, 1998; Carson et al., 2000; Milner and Smith, 1989).

Of these alternative explanations, intentional burial seems to us to be the most likely simply because intentional burial of deceased individuals is an exceedingly common cultural practice and rapid burial through catastrophic hydrologic processes is exceedingly rare. On the other hand, although the data are somewhat ambiguous, the match between a single fluvial stratum and the soil associated with the Kennewick burial is consistent with the hypothesis of rapid burial in a fluvial environment (Chatters, 2000).

One of the issues we were asked to explore concerns the origin of the reddish stain observed on some of the Kennewick bones (Powell and Rose, 1999). It has been suggested that this stain might be of cultural origin, perhaps resulting from the application of a red ochre pigment to the skin of the individual before burial. Although such pigments might be used for body painting in contexts unrelated to death, red ochre is commonly used in mortuary rituals and its presence would add some credence to the theory that this person was intentionally buried. Most of the Kennewick skeletal elements

had a light tan color and no indication of staining by a red pigment. Four bones had dark brown stains on more than 10% of their surface (Figure 6) and five bones had small areas with a reddish stain. The brown staining only affected cranial and hand bones, while the reddish stain was confined to one of the os coxae (hip bones) and a few hand bones. We carefully examined these stained areas and concluded that they are unlikely to be of cultural origin. Instead, they appear to be the result of natural processes operating after this person's burial. This conclusion is suggested by the absence of any evidence of superficial deposits of pigment and the dendritic pattern of some of the stained areas. In our experience, patterning of discolored areas in this way is often associated with the decomposition of roots that have come in contact with a burial.

Algal staining on some of the elements is probably due to exposure of the remains in shallow water just prior to their recovery along the Columbia River. This interpretation is reinforced by the fact that many of the bones with algae adhering to them also have bleached areas indicating several weeks or more of exposure to the sun.

Effects of the Depositional Environment

After burial, the Kennewick skeleton was subjected to several very significant post-depositional processes. Many of the bones are semi-fossilized with calcite deposits adhering to their surfaces (Figure 7). Most of the bones (82%) also have fractures that appear to have occurred in the depositional environment after a substantial amount of the collagen had been lost from the skeleton. These old fractures were diagnosed based upon two criteria: 1) the angle of the fracture (perpendicular to the cortical surface instead of at an acute angle) and, 2) the presence on the fractured surface of discoloration and, in many cases, adherent calcite indative of long

DOI 09766

residence in the depositional environment (Figure 7).

These ancient fractures are easy to differentiate from recent fractures produced during the erosion of the bones from the riverbank and through handling of the skeleton during and after recovery; recent fracture surfaces are devoid of adherent calcite, have sharp edges, and a clean, white appearance that contrasts with the darker color of the bone's cortical surface.

It is important to note that the current absence of calcite deposits on a bone is not necessarily an indication that such deposits were not originally present. Some skeletal elements, such as the cranium, underwent extensive post-recovery cleaning in preparation for casting that involved calcite removal. Additional calcite deposits exfoliated from the bones as they dried in the laboratory (Chatters, 2000).

The current distribution of the calcite in the Kennewick skeleton is heavily biased in favor of specific skeletal elements. More than 40% of the long bones, foot bones, and os coxae have heavy calcite deposits (>10% of the surface covered), while many of the hand bones, ribs, and vertebrae are little effected by calcite deposits (< 10% of the surface covered).

Ancient post-mortem factures are also unevenly distributed within the skeleton. Most of the Kennewick bones have one or more ancient, pre-recovery fractures. The only notable exceptions to this are the bones of the feet and especially the hands, which are remarkably well preserved with less than 20% of the skeletal elements showing pre-recovery fractures.

A few bones have tooth marks on their surfaces produced by rodent gnawing (Figure 8). These bones show the classic signs of rodent activity; namely, dents produced when the maxillary incisors were used to anchor the bone and long grooves that converge toward these dents that were produced by the gnawing movements of the mandibular incisors. These gnawed areas are clearly of considerable antiquity since the color of the gnawed area in

most cases closely approximates the color of the rest of the bone's surface (Figure 8).

Recent Pre-Recovery Changes

Clues to the process through which the Kennewick skeleton eroded from the riverbank are provided by differences in the condition of specific skeletal elements. These differences suggest that the erosion of the skeleton from the riverbank was a two-stage process. Twenty-seven of the bones are either bleached white through sun exposure, have algae growing on them, or exhibit both of these conditions (Figure 6). Some of these bones also have somewhat abraded fracture surfaces (Figure 9). . This is an indication of greater exposure to abrasion in the riverside environment than bones with sharp breaks lacking rounding.These signs of recent pre-recovery exposure to sunlight and abrasion are unevenly distributed within the skeleton and this provides evidence of the sequence of erosion episodes that redeposited the skeleton on the riverbank

In comparison to the other identifiable bones, the vertebrae and os coxae show significantly less evidence of prolonged pre-recovery exposure than the other identifiable skeletal elements ($x^2 = 4.3$, $p = 0.04$). This suggests that they eroded from the riverbank after the long bones. A reasonable interpretation of this pattern is that the skeleton originally rested on its side in a flexed position and that an initial episode of erosion resulted in the collapse of the portion of the riverbank that contained most of the appendicular skeleton. This initial episode may have been followed within a period of several weeks or months by a second riverbank collapse that deposited the remaining portions of the axial skeleton on the shore of the river.

The Kennewick remains were all recovered along the shore of the

DOI 09768

Columbia River at the base of the river's bank. Although none of them were recovered *in situ*, all of the larger fragments occurred within a 12m area (Chatters, 2000a). This suggests recent exposure. Bones rapidly become sorted through fluvial transport (Aslan and Behrensmeyer, 1996) with lighter, more porous bones, (e.g., vertebrae, patellae, and phalanges) being transported farther than heavy bones (e.g., limb bones and mandibles).

Post-Recovery Changes

The treatment of the Kennewick remains since their recovery from Columbia Park has substantially decreased the value of some skeletal elements for DNA research. A total of 54 transverse fractures have no soil adhering to them and are clearly of recent origin. These fractures either occurred at the discovery site a short time before the skeleton was recovered or in the laboratory after the recovery of the skeleton. Additional longitudinal cracking of long bones occurred in the laboratory during the drying process (Chatters, 2000). These changes included the loss of adherent calcite through cleaning and exfoliation as a result of drying. Additional alterations occurred because of the removal of material for radiocarbon analysis. One of the os coxae was treated with dilute hydrochloric acid to remove concretions that enclosed the projectile point embedded in it (Chatters, 2000). The neurocranium and some of the teeth were also treated with a diluted water-based acrylic polymer, and Elmer's glue was used to repair the cranium and mandible. During the process of producing a mold of the skull, it was treated with a release agent and then covered with a polyurethane mold. Finally, as part of Dr. Chatters' study, radiographs were taken of the right ilium and both distal femora and teeth (Chatters, 2000). Computed tomography (CT) scans were also made of the right ilium, right femur, and both humeri. Subsequent to this, Drs. Powell and Rose (1999) radiographed 31 bone fragments using

standard clinical cassettes, film, and procedures. Additional CT scans were also made of the point in the right pelvis, the calvarium, maxilla, left proximal femur, and left distal tibia.

These radiographic procedures have significant implication for the recovery of DNA owing to the wellknown, destructive effects that x-rays have on genetic material. This is especially significant in ancient DNA work owing to the highly degraded state of ancient biomolecules. X-rays havthe potential to further degrade whatever residual DNA remains inand ancient bone and thus reduce the chances for its recovery. On the other hand, both radiography and DNA extraction are routinely done on modern skeletal remains from forensic contexts, so it is clear that, at least in situations where DNA is well preserved, radiography does not preclude the subsequent retrieval of well preserved DNA

Selection Criteria

Based on the observations discussed above, we developed a set of selection criteria for ranking skeletal elements relative to their suitability for use in DNA analysis. Although few systematic studies have been done to provide an empirical basis for deciding what types of skeletal material are likely to contain well preserved DNA, anecdotal data from various laboratories working in the area of ancient DNA analysis, along with common sense, suggest thatbones with evidence of degradation through exposure to sunlight, weathering, fragmentation, and unstable environmental conditions are less likely to contain well-preserved DNA than intact bones from a stable depositional environment. Dr. David Glenn Smith an expert in anthropological genetics and analysis of ancient and modern human DNA participated in the discussion and selection process. Also involved in the selection and ranking from the perspectives of conservation and curation were Drs. Michael Trimble, Vicki Cassman, and Nancy Odegaard.

DOI 09770

Considering the full range of available information, we ranked sun-bleached bones and bones with significant cracks or other evidence of cortical bone deterioration as poor candidates for DNA analysis. Bones we considered to be good candidates for DNA analysis, in contrast, were those with well-preserved cortical surfaces lacking evidence of having been exposed to the elements for long periods of time during the process through which the Kennewick skeleton eroded from its original location.

A second important selection criterion was the type of skeletal tissue present in a specimen. Some skeletal elements have thin cortical layers with a high surface-to-cortical-bone-volume ratio and are thus less likely to provide a stable environment favorable to DNA preservation than other bones that have dense, thick, cortical layers. Although DNA has been successfully extracted from cancellous bone and bones with thin cortical layers such as ribs, most DNA analysts believe that long bones with dense cortical layers and the dentin of teeth provide a stable environment that is most favorable to the preservation of DNA.

The potential that a skeletal element has for yielding well preserved DNA needs to be balanced against the value that element has as a source of other types of bioarchaeological information. For example, because of their low diagnostic value in most types of osteological analysis, ribs are often used as DNA sources, even though their thin cortical layers make them less desirable than other bones as potential sources of ancient DNA. Teeth on the other hand, are both excellent DNA sources and extremely valuable owing to the detailed information they can provide on growth patterns, disease history, age, and other attributes. The final determination of which skeletal elements should be subjected to destructive analysis requires a careful balancing of such potential conflicts between different types of analyses.

Finally, there is evidence that the exposure of bones to x-rays can substantially degrade any DNA that is preserved within them. Our

DOI 09771

recommendations are predicated in part upon the recognition that much of the Kennewick skeleton has been subjected to radiographic analysis. Owing to this we eliminated most of the teeth and a number of other skeletal elements that otherwise might have been considered for our list of good candidates for DNA analysis.

Skeletal Elements Recommended for Use in DNA Analysis

Based on the selection criteria discussed above, in consultation with Drs. Smith, Trimble, Odegaard, and Cassman, we compiled a ranked list of skeletal elements that should be considered for DNA analysis (Table 1). This list was assembled considering the likelihood of intact organic material and the potential diagnostic characteristics of each element. With the exception of specimens 97.R.75a, 97.R.50a, 97.R.16 (MCc), micro-samples were taken from all of these bones. In addition, the data on the organic constituents of these micro-samples obtained by Dr. Taylor will obviously be of great value in determining which samples are finally selected for DNA analysis.

The third right mandibular molar (97.R.75a) and the third left maxillary molar (97.R.16 (MCc)) have not been micro-sampled and deserve special consideration if it is determined that they are the skeletal elements that should be subjected to DNA analysis. First, all sources of information regarding the radiography that has been performed on the skeleton should be consulted to insure that these specimens have not been x-rayed. Second, if it is determined that these molars should be subjected to destructive analysis, a sampling strategy should be devised that ensures the preservation of histological, trace element, and chronological information present in the microstructure of the tooth.

Summary

During our visit to the Burke Museum we conducted macroscopic and microscopic examinations of the Kennewick skeleton to determine the suitability

of specific skeletal elements for DNA analysis. As part of this work we consulted with other specialists working on the skeleton. Basing work on this research, we developed a selection criteria that were used to create a ranked list of skeletal elements that should be considered for DNA analysis (Table 1).

Our observations confirm the conclusion of Powell and Rose (1999). The preponderance of the evidence indicates that these are the remains of a single individual who was interred at the site instead of being left to decompose on the surface of the ground, or incorporated into the deposit through some catastrophic hydrologic event. This conclusion is consistent with the completeness of the skeleton and the absence of any clear indications of carnivore activity. Our taphonomic analysis clearly shows that the skeleton had been exposed on the riverbank for a relatively short period of time prior to discovery.

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DOI 09774

Table 1: Skeletal Elements Recommended for Use in DNA Analysis

DNA Sample Rank	Specimen Number	Skeletal Element	Micro-sample
1.	97.R.75a	3rd right mandibular molar	not micro-sampled due to diagnostic value.
2.	97.R.50a	3rd left maxillary molar	not micro-sampled due to diagnostic value.
3.	97.L.16(MCa)	3rd left metacarpal	Sample #1, piece from the distal end.
4.	97.I.12d(13)	Right 8th rib	Sample #2: vertebral end of rib fragment; Sample #3: sternal end of rib fragment
5.	97.U.4(C2.a)	2nd cervical vertebrae	not micro-sampled due to diagnostic importance.
6.	97.R.16(MCa)	3rd right metacarpal	Sample #4: proximal end piece; Sample #5: distal end piece.
7.	97.R.16(MCc)	2nd right metacarpal	not micro-sampled due to other micro-samples already taken of neighboring bone.
8.	97.L.16(MCb)	2nd left metacarpal	Sample #7, piece from distal end.
9.	97A.I.25c	2nd right metatarsal	mid-shaft metatarsal, Sample #6, piece from mid-shaft.
10.	97.L.20b	Left tibia	Sample #8, piece from proximal end adjacent to area from which one of the 1999 C14 samples was taken.

Figure 1: Examples of pathological conditions present in the Kennewick skeleton **Upper left:** Reactive area in the olecranon fossa of the left humerus; **Upper right**, Close-up view of reactive bone in the area of the septal aperture of the olecranon fossa of the left humerus; **Lower left**, Area of the head of the left radius showing evidence of localized trauma; **Lower Right**, A 6mm in diameter, well-healed depressed fracture to outer table of the left frontal bone. The photograph is of a high-resolution epoxy cast of the lesion (the black bar is 2mm long).

DOI 09776