Geochemical Studies on Archived Studies from the Kennewick Skeleton: Research Plan for 2005 Laboratory Analyses

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On December 14 and 15, 2004, I examined the Kennewick Man skeleton and associated samples**. One of the purposes of my examination was** to determine the **most appropriate** methods for making geochemical measurements on the skeleton and samples archived from previous radiocarbon and DNA analyses studies. The fossil bones are hard and will readily tolerate physical examination and measurement. Bone fragments and powders remaining from earlier (1999, 2000) government studies were more abundant, **of** larger mass and represented more different bones than **I had** expected. **They** are well suited for initial geochemical analyses. Using archived materials will decrease the amount of time and sample quantity needed for geochemical tests **and will reduce the amount of** intact bone **needed for test purposes**.

I examined all archived samples and selected a subset of those **that appear** best suited for the first geochemical work. These samples are listed in Tables 1 and 2. I am requesting that these bone fragments and bone powders be used for the chemical analyses listed below. I will attempt to save subsets of the archives for future comparisons and analyses.

Six geochemical tests will be performed on **the** archived samples**, although some of the Table 1 samples are too small for multiple analyses**. Nominally twenty to fifty milligrams of bone will be used for destructive analyses **in some cases**; replicate analyses **may be needed depending upon the** results **obtained from the initial tests**. The analyses and the general data obtained from each test are the following:

Proposed Geochemical Tests on Archived Samples

1) **CHN and Quantitative Amino Acid Analyses — e**stimate the amount of collagen present, its distribution within and among bones and the collagen's degree of chemical preservation.

2) **^{14}C Composition of Organic & Inorganic Phases** — AMS ^{14}C measurements on collagen and apatite will be indicators of the variability of $^{14}C/^{12}C$ ratios --- not ages --- in these organic and inorganic phases. The ^{14}C content varies depending upon bone diagenesis and contamination with foreign carbon. These data will be compiled to determine the best methods needed for redating the skeleton and **for** establishing why there is a **2600 year difference among previous radiocarbon dating analyses.**

3) **Elemental Analyses of Surface and Interior Elements**— electron microprobe analyses of external surfaces will determine how Fe and Mn, among other elements, are present as staining on **those** surfaces. Microprobe scans on polished cross sections will use organic nitrogen distributions to map collagen preservation within bones. Stable C, N, and O values will also be measured during the ion microprobe scans.

4) **SEM images and electron microprobe analyses of crystals filling bone & sediment voids** — the XYZ position and mineralogy of secondary crystal growths will help reconstruct vertical orientation of the skeleton **while it was in the ground** and quantify diagenetic changes that were caused by percolating waters and biological agents.

5) **High resolution (< 50 µm) CT scans of bones** — these data are applicable to porosity differences in sediment infillings, location of secondary crystal growths, and especially the distribution of collagen within a single bone. Because the technique maps the density and distribution of collagen, the method is a non-destructive **way to obtain proxy data concerning** DNA content and will **help** detect if suitable regions remain where DNA could be extracted.

6) **Petrographic thin sections ---** Standard petrographic thin sections examined under plain and polarized light, by cathode luminescence will give information on bone protein and bone mineral diagenesis, and secondary carbonate mineralization and foreign-crystal growth. Thin sections positioned along three planes at right angles will be combined with CT data and test these X-ray scans' reliability.

There are two reasons for the geochemical tests outlined above: 1) previous analyses were either incomplete or the results varied significantly between labs and 2) examining the skeleton during December 14 to 15, 2004 suggested new taphonomic and geochemical data that could be obtained from the archived samples.

Prior analyses **of the skeleton** that are either incomplete or lacking precision include:

1) Quantitative amino acid analyses — only 16 of 18 possible amino acids were measured; data for secondary (imino) amino acids, proline and hydroxyproline were not obtained and therefore exact collagen preservation **was** not determine**d**.

2) Radiocarbon measurements vary by 2660 RC years on five specimens from the same skeleton —an age variation that is forty-four times measurement precision. Whether the skeleton is 8410 RC years or older is not established.

3) Stable carbon isotope ($\partial^{13}C$) values range from –10.3‰ to –21.9‰, a 11.6‰ range that is fifty times measurement precision. Neither an accurate paleodiet estimate or ^{14}C correction can be made from these $\partial^{13}C$ values.

4) $\partial^{15}N$ values were not determined for distinguishing terrestrial and marine-based diets. $\partial^{18}O$ values would **provide information that could help in determining the** geographic origin for the human.

5) All skeletal elements were not examined **by** non-destructive and non-invasive instrumental techniques that would assess the large inter- and intra-bone variations in collagen content and DNA potential.

8) Iron and Mn oxides remain unanalyzed to determine their origin

New observations during **the** December 2004 **inspection visit** included the following, all of which can be quantified by analyses proposed in this summary**:**

1) The mineralogy and isotopic composition of calcium carbonate and carbonate-cemented sediments can be determined by using these archived bone fragments; no damage will occur to intact skeletal bone.

2) Sediments in the bone's medullary cavities contain vesicular structures and small (1 to 2 mm diameter) cavities filled with 10 to 50 μm long acicular crystals. Because both the vesicular structures and the secondary crystals occur in archived and intact skeletal bone, the origin and significance of sediment changes can be made without altering intact bone.

3) Collagen distribution is very heterogeneous and can be mapped by using non-destructive CT scans. These biogeochemical assessments will determine what bones are most suitable for ^{14}C and DNA analyses.

The geochemical studies on archived bones will provide: 1) more precise and accurate data about the distribution and preservation of collagen, and therefore DNA, in all bones, 2) reasons why ^{14}C and stable isotope data vary, 3) additional taphonomic tools for determining vertical orientation of the skeleton, 4) empirically-based assessments of the skeleton's actual potential for yielding valid genetic and paleodietary information**. Equally important, the amount of new intact bone that will be needed for future DNA and other tests** will be dramatically reduced by having first performed quantitative tests on bone already removed from the skeleton.