OXYGEN ISOTOPE ANALYSIS OF HUMAN BONE AND TOOTH

ENAMEL: IMPLICATIONS FOR FORENSIC INVESTIGATIONS

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Many times a day I realize how much my own life is built on the labors of my fellowmen, and how earnestly I must exert myself in order to give in return as much as I have received.

> *~ Albert Einstein*

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CHAPTER I

INTRODUCTION

Forensic anthropological investigations of human remains primarily focus on establishing the biological profile. The biological profile typically includes an assessment of age, ancestry, sex, stature, trauma, pathological conditions, and any potential individualizing characteristics present on the remains. Creating a biological profile can be challenging or even impossible in the case of highly fragmented or degraded remains, such as in cases of terrorism (e.g., September 11, 2001) or where remains have been exposed to environmental and taphonomic processes for an extended period of time (e.g., U.S. POW/MIAs from foreign conflicts). Even when remains are relatively complete and in good condition, the basic profile offered by the forensic anthropologist is sometimes not enough to ensure the successful identification of an unknown individual. Confounding the issue further is the fact that human beings have successfully developed transportation technologies that allow them a high degree of mobility, a situation complicating the identification of unknown remains.

No longer blocked by geographic barriers, modern populations have the choice of traveling to and relocating in nearly every conceivable corner of the earth with relative ease. Gone are the days when individuals are born and live out their days in one general location. Assuming that a set of discovered remains is of local origin is no longer an

assumption afforded those charged with the task of identification. It is precisely this situation that has led to increased difficulties in the identification of human remains.

Given the fact that mass transportation has liberated people of their geographic tethers, a demand exists for research and development of forensic techniques beyond the traditionally employed visual and morphological methods—methods that inherently hold assumptions and are limited in the information they provide. More sophisticated techniques providing additional evidence, particularly techniques that extract evidence of origin and recent movement, are toolkit essentials for the forensic anthropologist.

Such sophisticated techniques are found in the field of biomolecular research. Biology, geology, archaeology, chemistry, medicine, and numerous other fields of study and research are no strangers to molecular level research, nor is such research particularly novel. What is novel is the application of these sophisticated techniques in regards to modern cases of human identification. The past decade has witnessed an increase in the use of non-traditional methods such as DNA typing and trace element analysis. One trend has been the reliance on DNA typing, which is often thought of as the Holy Grail of criminal and forensic investigations. Unfortunately this is a myth perpetuated by Hollywood more than real life. DNA typing is often expensive, inapplicable, or useless (as is the case with highly degraded remains) and possessing a DNA sample is only as good as the database against which it is compared. Even the more than 6 million entries (June 2008) in the Combined DNA Index System (CODIS) maintained by the FBI are often not enough to provide identification in missing person cases. This problem results from the fact that the majority of the CODIS entries are those of very specific groups,

such as convicted criminals, military personnel, government employees, and certain health service professionals, not missing persons (Federal Bureau of Investigations 2008).

One potential source of assistance for forensic specialists is the largely untapped technique of elemental isotope analysis. Although isotopic analysis has become a cornerstone research tool for disciplines and sciences attempting to reconstruct the life-ways of ancient populations (Evans et al. 2000, Prowse et al. 2007, White et al. 1998, 2000), it has only recently been recognized as a potential and powerful part of research in the forensic sciences (Benson et al. 2006, Carter et al. 2005, Pye 2004). Practitioners of forensic anthropology have yet to completely embrace its growing research significance. The potential specificity of a multi-elemental, multi-isotopic analysis could theoretically yield a 1 in 1.47 billion specificity, a figure rivaling fingerprint analyses and low loci DNA matches (Meier-Augenstein and Liu 2004). Given such impressive figures, elemental isotope analysis should prove itself an integral tool in forensic anthropological research endeavors.

Research Purpose

This research will explore the utility of oxygen isotope chemistry as a means of providing information in regards to the geographic origin and residence of unidentified individuals. To facilitate this application, the bone and tooth enamel of six open forensic cases curated by the Forensic Anthropology Center, Texas State University-San Marcos will be analyzed for oxygen isotope composition and compared against the oxygen composition of corresponding tap water samples from their respective discovery locations. Given that research has demonstrated the oxygen in skeletal tissues is primarily incorporated from ingested water, the oxygen signatures of the skeletal tissues

are expected to match the oxygen signature of their respective discovery location's tap water (Longinelli 1984).

Research Assumptions

In this research, two assumptions are made: (1) the primary source of drinking water (including water used in food preparation) was secured from locally available municipal (city) or fresh water (e.g., well water) sources, and (2) the oxygen signature of the municipally available water is not significantly different than that of the local precipitation. Therefore, individuals consuming a fresh, non-municipal water source (e.g., well water) would reflect an oxygen signature similar to a municipal water source located at the discovery location. The second assumption is supported by Bowen et al.'s (2007) research demonstrating that municipally supplied tap water oxygen signatures across the continental United States are strongly correlated (R=0.74) with the oxygen signature of the corresponding local precipitation. In other words, municipal treatment processes do not significantly alter the isotopic signatures present in tap waters originally sourced by local precipitation. Therefore, fresh water sources such as well water will present oxygen signatures similar to those of local municipal water sources. This correlation accounts for any individuals within the sample set that may have consumed a fresh water source (well water) instead of municipally supplied water.

Research Hypothesis

Because the deposition locales of the forensic cases used in this research are documented, oxygen profiles resulting from the bone and tooth enamel of each case are expected to match the oxygen profiles of the drinking water from their respective

deposition locations (H_0). A match will suggest that the victim's birth and/or recent habitation is in the same general area as the deposition location. In the event the profiles are not in agreement (H_1), an inference will be made that the individual is not from the region of deposition, thus either supporting a local origin and/or residence or disproving it by way of exclusion. By demonstrating these matches, or mismatches, this research will provide support for the potential value and use of isotopic analysis in modern forensic investigations.

Research Contents

This research explores the potential forensic value of utilizing oxygen isotope analysis of human bone and tooth enamel as a means of placing constraints on the geographic origins and residential locales of unidentified individuals. However, to fully understand the concept of isotopic analysis, a basic understanding of the oxygen element and its manifestation within human skeletal tissue is necessary. An overview of the oxygen element and its relationship with skeletal tissue is provided in Chapters II and III. Chapter IV explores previous research that utilized oxygen isotope analysis of human skeletal tissue as a means for extracting information for inference of habitation, natal origin, and migration. Chapter V outlines the collection, preparation, and analysis of the bone and enamel samples utilized in this study. Chapter VI reports the results of the analyses, while Chapter VII discusses the inferences made from those results. Chapter VII also provides a discussion of the research assumptions, issues, and limitations. Finally, Chapter VIII concludes this research and offers suggestions for future research on this topic.

CHAPTER II

ELEMENTS AND ISOTOPES

Introduction

The earth and its atmosphere are composed of more than 90 known naturally occurring elements. An atom, the smallest unit of an element, is comprised of protons, neutrons, and electrons, together referred to as subatomic particles. The atom has essentially two parts, a nucleus and orbitals (pathways for the orbiting electrons). The protons and neutrons are housed within the atom's nucleus, while the electrons 'float' in orbit around the nucleus. The number of protons and electrons are always equal in an atom of any element, but the number of neutrons may vary. The number of protons in an atom is called its *atomic number*, e.g., the element carbon has an atomic number of 6 because it contains six protons in the nucleus. The sum of all three constituent components of an atom (protons, electrons, and neutrons) is its *atomic mass*, also often referred to as the *atomic weight*. For example, ^{12}C has 6 protons, 6 electrons and 6 neutrons, giving it an atomic mass of 12 (Emsley 2001, Hoefs 2004). Generally, electrons are not counted in the atomic mass since they contribute no significant amount of mass to the atom.

Of the more than 90 naturally occurring elements that make up the Earth and its atmosphere, approximately two-thirds are present in more than one form, called isotopes.

The number of isotopes each element presents varies. For example, oxygen has 17 known isotopes and more than 40 exist for the naturally occurring gas, xenon. Isotopes of an element contain the same number of protons but differ in their number of neutrons, i.e., isotopes differ in their atomic mass (weight). Isotopes are divided into two fundamental groups: stable and unstable. Stable isotopes retain their nuclear configuration (protons = neutrons) and do not decay (change their nuclear configuration) over time. In contrast, and because of their imbalanced nuclear configuration (protons ≠ neutrons), unstable isotopes will spontaneously decay into one or more decay products called daughter elements. The rate of decay of some unstable isotopes, such as ^{14}C, is known. Utilizing these known decay rates of unstable isotopes as a means of dating carbonaceous material has a long-standing history in many fields of research, particularly in the field of archaeology (Buikstra et al. 2004, Longinelli 1984, Prowse et al. 2007, Schwarcz et al. 1991, White et al. 1998). Similarly, stable isotopes, given that they do not change over time, have also been heavily utilized in research, most notably in paleoclimate research (Hoefs 2004).

Isotopic Variation

The earth's abundances and ratios of elemental isotopes were fixed when the Earth was formed and, globally, have not changed. This is untrue, however, of regional abundances of isotopes. Some isotopes, for example oxygen, are in a state of constant partitioning resulting in one isotope of oxygen existing more prevalently than another, a process termed fractionation (Hoefs 2004). Isotopic fractionation within living organisms results from both extraneous and intrinsic influences (Bryant and Froelich 1995, Hedges et al. 2005, Luz et al. 1984, Schoeller 1999). Extraneous influences that

can alter isotopic ratios include climatic and geographic conditions such as temperature, altitude, humidity, and continentality (distance from the sea) (Dansgaard 1964). In turn, and because organisms isotopically equilibrate with their environment, the tissues of the plants and animals living in a particular region will reflect the environmental oxygen ratio of that region.

Other non-climatic, human created, extraneous influences are also capable of obscuring isotopic signals. For example, when analyzing materials for carbon content it is imperative to know if the materials are pre- or post-1950s nuclear testing. The effects of which drastically altered the atmospheric carbon concentrations, and therefore, the concentrations incorporated into living organisms (Bowen and Wilkinson 2002, Dansgaard 1964, Gat 1996, Hoefs 2004, Yurtsever and Gat 1981).

Intrinsic physiological influences may also alter isotopic ratios in living tissues. It has been demonstrated that such factors as diet, metabolism, body size, and heat loss mechanisms may have significant fractionation effects in certain species (Bryant and Froelich 1995, Kohn 1996, Kohn et al. 1996). Thus, researchers must be aware of and take into account the dietary habits and physiological adaptations of the analyzed species.

Bone remodeling rates (discussed in Chapter III) also affect isotopic compositions due to the varying rates of bone turnover among the different skeletal elements and tissue types. Therefore, sampling the same elements and/or tissue types during an isotopic study is a sound research strategy and is implemented in this project (Martin et al. 1998, Pate 1994).

In humans researchers have shown that although metabolic fractionation of oxygen occurs as it travels from ingested water through the body water (e.g., blood), and

into skeletal tissues, a strong correlation between oxygen signatures of ingested water and skeletal tissue remains. Longinelli (1984) demonstrated that a linear relationship exists (R=0.98) between ingested water and body water. He further demonstrated a corresponding relationship (R=0.98) between body water and skeletal tissue, suggesting little change in the oxygen composition ingested via water and the oxygen composition eventually incorporated into bony tissues. Analyzing previously published data from Levinson et al. (1987), Luz and Kolodny (1985, 1989) indicate similarly strong relationships between ingested water, body water, and skeletal tissues (R=0.99 for bone and R=0.93 for teeth). Luz and Kolodny (1985, 1989) attribute the lower correlation (R=0.93 vs. R=0.98) between Levinson et al. (1987) and Longinelli (1984) to sample age difference. Longinelli's samples were from individuals living in the first half of this century, while Levinson's samples were derived from more recent individuals (1970s-1980s) whose drinking and dietary habits may have included water sources from imported beverages and foods. Luz and Kolodny (1985) and Levinson et al. (1987) have developed regression equations (Equation 1 and 2 respectively) for inferring the oxygen composition of the water ingested based on the oxygen composition of bone and enamel. The authors further suggested that an oxygen value from an isolated bone and/or tooth enamel may be used to infer the source of that individual's ingested water by working backwards through the metabolic fractionations of the human body.

In two recent studies Daux and colleagues (Daux et al. 2005, Daux et al. 2008) re-evaluated the regression equations of Luz and Kolodny (1985) and Levinson et al. (1987) utilizing more modern teeth (post 1980), while taking into account the use of tap water and modern consumption patterns. It must be noted here that the authors mentioned

above (Luz and Kolodny 1985 and Levinson et al. 1987) utilized naturally available

water (e.g., rain water) when formulating their regression equations, while Daux et al.

(2008) utilized tap water in their research. Daux et al. (2008) offer an updated regression

equation (Equation 3) they contend is more appropriate for modern skeletal samples

because of their use of tap water as a comparative sample (vs. natural sources such as

precipitation). Results indicate a strong correlation (R=0.87) between modern skeletal

tissue and tap water when utilizing the updated equation; the results of which equate to a

+1.05-1.20 per mil (‰) increase in the oxygen-18 (δ^{18}O) signature of skeletal tissue over

that of the ingested tap water.

$$\delta^{18}O_{bs} = 0.45(\delta^{8}O_{tw}) + 17.86 \qquad \text{(Eqn. 1: Luz and Kolodny 1985)}$$

$$\delta^{18}O_{es} = 0.46(\delta^{8}O_{tw}) + 19.4 \qquad \text{(Eqn. 2: Levinson et al. 1987)}$$

$$\delta^{18}O_{tw} = 1.54 \ (\pm0.09) \ (\delta^{18}O_{es, bs}) \ -33.72 \ (\pm1.51) \qquad \text{(Eqn. 3: Daux et al. 2008)}$$

(where: tw= tap water, bs=bone sample, es=enamel sample)

Another fractionation that may occur in human dental tissue is known as the

'nursing effect'. Previous research (White et al. 2004, Wright and Schwarcz 1998, 1999)

has indicated that because of metabolic fractionation in a nursing mother's body, breast

milk oxygen-18 is enriched by 0.7 parts per mil (‰). Given that the primary source of

water for breastfeeding infants is the mother's milk, the 0.7‰ level of enrichment is in

turn passed to the nursing infant. Therefore, this same level of enrichment is found in the

teeth mineralized during the breastfeeding period, e.g., the permanent incisors, canines,

and first molars. Research utilizing enamel formed prior to weaning should take into

consideration the possibility of altered isotope signatures due to the effects of nursing.

Measurement and Instrumentation

Isotopic analysis is performed utilizing an isotope ratio mass spectrometer (IRMS), a highly sensitive and specialized form of a mass spectrometer capable of delivering instrumental precision of <0.02‰ and a standard deviation of < ±0.01‰ (Hoefs 2004, Paul and Skrzypek 2006). As the name implies, a *mass* spectrometer is designed to measure the mass of the elements constituting a sample. Simplistically, mass spectrometry equipment performs three basic functions: (1) breakdown of the sample into its constituent elements, (2) separation of the elements by mass, and (3) collection and analysis of the proportions of each element. In the past, a sample intended for mass spectrometry analysis required conversion into a gaseous compound isotopically representative of the solid parent material prior to its manual introduction into the mass spectrometer. Mass spectrometry equipment at the time was equipped with only manual inlets; no automated introduction mechanisms yet existed (Brazier 1995). This meant that any sample not already in a gaseous state had to be manually converted and injected, thus adding time, expense, and the possibility of contamination. As an alternative to manual injection modern mass spectrometry equipment offers the option of interfacing with a variety of on-line gas preparation and/or combustion chambers to fully automate the solid to gas conversion and injection processes. Utilizing this type of technology eliminates external manipulation; thereby minimizing both expense and the potential for contamination (Meier-Augenstein and Liu 2004).

Although several methods of isotopic analysis exist, most fit into two general categories: (1) bulk analysis, and (2) compound specific analysis. Bulk analysis provides an averaged isotope signature for the sample as a whole, regardless of the isotopic

proportions housed within the constituent elemental compounds of the sample (see

Chapter III for a detailed description of the constituent compounds in bones and teeth).

In contrast, compound specific analysis separates the compounds within a complex

sample and delivers an isotopic signature for an individual compound (Carter et al. 2005,

Meier-Augenstein 2007). The bulk analysis method is utilized in this research.

Isotopes are generally measured as a ratio of the two most dominant forms of any

given element. For example, an oxygen isotope analysis would be reported as a ratio in

terms of the two most abundant oxygen isotopes, the heaver ^{18}O isotope over the lighter

^{16}O isotope (denoted as $^{18}O/^{16}O$). The difference in abundances of two isotopes is

typically quite small, often with the observable difference beginning in the third or fourth

decimal digit (Schoeller 1999). Mass spectrometry data are not generally reported in

their decimal form, but instead are converted to a simpler more manageable form through

the use of standardized equations. The result of an isotopic analysis is reported as a delta

value (δ) and expressed as a per mil (denoted as ‰) deviation relative to an

internationally recognized standard (Coplen 1996). In the case of oxygen, the delta value

is calculated using the equation:

$$\delta\ ^{18}O\ ‰\ =\ \left[\frac{[^{18}O/^{16}O]\ \text{sample}}{[^{18}O/^{16}O]\ \text{standard}} - 1 \right] 10^3 \qquad \text{(Eqn. 4)}$$

where $^{18}O/^{16}O$ $_{\text{sample}}$ is the isotopic ratio of the sample, and $^{18}O/^{16}O$ $_{\text{standard}}$ is the isotopic

ratio of the recognized standard (Paul et al. 2007). Resulting negative and positive delta

values (e.g., $\delta^{18}O$ -14.2‰ or $\delta^{18}O$ 3.8‰) indicate respectively depletion or enrichment of

the heavier ^{18}O isotope relative to the recognized standard (Brazier 1995, Coplen and

Kendall 2000, Mays 2000).

Standards and Best Practices

Various standards are utilized when reporting isotopic analyses results. Oxygen and hydrogen isotope ratios of liquids, for example, are reported relative to the Vienna Standard Mean Ocean Water 2 (VSMOW2) standard. The results of analyses of solid carbon materials, however, are generally compared against and reported relative to the Vienna Pee Dee Belemnite (VPDB) standard. For consistency, when mass spectrometry results are in both VPDB and VSMOW2 due to the analysis of different materials, conversion to one or the other standard using generally accepted equations (Equations 5 and 6) is necessary to make equivalent comparisons (Coplen 1983).

$$\delta^{18}O_{VSMOW2} = 1.03092(\delta^{18}O_{VPDB}) + 30.92 \qquad \text{(Eqn. 5: Coplen et al. 1993)}$$

$$\delta^{18}OV_{PDB} = 0.97002(\delta^{18}O_{VSMOW2}) - 29.98 \qquad \text{(Eqn. 6: Coplen et al. 1993)}$$

The VSMOW2 and VPDB standards are preferred over other existing standards because their use implies that the measurements have been calibrated according to the International Atomic Energy Agency (IAEA) guidelines for expression of delta values (Coplen 1996, Hoefs 2004).

Best practices for isotopic analysis involve not only comparison of results with datasets, maps, and calculation tools such as those publicly available from organizations and researchers (International Atomic Energy Agency (IAEA), Online Isotopes in Precipitation Calculator (OIPC), U.S. Geologic Survey (USGS)) (Appendix A and B), but also with a local sample of known origin. For example, if analyzing the oxygen composition of a human tooth discovered in a particular location, best practices suggest comparison with a human tooth documented to be from the same location. If no human

tooth of known origin is available, a tooth from local fauna may be substituted if species-specific fractionation effects are considered (Kohn 1996, Kohn et al. 1996, Pye 2004). Additionally, comparison of the oxygen composition of the human tooth with a water source from that discovery location is also an acceptable research design (given that the oxygen concentration in human tooth enamel is a function of ingested water, discussed below). However, the research design and sample selection depends on the research question under investigation and the element of focus, therefore the choice of a comparative sample will vary accordingly (Pye 2004).

Oxygen

Oxygen is a non-metallic element that exists in abundance on Earth as a colorless, odorless, two-atom gas (O_2). There are three naturally occurring, non-radioactive (stable) principal isotopes of oxygen existing in varying abundances: oxygen-16 (99.763%, ^{16}O), oxygen-17 (0.0375%, ^{17}O), and oxygen-18 (0.1995%, ^{18}O) (Hoefs 2004). Oxygen is the third most abundant element in the known Universe, and makes up 47% of the Earth's crust, 60% of the human body, 21% of the atmosphere (by volume), and 90% of the water we drink (Emsley 2001). Because the oxygen concentration in the in meteoric precipitation is geographically and climatically dependent, concentrations of this gas in water sources vary across geographic locations (Bowen and Wilkinson 2002, Dansgaard 1964, Gat 1996, Yurtsever and Gat 1981).

Oxygen in Skeletal Tissue

The oxygen isotope analysis of human bone and tooth enamel is based on the following underlying principles: (1) the strong linear relationship (R= 0.93–0.99) shown

to exist between the oxygen composition of skeletal tissues and the oxygen composition of drinking water, (2) the oxygen composition of drinking water, in turn, reflects its original source—local precipitation, and (3) the oxygen composition of precipitation is controlled by geographic and climatic factors such as temperature, altitude, and distance from the sea (Levinson et al. 1987, Longinelli 1984, Luz et al. 1984, Luz and Kolodny 1985, 1989).

In other words, as water evaporates from a body of water (e.g., the ocean), condenses to form clouds, and moves inland to varying locations, precipitation occurs and local water sources are imbued with the isotopic composition of the precipitation as determined by the geographic location. Generalizing, the closer to the body of water the more enriched the local precipitation is with the heavier ^{18}O isotope, given that it precipitates from condensed water preferentially over the lighter ^{16}O due to its heavier mass. As the evaporated moisture/condensation in the form of clouds moves farther inland, less and less ^{18}O is available, and therefore less is precipitated. In other words, the rainwater further inland has less ^{18}O compared to the lighter ^{16}O and is considered *depleted* of ^{18}O. Different regions, therefore, have distinct oxygen isotope signatures. Once deposited in the local water sources, the precipitated water—and its isotopic signature–is then ingested and incorporated into the body tissues, thereby creating a unique geo-location tracer within the organism (Hoefs 2004).

The oxygen composition of human bone and tooth enamel is a function of the oxygen sources consumed. For humans, incorporation of oxygen into body tissues is primarily (~60-70%) through drinking water (Bryant and Froelich 1995, Hedges et al. 2005, Podlesak et al. 2007, Sponheimer and Lee-Thorp 1999). As a result of this

incorporation process, the oxygen composition in the bones and teeth of an organism, as previously stated, correlate linearly to the oxygen composition of the organism's environmental water, and by extension, ingested water.

Several factors can affect the oxygen composition of human bone and teeth. Metabolically induced fractionation, individual consumption patterns, and diagenesis are three such factors. Researchers have found that although physiological adaptations, drinking habits, diet, and body size can significantly alter the linearity between body tissue and drinking water in the bone and teeth of some species, for humans, intra-population variation is low (1‰) and the linear relationship between body tissue and drinking water remains (Bryant and Froelich 1995, Kohn 1996, Kohn et al. 1996, Longinelli 1984). Additionally, it has been found that the inorganic mineral component of bone and teeth, the focus of this research, is resistant to post-depositional processes and preserves its original isotopic signature, particularly in remains not in prolonged burial contexts (Hedges et al. 2005, Koch et al. 1997, Kohn et al. 1999, Pate 1994, Sponheimer and Lee-Thorp 1999).

Given that the oxygen composition in the mineral component of bone and tooth enamel has been demonstrated to be linearly correlated with the oxygen composition of ingested water and is diagenetically resistant (discussed in Chapter III), bone and teeth make for highly suitable proxy materials for isotopic research in regards to natal origin and residential locale.

To fully understand the concept of oxygen isotope analysis of human bone and tooth enamel, a basic understanding of the element's presence within the composition of human skeletal tissue is necessary. Therefore, an overview of the relationship between

oxygen and human bone and tooth enamel and a detailed exploration of the composition,

structure, and function of bone is provided in the following chapter. Also provided is a

justification for the selection of tissues used in this study.

CHAPTER III

HUMAN BONE AND TOOTH ENAMEL

Introduction

The exceptional durability of bone and teeth makes these tissues the most often, and sometimes only, surviving substances of the human body subsequent to death. Their consistent presence in the paleoanthropological, archaeological, and forensic records makes them one of the most analyzed materials in anthropological research (Hedges 2005). Although the constituent components of bone and tooth enamel are similar, they vary dramatically in proportion, formation, structure, and reaction to post-deposition alteration (diagenesis). Given that isotopic analysis typically targets individual molecular compounds, a basic comprehension of the composition differences between bone and teeth is essential in the molecular level type research presented in this project. For this reason, a brief review of the structure, biochemistry, and potential diagenetic changes of human bone and tooth enamel is given.

Bone Structure and Composition

The average adult human skeleton is comprised of 206 bones, or elements, of four general forms: (1) long bones—characterized by a hollow, tubular shaft (diaphysis) with flaring, closed ends (epiphysis), found in the upper and lower extremities, and whose

main functions are weight bearing, locomotion, and manipulation, (2) flat bones–
characterized by thin, tabular shaped bones, found in the cranial vault, shoulder, rib
cage, and pelvis, and whose primary functions are protection and provision of large areas
for muscle attachment, (3) irregular bones—such as vertebrae, which exhibit a variety of
complex shapes and sizes depending on their location and function, and (4) short bones—
such as carpals and tarsals, are roughly cuboidal and like irregular bones exhibit a variety
of sizes, shapes, and functions (Bass 1995, White and Folkens 2000).

Based on porosity, two types of bone (osseous) tissue exist within adult human
skeletal elements: (1) *compact tissue*—also referred to as *cortical* tissue depending on if
the tissue is in the form of a thick or a thin layer—is a dense, tightly packed tissue of 5%-
10% porosity, and (2) *cancellous tissue*—also called *spongy* tissue, is a porous,
lightweight, honeycomb structured tissue, with porosity of 75%-95% (Martin et al. 1998).
Compact or cortical tissue forms the outer walls of the long and short bone diaphyses and
the external surfaces of the flat and irregular elements, varies in thickness depending on
the skeletal element, and comprises the bulk of the osseous tissue in the human skeleton.
Trabecular tissue makes up the portion of bone between the cortical layers of the flat and
irregular bones, the inner shafts of long bones, and is the major tissue constituting long
bone epiphyses (White and Folkens 2000). Some skeletal elements, such as the femur
and the tibia, have a high proportion of compact tissue, while others, such as vertebrae
and ribs, have higher proportions of cancellous tissue (Martin et al. 1998). In this
research the terms *compact* and *cancellous* will be used when referring to these two
osseous tissue types.

Although a wide array of shapes and sizes exist in the human skeleton, the basic structure of osseous tissue is that of a highly organized structural matrix of protein with mineral crystals interspersed within the gaps of the matrix, a composition producing a tough, structural integrity in human bone. Within this basic structure human bone is composed of two principal components: organic and inorganic (Martin et al. 1998).

The organic component makes up ~30% of dry bone by weight and is primarily comprised of collagen (Martin et al. 1998). Collagen—a structural protein found ubiquitously throughout vertebrates—is the most abundant protein found in the human body and exists in several forms. Type I collagen, the primary form of collagen found in human bone, is organized into strong, flexible fibers with alternating parallel and perpendicular orientation to the bone surface (Martin et al. 1998). This alternating organization provides the matrix for deposition of the mineral component of the inorganic portion of bone (Martin et al. 1998).

The inorganic component makes up ~70% of dry bone by weight and is comprised principally of a composite of calcium phosphate minerals closely resembling the naturally occurring mineral, hydroxyapatite ($Ca_{10}(PO_4)_6(OH)_2$) (Martin et al. 1998). Biological hydroxyapatite, or bioapatite, differs from the geological hydroxyapatite in its lack of stoichiometry (i.e., ratio of atoms remain the same before and after a chemical reaction), small crystal size, high rate of substitution, and structural disorganization (Budd et al. 2001, Martin et al. 1998). The hydroxyapatite in bone is considered impure due to its poor crystalline structure that allows for elemental substitutions, substitutions supplied by ingested food and water or resulting from diagenetic processes. In humans, the primary substitutions occur in the phosphate (PO_4) and hydroxyl (OH) sites of the

hydroxyapatite molecule, mainly substituted with carbonate CO_3 (although other substitutions such as fluoride and chloride can also occur) (Hedges et al. 2005, Kohn et al. 1999). The carbonate substitution renders a hydroxyapatite formula with a CO_3 molecule in place of either or both of the PO_4 or OH molecules (Shemesh 1990). Therefore, human bone contains three sites where oxygen, in varying content, may be found: 1) the phosphate site (PO_4), containing 35% oxygen, 2) the hydroxyl site (OH), containing 1.6% oxygen, and 3) the carbonate site (CO_3), containing 3.3% oxygen (Martin et al. 1998).

Although both the phosphate and carbonate sites within the mineral component of bone house oxygen available for analysis, a large proportion of studies have primarily utilized the phosphate site for several reasons. First is the high proportion of analytical oxygen available in the phosphate site (35%) as compared to the amount available in the carbonate site (3.3%) (Martin et al. 1998). Second, the P-O bond of the PO_4 molecule is stronger than the C-O bond of the CO_3 molecule, suggesting the phosphate site is more diagenetically resistant (Sponheimer and Lee-Thorp 1999). Third is the strong correlation (R=0.93–0.99) demonstrated to exist between the phosphate oxygen and the oxygen composition of ingested water (Longinelli 1984, Luz and Kolodny 1985, 1989). Taken together, these characteristics render the phosphate site the most likely to yield useful oxygen composition data, and therefore is a common analyte choice for oxygen isotope research (Budd et al. 2001, Hedges et al. 2005, Koch et al. 1997).

The carbonate site, although containing less oxygen, is sometimes the chosen analyte for two reasons. First, the strong correlation (R=0.98) demonstrated to exist between the carbonate oxygen and phosphate oxygen indicates that, after conversion

(Equation 7), the carbonate site reflects the same isotopic signature as the phosphate site, and therefore, is equally as informative in isotopic studies (Bryant et al. 1996, Iacumin et al. 1996). Second is the ease of oxygen extraction from the carbonate molecule compared to that of the more complex, lengthy process required for oxygen extraction from the phosphate molecule (Iacumin et al. 1996, Sponheimer and Lee-Thorp 1999).

$$\delta^{18}O \text{ (phosphate)} = 0.998* \delta^{18}O \text{ (carbonate)} -8.5 \qquad \text{(Eqn. 7: Iacumin et al. 1996)}$$

The structural integrity and high mineral content of bone render it one of the longest surviving and, therefore, most abundant tissues available for analysis following the death of a vertebrate organism.

Tooth Structure and Composition

Although typically not as abundant, human tooth enamel—the hardest substance is the human body–is also a tissue often chosen for isotopic analysis. Humans develop two sets of dentition throughout their lifetime. One set, the deciduous, or baby teeth, begin mineralizing (forming) *in utero* and erupt from early infancy though early childhood. Deciduous teeth start erupting at approximately 6 months of age and typically by the age of 2 years eruption is complete, resulting in a full set of 20 teeth. Beginning around the age of 6 years the deciduous teeth begin to be displaced by the emerging permanent teeth (Hillson 1996). The 20 deciduous teeth are progressively lost throughout the early childhood and pre-teenage years when they are replaced by a full set of permanent teeth (Anderson et al. 1976, Scheuer and Black 2004).

The second set of human dentition, the permanent teeth, mineralize from birth through the early teenage years, and erupt from early childhood through the late teenage years (see Table 1). Beginning around the age of 6 years the first permanent teeth

emerge and continue to emerge in a particular order until all 32 permanent teeth are fully erupted. The first 28 permanent teeth erupt at predictable intervals, with completion around the age of 12. The remaining four teeth, the third molars (wisdom teeth), exhibit highly variable eruption and, in some cases, never erupt. The typical eruption period for the third molars is approximately 18-21 years of age (Anderson et al. 1976, Hillson 1996, Scheuer and Black 2004).

Both deciduous and permanent teeth have three types of hard tissue: (1) enamel–the accreted layers of material covering the crown, (2) dentine—the bulk of the inner tooth, and (3) cementum—the outer layer of the roots (Scheuer and Black 2004). As with bone, these components contain varying organic and inorganic proportions. Tooth enamel, the focus of this study, is composed of ~1% organic protein (amelogenin), ~2% water, and ~97% inorganic minerals (hydroxyapatite) (Hillson 1996). The organic portion of tooth enamel is principally (90%) composed of the protein amelogenin functioning similarly to the collagen in bone, providing a structural matrix for the deposition of inorganic minerals (Hillson 1996). The inorganic mineral portion of tooth enamel functions similarly to bone, but is composed of larger crystals and a greater structural organization, a structure allowing fewer ionic substitutions than is found in bone. These properties, along with the high mineral content, afford enamel the characteristic of being the most durable and elementally stable substance in the human body.

Table 1: Permanent Dentition, Mineralization and Eruption

Tooth	Crown Mineralization Initiation[a]	Crown Mineralization Completion[a]	Eruption[b]
Central Incisor	0.25-0.3	4-5	6-7
Lateral Incisor	0.8-1	4-5	7-8
Canine	0.3-0.4	6-7	9-10
1st Premolar	1.5-2	5-6	10-12
2nd Premolar	2-2.5	6-7	11-12
1st Molar	0	2.5-3	6-7
2nd Molar	2.5-3	7-8	11-13
3rd Molar	7-10	12-16	17-21

[a] Hillson (1996) from Schour & Massler (1940), range in years.
[b] Scheuer and Black 2004, range in years.

Bone and Tooth Enamel Remodeling

Even though human bone and tooth enamel are highly durable materials, the human body continually replaces damaged, fatigued osseous tissue with new, structurally sound tissue, a process known as remodeling or *turnover*. Bones remodel by dissolution and replacement of bone structural units (BSU). Through a complex osteo cell-signaling system, weakened BSUs—resulting from injury, disease, or age—are destroyed by osteoclastic activity with subsequent osteoblastic deposition of new BSUs. A BSU's average lifetime ranges from 3 to 20 years (Pate 1994). The mean annual turnover rate for adult compact and cancellous tissue is 5% and 25% respectively, but is variable depending on the individual and the particular bony element in question (Martin et al. 1998). The turnover rate of compact bone (5%) is a function of damaged cell replacement and maintenance of structural integrity. The faster turnover rate of cancellous tissue (25%) is related to the support of mineral homeostasis—a sort of mineral reservoir for the body—a necessary condition for the maintenance of certain physiological functions in humans (Ortner 2003). Cancellous tissue, therefore,

continually and quickly turns over constituent elemental components while compact tissue does so more slowly.

In contrast to bone, tooth enamel is a dense, static tissue, and once formed undergoes no post-mineralization remodeling or element exchange with its environment (Hillson 1996). Therefore, enamel maintains its original elemental signature encoded during mineralization. The lack of remodeling and the miniature archival quality of teeth make them one of the most often chosen bio-materials for elemental isotopic analysis (Lee-Thorp 2002).

Diagenesis and Forensic Material

Although an elementally durable material that tends to resist external influences, the elemental analysis of bone and teeth has historically been riddled with difficulties, difficulties brought about by the effects of diagenesis. Anthropologically, diagenesis is defined as the chemical alteration of biological material, from both infiltration and leaching within the depositional context, that occurs from the time of death to discovery in the archaeological or forensic record (Darwent and Lyman 2002, Lyman 1994).

Although no skeletal tissue is immune to digenetic processes, the dense, highly mineralized structure of tooth enamel affords it a measure of protection. Bone, on the other hand, is a more porous tissue, allowing for greater potential of diagenetic exchange. According to Sorg and Haglund (2002), the initial biochemical response of bone to its depositional environment is similar to that of bone in a fresh, living state; a response afforded it by the protection of retained moisture and organic components. As the length of deposition increases and the organic components within and surrounding the skeletal tissue decompose, the chemical composition of the osseous tissue may change. The type

and extent of this change is largely dependent on the surrounding environmental conditions and whether the deposition is in a buried or surface context (Rodriguez 1997). Within the deposition environment such extrinsic factors as moisture, temperature, soil pH, and the presence of microorganisms are primary factors affecting the presence and rate of diagenetic alteration of bone and teeth (Gill-King 1997).

Potential diagenetic effects on bony tissue in regards to modern forensic material are often disregarded due to the depositional context and/or youth of the material being analyzed (Hedges et al. 2005). Although it is true that much of the material forensically analyzed is generally less than 60 to 70 years of age, these materials, as with archaeological materials, are subject to diagenetic processes. Skeletal remains deposited as the result of a crime are discovered in a wide variety of depositional contexts, many containing the same varying microenvironments found within archaeological sites (Hochrein 2002). It is important to keep in mind that diagenetic processes are not uniform and, therefore, no concrete timeline can be developed that delineates when osseous tissue becomes diagenetically susceptible (Haglund and Sorg 1997). Disregarding the potential of postmortem alteration based on temporal criteria, even for modern samples, is highly cautioned against (Hedges et al. 2005).

Hedges (2002) indicates a number of intrinsic factors of bone such as size, shape, condition, and age that may also contribute to its chemical integrity. For example, the highly porous, aged bones often found in the elderly may succumb to diagenetic processes more quickly than would healthy, dense tissue found in younger adults. Similarly, the incompletely mineralized bone of infants and children are also vulnerable

to diagenetic alteration and are highly susceptible to not only rapid chemical exchange, but to external mechanical damage as well.

A variety of techniques exist to assess diagenetic alteration including (1) consideration of the deposition context, (2) visual assessment for signs of color, weight, porosity, and structure changes, and (3) spectral techniques such as X-ray diffraction, infrared spectroscopy, and Fourier transform infrared spectroscopy (FTIR) (Hedges 2002, Sandford and Weaver 2000, Shemesh 1990). When postmortem alteration is suspected, various sample preparation treatments are utilized to remove diagenetically absorbed contaminants. For bone and tooth enamel, contaminant removal is typically performed via an acetic acid wash. (Garvie-Lok et al. 2004, Pate 1994). Once the acid wash is complete, the element signature remaining more accurately reflects the *in vivo* isotope condition.

Human bone and tooth enamel have been utilized extensively for research in a variety of academic disciplines. Reviewed in the following chapter are studies utilizing isotope analyses of human bone and tooth enamel to explore research questions, clarify historical events, and challenge long standing misconceptions regarding past peoples. In addition to formal research, a modern forensic homicide case in which isotopic analysis was utilized as an investigative tool is discussed.

PREVIOUS RESEARCH AND MODERN FORENSIC APPLICATION

Introduction

The use of oxygen isotope analysis for the acquisition of information to assist in the reconstruction of ancient climates, migration patterns, diet, habitation, and origins of both humans and animals is by no means a novel idea. Since their discovery by Thomson and Aston in 1913, and the subsequent identification of most existing stable isotopes in the 1930s, the use of isotopes in research has proven invaluable throughout a range of disciplines (Katzenberg 2000). Previously a research cornerstone in medicine, chemistry, and geology, the increased number of studies employing isotopic analysis from an array of disciplines indicates its growing significance as a routine research tool, particularly in the field of anthropology (Katzenberg and Harrison 1997, Sandford and Weaver 2000). Although the most frequently utilized elements in isotopic investigations have traditionally been carbon and nitrogen, oxygen is steadily becoming an analyte choice (Mays 2000, Pye 2004). Explored below are studies utilizing oxygen isotope analysis of human bone and tooth enamel for inference of habitation, natal origin, and migration.

Origin and Migration

Conventional methods for tracing the origins and migration of past peoples primarily rely on identifying the artifactual similarities of material culture. Sites

(including burials) with similar artifacts, but located some distance apart, are often linked via the causal explanation of migration or trade networks in existence between the sites (Evans et al. 2006). Unfortunately the impermanent nature of moveable objects renders concrete associations nearly impossible without corroborating evidence (e.g., hieroglyphic texts and iconography). The employment of isotopic studies in regards to the study of past peoples is beginning to delineate the hazy boundary between actual migration and what was simply trade or casual transport of material objects (Evans et al. 2006, Lee-Thorp 2002). Although isotopic studies investigating migration and origins have typically utilized strontium (e.g. Ezzo et al. 1997, Ezzo and Price 2002, Price et al. 1994, 2002, Sealy et al. 1991), researchers are beginning to use oxygen analysis as an additional means of elucidating the movement of past peoples (Lee-Thorp 2002).

The underlying principle of oxygen isotope analysis of human bone and teeth, as stated previously (Chapter II), is the strong linear relationship between the oxygen composition of body tissue formed at various stages of life, and that of the water ingested during the formation of that tissue. Therefore, it is reasonable to assume that geographically distinct isotopic signatures identifiable in human tissues are suggestive of the geolocations of an organism at different developmental stages.

Several early studies utilizing oxygen isotopes for geolocation determination were conducted by Keenleyside et al. (1997), Schwarcz et al. (1991), and White et al. (1998). Keenleyside and colleagues (1997) used oxygen and lead isotope analyses to investigate and determine the geographic origin of nearly 400 human skeletal elements and bone fragments. These represented a minimum of 11 individuals thought to be from, but inconclusively linked to, the ill-fated European crewed, America bound Franklin

Expedition of 1845-1848 (Melbye and Fairgrieve 1994). Results of the isotope analyses

reflected the birthplace and most recent habitation locale of two of the individuals

represented by the bones and fragments as Western Europe and not the Northern Arctic.

This, therefore, suggested the remains were potentially those of the Franklin Expedition

crew. Although not conclusive evidence of exact origin, the multi-elemental isotopic

evidence found in this study sufficiently allayed suspicions that the remains were of local

Inuit origin. To date, 3 of the represented 11 individuals have been positively identified

utilizing a multi-disciplinary approach combining both traditional and isotopic

techniques.

Schwarcz and associates (1991) conducted oxygen isotope analyses on human

skeletal remains from the War of 1812 interred in the Snake Hill military cemetery,

Ontario, Canada. This was done in an attempt to gain insight into the place of origin of 6

of the buried soldiers. Results of the study demonstrated a fairly uniform oxygen

signature, suggesting the interred individuals were from the same general region. Based

on isotopic values from local archaeological and modern reference samples, it was found

that the region, however, was not Ontario. Results instead indicated a lower latitude

region, most likely the Northeastern United States. This supported the authors' original

intent to demonstrate that at least some of the military interments at the Canadian

cemetery were in fact US soldiers.

Similarly, White and colleagues (1998) conducted oxygen isotope analyses on

archaeological samples from Tlailotlacan, Mexico, a culturally homogenous enclave on

the western edge of Teotihucan in the Valley of Mexico. The authors theorized that

immigrants from the southern Valley of Oaxaca originally populated Tlailotlacan. Using

Monte Alban (Valley of Oaxaca) and the Teotihucan peripheral site of Tlajinga (Valley of Mexico) as comparative baseline isotope ratios, the authors showed the isotopic signatures of the Tlailotlacan enclave more closely resembled the Oaxacan Valley ratios than those from the Valley of Mexico.   These finding supported the authors' contention of an immigrant enclave in the Valley of Mexico.

In a 2000 study, White and colleagues once more focused their attention on the Valley of Mexico, this time to determine the extent of Teotihuacan (Valley of Mexico) influence over a neighboring region, Kaminaljuyu, Guatemala. Again utilizing oxygen isotopes, White and associates (2000) analyzed enamel from the remains of 31 suspected "foreigners" (based on archaeological evidence) in positions of power—be it a ruler or an elite citizen—in the ancient Mayan city of Kaminaljuyu in an attempt to infer natal origins of the 31 individuals. Results demonstrated only a minor biochemical link (3 of 31 samples) to the Valley of Mexico, challenging previously held beliefs of a larger Teotihuacan presence in Kaminalijuyu. Instead, results indicated support for a more recent view (Braswell 2004) that local Kaminalijuyu elite appropriated Teotihuacan materials and symbols to elevate their personal status within the community. Authors White et al. caution, however, against broad interpretation of results based solely on tooth enamel since enamel is indicative of early childhood location only and provides no evidence of later life residence or mobility. The concluding results of this study simply suggest that the remains of what were believed to be foreign individuals were in fact born locally in the Kaminalijuyu area.

In perhaps one of the better-known studies employing isotopic techniques, Hoogewerff et al. (2001) and Muller et al. (2003) utilized data from strontium, carbon,

and oxygen isotope analyses to suggest the natal origins and adult residential locale in relation to the discovery site of the 5300 year old mummified remains of the famed Alpine iceman, "Otzi." Results based on the analyses performed on bone and enamel samples from Otzi's rib, femur, canine and first premolar indicate Otzi most likely spent the majority of his adult life at higher altitudes of the Alpine region, while his early years were spent at lower altitudes. This evidence, the authors suggest, may assist in resolving the still unanswered question of who really was this Late Neolithic individual that died an obviously unexpected death in the inhospitable environment of the high Alps.

Long held beliefs supported by tangible archaeological evidence have not escaped challenge when confronted with the results of isotopic analysis. Based on several lines of archaeological evidence, anthropologists have generally accepted that the Mayan king, K'inich Yax K'uk Mo (ruled A.D. 426-437), founder and first ruler of the Early Classic dynasty of Copan in the central Peten region (c. A.D 250-600), was originally from a region northwest of Copan, most likely the powerful political center at Teotihuacan in the Valley of Mexico (Sharer and Traxler 2005). Additionally, hieroglyphic texts deciphered at the Copan Acropolis also indicate that the Mayan lord originated from outside the Copan region. Utilizing both strontium and oxygen isotopes, Buikstra and colleagues (2004) investigated the natal origins of the Mayan king in relationship to his burial location at Copan. Results from the analyses indicated that the remains archaeologically identified as those of K'inich Yax K'uk Mo were more characteristic of Copan in the Central Peten than of the Valley of Mexico. These results effectively challenged the prevailing theory that the Mayan king was originally from the prestigious political site of the northern located Teotihuacan.

In another study originating from the Valley of Mexico, Spence and associates (2004) attempted to isotopically discern the origins of human maxillae trophy pendants worn by sacrificed soldiers who were excavated from a mass grave at the Feathered Serpent Pyramid (*c*. A.D. 200), Teotihuacan. Results of the oxygen analysis revealed two interesting outcomes. First, the victims represented by the trophy maxillae were from both nearby, perhaps even Teotihuacan, and distant regions, suggesting the soldiers may have killed local residents as well as foreigners. Second, the archaeological evidence reporting Teotihuacan's military interactions with other Mesoamerican regions does not appear until a century after the construction of the Feathered Serpent Pyramid. Evidence from the isotopically analyzed trophy maxillae excavated from the Pyramid suggests otherwise by demonstrating that interaction with more distant regions must have occurred prior to the time indicated by archaeological evidence. As in the Copan study discussed above, the utilization and results of isotopic analysis on the maxillae trophy pendants of Teotihuacan again challenged the archaeologically based acceptance of long standing theories concerning the timing of its military interactions with foreign regions.

Bentley and colleagues (2005) uniquely utilized isotopic analysis to support the genetic and linguistic evidence indicating the presence of matrilocality during the agricultural transition in the village of Ban Chiang, Thailand. Additionally, they utilized isotope data to lend clarity to the contradictory genetic results suggesting that Thai male ancestors originated elsewhere, while females shared a local maternal ancestor. The results of carbon, strontium, and oxygen isotope analyses of the remains of 44 individuals demonstrated support for the authors' contention that males during this period emigrated to Ban Chiang, while the females were of local origin. These data supported their theory

that the females of Ban Chiang shared a long, local maternal ancestry and were practitioners of matrilocality.

In two recent studies, Evans and associates (2006) demonstrated the utility of isotopic analysis as evidence for the inference of migratory behavior in Southern England. In the first of these studies, Evans and fellow researchers performed oxygen and strontium analysis on a set of 16 burials from a Late Roman cemetery in Winchester to test their hypothesis that the exotically interred individuals were immigrants from the distant Danube region in Central Europe. Although the isotopic results showed a varied set of values for the burials, none were consistent with Southern England, indicating support for the authors' contention that the burials were indeed of non-indigenous origin. The authors further suggested that such diverse results for the exotic interments provided support for an additional hypothesis that the individuals did not relocate as one large community group, but instead included small groups, families, and single individuals.

In a second study, Evans and Chenery (2006) utilized isotopic analysis to discern the origins of an individual interred in a particularly rich fashion for the early Bronze Age, the "Amesbury Archer," discovered three miles Southeast of Stonehenge. Resulting isotopic values from the Archer's teeth revealed a childhood location other than Amesbury or even England, but instead most likely a cooler locale in central Europe. The authors further contended that the lavishly buried Archer, dating to approximately 2300 BC, was of particular anthropological interest in regards to the chronological relationship with the large stone building phase of Stonehenge, raising question as to the Archer's involvement and potential 'foreign' influence on the construction of Stonehenge. In the two studies discussed above Evans and colleagues successfully

demonstrated the ability of isotopic analysis to cast doubt on the belief that migrations were most often a community or group affair.

Similarly, Prowse and colleagues (2007) attempted to demonstrate that migration to the Italian island of Isla Sacra was not the predominantly single, adult male activity as indicated by historical records. The authors theorize instead that movement of people to the island was potentially a family affair as well. The authors conducted oxygen isotope analysis of first and third molars of 61 individuals (both male and female) interred in the necropolis of Isla Sacra (1st-3rd century AD). Isotopic results indicated that approximately 23% of the individuals analyzed relocated during the time period between crown completion of the first and third molars (ca. 2-3 and ca. 10-16 years of age respectively). These results supported not only the authors' contention that migratory behavior was not limited to adult males but also lent credibility to the migration hypotheses tested by Evans and associates in the above mentioned studies conducted in Southern England.

Modern Forensic Application

Application of isotopic analysis to modern human material is a relatively recent development in regards to forensic investigations. Few studies conducted have been specifically concerned with forensic applications and utilization of isotopic signatures in modern remains, and fewer still have been conducted using the oxygen element for inference of origin and habitation of an unidentified individual (Pye 2004).

One researcher utilizing oxygen isotope analysis for the specific purpose of investigating natal origins is Henry P. Schwarcz of the Department of Anthropology, McMaster University, Ontario, Canada. Following is the detailed account of a recent

homicide case for which Schwarcz conducted oxygen analyses on bone and tooth enamel in an attempt to gain data regarding the origin and residence of the murdered individual (Schwarcz 2007).

Mammoth Lakes Homicide Case

In late May 2003 a hiker discovered skeletonized human remains along a trail in the Mammoth Lakes region of Central California. The remains were estimated to be those of a 30-40 year old female who stood approximately 4'6" at the time of death, with an estimated postmortem interval of 9 months. After the failure of several conventional identification techniques (e.g., DNA typing) investigators pursued alternative methods, inviting Schwarcz and his expertise in isotopic analysis into the case.

Schwarcz's analysis resulted in a determination that the victim had imbibed water as a child from a location other than where the remains were discovered. In fact, the oxygen signature in the tooth enamel could have come only from an area much farther south. Because other evidence connected the Mammoth Lakes victim with the southern Mexican village of San Mateo in the Oaxacan region, Schwarcz also conducted an oxygen analysis of a drinking water sample from that area. The drinking water analysis resulted in a match to the oxygen value found in the tooth enamel, supporting a San Mateo origin. Continuing, Schwarcz performed an oxygen analysis of a rib fragment— information that would indicate residential locale for approximately the last eight to ten years. The result of the rib analysis matched neither the tooth oxygen value (San Mateo) nor the oxygen signature of the Mammoth Lakes region (established through the analysis of a baby tooth from a lifelong resident). Knowing that the bone sample would reflect a fairly recent geolocation, and taking into account the enamel and San Mateo water

oxygen values, as well as other case evidence, Schwarcz concluded that the victim was most likely born and lived the majority of her life in the Oaxacan region. Additionally, because no oxygen signature of the Mammoth Lakes region registered in the victim's teeth or bones, Schwarcz was confident the victim's arrival to Central California was recent, most likely within the last two years (Dotsie 2007, Page 2007, Schwarcz 2007). Armed with this evidence investigators focused their efforts in the Southern Mexico region of Oaxaca and were eventually able to tentatively identify the remains as Barbara Pacheco Santiago; an identification currently awaiting DNA confirmation.

Previous Research Conclusion

Although this review illustrates a variety of archaeological applications for which isotope analysis has been utilized, it demonstrates only a fraction of the research conducted over the past several decades. Consequently, a wealth of comparative information exists for those attempting to reconstruct the lives of past peoples. To date, however, the use of isotope analysis in a modern forensic context has yet to be fully realized or extensively applied.

CHAPTER V

MATERIALS AND METHODS

Introduction

In an effort to test the efficiency of oxygen isotope analysis of modern forensic cases, fourteen unsolved forensic cases curated at the Forensic Anthropology Center at Texas State University-San Marcos were examined for potential inclusion in this study. Only adult remains (estimated age ≥18) with the required elements (see Justification for Tissues Used in This Study below) were selected for inclusion in this research. To minimize the possibility of erroneous isotope readings, cases exhibiting obvious pathology were not considered for this study. Three analyses (hereafter referred to as a 'set') were performed on each selected case. A set contained one sample each of cancellous tissue, compact tissue, and dental enamel. The cancellous and compact tissue samples were taken from the shaft of the femur and the dental sample from an intact maxillary molar (see Justification for Tissues Used in This Study below). To establish a local oxygen signature for the known discovery locations (counties) of the cases, a fourth analysis was performed utilizing a sample of municipally available drinking water collected from a city within each county (Chapter II) (Bowen 2007).

In an effort to minimize potential postmortem contaminants, all skeletal elements were visually assessed and any remains exhibiting signs of postmortem diagenetic

alteration were precluded from this study. Diagenetic alteration was assessed based on a

visual assessment for differential coloring, weight, porosity, structural abnormalities, and

weathering. The six cases chosen (see Table 2) for inclusion presented a uniform

appearance and exhibited no signs of structural damage or advanced weathering, e.g.,

cracking and/or flaking of the outer surfaces. These cases represent unidentified

individuals from four South Texas counties.

As previously discussed in Chapter III, a variety of methods exist for assessing

and accounting for postmortem alteration. The standard protocol most commonly

utilized is the acetic acid wash (Hedges 2002).   Although no diagenetic alteration was in

evidence, it is standard procedure to perform a contamination removal treatment as a

precaution even when no contamination is suspected. Therefore, an acetic acid

contamination removal treatment was utilized on a portion of the samples in this study.

Two sub-samples were created from each primary bone and enamel sample, heretofore

referred to as sub-sample A and B. Sub-sample A was treated with the acetic acid soak

described below, while sub-sample B was left untreated (see Table 3). As a side analysis,

isotope values from both sub-sample sets were compared to suggest the necessity of

diagenetic contaminant removal treatments for forensic material (see Chapter III).

Justification for Tissues Used in This Study

Although several tissues (bone, teeth, dry tissue, and hair) are often recovered

with a set of remains, the selection of tissue for elemental isotopic analysis is a function

of the research objectives. In this study, the primary objectives are the exclusionary

inference of: (1) most recent habitation locale, (2) long term habitation locale, and (3)

early childhood location based on a match/mismatch with the isotopic signature of the

local drinking water for each of the individuals analyzed. These objectives demand the analysis of tissues that provide supporting evidence. Therefore, the tissues selected for analysis in this study are: (1) compact and cancellous tissue from the proximal one-third of a femoral diaphysis and, (2) enamel from a first permanent maxillary molar.

The decision to utilize a tissue sample from the femur is based on the thickness of its proximal shaft. This portion of the element houses some of the thickest compact tissue (along with the tibia) in the human body, and therefore, provides the longest elemental life history for the individual. The femur, with its thick outer compact tissue layer and slow turnover rate discussed previously, can provide a life history extending to ~20 years prior to death (Hill and Orth 1998). The cancellous tissue of the femur is also highly useful as a residential indicator. The quick turnover rate of this tissue makes it an ideal choice for rendering evidence of recent habitation, on the order of $\leq$ 8-10 years (Simmons et al.1991). Taken in concert, the outer compact and inner cancellous tissue of a large, thick element such as the femur holds the potential to yield evidence for objectives #1 and #2, recent and long-term geo-habitation.

The choice of the first permanent maxillary molar (#3 or #14) for this study is derived from the fact that the chronological mineralization of individual teeth, although somewhat variable, is predictable and well known (Hillson 1996). Therefore, the selection of a tooth for analysis in this study is based on the objective of obtaining information to infer early childhood residence. This objective demands the analysis of one of the earliest forming adult teeth. The permanent first molars, incisors, and canines begin mineralization *in utero* and during early infancy, with crown (enamel) completion at 2-3, 4-5, and 6-7 years of age respectively (see Table 1), thus providing a static

elemental archive of early development (recalling that teeth do not recycle elements once formed) (Hillson 1996, Scheuer and Black 2004). Due the nature of the single root system of the incisors and canines, these teeth are more easily dislodged and lost post-mortem. In contrast, the triple root system of the first permanent maxillary molar affords it more postmortem security, and is therefore more often found with remains (Anderson et al. 1976, Hillson 1996). The early mineralization and postmortem survivability render the first permanent maxillary molar the best choice for yielding evidence for study objective #3.

Collection Procedures

One bone and tooth enamel sample was extracted from each of the six selected cases. Instruments utilized for sample collection include: 1) one electric Dremel® 200 Series high-speed rotary tool, 2) Dremel® reinforced cutting wheels, product number 426, and 3) stainless steel dental instruments (see Figure 1). To minimize cross contamination, all equipment was externally cleaned with a dilute bleach solution, the working area brushed clear, latex gloves changed, and an unused cutting wheel mounted prior to each extraction (Westen et al. 2008). Post extraction, each sample was immediately labeled with a Pigma® Micron® permanent archival quality pen and placed in a ventilated plastic envelope marked with the corresponding sample and case data (see Figure 2). No cleansing or processing occurred prior to packaging. The author personally collected all samples.

Table 2: Case Summary and Sample Data

Sample ID	Case Number	Case Location[a]	Case Year	Femur Sample	Tooth #3	Tooth #14	Water Sample
A	06-3784	Webb County, Texas	2006	X		X	X
B	0A0760	Cameron County, Texas	2007	X		X	X
C	DMG-9319	Starr Country, Texas	1993	X		X	X
D	06-5125	Webb County, Texas	2006	X	X		X
E	DMG-9821	Zapata County, Texas	1998	X	X		X
F	F2008-100	Webb County, Texas	2008	X	X		X

a   See Appendix C for Texas county map.

Table 3: Sub-sample A (white, acetic acid) and Sub-sample B (gray, no acetic acid)

Sub-sample Number	Sample Type	Bone or Tooth Sampled	County Case Number	Case Location (County)	Water Sample Location w/i County
A1 A	tooth enamel	Molar #14			-
A2 A	compact tissue	L. Femur	06-3784	Webb	-
A3A	cancellous tissue	L. Femur			-
A4 A	water	n/a			Laredo
A1 B	tooth enamel	Molar #14			-
A2 B	compact tissue	L. Femur	06-3784	Webb	-
A3 B	cancellous tissue	L. Femur			-
B1 A	tooth enamel	Molar #14			-
B2 A	compact tissue	R. Femur	0A0760	Cameron	-
B3 A	cancellous tissue	R. Femur			-
B4 A	water	n/a			Harlingen
B1 B	tooth enamel	Molar #14			-
B2 B	compact tissue	R. Femur	0A0760	Cameron	-
B3 B	cancellous tissue	R. Femur			-
C1 A	tooth enamel	Molar #14			-
C2 A	compact tissue	R. Femur	DMG-9319	Starr	-
C3 A	cancellous tissue	R. Femur			-
C4 A	water	n/a			Roma
C1 B	tooth enamel	Molar #14			-
C2 B	compact tissue	R. Femur	DMG-9319	Starr	-
C3 B	cancellous tissue	R. Femur			-
D1 A	tooth enamel	Molar #3			-
D2 A	compact tissue	L. Femur	06-5125	Webb	-
D3 A	cancellous tissue	L. Femur			-
D4 A	water	n/a			Laredo
D1 B	tooth enamel	Molar #3	06-5125		-
D2 B	compact tissue	L. Femur		Webb	-
D3 B	cancellous tissue	L. Femur			-
E1 A	tooth enamel	Molar #3			-
E2 A	compact tissue	L. Femur	DMG-9821	Zapata	-
E3 A	cancellous tissue	L. Femur			-
E4 A	water	n/a			Zapata City
E1 B	tooth enamel	Molar #3			-
E2 B	compact tissue	L. Femur	DMG-9821	Zapata	-
E3 B	cancellous tissue	L. Femur			-
F1 A	tooth enamel	Molar #3			-
F2 A	compact tissue	L. Femur	F2008-100	Webb	-
F3 A	cancellous tissue	L. Femur			-
F4 A	water	n/a			Laredo
F1 B	tooth enamel	Molar #3			-
F2 B	compact tissue	L. Femur	F2008-100	Webb	-
F3 B	cancellous tissue	L. Femur			-

Bone Sample Collection

One 2 centimeter (cm), semi-lunar shaped bone sample was removed from the proximal one-third of the femoral diaphysis from each of the aforementioned cases. Due to the incomplete nature of the remains of the six cases, both right and left femora are represented in the samples. Each femur was measured to locate the proximal one-third, and distally from that point a 2 cm semi-lunar section was measured, marked, and extracted. The semi-lunar section was further divided in two, one portion for compact tissue analysis and one portion for cancellous tissue analysis (see Figures 3a-3d).

Tooth Enamel Sample Collection

One first permanent molar (#3 or #14) was extracted from the maxilla of each case. Only teeth exhibiting no evidence of modification, fracture, restorative procedures, or obvious pathology were selected for inclusion in this research. To minimize damage to both the molar and the alveolar process, the surrounding alveolar tissue was worked away from the lingual surface of the selected tooth with dental instruments prior to extraction (see Figure 3e). Once the roots were partially exposed, a hooked dental pick was inserted between the root projections and the tooth was carefully worked from the maxilla.

Reference Water Sample Collection

As stated previously, the sample against which the bone and tooth enamel samples were compared was the local drinking water collected from the known discovery location of each case. The author visited each case locale and a water sample was obtained from an unfiltered municipally supplied water source (e.g., restaurants and

hotels). Unused 250ml Fisher® clear glass bottles with polyvinyl-lined caps (#02-911-732) were utilized for water collection (see Figure 4). To minimize contamination, the cold water tap was run for ~30 seconds after which each bottle rinsed three times with the water source prior to sample collection. Bottles were filled completely, leaving no air space between the water sample and the cap. After sampling, the bottles were immediately capped and labeled with relative location and case data. Samples were placed in dark, room temperature (~24°C) storage until the analyses were performed (~4 weeks) (following Bowen et al. 2007).

Bone and Enamel Sample Preparation

Bone and enamel sample preparation was performed at the Center for Archaeological Studies, The University of Texas-San Antonio following a protocol adapted from Garvie-Lok et al. (2004). Sub-sample A bone specimens were exteriorly cleaned of adhering matrix by mechanical abrasion using a Dremel® rotary tool fitted with a brush attachment. After exterior cleaning, specimens were ultrasonically cleaned with double distilled water (ddH$_2$0) for 60 minutes as many times as necessary to remove any remaining surface contaminants and dried at 50 C° for 2 hours. Utilizing ultrasonically cleaned drill bits and a Dremel® rotary tool, 100-250 milligram (mg) of bone powder was removed from each bone specimen. Following removal, the bone powder samples were placed in 20 ml test tubes with ddH$_2$0, centrifuged, supernatant removed, and dried at 50 C° until the ddH$_2$0 was evaporated. Once water was evaporated, 10 milliliter (ml) of 2% sodium hypochlorite (NaOCL) was added to the test tubes, agitated, and allowed to stand for 12 hours. After 12 hours, the supernatant was decanted and the process repeated every 12 hours for 48 hours. The bone powder

samples were then repeatedly rinsed with ddH_20 until neutrality (pH 7) was achieved and then dried at 50Cº until all water was evaporated. 0.1 molar (M) acetic acid at a rate of 0.04 ml per mg of sample was added to each test tube and left to soak for 4 hours and then centrifuged to neutrality with ddH_20. When neutrality was achieved, the bone powder samples were completely dried at 50 Cº, weighed, and recorded.

With the exception of the amount of sample collected (20 mg), sub-sample A enamel specimens were processed in the same manner as the sub-sample A bone specimens detailed above. It should be noted that enamel was removed from either the cusps of the tooth or the highest point available if the cusps exhibited wear.

Sub-sample B bone and enamel specimens were processed as outlined above with the exclusion of the acetic acid soak.

Water Sample Preparation

No preparation is necessary for water samples prior to mass spectrometry analysis.

Mass Spectrometry: Bone and Enamel

The mass spectroscopy analysis for the bone and enamel samples was conducted in the Laboratory for Stable Isotope Geochemistry, Department of Earth and Environmental Science, The University of Texas-San Antonio. Oxygen isotope analysis for these samples was performed using a Thermo Finnigan® Delta[Plus] XP isotope ratio mass spectrometer (IRMS) in continuous flow mode (see Figure 5), coupled to a Thermo Finnigan® GasBench II automated on-line gas preparation and introduction system (see Figure 6).

Bone and enamel powder was prepared for IRMS analysis following the protocol reported by Paul and Skrzypek (2007) as adapted from Spotl and Vennemann (2003). The bone and enamel powder samples (~200-400 µg each, µg=microgram=1/1,000,000 of a gram) were weighted into helium flushed (to remove air from the vials) 10 ml butyl rubber sealed glass vials where they were reacted with 100% ortho-phosphoric acid at 70ºC for 30 minutes to produce carbon dioxide gas (CO_2). The resulting CO_2 gas was automatically collected (via the GasBench II) and injected into the IRMS. Once introduced into the IRMS, samples were analyzed and referenced against CO_2 gasses derived from one internal (CO_2 tank at 99.995%) and three external (NBS 19, NBS 18, and LSVEC) standards. For quality assurance during each analysis, two replicates each of the external standards and two replicates of the internal standard were simultaneously run with each batch of unknown samples. Precision for the bone and enamel sample suite was $<\pm 0.05$ ‰.

Mass Spectrometry: Water

The mass spectroscopy analysis for the water samples was conducted at Coastal Science Laboratories, Austin, Texas. Oxygen isotope analysis for these samples was performed using one of two laboratory isotope ratio mass spectrometers (IRMS), a VG Micromass 602D or a VG Micromass SIRA Series II.

Oxygen isotope analysis of the water samples was prepared for IRMS analysis using the carbon dioxide (CO^2) equilibration method of Epstein and Mayeda (1953) as described by Yoshida and Yoshihiko (1986). Six ml of sample water was drawn into a common 20 ml syringe (the equilibration vessel) and then fitted with a 25 mm 22 gauge needle. Air bubbles were removed as the water volume was reduced to 5 ml. Seven ml of

99.9% CO_2 was then introduced into the syringe through a rubber septum attached to a mercury manometer. The CO_2 pressure in the syringe was adjusted to 1 atmosphere, removed from the manometer, and the needle sealed with a 000 rubber stopper. The syringe was then attached to a rack in a 25Cº water bath and agitated for 2 hours. After 2 hours the syringe was removed from the water bath and the equilibrated CO^2 gas manually introduced into an evacuated injection port on the IRMS. Once introduced into the IRMS, samples were analyzed and referenced against gasses derived from two carbonate (CO_2 tank at 99.9% and NBS 19) and one external (Austin Meteoric Water) standard. For quality assurance during each analysis, one standard was measured after every ten samples. Additionally, as a precision check ($\pm$ 0.2 ‰), replicate analyses were performed on 10% of the sample suite.

The sample δ values were calculated using Equation 1 (Chapter II) and reported in ‰ relative to the VSMOW2 standard for oxygen and VPDB standard for carbonates as established by the IAEA (Coplen 1996).

Data Adjustments and Comparisons

As discussed previously, researchers have estimated adjustments necessary to account for metabolically induced oxygen fractionation that occurs from the time the oxygen enters the body to the time it is incorporated into the bone and enamel tissues. One such fractionation correction, the nursing effect, as reported by White et al. (2004) and Wright and Schwarcz (1998, 1999) was applied to the enamel samples in the current study. In concert with the nursing effect adjustment, the regression equation developed by Daux et al. (2008) was also applied. Summarizing, the following data comparisons will be analyzed and discussed:

Bone

1. A comparison between the bone $\delta^{18}O$ and the corresponding local water $\delta^{18}O$ after application of regression equation (3) for estimating the likely drinking water $\delta^{18}O$ from skeletal tissue (Daux et al. 2008).

Tooth Enamel

1. A comparison between the enamel $\delta^{18}O$ and the corresponding local water $\delta^{18}O$ after application of regression equation (3) for estimating the likely drinking water $\delta^{18}O$ from skeletal tissue (Daux et al. 2008).

2. A comparison between the enamel $\delta^{18}O$ and the corresponding local water $\delta^{18}O$ after application of #1 above *and* of the nursing effect adjustment of -0.7‰ (White et al. 2004 and Wright and Schwarcz 1998, 1999).

As mentioned previously, an acetic acid treatment is commonly applied to bone and enamel to remove diagenetic contaminants prior to mass spectrometry analysis. To assess the necessity for such treatments in regards to forensic materials, an acetic acid treatment was applied to a portion of the samples in this study. The results of the acetic acid analyses along with the bone and enamel comparisons mentioned above are detailed in the following chapter.

Figure 1: Sample Extraction
Instruments. Photo by author.

Figure 2: Samples Post Extraction.
Photo by author.

Figure 3a-d: Sample Extraction Process: a) locate proximal one-third, b) mark proximal one-third, c) measure 2 cm from location found in step a, d) extract 2 cm sample. **Figure 3e**: Dentition Extraction. Photos by author.

Figure 4: Water Sample Collection Materials. Photos by author.
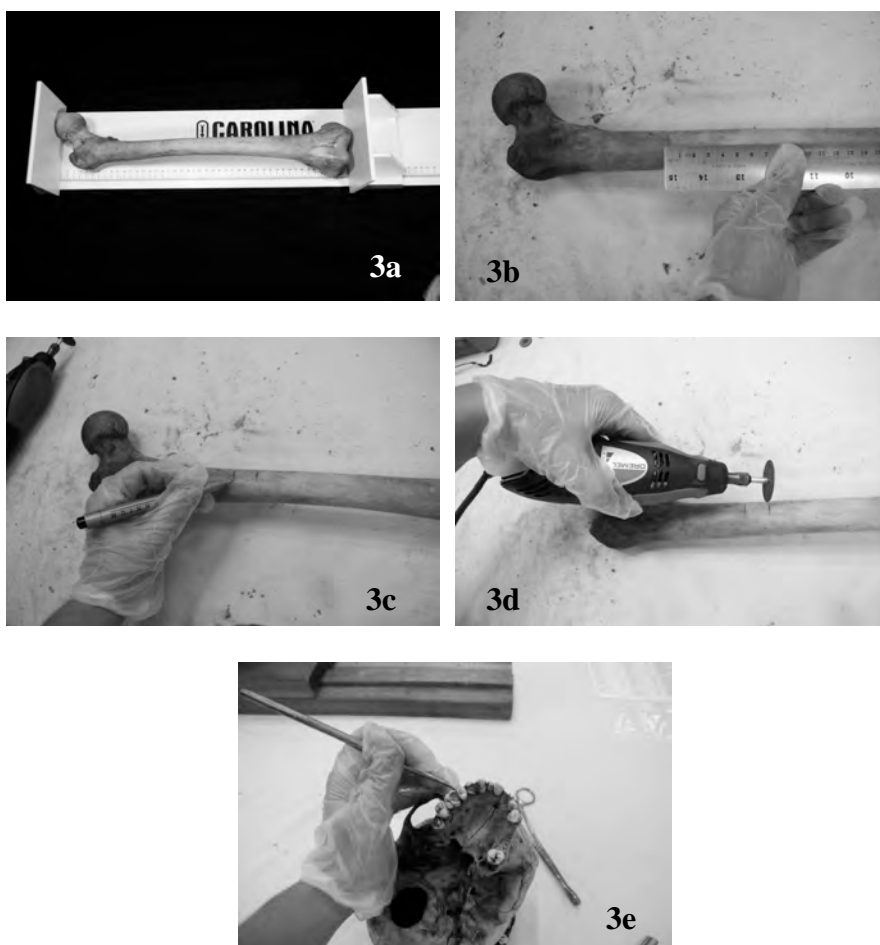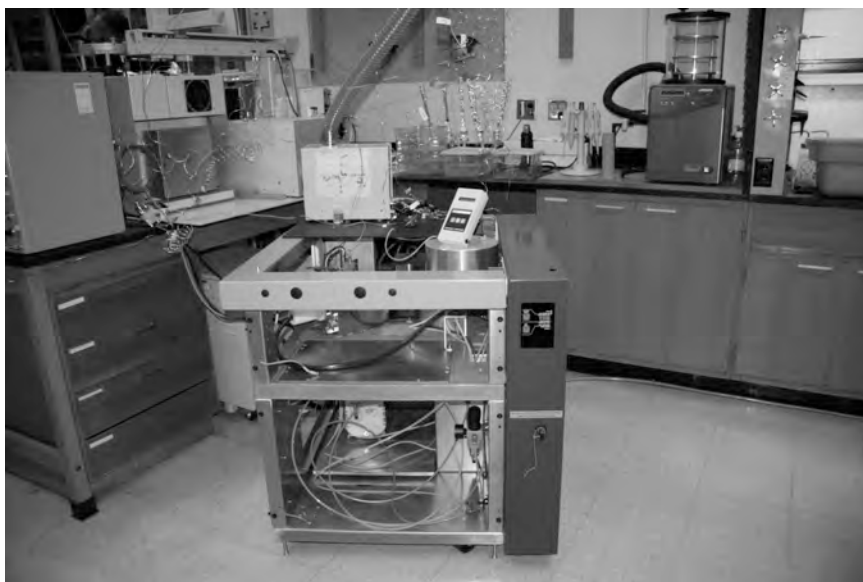
Figure 5: Thermo Finnigan DeltaPlus XP® Stable Isotope Ratio Mass Spectrometer. Photo by author.
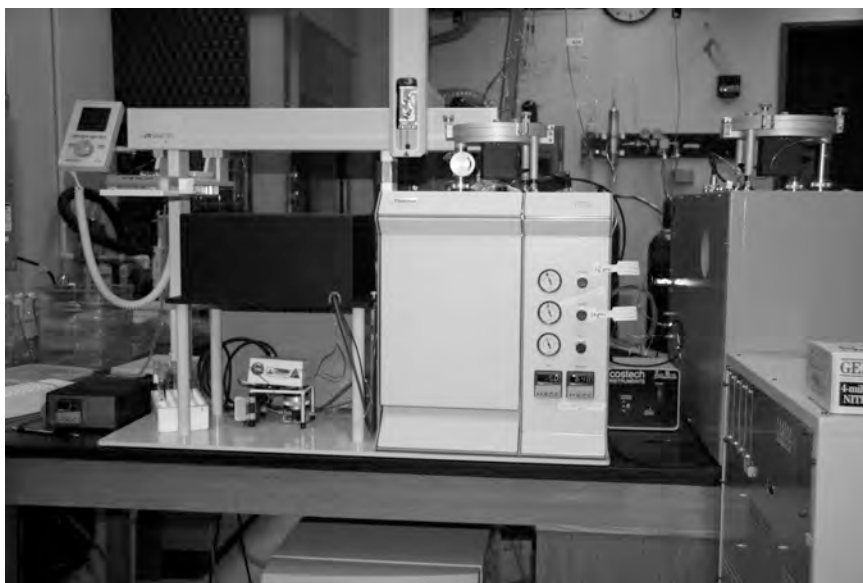
Figure 6: Thermo Finnigan® GasBench II Automated Gas Preparation System. Photo by author.

CHAPTER VI

RESULTS

Introduction

The results of oxygen isotope analyses of 18 human bone and tooth enamel and 4 corresponding tap water samples are shown in Table 4. Because it is standard protocol to treat bone and enamel with acetic acid to remove possible post-depositional contaminants, this study followed that protocol and utilized sub-sample A, the acetic acid treated samples, as the primary sample set. Only the results of sub-sample A are reported below. For completeness, however, the detailed calculations and results of sub-sample B, the samples not treated with acetic acid, are included in Appendix D. In addition to Table 4, the results of the oxygen isotope analysis for each case are summarized below. All values are in parts per million.

For clarification, in this study the term 'isotope region' does not correlate to 'geographic region'. An isotope region is any geographic region with the same isotope profile and may encompass multiple locations worldwide. Similar $\delta^{18}O$ isotope values as those found in the skeletal tissues of the below cases have been reported to exist elsewhere within the continental United State and worldwide. See Appendix A and B for maps and resources containing documented worldwide $\delta^{18}O$ isotope values.

Case 06-3784, Webb County

Three distinct $\delta^{18}O$ values are present for case 06-3784. The $\delta^{18}O$ values for the compact (-11.04) and cancellous (-9.31) tissues are clearly out of agreement with the corresponding tap water $\delta^{18}O$ value (-4.7). While the expected $\delta^{18}O$ value for the tooth enamel (-5.06) is also in disagreement with the corresponding tap water (-4.7), it is comparable with the low end of the enamel range at two standard deviations (-5.39 to -4.73).

Case 0A0760, Cameron County

The compact tissue sample for case 0A0760 was contaminated during processing and no data was available for analysis. The $\delta^{18}O$ values for the tooth enamel and cancellous tissues are isotopically similar to one another (-7.79 and -7.17 respectively), but clearly disagree with the corresponding tap water $\delta^{18}O$ value (-1.9). At two standard deviations, the enamel (-7.25 to -7.09) and cancellous (-7.80 to -7.78) tissue results remain in disagreement with the corresponding tap water.

Case DMG-9319, Starr County

The $\delta^{18}O$ values for the enamel (-6.75), compact (-7.39), and the cancellous (-9.19) tissues disagree with the corresponding tap water $\delta^{18}O$ value (-3.2). At two standard deviations, the enamel (-6.89 to -6.62), compact (-7.44 to -7.33), and cancellous (-9.34 to -9.04) tissue results remain in disagreement with the corresponding tap water. Although in disagreement with the local tap water, the enamel and compact tissue $\delta^{18}O$ values are isotopically similar to one another, differing by 0.64.

Case 06-5121, Webb County

The $\delta^{18}O$ values for the enamel (-11.50), compact (-12.27), and the cancellous (-14.04) tissues are clearly out of agreement with the corresponding tap water $\delta^{18}O$ value (-4.7). At two standard deviations, the enamel (-11.92 to -11.08), compact (-12.78 to -11.76), and cancellous (-14.76 to -13.32) tissue results remain in disagreement with the corresponding tap water. Although in disagreement with the local tap water, the enamel and compact tissue $\delta^{18}O$ values are isotopically similar to one another (0.77 difference) with overlapping ranges at two standard deviations.

Case DMG-9821, Zapata County

The $\delta^{18}O$ values for the tooth enamel and compact tissues are isotopically similar to one another (-6.17 and -6.37 respectively), but are in disagreement with the corresponding tap water $\delta^{18}O$ value (-4.7). The cancellous tissue value range (-5.72 to -5.14), although not in absolute agreement, is within 0.44 of the corresponding tap water $\delta^{18}O$ value (-4.7). At two standard deviations, the enamel (-6.37 to -5.97), compact (-6.55 to -6.19), and cancellous (-5.72 to -5.14) tissue results remain in disagreement with the corresponding tap water.

Case F2008-100, Webb County

Three distinct $\delta^{18}O$ values are present for case F2008-100. The $\delta^{18}O$ values for the enamel (-10.73), compact (-12.70), cancellous (-14.07) tissues are clearly out of agreement with the corresponding tap water $\delta^{18}O$ value (-4.7). At two standard deviations, the enamel (-11.06 to -10.40), compact (-13.26 to -12.14), and cancellous (-14.79 to -13.35) tissue results remain in disagreement with the corresponding tap water.

Minimal overlap (0.09) exists only between the compact and cancellous ranges at two

standard deviations

     A detailed discussion of the results for each case outlined above is given in the

following chapter. Also discussed are the possible consequences of the nursing effect,

acetic acid side analysis, and research assumptions, issues and limitations.

Table 4: Oxygen Isotope Analysis Results and Adjustments[a]

Sample	Case Number	County	Type	Carbonate δ¹⁸O‰[PDB]	Carbonate δ¹⁸O‰[b]	Phosphate δ¹⁸O‰[c]	Calculated Ingested Water[d]	Calculated Ingested Water Range[d] δ¹⁸O‰	2sd	With Nursing Effect[e] δ¹⁸O‰		Actual Tap Water δ¹⁸O‰
A1-A	06-3784	Webb	tooth enamel	-3.64	27.17	18.61	-5.06	-5.39	-4.73	-4.69	-4.03	
A2-A			compact	-7.42	23.27	14.73	-11.04	-10.67	-11.40			
A3-A			cancellous	-6.32	24.40	15.85	-9.31	-9.14	-9.48			
A4-A			tap water									(-4.7)
B1-A	0A0760	Cameron	tooth enamel	-5.36	25.39	16.84	-7.79	-7.80	-7.78	-7.10	-7.08	
B2-A			compact	*No data. Sample contaminated during processing.*								
B3-A			cancellous	-4.97	25.80	17.24	-7.17	-7.25	-7.09			
B4-A			tap water									(-1.9)
C1-A	DMG-9319	Starr	tooth enamel	-4.71	26.07	17.51	-6.75	-6.89	-6.62	-6.19	-5.92	
C2-A			compact	-5.11	25.65	17.10	-7.39	-7.44	-7.33			
C3-A			cancellous	-6.25	24.48	15.93	-9.19	-9.04	-9.34			
C4-A			tap water									(-3.2)
D1-A	06-5125	Webb	tooth enamel	-7.71	22.98	14.43	-11.50	-11.08	-11.92	-10.38	-11.22	
D2-A			compact	-8.19	22.47	13.93	-12.27	-11.76	-12.78			
D3-A			cancellous	-9.31	21.32	12.78	-14.04	-13.32	-14.76			
D4-A			tap water									(-4.7)
E1-A	DMG-9821	Zapata	tooth enamel	-4.34	26.45	17.89	-6.17	-6.37	-5.97	-5.67	-5.27	
E2-A			compact	-4.47	26.31	17.76	-6.37	-6.55	-6.19			
E3-A			cancellous	-3.88	26.92	18.37	-5.43	-5.72	-5.14			
E4-A			tap water									(-4.7)
F1-A	F2008-100	Webb	tooth enamel	-7.22	23.47	14.93	-10.73	-10.40	-11.06	-9.70	-10.36	
F2-A			compact	-8.47	22.19	13.65	-12.70	-12.14	-13.26			
F3-A			cancellous	-9.32	21.31	12.76	-14.07	-13.35	-14.79			
F4-A			tap water									(-4.7)

a   Unless otherwise stated all values are relative to VSMOW2.
b   Coplen et al. 1983.
c   Iacumin et al. 1996.
d   Daux et al. 2008.
e   White et al. 2004.

CHAPTER VII

DISCUSSION

Introduction

As discussed previously (Materials and Methods, page 45), a number of data comparisons, adjustments, and applications of regression formulae were made against the bone and tooth enamel sample data. The results of these comparisons are detailed for each case below. The nursing effect and the acetic acid side analysis mentioned earlier (Materials and Methods, page 38) are discussed separately from the individual cases. A discussion of the research assumptions, issues, and limitations is also provided.

Case 06-3784, Webb County

The enamel $\delta^{18}O$ value for case 06-3784 indicates that the individual likely ingested water during early infancy and childhood (0-3 years) with a similar isotopic signature as that found in the discovery location, Webb County. In contrast, the compact and cancellous values suggest the individual lived in at least two isotopically distinct locations as an adult. Both values are indicative of either a cooler or higher altitude climate where isotope values are more negative than in warmer or lower altitude areas.

Based on the three $\delta^{18}O$ values found within the tissues of case 06-3784, a suggestion is made that the individual represented by the remains resided in an

isotopically similar area as that found in Webb County during early childhood. The individual did not, however, live in said area recently (~8-10 years) or up to approximately 20 years prior to death. Because the discovery location $\delta^{18}O$ value was not found in any of the individual's bone tissues, the individual was most likely not from or had only recently (<2 years) relocated to the area. Because of the close correspondence between the enamel $\delta^{18}O$ value and the Webb County tap water $\delta^{18}O$ range at two standard deviation, an alternative explanation may be that the individual spent a portion of his or her early childhood in or near Webb County, but later moved from the area.

Case 0A0760, Cameron County

The compact tissue sample for case 0A0760 was contaminated during processing and no data for that tissue was available for analysis. Enamel and cancellous $\delta^{18}O$ values represent ingested water during early childhood (0-3 years) and recent residence (~8-10 years). Similar $\delta^{18}O$ values in these tissues, therefore, indicate the individual resided in an area during these stages with a similar $\delta^{18}O$ value to one another or in the same location for both stages of development. The enamel and cancellous $\delta^{18}O$ values for this case are similar to one another, but not similar to the discovery location, Cameron County. This suggests that the individual likely lived in the same area during early childhood and for approximately the last 8-10 years of his or her life. Due to the absence of the compact tissue, a suggestion cannot be given in regards to long-term residence (~20 yeas prior).

In summary, the individual represented by the remains of case 0A0760 resided in an isotopically similar area during the first three years of life and for approximately 8-10

years prior to death. That area was, however, most likely not the discovery location, Cameron County.   Although no discovery location $\delta^{18}O$ value was found in either of the individual's tissues, this does not preclude the possibility that the individual only recently (<2 years) relocated to the Cameron County region.

Case DMG-9319, Starr County

The enamel and compact tissue $\delta^{18}O$ values for case DMG-9319 are distinct from the tap water $\delta^{18}O$, but isotopically similar to one another (0.64 difference) and most likely represent the same or adjacent isotope regions of residence during early childhood (0-3 years) and ~20 years prior to death. The cancellous tissue $\delta^{18}O$ value is distinct from the other two tissues and the corresponding tap water $\delta^{18}O$ values. The highly negative cancellous tissue $\delta^{18}O$ value is indicative of either a cooler or higher altitude climate where isotope values are more negative than in warmer or lower altitude areas.

In summary, the individual represented by the remains of case DMG-9319 resided in an isotopically similar area during the first three years of life and for approximately 20 years prior to death. Isotope values further indicate that recent residence was in a cooler or higher altitude region. These areas were most likely not the discovery location, Starr County. Although no discovery location $\delta^{18}O$ value was found in any of the individual's tissues, this does not preclude the possibility that the individual only recently (<2 years) relocated to the Starr County region.

Case 06-5121, Webb County

The enamel and compact tissue $\delta^{18}O$ values for case 06-5121 are similar and most likely represent the same or adjacent isotope regions of residence during early childhood

(0-3 years) and ~20 years prior to death. In contrast, the cancellous $\delta^{18}O$ value is clearly

distinct from the other two tissues and the corresponding tap water $\delta^{18}O$ value. The

cancellous tissue value is indicative of residence that is either a cooler or higher altitude

climate where isotope values are more negative than in warmer or lower altitude areas.

In summary, the individual represented by the remains of case DMG-5121 resided

in an isotopically similar area during the first three years of life and for approximately 20

years prior to death. Isotope values further indicate that recent residence was in a cooler

or higher altitude region. These areas were, however, most likely not the discovery

location, Webb County. Although no discovery location $\delta^{18}O$ value was found in any of

the individual's tissues, this does not preclude the possibility that the individual only

recently (<2 years) relocated to the Webb County region.

Case DMG-9821, Zapata County

The enamel and compact tissue $\delta^{18}O$ values for case DMG-9821 are distinct from

the tap water $\delta^{18}O$, but isotopically similar to one another (0.20 difference) and most

likely represent the same or adjacent isotope regions of residence during early childhood

(0-3 years) and ~20 years prior to death. The cancellous $\delta^{18}O$ value, although not in

absolute agreement, is within 0.44 of the corresponding tap water $\delta^{18}O$ value at two

standard deviations. This value suggests a possible recent relocation to a region with an

isotope value similar to the Zapata County tap water.   If the relocation was in fact to the

Zapata area, the difference between the cancellous tissue and the tap water (0.44) further

indicates incomplete isotopic acclimation; therefore suggesting the relocation was likely

≤ 8 years.

In summary, the individual represented by the remains of case DMG-9821 resided in an isotopically similar area during early childhood and approximately 20 years prior to death. That area was, however, most likely not the discovery location, Zapata County. In contrast, recent residence was in an isotope region similar to that of the Zapata region, indicating the individual likely moved to the Zapata area in the last 8 years.

Case F2008-100, Webb County

Three distinct $\delta^{18}O$ values are present for case F2008-100. Although minimal overlap (0.09) exists between the compact and cancellous $\delta^{18}O$ ranges produced when applying two standard deviations, it is unlikely the values are indicative of residence in similar isotope regions. This is particularly true given that a standard deviation of .165 is significant when differences in $\delta^{18}O$ values begin registering at the 4th or 5th decimal position. Most likely the $\delta^{18}O$ values found in this case represent three distinct isotope regions. The highly negative values found (enamel:-10.73, compact:-12.70, and cancellous: -14.07) are more typical of $\delta^{18}O$ values found in cooler areas or regions of higher altitudes.

In summary, the individual represented by the remains of case F2008-100 resided in at least three isotopically distinct locations during his or her lifetime. That location was, however, not the discovery location, Webb County, but instead, a cooler and/or high altitude region.

Acetic Acid Treatment Analysis

Acetic acid treatments are typically used to remove contaminants in archaeological samples resulting from diagenetic activity within the deposition context.

Unlike most archaeological materials, modern human skeletal tissues are not environmentally exposed for great lengths of time and should, theoretically, hold little to no contamination. To test for the necessity of a contaminate removal treatment (acetic acid) in regards to modern human skeletal material, a separate analysis was performed on a sub-set of the bone and enamel samples in this study.

Half of each bone and enamel sample was processed utilizing the acetic acid treatment (sub-sample A) described in Chapter V, while the other half of the sample was processed with no acetic acid treatment (sub-sample B). The difference in each set, with versus without treatment, showed no overall trend toward more positive or more negative isotope values (see Table 4 and Appendix D). After the acid treatment on sub-sample A, seven of the 18 sample pairs (~39%) resulted in more negative isotope values, while 11 of the 18 were more positive (~61%).

A two-tailed, paired-samples t-test revealed no significant difference in the oxygen values before and after the acetic acid treatment, $t_{(17)} = 0.93$, p = 0.37. This indicates that the mean oxygen value after the acetic acid treatment (M = -6.53) was not significantly different than the mean before the acetic acid treatment (M = -6.71), i.e., a mean difference of 0.18 between treated and untreated samples.

Based on these few observations and small sample size, it is difficult to conclusively state the necessity of a contaminate removal treatment for modern, forensic bone and tooth enamel. Although statistically no difference is demonstrated, the fact that nearly ~39% of the samples resulted in more negative isotope values and ~61% in more positive values after the treatment prevents a definite conclusion based on this sample set. The mean difference between the acid and non-acid treated sets was 0.18. Interestingly,

both the percentage of samples affected (~39% and ~61%) and the mean difference (0.18) are similar to the results of a previous study utilizing a similar acetic acid test (30%, more negative, 60% more positive, 10% no change, and 0.19 mean difference, Regan 2006). Further research in regards to sample preparation is critical to the reliability and acceptance of isotope analysis as a valid technique in the pursuit of investigating the identity of modern human remains.

Nursing Effect

Researchers have demonstrated the existence of a metabolically induced fractionation of the oxygen composition of tooth enamel formed during nursing (Evans et al. 2006, White et al. 2000, Wright and Schwarcz 1998, 1999). Due to this fractionation, breast milk is oxygen enriched by 0.7‰ compared to the intake water of the mother. The breast milk enrichment is passed to the infant where it is subsequently incorporated into the bone and enamel tissues. Enamel is a static tissue and maintains its isotopic record through life and after death. In contrast, and because of the remodeling nature of bone, no nursing effect oxygen imprint will be found in the bony elements unless the infant perished during or shortly after the nursing period.

In this study the nursing effect adjustment was applied to the calculated ingested water ranges. Comparison of the results (Table 4) do not significantly alter the conclusions drawn in the above discussion. The results do, however, support the conclusion suggested in case 06-3784 that the individual represented by the remains most likely formed his or her first permanent molar enamel in an isotopic region similar to the Webb County discovery location.

Research Assumptions, Issues, and Limitations

Assumption: Consumption of Local Water

In this study the oxygen compositions within the skeletal tissues of unidentified

human remains were compared against the oxygen composition of their respective

discovery location tap waters. Utilizing only tap water as the sample against which the

skeletal tissue is compared does not take into account imported food supplies and cultural

dietary practices that may alter oxygen signature within the body. In today's global

economy and transportation of foodstuffs, it is likely that some individuals, such a those

living within large population centers located some distance from agricultural activity,

consume little, if any, of the local food oxygen signature. Confounding the issue further

is the possibility that individuals ingest a large portion of non-local water, either by

choice or because the municipally supplied water is piped from a distant source. For

these reasons, utilizing the oxygen composition of the locally supplied tap water as a

means of comparison to the oxygen composition found in the skeletal tissues may not be

the best choice of comparative samples. A more suitable candidate for comparison is a

bone and/or tooth sample from an individual known to have originated and continually

resided in the discovery location.

Assumption: Nursing

Discussed and applied in this study is the concept that nursing activity affects the

oxygen composition statically imprinted within the enamel of dentition formed during the

nursing period. The importance placed on the nursing effect is primarily determined by

the research intent and goals. If attempting to determine ages at which weaning may

have occurred in past populations, the application of the nursing effect is of the utmost

importance. In contrast, the amount of alteration demonstrated to exist (0.7, Wright and Schwarcz 1998, 1999) is unlikely to alter results to the extent that an origin from an entirely distinct isotope region is indicated. Examination of the oxygen values resulting from application of the nursing effect adjustment did not lead to alterations in the conclusions discussed above.

Issue: Fresh Water Versus Tap Water

As discussed in Chapter I, a strong correlation (R=0.70) exists between the oxygen composition of corresponding local fresh (e.g., precipitation or well water) and tap waters. Although Bowen et al. (2007) demonstrate this relationship, the authors recognize the fact that 30% of the variation between the two water sources remains unidentified. Responsible for the remaining variation, contend Bowen and colleagues, is principally the nature of the source (e.g., an open, shallow well vs. deep aquifers), local geology, and municipal water treatment processes.   Without detailed knowledge and incorporation of these factors into a research design, the acceptance of fresh and tap water oxygen compositions as equal factors in the determination of likely ingested water may result in erroneous data. Therefore, the assumption that the oxygen in the skeletal tissues from an individual consuming only fresh water (e.g., well water) is still indicative of the geolocation based on the oxygen in a corresponding tap water sample may lead to inaccurate results.

Issue: Ethics

An ethical conflict arises as a result of this methodology. That concern centers on the destructive nature of the process.   Even though advances in technology (e.g., direct

laser fluorination (Lindars et al. 2001) and thermal laser ablation (Passey and Cerling 2006) now allow for isotopic analysis of minute amounts of skeletal tissue, the fact remains that the process destroys the original sample. When skeletal tissue is limited or falls under protective legislation (e.g., NAGPRA), destructive procedures are unacceptable. Even when destructive analyses are allowed, the long-term integrity of the samples and/or collection must be considered and made a priority (Katzenberg 2000).

Although the integrity of human skeletal material should be a concern for all professionals dealing with human remains, the bigger picture must also be considered. One of the primary goals of modern forensic casework is the identification of the human remains and the eventual return of those remains to his or her family. Despite the current destructive nature of this method, the opportunity to provide potentially helpful information leading to the identification of an unknown individual may quite possibly outweigh the sample loss resulting from isotopic techniques.

Limitation: Unknown Deposition Context

The deposition contexts of the six cases in this study are unknown. This absence presents implications for the interpretation of the isotope data. As discussed in Chapter III, the potential for diagenetic alteration caused by the burial context (e.g., buried, frozen, submerged, or burned) can be substantial depending on the burial type and microenvironment (Sorg and Haglund 2002). Because of this, the interpretations based solely on the isotopic data of this study are limited. Therefore, future studies attempting interpretation of isotope data from bone and tooth enamel should include documented knowledge of the deposition context.

Limitation: Data Availability

A number of the researchers discussed in this study (Katzenberg and Harrison 1997, Prowse et al. 2007, Spence et al. 2004) have expressed concerns in regards to the dearth of isotope databases against which data may be compared. This study demonstrates well this limitation.   Although multiple pieces of information resulted from the isotope analyses conducted as part of this research, the usefulness and interpretation of the data is limited to the chosen comparative sample or to the few studies thus far conducted. Further research and database compilation is imperative if the utilization of isotope signatures is to be used, and even more importantly, accepted as viable evidence towards the identification of an unknown individual.

CHAPTER VIII

CONCLUSION

At death the human skeleton holds an *in vivo* molecular history of the stressors, diet, mobility, and health of the individual, a history that holds the potential of immense information and wide scientific exploration. The use of molecular approaches for extracting that recorded history, however, remains limited in forensic research applications. As reviewed in the previous research section of this study, the ability of biochemical techniques to extract the subtle clues left behind in skeletal tissue hold the potential as an informative and valuable avenue in future research, particularly in the field of forensic anthropology (Meier-Augenstein 2007, Ortner 2003).

Summary

The primary intent of this research was an exploration into the value of oxygen isotope chemistry as a means of providing information with regards to the identification of unknown individuals. To facilitate this application, the bone and tooth enamel of six open, unidentified forensic cases curated by the Forensic Anthropology Center, Texas State University-San Marcos were analyzed for oxygen isotope composition and compared together with the oxygen composition of corresponding tap water samples from their respective discovery locations. Given that the oxygen composition of skeletal tissues is strongly correlated with the oxygen composition of ingested water, oxygen

signatures of the skeletal tissues were expected to match the oxygen signature of their respective discovery location's tap water. Although the analyses resulted in additional information in regards to the individuals' residential histories, the data were too general in nature to formulate conclusive statements without further analysis of the remains and a broader research design.

Conclusion and Suggestions

As demonstrated in this study, oxygen isotope analysis has been used in attempts to answer a variety of research questions, clarify historical events, and even challenge prevailing theories. Strikingly few studies, however, have utilized isotopic analysis as a data gathering technique in modern forensic investigations of unidentified human remains. Those studies that do exist have primarily applied isotopic analyses in order to narrow down the geographic origin of the remains of missing American soldiers found on foreign soil (Beard and Johnson 2000, Regan 2006,). Such studies facilitate identification by providing scientists and investigators with a means to create a shortlist from the more than 88,000 missing American soldiers (Joint POW-MIA Accounting Command 2008). It stands to reason that applying a similar methodology to modern forensic investigations would result in the same: a shortlist of missing persons to which a set of unidentified remains may belong. Multiply this application by potential uses in mass fatality incidents, acts of terrorism, or human rights violations and the value of isotopic analysis is readily apparent.

Although this research demonstrated the predicted exclusionary value of oxygen isotope analysis in regards to unknown human remains, it is believed that future research utilizing a multiple elemental approach and documented modern bone and tooth samples–

–to account for contemporary food, water, and cultural dietary practices— will yield less broad results that allow for more conclusive interpretation.

Additionally, a concerted effort is needed among researchers to compile and make publicly available isotope databases against which data may be compared. Such a collaborative effort is imperative to the future success and acceptability of isotope research as viable means of exposing the valuable but hidden elemental information that resides within human skeletal material.

Finally, further research should be conducted to clarify the diagenetic effects on modern human remains. In addition to the primary intent, this research also conducted an analysis in an attempt to discern the necessity of treating modern skeletal material for diagenetic contamination. The small sample size and results preclude a conclusive statement concerning the need for such treatments. Future researchers would benefit from a larger study whose primary focus is to determine both the type and necessity of contamination removal treatments on modern skeletal material.

Implications

So why choose oxygen isotope analysis as a means of extracting information from a set of human remains? Although a number of answers are valid and were outlined in Chapter II, two additional reasons warrant attention. First, the cost of a single oxygen analysis is fairly inexpensive, ranging from $20-$40. This cost is likely affordable by most law enforcement agencies. In contrast, some laboratory techniques (DNA typing) can easily cost hundreds per sample. Such costs are often prohibitive to small law enforcement communities. Second, the time required for a lab to complete an elemental analysis is often only a few weeks depending on the lab chosen. During this research a

number of laboratories around the country were found to not only offer oxygen analysis but also offer an expedited service of less than two weeks if requested and the additional fees paid. Laboratory techniques such as DNA typing require a year or more for processing and the return of results. This turn around time causes delay in both the investigation and closure to the families. Given these reasons, perhaps the more appropriate question would be *why not* choose isotope analysis if even a small chance exists of obtaining information that may bring a lost individual home.

While it is true that the information gleaned from an oxygen analysis of skeletal material tends to be somewhat broad in nature, as the results in this research indicated, the data do in fact provide information about the origins and residence patterns of the represented individuals. Although the information appears to be primarily exclusionary, this does not mean that the information resulting from isotopic analysis, however small or broad, is of little value to those charged with the responsibility of identifying a set of human remains or to the family of the missing individual. Armed with the information provided through isotope profiling, investigators possess the ability to geographically constrain (or expand) investigations, thereby potentially narrowing what may be a vast list of possible victims. Elemental isotope analysis of human skeletal material, when used as one of the many lines of evidence in the pursuit of identification of an unknown individual, holds the potential to be a key factor in the identification of those who can no longer identify themselves.

APPENDIX A

OBSERVED $\delta^{18}O$ ISOTOPE RATIOS FOR TAP WATERS

IN THE CONTIGUOUS UNITED STATES

Observed δ¹⁸O Isotope Ratios for Tap Waters in the Contiguous United States (Bowen 2007)
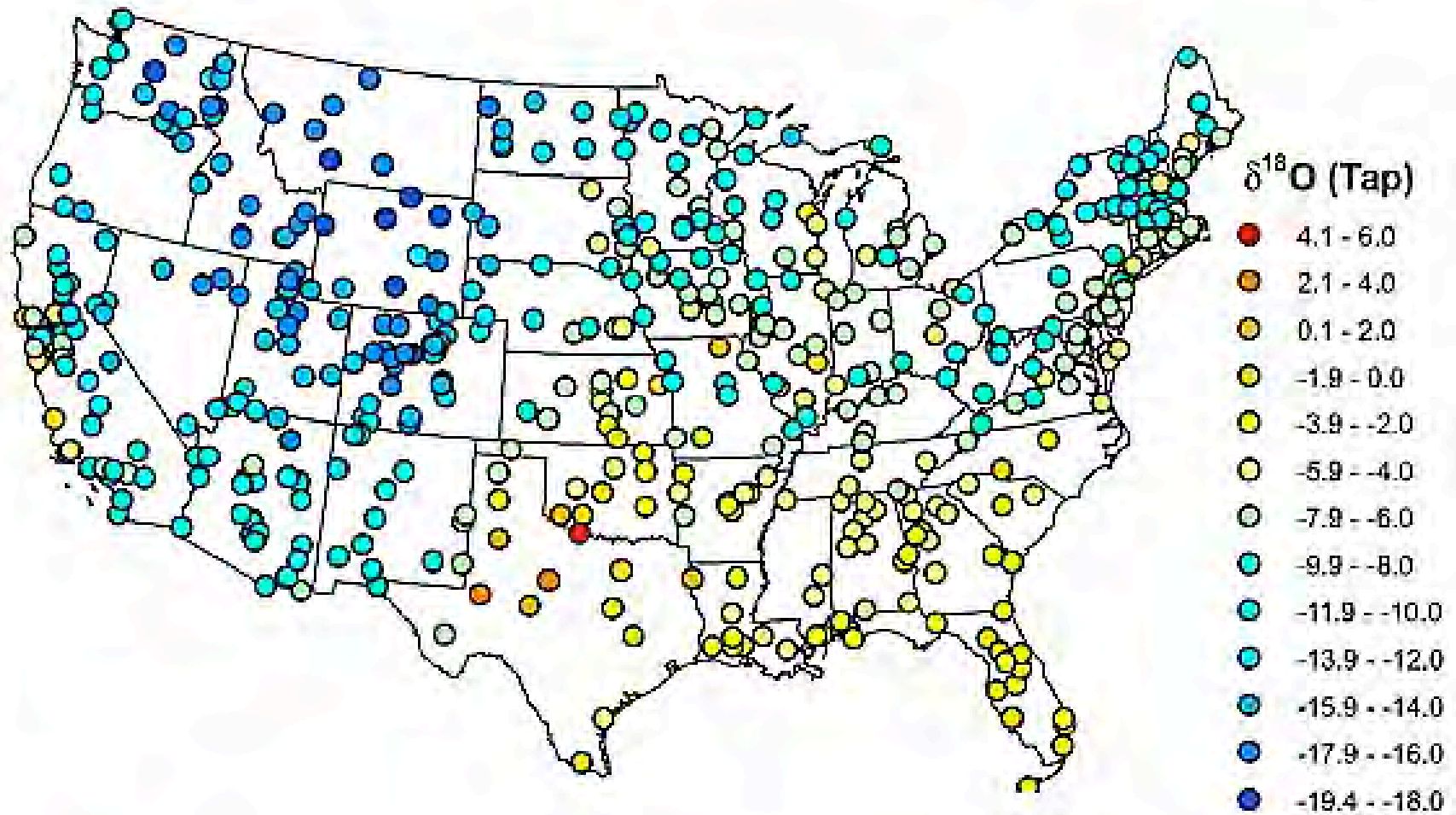
APPENDIX B

DOCUMENTED OXYGEN ISOTOPE VALUES

RESOURCES

Isotope Data Resources

- *Stable Hydrogen and Oxygen Isotope Ratios for Selected Sites of the U.S. Geological Survey's NASQAN and Benchmark Surface-water Networks.* Available at: http://pubs.usgs.gov/of/2000/ofr00-160.

- *Water Isotope System for Data Analysis, Visualization and Electronic Retrieval* (WISER). Available at: http://nds121.iaea.org/wiser.

- *Stable Isotope Ratios of Tap Water in the Contiguous United States.* Information available from the author at: gabe@purdue.edu.

- *Waterisotopes.org*. Provides information, data, and resources for scientific applications involving spatial variation in the isotopes of hydrogen and oxygen.

- *The Online Isotopes in Precipitation Calculator* (OIPC). An interactive tool allowing on-demand estimation of the stable water isotope ratios of precipitation at any site. Available at: http://wateriso.eas.purdue.edu/waterisotopes/pages/data_access/oipc.html

APPENDIX C

TEXAS COUNTY MAP

INDICATING

CASE COUNTIES, OXYGEN ISOTOPE SIGNATURE, AND

WATER COLLECTION LOCATIONS

Texas County Map

Boundaries as of January 1, 1990
www.lib.utexas.edu/maps/texas

SCALE

0   50   100   150   200 Kilometers

0   50   100   150   200 Miles

Webb County
$\delta 18O‰ = -4.7$
(Laredo)

Zapata County
$\delta 18O‰ = -4.7$
(Zapata City)

Starr County
$\delta 18O‰ = -3.2$
(Roma)

Cameron County
$\delta 18O‰ = -1.9$
(Harlingen)
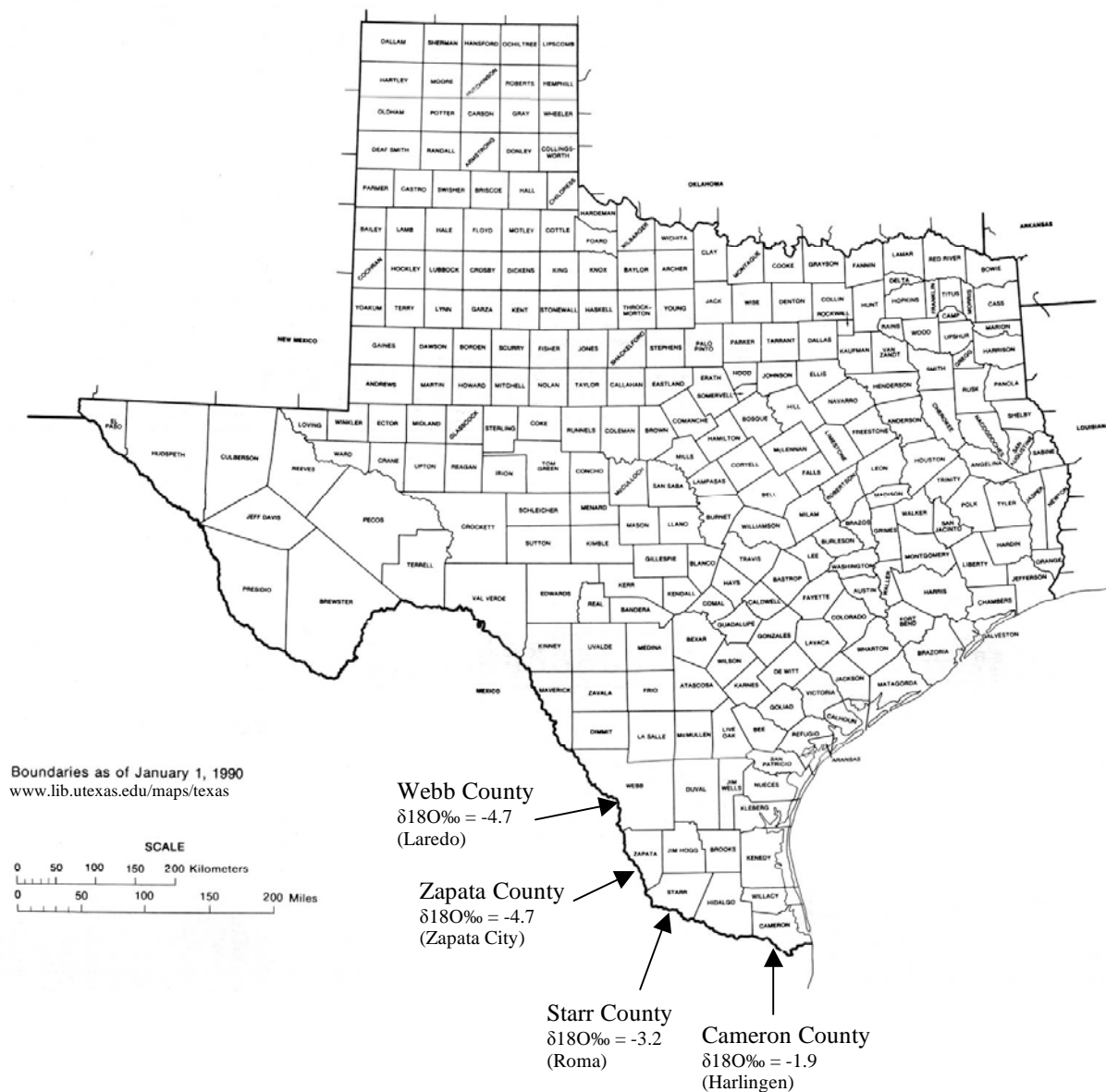
APPENDIX D

SUB-SAMPLE B OXYGEN ISOTOPE ANALYSIS

RESULTS AND ADJUSTMENTS

Sub-Sample B Oxygen Isotope Analysis Results and Adjustments[a]

Sample	Case Number	County	Type	Carbonate δ¹⁸O‰[PDB]	Carbonate δ¹⁸O‰[b]	Phosphate δ¹⁸O‰[c]	Calculated Ingested Water[d]	Calculated Ingested Water Range[d] δ¹⁸O‰ 2sd		With Nursing Effect[e] δ¹⁸O‰		Actual Tap Water δ¹⁸O‰
A1-B	06-3784	Webb	tooth enamel	-4.41	26.37	17.82	-6.28	-6.46	-6.09	-5.76	-5.39	
A2-B			compact	-7.42	23.27	14.73	-11.04	-10.67	-11.40			
A3-B			cancellous	-6.32	24.40	15.85	-9.31	-9.14	-9.48			
A4-B			tap water									(-4.7)
B1-B	0A0760	Cameron	tooth enamel	-5.79	24.95	16.40	-8.46	-8.40	-8.53	-7.70	-7.83	
B2-B			compact	-5.41	25.34	16.79	-7.86	-7.87	-7.86			
B3-B			cancellous	-5.31	25.44	16.89	-7.71	-7.73	-7.69			
B4-B			tap water									(-1.9)
C1-B	DMG-9319	Starr	tooth enamel	-5.43	25.32	16.77	-7.89	-7.89	-7.90	-7.19	-7.20	
C2-B			compact	-5.83	24.91	16.36	-8.53	-8.45	-8.60			
C3-B			cancellous	-5.38	25.37	16.82	-7.82	-7.82	-7.81			
C4-B			tap water									(-3.2)
D1-B	06-5125	Webb	tooth enamel	-7.25	23.44	14.90	-10.77	-10.44	-11.11	-9.74	-10.41	
D2-B			compact	-8.37	22.29	13.75	-12.55	-12.00	-13.09			
D3-B			cancellous	-8.79	21.86	13.32	-13.21	-12.58	-13.83			
D4-B			tap water									(-4.7)
E1-B	DMG-9821	Zapata	tooth enamel	-4.55	26.23	17.67	-6.51	-6.67	-6.35	-5.97	-5.65	
E2-B			compact	-4.48	26.30	17.74	-6.40	-6.57	-6.23			
E3-B			cancellous	-3.46	27.35	18.80	-4.77	-5.13	-4.40			
E4-B			tap water									(-4.7)
F1-B	F2008-100	Webb	tooth enamel	-7.86	22.81	14.27	-11.74	-11.29	-12.20	-10.59	-11.50	
F2-B			compact	-8.13	22.53	13.99	-12.18	-11.67	-12.68			
F3-B			cancellous	-8.55	22.11	13.56	-12.84	-12.26	-13.42			
F4-B			tap water									(-4.7)

a   Unless otherwise stated all values are relative to VSMOW2.
b   Coplen et al. 1983.
c   Iacumin et al. 1996.
d   Daux et al. 2008.
e   White et al. 2004.

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VITA

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