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#### PARAFFIN:

### AN ECONOMIC AND ECOLOGICAL ALTERNATIVE

### FOR BONE CONSERVATION

By

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**Professional Paper** 

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Anthropology

Paraffin: An Economic and Ecological Alternative for Bone Conservation

Chairperson: Kelly J. Dixon, Ph.D.

Committee member: David B. Bolstad, Ph.D.

Abstract:

Fragile bone materials are ubiquitous in archaeological museum and forensic settings. Although there are many chemical and industrial options for conservation, these may adversely affect the bone objects undergoing preservative treatment. Here, paraffin is explored as a biologically friendly alternative to bone material conservation. Modern domestic pig ribs were subjected to paraffin treatment and then to isotopic analysis to quantify and investigate the chemical effects of paraffin. Although the results showed that paraffin had a limited impact, the sample size proved too small to display the definitive parameters of paraffin's effects. The results were nevertheless compelling enough to warrant more research with a greater sample size.

#### **Acknowledgements**

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#### **Chapter 1: Introduction**

Bone is the predominant material of animal origin to survive within the archaeological environment (Child 1995:19).

Bone artifacts, skeletal remains, and other bone objects are frequently recovered during archaeological, bioarchaeological, and forensic investigations. Bone material is also common among specimens housed and displayed in museums and laboratories. However, as a biological material, bone decomposes, and undergoes constant changes while buried in the ground (Plenderlieth and Werner 1971; Cronyn 1990; Stone et al. 1990; Child 1995; Cronyn 2001; Raiswell 2001; Watkinson 2001; Pollard et al. 2007; Weiner 2010), when it is cleaned in a laboratory, and wherever it is placed in storage (Cronyn 1990; Johnson 1994). Therefore it is important to protect the integrity of the bone, keeping as much of the original material as intact as possible. In the age of science, there have been some solutions available for consolidating bone, but these are expensive and often require "state-of-the-art" laboratories (Smith 2003), which are often not available to smaller projects within the laboratory or out in the field. Therefore there is a problem – and challenge -- to supply a less costly and smaller scale set of alternatives. These alternatives require parameters: the treatment must not damage or destroy the integrity of the bone; the treatment has to be affordable to apply, store, and dispose of in order to be accessible to all projects; and the treatment should not hinder future research or analysis on the bone material.

Here, paraffin is presented as an alternative method for bone preservation. As a conservation tool, paraffin has not been explored to the depths needed to really flesh out its

potential. Thus, understanding paraffin's parameters may hold a key to preservation of bone. First, I present a brief survey of the literature pertinent to conservation treatment of bone. Then I discuss the structure of bone, what is known about paraffin, and the analytical process of isotopic analysis. After presenting this background, the history of bone conservation, along with paraffin's place in that realm, will be discussed. Finally, I summarize the methods and results of the experiments I conducted to test paraffin against the parameters noted above.

\* \*

Even though a bone ecofact or artifact may appear stable, studies have shown that bone is in a constant process of transition while it interacts with its environment (Plenderlieth and Werner 1971; Cronyn 1990; Stone et al. 1990; Child 1995; Cronyn 2001; Raiswell 2001; Watkinson 2001; Pollard et al. 2007; Weiner 2010). Given the nature of bone material, it is imperative to take responsibility for these items and store them in a manner that preserves their integrity.

What action should be taken, then, to preserve those fragile bone materials? Many studies have analyzed the various methods used for bone preservation (Lucas 1924; Plenderlieth and Werner 1971; Stone et al. 1990; Johnson 1994; Smith 2003). Most of these treatments require costly laboratory spaces (Smith 2003) and involve consolidants that would hold bone material together, but it is unclear for how long; moreover, what happens to the consolidants over time is unknown (Caldararo 1982; Johnson 1994; Watkinson 2001). It is, of course, essential that conservation treatments do not interfere with current and future analytical testing (Bourque et al. 1980; Caldararo 1987; Johnson 1994).

In addition to the concerns involved in the research side of bone preservation, we have an ecological and ethical responsibility to try to use biologically friendly materials. Many

of the chemicals involved with preservation treatments cannot be simply thrown away after the treatment procedure, but require hazardous materials handling and disposal, creating additional expenses. As conservators, and environmentally minded citizens, it is challenging to find a more effective, cost efficient way to preserve bone materials. During the beginning of the last century paraffin was employed as an effective and efficient preservation substance (Lucas 1924; Johnson 1994).

Additional considerations must be addressed when contemplating treatment of human remains. The choice to conserve human remains involves a plethora of difficulties, such as the cultural concerns of the descendant community, as well as, the social, political, and biological concerns involving such remains (McGowan and LaRoche 1996; Cassman and Odegaard 2004; Frigo 2008). It is always best to err on the side of caution when dealing with such culturally sacred and biologically invaluable materials, and paraffin has the potential to provide a non-invasive conservation option that can help navigate the sensitive milieu associated with human remains. Paraffin is non-invasive, colorless, and can be applied on location so that sensitive remains not have to be moved to inappropriate locations for treatment (Cassman and Odegaard 2004). If conservation treatment is allowed, paraffin's flexibility makes it a viable option for preserving these objects and remains.

The isotopic tests carried out as a part of this research showed that paraffin could be a preservative that would not dramatically impact analytical methods, and that would be able to preserve and support bone material regardless of the bone's condition. This means that bone artifacts would be submitted to a treatment process only once, thereby limiting their exposure to conservation procedures while in a fragile state. A single treatment would both preserve a bone object's condition and provide structural support and protection from the environment. Paraffin

treatment is reversible. While other conservation treatments, such as cellulous nitrate (Johnson 1994) or poly(vinyl) acetate (Plenderlieth and Werner 1971; Johnson 1994), would require harmful chemicals and / or temperature that can cause damage to bone material, no chemicals and low heat (at least 117 degree Fahrenheit / 47.2 degree Celsius) are required to apply or remove paraffin. Finally, paraffin can preserve the important cultural and scientific information held by these physical remains.

So far the term "bone" has been used in the broadest sense, because paraffin can be used on any bone object (e.g., artifacts, ecofacts, and osseous remains). The flexibility of paraffin and its interaction with bone are the same regardless of the shape, form, or historic use. The bone material subjected to this conservation treatment will retain its shape. Taking all of these qualities into consideration, the decision to utilize paraffin - or any other treatment - must lie in the hands of the conservator in concert with the archaeologist, bioarchaeologist, forensic scientist, faunal analysis, museum curator, or other field specialist dealing with the recovery and treatment of bone material (Caldararo 1982; Cronyn 1990; Johnson 1994; Child 1995; Rule 2006).

#### **Chapter 2: Paraffin and Bone, their Parallel Lives**

#### Paraffin as a Substance

*Paraffin: from the Latin* Parum affinis. Parum = too little and Affinis = attraction (The Free

Dictionary 2005).

Paraffin is an "old fashioned" name for alkanes (Oxford Dictionary 2010). The alkanes are a type of organic compound known as hydrocarbons (Ege 1994). These hydrocarbon molecules are made of only hydrogen and carbon atoms (Ege 1994; McMurry and Fay 2004). Hydrocarbons are considered saturated when the carbon atoms are connected by single bonds to as many hydrogen atoms as it has room for (Ege 1994; McMurry and Fay 2004), which is why these molecules are inert towards most reagents (Ege 1994). A way to alter alkanes is through heat; for example, 117 to 147 degrees F will melt paraffin. Paraffin is a mix of alkanes that contain a high amount of carbon and hydrogen (e.g.,  $C_{25}H_{52}$ ) in one substance.

The inert quality of paraffin has made it an attractive candidate for bone preservation. When solidified around an artifact, it can provide support and consolidation for fragile materials without interacting with the chemical integrity of the object, in this case, the bone. It simply lays on the surface, remaining chemically inactive to the elements of the bone; yet, by laying on that surface, it offers structural support and creates a barrier between the bone material and the surrounding environment.

Paraffin's history as a preservative is not completely clear; in the early 20<sup>th</sup> century (and possibly before) paraffin and beeswax were both used as preservatives for artifacts (Johnson 1994; Rivers and Umney 2003). It was observed that the two substances reacted differently to their storage environment, and this has confused the actual effects of each

substance (Rivers and Umney 2003). There was a vast chemical dissimilarity between beeswax and paraffin, as paraffin was 100% hydrocarbon, while beeswax was only 14% hydrocarbon. Beeswax contained 284 different compounds, 111 of which have been identified (Sivasubramaniam and Seshadri 2005). Neither has been submitted to any in-depth analytical tests in reference to preservation. Only the research done on beeswax was involved with furniture preservation and treatment; this recorded the effects and resulting physical responses that beeswax (along with other waxes) had (Rivers and Umney 2003).

#### The Nature of Bone

Bone is composed of three main components: inorganic mineral, organic protein, and water. This specialized organic and inorganic duality places bone in a composite material category. The inorganic mineral is primarily hydroxyapatite (a form of calcium phosphate) which comprises approximately 60-90% of bone, while the remainder is a mix of collagen, noncollagen proteins, lipids, mucopolysaccrides, carbohydrates, and water. Additionally, the ratio of the component levels in bone can fluctuate from bone to bone within and between living entities (Childs 1995; White and Folkens 2000; Weiner 2010).

Beyond the molecular structure of bone, the histology of mature bone falls into two categories: compact and spongy. The compact bone has a more solid and dense appearance and forms the external surface of bone. Spongy (cancellous, trabecular) bone looks more like a pumice stone or an actual sponge, and forms the interior of most bone (White and Folkens 2000; Pollard and Heron 2008; Weiner 2010).

Bone has the potential to reveal many things about the culture and timeframe from which it originated. Most excavated bone material may not contain analytical DNA samples

(Poinar and Stankieweiz 1999), or lend itself to visual assessment beyond the burial environment due to the surface or stratigraphic erosion and exposure to the atmosphere (Cronyn 1990; Child 1995; Poinar and Stankieweiz 1999; Cronyn 2001; Raiswell 2001; Watkinson 2001). However, there are still many things that can be learned from a physical and methodical examination. There are many different analytical tests that can involve bone collagen, tooth enamel, and bone mineral (Pollard et al. 2007; Weiner 2010). Some of these tests include: radiocarbon and electron spin resonance (dating), DNA sequencing (paleogenetics), as well as isotopic analysis of oxygen, nitrogen, carbon, strontium, and lead (paleodiet and paleoenvironmental reconstruction and paleomigration) (Ambrose 1993, 1998; Richards and Hedges 1999; Pollard and Wilson 2001; Sealy 2001; Jay and Richards 2006; Pollard et al. 2007; Pollard and Heron 2008; Weiner 2010). There are more examinations that can be done with biometrics (demography) (Sattenspiel and Harpending 1983; Gage 2000; Story 2007). These examples focus mainly on human remains, but faunal remains can also be subjected to such studies. The skeletal structure holds a cornucopia of information, be it molecular or visual.

Bone material is quite dynamic while still in the body and adhering to Wolf's Law (White and Folkens 2000); and yet, when its journey into the archaeological record begins, bone continues to change as it adjusts to the burial environment in concert with surrounding depositional processes (Cronyn 1990:277; Child 1995:21; Poinar and Stankieweiz 1999:8426; Pollard et al. 2007:27; Weiner 2010:110, 115-118). Diagenesis is the term used to discuss how any biological material interacts with the burial environment (Caple 2001; Pollard et al. 2007; Weiner 2010). In reference to bone material, this interaction can occur structurally and chemically (Dowman 1970; Cronyn 1990; Weiner 2010) depending on the density of the bone

object and the type of surrounding soil and environment. Dowman (1970:21) presented an example when she discussed what can occur in an acidic environment:

In general, though, in a sandy soil with a pH below 5.6 the phosphate will be leached out and there will be no trace of bone although in a clay some may remain in a pH as low as 3.5. It is therefore no proof that there was never a burial if no bones are found in a good state of preservation in a pH below neutrality (pH7) and where they are found in a slightly acid soil they will be in a weakened condition.

In relation to bone material, this exemplified one type of external force. Additionally, Cronyn (1990) discussed, in detail, how each environment could affect bone and how the surface may have appeared. Upon initial excavation, it may look like it is in good shape, but upon drying (if from a moist environment) or after continued exposure to the atmosphere and other external environmental forces that differ from the original burial environment, bone may become weak and split or become chalky (Cronyn 1990:277-279).

The burial or surface environment can do many things to bone materials as they come to equilibrium with that environment (Cronyn 1990:5; Caple 2001:588; Cronyn 2001:628; Raiswell 2001:595; Pollard et al. 2007:26; Weiner 2010:110). The best means to combat the forces of equilibrium is to know the agents active in that environment and to be aware of the diagenetic effects expected to occur on the bone materials residing in that setting.

#### **Chapter 3: Isotopic Analysis**

The search for and exploitation of preserved molecules from relatively protected niches requires an understanding of the structure of the material in which they are preserved, preservation conditions, and the application of analytical techniques capable of handling the small quantities of materials likely to be preserved. The challenge is therefore not only to find protected niches with preserved molecules, but also to be able to analyze the minute amounts of these molecular "treasures" that are likely to be available (Weiner 2010:207).

Isotopic analysis has been available as an analytical investigation since the beginning of the 1980s (Richards and Schulting 2006). The type of isotopic analysis used in this study is concerned with the ratios of carbon to nitrogen, a common tool for reconstructing paleodiets (Ambrose 1990:431, 1993:60; Ambrose and Norr 1993:1; Ambrose 1998:278; Richards and Hedges 1999; Pollard and Wilson 2001:191,195-198; Sealy 2001; Jay and Richards 2006:654-655; Pollard et al. 2007:21, 180-182; Pollard and Heron 2008:346-370). This methodological approach pieces together the dietary habits of past environmental consumers. The perspective of carbon and nitrogen ratios in particular, has not continued without scrutiny (Milner et al. 2004), however, there is still a firm belief in the practice of carbon and nitrogen ratios with the field of isotopic analysis to hold the capacity to continue to strongly contribute to archaeology, as well as to the greater discipline of anthropology, as a means of studying how humans interacted with their environments (Hedges 2004; Barberena and Borrero 2005; Richards and Schulting 2006).

A simplified image of an atom has a nucleus which contains proton(s) (positively charged) and neutron(s) (uncharged) with an electron(s) (negatively charged) circulating around the central nuclear ions. The number of protons and electrons gives an element its characteristics, yet the numbers of neutrons vary for some elements (Ege 1994; Pollard and Wilson 2001; Pollard et al. 2007; Pollard and Heron 2008; Weiner 2010). See Figure 3.1 for a sample of

standard isotope and its notation using carbon - 12 as an example. Since elements are defined by the number of protons in an atom's nucleus, isotopes occur when an element's atoms exist with different numbers of neutrons. The difference in the number of neutrons between atoms that have the same amount of protons will result in a difference in their weight. The lighter isotopes are more likely to enter into chemical reactions, while the heavier isotopes take longer to do the same (Pollard and Wilson 2001).



In nature, many elements have isotopic variables represented by the lighter and heavier isotopic range (Ege 1994), and the ones in particular that currently interest paleodiet reconstructionist archaeological scientists are carbon (<sup>12</sup>C, <sup>13</sup>C) and nitrogen (<sup>14</sup>N, <sup>15</sup>N) isotopes (Ambrose 1990, 1993; Ambrose and Norr 1993; Ambrose 1998; Richards and Hedges 1999; Pollard and Wilson 2001; Sealy 2001; Pollard et al. 2007; Pollard and Heron 2008). There are others that have been used by archaeological scientists in the past, and are still useful today. In particular, strontium (<sup>87</sup>Sr, <sup>86</sup>Sr, <sup>90</sup>Sr) and calcium (Ca) replacement are used in trace element

studies, while strontium and oxygen (<sup>18</sup>O, <sup>16</sup>O) help indicate location or migrating populations (Sealy 2001; Pollard et al. 2007; Pollard and Heron 2008). These isotopes are often separated into their lighter or heavier isotope groups.

Light isotopes are the lighter form of an element, such as <sup>16</sup>O in comparison to <sup>17</sup>O and <sup>18</sup>O. These lighter forms tend to circulate around the atmosphere and terrestrial levels faster and more often than their heavier counterparts (Pollard and Wilson 2001; Pollard and Heron 2008) thus, they enter into chemical reactions at a faster rate than heavier isotopes (Ambrose 1993). For example Pollard and Wilson (2001) and Pollard and Heron (2008) observed that through the process of evaporation, lighter isotopes of oxygen (<sup>16</sup>O) more readily enter in to the evaporation cycle, leaving the heavier isotopes behind in the water source from which they originated. These sources have a higher abundance of heavier isotopes. This process is called *fractionation* (Ege 1994; Ambrose 1993; Pollard and Wilson 2001; Pollard and Heron 2008). Higher ratios of heavier isotopes are consumed by the creatures that exist in that environment, storing the light and heavy ratio within their bodies. Since both light and heavy isotopes can exist in a source, there is an equation that can express these isotopic abundances within a sample:

$$\delta$$
 (‰) = [(X<sub>sample</sub>/X<sub>standard</sub>) -1]x 1000.

Here X represents the isotopic element; and the delta notation ( $\delta$ ) accompanied by the parts per thousand, expressed as per mil. (‰) represents the amount of a particular isotope in a sample (Ambrose 1993). Additionally, if an individual wanted to know where their sample stood in relation to the international standards, the equation takes a slight adjustment:

$$\delta = [(({}^{B}X/{}^{A}X)sample - ({}^{B}X/{}^{A}X)standard)/(({}^{B}X/{}^{A}X)standard)] * 1000$$

Here X stands for the element, the B symbolizes the heavier isotope, and A symbolizes the lighter isotope. If the ratio is the same as the standard, the  $\delta$  value would be zero. If the  $\delta$  value was positive, then the sample contained a greater number of heavier isotopes than the standard; and the reverse would also be true, a negative  $\delta$  value would mean the sample contained a greater amount of lighter isotopes than the international standard. The multiplication of everything by 1,000 allows for smaller changes to be exaggerated (Pollard and Heron 2008).

Isotopic ratios, namely those representing carbon and nitrogen, are important because of their intricate relationship to everything that exists in our environment and within our bodies. In relation to palaeodietary studies, carbon and nitrogen levels can state what living beings were consuming during any known timeframe (Ambrose 1990:431, 1993:60; Ambrose and Norr 1993:1; Ambrose 1998:278; Richards and Hedges 1999; Pollard and Wilson 2001:191,195-198; Sealy 2001; Jay and Richards 2006:654-655; Pollard et al. 2007:21, 180-182; Pollard and Heron 2008:346-370). Carbon and nitrogen are taken in during consumption and used by our bodies within our skeletal structure. Depending on the level of each element and their ratios in comparison to other consumers within the environment, along with the known levels in a particular region, it is possible to know what the consumers being studied (usually humans) were eating. These elements are stored in the skeletal structure and bone material observed in recoveries from archaeological – or other – contexts.

Given isotopes propensity to reveal stories about osseous materials, collagen needs to be considered as another source for isotopic studies. Bone collagen, because of its mineral structure (hydroxyapatite/carbonate hydroxyapatite) interwoven with collagenous fibers, show a great resistance to diagenetic process (Ambrose 1990:431, 1993:72; Sealy 2001:270; Pollard et al. 2007:182; Pollard and Heron 2008:347; Weiner 2010:211). With this resistance,

collagens preserve the telltale isotopes in ratios that provide information about diet, environment, and other palaeodietary inquiries.

There were concerns with using collagen's carbon to nitrogen ratios because of the possible contamination that can occur in the burial environment. The process of diagenesis alters the composition of bone material, in this case collagen, to reflect the burial environment primarily if not completely (Caple 2001; Pollard et al. 2007:26-30; Pollard and Heron 2008:10; Weiner 2010:110-118); as a result, the remaining information from a bone or bone object's "preburial" life is lost. The challenge of diagenesis does not render analysis impossible, but requires proving the purity of the collagen samples through controls or purifying processes (Weiner 2010:259). It is essential then, for the environment of the samples to be well documented so that any results that appear to be different from the depositional environment can be more thoroughly examined.

#### Chapter 4:

#### A Brief History of Bone Conservation and Paraffin as a Preservative Tool

No single type of matter is stable under all conditions (Plenderlieth and Werner 1971: 1).

#### From Wax to Polymers

Conservationists must identify the best preservatives for any given precious object, from document scrolls to works of art (Caldararo 1987). Lucas (1924) created one of the first artifact preservation manuals in English (Caldararo 1987) and in that early guide he recommended the use of paraffin; consequently, Lucas's application of paraffin is recommended for more than just the treatment of bone. Lucas (1924:19, 21, 56) suggests paraffin wax be used as a consolidant for Plaster of Paris, as a strengthener for a myriad of materials (e.g., fabric, gilt, or painted Gesso), as additional support for ivory items in poor shape, and finally for objects such as wood, horn, and bone.

Lucas (1924) felt that paraffin was incredibly flexible in its application and subsequently recommended it as a supporting substance for weak materials; since it could be heated to a liquid, which only changed its physical state, not its inert characteristics. When paraffin was in a liquid form, it could be mixed with other substances - which was not mentioned specifically by Lucas, but was practiced. An example of such a mixture would be to add other waxes, like beeswax or carnauba wax, along with a pigment dye for the repair of leather furniture (Rivers and Umney 2003). Upon drying, Lucas found that paraffin remained colorless, while not affecting the appearance of most artifacts, leaving only a darkening on wood and bone. Importantly, Lucas (1924) knew paraffin was removable (e.g., reversible) with the application of mineral spirits or heat.

However, paraffin was not always used when a wax was needed in conservation. Often the need for wax could have been supplied by either paraffin or beeswax (Johnson 1994; Rivers and Umney 2003). Yet, beeswax did not always behave in the same positive manner that paraffin had. As mentioned above, beeswax is not made of the same substances as paraffin and would interact with the environment more than paraffin did, causing shrinkage and off-gassing inside museum environments; in addition, beeswax was more brittle after cooling and continued to become fragile over time (Rivers and Umney 2003).

As conservation science advanced, there was a strong move toward the use of more "modern" consolidants. Plenderlieth and Werner (1971:155) clearly display this drive for modernization when they recommend an "impregnation of a 5 per cent solution of a suitable transparent synthetic resin, notably polyvinyl acetate or polymethacrylates, in toluene." These methods were employed to maintain the appearance of the bone artifact, often with aesthetics as the driving force rather than consideration of the authentic integrity of the artifact (Plenderlieth and Werner 1971; Caldararo 1987). The main problem with this approach is the fact that these substances are invasive and alter (or have the potential to alter) the specimens, limiting options for future research and analysis of samples (Bourque et al. 1980:795; Caldararo 1987; Johnson 1994). An example would be the effect that poly (vinyl) acetate emulsions have on bone. As Johnson (1994) explains, after drying many of these emulsions become crosslinked - meaning that they are no longer an emulsion (polymers floating free within a carrier substance) but are connected to one another through chemical bonds - causing the polymer to become fragile. In addition, once the polymer has been absorbed into the pores of the bone in its crosslinked state, it becomes impossible to remove without complete destruction of the bone material.

Current conservation techniques favor the treatment of artifacts with polymers via infusion, emulsions, or direct applications (Plenderlieth and Werner 1971; Johnson 1994; Cronyn 1990; Stone et al. 1990; Smith 2003). Often, these chemical methods have not been properly tested in a long-term storage environment and so their stability - and lifespan - is unknown. Moreover, these treatments can even be destructive to the artifact (e.g., the shrinkage of natural polymers like shellac pulling the surface of the bone with it, or the nature of cellulose nitrate becoming brittle and flaking away from the artifact, making it weak or unstable (Johnson 1994:225)) (Bourque et al. 1980; Johnson 1994).

To further complicate the matter, bone remains and artifacts are susceptible to change due to their storage environment, as is true with most artifacts and works of art (Rule 2006). The storage environment cannot mimic the dynamic or static nature of the strata with which they had already come to equilibrium (Plenderlieth and Werner 1971:1-2; Cronyn 1990:5; McGowan and LaRoche 1996:116, 117; Pollard et al. 2007:26, 27). Bone artifacts may appear in good condition upon initial recovery; however, they are more often in a less than stable condition, requiring an immediate evaluation and possible treatment if the bone object is to stay intact beyond field recovery (Lucas 1924:1; Plenderlieth and Werner 1971:2-3, 148-149; Cronyn 1990:5, 277; Stone et al. 1990:184; Smith 2003:112-113; Pollard et al. 2007:26, 27; Weiner 2010:111-118). Cronyn (1990:279-280) illustrated the occurrence of two fragments of antler that were damp in the burial environment, which dissolved some of the collagen fibrils, but (without any controls) they shrank, warped, and cracked after being allowed to dry after an extended period. Their original form was lost, and this condition worsened over time as the ambient relative humidity (RH) rose and fell in its storage environment. As was discussed above,

treatment with the wrong substance can be just as destructive as no consideration or care for the environment of the artifact.

#### Research (Re)Design

When archaeologists are faced with the need to treat excavated bone, and do not have the resources (Bourque et al. 1980) to dedicate to uncertain treatments (Caldararo 1987), it is important to have cost effective methods that have been put through some form of research and evaluation and are cost effective. The research presented here was inspired by these conservation quandaries. When smaller projects are faced with conservation needs but do not have the budget, resources, and laboratory space, there should be environmentally friendly options that have been tested and that yield dependable and consistent results. To resolve this issue, I suggest a revival of Lucas's (1924) recommendations of the use of paraffin and present my research dedicated to testing the parameters of paraffin to see if application on bone materials is a viable conservation alternative.

I tested three hypotheses. The first hypothesis was that the treatment of bone by paraffin may mute the isotopic signature of the bone, but that it would, nevertheless, be statistically accountable. The initial hypothesis was based on the assumption that paraffin would leave a chemical signature that was unique in some way and could be recognized and statistically removed through an additional mathematical equation. As an example, the paraffin might add a - 2‰ onto the carbon ratio (which is sample dependent), if that is known, it could be accounted for by including a step to the calculations that removed the -2‰ from the carbon result. Assuming that the paraffin treatment to the bones would likely change the chemical fingerprint of the material treated, this hypothesis was expected to determine the amount of isotopic information

skewed in the result from the bone. I will discuss (in the results chapter) how the post-paraffin treatment changes to the object could be statistically predictable, and that treatment of bone would not completely distort isotopic test results.

The second hypothesis was that it was not possible to statistically predict the effects of paraffin treatment, but that it would not affect the carbon and nitrogen ratios determined in spite of the paraffin chemical signature. If the second hypothesis was true, then the isotopic results would show no pattern, and appear random. To illustrate the second hypothesis, paraffin would affect each sample in a different way, showing significantly different results for each individual sample.

The third hypothesis states that paraffin would change the bones chemical composition in a way that useful isotopic data from analysis would be unattainable. If this was true, then the dominant result would be the chemical signature of paraffin, and each sample would show the same signature.

Given the importance of isotopic analysis on bone materials, I treated bone samples with paraffin and then sent in those (along with control samples) to the Max Planck Institute for isotopic analysis that focused on carbon and nitrogen ratios.

#### **<u>Chapter 5: Material and Methods</u>**

How is the truth of this determined? Through analysis: material and cause (Marcus Aurelius, translated by Hays 2002:42).

#### Sample Preparation

The items used for this experiment are rib bones from a domesticated pig - Sus scrofa. These ribs were purchased from Diamond Bar Meats, located in Missoula Montana. The pig was raised on a farm in Pendleton, Oregon. Every rib used in this study was from the same pig. Three ribs were cut off and a majority of their soft tissue was removed. The cleaned samples were then placed in a ziplock bag with Mortons Iodized Salt; salt was added to aid in the removal of the rest of the soft tissue without utilizing industrial chemicals. In addition to the uncooked ribs, three ribs had gone through a cooking process, to temperatures which did not exceed 482 degrees Fahrenheit / 250 degrees Celsius. These ribs also had the majority of their soft tissue removed and were placed in a plastic bag with salt. These samples were kept separate and stored in a refrigerator at 41 degrees Fahrenheit / 5 degrees Celsius.

Ten days later, the uncooked ribs still had small amounts of soft tissue that was still releasing liquids. The ribs were subsequently removed from their bag and placed in a square "Tupperware" container and covered in salt. Once the ribs were placed in the salt they were again covered in more salt. The lid was secured and they were returned to the refrigerator. The cooked ribs were showing less liquid release, as a result, more salt was added and they remained in their bag.

Fifteen days later, the remaining tissue had fully dried on the uncooked and cooked ribs and could be peeled off. It was removed by hand with the aid of a stainless steel knife (Figure 5.1) - J.A. Henckels International, with a stainless steel 12.3 cm blade.



Figure 5.1. The removal of excess tissue from bone.

Following their cleaning, the samples were placed in respective bags and they were moved to the Historical Archaeological Laboratory on the University of Montana, Missoula campus. One bone was selected from each group to be the control, meaning that it would not undergo any treatment. The control ribs were photographed, and numbered, while the remaining ribs were submerged in paraffin. The temperature of the melted paraffin was set at 200 degrees Fahrenheit / 93.3 degrees Celsius, and the ribs were kept in the paraffin until bubbles of air had ceased to emerge from the bone (Figure 5.2).



Figure 5.2. Illustration of the paraffin treatment process.

Figure 5.3. Illustration of the cooling period after paraffin

After their paraffin treatment, the bones were allowed to cool for ten minutes (Figure 5.3). The specimens were then evaluated for the two that approximately matched in size – one of the cooked matched with one of the uncooked. Those two were then placed in the oven - Oster countertop convection oven - for twenty minutes at 200 degrees Fahrenheit / 93.3 degrees Celsius; removing most of the paraffin. Again, the bones were withdrawn and allowed to cool (Figure 5.4).



Once all the treatment was complete, each bone had a sample of the shaft

removed using a craft saw - a Zona Junior Hack saw (Figure 5.5).



Figure 5.5. The shaft samples being removed by hack saw.

Once a sample was cut, it was labeled and placed in a bag with its corresponding label. The labeling rubric was:

U1 = uncooked, untreated (control); C1 = cooked, untreated (control);

U2 = uncooked, dipped in paraffin; C2 = cooked, dipped in paraffin;

U3 = uncooked, dipped in paraffin, processed for paraffin removal; C3 = cooked, dipped in

paraffin, and processed for paraffin removal.



Figure 5.6a. The uncooked specimens with their associated samples.

Figure 5.6b. The cooked specimens with their associated samples.

The samples were then grouped by either U to denote uncooked or C to denote cooked, and then placed together by that designation into two bags (Figure 5.6a and Figure 5.6b). These bags, plus a lab report were then sent to the Max Planck Institute in Leipzig, Germany to be submitted to carbon and nitrogen ratio isotopic analysis.

#### The Isotopic Analysis Testing Process

The bone samples were processed at the Max Planck Institute according to the modified Longin method put forth by Brown et al. (1988) and Richards and Hedges (1999:722). This method is summarized briefly here. The samples were ground into a powder form and yielded individual amounts (Table 5.1).

		Collagen	
Sample #	Sample [mg]	[mg]	
U1	116.7	2.4	
U2	157.2	1.2	
U3	151.7	6.9	
C1	98.9	0.4	
C2	131.4	2.8	
C3	107.7	1.1	

Table 5.1. The left column is the labeling rubric. The center column is the yield of sample material available for testing. The right column is the collagen that was derived from the sample material.

The powder samples were then placed in 10 ml of a 0.5 M solution of Hydrochloric acid (HCI). The samples were left for two to five days in 41 degrees Fahrenheit / 5 degrees Celsius for the collagen to be extracted. Once the collagen was separated from the impurities, the impurities were then rinsed away with distilled water. The collagen was then mixed with pH 3 HCI and put into a sealed tube and allowed to gelatinize for a full 48 hours at 167 degrees Fahrenheit / 75 degrees Celsius. Then, the solution was freeze dried. The solution was then put through an ultrafiltration process using filters and a centrifuge to separate the products from any impurities in the collagen gelatin solid. The resulting gelatinized purified collagen was burned in a CHN Anaylizer and the <sup>13</sup>C and <sup>15</sup>N values were measured in a continuous flow isotope ratio monitoring mass spectrometer. These methods are based off the methodology of Longin (1972), which was modified by Brown et al. (1988), with the complete process outlined in Hedges and Richards (1999:722). Each sample was analyzed for carbon 13 (<sup>13</sup>C) and nitrogen 15 (<sup>15</sup>N) per mil. amounts and then calculated for their delta ( $\delta$ ) values to see how they compared to the international standard.

#### **Chapter 6: Results and Discussion**

If the nature of materials is not simple, the nature of changes and decay that have taken place is still less simple. With care, however, and by means of a few elementary physical and chemical tests gross errors on both points may be avoided, and methods of testing will therefore be given

(Lucas 1924:2).

The isotopic analysis showed differences within the same sample, and between samples. Each sample was run twice to eliminate as much error as possible – except number 21938 / C1 because the sample yield was low (Table 6.1).

		Collagen
Sample #	Sample [mg]	[mg]
U1	116.7	2.4
U2	157.2	1.2
U3	151.7	6.9
C1	98.9	0.4
C2	131.4	2.8
C3	107.7	1.1

Table 6.1. This table displays the entire sample yield. The left column is the sample rubric. The center column is the sample yield for analysis, and the right column is the collagen yield. The highlighted area displays the significantly small yield from the C1 sample, the control for the cooked samples.

Sample	13C	15N	%C	%N	C:N
U1a	-13.73	4.49	43.74	11.26	4.53
U1b	-13.90	4.44	45.03	11.35	4.63
U2a	-13.76	4.51	44.52	12.20	4.26
U2b	-13.73	4.57	45.17	12.31	4.28
U3a	-13.72	4.49	41.98	12.07	4.06
U3b	-13.67	4.54	45.63	13.00	4.09
C1a	-13.85	4.38	39.42	10.05	4.58
C2a	-14.26	4.37	48.32	10.22	5.52
C2b	-14.13	4.41	48.36	10.87	5.19
C3a	-14.51	4.49	47.06	9.63	5.70
C3b	-14.47	4.40	47.87	9.78	5.71

Table 6.2. This table displays the complete results from the samples. The left column is the sample rubric, with the letter (a) and (b) determining the initial test run from the second test run. The second and third columns are referencing carbon 13 and nitrogen 15. The fourth and fifth columns show the parts per mil. of each isotope. The sixth column is the carbon to nitrogen ratio. This highlighted rows are the controls, with U1 representing the uncooked specimen, and C1 representing the cooked specimen; only one sample could be created and analyzed from the C1 sample.

The raw amount of the isotopes present is presented in the first two columns headed by 13C and 15N. Each sample's carbon 13 (<sup>13</sup>C) and nitrogen 15 (<sup>15</sup>N) per mil. amounts were calculated for their delta ( $\delta$ ) values to display how they compared to the international standard, which is displayed under the %C and %N headings. The last column shows their ratios of carbon to nitrogen and are beneath the C:N headings (Table 6.2).

The fluctuations in the data seemed small, yet it would be considered of some significance in the realm of palaeodietary reconstructions, where  $\pm 3\%$  or less is their realm of significance (Jay and Richards 2006). However, the results in comparison to the control, the uncooked, untreated sample (U1), are provocative.



The chart (Figure 6.1) displays a rise from the control and then descends when it comes to the cooked, untreated sample (C1a); unfortunately C1 did not yield much collagen for sampling and only one round of analysis could be completed. At this time it cannot be stated with certainty that the cooking process (not in excess of 482 degrees Fahrenheit / 250 degrees Celsius) may have

damaged some of the collagen. Having only one sample available for C1, interpreting its carbon data is inappropriate. However, viewing the remaining samples, in relation to the Control (U1) sample (Figure 6.1), it seems to appear more like an outlier.

The  $\delta$  carbon values (Figure 6.2a) show a similar effect, though not producing as great a decrease. The  $\delta$  nitrogen values (Figure 6.2b) are affected differently than the carbon values, mostly because the treatment applied along with the effects of cooking would influence the carbon more than the nitrogen because of the carbon in both paraffin and the cooking process (Michael P. Richards, personal communications 2011).



Figure 6.2a. The vertical axis shows the negative spectrum of delta values, which displays the distance from the international standard for these isotopes. The samples and their values are averaged and are along the horizontal axis.



Figure 6.2b The vertical axis shows the positive spectrum of delta values, which displays the distance from the international standard for these isotopes. The samples and their values are averaged are along the horizontal axis.

When the two values for each sample (except the C1a sample: its original value is used) are averaged (Table 6.3) and viewed in comparison (Figure 6.3), the difference from the control becomes more visible.



Figure 6.3. The graph above shows the difference between the averaged samples and the control. The averages are taken from the parts per mil (%) values. Those averages are seen in Table 6.3.

	Carbon	Nitrogen
Control	0	0
<b>U2</b>	0.462618232	0.954338512
<b>U3</b>	-0.575762541	1.231669288
C1	-4.962304287	-1.26
C2	3.959528916	-0.76010129
C3	3.084336698	-1.599091665

Table 6.3 The control is considered zero, but the amounts are subtracted from the original averages. The control is shown as zero to further exemplify the difference in the variances. The differences in the carbon values seem to increase and decrease following the treatment, removal, and cooking of the samples. There is a large jump between the uncooked samples and the cooked sample (Figure 6.4). This could be due to the exposure to heat, or to the paraffin. The uncooked samples are exposed to heat during the paraffin treatment; however, this temperature (200 degrees Fahrenheit / 93.3 degrees Celsius) is below what is considered dangerous for collagen samples (Ambrose 1993:73-74). The cooked samples are exposed to both the heat required for cooking and the paraffin processing heat. Thus, it is unknown if the cooking fires or the paraffin treatment altered the carbon values; future experiments must determine which process affected the carbon levels before paraffin is considered as a preservation option for samples that may be subjected for analyses such as radiocarbon dating.



Figure 6.4. The averaged parts per mil. (‰) between the uncooked and cooked carbon values, followed by the averaged per mil. (‰) between the uncooked and cooked nitrogen values.

The parts per mil. values were the main focus because they display the direct amount of carbon and nitrogen isotopes in the samples, and as stated above, they are what would be affected directly by paraffin treatment. Therefore, these values were expected to display the best response for interpretation. The deviation of .5 - 1‰ from the standard established by the surrounding samples holds importance in the field of paleodiets; however, it was difficult to apply in this circumstance since there is not an environmental signature associated with the sample or other consumers with which to compare and create a surrounding sample base line.

The carbon values, once averaged and compared to each other (Figure 6.4) show a possible linear relation, except for the extreme C1 value. That is, they show an increase in their carbon values with paraffin treatment and a decrease in their values with paraffin removal; therefore, this shows the potential for the effects of paraffin to be discounted from the result. The difference between U1 (uncooked untreated) and C1 (cooked untreated) could be due to two explanations: 1) the C1 value was somehow not representative of the sample due to some internal problem within the bone it came from, and thus represents an error in processing, or internal contamination. There seemed to be deviation from the rhythm - the baseline, small increase with paraffin, small decrease with removal of paraffin - of the rest of the sample<sup>1</sup>; 2) the second possible explanation for the dramatic decrease in the C1 sample could be that the remaining cooked samples may have mimicked the signature of the paraffin that was used to treat them. The second explanation can be ruled out because of approximately .5-1‰ except for C1. Given that the second explanation can be ruled out, it appears that the first

<sup>&</sup>lt;sup>1</sup> The samples seem to show an increase followed by a decrease of approximately 1‰ with each treatment. The only deviation from that pattern is the C1 sample. It creates a severe decrease that puts its result in question, and does not provide a trustable base line in accordance with the rest of the pattern.

explanation can be used to support a future larger sample size that would be needed to allow for the possible outliers that can occur in isotopic, or any kind of sample based method analysis.

#### Recommendations for Continued Research

These results are limited by the small sample size. They are also limited by the fact that the research presented here does not have the longevity to demonstrate the inert effects of paraffin over time. The long-term potential has been shown by a study (Luksha  $2010^2$ ) that involved a set of chicken bones treated with paraffin. Over the past two years, the stability of these chicken bones has not been altered, suggesting, at least in the short-term, that paraffin has the potential to hold up as an inert conservation treatment over time.

Given this potential and the preliminary nature of the study presented herein and elsewhere (Luksha 2010), paraffin treatment should be the subject of additional tests to determine whether larger sample sizes and more environmental factors will increase our understanding of paraffin's potential as a conservation treatment. The rubric for future research is recommended as follows. First, more isotopic analysis tests should be run on a larger sample of experimental bone material (i.e. not those from archaeological contexts) to better understand the nature of the anomalous C1 sample noted above. In addition, experimental bone materials should also be submitted for other analyses, such as DNA and bone histology, to see if paraffin alters the bone in any of these cases.

Once these trials are determined sound and the inert effects of paraffin are relatively certain on the experimental bone, then it is necessary to commence with a second step: to determine the long-term effects of paraffin on bone. As noted above, a period of two years has already been established with a small sample of experimental [chicken] bone. In addition, the

<sup>&</sup>lt;sup>2</sup> See Appendix A.

materials Lucas encased in paraffin (1924) might reveal a century's worth of data on paraffintreated bone or other objects. However, these items need to be located, identified, and examined. Thus, a major part of the second major step in future research will require contacting various museums to see if the materials are even available for examination and documentation.

If future investigations determine that paraffin does not cause any major changes over time, then a third phase of research can commence. This third phase will locate and treat objects from archaeological collections that have an abundance of bone material (e.g. faunal remains from buffalo jump sites, historic butcher shops, and/or unprovenienced collections) and that represent a variety of depositional and curation settings. Once additional tests are run on these objects, it will be possible to understand whether and how buried and storage environments may or may not influence the efficacy of paraffin treatments, as well as isotopic, DNA, radiocarbon, or histological analyses.

#### **Chapter 7: Conclusion**

Show me I'm making a mistake or looking at things from the wrong perspective – I'll gladly change. It's the truth I'm after, and the truth never harmed anyone (Marcus Aurelius, translated by Hays 2002:74).

#### Preserving Data Potential

One of the driving forces behind using paraffin as a preservative is its chemical inertia. Conservation treatment can be traumatic for artifacts, whether it is the actual treatment or whether it involves actions that occur years after that treatment (Cronyn 1990:5, 277, 279-280; Johnson 1994). As has been discussed, the conservation philosophy is dedicated to preserving these items for future researchers as technological advances and new research questions and techniques are adopted by archaeology, paleodiet studies, bioanthropology, forensics and the like.

To have samples that can survive the first excavation and subsequent analysis is of the most vital importance. For example, Hardesty (1997), using what was considered "cutting edge" radioimmunoassay analysis in the 1990s, successfully identified the species of a sample of bone fragments from the Donner Party's Donner Lake camp. The radioimmunoassay analysis revealed that some of the bones were human, but the analysis was destructive, which prevented the application of less destructive techniques in the future. Recently, new analyses were carried out on bone recovered from another Donner Party encampment (Alder Creek) that had not been subjected to radioimmunoassay techniques. These "future" examinations are just now being conducted, with histological techniques [drawing from microscopy] helping researchers identify species by only removing thin sections of the bone samples (e.g., Robbins et al.

2011). Additional microscopic advances are being used to interpret bone processing and trauma on the Donner (e.g., Novak 2011) and other skeletal materials (e.g., Novak 2008). Given the potential with bone from this one, small set of archaeological sites, it is clearly important to explore less destructive analytical and conservation options for bone from all contexts. This justifies the importance of subjecting the paraffin alternative to additional tests to determine its parameters, as well as its weaknesses. Paraffin may, indeed, represent a preservative that saves not only the structure, but the data potential that resides within the bone material.

#### Paraffin as a Conservation Option

The purpose of this research project was to conduct a preliminary evaluation of the efficacy of paraffin as an environmentally friendly conservation treatment for bone artifacts and ecofacts. Paraffin was chosen because it has a history as a preservation option for fragile and deteriorating artifacts made of various materials (Lucas 1924). Plus, paraffin is efficient and cost effective. The preliminary research here suggests that paraffin may have a future in preservation for bone materials due to its non-invasive effect on bone and its probable insignificant signature on analysis such as isotopic testing.

The original hypotheses posed for paraffin treatment are reiterated here to discuss the results of the experiments presented above: 1) The first hypothesis stated that the chemical signature (the isotopic per mil.) of bone would not be significantly altered, but muted in a way that could be mathematically accounted for; 2) The second hypothesis asserted that the signature will not be erased, but would be affected in an unpredictable way; 3) the third hypothesis proposed the chemical make-up is not measurable. Although the results suggest that the first hypothesis has the most evidence supporting it, the sample size was too small to confirm this – or

any of the other hypotheses. Even so, the evidence is compelling enough to support further research.

Increasing sample size and applying paraffin treatments to samples from archaeological collections (as opposed to the experimental archaeological collection created for this research) will provide additional lines of evidence to help understand paraffin's capabilities and its limits. Paraffin has already been applied as a conservation treatment on various materials, including bone (Lucas 1924; Rivers and Umney 2003), which means those collections, should, ideally, be available for study to determine the long-term effects of paraffin. In addition to examining those collections and subjecting them to the same isotopic tests here, it would be beneficial to conduct another series of trials on a larger archaeological sample of bone, representing various depositional settings and assorted storage environments, to determine whether there are other factors to be considered when making conservation decisions. Given bone's tendency to be in a constant state of deterioration, not treating bone objects is quickly becoming as much of a problem as faulty treatments. Paraffin is a non-chemical, economically and easily applied treatment that could provide a conservation option for many collections. With the possibility that this treatment is inert, analytical prospects for research would allow greater access to past, present and future collections.

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# Appendix A

