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BONE PRESERVATION FROM ARCHAEOLOGICAL SITES IN WEST-CENTRAL AFRICA

by

Dennis G. Mueller

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The undersigned certify that they have read, and recommend to the Faculty of Graduate Studies for acceptance, a thesis entitled, "BONE PRESERVATION FROM ARCHAEOLOGICAL SITES IN WEST-CENTRAL AFRICA" submitted by Dennis G. Mueller in partial fulfillment of the requirements for the degree of Master of Arts.

M. Anne Katzenberg

Supervisor, Dr. M.A. Katzenberg
Department of Archaeology

Dr. N. David
Department of Archaeology

R. Roy Brouse

Dr. R. Krouse
Department of Physics

DATE 1989-04-14

ABSTRACT

This thesis focuses on the preservation of bone collagen and the potential for stable isotope analyses of samples from two Northern Cameroonian sites, one Iron Age site and one Neolithic site. Two similar collagen extraction techniques were compared, one of which includes a step to eliminate base-soluble humate contaminants through a soak in a basic (NaOH) solution. This comparison involved several variables, including extractable yields of carbon and nitrogen, and ratios between the stable isotopes of these two elements.

The comparison of techniques suggests that humates artificially inflate extractable yields. Elimination of these contaminants produced lower 'collagen' yields, but higher yields of carbon and nitrogen and more negative $\delta^{13}C$ values. It was concluded that, while a certain amount of collagen may be lost during humate removal, the elimination of these contaminants is a necessary step for an acceptable analysis using stable isotopes.

Samples from the Iron Age site generally produced higher yields of 'collagen', carbon, and nitrogen when compared to the Neolithic site. Within the Iron Age site, bones from the lower levels produced higher yields compared to bones from the upper levels. It was concluded that past environmental conditions (i.e. increased groundwater flow), in concert with human activities, have influenced the

degree of bone preservation at the two sites. The few acceptable stable isotope values indicate that humans, cattle and sheep were primarily feeding on C4 vegetation while goats included some C3 vegetation in their diets.

The potential use of stable isotope analysis in West Africa is discussed and it is concluded that this technique can provide additional information to prehistorians. Recommendations for future research in stable isotope analysis are put forth.

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CHAPTER ONE

INTRODUCTION

1.1 Dietary Reconstruction

The field of archaeology has as its most basic activity the recovery of the remains of past hominid populations. These remains consist of cultural materials as well as the hominid skeletal elements. The interpretation of these remains enables the researcher to probe into the 'every-day life' of the people under study. For example, the archaeologist may be able to distinguish patterns indicative of specific religious beliefs or of various modes of interactions between groups. The analysis of materials thought to have been used in food procurement and preparation, as well as the physical remains of ancient meals themselves, allow an understanding of one of the most basic of human needs, the consumption of food. In past traditional hunting and gathering societies, food is obtained directly from the surrounding environment while pastoralists and agriculturalists utilize a more limited segment of their environment but control these particular resources to a greater degree. Direct archaeological evidence of the food items used by the group (e.g. carbonized seeds and butchered animal remains) can also provide the researcher with information concerning the paleoenvironmental conditions of the site. If remains

include or mainly consist of butchered bison bones and the tools used in the processing of the animals, it might be suggested that humans were utilizing these animals as sources of the raw materials for tools and/or for food. When used in concert with other sources of information, models may be developed which relate to certain aspects of the area under study such as paleoenvironmental conditions (e.g. McKinnon, 1986). Any model will depend on not only the type of information available, but also the quality of this information. If, for example, taphonomic processes play a role in determining the form in which the material is recovered, the model will not be representative of the actual conditions unless these factors are understood. Such a situation applies to remains composed of bone, a substance which is actively changing both during life and after burial (Henderson, 1987).

1.2 Methods of Dietary Reconstruction

The analysis of paleodiets has recently assumed a multidisciplinary dimension in that techniques from such fields as geochemistry, physics, biochemistry, biology, ethnography, and archaeology are now used. Traditional techniques have included the study of 1) skeletal remains of hominids, 2) faunal remains, 3) archaeobotanical samples, and 4) food procurement and preparation tools. Within the last few decades, emphasis has been placed on obtaining more precise dietary information through the use

of various chemical techniques. A primary example of this is the use of the stable isotopes of carbon and nitrogen.

Advances in the development of equipment in other fields of study have brought about a revolution in the ways archaeologists and anthropologists can analyze their study materials. Traditional methods have been expanded to include such techniques as the use of scanning electron microscopes to study microwear patterns on teeth and the analysis of trace elements in bone through the use of X-ray fluorescence spectrometry (Walker, 1981; Katzenberg, 1984). Stable isotope analysis has recently risen as a powerful tool with which to reconstruct not only the paleodiets of hominids and fauna but also the environmental conditions within which the hominids and animals lived. Stable isotopes have been for many years the subject of research in physics, geochemistry, and the biological sciences but it was only since the relatively recent work of researchers such as DeNiro and Epstein (1978) and Vogel and van der Merwe (1977; van der Merwe and Vogel, 1978) that their potential use in paleodietary studies have been recognized. These researchers discovered that the isotopic signature of an animal's dietary intake is reflected in its tissues, including the main protein of bone, collagen.

1.3 Stable Isotopes and Anthropology

The use of stable isotopes in an anthropological context is still in its infancy and there are many factors still to be investigated. One important factor is the variation in the preservation of collagen in archaeological samples. Environmental differences can profoundly influence the survival of collagen. How do diagenetic factors affect the elemental composition of bone and protein degradation and how does this in turn affect stable isotope ratios? Laboratory experiments have shown that protein loss is a function of numerous factors, the most important being soil water, pH and temperature (Von Endt, 1980; Carr, 1982). This thesis will examine the diagenetic factors which affect bone protein preservation using samples from two sites located in West-Central Africa.

A second factor involves the stable isotopic investigation of the African continent. While archaeologists have revealed a great deal of Africa's prehistory (Phillipson, 1985), there is a lesser amount of isotopic research, particularly for the West-Central region (van der Merwe and Vogel, 1983). Studies have concentrated on the southern and eastern portions of the continent and deal with the investigation of modern as well as prehistoric human and faunal populations (e.g. van der Merwe and Vogel, 1983; Ambrose, 1986; Ambrose and DeNiro, 1986a and 1986b; Sealy and van der Merwe, 1986).

The reasons for this discrepancy range from the practical (e.g. the difficulty of working for extended periods of time in a hostile environment, deficiencies in available materials or the poor understanding of an area's history) to the political (eg. the problems involved in obtaining permits to work in a 'politically sensitive' areas or the difficulty in obtaining sufficient funds to mount an expedition into West-Central Africa because of the general lack of interest in the area - Shaw, 1978 and 1981).

The scarcity of stable isotope studies in West Africa may also relate to a general lack in the understanding of the application of such studies to this area. This thesis will investigate this deficiency with particular attention directed at the problem of bone preservation in savanna environments of West-Central Africa.

1.4 The Current Study

This thesis will examine three main questions: 1) how can stable isotope and elemental analysis be applied to questions in the study of West-Central African prehistory, 2) how does a savanna environment affect the preservation of collagen and therefore the original stable isotope ratios of skeletal remains, and 3) of the methods currently utilized to extract collagen and to test for diagenetic change in bones, are there qualitative and quantitative

differences in the results that they produce and which have the most practical applications. Two sites from Northern Cameroon will be used in the investigation of these problems. The investigation of the first question will allow for the development of interpretations which will be useful to future researchers working in a savanna region. Descriptions of the two sites will be included along with a general overview of the cultural and environmental history of the study area in Chapter Two. In Chapter Three, the general background to stable isotope analysis is provided, as well as a discussion of the source material for such analysis, the bone protein collagen. Chapter Four includes a description of the sample material and methods utilized in this study and also includes a discussion of the two collagen extraction procedures utilized in this thesis. Chapter Five is a presentation of the results obtained in this study and Chapter Six is a discussion of these results with reference to the problems stated above and concludes with a summary statement.

CHAPTER TWO

A REVIEW OF THE PREHISTORY AND ENVIRONMENTAL SETTING OF NORTHERN CAMEROON

2.1 Introduction

This chapter provides a general background to the cultural history and environmental conditions of the southern portion of the Chad basin, and focuses specifically on the Mora plain of northern Cameroon situated between Lake Chad and the northern end of the Mandara Mountains (i.e. roughly between 11° - 16° north latitude and 10° - 15° east longitude; see Boutrais, 1984 and Figure 2.1). This is followed by a description of the sites from which materials making up the analytical component of this thesis were collected.

2.2 Temporal Setting

The Earliest Traces

Much of the archaeological research in this area has focused on sediments of the last 10,000 years. It is during this time period that major technological innovations appear in human cultures, for example, the domestication of animals and plants and the development of metallurgy as a means of tool production. Prior to this time, evidence for occupation of the Chad basin is scarce due to a lack of hominid skeletal and cultural remains. Archaeological

remains are more abundant during later time periods suggesting that 1) early hominid remains are not preserved due to environmental conditions, 2) poor archaeological visibility or the lack of any substantial investigation of the area have hampered research into the early history of this area, or 3) hominids did not inhabit this part of Africa until relatively recent times. The lack of skeletal remains will necessarily limit the degree to which anthropologists can reconstruct the lifeways of the prehistoric occupants of the Chad basin area using stable isotopes.

The earliest proto-negroid skeleton recovered in West Africa was found at the Iwo Eleru site in Nigeria and is dated at 11,200 +/- 200 years ago (Shaw and Daniels, 1984). Tillet (1985), however, provides evidence which indicates that hominids were in the northern part of the basin (north of 13° north latitude) during early Acheulian times i.e. earlier than 120,000 years ago. At the present time, researchers have been unable to recover hominid skeletal remains in association with the lithic materials.

The Stone, Neolithic, and Iron Ages

Africa in general appears to have followed a pattern of tool development similar to that found in Europe. According to early archaeologists, this validated the use of the traditional Three Age system (with minor changes) in their studies of African prehistory. Africanists presently

debate among themselves whether or not this system of classification is appropriate for their interest area and many have criticized its use with some suggesting other systems (e.g. Monod, 1963; T. Shaw, 1976 and 1977; Posnansky, 1981; Clark, 1982; Sutton, 1982). Since this system is still widely used in the current literature, it will also be utilized in this thesis.

The Stone Age

Information relating to Stone Age, in particular the early portion of this phase, is lacking throughout much of West-Central Africa (A. Smith, 1976; Clark, 1980a; Isaac, 1982; Van Noten, 1982) and is especially lacking for Northern Cameroon (David, 1980). Material has usually consisted of undated surface finds or material in a poor context which is similar in style to the East African Oldowan and Acheulian industries. The Middle and Later Stone Ages are characterized by various lithic industries which have been intensively studied relative to the meager collection of hominid remains. This has resulted in a plethora of names for the tools but relatively little interpretation of how the tools may have used in the procurement and preparation of food. Such indirect evidence for the diet of the ancient peoples is vital as supportive evidence for interpretations made using stable isotope analysis.

The Neolithic

The Neolithic period follows the Stone Age, however, it lacks a precise definition which can be generally accepted by all sub-Saharan archaeologists. The term has not been used in a consistent manner by African archaeologists and this has led at least one researcher to suggest that the term be replaced (e.g. Shaw, 1977). The various definitions do, however, contain several common themes i.e. the presence of pottery, the use of a groundstone tool manufacturing technique, and the appearance of some form of food production system (e.g. Shaw, 1977; Clark, 1980b; Posnansky, 1981a; Phillipson, 1985). The Neolithic arose in West-Central Africa between the fifth and third millennium B.C. and was replaced by an Iron Age period in the last few centuries B.C. (Sutton, 1982; Phillipson, 1985). It should be emphasized, however, that the opening and closing of this period depends on several factors - e.g. the region under study, the amount of contact between regions, local population concentration, and environmental factors such as climate and availability of raw materials (e.g. Porteres and Barrau, 1981). David (1981) emphasizes the fact that the term Neolithic is not a chronological ordering tool, that is, it does not imply a specific time period for the habitation of a site but rather the technological level of the former occupants.

In terms of the Neolithic in the Chad basin, the Borno 38 site in northern Nigeria is relevant. Radiocarbon dates for this site suggests this period began as early as the second millennium B.C. (David, 1980; Connah, 1981). Dates from other Neolithic sites are more recent (500-1 B.C.) but these may represent later parts of the fully developed Neolithic period. Connah (1981) places the transition between the Neolithic and Iron Ages at approximately the beginning of the Christian Era for the site of Daima, located 100 kms northwest of the study area.

The Iron Age

The end of the Neolithic corresponds to the introduction of a new tool technology based on the smelting of iron ore. This time period is better understood in the study area and Africa as a whole compared to the previous periods mainly because the remains of Iron Age settlements are much easier to locate and identify. This is especially true in the area around Lake Chad where older (i.e. greater than 3000 B.P.) deposits are deeply buried under more recent lacustrine sediments formed during the lake's enlargement phase before 3000 years ago (Connah, 1984). The enlarged Lake Chad, commonly referred to as "Lake MegaChad", did not reach far enough south to encompass site 523 of the present study, however, the fluviolacustrine sediments over-laying the single cultural layer of site

506A may have resulted from activities associated with the enlargement of the lake.

The subsistence patterns of the Iron Age populations appear to have been a continuation of the traditions begun in the Neolithic and which are still in evidence today i.e. the use of cereals as staples in the diet with occasional use of other plant and animal products (Ifemesia, 1965a). The presence of stone grinding equipment at site 523 supports this hypothesis. Caution is advised when interpreting the presence of grinding equipment as evidence for the use of grain since researchers are not entirely sure that all grindstones, for example, were used exclusively to grind grains for food preparation (e.g. Lubell, 1984).

2.3 Cultural Identity of the Former Occupants

Establishing the cultural identity of the former occupants of the study sites can provide important clues in the analysis of the recovered cultural remains. The sites can then be viewed within a wider regional perspective thus improving our knowledge of the cultural history of the area. Analogies with modern groups can also aid in understanding the common lifeways of the prehistoric groups. In the case of the present study sites, the materials available for such comparisons are limited and therefore, interpretations must be considered tenuous. Site 506A is particularly lacking in comparative material

although some is available. The pottery, ground and polished stone axes, and terracotta figurine of Bos all suggest a cultural similarity to materials found at sites such as Daima in Borno, west of Lake Chad (David and Sterner, 1987).

Site 523, a larger village site, provides more clues as to its former occupants. Modern informants suggest the site is locally attributed to a poorly known group called the "Sao" or "So" (David and MacEachern, 1988). This name was originally adopted by J. P. Lebeuf in the 1930's to describe the people responsible for a number of mound sites which he excavated in Chad and Cameroon (Lebeuf, 1962; Gauthier, 1973). The Sao are also known through oral traditions (they have been mentioned by Arab travellers although this term may have been used as a 'catch-all' phrase to describe unknown groups of people - see Hunwick, 1976). The informants may have used this term in the later context, that is, they do not know for certain which peoples are responsible for the mounds at site 523, only that they were at the site before the modern groups.

The origin and subsequent fate of the Sao is unknown, however, Gauthier (1973) maintains that this group existed as early as the fifth century B.C. and subsequently founded several kingdoms which reached their peak of power in the early part of the second millennium A.D. Written documents from the earliest travels into the area relate how this

group was in constant conflict with the Kanuri or Kanem/Bornu Empire during the thirteenth to seventeenth centuries A.D. (see Ifemesia, 1965b; Osae et al., 1973; A. Smith, 1976; Hunwick, 1976). Connah (1981) and Ifemesia (1965b) believe that after the Sao were defeated, they were assimilated into the Kanuri while Gauthier (1973) feels that this group dispersed over a wide area with some taking refuge in the Mandara Mountains, mixing with the resident populations who are represented today by the Kotoko people. The hypothesis that the Kotoko are remnants of the Sao has been supported elsewhere (e.g. Eyongetah and Brain, 1974; David, 1980).

The Sao may have been the original occupants of at least one of the study sites. Local Fulani informants living in the Mehe Djiddere hamlet believe that site 523 was originally used by the Sao (David and MacEachern, 1988). Unfortunately, more information could not be evoked from surrounding groups.

Little is known concerning the lifestyles of the Sao. Archaeologically recovered material includes terracotta figurines, stone ornaments, copper and bronze artifacts, and the large Sao pots, thought to have been used for the storage of water and/or grains or as burial containers (Lebeuf, 1962; Shaw, 1965; Andah, 1981). Unfortunately, direct evidence for agriculture is lacking in this area (the Daima site has provided evidence in the form of a

cluster of carbonized sorghum grains dated from approximately A.D. 800 - see Connah, 1981:188-189). Faunal remains recovered from Sao sites include domesticated animals such as cattle, sheep, and goats. It is likely that these people followed the typical dietary strategy of the times - cultivating various cereal grasses as well as raising livestock and fishing. Palynological studies of Sao sites and stable isotopic analysis of the few skeletal remains would aid in the interpretation of the diets of the former inhabitants of these sites. Stable isotopes have been used in East and South Africa to investigate whether or not the diets of various modern and archaeological groups can be identified (eg. Ambrose, 1986; Ambrose and DeNiro, 1986a; Sealy and van der Merwe, 1986). These researchers have demonstrated that groups exhibiting a pastoral economy can be differentiated from groups using different methods of subsistence. This fact may allow researchers to study the introduction of pastoralism into the present study area provided that an adequate skeletal population can be recovered.

2.4 Environmental Setting

Geography and Climate

The study area is located in the southern portion of the Chad basin, an area in West-Central Africa roughly located between 8° and 16° north latitude. A series of mountain ranges form the edges of the basin and include the

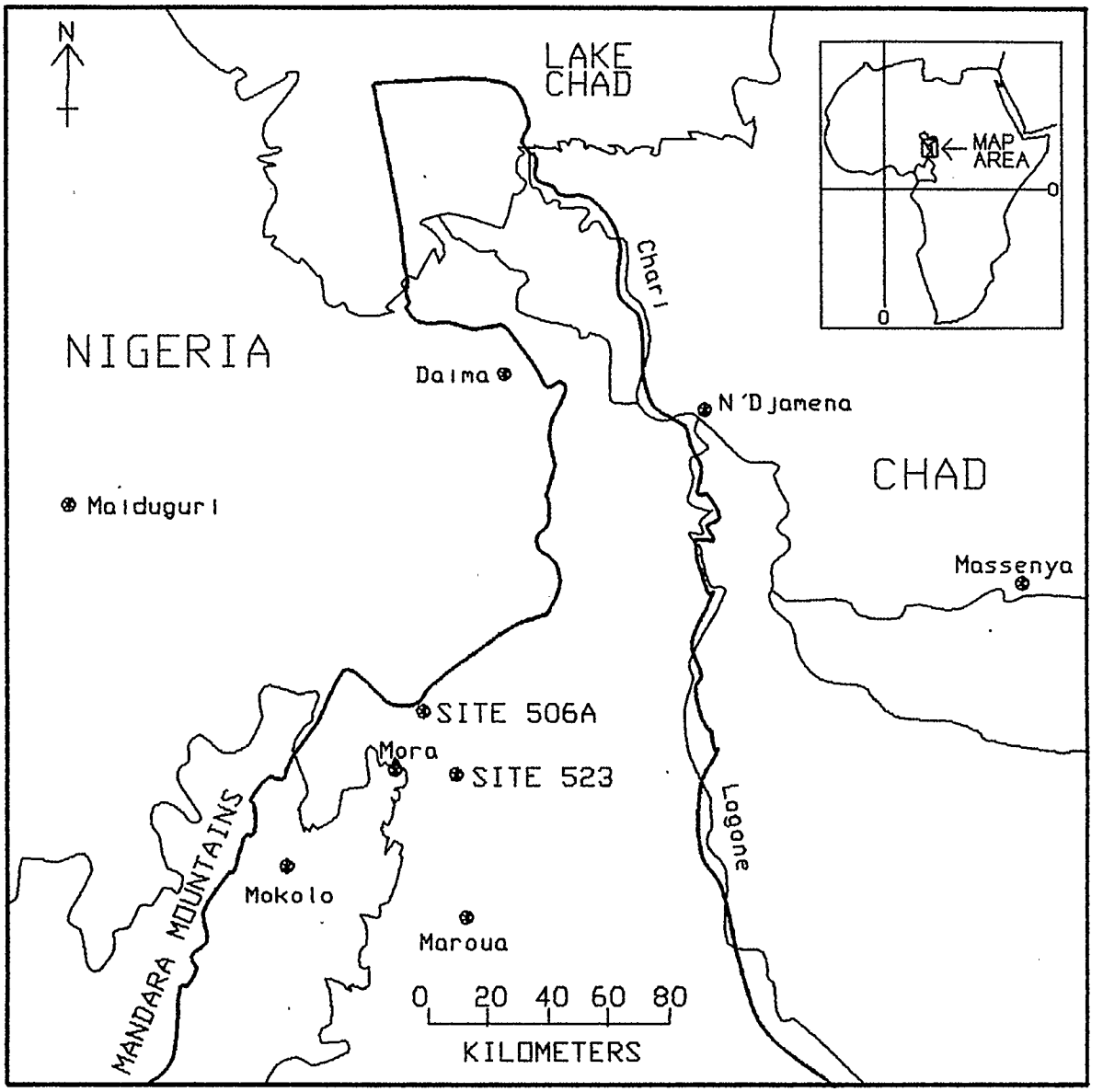


Figure 2.1 Northern Cameroon and surrounding areas. (based on Connah, 1981:20)

Mandara Mountains on the southwestern side. The present study area is situated between the lake and the Mandara Mountains, two different geographical entities which have had, and continue to have, significant effects on the climate of the area. The importance of the mountain range's influence, however, may have been lessened during the Holocene compared to factors which were causing major continent-wide climatic changes at the same time. The range was also important to the prehistoric inhabitants of the area since it served as a source of raw material for Later Stone Age peoples and may have assumed the role of a 'refuge area' for human groups during times of poor climatic conditions or during invasions from outside the area (Connah, 1981).

Lake Chad has played an important role in the last 10,000 years of West-Central African prehistory in part because of the major variations in its levels and extent brought about as a response to climatic fluctuations. The area containing the two sites under study is predominantly covered by alluvial soils (Boutrais, 1984). These include, among others, vertisols or hydromorphic soils (Jones and Wild, 1975; Kowal and Kassam, 1978; White, 1983), which are composed primarily of a mixture of sand and clay and which were laid down during past episodes of lake enlargement and increased output by its associated rivers (Pugh and Perry, 1960). This type of soil is relatively fertile allowing the

growth of various grain crops such as sorghum and other millets (Phillips, 1959). During the periods in which the lake was at its largest size, therefore, it would have had important effects on the study area e.g. increasing the humidity and rainfall, decreasing the amount of arable land surrounding the lake, and generally making some areas inhospitable to human habitation due to flooding.

This section will describe climatic conditions for approximately the last 100 years which, when combined with paleoecological data, allows the construction of a model of the paleoclimatic conditions (see section 2.5).

A general survey of available data indicates that the study area is in a highly variable rainfall zone: Mora and Mokolo, at the northern and western bases of the Mandara Mountains, average 706 and 960 mm/year respectively, Maroua to the east of the mountain range averages approximately 800 mm/year, N'Djamena has 613 mm/year, Massenya averages 788 mm/year, and Maiduguri in northern Nigeria has an annual rainfall of 640 mm (Lebedev, 1970; MacEachern, n.d.; White, 1983 - see Figure 2.1 for site locations). These average precipitation values are the result of measurements taken over a varying amount of time ranging from a few years to several decades, however, a comparison of the values recorded earlier in this century to those obtained more recently reveals few significant differences (Kowal and Kassam, 1978). The individual site records, along with

more general rainfall zone information, suggests that the study area receives approximately 750 to 800 mm of precipitation annually.

The distribution of the annual rainfall throughout the year is extremely important, particularly in relation to the plants which grow in the study area. Maiduguri is representative of the unimodal rainfall distribution pattern which is found in and around the study area. The rainfall slowly increases in June, reaches a peak during July and August, and declines during September with the remainder of the year being dry (Kowal and Kassam, 1978; White, 1983; Hayward and Oguntoyinbo, 1987).

The seasons are partially dependent on the winds that blow through the area during different parts of the year. Winds from the northeast (the 'Harmattan' winds) bring dry air into the area resulting in cool winters. Warmer winds from southwest, laden with moisture, bring most of the rain to the study area (Hopkins, 1965; Ojo, 1977; Kowal and Kassam, 1978).

Flora

Geographers and ecologists have long recognized that Africa is composed of a number of environmental zones which follow a relatively regular pattern from the equator towards the poles. The definition of these zones has been a subject of debate for many decades resulting in a plethora

of systems. All of these systems, however, rely on a central theme, the diagnostic flora found in a region. In schemes using generalized terms such as 'savanna' there frequently is a lack of a single, adequate definition for such terms (e.g. see Swami, 1973 pp. 16-20 for examples of West African 'savanna' boundaries utilized by various authors). The criteria used to define the terms can vary in each specific instance depending on the purposes of the investigator.

While there is disagreement among researchers on the boundaries of the various vegetational zones and the actual definitions of these zones, there is a general agreement on the flora found in the study area. The majority of grasses belong to the C4 photosynthetic group (see Chapter 3 for a discussion of the various modes of photosynthesis utilized by plants) and are used as a source of food for both animals and humans e.g. Cenchrus spp. (African foxtail), Chloris spp. (Rhodes grass), Echinochloa spp. (Antelope grass), and Panicum spp. for animal pasture and Pennisetum spp. and Sorghum spp. for human consumption (Letouzey, 1968a and 1968b, Downton, 1971 and 1975, Purseglove, 1972, Church, 1980). There are also a limited number of C3 grasses in the study area, however, they are not as important as food sources compared to the C4 grasses (Walker, 1980).

The study area also includes a large number of economically important shrub and tree species, the most important of which include species of the Acacia genus (Letouzey, 1968; Purseglove, 1968; White, 1968; Boutrais, 1984). The leaves of the Acacia albida tree are frequently used as animal fodder during the dry season and have been recognized as providing an additional source of nutrients for crop plants (Ahn, 1970; Harlan et al., 1976). Examples of other common shrubs and trees include Adansonia digitata (baobab), Balanites, Daniellia, Ficus, Hyphaene, Khaya, Philostigma, Terminalia, and Zizyphus species (Purseglove, 1968). For the local inhabitants, each of these plants serves a different function ranging from fodder for animals to acting as a source of shade. Trees and shrubs, however, are not generally considered to be a major source of food for humans in the study area.

Paleoecological studies suggest that the study area may have been more extensively forested in the past and that the current 'open savanna' environment may have been induced by the activity of humans (see David, 1980). These activities consist primarily of the use of fire to keep tracts of land clear for cultivation which in turn reduced the plant species diversity in favor of fire resistant forms such as annual grasses (Ahn, 1970; Trollope, 1982). The burning of large areas of grassland and the clearing of forests have been most important, however, in the last

several centuries (Moreau, 1966; Phillips, 1968) indicating that the present environmental conditions are not fully indicative of conditions during early periods of occupation nor of the modern natural climactic vegetation.

Fauna

The fauna which comprises the savannas of Africa has been seen to represent one of the largest collections of animal biomass in the world, in particular the ungulate group of mammals. The population sizes of native species were considerably greater in the past and have been reduced in recent times due to human alteration of the environment (Bourliere, 1963; Church, 1980). Hunters of three or four thousand years ago certainly faced a greater abundance of animals relative to modern hunters. Besides the large quantities of ungulates, other animals were also available to the prehistoric inhabitants e.g. birds, freshwater fish, reptiles, amphibians, and freshwater molluscs, gastropods and bivalves (LeMoine, n.d.). Unfortunately, there is a lack of detailed studies concerning the changes in species composition brought about by humans, a situation that archaeological research can help to alleviate (Bourliere, 1963; Sidney, 1965; Curry-Lindahl, 1968; Cloudsley-Thompson, 1969).

A preliminary analysis of approximately one quarter of the faunal assemblage recovered from site 523 revealed that the vast majority of the bones were from domesticated

cattle, sheep and goats (LeMoine, n.d.). If this sample is representative of the entire assemblage, and an cursory inspection of the remaining three quarters indicates that this is true, the former occupants of site 523 may have only hunted wild animals to supplement their diet or simply as a change from their usual reliance on grains, cattle, goats, and sheep. Wild animals, therefore, do not seem to have held a great deal of importance in the diets of the former occupants of the site.

The material from the Neolithic site 506A has fewer skeletal elements and these are of poorer quality relative to the 523 collection. Thus the species present at the site cannot be described in the same detail. David and MacEachern (1988), however, state that "(co-investigator) Wilson has identified bones that could be of domestic cattle ... and is confident of the presence of domestic sheep/goat." (p. 59). A clay figurine representing Bos which was recovered during the excavation supports this hypothesis. The proportion of domesticated versus wild animal species cannot be estimated for this site and so it is unknown to what extent the former occupants relied on the two forms of animals for food.

2.5 Paleoclimatological Reconstruction

The climate of the study area during the habitation of the sites investigated in this thesis obviously cannot be examined in the same manner as in our modern society.

Written records left by Arabic or European explorers are limited in that the descriptions can be ambiguous and the location names quite different from present names. Devices capable of recording weather patterns for any length of time have only been in use since the late 17th century (Lamb, 1972). Several approaches have been developed, however, in order to correct this situation. One such approach is the use of historical analogy (see Rognon 1979; Nicholson, 1980). While this approach is useful, it does not allow for unrefutable interpretations, and thus caution is advised when using the models generated by this approach.

While early researchers believed that Africa as a whole showed similar patterns of paleoclimatic conditions to those of Europe (van Zinderen Bakker, 1967a and 1967b; Butzer and Cook, 1982; cf. Evenari, 1984), further research has revealed that European sequences are not applicable to the sub-Sahara. Part of this research involves Lake Chad which has been the focus of a great deal of paleoclimatological analysis both in isolation and in relation to other areas of the continent. Much of this research has concentrated on periods of time which are much older and longer than the intervals associated with the two study sites (e.g. van Zinderen Bakker, 1967a and 1967b). There is, however, a reasonable amount of information dealing with the last 10,000 years. A further complication

involves what some researchers perceive as the interchangeable usage of the terms paleoenvironment and paleoclimate. These two terms have very different meanings - the former involves a reconstruction of all ecological factors in an area (e.g. floral and faunal patterns, relationships between these variables as well as climate). Paleoclimatological reconstructions, conversely, involve the study of the climatic conditions which prevailed in the past (e.g. temperature range, amount of rainfall and humidity). This thesis will follow the suggestion of B. Shaw (1976) in that information pertaining to the reconstruction of the paleoclimate will be used due to the lack of material fully describing the past flora and fauna of the study area.

Perhaps the most prominent researchers involved in the area of paleoclimatological reconstruction are S. Servant-Vildary, in association with M. Servant, and J. Maley (Maley, 1973; Servant-Vildary, 1979; Servant and Servant-Vildary, 1980; Maley, 1981: see also Moreau, 1966; Grove and Warren, 1968; Burke et al., 1971; Livingstone, 1975; B. Shaw, 1976; van Zinderen Bakker and Maley, 1979; Butzer and Cook, 1982; Sutton, 1982). These researchers have utilized an array of techniques in their studies including palynology of lake sediment cores, liminology, diatom studies, and geographical landforms. The dates should be viewed as approximations only. The changes in lake levels

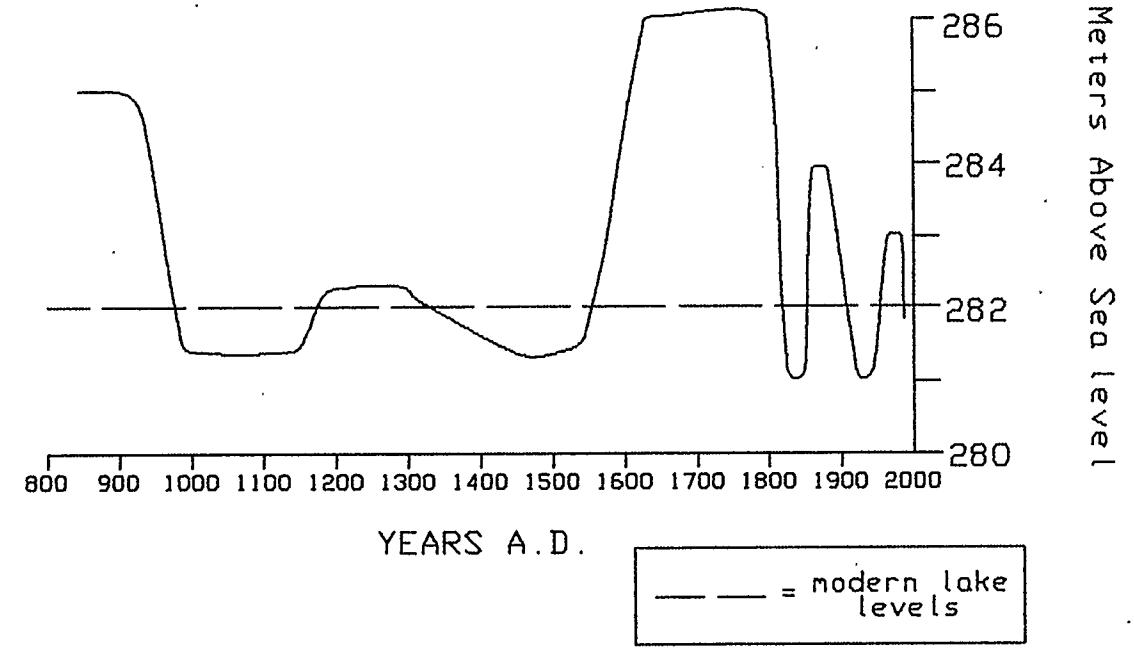
evident in more recent times do not necessarily indicate a shift to wetter or drier climatic conditions (see below).

Figure 2.2 illustrates that for the period of occupation of site 523, the Lake Chad levels have fluctuated around the present day level. This suggests that rainfall levels also varied around the lake or within the area of the Chari and Logone rivers, the major sources of water for the lake. The information pertaining to the occupational period for site 506A is less well understood than for more recent times. The fluviolacustrine deposits lying over top of the cultural level at site 506A may represent either the minor 'lacustrine transgression' which Maley (1981) describes at about 1750 years ago or a more localized occurrence. Site 523, in contrast to site 506A, is located on the southern side of the Bama ridge (a 400 km long ridge running through Maiduguri and Bama, Nigeria and continuing in a southeasterly direction through northern Cameroon). This ridge is commonly believed to represent a Lake MegaChad strandline of approximately 5000 to 6000 years ago (Grove and Pullan, 1963; van Zinderen Bakker and Maley, 1979; cf. Durand, 1982). It is situated about 40 m higher than the present-day lake level of 282 m above sea level suggesting that Lake MegaChad rose a considerable amount in the past.

Figure 2.2

Lake Chad levels since A.D. 900

(after Maley, 1973; Sutton, 1982)



Caution must be used when interpreting rising lake levels since this does not necessarily indicate a more humid climate for the area directly around the lake. It is equally possible that the increase in lake levels resulted from an increase in rainfall in the area of the rivers that feed the lake. Thus, the headwaters of the Logone and Chari rivers, the main suppliers of water to Lake Chad (Grove and Warren, 1968), may have received more rainfall rather than the lake itself (Monod, 1963; van Zinderen Bakker and Maley, 1979).

While the forces that caused the increased lake levels may be difficult to identify, the effects of increased lake area on the regions surrounding the lake can be more fully understood. An increase in humidity and rainfall are the most obvious effects of an enlarged lake and these would have in turn altered the floral (e.g. a shift to a higher proportion of C3 plants) and animal distribution patterns. Occupants of site 506A may have suffered if the lake reached the Bama ridge since the site would have been inundated by the rising lake or flooding rivers resulting in the abandonment of 506A. Flooding of sites would also have had a significant effect on the preservation of previously buried skeletal remains and this will discussed further in Chapter Three.

This discussion of the paleoclimatic conditions of the study area presents the general picture of a relatively

stable climate (in comparison, for example, to earlier periods of widespread, dramatic changes) for possibly 3000 years. Minor fluctuations would have had important local and temporary effects on the local environment but sudden, wholesale changes of the flora, for example, would not have occurred. Any changes would have been occurring at a gradual rate during the period after the last major expansion of Lake Chad some 5000 to 6000 years ago. Human intervention sped up this process, especially during the Iron Age when forests would have been cleared using iron tools instead of the less efficient stone axes of the previous periods as well as through the use of fire (David, 1980). Thus, the present savanna zones of the Sudan region may be secondary features which resulted in part from human activities of the last few hundred years (Hopkins, 1965; Ahn, 1970). It is possible that instead of human intervention being the main cause of the 'savannification' of the Sudan region, natural processes are to blame (see Cumming, 1982; Medina, 1982). Obviously, much more research is required to understand the role which humans have played in controlling the environments in which they live.

2.6 Site Descriptions

A long-term project was organized in 1984 which was to deal with the study of the cultural history and ethnology of several groups of people in an area of Northern Cameroon, West Africa (David and MacEachern, 1988). The

Mandara Archaeological Project, under the direction of Dr. N. David and with the assistance of Dr. M. Wilson (co-investigator), began with a season of archaeological fieldwork in the summer of 1984 and continued during 1986 with an ethnoarchaeological study. The initial field season involved archaeological surveying in and around a 2000 square kilometer area in and adjacent to the northern Mandara Mountains. Over 70 sites were discovered and two of these were test excavated: a Neolithic camp site (site 506A - UTM 1240.3/413.2) and an early Iron Age occupation site (site 523 - UTM 1215.5/426.0).

The Neolithic site, 506A is located approximately 1 km. north of the Bama Ridge and near the Nigerian border. It consists of a single cultural horizon 20 cm. thick which is positioned between fluviolacustrine deposits. The site may represent a camp over 1 hectare in size which was occupied for a relatively short period of time. Unfortunately, no charcoal for radiocarbon dating was recovered. A sufficient quantity of pottery was excavated allowing a series of thermoluminescence dates to be extracted by Alpha Analytic Inc., however, these produced rather erratic values (between 1940 +/- 190 b.p. and 640 +/- 70 b.p.). Based on various factors such as the nature of the site and the supposed time of arrival of iron working into the area, David and Sterner (1987) reject the two later dates and accept the earliest only as a minimum

age for the occupation. The faunal remains are highly fragmented, however several bones were found to be in an acceptable state of preservation such that an identification to the Class level could be made. Support for the presence of domesticated animals at the site was discovered in the form of a terracotta figurine of Bos.

Site 523, also known as the Mehé Djiddere site after the modern Fulani hamlet found nearby, is located approximately 10 kilometers to the northeast of Mémé on the Mora Plain in northern Cameroon (Figure 2.1). It is the largest of four 'Iron Age' complexes found at this locality and consists of at least 17 mounds grouped into an area of approximately 7 hectares. It has been suggested that if these mounds are contemporaneous, the site may represent a very large village or even a town (David and MacEachern, 1988).

The mounds themselves vary in size from less than 1 m to over 4 m in height and from 15 m to 50 m in diameter. Two test excavations were carried out at site 523, a 3 x 1.5 m trench on the largest of the mounds, number I, and a 3 x 1 m trench on the much smaller mound VII. A 1 x 1 m pit was also excavated between mound I and its closest neighbor. David and MacEachern (1988) describe the mounds:

Table 2.1

Radiocarbon Dates for Site 523
(from David and Sterner, 1987)

UNIT	LAYER(cm)	LAB NUMBER	DATE B.P.
IA:2	30-45	S-2677	230 ± 100
IA:3	60-75	Ly-3818	1160 ± 140
IA:4	90-105	S-2676	575 ± 95
IA:4	150-165	Ly-3819	1600 ± 110
VIIA:1	20-35	Ly-3817	790 ± 100
VIIA:3	0-15	S-2674	1020 ± 165

> Mound I gave a sequence over 4 m in depth - the base was not quite reached - consisting essentially of midden deposits with no evident stratification in its upper part, and of an extremely complex series of pits, other features and largely disturbed structural remains below.(p. 60)

The authors go on to describe mound VII as having a "similar but shorter and less complex sequence." (ibid, p. 61) suggesting that this mound was occupied for a shorter time period than mound I.

The mounds produced a large quantity of material consisting primarily of pottery, iron slag and burnt clay (including tuyere fragments and pieces of burnt daub). An analysis of the pottery is currently being undertaken by E. Wahome of the University of Calgary. A large quantity of material associated with iron production was recovered and this may indicate that the site was a major supplier of iron tools to the surrounding area (David and Sterner, 1987). Stone grinding equipment and other cultural remains were also recovered but in much smaller amounts relative to other materials. Faunal remains were also collected and partially analyzed by G. LeMoine of the University of Calgary (LeMoine, n.d.) the results of which will be discussed in Chapter 4.

Charcoal samples were collected from several levels of the two mounds and submitted to two different laboratories for radiocarbon analysis. The results, presented in Table 2.1, indicate that there is a degree of discrepancy between

the two laboratories, a condition which is difficult to explain. David and Sterner (1987) suggest that the Lyon (Ly) series of dates are acceptable while the Saskatchewan (S) dates are too young based on several factors:

> ...a) the lack of oral traditions of historical information about its (site 523) occupants, b) the considerable depth of the deposits (mound I has a depth of over 4 m.), and c) the paucity of substantial architectural remains that might account for a rapid buildup of the mounds. (p. 4)

The site's occupational period, however, can be confidently placed in the Iron Age.

CHAPTER THREE

THEORETICAL BACKGROUND

3.1 Introduction

The incorporation of stable isotope analysis into anthropological studies has benefitted from earlier research in areas such as physics, geochemistry, biology, and, more recently, medicine. The connection between isotopes and paleodietary reconstruction was established when researchers observed that 1) the different modes of photosynthesis which exist in the plant kingdom result in specific isotopic ratios in plant tissues (Bender, 1968 and 1971), and 2) animals retain the isotopic signature of their food within the collagen of bone (DeNiro and Epstein, 1978 and 1981). The 1970's witnessed a substantial increase in the number of studies being conducted on the application of such techniques to the study of past human populations. These studies have covered many different areas including North and South America, Denmark, and South and East Africa, however, there is a significant lack of isotopic work in West-Central Africa.

This chapter will provide the theoretical basis for the use of stable isotopes in paleodietary studies. The general characteristics of stable isotopes, with particular reference to the elements utilized in this thesis, carbon

and nitrogen, will be discussed. A description of the source material used in paleodietary studies, collagen, will follow.

3.2 Stable Isotopes

Elements occur in several forms called isotopes, containing the same number of protons and electrons but varying in the number of neutrons located in their nuclei. These variations in the neutron number result in different atomic weights and mass numbers. For example, in carbon the ^{13}C and ^{12}C isotopes both contain six protons and six electrons but differ in their number of neutrons (^{13}C contains seven neutrons while ^{12}C contains only six). Isotopes with larger atomic masses are known to react more slowly during chemical reactions relative to the 'lighter' isotopes. This results in fractionation or a change in the relative isotopic abundances from one step in a chemical reaction to another within the organism's tissues. Fractionation of carbon and nitrogen isotopes will vary between plant species and it is this which forms the basis of the isotopic analysis of paleodiets (Lerman, 1975 - see also the following sections). Isotopes also vary in their chemical stability with some remaining unchanged for long periods of time whereas others undergo a much more rapid change and are converted into other forms (i.e. they are radioactive). This has led to the incorporation of the terms stable and radioactive to describe these isotopes.

The later forms (e.g. ^{14}C - see review by Taylor, 1987) are of particular importance in the dating of organic material recovered during archaeological excavations.

In paleodietary studies, the relative masses of the ^{13}C, ^{12}C, ^{14}N, and ^{15}N in an sample are measured and the ratios of ^{13}C to ^{12}C and ^{15}N to ^{14}N are then compared to those found in a standard. These isotopes can then be compared by determining the $\delta^{13}C$ and $\delta^{15}N$ of the sample. These values are reported as per mille (o/oo) and determined through the use of the following formulae:

$$\delta^{13}C = \frac{^{13}C/^{12}C\ [sample] - ^{13}C/^{12}C\ [standard]}{^{13}C/^{12}C\ [standard]} \times 1000$$

and

$$\delta^{15}N = \frac{^{15}N/^{14}N\ [sample] - ^{15}N/^{14}N\ [standard]}{^{15}N/^{14}N\ [standard]} \times 1000$$

The universally accepted standards are a Cretaceous marine shell (belemnite) from the PeeDee formation of South Carolina (PDB for the $\delta^{13}C$ scale) and atmospheric nitrogen (AIR for the $\delta^{15}N$ scale). These standards have delta values of zero and all sample $\delta^{13}C$ and $\delta^{15}N$ values are given in relation to the values of these standards. All known $\delta^{13}C$ values from organic sources are negative since the standard usually has a higher ^{13}C content while the $\delta^{15}N$ values are

generally greater than zero (but can also be negative) due to samples having a higher ^{15}N content than air.

The isotopes are measured through the use of a mass spectrometer, a device which "... separates charged atoms and molecules on the basis of their masses based on their motions in magnetic and/or electrical fields." (Hoefs, 1987:19). Thus, molecules of $^{15}N^{15}N$ and $^{15}N^{14}N$ have different atomic masses which will in turn cause them to follow different paths in magnetic or electrical fields generated by the mass spectrometer. The two molecules are separated from each other, the amounts of each are determined, and from this, the quantities of the various isotopes can be calculated. A number of texts are available for a more detailed discussion of the operation of mass spectrometers (e.g. Hill, 1972).

3.3 Carbon

Carbon has been found to exist in a number of isotopic forms with ^{12}C, ^{13}C, and ^{14}C making up the majority of the earth's carbon reservoirs. The ratios of these isotopes vary with ^{12}C making up the majority of the environmental carbon and ^{14}C being the rarest of the three (the actual ratio is approximately $1:10^{-2}:10^{-12}$ or 98.9% ^{12}C, 1.1% ^{13}C, and 10^{-10}% ^{14}C - see Taylor, 1978 and Bolin *et al.*, 1977).

The Carbon Cycle

In order to fully appreciate the use of carbon in paleodietary studies, it is necessary to understand the way in which this element is cycled through the ecosystem. The biosphere contains only a small portion of the global carbon content and the carbon that is trapped within living organisms is eventually returned to one of the other reservoirs (e.g. the lithosphere, hydrosphere and atmosphere). Recently, human interference has caused the reservoirs to vary significantly in their carbon content e.g. the increase in the CO_2 content of the atmosphere since the Industrial Revolution of the mid-1800s (Bolin *et al.*, 1977). This can have important ramifications in stable isotope research since the isotope ratios may have also varied with the carbon content, thus altering the isotopic signature of the carbon source used by primary producers.

Researchers have also discovered that various components within each reservoir may contain different amounts of carbon as well. Whittaker and Likens (1973) and Ajtay *et al.* (1977) have shown that for any given ecosystem, plants make up a much larger portion of the total biomass relative to animals, although the ratios between the two groups can vary slightly. Since plants are much more abundant, they will also contribute more of the carbon to the ecosystem.

Plants, Animals, and Carbon Isotopes

Biologists have established the presence of at least three modes of photosynthesis in the plant kingdom. The first photosynthetic mode involves the manufacture of phosphoglycerate ($[C_3O_4H_4(PO_3)]$) in the initial step. The plants which utilize this mode (e.g. many trees, shrubs, legumes, and temperate grasses) are termed C3 plants because of the three carbon atoms making up phosphoglycerate. The second photosynthetic condition produces a four carbon molecule called oxaloacetic acid ($C_4O_5H_4$) in its initial step and the plants using this mode (e.g. tropical and arid environment grasses as well as many economically important grain crops) are termed C4 plants. The third mode, Crassulacean Acid Metabolism or CAM photosynthesis, occurs in succulent plants such as cacti. This mode uses both of the two previous modes, C3 during the day and C4 during the night (Raven et al., 1976).

During photosynthesis, plants discriminate against the ^{13}C isotope in a process called fractionation (Bender, 1968 - see below). It is this process which forms the basis of isotopic research in paleodietary studies since C3 and C4 plants differ in their discrimination of ^{13}C. The C3 plants generally have $\delta^{13}C$ values in the range -22 o/oo to -30 o/oo (mean = -26.5 o/oo) while C4 plants fall between -9 o/oo and -16 o/oo (mean = -12 o/oo)(van der Merwe and Vogel, 1983). These ranges vary slightly (cf. Bender et

al., 1981), however, the C3 and C4 ranges do not overlap. The range covered by CAM plants, on the other hand, includes both the C3 and C4 ranges. This separation of the isotopic values provides researchers with the means of identifying, in broad terms, the types of plants eaten by prehistoric animals (DeNiro and Epstein, 1978; van der Merwe and Vogel, 1978).

Studies have shown that there is an enrichment in $\delta^{13}C$ values when the isotopic signatures of an animal's diet and various body tissues are compared (DeNiro and Epstein, 1978; Vogel, 1978). Van der Merwe (1982) concluded that bone collagen is enriched by +5.1 o/oo over the animal's diet, that is, collagen is 5.1 o/oo lighter that the diet. This factor must be taken into account during the reconstruction of paleodiets.

Fractionation of Carbon Isotopes

Extrapolation of the isotopic data with the goal of producing a possible set of diets for an long dead animal is a complex undertaking. Both plants and animals discriminate in their uptake of the various isotopes. Many hypotheses have been presented dealing with the mechanism of these processes and the most widely accepted hypothesis involves the ribulose biphosphate carboxylase enzyme of plant photosynthesis (Park and Epstein, 1960; Abelson and Hoering, 1961; Black, 1973; O'Leary, 1981). Because of

their different atomic masses, ^{12}C and ^{13}C react differently during chemical reactions with ^{13}C reacting more slowly than ^{12}C. This will result in the formation of molecules with lower quantities of ^{13}C compared to ^{12}C and 'lighter' isotopic signatures (i.e. a more negative $\delta^{13}C$ value relative to the atmospheric CO_2). The degree to which the ^{13}C is discriminated against is dependent on local reaction conditions such as pH, temperature and other variables (O'Leary, 1981). The passage of CO_2 from the atmosphere into plant cells via the diffusion process will also play a role in isotopic fractionation, again due to the differences in atomic masses between the isotopes (Park and Epstein, 1960).

Food that is ingested by animals must pass through a variety of stages before it can be utilized and these stages may also result in a fractionation of the carbon isotopes. Further research pertaining to the possibility of trophic level effects on carbon isotope ratios is also required.

Uses of Carbon Isotopes in Paleodietary Studies

The uses of stable isotopes of carbon in archaeology have been summarized by Bumsted (1981), van der Merwe (1982) and DeNiro (1987). Carbon isotopes have been used in paleodietary reconstructions to determine the importance of marine versus terrestrial foods (e.g. Chisholm *et al.*, 1982

and 1983b) and of C3 versus C4 plants (e.g. Lovell et al., 1986a), to identify the introduction of various crop species into certain areas of the world (e.g. Schwarcz et al., 1985), or the migration of populations within an area (e.g. Sealy and van der Merwe, 1986). Herbivore diets have also been analyzed through the use of carbon isotopes since such an analysis will aid in the interpretation of the diets of the humans which used the herbivores as food items (e.g. Vogel, 1978; Tieszen et al., 1979a; Tieszen and Imbamba, 1980). Carbon isotopes are also useful in paleoenvironmental reconstruction (e.g. McKinnon, 1986).

3.4 Nitrogen

Nitrogen, like carbon, is vital in the maintenance of any living organism. This element is most abundant within the building blocks of all proteins, the amino acids and is important in the structure of the nucleic acids of D.N.A. and R.N.A. (Stewart et al., 1983). Like carbon, nitrogen is passed through the biosphere in the food that is consumed by animals (which in most cases is mainly proteinaceous in nature) and eventually it is returned to reservoirs outside the biosphere. Nitrogen has two naturally occurring stable isotopes, ^{14}N and ^{15}N, the former being more common than the later (approximately 99% of the stable isotopes of nitrogen are represented by ^{14}N - Parwel et al., 1957).

The Nitrogen Cycle

The cycling of nitrogen through the various components of the earth's ecosphere is not as well understood as is the cycling of carbon. The main reservoir for this element is the lithosphere which contains approximately 93.8% of all nitrogen, the remainder is contained within the atmosphere (6.2%), the hydrosphere (.04%) and the biosphere (.001%) (Hubner, 1980, Rosswall, 1981). Nitrogen obtained by plants from the lithosphere can be used directly, however, the N_2 gas in the atmosphere must first be changed into a form which plants can use and this is accomplished by the process of nitrogen fixation. The source of the nitrogen, whether it be from the lithosphere or the atmosphere, will eventually determine the isotopic value of the plant tissues.

Nitrogen Fixation

Nitrogen, unlike carbon, is primarily introduced into the biosphere through the action of various families of bacteria, some of which live freely in the soil (e.g. the Nitrosomonas and Nitrobacter families) and others which maintain a symbiotic relationship with plants (e.g. the Rhizobium genus). It is these microorganisms which convert or 'fix' the chemically unreactive nitrogen of the atmosphere and lithosphere into either nitrate (NO_3^-) or ammonium (NH_4^+), forms of nitrogen that plants can utilize

in the production of amino acids, proteins, nucleic acids, and chlorophyll (Raven et al., 1976). These compounds are absorbed into the root system of the plant (the exact mechanism is unknown - see Lewis, 1986) where they are either stored or shuttled to appropriate areas of the plant such as the shoots (Pate, 1983).

Plants, Animals, and Nitrogen Isotopes

The theory involved in stable nitrogen isotope analysis is similar to that of the carbon isotopes in that plants fractionate isotopes, with discrimination against ^{15}N. Leguminous plants such as peas, beans and alfalfa are able to fix atmospheric nitrogen indirectly due to the presence of the symbiotic nitrogen-fixing bacteria located on their roots (the N-fixers). Nonleguminous or non-fixing plants such as corn obtain their nitrogen from the surrounding soil where species of free-living bacteria have already fixed the atmospheric nitrogen. Ambrose and DeNiro (1986) state that legumes generally have lower (e.g. less positive) $\delta^{15}N$ values than nonleguminous plants and as such, stable nitrogen analysis may be useful in the determination of the relative importance of legumes or nonlegumes in a human or animal population. Legumes appear to have $\delta^{15}N$ values which average around +1.0 o/oo and range between -6.5 o/oo to +6.5 o/oo while nonlegume values have an average of +3.0 o/oo and range from -7.8 o/oo to +17 o/oo (Schoeninger and DeNiro, 1984). The large overlap

between the two different modes of nitrogen utilization suggests that this isotope will have a more restricted use in paleodietary studies compared to carbon isotopes although DeNiro (1987) maintains that this overlap is caused by modern fertilizer effects. Prehistoric plants would therefore have had a much more obvious distinction between the two modes since fertilizers were not as heavily used as in modern farming operations. Studies of archaeologically recovered plant material such as that by DeNiro and Hastorf (1985) would be useful in the investigation of the effects of fertilizer use on stable isotope ratios of nitrogen. Modern studies may involve the isotopic analysis of plants grown under controlled conditions without the use of fertilizers. Comparisons can then be made with plants grown by modern agriculturalists in order to determine the extent to which the $\delta^{15}N$ values have been altered. Unfortunately, many studies which have produced lists of plant isotopic values do not provide information on the sources from which the plants were obtained (e.g. Wickman, 1952; Smith and Epstein, 1971; Winter et al., 1976 - these examples deal with carbon isotopes since there are very few lists of the $\delta^{15}N$ values of plants).

Fractionation of Nitrogen Isotopes

Ambrose and Deniro (1986b) suggest that the differences in $\delta^{15}N$ values observed among various plant

species are the result of fractionation during the processes of deamination and transamination (i.e. the removal of amino groups during the catabolism of the amino acids - see Lehninger, 1975 pp. 562-567). DeNiro (1987), however, states that the variation

> ... reflects differences in the isotopic composition of the nitrogen sources the plants use, rather than isotopic fractionation during nitrogen uptake and conversion into plant matter ... (p. 184)

The major fractionation events may occur during the processes of nitrogen fixation and the biosynthesis and degradation of amino acids, although the contribution of both to the overall fractionation process may be unequal. Future studies must address this fractionation problem if nitrogen isotopes are to be fully exploited in paleodiet reconstruction.

Uses of Nitrogen Isotopes in Paleodietary Studies

The suggestion has been put forth that $\delta^{15}N$ values are useful as indicators of trophic level within a specific food web (Schoeninger, 1985). The enrichment factor associated with this passage from level to level has been found to be approximately 3 o/oo (Minigawa and Wada, 1984). Nitrogen isotopes, in conjunction with $\delta^{13}C$ values, have also been used in the differentiation of terrestrial and marine feeders (Norr, 1982; Schoeninger *et al.*, 1983; Schoeninger and DeNiro, 1984; Walker and DeNiro, 1986) or between pastoralists and farmers in East Africa (Ambrose,

1986; Ambrose and DeNiro, 1986a). Recently, several authors have suggested that $\delta^{15}N$ values may be affected by climatic conditions and specifically by water availability (see Heaton et al., 1986; Ambrose and DeNiro, 1987; Sealy et al., 1987).

3.5 Carbon and Nitrogen metabolism

All living organisms are primarily composed of an ordered collection of proteins. Molecules such as carbohydrates and fats are also important but more in terms of the energy that they provide to the organism than as structural elements. Proteins are composed of amino acids which originate from two main sources: 1) directly from the unaltered original food amino acid or, more importantly, 2) from the individual elements which once made up the food amino acids. An understanding of the mechanisms involved in the processing of food will therefore aid in understanding the fate of individual carbon and nitrogen atoms in terms of how they are incorporated into proteins such as collagen.

Food is primarily composed of a complex collection of carbohydrates, fats and proteins, each varying in quantity depending on the origin of the food item (e.g. meat or muscle tissue is mainly composed of protein while some plant grains are made up almost entirely of carbohydrates). Regardless of the elemental composition, each food item

must first be broken down into smaller units which can be absorbed by the gastrointestinal mucosa. This breakdown involves various enzymes, each of which acts upon a specific food element. Digestion begins in the mouth where the food is physically broken down into portions which can be acted upon by the salivary enzymes. The partially transformed food is then passed down the esophagus and into the stomach where it is again acted upon by a further series of enzymes such as pepsin. The vast majority of food digestion, however, takes place in the small intestine and it is here that food is broken down to its most basic parts e.g. simple sugars, fatty acids and amino acids.

Carbohydrates and fats are used by mammals in the production and storage of energy and are not important during the synthesis of proteins in the normal adult animal (Guyton, 1981; Alpers, 1987). In areas where dietary protein intake is poor, however, these components can play an important role in the synthesis of proteins. Under such conditions, carbohydrates and fats may act as an alternate, although limited, source of carbon and nitrogen. Experiments have shown that isotopic values can vary significantly between proteins, carbohydrates and fats obtained from the same food source, thus affecting the isotopic signature of the proteins making up the animal in question (Abelson and Hoering, 1961; Park and Epstein, 1961; DeNiro and Epstein, 1978).

3.6 Collagen

Use of the term 'collagen' in this thesis will follow the convention suggested by DeNiro (1985:808) to describe the residue produced by the extraction methods. This material may not be composed exclusively of collagen but until adequate characterization studies are undertaken, the material will be referred to as 'collagen'.

Biochemistry

Collagen is the most abundant structural protein in the animal kingdom and is the principle component of several organs of the human body including blood vessels, the cornea of the eye, skin, and tendons. This protein makes up approximately 90% of the organic component of bone and in doing so, gives bone a certain degree of flexibility (Vaughan 1981). Several review articles are available concerning the structure and biosynthesis of collagen and consequently, the following discussion will be brief (see articles by Bornstein and Traub, 1979, Miller and Gay, 1987 and edited volumes by Ramachandran, 1967, Ramachandran and Reddi, 1976, and Weiss and Jayson, 1982).

Collagen is a large protein with a molecular weight of approximately 100,000. The term 'collagen' refers to a large network of structural proteins which all share similar basic properties e.g. three peptide chains, each made up of about 1050 amino acids with glycine in every

third position, and with the three chains wrapped around each other in a triple helical arrangement (Miller, 1984 and 1985). The main differences between the collagen types relate to the amino acid sequence of the peptide chains. Bone, for example, is primarily composed of Type I collagen which consists of 2 α1(I) chains and a single α2(I) chain. The composition of this type includes several amino acids and four in particular - glycine, alanine, proline and hydroxyproline.

Collagen is of particular interest to researchers interested in paleodietary reconstruction because of several qualities: the relative ease with which it can be extracted from bone (due primarily to its great abundance in bone), its general tendency to preserve for long periods of time without undergoing large postmortem changes, and its relatively rapid turnover rate, on the order of several decades in humans (Libby et al., 1964; Wyckoff, 1972; Kanungo, 1980; Gurtler, et al., 1981; Robins, 1977; Armstrong, et al., 1983). Problems also arise because of these same properties e.g. only the 'average' diet for the last few decades of the individual's life is contained within the collagen's isotopes. Therefore changes in the diet during earlier parts of a person's lifetime will not be seen. Unless infant or juvenile remains are recovered, differences in diet due to age would be difficult to discern. Researchers have found that the isotopic ratios

themselves are independent of age and sex for populations subsisting on a monotonous diet (DeNiro and Schoeninger, 1983; Lovell et al., 1986b). Secondary sources of information such as archaeological or ethnological data would be useful when a correlation between age, sex and diet is indicated by the stable isotope data.

Collagen and Bone Preservation

The possibility of diagenetic change in the composition of the collagen must be considered when using archaeological material in a chemical analysis. Henderson (1987) states that factors affecting the preservation of bone include both extrinsic (e.g. environmental conditions) and intrinsic aspects (e.g. chemical composition, shape, size, density, and age of the bone). Soil factors such as groundwater, temperature and pH are the most important of the extrinsic group. Extremes in environmental conditions may result in substantial changes in both the bone's organic and inorganic composition. An example is that an extremely wet environment in combination with a fairly permeable soil type (e.g. a high level of groundwater) will not preserve bone as well as a drier environment and this results in lower collagen yields (Berger et al., 1964). While the study area does not receive a substantial amount of moisture (approximately 750 to 850 mm of rain annually), the rain that does fall is concentrated into a relatively short period of time during the summer months, thus

mimicking a wetter environment only briefly. Microbial activity within the soil is directly affected by the amount of rainfall, thus, the degradation of collagen by these organisms will have a seasonal pattern i.e. higher activities during the wet season (Jones and Wild, 1975). It follows from this that collagen yield would be a poor indicator of the length of time that a sample has been buried unless the environmental conditions in which the sample was deposited are well known.

Von Endt (1980) has proposed a model in which leaching of the bones by groundwater causes a disruption in the bond between the protein and mineral components. The inorganic component of bone acts as an 'anchor', holding the proteinaceous component within the bone and once this anchor is lost, the proteins are more susceptible to microbial degradation and leaching. Von Endt also discovered that there is an inverse relationship between the size of the bone and the loss of protein - the larger the bone, the slower protein is lost. This has important consequences in studies where the remains are highly fragmented. Since the bones of the present study do not appear to have been exposed to a continuous supply of groundwater, leaching would not be as steady as in an area with a more consistently reliable source of groundwater. Research involving the effects of variable amounts of

groundwater and of alternating wet and dry periods would aid in understanding bone preservation in the current study area.

Soil pH can also greatly affect the preservation of skeletal remains, although its importance in this regards is less than that of soil water. Preservation is generally better in an alkaline as opposed to an acidic environment (Wyckoff, 1980). This has led to major problems for faunal analysts in West tropical Africa where soils tend to be more acidic (Shaw, 1972). The savanna zone, on the other hand, is primarily composed of soils which are neutral or slightly alkaline in nature (Ahn, 1970), although soil pH levels can vary depending on local conditions (Jones and Wild, 1975). Acidic conditions lead to dissociation of the mineral component of bone and an increase in the hydrolytic breakdown of collagen (Von Endt, 1980). Tuross and Hare (1977) have determined that under acidic conditions, collagen is broken down to smaller peptide units which are then converted into humates, however, these researchers do not state whether this action would affect stable isotope ratios.

Temperature is a third environmental factor that can have a significant role in changing the composition of bone but once again, not to the same degree as soil water and pH. In vitro experiments have shown that as the ambient temperature increases, protein loss also increases (Ortner

et al., 1972; Von Endt and Ortner, 1984). These studies use temperatures which bones will not normally encounter under natural conditions, the underlying theory being that such conditions represent a compressed version of the true archaeological situation. The results are then "... projected back to more realistic temperature conditions." (Von Endt and Ortner, 1984:249).

It should be noted that local conditions are more important in bone preservation than overall habitat characteristics (Behrensmeyer 1978). Therefore, when it is possible, soil and climatic information should be obtained directly from the site under study rather than relying on regional descriptions.

The relationship between the intrinsic features of bone and their effect on preservation has not been extensively studied although a limited number of projects have been undertaken. An example of such a project, discussed previously in this section, is Von Endt's (1980) study of the relationship between bone protein preservation and the size of the bone sample. Factors such as bone shape and density have been examined more in terms of their pre-burial effects (cf. Shipman, 1981:26-28).

The age of the individual at death will also be important since researchers have found that bone composition changes with age. Garlick (1969) states that

the nitrogen content of human bone varies with age and Quelch et al. (1983) believes that this reflects a higher protein content in infants relative to adults. It was not reported whether this represented a decrease in much more abundant collagenous or the rarer noncollagenous proteins. The significance of the individual's age, therefore, seems to apply to the amount of protein which is available for preservation i.e. under similar conditions, bones with higher protein contents will produce higher 'collagen' yields than those with lower levels of proteins. Collagen is also known to increase in its insolubility with age, although the exact mechanism for this change is not fully understood but may relate to an increase in crosslinking between the collagen molecules (Hamlin and Kohn, 1971 and 1972; Kanungo, 1980; Klein and Rajan, 1984). The bones from older individuals will therefore contain more insoluble collagen (but a lesser amount of total bone protein) which may result in an increase in the protein's resistance to degradational forces. Thus, the 'collagen' yield would rise in samples from older individuals.

A final factor which also is not fully understood is the relationship between the organic and inorganic phases of bone. Von Endt's leaching model described above suggests that groundwater alters this relationship thereby allowing for an increased loss of the separate components. Research, however, has failed to determine the precise manner in

which proteins and mineral bond in living bone and until this is accomplished, the mechanism(s) by which this relationship changes after burial will remain unknown.

Preservation of the Stable Isotope Ratios in Collagen

The retention of the original stable isotope ratios will depend on the overall preservation of the parent collagen. Factors which allow differential loss of 'lighter' versus the 'heavier' isotopes, for example, will influence the ratios which are experimentally determined (i.e. since the 'heavier' isotopes react slower chemically, they may not be leached from the collagen as readily as the 'lighter' isotopes). This is the basis for a test which was recently developed by DeNiro (1985). DeNiro determined the ratio between the molar amounts of carbon and nitrogen in a series of modern and archaeological animal bone collagen samples. He found that the ratio between the elements carbon and nitrogen in the modern bone samples consistently fell in the range between 2.9 to 3.6 while the prehistoric samples produced highly erratic isotope values when their elemental ratios fell outside this range. DeNiro concluded that this ratio provided a useful test for determining whether or not a bone has undergone diagenetic change. The procedure has been generally accepted among researchers but has been criticized by some (e.g. Sealy and van der Merwe, 1986, Lovell et al., 1986b). DeNiro does not explain, for example, why C/N ratios may vary between different bones.

It is likely that many different processes interact in a complex manner in determining the amounts of carbon and nitrogen in a sample. Masters (1987) has suggested that C/N values slightly above the critical 3.6 level may in fact represent the inclusion of some noncollagenous proteins in the 'collagen' sample.

C/N ratios of the bone collagen samples were determined through the use of a modified Perkin-Elmer model 240 Elemental Analyzer. The operation of this machine begins with the combustion of the collagen sample at 950°C and the channelling of the resultant products through a reducing environment. The sample volume is then sent through water and carbon dioxide traps which extract the original sample hydrogen and carbon leaving nitrogen and supplementary compounds (e.g. silica from the glass wool used in the extraction procedure). Detectors then determine the specific amounts of each compound and these values are converted to percentages of the original sample weight.

This procedure results in C/N ratios which are different from those found DeNiro (1985). The actual weight percentage of each element in the sample was determined rather than the atomic ratio molar value. To convert actual weights to molar values, a common chemical principle is used - in order to obtain molar values of any solution, the actual weight of that element in a sample is divided by the element's atomic weight (Mortimer, 1975). The results in

Table 5.2 reflect this method of correction and are comparable to DeNiro's atomic C/N ratios.

CHAPTER FOUR

MATERIALS AND METHODS

4.1 Introduction

The theory and procedures involved in the reconstruction of paleodiets using stable isotopes had their origins in fields other than anthropology (Bumsted, 1984). Researchers interested in paleodiets have therefore had to adapt these techniques for use on the skeletal remains of prehistoric humans and animals. Bone is made up of organic and inorganic components which can be separated and studied individually. This chapter will describe the prehistoric remains utilized in this thesis and the various methods for isolating type I collagen, the major protein of bone (Vaughan, 1981).

4.2 Sample Description

The samples consist of human and faunal remains recovered from sites 506A and 523 as described in Chapter Two. The remains from site 506A have not been studied previously. The material from mound 1 of site 523 was the subject of a preliminary analysis by LeMoine (n.d.) and the following description of the remains is based upon her report. In this analysis, LeMoine analyzed 2244 fragments representing approximately one-quarter of the total sample set. LeMoine encountered two major difficulties with the

sample: 1) the highly fragmentary nature of the remains and 2) insufficient comparative materials for identification beyond the level of Class in most cases (Table 4.1).

Table 4.1
Levels of Identification for Faunal Sample
(after LeMoine, n.d.)

TAXONOMIC LEVEL	NUMBER OF BONES	PERCENTAGE OF TOTAL SAMPLE
CLASS	1864	83.1%
ORDER	26	1.2%
FAMILY	137	6.1%
SPECIES	71	3.2%

Of the 137 bones identifiable to family, all were placed within the Bovidae and 119 of these were believed to belong to the sheep/goat group. Thirty-one bones were identified as Bos, i.e. cattle, and a further twenty bones were identified as human. LeMoine also examined the taphonomic condition of the remains and found that the majority of the bones were reasonably well preserved, corresponding to stage two of the weathering scheme proposed by Behrensmeyer (1978). This scheme is based on a recent faunal assemblage recovered from the Amboseli Basin

in southern Kenya. Stage two of this scheme exhibits bones with the following characteristics:

> Outermost concentric thin layers of bone show flaking, usually associated with cracks, in that the bone edges along the cracks tend to separate and flake first. Long thin flakes, with one or more sides still attached to the bone, are common in the initial part of Stage 2. Deeper and more extensive flaking follows, until most of the outermost bone is gone. Crack edges are usually angular in cross-section. Remnants of ligaments, cartilage, and skin may be present. (Behrensmeyer, 1978:151).

During her analysis, LeMoine noted that 599 bones had carbonate concretions deposited on their surfaces and that these bones were distributed unevenly throughout the site with the upper four levels containing the highest frequency of carbonate encrusted bone. The causes of these concretions are not well understood at the present time but may be related to rainfall levels. If the levels were higher at a certain period relative to other times, more carbonate would be leached from the soils which would subsequently accumulate on the bones buried below the surface. Based on the hypothesis that the carbonate concretions are indicative of a humid past environment, the decreased number of carbonate encrusted bones in the lower levels may be suggestive of a drier climate during the earlier parts of the occupational history of site 523 (LeMoine, n.d.). These concretions can also have a significant effect on the stable carbon isotope and C/N

ratio values of the bone in that carbon with a different $\delta^{13}C$ signature maybe introduced into the sample thereby causing the bone values to deviate from their original values and artificially inflating the amount of carbon with respect to nitrogen.

The methods used in the present study take this factor into account - the initial pretreatment with hydrochloric acid extracts both the indigenous and intrusive mineral deposits. Behrensmeyer (1978) discovered that a portion of her faunal assemblage was covered by salt concretions, a factor which she attributes to the highly alkaline nature of the soil from which her sample was excavated. She describes her bones as showing a "...flaking or splitting caused by the force of crystallization (of the salt)."(p. 154), a feature not observed in the site 523 material. Carbonate crystal formation may be less destructive on bone and so the flaking and splitting features seen in Behrensmeyer's Kenyan sample would not be seen in the present study sample. Further research into the mechanisms of carbonate accumulation on bone would aid in the interpretation of these results.

Breakage patterns of the bones were examined by LeMoine (n.d.), however, the majority of the broken bones had patterns which were difficult to distinguish. Of the 1686 bones that were broken, 63.8% were placed in the 'indeterminable break' group, that is, their breakage

pattern was not distinct enough from other types of patterns to be categorized. Of the patterns that were discernible, 22.3% were green spiral fractures, 11.7% were broken through weathering processes, and the rest were fractured by other means. Other cultural modification activities, such as cutmarks, chop marks, and scrapes, were found on 102 bones.

The mound VIIA samples consisted of a group of three bones collected from the upper meter of the excavation. A radiocarbon date of 790 ± 100 B.P. was determined for the uppermost level (David and Sterner, 1987). This date is earlier than the accepted dates obtained for the first three layers of mound 1 (see Table 2.1). The close spatial and temporal proximities of the two mounds, however, suggests that their preservational histories were similar.

A cursory examination of the remains from site 506A revealed a sample which is smaller and more fragmented than the 523 remains. The bones are in the same general condition as those of 523 (i.e. at Behrensmeyer's stage 2). Due to the highly fragmented nature of the sample, however, an examination of species representation could not be undertaken to the same extent as for the mound 1 material (a sheep, equid, and large ungulate were found to be present - see Table 4.2). This material was not used in the

preliminary study (described below) but was used in subsequent tests.

4.3 Methods

A preliminary investigation was performed to determine whether the method developed by Longin (1971) was suitable for this collection. Eleven bones were selected from the mound 1 collection of site 523 previously examined by LeMoine. At least three bones from each of the three most frequent species (cattle, sheep/goats and human) were selected from levels 1 - 4. No human bone was recovered from level 4. Each bone sample weighed at least 3 grams to insure an adequate yield of collagen for analysis. The identification of each bone as to element and species was confirmed where possible using the faunal collections of the Departments of Archaeology and Biology of the University of Calgary. Difficulties were encountered at this stage due to the lack of an adequate African faunal collection. All bone samples were cleaned of any extraneous material and catalog numbers were thoroughly removed from the bone using a knife.

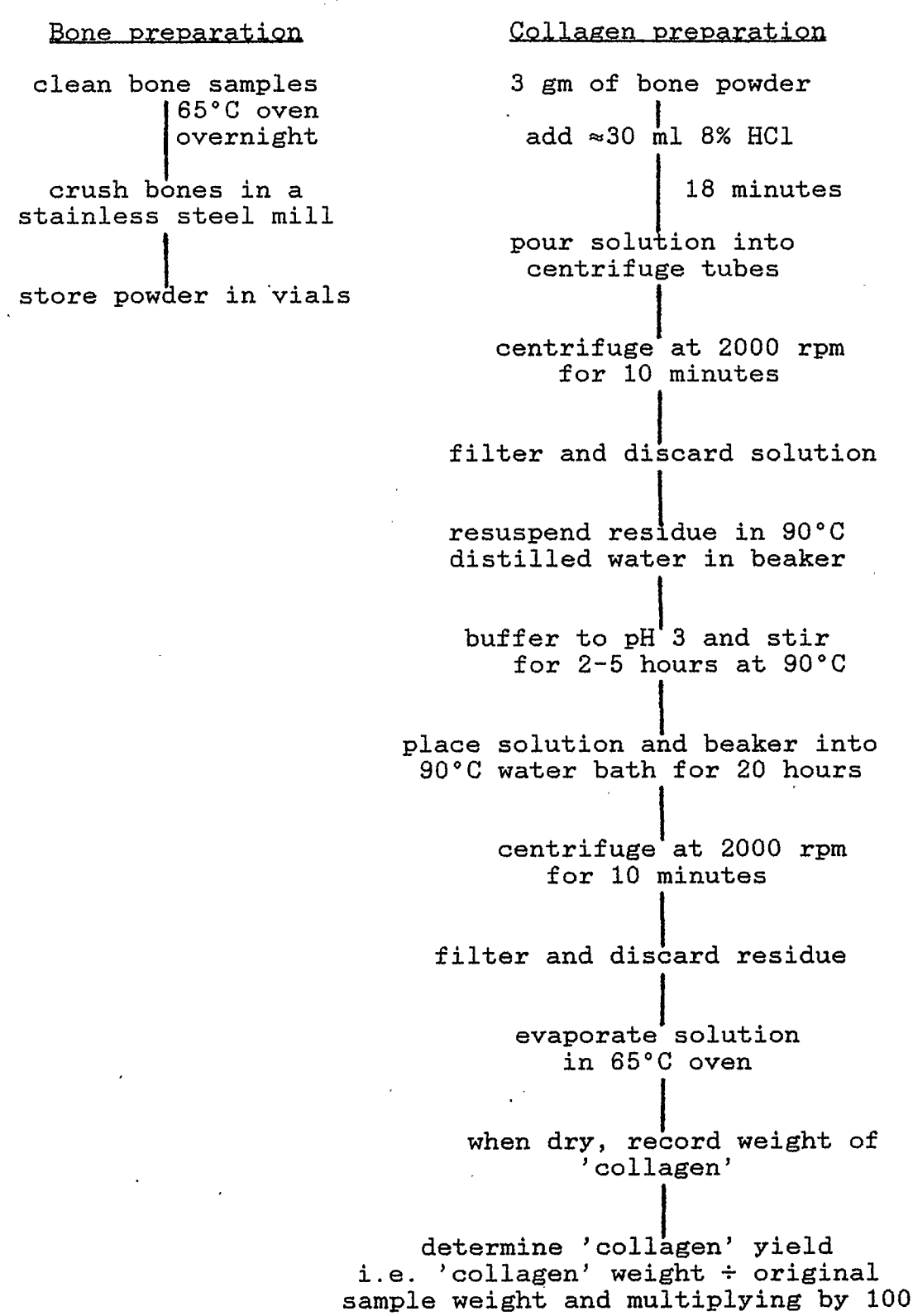
The bones were then cleaned ultrasonicly in cold distilled water. Katz and Man (1979) warn that ultrasonic treatment may adversely affect amino acid concentrations if the treatment is excessively long, i.e. longer than 30 minutes. The present samples were placed within the ultrasonic cleaner for a period of 5 minutes and thus, the

amino acid content of the samples should not be significantly affected. The samples were dried overnight in an oven set at 65°C and with an excess of silica gel. After drying, the samples were crushed into a powder using a stainless steel Bleuler-Mill in the Department of Geology, University of Calgary. The mill was thoroughly cleaned after each bone was crushed and handling of the bone samples was kept to a minimum. Contamination of sample carbon contents through direct handling can be a problem, particularly in isotopic research (e.g. in radiocarbon dating - see reviews by Kvenvolden, 1975, Taylor, 1987).

The collagen extraction method used in the preliminary study was based on the procedure developed by Longin (1971) and is illustrated in Figure 4.1. This procedure was chosen because it has been routinely used to obtain samples for radiocarbon dating, it is simple, and the necessary equipment was available in the Department of Archaeology's physical anthropology laboratory at the University of Calgary. Several problems arose during this operation which will be discussed in section 4.4.

The insoluble fractions obtained through this procedure were prepared for stable carbon isotopic analysis using the method described by Sofer (1980). Approximately 10 milligrams of the insoluble fraction, along with a limited amount of cupric oxide, were placed into a pyrex tube such that the copper oxide filled approximately 2.5 cm

Figure 4.1
Method 1 Collagen Extraction Procedure
(after Longin, 1971)



of the tube. Each tube had an outer diameter of 6 mm, was cut into 20 cm lengths and was sealed at one end. The tubes were evacuated to a level of 10 to 30 microns (.01 to .03 torr) of pressure, sealed and placed into an oven set for 550°C for 5 hours. After the samples were combusted, the tubes were allowed to cool gradually overnight. The samples were then analyzed for $\delta^{13}C$ by mass spectrometry in the laboratory of Dr. R. Krouse, Department of Physics, University of Calgary. The precision of this machine is ± 0.2 o/oo and all $\delta^{13}C$ values were recorded with reference to the PDB standard. The results are illustrated in Table 5.1.

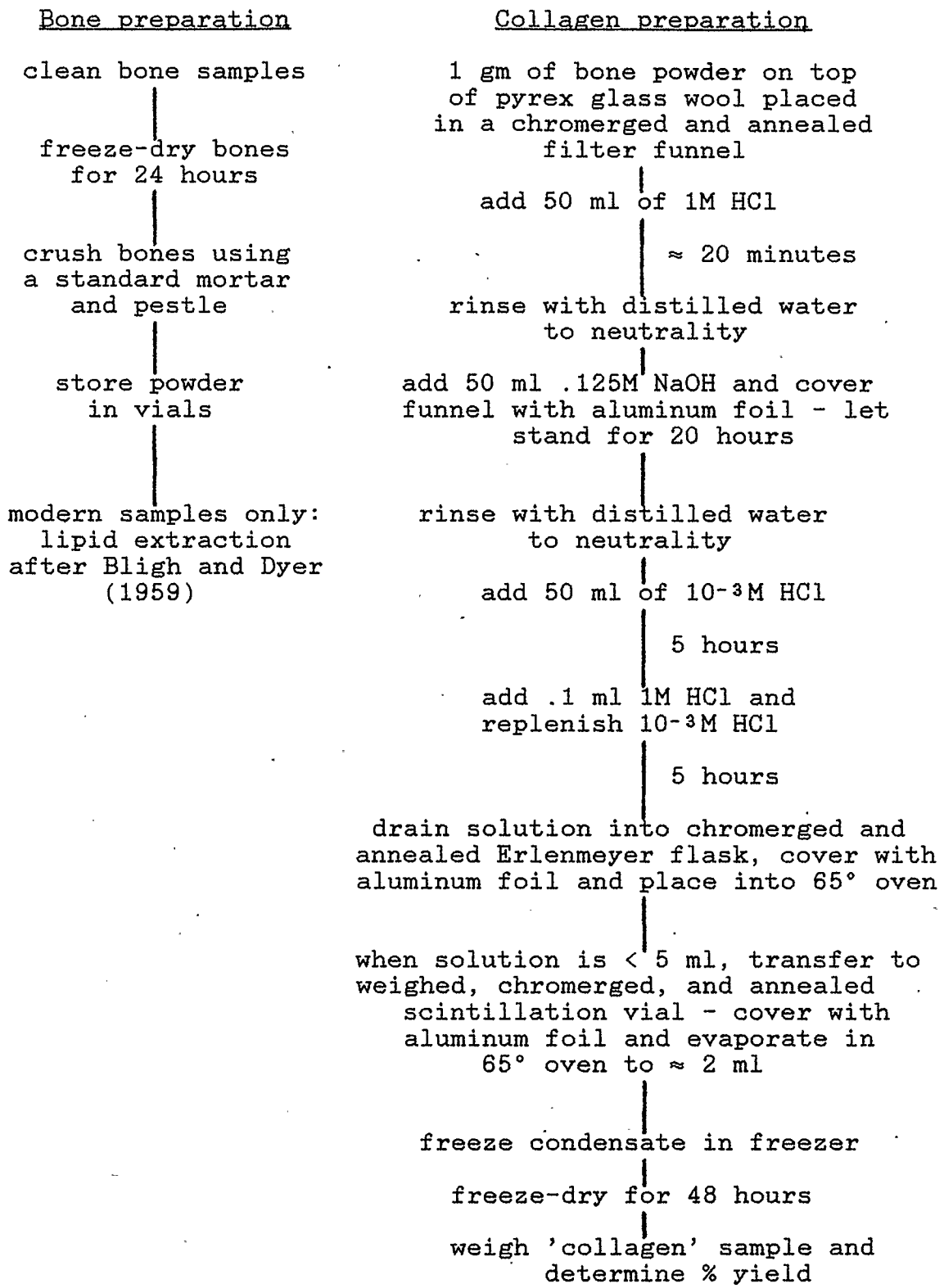
Based on the encouraging isotopic results, several more bone samples were prepared for extraction. The sample set was limited to twenty-three bones due primarily to the difficulty encountered in identifying the bones beyond the level of order. Animals within an order can vary significantly in their feeding modes and therefore, the identification of the remains to at least the family level is a requirement of a study such as this. Each level from one through four was now represented by at least seven bones including those from the preliminary study. Mound VIIA of site 523 was represented by three bones in order to provide a comparison with the samples from mound 1. The collagen extraction technique was changed to that described by DeNiro and Epstein (1981) and modified by Schoeninger

and DeNiro (1984) and illustrated in Figure 4.2. This procedure, unlike the method used in the preliminary study, incorporates a sodium hydroxide soak used to control contamination by certain humic acids (see section 4.4). It was found in later extractions that the use of a glass filter paper and the discontinued use of a vacuum during the filtering steps reduced the problem of clogging of the funnel's coarse filter.

Collagen samples were prepared for both stable carbon and nitrogen isotope analysis using the following procedure: quartz tubes were sealed at one end, annealed, and filled with approximately 1 gram each of copper and cupric oxide, 5 to 10 mg of collagen, and a small 9 mm^2 piece of silver foil. The tubes were evacuated to approximately .02 torr, then placed in an oven and heated to 800°C for 3 hours after which they were allowed to cool overnight. It was found that upon combustion at higher temperatures (900°C) for shorter periods of time (1 hour) and with collagen samples greater than 5 mg, some of the tubes burst. To overcome this problem, sample weights were reduced to less than 5 mg, the temperature was lowered but combustion was allowed to take place over an extended period of time and the oven door was kept closed overnight.

In later extractions, the collagen samples were freeze-dried to eliminate water vapor. Two blank samples were included in order to determine whether any

Figure 4.2
Method 2 Collagen Extraction Procedure
(after Schoeninger and DeNiro,1984)



contamination was produced using the equipment suggested by Schoeninger and DeNiro (1984). These blanks were also prepared for isotopic analysis and it was found that the residue produced during the extraction procedure did not produce detectable amounts of CO_2 or N_2 gases. Kennedy (1989), in a similar study, found that a residue averaging .026 ± .03 gms was recovered and that measurable amounts of CO_2 and N_2 gas were not produced when the samples were examined by mass spectrometery. It was therefore concluded that method 2 does not introduce contaminates capable of significantly altering carbon or nitrogen isotopic ratios. Carbon and nitrogen contents for samples of the present study were corrected for the addition of extraneous material as determined by Kennedy (1989). Results for the bone samples are presented in Table 5.2.

A third series of extractions on 35 samples was done in order to increase the sample size. The provenience of all samples is given in Table 4.2 along with the new level designations formulated for mound 1 of site 523. Bone samples prepared previously were completely used during the procedure. Sample B41 was extracted by both methods 1 and 2 (the method 1 yield was low, 2.9%, and a $\delta^{13}C$ value was not obtained) while sample B19 was extracted in the second batch of extractions. This third series of bones were prepared according to the method of Schoeninger and DeNiro

Table 4.2

Sample Provenience

SITE/ MOUND	SAMPLE	STRAT. UNIT	DBD (cm.)	ELEMENT
523/1	B14*	2	82.5	dist. long bone
523/1	B14	2	82.5	dist. long bone
523/1	H14*	2	82.5	cranial frag.
523/1	L1-14	2	82.5	vertebra
523/1	O14a*	2	82.5	radial shaft frag.
523/1	O14b	2	82.5	astragalus
523/1	H15	2	97.5	femur shaft frag.
523/1	O15a	2	97.5	dist. humerus
523/1	O15b	2	97.5	astragalus
523/1	L1-18	3	112.5	proximal humerus
523/1	B19	3	127.5	astragalus frag.
523/1	B19	3	127.5	astragalus frag.
523/1	O19	3	127.5	1st phalanx
523/1	B21a*	3	127.5	prox. metatarsal
523/1	B21b	3	127.5	sesamoid
523/1	B41*	3	142.5	vertebral frag.
523/1	B41	3	142.5	vertebral frag.
523/1	H41*	3	142.5	ulnar shaft frag.
523/1	O41*	3	142.5	astragalus
523/1	H46*	4	157.5	prox. radius frag.
523/1	L2-77	4	157.5	phalanx
523/1	B57*	4	172.5	rib frag.
523/1	B57	4	172.5	rib frag.
523/1	Bv57	4	172.5	cuneiform
523/1	O57*	4	172.5	horn core
523/1	E82	4	172.5	dist. radius
523/1	O62a	4	187.5	calcaneum
523/1	O62b	4	187.5	mandible frag.
523/1	L3-36	5	202.5	immature cannon
523/1	L3-130A	5	217.5	vertebra
523/1	L3-130B	5	217.5	cancellous bone
523/1	L3-137	5	217.5	magnum/cuneiform
523/1	L3-148	5	232.5	humerus head
523/1	B179	6	247.5	scaphoid
523/1	O179	6	247.5	radial shaft frag.
523/1	O220	6	262.5	dist. humerus
523/1	L4-224	6	277.5	vertebra frag.
523/1	B253	6	322.5	vertebra frag.

Figure 4.2

Provenience of Samples (cont'd)

SITE/ MOUND	SAMPLE	STRAT. UNIT	DBD (cm.)	ELEMENT
523/1	L5-263A	7	337.5	mandibular frag.
523/1	L5-263B	7	337.5	rib frag.?
523/1	L5-266A	7	352.5	cranial frag.
523/1	L5-266B	7	352.5	cancellous bone
523/1	S266	7	352.55	metapodial
523/1	B274*	8	382.5	phalanx frag.
523/1	B274	8	382.5	phalanx frag.
523/1	L6-274A	8	382.5	mandibular frag.
523/1	L6-274B	8	382.5	rib frag.?
523/1	O274*	8	382.5	mandible ramus
523/1	L6-290	8	382.5	metacarpal shaft
523/1	L7-278A	9	397.5	cancellous bone
523/1	L7-278B	9	397.5	cancellous bone
523/1	L7-278C	9	397.5	metapodial end?
523/1	L7-283A	9	412.5	tibial shaft frag.
523/1	L7-283B	9	412.5	cancellous bone
523/1	L9-309	11	453.5	acetabulum frag.
523/7	7-E160	1	?	phalanx
523/7	7-L174	1	?	astragalus frag.
523/7	7-O197	3	?	dist. femur
506A	TB1-18	1	?	shaft frag.
506A	TA1-31	1	?	cancellous bone
506A	N-Bv21	2	?	temporal bulla
506A	N-O21	2	?	tibial shaft
506A	TB2-21	2	?	cancellous bone
506A	T2-22	test 2	?	shaft frag.
506A	N-Ov23	2	?	calcaneous
506A	TA2-23	2	?	shaft frag.
	G1	-	?	metatarsal frag.
	M-Ov1	modern	-	rib-compact bone

NOTE: for sample numbers, B = Bos, O = Ovis/Capra,
H = Homo, Bv = Bovid, E = Equid, S = Suid,
L = unknown taxon, G = Giraffa, M-Ov = modern Ovis
* = used in preliminary extractions
DBD = depth below datum

(1984) in that the bones and gelatin samples were freeze-dried. When the bones were not undergoing analysis, they were stored in the freezer section of a conventional refrigerator.

All collagen samples were examined for their C/N ratios in order to determine whether they fit the criterion described by DeNiro (1985) for unaltered bone. Analysis was performed using a modified Perkin-Elmer model 240 Elemental Analyzer (precision of this machine was ± .3%). A preliminary collection of samples extracted during the first two series of experiments revealed the possibility that a number of the bones would not meet DeNiro's criterion and therefore, emphasis was placed on providing an explanation for this.

4.4 Methodological Discussion

The preliminary results in Table 5.1 demonstrate that the sample collection was capable of yielding results. No CO_2 was produced in the combustion phase for the B41, O14a and O41 samples. The lack of results for the three samples may relate to the amount of organic material in the samples. The yields of acid-insoluble material for two of these three samples were relatively low compared to the other samples (2.9% for B41, 4.8% for O14a, but 15.0% for O41). It is possible that samples B41 and O14a simply did not contain enough preserved organic material to study

isotopically. Sample 041 was a blackened color suggesting that it may have been burnt during food preparation or after being deposited. DeNiro and colleagues (1985) found that intense heating such as that produced by cooking over a fire, substantially alter the $\delta^{13}C$ and $\delta^{15}N$ values of the sample as well as the C/N ratios. These researchers also discovered that little if any of the insoluble fraction was extracted from experimental bones subjected to high temperatures. Sample 041, therefore, follows the pattern found by other researchers for a bone which has been burnt.

The method utilized in the preliminary study is simple and widely used within other disciplines, i.e. in the soil sciences where the initial acidification step dissolves a carbonate sample which allows carbon to be collected in the form of carbon dioxide. Difficulties arose in 1) handling of the samples and 2) the lack of control over certain contaminates found in archaeological organic samples. Loss of sample during the transfer of solutions between containers has been cited as one problem with this method (Schoeninger and DeNiro, 1984). These authors have suggested that the use of a glass funnel fitted with a coarse fritted glass filter and a stopcock will alleviate this problem since all of the steps take place in a single container. This allows the utilization of smaller bone

samples, an important consideration in a study such as this in which the bones are fragmented.

The second problem has an effect on the $\delta^{13}C$ results. It stems from the fact that bone buried in soil for any length of time will undergo degradation of the organic, and later inorganic, components (Carbone and Keel, 1985). Bacteria attack the proteinaceous portion of bone and groundwater leaches out more of the organic fractions (Berger et al., 1964; Von Endt, 1980). Enzymatic manipulation of these organic residues (eg, proteins, carbohydrates, fats, and lignins) results in the formation of humic and fulvic acids, among other compounds (Kononova, 1961; Bohn et al., 1979), and it is these substances, particularly the humic acids, which the method developed by Longin does not remove. Humates have the potential to alter the isotopic composition of a sample because of their significant carbon and nitrogen contents (45 to 60% C and 3 to 5% N) (Kononova, 1961; Hare and Estep, 1982; DeNiro and Hastorf, 1985). They can also alter amino acid concentrations in a sample due to their own amino acid compositions (Schnitzer and Khan, 1972). Since the majority of humates are derived from the bacterial degradation of plant material, humic substances will have their most important effects in areas with suitable environmental conditions for the bacteria and where there are large amounts of plant material, for example in a tropical

rainforest. Savanna soils are known to be poorer in terms of their organic matter and humus content (Ahn, 1970; Jones and Wild, 1975) and therefore, the effects of humates will be lessened somewhat in this region.

Fulvic acid is soluble in acids (Stevenson and Butler, 1969) and can be extracted in the initial demineralization step through the use of relatively strong hydrochloric acid, however, humic acids are base-soluble (Schnitzer and Khan, 1972). This fact led DeNiro and Epstein (1981) to suggest that samples should be treated with sodium hydroxide (NaOH), a solution widely used in the soil sciences to isolate humic acids (Schnitzer and Khan, 1972).

Chisholm et al. (1983a) have criticized the use of sodium hydroxide as a means of eliminating humate contaminants because it lowers the yield of collagen (see also Olsson et al., 1974). Chisholm and colleagues extracted the gelatin from 6 human bone samples using three separate methods: the first as described by Longin (1971), the second a variation of Longin's method where a 20 hour treatment with NaOH was utilized, and the third method being another modification of Longin's method where the initial demineralization step uses a weaker HCl solution in several extraction steps until a constant pH is reached. The remainder of the procedure is as in Longin's method.

Table 4.3
$\delta^{13}C$ Values of Different Collagen Extraction Techniques (in o/oo)
(from Chisholm et al., 1983A)

SAMPLE #	METHOD 1	DIFFERENCE 1 VS. 2	METHOD 2	DIFFERENCE 2 VS. 3	METHOD 3
1	-16.0	.3	-16.3	-	-
2	-15.6	.1	-15.7	.1	-15.6
3	-13.6	0	-13.6	.1	-13.5
4	-13.2	0	-13.2	.1	-13.1
5	-21.5	.4	-21.1	.4	-21.5
6	-11.5	.7	-10.8	.8	-11.7

(experimental error = +/- .08 o/oo)
for a description of methods, see text.

The $\delta^{13}C$ values for their samples are reported in Table 4.3.

These results indicate that of bones 2 through 6, three had values that were all were within or extremely close (e.g. within .02 o/oo) to the experimental error while the remaining two samples were outside the experimental error limits when comparing methods 1 and 3 to method 2. Since the ultimate source of each sample is not given, it is unknown whether the last two bones had different diagenetic histories or whether the differences are due to some other factor. A possible explanation for the greater differences seen in samples 5 and 6 may be the result of a higher humic acid contamination than in the other samples.

The authors were not able to obtain precise yield values, however, they state that "... in general, method 2 (with the NaOH step) appeared to give lower yields than either method 1 or method 3." (p. 358). This decrease may be due to a loss of specific amino acids such as serine and threonine. Olsson and colleagues (1974) found that a similar situation arose when they applied a NaOH-soak to their HCl-extraction procedure. Since it is known that individual amino acids from the same source can vary substantially in their $\delta^{13}C$ values (Hare and Estep, 1982), the loss of amino acids in the NaOH soak is an important consideration. Bone collagen is composed primarily of the

amino acids glycine, proline, alanine, hydroxyproline and to a lesser extent, glutamic acid, aspartic acid, and arginine (Eastoe, 1967; Jope, 1980; Weiss and Ayad, 1982). Serine and threonine are found in lower abundances and therefore are unlikely to play a substantial role in the determination of the protein's isotopic value. If, however, the NaOH causes the loss of one of the more abundant amino acids, this may have a significant effect on the $\delta^{13}C$ and $\delta^{15}N$ values of the sample.

Hare and Estep (1982), using the collagen of a modern bovine tendon, separated the amino acids and compared the $\delta^{13}C$ and $\delta^{15}N$ values of the amino acids with the overall tendon values. The extraction technique is not provided, however, since the tendon was of modern, non-archaeological origin, a NaOH soak may not have been used. Tendon contains the same collagen type as bone (type I - see Kanungo, 1980, Vaughan, 1981) implying that the results of such a study, presented in Table 4.4, have significance for bone research.

Table 4.4 indicates that of the amino acids used in the study, proline and hydroxyproline from the modern sample most accurately reflect the collagen $\delta^{13}C$ values. Alanine, on the other hand, reflects the $\delta^{15}N$ of both the modern and fossil whole collagen values in the most accurate manner of the listed amino acids. If the isotopic values of collagen are averages of their component amino

Table 4.4
Isotopic Values for Selected Amino Acids
(after Hare and Estep, 1982)

Amino acid	Source	$\delta^{13}C$(o/oo)	$\delta^{15}N$(o/oo)
ARGININE	MODERN	-15.3	5.3
ASPARTIC ACID	MODERN	-13.1	8.3
ASPARTIC ACID	FOSSIL	-12.5	9.4
GLUTAMIC ACID	MODERN	-10.2	9.0
GLUTAMIC ACID	FOSSIL	-10.2	9.9
PROLINE	MODERN	-12.1	9.0
PROLINE	FOSSIL	-12.4	10.2
HYDROXYPROLINE	MODERN	-12.5	9.5
HYDROXYPROLINE	FOSSIL	-11.7	10.5
GLYCINE	MODERN	-8.4	4.8
GLYCINE	FOSSIL	-8.8	6.7
ALANINE	MODERN	-14.8	7.7
ALANINE	FOSSIL	-16.0	7.5
LYSINE	MODERN	-13.7	8.1
HYDROXYLYSINE	MODERN	-13.9	7.1

NOTE: modern collagen from bovine tendon and fossil collagen from bison bone radiocarbon dated to 10,314 +/- 104 yrs. B.P.
whole bone isotopic values:
modern $\delta^{13}C$ = -12.0 and $\delta^{15}N$ = 7.0
fossil $\delta^{13}C$ = -15.0 and $\delta^{15}N$ = 7.6
standard deviation for delta values = ± .1 o/oo

acids' values, a change in the relative proportions of the amino acids would also cause a disruption in the values of the parent collagen. This has important ramifications if NaOH does indeed remove amino acids from the collagen sample as suggested by Chisholm et al. (1983a). Further research relating to the amino acids of collagen must include an isotopic analysis of all of the amino acids found in type I collagen, an examination of the relative contributions of individual amino acids to the overall isotopic signature, and the analysis of the sources of the carbon and nitrogen in each amino acid (i.e. do specific foods contribute elements to certain amino acids?).

An alternative method to the examination of whole bone collagen is the isolation and study of one specific amino acid from the protein. Such an amino acid must meet several criteria: it must be easily isolated, limited in its distribution throughout other tissues of the body, and the relationship between its isotopic value and that of the parent collagen must be understood. An amino acid which meets each of these requirements is hydroxyproline. This amino acid is formed from the hydroxylation of proline and makes up approximately 10% to 12% of the total amino acid complement of collagen in general (Weiss and Ayad, 1982). Hydroxyproline is not unique to collagen (Eastoe, 1967; Hauschka, 1980) but is found in its highest abundance in this protein. Adams and Frank (1980) have reviewed the

metabolism of this amino acid within plants and animals. Hydroxyproline can be isolated with the same techniques used in the isolation of other amino acids e.g. liquid chromatography, ion exchange chromotography or gas-liquid chromotography (Perrett, 1985; Engel and Hare, 1985; Hare et al., 1985; Macko et al., 1982). The previously mentioned study by Hare and Estep (1982) revealed that the $\delta^{13}C$ and $\delta^{15}N$ values for this amino acid were within .5 o/oo of the $\delta^{13}C$ value and within 2.5 o/oo of the $\delta^{15}N$ value for the overall modern collagen values (Table 4.4). The analysis of a 10,000 year old bison bone used in the same study revealed that these general conditions were retained although the ranges of isotopic values were slightly higher (e.g. 3.3 o/oo for the $\delta^{13}C$ and 2.9 o/oo for the $\delta^{15}N$). Based on these observations, hydroxyproline has the potential to increase the resources available to isotopic researchers interested in past diets. Future study of this amino acid must address important problems such as the way in which hydroxyproline is affected by different types of diagenetic conditions and the geochemical lifespan of this amino acid.

CHAPTER FIVE

RESULTS

5.1 Preliminary Results Discussion

The results obtained during the preliminary analysis of eleven bones from mound 1, site 523 are presented in Table 5.1. Five out of the seven samples for which C/N ratio were calculated yielded results which fell within the range of modern collagen, i.e. 2.9 to 3.6 (DeNiro, 1985). The carbon and nitrogen contents were, in general, higher for bones recovered from the lower levels. These results suggest that diagenetic factors have affected the preservation of bone collagen from the upper levels to a greater extent than collagen from the lower levels. This collection of bones is not representative of all levels in the excavation, however, the results do indicate the potential for more detailed analysis of the effects of diagenetic factors on bone preservation at the study sites.

Ambrose (1986) has suggested that sheep and goats may be distinguishable at an isotopic level due to their different feeding regimes (sheep are grazers of C4 grasses while goats tend to rely more on a combination of C4 and C3 plants). Based on this, sample 057 may represent a sheep since the $\delta^{13}C$ value of its food (-14.2 o/oo when the enrichment factor of +5.1 o/oo is taken into account - see

Table 5.1 Preliminary Study Results

SAMPLE	STRAT. UNIT	% COLLAGEN YIELD	$\delta^{13}C$	ATOMIC C/N	%C	%N
B14	2	14.2	-6.4	3.3	4.8	1.7
H14	2	6.0	-12.5	-	1.6	0.0
O14a	2	4.8	-	-	.6	0.0
B41	3	2.9	-	-	.5	0.0
H41	3	17.4	-4.8	2.0	4.3	2.5
O41	3	15.0	-	-	.3	0.0
H46	4	19.1	-4.1	3.3	5.3	1.9
B57	4	5.0	-8.2	13.6	7.0	.6
O57	4	4.8	-19.3	3.5	7.6	2.5
B274	8	18.8	-8.4	3.1	18.2	6.8
O274	8	27.0	-12.9	3.6	14.2	4.6

NOTE: for sample numbers, H = Homo, B = Bos, O = Ovis/Capra

Chapter Four) is suggestive of a reliance on grasses of the lower part of the C4 range. It is also possible that this sample represents a goat which relied on a combination of grasses in the upper region of the C4 range and a certain amount of C3 plants. The value of food eaten by the O274 individual, conversely, yielded a $\delta^{13}C$ value (-8.8 o/oo with the enrichment factor) which fell close to the upper limit for the C4 range suggesting that this was a sheep. These two samples illustrate that further analysis of the use of stable isotopes in animal identification is required.

5.2 Combination of Data Sets

Chapter Four provided a description of the two methods of collagen extraction utilized in the present study, the method developed by Longin (1971; referred to as the number 1 extraction group) and that utilized by Schoeninger and DeNiro (1984; referred to as the number 2 group). The latter set of extractions were further subdivided into two groups based on minor differences in sample preparation. The first collection, consisting of 23 bones, was dried in a 65°C oven for approximately 24 hours after initial cleaning. The 32 bones of the second group were freeze-dried after cleaning. The later method utilizes a cold vacuum in order to dry the samples while the former method uses the less efficient method of using heat to perform this function. The method of drying can influence

Table 5.2

Results of Stable Isotope and C/N Analyses

sample number	collagen yield (%)	extraction method	$\delta^{13}C$	$\delta^{15}N$	Atomic C/N	%C	%N
*B14	14.2	1	-6.4		3.3	4.8	1.7
B14	5.3	2	-9.2	4.1	4.6	14.0	3.6
H14	6.0	1	-12.5		-	1.6	0.0
L1-14	5.2	2					
O14a	4.8	1			-	0.6	0.0
O14b	2.8	2	-10.8		-	4.6	0.0
H15	4.8	2	-8.8	4.1	6.1	9.4	1.8
O15a	2.8	2			4.7	14.1	3.5
O15b	3.8	2	-15.3		4.0	14.2	4.1
L1-18	2.7	2					
B19	5.7	2.1	-11.9				
B19	2.3	2.2			-	0.5	0.0
O19	7.0	2	-13.9		3.9	20.8	6.2
B21a	3.7	2			-	4.6	0.0
B21b	5.9	2	-11.6	10.1	8.4	7.9	1.1
B41	2.9	1			-	0.5	0.0
B41	3.5	2			-	0.8	0.0
H41	17.4	1	-4.8		2.0	4.3	2.5
O41	15.0	1				0.3	0.0
*H46	19.1	1	-4.1		3.3	5.3	1.9
B57	5.0	1	-8.2		13.6	7.0	0.6
B57	7.7	2	-12.0	0.9	5.3	4.5	1.0
Bv57	8.4	2	-6.0	4.0	4.0	16.3	4.8
*O57	4.8	1	-19.3		3.5	7.6	2.5
L2-77	7.3	2					
O62a	6.9	2	-14.3	3.0	4.4	16.6	4.4
O62b	4.9	2	-18.5	2.8	4.0	16.8	4.9
E82	6.1	2	-9.8	3.4	7.3	9.4	1.5
L3-36	3.6	2			3.4	4.6	1.6
L3-130A	8.0	2			3.2	28.2	10.2
L3-130B	2.7	2			3.4	2.6	0.9
L3-137	1.4	2			3.6	5.0	1.6
L3-148	2.9	2			4.0	3.4	1.0
B179	3.7	2	-13.1		-	4.7	0.0
O179	5.4	2	-18.3	5.0	4.3	15.8	4.3
O220	3.8	2	-18.4	5.0	3.7	15.6	4.9
L4-224	3.7	2			3.4	9.9	3.4
B253	24.5	2	-9.4	3.6	3.9	17.1	5.1

Table 5.2

Results of Stable Isotope and C/N Analysis (cont'd)

sample number	collagen yield (%)	extraction method	$\delta^{13}C$	$\delta^{15}N$	Atomic C/N	%C	%N
L5-263A	9.7	2			3.2	29.8	10.8
L5-263B	5.8	2			3.2	21.9	7.9
L5-266A	5.7	2			3.2	27.2	10.0
L5-266B	5.1	2			3.3	11.2	4.0
*S266	8.1	2	-9.1	4.2	3.5	22.2	7.3
*B274	18.8	1	-8.4		3.1	18.2	6.8
*B274	11.0	2	-9.0	4.1	3.4	26.0	8.8
L6-274A	6.9	2			3.2	26.4	9.5
L6-274B	6.7	2			3.2	23.4	8.5
*O274	27.0	1	-12.9		3.6	14.2	4.6
L6-290	10.2	2			3.2	32.8	12.0
L7-278A	7.9	2			3.2	26.6	9.8
L7-278B	9.0	2			3.2	30.5	11.2
L7-278C	5.0	2			3.2	17.6	6.4
L7-283A	10.5	2			3.2	30.0	11.0
L7-283B	8.8	2			3.2	32.1	11.8
L9-309	4.2	2			3.5	3.3	1.1
7-E160	5.8	2	-11.5	0.5	-	4.2	0.0
7-L174	5.3	2	-10.8	4.1	4.8	11.2	2.7
7-O197	5.5	2	-16.8	2.8	5.3	10.4	2.3
TB1-18	2.3	2			-	0.3	0.0
TA1-31	2.7	2			-	0.3	0.0
N-Bv21	2.9	2			-	0.0	0.0
N-O21	3.8	2			-	0.5	0.0
TB2-21	2.5	2			-	0.3	0.0
T2-22	2.7	2			-	0.3	0.0
N-Ov23	3.6	2			-	0.3	0.0
TA2-23	2.8	2			-	0.3	0.0
N-G1	4.2	2	-20.0		3.1	19.4	7.2
M-Ov1	9.1	2	-10.8		3.2	30.7	11.3

NOTE 1: sample numbers preceded by a '*' have C/N ratios falling within the range of modern bone in combination with stable isotope results

Note 2: for extraction methods, 1 = Longin (1971) and 2 = Schoeninger and DeNiro (1984)

Note 3: for sample B19, method 2.1 = oven dried and method 2.2 = freeze dried

'collagen' yields by determining the amount of water that is retained.

The results from the two drying groups were analyzed in order to determine the validity of combining the two data sets (see Table 5.2 for complete results). This is desirable since it would increase the total sample size from mound 1 of site 523 which would in turn allow a more confident interpretation of the diagenetic factors that influenced this particular site. Statistical tests can be useful in this situation but only if they meet certain criteria: They must 1) be able to use data at an ordinal level, 2) be able to test non-normally distributed samples, and 3) test whether or not two independent samples originated from the same population. The Mann-Whitney U-test, a nonparametric test, fulfills these criteria and consequently was used in this analysis (Siegel, 1956:116-127; Conover, 1980:216-223).

It was found that the two sample preparation groups did not differ significantly in their collagen yields or % carbon values. They did, however, differ significantly in their % nitrogen and carbon-to-nitrogen ratios (Table 5.5A). These results suggest that some factors have influenced the amount of nitrogen that is recovered from the bones. Further analysis was undertaken to determine whether this unknown factor involves the method of sample preparation or some other factor.

Samples from a single bone (B19) were prepared by the two drying methods. The oven-dried sample produced a higher yield than the freeze-dried sample (5.7% vs. 2.3%) but the actual amount was insufficient to supply a sample for stable isotope and C/N analysis. The larger yield produced by the oven-dried sample may be the result of incomplete water extraction from the sample. The yield would, therefore, represent 'collagen' and water, a combination not found in freeze-dried specimens where water is removed in a more efficient manner. This factor must be taken into account when examining Tables 5.1 and 5.2.

With the development of chemical techniques useful in the analysis of archaeological bones, research has begun on the specific chemical reactions involved in the diagenesis of the bone as well as on the factors which affect the rates of these reactions. Researchers have concentrated on a number of factors including ambient temperatures. DeNiro, Schoeninger and Hastorf (1985) have shown that heating bones at 200°C for 4 hours does not affect the C/N ratios of the samples while heating the bones at the same temperature for 12 hours increased the C/N ratios by approximately 2 (indicating a greater loss of nitrogen). These researchers did not use temperatures lower than 200°C but given the fact that chemical reactions double their reaction time for every 10°C rise in temperature (Mortimer, 1975:426-429), reactions at 200°C would be occurring at a

much faster rate than at 65°C. This suggests that any alteration of the organic component and subsequently the C/N ratios of the bone samples placed in at 65°C oven for 24 hours would be insignificant. The method of sample preparation, therefore, does not provide an adequate explanation for the differences in nitrogen recovery and thus, the two sample sets may be combined.

An alternative explanation for the differences in the total nitrogen content, and subsequently in the C/N ratios, may relate to the level from which the bones were extracted. This factor was not controlled for in the initial investigation due to the limited sample size. The oven dried subgroup consisted primarily of samples recovered from the upper two units of the excavation (i.e. within 2 m of the surface) while the freeze dried collection was mainly recovered from beneath this level. It is possible that water flowing over the site in recent times may have affected the upper 2 meters of mound 1 while affecting the deeper levels to a lesser degree (see Chapter Three for a discussion of the effects of groundwater on bone preservation). In order to test this hypothesis of the effects of depth of bone burial, the results were divided into two groups according to the depth below datum. The first group consisted of all extraction group 2 samples from the upper 2 m while the second included bones from below this level. Statistical analysis of the two groups'

C/N ratios, %C, %H, and %N support the hypothesis that depth of burial is a significant factor (all of these factors show a significant difference between the two groups - see Table 5.5A).

Based on these results and the research delaing with the effects of temperature on bone preservation, the two number 2 extraction batches were combined into a single sample. As a further test of the extraction group 2 method, a modern sheep rib was prepared by this method in combination with the lipid extraction procedure developed by Bligh and Dyer (1959). The sample was collected from a modern village located within the present study area. The results (sample M-Ov1, Table 5.4) indicate that this method extracts a significant amount of the organic material from the bones and confirms the atomic C/N ratio range (2.9 - 3.6) established by DeNiro (1985).

5.3 Data Presentation

Summary statistics for the two extraction methods representing the material from the two study sites are presented in Table 5.4. The results of the Mann-Whitney and Spearman's Rank Correlation Coefficient tests are presented in Table 5.5.

Figures 5.1 through 5.8, to be described later in this chapter, graphically portray the relationships between the depth below datum, yield, atomic C/N ratio, % carbon, and

% nitrogen variables in the extraction groups 1 and 2 samples from Mound 1 exclusively. Best-fit lines are presented for each graph but because all of the data are not at an appropriate level (at least interval data), regression formulae and the accompanying correlation coefficients were not calculated (Thomas, 1976:390).

Comparison of Extraction Methods

Four bones were subjected to both extraction methods in order to further expose any differences between the methods (Table 5.3). It was found that the extraction method 1 group had a higher yield, enriched $\delta^{13}C$ values, higher C/N ratios but lower quantities of carbon and nitrogen in comparison to the method 2 samples. The enriched $\delta^{13}C$ values suggest humic substances which have contaminated the samples arose from degradation of the predominantly C4 grass cover. The small number of samples pose certain problems in the interpretation of these results, however, they do provide an interesting contrast to the study of Chisholm et al. (1983a)(see Chapter Four).

Table 5.4 indicates that the two extraction methods differ significantly in several variables. The method 2 extractions from Mound 1 of site 523 have, on average, lower yields, $\delta^{13}C$ (this maybe an artifact of the difference in animal diets), and C/N ratios than the method 1 extractions from the same site. The method 2 samples also

Table 5.3

Results of a Comparison Study between the Two Extraction Methods

SAMPLE	YIELD		$\delta^{13}C$		C/N		%C		%N	
	M1	M2	M1	M2	M1	M2	M1	M2	M1	M2
B14	14.2	5.3	-6.4	-9.2	3.3	4.5	4.8	14.0	1.7	3.6
B41	2.9	3.5	-	-	-	-	.5	.8	.0	.0
B57	5.0	7.7	-8.2	-12	13.6	5.3	7.0	4.5	.6	1.0
B274	18.8	11	-8.4	-9.0	3.1	3.4	18.2	26.0	6.8	8.8

NOTE: M1 = method 1 extractions, M2 = method 2 extractions
AVG = averages, SD = standard deviations
sample B41, M2 extraction was freeze dried while the rest were oven dried

Table 5.4

Summary Statistics For Various Sample Sets

SAMPLE SET	VARIABLE	# OF SAMPLES	MEAN	STANDARD DEVIATION
	yield	11	12.3	8.0
extraction	$\delta^{13}C$	8	-9.6	5.1
group 1	C/N	7	4.6	4.0
mound 1	%C	11	5.9	5.8
	%N	11	1.9	2.2
	yield	43	5.7	2.4
extraction	$\delta^{13}C$	17	-12.4	3.7
group 2	$\delta^{15}N$	12	4.2	2.2
mound 1	C/N	34	4.0	1.2
	%C	39	15.5	10.1
	%N	39	5.0	4.0
extraction	yield	20	5.1	1.8
group 2,	$\delta^{13}C$	12	-11.8	3.3
levels 1+2	$\delta^{15}N$	8	0.8	2.6
(without	C/N	11	5.2	1.5
neolithic	%C	16	9.7	6.6
and mound 7)	%N	16	2.3	2.2
extraction	yield	25	7.0	4.5
group 2,	$\delta^{13}C$	6	-12.9	4.5
levels 3-9	$\delta^{15}N$	5	4.4	0.6
(without	C/N	24	3.4	0.3
neolithic	%C	25	18.7	10.0
and mound 7)	%N	25	6.5	4.0
extraction	yield	9	9.9	6.4
group 1,	$\delta^{13}C$	6	-9.2	5.8
levels 1+2	C/N	5	5.1	4.8
mound 1	%C	9	3.6	2.9
	%N	9	1.0	1.1

NOTE: Statistics were not calculated for Longin levels 3 through 9 since only two samples were analyzed from these levels.

Table 5.5A

Results of Mann-Whitney Test

Variables Used	Method	Depth (m)	Significant differences in distributions?
yield	2.1 vs. 2.2	all	no
C/N	2.1 vs. 2.2	all	yes
%C	2.1 vs. 2.2	all	no
%N	2.1 vs. 2.2	all	yes
%H	2.1 vs. 2.2	all	yes
yield	2	>2 vs. <2	no
C/N	2	>2 vs. <2	yes
%C	2	>2 vs. <2	yes
%N	2	>2 vs. <2	yes
yield	2	M1 vs. M7	no
C/N	2	M1 vs. M7	no
%C	2	M1 vs. M7	no
%N	2	M1 vs. M7	no
C/N	1 vs. 2	<2 vs. <2	no
C/N	1 vs. 2	<2 vs. >2	no
%C	1 vs. 2	<2 vs. >2	no
%N	1 vs. 2	<2 vs. >2	no
%C	1 vs. 2	<2 vs. >2	yes
%N	1 vs. 2	<2 vs. >2	yes

NOTE: 2.1 = oven dried group 2 extractions
2.2 = freeze-dried group 2 extractions
1 = Longin extractions
M1 = mound 1, site 523 samples
M7 = mound VII, site 523 samples
>2 = upper 2 meters
<2 = below 2 meters

all H_o = no significant differences in distributions of the 2 samples (i.e. they are from the same population)
all significance levels (α) = .05
samples G1 and M-Ov1 not included in calculations

Table 5.5B

Results of Spearman's Rho Test
for Mound 1 Material Only

Variables used	Method	Correlation	Number of samples
yield, C/N	1	- (r_s=-.198)	7
yield, C/N	2	- (r_s=-.382)	34
yield, %C	1	- (r_s=.456)	11
yield, %C	2	* (r_s=.805)	39
yield, %N	1	* (r_s=.626)	11
yield, %N	2	* (r_s=.787)	39
δ^{13}C, C/N	1	- (r_s=-.468)	7
δ^{13}C, C/N	2	- (r_s=.095)	14
δ^{15}N, C/N	2	* (r_s=-.085)	12
%C, %N	1	* (r_s=.877)	11
%C, %N	2	* (r_s=.978)	39
dbd,yield	1	- (r_s=.431)	11
dbd, C/N	1	- (r_s=.367)	7
dbd, %C	1	* (r_s=.782)	11
dbd, %N	1	* (r_s=.742)	11
dbd, yield	2	* (r_s=.472)	42
dbd, C/N	2	* (r_s= -.802)	34
dbd, %C	2	* (r_s=.565)	39
dbd, %N	2	* (r_s=.629)	39

NOTE: 1 = Longin (1971) method extractions
2 = Schoeninger and Deniro (1984) method extractions excluding sample B253 and the oven dried B19
dbd = depth below datum in cms.
\- = no significant correlation (α = .05)
* = significant correlation (α = .05)

H_o = no significant correlation between the samples

samples G1 and M-Ov1 not included in calculations

produced higher average yield of both carbon and nitrogen compared to the method 1 samples. The method 1 specimens were not subjected to a nitrogen isotope analysis and therefore, comparisons of $\delta^{15}N$ values with those of the method 2 collection were not possible. It must be emphasized that the method 1 extractions consisted of fewer samples relative to the method 2 collection and therefore, conclusions involving this sample set must be viewed with caution.

The Mann-Whitney test, described above, was used to compare the two extraction methods (Table 5.5A). In order to test the hypothesis that the depth of burial of the bone plays a significant role in preservation, the group 1 samples from the upper 2 m of mound 1 were compared first to the group 2 'collagen' samples from the upper 2 m of mound 1 and subsequently to the rest of the extraction group 2 samples. The results indicate that in both cases, there is no statistically significant difference in the distributions of the C/N ratios of the two groups. Analysis of elemental compositions revealed that the group 1 (upper 2 m) and group 2 (upper 2 m) collections did not differ significantly in their carbon and nitrogen contents while the group 1 (upper 2 m) and group 2 (below 2 m) groups did differ in this regards. The later group also contained a significantly higher proportion of these elements compared to the former group.

Comparisons of Variables

Comparisons were also undertaken in order to determine whether results from the method 2 sample set were consistent. The samples from the upper two meters of site 523, mound 1 produced lower average yields, $\delta^{15}N$ values, % carbon and % nitrogen and higher average $\delta^{13}C$ and C/N ratios. The different $\delta^{13}C$ and $\delta^{15}N$ values may again reflect differences in animal diet while the other factors reflect the influence of the preservational factors discussed above.

Figures 5.1 through 5.8 illustrate that: 1) the amount of 'collagen' which can be extracted from any given bone, regardless of the method used, is related to the depth from which it was recovered, and 2) the amounts of extractable carbon and nitrogen, as well as the ratio between these two elements, are inversely related to the level from which the sample was recovered (e.g. the lower the level, the higher the elemental content).

To determine whether the relationships that are implied by the graphs are statistically significant, the data was analyzed using Spearman's Rank Correlation Coefficient (r_s) (Siegel, 1956:202-213). The selection of this nonparametric test was based on the fact that the data was at an ordinal level. The results of this test are presented in Table 5.5B.

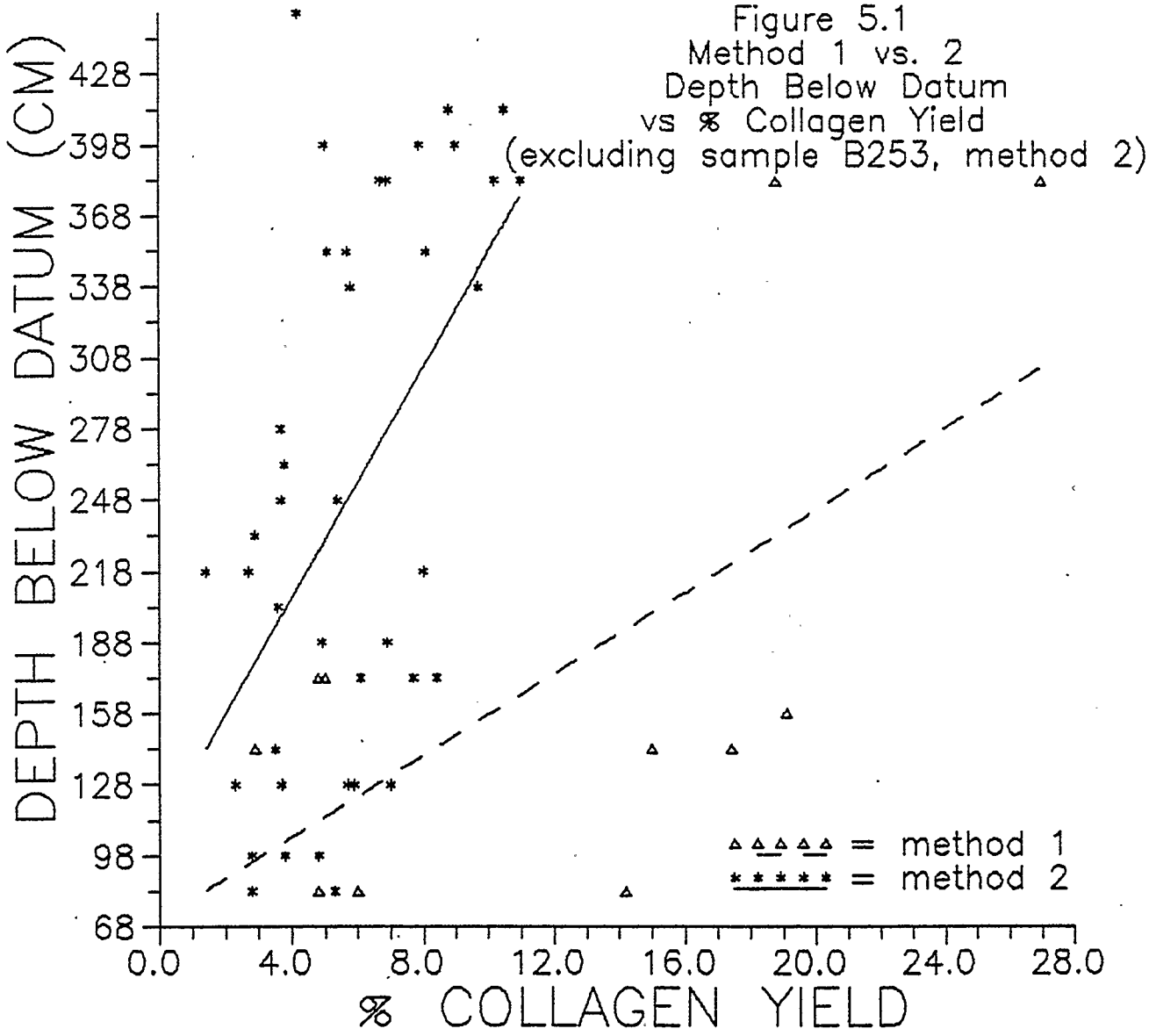


Figure 5.1
Method 1 vs. 2
Depth Below Datum
vs % Collagen Yield
(excluding sample B253, method 2)

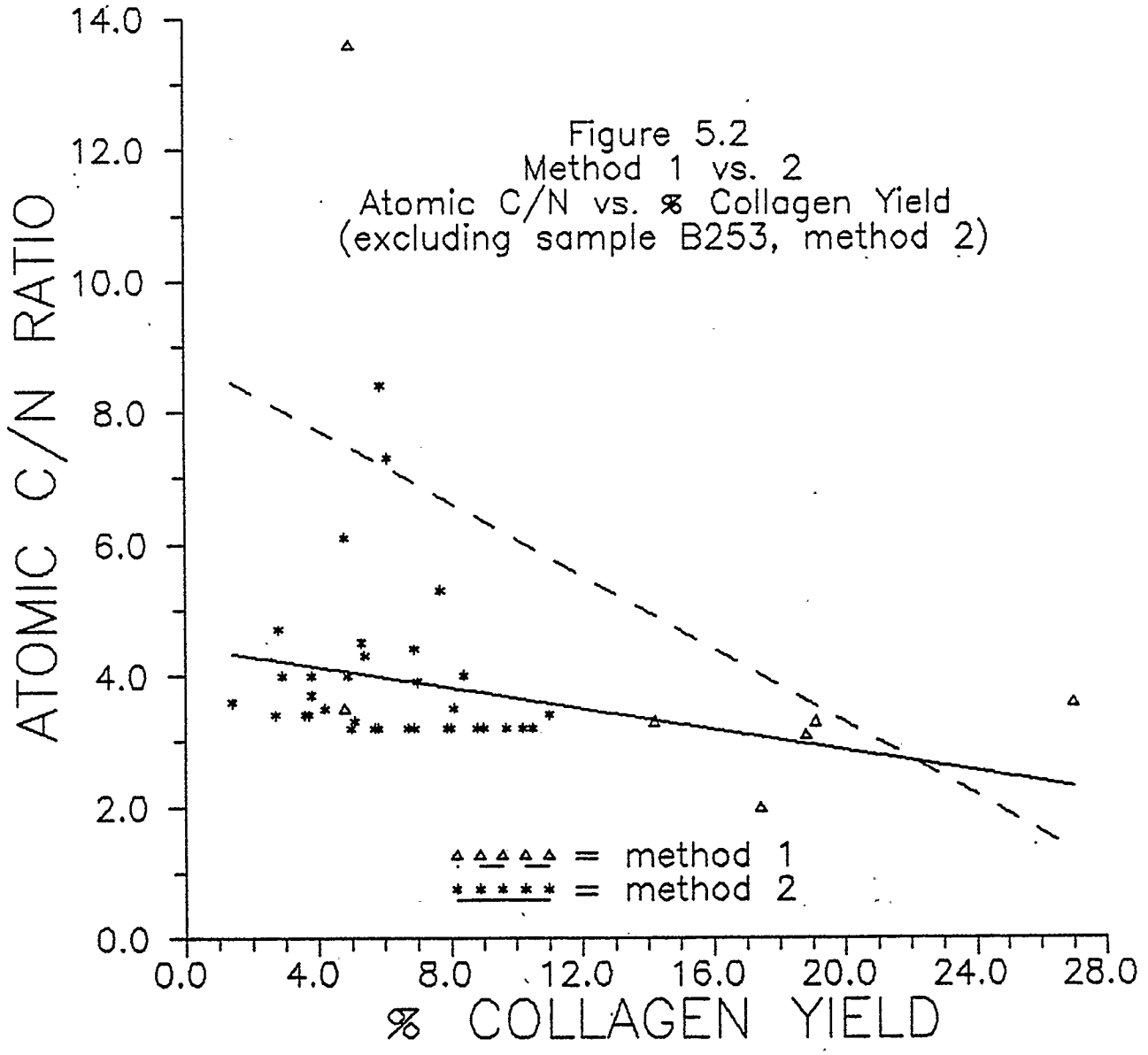


Figure 5.2
Method 1 vs. 2
Atomic C/N vs. % Collagen Yield
(excluding sample B253, method 2)
ATOMIC C/N RATIO
14.0
12.0
10.0
8.0
6.0
4.0
2.0
0.0
0.0
4.0
8.0
12.0
16.0
20.0
24.0
28.0
% COLLAGEN YIELD
△△△△△ = method 1
***** = method 2

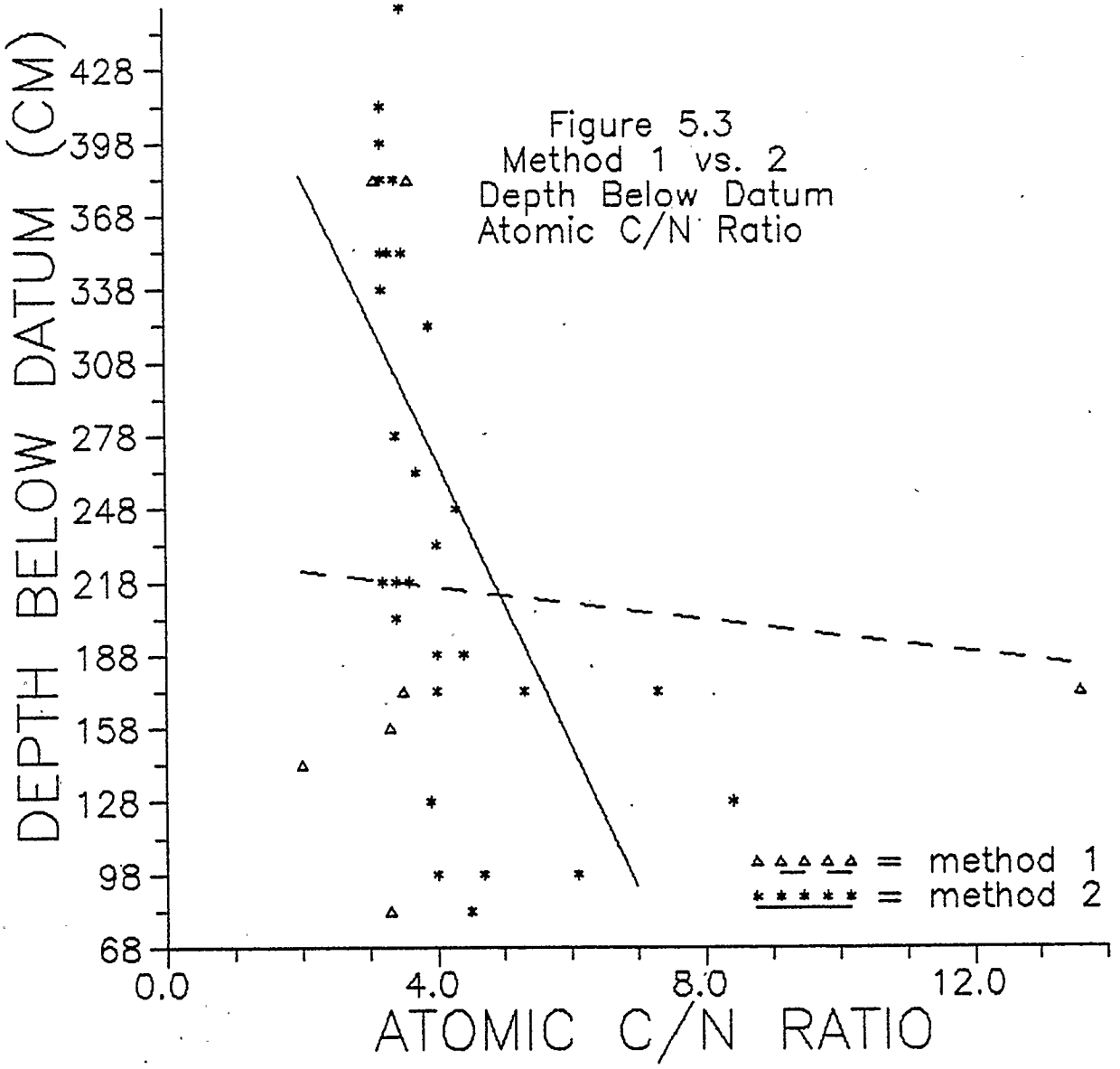


Figure 5.3
Method 1 vs. 2
Depth Below Datum
Atomic C/N Ratio

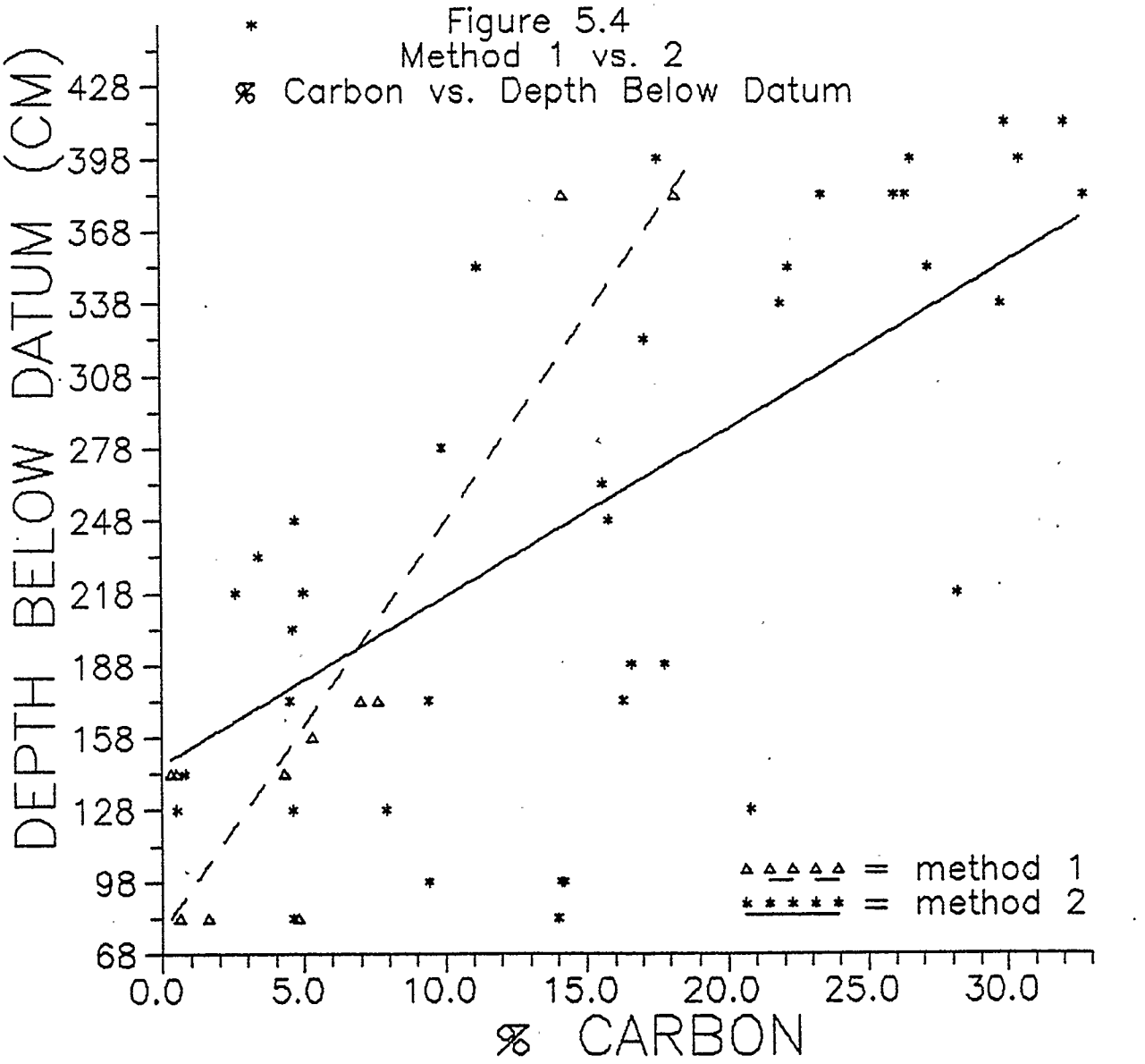


Figure 5.4
Method 1 vs. 2
% Carbon vs. Depth Below Datum
DEPTH BELOW DATUM (CM)
428
398
368
338
308
278
248
218
188
158
128
98
68
0.0
5.0
10.0
15.0
20.0
25.0
30.0
% CARBON
= method 1
= method 2

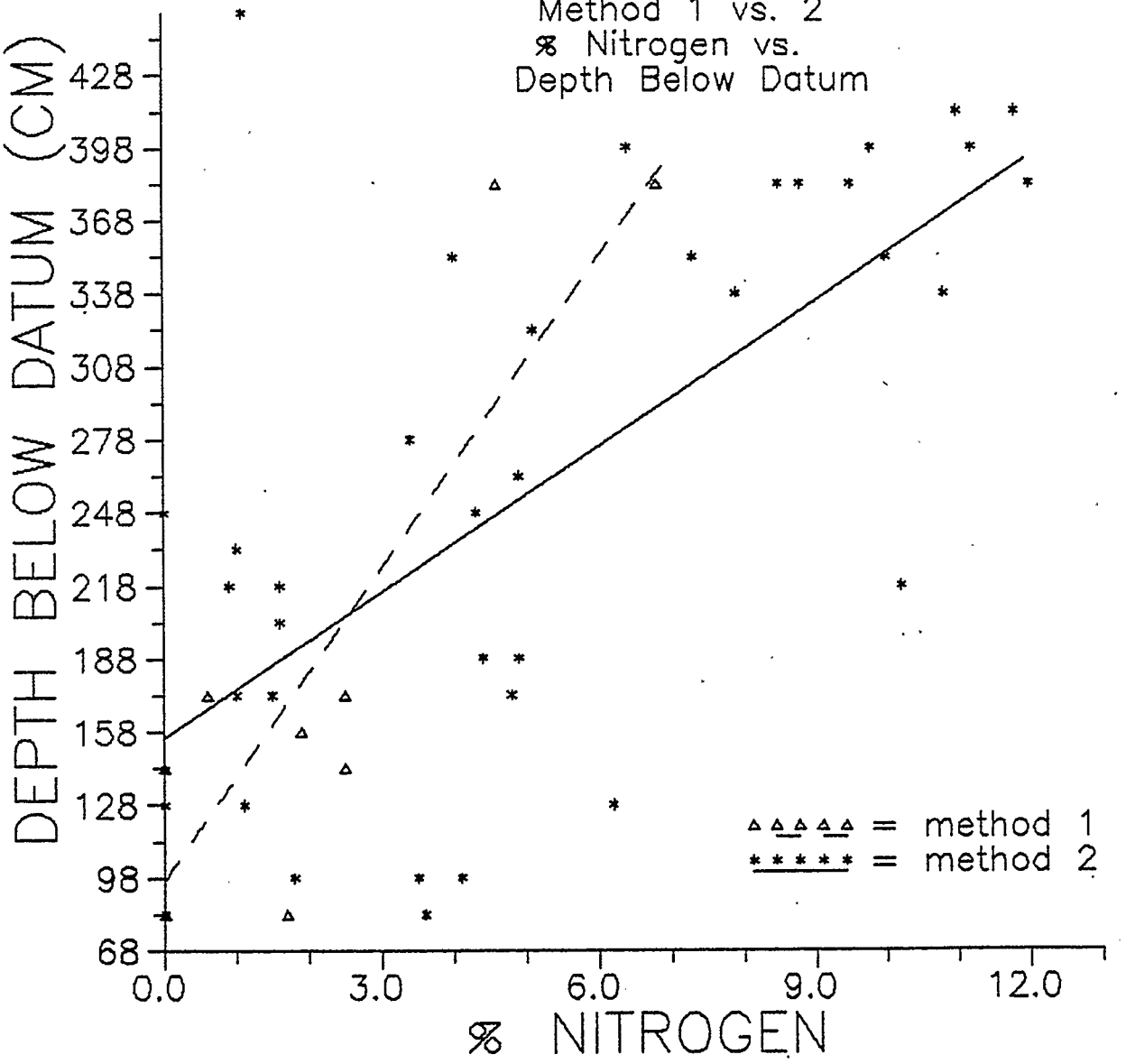
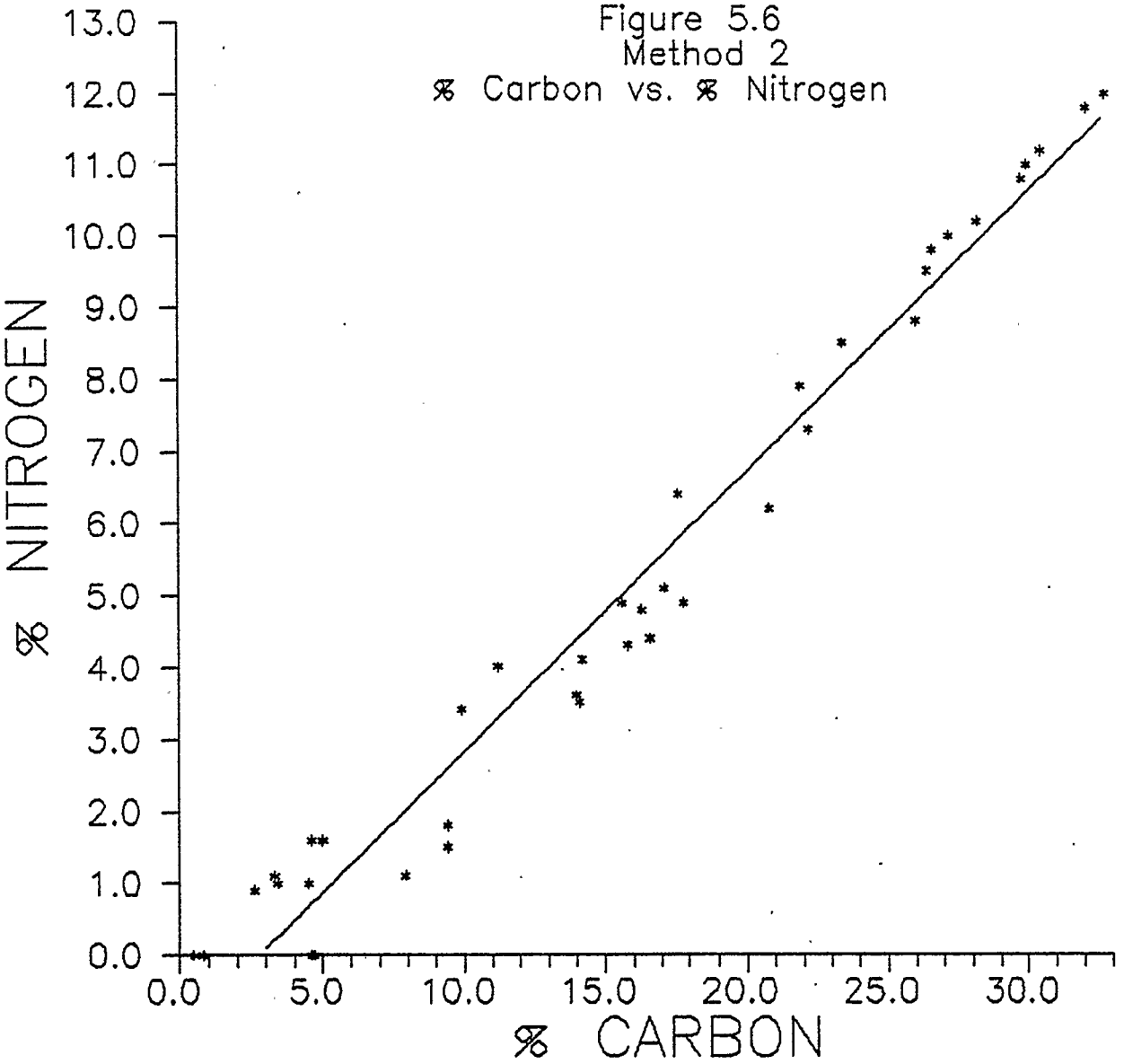


Figure 5.5
Method 1 vs. 2
% Nitrogen vs.
Depth Below Datum
DEPTH BELOW DATUM (CM)
428
398
368
338
308
278
248
218
188
158
128
98
68
0.0
3.0
6.0
9.0
12.0
% NITROGEN
= method 1
= method 2

Figure 5.6
Method 2
% Carbon vs. % Nitrogen



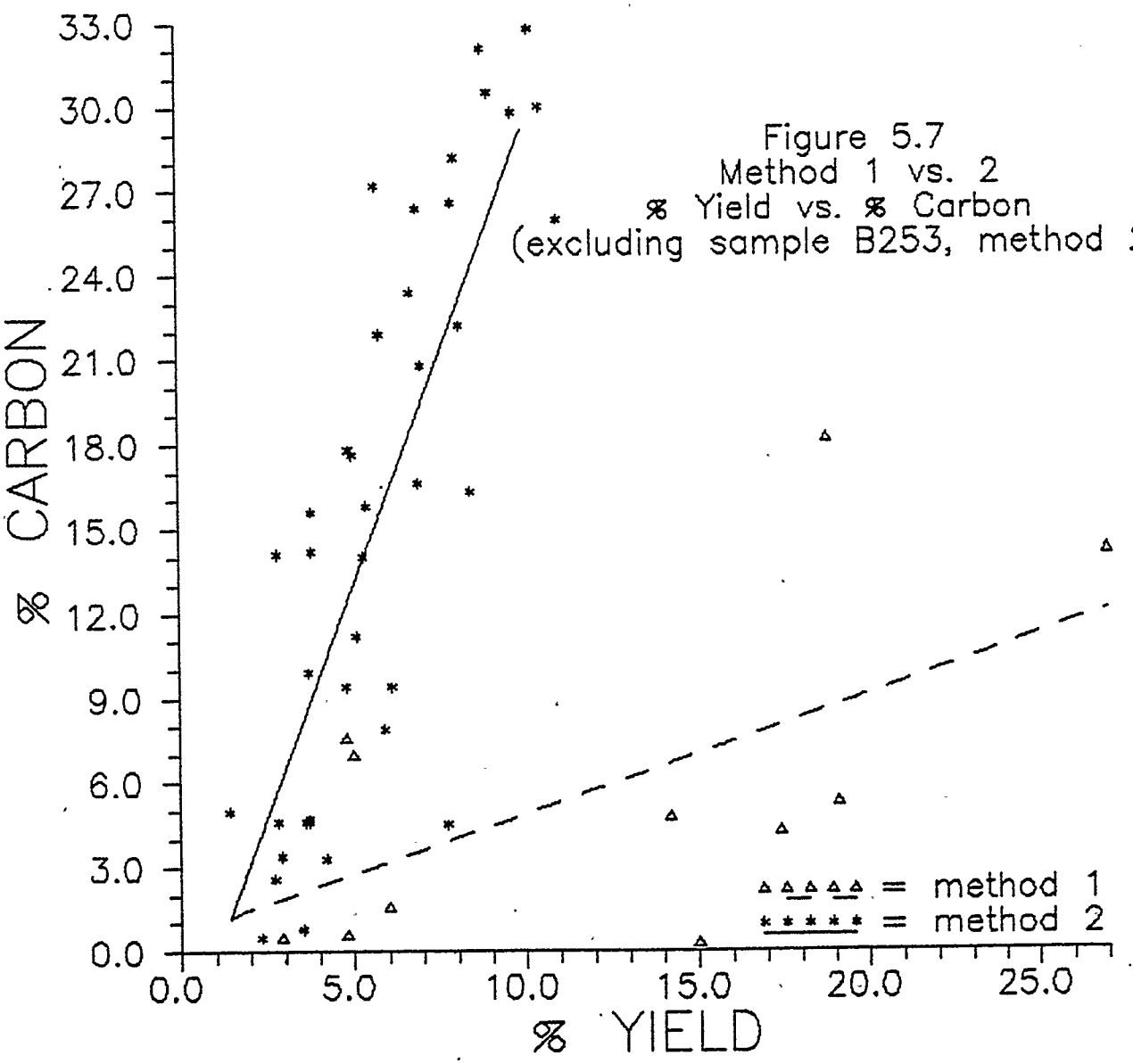
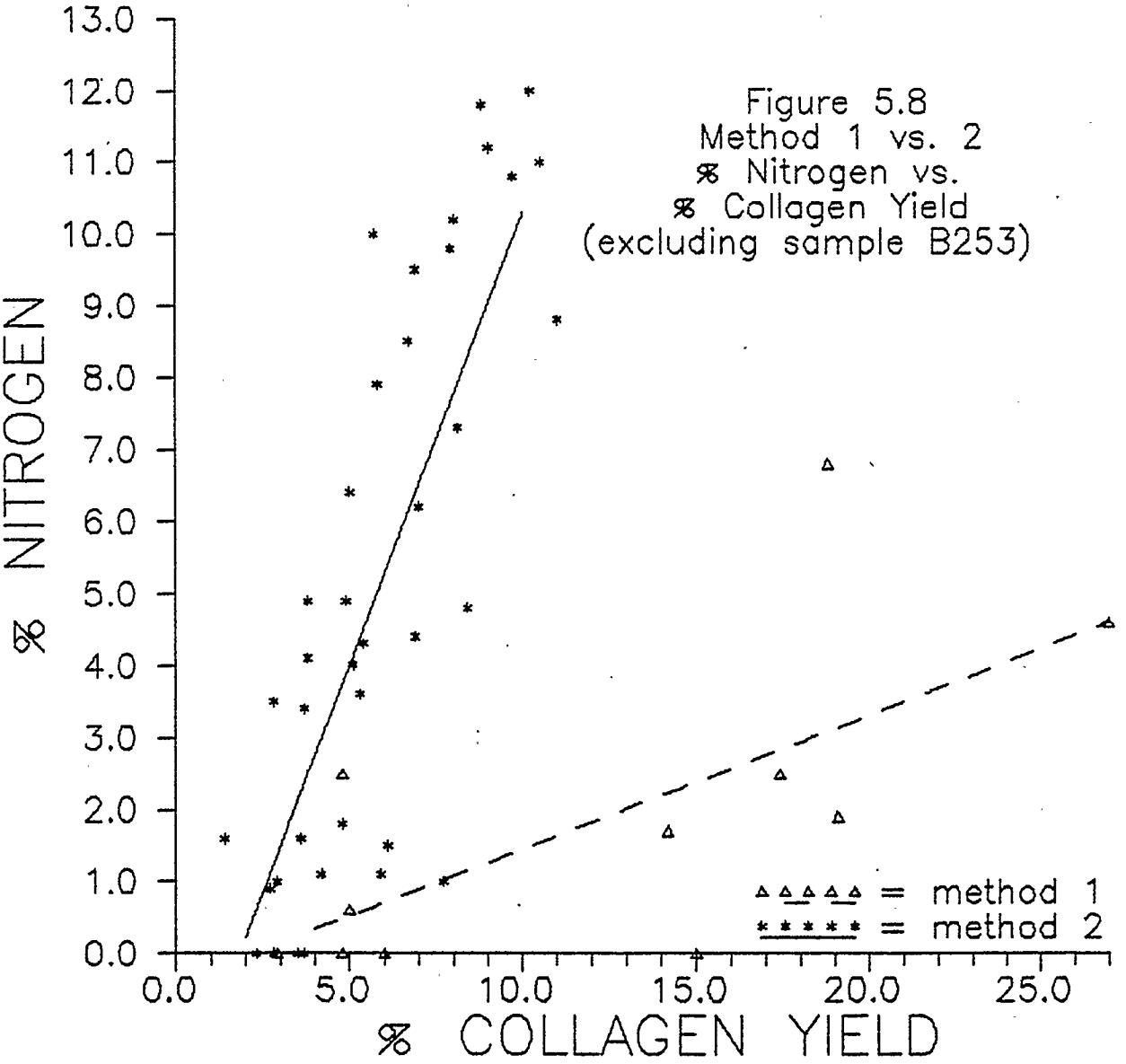


Figure 5.7
Method 1 vs. 2
% Yield vs. % Carbon
(excluding sample B253, method 2)


13.0
12.0
11.0
10.0
9.0
8.0
7.0
6.0
5.0
4.0
3.0
2.0
1.0
0.0
% NITROGEN
0.0
5.0
10.0
15.0
20.0
25.0
% COLLAGEN YIELD
Figure 5.8
Method 1 vs. 2
% Nitrogen vs.
% Collagen Yield
(excluding sample B253)
= method 1
= method 2

The statistical results suggest that there is a significant correlation between 'collagen' yield and the amount of carbon and nitrogen extracted from the samples using method 2 but only between yield and %N for method 1. There is a highly significant correlation between the amounts of extractable carbon and nitrogen indicating that there is a lack of bias towards the loss of one element over the other during the diagenetic alteration of the bone. The amount of extractable 'collagen' was not significantly correlated with the C/N ratio of the sample which also supports the hypothesis of a lack of bias in elemental retention.

The results from the method 1 group indicate that the depth from which a bone is recovered plays a significant role in the amount of carbon and nitrogen which can be recovered from the sample, however, depth is not important in the determination of the C/N ratio of the sample. For the group 2 method, the amounts of carbon and nitrogen as well as the C/N ratio are all dependent on depth below surface. These results provide contradictory evidence with relation to the hypothesis of differential preservation of carbon and nitrogen - if there was a bias in the preservation of these elements based on the depth of burial, one would expect that the C/N ratio would also be dependent on the depth as in the method 2 results (see Chapter Six for further discussion).

Site 506A Sample

The Neolithic samples (site 506A) presented in Table 5.2 can be seen to have produced much lower quantities of 'collagen', carbon, and nitrogen in comparison to the site 523 samples (both extraction methods). Several of the C/N ratios from this older site did, however, fall within the ranges of the site 523 samples.

Preservation Across Site 523

In order to test whether preservational histories differ across site 523, samples from mounds 1 and VIIA were compared. The results support the hypothesis that the two mounds have shared a similar preservational history - the mound VIIA material is not significantly different in any of the variables relative to the those from the upper meter of mound 1 (Table 5.5A).

CHAPTER SIX

DISCUSSION AND CONCLUSION

6.1 Introduction

Researchers frequently incorporate several different methods in their investigations and the isotopic analysis of paleodiets is no exception. In this chapter, the following topics will be discussed: 1) the results from two collagen extraction techniques, 2) an examination of the relevance of isotopic analysis to West-Central African prehistory, and 3) a presentation of several recommendations for future research. A summary of the present study will conclude the chapter.

6.2 Extraction Method Analysis

Collagen Yield

The group 2 method was found to extract, on average, a lower quantity of 'collagen' from a bone sample relative to the extraction 1 method. This observation may be explained through an examination of the steps used in the two methods. Both procedures utilize the same basic steps of dissolving bone mineral and gelatinizing and hydrolyzing the 'collagen' but deviate after this point in that the group 2 method includes a sodium hydroxide soak to eliminate humic contaminants while the group 1 method does not. This purification step may account for the yield

differences in that the group 1 'collagen' samples may include humic substances which artificially increase the average yield. The color of the 'collagen' samples tend to support this interpretation - the group 1 samples were a brownish color which is characteristic of humates (Stevenson and Butler, 1969; Limbrey, 1975), while the group 2 samples were lighter in color. Both methods were applied to four separate bone samples and it was found that two group 1 extractions produced significantly higher 'collagen' yields while the group 2 method of the other two samples produced slightly higher yields (Table 5.3 and see below).

Chisholm and colleagues (1983a) produced similar yield results in their comparisons of various extraction techniques. They believe that the lower yields may be "... due to the solubility and loss of some of the amino acids (e.g. serine and threonine) to the NaOH ..."(Chisholm et al., 1983a:357). Such amino acids, however, make up only a relatively small proportion of the collagen molecule (together, these two amino acids make up approximately 5% of the total collagen composition - see Eastoe, 1967). Due to their low representation in the collagen molecule, the loss of these amino acids would not affect the final quantity of 'collagen' as much as the elimination of humates. This argument also applies to the effects of

serine and threonine loss on the overall isotopic values of the bone collagen (Tuross et al., 1988:931).

Several researchers have investigated this problem through an analysis of the amino acid composition of the base-soluble extract (Boutton et al., 1984, Katzenberg, in press). The studies found collagen-like amino acid profiles for the extract and this led the researchers to suggest that some 'collagen' may be lost during the NaOH soak. Further studies must focus on the processes involved in this loss. For example, are specific portions of the collagen molecule lost or is the loss random? To what degree do the amino acid profiles of the base-soluble humates resemble that of collagen and how much do they contribute to the results found by Boutton et al. and Katzenberg?

The 'collagen' yield ranges reported by other studies have varied from 3% - 12% (DeNiro and Epstein, 1981) to as high as 20% - 30% (Hare, 1980). The yields for the present study ranged from 2.9% - 27% (average = 12.3%) for the group 1 extractions and 1.4% - 24.5% (average = 6.13%) for the group 2, mound 1 samples (excluding sample B253, the range is 1.4% - 11% and averages 5.7%). The modern sheep sample produced a yield of 9.1% suggesting that many of the group 2 archaeological samples have lost a certain amount of organic material. This loss may relate to taphonomic factors. Yields which were higher than the modern sheep

sample's result may be a result of the inclusion of contaminants.

A significant positive correlation was found to exist between sample burial depth and 'collagen' yield for the entire group 2 collection (Table 5.5). Taphonomic factors have affected the bone samples and the strength of the correlation between depth and yield suggests the samples were affected equally in terms of their 'collagen' contents, a conclusion similar to the linear collagen degradation model proposed by Dennison (1980). The strong correlation between the amount of 'collagen' and the amounts of both carbon and nitrogen yields is understandable - the more 'collagen' that is available, the greater the quantity of carbon and nitrogen which is available for extraction.

Isotopic Values

It was found that the group 1 extraction samples produced enriched (i.e. more positive) $\delta^{13}C$ values and lower $\delta^{15}N$ values relative to the group 2 samples. Caution must be used, however, when conclusions are based on a heterogeneous archaeological sample (i.e. many bones from different individuals). Under such conditions, the difference in isotopic values may reflect a dietary difference rather than factors related to the method of extraction. In an attempt to avoid this lack of control over sample composition, a comparison was undertaken

involving the three bones extracted by both methods for which $\delta^{13}C$ values were obtained (Table 5.3). The results suggest that humates produced at these particular sites tend to cause $\delta^{13}C$ values to appear artificially enriched. Stafford, Brendel, and Duhamel (1988) found that humates do indeed affect the isotopic values of the collagen sample, although the nature of the shift in $\delta^{13}C$ values can vary depending on the humic substance involved. Katzenberg (in press) found similar results in a recent study using three separate bones. $\delta^{15}N$ values were not determined for the group 1 samples and thus it was not possible to interpret the effects that humate contamination has upon the ^{15}N and ^{14}N isotopes.

Carbon and Nitrogen Contents

The differences in the carbon and nitrogen contents of the samples are likely due to taphonomically-induced loss of these elements, particularly for the bone samples of the upper 2 meters of site 523. The group 1 samples were derived primarily from the upper two meters of mound 1 of the 523 site while the group 2 sample set is essentially composed of samples from below this level. No bone fragment in the entire data set was found to contain comparable amounts of carbon and nitrogen as found in modern collagen ($\approx$ 45% C and $\approx$18.5% N - Krueger and Sullivan, 1984; Eastoe, 1961) indicating that organic matter has been lost from the bones (the low levels of these elements from the modern

sheep sample maybe related to the loss of parts of the collagen molecule during the sodium hydroxide soak - see Chapter Four). This loss is more extensive in the upper two meters based on a comparison of the average elemental yields (Table 5.4) and statistical analysis (significant differences exist between the upper two meters and those below this limit in terms of the total carbon and nitrogen contents - see Table 5.5A). If only the larger method 2 collection is examined, the loss appears to preferentially favor one element over the other. C/N ratios are significantly correlated with depth of burial and for this to take place, the individual rates at which carbon and nitrogen are lost must be different. Several C/N ratios are large and may be the result of either 1) a loss of certain components containing larger amounts of nitrogen e.g. the amino acid glycine (Masters, 1987:3210; Tuross, Fogel, and Hare, 1988:931) or 2) the addition of contaminants made up of larger amounts of carbon as opposed to nitrogen. The later factor is of unknown importance, however, since only a limited number of experiments have dealt with the problem of collagen exchanging carbon with the burial environment (Berger et al., 1964; Hassan and Hare, 1978; Gurtler et al., 1981).

A factor which likely does not play an important role in the group 1 samples is the inclusion of humate contaminates. Humic substances derived from organic sources

such as decaying plants generally contain more carbon (≈ 50-60%) and less nitrogen (≈ 8.5%) in comparison to bone collagen (Kononova, 1961; Schnitzer and Khan, 1972). Carbon percentages should therefore be higher and nitrogen levels lower in the group 1 extractions, which contain more humic contaminants, than in the group 2 samples. The present study, however, does not entirely support this hypothesis since the elemental levels are much lower than the hypothesis predicts. This also holds true for the four bones extracted by both methods, although sample B57 yielded more carbon in the group 1 sample than in the group 2 extraction (the $\delta^{13}C$ value of this sample also shows the largest variation of all four samples suggesting that the sample has been contaminated to a greater extent than the others). Humate contamination, while likely being of some importance in the preservational history of the bones of the present study, does not by itself provide an adequate explanation for the differences in elemental compositions.

It can be concluded from these results that diagenetic factors are responsible for an increased loss of organic matter from the bones of the upper two meters of mound 1, site 523. One such diagenetic factor may relate to the lack of an evident stratification in the upper part of this mound (David and MacEachern, 1988). The investigators believe that this disorganization was caused by activities associated with the building of structures, i.e. from the

planting and replanting of house posts, and agriculture. If bones were included in the soil that was dug from the post-hole, those of the upper levels would have been exposed to weathering factors more frequently and for a longer period of time than bones of the lower levels. This would in turn lead to an increased degradation of the bone and the subsequent loss of organic components such as amino acids (Kvenvolden, 1975; Carr, 1982; Lambert et al., 1985). The final result would be a decrease in the elemental contents of the bone fragments.

Extraction Method Comparison

Table 5.3 provides a contrast between the two methods of collagen extraction based on four distinct bones. As has been mentioned previously, the sample size is small which makes it difficult to build interpretations, however, certain factors can be elicited from this exercise. The methods were divided in terms of producing a larger 'collagen' yield relative to the other. The group 2 extractions, on the other hand, yielded a higher percentage of carbon and nitrogen for every sample except in one instance (one sample also did not yield any significant amounts of nitrogen by either method). The carbon yields of the two methods generally appear to have varied more between the methods than did the nitrogen values. Only the atomic C/N ratio of sample B274 fell within the range for modern collagen. These observations suggest that there is

no consistent pattern which explains the differences between the results of the two methods. The data do, however, support the depth of burial hypothesis with the sample from the lowest level (B274) yielding the highest quantity of 'collagen', carbon and nitrogen. Factors intrinsic to the bones themselves in association with their individual taphonomic histories (as opposed to the extraction method) appear to have determined the quantity of organic materials which survive in the bones.

Stable Isotopes and the Present Study

Stable isotope ratios were determined for a limited number of samples. Of these samples, only six display atomic C/N ratios which fall within the range of modern bone (see note 1 in Table 5.2). The two Bos (B14 and B274), the Suid (S266) and the Homo (H46) samples all yielded $\delta^{13}C$ values which indicate a diet which consisted entirely of C4 plants. This suggests that the human may have subsisted primarily on sorghum and/or millets and that the animals fed on the same plants or on wild C4 plants growing around the site. Stable isotopes cannot differentiate between individual plants within the gross C4 category but the data tend to support modern ethnographic material.

Two samples (S266 and B274) yielded $\delta^{15}N$ values which, along with their respective $\delta^{13}C$ values, fall within the range of animals grazing on C4 (savanna) plants as

established by Ambrose (1986:710) and DeNiro (1987:190). Sample 057 produced a $\delta^{13}C$ value which suggests that this individual relied somewhat on C3 plants while the second Ovis/Caprid sample (0274) yielded a $\delta^{13}C$ value which is in the C4 range. Ambrose (1986) suggests that sheep and goats can be distinguished isotopically due to their different feeding regimes - sheep are primarily grazers of low-lying C4 grasses whereas goats are mixed feeders of both C4 grasses and C3 browse. Based on this hypothesis, the 057 sample likely originated from a goat which fed on both C3 and C4 vegetation while specimen 0274 may have been representative of a sheep.

These few samples, while not providing an adequate sample size for the reconstruction of paleodiets, do indicate that C4 plants were of primary importance to at least some of the former inhabitants of site 523. The goat sample, however, does indicate that C3 plants were also available at this site. Indications of the relative importance of C3 versus C4 plants, and of meat in the humans' diet, as well as interpretations of paleoenvironmental conditions must await the analysis of a larger collection of well preserved bones from this site.

6.3 Stable Isotopes, C/N Ratios, and African Prehistory

Iron Age versus Neolithic sites

The current study illustrates a potential problem which African researchers face when studying older sites. None of the bones from the Neolithic site 506A yielded significant quantities of nitrogen (i.e. greater than .1%) while the carbon yields averaged .3% (maximum = .5%). The site 506A material appears to have lost a large amount of organic material, likely from leaching by groundwater as evidenced by the two surrounding fluviolacustrine deposits. The taphonomic history of the giraffe metatarsal is unknown but appears to have been much more conducive to the retention of organic matter. The metatarsal fragment may have been buried in undisturbed deposits for most of its history since the elemental yields are relatively large when compared to the samples from the group 2 collection. The bone may only have been exposed recently which would not allow sufficient time for weathering factors to affect its organic composition.

Stable Isotopes and West-Central Africa

The present study illustrates the problems faced by researchers in their attempts to apply stable isotopic analysis to West-Central African prehistory. One obvious point is that the taphonomic history of the bones will play a major role in determining whether or not stable isotope analysis will provide meaningful results. Investigation of

the bones' preservational history can be accomplished through an examination of the bones for obvious signs of contamination, by looking at the burial environment from which the bones were recovered, and by subjecting a representative collection of 'collagen' samples to the series of diagenetic tests currently available.

Information gathered from stable isotopic analysis is useful in the examination of the origins and spread of certain types of food strategies, for example, the use of freshwater species of plants and animals in the diet. While the relationship between freshwater biomes and isotopes is still not fully understood (see review by DeNiro, 1987), freshwater plants and the animals feeding on them appear to exhibit carbon isotopic signatures similar to C3 plants (van der Merwe and Vogel, 1983:39). If further research substantiates this observation, it will have important consequences for the present study area. The majority of plants making up the savanna vegetational pattern are C4 (van der Merwe and Vogel, 1983:44) and thus, individuals who primarily fed on freshwater molluscs and fish, for example, should exhibit carbon isotopic ratios which are more negative than populations relying on C4 grasses. The general proportions of C3 vs. C4 foods in the diet can theoretically be estimated and any changes in these proportions can be used as evidence of a changing diet,

assuming that the environment remained constant (see Lovell et al., 1986a).

Such an analysis would be applicable to the Lake Chad area, for example at the Daima site in Northern Nigeria. Connah (1981) found that this site produced a number of bone harpoons and freshwater fish remains and, at the same time, evidence for grain cultivation and stock raising. The remains by themselves do not allow researchers to determine the importance of each food item in the former inhabitant's diet. Taphonomic factors such as increased rainfall will bias the samples by selecting against smaller bone fragments and those fragments buried at shallower depths. Stable isotopes, on the other hand, would be of use in resolving the Daima problem. If, for example, the human remains were found to exhibit carbon isotope ratios indicative of a C3 plant diet, the hypothesis that these individuals relied heavily on freshwater animals and plants is strengthened. Seasonal movements between areas around the ancient enlarged lake and the savanna zone may also be investigated in the same manner used by Sealy and van der Merwe (1986), who examined the hypothesis concerning the movement of populations between the coastal and interior regions of South Africa. Questions such as 'were there major migrations throughout the savanna zone' and 'did the "Sao" have a seasonal pattern of resource usage?' can then be examined.

Carbon isotopes are also useful in tracing the origin and spread of certain crops. Van der Merwe and Vogel (1983) cite two examples, the first involves the spread of cereal agriculture into the West African tropical forest zone. Forest vegetation primarily consists of C3 plants and this fact would allow researchers to trace the movement of a C4 grain into the forest zone. Difficulties arise when the savanna zone is examined, however, due to the fact that the isotopic ranges of some types of the plants can overlap with the ranges of other crop species (Smith and Brown, 1973; Winter, Troughton and Card, 1975). This leads to the conclusion that while the general photosynthetic grouping of the main crops consumed by the individual can be determined, the specific plants within these groupings which are eaten can only be determined using other information such as paleobotanical remains.

The second example which van der Merwe and Vogel provide involves tracing the recent introduction of C3 plants into the savanna. In this example, it is not a necessity that the population should have depended on a single type of food (i.e. sorghum) since it is the changing proportions of the rarer plant group (e.g. the amount of C3 plants eaten by a population relying mainly on sorghum) which is important (Lovell et al., 1986a).

The combination of carbon and nitrogen isotopes have been used to determine the relative importance of meat in

the diet of prehistoric peoples (Ambrose, 1986; Ambrose and DeNiro, 1986a). Ambrose and DeNiro have convincingly demonstrated that for groups in East Africa, stable isotopes can distinguish "... pastoralists from farmers, camel pastoralists from capri-bovine pastoralists, and grain framers from non-grain farmers."(Ambrose, 1986:707 and figure 1, p. 710). Groups relying on animal protein tend to have more positive $\delta^{15}N$ values while groups subsisting on grains will have less negative $\delta^{13}C$ values. Conclusions based on $\delta^{15}N$ values must be used with caution, however, since factors such as the physiological adaptation by an animal to an arid environment may have a significant influence on bone collagen $\delta^{15}N$ values (Ambrose and DeNiro, 1986b; Sealy et al., 1987).

A final example involves the reconstruction of paleoenvironmental conditions in the study area. It was mentioned in Chapter Three that the C3 and C4 plant groups represent adaptations to different environmental conditions. The environmental requirements of the dominant crop and fodder of an area must be known, however, before it is possible to build a model of the area's paleoenvironmental conditions using isotopic values. For example, skeletal remains producing $\delta^{13}C$ values which cluster around -7 o/oo indicate that not only did the animals fed primarily on C4 plants or on the meat of other C4 feeders, but the environment may have exhibited

conditions similar to a modern savanna. Such conclusions must be viewed as being tentative, however, since isotopic values cannot provide information about the plants which were not consumed by the individual (for the example above, the animal may have been selectively feeding on C4 plants exclusively while ignoring the equally abundant C3 plant cover).

Paleoenvironmental models based on stable isotope values of contemporaneous organisms must also be viewed as tentative since many factors other than the environment can influence the distribution of plants. Stable isotopes when used in conjunction with other forms of information can, however, provide valuable information for the reconstruction of past environmental conditions.

This section has presented several suggestions on how the stable isotopes of carbon and nitrogen can be used in the study of West-Central African prehistory. While the usefulness of the information that stable isotopic analysis can provide will vary depending on the problem under examination, all investigations have a common requirement - the need for adequate samples of study materials. Chapter Two described the general lack of prehistoric human skeletal material which characterizes the study area as well as the qualities which make the available collections unsuitable for isotopic analysis. The large collection of human skeletal remains from Daima and the older Kursakata

site of northeastern Nigeria illustrate this problem (Connah, 1971 and 1981). The Daima material is of particular importance since it represents a fairly large early Iron Age population which was recovered from a well studied site. The Daima and Kursakata material have not been thoroughly analyzed, although Pfeiffer (1988) has examined the dentition of the Daima collection. Upon recovery, these collections were treated with Bedacryl, a deep penetrating acrylic resin containing a relatively large amount of carbon (Dowman, 1970). Because of this later feature, Bedacryl would introduce foreign carbon into the samples which may significantly alter the isotopic ratios of the samples. Extraction of this preservative may be possible, however, further research is required to determine the methods which can be used. Because of th e Bedacryl, the Daima and Kursakata collections were not used in the present study.

6.4 Future Research

Upon the introduction of stable isotopic analysis to the anthropological field, researchers realized its power as a tool for the reconstruction of the paleodiets of humans and animals as well as for general indications of past environmental conditions. There are still, however, a number of factors which have not been fully investigated, and which can be grouped into two main categories -

1) bones, collagen and diagenetic factors, and 2) methods of analysis.

Bones, Collagen, and Diagenetic Factors

This category includes the effects of various intrinsic and extrinsic factors, diagenetic changes in bone and collagen as well as the effects of these changes on the carbon and nitrogen isotope ratios, the degradation pathways of collagen in an archaeological situation, the importance of noncollagenous proteins, and the accuracy with which archaeologically-derived collagen reflects the original conditions of the individual during life. Chapter Three described the different factors associated with the bone (intrinsic) and its burial environment (extrinsic) which can affect the preservation of the remains. Several important intrinsic factors have been investigated but few deal with the relationship between the individual factor and stable isotope ratios: What effect will the loss of a portion of a skeletal element have on the overall isotopic ratio of that element after it is crushed into powder i.e. are stable isotopes equally distributed throughout a given bone or will specific areas reflect certain times in the individual's life? Bone is known to be a dynamic tissue which is constantly being remodeled in response to various pressures placed on it during the individual's life (this is known as Wolff's Law of bone remodelling). Bone is deposited or resorbed from muscle attachment points, for example, depending on the activities of the associated

muscles. If the individuals not only changed their activities during the later part of their lives but also changed their diet, the final interpretation of the individual's 'average' diet will depend on which part of the bone is represented i.e. that which was deposited near the time of the individual's death or chronologically 'older' bone.

Extrinsic factors are perhaps of greater importance to the archaeologist since these factors are more site specific compared to the intrinsic features of bone. Laboratory studies have tended to be rather limited in their scope, e.g. they have virtually ignored the interactions between different extrinsic factors and bone preservation. The results from these studies have not been used to examine 'real world' situations, for example what groundwater activities, soil pH, and soil temperatures are most conducive to the preservation of bone and what environments are characterized by these features? It may be possible to build a database consisting of combinations of different environmental conditions, their effects on bone preservation, and the correlation of these hypothetical environmental types with real study sites. Such factors, would allow archaeologists to determine whether or not a site will produce adequate bone collections and if they decide to continue with bone recovery, a limited number of test extractions can then be undertaken.

Many questions still exist with relation to the post-burial degradation of collagen. For example, what are the specific roles of soil microorganisms and how important are they in the breakdown of collagen? How closely do their activity levels correspond to variations in environmental conditions? Is there a basic pattern to collagen breakdown in the soil which varies slightly due to varying environmental conditions? How do these different environmental conditions affect the bone collagen stable isotope concentrations and is collagen lost at a consistent rate such that stable isotope ratios will remain the same as during the individual's life?

The question of the rate of collagen loss is perhaps the most immediate problem which must be investigated. If collagen is first broken into peptide segments (Bada, 1985:243; Henderson, 1987:44; cf. Hare, 1980), isotope ratios will not accurately reflect the individual's diet. Different peptide sequences contain varying amounts of amino acids which contain different proportions of carbon and nitrogen atoms. The loss of individual peptide segments through leaching by groundwater, for example, will change the carbon/nitrogen and stable isotope ratios of the extractable 'collagen' from its original condition. Thus, inconsistent collagen degradation may substantially influence the elemental and stable isotope compositions of archaeological remains. If, on the other hand, protein loss

is consistent (Dennison, 1980), isotopic ratios should theoretically remain constant. This example illustrates the need for a greater understanding of the degradation pathways of bone collagen in the burial environment.

Another problem relates to the turnover rate of bone collagen in living animals. Extrapolations for this rate have ranged from one or two decades to several centuries, the former being the popular choice in anthropological studies (see Chapter 3, section 3.6). Depending on the accepted range, collagen will reflect either the average diet over the individual's entire lifetime or the diet over the last few decades of life. This can have important consequences under certain circumstances such as when individuals change their dietary habits. Short term changes in diet may only be resolved if bone collagen is turned over in a short time span since a longer turnover rate will not be able to distinguish this type of change.

An area which has been ignored in anthropological stable isotope analyses involves the general metabolism of food and the relationship between food components and the manufacture of bone collagen. Do all food elements contribute equally to the synthesis of bone collagen or are the amino acids used in the synthesis process derived from a general storage pool in the body? How do fluctuations in the individual's protein intake affect the manufacture of bone collagen, i.e. in nutrient poor situations, do

carbohydrates and fats compensate for a decreased protein intake? In order to resolve such questions, studies may involve the radioactive labelling of amino acids with known quantities of ^{12}C and ^{13}C and following such components through the digestive process. Examining the bone collagen of laboratory animals raised on high carbohydrate and high fat diets versus those raised on high protein diets may provide additional information (see Kennedy, 1989).

A final example of potential future research involves the importance of noncollagenous proteins in stable isotope ratios. Masters (1987) has drawn attention to the possibility of utilizing these other bone proteins as a source for stable isotope analysis. Masters states that several noncollagenous proteins have higher affinities for bonding to bone mineral compared to collagen which suggests that such proteins may survive unaltered for longer periods of time relative to collagen. The main problems associated with these proteins are their rarity in bone when compared to collagen and the poor understanding of the meaning of their isotopic ratios. Proteins such as osteocalcin have recently been the focus of intensive study and are reasonably well understood (Hauschka, 1980; Gundberg et al., 1984). Their use in paleodietary reconstruction, however, has not been examined in any detail. Hauschka (1980) states that:

> Although osteocalcin has not yet been isolated from fossil bone, there is no question that fragments of the protein ... are preserved for at least 50,000 years. (p. 80)

Preservation of this protein is not as good as bone collagen, which has been reported to have survived for millions of years (Wyckoff, 1972), however, 50,000 years is still a considerable length of time with which to work. Modern isolation techniques alleviate the problem of their low concentrations and this will allow researchers to investigate the second problem.

Methods of Analysis

A characteristic of many scientific disciplines is the use of a standardized set of procedures. Anthropological stable isotope analysis has not, for the most part, followed this example but has instead relied on several different procedures for collagen extraction, each with its own advantages and disadvantages (e.g. Olsson et al., 1974; Chisholm et al., 1983a; Schoeninger and DeNiro, 1984; Sealy and van der Merwe, 1986). For example, the use of EDTA (ethylenediamine tetraacetate) produces a collagen sample with a relatively high purity but the technique is time consuming (Olsson et al., 1974:178). The method used in the present study is not as time-consuming but may instead release parts of the collagen molecule which are not subsequently recovered.

Taylor (1983) proposes a solution to the problem of collagen loss in the NaOH soak procedures. This researcher uses an extraction procedure similar to that used by Schoeninger and DeNiro (1984) with an important modification: the collection and analysis of the products ordinarily rejected in other methods (Taylor, 1983:649). This additional step will enable researchers to collect indigenous organic material extracted by the NaOH depending on whether the humates and collagen segments can be distinguished from one another, a task made more difficult by the fact that these collagen segments can vary in size and amino acid content. Thus, small collagen polypeptide segments may not be distinguishable from other amino acid sequences such as those originating from humates or noncollagenous proteins. Segments containing hydroxyproline, however, likely originate from collagen since this amino acid is so prevalent in this protein but rare in other organic compounds (see Chapter 4).

Besides extraction methods, the main area requiring standardization in anthropological stable isotope analyses involves the tests for diagenetic alteration of the 'collagen'. Michael DeNiro first developed a test expressly designed for the examination of diagenetic changes in a bone's collagen during the mid-1980s (DeNiro, 1985). The test has been widely accepted although problems still remain, for example, what mechanisms are responsible for

the change in the elemental contents of collagen (see Masters, 1987)? Three additional tests have recently been proposed by DeNiro and Weiner (1988) including the use of collagenases to measure 'collagen' concentrations, determinations of the amino acid composition (a technique which has been used in past studies - Wyckoff, 1972 and 1980; Ho, 1965 and 1967) as well as the infrared spectra of the 'HCl insoluble fractions'. If test extractions indicate that there is a sufficient amount of collagen in the sample to allow further analysis:

> Elemental and amino acid compositions and infrared spectra of "collagen" samples from bones not eliminated during such prescreening should be determined ...(ibid., p. 2205)

These procedures are advantageous in that they make use of extraction products which are easier to produce than the final 'collagen' component. The main disadvantages involve the ability and opportunity to use the necessary equipment required by the procedures and the difficulty of applying such tests to large sample sets. DeNiro and Weiner suggest that for large sample sets:

> ... it might be appropriate to analyze a few samples from each depositional environment. However, we urge caution in extrapolating results of analyses of the few to the many, since our observations indicate that different bones from the same site do not respond to diagenetic processes in the same fashion ... (ibid., p.2205)

These researchers have demonstrated that additional diagenetic tests are available, although they may not always be practical. Further tests may be developed which rely on a single amino acid, such as hydroxyproline or gamma-carboxyglutamate, on a specific protein such as osteocalcin, or on a more diagenetically stable element. Refining the tests currently available should be a major topic of investigation in stable isotope analysis of paleodiet reconstruction.

6.5 Conclusions and Summary

This thesis has demonstrated that past environmental conditions, such as an increase in rainfall, as well as human factors (e.g. disturbance of former occupational levels at site 523) can significantly affect the retention of carbon and nitrogen compounds as well as the stable isotope ratios of these two elements. With respect to the African material utilized in this study, bones from the deeper levels of site 523 appear to have been better preserved than samples from the upper levels. This depth effect in bone preservation relates primarily to paleoenvironmental and, to a lesser extent, human factors. The current analysis indicates that archaeological sites located in savanna zones can yield material which has been adequately preserved so as to allow chemical analysis of the bones. It must be remembered, however, that the definition of 'well preserved remains' utilized in this

thesis is based on a single diagenetic test (the atomic C/N ratio).

Two methods for the extraction of 'collagen' from the skeletal remains were examined, the major difference between the two being the addition to one method of a sodium hydroxide soak for the purpose of base-soluble humic contaminant removal. In relation to the present study material, the method lacking the NaOH soak was found to produce higher yields and C/N ratios but lower quantities of both carbon and nitrogen relative to the second method. It was suggested that, except for the yield (which is likely due to the retention of humate contaminates), these differences relate more to preservational than methodological factors (bones of the upper two meters of the mound 1 excavation were found to differ significantly in several variables when compared to the lower levels). Human interference in the form of periods of increased building activities was postulated as one possible cause for this 'depth' effect.

The use of stable isotopes in the investigation of West-Central African prehistory was briefly explored and it was concluded that such analyses can yield important results. Examination of the spread of crops and the reliance on certain types of food sources are two areas which can more fully explored using the stable isotopes of carbon and nitrogen. The main obstacle to be overcome is

the lack of skeletal populations large enough and in an adequate state of preservation so as to allow investigations to be undertaken.

Stable isotope analysis is a valuable tool in the reconstruction of the diets of prehistoric humans and animals. If it is to be used on a continuing basis in anthropological studies, however, several problems must be overcome and an equal number of basic concepts must be more fully explored. Scientific method and theory have become a vital part of anthropological research and while some may debate whether or not archaeology is a "science", the fact that the use of scientific methods does not equate to a scientific field of investigation cannot be ignored. Borrowing methods from other areas of study necessitates a familiarity with the method, the confirmation that the application of the method to the problem is appropriate, and the identification and correction of the problems associated with the method. The blind use of a single method can lead to incorrect interpretations of the archaeological record and thus, results produced by stable isotopic analysis must be used in concert with more traditional methods of paleodietary reconstruction. Within these boundaries, interpretations of the lifeways of our ancestors can be elevated from fiction to historical fact.

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