UNIVERSITY OF ALBERTA

ELEMENTAL VARIATION IN MODERN HUMAN BONE: CALCIUM, MAGNESIUM AND ZINC CONCENTRATIONS IN NORMAL AND PATHOLOGICAL FIBULAE

BY

PING LAI

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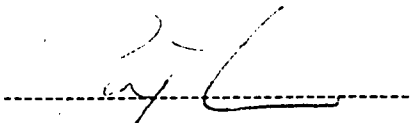

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The undersigned certify that they have read, and recommend to the Faculty of Graduate Studies and Research for acceptance, a thesis entitled Elemental Variation in Modern Human Bone: Calcium, Magnesium and Zinc Concentrations in Normal and Pathological Fibulae submitted by PING LAI in partial fulfillment of the requirements for the degree of MASTER OF ARTS.

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ABSTRACT

Samples of modern human fibulae, representative of normal and pathological bone, were examined for concentrations of calcium, magnesium and zinc using the analytical method of instrumental neutron activation analysis. Analyses and interpretation of the results are presented in two parts relating to the problem of elemental distribution at three samplings sites within a single normal and pathological bone. Results of the normal fibuale analysis reveal that the distribution of calcium, magnesium and zinc at the proximal, midshaft and distal sampling sites is variable although magnesium shows the most consistency in concentration. Moreover, the analysis provided evidence to support the midshaft as a representative sampling site. Results from the analysis of the pathological fibulae show that elemental concentrations at the pathological sample are not abnormal regarding normal physiological levels. Also, calcium and magnesium have been shown in a synergistic relationship. Finally, the distribution of zinc was extremely variable for both normal and pathological fibulae in this study. The results demonstrate that further research is needed in this area as elemental distribution in both normal and pathological bone was generally variable between sampling sites.

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CHAPTER ONE
INTRODUCTION

Trace element analysis of ancient human tissue has been applied in anthropology to investigate three central issues: the reconstruction and interpretation of paleodiet; the determination of the relationship between the geochemical and biochemical environment for an understanding of the impact of diagenesis; and the establishment of the relationship between trace elements and human health. Although trace element analysis has been widely used in anthropology, it has not been applied to the problem of trace element distribution. This thesis is a contribution to trace element research in that it provides data concerning the patterns of elemental distribution within modern fibulae, both in normal and pathological specimens.

This thesis project was approached with two goals in mind. The first objective was to examine trace element distribution in modern human cortical bone to test whether distribution is variable or consistent throughout a single bone tissue. Elemental variability has been reported previously in archaeological bone with the conclusion that trace element concentration varies between different bones and different bone tissues (Brätter *et al.* 1977; Grupe, 1988a; Klepinger *et al.* 1986; Lambert *et al.*, 1979). In comparison to archaeological bone, studies of modern bone have been limited, however. Modern bone is herein defined as skeletal material that has not been incorporated into any archaeological context. This may include clinical autopsy material or biological prepared specimens, bone that has not been buried. Inclusion of modern bone in anthropological studies has consisted of comparisons between modern and archaeological for dietary reconstruction and as a method of detecting diagenetic changes (Edward and Benfer, 1993; Hancock *et al.* 1989).

The second objective involved the analysis of modern pathological bone to see whether elemental distribution is affected by disease or trauma. A comparison of the elemental concentrations was made between pathological tissue and "normal" tissue taken from a single bone specimen to test the hypothesis of elemental variation. Previous analyses of pathological bone from archaeological contexts have concentrated on the assessment of nutritional status and the determination of etiology in light of trace element data (Fornaciari *et al.* 1983; Grupe, 1988b).

The samples used in this study were obtained from the osteology collection in the department of Anthropology, University of Alberta. A total of seven fibulae were selected for analysis, three normal and four pathological specimens. The samples were analyzed at the University of Alberta's S'owpoke nuclear reactor. The results of the analyses are presented in Chapters three and four.

This thesis consists of five chapters in all including this brief introductory one. Chapter two provides a historical review of the applications of trace element research in anthropology including a description of trace elements and bone structure and function. In addition, Chapter two provides a summary of the analytical method, neutron activation. Chapter three presents the results of an analysis of the distribution of calcium, magnesium and zinc at three sampling sites in three different fibulae. In Chapter four the relationship of trace elements and human health was investigated in that four pathological fibulae were tested for elemental variability with samples taken from two "normal" and one pathological site. Chapter five is a general discussion and conclusion of the thesis with some suggestions for future research.

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CHAPTER TWO
APPLICATIONS OF ELEMENTAL ANALYSIS IN ANTHROPOLOGY: A REVIEW

INTRODUCTION

Trace element analysis of bone has increased since its initial application over twenty years ago (Brown, 1973). The seminal work by Brown (1973) provided the impetus for others to use trace element analysis as a tool for paleodietary reconstruction (Gilbert, 1975; Lambert *et al.* 1979 and Schoeninger, 1979). Through the 1980's, paleodietary reconstruction continued as the focus of many bone chemistry studies (Edward *et al.*, 1984; Katzenberg, 1984; Runia, 1987; Schoeninger, 1981), but a new direction had emerged for trace element analysis. It was an interest in the relationship of bone to its post-deposition environment that fostered studies on diagenesis (Klepinger *et al.*, 1986; Kyle, 1986; Lambert *et al.* 1982, 1983, 1984; Pate and Hutton, 1988). Anthropologists define diagenesis as "postmortem alterations in the chemical constituents of bone following deposition in soil" (Sandford, 1992:86). Investigation into the impact of diagenetic processes on elemental concentrations is, at present, the most frequent application of trace element analysis (e.g. Edward and Benfer, 1993; Link and Lovell, n.d.). However, a third application emerged that involved the effects of trace elements on human health and disease (see Armelagos *et al.*, 1989). Although studies of paleodietary reconstruction and diagenetic effects have provided a large body of knowledge of the behavior of trace elements, the potential of the last application has yet to be fully recognized (Aufderheide, 1989; Sandford 1992, 1993).

The present chapter is a historical review of trace element research in anthropology, with special emphasis placed on previous bone analyses. In attempting to provide the proper background for subsequent chapters the ensuing review will include a discussion of both trace elements and the chemistry and structure of bone because "one cannot hope to interpret elemental concentrations without first achieving basic understanding of the chemical elements themselves and the skeleton as a dynamic system" (Sandford, 1993:20). Particular attention will be given to three elements; calcium, magnesium and zinc, since they are the elements of interest in the succeeding chapters.

DESCRIPTION OF TRACE ELEMENTS

All biological organisms contain major and trace elements, a classification based upon their concentration. Major elements include calcium, carbon, chlorine, hydrogen, magnesium, nitrogen, oxygen, phosphorous, sodium, and sulfur (Schrauzer, 1984); and are usually expressed in grams per kilogram. While major elements are the most abundant, trace elements are present in smaller amounts, usually making up less than 0.1% of the body's mass (Schroeder, 1973). Trace elements are distinguished as either essential, elements which are necessary for the maintenance of life (growth, health and reproduction) or nonessential, elements which lack demonstrable functions (Mertz, 1981).

An element is considered essential if it meets the following biochemical criteria set by Cotzias (1967:7): a) it is present in all healthy tissues of all living things; (b) its concentration in different species is fairly constant; (c) its absence produces the same physiological or structural anomalies regardless of species; (d) its addition prevents or reverses the abnormalities; (e) the abnormalities brought on by its deficiency are accompanied by biochemical changes and (f) the biochemical changes are prevented or cured as the deficiency is prevented or cured.

Fifteen trace elements have been established as essential and they are arsenic, chromium, cobalt, copper, fluorine, iodine, iron, manganese, molybdenum, nickel, selenium, silicon, tin, vanadium and zinc (Mertz, 1981; Underwood, 1971). These essential elements are functionally important to biological systems as outlined by Neilsen (1991:20) because they:

(1) serve as a catalyst in enzyme reactions by attracting substrate molecules,
(2) participate in reduction and oxidation reactions by accepting or donating electrons,
(3) bind, transport and release oxygen through the body, and
(4) maintain the structural integrity of molecules.

The principle functions of zinc, for example, include the synthesis of ribonucleic acid and protein, involvement in bone growth and mineralization and the development of reproductive organs (Cunnane, 1988; Leichtmann and Sitrin, 1991; Prasad, 1978; Underwood, 1971). Similarly, magnesium and calcium are also essential to normal bodily functions but can not be classified as trace elements since they occur in high concentrations in the body. Magnesium is essential for proper growth and development

as it is also responsible for RNA and DNA synthesis (Prasad, 1978). Furthermore, magnesium is fundamental as a catalyst for many enzymes (Aikawa, 1981). Calcium is particularly important in that it functions in bone metabolism, blood clotting, cell proliferation, enzyme activity and muscle contraction (Miller, 1983; Mundy, 1989).

Trace Element Metabolism

The essential trace elements are under homeostatic control, through a regulatory system that involves absorption, excretion and storage. Trace elements may be incorporated into the body through dietary and environmental exposure, with dietary intake via numerous trophic pathways involving the geochemical environment (Underwood, 1971). Because trace elements are nutritionally important, inadequate intake could lead to deficiency which would eventually result in dysfunction if the condition is chronic. Although homeostatic mechanisms in the human body should make deficiencies uncommon, they do occur because of reactions to disease, malnutrition, and stress. Changes in hormonal, physiological, metabolic and nutritional systems are contributory factors to deficiency or toxicity of trace elements (Neilsen, 1990).

Absorption and Excretion of Trace Elements

The maintenance of mineral homeostasis is dependent upon two mechanisms, absorption and excretion, which directly influence elemental concentrations. "True" absorption is distinguished from "apparent" absorption on the basis that a proportion of an element is taken up by the gastrointestinal tract (O'Dell, 1985). True absorption is measured through tracer studies in which the pattern and pathway of radioisotope and stable isotope labels are discerned *in vivo* . Absorption is often confused with bioavailability, which refers to nutrients that are absorbed and metabolically used by the body (O'Dell, 1985; Valkovic, 1980; Zumkley and Spieker, 1988). Many factors influence bioavailability of trace elements and they include: (1) dietary intake and absorption; (2) excretion; (3) carrier mechanisms and (4) physiological state of the individual (Zumkley and Spieker, 1988:147).

While absorption can affect trace element bioavailability, it is the diversity in the mechanisms, sites and efficiency of absorption for each element that ultimately results in variability in elemental concentration (Sandford, 1993). Carrier mechanisms include plasma protein molecules such as transferrin and albumin which recognize a specific

element upon entry into the body (Mertz, 1981). The function of carriers is to transport the selected elements to sites of biological action; the action of each trace element is specific at these sites. The manner in which carriers associate with specific elements is unknown, however (Mertz, 1981). The sites of biological activity for absorbed elements include hepatic (liver) cells, the primary site of chemical food processing, and renal (kidney) cells.

Another factor that can influence absorption, and hence bioavailability, is the interaction between the elements themselves. Elemental interactions are characterized as either synergistic or antagonistic. The presence of one or more elements or chemical substances which enhance absorption of a element are often recognized as synergists (Kirchgessner *et al.*, 1982; Sandford, 1992). The number of elements identified as synergists is few and many of these interactions have not been shown in human populations (Underwood, 1971). For example, zinc has synergistic relationships with magnesium and vitamin A (Cunnane, 1988) while zinc's interaction with vitamin D, a possible synergist, has only been reported in rats (Underwood, 1971).

In contrast, chemical substances or elements which inhibit elemental uptake of a element are defined as antagonists (Kirchgessner *et al.*, 1982; Sandford, 1993; Underwood, 1971). Antagonism often implies competition for protein binding sites such that elemental deficiency may occur (Prasad, 1978). If elemental deficiency is already occurring, then the presence of antagonists can further aggravate the degree of deficiency. Zinc antagonists include calcium, cadmium, copper and iron (Cunnane, 1988; O'Dell, 1985; Prasad, 1978; Underwood, 1971). Another zinc antagonist of particular importance to human populations is phytate because of its consumption in the form of unleavened bread (Klepinger, 1993; O'Dell, 1985; Sandford 1992, 1993). Clinical cases of severe zinc deficiency have been documented in several Middle Eastern populations where phytate intake is high (Prasad, 1978). Phytate binds to zinc in a form which is not readily released or absorbed, and the presence of excessive calcium and fiber can exacerbate the effects on zinc (Klepinger, 1993; O'Dell, 1985; Sandford, 1992; Underwood, 1971).

Elemental excretion for a majority of the elements is mainly through feces elimination followed by urine and sweat (Leichtmann and Sitrin, 1991). Excretion functions in maintaining mineral homeostasis; and for some elements, it provides a way to maintain their normal concentration in plasma, soft tissue or bone This is especially important to major elements such as magnesium and calcium because elemental

imbalance may lead to detrimental health problems (Aikawa, 1981). Apparent excretion involves elimination of unabsorbed nutrients. By contrast, however, true or endogenous excretion refers to absorbed quantities of an element that are lost through bile, urine or sweat.

Elemental homeostasis is often regulated by hormones and vitamins. Specifically, the concentration of calcium is regulated by parathyroid hormone, thyrocalcitonin and vitamin D (Lane and Werntz, 1984; Vaughan, 1981). Parathyroid hormone or PTH promotes calcium and phosphate reabsorption in bone in order to have these elements in plasma. The plasma calcium concentration is maintained at the expense of bone mineral (Buchinsky and Krieger, 1992). In contrast, calcitonin decreases both calcium reabsorption and calcium absorption across the gut by inhibiting the production of the vitamin D metabolite, (1,25 $(OH)_2$) which is responsible for stimulation of calcium absorption (Favus, 1992; Glimcher, 1992; Lane and Werntz, 1984).

Structure and Function of Bone

The structure of bone on a macroscopic level has been identified as either cortical (compact) or trabecular (cancellous) in the mature skeleton. Cortical bone is typical of long bone shafts; it is dense with few visible spaces unlike trabecular bone which is characterized by cavities within an intertwined network of irregular thin rods of bone called trabeculae (Shipman *et al.* 1985; Vaughan, 1981). It is in these cavities that red or fatty marrow is contained, providing trabecular bone with hematopoietic responsibility. Trabecular bone is predominantly found at the end of long bones and, due to its vascularity, this bone type functions as the metabolic center for bone (Shipman *et al*, 1985). Trabecular tissue has higher rates of bone turnover than does cortical bone, although the rates vary between different bones (Curzon and Cutress, 1983; Vaughan, 1981). Because of increased metabolic activity and greater surface area, the number and concentration of trace elements are higher in trabecular than cortical bone (Behne, 1976; Gawlik *et al.*, 1982). According to Brätter *et al.* (1977), this disparity between trabecular and cortical bone is related to differences in their structure and function.

Bone has several functions but the most important is that "it acts as an ion reservoir and, as such, is the body's major storage site for calcium, magnesium, sodium and other ions" (Glimcher, 1984:39). Approximately 62% of the body's zinc is found in bones and muscles (Cunnane, 1988), while 99% of total body calcium and 89% of

magnesium are contained in the skeleton (Aikawa, 1981; Martin and Burr, 1989). As a result of this ion reservoir storage property, "[the inorganic, solid mineral phase] plays a critical role in maintaining the concentration of these ions in the extracellular fluid that in turn is vital for a number of important biochemical reactions and physiological activities" (Glimcher, 1984:39). Almost 70% of bone is comprised of minerals. Although some elements such as copper and iron do bind to the organic matrix, the majority of elements are found in the mineral fraction (Becker *et al.*, 1968; Spadaro *et al.*, 1970). Bone mineral gives the skeleton its hardness and rigidity, which permit it to withstand mechanical forces, strains and stresses (Glimcher, 1992; Martin and Burr, 1989). Furthermore, the skeleton protects the vital organs and plays a role in hematopoiesis.

Bone is a very specialized complex tissue comprised of both organic and inorganic constituents. The organic component consists chiefly of Type I collagen, the major protein of bone (Triffitt, 1980). Collagen is a protein that provides tensile strength to bone because of its arrangement in parallel fibril bundles (Triffitt, 1980). Collagen makes up approximately 90% of the weight of the organic component while the remaining consists of a "ground substance" of mucopolysaccharides and noncollagenous proteins (Neuman, 1980; Pritchard, 1972; Triffitt, 1980).

While the organic component provides tensile strength, it is the inorganic portion that gives bone its compressional strength. The mineral phase consists of two calcium phosphate pools; a crystalline and an amorphous noncrystalline phase (Neuman and Neuman, 1958; Neuman, 1980; Sillen, 1989). Calcium phosphate in its crystalline form is commonly known as hydroxyapatite with the following chemical formula $[Ca_{10}(PO_4)_6(OH)_2]$. These crystals precipitate into the organic matrix along, and perhaps within, the collagen fibrils (Neuman, 1980; Sandford, 1993). In addition to the principal crystalline structure of hydroxyapatite, other forms of calcium phosphate compounds exist and they vary according to acidity, solubility and thermodynamic stability (Neuman, 1980). It has also been shown that the constituents of bone mineral vary with age and intrabone location (Neuman, 1980; Posner, 1969).

A major reason for compositional differences in hydroxyapatite crystals is the lack of stoichiometry in the calcium and phosphate ratios (Neuman and Neuman, 1958; Sandford, 1993; Sillen, 1989). Deviations from the expected stoichiometric formula is due to tonic exchange, ionic substitutions and recrystallization (Neuman and Neuman, 1958). Ionic exchange involves three different crystal zones: the crystal interior, crystal

surface and the hydration shell (Vaughan, 1981). The hydration shell is a layer of water that is bound to the surface of the crystals, and its function is to maintain ionic equilibrium between crystals and surrounding aqueous environment. Ions that are surface bound are more readily exchanged than ions which have penetrated the crystal interior. For example, magnesium is a surface-limited ion, and under normal circumstances, one-third is absorbed on the surface of the apatite crystal while the rest of the bone magnesium replaces calcium in the crystal (Vaughan, 1981).

Ionic substitutions involve the displacement of lattice ions on the surface of the crystal and within the crystal. For example, strontium, lead, magnesium and sodium ions substitute for calcium (Sandford, 1993:29). Substitution is one of four types of ionic exchange reported by Neuman and Neuman (1958:82-83), based on research that was carried out to document how ions interact with the hydroxyapatite crystal. The other three forms of exchange include:

1. Diffusion of ions into the hydration shell with ion concentration proportional to the surrounding solution.

2. Ions that enter the hydration shell and replace ions in the crystal surfaces.

3. Ions that penetrate the hydration shell to neutralize surface change asymmetry.

A discussion of bone's structure and function and the behavior of trace elements would not be complete without mention of the bone cells. Osteoblasts and osteoclasts are integral to the chemical structure and function of the skeleton. Osteoblasts are responsible for the formation of the organic matrix (Type I collagen and bone proteins) and its mineralization (Dempster, 1992; Matthews, 1980). Osteoblasts are regulated by calcitonin which functions to decrease their activity (Vaughan, 1981). Osteoclasts, on the other hand, are specialized for bone resorption (Mundy, 1989). As osteoclasts resorb bone, minerals are released and the bone matrix becomes demineralized. Parathyroid hormone regulates osteoclasts by increasing their activities (Lane and Werntz, 1984). Together, osteoblast and osteoclast activity is responsible for growth, remodeling and repair within the skeleton.

PALEODIETARY RECONSTRUCTION

Dietary reconstruction of ancient populations through skeletal remains was made possible because of the field of archaeometry, an area that involved application of scientific methods to archaeological problems. In this area, trace element analysis of organic matter such as bone and hair logically developed from the analysis of inorganic materials. In attempts to formulate past behaviors and due to the wealth of information available from skeletal remains, discerning diet and subsistence became a major concern of anthropologists and archaeologists alike. Trace element data from human skeletons was considered a direct means of reconstructing diet as an alternative to the analysis of flora and fauna remains. This is based on the premise that elemental concentrations accurately reflect dietary consumption (Sandford, 1993:3). However, it has been subsequently discovered that individual variation, health status and physiology can affect trace element concentrations, and that elemental data can not be interpreted in a straightforward manner (Radosevich, 1993). Although many variables are now known or suspected to contribute to elemental variability, trace element analysis continues to be used for paleodietary reconstruction.

Strontium and the Beginnings of Paleodietary Reconstruction

Strontium was the first element analyzed for dietary reconstruction in anthropology (Brown, 1973). The pioneering work of Brown involved the examination of strontium in four skeletal populations each from a geographically distinct region. Brown concluded, through analysis of trophic levels in the food chain, that strontium's concentration is higher in skeletons of individuals whose diets consist primarily of plants as compared to a diet composed mostly of meat. This conclusion is based on the premise that strontium is discriminated against in the food chain (Sandford, 1992). The dietary discrimination involves a decline in the amount of strontium that can be absorbed at each trophic level in an ascending food chain, so that the highest strontium can be found in plants which are at the bottom and the lowest strontium in carnivores which are at the top of the food chain.

As a result of Brown's work, subsequent strontium studies have attempted to assess the dietary importance of animal protein and plants (Katzenberg, 1984; Runia, 1987; Schoeninger, 1979, 1981, 1982; Sillen, 1981) and to differentiate between consumption patterns between coastal and inland sites (Kyle, 1986). Although animal protein can be distinguished from plant resources on the basis of strontium, dietary

interpretations are confounded by the presence of strontium in shellfish (Schoeninger and Peebles, 1981), nuts (Katzenberg, 1984) and dairy foods (Sillen and Kavanagh, 1982). Furthermore, the quantity of strontium in plant resources is not uniform. Grasses such as maize, for example, are lower in strontium than are leafy plants (Katzenberg, 1984; Sandford, 1993).

Paleodietary reconstruction based on the specific presence and concentration of a single element is, at best, speculative because of the effects of diagenesis (to be discussed later in this chapter) and individual variation (see Radosevich, 1993; Sillen and Kavanagh, 1982). Strontium was once thought to be impervious (Parker and Toots, 1970, 1980) and less resistant to diagenesis (Lambert *et al.*, 1979, 1984), but these concepts were soon abandoned as diagenetic changes to skeletal strontium have been reported for various sites and geochemical environments (Klepinger *et al.*, 1986; Price, 1989; Radosevich, 1993).

Multielement Analysis: Diet Assessment and More

While strontium as a dietary indicator has been proven to be less reliable than other elements because of postmortem alterations, it is still the most important trace element in paleodietary research (Runia, 1987). However, the incorporation of multielement analysis in anthropology has provided more complete assessments of ancient diets (Buikstra *et al.*, 1989; Lambert *et al.*, 1979; Price, 1989). Dietary discrimination of meat versus plant consumption has been improved by the analysis of elements that are diagnostic of specific foods (see Edward *et al.*, 1984; Geidel, 1982; Gilbert, 1975; Hatch and Geidel, 1983). For example, zinc is higher in concentration in animal protein than in vegetables (Table 2.1). Based on the elemental concentrations in Table 2.1, discrimination between meat and plant resources should be relatively straightforward. In contrast, dietary interpretations that focus on single element analysis may be unreliable because several food sources can contribute to the concentration of the element. For instance, it would be difficult to identify the specific food source to explain a high zinc concentration since both nuts and animal protein are high in zinc (Table 2.1). The preservation of archaeological evidence in the form of flora, fauna and artifacts might elucidate the proper reconstruction.

The use of trace elements is not limited simply to reconstruction of diet and dietary proportions but is also used to examine social dynamics. Several studies have

investigated the relationship between skeletal concentrations of trace elements and society status differences with specific food access (Blakely and Beck, 1981; Fornaciari *et al.*, 1984; Geidel, 1982; Hatch and Geidel, 1985; Lambert *et al.*, 1979; Schoeninger, 1979). These studies have assumed that access to meat over plants in stratified societies was limited to the elite, and that higher concentrations of skeletal zinc and lower skeletal strontium was indicative of status. However it is important that the archaeological record, based on burial context, provide evidence for the association of diet and status. For instance, Fornaciari *et al.* (1984) found zinc levels to be higher (221 ppm) in the skeletal remains of individuals buried in a Roman mausoleum as compared to skeletons buried in a paleo-Christian basilica (160 ppm). An inference of high status was attributed to the mausoleum skeletons based on the quality of the associated grave goods.

While archaeological evidence can strengthen interpretations of status, as was the case in the work of Fornaciari *et al* (1984), it is important that the elemental data support dietary differences based on status. Blakely and Beck (1981) found no differences in the concentrations of strontium, zinc, copper or magnesium between two burial situations at Etowah, a Mississippian site in Georgia, that showed definite status differentiation. They concluded that status was earned and not ascribed because status differences could not be detected in the elemental profiles of the Mississippian individuals. According to Blakely and Beck, if status was ascribed then the skeletal strontium concentration would be lower in the mound burials than the village burials since these individuals would have had access to meat since childhood. The conclusion drawn by Blakely and Beck is reasonable but they assumed that diagenesis was minimal and that these two burial settings were contemporary (Buikstra *et al.*, 1989). Further analysis of the Etowah skeletal series by Beck (1985) revealed that the village burials were later than the mound burials and that there was greater reliance on maize consumption.

The application of trace element data to document the dietary transition from hunting and gathering to maize agriculture has been the focus of other studies as well (Buikstra *et al.*, 1989; Gilbert, 1977; Katzenberg, 1984; Lambert *et al.*, 1979; Price and Kavanagh, 1982). Gilbert (1977) found that the concentration of strontium had increased during the transition from a hunter-gatherer diet to maize cultivation. Similar changes in strontium were observed in Katzenberg's (1984) study of Late Woodland times in southern Ontario.

Although elemental variation may be attributed solely to status-based differences within a population, it is wise to consider also the effects of age, sex, health and cultural practices (Klepinger, 1993; Radosevich, 1993). Gender-based differences in diet have been reported in several archaeological populations (Gilbert, 1975; Lambert *et al.* 1979, 1982). Lambert *et al.* (1979) found that females at the Late Woodlands Ledders site (A.D. 1000-1200) in Illinois had higher strontium and lower zinc concentrations, suggesting that males had differential access to specific zinc-rich foods. In contrast, however, the same researchers did not find any significant sex differences in elemental concentrations for the Middle Woodland Gibson site (150 B.C.-A.D. 400). They concluded that either the diets were similar between the sexes or elemental contamination had occurred. Subsequent research revealed, through soil analysis, that strontium, zinc, calcium and sodium were not affected by diagenesis at the Middle Woodland Gibson site (Lambert *et al.*; 1984). Therefore, gender specific access to food was not occurring at this site.

While dietary differences may contribute to elemental variation between the sexes, this explanation is not completely satisfactory because of the reported effects of pregnancy and lactation on elemental concentration (Brätter *et al.* 1980; Klepinger 1984, 1993; Sillen and Kavanagh, 1982). During these periods, the concentration of both calcium and zinc decrease in bone to compensate for loss of these elements into plasma (Cunnane, 1988; Irving, 1973). Because of the increased demands from the fetus and placenta, bone minerals are easily mobilized to restore homeostasis. In particular, the needs of plasma calcium take precedence over the skeletal need (Recker, 1992). In addition, higher turnover rates have been recognized in pregnant and lactating women (Kent *et al.*, 1990). Although it may be difficult to provide evidence of pregnancy or lactation in archaeological skeletons, it is necessary that physiological factors be included in any interpretation of elemental differences.

In addition to pregnancy and lactation, age contributes to the variability in elemental concentration. Lambert *et al.* (1979) found that strontium and zinc decreased in childhood, increased in adolescence, stabilized at age 20 to 50 years and then increased slightly past 50 years. In contrast, the absolute concentration of strontium was reported to increase gradually with age (Tanaka *et al.*, 1981). Because elemental concentration in infants and children are derived originally from maternal stores, concentration levels drop if these elements are not replenished in the diet. Therefore, elemental concentration is a

reflection of both diet and age although other variables such as health and sex also affect elemental variability.

An alternative explanation is that the low strontium level observed in the juvenile skeletons by Lambert and associates is related to poor crystallinity as the amorphous phase is more soluble than apatite (Buikstra *et al.*, 1989). The crystallinity of bone apatite varies with age since bone progresses from a poorly crystalline amorphous state which is indicative of immature bones to a well crystalline apatite seen in adult skeletons (Katzenberg, 1984; McLean and Urist, 1961). The size of the crystals also increases with age (Budy, 1967; Lambert *et al.*, 1979). Furthermore, strontium substitution for calcium explains the increasing concentration of strontium as calcium decreases with age (Aitken, 1976). Another calcium substitute is magnesium and it has also been found to increase with age (Aikawa, 1981).

It has been well established that the prevalence of postmenopausal women who suffer from osteoporosis, a condition characterized by decreasing bone mass and increased porosity, is higher than in males of the same age (Lindsay and Cosman, 1992). The loss of calcium occurs in both sexes but is more rapid in females due to the decrease in the level of hormones that accompany age. As discussed previously, several hormones regulate calcium's concentration and any changes in hormone levels will contribute to elemental differences.

TRACE ELEMENTS IN HEALTH AND DISEASE

Although the link between trace elements and disease has only been recently established, the potential of this relationship is nonetheless promising because elemental data provide a useful tool to investigate and interpret the etiology of pathological conditions which afflict the skeleton (Armelagos *et al.*, 1989; Grupe, 1988b; Oster, 1987). In addition, elemental data can be used to assess nutritional status to determine subsequent effects on health (Klepinger, 1993), and it can be used to investigate health and cultural practices of past populations that were exposed to toxic elements (Aufderheide, 1989).

Iron

Studies which have used trace element analysis to investigate skeletal iron status of past populations include Edward and Benfer (1993), Fornaciari *et al.* (1983), Sandford *et al.* (1988) and Zaino (1968). Zaino was the first to employ elemental data in assessing nutritional status among the Anasazi. He concluded that the Anasazi diet was not deficient in iron since the skeletal iron concentration was similar to that observed in modern populations. This work was followed by the application of trace element analysis to the determination of the cause of gross morphological lesions such as porotic hyperostosis.

Iron deficiency anemia has often been considered responsible for the manisfestation of cribra orbitalia and porotic hyperostosis, nutritional "stress indicators" (Carlson *et al.*, 1974; Cybulski, 1977; Lallo *et al.*, 1977). The skeletal changes diagnostic of cribra orbitalia are porous lesions or pitting of the orbital roofs while the same lesion located in the parietals is termed porotic hyperostosis (Angel, 1966). Both cribra orbitalia and porotic hyperostosis refer to the same condition and generally the terms are interchangeable although they are used in reference to specific sites in bone (Ortner and Putschar, 1985; Steinbock, 1977). The prevalence of these skeletal lesions in the archaeological record have prompted researchers to investigate nutritional status in attempts to identify the etiology of these "stress indicators".

For example, Fornaciari *et al.* (1983) in their analysis of Punic skeletons from Carthage in Tunisia found lower iron concentrations in skeletons afflicted with cribra orbitalia. Similar results have been found by Sandford *et al.* (1983) in their study of Kulubnarti subadults. Although they analyzed hair rather than bone, the iron concentration was definitely lower in the subadults with cribra orbitalia. However, they also found the absolute iron levels to be high for archaeological remains in comparison to contemporary iron levels among the !Kung. The higher iron concentration seen in the archaeological remains may be indicative of diagenetic contamination.

While elemental analyses with respect to iron status has been concerned mainly with bone, chemical analysis of hair has received some attention (reviewed by Sandford and Kissling, 1993). Elemental analysis of hair has been restricted because of poor preservation in the archaeological record. In addition, hair analysis is relatively new and controversial since it is unclear the degree to which elemental concentration reflects endogenous or exogenous factors (Sandford and Kissling, 1993). Similar problems exist for bone as well, since both intrinsic and extrinsic factors (discussed below) affect

element concentration. It may be for this reason that few studies have taken advantage of the potential offered from the analysis of trace elements to predict or identify the etiology of nutritional "stress indicators" (Aufderheide, 1989).

Lead

Despite the number of cases of cribra orbitalia and porotic hyperostosis available to investigate iron deficiency, elemental analyses with respect to health issues have emphasized lead and its exposure in past populations (Aufderheide *et al.*, 1981; Corruccini *et al.*, 1987; Reinhard and Ghazi, 1992). Lead is an "ultratrace" element which can accumulate in osseous tissue, acting as a substitute for calcium. Lead concentration in the skeleton is dependent upon the length of exposure as well as bone chemistry. The residence time of lead is extremely long although bone remodeling does result in some lead loss (Aufderheide, 1989). It is assumed that skeletal lead content reflects lifetime exposure.

Lifetime exposure to lead was higher in the past than it is today because of its extensive and habitual use in ancient and historic populations (Nriagu, 1983). The Romans, for example, used lead in the manufacture of cosmetics, contraceptives, cooking vessels, aqueducts and even in the production of wine (Gillfillan, 1965; Nriagu, 1983; Waldron, 1973). It has been suggested that lead contributed to the decline and fall of the Roman Empire because the aristocracy, who supposedly ruled the Empire and had more exposure to lead, became infertile thus leading to a general decline in the ruling class (Gillfillan, 1965). Similar findings of status differences in lead exposure have been reported by Aufderheide and associates (1981). In their analysis, Colonial American plantation owners had elevated lead levels in comparison to their slaves due to the former's use of pewter tableware and food containers.

The inadvertent consumption of lead and the subsequent toxicity has also been documented by Corruccini *et al.* (1987) and Kowal *et al.* (1991). Corruccini and colleagues reported high skeletal lead levels in Barbadian slaves from the 17th and 18th centuries. They concluded that the high lead content was due to heavy rum consumption because the rum had been distilled through lead-containing devices. Furthermore, they suggested that the sugar manufacturing process also contributed to lead exposure. Similar lead toxicity has been described in the sailors from the Franklin Arctic Expedition, although the source of lead came from tinned foods (Kowal *et al.*, 1991). It

was evident that lead contamination was introduced into the food from the lead solder on the inside seam of the tin can.

Further Investigations of Human Health and Disease

Trace element analysis has also been applied to the investigation of the etiology of specific pathological conditions. Grupe (1988b) analyzed a case of metastasizing carcinoma in a medieval skeleton. She diagnosed the condition as malignant bronchogenetic carcinoma. Elemental analysis revealed elevated levels of caesium and antimony and she suggested that this individual was exposed to carcinogenetic elements, perhaps in a metal smelting environment. Although caesium and antimony are not known to be carcinogenetic, their presence may be indicative of cultural activity such as lead and copper smelting. However, there is no direct evidence from skeletal material belonging to individuals associated with metal smelting in the vicinity of this skeleton to confirm environmental pollutants. While Grupe did not positively identify the etiology of the carcinoma, she nevertheless provided evidence that elemental data could used for purposes other than dietary reconstruction.

In addition, Blondiaux *et al.* (1992) examined elemental concentrations in bone tissue affected with hypertrophic osteoarthropathy. They analyzed eleven elements to test for differences in elemental concentration between compact, cancellous and periosteal new bone. They found that zinc concentration was higher in new bone formation due to the pathological condition. This result agrees with documented evidence that zinc functions in bone mineralization and bone repair (Okada *et al.*, 1990). The work of Blondiaux and associates is important because it illustrates the potential of examining pathological material to identify sources of elemental variability.

DIAGENESIS: CONSIDERATIONS AND PROBLEMS

Diagenesis as defined by anthropologists refers to the postmortem changes to bone chemistry following deposition. The emphasis on investigating the impact of diagenesis is due to the recognition that diagenetic processes have an effect on elemental variation, and as such it could result in misinterpretation of elemental data (Buikstra *et al.*, 1989; El-Kammar *et al.*, 1989; Katzenberg, 1984; Keeley *et al.*, 1977; Klepinger *et al.*, 1986; Kyle, 1986; Lambert *et al.* 1982, 1983, 1984, 1985a, b; Link, 1991; Nelson and

Sauer, 1984; Parker and Toots, 1980; Pate and Hutton, 1988; Price *et al.*, 1989; Sillen, 1989; Vlasak, 1983; Von Endt and Ortner, 1984).

Mechanisms of Postmortem Change to Bone

Three mechanisms of diagenetic change in bone mineral have been identified (Pate and Hutton, 1988; Pate *et al.*, 1989; Sillen, 1989; Sandford, 1993):

1. Precipitation of elements as separate void-filling mineral phases in the small fractures and pores of bone. Calcite is an example of such a void-filling mineral;

2. Ionic exchange between soil solution and calcium phosphate lattice positions; various cations and anions are substituted for those normally present in hydroxyapatite;

3. Recrystallization and growth of apatite crystals; apatite can behave as a template for new crystals of diagenetic origin.

Other contributing factors to diagenetic change involve the geochemical and biochemical environments; also referred to as intrinsic and extrinsic variables (Von Endt and Ortner, 1984). Intrinsic factors involve the characteristics of bone which include bone size, porosity, volume and chemistry. For example, trabecular bone is more susceptible than cortical bone to diagenesis because trabecular tissue favors heteroionic exchange between bone mineral and soil due to larger surface area and multiple cavities (Grupe, 1988a). In contrast, extrinsic factors are related to the immediate bone environment after death and these include soil pH, water, temperature and microorganisms (Gordon and Buikstra, 1981; Grupe and Piepenbrink, 1988; Newesely, 1989; Pate *et al.*, 1989; Von Endt and Ortner, 1984).

Detection of Diagenesis

Several methods have been applied to elemental analysis to identify diagenetic changes. One approach has been to compare elemental concentrations of the bone to those of the soil surrounding it (Edward and Benfer, 1993; ; Katzenberg, 1984; Keeley *et al.*, 1977; Link, 1991; Nelson and Sauer, 1984). In theory, soil should reflect elemental exchange between soil and bone either from leaching or enrichment. For example, soil rich elements such as manganese and iron were found in higher concentrations in two geographically distinct sites, one in Illinois and the other in Ontario (Katzenberg, 1984;

Lambert *et al.*, 1979). Generally, the same elements have been consistently identified as contaminants regardless of soil chemistry or geographical location.

Another method of detecting diagenesis is the examination of elemental profiles in bone cross-sections. This method is based on the assumption that elemental concentration decreases from periosteal to endosteal surfaces, and that higher concentrations at either the interior or exterior indicate diagenesis (Edward and Benfer, 1993; Jaworowski *et al.*, 1985; Klepinger *et al.*, 1986). Contaminants generally accumulate on the cortical exterior because this surface is in direct contact with the soil and groundwater (Badone and Farquhar, 1982; Gilbert, 1975; Lambert *et al.*, 1983; Pate *et al.*, 1989; Vlasak, 1983). Elements found to be consistent contaminants to the cortical surface are iron, manganese, aluminum and potassium.

Due to the differences in elemental concentration in depth from surface, researchers have attempted to circumvent contamination by physical abrasion of the periosteal and endosteal surfaces (e.g Link, 1991). This type of sample preparation ensures a relatively homogenous sample although contamination from the abrasion process may also occur.

Another approach concerns a comparison between modern and archaeological bone (Byrne and Parris, 1987; Edward *et al.*, 1984; Hancock *et al.* 1987, 1989; Spzunar *et al.*, 1978). If elements are higher in concentration in ancient bone then they are assumed to be contaminants. However, the assumption does not take into account diet and disease as factors which could contribute to elevated concentrations (Edward and Benfer, 1993). Another drawback of this comparison is that normal elemental levels have not been well documented for modern bone. While some data is available, discrepancies are apparent because values have been derived from different bones and bone tissue with no separation according to sex and age. It is difficult to compare element concentration in light of these variations. However, substantial elemental differences between modern and archaeological bone may indicate diagenesis and thus prove worthwhile (Radosevich, 1993).

Trabecular and cortical tissues have been compared to assess the effects of diagenesis, since the degree of susceptibility varies for each tissue (Lambert *et al.*, 1982; Vlasak, 1983). Lambert and colleagues compared ribs to femora and found ribs to be more susceptible to diagenesis because of greater porosity. In addition, different bones

analyzed from the same individual have provided indications of different degrees of diagenesis (Buikstra *et al.*, 1989; Lambert *et al.*, 1982). However, intra-individual variation could account for the elemental differences between and within bones (Francalacci *et al.*, 1988; Klepinger *et al.*, 1986).

Bone color has been advocated by Edward and Benfer (1993) as an indicator of diagenesis. Presumably, the longer a bone is exposed to diagenetic effects the darker it becomes. Moreover, the darkness of the bone is thought to be correlated with the duration of burial and thus exposure to contaminants such as bacteria and fungi (Piepenbrink, 1986). This method is only useful as a visual check, however.

ANALYTICAL METHODS USED IN ELEMENTAL DETERMINATION

Many methods have been applied in the determination of elements (major, trace and ultratrace) in bone including atomic absorption spectroscopy (e.g. Szpunar *et al.*, 1978), electron microprobe analysis (e.g. Vlasak, 1983), optical emission spectroscopy (e.g. Iscan *et al.*, 1989), inductively-coupled plasma (ICP) atomic emission spectrometry (e.g. Bethell and Smith, 1989) and neutron activation analysis (e.g. Badone and Farquhar, 1982). While each method has its advantages and disadvantages, the following discussion will be limited to neutron activation since it was the analytical method used in succeeding chapters.

Neutron Activation Analysis

Neutron activation has a long history with its discovery in 1936 by Hevesy and Levi (Guinn, 1990). In the early years (1936-1944), however, the potential of neutron activation to determine the elemental concentrations of a variety of substances was limited due to the requirement of a nuclear reactor as a source of radioactivity. The increasing availability of research-type nuclear reactors and the development of gamma-ray spectrometry equipment, have made neutron activation an extremely attractive method for anthropologists to assess elemental concentrations in human bone. There are several reasons for the popularity of instrumental neutron activation analysis:

a) it involves minimal sample preparation beyond cleaning, drying and weighing of samples;

b) it is nondestructive in that it allows repeat measurements of samples unlike atomic absorption which involves chemical dissolution of samples;

c) it provides analysis of the complete specimen unlike X-ray diffraction which only penetrates the surface;

d) it is a multielemental method that allows analysis of several elements simultaneously unlike atomic absorption which only measures one element at a time and finally

e) it is a sensitive method which can detect elemental concentrations as low as 1 ppm although ICP surpasses it in the degree of sensitivity (Lyon, 1970;Rakovic, 1970; Tite, 1972; Willard *et al.*, 1988).

The Principles of Neutron Activation Analysis

Neutron activation involves the excitation of the nuclei of stable isotopes by bombarding the nuclei with neutrons producing radioisotopes (Tite, 1972; Willard *et al.*, 1988). These radioisotopes begin to decay, according to a well defined time constant known as half-life, to form stable isotopes. During the decay process, gamma-rays are emitted and it is the energies of the gamma-rays that are used in the identification of elements. Detection, counting and identification of these gamma-rays (seen as photopeak energies) affords a means of elemental analysis. The basic equation for neutron activation analysis is as follows:

$$A = \frac{m \times N \times \theta \times \sigma \times \emptyset \times I \times \varepsilon}{M \times \lambda} (1-e^{-\lambda ti}) \times e^{-\lambda td} \times (1-e^{-\lambda tc})$$

where

A = photopeak area (activity)
I = fractional gamma yield
m = mass of element
N = Avogadros number, 6.02×10^{23} atoms/mole
θ = fractional isotopic abundance of isotope
M = molecular mass of target element
λ = decay constant ($0.693/ T^1/_2$)
σ = nuclear reaction cross-section
$\emptyset$ = neutron flux (n cm^{-2} s^{-1})
ε = fractional dector efficiency
ti = irradiation time in seconds
td = decay time in seconds
tc = count time in seconds

This equation requires that all variables are known in order to determine elemental concentrations. An alternative equation that is much simpler and more accurate because less variables are involved arose from the comparative method. In this method, the "comparator" or"standard" is similar in form and composition to the sample but contains a known quantity of the element to be determined. The element in the sample and standard are irradiated under exactly the same nuclear conditions and are counted by the same radiation detector. Therefore, the equation above is reduced to a simpler form shown below:

$$\frac{\text{Radioactivity in sample (a)}}{\text{Radioactivity in standard (b)}} = \frac{\text{Quantity of element in sample (c)}}{\text{Quantity of element in std (d)}}$$

Three variables, (a), (b), (d), as shown in the above equation must be known to calculate the amount of the element in the sample (c). The first step in the determination of radioactivity requires calculation of the rate of decay. Radioactive decay is expressed mathematically by the following equation:

$$e^{\lambda t},$$

Where $\lambda = 1n2/T_{1/2}$
1n2 = the natural logarithm of 2
t = delay time
and $T_{1/2}$ = half-life

Induced activity through neutron bombardment decreases with time, and the rate of decrease is exponential and unique to each isotope. The delay time represents the elapsed time between the end of irradition and the beginning of the activity measurement (count time). The counting procedure involves, with the aid of a lithium- drifted germanium semiconductor detector, the location of photopeaks, identification of photopeak energies and the measurement of net photopeak areas (total counts). Corrected peak area refers to the application of decay correction factors which mathematically adjust the count number (photopeak area) to reflect the counts lost during the time elapsed from the end of irradiation to the beginning of the counting process. Once the activities in the sample and standard have been established and the concentration of an element in the standard is known, the concentration of the element in a given weight of the sample is then calculated by the comparative method.

In the following chapters (three and four), the comparator equation was used to calculate elemental concentration. Due to the advantages listed above, neutron activation was the method of choice in this study.

CONCLUSION

In this chapter I provided the background to applications of trace element analysis of bone in anthropology. The elemental analysis of bone includes studies concerned with dietary reconstruction, diagenesis and disease. It is evident that trace element analysis is very complex with many factors contributing to variation in element concentration. While dietary analysis remains at the forefront of elemental studies, it is diagenesis that leads as the topic of present research. Paleodietary reconstruction was discussed in a historical framework providing accounts of the beginnings of elemental analysis with strontium as the element of choice, followed by other single element studies and then finally culminating in multielemental analyses.

With advances in method and instrumentation, elemental analysis has became focused largely on the issue of diagenesis. Several methods have been applied in the detection of diagenetic change, from soil analysis to comparisons of bone tissue types. It has been recognized that investigating and understanding the impact of diagenesis should precede other avenues of trace element research, especially palaeodiet since there are several mechanisms of chemical changes. For example, archaeological, bone is more susceptible to diagenesis because of void-filling minerals from the surrounding burial environment.

A relatively new application of elemental research which has not reached its potential is the relationship of human health and elemental distribution. Specific elemental deficiencies and toxicities were discussed in this chapter. Although little research has been done in this area so far, future investigations of human health and trace elements should provide much needed information on elemental properties and behaviors.

This chapter also stressed the importance of considering all data such as age, sex,diet and health before embarking on any analysis of trace elements in bone. Individualizing variables need to examined in order to assess the impact of these factors on elemental concentration. Trace element concentration and distribution are subject to

other factors including bone size, chemical interaction and diagenesis (Klepinger, 1993; Sandford, 1993; Von Endt and Ortner, 1993). Thus, it is necessary that all these variables are considered in any interpretation of trace element data.

TABLE 2.1 Elemental Concentrations in Various Food Groups (in parts per million)

Elements	Grains and Cereals	Vegetables	Meats	Nuts
copper	2.00	1.20	3.90	14.80
cobalt	0.43	0.14	0.22	0.26
magnesium	805.00	307.00	267.00	1970.00
manganese	7.00	2.50	0.20	17.00
molybdenum	2.07	0.51	6.00	---
selenium	0.15	---	0.92	0.05
strontium	3.00	1.90	2.00	60.00
zinc	17.70	6.00	30.60	34.00

--- No data

Source: Gilbert (1977)

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CHAPTER THREE
INVESTIGATION OF CALCIUM, MAGNESIUM AND ZINC DISTRIBUTION IN MODERN FIBULAE

INTRODUCTION

Many bioarchaeological projects have used trace element analysis to address problems that involve the examination of human skeletal material. Two principle applications of elemental analysis have been the determination of paleodiets and the investigation of diagenesis. The earliest application of elemental analysis emphasized dietary reconstruction based on a single element, strontium (Brown, 1973; Toots and Voorhies, 1965). Subsequent researchers recognized the necessity of including more elements such as zinc, manganese and copper in their investigations (Gilbert, 1975; Schoeninger, 1979; Sillen, 1981). In the years following, advances in analytical methods and instrumentation associated with elemental assay have led trace element analysis to progress beyond the problem of paleodietary reconstruction. As a result, much of the current anthropological research is aimed at understanding the impact of diagenesis on the biogenic nature of archaeological bone (Buikstra *et al.*, 1989; Edward and Benfer, 1993; El-Kammar *et al.*, 1989; Kyle, 1986; Lambert *et al.*, 1982, 1983, 1984a, b, c, 1985a, b; Link, 1991; Link and Lovell, n.d., Pate and Hutton, 1988). While the preservation of human skeletal remains of past populations provides opportunities to gather specific information pertaining to lifestyle, health and postdepositional processes, the purpose of this study is to examine elemental concentration within modern bone to determine whether elemental distribution is variable or homogeneous. The main objective of this study is to analyze the distribution of calcium, magnesium and zinc at proximal, midshaft and distal sampling sites in three modern fibulae to see how these elements are distributed.

Background to the Study

Since its initial application in anthropology twenty years ago, the primary use of trace element analysis was to reconstruct diet (Lambert *et al.*, 1979; Schoeninger, 1979; Sillen, 1981). However, over the years trace element analysis has been diversely employed to include such diet-related issues as diachronic shifts in subsistence patterns

(Beck, 1985; Katzenberg, 1984; Price and Kavanaugh, 1982; Schoeninger, 1981) and status differences in access to specific foods (Blakely and Beck, 1981; Geidel, 1982; Hatch and Geidel, 1985; Lambert *et al.*, 1979).

Other studies have looked at differences in elemental concentration due to sex (Beattie, 1981; Lambert *et al.*, 1979) and questioned whether these elemental differences might have been attributable to the influence of physiological processes such as pregnancy and lactation (Blakely, 1989; Klepinger, 1984). In addition to interest in gender-based variability, age has been examined to see whether it had an effect on element concentration (Lambert *et al.*, 1979; Tanaka *et al.*, 1981; Yoshinaga and Suzuki, 1989). While individualizing characteristics should be included in elemental interpretations, inclusion of these variables is based on the premise that diagenetic changes are not distorting or masking this individual information. A major concern in trace element research is the determination of the biochemical changes that bone undergoes once deposited within an archaeological setting. It is unwise to interpret elemental data if the effects of diagenesis have not been distinguished especially if other variables such as health and sex are affecting elemental concentrations in addition to diagenetic factors (Radosevich, 1993).

In attempts to detect diagenetic change, several methods have been applied including soil analysis from burial context (Katzenberg, 1984; Keeley *et al.*, 1977; Nelson and Sauer, 1984), comparison of elemental concentration between trabecular and compact bone (Lambert *et al.*, 1982; Vlasak, 1983) and comparison of modern and archaeological bone (Byrne and Parris, 1987; Hancock *et al.*, 1987; Lambert *et al.*, 1983). While investigations of diagenesis have provided information regarding the effects of the geochemical environment onarchaeological bone, many areas concerning bone chemistry have been unexplored. One such topic is the distribution of trace elements within a single bone.

This chapter examines elemental distributions within modern human bone to determine the extent to which elemental concentrations are variable or consistent. Modern bone is herein defined as skeletal material that has not been incorporated into any archaeological context. This may include clinical autopsy material or biological prepared specimens, bone that has not been buried. Very few anthropological studies have undertaken analyses of elemental distribution beyond differences in elemental concentration in bone cross-sections (Klepinger *et al.*, 1986). Generally, researchers

consistently sample long bones at the midshaft because it is considered the least likely to be diagenetically altered due to its greater thickness (Grupe, 1988). Clearly, single site sampling does not provide any indication of intrabone elemental distribution.

One consequence of single site sampling is that the reliability of the elemental data for paleodiet reconstruction becomes suspect because elemental concentration may not reflect dietary intake if element distribution varies within a bone. Thus it becomes necessary to establish intrabone distribution of elements especially if we intend to draw comparisons within and between studies. Without intrabone comparisons, pathological conditions might go undetected or alternatively thought to be present where they are not. This is based on the assumption that elemental concentrations may be used to indicate a pathological condition in light of the absence of gross morphological changes. Moreover, it is assumed that the presence of pathological changes to bone affect the entire bone. Certainly, variations in elemental concentration could be detected by sampling at other sites in addition to the midshaft, and this data could be used to support or dispel some of the assumptions associated with trace element analysis. Therefore, it is the intent of the present work to test the hypothesis that modern human cortical bone has a homogeneous distribution of elements when sampled at various locations.

If elemental distribution is not variable among different sampling sites then it is possible that samples could be taken at any location since all sites reflect normal physiological levels. The value of this is that fragmentary skeletal remains could be analyzed. Although diagenetic changes would have to be considered, the potential to analyze archaeological bone that is often considered unsatisfactory for elemental studies would be substantial. If, however, elemental distribution is variable among the sampling sites then this suggests a serious problem with elemental studies which attempt to reconstruct diet. The potential to misinterpret paleodiet is extensive and extremely dependent upon sampling choice. Even though the variation may be only slightly statistically significant , it would nevertheless be wiser to conclude that absolute elemental concentration is variable and affected by other factors in addition to diet. Until more knowledge of the characteristics, properties and behavior of elements in bone have been established, attempts to investigate and explain any variability in elemental distribution should be made.

It is important that modern bone be analyzed before archaeological bone because it is difficult to make any interpretations of diet or diagenesis without knowledge of the

"normal" physiological concentration and pattern of elements. An erroneous assumption, identified by Radosevich (1993) as the fourth deadly sin of early trace element studies, is that elemental levels of modern bone could not be compared to archaeological bone because of geographical, geological and cultural differences (Lambert *et al.*, 1979; Schoeninger and Peebles, 1981). In fact, this comparison is valuable in that it allows for detection of diagenesis in archaeological bone (Edward and Benfer, 1993). Yet, few studies have carried out elemental analysis on contemporary bone (see Aitken, 1976; Behne, 1976; Hancock et *al.*, 1987).

MATERIALS AND METHODS

Specimens

Modern human fibulae of South Asian origin, part of the human osteological collection at the University of Alberta, Department of Anthropology, were analyzed for calcium, magnesium and zinc. The precise origins of the specimens are unknown although they had been acquired through a biological supply company in Calcutta, India. Three adult right fibulae, all males, were chosen for instrumental neutron activation analysis.

Three samples were removed from each fibula with a jeweller's saw. The saw blade was cleaned with ethanol after each use to ensure that no interbone and intersite contamination occurred. Three cross-section cuts were made into the fibulae at proximal, midshaft and distal locations in the shaft. Thus, a total of nine samples were obtained with each cross-section sample approximately 5 to 8 millimeters in length. Only cortical bone was sampled because trabecular bone is subject to more chemical changes due to its greater surface area and higher turnover rate (Aitken, 1976; Brätter *et al.*, 1977). Furthermore, cortical bone shows less intraindividual variation in elemental concentration than trabecular bone (Grupe, 1988). It was suggested that to maintain sampling consistency and to provide a comparative framework between trace element studies, all samples should be taken from cortical tissue (Grupe, 1988).

Sample Preparation

The samples were cleaned in an ultrasonic bath of distilled water for 5 minutes to remove any extrinsic contaminants. Following air drying, the samples were manually reduced to bone chips by wrapping each sample in aluminum foil and crushing it with a hammer. The samples were dried for 20 hours at a calibrated temperature of 104 degrees Celsius, following which the mass of each sample was determined to the nearest 0.0001 g (Table 3.1). Afterwards, the samples were encapsulated within small polyethylene vials which had been cleaned previously with 5% nitric acid. The vials were heat seared according to the standard sample preparation protocol established by the University of Alberta's Slowpoke nuclear reactor. During the preparation of the vials, surgical gloves were worn to ensure that skin and sweat did not contaminate the samples.

In addition to the human bone samples, four sample vials were prepared containing ground bovine bone powder. The powder, designated as bovid bone H-5, was supplied by the International Atomic Energy Agency and used as a reference standard to permit quantification of elements within bone. Table 3.2 presents the masses of the standard used in this study and Table 3.3 shows the established elemental composition of IAEA H-5.

The samples were assayed for calcium, magnesium and zinc because these elements are consistently present and relatively abundant in bone. More importantly, these elements are essential for the normal metabolic and physiologic functions of bone. Calcium is responsible for bone mineralization, blood clotting, cell growth and muscle contraction (Bourne, 1971; Mundy, 1989; Vaughan, 1981). Magnesium is essential for proper growth and development and serves as a catalyst for many enzyme reactions (Aikawa, 1981; Underwood, 1971). Similarly, zinc is necessary for bone growth and mineralization but is also essential for protein and RNA synthesis (Cunnane, 1988; Prasad, 1979; Underwood, 1971).

Elemental Assay

Elemental determination was accomplished using the Slowpoke nuclear reactor at the University of Alberta. The use of neutron activation for element analysis has previously proven to be appropriate, especially for paleodietary reconstruction (Edward *et al.*, 1984; Schoeninger and Peebles, 1981) and the investigation of diagenesis (Badone

and Farquhar, 1982; Hancock *et al.*, 1987), since several elements can be analyzed simultaneously without risk of destruction of the sample (Guinn, 1990).

Neutron activation analysis involves irradiating the samples by bombarding the nuclei of stable isotopes with neutrons for predetermined durations, according to the nuclear properties of the elements of interest. The result is the formation of new compound nuclei that are in a highly excited state. These new nuclei (radioactive isotopes) do not remain excited but pass (decay) to a lower energy state by releasing gamma (λ)-rays. The radioactive decay occurs according to a equation with a well-defined decay constant known as the half-life. Each isotope has a characteristic half-life ($t_{1/2}$) which is the time it takes for half the nuclei to decay. The decay is accompanied by the emission of gamma-rays whereby the energy (keV) of the gamma peak is characteristic of a radionuclide. These peak energies are used in the detection and quantification of radioisotopes.

All the samples were irradiated following ttwo irradiation schemes corresponding to the different isotopes involved. The duration of the first irradiation, used to identify the ^{49}Ca isotope, was 300 seconds (s) with an initial counting period of 300 s after a minimum 300 s decay. At the end of the same irradiation day, another count of 600 s was taken to detect ^{27}Mg. The samples were irradiated a second time for 1 hour to determine the ^{65}Zn isotope. A counting period of 20,000 s was established after a decay of 1 month. Both irradiations had a neutron flux of 1×10^{12} neutrons/cm^2/s.

RESULTS AND DISCUSSION

Our knowledge of major, trace and ultratrace elements and their distribution in human bone is very meager and inconsistent. Inconsistencies can be seen, for example, in Table 3.4 which presents reported concentrations for the elements calcium, magnesium and zinc. Furthermore, some researchers have provided element concentration as absolute figures while others have given ranges. Also the "normal" data are not comparable because different bones were sampled. The concentrations presented in Table 3.4 are assumed to be indicative of "normal" physiological levels in bone; however, they only provide a general guide. In fact, this assumption is inherently inaccurate because it presupposes that biopsy material or sections of tissues have elemental

concentrations which reflect quantities in other parts of the same tissue (cortical or trabecular) and in different individuals (Jackson, 1989).

Table 3.5 presents the results of the present analysis. It gives the concentrations of calcium, magnesium and zinc in cortical fibulae samples of three contemporary adults. The concentrations are reported with their associated error of 2 standard deviations.

Calcium

The results for calcium show that its distribution at the midshaft and distal sampling sites in specimens 58 and 79 are similar, with concentrations that are not statistically different between the midshaft and distal samples in each specimen. Figure 3.1 illustrates the similarity in elemental concentration between midshaft and distal sampling sites in specimens 58 and 79. Although the actual patterns are different between the two specimens, the absolute concentrations from the midshaft and distal samples are close in both specimens (Table 3.5). In contrast, specimen 37 provided evidence of elemental variability between all three sampling sites as shown in Figure 3.2. This figure shows that the concentration at the distal sampling site is slightly elevated beyond the published calcium values reported in Table 3.4. Similarly, an elevated calcium concentration is observable in specimen 58 except that the proximal sample is affected rather than the distal (Figure 3.1).

One interesting pattern that has emerged from the calcium results is that all the midshaft samples are within published physiological levels. Based on this evidence it is reasonable to conclude that the midshaft is an appropriate sampling site, which may provide representative samples of "normal" elemental concentration. It is possible that the physiological activity at midshaft is minimal compared to the "ends" although the reason for this is not completely clear. In addition, it is known that the annual turnover rate for cortical bone is approximately 1% (Curzon and Cutress, 1983) but whether this rate is consistent for the length of a long bone remains unclear. It has also been established that turnover rates are variable in different bones (e.g. 10-19% for ribs compared to 0.9% for tibiae (Molnar, 1990; Vaughan, 1981) and perhaps this rate varies between different parts of a bone. In fact, the present study may provide evidence for the variability in turnovers rates within cortical bone as all three specimens had different calcium patterns.

Based on the correlation between age and quantity of osteoblasts and the relationship of calcium ions to osteoblasts, the results are consistent with specimens 58 and 37 representing younger adults compared to specimen 79. It was not possible to determine the specific age of each specimen although all three specimens represented adults. Since the concentration of calcium is above "normal" physiological levels at the proximal site in specimen 58 and at the distal site in specimen 37, it is likely that more osteoblasts are present at those locations, and that new bone is being deposited. Calcium is continually deposited in new bone tissue because it is responsible for mineralization -- the hardening of bone tissue (Bourne, 1971). Mineralization occurs in different areas of a bone at different rates depending upon the activation of osteoblasts. The division between calcified matrix and uncalcified matrix is referred to as the calcification front. It has been found that calcium tends to accumulate in the vicinity of the calcification front (Irving, 1973). A source of calcium adjacent to the osteoid definitely facilitates the process of calcification (Vaughan, 1981).

Perhaps these elevated concentrations represent sites of active bone remodeling at the bone ends. Remodeling is the collective effects of resorption and apposition, with osteons as the sites of remodeling. The process of cortical remodeling occurs during and after the cessation of growth, and it is not uniform (Ortner and Putschar, 1985). The rate of remodeling is affected by age and disease; it decreases with increasing age and increases due to pathology (Martin and Burr, 1989; Molnar, 1990). According to Steinbock (1976), the number of osteoblasts is considerably decreased in adults, and presumably the older the individual the fewer the osteoblasts. It has been found that osteoblasts play a role in the movement of calcium ions from the circulatory system to bone matrix (Vaughan, 1981). In addition, the mitochondria associated with osteoblasts have a capacity to store calcium. Since 99% of the body's calcium is stored in the skeleton, the relationship between cortical remodeling and calcium is clearly significant.

Although an age difference between the specimens has been postulated, no published data concerning differential accumulation of osteoblasts at the bone "end" is available. However, more blood is circulated to the "ends" of a bone via the metaphyseal arteries as the vascular supply must maintain the greater metabolic activity in trabecular bone (Bourne, 1971). In contrast, it has been reported that the circulation in the midshaft is reduced in mature bones (McLean and Urist, 1961). Since the blood supply is

increased distally and proximally, it can be expected that there are more active osteons as well as active osteoblasts located at the bone "ends".

While the number of osteoblasts may explain the elevated calcium concentrations in specimens 58 and 37, it does not clarify the discrepancy in the location of these elevated figures. The difference in the calcium pattern between the two specimens may be attributable to individual variation. Factors such as sex, age, health, physiology, metabolic rate and dietary intake can affect the behavior and distribution of elements (Radosevich, 1993). Inter-individual variation is often expected to be greater than intra-individual variation due to the factors listed above. Therefore, it is not surprising that the results show calcium behaving inconsistently between the two specimens.

Magnesium

An examination of the magnesium results reveals that it is homogeneously distributed in two specimens 37 and 79 at all three sampling sites (Figure 3.3). In contrast, specimen 58 shows homogeneity only at the midshaft and distal locations while magnesium's concentration at the proximal end is higher than reported levels in healthy individuals (Figure 3.4). With the exception of the proximal 58 sample all other samples have magnesium concentrations that are within range of "normal" physiological levels presented in Table 3.4.

The results provide positive evidence of the consistency in the distribution of magnesium in 2 of the 3 specimens. In contrast, the evidence for calcium was not as consistent. Although both elements share the same homeostatic control mechanisms -- intestinal absorption and renal excretion.-- they differ with respect to their physiological roles. According to Aikawa (1981) and Schwartz (1990), calcium and magnesium have both an antagonistic and synergistic relationship although it has been suggested that the relationship tends to be more antagonistic as magnesium has the effect of inhibiting the absorption of calcium. The present results show no evidence of an antagonistic relationship, in fact the elevated proximal concentration is seen in specimen 58 for both elements (Figure 3.4).

It has been well documented that an excess or deficiency of magnesium causes mineral and morphological abnormalities such as growth retardation and defective mineralization (Aikawa, 1971; Schwartz, 1990). Therefore, it would be reasonable to

expect to see skeletal defects in light of the elevated magnesium concentration in specimen 58 (Figure 3.4). However, no manifestation of any abnormalities have been observed. This is interesting especially considering that the proximal concentration is much higher than the reported concentrations from femoral head samples in Table 3.4. It has been documented that trabecular bone has higher elemental concentrations than cortical tissue (Gawlik *et al.*, 1982). This result may indicate that the cortical tissue of bone "ends" is normally higher due to proximity to trabecular bone. However, too little is known of the "normal" intrabone magnesium levels to support this theory. A pathological condition may explain the anomaly between the proximal concentration and the other two samples.

One pathological condition that could explain the elevated bone magnesium or hypermagnesemia is a failure on the part of the kidney to properly excrete magnesium. A consequence of impaired renal function is plasma magnesium toxicity which can affect the central nervous system and the cardiovascular system (Aikawa, 1981). It has been suggested that an equilibrium exists between the magnesium of plasma and bone (Aikawa, 1971). If the concentration of plasma magnesium is high it might explain the elevated bone magnesium in specimen 58. Although bone magnesium is mobilized to extracellular fluid during magnesium deficiency, it is unlikely that ionic exchange occurs from plasma to bone in the presence of excess magnesium. Furthermore, this should result in abnormal magnesium levels at all sampling sites. Since the circulatory system is not restricted to the proximal end, the explanation of a malfunction in the homeostatic control mechanisms is unsatisfactory. Therefore, the most plausible interpretation for the elevated magnesium concentration would be a pathological condition.

Several possible pathological conditions could be responsible for the abnormal magnesium concentration. Perhaps a metabolic condition is involved which would also explain the elevated calcium concentration as well. It is suspected that there may be a defect in hormone control, especially the release of parathyroid hormone and calcitonin. These hormones regulate the absorption and excretion of both calcium and magnesium (Bourne, 1971, Vaughan, 1981). Although hormonal imbalance is possible, it does not adequately explain why only the proximal sampling site showed elevated bone magnesium. If homeostatic control had been lost then we would expect to see abnormal concentrations at all sites within specimen 58. Yet, the midshaft and distal samples have "normal" magnesium concentrations.

It may also be possible that the dietary intake of this individual was high in magnesium-rich foods. Many edible plants, marine fish and animal protein contain substantial quantities of magnesium. A diet that is high in protein may account for the elemental pattern in specimen 58 because magnesium accumulates in skeletal muscle and bone. If meat consumption was high, it would not be surprising to see a concomitant rise in the body's concentration of magnesium. While a dietary interpretation of magnesium's results for specimen 58 is the most satisfactory; it is unlikely that nutrition played a role given the cultural and geographical background of the individual. The only reasonable dietary source of magnesium in South Asia would have been soil. Geophagia (eating of dirt) is not unknown is some parts of the world among contemporary peoples, but whether this was practiced by this individual is difficult to interpret. Furthermore, a dietary explanation does not explain the proximal site phenomenon.

Zinc

The results show that the distribution of zinc is variable in all three specimens. In Figure 3.5 the zinc concentrations are either depressed or elevated in comparison to the midshaft values for each specimen. In all the specimems, the midshaft sample has a consistently lower concentration than the proximal sampling site whereas the midshaft-distal relationship is not as apparent. Specimens 37 and 79 provided the only evidence for zinc levels that fell within "normal" physiological parameters. It is interesting that both examples of "normal" zinc concentration are from the proximal sampling sites. for zinc, unlike calcium or magnesium, a case could not be made for the midshaftIt to be representative of "normal" physiological levels.

The unusual pattern or lack of pattern seen in specimen 79 (Figure 3.5) is difficult to interpret since any plausible solutions need to take into account the activity of calcium and magnesium as well. The low zinc concentrations at the midshaft and distal sites may not be attributable to a dietary cause. It has been shown that zinc is a necessary mineral for growth, and that zinc deficiency results in growth impairment (Prasad, 1979). According to Wallwork and Sandstead (1990) zinc deficiency may be associated with human protein malnutrition and delayed or inhibited growth. Although growth has ceased in specimen 79, the low zinc concentration may be due to insufficient protein intake. This explanation is appropriate considering the cultural and geographical

background of the individual. Given that this individual is South Asian, consumption of animal protein would have been minimal.

It is evident that zinc is the most variable of the elements analyzed as no discernible pattern could be distinguished in elemental distribution among the three specimens. However, some discernible patterns are present in the form of a synergistic relationship between zinc and calcium in specimen 37 and as elevated concentrations for all three elements at the same sampling site in specimen 58. The first pattern concerns the configuration of zinc and calcium in specimen 37, both elements have the same distribution site for site (Figure 3.6). It has been reported that at a dietary level, zinc and calcium have an antagonistic relationship (Cunnane, 1988). It has been suggested that calcium inhibits zinc absorption and bioavailability; however, the data has been taken from animal studies (Underwood, 1971). It is assumed that information obtained on animal metabolism is applicable to humans, but results of this study suggests that extrapolation may be inappropriate. If calcium inhibits zinc, then a low zinc concentration would be expected at the distal end in specimen 37 because the calcium concentration is above "normal" levels. Rather than seeing the expected decrease distally, there is an elevated zinc concentration instead. Therefore, it is apparent from specimen 37 that calcium and zinc are not antagonists in this case.

It is too simplistic to make interpretations of zinc's distribution based solely on its interactions with calcium. Zinc has other elemental interactions, including potential antagonistic relationships with cadmium, chromium, copper and iron (Underwood, 1971). Elements that are presumed to be antagonists to zinc might have had some effect on the results but it is believed that the affects were minimal. Eelemental interactions are so complex that any antagonism from other elements on zinc would effect the behavior of calcium' and magnesiu as well. In addition to elemental interactions, phytate, a hexaphosphoric acid of inosital present in unleavened bread, can reduce the bioavailability of zinc . Phytate, however, is also known to reduce magnesium's bioavailability (Sandford 1992, 1993), and since the only specimen with a low zinc concentration has a "normal" magnesium content a dietary intake of phytate is unlikely.

A second pattern concerns the similarity in distribution for zinc, calcium and magnesium in specimen 58 (Figure 3.7). The elemental concentrations at the proximal site are all elevated in comparison to the midshaft and distal sampling sites, which seem to show consistency in concentration. For zinc, the proximal sample is higher than

"normal" physiological levels in Table 3.4 while calcium and magnesium have concentrations that are close to the extreme end of the range. The possibility of a pathological condition, mentioned previously, is still the most reasonable explanation for the similarity seen in the distribution of calcium, magnesium and zinc. Although the specific condition is unclear , it is obvious from the results that the condition has an effect on all the elements. However, the elevated proximal concentration of zinc may reflect a combination of the effects of a pathology and normal physiological distribution of zinc. This is only speculative since information concerning the distribution of elements is lacking.

CONCLUSION

In summary, this study analyzed three modern fibulae using neutron activation to examine the distribution of calcium, magnesium and zinc at three different sampling sites. Although the results reveal that intrabone elemental distribution is not consistent, they do show some interesting patterns. Of the three elements analyzed, magnesium provided the most convincing support for intrabone elemental consistency with evidence of homogeneity in two of the three specimens. In contrast, zinc provided the least evidence with distribution patterns that displayed the most variability. In addition, all the elements in specimen 58 displayed the same distribution pattern -- an elevated proximal value with almost equivalent midshaft and distal concentrations. The reason for this pattern is not clear because the results contradict published data suggesting that the chemical interaction of calcium and zinc is antagonistic.

One important result that has come from this study concerns midshaft sampling Samples taken from midshaft do reflect "normal" physiological levels thus the midshaft is an appropriate sampling site; however, this only applies to an analysis of calcium and magnesium. The midshaft concentration of calcium was consistently in the range of 20 % while magnesium also displayed relative consistency with concentrations between 2000 and 3000 parts per million. Zinc could not be included in the interpretation because its concentration at midshaft was variable. However, the variability might be related zinc's concentration at midshaft did not fall within "normal" physiological levels.

Another finding from this study is that the concentrations of calcium and magnesium compared well to published elemental values compiled in Table 3.4 . In

contrast, zinc concentrations generally fell outside the normal range for all samples except for the proximal samples of specimens 37 and 79. Throughout this paper emphasis was placed on making comparisons of the results to "normal" physiological concentrations of trace elements. Although the results were relatively consistent with published data, it is apparent that more research is needed to determine normal physiological levels in different bones for various ages and sex. Thus it is important that more analyses should be performed on modern bone specimens rather than archaeological bone because of diagenesis.

Furthermore, it was recognized that individual variation was a confounding factor; and that sex, age, health and physiology may affect trace element concentration and perhaps distribution. Although the effects of individual variation have been documented for such elements as calcium and zinc, anthropologists still assume that elemental concentration is an absolute reflection of diet. Yet, it has been established that age, sex, health and physiology can influence mechanisms of absorption, excretion and storage of elements. Certainly, it is possible to reconstruct diet from elemental profiles but caution must be exercised with regard to the accuracy of elemental analysis. Indeed, a major concern of dietary reconstruction is whether the sample is representative of the population and whether the individuals sampled provide complete profiles of dietary habit. Because individual variation has an impact on elemental concentration, it should not be assumed that the samples are representative of the entire population.

TABLE 3.1 Sample Mass of Modern Fibulae at Three Sampling Sites (expressed in grams)

Specimen	Sampling Site Proximal	Midshaft	Distal
37	0.385	0.924	0.464
58	0.214	0.857	0.661
79	0.650	0.862	0.911

TABLE 3.2. Sample Mass of IAEA H-5 Bovid Bone (g)

	Sample			
	#1	#2	#3	#4
Mass	0.156	0.196	0.252	0.271

TABLE 3.3 Elemental Composition of IAEA H-5 Bovid Bone. (published IAEA report cited in Link, 1991:64)

Element	Unit	Content	% error
Ba	mg/kg	79	16
Br	mg/kg	3.5	14
Ca	g/kg	212	3.8
Cl	mg/kg	550	18
Fe	mg/kg	79	7.5
K	mg/kg	680	17
Mg	g/kg	3.55	2.5
Na	g/kg	5.0	5.6
P	g/kg	102	8.4
Pb	mg/kg	3.1	18
Sr	mg/kg	96	8.6
Zn	mg/kg	89	5.9

TABLE 3.4. A Selection of Reported "Normal" Element Concentrations of Contemporary Adult Bones

Author(s)	Element	Concentration	Bone Sample
Hancock *et al.* (1989)	Ca	25.4±1.4%	Femoral Head
Hancock *et al.* (1989)	Ca	40.2±0.6%*	Femoral Head
Tipton and Shafer (1964)	Ca	37%*	unspecified
Bowen (1979)	Ca	17%	unspecified
Goode *et al.* (1972)	Mg	7000 ppm	unspecified
Tipton and Shafer (1964)	Mg	4600±1000 ppm*	unspecified
Bowen (1979)	Mg	700-1800 ppm	unspecified
Hancock *et al.* (1989)	Mg	2200±200 ppm	Femoral Head
Hancock *et al.* (1989)	Mg	3800±400 ppm*	Femoral Head
Aisling *et al.* (1963)	Zn	100-300 ppm	unspecified
Underwood (1971)	Zn	150-250 ppm	unspecified
Cunnane (1988)	Zn	218 ppm	unspecified
Edward *et al.* (1984)	Zn	107.4±1.6 ppm	Femur
Hancock *et al.* (1989)	Zn	210±20 ppm*	Femoral Head
Gawlik *et al.* (1982)	Zn	151±22 ppm	Iliac Crest
O'Connor *et al.* (1980)	Zn	155-287 ppm	Vertebrae
Tipton and Shafer (1964)	Zn	210±46 ppm*	unspecified

* concentration for ashed bone

TABLE 3.5. Elemental Concentration of Three Modern Fibulae

Specimen	Element	Sampling Site: Midshaft	Distal
58	Ca %	21.8±0.87	19.5±0.79
	Zn ppm	339±23	371±26
	Mg ppm	2922±479	2825±552
79	Ca %	20.2±0.82	22.8±0.92	23.1±0.92
	Zn ppm	109±10	72±7	47±5
	Mg ppm	2544±470	2997±497	2840±482
37	Ca %	26.3±1.08	19.9±0.79	33.5±1.33
	Zn ppm	249±22	72±7	402±30
	Mg ppm	2409±597	2514±444	2935±622

FIGURE 3.1 Similarity in Calcium Distribution Between Midshaft and Distal in Specimens 58 and 79

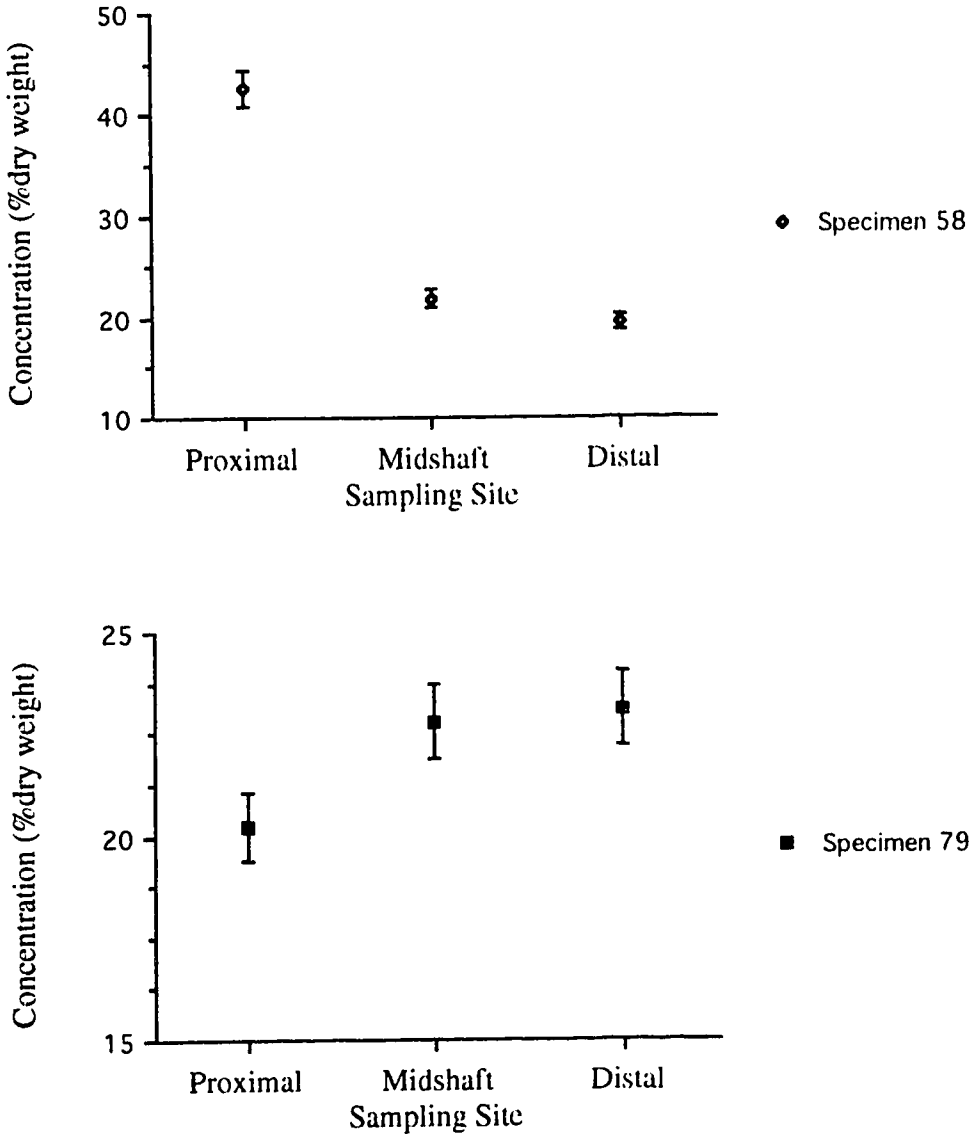


FIGURE 3.2 Variability in Calcium Distribution Between All Sampling Sites in Specimen 37

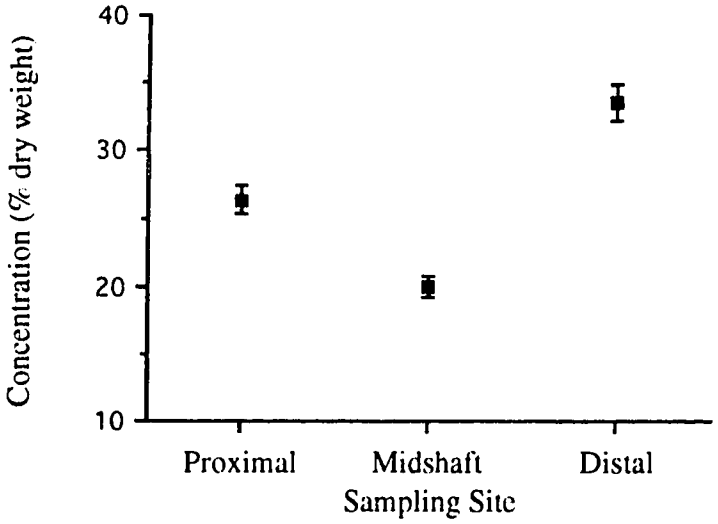


FIGURE 3.3 Proximal, Midshaft and Distal Distribution of Magnesium in Specimens 37 and 79

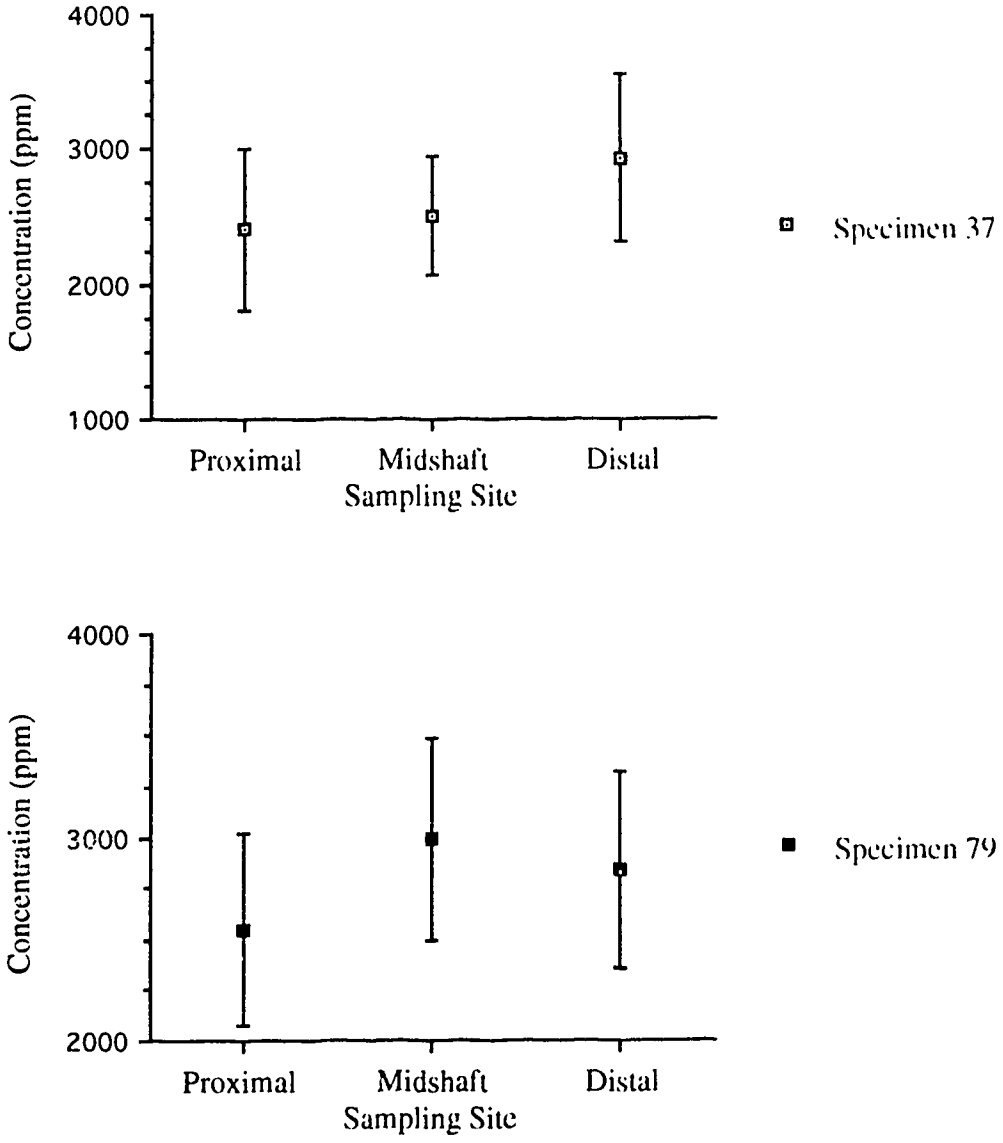
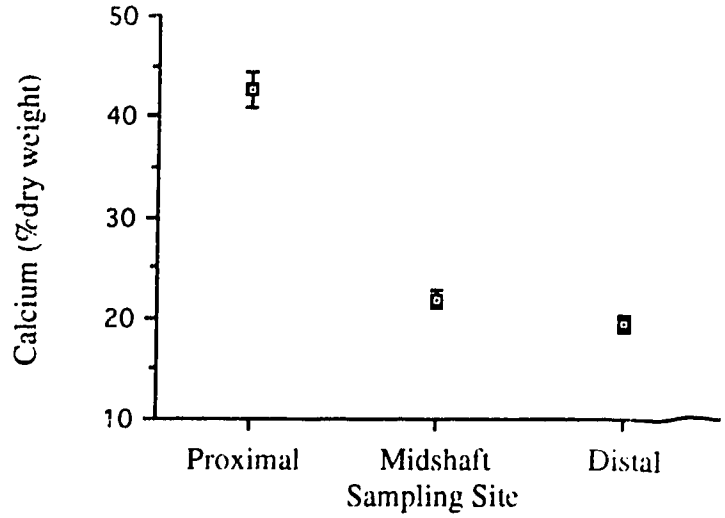


FIGURE 3.4 Similarity in Distribution of Calcium and Magnesium in Specimen 58



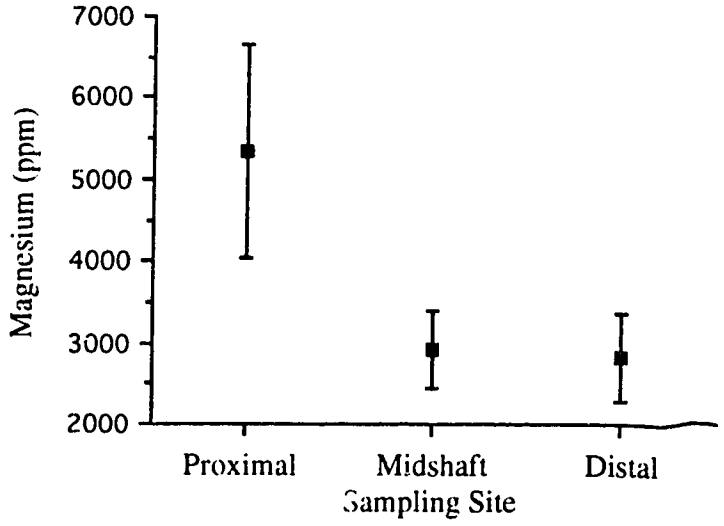


FIGURE 3.5 Variability in Zinc Distribution in Three Fibulae

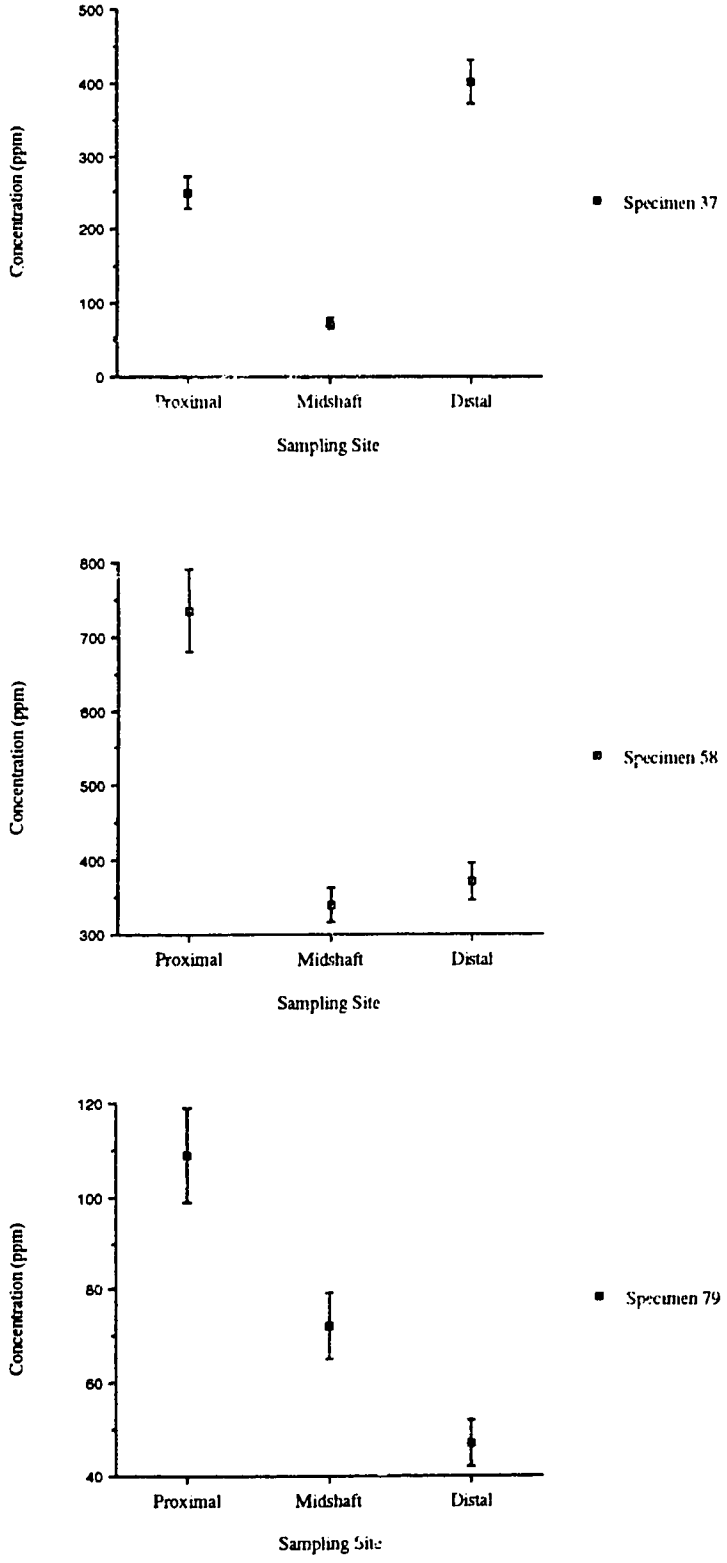


FIGURE 3.6 Zinc and Calcium Distribution in Specimen 37

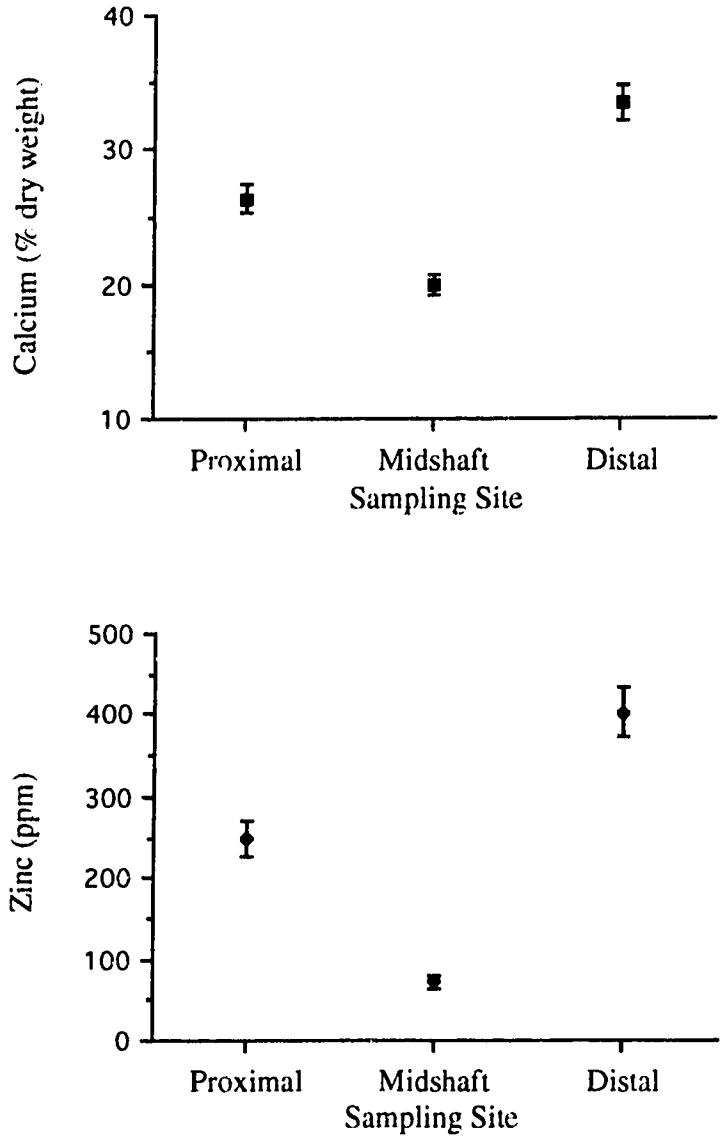
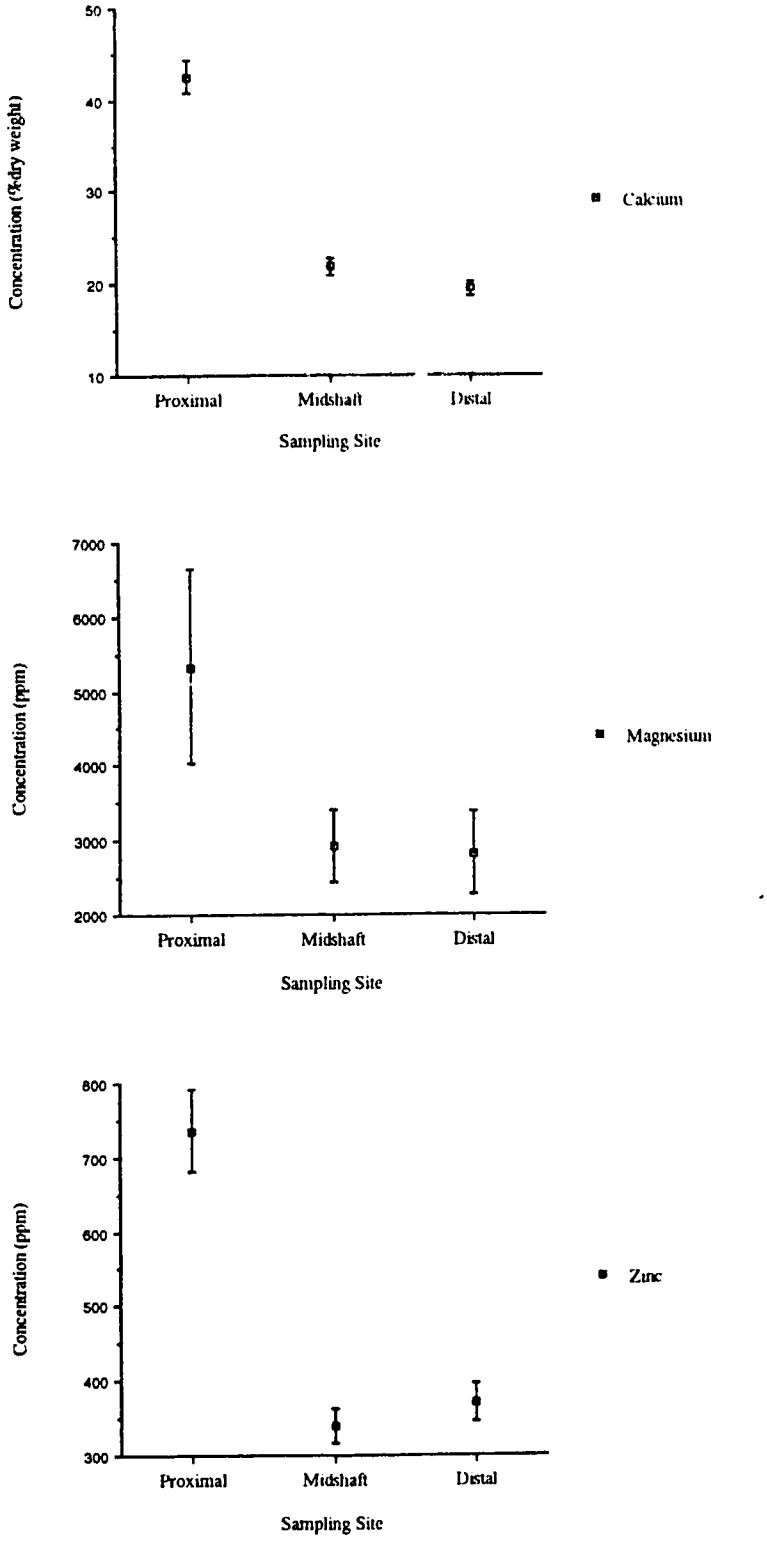


FIGURE 3.7 Calcium, Magnesium and Zinc Distribution in Specimen 58



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CHAPTER FOUR
CALCIUM, MAGNESIUM AND ZINC IN MODERN PATHOLOGICAL FIBULAE

INTRODUCTION

In the last twenty years of physical anthropological research, it has become routine to incorporate chemical techniques in the investigation of ancient human tissue (see reviews by Klepinger, 1984; Price *et al.*, 1985; Sandford 1992, 1993; Sillen *et al.*, 1989 and Sillen and Kavanaugh, 1982). Of the two approaches to chemical analysis, isotopic and elemental, it is the quantification of trace element concentration that has been most often studied. At the forefront of trace element research has been and continues to be paleodietary reconstruction (Antoine *et al.*, 1988; Price and Kavanaugh, 1982; Runia, 1987; Schoeninger, 1979; Sillen, 1981). In addition, much research has been concerned with the effects of diagenesis on trace element concentrations (Bumsma *et al.* 1989; Edward and Benfer, 1993; El-Kammar *et al.* 1989; Kyle, 1986; Lambert *et al.* 1982, 1983, 1984; 1985a, b; Lambert and Xue, 1988; Link, 1991; Link and Lovell, n.d.; Pate and Hutton, 1988). In contrast, this paper does not address either issue but focuses on elucidating the relationship of trace elements to pathology.

Emphasis on dietary reconstruction may stem from our need to characterize human behavior. If one aspect of a past population, such as diet, can be established and combined with other aspects of life, a complete reconstruction of lifestyle may be possible. Furthermore, the search for evidence of the consumption of different food resources, such as plant versus meat, has been stimulated by interest in evolutionary models of early human behavior. The 'man the hunter' versus 'woman the gatherer' models provided the initial basis for the use of trace element analysis; to distinguish between the consumption of meat and plants in past populations. In addition, many researchers have assumed that status differentiation is inherently linked to this dichotomy in food resources (Blakely and Beck, 1981; Geidel, 1982; Lambert, 1979). If specific food groups could be identified on the basis of trace element content alone then the determination of status becomes possible. However, the trophic distribution of food resources and the nature of food pathways are complex and as such the sources of trace elements can be obscure. Thus, for example, the simple interpretation that the presence

of zinc in bone is indicative of meat consumption does not take into account other possible sources of zinc. Furthermore, not all dietary sources of zinc, and for that matter other elements, are absorbed or utilized (O'Dell, 1985).

Perhaps the most important reason for the emphasis on dietary reconstruction is that it is easier than investigating the impact of diagenesis because the determination of paleodiet does not require any formal training in the biochemistry and physiology of trace elements (Sandford, 1992). Although dietary reconstruction requires some familiarity with the properties and behavior of trace elements, it often involves only the identification of elements that form the broad spectrum of nutrition. It seems that more importance is attributed to knowing whether zinc's concentration is due to the presence of meat or nuts in the diet, for example. At present, knowledge and understanding of the biochemical, metabolic and physiological properties and characteristics of trace elements are far from complete. In fact, no fewer than fifteen elements are considered to be essential, with an essential element defined as one that is necessary for the maintenance of life (Iyengar, 1991; Mertz, 1981). However, only a few of the fifteen are truly understood while relatively little is known of the remaining elements with respect to normal human physiology (Leichtmann and Sitrin, 1991). It becomes apparent that much research is needed and that dietary reconstruction should not be pursued until further information is obtained.

Although paleodietary research continues to proliferate, a phenomenon of the 1990's is the increasing number of studies concerned with understanding the interaction between the geochemical and biochemical environment in archaeological settings (e.g. Edward and Benfer, 1993; Link and Lovell, n.d.; Radosevich, 1993). The interest on diagenesis stems from the realization that post-mortem changes to bone might make dietary interpretations meaningless. One approach has been to use trace element analysis to assess the similarity or dissimilarity of element content between archaeological and modern bone samples in order to detect any diagenetic changes (El-Kammar *et al.*, 1989; Hancock *et al.*, 1987). Despite attempts to caution researchers of the inherent problems associated with paleodietary reconstruction (Hancock *et al.*, 1989), the assumption that elemental concentrations in bone are accurate reflections of dietary intake continues to be made all too often. It is now clear that the notion of "we are what we eat" first advocated by Hippocrates (Iyengar, 1991) indicates a lack of basic knowledge concerning trace

element metabolism. In fact, not only is trace element metabolism not well described or understood, but it is not the same for every individual (Francalacci and Tarli, 1988).

Dietary intake does not necessarily imply intestinal absorption and utilization of minerals because differences exist between bioavailability, "true" absorption and "apparent" absorption (O'Dell, 1985). O'Dell views "true" absorption as the movement of food nutrients from the intestinal lumen into the body while "apparent" absorption is a measure of the nutrient content that is found through excretion (1985:42). Once elements are absorbed they are transported to sites where they will be metabolized or stored. Many elements are stored within the skeleton as opposed to other tissues because of the ion reservoir storage property of bone. Skeletal analysis of trace elements has the potential to predict or identify the etiology of pathological conditions but very few studies to date have taken advantage of the possible benefits to paleopathology (Aufderheide, 1989). In fact, little is known of the exact relationship between trace elements and disease because evidence from human examples is lacking (Aufderheide, 1989). Rather, most of the information regarding elemental deficiency or toxicity and their effects on the skeletal system has been acquired through non-human studies (Doyle, 1979; Underwood, 1971). While much of the data can be extrapolated to humans, it is unclear whether the same biological changes will be seen in the human counterpart. However, real data from human subjects do exist, albeit meagerly, for such elements as zinc and selenium. For example, in the Middle East, a high phytate diet which results in zinc deficiency has been recognized as having detrimental effects on health (Prasad, 1982). Zinc deficiency can cause such skeletal changes as retarded growth, even to the point of dwarfism, and delayed maturation (Cunnane, 1988; Klepinger, 1993). Selenium and its deficiency has been reported to be responsible for Kaschin-Beck disease, a form of degenerative osteoarthritis (Burke, 1976; Levander, 1982).

Despite the clinical evidence which shows conclusively that elemental deficiency and excess can have effects on the skeleton such as delayed maturation and poor mineralization (Mundy, 1989; Prasad, 1978; Yang, 1985), it is unclear how elemental concentration is affected by the presence of disease or trauma. For example, while elemental deficiency can cause gross morphological changes to the skeleton, it has not been clearly established whether trauma or disease can alter the chemical profile of bone by affecting the concentration and distribution of trace elements.

A major contrast between clinical and anthropological evidence, with respect to the link between pathological conditions and trace elements in skeletal tissue, is that anthropologists generally do not analyze bones from extant populations. Archaeological remains that exhibit skeletal lesions have been chemically tested to assess the likelihood of nutritional deficiencies such as anemia (Fornaciari *et al.*, 1983; Sandford, 1988; Zaino, 1968). Iron deficiency anemia has often been considered to be the causative agent for the manifestation of cribra orbitalia and porotic hyperostosis (Carlson *et al.*, 1974; Cybulski, 1977; Lallo *et al.*, 1977). However, it is more likely that anemia involves more than simple dietary deficiency and that the presence of nutritional stress indicators might be evidence of one or more of a myriad of factors relating to other health problems (Stuart-Macadam, 1992). Cultural practices such as weaning patterns and poor hygiene and health care, leading to a susceptibility to infection, may contribute further to anemia (Price *et al.*, 1985; Sandford, 1992).

Researchers who have applied trace element analysis to examine pathological bone include Blondiaux *et al.* (1992), Gilbert (1975) and Grupe (1988a). Although these studies do not provide conclusive evidence of etiology for the skeletal lesions present in their samples, it is notable that they do show a relationship between trace elements and pathology. However, the precise nature of this relationship is unclear because elemental studies are often vague as to sampling procedures (e.g location of bone sample). This creates difficulties in the interpretation of elemental data since it is unknown whether elemental concentration is actually indicative of a localized pathological condition in the bone or reflective of the normal elemental variability within (as shown in Chapter 3).

Due to the limited data that is available concerning the relationship of trace elements and pathology in bone, the objective of this study is to analyze samples of modern pathological bone to describe and identify the behavior of zinc, magnesium and calcium. More importantly, it is hoped that it will be possible to determine the elemental responses to disease or trauma, and whether they are likely to be the same in three different samples taken from a single pathological bone specimen. Although elemental concentration may become elevated or depressed as a result of disease or trauma, it is important to know whether these changes to element levels are site specific. For this reason the elemental concentration of pathological tissue has been compared to samples of normal bone from the same specimen.

Despite the scarcity of studies analyzing the behavior of trace elements in human health and disease, little attention has been paid to the analysis of modern bone. In many ways modern bone is more suitable than archaeological bone for elemental analysis because modern bone has not been subjected to certain forms of diagenesis. Investigation into the relationship of trace elements and paleopathological bone can be marred by heteroionic and isoionic exchange with elements in the geochemical environment. For example, groundwater can leach elements out of skeletal material whi[illegible] at the same time it can contaminate bone by deposition of elements found in soil. In addition, microbial activity may alter elemental concentration because it is highly invasive to the chemical and structural integrity of bone tissue (Grupe and Piepenbrink, 1988). While it is acknowledged that soft tissue decomposition may chemically alter modern bone, changes to elemental concentration should be greater from interred bones since they also suffer ionic exchange with the surrounding soil .

Modern bone deserves further investigation because it provides a basis for comparison of the "normal" trace elemental concentrations with those of archaeological bone. Furthermore, due to the often fragmentary nature of archaeological [illegible]ne, modern bone has a distinct advantage in that skeletal remains obtained from cadav[illegible] are seldom incomplete. Although chemical analysis of diseased soft tissue and its elemental concentration is done routinely in clinical medicine (Heydorn, 1984; Zwanziger, 1989), the investigation of intra-bone element concentrations of modern pathological samples has not received the same attention.

MATERIALS AND METHODS

Specimens

The samples used in this study were taken from modern adult fibulae of South Asian origin, part of the human osteology collection at the Department of Anthropology, University of Alberta. Precise provenance is unavailable for the samples, but they are known to have been acquired from a biological supply company which distributed human skeletal material from Calcutta, India. While no biological data accompanied the specimens, sex assessment was based on the criteria of sexual dimorphism. Individuals that had large articular ends, long fibular length and rugose muscle origins were

considered to be males. In this study, two males, one female and one specimen of undetermined sex were selected for neutron activation analysis.

Each of the four fibulae displayed a different pathological condition, namely trauma (specimen 59), infection (specimen 49) and skeletal malformation (specimen 16). The remaining fibula (specimen 85) is an example of periostitis which is an ambiguous condiition because periosteal deposition is not a disease in itself but a response to either trauma or infection (Ortner and Putschar, 1985). According to Putschar "it is often impossible to determine which of these two conditions gave rise to a given lesion in an archeological skeleton" (1966:60). While the periostitic specimen is not from any archaeological context, it is nevertheless difficult to attribute etiology to the bony changes because the periostitis is localized and perhaps secondary to a specific disease process. The periosteal bone formation covers approximately 8 centimeters of the anteriolateral surface of the distal end, including the lateral malleolus (Figure 4.1). In most infections, one of the significant changes to the skeleton is periosteal bone formation. Inflammatory periostitis involves uneven distribution of periosteal bone, sometimes plaque-like, that is very porous in appearance due to hypervascularity of the periosteum (Ortner and Putschar, 1985). Similarly, periostitis that is secondary to trauma has the same appearance and is the result of sudden or chronic insult to bone. Due to the localization of the periostitis it is extremely likely that specimen 85 is indicative of a post-traumatic event. However, the possibility that the periosteal bone deposition is a response to some nonspecific infectious disease cannot be dismissed.

In contrast, specimen 59 has definite indications of a traumatic condition, namely a healed spiral fracture located at the distal end approximately 3.4 centimeters superior to the lateral malleolus (Figure 4.2). The fracture is macroscopically evident due to the presence of an incompletely remodeled callus which is approximately 3 centimeters in length. The fracture is well aligned with no angular distortion. The fracutre represents the only female in the study.

Specimen 49 is a male with evidence of osteomyelitis that involves moderate to extreme new bone formation which covers approximately one-third of the fibula's distal length (Figure 4.3). Osteomyelitis, by definition, is an infection of bone involving the marrow, whereby the infection is due to the introduction of pyogenic bacteria (Ortner and Putshcar, 1985). Although the periosteal reaction is moderate there are no signs of any sequestra. The periosteal response in the osteomyelitis is similar to the periostitis in that

it is predominately anteriolateral. However, osteomyelitis is distinguished from superficial periostitis because of its endosteal involvement. The endosteal response is visible but only minor bony changes have occurred.

The remaining fibula, specimen 16, had been catalogued in the pathological collection as an idiopathic anomaly because of its abnormal thinness and curvature of its shaft, this specimen may provide evidence of club foot or post paralytic deformity (Figure 4.4.). Ortner and Putschar (1985) provide an illustrated example of a case of bilateral club foot (see p. 326) with a similar torsion of the distal fibular shaft due to articulation with an uptilted calcaneus. The diagnosis of club foot or post-paralytic deformity would explain the gracile appearance of the shaft since little biomechanical stress is placed on it. Instead, the lateral malleolus must receive all the biomechanical stress related to the abnormal movement of the lower limbs. This application of stress on the lateral malleolus is evident by the torsion of the distal "end" in a posteriolateral angle. The post-paralytic specimen could not be sexed because no distinct sexually dimorphic features were identified. It is likely that the pathological condition could have contributed to the ambiguity of morphological sex traits.

Three samples were removed from each fibula with a jeweller's saw. The saw blade was cleaned with ethanol after each use to ensure that no interbone and intersite contamination would occur. Three cross-section cuts were made into the fibulae at pathological, midshaft and distal locations in the shaft. Thus, a total of twelve samples were obtained with each cross-section sample approximately 5 to 8 millimeters in length. Only cortical bone was sampled because trabecular bone is subject to more chemical changes due to its greater surface area and higher turnover rate (Aitken, 1976; Brätter *et al.*, 1977). Furthermore, cortical bone shows less intraindividual variation in elemental concentration than trabecular bone (Grupe, 1988b). It was suggested that to maintain sampling consistency and to provide a comparative framework between trace element studies, all samples should be taken from cortical tissue (Grupe, 1988b).

Sample Preparation

The samples were cleaned in an ultrasonic bath of distilled water for 5 minutes to remove any extrinsic contaminants. Following air drying, the samples were manually reduced to bone chips by wrapping each sample in aluminum foil and crushing it with a hammer. The samples were dried for 20 hours at a calibrated temperature of 104 degrees

Celsius, following which the mass of each sample was determined to the nearest 0.0001 g (Table 4.1). Afterwards, the samples were encapsulated within small polyethylene vials which had been cleaned previously with 5% nitric acid. The vials were heat seared according to standard sample preparation protocol established by the University of Alberta's Slowpoke nuclear reactor. During the preparation of the vials, surgical gloves were worn to ensure that skin and sweat did not contaminate the samples.

In addition to the human bone samples, four sample vials were prepared containing ground bovine bone powder. The powder, designated as bovid bone H-5, was supplied by the International Atomic Energy Agency and used as a reference standard to permit quantification of elements within bone. Table 4.2 presents the masses of the standard used in this study and Table 4.3 shows the established elemental composition of IAEA H-5.

The samples were assayed for calcium, magnesium and zinc because these elements are consistently present and relatively abundant in bone. More importantly these elements are essential for the normal metabolic and physiologic functions of bone. Calcium is responsible for bone mineralization, blood clotting, cell growth and muscle contraction (Bourne, 1971; Mundy, 1989; Vaughan, 1981). Magnesium is essential for proper growth and development and serves as a catalyst for many enzyme reactions (Aikawa, 1981; Underwood, 1971). Similarly, zinc is necessary for bone growth and mineralization but is also essential for protein and RNA synthesis (Cunnane, 1988; Prasad, 1979; Underwood, 1971).

Elemental Assay

Elemental determination was accomplished using the Slowpoke research nuclear reactor located at the University of Alberta. The use of neutron activation for element analysis has previously proven to be appropriate, especially for paleodietary reconstruction (Edward *et al.*, 1984; Schoeninger and Peebles, 1981) and the investigation of diagenesis (Badone and Farquhar, 1982; Hancock *et al.*, 1987), since several elements can be analyzed simultaneously without risk of destruction of the sample (Guinn, 1990).

Neutron activation analysis involves irradiating samples by bombarding the nuclei of staple isotopes with neutrons for predetermined duration, according to the nuclear

properties of the elements of interest. The result is the formation of new compound nuclei that are in a highly excited state. These new nuclei (radioactive isotopes) do not remain excited but pass (decay) to a lower energy state by releasing gamma (λ)-rays. The radioactive decay occurs according to a equation with a well-defined decay constant known as the half-life. Each isotope has a characteristic half-life ($t_{1/2}$) which is the time it takes for half the nuclei to decay. The decay is accompanied by the emission of gamma-rays whereby the energy (keV) of the gamma peak is characteristic of a radionuclide. These peak energies are used in the detection and quantification radioisotopes.

All the samples were irradiated following two irradiation schemes in order to measure the isotopes of selected elements. The duration of the first irradiation, used to identify the ^{49}Ca isotope, was 300 seconds (s) with an initial counting period of 300 s after a minimum 300 s decay. At the end of the same irradiation day, another count of 600 s was taken to detect ^{27}Mg. The samples were irradiated a second time for 1 hour to determine the ^{65}Zn isotope. A counting period of 20,000 s was established after a decay of 1 month. Both irradiations had a neutron flux of 1 x 10^{12} neutrons/cm^2/s.

RESULTS

Table 4.4 presents the elemental concentrations of calcium, magnesium and zinc at three sampling sites in each of the four fibulae, with their associated error of 2 standard deviations. Table 4.5 gives a selection of published elemental values in normal modern bone for the three elements.

It is apparent from these results that the elemental concentrations of the pathological site samples are all within "normal" physiological levels, independent of the specific pathological condition. This is also the case for most but not all of the results from the other two sampling sites, where the bone is taken to be normal, not pathological. The three exceptions to this are at the proximal sampling location in the fracture where the concentration of calcium is lower than the "normal" physiological values, and at the proximal site in the specimens with osteomyelitis and post-paralytic deformity where the zinc concentrations are slightly elevated.

Examination of the elemental concentrations with the aim of determining patterns in the distributions also yielded interesting results. For example, it is evident that zinc's

distribution pattern is similar in appearance in two pathological conditions, the fracture and periostitis (Figure 4.5). In both specimens, the concentration of the midshaft samples are lower than that observed at the other two sampling locations. Furthermore, with respect to absolute zinc concentration the overall difference between the highest and lowest values did not exceed 70 parts per million in either specimen.

Another pattern, as illustrated by Figure 4.6, is the similar pattern for magnesium distribution in the cases of osteomyelitis and post-paralytic deformity. Furthermore, the calcium distributions for the same two conditions are also similar (Figure 4.7). The midshaft is consistently higher in concentration than the pathological and proximal sampling sites for both calcium and magnesium in these two bones. However, the most remarkable result is that in each bone calcium and magnesium exhibit the same pattern at each sampling site regardless of the type of pathological condition involved. Although the actual concentrations differ for each pathological condition, it can be seen in Figures 4.8, 4.9, 4.10, and 4.11 that the distribution of these two elements is similar.

The patterns in the osteomyelitic and post-paralytic specimens have been mentioned previously and are illustrated by Figures 4.8 and 4.10. Figure 4.9 illustrates that in the fracture specimen the highest concentration of both calcium and magnesium is found in the pathological sample. In addition, the same figure shows that the proximal sampling location provided the lowest calcium and magnesium concentrations for the fracture. In contrast, Figure 4.11 shows that the periostitic bone has a high concentration of both elements in the proximal sample while the pathological and midshaft samples are relatively similar in concentration. Furthermore, the periostitic specimen is dissimilar to the other three pathological bones in that it is the only one to have a proximal concentration that is higher than the midshaft.

Finally it is important to recognize that the results provide no evidence that pathological regions differ from other regions in bone in any regular manner. In some cases, the pattern of elemental distribution of the pathological samples is similar to the midshaft and in others it is similar to the proximal sites.

DISCUSSION

An important step in evaluating any relationship of trace elements to pathology is the determination of their "normal" physiological concentration. The problem with establishing "normal" physiological values is that human variation, in terms of sex, age and health status, contribute to different elemental levels. Furthermore, physiological differences in the skeleton also add to the variability as elemental content is different in different bones (Klepinger *et al.*, 1986). While these conditions make it difficult to assess changes due to disease or trauma, it has been demonstrated (see Chapter 3) that the midshaft sample may provide the most representative example of elemental concentration. Using the midshaft concentration as reference, it became clear that areas affected with disease and trauma are associated with both higher and lower trace element concentrations.

It is immediately apparent that this study provides evidence on two points. The first is that the elemental concentrations of pathological bone tissue were not abnormal but, on the contrary, were well within the "normal" physiological range (see Table 4.5). An abnormal elemental concentration was expected at the site of disease or trauma because of the reported relationship between element concentration and pathological conditions (Prasad, 1986; Underwood, 1971). These reports refer to soft tissue analysis, and it appears that skeletal tissue is not as profoundly affected as soft tissue. A reason for this may reside in the fact that while the skeleton acts as a mineral reservoir, the concentration of minerals may be relatively stable since ions are not mobilized unless the elemental concentration of the plasma and extra cellular fluid is in jeopardy. Although isoionic and heteroionic exchange occurs in periods of physiological stress such as pregnancy and lactation, it is possible that the mineral integrity of bone is kept relatively constant.

The results revealed that all the pathological samples had "normal"" concentrations of calcium, magnesium and zinc; and that some of the samples that were representing "normal" bone showed slight variability from the physiological levels. An explanation of this phenomena is that some elemental change most likely occurs in the area of the pathological condition, but unless the pathology involves uncoupling bone formation from bone resorption an abnormal elemental concentration should not be observed (Mundy, 1989). An imbalance in elemental concentration only occurs when the A-R-F (activation-resorption -formation) sequence no longer functions properly (Frost,

1980). The results of this study show that the osteoblastic and osteoclastic activity at the pathological sampling sites were coupled.

The second point involves the chemical interactions between elements which have been described elsewhere but need to be reviewed briefly here (see Aikawa, 1981; Becker *et al.*, 1968; O'Dell, 1985; Prasad, 1978; Sandford, 1992; Spadaro *et al.*, 1970; Underwood, 1971). The interactions between the elements themselves are often described as either synergistic or antagonistic. Synergism is defined as the need of one element for another to maintain normal efficacy in metabolism whereas antagonism refers to the impairment in function due to the presence of another element (Kirchgessner *et al.*, 1982). A high intake of zinc that interferes with iron absorption because of competition for protein-binding sites in the intestinal mucosa is an example of antagonism (Prasad, 1978). Despite the information available on the relationship between and among elements, no study has conclusively shown that antagonistic and synergistic interactions are consistent within and between individuals.

The results of the present study demonstrate unequivocally that calcium and magnesium behave synergistically. For each sampling site and regardless of the pathological condition involved, the distribution pattern of both calcium and magnesium are identical, as is true for normal bone (Chapter 3). According to Aikawa (1981), calcium and magnesium have both a synergistic and antagonistic relationship but the results here provide no indication of any antagonism. The precise nature of the suggested antagonism is unclear yet it might concern the fact that calcium and magnesium share the same homeostatic mechanisms. Magnesium is not directly incorporated into apatite crystal but binds to the hydration shell and thus it is unlikely that magnesium and calcium compete for specific binding sites since calcium ions are incorporated into the crystal. According to this logic, a synergistic interrelationship would be expected to be the normal condition.

Further evidence of chemical interactions is seen between zinc and calcium. These two elements have an antagonistic relationship which is definitely visible in Figure 4.11 at the pathological and proximal sampling sites. Antagonism is also visible in the post-paralytic specimen at the proximal site (Figure 4.10). Although the relationship is not completely antagonistic site for site in the four pathological conditions, there is definite evidence of the interactive relationship between calcium and zinc. It should be noted that previous data from non-human studies concerning the interaction of calcium

and zinc cite the inhibitory effect of calcium on zinc's absorption and bioavailability (Cunnane, 1988).

Zinc shows the most variability of the three elements, with elevated concentrations seen in the osteomyelitis and post-paralytic deformity (Figure 4.12). The abnormal elemental content in these conditions are located at and near the proximal "end". Indeed, an examination of all proximal concentrations shows that this site is consistently higher than midshaft for all pathological conditions. This pattern is not only evident in this study but can be seen in normal bone as well (Chapter 3). It is reasonable to infer that the proximal end is more physiologically active than other areas within the fibula. The reasons for this remain unclear, although zinc's predominance at the proximal end might be related to bone biomechanics. Biomechanical stress is distributed mainly through the proximal head in the fibula due to its anatomical position in relation to the tibia, which is a major weight bearing bone. Stress is distributed along the longitudinal axis of the bone (Martin and Burr, 1989). Zinc's concentration may be extremely important at the proximal end because of zinc's roles in mineralization and protein synthesis. A higher concentration of zinc at the proximal end may aid in strengthening the bone to handle compressive and tensile forces.

This study also provides further evidence of the inherent importance of zinc to the healing process. Based on the clinical literature (Okado *et al.*, 1990; Pories and Strain, 1966; Wacker 1976), it was expected that zinc would be higher at the fracture site since it tends to accumulate at sites of bone repair (Blondiaux *et al.*, 1992). From experimental studies done with rats, the rate of fracture healing increased with the introduction of zinc supplements (Pories and Strain, 1966). The acceleration in fracture healing was attributed to the accumulation of zinc at the fracture site because of zinc's role in cell division.

According to Lane and Werntz (1984) there are six stages involved in the osseous repair process and they are impact, induction, inflammation, soft callus, hard callus and finally remodeling. Induction involves the activation of such cellular components as endosteal cells, periosteal cells and osteocytes. Inflammation refers to the disruption of blood supply resulting in bone necrosis and cell death. Following the inflammation stage, osteoclasts start to appear in order to remove dead bone. Osteoclastic activity occurs in the callus stage which is also marked by an increase in vascularity and cellularity. The soft callus is fibrocartilaginous and functions to join the ends of the fracture. Due to

increase blood flow and hence oxygen, the microenvironment becomes conducive for the calcification of the soft callus (Simmons, 1980). The conversion of the fibrocartilaginous tissue to bone marks the hard callus stage. In the remodeling stage, the diameter of the bony callus decreases with subsequent endosteal bone formation and periosteal bone resorption.

While the fracture site displays a higher concentration of zinc than the midshaft, the absolute concentration is normal. This concentration signifies that little remodeling is occurring in the fracture, which indicates that it is in or past the final stage of the repair process. This is consistent with the order of events in the list of stages for fracture repair. Because little to no remodeling is occurring at the fracture site, osteoblastic activity appears to have ceased. Osteoclastic activity is still occurring, although probably at a low level since the diameter of the fracture callus has not been obliterated by the remodeling process. The functional lifespan of osteoblasts at a given remodeling site may range from 3-4 months to 1.5 years with an average of 5-6 months (Recker, 1992). Given that there is no osteoblastic activity, the fracture is estimated to be at least a year old.

The relationship between zinc and trauma is also evident in the periostitis case (Figure 4.11). The zinc distribution in the periostitis is similar in configuration to the fracture suggesting that the periostitis represents another case of trauma rather than infection. It has been found that the elemental response to infection is contrary to the response elicited by zinc for trauma (Prasad, 1982). An accumulation of zinc is considered the proper physiological response to trauma while zinc deficiency can impair immune responses and enhance the risk of infection (Chandra, 1991). Infection arises due the body's susceptibility to infectious agents because the immunological system no longer responds normally in protein-energy malnutrition (Chandra, 1991). One of the most significant changes to the immune system is the decrease in the number of lymphocytes which act as mediators against microbial invasion (Chandra, 1991; Prasad, 1985).

Although the concentration of zinc in the periostitis did not indicate infection, positive evidence of zinc deficiency and infection can be seen in the osteomyelitic case (Figure 4.8). The osteomyelitic specimen supports the relationship between zinc and infection as the pathological sample has a low zinc concentration as compared to the other two sampling sites. In contrast, the post-paralytic deformity is not associated with infection but indicates skeletal malformity. This explains the abnormal shaft thinness

seen in this specimen and the low zinc concentration . It is unclear whether defective bone mineralization is the cause of the low zinc concentration or that dietary deficiency of zinc caused the atrophy. The appearance of defective bone mineralization is considered to be a manisfestation of zinc deficiency (Klepinger, 1993; Prasad, 1978; Underwood, 1971). It appears that the uncoupling occurred endosteally and periosteally because bone removal exceeded mineralization of new bone thereby contributing to cortical thinness.

It is not surprising that the fracture and periostitis have the same distribution pattern for zinc if they are related to the same traumatic processes. The fracture specimen is from a female but this does not affect elemental distribution as evident by the similarity to the pattern of the periostitic bone, which is male. If sex has any affect on elemental distribution it is not apparent in these results. In contrast, the low calcium level at the proximal site in the fracture may be sex related because a similar concentration is seen in the post-paralytic deformity. Although the sex of the post-paralytic deformity is ambiguous, it may be female because of the similarity to the fracture specimen in the proximal site for calcium and zinc. According to Sevitt (1981) many fractured bones become osteoporotic to some degree because bone tissue and minerals are lost. While the similarity of the elemental pattern seen in the fracture and post-paralytic deformity may be attributable to sex differences, it is not conclusive since the element to element comparisons between the two conditions are variable.

CONCLUSION

Elemental analysis of pathological bone has seldom been the objective of research in anthropology as evident by the scarcity of studies. To understand the relationship between trace elements and disease or trauma, this study analyzed four pathological conditions for three elements at three different sampling locations and the results are extremely interesting. The most surprising discovery was that the presence of disease or trauma did not affect elemental concentration abnormally. Some elevation or depression of concentration was seen but the absolute concentrations remained within the range of normal physiological levels. This is an important fact because it indicates that even in the presence of disease or trauma the chemical integrity of bone is maintained.

The results also provide support for specific elemental interrelationships. This study shows conclusively that magnesium and calcium behave similarly. The elemental patterns of these two elements were almost the same for each pathological condition. This phenomenon is important because if any variation is detected in the behavior of calcium and magnesium there must some cultural or biological explanation since both elements are suppose to have the same pattern of distribution. Thus, it is necessary to consider what may be causing the variability.

While sex has been reported to affect elemental concentration, no differences in elemental distribution due to sex could be detected, however, only one of the specimens in the study was female (fracture). The fracture and periostitic specimens had similar patterns which would not be expected if sex was a contributing factor to elemental variation.

Another discovery from this study was that zinc is a highly unpredictable element in its distribution. However, a recommendation for future research would be to analyze a larger sample to see if zinc's concentration is always higher in the proximal end. It was suggested that a higher zinc content proximally is normal but because of the small sample size this interpretation needs substantiation.

TABLE 4.1 Sample Mass of Pathological Fibulae at Three Sampling Sites (expressed in grams)

Specimen	Sampling Site		
	Pathological	Midshaft	Distal
85	0.694	0.614	1.044
59	0.502	0.548	0.541
49	0.459	1.156	0.842
16	0.48.	0.350	0.438

TABLE 4.2. Sample Mass of IAEA H-5 Bovid Bone (g)

	Sample			
	#1	#2	#3	#4
Mass	0.156	0.196	0.252	0.271

TABLE 4.3 Elemental Composition of IAEA H-5 Bovid Bone. (published IAEA report cited in Link, 1991:64)

Element	Unit	Content	% error
Ba	mg/kg	79	16
Br	mg/kg	3.5	14
Ca	g/kg	212	3.8
Cl	mg/kg	550	18
Fe	mg/kg	79	7.5
K	mg/kg	680	17
Mg	g/kg	3.55	2.5
Na	g/kg	5.0	5.6
P	g/kg	102	8.4
Pb	mg/kg	3.1	18
Sr	mg/kg	96	8.6
Zn	mg/kg	89	5.9

TABLE 4.4. Elemental Concentration of Four Pathological Fibulae at Three Sampling Sites

		Sampling Site		
		Pathological	Midshaft	Proximal
Specimen	Element			
Osteomyelitis	Zn ppm	154±12	222±18	350±25
	Mg ppm	2214±354	3398±500	2294±389
	Ca %	22.3±0.88	29.2±1.16	22.3±0.89
Fracture	Zn ppm	242 ±19	173±15	251±21
	Mg ppm	2254±680	2088±526	1425±514
	Ca %	26.1±1.06	21.9±0.89	14.4±0.62
Periostitis	Zn ppm	252±18	182 ±13	219±19
	Mg ppm	2635±457	2652±385	4094±772
	Ca %	21.9±0.88	20.5±0.81	30.2±1.23
Post-paralytic	Zn ppm	174±16	352±29	370±28
	Mg ppm	3475±673	5627±872	3063±523
	Ca %	29.8±1.21	39.8±1.57	18.3±0.76

TABLE 4.5 A Selection of Reported Elemental Concentrations in "Normal" Modern Adult Bones

Author(s)	Element	Concentration	Bone Sample
Hancock *et al.* (1989)	Ca	25.4±1.4%	Femoral Head
Hancock *et al.* (1989)	Ca	40.2±0.6% *	Femoral Head
Tipton and Shafer (1964)	Ca	37% *	unspecified
Bowen (1979)	Ca	17%	unspecified
Goode *et al.* (1972)	Mg	7000 ppm	unspecified
Tipton and Shafer (1964)	Mg	4600±1000 ppm*	unspecified
Bowen (1979)	Mg	700-1800 ppm	unspecified
Hancock *et al.* (1989)	Mg	2200±200 ppm	Femoral Head
Hancock *et al.* (1989)	Mg	3800±400 ppm*	Femoral Head
Aisling *et al.* (1963)	Zn	100-300 ppm	unspecified
Underwood (1975)	Zn	150-250 ppm	unspecified
Cunnane (1988)	Zn	218 ppm	unspecified
Edward *et al.* (1984)	Zn	107.4±1.6 ppm	Femur
Hancock *et al.* (1989)	Zn	210±20 ppm*	Femoral Head
Gawlik *et al.* (1982)	Zn	151±22 ppm	Iliac Crest
O'Connor *et al.* (1980)	Zn	155-287 ppm	Vertebrae
Tipton and Shafer (1964)	Zn	210±46 ppm*	unspecified

* concentration for ashed bone

FIGURE 4.1 Photographic Image of Periostitis in Specimen 49 (lateral view)

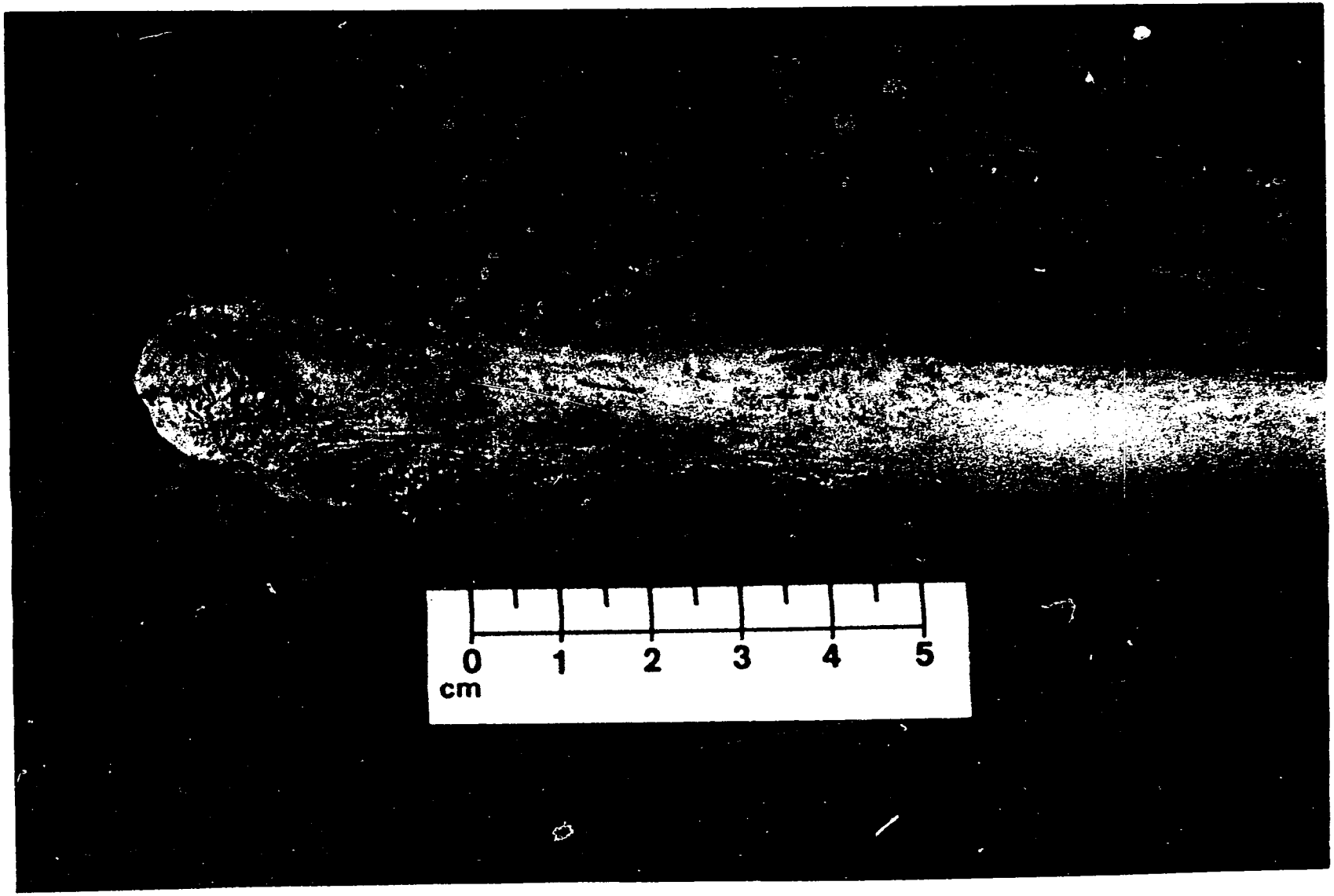

0
1
2
3
4
5
cm

FIGURE 4.2 Photographic Image of a Fracture in Specimen 59 (lateral view)

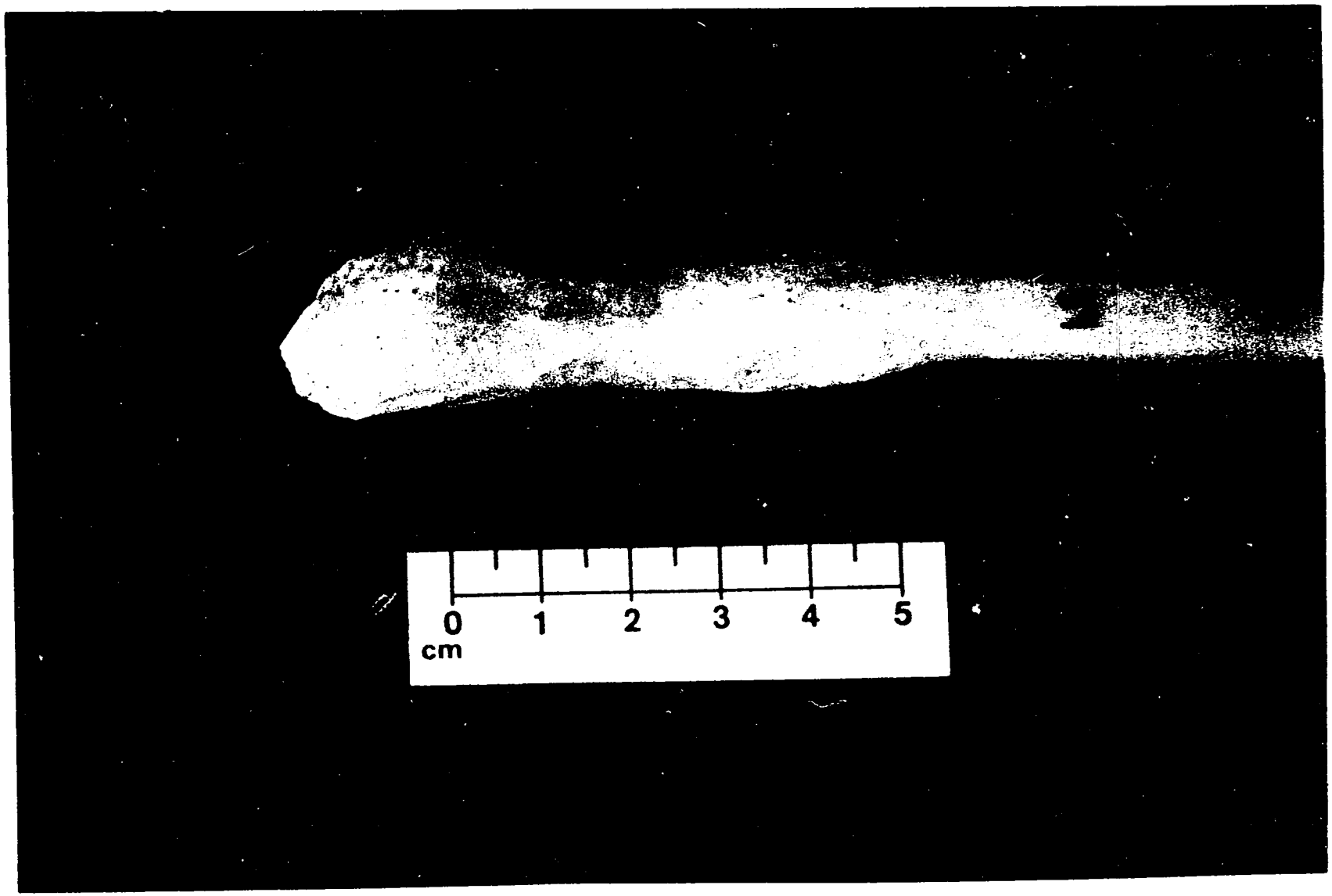

0
1
2
3
4
5
cm

FIGURE 4.3 Photographic Image of Osteomyelitis in Specimen 85 (posterior view)

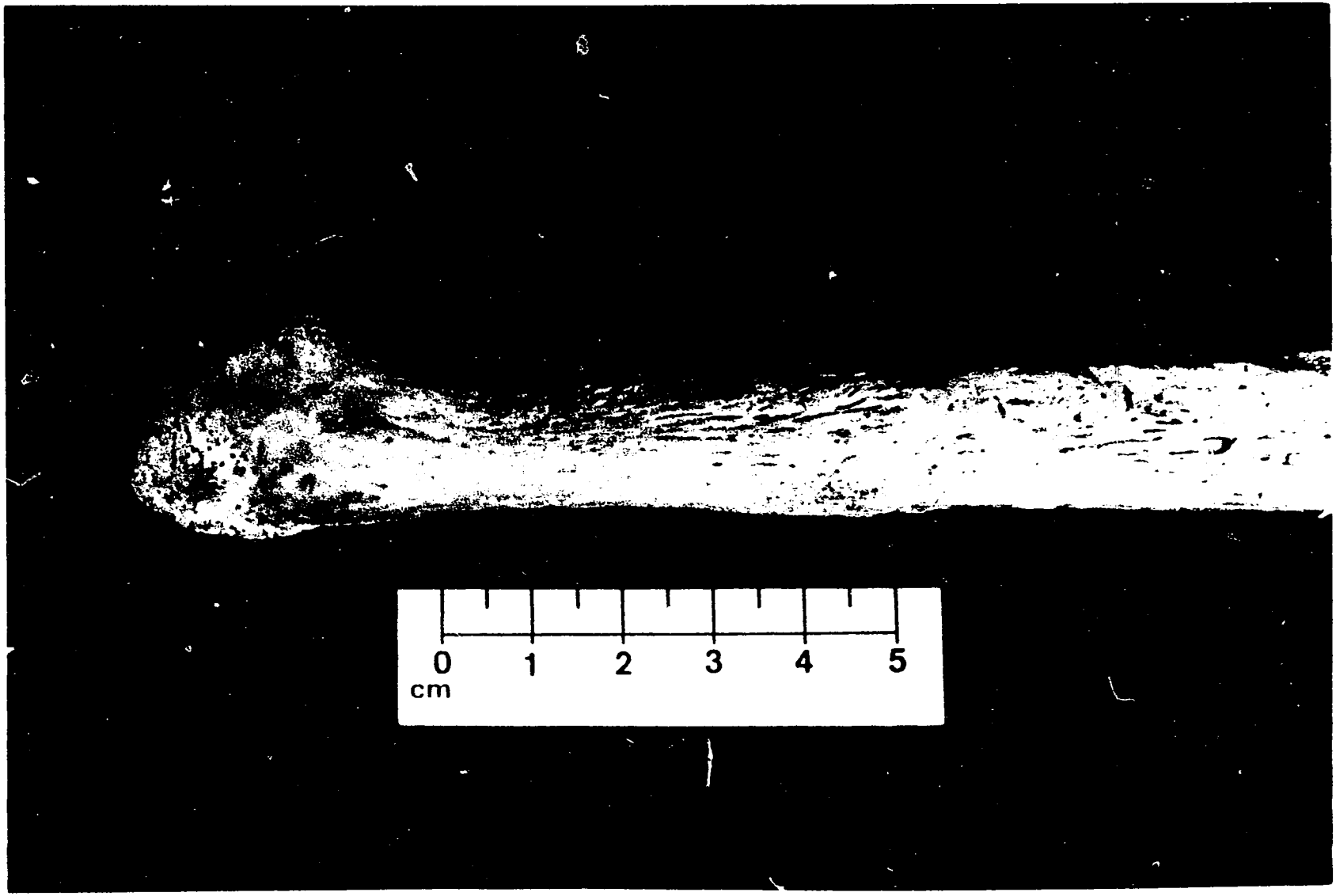

0
1
2
3
4
5
cm

FIGURE 4.4 Photographic Image of Post-paralytic Deformity in Specimen 16 (R) (posterior view)

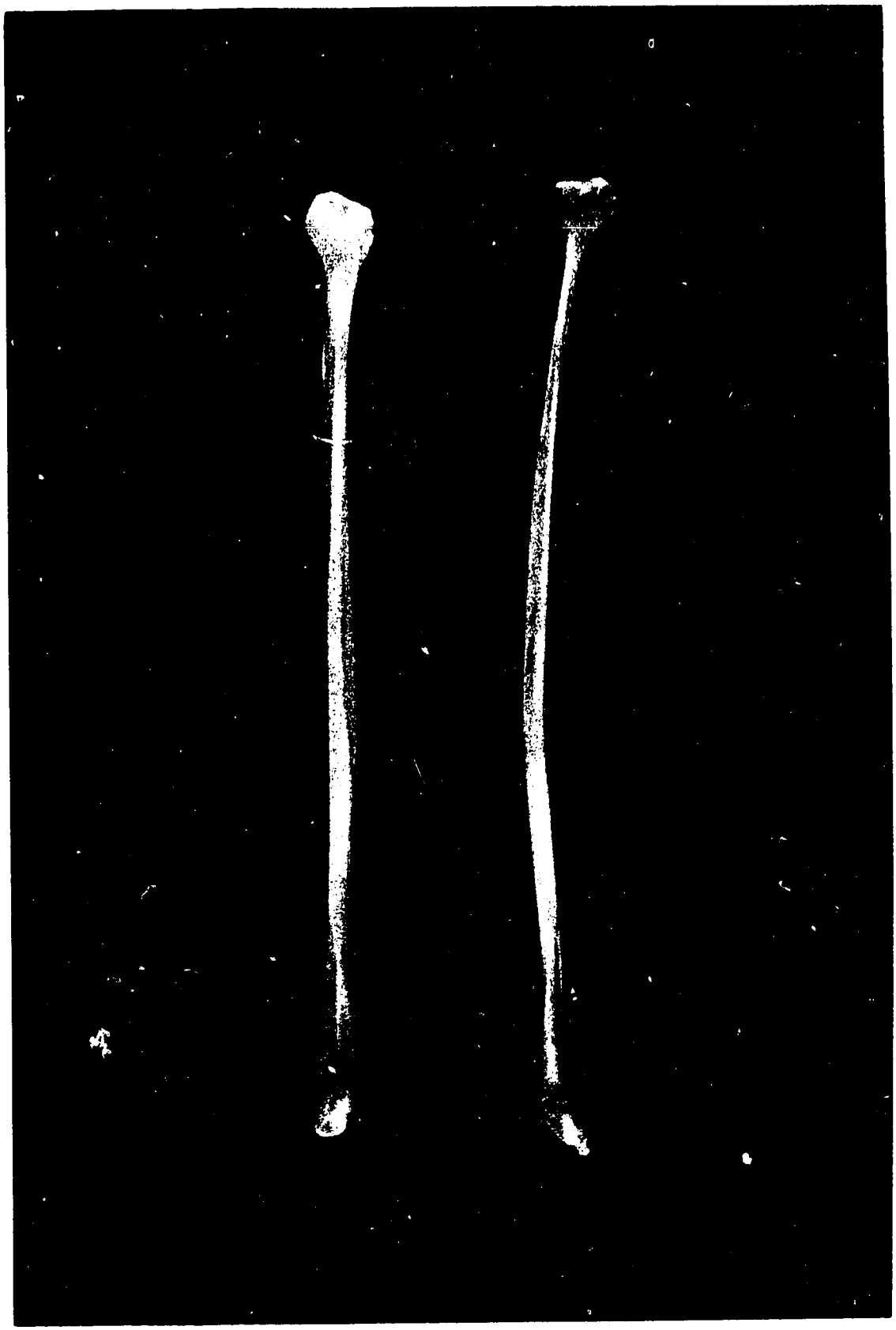


FIGURE 4.5 Zinc Distribution in Two Fibulae: Fracture and Periostitis

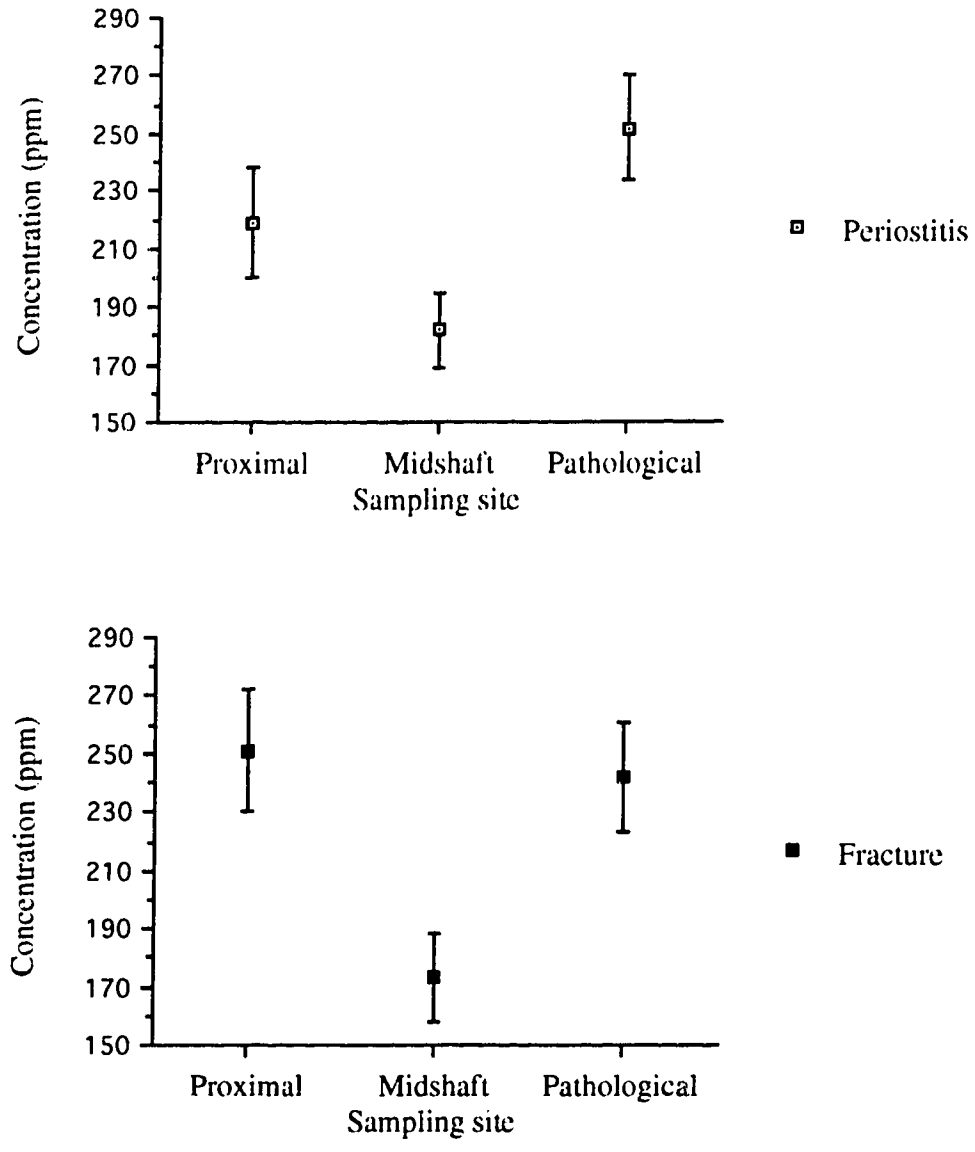


FIGURE 4.6 Similarity in Distribution of Magnesium in Osteomyelitic and Post-paralytic Fibulae

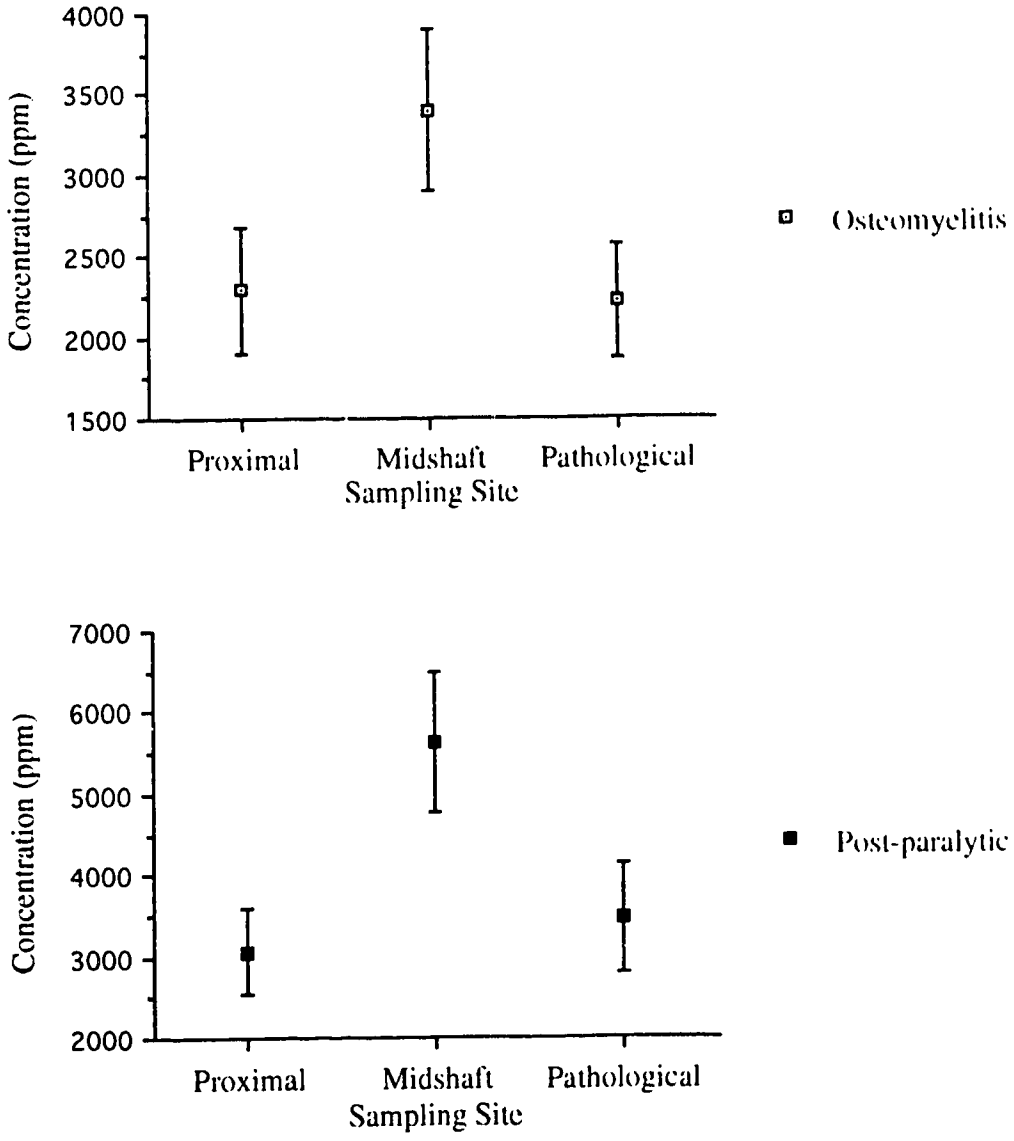


FIGURE 4.7 Calcium Distribution in Two Different Pathological Conditions

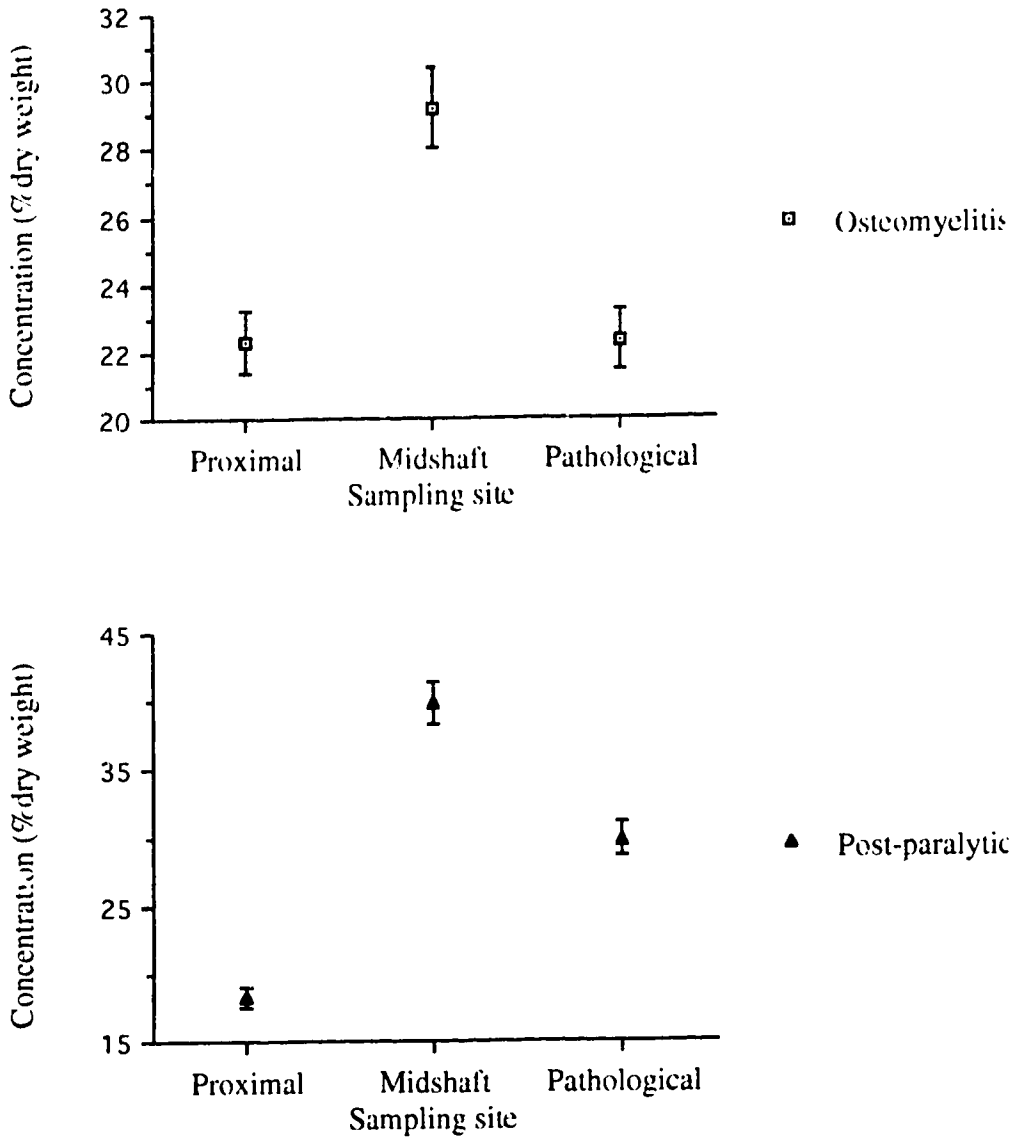


FIGURE 4.8 Elemental Distribution and Osteomyelitis

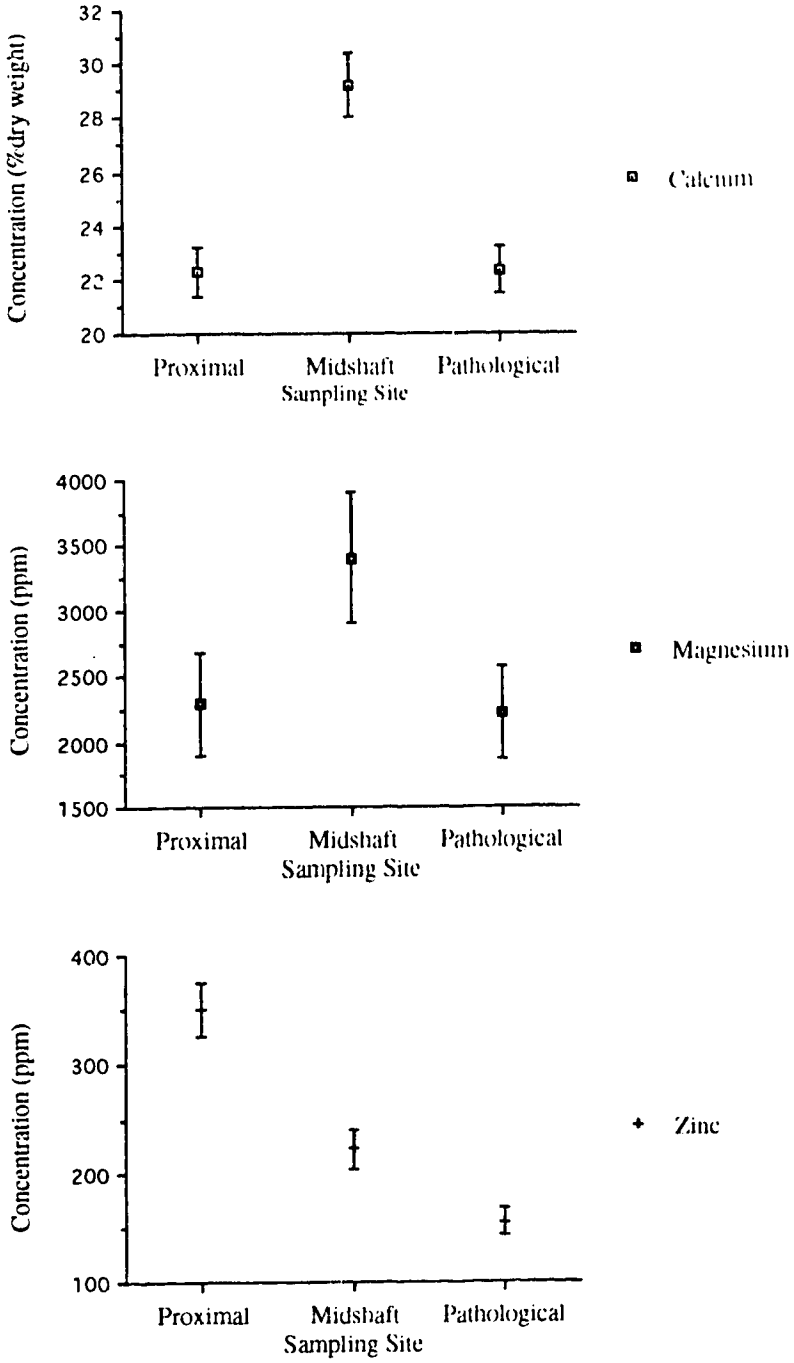


FIGURE 4.9 Calcium, Magnesium and Zinc Distribution in a Fracture

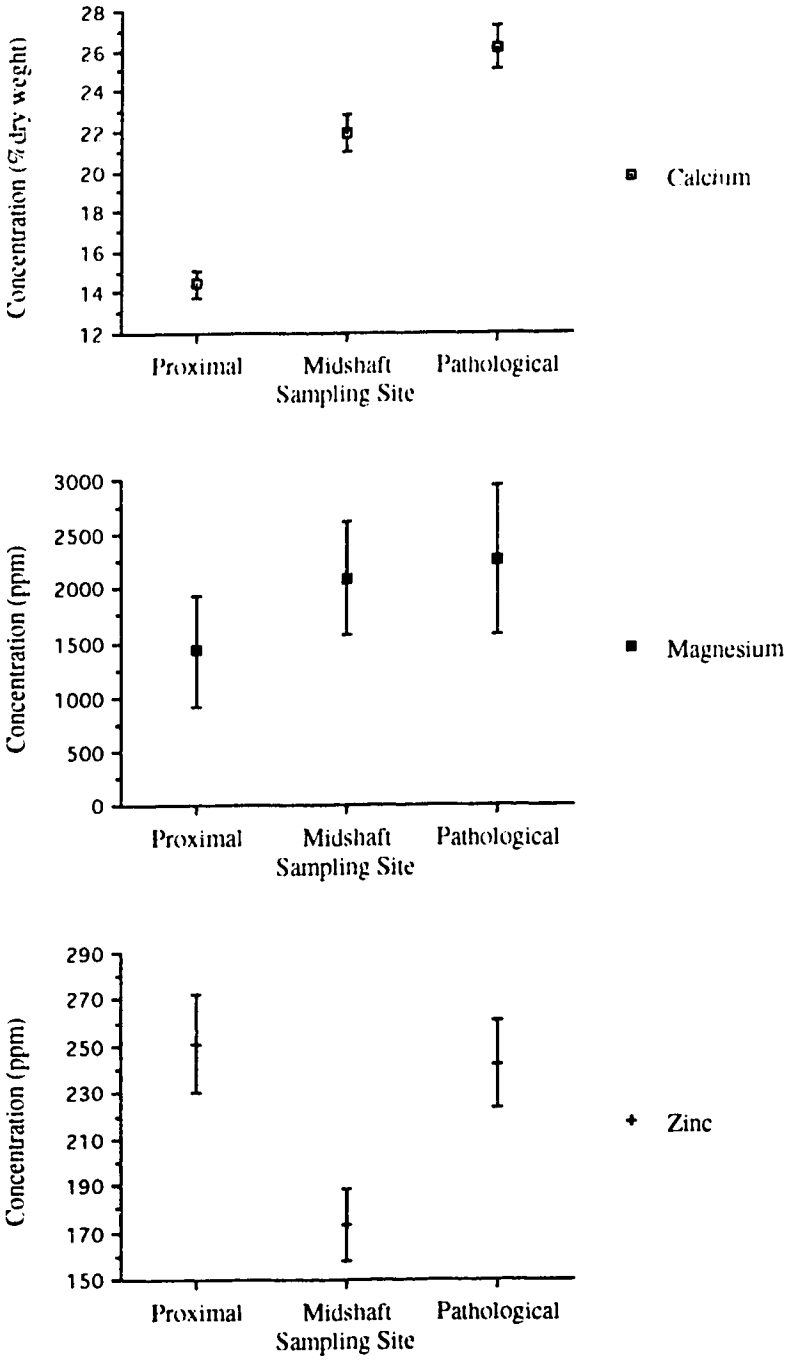


FIGURE 4.10 Calcium, Magnesium and Zinc Distribution in a Fibula with Post-paralytic Deformity

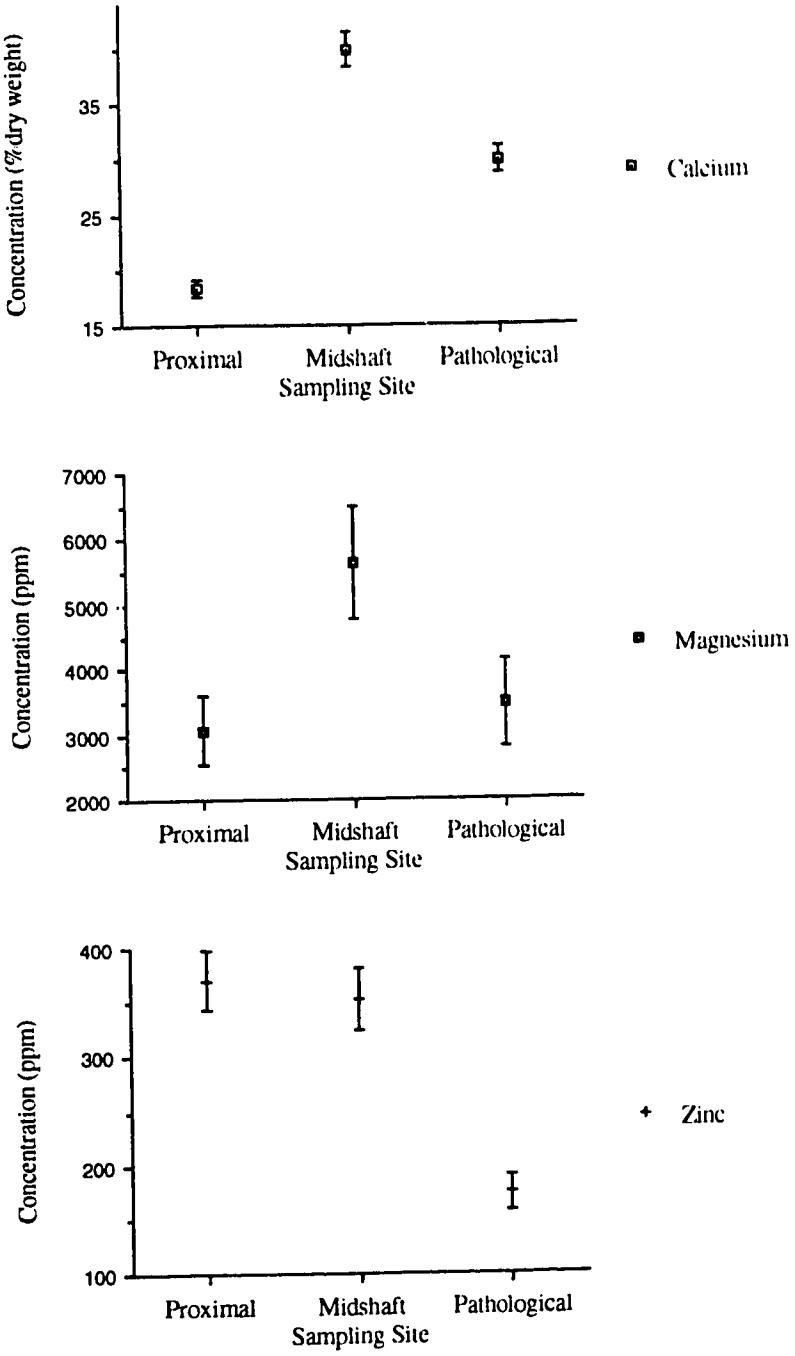


FIGURE 4.11 Calcium, Magnesium and Zinc Distribution in a Fibula with Periostitis

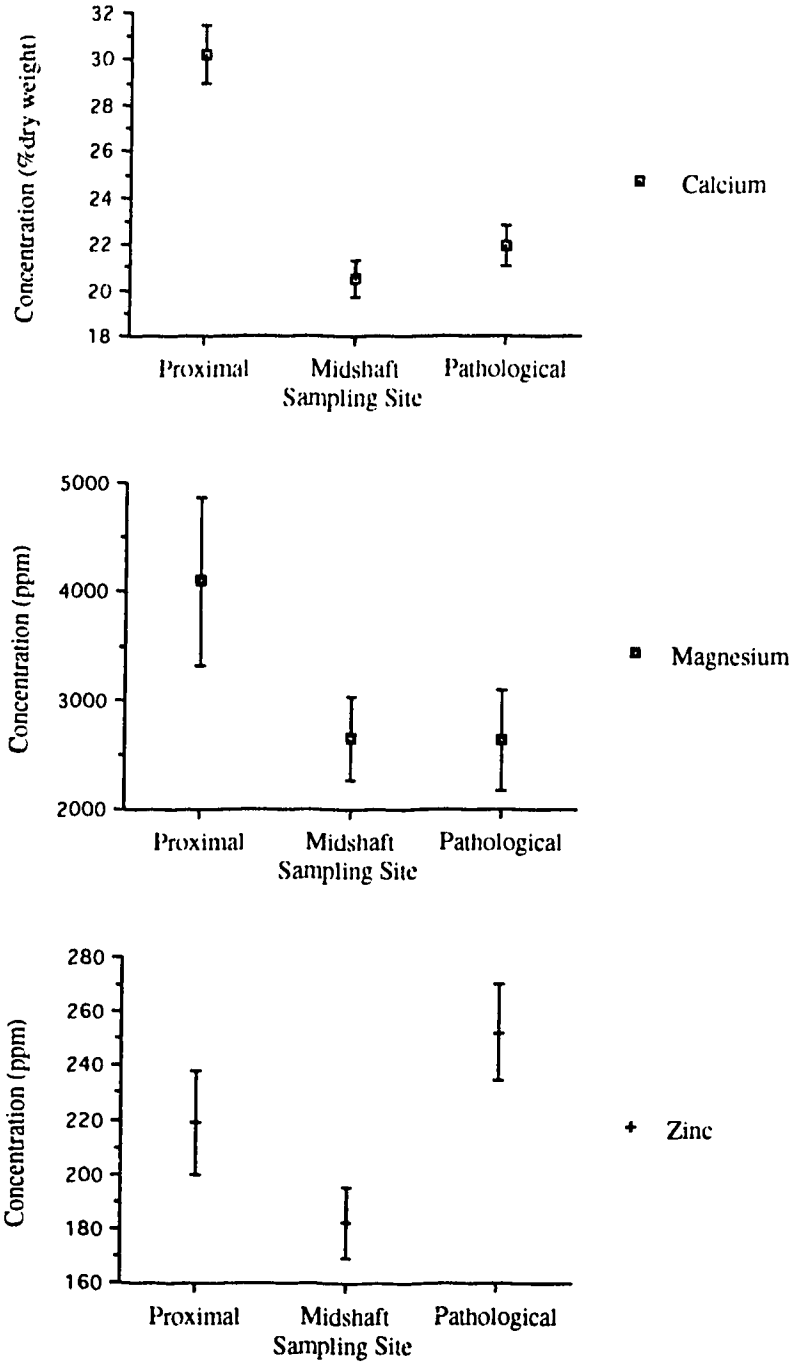
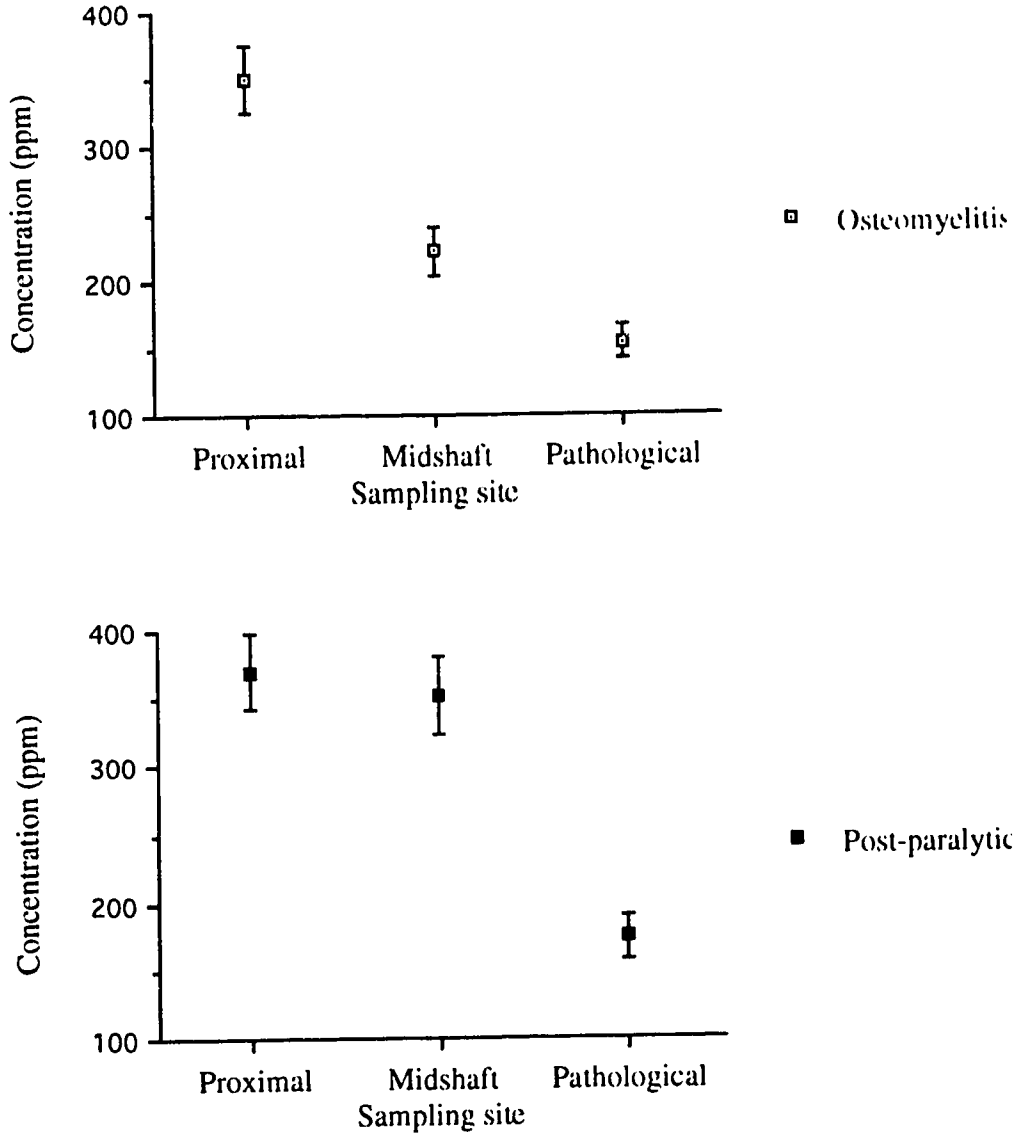


FIGURE 4.12 Elevated Zinc in Fibulae with Osteomyelitis and Post-paralytic Deformity



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CHAPTER FIVE
GENERAL DISCUSSION AND CONCLUSIONS

Trace element analysis in anthropology has become a very popular tool in the examination of ancient human tissue. Three principal applications of trace element analysis have been the reconstruction of ancient diets, investigation of chemical changes to bone due to diagenesis, and establishment of the relationship of human health and disease to trace element concentration. Because human bone preserves better than soft tissue, bone is often the subject of trace element research. While anthropologists have focused mainly on the analysis of archaeological bone, this study showed that modern bone is an appropriate sample choice because the elemental concentrations are representative of diagenetic unaltered bone.

A major hypothesis of this thesis was that elemental concentrations of modern cortical tissue would be similar in samples taken from different locations in one bone. In Chapter three, there was evidence of consistency in elemental concentrations at the proximal, midshaft and distal site for magnesium. Although the results are not conclusive for calcium and zinc, this study has provided some evidence towards determining elemental distribution. Chapter three also shows that the midshaft is the most consistent and representative site of the normal physiological concentration of calcium and magnesium. This is a valuable finding in that future trace element studies will be able to maintain sampling consistency since much of the existing variability in trace element data is related to sample choice (Grynpas *et al.*, 1987).

Although elemental variability was shown to be substantial in some specimens, especially for zinc, several important patterns emerged. In Chapter three, the results contradicted existing information regarding the chemical interaction of calcium and zinc as being antagonistic (Cunnane, 1988). In contrast, there were several examples in Chapter four which substantiated the reported relationship of antagonism between calcium and zinc. In addition, the present research provided conclusive evidence of a synergistic interaction between calcium and magnesium (Aikawa, 1981). The interaction of calcium and magnesium was such that they had the same distribution pattern for almost every sampling site regardless of specimen or pathological condition.

Another interesting result was shown in Chapter four where pathological bone samples had elemental concentrations that were within normal physiological levels. This unexpected result suggests that homeostatic control mechanisms maintain the elemental environment of bone. On the other hand, it could indicate that pathological conditions do not contribute to site specific changes in elemental concentration but affect the entire chemical composition of bone so that sampling the site with the most obvious changes to gross appearance would not reveal an abnormal elemental concentration. This leads to the importance of establishing normal elemental concentrations in bone since these concentrations provide the basis for comparison. A shortcoming of attempting to determine normal elemental concentrations in bone is that factors such as age and sex, physiological state, and health status contribute to elemental variation . For future research, it is recommended that all personal data of the specimens be acquired. To control for all this data, modern bone is recommended because clinical information would be available.

While this thesis has made some contributions to trace element knowledge, it is apparent that more research is required. It is suggested that other elements which are in relative abundance in bone be analyzed as well. This was a major shortcoming of the present work since elements like phosphorous and sodium are biochemically important to bone. In addition, it may be appropriate to use other analytical method especially in the analysis of phoshorous since neutron activation can not detect this element. Furthermore, larger sample sizes would have allowed the application of statistical tests that are important in attempts to quantify the degree of elemental variability .

Other recommendations for future research would include the analysis of the aluminum foil as this may have contributed to some of the results. Aluminum has been reported to cause interference in the detection of selenium and magnesium. In Chapter three, the sample preparation of aluminum foil does not appear to have affected the magnesium results as all concentrations were normal. In addition, a piece of the jeweler's blade could also be included in the analysis as done previously by Link (1991). Another suggestion is to analyze elemental behavior in a large sample containing only one type of pathological condition to elucidate whether elemental distribution is consistent for a particular condition. For example, it would be interesting to analyze fractures to determine if zinc behaves the same with respect to the healing process.

On final analysis it is clear that the relationship between bone and trace elements is far from being understood and that the analysis of elemental distribution in a single bone is the first step to acquiring the necessary information of this relationship. While this thesis only touched upon the surface of such a relationship, it nonetheless provided ample evidence of elemental interactions and interrelationship, elemental responses to disease and normal distribution of calcium, magnesium and zinc in modern fibulae.

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