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Bone Chemistry and Past Behavior: an Overview

Human bone is a complex amalgam of compounds and chemicals—a variety of elements and isotopes—arranged in both organic and inorganic phases. In addition to the major components—calcium, phosphate and water—a number of minor and trace elements are also incorporated during the manufacture of bone tissue. These building materials are obtained by ingestion and the chemical composition of bone is thus in part a reflection of the local environment from which foods are obtained. Both isotopes and trace elements in prehistoric bone have been used to obtain information on human diet and the local environment. These new techniques are outlined here as a means for studying questions such as subsistence, status, and residence. Bone mineralization processes are also discussed as a means for the discovery of paleopathology and disease. Example applications are reviewed to document the potential of such techniques for the reconstruction of the past.

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1. Introduction

Bone is a complex tissue formed of mineralized fibers—"a vascular network lying in a collagen matrix that is filled with calcium phosphate crystals" (Jowsey, 1977, p. 15). This tissue consists of three major components: an inorganic fraction (bone ash), the organic matrix and water. These fractions occur in the approximate proportions of 17:20:15 in fresh bone powder (Engström *et al.*, 1957, p. 28). By dry weight, organic materials constitute about 30% and minerals about 70% of bone (Leblond & Weinstock, 1976, p. 536).

Collagen, a protein, comprises 90% of the organic portion of dry, fat-free bone (McLean & Urist, 1968, p. 24). Other organic materials include various proteins, reticulin, ground substances (protoeoglycans) and water (Table 1). At the histological level, bone comprises primarily multi-cellular units called osteons (Martin & Armelagos, p. 528 this issue). Bone has a cellular matrix which is composed of collagen fibers embedded in a ground substance of muco-polysaccharides (Hancox, 1972). The function of collagen is to provide nucleation centers for initiating the calcification of bone.

Mineralized layers of bone are arranged concentrically around a central vascular canal (Vaughn, 1975). Bound to the protein fibers is the bone mineral in the form of hydroxyapatite crystals, composed of calcium, phosphate, hydroxyl ions and trace elements, to form the crystal lattice (Figure 1). Bone mineral consists of Ca^{++}, PO_4^{-3} and OH^-, carbon dioxide, citrate and bound water, with an admixture of small amounts of other ions such as Na^+, K^+, Mg^{++}, Sr^{++}, Cl^- and F^- among others (Table 2). The formula for the hydroxyapatite mineral resembles $3Ca_3(PO_4)\cdot Ca(OH)_2$ (McLean & Urist, 1968, p. 31). A variety of elements may substitute in this arrangement and are bound to skeletal tissue.

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Table 1 **Components of the organic matrix of cortical bone as percentage of dry weight of organic fraction (after Urist, 1976, pp. 18–19).**

Primary	%	Secondary	%
Collagen	79·2–88·9		
Noncollagenous protein	4·7–9·5	Peptides	0·48–0·53
		Albumin	0·60–1·79
		Lipoprotein	0·30–0·98
		Protein polysaccharides	0·24–1·66
		Phosphoprotein	0·20
		Sialo proteins	0·36
		Glycoproteins, other associated proteins, errors, etc.	4·95–8·35
		Carboxyglutamic acid rich protein	1·00
Insoluble collagenase-resistant material and insoluble material resistant to gelatinization	1·56–4·90		

Post-mortem changes in fossil or archeological bone are also of significance. Diagenetic processes alter the original composition of bone following its deposition in the earth, through activities such as leaching, decomposition and exposure to ground water. These activities serve to enrich, deplete or substitute for original elements in the bone.

Analysis of the content of fossil bone began almost two centuries ago: Morichine, in 1802, published an analysis of the chemical composition of fossil teeth (cited in Cook, 1960). The majority of early studies dealt with either the processes of fossilization (e.g.,

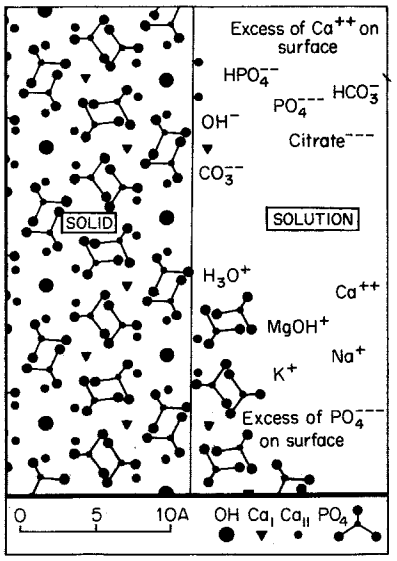


Figure 1. Schematic model of structure and formation of hydroxyapatite in bone. (From Engstrom *et al.*, 1957.)

Table 2 **Components of the mineral portion of cortical bone as percentage of dry weight (after Armstrong & Singer 1965)**

Cations	
Calcium	26·70 ± 0·15
Magnesium	0·436 ± 0·009
Sodium	0·731 ± 0·015
Potassium	0·055 ± 0·0009
Strontium	0·035
Anions	
Phosphorus as PO_4^{3-}	12·47 ± 0·013
Carbon Dioxide as CO_3^{2-}	3·48 ± 0·022
Citric Acid as Cit^{3-}	0·863 ± 0·004
Chloride	0·077 ± 0·004
Fluoride	0·072 ± 0·003

Scheurer-Estner, 1870; Barber, 1939) or the use of the fluorine content of bone for purposes of relative dating (cf., Middleton, 1844; Carnot, 1893; van Bemmelen, 1897; Oakley, 1945). Cook & Heizer were responsible for much of the more recent analyses of archeological bone bone (cf., 1947, 1951, 1952; Cook, 1951). Multi-element analyses were carried out in the hope of obtaining information on the age of the material and past environmental conditions. Their investigations, however, "yielded results of no greater direct value for archaeological dating" because of difficulties "inherent in the nature of the material" (Cook & Heizer, 1953, p. 238). Since the advent of radiocarbon dating, much of the interest in the composition of bone has focused on its suitability as a material for isotopic assay (e.g., Lerman, 1972; Hassan & Ortner, 1977).

Most recently, however, the development of highly accurate and readily available analytical equipment for the analysis of skeletal tissue has permitted the diversification of bone chemistry studies and the examination of a wide variety of elements and isotopes in bone as indicators of past conditions. Such investigations have demonstrated the potential of bone chemistry to provide objective information on diet and subsistence, environment, status, disease and stress, residence and the like.

In the following pages we describe three categories of bone studies: element analysis, isotopic analysis and paleopathological investigations. We consider both the methods and theories for these approaches as well as certain problems that remain.

2. Element Analysis

The majority of living tissue is composed of hydrogen, carbon, nitrogen, oxygen and sulfur—the "bulk elements" (Mertz, 1981, p. 1332). The "macrominerals"—sodium, magnesium, phosphorus, chlorine, potassium and calcium—provide essential components for structural tissues and body fluids. Other naturally-occurring elements in the periodic table are found in low concentrations in living matter. These elements were not easily measured by earlier analytical techniques and were therefore reported in "trace" levels.

Although a variety of elements have been examined as potential indicators of pre-mortem conditions in prehistoric bone, only a few appear to be both reliable and reasonably stable through time. Investigations by Gilbert (1975) suggested that copper, manganese and magnesium did not provide consistent results. Lambert *et al.* (1979) demonstrated that iron, aluminum, manganese and potassium in bone were significantly

altered by post-depositional processes and were unsuitable for the analysis of pre-mortem conditions. Lambert *et al.* (1982) argued that only a few elements were stable through diagenetic activities. Zinc, sodium and strontium did not appear to undergo significant alteration after burial and thus offered more potential for the study of the past. Nelson and Sauer (1984) suggested that zinc and manganese did not permeate bone and thus might be of interest for elemental studies.

Most investigations to date have focused on strontium as a stable indicator of past conditions. Strontium analysis will be discussed in detail here as the best example of elemental studies of bone composition.

Recognition of the importance of strontium in human bone as an indicator of past conditions is largely a result of the testing of nuclear weapons during the 1950s and the subsequent investigations of the products of atomic fallout. ^{90}Sr, a dangerous radioactive isotope produced in nuclear explosions, appeared in relatively high concentrations in milk and other foods following intensive periods of weapons testing. Study of the movement of ^{90}Sr through the atmosphere and into soils, water, plants, and the food chain demonstrated that there was a reduction in the concentration of strontium as it passed through various trophic levels. This information was used by Toots & Voorhies (1965) in an examination of strontium in fossil bone and the reconstruction of the eating habits of certain herbivores. The strontium analysis of prehistoric human remains was begun by A. B. Brown (1973) in a study of several areas for information on diet.

Strontium studies have been applied to past human populations to examine differences in diet due to status (Brown, 1973; Schoeninger, 1979*a*,*b*; Lambert *et al.*, 1979; Hatch & Geidel, 1982), gender (Brown, 1973; Lambert *et al.*, 1979) and changes in the subsistence base (Lambert *et al.*, 1979; Schoeninger, 1982; Sillen, 1981; Price & Kavanagh, 1982). A recent review of strontium analysis and paleodietary research is available and offers a summary of the current state of investigations (Sillen & Kavanagh, 1982).

Brown (1973) analyzed prehistoric burials from the Midwestern U.S., Mesoamerica and the Near East. Variation in strontium levels within these areas reflected differences due to sex, age and status. Schoeninger (1979*a*,*b*) examined a large burial population from the site of Chalcatzingo in Mexico, documenting dietary differences by status position. High status burials, indicated by the presence of jade in the grave, had lower bone strontium levels than individuals accompanied only by pottery or lacking any grave goods. Greater meat consumption by higher status individuals would explain the observed differences.

Sillen (1981) investigated strontium levels in bone samples from the site of Hayonim Cave in Israel. The strontium/calcium ratio effectively discriminated herbivores and carnivores in the Natufian levels at the site. The average ratio for a herbivore (gazelle) was 0·98 and for carnivore (fox) the ratio was 0·63. Human bone samples exhibited an intermediate value averaging 0·77. The omniverous nature of the human diet is clearly reflected in these ratios. The trace element analysis suggests that plants and animals were consumed in roughly equivalent amounts by the inhabitants of Hayonim Cave. In another study, Price & Kavanagh (1982) examined trace elements in human bone samples from prehistoric populations in Wisconsin. A clear increase in the Sr/Ca ratio through time was observed, suggesting that plants increased in importance in the diet from the late Archaic to the Mississippian period.

The chemical and physiological basis of the method is relatively straightforward. Strontium is an alkaline earth metal that is unevenly distributed in trace amounts in the

lithosphere (Odum, 1951). The strontium in ground water is a mixture of strontium levels in different soils in a region and it is this mixture that is taken up by plants (Menzel & Heald, 1959). Although strontium values in plants vary considerably by species, body part and season, strontium/calcium ratios are generally of the same order of magnitude (Bowen & Dymond, 1955).

More than 99% of the strontium in vertebrate animal tissue is found in the mineral component of the bone (Schroeder *et al.*, 1972). Animals discriminate against strontium in favor of calcium in the manufacture of bone tissue (Comar *et al.*, 1957). The ratio of strontium to calcium in the bones of a herbivore, for example, is approximately five times greater than in an equivalent amount of plant tissue. Animals also show distinct variation between species in bone strontium levels. Carnivores exhibit lower concentrations of strontium in bone tissue than herbivores due to the reduced amounts in their diets and to similar metabolic discrimination against strontium in favor of calcium. Omnivores manifest levels of strontium that are intermediate to those in herbivores and carnivores and in proportion to the relative intake of plants and animals in their diets (Figure 2). It is this latter relationship that is the key to the reconstruction of past human diets. Human bone strontium levels should fall somewhere between herbivores and carnivores, depending upon the relative contribution of plant and animal food to the diet.

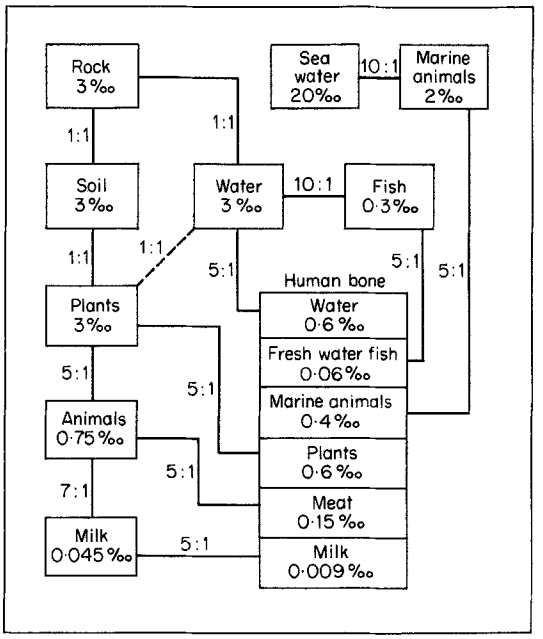


Figure 2. Movement of stable strontium from the environment and diet into human bone. Values are approximate. (After Kavanagh, 1979.)

Marine organisms behave somewhat differently with respect to strontium. Mineral levels are concentrated in oceanic waters and thus higher amounts of strontium show up in marine plants and animals (Odum, 1957; Rosenthal, 1963). Application of bone composition analysis to the study of past populations from coastal Alaska has indicated that strontium levels can be used to distinguish the importance of terrestrial *vs* marine organisms in subsistence (Connor & Slaughter, 1984). Strontium may also be

concentrated in certain organisms such as freshwater shellfish (e.g., Schoeninger & Peebles, 1981).

Procedures for the measurement of strontium levels in bone involve cleaning, ashing, grinding and solution of the sample (Brown & Keyser 1978). Szpunar *et al.* (1978) have suggested an additional step for the complete digestion of the sample that may provide more accurate results than simple dissolution. Strontium concentrations are measured by various means of neutron activation (cf., Wessen *et al.* 1977; Schoeninger, 1979*b*) or spectrometry. A comparison of neutron activation and atomic absorption spectrometry indicated only slight differences in the results of the two methods (Schoeninger, 1979*b* p. 303; Nelson & Sauer, 1984 p. 143). Methods of spectrometry, while destructive, are generally less expensive and more widely available.

Strontium concentrations are reported in parts per million (p.p.m.) per sample. For comparative purposes, however, a ratio of strontium p.p.m to 1000 p.p.m. calcium may be used. The Sr/1000Ca ratio should reflect the amounts of these two elements available to the organism in its dietary intake of strontium and metabolism into the bone structure (Comar *et al.*, 1957).

While strontium studies of bone can offer insight into prehistoric human behavior, the method is still in a developmental stage and should be regarded as experimental. A number of questions remain to be resolved before the technique is fully operational. Specifically, the sources of variation in bone strontium levels must be better understood. In addition to diet, strontium levels are affected by differences between individuals, differences between local environments, and post-mortem alterations of the bone. Each of these sources of variation will be considered below.

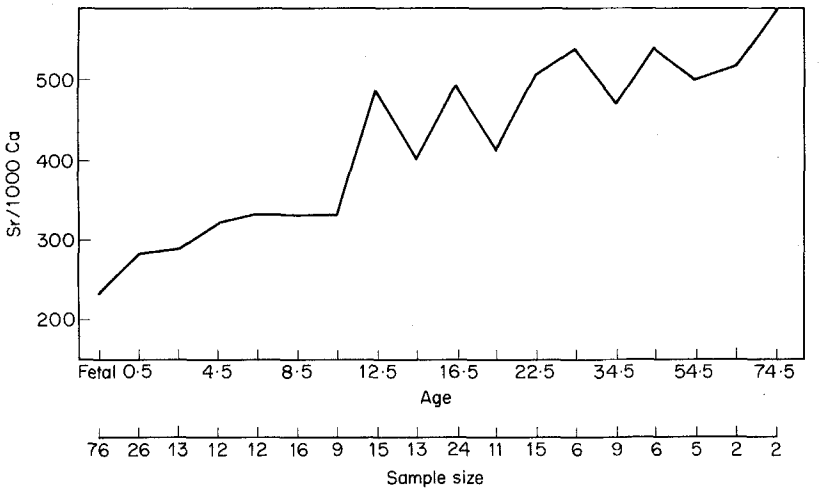
Individual Variation

A number of factors may affect strontium levels in individuals from the same population and contribute to variability. Schoeninger (1979*b*, p. 299) has suggested that a coefficient of variability (c.v.) of approximately 20% may be appropriate for populations ingesting the same diet. (This coefficient is a measure of sample variability independent of the size of the sample mean.) This individual variability is due for the most part to age, gender and metabolic differences.

Age-related variation in bone strontium has been investigated in depth. Comar *et al.* (1955) observed no differences in the strontium levels of different age groups from several species. Study of human subjects (Comar *et al.*, 1957) indicated no changes in strontium levels among persons aged nine to 73 years. Sowden & Stitch (1957), however, reported a slight increase in strontium levels with age in individuals between six and 74 years of age. Lambert *et al.* (1979), using a large sample of prehistoric individuals, found that values for strontium, sodium and zinc decreased in childhood, increased during adolescence, and remained more or less stable between the ages of 20 and 50. Tanaka *et al.* (1981) have reported a gradual increase in strontium levels with age (Figure 3). The average coefficient of variation for the population reported in their study was 19·1%. Individual variation, as measured by standard deviations, was much higher among children and adolescents. Because of the high variability among sub-adults, only adult individuals have been used in the majority of recent paleodietary studies.

Sex-related differences within a population are reported in some studies and not in others. Early tests on modern bone samples indicated no differences in the Sr/1000Ca ratio

Figure 3. Distribution of stable strontium concentrations in bone by age classes. (Data from Tanaka *et al.*, 1981.)



due to sex (Turekian & Kulp, 1956). Gilbert (1975) found strontium values to be slightly higher for males in the prehistoric population at Dickson Mounds, Illinois. Lambert *et al.* (1979) observed statistically significant higher strontium values in males at the Late Woodland site of Ledders in southern Illinois. However, they did not observe this difference at the earlier Middle Woodland site of Gibson in the same area. Tanaka *et al.* (1981) report very little difference between modern males and females of all ages in Japan. However, Snyder *et al.* (1964) in a study of U.S. males and females between the ages of 20 and 59 observed significant differences between the sexes.

Differences in strontium levels between males and females may be related to both physiology and diet. Some differences may be expected between sexes due to strontium loss during pregnancy and lactation (Comar *et al.*, 1957). Atkinson & West (1970) have shown that modern human females can lose up to 2·2% of their bone during 100 days of lactation. There is no reason to assume that strontium does not follow calcium in this depletion process among pregnant and lactating females. This question is considered further by Price (this issue).

Other factors do not appear to play major roles in the determination of strontium levels within a single population. Electron microprobe studies of bone sections indicate that there is no significant patterning of strontium across the tissue structure (Schoeninger, 1979*b*; Vlasak, 1982). Although strontium values do not vary greatly among the different bones of the skeleton, there is significant variability present both between different skeletal parts (Snyder, *et al.*, 1964, p. 183; Tanaka *et al.*, 1981) and within the same bone (Tanaka *et al.* 1981, pp. 605–607). Table 3 reproduces the information presented by Tanaka *et al.* (1981) in which the strontium/calcium ratio of various skeletal parts is reported relative to the average strontium/calcium content of the vertebral column.

Environmental Variation

Toots & Voorhies (1965)—originators of the use of strontium analysis for the reconstruction of diet—caution that comparison of materials from different locales is unreliable unless it can be demonstrated that natural strontium concentrations in the two local environments are similar. Environmental variation in strontium levels can be pronounced over large areas. Schacklette *et al.* (1950) reported high levels of natural strontium in the southwestern U.S. and low levels in the Great Lakes region. Strontium and calcium values generally tend to be consistent within moderately-sized drainage systems and are ultimately dependent upon local lithology, soils and climate (Skougstadt & Horr, 1966). Thus, the amount of strontium deposited in bone is ultimately dependent upon local environmental levels. However, this source of variation in strontium levels can be controlled through the use of a baseline species for comparison with the human remains (Katzenberg, 1984; Price *et al.*, 1985).

Post-mortem Variation

Another major potential source of variation in bone strontium concerns post-mortem physical and chemical changes in bone. Toots & Voorhies (1965) observed that the bones of fossil animals in Wyoming were enriched with iron, manganese and barium. They did not observe comparable changes in levels of strontium. Parker & Toots (1970) examined the question of diagenetic changes in strontium and argued that fossil concentrations were consistent with amounts in bones from modern populations of similar animals from the same area. In 1980, Parker & Toots compared strontium levels in enamel, dentin and bone from fossil *Subhyracodon.* No differences were observed among these diverse materials and they argue that post-mortem changes, if operating, should have created some variation since the harder enamel would have been less subject to contamination.

Contradictory information comes from other studies of fossil and prehistoric remains. Sillen (1981) has argued that changes in strontium and calcium content in bone can be observed through time. Sillen examined both Natufian and Aurignacian remains from Hayonim Cave in Israel. The bones of herbivores and carnivores showed Sr/1000Ca values distinct from the Natufian levels but no significant difference was found from the older Aurignacian levels. Sillen concluded that diet was unlikely to have been responsible for the observed shift in Sr/1000Ca values and that the lack of differentiation in the Aurignacian levels was due to time-dependent changes in the composition of bone.

One of the more detailed studies of the potential effects of diagenesis on strontium levels has been carried out by Lambert *et al.* (1979). They examined a suite of 12 elements in both soil and prehistoric human bone. Comparison of the various elements indicated that the bone had been contaminated by iron, manganese and potassium in the soil. Strontium, zinc, magnesium, calcium, sodium and copper did not occur in the same concentrations in both the soil and bone. The authors concluded that these latter elements are less subject to contamination and thus are more reliable for the study of the pre-depositional condition of the bone.

A recent follow-up to this work has been reported (Lambert *et al.*, 1982). This more recent study focused on the differences between ribs and femurs from prehistoric human burials. The authors reasoned that the porous, trabecular bone of the rib should be more susceptible to contamination than the denser, cortical bone of the femur. Results indicated that the elements generally associated with soil contamination (iron, potassium, aluminum

and manganese) were found in slightly higher proprotions in ribs. levels of calcium and sodium were lower in ribs than in femurs. Elements most closely associated with diet—strontium, zinc and magnesium—were recorded at similar levels in both the ribs and femurs. The results suggested that "diagenetic loss of materials appears not to be as serious a problem as the incorporation of extraneous elements" (Lambert *et al.*, 1982, p. 291), and further:

> Our observation of possible diagenetic depletion of Ca in ribs calls into question the use of the Sr/Ca ratios in archaeological samples. Although the Sr/Ca ratio is of undoubted use in biochemical discussion of modern bone, differential diagenetic effects on the two elements require that their proportions should be discussed separately for archaeological studies (1982, p. 291).

Some aspects of this study require further discussion. The ribs and femurs that were analyzed do not always come from the same individual. Although sample sizes were generally sufficient, some of the observed differences between ribs and femurs may be due to individual variation. More importantly, there are significant differences between these parts of skeleton as noted earlier (Table 3). The data from Tanaka *et al.* (1981, p. 613) indicate that the Sr/1000Ca ratio in bone has a non-uniform distribution with the following order: vertebra<rib<long bone (excluding the femur)<femur<skull. Thus, the lower levels of calcium in the ribs observed by Lambert *et al.* may not be a result of diagenesis, but rather a reflection of natural variation.

Table 3

Estimated skeletal content of stable strontium/calcium ratio in the Japanese adult male. (From Tanaka *et al.*, 1981 p. 610). Relative values are calculated with respect to the vertebral column

Bone	Sr/Ca Ratio (mg/g)	Relative Sr/Ca Ratio
Skull (including mandible and teeth)	0·661	1·80
Chest cage (scapula, clavicle, rib and sternum)	0·422	1·15
Vertebral column	0·367	1·00
Sacrum	0·385	1·05
Innominates	0·404	1·10
Humerus	0·514	1·40
Ulna	0·514	1·40
Radius	0·514	1·40
Hands	0·477	1·30
Femur	0·587	1·60
Patella	0·514	1·40
Tibia	0·514	1·40
Fibula	0·514	1·40
Feet	0·477	1·30

Vlasak (1982) continued the analysis of post-mortem element mobility. Electron microprobe analysis of the cross-sections of prehistoric femurs showed Fe, Al, K and Mn concentrated along the margins of the bone, suggesting that these elements were added to the bone as post-depositional contaminants from the soil (Lambert *et al.*, 1983). Homogeneous distributions of Zn, Sr, Pb and Na throughout the bone section indicated that no post-mortem enrichment or depletion had taken place. Ambiguous results were

found for calcium and magnesium. Calcium showed a homogeneous distribution in section, indicative of stability. Magnesium was concentrated along the edges of the bone, indicating extraneous contamination. These results contradicted another phase of the study, the analysis of elemental distributions through in situ sections of bone and into the surrounding soil. In this phase, high concentrations of calcium were found directly under the bone in the soil and the calcium content of the soil decreased with distance from the bone. Both of these observations suggest that calcium is mobile under post-mortem conditions and is depleted from bone. Magnesium was found in higher concentrations in areas adjacent to the bone, suggesting that it also was depleted. Other elements generally conformed to the observations reported in the electron microprobe study.

In sum, while rib and femur comparisons and the electron microprobe analysis demonstrate no significant post-mortem changes in the calcium and strontium content of bone, evidence from the bone/soil distribution study suggests that calcium is depleted through time. Clearly, as bone decays its constituent elements will gradually move into the surrounding soil matrix while other elements replace the bone material. In essence, however, it appears that as long as the bone is not completely demineralized, strontium levels should be reflective of pre-mortem conditions (cf., Lambert *et al.*, this issue).

Nelson *et al.* (1983) have taken a different approach to the question of diagenesis. Post-mortem alteration was studied through the comparison of modern and prehistoric material from the same area. Marine seal bone and terrestrial reindeer bone from western Greenland were analyzed. While modern animals show large differences in strontium concentrations between seals and reindeer, the prehistoric values from these two classes of animals overlap completely. Isotopic analysis of this material suggests that as much as 80% of the stable strontium may have been added post-mortem to the bone in this specific depositional context.

A number of essential questions regarding potential changes in the proportions of both strontium and calcium during the process of diagenesis remain to be answered. Existing evidence would appear to indicate that pre-mortem values generally do not change dramatically except in unusual depositional contexts. Measurement of diagenetic loss and modification is critical to the advancement of trace element analysis for paleonutritional information. Certainly the stability of strontium and calcium concentrations is a function of the length and situation of burial and additional information is needed on the variation introduced in a variety of such contexts.

Summary

Strontium analysis of prehistoric human bone offers a powerful new technique for the study of past diet, status, environment and other conditions. Comparison of prehistoric human bone with herbivores and carnivores can indicate the relative importance of plants and animals in the diet—information that has not previously been available. Comparison of strontium levels in sex, age or status groups within a population can provide information on variation in diets along such divisions. Information on weaning age (Sillen & Smith, 1984), environmental levels of strontium, and many other aspects of the past can also be pursued.

Several major obstacles remain however. Problems with strontium analysis continue to arise from individual variation, environmental differences and diagenetic changes. Some of

changes. Some of these sources of variability can be controlled. Differences due to age and sex can be eliminated by the use of adult-only samples of one sex. Variation among the various parts of the skeleton can be reduced through the use of a particular bone for analyses. Individual variation is generally not large, however, and can be reduced through the use of mean estimates from large sample sizes. Single or a few analyses of the trace element content for a population are insufficient because of the variability that is present.

Environmental variation in strontium levels among different areas can be controlled through the measurement of a baseline species present at the sites to be compared. For example, white-tailed deer (*Odocoileus virginianus*) is found at virtually all archeological sites in the Eastern United States that contain bone. Strontium levels in this herbivore can be used as a baseline for the variation in natural strontium levels and the difference in strontium values between humans and deer used as a measure of comparability between different sites (Price *et al.*, 1985). Such a procedure eliminates the variation in strontium levels introduced by environmental differences.

Variation in strontium levels due to post-mortem changes in bone is more difficult to control because it is less well understood at present. Evidence suggests that as long as the mineral portion of the bone is intact that strontium levels should reflect pre-depositional conditions. The specific effects of leaching and exposure to ground water will raise variability in elemental levels on a local level. Through time the general trend of such alteration appears to be a homogenization of values (Sillen, 1981). Study of the effects of diagenesis on bone mineral levels is the most critical aspect of bone chemistry analysis at the present time.

Strontium has been the focus of investigations because of a long history of study of its behavior in the food chain during the period of the atmospheric testing of nuclear weapons. In addition to strontium, a number of other elements may also provide information on past diet or behavior. As more is learned regarding the physiology and stability of other elements, a new suite of indicators of prehistoric conditions may become available. Elias *et al.* (1982) suggest that barium may be a better indicator of trophic position than strontium. Zinc and magnesium have also been suggested as useful candidates for indicators of past diet (Lambert *et al.*, 1979; Beck, this issue; Hatch & Geidel, this issue). Trace element analysis, along with isotopic studies, as a tool in the study of the past is in its infancy.

3. Isotopic Analyses

Carbon and nitrogen, two of the "bulk elements" (Mertz, 1981, p. 1332) found in living tissue, also record dietary information. The concentration of each of these *elements* in an animal's tissues (specifically in bone collagen) is under strict metabolic control, but the ratio of the stable isotopes of each of these elements ($^{13}C/^{12}C$ and $^{15}N/^{14}N$) reflects the same stable isotope ratio as the animal's diet. This conclusion is supported by results from laboratory studies in which animals were raised on diets of known isotopic composition (DeNiro & Epstein, 1979, 1981; Bender *et al.*, 1981; Macko *et al.*, 1982; Tieszen *et al.*, 1983) as well as from field studies (Tieszen *et al.*, 1979; Van der Merwe & Vogel, 1983). The levels of ^{13}C and ^{15}N in bone collagen are low and the differences in ^{13}C and ^{15}N concentration between biogenic materials are small (see discussion in Van der Merwe & Vogel, 1983). Thus, the stable isotope ratios in the sample of bone collagen are expressed

relative to the stable isotope ratios in a standard and the expression is represented as a delta value in parts per thousand (‰)

$$\delta^{15}N = \left[\frac{(^{15}N/^{14}N) \text{ sample}}{(^{15}N/^{14}N) \text{ standard}} - 1 \right] \times 1000‰.$$

$$\delta^{13}C = \left[\frac{(^{13}C/^{12}C) \text{ sample}}{(^{13}C/^{12}C) \text{ standard}} - 1 \right] \times 1000‰.$$

The standards are Pee Dee Belemnite (PDB) carbonate for $\delta^{13}C$ values and atmospheric nitrogen (AIR) for $\delta^{15}N$ values. The delta value for both standards is, by definition, 0‰ The PDB carbonate is more positive (is more enriched in ^{13}C) than most biogenic materials. Consequently, most bone collagen $\delta^{13}C$ values are negative. A $\delta^{13}C$ value of $-10‰$ means that the sample contains 10 parts per thousand (1%) less ^{13}C than the standard carbonate. Conversely, the nitrogen standard, AIR, has less ^{15}N than most biogenic materials. Hence, bone collagen $\delta^{15}N$ values are positive. A nitrogen delta value of $+10‰$ means that the sample has 10 parts per thousand (1%) more ^{15}N than atmospheric nitrogen.

Although both the experimental data and the results from field studies indicate that diet carbon and nitrogen delta values determine bone collagen delta values, the magnitude of the relationship between the two ratios is unclear (Bumsted, 1983). DeNiro & Epstein (1978) found that bone collagen $\delta^{13}C$ values of two sets of laboratory raised mice were 3·8 and 2·8‰ less negative than were the values in the animals' diet. However, the difference between bone collagen and diet $\delta^{13}C$ values appears to be closer to 5‰ in the large ungulates studied by Vogel (1978) and by Tieszen (pers. comm.). In the case of nitrogen, DeNiro & Epstein (1981) report an enrichment of $+3·0 \pm 2·6‰$ ($n = 13$) and Minagawa & Wada (in press) report a range of enrichment of +2·9 to 4·9‰ of animals' tissues relative to diet based on a wide variety of laboratory raised animals. Inferences from field studies in which various animal tissues were analyzed suggest an enrichment around +3‰ but with a range of at least ± 2‰ (Wada, 1980; Macko, *et al.* 1982; Schoeninger & DeNiro, 1984). Bone collagen appears to be enriched about 3‰ relative to diet (DeNiro & Epstein, 1981, Schoeninger & DeNiro, 1984).

The reason that the delta values of bone collagen differ from those in the diet has to do with fractionation, the preferred incorporation or exclusion of one versus the other isotope in the products of a chemical reaction, or metabolic bias (cf. Bumsted, this issue), different sources of carbon for different tissues. Both carbon and nitrogen have small masses, thus the difference in mass between their stable isotopes (^{12}C *vs* ^{13}C and ^{14}N *vs* ^{15}N) is relatively large. The lighter isotopes react more quickly in chemical reactions than do the heavier isotopes. Therefore, it is reasonable to expect that, as a result of bone collagen synthesis, the collagen would contain a different proportion of either the heavier isotopes (^{13}C and ^{15}N) or the lighter isotopes (^{12}C and ^{14}N) than exists in the products excreted (urea, CO_2, N_2, etc.). The observation that bone collagen has $\delta^{13}C$ values less negative than diet, and $\delta^{15}N$ values more positive than diet (DeNiro & Epstein, 1978; Bender *et al.*, 1981; Tieszen *et al.*, 1983), means that a greater proportion of the heavier isotopes (^{13}C and ^{15}N) is retained in collagen relative to the products excreted.

Thus, although specifics remain to be worked out, a relationship has been established between diet and bone collagen stable isotope ratios for the elements carbon and nitrogen. The second major key to demonstrating the usefulness of stable isotope ratios in bone collagen for diet reconstruction has been the observation that groups of potential food products have consistent differences in their carbon and nitrogen stable isotope comopsition.

Nitrogen

Within the biogenic environment, organisms can be grouped into three major divisions based on the stable nitrogen isotope ratio of their tissues. The first of these divisions comprises all plants that fix atmospheric nitrogen (N_2-fixing) and the animals that feed on those plants. Since atmospheric nitrogen has a defined $\delta^{15}N$ value of 0‰, and little isotopic fractionation occurs during N_2-fixation, plants that fix nitrogen from the air should have ^{15}N values near zero. This should be true whether the plants are terrestrial or marine. The mean of published delta values for nitrogen fixing terrestrial plants is close to +1‰ with a standard deviation of ±2‰, that for nitrogen fixing marine plants is 0‰ with a standard deviation of ±3‰ (Schoeninger & DeNiro, 1984). There are no published data on the $\delta^{15}N$ values of terrestrial animals that feed on nitrogen fixing plants, but there are a few data from the marine system. Nitrogen fixing blue-green algae and zooplankton feeding on them have lower $\delta^{15}N$ values than phytoplankton which do not fix nitrogen and their associated zooplankton (Wada & Hattori, 1976; Wada, 1980). Fish feeding in coral reefs, areas noted for a large amount of nitrogen fixation by blue green algae (Stewart, 1978), have $\delta^{15}N$ values that are much lower than fish of equivalent trophic position in the open ocean (Schoeninger & DeNiro, 1984).

The second major division includes the remaining majority of terrestrial plants and those animals which feed on them. The major sources of inorganic nitrogen available to terrestrial plants are soil nitrates and ammonium ions (Sweeney *et al.*, 1978). Uptake of nitrogen by plants occurs with small amounts of isotopic fractionation (Hoering & Ford, 1960; Delwiche & Steyn, 1970; and Wada *et al.*, 1975), thus most modern non-N_2-fixing terrestrial plants have $\delta^{15}N$ values between 0 and +6‰ (Parwel *et al.* 1957; Sweeney *et al.*, 1978; DeNiro & Hastorf, in press). Results from analyses of prehistoric plants from Peru suggest that this range may be low (DeNiro & Hastorf, in press). If the modern plants were fertilized with nitrates produced from atmospheric nitrogen they would have lower than normal $\delta^{15}N$ values (Freyer & Aly, 1974). As discussed more thoroughly below, animals that feed on terrestrial plants have bone collagen $\delta^{15}N$ values that are enriched in ^{15}N relative to the plants, yet, reflect dietary dependence on non-N_2-fixing plants.

The third major division includes all marine organisms excluding those in trophic systems with N_2-fixing blue-green algae at the base of the food chain. In the ocean the process of denitrification and, consequently, the nitrogen isotopic composition of the resulting nitrates, is different than in the terrestrial system. Although the magnitude varies geographically, denitrification in oceanic depths occurs with a relatively large fractionation factor (Cline & Kaplan, 1975; Wada *et al.*, 1975). Thus, nitrates utilized by plankton at the base of the food chain are enriched in ^{15}N relative to nitrates utilized by terrestrial plants. This ^{15}N enrichment is carried up the food chain causing marine phytoplankton and fish to have $\delta^{15}N$ values more positive than those of terrestrial plants and animals (Miyake & Wada, 1967; Minagawa & Wada, in press; Schoeninger & DeNiro, 1984).

Freshwater systems may constitute a fourth division but they are not well understood. Denitrifying bacteria occur mainly in areas with low oxygen levels (e.g., rice paddies and stagnant ponds), yet, in completely anoxic conditions denitrification can occur without isotopic fractionation (Sweeney *et al.*, 1978). Complicating the issue, it appears that animals living in freshwater feed on material originating both in water and on land (Rau, 1980), therefore, their body nitrogen would come from both systems.

It follows from the above discussion that bone collagen $\delta^{15}N$ values reflect diet $\delta^{15}N$ values and that $\delta^{15}N$ values are different in three major groups of potential food sources: nitrogen fixing plants, terrestrial food products not based on N_2-fixation, and marine food products not based on N_2-fixation. Thus, it should be possible to distinguish between human groups whose diets are based mainly on legumes (N_2-fixing terrestrial plants), marine, or terrestrial food products. No thorough study of the effects of a diet of legumes has yet been completed and this remains an area for study. On the other hand, the marine/terrestrial aspect has been investigated. Within recent groups of coastal Eskimos, Haida and Tlingit, all of whom depended on marine products for a majority of their food, the bone collagen $\delta^{15}N$ values are, on average, 10% more positive than those of Peublo agriculturalists from the U.S. southwest (Schoeninger *et al.*, 1983). The same distinction in $\delta^{15}N$ values was apparent between prehistoric human groups identified by thorough midden analyses as agriculturalists or dependent on marine molluscs, fish, and mammals (Schoeninger *et al.*, 1983). Applications to the archeological record are discussed below.

In addition to the marine/terrestrial/leguminous plants division, there is some evidence that $\delta^{15}N$ values become more positive as nitrogen is transferred along the continuum from plants, to herbivores, to primary carnivores, and finally to secondary carnivores. As mentioned previously, laboratory experiments indicate that $\delta^{15}N$ values of an animal's bone collagen is on average about 3‰ more positive than that of the animal's diet (DeNiro & Epstein, 1981). Marine and freshwater zooplankton appear to have $\delta^{15}N$ values that are, on average, 3‰ more positive than associated phytoplankton (Miyake & Wada, 1967; Wada and Hattori, 1976; Pang & Nriagu, 1977; Minagawa and Wada, in press). Analyses of a large number of primary and secondary vertebrate marine carnivores support the experimental results (Schoeninger & DeNiro, 1984) although analyses of terrestrial vertebrate hervibores and carnivores are equivocal (Schoeninger, this issue). Hypothetically this could be applied in the analysis of prehistoric human diet; it may be possible to estimate the amount of meat in the diet on the basis of the $\delta^{15}N$ values of bone collagen.

Carbon

Potential foods can also be divided into several groups based on their $^{13}C/^{12}C$ ratios. Within the terrestrial system the $\delta^{13}C$ values of many grasses are distinct from non-grasses because the two plant types utilize different photosynthetic pathways and fractionation occurs during photosynthesis (Park & Epstein, 1961). Many grasses, and some other plants, metabolize carbon dioxide by conversion to a four-carbon compound in the first step. Thus, these plants (which include maize, sorghum, millet, amaranth, among others) are referred to as C_4 plants. Plants in the second major group (including bushes, leafy plants, and some non-tropical grasses such as barley and wheat) are referred to as C_3 because the first compound formed during photosynthesis contains three carbon atoms. C_4 plants appear to utilize CO_2 more efficiently than do C_3 plants and incorporate relatively

more of the available ^{13}C (Farquhar *et al.*, 1982). The $\delta^{13}C$ values of C_4 plants are, therefore, less depleted in ^{13}C or, in other words, are less negative than the values for C_3 plants (Bender, 1971).

The mean $\delta^{13}C$ value for C_4 plants is close to $-12{\cdot}5‰$, that for C_3 plants is close to $-27‰$ (Smith & Epstein, 1971; Vogel *et al.*, 1978; Deines, 1980). The bone collagen $\delta^{13}C$ values of animals feeding on either C_3 or C_4 plants reflect the differences in $\delta^{13}C$ values of the plants (Vogel & Van der Merwe, 1977; Vogel, 1978; Bender *et al.*, 1981). There is a third photosynthetic pathway called Crassulacean acid metabolism (CAM) in which plants fix CO_2 using some combination of both C_3 and C_4 pathways (Osmond, 1978). Even so, the resulting distribution of $\delta^{13}C$ values for such plants is similar to that for C_4 plants (Bender *et al.*, 1973). In theory, this could cause some problem in attempting to reconstruct diet on the basis of $\delta^{13}C$ values in bone collagen. However, most CAM plants are cacti and succulents which formed dietary components in only a few human groups (e.g., Southwestern U.S. hunter/gatherers). Therefore, the potential use of CAM plants by human groups is not considered to be a major difficulty in using this method for diet reconstruction is most areas.

There also appears to be a difference in $\delta^{13}C$ values between marine and terrestrial organisms that might serve as human food. The $7‰$ difference between seawater bicarbonate and atmospheric CO_2 (Craig, 1953) should be reflected in the tissues of organisms feeding exclusively in one environment (Chisholm *et al.*, 1982). There is, however, a great deal of overlap in $\delta^{13}C$ values between organisms in the two environments (Schoeninger & DeNiro, 1984). Results from the analyses of over 100 marine and terrestrial vertebrates suggest that although there is a $7‰$ difference of means, the overlap is large enough that reconstruction of this aspect of diet on the basis of $\delta^{13}C$ values can be accomplished only in certain geographic areas. Mixed feeding on C_3 and C_4 plants or feeding on animals that consumed a mixed diet of C_3 and C_4 plants would result in bone collagen $\delta^{13}C$ values similar to those resulting from a diet based on marine foods (Chisholm *et al.*, 1982). Reconstructions of human diets using bone collagen $\delta^{13}C$ values in geographic areas where C_4 plants are not present have proven to indicate accurately the marine component in the diet (Tauber, 1981; Chisholm, *et al.*, 1982; Schoeninger *et al.*, 1983).

Finally, there have been suggestions that there is a trophic level effect on $\delta^{13}C$ values of organisms (Fry *et al.*, 1983; Rau *et al.*, in press; McConnaughy & McRoy, 1979). Among organisms within single trophic systems there appears to be an enrichment on the order of 1% in $\delta^{13}C$ values between each trophic level through the continuum of primary producers, herbivores, primary carnivores, and then to secondary carnivores. A recent study of bone collagen $\delta^{13}C$ values from modern fish, birds, and mammals indicates that the proposed 1% enrichment per trophic level is obscured when the animals orginate from multiple food webs (Schoeninger & DeNiro, 1984).

In sum, $\delta^{13}C$ values of bone collagen should prove useful indicators of dependence on C_3 *vs* C_4 grasses when the use of marine foods can be discounted. Further, these values are indicative of dietary dependence on marine versus terrestrial foods when C_4 grasses are not present in the environment.

Applications

Before dietary reconstruction in prehistoric human populations can be attempted, certain potential sources of variability must be evaluated. Variation in stable carbon and nitrogen

delta values of bone collagen among animals raised on a single diet is less than 1% and the variation between bones of a single individual is also less than 1% (DeNiro & Schoeninger, 1983). Thus, choice of bone for analysis will not adversely affect the final result. Further, individual metabolic differences will probabily not pose a problem although not enough is yet known about the influence of age, lactation and seasonality of diet to be confident in the case of humans that one individual's bone collagen $\delta^{15}N$ and $\delta^{13}C$ values are representative of all other individuals in that person's social group.

Diagenetic alteration of carbon and nitrogen stable istope ratios in bone collagen does not occur in most cases. Experimental degradation of collagen through heating (DeNiro *et al.*, in press) and flushing with water (DeNiro & Schoeninger, in prep.) has not produced any significant shift in delta values as long as the proportion of collagen in bone is 5% or greater. The same is true for bone powder that has been immersed in a microbial bath for two years (DeNiro & Schoeninger, in prep.) although in this latter case no change in percent collagen occurred. Hare (pers. comm.) has noted that not all amino acids in collagen have the same $\delta^{13}C$ and $\delta^{15}N$ values. Since experimental degradation of collagen does not result in alteration of nitrogen or carbon delta values until the large majority of collagen is removed, it is likely that alteration of portions of collagen strands does not occur. Instead, it seems that complete strands are removed.

The same does not appear to be true consistently for the other form in which carbon is bound in bone. The $\delta^{13}C$ value of the carbon attached to the apatite (mineral) portion in bone is also reflective of the diet $\delta^{13}C$ (DeNiro & Epstein, 1978). In some cases prehistoric and fossil bone apatite retains carbon with the isotopic ratio present during the life of the individual (Sullivan & Krueger, 1981) but this is not always true (Schoeninger & DeNiro, 1982*a*). Until an independent means of identifying altered bone is developed, the carbon in bone apatite cannot be accepted as representative of the original biogenic carbon (Hassan, 1975).

Another potential source of variation in carbon isotope ratios is due to latitudinal, hemispheric, and polar discrepancies in the $\delta^{13}C$ values of plankton (Rau *et al.*, 1982) that form the base of the food chain for marine organisms. Van der Merwe & Vogel (1983) recommend treatment of each marine environment separately in stable carbon isotope studies. For example, their average value for fish and shellfish recovered off the west coast of South Africa is about $-16‰$ (Van der Merwe & Vogel, 1983). This compares well with the average $\delta^{13}C$ values of $-16‰$ in bone collagen of six birds collected on the Falkland Islands, but in the same study the average value in bone collagen for ten fish collected off the California coast was $-12{\cdot}5‰$ (Schoeninger & DeNiro, 1984).

Archeological Applications

The use of carbon and nitrogen stable isotope ratios in the reconstruction of human diet has been limited to date and the majority of studies thus far have involved only carbon. A paper by Van der Merwe & Vogel, presented at the 1976 meeting of the Society for American Archaeology, on maize introduction into North America stimulated many of the archeological applications of ^{13}C values in monitoring the spread of agriculture in the New World (Vogel & Van der Merwe, 1977; Van der Merwe & Vogel, 1978; Van der Merwe, 1982). These studies evolved from earlier investigations of problems with radiocarbon dating concerning isotopic fractionation (Bender, 1968; Vogel & Lerman, 1969; Lerman, 1972; Andersen & Levi, 1952; Wickham, 1952).

DeNiro & Epstein (1981) presented somewhat controversial results from their analyses of human bone collagen from the Tehuacan Valley of Mexico. They suggested that human populations in that area were heavily dependent on maize some 6000 years ago. The evidence from plant and animal remains however indicates that such dependence did not occur until several thousand years later (MacNeish, 1967). The number of samples available to DeNiro & Epstein for the earlier phases in Tehuacan was very small ($n = 1$ for the Coxcatlan phase and $n = 2$ for the Santa Maria phase) and, in addition, these three cases had the lowest collagen concentration (3–4%) of all the human samples assayed from Tehuacan. The Tehuacan Valley samples that have greater than 5% collagen have $\delta^{13}C$ values similar to those expected from the archeological evidence for diet. Both the human bone collagen $\delta^{13}C$ values from the samples and the archeological plant remains indicated that maize was an important dietary component between 1000 and 2000 years ago. Thus, the three problemetical values are the three with the lowest collagen. If alteration occurs in those samples with very low collagen as has been observed in some, limited experimental situations (Schoeninger & DeNiro, 1982*b*; Farnsworth *et al.*, 1985) then these three are the most likely to be in error. More investigation of collagen diagenesis is necessary before the likelihood of this possibility can be evaluated. Norr (1981) reported increasing $\delta^{13}C$ values in human bone collagen from inland sites in Costa Rica between 1600 and 500 years ago. She interpreted this trend as a reflection of increasing dependence on maize as a dietary staple.

Two major studies (Van der Merwe & Vogel, 1978; Bender *et al.*, 1981) in combination serve to outline the introduction of agriculture into the middle portion of the U.S. Their results indicate that large scale dependence on maize as a dietary staple began around 1000 years ago. This timing is somewhat more recent than that noted for the Tehuacan Valley in Mexico or inland Costa Rica. Katzenberg & Schwarcz (1984; Katzenberg, 1984) are investigating the introduction of maize into southern Ontario.

Van der Merwe *et al.* (1981) have also used bone collagen $\delta^{13}C$ values to investigate the shift to a dependence on maize agriculture in one area of Amazonia along the Orinoco River. They concluded that this shift occurred around 2000 years ago and that maize was a more important staple than had been previously recognized. In their application of $\delta^{13}C$ values, Burleigh & Brothwell (1978) ascertained the presence of maize in prehistoric human living areas by analyzing the hair of domestic dogs in prehistoric Peru and Ecuador.

Other studies have demonstrated human dietary dependence on marine foods in geographic areas that do not include C_4 plants. Tauber (1981) determined that during the Mesolithic period in Denmark human populations in the area included a large percentage of marine animals in their diet. Further, there was a change in dietary adaptation in the subsequent Neolithic period. The $\delta^{13}C$ values of the bone collagen in the Neolithic period people were more negative than those in the preceding Mesolithic period people suggesting a shift to the use of terrestrial food products (plant as well as animal). As mentioned previously, two other studies (Chisholm *et al.*, 1982; Schoeninger *et al.*, 1983) have demonstrated the applicability of $\delta^{13}C$ values to determine diet in human populations from the N.W. coast of North America. In a subsequent paper, Chisholm *et al.* (1983) determined that prehistoric people from coastal British Columbia obtained a large majority of their diet from marine sources. Their conclusion and the $\delta^{13}C$ values for human bone collagen that they obtained agree well with the results of another study that included

coastal Eskimos and N.W. Coast Haida and Tlingit (Schoeninger *et al.*, 1983). Hobson & Collier (1984) report human bone collagen $\delta^{13}C$ values from the prehistoric site of Broadbeach in Australia. The values are equivalent to bone collagen $\delta^{13}C$ values of fish from two other areas in the southern hemisphere (Van der Merwe & Vogel, 1983; Schoeninger & DeNiro, 1984). The authors assume that use of C_4 plants was minimal; thus, the results suggest a heavy dependence on marine foods by the human populations.

The use of nitrogen stable istope ratios in estimating prehistoric human diet has been more limited than that of carbon stable isotope ratios. DeNiro & Epstein (1981) concluded that decreasing $\delta^{15}N$ values in human bone collagen through the sequence at the MesoAmerican site of Tehuacan could best be explained by an increasing dependence on legumes in the diet. With good reason they caution that more data are necessary before a firm conclusion can be reached. They argue that more knowledge on the effects of diagenesis on skeletal and plant proteins is needed. In addition, since the range of published $\delta^{15}N$ values for nitrogen fixing plants overlap that of non-N_2-fixing plants (Schoeninger & DeNiro, 1984) further study of $\delta^{15}N$ values in legumes and in organisms that feed on legumes is essential.

The reconstruction of dependence on marine or terrestrial foods will probably prove the most fruitful application of $\delta^{15}N$ values in bone collagen. The greater than five per mil difference in means between terrestrial and marine plants increases at higher trophic levels (Schoeninger & DeNiro, 1984) and is reflected in a ten per mil difference between recent North American agriculturalists and marine mammal hunters (Schoeninger *et al.*, 1983). Based on decreasing $\delta^{15}N$ values in bone collagen Norr (1982) has proposed that a lessening in the dietary dependence on marine products occurred in Central America between 7000 years ago and the ceramic period. In both this case and the one mentioned previously (Schoeninger *et al.*, 1983) $\delta^{15}N$ values were used in conjunction with $\delta^{13}C$ values for distinguishing between the contribution to the diet of C_4 plants versus marine food products.

Finally, it is possible that the $\delta^{15}N$ values of bone collagen may prove useful in conjunction with bone strontium levels in estimating the dietary dependence on meat by human populations. This possibility is discussed more thoroughly in the paper by Schoeninger in this issue.

4. Paleopathology and Diet

The development of trace mineral and stable isotope analysis provides skeletal biologists with important techniques for understanding past dietary behavior. Although earlier chemical studies were undertaken without reference to other nutritional indicators, it is essential that chemical analyses be integrated with macroscopic and microscopic evidence. Nutritional status determined from morphological indicators can complement chemical analyses. Paleopathological evidence can be used to establish the nutritional status of prehistoric populations. Chemical evidence of changes in the diet may result in deficiencies which do not cause observable changes in bone. The paleopathological evidence of dietary deficiency often requires biochemical analyses to understand their underlying causes.

The analysis of nutritional disease in prehistory has been enhanced by the use of multiple stress indicators. In the past, analysis relied on skeletal evidence for a single dietary deficiency. For example, if an individual were deficient in vitamin D (rickets), there

would be very distinct skeletal changes. Rickets often results in bowing and twisting of the long bones. Radiographically, bone appears to be very thin, especially at metaphyses.

There are, however, difficulties in the diagnosis of specific nutritional deficiences in archeological poulations. Steinbock (1976, p. 332) states that "it should be emphasized that malnutrition is rarely selective for only one vital component. Malnutrition (including malabsorption and excessive loss of nutrients) is almost always multiple, resulting in deficiency of several or many nutrients to varying degrees".

Other factors also argue for the use of multiple indicators of nutritional stress. Calvin Wells (1975, p. 758) states that "no indubitable examples of kwashiorkor are known from ancient burial grounds and it is unlikely that any will be recognized unless some wholly unexpected method of identification can be devised". The implication inherent in this statement is that a single diagnostic technique will uncover specific nutritional deficiencies.

While Wells' observation is correct with regard to the failure of traditional methods to identify the existence of protein-energy malnutrition in prehistory, it is not necessary to await further scientific breakthroughs to see a solution. The problem of diagnosing protein-energy malnutrition and other nutritional deficiencies lies in the systematic application of diagnostic techniques that currently are available and have been known for some time.

The lack of success in understanding the nutrition of prehistoric groups is due to the generalized systemic nature of nutritional problems, but it is this response which can be used to interpret the stressors involved (factors which cause physiological disruption—stress). Instead of applying single diagnostic criteria, mutliple indicators are used to reveal a pattern of nutritional deficiency (Huss-Ashmore *et al.*, 1982). Patterns of skeletal remodeling (the deposition and resorption of bone), evidence of infection and the degeneration of bones can be used to elucidate nutritional problems. For example, the existence of porotic hyperostosis (an indicator of iron deficiency anemia), periosteal reaction (a response to infection), osteoporosis (a disease in bone mass), long bone growth patterns, sexual dimorphism, Harris Lines (lines of increased density revealed by X-ray), and enamel hypoplasia (a defect in calcification of dental enamel) can be used in conjunction with chemical analysis of stable isotopes and of the major, minor, and trace minerals to establish the consequences of dietary behavior on the skeleton.

Porotic Hyperostosis

Porotic hyperostosis is one of the most frequently used nutritional stress indicators in bioarchaeology. Although the condition had been reported early in this century, it was only in the 1960s that dietary deficiencies were understood to be a factor in its etiology. Porotic hyperostosis occurs when there is an expansion of the diploe (inter-portion of thin bones of the skull) and a thinning of the outer table of bone resulting is a sieve-like (porous) appearance. This condition results from the increased red blood cell production which is a response to anemia. While there are many anemias which will cause porotic hyperostosis (thalassemia, sickle cell anemia, hereditary non-spherocytic anemia), the pattern of the lesion and its age distribution can aid in differential diagnosis (Mensforth *et al.*, 1978). Hereditary anemias are usually much more severe and affect cranial and postcranial skeletal elements. The nutritional anemias are less severe and usually involve the roof of the orbits (cribra orbitalia), the frontals and parietals (cribra crania). The nutritionally

caused anemia would most likely be found in very young children (beginning at six months and peaking at two) and among young adult females.

When the pattern of porotic hyperostosis suggests dietary involvement (lesions restricted to the orbits, frontals, and parietals), an analysis of ecological factors is essential to an understanding of the primary cause. It is possible that the anemia is a secondary response to infection which decreases the bioavailability of nutrients. In order to clarify this relationship of iron deficiency anemia and infection, it is necessary to evaluate the frequency and severity of periosteal reaction in a population.

Chronic infection often affects the normal bone development on the outer periosteal of bone. Toxins and fluids produced by the pathogen will cause the death of some bone cells and raise the periosteum from the bone. Although new bone is being formed, it is rough and irregular in appearance.

Iron Deficiency Anemia, Infection and Diet

The etiology of porotic hyperostosis has been studied extensively. Carlson *et al.* (1974) argue that a reliance on cereal grains such as millet and wheat (poor sources of iron), weaning practices, and parasitic infection predisposed prehistoric Nubians to iron deficiency anemia. El-Najjar *et al.* (1976) reached similar conclusions in their analysis of porotic hyperostosis in prehistoric groups from the southwestern United States. Reliance on maize (also a poor source or iron) was the primary cause of the condition in this area as well.

The analysis of porotic hyperostosis at the Libben Site (in Ohio) suggests another cause (Mensforth *et al.* 1978). Dietary reconstruction at Libben shows that adequate sources of protein were available. However, members of the Libben population was exposed to infectious pathogens early in life. Over 50% of the children who died during their first three years show evidence of periostitis. Mensforth and co-workers suggest that the iron deficiency anemia (porotic hyperostosis) was a secondary response to the infection which decreased the bioavailability of iron.

The analysis of porotic hyperostosis and periosteal lesions at the Dickson Mounds (Illinois) is more problematical. There is evidence of infection and iron deficiency anemia in the younger individuals in the population (Lallo *et al.*, 1977). The dietary change (increased reliance on maize) has been suggested as a primary factor for the increase in porotic hyperostosis. Trace element analysis (Gilbert, 1975) supports this hypothesis. Gilbert (1975) and Bahou (1975) found a correlation between the decrease in zinc and an increase in infectious lesions. Unfortunately, the iron levels were not determined in the Dickson Mounds sample. Von Endt and Ortner (1982) have demonstrated a decrease in iron levels in individuals with porotic hyperostosis.

Growth and Development

While growth is a primary indicator of health in modern medicine, its use in evaluating the health of prehistoric populations has only begun. The delay in applying growth parameters to the past is easily understood when we realize the difficulties of the analysis. In living populations, longitudinal studies can be used to establish standards. In prehistoric samples, however, the cross-sectional nature of the population complicates the analysis. Johnston (1962), Merchant & Ubelaker (1977), Uberlaker (1978), and Armelagos *et al.* (1972) have compiled growth curves for North American and Nubian populations using

long bone lengths. Many of these studies demonstrate growth retardation between the ages of two and six (the critical period of weaning), but the small sample sizes and the cross-sectional nature of the data make it difficult to interpret. Huss-Ashmore (1978) was able to clarify the growth problems in prehistoric Nubians by examining not only long bone lengths, but also by considering the cortical thickness of developing bone. While Huss-Ashmore found it difficult to show the effects of growth retardation using bone length, her analysis of cortical thickness shows indisputable evidence of growth retardation. The cortical thickness of many individuals under age 14 did not increase after the second year of life. There appears to be a compensatory response to nutritional deprivation. Long bone growth is maintained at the expense of cortical thickness.

Adult stature in prehistoric populations is often used as an index of relative health. The assumption is that small stature is a reflection of chronic deficiencies. Thomas (1973), however, has proposed that small body size may be an energy efficient adaptive response in situations with low nutrient availability. While stature may not be a useful indicator of individual dietary status, the relative size difference between males and females (the degree of sexual dimorphism) may reveal dietary stress.

Sexual Dimorphism

The use of sexual dimorphism as a dietary indicator is another complex problem. Brues (1975), for example, argues that subsistence activity affects body size, proportion, and sexual dimorphism. Using the example of the morphology of the spearman and the archer, Brues' hypothesis is that linear body build (tall stature and long limbs) would be more adaptive to the needs of a hunter using a spear. Hunters with a shorter and more robust body build would use the bow and arrow more effectively.

Frayer (1980, 1981), expanding on Brues' initial observation, suggests that the dangers involved in hunting large prey would select for larger body size in males. With a decrease in the size of prey in later phases of hominid evolution, there would be a concomitant decrease in male stature and a decrease in sexual dimorphism (assuming that female stature would remain the same).

Stini (1969, 1972, 1975) suggests a more direct relationship between nutrient availability and the degree of sexual dimorphism. He argues that males are more likley to be adversely affected by severe malnutrition than females. Female hormonal factors act as buffers against nutritional stress, allowing them to more effectively maintain their growth trajectory. In a population undergoing nutritional deprivation, there would be a significant decrease in sexual dimorphism.

Lallo (1973) has tested Stini's hypothesis using the Dickson Mounds population from the Illinois River Valley. Although the population underwent a dramatic shift in diet (due to an intensification in agriculture), he did not find any indication of change in sexual dimorphism. Larsen (1981) and Hamilton (1975) found reduced robusticity in American Indian populations which shifted to agriculture, but argued that differential work activity in males and females was the major factor involved in this change.

Osteoporosis

Osteoporosis, defined by a decrease in bone mass, has been studied as an age-related phenomenon and the dietary component of bone loss has been overlooked. The original study of osteoporosis in prehistory (Dewey *et al.*, 1969) considered aging as the major factor

in the decrease of bone mass. Perzigian (1973) did test the hypothesis that diet would affect bone loss. He found that the farmers, compared with foragers, showed an increase in the medullary cavity (an indication that bone is being lost). However, he did not believe that this loss was nutritionally related. He assumed that agricultural populations were better nourished and therefore that bone loss could not be related to diet.

There are a number of methods for determining osteoporosis. The most successful technique is the direct measurement of femoral cortical thickness or area. Interpretation often requires an analysis of microstructure to establish the underlying causes. Martin and Armelagos (this volume) provide information on the potential of histomorphology for clarifying the nutritional aspect of premature osteoporosis and the age-related aspects of the condition.

Harris Lines and Enamel Hypoplasia

Harris lines and enamel hypoplasia are generalized indicators of stress. While is it not possible to relate these features to specific dietary deficiencies, the analysis of each in conjunction with other nutritional disease measures can be very useful.

Harris lines are defined as increased radiopacity on the X-rays of long bones. Although there is considerable controversy regarding their cause, the lines do appear to represent growth cessation and recovery. Anthropologists have correlated the occurance of Harris lines with gender (Wells, 1967; Woodhall, 1968), stature (Blanco *et al.*, 1974; Perzigian, 1977; Goodman & Clark, 1981), and culture, but the significance of these differences has yet to be determined.

Clarke (1978), using known standards of growth in long bones, developed a method for determining the age of onset for Harris lines. Knowing the yearly increments of growth for the tibia, Clarke was able to examine radiographs and demonstrate the age at which an adult experienced growth arrest and recovery. A number of studies show that adults who survived childhood were frequently stressed around two years of age (the period of weaning) and at age 13 (the period of the adolescent growth spurt). The premanency of Harris lines, however, is problematical. Bone remodeling can often erase their resistance and visibility.

Enamel hypoplasia—seen as a thin line of decreased mineralization in the enamel—is a permanent record of stress. Since enamel does not remodel, the defect will remain. Cook & Buikstra (1979) have analyzed the occurence of hypoplasia and related their appearance to nutritional problems associated with weaning. Goodman *et al.* (1980) have shown a significant increase in hypoplasia with the intensification of agriculture in the Dickson population.

The chronology of enamel hypoplasia has been established by a number of researchers. The rate of enamel crown development can be used to establish the age in childhood that an adult was stressed. The pattern in three archeological populations—from California (Schulz & McHerry, 1975), Sweden (Swärdstedt, 1966), and Dickson Mounds (Goodman *et al.* (1980)—demonstrates childhood stress after the second year (the period of weaning).

The use of enamel hypoplasia may also provide information on the seasonality of stress. Swärstedt (1966) and Goodman *et al.* (1980) have attempted to establish yearly cycles of stress. By comparing the occurrence of enamel hypoplasia in half year or whole year intervals, Swärdstedt found no indications in his sample from Sweden, while Goodman and co-workers noted annual cycles of stress at Dickson.

Finally there appears to be a relationship between stress, as measured by hypoplasia, and longevity. Swärdstedt (1966) and Goodman & Armelagos (unpubl.) found that individuals who suffered stress as children (as evidenced by enamel hypoplasia) did not live as long as those who did not.

Serial Extraction of Collagen in Archeological Bone

The importance of collagen in the normal development of bone has been well established. If there are abnormalities in collagen synthesis, it can affect the calcification process. Furthermore, changes in the maturation of collagen may provide some insights into the diet. Collagen is not synthesized *in toto*, but is produced as a primitive monomer which is then polymerized through the formation of inter- and intra-molecular cross-links. The immature and mature fractions are differentially soluble and the ratio of these fractions can be an important diagnostic tool. Since these fractions represent specific metabolic pools, changes in these fractions can be used to investigate differences in aging or evidence of diseases interference (Conroy *et al.*, 1984).

Collagen that is not highly cross-linked can be extracted with solvents such as cold salt solutions at neutral pH or dilute acetic acid, while varying amounts of more highly cross-linked collagen can be removed by extraction with denaturing solutions such as 5M guanidine hydrochloride and boiling water. From this serial extraction we can determine the relative time required for the fraction to be synthesized. The half-life for the salt soluble extract is 14–20 days, that of the acid soluble portion is two to three months, and the insoluble extracts are synthesized over a period of up to 10 years (Miller *et al.*, 1967; R. G. Brown, University of Massachusetts, Amherst, Department of Food Sciences and Nutrition, pers. comm.).

For the analysis, the bone sample is ground to a powder and a total extract of collagen by hydrolysis is undertaken. This procedure provides a good yield of pure collagen, depending on the solubility of the sample in acidic hot water. HCl solution is used to eliminate most mineral substances from the crushed bones and also certain organic pollutants. The acid also breaks some hydrogen bonds, so that the collagen becomes soluble in hot water. Since the amino acid hydroxyproline is found only in the collagen tissues of the body and in constant proportions, it is possible to determine the amount of collagen in bone by measuring the amount of hydroxyproline in the sample. This is done by a standard colorimetric analysis.

In a small sample of well-preserved bones from Sudanese Nubia, an individual with a high percentage of immature collagen (from the acid soluble faction) was uncovered. We are in the process of determining the cause but a high percentage of immature collagen is indicative of osteolathryrism, a copper-zinc—or vitamin E—deficiency.

Serial extraction of collagen can provide information on the impact of diet on disease at the level of skeletal development. In well-preserved bone, it may be possible to determine nutritional diseases which do not alter the overt skeletal morphology. In addition, serial extraction may be extremely useful in stable isotope analysis where the composition of collagen can be examined at various stages in an individual's life cycle (P. Bumsted, pers. comm.).

The relationship of trace minerals and stable isotopes to nutritional deficiency requires more basic research. Although most researchers are well aware of the need to understand the impact of diagenetic factors which may affect the biological interpretation of the

chemical constituents of bone, they seem less willing to consider the affect that the disease process may have on the sample of bone used for analysis. Various nutritional diseases may affect collagen synthesis and mineralization and the impact of these changes on stable isotope and elemental analyses is yet to be determined.

The potential of the chemical analysis of dietary behavior also requires an understanding of variation in concentrations of stable isotopes and major/minor/trace elements. The use of archeological populations will aid our understanding of diet in prehistory and will also help in interpreting these chemical components in modern populations. Baseline data for many chemical features need to be established and archeological populations may provide an important means to this end.

Clearly, the analysis of dietary behavior is enhanced by the integration of gross measures of nutritional pathology, microstructure of bone, and chemical analysis. The systematic analysis of diet using pathology, morphology and chemistry may well herald a new era in nutritional anthropology.

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