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Ancient Human Diet from Inorganic Analysis of Bone

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Diet is the key to understanding many aspects of the development of human culture. Changes in diet must have occurred along with alteration of subsistence strategies, such as the evolution of farming cultures from those based on hunting, gathering, or fishing. Cultural development often brought about social stratification, which in turn led to preferential diet for the favored individuals. Divergence or convergence of sexual roles could be accompanied by changes in the diets of one sex relative to the other. The development of metal and glass technology brought increased exposure to heavy metals, not only for the artisan who made arsenical or leaded bronzes, pewter, or lead-glazed pottery but also for the individuals who may have eaten from containers made from such materials. Thus knowledge of the diet of ancient cultures can provide information on subsistence strategy, social stratification, sexual roles, and technology.

The traditional approach to obtaining information on diet was through identification of excavated plant and animal remains or analysis of coprolites (fossilized excrement). During the past decade, chemical analysis of bone has provided new approaches to understanding ancient diet. Analysis of bone provides the proportion of elements such as strontium (Sr) or zinc (Zn). If concentration of the element in bone accurately reflects its proportion in the diet, and if the element varies from food to food, then inorganic analysis leads directly to inferences about diet.

The first successful application of this method to paleontology was by Toots and Voorhies[1] in studies of trophic (food-chain) relationships of nonhuman animals. Their work was based on earlier studies by Comar, Wasserman, and Lengemann.[2] Toots and Voorhies detected differences in the Sr levels of various herbivores. At the lower end of the food chain, plants absorb Sr essentially at the geological background level. Herbivores consume Sr through plants, but very little Sr is lodged permanently in any part of the body except the skeleton. Because flesh contains very low levels of Sr, a carnivore has a much lower level of Sr consumption. Only about 3–4% of dietary Sr is thought to come from water directly.[3] Thus a carnivore should have less bone Sr than a herbivore, and an omnivore would be intermediate.

This simple argument suggests that analysis of bone for Sr could provide information about meat intake. It is doubtful, however, that such a simple relationship between one component of diet and one element exists, but analysis for several elements, which respond to different components of the diet, might provide a pattern analysis for the ancient human diet. Development of such a procedure requires answers to several questions. (1) Can factors other than diet be controlled or neglected? (2) Are elemental differences within or between bones of a single individual too great to permit useful analysis? (3) What are the effects of burial on the constitution of bone? (4) Which elements respond to dietary input? The first applications of inorganic analysis of human bone to obtain information on diet were the doctoral dissertations of Brown[4] and of Gilbert.[5] These two studies addressed these questions and laid much of the groundwork for all future studies. In the following sections, we will discuss various aspects of these questions, and then describe some of the results on diet from the inorganic analysis of human bone.

The mechanics of analysis have now been well worked out.[6,7] Instrumentation may be atomic absorption, neutron activation, X-ray fluorescence, inductively coupled plasma, or mass spectrometry. Samples should be powdered and ashed in a muffle furnace or by a similar procedure, so that analyses may be based on the weight of dry ash. Bone contains variable amounts of water and of organic material that are removed by ashing. The variability of these components, if not removed, would lead to systematic errors in reported proportions. For this reason, analyses of whole bone by X-ray fluorescence, for example, cannot be compared with analyses of ashed bone by atomic absorption.

Which Bone and Whose?

Almost every bone in the human body has been used for drawing dietary inferences: skull, teeth, jaw, vertebra, rib, ilium, femur, tibia, and even undefined bone fragments. It was the explicit assumption of most workers that dietary conclusions would not depend on the choice of bone, so long as all the samples were of the same type, e.g., rib.

It was appreciated very early that teeth represent a special situation. Tooth enamel is much more compact than bone, and elemental turnover is kinetically much slower.[8,9] Thus teeth undergo little remodeling, and

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(1) H. Toots and M. R. Voorhies, *Science (Washington D.C.)*, **149**, 854 (1965).

(2) C. L. Comar and R. H. Wasserman, in "Mineral Metabolism", C. L. Comar and F. Bronner, Eds., Academic Press, New York, 1963, Vol. 2, pp 523–572; F. W. Lengemann, in "The Transfer of Calcium and Strontium across Biological Membranes", R. H. Wasserman, Ed., Academic Press, New York, 1963, pp 85–96.

(3) G.-I. Tanaka, H. Kawamura, and E. Nomuro, *Health Phys.*, **40**, 601 (1981).

(4) A. B. Brown, Ph.D. Dissertation, University of Michigan, Ann Arbor, MI, 1973; A. B. Brown, *Contrib. Geol.*, **13**, 47 (1974).

(5) R. I. Gilbert, Jr., Ph.D. Dissertation, University of Massachusetts, Amherst, MA, 1975.

(6) A. B. Brown and H. Keyzer, *Contrib. Geol.*, **16**, 85 (1978).

(7) C. B. Szpunar, J. B. Lambert, and J. E. Buikstra, *Am. J. Phys. Anthropol.*, **48**, 199 (1978).

(8) R. B. Parker, J. W. Murphy, and H. Toots, *Archaeometry*, **16**, 98 (1974).

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elemental levels in enamel tend to be determined by dietary conditions at the time of formation. Juvenile diet can be quite different from adult diet, so that conclusions based on teeth may differ from those based on bone. Elemental turnover in bone, on the other hand, is on the order of months, so that elemental concentrations should reflect adult diet. Most workers now exclude teeth from their studies, unless juvenile diet is a specific desideratum.

In order to test the compatibility of data sets based on different skeletal components, we compared the concentrations of 11 elements in 47 femurs and 118 ribs from Woodland populations of Illinois (AD 200–1000).[10] Sr and Zn showed almost no differences between rib and femur, either for the overall populations or for male and female subgroups. The identity of Zn and of Sr levels confirmed their reliability for dietary conclusions and suggested that at least these two elements are constant within archaeological variance for these two bone types. The skeletal set, however, was heterogeneous with respect to age and sex, and small differences in Sr and Zn between rib and femur could have been erased by the large value of the standard deviations.

It is now certain that earlier hopes were too optimistic and that even Sr varies inherently to a small extent from bone to bone. Modern cadavers provide better controls. Extremely well-designed experiments on a modern Japanese population showed that Sr varies by about a factor of two throughout the body,[3] less than the range for typical archaeological samples[10] but still significantly. This result is notable, since many workers have traditionally cited Sr as constant throughout the body.[11] Variance with other results may be attributed to the high precision of the Japanese study,[3] much higher than is possible in studies of buried bone.

In an investigation of lead (Pb) distributions, Aufderheide and Wittmers confirmed that there are inherent differences between skeletal components but suggested that possibly there are only three broad groupings:[12] (1) skull, femur, tibia, humerus, radius, ulna, hand, patella, fibula, foot, mandible, and hyoid; (2) vertebra, rib, and sternum; and (3) ilium, scapula, and clavicle. It is not known whether these classifications can carry over to Sr or Zn. In agreement with the Japanese work,[3] Aufderheide's study showed that there is concern in mixing bone types, although it is possible that groupings of cortical or of cancellous bones can be made. A well-controlled study probably should be limited to a single skeletal component, although for archaeological samples, with their large standard deviations, even cortical and cancellous bones have identical amounts of Sr and Zn, as shown in the comparison of ribs and femurs.[10]

If elemental levels vary from bone to bone, what sort of variation is there within a single bone? In an unpublished study on a Gibson sample (Middle Woodland, about AD 200), we divided a single rib into six parts and analyzed it for 11 elements.[13] The variance (standard deviation as a percentage of the mean) for Zn was 5%. Schoeninger[14] found that 14 samples from a single rabbit showed a variance for Sr of 9% and 19 samples from farm-raised mink (several animals) showed a variance of 21%. Tanaka et al.[3] cut 11 pieces from the ulna of a 42-year-old male cadaver. The Sr content varied almost monotonically from 125 ppm in the epiphysis (end) to 165 ppm in the diaphysis (middle), a range of about 15%. The calcium (Ca) percentage was almost constant for the same 11 samples. It seems that even for single bones, Sr varies by 5–15%.

Other factors that may contribute to variance in bone analyses are species, sex, pathological conditions, age at death, climate, and geology. Nondietary differences between species are not well understood, but are not relevant to the present subject of human diet. Differences between sexes can arise from dietary causes and hence are a legitimate subject in the present context. Several such differences will be discussed later. Sillen and Kavanagh have cautioned that some of these differences may be caused by pregnancy and lactation in the female,[15] particularly since the age group 18–40 usually is represented strongly in burials. These factors have not been tested directly, but it should be appreciated that they may contribute to the overall variance in female bone composition. Pathologies may give rise to unusual levels for certain elements, as will be described in a later section. Often, however, such conditions can be recognized during examination of the skeleton, so that pathological cases can be excluded or studied separately.

There are definite trends with the age of the individual at death. We found that bones of young children and older adults tend to be more easily contaminated by soil elements.[16] We observed only small changes with age, however, for Sr, Zn, and Na. Tanaka et al.[3] again have supplied the definitive study. With a very large sample size ($n = 272$), they found increases in the Sr level from fetal samples through about age 25 and relatively constant levels thereafter. Fetal levels are about half the adult levels, and changes after age 12 are only about 20%. Although some workers have advocated excluding all juveniles because of possible systematic differences from adults, it appears, at least for Sr, that the differences from age 12 are within the expected overall variance. Finally, variation caused by climate or geology can be controlled by studying groups from the same or similar sites.

Although it was hoped that elemental levels of trace and minor elements would be determined primarily by dietary factors, it now appears that elemental levels, even that of Sr, can vary with the choice of bone, within a single bone, with pathological conditions, with the age of the individual at death, and possibly with the history of maternity and lactation in females. Extraction of dietary information from this background of statistical noise requires a large sample for any study. Comparisons should be made only between means of substantial

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(9) R. B. Parker and H. Toots, *Geol. Soc. Am. Bull.*, 81, 925 (1970); R. B. Parker and H. Toots, in "Fossils in the Making", A. K. Behrens-Mayer and A. P. Hill, Eds., University of Chicago Press, Chicago, 1980, pp 197–207.

(10) J. B. Lambert, S. M. Vlasak, A. C. Thometz, and J. E. Buikstra, *Am. J. Phys. Anthropol.*, 59, 289 (1982).

(11) D. L. Thurber, J. L. Kulp, E. Hodges, P. W. Gast, and J. M. Wampler, *Science (Washington, D.C.)* 128, 256 (1958).

(12) A. C. Aufderheide and L. E. Wittmers, Jr., in "Chemical Analyses of Ancient Skeletal Material", L. Klepinger, Ed., submitted for publication.

(13) C. B. Szpunar, Ph.D. Dissertation, Northwestern University, Evanston, IL, 1978, Appendix I, p 167.

(14) M. J. Schoeninger, *Am. J. Phys. Anthropol.*, 58, 37 (1982).

(15) A. Sillen and M. Kavanagh, *Yearb. Phys. Anthropol.*, 25, 67 (1982).

(16) J. B. Lambert, C. B. Szpunar, and J. E. Buikstra, *Archaeometry*, 21, 115 (1979).

subgroups by standard statistical tests. Conclusions based on single individuals or small groups should be resisted.

Diagenesis

There are two necessary requirements for inorganic analysis of bone to give useful dietary information. First, dietary input of a given element must influence the level in bone (next section). Second, levels measured in excavated bone must be equal to or proportional to levels at the time of death. Diagenesis is the general term for the process of alteration of bone after burial. The extent of diagenesis and its influence on levels of chemical elements must be established before dietary inferences can be made. The amount of alteration during burial can range from nil to complete disappearance of the bone. Normally, bone analyses are carried out only on well-preserved specimens, for which decomposition is minimal. Such specimens come from sites at which the soil is alkaline (high pH), flooding is infrequent, and the climate is dry. Nonetheless, ion movement involving the very elements of dietary interest can occur and vitiate any conclusions. It therefore is necessary to ask when contamination has occurred and which elements are involved.

Several approaches have been used to test for soil contamination. We will discuss three that we have used: comparison of different skeletal components, analysis of soil associated with burials, and examination of elemental concentrations in bone as a function of depth from the bone surface.

Because skeletal components differ in density and crystallinity, they can be contaminated at different rates. This approach was first used by Parker and Toots, who compared bone, tooth dentine, and tooth enamel.[9] Bone is the most porous, enamel the most impermeable, and dentine intermediate. They found that the concentration of Sr was equal in all three skeletal components, indicating no diagenetic effect.

Comparisons between ribs and femurs by our group[10] have led to conclusions that are similar to those of Parker and Toots. The denser, cortical femur should be less sensitive to diagenesis than the more porous, cancellous rib. In our comparison of femurs and ribs from Woodland populations, the elements fell into three categories. As already noted, Sr, Zn, and also Mg showed almost no differences between rib and femur. Thus these elements seem to have been subjected to little flux with the environment at this site. Ca, Na, and Pb showed lower concentrations in rib than femur, probably caused by more rapid leaching of the element from rib to soil than from femur to soil or by inherent differences. Finally, Fe, Al, Mn, K, and Cu showed higher levels in the rib, characteristic of contamination. Another group similarly found higher concentrations of barium (Ba) in the rib than in the fibula.[17] Thus, at least for these samples, various elements were unaffected (Sr, Zn, Mg), leached out of bone (Ca, Na), or moved into bone (Fe, Al, Mn, K, Cu, Ba). For older samples or in more acidic soil, it is possible that even Sr, Zn, and Mg could be affected.

In a second approach, we analyzed elements in soil around buried human bone from Woodland sites.[18] Natural concentrations of Mn, Cu, Fe, Al, and K in soil are similar to or much higher than those in bone, whereas bone has higher concentrations of Sr, Zn, Ca, and Na.[16] More useful than these measurements of absolute levels in soil was the discovery of elemental gradients in soil within a few centimeters of the bone.[18] Leaching of an element into or out of bone should result in concentration changes primarily right around the bone. We found that Sr and Zn are distributed homogeneously around and away from femurs of Woodland burials, characteristic of no flux.[18] Ca showed higher concentrations in regions around the bone, indicative of loss from the bone. Fe, Al, and K showed lower concentrations around the bone, indicative of removal from soil to bone. Phosphate in the femur may be fixing these elements.

The concentration of Pb in soil has been studied extensively. Although in some cases there may be no movement of Pb from soil to bone,[19] in others there is clear evidence for diagenetic effects.[20] Waldron et al. analyzed rib and associated soil.[20] From the proportionality between Pb concentrations in bone and soil, they concluded that contamination must have occurred. Because contamination is not always the case for Pb, analysis of soil is imperative.

Both analysis of soil and comparisons of ribs with femurs yielded the same three groups of elements. The diagenetic loss of Ca is important in the context of use of the Sr/Ca ratio by some authors. Although use of the ratio in geological or biochemical contexts is fully justified, it appears not to be justified in the archaeological context. When one member of a ratio is subject to diagenesis, but the other is not, clearly the ratio can give false trends. Examination of the absolute level of Sr is necessary. In studies that did utilize the Sr/Ca ratio, variance between subgroups often was due entirely to the Sr component, Ca being essentially constant. Ca is found typically at the 30% level, Sr at the 0.01% level. Thus any dietary effect on Sr will have a negligible effect on Ca, and variances between means should be caused only by Sr effects. The concentraiton of Ca in the hydroxyapatite matrix of a given bone should be relatively constant. In the presence of diagenetic effects on Ca, the Sr/Ca ratio would give false results. In the absence of diagenetic effects, the Sr/Ca ratio gives results that come primarily from Sr variation.

Our third approach for determining the effects of diagenesis involved examination of elemental concentrations in bone as a function of depth from the bone surface. Parker and Toots recognized two distinct types of contamination.[9] Some elements can actually replace components in the hydroxyapatite matrix, e.g., F^- for HO^- or Y for Ca. Other elements (Fe, Si, Mn) move into voids in bone caused by loss of organic material. These authors also recognized that some elements (Na, Mg, Cl, K) could be lost from the bone matrix. Our recent electron microprobe studies of Woodland femurs showed clear distinctions between stable and contaminative elements.[21] For example, the distributions of

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(17) L. Ahlgren, J.-O. Christoffersson, and S. Mattsson, *Adv. X-Ray Anal.*, **24**, 377 (1981).

(18) J. B. Lambert, S. V. Simpson, J. E. Buikstra, and D. K. Charles, in "Archaeological Chemistry III", J. B. Lambert, Ed., American Chemical Society, Washington, DC, 1984, Adv. Chem. Ser. No. 205, pp 97–116.

(19) H. A. Waldron, A. Khera, G. Walker, G. Wibberley, and C. J. S. Green, *J. Archaeol. Sci.*, **6**, 295 (1979); A. Mackie, A. Townshend, and H. A. Waldron, *ibid.*, **2**, 235 (1975).

(20) H. A. Waldron, *Am. J. Phys. Anthropol.*, **55**, 395 (1981); H. A. Waldron, *J. Archaeol. Sci.*, **10**, 35 (1983).

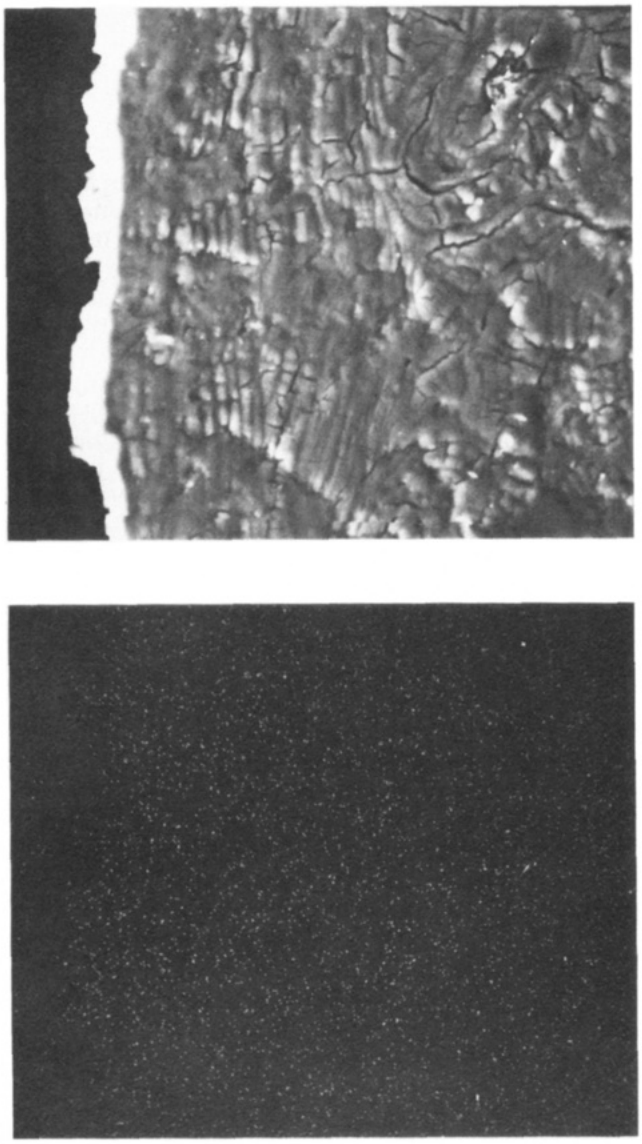

Figure 1. (Top) Electron micrograph of the femur from Ledders 1-41 by SEM. The outer surface is clearly visible at the left side. The width of the scan is 380 μm and the expansion is 240×. (Bottom) The zinc distribution in the same sample is seen to be homogeneous throughout the region, as indicated by the dot distribution.

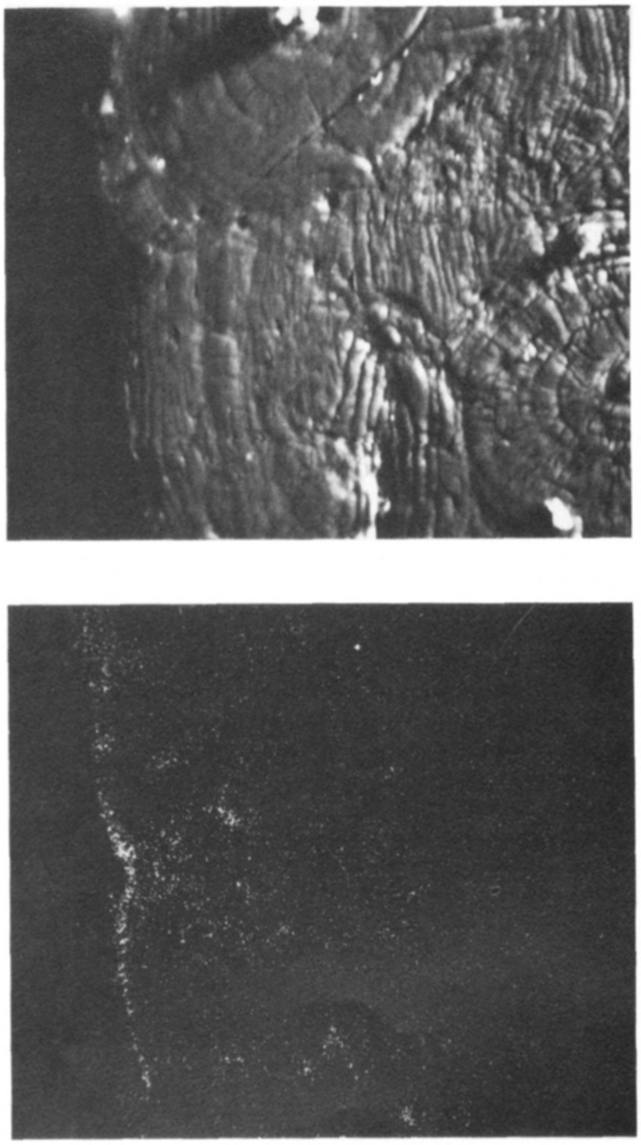

Figure 2. (Top) Electron micrograph of the femur from Ledders 1-16 by SEM. The outer surface is clearly visible at the left side. The width of the scan is 380 μm and the expansion is 240×. (Bottom) The manganese distribution in the same sample is seen to build up along the outer surface and in pockets near the surface, as indicated by the higher density of dots.

Sr and Zn are homogeneous from one surface of the femur to the other. Figure 1 shows a typical micrograph of the Zn distribution. On the other hand, Fe, Al, K, Mn, Cu, Ba, and sometimes Mg show clear evidence of buildup on the surfaces and 10–400 μm into the femur,[21] as shown in Figure 2 for Mn. Surface contamination by Pb has been found by other workers.[20] Sequential sampling and neutron activation analysis of fossil bison samples yielded similar results.[22] Whereas Sr showed no concentration gradients, U, Mn, Ba, V, and F clearly showed higher concentrations at the surfaces.[22] Both the microprobe[21] and the NAA study[22] showed no gradients for Ca and Na. These are major elements, for which considerable loss into the soil can occur in absolute terms, although the loss is small relative to the total amount present.

Contamination of rib by elements in the soil has also been seen in plots of concentration vs. age of the individual at death.[16] Increases are seen for Mn, K, Al, and Fe for older individuals and for the very young.[16] More porous bones for both groups may cause these observations. Other workers have used comparisons of herbivores with carnivores[23] and isotope analysis[24] to assess the effects of diagenesis.

These studies on diagenesis have provided a reasonably firm picture for quite a few elements. Sr and Zn are stable for the most part to diagenetic effects and therefore should be useful in dietary studies. Although Na and Ca are subject to leaching, the remaining amounts should be proportional to lifetime levels, so that these elements too may be useful. The cases of Pb, Mg and K seem to vary from sample to sample. These elements may be useful if diagenetic effects can be excluded. Finally, Fe, Al, and Mn and possibly Cu and

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(21) J. B. Lambert, S. V. Simpson, J. E. Buikstra, and D. Hanson, *Am. J. Phys. Anthropol.*, **61**, 409 (1983).

(22) E. Badone and R. M. Farquhar, *J. Radioanal. Chem.*, **69**, 291 (1982).

(23) A. Sillen, *Am. J. Phys. Anthropol.*, **56**, 131 (1981).

Ba appear to be contaminated in all cases so far observed. Measured amounts have little or nothing to do with lifetime levels, so these elements are not useful.

Caution must be exerted, even with the most reliable elements. Various authors have failed to find distinguishing properties for Sr.[23-26] In two such cases,[23,25] the sample number was very small. In another,[26] the materials were of Pliocene or Pleistocene age, much older than any of the human samples discussed herein. In these studies,[25,26] no direct evidence was offered for diagenetic effects on strontium. The conclusions were based on failures to find expected differences in strontium concentrations.

Although caution must be exhibited with all elements, it is still possible that some of the contaminative elements will yield useful dietary information in the future. Our SEM studies[21] and the neutron activation studies[22] showed that the preponderance of contamination is near the surface. Removal of the surface of the bone, to a depth determined by associated SEM studies, may remove contamination to the extent that other elements may prove to be reliable.

Which Elements?

Strontium. Although there is a vast array of elements available, some may not be relevant in the context of diet, others may be particularly sensitive to pathological conditions, and still others may lose significance by the operation of diagenesis. Sr was established early as the most important element for the analysis of diet. By electron microscopy, Parker and Toots[9] showed that Sr is present within the hydroxyapatite crystal lattice, rather than in voids that develop after death, so that in all likelihood measured percentages reflect antemortem levels. We confirmed this observation by our findings that Sr is identical in archaeological ribs and femurs,[10] that it is distributed homogeneously in the soil around bone,[18] and that there is no buildup of Sr on the bone surface.[21] Thus Sr has passed all the tests for the absence of diagenetic effects.

Because of concerns over Sr-90 fallout during the 1950s and 1960s, considerable research was carried out on the relationship of Sr to diet. As a result, this element became the focus of archaeological studies. As mentioned earlier, it is found at a relatively high level in plants and at a low level in meat. Unfortunately, it is not a perfect measure of animal protein, as it also is found at high levels in nuts and marine invertebrates such as molluscs.[27] High consumption of these materials could give rise to high levels of bone Sr and be interpreted incorrectly in terms of an agriculturist diet. It is also possible that disease may influence Sr levels.[15] Finally, Ca is an antagonist to Sr, so that low Sr may reflect high Ca diets. For these reasons, it is simplistic to consider that Sr is determined solely by intake of animal protein vs. plants. Diet is a complex and multidimensional subject that should be approached with as many measurables as possible. With an array of elements, it would become possible to apply the methods of pattern (cluster) analysis and hence to define specific dietary components more reliably. At the same time, elements should not be included indiscriminately. Each must be tested for its sensitivity to diet and diagenesis within the context of a given excavation.

Barium. The element just below Sr in the periodic table is a natural choice. Since its atomic properties, such as ionic radius, differ more from Ca than those of Sr, physiological discrimination against Ba should be even higher. Recent studies of Wessen et al. confirmed this expectation.[28] Comparison of Sr and Ba levels in recent kills of fur seals (carnivore) and deer (herbivore) showed that Sr is much less sensitive than Ba to dietary differences.

Although Ba is clearly a better discriminator than Sr, there are serious reasons for concern over diagenetic effects. Ahlgren et al. found that Ba levels are much higher in rib than in fibula.[17] Such differences are suggestive of greater contamination in the rib. We compared elemental composition between subgroups at Woodland sites and found Ba to be insensitive to dietary changes, whereas Sr provided good distinctions.[29] We also found that Ba concentrates on the surface of bone, unlike Sr and Zn but like Mn and other contaminative elements.[29] Parker and Toots[9] and Badone et al.[22] also found evidence for Ba contamination. Thus, we conclude that Ba is an excellent discriminator for diet but that archaeological results probably will be vitiated by diagenetic effects.

Zinc. As described above, Zn appears to be free of diagenetic effects, at least for burials in nonacidic soil for a few thousand years. Rheingold et al.[30] established that Zn, like Sr, is related to diet. Whereas Sr decreases in the order herbivore (400–500 ppm for Rheingold's samples) to omnivore (150–400 ppm) to carnivore (100–300 ppm), Zn increases over the same series (90–150, 120–220, 175–250 ppm, respectively). Unlike Sr, Zn occurs in relatively high levels in meat.[27] It also is found at high levels in nuts and molluscs.[27] Thus two-dimensional plots of Sr vs. Zn provide a rudimentary pattern analysis and a better approach to dietary problems than use of Sr alone. Beck[31] has exploited this approach with several data sets.

Lead. In our studies of diagenesis, we found Pb to be relatively free of diagenetic effects.[10,81,21] Waldron et al.[19,20] found deleterious effects of contamination in some, but not all, cases. In samples known to be free of diagenetic effects, bone analysis can give useful information about the intake of dietary Pb. Unlike Sr and Zn, sources of Pb in general are unnatural, such as pewter containers, lead-glazed pottery, or Pb pipes. Hence, analysis for Pb will give pathological information that is still useful in the anthropological context.[12]

Sodium. Parker and Toots reported that Na, like Sr, is found only in the hydroxyapatite matrix, rather than in voids.[9] We also found no evidence for contamination of bone by Na from the soil, although levels of Na may decrease slightly by loss to the soil.[10,18,21] To

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(24) J. E. Ericson, paper delivered at the 82nd Annual Meeting of the American Anthropological Association, Chicago, IL, Nov 1983.

(25) M. Elias, *Am. J. Phys. Anthropol.*, **53**, 1 (1980).

(26) N. T. Boaz and J. Hampst, *J. Paleontol.*, **52**, 928 (1978).

(27) R. I. Gilbert, Jr., in "Biocultural Adaptation in Prehistoric America", R. L. Blakely, Ed., University of Georgia Press, Athens, 1977, pp 85–100.

(28) G. Wessen, F. H. Ruddy, C. E. Gustafson, and H. Irwin, *Archaeometry*, **19**, 200 (1977); G. Wessen, F. H. Ruddy, C. E. Gustafson, and H. Irwin, in "Archaeological Chemistry II", G. F. Carter, Ed., American Chemical Society, Washington, DC, 1978, Adv. Chem. Ser. No. 171, pp 99–108.

(29) J. B. Lambert, S. V. Simpson, C. B. Szpunar, and J. E. Buikstra, *Archaeometry*, **26**, 000 (1984).

(30) A. L. Rheingold, S. Hues, and M. N. Cohen, *J. Chem. Educ.*, **60**, 233 (1983).

(31) L. A. Beck, submitted for publication.

our knowledge, no studies have yet been carried out to establish that Na levels in the diet are reflected directly by levels in the bone. Nonetheless, its low sensitivity to diagenetic effects and its undoubted physiological importance make Na a prime subject for study.

Copper, Magnesium, and Vanadium. These three elements are probably indicative of specific components of the diet and have been used in some studies.[16,27,32,33] Unfortunately, all three have been associated with diagenetic effects.[10,18,21,22,28] Because contamination seemed lower than for Fe, Al, Mn, or K, or because diagenetic effects were not always present, these elements may prove useful in certain cases.

Carbon and Nitrogen. Although this *Account* focuses on the inorganic analysis of bone, dietary information also is available from the organic components. Isotope analysis, either of $^{13}C/^{12}C$ or of $^{15}N/^{14}N$, can provide useful information.[34,35]

Results on Dietary Elements

The early work of Brown[4,6] and of Gilbert[5,27] laid the foundation for the study of human diet by inorganic analysis of bone. Brown studied several sites and attributed some differences in Sr levels to differences in status.[4] Gilbert[5] studied Late Woodland, Transitional, and Middle Mississippian populations (AD 400–1300) at the Dickson Mounds, IL, in order to determine effects of the arrival of agriculture. He measured Sr, Zn, Mg, Mn, and Cu but did not make allowance for diagenesis except to remove the outer portions of the bone. He found significant differences (99% level) in Zn between the Late Woodland and either the Transitional or the Middle Mississippian populations. The earlier Woodland group had higher levels of Zn, possibly indicating higher consumption of meat or nuts. He found some changes, though less definitive, in the Sr levels. The lack of intelligible trends with Cu, Mg, and Mn probably can be attributed now to diagenetic effects. Gilbert's study was significant because he was able to look at changes in subsistance strategies at a common geographical site and because he was the first to use several elements with human samples. In fact, his best results were with Zn rather than with the later more popular Sr.

Our early study, also on Illinois populations,[16] compared the earlier Middle Woodland population from Gibson (hunter–gatherer) with the Late Woodland population from Ledders (beginnings of maize cultivation). We used the first large array of elements (Sr, Zn, Mg, Ca, Na, Cu, Fe, Al, Mn, K) and introduced the method of total dissolution.[7] By analysis of soil and of content as a function of age at death, we were able to suggest that Fe, Al, Mn, and K levels were primarily the result of contamination. Whereas the earlier Gibson site showed no differences between the sexes, there were differences at Ledders for both Sr and Zn. Males had lower Sr and higher Zn at Ledders, suggestive of a higher meat diet. The earlier Gibson burials could be classified according to the status of the burial in the mound. The higher status individuals differed from the lower status individuals in levels of Sr, Zn, Mg, and Na. Pattern analysis was used to distinguish subgroupings on the basis of the inorganic analyses. A defect of this study was the mixing of juveniles and adults, although the conclusions should not have been affected.

Schoeninger[36] studies the Chalcatzingo site in Morelos, Mexico. She used both atomic absorption and neutron activation analysis to show that the analytical results were not dependent on the choice of technique. Her sample size was large, but she analyzed only for Sr. Using pattern analysis based on types of burial goods, she was able to define three distinct groups that correlated well with the status of the grave goods: burials with jade, with a shallow dish, and with no goods (or nondiagnostic). She found the lowest levels of Sr in the highest status jade group. Her study was based entirely on adults but did not distinguish the sexes.

Schoeninger and Peebles[37] demonstrated the importance of careful examination of the archaeological data in conjunction with inorganic analysis. In a comparison of three ribs each from Late Archaic and Mississippian levels from Seven Mile Island, Lauderdale County, AL, they found much higher levels of Sr for the Archaic hunter–gatherers than for the Mississippian agriculturalists, an inversion of the expected trend. On the basis of archaeological observation of molluscs associated with the Archaic level but not with the Mississippian level, they concluded that these invertebrates comprised a large part of the diet of the Archaic population and caused high Sr levels. This study suffered from the very small size of the sample (three each) and would have benefited from analysis for Zn.

Sillen's study of HaYonim Cave, Israel,[23] was important in establishing the shortcomings of tooth enamel analyses. It suffered from a small sample size ($n = 14$) but attempted to examine much earlier human populations than any of the previous studies (Natufian, 9970 ± 90 BC, and Aurignacian, 16000–20000 BP). He was the first to introduce serious examination of associated carnivores and herbivores as controls for conclusions concerning humans. The Natufian-level carnivores had a clearly lower proportion of Sr than the herbivores. The equality at the earlier Aurignacian level suggested that diagenesis was important over the longer term. There were no specific conclusions concerning human Sr levels.

Price and Kavanagh[38] examined several populations in Wisconsin, ranging from Late Archaic to Mississippian. They used inductively coupled plasma techniques and analyzed 12 elements (P, K, Ca, Mg, Na, Al, Ba, Fe, Sr, B, Cu, and Zn), although commenting only on Sr levels. They found a trend of increasing levels of Sr from the Archaic Reigh site, to the Middle Woodland Trempealeau and Millville sites, and to the Middle Mississippian Aztalan site. Only 13 samples were reported altogether, however.

Schoeninger[14] examined the decrease in robustness from Neanderthals to modern populations, by measurement of Sr levels at Levantine (eastern Mediterranean) sites. She also used associated fauna for the purpose of calibration. There appeared to be no difference in Sr levels between the earlier robust popula-

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(32) R. L. Blakely and L. A. Beck, *Ann. N. Y. Acad. Sci.*, **376**, 417 (1981).

(33) J. W. Hatch and R. A. Geidel, *Archaeology*, **36** (1), 56 (1983).

(34) N. J. van der Merwe and J. C. Vogel, *Nature (London)*, **276**, 815 (1978).

(35) M. J. Schoeninger, M. J. DeNiro, and H. Tauber, *Science (Washington, D.C.)*, **220**, 1381 (1983).

(36) M. J. Schoeninger, *Am. J. Phys. Anthropol.*, **51**, 295 (1979).

(37) M. J. Schoeninger and C. S. Peebles, *J. Archaeol. Sci.*, **8**, 391 (1981).

(38) T. D. Price and M. Kavanagh, *Midcont. J. Arch.*, 7, 61 (1982).

tions at Tabûn (70 000 BP), Skhūl (30–35 000 BP), or Qafzeh (30–35 000 BP) and the early modern populations at Kebara C (15 000 BP). Kebara B and El Wad (10 000 BP), however, did show higher Sr levels (as a ratio to fauna). She suggested that dietary changes associated with differences in food procurement must have occurred in the period 15 000–10 000 BP, after morphological changes that included loss of robustness.

Blakely and Beck[32] compared a high-status population at Mound C of the Etowah site, GA (Mississippian, after AD 1000), with a village population from the same site. They reported levels for Sr, Zn, Cu, and Mg. Finding no significant differences between the means of the two groups, they concluded that higher social status of the Mound C group had been achieved rather than inherited. Their population was a large group ($n = 51$) and was identified by sex. Analyses were by inductively coupled plasma. They found slightly higher levels of Zn and lower levels of Sr in the Mound C group, but differences from the villagers were at only the 80–90% level. No direct measures of diagenesis were reported, so the results on Cu and Mg cannot be assessed.

Hatch and Geidel[33] reported briefly on a study of the Hixon and Dallas sites (1300–1550 AD) of East Tennessee. They assessed status by grave goods, had a complete identification by sex, and analyzed for Sr, Zn, Mn, and V. Like at Etowah, there were high-status mound burials and low-status village burials. Unfortunately, actual elemental levels were not reported nor were specific statistical tests described. The quality of the data could not be assessed without knowledge of the number of samples. Differences between mound and village subadults were found for Sr, V, and Mn. Although no differences were found for adult females, adult males showed higher V and Zn for the village group, possibly indicating a diet high in nuts and plants rather than in meats. The absence of differences between the females was interpreted as indicative of higher social mobility for females. Thus a high-status female might have come from a village family. This conclusion, however, is not warranted. Unlike tooth enamel, most bones have a reasonably rapid turnover of the elemental constituents,[3] on the order of months. If a village female had married into a high-status family, her improved diet in later life would have been reflected in the inorganic content of her skeleton. It is not possible to draw conclusions about early life from skeletal analysis of adults. This same criticism can be made of the Etowah work.[32] The conclusions about achieved vs. inherited status are not warranted from skeletal analysis alone, as the improved diet of the individual who achieved high status would cause the skeletal inorganic content, in a matter of months, to approach that of the individual who inherited status. The study of the Dallas and Hixon sites also drew upon levels of Mn and V, whose proportions are known to be strongly influenced by diagenesis.[10,18,21,22] This report was preliminary, so that further discussion of the results is expected.

In still unpublished work, Beck[31] has obtained the first rudimentary approach to pattern analysis of diet, using two-dimensional plots of Sr vs. Zn. She reexamined the Gibson,[16] Ledders,[16] and Etowah[32] data and analyzed new data from Moundville, AL, obtained by Peebles. In all cases, the two-dimensional plots were more revealing than analysis of Sr alone. Hunter–gatherers were distinguished by high Zn and low Sr, agriculturalists by low Zn and high Sr, and high nut and mollusc consumption by high Zn and high Sr. The Gibson and protoagriculturist Moundville populations gave excellent patterns for hunter–gatherer societies. The agriculturist Moundville population produced the completely different pattern expected for a low meat diet. The Etowah population showed the agriculturist pattern for both Mound C and villager subgroups, although higher Zn diet was evident for the village group. The Middle Woodland Gibson site also had a hunter–gatherer pattern with a skew toward high Sr for some high Zn individuals. Beck's results clearly point toward more successful dietary studies from a multielement pattern analysis.

Results on Pathological Elements

The most revealing results to date on elements in bones associated with pathologies have come from Pb analysis. Early results were directed toward establishing Pb levels for preindustrial societies.[39] Waldron and co-workers[19,20] analyzed Romano–British and medieval British samples but found clear evidence for soil contamination in some cases. Analysis of Pb levels in terms of environmental exposure thus is difficult. Ericson et al.[40] examined Pb levels in Peruvian (4500–1400 BP) and Egyptian (2200 BP) skeletons. They also found evidence for soil contamination but were able to establish levels of Pb for comparison with modern populations.

Aufderheide et al.[41] reported a very successful study of Pb levels in burials at the Clifts Plantation site, Westmoreland County, VA (AD 1670–1730). Although diagenetic effects may have been present, their consequences were avoided by comparing two separate cemetery populations, which presumably had been subjected to similar contaminative processes. The north group contained five whites, the south group ten blacks and one white. The former group was associated with the planter population, the latter with the laborers. The mean for the planter group was 185 ppm, more than 5 times as large as that for the laborers, 35 ppm. Although the sample is quite small, there was no overlap in the proportions, 128–258 and 8–96 ppm. Analyses were by graphite furnace atomic absorption on several different skeletal components. The coffins were of pine, so that the differences could not have come from burial practices. The authors attributed the very high levels of Pb to the use of pewter and lead-glazed earthenware by the planter class. By comparison, a modern American carries about 50 ppm of Pb in the skeleton, and the ancient Peruvians had 1 ppm or less.[40] It was not possible to determine whether the high Pb levels in the planter population might have had a clinical effect.

Bahou[42] suggested the examination of several other elements to obtain information about pathological conditions, such as infectious diseases, developmental anomalies, degenerative conditions, tumors, traumas,

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(39) S. Jarcho, *Am. Antiq.*, **30**, 94 (1964); Z. Jaworowski, *Nature (London)*, **217**, 152 (1968).

(40) J. E. Ericson, H. Shirahata, and C. C. Patterson, *N. Engl. J. Med.*, **300**, 946 (1979).

(41) A. C. Aufderheide, F. D. Neiman, L. E. Wittmers, Jr., and G. Rapp, *Am. J. Phys. Anthropol.*, **55**, 285 (1981).

(42) W. F. Bahou, *Paleopath. Newsletter*, No. 11, 15 (1975).

and anemia. Although there are known dietary correlations, such as Fe deficiency with anemia or Zn deficiency with growth and maturation retardation, there have been no thorough studies on archaeological populations with appropriate controls to relate pathological conditions with elements other than Pb.

Conclusions and Prospectus

Sufficient data have now been accumulated to warrant the conclusion that inorganic analysis of archaeological bone provides a valid but crude measure of the ancient diet and of pathological conditions, provided that a certain protocol is followed. Skeletons must be in good condition and fully characterized according to sex, age, and, if available, status. Normally, individuals under the age of 12 should be excluded or analyzed as a distinct subgroup. For information about adult diet, tooth samples should be avoided. A given study should be limited to use of a single skeletal component, since elemental levels vary over the skeleton. Although most bones are acceptable, the cortical bones, such as the femur, are least sensitive to diagenetic effects. Samples should be ashed to remove organic material and water and to provide a common basis for comparison of analyses.

The number of samples should be large, because variance is large, on account of natural variation of elemental levels within a single bone, with the age of the individual at death, with pathological conditions, with the maternity history of females, and with other nondietary factors such as burial practices. As a possible guideline, no subgroup should have fewer than about a dozen members, so that its mean can be compared reliably with that of others. Comparison of single individuals or of statistically small groups should be avoided. It is important to obtain some information on the diagenetic status of the bone sample. Finally, as large an array of elements as possible is needed in order to have several handles on the ancient data. The elements Sr, Zn, Na, and Ca have proved to be most useful and least sensitive to diagenesis.

When studied in this manner, elemental levels can provide useful information on dietary differences between subgroups of a single culture, based for example on sex or status, or between temporally separated cultures that inhabited a single locale. Internal differences in turn may be compared on a more general basis between more distant cultures. Future studies should include a multielement comparison of juvenile and adult diet by parallel analysis of tooth enamel and cortical bone in the same population. Questions about acquired and inherited status might be answered. Multielement analysis of skeletons with observed pathologies, based on physical examination, should be useful in assessing diet-based and other health factors in ancient populations, provided statistically significant samples can be obtained.

As of the moment, the Sr–Zn two-dimensional plot offers the closest approach we have to pattern analysis. Na may provide a third dimension. Two additional advances must be made in order to achieve a better definition of the ancient diet. (1) More elements must be found that are free of diagenetic effects and have a proved dependence on diet. (2) The relationship between specific elements (Sr, Zn, Na, etc.) and specific components of the diet (leafy vegetables, nuts, meat, etc.) must be established through the use of laboratory animals. More sophisticated clustering procedures then could be used, based on the multidimensional data set of several elemental concentrations, to define more accurately the multidimensional diet of ancient populations.

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Stereoelectronic Effects on Acetal Hydrolysis

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This work has its origins in the pioneering X-ray structural studies of Phillips and his co-workers on the enzyme lysozyme,[1,2] as seen in the light of the stereoelectronic theory of Deslongchamps.[3,4] From the protein crystallography emerged a uniquely detailed picture of an enzyme–substrate interaction, which has inspired a generation of mechanistic studies on acetals.[5] The stereoelectronic theory has made chemists think explicitly about the way nonbonding (lone-pair) electrons can control reactivity. I describe in this *Account* some evidence that the remarkable way lysozyme binds its substrate can be explained as a predictable response to stereoelectronic factors, which control acetal hydrolysis

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(1) Imoto, T.; Johnson, L. N.; North, A. C. T.; Phillips, D. C.; Rupley, J. A. In "The Enzymes"; Boyer, P. D., Ed.; Academic Press: New York, 1972; Vol. VII, p 665.

(2) Ford, L. D.; Johnson, L. N.; Machin, P. A.; Phillips, D. C.; Tjian, R. *J. Mol. Biol.* 1974, *88*, 349.

(3) Deslongchamps, P. "Stereoelectronic Effects in Organic Chemistry"; Pergamon Press: Oxford, 1983; *Tetrahedron* 1975, *31*, 2463.

(4) Kirby, A. J. "The Anomeric Effect and Related Stereoelectronic Effects at Oxygen"; Springer-Verlag: Heidelberg–Berlin–New York, 1983.

(5) Dunn, B. M.; Bruice, T. C. *Adv. Enzymol.* 1973, *37*, 1.

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