

## Bone Stable Isotope Studies in Archaeology

Margaret J. Schoeninger<sup>1,3</sup> and Katherine Moore<sup>2</sup>

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*Stable isotope ratios of carbon and nitrogen in bone have become increasingly frequent inclusions in archaeological reports over the past few decades. The majority of such studies has been done in North America, where the use of marine foods and the introduction of maize have been monitored. Similar questions have been addressed in Mesoamerica and South America. In Europe, stable isotope ratios have documented the shift from marine fishing and gathering to agriculture in coastal areas and the introduction of millet in parts of eastern and southern Europe. Much work remains to be done in Asia, where millet replaced early C3 plant foods and, in turn, was replaced by rice. In Africa marine adaptations, freshwater fishing, agriculture, and pastoralism all yield diagnostic isotopic signatures. We review these studies, discuss areas requiring further study, and close with discussions of areas promising interesting future developments.*

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**KEY WORDS:** carbon; nitrogen; stable isotopes; paleodiet; subsistence; metabolism.

### INTRODUCTION

How do we gain understanding of the dynamics of prehistoric economic systems? Many methods have been applied, from the large scale of site distribution analysis and trading patterns to the smaller scale of seed and bone identification, phytolith analysis, and the study incremental structures in teeth. The focus of this paper is a method of even smaller scale, i.e., stable isotope analysis. This method traces prehistoric food intake using human skeletal material. In conjunction with other methods of analysis, it promises to extend our capacity

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<sup>1</sup>Department of Anthropology, University of Wisconsin—Madison, Madison, Wisconsin 53706.

<sup>2</sup>Department of Behavioral Science, Bentley College, Waltham, Massachusetts 02154.

<sup>3</sup>To whom correspondence should be addressed.

for investigating the lives of people long gone. The unit of analysis is the individual rather than the broader scale offered by studying site organization, features within a site, individual living floors, or trash deposits. The focus is on diet; it is up to the analyst to make the translation into economies and life-styles.

Many methods have been applied to the study of diet including several which focus on human biological remains. The scale of these also varies from the gross morphological assessment of skeletal pathologies to the microscopic analyses of fecal remains to the submicroscopic analysis of bone composition. The information obtained varies as well. When a human skeleton is sampled several years of consumption are represented. Both seasonal and longer-term variation are averaged over this time. Differences in diet between social and economic strata can be visible using stable isotopes, whereas they are difficult to confirm from middens or coprolites. On the other hand, because several food combinations can produce identical stable isotope values, midden and coprolite analyses are critical for identifying particular items of food. Thus, even though many methods reflect behavior relating to food, in combination they provide more information about behavior than any of them can separately. We urge the reader to keep this in mind even though our focus must, of necessity, be on one method.

Stable isotope analysis began as a geochemical technique. Initially both the archaeological and the geochemical communities accepted results as infallible for reconstructing diet in prehistory. In cases of disagreement between the archaeological and the bone composition evidence, some authors went so far as to say that the archaeological evidence must be incorrect (DeNiro and Epstein, 1981; Sealy and van der Merwe, 1985). Since that first flush it has become obvious that the situation is far more complicated than expected from simplistic mathematical models (Sillen *et al.*, 1989). The biological system is more "messy" than the geochemical one. The analysis of bone stable isotope composition does not provide a clear window to the past but, rather, permits one to look "through a glass darkly." Shading is produced by bone turnover rates, metabolic variation, and diet complexity, among other sources of biological variability.

The transformation of stable isotope analysis from a geochemical technique to an anthropological one has included accommodation of poorly preserved bone, long-curved museum specimens, and small sample sizes. This process has resulted from intense communication among scholars in anthropology, biology, chemistry, and geology. Now that anthropologists are familiar with the potentials of the technique, the process of applying the method to questions of interest has begun.

In this paper, written by a biological anthropologist (M.S.) and an archaeologist (K.M.), we provide an introduction to the method of stable isotope

analysis, an overview of previous work, and suggestions for the future. Due to space constraints and our particular area of expertise, we have restricted our discussions to stable isotope analysis of elements in bone. There is a great deal of fascinating work being done on paleoclimate and paleoenvironmental reconstructions using other materials and we direct the interested reader to those references (e.g., Ambrose and Sikes, 1991; Marino and McElroy, 1991).

### **The Need for Dietary Information: Why Study Diet?**

Human diets have expanded and changed dramatically over the course of human evolution. Due to the recognized importance of food for survival, the pattern of this evolutionary change and its social and economic consequences are of fundamental importance in studying human adaptation. In particular, three basic problems can be addressed through the reconstruction of past subsistence strategies. First, the response of human adaptations to climatic and environmental changes. Dietary change is often postulated as a bridge between changes in prehistoric environments and changes in prehistoric technologies. This link may be tested by demonstrating the actual foods eaten and by documenting the relative importance of each food. Second, the diffusion or expansion of new economic adaptations. The most striking case is the replacement of foragers by food-producers in many locations, but others, such as the incorporation of marine foods (Yesner, 1987), are also of interest. Knowledge of dietary intake permits evaluation of the causes and nutritional consequences of such a shift. Third, the emergence of stratified societies when access to foods may be restricted to members of a particular sex, age class, religious status, occupation, caste, or descent group (e.g., see Bogan, 1983; Huelsbeck, 1988). Such restrictions may have resulted in differences in health status and reproductive success.

### **Midden Analysis**

The incorporation of food remains into archaeological sediments may be deliberate or accidental but rarely appears to reflect use of those foods in any systematic fashion. Because the remains of plant and animal foods are heterogeneous in composition, they react differently to the conditions of burial in an archaeological sediment. When these midden deposits are sampled, the plant and animal remains recovered are an altered and biased sample of the original deposit, which was an imperfect sample of the prehistoric behavior in the first place.

Even so, the trend of dietary reconstructions from plant remains is one of growing sophistication within specific archaeological contexts. The problems of quantification of the relative items of plant foods in the diet have been explicitly considered (Hastorf and Popper, 1988; Pearsall, 1989b) and as well-studied

assemblages of plant remains accumulate for specific regions, the patterns of change in plant use at individual sites have been strengthened and clarified (e.g., Johannsen, 1988). The resolution of these regional patterns is usually limited to consistent changes in the frequency ranking of different plant foods or types of plant foods or, even less precise, the ubiquity of plants on a presence/absence basis, but they provide the best indication of the suite of foods used by early people.

Very few samples of animal food (that is, meat and fat) are recovered archaeologically, yet there is abundant information on the prehistoric use of such foods from the associated bones, teeth, shells, exoskeletons, eggshell, and occasional samples of hair and skin. The toughest of these materials (teeth, bones, and mollusk shell) are more resistant to decay than are plant remains. They also reflect the original discarded assemblage at a particular site much more accurately than do plant remains. Processes of destruction that take place before and after burial can still strongly bias the reconstruction of the original food represented, but animals occur in nature as predictable units (i.e., whole individuals), so when parts of an animal are recovered, the total original amount of food can be reconstructed.

Recent studies have been carried out to refine models for the food value represented by bones and shells. These take into account the size and condition of food animals, the butchering and processing practices used, the destruction of bones by dogs, and the damage from burial in different soils (Binford and Bertram, 1977; Bunn, 1991; Metcalfe and Jones, 1988). In addition, subtle changes in preferential utilization of specific parts of animal herds have been considered in relation to nutritional consequences (Speth, 1983). Even so, in a situation similar to that of plant remains, quantification of animal remains most often resolves at the level of order or percentage of abundance, although it, too, is critical as an indication of foods selected by people.

### Archaeological Implements

Tools are tied most closely to processing not to consumption, although they may indicate the use of previously unrecognized resources (Hill and Evans, 1989). At times, residues on the inner surfaces of ceramic vessels can be identified as a specific food on morphological grounds, and when residues are too amorphous due to processing or burial, identification may be made on chemical composition (e.g., Hall *et al.*, 1990). The identifications of foods and the association of foods with distinctive vessels and implements are important, but individual sherds represent very small samples of behavior. Only if many vessels or implements can be analyzed will the dietary importance of different foods be recognized.

### Nondestructive Analysis of Human Remains

In contrast to techniques based on food remains, analysis of human skeletal material revolves around the individual. Among these methods are microwear analysis of teeth, analysis of fecal material and intestinal content, paleopathology, bone shape assessment, and demographic analyses (for a recent review see Larsen, 1987). Some of these have served to discriminate between activity patterns such as agriculture and hunting/gathering (Bridges, 1989; Ruff *et al.*, 1984). In such cases, generalized information concerning subsistence is produced.

For the most part, however, the methods have been used to assess the health status of individuals. The demographic profile, degree of sexual dimorphism, and pathological conditions (including trauma) are responses to a combination of stimuli including infectious disease, nutrition, and sociocultural practices. Because these function as a unit, it is difficult to separate individual stressors; thus, dietary information is difficult to isolate. Similarly, micromorphology of bone cross sections have been used to infer nutritional adequacy, not specific diet items (Martin *et al.*, 1985).

Tooth microwear has been monitored for compression fractures associated with nut consumption, surface polishing with vegetable fiber, and the disappearance of polishing with the introduction of maize agriculture (Rose and Harmon, 1986). If this method can be refined, it may prove useful in other situations. It provides a record of foods processed in the mouth over several months preceding the death of the individual (Walker *et al.*, 1978). Thus, it could serve to indicate seasonal diets or in cases where seasonality does not occur, the method may provide specific dietary information.

Similar limitations apply to human fecal remains. They appear to indicate pollen-bearing and fibrous plants consumed over the course of a day or so but provide little evidence for animal foods except for odd hair, feathers, bones, and skin. Chemical analysis of lipids diagnostic of meat, leaves, and fruits may provide further qualitative evidence for dietary composition (Marchbanks, 1989). Although they have been used to study diet, seasonality of occupation, and health status (Fry, 1985), the rarity of preservation limits the number of applications.

### Combining Techniques

The goal in dietary analysis is to identify both the different foods and the proportions of foods in the diet. As indicated above, archaeological remains provide strong qualitative reconstructions of diet, biased estimates of relative proportions of diet within a class of similar food items, and limited indication of the relative proportions of some fundamental dietary categories. The scale of

reconstruction possible in time, space, and generality is summarized in Table 1.

Though it is difficult to confirm field impressions, excavators often sense that the remains from archaeological features and layers represent very small samples of food processing or discard behavior (e.g., Wright *et al.*, 1981). Fine-scale recovery techniques can produce seasonal or functional assemblages of remains (e.g., Pearsall, 1989a), though this level of precise recovery is rare. Combining seasonal with spatial variability allows estimation of a regional settlement-subsistence system. The resolution of bone composition analysis, based on stable isotope ratios of specific elements, is quite different. Bone composition analysis offers a quantitative estimate of food intake at the level of the individual; samples including larger numbers of individuals can provide views of community and regional variability. It also represents long-term averaging of individual diets; the seasons and places in which foods were obtained are usually not reflected in this average.

The greatest progress in interpreting bone composition has been made where assemblages of food remains have also been studied. The early anticipation that new analytical techniques would replace traditional analyses or allow dietary estimates in the absence of plant and animal remains has not been generally met. In some cases, bone composition studies have provided an apparent confirmation of a dietary reconstruction based on traditional techniques of midden

**Table 1.** Comparison of Techniques for Diet Reconstruction

Technique	Generality of analytical results	
	Resolution within the social unit	Resolution of time
Stable isotopes	Individual to population	10 to 20 years, reflects average of lifetime and ontogenetic diet changes
Midden/trash analysis	Community	Short-term to hundreds of years <sup>a</sup>
Midden/feature analysis	Household/household cluster	Short-term use life of each feature, no more than several years in most cases <sup>b</sup>
Residues on pots, tools	Household	Days to weeks—residue reflects last use of implement
Coprolites	Household/structure	Daily variation, observations jumbled in latrine areas
Skeletal analysis	Individual to population	Individual lifetime or shorter, healed traces may reflect period long before death
Paleoenvironmental reconstruction	Community/population	Tens to hundreds of years, depending on source of data: tree rings, pollen, etc.

<sup>a</sup>The longer a site is occupied, the more difficult it is to relate specific occupations to trash deposits. Regional differences between contemporary sites can reflect specific resources production and use.

<sup>b</sup>Sealed feature deposits provide similar regional and spatial differentiation to open midden deposits but provide better chronological control.

and skeletal analysis. In other cases, the lack of fit between reconstructions based on archaeological remains and bone composition data have highlighted the weaknesses of one or the other technique. On one side is the imprecision of archaeological remains, and on the other, the incomplete understanding we have of variability in stable isotope ratios. In individual studies, it has been difficult, at times, to evaluate where the greater problem lies. It is our hope that the following will serve to stimulate new scholars to begin their own investigations, thereby increasing our overall understanding of prehistoric subsistence systems.

## STABLE ISOTOPE ANALYSIS

### Background

Most elements exist as composites of two or more isotopes. Although isotopes can be stable or unstable radioactively, the discussion in this paper is limited to stable isotopes, of which approximately 300 have been recognized across all elements. Of these, over 10 elements of biological interest have more than one stable isotope, but only 6 (C, N, O, H, S, and Sr) are mentioned in this paper. Of these six, the focus is on C and N; the discussion of O, H, S, and Sr is limited to the section on Future Potential.

Isotopes of a single element are atoms that differ in the number of neutrons in their nuclei but which contain the same number of protons and electrons (for an excellent introduction to the subject, see Hoefs, 1987). Because of this equality in the number of protons, all isotopes of a single element have the same atomic number. Because of the equality in the number of electrons, all isotopes of a given element behave similarly in chemical reactions. They differ, however, in mass, which can result in a difference in the rate of reaction between isotopes of an element. The magnitude of the effect is determined by relative differences in mass between reactive species so that the greatest effect is seen among small molecules of the lighter elements (e.g.,  $^{13}\text{CO}_2$  versus  $^{12}\text{CO}_2$ ). The difference in rates often results in reaction products that have different isotopic compositions than that of the source material. For example, atmospheric  $\text{CO}_2$  (the product, in this case) has a smaller  $^{13}\text{C}/^{12}\text{C}$  ratio (i.e., there is less  $^{13}\text{C}$  relative to  $^{12}\text{C}$ ) than does the ocean (the source, in this case). This occurs because the bonds containing  $^{12}\text{C}$  break and form more rapidly than do bonds containing  $^{13}\text{C}$  during the transfer of carbon from the oceanic pool to the atmosphere.

The method of measuring the abundance of isotopes of an element uses a mass spectrometer. The material of interest (collagen or plant material) is either combusted at high heat or treated chemically to liberate gases such as carbon dioxide ( $\text{CO}_2$ ) and nitrogen gas ( $\text{N}_2$ ). When a volume of  $\text{CO}_2$  is released into the mass spectrometer, its electronic detector distinguishes between the stable

isotopes of carbon by measuring the concentration of molecules of mass 44 ( $\text{CO}_2$  composed of  $^{12}\text{C} + ^{16}\text{O} + ^{16}\text{O}$ ) versus those of mass 45 ( $\text{CO}_2$  composed of  $^{13}\text{C} + ^{16}\text{O} + ^{16}\text{O}$ ) and those of mass 46 ( $\text{CO}_2$  composed of  $^{12}\text{C} + ^{18}\text{O} + ^{16}\text{O}$ ). Similarly in a volume of  $\text{N}_2$ , the mass spectrometer's electronic detector records the impact of mass 28 ( $^{14}\text{N}/^{14}\text{N}$ ) and of mass 29 ( $^{15}\text{N}/^{14}\text{N}$ ) in distinguishing between the two isotopes ( $^{15}\text{N}$  and  $^{14}\text{N}$ ). The other possibility ( $^{15}\text{N}/^{15}\text{N}$ ), with a mass of 30, occurs so rarely that most mass spectrometers do not record the impact.

During the process of measurement, the gas from the sample is compared with that from a laboratory standard (usually tank  $\text{CO}_2$  and processed lab air for  $\text{N}_2$ ). These lab gas standards must be calibrated relative to international standards. For carbon the internationally recognized standard is PeeDee Belemnite Carbonate (PDB), a marine carbonate. For nitrogen, the sample ratio is reported relative to AIR (ambient inhalable reservoir), which became the internationally recognized standard following the demonstration that the isotope ratio of  $\text{N}_2$  in the atmosphere is constant across the globe (Mariotti, 1983). Today's mass spectrometers are attached to computers programmed to compare the sample mass to the lab standard mass and to perform the calculations necessary to translate it into a comparison with the international standard. Thus, although few laboratories actually have any PDB for measurement, they can provide analyses which have been standardized to PDB. These comparisons are presented as delta ( $\delta$ ) values in parts per thousand ("per mil," represented by the symbol ‰), as shown in the equations below.

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

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

Since the majority of biological materials have less  $^{13}\text{C}$  relative to  $^{12}\text{C}$  (i.e., lower  $^{13}\text{C}/^{12}\text{C}$  ratios) than does PDB, most biological samples have negative  $\delta^{13}\text{C}$  values. This is due to historical accident. The first geochemical investigations were carried out at the University of Chicago. When a standard was needed, they used a material that was on hand and available in abundance: PDB. The standard is still referred to by some as the Chicago limestone (Cai and Qiu, 1984). The majority of biological materials have larger  $^{15}\text{N}/^{14}\text{N}$  ratios than the abundance ratio in the atmosphere, thus, most biological samples have positive  $\delta^{15}\text{N}$  values.

As mentioned above, in most cases the laboratory gas standard is  $\text{N}_2$  collected from air. Producing nitrogen isotope standards is a nontrivial yet critical problem, and when sending samples out for analyses, one should be aware of the difficulties. In addition, there are two major difficulties in producing and

handling  $N_2$ : incomplete combustion of the sample and errors in vacuum-line collection. Although most readers of this paper will not be preparing and analyzing their own samples, they should be aware of the problems and request that the analyst do the following. Comparative data across several laboratories should be available; in other words, samples should have been sent to several laboratories and the same values obtained. As a check for both preparation problems mentioned above, duplicate samples (approximately 10% of the sample set) must be combusted and analyzed. These duplicates should be prepared separately and run on the mass spectrometer on different days. In addition, a laboratory combustion standard should be included in each set of samples prepared for combustion. The analyst should report the replicability of measurements on the laboratory combustion standard. It should be of the order of 0.2‰ for  $\delta^{13}C$  and 0.3‰ for  $\delta^{15}N$ .

### Carbon Sources and Stable Isotope Ratios

There are two stable isotopes of carbon,  $^{13}C$  and  $^{12}C$ , with natural abundances of approximately 1.1 and 98.9%, respectively (Hoefs, 1987; Schoeninger, 1990). The majority of the world's carbon is nonbiological and is contained in the oceans at the natural abundance ratio. As mentioned above, during the transfer of carbon from the oceanic pool to the atmosphere, carbon dioxide ( $CO_2$ ) in the atmosphere is depleted in  $^{13}C$  relative to the oceanic pool. Both atmospheric carbon and oceanic carbon are transferred into the biological system largely through photosynthesis by green plants and chemosynthesis by bacteria in symbiotic relation with deep sea vent organisms. Both processes increase the concentration of  $^{12}C$  relative to  $^{13}C$  in living organisms compared with source carbon.

Atmospheric  $CO_2$  is the major carbon source for all terrestrial plants. The  $\delta^{13}C$  value today is about  $-7‰$  but would have been closer to  $-5$  to  $-6‰$  prehistorically due to the lack of fossil fuel carbon contribution (Keeling, 1961; Bada *et al.*, 1990; Marino and McElroy, 1991). Plant  $\delta^{13}C$  values are determined by the isotopic composition of atmospheric  $CO_2$  and the plant's particular photosynthetic pathway. Three such pathways occur among terrestrial plants, commonly referred to as the C3, C4, and CAM pathways. C3 plants are those that undergo C3 photosynthesis, so named because the first product contains three carbon atoms; C4 plants undergo C4 photosynthesis, in which the first product is a compound containing four carbon atoms. The CAM pathway is named for crassulacean acid metabolism, which has been found and studied in succulents; these plants utilize either a C3 or a C4 photosynthetic pathway, depending on environmental conditions. Certain CAM species, such as *Opuntia*, however, appear to use the C4 pathway fairly consistently. The C4 plants include such crops as maize, millet, and sorghum, which have  $\delta^{13}C$  values ranging from  $-9$

to  $-16\text{‰}$ , with the mode at about  $-12\text{‰}$  (see Fig. 1). The C3 plants, which are more common in the higher latitudes, have  $\delta^{13}\text{C}$  values between  $-20$  and  $-34\text{‰}$ , with most species about  $-26\text{‰}$ . The CAM plants under hot, arid conditions use a C4-like pathway resulting in  $\delta^{13}\text{C}$  values like those of C4 plants (for a recent review see O'Leary, 1988).

Marine organisms use several carbon sources (Hoefs, 1987; Peterson *et al.*, 1980) including terrestrial detritus washed into the oceans by rivers (with  $\delta^{13}\text{C}$  values representative of a mixture of local terrestrial plants), dissolved  $\text{CO}_2$  with  $\delta^{13}\text{C}$  values of atmospheric  $\text{CO}_2$  ( $-7.0\text{‰}$ ), and dissolved carbonic acid with  $\delta^{13}\text{C}$  values close to zero ( $0.0\text{‰}$ ). For this reason, the  $\delta^{13}\text{C}$  values in marine plants can overlap the values in terrestrial plants. Sea grasses have  $\delta^{13}\text{C}$  values like those of C4 plants, whereas cold water plankton values are closer to those of C3 plants. Most planktonic species, which provide the principal source of carbon for higher organisms in the marine system, are intermediate in  $\delta^{13}\text{C}$  values between C3 and C4 plants. For this reason, the majority of marine vertebrates have  $\delta^{13}\text{C}$  values between the extremes delineated by terrestrial C3 and C4 plants. Freshwater organisms use a mixture of carbon from terrestrial detritus and dissolved  $\text{CO}_2$ . Their  $\delta^{13}\text{C}$  values reflect the relative contribution of carbon sources (Rau, 1978; Schell, 1983).

### Nitrogen Sources and Stable Isotope Ratios

Nitrogen also consists of two stable isotopes,  $^{15}\text{N}$  and  $^{14}\text{N}$ , with a natural abundance ratio of 0.36 to 99.64% (Hoefs, 1987). Over 99% of the world's nitrogen is bound as  $\text{N}_2$  in the atmosphere or dissolved in the ocean. Two major processes account for the transfer of nitrogen into the biological realm. The first depends on  $\text{N}_2$ -fixing organisms, i.e., blue/green algae in aqueous situations (both marine and freshwater) and bacterial nodules on terrestrial plant roots. This process results in synthesized tissues with  $\delta^{15}\text{N}$  values similar to atmospheric  $\text{N}_2$  (i.e., close to zero). The second major process involves bacterial breakdown of complex nitrogen-containing molecules in organic matter following death of organisms; nitrates are produced which can be used directly by vascular plants. The  $^{15}\text{N}/^{14}\text{N}$  ratios of these nitrates contain more  $^{15}\text{N}$  relative to  $^{14}\text{N}$  than is true in the atmosphere. For this reason, in part, the plants using these nitrates tend to have slightly more positive  $\delta^{15}\text{N}$  values than do  $\text{N}_2$ -fixing plants. A broad range of values is observed in terrestrial plants overall, although the majority of terrestrial plants have values similar to atmospheric  $\text{N}_2$  (Virginia and Delwiche, 1982; Wada *et al.*, 1975).

The vast majority of available nitrogen in the marine system is produced by bacterial denitrification, with greater amounts of  $^{15}\text{N}$  than is true for dissolved  $\text{N}_2$ . Thus, although there is considerable variation in marine  $\delta^{15}\text{N}$  values, the majority is more positive than atmospheric nitrogen (Wada, 1980). Marine orga-

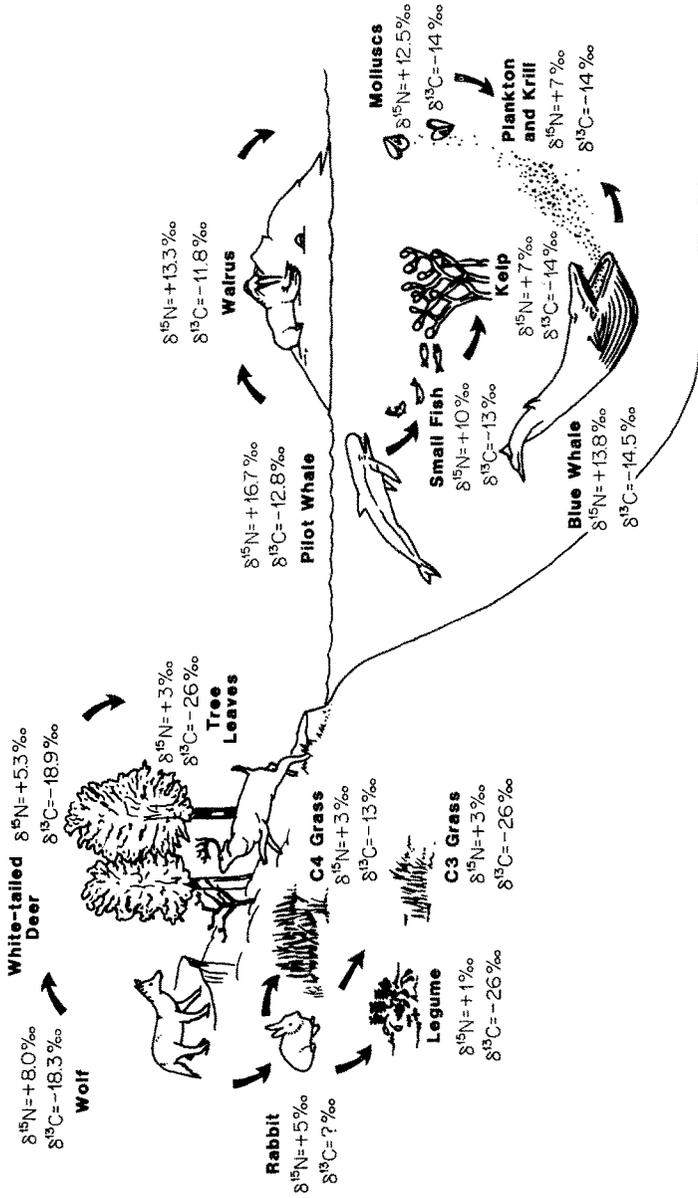


Fig. 1. Simplified drawing of the distribution of stable isotope ratios of carbon and nitrogen in the biosphere.

nisms at the base of food chains are, for the most part, more positive in  $\delta^{15}\text{N}$  values than are terrestrial plants. In most areas of the world, marine vertebrates are significantly more positive than are terrestrial vertebrates (Schoeninger and DeNiro, 1984).

### Trophic-Level Effects

Within individual trophic systems, there is an increase in  $\delta^{13}\text{C}$  and  $\delta^{15}\text{N}$  values between trophic levels (McConnaughey and McRoy, 1979; Wada, 1980). Because it is of the order of only 1‰ in carbon, it is not discernible in any but the best-controlled systems. For nitrogen, on the other hand, it is approximately 3‰ (see Fig. 1). The magnitude of this spacing holds true among invertebrates (Wada, 1980), marine vertebrates (Minagawa and Wada, 1984; Schoeninger and DeNiro, 1984; Wada, 1980), and terrestrial vertebrates (Schoeninger and DeNiro, 1984; Schoeninger, 1985, 1989). When herbivores feed on plants there is a concentration (enrichment) of the  $^{15}\text{N}$  isotope relative to  $^{14}\text{N}$ . Thus, the tissues of the herbivores have more positive  $^{15}\text{N}/^{14}\text{N}$  ratios than that in the plants upon which they feed. This continues into the carnivore level; i.e., carnivore tissues contain more  $^{15}\text{N}$  relative to  $^{14}\text{N}$  than is true for herbivore tissues. This occurs because during metabolism, the bonds between  $^{12}\text{C}$  and  $^{14}\text{N}$  break more readily than those between  $^{12}\text{C}$  and  $^{15}\text{N}$ . Relatively more  $^{14}\text{N}$  is excreted in urea, leaving relatively more  $^{15}\text{N}$  available for use in tissue synthesis.

The stepwise enrichment is obvious, however, only when "the original nitrogen sources are all equivalent" (Minagawa and Wada, 1984, p. 1137). It has become clear that comparisons across systems are not valid since the isotopic composition of the sources is not necessarily equivalent across systems. For example, the marine system and the terrestrial systems have significantly different source nitrogen in most geographic areas and, thus, cannot be compared directly. Even so, within trophic systems the 3‰ enrichment appears to hold constant across a wide variety of situations (Vogel *et al.*, 1990).

### Dietary Information from Stable Isotope Analysis

The results from two laboratory studies (Bender *et al.*, 1981; DeNiro and Epstein, 1978; DeNiro and Epstein, 1981) and a field study (Vogel, 1978) in the late 1970s form the basis of all present attempts (initiated by van der Merwe and Vogel, 1978) to extract human dietary information from stable isotope ratios. The laboratory and field studies demonstrated that the isotopic composition of an animal's tissues reflects the isotopic composition of the animal's diet. Although the  $\delta^{13}\text{C}$  and  $\delta^{15}\text{N}$  values differ across an animal's tissues (DeNiro and Epstein, 1978, 1981; Sealy *et al.*, 1987; Tieszen *et al.*, 1983), it was assumed, based on initial results, that the choice of a single tissue for analysis

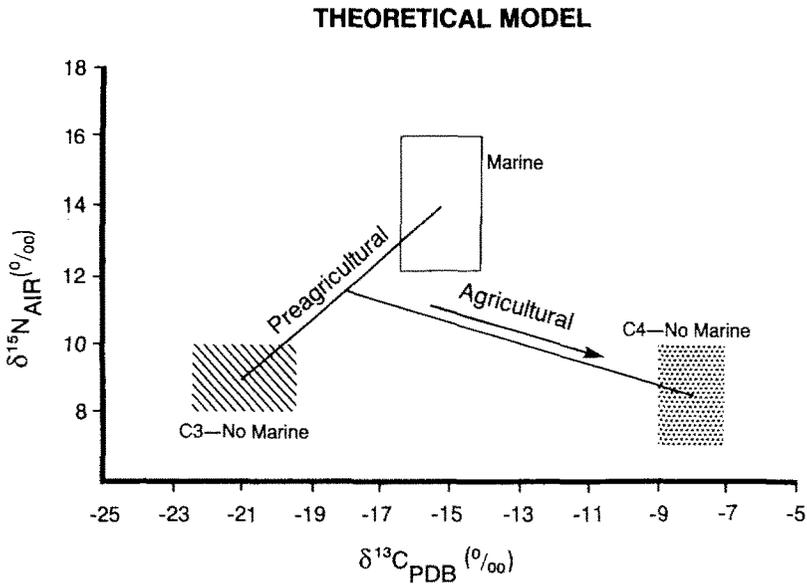
(e.g., bone collagen) would ensure a consistent spacing between the tissue and the diet. The isotopic composition of the diet could, therefore, be calculated from the isotopic composition measured in the animal's tissues. The situation has proven to be more complicated.

In carbon, small animals (mice, chickens, and gerbils) raised on diets of known isotopic compositions (Bender *et al.*, 1981; DeNiro and Epstein, 1978; Tieszen *et al.*, 1983) had  $\delta^{13}\text{C}$  values which differed from that of the diet by 1 to 3‰. Large, free-ranging ruminants with estimated, not measured, diet  $\delta^{13}\text{C}$  values had collagen 5–6‰ less negative than the estimated diet. There is a great deal of uncertainty in both types of studies. At the present time the most reasonable estimate of the difference between diet and collagen for carbon appears to be of the order of 3–5‰. The absolute magnitude is important only in those cases where proportions of particular diet components are being estimated.

The magnitude of the difference in  $\delta^{15}\text{N}$  values between diet and bone collagen also remains somewhat uncertain. Laboratory studies on mice (DeNiro and Epstein, 1981) and pigs (Hare *et al.*, 1991) and some field studies (Vogel *et al.*, 1990; Wada, 1980) indicate that bone collagen and other proteins are approximately 3‰ more positive than diet. In an intriguing comparison of fingernails from mothers and their nursing babies, the babies' fingernails were approximately 2‰ enriched over those of the mothers' fingernails (Tuross *et al.*, 1991). Other analyses of bone collagen from free ranging ruminants (Ambrose and DeNiro, 1986a; Heaton *et al.*, 1986; Schoeninger, 1989; Sealy *et al.*, 1987), however, suggest that in some cases bone collagen is enriched far more than 3‰. Additionally, indirect evidence suggests that the bone collagen value in adult humans is often greater than 3‰ more positive than that of diet (Schoeninger, 1989). Reasons for these results are discussed below, under Knowledge of Metabolism.

Although it may not be possible at present to calculate absolute diet isotopic ratios based on measured isotope ratios in collagen, there are several aspects of diet that can be determined based on the ratios in collagen. As discussed above in the section on carbon isotope ratios, the two major photosynthetic pathways in plants result in significantly different  $\delta^{13}\text{C}$  values. Because the carbon isotope ratios in collagen are determined by diet values, the dependence on C3 versus C4 plants is recorded in collagen even though the exact percentages of each cannot be estimated precisely at this time. Further, the dependence on marine versus terrestrial foods is also recorded in the carbon isotope signal because of the difference in carbon sources in the two environments. A complication arises when a C4 plant and marine foods are both possible diet items. As depicted in Fig. 2, nitrogen isotope ratios should also be used when this is a possibility because marine foods have  $\delta^{15}\text{N}$  values that are distinguishable from those of terrestrial foods in most geographic areas.

Following this line of reasoning, it should also be possible to separate



**Fig. 2.** Theoretical model of expected stable isotope values in human bone collagen from preagricultural populations using no marine foods, preagricultural populations using a significant amount of marine foods, and maize agriculturalists with decreased or no use of marine foods. (Redrawn from Schoeninger *et al.*, 1990).

dependence on plants that fix atmospheric nitrogen (e.g., legumes, such as beans) from dependence on plants that do not. There has, however, been neither a laboratory experiment nor a field study that demonstrates that feeding on leguminous plants necessarily results in collagen with  $\delta^{15}\text{N}$  values less positive than normal. Further, it is striking that low  $\delta^{15}\text{N}$  values indicative of beans have never been detected as food residues on cooking utensils in archaeological studies (Morton *et al.*, 1991). Archaeological beans that have been analyzed have had  $\delta^{15}\text{N}$  values more positive than zero (Spielmann *et al.*, 1990). It seems possible, therefore, that domesticated beans grown in agricultural plots do not fix nitrogen but, instead, make use of nitrates available as the result of organic decay. A further complication is that many nonleguminous plants have  $\delta^{15}\text{N}$  values similar to those of  $\text{N}_2$ -fixing plants (Minagawa and Akazawa, 1991; Virginia and Delwiche, 1982).

Because of the trend of increasingly positive  $\delta^{15}\text{N}$  values (Schoeninger and DeNiro, 1984) from the level of primary producers to that of top consumers, it was hoped that the values might prove useful in identifying meat consumption among prehistoric peoples. Despite early enthusiasm (Schoeninger, 1985), however, this has been largely unsuccessful (Spielmann *et al.*, 1990; but see claims of Bocherens *et al.*, 1991). In part, the reason for this lies in the relatively small

difference in  $\delta^{15}\text{N}$  values between trophic levels. Most questions involving humans do not revolve around whether or not humans were carnivores or herbivores, a difference expected to produce a 3‰ difference in bone collagen  $\delta^{15}\text{N}$  values. In most cases, the question is more likely to be whether humans ate 10 versus 25% meat. With few exceptions, human groups do not consume greater than 25% of their calories as meat protein (Noli and Avery, 1988) and estimates suggest that humans could not survive on greater than 40% meat protein. The contradictions, such as Eskimos (Draper, 1977) and the Dogrib (Szathmary *et al.*, 1987), eat the vast majority of their calories (>60%) as fat and, thereby, survive on a diet that contains up to 40% of total calories from meat protein. Assuming a constant linear relationship between diet  $\delta^{15}\text{N}$  and bone collagen  $\delta^{15}\text{N}$ , a difference of 15% meat in diet between 10 and 25% would be expected to produce less than a 0.5‰ difference in bone collagen  $\delta^{15}\text{N}$ . Such a difference would be obvious only as a difference in means between two groups, each with a large number of individuals, where there is little variation within each group. It may be possible to use  $\delta^{15}\text{N}$  values among human groups with comparable diets to indicate greater or lesser dependence on meat but the estimate will necessarily be crude.

One possible application of the trophic level effect was demonstrated in a prehistoric group (Tuross *et al.*, 1991) where youngsters under the age of 4 years had, on average, bone collagen  $\delta^{15}\text{N}$  values 2.0 to 2.5‰ more positive than those in adults. These children were presumably nursing or recently weaned. It appears possible to use  $\delta^{15}\text{N}$  values to determine weaning age across different populations and between groups within populations.

### Material Used in Analyses

The majority of studies in archaeology have used bone for analysis because it is the material most often preserved. Bone is a complex tissue composed of several constituents, several of which contain carbon and/or nitrogen, which could, potentially, be useful for human dietary studies. Collagen, the main protein found in bone, contains both carbon and nitrogen and is relatively insoluble due to extensive linkages between each of three equal-sized chains that comprise the molecule. Because of this stability, even degraded bone often contains some collagen residues. This, in addition to the large fraction of bone (approximately 25% by weight) that it represents, has resulted in its use for most dietary studies and it receives the majority of attention in the pages which follow.

Noncollagenous proteins (NCPs) contain both carbon and nitrogen but represent a much smaller fraction of bone (approximately 2% by weight). Their potential in diet studies has received some attention because there is evidence that these noncollagenous proteins are preserved preferentially relative to col-

lagen in archaeological and fossil bone (Hare, 1980; Tuross *et al.*, 1989). The carbon and nitrogen in these proteins have stable isotope ratios indicative of diet in the cases where it has been studied (Masters, 1987). More work is necessary because of the technical difficulties of separating pure NCPs from collagen degradation products (DeNiro and Weiner, 1988; Solanko, 1989) and because of their low concentration.

The remaining portions of bone contain carbon but not nitrogen. The small carbohydrate and lipid fractions are rapidly removed from bone after burial (Evershed, 1990) and, thus, are unusable for diet studies. The mineral fraction of bone contains a measurable amount of carbon as carbonate, which appears to record dietary information, although there has been much controversy over the exact message (Krueger and Sullivan, 1984; Sullivan and Krueger, 1981). The spacing between diet and apatite carbon and, thus, between apatite carbon and collagen carbon appears to differ slightly depending on the trophic position of the animal. The spacing between apatite carbon and collagen carbon appears to be about 8‰ for herbivores and 4‰ for carnivores (Koch *et al.*, 1990; Krueger and Sullivan, 1984; Lee Thorp *et al.*, 1989), although this has not been demonstrated conclusively experimentally or empirically. Theoretically, a plot of collagen  $\delta^{13}\text{C}$  against apatite  $\delta^{13}\text{C}$  could be used to indicate the trophic position of an animal. In reality, it has proven useful only for the C4 end of the distribution between "pure" carnivores and herbivores due to the "overlap in the distribution of values for herbivores and carnivores, . . . in the C3 range" (Lee Thorp *et al.*, 1989, pp. 590–591). Within the C4 range, the method is applicable to only the last 20,000 years or so because collagen is normally retained for less than 20,000 years in bone. One attempt to apply it to human omnivory was not successful (Lee Thorp *et al.*, 1989), although another has produced promising results (Roksandic *et al.*, 1988).

## PREVIOUS INVESTIGATIONS

### North America

Several studies using stable isotope data have focused on areas in North America. As these can be separated into major geographic areas, they are discussed in turn below. Figure 3 presents data from some of these studies. Examples of distinctly different dietary adaptations were chosen to demonstrate the extremes of stable isotope ratios in bone collagen.

*West Coast.* In this area, the stable isotope method has been used to determine the use of marine foods throughout prehistory and the Contact period from the northernmost portion of Alaska to the southernmost part of California. Given

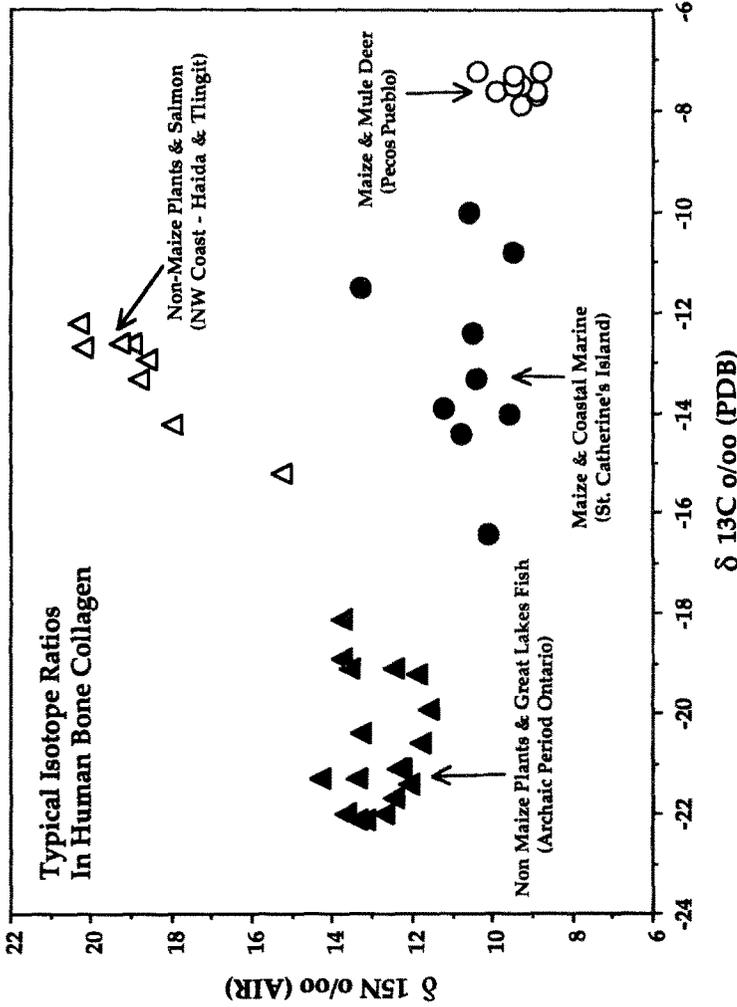


Fig. 3. Examples of stable isotope values produced by various dietary adaptations across North America. The northwest coast populations represent complete marine adaptation, the southwest population represents complete C4 adaptation (maize), the Ontario population represents complete C3 adaptation (premaize), and the St. Catherine's Island population represents a mix of C4 (maize), C3 (deer), and marine. Data redrawn from Schoeninger and DeNiro (1984), Schoeninger *et al.* (1990), Schwarcz *et al.* (1985), and Spielmann *et al.* (1990).

the absence of maize as an agricultural product, both carbon and nitrogen isotope values serve to indicate dependence on marine foods in the absence of C4 plants, thereby providing baseline values for comparison with other areas. The earliest work by Chisholm (1982) and a subsequent study by Lovell (1986) presented only carbon data, but the remainder have presented both carbon and nitrogen.

Significantly, the results among the studies are very consistent. Prehistoric and recent dwellers of the Northwest coast had a range of  $\delta^{13}\text{C}$  values between  $-14$  and  $-13\text{‰}$  (Chisholm *et al.*, 1982) in one study and  $-15$  and  $-13\text{‰}$  in another (Schoeninger *et al.*, 1983). In southern California late prehistoric coastal dwellers had similar carbon values [ $-15$  to  $-14\text{‰}$  (Walker and DeNiro, 1986) and  $-16$  to  $-13\text{‰}$  (Schoeninger *et al.*, 1983)]. Modern Eskimo displayed a slightly larger range [ $-17$  to  $-11\text{‰}$  (Schoeninger *et al.*, 1983)] in one study but not in another [ $-13\text{‰}$  (Chisholm *et al.*, 1982)].

The nitrogen values are also very consistent with modern Eskimo and northwest fishing people [ $17$  to  $20\text{‰}$  (Schoeninger *et al.*, 1983)] very similar to late Prehistoric dwellers on the southern California coast [ $16$  to  $18\text{‰}$  (Walker and DeNiro, 1986) and  $14$  to  $19\text{‰}$  (Schoeninger *et al.*, 1983)]. Prehistoric people from the interior of California have isotope values indicative of less use of marine products [range of  $\delta^{13}\text{C}$  between  $-19$  and  $-17\text{‰}$  and range of  $\delta^{15}\text{N}$  between  $7$  and  $13\text{‰}$  (Walker and DeNiro, 1986)].

Few reports have attempted to translate the isotope values into percentage of different foods, but based on  $\delta^{13}\text{C}$  results, Chisholm suggested that 85–100% of the carbon in bone collagen of people between 2000 B.P. and recent times in British Columbia was derived from marine sources. Previous estimates based on archaeological evidence had been of the order of 55% of the total calories from marine sources. Chisholm's work first pointed to the possible partitioning of diet sources of carbon (carbohydrate, lipid, and protein) in terms of uptake during tissue synthesis (discussed below, under Knowledge of Metabolism). Although the final conclusions have not been reached, it is becoming apparent that some partitioning is occurring.

For the most part, the data have been used to indicate relative diet differences between areas and through time. The use of anadromous salmon by the inhabitants of interior sites in British Columbia was monitored by demonstrating a decrease in  $\delta^{13}\text{C}$  values in human bone collagen from coastal to more inland sites (Lovell *et al.*, 1986). In skeletal remains from the Channel Islands, the mainland coast, and the mainland interior (Walker and DeNiro, 1986), the mean isotopic ratios for both carbon and nitrogen decreased progressively from the islands (range,  $-15$  to  $-14\text{‰}$  in carbon and  $15$  to  $18\text{‰}$  in nitrogen) to the mainland coast (range,  $-18$  to  $-13\text{‰}$  in carbon and  $8$  to  $19\text{‰}$  in nitrogen) to the inland sites (range,  $-19$  to  $-17\text{‰}$  in carbon and  $7$  to  $13\text{‰}$  in nitrogen).

Interestingly, there was a significant amount of variation within each of

these areas. Two of the individuals from the coast exhibit more positive carbon and nitrogen isotopic ratios than occurred among any of the people from the islands. Walker suggested that this might be due to sexual division of labor and exchange of marriage partners (Walker and Erlandson, 1986) but noted that too few skeletons could be sexed to permit testing his hypotheses. There is also an increase in the isotopic ratios through time along the coast and in the interior, confirming previous evidence for increased dependence on marine foods through the late prehistoric period (Walker and Erlandson, 1986).

*East Coast.* As with the west coast studies, the emphasis on the east coast has been the dependence on marine products throughout prehistory and into the protohistoric period. The use of maize as an agricultural product in the eastern United States is a complicating factor for carbon isotope studies along this coast, and for this reason, these studies have included stable nitrogen isotope analysis. Figure 2 indicates the theoretical expectations for a shift in collagen stable isotope values resulting from the inclusion of maize in two model diets. One of these is a marine adaptation in an otherwise C3 environment; the other is an inland adaptation without C4 plants.

In the northern sector, a study of Nantucket Island off the Massachusetts coast in the period around 1000 A.D. (Medaglia *et al.*, 1990) revealed less negative carbon values ( $-11$  to  $-10\text{‰}$ ) coupled with less positive nitrogen values ( $14$  to  $16\text{‰}$ ) than those found on the west coast even though the modern fauna and flora on both coasts are similar in isotopic composition (Little *et al.*, 1991). This period of time on Nantucket provides no convincing archaeological evidence for maize agriculture, but the  $\delta^{13}\text{C}$  values of human bone collagen indicate either that the majority of the carbon in human collagen came from marine foods or that a significant amount of a C4 plant (or a CAM plant such as *Opuntia* with a C4 signature) was being consumed. The  $\delta^{15}\text{N}$  values can be interpreted as indicating a major dependence on marine foods, although the values are not as positive as those measured among Eskimo populations (compare with Fig. 3).

Farther to the south, isotopic analyses of samples were carried out as part of a study on the prehistoric life of the Guale Indians on the Georgia coast and on the impact by Spaniards in the 15th and 16th centuries (Larsen, 1990; Thomas, 1990). In combination, less negative  $\delta^{13}\text{C}$  values and stable  $\delta^{15}\text{N}$  values (Schoeninger *et al.*, 1990) documented the shift to maize agriculture with continuing dependence on marine foods around 1000 A.D. (preagricultural  $\delta^{13}\text{C}$  values,  $-19$  to  $-14\text{‰}$ ; agricultural,  $-16$  to  $-10\text{‰}$ ; and preagricultural  $\delta^{15}\text{N}$  values,  $11$  to  $14\text{‰}$ ; agricultural,  $10$  to  $13\text{‰}$ ). During the time of Spanish control, the diet of the native populations changed again. This time there was a further increase in dependence on maize accompanied by a virtual abandonment of marine foods evidenced by less negative  $\delta^{13}\text{C}$  values ( $\delta^{13}\text{C}$  values,  $-14$  to  $-10\text{‰}$ ) and less positive  $\delta^{15}\text{N}$  values ( $7$  to  $11\text{‰}$ ). The decrease in the use of

marine foods is associated with demographic shifts and changes in pathological indicators, all of which suggest a decrease in the overall health of the population (Larsen *et al.*, 1990).

*Eastern Interior.* Because there are no confounding influences from marine foods or alternative C4 plants, the  $\delta^{13}\text{C}$  values of human collagen were used first in this area in tracing the introduction of maize agriculture into North America. These first applications used only carbon, but they served as models for all subsequent studies of prehistoric dietary adaptation (van der Merwe and Vogel, 1978; Bender *et al.*, 1981). As in the other areas, the consistency across studies is striking. Within the Archaic period of Arkansas, Illinois, Missouri, New York, Wisconsin, and Ohio, the  $\delta^{13}\text{C}$  values fall between  $-23$  and  $-21\text{‰}$  (van der Merwe and Vogel, 1978; Bender *et al.*, 1981; Lynott *et al.*, 1986; Vogel and van der Merwe, 1977). The Early Woodland period in New York and Arkansas, the Middle Woodland period of Illinois, West Virginia, Wisconsin, Iowa, and Ohio, and the Late Woodland period of Missouri, Illinois, Wisconsin, New York, and Ontario are also relatively consistent, with  $\delta^{13}\text{C}$  values between  $-22$  and  $-18\text{‰}$  (Vogel and van der Merwe, 1977; Lynott *et al.*, 1986; van der Merwe and Vogel, 1978; Schwarcz *et al.*, 1985; Pratt, 1991; Bender *et al.*, 1981). There are few exceptions and, significantly, all are in the Late Woodland period. Bender *et al.* (1981) report a value from Illinois (A.D. 1000) of  $-15\text{‰}$ , Vogel and van der Merwe (1977) and Stothers and Bechtel (1987) report values from New York (A.D. 1000 to 1300) of up to  $-14\text{‰}$ , and Schwarcz *et al.* (1985) report similar values from Ontario. It is possible that the attribution to the Late Woodland period is incorrect, but perhaps it is our understanding of Late Woodland period subsistence strategies that is incorrect or incomplete.

The Upper Mississippian has relatively consistent values reported for Illinois, Ohio, Minnesota, and Ontario, with the majority of the range of  $\delta^{13}\text{C}$  values between  $-15$  and  $-11\text{‰}$  (Vogel and van der Merwe, 1977; van der Merwe and Vogel, 1978; Stothers and Bechtel, 1987; Pratt, 1991). Even so, some of the values from Ontario (Farrow, 1986; Schwarcz *et al.*, 1985) and from New York (Broida, 1984) are less negative (i.e.,  $-10$  to  $-9\text{‰}$ ). At this time, it is not clear what these differences mean. A similar range is observed in samples attributed to the Middle Mississippian. Some, from Arkansas (Lynott *et al.*, 1986), Wisconsin, and Illinois (Bender *et al.*, 1981), fall between  $-21$  and  $-12\text{‰}$ , whereas others from the same states (van der Merwe and Vogel, 1978; Lynott *et al.*, 1986; Buikstra *et al.*, 1988) and from Indiana (Schurr, 1989) are consistently less variable and less negative (e.g.,  $-16$  to  $-9\text{‰}$ ). In both the Upper and the Middle Mississippian there may be some misidentification, but more likely, this represents differential uptake of maize as a major agricultural product. Attention to compiling data from large numbers of samples from numerous sites representing both Middle and Upper Mississippian adaptations is necessary to sort out the subsistence strategies used by these peoples.

Most importantly, the work thus far has served to indicate that the first introduction of maize took place later than had been assumed prior to the first stable isotope studies. Further, the shift toward less negative  $\delta^{13}\text{C}$  values occurs somewhat later in more northern regions than it does in the southern sections (Katzenberg, 1988). This pattern has been interpreted as being due to the time required in developing maize varieties that could survive and be productive in the shorter growing season in the northern regions. It appears that the intensification upon maize was a relatively late, Mississippian phenomenon across the majority of the eastern interior. In the western Lake Erie region, on the other hand, the use of maize has been reported as rising from providing essentially 0 to more than 50% of incorporated carbon, and we assume calories, derived from the crop between 0 and 600 A.D. (Stothers and Bechtel, 1987). The patterning of the introduction of this important crop thus requires further attention. The Protohistoric and Historic period skeletons all reflect a heavy dependence upon maize as a food source [-15 to -11 (Schwarcz *et al.*, 1985; Pratt, 1991; Bumsted, 1984; Lynott *et al.*, 1986; Vogel and van der Merwe, 1977)]. These data suggest that a major diet shift accompanied the influx of Europeans and the concomitant disruption of Native American life.

Other studies in this geographic area have included the analysis of nitrogen ratios in attempts to trace the introduction of beans into the area (Schwarcz *et al.*, 1985). Beans were expected to display  $\delta^{15}\text{N}$  values similar to atmosphere since, as mentioned previously, leguminous plants are capable of fixing atmospheric nitrogen. A decrease in  $\delta^{15}\text{N}$  values in human bone collagen would serve as an indication of bean domestication, but such an effect has not been observed. One possible explanation is that beans were a very small proportion of the diet (Schwarcz *et al.*, 1985), and thus, the isotopic signal would not be affected. Another possibility, discussed above, is that beans do not fix atmospheric nitrogen in situations, like burned, agricultural fields, where nitrates are available in the soil.

Very few nitrogen isotope data have been reported from the eastern interior. Even so, those which have are quite interesting. Human skeletons from near the Great Lakes have  $\delta^{15}\text{N}$  values that are more positive (12 to 14‰) than usual for terrestrial-based human groups (Schwarcz *et al.*, 1985; Pratt, 1991). This is true in the Middle Woodland, Late Woodland, Protohistoric, and Historic periods and reflects use of freshwater fish as a major protein source (Katzenberg, 1989). Those skeletons from farther away from the lakes have values more typical of inland dwellers [8 to 10‰ (Pratt, 1991)].

*Western Interior.* Several studies have attempted to use carbon isotope ratios to monitor the introduction of maize agriculture and to estimate the dependence on maize in the southwestern portion of the United States. There are complications with such applications in these areas, because other food items (e.g., bison meat, amaranth, various types of cactus) all have  $\delta^{13}\text{C}$  values similar

to that of maize (as discussed by Schoeninger and Schurr, 1991). These problems have been demonstrated in the Late Archaic period of the Lower Pecos (Huebner, 1991), where  $\delta^{13}\text{C}$  values of  $-16$  to  $-12\text{‰}$  indicated a major dependence on various CAM plants, especially compared to other people nearby who were not eating such foods ( $-20$  to  $-18\text{‰}$ ). A similar adaptation was proposed for the Panhandle region of Texas and Oklahoma. An average carbon value of  $-8\text{‰}$  in human populations indicated that the people depended to a great extent on gathered cactus and other wild C4 plants, on bison meat, and, to a varying extent, on maize grown in their own fields or traded from other groups (Habicht-Mauche *et al.*, 1991). Studies across Texas have revealed a variety of adaptations. In the central coastal plain and in the southeasternmost portion in the Late Archaic period, the emphasis must have been on browsing mammals such as deer and on nuts or other C3 plants (Huebner and Comuzzie, 1991; Huebner and Boutton, 1991). During the Historic period, at one of the Spanish Missions, however, the emphasis was obviously on maize (Evans, 1989).

In New Mexico, at Pecos pueblo (Spielmann *et al.*, 1990), a  $\delta^{13}\text{C}$  value ( $-7\text{‰}$ ) similar to that in the Panhandle region of Texas was interpreted to indicate that the majority of calories and plant protein came from maize. The archaeological evidence argued against large-scale gathering of C4 plants and for agriculture. The isotope evidence also served to support Spielmann's (1983) hypothesis of a disruption in trade between pueblo and plains groups during the time of Spanish control in the area. Decreasing  $\delta^{13}\text{C}$  values indicated a shift toward C3-based foods and away from bison, the product traded by Plains groups.

### Mesoamerica

One of the first applications of stable carbon and nitrogen isotope ratios to an archeological problem included the analyses of samples from the Tehuacan valley of Mexico (DeNiro and Epstein, 1981). Although midden analyses indicated little dependence on maize, the  $\delta^{13}\text{C}$  values in human bone collagen shifted toward a C4 signature at about 4000 BC (from  $-13\text{‰}$  at 6000 B.C. to  $-6\text{‰}$  at 4000 B.C.). The authors concluded that "the isotopic ratios of bone collagen may be more reliable than that based on archaeological analysis of plant and animal remains from the deposits in the Tehuacan Valley" (DeNiro and Epstein, 1981, p. 349). A subsequent attempt to reconcile the differences managed to change slightly the expectation derived from midden analyses, but the bone collagen isotope values still indicated far greater dependence on maize in the early period than more traditional archaeological reconstruction suggested (Farnsworth *et al.*, 1985). Further, DeNiro and Epstein interpreted a decrease in bone collagen  $\delta^{15}\text{N}$  values from  $+10$  to  $+9\text{‰}$  as indicating a change in dependence on beans. In retrospect, however, the nitrogen values in the earlier

samples were most likely diagenetically altered as these samples had very poor collagen preservation. Recalculation of the C:N ratios indicated an amino acid composition dissimilar to that of collagen (Schoeninger and DeNiro, 1981). The carbon and nitrogen stable isotope ratios in a population from the west coast of Panama dating to the same time period (5000–3000 BC) indicated that maize was not a major diet item, if used at all (Norr, 1981, 1982, 1991). There was, however, increased dependence on maize, in both inland and coastal Costa Rica between AD 300 and AD 1550 (Norr, 1981, 1982, 1991).

In Belize during the Classic, Post Classic, and Historic periods, people used maize to a lesser extent than in the Tehuacan valley (White and Schwarcz, 1989). For example, the Post Classic period people who exhibited the least negative  $\delta^{13}\text{C}$  values ( $-9\text{‰}$ ) are  $3\text{‰}$  more negative (indicating less use of maize) than the skeletons from the Tehuacan Valley. Further, in Belize, there is a decrease in use of maize through the Classic period. At the beginning of the Classic period the values average  $-12\text{‰}$ , but by the Terminal Classic the average is  $-15\text{‰}$ . These differences between Belize and the Tehuacan valley may be due to a greater dependence on marine products as a protein source in Belize than was true in the Tehuacan valley. If true, however, the people in Belize must have been choosing invertebrates that are low in the trophic pyramid because the nitrogen isotope ratios are not very high. The  $\delta^{15}\text{N}$  values remained constant through time at about  $+10\text{‰}$  except for one male buried in a stone tomb, who had a value of  $+13\text{‰}$ . The authors suggest that this is due to status-based access to marine foods, not available to females since the female buried in the same tomb had the lower value. It is also possible that the male came from a different area as an adult and that his bone collagen nitrogen isotope ratios reflects his diet from an earlier period.

Keegan's archaeological study of prehistoric island adaptation in the Bahamas was based on previous observations that certain reef-dwelling fish exhibited relatively positive carbon and relatively negative nitrogen isotope ratios (Schoeninger and DeNiro, 1984). He anticipated being able to distinguish dependence on terrestrial foods versus reef resources versus deep-water fish. In a baseline study, he included the analysis of dozens of potential and actual food items from both the terrestrial and the marine (both reef and deep ocean) environments (Keegan and DeNiro, 1988). The results indicate that the pattern of less negative  $\delta^{13}\text{C}$  values and less positive  $\delta^{15}\text{N}$  values is found in the humans, reflecting their dependence on marine foods from the seagrass and coral-reef communities.

### South America

Dietary reconstruction using stable isotope analysis in South America is similar to studies in other parts of the New World in the emphasis upon the relative importance of maize to other plant foods and the importance of marine

products. Because of the possible confusion between marine and C4 sources of carbon in dietary reconstructions, the use of nitrogen stable isotopes together with carbon has been essential. In a few areas, the use of succulents (CAM plants) also could confuse an interpretation of maize consumption based on carbon signatures.

In humid parts of South America, where preserved plant remains are rare, stable isotope studies have provided critical indications of a shift in plant food staples, in one case from non-C4 plants ( $-26\text{‰}$ ) at 800 B.C. in Venezuela to maize ( $-10\text{‰}$ ) by A.D. 400 (van der Merwe *et al.*, 1981). In Andean South America, plant and animal remains are abundant, which allows important insights into the potentials of stable isotope reconstructions. Working in the highland valley of Jauja, in central Peru, Hastorf (1985, 1988; DeNiro and Hastorf, 1985; Hastorf and DeNiro, 1985) investigated the effects of food processing and diagenesis on isotope ratios of archaeological and modern plants. The results suggest that such effects are negligible. Applications of these results to a series of late prehistoric archaeological skeletons allowed Hastorf (1985, 1988) to follow meat and maize intake between different status groups in a time period where rapid political change was altering production and distribution of food in the area. This work demonstrated a lack of significant use of C4 plants prior to A.D. 1470 ( $-18\text{‰}$ ) and then a change in the last three decades of the century ( $-14\text{‰}$ ; A.D. 1470–1532). Plant remains, animal remains, and the remains of food residues from cooking vessels have all been available to test and to confirm this reconstruction (Earle *et al.*, 1987).

Nearby, but at an altitude above the limit of modern agriculture, Moore and Schoeninger (unpublished data) investigated the isotope ecology at Panau-luaca, where hunting and herding populations may have had an unusually high meat intake. Because of the potential importance of animal foods, care was taken to investigate the stable isotopes of the animal bone collagen as well as the important forage plants, confirming values within the food web. Nitrogen stable isotope ratios for humans (10 to  $11\text{‰}$ ) were more positive than those for carnivores (average of  $9.6\text{‰}$ ) specializing on the same animals hunted and herded by the humans. It is difficult to reconcile the reconstruction of a meat-dominated diet with human dietary needs and limitations. In comparison, these nitrogen data have the same range of values as those of the agriculturalists studied by Hastorf. It is not yet clear what the nitrogen data are telling us. Even so, the reconstruction from stable isotope ratios coincides with the picture from plant (Pearsall, 1989a) and animal remains (Moore, 1989), that the input of foods from lower altitudes was extremely limited because no marine signal can be detected in the nitrogen isotope values.

Adaptations on the northern coast of Peru have been investigated (Ericson *et al.*, 1989) as well. Preserved archaeological remains were compared with stable isotope ratios in skeletons from looted cemeteries. Comparisons of coastal

and inland sites confirm the expected drop-off in importance of marine foods away from the coast (coastal nitrogen values are 10 to 14‰; inland nitrogen values are 9 to 10‰) and allowed estimation of the amount of diet provided by C4 plants such as maize. The carbon data indicate a significant use of maize from 250 B.C. [ $-12‰$  (Schoeninger and DeNiro, 1982)] to the later period of A.D. 900 [ $-12$  to  $-11‰$  (Ericson *et al.*, 1989)]. Data also suggest some variation in marine food intake between different coastal communities during the Middle Horizon (A.D. 500–900), the period with the most abundant samples. In the northern Peruvian highlands, in an area with few recovered plant remains, stable carbon isotope ratios indicate limited importance of maize in Early Horizon times [ca. 400 B.C. (Burger and van der Merwe, 1990)].

A particularly interesting possibility in the Andes is that of following diets of important domesticated animals using stable isotope ratios. Several studies have reconstructed diets for the native camelids in quite different environments, including coastal, highland valley, and high grassland (DeNiro, 1988; Burger and van der Merwe, 1990; Moore and Schoeninger, unpublished data). The human influence in the feeding ecology of the camelids seems to be particularly strong on the coast, where a marine signature was inferred (DeNiro, 1988).

## Europe

One of the early studies using carbon stable isotope ratios (Tauber, 1981) monitored a shift from marine fishing and gathering to agriculture in Denmark (Mesolithic period range,  $-15$  to  $-12‰$ ; Neolithic period range,  $-22$  to  $-18‰$ ). The same pattern, with almost identical stable isotope values, is seen in Britain, with a change from the Mesolithic period [ $-15$ ,  $-14‰$  (Clutton-Brock and Noe-Nygaard, 1990)] to the Neolithic period [ $-21$ ,  $-19‰$  (Schoeninger *et al.*, 1983)].

Using an innovative approach, Mesolithic period dietary patterns in Denmark and Britain (Noe-Nygaard, 1988; Clutton-Brock and Noe-Nygaard, 1990) have been inferred from domestic dogs because of an absence of human skeletal remains. They indicate a strong marine component in the diet ( $-17$  to  $-14‰$ ), even some distance from the contemporary coast. Data reported from another area of Denmark have been interpreted to indicate less dependence on marine foods [ $-20$  to  $-17‰$  (Price, 1989; based on Hakansson, 1982, 1984)]. These data, however, were calculated based on a C-14 correction with an accuracy no better than 4 to 5‰ (Gillespie, 1984). Therefore, these data probably fall within the same range as those reported by Tauber and Noe-Nygaard. Such an interpretation is strengthened by the observation that a similar adaptation has been documented based on human remains from the same period in coastal Portugal

[ $-15\text{‰}$  in humans (Lubell *et al.*, 1989)] and in Britain [ $-15$  to  $-14\text{‰}$  (Clutton-Brock and Noe-Nygaard, 1990)].

Murray and Schoeninger (1988), while testing for differential meat eating among high-ranking males, females, and low-ranking males in Iron Age Slovenia unexpectedly found that a C<sub>4</sub> food item was being eaten ( $-14\text{‰}$ ). A further study of plant foods available as human food items (Murray and Schoeninger, 1989) identified millets as the only C<sub>4</sub> plants in the area. In contrast, previous studies had assumed that millet was used as forage for animals, not consumed by prehistoric Europeans.

A recent study purports to identify almost virtual carnivory among archaic members of our species (Bocherens *et al.*, 1991). Regrettably the authors have not taken account of the change in atmospheric CO<sub>2</sub> or the change in vegetation cover in their comparisons between modern and archaic fauna. It is very possible that the archaic fauna resemble the modern fauna in carbon isotope values due to a fortuitous combination of diagenetic alteration in the archaic fauna and changes in vegetation cover and atmospheric CO<sub>2</sub> that affect the modern fauna (see Bada *et al.*, 1990). Further, based on multiple studies, it is clear that a direct comparison of human collagen nitrogen isotope ratios with those of carnivores is not valid (see discussion below, under Knowledge of Metabolism).

### Asia

Very little has been published on prehistoric human samples from China. One survey (Cai and Qiu, 1984) demonstrates the applicability of the method to various archaeological problems within China. Their carbon isotope data ( $-14$  to  $-13\text{‰}$ ) corroborate archaeological evidence for early (7000 years B.P.) millet domestication in northern China and, further, discriminates between the millet-based economies of northern China and the rice-based ( $-20$  to  $-19\text{‰}$ ) economies in the southern region. The authors recommend expansion of such studies in order to address several questions. First, they would like to document the spread of millet-based economies throughout China. Second, stable isotope studies could monitor the shift from millet-based to rice-based diets throughout China. Third, such studies could be used to determine the timing of animal domestication in those areas where millet was the major forage crop. Fourth, stable carbon isotope ratios might help in assessing dietary differences between social strata where poorer people would have eaten millet and people in the higher strata would have had access to rice and/or wild animals feeding on C<sub>3</sub> plants.

On the islands of Japan, the stable isotope ratios of carbon and nitrogen have been used to determine the dependence on marine and terrestrial foods by several prehistoric groups (Chisholm *et al.*, 1988; Minagawa and Akazawa,

1991; Roksandic *et al.*, 1988). Preliminary baseline studies (Koike and Chisholm, 1988; Minagawa and Akazawa, 1991) have demonstrated the isotopic distinctiveness of marine and terrestrial resources. There are few C4 plants available for human consumption, and the leguminous and nonleguminous plants both had  $\delta^{15}\text{N}$  values close to zero, which simplified the interpretation of results. The archaeological evidence indicated significant dependence on varying combinations of marine mammal, marine fish, and marine shellfish with the exception of inland sites. The isotopic data support this interpretation ( $-22$  to  $-20\text{‰}$  at the inland site,  $-15$  to  $-12\text{‰}$  at the coastal sites), with a significant amount of variation across the islands (Chisholm *et al.*, 1988). Further, they demonstrate a significant difference between the northern islands of Sakhalin and Hokkaido ( $-15$  to  $-13\text{‰}$ ) and the more southerly island ( $-18\text{‰}$  on the coast of Honshu). This distinction is consistent through time; the historic Ainu on Hokkaido and on Sakhalin are similar to the Early Jomon period and Epi Jomon period residents of Hokkaido. The Honshu populations depended far more on terrestrial plants and animals than did Hokkaido and Sakhalin people. Further, the inland site on Honshu exhibited less positive  $\delta^{15}\text{N}$  values and more negative  $\delta^{13}\text{C}$  values than the coastal site from the same period (Late Jomon). This pattern is similar to those observed by Walker and DeNiro (1986) in coastal and inland sites in southern California and by Chisholm and colleagues (1982) in British Columbia.

## Africa

*South Africa.* The stable isotope approach was used to test an archaeological hypothesis concerning seasonal movements by the prehistoric inhabitants of the southernmost portion of this region (Sealy and van der Merwe, 1985, 1986). The hypothesis, based on archaeological evidence from middens and on knowledge of food availability in the area, stated that between 4000 and 2000 years B.P. hunting and gathering groups on the South African Cape moved seasonally from the coast to the interior (Parkington, 1972). The isotope analyses included a baseline study of more than 200 plants, animal meat, animal bone collagen, and human bone collagen. The results supported the expectation that coastal foods had different carbon isotope ratios from those collected inland but did not necessarily support the seasonal movement hypothesis. As outlined by Sealy and van der Merwe, such seasonal movements between the coast and the interior should result in similar carbon isotope signatures in people buried on the coast and people buried inland. The results, however, demonstrated that inland people had bone collagen carbon isotope ratios that differed, on average, from those of people buried on the coast (coastal,  $-18$  to  $-11\text{‰}$ ; inland,  $-19$  to  $-16\text{‰}$ ; coastal nitrogen,  $10$  to  $18\text{‰}$ ; and inland,  $10$  to  $16\text{‰}$ ), although there is overlap

in both carbon and nitrogen distributions. They concluded that “a significant proportion of the coastal population consumed an entirely marine diet and so must have spent all its time on the shore” (Sealy and van der Merwe, 1985, p. 140). Some coastal people may have moved inland seasonally since among some of them the isotope ratios indicated that some 40% of their diet carbon came from a C3 source that may have been coastal or interior. The inland people, on the other hand, did not move to the coast seasonally in all likelihood. They conclude that their results strongly call the seasonal movement hypothesis into question and call for reexamination of the proposal in other areas of the world. There also appears to have been a shift toward including greater amounts of terrestrial foods through time on the coast ( $-13\text{‰}$  at 2000 BP and  $-15\text{‰}$  at 1000 BP); patterns supported by the available midden analyses (Sealy and van der Merwe, 1988). The authors propose a difference in diet between the sexes and differential change between the sexes through time, but given the scatter within the data and the small sample size, this seems less than conclusive.

The large baseline study also included nitrogen isotope analysis (Sealy *et al.*, 1987). In many cases, there was no difference between marine and terrestrial organisms in  $\delta^{15}\text{N}$  values due to the unusually positive  $\delta^{15}\text{N}$  values in the terrestrial fauna. They attribute the terrestrial values to water stress, with some influence of ruminant microbial activity (Sealy *et al.*, 1987). This aspect is discussed in greater detail below. The complications within the system made interpretation of the human  $\delta^{15}\text{N}$  values extremely difficult. Unlike an earlier study of other inland-dwelling South African groups (Ambrose and DeNiro, 1986b), they were not able to separate pastoralists from nonpastoralists on the basis of nitrogen stable isotope ratios. Their study made it apparent that baseline values must be determined prior to application of stable isotope values in diet estimation.

*East Africa.* Two large ecological studies with applications to human adaptations have been undertaken in East Africa. Ambrose was able to demonstrate distinct stable isotope signatures in pastoralists ( $14\text{‰}$  in nitrogen) versus agriculturalists ( $10\text{‰}$  in nitrogen) versus hunter-gatherers similar to the pattern he observed in South Africa (Ambrose and DeNiro, 1986b). The nitrogen isotope data reflected ethnographic reports on diet. Those groups most dependent on plant foods have  $\delta^{15}\text{N}$  values that are, on average,  $4\text{‰}$  less positive than those reported as most dependent on animal products. The relative dependence on C3- and C4-based foods expected from the reports is also reflected in the  $\delta^{13}\text{C}$  values of human bone collagen (C4 agriculture,  $-6.5\text{‰}$ , versus a mix of C3 and C4,  $-15\text{‰}$ ).

Stable nitrogen isotope ratios allowed previously unidentified human skeletons from stone cairns to be identified as pastoral nomads rather than as fisher-gatherers in the region east of Lake Turkana (Schoeninger, 1991). The food chain in the lake is based on nitrogen-fixing blue/green algae; thus, all of the

fish and crocodiles have  $\delta^{15}\text{N}$  values that are significantly less positive than those in land animals. The human skeletons displayed a signature indicative of dependence on land animals, not on fish.

### Australia

Very little work of this nature has been done in Australia, due largely to the difficulties of working with aboriginal skeletal material (Gould, personal communication). One project with 28 human samples has been published (Collier and Hobson, 1987; Hobson and Collier, 1984; see Lee Thorp and Sealy, 1986). Two sites were analyzed. Broadbeach, on the coast of southeastern Queensland, was inhabited over the last thousand years. The second site at Swanport on the lower Murray river in South Australia provided a sample of people assumed to have died of smallpox in 1830. The results of the Broadbeach sample ranged from  $-18.6$  to  $-14.8\text{‰}$ , but because nitrogen isotopic analyses were not performed, it is not possible to determine whether the signature is due to ingestion of marine foods or of C4 plants. Results of analyses on 11 animals (5 terrestrial, 6 marine) and 2 plants were included, although none of these were C4 plants. The carbon isotope signature is such that the maximum amount of marine foods would have been less than 50% at Broadbeach, assuming that no C4 plants were eaten. If C4 plants were eaten, then the dependence on marine foods must have been lower. The sample from Swanport has an isotope signature that is basically C3, which was unexpected; the Swanport sample may not be representative of precontact populations. Further, it is not reported what section of the 1000-year span of occupation at Broadbeach is represented by the eight humans analyzed in this study. Further work is needed in the area in order truly to understand human adaptation in this portion of Australia. A broader study of plants, animals, and humans from South Australia is currently under way (D. Pate, personal communication) and it should serve to elucidate adaptation in this fascinating geographical area.

## REQUIREMENTS AND LIMITATIONS

### Knowledge of Metabolism

*Age.* No intensive study of the association between age and isotopic composition of bone collagen has yet been undertaken for any long-lived mammal. Thus, no direct information concerning the potential effects in humans is avail-

able. The available data on invertebrates and fish (Minagawa and Wada, 1984) suggest that no such effect should be expected in mammals. A cross-sectional study of elephants from Tsavo Park in South Africa displays no association between  $\delta^{13}\text{C}$  values and age, and “delta  $^{15}\text{N}$  values were marginally related to age ( $P = 0.04$ ) with an  $R^2$  of only 0.08” (Tieszen *et al.*, 1989, p. 22). A similar cross-sectional study of macaque bone collagen (Koike and Chisholm, 1988) and of hair in modern Japanese (Minagawa, 1991) showed constancy across ages. Prehistoric skeletons assigned different ontogenetic ages and with assumed diet constancy also showed no relation between age and isotope ratios (Lovell *et al.*, 1986). An increase in  $\delta^{13}\text{C}$  values with age among prehistoric agriculturalists was interpreted as indicating the use of maize as a weaning diet (Katzenberg, 1990). A similar increase among Holocene hunter-gatherers in the southwestern Cape in South Africa suggested a lack of marine food in infant diets (Sealy and van der Merwe, 1988). Given the paucity of data, the question of isotopic variation with age is not settled and further studies directed toward elucidating this point would be welcomed.

*Sex.* Although limited in number, the available data suggest that there is little, if any, variation among individuals that can be attributed to differences in metabolism between the sexes. Mink raised on monotonous diets (DeNiro and Schoeninger, 1983), wild elephants (Tieszen *et al.*, 1989), and Japanese macaques (Koike and Chisholm, 1988) showed no difference between males and females in either  $\delta^{15}\text{N}$  values or  $\delta^{13}\text{C}$  values in bone collagen. Hair from modern Japanese also showed no variation along sex lines (Minagawa, 1991).

Studies of prehistoric samples support these findings. A large number of human bone samples recovered from a common grave, the result of a massacre in the 15th century at Crow Creek in South Dakota, displayed little variation in  $\delta^{13}\text{C}$  values (Bumsted, 1983, 1984). Ethnographic sources indicated that all of the adults recovered had eaten the same diet during life. Only data on carbon have been published, but these show only a 0.5‰ difference between the sexes. This amount is only about 0.3‰ above the level of precision expected in re-analyses of the laboratory combustion standard and therefore is not large enough to be used in separating individuals of unknown sex (*contra* Ericson *et al.*, 1989). Similar results are reported for a site in Ontario (Lovell *et al.*, 1986). An Iron Age sample from Yugoslavia (Murray and Schoeninger, 1988) revealed no difference between men and women, nor did part-time horticulturalists and settled agriculturalists in the southwestern and southeastern United States (Spielmann *et al.*, 1990; Habicht-Mauche *et al.*, 1991), agriculturalists in prehistoric Belize (White and Schwarcz, 1989), hunter-gatherers in prehistoric Japan (Minagawa and Akazawa, 1991), or hunter-herders in highland Peru (Moore and Schoeninger, unpublished data). Given these data, the most reasonable interpretation of reported differences between males and females when they occur in

archaeological populations is that there is a real dietary difference between them (Chisholm *et al.*, 1988; Sealy and van der Merwe, 1988).

*Water Stress/Nutrient Stress: Effect on  $\delta^{15}\text{N}$  Values.* There are some indications that variation in  $\delta^{15}\text{N}$  values may be due to factors other than diet. Higher than expected  $\delta^{15}\text{N}$  values in bone collagen have been reported from some areas where the animals may have been water stressed (Schoeninger and DeNiro, 1984; Ambrose and DeNiro, 1986a; Heaton *et al.*, 1986; Sealy *et al.*, 1987; Schoeninger, 1989). The situation is complicated, however, since low  $\delta^{15}\text{N}$  values have been reported for ruminants from the desert areas of the U.S. Southwest (Spielmann *et al.*, 1990; Habicht-Mauche *et al.*, 1991).

Protein restriction in conjunction with water stress has been suggested as an important factor (Ambrose and DeNiro, 1986a). The empirical data are from ruminants, however, and their ability to recycle nitrogen within their gut has been noted as a complicating factor (Sealy *et al.*, 1987), preventing direct comparisons with humans. Recently, very positive  $\delta^{15}\text{N}$  values have been reported for plants in one desertic area, suggesting that the plants may be the source of the elevated isotope ratios (Vogel *et al.*, 1990), although this is not true in all cases (Ambrose and DeNiro, 1986a; Sealy *et al.*, 1987). Animals, such as carnivores, that consume excess protein also display elevated  $\delta^{15}\text{N}$  values, perhaps because such a large amount of nitrogen is excreted in urea when amino acids serve as energy sources rather than nitrogen sources (Schoeninger and Bada, 1989). In sum, it is still far from clear what role water intake, protein levels, gastrointestinal bacteria, and plant variation play in determining the final  $\delta^{15}\text{N}$  value of an animal's tissues. The area is one open for study.

*Carbon and Nitrogen Source Effects on  $\delta^{13}\text{C}$  and  $\delta^{15}\text{N}$  Values.* A major question has surrounded the source of the carbon and nitrogen incorporated into bone collagen. In theory, three major food components, carbohydrate, protein, and lipid, all could provide carbon available for tissue synthesis, while only protein can supply nitrogen. For most humans, protein can come from plants and/or animals. For nitrogen, therefore, the question becomes whether that nitrogen incorporated into bone reflects all sources of protein or animal protein, solely.

The general model of synthesis from fundamental components (Lehninger, 1975) supports the interpretation that all diet components are equally represented. Some researchers (Krueger and Sullivan, 1984), on the other hand, have suggested that lipid carbon is not available for tissue synthesis. In an exploratory study of dietary adaptations by two recent Eskimo groups, Hausler and Schoeninger (unpublished data) tested whether a difference in fat intake recorded ethnographically might be reflected in bone collagen  $\delta^{13}\text{C}$  values. There was no difference between the groups, providing indirect support of the model.

Further, the model proposed by Krueger and Sullivan (1984) suggests that protein is the major source of carbon as well as nitrogen incorporated into

collagen. The extremely positive  $\delta^{13}\text{C}$  values from human samples collected on marine coasts suggest that this may be true in some cases. The carbon isotope signature can be interpreted as indicating that almost 100% of the carbon is coming from marine sources (Chisholm *et al.*, 1982; Medaglia *et al.*, 1990; Sealy and van der Merwe, 1988). In these areas either there are no plants with a C4 signature or the archaeological record indicates that the sites are premaize. No marine plants have ever provided a major food source for humans. Based on nutritional constraints (Noli and Avery, 1988; Shils and Young, 1988), it is unlikely that 100% of the calories in diet came from marine foods, but perhaps 100% of the carbon that was incorporated into collagen came from marine foods. The composition of foods provided by marine vertebrates is largely lipid and protein, although some marine invertebrates contain minimal amounts of carbohydrate. Such a weighting of the isotope signal in favor of protein over other diet components may occur only in cases where an excess of protein is available and where sufficient calories are available. In such a case, both carbon and nitrogen in bone collagen should reflect the isotopic signature of the source protein. Obviously further study is required on this subject, we anticipate interesting results from several laboratories in the near-future (Fogel, personal communication; Ambrose, personal communication).

*Variation Across Tissues of an Animal and Across Skeletal Elements.* Tissues containing lipids (muscle, kidney, and liver) usually have more negative  $\delta^{13}\text{C}$  values than tissues without lipids (hair, skin, and bone collagen) (Tieszen *et al.*, 1983). The offset between muscle and collagen appears to be relatively consistent at about 2‰ (Medaglia *et al.*, 1990; van der Merwe, 1989), the effect being due to the preferential incorporation of  $^{12}\text{C}$  in the lipid fraction (DeNiro and Epstein, 1977). Hair and skin appear to be similar to bone collagen values (Schoeninger and Robinson, unpublished data), although differences occur in some cases (White, 1991). There is variation in the  $\delta^{15}\text{N}$  values across tissues of an animal, although it appears that tissues with long turnover times have values within 1‰ of each other.

Thus far, the evidence suggests that there are no patterned differences in isotopic ratios across skeletal elements even though turnover rates differ. Among bones of individual mink, all of which had been raised on the same diet, the range of variation among skeletal elements in 19 individuals was 0.2‰ for carbon and 0.3‰ for nitrogen (DeNiro and Schoeninger, 1983). These ranges of variation are similar to those that result from repeated analyses of the laboratory combustion sample. Additional support comes from the analyses of vertebra and femur samples from recent humans buried in permafrost (Schoeninger, 1989), where variation due to diagenetic alteration can be discounted. Both bones gave identical carbon and nitrogen isotopic ratios, even though written records about these people indicated that they probably had diets that varied seasonally (Maat, 1981; van Wijngaarden-Bakker and Pals, 1981) and even

though the cancellous bone in a vertebrae turns over more rapidly than the cortical bone in the femur.

### Sample Selection

The design of a study of prehistoric diet from bone collagen stable isotopes involves considerations of the pragmatic and ideal. Where unlimited numbers of human skeletons are available for study, subsamples of chronological and spatial variability in the study set should be chosen. Samples of both males and females are recommended to screen for dietary differences between the sexes. Studies of health and nutritional status for each skeleton are a desirable complement to isotopic analyses. Where plants and animals from the site have been recovered, it is important to include samples of these food sources in the study. Modern equivalents should be used if archaeological specimens are not adequate, keeping in mind that the carbon isotope signature in modern plants will be somewhat more negative due to the more negative isotope composition of today's atmospheric carbon dioxide versus the prehistoric. Where the skeletons are few in number, poorly preserved, or not well documented, the value of the sample to a research question may still justify its analysis. Such cases include those where other samples from the same region provide detailed background on baseline conditions and expected human stable isotope values and those cases where the cultural problem of interest, not addressable by other means, could be resolved by a relatively few determinations. Overall, it is best that samples of 5–10 individuals be included in order to assess the variation between members of skeletal population.

As mentioned above, there are few constraints upon which bone of the skeleton is chosen for analysis though considerations specific to a particular sample set may demand that only a certain bone such as ribs be used. If the surface of the bone appears to indicate poor preservation, the organic component could still be largely intact, and we recommend that questionable samples be subject to screening for diagenetic changes (e.g., Moore *et al.*, 1989; Schoeninger *et al.*, 1989). The highest-quality samples are those where age and sex determinations have been made, though material may be too fragmentary to permit this. Where unlimited bone is available, it is useful to sample at least 10 g to allow for loss of material in cleaning, to allow for replicate samples to be prepared, and to allow for other analysis such as preparation of thin sections. Smaller samples (down to 1 g) are usable for stable isotope analysis, but other analyses such as histomorphometry on thin sections will not be possible.

Little special care need be taken while handling samples though the application of consolidants, solvents, ink labels, and adhesives should be avoided. Material should be stored and shipped in plastic bags, vials, or aluminum foil rather than in paper or fiber packing material. The most critical consideration

is the secure association of the sample identity with the sample package. Pre-historic bone samples and dried or carbonized plant material are stored in sealed containers at room temperature, while fresh plant samples, tissue samples, and collagen (or "gelatin") samples should be stored in freezers or desiccators.

### Diagenesis

As most recently pointed out by several presenters at the First International Workshop on Fossil Bone, bone recovered from archaeological contexts has been subjected to any number of possible events after burial and also after deposition in a storage facility (Schwarcz *et al.*, 1989). These events may result in the addition of contaminants such as humic acids or nitrogen-containing compounds (organic and inorganic) or the loss of portions of the initial organic material (Hedges and Law, 1989). These processes may be mediated by fungi or bacteria (for effect on bone material, see Grupe and Piepenbrink, 1989), which in turn may leave fragments that are themselves contaminants. Such contaminants (humic acids or fungi) must be removed, either chemically or mechanically. Although not clear as yet whether fungi or bacteria directly affect the stable isotope ratios (Grupe and Piepenbrink, 1989), they may be the cause of differential degradation of the collagen cross-linked chains.

Applications of consolidants can add carbon and nitrogen in which the stable isotope ratios bear no relation to that of the bone. In some cases, removal of such contaminants can be accomplished with the proper solvents, although care must be taken to ensure that the solvent itself does not contain carbon and nitrogen that remains in the sample. The first step should be analysis of the consolidant to determine how much carbon and nitrogen it contains and the isotope ratios of the elements (Moore *et al.*, 1989). After evaluating the potential contamination, the choice of sample preparation must be made in consultation with the analyst. Removing the external surface of the bone sample successfully removes the majority of consolidants when bone has been painted superficially or dipped quickly into a vat. In rare cases where the bone has been treated under pressure with consolidant, it is virtually impossible to assure complete removal.

Once the potential for contamination by consolidants has been assessed, the preservation of the sample must be evaluated. The composition of collagen is extremely ordered (Eyre, 1980) and is not replicated by nonbiogenic sources, thus the amino acid composition of an archaeological or fossil bone sample can serve as a check for diagenetic alteration (Tuross *et al.*, 1988; Schoeninger *et al.*, 1989). Each collagen chain has approximately 1000 amino acid residues, of which every third one is glycine. Whereas most amino acids have four or more carbon atoms to each atom of nitrogen, glycine has only two carbon atoms to one atom of nitrogen. Because over 30% of the amino acid residues in collagen are glycine, the atom-to-atom ratio of carbon to nitrogen (C:N) in

collagen is about 3 to 1. In other proteins, this C:N ratio is closer to 5 to 1. In addition, collagen contains relatively large amounts of proline and hydroxyproline (one-fifth to one-fourth of all amino acid residues). The low C:N ratio, large relative amount of glycine, and large amounts of proline plus hydroxyproline are considered diagnostic features of collagen.

The criterion for acceptance of reliable specimens, then, is the identification of intact proteins or ones with amino acid compositions similar to intact proteins. The suggestion of the use of C:N ratios, in isolation, as a screening procedure (DeNiro, 1985; DeNiro and Epstein, 1981; Schoeninger and DeNiro, 1984) has proven ineffective. In several instances, acceptable C:N ratios have been recovered from material identified as noncollagenous by amino acid composition (Tuross, personal communication; Schoeninger *et al.*, 1989). It appears that a C:N ratio outside the acceptable range of 2.7–3.6 indicates a noncollagenous material that should not be used for isotopic analysis. Even so, a C:N ratio within the range imparts no assurance of acceptable collagen. In bone that appears well preserved superficially, has been cleaned chemically and mechanically, and retains a percentage of organic residue equal to 50% or more of the original organic (organic residue >10% of the original dry bone weight), the C:N ratio is superfluous. In bone that retains organic residue equal in weight to 25% or less of original protein (i.e., <5% of original bone weight), a good C:N cannot be depended upon, although a bad C:N would indicate rejecting the sample for analysis. In samples with such low amounts of retained organic residue, it is imperative to determine the amino acid composition of the residue in order to determine if it contains a collagen-like pattern. Although not practical for every sample, a subset from each locale should be screened in this manner.

One final caveat is necessary. Up to this point, it has been assumed that analyses of material displaying a collagen-like composition will retain a dietary isotopic signature. A theoretical model and preliminary data (Bada *et al.*, 1989) suggest that this may not be true in cases where the amount of retained protein is about 1% (about 5% of original organic of bone) of the dry bone weight. During peptide bond hydrolysis a difference of only 0.005 in the rate constants for the peptide bond containing  $^{14}\text{N}$  and the bond containing  $^{15}\text{N}$  can be expected to result in an enrichment of 15‰ in the most insoluble collagen relative to the first 99% of collagen to be hydrolyzed. This effect is expected to be far less for carbon than for nitrogen because the majority of carbon atoms in an amino acid are not part of the bond between amino acids and thus would not be affected. At this time, the safest route is to limit isotopic analyses to samples retaining >4% of original protein (i.e., organic residue equals >1% of the original dry bone weight).

The assessment of diagenetic alteration of carbonate in apatite is more difficult than is true of collagen. Carbonate can be produced by geochemical processes as well as by biogenic processes. Unlike collagen where elemental

exchange cannot occur, exchange of carbonate for original bone phosphate and exchange of carbonate from groundwater for original bone carbonate occur readily. The addition of calcium carbonate in bone interstices can be detected by X-ray diffraction and removed with acetic acid washes (Schoeninger, 1980; Koch *et al.*, 1990; Sillen, 1986), but identification and removal of carbonate contamination within apatite remain a serious problem. The best means of dealing with secondary carbonate appears to be identification of its occurrence with evaluation of the magnitude of effect on the original isotope signal. If the effect is small relative to the biogenic signal, then meaningful data can be collected. For example, Lee Thorp and van der Merwe (1987) measured carbon isotope ratios in enamel apatite from Pliocene vertebrates and in soil carbonate. Although some exchange toward the soil isotope ratio had occurred, it was minimal. Differences in carbon isotope ratios in the vertebrate tooth enamel were discernible and were not in the direction expected from soil carbonate contamination. Koch *et al.* (1990) suggests measuring the oxygen isotope ratio as a check for carbonate exchange. This sounds extremely promising and it is hoped that someone will decide to pursue this line of inquiry.

## FUTURE POTENTIAL

### Food Stress

The meaning of the large range of variation in  $\delta^{15}\text{N}$  values across species is still uncertain. As discussed previously, the explanations have included water stress (Schoeninger and DeNiro, 1984; Heaton *et al.*, 1986), water and protein stress (Ambrose and DeNiro, 1986a), recycling of nitrogen by ruminant bacteria (Sealy *et al.*, 1987) and unexplained positive  $\delta^{15}\text{N}$  values in plants (Vogel *et al.*, 1990). A great deal of variation in  $\delta^{15}\text{N}$  values across human groups has also been reported in the literature. Human groups in three adaptive situations (marine fishers, southwest maize agriculturalists, and Kenyan pastoralists) all had  $\delta^{15}\text{N}$  values significantly more positive than expected based on their trophic position (Schoeninger, 1989). In each case, the human values are more positive than the average value for carnivores in the same region; thus, trophic position is not an adequate explanation. These positive values may be due to diet quality. In a recent "personal" experiment, N. Tuross (personal communication) succeeded in driving her fingernail  $\delta^{15}\text{N}$  value up to +16‰ from a normal of +9‰ after 9 months on a diet that provided about 80% of the daily requirements of protein with adequate calorie intake. Experimental studies of animals on controlled diets and surveys of nitrogen isotope values in plants are critical to clarify the situation.

### Other Elements and New Applications

Isotopic variation in other elements such as hydrogen (H), oxygen (O), sulfur (S), and strontium (Sr) has potential for providing information about aspects of human adaptation, the environment, and bone preservation. These, rather than direct information on diet, appear to be the most likely future directions for research involving bone isotopic composition. For example, the isotopic ratios of hydrogen (deuterium/hydrogen or D/H) and oxygen ( $^{18}\text{O}/^{16}\text{O}$ ) of terrestrial water vary as a result of the ratios in rainfall, which, in turn, are related to temperature (Hoefs, 1987). Thus, if the isotopic ratios in prehistoric terrestrial waters were known, the temperature at the time could be calculated. Further, both ratios in leaf water are dependent upon humidity, thus, the ratios in prehistoric leaf water would indicate the humidity at the time the leaves were alive. Publications by Longinelli (1984; Longinelli and Nuti, 1973), by Kolodny (Kolodny *et al.*, 1983), and by Luz (Luz *et al.*, 1990; Luz and Kolodny, 1989) with their colleagues suggest a method for obtaining the oxygen isotope ratios in these prehistoric waters. The  $^{18}\text{O}/^{16}\text{O}$  ratio in bone and tooth apatite (Luz and Kolodny, 1989; Koch *et al.*, 1990) is in equilibrium with the water ingested by an animal at the time of apatite synthesis. In those animals that must drink, this water is terrestrial, surface water. Among other animals that obtain the majority of their water from food, especially leaves, this water is leaf water. Thus, apatite  $^{18}\text{O}/^{16}\text{O}$  ratios can be used to estimate the ratio in source water, depending on how the animal obtains water, either the temperature or the humidity of the climate can be calculated. Similarly, the H/D ratios in herbivore collagen appear to depend on temperature and humidity (Cormie and Schwarcz, 1985). Both of these methods require further investigation.

Both sulfur and strontium isotope ratios offer the potential for estimating the locale in which people lived for the majority of their lives and in certain circumstances, where they obtained their food. In the case of strontium there is no fractionation during metabolism, but there is significant variation in the rock/soil stable strontium isotope ratio across geographical regions. Between regions where such differences occur, it should be possible to identify the area in which people were born in those cases where they have moved as adults to areas with different baseline isotope ratios (Ericson, 1989). Sulfur isotope ratios also vary significantly across the landscape (Fry *et al.*, 1982). Theoretically, it should be possible to sort foods from deep sea, from reef, and from land, although the system is very complicated (Keegan and DeNiro, 1988).

### Plant/Animal Domestication

One powerful indication of the domestication of animals is the control of their diet by humans. Evidence from South America documents the addition of marine food to diet of terrestrial animals (DeNiro, 1988) and the possible addi-

tion of C4 food to the diet of animals with natural forage upon C3 (Burleigh and Brothwell, 1978). The greatest differences that have been observed are for dogs in Europe and elsewhere (Clutton-Brock and Noe-Nygaard, 1990; Noe-Nygaard, 1988), pigs (several unpublished studies), and llamas and alpacas in Peru (DeNiro, 1988).

Because of the interest in North America on the rapid intensification of maize cultivation, there has been some speculation that wild animals would also reflect this alteration of the environment. Up to this point, this search for less negative carbon isotope ratios among wild herbivores has been largely unsuccessful, though relatively few determinations have been made in the Mississippi drainage. Deer, even though they are pests in maize fields today, seem to have spent little time there in the Mississippian period (Katzenberg, 1988; Buikstra *et al.*, 1988) or in the Anasazi southwest (Spielmann *et al.*, 1990).

### Other Tissues

Hair, skin, and desiccated or frozen flesh may also be available for analysis from humans who were buried in hyperarid or arctic environments where biological decay was inhibited. White and colleagues (1991) have found characteristic differences in composition among hair, skin, bone collagen, and bone lipids from Nubian skeletons. Studies thus far indicate that isotope ratios in hair reflect the diet isotope ratios (Minagawa, 1991; Schoeller *et al.*, 1986).

### Individual Amino Acids

The transition of dietary components into body proteins is understood in only the most general sense. The large, relatively complex nutrient molecules (protein, carbohydrates, and lipids) are broken down into smaller components, which are broken down further and enter the citric acid cycle, where energy is produced. A large portion of the nitrogen and carbon from the diet is excreted in urine and feces or, in the case of carbon, exhaled CO<sub>2</sub>. The remainder are available for synthesis of body tissues including such proteins as collagen. For the most part, amino acids from diet do not transfer directly into amino acids in newly synthesized proteins. In theory, the synthesis of amino acids prior to the synthesis of collagen may combine a carbon backbone originally derived from a dietary carbohydrate or lipid with an amine group (a nitrogen-containing molecule).

One suggestion was that essential amino acids would have isotopic ratios similar to those in diet because these amino acids would be taken up directly from diet (Gaebler *et al.*, 1966). Recent work (Macko and Estep, 1984; Macko *et al.*, 1986, 1987) indicates that the system is more complicated. Based largely on microbial studies, it appears that fractionation is related to the chemical nature

of particular amino acids. In other words, collagen is made up of a series of amino acids, each of which may have a delta value that is unique relative to its precursor.

The necessary studies of fractionation between diet and the individual amino acids in proteins, in general, and collagen, specifically, have not been completed as yet. In part this is due to the technical difficulties of separating amino acids without cross contamination, the first step in producing the sample necessary for stable isotope analysis (Hare and Estep, 1983; Tuross *et al.*, 1988). Another difficulty, related to the first, is extracting enough material for analysis. The latter problem has been minimized with new mass spectrometers capable of accurately analyzing samples composed of less than a micromole of gas. More information on this crucial issue can be anticipated in the near-future.

Based on the available data, it appears that the isotope ratios vary predictably between different amino acids of collagen. As reported by Tuross *et al.* (1988) and recently supported by data from controlled feeding experiments (Hare *et al.*, 1991), glycine, serine, and threonine have less positive nitrogen isotope ratios and more positive carbon isotope ratios than does collagen as a whole. Hydroxyproline (and, therefore, proline), glutamic acid, aspartic acid, and alanine, on the other hand, are either equal to or more negative in carbon isotope ratios than is true of collagen as a whole. The same amino acids are also equal to or more positive in nitrogen isotope ratios than those of collagen. If these results are duplicated in other samples from different environments, it will suggest that individual amino acids known to have derived from collagen could be analyzed for stable isotope ratios and thus avoid diagenetic problems. Additionally, if it is shown that different information is being recorded, perhaps more specific dietary information can be obtained by comparing the analysis of one group of amino acids with another.

## CONCLUSIONS

The preceding pages have outlined what we see to be the present state of paleodiet studies. We have presented the method with suggestions for sampling handling and analysis. In addition, we have summarized those data that could be compiled for the various areas of the world. There are several areas, both geographical and in terms of problem orientation, that promise great potential. The study of coastal adaptations, particularly the transition from fishing/gathering to dependence on plant and/or animal domesticates, is an obvious problem to be addressed by stable isotope studies. In terms of geographic areas, the dependence on C4 plants in mainland Asia needs to be studied, as does the expansion of C4 plants from Africa. Although we devoted a great deal of discussion to diagenesis, the emphasis should be placed on the recognition of which

samples are preserved well enough to be analyzed. It is this recognition that has been achieved most recently and will be the most useful.

Perhaps the best comparison of the present state of paleodiet studies is with the state of radiocarbon dating at the same stage in its history of use. The first radiocarbon dates were published in the early 1950s; Williard Libby won a Nobel Prize in Chemistry in 1960 for his discoveries that led to the dating technique. Many of these dates were used to address the timing of the arrival of the earliest humans into the New World. As more work was done, however, several complications arose. Dates run on bone were often not in line with expectations. In part, the investigations surrounding this difficulty led to an understanding of bone composition, bone diagenesis, and bone mineral uptake of ionic species. Greater implications for understanding our world, however, came from the recognition that dates run on maize and on marine shell yielded spurious results. The latter observation led to the recognition of the significance of a previously reported observation of distinctive stable carbon ratios in marine organisms (Parker, 1964), which in turn led to one of the early diet studies (Tauber, 1981).

The maize story is more complicated. During the 1960s several papers were published reporting a difference in isotopic fractionation in maize versus other plants (Hall, 1967; Bender, 1968; Lowdon, 1969), yet they did not recognize the significance of the difference. In 1961, Melvin Calvin won a Nobel Prize in Chemistry for his discovery of the C<sub>3</sub> mode of photosynthesis. At about the same time, the influence of photosynthesis on the fractionation of the stable isotopes of carbon was recognized by geochemists (Park and Epstein, 1960). In the mid-1960s, a second photosynthetic pathway was recognized, this time in a C<sub>4</sub> plant, sugarcane (Hatch and Slack, 1966). It was not until 1970, however, that a formal publication appeared that explained the significance of the difference in photosynthetic pathway on the carbon isotope ratios in the two groups of plants (Smith and Epstein, 1970).

The different threads, from chemistry, geochemistry, biology, and anthropology, were pulled together during the 1970s, over 20 years after the earliest publications. Once again radiocarbon dating is back on track. With the recent work by the Oxford Laboratory, among others (Hedges and Law, 1989), even the dating of bone is achieving new respect. Older dates are being rerun or, at least, reinterpreted (e.g., see Holliday and Johnson, 1986).

We suspect that our own field of paleodiet studies will also find its own track, now, as it enters its third decade. We can only hope that as much information about the world can be compiled during the sorting as was true for the radiocarbon dating studies. In the meantime, however, it is worth emphasizing that these stable isotope studies are real archaeological/anthropological techniques. Archaeologists have had to become increasingly sophisticated about the possibilities and limits of radiocarbon dating to use it as a tool in their research.

In the same manner, archaeologists should be prepared to include stable isotope analysis in their research design following discussion with the analyst. The role of the archaeologist in designing studies and collaborating on the interpretation of results will be central to the continuing development of this area of stable isotope studies. The coordination of stable isotope data with other archeological remains has transformed the questions that are now being asked of the prehistoric record. As the understanding of stable isotope systems improves, the challenge will be to continue to redefine the questions that are being asked of both stable isotope and archeological data.

To decide if the stable isotope techniques are appropriate tools for a particular research program, a few preliminary questions should be pondered. First, does the isotopic ecology of the environmental or cultural setting suggest that variation in isotope signatures is likely to be the result from differences in dietary intake? Is this variation likely to be interesting or relevant from a social or biological point of view? Second, are the suspected dietary patterns distinctive enough to be diagnosed using the precision available from stable isotope studies? Third, are the samples from well-understood, well-documented archaeological contexts? Fourth, how will stable isotope results integrate with other paleodietary data sets in the study? Are other data available? Fifth, are the bones well enough preserved to produce reliable stable isotope data?

After the answers to these questions are assessed, active collaboration with a specialist in stable isotope studies should begin. There may be certain areas of study that may not benefit from such analysis, but it would be difficult to decide which areas to ignore when there remains so much to be learned!

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