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Stable Isotope Analyses in Human Nutritional Ecology

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ABSTRACT Extracting nutrients is of upmost importance to the survival of any individual or species. One of the distinguishing characteristics of the order Primates is the vast range of nutritional adaptations it exhibits. Within our own species all manner of adaptations are practiced and it has been a major focus of research to determine when and where these various patterns originated. We present one method based on stable isotope analysis in human tissues and discuss its contributions. The ratios of $^{13}C/^{12}C$ and $^{15}N/^{14}N$ vary among various pools (i.e., the atmosphere, the oceans, plant communities, trophic levels). These differences are transferred to humans via the foods they eat. The major differences in carbon occur between two photosynthetic pathways (C3 and C4), which in the New World permits tracing the introduction of maize (a C4 plant) and in Asia permits tracing the introduction of millet (also a C4 plant). The marine and terrestrial systems have distinctive isotope ratios of both carbon and nitrogen. Thus, the dependence on marine resources has been traced throughout several areas of the New and Old Worlds. We discuss several potential sources of variation including sex, age, nutritional status, among others. We conclude with some suggestions for future research.

Food and sex, i.e., the ways in which animals obtain access to necessary nutrients and to mates, are the most basic aspects of animal behavior. One can argue that the study of food is primary since without the former the latter becomes irrelevant. In the most recent edition of *Human Biology* (Harrison et al., 1988), Baker's overview of the variety of methods employed by humans to extract nutrients suggests that dietary adaptability may be the key to the overall success of our species. Humans are able to survive as complete carnivores (e.g., Eskimos), herbivores (e.g., certain cultural/religious groups in India), and almost any combination of these two extremes. The order Primates displays an enormous range of dietary adaptations and it does not seem too extreme to suggest that such dietary flexibility may have been an important component in the spread of our order, the emergence of our genus, *Homo,* the evolution of our own species, *Homo sapiens,* and the variety of social/economic/cultural modes of organization evident among humans today.

Physical anthropologists have focused on the diets of living humans and other primates and also on recent and not so recent human and primate ancestors. The methods utilized for assessing varieties of foods consumed have been extremely diverse, but can be categorized into two basic types. The first applies to groups, whether the group be a population or species. Group assessment methods include consideration of body size constraints on nutritional requirements (e.g., Fleagle,

1985), optimal foraging models (e.g., Winterhalder and Smith, 1981), site catchment analysis (e.g., Styles, 1985), and analyses of recovered floral and faunal materials and various cultural residues such as pottery or stone tools.

Body size constraints have been useful in reconstructing general nutritional strategies of primates from earlier epochs (Kay and Hylander, 1978; Kay, 1984), but between groups of humans or species of the genus *Homo,* its usefulness is limited for obvious reasons. Development of optimal foraging models assumes that choices among possible activities are made to optimize the acquisition of a particular currency, usually energy. Developed by animal ecologists, the application to human groups has been most successful among foragers. Part-time horticulturalists and full-time agriculturalists present systems too complicated for the models as they exist presently. Site catchment analysis requires the assumption that the landscape and its productivity have remained unchanged or can be accurately reconstructed.

The analysis of floral (Hastorf and Popper, 1988) and faunal (Binford, 1981) remains and cultural residues left in middens by human groups or discarded by primates as they move throughout the day are invaluable because they identify the available dietary components. However, the relative importance of any individual item in the diet is difficult to assess (Smith, 1979) even though items may be ranked by abundance in middens or time spent in feeding. Cut-marks on bones of discarded fauna serve to indicate butchering patterns (Bunn and Kroll, 1986; Potts and Shipman, 1981; Shipman and Rose, 1983), but for the most part, reveal more about behavioral patterns than about diet. For instance, Marshall (1986) discovered that among recent pastoralists in northern Kenya, most meat was eaten in stews. The behavior was reflected in the bone breakage pattern where all bones were broken to a size that would fit a normal cooking pot. The analyses of residues on pots by a variety of techniques such as gas chromatography and stable isotope analysis can reveal processing of certain products and use of particular food items (Hall et al., 1990; Hastorf and DeNiro, 1985; Morton et al., 1991; Marchbanks, 1989; Marchbanks and Quigg, 1990). Travelers' accounts have also provided interesting information on recent human groups (de Champlain, 1968; Swanton, 1911), although these have the obvious drawback of being observations limited in time and scope.

The second basic set of analytical methods used to identify the varieties of food consumed gives information about individuals rather than groups. Among these methods are microwear analysis on teeth, analysis of fecal material and intestinal content, paleopathology, bone shape assessment, and demographic analyses (see Larsen, 1987, for a recent review). Tooth microwear has been monitored on animals as diverse as Miocene apes (Teaford and Walker, 1984), Plio-Pleistocene hominids (Grine and Kay, 1988), and recent hunter-gatherers (Rose and Harmon, 1986). Across the order Primates, microwear serves to discriminate between hard-object and soft-object feeding. Among human groups it has been monitored for compression fractures associated with nut consumption, surface polishing from vegetable fiber, and the disappearance of such polishing with the introduction of maize agriculture (Rose and Harmon, 1986). This method provides a valuable record of foods processed in the mouth over the several months preceding the individual's death (Walker et al., 1978; Covert and Kay, 1981) and, thus, could indicate seasonal diets. Fecal and stomach/intestinal content analyses provide information limited to single or, at most, a few meals (Fry, 1985). Only foods recognizable after some digestion has occurred can be identified, and, in addition, such materials, however informative, are preserved only rarely. Chemical analyses of lipids diagnostic of meat, leaves, or fruits may provide further qualitative evidence for dietary composition in more recent specimens (Marchbanks, 1989; Marchbanks and Quigg, 1990).

Paleopathology and micromorphology of bone cross-sections (Martin et al., 1985) have been used to infer nutritional adequacy, not specific diet items. The protein composition of the organic fraction of bone has also received some attention. Al-

bumin has been identified in fossil bone (Tuross, 1989), but has not been used in diet reconstruction. Instead, the approach demonstrates the potential of obtaining information about other aspects of adaptation.

This paper is also concerned with diet in the broad sense. We focus on the application of stable isotope ratios of several elements in bone to trace prehistoric food intake. Stable isotope ratios of carbon and nitrogen from bone collagen and of carbon from bone and tooth enamel apatite reflect consumption of certain categories of foods. The unit of analysis is the individual; thus, variation within groups may be assessed in addition to differences between groups. Differences in diet between social and economic strata can be investigated using stable isotopes. The diet information obtained is an average of several years' consumption because the turnover rate of human collagen is on the order of ten years (Stenhouse and Baxter, 1979). An as yet undetermined period is represented by the apatite fraction of bone. Therefore, seasonal variation cannot be determined using bone collagen. Other tissues (e.g., skin and hair) that are sometimes preserved have much faster turnover rates and may provide data on food consumed over a short period prior to death.

As with any review of this sort there are many topics that we cannot include due to space limitations. We restricted the review to studies of human bone or to studies with data directly influencing our interpretation of diet in humans. Thus, many interesting papers on climate reconstruction and ecology that use stable isotope data are not included (e.g., Marino and DeNiro, 1987; DeNiro et al., 1988; Bada et al., 1990; Marino and McElroy, 1991). Further, we decided not to include those studies in which the isotope ratio is only indirectly an indicator of diet. Most notable of this type of study is the use of strontium isotope ratios (e.g., Ericson, 1985, 1989; Sealy et al., 1991) where the ratios reflect those of the soils in which plants grow. We direct the interested reader to these papers.

ANALYTICAL STUDIES OF HUMAN REMAINS

Basic assumptions

The total composition of animal tissues is determined by the balance between input and output variables. In the former category we include the normal components of the diet, drinking water, and any atmospheric constituents that may be incorporated into the body (particularly oxygen). Outputs are determined by the balance between tissue breakdown and synthesis, i.e., catabolic versus anabolic processes. In general most animals are close to a steady state condition in which the inventory of chemical substances in the body does not fluctuate greatly on a day-to-day time scale (Shils and Young, 1988).

This assumption is the basis for the use of the composition of an animal's tissues for inferring dietary inputs. The approach requires calibration of data, relating inputs to the steady state compositions of individual tissues. Ideally, for human diet studies such data would be obtained by analysis of animals serving as human models, or of humans living under controlled conditions. In such instances, diets could be ascertained and modified to assess the effect of changes on tissue composition. Ethical constraints prevent extensive studies using humans in most experimental situations and the difficulties encountered in identifying appropriate animal models for specific studies are not trivial. Even so, valuable information summarized later in this paper has been obtained as a result of both controlled experimental studies of animals as well as the study of natural populations of humans and free-ranging animals.

For the purposes of paleoanthropology, the main focus of analysis must be on those tissues that frequently survive long after death of the human or animal. For the most part we are therefore concerned with the composition of fresh and fossil bone. The relationship between diet and bone composition is modified by the anabolic processes that mediate construction of bone tissue. Thus, characteristic differences between diet composition and tissue composition often occur. Below, we address the possibility that differences between the two may also vary as a func-

tion of the state of health of the animal, the quality of the diet, or other biological parameters (age, sex, etc.). Various constituents of bone have been proposed as specific indicators of diet (see papers and references in Price, 1989a; Schwarcz et al., 1989; Sillen and Armelagos, 1991), but this review will focus on only one type, stable isotopes. Other chemical and isotopic tracers of diet are also possible; these will be introduced briefly in the next section. Some of these, such as isotopes and certain nonessential trace elements (e.g., strontium), can be considered as essentially *passive* markers that are fortuitously incorporated into foods. Other dietary tracers (e.g., zinc, iron) are biologically *active* substances, either as nutritional requirements or as toxic substances. It is possible that their presence in bone or other preserved tissues may indicate dietary deficiency or excess of the component, but conclusive evidence has not yet been presented.

Theory and limitations

The major element content of bone mineral and protein is largely controlled by biological factors that do not vary as a function of diet. The mineral phase is carbonate-hydroxyl apatite with compositional variability in the proportion and structural distribution of carbonate and hydroxyl groups in the crystals (McConnell, 1962). Minor amounts of magnesium are also present in living bone (tenths of a percent) while traces of a large number of elements have been reported (Underwood, 1977).

The organic matrix of bone is largely composed of collagen, a protein with a well-defined amino acid sequence that does not vary as a function of diet (Stryer, 1975). Approximately 5% of bone organic matter is a mixture of noncollagenous proteins whose amino acid sequences are also independent of diet (Boskey and Posner, 1984). Some diet-derived organic chemicals can be found in bone at trace concentrations, if they resist metabolic processes and are deposited in an unmodified state in a tissue. An example is tetracycline, which is deposited in bones and teeth (Bassett et al., 1980).

Of the various chemical constituents of bone, the only ones that have proven useful as indicators of past diet are the trace and minor elements. Some of these are chemically similar to the major element components. For example, the alkaline earth elements (i.e., magnesium, strontium, and barium) can substitute for calcium. In addition, arsenate can substitute for phosphate, and fluoride substitutes for the hydroxide group. A large number of other trace metals can also enter the structure of the bone mineral crystal (i.e., the crystal lattice) presumably by substituting for calcium. Among these, the most commonly reported are copper, zinc, manganese, and lead. The latter is presumed to be a toxic substance for which there is no minimum dietary requirement. Some of the others are essential components of the diet although their entrapment in apatite is probably an accident of their chemical similarity to calcium.

For the apparently nonessential metals, such as strontium, barium, and lead, that substitute for calcium, it appears that their concentrations with respect to calcium are highest in herbivores, and are lowest in carnivores at the top of the trophic pyramid. This phenomenon of "biopurification," demonstrated in a field situation by Elias et al. (1982), results in a decrease in the concentration of these elements relative to calcium (i.e., strontium:calcium, barium:calcium, or lead:calcium) at each subsequent trophic level. In other words, the ratio in the consumer is depleted with respect to the elemental ratio in the food by a constant fraction. It should be possible in this way to learn about the trophic level of an organism by comparing these ratios in bones of known herbivores and carnivores from the same region (Schoeninger, 1980; Sillen, 1981a,b). It is essential to normalize to local element ratios, since these can vary widely depending on the geochemical makeup of the environment (Schoeninger, 1979; Sillen and Kavanagh, 1982).

These techniques have been successful in specific cases. The ratio of barium to strontium in bone mineral may indicate the amount of marine foods in human diet due to the extreme depletion of barium in seawater (Burton and Price, 1990). The

concentration of strontium and zinc in bone mineral has served to indicate the proportion of meat in human diet (Sillen and Kavanagh, 1982; Schoeninger, 1979, 1981; Sillen, 1981b; Blakely and Beck, 1981; Morgan and Schoeninger, 1989). Other cases have not proven to be as successful because the expected separation among herbivores, carnivores, and humans was not apparent (Decker, 1986; Katzenberg and Schwarcz, 1986). Some of the difficulties encountered in these applications appear related to the complexity of food web structures (Sealy and Sillen, 1988), where identical ratios may result from a variety of trophic positions (Schoeninger, 1989).

The applicability of trace element analysis to ancient bones is limited principally by the fact that the concentrations of trace elements change as a result of diagenesis of the bone (Nelson et al., 1986). This concept, which has wide implications to our studies of paleodiet, refers to the wide variety of chemical and mineralogical changes that affect fossil bone as a result of burial. One of these processes is recrystallization of bone mineral, which can result in uptake of trace elements from the surrounding soil, or loss of some elements to the pore water of this soil (Pate and Hutton, 1988, Pate et al., 1989).

Isotopic analyses

Most elements exist as mixtures of two or more isotopes that have the same number of electrons and protons but differ from each other in the number of neutrons in their nuclei (Hoefs, 1987). Since chemical reactions for the most part are determined by the electron configuration of an ion or atom, the different isotopes of a single element have the same chemical properties. Isotopes may be stable or unstable radioactively and also may be radiogenic or nonradiogenic. These are introduced in the following sections.

Radiogenic isotopes

Some elements vary in their isotopic makeup because one or more of the isotopes of the element are radiogenic, that is, the product of the decay of a long-lived ("primordial") radioactive isotope. The main isotopes used in this way are ^{87}Sr (strontium), and the various isotopes of lead, ^{206}Pb, ^{207}Pb, and ^{208}Pb. These isotopes vary considerably in abundance with respect to the associated non-radiogenic isotopes (^{86}Sr and ^{204}Pb), and these variations can be used to determine the source of a particular sample of bone or other material. That is, the strontium and lead isotope ratios act as "fingerprints" of the source of these elements. Strontium isotope measurements were used, for example, to demonstrate the mobility of strontium in buried bones and thus the potential unreliability of strontium concentration analyses (Nelson et al., 1986). Lead isotope analyses have been used to demonstrate the source of lead found as a trace constituent of buried human bones (Ericson et al., 1979). The use of more than one isotope can permit more precise definition of possible sources of strontium or lead. As Nelson et al. (1986) showed, however, continuous uptake of strontium from the soil or pore water can alter or obliterate an indigenous, diet-derived signal. As with trace element concentration studies, a useful safeguard is to analyze associated bones of fauna whose diet can be assumed not to have changed through time.

Natural radioactive isotopes

Some short-lived radioactive isotopes occur in nature and can be assimilated into human tissues through ordinary metabolic processes. The foremost example is ^{14}C (carbon 14), which, like ^{13}C to be discussed below, enters the photosynthetic cycle from atmospheric carbon dioxide (CO_2), and thereafter passes through all trophic levels, finally entering the human diet as plant or animal foods. The level of ^{14}C activity in prehistoric human tissue is usually taken as an estimate of the age of the tissue. For tissues (e.g., collagen) of known age, however, the ^{14}C activity can be used as a dietary tracer. For example, marine foods are known to be depleted in ^{14}C due to the finite residence time of ^{14}C in seawater, although information on

TABLE 1. Natural abundance of stable isotopes

Isotope	Abundance (percent)
^{1}H	99.985
^{2}H	0.015
^{12}C	98.900
^{13}C	1.100
^{14}N	99.640
^{15}N	0.360
^{16}O	99.800
^{17}O	0.040
^{18}O	0.200
^{32}S	95.000
^{33}S	0.760
^{34}S	4.200
^{36}S	0.020

Taken from Burlingame and Schnoes, 1969.

marine foods in an organism's diet can usually be determined more precisely by stable isotope analyses.

Other naturally occurring radioactive isotopes are the daughter isotopes of ^{238}U (uranium), such as ^{226}Ra (radium) or ^{210}Pb (lead), which can be used to estimate the mobility of these elements in buried bone, as well as ^{230}Th (thorium), which can be used to estimate the age of the bone (Rae et al., 1989). In general, the short-lived radioisotopes (including other "cosmogenic" ones like ^{36}Cl [chlorine]) are principally of interest for the information they provide about timing of burial and diagenetic processes. In rare instances, there may exist significant differences in the activity level of possible food sources, which could be passed onto the consumer as a distinctive isotopic label in either the mineral phase or bone collagen. For example, as mentioned above, marine foods are intrinsically depleted in ^{14}C with respect to most terrestrial sources (Taylor and Slota, 1988) and would cause unaltered bone collagen to have an anomalously high apparent ^{14}C age.

Stable, nonradiogenic isotopes.

All the biochemically important elements except fluorine have more than one stable isotope. Little is known about the isotopic distribution in many of these elements (e.g., calcium, chlorine, magnesium, potassium, and silicon; Ehleringer and Rundel, 1989), although they appear to have potential in clinical studies of metabolism (Janghorbani and Ting, 1990). As discussed more thoroughly in the remainder of this paper, however, the isotope distributions of carbon (C), nitrogen (N), oxygen (O), hydrogen (H), and sulfur (S) have proven invaluable in many studies of biological and ecological interest.

The relative abundances of all the stable isotopes of a given element are essentially constant from one sample of the element to another. Carbon has two stable isotopes, ^{13}C and ^{12}C, with natural abundances of approximately 1.1% and 98.9%, respectively. Nitrogen has two stable isotopes, ^{15}N and ^{14}N, with a natural abundance of 0.36% and 99.64%, respectively (Hoefs, 1987). In general, the other light elements have two or more stable isotopes, of which the lowest-mass species is the most abundant (Table 1).

Because different isotopes of a single element have different masses, due to the difference in numbers of neutrons, they can have different kinetic and thermodynamic properties (i.e., differences in rates of reactions and in heat capacity) when they undergo chemical reactions (Urey, 1947). These differences mean that isotopically labeled molecules can be fractionated from one another in chemical processes. The term "isotopic fractionation" refers to any measurable difference in the isotope ratio between two associated substances, e.g., a reaction product and its substrate. For example, the $^{13}C/^{12}C$ ratio of atmospheric CO_2 is larger than that of photosynthetically produced cellulose. There is a fractionation between the CO_2 (substrate) and cellulose (product). The magnitude of the isotopic fractionation

generally decreases with increasing temperature, and is greater the larger the proportional difference in molecular weight of the isotopically labeled atoms, or, as we call them, *isotopic species* (e.g., $H_2{}^{18}O$ and $H_2{}^{16}O$). Isotopic paleodiet studies must take into account fractionations between various foods and the animal tissues that are the product of the food. For further reading on the theory of stable isotopic fractionations, see Hoefs (1987) and the volume edited by Rundel et al. (1989).

THEORY OF STABLE ISOTOPE DISTRIBUTIONS IN NATURE

Isotope ratios: Notation

Because the observed fractionations between isotopic species during chemical reactions are very small, they are measured in fractions of a percent. These small differences can be readily measured by modern mass spectrometers (see below). As we are only interested in relative and not absolute differences in isotope ratio, we express the isotopic abundance in a substance by the difference of its isotope ratio (R) from that of a standard, using the δ notation:

$$\delta\,E\,(x) = \left(\frac{R_x}{R_s} - 1\right) \times 1{,}000$$

where R is the isotope ratio (e.g., $^{18}O/^{16}O$) in a sample (x) or standard (s) for an element E. The units of δ are per mil (‰). A substance whose isotope ratio is less than the standard gives a negative δ value, and is said to be "less positive" than the standard. The difference in isotopic composition between any two substances A and B is given by $\Delta_{AB} = \delta(A) - \delta(B)$. δ values are approximately additive for small differences in δ. Significant variations in stable isotope ratios are only observed for elements of mass less than 40. For heavier atoms, the difference in kinetic and thermodynamic properties of the isotopes appears to be too small to induce significant fractionation (e.g., calcium isotope effects are barely detectable in natural systems).

Sources of isotopic variation

Equilibrium fractionation

As mentioned above, two isotopically labeled species of the same compound (e.g., $C^{16}O_2$ and $C^{18}O_2$) are slightly different thermodynamically. Therefore, we can always write a possible isotopic exchange reaction between them, as, for example, between carbon monoxide and carbon dioxide:

$$1/2\,C^{16}O_2 + C^{18}O = 1/2\,C^{18}O_2 + C^{16}O \qquad (1)$$

Note that the molecules are the same on both sides of this reaction and only isotopic labels have been shifted. Isotopic equilibrium between the left and right sides of this reaction will result in a particular value of $\Delta CO_2\text{-}CO$ at a particular temperature, which can be determined experimentally or, for simple molecules, can be calculated from statistical mechanics (Bottinga, 1969). Such equilibrium exchange reactions are important in determining isotopic partitioning of simple molecules such as CO_2, H_2O, or HCO_3^- (bicarbonate). Isotopic equilibrium is seldom attained between organic molecules in biochemical systems.

Kinetic isotope effects

For any chemical reaction involving isotopic species, it is possible to write two isotopic reactions. For example, in the chemical reaction:

$$CH_4 + 3/2\,O_2 \rightarrow CO_2 + H_2O \qquad (2)$$

the two isotopic reactions are:

$$^{12}CH_4 + 3/2\,O_2 \rightarrow {}^{12}CO_2 + H_2O \qquad (2a)$$

$$^{13}CH_4 + 3/2\ O_2 \rightarrow {}^{13}CO_2 + H_2O \quad (2b)$$

In general, the rate constant 'k' (the rate of reaction under a set of defined conditions) will differ for the two isotopic reactions, typically by a few tenths of a percent. As a result, in reaction (2) the product of the reaction (CO_2) will differ slightly in its isotope ratio from that of the substrate (CH_4) in those cases where the reaction does not go to completion. If the reaction goes to completion, the final product will, of course, have the same isotope ratio as the substrate. If the substrate constitutes a large pool of which only a small part is ever consumed (e.g., atmospheric CO_2 used in photosynthesis), then there will be a persistent difference in isotope ratio between substrate and product. Such differences can be quite large, up to several tens of per mil. We call such differences kinetic isotope effects (KIE) (Melander and Saunders, 1980). The magnitude of the differences in isotope ratio decreases with increasing temperature. In many reactions the fractionation is such that the lighter species has the higher rate constant and as a result the product is depleted in the heavy isotope. For example, during the formation of cellulose from CO_2, there is a KIE of about 17‰ in the majority of leafy plants.

Natural isotopic tracers

Substances produced in nature acquire their isotopic label from their parent chemical species. As a result of various isotopic fractionation processes (e.g., equilibrium fractionations or kinetic isotope effects) we find that certain natural systems have characteristic isotopic labels, which are then systematically passed on to substances formed from them. For example, meteoric (i.e., surface) waters are always depleted in ^{18}O and ^{2}H (deuterium [D]) with respect to seawater (Gat and Gonfiantini, 1981). Further, in relation to each other, it is found that

$$\delta D = 8\delta^{18}O + 10‰ \quad (3)$$

in virtually all meteoric waters. Therefore, any substance that acquires its oxygen or hydrogen atoms from meteoric water will tend to reflect that relationship unless it has also experienced some other isotopic fractionation process. We can thus use the stable isotope ratios of natural substances as tracers to determine the source of the material. Due to the additivity of δ values, we can assume that normal mixing relations exist. That is, a substance S that derives its atoms from, say, two natural sources will have an isotopic composition

$$\delta(S) = X_A\,\delta(A) + (1-X_A)\,\delta(B) \quad (4)$$

$$X(B) \quad 1 \quad - \quad X(A)$$

where X(A) and X(B) are the atom fractions derived from the two sources A and B, with isotopic compositions δ(A) and δ(B), respectively. Note, however, that these mixing rules will, in general, have superimposed on them characteristic fractionations with respect to the sources, which must be included in equations of the form of (4). These fractionations will be described more fully as we deal with individual isotopic systems.

Analytical methods and precision

Stable isotope analyses are done on mass spectrometers, which automatically measure the isotope ratio of a sample and then compare it with the isotope ratio of a standard. That is, the sample and standard are alternately introduced into the source of the mass spectrometer in the form of a gas, through an appropriate set of electrically operated valves. For each element there is an appropriate gas, which is conventionally analyzed to determine isotope ratios: CO_2 for $\delta^{13}C$ and $\delta^{18}O$; N_2 for $\delta^{15}N$; and H_2 for δD (also written $\delta^{2}H$). Sulfur isotope ratios can be determined on either SO_2 or SF_6.

Analysis of organic materials for carbon, nitrogen, or hydrogen isotope ratios is relatively simple. Once the organic substance (collagen, amino acid, cellulose, etc.)

has been isolated and purified, it is placed in a quartz or Vycor (synthetic quartz) tube, which is sealed at one end, and to which is added an excess of cupric oxide (CuO); copper and silver metals are also added to ensure that sulfite (SO_2) and various oxides of nitrogen are not produced in the reaction. The tube is evacuated and sealed with a flame; it is then placed in a furnace and heated at 850–875°C for one hour, and then slowly cooled. The carbon, hydrogen, and nitrogen of the sample is converted quantitatively to CO_2, N_2, and H_2O. These species can then be separated by cryogenic distillation in a vacuum line and analyzed on a mass spectrometer. The H_2O can be either passed over hot uranium to convert it to H_2 gas, or reduced by zinc catalyst.

Specialized techniques are used for isotopic analysis of organic matter for oxygen (Thompson and Gray, 1977) and sulfur (Fry et al., 1986). Oxygen isotopic analysis of phosphate of bone and carbon and oxygen analysis of carbonate of bone mineral are discussed below. In each technique, it is essential to obtain 100% of the atoms from the sample being analyzed as CO_2, N_2, H_2O, etc. Partial yields (as in the production of CO and N_xO_y) commonly result in isotopic fractionation and errors in the isotope ratio.

The precision of isotopic analyses (repeated combustion, purification, and mass spectrometric measurement of a single organic or inorganic sample) is generally $\pm 0.1–0.2‰$ for all elements except H, for which it is $\pm 1‰$. Data are reported relative to international standards: for carbon and oxygen the standard is PDB (PeeDee Belemnite carbonate); for nitrogen it is AIR (Ambient Inhalable Reservoir); for sulfur it is CDT (Cañon Diablo meteorite); and for hydrogen it is SMOW (Standard Mean Ocean Water). Descriptions of these standards and their availability are given in Hoefs (1987). Normally, each laboratory establishes an internal standard, which is periodically calibrated against these international standards.

ISOTOPICALLY LABELED MATERIALS IN HUMAN REMAINS

The various tissues of the human body are principally composed of atoms of a few elements: carbon, hydrogen, nitrogen, sulfur, oxygen, and phosphorus. Of these, all but the last have stable isotopes, and there are significant variations in all of the isotope ratios. If a human has been eating a uniform diet for a period of time that is long compared with the rate of turnover of a given tissue, then the isotope ratio of each tissue and organic molecule in the body will achieve a steady state value. That value will generally differ from that of the dietary intake (i.e., there is a fractionation between the tissue and the diet). The overall isotopic composition (weighted mean of all components) itself represents a steady state between intake (principally diet) and outputs (urine, feces, expired CO_2 and H_2O). In this section we shall only discuss those tissues that are actually found preserved in normal circumstances. Later we discuss the characteristic isotopic differences observed among these tissues and the characteristic fractionation between them and the diet.

The majority of the studies thus far have used bone as the material for analysis. Bone is a complex tissue composed of three components: water, an organic matrix, and an inorganic mineral fraction closely bound to the organic matrix. The relative proportions of these three components vary somewhat across species, among bone types, and throughout the lifetime of an individual (Lowenstam and Weiner, 1989). In general, the mineral portion accounts for approximately 55–75% of dry weight; the remainder is partitioned as approximately 90% collagen, about 5% noncollagenous proteins, and $<5\%$ a combination of lipids and carbohydrates (Boskey and Posner, 1984). The organic and inorganic components are extracellular for the most part.

Several of these components contain carbon and/or nitrogen and are useful for human dietary studies. Collagen contains both carbon and nitrogen and is relatively insoluble due to extensive linkages between each of three equal-sized chains. Because of this, even degraded bone often contains some collagen residues. This, in

addition to the large fraction of bone that it represents, has resulted in its use for most dietary studies. Noncollagenous proteins contain both carbon and nitrogen and their potential in diet studies has received some attention (Masters, 1987). The small carbohydrate and lipid fractions contain carbon but no nitrogen and, in most cases, are rapidly removed from bone after burial (Evershad, 1990). The carbon in bone apatite records dietary information although there has been much controversy over the exact message (Sullivan and Krueger, 1981; Krueger and Sullivan, 1984; Lee Thorp et al., 1989; Koch et al., 1990). One complication is the vulnerability of bone carbonate to exchange with carbonate dissolved in groundwater (Hassan et al., 1977; Hassan and Ortner, 1977; Schoeninger and DeNiro, 1982; but see Krueger, 1991). Recent work, however, suggests that apatite may be useful in some diet studies (Lee Thorp and van der Merwe, 1987, 1991; Lee Thorp et al., 1989).

Collagen

Nature of the material

Collagen is a fibrous protein that constitutes approximately one-quarter of all proteins occurring in mammals (Stryer, 1975). At least five different types of collagen have been identified as unique gene products; the one found in bone, skin, dentin, and tendon is called Type I. The following description applies to Type I collagen.

The most important and unique properties of collagen are its great tensile strength and its relative insolubility due to extensive linkages between each of its three equal-sized chains. Each chain has approximately 1,000 amino acid residues, and is approximately 300 nanometers (nm = 10^{-9} m) in length. Molecules are ordered functionally into fibrils 10–200 nm in diameter (Eyre 1980), aggregates of which can be observed by light microscopy. In comparison, single crystals of hydroxyapatite are approximately 20 nm × 0.5 nm (Lowenstam and Weiner, 1989).

The amino acid composition of collagen is extremely ordered. Approximately one-third of the residues are glycine, and two other amino acids, proline and hydroxyproline, together constitute another one-fifth to one-fourth of the total. Hydroxyproline is synthesized directly from proline by posttranslation enzyme reactions (Eyre, 1980) and, thus, there is no accompanying fractionation.

The high frequency of glycine is most unusual among proteins, as is the presence of hydroxyproline. The regular glycine-X-Y sequence in collagen accounts both for collagen's structural integrity and its triple helical form because only glycine is small enough to fit in the center of the triple helix (Stryer, 1975; Boskey and Posner, 1984). 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) is about 3:1. In other proteins, this C:N ratio is closer to 5:1. In sum, the low C:N ratio and the large relative amount of glycine plus proline/hydroxyproline are considered diagnostic features of collagen. These features are the ones used in assessing the preservation of bone prior to isotopic analysis of the organic fraction.

Methods of preparation for analysis

Several methods for the preparation of "collagen" for analysis have been outlined by various authors. The term "collagen" is placed in quotes because in most cases the amino acid composition of the material has not been determined. Three common methods are outlined below. In the first, the material extracted for analysis is best termed "gelatin." It is that portion of preserved organic material in bone that dissolves in weak acid at slightly elevated temperatures. In the second and third methods, the material extracted for analysis is best termed "organic residue." It is the insoluble fraction that remains following demineralization of the bone with a calcium-chelating agent (method 2) or with weak acid (method 3).

Method 1 was developed by DeNiro and Epstein (1978, 1981) based on Longin's

(1971) work. Later the approach was modified by Chisholm et al. (1983) and Schoeninger and DeNiro (1984). The method works well on fresh bone, and on well-preserved archaeological material, but is less effective on some archaeological bone. The bone is ground at the temperature of liquid nitrogen to a size less than 0.71 mm, demineralized by soaking in 1 M HCl (hydrochloric acid) for 20 minutes and then washed to neutrality with distilled, deionized water. The sample, composed mainly of original bone organics found in fresh bone and, additionally, of soil contaminants such as humic acids in buried bone, is placed in 0.125 M NaOH (sodium hydroxide) at room temperature for 20 hours in order to remove contaminants. Because the NaOH treatment affects the isotope ratios in fresh bone by approximately 1‰ (DeNiro and Epstein, 1981), it must be applied equally to both fresh and buried bone. After washing the NaOH-soaked material to neutrality with distilled, deionized water, it is dissolved in 0.001 M HCl for ten hours at 90°C. Following this, the solution is filtered. Often, in the case of fresh bone, organic material of unknown composition is retained on the filter. The solution drawn through the filter is freeze-dried; the resulting substance is referred to as "gelatin." This is the sample that is placed in a quartz or Vycor tube, as described above.

Method 2 was developed by Tuross et al. (1988). Powdered bone (produced as described above) is placed in dialysis tubing with a high molecular weight cut-off and dialyzed against 0.5 M EDTA (ethylenediaminetetraacetic acid) at 4°C. The solution is changed at least once a day for ten days. At the end of this period, it is dialyzed against distilled water for five days, changing the water twice per day. Alternatively, larger (cleaned) bone chunks can be put in stoppered tubes filled with 0.5 M EDTA solution, and stored at 4°C, agitating continuously. The solution is changed at least once a day for ten days. This generates gelatinous "pseudomorphs" from the bone fragments, which can be isolated by centrifugation. The EDTA solution is removed and replaced by distilled water. The bone pieces are rinsed several times with distilled water and centrifuged, discarding the solution between each rinse. The remaining insoluble "organic residue" is freeze-dried.

Method 3 was developed by Sealy (Sealy and van der Merwe, 1986). This is the simplest and appears to produce an "organic residue" adequate for analysis. Cleaned chunks of bone are kept in closed vials containing 1% HCl at room temperature for seven to ten days, replacing with fresh acid several times during the period. Demineralization is complete when the sample is translucent. It can be soaked in an NaOH wash to remove contaminating humic acids, following the demineralization step. The "organic residue" is freeze-dried. The advantages of this method are extreme ease of preparation and minimal laboratory requirements. The disadvantage is that it may be difficult to determine when demineralization is complete. This becomes easier to determine by sight and texture with experience. Alternatively, the sample can be dried and if the dry weight exceeds 25% of the original dry bone weight it should be replaced in acid.

Although all three methods have proven successful on fresh bone (Tuross et al., 1988), evidence is mounting that the last two methods are preferable to the first. All of the organic material is available for analysis using methods 2 and 3, whereas in method 1, an amount is often retained on the filter in the last step. In addition, collagen fragments of high molecular weight (discussed below) will be lost in the original strong acid (1 M HCl) demineralization wash of method 1, whereas they are often retained in methods 2 and 3 because a strong acid is not used. In a comparison of method 1 with method 3 (Schoeninger et al., 1989), the latter method consistently produced a residue with an amino acid composition similar to collagen, whereas in certain cases method 1 did not retain amino acids representative of the complete protein. The problems with method 1 become most apparent when bone is degraded, containing less than 10% of its original collagen. Chisholm (1989) has suggested modifications of method 1 that may avoid these problems; the obvious drawback is the amount of time required by laboratory personnel. Methods 2 and 3 appear to produce similar results (Tuross et al., 1988; Schoeninger, unpublished data). At this time method 3 is the easiest preparation method that is

also reliable. For special samples such as fossils with small amounts of organic material, the EDTA extraction method (method 2) should probably be the method of choice. The only drawbacks with this method are (1) the EDTA must be removed completely or nitrogen from the EDTA will contribute to the measured nitrogen isotopic ratio and (2) the need to carry out the process at a constant temperature of 4°C.

Diagenetic effects; Criteria of acceptance

As most recently pointed out at the First International Workshop on Fossil Bone (see Schwarcz et al., 1989), bone recovered from archaeological contexts has been subjected to a complex postburial history that may result in the addition of contaminants such as humic acids or certain 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 (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 it is not clear as yet whether fungi or bacteria directly affect the stable isotope ratios (Grupe et al., 1989), they may be the cause of differential degradation of the collagen cross-linked chains.

In theory, archaeological bone can contain whole collagen, peptide fragments composed of various numbers of amino acid residues and free amino acids. When a substantial amount of organic material remains in bone (≈5% of dry bone weight), these other components comprise such a small percentage of the total that the original biogenic isotope ratios are not altered (DeNiro et al., 1985). The situation is different when the total organic residue is around 1% of the bone's dry weight. In practice, free amino acids are uncommon in recovered bone (Hare, 1980), suggesting that *external* contamination by amino acid segments is not a major problem (Tuross, 1989). The majority of organic fragments found in buried bone are from the bone itself (DeNiro and Weiner, 1989a), either from degraded collagen or noncollagenous proteins. It is possible, however, for fragments of degraded collagen to be composed of amino acid residues in proportions dissimilar to protein normally found in bone. As we shall discuss, there are significant isotopic fractionations between individual amino acids. Therefore, a selective loss may influence the isotopic composition of degraded collagen. For this reason, only complete proteins or protein fragments retaining the original amino acid composition are reliable.

The criterion for acceptance, then, is the identification of intact proteins or ones with amino acid compositions similar to intact proteins. The use of C:N ratios as a screening procedure (DeNiro and Epstein, 1981; Schoeninger and DeNiro, 1981; Schoeninger and DeNiro, 1984; DeNiro, 1985) 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). A C:N ratio outside the acceptable range of 2.7–3.6 indicates noncollagenous material, but a C:N ratio within that range imparts no assurance of acceptable collagen. If bone is well preserved superficially, has been chemically and mechanically cleaned, and retains at least 5% by weight of organic residue, the C:N ratio is superfluous. In bone retaining organic residues that weigh less than 5% of original bone weight, one cannot depend on a good C:N ratio. In such cases, it is imperative to determine the amino acid composition of the residue in order to determine if it contains a collagen-like pattern.

In cases where the amount of retained protein is less than 1% of the dry bone weight there can be an enrichment in nitrogen of 15‰ relative to the original collagen (Bada et al., 1989). This effect is due to the difference in rate constants for the peptide bond containing ^{14}N and the bond containing ^{15}N and is expected to be far less for carbon than for nitrogen. At this time, isotopic analyses should be limited to samples where organic residue is >1% of the original dry bone weight.

Noncollagenous proteins

Nature of the material

About 5% of the organic material in fresh bone consists of proteins other than collagen. Some of these proteins may be preserved preferentially relative to collagen (Hare, 1969, 1980; Tuross, 1989; Tuross et al., 1989). Masters (1987) demonstrated that they display an isotopic signature reflecting the same dietary signal as collagen. This may, however, not be true of degraded fragments of noncollagenous proteins (NCPs). Because of their greater stability, they show promise of providing isotopic paleodiet signals for older samples, especially where collagen has been removed diagenetically (DeNiro and Weiner, 1988c). Although noncollagenous proteins have been identified in fossil bone (Masters, 1987; Solanko, 1989; Tuross et al., 1989), no dietary applications have as yet been published.

Method of preparation for analysis

One difficulty has been the separation of these proteins from collagen degradation products. Recent attempts at using collagenase for removing degraded fragments of collagen have produced promising results (DeNiro and Weiner, 1988c; Solanko, 1989). However, commercially available collagenase may be contaminated, resulting in unreliable removal of collagen. A further difficulty is the low concentration of these proteins; extracting enough material for isotopic analyses can be problematical. Once the material has been separated from collagen, it should be identified as specific noncollagenous proteins by gel electrophoresis (Tuross, 1989; Solanko, 1989). Once the identity is established as a noncollagenous protein derived from bone, the material can be subjected to combustion, cryogenic purification, and mass spectrometry as described for collagen. Further identification of the material can be done by amino acid analysis. These proteins are at present compositionally less well defined than collagen and it is consequently more difficult to define their degree of preservation.

Bone mineral:Carbon

The mineral portion of bone (hydroxyapatite) is a calcium phosphate in which less than 10% carbonate is substituted for the hydroxyl and phosphate groups. This carbonate is derived from dissolved CO_2 in the plasma and therefore samples the total metabolic carbon pool, and ultimately, diet. The $\delta^{13}C$ of bone carbonate is about 12‰ more positive than that of plasma CO_2 (DeNiro and Epstein, 1978). The magnitude is close to the expected equilibrium isotopic fractionation between a carbonate mineral and CO_2 at body temperature. The fractionation between diet and apatite carbon and, thus, between apatite carbon and collagen carbon may differ depending on the trophic level of the animal. In theory, the fractionation between carbonate carbon and collagen carbon is on average about 8‰ for herbivores and 4‰ for carnivores (Koch et al., 1990, Land et al., 1980; Krueger and Sullivan, 1984; Lee Thorp et al., 1989). Krueger and Sullivan account for this difference by noting that carnivores derive a significant part of their metabolic energy from oxidation of animal fats, which are about 3‰ lighter than herbivore flesh. In principle, the observed fractionation between collagen and apatite could be used to indicate the trophic position of an animal. In practice, however, the difference has been discernible only statistically in populations of carnivores and herbivores and only among grazing ungulates and their predators (Lee Thorp et al., 1989). Even in those cases where carbonate conserves its initial $\delta^{13}C$ value, this method of trophic level analysis can only be used as long as collagen survives (ca. 10–20 ka). Nevertheless, Roksandic et al. (1988) have obtained some promising results by this method, and further investigation is necessary.

Method of preparation for analysis

The following is based on several published methods of preparation (Sullivan and Krueger, 1981; Schoeninger and DeNiro, 1982; DeNiro and Weiner, 1988b; Lee

Thorp and van der Merwe, 1987; Lee Thorp et al., 1989; Koch et al., 1990). Bone samples are deproteinated over several days in a weak solution of clorox (sodium hypochlorite). Following several rinses with double-distilled water to remove all traces of the clorox, the sample is soaked in a weak acetic acid solution to remove diagenetic minerals such as calcite. After two days in acetic acid, the sample is rinsed with double-distilled water and then dried. The dry sample is loaded into a carbonate reaction vessel with degassed phosphoric acid. The reaction vessel is sealed under vacuum and then placed in a constant temperature water bath at 50°C overnight, after which it is evacuated and sealed again. The acid and sample are combined and the vessel is placed back in the water bath at 50°C for five hours. The evolved CO_2 from the reaction is purified cryogenically, collected on the vacuum line, and analyzed by mass spectrometry.

Diagenetic effects

The assessment of diagenetic alteration of carbonate in apatite is more difficult than it is for collagen. Carbonate can be produced by geochemical processes as well as by biogenic processes. Unlike collagen, where exchange of individual elements cannot occur, heteroionic exchange of geochemical carbonate for original bone phosphate and isoionic exchange of geochemical carbonate for original biogenic carbonate occurs readily. Addition of calcium carbonate into bone interstices can be detected by X-ray diffraction (Schoeninger, 1980) and removed with acetic acid washes (Koch et al., 1990; Sillen, 1986), but identification and removal of carbonate contamination within the apatite mineral remains a serious problem. Analyses of a taphonomic series in eastern Africa (Koch et al., 1990) demonstrated that alteration can occur prior to burial, presumably in the absence of groundwater exchange, merely as the result of surface weathering.

If the effect is small relative to the biogenic signal, however, 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 enamel were discernible and were not in the direction expected from soil carbonate contamination. Koch et al. (1990) suggest 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.

Bone mineral: Oxygen

The $^{18}O/^{16}O$ ratio of the phosphate of bone mineral has been shown to be very resistant to exchange during bone diagenesis (Kolodny et al., 1983). The $\delta^{18}O$ of bone phosphate is, therefore, one of the best preserved records of the original composition of the organism. Kolodny et al. (1983) showed that this value reflects the $\delta^{18}O$ of blood plasma, and therefore is largely determined by the aqueous component of diet. There is a small influence from metabolic CO_2, whose oxygen is derived from air and has a constant $\delta^{18}O$ value of 23‰. The principal use of $\delta^{18}O$ analyses of animal bones so far has been to define changes in climate. Shifts in the ratios have been found in fossil deer bones from cave sediments in France that correlate with a glacial/interglacial transition (Schwarcz and Luz, unpublished). $\delta^{18}O$ of environmental water varies strongly with temperature, and leaf water (the principal source of water in some herbivores) is dependent on humidity (Luz et al., 1990). No direct application to paleodiet has yet been undertaken. Schwarcz et al. (unpublished) used $\delta^{18}O$ of a population of American soldiers from the Fort Erie (Canada) site to show that they had all originated in the same climatic region, probably in the northeastern United States. Such studies of oxygen isotopes in bone phosphates may be widely applicable to testing for geographic outliers in an otherwise homogeneous population (e.g., slaves, prisoners of war, exogamous mates).

The analysis of $\delta^{18}O$ in bone phosphate is, however, rather complex and tedious.

Apatite as such cannot be analysed because it contains oxygen atoms bound to carbon and hydrogen as well as phosphorous. These other oxygen atoms are less stable to diagenesis, and must be excluded. Typically, bone is converted to bismuth phosphate, and this substance is reacted with bromine pentafluoride to liberate O_2 gas for isotopic analysis (Shemesh, et al., 1983).

Bone mineral and collagen: Hydrogen

Hydrogen is bound in bone in at least two ways: (1) as hydroxyl groups in the bone mineral (inorganic hydrogen) and (2) as a component of the organic phase, especially collagen. There have been no significant studies of the D:H ratio of inorganic hydrogen in bone. Cormie and Schwarcz (1985) found that approximately 22% of the hydrogens (oxygen- and nigrogen-bound) in collagen are rapidly exchangeable with atmospheric water vapor, and will attain equilibrium with laboratory atmosphere in a few minutes. The remaining, carbon-bound hydrogens may be stable on the scale of thousands of years and can be used to estimate the δD of environmental water, a ratio largely determined by temperature. The effect of humidity on δD of leaf water is smaller than for $\delta^{18}O$ (Edwards et al., 1985), but Cormie and Schwarcz (1985) showed a parallel relation between $\delta^{18}O$ of bone phosphate and δD of collagen for North American white-tailed deer. No tests of δD in human collagen have been done.

Skin and hair

Some archaeological human remains include tissues other than bone, as a result either of intentional preservation practices at the time of internment (mummification), or the fortuitous environment of burial. Environments in which such preservation can occur include permafrost, anaerobic swamps, and hyperarid (desert) regions. Spontaneous mummification is commonly encountered in burials in arid regions such as the coastal deserts of Chile and Peru and the Sahara.

Dessicated soft tissues should also preserve a paleodiet signal. They have the particular advantage that the turnover rate for all isotopes is much faster than in bone, and therefore we should see a sample of the diet consumed in the last few months or weeks of life. A study of skin and hair samples from Nubians of the Meroitic, "X," and Christian periods of northern Sudan revealed uniform offsets in $\delta^{13}C$ and $\delta^{15}N$ between skin and bone collagen for these individuals (White, 1991). Further, successive portions cut from single bunches of hair showed enrichment in ^{13}C just prior to death. This was correlated with increased consumption of millet, typically harvested in the summer. It was inferred that death occurred more commonly in the summer, a time of climatic stress.

ENDOGENOUS SOURCES OF ISOTOPIC VARIABILITY

Age

Up to the present time, no intensive study of the association between age and isotopic composition has been undertaken for any long-lived mammal. The available data on living animals, however, suggests a lack of any association. Minagawa and Wada (1984) studied two species of mussels with different growth rates and observed no change in the stable nitrogen isotope ratio of the whole animal body relative to the diet up to an estimated age of 7 years. These authors also cite unpublished data on fish (*Tilapia*) that also lack any evidence for directional change in nitrogen isotope ratios with age when diet is held constant. In contrast, Rau et al. (1981) report a positive correlation between nitrogen isotopic ratios and body weight in a marine fish, Denver sole. It seems likely that dietary change through the life of the individual may account for this observation. The few data on mammals are consistent with the above. Elephants from Tsavo Park in South Africa display no association between $\delta^{13}C$ values and age and "$\delta^{15}N$ values were marginally related to age ($P = 0.04$) with an R^2 of only 0.08" (Tieszen et al., 1989:22). The isotope ratios in hair of modern Japanese were constant across all ages (Minagawa, 1991).

Studies of archaeological human skeletal material also indicate a lack of any age effect. The remains of 51 individuals (newborn to +50 years old) from Saskatchewan showed a range of variation in $\delta^{13}C$ values of only 1‰ even though the site was used for about 2,000 years (Lovell et al., 1986b). An early 15th-century massacre site in South Dakota had a greater range of variation in $\delta^{13}C$ values (≅3‰), but no association is obvious between the values and the age of the individuals at death (Bumsted, 1984). Neither of these studies included nitrogen isotope data. Variation similar to that observed in the Tsavo Park elephants occurred among the nitrogen stable isotope ratios of prehistoric agriculturalists in Ontario (Katzenberg, 1990). Whether this reflects a true age-dependent difference or a change in nitrogen availability through time is uncertain. The similarity of the data to those in modern Japanese hair argues against the former interpretation.

In cases in which significant age-associated variation has been observed, the archaeological evidence indicates a change in diet. An increase in $\delta^{13}C$ values in young children compared with infants from Ontario was interpreted to indicate 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 diet (Sealy and van der Merwe, 1988).

Thus, the data on humans are either cross-sectional (e.g., on modern hair) or from archaeological populations where constancy of diet is inferred, not known. Even so, they suggest strongly that there is no age-dependent physiological change in the way that carbon and nitrogen isotopes are processed. If diet is held constant throughout the life of an individual, an increase in isotope values could be produced through the recycling and/or reutilization of existing bone collagen in newly synthesized collagen. This process of recycling carbon and nitrogen from the body tissues occurs (Shils and Young, 1988) although the magnitude is unknown. It appears that such remodeling does not result in a shift in isotopic ratios, but a study directed toward elucidating this point would be most welcome.

Sex

Theoretical considerations (Chisholm, 1989) and the available data, which are admittedly limited in number, suggest that there is little, if any, variation among individuals that can be attributed to sexual differences. Mink raised on monotonous diets (DeNiro and Schoeninger, 1983) and the population of wild-shot elephants mentioned previously (Tieszen et al., 1989) had no difference in either carbon or nitrogen isotope ratios. Hair from the modern Japanese sample also showed no variation along sex lines (Minagawa, 1991).

Many archaeological samples analyzed thus far follow the above pattern. The samples from the massacre site mentioned above show a 0.5‰ difference in $\delta^{13}C$ between the mean values of the sexes (Bumsted, 1983, 1984). The amount is only about 0.3‰ above the level of precision of the measurement and so cannot be used in separating individuals of unknown sex. In comparison, the average $\delta^{13}C$ value in collagen of nine males differed from that of nine females by less than 0.1‰ in the Saskatchewan site (Lovell et al., 1986b). Studies of an Iron Age sample from Yugoslavia (Murray and Schoeninger, 1988), recent prehistoric pueblo agriculturalists (Spielmann et al., 1990), part-time horticulturalists in the Panhandle region of Texas (Habicht-Mauche et al., 1991) and the southeastern United States (Schoeninger et al., 1990), agriculturalists in prehistoric Belize (White and Schwarcz, 1989), and hunter-gathers in prehistoric Japan (Minagawa and Akazawa, 1991) and in highland Peru (Moore and Schoeninger, unpublished data) revealed no difference between men and women. Based on the above, 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 (Sealy and van der Merwe, 1988).

Trophic level

Based on several lines of evidence, there appears to be a trend of increasing δ values in both carbon and nitrogen from the level of primary producers to that of top consumers within individual trophic systems. The increase in $\delta^{13}C$, however, is too small (<1‰; Schoeninger and DeNiro, 1984) to be of use in estimating trophic position. Nitrogen, on the other hand, holds some promise, for ecologists and possibly for anthropologists.

Studies focused on both invertebrates (Wada, 1980) and marine (Wada, 1980; Minagawa and Wada, 1984; Schoeninger and DeNiro, 1984) and terrestrial vertebrates (Schoeninger and DeNiro, 1984; Schoeninger, 1985, 1989; Schwarcz and Knyf, 1991) agree that there is an enrichment of approximately 3‰ between trophic levels. The step-wise enrichment is obvious only when "the original nitrogen sources are all equivalent" (Minagawa and Wada, 1984:1137). As more systems are studied, it has become clear that comparisons across systems are not valid since the isotopic composition of the sources are not equivalent across systems. For example, the marine system and the terrestrial system have significantly different source nitrogen and therefore cannot be compared directly. Further, as discussed more thoroughly below, animals at the base of a terrestrial trophic system in desertic areas may not be equivalent to those at the base of a liberally watered area (Schoeninger and DeNiro, 1984, Schoeninger, 1989; Ambrose and DeNiro, 1986a; Heaton et al., 1986; Sealy et al., 1987).

Even so, within trophic systems, the 3‰ enrichment appears to hold constant across a wide variety of situations. This enrichment can be accounted for by noting that urea is significantly depleted in ^{15}N with respect to the whole body (Steele and Daniel, 1978), presumably as a result of a kinetic isotope effect during the conversion of amines to urea. With nitrogen loss as urea, the residual amine nitrogens in the body are enriched in ^{15}N; this signal is passed on to all the proteins during transamination.

A particularly intriguing study of humans included analyses of the fingernails of nursing mothers and the fingernails of their babies (Tuross et al., 1991). During the period of time that the babies were fed only a diet of mother's milk, the babies' fingernails were approximately 3‰ enriched over those of the mothers. After weaning, their fingernails decreased to an isotopic ratio equivalent to the mother. In application to a prehistoric group, children under the age of 4 years (presumably nursing or recently weaned) had, on average, bone collagen $\delta^{15}N$ values 1.5 to 2.0‰ more positive that those in adults (Tuross et al., 1991).

Despite early enthusiasm (Schoeninger, 1985), subsequent efforts to use $\delta^{15}N$ values to estimate the amount of meat in human diet have been largely unsuccessful (Spielmann et al., 1990). The explanation lies in the relatively small difference in $\delta^{15}N$ values between trophic levels, i.e., 3‰. Modern human groups appear to have an upper limit of about 35–40% of total calories ingested as meat protein (Szathmary et al., 1987; Noli and Avery, 1988). Eskimos, which are an apparent exception, traditionally ate the vast majority of their calories (>60%) as fat (Draper, 1977); today, processed carbohydrates have been substituted for the fat. Assuming a linear relationship between diet $\delta^{15}N$ and bone collagen $\delta^{15}N$, the difference between 10% and 35% of meat in diet would produce approximately a 0.75‰ difference in bone collagen $\delta^{15}N$. Given the variation in $\delta^{15}N$ of possible foods, even this example of an extreme difference in diet would not be discernible in most cases.

Water stress: Effect on ^{15}N

Although there appears to be little association between age or sex and carbon and nitrogen stable isotope ratios, worldwide variation in $\delta^{15}N$ values appears to be due to other factors in addition to diet. The first empirical study noted higher than expected $\delta^{15}N$ values in nondrinking rodents (Schoeninger and DeNiro, 1984) and, subsequently, a survey of humans and other animals from South Africa

showed a strong correlation between $\delta^{15}N$ value of collagen and rainfall (Heaton et al., 1986). Another study in eastern Africa (Ambrose and DeNiro, 1986a, 1989) appeared to reinforce the importance of water intake, although in some cases drought-tolerant mammals included in the study were not more positive than obligate drinkers.

The complexity of the system has become all too obvious in recent years. Among a wide variety of organisms in South Africa, terrestrial mammals from areas of low rainfall (<400 mm/year) had $\delta^{15}N$ values far more positive than expected from the isotopic ratios of plants at the base of the food chain (Sealy et al., 1987, contra Vogel et al., 1990). Even so, the more positive $\delta^{15}N$ values in collagen were not always obtained from animals living in areas that would have produced the greatest water stress. Low $\delta^{15}N$ values in ruminants from desert areas of the southwestern United States (Spielmann et al., 1990) also indicate that other factors are operating. Interestingly, Cormie and Schwarcz (unpublished data), in a study of white-tailed deer from North America, found that only consumers of C4 plants exhibit the low-rainfall, high-$\delta^{15}N$ relationship. In contrast, in northern Kenya all terrestrial fauna are more positive than expected; there is no unique association between consumers of C4 plants and exceptionally high $\delta^{15}N$ values (Schoeninger, 1989). Adding yet another complicating set of data, Vogel et al. (1990) present data from plants in South Africa in which some have extremely positive $\delta^{15}N$ values while others are within the normal range. Taken together, no consistent patterns appear obvious.

The balance between water- and protein-intake levels and their effect on urea production have been suggested as critical factors (Ambrose and DeNiro, 1986a; Ambrose, 1991). Since urea is depleted in ^{15}N relative to diet (Steele and Daniel, 1978), factors affecting the amount of dietary nitrogen crossing the gut (and thus available for urea synthesis) or affecting the amount of absorbed nitrogen excreted as urea should affect the nitrogen isotope ratio of synthesized proteins as well. Animals under water stress, but with adequate protein, excrete greater amounts of urea in order to maintain constant osmolality in the plasma of the kidney (Maloiy, 1973). However, there is some evidence that restricting both water and protein has an alternative effect. Livingston et al. (1962) report a drop in urea to as little as 10% of normal and reduction of the amount of urea nitrogen as percent of total urine nitrogen to as little as 25% of normal. In part this may be due to lower nitrogen availability. Amino acid absorption across the small intestine appears to be positively associated with the amount of protein in diet (Karasov and Diamond, 1983). Approximately 40% of dietary nitrogen is excreted in urine (Schoenheimer, 1964) and it appears that this figure does not change under protein stress.

Recycling of urea by bacteria in the gut of ruminants may also play a role (Sealy et al., 1987), since it increases the availability of a greater proportion of dietary nitrogen for inclusion in body proteins. The bacteria effectively add another trophic level to the organism. We suggest, however, that such recycling might not actually increase $\delta^{15}N$ (contra Sealy et al., 1987) in bacterially synthesized proteins. Because a greater proportion of dietary nitrogen is being used in protein synthesis, the $\delta^{15}N$ of protein should be closer to the dietary $\delta^{15}N$ value. It is possible, however, that selective utilization of particular amino acids by bacteria could result in an enrichment.

Animals with excess protein (such as carnivores) may also display elevated $\delta^{15}N$ values because of the larger proportion of nitrogen excreted in urea when amino acids serve as energy sources (Schoeninger and Bada, 1989). In sum, it is still far from clear what role water intake, protein levels, and gastrointestinal bacteria play in determining the final $\delta^{15}N$ value of an animal's tissues.

Intraorganism fractionations

Intertissue effects

Tissues containing lipids (muscle, kidney, and liver) usually have more negative $\delta^{13}C$ values than tissues without lipids (DeNiro and Epstein, 1978; van der Merwe,

TABLE 2. Variation in $\delta^{15}N$ values across tissues[1]

Animal	Muscle	Collagen	Hair	Nails	Source
Springbok	17.3	16.3			Sealy et al., 1987
Springbok	13.8	15.4			"
Steenbok	3.2	3.4			"
Steenbok	8.2	7.8			"
Hare	7.4	11.0			"
Hare	13.7	11.4			"
Hare	12.1	11.6			"
Springhare	8.8	8.0			"
Mouse (lab)	7.9	7.6	8.0		(DeNiro and Epstein, 1981)
Human #1			9.3	10.2	Robinson and Schoeninger, (unpublished data)
Human #2			8.5	9.1	"
Human #3			10.5	10.5	"
Human #4			10.2	10.9	"

[1]Values given in ‰ AIR. (Ambivalent Inhalable Reservoir.)

1982; Tieszen et al., 1983). The offset between bone collagen and muscle appears to be approximately 2‰ (Sealy and van der Merwe, 1987; Medaglia et al., 1990), but the effect of lipid incorporated in muscle has not been determined.

$\delta^{15}N$ values across tissues vary, although it appears that tissues with long turnover times have values within 1‰ of each other (see Table 2).

Intraskeletal effects

Thus far, the evidence suggests that there are no differences in isotope ratios across skeletal elements even though turnover rates vary. In a study of mink bones where all of the animals 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). The precision on repeated analyses of the same sample results in variation on the same order of magnitude (see section on analytical methods and precision). Further, in the analysis of vertebrae and femoral samples from recent humans buried in permafrost (Schoeninger, 1989), both bones gave identical carbon and nitrogen isotope ratios. Written records about these people indicated that they probably had diets that varied seasonally, and though cancellous bone in a vertebrae turns over more rapidly than the cortical bone in the femur, the two were identical.

Isotopic fractionation between individual amino acids

Few data exist on fractionations between constitutent amino acids of proteins, due to the difficulty in obtaining sufficient quantities of the individual amino acids for analysis. High-pressure liquid chromatography has been used to separate milligram quantities from some proteins (Tuross et al., 1988; 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 and equal to or more positive in nitrogen isotope ratios than is true of collagen as a whole.

The significance of these fractionations is that differential loss of particular amino acids could result in a shift in the isotope ratio of an organic residue. For example, Tuross et al. (1988) note that if glycine (which contributes 30% of total nitrogen to protein) is removed preferentially, this would increase the $\delta^{15}N$ of the residual material, and in turn could be mistakenly interpreted as having dietary significance.

Of further significance is the potential of extracting specific amino acids for analysis in place of the total organic residue. For example, if proline and hydroxyproline could be isolated, the analyst could be much more certain that the amino acids originated from bone. The two must have the same isotope ratio because

hydroxyproline is formed from proline following collagen synthesis. The sample size would be small, but some bone may provide enough for analysis.

Transamination

Nonessential amino acids are those that are synthesized from the body's pool of carbon, nitrogen, hydrogen, and oxygen. The isotopic compositions of these amino acids are dependent on the body's total intake of these elements, and they do not directly inherit the isotopic compositions of their sources. Essential amino acids, on the other hand, are acquired directly from the diet; the isotopic composition of their carbon-hydrogen backbone as well as some other functional groups are inherited (Abelson and Hoering, 1961; Nelson, 1991) from the plant that synthesized the amino acid; these acids could therefore conserve some isotopic record of the source (Gaebler et al., 1966). Even essential amino acids are, however, broken down to metabolites and, thus, the ones included in new proteins do not retain an isotopic signal identical to that in the original diet pool (based on Macko et al., 1986; 1987; Macko and Estep 1984).

The amine groups (the nitrogen-containing groups) on most essential amino acids are homogenized with the body's pool of amines (Stryer, 1975). As a result of this transamination process, the $\delta^{15}N$ values of most amino acids are equilibrated with the body's nitrogen pool although there are still intrinsic interacid isotopic fractionations (Hare et al., 1991).

At least one amino acid, threonine, is not transaminated, due to the lack of an appropriate transaminase enzyme. This should result in the conservation of the $\delta^{15}N$ signal from the source nitrogen in this amino acid. Hare et al. (1991) found that, whereas most of the essential and nonessential amino acids are enriched by 2 to 3‰ (as expected from the effect of urea-loss), threonine is depleted in ^{15}N by about 5‰ with respect to the diet. The authors attribute this depletion to a kinetic isotope effect during the degradation of threonine to urea. In principle, it seemed possible to use threonine's isotopic signal as a diet indicator. In practice, there is too little threonine in collagen for this to be a practical approach at this time. In sum, it appears that the nitrogen isotopic signal reflects an average of the total pool of nitrogen from the diet. Thus, specific diet sources of nitrogen cannot be identified using individual amino acids, but an overall average estimate is possible by analyzing a complete protein such as collagen.

Fractionation during excretion

Most carbon is excreted as respiratory CO_2, which is isotopically similar to the oxidative energy sources in the body (principally blood glucose); little shift in the body's $\delta^{13}C$ value is expected to occur as a result of CO_2 loss and, in any case, the organism is presumably in a steady state so that $\delta^{13}C$ of the glucose/fatty acid pool is constant (Schoeller et al., 1986).

Little work has been done on carbon isotope ratios in feces and urine. In the case of nitrogen, urine is less positive isotopically than are body tissues (Steele and Daniel, 1978; Minagawa and Wada, 1984), which can account for the enrichment of body tissues over diet. This relationship holds true irrespective of whether the nitrogen is excreted as urea, uric acid, amino acids, or ammonia (Minagawa and Wada, 1984).

EXOGENOUS SOURCES OF ISOTOPIC VARIATION: FOODS

Carbon sources

The utility of stable isotopes as paleodiet indicators arises principally from the differences in isotope ratios of various dietary substrates (food and drink). DeNiro and Epstein (1978, 1981) showed that, at least for carbon and nitrogen, such differences were linearly related to differences in $\delta^{13}C$ and $\delta^{15}N$ of tissues and whole organism. Such differences in foods arise from: (1) intrinsic fractionations associated with particular biochemical or physiological pathways, or (2) differences in isotope ratios of element pools (e.g., seawater versus fresh water).

The majority of carbon available in the global exchange system occurs as dissolved bicarbonate in the ocean, with $\delta^{13}C \cong 0‰$. Atmospheric CO_2, with a $\delta^{13}C$ between -7 and $-8‰$, is in isotopic equilibrium with the ocean. Both atmospheric and oceanic carbon are transferred into the biological system through photosynthesis by green plants and chemosynthesis by bacteria in symbiotic relation with deep sea vent organisms. The latter process accounts for an as yet unquantified amount of biological carbon. 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. Plant $\delta^{13}C$ values are determined by the plant's particular photosynthetic pathway (as discussed more thoroughly below). Marine organisms use several carbon sources (Peterson et al., 1980; Hoefs, 1987), including terrestrial detritus washed into the oceans by rivers with $\delta^{13}C$ values representative of a mixture of local terrestrial plants, dissolved CO_2 with $\delta^{13}C$ of atmospheric CO_2, and dissolved carbonic acid with $\delta^{13}C$ values close to zero. For this reason, the $\delta^{13}C$ values in marine plants can overlap the values in terrestrial plants (Fry and Sherr, 1984).

Carbon isotope fractionation during photosynthesis

All animals ultimately obtain their nutrients from plants, and thus inherit the isotopic differences that arise from the different possible photosynthetic pathways: C3, C4, and CAM (crassulacean acid metabolism) (see O'Leary, 1988, for a recent review). The first two pathways are named according to the number of carbon atoms in the earliest metabolites that appear after CO_2 fixation. In C3 and C4 plants these contain three and four carbon atoms, respectively. Different enzymes catalyze each of these pathways and, as a result, the kinetic isotope effects differ greatly. In general, C3 plants are depleted in ^{13}C with respect to C4 plants by about 14‰, as a result of the differences in the kinetic isotope effects associated with the two photosynthetic cycles. In principle, the $\delta^{13}C$ of each plant type is controlled by these kinetic isotope effects and $\delta^{13}C$ of atmospheric CO_2. However, a substantial variation in $\delta^{13}C$ is observed in both C3 and C4 plants as a result of other factors, including variable light level, moisture stress, and recycling of CO_2 from decomposing plant material in the soil (Sasakawa et al., 1989; Tieszen and Boutton, 1989; Tieszen, 1991; van der Merwe and Medina, 1991). CAM plants utilize both the C3 and C4 photosynthetic pathways; the relative amount of use depends on environmental conditions. Some CAM plants such as *Opuntia* use only the C4 pathway (Bender, personal communication).

The majority of cultivated and wild plants in temperate regions are C3. Some C4 plants of subtropical origin have been adapted to temperate conditions through human selection practices; the most notable prehistoric example in North America is maize. Millet and sorghum, also important agriculturally, are also C4. Maize tends to have exceptionally high $\delta^{13}C$ values ranging up to $-9‰$ (DeNiro and Hastorf, 1985; Schwarcz et al., 1985), while other C4 cultigens have $\delta^{13}C$ values between -10 and $-14‰$. Some of these analyses have been performed on preserved plants more than 40 years old. In such cases, some of the variation across the samples (prehistoric, preserved, and modern) may be due to the recent increase in anthropogenic CO_2 in the atmosphere, which has resulted in a decrease in the $\delta^{13}C$ value of atmospheric CO_2 of more than 1‰ (Keeling, 1961; Bada et al., 1990; Marino and McElroy, 1991).

As a result of these differences in $\delta^{13}C$ between plants using different photosynthetic pathways, it is possible to determine the proportion of C3 and C4 plant foods in the diet of an individual from the $\delta^{13}C$ of a preserved tissue such as collagen. In addition, ^{13}C-enriched carbon from C4 plants enters the human diet indirectly through the flesh or grazing herbivores, because many C4 grasses are found in areas of low rainfall. Indeed, the $\delta^{13}C$ of the collagen of some grazers is found to vary continuously in regions of varying rainfall, reflecting the varying proportions of C4 grasses consumed by the grazers (Chisholm et al., 1986; Ambrose and DeNiro, 1986a).

The majority of carbon available to marine organisms derives ultimately from the photosynthetic activity of phytoplankton. The magnitude of chemosynthetic fixation is unknown and of carbon from terrestrial detritus is probably a small input except in coastal areas. $\delta^{13}C$ values in marine plants can overlap the values in terrestrial plants (Sackett, 1989). Sea grasses have $\delta^{13}C$ 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, are intermediate in $\delta^{13}C$ values between C3 and C4 plants; the value generally decreases with increasing latitude (Fontugne and Duplessy, 1981). The majority of marine vertebrates have $\delta^{13}C$ values that indicate that their diet $\delta^{13}C$ values are intermediate between the extremes delineated by terrestrial C3 and C4 plants.

Aquatic plants (from lakes and rivers) derive their carbon mainly from dissolved inorganic carbon (DIC) in the water. The DIC is derived in turn from a mixture of sources: dissolution of atmospheric CO_2, decomposition of terrestrial plants (which enters into streams), and reaction with limestone in the bedrock. As a result, the $\delta^{13}C$ of these plants and the fish that feed on them may vary widely, reflecting the contribution of carbon sources (Rau, 1978; Schell, 1983). In general both seem to be close to the values of C3 plants (approximately $-25‰$).

Nitrogen isotopic variation in plants and animals

Over 99% of exchangeable nitrogen is bound as N_2 in the atmosphere or dissolved in the ocean. The major reservoir is the atmosphere, which has a constant worldwide $^{15}N/^{14}N$ ratio (Mariotti, 1983). Two major processes are involved in the transfer of nitrogen into the biological realm. The first depends on N_2-fixing organisms, i.e., blue/green algae in aqueous situations (both marine and freshwater) and bacterial nodules on terrestrial plant roots. This is an inefficient process, which results in synthesized tissues that have $\delta^{15}N$ values similar to atmospheric N_2 (i.e., close to zero). Complex nitrogen-containing molecules in organic matter are broken down by bacterial action following death of organisms. Through a series of steps involving nitrification and denitrification, nitrates that can be used directly by vascular plants are produced and decomposed, with varying isotopic fractionations. The $\delta^{15}N$ values of these nitrates and of the plants using them are more positive than the atmosphere.

The $\delta^{15}N$ values of higher organisms (plant and animal) depend on source nitrogen (N_2-fixing or bacterial degradation) and on metabolic processes within the organism. "Nitrogen uptake in terrestrial plants is primarily implemented by the fixation of atmospheric N_2 mediated by soil bacteria" (Hoefs, 1987: 156). Thus, although two processes (N_2 fixation and nitrate production) are involved, the majority of terrestrial plants have $\delta^{15}N$ values close to atmospheric N_2 (Virginia and Delwiche, 1982; Wada et al., 1975), although a broad range of $\delta^{15}N$ values is observed in terrestrial plants overall. Legumes which obtain most of their nitrogen by bacterial fixation, often have $\delta^{15}N$ values (near $0‰$). In contrast, the major source of available nitrogen in the marine system is bacterial and algal fixation producing N-containing compounds (nitrates and ammonia) with higher $\delta^{15}N$ values than dissolved N_2. As a result, $\delta^{15}N$ of marine organisms is typically higher than that for terrestrial ones, with some values ranging above $20‰$ (Wada, 1980). A similar effect is seen in aquatic systems (rivers, lakes, swamps), in which organisms may have $\delta^{15}N$ values elevated with respect to terrestrial organisms (Schwarcz et al., 1985; Katzenberg, 1989).

Marine/terrestrial effects

As has been noted, terrestrial C3 plants are commonly depleted in ^{13}C by about $7‰$ with respect to marine plants and animals. This difference is reflected in the difference in $\delta^{13}C$ between the flesh of the animals feeding on these plants. It is therefore possible to use carbon isotopes to distinguish between consumers of marine and terrestrial foods, and even to determine the proportions of these two

sources in the diet of individual humans (Chisholm et al., 1982; Schoeninger et al., 1983). The specific marine foods and their isotopic compositions must be known, however, because there may be significant regional variations in $\delta^{13}C$ of marine animals reflecting variation in their planktonic food sources.

A similar consistent enrichment in ^{15}N is seen in marine animals (Wada, 1980), partly because phytoplankton utilize ^{15}N-enriched dissolved nitrate in seawater, and partly because of the high trophic level of the larger marine carnivores (Schoeninger and DeNiro, 1984), which are common marine food sources for humans. Therefore, $\delta^{15}N$ of preserved human tissues can also be used to determine the proportion of marine food in the diet (Schoeninger et al., 1983; Schwarcz, 1991). The simultaneous use of both $\delta^{15}N$ and $\delta^{13}C$ should yield the most precise estimate of the dietary dependence on marine foods (Schoeninger, 1989).

REQUIREMENTS AND LIMITATIONS OF ISOTOPE NUTRITIONAL ECOLOGY

Knowledge of δ values of potential foods

In general, the isotopic paleodiet method assumes that the δ values (e.g., $\delta^{13}C$) of a human tissue are linearly related to the proportions of the various foods consumed that differ in their respective δ values. Thus, if we have two foods F_1 and F_2 that have δ values δ_1 and δ_2, respectively, and a tissue with δ_T, then we assume that the proportion (P) of F_1 in the diet is given by

$$P_1 = \frac{\delta_2 - (\delta_T + \Delta_{DT})}{\delta_2 - \delta_1}$$

where Δ_{DT} is the fractionation between diet and tissue; DT is here assumed to be independent of the food type, but see below for further discussion of this point. The error in the estimate of P_1 depends not only on the precision of δ_T but also on the precision of our estimates of δ_1 and δ_2, the isotopic compositions of the food sources. Schwarcz (1991) has generalized this linear mixing model to a multicomponent system. If the δ values of both foods and preserved tissues are known for nitrogen isotopic elements (e.g., carbon, hydrogen, nitrogen, oxygen), and if the foods have well-defined isotopic compositions for each element, then it should be possible to determine the proportions of N + 1 foods in the diet. Thus, for example, we can use the $\delta^{13}C$ and $\delta^{15}N$ values of collagen to determine the proportions of three food sources.

In order to apply the method it is important to identify the foods actually consumed, and their isotopic compositions. For the identification of potential dietary constituents, we can use analyses of charred or preserved organic residues, seeds, phytoliths, bones, etc. Estimates of the δ values of these foods can be obtained by analyzing modern samples of the same species. However, modern foods may have different δ values than their ancient equivalents. For example, the use of artificial fertilizers can affect the $\delta^{15}N$ value of proteins. Also, some modern herbivores may graze on crops including C4 plants (principally maize) that were not part of their ancient diet. Hastorf and Deniro (1985) have shown that carbonized samples of ancient plant materials may also be reliably used to estimate $\delta^{13}C$ and $\delta^{15}N$ of the original plants. The δ values of collagen from fossil animal, bird, and fish bones can be used to estimate the δ values of their flesh, where the appropriate fractionations are known. Charred food residues from ceramic can be used to put some limits on the range of δ values of foods actually consumed.

Fractionations between diet and tissues; Routing of isotopes

Based on the original work of Deniro and Epstein (1978, 1981), it is well known that there are characteristic fractionations between various tissues and the diet of the consumer (see Schoeller et al., 1986, for an interesting application). The fractionations of most interest to us here are between diet and preserved components, especially collagen and bone carbonate. Various estimates of these fractionations have been made, but a well-controlled study of the fractionation between human

bone collagen and diet has not been carried out, largely due to the difficulty of obtaining bones from humans whose total diet is sufficiently well controlled.

The results from studies on carbon have not been completely consistent. Small animals (mice, chickens, and gerbils) raised on diets of known isotopic compositions (DeNiro and Epstein, 1978; Bender et al., 1981; Tieszen et al., 1983) have $\delta^{13}C$ values that differ from diet by 1–3‰. Large, free ranging ruminants whose diet $\delta^{13}C$ values were estimated based on observations of diet selection followed by analyses of particular diet constituents (Vogel, 1978) had collagen 5–6‰ more positive than diet. There is a great deal of uncertainty in both types of studies. In the first group, the animals are small, short-lived, and fed on diets with calories and protein adequate to meet energy and growth needs. In the second group, the adequacy of the diet is unknown. Further, the comparison of nonruminant laboratory animals with free-ranging ruminants is questionable. At the present time the most reasonable estimate of the difference between diet and collagen appears to be on the order of 3–5‰. The absolute magnitude is important for cases where proportions of particular diet components are being estimated.

The magnitude of the difference in $\delta^{15}N$ 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 (Wada, 1980; Vogel et al., 1990) indicate that bone collagen and other proteins are approximately 3‰ more positive than diet. As noted above, prior to weaning, infants are enriched by about 3‰ with respect to their mothers. However, other analyses of bone collagen from free-ranging ruminants (Heaton et al., 1986; Ambrose and DeNiro, 1986a; Sealy et al., 1987; Schoeninger, 1989) suggest that in some cases bone collagen is enriched far more than 3‰ with respect to plant foods, due to water stress and/or other effects. 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).

The difference estimate of $\Delta^{15}N$ above assumes that the atoms of a particular food are homogeneously distributed among all the tissues of the body of the consumer (with the appropriate fractionations). It is possible, however, that the atoms of some molecular species do not homogenize with the rest of the body's atoms, but that instead the molecule is directly assimilated into tissues. We can speak of the molecule being "routed" directly to a tissue. This is true, for example, of the carbon skeleton of essential amino acids. This may lead to overestimates of the proportion of a given food in the diet. This problem has been discussed by Parkington (1987, 1991) and Krueger and Sullivan (1984). The latter proposed that the systematic differences in $\delta^{13}C$ values between bone collagen and bone carbonate could be accounted for by assuming that the latter had formed in isotopic equilibrium with blood bicarbonate, which was largely derived, in turn, from metabolism of carbohydrates. Following this line of reasoning carbohydrates would be less strongly represented in collagen, which would obtain its carbon atoms from amino acids. While some of these (the nonessential amino acids) could exchange with the total carbon-atom pool of the body, the essential amino acids would be derived directly from the plant foods where they had been synthesized. Also, as Parkington notes, in humans on high-protein diets even the nonessential amino acids will tend to be routed to the collagen molecules. In theory, differences between $\delta^{13}C$ of collagen and bone carbonate may thus be useful in recognizing humans who had lived on high-protein diets. Attempts, thus far, have not been particularly successful (Lee Thorp et al., 1989).

PREVIOUS APPLICATIONS

Introduction of agriculture

To the extent that there was an agricultural "revolution" in the New World, it was marked by the arrival of a C4 plant, maize ("corn" in American usage), which replaced several other previously domesticated plants (Ford, 1985). The majority of early stable isotope studies were directed toward determining the temporal and

geographic patterning of the introduction of maize, particularly into North America. It will become obvious in the following sections that tracing the use of maize continues to be a major focus.

Very few such studies have been undertaken in areas other than the New World. Largely this is because few plants in other areas have distinctive isotopic signatures. One exception to this is the introduction of millets into parts of Asia. We end this section with a reference to the only study published thus far.

North America

Considering the importance of the change in subsistence orientation that occurred with the introduction of maize, it is not surprising that it was the focus of the first applications of stable isotope data (carbon) to an anthropological problem (Vogel and van der Merwe, 1977; van der Merwe and Vogel, 1978; Bender et al., 1981). The results were intriguing because they indicated that large scale dependence on maize agriculture in the middle and eastern portions of the continent did not occur until around A.D. 900–1000, several hundred years later than previously proposed. Many subsequent studies of $\delta^{13}C$ and $\delta^{15}N$ in prehistoric human skeletons from the United States and Canada have been made.

These studies have served to refine our knowledge of maize usage in specific areas of North America and they indicate temporal variation in the switch to maize agriculture. In the western Lake Erie area, significant amounts of maize (30–60%) were being eaten, and presumably grown, by A.D. 500 (Stothers and Bechtel, 1987). In contrast, it was not until after A.D. 1000 that such a shift took place in southeastern Missouri (Lynott et al., 1986). Across the southeast, the stable carbon isotope data confirm that the intensification of maize was relatively late (Broida, 1984; Farrow, 1986; Buikstra et al., 1988; Schurr, 1989; Schoeninger et al., 1990). A reexamination of the data from the northeastern section of the continent (Katzenberg and Schwarcz, 1986; Katzenberg et al., 1991) indicates that the increase in $\delta^{13}C$ values occurs somewhat later as one progresses farther north, suggesting a lag in the development of a variety of corn adapted to the area's shorter and colder growing season. The archaeologically defined northern limit of corn usage in Ontario is confirmed by the disappearance of ^{13}C-enrichment in bone and pot-sherd residues (Morton and Schwarcz, 1988). More recent studies are beginning to investigate assumed differential use of maize among different types of communities (Buikstra and Milner, 1991).

There are still some unresolved questions. Native Americans on Nantucket Island dated to about A.D. 1000, considered premaize by many archaeologists, had stable carbon isotope ratios more positive than expected based on a presumed hunting, gathering, and fishing adaptation (Medaglia et al., 1990). It is possible that they had maize earlier than expected or that the date is artificially old, affected by inclusion of marine foods depleted in ^{14}C (see above).

Other important domesticates such as beans and squash were introduced somewhat later. Squashes are C3 plants and thus indistinguishable isotopically from most wild plants of North America. Beans, like most legumes, should be depleted in ^{15}N with respect to other C3 or C4 plants. The twin effects of ^{13}C enrichment (from corn) and ^{15}N depletion was expected to be detectable in the bone collagen of agriculturalists. Curiously, the nitrogen isotopic effect of increased consumption of beans has not yet been detected, either in North America (Schwarcz et al., 1985; Katzenberg et al., 1991; Morton and Schwarcz, 1988, food residues only) or in Central America (White and Schwarcz, 1989). One possible explanation is that beans constitute such a small proportion of the diet that no effect is observable. Schwarcz et al. (1985) estimate that less than 20% of the protein content of the diet could be derived from beans assuming that the principal sources of protein (fish and herbivore flesh) remained stable across the transition to agriculture (see also White and Schwarcz, 1989).

Another possible explanation is related to the observation by Mariotti (personal communication, and see Mariotti et al., 1980) that $\delta^{15}N$ of agricultural soils is

higher than that of nonagricultural soils, and increases with duration of use. Also, Shearer et al. (1983) have shown that legumes do not fix atmospheric nitrogen when soil nitrogen is available. The $\delta^{15}N$ value of domestic beans from middens at Pecos pueblo in New Mexico was as positive as that in the other terrestrial plants measured (Spielmann et al., 1990). Modern legumes in Japan were also equivalent to other plants (Minagawa and Akazawa, 1991). These results suggest that an intensive survey of nitrogen stable isotope values and of animals eating them is required.

Some unexpected results have been found in these stable isotope studies. Collagen from domestic dogs from a postmaize site in Ontario was isotopically similar to humans, i.e., enriched in both ^{13}C and ^{15}N. Their high $\delta^{13}C$ values suggest that they were being fed significant amounts of maize. The ^{15}N enrichment indicates consumption of fish and herbivore flesh (Katzenberg, 1989). In the Panhandle region of Texas, the average $\delta^{13}C$ value of $-7‰$ in human bone collagen indicated an adaptation based on bison, cactus, and varying amounts of maize, rather than consistent maize agriculture, since archaeological evidence indicates that agriculture could not have been maintained in the area (Huebner, 1991; Habicht-Mauche et al., 1991).

Mesoamerica

In Mesoamerica, the analyses of samples from the Tehuacan Valley of Mexico indicated a shift toward C4 plants around 4000 B.C., although midden analyses indicated little dependence on maize (DeNiro and Epstein, 1981). 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: 349). A subsequent attempt to reconcile the differences managed to change slightly the expectation derived from midden analyses, but, even so, the bone collagen isotope values 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 shift in bone collagen $\delta^{15}N$ values from 10 to 9‰ as indicating a change in dependence on beans. In retrospect, however, the nitrogen isotope values in the earlier samples were most likely diagenetically altered. These samples had very poor collagen preservation and recalculation of the C:N ratios indicated an amino acid composition dissimilar to 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 B.C.) indicated that maize was not a major diet item if used at all (Norr, 1981, 1982, 1991). Increased dependence on maize in both inland and coastal Costa Rica did not occur until some time between A.D. 300 and A.D. 1550 (Norr, 1981, 1982, 1991).

White and Schwarcz (1989) showed major changes in the use of maize in the Maya of Belize. Preclassic people used maize, but less intensively than at Tehuacan. Collagen $\delta^{13}C$ values fell through the Classic period, indicating a decrease in maize consumption, but rose abruptly at the beginning of the Postclassic, reaching a maximum of $-9‰$ (still 3‰ lower than observed in the Tehuacan Valley). $\delta^{15}N$ values remained generally constant through time at around 10‰, implying no change in the source of protein (presumably herbivore flesh).

South America

In South America, stable isotope studies have been critical in documenting the shift to maize (van der Merwe et al., 1981; Ericson et al., 1989; Burger and van der Merwe, 1990), the use of maize and marine foods (DeNiro, 1988; Ericson et al., 1989), and in tracing patterns of food distribution (Hastorf and DeNiro, 1985; Hastorf, 1988). Further, the effects of food processing and diagenesis upon isotope ratios of archaeological and modern plants have been tested using South American materials (DeNiro and Hastorf, 1985).

Asia

One survey has been published on prehistoric human samples from China (Cai and Qiu, 1984; see Weld, 1985; for English translation). The study traced millet domestication in an area of northern China approximately 7,000 years ago. The authors recommend expansion of such studies in order to address several questions. This would document the spread of millet-based economies throughout China and monitor the shift from millet-based to C3 rice-based diets throughout China. It would also determine the timing of animal domestication in those areas where millet was the major forage crop and assess dietary differences between social strata where poorer people would have eaten millet and higher status individuals would have had access to rice and/or wild animals feeding on C3 plants.

Dependence on marine versus terrestrial resources

Carbon

The second major area of application of stable isotope data was in using the $\delta^{13}C$ of human bone collagen to indicate the proportion of marine foods in the diet of prehistoric populations in Europe and North America (Tauber, 1981; Chisholm et al., 1982). In Europe, Tauber (1981) found a high proportion of marine foods in Mesolithic period residents of Denmark, and a shift through time toward less positive carbon isotope values in the Neolithic period with the introduction of farming and domestic animals. The stable isotope ratios of early domestic dogs from British and Danish sites lacking human remains (Noe-Nygaard, 1988, 1990; Clutton-Brock and Noe-Nygaard, 1990) were interpreted as indicating a strong marine foods component in human diet even in sites that are some distance from the contemporary coast. A similar pattern was observed in Portugal in human skeletons from a Mesolithic site located somewhat inland (Lubell et al., 1989). In other areas of Mesolithic Denmark, stable isotope ratios from human skeletons used in radiocarbon dating (Price, 1989b; based on Hakansson, 1982, 1984) indicate mixed dependence on marine and terrestrial foods.

In North America, Chisholm et al. (1982) examined coastal populations in British Columbia, dating from 2,000 B.P. to recent times, and concluded that the protein portion of the diet of all the individuals consisted of 85–100% marine foods. Archaeologists had estimated the quantity of overall caloric dependence to be approximately 55%, based on relative frequency of marine and terrestrial faunal remains. At interior sites in British Columbia, the use of anadromous salmon as a major food source by people living along the major streams was detected from the gradual decrease inland in ^{13}C of bone collagen (Lovell et al., 1986a).

A similar study was initiated in Australia. One small project has been published (Hobson and Collier, 1984; Collier and Hobson, 1987) in which two sites were analyzed. The first, Broadbeach, is on the coast of southeastern Queensland and was inhabited over a period of 1,000 years. The $\delta^{13}C$ values averaged $-16.6 \pm 1.2‰$, which is relatively positive. It is not possible, however, to determine whether this is due to the ingestion of C4 plants or marine foods (Lee Thorp and Sealy, 1986). It is not known, or reported, whether any of the plant dietary staples are C4. The other site, Swansport, an historic period settlement (around 1830) on the Murray River in South Australia, has a carbon isotopic signature that is basically C3 even though a greater dependence on marine foods was expected. The peopling of Australia is still a subject of much debate. The migration to interior areas should be discernible in the stable isotope ratios as people change from marine protein to terrestrial protein. It is hoped that such a study will be possible at some future time.

Nitrogen and carbon

In many areas of the world, the stable nitrogen isotope ratios are better discriminators between marine and terrestrial foods than are stable carbon isotope ratios (Schoeninger et al., 1983; Schoeninger and DeNiro, 1984). This is true especially in

those areas of the world where C4 plants such as maize or millet form a major portion of the subsistence base. It is unfortunate that the early studies did not report nitrogen isotope ratios since the data would have provided an important comparative base for other data sets. Recently, Schwarcz and colleagues have found a correlation between the nitrogen and carbon isotope ratios in the early studied British Columbian populations (see Schwarcz, 1991).

One of the first applications of a bivariate approach was included as part of a larger study of adaptation on the California coast (Walker and DeNiro, 1986), where maize was not used. The study included analyses of bone collagen from people buried on the Channel Islands, the mainland coast, and the mainland interior. The mean isotopic ratios for both carbon and nitrogen decreased progressively from the islands to the mainland coast to inland sites. Interestingly, there was a significant amount of variation within each of these areas. Two of the individuals from the coast exhibited more positive carbon and nitrogen isotopic ratios than occurred among any of the people from the islands. Walker suggested possible explanations largely based on sexual division of labor and exchange of marriage partners (Walker and Erlandson, 1986), but noted that so few skeletons could be sexed that testing such hypotheses was impossible at the time of their study. There was also an increase in the isotopic ratios through time along the coast and the interior, confirming previous archaeological evidence for increased dependence on marine foods through the late prehistoric period (Walker and Erlandson, 1986).

Prehistoric adaptations in the Bahamas were amenable to a similar approach (Keegan and DeNiro, 1988). Dozens of potential and actual human foods from the terrestrial, reef, and deep ocean environments were analyzed. The sampling strategy was based on a previous observation that certain reef-dwelling fish in the Bahamas exhibited carbon isotope ratios more enriched in ^{13}C than most marine fish coupled with nitrogen isotope ratios depleted in ^{15}N compared with other marine fish (Schoeninger and DeNiro, 1984). The pattern of enriched $\delta^{13}C$ values and depleted $\delta^{15}N$ values was found in the humans, reflecting their dependence on marine foods from the seagrass and coral-reef communities.

In a study mentioned previously, Medaglia et al. (1990) investigated human adaptation on Nantucket Island off the Massachusetts coast in the period around A.D. 1000. This date is prior to the time of convincing archeological evidence for maize agriculture in the area. The $\delta^{13}C$ values of human bone collagen, however, indicated that either 95% of the carbon in human collagen came from marine foods or that a significant amount of a C4 plant (or a CAM plant with a C4 signature) was being consumed. An isotopic survey of marine and terrestrial foods, recorded ethnographically and recovered archaeologically, revealed only one potential food plant (prickly pear cactus) other than maize with a C4 signature. The $\delta^{15}N$ values indicate a significant dependence on marine foods, but, even so, human nutritional limitations argue against 95% of dietary calories extracted from marine foods. It is possible that the bone collagen is recording the carbon isotopic signal from certain marine foods preferentially over the signal from the remainder of the diet. Further study is necessary.

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, a shift toward less negative $\delta^{13}C$ values combined with stable $\delta^{15}N$ values documented the shift to maize agriculture with continuing dependence on marine foods around A.D. 1000. 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, but a virtual abandonment of marine foods, evidenced by less negative $\delta^{13}C$ values and less positive $\delta^{15}N$ values (Schoeninger et al., 1990). 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 overall health of the population (Larsen et al., 1990).

In Mesoamerica also, use of nitrogen isotope ratios has served as control for use of marine foods in order to estimate the dependence on maize. As discussed above, Norr (1981, 1982, 1991) monitored the dependence on maize through time in Panama and Costa Rica. Similarly, White and Schwarcz (1989) demonstrated little dependence on marine foods except for one male buried in a stone tomb who had a value of +13‰. The authors suggested that this was due to status-based access to marine foods not available to females, since the female buried in the same tomb had a lower value. It also seems possible that the male came from the coast as an adult and that his bone collagen nitrogen isotope ratios reflects his diet from an earlier period.

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 in several prehistoric groups (Minagawa and Akazawa, 1991; Roksandic et al., 1988). A preliminary baseline study demonstrated the isotopic distinctiveness of marine and terrestrial resources in the area. There are few C4 plants available for human consumption and the leguminous and nonleguminous plants both had $\delta^{15}N$ values close to zero, which simplified the interpretation of results (Minagawa and Akazawa, 1991). The archaeological evidence with one exception (an inland site) indicated significant dependence on varying compositions of marine mammal, marine fish, and marine shellfish. The isotopic data for the northern islands of Sakhalin and Hokkaido are distinct from those for Honshu, the more southerly island. 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 $\delta^{15}N$ values and more negative $\delta^{13}C$ values than the coastal site from the same period (Late Jomon). This pattern is the same as that observed by Walker and DeNiro (1986) in coastal and inland sites in southern California.

Migratory populations (transhumance)

The potential of isotopic paleodiet analyses to detect migratory behavior in past human populations was first tested by Sealy (Sealy and van der Merwe, 1985, 1986). It had been proposed that prior to 2,000 B.P., hunting and gathering groups moved seasonally from the coast to the interior in areas of the South African coast (Parkington, 1972). Such seasonal movement should result in people buried on the coast having similar carbon isotope signatures to those buried inland. Analyses of more than 200 plant, animal meat, and animal human bone collagen samples supported the expectation that coastal foods had different carbon isotope ratios from inland foods. Even so, inland people had bone collagen carbon isotope ratios that differed from those people buried on the coast. Sealy and van der Merwe (1985, 1986) concluded that their results questioned the seasonal movement hypothesis and called for reexamination of the proposal in other areas of the world. The results of this study and the reaction to it (Parkington, 1987, 1991) have served to focus interest on the metabolic pathways of nutrient components, an issue raised simultaneously based on theoretical considerations (Krueger and Sullivan, 1984; Chisholm, 1989; Schoeninger, 1989).

Another intriguing application of isotope ratios to the study of transhumance has been proposed by Reddy (unpublished Dissertation). She is attempting to trace the pattern of transhumance in Gujarat in northwestern India. While at home the fodder for animals includes millet (a C4 plant) as a by-product of processing for human consumption. The major forage plants available during the time away from home are all C3 plants. A similar approach was used in identifying the diets of domestic camelids in Peru where, in some cases, it appears that these animals were moved to the coast and given kelp as forage (DeNiro, 1988).

At an altitude above the limit of modern agriculture, Moore and Schoeninger (unpublished data) investigated the isotope ecology of hunting and herding popu-

lations. Nitrogen stable isotopes for humans were more positive than were those for carnivores specialized upon the same animals hunted and herded by the humans, but it is difficult to reconcile this reconstruction with human dietary needs and limitations. The reconstruction from carbon stable isotope ratios coincides with the picture from plant (Pearsall, 1989) and animal (Moore, 1989) remains that the input of foods from the coast was extremely limited.

In eastern Africa, Ambrose was able to demonstrate distinct stable isotope signatures in pastoralists versus agriculturalists versus hunter-gatherers (Ambrose and DeNiro, 1986b). The nitrogen and carbon isotope data in combination reflected ethnographic reports on diet. Those groups most dependent on plant foods have $\delta^{15}N$ values that are, on average, 4‰ 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 $\delta^{13}C$ values of human bone collagen. Other studies have investigated adaptations in the Middle Paleolithic (Bocherens et al., 1991) and the Upper Paleolithic of Europe (Hayden et al., 1987).

Aquatic resource use in terrestrial environments

The combined use of $\delta^{15}N$ and $\delta^{13}C$ has allowed the discrimination of other dietary adaptations. In the specific case of terrestrial populations in North America, Schwarcz et al. (1985) found that $\delta^{15}N$ values of preagriculture peoples were significantly higher than expected. Further investigation revealed that the flesh of fish in this region is significantly more enriched in ^{15}N than is herbivore flesh (Katzenberg, 1989). Because the $\delta^{13}C$ values of the fish are similar to that of C3-consuming herbivores, no obvious difference is apparent in the people's carbon isotopic signature. MacDonald and Schwarcz (in preparation) studied burials from a fishing site on the north shore of Lake Erie dating from about A.D. 1100, when maize would have been available as a cultigen. Collagen showed no ^{13}C enrichment, while ^{15}N was enriched, although not exceptionally compared with other inland sites. They infer that much of the total protein intake came from fish.

The sort of baseline study initiated by Katzenberg is necessary before estimates of diet components can be attempted, because the isotopic ratio of source nitrogen can vary between ecosystems. For example, in the Black Warrior River of west central Alabama modern and archaeological fish have $\delta^{15}N$ values that are significantly less positive than those in the region of the Great Lakes (Schoeninger and Peebles, unpublished data). Similarly, modern fish and crocodiles from Lake Turkana in northern Kenya are also less positive (Schoeninger, 1991) because the main source of nitrogen is that fixed from the atmosphere by blue green algae (hence the name "Jade Sea" for Lake Turkana). In this case, Schoeninger (1991) could attribute previously unidentified human skeletons from stone cairns as pastoral nomads rather than fisher-gatherers in the region east of Lake Turkana.

THE FUTURE

Seeing through diagenesis: The diet of early hominids

The content of collagen in bone generally tends to decrease with time, due to hydrolysis and subsequent leaching of soluble amino acids or polypeptides. The "half-life" of collagen in bone is extremely variable, depending on conditions of burial, and for arctic environments it may range up to several hundred thousand years. In temperate and tropical environments collagen has usually disappeared after a few tens of thousands of years. Well before that time, what remains is so badly degraded that it may be useless for isotopic analysis. This creates a time limit beyond which it is impossible to use conventional isotopic methods to reconstruct paleodiet. Some of the most interesting stages in human biological evolution are thus inaccessible. At present, our knowledge about dietary preferences for early modern and premodern hominids comes from nonisotopic studies such as tooth wear, artifact assemblages, or faunal/floral remains. Perhaps other target materials could be studied. Noncollagenous protein and lipid, present in dentine

and tooth-enamel, respectively, both appear to be more stable than bone collagen and may prove more useful for such studies.

Pathways in the body: Residence times

Implicit in the use of isotopic ratios as paleodiet signatures is the assumption that isotopes supplied by nutrients are randomly distributed through all tissues (or at least those that are sampled, such as collagen). As noted earlier, it is likely that some portions of amino acids are transferred directly (routed) to collagen from dietary intake. Similarly, some lipids are known to conserve their carbon skeleton and are deposited intact in fat globules. Therefore, isotopic analyses of some preserved tissue components (collagen, lipids) may be biased toward nutrient sources rich in the particular component being analysed (e.g., fat- or protein-rich foods). This problem has not been carefully studied, and some published conclusions regarding the balance of nutrients in the diet may have to be modified when this aspect comes to be better understood.

Related to this is the question of the residence time (i.e., the period of time reflected by the isotopic signal) of isotopic labels. No systematic studies have been made since the 1970s (Stenhouse and Baxter, 1979). The effect of such factors as remodeling, infectious disease, or nutritional stress (starvation, malnutrition) have not been evaluated. While we know that different skeletal elements do not normally differ isotopically, it would be worthwhile to study variations in skeletal tissues that have undergone obvious modification after skeletal maturity.

Degeneracy: Nonunique solutions

The use of isotopic labels to identify sources of nutrients also assumes that the nutrients are themselves isotopically unique (e.g., have unique combinations of δ values). In reality, some combinations of nutrients have indistinguishable isotopic signatures. Ideally, the solution to such problems is to add other isotope labels (e.g., $\delta^{34}S$ or δD). In such cases, however, it is especially important to have analyses of the actual nutrients consumed, rather than using generalized values.

Isotopic analysis of single amino acids

Individual essential amino acids conserve their carbon-hydrogen skeleton as they are assimilated, and can be used to record the isotopic composition of their source (Hare et al., 1991). The carbon and hydrogen atoms of nonessential amino acids are derived from the body's total nutrient pool. It may be useful to trace the sources of some essential amino acids in populations, especially where there has been some isotopic ambiguity in the interpretation of analyses of total collagen (see Nelson, 1991). This may become particularly useful where a given nutrient contains exceptionally high (or low) proportions of the given amino acid. The small amounts of lysine in most maize varieties, for example, could translate into collagen lysine having a non-C4 $\delta^{13}C$ value in those cases where an alternative lysine source (e.g., beans) is available in diet. Due to the prevalence of transamination, the $\delta^{15}N$ signal of most amino acids will not reveal their source. Also, since urea is generated from free amine and not from amino acid-bound nitrogen, such conservative amino acids should not show a trophic level effect.

CONCLUSIONS

The preceding pages have outlined what we see to be the present state of stable isotope studies in human nutritional ecology. We have presented the geochemical basis for the method and the biological responses by organisms to isotopic distributions within the environment. We have summarized data from various areas of the world, emphasizing the achievements thus far. On a broad scale, the method has already indicated patterns of nutrient use. The use of maize has been monitored through stable isotope ratios of carbon in bone collagen and carbonate in large areas of central North and South America, where marine foods and other C4 plants are not included in diet. The dependence on marine versus terrestrial foods

is clearly indicated in both the stable carbon and nitrogen isotope ratios of bone collagen. Thus, in coastal regions, the $\delta^{15}N$ values have served as controls for marine dependence, thereby permitting use of $\delta^{13}C$ to indicate maize. In regions including alternative C4 plants, the archaeological record has guided interpretation of carbon stable isotope results. In specific regions, unique isotopic signals have indicated the importance of certain foods. For example, unique $\delta^{15}N$ values of Great Lakes fish and of Lake Turkana fish were targeted in relation to seemingly odd values in human bone collagen.

We have also noted several areas, both geographical and general, that hold promise for future investigations. Two obvious geographical areas needing study are mainland Asia and Australia. But much remains to be learned about physiological aspects as well. Final understanding of the transfer of the isotopic signal of specific nutrients and/or of specific amino acids requires further experimentally and empirically based studies before precise percentages of diet items can be calculated. Other elements, especially oxygen, hydrogen, and sulfur, have stable isotopes that could provide more general ecological and behavioral information.

In closing, we would like to restate a point from our opening discussion. The stable isotope method is complementary to other methods. For archaeological studies, the faunal and floral remains from sites are needed to identify dietary options. The isotope signal then serves to refine estimates of levels of dependence.

We are now entering the third decade of stable isotope studies in human nutritional ecology. The present state of these studies may best be compared with that of radiocarbon dating at the same period in its history of use. If a comparable amount of information is compiled during the development of this method as was compiled during the development of radiocarbon dating, we will be justified in our enthusiasm in pursuing this area of science.

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