PART II

STABLE ISOTOPE ANALYSIS AND HUMAN DIET

CHAPTER

10

Isotope Analyses and the Histories of Maize

ROBERT H. TYKOT

Department of Anthropology, University of South Florida, Tampa, Florida

Glossary

Apatite (bone, tooth enamel) Inorganic portion reflecting whole diet.

Carbon, nitrogen, and oxygen isotopes Stable isotopes representing the main dietary components of skeletal tissues.

Collagen Organic portion of bone mainly reflecting dietary protein.

Fractionation The change in isotope ratios caused by chemical processes such as photosynthesis and metabolism.

Isotopes Elements with different numbers of neutrons in the atomic nucleus.

Mass spectrometer Instrument used to produce precise measurements of isotope ratios.

PDB International standard for C and O isotope analysis, based on *Belemnitella americana* from the Peedee formation in South Carolina.

Strontium isotopes Heavy stable isotope representing geological area of food acquisition.

The contribution of isotope analyses to archaeology has significantly increased ever since the introduction of radiocarbon dating by Willard Libby [60], with applications of isotope analyses developing ever since. Today, stable isotope analysis is regularly applied to address questions concerning human diets around the world, and this is illustrated in this book with the large number of studies on the origins, importance, and spread of maize throughout the Americas. In this introductory chapter, an overview of the history and principles of isotope analyses is provided, while the individual chapters in this section provide specific details on the samples and procedures used in each study, and how their results have contributed to our understanding the histories of maize. In particular, "we are what we eat," and isotope analyses provide quantitative data that complement floral, faunal, ethnohistoric, and other information about dietary practices. The details that follow include the contributions of methodological and interpretive isotope studies, but like some of the previous synthetic publications on isotopic analysis applied to archaeology, focus on relevance to maize studies [1, 2, 6, 8, 23, 44, 45, 46, 54, 65, 81, 93, 94, 97, 99, 107, 108, 111, 112, 114].

ISOTOPE DEFINITIONS

Isotopes are defined as one of the two or more forms of an element (e.g., carbon) that have the same number of protons in the nucleus (known as the atomic number) of an atom but different numbers of neutrons in the nucleus, resulting in different atomic weights. Radioactive isotopes (e.g., carbon-14) decay over time, whereas stable isotopes (e.g., carbon-12, carbon-13) do not. Carbon occurs in three isotopic forms: ^{12}C, ^{13}C, and ^{14}C, where the superscript number is the sum of the protons (6) and neutrons (6, 7, 8) in the carbon nucleus. Carbon dioxide (CO_2) in the atmosphere consists of about 98.9% $^{12}CO_2$ and 1.1% $^{13}CO_2$ (with radioactive $^{14}CO_2$ a tiny fraction of about 1×10^{-10}%). The isotopic composition of atmospheric CO_2 is established by equilibration with the much more abundant pool of dissolved inorganic carbon (mainly bicarbonate, $HCO3^-$) in sea water. There is also considerable carbon in calcium

Copyright © 2006 by Academic Press.
All rights of reproduction in any form reserved.

carbonate ($CaCO_3$), the main component of limestone and marble. Atmospheric carbon dioxide is photosynthesized by plants and metabolized into complex molecular compounds that we categorize as carbohydrates, proteins, and lipids, whereas many plants (wild and domesticated) are consumed by organisms that then appropriate or convert, or both, these compounds into their body tissues. In biosynthetic chemical reactions, especially photosynthesis [76], the lighter weight isotopic compounds react faster, using less energy. If, as in the case of photosynthesis, the reaction is incomplete, the product (e.g., maize) will be enriched in ^{12}C relative to the substrate (e.g., atmospheric CO_2), resulting in changes in the $^{13}C/^{12}C$ ratio. This is called isotopic **fractionation.** When plants are consumed by herbivorous animals, the metabolic processes involved reverse the direction of fractionation, increasing the proportion of the heavier carbon (and nitrogen) isotope in the body tissues. Laboratory-based and other isotope studies have specifically determined that bone **collagen** is produced mainly from dietary protein, whereas **bone apatite** and **tooth enamel** represent the whole diet [7, 106]. In addition, it is also clear that turnover rates for bone are slow, so that the isotope values obtained represent at least the last several years of an individual's life.

Because of the small difference in $^{13}C/^{12}C$ ratios between all samples, these and other isotope abundances, instead of being reported as simple ratios (e.g., $^{13}C/^{12}C$) they are converted to ratios relative to international standards using the delta notation:

$$\delta^{13}C \text{ (in ‰ or per mil)} = \left[\left\{\left(\text{sample } {}^{13}C/{}^{12}C\right)/\left(\text{standar } {}^{13}C/{}^{12}C\right)\right\} - 1\right] \times 1000$$

The standard reference material, **PDB**, was originally derived from a Cretaceous marine fossil sample, *Belemnitella americana,* recovered from the Peedee formation in South Carolina [19, 20]. Because the original material is no longer available, sample **carbon isotope** ratios are now reported relative to Vienna Peedee belemnite (VPDB), with raw values typically calibrated using the NBS National Bureau of Standards (NBS) 19 reference sample so that results from different laboratories may be reliably compared [18].

The same mathematical formula is used for reporting nitrogen ($^{15}N/^{14}N$) and oxygen ($^{18}O/^{16}O$) isotope ratios, with the standard reference materials being AIR for nitrogen and VPDB for oxygen. Because the $^{13}C/^{12}C$ ratio of all photosynthesized carbon is lower than that of the reference VPDB material, the ^{13}C values for plants and animals are mostly negative (see equation).

HISTORY OF ISOTOPE STUDIES

Variation in the relative proportions of stable carbon isotopes was first measured in 1939 by Nier and Gulbransen [71], and the general distribution of carbon isotopes in nature was explored by Craig in 1953 [19]. It was less than 20 years later then that the archaeological significance of such analyses was recognized, both for studying prehistoric human diets [110] and for sourcing marble [21, 41]. In between, important advances had been made by Calvin and Benson [16] in the understanding of the chemical pathway for carbon during photosynthesis, followed by the realization that there were multiple photosynthetic pathways, commonly referred to as C_3, C_4, and crassulacean acid metabolism (CAM) by Hatch and Slack [38] and Ransom and Thomas [85]. At about the same time that it was determined that maize followed the C_4 photosynthetic pathway [39], it was realized by archaeologists that radiocarbon dates on preserved carbonized maize samples were consistently about 200 years younger than expected [36], with the result that stable isotope measurements were made and correction factors were applied to radiocarbon dates being done on maize and other nonwood charcoal samples [11]. A conference held in Australia in 1970 led to the explicit realization that plants following the C_3 photosynthetic pathway had different stable carbon isotope ratios (ave. $\delta^{13}C = -26.5‰$) than those following the C_4 pathway (ave. $\delta^{13}C = -12.5‰$), which includes maize, sugarcane, millet, sorghum, and certain species of amaranth and chenopodium [101]. These now domesticated C_4 plants, as well as a range of wild C_4 grasses, are originally native to areas of hot and dry climates, whereas CAM plants switch between C_3 and C_4 depending on their actual location and environmental circumstances.

The idea that carbon isotope values could be used for dietary information was developed at least by 1964, when Parker [77] published an article on carbon isotope analysis of marine plants and animals, but it was not until 1971 when a dietary study was done on humans. Van der Merwe and Vogel [111] specifically tested an Iron Age Khoi skeleton from the Transvaal of South Africa, whose bone collagen (protein made of multiple amino acids) carbon isotope value ($\delta^{13}C = -10.4$) was interpreted then as indicating dependence on sorghum (or other C_4 plants) in the Transvaal Lowveld. It should also be noted that although the earliest radiocarbon dating of bone was done on whole bone samples, by 1970 demineralization of bone and extraction of humic and fulvic acids had been developed to produce much more accurate dating results on bone collagen [51, 62], and this was the specific sample material tested by van der Merwe and Vogel.

Shortly afterwards, discussions in van der Merwe's [110] 1973 seminar at SUNY-Binghamton led to the isotopic analysis of human skeletal remains from sites in New York to address the question of when maize agriculture was introduced to that region [110]. The results were first published in *American Antiquity* in 1977 [116]. This was followed by a larger study of Archaic, Woodland, and Mississippian period sites in North America, which achieved greater sci-

entific recognition with a publication in *Nature* in 1978 [115]. Both studies clearly showed that the introduction of maize agriculture in North America dramatically changed the isotope values on human bone collagen in measurable, quantita-tive ways, demonstrating that isotope analyses could strongly complement botanical and other evidence for dietary interpretations. These early studies also demonstrated that for collagen $\delta^{13}C$ values, "You are what you eat, plus 5‰." The percentage of C_4 foods in the diet could also be estimated by simple interpolation of the adjusted collagen value between the C_3 and C_4 endpoints (e.g., a collagen result of −14‰ suggests about 50% C_4 in the diet) (Figure 10-1).

This initial work was quickly followed by other studies by several scholars, with DeNiro and Epstein, who had already studied isotopic variation among animals [24], reporting results for early maize consumption at Tehuacan in highland Mexico [25], at the same time that a study was done on maize in the Hopewell Period of the midwestern United States by Bender, Baerreis and Steventon [12], while yet another study was done by van der Merwe and Vogel, with Roosevelt on maize in the rain forests of the Orinoco Valley of Venezuela [113]. This particular project emphasized the importance when interpreting isotopic results for humans of establishing baseline values for the plants and animals in a given region, which in this case were especially negative because of the recycling of CO_2 under a dense forest canopy [66]. Another early study specifically addressed maize in the diet of domestic dogs in Peru and Ecuador [15].

Stable isotope analysis of nitrogen also developed in the early 1980s, with clear indications of differences caused by trophic level effects, especially in marine systems [5, 25, 26, 90, 91, 96, 100]. More studies have added significantly to

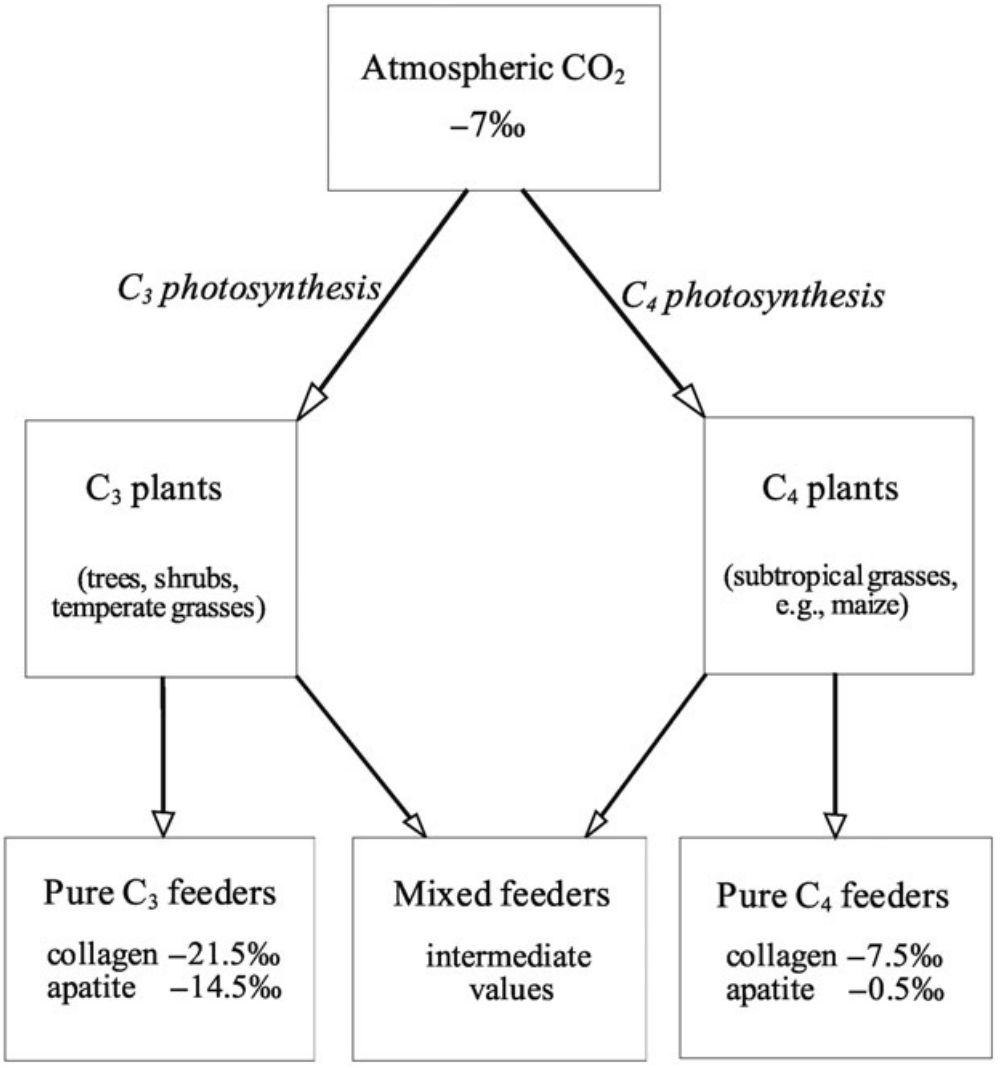

FIGURE 10-1 C_3 and C_4 pathways, showing isotopic differences passed on to skeletal tissues.

our understanding and interpretation of **nitrogen isotope** ratios, in particular the effects of climate and environment on both plant and animal values and trophic level increases in both terrestrial and marine ecosystems [3, 14]. Nitrogen isotope analysis has also been demonstrated to be useful for investigation of ancient weaning practices [94, 122, 125]. In general, nitrogen isotope ratios increase 2 to 3‰ with each trophic level, with values for terrestrial plants and animals generally much lower than for fish and mammals in most freshwater or marine ecosystems (Figure 10-2).

Other major advances to bone chemistry studies came in the 1980s regarding the preparation and carbon isotope analysis of inorganic bone apatite (calcium hydroxyphosphate, $Ca_5[PO_4]_3OH$, with some carbonate substitution). Because this mineral, which accounts for more than 75% of whole bone, is inorganic, it is much more susceptible to weathering and chemical reactions with soil deposits and, thus, alteration (diagenesis) of its original isotopic signature. This was quite clear from early radiocarbon dating of whole bone samples, which produced unreliable results, so that when stable isotope analysis of bone apatite for dietary purposes was first proposed by C. H. Sullivan and H. W. Krueger in 1981 [53, 105], it was not accepted by other researchers [89]. Since then, however, bone apatite sample pretreatment procedures have matured [48, 52, 58], and analysis of nonporous tooth enamel has been shown to be reliable going back millions of years [49, 55, 56, 59, 102]. Increased understanding of fossilization pathways is also now leading to better assessment of the isotopic integrity of bone apatite samples [57, 117]; yet, there does seem to be some systematic offsets in isotope values produced using different sample preparation methods [34].

Overall, isotopic analysis of archaeological materials has increased tremendously since the early studies in the 1970s, when carbon isotope analysis of bone collagen were regularly done by radiocarbon laboratories to properly correct C14 dates, with both carbon and nitrogen isotope analysis now done in many other laboratories to address dietary

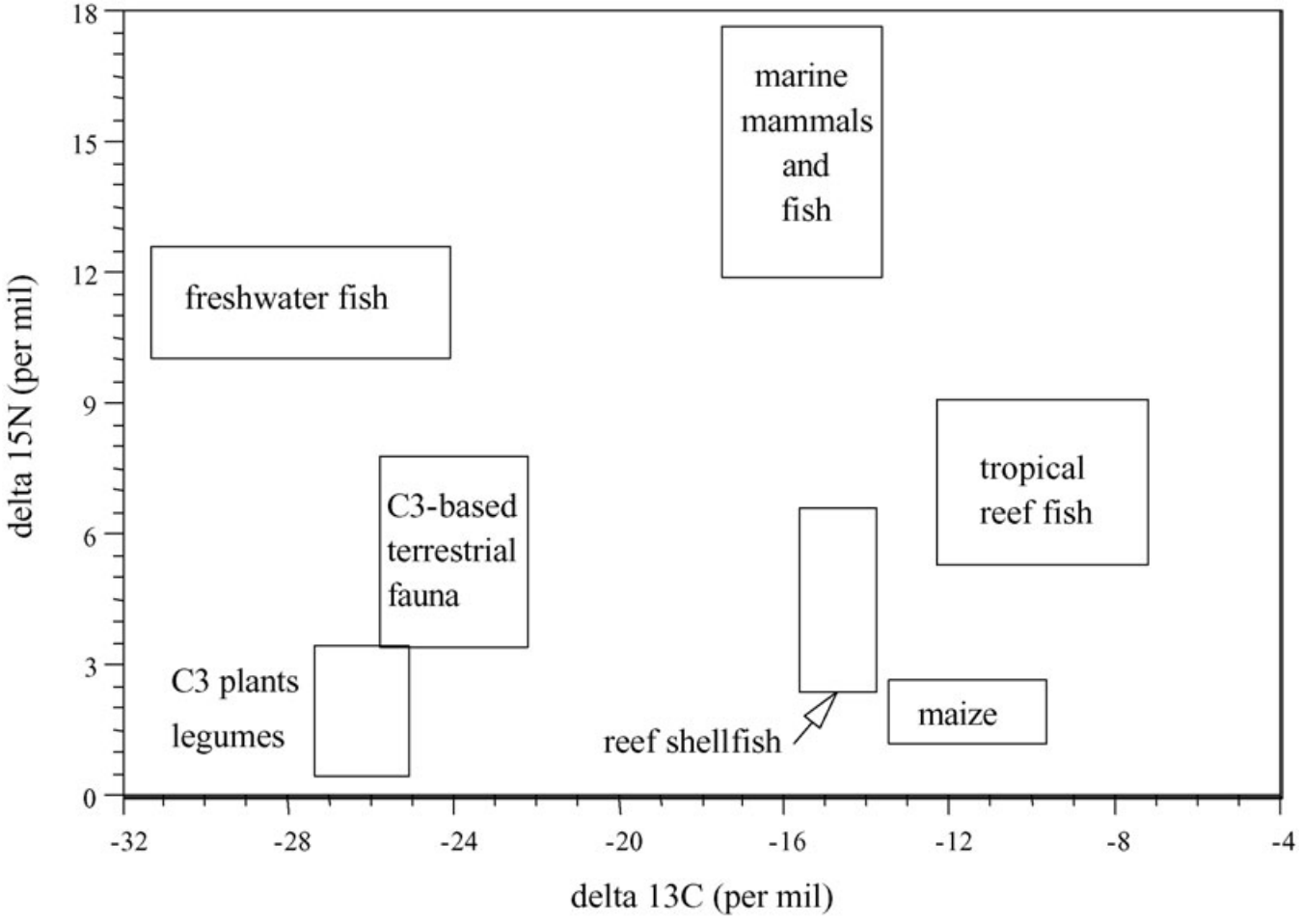

FIGURE 10-2 A Gulf Coast example of stable carbon versus nitrogen isotope ratios for plant and animal groups. Faunal bone collagen carbon corrected –2% to simulate flesh; modern samples corrected +1.5% for industrial effect. Although values for plants and terrestrial consumers are similar in other regions, values for aquatic resources may be different.

questions. Until recently, I have personally maintained a detailed database of all published isotope studies in the New World, but the feasibility of this is steadily decreasing as the number of studies exponentially increase, not to mention more studies being done by graduate students or for cultural resource management (CRM) firms, without widespread publication of the results.

SAMPLE PREPARATION AND ISOTOPIC ANALYSIS

Archaeological isotope studies, in contrast to most forensic studies of the recently deceased, must deal first with issues of preservation and contamination to produce reliable scientific results with which to address ancient dietary questions. Over time, slightly different laboratory preparation procedures have been developed for preparing bone collagen and bone apatite samples. In most cases, a single 1 gram sample of reasonably preserved bone is sufficient for the preparation of both collagen and apatite (Figure 10-3); the actual amount of sample analyzed by most **mass spectrometer**s is no more than a few milligrams, and as little as several hundred micrograms. For preparation of collagen, most techniques use an acidic solution to demineralize the bone and other chemicals to remove contaminants and residual lipids. For preparation of bone apatite samples, sodium hypochlorite (bleach) is typically used to dissolve any preserved organic components, and a weak acid solution is employed to remove any nonbiogenic carbonates. The specific procedures used are discussed in each of the chapters on isotope studies, as well as the particular mass spectrometer used and the precision of the measurements obtained. The precision of carbon and nitrogen isotopic results is almost always ≤0.3‰, and in many cases about 0.1‰, so that actual dietary isotope differences of just a few percent are noticeable.

Even more important is assessment of the reliability of the isotope results produced. Although mass spectrometers have significantly changed over time, from manually operated systems in which solid samples were converted to CO_2 and N_2 gas off-line (Figures 10-4A, 10-4B), to modern, largely computer-operated instruments with automated in-line sample introduction systems (CHN analyzers for organic samples, common or individual acid baths for apatite-enamel samples) (Figures 10-5A, 10-5B), the users are always responsible for the type, quality, and purity of the sample being tested. For bone collagen, this is typically dealt with by measuring the percentage of collagen produced from the original whole bone sample (<1% is usually considered unreliable because bone originally is more than 20% collagen); the amount of C and N measured by the mass spec-

FIGURE 10-3 Typical bone sample, and collagen and apatite produced in Tykot's Laboratory for Archaeological Science at the University of South Florida.

FIGURE 10-4A Off-line gas sample preparation system for stable isotope samples, with Nikolaas J. van der Merwe. University of Cape Town, Stable Light Isotope Laboratory. Photo by R. H. Tykot.

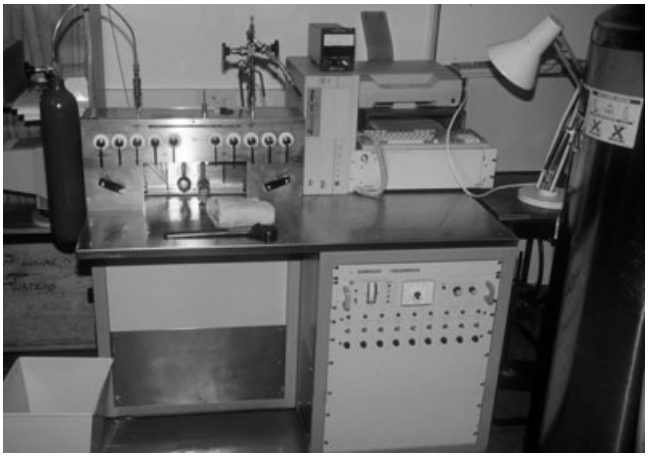

FIGURE 10-4B A venerable stable isotope ratio mass spectrometer (VG602E), which requires manual input of CO_2 and N_2 gas samples. University of Cape Town, Stable Light Isotope Laboratory. Photo by R. H. Tykot.

trometer, relative to the size of the sample introduced; and the actual C : N ratio (which should be the same as in living organisms). Problematic results mainly occur when preservation has not been so poor that no collagen remains (and thus no isotope data produced) but where degradation has resulted in unequal breakdown and loss of the different amino acids, which individually would have different isotope values because of the different chemical reactions involved in their initial production. For bone apatite and tooth enamel samples, other than measures of sample loss during the preparation procedures and CO_2 yield during the mass spectrometry analysis, more complex tests of sample reliability, using Fourier transform infrared spectroscopy (FTIR), have been developed [34, 48, 57, 69 70, 125]. One logistical approach to producing reliable isotope results, in addition to measures of sample preparation and analysis, is the need for performing repeat isotope analyses for "outliers" when testing a group of individuals thought to have had similar diets.

INTERPRETATION AND SIGNIFICANCE OF CARBON AND NITROGEN ISOTOPE DATA

From the time of the earliest isotope studies in the 1970s, it was realized that bone collagen has a slow turnover rate, at least for adults, so that the isotope results obtained would represent the average diet over at least the last several years of an individual's life. Although the exact rate of turnover is still unknown, it is estimated to be at least 5 to 7 years if not more and varies depending on the density of each bone. In any case it has become evident that isotope studies generally may be done on any available part of skeletal remains, although microsampling of interior and exterior parts of long bones could produce different values if diet isotopically changed significantly during the time of their recent production. Although the same lengthy turnover time applies to bone apatite as well, it is clear that tooth enamel, tooth roots (dentin), hair, fingernails, and flesh are different. Teeth form only once and, thus, represent at most a few years of diet,

FIGURE 10-5A Example of a modern stable isotope ratio mass spectrometer (Finnigan MAT Delta Plus XL), run by computer (far right), and connected with several input devices including a Carlo Erba CHN analyzer (upper right corner) that converts organic samples into CO_2 and N_2 gas. University of South Florida, Paleolab. Photo by R. H. Tykot.

FIGURE 10-5B Another stable isotope ratio mass spectrometer, connected to an automated Kiel III individual acid bath system (left) that converts inorganic carbonates into CO_2. University of South Florida, Paleolab. Photo by R. H. Tykot.

at the age when the tooth formed, with third molars the last to form, between ages 10–12 for the enamel and by young adulthood for the tooth dentin (roots). Analysis of other permanent teeth can reveal the age of weaning, at which point there is no longer a trophic level increase in isotope values resulting from breast-feeding [125]. Analysis of sequential samples in tooth dentin may be used to investigate seasonal isotope variation in diet, as can microsampling of tooth enamel layers [9, 10, 122]. When preserved, sequential hair and fingernail samples may also be used to assess short-term dietary change [32, 64, 73–75, 88, 98].

As for specific aspects of diet that are revealed through these different isotope studies, it became clear from studies of animals raised on controlled diets that bone collagen is produced primarily from dietary protein, whereas bone apatite and tooth enamel are products of the whole diet [7, 106]. For collagen, it appears that essential amino acids are transferred directly from appropriate foods, whereas nonessential amino acids may be transferred or produced independently and, thus, represent the whole diet, including maize [33, 37, 43]. Isotopic analysis of individual amino acids would be especially useful for studies of the importance of maize in coastal environments.

Controlled diet studies have also provided a more precise assessment of the difference in $\delta^{13}C$ values between dietary resources and bone apatite, with variation in fractionation seemingly attributable to differences in body size, digestive physiology, and amount of methanogenesis [9, 17, 43, 58, 78]. For humans (not directly tested with controlled diets), the fractionation between diet and bone apatite is estimated to be at least 9.5‰ [7] and as much as 11 to 12% [40]. Although it is currently possible to quantitatively *compare* human individuals and groups of individuals, further studies are necessary if we are to have better precision on the *quantity* of maize and other C_4 plants in the diet.

Overall, interpretations are currently as follows. For herbivores, where the entire diet is based on carbohydrate dominated plant foods, any mixtures of C_3, C_4, and CAM plants would contribute equally to both collagen and apatite, unless there were significant differences in the percentage of protein in those plants (e.g., beans with about 20% protein versus maize with about 10% protein). For omnivores (including humans), animal food—whether wild or domestic, terrestrial or aquatic—would make a much greater contribution to bone collagen. So for a hypothetical New World inland society with maize agriculture, where dogs are the only domesticated animals, it would be expected to have isotope values for both collagen and apatite that indicate maize consumption, with the percentage represented at least somewhat higher for bone apatite than for bone collagen, unless the dogs (or wild hunted animals) ate more maize than the humans (one reason for testing the isotope values of animals that were consumed). Differences between individuals in the percentage of maize versus C_3 plants in the diet would be far more apparent in apatite than in collagen, thus making analysis of bone and tooth apatite much more effective than collagen for addressing questions about variation within a population or at a single site; comparison of individuals based on sex, status, and/or specialization; and changes over time [e.g., 4, 35, 109].

For studies where C_4 (or CAM) plants other than maize existed or where seafood may have been consumed, it is strongly recommended to test archaeological faunal remains to learn whether they may have contributed more positive carbon isotope values to human consumers, as well as to use other archaeological and ethnohistoric information to interpret the isotope results. Marine plants and their consumers have more positive $*^{13}C$ values because their original sources of carbon include dissolved CO_2 and carbonic acid, as well as detritus from local terrestrial plants [93]. Because of the many trophic levels for marine fauna, there are continuous increases in both carbon and nitrogen isotope ratios from level to level, resulting in many cases with large fish and marine mammals with $*^{13}C$ values similar to C_4 plants such as maize—but also enriched $*^{15}N$ values. Analysis of nitrogen isotopes in bone collagen is, thus, necessary for looking at diets in coastal regions, and in conjunction with bone carbonate carbon isotope analysis, it may be used to assess the relative contributions of maize and marine foods in the whole diet and in protein in particular. Most freshwater aquatic fish from inland lakes and rivers also have much higher $*^{15}N$ values than terrestrial foods, but $*^{13}C$ values similar to C_3 plants, so that consumption of maize or other C_4 plants is readily apparent. It should be noted that some scholars have developed and used mathematical mixing models to interpret isotope results [61, 68, 79, 80].

OXYGEN AND STRONTIUM ISOTOPES

Even more significant has been the expansion of isotope analyses from carbon and nitrogen in bone collagen and apatite to charred ceramic residues [e.g., 67], carbonates and humic matter in soils [72, 118], and to carbon isotope analysis of cholesterol preserved in bones and teeth, of animal fats, and of lipid residues in ceramics [29, 30, 86, 87, 104]. Because the turnover time of cholesterol in bone samples is much greater than that of collagen and apatite, cholesterol analyses may be specifically used to address dietary change during the time more immediately preceding death, whereas analyses of residues and soils provide information on cultural and spatial landscape practices.

Oxygen and **strontium isotope** analyses have also been added to the isotope repertoire applied to ancient dietary issues. **Oxygen isotope** values relate directly to local climate, temperature, and humidity [50, 63] and, thus, have

been used not only for determining the seasonality of shells and presumably their consumers [22] but also for climate studies [92, 103] and mobility [119–121], with appropriate changes in dietary patterns. Isotope ratios of strontium, which does not isotopically fractionate like biological C, N, and O, directly represent the geographic area of food production/acquisition and, thus, the mobility of dietary resources or their consumers, or both [27, 28, 82]. Strontium isotope analysis has, thus, been applied to significant migration periods in Europe [13, 83], immigration to Mesoamerican sites such as Teotihuacan and Tikal [84, 42, 124], and migration and mortuary patterns in South America [47].

ISOTOPE STUDIES IN THIS VOLUME

The chapters in the following part of this volume all focus on isotope studies of maize acquisition and consumption. A broad range of geographic areas are covered, including White and colleagues (Chapter 11), Chisholm and Blake (Chapter 12), and Mansell and colleagues (Chapter 13) on Mesoamerica; Tykot and colleagues (Chapter 14) and Gil and colleagues (Chapter 15) on parts of Andean South America; and Greenlee (Chapter 16), Reber (Chapter 17), Kelly and colleagues (Chapter 18), Katzenberg (Chapter 19), Coltrain and colleagues (Chapter 20), and Benson (Chapter 21) on many areas throughout North America, followed by a synthesis by Schwarcz (Chapter 22). Isotope studies such as these continue to make significant contributions to our understanding of the histories of maize.

References Cited

1. S. H. Ambrose. (1990). Preparation and characterization of bone and tooth collagen for isotopic analysis. *Journal of Archaeological Science, 17,* 431–451.
2. S. H. Ambrose. (1993). Isotopic analysis of paleodiets: Methodological and interpretive considerations, In: M. K. Sandford, (Ed.), *Investigations of ancient human tissue: Chemical analysis in anthropology.* Langhorne, PA: Gordon and Breach Science Publishers. pp. 59–129.
3. S. H. Ambrose. (2000). Controlled diet and climate experiments on nitrogen isotope ratios of rats, In: S. H. Ambrose, M. A. Katzenberg, (Eds.), *Biogeochemical approaches to paleodietary analysis.* New York: Plenum. pp. 243–267.
4. S. H. Ambrose, J. Buikstra, H. Krueger. (2003). Status and gender difference in diet at Mound 72, Cahokia, revealed by isotopic analysis of bone. *Journal of Anthropological Archaeology, 22,* 217–226.
5. S. H. Ambrose, M. J. DeNiro. (1986). Reconstruction of African human diet using bone collagen carbon and nitrogen isotope ratios, *Nature, 319,* 321–324.
6. S. H. Ambrose, J. Krigbaum. (2003). Bone chemistry and bioarchaeology. *Journal of Anthropological Archaeology, 22,* 193–199.
7. S. H. Ambrose, L. Norr. (1993). Experimental evidence for the relationship of the carbon isotope ratios of whole diet and dietary protein to those of bone collagen and carbonate, In: J. B. Lambert, G. Grupe, (Eds.), *Prehistoric human bone: Archaeology at the molecular level.* New York: Springer-Verlag. pp. 1–37.
8. G. J. Armelagos. (1994). You are what you eat, In: K. D. Sobolik, (Ed.), *Paleonutrition: The diet and health of prehistoric Americans*. Carbondale, IL: Southern Illinois University. pp. 235–244.
9. M. Balasse. (2002). Reconstructing dietary and environmental history from enamel isotopic analysis: time resolution of intra-tooth sequential sampling. *International Journal of Osteoarchaeology, 12,* 155–165.
10. M. Balasse. (2002). Potential biases in sampling design and interpretation of intra-tooth isotope analysis. *International Journal of Osteoarchaeology, 13,* 3–10.
11. M. M. Bender. (1968). Mass spectrometric studies of carbon 13 variations in corn and other grasses. *Radiocarbon, 10,* 468–472.
12. M. M. Bender, D. A. Baerreis, R. L. Steventon. (1981). Further light on carbon isotopes and Hopewell agriculture. *American Antiquity, 46,* 346–353.
13. A. Bentley, T. D. Price, J. Luning, D. Gronenborn, J. Wahl, P. Fullagar. (2002). Prehistoric migration in Europe: Strontium isotope analysis of Early Neolithic skeletons. *Current Anthropology, 43,* 799–804.
14. H. Bocherens, D. Drucker. (2003). Trophic level isotopic enrichment of carbon and nitrogen in bone collagen: case studies from recent and ancient terrestrial ecosystems. *International Journal of Osteoarchaeology, 13,* 46–53.
15. R. Burleigh, D. Brothwell. (1978). Studies on American dogs, 1. Carbon isotopes in relation to maize in the diet of domestic dogs from early Peru and Ecuador. *Journal of Archaeological Science, 5,* 355–362.
16. M. Calvin, A. A. Benson. (1948). The path of carbon in photosynthesis. *Science, 107,* 476–480.
17. T. E. Cerling, J. M. Harris. (1999). Carbon isotope fractionation between diet and bioapatite in ungulate mammals and implications for ecological and paleoecological studies. *Oecologia, 120,* 247–363.
18. T. B. Coplen. (1994). Reporting of stable hydrogen, carbon, and oxygen isotopic abundances. *Pure & Applied Chemistry, 66,* 273–276.
19. H. Craig. (1953). The geochemistry of the stable carbon isotopes. *Geochimica et Cosmochimica Acta, 3,* 53–92.
20. H. Craig. (1957). Isotope standards for carbon and oxygen and correction factors for mass-spectrometric analyses of carbon dioxide. *Geochimica et Cosmochimica Acta, 12,* 133–149.
21. H. Craig, V. Craig. (1972). Greek marbles: Determination of provenance by isotopic analysis. *Science, 176,* 401–403.
22. M. Deith. (1988). Shell seasonality: An appraisal of the oxygen isotope technique, In: R. E. Webb, (Ed.), *Recent developments in environmental analysis in Old and New World Archaeology.* Oxford, UK: BAR International Series 416. pp. 37–49.
23. M. J. DeNiro. (1987). Stable isotopy and archaeology. *American Scientist, 75,* 182–191.
24. M. J. DeNiro, S. Epstein. (1978). Influence of diet on the distribution of carbon isotopes in animals. *Geochimica et Cosmochimica Acta, 42,* 495–506.
25. M. J. DeNiro, S. Epstein. (1981). Influence of diet on the distribution of nitrogen isotopes in animals. *Geochimica et Cosmochimica Acta, 45,* 341–351.
26. M. J. DeNiro, M. J. Schoeninger. (1983). Stable carbon and nitrogen isotope ratios of bone collagen: Variations within individuals, between sexes, and within populations raised on monotonous diets. *Journal of Archaeological Science, 10,* 199–203.
27. J. E. Ericson. (1985). Strontium isotope characterization in the study of prehistoric human ecology, *Journal of Human Evolution, 14,* 503–514.
28. J. E. Ericson. (1989). Some problems and potentials of strontium isotope analysis for human and animal ecology, In: P. W. Rundel, J. R. Ehleringer, K. A. Nagy, (Eds.), *Stable isotopes in ecological research*. New York: Springer-Verlag. pp. 252–259.

29. R. P. Evershed, C. Heron, S. Charters, L. J. Goad. (1992). The survival of food residues: New methods of analysis, interpretation and application, In: A. M. Pollard, (Ed.), *New developments in archaeological science.* New York: Oxford University Press. pp. 187–208.
30. R. P. Evershed, S. N. Dudd, M. S. Copley, R. Berstan, A. W. Stott, H. Mottram, S. A. Buckley, Z. Crossman. (2002). Chemistry of archaeological animal fats. *Accounts of Chemical Research, 35,* 660–668.
31. P. Farnsworth, J. E. Brady, M. DeNiro, R. S. MacNeish. (1985). A reevaluation of the isotopic and archaeological reconstructions of diet in the Tehuacan Valley. *American Antiquity, 50,* 102–116.
32. J. N. Fenner. (2002). *Putting a hair in the soup: An investigation into the archaeological analysis of single hairs.* Master's Thesis, Eastern New Mexico University.
33. M. L. Fogel, N. Tuross. (2003). Extending the limits of paleodietary studies of humans with compound specific carbon isotope analysis of amino acids. *Journal of Archaeological Science, 30,* 535–545.
34. S. Garvie-Lok, T. Varney, M. Katzenberg. (2004). Preparation of bone carbonate for stable isotope analysis: The effects of treatment time and acid concentration. *Journal of Archaeological Science, 31,* 763–776.
35. J. P. Gerry. (1997). Bone isotope ratios and their bearing on elite privilege among the Classic Maya. *Geoarchaeology, 12,* 41–69.
36. R. A. Hall. (1967). Those late corn dates: Isotopic fractionation as a source of error in carbon-14 dates. *Michigan Archaeologist, 13,* 171–180.
37. P. E. Hare, M. L. Fogel, T. W. Stafford Jr., A. D. Mitchell, T. C. Hoering. (1991). The isotopic composition of carbon and nitrogen in individual amino acids isolated from modern and fossil proteins. *Journal of Archaeological Science, 18,* 277–292.
38. M. D. Hatch, C. R. Slack. (1966). Photosynthesis by sugarcane leaves. A new carboxylation reaction and the pathway of sugar formation. *The Biochemical Journal, 101,* 103–111.
39. M. D. Hatch, C. R. Slack, H. S. Johnson. (1967). Further studies on a new pathway of photosynthetic carbon dioxide fixation in sugarcane, and its occurrence in other species. *The Biochemical Journal, 102,* 417–422.
40. R. E. M. Hedges. (2003). On bone collagen-apatite carbonate isotopic relationships. *International Journal of Osteoarchaeology, 13,* 66–79.
41. N. Herz, D. B. Wenner. (1978). Assembly of Greek marble inscriptions by isotopic methods, *Science, 199,* 1070–1072.
42. D. A. Hodell, R. L. Quinn, M. Brenner, G. Kamenov. (2004). Spatial variation of strontium isotopes (87Sr/86Sr) in the Maya region: A tool for tracking ancient human migration. *Journal of Archaeological Science, 31,* 585–601.
44. M. A. Katzenberg. (1992). Advances in stable isotope analysis of prehistoric bones, In: S. R. Saunders, M. A. Katzenberg, (Eds.), *Skeletal biology of past peoples: Research methods.* New York: Wiley-Liss. pp. 105–120.
45. M. A. Katzenberg. (2000). Stable isotope analysis: a tool for studying past diet, demography, and life history, In: M. A. Katzenberg, S. R. Saunders, (Eds.), *Biological anthropology of the human skeleton.* New York: Wiley-Liss. pp. 305–328.
46. M. A. Katzenberg, R. G. Harrison. (1997). What's in a bone? Recent advances in archaeological bone chemistry. *Journal of Archaeological Research, 5,* 265–293.
47. K. J. Knudson, T. D. Price, J. E. Buikstra, D. E. Blom. (2004). The use of strontium isotope analysis to investigate Tiwanaku migration and mortuary ritual in Bolivia and Peru. *Archaeometry, 46,* 5–18.
48. P. L. Koch, N. Tuross, M. L. Fogel. (1997). The effects of sample treatment and diagenesis on the isotopic integrity of carbonate in biogenic hydroxylapatite. *Journal of Archaeological Science, 24,* 417–429.
49. M. Kohn, M. J. Schoeninger, W. W. Barker. (1999). Altered states: Effects of diagenesis on fossil tooth chemistry. *Geochimmica et Cosmochimica Acta, 63,* 2737–2747.
50. Y. Kolodny, B. Luz, O. Navon, (1983). Oxygen isotope variations in phosphate of biogenic apatites, *Earth and Planetary Science Letters, 64,* 398–404.
51. H. W. Krueger. (1965). The preservation and dating of collagen in ancient bones, In: R. M. Chatters, E. A. Olson, (Eds.), *Proceedings of the 6th International Conference on Radiocarbon and Tritium Dating.* Washington, D.C.: National Bureau of Standards. pp. 332–337.
52. H. W. Krueger. (1991). Exchange of carbon with biological apatite. *Journal of Archaeological Science, 18,* 355–361.
53. H. W. Krueger, C. H. Sullivan. (1984). Models for carbon isotope fractionation between diet and bone, In: J. Turnlund, P. E. Johnson, (Eds.), *Stable isotopes in nutrition.* ACS Symposium Series 258. Washington, D.C.: American Chemical Society. pp. 205–222.
54. C. S. Larsen. (1997). *Bioarchaeology: Interpreting behavior from the human skeleton.* Cambridge, UK: Cambridge University Press.
55. J. A. Lee-Thorp. (1989). *Stable carbon isotopes in deep time: The diets of fossil fauna and hominids.* Ph.D. thesis, University of Cape Town.
56. J. A. Lee-Thorp. (2000). Preservation of biogenic carbon isotopic signals in Plio-Pleistocene bone and tooth mineral, In: S. H. Ambrose, M. A. Katzenberg, (Eds.), *Biogeochemical approaches to paleodietary analysis.* New York: Plenum. pp. 89–116.
57. J. A. Lee-Thorp, M. Sponheimer. (2003). Three case studies used to reassess the reliability of fossil bone and enamel isotope signals for paleodietary studies. *Journal of Anthropological Archaeology, 22,* 208–216.
58. J. A. Lee-Thorp, N. J. van der Merwe. (1987). Carbon isotope analysis of fossil bone apatite. *South African Journal of Science, 83,* 712–715.
59. J. A. Lee-Thorp, N. J. van der Merwe. (1991). Aspects of the chemistry of modern and fossil biological apatites. *Journal of Archaeological Science, 18,* 343–354.
60. W. F. Libby. (1955). *Radiocarbon dating.* Chicago, IL: University of Chicago Press.
61. J. D. C. Little, E. A. Little. (1997). Analysing prehistoric diets by linear programming. *Journal of Archaeological Science, 24,* 741–747.
62. R. Longin. (1970). New method of collagen extraction for radiocarbon dating. *Nature, 230,* 241–242.
63. B. Luz, Y. Kolodny, M. Horowitz. (1984). Fractionation of oxygen isotopes between mammalian bone-phosphate and environmental drinking water. *Geochimica et Cosmochimica Acta, 48,* 1689–1693.
64. S. A. Macko, M. H. Engel, V. Andrusevich, G. Lubec, T. C. O'Connell, R. E. M. Hedges. (1999). Documenting the diet in ancient human populations through stable isotope analysis of hair. *Philosophical Transactions of the Royal Society of London B, 354,* 65–76.
65. S. Mays. (1998). *The archaeology of human bones.* London and New York: Routledge.
66. E. Medina, P. Minchin. (1980). Stratification of $\delta^{13}C$ values of leaves in Amazonian rain forests. *Oecologia*, *45*, 377–378.
67. J. Morton, H. P Schwarcz. (2004). Paleodietary implications from isotopic analysis of food residues on prehistoric Ontario ceramics. *Journal of Archaeological Science, 31,* 503–517.
68. S. D. Newsome, D. L. Phillips, B. J. Culleton, T. P. Guilerson, P. L. Koch. (2004). Dietary reconstruction of an early to middle Holocene human population from the central California coast: Insights from advanced stable isotope mixing models. *Journal of Archaeological Science, 31,* 1101–1115.
69. C. M. Nielsen-Marsh, R. E. M. Hedges. (2000). Patterns of diagenesis in bone I: The effects of site environments. *Journal of Archaeological Science, 27,* 1139–1150.
70. C. M. Nielsen-Marsh, R. E. M. Hedges. (2000). Patterns of diagenesis in bone II: The effects of acetic acid treatment and the removal of diagenetic CO_3^{2-}. *Journal of Archaeological Science, 27,* 1151–1159.

43. Howland, M. R., L. T. Corr, S. M. M. Young, V. Jones, S. Jim, N. J. van der Merwe, A. D. Mitchell, and R. P. Evershed. (2003). Expression of the dietary isotope signal in the compound-specific C13 values of pig bone lipids and amino acids. *International Journal of Osteoarchaeology*, 13, 54-65.

71. A. O. Nier, E. A. Gulbransen. (1939). Variations in the relative abundance of the carbon isotopes. *Journal of the American Chemical Society, 61,* 697–698.
72. L. C. Nordt. (2001). Stable carbon and oxygen isotopes in soils, In: P. Goldberg, V. T. Holliday, C. R. Ferring, (Eds.), *Earth sciences and archaeology.* New York: Kluwer. pp. 419–448.
73. T. C. O'Connell, R. E. M. Hedges. (1999). Investigations into the effect of diet on modern human hair isotopic values. *American Journal of Physical Anthropology, 108,* 409–425.
74. T. C. O'Connell, R. E. M. Hedges. (1999). Isotopic comparison of hair and bone: Archaeological analyses. *Journal of Archaeological Science, 26,* 661–665.
75. T. C. O'Connell, R. E. M. Hedges, M. A. Healey, A. H. R. W. Simpson. (2001). Isotopic comparison of hair, nail and bone: Modern analyses. *Journal of Archaeological Science, 28,* 1247–1255.
76. R. Park, S. Epstein. (1960). Carbon isotope fractionation during photosynthesis. *Geochimica et Cosmochimica Acta*, 21, 110–126.
77. P. L. Parker. (1964). The biogeochemistry of the stable isotopes of carbon in a marine bay. *Geochimica et Cosmochimica Acta, 28,* 11755–1164.
78. B. H. Passey, T. F. Robinson, L. K. Ayliffe, T. E. Cerling, M. Sponheimer, M. D. Dearing, B. L. Roeder, J. R. Ehleringer. (2005). Carbon isotope fractionation between diet, breath CO2, and bioapatite in different mammals. *Journal of Archaeological Science, 32,* 1459–1470.
79. D. L. Phillips, P. L. Koch. (2002). Incorporating concentration dependence in stable isotope mixing models. *Oecologia, 130,* 114–125.
80. D. L. Phillips, J.W. Gregg. (2003). Source partitioning using stable isotopes: coping with too many sources. *Oecologia, 136,* 261–269.
81. A. M. Pollard. (1998). Archaeological reconstruction using stable isotopes, In: H. Griffiths, (Ed.), *Stable isotopes: Integration of biological, ecological and geochemical processes*. Oxford, UK: Bios Scientific Publishers. pp. 285–302.
82. T. D. Price, J. H. Burton, R. A. Bentley. (2002). The characterization of biologically available strontium isotope ratios for the study of prehistoric migration. *Archaeometry, 44,* 117–135.
83. T. D. Price, G. Grupe, P. Schröter. (1994). Reconstruction of migration patterns in the Bell Beaker period by stable strontium isotope analysis. *Applied Geochemistry, 9,* 413–417.
84. T. D. Price, L. Manzanilla, W. D. Middleton. (2000). Immigration and the ancient city of Teotihuacan in Mexico: A study using strontium isotope ratios in human bone and teeth. *Journal of Archaeological Science, 27,* 903–913.
85. S. L. Ransom, M. Thomas. (1960). Crassulacean acid metabolism. *Annual Review of Plant Physiology, 11,* 81–110.
86. E. A. Reber, R. P. Evershed. (2004). How did Mississippians prepare maize? The application of compound-specific carbon isotope analysis to absorbed pottery residues from several Mississippi Valley sites. *Archaeometry, 46,* 19–33.
87. E. A. Reber, R. P. Evershed. (2004). Identification of maize in absorbed organic residues: A cautionary tale. *Journal of Archaeological Science, 31,* 399–410.
88. M. K. Sandford, G. E. Kissling. (1993). Chemical analyses of human hair: Anthropological applications, In: M. K. Sandford, (Ed.), *Investigations of ancient human tissue: Chemical analyses in anthropology.* Langhorne, PA: Gordon and Breach Science Publishers. pp. 131–167.
89. M. J. Schoeninger, M. J. DeNiro. (1982). Carbon isotope ratios of apatite from bone cannot be used to reconstruct diets of animals. *Nature, 197,* 577–578.
90. M. J. Schoeninger, M. J. DeNiro. (1983). Stable nitrogen isotope ratios of bone collagen reflect marine and terrestrial components of prehistoric human diet. *Science, 220,* 1381–1383.
91. M. J. Schoeninger, M. J. DeNiro. (1984). Nitrogen and carbon isotopic composition of bone collagen from marine and terrestrial animals. *Geochimica et Cosmochimica Acta, 48,* 625–639.
92. M. J. Schoeninger, M. J. Kohn, J. W. Valley. (2000). Tooth oxygen isotope ratios as paleoclimate monitors in arid ecosystems, In: S. H. Ambrose, M. A. Katzenberg, (Eds.), *Biogeochemical approaches to paleodietary analysis.* New York: Plenum. pp. 117–140.
93. M. J. Schoeninger, K. Moore. (1992). Bone stable isotope studies in archaeology. *Journal of World Prehistory, 6,* 247–296.
94. M. J. Schoeninger, M. R. Schurr. (1994). Interpreting carbon stable isotope ratios, In: S. Johannessen, C. A. Hastorf, (Eds.), *Corn and culture in the prehistoric New World*, Boulder, CO: Westview Press. pp. 55–66.
95. M. R. Schurr. (1998). Using stable nitrogen-isotopes to study weaning behavior in past populations. *World Archaeology, 30,* 327–342.
96. H. P. Schwarcz, J. Melbye, M. A. Katzenberg, M. Knyf. (1985). Stable isotopes in human skeletons of southern Ontario: Reconstructing paleodiet. *Journal of Archaeological Science, 12,* 187–206.
97. H. P. Schwarcz, M. J. Schoeninger. (1991). Stable isotope analysis in human nutritional ecology. *Yearbook of Physical Anthropology, 34,* 283–321.
98. H. P. Schwarcz, C. D. White. (2004). The grasshopper or the ant?: Cultigen-use strategies in ancient Nubia from C-13 analyses of human hair. *Journal of Archaeological Science, 31,* 753–762.
99. J. Sealy, R. Armstrong, C. Schrire. (1995). Beyond lifetime averages: tracing life histories through isotopic analysis of different calcified tissues from archaeological human skeletons. *Antiquity, 69,* 290–300.
100. J. C. Sealy, N. J. van der Merwe, J. A. Lee-Thorp, J. Lanham. (1987). Nitrogen isotopic ecology in southern Africa: Implications for environmental and dietary tracing. *Geochimica et Cosmochimica Acta, 51,* 2707–2717.
101. B. N. Smith, S. Epstein. (1970). Two categories of $^{13}C/^{12}C$ for higher plants. *Plant Physiology, 47,* 380–384.
102. M. Sponheimer, J. Lee-Thorp. (1999). Isotopic evidence for diet of an early hominid, *Australopihecus africanus. Science, 283,* 368–370.
103. E. Stephan. (2000). Oxygen isotope analysis of animal bone phosphate: Method refinement, influence of consolidants, and reconstruction of palaeotemperatures for Holocene sites. *Journal of Archaeological Science, 27,* 523–535.
104. A. W. Stott, R. P. Evershed. (1996). $\delta^{13}C$ analysis of cholesterol preserved in archaeological bones and teeth. *Analytical Chemistry, 68,* 4402–4408.
105. C. H. Sullivan, H. W. Krueger. (1981). Carbon isotope analysis of separate chemical phases in modern and fossil bone. *Nature, 292,* 333–335.
106. L. L. Tieszen, T. Fagre. (1993). Effect of diet quality and composition on the isotopic composition of respiratory CO_2, bone collagen, bioapatite, and soft tissues, In: J. B. Lambert, G. Grupe, (Eds.), *Prehistoric human bone: Archaeology at the molecular level.* New York: Springer-Verlag. pp. 121–155.
107. J. R. Turnlund, P. E. Johnson, (Eds.). (1984). *Stable isotopes in nutrition.* Washington, D.C.: American Chemical Society.
108. R. H. Tykot. (2004). Stable isotopes and diet: You are what you eat, In: M. Martini, M. Milazzo, M. Piacentini, (Eds.), *Physics methods in archaeometry. Proceedings of the International School of Physics "Enrico Fermi."* Bologna, Italy: Società Italiana di Fisica. pp. 433–444.
109. D. H. Ubelaker, M. A. Katzenberg, L. G. Doyon. (1995). Status and diet in precontact highland Ecuador. *American Journal of Physical Anthropology, 97,* 403–411.
110. N. J. van der Merwe. (1973). New wrinkles in radiocarbon. Paper presented at the Plains Anthropology Conference in Columbia, Missouri, November, 1973.
111. N. J. van der Merwe. (1982). Carbon isotopes, photosynthesis, and archaeology. *American Scientist, 70,* 596–606.
112. N. J. van der Merwe. (1992). Light stable isotopes and the reconstruction of prehistoric diets. *Proceedings of the British Academy, 77,* 247–264.

113. N. J. van der Merwe, A. C. Roosevelt, J. C. Vogel. (1981). Isotopic evidence for prehistoric subsistence change at Parmana, Venezuela. *Nature, 292,* 536–538.
114. N. J. van der Merwe, H. Tschauner. (1999). C4 plants and the development of human societies. In: R. F. Sage, R. K. Monson, (Eds.), *C4 plant biology.* New York: Academic Press. pp. 509–549.
115. N. J. van der Merwe, J. C. Vogel. (1978). 13C content of human collagen as a measure of prehistoric diet in woodland North America. *Nature, 276,* 815–816.
116. J. C. Vogel, N. J. van der Merwe. (1977). Isotopic evidence for early maize cultivation in New York State. *American Antiquity, 42,* 238–242.
117. Y. Wang, T. E. Cerling. (1994). A model of fossil tooth and bone diagnesis: Implications for paleodiet reconstruction from stable isotopes. *Palaeogeography, Paleoclimatology, Palaeoecology, 107,* 281–289.
118. E. A. Webb, H. P. Schwarcz, P. F. Healy. (2004). Detection of ancient maize agriculture in the Maya lowlands using the stable carbon isotope compositions of soil organic matter: Evidence from Caracol, Belize. *Journal of Archaeological Science, 31,* 1039–1052.
119. C. D. White, F. J. Longstaffe, K. R. Law. (2004). Exploring the effects of environment, physiology and diet on oxygen isotope ratios in ancient Nubian bones and teeth. *Journal of Archaeological Science, 31,* 233–250.
120. C. D. White, M. W. Spence, F. J. Longstaffe, K. R. Law. (2000). Testing the nature of Teotihuacán imperialism at Kaminaljuyú using phosphate oxygen-isotope ratios. *Journal of Anthropological Research, 56,* 535–558.
121. C. D. White, M. Spence, F. J. Longstaffe, K. R. Law. (2004). Demography and ethnic continuity in the Tlailotlacan enclave of Teotihuacan: The evidence from stable oxygen isotopes. *Journal of Anthropological Archaeology, 23,* 385–403.
122. J. W. Wilson, R. H. Tykot, D. H. Ubelaker. (2001). Intradental variation in stable carbon isotope ratios in human tooth enamel. *American Journal of Physical Anthropology, 114,* 166.
123. L. E. Wright. (1999). Correspondence between stable carbon, oxygen and nitrogen isotopes in human tooth enamel and dentine: Infant diets at Kaminaljuyu. *Journal of Archaeological Science, 26,* 1159–1170.
124. L. E. Wright. (2005). Identifying immigrants to Tikal, Guatemala: Defining local variability in strontium isotope ratios of human tooth enamel. *Journal of Archaeological Science, 32,* 555–566.
125. L. E. Wright, H. P. Schwarcz. (1996). Infrared and isotopic evidence for diagenesis of bone apatite at Dos Pilas, Guatemala: Palaeodietary implications. *Journal of Archaeological Science, 23,* 933–944.
126. L. E. Wright, H. P. Schwarcz. (1998). Stable carbon and oxygen isotopes in human tooth enamel: Identifying breastfeeding and weaning in prehistory. *American Journal of Physical Anthropology, 106,* 1–18.