CHAPTER 13

STABLE ISOTOPE ANALYSIS: A TOOL FOR STUDYING PAST DIET, DEMOGRAPHY, AND LIFE HISTORY

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INTRODUCTION

This chapter is about chemical variation in the various components that make up bones and teeth and its application to studies of diet, demography, and life history. Bones and teeth provide direct evidence of past diets, including infant diets. Knowledge gained from bone chemistry relates to other sources of evidence for diet and, in turn, the interaction of diet, nutrition, and disease. Understanding infant diets and the duration of nursing relates to demographic variables such as birth spacing and population growth. Chemical variation in bones and teeth can also be linked to chemical variation in the environment and, thus, reveals information about place of residence and migration. Although stable isotope analysis may be viewed as a fairly technical research specialty, the results of such analyses make a significant contribution to the reconstruction of past human life.

Uses of Stable Isotope Analysis in Skeletal Biology

The routine use of stable carbon isotopes in current skeletal studies is very different from the excitement generated by the first applications of stable carbon isotopes to human paleodiet reconstruction. The idea that one could determine whether prehistoric peoples of North America consumed corn (maize) by performing chemical tests on their bones seemed like science fiction to most archaeologists and physical anthropologists in the mid-1970s when it was first attempted (Vogel and van der Merwe, 1977). Today, stable carbon isotope analysis is part of a suite of technical specialties performed on remains from archaeological sites. In addition to studying stable carbon and nitrogen isotopes in preserved protein, it is now possible to study stable isotopes of carbon, oxygen, and strontium from the mineral portion of bones and teeth. This chapter provides some background to the use of stable isotopes in bioarchaeological studies. It includes technical information on how such analyses are performed, with a sampling of applications, problems, and finally, promises for the future. This chapter is not intended to be a review of the now vast literature on methods and applications of stable isotope analysis in studies of past peoples. Reviews of the earlier literature are available elsewhere (Katzenberg and Harrison, 1997; Pate, 1994;

Biological Anthropology of the Human Skeleton, Second Edition. Edited by M. Anne Katzenberg and Shelley R. Saunders

Schoeninger and Moore, 1992; Schwarcz and Schoeninger, 1991), while more recent comprehensive articles focus on particular aspects of stable isotope applications (e.g., Katzenberg et al., 1996 on weaning; Lee-Thorp and Sponheimer, 2006 on early hominins). The field has grown enormously with many more laboratories training many more students, applying stable isotope analysis to an ever increasing array of interesting problems. Hopefully the various discussions herein will guide the reader to the relevant literature if more detailed information is desired.

Developments in Stable Isotope Analysis

From the perspective of the archaeologist, the first stable isotope studies of past diet were carried out in the 1970s (DeNiro and Epstein, 1978, 1981; van der Merwe and Vogel, 1978; Vogel and van der Merwe, 1977). However, studies of stable isotopes began in the early years of the twentieth century in the laboratories of chemists and physicists. After the discovery of stable isotopes in 1913, improvements in instrumentation and intensive study resulted in the identification of most stable isotopes by the mid-1930s. The first commercial mass spectrometer was used to analyze petroleum in 1942 (Gross, 1979). Throughout the 1950s and 1960s mass spectrometers and the applications of stable isotope studies advanced rapidly in chemistry, biology, and geochemistry. Efforts were directed toward understanding variation in the relative abundances of stable isotopes of the various elements. For example, geochemists explored oxygen isotope variation and its potential for studies of past climate (reviewed by Luz and Kolodny, 1989). Major advances in understanding stable isotope variation in the biosphere and geosphere occurred during the 1950s and 1960s. Botanists and geochemists explored stable carbon isotope variation in plants (Craig, 1954; Smith and Epstein, 1971), and researchers in radiocarbon dating laboratories shared their interest (Bender, 1968; Hall, 1967; Lowdon, 1969) since this variation is relevant to radiocarbon dating methods.

Along with advances in understanding the processes that cause variation in stable isotope abundance ratios in different substances, major advances have occurred in instrumentation. Although improvements in resolution, detection, and overall design of mass spectrometers occurred throughout the twentieth century, advances in the last 15 years have had an enormous impact on the use of stable isotope methods because of the ability to run more samples at a much lower cost. Stable isotope analysis used to be a time-consuming and, therefore, expensive method. Preparation of samples, isolation of gasses containing specific elements of interest, and the actual analysis and corrections to standards were very laborious processes. Only a small number of samples per day could be analyzed, and analyses required constant attention by lab personnel. In the late 1980s new instrumentation was developed that simplifies sample preparation, automates introduction of samples into the system, requires much smaller samples, is much faster, and therefore is much less expensive. This development has opened up many more applications of stable isotope analysis, most notably in ecological research (Barrie et al., 1989; Fry, 2006; Griffiths, 1998; Rundel et al., 1989). It has also made it possible for many more samples to be analyzed in archaeological studies. Instead of selecting a few human bones for analysis, as was true of many studies carried out before 1990, researchers now routinely analyze nonhuman faunal bone and either prehistoric or modern plants to compare potential foods with human stable isotope data. These newer methods have allowed researchers to analyze many more human samples, revealing previously unknown variation within populations, including age differences and individuals who are outliers with respect to their chemical signatures. Stable isotope methods have now been applied to studies of demography, residence patterns, and disease in addition to studies of diet. Although newer instruments simplify analyses, it is imperative to have

trained personnel in reputable laboratories running the instruments to ensure good accuracy and precision.

History of Applications to Analysis of Past Peoples

The realization that stable isotopes of carbon could be used to investigate past diets can be traced to two different but related fields of study (reviewed by van der Merwe, 1982). Scientists working to determine ^{14}C dates on ancient organic remains noted variation in dates derived from some human skeletal remains. Also, maize from archaeological sites gave anomalous dates relative to wood charcoal (Bender, 1968; Hall, 1967). These findings coincided with research on different biochemical pathways of photosynthesis among plants (Smith and Epstein, 1971). Maize fixes carbon by a different pathway and, as a result, contains more ^{13}C relative to ^{12}C than most other plants from temperate regions. Since samples for radiocarbon dating were assumed to have the same stable carbon isotope composition as the standard, those samples containing relatively more ^{13}C, such as maize cobs and kernels, gave erroneous dates. In addition to its use in differentiating human consumption of plants with different photosynthetic pathways, carbon isotopes have been shown to differentiate marine from terrestrial-based diets in humans (Chisholm et al., 1982; Tauber, 1981).

Carbon was the first element for which stable isotope variation was used in archaeology, which follows from archaeologists' familiarity with radiocarbon. Once the potential of studying stable carbon isotopes in preserved protein was understood, interest in other elements such as nitrogen, oxygen and sulfur flourished. Each of these elements and their stable isotopes have been studied extensively in geological and ecological systems as well. In fact, archaeologists are relative latecomers to the study of stable isotope variation of the elements, with the exception of carbon.

The second element to be used in paleodiet research was nitrogen. DeNiro and Epstein (1978, 1981) carried out controlled feeding experiments on several species to study the relationship between the stable carbon and stable nitrogen isotope ratios in diet and in animal tissues. Shortly after that, DeNiro, working with two postdoctoral researchers, explored trophic level and regional variation in nitrogen isotopes (Schoeninger and DeNiro, 1984) and trophic level variation and dietary differences in east Africa (Ambrose and DeNiro, 1987).

Carbon and nitrogen stable isotopes are the isotopes most commonly studied in human remains. More recently, stable oxygen isotopes and strontium isotopes have been studied in bone and in tooth enamel. Stable isotopes of sulphur have been studied in hair and to a lesser degree, bone, since hair keratin contains more sulphur than bone collagen, which contains very little sulphur.

Basic Concepts of Stable Isotope Variation

Isotopes are atoms of the same element with the same number of protons but different numbers of neutrons. Since the atomic mass is determined by the number of protons and neutrons, isotopes of an element vary in their masses. Table 13.1 shows some of the chemical elements that have several isotopes and the abundances of those isotopes. In contrast to unstable (radioactive) isotopes, stable isotopes do not decay over time. For example, ^{14}C in a dead organism decays to ^{14}N, whereas the amounts of ^{12}C and ^{13}C in the same organism will remain constant.

In chemical reactions, such as the conversion of atmospheric CO_2 into glucose by plants, the relative amounts of ^{12}C and ^{13}C differ in plant tissue relative to that of atmospheric CO_2. This variation is due to the fact that isotopes vary in mass and therefore have slightly different chemical and physical properties. Isotopes with higher mass (heavier isotopes) such as ^{13}C usually react slightly more slowly than lighter isotopes such as ^{12}C. Physical phenomena that occur during chemical reactions as a

TABLE 13.1 Average Terrestrial Abundances of Stable Isotopes of Elements Used in Analyses of Ancient Human Tissues

Element	Isotope	Abundance (%)
Hydrogen	^{1}H	99.985
	^{2}H	0.015
Carbon	^{12}C	98.89
	^{13}C	1.11
Nitrogen	^{14}N	99.63
	^{15}N	0.37
Oxygen	^{16}O	99.759
	^{17}O	0.037
	^{18}O	0.204
Sulfur	^{32}S	95.00
	^{33}S	0.76
	^{34}S	4.22
	^{36}S	0.014
Strontium	^{84}Sr	0.56
	^{86}Sr	9.86
	^{87}Sr	7.02
	^{88}Sr	82.56

Extracted from Table 1.1, Ehleringer and Rundel, 1989.

result of the mass differences in isotopes are referred to as "isotope effects." The resulting difference in the isotope ratio of the carbon in the plant tissues as compared with the carbon in atmospheric CO_2 caused by isotope effects is termed "fractionation." Fractionation is the basis for stable isotope variation in biological and geochemical systems, and gaining an understanding of the chemical reactions that result in stable isotope variation allows the biological anthropologist, archaeologist, geochemist, or ecologist to put stable isotope analysis to work to solve a wide range of interesting problems. More detailed discussions of isotope effects and fractionation can be found in textbooks such as that by Hoefs (1997) and Fry (2006), with the latter providing an entire chapter on fractionation.

Tissues Used in Stable Isotope Studies

The first tissue to be used in archaeological stable isotope studies of human paleodiet was the collagen of bone. Methods for isolating collagen had already been developed in radiocarbon dating labs since collagen was used in dating. Information on isolating collagen from bones and teeth is provided by Ambrose (1990), who critically reviews the various methods available.

Bone is composed of an organic matrix of the structural protein, collagen, which is studded with crystals of calcium phosphate, largely in the form of hydroxyapatite. Dry bone is approximately 70% inorganic and 30% organic by weight. Most of the organic portion (85–90%) is collagen. The remainder includes noncollagenous proteins, proteoglycans, and lipids (Triffit, 1980). Because of the intimate structural relationship between collagen and hydroxyapatite, collagen may survive for thousands of years (Tuross et al., 1980), and protein that is probably degraded collagen has even been recovered from dinosaur fossils (Wyckoff, 1980). Collagen contains approximately 35% carbon and 11–16% nitrogen by weight (van Klinken, 1999), and it is the tissue of choice for stable carbon and nitrogen isotope analysis. The detection of postmortem degradation of collagen has been an active area of research (e.g., Child, 1995; Collins et al., 1995, 2002; DeNiro, 1985; Hedges, 2002; Schoeninger et al., 1989; Tuross, 2002).

Because collagen does degrade over time and at varying rates depending on the burial environment, researchers have sought other sources of carbon that are representative of lifetime carbon intake. Another biological source of carbon in bones and teeth is in the form of carbonate (CO_3), which occurs in the mineral portion of bone. Bone mineral is largely composed of hydroxyapatite, $Ca_{10}(PO_4)_6OH_2$. However, several ions can substitute for the constituent ions of the hydroxyapatite crystals (see Burton, this volume). Well known from the trace element literature are the substitutions of Sr^{++} (strontium) or Pb^{++} (lead) for Ca^{++} (calcium). Another common substitution is $CO_3^{=}$ for $PO_4^{\equiv}$ (phosphate) (LeGeros et al., 1967). Sullivan and Krueger (1981) proposed using the carbon in bone mineral for stable carbon isotope studies in fossil bone, when

collagen was too badly degraded to be used. This proposal was challenged by Schoeninger and DeNiro (1982), and a debate in the literature followed (reviewed by Krueger, 1991 and Lee-Thorp and van der Merwe, 1991). The challenge centered on whether bone carbonate was altered in the postmortem environment by exchange between constituents of buried bone and carbonates in sediments such that the carbon isotope ratios would not reflect lifetime carbon deposition. These concerns were addressed by Lee-Thorp (1989), who developed preparation methods to remove the more soluble carbonates, which are those most likely to be diagenetic in origin. More recent debate has centered on the use of carbonate in tooth enamel versus dentin and bone (Koch et al., 1997). There are two compelling reasons to pursue the use of carbonate in biological apatite as a source of carbon isotope ratios. It allows stable isotope studies to be applied to much older materials where it is no longer possible to isolate collagen (e.g., Lee-Thorp et al., 1989; Lee-Thorp and Sponheimer, 2006; Sponheimer and Lee-Thorp, 1999), and carbon from biological apatite records slightly different dietary information than does collagen.

The idea that the carbon in the carbonate of bones and teeth comes from different dietary components than the carbon in collagen was first proposed by Krueger and Sullivan (1984). Ambrose and Norr (1993) and Tieszen and Fagre (1993), in two separate controlled feeding experiments, demonstrated that Krueger and Sullivan were correct in suggesting that collagen carbon comes mainly from ingested protein in the diet, whereas the carbon in biological apatite reflects whole diet. The reason is that collagen is composed of a mixture of essential and nonessential amino acids. The essential amino acids come from ingested protein. The nonessential amino acids may come from ingested protein, or they may be formed from other dietary sources and breakdown products within the body. Carbonate in bone is formed from dissolved bicarbonate in the blood, and this comes from dietary carbohydrate, lipid, and protein. Therefore, the carbon in biological apatite provides a picture of the total diet, whereas collagen is more reflective of dietary protein.

The mineral portion of bone is also the source of oxygen and strontium used in isotope studies. Oxygen isotopes are most often isolated from PO_4 (Luz and Kolodny, 1989; Stuart-Williams and Schwarcz, 1997) and have been used in paleoclimate and, more recently, paleodemographic studies. The oxygen in bone and tooth carbonate has also been analyzed for stable oxygen isotopes (Koch et al., 1997), and although it is a technically less demanding procedure relative to isolating phosphate, carbonate is more likely to be altered by diagenetic processes, more so for bone than for tooth enamel carbonate (see Garvie-Lok et al., 2004; Nielsen-Marsh and Hedges, 2000a, 2000b; for more detailed discussions of diagenesis and bone carbonate). Strontium is a common trace element in bone where it substitutes for calcium. Strontium isotopes have been used in paleodiet and residence studies (e.g., Bentley et al., 2005; Ericson, 1989; Ericson et al., 1989; Ezzo et al., 1997; Price et al., 1994a, 1994b; Sealy et al., 1991, 1995).

Methods for Isolating Specific Components for Stable Isotope Analysis

Specific instructions for isolating various components of bone can be found in the sources cited throughout this section. It is important to understand the chemical principals of each method. A wide range of variation exists in postmortem environments, duration of interment, and therefore the preservation of hard tissues. It is sometimes necessary to vary the methods for poorly preserved bone samples. For example, by diluting the acid solution, the process of dissolving the bone mineral is less harsh and proceeds more slowly so that partially degraded collagen may be recovered. Tropical environments often have poor preservation of bone with extensive collagen degradation; however, teeth may be better preserved, allowing extraction of collagen from dentine. Sometimes

postmortem alteration is so extensive that specific analyses are not possible.

Collagen. Most researchers use one of three methods for isolating collagen from bones and tooth dentine. Sealy (1986) describes a simple method in which small chunks of bone (1–3 g in total) are decalcified in a hydrochloric acid solution (between 1% and 5%, depending on the density of the sample and its outward appearance). An additional soak in sodium hydroxide (0.1 molar) may follow to remove decayed organic matter from the burial environment. The remaining collagen is freeze-dried.

In another method, (Bocherens et al., 1995; Tuross et al., 1988) small chunks of bone are demineralized in EDTA (ethylenediaminetetracetic acid), a sodium salt, which separates collagen from bone mineral. A third method was originally developed by Longin (1971) and later modified by Schoeninger and DeNiro (1984) and by Brown et al. (1988). In this method, powdered bone is demineralized in 8% hydrochloric acid for a short period of time (around 18 minutes). This process is followed by a slow hydrolysis in weakly acidic hot water (pH 3). This method is preferable for poorly preserved bone; however, the risk is that one may obtain other organic matter in addition to collagen. Thus, an additional soak in sodium hydroxide (0.1 molar) to remove decayed organic matter is usually recommended.

Several researchers have compared methods and their yields (Chisholm et al., 1983; Schoeninger et al., 1989). Boutton et al. (1984) and Katzenberg (1989; Katzenberg et al., 1995) have demonstrated that some collagen is lost when demineralized bone is soaked in sodium hydroxide, but that the other material removed in the soak contains humic contaminants (decayed organic matter) that may skew $\delta^{13}C$ values. For example, Katzenberg et al. (1995) demonstrated that the residue removed during the sodium hydroxide soak of prehistoric human bones from southern Ontario had a much lighter $\delta^{13}C$ than the collagen, indicating that the residue contained decayed C_3 plant remains.

It is essential to demonstrate that the material being analyzed is collagen, and various criteria have been used for this purpose. DeNiro (1985) proposed that extracted material with a carbon-to-nitrogen (C/N) ratio in the range of 2.9 to 3.6 should preserve reasonable stable isotope ratios to those from the lifetime of the organism. Since the ratio of carbon to nitrogen in modern (never buried) bone collagen is 3.2, this range was suggested to take into consideration analytical error and some slight alteration over long periods of time. These figures are based on the atomic ratio of carbon to nitrogen in collagen. Modern light isotope mass spectrometers are usually interfaced with gas analyzers that provide data on the content of carbon and nitrogen in samples, and they also calculate carbon-to-nitrogen ratios. However, these are weight ratios and will be slightly lower (by a factor of 1.16667) than the atomic ratios given by DeNiro (1985, and see Ambrose, 1990). Recent publications often include data on percentage carbon and nitrogen in samples as well as the C/N ratios. These data are important for evaluating the validity of stable isotope results. For example, recent samples that contain lipids will have more carbon and erroneous stable carbon isotope ratios since lipid is less enriched in the heavier isotope than is collagen. Samples in which collagen is degraded often have low nitrogen content and may provide results for stable carbon isotopes but not stable nitrogen isotopes, since collagen contains much more carbon than nitrogen (thus the expected 3.2 ratio). In all of these procedures, the objective is to isolate collagen from bone mineral and any organic matter introduced in the postmortem environment.

Compound-Specific Analyses. With advances in the field, researchers have focused their attention on isolating individual amino acids, initially for accelerator radiocarbon dating (Stafford et al., 1991). Stable isotope values vary among the different amino acids, so preferential loss of certain amino acids from diagenesis can alter the overall $\delta^{13}C$ of a protein such as collagen (Hare and Estep, 1982).

Interest also exists in isolating the indispensable (essential) amino acids (those amino acids that must be obtained from the diet) from collagen for stable isotope analysis for dietary reconstruction (Hare et al., 1991). Since these amino acids come from dietary protein and are incorporated into human proteins such as collagen, they provide a more direct tracer than bulk collagen, which is made up of both dispensable amino acids (those that may be synthesized by the organism) and indispensable amino acids. Methods used for such study are much more complex than those for simply isolating collagen and are described by Stafford et al. (1991) and Hare et al. (1991). There is considerable promise in pursuing these methods, particularly with the development of GC/C/IRMS (gas chromatography/combustion/isotope ratio mass spectrometry) (Lichtfouse, 2000; Macko et al., 1997). This method allows the researcher to isolate specific organic compounds and then to introduce them into the mass spectrometer. Evershed pioneered this research in paleodiet studies by focusing on lipids and demonstrating the presence of specific substances in residues from prehistoric ceramic vessels (Evershed, 1993; Stott and Evershed, 1996), including dairy fats (Copley et al., 2005a, 2005b). Corr et al. (2005) have demonstrated that compound-specific stable carbon isotope analysis of collagen amino acids allows one to differentiate diets with marine protein and C_4 plants in arid regions where $\delta^{15}N$ values are unpredictable. Students interested in graduate study in this area are well advised to become familiar with the principles and methods of organic chemistry and biochemistry.

Biological Apatite. A method for isolating the carbonate fraction of bone mineral was developed by Lee-Thorp (1989; Lee-Thorp and van der Merwe, 1991; Lee-Thorp et al., 1989). Ground bone is soaked in sodium hypochlorite to remove organic material. Carbonate adsorbed from the burial environment is removed with one molar acetic acid. Samples are then reacted with phosphoric acid to release the structural carbonate. CO_2 is collected by cryogenic distillation. Specific steps are described in the references cited above and by Tieszen and Fagre (1993) and Ambrose and Norr (1993).

More recently, carbon in the carbonate fraction of tooth enamel has been analyzed using laser ablation stable isotope analysis (Sponheimer et al., 2006). The use of the laser allows sampling of such small quantities that sampling is nearly imperceptible for purposes of long-term curation, and seasonal variation can be explored, as was done by Sponheimer et al. (2006) on teeth from *Paranthropus robustus*.

Oxygen isotope measurements in bone usually make use of the oxygen in phosphate, which is less affected by diagenetic processes than carbonate. Stuart-Williams (1996; Stuart-Williams and Schwarcz, 1995, 1997) developed a method of isolating organic phosphate from bone that is simpler and safer than previous procedures. Bryant et al. (1996) compared the oxygen isotope results from phosphate and carbonate of tooth enamel and demonstrated that it is possible to estimate one from the other.

Hydroxyapatite. Strontium substitutes for calcium in the hydroxyapatite crystals of bone mineral. Two different methods of isolating bone mineral for analysis of strontium isotopes have been described in the recent literature. The method used by Sealy et al. (1995) and by Sillen et al. (1998) makes use of Sillen's 1986 solubility profile method. Bone or tooth powder is washed in acetic acid and sodium acetate buffer solution repeatedly, saving each wash. The various washes are analyzed by ICP (inductively coupled plasma emission spectrometry). The first few washes presumably contain recently deposited contaminants from the burial environment and show variation in trace element concentrations. Later washes tend to show less variation in the concentration of strontium and other trace elements. It is these later washes, which are thought to contain the biologically deposited strontium, that are used for strontium isotope analysis by mass spectrometry.

A second method of preparation for strontium isotope analysis, described by Price and colleagues (1994a, 1994b) begins with

mechanical cleaning of the outer surface of bone, followed by an overnight soak in one normal acetic acid. The acid removes soluble carbonates and the portion of bone most likely to contain elements from the burial environment. The residue is then wet-ashed in nitric acid in preparation for mass spectrometry. Both methods attempt to isolate bone mineral that has not been diagenetically altered, and although Sillen's method is more conservative, it is also more labor intensive. Sillen and Sealy (1995) have demonstrated that the solubility profile method does not result in any recrystallization of bone mineral, unlike methods that employ dry ashing (heating to high temperature to destroy the organic component).

Lipids. Methods for treating lipids from bone samples depend on whether one intends to remove the lipids so that only protein or carbonate is analyzed, or whether one wants to determine the $\delta^{13}C$ of lipid. Lipids are less enriched in the heavier isotope (i.e., $\delta^{13}C$ is more negative) than bone collagen. Therefore it is necessary to remove lipids from bone samples before analysis. This process is particularly important when analyzing bones of recent origin for comparative purposes. Liden et al. (1995) discuss methods of removing lipids. The most commonly used method involves soaking the bone in a mixture of chloroform and methanol (Bligh and Dyer, 1959; Folch et al., 1957) after demineralization. The residue must be rinsed carefully since these are organic solvents, which may contaminate the sample. Bone may also be soaked in diethyl ether before demineralization. The normal sodium hydroxide soak then follows demineralization (Ambrose and Norr, 1993). These methods will effectively eliminate lipids from collagen preparations. Bligh and Dyer (1959) describe a method for extracting and purifying lipid from biological materials. Recent work by Evershed and colleagues (Evershed, 1993; Stott and Evershed, 1996) on characterization of lipid extracts from ancient materials, such as ceramic vessels, has added another source of evidence for paleodiet studies by allowing $\delta^{13}C$ determinations of lipids and, more recently, of specific compounds within lipids (see the previous section on compound-specific analyses).

Mass Spectrometry

Stable isotope abundance ratios are measured in isotope ratio mass spectrometers (IRMS), which should not be confused with organic mass spectrometers that are used to characterize complex organic molecules. Isotope ratio mass spectrometers have four components: an inlet system, an ion source, a mass analyzer, and a series of ion detectors (Fig. 13.1). For most elements of interest (H, O, N, C), the sample is introduced to the mass spectrometer as a gas (H_2, CO_2, N_2, and CO_2, respectively). Until recently, most stable isotope work performed with collagen or carbonate required combustion of the sample in sealed tubes. After combustion, the resultant CO_2 and H_2O were separated offline before the CO_2 was let into the mass spectrometer. Modern instruments now interface combustion furnaces and gas analyzers with mass spectrometers to simplify and ease the conversion of the sample into the requisite gaseous form. In such a setup, collagen is weighed into tin sample holders, which are then placed into an automated sample tray. The revolving tray drops samples into the furnace where N_2, CO_2, and H_2O are produced. These gasses, carried by helium carrier gas, are separated before being swept into the mass spectrometer. (For a detailed presentation of continuous-flow stable isotope analysis, see Barrie et al., 1989 and Barrie and Prosser, 1996.)

Once in the mass spectrometer, the gas of interest is let into the second component of the mass spectrometer, the ion source. In the ion source, some gas molecules are ionized by electron bombardment, which allows them to be controlled and focused into a beam. The ion beam is then directed, via a flight tube, into the mass analyzer zone of the mass spectrometer. As the name suggests, the mass analyzer separates the ion beam into several smaller beams by passing it between the poles of a magnet. This process is directly analogous to the separation

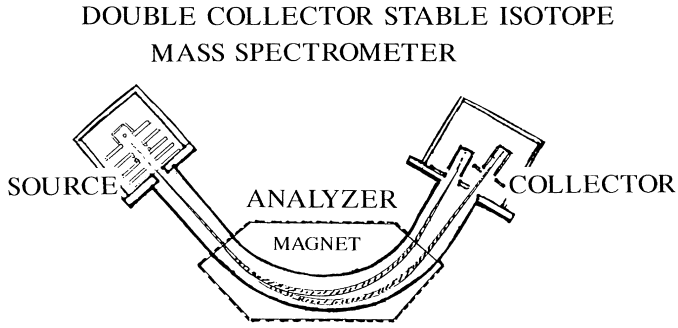

Figure 13.1 Diagram showing three of the four components of the mass spectrometer: Gas is introduced into the source where molecules are ionized and then accelerated; the resulting ion beam is directed into the mass analyzer where ions of different masses are separated; ion collectors measure intensities of the separated ion beams. (Courtesy of H.R. Krouse.)

of white light into its constituent wavelengths through a prism. The separation of one ion beam into several beams according to mass results in the desired "mass spectrum." The beam intensities of the respective ion beams can then be measured in the ion collector section of the instrument. The relative intensities of the individual isotope ion beams are then reported as isotope ratios, for example, $^{13}CO_2$:$^{12}CO_2$. To report a meaningful value, the mass spectrometer alternately analyzes aliquots of the unknown sample and a known standard gas, thereby providing a ratio of the stable isotopes in the sample relative to that same ratio in the standard.

Because the element of interest cannot always be converted into an easily handled gas, it is sometimes necessary to introduce the sample in solid form. For strontium isotope analysis, the sample is deposited directly on a filament near the ion source where it is heated to evaporation and ionized under vacuum. This process is referred to as thermal ionization mass spectrometry (TIMS) and requires a different ion source (solid source) for analysis. Many labs will have separate instruments for analyzing gasses and solids. More recently, laser ablation ICP-MS (inductively coupled plasma–mass spectrometery) has been used for pinpoint sampling to analyze isotopes of heavier elements such as strontium (Latkoczy et al., 2001; Prohaska et al., 2002).

For compound-specific analyses, a gas chromatograph (GC) can be interfaced with an IRMS resulting in GC-C-IRMS (gas chromatography combustion isotope ratio mass spectrometry). This configuration allows one to determine the isotope ratio of a specific compound, such as cholesterol, that has been isolated using gas chromatography (Stott et al., 1999; Tripp and Hedges, 2004).

It is helpful to know enough about mass spectrometry to be able to discuss one's needs with various laboratory personnel at the beginning of a research project. It is important to be able to understand problems that may develop when the data have been collected. Considerations include the general composition of the sample and the range of the expected results. For example, departures from the expected range of carbon to nitrogen in bones and teeth may signal poor preservation or contamination. It is necessary to have some idea of the range of isotope compositions expected since the standards used should bracket the expected values.

Mass spectrometers are analytical instruments that have many uses in chemistry, biochemistry, geochemistry, and ecology. Instruments vary in their setup depending on the needs of the researcher. A laboratory may be set up to perform analyses on certain types of samples and may specialize in certain elements. Laboratories carrying out ecological research are most likely able to accommodate most analyses of interest to the archaeologist and physical anthropologist interested in light isotopes (hydrogen, carbon, nitrogen, oxygen,

and sulphur), whereas geochemists are more likely to have TIMS and laser ablation setups. Organic chemists and biochemists may have GC-C-IRMS capabilities, and enterprising biological anthropologists and archaeologists may have their own instruments. Given the high cost of mass spectrometers and the need for full-time technical support, most institutions have shared facilities that serve the needs of multiple users.

Standards, Precision, and Accuracy

Stable isotope abundance ratios are determined relative to the ratios of those same isotopes in standard materials. The mass spectrometer compares the stable isotope abundance ratio in the sample with the stable isotope ratio in a standard. Thus, the reported value uses the following notation:

$$\delta \text{ in } ‰ = \frac{R_{(\text{sample})} - R_{(\text{standard})}}{R_{(\text{standard})}} \times 1000$$

where R = the ratio of the number of heavier to lighter isotopes, so that for carbon isotopes, the equation is

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

The ‰ (permil sign) means "per thousand" since the ratio is multiplied times 1000.[1] This calculation is done to amplify the difference between the ratio of the stable isotopes in the sample and the ratio of stable isotopes in the standard, which is usually a very small number.

[1]The term "permil," meaning per thousand, is similar to the term "percent," meaning per hundred. In the isotope literature, using the delta notation, values are reported as some number permil (‰). In the trace element literature, elements may be measured in parts per million (ppm). This measure is very different in that it is not a ratio and it refers to a much smaller proportion (out of one million). It is best to avoid using the phrase "parts permil," which is sometimes heard from anthropologists. Although not incorrect, is it awkward and incongruous, since people do not say parts per cent. Standard terminology is "permil."

International standards are available through the National Bureau of Standards (NBS) and the International Atomic Energy Agency (IAEA), Vienna. The circulation of these standards among laboratories allows comparison of results from different researchers working in different laboratories. However, because of the high cost, individual laboratories normally also have internal standards. These standards are substances whose isotopic ratio is well characterized relative to an international standard and that are run routinely with batches of unknowns to check for consistency in the instrument. Internal and reference standards are reported relative to a primary reference standard for a particular element, which by definition has a δ value of 0. Absolute isotope abundances of some primary reference standards are available in textbooks such as that of Hoefs (1997) and Fry (2006). Primary and other reference standards for elements discussed in this chapter are listed in Table 13.2.

The sensitivity of mass spectrometers varies, and it is important to know the precision of the instrument used before making interpretations from the data. Most light isotope mass spectrometers can measure $\delta^{13}C$ values with a precision of $\pm 0.1‰$ and $\delta^{15}N$ values with a precision of $\pm 0.2‰$. Newer models have improved sensitivity, although some newer continuous flow systems that simultaneously measure more than one element in a sample sacrifice precision for speed and economy of sample size. Precision should be determined for individual instruments using multiple analyses of samples with similar composition to those of interest. Data should be reported in a manner that is consistent with the precision of the instrument. If the precision is $\pm 0.2‰$, then it is incorrect to report results to 0.01‰.

APPLICATION OF STABLE ISOTOPE ANALYSIS TO SELECTED PROBLEMS IN SKELETAL BIOLOGY

During the 1990s, with the growing use of stable isotopes to address problems in biological

TABLE 13.2 Primary (International) and Reference Standards for Selected Elements

Element	Primary Standard	Other Reference Standards
Hydrogen	Vienna Standard Mean Ocean Water (VSMOW)	V-GISP, V-SLAP, NBS-30
Oxygen	Standard Mean Ocean Water (VSMOW)	NBS 19, 20, 18, 28, 30, V-GISP, V-SLAP
Carbon	PeeDee Belemnite (VPDB)	NBS 18, 19, 20, 21
Nitrogen	Atmospheric Nitrogen (Air)	
Sulfur	Canyon Diablo meteorite troilite (VCDT)	

Adapted From Hoefs, 1997 and Coplen, 1994.

anthropology and archaeology, several review articles were published. Katzenberg and Harrison (1997) discuss developments and review the literature since 1989. Other reviews, which also cover basic concepts in stable isotope studies, include Schwarcz and Schoeninger (1991), Schoeninger and Moore (1992), and Pate (1994). A series of conferences entitled "Advanced Seminars in Paleodiet" has resulted in several publications (edited books as well as guest-edited issues of journals) of papers presented at those conferences (Price, 1989; Sillen and Armelagos, 1991; Lambert and Grupe, 1993; Bocherens et al., 1999; Ambrose and Katzenberg, 2001; Koch and Burton, 2003;). A symposium in honor of the work of Dr. Harold W. Krueger, held at the 2001 meetings of the Society for American Archaeology, was also published as a special issue of a journal (Ambrose and Krigbaum, 2003). Collectively, these volumes provide a detailed look at the history and developments in the use of stable isotopes for studying archaeological human remains. For a similar review of developments in research on diagenesis, a series of conferences have been held on bone diagenesis, and these are also published as special issues of journals (Schwarcz et al., 1989; Hedges and van Klinken, 1995; Bocherens and Denys, 1997; Fernandez-Jalvo et al., 2002).

Initially, most applications of stable isotope analysis to human remains were concerned with reconstructing diet. Subsequently other research questions have been addressed with stable isotope methods. These questions include determining the duration of breastfeeding, effects of disease processes, and determination of residence and migration patterns. The following sections highlight some of these applications in addition to more traditional approaches to paleodiet studies.

Paleodiet

C_3 and C_4 plants. Maize is one of several tropical grasses that fixes carbon by a different photosynthetic pathway (referred to as the Hatch–Slack or C_4 pathway) than most plants found in temperate regions. C_4 plants, which also include sorghum, millet, and sugar cane, adapt to heat and aridity by minimizing the amount of time that the leaf pores (stomata) are open, thereby minimizing water loss. These plants discriminate less against the heavier isotope, ^{13}C, than do temperate plant species, which use the C_3 (Calvin) photosynthetic pathway. Atmospheric CO_2 has a $\delta^{13}C$ value of $-8‰$ today, but before the widespread burning of fossil fuels, the value was around $-7‰$.[2] C_4 plants range from -9 to $-14‰$, whereas C_3 plants range from -20 to $-35‰$ (Deines, 1980). The non-overlapping ranges of C_3 and C_4 plants provide the basis for using stable isotopes of carbon in preserved human tissue for revealing diet.

Several studies have been carried out to determine the difference between the $\delta^{13}C$ of the diet and the $\delta^{13}C$ value of various body

[2]Fossil fuels are depleted in ^{13}C, and their use has resulted in a decrease in atmospheric $\delta^{13}C$ of approximately 1‰. The value of $-7‰$ is used in archaeological research to reflect the conditions present during the lifetime of the individuals being studied.

tissues (Ambrose and Norr, 1993; Lyon and Baxter, 1978; Tieszen and Fagre, 1993; Vogel, 1978). Bone collagen $\delta^{13}C$ is approximately 5‰ greater than $\delta^{13}C$ of the diet, which is the basis for the expression, "you are what you eat +5‰." Interestingly, this number was first suggested by van der Merwe and Vogel (1978) based on measurements of free-ranging large mammals and their diets and then was confirmed recently by Ambrose and Norr (1993). In controlled feeding studies of rats, the diet to collagen spacing was only 5‰ when the dietary protein, carbohydrate, and fats were all from similar sources (C_3 or C_4). In situations where the protein source differed from that of the carbohydrates and fats, the spacing value varied (Ambrose and Butler, 1997). Such a situation in humans might occur if people were consuming the meat of C_3 browsers such as deer and C_4 plants such as maize. In the controlled feeding experiments on rats, such a situation results in a diet to collagen spacing of less than 5‰. Thus, although the spacing factor of 5‰ may be used as a guide, it should not be taken as an absolute value.

After the demonstration that stable carbon isotopes in bone collagen could be used to document the consumption of C_4 plants such as maize against a background of C_3 plants (Vogel and van der Merwe, 1977; van der Merwe and Vogel, 1978), several other researchers applied these same principles to other regions where maize was the major introduced cultigen (e.g., Buikstra and Milner, 1991; Katzenberg et al., 1995; Larsen et al; 1992; Schurr and Redmond, 1991; Schwarcz et al., 1985). The method works very nicely in eastern North America where maize is the predominant and, in some places, the only C_4 plant consumed in any quantity. Follow-up studies took into consideration the fact that if the animals exploited by human groups consumed C_4 plants, then their tissues would be enriched in the heavier isotope and this would show up in human bone collagen carbon (Katzenberg, 1989). Subsequent work on the differential routing of protein and nonprotein nutrients to the synthesis of collagen reinforces the importance of understanding the diets of the animals consumed by people (Ambrose and Norr, 1993; Tieszen and Fagre, 1993).

Several regions of archaeological interest exist where stable isotope analysis was not attempted because it did not seem that such analysis could provide any additional information. If all potential food sources have similar stable isotope ratios, then little can be learned, or if the central research question focuses on a C_4 plant, such as maize, but there are other C_4 plants in the region, it will not be possible to distinguish when maize was first introduced. The application of stable isotope methods came somewhat later to the American Southwest, where maize made an early appearance, but there are also several indigenous C_4 and CAM plants (a third photosynthetic pathway with values intermediate to C_3 and C_4 plants) in addition to human exploitation of animals that consume C_4 plants. Nevertheless, researchers did tackle this more complex region (Benson et al., 2006; Coltrain et al., 2006; Decker and Tieszen, 1989; Katzenberg and Kelley, 1991; Matson and Chisholm, 1991; Spielmann et al., 1990) with useful results in terms of documenting the intensity of maize use.

Many recent studies include stable carbon isotope data from both bone collagen and bone carbonate or, in cases where collagen is not preserved, just bone carbonate. Since bone carbonate is thought to better reflect the whole diet (Ambrose and Norr, 1993; Tieszen and Fagre, 1993) and collagen preferentially reflects dietary protein, analyses of both components provide additional information. For example, Harrison and Katzenberg (2003) used stable carbon isotope data from bone apatite to detect small amounts of maize in the diet that do not show up in collagen, when it was first introduced into southern Ontario. Ambrose et al. (2003) could better differentiate the diets of high and low status burials from Cahokia (in Illinois) using stable carbon isotope ratios from both collagen and carbonate and stable nitrogen isotope ratios from collagen.

Marine Versus Terrestrial Based Diets. Stable carbon isotopes are also useful in studies of people inhabiting or exploiting coastal areas where it is possible to test hypotheses about the relative importance of marine and terrestrial foods in the diet (Blake et al., 1992; Chisholm et al., 1982; Hayden et al., 1987; Keegan and DeNiro, 1988; Lubell et al., 1994; Norr, 1991; Walker and DeNiro, 1986; Tauber, 1981). Such studies now span the globe and include research in the Arctic (Coltrain et al., 2004), Tierra del Fuego (Yesner et al., 2003), and the Pacific (Ambrose and Butler, 1997; Pate et al., 2001). The main source of carbon for marine organisms is dissolved carbonate, which has a $\delta^{13}C$ value of 0‰, whereas the main source of carbon for terrestrial organisms is atmospheric CO_2, which had a $\delta^{13}C$ value of -7‰ in pre-industrial times. Tauber (1981) and Chisholm and colleagues (1982, 1983) demonstrated that this 7‰ difference is reflected in mammals, including humans, feeding from these two different ecosystems. Potential dietary change in northern Europe from the Mesolithic Period to the Neolithic Period has been investigated using stable carbon and nitrogen isotopes. Richards et al. (2003) have suggested that use of marine foods stopped when domesticated plants and animals were introduced into Britain, based on $\delta^{13}C$ in bone collagen. Others working in northern Europe have argued that such an abrupt transition did not occur and that the exploitation of marine foods continued into the Neolithic Period (Liden et al., 2004; Milner et al., 2004). This debate provides a good illustration of both the promises and the limitations of stable isotope analysis (Hedges, 2004), and it emphasizes the need to use the results of stable isotope data in the larger context of other archaeological evidence (Milner et al., 2004).

Nitrogen Isotopes and Diet

Trophic Level Distinctions. At the same time that DeNiro and Epstein (1981) demonstrated that carbon isotope ratios of diet are reflected in the tissues of an animal, they carried out a study of the relationship of diet and tissues for stable isotopes of nitrogen. Nitrogen isotopes vary depending on trophic level. Atmospheric nitrogen (N_2) is the primary standard, and its value is set at 0‰. Some plants (legumes) have a symbiotic relationship with bacteria of the genus *Rhizobium*. The bacteria live in the roots and can fix nitrogen (combine it with other elements such as hydrogen or oxygen), thereby making it available to the plant (Brill, 1977). Other plants must get their nitrogen from decomposed organic matter, which breaks down to compounds such as ammonia (NH_3) or nitrate (NO_3). Legumes have $\delta^{15}N$ values closer to that of atmospheric nitrogen, whereas nonleguminous plants are more enriched in ^{15}N and therefore have higher $\delta^{15}N$ values. Herbivore $\delta^{15}N$ values are approximately 3‰ higher than the $\delta^{15}N$ of their diet; thus, herbivores consuming legumes will have lower $\delta^{15}N$ values than those consuming nonleguminous plants. Carnivore tissues are again enriched in the heavier isotope resulting in $\delta^{15}N$ values approximately 3‰ higher than their diet. This principal of enrichment through successively higher trophic levels, which was first pointed out by Minagawa and Wada (1984) and Schoeninger and DeNiro (1984), provides the basis for using stable nitrogen isotopes to infer trophic level. Variation occurs in the magnitude of the trophic-level effect in stable nitrogen isotopes both among different tissues within the same organism and among different taxa (Vanderklift and Ponsard, 2003). It is important to understand this variation when working with tissues other than bone collagen and with fauna other than mammals.

Ideally, a range of animals and plants from the environment under study is analyzed and humans are viewed relative to the other organisms in their environment. An example is provided in Fig. 13.2, which shows carbon and nitrogen stable isotope ratios for humans from several prehistoric sites in New Mexico relative to other mammals from the region (from Katzenberg and Kelley, 1991). Humans have

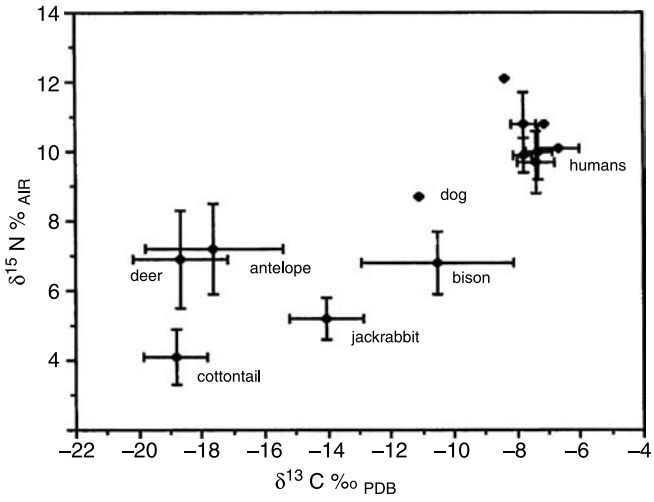

Figure 13.2 $\delta^{13}C$ and $\delta^{15}N$ values (mean $\pm$ one standard deviation) for collagen from human and faunal bone samples from sites in the Sierra Blanca region of New Mexico, dating from A.D. 800 to A.D. 1400. (Reproduced from Katzenberg and Kelley (1991) with permission of the publisher.)

the highest $\delta^{15}N$ and $\delta^{13}C$ values. In this region, humans consumed maize as well as animals that fed on C_4 plants. The figure indicates that both jackrabbits and bison consumed some C_4 plants. Humans are approximately 3‰ higher than deer, antelope, and bison for $\delta^{15}N$. The elevated $\delta^{15}N$ for deer and antelope relative to cottontail may reflect consumption of some legumes by cottontail, but it may also be related to habitat. Cottontail and deer prefer forested, moister habitats, whereas antelope and jackrabbit are adapted to open range habitat. Heaton et al. (1986) and Ambrose (1991) have demonstrated that $\delta^{15}N$ is sensitive to climate and is elevated in arid regions. For this reason, Ambrose (1991) suggests that species from different ecosystems cannot be compared directly without considering the isotopic composition of the local food web. This caution has been verified in subsequent studies.

Freshwater Resources

Initially, it was assumed by archaeologists that freshwater fish had $\delta^{13}C$ values similar to those of terrestrial C_3-consuming organisms. Little was known about stable nitrogen isotope ratios in freshwater systems. In 1989, Katzenberg explored this question by analyzing bones of freshwater fish from archaeological sites around the Great Lakes. It was discovered that freshwater fish exhibit a trophic level effect, which results in higher $\delta^{15}N$ and slightly increased $\delta^{13}C$ in carnivorous fish. Thus, it is possible to estimate reliance on fish in regions like the Great Lakes where they are an abundant resource. This reliance is particularly significant since fish are frequently underrepresented in the zooarchaeological record becasue of cultural practices and, in earlier excavations, because of methods of recovery. Cultural practices, such as filleting and drying fish where they are caught and then transporting the dried fillets back to the village, will result in underrepresentation of fish bones relative to their dietary importance. Excavations carried out without fine screening will miss bones of fish as well as other small skeletal elements.

Freshwater fish also exhibit more variation in $\delta^{13}C$ than had been assumed. Several studies of freshwater ecosystems by ecologists have provided the background to understanding the sources of this variation (France, 1995; Hecky and Hesslein, 1995; Kiyashko et al., 1991;

Zohary et al., 1994). Freshwater plants have numerous sources of carbon unlike terrestrial plants whose source is atmospheric CO_2. In freshwater ecosystems, carbon comes from atmospheric CO_2, CO_2 in the water, bicarbonate and carbonate from rocks and soils, and organic carbon as waste and decomposition products from plants and animals living in the water (Zohary et al., 1994). The result is that fish living in different habitats within freshwater lakes display widely varying $\delta^{13}C$. This is illustrated in Fig. 13.3, which shows a reconstruction of the stable isotope ecology of the region around Lake Baikal, Siberia (from Katzenberg and Weber, 1999). The $\delta^{13}C$ of fish bones ranges from -14.2 to $-24.6‰$ with higher $\delta^{13}C$ in species inhabiting the shallow waters and lower $\delta^{13}C$ for fish inhabiting the deeper, open waters of the lake (Katzenberg and Weber, 1999). The higher $\delta^{13}C$ for some fish explains the observed variation in human bone collagen $\delta^{13}C$ from the region. This finding is important since there are no C_4 plants in the area and yet some human samples analyzed showed evidence of food that was enriched in the heavier isotope. In other words, stable isotope evidence from humans and their presumed food sources can indicate when something has been missed.

Figure 13.3 also illustrates the trophic-level effect in $\delta^{15}N$ in both terrestrial and freshwater organisms. Large terrestrial herbivores have $\delta^{15}N$ around 4‰ to 5‰. Humans from a large number of sites in the region range from 10.1‰ to 14.4‰. Fish and the freshwater seals of Lake Baikal vary according to their trophic position. Carp (*Caras a.*) are bottom-feeders and have the lightest $\delta^{15}N$. Lenok (*Branchimystax l.*) are highest in the littoral food web and have higher $\delta^{15}N$. Seals occupy the highest position in the freshwater food web with $\delta^{15}N$ around 14‰.

Nitrogen Isotopes and Water Stress

Environmental variation in $\delta^{15}N$ of plants has been demonstrated in coastal versus inland regions and in arid versus wetter regions

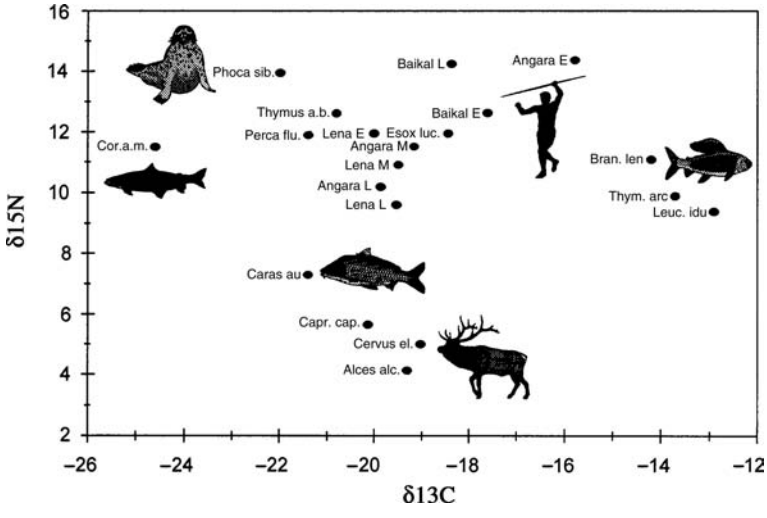

Figure 13.3 $\delta^{13}C$ and $\delta^{15}N$ values (means) for collagen from human and faunal bone samples from sites from the western shore of Lake Baikal, the Angara River Valley, and the Upper Lena River Valley, Siberia, dating from the Early Neolithic (5800–4900 B.C.) to the Early Bronze Age (3400–1700 B.C.). Fish bones are from modern specimens. Mean values for human bones are plotted by site and location (e.g., Baikal E refers to Early Neolithic Lake Baikal site; Lena M refers to Middle Neolithic Lena Valley site, and Angara L refers to Early Bronze Age Angara Valley site). (Reprinted from Katzenberg and Weber (1999) with permission.)

(Heaton, 1987; Shearer et al., 1983; Virginia and Delwiche, 1982). Heaton et al. (1986) and Sealy et al. (1987) have also shown that $\delta^{15}N$ varies in animals of the same species from arid versus wetter regions. Sealy et al. (1987) point out the importance of recognizing reasons for elevated $\delta^{15}N$ in arid regions when those regions are also in close proximity to coastal resources, as is the case on the southern cape of Africa. Incorrect dietary interpretations for human samples can result if higher $\delta^{15}N$ is attributed to the use of marine resources without recognizing that the same $\delta^{15}N$ might result from consumption of water-stressed terrestrial animals. Ambrose (1991) has explored the physiologic basis for $\delta^{15}N$ variation in mammals living in arid regions. He and DeNiro (1986) proposed a model based on varying nitrogen loss in urea, which is excreted in urine. Urea is depleted in ^{15}N relative to diet. Under conditions of water stress, more urea is excreted relative to the total volume of urine, and therefore, more of the lighter isotope, ^{14}N, is lost. Therefore, more ^{15}N is retained in the body where it is available for tissue synthesis. The result is that tissue $\delta^{15}N$ will increase under prolonged water stress conditions.

Nitrogen Isotopes and Protein Stress

Another cause of elevated $\delta^{15}N$, relative to expectations from diet, is protein stress. This stress is related to the model above in that insufficient protein intake results in the breakdown and reutilization of existing tissues in the body that are already enriched in ^{15}N because of preferential excretion of ^{14}N. Hobson and colleagues, in two different studies on birds (Hobson and Clark, 1992; Hobson et al., 1993), have found that under conditions of nutritional stress, new protein is synthesized from the products of catabolism of existing protein. Katzenberg and Lovell (1999) have presented evidence that suggests this may be detected in humans. They compared $\delta^{13}C$ and $\delta^{15}N$ in normal versus pathological segments of bones from individuals of known medical history. One individual, who died of AIDS and experienced some new bone deposition because of osteomyelitis, showed elevated $\delta^{15}N$ in the diseased segment relative to the two unaffected segments of bone. This case suggests that the recently deposited bone collagen was synthesized, at least in part, from amino acids liberated from the catabolism of existing proteins in the body. White and Armelagos (1997) reported elevated $\delta^{15}N$ in bones from individuals with osteoporosis from an arid region of Sudan. They discuss water stress as a possible factor to explain the nitrogen isotope data, but it is possible that protein stress was also a factor. The use of stable isotopes to address questions in paleopathology is a relatively new area of inquiry that promises to add to our understanding of disease processes as well as to our understanding of stable isotope variation in metabolism.

Infant Feeding and Weaning Studies

One of the big questions that carries through many approaches to studying past peoples from their skeletal and cultural remains is the timing and rate of population growth. Buikstra et al. (1986) presented an interesting hypothesis that draws together the adoption of agriculture, the development of thin-walled ceramic vessels, and therefore the ability to prepare cereal pap as a weaning food. The result would be earlier weaning (defined here as the introduction of non-milk foods rather than the complete cessation of breastfeeding) and thus decreased inter-birth intervals, increased population growth, and larger population size. This result is based on the fact that nursing suppresses ovulation so that supplementing mother's milk with cereal gruel will result in a shorter period of infertility after childbirth. The hypothesis that weaning occurs sooner in agricultural societies was tested by Fogel and colleagues (1989) in a study that included stable nitrogen isotopes from fingernails of nursing mothers and their infants. They found a trophic-level increase in $\delta^{15}N$ (+2.4‰ on

average) of infant protein beginning shortly after birth (around three months) and decreasing when supplemental foods were introduced. By three to five months after nursing ceased, fingernail $\delta^{15}N$ for mothers and babies was the same. Fogel and colleagues (1989, 1997) then analyzed bone collagen from preagricultural and agricultural sites to see whether the latter children were weaned at an earlier age. In both groups, $\delta^{15}N$ decreased at 18–20 months of age. This study demonstrated that the trophic-level effect of increased $\delta^{15}N$ is reflected in protein, including bone collagen, and that the potential exists to apply this method to skeletal samples. In a more recent study, Schurr and Powell (2005) analyzed stable carbon and nitrogen isotopes from individuals from four archaeological sites in eastern North America, two preagricultural and two agricultural, and found no difference in the duration of nursing among the four samples.

Others have applied this method and have attempted to refine estimates of the duration of nursing in the past (reviewed by Katzenberg et al., 1996; Schurr, 1998). For example, Herring et al. (1998) studied the process of weaning (introduction of non-breast milk foods and the gradual cessation of breastfeeding) in a nineteenth-century skeletal sample from Ontario by using stable nitrogen isotopes, parish records, census data, and skeletal evidence. The sample is from an Anglican Church cemetery (see Saunders, this volume, for additional background information on the cemetery). Many skeletons of infants and young children were included in the sample, and parish records as well as the regional censuses could be consulted to obtain multiple sources of mortality data. Careful age determination of the individuals allowed the construction of a mortality profile, which could be compared with the parish records. Since mortality increases as infants are weaned, becasue of exposure to infectious agents and loss of passive immunity, it was possible to compare mortality with the nitrogen isotope evidence for weaning. The combined data show that non-breast milk foods were introduced around the age of 5 months, as indicated by increasing mortality, and that milk continued to be the major source of protein until around 14 months, as indicated by decreasing $\delta^{15}N$.

Fuller et al. (2006) carried out a longitudinal study of $\delta^{13}C$ and $\delta^{15}N$ in fingernails and hair of eight modern mother–infant pairs. Five infants were exclusively breastfed, whereas two were breastfed initially and then formula-fed and one was fed only formula. The results of this study provide confirmation for the use of stable nitrogen isotopes to document the duration of nursing and show the smaller trophic-level effect in stable carbon isotopes. Such carefully controlled studies are very important for providing a more precise understanding of the information conveyed through stable isotope analysis.

A potential problem with using stable isotopes of nitrogen to study past breastfeeding patterns is that the subjects under study died in infancy and early childhood of unknown causes. Since $\delta^{15}N$ may be increased in situations of nutritional stress, it may not be possible to separate the trophic effect of breastfeeding from cause of death in some cases. This possibility has been examined, but it is not yet clear that it is a serious problem (Katzenberg, 1999).

One way to avoid the problem of analyzing bones from individuals who died in infancy or early childhood is to analyze tissues that were formed early in postnatal development that can be isolated from older children and adults. The permanent tooth crowns are formed between the ages of three months (incisors) and seven years (second premolars and second molars) (Hillson, 1996). The third molar crown forms later but is highly variable. Analysis of nitrogen isotopes in dentine collagen and oxygen and carbon isotopes in enamel apatite can provide information about nursing and weaning for individuals who survived into at least late childhood. This approach has been followed by Wright and Schwarcz (1998). They analyzed stable isotopes of carbon and oxygen from the carbonate in tooth enamel. Oxygen isotope

measurements are normally performed on the phosphate of bones and teeth since phosphate is less likely to undergo diagenetic change than is carbonate. Wright and Schwarcz (1998) point out that the procedure for isolating phosphate is more complex than that for isolating carbonate, and that tooth enamel is less likely to be affected by diagenetic processes than is bone mineral. The principle of using stable isotopes of carbon and oxygen is as follows. Carbon isotopes reflect the introduction of weaning foods, which, in the Americas, are usually maize-based gruel. Therefore, $\delta^{13}C$ is expected to increase as this C_4-based weaning food is introduced into the diet. Oxygen isotopes reflect water source. Body water has a higher $\delta^{18}O$ than ingested water because more ^{16}O relative to ^{18}O is lost in expired water vapor. Since breast milk incorporates body water, it is enriched in the heavier isotope in comparison to other water sources for the infant. Therefore as the infant is weaned, $\delta^{18}O$ should decrease and breastfed infants should have higher $\delta^{18}O$ than infants who are not breastfed. Wright and Schwarcz (1998) compared $\delta^{13}C$ and $\delta^{18}O$ in enamel carbonate from tooth crowns formed at different ages and showed that, as expected, $\delta^{13}C$ increases with age, whereas $\delta^{18}O$ decreases with age.

RESIDENCE AND MIGRATION STUDIES

From the preceding information, it is obvious that some principles regarding stable isotope variation may be used to indicate place of residence. Stable carbon isotope abundance ratios vary based on diet, whereas stable nitrogen isotope abundance ratios vary based on diet and habitat. Oxygen isotope abundance ratios vary based on climate and water source. In general, $\delta^{18}O$ decreases with increasing latitude, increasing distance from the coast, and increasing altitude, because more of the heavy isotope, ^{18}O, falls in precipitation (Dansgaard, 1964). Other variables that affect the $\delta^{18}O$ of human bone phosphate include humidity and the plants and animals consumed (Luz and Kolodny, 1989). Comparisons of stable isotope ratios in tissues laid down early in life and those that turn over throughout life may be used to determine whether individuals have moved. An elegant example of this type of study is that of Sealy et al. (1995), who compared enamel, dentine, and bone from five individuals from the southern Cape of Africa. Two individuals are prehistoric Khoisan hunter-gatherers, and three others date to the historic period. Of the historic burials, two were thought to be males of European ancestry and one was thought to be a female who was brought to the Cape as a slave. Enamel, dentine, and bone were analyzed for stable isotopes of carbon, nitrogen, and strontium. The prehistoric individuals have similar isotope values for all tissues analyzed. However, two historic individuals show significant variation in stable isotope values for the different tissues, indicating that they moved between their childhood and several years before their death as adults.

The use of stable oxygen isotopes to identify the geographical origin of prehistoric peoples has been carried out on prehistoric human bones from Mexico (White et al., 1998, 2004) and on historic soldiers from northeastern North America (Schwarcz and Schoeninger, 1991). The method becomes problematic in more recent peoples because our food and water comes from many sources (e.g., Perrier water from France is popular in North America, as is New Zealand lamb). However, in people who were unlikely to move frequently, and who had fairly monotonous diets, oxygen isotope analysis is a useful indicator of residence.

Strontium isotopes are also useful in residence and migration studies since their variation is tied to local geology. Strontium isotopes vary depending on the underlying bedrock that gives rise to soils, and they vary between people feeding on marine versus on terrestrial resources (Sealy et al., 1991). In addition to the study described above by Sealy et al. (1995), strontium isotopes have been used in studies of

residence at the Grasshopper Pueblo in Arizona (Ezzo et al., 1997; Price et al., 1994b) and in a Bell Beaker sample from Bavaria (Price et al., 1994a). They have also been used to identify immigrants to large ceremonial sites such as the Mayan site of Tikal (Wright, 2005), and to investigate residential mobility in the Andes (Knudson et al., 2004). The potential of using strontium isotopes for residence studies was first pointed out by Ericson (1989). Because of the strontium isotope differences between marine and terrestrial foods, they can also be used in paleodiet studies (Sealy et al., 1991).

A DAY WITHOUT STABLE ISOTOPES: WHAT HAS THEIR USE ADDED TO OUR KNOWLEDGE?

The use of stable isotope methods to address archaeological questions now dates back over 30 years. Do we really need these sophisticated analyses to tell us what people ate, where they lived, and whether they migrated? Other sources of evidence exist in the archaeological record for all of these questions. The presence of a food item in an archaeological assemblage does not necessarily imply that it was locally produced or that it was eaten. Differential preservation of food remains may confuse interpretations of the relative importance of particular foods. Charred maize cobs and kernels may be well preserved in comparison with other plants, and this has resulted in situations where the importance of maize was overestimated, as shown by stable isotope analyses. The use of nitrogen isotopes has allowed researchers to have a clearer picture of the importance of freshwater fish in the diets of inland populations. Collectively the use of stable isotopes for dietary reconstruction refines estimates of the relative importance of various foods and therefore leads to more accurate interpretations of the effects of changing diet on health and demography. It is also the only means currently available of detecting sex and age differences in diet within past human groups. The use of nitrogen as well as carbon and oxygen isotopes to estimate the duration of breastfeeding also ties into demographic reconstruction. Residence and migration studies help explain population interaction and movement in the past.

With advances in instrumentation and the increasing use of stable isotopes in ecological studies, there has been a very fruitful exchange of information between bioarchaeologists and ecologists. This exchange is illustrated in the example of the Lake Baikal study by Katzenberg and Weber (1999), where much of the information on stable carbon isotope values for freshwater fish was obtained from ecological studies. The exchange has also been beneficial in the other direction in that ecological studies have benefited from the extensive stable isotope ecology reconstructions carried out by archaeologists such as Ambrose, working in East Africa (1986, 1991; Ambrose and DeNiro, 1986) and Sealy, working in Southern Africa (1986; Sealy and van der Merwe, 1988; Sealy et al., 1987).

There are still problems to solve, but many of the problems identified in 1989, in an important paper by Sillen et al. entitled "Chemistry and Paleodietary Research: No More Easy Answers" have seen significant progress. Controlled feeding studies such as those carried out by Ambrose and Norr (1993; Ambrose, 2001) and Tieszen and Fagre (1993) have addressed questions about $\delta^{13}C$ differences in collagen and bone carbonate. Others (Sponheimer et al., 2003) have focused on nitrogen isotopes in the diet and tissues of mammals. Research has become more sophisticated in terms of understanding the underlying biochemical processes that affect stable isotopes. Examples include Ambrose's (2001) work on nitrogen isotopes and water stress, Fogel et al.'s (1997) insights into protein stress and amino acid metabolism, and Evershed's (1993, Stott and Evershed 1996) research on stable isotopes in lipids. Research carried out on living subjects, using hair and fingernails as sources of protein for analysis, allows much more control over

diet, dietary change, and variation (Fuller et al., 2006; O'Connell and Hedges, 1999).

Ethical issues have an impact on all skeletal studies of past peoples (see Walker, this volume). In some situations, visual study is permitted but no destructive analyses are allowed. Even though analytical methods have increasingly been improved and refined so as to require milligram quantities of material, some legislation completely prohibits any destructive analyses of human remains. Even when destructive analyses are permitted on skeletal samples, one must be careful to consider the long-term integrity of collections. Are there other ways to obtain the information? Is the method likely to be successful and informative? Biological anthropologists must continue to work with concerned groups and to explain the value of such studies to everyone.

It is also important to be aware of limitations on the information that may be provided. This awareness is particularly important with paleodiet studies where consumption of several different combinations of foods with varying emphasis on each one may result in the same stable isotope ratios. Use of multiple elements and familiarity with other archaeological sources of dietary information can go a long way toward unraveling this problem. Recent developments using GC/IRMS in which specific amino acids, or other specific biomolecules, are isolated and analyzed can also help to solve this problem.

The future of stable isotope analysis will include many applications that have become routine over the last 30 years, but the field is also moving forward very quickly. It is increasingly important for individuals wishing to pursue this area of study to have training in chemistry and biochemistry, since the level of sophistication has increased substantially. It is true of both the methods for isolating specific components in bone samples as well as the interpretation of the results. Indeed many recent advances have been made by individuals with backgrounds in biochemistry, geochemistry, and ecology who have become interested in archaeological applications. At the same time, some of the most fruitful collaborations have been between individuals trained in the physical sciences and those trained in archaeology and physical anthropology.

BOX 13.1

When working with prehistoric skeletal remains, actual age at death is not known nor are actual familial relationships. However, when studying past diet in individuals buried in historic cemeteries, analyses may be carried out on individuals of known sex, age at death, and familial relationship since grave markers or coffin plates may be present. Such was the case with a small historic cemetery in southern Ontario (Katzenberg, 1991). Stable carbon and nitrogen[3] isotopes were analyzed in collagen from 15 individuals, of whom 9 were adults and 6 were children ranging in age from around the time of birth to six years. The oldest adults probably spent their earlier years in Great Britain before migrating to Canada. In some cases, this migration is also known since year of death is known (Saunders and Lazenby, 1991). Thus, it is possible in this small sample to view stable isotope data from migrants and those born in Canada, to assess diet, and to look for evidence of nursing and weaning in the bones of infants. In one particular case, a mother and child were buried together. Two presumed mother/infant pairs were also buried in the small cemetery.

[3]Reanalysis of stable isotopes of nitrogen was carried out after the 1991 publication to obtain a complete set of data. The more recent results are provided herein.

The data are presented in the table below:

Burial Number	Sex	Age	$\delta^{13}C$	$\delta^{15}N$
1	Female	25 years	−18.3	12.0
1a	Female	1 year, 6 months	−18.9	14.1
2	Male	1 year	−18.7	13.1
3	Male	31 years	−18.6	12.0
4	Male	71 years	−18.9	11.2
5		neonate	−16.5	12.0
6	Male	71 years	−19.5	11.5
7	Female	98 years	−17.8	12.2
8	Male	71 years	−20.9	10.7
9		1 year	−18.9	12.7
11	Female	34 years	−18.7	11.1
12	Female	6 years	−19.5	10.3
13	Female	31 years	−18.4	11.8
14	Male	57 years	−17.7	11.7
15		neonate	−18.9	12.0

The known mother and child are burials 1 and 1a, respectively. Notice that the $\delta^{15}N$ of the child, aged one year and six months at death, is 2.1‰ greater than that of the mother, indicating that the child nursed and may have still been nursing at the time of death. There is some time lag between the cessation of nursing and the deposition of new collagen that no longer reflects the trophic-level effect of nursing. In other studies, a small trophic-level shift in $\delta^{13}C$ has been observed, but it is not present here. The $\delta^{13}C$ of the child is 0.6‰ less enriched than that of the mother.

One of the presumed mother–infant pairs is burial 13 and burial 15. In this case, the infant died within a month of birth and the difference in $\delta^{15}N$ between mother and infant is only 0.2‰. Given that the precision of the analysis is $\pm$ 0.2‰, this difference is not significant, and repeat analyses may result in the numbers being the same or in the mother having a 0.1% or 0.2‰ difference from the infant in the other direction. The third presumed mother–infant pair (and the least certain, based on other sources of evidence) includes burials 5 and 11. Once again, the infant died within the first month of life. Here the $\delta^{15}N$ of mother and infant differ by 0.9‰. If we compare the $\delta^{13}C$ for these mother and infant pairs, we see that they are similar for burials 13 and 15 (difference of 0.5‰) but that they differ by 2.2‰ for burials 5 and 11. One would expect a newborn baby to have similar isotope ratios to its mother, so we might suggest that this is not a mother–infant pair, based on the stable isotope evidence. (In a matched study of fingernails from living mother–infant pairs, Fuller et al. (2006) found that $\delta^{13}C$ did not differ by more than 1‰ between mothers and their infants.)

The diet of people from Great Britain is based exclusively on C_3 plants such as wheat, oats, and barley and on domesticated animals that also consumed C_3 plants. The average $\delta^{13}C$ is around −20% to −21‰ (van Klinken et al., 2001). Settlers to southern Ontario brought that diet with them and characteristically have $\delta^{13}C$ values around −19% to −18‰, as seen here (Katzenberg et al., 2001). The slight enrichment is undoubtedly from the incorporation of the New World domesticate, maize, which is a C_4 plant, that was used in bread. Another C_4 plant product, cane sugar, was used in baked goods (Katzenberg et al., 2001).

The people buried in the cemetery came from Scotland and moved first to New York in 1810 and then to Ontario in 1817. Is it possible to see any dietary differences between the oldest adults, who would have spent some time in Scotland, and the younger individuals? At

first glance, only one individual stands out and that is burial 8, a 71-year-old man. His $\delta^{13}C$ value is $-20.9‰$, the most depleted in the heavier isotope of carbon. Burial 8 died in 1825, so he would have spent the first 56 years of his life in Scotland and only the last 15 years in North America. Collagen turnover is estimated to be about 10–20 years so it is possible that his bones still contained some collagen that formed during his many years in Scotland. Burial 7 is the oldest individual in the cemetery, and her $\delta^{13}C$ value is $-17.8‰$, which is different from burial 8 and more in line with a North American diet. However, she died in 1894 so she spent almost her entire life (the last 84 years) in North America and only the first 14 in Scotland. Therefore, her bone collagen reflects her North American diet.

This example illustrates some of the diverse information that may be obtained from stable isotope data. Obviously, some information in a historic sample will not be available in other situations; however, the certainty that is gained through such analyses is important in strengthening interpretations in less well-documented samples.

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