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Carbon Isotopes, Photosynthesis, and Archaeology

Different pathways of photosynthesis cause characteristic changes in carbon isotope ratios that make possible the study of prehistoric human diets

In 1960 and 1961 two American scientists were awarded the Nobel Prize in Chemistry for their research on the chemistry of carbon. The 1960 laureate, Willard F. Libby, had discovered the natural radioactive isotope of carbon, ^{14}C, and developed the radiocarbon-dating method for determining the age of organic materials up to about 40,000 years—a technique that has revolutionized the archaeological study of *Homo sapiens sapiens* (Libby et al. 1949; Libby 1955). The 1961 prize went to Melvin Calvin for his description of the chemical pathway followed by carbon during photosynthesis in plants (Calvin and Benson 1948; Calvin and Bassham 1962). Some twenty years later, these two lines of research have fused into a single strand to provide archaeologists with yet another technique for studying the past: using the behavior of carbon isotopes during photosynthesis as a means to measure the diet of prehistoric peoples. Ironically, this method is based on characteristics of carbon and plants of which both Libby and Calvin were unaware at the time of their discoveries. The path of discovery which has led to the development of the method is the subject of this article.

Carbon occurs in three isotopic

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forms: ^{12}C, ^{13}C, and ^{14}C. The first two isotopes are stable, whereas ^{14}C disintegrates radioactively over time. The three react chemically in the same way, but because their atoms have different atomic weights and are of different sizes, they react at different rates. Thus different chemical and metabolic processes change the ratios between the isotopes in characteristic ways. Not all plants follow the photosynthetic pathway described by Calvin; in fact, it is the different pathways, with the different ratios they yield, that make dietary tracing possible.

Before nuclear weapons tests added large amounts of radioactive ^{14}C to the atmosphere, the average ratio of ^{12}C, ^{13}C, and ^{14}C on the earth was approximately 100 to 1.1 to 1×10^{-12}. Since ^{12}C and ^{13}C are stable, the worldwide average ratio between them has remained unchanged over time, but it has been found to differ from one carbon-bearing material to another. Marine limestone, for example, when decomposed with acid, generates carbon dioxide which contains more ^{13}C than does an equal volume of carbon dioxide obtained by burning wood.

To specify the small differences in isotopic composition between such materials, the $^{13}C/^{12}C$ ratio of a given sample is compared in a mass spectrometer with the ratio of an agreed standard. The difference between the sample and the standard is known as the relative ^{13}C content, designated by δ, and is measured in parts per thousand, or per mil (‰). Thus, if a sample of carbon dioxide proves to have a $^{13}C/^{12}C$ ratio which is less than that of the standard by 5 per mil, it is said to have a $\delta^{13}C$ value of −5‰ (minus 5 per mil).

The commonly agreed reference for $\delta^{13}C$ measurements, known as the Chicago PDB marine carbonate standard, is derived from a piece of Cretaceous marine fossil, *Belemnitella americana*, from the Peedee formation in South Carolina (Craig 1953, 1957). Because this piece of carbonate has long since been used up, current measurements are related to this near-mythical fossil through secondary standards prepared by the National Bureau of Standards. A marine carbonate makes a convenient standard, because it has a higher $^{13}C/^{12}C$ ratio than nearly all other natural carbon-based materials: the PDB standard is assigned a $\delta^{13}C$ value of zero, and most natural materials have negative values.

Isotopic fractionation—change in isotopic ratios between materials, due to the different rates at which various isotopes undergo chemical reactions—is a well-established phenomenon. The first measurements were made in 1939 by Alfred O. Nier and Earl A. Gulbransen, who compared, among other things, the $^{13}C/^{12}C$ ratios of Boston clamshells, Massachusetts pine, and the air over Cambridge (Nier and Gulbransen 1939). We now know that the $\delta^{13}C$ values for natural carbon-bearing materials vary from about +4‰ to −50‰, relative to the PDB standard. Table 1 gives a sampling of values from carbon dioxide in the atmosphere, from plants, and from herbivores.

Carbon isotopes are strongly fractionated during photosynthesis, when plants metabolize carbon dioxide (Park and Epstein 1960). Three types of photosynthesis occur in the plant world, commonly referred to as the C_3, C_4, and CAM pathways. The first to be described was C_3 photosynthesis, discovered in experiments with algae, spinach, and barley (Calvin and Benson 1948). During the first step of photosynthesis, C_3 plants convert carbon

dioxide from the air to a phospho-glycerate compound with three carbon atoms.

A different photosynthetic pathway was identified in Hawaiian sugarcane: the conversion of carbon dioxide to dicarboxylic acid, a four-carbon compound (Kortshack et al. 1965). The details of the C_4 photosynthetic pathway were described in Australian sugarcane (Hatch and Slack 1966) and shown to occur in several other species of grasses (Hatch et al. 1967). Of these, the most important to humans are maize, sorghum, and millet—the staple cereals of the Americas and Africa. This discovery was rapidly followed by the realization that those grass species with so-called Kranz leaf anatomy all have C_4 photosynthesis (Downton and Tregunna 1968). *Kranz*, the German word for "wreath," refers to the circular arrangement of bundle sheath cells in a leaf, visible in cross section under a microscope.

With further research, yet another photosynthetic pathway was discovered: CAM, for "crassulacean acid metabolism." The CAM pathway, found in succulents such as the cactus, has little bearing on this discussion. More important for the development of dietary tracing was the finding that C_3 and C_4 plants fractionate carbon isotopes in distinctly different ways.

During the 1960s, information about isotopic fractionation in plants was built up in radiocarbon laboratories around the world. The goal was not to study photosynthesis but to calibrate the $^{14}C/^{12}C$ ratios of radiocarbon-dated samples to the same isotopic ratio as the "modern standard carbon" that served as a common reference. Modern standard carbon, still in use today, imitates the isotopic ratio of carbon in oak as it might have been produced in 1950, if no nuclear explosions had occurred before that date; it has a $\delta^{13}C$ value of $-25‰$. If a carbon sample proves to have a $\delta^{13}C$ value of, say, $-15‰$ it is said to be isotopically too heavy by $10‰$, or 1%. This fractionation effect is doubled for ^{14}C (Libby 1955); hence, such a sample will have begun its life with 2% more ^{14}C than standard carbon. The average lifetime of a ^{14}C atom is about 8,000 years, 2% of which is 160 years. Our hypothetical sample will consequently appear to be 160 years
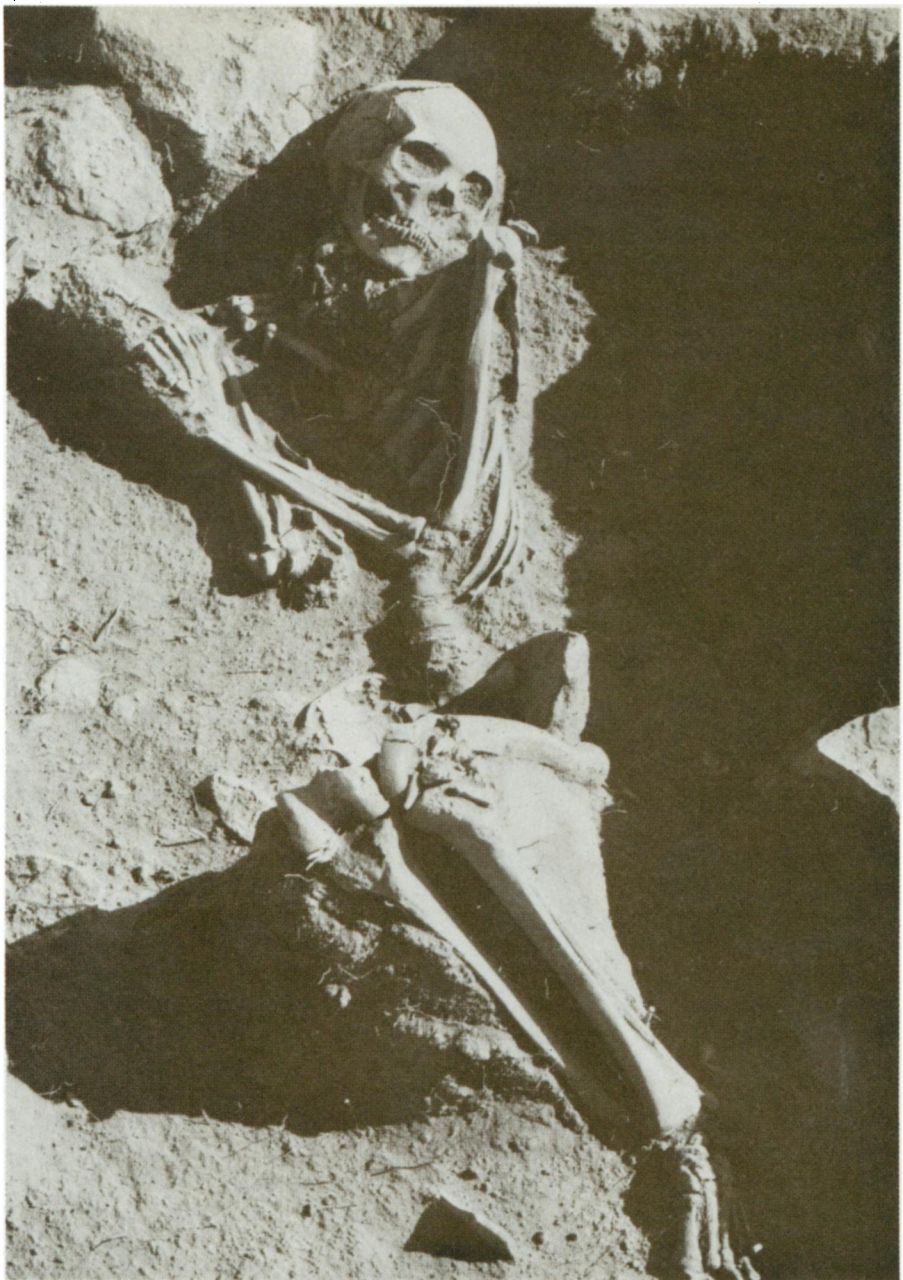

Figure 1. This eleventh-century inhabitant of an Iron Age village, excavated by the author in 1970 at Kgopolwe 3, near Phalaborwa, South Africa, posed an anthropological puzzle, because he was of a different physical type (Khoisan) from the other inhabitants (Negro). However, a $\delta^{13}C$ value of $-10‰$ obtained from his skeleton suggested that he had shared the standard diet of the village, which included the C_4 plant sorghum as a staple food. This evidence was later cast into doubt by the discovery that many other C_4 plants grow in the area and could account for the rather high $\delta^{13}C$ value of the skeleton; nevertheless, this case is credited with having led to many later, more successful tracings of C_4 plants in human diets. (Photo by the author.)

younger, in terms of ^{14}C activity, than it really is in calendar years. Its radiocarbon age must be adjusted accordingly; the correction factor is based on the $^{13}C/^{12}C$ ratio of the sample.

As a result of such corrections for fractionation, it became known that human skeletons have erratic $\delta^{13}C$ values and hence erratic radiocarbon dates. In North America, archaeologists came to realize that the

carbonized maize remains often excavated from the settlements of agricultural peoples consistently gave ages that were 200 years too young in radiocarbon years, if left uncorrected (Hall 1967). Several radiocarbon laboratories consequently added mass spectrometric readings of $\delta^{13}C$ values to their repertoire, particularly when the sample material was something other than wood charcoal. It is probably fair to say that these

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calibrations were done on a purely empirical basis, without regard to the systematics which lay behind deviations in $\delta^{13}C$ values. It soon became apparent, however, that several American grass species have $\delta^{13}C$ values similar to that of maize (Bender 1968). The stage was now set for the combination of many small discoveries into a single elegant statement about plants.

These grasses were, of course, C_4 plants, although they were not at first categorized in that way. However, the correlation of photosynthetic pathway, leaf anatomy, carbon dioxide absorption rate, and $\delta^{13}C$ value was not long in coming. A conference on photosynthesis and photorespiration held in Australia in 1970 was instrumental in bringing together scientists from many different disciplines and making all the accumulated information common cause. The point was made explicit that plants with C_4 photosynthesis, Kranz leaf anatomy, and rapid carbon dioxide uptake all exhibit $\delta^{13}C$ values near a mean of -12.5%, and that any one of these factors predicts the other three without fail (Smith and Epstein 1970).

In contrast, plants with C_3 photosynthesis all lack Kranz leaf anatomy, have slow rates of CO_2 uptake, and average about -26.5% in $\delta^{13}C$ value. Of these characteristics, the leaf anatomy and carbon isotope ratio were relatively easy to determine, and they contributed quickly to the identification of C_4 plants. The list of known C_4 species mounted rapidly into the hundreds—with attendant repercussions when they turned up inconveniently in 16 families of flowering plants, including *Amaranthaceae, Chenopodiaceae*, and *Graminae*. At least 13 genera were found to include both C_3 and C_4 species. This apparently haphazard distribution has upset many plant taxonomists, but on the other hand it has contributed to a better understanding of the adaptive characteristics and evolution of C_4 plants (Moore 1982).

Carbon isotopes and the environment

Part of the reason that the characteristics of C_4 plants took so long to be recognized was that the scientists working on photosynthesis were separated by great distances from most of the C_4 plant species on earth.

North America and Europe happen to contain C_3 plants almost exclusively, with the obvious exception that they grow maize and, to a lesser extent, sugarcane. It is not surprising, therefore, that a significant share of basic and applied research on C_4 plants has been done in Australia and South Africa, countries with both scientific capability and a lot of C_4 plants. Most of the grasses in the Australian and South African interiors are of the C_4 type; so also are most grasses and a few shrubs in subtropical, savannah, and arid regions. Grasses growing in salt marshes or along the ocean are often C_4 species as well, even though the climate may be temperate. In contrast, all trees and most shrubs are C_3 plants, as are grasses from temperate regions and tropical forests.

Table 1. A sampling of relative ^{13}C values (in mils)

PDB marine carbonate standard	0
Air (carbon dioxide)	
normal atmosphere	−7
urban atmosphere, Los Angeles (diluted by fossil fuel combustion)	−8
Amazon rain forest at ground level (diluted by rotting leaf litter)	−15.5
C_3 plants (leaves)	
Beta vulgaris (sugar beet)	−30.1
Raphanus sp. (radish)	−28.8
Pisum sativum (pea)	−26.1
Triticum aestivum (bread wheat)	−23.7
C_4 plants (leaves)	
Atriplex vesicara (goosefoot herbs)	−15.1
Zea mays (maize)	−14.0
Saccharum sp. (sugarcane)	−13.9
Thallasia testudinum (water grass)	−9.3
Herbivores (bone collagen)	
browsers	
grey duiker	−23.8
tropical grysbuck	−22.6
kudu	−21.1
giraffe	−20.8
mixed feeders[a]	
sable antelope	−13.6
mountain zebra	−11.8
hippopotamus	−9.1
tsesabe	−6.8

[a] This group ranges from truly mixed feeders (at top of list) to pure grazers (at bottom).

SOURCES: Smith and Epstein 1970; Vogel 1978b

On the face of it, most C_4 plants seem to be grasses from hot climates. However, tropical forests, which grow in some of the hottest places on earth, have essentially no C_4 plants in them. It appears instead that C_4 photosynthesis is a complex adaptation to environments in which the growing season is subject to strong radiation from the sun; a tropical forest, though hot, provides shade.

C_4 plants are highly efficient carbon processors, using less time and less water to convert a given volume of carbon dioxide into plant matter. It is generally known that plants absorb carbon dioxide in light, while releasing oxygen and water vapor; in darkness, plants absorb oxygen and release carbon dioxide. This is only the net result of plant respiration, however. In light, many plants not only absorb carbon dioxide and convert it to plant matter but also absorb oxygen and release large amounts of carbon dioxide through the breakdown of their own tissues; this second process, known as photorespiration, occurs at a lower rate in C_4 plants, which accounts for their efficiency in processing carbon (Zelitch 1971). These photosynthetic advantages obviously give C_4 plants the adaptive edge in environments with strong sun, lack of water, or some combination of the two. (A salt marsh is a special case of lack of water.) The appearance of C_4 plants in so many parts of the world and in so many plant families also means that this syndrome has evolved several times in different places in response to various environmental pressures.

Of particular interest to this discussion, of course, is the way in which different photosynthetic systems fractionate the carbon isotopes under different circumstances. The mean $\delta^{13}C$ values of C_3 and C_4 plant foliage (-26.5% and -12.5%, respectively) differ considerably. These averages are based on hundreds of isotopic readings which have normal distributions (bell curves) around the mean values. Figure 2 shows the normal distribution for a sample of 351 South African grass species that includes both the C_3 and the C_4 type. Under different environmental conditions the $\delta^{13}C$ values of C_3 plants may vary from -20% to -35%, whereas C_4 plants may vary from -9% to -16%. The two ranges do not overlap—a fortunate circum-

stance which makes possible a number of applications. Before we discuss these, however, it is necessary to look at some special circumstances which cause deviations from the mean values.

In the terrestrial carbon cycle, the primary source of carbon is atmospheric carbon dioxide; the $\delta^{13}C$ value of well-mixed air is $-7‰$. C_3 plants deplete this value by $-19.5‰$ on the average, producing foliage with a $\delta^{13}C$ value of $-26.5‰$. If the isotopic composition of the carbon dioxide changes, the isotopic composition of the foliage will change by a corresponding amount. This phenomenon can be observed in dense forests, for example, where rotting leaf litter on the ground releases large amounts of carbon dioxide that is isotopically light, while the forest canopy traps the air and prevents it from mixing effectively with the free atmosphere. The result is a gradient of isotopic values from the forest floor to the open air high above it, with a corresponding gradient in the plant foliage. This "canopy effect" is demonstrated by isotopic values in the forests of Bavaria, for example, where $\delta^{13}C$ values of leaves have been shown to vary from about $-31‰$ at 2 m above the ground to about $-28‰$ at 19 m, even on the same tree. Air in an upturned barrel on the forest floor had a $\delta^{13}C$ value of $-27‰$ (Vogel 1978a).

In the case of the Bavarian forest, the fractionation factor remains constant. However, in a less well understood phenomenon, the fractionation factor may vary slightly, causing variations in isotopic composition. Different plant species with the same photosynthetic pathway may have different $\delta^{13}C$ values, though they grow side by side. Plants of the same species growing in different environments have slightly different $\delta^{13}C$ values as well. These variations are more marked in C_3 plants, which exhibit a wider range of isotopic values than C_4 plants. C_3 plants grow in a much wider range of habitats, of course, and it may prove that the fractionation factor varies with photorespiration, which varies with sunlight.

So far, we have been discussing terrestrial plants, but what of life in the water? Carbon sources in the oceans include dissolved carbon dioxide as well as bicarbonates and carbonates. The latter, it will be re-

called, have a $\delta^{13}C$ value of zero, which makes it possible for some marine organisms to be isotopically heavier than terrestrial ones. Marine $\delta^{13}C$ values vary from $-7‰$ to $-31‰$, with warm-water organisms usually showing a greater relative ^{13}C content than cold-water organisms. The division of terrestrial plants on the basis of photosynthetic system does not apply in the ocean, but marine planktons tend to resemble C_3 plants in isotopic composition, whereas some intertidal kelps and seaweeds tend to resemble C_4 plants (Sackett et al. 1965). These facts become important when we come to consider seafood in human diets.

In freshwater systems, as in the oceans, the carbon sources include carbonates, bicarbonates, and dissolved carbon dioxide. The latter exhibits $\delta^{13}C$ values which vary from $-4‰$ in hard waters to $-8‰$ in soft waters. In general, plants in soft waters resemble C_3 plants in $\delta^{13}C$ value, whereas plants in hard waters resemble C_4 plants.

Terrestrial food webs

As early as 1964, P. L. Parker noted that marine animals cover the same range of isotopic values as the foods they eat and suggested that stable

Figure 2. The $\delta^{13}C$ values of 351 species of grasses, plotted in a histogram, fall into two separate clusters, illustrating the distinction between C_3 and C_4 plants. The values for C_3 plants show an average of $-26.5‰$; for C_4 plants, the average is $-12.5‰$. (After Vogel et al. 1978.)

carbon isotopes could be used as diet tracers (Parker 1964). We now know that the "isotopic signature" of food is in fact passed on to consumers. In the course of metabolism, the carbon isotopes in the food may be fractionated yet again before storage in the tissues. These facts make it possible to determine, for example, the proportions of C_3 and C_4 plants in the diet of a herbivorous animal, through measurement of the $\delta^{13}C$ value of one or more of its storage tissues. The sample material can be hair, skin, meat, horn, or bone, provided that the fractionation factor of the material is known. These factors are still being investigated and are by no means as well understood as one would like them to be, but enough is known about them to permit the study of food webs of animals and humans, with some success.

Part of the information used to assess diet on the basis of $\delta^{13}C$ values in animal tissues has been contributed by Michael J. De Niro, who conducted a number of experiments with insects and animals fed on diets of known isotopic composition (De Niro and Epstein 1978). These included such cases as snails feeding on lettuce, flies eating meat, and mice raised on Purina Rat Chow. For the smallest creatures, he was able to measure the $\delta^{13}C$ value of the whole organism and found it to be very similar to that of the food. In the mice, the body tissues and excreta were enriched by about $1‰$ relative to the food—that is, their $\delta^{13}C$ value was more positive. The carbon dioxide breathed out by the mice was depleted by $1‰$ relative to the food. De Niro thus showed quantitatively that each organism maintains a carbon balance sheet. On one side of the account is the total number of ^{12}C and ^{13}C atoms taken in from food; on the other side is the total number of ^{12}C and ^{13}C atoms stored in tissues, excreted, and breathed out as carbon dioxide. This means that the $\delta^{13}C$ enrichment factor in the storage tissues and excreta of a mouse, multiplied by the total weight, must equal the ^{13}C depletion in the carbon dioxide the mouse breathes out over its lifetime.

When living organisms are considered in this manner, as carbon isotope separaters, it becomes easy to understand that different storage tissues in the same animal (flesh, fat, bone, hair) may have slightly differ-
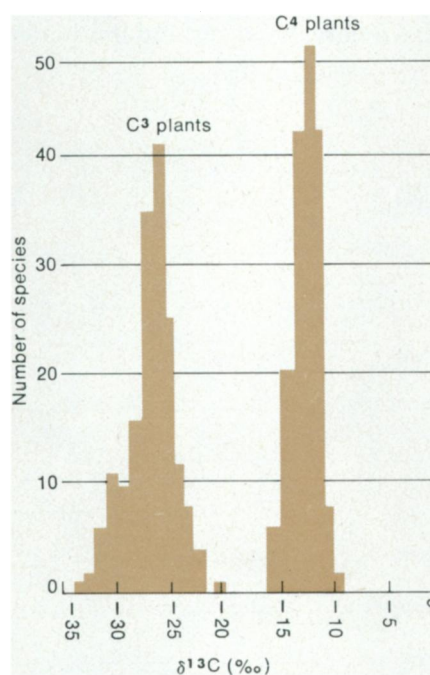
ent $\delta^{13}C$ values. Each tissue type has its own characteristic fractionation factor. De Niro's work shows that these fractionation factors may have two components: fractionation resulting from the formation of the tissue itself, and inheritance of the different isotopic values of food components such as soluble protein, carbohydrates, and fats. In other words, fats in the diet tend to translate directly into fat in the consumer, as most diet-conscious humans know only too well, and in doing so transmit the isotopic composition of food fat directly to fatty tissue in the consumer. Of course, fattening foods include many other things beside fats per se, and therefore the situation must be more complicated than this. What De Niro's results imply, however, is that the different isotopic ratios in different tissues of an animal may be due directly to the isotopic ratios of the food components from which the tissues are built up. An opposite view would be a "scrambled egg" theory of metabolism, in which the animal breaks all its food down into atoms or small molecules and reassembles them to build tissues.

The way in which larger animals fractionate the carbon isotopes was measured by J. C. Vogel (1978b). In collaboration with botanists A. Fuls and R. P. Ellis, he first identified the C_4 grass species in South Africa on the basis of their $\delta^{13}C$ values and found that they occurred in the summer rainfall areas of the country, with a gradient between the C_3 plant environment of the winter rainfall areas around Cape Town and the arid interior, which has nearly exclusively C_4 grasses (Vogel et al. 1978). The isotopic ratios of large ungulates followed the same gradient, but with definite fractionation of the isotopes

Figure 3. The different tissues of an animal may each have a characteristic $\delta^{13}C$ value, owing to their different fractionation of the carbon isotopes. When the values of fat, flesh, hide, and bone collagen are plotted for animals from different areas of South Africa, it can be seen that the average difference of $\delta^{13}C$ values between bone and hide is 0.6‰; between bone and flesh, 2.3‰; and between bone and fat, 7.9‰. At the points marked by dashed lines, values were unobtainable and have been interpolated on the basis of the average differences among tissues. Note that even within species (e.g., springbok and sheep) the $\delta^{13}C$ value for one type of tissue can vary considerably, owing to differences in local plant cover. (After Vogel 1978b.)

for different tissues. A sampling of 13 animals is plotted in Figure 3 to show the different $\delta^{13}C$ values of fat, flesh, hide, and bone.

Of particular interest were the $\delta^{13}C$ values of browsing animals like the kudu, which feeds almost exclusively on the leaves of trees and shrubs—that is, C_3 plants. Using the average $\delta^{13}C$ value of −26.5‰ for C_3 foliage and the average $\delta^{13}C$ values of different tissues in the kudu, it is possible to calculate fractionation factors of +5.3‰ for bone collagen, +3‰ for flesh, and −3‰ for fat. These values differ, of course, from those obtained for laboratory mice by De Niro; one assumes that the discrepancy is explained by differences in metabolism between the long-lived kudu and the short-lived mice, and by their different diets.

Vogel also noticed that there is little or no difference in $\delta^{13}C$ values for the same tissue type on different trophic levels: the isotopic composition of the bone collagen of a carnivore is closely similar to that of the bone collagen of the animals it consumes. This observation tends to confirm the deduction I have made from De Niro's results: the most important fractionation of the carbon isotopes takes place in plants at the base of the food web. Few measurements have been done to confirm this theory, however, and the matter
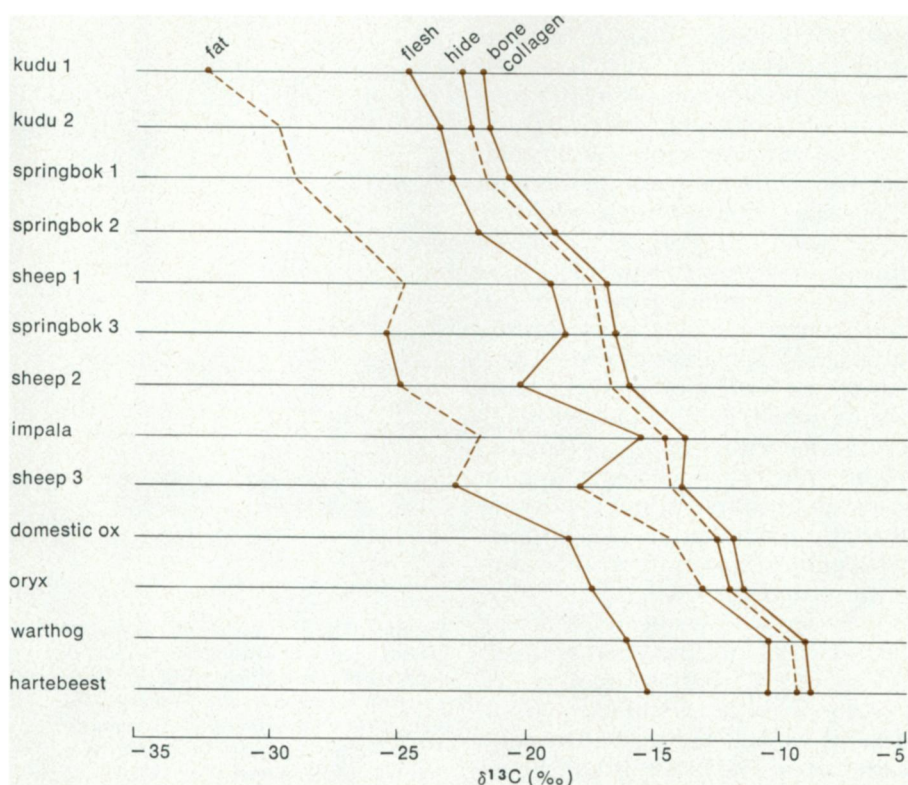
must be considered to be far from settled.

Human diet

The application of stable carbon isotope measurements to the study of human diet came about more or less by accident. In 1970 I was excavating a group of Iron Age sites at Phalaborwa in the Transvaal Lowveld, South Africa. Among the excavations was Kgopolwe 3, at that time the oldest known Iron Age site in South Africa, with radiocarbon dates on charcoal ranging approximately from A.D. 950 to 1100. In an ash heap we found a human male skeleton, shown in Figure 1, which appeared out of place on various counts. The body had been rather casually disposed of, instead of being tightly flexed and buried under the floor of the owner's hut, as is customary for men buried inside Iron Age settlements in this area. Furthermore, the skull looked like that of a large Khoisan (Bushman or Hottentot) rather than a Negro.

On examining the skeleton, my colleague Philip Rightmire identified it as a male Hottentot (Rightmire and van der Merwe 1976). This threw us into a real quandary: What was a Hottentot doing in a Transvaal Iron Age village? In 1970 it was not well known that the Stone Age Khoisan

people of South Africa were often absorbed into the settlements of their Iron Age neighbors, and therefore it was difficult to account for this skeleton. We went to some lengths to find out more. It had been established that maize and sorghum, the staple foods of contemporary Africa, have very positive $\delta^{13}C$ values, which translate into too-young dates on African skeletons. Maize was not yet present in Africa in A.D. 1000, but sorghum should have been a significant component in the diet of a proper Iron Age villager. A mass spectrometer reading by Vogel on the Kgopolwe 3 skeleton yielded a $\delta^{13}C$ value of −10‰. This result, we thought, proved that our problem individual had been a sorghum eater. Voilà, a Hottentot agriculturist in the Iron Age of South Africa! The case seemed closed, even though the result was most unorthodox. Shortly thereafter, however, Vogel reported that nearly all the grasses in the Transvaal Lowveld were C_4 plants. Anyone who lived in that area, whether a hunter-gatherer, a pastoralist, or an agriculturist, could be expected to have a $\delta^{13}C$ value as positive as that of the Kgopolwe 3 skeleton. Thus we knew less about our specimen than we had thought: he was a Hottentot, but he may or may not have been a sorghum eater.

The story does not end there,

however. The subject of how the isotopic composition of plants registers in human skeletons was discussed at length in a seminar I was teaching at SUNY-Binghamton. A number of American Indian skeletons had been excavated nearby, and the question of when maize agriculture was introduced to New York was very much on our minds. Presumably, the arrival of maize in an environment dominated by C_3 plants had made a substantial difference in the isotopic composition of the inhabitants. We tested this idea on a series of human ribs from sites in New York dated to about 2500–100 B.C. (Archaic and Early Woodland), well before maize could have been present, and from sites dated to about A.D. 1000–1500, a period during which carbonized maize kernels occur in the archaeological deposits. The results were quite startling: the early, pre-maize skeletons all had $\delta^{13}C$ values clustered near the prehistoric Western European average of about −21‰, whereas the later group showed a change over time from −16.5‰ to −13.5‰, correlated with an increase in maize consumption (Vogel and van der Merwe 1977). This meant not only that one could detect the arrival of maize in a C_3 environment such as the North American woodlands, but also that the amount of maize in the diet of the

inhabitants could be measured. The path was now open toward a study of the history of human diet by means of carbon isotope measurements.

Archaeological applications

Since those first carbon isotope measurements on New York skeletons, Vogel and I have made several hundred measurements on human skeletons from selected sites in the Americas and Africa. During the latter half of the 1970s, other investigators added a large body of information on the isotopic composition of plants, animals, and humans. The result is a much better understanding of how carbon isotopes can be used to study food webs in a variety of environmental circumstances.

In order to assess the proportion of C_3 and C_4 plants in human food webs, it is necessary to know how human metabolism affects isotope ratios. The sample material an archaeologist is most likely to encounter is human bone, which consists of collagen—a soft protein tissue—and apatite (calcium phosphate). In adults, collagen has a very slow turnover rate (Stenhouse and Baxter 1976), and the carbon in it therefore comes from food eaten over a long period, perhaps several decades. Human adult collagen is also an inert material: it does not exchange carbon with air or other organic materials in archaeological deposits, which adds to its usefulness for analysis.

Reconstructing human diet from the isotopic ratio of collagen requires an accurate value for the fractionation caused by collagen formation. Several investigators have measured collagen fractionation, with results ranging from about +3‰ for experimental mice and chickens (De Niro and Epstein 1978; Bender et al. 1981) to +5.3‰ for African ungulates in their natural habitat (Vogel 1978b). The discrepancy may be due in part to different metabolic rates between small and large animals, or to an error in the assumption that the diet of a browsing animal has a $\delta^{13}C$ value of −26.5‰.

Figure 4. The warthog (*Phacochoerus africanus*) is a mixed feeder, whose diet includes both C_3 and C_4 plants. The warthogs shown here are feeding in the Nairobi Game Park, Kenya. (Photo by Melvin Konner, courtesy of Anthro-Photo.)

Alternatively, this discrepancy could be due to the nature of the food itself. Proteins extracted from the foliage of C_3 plants have $\delta^{13}C$ values that are 3‰ to 5‰ more positive than those of whole leaves (Vogel 1982). This could mean that little or no fractionation takes place when protein tissues such as collagen and muscle are formed; instead, there may be merely some sorting and storage of amino acids with different isotopic ratios. The difference in protein content between laboratory feed and African plants may, therefore, account for the observed differences. The question remains unanswered, and for this discussion I will continue to use the term "fractionation" to describe the isotopic shift between diet and collagen.

To measure the fractionation of the carbon isotopes in human collagen, Vogel and I decided to confine our measurements to the skeletons of adults from populations that had lived for centuries in a C_3 plant environment, with no access to C_4 plants (van der Merwe and Vogel 1978). The closest approximation to this ideal proved to be some of the early populations of North America.

In the woodlands of the North American interior, Archaic peo-ples—to give them their archaeolo-gical name—practiced a way of life which remained essentially unchanged for several millennia before the Christian era. Their diet consisted largely of wild plant foods, supplemented by hunting, fishing in the rivers and lakes, and the collecting of freshwater shellfish. The terrestrial plants in this environment are overwhelmingly of the C_3 type, and the freshwater plants resemble them closely in isotopic composition. A few C_4 plant species do occur in the woodlands (*Panicum virgatum* is one example), but they are sufficiently rare to have minimal influence. As for the intermediate trophic levels in the food web, the animals eaten by humans, these add little or no fractionation of the isotopes. For our purpose it can therefore be assumed that the Archaic food web was firmly based on the foliage of C_3 plants.

Vogel and I analyzed 31 Archaic skeletons and obtained an average $\delta^{13}C$ value of -21.4‰ ± 0.78. Assuming that the average value of plant foliage in the woodlands is -26.5‰, we now have a fractionation value for human bone collagen of $+5.1$‰—remarkably similar to the fractionation value of $+5.3$‰ obtained for a small number of browsing animals in South Africa. In fact,

further unpublished measurements on African animals show that this value is nearer to 5.1‰ (see Vogel 1982). In calculating the isotopic ratios of diet from collagen, therefore, I will consistently use a shift of 5.1‰. A hypothetical diet consisting exclusively of C_4 plants should yield human bone collagen with a $\delta^{13}C$ value of -7.4‰, whereas a mixed diet should yield values between -7.4‰ and -21.4‰. This calculation assumes that the same value holds for C_3 and C_4 plants, although there is some evidence that the shift from C_4 plants may be 6‰ (Sullivan and Krueger 1981). Figure 5 shows the carbon pathway for different diets in the form of a flow chart.

Marked changes in $\delta^{13}C$ values of human collagen resulted from the introduction of maize to the North American woodlands. During the period around A.D. 1000–1200, these values changed from about -21.4‰ to -12‰, which means that the proportion of carbon from C_4 plants in the collagen went from zero to more than 70%; these data are plotted in Figure 6. This is not quite the same as saying that maize formed more than 70% of the diet by A.D. 1200, but close enough for the purpose of this discussion. The rapid acceptance of agriculture in exchange for a former hunter-gatherer way of life which had endured for thousands of years brought other revolutionary changes in its wake. People moved from their villages along river edges to more open ground on the uplands, the population increased, and larger-scale societies developed.

The effect of maize agriculture on population size is aptly demonstrated by an example of the Orinoco Valley of Venezuela. Maize is commonly assumed not to have played a major role in the prehistory of South American rain forests. The orthodox view is that the "tropical forest system" was the major means of livelihood of forest tribes until historic times. In this system most calories are provided by cultivated manioc, or cassava, and other forest root crops, and protein is obtained by hunting and fishing. It is associated ethnographically with small, autonomous villages as a logical outcome of the scarcity of animals to sustain the population during the rainy season, when fishing is impossible in the raging rivers. The large, populous chiefdoms of the Orinoco and Ama-
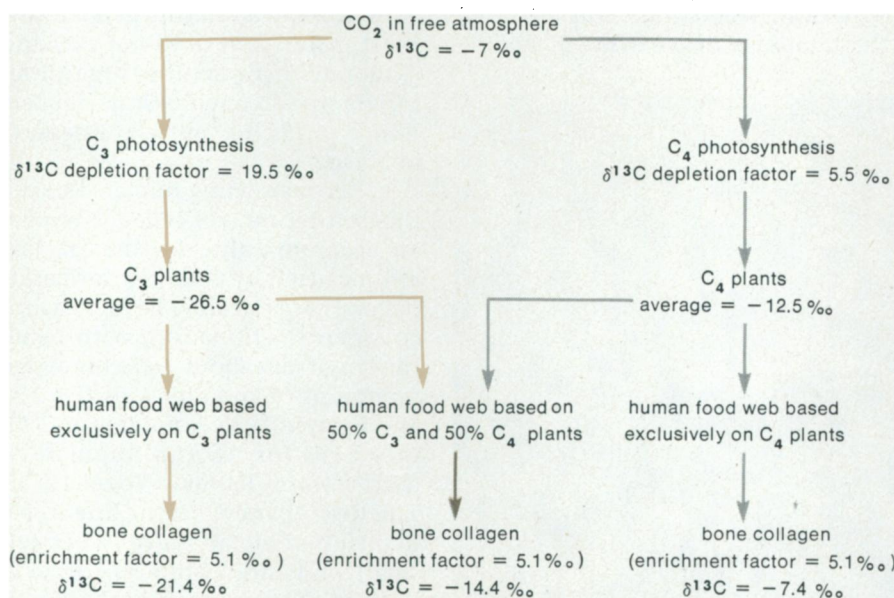

Figure 5. Three different human diets — one based entirely on C_3 plants, one with a mixture of C_3 and C_4 plants, and one based entirely on C_4 plants — entail three distinct pathways of carbon isotope fractionation. Each pathway ultimately leaves its signature in the $\delta^{13}C$ value of human bone collagen. The material of bone collagen itself enriches the $\delta^{13}C$ value by 5.1‰, but because this enrichment factor is constant, it does not interfere with the making of comparative measurements. Values of -21.4‰ and -14.4‰ have been observed, from skeletons of North American Archaic peoples and Europeans and from present-day North Americans, respectively; a value of -7.4‰, which would result from a diet based exclusively on C_4 plants, has not been observed anywhere in humans but is common in grazing animals in the grasslands of Africa.

zon mainstreams of late prehistoric times are usually explained as an elaboration of the tropical forest system, but one based on intensive manioc cultivation on the fertile river floodplains rather than in the infertile forests.

An alternate explanation for the large communities of people in prehistoric Amazonia has been provided by Anna Curtenius Roosevelt (1980). She argues that manioc cultivation is not appropriate to the floodplains, since the roots would rot during the rainy season; that the tropical forest system cannot support populations larger than village-size, since its upper limit is set by a scarcity of animal protein during the rainy season; and that larger populations were due to the cultivation of seed crops (maize and beans) in the floodplains. To test this theory she excavated in the area of Parmana, in the middle Orinoco Valley, focusing particularly on the remains of food and food-processing tools, and estimated the population of the area (500 km^2) at different times on the basis of the size of the refuse mounds created by prehistoric villages.

The prehistory of the Orinoco had been studied earlier by Irving Rouse and José Cruxent (1963). When Roosevelt's findings are added to theirs, the outline which emerges is that highland maize became known to the people in Parmana in 600–400 B.C.; that a lowland-adapted variety was developed, probably around A.D. 100–200; and that maize had eclipsed manioc as a staple by about A.D. 400. Since maize has a protein content of up to 10%, it removed the limit on population imposed by the scarcity of forest animals, and the population increased fifteenfold in the process.

Roosevelt's explanation was eminently sensible, but also controversial, because it challenged the long-held views of several other archaeologists and was based in part on inference from stone tools. Rouse suggested measuring the isotopic composition of the skeletons to settle the matter. To provide the correct context for the measurements, we first had to establish on theoretical grounds that the introduction of maize to Parmana would in fact have had a measurable effect on the isotopic composition of the human inhabitants. The plants of the tropical forest are all of the C$_3$ type, but some C$_4$ grasses grow in drier savannah

areas in the vicinity and could have been incorporated into the human food web through grazing animals such as the capybara, a large rodent. A check of the animal remains associated with the period 2100–1800 B.C. showed that the plant foods of most animals in the human diet, even the fish and turtles, derived ultimately from the tropical gallery forests fringing the waterways of the region.

The next step was to determine the isotopic composition of manioc. The best we could do in this regard was to analyze two manioc cakes bought by Roosevelt in the Puerto Rican market of New York and a root I collected in Malawi. Their δ^{13}C

values averaged about −26‰, confirming that manioc is a C$_3$ plant (van der Merwe et al. 1981).

At this point we analyzed two human skeletons at Parmana dating from about 800 B.C.—that is, contemporary to or earlier than the first appearance of maize in the area. The resulting δ^{13}C average of −26‰ for their collagen puzzled us at first. We had never seen a value so strongly negative for human collagen before: if the collagen enrichment factor of 5.1‰ is subtracted, the result is a δ^{13}C value of −31.1‰ for the plant base of the Parmana food web. Fortuitously, E. Medina and P. Minchin (1980) produced a series of measurements which solved our problem. They
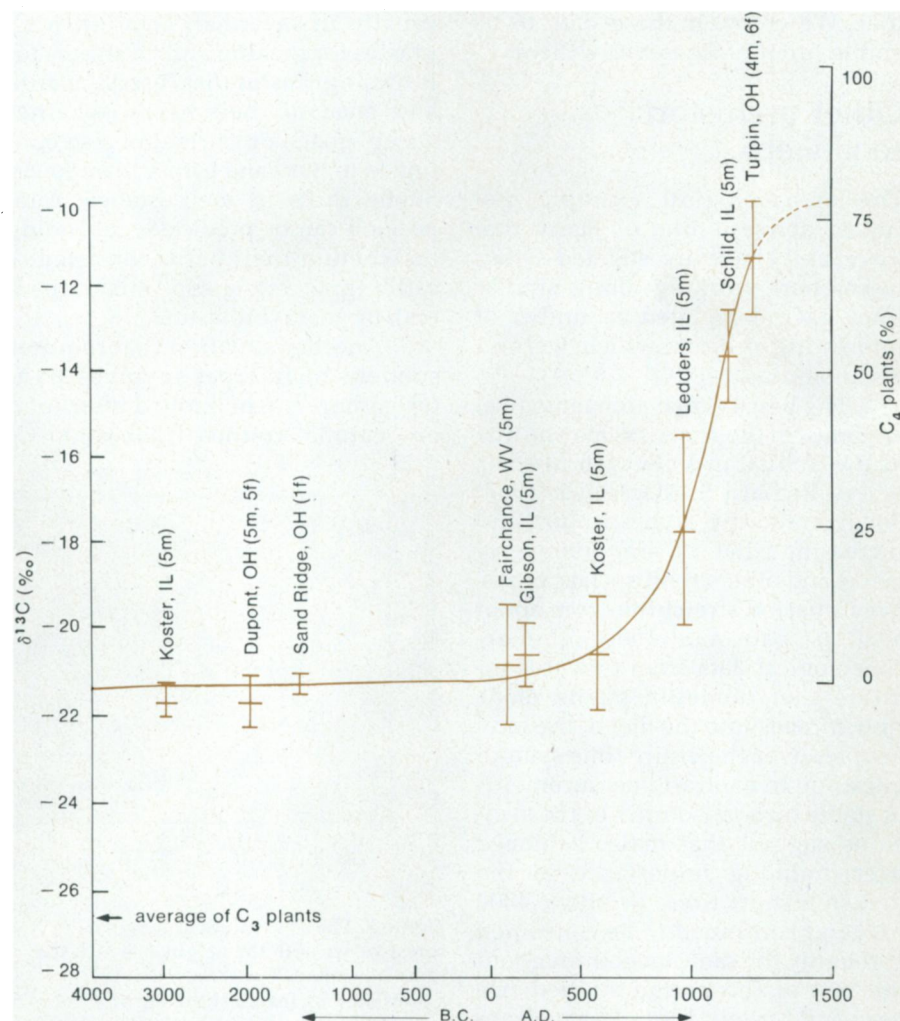

Figure 6. Sharp changes in δ^{13}C values of human bone collagen tell of a food-producing revolution among the hunter-gatherer peoples of North American woodlands: the cultivation of maize. These measurements are taken from adult skeletons, both male and female, in Illinois, Ohio, and West Virginia; the number and gender of each sample is indicated in parentheses. Previous to about A.D. 200, the environment of these peoples had included virtually no C$_4$ plants, and the δ^{13}C value of their bone collagen had consequently been stable over a long period; with the introduction of maize and the rapidly growing dependence on it as a staple food, δ^{13}C values rose steeply. This dietary revolution was accompanied by increases in population, changes in settlement patterns, the occurrence of diet-related anemias, and the development of larger-scale societies. (After van der Merwe and Vogel 1978.)

showed that the $\delta^{13}C$ value of leaves in the Amazon forest can vary from about $-30‰$ in the upper canopy to about $-35‰$ near the ground, owing to the recycling of carbon dioxide under the dense canopy. Such a range is plotted for two kinds of forests in Figure 7. The gallery forests at Parmana are less dense than the Amazon forest, and a food web based on its leaf litter and on manioc grown in small swidden fields in the forest could well average $-31.1‰$; this would give a value of $-26‰$ for humans subsisting primarily on forest root crops and fauna. In contrast, the three skeletons from Parmana dating to about A.D. 400 showed an average value of $-10.3‰$, which means that maize comprised almost 80% of their diet. We consider these data to be ample proof of Roosevelt's theory!

Other prehistoric food webs

The archaeological example discussed above is one of many that Vogel and I have investigated. Other researchers working along similar lines have completed a number of interesting projects which warrant discussion.

The best-known archaeological sequence in the Americas may be that of the Tehuacan Valley, in Mexico, where Richard S. MacNeish (1967) documented the domestication and increasing dietary importance of maize and other crops, such as beans, over a period stretching from about 6000 B.C. to A.D. 1000. The archaeological data from this area indicate that domestic plants made slow inroads into the diet of the local peoples over the entire time span in question. In contrast, measurements of stable carbon isotopes of the skeletons suggest that maize achieved overwhelming importance in the diet in a short time, by about 4000 B.C., and continued to be consumed at roughly the same level throughout the rest of the period studied (De Niro and Epstein 1981). In a new application of stable isotopes, De Niro and Epstein also measured the ratio of two isotopes of nitrogen, ^{14}N and ^{15}N, in skeletons and showed that the amount of pulses (beans, in this case) in a diet can be determined by this technique. The consumption of beans in the Tehuacan Valley actually decreased slowly between 6000 B.C. and A.D. 1000.

So far, the archaeological examples have been confined to the effects of maize agriculture on the isotopic composition of humans and animals. This is the most dramatic sort of change that one is likely to encounter, especially when maize enters what has previously been an exclusively C_3 environment, and it is the easiest to measure and interpret. More subtle changes in diet can also be investigated, however, and therein lies the promise of a rich research harvest for the future. One example of such a change would be the adoption of herding animals by a hunter-gatherer group as a means of food production. This sort of change is known to have happened many times in African prehistory, usually in savannah areas with C_4 grasses for grazing and C_3 shrubs for browsing. Pastoralists used the milk and meat of their herds, whether sheep, goats, or cattle, but also continued to hunt and gather plant foods. Subtle shifts in their isotopic composition can be predicted, depending on whether their herds consisted of cattle (grazers), sheep (mixed feeders), or goats (browsers).

Another situation that requires subtlety to interpet involves hunter-gatherers who moved after food on seasonal rounds from C_4 to C_3

Figure 7. The $\delta^{13}C$ values of leaves in a forest varies with the height at which the sample is taken. The carbon dioxide assimilated by leaves at the top of a tree undergoes some mixing with the carbon dioxide of the atmosphere, giving values less negative than those of carbon dioxide produced by leaves rotting on the forest floor. In this sampling of a Bavarian forest and an Amazon podsol forest in Venezuela, the Amazon forest shows more negative ranges of value throughout than does the Bavarian forest; this is owing to thicker tree growth in the Amazon forest, which inhibits the mixing of carbon dioxide from the atmosphere. (After Medina and Minchin 1980; Vogel 1978a.)

environments or who included seafoods in their diet. The isotopic composition of marine animals varies with sea temperature, but depends above all on the food of the animal. Filter-feeding bivalves such as clams and oysters live on plankton and tend to resemble C_3 plants in isotopic composition, whereas grazing univalves such as abalone and limpets tend to resemble C_4 plants. Higher in the food chain, fish and seals integrate these organisms into their diet in various ways. The first study of a situation with this sort of complexity was carried out by Frank B. Silberbauer (1979 thesis), who analyzed the skeletons of Holocene peoples (i.e., dating from about 12,000 to 500 years ago) in the eastern Cape Province of South Africa. He was able not only to separate hunter-gatherers from pastoralists but also to comment on environmental and cultural changes which took place during the Holocene. Canadian investigators have successfully measured the importance of salmon in the diet of prehistoric Pacific Coast peoples (Chisholm et al. 1982).

Reconstructing past environments

Archaeologists are interested in finding out more about past environments and how people adapted to environmental change. Environments can be reconstructed by means of a variety of techniques, including the interpretation of faunal remains from archaeolgical sites. For example, the presence of skeletons of many grazing herd animals in an African site could be taken to mean that grassland predominated in the area at the time that the bones were deposited. In contrast, the bones of forest hogs and bushbuck could indicate a forest environment. These interpretations are necessarily somewhat crude, given the complexity of animal and human behavior. They can be considerably refined by measuring the $\delta^{13}C$ values of identified bones from species that feed on both grasses and shrubs and that can adapt to changing circumstances by eating more of one or the other. African elephants and buffalo seem to be such species, but their isotopic composition will have to be studied in more detail in different environments before solid conclusions can be reached.
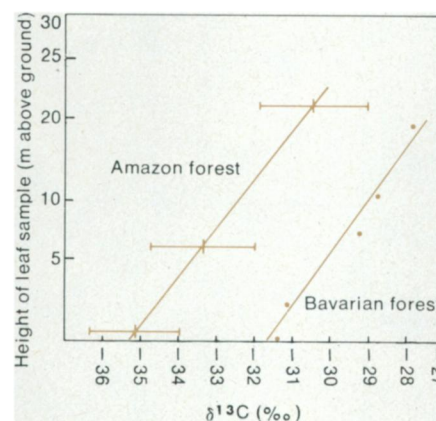
An interesting example of a mixed feeder is the African ostrich, which has a reputation for swallowing anything that will pass down its capacious gullet. Yasmin von Schirnding has measured the $\delta^{13}C$ value of ostrich eggshells to determine whether the birds are really such indiscriminate feeders (von Schirnding et al. 1982). She found that ostriches register the isotopic composition of food eaten during the breeding season (winter) in their eggshells and that, since the grasses of the South African karoo, or interior plateau, are dry in the winter, the ostriches have a marked preference for shrubs. Where the environment does not include many shrubs, they eat dry grasses. An important conclusion from this study is that the calcium carbonate of eggshell registers the isotopic composition of the bird's food, with an enrichment factor of +16.2‰, and that the information is apparently preserved unchanged for at least 12,000 years (the period included in von Schirnding's collection). If this result can stand up under further testing it might mean that archaeologists could draw conclusions about the distribution and extent of karoo-type vegetation from as long ago as the time people began collecting ostrich eggs—about 50,000 years.

Grazing animals provide a means of assessing the proportion of C_3 and C_4 grasses in an area. The relative success of the two photosynthetic pathways can be a sensitive indicator of climate. This is particularly noticeable in the mountainous regions of tropical Africa, where the ratio of C_3 to C_4 species is accurately correlated with altitude and thus with temperature (Livingstone and Clayton 1980). In Kenya, for example, the average $\delta^{13}C$ value of the grass cover at a given place between 2,000 m and 3,000 m can be used to predict its altitude to within 100 m (Tieszen et al. 1979). These vegetation mixtures, which have moved up and down the mountains as the climate changed, are recorded in the tissues of pure grazers. Vogel (in press) has used the $\delta^{13}C$ values of zebra teeth from Lesotho to show that the proportion of C_3 species in the grass cover declined from a high of 85% during the last glaciation, about 35,000 years ago, to less than 40% today.

To conclude this paper it is appropriate to look into the future. The most obvious contexts in which $\delta^{13}C$ measurements are useful to archaeologists are rapidly being investigated by a number of research workers. Foremost of these topics is the spread of maize agriculture through the woodlands and forest of the Americas. The penetration of African crops such as sorghum into the Balkan countries during medieval times is also receiving attention (Burleigh, pers. comm.). The reverse situation—a C_3 cultigen entering a C_4 plant environment—is represented by wheat and barley in Egypt and the Sahara. I have tried to investigate this situation, so far without success, since the bones from desert sites are heavily mineralized and all their collagen decomposes within a few thousand years.

Two other obvious lines of future research for archaeological purposes suggest themselves. The first is to measure the isotopic composition of human skeletons in situations less clear-cut than that of the spread of agriculture. This is already being done, particularly in Africa, but many other situations await study. A related topic is the refinement of our knowledge of the feeding behavior of animals, as a means toward studying past environments.

The second line of research is much more difficult but is potentially of great importance. Bone collagen has a limited lifetime, particularly in open African sites—precisely the area of the world in which the earliest known human forms can be studied. We know that the small amount of calcium carbonate in bone registers the isotopic composition of the diet, but it may also exchange carbon with atmospheric carbon dioxide over time, while additional calcium carbonate may precipitate in bone fissures from groundwater. If uncontaminated carbonate can be isolated by appropriate chemical treatment, it should be possible to study the diet of early hominids. Some success toward this end has been reported with bone carbonate older than 10,000 years (Sullivan and Krueger 1981), but other investigators have been less successful with the same chemical procedures applied to bone from more difficult environmental situations.

The examples discussed so far have all involved a study of the past.

Modern populations and their diet are also susceptible to study, of course. The purity of certain foods is currently monitored by carbon isotope measurements in several commercial laboratories. Pure clover honey and unsweetened orange juice, both derived from C_3 plants, had better not show suspiciously high $\delta^{13}C$ values, which could derive from cane sugar or corn syrup. Brandy and fortified wines are supposed to contain only products of the grape, a C_3 plant, and not the less expensive grain spirit distilled from maize. Medical studies of human metabolism and diets based on isotopic ratios are also emerging.

The applications of stable carbon isotope analysis are in fact as all-pervasive as the presence of carbon, the basic building block of life. This elegant and simple method for studying food chains promises to have wide-ranging effects in many areas of the life sciences. At a time when students of the past are turning to the view that a preoccupation with the stomach was, after all, the most important force that shaped human evolution, this comes as good news for archaeologists.

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"The computer can talk to terminals all over the country. Bentley is convinced it's talking about him."