STABLE ISOTOPES IN PALEONTOLOGY AND ARCHEOLOGY

INTRODUCTION

The isotopic composition of a given element in living tissue depends on: (1) the source of that element (e.g., atmospheric CO$_2$ versus dissolved CO$_2$; seawater O$_2$ vs. meteoric water O$_2$), (2) the processes involved in initially fixing the element in organic matter (e.g., C$_3$ vs. C$_4$ photosynthesis), (3) subsequent fractionations as the organic matter passes up the food web. Besides these factors, the isotopic composition of fossil material will depend on any isotopic changes associated with diagenesis, including microbial decomposition. In this lecture, we will see how this may be inverted to provide insights into the food sources of fossil organisms, including man. This, in turn, provides evidence about the environment in which these organisms lived.

ISOTOPES AND DIET: YOU ARE WHAT YOU EAT

In Lecture 29 we saw that isotope ratios of carbon and nitrogen are fractionated during primary production of organic matter. Terrestrial C$_3$ plants have $\delta^{13}$C values between -23 and -34‰, with an average of about -27‰. The C$_4$ pathway involves a much smaller fractionation, so that C$_4$ plants have $\delta^{13}$C between -9 and -17‰, with an average of about -13‰. Marine plants, which are all C$_3$, can utilize dissolved bicarbonate as well as dissolved CO$_2$. Seawater bicarbonate is about 8.5‰ heavier than atmospheric CO$_2$; as a result, marine plants average about 7.5‰ heavier than terrestrial C$_3$ plants. In contrast to the relatively (but not perfectly) uniform isotopic composition of atmospheric CO$_2$, the carbon isotopic composition of seawater carbonate varies due to biological processes. Because the source of the carbon they fix is more variable, the isotopic composition of marine plants is also more variable. Finally, marine cyanobacteria (blue-green algae) tend to fractionate carbon isotopes less during photosynthesis than do true marine plants, so they tend to average 2 to 3 ‰ higher in $\delta^{13}$C.

Nitrogen isotopes are, as we saw, also fractionated during primary uptake. Based on their source of nitrogen, plants may also be divided into two types: those that can utilize N$_2$ directly, and those utilize only “fixed” nitrogen as ammonia and nitrate. The former include the legumes (e.g., beans, peas, etc.) and marine cyanobacteria. The legumes, which are exclusively C$_3$ plants, utilize both N$_2$ (through symbiotic nitrogen-fixing bacteria in their roots) and fixed nitrogen, and have an average $\delta^{15}$N of +1‰, whereas modern nonleguminous plants average about +3‰. However, it seems likely that prehistoric nonleguminous plants were more positive, averaging perhaps +9‰, because the isotopic composition of present soil nitrogen has been affected by the use of chemical fertilizers. For both groups, there was probably a range in $\delta^{15}$N of ±4 or 5‰, because the isotopic composition of soil nitrogen varies and there is some fractionation involved in uptake.

![Figure 35.1](image-url)
Marine plants have $\delta^{15}N$ of $+7 \pm 5\%_o$, whereas marine cyanobacteria have $\delta^{15}N$ of $-1\pm 3\%_o$. Figure 35.1 summarizes the isotopic composition of nitrogen and carbon in the various classes of photosynthetic organisms (autotrophs).

DeNiro and Epstein (1978) studied the relationship between the carbon isotopic composition of animals and their diet. (Most of the animals in this study were perhaps of little direct paleontological interest, being small and soft-bodied. DeNiro and Epstein studied small animals for a practical reason: they are easier to analyze than a large animal such as a horse.) Figure 35.2 shows that there is little further fractionation of carbon by animals and thus the carbon isotopic composition of animal tissue closely reflects that of their diet. Typically, carbon in animal tissue is about $1\%_o$ heavier than their diet. Mice, although not analyzed whole and not shown in Figure 35.2, were also included in the study. Various tissues from mice had $\delta^{13}C$ within $\pm 2\%_o$ of their diet, so the relationships in Figure 35.2 extend to vertebrates as well. DeNiro and Epstein found that the same species has a different isotopic composition when fed a different diet. Conversely, different species had similar isotopic compositions when fed the same diet. Thus diet seems to be the primary control on the isotopic composition of animals.

The small fractionation between animal tissue and diet is a result of the slightly weaker bond formed by $^{12}C$ compared to $^{13}C$. The weaker bonds are more readily broken during respiration, and, not surprisingly, the CO$_2$ respired by most animals investigated was slightly lighter than their diet. Thus only a small fractionation in carbon isotopes occurs as organic carbon passes up the food chain, about $+1\%_o$ at each step in the chain. Terrestrial food chains are usually not more than 3 trophic levels long, implying a maximum further fractionation of $+3\%_o$. Marine food chains can have up to 7 trophic levels, implying a maximum carbon isotopic difference between primary producers and top predators of $7\%_o$. These differences are smaller than the range observed in primary producers. In a similar study, DeNiro and Epstein (1981) found that $\delta^{15}N$ of animal tissue is related to the $\delta^{15}N$ of the animal’s diet, but is typically 3 to $4\%_o$ higher than that of the diet (Figure 35.3). Thus in contrast to carbon, significant fractionation of nitrogen isotopes will occur as nitrogen passes up the food chain.

Schoeninger and DeNiro (1984) studied the carbon and nitrogen isotopic composition of bone collagen in
animals. Their findings reflected just the relationships expected from the work of DeNiro and Epstein (1978, 1981): in primary herbivores, carbon in bone collagen was a few per mil heavier than the isotopic composition of plants, and $\delta^{15}N$ increased by about 3‰ at each trophic level. Marine vertebrates tend to have more positive $\delta^{15}N$ than do their terrestrial counterparts because they generally feed at a higher trophic level. The primary produces in the ocean are generally microscopic algae. Most marine herbivores are also microscopic (zooplankton); there are very few marine vertebrate herbivores (anchovies are an example). Most terrestrial food chains have only three levels: primary producers, herbivores, and predators. Marine food chains, by contrast, can have up to seven levels. Since nitrogen isotope fractionation occurs at each level, the top marine predators have more positive $\delta^{15}N$. These relationships are summarized in Figure 35.4.

Apatite in bone appears to undergo isotopic exchange with meteoric water once it is buried, but bone collagen and tooth enamel appear to be robust and retain their original isotopic compositions. Tooth enamel, in which carbon is present as carbonate, however, is systematically 12 to 15‰ heavier than carbon in organic tissue. (Such a fractionation is, of course, expected, and consistent with the observation that carbonate is always heavier than organic carbon.) Collagen typically has carbon about 4‰ heavier than diet. These results mean that the nitrogen and carbon isotopic composition of fossil bones and teeth can be used to reconstruct the diet of fossil animals.

**Carbon Isotopes and the Evolution of Horses and Grasslands**

Horses (Family Equidae) have been around for 58 million years. Beginning in the early Miocene, a major radiation took place and the number of genera in North America increased from three at 25 Ma to twelve at 10 Ma. It subsequently fell at the end of the Miocene, and the last North American species became extinct in the Holocene. A major change in dental morphology, from low-crowned to high crowned, accompanied the Miocene radiation. For nearly 100 years, the standard textbook explanation of this dental change was that associated with a change in feeding from leaf browsing to grass grazing. Grasses contain enough silica to make them quite abrasive, thus a high crowned tooth would last longer in a grazing animal and would therefore be favored in horse’s evolution as it switched food sources. The change in horse diet was thought to reflect the evolution of grassland ecosystems (or bi-
omes). This line of reasoning led to the conclusion that grasslands first became important biomes in the Miocene.

Carbon isotope ratios provide the first opportunity to test this hypothesis. Grasses of tropical and temperate regions are almost exclusively $C_4$ plants. $C_3$ grasslands occur only in high latitude regions. In the North American prairie, for example, $C_4$ grasslands become important only north of the US-Canadian border (Figure 35.5). The appearance of grasslands inferred from the evolution of horse teeth implies a change from a predominantly $C_3$ to a predominantly $C_4$ photosynthetic pathway and a corresponding shift in the $\delta^{13}C$ of the biomass in these regions. Since the carbon isotopic composition of animals reflects that of their diet, and since the $\delta^{13}C$ of dental enamel appears to record the $\delta^{13}C$ of the animal, the change in horse dentition should also be reflected in a change in the carbon isotopic composition of those teeth if the change in dentition were related to a change in diet.

Wang et al. (1994) carried out such a test by analyzing the carbon isotopic composition of dental enamel from fossil horse teeth of Eocene through Pleistocene age. They found a sharp shift in the isotopic composition of the teeth consistent with a change in diet from $C_3$ to $C_4$ vegetation, but it occurred later than the change in dental morphology (Figure 35.6). The change in dental morphology begins in the mid-Miocene (about 18 Ma), while shift in $\delta^{13}C$ occurs at around 7 Ma. This leads to an interesting dilemma. Which change, that in morphology or that in carbon isotopic composition, actually reflects the appearance of the grassland biome? It is possible that the change in dental morphology is unrelated to the evolution of grasslands? If that is the case, it is difficult to understand the change in dental mor-
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Figure 35.6. δ13C and crown height in North American fossil horses as a function of age. From Wang et al. (1994).

phyology. Alternatively, grasslands may have appeared in mid-Miocene and only subsequently become dominated by C₄ grasses.

If the latter interpretation is correct, it raises the question of what evolutionary pressure caused the change from C₃ to C₄ photosynthesis in tropical and temperate grasses. An important observation in that respect is that C₄ grasslands appear to have become important in both North America and Asia at about the same time (7 Ma). Indeed, the first evidence for a shift from C₃ dominant to C₄ dominant ecosystems came from an observed change in the δ13C of soil carbonate in Pakistan (Quade et al., 1989). Quade et al. (1989) first interpreted this as a response to the uplift of the Tibetan Plateau and the development of the Monsoon. However, other evidence, including oxygen isotope data from Pakistani soil carbonates, suggests the Monsoons developed about a million years earlier (about 8 Ma). The synchronicity of the dominance of C₄ grasslands in Asia and North America (Figure 35.7) suggests a global cause, while the Monsoons are a regional phenomenon.

Though there has been some speculation that the C₄ photosynthetic pathway may have evolved as early as the Cretaceous, the oldest direct fossil evidence for C₄ plants (plants with enlarged bundlesheath cells) is late Miocene; i.e., the same age as the observed δ13C increase. Thus the isotopic shift may date the evolution of C₄ photosynthesis. C₄ photosynthesis involves only relatively minor modification of plant enzymes and structures and it occurs in diverse, distantly related families. It may, therefore, have evolved independently in many families (Ehleringer, et al. 1991). This also suggests some global environmental change that favored C₄ photosynthesis.

Several groups have now suggested that the appearance of C₄ grasses reflects a drop in the concentration of atmospheric CO₂ in the Miocene. In the C₃ photosynthetic pathway, Rubisco can catalyze not only the fixation of carbon in phosphoglycerate, but also the reverse reaction where CO₂ is released, a process called photorespiration. When concentrations of CO₂ are high, the forward reaction is favored and the C₃ pathway is more efficient overall than the C₄ path-

Figure 35.7. δ13C in carbonates from paleosols of the Potwar Plateauin Pakistan. The change in δ13C may reflect the evolution of C₄ plants. From Quade et al. (1989).
way. At low CO₂ concentrations, however, the C₄ pathway, in which CO₂ is first transported into bundle- sheath cells, is more efficient, as the concentration in bundle-sheath cells is maintained at around 1000 ppm (Figure 35.8). Thus under present conditions, C₄ plants have a competitive advantage. At higher CO₂ conditions, C₃ plants are more efficient. There is some evidence that Eocene CO₂ concentrations were much higher than present (perhaps 800 ppm as opposed to 250 ppm pre-Industrial Revolution), and that concentrations dropped dramatically during the Miocene. Such a drop would give C₄ plants a competitive advantage. This would be particularly true in the warm climates where C₃ plants dominate because the rate of photorespiration is temperature dependent.

**ISOTOPES AND PALEODIETS**

The differences in nitrogen and carbon isotopic composition of various foodstuffs and the preservation of these isotope ratios in bone collagen provides a means of determining what ancient peoples ate. In the first investigation of bone collagen in human remains, DeNiro and Epstein (1981) concluded that Indians of the Tehuacan Valley in Mexico probably depended heavily on maize (a C₄ plant) as early as 4000 BC, whereas archeological investigations had concluded maize did not become important in their diet until perhaps 1500 BC (Figure 35.9a). In addition, there seemed to be steady increase in the dependence on legumes (probably beans) from 6000 BC to 1000 AD and a more marked increase in legumes in the diet after 1000 AD (Figure 35.9b).

Mashed grain and vegetable charred onto pot sherds provides an additional record of the diets of ancient peoples. From Ehleringer et al. (1991).

![Figure 35.8. Rate of photosynthesis as a function of intercellular CO₂ concentrations in C₄ and C₃ plants. At concentrations of atmospheric CO₂ that prevailed before the Industrial Revolution, C₄ plants would have had a competitive advantage. At concentrations above the present level, C₃ plants are more efficient. From Ehleringer et al. (1991).](image-url)

![Figure 35.9. δ¹³C and δ¹⁵N in human bone collagen (open symbols) and calculated values in the diet (closed symbols) of the Tehuacan Indians as a function of age. The δ¹³C data indicate a predominance of C₄ plants (probably maize) in all phases after the El Riego period. The δ¹⁵N data indicate the importance of legumes (beans?) in the diet became increasingly important with time. After DeNiro and Epstein (1981).](image-url)
ancient peoples. DeNiro and Hasdorf (1985) found that vegetable matter subjected to conditions similar to burial in soil underwent large shifts in δ15N and δ13C but that vegetable matter that was burned or charred did not. The carbonization (charring, burning) process itself produced only small (2 or 3‰) fractionations. Since these fractionations are smaller than the range of isotopic compositions in various plant groups, they are of little significance. In the process of cooking, plant remains can become charred onto the pots in which they are cooked. Since pot sherds are among the most common artifacts recovered in archaeological sites, this provides a second value means of reconstructing the diets of ancient peoples.

Figure 35.10 summarizes the results obtained in a number of studies of bone collagen and pot sherds (DeNiro, 1987). Studies of several historical populations, including Eskimos and the Tlingit Indians of the Northwest US, were made as a control. The isotope data show that the diet of Neolithic Europeans consisted entirely of C3 plants and herbivores feeding on C3 plants, in contrast to the Tehuacan Indians, who depended mainly on C4 plants. Prehistoric peoples of the Bahamas and Denmark depended both on fish and on agriculture. In the case of Mesolithic Denmark, other evidence indicates the crops were C3, and the isotope data bear this out. Although there is no corroborating evidence, the isotope data suggest the Bahamains also depended on C3 rather than C4 plants. The Bahamains had lower δ15N because the marine component of their diet came mainly from coral reefs. Nitrogen fixation is particularly intense on coral reefs, which leads to 15N depletion of the water, and consequently, of reef organisms.

**Carbon Isotopes and the Earliest Life**

We saw in Lecture 29 that carbon fixation, whether through chemosynthesis or photosynthesis, produces a large negative isotopic fractionation of carbon. This large fractionation occurs in the Benson-Calvin cycle that is common to all autotrophs. Consequently, δ13C values of -20‰ or less in reduced carbon compounds is generally interpreted as evidence of biologic origin of those compounds. Schidlowski (1988) first reported δ13C as low as -26‰ in samples from Godthåbfsford region of West Greenland that are ostensibly older than 3.5 Ga. In 1996, Mojsis and others reported δ13C between -20 to -50‰ graphite inclusions in grains of apatite in 3.85 Ga in banded-iron formations (BIFs) from Akilia Island in that same area of West Greenland. In 1999, Rosing reported δ13C of -19‰ from graphite in tubiditic and pelagic metasedimentary rocks from the Isua greenstone belt in the same area. These rocks are thought to be older than 3.7 Ga.
Schidlowski (1988, Mojzsis et al. (1996), and Rosing (1999) interpreted these negative δ^{13}C as evidence of a biogenic origin of the carbon, and therefore that life existed on Earth at this time. This is older that the earliest physical fossil evidence of life, which occurs in rocks 3.5 Ga old. Furthermore, the earliest part of this time coincides with the late heavy bombardment of the Moon, and a similar asteroid bombardment is thought to have affected the Earth at this time – presumably, making life difficult for an organisms that did exist. Perhaps not surprising, the interpretation of life older than 3.7 Ga has been controversial.

There are several reasons for the controversy, but all ultimately relate to the extremely complex geological history of the area. The geology of the Godthåbsfjord region includes not only the early Archean Isua greenstone belt and Itsaq gneiss complex, but also rocks of middle and late Archean age as well. Most rocks are multiply and highly deformed and metamorphosed and the exact nature, relationships, and structure of the precursor rocks are difficult to decipher.

Rosing et al. (1996) demonstrated that at least some of the carbonates sampled by Schidlowski (1988) cross-cut pre-existing lamination and argued that they are veins deposited by metamorphic fluid flow. Van Zuilen et al. (2003) argue that the graphite originated by thermal decomposition of siderite, in reactions such as:

\[ 3 \text{FeCO}_3 = \text{Fe}_3\text{O}_4 + 2\text{CO}_2 + \text{CO} \]

followed by disproportionation of the CO:

\[ 2 \text{CO} = \text{C} + \text{CO}_2 \]

The difference in δ^{13}C they observed between the graphite and carbonate phases, roughly 5 to 10‰, is consistent with equilibrium fractionation in the range of 300-600°C.

On Akilia Island, the locality studied by Mojzsis et al. (1996), the predominant Amitsoq gneisses are metamorphosed dioritic to granitic plutons that surround fragments of metasedimentary rocks which include conglomerates, BIFs, metacherts, and felsic metasediments with graded bedding. The precursors are thought to have been deposited in a shallow marine environment; they were subsequently metamorphosed to nearly granulite facies. Both the ortho- and paragneisses were intruded by the maﬁc Ameralik dikes, which are now amphibolites. The region was again deformed, folded and metamorphosed in the late Archean. Structural relationships are unclear and although some dates are as old as 3.87 Ga, the age of the metasediments is far less clear. Indeed, the apatites themselves have Pb-Pb ages of 1.5 Ga, but it is unclear whether this is a crystallization or metamorphic age. Even if this is a recrystallization age, it is possible that (1) the depositional age of the metasediments is as young as 3.6 or 3.7 Ga and (2) the apatites and their graphite inclusions are secondary and grew during the early Archean metamorphism.

Samples studied by Rosing (1999) come from some of the least deformed rocks in the region. The rocks have been metamorphosed only to schist facies and original sedimentary structures are apparent. The δ^{34}S in these rocks is fairly uniform, and distinctly more negative than δ^{34}S of graphite (-11‰) and carbonate (-2.5‰) from carbonate veins in the region. Rosing argues that not only that the negative δ^{34}S is evidence of a biogenic origin of the carbon, but, from sedimentological considerations, that source of the carbon was photosynthetic planktonic organisms. Although Rosing (1999) reported a Sm-Nd age of 3.78±0.81 Ga for the sediments and the volcanic rocks upon which they were deposited, the scatter in the isochron is relatively large. Cross-cutting intrusive rocks, however, are well dated at 3.71 Ga and provide a minimum age.

While Rosing’s data do perhaps reflect the best case for life from the Isua area, this age remains 160 Ma younger than the age claimed by Mojzsis et al. (1996). Furthermore, δ^{34}S values are not as negative as the data of Mojzsis (1996) and it remains unclear whether the fractionation of ~16.5‰ between the graphite and local carbonate might have resulted from abiologic processes.
REFERENCES AND SUGGESTIONS FOR FURTHER READING


