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12 Stable Isotope Compositions of Biological Apatite

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INTRODUCTION

The stable isotope compositions of biogenic materials record a combination of environmental parameters and biological processes. In general, the environment provides a range of isotopic compositional inputs, and an animal processes those signals through dietary preference, physiology, behavior, etc. Geochemists then use isotope signals preserved in biogenic materials to infer either what the biologic filter was (i.e., a species-specific biologic process), or the environment in which the animal lived (e.g., see review of Koch 1998). Although stable isotopes of hydrogen, carbon, nitrogen, oxygen, and strontium in modern-day animals have provided fruitful information on environment or biology, preservation of hydrogen and nitrogen is poor for most fossil materials, especially those older than a few million years. Consequently, nearly all stable isotope studies focus on the best preserved tissues, which are biological apatites (or bioapatites) - bone, dentin, enamel, scales, etc. - and on the most diagenetically resistant isotopes - oxygen, carbon, and occasionally strontium, which occur as principal or substitutional components in bioapatite. Because of the inherent synergy between biology and environment, the scientific scope of stable isotope research on bioapatites is quite broad. In addition to studies of terrestrial paleoclimate and dietary preference, stable isotopes in bioapatite have helped elucidate such diverse issues as dinosaur thermoregulation (Barrick and Showers 1994, 1995; Barrick et al. 1996, 1998; Fricke and Rogers 2000), the global rise of C_4 plants (Cerling et al. 1993, 1997), pinniped migration (Burton and Koch 1999), cetacean osmoregulation (Thewissen et al. 1996, Roe et al. 1998), herding practices (Bocherens et al. 2001), topographic uplift (Dettman et al. 2001, Kohn et al. 2003), the demise of Norse colonies in Greenland (Fricke et al. 1995, Arneborg et al. 1999), Miocene "rhinoceros" ecology (MacFadden 1998), and mastodon migration (Hoppe et al., 1999)! In this chapter, we first describe some analytical basics, and discuss the different materials that can be analyzed, focusing on special concerns when dealing with fossils. We then outline the principles underlying C, O, and Sr isotopic variations in bioapatites, and conclude with a few examples from the literature.

ANALYSIS

CO_3 component

The CO_3 component of bioapatites is analyzed for C and O isotopes by dissolution in H_3PO_4 (McArthur et al. 1980, Land et al. 1980), similar to the analysis of carbonates (McCrea 1950). Phosphates contain several CO_3 components, including structural CO_3 that substitutes for PO_4 and OH, as well as "labile" components whose structural identity is ambiguous (e.g., Rey et al. 1991). Only the PO_4- and OH-substituting CO_3 is believed to have any diagenetic resistance, so the labile CO_3 is removed by acid pretreatment,

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usually in a (buffered) acetic acid solution (e.g., see Koch et al. 1997), which also removes any calcite or dolomite contamination. Dissolution of the purified bioapatite ordinarily produces ~0.5 μmole of CO_2/mg, so commonly ~1 mg of bioapatite is analyzed in dual-inlet machines. Sulfur compounds may be liberated by reaction of contaminants in fossils, and their masses can interfere with those of CO_2, biasing analyses. Sulfur contamination can be avoided if Ag metal is present during dissolution or (in off-line systems) by reacting the mixed CO_2 and sulfur gases with Ag foil (Cerling and Sharp 1996; MacFadden et al. 1996).

PO_4 component

The PO_4 component is more difficult to analyze, and several decades of research have been required to develop techniques for its routine O isotope analysis. PO_4 is separated from other O-bearing components by dissolution in acid (usually HNO_3 or HF), and further purified on ion exchange columns and/or directly chemically processed to produce either $BiPO_4$ (Tudge 1960; Longinelli 1965; Longinelli and Nuti 1973a,b; Longinelli et al. 1976; Kolodny et al. 1983; Karhu and Epstein 1986) or Ag_3PO_4 (Firsching 1961, Wright and Hoering 1989, Crowson et al. 1991, Lécuyer et al. 1993, O'Neil et al. 1994, Dettman et al. 2001). Ag_3PO_4 is now the material of choice because it is much less hydroscopic (Wright and Hoering 1989) and conveniently decomposes at > 1000 ˚C to produce O_2 gas (Anbar et al. 1960, O'Neil et al. 1994). The $BiPO_4$ or Ag_3PO_4 is then fluorinated (Longinelli 1965, Longinelli and Sordi 1966, Luz et al. 1984a, Wright and Hoering 1989, Crowson et al. 1991), brominated (Stuart-Williams and Schwarcz 1995), or thermally decomposed in the presence of C using furnaces or lasers, to produce either CO_2 or CO (O'Neil et al. 1994, Farquhar et al. 1997, Wenzel et al. 2000, Vennemann et al. 2002), which is then analyzed in a mass spectrometer. The amount of Ag_3PO_4 needed for analysis ranges from <<1 to ~20 mg. Although small quantities of Ag_3PO_4 appear to be precisely measurable by continuous-flow mass spectrometry, it is unclear whether small amounts of PO_4 can be reproducibly precipitated as Ag_3PO_4.

Combined/bulk components. Bioapatites may also be analyzed in bulk for O and/or C isotopes via laser fluorination (CO_2 laser: Kohn et al. 1996; UV laser: Jones et al. 1999), direct thermal decomposition (Cerling and Sharp 1996, Sharp and Cerling 1996, Sharp et al. 2000), or ion probe (Eiler et al. 1997). These techniques all offer reduced sample size, and the ion probe and UV laser accommodate *in situ* analysis. Bulk analysis has disadvantages that include accuracy corrections (Kohn et al. 1996, Eiler et al. 1997), pretreatment requirements that can potentially bias compositions (Lindars et al. 2001), and, most importantly, a difficulty in assessing diagenetic alteration and contamination of fossils. Although ~85% of oxygen in bioapatite is P-bound, bulk analysis includes all other oxygen components (OH, CO_3, diagenetic oxides and silicates, etc.). If different components react differently to diagenesis, then samples will be isotopically biased, possibly by several permil, depending on the diagenetic environment (Kohn et al. 1999).

MATERIALS AND DIAGENESIS

Basically, five bioapatite materials may be analyzed: bone, dentin, enamel(oid), fish scales, and invertebrate shells. Cementum is very rarely analyzed. Each material has its proponents and critics, in part depending on whether PO_4 or CO_3 is analyzed. The mineralogy of bone, dentin, and enamel is reviewed in Elliott (this volume), and crystallite size is briefly discussed here because it influences diagenetic susceptibility. Bones and shells are extremely common, but small crystallite size (a few nm wide, a few tens of nm long, and possibly less than 1 nm thick) and high porosity and organic content (for bone) make them extremely susceptible to recrystallization and isotopic alteration. Different types of bone do have different porosities, however, and cancellous ("spongy")

bone is likely far more susceptible to alteration than cortical ("compact") bone (e.g., Sealy et al. 1991). Dentin has a similar crystallite size to bone, but much lower porosity, potentially reducing susceptibility to alteration. In contrast to dentin and bone, enamel is extremely compact and has larger crystallites (tens of nm wide and thick, and possibly hundreds of nm long), so it is least likely to be affected diagenetically. Fish scales are rarely analyzed (but see Fricke et al. 1998a), so diagenetic concerns are less clear.

The different chemical components in bioapatite also exhibit different chemical susceptibilities to alteration. F-, U- and REE-substitution for OH and Ca is thousands of times greater in fossils than in original bioapatites (e.g., see Tuross and Trueman, this volume) attesting to extreme alteration potential for some components, but at issue is whether other chemical constituents of bioapatite are affected, specifically heavy isotope ratios, and the CO_3 and PO_4 components. Because of the strength of P-O bonds, the PO_4 component is widely believed to have great resistance to diagenetic alteration, whereas the CO_3 component is less resistant (Shemesh et al. 1983; Kolodny et al. 1983; Luz et al. 1984b; Nelson et al. 1986; Kolodny and Luz 1991; Barrick and Showers 1994, 1995; Barrick et al. 1996; Fricke et al. 1998a; Kohn et al. 1999). Bulk strontium is known to be altered in virtually all fossil materials, and retrieval of a biologic signal rests solely on a chemical separation of components, based on differential solubilities.

Identifying physical or chemical alteration of bioapatite is extremely contentious and scientifically polarized, as some research is based on either the retrieval of pristine biogenic signals, or the assumption that diagenetic alteration overwhelms original compositions. Table 1 summarizes 46 studies of diagenesis. Generally six diagenetic tests have been applied:

(1) Do different samples from a single locality exhibit unexpected compositional heterogeneity or homogeneity? Depending on the environment and species, different animals may be expected to have similar or different compositions, and so unexpected compositional heterogeneity or homogeneity has been explained by diagenetic alteration. Such an approach is, of course, predicated on a previous knowledge of "normal" variability. The growth of different tissues at different times in an animal may compromise definitive identification of diagenesis. In the case of Bryant et al. (1994), a compositional difference between enamel and bone from the same quarry was ascribed to diagenetic alteration of enamel, whereas later Bryant et al. (1996) interpreted compositional variation in enamel from different teeth as reflecting excellent preservation of biological signals. The reason for this interpretational disparity was Bryant et al.'s later realization that enamel *should* preserve compositional variation and could exhibit compositional differences compared to other tissues. Generally, interpretations based on homogeneity and/or heterogeneity have suggested that bone, dentin, and cementum can be readily altered even for Pleistocene samples (Ayliffe et al. 1992), whereas there is no evidence for alteration of enamel CO_3 or PO_4 (Ayliffe et al. 1992, Bryant et al. 1996). Extremely young bone can sometimes preserve original CO_3 and PO_4 isotopic compositions (Iacumin et al. 1996a). Work by Barrick and coworkers suggests that cortical bone PO_4 may preserve isotopic integrity, even for Cretaceous samples (Barrick 1998; Barrick and Showers 1994, 1995, 1999; Barrick et al. 1996).

(2) Do different sympatric animals preserve expected isotopic differences? Some animals have known isotopic compositional differences, because of diet (for C) or habitat (terrestrial vs. marine, for Sr), and a change in the magnitude of original compositional offset is ascribed to alteration. Bone CO_3 $\delta^{13}C$ can be affected for Pleistocene and younger samples (Nelson et al. 1986, Lee-Thorpe and van der Merwe 1991), although some Pleistocene samples apparently retain original com-

Table 1. Summary of diagenetic tests and results.

Test	*Summary of results*	*References*
Multi-sample heterogeneity or homogeneity	Pleistocene bone, dentin and cementum PO_4 altered, but Cretaceous bone PO_4 may be unaltered. No evidence for enamel PO_4 alteration	1, 2, 4-8 11, 12, 20, 22
Different sympatric animals retain expected isotopic differences	Bone and dentin CO_3 affected for Pliocene samples, no evidence for enamel CO_3 alteration. Sr in Miocene teeth is not preserved.	3, 10, 13, 15, 16, 26, 30, 32, 33, 36, 43, 46
Different tissues from a single animal retain biological fractionations	Bone CO_3 altered for archeological and Pleistocene bulk samples, and for some pretreated Pliocene samples; Pleistocene bone PO_4 and dentin PO_4 and CO_3 may be altered; no evidence for enamel CO_3 or PO_4 alteration	1, 2, 11, 14, 23, 25, 26, 28, 29, 30, 37, 39, 40, 43, 45
Crystallinity	Bone altered within years; Pleistocene dentin may be altered; enamel unaltered	2, 9, 11, 14, 19, 27, 30, 31, 34, 35, 38, 41, 42, 44
Comparison to surrounding sediment or expected value	Some Pleistocene bone CO_3 altered; some Plio-Pleistocene bone PO_4 may be altered, but Cretaceous bone PO_4 may be unaltered; bone Sr altered immediately; late Pliocene bulk tooth Sr altered	3, 4-8, 24, 28, 29, 30, 33, 36, 37, 42, 44, 45
Isotopic correlation between different chemical components	Most bone CO_3 altered, but some Recent bone CO_3 unaltered; Eocene enamel CO_3 may be altered	4-8, 17, 18, 20, 21

References: 1. Ayliffe et al. (1992); 2. Ayliffe et al. (1994); 3. Barrat et al. (2000); 4. Barrick (1998); 5. Barrick and Showers (1994); 6. Barrick and Showers (1995); 7. Barrick and Showers (1999); 8. Barrick et al. (1996); 9. Bartsiokas and Middleton (1992); 10. Bocherens et al. (1996); 11. Bryant et al. (1994); 12. Bryant et al. (1996); 13. Cerling et al. (1997); 14. Delgado-Huertas et al. (1997); 15. Ericson et al. (1981); 16. Feranec and MacFadden (2000); 17. Fox and Fisher (2001); 18. Fricke et al. (1998a); 19. Hedges et al. (1995); 20. Iacumin et al. (1996a); 21. Iacumin et al. (1996b); 22. Iacumin et al. (1996c); 23. Iacumin et al. (1997); 24. Koch et al. (1992); 25. Koch et al. (1997); 26. Koch et al. (1998); 27. Kyle (1986); 28. Land et al. (1980); 29. Lee-Thorpe and van der Merwe (1987); 30. Lee-Thorpe and van der Merwe (1991); 31. Michel et al. (1996); 32. Morgan et al. (1994); 33. Nelson et al. (1986); 34. Person et al. (1995); 35. Person et al. (1996); 36. Quade et al. (1992); 37. Sánchez-Chillón et al. (1994); 38. Schoeninger (1982); 39. Schoeninger and DeNiro (1982); 40. Sharp et al. (2000); 41. Sillen (1986); 42. Stuart-Williams et al. (1996); 43. Sullivan and Krueger (1981); 44. Tuross et al. (1989); 45. Wang and Cerling (1994); 46. Zazzo et al. (2000).

positions (Ericson et al. 1981, Sullivan and Krueger 1981, Bocherens et al. 1996). There is no evidence for isotopic alteration of enamel CO_3 for samples up to Miocene in age (Quade et al. 1992, Morgan et al. 1994, Bocherens et al. 1996, Cerling et al. 1997, Koch et al. 1998, Feranec and MacFadden 2000, Zazzo et al. 2000). Strontium isotopes are affected for Miocene teeth (Barrat et al. 2000).

(3) Do different tissues from the same individual retain biological fractionations? Different tissues have known isotopic offsets in modern samples. Intercomparison of different tissues (e.g., $\delta^{13}C$ of collagen vs. bone CO_3) indicates that bone and possibly dentin CO_3 is already altered for Pleistocene samples (Land et al. 1980, Lee-Thorpe and van der Merwe 1991), although some Plio-Pleistocene bone CO_3 may preserve isotopic integrity (Ericson et al. 1981; Sullivan and Krueger 1981; Lee-Thorpe and van der Merwe 1987). Pleistocene bone, dentin, and cementum PO_4

can also be altered (Ayliffe et al. 1992, 1994; Sánchez-Chillón et al. 1994). There is no evidence for CO_3 or PO_4 alteration in enamel as old as the Oligocene (Wang and Cerling 1994).

(4) Do samples retain biological crystal size distributions? Because of their small size, bioapatite crystallites have a strong tendency to coarsen post-mortem. Because coarser crystals have sharper X-ray diffraction peaks, Shemesh (1990) proposed using peak sharpness as a crystallinity index, or semiquantitative measure of recrystallization. Crystallinity index does depend on sample preparation (Roe et al. 1998), and furthermore a change in crystallinity will not affect oxygen isotopes in PO_4 if it occurs abiotically at T < 75-100 ˚C and does not involve incorporation of exogenous material (Tudge 1960; Kolodny et al. 1983; Blake et al. 1997, 1998; Lécuyer et al 1999). Nonetheless bone undergoes recrystallization within a few years post-mortem (Tuross et al. 1989), and dentin and cementum are affected in Pleistocene samples (Ayliffe et al. 1994; Fig. 1). Fresh enamel is essentially fully crystalline, and so no change in crystallinity is apparent (Ayliffe et al. 1994; Fig. 1).

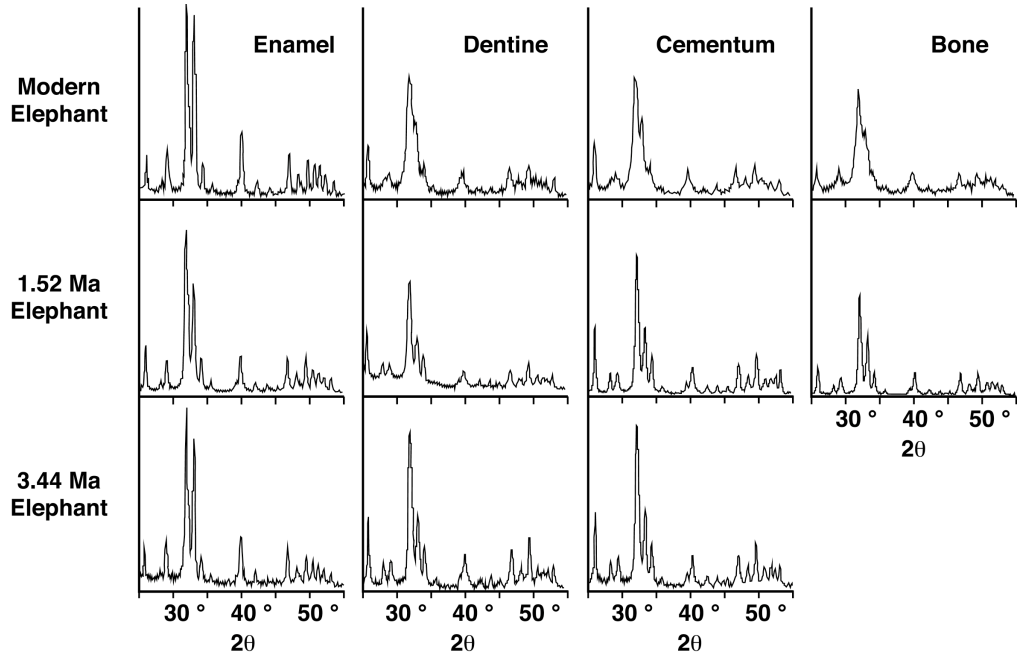


Figure 1. XRD traces of different bioapatite materials from modern and fossil elephants. Fossil dentin, cementum and bone all show a decrease in peak width compared to modern materials, indicating diagenetic recrystallization and coarsening of original apatite. Enamel peaks are essentially unaffected. From Ayliffe et al. (1994).

(5) Do compositions of fossils remain distinct from surrounding sediments or retain a known original composition? Diagenetic alteration is expected to shift the compositions of a fossil towards equilibrium with the surrounding sediment. Such a shift is quite evident for Sr in archeological bone (e.g., Nelson et al. 1986). Marine teeth of known age have a known original Sr isotope composition, and whereas modern fish teeth have isotopic compositions indistinguishable from modern-day seawater (e.g., Schmitz et al. 1991, Vennemann and Hegner 1998, Vennemann et al. 2001), fish teeth as young as Plio-Pleistocene deviate significantly from expected values (Staudigel et al. 1985). Pleistocene or even archeological bone CO_3 can be altered (Land et al. 1980, Schoeninger and DeNiro 1982, Nelson et al. 1986), but Cretaceous cortical bone PO_4 may be unaltered (Barrick 1998; Barrick and Showers

1994, 1995, 1999; Barrick et al. 1996).

(6) Are compositions of different chemical components of the same tissue correlated and do they retain a biological offset? As discussed below, the $\delta^{18}O$ of CO_3 and PO_4 components have a systematic offset that is not commonly retained in bone (Iacumin et al. 1996a) except for Recent materials in extraordinary settings (Iacumin et al. 1996b). Tooth enamel preserves the expected offset for Miocene samples (Fox and Fisher 2001), but not for Eocene samples (Fricke et al. 1998a).

In summary, bone hardly ever preserves original isotopic compositions, except in young and/or exceptionally well-preserved samples, although the work of Barrick and coworkers implies that some bone PO_4 may be diagenetically resistant. The CO_3 component of dentin is not likely to be reliable in samples older than the Plio-Pleistocene. There is no evidence for isotopic change to the PO_4 component of enamel for any sample, and enamel CO_3 is apparently resistant for Oligocene samples, albeit not for some Eocene samples. Strontium isotopes are highly suspect in bone of any age, and in dentin that is Pliocene or perhaps younger in age. Enamel $^{87}Sr/^{86}Sr$ has not been investigated thoroughly, but is likely to be much better preserved than in other tissues. Because enamel and PO_4 are physically and chemically most resistant to alteration, many researchers now restrict analysis and research scope to fossil enamel and/or the PO_4 component. Other researchers persevere with other phosphatic tissues and components, burdened as they are with diagenetic concerns.

CARBON ISOTOPES IN BIOAPATITES

Carbon isotope analysis of bioapatites was first applied to terrestrial mammals in the early 1980s (Land et al. 1980, Ericson et al. 1981, Sullivan and Krueger 1981). The work on fossils by Ericson, Sullivan, Krueger and coworkers was immediately criticized as having ignored diagenesis because results from collagen did not agree with results from bone from archeological sites (Schoeninger and DeNiro 1982). While it is now known that some bone does undergo C-isotope exchange extremely readily, collagen, bone, and enamel record different periods of time in the life of a single individual, and diet may change. That is, there is a fundamental ambiguity (preservation vs. normal intra-individual differences) in interpreting isotopic differences among different tissues. Unfortunately, early results were taken to imply that all bioapatites are unreliable, and it was not until the 1990s that it became accepted that tooth enamel, at least, is a robust recorder of diet. Thus, the early work of Lee-Thorp and van der Merwe (Lee-Thorp and van der Merwe 1987, 1991; Lee-Thorp et al. 1989) was a struggle against the tide of misplaced opinion.

Ecological trends

One interesting story involving carbon isotopes in bioapatites concerns the different photosynthetic pathways used by terrestrial plants (Fig. 2) and the animals that eat them. Carbon-isotope discrimination among plants has been described well in a pair of comprehensive reviews by O'Leary (1988) and Farquhar et al. (1989). The main controls on fractionation are the action of a particular enzyme and the "leakiness" of cells.

Atmospheric CO_2 first moves through the stomata, dissolves into leaf water, and so enters the mesophyll cell. Most dicotyledonous plants (dicots) as well as monocotyledenous plants (monocots) in regions with cool growing seasons use the C_3-photosynthetic pathway. Mesophyll CO_2 is directly combined with ribulose bisphosphate (RuBP - a 5-carbon molecule), in a reaction catalyzed by the enzyme ribulose bisphos-

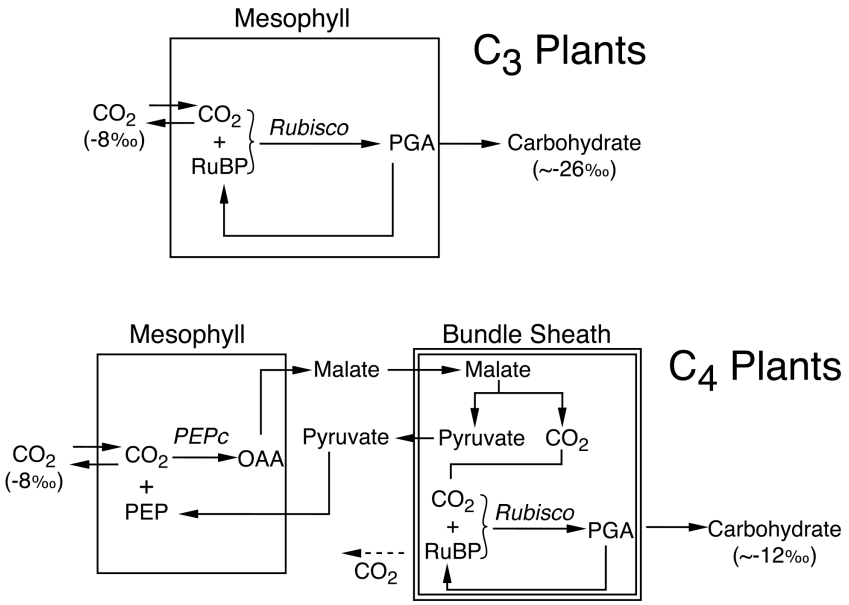


Figure 2. Schematic diagram showing differences in photosynthetic pathways for C_3 vs. C_4 terrestrial plants, which yield different C-isotope fractionations in plant tissue. Phosphoglycerate (PGA), oxaloacetate (OAA), malate, and pyruvate are organic compounds that participate in photosynthesis, and Rubisco and PEPc are enzymes that catalyze reactions among these organic compounds and CO_2. Solid arrows show flow of organic molecules within and across cell; dashed arrow shows that some CO_2 can leak out of bundle sheath cells. See text for more detail. Permil values are all relative to Vienna Peedee Belemnite (V-PDB).

phate carboxylase/oxygenase ("Rubisco"). The resulting 6-carbon molecule is then cleaved into 2 molecules of phosphoglycerate (PGA), each with 3 carbon atoms (hence "C_3"). Most PGA is recycled to make RuBP, but some is used to make carbohydrates. There is fairly free exchange between external and mesophyll CO_2 (i.e., the mesophyll cell is very "leaky") so the large isotope fractionation associated with the carboxylation of RuBP (~-25‰) contributes directly to the isotope composition of end-product phosphoglycerate (PGA) and carbohydrates.

Many monocots use the C_4 photosynthetic pathway. CO_2 in mesophyll cells is first combined with phosphoenolpyruvate (PEP) via the enzyme PEP carboxylase (PEPc) to make the molecule oxaloacetate (OAA), which has 4 carbon atoms (hence "C_4"). The OAA is usually transformed into malate, which is transported into bundle sheath cells and cleaved to pyruvate and CO_2 again. The pyruvate is recycled back into the mesophyll cells to reform PEP. Unlike mesophyll cells, the bundle sheath cells in C_4 plants are able to concentrate CO_2 (i.e., they are not very leaky), so that most of the CO_2 is fixed, and less fractionation occurs in forming PGA. If bundle sheath cells were perfectly gas tight, there would be zero fractionation from Rubisco, whereas if the cells were completely permeable to CO_2, the isotope fractionation from Rubisco would be ~-25‰. In reality, bundle sheath cells can exhibit some "leakiness," so there can be some Rubisco discrimination, but far less than in mesophyl cells.

Other photosynthetic steps also have isotope fractionations, particularly diffusion of CO_2 across stomata (~4‰). However, the isotopic difference between C_3 vs. C_4 plants is largely influenced by the degree to which the Rubisco isotope discrimination is dominant, which depends on the degree to which cells are "CO_2-leaky." Because mesophyll cells are very "leaky" but bundle sheath cells are not, C_3 vs. C_4 plants have ^{13}C depletions of ~18‰ vs. ~4‰ relative to atmospheric CO_2. Therefore, in the modern world ($\delta^{13}C$ of atmospheric CO_2 is -8‰), most C_3 dicots have $\delta^{13}C$ values from about -25 to -30‰

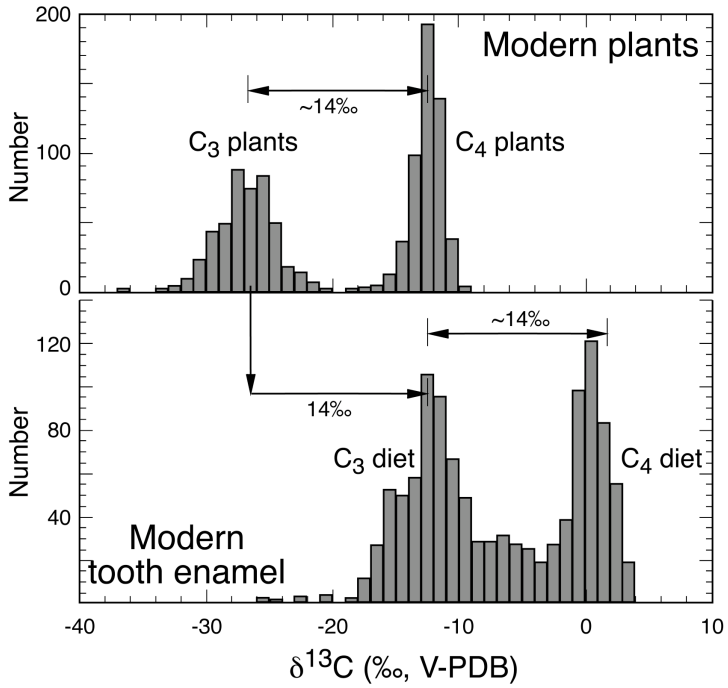


Figure 3. Histograms of $\delta^{13}C$ of modern plants (above) and the CO_3 component of African mammal tooth enamel (below). C_3 plants have an average isotope composition of ~-26‰, whereas C_4 plants cluster ~14‰ higher at ~-12‰. This isotope difference is passed onto herbivores: C_4 consumers have $\delta^{13}C$ values that cluster ~14‰ higher than C_3 consumers (0 to +2‰ vs. ~-12‰). The 14‰ offset between plant and enamel $\delta^{13}C$ (e.g., C_3 plants vs. enamel of C_3 plant consumers) reflects two components: the bulk $\delta^{13}C$ enrichment of an animal over its food source (2-4‰, DeNiro and Epstein 1978) and a large isotopic fractionation between the bulk $\delta^{13}C$ of an animal and the $\delta^{13}C$ of the carbonate component of bioapatite. Modern tooth compositions that fall between C_3 and C_4 diet end-members probably reflect mixed feeding.

(depending on local conditions) whereas most C_4 monocots have $\delta^{13}C$ values between -11 and -13‰ (Fig. 3).

In general, an animal's isotope composition depends on what it consumes (i.e., "You are what you eat" isotopically; DeNiro and Epstein 1976, 1978), and herbivores have dietary preferences-given a choice, some eat monocots, others eat dicots, and still others eat a mix. The resulting dietary C-isotope composition is transmitted to and recorded in all body tissues, including bioapatites. Thus monocot (C_4) consumers in warm regions will have a higher $\delta^{13}C$ than dicot (C_3) consumers, with mixed (C_3 + C_4) feeders in between (Fig. 3). Bone, tooth dentin, and tooth enamel are possible recorders of diet preferences in mammals, but the $\delta^{13}C$ of bioapatite is offset from the $\delta^{13}C$ of animal tissues. Studies of the isotope enrichment from diet to bioapatite are still underway. Field studies of large herbivores suggest an isotopic enrichment of ~14‰ (see summary of Koch 1998; Cerling and Harris 1999), whereas controlled feeding studies of mice, rats, and pigs suggest a smaller enrichment of ~9‰ (see summary of Koch 1998). No single herbivore has been studied both in the laboratory and field to reconcile these differences. The few data for carnivores suggest an isotopic enrichment of ~9‰ (Lee-Thorp et al. 1989). Because most C_4 plants are tropical grasses, at lower latitudes (< ~40° N or S latitude), stable carbon isotopes can readily distinguish between grazing mammals, which consume grass, from browsing mammals, which do not consume grass. At increasingly higher latitudes, temperate grasses use the C_3 photosynthetic mechanism, which ultimately limits identification of browsing vs. grazing.

Other factors can influence the $\delta^{13}C$ of plant tissues and hence bioapatite. CO_2 in closed canopy forests has a lower $\delta^{13}C$ because of degrading low-$\delta^{13}C$ plant material at the forest floor, and this ultimately stratifies the $\delta^{13}C$ of leaves within the forest (e.g., Medina and Minchin 1980, Medina et al. 1986). There are also physiological and isotopic responses to drought, salinity and light levels (e.g., see Farquhar et al. 1989), and ocean organic matter exhibits ^{13}C variations, both latitudinal and onshore vs. offshore, which ultimately impacts the isotopic compositions of marine animals farther up the food chain (Burton and Koch 1999). Because of these links among environment, diet, and isotopic compositions, $\delta^{13}C$ in bioapatite has enjoyed extensive application in a variety of paleoecological and paleodietary studies, including investigation of (a) feeding ecology, specifically C_3 vs. C_4 consumption (Ericson et al. 1981, Lee-Thorp et al. 1994, MacFadden and Cerling 1996, Koch et al. 1998, MacFadden 1998, MacFadden et al. 1999a, Sponheimer and Lee-Thorp 1999, Feranec and MacFadden 2000, Bocherens et al. 2001), (b) other aspects of animal feeding ecology, including marine vs. terrestrial components, canopy effects, etc. (Iacumin et al. 1997, Koch et al. 1998, Roe et al. 1998, Clementz and Koch 2001, Gadbury et al., 2000), and (c) terrestrial ecosystems or environmental conditions, using $\delta^{13}C$ of fossil bioapatites as a monitor (Thackeray et al. 1990; Koch et al. 1992, 1995b; Quade et al. 1992, 1994; Wang et al. 1993, 1994; Bocherens et al. 1996; MacFadden et al. 1996, 1998b; Cerling et al. 1997; Iacumin et al. 1997; Latorre et al. 1997; Sponheimer and Lee-Thorp 1999; Gadbury et al. 2000; MacFadden 2000; Luyt et al. 2000; Zazzo et al. 2000; Passey et al. 2002)

Tooth growth and isotopic zoning

Some phosphatic tissues such as bone can remodel during an animal's lifetime and hence provide an average composition, albeit possibly biased towards seasons of preferential tissue growth and/or bioapatite mineralization (Luz et al. 1990, Stuart-Williams and Schwarcz 1997). However other tissues such as teeth and shells precipitate progressively and are chemically and physically invariant after formation (aside from normal wear). Teeth are particularly well studied, and the following description of tooth growth is based on Hillson (1996). Enamel and dentin first nucleate at the dentin-enamel junction at the crown of a tooth, and grow away from each other (inward for dentin, outward for enamel), towards the tooth base through time (Fig. 4). In both materials an organic matrix seeded with crystallites is laid down, with an average organic content of about 30%. In enamel, the organic matrix is progressively replaced, ultimately creating a dense, compact structure, whereas dentin retains a high organic content. Tubular holes (tubules), ~1 μm in diameter, are moderately abundant in dentin but virtually absent in enamel. Once formed, enamel and dentin are nearly invariant chemically and structurally except for wear, and (unfortunately) dissolution of enamel to form caries. Later dentin can precipitate interior to tubules in some mammal orders (e.g., primates, but not rodents) and on the inner boundary with the pulp chamber.

A single tooth from a small animal may take a few weeks to months to form; a single tooth from a large herbivore such as an elk or horse may require over a year to form fully; and elephant tusks can grow for decades. Thus, by virtue of excellent preservation, teeth have the potential to record seasonal changes in diet long into the geological past. Blood chemistry changes very quickly, so that some tissues (e.g. hair) can record significant changes in diet over a few days time (Cerling and Cook 1998). Although it is attractive to suppose that diets could be recorded with such high fidelity in teeth, each volume of enamel or dentin forms over a significant time period, so that the diet signal is attenuated over weeks or months (Fisher and Fox 1998; Hoppe et al. 2001, Passey and Cerling, 2002). Despite these limitations, useful seasonal isotope changes have been identified using tooth enamel and dentin (Koch et al. 1989, 1998; Cerling and Sharp 1996; Fricke and O'Neil 1996; Sharp and Cerling 1996; Fricke et al. 1998a,b; Kohn et al. 1998, 2003;

Sharp and Cerling 1998; Feranec and MacFadden 2000, Gadbury et al. 2000, MacFadden 2000, Dettman et al. 2001; Bocherens et al. 2001; Fox and Fisher 2001).

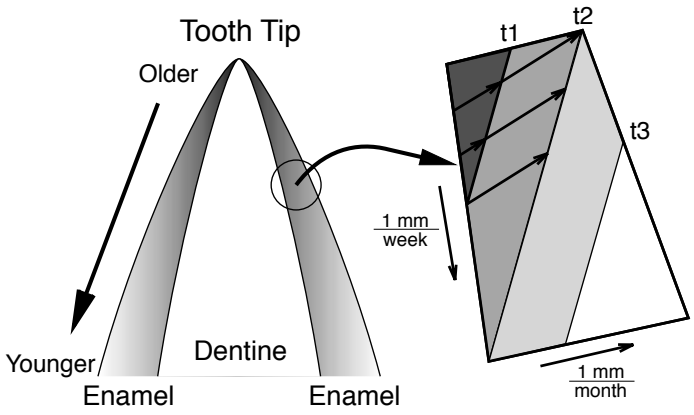


Figure 4. Sketch of how teeth from large herbivores grow. Enamel nucleates at dentin-enamel interface (DEI) at crown of tooth, and grows downward and outward through time. Crown-root growth rate is ~1 mm/week (Fricke et al. 1998b, Kohn et al. 1998). Angle between DEI and incremental growth lines in enamel is ~15˚ (e.g., Kierdorf and Kierdorf 1997), implying an outward growth rate of ~1 mm/25 days. From Kohn et al. (2003).

OXYGEN ISOTOPES IN BIOAPATITES

The oxygen isotopic composition of bioapatite depends on temperature and the isotopic composition of the fluid from which it precipitates. For mammals, there is a constant offset between the $\delta^{18}O$ of body water and PO_4 (~18‰ for mammals), and between PO_4 and CO_3 components of bioapatite (~8‰; Bryant et al. 1996, Iacumin et al. 1996a). Therefore, for modern samples or isotopically unaltered fossils, the PO_4 and CO_3 components are both equivalent measures of the $\delta^{18}O$ of the contributing organism. Both have been used to delineate modern isotopic trends.

Ecological trends

Oxygen isotope systematics in bioapatite were first investigated with carbonate shells (Longinelli 1965), which contain a small percentage of crystalline bioapatite. These data showed a clear dependence of isotope composition on temperature (Fig. 5a). It had been hoped that the isotope fractionations of PO_4 and CO_3 would have different temperature dependencies, so that a single shell could be used to determine both local water composition and temperature. Unfortunately, temperature dependencies of PO_4 and CO_3 fractionations are too similar (~0.23‰/˚C). However, later work (Longinelli and Nuti 1973a, Kolodny et al. 1983, Luz et al. 1984, Luz and Kolodny 1985, Lécuyer et al. 1996a) also showed that the temperature-dependence of PO_4 fractionations is indistinguishable among invertebrates, fish, and mammals. In general it is believed that rapid isotopic equilibration of PO_4 with body water at body temperature is achieved intracellularly via mechanisms involving ATP, which is common to all organisms. Certainly, studies of microbial use of PO_4 indicate very rapid changes to dissolved PO_4 $\delta^{18}O$ (Blake et al. 1997, 1998). Precipitation of Ca-phosphates then preserves the equilibrated PO_4 composition. Although there has been some investigation of ancient conodonts and brachiopods (Luz et al. 1984b, Picard et al. 1998, Wenzel et al. 2000), most work has focused on systematics in thermoregulating terrestrial vertebrates, because the known temperature of precipitation simplifies isotope interpretation.

The first bioapatite work on thermoregulators indicated, as expected, that isotope composition varies systematically with local water composition (Longinelli 1984, Luz et

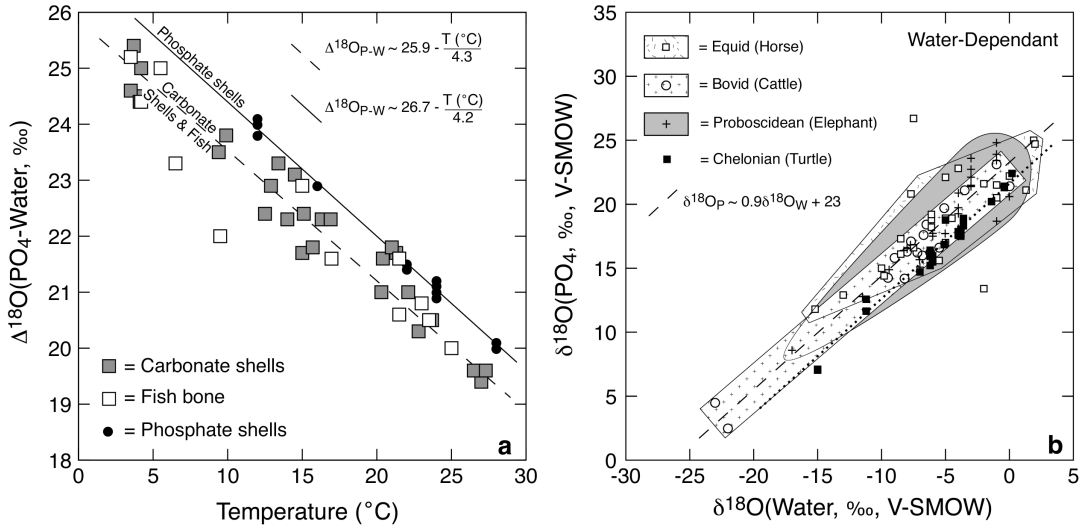


Figure 5. Plots of $\delta^{18}O$ of bioapatite vs. various environmental parameters. Almost all compositions for vertebrates are from modern bone PO_4. (a) Dependence of the $\delta^{18}O$ of the PO_4 component on temperature for invertebrates and fish. Isotopic offset between phosphate shells and other invertebrate bioapatite could be a species-specific difference, or could be an analytical artifact, as Lécuyer et al. (1996a) used the Ag_3PO_4 method, whereas Longinelli and Nuti (1973) and Kolodny et al. (1983) used the $BiPO_4$ method. Solid line is regression for phosphatic shells; dashed line is regression for carbonate shells and fish bone. Data from Longinelli and Nuti (1973a), Kolodny et al. (1983), and Lécuyer et al. (1996). (b-d) Plot of the $\delta^{18}O$ of the PO_4 component of bioapatite vs. local water for selected animals. Dashed line is regression of global data set of vertebrates, omitting turtles, deer, rabbits, and macropodids. (b) Obligate drinkers and turtles with greatest water dependence show a strong positive correlation between $\delta^{18}O$ of bioapatite and local water. The similar slope for turtles (dotted line) compared to mammals (dashed line) probably reflects behavioral thermoregulation and/or shell precipitation over a restricted temperature range. Data from Luz and Kolodny (1985), D'Angela and Longinelli (1990), Koch et al. (1991), Ayliffe et al. (1992), Bryant et al. (1994), Sánchez-Chillón et al. (1994), Delgado Huertas et al. (1995), Bocherens et al. (1996), Iacumin et al. (1996a), Barrick et al. (1999).

al. 1984a, Luz and Kolodny 1985). Humans were the first sources analyzed, but soon other animals were investigated, including cattle, pigs, sheep, mice, or whatever else was convenient and available (Longinelli 1984, Luz et al. 1984a, D'Angela and Longinelli 1990). These data all showed a reasonably simple relationship between bioapatite PO_4 and local water $\delta^{18}O$ (Fig. 5b-c). Bioapatite PO_4 calibration was solved and paleowater research could begin! However, three papers published nearly simultaneously and independently (Luz et al. 1990, Ayliffe and Chivas 1990, Koch et al. 1991) indicated that bioapatite isotopic systematics were not so simple after all. Bioapatite compositions can also depend critically on humidity (*h*, Fig. 5d-e), and different animals living in the same environment (i.e., same water and *h*) can have different isotope compositions (Fig. 5f). The variation seemed to be linked to the response of food source (plant) or surface water compositions to *h*, and possibly isotopic differences attending C_3 vs. C_4 diets.

More recent work has corroborated previous results and expanded the list of organisms. Rabbits show a strong *h*-dependence (Delgado Huertas et al. 1995), and sympatric animals with different diets and physiologies do have different isotope compositions, especially in arid settings (Kohn et al. 1996; Fig. 5f). Although an *h*-dependence is not obvious for some species, this may simply reflect small data sets. Humidity-dependencies have been found wherever they have been sought out, especially where data sets are large, although emphasis has been placed on drought-tolerant animals where *h*-dependencies should be highest. In the case of C_3 vs. C_4 consumption, the data of Bocherens et al. (1996) support a general trend towards a lower $\delta^{18}O$ with increased C_3

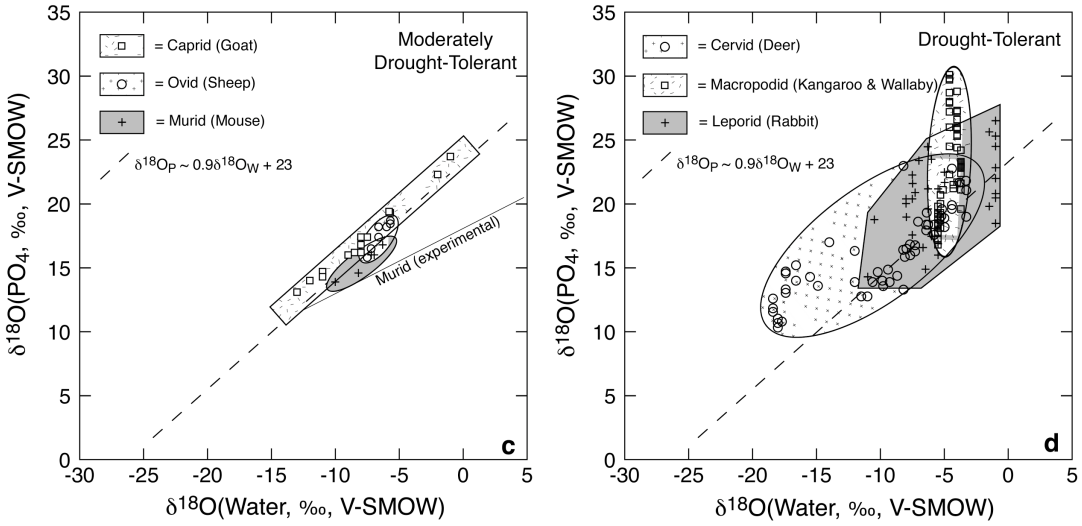
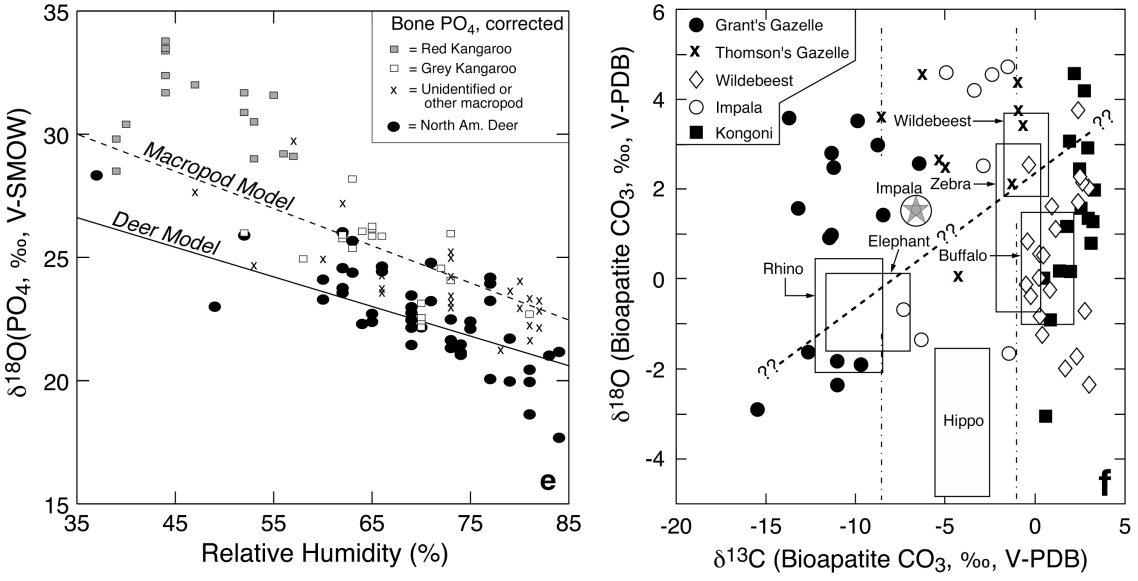


Figure 5, continued. Plots of $\delta^{18}O$ of bioapatite vs. various environmental parameters. Almost all compositions for vertebrates are from modern bone PO_4. (c and d) are plots of the $\delta^{18}O$ of the PO_4 component of bioapatite vs. local water for selected animals. Dashed line is regression of global data set of vertebrates, omitting turtles, deer, rabbits, and macropodids. (c) Moderately drought-tolerant animals show a strong positive correlation between $\delta^{18}O$ of bioapatite and local water. The thin solid line represents experimental results from laboratory mice; deviation from natural data may reflect how human-controlled settings cause isotopic deviations compared to natural settings. Data from Delgado Huertas et al. (1995) and D'Angela and Longinelli (1990). (d) Drought-tolerant animals show a poor correlation between $\delta^{18}O$ of bioapatite and local water. Data from Luz et al. (1990), Ayliffe and Chivas (1990), D'Angela and Longinelli (1990), and Delgado Huertas et al. (1995). (e) The $\delta^{18}O$ of the PO_4 component of bone for deer and macropodids re-plotted vs. humidity (*h*) after factoring out local water composition. The strong, negative correlation between $\delta^{18}O$ and *h* reflects influence of leaf water and leaf tissue composition on herbivore $\delta^{18}O$, as well as evaporative enrichment of local water at low *h*. The large variability in bioapatite composition at low humidity probably reflects uncertainties in assumed values of local water $\delta^{18}O$. Data from Luz et al. (1990) and Ayliffe and Chivas (1990). Lines show model predictions of humidity-dependencies (Kohn 1996). (f) $\delta^{18}O$ vs. $\delta^{13}C$ for the CO_3 component of bone and enamel for different sympatric species from East Africa. Boxes (multiple analyses) and circled star (single analysis) are for animals from Amboseli National Park (Koch et al. 1991, Bocherens et al. 1996), and illustrate a weak trend towards decreasing $\delta^{18}O$ of bioapatite CO_3 with decreasing $\delta^{13}C$, assuming that anomalously low $\delta^{18}O$ for hippos reflects aquatic adaptation or feeding ecology (Bocherens et al. 1996). More extensive data from tooth enamel for the Athi plains (individual points; T. Cerling, unpublished data) show greater scatter, and a tendency for some animals, such as gazelle, to plot far from the Amboseli line.



consumption. Because C_3 plants have a lower $\delta^{18}O$ than coexisting C_4 plants (Sternberg et al. 1994), the isotope trend was assumed to result from diet and incorporated in theoretical models (Kohn 1996). However, deviations from a simple correlation between $\delta^{18}O$ and $\delta^{13}C$ (Kohn et al. 1996) and the extreme variability exhibited by other animals (Fig. 5f) suggest that other factors play a major role. Data are now available for horses, goats, elephants, and turtles (Fig. 5b-c), as well as cetaceans (Yoshida and Miyazaki 1991, Barrick et al. 1992) and sharks (Vennemann and Hegner 1998, Vennemann et al. 2001). Interestingly, if bioapatite PO_4 compositions of turtles reflected mean annual temperature, then the turtle slope should be much shallower than that of mammals (Kohn 1996), because $\Delta^{18}O(PO_4\text{-water})$ increases with decreasing temperature (Fig. 5a), and lower temperatures correlate with lower meteoric water $\delta^{18}O$ (Dansgaard 1964). The steep turtle slope probably reflects behavioral maintenance of nearly constant body temperature, and/or preferential shell precipitation over a restricted temperature range. Thus it should not be assumed that heterothermic ("cold-blooded") animals can record different temperatures in different environments (i.e., contra models of Kohn 1996).

In sum, modern studies show that bioapatite $\delta^{18}O$ depends on: (1) water composition and temperature for non-thermoregulating aquatic organisms (as is well described for carbonate-secreters), or (2) water composition, humidity and, in some terrestrial vertebrates, diet.

Mass balance

Mass balance models (Luz and Kolodny 1985, Luz et al. 1990, Ayliffe and Chivas 1990, Cormie et al. 1994, Bryant and Froehlich 1995, Kohn 1996) provide a simple way of understanding oxygen isotope systematics in animal body water. Body water is the dominant control on bioapatite $\delta^{18}O$, because the isotopic compositions of the PO_4 and CO_3 components have a constant offset relative to the fluid from which the bioapatite precipitates. The key to these models is the identification of input and output oxygen components, and fractionations of these components with respect to two variables: local water [$\delta^{18}O(LW)$] and body water [$\delta^{18}O(BW)$]. The main input and output components are listed in Table 2, and are based on water turnover and energy expenditure studies of wild terrestrial herbivores. There are many other components, including water in plant stems (which is not very fractionated), oxygen in plant tissues (which is extremely fractionated), water vapor in inspired air, oxygen in urea, etc., but the seven factors listed in Table 2, normalized to 100%, are sufficient for illustration.

To maintain isotope equilibrium, $M(in)*\delta^{18}O(in) = M(out)*\delta^{18}O(out)$, where M is moles of oxygen. Summing terms in Table 2:

$$0.25 * [\delta^{18}O(BW) + 38.65] + 0.4 * [\delta^{18}O(BW) + 0] + 0.25 * [\delta^{18}O(BW) - 8.5] =$$
$$0.25 * 15 + 0.45 * [\delta^{18}O(LW) + 26.2 * (1 - h)] + 0.3 * \delta^{18}O(LW) \quad (1)$$

or, propagating physiological variability and uncertainties,

$$\delta^{18}O(BW) = 9.85 \pm 4.6 - 11.8 \pm 3.9 * h + 0.75 \pm 0.3 * \delta^{18}O(LW) \quad (2)$$

Several points are worth noting: (1) Predicting an isotope composition a priori is extremely uncertain for an animal whose physiology is poorly known, such as extinct taxa. Theoretically, $\delta^{18}O$(Bioapatite) for a species could be distinct, due to different offsets, *h* dependencies, or slopes on a $\delta^{18}O$(Bioapatite) vs. $\delta^{18}O$(LW) plot. However, the fact that many animals have similar compositions (Fig. 5b-c) indicates that physiological factors commonly offset each other. (2) There is a linear relationship among local water composition, *h*, and body water composition, with a direct *h*-dependence reflecting a leaf water source. (3) The slope of $\delta^{18}O$(BW) or $\delta^{18}O$(Bioapatite) vs. $\delta^{18}O$(LW) will be essentially 1 - (% air O_2), and so should be ~0.75 (Kohn 1996). The occurrence of many

isotope slopes ~0.9 (Fig. 5b-c) could indicate that animals take in more water than expected. In fact, domestic animals do consume more water per mole of air O_2 than their wild counterparts (e.g., Nagy and Petersen 1988), which would steepen slopes. Alternatively, not accounting for *h* could bias slopes if most of the high $\delta^{18}O$(Bioapatite) values occur in drier environments. (4) Measured *h*-dependencies are more extreme than predicted for some species (Fig. 5e). This probably reflects a *h*-dependency to the $\delta^{18}O$ of local water compositions, as local water will be enriched by evaporation in dry environments, a feature which is not considered in the predictive models.

Table 2. Most important oxygen input and output components affecting animal isotope compositions

Inputs	*Air oxygen*	*Leaf water*	*Drinking water*	
% of total[1]	25 ± 5	45 ± 15	30 ± 30	
$\Delta^{18}O$[2]	(fixed ~15‰)[3]	~26.2[4] * (1 - *h*)	0	
Outputs	*CO_2*	*Urine + sweat + fecal water*	*Exhaled water vapor*	*Transcutaneous water vapor*
% of total[1]	25 ± 5	40 ± 15	25 ± 10	10 ± 5
$\Delta^{18}O$[5]	38.65[6]	0	-8.5[7]	-18 ± 6[8]

[1]The percentage variability corresponds to actual different physiologies of animals (e.g., some are drought-tolerant, others require drinking water).

[2]Isotope fractionation of input component with respect to local water.

[3]Air O_2 has a composition of ~22.5‰, but there is a kinetic isotope fractionation of ~-7.5‰ associated with O_2 uptake in the lungs (Epstein and Zeiri 1988, Zanconato et al. 1992).

[4]Recent research is working to understand humidity dependencies better.

[5]Isotope fractionation of output component with respect to body water.

[6]Equilibrium fractionation between CO_2 and liquid water at 37°C.

[7]Assumes most loss is oral, but some is nasal.

[8]Virtually unmeasured; range indicates uncertainty in the correct value(s).

Isotopic zoning

Any perturbation to oxygen input or output amounts or $\delta^{18}O$ compositions will change the isotopic composition of bioapatite (Table 2, Eqn. 1, Eqn. 2) and will induce isotope zoning in teeth or other progressively precipitated phosphatic tissues. The most important perturbations are seasonal variations in local water composition and *h*. Other factors, such as temperature, do affect physiology (e.g., water turnover), but their isotopic effects are not well characterized. The isotopic expression of seasonal variations provides an isotopic proxy for seasonal climate, both in modern and fossil materials. This isotope zoning is extremely important, both because it must be accounted for when analyzing fossils (i.e., a single analysis is inadequate for average compositions unless it samples the entire length of the tooth), and because isotope seasonality can be a useful paleoclimatic indicator. Zoning studies have been conducted using both modern and fossil teeth (Koch et al. 1989, 1998; Fricke and O'Neil 1996; Fricke et al. 1998b; Kohn et al. 1998; Sharp and Cerling 1998; Feranec and MacFadden 2000, Gadbury et al. 2000, MacFadden 2000, Dettman et al. 2001; Bocherens et al. 2001; Fox and Fisher 2001). In general, large teeth can require an entire year to grow, whereas smaller teeth may form in only a single season. However, because different teeth may start growing at different times, analysis of several smaller teeth can sometimes retrieve the yearly seasonal signal (Kohn et al. 1998, Bocherens et al. 2001). Tusks are especially useful for seasonal isotope studies (Koch et

al. 1989, Koch et al. 1995b, Fox 2000, Fox and Fisher 2001) because the dentin (e.g., for elephants) and enamel (e.g., for gomphotheres) can potentially record decades of isotopic information.

Several factors damp climatically induced isotope seasonality. The most important are reservoir effects within the local environment and within the organism itself. Large water bodies such as rivers and lakes have less isotopic variability than seasonal precipitation. Drinking water from rivers and lakes or plants growing nearby will show less isotopic variability than would be predicted solely on the basis of seasonal variations in h or precipitation $\delta^{18}O$. Similarly, an organism cannot respond instantaneously to a change in the environment. The oxygen flux per day through an organism is ordinarily a small percentage (5-10%) of its total oxygen content. Therefore, compositional extremes within the environment will be smoothed, and a time-lag will develop between the predicted (instantaneous) and actual isotope composition of the species. For a sinusoidal variation in environmental composition with compositional amplitude ΔC, and a residence time τ, the damping factor D and time lag Δt will be (Albar de 1995),

$$D = \frac{\Delta C_{out}}{\Delta C_{in}} = \frac{1}{\left[\frac{2\pi\tau}{365} + 1\right]^{1/2}} \quad (1)$$

$$\Delta t = \frac{365}{2\pi}\tan^{-1}\left[\frac{2\pi\tau}{365}\right] \quad (2)$$

where τ (in days) is the total oxygen pool divided by the daily flux. If 5-10% of total oxygen is turned over daily, then τ is 10-20 days. Applying Equation (2), this implies that the isotope composition of an animal varies by only 85-90% of the total seasonal signal, and has a time lag of 2-3 weeks. Drought-tolerant animals (e.g., camels with τ~50 days) will have greater damping and a longer time lag, whereas extremely water-dependent animals (e.g., humans with τ~7 days) can potentially record climate in their phosphatic tissues with higher fidelity. However, a last damping factor is the precipitation of the enamel itself. The enamel maturation process can lead to significant time averaging, and thick enamel crowns can take months or even a year to form (Hillson 1996). Thus the highest fidelity can be obtained only for relatively thin enamel, or by using sampling techniques that remove a shallow band of enamel, where maturation is relatively fast.

Modern gazelle teeth from near Nairobi illustrate how teeth record yearly seasonality (Kohn et al. 1998; Fig. 6). In Kenya, the gazelle-birthing season and timing of tooth eruptions are well known, which allows the zoning patterns to be placed in a rough chronology. International Atomic Energy Agency (IAEA) stations provide seasonal water compositions for East Africa. Most locations in the world have either a single rainy or cold season, leading to a single isotope low, and a quasi-sinusoidal yearly isotope variation. However, this part of East Africa is a little unusual because it experiences a double rainy season, so that each year there are two isotope lows. Combining IAEA data with observations on gazelle diet, physiology, etc., permits construction of a theoretical isotope model. Such a model has large absolute uncertainties (e.g., predicted average composition for the year could vary by a couple permil), but relative differences among seasons are well resolved (uncertainties of much less than 1 permil). There are also uncertainties in the temporal placement of compositions (within the dashed line plus solid bar), because the same tooth can grow at different times in different individuals. Nonetheless, the data can be reconciled extremely well with the seasonal model, implying that seasonal climate change likely drives compositional changes recorded in this tooth enamel. Fricke et al. (1998b) also analyzed teeth from areas close to IAEA stations, and although they found that tooth enamel is ubiquitously zoned, the compositional zoning

could not always be modeled based solely on seasonal climate factors. Many teeth recorded smaller variability than predicted, especially in settings where water compositions changed very rapidly. The damping likely reflects reservoir effects, within the environment, the animal, or both. Apparently terrestrial vertebrates do not always record extremely rapid environmental changes well.

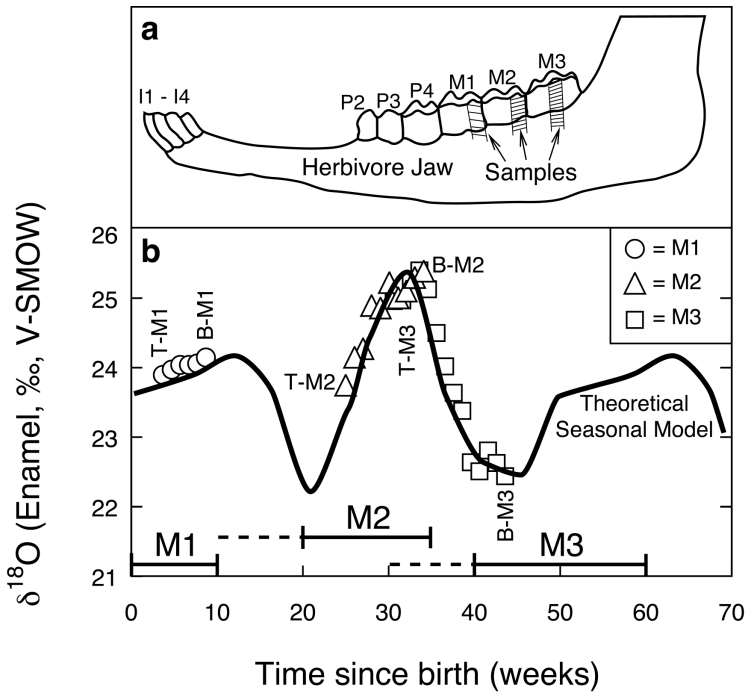


Figure 6. (a) Identity of teeth in a typical herbivore jaw. P2-P4 are premolars, M1-M3 are molars, and I1-I4 are incisors. Relative order of premolar and molar formation is: M1 < M2 < M3 < P2 ≤ P3 ≤ P4. Samples were collected by cutting a strip of enamel from molars, and sub-sampling at ~1-2 mm resolution. (b) Oxygen isotope zoning for bulk enamel from molars of a modern Nairobi gazelle vs. time since birth. By knowing the birth season of gazelles and when different teeth first erupt (i.e., appear above the gum line; solid bars), and by accounting for earlier growth prior to eruption (dashed line), data can be reconciled with a theoretical model of seasonal isotope compositions (thick black line). "T" and "B" refer to compositions from the top and base of each tooth crown. From Kohn et al. (1998), but with modifications to include reservoir effects within gazelles (Eqn. 1 and 2).

STRONTIUM ISOTOPES IN BIOAPATITES

Ordinarily, strontium isotope ratios ($^{87}Sr/^{86}Sr$) are considered radiogenic, because of the decay of ^{87}Rb to produce ^{87}Sr. However, because apatite accepts virtually no Rb, there is no radiogenic source able to change $^{87}Sr/^{86}Sr$ *in situ*. Thus, strontium can be considered a stable isotope, useful as an ecological or environmental tracer. Strontium is taken up significantly when bioapatite forms, but concentrations and isotopic compositions can also change after burial, with dentin and bone more strongly affected than enamel (see Trueman and Tuross, this volume). Therefore, applications must either (a) separate the original unaltered bioapatite crystallites, using chemical leaching (e.g., Sillen 1986, Sealy et al. 1991), or (b) assume that burial and diagenesis have no isotope effect or completely reset isotope compositions to pore-fluid compositions (e.g., Staudigel et al. 1985, Schmitz et al. 1991).

One application of Sr isotopes makes use of the change in seawater Sr isotope composition through time for age determinations (e.g., DePaolo and Ingram 1985). The

isotope composition of modern marine bioapatite is indistinguishable from modern seawater (e.g., Staudigel et al. 1985, Schmitz et al. 1991, Koch et al. 1992, Vennemann et al. 2001), so if unaltered material is analyzed, it can in theory be matched to the seawater Sr curve to define an age (Staudigel et al. 1985, Koch et al. 1992, Ingram 1995, Holmden et al. 1996, Vennemann and Hegner 1998, Martin and Haley 2000; Fig. 7). However, Sr isotope compositions of some bioapatites give erratic or inconsistent results compared to expected values for samples of known age, or in comparison with coexisting carbonate, possibly due to PO_4 recycling and Sr overprinting (Staudigel et al. 1985; Schmitz et al. 1991, 1997; Denison et al. 1993). Chemical leaching does not always remove extraneous Sr, even for enamel (Tuross et al. 1989, Koch et al. 1992, Elliot et al. 1998, Barrat et al. 2000). The solubility product of fluorapatite is less than that of hydroxyapatite (Moreno et al. 1977), and so the last material to be dissolved may well be diagenetic, rather than biologic.

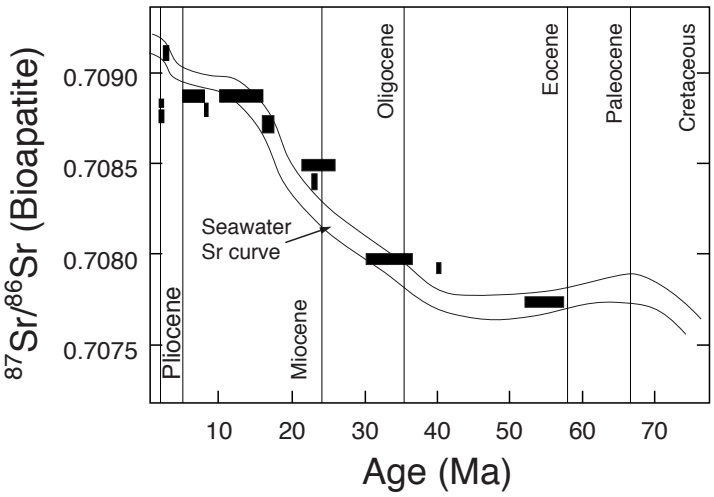


Figure 7. Strontium isotope compositions of fossil teeth and phosphatic debris vs. time, with the carbonate-based seawater Sr curve for reference. General correspondence between bioapatite and seawater $^{87}Sr/^{86}Sr$, and large variation in seawater $^{87}Sr/^{86}Sr$ suggests that isotope compositions of fossil marine bioapatites could be used geochronologically. However, several bioapatite compositions depart significantly from the seawater curve, even for Plio-Pleistocene samples, suggesting diagenetic alteration in many cases. From Staudigel et al. (1985).

Terrestrial $^{87}Sr/^{86}Sr$ is commonly elevated compared to marine $^{87}Sr/^{86}Sr$, because of radiogenic ^{87}Sr from old continental crust, and it is possible to use this $^{87}Sr/^{86}Sr$ difference as a measure of fish origin (Schmitz et al. 1991, 1997). Ocean water has a Sr concentration that is ~100 times higher than fresh water, so even a small marine component would overwhelm any freshwater isotope composition. However, freshwater fish consistently show higher $^{87}Sr/^{86}Sr$ compared to marine fish, permitting a freshwater vs. brackish/marine distinction (Schmitz et al. 1991, 1997). Similarly, fish living in different drainages exhibit isotopic differences correlated with bedrock $^{87}Sr/^{86}Sr$ (Kennedy et al. 1997).

Several studies have used isotope zoning, or differences in isotope compositions of samples of different individuals found together, to identify the occurrence and geographic patterns of migration (Ericson 1985; Sealy et al. 1991; Price et al. 1994a,b, 2000; Koch et al. 1995a; Ezzo et al. 1997; Grupe et al. 1997; Sillen et al. 1998; Hoppe et al. 1999). The main problem in validating such studies for fossil materials is whether the diagenetic overprint can be convincingly eliminated, and the general consensus is that it cannot for

bone (e.g., see Tuross and Trueman, this volume), except perhaps in some extraordinary settings. Enamel has greater potential, as it is less susceptible to diagenetic alteration. However, it is not immune to changes in trace element concentrations either (e.g., Kohn et al. 1999).

EXAMPLES AND APPLICATIONS

The Rapid Increase in C_4 Ecosystems (RICE)

The C_4 photosynthetic mechanism is a geologically rather recent development. Grasses had already evolved possibly as early as the Late Cretaceous and certainly by the early Eocene (Linder 1986, Crepet and Feldman 1991, Crepet and Herendeen 1992; see review of Jacobs et al. 1999). However, the earliest definitive C_4 macrofossils are from ~12 Ma (Dove Spring Formation, middle of Chron C5A; Nambudiri et al. 1978, Whistler and Burbank 1992, Jacobs et al. 1999; D. Whistler, pers. comm., 2001). All pre-mid-Miocene grasses apparently were C_3, yet today C_4 grasses dominate many low latitude ecosystems. Thus the origins and rate(s) of increased C_4 abundance worldwide constitute a major paleoecological issue. Because of the $\delta^{13}C$ difference attending C_3 vs. C_4 photosynthesis and because isotopic compositions of animals reflect the available plant ecosystem (i.e., their diet), $\delta^{13}C$ compositions of fossil bioapatites provide a critical line of evidence regarding when C_4 plants arose and how rapidly they spread. Reconstructing plant biomass from herbivore diet is not completely straightforward, however, because dietary selectivity filters the ecological signal (e.g., MacFadden et al. 1999). An absence of high $\delta^{13}C$ values does not imply an absence of C_4 plants, if animals simply chose not to eat them, or if they were not abundant. Nonetheless, if high $d^{13}C$ values are found, especially for a large animal with large daily food requirements, then C_4 plants must have been locally abundant.

Identifying clear C_4 consumption from stable isotopes in teeth first requires delineating the range of $\delta^{13}C$ values expected for pure C_3 consumers: only if $\delta^{13}C$ values exceed the range for a pure C_3 diet can a clear C_4 dietary component be inferred. The highest $\delta^{13}C$ values for enamel of modern-day large C_3 consumers is ~-8‰ [$\delta^{13}C(C_3$ plant) ≤ -22‰ plus a 14‰ offset; Fig. 3]. However fossil fuel burning since the industrial revolution has decreased the $\delta^{13}C$ of atmospheric CO_2 and modern plant tissues by at least 1‰ (Friedli et al. 1986, Marino and McElroy 1991). Thus a conservative isotopic cutoff for identifying ancient C_4 consumption from the $\delta^{13}C$ of herbivore tooth enamel is ~-7‰. This cutoff is extremely conservative because modern C_3 plants with $\delta^{13}C \geq$ -22‰ are rare. A pre-industrial bioapatite $\delta^{13}C$ of ~-7‰ for a pure C_3 feeder would imply either that an entire ecosystem was anomalous, or that an animal selectively fed on the anomalous plant(s) within an ecosystem.

Figure 8 summarizes work of Cerling et al. (1997) on the $\delta^{13}C$ of fossil herbivore teeth from ~20 Ma to the present, emphasizing equids and proboscideans, which consume C_4 plants if available. Clearly C_4 plants must have been rare on Earth before 8 Ma, as herbivore $\delta^{13}C$ values are all lower than -7‰ and all but one are lower than -8‰. Of course, if C_3 plants had $\delta^{13}C$ values of -24 to -26‰, then a small dietary component of C_4 may have been present since 14-19 Ma (Fig. 8; Morgan et al. 1994). However, by 6-8 Ma, herbivores in Pakistan, Africa, South America, and southern North America show a significant C_4 dietary component, indicating a Rapid Increase in C_4 Ecosystems (RICE[1])

[1] Ironically, rice (*Oryza sativa*) is C_3, but efforts are underway to genetically modify it to be more C_4-like, to improve grain yields.

worldwide. It is especially important that RICE was geologically rapid, but apparently not completely synchronous. C_4 plants were first evident at low latitudes and appeared later at higher latitudes (Cerling et al. 1997, Fig. 8).

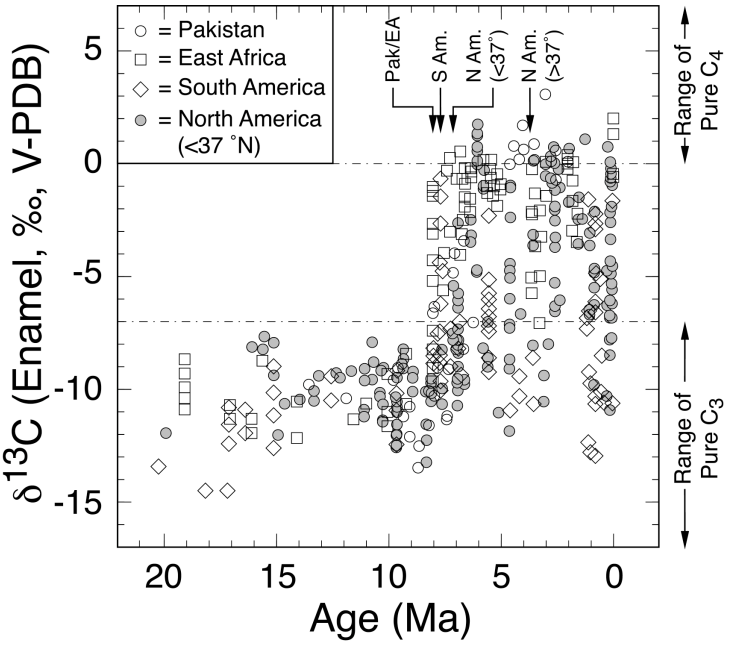


Figure 8. $\delta^{13}C$ of the CO_3 component of tooth enamel vs. age for global dataset of equids and elephantids at low latitudes, showing significant dietary change at 7-8 Ma. Arrows show times of shift to C_4-rich diets. High latitude data from western Europe show no isotopic shift, and data from North America north of 37 ˚N show a smaller shift at 3-4 Ma (data not shown). From Cerling et al. (1997).

Despite the occurrence of RICE during the latest Miocene, the mid-Miocene C_4 grass fossils (Nambudiri et al. 1978) and sporadic high $\delta^{13}C$ values up to -4‰ reported recently for 10-12 Ma teeth (Clementz and Koch 2001) imply that C_4 plants were already present on Earth before ~8 Ma. Thus the time between 8 and 6 million years ago evidently was a period of rapid C_4 biomass expansion, and not initial evolution. Although the shift observed in the first data from Pakistan was originally ascribed to tectonism (Quade et al. 1989, 1992), the later recognition of RICE's global character shifted focus to a global rather than regional chemical and/or climatic causes. It is extremely important that one of the main differences in C_3 vs C_4 plant physiology is the ability of C_4 plants to concentrate CO_2 in bundle sheath cells (Fig. 2). Because of this unique capability, decreasing CO_2 levels are a particularly attractive explanation for RICE (e.g., Ehleringer et al. 1991, Ehleringer and Cerling 1995, Cerling et al. 1997). If CO_2 levels dropped below some critical threshold, C_4 plants would have had a photosynthetic advantage, and could have spread rapidly. Furthermore, because C_4 plants compete better with C_3 plants at higher light levels, the CO_2 hypothesis also explains why RICE occurred first at low latitudes and spread to higher latitudes (Cerling et al. 1997).

The decreasing CO_2 hypothesis for RICE remains controversial. Recent work supports low CO_2 levels during the Miocene (Pagani et al. 1999a,b; Pearson and Palmer 2000) so that CO_2-limitation may well have promoted Miocene C_4 diversification and increased abundance. However, some studies suggest that CO_2 levels have been uniformly low since at least 20-25 Ma, prior to RICE, with no pronounced dip or trend to lower values at 6-8 Ma, (Pagani et al. 1999a,b; Pearson and Palmer 2000). So whereas a critical threshold may have been crossed at 7-8 Ma, other climatic triggers besides CO_2

levels continue to be considered. Two possibilities (Freeman and Colarusso 2001) are increased aridity, as C_4 plants are more drought-tolerant than C_3 plants, or increased seasonality with longer or warmer growing seasons. For the western US and Indian subcontinent, regional tectonics strongly influences aridity and climate seasonality, and mountain ranges and plateaus in these areas were evolving in the late Miocene. Thus, the rise of C_4 plants may perhaps in part reflect tectonic factors after all (Quade et al. 1989, 1992), although such a mechanism still has difficulty explaining the nearly simultaneous occurrence of RICE on several continents.

Terrestrial-marine climate coupling

An important goal in Earth science is to characterize past climates and to identify chemical or physical causes of their change (e.g., atmospheric chemistry, mountain building, ocean circulation patterns, orbital forcing, etc.). Most global climate research has focused on the marine record for two reasons: (1) climate directly impacts sea surface temperatures, ice volume, and carbon balance, and (2) marine carbonates are extremely common and well-preserved, and so provide a continuous record of Cenozoic climate via species abundances and stable isotopes of carbon and oxygen. However it is difficult to extrapolate from the oceans to the continents, and biogenic carbonates simply are not well represented or preserved in terrestrial sequences. In contrast, terrestrial bioapatites have been sampled on the continents for centuries in the form of fossil bones and teeth, and are well curated in paleontological collections. Thus, bioapatites provide an unparalleled resource for investigating continental paleoclimate, and allow the terrestrial and marine records of global climate to be linked.

One of the best-characterized links between marine and terrestrial paleoclimate concerns the Paleocene-Eocene thermal maximum (PETM). High-resolution marine cores delineated a rapid drop in benthic foraminifera $\delta^{13}C$ and $\delta^{18}O$ at ~55 Ma. The $\delta^{18}O$ drop corresponds to a deep-sea temperature rise of 5-6˚C in ten thousand years or less (Kennett and Stott 1991, Bralower et al. 1995, Thomas and Shackleton 1996). The cause is now inferred to be catastrophic methane release from clathrates on the continental margins (Dickens et al. 1995, 1997; Dickens 1999). Because methane is a greenhouse gas and has an extremely low $\delta^{13}C$ value (~-60‰), a large release would affect both temperature (oxygen isotopes) and carbon isotopes.

To examine the terrestrial record during the latest Paleocene, fossil teeth from the Paleocene-Eocene sequence of the Bighorn Basin, Wyoming were analyzed for $\delta^{13}C$ and $\delta^{18}O$ (Koch et al. 1992, 1995b; Fricke et al. 1998a). These data (Fig. 9) show that there was a simultaneous dip in carbon isotopes and bump in oxygen isotopes at the end of the Paleocene. Paleosol carbonates show similar trends. These isotope dips and bumps correspond temporally with the $\delta^{13}C$ and $\delta^{18}O$ marine spikes, as expected if terrestrial and marine climates responded in concert with a global PETM event. Specifically, if the global bioreservoir $\delta^{13}C$ dipped at 55 Ma, then all organic materials should record this event, including plant communities, herbivores, and marine carbonates. The increase in temperature should have caused a decrease in the $\delta^{18}O$ of marine carbonate, assuming ocean water composition did not change significantly, because Δ(carbonate-water) decreases with increasing temperature. However, because meteoric water $\delta^{18}O$ increases with increasing temperature, a global rise in temperature should have caused an increase in terrestrial bioapatite $\delta^{18}O$, as shown by the teeth. The PETM event also corresponds to an enormous faunal radiation at the boundary between the Clarkforkian and Wasatchian land mammal ages (Gingerich 1989). In North America, this boundary marks the first appearance of artiodactyls (antelopes, pigs, cattle, etc.), perissodactyls (horses, tapirs and rhinos), and true primates. This radiation implies that global climate change either helped drive evolution directly, or promoted major immigration and intercontinental faunal

exchange (Koch et al. 1995b). Terrestrial-marine paleoclimate links have also been investigated at the Permo-Triassic boundary (Thackeray et al. 1990, MacLeod et al. 2000)

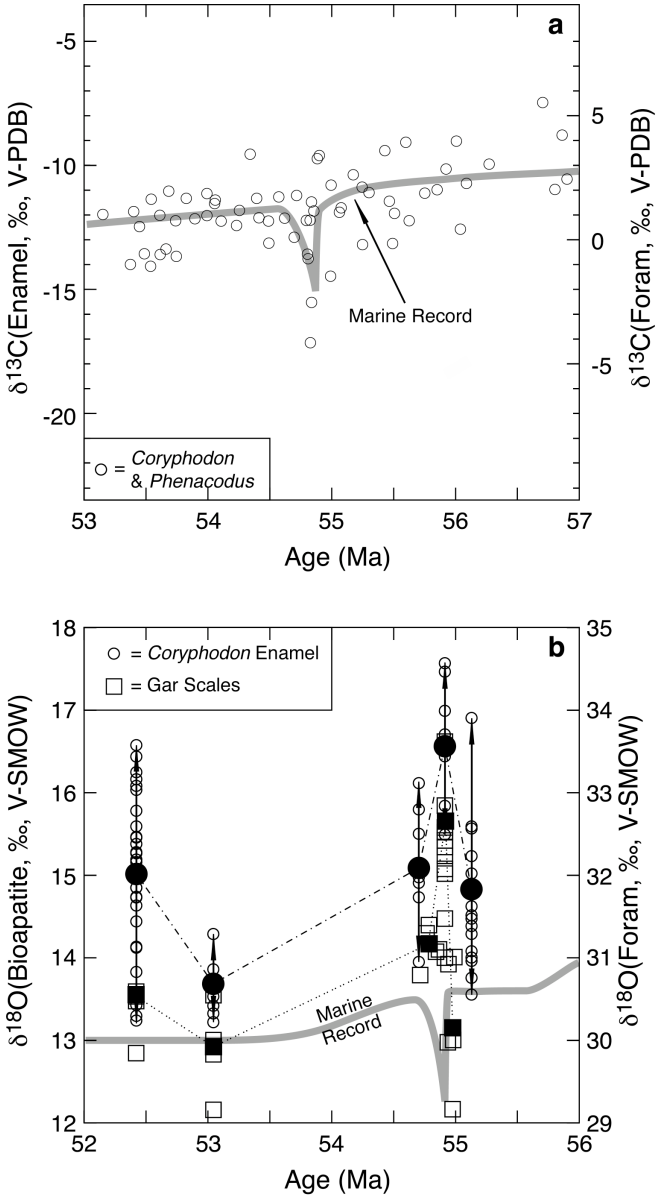


Figure 9. Marine vs. terrestrial isotope variations across the Paleocene-Eocene thermal maximum for (a) $\delta^{13}C$ of the CO_3 component of tooth enamel, and (b) $\delta^{18}O$ of the PO_4 component of tooth enamel and fish scales. Solid symbols in (b) are average $\delta^{18}O$. Note correlated dip in $\delta^{13}C$ for marine carbonates and for the large herbivores *Phenacodus* and *Coryphodon*, and correlated drop in marine carbonate $\delta^{18}O$ and rise in *Phenacodus* and gar $\delta^{18}O$. Terrestrial data from Koch et al. (1992, 1995b) and Fricke et al. (1998a). Marine data averaged from Shackleton et al. (1985), Kennett and Stott (1991), Bralower et al. (1995), and Thomas and Shackleton (1996).

Tectonics

Stable isotopes of bioapatites can be used to investigate tectonics via a paleoclimate link. One direct climate-tectonics link is the generation of rain shadows by mountain ranges (Kohn et al. 2003). Mountain ranges in western North America profoundly affect isotope compositions of meteoric water. The N-S trending Sierra Nevada, Cascades, and

Coast Ranges force east-traveling weather systems upward, cooling clouds and causing intense precipitation on western slopes, which produces a rain shadow on the eastern slopes and inland areas. Rainfall can be five times higher and humidity 30% greater on the coast compared to the eastern interior. Rayleigh distillation during rainout depletes clouds and subsequent rainfall in ^{18}O (Dansgaard 1964), so that precipitation in the eastern interior has a much lower $\delta^{18}O$ than along the coast (e.g., Sheppard et al. 1969, Coplen and Kendall 2000; Fig. 10). The degree of distillation (isotope depletion) is principally dependent on range height and lapse rate, but for modern heights, the difference in meteoric water compositions across these ranges is ~7-8‰.

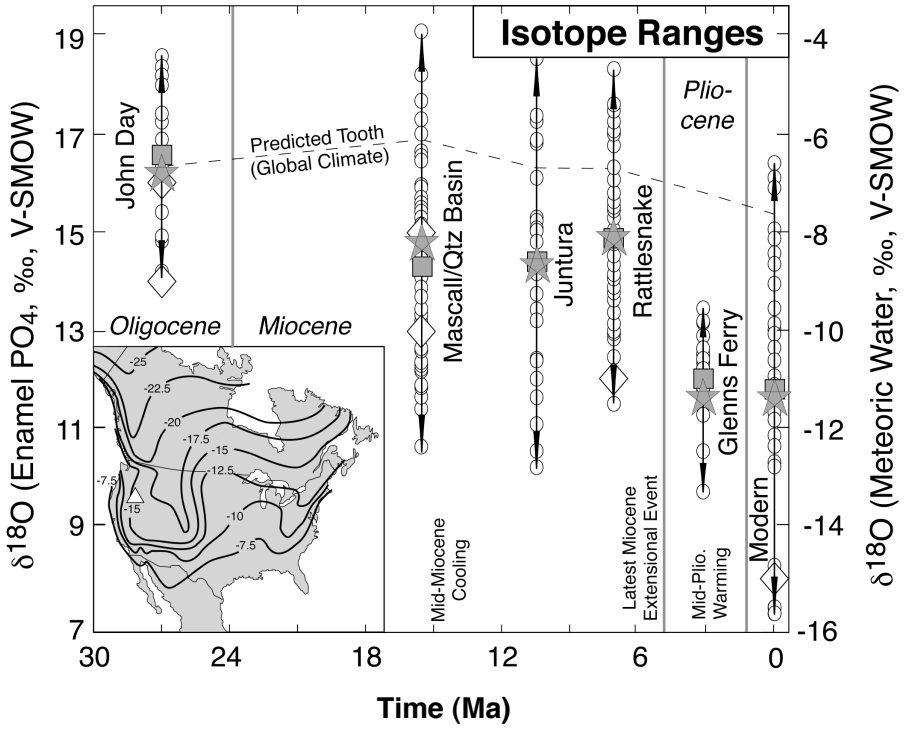


Figure 10. $\delta^{18}O$ of enamel PO_4 for fossil equids from central Oregon and western Idaho, illustrating a systematic shift through time. Open circles are individual measurements, shaded squares are mean and median compositions (indistinguishable), and stars are midpoint compositions. Compositional ranges reflect isotopic zoning within teeth as well as isotopic differences among teeth of same age. Water compositions (open diamonds) are inferred based on bioapatite compositions and humidity estimates from paleosols and paleoflora. Dashed line shows predicted shift to tooth $\delta^{18}O$ resulting solely from secular changes to global climate. Data from Kohn et al. (2003). Inset shows $\delta^{18}O$ isopleths for meteoric water across North America (modified from Sheppard et al. 1969, Coplen and Kendall 2000). Triangle on the map shows the approximate study area.

Kohn et al. (2003) investigated the development of the Cascade rain shadow by analyzing fossil equid teeth in interior Oregon and western Idaho. These teeth show a ~5‰ decrease in midpoint and mean $\delta^{18}O$ compositions since the late Oligocene, essentially in 2 periods: between 27 and 15.4, and between 7.2 and 0 Ma (Fig. 10). In the intervening mid- to late-Miocene, tooth $\delta^{18}O$ essentially stabilized. Large changes in isotope ranges are also apparent, with ≥7‰ seasonal and yearly variability in the mid-Miocene, decreasing to only 3-4‰ in the mid-Pliocene, and expanding to ~8‰ in modern samples.

In contrast to the PETM, there is little evidence for a global climate signal in these data. Marine records of warming from the Oligocene to Miocene are not reflected in any increase in bioapatite $\delta^{18}O$, and cooling through the Miocene caused no obvious change

to mean or median compositions. Semiquantitative modeling of predicted meteoric water and tooth isotope compositions, accounting for both temperature and ice volume effects suggests that global climate change since 27 Ma should have decreased tooth $\delta^{18}O$ by only ~-1‰. That is, of the 5‰ decrease in tooth $\delta^{18}O$ that occurred since 27 Ma, only ~1‰ can be ascribed to changes in source (coastal) water compositions resulting from global climate change. The remaining ~4‰ must instead result from monotonic uplift in topography, first between 27 and 15.4 Ma, and then further between 7.2 and 0 Ma. One important feature is the constancy of mean isotope compositions between 15.4 and 7.2 Ma, which corresponds with a change in volcanic style and/or abundance (e.g., Priest 1990). Arc volcanism was abundant from ~35 to ~18 Ma, and ~8 to 0 Ma. During 18 and 8 Ma, the Columbia River flood basalts erupted (mainly 18-14 Ma). Thus, the Cascades appear to have gained height and impacted isotope compositions when the arc was volcanically active, but changed height very little when the Columbia River Basalts erupted.

The observed isotope shift in tooth compositions (~4‰) is much smaller than the modern-day isotope difference for rainwater across the Cascades (~7‰). This could be because the range had already begun forming prior to the oldest samples at 27 Ma, but a more likely explanation involves the dependence of bioapatite $\delta^{18}O$ on water composition and relative humidity. Global correlations between bioapatite and local water $\delta^{18}O$ suggest a ~4‰ shift in bioapatite composition corresponds to a larger ~4 1/2‰ shift in water composition (Fig. 5b). Development of the rain-shadow over central Oregon also caused a decrease in relative humidity of ≥ ~15%, as indicated by paleoflora and paleosols (e.g., Ashwill 1983, Retallack et al. 2000). Theoretical models (Kohn 1996) and observations for deer (Luz et al. 1990; Fig. 5e) indicate a ~0.1-0.2‰ increase in bioapatite $\delta^{18}O$ for each 1% decrease in relative humidity. The ≥ ~15% decrease in humidity documented by flora and paleosols translates into a ≥ 1 1/2 - 3‰ increase in bioapatite $\delta^{18}O$ since 27 Ma. Subtracting off the humidity effect and accounting for the fact that bioapatite and local water do not have a 1:1 correlation implies that the 4‰ decrease in bioapatite $\delta^{18}O$ likely reflects a decrease in meteoric water $\delta^{18}O$ of ≥ 6-8‰. The similarity of the inferred shift to the modern isotope effect across the range implies that most topographic uplift occurred subsequent to 27 Ma.

Another link between climate and tectonics is the generation of monsoons by plateaus. The three major plateaus on Earth (Tibet, Puna/Altiplano, and Colorado) are each associated with a strong monsoon (in southeast Asia, central South America, and southwestern North America, respectively). The two other monsoons on Earth are either very weak (West Africa), or arguably driven by the Tibetan plateau (northern Australia). So investigations of isotope seasonality as a proxy for climatic seasonality can potentially elucidate the rates of development of a seasonal monsoon, and its attendant plateau. In general this climate-tectonics relationship has been rarely exploited, but Dettman et al. (2001) did show that isotope seasonality in fossil equid teeth and freshwater clam shells of the Indian subcontinent has remained essentially unchanged for over 10 million years, implying that the Tibetan plateau was already sufficiently broad and high by then to cause a monsoon. Thus, Dettman et al.'s data contradict popular models of geologically rapid rise at 7-8 Ma or later (although the plateau may have gained additional height or extent after 10 Ma), and limit the range of tectonic processes that led to its formation.

Dinosaur thermoregulation

A perennial physiological question concerning dinosaurs is whether they were capable of maintaining relatively uniform temperatures throughout their bodies (homeothermy; nominally taken to be ±2˚C) via intrinsic mechanisms (endothermy). Thermoregulation in fact can be achieved by a variety of mechanisms. Even ectotherms

(animals that are incapable of internally maintaining temperature) can sometimes roughly regulate temperature via behavior (e.g., turtles, crocodiles, etc.), or high thermal mass (gigantothermy; Spotila et al. 1991). Therefore, assessing endothermy is not simple. Two isotopic approaches have been used.

Barrick and Showers (1994) noted that endotherms maintain a nearly constant temperature *throughout* their bodies, whereas homeothermic ectotherms maintain uniform temperatures only in their body cores. Temperatures in ectotherms could be significantly different in their core vs. extremities, in contrast to endotherms. Because oxygen isotopic compositions of bioapatites precipitated from a uniform fluid reservoir are temperature-dependent (Longinelli 1965), ectotherms are expected to have more variable compositions, whereas endotherms should have uniform compositions. Thus, Barrick and Showers (1994) suggested that isotopic differences within and among bones of an individual could be used to identify endothermy. Application to several different dinosaur genera shows relatively uniform isotopic compositions, at least within their cores (core homeothermy), and similar but somewhat cooler or more variable temperatures in their limbs (regional heterothermy), in contrast to lizards that showed variable temperatures throughout their bodies (Barrick and Showers 1994, 1995, 1999; Barrick et al. 1996, 1998; Fig. 11). From these data Barrick and coworkers conclude that dinosaurs were basically endothermic, and that endothermy was accomplished by high metabolisms, but that metabolisms were perhaps not as high as in modern day birds or mammals. Others have criticized their results, partly because of issues regarding the potential for alteration of PO_4 oxygen in bones (Kolodny et al. 1996), and partly because a > ± 2 ˚C temperature variability for the "dinosaurian endotherms" cannot be rejected statistically (Ruxton 2000). Nonetheless, the apparent preservation of isotopic differences among bones of an ecotherm, but not in dinosaurs, does suggest that dinosaurs had a different metabolism, and were capable of at least crude endothermy.

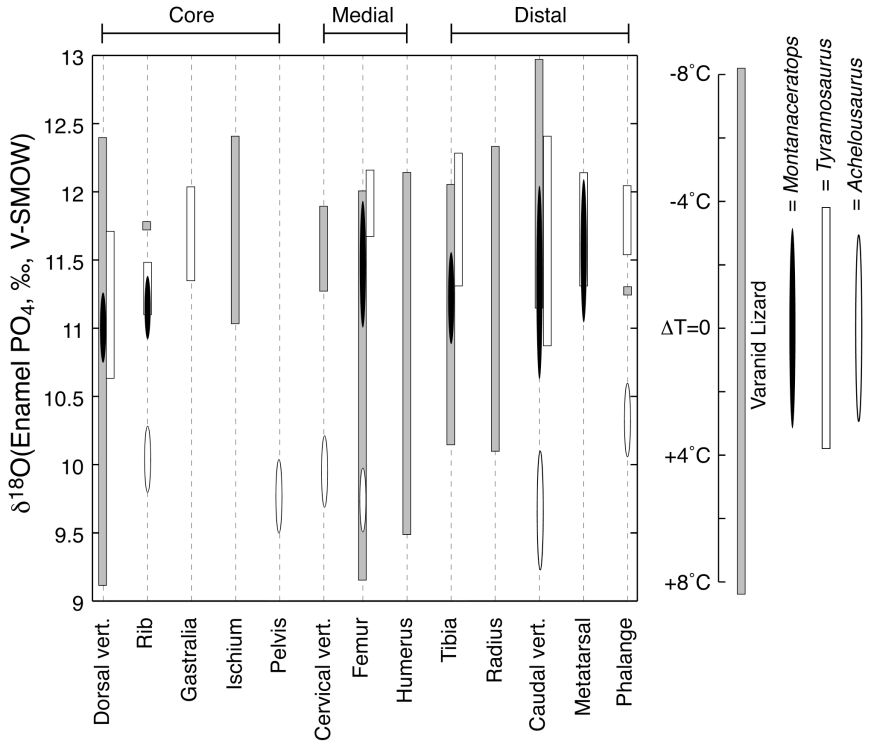


Figure 11. $\delta^{18}O$ of the PO_4 component of bone vs. bone identity, from core to extremities for a ~1 m long heterothermic lizard (Varanid) and three dinosaurs: a 1-2 m *Montanaceratops*, a <6 m juvenile *Achelousaurus*, and a ~12 m *Tyrannosaurus*. Large variation in bioapatite composition for lizard corresponds to ~±4˚C (1σ) variability (right side) and is consistent with heterothermy. Smaller →→

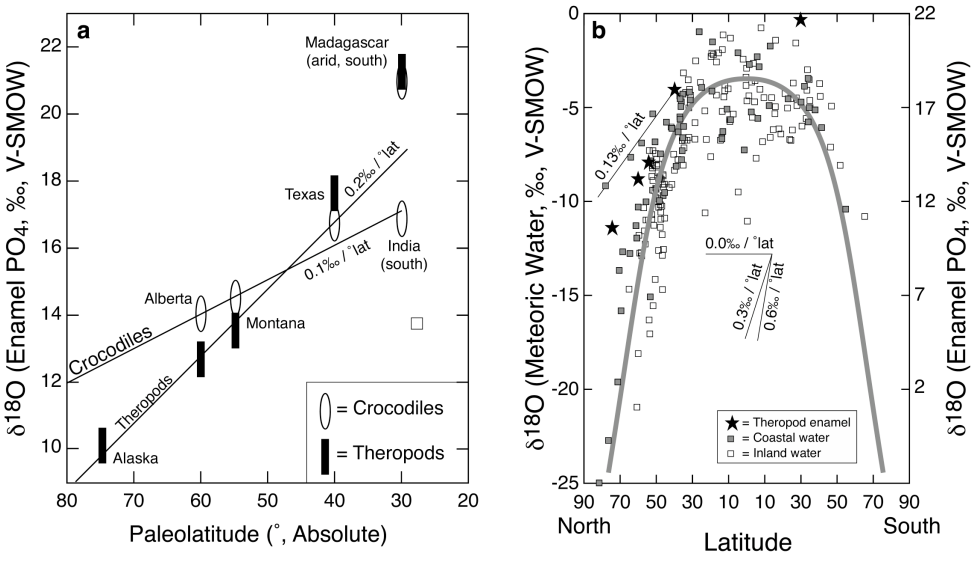


Figure 12. (a) $\delta^{18}O$ of the PO_4 component of theropod and crocodile teeth vs. latitude. Fossil theropod dinosaur enamel shows a steeper isotope gradient than do coexisting crocodile teeth. $\delta^{18}O$ should be shifted to higher values at colder temperatures (Fig. 5a). Assuming that heterothermic crocodile body temperature parallels local temperature, the different slopes imply theropods were better able to regulate body temperature than crocodiles, i.e., dinosaurs may have been homeotherms. (b) $\delta^{18}O$ of meteoric water and the PO_4 component of theropod enamel vs. latitude. Assuming homeothermy, the shallower slope for theropods (~0.2‰/° lat.) compared to average meteoric water at similar latitudes today (~0.3-0.6‰/° lat.) implies greater poleward heat transport in Cretaceous oceans and atmosphere compared to today. The shallowest modern slope for modern meteoric water at intermediate to high latitudes (0.13‰/° lat.; thin line) reflects unusually high poleward heat transport in the north Atlantic. Fossil tooth data from Fricke and Rogers (2000). Squares are meteoric water data from Rozanski et al. (1993).

Fricke and Rogers (2000) proposed that tooth compositions of dinosaurs at different latitudes (i.e., at differing mean annual temperatures) could be compared to those of sympatric crocodiles, which are known ectotherms. They reasoned that because bioapatite $\delta^{18}O$ fractionations increase with decreasing temperature (Longinelli 1965; Fig. 5a), a variable-temperature ectotherm should have a higher $\delta^{18}O$ relative to a constant-temperature endotherm at progressively colder temperatures. A similar isotopic offset, independent of latitude would indicate that dinosaurs had similar average temperatures as crocodiles, so dinosaurs probably were ectotherms. If instead dinosaurs had progressively lower $\delta^{18}O$ compositions at higher latitudes in comparison to crocodiles, then dinosaurs were probably endotherms. Fricke and Rogers indeed found that Cretaceous theropod dinosaurs show less isotopic change with latitude than crocodiles (Fig. 12), implying a greater degree of homeothermy. Criticism (Barrick and Kohn 2001) has focused on some of the details of interpreting isotope compositions for different species: do crocodile compositions accurately reflect mean annual temperatures, could behavioral or adaptive strategies produce different isotope slopes for different species, etc.? Nonetheless, data from Barrick, Fricke, and coworkers have generally supported dinosaurian endothermy. Certainly compositional comparisons of dinosaurs vs. known ectotherms show clear differences (Fig. 11, 12).

variation in bioapatite composition for dinosaurs corresponds to $<\pm 2^{\circ}C$ (1σ) temperature variability (right side) and is consistent with homeothermy. Bioapatite compositional variability in dinosaurs is independent of both body size and ontogeny, strongly suggesting that dinosaurs were endotherms. Data from Barrick and Showers (1994, 1995) and Barrick et al. (1996).

Fricke and Rogers' work also has implications for global heat distributions in the past. The Cretaceous latitudinal gradient in theropod isotope composition (~0.2‰ per degree latitude) is shallower than observed for meteoric water on Earth today, except in settings such as the North Atlantic, where ocean currents transport an unusual amount of heat pole-ward (Fig. 12). If Fricke and Rogers' results are typical for the Cretaceous, then bioapatite isotope compositions can be used to identify increased or reduced pole-ward heat transport in the past and possibly latitudinal temperature gradients within the continents.

CONCLUSIONS

The isotope compositions of bioapatite depend on ecology, physiology, climate, and geology, and consequently bioapatite $\delta^{13}C$, $\delta^{18}O$, and $^{87}Sr/^{86}Sr$ compositions have the potential to inform processes within and at the interfaces of all these disciplines. In general, the basic controls on isotopic compositions of bioapatites are known. Carbon isotopes closely reflect diet, and can be used in a variety of (paleo)ecological studies. Oxygen isotopes are more complicated, but are known to depend on local water compositions, climate (humidity), and diet. In some instances, these dependencies are sufficiently well known to investigate ecology and climate. Original strontium isotopes reflect the soil/bedrock substrate on which an animal lives, but can be rapidly altered diagenetically, at least for bone and dentin. The original biogenic signal can potentially help delineate sample age (for marine settings), migratory patterns, and/or habitat use. In terms of materials, diagenetic bias is common for bone and dentin, but by all indications enamel is extremely resistant to isotopic exchange. Consequently, most workers are focusing on enamel for retrieving original compositions, although this does require assessing seasonal isotope variations. Note, however, that the diagenetic imprint has its own usefulness (Tuross and Trueman, this volume).

Lingering unresolved issues remain regarding isotope studies of bioapatite. The biological fractionation of carbon isotopes remains ambiguous, and whereas a relatively pure C_3 diet can be readily distinguished from a relatively pure C_4 diet, definitive identification of differing ecosystems (e.g., closed canopy vs. savannah) is not always possible. A reconciliation of laboratory and field observations is necessary. The dependence of oxygen isotope compositions on water composition, climate, and diet is rather rudimentary, which essentially restricts work to comparisons of one taxon through time (to eliminate physiological effects), or of sympatric taxa at selected times. There are ongoing studies of large animals, both wild and domestic (e.g., Hoppe and Amundsen, 2001), but complete results are not yet available because of the long times necessary for the animals to mature. Last, diagenesis is obvious both physically and chemically in virtually all fossils, yet the mechanisms by which bioapatite crystallites change size and composition post-mortem is only vaguely understood. A detailed understanding of the crystal-chemical mechanisms of alteration might permit improved methods of retrieving original biologic signals, and help fully realize the potential of bioapatites in studying (paleo)ecology, (paleo)climate, and (paleo)diet.

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